09/194957

ISSUE CLASSIFICATION	
Class	Subclass
514	16

PATENT NUMBER

6362164

6362164

U.S. UTILITY PATENT APPLICATION

3 PUP O.I.P.E.
SCANNED ____ Q.A. mc

PATENT DATE
MAR 26 2002

SECTOR	CLASS	SUBCLASS	ART UNIT	EXAMINER
	514	16	1631	Borin

FILED WITH: ☐ DISK (CRF) ☐ FICHE
(Attached in pocket on right inside flap)

BEST COPY

PREPARED AND APPROVED FOR ISSUE

ISSUING CLASSIFICATION

ORIGINAL		CROSS REFERENCE(S)	
CLASS	SUBCLASS	CLASS	SUBCLASS (ONE SUBCLASS PER BLOCK)
514	16	530	311
INTERNATIONAL CLASSIFICATION		514	02
A61K 38/31		540	456
A61K 38/08			
/			
/			
/			

☐ Continued on Issue Slip Inside File Jacket

☐ TERMINAL DISCLAIMER	DRAWINGS			CLAIMS ALLOWED	
	Sheets Drwg.	Figs. Drwg.	Print Fig.	Total Claims	Print Claim for O.G.
	N/A	N/A	—	13	1

☐ a) The term of this patent subsequent to _____ (date) has been disclaimed.

_____ (Assistant Examiner) _____ (Date)

NOTICE OF ALLOWANCE MAILED

10-19-01

☐ b) The term of this patent shall not extend beyond the expiration date of U.S. Patent No. _____.

MICHAEL BORIN, PH.D
PRIMARY EXAMINER
_____ (Primary Examiner) 10/05/01 (Date)

ISSUE FEE (W)

Amount Due	Date Paid
1280.00	1-15-02

☐ c) The terminal ____ months of this patent have been disclaimed.

_____ (Legal Instruments Examiner) 10/9/01 (Date)

ISSUE BATCH NUMBER

N103

WARNING:
The information disclosed herein may be restricted. Unauthorized disclosure may be prohibited by the United States Code Title 35, Sections 122, 181 and 368. Possession outside the U.S. Patent & Trademark Office is restricted to authorized employees and contractors only.

Form PTO-436A
(Rev. 6/98)

ISSUE FEE IN FILE

(LABEL AREA)

6,362,164

COMBINATION OF A SOMATOSTATIN ANALOGUE AND A RAPAMYCIN

Transaction History

Date	Transaction Description
12-07-1998	Initial Exam Team nn
12-07-1998	Preliminary Amendment
12-07-1998	Receipt of 371 Request
12-14-1998	371 Application Preexamination Docketing
12-15-1998	IB Paper Match
01-20-1999	371 Application Preexamination Docketing
01-20-1999	371 Application Preexamination Docketing
05-03-1999	371 Application Preexamination Docketing
05-04-1999	Released to OIPE
05-05-1999	Notice of DO/EO Acceptance Mailed
05-12-1999	Preliminary Amendment
05-19-1999	Application Dispatched from OIPE
05-19-1999	IFW Scan & PACR Auto Security Review
06-03-1999	Case Docketed to Examiner in GAU
12-23-1999	Case Docketed to Examiner in GAU
02-14-2000	Mail Non-Final Rejection
02-14-2000	Non-Final Rejection
06-13-2000	Response after Non-Final Action
06-22-2000	Date Forwarded to Examiner
08-22-2000	Mail Non-Final Rejection
08-22-2000	Non-Final Rejection
01-25-2001	Response after Non-Final Action
01-25-2001	Request for Extension of Time - Granted
02-01-2001	Date Forwarded to Examiner
04-03-2001	Final Rejection
04-04-2001	Mail Final Rejection (PTOL - 326)
07-05-2001	Response after Final Action
07-11-2001	Date Forwarded to Examiner
07-16-2001	Examiner Interview Summary Record (PTOL - 413)
08-06-2001	Amendment/Argument after Notice of Appeal
08-06-2001	Notice of Appeal Filed
08-06-2001	Request for Extension of Time - Granted
08-10-2001	Advisory Action (PTOL-303)
08-13-2001	Mail Advisory Action (PTOL - 303)
09-19-2001	Examiner Interview Summary Record (PTOL - 413)
10-19-2001	Receipt into Pubs
10-19-2001	Receipt into Pubs
10-19-2001	Mail Notice of Allowance
10-19-2001	Notice of Allowance Data Verification Completed
11-02-2001	Workflow - File Sent to Contractor
12-14-2001	Receipt into Pubs
01-15-2002	Issue Fee Payment Verified
01-15-2002	Issue Fee Payment Received
02-18-2002	Application Is Considered Ready for Issue
02-19-2002	Receipt into Pubs
03-08-2002	Issue Notification Mailed
03-26-2002	Recordation of Patent Grant Mailed
03-26-2002	Patent Issue Date Used in PTA Calculation
09-21-2005	Post Issue Communication - Certificate of Correction

MB

09/194957

10

PATENT APPLICATION

09194957

INITIALS _____

CONTENTS

		Date received (Incl. C. of M.) or Date Mailed			Date received (Incl. C. of M.) or Date Mailed
1.	Application _____ papers.		42.		
2.	903	05 MAY 1999	43.		
3.	Rul Amdt A	12-7-98	44.		
4.	Re amdt B	05-12-99	45.		
5.	Rej 3 MOS	2/14/00 2/14	46.		
6.	Amdt C	6/13/00 CFM 6/9	47.		
7.	Rej 3 mos	9-22-00 8/22	48.		
8.	2mo ext of time/amdt D	1.25.01	49.		
9.	final Rej 3mos	4-4-01 4/3	50.		
10.	of amend E (~~RCE~~)	7.5.01	51.		
11.	int summ	7.16.01	52.		
12.	Advisory Action	8-13-01 8/10	53.		
13.	cont'd	8.6.01	54.		
14.	Notice of Appeal	8-6-01	55.		
15.	request f reconsideration	8/[illegible]/01	56.		
16.	int summ	9/19/01	57.		
17.	Allowance	10.19.01 10/	58.		
18.	Req. for corr	7/15/02	59.		
19.			60.		
20.			61.		
21.			62.		
22.			63.		
23.			64.		
24.			65.		
25.			66.		
26.			67.		
27.			68.		
28.			69.		
29.			70.		
30.			71.		
31.			72.		
32.			73.		
33.			74.		
34.			75.		
35.			76.		
36.			77.		
37.			78.		
38.			79.		
39.			80.		
40.			81.		
41.			82.		

(LEFT OUTSIDE)

ISSUE SLIP STAPLE AREA (for additional cross references)

POSITION	INITIALS	ID NO.	DATE
FEE DETERMINATION			
O.I.P.E. CLASSIFIER			
FORMALITY REVIEW			

INDEX OF CLAIMS

✔ ..... Rejected
= ..... Allowed
— (Through numeral)... Canceled
÷ ..... Restricted
N ..... Non-elected
I ..... Interference
A ..... Appeal
O ..... Objected

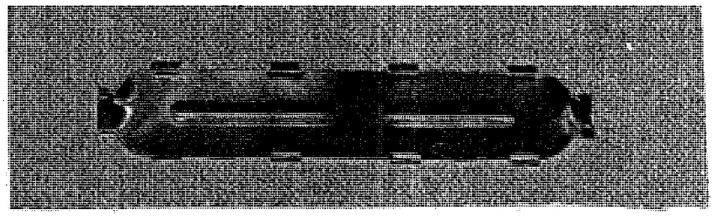
Claim Final	Claim Original	Date
	1	02/08/00 04/10/01
	2	
	3	
	4	
	5	
	6	✓ ✓ ✓
	7	
	8	✓
	9	✓
	10	✓
	11	
	12	✓
	13	✓
	14	
	15	✓ ✓ ✓
	16	
	17	
	18	✓
1	19	=
2	20	
3	21	
4	22	
5	23	
6	24	
7	25	
8	26	
9	27	
10	28	
11	29	
12	30	
13	31	=
	32	
	33	
	34	
	35	
	36	
	37	
	38	
	39	
	40	
	41	
	42	
	43	
	44	
	45	
	46	
	47	
	48	
	49	
	50	

Claim Final	Claim Original	Date
	51	
	52	
	53	
	54	
	55	
	56	
	57	
	58	
	59	
	60	
	61	
	62	
	63	
	64	
	65	
	66	
	67	
	68	
	69	
	70	
	71	
	72	
	73	
	74	
	75	
	76	
	77	
	78	
	79	
	80	
	81	
	82	
	83	
	84	
	85	
	86	
	87	
	88	
	89	
	90	
	91	
	92	
	93	
	94	
	95	
	96	
	97	
	98	
	99	
	100	

Claim Final	Claim Original	Date
	101	
	102	
	103	
	104	
	105	
	106	
	107	
	108	
	109	
	110	
	111	
	112	
	113	
	114	
	115	
	116	
	117	
	118	
	119	
	120	
	121	
	122	
	123	
	124	
	125	
	126	
	127	
	128	
	129	
	130	
	131	
	132	
	133	
	134	
	135	
	136	
	137	
	138	
	139	
	140	
	141	
	142	
	143	
	144	
	145	
	146	
	147	
	148	
	149	
	150	

If more than 150 claims or 10 actions staple additional sheet here

(LEFT INSIDE)



SEARCHED

Class	Sub.	Date	Exmr.
514	15 ~~18~~	02/00	MG
updated	08/00	MB	
updated	04/01	MB	
updated	10/01	MMS.	

INTERFERENCE SEARCHED

Class	Sub.	Date	Exmr.
514	16	10/01	MMS.

SEARCH NOTES
(INCLUDING SEARCH STRATEGY)

	Date	Exmr.
STN Van Line reports enclosed	02/00	MB

(RIGHT OUTSIDE)

04/02/01 M. Borin

CAPLUS COPYRIGHT 2001 ACS
AN 1997:37048 CAPLUS
DN 126:54378
TI Paclitaxel combination therapy in the treatment of metastatic breast cancer
AU Holmes, Frankie Ann
CS The University of Texas M.D. Anderson Cancer Center, Houston, TX, 77030-4009, USA
SO Semin. Oncol. (1996), 23(5, Suppl. 12), 29-39
CODEN: SOLGAV; ISSN: 0093-7754
PB Saunders
DT Journal; General Review
LA English
AB A review with 56 refs. After the single-agent activity of paclitaxel (Taxol; Bristol-Myers Squibb Company, Princeton, NJ) was confirmed, trials to develop a **synergistic** combination began. Doxorubicin, the most active agent for breast cancer, was studied first. As paclitaxel became more available, other combinations, including high-dose regimens and adjuvant therapies, have been studied. No optimal combination regimen has been defined. Recent and/or ongoing trials are looking at paclitaxel in combination with cisplatin, cyclophosphamide, 5-fluorouracil/folinic acid, and mitoxantrone combinations, as well as with high-dose regimens and as adjuvant therapy. This review describes a plethora of combination studies finally under way to better define the optimal use of paclitaxel in breast cancer therapies, both as adjuvant treatment and for metastatic disease. Because of the **unpredictable** nature of drug interactions related to schedule and sequence, ad hoc combinations should not be undertaken outside the context of a well-designed trial.

04/02/01 M. Borin

CAPLUS COPYRIGHT 2001 ACS
AN 1990:512336 CAPLUS
DN 113:112336
TI In vitro bactericidal activity of tobramycin and amikacin alone and in combination against isolates of Pseudomonas aeruginosa from patients with cystic fibrosis
AU Bertrou, A.; Marty, N.; Henry, S.; Agueda, L.; Chabanon, G.
CS Lab. Bacteriol.-Virol., CHU Rangueil, Toulouse, 31054, Fr.
SO Pathol. Biol. (1990), 38(5), 366-75
CODEN: PTBIAN; ISSN: 0031-3009
DT Journal
LA French
AB The bactericidal kinetics on 60 P. aeruginosa isolates were studied by the liq. medium micromethod. Bacteria were incubated with tobramycin and amikacin alone at several concns. and combined with piperacillin, cefsulodin, ceftazidime, imipenem, and ciprofloxacin at concns. obtained in vivo. When used alone, tobramycin showed the most rapid bactericidal activity, whatever the concn. used. The bactericidal activity (.gtoreq.99.99% killing) was obtained in 5 h with 1 or 2 .times. MIC against the majority of the strains with the 2 aminoglycosides. No difference was found between tobramycin and amikacin when combined with an antibiotic which notably increases the bactericidal activity. The combination of amikacin plus imipenem was **synergistic** against 48% of the strains; 26% were synergistically inhibited by amikacin plus ciprofloxacin. When correlated with susceptibility patterns, the results were rather **unpredictable**.

04/02/01 M. Borin

AN 90140745 MEDLINE
DN 90140745
TI Design of combination biotherapy studies: future goals and challenges.
AU Gilewski T A; Golomb H M
CS Section of Hematology/Oncology, University of Chicago Medical Center, IL 60637..
SO SEMINARS IN ONCOLOGY, (1990 Feb) 17 (1 Suppl 1) 3-10; discussion 38-41.
Ref: 83
Journal code: UN5. ISSN: 0093-7754.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
General Review; (REVIEW)
(REVIEW, TUTORIAL)
LA English
FS Priority Journals; Cancer Journals
EM 199005
AB The recent large-scale production of biomodulators, also known as biologic response modifiers, made possible through recombinant DNA technology, offers the potential for significant advances in the treatment of cancer. The antitumor activity of these agents, such as interferons, interleukins, and tumor necrosis factor, have generated enthusiasm for further investigation. In an effort to improve response rates, combinations of these agents both with and without conventional therapies are currently being examined. Clinical trials have been conducted with various therapeutic combinations, including a biomodulator plus chemotherapy, combinations of different biomodulators, a biomodulator with concomitant chemotherapy and radiation, and multiple combinations of chemotherapies and biomodulators. These approaches are promising and some limited successes have been reported; however, the goal of increased anticancer activity without greater toxicities or antagonism between various agents is not always achieved. **Synergism** among active agents is not necessarily assured and quite unexpected and **unpredictable** toxicities have been noted. The studies to date suggest that important new therapies will emerge, but many questions have to be answered before the specific roles of these new treatments are defined.

04/02/01 M. Borin

```
     (FILE 'CAPLUS' ENTERED AT 10:54:01 ON 03 APR 2001)
L3        58245 S SYNERGI####
L4           18 S L3 AND UNPREDICTAB?

     FILE 'MEDLINE, BIOSIS, EMBASE' ENTERED AT 11:01:25 ON 03 APR 2001
L5           42 FILE MEDLINE
L6           13 FILE BIOSIS
L7           24 FILE EMBASE
     TOTAL FOR ALL FILES
L8           79 S L3 AND UNPREDICTAB?
L9            0 FILE MEDLINE
L10           0 FILE BIOSIS
L11           0 FILE EMBASE
     TOTAL FOR ALL FILES
L12           0 S L8 NOT L4
L13          52 DUPLICATE REMOVE L8 (27 DUPLICATES REMOVED)
L14          42 S L13
L15          11 FILE MEDLINE
L16           3 S L13
L17           0 FILE BIOSIS
L18           7 S L13
L19           0 FILE EMBASE
     TOTAL FOR ALL FILES
L20          11 S L13 AND REVIEW/DT
L21         250 FILE MEDLINE
L22         134 FILE BIOSIS
L23         145 FILE EMBASE
     TOTAL FOR ALL FILES
L24         529 S SYNERGI? AND SOMATOSTATIN?
L25           0 FILE MEDLINE
L26           0 FILE BIOSIS
L27           0 FILE EMBASE
     TOTAL FOR ALL FILES
L28           0 S L24 AND RAPAM?
L29           0 FILE MEDLINE
L30           0 FILE BIOSIS
L31           0 FILE EMBASE
     TOTAL FOR ALL FILES
L32           0 S L24 AND UNPREDICT?
L33          23 FILE MEDLINE
L34           0 FILE BIOSIS
L35           0 FILE EMBASE
     TOTAL FOR ALL FILES
L36          23 S L24 AND REVIEW/DT
```

04/02/01 M. Borin

(FILE 'CAPLUS' ENTERED AT 10:54:01 ON 03 APR 2001)
L3 58245 S SYNERGI####
L4 18 S L3 AND UNPREDICTAB?

=> d bib,abs 4,7,9,11,13,17

L4 ANSWER 4 OF 18 CAPLUS COPYRIGHT 2001 ACS
AN 1997:37048 CAPLUS
DN 126:54378
TI Paclitaxel combination therapy in the treatment of metastatic breast cancer
AU Holmes, Frankie Ann
CS The University of Texas M.D. Anderson Cancer Center, Houston, TX, 77030-4009, USA
SO Semin. Oncol. (1996), 23(5, Suppl. 12), 29-39
CODEN: SOLGAV; ISSN: 0093-7754
PB Saunders
DT Journal; General Review
LA English
AB A review with 56 refs. After the single-agent activity of paclitaxel (Taxol; Bristol-Myers Squibb Company, Princeton, NJ) was confirmed, trials to develop a **synergistic** combination began. Doxorubicin, the most active agent for breast cancer, was studied first. As paclitaxel became more available, other combinations, including high-dose regimens and adjuvant therapies, have been studied. No optimal combination regimen has been defined. Recent and/or ongoing trials are looking at paclitaxel in combination with cisplatin, cyclophosphamide, 5-fluorouracil/folinic acid, and mitoxantrone combinations, as well as with high-dose regimens and as adjuvant therapy. This review describes a plethora of combination studies finally under way to better define the optimal use of paclitaxel in breast cancer therapies, both as adjuvant treatment and for metastatic disease. Because of the **unpredictable** nature of drug interactions related to schedule and sequence, ad hoc combinations should not be undertaken outside the context of a well-designed trial.

L4 ANSWER 7 OF 18 CAPLUS COPYRIGHT 2001 ACS
AN 1994:153032 CAPLUS
DN 120:153032
TI **Synergistic** effects of monensin in combination with permethrin or neomycin on neuronal activity
AU Nation, Patrick N; Roth, Sheldon H
CS Heritage Med. Res. Cent., Univ. Alberta, Edmonton, AB, T6G 2S2, Can.
SO Vet. Hum. Toxicol. (1993), 35(5), 414-8
CODEN: VHTODE; ISSN: 0145-6296
DT Journal
LA English
AB Drug combinations have the potential to produce novel and **unpredictable** responses on nervous tissue. This study was designed to test the hypothesis that the effects of combinations of monensin (an ionophore antibiotic) and either neomycin (an aminoglycoside antibiotic) or permethrin (synthetic pyrethroid) are **synergistic**. Effects of the drug combinations upon the elec. properties and membrane

activities of an in vitro sensory neuron prepn. were found to be greater than expected from addn. of the effects of the same drugs acting individually, ind' ting **synergism** and thus suppor' g the hypothesis. It wa. concluded that drugs acting at ..fferent neuronal membrane sites and applied in combination produce **unpredictable** responses. Such drug combinations behave as if they were novel drugs.

L4 ANSWER 9 OF 18 CAPLUS COPYRIGHT 2001 ACS
AN 1990:512336 CAPLUS
DN 113:112336
TI In vitro bactericidal activity of tobramycin and amikacin alone and in combination against isolates of Pseudomonas aeruginosa from patients with cystic fibrosis
AU Bertrou, A.; Marty, N.; Henry, S.; Agueda, L.; Chabanon, G.
CS Lab. Bacteriol.-Virol., CHU Rangueil, Toulouse, 31054, Fr.
SO Pathol. Biol. (1990), 38(5), 366-75
CODEN: PTBIAN; ISSN: 0031-3009
DT Journal
LA French
AB The bactericidal kinetics on 60 P. aeruginosa isolates were studied by the liq. medium micromethod. Bacteria were incubated with tobramycin and amikacin alone at several concns. and combined with piperacillin, cefsulodin, ceftazidime, imipenem, and ciprofloxacin at concns. obtained in vivo. When used alone, tobramycin showed the most rapid bactericidal activity, whatever the concn. used. The bactericidal activity (.gtoreq.99.99% killing) was obtained in 5 h with 1 or 2 .times. MIC against the majority of the strains with the 2 aminoglycosides. No difference was found between tobramycin and amikacin when combined with an antibiotic which notably increases the bactericidal activity. The combination of amikacin plus imipenem was **synergistic** against 48% of the strains; 26% were synergistically inhibited by amikacin plus ciprofloxacin. When correlated with susceptibility patterns, the results were rather **unpredictable.**

L4 ANSWER 11 OF 18 CAPLUS COPYRIGHT 2001 ACS
AN 1985:3110 CAPLUS
DN 102:3110
TI **Unpredictable** interactions between cefotetan and penicillins or aminoglycosides
AU Barry, A. L.; Packer, R. R.; Jones, R. N.
CS Clin. Microbiol. Inst., Tualatin, OR, 97062, USA
SO Diagn. Microbiol. Infect. Dis. (1984), 2(4), 347-51
CODEN: DMIDDZ; ISSN: 0732-8893
DT Journal
LA English
AB The bacteriostatic and bactericidal activity of gentamicin, amikacin, piperacillin, azlocillin, ampicillin, benzylpenicillin, and oxacillin against 85 selected isolates was measured alone and in the presence of subinhibitory concns. of cefotetan. A potential for antagonistic interactions between cefotetan and acylamino penicillins (piperacillin > azlocillin > ampicillin) were obsd. with some strains; synergy was suggested with other isolates, however. Bacteriostatic and bactericidal activity of the 2 aminoglycosides was enhanced by cefotetan with most gram-neg. bacilli, but bactericidal activity was significantly diminished with 4 of 65 strains. Piperacillin and cefotetan were clearly antagonistic with 11 of 44 strains and **synergistic** with 4 of 44 strains of gram-neg. bacilli: the remaining 21 strains gave off scale end points and could not be analyzed in that way.

L4 ANSWER 13 OF 18 CAPLUS COPYRIGHT 2001 ACS
AN 1983:591500 CAPLUS
DN 99:191500
TI In vitro study of the combination of rifampin with oxacillin against

Staphylococcus aureus
AU Van der Auwera, P.; Klastersky, J.
CS Inst. Jules Borde' Univ. Bruxelles, Brussels, Bel(
SO Rev. Infect. Dis. (1983), 5(Suppl. 3), S509-14
CODEN: RINDDG; ISSN: 0162-0886
DT Journal
LA English
AB The interaction of rifampin and oxacillin in various concns. was examd. in
vitro by a dynamic biophotometric technique. While **synergism** was obsd. occasionally, most concns. studied exhibited either antagonism or indifference. No antagonism was obsd. with rifampin-resistant strains.
Rifampin-resistant strains often emerged when the drug was tested alone. These findings again illustrate the complex and often **unpredictable** effect of combining rifampin with .beta.-lactam antibiotics.

L4 ANSWER 17 OF 18 CAPLUS COPYRIGHT 2001 ACS
AN 1972:413925 CAPLUS
DN 77:13925
TI Interaction of drugs inhibiting different steps in the synthesis of DNA
AU Grindey, Gerald B.; Nichol, Charles A.
CS Dep. Exp. Ther., Roswell Park Mem. Inst., Buffalo, N. Y., USA
SO Cancer Res. (1972), 32(3), 527-31
CODEN: CNREA8
DT Journal
LA English
AB In leukemia L1210 suspension cultures, combinations of 1-.beta.-D-arabinofuranosylcytosine (ara-C) [147-94-4] with either methotrexate (I) [59-05-2] or 5-fluorodeoxyuridine (FUdR) [50-91-9] showed strong antagonistic effects compared to their independent inhibitions of DNA synthesis. Combination of 1-formylisoquinoline thiosemicarbazone (IQ-1) [2365-26-6] with I yielded slight antagonism, while the combination of IQ-1 with FUdR was additive or slightly **synergistic**. The combination of 9-.beta.-D-arabinofuranosyladenine [5536-17-4] with I was antagonistic, whereas the combination of 9-.beta.-D-arabinofuranosyladenine with FUdR was additive. Also, the combination of 9-.beta.-D-arabinofuranosyladenine with either IQ-1 or ara-C was additive.
The primary site of action of ara-C in these growing cells is not inhibition of ribonucleotide reductase [9040-57-7]. The various interactions obsd. in this study were **unpredictable** and could not be explained by the present concepts concerning combination chemotherapy.

```
Trying 3106000224...Open

Welcome to STN International!  Enter x:x
LOGINID:ssspta1811mxb
PASSWORD:
TERMINAL (ENTER 1, 2, 3, OR ?):2


* * * * * * * * * *   Welcome to STN International   * * * * * * * * * *

 NEWS  1  Feb  2  Web Page URLs for STN Seminar Schedule - N. America
 NEWS  2  Aug  9  Expanded CAplus Coverage of US, Japanese, WIPO
                  and EPO Patents
 NEWS  3  Sep  7  ESBIOBASE - Elsevier Biobase now on STN
 NEWS  4  Sep 20  ARCHIVE, REDISTRIBUTE SEARCH RESULTS WITH
                  STN KEEP AND SHARE
 NEWS  5  Sep 29  Aluminum Industry Abstracts Now on STN
 NEWS  6  Oct  5  Elsevier's World Textiles now available on STN
 NEWS  7  Oct 27  EUROPATFULL - backlog data being added
 NEWS  8  Oct 27  DATA ELEMENTS TO BE REMOVED FROM JAPIO
 NEWS  9  Nov  8  Derwent World Patents Index: Technology Focus
                  To Be Added to Basic Index

 NEWS EXPRESS  STN Express 5.0 Now Available
 NEWS HOURS    STN Operating Hours Plus Help Desk Availability
 NEWS INTER    General Internet Information
 NEWS LOGIN    Welcome Banner and News Items
 NEWS PHONE    Direct Dial and Telecommunication Network Access to STN
 NEWS WWW      CAS World Wide Web Site (general information)

Enter NEWS followed by the item number or name to see news on that
specific topic.

  All use of STN is subject to the provisions of the STN Customer
  agreement.  Please note that this agreement limits use to scientific
  research.  Use for software development or design or implementation
  of commercial gateways or other similar uses is prohibited and may
  result in loss of user privileges and other penalties.

* * * * * * * * * * * * * * * * STN  Columbus  * * * * * * * * * * * * * * * *

FILE 'HOME' ENTERED AT 09:47:47 ON 11 NOV 1999

=> file caplus

COST IN U.S. DOLLARS                                SINCE FILE      TOTAL
                                                       ENTRY    SESSION
FULL ESTIMATED COST                                     0.15       0.15
```

```
FILE 'CAPLUS' ENTERED AT 09:47:57 ON 11 NOV 1999
USE IS SUBJECT TO THE TERMS OF YOUR STN CUSTOMER AGREEMENT.
PLEASE SEE "HELP USAGETM 3" FOR DETAILS.
COPYRIGHT (C) 1999 AMERICAN CHEMICAL SOCIETY (ACS)
```



```
FILE COVERS 1967 - 11 Nov 1999  VOL 131 ISS 20
FILE LAST UPDATED: 10 Nov 1999  (19991110/ED)

  This file contains CAS Registry Numbers for easy and accurate
  substance identification.

  This file supports REGISTRY for direct browsing and searching of
  all substance data from the REGISTRY file.  Enter HELP FIRST for
  more information.


=> s ?rapamycin or (lactam macrolide)

          1746 ?RAPAMYCIN
         19304 LACTAM
         11444 LACTAMS
         23695 LACTAM
                 (LACTAM OR LACTAMS)
          5282 MACROLIDE
          2678 MACROLIDES
          6274 MACROLIDE
                 (MACROLIDE OR MACROLIDES)
            24 LACTAM MACROLIDE
                 (LACTAM(W)MACROLIDE)
L1         1770 ?RAPAMYCIN OR (LACTAM MACROLIDE)

=> s somatostatin or ostreotide or vapreotide or lanreotide

         14445 SOMATOSTATIN
           119 SOMATOSTATINS
         14451 SOMATOSTATIN
                 (SOMATOSTATIN OR SOMATOSTATINS)
             0 OSTREOTIDE
            26 VAPREOTIDE
            88 LANREOTIDE
L2       14466 SOMATOSTATIN OR OSTREOTIDE OR VAPREOTIDE OR LANREOTIDE

=> del l2

DELETE L2? (Y)/N:y


=> s somatostatin or octreotide or vapreotide or lanreotide

         14445 SOMATOSTATIN
           119 SOMATOSTATINS
         14451 SOMATOSTATIN
                 (SOMATOSTATIN OR SOMATOSTATINS)
          1053 OCTREOTIDE
             5 OCTREOTIDES
          1053 OCTREOTIDE
                 (OCTREOTIDE OR OCTREOTIDES)
```

```
            26 VAPREOTIDE
            88 LANREOTIDE
L2       14749 SOMATOST   N OR OCTREOTIDE OR VAPREOTIDE    LANREOTIDE

=> s l2 and l1

L3           3 L2 AND L1

=> d bib, kwic 1-3


L3   ANSWER 1 OF 3  CAPLUS  COPYRIGHT 1999 ACS
AN   1998:13858  CAPLUS
DN   128:84386
TI   Combination of a somatostatin analog and a rapamycin
     for the prevention or treatment of cell hyperproliferation
IN   Weckbecker, Gisbert
PA   Novartis A.-G., Switz.; Weckbecker, Gisbert
SO   PCT Int. Appl., 31 pp.
     CODEN: PIXXD2
DT   Patent
LA   English
FAN.CNT 1
     PATENT NO.        KIND  DATE           APPLICATION NO.  DATE
     ----------------  ----  --------       ---------------  --------
PI   WO 9747317         A1   19971218       WO 1997-EP3036   19970611
         W:  AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE,
             DK, EE, ES, FI, GB, GE, GH, HU, IL, IS, JP, KE, KG, KP, KR, KZ,
             LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL,
             PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, US, UZ,
             VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM
         RW: GH, KE, LS, MW, SD, SZ, UG, ZW, AT, BE, CH, DE, DK, ES, FI, FR,
             GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA,
             GN, ML, MR, NE, SN, TD, TG
     CA 2249439         AA   19971218       CA 1997-2249439  19970611
     AU 9732572         A1   19980107       AU 1997-32572    19970611
     EP 896544          A1   19990217       EP 1997-928175   19970611
         R:  AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, PT, IE, FI
PRAI GB 1996-12171     19960611
     GB 1996-19310     19960916
     WO 1997-EP3036    19970611
OS   MARPAT 128:84386
TI   Combination of a somatostatin analog and a rapamycin
     for the prevention or treatment of cell hyperproliferation
AB   A combination of a compd. of the somatostatin class (e.g.
     octreotide) and a rapamycin macrolide is useful for the
     prevention or treatment of cell hyperproliferation.  The combination is
     synergistic.
ST   somatostatin analog rapamycin macrolide combination
     antiproliferative; hyperproliferation somatostatin analog
     rapamycin synergistic combination; octreotide
     rapamycin macrolide antiproliferative
IT   Pancreatic tumors
        (inhibitors; somatostatin analog-rapamycin
        macrolide combination for prevention or treatment of cell
        hyperproliferation)
IT   Injections (drug delivery systems)
     Solutions (drug delivery systems)
        (injection solns.; somatostatin analog-rapamycin
        macrolide combination for prevention or treatment of cell
        hyperproliferation)
IT   Antitumor agents
        (pancreatic; somatostatin analog-rapamycin
```

macrolide combination for prevention or treatment of cell hyperproliferation)

IT Ampuls
Antiproliferative agents
Antitumor agents
Capsules (drug delivery systems)
Drug delivery systems
Sustained release drug delivery systems
Synergistic drug interactions
(**somatostatin** analog-**rapamycin** macrolide combination for prevention or treatment of cell hyperproliferation)

IT Macrolides
RL: BAC (Biological activity or effector, except adverse); THU (Therapeutic use); BIOL (Biological study); USES (Uses)
(**somatostatin** analog-**rapamycin** macrolide combination for prevention or treatment of cell hyperproliferation)

IT 51110-01-1D, **Somatostatin**, analogs 53123-88-9, **Rapamycin** 53123-88-9D, **Rapamycin**, derivs. 83150-76-9, **Octreotide** 103222-11-3, **Vapreotide** 108736-35-2, **Lanreotide** 135467-16-2, **Octreotide** pamoate 159351-69-6
RL: BAC (Biological activity or effector, except adverse); THU (Therapeutic use); BIOL (Biological study); USES (Uses)
(**somatostatin** analog-**rapamycin** macrolide combination for prevention or treatment of cell hyperproliferation)

L3 ANSWER 2 OF 3 CAPLUS COPYRIGHT 1999 ACS
AN 1993:573820 CAPLUS
DN 119:173820
TI Inhibition of cAMP-responsive element-mediated gene transcription by cyclosporin A and FK506 after membrane depolarization
AU Schwaninger, Markus; Blume, Roland; Oetjen, Elke; Lux, Gundula; Knepel, Willhart
CS Cent. Pharmacol. Toxicol., Univ. Goettingen, Goettingen, 37070, Germany
SO J. Biol. Chem. (1993), 268(31), 23111-15
CODEN: JBCHA3; ISSN: 0021-9258
DT Journal
LA English
AB . . . than CsA. CsA/FK506 responsiveness was mediated by the glucagon CRE and also by well characterized CREs of the choriogonadotropin and **somatostatin** gene. **Rapamycin** antagonized the inhibitory effect of FK506 but not CsA, suggesting that FK506 and CsA may act through complex formation with. . .

L3 ANSWER 3 OF 3 CAPLUS COPYRIGHT 1999 ACS
AN 1992:99301 CAPLUS
DN 116:99301
TI Maleic anhydride copolymers as antidotes for the cytotoxicity of neoplasm inhibitors
IN Bach, Ardalan; Shanahan, William R., Jr.
PA Searle, G. D., and Co., USA
SO Eur. Pat. Appl., 27 pp.
CODEN: EPXXDW
DT Patent
LA English
FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	EP 393575	A1	19901024	EP 1990-107246	19900417
	EP 393575	B1	19940316		
	R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE				
	CA 2014732	AA	19901017	CA 1990-2014732	19900417
	JP 02292227	A2	19901203	JP 1990-101530	19900417

AT 102838 E 19940415 AT 1990-107246 19900417
ES 2062155 T* 19941216 ES 1990-107246 19900417
PRAI US 1989-339503 1. 0417
EP 1990-107246 19900417
OS MARPAT 116:99301
IT 50-18-0, Cyclophosphamide 50-76-0, Dactinomycin 50-91-9, Floxuridine 51-21-8, 5-Fluorouracil 51-21-8D, conjugates with fibrinogens 53-19-0, Mitotane 54-42-2, NSC 39661 56-18-8, Norspermidine 57-22-7 59-05-2, Methotrexate 75-19-4D, Cyclopropane, spiro derivs. 113-15-5, ERgotamine 127-07-1 143-67-9, Vinblastine sulfate 147-94-4, Cytarabine 147-94-4D, Cytarabine, conjugates 154-42-7, Thioguanine 154-93-8, Carmustine 302-79-4, Retinoic acid 305-03-3, Chlorambucil 432-70-2, .alpha.-Carotene 636-65-7, Isoglutamine 642-18-2 645-05-6, Altretamine 671-16-9, Procarbazine 1149-99-1, Illudin 1404-00-8, Mitomycin 1404-64-4, Sparsomycin 1948-56-7D, Dehydroalanine, N-acyl deriv. 2353-33-5, NSC 127716 3073-59-4, NSC 95580 3094-09-5, Doxifluridine 3778-73-2, Ifosfamide 4005-51-0, Aminothiadiazole 4342-03-4 4759-48-2, Isotretinoin 5373-42-2, Thaliblastine 6620-60-6, Proglumide 6829-55-6 7440-06-4D, Platinum, derivs., complexes 7481-89-2, Dideoxycytidine 7534-61-4, NSC 145813 9014-02-2D, Neocarzinostatin, conjugates with styrene-maleci acid copolymer 9015-68-3, Asparaginase 9041-93-4, Bleomycin sulfate 9054-89-1, Superoxide dismutase 10318-26-0, Mitolactol 12633-27-1, T 680 13010-47-4, Lomustine 13494-90-1, Gallium nitrate 13665-88-8, Mopidamol 13909-02-9 13909-09-6, Semustine 14459-29-1D, polymers 14930-96-2, Cytochalasin B 15219-97-3, Oxalysine 15663-27-1, Cisplatin 18378-89-7, Plicamycin 19624-67-0, SKF 101772 20830-81-3 21416-87-5, Razoxane 22862-76-6, Anisomycin 23214-92-8, Doxorubicin 23214-92-8D, conjugates with fibrinogens 24584-09-6, ICRF 187 25300-64-5D, conjugates with neocarzinostatin 26833-87-4, Homoharringtonine 27686-84-6, CHX 100 29069-24-7, Prednimustine 29767-20-2 31430-18-9D, Nocodazole, N-acyl deriv. 33069-62-4 33419-42-0 34140-52-8D, Aeroplysinin, derivs. 35144-64-0D, Aldophosphamide, analogs 38077-12-2 39389-47-4, Distamycin 39544-74-6, Benzotript 40919-33-3, Uricytin 41575-94-4, Carboplatin 41729-52-6, Dezaguanine 41992-23-8, Spirogermanium 50264-69-2, Lonidamine 51213-99-1, Clanfenur 51264-14-3, Amsacrine 51321-79-0, PALA 51350-19-7, EHNA 52128-35-5, Trimetrexate 52205-73-9, Estramustine phosphate sodium 53123-88-9, **Rapamycin** 53643-48-4, Vindesine 53910-25-1, Pentostatin 54083-22-6, Zorubicin 54350-48-0, Etretinate 54526-94-2, Steffimycin B 54824-17-8, Mitonafide 55073-32-0, Genkwadaphnin 55079-83-9, Acitretin 55303-98-5, Avarol 56281-36-8, Motretinide 56420-45-2, Epirubicin 56605-16-4, Spiromustine 56973-26-3, SM 108 57248-88-1 57576-44-0, Aclarubicin 58066-85-6, Hexadecylphosphocholine 58196-43-3 58337-35-2, Elliptinium acetate 58338-59-3, Dinaline 58957-92-9 58994-96-0, Ranimustine 59040-30-1, Nafazatrom 59653-73-5, Teroxirone 60084-10-8, Tiazofurin 60784-46-5, Elmustine 61251-97-6 61422-45-5, Carmofur 61825-94-3, Oxaliplatin 62396-95-6 62488-57-7, NSC 26480 62816-98-2, Tetraplatin 62928-11-4, Iproplatin 63521-85-7, Esorubicin 64124-21-6, Trimelamol 65222-35-7, Pazelliptine 65271-80-9, Mitoxantrone 65646-68-6, Fenretinide 65794-79-8, Gregatin A 65886-71-7, Fazarabine 66052-62-8, NSC 264394 67199-66-0, Batracylin 67699-40-5 67995-68-0, Tallysomycin 68247-85-8 69408-81-7, Amonafide 69772-39-0, Neoenactin 69839-83-4, Didox 69955-43-7, .alpha.-Difluoromethylarginine 70189-62-7, TA 077 71103-05-4 71240-74-9, GYKI 17230 71486-22-1, Vinorelbine 71628-96-1, Menogaril 72238-02-9 72496-41-4, Pirarubicin 72732-56-0, Piritrexim 72880-48-9, K-AM 73387-70-9 73612-99-4, CA 102 74427-64-8, NSC 342215 75219-46-4, Bestrabucil 75607-67-9, Fludarabine phosphate 76520-52-0, Spatol 77327-05-0 77699-47-9, Herbimycin 77739-71-0, Acanthifolic acid 78186-34-2, Bisantrene 78287-27-1, SN 22 80205-24-9, Cyplatate 80427-58-3, Benfluron 80576-83-6, CGP 30694 80667-13-6, FO-152 80681-73-8 80790-68-7, ADR 456 80841-47-0, CI-921

81424-67-1, Caracemide 81485-25-8 81600-06-8, Vintriptol 83150-76-9,
Octreotide 83314-[illegible]-6, Bryostatin 1 83373-60-8, D 609
83519-04-4, Ilmofos[illegible] 83947-09-5, Antibiotic AN 3 83996-50-3
83997-75-5, FCE 21954 84396-34-9, SS 554 85233-2[illegible]-0, BMY-28438
85326-06-3 85622-93-1, Temozolomide 85700-43-2 85969-07-9,
Budotitane 85977-49-7, Tauromustine 86229-97-2, RA 700 87385-19-1
87790-39-4, SUN 0237 87810-56-8, Fostriecin 87859-14-1 88266-67-5,
NK-313 88303-61-1, CI-941 88859-04-5, Mafosfamide 88895-06-1
89196-07-6, NSC 357704 90072-82-5 90996-54-6, Rhizoxin 91265-19-9
91441-23-5, Piroxantrone 91441-48-4, CI-937 91531-30-5, Antineoplaston
A10 91531-98-5, Amphethinile 91753-07-0, Mitoquidone 92090-94-3,
Kazusamycin 92118-27-9, Fotemustine 92211-45-5, SUN 2071 92455-12-4,
AD 5 92803-82-2 95056-36-3, BMY-25067 95058-81-4, LY 188011
95458-43-8, MZPES 95604-83-4, Probimane 95693-76-8, DATHF
95734-82-0, 254S 96086-68-9, DN-9693 96201-88-6, Brequinar sodium
96203-70-2, Pancratistatin 96389-68-3, Crisnatol 96497-67-5
96892-57-8, Hepsulfam 97068-30-9, Elsamicin-A 97534-21-9, Merbarone
97919-22-7 98227-64-6, SS 9816B 98383-18-7, CY 233 98631-95-9,
MST-16 98985-36-5, DM-75 99009-20-8, PD-115934 99107-03-6
99107-06-9, BMY-26605 99212-42-7, Anaxirone 99674-26-7 99755-38-1
100286-90-6 100415-25-6, Sorangicin A 100438-92-4, Normosang
100440-25-3, Terpentecin 100753-80-8, SN 07 100827-28-9, Erbstatin
101156-09-6, Chromoximycin 102363-08-6, FR-900482 102636-25-9, TAC-788
103775-75-3, DWA 2114R
RL: PRP (Properties)
(cytotoxicity of, maleic anhydride copolymer antidote for)

US006362164B1

(12) **United States Patent**
Weckbecker

(10) **Patent No.: US 6,362,164 B1**
(45) **Date of Patent: Mar. 26, 2002**

(54) **COMBINATION OF A SOMATOSTATIN ANALOGUE AND A RAPAMYCIN**

(75) Inventor: **Gisbert Weckbecker**, Biel-Benken (CH)

(73) Assignee: **Novartis AG**, Basel (CH)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.

(21) Appl. No.: **09/194,957**

(22) PCT Filed: **Jun. 11, 1997**

(86) PCT No.: **PCT/EP97/03036**

§ 371 Date: **Dec. 7, 1998**

§ 102(e) Date: **Dec. 7, 1998**

(87) PCT Pub. No.: **WO97/47317**

PCT Pub. Date: **Dec. 18, 1997**

(30) **Foreign Application Priority Data**

Jun. 11, 1996 (GB) 9612171
Sep. 16, 1996 (GB) 9619310

(51) **Int. Cl.**[7] **A61K 38/31**; A61K 38/08

(52) **U.S. Cl.** **514/16**; 530/311; 514/2; 540/456

(58) **Field of Search** 514/15

(56) **References Cited**

FOREIGN PATENT DOCUMENTS

GB 2 239 178 A 6/1991
WO WO 93 11130 A 6/1993

OTHER PUBLICATIONS

Shi E.A., Cancer Research, vol. 55, pp. 1982–19088 (1995). *

Grant et al., Circulation, vol. 89, No. 4, pp. 1511–1517 (1994).

Demoliou–Mason, Exp. Opin.Ther. Patents, vol. 4, No. 7, pp. 813–829 (1994).

Primary Examiner—Michael Borin
(74) *Attorney, Agent, or Firm*—Joseph J. Borovian

(57) **ABSTRACT**

A combination of a compound of the somatostatin class and a rapamycin macrolide is useful for the prevention or treatment of cell hyperproliferation.

13 Claims, No Drawings

COMBINATION OF A SOMATOSTATIN ANALOGUE AND A RAPAMYCIN

The present invention relates to a pharmaceutical combination and its use in the treatment of disorders associated with excess benign and malignant cell proliferation, e.g. tumors or intimal cell proliferation.

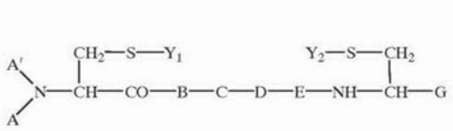
There is a continuing need for the development of drugs having increased effectiveness in inhibiting or slowing down undesired cell proliferation, particularly in the cancer field and in vasculopathies.

Accordingly, there is provided a pharmaceutical combination comprising a compound of the somatostatin class, and a rapamycin macrolide.

The somatostatin class is a known class of small peptides comprising the naturally occurring somatostatin-14 and analogues having somatostatin related activity, e.g. as disclosed by A. S. Dutta in Small Peptides, Vol. 19, Elsevier (1993). By "somatostatin analogue" as used herein is meant any straight-chain or cyclic polypeptide having a structure based on that of the naturally occurring somatostatin-14 wherein one or more amino acid units have been omitted and/or replaced by one or more other amino radical(s) and/or wherein one or more functional groups have been replaced by one or more other functional groups and/or one or more groups have been replaced by one or several other isosteric groups. In general, the term covers all modified derivatives of the native somatostatin-14 which exhibit a somatostatin related activity, e.g. they bind to at least one somatostatin receptor (hSST-1, hSST-2, hSST-3, hSST4 or hSST-5), preferably in the nMolar range, more preferably to at least the hSST-2 receptor in the nMolar range.

Cyclic, bridge cyclic and straight-chain somatostatin analogues or derivatives are known and have been described together with processes for their production e.g. in U.S. Pat. Nos. 4,310,518 and 4,235,886, in European Patent Specifications EP-A-1295; 23,192; 29,310; 29,579; 30,920; 31,303; 63,308; 70,021; 83,305; 215,171; 203,031; 214,872; 143, 307; 298,732; 277,419; 389,180; 395,417; 450,480A2; in Belgian Patent Specification BE-A-900,089; and in WO 91/09056; WO 97/01579; WO 97/14715, the contents thereof, in particular with respect to the compounds, being incorporated herein by reference.

Preferred somatostatin analogues are e. g. compounds of formula I



wherein

A is C_{1-12}alkyl, C_{7-10}phenylalkyl or a group of formula RCO—, whereby

i) R is hydrogen, C_{1-11}alkyl, phenyl or C_{7-10}phenylalkyl, or

ii) RCO— is

a) a D-phenylalanine residue optionally ring-substituted by halogen, NO_2, NH_2, OH, C_{1-3}alkyl and/or C_{1-3}alkoxy; or

b) the residue of a natural or a synthetic α-amino-acid other than defined under a) above, or of a corresponding D-amino acid, or

c) a dipeptide residue in which the individual amino acid residues are the same or different and are selected from those defined under a) and/or b) above, the α-amino group of amino acid residues a) and b) and the N-terminal amino group of dipeptide residues c) being optionally mono- or di-C_{1-12}alkylated or substituted by C_{1-8}alkanoyl;

A' is hydrogen or C_{1-3}alkyl,

Y_1 and Y_2 represent together a direct bond or each of Y_1 and Y_2 is hydrogen

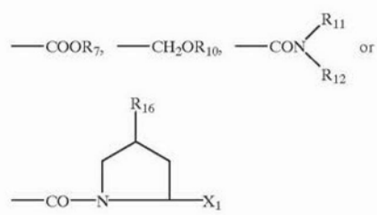
B is -Phe- optionally ring-substituted by halogen, NO_2, NH_2, OH, C_{1-3}alkyl and /or C_{1-3}alkoxy (including pentafluoroalanine), naphthylalanine or pyridylalanine,

C is (L)-Trp- or (D)-Trp- optionally α-N-methylated and optionally benzene-ring-substituted by halogen, NO_2, NH_2, OH, C_{1-3}alkyl and/or C_{1-3}alkoxy,

D is Lys, 4-aminocyclohexylAla or 4-aminocyclohexylGly,

E is Thr, Ser, Val, Tyr, lie, Leu or an aminobutyric or aminoisobutyric acid residue,

G is a group of formula



wherein

R_7 is hydrogen or C_{1-3}alkyl,

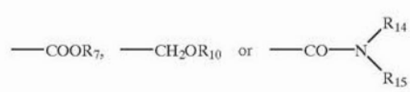
R_{10} is hydrogen or the residue of a physiologically acceptable, physiologically hydrolysable ester, e.g. formnyl, C_{2-12}alkylcarbonyl, benzoyl,

R_{11} is hydrogen, C_{1-3}alkyl, phenyl or C_{7-10}phenylalkyl

R_{12} is hydrogen, C_{1-3}alkyl or a group of formula —CH(R_{13})—X_1,

R_{13} is CH_2OH, —$(CH_2)_2$—OH, —$(CH_2)_3$—OH, —CH(CH_3)OH, isobutyl, butyl, benzyl, naphthyl-methyl or indol-3-yl-methyl, and

X_1 is a group of formula



wherein

R_7 and R_{10} have the meanings given above,

R_{14} is hydrogen or C_{1-3}alkyl,

R_{15} is hydrogen, C_{1-3}alkyl, phenyl or C_{7-10}phenylalkyl, and

R_{16} is hydrogen or hydroxy,

with the proviso that

when R_{12} is —CH(R_{13})—X_1, then R_{11} is hydrogen or methyl,

wherein the residues B, D and E have the L-configuration, and the residues in the 2- and 7-position each independently have the (L)- or (D)-configuration,

in free form or in pharmaceutically acceptable salt or complex form.

Individual compounds of formula I suitable in accordance with the present invention are the following somatostatin analogues:

a. (D)Phe-Cys-Phe-(D)Trp-Lys-Thr-Cys-Thr-ol
also known as octreotide

b. (D)Phe-Cys-Tyr-(D)Trp-Lys-Val-Cys-ThrNH_2

c. (D)Phe-Cys-Tyr-(D)Trp-Lys-Val-Cys-TrpNH_2
also known as vapreotide

d. (D)Trp-Cys-Phe-(D)Trp-Lys-Thr-Cys-ThrNH_2

e. (D)Phe-Cys-Phe-(D)Trp-Lys-Thr-Cys-ThrNH_2

f. 3-(2-(Naphthyl)-(D)Ala-Cys-Tyr-(D)Trp-Lys-Val-Cys-ThrNH_2
also known as lanreotide

g. (D)Phe-Cys-Tyr-(D)Trp-Lys-Val-Cys-β-Nal-NH_2

h. 3-(2-naphthyl)-Ala-Cys-Tyr-(D)Trp-Lys-Val-Cys-β-Nal-NH_2

i. (D)Phe-Cys-β-Nal-(D)Trp-Lys-Val-Cys-Thr-NH_2

j. (D)Phe-Cys-Tyr-(D)Trp-Lys-Leu-Cys-Thr-NH_2

k. (D)Phe-Cys-Tyr-(D)Trp-Lys-Cys-Thr-NH_2.

A preferred compound of formula I is octreotide.

Compounds of formula I may exist e.g. in free form, salt form or in the form of complexes thereof. Acid addition salts may be formed with e.g. organic acids, polymeric acids and inorganic acids. Such acid addition salt forms include e.g. the hydrochlorides and acetates. Complexes are e.g. formed from compounds of the invention on addition of inorganic substances, e.g. inorganic salts or hydroxides such as Ca- and Zn-salts, and/or on addition of polymeric organic substances.

Further somatostatin analogues suitable for use in accordance with the present invention are:

cyclo [-Asn-Phe-Phe-DTrp-Lys-Thr-Phe-Gaba-], cyclo (Asu-Lys-Asn-Phe-Phe-Trp-Lys-Thr-Tyr-Thr-Ser), and

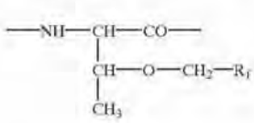
(D)Nal-Glu-Tyr-(D)Trp-Lys-Val-Lys-Thr-NH_2.

According to an alternatively preferred embodiment of the invention, the somatostatin component of the combination is a somatostatin analogue comprising the amino acid sequence of formula (II)

—(D/L)Trp-Lys-X_2—X_3— (II)

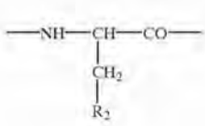
wherein

X_2 is a radical of formula (a) or (b)



(a)

or

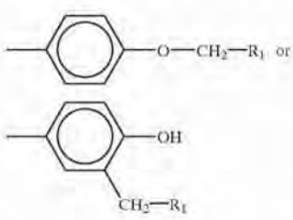


(b)

wherein

R_1 is optionally substituted phenyl,

R_2 is —Z_1—CH_2—R_1, —CH_2—CO—O—CH_2—R_1,



wherein

Z_1 is O or S, and

X_3 is an α-amino acid having an aromatic residue on the C_α side chain, or an amino acid unit selected from Dab, Dpr, Dpm, His,(Bzl)HyPro, thienyl-Ala, cyclohexyl-Ala and t.-butyl-Ala,

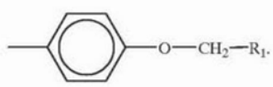
the residue Lys of said sequence corresponding to the residue Lys^9 of the native somatostatin-14.

Such somatostatin analogues are e.g. disclosed in WO/97/01579, the contents thereof, in particular with respect to the specifically exemplified compounds, being incorporated herein by reference.

Preferably the sequence of formula II as defined above corresponds to the residues at positions 8 through 11 of the somatostatin-14. More preferably the somatostatin analogue as disclosed above comprises a hexapeptide unit, the residues at positions 3 through 6 of said hexapeptide unit comprising the sequence of formula II. More particularly the hexapeptide unit is cyclic, e.g. having a direct peptide linkage between the α-carbonyl group of the residue at position 6 and the α-amino group of the residue at position 1.

While Lys, X_2 and X_3 in the sequence of formula II have the L-configuration, Trp may have the D- or L-configuration, preferably the D-configuration.

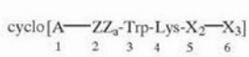
X_2 is preferably a residue of formula (a) or (b), R_2 being preferably —Z_1—CH_2—R_1 or



When X_3 comprises an aromatic residue on the C_α side chain, it may suitably be a natural or unnatural α-amino acid, e.g. Phe, Tyr, Trp, Nal, Pal, benzothienyl-Ala, Tic and thyronin, preferably Phe or Nal, more preferably Phe. X_3 is preferably an α-amino acid bearing an aromatic residue on the C_α side chain.

When R_1 is substituted phenyl, it may suitably be substituted by halogen, methyl, ethyl, methoxy or ethoxy e.g. in ortho and/or position. More preferably R_1 is unsubstituted phenyl. Z_1 is preferably O.

Representative somatostatin analogues comprising a residue of formula II are e.g compounds of formula (III)

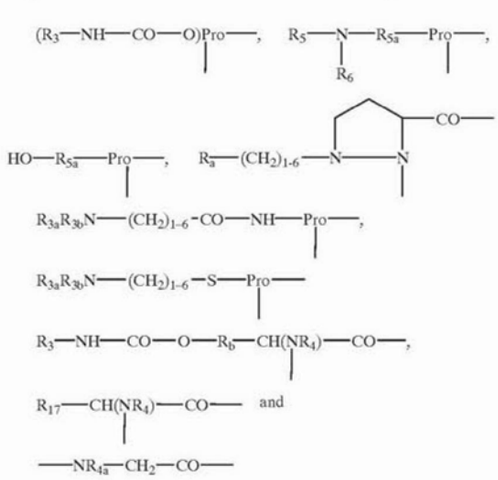


(II)

wherein

X_2 and X_3 are as defined above,

A_1 is a divalent residue selected from Pro,

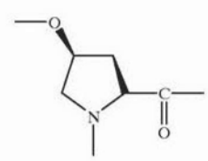


wherein R_3 is NR_8R_9—C_{2-6}alkylene, guanidino-C_{2-6}alkylene or C_{2-6}alkylene-COOH, R_{3a} is H, C_{1-4}alkyl or has independently one of the significances given for R_3 $R_{3b}$is H or C_{1-4}alkyl, R_a is OH or NR_5R_6, R_b is —$(CH_2)_{1-3}$— or —$CH(CH_3)$—, R_4 is H or CH_3, R_{4a} is optionally ring-substituted benzyl, each of R_5 and R_6 independently is H, C_{1-4}alkyl, ω-amino-C_{1-4}alkylene, ω-hydroxy-C_{1-4}alkylene or acyl, $R_{5a}$is a direct bond or C_{1-6}alkylene, each of R_8 and R_9 independently is H, C_{1-4}alkyl, ω-hydroxy-C_{2-4}alkylene, acyl or CH_2OH—$(CHOH)_c$—CH_2— wherein c is 0, 1, 2, 3 or 4, or R_8 and R_9 form together with the nitrogen atom to which they are attached a heterocyclic group which may comprise a further heteroatom, and R_{17} is optionally ring-substituted benzyl, —$(CH_2)_{1-3}$—OH, CH_3—CH(OH)— or —$(CH_2)_{1-5}$—NR_5R_6, and

ZZ_a is a natural or unnatural α-amino acid unit.

ZZ_a may have the D- or L-configuration. When ZZ_a is a natural or unnatural α-amino acid unit, it may suitably be e.g. Thr, Ser, Ala, Val, Ile, Leu, Nle, His, Arg, Lys, Nal, Pal, Tyr, Trp, optionally ring-substituted Phe or N^α-benzyl-Gly. When ZZ_a is Phe, the benzene ring thereof may be substituted by e.g. NH_2, NO_2, CH_3, OCH_3 or halogen, preferably in para position. When ZZ_a is Phe, the benzene ring thereof is preferably unsubstituted.

When A_1 comprises a Pro amino acid residue, any substituent present on the proline ring, e.g. R_3—NH—CO—O— etc., is preferably in position 4. Such substituted proline residue may exist in the cis form, e.g.

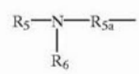


as well as in the trans form. The present invention covers each geometric isomer individually as well as mixtures thereof.

When A_1 is (NR_8R_9—C_{6-2}alkylene-NH—CO—)Pro- where NR_8R_9 forms a heterocyclic group, such group may be aromatic or saturated and may comprise one nitrogen or one nitrogen and a second heteroatom selected from nitrogen and oxygen. Preferably the heterocyclic group is e.g. pyridyl or morpholino. C_{2-6}Alkylene in this residue is preferably —CH_2—CH_2—.

Any acyl as R_5, R_6, R_8 and R_9 in A_1 may be e.g. $R_{18}CO$— wherein R_{18} is H, C_{1-4}alkyl, C_{2-4}alkenyl, C_{3-6}cycloalkyl or benzyl, preferably methyl or ethyl. When R_{4a}, or R_{17} in A_1 is ring-substituted benzyl, the benzene ring may be substituted as indicated above for ZZ_a.

A preferred group of compounds of formula III are such wherein A_1 is free of a lateral —NH—CO—O— moiety. A further group of preferred compounds of formula III are such wherein A_1 comprises a basic lateral radical, e.g. a R_3—NH—CO—O— or



moiety.

A still further group of preferred compounds of formula III are such wherein the N-terminal amino acid comprises a substituted Pro, particularly 4-substituted Pro, e.g. compounds of formula III wherein A_1 is 4-substituted Pro.

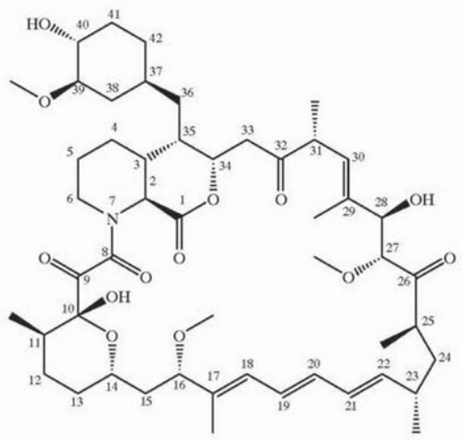
Preferably A_1 is 4-(R_3—NH—CO—O)Pro.

Examples of somatostatin analogues comprising a residue of formula II include e.g. cyclo [4—(NH_2—C_2H_4—NH—CO—O—)Pro-Phe-DTrp-Lys-Ser(Benzyl)-Phe].

The term "macrolide" as used herein, refers to a macrocyclic lactone, for example a compound having a 12-membered or larger lactone ring. Of particular interest are the "lactam macrolides", i.e. macrocyclic compounds having a lactam (amide) bond in the macrocycle in addition to a lactone (ester) bond, for example rapamycin and its numerous derivatives and analogues. Rapamycin is an immunosuppressive lactam macrolide that is produced by

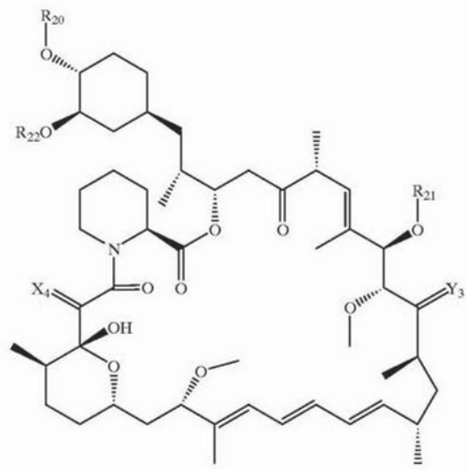
Streptomyces hygroscopicus, and having the structure depicted in Formula

(A)



See, e.g., McAlpine, J. B., et al., J. Antibiotics (1991) 44: 688; Schreiber, S. L., et al., J. Am. Chem. Soc. (1991) 113: 7433; U.S. Pat. No. 3 929 992. One group of rapamycin derivatives are 40-0-substituted derivatives of rapamycin having the structure of Formula IV:

IV



wherein

X_4 is (H,H) or O;

Y_3 is (H,OH) or O;

R_{20} and R_{21} are independently selected from H, alkyl, arylalkyl, hydroxyalkyl, dihydroxyalkyl, hydroxyalkoxycarbonylalkyl, hydroxyalkylaryalkyl, dihydroxyalkylarylalkyl, acyloxyalkyl, aminoalkyl, alkylaminoalkyl, alkoxycarbonylaminoalkyl, acylaminoalkyl, arylsulfonamidoalkyl, allyl, dihydroxyalkylallyl, dioxolanylallyl, dialkyl-dioxolanylalkyl, di(alkoxycarbonyl)-triazolyl-alkyl and hydroxyalkoxy-alkyl; wherein "alk-" or "alkyl" refers to C_{1-6}alkyl, branched or linear, preferably C_{1-3}alkyl,; "aryl" is phenyl or tolyl; and acyl is a radical derived from a carboxylic acid; and

R_{22} is methyl or R_{22} and R_{20} together form C_{2-6}alkyl; provided that R_{20} and R_{21} are not both H; and hydroxyalkoxyalkyl is other than hydroxyalkoxymethyl.

Such compounds are disclosed in WO 94/09010 the contents of which, in particular with respect to the specifically exemplified compounds, are incorporated herein by reference.

A preferred compound is e.g. 40-O-(2-hydroxy)ethyl-rapamycin (referred thereafter as Compound B).

Further preferred rapamycin derivatives are e.g. those disclosed in WO 96/41807, the contents thereof, in particular with respect to the specifically exemplified compounds of formula I disclosed therein, being incorporated herein by reference. Particularly preferred are 32-deoxo-rapamycin, 16-O-pent-2-ynyl-32-deoxo-rapamycin, 16-O-pent-2-ynyl-32-deoxo-40-O-(2-hydroxyethyl)-rapamycin, 16-O-pent-2-ynyl-32-(S)-dihydro-rapamycin and 1 6-O-pent-2-ynyl-32-(S)-dihydro-40-O-(2-hydroxyethyl)-rapamycin.

Further rapamycin derivatives are known, e.g. carboxylic acid esters such as disclosed in WO 92/05179, amide esters such as disclosed in U.S. Pat. No. 5,118,677, carbamates such as described in U.S. Pat. No. 5,118,678, fluorinated esters such as disclosed in U.S. Pat. No. 5,100,883, acetals, e.g. in U.S. Pat. No. 5,151,413, silyl ethers, e.g. in U.S. Pat. No. 5,120,842, arylsulfonates and sulfamates, e.g. in U.S. Pat. No. 5 177 203, derivatives wherein the methoxy group at the position 16 is replaced with alkynyloxy, e.g. in WO 95/16691 and further derivatives such as disclosed in WO 93/11130, WO 94/02136, WO 94/02385 and WO 95/14023, all incorporated herein by reference.

Rapamycin and above mentioned derivatives have been shown to have potent immunosuppressant properties. Rapamycin has also been shown to inhibit smooth muscle cell proliferation and to inhibit cancer growth.

Somatostatin analogues, e.g. octreotide, vapreotide and lanreotide, have been disclosed i.a. to inhibit growth hormone secretion and to have an inhibiting effect on malignant tumor growth, e.g. in breast cancer. Octreotide and lanreotide have also been disclosed to inhibit smooth muscle cell proliferation.

In accordance with the invention, it has now surprisingly been found that a combination of 2 active ingredients believed to act on basically different mechanisms such as a somatostatin analogue and rapamycin or a derivative thereof, can be combined and synergistically inhibit cell hyperproliferation.

In accordance with the particular findings of the present invention, there is provided in a first aspect:

1. Use of a compound of the somatostatin class, in free form or in pharmaceutically acceptable salt form, for manufacturing a pharmaceutical composition for use in synergistically effective amounts in the prevention or treatment of cell hyperproliferation in combination with a rapamycin macrolide, e.g. for the manufacture of a kit as disclosed hereinafter.
2. Use of a compound of the somatostatin class, in free form or in pharmaceutically acceptable salt form, in combination in synergistically effective amounts with a rapamycin macrolide for the prevention or treatment of cell hyperproliferation.
3. A method for preventing or treating cell hyperproliferation in a subject in need of such treatment which comprises administering to such subject a synergistically

effective amount of a compound of the somatostatin class in free form or in pharmaceutically acceptable salt form, and a rapamycin macrolide.

4. A kit or package for the treatment or prevention of cell hyperproliferation, said kit or package including a pharmaceutical composition comprising a compound of the somatostatin class in free form or in pharmaceutically acceptable salt form, and a pharmaceutical composition comprising a rapamycin macrolide. The kit or package may also contain instructions to use the pharmaceutical compositions in accordance with the present invention.

According to the invention, the combination of a compound of the somatostatin class and a rapamycin macrolide is indicated for the prevention or treatment of malignant tumor growth, e.g. breast, lung, GEP tumors, pituitary adenomas, lymphomas, etc., for the prevention or treatment of proliferative vascular diseases, e.g. biologically or mechanically induced vascular injury causing intimal thickening, e.g. restenosis, atherosclerosis, vascular occlusion, injury following percutaneous transluminal coronary angioplasty, vascular surgery or transplantation surgery, transplant vasculopathies, for example chronic rejection of various tissues and organs such as heart, kidney, pancreas, lung, liver, bowel, trachea and combined heart-lung.

The combination is particularly indicated for preventing intimal smooth muscle cell hyperplasia, restenosis and vascular occlusion in a mammal.

Utility of the combination in the treatment of disorders and diseases as hereinbefore specified, may be demonstrated for example in accordance with the method hereinafter described.

A. In vitro Assay

AR42J cell cultures are propagated in DMEM supplemented with 10% fetal calf serum (FCS) at 5% CO_2. Cells are grown in the absence of antibiotics or antifungal agents. Subconfluent AR42J cells growing in DMEM and supplemented with 10% FCS are trypsinized, diluted in DMEM+ 2.5% FCS and seeded in uncoated 96-well plates (5,000 to 10,000 cells per well in 180 μl). After a 48-hr incubation period (Day O), the number of cells in a separate control plate is determined both by counting cells in a Coulter counter and by the sulforhodamine B (SRB) staining assay. The cells are then exposed either to the somatostatin analogue alone, e.g. octreotide, or to rapamycin or a derivative thereof alone or to a combination of the somatostatin analogue and rapamycin or its derivative up to 5 days at various concentrations. Total drug exposure lasts for up to 5 days following the first addition and SRB analysis as described above is performed e.g. on day 2 and day 5. Growth is determined as difference in absorbance (OD) between day 0 and day x values (=delta OD). Calculations are made based on the fractional product method of Webb (Valeriote and Lin, 1975; Cory and Carter, 1986; Berenbaum, J. Theor. Biol. 114: 413–431, 1985) and the method by Chou and Talalay (Adv. Enz. Regul. 22: 27–55, 1984). If the measured cell growth (% of control) is <compared to the calculated cell growth, this shows evidence for a synergistic effect. Under these conditions a combination of a somatostatin analogue at a concentration of from 10^{-10} to 10^{-6} M with a rapamycin macrolide thereof at a concentration of from 1 to 1000 nM significantly inhibits the growth of the tumor cells.

In this assay, the following results are obtained with octreotide alone, Compound B alone and a combination of octreotide and Compound B. The synergy according to the Webb Method is confirmed by using the Chou-Talalay Method.

	Cell Growth (% of CONTROL)			
	Concentration (nM)	Cell Growth (ΔOD) (%)	Observed (%)	Calculated (Webb Method) (%)
Control		664 ± 9	100	
Octreotide	1.2	397 ± 16	59.8	
Compound B	12.0	420 ± 12	63.3	
Octreotide + Compound B	1.2 + 12.0	103 ± 5	15.6	37.9

B. In Vivo Assay

The AR42J (AR4-2J) rat pancreatic tumor cell line is derived from an azaserine-induced exocrine pancreatic tumor (Jessop and Hay, 1980). It was obtained from ATCC. Cultures are propagated in DMEM supplemented with 10% fetal calf serum (FCS) at 5% CO_2. Cells are grown in the absence of antibiotics or antifungal agents. Female nude mice (nu/nu Balbc-A from Iffa Credo, Lyon, France) weighing 19–22 g, are kept in groups of 5 animals in macrolon cages (type III, 16×22×11 cm). The cages are placed in ventilated cabinets (Iffa Credo) that are maintained at 24±1° C. The animals have free access to drinking water and a pathogen-free rodent diet (Diet A, Kliba, Basel, Switzerland). To initiate tumors from cultured cells, AR42J cells are trypsinized and 10×10^6 tumor cells (in 0.2 ml) are injected subcutaneously (s.c.) into both flanks of nude mice. When tumors have reached a volume of 0.03 cm^3, animals are randomized into control and treatment groups. Control animals receive placebo. Animals are treated as indicated below for 3 weeks with single agents or the drug combination. The somatostatin analogue is given as a single injection of a slow release form at 30 mg/kg s.c. The size of the tumors is determined with a caliper. To calculate the tumor volume in ml, the equation "volume (ellipsoid)=length×depth×height×0.52" was used.

Results

After 4 weeks, the following tumor sizes were determined.

(Please note that values in the control group correspond to 3 week values, since animals were killed afterwards for tumors that became excessively large.)

Treatment	Volume mm^3	SE
Control	4020	579
A) Compound B, 5 mg/kg p.o.	3685	263
B) Rapamycin, 5 mg/kg p.o.	2748	325
C) Octreotide pamoate (biodegradable, sustained release formulation), 30 mg/kg, single inj.	2205	339
Compound B + octreotide (C)	130	75
Rapamycin + octreotide (C)	106	44

C. Clinical trial

Patients are included who have breast cancer as evidenced by histological biopsy (glandular analysis—EOA). They present a metastatic illness and/or loco-regional localization

which is measurable and evaluable. If desired, patients may be included who are resistant to other treatment to conventional therapy such as surgery, radiotherapy, other chemotherapy and/or hormone therapy.

The patients present at least one target, on X-ray analysis, which is measurable or evaluable such as a primitive metastatic tumor which is cutaneous or sub-cutaneous. It may be gangliar or visceral. Preferably, the patients have lesions which have progressed within the month preceding the trial and have an estimated survival time of at least 3 months.

The rapamycin macrolide, e.g rapamycin or compound B is administered orally. The treatment is for at least 3 months or until complete remission. The response may be followed by conventional methodology, e.g. according to IUCC response criteria, e.g. progression, stabilization, partial or complete remission.

The somatostatin analogue, e.g. octreotide, is administered parenterally, e.g. subcutaneous, particularly in a continuous subcutaneous way by means of a portable syringe pump (infusion pump).

According to the invention, the somatostatin analogue and the rapamycin macrolide are preferably administered in the form of a pharmaceutical composition. Rapamycin and its derivatives, e.g. Compound B, may be administered by any conventional route, in particular enterally, e.g. orally, e.g. in the form of tablets, capsules, drink solutions, emulsions or microemulsion preconcentrates, nasally, pulmonary (by inhalation), parenterally, e.g. in the form of injectable solutions or suspensions, or topically. Rapamycin and its derivatives are preferably administered per os and the somatostatin analogue is preferably administered parenterally, e.g by infusion. The somatostatin analogue may also be administered in a slow release form, e.g. as disclosed in UK Patent Specification 2,265,311B. The administration of each component of the combination may take place either separately, simultaneously or sequentially, e.g. rapamycin or Compound B may be administered at first followed later, e.g. 8 to 24 hours later, by the somatostatin analogue.

The amount of each component administered is determined taking into account various factors such as the etiology and severity of the disease, and the patient's condition. Rapamycin or its derivatives may conveniently be administered at doses which are in the range used in immunosuppressive applications such as prevention and treatment of graft vs. host disease, transplant rejection or autoimmune diseases e.g. at a daily dosage from about 0.5 to 500 mg as a single dose or in divided doses. Such doses may also be given intermittently, for example, every other day or every third day. The somatostatin analogue may be administered, e.g. subcutaneously, in a dosage range of about 100 μg to 10 mg per day as a single dose or in divided doses. Thus octreotide may be administered at a dose of from 0.2 mg to 10 mg twice or three times daily. When administered as a slow release form, such formulation may comprise the somatostatin peptide in a concentration from 2.0 to 10% by weight. The release period of such a formulation may be from 1 week to about 2 months. The combination of the somatostatin analogue with rapamycin or its derivative allows to maximize the antiproliferative effect.

The invention contemplates that the active ingredients discussed herein may be utilized in combination with pharmaceutically acceptable diluents and carriers.

FORMULATION EXAMPLES

A. Somatostatin Formulations

1. Ampoules

Octreotide	0.5 mg
Mannitol	45.0 mg
Lactic acid (88%)	3.4 mg
Sodium hydrogenocarbonate	to pH 4.2
Water (inject. grade)	to 1 ml
Carbon dioxide	q.s.

2. Biodegradable Sustained Release Formulation

Octreotide Acetate	4.65% (by weight)
Poly(DL-lactide-co-glycolide)	78.35%
Sterile Mannitol	17%
Vehicle	
Carboxymethylcellulose	0.5% (by weight)
Mannitol	0.6%
Water for injection	98.9%

B. Rapamycin (or Derivative thereof) Formulation: e.g. Capsules

Ethanol	20.0 mg
1,2-propylene glycol	81.0 mg
Refined oil	121.5 mg
Cremophor RH40	202.5 mg
Rapamycin or Compound B	20.0 mg
Total	500 mg

What is claimed is:

1. A kit or package for the inhibition of cell hyperproliferation, said kit or package including a pharmaceutical composition comprising an analogue of somatostatin-14 binding to at least the hSST-2 receptor in the nMolar range selected from

a) (D)Phe-Cys-Phe-(D)Trp-Lys-Thr-Cys-Thr-ol

b) (D)Phe-Cys-Tyr-(D)Trp-Lys-Val-Cys-ThrNH$_2$

c) (D)Phe-Cys-Tyr-(D)Trp-Lys-Val-Cys-TrpNH$_2$

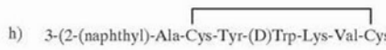
d) (D)Trp-Cys-Phe-(D)Trp-Lys-Thr-Cys-ThrNH$_2$

e) (D)Phe-Cys-Phe-(D)Trp-Lys-Thr-Cys-ThrNH$_2$

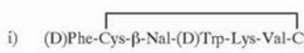
f) 3-(2-(Naphthyl)-(D)Ala-Cys-Try-(D)Trp-Lys-Val-Cys-ThrNH$_2$

g) (D)Phe-Cys-Tyr-(D)Trp-Lys-Val-Cys-β-Nal-NH$_2$

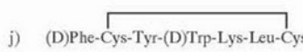
-continued



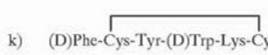
h) 3-(2-(naphthyl)-Ala-Cys-Tyr-(D)Trp-Lys-Val-Cys-β-Nal-NH_2



i) (D)Phe-Cys-β-Nal-(D)Trp-Lys-Val-Cys-Thr-NH_2



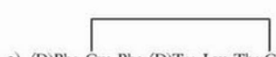
j) (D)Phe-Cys-Tyr-(D)Trp-Lys-Leu-Cys-Thr-NH_2 and



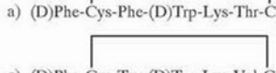
k) (D)Phe-Cys-Tyr-(D)Trp-Lys-Cys-Thr-NH_2,

in free form or in pharmaceutically acceptable salt form, and a pharmaceutical composition comprising a rapamycin macrolide selected from rapamycin and 40-O-(2-hydroxyethyl)-rapamycin, said compositions being present in synergistic effective amounts, together with instructions for use.

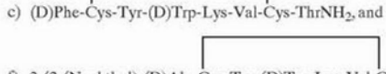
2. A kit or package according to claim **1** wherein the analogue of somatostatin-14 is selected from



a) (D)Phe-Cys-Phe-(D)Trp-Lys-Thr-Cys-Thr-ol



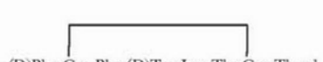
c) (D)Phe-Cys-Tyr-(D)Trp-Lys-Val-Cys-ThrNH_2, and



f) 3-(2-(Naphthyl)-(D)Ala-Cys-Tyr-(D)Trp-Lys-Val-Cys-ThrNH_2,

in free form or pharmaceutically acceptable salt form.

3. A kit or package according to claim **2** wherein the analogue of somatostatin-14 is



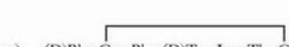
(D)Phe-Cys-Phe-(D)Trp-Lys-Thr-Cys-Thr-ol

in free form or in pamoate salt form.

4. A kit or package according to claim **3** wherein the somatastatin-14 analogue is in sustained release form and the rapamycin macrolide is 40-O-(2-hydroxyethyl)-rapamycin.

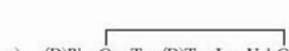
5. A kit or package according to claim **1** for simultaneous or sequential use in synergistically effective amounts.

6. A pharmaceutical composition comprising a pharmaceutically acceptable carrier and a therapeutically effective amount of: 1) an analogue of somatostatin-14 binding to at least the hSST-2 receptor in the nMolar range selected from

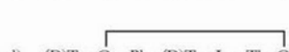


a) (D)Phe-Cys-Phe-(D)Trp-Lys-Thr-Cys-Thr-ol

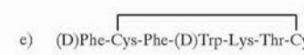
b) (D)Phe-Cys-Tyr-(D)Trp-Lys-Val-Cys-ThrNH_2



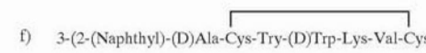
c) (D)Phe-Cys-Tyr-(D)Trp-Lys-Val-Cys-TrpNH_2



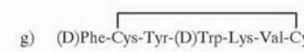
d) (D)Trp-Cys-Phe-(D)Trp-Lys-Thr-Cys-ThrNH_2



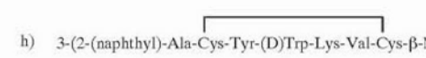
e) (D)Phe-Cys-Phe-(D)Trp-Lys-Thr-Cys-ThrNH_2



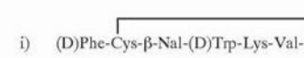
f) 3-(2-(Naphthyl)-(D)Ala-Cys-Try-(D)Trp-Lys-Val-Cys-ThrNH_2



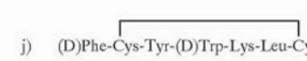
g) (D)Phe-Cys-Tyr-(D)Trp-Lys-Val-Cys-β-Nal-NH_2



h) 3-(2-(naphthyl)-Ala-Cys-Tyr-(D)Trp-Lys-Val-Cys-β-Nal-NH_2



i) (D)Phe-Cys-β-Nal-(D)Trp-Lys-Val-Cys-Thr-NH_2



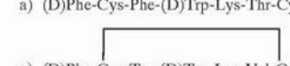
j) (D)Phe-Cys-Tyr-(D)Trp-Lys-Leu-Cys-Thr-NH_2 and

k) (D)Phe-Cys-Tyr-(D)Trp-Lys-Cys-Thr-NH_2,

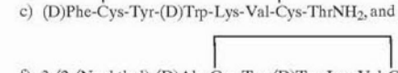
in free form or pharmaceutically acceptable salt form; and 2) a rapamycin macrolide selected from rapamycin and 40-O-(2-hydroxyethyl)-rapamycin, said somatastatin-14 analogue and macrolide being present in synergistic effective amounts.

7. A composition according to claim **6** wherein the analogue of somatostatin-14 is selected from

a) (D)Phe-Cys-Phe-(D)Trp-Lys-Thr-Cys-Thr-ol



c) (D)Phe-Cys-Tyr-(D)Trp-Lys-Val-Cys-ThrNH_2, and



f) 3-(2-(Naphthyl)-(D)Ala-Cys-Tyr-(D)Trp-Lys-Val-Cys-ThrNH_2,

in free form or pharmaceutically acceptable salt form.

8. A composition according to claim **7** wherein the analogue of somatostatin-14 is

(D)Phe-Cys-Phe-(D)Trp-Lys-Thr-Cys-Thr-ol

in free form or in pamoate salt form.

9. A composition according to claim **8** wherein the somatostatin-14 analogue is in sustained release form and the rapamycin macrolide is 40-O-(2-hydroxyethyl)-rapamycin.

10. A method of inhibiting cell hyperproliferation comprising administering to a subject in need of such treatment a therapeutically effective amount of: 1) an analogue of somatostatin-14 binding to at least the hSST-2 receptor in the nMolar range selected from

a) (D)Phe-Cys-Phe-(D)Trp-Lys-Thr-Cys-Thr-ol

-continued

b) (D)Phe-Cys-Tyr-(D)Trp-Lys-Val-Cys-ThrNH$_2$

c) (D)Phe-Cys-Tyr-(D)Trp-Lys-Val-Cys-TrpNH$_2$

d) (D)Trp-Cys-Phe-(D)Trp-Lys-Thr-Cys-ThrNH$_2$

e) (D)Phe-Cys-Phe-(D)Trp-Lys-Thr-Cys-ThrNH$_2$

f) 3-(2-(Naphthyl)-(D)Ala-Cys-Try-(D)Trp-Lys-Val-Cys-ThrNH$_2$

g) (D)Phe-Cys-Tyr-(D)Trp-Lys-Val-Cys-β-Nal-NH$_2$

h) 3-(2-(naphthyl)-Ala-Cys-Tyr-(D)Trp-Lys-Val-Cys-β-Nal-NH$_2$

i) (D)Phe-Cys-β-Nal-(D)Trp-Lys-Val-Cys-Thr-NH$_2$

j) (D)Phe-Cys-Tyr-(D)Trp-Lys-Leu-Cys-Thr-NH$_2$ and

k) (D)Phe-Cys-Tyr-(D)Trp-Lys-Cys-Thr-NH$_2$,

in free form or pharmaceutically acceptable salt form; and 2) a rapamycin macrolide selected from rapamycin and 40-O-(2-hydroxyethyl)-rapamycin, said somatostatin-14 analogue and macrolide being present in synergistic effective amounts.

11. A method according to claim **10** herein the analogue of somatostatin-14 is selected from

a) (D)Phe-Cys-Phe-(D)Trp-Lys-Thr-Cys-Thr-ol

c) (D)Phe-Cys-Tyr-(D)Trp-Lys-Val-Cys-ThrNH$_2$, and

f) 3-(2-(Naphthyl)-(D)Ala-Cys-Tyr-(D)Trp-Lys-Val-Cys-ThrNH$_2$,

in free form or pharmaceutically acceptable salt form.

12. A method according to claim **11** wherein the analogue of somatastatin-14 is

(D)Phe-Cys-Phe-(D)Trp-Lys-Thr-Cys-Thr-ol

in free form or in pamoate salt form.

13. A method according to claim **12** wherein the somatostatin-14 analogue is in sustained release form and the rapamycin macrolide is 40-O-(2-hydroxyethyl)-rapamycin.

* * * * *

PATENT APPLICATION SERIAL NO. 09/194957

U.S. DEPARTMENT OF COMMERCE
PATENT AND TRADEMARK OFFICE
FEE RECORD SHEET

12/10/1998 WCLAYBRO 00000020 190134 09194957

01 FC:970 840.00 CH
02 FC:964 78.00 CH

PTO-1556
(5/87)

*U.S. GPO: 1998-433-214/80404

SERIAL NUMBER	FILING DATE	CLASS	GROUP ART UNIT	ATTORNEY DOCKET NO.
09/194,957	12/07/98	540	1611	4-100-8322/A

APPLICANT

GISBERT WECKBECKER, BIEL-BENKEN, SWITZERLAND.

CONTINUING DOMESTIC DATA********
VERIFIED

371 (NAT'L STAGE) DATA********
VERIFIED THIS APPLN IS A 371 OF PCT/EP97/03036 06/11/97

FOREIGN APPLICATIONS********
VERIFIED UNITED KINGDOM 9612171.0 06/11/96
UNITED KINGDOM 9619310.7 09/16/96

IF REQUIRED, FOREIGN FILING LICENSE GRANTED 05/11/99

Foreign Priority claimed / 35 USC 119 (a-d) conditions met	STATE OR COUNTRY	SHEETS DRAWING	TOTAL CLAIMS	INDEPENDENT CLAIMS
☑ yes ☐ no / ☑ yes ☐ no ☐ Met after Allowance Verified and Acknowledged _____ Examiner's Initials _____ Initials	CHX	0	9	4

ADDRESS

SEE CUSTOMER NUMBER: 001095

TITLE

COMBINATION OF A SOMATOSTATIN ANALOGUE AND A RAPAMYCIN

FILING FEE RECEIVED	FEES: Authority has been given in Paper No. _____ to charge/credit DEPOSIT ACCOUNT NO. _____ for the following:	☐ All Fees ☐ 1.16 Fees (Filing) ☐ 1.17 Fees (Processing Ext. of time) ☐ 1.18 Fees (Issue) ☐ Other _____ ☐ Credit
$918		

303 Rec'd PCT/PTO 07 DEC 1998

NG BY "EXPRESS MAIL" UNDER 37 CFR 1.10

EL239200900US	7 December 1998
Express Mail Label Number	Date of Deposit

Form PTO-1390-MOD (REV 10-96) — U. S. Department of Commerce Patent and Trademark Office

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371

ATTORNEY'S DOCKET NUMBER: 4-100-8322/A/PCT

U.S. APPLICATION NO. (If known, see 37 CFR 1.5): 09/194957

INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/EP 97/03036	11 June 1997 (11.06.97)	11 June 1996 (11.06.96)

TITLE OF INVENTION
COMBINATION OF A SOMATOSTATIN ANALOGUE AND A RAPAMYCIN

APPLICANT(S) FOR DO/EO/US
GISBERT WECKBECKER

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. ☐ This express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1).
4. ☒ A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.
5. ☒ A copy of the International Application as filed (35 U.S.C. 371(c)(2))
   a. ☐ is transmitted herewith (required only if not transmitted by the International Bureau).
   b. ☒ has been transmitted by the International Bureau. (See attached Form PCT/IB/308)
   c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US).
6. ☐ A translation of the International Application into English (35 U.S.C. 371(c)(2)).
7. ☒ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C.371(c)(3)).
   a. ☐ are transmitted herewith (required only if not transmitted by the International Bureau).
   b. ☐ have been transmitted by the International Bureau.
   c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
   d. ☒ have not been made and will not be made.
8. ☐ A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371 (c)(3)).
9. ☒ An executed Declaration and Power of Attorney (original or copy) (35 U.S.C. 371(c)(4)).
10. ☐ A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

Items 11. to 16. below concern document(s) or information included.

11. ☐ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.

12. ☐ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.

13. ☒ A FIRST preliminary amendment.
    ☐ A SECOND or SUBSEQUENT preliminary amendment.

14. ☐ A substitute specification.

15. ☐ A change of power of attorney and/or address letter.

16. ☒ Other items or information: (See attached Form PCT/ISA/210).

U.S. APPLICATION NO. (if known, see 37 CFR 1.5)	INTERNATIONAL APPLICATION NO.	ATTORNEY'S DOCKET NUMBER
	PCT/EP 97/03036	4-100-8322/A/PCT

17. ☒ The following fees are submitted:

BASIC NATIONAL FEE (37 CFR 1.492(a) (1)-(5)):

		CALCULATIONS PTO USE ONLY
Search Report has been prepared by the EPO or JPO	$840	
International preliminary examination fee paid to USPTO (37 CFR 1.482)	$670	
No international preliminary examination fee paid to USPTO (37 CFR 1.482) but international search fee paid to USPTO (37 CFR 1.445(a)(2)).	$760	
Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO.	$970	
International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(2)-(4).	$96	

ENTER APPROPRIATE BASIC FEE AMOUNT =							$ 840
Surcharge of **$130** for furnishing the oath of declaration later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492(e)).							$
CLAIMS	NUMBER FILED		NUMBER EXTRA	RATE			
Total claims	9	- 20 =	0	X	$	18	$
Independent claims	4	- 3 =	1	X	$	78	$ 78
MULTIPLE DEPENDENT CLAIM(S) (if applicable)				+	$	260	$
TOTAL OF ABOVE CALCULATIONS =							$ 918
Reduction of 1/2 for filing by small entity, if applicable. Verified Small Entity Statement must also be filed (Note 37 CFR 1.9, 1.27, 1.28).							$
SUBTOTAL =							$ 918
Processing fee of **$130** for furnishing the English translation later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492(f)).						+	$
TOTAL NATIONAL FEE =							$ 918
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). **$40** per property						+	$
TOTAL FEES ENCLOSED =							$ 918
							Amount to be: refunded $
							charged $

a. ☐ A check in the amount of $_____ to cover the above fees is enclosed.

b. ☒ Please charge Deposit Account No. 19-0134 in the amount of $918 to cover the above fees. A duplicate copy of this form is enclosed.

c. ☒ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 19-0134.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

Send all correspondence to the address associated with Customer No. 001095, which is currently:

Michael W. Glynn
Novartis Corporation
Patent and Trademark Dept.
564 Morris Avenue
Summit, NJ 07901-1027

Joseph J. Borovian
Agent for Applicant
Reg. No. 26,631
(908) 522-6921

FILING BY "EXPRESS MAIL" UNDER 37 CFR ...10

EL239200900US	7 December 1998
Express Mail Label Number	Date of Deposit

Form PTO-1390-MOD (REV 10-96) — U. S. Department of Commerce Patent and Trademark Office		ATTORNEY'S DOCKET NUMBER 4-100-8322/A/PCT
TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371		U.S. APPLICATION NO. (If known, see 37 CFR 1.5) 09/194957
INTERNATIONAL APPLICATION NO. PCT/EP 97/03036	INTERNATIONAL FILING DATE 11 June 1997 (11.06.97)	PRIORITY DATE CLAIMED 11 June 1996 (11.06.96)
TITLE OF INVENTION COMBINATION OF A SOMATOSTATIN ANALOGUE AND A RAPAMYCIN		
APPLICANT(S) FOR DO/EO/US GISBERT WECKBECKER		

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. ☐ This express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1).
4. ☒ A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.
5. ☒ A copy of the International Application as filed (35 U.S.C. 371(c)(2))
   a. ☐ is transmitted herewith (required only if not transmitted by the International Bureau).
   b. ☒ has been transmitted by the International Bureau. (See attached Form PCT/IB/308)
   c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US).
6. ☐ A translation of the International Application into English (35 U.S.C. 371(c)(2)).
7. ☒ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C.371(c)(3)).
   a. ☐ are transmitted herewith (required only if not transmitted by the International Bureau).
   b. ☐ have been transmitted by the International Bureau.
   c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
   d. ☒ have not been made and will not be made.
8. ☐ A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371 (c)(3)).
9. ☒ An executed Declaration and Power of Attorney (original or copy) (35 U.S.C. 371(c)(4)).
10. ☐ A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

Items 11. to 16. below concern document(s) or information included.

11. ☐ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.

12. ☐ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.

13. ☒ A FIRST preliminary amendment.
    ☐ A SECOND or SUBSEQUENT preliminary amendment.

14. ☐ A substitute specification.

15. ☐ A change of power of attorney and/or address letter.

16. ☒ Other items or information: (See attached Form PCT/ISA/210).

U.S. APPLICATION NO. (if known, see 37 CFR 1.5)	INTERNATIONAL APPLICATION NO.	ATTORNEY'S DOCKET NUMBER
	PCT/EP 97/03036	4-100-8322/A/PCT

17. ☒ The following fees are submitted:

BASIC NATIONAL FEE (37 CFR 1.492(a) (1)-(5)):

		CALCULATIONS PTO USE ONLY
Search Report has been prepared by the EPO or JPO	$840	
International preliminary examination fee paid to USPTO (37 CFR 1.482)	$670	
No international preliminary examination fee paid to USPTO (37 CFR 1.482) but international search fee paid to USPTO (37 CFR 1.445(a)(2)).	$760	
Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO.	$970	
International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(2)-(4).	$96	

ENTER APPROPRIATE BASIC FEE AMOUNT = $ 840

Surcharge of **$130** for furnishing the oath of declaration later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492(e)). $

CLAIMS	NUMBER FILED		NUMBER EXTRA	RATE			
Total claims	9	- 20 =	0	X	$	18	$
Independent claims	4	- 3 =	1	X	$	78	$ 78
MULTIPLE DEPENDENT CLAIM(S) (if applicable)				+	$	260	$

TOTAL OF ABOVE CALCULATIONS = $ 918

Reduction of 1/2 for filing by small entity, if applicable. Verified Small Entity Statement must also be filed (Note 37 CFR 1.9, 1.27, 1.28). $

SUBTOTAL = $ 918

Processing fee of **$130** for furnishing the English translation later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492(f)). + $

TOTAL NATIONAL FEE = $ 918

Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). **$40** per property + $

TOTAL FEES ENCLOSED = $ 918

Amount to be: refunded	$
charged	$

a. ☐ A check in the amount of $_____ to cover the above fees is enclosed.

b. ☒ Please charge Deposit Account No. 19-0134 in the amount of $918 to cover the above fees. A duplicate copy of this form is enclosed.

c. ☒ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 19-0134.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

Send all correspondence to the address associated with Customer No. 001095, which is currently:

Michael W. Glynn
Novartis Corporation
Patent and Trademark Dept.
564 Morris Avenue
Summit, NJ 07901-1027

Joseph J. Borovian
Agent for Applicant
Reg. No. 26,631
(908) 522-6921

PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : A61K 38/31 — A1

(11) International Publication Number: WO 97/47317

(43) International Publication Date: 18 December 1997 (18.12.97)

(21) International Application Number: PCT/EP97/03036

(22) International Filing Date: 11 June 1997 (11.06.97)

(30) Priority Data:

9612171.0	11 June 1996 (11.06.96)	GB
9619310.7	16 September 1996 (16.09.96)	GB

(71) Applicant *(for all designated States except US)*: NOVARTIS AG [CH/CH]; Schwarzwaldallee 215, CH-4058 Basel (CH).

(72) Inventor; and

(75) Inventor/Applicant *(for US only)*: WECKBECKER, Gisbert [DE/CH]; Loeliring 31, CH-4105 Biel-Benken (CH).

(74) Agent: ROTH, Bernhard, M.; Novartis AG, Patent- und Markenabteilung, Klybeckstrasse 141, CH-4002 Basel (CH).

(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).

Published
With international search report.

(54) Title: COMBINATION OF A SOMATOSTATIN ANALOGUE AND A RAPAMYCIN

(57) Abstract

A combination of a compound of the somatostatin class and a rapamycin macrolide is useful for the prevention or treatment of cell hyperproliferation.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	**ES**	Spain	**LS**	Lesotho	**SI**	Slovenia
AM	Armenia	**FI**	Finland	**LT**	Lithuania	**SK**	Slovakia
AT	Austria	**FR**	France	**LU**	Luxembourg	**SN**	Senegal
AU	Australia	**GA**	Gabon	**LV**	Latvia	**SZ**	Swaziland
AZ	Azerbaijan	**GB**	United Kingdom	**MC**	Monaco	**TD**	Chad
BA	Bosnia and Herzegovina	**GE**	Georgia	**MD**	Republic of Moldova	**TG**	Togo
BB	Barbados	**GH**	Ghana	**MG**	Madagascar	**TJ**	Tajikistan
BE	Belgium	**GN**	Guinea	**MK**	The former Yugoslav Republic of Macedonia	**TM**	Turkmenistan
BF	Burkina Faso	**GR**	Greece			**TR**	Turkey
BG	Bulgaria	**HU**	Hungary	**ML**	Mali	**TT**	Trinidad and Tobago
BJ	Benin	**IE**	Ireland	**MN**	Mongolia	**UA**	Ukraine
BR	Brazil	**IL**	Israel	**MR**	Mauritania	**UG**	Uganda
BY	Belarus	**IS**	Iceland	**MW**	Malawi	**US**	United States of America
CA	Canada	**IT**	Italy	**MX**	Mexico	**UZ**	Uzbekistan
CF	Central African Republic	**JP**	Japan	**NE**	Niger	**VN**	Viet Nam
CG	Congo	**KE**	Kenya	**NL**	Netherlands	**YU**	Yugoslavia
CH	Switzerland	**KG**	Kyrgyzstan	**NO**	Norway	**ZW**	Zimbabwe
CI	Côte d'Ivoire	**KP**	Democratic People's Republic of Korea	**NZ**	New Zealand		
CM	Cameroon			**PL**	Poland		
CN	China	**KR**	Republic of Korea	**PT**	Portugal		
CU	Cuba	**KZ**	Kazakstan	**RO**	Romania		
CZ	Czech Republic	**LC**	Saint Lucia	**RU**	Russian Federation		
DE	Germany	**LI**	Liechtenstein	**SD**	Sudan		
DK	Denmark	**LK**	Sri Lanka	**SE**	Sweden		
EE	Estonia	**LR**	Liberia	**SG**	Singapore		

COMBINATION OF A SOMATOSTATIN ANALOGUE AND A RAPAMYCIN

The present invention relates to a pharmaceutical combination and its use in the treatment of disorders associated with excess benign and malignant cell proliferation, e.g. tumors or intimal cell proliferation.

There is a continuing need for the development of drugs having increased effectiveness in inhibiting or slowing down undesired cell proliferation, particularly in the cancer field and in vasculopathies.

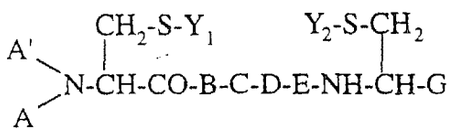
Accordingly, there is provided a pharmaceutical combination comprising a compound of the somatostatin class, and a rapamycin macrolide.

The somatostatin class is a known class of small peptides comprising the naturally occurring somatostatin-14 and analogues having somatostatin related activity, e.g. as disclosed by A.S. Dutta in Small Peptides, Vol.19, Elsevier (1993). By "somatostatin analogue" as used herein is meant any straight-chain or cyclic polypeptide having a structure based on that of the naturally occurring somatostatin-14 wherein one or more amino acid units have been omitted and/or replaced by one or more other amino radical(s) and/or wherein one or more functional groups have been replaced by one or more other functional groups and/or one or more groups have been replaced by one or several other isosteric groups. In general, the term covers all modified derivatives of the native somatostatin-14 which exhibit a somatostatin related activity, e.g. they bind to at least one somatostatin receptor (hSST-1, hSST-2, hSST-3, hSST-4 or hSST-5), preferably in the nMolar range, more preferably to at least the hSST-2 receptor in the nMolar range.

Cyclic, bridge cyclic and straight-chain somatostatin analogues or derivatives are known and have been described together with processes for their production e.g. in US Patent Specifications 4,310,518 and 4,235,886, in European Patent Specifications EP-A-1295; 23,192; 29,310; 29,579; 30,920; 31,303; 63,308; 70,021; 83,305; 215,171; 203,031; 214,872; 143,307; 298,732; 277,419; 389,180; 395,417; 450,480A2; in Belgian Patent Specification BE-A-900,089; and in WO 91/09056; WO 97/01579; WO 97/14715,

the contents thereof, in particular with respect to the compounds, being incorporated herein by reference.

Preferred somatostatin analogues are e. g. compounds of formula I



(I)

wherein

A is C_{1-12}alkyl, C_{7-10}phenylalkyl or a group of formula RCO-,

whereby

i) R is hydrogen, C_{1-11}alkyl, phenyl or C_{7-10}phenylalkyl, or

ii) RCO- is

a) a D-phenylalanine residue optionally ring-substituted by halogen, NO_2, NH_2, OH, C_{1-3}alkyl and/or C_{1-3}alkoxy; or

b) the residue of a natural or a synthetic α-amino-acid other than defined under a) above, or of a corresponding D-amino acid, or

c) a dipeptide residue in which the individual amino acid residues are the same or different and are selected from those defined under a) and/or b) above,

the α-amino group of amino acid residues a) and b) and the N-terminal amino group of dipeptide residues c) being optionally mono- or di-C_{1-12}alkylated or substituted by C_{1-8}alkanoyl;

A' is hydrogen or C_{1-3}alkyl,

Y_1 and Y_2 represent together a direct bond or each of Y_1 and Y_2 is hydrogen

B is -Phe- optionally ring-substituted by halogen, NO_2, NH_2, OH, C_{1-3}alkyl and /or

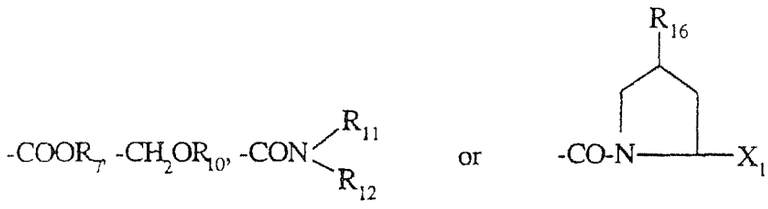
C_{1-3}alkoxy (including pentafluoroalanine), naphthylalanine or pyridylalanine,

C is (L)-Trp- or (D)-Trp- optionally α-N-methylated and optionally benzene-ring-substituted by halogen, NO_2, NH_2, OH, C_{1-3}alkyl and/or C_{1-3}alkoxy,

D is Lys, 4-aminocyclohexylAla or 4-aminocyclohexylGly,

E is Thr, Ser, Val, Tyr, Ile, Leu or an aminobutyric or aminoisobutyric acid residue,

G is a group of formula



wherein

R_7 is hydrogen or C_{1-3}alkyl,

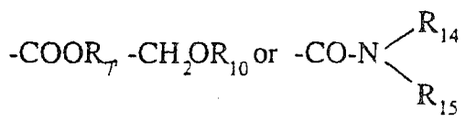
R_{10} is hydrogen or the residue of a physiologically acceptable, physiologically hydrolysable ester, e.g. formyl, C_{2-12}alkylcarbonyl, benzoyl,

R_{11} is hydrogen, C_{1-3}alkyl, phenyl or C_{7-10}phenyl-alkyl,

R_{12} is hydrogen, C_{1-3}alkyl or a group of formula $-CH(R_{13})-X_1$,

R_{13} is CH_2OH, $-(CH_2)_2-OH$, $-(CH_2)_3-OH$, $-CH(CH_3)OH$, isobutyl, butyl, benzyl, naphthyl-methyl or indol-3-yl-methyl, and

X_1 is a group of formula



wherein

R_7 and R_{10} have the meanings given above,

R_{14} is hydrogen or C_{1-3}alkyl ~~and~~

R_{15} is hydrogen, C_{1-3}alkyl, phenyl or C_{7-10}phenylalkyl, and

R_{16} is hydrogen or hydroxy,

with the proviso that

when R_{12} is $-CH(R_{13})-X_1$, then R_{11} is hydrogen or methyl,

wherein the residues B, D and E have the L-configuration, and the residues in the 2- and 7-position each independently have the (L)- or (D)- configuration,

in free form or in pharmaceutically acceptable salt or complex form.

Individual compounds of formula I suitable in accordance with the present invention are the following somatostatin analogues:

a. (D)Phe-Cys-Phe-(D)Trp-Lys-Thr-Cys-Thr-ol
also known as octreotide

b. (D)Phe-Cys-Tyr-(D)Trp-Lys-Val-Cys-ThrNH_2

c. (D)Phe-Cys-Tyr-(D)Trp-Lys-Val-Cys-TrpNH_2
also known as vapreotide

d. (D)Trp-Cys-Phe-(D)Trp-Lys-Thr-Cys-ThrNH_2

e. (D)Phe-Cys-Phe-(D)Trp-Lys-Thr-Cys-ThrNH_2

f. 3-(2-(Naphthyl)-(D)Ala-Cys-Tyr-(D)Trp-Lys-Val-Cys-ThrNH_2
also known as lanreotide

g. (D)Phe-Cys-Tyr-(D)Trp-Lys-Val-Cys-β-Nal-NH_2

h. 3-(2-naphthyl)-Ala-Cys-Tyr-(D)Trp-Lys-Val-Cys-β-Nal-NH_2

i. (D)Phe-Cys-β-Nal-(D)Trp-Lys-Val-Cys-Thr-NH_2

j. (D)Phe-Cys-Tyr-(D)Trp-Lys-Leu-Cys-Thr-NH_2

k. (D)Phe-Cys-Tyr-(D)Trp-Lys-Cys-Thr-NH_2.

A preferred compound of formula I is octreotide.

Compounds of formula I may exist e.g. in free form, salt form or in the form of complexes thereof. Acid addition salts may be formed with e.g. organic acids, polymeric acids and inorganic acids. Such acid addition salt forms include e.g. the hydrochlorides and acetates. Complexes are e.g. formed from compounds of the invention on addition of inorganic substances, e.g. inorganic salts or hydroxides such as Ca- and Zn-salts, and/or an addition of polymeric organic substances.

Further somatostatin analogues suitable for use in accordance with the present invention are:

cyclo [-Asn-Phe-Phe-DTrp-Lys-Thr-Phe-Gaba-],

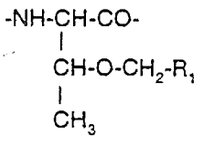
cyclo(Asu-Lys-Asn-Phe-Phe-Trp-Lys-Thr-Tyr-Thr-Ser), and

(D)Nal-Glu-Tyr-(D)Trp-Lys-Val-Lys-Thr-NH_2.

According to an alternatively preferred embodiment of the invention, the somatostatin component of the combination is a somatostatin analogue comprising the amino acid sequence of formula (II)

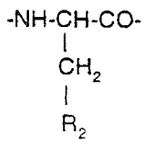
-(D/L)Trp-Lys-X_2-X_3- (II)

wherein X_2 is a radical of formula (a) or (b)



(a)

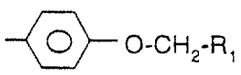
or



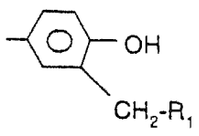
(b)

wherein R_1 is optionally substituted phenyl,

R_2 is $-Z_1-CH_2-R_1$, $-CH_2-CO-O-CH_2-R_1$,



or



wherein Z_1 is O or S,

and

X_3 is an α-amino acid having an aromatic residue on the C_α side chain, or an amino acid unit selected from Dab, Dpr, Dpm, His,(Bzl)HyPro, thienyl-Ala, cyclohexyl-Ala and t.-butyl-Ala,

the residue Lys of said sequence corresponding to the residue Lys^9 of the native somatostatin-14.

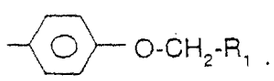
Such somatostatin analogues are e.g. disclosed in WO/ 97/01579, the contents thereof, in particular with respect to the specifically exemplified compounds, being

incorporated herein by reference.

Preferably the sequence of formula II as defined above corresponds to the residues at positions 8 through 11 of the somatostatin-14. More preferably the somatostatin analogue as disclosed above comprises a hexapeptide unit, the residues at positions 3 through 6 of said hexapeptide unit comprising the sequence of formula II. More particularly the hexapeptide unit is cyclic, e.g. having a direct peptide linkage between the α-carbonyl group of the residue at position 6 and the α-amino group of the residue at position 1.

While Lys, X_2 and X_3 in the sequence of formula II have the L-configuration, Trp may have the D- or L-configuration, preferably the D-configuration.

X_2 is preferably a residue of formula (a) or (b), R_2 being preferably $-Z_1-CH_2-R_1$ or



When X_3 comprises an aromatic residue on the C_α side chain, it may suitably be a natural or unnatural α-amino acid, e.g. Phe, Tyr, Trp, Nal, Pal, benzothienyl-Ala, Tic and thyronin, preferably Phe or Nal, more preferably Phe. X_3 is preferably an α-amino acid bearing an aromatic residue on the C_α side chain.

When R_1 is substituted phenyl, it may suitably be substituted by halogen, methyl, ethyl, methoxy or ethoxy e.g. in ortho and/or ~~para~~ position. More preferably R_1 is unsubstituted phenyl. Z_1 is preferably O.

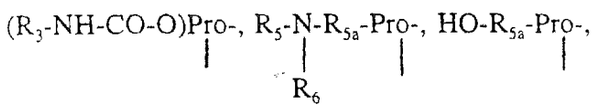
Representative somatostatin analogues comprising a residue of formula II are e.g compounds of formula (III)

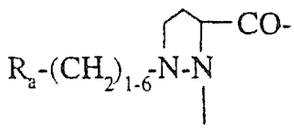
$$\text{cyclo}[\underset{1}{A} - \underset{2}{Z}\underset{a}{Z} - \underset{3}{Trp} - \underset{4}{Lys} - \underset{5}{X_2} - \underset{6}{X_3}] \qquad (II)$$

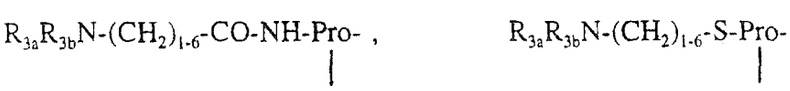
wherein

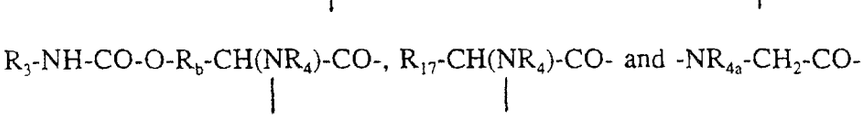
X_2 and X_3 are as defined above,

A_1 is a divalent residue selected from Pro,









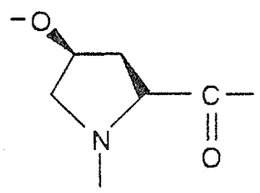
wherein R_3 is NR_8R_9-C_{2-6}alkylene, guanidino-C_{2-6}alkylene or C_{2-6}alkylene-COOH, R_{3a} is H, C_{1-4}alkyl or has independently one of the significances given for R_3, R_{3b} is H or C_{1-4}alkyl, R_a is OH or NR_5R_6, R_b is $-(CH_2)_{1-3}-$ or $-CH(CH_3)-$, R_4 is H or CH_3, R_{4a} is optionally ring-substituted benzyl, each of R_5 and R_6 independently is H, C_{1-4}alkyl, ω-amino-C_{1-4}alkylene, ω-hydroxy-C_{1-4}alkylene or acyl, R_{5a} is a direct bond or C_{1-6}alkylene, each of R_8 and R_9 independently is H, C_{1-4}alkyl, ω-hydroxy-C_{2-4}alkylene, acyl or CH_2OH-$(CHOH)_c$-CH_2- wherein c is 0, 1, 2, 3 or 4, or R_8 and R_9 form together with the nitrogen atom to which they are attached a heterocyclic group which may comprise a further heteroatom, and R_{17} is optionally ring-substituted benzyl, $-(CH_2)_{1-3}$-OH, CH_3-CH(OH)- or $-(CH_2)_{1-5}-NR_5R_6$, and

ZZ_a is a natural or unnatural α-amino acid unit.

ZZ_a may have the D- or L-configuration. When ZZ_a is a natural or unnatural α-amino acid unit, it may suitably be e.g. Thr, Ser, Ala, Val, Ile, Leu, Nle, His, Arg, Lys, Nal, Pal,

Tyr, Trp, optionally ring-substituted Phe or N^{α}-benzyl-Gly. When ZZ_a is Phe, the benzene ring thereof may be substituted by e.g. NH_2, NO_2, CH_3, OCH_3 or halogen, preferably in para position. When ZZ_a is Phe, the benzene ring thereof is preferably unsubstituted.

When A_1 comprises a Pro amino acid residue, any substituent present on the proline ring, e.g. R_3-NH-CO-O- etc., is preferably in position 4. Such substituted proline residue may exist in the cis form, e.g.



as well as in the trans form. The present invention covers each geometric isomer individually as well as mixtures thereof.

When A_1 is (NR_8R_9-C_{2-6}alkylene-NH-CO-O)Pro- where NR_8R_9 forms a heterocyclic group, such group may be aromatic or saturated and may comprise one nitrogen or one nitrogen and a second heteroatom selected from nitrogen and oxygen. Preferably the heterocyclic group is e.g. pyridyl or morpholino. C_{2-6}Alkylene in this residue is preferably -CH_2-CH_2-.

Any acyl as R_5, R_6, R_8 and R_9 in A_1 may be e.g. R_{18}CO- wherein R_{18} is H, C_{1-4}alkyl, C_{2-4}alkenyl, C_{3-6}cycloalkyl or benzyl, preferably methyl or ethyl. When R_{4a} or R_{17} in A_1 is ring-substituted benzyl, the benzene ring may be substituted as indicated above for ZZ_a.

A preferred group of compounds of formula III are such wherein A_1 is free of a lateral -NH-CO-O- moiety. A further group of preferred compounds of formula III are such wherein A_1 comprises a basic lateral radical, e.g. a R_3-NH-CO-O- or R_5-N-R_{5a}-
|
R_6

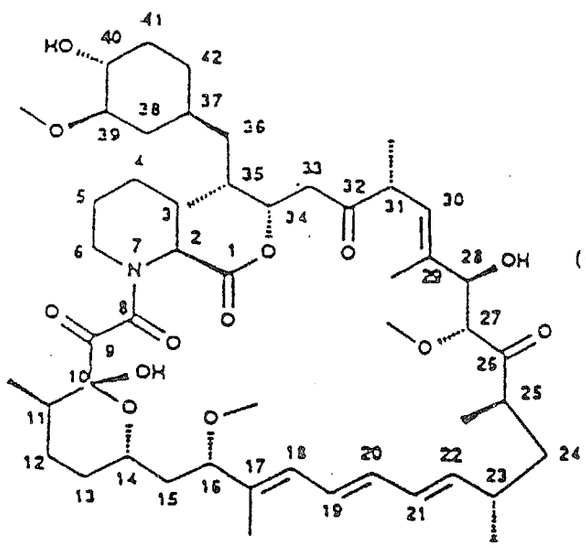
moiety.

A still further group of preferred compounds of formula III are such wherein the N-terminal amino acid comprises a substituted Pro, particularly 4-substituted Pro, e.g. compounds of formula III wherein A_1 is 4-substituted Pro.

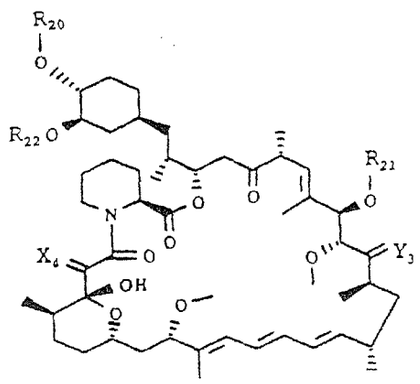
Preferably A_1 is 4-(R_3-NH-CO-O)Pro.

Examples of somatostatin analogues comprising a residue of formula II include e.g. cyclo[4-(NH_2-C_2H_4-NH-CO-O-)Pro-Phe-DTrp-Lys-Ser(Benzyl)-Phe].

The term "macrolide" as used herein, refers to a macrocyclic lactone, for example a compound having a 12-membered or larger lactone ring. Of particular interest are the "lactam macrolides", i.e. macrocyclic compounds having a lactam (amide) bond in the macrocycle in addition to a lactone (ester) bond, for example rapamycin and its numerous derivatives and analogues. Rapamycin is an immunosuppressive lactam macrolide that is produced by Streptomyces hygroscopicus, and having the structure depicted in Formula A:



See, e.g., McAlpine, J.B., et al., J. Antibiotics (1991) 44: 688; Schreiber, S.L., et al., J. Am. Chem. Soc. (1991) 113: 7433; US Patent No. 3 929 992. One group of rapamycin derivatives are 40-0-substituted derivatives of rapamycin having the structure of Formula IV:



IV

wherein

X_4 is (H,H) or O;

Y_3 is (H,OH) or O;

R_{20} and R_{21} are independently selected from H, alkyl, arylalkyl, hydroxyalkyl, dihydroxyalkyl, hydroxyalkoxycarbonylalkyl, hydroxyalkylarylalkyl, dihydroxyalkylarylalkyl, acyloxyalkyl, aminoalkyl, alkylaminoalkyl, alkoxycarbonylaminoalkyl, acylaminoalkyl, arylsulfonamidoalkyl, allyl, dihydroxyalkylallyl, dioxolanylallyl, dialkyl-dioxolanylalkyl, di(alkoxycarbonyl)-triazolyl-alkyl and hydroxyalkoxy-alkyl; wherein "alk-" or "alkyl" refers to C_{1-6}alkyl, branched or linear, preferably C_{1-3}alkyl,; "aryl" is phenyl or tolyl; and acyl is a radical derived from a carboxylic acid; and

R_{22} is methyl or R_{22} and R_{20} together form C_{2-6}alkyl;

provided that R_{20} and R_{21} are not both H; and hydroxyalkoxyalkyl is other than hydroxyalkoxymethyl.

Such compounds are disclosed in WO 94/09010 the contents of which, in particular with respect to the specifically exemplified compounds, are incorporated herein by reference.

A preferred compound is e.g. 40-O-(2-hydroxy)ethyl-rapamycin (referred thereafter as Compound B).

Further preferred rapamycin derivatives are e.g. those disclosed in WO 96/41807, the contents thereof, in particular with respect to the specifically exemplified compounds of formula I disclosed therein, being incorporated herein by reference. Particularly preferred are 32-deoxo-rapamycin, 16-O-pent-2-ynyl-32-deoxo-rapamycin, 16-O-pent-2-ynyl-32-deoxo-40-O-(2-hydroxyethyl)-rapamycin, 16-O-pent-2-ynyl-32-(S)-dihydro-rapamycin and 16-O-pent-2-ynyl-32-(S)-dihydro-40-O-(2-hydroxyethyl)-rapamycin.

Further rapamycin derivatives are known, e.g. carboxylic acid esters such as disclosed in WO 92/05179, amide esters such as disclosed in US 5 118 677, carbamates such as described in US 5 118 678, fluorinated esters such as disclosed in US 5 100 883, acetals, e.g. in US 5 151 413, silyl ethers, e.g. in US 5 120 842, arylsulfonates and sulfamates, e.g. in US 5 177 203, derivatives wherein the methoxy group at the position 16 is replaced with alkynyloxy, e.g. in WO 95/16691 and further derivatives such as disclosed in WO 93/11130, WO 94/02136, WO 94/02385 and WO 95/14023, all incorporated herein by reference.

Rapamycin and above mentioned derivatives have been shown to have potent immunosuppressant properties. Rapamycin has also been shown to inhibit smooth muscle cell proliferation and to inhibit cancer growth.

Somatostatin analogues, e.g. octreotide, vapreotide and lanreotide, have been disclosed i.a. to inhibit growth hormone secretion and to have an inhibiting effect on malignant tumor growth, e.g. in breast cancer. Octreotide and lanreotide have also been disclosed to inhibit smooth muscle cell proliferation.

In accordance with the invention, it has now surprisingly been found that a combination of 2 active ingredients believed to act on basically different mechanisms such as a somatostatin analogue and rapamycin or a derivative thereof, can be combined and synergistically inhibit cell hyperproliferation.

In accordance with the particular findings of the present invention, there is provided in a first aspect:

1. Use of a compound of the somatostatin class, in free form or in pharmaceutically acceptable salt form, for manufacturing a pharmaceutical composition for use in synergistically effective amounts in the prevention or treatment of cell hyperproliferation in combination with a rapamycin macrolide, e.g. for the manufacture of a kit as disclosed hereinafter.

2. Use of a compound of the somatostatin class, in free form or in pharmaceutically acceptable salt form, in combination in synergistically effective amounts with a rapamycin macrolide for the prevention or treatment of cell hyperproliferation.

3. A method for preventing or treating cell hyperproliferation in a subject in need of such treatment which comprises administering to such subject a synergistically effective amount of a compound of the somatostatin class in free form or in pharmaceutically acceptable salt form, and a rapamycin macrolide.

4. A kit or package for the treatment or prevention of cell hyperproliferation, said kit or package including a pharmaceutical composition comprising a compound of the somatostatin class in free form or in pharmaceutically acceptable salt form, and a

pharmaceutical composition comprising a rapamycin macrolide. The kit or package may also contain instructions to use the pharmaceutical compositions in accordance with the present invention.

According to the invention, the combination of a compound of the somatostatin class and a rapamycin macrolide is indicated for the prevention or treatment of malignant tumor growth, e.g. breast, lung, GEP tumors, pituitary adenomas, lymphomas, etc., for the prevention or treatment of proliferative vascular diseases, e.g. biologically or mechanically induced vascular injury causing intimal thickening, e.g. restenosis, atherosclerosis, vascular occlusion, injury following percutaneous transluminal coronary angioplasty, vascular surgery or transplantation surgery, transplant vasculopathies, for example chronic rejection of various tissues and organs such as heart, kidney, pancreas, lung, liver, bowel, trachea and combined heart-lung.

The combination is particularly indicated for preventing intimal smooth muscle cell hyperplasia, restenosis and vascular occlusion in a mammal.

Utility of the combination in the treatment of disorders and diseases as hereinbefore specified, may be demonstrated for example in accordance with the method hereinafter described.

A. In vitro Assay

AR42J cell cultures are propagated in DMEM supplemented with 10 % fetal calf serum (FCS) at 5 % CO_2. Cells are grown in the absence of antibiotics or antifungal agents. Subconfluent AR42J cells growing in DMEM and supplemented with 10 % FCS are trypsinized, diluted in DMEM + 2.5 % FCS and seeded in uncoated 96-well plates (~~5'000 to 10'000~~ 5,000 to 10,000 cells per well in 180 µl). After a 48-hr incubation period (Day O), the number of cells in a separate control plate is determined both by counting cells in a Coulter counter and by the sulforhodamine B (SRB) staining assay. The cells are then exposed either to the somatostatin analogue alone, e.g. octreotide, or to rapamycin or a derivative thereof alone or to a combination of the somatostatin analogue and rapamycin

or its derivative up to 5 days at various concentrations. Total drug exposure lasts for up to 5 days following the first addition and SRB analysis as described above is performed e.g. on day 2 and day 5. Growth is determined as difference in absorbance (OD) between day 0 and day x values (= delta OD). Calculations are made based on the fractional product method of Webb (Valeriote and Lin, 1975; Cory and Carter, 1986; Berenbaum, J. Theor. Biol. 114: 413-431, 1985) and the method by Chou and Talalay (Adv. Enz. Regul. 22: 27-55, 1984). If the measured cell growth (% of control) is < compared to the calculated cell growth, this shows evidence for a synergistic effect. Under these conditions a combination of a somatostatin analogue at a concentration of from 10^{-10} to 10^{-6} M with a rapamycin macrolide thereof at a concentration of from 1 to 1000 nM significantly inhibits the growth of the tumor cells.

In this assay, the following results are obtained with octreotide alone, Compound B alone and a combination of octreotide and Compound B. The synergy according to the Webb Method is confirmed by using the Chou-Talalay Method.

	Cell Growth (% of CONTROL)			
	Concentration (nM)	Cell Growth (ΔOD) (%)	Observed (%)	Calculated (Webb Method) (%)
Control		664 ± 9	100	
Octreotide	1.2	397 ± 16	59.8	
Compound B	12.0	420 ± 12	63.3	
Octreotide + Compound B	1.2 + 12.0	103 ± 5	15.6	37.9

B. In Vivo Assay

The AR42J (AR4-2J) rat pancreatic tumor cell line is derived from an azaserine-induced exocrine pancreatic tumor (Jessop and Hay, 1980). It was obtained from ATCC.

Cultures are propagated in DMEM supplemented with 10% fetal calf serum (FCS) at 5% CO_2. Cells are grown in the absence of antibiotics or antifungal agents. Female nude mice (nu/nu Balbc-A from Iffa Credo, Lyon, France) weighing 19-22 g, are kept in groups of 5 animals in macrolon cages (type III, 16 x 22 x 11 cm). The cages are placed in ventilated cabinets (Iffa Credo) that are maintained at 24 ± 1° C. The animals have free access to drinking water and a pathogen-free rodent diet (Diet A, Kliba, Basel, Switzerland). To initiate tumors from cultured cells, AR42J cells are trypsinized and $10x10^6$ tumor cells (in 0.2 ml) are injected subcutaneously (s.c.) into both flanks of nude mice. When tumors have reached a volume of 0.03 cm^3, animals are randomized into control and treatment groups. Control animals receive placebo. Animals are treated as indicated below for 3 weeks with single agents or the drug combination. The somatostatin analogue is given as a single injection of a slow release form at 30 mg/kg s.c.. The size of the tumors is determined with a caliper. To calculate the tumor volume in ml, the equation "volume (ellipsoid) = length x depth x height x 0.52" was used.

Results

After 4 weeks, the following tumor ~~size~~ sizes were determined.

(Please note that values in the control group correspond to 3 week values, since animals were killed afterwards for tumors that became excessively large.)

Treatment	**Volume** mm^3	**SE**
Control	4020	579
A) Compound B, 5 mg/kg p.o.	3685	263
B) Rapamycin, 5 mg/kg p.o.	2748	325
C) Octreotide pamoate (biodegradable, sustained release formulation), 30 mg/kg, single inj.	2205	339

Compound B + octreotide (C)	130	75
Rapamycin + octreotide (C)	106	44

C. Clinical trial

Patients are included who have breast cancer as evidenced by histological biopsy (glandular analysis - EOA). They present a metastatic illness and/or loco-regional localization ~~localisation~~ which is measurable and evaluable. If desired, patients may be included who are resistant to other treatment to conventional therapy such as surgery, radiotherapy, other chemotherapy and/or hormone therapy.

The patients present at least one target, on X-ray analysis, which is measurable or evaluable such as a primitive metastatic ~~tumour~~ tumor which is cutaneous or sub-cutaneous. It may be gangliar or visceral. Preferably, the patients have lesions which have progressed within the month preceding the trial and have an estimated survival time of at least 3 months.

The rapamycin macrolide, e.g rapamycin or compound B is administered orally. The treatment is for at least 3 months or until complete remission. The response may be followed by conventional methodology, e.g. according to IUCC response criteria, e.g. progression, stabilization, partial or complete remission.

The somatostatin analogue, e.g. octreotide, is administered parenterally, e.g. subcutaneous, particularly in a continuous subcutaneous way by means of a portable syringe pump (infusion pump).

According to the invention, the somatostatin analogue and the rapamycin macrolide are preferably administered in the form of a pharmaceutical composition. Rapamycin and its derivatives, e.g. Compound B, may be administered by any conventional route, in particular enterally, e.g. orally, e.g. in the form of tablets, capsules, drink solutions, emulsions or microemulsion preconcentrates, nasally, pulmonary (by inhalation),

parenterally, e.g. in the form of injectable solutions or suspensions, or topically. Rapamycin and its derivatives are preferably administered per os and the somatostatin analogue is preferably administered parenterally, e.g by infusion. The somatostatin analogue may also be administered in a slow release form, e.g. as disclosed in UK Patent Specification 2,265,311B. The administration of each component of the combination may take place either separately, simultaneously or sequentially, e.g. rapamycin or Compound B may be administered at first followed later, e.g. 8 to 24 hours later, by the somatostatin analogue.

The amount of each component administered is determined taking into account various factors such as the etiology and severity of the disease, and the patient's condition. Rapamycin or its derivatives may conveniently be administered at doses which are in the range used in immunosuppressive applications such as prevention and treatment of graft vs. host disease, transplant rejection or autoimmune diseases e.g. at a daily dosage from about 0.5 to 500 mg as a single dose or in divided doses. Such doses may also be given intermittently, for example, every other day or every third day. The somatostatin analogue may be administered, e.g. subcutaneously, in a dosage range of about 100 µg to 10 mg per day as a single dose or in divided doses. Thus octreotide may be administered at a dose of from 0.2 mg to 10 mg twice or three times daily. When administered as a slow release form, such formulation may comprise the somatostatin peptide in a concentration from 2.0 to 10% by weight. The release period of such a formulation may be from 1 week to about 2 months. The combination of the somatostatin analogue with rapamycin or its derivative allows to maximize the antiproliferative effect.

The invention contemplates that the active ingredients discussed herein may be utilized in combination with pharmaceutically acceptable diluents and carriers.

Formulation Examples:

A. Somatostatin Formulations:

1. Ampoules

Octreotide	0.5 mg
Mannitol	45.0 mg
Lactic acid (88%)	3.4 mg
Sodium hydrogeno-carbonate	to pH 4.2
Water (inject.grade)	to 1 ml
Carbon dioxide	q.s.

2. Biodegradable sustained release formulation:

Octreotide Acetate	4.65 %	(by weight)
Poly(DL-lactide-co-glycolide)	78.35 %	
Sterile Mannitol	17 %	

Vehicle:	Carboxymethylcellulose	0.5 %	(by weight)
	Mannitol	0.6 %	
	Water for injection	98.9 %	

B. Rapamycin (or derivative thereof) formulation: e.g. capsules

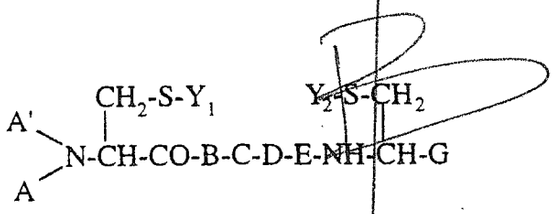
Ethanol	20.0 mg
1,2-propylene glycol	81.0mg
Refined oil	121.5mg
Cremophor RH40	202.5mg
Rapamycin or Compound B	20.0mg
Total	500 mg

CLAIMS

1. Use of a compound of the somatostatin class, in free form or in pharmaceutically acceptable salt form, for manufacturing a pharmaceutical composition for use in synergistically effective amounts in the prevention or treatment of cell hyperproliferation in combination with a rapamycin macrolide.

2. Use of a compound of the somatostatin class, in free form or in pharmaceutically acceptable salt form, in combination in synergistically effective amounts with a rapamycin macrolide for the prevention or treatment of cell hyperproliferation.

3. Use ~~according to claim 1 or~~ 2, wherein the compound of the somatostatin class is a compound of formula I



(I)

wherein

A is C_{1-12}alkyl, C_{7-10}phenylalkyl or a group of formula RCO-,

whereby

i) R is hydrogen, C_{1-11}alkyl, phenyl or C_{7-10}phenylalkyl, or

ii) RCO- is

a) a D-phenylalanine residue optionally ring-substituted by halogen, NO_2, NH_2, OH, C_{1-3}alkyl and/or C_{1-3}alkoxy; or

b) the residue of a natural or a synthetic α-amino-acid other than defined under a) above, or of a corresponding D-amino acid, or

c) a dipeptide residue in which the individual amino acid residues are the same or different and are selected from those defined under a) and/or b) above,

the α-amino group of amino acid residues a) and b) and the N-terminal amino group of dipeptide residues c) being optionally mono- or di-C_{1-12}alkylated or substituted by C_{1-8}alkanoyl;

A' is hydrogen or C_{1-3}alkyl,

Y_1 and Y_2 represent together a direct bond or each of Y_1 and Y_2 is hydrogen

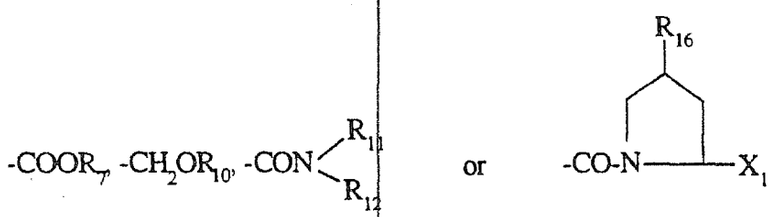
B is -Phe- optionally ring-substituted by halogen, NO_2, NH_2, OH, C_{1-3}alkyl and /or C_{1-3}alkoxy (including pentafluoroalanine), naphthylalanine or pyridylalanine,

C is (L)-Trp- or (D)-Trp- optionally α-N-methylated and optionally benzene-ring-substituted by halogen, NO_2, NH_2, OH, C_{1-3}alkyl and/or C_{1-3}alkoxy,

D is Lys, 4-aminocyclohexylAla or 4-aminocyclohexylGly

E is Thr, Ser, Val, Tyr, Ile, Leu or an aminobutyric or aminoisobutyric acid residue

G is a group of formula



wherein

R_7 is hydrogen or C_{1-3}alkyl,

R_{10} is hydrogen or the residue of a physiologically acceptable, physiologically hydrolysable ester,

R_{11} is hydrogen, C_{1-3}alkyl, phenyl or C_{7-10}phenyl-alkyl,

R_{12} is hydrogen, C_{1-3}alkyl or a group of formula $-CH(R_{13})-X_1$,

R_{13} is CH_2OH, $-(CH_2)_2-OH$, $-(CH_2)_3-OH$, $-CH(CH_3)OH$, isobutyl, butyl, benzyl, naphthyl-methyl or indol-3-yl-methyl, and

X_1 is a group of formula

$$-COOR_7, -CH_2OR_{10} \text{ or } -CO-N\begin{matrix}R_{14}\\R_{15}\end{matrix}$$

wherein

R_7 and R_{10} have the meanings given above,

R_{14} is hydrogen or C_{1-3}alkyl and

R_{15} is hydrogen, C_{1-3}alkyl, phenyl or C_{7-10}phenylalkyl, and

R_{16} is hydrogen or hydroxy,

with the proviso that

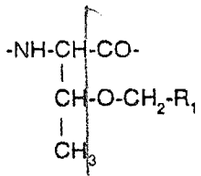
when R_{12} is $-CH(R_{13})-X_1$ then R_{11} is hydrogen or methyl,

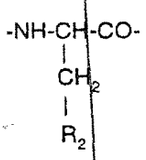
wherein the residues B, D and E have the L-configuration, and the residues in the 2- and 7-position each independently have the (L)- or (D)- configuration

or a somatostatin analogue comprising the amino acid sequence of formula II

$$-(D/L)Trp-Lys-X_2-X_3- \qquad (II)$$

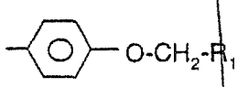
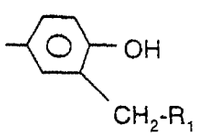
wherein X_2 is a radical of formula (a) or (b)

or  (a)

 (b)

wherein R_1 is optionally substituted phenyl,

R_2 is $-Z_1-CH_2-R_1$, $-CH_2-CO-O-CH_2-R_1$,

 or 

wherein Z_1 is O or S,

and

X_3 is an α-amino acid having an aromatic residue on the C_α side chain, or an amino acid unit selected from Dab, Dpr, Dpm, His,(Bzl)HyPro, thienyl-Ala, cyclohexyl-Ala and t.-butyl-Ala,

the residue Lys of said sequence corresponding to the residue Lys^9 of the native somatostatin-14,

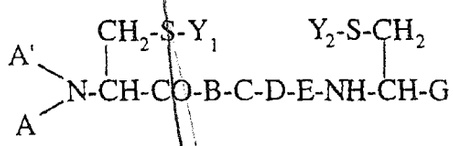
in free form or in pharmaceutically acceptable salt form.

4. Use according to claim 3, wherein the compound of the somatostatin class is octreotide, lanreotide or vapreotide.

5. A method for preventing or treating cell hyperproliferation in a subject in need of such treatment which comprises administering to such subject a synergistically effective amount of a compound of the somatostatin class in free form or in pharmaceutically acceptable salt form, and a rapamycin macrolide.

6. A kit or package for the treatment or prevention of cell hyperproliferation, said kit or package including a pharmaceutical composition comprising a compound of the somatostatin class in free form or in pharmaceutically acceptable salt form, and a pharmaceutical composition comprising a rapamycin macrolide, together with instructions for use.

7. A kit or package according to claim 6, wherein the compound of the somatostatin class is a compound of formula I



(I)

wherein

A is C_{1-12}alkyl, C_{7-10}phenylalkyl or a group of formula RCO-,

whereby

i) R is hydrogen, C_{1-11}alkyl, phenyl or C_{7-10}phenylalkyl, or

ii) RCO- is

a) a D-phenylalanine residue optionally ring-substituted by halogen, NO_2, NH_2, OH, C_{1-3}alkyl and/or C_{1-3}alkoxy; or

b) the residue of a natural or a synthetic α-amino-acid other than defined under a) above, or of a corresponding D-amino acid, or

c) a dipeptide residue in which the individual amino acid residues are the same or different and are selected from those defined under a) and/or b) above,

the α-amino group of amino acid residues a) and b) and the N-terminal amino group of dipeptide residues c) being optionally mono- or di-C_{1-12}alkylated or substituted by C_{1-8}alkanoyl;

A' is hydrogen or C_{1-3}alkyl,

Y_1 and Y_2 represent together a direct bond or each of Y_1 and Y_2 is hydrogen,

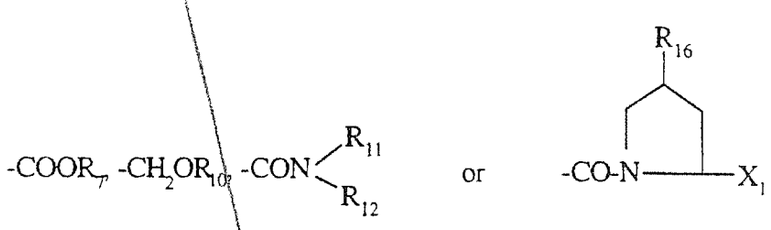
B is -Phe- optionally ring-substituted by halogen, NO_2, NH_2, OH, C_{1-3}alkyl and /or C_{1-3}alkoxy (including pentafluoroalanine), naphthylalanine or pyridylalanine,

C is (L)-Trp- or (D)-Trp- optionally α-N-methylated and optionally benzene-ring-substituted by halogen, NO_2, NH_2, OH, C_{1-3}alkyl and/or C_{1-3}alkoxy,

D is Lys, 4-aminocyclohexylAla or 4-aminocyclohexylGly,

E is Thr, Ser, Val, Tyr, Ile, Leu or an aminobutyric or aminoisobutyric acid residue,

G is a group of formula



wherein

R_7 is hydrogen or C_{1-3}alkyl,

R_{10} is hydrogen or the residue of a physiologically acceptable, physiologically hydrolysable ester,

R_{11} is hydrogen, C_{1-3}alkyl, phenyl or C_{7-10}phenyl-alkyl,

R_{12} is hydrogen, C_{1-3}alkyl or a group of formula $-CH(R_{13})-X_1$,

R_{13} is CH_2OH, $-(CH_2)_2-OH$, $-(CH_2)_3-OH$, $-CH(CH_3)OH$, isobutyl, butyl, benzyl, naphtyl-methyl or indol-3-yl-methyl, and

X_1 is a group of formula

$-COOR_7$, $-CH_2OR_{10}$ or $-CO-N(R_{14})(R_{15})$

wherein

R_7 and R_{10} have the meanings given above,

R_{14} is hydrogen or C_{1-3}alkyl and

R_{15} is hydrogen, C_{1-3}alkyl, phenyl or C_{7-10}phenylalkyl, and

R_{16} is hydrogen or hydroxy,

with the proviso that

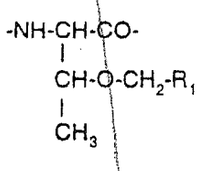
when R_{12} is $-CH(R_{13})-X_1$ then R_{11} is hydrogen or methyl,

wherein the residues B, D and E have the L-configuration, and the residues in the 2- and 7-position each independently have the (L)- or (D)- configuration

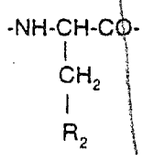
or a somatostatin analogue comprising the amino acid sequence of formula II

$$-(D/L)Trp-Lys-X_2-X_3- \qquad (II)$$

wherein X_2 is a radical of formula (a) or (b)



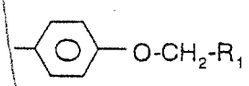
(a)



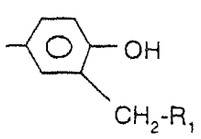
(b)

wherein R_1 is optionally substituted phenyl,

R_2 is $-Z_1-CH_2-R_1$, $-CH_2-CO-O-CH_2-R_1$,



or



wherein Z_1 is O or S,

and

X_3 is an α-amino acid having an aromatic residue on the C_α side chain, or an amino acid unit selected from Dab, Dpr, Dpm, His,(Bzl)HyPro, thienyl-Ala, cyclohexyl-Ala and t.-butyl-Ala,

the residue Lys of said sequence corresponding to the residue Lys^9 of the native somatostatin-14,

in free form or in pharmaceutically acceptable salt form.

8. A kit or package according to claim 7, wherein the compound of the somatostatin class is octreotide, lanreotide or vapreotide.

9. A kit or package according to claim 6 for simultaneous, ~~separate~~ or sequential use in synergistically effective amounts.

PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 :
A61K 38/31

A1

(11) International Publication Number: WO 97/47317

(43) International Publication Date: 18 December 1997 (18.12.97)

(21) International Application Number: PCT/EP97/03036

(22) International Filing Date: 11 June 1997 (11.06.97)

(30) Priority Data:
9612171.0 11 June 1996 (11.06.96) GB
9619310.7 16 September 1996 (16.09.96) GB

(71) Applicant *(for all designated States except US)*: NOVARTIS AG [CH/CH]; Schwarzwaldallee 215, CH-4058 Basel (CH).

(72) Inventor; and
(75) Inventor/Applicant *(for US only)*: WECKBECKER, Gisbert [DE/CH]; Loeliring 31, CH-4105 Biel-Benken (CH).

(74) Agent: ROTH, Bernhard, M.; Novartis AG, Patent- und Markenabteilung, Klybeckstrasse 141, CH-4002 Basel (CH).

(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).

Published
With international search report.

(54) Title: COMBINATION OF A SOMATOSTATIN ANALOGUE AND A RAPAMYCIN

(57) Abstract

A combination of a compound of the somatostatin class and a rapamycin macrolide is useful for the prevention or treatment of cell hyperproliferation.

COMBINATION OF A SOMATOSTATIN ANALOGUE AND A RAPAMYCIN

The present invention relates to a pharmaceutical combination and its use in the treatment of disorders associated with excess benign and malignant cell proliferation, e.g. tumors or intimal cell proliferation.

There is a continuing need for the development of drugs having increased effectiveness in inhibiting or slowing down undesired cell proliferation, particularly in the cancer field and in vasculopathies.

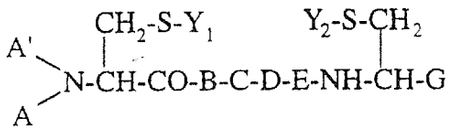
Accordingly, there is provided a pharmaceutical combination comprising a compound of the somatostatin class, and a rapamycin macrolide.

The somatostatin class is a known class of small peptides comprising the naturally occurring somatostatin-14 and analogues having somatostatin related activity, e.g. as disclosed by A.S. Dutta in Small Peptides, Vol.19, Elsevier (1993). By "somatostatin analogue" as used herein is meant any straight-chain or cyclic polypeptide having a structure based on that of the naturally occurring somatostatin-14 wherein one or more amino acid units have been omitted and/or replaced by one or more other amino radical(s) and/or wherein one or more functional groups have been replaced by one or more other functional groups and/or one or more groups have been replaced by one or several other isosteric groups. In general, the term covers all modified derivatives of the native somatostatin-14 which exhibit a somatostatin related activity, e.g. they bind to at least one somatostatin receptor (hSST-1, hSST-2, hSST-3, hSST-4 or hSST-5), preferably in the nMolar range, more preferably to at least the hSST-2 receptor in the nMolar range.

Cyclic, bridge cyclic and straight-chain somatostatin analogues or derivatives are known and have been described together with processes for their production e.g. in US Patent Specifications 4,310,518 and 4,235,886, in European Patent Specifications EP-A-1295; 23,192; 29,310; 29,579; 30,920; 31,303; 63,308; 70,021; 83,305; 215,171; 203,031; 214,872; 143,307; 298,732; 277,419; 389,180; 395,417; 450,480A2; in Belgian Patent Specification BE-A-900,089; and in WO 91/09056; WO 97/01579; WO 97/14715,

the contents thereof, in particular with respect to the compounds, being incorporated herein by reference.

Preferred somatostatin analogues are e. g. compounds of formula I



(I)

wherein

A is C_{1-12}alkyl, C_{7-10}phenylalkyl or a group of formula RCO-,

whereby

i) R is hydrogen, C_{1-11}alkyl, phenyl or C_{7-10}phenylalkyl, or

ii) RCO- is

a) a D-phenylalanine residue optionally ring-substituted by halogen, NO_2, NH_2, OH, C_{1-3}alkyl and/or C_{1-3}alkoxy; or

b) the residue of a natural or a synthetic α-amino-acid other than defined under a) above, or of a corresponding D-amino acid, or

c) a dipeptide residue in which the individual amino acid residues are the same or different and are selected from those defined under a) and/or b) above,

the α-amino group of amino acid residues a) and b) and the N-terminal amino group of dipeptide residues c) being optionally mono- or di-C_{1-12}alkylated or substituted by C_{1-8}alkanoyl;

A' is hydrogen or C_{1-3}alkyl,

Y_1 and Y_2 represent together a direct bond or each of Y_1 and Y_2 is hydrogen

B is -Phe- optionally ring-substituted by halogen, NO_2, NH_2, OH, C_{1-3}alkyl and /or

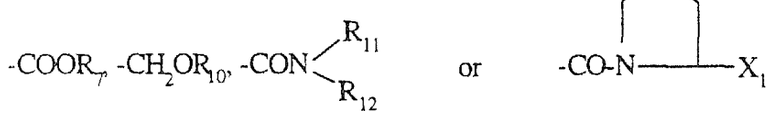
C_{1-3}alkoxy (including pentafluoroalanine), naphthylalanine or pyridylalanine,

C is (L)-Trp- or (D)-Trp- optionally α-N-methylated and optionally benzene-ring-substituted by halogen, NO_2, NH_2, OH, C_{1-3}alkyl and/or C_{1-3}alkoxy,

D is Lys, 4-aminocyclohexylAla or 4-aminocyclohexylGly

E is Thr, Ser, Val, Tyr, Ile, Leu or an aminobutyric or aminoisobutyric acid residue

G is a group of formula



wherein

R_7 is hydrogen or C_{1-3}alkyl,

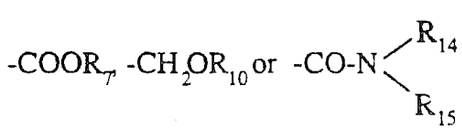
R_{10} is hydrogen or the residue of a physiologically acceptable, physiologically hydrolysable ester, e.g. formyl, C_{2-12}alkylcarbonyl, benzoyl,

R_{11} is hydrogen, C_{1-3}alkyl, phenyl or C_{7-10}phenyl-alkyl,

R_{12} is hydrogen, C_{1-3}alkyl or a group of formula $-CH(R_{13})-X_1$,

R_{13} is CH_2OH, $-(CH_2)_2-OH$, $-(CH_2)_3-OH$, $-CH(CH_3)OH$, isobutyl, butyl, benzyl, naphthyl-methyl or indol-3-yl-methyl, and

X_1 is a group of formula



wherein

R_7 and R_{10} have the meanings given above,

R_{14} is hydrogen or C_{1-3}alkyl and

R_{15} is hydrogen, C_{1-3}alkyl, phenyl or C_{7-10}phenylalkyl, and

R_{16} is hydrogen or hydroxy,

with the proviso that

when R_{12} is $-CH(R_{13})-X_1$ then R_{11} is hydrogen or methyl,

wherein the residues B, D and E have the L-configuration, and the residues in the 2- and 7-position each independently have the (L)- or (D)- configuration,

in free form or in pharmaceutically acceptable salt or complex form.

Individual compounds of formula I suitable in accordance with the present invention are the following somatostatin analogues:

a. (D)Phe-Cys-Phe-(D)Trp-Lys-Thr-Cys-Thr-ol
   also known as octreotide

b. (D)Phe-Cys-Tyr-(D)Trp-Lys-Val-Cys-ThrNH_2

c. (D)Phe-Cys-Tyr-(D)Trp-Lys-Val-Cys-TrpNH_2
   also known as vapreotide

d. (D)Trp-Cys-Phe-(D)Trp-Lys-Thr-Cys-ThrNH_2

e. (D)Phe-Cys-Phe-(D)Trp-Lys-Thr-Cys-ThrNH_2

f. 3-(2-(Naphthyl)-(D)Ala-Cys-Tyr-(D)Trp-Lys-Val-Cys-ThrNH_2
   also known as lanreotide

g. (D)Phe-Cys-Tyr-(D)Trp-Lys-Val-Cys-β-Nal-NH_2

h. 3-(2-naphthyl)-Ala-Cys-Tyr-(D)Trp-Lys-Val-Cys-β-Nal-NH_2

i. (D)Phe-Cys-β-Nal-(D)Trp-Lys-Val-Cys-Thr-NH_2

j. (D)Phe-Cys-Tyr-(D)Trp-Lys-Leu-Cys-Thr-NH_2

k. (D)Phe-Cys-Tyr-(D)Trp-Lys-Cys-Thr-NH_2.

A preferred compound of formula I is octreotide.

Compounds of formula I may exist e.g. in free form, salt form or in the form of complexes thereof. Acid addition salts may be formed with e.g. organic acids, polymeric acids and inorganic acids. Such acid addition salt forms include e.g. the hydrochlorides and acetates. Complexes are e.g. formed from compounds of the invention on addition of inorganic substances, e.g. inorganic salts or hydroxides such as Ca- and Zn-salts, and/or an addition of polymeric organic substances.

Further somatostatin analogues suitable for use in accordance with the present invention are:

cyclo [-Asn-Phe-Phe-DTrp-Lys-Thr-Phe-Gaba-],

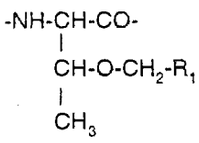
cyclo(Asu-Lys-Asn-Phe-Phe-Trp-Lys-Thr-Tyr-Thr-Ser), and

(D)Nal-Glu-Tyr-(D)Trp-Lys-Val-Lys-Thr-NH_2

According to an alternatively preferred embodiment of the invention, the somatostatin component of the combination is a somatostatin analogue comprising the amino acid sequence of formula (II)

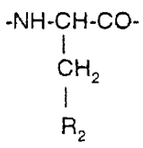
-(D/L)Trp-Lys-X_2-X_3- (II)

wherein X_2 is a radical of formula (a) or (b)



(a)

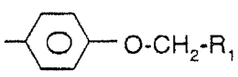
or



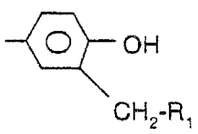
(b)

wherein R_1 is optionally substituted phenyl,

R_2 is $-Z_1-CH_2-R_1$, $-CH_2-CO-O-CH_2-R_1$,



or



wherein Z_1 is O or S,

and

X_3 is an α-amino acid having an aromatic residue on the C_α side chain, or an amino acid unit selected from Dab, Dpr, Dpm, His,(Bzl)HyPro, thienyl-Ala, cyclohexyl-Ala and t.-butyl-Ala,

the residue Lys of said sequence corresponding to the residue Lys^9 of the native somatostatin-14.

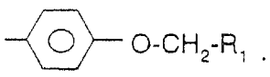
Such somatostatin analogues are e.g. disclosed in WO/ 97/01579, the contents thereof, in particular with respect to the specifically exemplified compounds, being

incorporated herein by reference.

Preferably the sequence of formula II as defined above corresponds to the residues at positions 8 through 11 of the somatostatin-14. More preferably the somatostatin analogue as disclosed above comprises a hexapeptide unit, the residues at positions 3 through 6 of said hexapeptide unit comprising the sequence of formula II. More particularly the hexapeptide unit is cyclic, e.g. having a direct peptide linkage between the α-carbonyl group of the residue at position 6 and the α-amino group of the residue at position 1.

While Lys, X_2 and X_3 in the sequence of formula II have the L-configuration, Trp may have the D- or L-configuration, preferably the D-configuration.

X_2 is preferably a residue of formula (a) or (b), R_2 being preferably $-Z_1-CH_2-R_1$ or



When X_3 comprises an aromatic residue on the C_α side chain, it may suitably be a natural or unnatural α-amino acid, e.g. Phe, Tyr, Trp, Nal, Pal, benzothienyl-Ala, Tic and thyronin, preferably Phe or Nal, more preferably Phe. X_3 is preferably an α-amino acid bearing an aromatic residue on the C_α side chain.

When R_1 is substituted phenyl, it may suitably be substituted by halogen, methyl, ethyl, methoxy or ethoxy e.g. in ortho and/or para. More preferably R_1 is unsubstituted phenyl. Z_1 is preferably O.

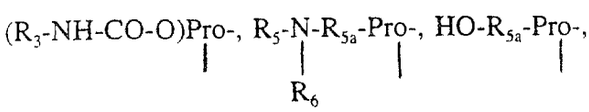
Representative somatostatin analogues comprising a residue of formula II are e.g compounds of formula (III)

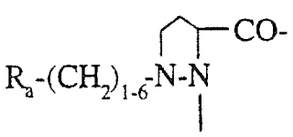
$$\underset{1}{\text{A}} - \underset{2}{\text{ZZ}_a} - \underset{3}{\text{Trp}} - \underset{4}{\text{Lys}} - \underset{5}{\text{X}_2} - \underset{6}{\text{X}_3} \quad \text{(II)}$$

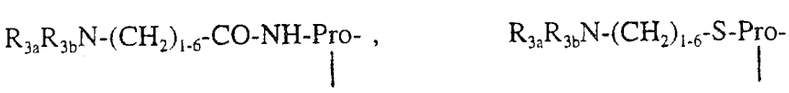
wherein

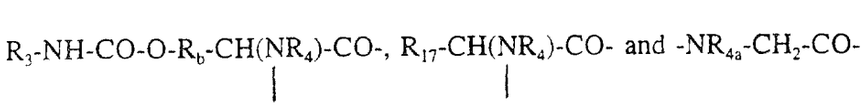
X_2 and X_3 are as defined above,

A_1 is a divalent residue selected from Pro,









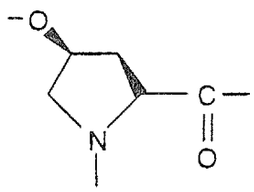
wherein R_3 is NR_8R_9-C_{2-6}alkylene, guanidino-C_{2-6}alkylene or C_{2-6}alkylene-COOH, R_{3a} is H, C_{1-4}alkyl or has independently one of the significances given for R_3, R_{3b} is H or C_{1-4}alkyl, R_a is OH or NR_5R_6, R_b is $-(CH_2)_{1-3}-$ or $-CH(CH_3)-$, R_4 is H or CH_3, R_{4a} is optionally ring-substituted benzyl, each of R_5 and R_6 independently is H, C_{1-4}alkyl, ω-amino-C_{1-4}alkylene, ω-hydroxy-C_{1-4}alkylene or acyl, R_{5a} is a direct bond or C_{1-6}alkylene, each of R_8 and R_9 independently is H, C_{1-4}alkyl, ω-hydroxy-C_{2-4}alkylene, acyl or CH_2OH-$(CHOH)_c$-CH_2- wherein c is 0, 1, 2, 3 or 4, or R_8 and R_9 form together with the nitrogen atom to which they are attached a heterocyclic group which may comprise a further heteroatom, and R_{17} is optionally ring-substituted benzyl, $-(CH_2)_{1-3}$-OH, CH_3-CH(OH)- or $-(CH_2)_{1-5}-NR_5R_6$, and

ZZ_a is a natural or unnatural α-amino acid unit.

ZZ_a may have the D- or L-configuration. When ZZ_a is a natural or unnatural α-amino acid unit, it may suitably be e.g. Thr, Ser, Ala, Val, Ile, Leu, Nle, His, Arg, Lys, Nal, Pal,

Tyr, Trp, optionally ring-substituted Phe or N^{α}-benzyl-Gly. When ZZ_a is Phe, the benzene ring thereof may be substituted by e.g. NH_2, NO_2, CH_3, OCH_3 or halogen, preferably in para position. When ZZ_a is Phe, the benzene ring thereof is preferably unsubstituted.

When A_1 comprises a Pro amino acid residue, any substituent present on the proline ring, e.g. R_3-NH-CO-O- etc., is preferably in position 4. Such substituted proline residue may exist in the cis form, e.g.



as well as in the trans form. The present invention covers each geometric isomer individually as well as mixtures thereof.

When A_1 is $(NR_8R_9\text{-}C_{2\text{-}6}\text{alkylene-NH-CO-O})$Pro- where NR_8R_9 forms a heterocyclic group, such group may be aromatic or saturated and may comprise one nitrogen or one nitrogen and a second heteroatom selected from nitrogen and oxygen. Preferably the heterocyclic group is e.g. pyridyl or morpholino. $C_{2\text{-}6}$Alkylene in this residue is preferably $-CH_2-CH_2-$.

Any acyl as R_5, R_6, R_8 and R_9 in A_1 may be e.g. $R_{18}CO$- wherein R_{18} is H, $C_{1\text{-}4}$alkyl, $C_{2\text{-}4}$alkenyl, $C_{3\text{-}6}$cycloalkyl or benzyl, preferably methyl or ethyl. When R_{4a} or R_{17} in A_1 is ring-substituted benzyl, the benzene ring may be substituted as indicated above for ZZ_a.

A preferred group of compounds of formula III are such wherein A_1 is free of a lateral -NH-CO-O- moiety. A further group of preferred compounds of formula III are such wherein A_1 comprises a basic lateral radical, e.g. a R_3-NH-CO-O- or R_5-N-R_{5a}-
R_6

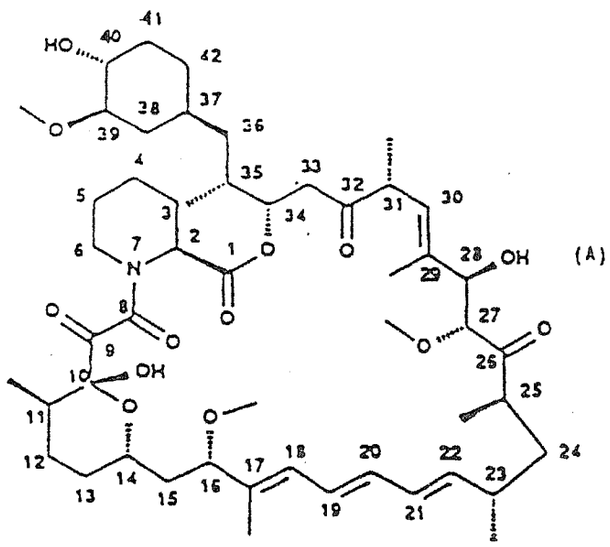
moiety.

A still further group of preferred compounds of formula III are such wherein the N-terminal amino acid comprises a substituted Pro, particularly 4-substituted Pro, e.g. compounds of formula III wherein A_1 is 4-substituted Pro.

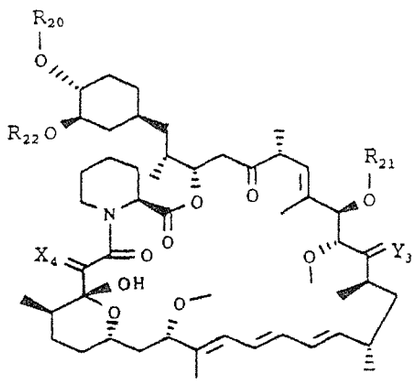
Preferably A_1 is 4-(R_3-NH-CO-O)Pro.

Examples of somatostatin analogues comprising a residue of formula II include e.g. cyclo[4-(NH_2-C_2H_4-NH-CO-O-)Pro-Phe-DTrp-Lys-Ser(Benzyl)-Phe].

The term "macrolide" as used herein, refers to a macrocyclic lactone, for example a compound having a 12-membered or larger lactone ring. Of particular interest are the "lactam macrolides", i.e. macrocyclic compounds having a lactam (amide) bond in the macrocycle in addition to a lactone (ester) bond, for example rapamycin and its numerous derivatives and analogues. Rapamycin is an immunosuppressive lactam macrolide that is produced by Streptomyces hygroscopicus, and having the structure depicted in Formula A:



See, e.g., McAlpine, J.B., et al., J. Antibiotics (1991) 44: 688; Schreiber, S.L., et al., J. Am. Chem. Soc. (1991) 113: 7433; US Patent No. 3 929 992. One group of rapamycin derivatives are 40-0-substituted derivatives of rapamycin having the structure of Formula IV:



IV

wherein

X_4 is (H,H) or O;

Y_3 is (H,OH) or O;

R_{20} and R_{21} are independently selected from H, alkyl, arylalkyl, hydroxyalkyl, dihydroxyalkyl, hydroxyalkoxycarbonylalkyl, hydroxyalkylarylalkyl, dihydroxyalkylarylalkyl, acyloxyalkyl, aminoalkyl, alkylaminoalkyl, alkoxycarbonylaminoalkyl, acylaminoalkyl, arylsulfonamidoalkyl, allyl, dihydroxyalkylallyl, dioxolanylallyl, dialkyl-dioxolanylalkyl, di(alkoxycarbonyl)-triazolyl-alkyl and hydroxyalkoxy-alkyl; wherein "alk-" or "alkyl" refers to C_{1-6}alkyl, branched or linear, preferably C_{1-3}alkyl,; "aryl" is phenyl or tolyl; and acyl is a radical derived from a carboxylic acid; and

R_{22} is methyl or R_{22} and R_{20} together form C_{2-6}alkyl;

provided that R_{20} and R_{21} are not both H; and hydroxyalkoxyalkyl is other than hydroxyalkoxymethyl.

Such compounds are disclosed in WO 94/09010 the contents of which, in particular with respect to the specifically exemplified compounds, are incorporated herein by reference.

A preferred compound is e.g. 40-O-(2-hydroxy)ethyl-rapamycin (referred thereafter as Compound B).

Further preferred rapamycin derivatives are e.g. those disclosed in WO 96/41807, the contents thereof, in particular with respect to the specifically exemplified compounds of formula I disclosed therein, being incorporated herein by reference. Particularly preferred are 32-deoxo-rapamycin, 16-O-pent-2-ynyl-32-deoxo-rapamycin, 16-O-pent-2-ynyl-32-deoxo-40-O-(2-hydroxyethyl)-rapamycin, 16-O-pent-2-ynyl-32-(S)-dihydro-rapamycin and 16-O-pent-2-ynyl-32-(S)-dihydro-40-O-(2-hydroxyethyl)-rapamycin.

Further rapamycin derivatives are known, e.g. carboxylic acid esters such as disclosed in WO 92/05179, amide esters such as disclosed in US 5 118 677, carbamates such as described in US 5 118 678, fluorinated esters such as disclosed in US 5 100 883, acetals, e.g. in US 5 151 413, silyl ethers, e.g. in US 5 120 842, arylsulfonates and sulfamates, e.g. in US 5 177 203, derivatives wherein the methoxy group at the position 16 is replaced with alkynyloxy, e.g. in WO 95/16691 and further derivatives such as disclosed in WO 93/11130, WO 94/02136, WO 94/02385 and WO 95/14023, all incorporated herein by reference.

Rapamycin and above mentioned derivatives have been shown to have potent immunosuppressant properties. Rapamycin has also been shown to inhibit smooth muscle cell proliferation and to inhibit cancer growth.

Somatostatin analogues, e.g. octreotide, vapreotide and lanreotide, have been disclosed i.a. to inhibit growth hormone secretion and to have an inhibiting effect on malignant tumor growth, e.g. in breast cancer. Octreotide and lanreotide have also been disclosed to inhibit smooth muscle cell proliferation.

In accordance with the invention, it has now surprisingly been found that a combination of 2 active ingredients believed to act on basically different mechanisms such as a somatostatin analogue and rapamycin or a derivative thereof, can be combined and synergistically inhibit cell hyperproliferation.

In accordance with the particular findings of the present invention, there is provided in a first aspect:

1. Use of a compound of the somatostatin class, in free form or in pharmaceutically acceptable salt form, for manufacturing a pharmaceutical composition for use in synergistically effective amounts in the prevention or treatment of cell hyperproliferation in combination with a rapamycin macrolide, e.g. for the manufacture of a kit as disclosed hereinafter.

2. Use of a compound of the somatostatin class, in free form or in pharmaceutically acceptable salt form, in combination in synergistically effective amounts with a rapamycin macrolide for the prevention or treatment of cell hyperproliferation.

3. A method for preventing or treating cell hyperproliferation in a subject in need of such treatment which comprises administering to such subject a synergistically effective amount of a compound of the somatostatin class in free form or in pharmaceutically acceptable salt form, and a rapamycin macrolide.

4. A kit or package for the treatment or prevention of cell hyperproliferation, said kit or package including a pharmaceutical composition comprising a compound of the somatostatin class in free form or in pharmaceutically acceptable salt form, and a

pharmaceutical composition comprising a rapamycin macrolide. The kit or package may also contain instructions to use the pharmaceutical compositions in accordance with the present invention.

According to the invention, the combination of a compound of the somatostatin class and a rapamycin macrolide is indicated for the prevention or treatment of malignant tumor growth, e.g. breast, lung, GEP tumors, pituitary adenomas, lymphomas, etc., for the prevention or treatment of proliferative vascular diseases, e.g. biologically or mechanically induced vascular injury causing intimal thickening, e.g. restenosis, atherosclerosis, vascular occlusion, injury following percutaneous transluminal coronary angioplasty, vascular surgery or transplantation surgery, transplant vasculopathies, for example chronic rejection of various tissues and organs such as heart, kidney, pancreas, lung, liver, bowel, trachea and combined heart-lung.

The combination is particularly indicated for preventing intimal smooth muscle cell hyperplasia, restenosis and vascular occlusion in a mammal.

Utility of the combination in the treatment of disorders and diseases as hereinbefore specified, may be demonstrated for example in accordance with the method hereinafter described.

A. In vitro Assay

AR42J cell cultures are propagated in DMEM supplemented with 10 % fetal calf serum (FCS) at 5 % CO_2. Cells are grown in the absence of antibiotics or antifungal agents. Subconfluent AR42J cells growing in DMEM and supplemented with 10 % FCS are trypsinized, diluted in DMEM + 2.5 % FCS and seeded in uncoated 96-well plates (5'000 to 10'000 cells per well in 180 µl). After a 48-hr incubation period (Day O), the number of cells in a separate control plate is determined both by counting cells in a Coulter counter and by the sulforhodamine B (SRB) staining assay. The cells are then exposed either to the somatostatin analogue alone, e.g. octreotide, or to rapamycin or a derivative thereof alone or to a combination of the somatostatin analogue and rapamycin

or its derivative up to 5 days at various concentrations. Total drug exposure lasts for up to 5 days following the first addition and SRB analysis as described above is performed e.g. on day 2 and day 5. Growth is determined as difference in absorbance (OD) between day 0 and day x values (= delta OD). Calculations are made based on the fractional product method of Webb (Valeriote and Lin, 1975; Cory and Carter, 1986; Berenbaum, J. Theor. Biol. 114: 413-431, 1985) and the method by Chou and Talalay (Adv. Enz. Regul. 22: 27-55, 1984). If the measured cell growth (% of control) is < to the calculated cell growth, this shows evidence for a synergistic effect. Under these conditions a combination of a somatostatin analogue at a concentration of from 10^{-10} to 10^{-6} M with a rapamycin macrolide thereof at a concentration of from 1 to 1000 nM significantly inhibits the growth of the tumor cells.

In this assay, the following results are obtained with octreotide alone, Compound B alone and a combination of octreotide and Compound B. The synergy according to the Webb Method is confirmed by using the Chou-Talalay Method.

	Cell Growth (% of CONTROL)			
	Concentration (nM)	Cell Growth (ΔOD) (%)	Observed (%)	Calculated (Webb Method) (%)
Control		664 ± 9	100	
Octreotide	1.2	397 ± 16	59.8	
Compound B	12.0	420 ± 12	63.3	
Octreotide + Compound B	1.2 + 12.0	103 ± 5	15.6	37.9

B. In Vivo Assay

The AR42J (AR4-2J) rat pancreatic tumor cell line is derived from an azaserine-induced exocrine pancreatic tumor (Jessop and Hay; 1980). It was obtained from ATCC.

Cultures are propagated in DMEM supplemented with 10% fetal calf serum (FCS) at 5% CO_2. Cells are grown in the absence of antibiotics or antifungal agents. Female nude mice (nu/nu Balbc-A from Iffa Credo, Lyon, France) weighing 19-22 g, are kept in groups of 5 animals in macrolon cages (type III, 16 x 22 x 11 cm). The cages are placed in ventilated cabinets (Iffa Credo) that are maintained at 24 ± 1° C. The animals have free access to drinking water and a pathogen-free rodent diet (Diet A, Kliba, Basel, Switzerland). To initiate tumors from cultured cells, AR42J cells are trypsinized and $10x10^6$ tumor cells (in 0.2 ml) are injected subcutaneously (s.c.) into both flanks of nude mice. When tumors have reached a volume of 0.03 cm^3, animals are randomized into control and treatment groups. Control animals receive placebo. Animals are treated as indicated below for 3 weeks with single agents or the drug combination. The somatostatin analogue is given as a single injection of a slow release form at 30 mg/kg s.c.. The size of the tumors is determined with a caliper. To calculate the tumor volume in ml the equation "volume (ellipsoid) = length x depth x height x 0.52" was used.

Results

After 4 weeks the following tumor size were determined.

(Please note that values in the control group correspond to 3 week values, since animals were killed afterwards for tumors became excessively large.)

Treatment	**Volume** mm^3	**SE**
Control	4020	579
A) Compound B, 5 mg/kg p.o.	3685	263
B) Rapamycin, 5 mg/kg p.o.	2748	325
C) Octreotide pamoate (biodegradable, sustained release formulation), 30 mg/kg, single inj.	2205	339

Compound B + octreotide (C)	130	75
Rapamycin + octreotide (C)	106	44

C. Clinical trial

Patients are included who have breast cancer as evidenced by histological biopsy (glandular analysis - EOA). They present a metastatic illness and/or loco-regional localisation which is measurable and evaluable. If desired, patients may be included who are resistant to other treatment to conventional therapy such as surgery, radiotherapy, other chemotherapy and/or hormone therapy.

The patients present at least one target, on X-ray analysis, which is measurable or evaluable such as a primitive metastatic tumour which is cutaneous or sub-cutaneous. It may be gangliar or visceral. Preferably the patients have lesions which have progressed within the month preceding the trial and have an estimated survival time of at least 3 months.

The rapamycin macrolide, e.g rapamycin or compound B is administered orally. The treatment is for at least 3 months or until complete remission. The response may be followed by conventional methodology, e.g. according to IUCC response criteria, e.g. progression, stabilization, partial or complete remission.

The somatostatin analogue, e.g. octreotide, is administered parenterally, e.g. subcutaneous, particularly in a continuous subcutaneous way by means of a portable syringe pump (infusion pump).

According to the invention, the somatostatin analogue and the rapamycin macrolide are preferably administered in the form of a pharmaceutical composition. Rapamycin and its derivatives, e.g. Compound B, may be administered by any conventional route, in particular enterally, e.g. orally, e.g. in the form of tablets, capsules, drink solutions, emulsions or microemulsion preconcentrates, nasally, pulmonary (by inhalation),

parenterally, e.g. in the form of injectable solutions or suspensions, or topically. Rapamycin and its derivatives are preferably administered per os and the somatostatin analogue is preferably administered parenterally, e.g by infusion. The somatostatin analogue may also be administered in a slow release form, e.g. as disclosed in UK Patent Specification 2,265,311B. The administration of each component of the combination may take place either separately, simultaneously or sequentially, e.g. rapamycin or Compound B may be administered at first followed later, e.g. 8 to 24 hours later, by the somatostatin analogue.

The amount of each component administered is determined taking into account various factors such as the etiology and severity of the disease, and the patient's condition. Rapamycin or its derivatives may conveniently be administered at doses which are in the range used in immunosuppressive applications such as prevention and treatment of graft vs. host disease, transplant rejection or autoimmune diseases e.g. at a daily dosage from about 0.5 to 500 mg as a single dose or in divided doses. Such doses may also be given intermittently, for example, every other day or every third day. The somatostatin analogue may be administered, e.g. subcutaneously, in a dosage range of about 100 µg to 10 mg per day as a single dose or in divided doses. Thus octreotide may be administered at a dose of from 0.2 mg to 10 mg twice or three times daily. When administered as a slow release form, such formulation may comprise the somatostatin peptide in a concentration from 2.0 to 10% by weight. The release period of such a formulation may be from 1 week to about 2 months. The combination of the somatostatin analogue with rapamycin or its derivative allows to maximize the antiproliferative effect.

The invention contemplates that the active ingredients discussed herein may be utilized in combination with pharmaceutically acceptable diluents and carriers.

Formulation Examples:

A. Somatostatin Formulations:

1. Ampoules

Octreotide	0.5 mg
Mannitol	45.0 mg
Lactic acid (88%)	3.4 mg
Sodium hydrogeno-carbonate	to pH 4.2
Water (inject.grade)	to 1 ml
Carbon dioxide	q.s.

2. Biodegradable sustained release formulation:

Octreotide Acetate	4.65 %	(by weight)
Poly(DL-lactide-co-glycolide)	78.35 %	
Sterile Mannitol	17 %	

Vehicle:	Carboxymethylcellulose	0.5 %	(by weight)
	Mannitol	0.6 %	
	Water for injection	98.9 %	

B. Rapamycin (or derivative thereof) formulation: e.g. capsules

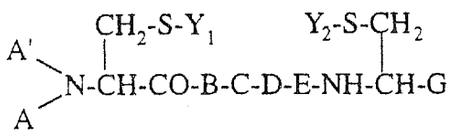
Ethanol	20.0 mg
1,2-propylene glycol	81.0mg
Refined oil	121.5mg
Cremophor RH40	202.5mg
Rapamycin or Compound B	20.0mg
Total	500 mg

CLAIMS

1. Use of a compound of the somatostatin class, in free form or in pharmaceutically acceptable salt form, for manufacturing a pharmaceutical composition for use in synergistically effective amounts in the prevention or treatment of cell hyperproliferation in combination with a rapamycin macrolide.

2. Use of a compound of the somatostatin class, in free form or in pharmaceutically acceptable salt form, in combination in synergistically effective amounts with a rapamycin macrolide for the prevention or treatment of cell hyperproliferation.

3. Use according to claim 1 or 2, wherein the compound of the somatostatin class is a compound of formula I



(I)

wherein

A is C_{1-12}alkyl, C_{7-10}phenylalkyl or a group of formula RCO-,

whereby

i) R is hydrogen, C_{1-11}alkyl, phenyl or C_{7-10}phenylalkyl, or

ii) RCO- is

a) a D-phenylalanine residue optionally ring-substituted by halogen, NO_2, NH_2, OH, C_{1-3}alkyl and/or C_{1-3}alkoxy; or

b) the residue of a natural or a synthetic α-amino-acid other than defined under a) above, or of a corresponding D-amino acid, or

c) a dipeptide residue in which the individual amino acid residues are the same or different and are selected from those defined under a) and/or b) above,

the α-amino group of amino acid residues a) and b) and the N-terminal amino group of dipeptide residues c) being optionally mono- or di-C_{1-12}alkylated or substituted by C_{1-8}alkanoyl;

A' is hydrogen or C_{1-3}alkyl,

Y_1 and Y_2 represent together a direct bond or each of Y_1 and Y_2 is hydrogen

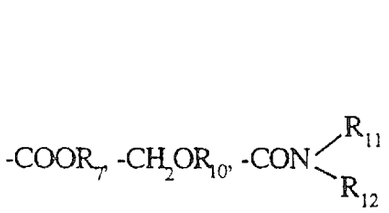
B is -Phe- optionally ring-substituted by halogen, NO_2, NH_2, OH, C_{1-3}alkyl and /or C_{1-3}alkoxy (including pentafluoroalanine), naphthylalanine or pyridylalanine,

C is (L)-Trp- or (D)-Trp- optionally α-N-methylated and optionally benzene-ring-substituted by halogen, NO_2, NH_2, OH, C_{1-3}alkyl and/or C_{1-3}alkoxy,

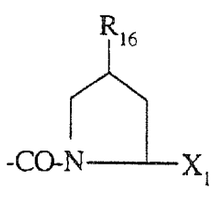
D is Lys, 4-aminocyclohexylAla or 4-aminocyclohexylGly

E is Thr, Ser, Val, Tyr, Ile, Leu or an aminobutyric or aminoisobutyric acid residue

G is a group of formula



or



wherein

R_7 is hydrogen or C_{1-3}alkyl,

R_{10} is hydrogen or the residue of a physiologically acceptable, physiologically hydrolysable ester,

R_{11} is hydrogen, C_{1-3}alkyl, phenyl or C_{7-10}phenyl-alkyl,

R_{12} is hydrogen, C_{1-3}alkyl or a group of formula $-CH(R_{13})-X_1$,

R_{13} is CH_2OH, $-(CH_2)_2-OH$, $-(CH_2)_3-OH$, $-CH(CH_3)OH$, isobutyl, butyl, benzyl, naphthyl-methyl or indol-3-yl-methyl, and

X_1 is a group of formula

$$-COOR_7 \quad -CH_2OR_{10} \quad \text{or} \quad -CO-N\begin{matrix}R_{14}\\R_{15}\end{matrix}$$

wherein

R_7 and R_{10} have the meanings given above,

R_{14} is hydrogen or C_{1-3}alkyl and

R_{15} is hydrogen, C_{1-3}alkyl, phenyl or C_{7-10}phenylalkyl, and

R_{16} is hydrogen or hydroxy,

with the proviso that

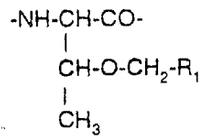
when R_{12} is $-CH(R_{13})-X_1$ then R_{11} is hydrogen or methyl,

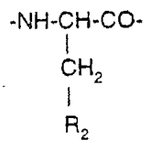
wherein the residues B, D and E have the L-configuration, and the residues in the 2- and 7-position each independently have the (L)- or (D)- configuration

or a somatostatin analogue comprising the amino acid sequence of formula II

$$-(D/L)Trp-Lys-X_2-X_3- \qquad (II)$$

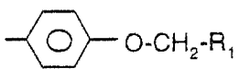
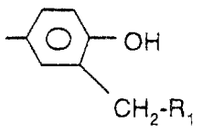
wherein X_2 is a radical of formula (a) or (b)

or  (a)

 (b)

wherein R_1 is optionally substituted phenyl,

R_2 is $-Z_1-CH_2-R_1$, $-CH_2-CO-O-CH_2-R_1$,

 or 

wherein Z_1 is O or S,

and

X_3 is an α-amino acid having an aromatic residue on the C_α side chain, or an amino acid unit selected from Dab, Dpr, Dpm, His,(Bzl)HyPro, thienyl-Ala, cyclohexyl-Ala and t.-butyl-Ala,

the residue Lys of said sequence corresponding to the residue Lys^9 of the native somatostatin-14,

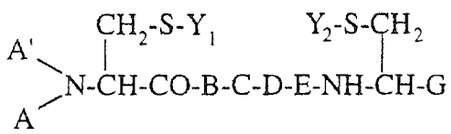
in free form or in pharmaceutically acceptable salt form.

4. Use according to claim 3, wherein the compound of the somatostatin class is octreotide, lanreotide or vapreotide.

5. A method for preventing or treating cell hyperproliferation in a subject in need of such treatment which comprises administering to such subject a synergistically effective amount of a compound of the somatostatin class in free form or in pharmaceutically acceptable salt form, and a rapamycin macrolide.

6. A kit or package for the treatment or prevention of cell hyperproliferation, said kit or package including a pharmaceutical composition comprising a compound of the somatostatin class in free form or in pharmaceutically acceptable salt form, and a pharmaceutical composition comprising a rapamycin macrolide, together with instructions for use.

7. A kit or package according to claim 6, wherein the compound of the somatostatin class is a compound of formula I



(I)

wherein

A is C_{1-12}alkyl, C_{7-10}phenylalkyl or a group of formula RCO-,

whereby

i) R is hydrogen, C_{1-11}alkyl, phenyl or C_{7-10}phenylalkyl, or

ii) RCO- is

a) a D-phenylalanine residue optionally ring-substituted by halogen, NO_2, NH_2, OH, C_{1-3}alkyl and/or C_{1-3}alkoxy; or

b) the residue of a natural or a synthetic α-amino-acid other than defined under a) above, or of a corresponding D-amino acid, or

c) a dipeptide residue in which the individual amino acid residues are the same or different and are selected from those defined under a) and/or b) above,

the α-amino group of amino acid residues a) and b) and the N-terminal amino group of dipeptide residues c) being optionally mono- or di-C_{1-12}alkylated or substituted by C_{1-8}alkanoyl;

A' is hydrogen or C_{1-3}alkyl,

Y_1 and Y_2 represent together a direct bond or each of Y_1 and Y_2 is hydrogen

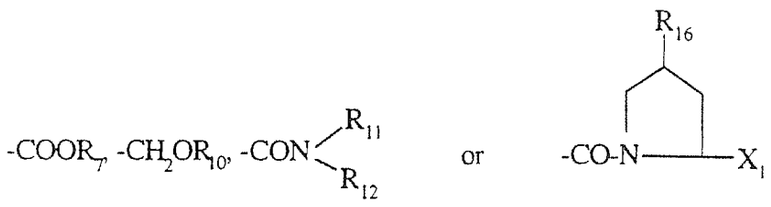
B is -Phe- optionally ring-substituted by halogen, NO_2, NH_2, OH, C_{1-3}alkyl and /or C_{1-3}alkoxy (including pentafluoroalanine), naphthylalanine or pyridylalanine,

C is (L)-Trp- or (D)-Trp- optionally α-N-methylated and optionally benzene-ring-substituted by halogen, NO_2, NH_2, OH, C_{1-3}alkyl and/or C_{1-3}alkoxy,

D is Lys, 4-aminocyclohexylAla or 4-aminocyclohexylGly

E is Thr, Ser, Val, Tyr, Ile, Leu or an aminobutyric or aminoisobutyric acid residue

G is a group of formula



wherein

R_7 is hydrogen or C_{1-3}alkyl,

R_{10} is hydrogen or the residue of a physiologically acceptable, physiologically hydrolysable ester,

R_{11} is hydrogen, C_{1-3}alkyl, phenyl or C_{7-10}phenyl-alkyl,

R_{12} is hydrogen, C_{1-3}alkyl or a group of formula $-CH(R_{13})-X_1$,

R_{13} is CH_2OH, $-(CH_2)_2-OH$, $-(CH_2)_3-OH$, $-CH(CH_3)OH$, isobutyl, butyl, benzyl, naphtyl-methyl or indol-3-yl-methyl, and

X_1 is a group of formula

$$-COOR_7,\ -CH_2OR_{10}\ or\ -CO-N\begin{matrix}R_{14}\\ \diagdown\\ R_{15}\end{matrix}$$

wherein

R_7 and R_{10} have the meanings given above,

R_{14} is hydrogen or C_{1-3}alkyl and

R_{15} is hydrogen, C_{1-3}alkyl, phenyl or C_{7-10}phenylalkyl, and

R_{16} is hydrogen or hydroxy,

with the proviso that

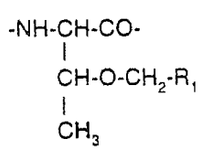
when R_{12} is $-CH(R_{13})-X_1$ then R_{11} is hydrogen or methyl,

wherein the residues B, D and E have the L-configuration, and the residues in the 2- and 7-position each independently have the (L)- or (D)- configuration

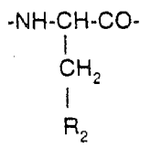
or a somatostatin analogue comprising the amino acid sequence of formula II

$$-(D/L)Trp-Lys-X_2-X_3- \qquad (II)$$

wherein X_2 is a radical of formula (a) or (b)



(a)

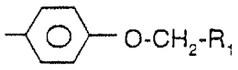


(b)

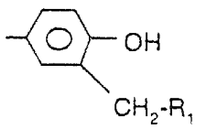
or

wherein R_1 is optionally substituted phenyl,

R_2 is $-Z_1-CH_2-R_1$, $-CH_2-CO-O-CH_2-R_1$,



or



wherein Z_1 is O or S,

and

X_3 is an α-amino acid having an aromatic residue on the C_α side chain, or an amino acid unit selected from Dab, Dpr, Dpm, His,(Bzl)HyPro, thienyl-Ala, cyclohexyl-Ala and t.-butyl-Ala,

the residue Lys of said sequence corresponding to the residue Lys^9 of the native somatostatin-14,

in free form or in pharmaceutically acceptable salt form.

8. A kit or package according to claim 7, wherein the compound of the somatostatin class is octreotide, lanreotide or vapreotide.

9. A kit or package according to claim 6 for simultaneous, separate or sequential use in synergistically effective amounts.

US Case **4-100-8322/A**

DECLARATION AND POWER OF ATTORNEY FOR UNITED STATES PATENT APPLICATION

☒ Original ☐ Supplemental ☐ Substitute

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name, and

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if more than one name is listed below) of the subject matter which is claimed and for which a United States patent is sought on the invention entitled

Combination of a Somatostatin Analogue and a Rapamycin

the specification of which:

☐ is attached hereto.

☐ was filed on _____ (day/month/year) as Application No. _____

and, if this box (☐) contains an ✕

☐ was amended on _____ (day/month/year)

☒ was filed as Patent Cooperation Treaty international Application No.

PCT/EP 97/03036/A on **11/06/97** (day/month/year)

and, if this box (☐) contains an ✕

☐ entered the national stage in the United States and was accorded Application No.

_____

and, if this box (☐) contains an ✕

☐ was amended, subsequent to entry into the national stage, on _____ (day/month/year)

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment(s) referred to above and, if this application was filed as a Patent Cooperation Treaty international application, by any amendments made during the international stage (including any made under Patent Cooperation Treaty Rule 91, Article 19 and Article 34).

I acknowledge my duty to disclose all information which is known by me to be material to the patentability of this application as defined in 37 C.F.R. § 1.56.

I hereby claim the benefit under 35 U.S.C. §119(a)-(d) or §365(b) of any foreign application(s) for patent or inventor's certificate listed below and under 35 U.S.C. §365(a) of any Patent Cooperation Treaty international application(s) designating at least one country other than the United States listed below and have also listed below any foreign application(s) for patent or inventor's certificate and Patent Cooperation Treaty international application(s) designating at least one country other than the United States for the same subject matter and having a filing date before that of the application the priority of which is claimed for that subject matter:

COUNTRY/REGION (OR P.C.T.)	APPLICATION No.	FILING DATE (day/month/year)	PRIORITY CLAIMED	
Great Britain	**9612171.0**	**11/06/1996**	☒ Yes	☐ No
Great Britain	**9619310.7**	**16/09/1996**	☒ Yes	☐ No
			☐ Yes	☐ No
			☐ Yes	☐ No
			☐ Yes	☐ No

I hereby claim the benefit under 35 U.S.C. § 119 (e) of any United States provisional application(s) listed below:

APPLICATION NO.	FILING DATE (day/month/year)

I hereby claim the benefit under 35 U.S.C. §120 of any United States application(s) listed below and under 35 U.S.C. §365(c) of any Patent Cooperation Treaty international application(s) designating the United States listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in said prior application(s) in the manner required by the first paragraph of 35 U.S.C. §112, I acknowledge my duty to disclose all information known to me to be material to patentability as defined in 37 C.F.R. §1.56 which became available between the filing date(s) of the prior application(s) and the national or Patent Cooperation Treaty international filing date of this application:

United States Application No.	United States Filing Date (day/month/year)	Status (Pending, Abandoned or U.S. Patent No.)	International Application No.	and Filing Date

I hereby appoint the registered practitioners associated with Customer No. 001095, respectively and individually, as my attorneys and agents, with full power of substitution and revocation, to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith.

If these brackets contain an X [X], I hereby authorize the registered practitioners associated with Customer No. 001095 and any others acting on my behalf to take any action relating to this application based on communications from the Patents and Trademarks Division of Novartis Services AG, Basle, Switzerland, or an affiliate thereof or a successor thereto, without direct communication from me.

Please address all communications to Michael W. Glynn, Novartis Corporation, Patent and Trademark Department, 564 Morris Avenue, Summit, NJ 07901-1027.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under 18 U.S.C. §1001 and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

1-00

Full name of sole or first joint inventor: **Gisbert WECKBECKER**

Inventor's signature: _____ Date: Sept. 2, 1998 (day/month/year)

Residence: **4105 Biel-Benken, Switzerland** CHX

Citizenship: **Germany**

Post Office Address: **Löliring 31**
4105 Biel-Benken
Switzerland

IMPORTANT: Before this declaration is signed, the patent application (the specification, the claims and this declaration) must be read and understood by each person signing it, and no changes may be made in the application after this declaration has been signed.

CASE 4-100-8322/A/PCT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

IN RE PCT NATIONAL STAGE APPLICATION OF

GISBERT WECKBECKER

INTERNATIONAL APPLICATION NO: PCT/EP 97/03036

FILED: 11 JUNE 1997

U.S. APPLICATION NO: Not Yet Known

35 USC §371 DATE: Herewith

FOR: COMBINATION OF A SOMATOSTATIN ANALOGUE AND A RAPAMYCIN

Assistant Commissioner for Patents
Washington, D.C. 20231

PRELIMINARY AMENDMENT

Sir:

Prior to calculation of the national filing fees, please amend the application as follows:

IN THE CLAIMS

Please re-number the claim pages as -- Pages 20-27 --, respectively.

Claim 3, line 1; replace "according to claim 1 or 2" by -- claim 1 --.

REMARKS

The "claim" pages have been re-numbered so as to be consecutive with the last page of the specification.

Claim 3 has been amended to remove its multiple dependency.

Respectfully submitted,

Novartis Corporation
Patent and Trademark Dept.
564 Morris Avenue
Summit, NJ 07901-1027
(908) 522-6921

Joseph J. Borovian
Agent for Applicant
Reg. No. 26,631

Date: December 7, 1998

PATENT COOPERATION TREA[illegible]

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference	FOR FURTHER ACTION	see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.
100-8322		
International application No.	International filing date *(day/month/year)*	(Earliest) Priority Date *(day/month/year)*
PCT/EP 97/03036	11/06/1997	11/06/1996
Applicant		
NOVARTIS AG et al.		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 3 sheets.

[X] It is also accompanied by a copy of each prior art document cited in this report.

1. [X] **Certain claims were found unsearchable** (see Box I).

2. [ ] **Unity of invention is lacking** (see Box II).

3. [ ] The international application contains disclosure of a **nucleotide and/or amino acid sequence listing** and the international search was carried out on the basis of the sequence listing
   - [ ] filed with the international application.
   - [ ] furnished by the applicant separately from the international application,
     - [ ] but not accompanied by a statement to the effect that it did not include matter going beyond the disclosure in the international application as filed.
   - [ ] Transcribed by this Authority

4. With regard to the **title,**
   - [X] the text is approved as submitted by the applicant.
   - [ ] the text has been established by this Authority to read as follows:

5. With regard to the **abstract,**
   - [X] the text is approved as submitted by the applicant.
   - [ ] the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this International Search Report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is:

   Figure No. _____
   - [ ] as suggested by the applicant.
   - [ ] because the applicant failed to suggest a figure.
   - [ ] because this figure better characterizes the invention.
   - [X] None of the figures.

Form PCT/ISA/210 (first sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP 97/03036

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Remark: Although claim(s) 2 - 5
is(are) directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 97/03036

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61K38/31

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	GB 2 239 178 A (SANDOZ LTD) 26 June 1991 see the whole document ---	1-9
A	WO 93 11130 A (SMITHKLINE BEECHAM PLC) 10 June 1993 cited in the application see the whole document ---	1-9
A	SHI E.A.: "Rapamycin enhances apoptosis and increases sensitivity to cisplatin in vitro" CANCER RESEARCH, vol. 55, 1 May 1995, pages 1982-1988, XP002040888 see the whole document -----	1-9

[ ] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
17 September 1997	01. 10. 97

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+ 31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+ 31-70) 340-3016	Groenendijk, M

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

mation on patent family members

International Application No
PCT/EP 97/03036

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
GB 2239178 A	26-06-91	AT 177788 A	15-02-97
		AU 618270 B	19-12-91
		BE 1001079 A	04-07-89
		CA 1328402 A	12-04-94
		CH 677449 A	31-05-91
		CS 9104115 A	17-06-92
		CY 1631 A	10-07-92
		CY 1632 A	10-07-92
		DE 3822557 A	19-01-89
		FI 93308 B	15-12-94
		FR 2617714 A	13-01-89
		GB 2208200 A,B	15-03-89
		GR 1000172 B	15-11-91
		HK 16492 A	06-03-92
		HK 16592 A	06-03-92
		IE 61908 B	30-11-94
		JP 7048272 A	21-02-95
		JP 1031728 A	02-02-89
		JP 2578477 B	05-02-97
		LU 87268 A	08-03-89
		NL 8801734 A	01-02-89
		NO 179359 B	17-06-96
		PT 87957 B	01-03-95
		SE 503191 C	15-04-96
		SE 8802569 A	11-01-89
WO 9311130 A	10-06-93	AU 2954392 A	28-06-93
		EP 0621865 A	02-11-94
		JP 7501804 T	23-02-95
		US 5491229 A	13-02-96

PATENT COOPERATION TRL. .TY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference	**FOR FURTHER ACTION** see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.	
100-8322		
International application No.	International filing date *(day/month/year)*	(Earliest) Priority Date *(day/month/year)*
PCT/EP 97/03036	11/06/1997	11/06/1996
Applicant		
NOVARTIS AG et al.		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 3 sheets.

[X] It is also accompanied by a copy of each prior art document cited in this report.

1. [X] **Certain claims were found unsearchable** (see Box I).

2. [ ] **Unity of invention is lacking** (see Box II).

3. [ ] The international application contains disclosure of a **nucleotide and/or amino acid sequence listing** and the international search was carried out on the basis of the sequence listing
   - [ ] filed with the international application.
   - [ ] furnished by the applicant separately from the international application,
     - [ ] but not accompanied by a statement to the effect that it did not include matter going beyond the disclosure in the international application as filed.
   - [ ] Transcribed by this Authority

4. With regard to the **title,**
   - [X] the text is approved as submitted by the applicant.
   - [ ] the text has been established by this Authority to read as follows:

5. With regard to the **abstract,**
   - [X] the text is approved as submitted by the applicant.
   - [ ] the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this International Search Report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is:

   Figure No. _____
   - [ ] as suggested by the applicant.
   - [ ] because the applicant failed to suggest a figure.
   - [ ] because this figure better characterizes the invention.
   - [X] None of the figures.

Form PCT/ISA/210 (first sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP 97/03036

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Remark: Although claim(s) 2 - 5
is(are) directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

J ERNATIONAL SEARCH REPORT

International Application No
PCT/EP 97/03036

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61K38/31

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	GB 2 239 178 A (SANDOZ LTD) 26 June 1991 see the whole document ---	1-9
A	WO 93 11130 A (SMITHKLINE BEECHAM PLC) 10 June 1993 cited in the application see the whole document ---	1-9
A	SHI E.A.: "Rapamycin enhances apoptosis and increases sensitivity to cisplatin in vitro" CANCER RESEARCH, vol. 55, 1 May 1995, pages 1982-1988, XP002040888 see the whole document -----	1-9

☐ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

3

Date of the actual completion of the international search	Date of mailing of the international search report
17 September 1997	01/10/97

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Groenendijk, M

Form PCT/ISA/210 (second sheet) (July 1992)

'ERNATIONAL SEARCH REPOR'

Information on patent family members

International Application No
PCT/EP 97/03036

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
GB 2239178 A	26-06-91	AT 177788 A	15-02-97
		AU 618270 B	19-12-91
		BE 1001079 A	04-07-89
		CA 1328402 A	12-04-94
		CH 677449 A	31-05-91
		CS 9104115 A	17-06-92
		CY 1631 A	10-07-92
		CY 1632 A	10-07-92
		DE 3822557 A	19-01-89
		FI 93308 B	15-12-94
		FR 2617714 A	13-01-89
		GB 2208200 A,B	15-03-89
		GR 1000172 B	15-11-91
		HK 16492 A	06-03-92
		HK 16592 A	06-03-92
		IE 61908 B	30-11-94
		JP 7048272 A	21-02-95
		JP 1031728 A	02-02-89
		JP 2578477 B	05-02-97
		LU 87268 A	08-03-89
		NL 8801734 A	01-02-89
		NO 179359 B	17-06-96
		PT 87957 B	01-03-95
		SE 503191 C	15-04-96
		SE 8802569 A	11-01-89
WO 9311130 A	10-06-93	AU 2954392 A	28-06-93
		EP 0621865 A	02-11-94
		JP 7501804 T	23-02-95
		US 5491229 A	13-02-96

PATENT COOPERATION TRI TY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference	FOR FURTHER ACTION see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.	
100-8322		
International application No.	International filing date *(day/month/year)*	(Earliest) Priority Date *(day/month/year)*
PCT/EP 97/03036	11/06/1997	11/06/1996
Applicant		
NOVARTIS AG et al.		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 3 sheets.

[X] It is also accompanied by a copy of each prior art document cited in this report.

1. [X] **Certain claims were found unsearchable** (see Box I).

2. [ ] **Unity of invention is lacking** (see Box II).

3. [ ] The international application contains disclosure of a **nucleotide and/or amino acid sequence listing** and the international search was carried out on the basis of the sequence listing

   [ ] filed with the international application.

   [ ] furnished by the applicant separately from the international application,

      [ ] but not accompanied by a statement to the effect that it did not include matter going beyond the disclosure in the international application as filed.

   [ ] Transcribed by this Authority

4. With regard to the **title,**

   [X] the text is approved as submitted by the applicant.

   [ ] the text has been established by this Authority to read as follows:

5. With regard to the **abstract,**

   [X] the text is approved as submitted by the applicant.

   [ ] the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this International Search Report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is:

   Figure No. _____

   [ ] as suggested by the applicant.

   [ ] because the applicant failed to suggest a figure.

   [ ] because this figure better characterizes the invention.

   [X] None of the figures.

Form PCT/ISA/210 (first sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP 97/03036

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Remark: Although claim(s) 2 - 5
is(are) directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 97/03036

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61K38/31

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	GB 2 239 178 A (SANDOZ LTD) 26 June 1991 see the whole document ---	1-9
A	WO 93 11130 A (SMITHKLINE BEECHAM PLC) 10 June 1993 cited in the application see the whole document ---	1-9
A	SHI E.A.: "Rapamycin enhances apoptosis and increases sensitivity to cisplatin in vitro" CANCER RESEARCH, vol. 55, 1 May 1995, pages 1982-1988, XP002040888 see the whole document -----	1-9

☐ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

3

Date of the actual completion of the international search	Date of mailing of the international search report
17 September 1997	01. 10. 97

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Groenendijk, M

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/EP 97/03036

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
GB 2239178 A	26-06-91	AT 177788 A	15-02-97
		AU 618270 B	19-12-91
		BE 1001079 A	04-07-89
		CA 1328402 A	12-04-94
		CH 677449 A	31-05-91
		CS 9104115 A	17-06-92
		CY 1631 A	10-07-92
		CY 1632 A	10-07-92
		DE 3822557 A	19-01-89
		FI 93308 B	15-12-94
		FR 2617714 A	13-01-89
		GB 2208200 A,B	15-03-89
		GR 1000172 B	15-11-91
		HK 16492 A	06-03-92
		HK 16592 A	06-03-92
		IE 61908 B	30-11-94
		JP 7048272 A	21-02-95
		JP 1031728 A	02-02-89
		JP 2578477 B	05-02-97
		LU 87268 A	08-03-89
		NL 8801734 A	01-02-89
		NO 179359 B	17-06-96
		PT 87957 B	01-03-95
		SE 503191 C	15-04-96
		SE 8802569 A	11-01-89
WO 9311130 A	10-06-93	AU 2954392 A	28-06-93
		EP 0621865 A	02-11-94
		JP 7501804 T	23-02-95
		US 5491229 A	13-02-96

INTERNATIONAL SEARCH REPORT

Inte nal Application No
PCT/EP 97/03036

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61K38/31

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	GB 2 239 178 A (SANDOZ LTD) 26 June 1991 see the whole document ---	1-9
A	WO 93 11130 A (SMITHKLINE BEECHAM PLC) 10 June 1993 cited in the application see the whole document ---	1-9
A	SHI E.A.: "Rapamycin enhances apoptosis and increases sensitivity to cisplatin in vitro" CANCER RESEARCH, vol. 55, 1 May 1995, pages 1982-1988, XP002040888 see the whole document -----	1-9

☐ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

3

Date of the actual completion of the international search	Date of mailing of the international search report
17 September 1997	0 1. 10. 97

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax (+31-70) 340-3016	Groenendijk, M

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

ernational application No.
PCT/EP 97/03036

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Remark: Although claim(s) 2 - 5
is(are) directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

information on patent family members

Inte nal Application No
PCT/EP 97/03036

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
GB 2239178 A	26-06-91	AT 177788 A	15-02-97
		AU 618270 B	19-12-91
		BE 1001079 A	04-07-89
		CA 1328402 A	12-04-94
		CH 677449 A	31-05-91
		CS 9104115 A	17-06-92
		CY 1631 A	10-07-92
		CY 1632 A	10-07-92
		DE 3822557 A	19-01-89
		FI 93308 B	15-12-94
		FR 2617714 A	13-01-89
		GB 2208200 A,B	15-03-89
		GR 1000172 B	15-11-91
		HK 16492 A	06-03-92
		HK 16592 A	06-03-92
		IE 61908 B	30-11-94
		JP 7048272 A	21-02-95
		JP 1031728 A	02-02-89
		JP 2578477 B	05-02-97
		LU 87268 A	08-03-89
		NL 8801734 A	01-02-89
		NO 179359 B	17-06-96
		PT 87957 B	01-03-95
		SE 503191 C	15-04-96
		SE 8802569 A	11-01-89
WO 9311130 A	10-06-93	AU 2954392 A	28-06-93
		EP 0621865 A	02-11-94
		JP 7501804 T	23-02-95
		US 5491229 A	13-02-96

19

PATENT COOPERATION TRL .IY

PCT

REC'D 3 0 JUN 1998

WIPO PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
4-100-8322/A		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/EP 97/ 03036	11/06/1997	11/06/1996
International Patent Classification (IPC) or national classification and IPC		
A61K38/31		
Applicant		
NOVARTIS AG et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 4 sheets, including this cover sheet.

   ☐ This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consists of a total of _____ sheets.

3. This report contains indications and corresponding pages relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☐ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
12/12/1997	2 6. 06. 98
Name and mailing address of the IPEA/	Authorized officer
European Patent Office D-80298 Munich Tel. (+49-89) 2399-0, Tx: 523656 epmu d Fax: (+49-89) 2399-4465	A. Klaassen
	Telephone No.

Intern. application No.

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

PCT/EP97/03036

I. Basis of the report

1. This report has been drawn up on the basis of (Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments.):

[x] the international application as originally filed.

[ ] the description, pages _____, as originally filed,
pages _____, filed with the demand,
pages _____, filed with the letter of _____,
pages _____, filed with the letter of _____,

[ ] the claims, Nos. _____, as originally filed,
Nos. _____, as amended under Article 19,
Nos. _____, filed with the demand,
Nos. _____, filed with the letter of _____,
Nos. _____, filed with the letter of _____,

[ ] the drawings, sheets/fig _____, as originally filed,
sheets/fig _____, filed with the demand,
sheets/fig _____, filed with the letter of _____,
sheets/fig _____, filed with the letter of _____.

2. The amendments have resulted in the cancellation of:

[ ] the description, pages _____.
[ ] the claims, Nos. _____.
[ ] the drawings, sheets/fig _____.

3. [ ] This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

4. Additional observations, if necessary:

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step and industrial applicability; citations and explanations supporting such statement

1. STATEMENT

Novelty (N)	Claims 1-9	YES
	Claims	NO
Inventive Step (IS)	Claims 1-5,9	YES
	Claims 6-8	NO
Industrial Applicability (IA)	Claims 1-4,6-9	YES
	Claims 5	NO

2. CITATIONS AND EXPLANATIONS

Reference is made to the following documents:
D1:GB-A-2 239 178
D2:WO-A-93 11130

1). None of the documents cited in the International Search Report discloses the subject matter of the present claims. Therefore, the novelty can be acknowledged.

2). D1 discloses the use of somatostatin analogues for the treatment of cell hyperproliferation.
D2 discloses the use of a rapamycine derivative for the treatment of cell hyperproliferation, see claims 9-10 and page page 13.
The combined teachings of D1 and D2 do not suggest a synergistic effect of a combination of somatostatin and rapamycin.
Therefore, the subject matter of claims 1-5 and 9 appears to involve an inventive step with respect to the prior art cited in the International Search Report.
Claims 6-8, however, are not restricted to synergistic combinations. In view of the fact that the antihyperproliferative effect of both somatostatin and

rapamycin are already known and, moreover, the somatostatin derivatives used in accordance with claims 6-8 do not appear to be generally novel (cf. page 4 of the description and claim 8), it appears that the treatment of cell hyperproliferation with a combination of somatostatin and rapamycin is obvious. Therefore, the subject matter of claims 6-8 does not appear to involve an inventive step.

3). For the assessment of the present claim 5 on the question whether it is industrially applicable, no unified criteria exist in the PCT. The patentability can also be dependent upon the formulation of the claims. The EPO, for example, does not recognize as industrially applicable the subject-matter of claims to the use of a compound in medical treatment, but may allow, however, claims to a known compound for first use in medical treatment and the use of such a compound for the manufacture of a medicament for a new medical treatment.

PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

United States Patent and Trademark Office
(Box PCT)
Crystal Plaza 2
Washington, DC 20231
ETATS-UNIS D'AMERIQUE

in its capacity as elected Office

Date of mailing (day/month/year) 07 January 1998 (07.01.98)	
International application No. PCT/EP97/03036	**Applicant's or agent's file reference** 100-8322
International filing date (day/month/year) 11 June 1997 (11.06.97)	**Priority date** (day/month/year) 11 June 1996 (11.06.96)
Applicant WECKBECKER, Gisbert	

1. The designated Office is hereby notified of its election made:

[X] in the demand filed with the International Preliminary Examining Authority on:

12 December 1997 (12.12.97)

[ ] in a notice effecting later election filed with the International Bureau on:

2. The election [X] was

[ ] was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO **34, chemin des Colombettes** **1211 Geneva 20, Switzerland**	Authorized officer Aino Metcalfe
Facsimile No.: (41-22) 740.14.35	Telephone No.: (41-22) 338.83.38

Form PCT/IB/331 (July 1992)

1826436

WO 97/47317
PCT/EP97/03036

PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

M/D
F/E
Specialist

PCT

NOTICE INFORMING THE APPLICANT OF THE COMMUNICATION OF THE INTERNATIONAL APPLICATION TO THE DESIGNATED OFFICES

(PCT Rule 47.1(c), first sentence)

To:

ROTH, Bernhard, M.
Novartis AG
Patent- und Markenabteilung
Klybeckstrasse 141
CH-4002 Basel
SUISSE

P + TM Dept.
23. Dez. 1997 FD4

Date of mailing (day/month/year) 18 December 1997 (18.12.97)	
Applicant's or agent's file reference 100-8322	IMPORTANT NOTICE

International application No.	International filing date (day/month/year)	Priority date (day/month/year)
PCT/EP97/03036	11 June 1997 (11.06.97)	11 June 1996 (11.06.96)

Applicant
NOVARTIS AG et al

1. Notice is hereby given that the International Bureau has communicated, as provided in Article 20, the international application to the following designated Offices on the date indicated above as the date of mailing of this Notice:
   AU,BR,CA,CN,EP,IL,JP,KP,KR,NO,PL,SK,US

   In accordance with Rule 47.1(c), third sentence, those Offices will accept the present Notice as conclusive evidence that the communication of the international application has duly taken place on the date of mailing indicated above and no copy of the international application is required to be furnished by the applicant to the designated Office(s).

2. The following designated Offices have waived the requirement for such a communication at this time:
   AL,AM,AP,AT,AZ,BA,BB,BG,BY,CH,CU,CZ,DE,DK,EA,EE,ES,FI,GB,GE,GH,HU,IS,KE,KG,KZ,LC,
   LK,LR,LS,LT,LU,LV,MD,MG,MK,MN,MW,MX,NZ,OA,PT,RO,RU,SD,SE,SG,SI,TJ,TM,TR,TT,UA,UG,
   UZ,VN,YU,ZW
   The communication will be made to those Offices only upon their request. Furthermore, those Offices do not require the applicant to furnish a copy of the international application (Rule 49.1(a-bis)).

3. Enclosed with this Notice is a copy of the international application as published by the International Bureau on
   18 December 1997 (18.12.97) under No. WO 97/47317

REMINDER REGARDING CHAPTER II (Article 31(2)(a) and Rule 54.2)

If the applicant wishes to postpone entry into the national phase until 30 months (or later in some Offices) from the priority date, **a demand for international preliminary examination** must be filed with the competent International Preliminary Examining Authority before the expiration of 19 months from the priority date.

It is the applicant's sole responsibility to monitor the 19-month time limit.

Note that only an applicant who is a national or resident of a PCT Contracting State which is bound by Chapter II has the right to file a demand for international preliminary examination.

REMINDER REGARDING ENTRY INTO THE NATIONAL PHASE (Article 22 or 39(1))

If the applicant wishes to proceed with the international application in the **national phase**, he must, within 20 months or 30 months, or later in some Offices, perform the acts referred to therein before each designated or elected Office.

For further important information on the time limits and acts to be performed for entering the national phase, see the Annex to Form PCT/IB/301 (Notification of Receipt of Record Copy) and Volume II of the PCT Applicant's Guide.

30.12.97

ERSTERFASSUNG Visum:
ZWEITERFASSUNG Visum:

The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland	Authorized officer J. Zahra
Facsimile No. (41-22) 740.14.35	Telephone No. (41-22) 338.83.38

Form PCT/IB/308 (July 1996) 1806708

09/194957
PCT/EP 97/03036

The Patent Office

The Patent Office
Cardiff Road
Newport
Gwent
NP9 1RH

PRIORITY DOCUMENT

I, the undersigned, being an officer duly authorised in accordance with Section 74(1) and (4) of the Deregulation and Contracting Out Act 1994, to sign and issue certificates on behalf of the Comptroller-General, hereby certify that annexed hereto is a true copy of the documents as originally filed in connection with the patent application identified therein.

In accordance with the Patents (Companies Re-registration) Rules 1982, if a company named in this certificate and any accompanying documents has re-registered under the Companies Act 1980 with the same name as that with which it was registered immediately before re-registration save for the substitution as, or inclusion as, the last part of the name of the words "public limited company" or their equivalents in Welsh, references to the name of the company in this certificate and any accompanying documents shall be treated as references to the name with which it is so re-registered.

In accordance with the rules, the words "public limited company" may be replaced by p.l.c., plc, P.L.C. or PLC.

Re-registration under the Companies Act does not constitute a new legal entity but merely subjects the company to certain additional company law rules.

Signed

Dated 21 MAY 1997

Patents Form 1/77

1/77

Patents Act 1977
(Rules 16)

12JUN96 E199929-1 000524
P01/7700 25.00

Request for grant of a patent

(See the notes on the back of this form. You can also get an explanatory leaflet from the Patent Office to help you fill in this form)

The Patent Office

THE PATENT OFFICE
G
11 JUN 1996
RECEIVED BY HAND

The Patent Office

Cardiff Road
Newport
Gwent NP9 1RH

1.	Your reference	100-8322
		11 JUN 1996
2.	Patent application number *(The Patent Office will fill in this part)*	9612171.0
3.	Full name, address and postcode of the or of each applicant *(underline all surnames)*	SANDOZ LTD. 35 Lichtstrasse CH-4002 Basel Switzerland
	Patents ADP number *(if you know it)*	00703207001
	If the applicant is a corporate body, give the country/state of its incorporation	Switzerland
4.	Titel of the invention	ORGANIC COMPOUNDS
5.	Name of your agent *(if you have one)*	B. A. Yorke & Co.
	"Address for service" in the United Kingdom to which all correspondence should be sent *(including the postcode)*	Coomb House 7 St. John's Road Isleworth, Middlesex TW7 6NH
	Patents ADP number *(if you know it)*	1800001

6.	If you are declaring priority from one or more earlier patent applications, give the country and the date of filing of the or of each of these earlier applications and *(if you know it)* the or each application number	Country	Priority application number *(if you know it)*	Date of filing *(day / month / year)*
7.	If this application is divided or otherwise derived from a earlier UK application, give the number and the filing date of the earlier application	Number of earlier application		Date of filing *(day / month / year)*

8. Is a statement of inventorship and of right to grant of a patent required in support of this request? *(Answer "Yes" if:*

a) any applicant named in part 3 is not an inventor , or

b) there is an inventor who is not named as an applicant, or

c) any named applicant is a corporate body.

See note (d))

Yes

Patents Form 1/77

Patents Form 1/77

9. Enter the number of sheets for any of the following items you are filing with this form. Do not count copies of the same document

Continuation sheets of this form	
Description	12
Claim(s)	1
Abstract	
Drawing (s)	

10. If you are also filing any of the following, state how many against each items.

Priority documents	
Translations of priority documents	
Statemtent of inventorship and right to grant of a patent *(Patents Form 7/77)*	
Request for preliminary examination and search *(Patents Form 9/77)*	
Request for substantive examination *(Patents Form 10/77)*	
Any other documents *(please specify)*	

11. I/We request the grant of a patent on the basis of this application.

Signature B. A. Yorke & Co. Date 07/06/1996

B. A. Yorke & Co

12. Name and daytime telephone number of person to contact in the United Kingdom

Warning

After an application for a patent has been filed, the Comptroller of the Patent Office will consider whether publication or communication of the invention should be prohibited or restricted under Section 22 of the Patents Act 1977. You will be informed if it is necessary to prohibit or restrict your invention in this way. Furthermore, if you live in the United Kingdom, Section 23 of the Patent Act 1977 stops you from applying for a patent abroad without first getting written permission form the Patent Office unless an application has been filed at least 6 weeks beforehand in the United Kingdom for a patent for the same invention and either no direction prohibiting publication or communication has been given, or any such direction has been revoked.

Notes

a) *If you need help to fill in this form or you have any questions, please contact the Patent Office on 0645 500505*

b) *Write your answers in capital letters using black ink or you may type them.*

c) *If there is not enough space for all the relevant details on any part of this form, please continue on a separate sheet of paper and write "see continuation sheet" in relevant part(s). Any continuation sheet should be attached to this form.*

d) *If you have answered "Yes" Patents Form 7/77 will need to be filed.*

e) *Once you have filed in the form you must remember so sign and date it.*

f) *For details of the fee and ways to pay please contact the Patent Office.*

ORGANIC COMPOUNDS

The present invention relates to a pharmaceutical combination and its use in the treatment of disorders associated with excess benign and malignant cell proliferation, e.g. tumors or myointimal cell proliferation.

There is a continuing need for development of drugs having increased effectiveness in inhibiting or slowing undesired cell proliferation, particularly in the cancer field and in vasculopathies.

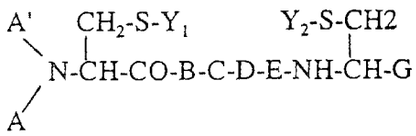
Accordingly, there is provided a composition comprising a somatostatin analogue or derivative and rapamycin or a derivative thereof.

By "somatostatin analogues or derivatives" as used herein is meant any straight-chain or cyclic polypeptide derived from that of the naturally occurring tetradecapeptide somatostatin wherein one or more amino acid units have been omitted and/or replaced by one or more other amino radical(s) and/or wherein one or more functional groups have been replaced by one or more other functional groups and/or one or more groups have been replaced by one or several other isosteric groups. In general, the term covers all modified derivatives of the biologically active somatostatin tetradecapeptide which exhibit a somatostatin-like activity, e.g. they bind to somatostatin receptors, particularly somatostatin octapeptides.

Cyclic, bridge cyclic and straight-chain somatostatin analogues or derivatives are known and have been described together with processes for their production e.g. in US Patent Specifications 4,310,518 and 4,235,886, in European Patent Specifications EP-A-1295; 23,192; 29,310; 29,579; 30,920; 31,303; 63,308; 70,021; 83,305; 215,171; 203,031; 214,872; 143,307; 298,732; 277,419 and in Belgian Patent Specification BE-A-900,089, the contents thereof, in particular with respect to the compounds, being incorporated herein by reference.

Preferred somatostatin analogues or derivatives are:

A. Compounds of formula I



(I)

wherein

A is C_{1-12}alkyl, C_{7-10}phenylalkyl or a group of formula RCO-,

whereby

i) R is hydrogen, C_{1-11}alkyl, phenyl or C_{7-10}phenylalkyl, or

ii) RCO- is

a) a D-phenylalanine residue optionally ring-substituted by halogen, NO_2, NH_2, OH, C_{1-3}alkyl and/or C_{1-3}alkoxy; or

b) the residue of a natural or a synthetic α-amino-acid other than defined under a) above, or of a corresponding D-amino acid, or

c) a dipeptide residue in which the individual amino acid residues are the same or different and are selected from those defined under a) and/or b) above, the α-amino group of amino acid residues a) and b) and the N-terminal amino group of dipeptide residues c) being optionally mono- or di-C_{1-12}alkylated or substituted by C_{1-8}alkanoyl;

A' is hydrogen or C_{1-3}alkyl,

Y_1 and Y_2 represent together a direct bond or

each of Y_1 and Y_2 is hydrogen

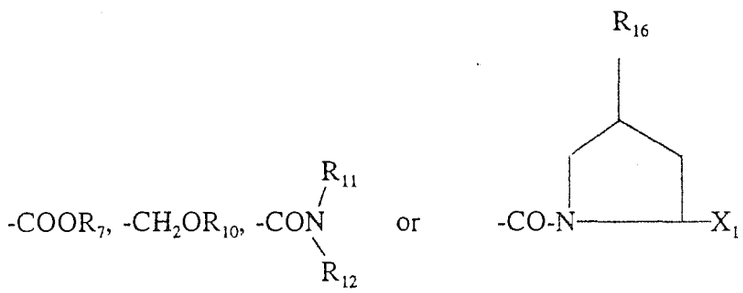
B is -Phe- optionally ring-substituted by halogen, NO_2, NH_2, OH, C_{1-3}alkyl and /or C_{1-3}alkoxy (including pentafluoroalanine), or naphthylalanine

C is (L)-Trp- or (D)-Trp- optionally α-N-methylated and optionally benzene-ring-substituted by halogen, NO_2, NH_2, OH, C_{1-3}alkyl and/or C_{1-3}alkoxy,

D is Lys, 4-aminocyclohexylAla or 4-aminocyclohexylGly

E is Thr, Ser, Val, Tyr, Ile, Leu or an aminobutyric or aminoisobutyric acid residue

G is a group of formula



wherein

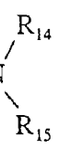
R_7 is hydrogen or C_{1-3}alkyl,

R_{10} is hydrogen or the residue of a physiologically acceptable, physiologically hydrolysable ester,

R_{11} is hydrogen, C_{1-3}alkyl, phenyl or C_{7-10}phenyl-alkyl,

R_{12} is hydrogen, C_{1-3}alkyl or a group of formula $-CH(R_{13})-X_1$,

R_{13} is CH_2OH, $-(CH_2)_2-OH$, $-(CH_2)_3-OH$, or $-CH(CH_3)OH$ or represents the substituent attached to the α-carbon atom of a natural or synthetic α-amino acid (including hydrogen) and

X_1 is a group of formula $-COOR_7$, $-CH_2OR_{10}$ or 

wherein

R_7 and R_{10} have the meanings given above,

R_{14} is hydrogen or C_{1-3}alkyl and

R_{15} is hydrogen, C_{1-3}alkyl, phenyl or C_{7-10}phenylalkyl, and

R_{16} is hydrogen or hydroxy,

with the proviso that

when R_{12} is $-CH(R_{13})-X_1$ then R_{11} is hydrogen or methyl,

wherein the residues B, D and E have the L-configuration, and the residues in the 2- and 7-position each independently have the (L)- or (D)- configuration,

in free form or in pharmaceutically acceptable salt or complex form.

Individual compounds of formula I suitable in accordance with the present invention are the following somatostatin analogues:

a. (D)Phe-Cys-Phe-(D)Trp-Lys-Thr-Cys-Thr-ol
also known as octreotide

b. (D)Phe-Cys-Tyr-(D)Trp-Lys-Val-Cys-ThrNH_2

c. (D)Phe-Cys-Tyr-(D)Trp-Lys-Val-Cys-TrpNH_2
also known as vapreotide

d. (D)Trp-Cys-Phe-(D)Trp-Lys-Thr-Cys-ThrNH_2

e. (D)Phe-Cys-Phe-(D)Trp-Lys-Thr-Cys-ThrNH_2

f. 3-(2-(Naphthyl)-(D)Ala-Cys-Tyr-(D)Trp-Lys-Val-Cys-ThrNH_2
also known as lanreotide

g. (D)Phe-Cys-Tyr-(D)Trp-Lys-Val-Cys-β-Nal-NH_2

h. 3-(2-naphthyl)-Ala-Cys-Tyr-(D)Trp-Lys-Val-Cys-β-Nal-NH_2

i. (D)Phe-Cys-β-Nal-(D)Trp-Lys-Val-Cys-Thr-NH_2

j. (D)Phe-Cys-Tyr-(D)Trp-Lys-Leu-Cys-Thr-NH_2

k. (D)Phe-Cys-Tyr-(D)Trp-Lys-Cys-Thr-NH_2

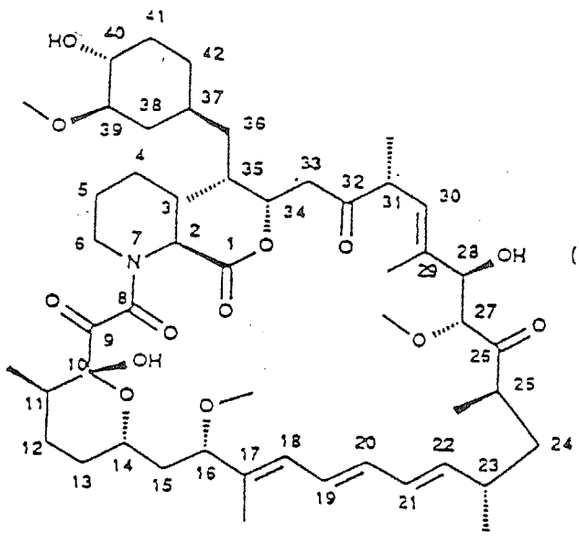
B. Further compounds suitable for use in accordance with the present invention are:
cyclo [-Asn-Phe-Phe-DTrp-Lys-Thr-Phe-Gaba-],
cyclo(Asu-Lys-Asn-Phe-Phe-Trp-Lys-Thr-Tyr-Thr-Ser), and

(D)Nal-Glu-Tyr-(D)Trp-Lys-Val-Lys-Thr-NH_2

Octreotide is preferred.

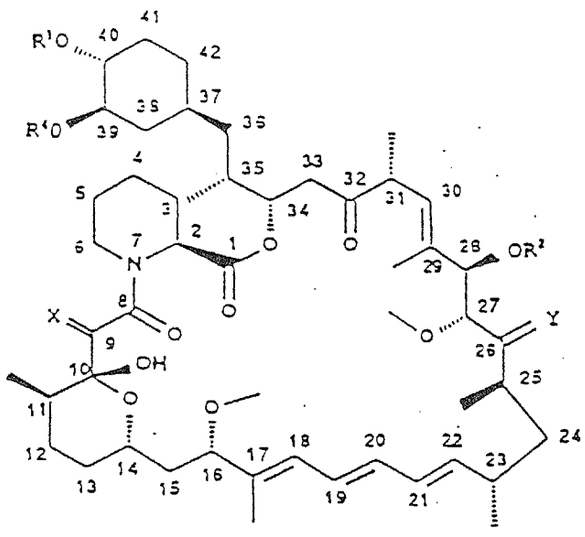
Compounds of formula I may exist e.g. in free form, salt form or in the form of complexes thereof. Acid addition salts may be formed with e.g. organic acids, polymeric acids and inorganic acids. Such acid addition salt forms include e.g. the hydrochlorides and acetates. Complexes are e.g. formed from compounds of the invention on addition of inorganic substances, e.g. inorganic salts or hydroxides such as Ca- and Zn-salts, and/or an addition of polymeric organic substances.

Rapamycin is a known macrolide antibiotic produced by Streptomyces hygroscopicus, having the structure depicted in Formula A:



(A)

See, e.g., McAlpine, J.B., et al., J. Antibiotics (1991) 44: 688; Schreiber, S.L., et al., J. Am. Chem. Soc. (1991) 113: 7433; US Patent No. 3 929 992. Numerous derivatives of rapamycin are known. One group of derivatives are alkylated derivatives of rapamycin having the structure of Formula I:



(I)

X is (H,H) or O;

Y is (H,OH) or O;

R^1 and R^2 are independently selected from

H, alkyl, thioalkyl, arylalkyl, hydroxyalkyl, dihydroxyalkyl, hydroxyalkylarylalkyl, dihydroxyalkylarylalkyl, alkoxyalkyl, acyloxyalkyl, aminoalkyl, alkylaminoalkyl, alkoxycarbonylaminoalkyl, acylaminoalkyl, arylsulfonamidoalkyl, allyl, dihydroxyalkylallyl, dioxolanylallyl, and $(R^3)_3Si$ where each R^3 is independently selected from H, methyl, ethyl, isopropyl, t-butyl, and phenyl; wherein "alk-" or "alkyl" refers to C_{1-6}alkyl, branched or linear preferably C_{1-3}alkyl, in which the carbon chain may be optionally interrupted by an ether (-O-) linkage; and

R^4 is methyl or R^4 and R^1 together form C_{2-6}alkyl;

provided that R^1 and R^2 are not both H; and
provided that where R^1 is $(R^3)_3Si$, X and Y are not both O.

Such compounds are disclosed in WO 94/09010 the contents of which, in particular with respect to the compounds, are incorporated herein by reference.

A preferred compound is e.g. 40-O-(2-hydroxy)ethyl-rapamycin (referred thereafter as Compound B).

Further rapamycin derivatives are known, e.g. carboxylic acid esters such as disclosed in WO 92/05179, amide esters such as disclosed in US 5 118 677, carbamates such as described in US 5 118 678, fluorinated esters such as disclosed in US 5 100 883, acetals, e.g. in US 5 151 413, silyl ethers, e.g. in US 5 120 842, arylsulfonates and sulfamates, e.g. in US 5 177 203, derivatives wherein the methoxy group at the position 16 is replaced with alkynyloxy, e.g. in WO 95/16691 and further derivatives such

as disclosed in WO 95/14023, all incorporated herein by reference.

Rapamycin and above mentioned derivatives have been shown to have potent immunosuppressant properties. Rapamycin has also been shown to inhibit smooth muscle cell proliferation and to inhibit cancer growth..

Somatostatin analogues, e.g. octreotide, vapreotide and lanreotide, have been disclosed i.a. to inhibit growth hormone secretion and to have an inhibiting effect on malignant tumor growth, e.g. in breast cancer. Octreotide and lanreotide have also been disclosed to inhibit smooth muscle cell proliferation.

In accordance with the invention, it has now surprisingly been found that a combination of 2 active ingredients believed to act on basically different mechanisms such as a somatostatin analogue or derivative and rapamycin or a derivative thereof, can be combined and synergize in the inhibition of cell proliferation.

In accordance with the particular findings of the present invention, there is provided in a first aspect:

1. A method for inhibiting cell proliferation in a subject in need of such treatment which comprises administering to such subject a therapeutically effective amount of a somatostatin analogue or derivative and rapamycin or a derivative thereof.

   In the method of the invention as defined above, the composition of the invention is particularly useful for the treatment of malignant tumor growth, e.g. breast, lung, GEP tumors, pituitary adenomas, lymphomas, etc., for the treatment of proliferative diseases, e.g. restenosis, injury after a procedure such as angioplasty, transplant vasculopathies, for example atherosclerosis, and chronic rejection of various tissues and organs such as heart, kidney, pancreas, lung, liver and combined heart-lung.

2. A kit or package for inhibiting cell proliferation, e.g. for use in a method as indicated

above, said kit or package including a pharmaceutical composition comprising a somatostatin analogue or derivative and a pharmaceutical composition comprising rapamycin or a derivative thereof. The kit or package may also contain instructions to use the pharmaceutical compositions in accordance with the present invention.

3. A therapeutic composition for inhibiting cell proliferation, e.g. for use in a method as indicated above, in a subject which comprises a somatostatin analogue or derivative and rapamycin or a derivative thereof.

Utility of the composition in the treatment of disorders and diseases as hereinbefore specified, may be demonstrated for example in accordance with the method hereinafter described.

Method

AR42J cell cultures are propagated in DMEM supplemented with 10 % fetal calf serum (FCS) at 5 % CO_2. Cells are grown in the absence of antibiotics or antifungal agents. Subconfluent AR42J cells growing in DMEM and supplemented with 10 % FCS are trypsinized, diluted in DMEM + 2.5 % FCS and seeded in uncoated 96-well plates (5'000 to 10'000 cells per well in 180 µl). After a 48-hr incubation period (Day O), the number of cells in a separate control plate is determined both by counting cells in a Coulter counter and by the sulforhodamine B (SRB) staining assay. The cells are then exposed either to the somatostatin analogue alone, e.g. octreotide, or to rapamycin or a derivative thereof alone or to a combination of the somatostatin analogue and rapamycin or its derivative up to 5 days at various concentrations. Total drug exposure lasts for up to 5 days following the first addition and SRB analysis as described above is performed e.g. on day 2 and day 5. Growth is determined as difference in absorbance (OD) between day 0 and day x values (= delta OD). Calculations are made based on the fractional product method of Webb (Valeriote and Lin, 1975; Cory and Carter, 1986; Berenbaum, J. Theor. Biol. 114: 413-431, 1985) and the method by Chou and Talalay (Adv. Enz. Regul. 22: 27-55, 1984). If the measured cell growth (% of control)

is < to the calculated cell growth, this shows evidence for a synergistic effect. Under these conditions a combination of a somatostatin analogue at a concentration of from 10^{-10} to 10^{-6} M with rapamycin or its derivative at a concentration of from 1 to 1000 nM significantly inhibits the growth of the tumor cells.

In this assay, the following results are obtained with octreotide alone, Compound B alone and a combination of octreotide and Compound B. The synergy according to the Webb Method is confirmed by using the Chou-Talalay Method.

	Cell Growth (% of CONTROL)			
	Concentration (nM)	Cell Growth (ΔOD) (%)	Observed (%)	Calculated (Webb Method) (%)
Control		664 ± 9	100	
Octreotide	1.2	397 ± 16	59.8	
Compound B	12.0	420 ± 12	63.3	
Octreotide + Compound B	1.2 + 12.0	103 ± 5	15.6	37.9

In this invention, the somatostatin analogue or derivative and rapamycin or a derivative are preferably administered as pharmaceutical compositions. Rapamycin and its derivatives are preferably administered per os and the somatostatin analogue or derivative is preferably administered parenterally, e.g by infusion. The somatostatin analogue or derivative may also be administered in a slow release form. The administration of each component of the combination may take place either simultaneously or sequentially, e.g. rapamycin or Compound B may be administered at first followed later, e.g. 8 to 24 hours later, by the somatostatin compound.

The amount of each component administered is determined taking into account various factors such as the etiology and severity of the disease, and the patient's condition. Rapamycin and its derivatives may conveniently be administered at doses which are in the range used in immunosuppressive applications such as prevention and treatment of graft vs. host disease, transplant rejection or autoimmune diseases e.g. at a daily dosage from about 0.5 to 500 mg as a single dose or in divided doses. The somatostatin analogue or derivative may be administered, e.g. subcutaneously, in a dosage range of about 100 μg to 10 mg per day as a single dose or in divided doses. Thus octreotide may be administered at a dose of from 0.2 mg to 10 mg twice or three times daily. The combination of the somatostatin analogue with rapamycin or its derivative enables to maximize the antiproliferative effect.

The invention contemplates that the active ingredients discussed herein may be utilized in combination with diluents and other carriers, for oral or parenteral administration, or may be delivered by any conventional delivery system.

Formulation Examples:

A. Somatostatin Formulations:

1. Ampoules

Octreotide	0.5 mg
Mannitol	45.0 mg
Lactic acid (88%)	3.4 mg
Sodium hydrogeno-carbonate	to pH 4.2
Water (inject.grade)	to 1 ml
Carbon dioxide	q.s.

2. Biodegradable sustained release formulation:

Octreotide Acetate	4.65 %	(by weight)
Poly(DL-lactide-co-glycolide)	78.35 %	
Sterile Mannitol	17 %	

Vehicle:	Carboxymethylcellulose	0.5 %	(by weight)
	Mannitol	0.6 %	
	Water for injection	98.9 %	

B. Rapamycin (or derivative thereof) formulation: e.g. capsules

Ethanol	20.0 mg
1,2-propylene glycol	81.0mg
Refined oil	121.5mg
Cremophor RH40	202.5mg
Rapamycin	20.0mg
Total	500 mg

CLAIMS

1. A method for inhibiting cell proliferation in a subject in need of such treatment which comprises administering to such subject a therapeutically effective amount of a somatostatin analogue or derivative and rapamycin or a derivative thereof.

2. A kit or package for inhibiting cell proliferation, said kit or package including a pharmaceutical composition comprising a somatostatin analogue or derivative and a pharmaceutical composition comprising rapamycin or a derivative thereof.

3. A therapeutic composition for inhibiting cell proliferation which comprises a somatostatin analogue or derivative and rapamycin or a derivative thereof.

4. The use of a somatostatin analogue or derivative in combination with rapamycin or a derivative thereof for inhibiting cell proliferation.

5. A method, kit, composition or use according to claim 1, 2, 3 or 4, wherein the somatostatin analogue or derivative is a somatostatin octapeptide, preferably a compound of formula I substantially as hereinbefore defined and/or described.

6. A method, kit, composition or use according to claim 1, 2, 3 or 4, wherein the rapamycin derivative is Compound B substantially as hereinbefore defined and/or described.

PCT/EP 97/03036

The Patent Office

The Patent Office
Concept House
Cardiff Road
Newport
South Wales
NP9 1RH

PRIORITY DOCUMENT

I, the undersigned, being an officer duly authorised in accordance with Section 74(1) and (4) of the Deregulation & Contracting Out Act 1994, to sign and issue certificates on behalf of the Comptroller-General, hereby certify that annexed hereto is a true copy of the documents as originally filed in connection with the patent application identified therein.

In accordance with the Patents (Companies Re-registration) Rules 1982, if a company named in this certificate and any accompanying documents has re-registered under the Companies Act 1980 with the same name as that with which it was registered immediately before re-registration save for the substitution as, or inclusion as, the last part of the name of the words "public limited company" or their equivalents in Welsh, references to the name of the company in this certificate and any accompanying documents shall be treated as references to the name with which it is so re-registered.

In accordance with the rules, the words "public limited company" may be replaced by p.l.c., plc, P.L.C. or PLC.

Re-registration under the Companies Act does not constitute a new legal entity but merely subjects the company to certain additional company law rules.

Signed Andrew Gerrey

Dated 23 May 1997

Patents Form 1/77

Patents Act 1977
(Rules 16)

THE PATENT OFFICE
16 SEP 1996
RECEIVED BY

The Patent Office

1/77

16SEP96 E220924-3 000524
P01/7700 25.00

Request for grant of a patent

(See the notes on the back of this form. You can also get an explanatory leaflet from the Patent Office to help you fill in this form)

The Patent Office

Cardiff Road
Newport
Gwent NP9 1RH

1. Your reference

100-8348

16 SEP 1996

2. Patent application number
(The Patent Office will fill in this part)

9619310.7

3. Full name, address and postcode of the or of each applicant *(underline all surnames)*

SANDOZ LTD.
35 Lichtstrasse
CH-4002 Basel
Switzerland

Patents ADP number *(if you know it)*

00703207001

If the applicant is a corporate body, give the country/state of its incorporation

Switzerland

4. Title of the invention

ORGANIC COMPOUNDS

5. Name of your agent *(if you have one)*

B. A. Yorke & Co.

"Address for service" in the United Kingdom to which all correspondence should be sent *(including the postcode)*

Coomb House
7 St. John's Road
Isleworth, Middlesex TW7 6NH

Patents ADP number *(if you know it)*

1800001

6. If you are declaring priority from one or more earlier patent applications, give the country and the date of filing of the or of each of these earlier applications and *(if you know it)* the or each application number

Country	Priority application number *(if you know it)*	Date of filing *(day / month / year)*

7. If this application is divided or otherwise derived from a earlier UK application, give the number and the filing date of the earlier application

Number of earlier application	Date of filing *(day / month / year)*

8. Is a statement of inventorship and of right to grant of a patent required in support of this request? *(Answer "Yes" if:*

a) any applicant named in part 3 is not an inventor , or
b) there is an inventor who is not named as an applicant, or
c) any named applicant is a corporate body.
See note (d))

Yes

Patents Form 1/77

9. Enter the number of sheets for any of the following items you are filing with this form. Do not count copies of the same document

Continuation sheets of this form	
Description	14
Claim(s)	1
Abstract	
Drawing (s)	

10. If you are also filing any of the following, state how many against each items.

Priority documents	
Translations of priority documents	
Statemtent of inventorship and right to grant of a patent *(Patents Form 7/77)*	
Request for preliminary examination and search *(Patents Form 9/77)*	
Request for substantive examination *(Patents Form 10/77)*	
Any other documents *(please specify)*	

11. I/We request the grant of a patent on the basis of this application.

Signature B. A. Yorke & Co. Date 13 Sept 96

B. A. Yorke & Co

12. Name and daytime telephone number of person to contact in the United Kingdom

Warning

After an application for a patent has been filed, the Comptroller of the Patent Office will consider whether publication or communication of the invention should be prohibited or restricted under Section 22 of the Patents Act 1977. You will be informed if it is necessary to prohibit or restrict your invention in this way. Furthermore, if you live in the United Kingdom, Section 23 of the Patent Act 1977 stops you from applying for a patent abroad without first getting written permission form the Patent Office unless an application has been filed at least 6 weeks beforehand in the United Kingdom for a patent for the same invention and either no direction prohibiting publication or communication has been given, or any such direction has been revoked.

Notes

a) *If you need help to fill in this form or you have any questions, please contact the Patent Office on 0645 500505*

b) *Write your answers in capital letters using black ink or you may type them.*

c) *If there is not enough space for all the relevant details on any part of this form, please continue on a separate sheet of paper and write "see continuation sheet" in relevant part(s). Any continuation sheet should be attached to this form.*

d) *If you have answered "Yes" Patents Form 7/77 will need to be filed.*

e) *Once you have filed in the form you must remember so sign and date it.*

f) *For details of the fee and ways to pay please contact the Patent Office.*

ORGANIC COMPOUNDS

The present invention relates to a pharmaceutical combination and its use in the treatment of disorders associated with excess benign and malignant cell proliferation, e.g. tumors or myointimal cell proliferation.

There is a continuing need for development of drugs having increased effectiveness in inhibiting or slowing undesired cell proliferation, particularly in the cancer field and in vasculopathies.

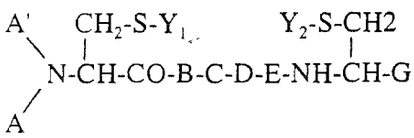
Accordingly, there is provided a composition comprising a somatostatin analogue or derivative and rapamycin or a derivative thereof.

By "somatostatin analogues or derivatives" as used herein is meant any straight-chain or cyclic polypeptide derived from that of the naturally occurring tetradecapeptide somatostatin wherein one or more amino acid units have been omitted and/or replaced by one or more other amino radical(s) and/or wherein one or more functional groups have been replaced by one or more other functional groups and/or one or more groups have been replaced by one or several other isosteric groups. In general, the term covers all modified derivatives of the biologically active somatostatin tetradecapeptide which exhibit a somatostatin-like activity, e.g. they bind to somatostatin receptors.

Cyclic, bridge cyclic and straight-chain somatostatin analogues or derivatives are known and have been described together with processes for their production e.g. in US Patent Specifications 4,310,518 and 4,235,886, in European Patent Specifications EP-A-1295; 23,192; 29,310; 29,579; 30,920; 31,303; 63,308; 70,021; 83,305; 215,171; 203,031; 214,872; 143,307; 298,732; 277,419 and in Belgian Patent Specification BE-A-900,089, the contents thereof, in particular with respect to the compounds, being incorporated herein by reference.

Preferred somatostatin analogues or derivatives are:

Compounds of formula I



(I)

wherein

A is C_{1-12}alkyl, C_{7-10}phenylalkyl or a group of formula RCO-,

whereby

i) R is hydrogen, C_{1-11}alkyl, phenyl or C_{7-10}phenylalkyl, or

ii) RCO- is

a) a D-phenylalanine residue optionally ring-substituted by halogen, NO_2, NH_2, OH, C_{1-3}alkyl and/or C_{1-3}alkoxy; or

b) the residue of a natural or a synthetic α-amino-acid other than defined under a) above, or of a corresponding D-amino acid, or

c) a dipeptide residue in which the individual amino acid residues are the same or different and are selected from those defined under a) and/or b) above, the α-amino group of amino acid residues a) and b) and the N-terminal amino group of dipeptide residues c) being optionally mono- or di-C_{1-12}alkylated or substituted by C_{1-8}alkanoyl;

A' is hydrogen or C_{1-3}alkyl,

Y_1 and Y_2 represent together a direct bond or

each of Y_1 and Y_2 is hydrogen

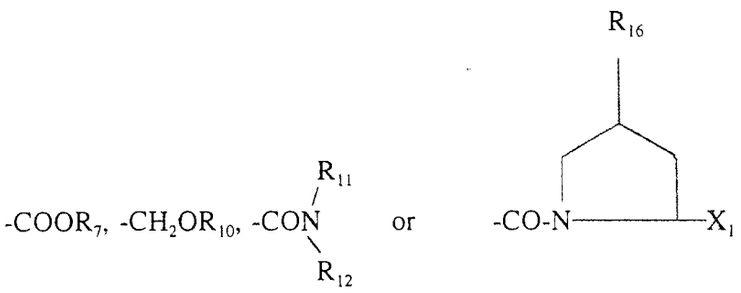
B is -Phe- optionally ring-substituted by halogen, NO_2, NH_2, OH, C_{1-3}alkyl and /or C_{1-3}alkoxy (including pentafluoroalanine), or naphthylalanine

C is (L)-Trp- or (D)-Trp- optionally α-N-methylated and optionally benzene-ring-substituted by halogen, NO_2, NH_2, OH, C_{1-3}alkyl and/or C_{1-3}alkoxy,

D is Lys, 4-aminocyclohexylAla or 4-aminocyclohexylGly

E is Thr, Ser, Val, Tyr, Ile, Leu or an aminobutyric or aminoisobutyric acid residue

G is a group of formula



wherein

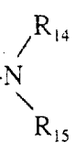
R_7 is hydrogen or C_{1-3}alkyl,

R_{10} is hydrogen or the residue of a physiologically acceptable, physiologically hydrolysable ester,

R_{11} is hydrogen, C_{1-3}alkyl, phenyl or C_{7-10}phenyl-alkyl,

R_{12} is hydrogen, C_{1-3}alkyl or a group of formula $-CH(R_{13})-X_1$,

R_{13} is CH_2OH, $-(CH_2)_2-OH$, $-(CH_2)_3-OH$, or $-CH(CH_3)OH$ or represents the substituent attached to the α-carbon atom of a natural or synthetic α-amino acid (including hydrogen) and

X_1 is a group of formula $-COOR_7$, $-CH_2OR_{10}$ or 

wherein

R_7 and R_{10} have the meanings given above,

R_{14} is hydrogen or C_{1-3}alkyl and

R_{15} is hydrogen, C_{1-3}alkyl, phenyl or C_{7-10}phenylalkyl, and

R_{16} is hydrogen or hydroxy,

with the proviso that

when R_{12} is $-CH(R_{13})-X_1$ then R_{11} is hydrogen or methyl,

wherein the residues B, D and E have the L-configuration, and the residues in the 2- and 7-position each independently have the (L)- or (D)- configuration,

in free form or in pharmaceutically acceptable salt or complex form.

Individual compounds of formula I suitable in accordance with the present invention are the following somatostatin analogues:

a. (D)Phe-Cys-Phe-(D)Trp-Lys-Thr-Cys-Thr-ol
also known as octreotide

b. (D)Phe-Cys-Tyr-(D)Trp-Lys-Val-Cys-ThrNH_2

c. (D)Phe-Cys-Tyr-(D)Trp-Lys-Val-Cys-TrpNH_2
also known as vapreotide

d. (D)Trp-Cys-Phe-(D)Trp-Lys-Thr-Cys-ThrNH_2

e. (D)Phe-Cys-Phe-(D)Trp-Lys-Thr-Cys-ThrNH_2

f. 3-(2-(Naphthyl)-(D)Ala-Cys-Tyr-(D)Trp-Lys-Val-Cys-ThrNH_2
also known as lanreotide

g. (D)Phe-Cys-Tyr-(D)Trp-Lys-Val-Cys-β-Nal-NH_2

h. 3-(2-naphthyl)-Ala-Cys-Tyr-(D)Trp-Lys-Val-Cys-β-Nal-NH_2

i. (D)Phe-Cys-β-Nal-(D)Trp-Lys-Val-Cys-Thr-NH_2

j. (D)Phe-Cys-Tyr-(D)Trp-Lys-Leu-Cys-Thr-NH_2

k. (D)Phe-Cys-Tyr-(D)Trp-Lys-Cys-Thr-NH_2

Further compounds suitable for use in accordance with the present invention are:

cyclo [-Asn-Phe-Phe-DTrp-Lys-Thr-Phe-Gaba-],

cyclo(Asu-Lys-Asn-Phe-Phe-Trp-Lys-Thr-Tyr-Thr-Ser), and

(D)Nal-Glu-Tyr-(D)Trp-Lys-Val-Lys-Thr-NH_2

Octreotide is preferred.

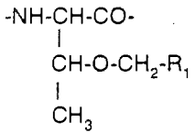
Compounds of formula I may exist e.g. in free form, salt form or in the form of complexes thereof. Acid addition salts may be formed with e.g. organic acids, polymeric acids and inorganic acids. Such acid addition salt forms include e.g. the hydrochlorides and acetates. Complexes are e.g. formed from compounds of the invention on addition of inorganic substances, e.g. inorganic salts or hydroxides such as Ca- and Zn-salts, and/or an addition of polymeric organic substances.

Further preferred somatostatin analogues or derivatives are e.g. the somatostatin compounds disclosed in PCT/EP96/02840 (filed on 28.6.1996), the contents thereof, in particular with respect to the compounds, being incorporated herein by reference.

Examples of such compounds are somatostatin analogues comprising the amino acid sequence of formula (III)

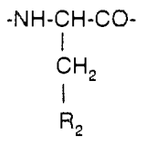
$$-(D/L)Trp-Lys-X_1-X_2- \qquad (III)$$

wherein X_1 is a radical of formula (a) or (b)



(a)

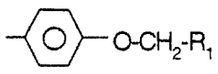
or



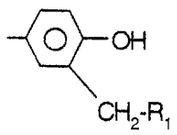
(b)

wherein R_1 is optionally substituted phenyl,

R_2 is $-Z_1-CH_2-R_1$, $-CH_2-CO-O-CH_2-R_1$,



or



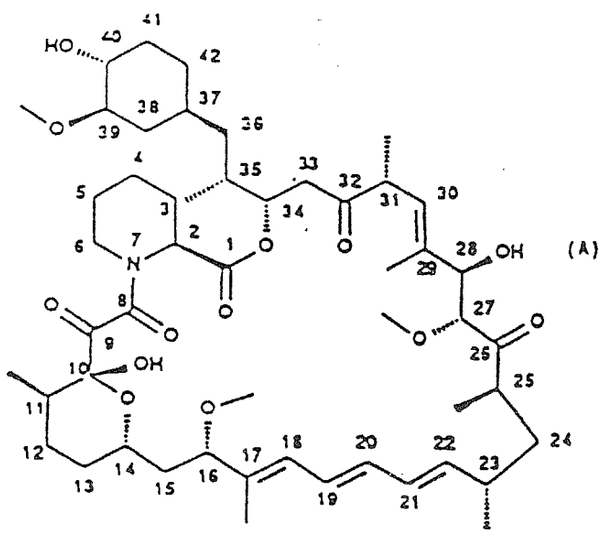
wherein Z_1 is O or S,

and

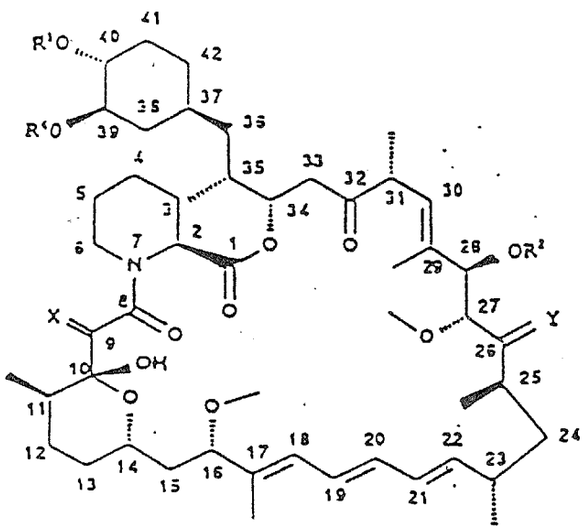
X_2 is an α-amino acid having an aromatic residue on the C_α side chain, or an amino acid unit selected from Dab, Dpr, Dpm, His,(Bzl)HyPro, thienyl-Ala, cyclohexyl-Ala and t.-butyl-Ala,

the residue Lys of said sequence corresponding to the residue Lys^9 of the native somatostatin-14.

Rapamycin is a known macrolide antibiotic produced by Streptomyces hygroscopicus, having the structure depicted in Formula A:



See, e.g., McAlpine, J.B., et al., J. Antibiotics (1991) 44: 688; Schreiber, S.L., et al., J. Am. Chem. Soc. (1991) 113: 7433; US Patent No. 3 929 992. Numerous derivatives of rapamycin are known. One group of derivatives are alkylated derivatives of rapamycin having the structure of Formula II:



(II)

X is (H,H) or O;

Y is (H,OH) or O;

R^1 and R^2 are independently selected from

H, alkyl, thioalkyl, arylalkyl, hydroxyalkyl, dihydroxyalkyl, hydroxyalkylarylalkyl, dihydroxyalkylarylalkyl, alkoxyalkyl, acyloxyalkyl, aminoalkyl, alkylaminoalkyl, alkoxycarbonylaminoalkyl, acylaminoalkyl, arylsulfonamidoalkyl, allyl, dihydroxyalkylallyl, dioxolanylallyl, and $(R^3)_3Si$ where each R^3 is independently selected from H, methyl, ethyl, isopropyl, t-butyl, and phenyl; wherein "alk-" or "alkyl" refers to C_{1-6}alkyl, branched or linear preferably C_{1-3}alkyl, in which the carbon chain may be optionally interrupted by an ether (-O-) linkage; and

R^4 is methyl or R^4 and R^1 together form C_{2-6}alkyl;

provided that R^1 and R^2 are not both H; and

provided that where R^1 is $(R^3)_3Si$, X and Y are not both O.

Such compounds are disclosed in WO 94/09010 the contents of which, in particular with respect to the compounds, are incorporated herein by reference.

A preferred compound is e.g. 40-O-(2-hydroxy)ethyl-rapamycin (referred thereafter as Compound B).

Further preferred rapamycin derivatives are e.g. those disclosed in PCT/EP96/02441 (filed on 5.6.1996), the contents thereof, in particular with respect to the compounds, being incorporated herein by reference. Particularly preferred are 32-deoxo-rapamycin, 16-O-pent-2-ynyl-32-deoxo-rapamycin and 16-O-pent-2-ynyl-32-(S)-dihydro-rapamycin.

Further rapamycin derivatives are known, e.g. carboxylic acid esters such as disclosed in WO 92/05179, amide esters such as disclosed in US 5 118 677, carbamates such as described in US 5 118 678, fluorinated esters such as disclosed in US 5 100 883, acetals, e.g. in US 5 151 413, silyl ethers, e.g. in US 5 120 842, arylsulfonates and sulfamates, e.g. in US 5 177 203, derivatives wherein the methoxy group at the position 16 is replaced with alkynyloxy, e.g. in WO 95/16691 and further derivatives such as disclosed in WO 95/14023, all incorporated herein by reference.

Rapamycin and above mentioned derivatives have been shown to have potent immunosuppressant properties. Rapamycin has also been shown to inhibit smooth muscle cell proliferation and to inhibit cancer growth..

Somatostatin analogues, e.g. octreotide, vapreotide and lanreotide, have been disclosed i.a. to inhibit growth hormone secretion and to have an inhibiting effect on malignant tumor growth, e.g. in breast cancer. Octreotide and lanreotide have also been disclosed to inhibit smooth muscle cell proliferation.

In accordance with the invention, it has now surprisingly been found that a combination of 2 active ingredients believed to act on basically different mechanisms such as a somatostatin analogue or derivative and rapamycin or a derivative thereof, can be

combined and synergize in the inhibition of cell proliferation.

In accordance with the particular findings of the present invention, there is provided in a first aspect:

1. A method for inhibiting cell proliferation in a subject in need of such treatment which comprises administering to such subject a therapeutically effective amount of a somatostatin analogue or derivative and rapamycin or a derivative thereof.

   In the method of the invention as defined above, the composition of the invention is particularly useful for the treatment of malignant tumor growth, e.g. breast, lung, GEP tumors, pituitary adenomas, lymphomas, etc., for the treatment of proliferative diseases, e.g. restenosis, injury after a procedure such as angioplasty, transplant vasculopathies, for example atherosclerosis, and chronic rejection of various tissues and organs such as heart, kidney, pancreas, lung, liver and combined heart-lung.

2. A kit or package for inhibiting cell proliferation, e.g. for use in a method as indicated above, said kit or package including a pharmaceutical composition comprising a somatostatin analogue or derivative and a pharmaceutical composition comprising rapamycin or a derivative thereof. The kit or package may also contain instructions to use the pharmaceutical compositions in accordance with the present invention.

3. A therapeutic composition for inhibiting cell proliferation, e.g. for use in a method as indicated above, in a subject which comprises a somatostatin analogue or derivative and rapamycin or a derivative thereof.

Utility of the composition in the treatment of disorders and diseases as hereinbefore specified, may be demonstrated for example in accordance with the method hereinafter described.

Method

AR42J cell cultures are propagated in DMEM supplemented with 10 % fetal calf serum (FCS) at 5 % CO_2. Cells are grown in the absence of antibiotics or antifungal agents. Subconfluent AR42J cells growing in DMEM and supplemented with 10 % FCS are trypsinized, diluted in DMEM + 2.5 % FCS and seeded in uncoated 96-well plates (5'000 to 10'000 cells per well in 180 µl). After a 48-hr incubation period (Day O), the number of cells in a separate control plate is determined both by counting cells in a Coulter counter and by the sulforhodamine B (SRB) staining assay. The cells are then exposed either to the somatostatin analogue alone, e.g. octreotide, or to rapamycin or a derivative thereof alone or to a combination of the somatostatin analogue and rapamycin or its derivative up to 5 days at various concentrations. Total drug exposure lasts for up to 5 days following the first addition and SRB analysis as described above is performed e.g. on day 2 and day 5. Growth is determined as difference in absorbance (OD) between day 0 and day x values (= delta OD). Calculations are made based on the fractional product method of Webb (Valeriote and Lin, 1975; Cory and Carter, 1986; Berenbaum, J. Theor. Biol. 114: 413-431, 1985) and the method by Chou and Talalay (Adv. Enz. Regul. 22: 27-55, 1984). If the measured cell growth (% of control) is < to the calculated cell growth, this shows evidence for a synergistic effect. Under these conditions a combination of a somatostatin analogue at a concentration of from 10^{-10} to 10^{-6} M with rapamycin or its derivative at a concentration of from 1 to 1000 nM significantly inhibits the growth of the tumor cells.

In this assay, the following results are obtained with octreotide alone, Compound B alone and a combination of octreotide and Compound B. The synergy according to the Webb Method is confirmed by using the Chou-Talalay Method.

	Cell Growth (% of CONTROL)			
	Concentration (nM)	Cell Growth (ΔOD) (%)	Observed (%)	Calculated (Webb Method) (%)
Control		664 ± 9	100	
Octreotide	1.2	397 ± 16	59.8	
Compound B	12.0	420 ± 12	63.3	
Octreotide + Compound B	1.2 + 12.0	103 ± 5	15.6	37.9

In this invention, the somatostatin analogue or derivative and rapamycin or a derivative are preferably administered as pharmaceutical compositions. Rapamycin and its derivatives are preferably administered per os and the somatostatin analogue or derivative is preferably administered parenterally, e.g by infusion. The somatostatin analogue or derivative may also be administered in a slow release form. The administration of each component of the combination may take place either simultaneously or sequentially, e.g. rapamycin or Compound B may be administered at first followed later, e.g. 8 to 24 hours later, by the somatostatin compound.

The amount of each component administered is determined taking into account various factors such as the etiology and severity of the disease, and the patient's condition. Rapamycin and its derivatives may conveniently be administered at doses which are in the range used in immunosuppressive applications such as prevention and treatment of graft vs. host disease, transplant rejection or autoimmune diseases e.g. at a daily dosage from about 0.5 to 500 mg as a single dose or in divided doses. The somatostatin analogue or derivative may be administered, e.g. subcutaneously, in a dosage range of about 100 μg to 10 mg per day as a single dose or in divided doses. Thus octreotide may be administered at a dose of from 0.2 mg to 10 mg twice or three times daily. The combination of the somatostatin analogue with rapamycin or its derivative enables to

maximize the antiproliferative effect.

The invention contemplates that the active ingredients discussed herein may be utilized in combination with diluents and other carriers, for oral or parenteral administration, or may be delivered by any conventional delivery system.

Formulation Examples:

A. Somatostatin Formulations:

1. Ampoules

Octreotide	0.5 mg
Mannitol	45.0 mg
Lactic acid (88%)	3.4 mg
Sodium hydrogeno-carbonate	to pH 4.2
Water (inject.grade)	to 1 ml
Carbon dioxide	q.s.

2. Biodegradable sustained release formulation:

Octreotide Acetate	4.65 %	(by weight)
Poly(DL-lactide-co-glycolide)	78.35 %	
Sterile Mannitol	17 %	

Vehicle: Carboxymethylcellulose	0.5 %	(by weight)
Mannitol	0.6 %	
Water for injection	98.9 %	

B. Rapamycin (or derivative thereof) formulation: e.g. capsules

Ethanol	20.0 mg
1,2-propylene glycol	81.0mg
Refined oil	121.5mg
Cremophor RH40	202.5mg
Rapamycin	20.0mg
Total	500 mg

CLAIMS

1. A method for inhibiting cell proliferation in a subject in need of such treatment which comprises administering to such subject a therapeutically effective amount of a somatostatin analogue or derivative and rapamycin or a derivative thereof.

2. A kit or package for inhibiting cell proliferation, said kit or package including a pharmaceutical composition comprising a somatostatin analogue or derivative and a pharmaceutical composition comprising rapamycin or a derivative thereof.

3. A therapeutic composition for inhibiting cell proliferation which comprises a somatostatin analogue or derivative and rapamycin or a derivative thereof.

4. The use of a somatostatin analogue or derivative in combination with rapamycin or a derivative thereof for inhibiting cell proliferation.

5. A method, kit, composition or use according to claim 1, 2, 3 or 4, wherein the somatostatin analogue or derivative is a somatostatin octapeptide, preferably a compound of formula I, substantially as hereinbefore defined and/or described.

6. A method, kit, composition or use according to claim 1, 2, 3 or 4, wherein the rapamycin derivative is Compound B or a somatostatin analogue comprising the amino acid sequence of formula III substantially as hereinbefore defined and/or described.

UNITED STATES DEPARTMENT OF COMMERCE
Patent and Trademark Office
Address: ASSISTANT COMMISSIONER FOR PATENTS
Washington, D.C. 20231

09/194957

U.S. APPLICATION NO.	FIRST NAMED APPLICANT	ATTY. DOCKET NO.
09/194,957	WECKBECKER G	4-100-8322/A

INTERNATIONAL APPLICATION NO.
PCT/EP97/03036

001095
MELVYN M KASSENOFF
NOVARTIS CORPORATION
PATENT AND TRADEMARK DEPT
564 MORRIS AVENUE
SUMMIT NJ 07901-1027

5611

I.A. FILING DATE	PRIORITY DATE
06/11/97	06/11/96

DATE MAILED: 05/05/99

NOTIFICATION OF ACCEPTANCE OF APPLICATION UNDER 35 U.S.C. 371 AND 37 CFR 1.494 OR 1.495

1. The applicant is hereby advised that the United States Patent and Trademark Office in its capacity as ☐ a Designated Office (37 CFR 1.494), ☑ an Elected Office (37 CFR 1.495), has determined that the above identified international application has met the requirements of 35 U.S.C. 371, and is **ACCEPTED** for national patentability examination in the United States Patent and Trademark Office.

2. The United States Application Number assigned to the application is shown above and the relevant dates ai

07 DEC 1998
35 U.S.C. 102(e) DATE

07 DEC 1998
DATE OF RECEIPT OF
35 U.S.C. 371 REQUIREMENTS

A Filing Receipt (PTO-103X) will be issued for the present application in due course. **THE DATE APPEARING ON THE FILING RECEIPT AS THE "FILING DATE" IS THE DATE ON WHICH THE LAST OF THE 35 U.S.C. 371(C) REQUIREMENTS HAS BEEN RECEIVED IN THE OFFICE. THIS DATE IS SHOWN ABOVE.** *The filing date of the above identified application is the international filing date of the international application (Article 11(3) and 35 U.S.C. 363).* Once the Filing Receipt has been received, send all correspondence to the Group Art Unit designated thereon.

3. ☐ A request for immediate examination under 35 U.S.C. 371(f) was received on _____ and the application will be examined in turn.

4. The following items have been received:
   - ☑ U.S. Basic National Fee.
   - ☑ Copy of the international application in:
     - ☐ a non-English language.
     - ☑ English.
   - ☐ Translation of the international application into English.
   - ☑ Oath or Declaration of inventors(s) for DO/EO/US.
   - ☐ Copy of Article 19 amendments. ☐ Translation of Article 19 amendments into English.
     The Article 19 amendments ☐ have ☐ have not been entered.
   - ☑ The International Preliminary Examination Report in English and its Annexes, if any.
   - ☐ Copy of the Annexes to the International Preliminary Examination Report (IPER).
     ☐ Translation of Annexes to the IPER into English.
     The Annexes ☐ have ☐ have not been entered.
   - ☑ Preliminary amendment(s) filed 7 DEC 1998 and _____.
   - ☐ Information Disclosure Statement(s) filed _____ and _____.
   - ☐ Assignment document.
   - ☐ Power of Attorney and/or Change of Address.
   - ☐ Substitute specification filed _____.
   - ☐ Verified Statement Claiming Small Entity Status.
   - ☑ Priority Document.
   - ☑ Copy of the International Search Report ☑ and copies of the references cited therein.
   - ☑ Other: IPB 308

Applicant is reminded that any communication to the United States Patent and Trademark Office must be mailed to the address given in the heading and include the U.S. application no. shown above. (37 CFR 1.5)

Francine Young

FORM PCT/DO/EO/903 (December 1997)

Telephone (703) 305-3402

DO/EO BIBLIOGRAPHIC DATA ENTRY

'RIAL NUMBER: 09 / 194957
NUMBER: PCT/ EP97 / 03036
MILY NAME: WECKBECKER
'VEN NAME: GISBERT
IORITY CLAIMED (Y/N): Y
BASIC FEE (Y/N): N
'TORNEY DOCKET NUMBER: 4-100-6322/A

RECEIPT DATE: 12 / 07 / 99
IA FILING DATE: 06 / 11 / 97
DELAY WAIVED (Y/N): N
DEMAND RECEIVED (Y/N): Y
PRIORITY DATE: 06 / 11 / 96
US DESIGNATED ONLY (Y/N): N
COUNTRY: EPX

>RRESPONDENCE NAME/ADDRESS: CUSTOMER NUMBER: 001095 TELEPHONE 9085122695
FAX

ME: MICHAEL W GLYNN
NOVARTIS CORPORATION
'REET: 564 MORRIS AVENUE

ITY: SUMMIT
'ATE/COUNTRY: NJ ZIP: 07901
UNE..
'E INVENTION TITLE:
COMBINATION OF A SOMATOSTATIN ANALOGUE AND A RAPAMYCIN

TAB TO LAST POSITION, PUSH SEND

DO/US WORKSHEET

U.S. Appl. No. 09/194,959 International Appl No. PCT/EP97/03036

Application filed by: ☐ 20 months ☑ 30 months

INTERNATIONAL APPLICATION PAPERS IN THE APPLICATION FILE:

- ☑ International application (RECORD COPY)
- ☐ Article 19 amendments
- ☑ PCT/IB/331
- ☑ PCT/IPEA/409 IPER (PCT/IPEA/416 on front)
- ☐ Annexes to 409
- ☑ Priority document(s) No. 1
- ☐ INTERNATIONAL APPLICATION ON DOUBLE SIDED PAPER (COPIES MADE)
- ☐ Request form PCT/RO/101
- ☐ PCT/IB/302
- ☑ PCT/ISA/210-Search Report
- ☑ Search Report references
- ☐ Other _____

RECEIPTS FROM THE APPLICANT: (other than checked above)

- ☐ Basic National Fee (paid or authorized to charge)
- Translation of international application as filed:
  - ☐ Description
  - ☐ Claims
  - ☐ Words in the drawing figure(s)
  - ☐ Article 19 amendments
  - ☐ Annexes to 409
- ☑ Oath / Declaration 7 Dec 98
- ☐ DNA diskette
- ☑ Preliminary amendment(s) filed 7 Dec 98
- ☐ Information Disclosure Statement
- ☐ Assignment document
- ☐ Power of attorney/Change of address
- ☐ Substitute specification
- ☐ Verified small status claim
- ☐ Other _____

Notes:

35 U.S.C. 371 - Receipt of Request (PTO-1390)	07 DEC 1998
Date acceptable oath / declaration received	07 DEC 1998
Date complete 35 U.S.C 371 requirements met	07 DEC 1998
102(e) Date	07 DEC 1998
Date of completion of DO/EO 906 - Notification of Missing 102(e) Requirements	
Date of completion of DO/EO 907 - Notification of Acceptance for 102(e) date	
Date of completion of DO/EO 911 - Application accepted under 35 U.S.C. 1.11	
Date of completion of DO/EO 905 - Notification of Missing Requirements	
Date of completion of DO/EO 916 - Notification of Defective Response	
Date of completion of DO/EO 903 - Notification of Acceptance	16 April 99
Date of completion of DO/EO 909 - Notification of Abandonment	

WIPO Publication

Publication No. WO 97/47317

Publication Date 18 Dec. 97

Publication Language English

Not Published
- ☐ U.S. only
- Designated
- ☐ EP request

Screening done by: Frederick Smith

CASE 4-100-8322/A/PCT

CERTIFICATE OF MAILING

I hereby certify that this paper (along with any paper referred to as being attached or enclosed) is being deposited with the United States Postal Service on the date shown below with sufficient postage as first class mail in an envelope addressed to the: Assistant Commissioner for Patents, Washington, D.C. 20231.

Joseph J. Borovian	Joseph Borovian	May 10, 1999
Type or print name	Signature	Date

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

IN RE PCT NATIONAL STAGE APPLICATION OF

GISBERT WECKBECKER

INTERNATIONAL APPLICATION NO: PCT/EP 97/03036

FILED: 11 JUNE 1997

U.S. APPLICATION NO: 09/194,957

35 USC §371 DATE: 7 DECEMBER 1998

FOR: COMBINATION OF A SOMATOSTATIN ANALOGUE AND A RAPAMYCIN

Assistant Commissioner for Patents
Washington, D.C. 20231

SECOND PRELIMINARY AMENDMENT AND CITATION OF PRIOR ART

Sir:

Please amend the subject application as follows:

IN THE SPECIFICATION

Page 2, line 20; after "hydrogen", insert a comma.

Page 3, line 4; after "4-aminocyclohexylGly", insert a comma.

Page 3, line 5; after "residue", insert a comma.

Page 3, line 11; change "hydrolysable" to -- hydrolyzable --.

Page 3, line 12; after "or", change "C_{7-10}phenyl-alkyl" to --C_{7-10}phenylalkyl --.

Page 4, line 3; after "C_{1-3}alkyl", delete "and" and insert a comma.

Page 4, line 7; before "then", insert a comma.

Page 5, line 12; change "an" to -- on--.

Page 5, line 17; after the amino acid sequence set forth, insert a period.

Page 7, line 18; after "para", insert -- position --.

Page 14, line 23; change "5'000 to 10'000" to -- 5,000 to 10,000 --.

Page 15, line 8; after "<", insert -- compared --.

Page 16, line 13; after "ml", insert a comma.

Page 16, line 16; after "weeks", insert a comma, and after "tumor", change "size" to -- sizes --.

Page 16, line 18; after "tumors", insert --that --.

Page 17, line 6; change "localisation" to --localization --.

Page 17, line 10; change "tumour" to -- tumor --.

Page 17, line 11; after "Preferably", insert a comma.

Abstract page; re-number said page as -- Page 28 --.

IN THE CLAIMS

Please cancel Claims 1-5.

Claim 7; please insert a comma at the following occurrences:

Page 25, line 2, after "hydrogen";
Page 25, line 7, after "4-aminocyclohexylGly";
Page 25, line 8, after "residue";
Page 26, line 8, before "then"; and
Page 26, line 10, after "configuration".

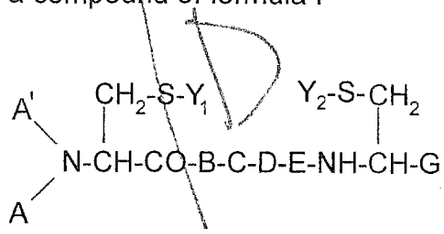
Claim 7, Page 26, line 4; after "C_{1-3}alkyl", delete "and" and insert a comma.

Claim 7, Page 26; relocate the "or" at the bottom of the page to between the formulae.

Please add the following new claims:

-- 10. A pharmaceutical composition comprising a pharmaceutically acceptable carrier and a therapeutically effective amount of: 1) a compound of the somatostatin class, in free form or in pharmaceutically acceptable salt form; and 2) a rapamycin macrolide. --

-- 11. A pharmaceutical composition according to claim 10 wherein the compound of the somatostatin class is a compound of formula I



(I)

wherein

A is C_{1-12}alkyl, C_{7-10}phenylalkyl or a group of formula RCO-, where

i) R is hydrogen, C_{1-11}alkyl, phenyl or C_{7-10}phenylalkyl, or

ii) RCO- is

a) a D-phenylalanine residue optionally ring-substituted by halogen, NO_2, NH_2, OH, C_{1-3}alkyl and/or C_{1-3}alkoxy; or

b) the residue of a natural or a synthetic α-amino-acid other than defined under a) above, or of a corresponding D-amino acid, or

c) a dipeptide residue in which the individual amino acid residues are the same or different and are selected from those defined under a) and/or b) above,

the α-amino group of amino acid residues a) and b) and the N-terminal amino group of dipeptide residues c) being optionally mono- or di-C_{1-12}alkylated or substituted by C_{1-8}alkanoyl;

A' is hydrogen or C_{1-3}alkyl,

Y_1 and Y_2 represent together a direct bond or each of Y_1 and Y_2 is hydrogen,

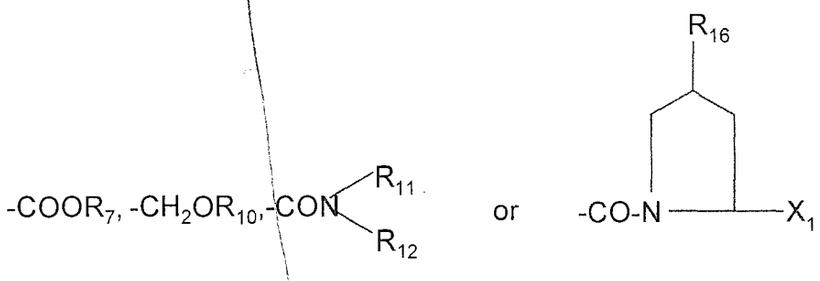
B is -Phe- optionally ring-substituted by halogen, NO_2, NH_2, OH, C_{1-3}alkyl and/or C_{1-3}alkoxy (including pentafluoroalanine), naphthylalanine or pyridylalanine,

C is (L)-Trp- or (D)-Trp- optionally α-N-methylated and optionally benzene-ring-substituted by halogen, NO_2, NH_2, OH, C_{1-3}alkyl and/or C_{1-3}alkoxy,

D is Lys, 4-aminocyclohexylAla or 4-aminocyclohexylGly,

E is Thr, Ser, Val, Tyr, Ile, Leu or an aminobutyric or aminoisobutyric acid residue, and

G is a group of formula



where

R_7 is hydrogen or C_{1-3}alkyl,

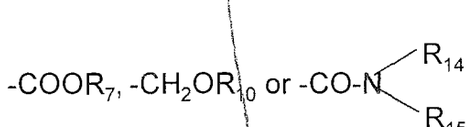
R_{10} is hydrogen or the residue of a physiologically acceptable, physiologically hydrolyzable ester,

R_{11} is hydrogen, C_{1-3}alkyl, phenyl or C_{7-10}phenylalkyl,

R_{12} is hydrogen, C_{1-3}alkyl or a group of formula $-CH(R_{13})-X_1$, where

R_{13} is CH_2OH, $-(CH_2)_2-OH$, $-(CH_2)_3-OH$, $-CH(CH_3)OH$, isobutyl, butyl, benzyl, naphthyl-methyl or indol-3-yl-methyl, and

X_1 is a group of formula



where

R_7 and R_{10} have the meanings given above,

R_{14} is hydrogen or C_{1-3}alkyl and

R_{15} is hydrogen, C_{1-3}alkyl, phenyl or C_{7-10}phenylalkyl, and

R_{16} is hydrogen or hydroxy,

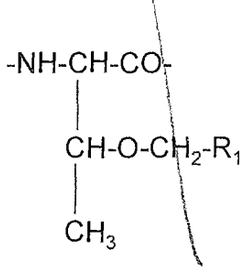
with the proviso that

when R_{12} is $-CH(R_{13})-X_1$, then R_{11} is hydrogen or methyl,

wherein the residues B,D and E have the L-configuration, and the residues in the 2- and 7-position each independently have the (L)- or (D)- configuration;
or a somatostatin analogue comprising the amino acid sequence of formula II

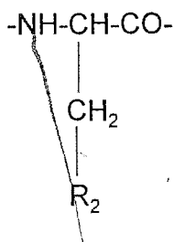
$$-(D/L)Trp-Lys-X_2-X_3- \qquad (II)$$

wherein X_2 is a radical of formula (a) or (b)



(a)

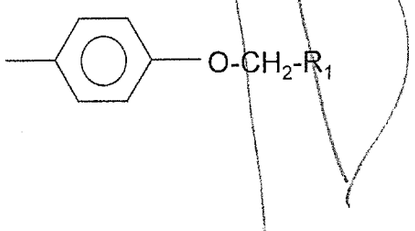
or



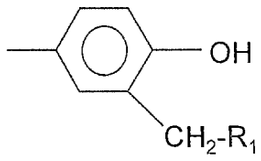
(b)

where R_1 is optionally substituted phenyl,

R_2 is $-Z_1-CH_2-R_1$, $-CH_2-CO-O-CH_2-R_1$,



or



wherein Z_1 is O or S,

and

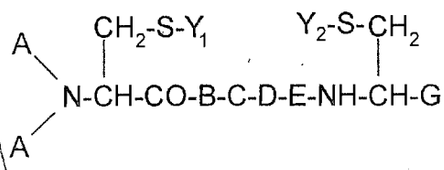
X_3 is an α-amino acid having an aromatic residue on the C_α side chain, or an amino acid unit selected from Dab, Dpr, Dpm, His, (Bzl)HyPro, thienyl-Ala, cyclohexyl-Ala and t.-butyl-Ala,

the residue Lys of said sequence corresponding to the residue Lys^9 of the native somatostatin-14, in free form or in pharmaceutically acceptable salt form. --

-- 12. A pharmaceutical composition according to claim 11 wherein the compound of the somatostatin class is selected from octreotide, lanreotide and vapreotide. --

-- 13. A method of preventing or treating cell hyperproliferation comprising administering to a subject in need of such treatment a therapeutically effective amount of: 1) a compound of the somatostatin class, in free form or in pharmaceutically acceptable salt form; and 2) a rapamycin macrolide. --

-- 14. A method according to claim 13 wherein the compound of the somatostatin class is a compound of formula I



(I)

wherein

A is C_{1-12}alkyl, C_{7-10}phenylalkyl or a group of formula RCO-, where

i) R is hydrogen, C_{1-11}alkyl, phenyl or C_{7-10}phenylalkyl, or

ii) RCO- is

a) a D-phenylalanine residue optionally ring-substituted by halogen, NO_2, NH_2, OH, C_{1-3}alkyl and/or C_{1-3}alkoxy; or

b) the residue of a natural or a synthetic α-amino-acid other than defined under a) above, or of a corresponding D-amino acid, or

c) a dipeptide residue in which the individual amino acid residues are the same or different and are selected from those defined under a) and/or b) above,

the α-amino group of amino acid residues a) and b) and the N-terminal amino group of dipeptide residues c) being optionally mono- or di-C_{1-12}alkylated or substituted by C_{1-8}alkanoyl;

A' is hydrogen or C_{1-3}alkyl,

Y_1 and Y_2 represent together a direct bond or each of Y_1 and Y_2 is hydrogen,

B is -Phe- optionally ring-substituted by halogen, NO_2, NH_2, OH, C_{1-3}alkyl and/or

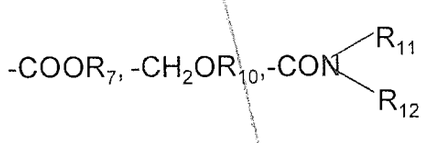
C_{1-3}alkoxy (including pentafluoroalanine), naphthylalanine or pyridylalanine,

C is (L)-Trp- or (D)-Trp- optionally α-N-methylated and optionally benzene-ring-substituted by halogen, NO_2, NH_2, OH, C_{1-3}alkyl and/or C_{1-3}alkoxy,

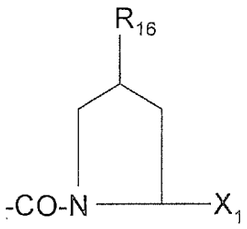
D is Lys, 4-aminocyclohexylAla or 4-aminocyclohexylGly,

E is Thr, Ser, Val,Tyr, Ile, Leu or an aminobutyric or aminoisobutyric acid residue, and

G is a group of formula



or



where

R_7 is hydrogen or C_{1-3}alkyl,

R_{10} is hydrogen or the residue of a physiologically acceptable, physiologically hydrolyzable ester,

R_{11} is hydrogen, C_{1-3}alkyl, phenyl or C_{7-10}phenylalkyl,

R_{12} is hydrogen, C_{1-3}alkyl or a group of formula $-CH(R_{13})-X_1$, where

R_{13} is CH_2OH, $-(CH_2)_2-OH$, $-(CH_2)_3-OH$, $-CH(CH_3)OH$, isobutyl, butyl, benzyl, naphthyl-methyl or indol-3-yl-methyl, and

X_1 is a group of formula

$$-COOR_7,\ -CH_2OR_{10} \text{ or } -CO-N\begin{matrix}R_{14}\\R_{15}\end{matrix}$$

where

R_7 and R_{10} have the meanings given above,

R_{14} is hydrogen or C_{1-3}alkyl and

R_{15} is hydrogen, C_{1-3}alkyl, phenyl or C_{7-10}phenylalkyl, and

R_{16} is hydrogen or hydroxy,

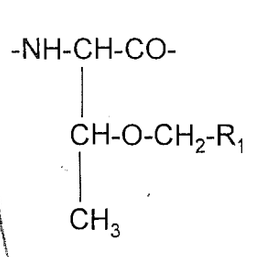
with the proviso that

when R_{12} is $-CH(R_{13})-X_1$, then R_{11} is hydrogen or methyl,

wherein the residues B,D and E have the L-configuration, and the residues in the 2- and 7-position each independently have the (L)- or (D)- configuration;
or a somatostatin analogue comprising the amino acid sequence of formula II

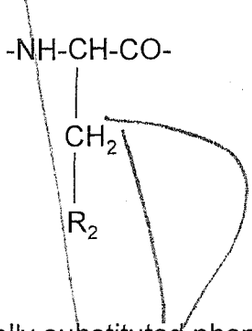
$$-(D/L)Trp-Lys-X_2-X_3- \qquad (II)$$

wherein X_2 is a radical of formula (a) or (b)



(a)

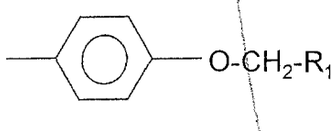
or



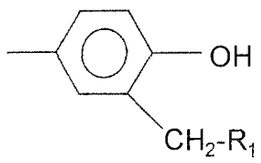
(b)

where R_1 is optionally substituted phenyl,

R_2 is $-Z_1-CH_2-R_1$, $-CH_2-CO-O-CH_2-R_1$,



or



wherein Z_1 is O or S,

and

X_3 is an α-amino acid having an aromatic residue on the C_α side chain, or an amino acid unit selected from Dab, Dpr, Dpm, His, (Bzl)HyPro, thienyl-Ala, cyclohexyl-Ala and t.-butyl-Ala,

the residue Lys of said sequence corresponding to the residue Lys^9 of the native somatostatin-14, in free form or in pharmaceutically acceptable salt form. --

-- 15. A method according to claim 14 wherein the compound of the somatostatin class is selected from octreotide, lanreotide and vapreotide. --

REMARKS

By the foregoing amendments to the specification: 1) the text has been Americanized; 2) editorial errors noted have been corrected; and 3) the "Abstract" page has been re-numbered so as to be consecutive with the last page of the claims.

Claims 1-5 have been cancelled and replaced by new Claims 10-15, which claims are believed to be in more acceptable form.

Claim 7 has been editorially amended.

In compliance with the duty of disclosure set forth in 37 CFR 1.56, the Examiner's attention is respectfully invited to all of the references that are listed on the enclosed completed PTO-1449 form. Since a copy of the asterisked references accompanied the International Search Report, copies of only the remaining references are enclosed. In addition, a copy of the International Search Report is also enclosed for the Examiner's convenience.

Although this Second Preliminary Amendment and Citation of Prior Art is being filed more than three months from the 35 USC 371 date, it is being filed prior to receipt of a first Office Action and, therefore, no additional fee is deemed to be necessitated by making the relevant prior art of record at this time. However, on the chance that this Second Preliminary Amendment and Citation of Prior Art crosses in the mail with a first Office Action that bears a date prior to the date of this Second Preliminary Amendment and

Citation of Prior Art (the benefit of which Applicant is entitled to by virtue of the "Certificate of Mailing" stamp on Page 1 thereof), please charge the $240.00 fee required by 37 CFR 1.97(c) to Deposit Account No. 19-0134 in the name of Novartis Corporation.

Respectfully submitted,

Novartis Corporation
Patent and Trademark Dept.
564 Morris Avenue
Summit, NJ 07901-1027
(908) 522-6921

Joseph J. Borovian
Agent for Applicant
Reg. No. 26,631

JJB:mjl

Encls.: Copy of completed PTO-1449 form
Copies of all references not listed on the International Search Report
Copy of International Search Report
Postcard

Date: May 10, 1999

FORM PTO-1449 (REV. 7-85)

U.S. DEPARTMENT OF COMMERCE
PATENT AND TRADEMARK OFFICE

INFORMATION DISCLOSURE CITATION

(Use several sheets if necessary)

ATTY. DOCKET NO.
4-100-8322/A/PCT
APPLICATION NO.
09/194,957
APPLICANT
Gisbert Weckbecker
35 USC 371 DATE
December 7, 1998

Group

U.S. PATENT DOCUMENTS

EXAMINER INITIAL		DOCUMENT NUMBER	DATE	NAME	CLASS	SUBCLASS	FILING DATE
	AA						
	AB						
	AC						
	AD						
	AE						
	AF						
	AG						
	AH						
	AI						
	AJ						
	AK						
	AL						

FOREIGN PATENT DOCUMENTS

		DOCUMENT NUMBER	DATE	OFFICE	CLASS	SUBCLASS	TRANSLATION YES	TRANSLATION NO
	AM	GB 2 239 178 A *	6/26/91	Great Britain			☐	☐
	AN	WO 93 11130 A *	6/10/93	PCT			☐	☐
	AO						☐	☐
	AP						☐	☐
	AQ						☐	☐

OTHER DOCUMENTS (Including Author, Title, Date, Pertinent pages, Etc.)

	AR	Shi E.A., Cancer Research, Vol. 55, pgs. 1982-19088 (1995). *
	AS	Grant et al., Circulation, Vol. 89, No. 4, pgs 1511-1517 (1994).
	AT	Demoliou-Mason, Exp. Opin.Ther. Patents, Vol. 4, No. 7, pgs. 813-829 (1994).

EXAMINER	DATE CONSIDERED

*EXAMINER: Initial of reference considered, whether or not citation is in conformance with MPEP 609: Draw a line through citation if not in conformance and not considered. Include a copy of this form with the next communication to applicant.

[CANCER RESEARCH 55, 1982–1988, May 1, 1995]

Rapamycin Enhances Apoptosis and Increases Sensitivity to Cisplatin *in Vitro*[1]

Yufang Shi, Andrea Frankel, Laszlo G. Radvanyi, Linda Z. Penn, Richard G. Miller, and Gordon B. Mills[2]

Oncology Research, The Toronto Hospital, Toronto M5G 2C4, Canada [Y. S., A. F., G. B. M.], Ontario Cancer Institute, Princess Margaret Hospital, Toronto M4X 1K9, Canada [L. G. R., R. G. M.], and Department of Microbiology, Immunology and Cancer, The Hospital for Sick Children, Toronto M5G 1X8, Canada [L. Z. P.]

ABSTRACT

Apoptosis can be regulated in a number of different systems by the actions of cytokines. Rapamycin has been shown to exert its effects on growth factor-induced cell proliferation, at least in part, by blocking the activation of the p70 S6 kinase and thus preventing the downstream signaling process, such as the activation of the members of the cdk family. To determine whether this pathway plays a role in the regulation of apoptosis, we assessed the effect of rapamycin on apoptosis induced by interleukin 2 deprivation in murine T-cell lines, by T-cell receptor ligation in a murine T-cell hybridoma, by enforced c-*myc* expression in murine fibroblasts, and by corticosteroids in murine T-lymphoma cell lines. Although rapamycin did not induce apoptosis on its own, rapamycin augmented apoptosis in each of the cell lines used as indicated by increased genomic DNA fragmentation, decreased cell viability, and characteristic apoptotic changes in morphology. These results suggest that a signal transduction pathway(s) inhibited by rapamycin plays an important role in the susceptibility of cells to apoptosis. Many chemotherapeutic agents kill cancer cells through the induction of apoptosis. Strikingly, rapamycin increased the ability of the alkylating agent, cisplatin, to induce apoptosis in the human promyelocytic leukemia cell line HL-60 and the human ovarian cancer cell line SKOV3. These data suggest that a signal transduction pathway, likely related to p70 S6 kinase, inhibited by rapamycin may be an important component of the pathway which prevents cell death in many cell lineages and also indicate that rapamycin has the potential to augment the efficacy of selected anticancer therapies.

INTRODUCTION

The discovery of the immunosuppressive drugs CsA,[3] FK506, and rapamycin has revolutionized organ transplantation and the treatment of autoimmune diseases (1). These immunosuppressants exert their effects by binding to a class of intracellular proteins called immunophilins, specifically interfering with the signaling pathways leading to cytokine production or proliferation of T lymphocytes upon activation (2). CsA binds to an immunophilin called cyclophilin A, while FK-506 and rapamycin bind to FKBP (2). Both cyclophilin and FKBP are peptidyl-prolyl *cis-trans* isomerases, the enzymatic activity of which is inhibited by the immunosuppressants. However, inhibition of the peptidyl-prolyl *cis-trans* isomerase does not account for the demonstrated immunosuppressive activity (3). The complex of cyclophilin A and cyclosporin A, as well as the complex of FK506 and FKBP, binds to and inactivates calcineurin, an intracellular calcium/calmodulin-activated protein phosphatase (2); while the complex of rapamycin and FKBP exerts its effect, at least in part, on the p70 S6 kinase pathway (4). Studies of the mechanisms by which these immunosuppressive drugs act have not only demonstrated how they function in cells but also have proved that they are useful tools to dissect cell signaling pathways (5).

Unlike cyclosporin A and FK506, which suppress a calcium-dependent pathway in the early stages of T-cell activation, rapamycin does not alter the early events following the activation of T cells through the T-cell antigen receptor, although it binds to and competes with FK506 for the same protein, FKBP (6). Instead, rapamycin inhibits signal transduction from the IL-2, epidermal growth factor, and other cytokine receptors, thus blocking the G_1 to S phase transition required for cell cycle progression (6). In addition, rapamycin also inhibits the proliferation of 3T3 cells (7) and the hepatoma cell line H4 (8), presumably by blocking the effects of the growth factor-like activity of serum. The signal transduction pathway inhibited by the rapamycin-FKBP complex is not completely understood. Regardless of the mechanism, rapamycin blocks the activation of p70 S6 kinase by diverse agents. It has also been shown that rapamycin blocks the activation of p34cdc2 kinase in T cells and in the myogenic cell line BC3H1 (9), presumably because p34cdc2 is a downstream target of p70 S6 kinase. This, combined with the evidence that p34cdc2 deregulation is an obligatory component of the induction of apoptosis by natural killers in target cells (10), suggests that rapamycin could potentially alter the induction of apoptosis.

Apoptosis, a physiologically programmed event, is an active suicide process requiring energy-dependent participation of the dying cells (11). Apoptosis can be induced in T cells by activation, or in growth factor- and hormone-dependent cells by deprivation of the dependent factors, or in malignant cells by chemotherapeutic agents. Cyclosporin A and FK-506 have been shown to block activation-induced apoptosis in T-cell lines (12), while rapamycin does not (13). Herein, we report that rapamycin augments apoptosis in a number of different systems. This effect can be demonstrated in the murine T-cell line CTLL-2 induced by IL-2 withdrawal, in T-cell hybridomas induced by activation through the T-cell antigen receptor, in murine S49 cells treated with steroids, and in *myc*-transformed RAT-1 fibroblasts induced by culturing under low serum conditions. Strikingly, rapamycin also promotes apoptosis in the human promyelocytic line HL-60 cells and the human ovarian cancer SKOV3 cells induced by the chemotherapeutic drug, cisplatin, indicating potential clinical applications.

MATERIALS AND METHODS

Reagents. MTT and dexamethasone were purchased from Sigma Chemical Co. (St. Louis, MO). Rapamycin was provided by the National Cancer Institute, NIH (Bethesda, MD). Recombinant human IL-2 was obtained from the former Cetus Corp. (La Jolla, CA). Cisplatin was from David Bull Laboratories Pty Ltd. (Mulgrave, Victoria, Australia). FK520 was a gift of Dr. N. H. Sigal (Merck, Sharp and Dohme Research Laboratories, Rahway, NJ). Unless otherwise indicated, all other chemicals were the purest grade available and were obtained from Sigma.

Cell Lines. The T-cell hybridoma A1.1 was a gift of Dr. B. Singh (University of Western Ontario, London, Ontario, Canada; Ref. 14). They were recloned and selected for responsiveness to activation stimuli and T-cell receptor expression. A hamster anti-murine CD3 B cell hybridoma, 145–2c11 (15), was used as sources of antibodies to the T-cell receptor complex. The IL-2-dependent mouse T cell line, CTLL-2, human promyelocytic leukemia

Received 11/4/94; accepted 3/1/95.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

[1] This project was supported by grants from the Medical Research Council of Canada and the National Cancer Institute of Canada (to G. B. M.). G. B. M. is a Medical Research Council of Canada Scientist, Y. S. is a recipient of a postdoctoral fellowship of the National Cancer Institute of Canada, and L. G. R. was supported in part by an Ontario Graduate Studentship.

[2] To whom requests for reprints should be addressed, at Section of Molecular Therapeutics, M. D. Anderson Cancer Center, University of Texas, C5.001, 1515 Holcombe Boulevard, Houston, TX 77030.

[3] The abbreviations used are: CsA, cyclosporin A; FKBP, FK-506/rapamycin binding protein; IL-2, interleukin 2; MTT, 3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide; ^{125}IUDR, ^{125}I-labeled deoxyuridine.

cell line HL-60, and human ovarian cancer cell line SKOV3 were obtained from American Type Culture Collection (Rockville, MD). The mouse lymphoma, S49, was kindly provided by Dr. G. T. Williams (University of Birmingham, Birmingham, United Kingdom). Rat-1 cells constitutively expressing c-*myc* under the control of the muLV retroviral promoter have been described previously (16). All cells were cultured at 37°C in humidified atmosphere containing 5% CO_2 in RPMI 1640 (GIBCO Laboratories, Grand Island, NY) supplemented with 2 mM L-glutamine, 10 mM HEPES, 50 μM 2-mercaptoethanol, 5–10% heat-inactivated fetal bovine serum (Sigma), and 10 μM gentamicin (GIBCO).

Apoptosis Induction. The T-cell hybridoma A1.1 was activated by anti-CD3 coated on tissue culture plastic by incubating with 0.05 M Tris-HCl (pH 9.0) overnight at 4°C or for 1 h at 37°C. Plates were washed with PBS to remove unbound antibody before A1.1 cells were added. Cells were harvested for DNA fragmentation analysis after a 12-h incubation at 37°C in humidified 5% CO_2. Apoptosis in CTLL-2 cells was induced by IL-2 starvation, in S49 cells by dexamethasone treatment, in Rat-1-*myc* cells by low serum, and in HL-60 cells and SKOV3 cells by cisplatin treatment as indicated.

Genomic DNA Fragmentation Assay. Cells ($4-6 \times 10^5$) were harvested and resuspended in an Eppendorf tube in 30 μl PBS and lysed with 30 μl of lysis buffer [80 mM EDTA, 200 mM Tris (pH 8.0), 1.6% (w/v) sodium lauryl sarcosinate, and 5 mg proteinase K/ml]. The lysate was mixed and then incubated in a 50°C water bath for 1.5 h. After adding 0.2 mg/ml RNase A, the mixture was incubated in a 37°C water bath for an additional 30 min. The resulting DNA solution was analyzed on 1% agarose gels in TAE buffer (10 mM Tris and 1 mM EDTA).

Alternatively, genomic DNA fragmentation was quantitated by labeling actively dividing cells with ^{125}IUDR at the concentration of 10^6 cells/ml with 1 μCi/ml of ^{125}IUDR at 37°C for 8–10 h. Labeled cells were harvested and washed with cold media at least three times. Treatments were then carried out in 200 μl media in 96-well tissue culture plates; genomic DNA fragmentation was then assayed as follows. Cells were harvested in 1.5-ml Eppendorf tubes and lysed by adding 900 μl lysis buffer [5 mM Tris (pH 7.4), 2 mM EDTA, and 0.5% Triton X-100 (nonionic detergent); total volume, 1.1 ml]. The Eppendorf tubes were then vortexed vigorously to ensure complete lysis of cells. After incubating on ice for 20 min, the tubes were centrifuged at 14,000 cpm for 20 min in a microfuge. One ml of supernatant (containing fragmented DNA) was transferred to a new Eppendorf tube, leaving 100 μl supernatant with the pellet to ensure that the pellets were not cotransferred. The radioactivity of the supernatant and the pellet were measured with a gamma counter. The percentage of fragmentation was calculated by:

$$\% \text{ DNA fragmentation} = \frac{\text{Supernatant cpm} \times 1.1}{\text{Supernatant cpm} + \text{pellet cpm}} \times 100$$

MTT Staining. Cell viability was assessed essentially as described by Mosmann (17). Briefly, cells were incubated in 100 μl media in 96-well plates with additions as indicated. Following incubation, 10 μl of MTT solution (5 mg MTT/ml in H_2O) were added and incubated at 37°C for 4 h. One hundred μl of acid-isopropanol (0.04 N HCl in isopropanol) were added to each culture and mixed by pipetting or shaking on a plate shaker to dissolve the reduced MTT crystals; the relative cell viability was obtained by scanning with an ELISA reader with a 570-nm filter.

Cell Proliferation Assay. Cells were incubated in 96-well plates with appropriate treatments. The proliferative response was determined by [^{3}H]thymidine incorporation in which 1 μCi of [^{3}H]thymidine was added to each well and incubated for 3 h. Cells were then harvested onto glass-fiber filter paper, and the rate of [^{3}H]thymidine uptake was quantitated by liquid scintillation counting.

RESULTS

The immunosuppressive effect of rapamycin is not through its effect on the signals from the T-cell antigen receptor but rather through the effects on the signals induced by IL-2 produced after T-cell activation (18). We tested the effect of rapamycin on IL-2-induced proliferation of a murine T-cell line, CTLL-2, by varying the concentrations of both IL-2 and rapamycin. As indicated by [^{3}H]thymidine incorporation shown in Fig. 1, IL-2 and rapamycin showed a

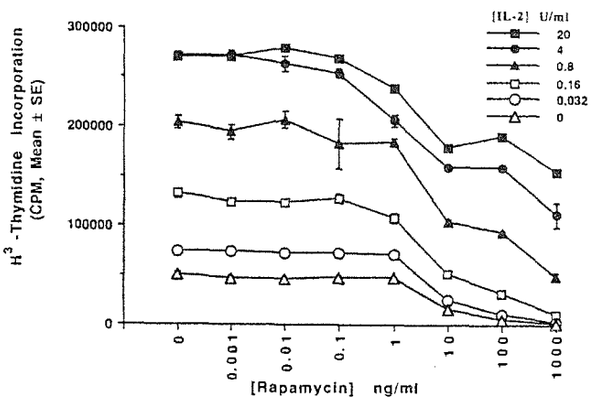


Fig. 1. Effect of rapamycin on IL-2-induced cell proliferation. CTLL-2 cells were plated in 96-well plates at 1×10^4 cells/well with different concentrations of rapamycin and recombinant human IL-2. After 20 h, 1 μCi of [^{3}H]thymidine was added to each well for 3 h. Cell proliferation was determined by measuring [^{3}H]thymidine incorporation by scintillation counting. Results represent the mean of six wells; *bars*, SE.

reciprocal effect; the higher the concentration of IL-2 in the culture medium, the higher the concentration of rapamycin required to suppress the proliferation of CTLL-2 cells to background levels. (The effect of rapamycin on IL-2-induced proliferation was analyzed by two-way ANOVA, $P < 0.0001$.) This suggests that the concentration of IL-2 determines the quantity or quality of the transmembrane signals and that rapamycin is able to completely block IL-2-induced proliferation only in the presence of relatively low concentrations of IL-2.

Since IL-2 induces proliferation in responsive cells, IL-2 must provide both mitogenic and cell survival signals upon the interaction with its receptor. It is conceivable that the effect of rapamycin on IL-2-induced proliferation of CTLL-2 cells might be due to the inhibition of cell survival signals rather than on the mitogenic signal induced by IL-2. We tested this hypothesis by using a model in which IL-2 deprivation induces apoptosis in CTLL-2 cells. As shown previously (19), 24 to 48 h after IL-2 withdrawal, the majority of CTLL-2 cells undergo apoptosis. When rapamycin was added, there was a significant increase of apoptosis in CTLL-2 cells as indicated by genomic DNA fragmentation assessed on agarose gels (Fig. 2) and by release of label from ^{125}IUDR-labeled cells (data not presented). However, this effect was only apparent when limiting concentrations of IL-2 were present. Indeed, in the presence of IL-2 (10 units/ml), rapamycin did not induce apoptosis at any concentration tested (Fig. 2 and data not shown). This is apparently discordant to Fig. 1, in which cell proliferation induced by as much as 20 units/ml of IL-2 can still be decreased by high concentrations of rapamycin. The most likely explanation for this discrepancy is that apoptosis only occurs when IL-2 signals are decreased below a critical threshold. The immunosuppressant FK-520, an analogue of FK506 which competes with rapamycin for FKBP, did not promote apoptosis, but rather reversed the effect of rapamycin when FK520 was present at a 20-fold excess (Fig. 3), indicating that the apoptosis-promoting effect of rapamycin depends on the binding of rapamycin to FKBP.

The distinction between cell survival and cell proliferation signals has been well established by genetic complementation of bcl-2 and *myc* (20, 21). The proto-oncogene c-*myc*, which plays an important role in cell proliferation and transformation, is also required for the induction of apoptosis in some cell lineages (22, 23). When cells constitutively expressing *myc* are cultured under reduced serum conditions, they undergo apoptosis (23). In this case, apoptosis can be suppressed by cell survival signals provided by the constitutive

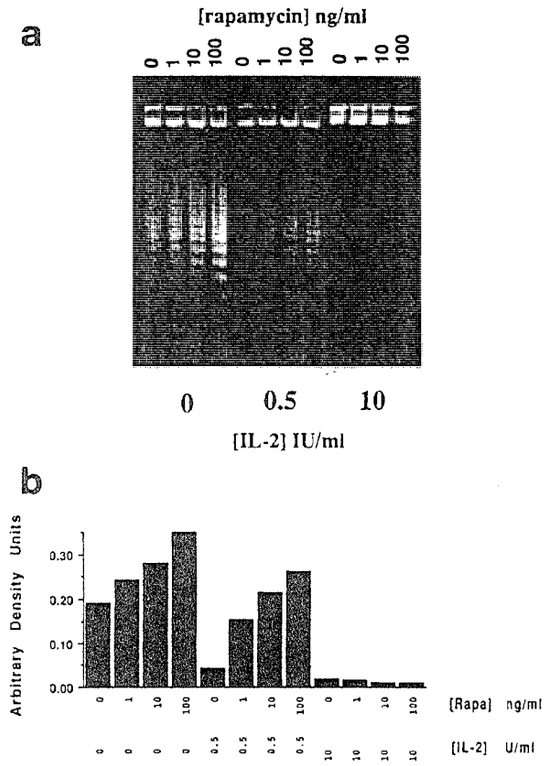


Fig. 2. Rapamycin increases IL-2 deprivation-induced genomic DNA fragmentation. CTLL-2 cells (1×10^6) were cultured without or with IL-2 at 0.5 or 10 units/ml, and rapamycin at 0, 1, 10, or 100 ng/ml. After 20 h, cells were harvested and lysed, and genomic DNA was extracted and analyzed by agarose gel electrophoresis (*a*). Densitometric analysis of the fragmented DNA of the second bands in the agarose gel is also presented (*b*).

expression of *bcl-2* or the presence of high concentrations of serum (20, 23). In accordance with previous studies, when Rat-1 cells constitutively expressing c-*myc* were cultured in media containing 0.5% serum, there was a dramatic decrease in cell viability as indicated by MTT reduction. This decrease in cell viability under low serum conditions was augmented by the addition of 10 ng/ml rapamycin (Fig. 4). Thus, rapamycin increases programmed cell death induced by the constitutive expression of c-*myc*, likely by interfering with cell survival signals mediated by the low concentration of serum present in the assays (one-way ANOVA, $P < 0.001$). As with CTLL-2 cells incubated with high concentrations of IL-2 (Figs. 1 and 2), rapamycin exerted little effect on apoptosis in RAT-1 cells in the presence of high concentrations of serum ($P < 0.05$).

Activation-induced apoptosis in T-cell hybridoma cells has been used as a model system to explore the mechanism regulating negative selection during T-cell development in the thymus (24). Cyclosporin A and FK-506 can completely block activation-induced apoptosis in T-cell hybridoma A1.1 cells (12, 13) and other cells (data not shown). However, when the effect of rapamycin was tested, it was found that, unlike CsA or FK506, rapamycin does not block activation-induced apoptosis (13). We sought to determine whether rapamycin would promote apoptosis in this system following activation of A1.1 cells with varying concentrations of anti-CD3. At an optimal dose of anti-CD3 (2 μg/ml), rapamycin did not alter activation-induced apoptosis in A1.1 cells, likely because the majority of the cells were committed to undergo apoptosis by anti-CD3 alone (25). However, at lower doses of anti-CD3 (0.5 μg/ml or lower), rapamycin dramatically promoted anti-CD3-induced apoptosis in A1.1 cells as indicated by genomic DNA fragmentation detected by agarose gel electrophoresis (Fig. 5, *a* and *b*) and by the release of ^{125}IUDR from labeled cells (Fig. 5*c*). Rapamycin alone did not induce apoptosis in the T-cell hybridoma cells (Fig. 5).

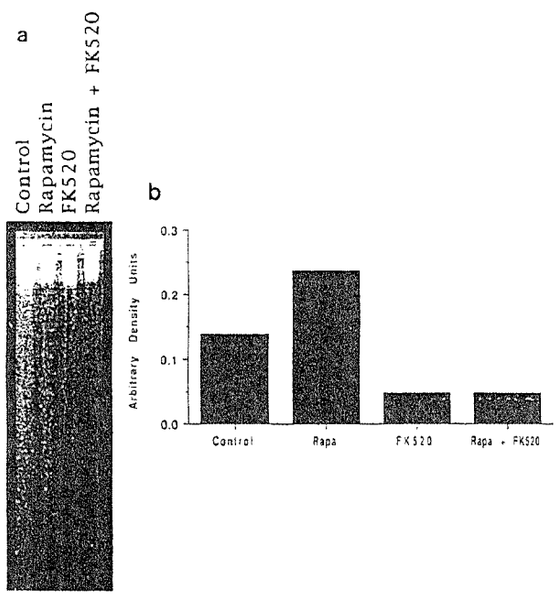


Fig. 3. FK520 reverses the effect of rapamycin on genomic DNA fragmentation induced by IL-2 deprivation. CTLL-2 cells (1×10^6) were cultured with or without rapamycin at 1 ng/ml in the presence or absence of FK520 at 20 ng/ml. Cells were harvested and assessed for genomic DNA fragmentation on an agarose gel following a 20-h incubation (*a*). Densitometric analysis of the second bands in the gel is presented in *b*.

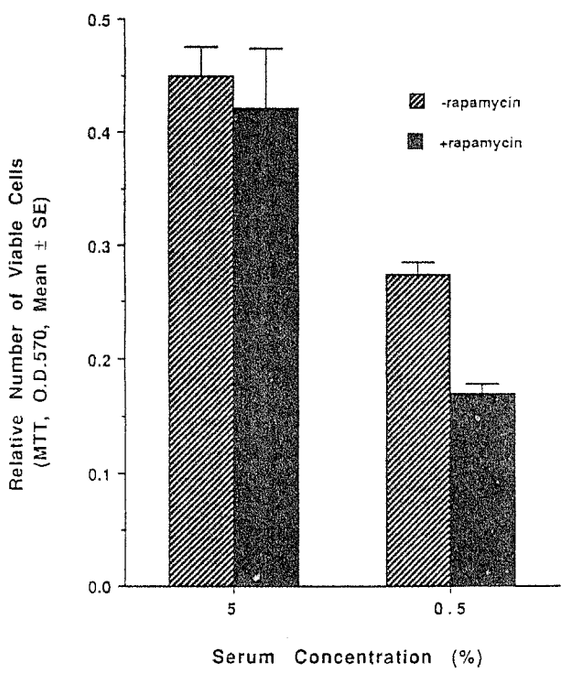


Fig. 4. Rapamycin enhances programmed cell death in *myc*-transformed fibroblasts induced by serum deprivation. *myc*-transformed Rat-1 cells were cultured in RPMI supplemented with 5 or 0.5% fetal bovine serum for 60 h with or without rapamycin at 10 ng/ml. The relative number of viable cells was determined by the reduction of MTT.

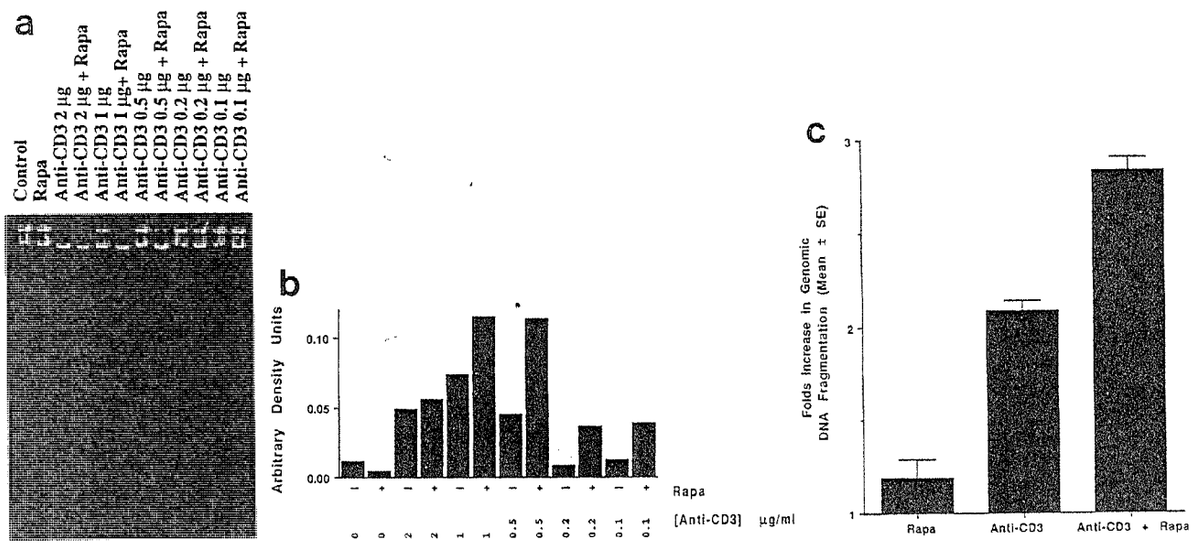


Fig. 5. Rapamycin augments activation-induced genomic DNA fragmentation in mouse T-cell hybridoma cells. T-cell hybridoma A1.1 cells were stimulated with different concentrations of anti-CD3 (145-2c11) coated on tissue culture plastic with or without rapamycin at 10 ng/ml. Cells were harvested 12 h after culture and assessed for genomic DNA fragmentation by agarose gel electrophoresis (*a*). Densitometric analysis of the fragmented genomic DNA is presented in *b*. A1.1 cells were also labeled with ^{125}IUDR at 1 μCi/1 × 10^6 cells/ml. After 10 h, cells were washed and cultured with 0.5 μg/ml anti-CD3 for 12 h. The percentage of genomic DNA fragmentation was assessed by the release of ^{125}IUDR-labeled fragmented DNA (*c*); *bars*, SE.

Corticosteroid-induced apoptosis in T cells and thymocytes is the best characterized model system for studying programmed cell death (26). The corticosteroid-sensitive mouse lymphoma cell line S49 undergoes characteristic apoptosis upon treatment with dexamethasone. When ^{125}IUDR-labeled S49 cells were incubated with a suboptimal concentration of dexamethasone (10^{-7} M), rapamycin significantly increased genomic DNA fragmentation (Fig. 6; one-way ANOVA; dexamethasone *versus* dexamethasone plus rapamycin; $P < 0.001$).

It has been demonstrated that chemotherapeutic reagents can induce apoptosis in target cells (27). Rapamycin has already been tested clinically as an immunosuppressant in patients and is relatively nontoxic in short-term administration (28). If rapamycin can augment apoptosis induced by chemotherapeutic reagents, then the addition of rapamycin to chemotherapy protocols could potentially increase the efficacy of chemotherapy. Cisplatin, an effective chemotherapy agent, has been shown to induce apoptosis in a number of cell lineages (29). A 24-h incubation with cisplatin at 30 μM or more induced DNA fragmentation in the human promyelocytic leukemia cell line HL-60, and as predicted, rapamycin augmented cisplatin-induced DNA fragmentation (Fig. 7*a*). The densitometric analysis of the gel is presented in Fig. 7*b*. However, as before, rapamycin did not augment the effect of cisplatin at doses which already induced maximal apoptosis (*i.e.*, 100 μM cisplatin). When HL-60 cells were incubated with cisplatin for 96 h, there was a dose-dependent reduction of cell viability, as determined by MTT reduction, which was detectable at 1 μM and reached maximal effects at 5 to 10 μM (Fig. 7*c*; $R^2 = 0.995$ from 0.625 μM to 5 μM; note: the different concentrations of cisplatin in the experiments in Fig. 7, *a* and *b*, reflect the different periods of incubation). We selected 2.5 μM cisplatin as a suboptimal dose and determined the effect of rapamycin. In this system, rapamycin induced a dose-dependent augmentation of cisplatin-induced reduction of cell viability (Fig. 7*d*; two-way ANOVA, $P < 0.001$ and $R^2 = 0.982$ for rapamycin from 0.01 nM to 10 nM). In contrast, rapamycin alone did not significantly alter HL-60 viability at several concentrations tested (Fig. 7). The effect of rapamycin on cell death induced by suboptimal doses of cisplatin was also readily observed by changes in morphology at the microscopic level (Fig. 7*e*). Cisplatin at 2.5 μM induced morphological changes consistent with apoptosis in only a small proportion of cells; rapamycin alone had no effect. However, when cells were coincu-

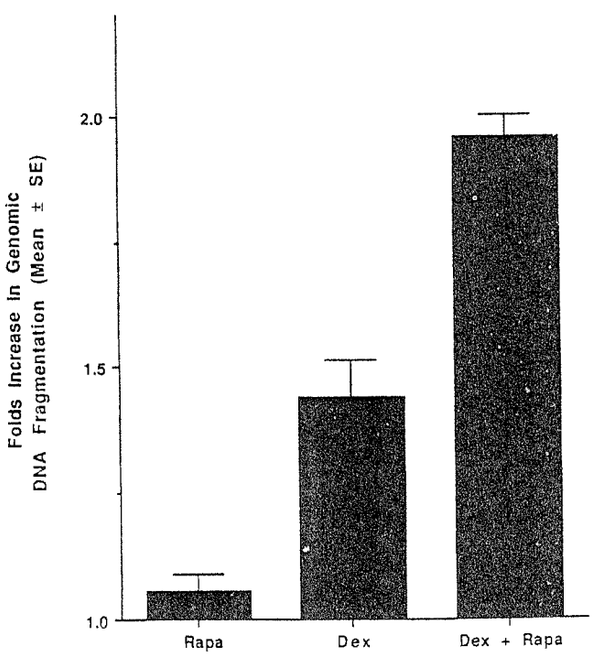


Fig. 6. Rapamycin increases genomic DNA fragmentation in steroid-sensitive cells. S49 cells were labeled with ^{125}IUDR at 1 μCi/1 × 10^6 cells/ml. After 10 h, cells were washed and cultured with dexamethasone at 10^{-7} M for 36 h. Percentage of genomic DNA fragmentation was assessed by the release of ^{125}IUDR-labeled fragmented DNA. *Bars*, SE.

Fig. 7. Rapamycin augments cisplatin-induced apoptosis in human cancer cells. *a*, human promyelocytic leukemia HL-60 cells were incubated with different concentrations of cisplatin with or without rapamycin at 10 ng/ml for 24 h. Genomic DNA was extracted and analyzed by agarose gel electrophoresis. *b*, the density of the second bands of the fragmented genomic DNA in *a* is analyzed by densitometry. *c*, dose-response of the effect of cisplatin on HL-60 viability. HL-60 cells were cultured with or without 2.5 μM cisplatin in the presence or absence of different concentrations of rapamycin. Cell viability was assessed at 72 h by reduction of MTT; *bars*, SE. *d*, rapamycin augments cell viability reduction induced by cisplatin. HL-60 cells were cultured with different concentrations of cisplatin for 72 h, and cell viability was determined by the reduction of MTT; *bars*, SE. *e*, rapamycin enhances cisplatin-induced morphological changes. HL-60 cells were cultured with or without rapamycin at 1 ng/ml in the presence or absence of 2.5 μM cisplatin. Morphological changes were assessed 72 h after the initiation of culture. *f*, the dose-response of the effect of cisplatin on the viability of the human ovarian cancer cell line SKOV3 cells. MTT assay was carried out at 72 h after treatment; *bars*, SE. *g*, the effect of rapamycin on cisplatin-induced cell viability reduction in SKOV3 cells. Cells were treated with or without 5 μM cisplatin in the presence or absence of different concentrations of rapamycin. Cell viability was assessed by MTT staining at 72 h; *bars*, SE.

bated with 2.5 μM cisplatin and 1 ng/ml rapamycin, nearly all of the cells underwent apoptosis.

Cisplatin is currently the drug of choice for the treatment of ovarian cancer. Therefore, we examined the effect of rapamycin on the induction of cell death in a relatively cisplatin-resistant ovarian cancer cell line, SKOV3. Since ovarian cancer cells frequently do not demonstrate cisplatin-induced DNA ladders despite clearly undergoing apoptosis (30), we used cell viability as assessed by MTT reduction as a measure of apoptosis in these cells. As shown in Fig. 7*f*, cisplatin induced a dose-dependent reduction in cell viability of SKOV3 cells as determined by MTT reduction. The effect of cisplatin was detectable at 3 μM and reached maximal effects at 25 to 50 μM (R^2 = 0.967 from 1.56 μM to 25 μM). We selected 5 μM cisplatin as a suboptimal dose and studied the effect of rapamycin on cisplatin-induced cell death. We found that rapamycin induced a concentration-dependent enhancement of cisplatin-induced cell death (Fig. 7*g*; two-way ANOVA, $P < 0.01$ and $R^2 = 0.954$ for rapamycin from 0.1 nM to 100 nM). Thus, rapamycin could also enhance cell death induced by cisplatin in ovarian cancer cells.

DISCUSSION

Unlike cyclosporin and FK506, which inhibit activation-induced apoptosis in T cells (12, 13), rapamycin enhances the induction of apoptosis in a number of cell lineages mediated by a number of different mechanisms. Many cancer chemotherapeutic agents have been shown to exert their effects by inducing apoptosis in cancer cells. Our observation that low doses of rapamycin increase apoptosis induced by suboptimal doses of cisplatin, at least *in vitro*, in the human promyelocytic leukemia cell line HL-60 and the human ovarian cancer cell line SKOV3 suggests a potential clinical application, as both drugs are currently in clinical use.

Studies of the mechanisms of malignant transformation have led to the identification of genes that regulate cell proliferation, cell viability, or both. Genes with the capacity to regulate cell viability include *bcl-2, bcl-x, bax, p53,* retinoblastoma gene, *raf, ras, abl, Fas*/APO-1, and c-*myc* (reviewed in Refs. 31 and 32). The hypothesis that cells require both proliferative and survival signals is best supported by genetic complementation experiments in which *myc* overexpression-induced cell death is suppressed by *bcl-2* expression (20, 21). Our results demonstrating that rapamycin promotes serum deprivation-induced cell death in c-*myc* transfected RAT-1 cells suggest that rapamycin promotes cell death through the inhibition of cell survival signal(s). In IL-2-dependent cell lines, IL-2 provides both survival and proliferative signals. It has been shown that p70 S6 kinase is activated in CTLL-2 cells after IL-2 stimulation, and rapamycin specifically inhibits p70 S6 kinase activation (6). Therefore, p70 S6 kinase might be important in providing survival signals. Recent reports show that one of the downstream targets of p70 S6 kinase is p34cdc2 kinase, which has been shown to be important in the regulation of apoptosis in target cells by cytotoxic killing (10).

Cisplatin is a potent antitumor agent. It is currently used in the treatment of many malignancies, including small cell lung, testicular, ovarian, head and neck, bladder, and esophageal cancers (33). Cisplatin induces the formation of DNA adducts, including cross-links between DNA and protein or inter- and intrastrand cross-links in DNA (29). However, there is no correlation between the amount of cisplatin required to induce cell death and to inhibit DNA synthesis in DNA repair-deficient and DNA repair-proficient cells. Thus, the cytotoxic effect of cisplatin is not solely due to the inhibition of DNA synthesis or DNA damage. Instead, the ability of cisplatin to decrease cell viability was inhibited by cyclohexamide and was accompanied by genomic DNA fragmentation, characteristic of cells dying by apoptosis (33). Our results demonstrating that rapamycin enhances the cytotoxic effects of cisplatin suggests that intracellular signaling molecules inhibited by rapamycin, such as p70 S6 kinase, may play a generalized role in regulating apoptosis induced by chemotherapeutic agents.

Although cisplatin has been widely used as an antitumor agent, high doses lead to severe multiorgan toxicities including kidney and bone marrow failure, intractable vomiting, peripheral neuropathy, deafness, seizures, and blindness, preventing dose intensification (34). Potentially, rapamycin could reduce the dosage and augment antitumor activity of cisplatin without increasing toxic side effects. Furthermore, the amount of rapamycin required for this effect *in vitro* is only 1 ng/ml, which is readily achieved in patients. For example, in renal transplant recipients, serum rapamycin concentrations as high as 10 ng/ml have been achieved 72 h after administration without toxicity (35). Thus, if rapamycin potentiates the effects of cisplatin or other drugs on tumor cells without increasing multiorgan toxicity of the chemotherapeutic agents, the combination of rapamycin with conventional chemotherapeutic agents may result in functional "dose intensity," perhaps increasing survival rates.

ACKNOWLEDGMENTS

We are indebted to Mary Hill, Feng Wang, and Ajay Sharma for technical support.

REFERENCES

1. Schreiber, S. L. Immunophilin-sensitive protein phosphatase action in cell signaling pathways. Cell, *70:* 365–368, 1992.
2. McKeon, F. When worlds collide: immunosuppressants meet protein phosphatases. Cell, *66:* 823–826, 1991.
3. Cyert, M. S. Immunosuppressants hit the target. Current Biol., *2:* 18–20, 1992.
4. Kuo, C. J., Chung, J., Fiorentino, D. F., Flanagan, W. M., Blenis, J., and Crabtree, G. R. Rapamycin selectively inhibits interleukin-2 activated p70 S6 kinase. Nature (Lond.), *358:* 70–73, 1992.
5. Sigal, N. H., and Dumont, F. J. Cyclosporin A, FK-506, and rapamycin: pharmacological probes of lymphocyte signal transduction. Annu. Rev. Immunol., *10:* 519–560, 1992.
6. Chung, J., Kuo, C. J., Crabtree, G. R., and Blenis, J. Rapamycin-FKBP specifically blocks growth-dependent activation of and the signaling by the 70 kd S6 protein kinases. Cell, *69:* 1227–1236, 1992.
7. Jefferies, H. B, Reinhard, C., Kozma, S. C., and Thomas, G. Rapamycin selectively represses translation of the polypyrimidine tract mRNA family. Proc. Natl. Acad. Sci. USA, *91:* 4441–4445, 1994.
8. Price, D. J., Grove, J. R., Calvo, V., Avruch, J., and Bierer, B. E. Rapamycin-induced inhibition of the 70-kilodalton S6 protein kinase. Science (Washington DC), *257:* 973–977, 1992.
9. Jayaraman, T., and Marks, A. R. Rapamycin-FKBP12 blocks proliferation, induces differentiation, and inhibits cdc2 kinase activity in a myogenic cell line. J. Biol. Chem., *268:* 25385–25388, 1993.
10. Shi, L., Nishioka, W. K., Th'ng, J., Bradbury, E. M., Litchfield, D. W., and Greenburg, A. H. Premature p34cd32 activation required for apoptosis. Science (Washington DC), *263:* 1143–1145, 1994.
11. Wyllie, A. H., Kerr, J. F. R., and Currie, A. R. Cell death: the significance of apoptosis. Int. Rev. Cytol., *68:* 251–305, 1980.
12. Shi, Y., Sahai, B. M., and Green, D. R., Cyclosporin A inhibits activation-induced cell death in T-cell hybridomas and thymocytes. Nature (Lond.), *339:* 625–626, 1989.
13. Birer, B. E., Matilla, P. S., Standaert, R. F., Herzenburg, L. A., Burakoff, S. J., Crabtree, G., and Schreiber, S. L. Two distinct signal transmission pathways in T lymphocytes are inhibited by complexes formed between an immunophilin and either FK-506 or rapamycin. Proc. Natl. Acad. Sci. USA, *87:* 9231–9235, 1990.
14. Fotedar, A., Boyer, M., Smart, W., Widtman, J., Fraga, E., and Singh. B. Fine specificity of antigen recognition by a T cell hybridoma clone specific for poly18: a synthetic polypeptide of defined sequence and conformation. J. Immunol., *135:* 3028–3033, 1985.
15. Leo, O., Foo, M., Sachs, D. H., Samelson, L. E., and Bluestone, J. A.. Identification of a monoclonal antibody specific for a murine T3 polypeptide. Proc. Natl. Acad. Sci. USA, *84:* 1374, 1987.
16. Penn, L. J. Z, Brooks, M. W., Laufer, E. M., Littlewood, T. D., Morgenstern. J. P., Evan, G. I., Lee, W. M. F., and Land, H. Domains of human c-myc protein required for autosuppression and cooperation with *ras* oncogenes are overlapping. Mol. Cell. Biol., *10:* 4961–4966, 1990.
17. Mosmann, T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J. Immunol. Methods, *65:* 55–63, 1983.

18. Dumont, F. J., Staruch, M. J., Koprak, S. L., Melino, M. R., Sigal, N. H. Distinct mechanisms of suppression of murine T cell activation by the related macrolides FK-506 and rapamycin. J. Immunol., *144:* 251–258, 1990.
19. Duke, R. C., and Cohen, J. J. IL-2 addiction: withdrawal of growth factor activates a suicide program in dependent T cells. Lymph. Res., *5:* 289- 299, 1986.
20. Bissonnette, R. P., Echeverrie, F., Mahboubi, A., and Green, D. R. Apoptotic cell death-induced by c-myc is inhibited by bcl-2. Nature (Lond.), *359:* 552–554, 1992.
21. Fanidi, A., Harrington, E. A., and Evan, G. I. Cooperative interaction between c-*myc* and *bcl-2* protooncogenes. Nature (Lond.), *359:* 554–556, 1992.
22. Shi, Y., Glynn, J. M., Guilbert, J. L., Cotter, T. G., Bissonnette, R. P., and Green, D. R. Role for c-myc in activation-induced apoptotic cell death in T cell hybridomas. Science (Washington DC), *257:* 212–214, 1992.
23. Evans, G. I., Wyllie, A. H., Guilbert, C. S., Littlewood, T. D., Land, H., Brooks, M., Waters, C. M., Penn, L. Z., and Hancock, D. C. Induction of apoptosis in fibroblasts by c-myc protein. Cell, *69:* 119–128, 1992.
24. Shi, Y., Bissonnette, R. P., Parfrey, N., Szalay, M. G., Kubo, R. T., and Green, D. R. *In vivo* administration of monoclonal antibodies to the CD3/T cell receptor complex induces cell death in immature thymocytes. J. Immunol., *146:* 3340–3346, 1991.
25. Shi, Y., Szalay, M. G., Paskar, L., Boyer, M., Singh, B., and Green, D. R. Activation-induced cell death in T cell hybridoma is due to apoptosis: morphological aspects and DNA fragmentation. J. Immunol., *144:* 3326–3333, 1990.
26. Wyllie, A. H. Glucocorticoid-induced thymocyte apoptosis is associated with endogenous endonuclease activation. Nature (Lond.), *284:* 555–557, 1980.
27. Hickman, J. A. Apoptosis induced by anticancer drugs. Cancer Metastasis Rev., *11:* 121–139, 1992.
28. Kahan, B. D., Chang, J. Y., and Segal, S. N. Preclinical evaluation of a new potent immunosuppressive agent, rapamycin. Transplantation (Baltimore), *52:* 185–191, 1991.
29. Chu, G. Cellular response to cisplatin. J. Biol. Chem., *269:* 787–790, 1994.
30. Ormerod, M. G., O'Neill, C. F., Robertson, D., and Harrap, K. R. Cisplatin induces apoptosis in a human ovarian carcinoma cell line without concomitant internucleosomal degradation of DNA. Exp. Cell Res., *211:* 231–237, 1994.
31. Allen, P. D., Bustin, S. A., MaCey, M. G., Johnston, D. H., Williams, N. S., and Newland, A. C. Programmed cell death (apoptosis) in immunity and haematological neoplasia. Br. J. Biochem. Sci., *50:* 135–149, 1993.
32. White, E. Death-defying acts: a meeting review on apoptosis. Genes Dev., *7:* 2277–2284, 1993.
33. Barry, M. A., Behnke, C. A., and Eastman, A. Activation of programmed cell death (apoptosis) by cisplatin, other anti-cancer drugs, toxins and hyperthermia. Biochem. Pharmacol., *40:* 2353–2362, 1990.
34. Gateley, D. P., and Howell, S. B. Cellular accumulation of the anticancer agent cisplatin: a review. Br. J. Cancer, *67:* 1171–1176, 1993.
35. Napoli, K. L., and Kahan, B. D. Sample clean-up and high-performance liquid chromatographic techniques for measurement of whole blood rapamycin concentrations. J. Chromatog. B, *654:* 111–120, 1994.

Localization of Insulin-Like Growth Factor I and Inhibition of Coronary Smooth Muscle Cell Growth by Somatostatin Analogues in Human Coronary Smooth Muscle Cells

A Potential Treatment for Restenosis?

M.B. Grant, MD; T.J. Wargovich, MD; E.A. Ellis, MS; S. Caballero, BSc; M. Mansour, MD; C.J. Pepine, MD

Abstract In this study, we demonstrate, for the first time, the localization of insulin-like growth factor I (IGF-I) in de novo and restenotic human coronary atherectomy plaques by using immunocytochemical techniques. Smooth muscle cells (SMCs) exhibiting the synthetic phenotype contained a statistically significant higher concentration of IGF-I than SMCs of the contractile phenotype or SMCs from normal coronary arteries. In addition, we provide data to suggest that the long-acting somatostatin analogues octreotide and angiopeptin inhibit IGF-I– and basic fibroblast growth factor (b-FGF)–induced human coronary artery SMC proliferation. Platelet-derived growth factor (PDGF)-stimulated cultures were minimally affected by the addition of octreotide but were significantly inhibited by angiopeptin. All three growth factors stimulated SMC migration in a dose-dependent manner. The somatostatin analogues tested had no effect on growth factor–stimulated SMC migration. Our data suggest that by reducing SMC proliferation, somatostatin analogues may have clinical usefulness in reducing the high incidence of restenosis observed after percutaneous transluminal coronary artery interventions. (*Circulation*. 1994;89:1511-1517.)

The major limitation of percutaneous transluminal coronary artery interventions remains the high restenosis rate, occurring in as many as 57% of patients.[1] Despite advances in catheter-based technology such as directional, rotational, or excimer laser atherectomy, the recurrence rate has not been reduced.[2,3] A major breakthrough in understanding the pathophysiological processes that determine restenosis was recognition that a critical step in the chain of events is injury-induced activation of vascular smooth muscle cells (SMCs), resulting in cell proliferation and migration into the subintima.[4-6] These same cellular and molecular mechanisms may be responsible for the excessive SMC proliferation observed in transplant arteriopathy.[7]

Growth factors such as platelet-derived growth factor (PDGF), basic fibroblast growth factor (b-FGF), and insulin-like growth factor (IGF-I) have been implicated in the regulation of SMC proliferation and migration because all are potent SMC mitogens in vitro and induce SMC chemotaxis.[8-10] PDGF and b-FGF have been localized in human coronary atheroma by immunocytochemical techniques. Nikol and coworkers[11] recently demonstrated expression of transforming growth factor–β_1 (TGF-β) in human coronary primary atherosclerotic or restenotic lesions by in situ hybridization, suggesting that expression for TGF-β mRNA was significantly higher in restenotic compared with de novo lesions. These observations were corroborated by Rakugi et al,[12] who demonstrated the localization of TGF-β to discrete areas of mesenchymal-appearing intimal cells adjacent to foamy macrophages. The presence of IGF-I in human coronary lesions has not been reported.

IGF-I, b-FGF, and PDGF receptors belong to an expanded family of growth factor receptors, each sharing the common feature of a tyrosine kinase domain in the cytoplasmic portion of the molecule.[13] Binding of growth factors induces autophosphorylation of the β-subunit of the receptor and activation of tyrosine kinase. Deactivation of these growth factor receptors involves specific protein tyrosine phosphatases (PTPases).[14] Somatostatin, a growth-inhibitory peptide found throughout the body, activates PTPases and can inhibit the stimulatory effects of selected growth factors. Native somatostatin, however, has limited clinical use due to its extremely short half-life and overly broad range of inhibitory activities. Somatostatin analogues with longer half-lives, such as octreotide and angiopeptin, have been shown to have direct antiproliferative effects in a wide range of cell types in vitro and in vivo, and both agents have been used therapeutically in the treatment of gastrointestinal neoplasms, pituitary tumors, and prostatic cancer.[15]

Merimee[16] demonstrated that growth hormone–deficient dwarfs with diabetes who were followed for 25 years were free of atherosclerotic disease, suggesting a role for growth hormone in initiating or propagating

Received November 29, 1993; revision accepted February 3, 1994.

From the Divisions of Endocrinology (M.B.G., E.A.E., S.C.) and Cardiology (T.J.W., M.M., C.J.P.), Department of Medicine, College of Medicine, University of Florida (Gainesville).

Reprint requests to M.B. Grant, MD, PO Box 100226, Division of Endocrinology, Gainesville, FL 32610-0226.

atherogenesis. Others have noted acceleration of coronary artery disease and its complications in acromegalic patients.[17] Studies by Lundergan and coworkers[18] suggest that the absence of growth hormone or IGF-I, the mediator of the mitogenic actions of growth hormone, may attenuate restenosis. These researchers used angiopeptin and demonstrated inhibition of myointimal proliferation in response to balloon-induced endothelial cell injury in the rat carotid artery, rabbit aorta, and iliac and coronary arteries.[18] Also, hypophysectomy has been shown to inhibit arterial neointimal plaque formation in response to endothelial cell injury.[19] Santoian and coworkers[20] recently demonstrated that angiopeptin inhibits the development of intimal hyperplasia in swine coronary arteries after balloon injury.

The purpose of these studies was twofold. First, we determined whether IGF-I was present in human coronary atherectomy specimens. Second, we sought to determine whether octreotide and angiopeptin inhibited the growth-stimulatory and migratory effects of IGF-I, PDGF, and b-FGF in cultured human coronary SMCs.

Methods

Immunocytochemical Localization of IGF-I

Human coronary atheromatous tissue was obtained from patients undergoing directional coronary atherectomy in our cardiac catheterization laboratory as treatment for symptomatic coronary artery disease. Normal coronary arteries were obtained from three individuals between the ages of 18 and 26 years who had died accidentally in motor vehicle accidents. These controls were not matched for age, sex, or cardiovascular history to the atheromatous tissue donors. Atherectomy samples were fixed within 5 minutes after removal from the individual and placed in cold 5% acrolein, 0.1 mol/L Na cacodylate–HCl buffer (pH 7.4) for 30 minutes; washed in buffer 4×15 minutes; postfixed in 1% osmium tetroxide, 0.1 mol/L Na cacodylate–HCl buffer (pH 7.4); dehydrated in an ethanol series; infiltrated; and embedded in epoxy resin. Normal coronary arteries were fixed within 6 hours after death. Gold sections on nickel grids were oxidized for 5 minutes with 1% periodic acid, washed with water, and treated with 0.05% trypsin for 30 minutes at room temperature followed by a phosphate-buffered saline (PBS) wash. Grids were treated for 15 minutes with blocker (PBS containing 0.1 mol/L NaCl, 1% bovine serum albumin [BSA], 1% cold water fish gelatin [CWFG], and 1% nonfat dry milk [NDM]) followed by a 2-hour incubation at room temperature in affinity purified, polyclonal rabbit anti-human IGF-I antibodies (kind gift of Dr Jergen Zapf) diluted 1:200 in PBS plus the same additives as contained in the blocker. After 2×5-minute washes in PBS containing the same additives as used above, grids were washed 2×5 minutes in Tris-buffered saline (TBS) (TBS containing 0.1 mol/L NaCl, 1% BSA, 1% CWFG, and 1% NDM) followed by incubation for 1 hour at room temperature in goat anti-rabbit IgG secondary antibodies labeled with either 10 nm or 15 nm colloidal gold. Grids were washed 3×5 minutes in TBS followed by 3×5-minute washes in deionized water. Grids were examined and photographed in the electron microscope at 75 kV without poststaining. Controls for nonspecific labeling consisted of reaction with primary antibodies absorbed with IGF-I and reaction with secondary antibody without exposure to primary antibody.

Semiquantitation of the localization of IGF-I was done from representative electron micrographs at a final magnification of ×25 000 by two observers blind to the identity of the type of plaque (de novo or restenotic). Colloidal gold particles were counted, and final counts were expressed per unit area. Comparisons were made between synthetic SMCs[21] (sSMCs) and contractile SMCs (cSMCs) within the same plaque.

Preparation of SMC Cultures

For preparation of human coronary SMCs, recipient hearts explanted at the time of orthotopic heart transplantation were obtained, and a segment of the coronary artery was dissected under sterile conditions. The endothelium was first removed by scraping with a rubber policeman, and then the tissue was cut into uniform 1-mm^2 pieces using a McIlwain tissue chopper (Mickle Engineering, Surrey, UK). Each piece was placed in one well of a 2% gelatin–coated 96-well plate and covered with 100 μL of Dulbecco's modified Eagle's medium (DMEM) containing 20% fetal bovine serum (FBS), 100 U/mL penicillin, 100 μg/mL streptomycin, and 0.25 μg/mL amphotericin B. Cultures were placed in a humidified incubator containing 5% CO_2 at 37°C. Fresh media with 15% FBS were added by drops every 3 days until the tissue was just covered. By day 10, SMCs were radiating from the explant. Once cultures were established, tissue was removed, and cells within wells were allowed to reach confluence. Cells were expanded to 75-cm^2 flasks for continued growth in media with 15% FBS. SMCs were verified by typical "hill-and-valley" morphology, as well as by immunocytochemical staining with α-actin antiserum.[22] Cultures between passage levels 2 through 6 were used for these studies.

Proliferation and Cell Migration Studies

Single-cell suspensions of SMCs were plated in 24-well plates (5000 cells per well) in media with 2.5% FBS and allowed to adhere for 24 hours. After we washed the wells, media containing 2.5% FBS with the appropriate amount of growth factor alone or in combination with either octreotide (kind gift of Sandoz Pharmaceuticals) or angiopeptin (kind gift of Henri Beaufour Institute) were added to quadruplicate wells. Cells were counted on days 0, 2, 4, 6, 8, and 10. Day 0 represented 24 hours after initial seeding, ie, when growth factor and/or somatostatin analogue was added. Cells were enzymatically dissociated, and the entire well contents were counted using a model Z_F Coulter Counter (Coulter Electronics, Hialeah, Fla).

For thymidine incorporation experiments, cells were plated as above; 10 nmol/L IGF-I or 10 nmol/L b-FGF, and increasing concentrations of octreotide were added to wells along with 5 μCi per well of [^{3}H]thymidine. After an 18-hour incubation, wells were aspirated and washed three times. DNA was precipitated with trichloroacetic acid (TCA), solubilized with 0.3 N NaOH, and then counted in scintillation fluid.

Chemotaxis and chemokinesis assays were performed as previously described except that the assay duration was 12 hours and the optimal pore size for the porous polyvinyl- and pyrrolidone-free polycarbonate membrane (Nucleopore, Pleasanton, Calif) was 8 μm.[23] Each migration experiment was repeated a minimum of three times.

Statistical Analysis

The mean±SEM value for each set of studies was determined, and Student's *t* test was used to determine significance.

Results

IGF-I was localized in SMCs, macrophages, and foam cells, as well as in the extracellular matrix (ECM) of all plaques examined (n=10: 7 de novo and 3 restenotic). Localization was most intense in sSMCs and often associated with endoplasmic reticulum–derived vesicles and cell processes (Fig 1A). Specificity of the immunoreactivity for IGF-I was confirmed by the lack of labeling in an adjacent section of the same plaque reacted with IGF-I antibody absorbed with recombinant IGF-I (Fig 1B). The intensity of IGF-I localization was markedly reduced in quiescent cSMCs compared with

sSMCs in the same section of a given plaque (Fig 1C and 1D), but there was localization in the surrounding ECM. Mean colloidal gold particle counts per square micrometer for sSMCs and cSMCs were as follows: plaque 1—de novo (2.17±0.43 sSMCs, 0.42±0.06 cSMCs [$P<.01$]); plaque 2—de novo (2.89±0.78 sSMCs, 0.57±0.17 cSMCs [$P<.01$]); plaque 3—de novo (1.67±0.15 sSMCs, 0.51±0.20 cSMCs [$P<.05$]); plaque 4—restenotic (5.78±1.54 sSMCs, 0.51±0.20 cSMCs [$P<.001$]); plaque 5—restenotic (2.56±0.65 sSMCs, 0.28±0.05 cSMCs [$P<.05$]); and plaque 6—restenotic (1.78±0.29 sSMCs, 0.67±0.15 cSMCs [$P<.01$]). There was no significant difference between de novo and restenotic lesions because of the limited sample size. The mean colloidal gold particle count for SMCs of normal coronary arteries (n=3) is 0.08±0.02 counts/μm^2, which is significantly less than for SMCs of either synthetic ($P<.01$) or contractile phenotype ($P<.05$). The colloidal gold particle count in sections where the antibody was preabsorbed with IGF-I before exposure to the grids and in sections in which the primary antibody was omitted was 0.06±0.02 and 0.05±0.01 counts/μm^2, respectively.

The effects of IGF-I, b-FGF, and PDGF on human coronary SMC proliferation were assessed for 10 days. IGF-I induced a dose-dependent increase in SMC proliferation in the range of 1 to 100 nmol/L (data not shown). Time course studies, performed at 10 nmol/L, demonstrated a proliferative effect of IGF-I that began to plateau by days 8 through 10 (Fig 2a, top, open bars). b-FGF–stimulated cells demonstrated a pronounced proliferative response at day 2, which plateaued at days 6 through 8 (Fig 2a, middle, open bars). In a similar fashion, 10 nmol/L PDGF stimulated SMC proliferation, reaching maximal cell density on day 10 (Fig 2a, bottom, open bars).

SMC proliferation studies performed in the presence of IGF-I and octreotide are shown in the top panel of Fig 2a (filled bars). On days 2, 4, and 6, octreotide induced a 15% to 20% inhibition of IGF-I–stimulated SMC proliferation, which achieved statistical significance ($P<.01$). Addition of octreotide to b-FGF–stimulated SMCs resulted in a 40% to 45% inhibition of cell proliferation on days 6 and 8 (Fig 2a, middle, filled bars) ($P<.01$). In contrast, PDGF-stimulated cell growth was not inhibited by octreotide (Fig 2a, bottom, filled bars).

The effect of octreotide on rapidly proliferating SMCs treated with either IGF-I (10 nmol/L) alone or b-FGF (10 nmol/L) alone was tested, and the results are shown in Fig 2b. Tritiated thymidine incorporation in the IGF-I–stimulated cells was inhibited by 12±3% at a concentration of 1 nmol/L, 30±4% at a concentration of 10 nmol/L, and 43±3% at a concentration of 100 nmol/L, clearly demonstrating that octreotide inhibited DNA synthesis in a dose-dependent manner. The b-FGF–stimulated cells responded to octreotide with a 20±4%, 38±6%, and 61±4% decrease at a concentration of 1, 10, or 100 nmol/L, respectively.

Identical studies as performed with octreotide were performed using angiopeptin at a concentration of 30 nmol/L (Fig 2c). This dose was chosen based on clinical studies that demonstrated that threefold higher concentrations of angiopeptin are required to induce the identical clinical effect as octreotide.[15]

Interestingly, although octreotide showed no inhibitory effect on PDGF-stimulated cells, angiopeptin did show a 15% reduction in SMC growth compared with cultures stimulated with PDGF alone, and this reached statistical significance ($P<.05$).

Both IGF-I and b-FGF induced human coronary artery SMC migration in a dose-dependent manner that began to plateau at approximately 125 ng/mL. The maximal response to IGF-I was 28-fold greater than in the BSA control, whereas the b-FGF response was 37-fold greater than the control response. Chemotactic activity was inhibited by the addition of either IGF-I or b-FGF antibodies. Using the concentrations of each growth factor shown to be effective in the cell proliferation assays, 25 to 100 ng, the effect of each somatostatin analogue on inhibition of SMC migration was assessed. The simultaneous addition of octreotide or angiopeptin and IGF-I or b-FGF to SMCs resulted in apparently fewer cells migrating compared with the wells containing growth factor alone; however, this did not achieve statistical significance (data not shown).

Discussion

We describe, for the first time, the ultrastructural localization of immunoreactive IGF-I in coronary atherectomy plaques. Intense localization of IGF-I occurred within the endoplasmic reticulum and cell processes of SMCs exhibiting the synthetic phenotype. SMCs exhibiting the synthetic phenotype contained a significantly greater number of gold particles than SMCs exhibiting the contractile phenotype. This observation suggests that SMCs are synthesizing and secreting IGF-I and that IGF-I protein expression is a function of the proliferative state of the SMCs within the atheromatous plaque. IGF-I also localizes in the ECM; the origin of this IGF-I could be secretion by SMCs within the plaque. However, SMCs in the adjacent vessel wall could also be releasing IGF-I into the ECM. A third source of IGF-I could be serum as IGF-I has been shown to be taken up by vascular cells.[24] Immunocytochemical localization of IGF-I also occurs in foam cells and in the ECM of the fibrous cap. In agreement with the studies of Hansson and coworkers,[25] normal vessels did not show IGF-I immunoreactivity. These data should be interpreted with caution because of the time interval between fixation and the death of the donor, as well as the inability to match for variables such as age, sex, and cardiovascular history between the control and atheromatous tissues. Hansson et al demonstrated that injury to the tissue rapidly induced extensive blood vessel formation, and these new blood vessels transiently expressed IGF-I immunoreactivity. Our results also support the results of previous studies[26] showing that SMCs can produce their own growth factors, allowing growth to be sustained in an autocrine fashion.[27]

Because IGF-I–binding proteins (IGFBPs) regulate IGF-I bioactivity, their involvement in SMC proliferation must be considered. Elgin et al[28] purified IGFBP-1 from conditioned medium of porcine aortic SMCs and demonstrated that this IGFBP potentiated IGF-I–induced DNA synthesis and cell growth in these same cells. Studies by Cohick and Gockerman[29] demonstrated that porcine SMCs secrete IGFBP-2 and IGFBP-4. Insulin and forskolin induced a 41% increase in IGFBP-2 levels by radioimmunoassay in their cell sys-

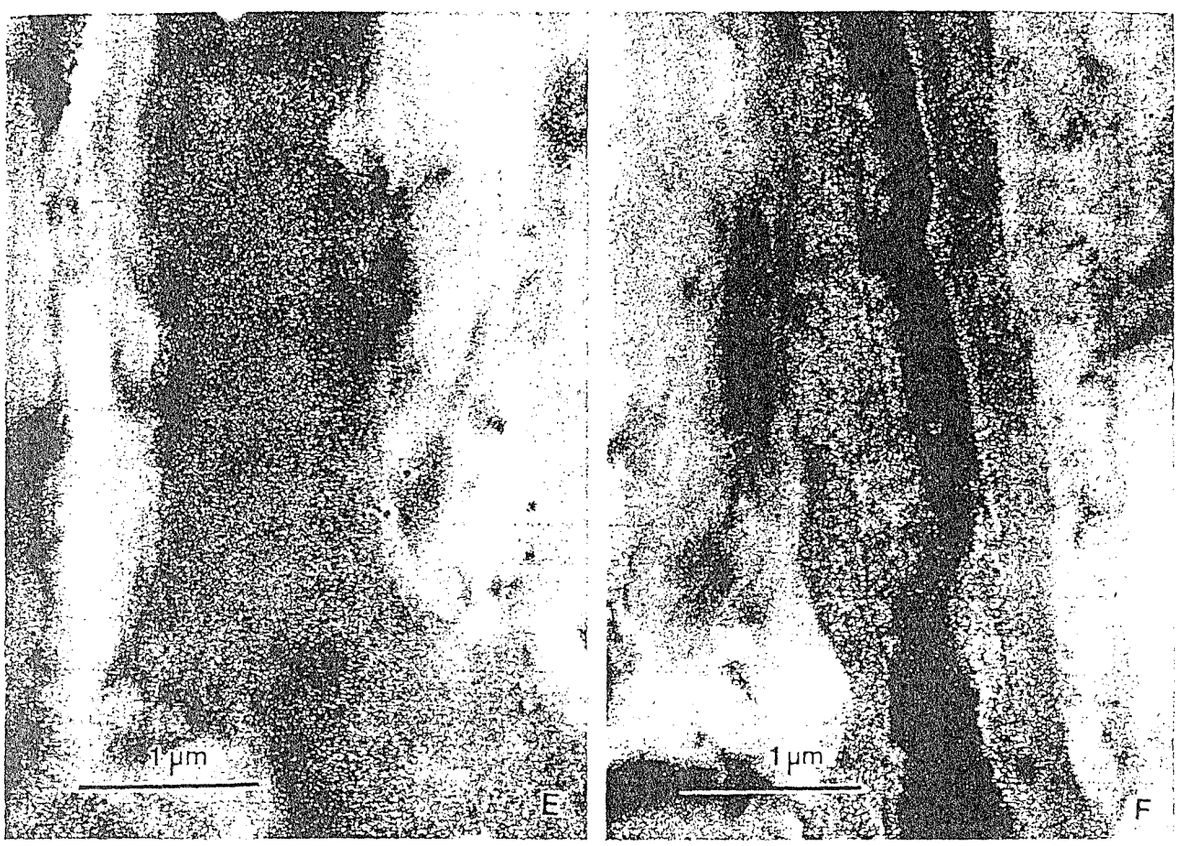


FIG 1. This and facing page. Photomicrographs. A, Localization of insulin-like growth factor (IGF-I) in synthetic smooth muscle cells (sSMC) in a restenotic atherectomy plaque. The secondary antibody is labeled with 15 nm colloidal gold. IGF-I localizes throughout the cytoplasm in endoplasmic reticulum–derived vesicles (arrowheads) and on cell processes. Original magnification, ×25 000. B, Control for specificity of IGF-I labeling. Comparable area in a section adjacent to that shown in Fig 1A that was reacted with IGF-I antibody absorbed with recombinant human IGF-I. Original magnification, ×25 000. C, Localization of IGF-I in a de novo plaque in the extracellular matrix (ECM) around contractile smooth muscle cells (cSMCs). The secondary antibody is labeled with 10 nm colloidal gold. IGF-I localizes predominantly in the ECM (arrowheads). Original magnification, ×25 000. D, Localization of IGF-I in sSMC in the same section as Fig 1D. There is increased localization in this sSMC compared with the cSMC. Original magnification, ×25 000. E, Localization of IGF-I in SMC of contractile phenotype from a coronary artery of a healthy individual. Original magnification, ×25 000. F, Control for specificity of IGF-I labeling. Comparable area in a parallel section to Fig 1E that was reacted with IGF-I antibody absorbed with recombinant human IGF-I. Original magnification, ×25 000.

tem. Insulin also increased the abundance of IGFBP-4. Exposure of SMCs to either PDGF, TGF-β, or b-FGF did not affect levels of either IGFBP-2 or IGFBP-4. Addition of IGFBP-4 to SMC cultures containing IGF-I had no effect on thymidine incorporation, whereas addition of IGFBP-4 to human fibroblasts with IGF-I resulted in near-complete inhibition of IGF-I–stimulated DNA synthesis.[29] Taken together, these studies suggest that the factors that regulate IGFBPs vary and that this differential regulation may be an important mechanism by which SMC growth is controlled.

Our immunocytochemical studies and in vitro studies provide a firm basis for using somatostatin analogues for inhibition of SMC proliferation in accelerated atherogenesis. They also establish that somatostatin analogues could be more effective in inhibiting proliferation of rapidly proliferating SMCs rather than quiescent SMCs.

Our in vitro studies have shown that IGF-I stimulates human coronary SMC proliferation in a dose- and time-dependent manner. IGF-I and PDGF act synergistically to stimulate SMC proliferation. IGF-I has been called a progression factor; ie, when quiescent cells are exposed to mitogens like PDGF, they become competent to replicate but cannot proceed through the cell cycle without a progression factor like IGF-I. Both growth factors increase the level of *c-myc* RNA in SMCs,[27] and the translated product is a DNA-binding protein associated with cellular growth that regulates the entry of cells into the S-phase of the cell cycle.

Somatostatin and its analogues octreotide and angiopeptin inhibit cellular proliferation in a wide variety of tumors. Protein tyrosine phosphorylation plays a crucial role in the cellular regulation of proliferation, differentiation, and transformation and is controlled by two sets of enzymes: protein tyrosine kinases (PTKs) and PTPases. Various PTPases have been shown to dephosphorylate the phosphorylated form of the insulin receptor in vitro, suggesting that PTPases can control signal transduction mediated by PTKs. Following IGF-I, b-FGF, or PDGF binding to their receptors, autophosphorylation of the tyrosine kinase domain occurs and activates PTK to phosphorylate exogenous proteins. Autophosphoryla-

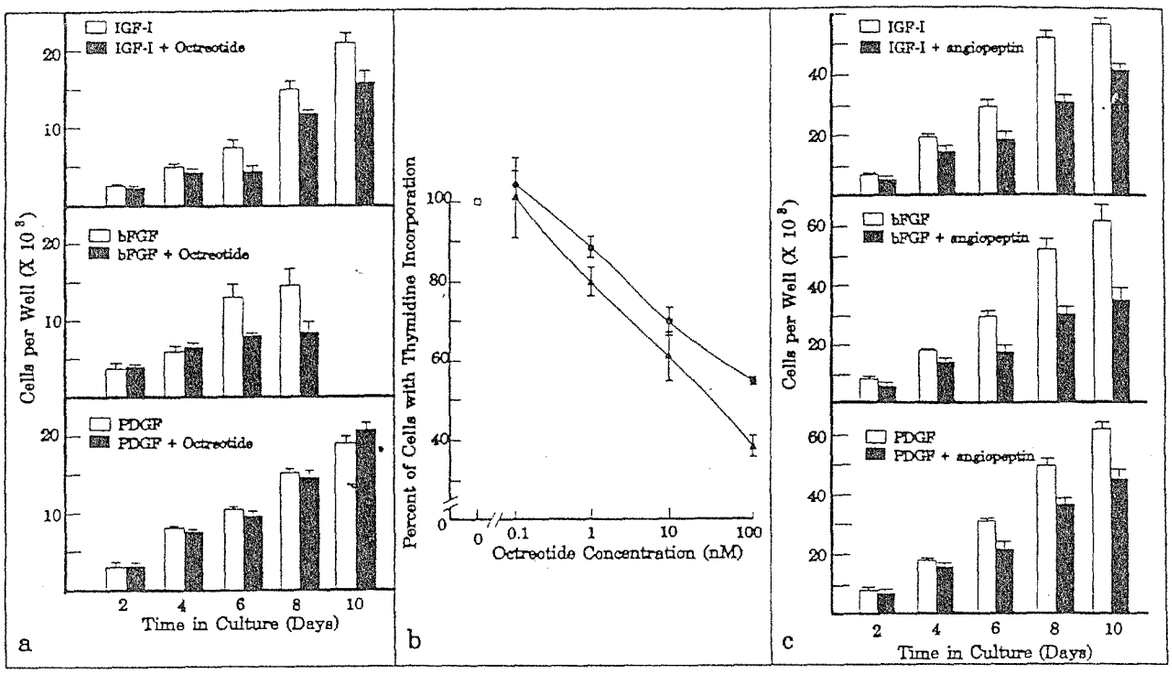


FIG 2. a, Bar graphs of growth factor–induced proliferation and octreotide inhibition of cultured human coronary smooth muscle cells (SMCs) grown for 8 to 10 days. Top, Inhibition of insulin-like growth factor (IGF-I)–induced proliferation by 10 nM octreotide (closed bars) versus IGF-I alone (open bars). Middle, Inhibition of basic fibroblast growth factor (b-FGF)–induced proliferation by 10 nM octreotide (closed bars) versus b-FGF alone (open bars). Bottom, Platelet-derived growth factor (PDGF) stimulation of proliferation (open bars) versus cells exposed to 10 nM PDGF and octreotide (closed bars). These data represent the mean±SEM cell count of quadruplicate wells. b, Plot of reduction in [^{3}H]thymidine uptake by cultured SMCs in response to growth factors and octreotide exposure. Cells were exposed to serial dilutions of octreotide along with either 10 nM IGF-I (●) or 10 nM b-FGF (▲) with 5 μCi [^{3}H]thymidine for 18 hours. Each point is the mean±SEM trichloroacetic acid precipitable counts for triplicate wells. The data are expressed as a percent of basal (100%) incorporation, ie, cells not exposed to octreotide (□). c, Bar graphs of growth factor–induced proliferation and angiopeptin inhibition of cultured human coronary SMCs. Top, Inhibition of IGF-I–induced proliferation by 30 nM angiopeptin (closed bars) versus IGF-I alone (open bars). Middle, Inhibition of b-FGF–induced proliferation by 30 nM angiopeptin (closed bars) versus b-FGF alone (open bars). Bottom, Inhibition of PDGF-induced proliferation by 30 nM angiopeptin (closed bars) versus PDGF alone (open bars). These data represent the mean±SEM cell count of quadruplicate wells.

tion renders PTK constituitively active, even when growth factor is subsequently removed from the binding site. Consequently, dephosphorylation, and not merely dissociation of the growth factor, is required to terminate PTK activity. In addition to the state of tyrosine autophosphorylation of each growth factor receptor, its degree of activation in vivo will depend on the relative activities of the PTPases involved in dephosphorylation.[30] Somatostatin analogues stimulate PTPases, which then inactivate the mitogenic potential of each of these growth factors.

In this in vitro study, we demonstrate that IGF-I, b-FGF, and PDGF stimulate DNA synthesis and cell proliferation in human coronary SMCs. The stimulating effect of IGF-I and b-FGF is blocked by the somatostatin analogue octreotide, and this inhibition occurs at a low concentration (10 nmol/L) of octreotide. The effective dose is comparable to that used in studies of other cell types. Octreotide also exerted a dose-dependent inhibition of thymidine incorporation in human coronary SMCs. Angiopeptin demonstrates a similar effect but also blocks the effect of PDGF on SMC proliferation. The reason for this is not entirely clear but may be related to differences in each analogue's ability to stimulate PTPases or differences in the PTPases that modulate these three growth factors.

Although antibodies to IGF-I and b-FGF inhibited migration induced by these growth factors, the somatostatin analogues did not. This may be due to the requirement of this assay that a single-cell suspension of SMC be prepared, as only single cells can migrate through the pores of the filters used for these studies. This necessitates aggressive trypsinization. Somatostatin receptors may be particularly sensitive to trypsin treatment, and the 12-hour duration of the migration assay may not permit adequate recovery of the somatostatin receptor. The proliferation assays, in contrast, were performed over 10 days, giving ample time for the receptors to recover or regenerate. For these reasons, the modified Boyden chamber assay may not adequately assess the effect of somatostatin analogues on in vitro SMC migration.

All three growth factors considered in this study have been shown to be produced by SMCs.[27,31-33] The in vivo mitogenic potential of these growth factors for SMCs is complex. Local cell injury, caused by interventional techniques, is required for release of b-FGF, as b-FGF is matrix bound. b-FGF can be released by plasminogen activators that are synthesized by cells several days after balloon injury.[34] Although additional IGF-I localization in restenotic lesions remains to be performed, our results in the de novo lesions support the involvement of

IGF-I in the pathogenesis of atherosclerosis. Local production of IGF-I by SMCs could facilitate SMC proliferation, thus contributing to the cellularity commonly seen in restenotic lesions.

Although extensive studies in the literature are available using aortic SMCs, studies using human coronary SMCs are extremely limited. Our results support the individual stimulatory effects of PDGF, IGF-I, and b-FGF on human coronary artery. Our data provide the basis to suggest the use of somatostatin analogues in the clinical setting to modify the high incidence of restenosis observed after coronary interventions by reducing SMC proliferation induced by IGF-I, b-FGF, and PDGF.

Acknowledgments

This work was supported in part by a grant from the American Heart Association (92GIA/862), the National Institutes of Health (EY07739-0181), and DVI Simpson Atherectomy Research Foundation.

References

1. Topol EJ, and the CAVEAT Investigators. Results of the CAVEAT trial. American College of Cardiology, Scientific Session; March 13-16, 1993; Anaheim, Calif.
2. Margolis JR, Mehta S. Excimer laser coronary angioplasty. *Am J Cardiol.* 1992;69:3F-11F.
3. Teirstein PS, Warth DC, Haq N, Jenkins NS, McCowan LC, Aubanel-Reidel P, Morris N, Ginsberg R. High speed rotational coronary atherectomy for patients with diffuse coronary artery disease. *J Am Coll Cardiol.* 1991;18:1694-1701.
4. Austin GE, Ratliff NB, Hollman J, Tabei S, Phillips DF. Intimal proliferation of smooth muscle cells as an explanation for recurrent coronary artery stenosis after percutaneous transluminal coronary angioplasty. *J Am Coll Cardiol.* 1985;6:369-375.
5. Essed CF, Brand MVD, Becker AE. Transluminal coronary angioplasty and early restenosis: fibrocellular occlusion after wall laceration. *Br Heart J.* 1983;49:393-396.
6. Ohara T, Kodama K, Mishima M, Nanto S, Hirayama A, Yutani C. Ultrastructural findings of proliferating and migrating smooth muscle cells at the site of percutaneous transluminal coronary angioplasty. *J Am Coll Cardiol.* 1988;11:131A.
7. Foegh ML. Chronic rejection-graft arteriosclerosis. *Transplant Proc.* 1990;22:119-122.
8. Raines E, Ross R. Mechanisms of plaque formation: cellular changes and possible role of growth-regulatory molecules. *Atheroscler Rev.* 1991;23:143-152.
9. Klagsbrun M, Edelman ER. Biological and biochemical properties of fibroblast growth factors: implications for the pathogenesis of atherosclerosis. *Arteriosclerosis.* 1989;9:269-278.
10. Grant MB, Caballero S, Wargovich TJ. Inhibition of smooth muscle cell proliferation by the somatostatin analogue, octreotide. *Endocrine Soc.* 1992:191A.
11. Nikol S, Isner JM, Pickering JG, Kearney M, Leclerc G, Weir L. Expression of transforming growth factor β 1 is increased in human vascular restenosis lesions. *J Clin Invest.* 1992;90:1582-1592.
12. Rakugi H, Gibbons G, Wang D, Billingham M, Vershave K, Ferguson J, Angellini P, Hogan P, Mussumy A, Buja LM, Clubb F, McAllister H, Willerson J, Dzau V. Expression of transforming growth factor beta 1 in human atherectomy specimens from primary and restenotic coronary artery lesions. *J Am Coll Cardiol.* 1992;19:329A.
13. Yarden Y, Ullrich A. Growth factor receptor tyrosine kinases. *Annu Rev Biochem.* 1988;57:443-478.
14. Cool DE, Andreassen PR, Tonks NK, Krebs EG, Fischer EH, Morgolis RL. Cytokinetic failure and asynchronous nuclear division in BHK cells overexpressing a truncated protein-tyrosine-phosphatase. *Proc Natl Acad Sci U S A.* 1992;89:5422-5426.
15. Parmar H, Bogden A, Mollard M, de Rouge B, Phillips RH, Lightman SL. Somatostatin and somatostatin analogues in oncology. *Cancer Treat Rev.* 1989;16:95-115.
16. Merimee TJ. A follow-up study of vascular diabetes in growth hormone deficient dwarfs with diabetes. *N Engl J Med.* 1978;298:1217-1222.
17. Martins JB, Kerber RE, Sherman BM, Marcus ML, Ehrhardt JC. Cardiac size and function in acromegaly. *Circulation.* 1977;56:863-869.
18. Lundergan CF, Foegh ML, Ramwell PW. Peptide inhibition of myointimal proliferation by angiopeptin, a somatostatin analogue. *J Am Coll Cardiol.* 1991;17:132B-136B.
19. Tiell ML, Stemerman MB, Spaet TH. The influence of the pituitary on arterial intimal proliferation in the rat. *Circ Res.* 1978;42:644-649.
20. Santoian EC, Schneider JE, Gravanis MB, Foegh M, Tarazona N, Cipolla GD, King SB. Angiopeptin inhibits intimal hyperplasia after angioplasty in porcine coronary arteries. *Circulation.* 1993;88:11-14.
21. Thyberg J, Hedlin U, Sjolund M, Palmberg L, Bottger BA. Regulation of differentiated properties and proliferation of smooth muscle cells. *Arteriosclerosis.* 1990;10:966-990.
22. Clowes AW, Clowes MM, Kocher O, Ropraz P, Choponnier C, Gabbian G. Arterial smooth muscle cells in vivo: relationship between actin isoform expression and mitogenesis and their modulation by heparin. *J Cell Biol.* 1988;107:1339-1345.
23. Grant MB, Khaw PT, Schultz GS, Adams JL, Shimizu RW. Effects of epidermal growth factor, fibroblast growth factor and transforming growth factor-β on corneal cell chemotaxis. *Invest Ophthalmol Vis Sci.* 1992;33:3292-3301.
24. Banskota NK, Carpentier JL, King GL. Processing and release of insulin and insulin-like growth factor I by macro- and microvascular endothelial cells. *Endocrinology.* 1986;119:1904-1913.
25. Hansson H-A, Brandsten C, Lossing C, Petruson K. Transient expression of insulin-like growth factor I immunoreactivity by vascular cells during angiogenesis. *Exp Mol Pathol.* 1989;50:125-138.
26. Libby P, Schwartz D, Brogi E, Tanaka H, Clinton K. A cascade model for restenosis: a special case of atherosclerosis progression. *Circulation.* 1992;86(suppl III):III-47-III-52.
27. Banskota NK, Taub R, Zeller K, King GL. Insulin, insulin-like growth factor I and platelet derived growth factor interact additively in the induction of the protooncogene c-myc and cellular proliferation in cultured bovine aortic smooth muscle cells. *Mol Endocrinol.* 1989;3:1183-1190.23.
28. Elgin RG, Busby WH Jr, Clemmons DR. An insulin-like growth factor (IGF) binding protein enhances the biological response to IGF-I. *Proc Natl Acad Sci U S A.* 1987;84:3254-3258.
29. Cohick WS, Gockerman A. Regulation of IGF binding protein synthesis and bioavailability in cultured vascular smooth muscle cells. *Endocrine Soc.* 1993:1484A.
30. Colas B, Cambillau C, Buscail L, Zeggari M, Esteve JP, Lautre V, Thomas F, Vaysse N, Susini C. Stimulation of a membrane tyrosine phosphatase activity by somatostatin analogues in rat pancreatic acinar cells. *Eur J Biochem.* 1992;207:1017-1024.
31. Libby P, Warner SJC, Salomon RN, Birinyi LK. Production of platelet-derived growth factor-like mitogen by smooth-muscle cells from human atheroma. *N Engl J Med.* 1988;318:1493-1496.
32. Winkles JA, Friesel R, Burgess WH, Howk R, Mehlman T, Weinstin R, Maciag T. Human vascular smooth muscle cells both express and respond to heparin-binding growth factor I (endothelial cell growth factor). *Proc Natl Acad Sci U S A.* 1987;84:7124-7128.
33. Cercek B, Fishbein MC, Forrester JS, Helfant RH, Fagin JA. Induction of insulin-like growth factor I messenger RNA in rat aorta after balloon denudation. *Circ Res.* 1990;66:1755-1760.
34. Kirschstein W, Simianer S, Dempfle CE, Keller H, Stegaru B, Rentrop P, Heene DL. Impaired fibrinolytic capacity and tissue plasminogen activator release in patients with restenosis after percutaneous transluminal coronary angioplasty (PTCA). *Thromb Haemost.* 1989;62:772-775.

Overview

Cardiovascular & Renal

Inhibition of vascular smooth muscle cell proliferation

Catherine D Demoliou-Mason

Thrombosis Research Institute, National Heart and Lung Institute, Emmanuel Kaye Building, Manresa Road, London, SW3 6LR, UK

Exp. Opin. Ther. Patents 4(7):813-829

Introduction

Recent studies and reviews on the aetiology and pathogenesis of atherosclerosis, have emphasised the need for this disease to be considered under the wider spectrum of the protective mechanisms associated with wound healing and inflammation [reviewed in ref 1]. These mechanisms involve the recruitment and interactions of different cells and the production of a large number of growth factors, cytokines and vasoregulatory molecules which function as mediators in these interactions. A critical event in the pathogenesis of atherosclerosis and in vessel wall repair, is the induction of proliferation and migration of vascular smooth muscle cells (VSMC). The changes in the major function of VSMC from that of maintaining vascular tone to that of proliferation and vascular wall repair, are associated with changes in their capacity to respond locally to chemoattractants and growth modulators. These changes are attributed to the ability of cells to adopt a different phenotype according to local demands. The phenotype state associated with repair processes has been denoted as "synthetic". This cell phenotype is characterised by the loss of VSMC contractile response and by the gain of the ability to respond to and to produce growth modulators/chemoattractants such as growth factors, cytokines (Table 1), extracellular matrix (ECM) and ECM degrading enzymes .

The molecules secreted by VSMC in the synthetic state can function in an autocrine as well as paracrine manner and, therefore, can stimulate in turn neigh-

Table 1:

Endothelial	VSMC	Macrophages	Platelets	T-Lymphocytes	Plasma
PDGF*	VEGF*	HB-EGE	PD-ECGF	IFN-γ^S	Thrombin*
bFGF*	bFGF	bFGF	EGF*	TGF-β	Factor Xa
IL-1*,^{S}	TGF-ß*	TGF-α	TGF-α	TGF-α	LDL
TGF-ß*,^{S}	IL-1	TGF-β	TGF-β	IL-1	Angiotensin
PGI_2	TNF-α	PDGF	TXA_2		
TNF-α*	M-CSF*	TNF-α	IGF-1		
IGF-1*	IGF-1	PGE			
NO	PGE	IL-1			
oxLDL*	HB-EGF	oxLDL			
	MCP-1*				
	GM-CSF*				
	collagen, elastin proteoglycans				

Cell source, growth factors (chemoattractants*, growth antagonists^S) as well as plasma components which can affect the proliferative response of VSMC in the vasculature. The list also includes growth modulators produced by VSMC in the interactions with cells involved in repair responses to injury/inflammation [1]. PDGF, platelet-derived growth factor; bFGF, basic fibroblast growth factor; IL-1, interleukin-1; TGF-ß, transforming growth factor-β; PGI_2, prostacyclin; IGF-1, insulin-like growth factor-1; NO, nitric oxide; oxLDL, oxidized low-density lipoprotein; M-CSF, macrophage-colony stimulating factor; TNF-α, tissue necrosis factor-α; PGE, prostaglandin E; HB-EGF, heparin-binding epidermal growth factor-like growth factor, MCP-1, monocyte chemotactic protein-1; GM-CSF, granulocyte-macrophage colony stimulating factor; VEGF, vascular endothelial growth factor; INF-γ , interferon-γ; TXA_2 , thromboxane A_2.

bouring SMC as well as other cells in the vasculature. Local high production of growth activators and chemoattractants due to the persistent activation of the fibroproliferative responses of VSMC and the activation of other cells (platelets, monocytes/macrophages, T lymphocytes, endothelial cells) are considered to create an imbalance in the feed back control mechanisms of wound repair/inflammatory processes and to lead to lesion progression [1-5].

Fibrous intimal SMC proliferation and recurrent narrowing of the lumen [6], are also the major contributors to the high rate of failure (30 - 50 %) of surgical interventions like angioplasty, arterial bypass grafting and entarterectomy which are used in the treatment of atherosclerosis. Similarly, occlusion at the perianastomotic site of vascular grafts due to myointimal proliferation is often responsible for vein graft failure; furthermore, VSMC proliferation may also contribute to the rejection of organ transplants [7-11]. Control of proliferation of VSMC is, therefore, believed to be the key to modifying or inhibiting lesion progression. The inhibition of VSMC proliferation has, thus, become the major target for the development of specific means in the prevention and/or treatment of restenosis and of atherosclerosis. Pharmacological approaches include the targeting of growth modulators for VSMC; the targeting of intracellular components essential for the induction of chemotaxis and of growth signals and the targeting of intracellular molecules which regulate cell growth and cell division. In this article, the current literature related to the approaches taken towards inhibition of VSMC proliferation *in vitro*, and towards myointimal hyperplasia and atherosclerosis *in vivo* , are reviewed. The sections are organised into categories according to the site of target used.

Growth Modulators and their Receptors

Platelet-derived growth factor and platelet-derived growth factor receptors

The important role of platelet-derived growth factor (PDGF) in wound healing is reflected in the mitogenic, chemotactic and angiogenic properties of this molecule. PDGF has also been identified to play an important role in embryonic development and in the development of the central and peripheral nervous system as well as in certain malignant disorders. It is a dimeric protein consisting of two disulphide linked polypeptide chains which are the products of two different genes. The type of PDGF isoform formed (i.e. PDGF-AA, PDGF-BB, PDGF-AB) is a result of a random assembly process and depends on the cell-type specific expression of the PDGF chain genes. Studies on the secretion of PDGF isoforms when expressed in CHO cells, have shown that most of PDGF-BB remains associated with the producer cell. PDGF-A chain is expressed in two differentially spliced variants and only the short A-chain variant is secreted [for reviews see Refs 12-15].

The mitogenic and chemotactic properties of the PDGF isoforms are transmitted via interactions with the transmembrane homo- and heterodimeric complexes of the PDGF α- and β-receptors. The two receptor proteins are structurally similar, and appear to have common as well as different functions. The PDGF receptor(s), like that of epidermal growth factor (EGF) receptor, is a protein tyrosine kinase [13,15]. Receptor dimerisation leads to autotransphosphorylation which induces the kinase to adopt the conformation for attachment of putative substrate molecules. Studies on the PDGF β-receptor substrates have shown that the receptor associates with most of SH2 (Scr homology region 2) containing substrates common to protein tyrosine kinase receptors [16-18]. For studies on the structure/function of the PDGF receptor, a number of PDGF receptor mutants have been constructed and characterised including those with mutations at specific autophosphorylation sites. Although the substrate specificity of each autophosphorylation site have been identified, it is not as yet known which of these substrates participates in the induction of signal transduction for PDGF-induced chemotaxis and cell division [12-15]. Recent evidence suggests that phospholipase C-γ2 is involved in the PDGF-induced signal transduction [19] and that the PDGF-induced chemotaxis is possibly the result of a balance between the activities of enzymes such as phospholipase C-γ and phosphatidylinositol-3-OH kinase which promote cell migration and of the Ras-GTPase activating protein which suppresses migration [20].

The development of PDGF antagonists and of PDGF or PDGF-receptor neutralising antibodies has attracted a lot of attention in the attempt to establish a treatment to be used *in vivo*, for the inhibition of the PDGF adverse effects in disease states of the vasculature and particularly in atherosclerosis. PDGF has also been implicated in the early response to vessel injury [21]. After sequencing and cloning of the PDGF chain genes, the production of PDGF antagonists has been primarily concerned with the chemical synthesis or the *in vivo* expression of PDGF peptide analogues derived mainly from the B-chain sequence. Such analogues, such as the *v-sis* oncogene product were shown to be expressed and secreted from transformed yeast cells [22]. The methods claimed [101] by Zymogenetics Inc include the expression of PDGF using expression vectors in which were incorporated: the PDGF B-chain homologous sequence of the *v-sis* gene, the yeast triose phosphate isomerase (TPI) promoter and terminator and the yeast mating pherormone alpha-factor

secretory signal. The construct was transformed into the yeast host cells with a TPI deletion. Growth media of selected transformants were shown to contain the *v-sis* related gene product which possesses biochemical properties and biological activities (mitogenic/chemotactic) in 3T3 mouse fibroblasts, identical to those of native human PDGF-BB. Claims for the use of this expression system, include the development of competitive PDGF agonists to be used in *in vivo* pharmacological studies of wound healing and of PDGF antagonist which are able to bind and inactivate specifically the PDGF-receptor(s) via either inhibition of receptor autophosphorylation or receptor dimerisation. The use of these peptide analogues and recombinant growth factor(s) for the purpose of raising antibodies is also claimed [201].

The crystal structure of the homodimeric BB isoform of human recombinant PDGF has been determined only recently, by X-ray analysis to 3.0 Å resolution [23]. On the basis of the data obtained, it has been proposed that each chain is able to fold into two highly twisted antiparallel pairs of ß-strands held by three intramolecular disulphide bonds. Chain dimerisation results in the formation of three surface loops clustered at each end. Combination of the results from studies with deletion and substitution mutants of the *v-sis* gene product and of results from PDGF A/B chimeras as well as the structural results have led to the proposal that the three loops may form the putative receptor recognition sites. The extended receptor binding site is proposed to include loop I (Ile25-Leu38) together with mainly non-polar residues at either end of loop III (Val78-Lys81) and possibly some residues from loop II (Cys53-Val58) of the mature dimeric molecule. Researchers from the Ludwig Inst for Cancer Research (Sweden), have developed a series of PDGF peptide derivatives which can bind to and prevent activation of the PDGF receptor by native PDGF. The peptides (monomers or dimers) are small and contain 15 or 16 amino acid residues of specific regions of PDGF [102]. A systematic study on the activity of a series of such peptides derived from the B-chain sequence have identified a chimeric peptide containing the amino acid residues 35-40 and 78-82 (ANFLVWEIVRKKP) which is able to inhibit binding to both α and β-receptors with the same efficiency suggesting that it may recognise a conserved structural element of the receptor [24]. It is of interest that loop III contains three basic residues which are conserved in both A- and B- chain of the human PDGF molecule, in agreement with the previous suggestion that basic amino acid residues do play a role in the binding of PDGF and the findings that the highly basic polypeptide protamine sulphate is a competitive inhibitor of PDGF binding [25]. Modification of the tryptophan residue of the PDGF chimeric peptide analogue [24] by a thioanisole group or with 2-nitophenylsulphenyl chloride, were found to increase its efficiency. This peptide was shown to be an antagonist able to inhibit receptor dimerisation and autophosphorylation. However, due to associated toxicity problems it requires further development before the peptide can be used for *in vivo* studies [24].

Another approach has included the use of a modified monomeric PDGF B-chain. Since this modification inhibits interdisulphide bond formation, it was hoped that it may act as an antagonist by preventing receptor dimerisation. However, the monomeric PDGF molecule was found to be able to bind to the receptor and induce both receptor dimerisation as well as receptor autophosphorylation suggesting that ligand-induced dimerisation of the receptor occurs via induction of receptor conformational changes rather than via receptor subunit bridging with the dimeric PDGF molecule [26]. PDGF chain-specific antibodies and mutagenesis were used to establish that although the PDGF molecule is bivalent, high affinity binding to the receptor can occur through a single subunit [27]. Further developments along similar lines include the Ludwig Inst for Cancer Research claim of disulphide linked dimers of two A or two B chains [102] and the proposed construction of a dimeric PDGF molecule composed of mixed wild type-mutant chains for the purpose of actively inhibiting receptor dimerisation [12]. Researchers from Dana-Faber Cancer Inst [203], are claiming the use of genetic engineering for the production of trans-dominant suppressor genes encoding mutated subunits of growth factors (i.e. PDGF, TGF-β). Mutations include those which prevent the maturation of the growth factor subunit or the formation of intersubunit disulphide bond required for activity. In the case of a dimeric growth factor such as PDGF, the mutated subunit can compete with the wild-type subunits during dimerisation to form an inactive complex. The PDGF mutants claimed by British Bio-Technology together with Pfizer [204], include the production of protease resistant PDGF isoforms with increased specific activity. The claim refers to PDGF-BB mutants which have an amino acid residue at a protease sensitive site, replaced with the corresponding amino acid residue from PDGF-A chain. Replacement of Arg^{28} with Ser or Arg^{32} with Pro are the preferred mutation. In addition, an interest has been expressed with regard to using the soluble form of the PDGF receptor(s) for the binding and inactivation of PDGF molecules secreted by various cells at the site of vascular injury. The extracellular region of PDGF β receptor expressed in CHO cells was shown to be able to block PDGF-BB- but not PDGF-AA-induced DNA synthesis in fibroblast cells. This inhibition was associated with inhibition of PDGF-BB binding (K_d = 0.4

nM) and of PDGF-receptor tyrosine kinase activity suggesting that the soluble receptor can function as a potent PDGF-BB antagonist [28].

With regard to the PDGF A-chain, specific interest has been expressed for the hydrophilic carboxyl terminus of the longer chain which consists of 18 amino acid residues (A194-211) because of alternative splicing of an exon of PDGF A-chain mRNA. The functional significance of this region has been associated with extracellular transport, nuclear transport and secretion of the PDGF AA isoform. The age dependent proliferation of rat aortic SMC was shown to be independent of differential splicing of PDGF-A chain mRNA [29]. A synthetic peptide corresponding to the A194-211 region and its tyrosinated form have been shown to have partial agonist activity as well as to inhibit the mitogenic activity of serum and of various growth factors which are not structurally related to PDGF. This peptide was also found to bind heparin and it has been proposed that the mechanism of action may involve blocking growth factor access to their corresponding receptor molecules on the cell surface by binding to extracellular glycosaminoglycans [30, 31]. Heparin has been shown to reversibly bind and inactivate the mitogenic activity of PDGF [32]. The production of recombinant PDGF-A chain monomers in *E. Coli*, together with methods to prevent dimerisation and to produce PDGF-A chain fragments are claimed by Creative Biomolecules [205]. The PDGF receptor binding site is claimed to reside between residues 80 and 110 of the C-terminus of the mature PDGF-A chain as exemplified by a series of *in vitro* studies using the polypeptide fragments to antagonise the PDGF-induced mitogenic response in human foreskin fibroblasts.

The unique biological activity of suramin has increased interest in it as an anticancer drug [33, 34], although recent studies have noted that it can cause thrombocytopaenia [35]. Farmitalia Carlo Erba is claiming the use of suramin alone or in unspecified combination for blocking TNF-α binding to cell surface receptors [36,206]. Suramin and non-anticoagulant heparin or suramin type compounds proposed by UpJohn [207], are claimed for use in directing specific compounds such as conjugated steroids to vascular (endothelial) cells. Low molecular weight compounds such as suramin [37] and neomycin [38] have been shown to interfere with PDGF membrane receptors binding in VSMC. However, the former was found to act in a non-specific manner; although neomycin was found to be specific for the PDGF α receptor, its potency was far too low for *in vivo* studies. At present, it would appear that further designing of similar compounds is required towards improving specificity and potency.

A number of antibodies to native PDGF and PDGF peptides have been raised with the purpose to study structure/function activities of the PDGF molecule and use of PDGF antibodies in immunotherapy for the treatment of malignancies, atherosclerosis and prevention of myointimal hyperplasia. Antibodies to the native PDGF have been shown to inhibit the stimulation of SSV-transformed cells via autocrine production of PDGF-BB *in vitro* [39], and to neutralise both the PDGF-induced chemotactic and mitogenic responses of VSMC [40]. In the latter case, the polyclonal antibody raised in goats against human platelet PDGF is claimed by the University of Washington to inhibit *in vivo* neointimal smooth muscle accumulation after angioplasty in athymic nude rats [208]. Administration of anti-PDGF-IgG before and for eight days after de-endothelialisation of the carotid artery in rats, reduced the area of the neointima by about 40 %. This reduction appeared to be due primarily to the inhibition of VSMC migration. Further developments in using immunotherapy for the inhibition of tumour cell growth and smooth muscle myointimal hyperplasia, have concentrated in the production of antibodies to the PDGF-β receptor for the purpose of inhibiting PDGF binding and PDGF induced mitogenic and chemotactic responses. Researchers from COR Therapeutics [209], are claiming the production of monoclonal antibodies against the PDGF-β receptor using recombinant DNA technology to make peptides to the various domains of the receptor. Monoclonal antibodies of any class which bind to the fifth immunoglobulin-like domain of the receptor and the production of recombinant versions of such antibodies using expression vector systems are also claimed. The monoclonal antibody raised (2A1E2; an IgG_1 isotype) was tested *in vitro* and shown to inhibit the mitogenic response of baboon smooth muscle cells to PDGF, via inhibition of PDGF binding (95 % inhibition at 1 nM), receptor dimerisation and receptor phosphorylation [41].

Other growth factors

As shown in Table 1, other factors can be secreted or become available in the vascular wall, at the site of injury. A survey of growth factor effects on cells of the vascular wall has been published recently [42]. A report on a VSMC autocrine migration factor [43], suggests that there are possibly more factors to be identified. VSMC in the activated "synthetic" state, can themselves produce and proliferate in response to many of these growth factors in addition to PDGF, such as the fibroblast growth factor which has been expressed in *E. Coli* [44,45]. Potential approaches claimed for the prevention of clinical restenosis, therefore, include bFGF polyclonal or monoclonal antibodies as well as the combination of growth factor antibodies (i.e. anti-

PDGF/anti-bFGF) for a more effective inhibition of both, VSMC migration and proliferation [40, 208].

Using a different therapeutic approach, Salk Inst & US Dept of Health claimed the use of growth factor receptors [210] as targets for the intracellular delivery of toxic agents. Saporin is a ribosome-inactivating protein from the plant *Saponaria officinalis* and is currently used for the construction of immunotoxins and growth factor toxins (mitotoxins). Recombinant saporin has been expressed in *E. coli*, and this system is claimed as suitable for the production of fusion proteins with saporin and ligands for cell surface receptors, e.g. bFGF, using recombinant DNA technology [46, 210]. Use of other cytotoxic agents including ricin, diptheria toxin and exotoxins are also claimed for the preparation of chimeras. The up-regulation of FGF receptors on the surface of VSMC of injured rat arteries, was used to specifically deliver the ribosome inactivator saporin in the form of a bFGF-saporin conjugate via bFGF receptor binding and internalisation. Whereas saporin alone had no effect, the bFGF-saporin conjugate was shown to be lethal for proliferating but not for quiescent SMC, and when administered *iv* to prevent (24 % inhibition) myointimal hyperplasia with no evidence of thrombosis after balloon catheterisation of rat aortas [47, 48, 210]. Recent studies have demonstrated the use of a recombinant cytotoxin specific for the EGF-receptor, to prevent VSMC outgrowth from human atherosclerotic plaques [49]. A number of other mitotoxins that have been created for various cell systems are reviewed in ref 50.

Growth factors and cytokines also have a role in inhibiting VSMC proliferation. These include transforming growth factor-β1 (TGF-β1) which has been shown to cause arrest of VSMC cells in late G1 phase [51] and to inhibit human VSMC growth and migration [52]. There is some controversy, however, with regard to the antiproliferative effect of TGF-β1 for VSMC. Activated α-macroglobulin with TGF-β1 were shown to induce a synergistic VSMC-proliferative response [53]. Adult human aortic SMC in culture, produce TGF-β1 [54] and recent evidence suggests that the VSMC antiproliferative response may be age related [55]. Five isoforms of TGF-β and TGF-β receptors have been described. In addition to cells of mesenchymal origin, TGF-β1 has been shown to exhibit an antiproliferative effect *in vitro* and *in vivo* for other cell types of epithelial, myeloid and lymphoid origin [56]. It has also been shown to have growth stimulating effects for various cells e.g. endothelial cells and epidermal keratinocytes. The mitogenic effect of TGF-β1 in human fibroblasts has been shown to involve the induction of the PDGF-receptor expression [57]. In VSMC, TGF-β1, and PDGF-AA and -BB have been shown to inhibit the production of nitric oxide [58, 59]. Genentech is claiming the administration of TGF-β isoforms prior to tissue damage for the acceleration of wound healing [211]; the administration of TGF-α in a bioadhesive mineral oil emulsion for acceleration of wound healing is also claimed by researchers from Berlix Labs [212]. In addition, Squibb is claiming a biodegradable TGF-β1 delivery system for bone healing, consisting of TGF-β1 dissolved in a degradable homopolymer or copolymer mix and of demineralised bone-matrix [213]. At present, *in vivo* data are limited to identify possibilities for therapeutic intervention in the inhibition of atherosclerosis and myointimal hyperplasia. Since atherosclerosis is a fibroproliferative disease, and since synthetic phenotype VSMC produce ECM components in response to growth factors and cytokines [1], agents which inhibit the activity of TGF-β1 in other systems may also have potential for use as therapeutics at least in preventing restenosis after angioplasty. PDGF and TGF-β1 have been shown to differentially affect the synthesis of biglycan and decorin in monkey arterial SMC [60]. Biglycan and decorin appear to play a role in the availability of active TGF-β1 and in the binding of TGF-β1 to cell surface receptors [61-65]; in a feedback mechanism ECM components appear to play a role in the negative regulation of expression of the TGF-β1 gene [66]. Whittier Inst claimed the use of anti-TGF-β antibodies, decorin, biglycan and Arg-Gly-Asp containing peptides for the reduction of fibrous scar tissue formation in brain lesions in rats [214]; antibodies to TGF-β and PDGF are also claimed by Victoria University for the treatment of other fibrotic disorders [215]. Furthermore, upon sequencing, isolation and characterisation of the TGF-β receptor(s), the production of antibodies to TGF-β receptor(s) and cDNA clones of the TGF-β receptor are claimed for use in therapeutic and diagnostic applications by General Hospital Corp [217], Whitehead Inst [218] and Salk Inst [219]. TGF-α treated with chymotrypsin or pepsin (0 - 2 °C; one week) is claimed to have cytostatic activity without the transformation activity associated with TGF-β [116]. Recently, the genes for TGF-β and those of a number of other growth factors and growth regulating molecules as well as molecules involved in coagulation/fibrinolysis have been proposed as candidates for gene therapy intended to modulate VSMC proliferation and inhibit intravascular blockages (see below).

A novel growth factor betacellulin (BTC-GF) has been isolated, recently, from the conditioned medium of a pancreatic tumour cell line initially derived from transgenic mice (RIPI-Tag2) where every beta cell expressed the oncogene SV40 large T antigen. This growth factor (Mr 32,000) was shown to have mitogenic activity for bovine aortic smooth muscle cells,

3T3 fibroblasts and retinal pigment epithelial cells but not endothelial cells [67]. Sequencing and cloning of this growth factor and its human equivalent [68], have enabled the production of recombinant non-glycosylated forms (Takeda) [220]. The recombinant non-glycosylated human BTC-GF proteins claimed by Takeda [220] have amino acid sequences between residues 1-80 and 1-147. The potential of their use in the treatment of wounds and ulcers has been claimed only from *in vitro* studies. It is also claimed that this growth factor sequence can be used to generate antibodies and as a template for the production of growth factor peptides with competitive antagonist properties for inhibition of growth factor-induced VSMC proliferation.

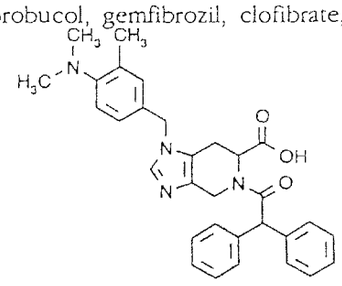
A novel angiogenic inhibitor and methods for its extraction and purification from embryonic or adult brain tissue from birds or mammals has been claimed by Max Planck [221]. Protocols for the inhibition of VSMC are provided but no biological data are disclosed. Neuropeptide Y has been shown recently to have mitogenic activity for rat VSMC [69].

Somatostatin analogues

Angiopeptin is a cyclic octapeptide analogue of somatostatin [70]. Angiopeptin together with other peptide analogues of somatostatin have been shown to be potent inhibitors of growth hormone release and of IGF-1 production and are claimed by Société d'étude as inhibitors of VSMC proliferation [222]. Of the various somatostatin-like peptides tested, angiopeptin and its congener, BIM 23034, were found to be the only peptides which were effective in inhibiting both, myointimal thickening and DNA synthesis in rat carotid arteries (injured by air-drying of the endothelium), when administered at 20-100 µg/kg/day *sc*, for 2 days prior to and for 5 days after injury. Angiopeptin was also equally effective when the pre-treatment period was reduced to 30 min. Since all somatostatin-like peptides tested inhibited growth hormone release, it has been suggested that angiopeptin and its congeners may inhibit VSMC proliferation via a local effect on the autocrine and paracrine mechanisms regulating cell growth. Angiopeptin was also shown to markedly inhibit myointima proliferation and DNA synthesis in rabbits, after balloon angioplasty and to markedly reduce intimal hyperplasia in transplanted hearts, in the rabbit heterotropic cardiac transplant model. In this model, angiopeptin also reduced lipid deposition although it was found that it did not affect the lipid profile of the animal [70-74]. The somatostatin analogue lanreotide has been shown to inhibit replication in allograft arteriosclerosis [75].

Vasoconstrictors/vasodilators and antithrombotic agents

Vascular remodelling is central to the pathophysiology of hypertension and atherosclerosis [76]. Recent evidence suggest that vasoconstrictors such as angiotensin II (AII) may function as a growth promoting substance for VSMC. Vascular injury, like that by balloon catheter, induces the expression of AII gene in the media and myointima in animal models; AII infusion after balloon catheter injury was shown to enhance VSMC proliferation and *in vitro* studies have shown that AII promotes growth of VSMC via induction of autocrine growth factor (i.e. PDGF, bFGF, TGF-β) production [77, 78]. AII has been shown to synergise with EGF in the proliferative response of porcine VSMC [79]. Consistent with the findings that the renin angiotensin system is involved in vascular growth control [80], AII antagonists have been shown to prevent myointimal thickening after carotid injury in rats [81]. Janssen Pharmaceuticals claims AII antagonists for the prevention of myointimal proliferation [82, 223]. Imidazopyridine compounds (62 in total) exemplified by the preferred compound (*S*)-1-((4-(dimethylamino)-3-methylphenyl)meth-1-yl)-5-(diphenylacetyl)-4,5,6-7tetrahydro-1-H-imidazo(4,5-*c*)-pyridine-6-carboxylic acid (**1**), are claimed to have an antiproliferative effect in the rat balloon angioplasty model. The use of angiotensin converting enzyme (ACE) inhibitors, such as cilazapril, captopril, enalapril, fosinopril and others are also claimed as a treatment against restenosis after vascular injury as well as for the treatment of hypertension by Hoffmann-La Roche [224]. Treatments claimed by Janssen Pharmaceuticals [225] and Schering [226] are primarily for their synergistic effects in lowering blood pressure, and include combinations of an ACE inhibitor with ketanserine or with novel mercaptoacylamino derivatives, respectively. For the treatment and prevention of atherosclerosis, Squibb & Sons [227] claims the combination of a squalene synthetase inhibitor such as an isoprenoid (phosphinylmethyl) phosphonate derivative, and an agent which reduces serum cholesterol or its biosynthesis via mechanisms other than those which inhibit production of HMG-CoA reductase or squalene synthetase. Such serum cholesterol reducing agents include probucol, gemfibrozil, clofibrate, dextrothy-



1

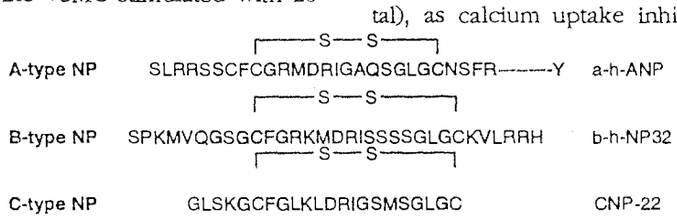
roxine, colestipol, cholestyramine, nicotinic acid, neomycin, *p*-aminosalicylic acid and aspirin. However, treatment with cilazapril [83], after percutaneous transluminal coronary angioplasty in humans, was shown recently to be ineffective in preventing restenosis [84], suggesting species variation and that current animal models used, may not adequately predict the efficacy of inhibitors of the renin-angiotensin system in man.

The atrial nitriuretic peptide (ANP) is known to be a physiological antagonist to AII and to inhibit VSMC proliferation [85]. Natriuretic peptides have been classified according to the route of biosynthesis. They are characterised by an endocyclic domain composed of 17 amino acids as the result of intramolecular -S-S-bonding, an exocyclic N-terminal domain and an exocyclic C-terminal domain. The C-type natriuretic peptides (CNP) have been isolated from brain tissue and their amino acid sequence has been shown to be highly conserved in pig, human and rat. The primary structure of peptides representative of the various types is shown in (**2**). CNP has been shown to possess pharmacological features distinct from those of the alpha-h-ANP by acting selectively with one of the two known natriuretic peptide receptor guanylyl cyclases (NPR-B). CNP has been shown to stimulate cGMP production and inhibit serum-induced DNA synthesis in cultured rat VSMC. Elevation of intracellular cGMP levels has been shown to result in the inhibition of proliferation of rabbit aorta smooth muscle cells [86]. These properties have been considered as having important implications in the use of this peptide for the development of potent CNP-analogues which can be used for the inhibition of myointimal hyperplasia [87]. A series of synthetic peptide analogues are claimed by Suntory [228] in which the C-terminal domain of alpha-h-ANP was deleted or part of its amino acid sequences were interchanged with CNP-22 or the N-terminal domain of CNP-22 was removed. When these peptides were tested for their ability to stimulate cGMP accumulation and to inhibit DNA synthesis in rat or bovine VSMC stimulated with 20 ng/ml PDGF, the results showed that there was correlation between the ability of peptides to stimulate cGMP production and inhibition of DNA synthesis. Three amino acid residues (Leu^9-Lys^{10}-Leu^{11}) in the ring portion of the peptide were shown to be particularly important since they appear to be involved in the binding and activation of the guanylyl cyclase enzyme. Derivatives exhibiting even stronger cGMP production were made by replacing Cys^6 with pentacyclomercaptopropionyl and/or Phe^7 with *p*-chlorophenylalanine in the peptide derivatives CNP(6-22) or [Leu^{10}, Lys^{11}, Leu^{12}]alpha-h-ANP(7-28). The peptides shown (**3**) were found to have the highest activity [88].

Thromboxane A_2 antagonists normally have been used as antithrombotic and anti-asthmatic agents. Recent studies, however, indicate that thromboxane A_2 may have additional roles. VSMC hypertrophy can be stimulated by Thromboxane A_2 via up-regulation and release of endogenous bFGF [89]; growth factors have been shown to down-regulate thromboxane receptors in VSMC, independent of cell growth [90]. The use of thromboxane A_2-receptor antagonists (eight in total) is claimed by Boehringer Mannheim for the treatment of VSMC proliferative responses to vessel wall injury [229]. The claim is based on the ability of these antagonists to inhibit DNA synthesis. The preferred compound (**4**) tested at concentrations between 10 nM and 1 µM, was able to inhibit DNA synthesis by 34 to 86 % in rat aorta smooth muscle.

Calcium antagonists have been reported to inhibit the proliferation of VSMC in response to pulsatile stretch and to PDGF [91]. In a recent study on their effect on VSMC proliferation in rabbit aortas, the new calcium antagonists, clentiazem, has been shown to inhibit myointimal thickening after balloon catheter injury [92].

Calcium uptake inhibitors have been reported to inhibit AII and PDGF induced VSMC proliferation [93]. Isoquinoline-5yl sulphonamide derivatives (34 in total), as calcium uptake inhibitors with vasodilatory

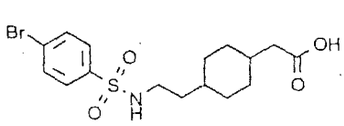


2

Peptide C pCl F G L K L D R I G A Q S G L G C N S F R Y

Peptide C pF F G L K L D R I G A Q S G L G C N S F R Y

3



4

activity for rat vascular smooth muscle, are claimed by Adair et Co as inhibitors for VSMC proliferation. The preferred compound is *N*-methyl-*N*-((1-(3-(*p*-fluorophenoxy) propyl) piperid-4-yl) methyl)(isoquinoline-5-yl)sulphonamide dihydrochloride [230].

Extracellular matrix /glycosaminoglycans

Recent experimental findings have altered our conception regarding the role of the components of ECM in cell differentiation and cell growth. The ECM has been shown to be composed of a variety of molecules secreted by cells including adhesive and anti-adhesive proteins, structural proteins and proteoglycans which carry glycosaminoglycan side chains (heparan sulphate or chondroitin sulphate). Glycosaminoglycans appear to provide a storage depot for secreted growth factors and enzyme molecules so that they are readily available locally when required as, for example, in the case of vascular injury. The distribution of glycosaminoglycans in the intima of human aortas, appears to change in atherosclerosis and in diabetes mellitus [94]. In addition, heparan sulphate and integral membrane heparan sulphate proteoglycans are considered to also have a cell-growth regulatory role by their involvement in the transduction signals generated from the interactions of matrix components, growth factors and proteinases. Regarding the interactions of VSMC with ECM components, recent studies have shown that the responsiveness of aortic SMC to soluble growth modulators is influenced by cell-matrix contact [95]. ECM components and plasma proteins which bind ECM have recently attracted the attention of academic researchers and companies for targeting therapies for restenosis and for thrombosis [96].

Thrombospondin has been shown to mediate and to potentiate the PDGF-induced migration of calf pulmonary artery SMC [97]. Vitronectin, the multifunctional plasma adhesive-glycoprotein that binds to ECM components [98], in its extended conformation (induced by urea treatment/dialysis which stabilises the molecule in its oligomeric form), is claimed by Thrombosis Res Inst as an antiproliferative agent for serum mitogen (i.e. PDGF)-induced VSMC proliferation [231]. The administration in its oligomeric form or in its native conformation (i.e. the conformation of circulating vitronectin in plasma), together with an agent which converts native vitronectin to its extended form, are claimed as methods for inhibiting human VSMC proliferation. Claims also include its use in diagnostic kits and assays for identifying individuals with high risk of atherosclerosis. Claims are based on the vitronectin antiproliferative effects on mitogen-induced DNA synthesis in human VSMC *in vitro*, and on binding data of radiolabelled vitronectin to VSMC monolayers.

Hadassah Medical Org claims polyaromatic compounds (18 in total), especially derivatives of triphenylmethane, such as aluminon and its halogenated and/or sulphonated forms as well as various dyes which are able to compete with the binding of bioactive compounds to glycosaminoglycans and thus change the tissue distribution of glycosaminoglycan-associated molecules involved in pathophysiological processes [232]. Applications of this claim include a concentration dependent increase in LPL activity in heart cells with the dye Evan's blue (139 % at 1 mM); inhibition of heparanase released products (78 %) with aluminol and inhibition of thrombin- (10^{-6} M) or bFGF- (250 ng/ml) induced DNA synthesis (>40 %) in bovine aortic SMC. Reference is also made on the anticoagulant activity of aluminol (>300 PTT (sec) *vs* 30.5 of control) and on the advantage that it can be absorbed through the gastrointestinal track for oral administration. The method claimed by researchers from La Jolla Cancer Research Fdn, for the treatment of disorders associated with the accumulation of ECM, involves the inhibition of synthesis of proteoglycans by using anti-growth factor antibodies (i.e. anti-TGF-β antibodies) and Arg-Gly-Asp-containing peptides [233]. The same group are also claiming the use of recombinant decorin and decorin peptide analogues expressed in *E. coli* as inhibitors of TGF-β binding and of TGF-β effects [66].

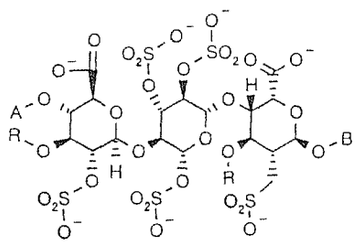
In vivo and *in vitro* studies have established that the highly sulphated glycosaminoglycan, heparin, is a potent inhibitor of VSMC proliferation [99-104,105]. The pharmacological role of unfractionated or low-molecular-weight heparins [106] in the prevention of myointimal hyperplasia after angioplasty or vascular surgery, has been recognised but its use in this context has been limited because of the heparin anticoagulant activity which can lead to haemorrhaging as well as other complications, such as electrolyte shifts and thrombocytopaenia [101]. A short course of heparin at anticoagulant dose, was found to be inadequate to prevent restenosis in human patients who had undergone angioplasty [107]; abnormal regulation of growth of VSMC by heparin has been observed in patients with restenosis [108]. Much effort has been expended, in producing non-anticoagulant heparin derivatives with enhanced potency in inhibiting VSMC proliferation [109]. Studies have shown that there is a strong interdependence between size and charge for the antiproliferative activity of heparin [110]. Heparin fragments made by periodate treatment were acylated with 2-, 4-, or 6-carbon chain lengths. At approximately equal mean molar concentration the 4- and 6-carbon acylated compounds were found to be more effective than whole heparin in inhibiting serum stimulated rat aortic SMC growth. In *in vivo* studies using the rat carotid balloon injury model, the 4-carbon acylated com-

pound was as effective as heparin in inhibiting intimal VSMC proliferation when used at the same mass dose. This derivative had no anticoagulant effect *in vivo* [111]. Acylated heparin derivatives and in particular, *O*-acylated heparin derivatives with low anticoagulant activity have been shown to have VSMC antiproliferative activity [112].

Glycomed Inc claims a method which can inactivate the anticoagulant activity of heparin without destruction of its antiproliferative activity [234,235]. This method is also claimed to have the advantage that the size distribution of heparin is maintained as in the naturally-occurring heparin/heparan sulphate preparations. The method includes first the deacetylation of commercially available heparin with hydrazine and then treatment of the heparin/heparan sulphate molecules with periodate under conditions which ensure the complete conversion of adjacent diols and alcohol-free amines to aldehydes, followed by reduction of the aldehyde moieties under conditions which minimise fragmentation. It is claimed also that the product can be used for radiolabelling or to raise antibodies. Its antiproliferative activity was measured *in vitro*, using bovine aorta SMC or *in vivo*, using the carotid rat model and endothelium denudation with a balloon catheter. Delivery using implanted EVAC discs (12 mg/disc) was more effective than *iv* administration and resulted in 18.1 % occlusion (n=19) as compared to 43.4 % occlusion of cross sectional area in control animals (n=10). The relative agglutination of platelets mediated by von Willebrand Factor fell to 50 % of control at non-anticoagulant heparin concentration of less than 0.01 mg/ml. The synthesis of highly sulphated oligosaccharides at particular positions other than the O-3 of the glycosaminoglycan moiety, using a novel blocking group strategy during retrosynthesis permitting different sulphated patterns from the same projected derivative, is also claimed by Glycomed Inc [236]. These derivatives are claimed for their decreased anticoagulant activity and increased antiproliferative activity as exemplified by the hexasaccharide:

IdoA-GlcNS-IdoA-GlcNS-IdoA-Man(2,5)
2S 6S 2S 6S 2S 6S

claimed to be most effective in inhibiting bovine aortic SMC proliferation (86 % inhibition at 15 µg/ml). The hexasaccharide has been shown to be as effective as heparin in antagonising bFGF-mediated mitogenesis (213). No biological data are given to substansiate the claim of VSMC antiproliferative activity of a number of novel heparin derivatives, disclosed by AKZO & Sanofi [237]. These novel sulphated glycosaminoglycanoid derivatives are claimed to have also high binding activity for antithrombin III and superior heparin cofactor II-mediated antithrombin III activity. The derivatives are composed of saccharide units in which the free hydroxyl or acetamido groups of the natural molecule are replaced with an alkyl or methyl group whereas the sulphate groups are preserved. Preferred compounds are those with five to eight saccharides. AKZO & Sanofi disclose carbohydrate derivatives comprising a trisaccharide unit which are claimed to have VSMC antiproliferative activity but no biological data are given [238]. The trisaccharide unit (5) corresponds to the EFG unit of the pentasaccharide DEFGH (EP-0301618) but differs in that the free hydroxyl groups of the uronic acids E and G are alkylated, arylated or ararkylated. These compounds are also claimed to have anti-Xa activity without activating thrombin. Factor X, Xa and protein S have been shown to induce DNA synthesis in VSMC [114].



R = alkyl, aryl or ararkyl

Q = alkyl, aryl, ararkyl or SO_3^-

A, B = H, alkyl, aryl, ararkyl or a carbohydrate

(A, B are selected independantly)

5

Rapamycin, a macrocyclic triene antibiotic produced by *Streptomyces hygroscopicus* has been shown to prevent proliferation of VSMC *in vitro*, in response to mitogenic and heterotrophic factors, and *in vivo*, after balloon catheterisation of the rat carotid artery [115, 116]. The administration of rapamycin and heparin simultaneously, separately or sequentially is claimed by American Home Products Corp as a method of treatment after biologically or mechanically mediated vascular injury by American Home Products [239]. The combination of 10 µg/ml heparin and 1.0 nM rapamycin inhibited DNA synthesis in rat aortic SMC by 76 %, and 50 µg/ml heparin with 10 nM rapamycin gave 96 % inhibition in support of the claim that this combination may prove to be more effective than administration of heparin alone.

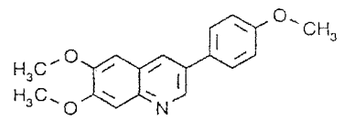
Differences in the mechanisms of glycation have been proposed to play a role in atherogenesis [117]. Beta-cyclodextrin tetradecasuphate has been shown to in-

hibit VSMC proliferation and restenosis after angioplasty in rodents [118].

Inhibitors of Growth Factor-signal Transduction

Transmembrane signal generation and transmission of growth factor-induced effects via receptor autophosphorylation and induction of receptor tyrosine kinase activity have been considered as potentially useful targets for the development of antiproliferative drugs [119]. Furthermore, overexpression of growth factor receptor (i.e. EGF) has been correlated with the loss of normal regulation of the receptor signal transduction pathway. Barret *et al.* [120] have reported an integrated system for the screening of the specificity of protein kinase inhibitors.

The class of compounds, tyrphostins, have been described as antiproliferative agents for cancer cells [121] with the potential to be used in the treatment of abnormal VSMC proliferation by inhibition of receptor tyrosine kinase activity. They have been shown to inhibit bombesin-induced tyrosine phosphorylation, c-fos expression and DNA synthesis in Swiss 3T3 cells [122], and to inhibit the cellular action of nerve growth factor [123]. Lavendustin-A has also been reported to be a potent tyrosine kinase inhibitor of the EGF receptor [124]. Erbstatin and its analogue RG 14921 have been used to study the inhibition mechanisms of the EGF-receptor kinase and cAMP dependent kinase activities [125]. The effect of erbstatin was shown to result in the inhibition of serum-induced M-phase progression [126]. Erbstatin has been reported to also inhibit angiogenesis [127]. Herbimycin, also a tyrosine kinase inhibitor, has been shown recently to inhibit the response of VSMC to PDGF [128]. Similar drugs include the development of growth factor specific (i.e. PDGF, EGF, insulin) receptor tyrosine kinase inhibitors exemplified by a series of novel (93 in total), bis mono- and/or bicyclic aryl and/or heteroaryl compounds, claimed by Rhône-Poulenc Rorer [241]. Efficacy was determined by measuring their ability to inhibit PDGF-, EGF- and insulin-receptor kinase activity as well as DNA synthesis induced in A431 cells by these growth modulators, or by measuring inhibition of PDGF-receptor autophosphorylation in a cell-free system (0.003 - 20 μM). Fourty-two compounds are specifically claimed including (**6**) which was shown to be most effective in inhibiting PDGF-receptor autophosphorylation. Although these studies indicated that these compounds do not function as general tyrosine kinase inhibitors and thus, they may be potentially useful in inhibiting VSMC proliferation, *in vivo* targeting of these compounds to VSMC has not been addressed, and their



6

in vivo antiproliferative effects and toxicity have yet to be determined.

Similar reservations apply for the use of a series of more than 260 novel quinoline-sulphonamido derivatives disclosed by Hiroyoshi Hidaka and Tobishi Yakuhin [242]. In addition to being inhibitors of platelet aggregation and possessing smooth muscle relaxation activity, these compounds are also claimed to have inhibitory activity for protein kinase A, myosin light chain kinase and protein kinase C and thus, to have the potential to be used as antiproliferative agents; no biological data for their anti-growth activity are given. However, the PDGF-induced mitogenic signals in VSMC has been shown not to be mediated through the protein kinase C and *c-fos* pathways [129]. However, protein kinase C isoforms may act at the G1/S transition phase in VSMC [130]. Inhibitors of cAMP phosphodiesterase (i.e. cilostazol) and their effect on the proliferation of rat aortic SMC has been reported [131].

Cell Growth and Division

Inhibitors of DNA synthesis and cell cycle progression

Psoralen (7*H*-furo[3,2-γ][1]benzopyran-7-one) belongs to the group of furocoumarins, occurring naturally in several plants. Psoralens are phytoalexins; they are used by plants in a defensive response to fungal and insect attacks [132]. They have also shown photosensitising and phototoxic effects and are used for the treatment of skin diseases and as photochemical probes in biological systems [133]. Ultraviolet light in the A band (UVA) in combination with psoralens (PUVA), are disclosed by Indiana University for the inhibition of proliferation of bovine aortic SMC [243]. PUVA with 8-methoxypsoralen (8-MOP) were found to be potent inhibitors of DNA synthesis with EC_{50} values in combination, ranging from 7 μM at 0.35 J/cm^2 to 0.2 μM at 2.1 J/cm^2. Similar antiproliferative effects were obtained using inverse variation of dose and energy delivery. Furthermore, a single exposure to PUVA (1μM, 1.5 J/cm^2) was found to completely inhibit cell growth without causing cell death. The inhibitory effect on serum-induced DNA synthesis, was the same with either an immediate or delayed (16h) application of PUVA as a result of inhibition of progression of cell cycle irrespective of cell phase. This antimitogenic effect was not due to changes in either the number or the affinity of PDGF or EGF binding

sites. The clinical application claimed for the treatment of myointimal hyperplasia includes the systematic administration of 8-MOP during vascular recanalisation and the subsequent UV irradiation (320-400 nm) of the region [72, 243]. The use of a lazer at a fixed wavelength for the crosslinking of 8-MOP or of another psoralen, angelicin, with DNA at the site of vascular injury, is also claimed by General Hospital Corp as a treatment for restenosis after vascular surgery [244]. The photoinhibition of SMC migration has been reported recently [135]. It does appear that use of photodynamic therapy/external beam irradiation of vessel segments with myointimal hyperplasia [137, 138], are gaining popularity in recent years as a potential therapy for restenosis.

A subclass of thiol protease inhibitors, benzyloxycarbonyl-Leu-norleucinal (calpeptin) (100 μM) and acetyl-Leu-Leu-norleucinal (TPI-1) (50 μM) the synthesis of which is claimed by Res Corp Technologies [245], were shown to reversibly block PDGF-BB- or serum-induced cell cycle progression at late G1-phase and at the transition from G2 to M phase in bovine aortic SMC. Inhibition of cell cycle progression was lost if TPI-1 was added more than 6h after serum addition. TPI-1 caused a 4-fold inhibition of the transient expression of *c-fos* and *c-myc* proto-oncogenes and a decrease in the levels of muscle and non-muscle actin. From studies in rabbits, the growth inhibitory effect of thiol protease inhibitors for VSMC [138] is claimed to have therapeutic implications in the prevention of restenosis after angioplasty [245].

For the inhibition of VSMC proliferation Medea Res is claiming the compound 3-acetoxy-thiophene-2-carboxylic acid. A process for its preparation is claimed by but no biological data are disclosed [246].

Gene therapy for the treatment of cardiovascular diseases

Gene therapy and antisense therapy are recently developed applications in biotechnology, and involve the introduction of foreign DNA into the target tissue in order to correct an inherited or acquired disorder *in vivo* through the synthesis of the desired gene product or to inhibit the expression of an unwanted gene product [139], respectively. *Ex-vivo* genetically engineered cells, e.g. endothelial cells, with recombinant genes encoding a number of heterologous proteins of therapeutic potential in the context of the treatment or prevention of cardiovascular and other diseases (i.e. soluble CD-4, Factor VIII, Factor IX, von Willebrand Factor, tissue plasminogen activator (t-PA), urokinase, hirudin, interferons, TNF, interleukins, haematopoietic growth factor, antibodies, glucocerebrosidase, adenosine deaminase, phenylalanine, hydroxylase, human growth hormone, insulin and erythropoietin) are claimed by Thomas Jefferson University for implantation in the vessel walls of mammals via vascular grafts [247]. The same institution also disclosed the direct delivery to discrete blood vessel segments via double balloon catheterisation of *ex-vivo* genetically transformed endothelial cells with genes for the above proteins as well as additional proteins such as streptokinase, aFGF, bFGF, TGF-β, TGF-α, ANP, PDGF, endothelin, diphtheria toxin, pertussis toxin and cholera toxin [248]. Porcine endothelial cells transfected with a murine amphotropic retroviral vector expressing recombinant β-galactosidase, were found to express the marker gene 2 to 4 weeks after introduction into the denuded iliofemoral arteries of mini-pigs using a double balloon catheter [140]. The *in vivo* introduction of retroviral vectors containing recombinant genes of any of all above proteins, is also claimed [248]. In a third patent, Thomas Jefferson University claims methods, kits and their composition for the application of gene therapy to specifically control the proliferation of VSMC-associated restenosis of the vessel wall [249]. The specific transformation of SMC and methodologies include the use of gene constructs coding for one or more interferon polypeptides [141] with or without coadministration of other therapeutics found effective in limiting or eliminating stenosis (i.e. anti-platelet, anti-coagulation, anti-inflammatory and vasodilation therapeutics). The claims also include use of a pharmaceutically acceptable DNA-carrier solution containing lipofectin and/or proteolytic enzymes and/or lipases and/or mild detergents, and the use of a perforated balloon catheter as methodologies for the delivery of DNA. No biological data are given for evaluation of these claims [249].

The development of a perforated balloon catheter has been based on the investigations of using microparticles as a potential fast delivery system of medication to the vessel wall in the treatment to reduce postangioplasty restenosis. Femoral arteries of atherosclerotic rabbits were injected with a 5 microns microparticle suspension for 45 sec at either 3 or 5 atm of infusion pressure, using a perforated balloon catheter immediately following angioplasty. Out of 34 excised arteries immediately or at 1, 3, 7 or 14 days after infusion, 30 contained retained microparticles with 21 exhibiting microparticles in the neointima, 12 in the media and 25 in the adventitia. Microparticles were retained as long as 14 days suggesting that biodegradable microparticles could serve as a vehicle for intramural drug delivery in the treatment of restenosis [142]. However, recent investigations suggest that using a perforated balloon catheter may not be effective for the delivery of liposome particles containing DNA to the injured site. A perforated balloon (Wolinsky) catheter [143] was used to inject (<1 min injection time), a

vector expressing the β-galactosidase marker gene into rabbit aortas. The use of this catheter was intended to reduce the much longer time period, e.g. 30 min, required with the double balloon catheter, since these long injection periods fall outside the time frame required for clinical application. However, less than 100 cells/2 cm length of aorta were found to be transduced showing that, at present, this is not an effective method for the transfer of genes to the vasculature. Use of a double balloon catheter, although more effective, remains a limitation to this approach [144].

Although the expression of a number of genes have been claimed as having the potential for gene therapy of cardiovascular diseases including myointimal hyperplasia, only the localised use of antisense oligonucleotides has been demonstrated by MIT to have a subsequent inhibitory effect on VSMC proliferation *in vivo*. This claim is based on using antisense oligonucleotides (15 to 30 nucleotides in length) specific for the mRNA transcribed from the gene of interest or on using antisense DNA to block non-encoding regulatory DNA sequences involved in gene transcription with the purpose to inhibit transcription under *in vivo* conditions in mammals [250]. Application of this strategy is exemplified by the use of antisense *c-myb* phosphorothioate oligonucleotides to inhibit the expression of the proto-oncogene which resulted in the inhibition of rat VSMC proliferation *in vitro* [145] and the prevention of myointimal hyperplasia *in vivo* [146] after balloon injury of the rat carotid artery. The advantages claimed for this method include using modified oligonucleotides for the purpose of reducing the rate of their degradation *in vivo*, and the direct application of DNA mixtures to cells at the site required to be treated. The latter includes the use of a biodegradable mixture (pluronic™ gel and ethylene vinyl acetate matrix) which is in liquid form below body temperature but reforms to a semisolid hydrogel at or near body temperatures. Oligonucleotides are claimed to be released from the gel and enter the cells in <1 h. Treatment for as little as 2 h was shown to cause maximal inhibition of cell growth over the following 72 h. Morphological examination of tissue after ten days to two weeks post angioplasty, showed that myointimal accumulation was minimal upon application of *c-myb* antisense nucleotide whereas extensive proliferation occured when the injured site was treated with sense or mismatch oligonucleotides. In addition to *c-myb* antisense inhibition [145, 146], there are several targeting studies with DNA for mRNA species which encode nuclear antigens from proliferating cells [147]. These include human arterial SMC [148], or DNA sequences encoding growth factor mRNA which will block autocrine growth factor induced cell proliferation [149]. Antisense oligonucleotides modified with phosphothionate or alkylphosphonate groups against the human *c-kit* proto-oncogene have also been claimed by Temple University, though for the inhibition of proliferative disorders related to erythroid cell lineages [151].

Conclusions

The present review has concentrated on some of the most recent developments in approaches regarding mainly the direct inhibition of VSMC proliferation, considered to be the key to inhibiting vascular lesion growth. However VSMC interact with a number of other cells in the vasculature, and contributions of additional cells and factors participating in wound healing and in inflammatory responses, have attracted equal attention as areas for the development of therapeutics and methods for the treatment of atherosclerosis and of restenosis. For example, lipoprotein (a) has been shown to promote proliferation of human VSMC [150]; high plasma concentrations of low density lipoprotein-associated cholesterol is considered to be an important causative factor in the pathogenesis of atherosclerosis and to contribute to restenosis [151, 152], whereas high density lipoprotein has been shown to inhibit EGF-induced DNA synthesis in VSMC [153]. Janssen [225] and Nissan Chem Ind [252] have claimed the uses of lipid lowering agents which may directly affect VSMC proliferation and of lipid antioxidants, although primarily in the context of secondary prevention [154]; clinical studies using such drugs are currently in progress. From studies in transgenic mice, lipoprotein (a), apoA-I, the microsomal triglyceride transfer protein and their genes have been proposed as primary targets for the pharmaceutical industry [155] and further developments in these areas can be anticipated. Abnormalities of endothelium functions associated with hypertension and the L-argenine: nitric oxide pathway [156 -159] and inhibition of Na^+-H^+ exchange in VSMC [160], as well as the platelet system and metalloproteinases [161] have also been considered as important targets for the prevention and treatment of atherosclerosis and of myointimal hyperplasia. Furthermore, recent findings on the mitogenic activity of thrombin for human VSMC [162] and its involvement in inflammation, cancer and neurodegenerative diseases have extended the development of novel thrombin inhibitors and their applications [163]. In addition, the recent identification of thrombin receptors in various cells including VSMC, has opened up a new area for the future synthesis of alternative type of thrombin inhibitors to regulate thrombin functions other than those involved in haemostasis [163]. Antitumour drugs, as demonstrated by the examples

reviewed here, and their potential for preventing myointimal hyperplasia is also another area with an interesting future [164].

It should be noted, however, that the pharmacological approaches of the 1980s using anticoagulants including heparin or antiplatelet agents, Ca^{2+}-channel blockers and corticosteroids proved to be ineffective in preventing restenosis in man. In view of the recent findings regarding differences in the response to angiotensin converting enzyme inhibitors between animals and humans, there may be a need for reassessment of animal models currently used.

Validation in man will also be required regarding the claims reviewed here for the direct inhibition of VSMC-fibroproliferative responses. Use of growth factors or growth factor-receptor peptide antagonists and their antibodies as antiproliferative agents may carry the risk of autoimmune responses which may limit their application. Cell Genesys [253], is claiming a method for the production of humanised antibodies in transgenic mice as a means to prevent the immunogenic response to murine antibodies when used to treat humans. Targeting of VSMC also remains of paramount importance in all approaches. Intravenous or local delivery of the anti-growth agent at the site of vascular injury are more likely to have first application in the treatment of man. In addition to delivery systems, like those used for TGF-β (Berlix) [212], (Bristol-Myers Squibb) [213], a lyophilised protein/peptide formulation using cellulose derivatives is claimed by Genentech [254]. Otsuka Pharmaceutical [255], is claiming a delivery system comprised of proteins attached to liposomes.

In the case of gene therapy, in addition to problems of targeting VSMC, this approach is still associated with health risks from the use of viral vectors and with problems of high expression levels of the desired product and stability of the foreign DNA. The rapid progress made in biotechnology, may soon lead to the development of new mammalian expression vectors carrying strong cell-specific promoters, and to the development of cell-targeted DNA delivery systems with higher uptake efficiency and higher stability. The construction of carrier cells capable of delivery of genetic material to a target tissue is claimed by researchers at Ohio University [256]. Cell Genesys [257], is claiming the technique for the inactivation of the major histocompatibility complex (MHC) genes in *ex-vivo* modified cells for use in gene therapy. They also claim targeting of DNA sequence to the specific position in the genome which has homology with the introduced gene, resulting in replacement of the mutated copy with good copy at the exactly correct position [258]. Both, gene therapy and antisense therapy will also require validation as a treatment for cardiovascular diseases and for the prevention of restenosis in man. It can be anticipated that further developments regarding methods of administration will be needed as well as further research for the identification of key gene(s) involved in the regulation of the fibroproliferative response of VSMC. Considering that atherogenesis and the restenosis process are multifactorial as a result of contributions not only from VSMC but from other cells in the vasculature, and of a large number of yet unknown biochemical events, the use of gene therapy in the wider context of treating or preventing atherosclerosis in humans, may not prove to be an easy task.

References to primary literature

References of interest are highlighted as:
- • of interest
- •• of considerable interest

1. ROSS R: *Nature* (1993) 362:801
•
A review article giving a perspective of the pathogenesis of atherosclerosis for the 1990s discussing cells and growth factors, cytokines and vasoregulatory molecules involved.

2. PICKERING JG, WEIR L, ROSENFIELD K, STETZ J, JEKANOWSKI J, ISNER JM: *J. Am. Coll. Cardiol.* (1992) 20:1430
•
An article on smooth muscle cell outgrowth from human atherosclerotic plaque.

3. JACKSON CL, SCHWARTZ SM: *Hypertension* (1992) 20:713
•
A review on the pharmacology of smooth muscle cell replication.

4. REIDY MA: *Arch. Pathol. Lab. Med.* (1992) **116**:1276
•
A review on factors controlling smooth muscle cell proliferation.

5. BABAEV VR, ANTONOV AS, DOMOGATSKY SP, KAZANTSEVA IA: *Atherosclerosis* (1992) 96:189

6. CHAN P, MUNRO E, PATEL M, BETTERIDGE L, SCHACHTER M, SEVER P, WOLFE J: *Eur. J. Vasc. Surg.* (1993) 7: 129

7. MARZOCCHI A, MARROZZINI C, PIOVACCARI G, FATTORI R, CASTIOTA F, D'ANNIBALLE G, BRANZI A, MAGNANI B: *Cardiologia* (1991) 36(12 Suppl 1):309

8. DILLEY RJ, McGEACHIE JK, TENNANT M: *Cell. Tissue Res.* (1992) 269:281

9. SCHWARTZ RS, EDWARDS WD, HUBER KC, ANTONIADES LC, BAILEY KR, CAMRUD AR, JORGENSON MA, HOLMES DR Jr: *Mayo Clin. Proc.* (1993) 68:54
•
An article on prospects for solution and new perspectives for coronary restenosis.

10. REIDY MA, FINGERLE J, LINDNER V: *Circulation* (1992) 86(6 Suppl):III43

11. LIBBY P, SCHWARTZ D, BROGI E, TANAKA H, CLINTON SK: *Circulation* (1992) 86(6 Suppl):III47

12. HELDIN C-H: EMBO J. (1992) 11:4251
••
A comprehensive review on structural and functional studies on PDGF.

13. WILLIAMS LT: *Science* (1989) 243:1504
••

A comprehensive review on the PDGF receptor and signal transduction mechanisms.

14. HELDIN C-H, WESTERMARK B: *Cell Reg.* (1990) **1**:555
•• A thorough review on PDGF structure/function.

15. RAINES EW, BOEN-POPE DF, ROSS R: **Peptide Growth Factors and Their Receptors**: In: *Handbook of Experimental Pharmacology* (1990). SPORN MB, ROBERTS AB, (Eds), Springer-Verlag, Heidelberg **95**(Part I) p. 173

16. ULLRICH A, SCHLESSINGER J: *Cell* (1990) **61**:203

17. CANTLEY LC, ANGER KR, CARPENTER C, DUCKWORTH B, GRAZIANI A, KAPELLER R, SOLTOFF S: Cell (1991) **64**: 281

18. KOCH CA, ANDERSON D, MORAN MF, ELLIS C, PAWSON T: *Science* (1991) **252**:668

19. HOMMA Y, SAKAMOTO H, TSUNODA M, AOKI M, TAKENAWA T, OOYAMA T: *Biochem. J.* (1993) **142**:715

20. KUNDRA V, ESCOBEDO JA, KAZLAUSKAS A, KIM HK, RHEE
• SG, WILLIAMS LT, ZETTER BR: *Nature* (1994) **367**:474
Key paper discussing PDGF-induced signal transduction mechanisms and cell migration.

21. MARMUR JD, POON M, ROSSIKHINA M, TAUBMAN MB: *Circulation* (1992) **86**(6 Suppl):II53

22. KELLY JD, RAINES EW, ROSS R, MURRAY MJ: *Embo J.* (1985) **4**:3399

23. OEFNER C, D'ARCY A, WINKLER FK, EGGIMANN B,
•• HOSANG M: *Embo J.* (1992) **11**: 3921
The first x-ray analysis study of the crystal structure of PDGF-BB.

24. ENGSTROM U, ENGSTROM A, ERLUND A, WESTERMARK B, HELDIN C-H: *J. Biol. Chem.* (1992) **267**:16581

25. HUANG JS, HUANG SS, KENNEDY B, DEUEL TF: *J. Biol. Chem.* (1982) **257**:8130

26. ANDERSSON M, OSTMAN A, BACKSTROM G, HELLMAN U, GEORGE-NASCIMENTO C, WESTERMARK B, HELDIN C-H: *J. Biol. Chem.* (1992) **267**:11260

27. FRETTO LJ, SNAPE AJ, TOMLINSON JE,SEROOGY JJ, WOLF DL, LAROCHELLE WJ, GIESE NA: *J. Biol. Chem.* (1993) **268**:3625

28. DUAN D-SR, RAZIN MJ, FRETTO LJ, WILLIAMS LT: *J. Biol. Chem.* (1991) **266**:413

29. SZABO P, WEKSLER D, WHITTINGTON E, WEKSLER BB: *Mech. Ageing Dev.* (1993) **67**:79

30. KHACHIGIAN LM, CHESTERMAN CN: *J. Biol. Chem.* (1992) **267**:7478

31. KHACHIGIAN LM, OWENSBY DA, CHESTERMAN CNJ: *J. Biol. Chem.* (1992) **267**:1660

32. FAGER G,CAMEJO G, BONDJERS G: *In Vitro Cell Develop. Biol.* (1991) **28A**(3 Pt 1):168

33. LARSEN AK: *Cancer Chemother. Pharmacol.* (1993) **32**: 96
• Comprehensive article on the unique biological effects of suramin.

34. VOOGD TE, VANSTERKEUBURG EL, WITTING J, JANSSEN LH: *Pharmacol. Rev.* (1993) **45**:177
A review article on the biological activity of suramin.

35. SEIDMAN AD, SCHWARTZ M, REICH L, SCHER HI: *Cancer* (1993) **71**: 851

36. ALZANI R. CORTI A, CZAZIOLI L, COZZI E, CHEZZI P, MARCUCCI F: *J. Biol. Chem.* (1993) **268**:12526

37. WILLIAMS LT, TREMBLE PM, LAVIN MF, SUNDAY ME: *J. Biol. Chem.* (1984) **259**:5287

38. VASSVOTH FS, OSTMAN A, SIEGBAHN A, HOLMSEN H, HELDIN C-H: *J. Biol. Chem.* (1992) **267**:15635

39. JOHNSSON A, BETSHOLTZ C, VON DER HELM K, HELDIN C-H, WESTERMARK B: *Proc. Natl. Acad. Sci.* USA (1985) **82**:1721

40. REDDY KB, HOWE PH: *J. Cell Physiol.* (1993) **156**:48

41. FERNS GAA, RAINES EW, SPRUGEL KH, MOTANI AS, REIDY MA, ROSS R: *Science* (1991) **253**:1129

42. D'AMORE PA, SMITH SR: *Growth Factors* (1993) **8**:61
• A survey on growth factor effects on cells of the vascular wall.

43. KOYAMA N, HARADA K, YAMAMOTO A, MORISAKI N, SAITO Y, YOSHIDA S: *J. Biol. Chem.* (1993) **268**:13301

44. KLAGSBRUN M, BAIRD A: *Cell* (1991) **67**:229

45. KNOERZER W, BINDER HP, SCHNEIDER K, GRUSS P, McCARTHY JE, RISAU W: *Gene* (1989) **75**:21

46. BARTHELEMY I, MARTINEAU D, ONG M, NATSUNAMI R, LING N, BENATTI L, CAVALLARO U, SORIA M, LAPPI DA: *J. Biol. Chem.* (1993) **268**:6541

47. CASSCELLS W, LAPPI DA, OLWIN BB, WAIC, SIEGMAN M, SPEIR EH, SASSE J, BAIRD A: *Proc. Natl. Acad. Sci. USA* (1992) **89**:7159

48. LINDNER V, LAPPI DA, BAIRD A, MAJACK RA, REIDY MA: *Circulation Res.* (1991) **68**:106

49. PICKERING JG, BACHA PA, WEIR L, JEKANOWSKI J, NICHOLS JC, ISNER JM: *J. Clin. Invest.* (1993) **91**:724

50. LAPPI DA, BAIRD A: *Progress in Growth Factor Res.* (1990)
• **2**:223
Review article on mitotoxins.

51. REDDY KB, HOWE PH: *J. Cell Physiol.* (1993) **156**:48

52. MII S, WARE JA, KENT AC: *Surgery* (1993) **114**:464

53. STOUFFER GA, LAMARRE J, GONIAS SL, OWENS GK: *J. Biol. Chem.* (1993) **268**:18340

54. KIRSCHENLOHR HL, METCALFE JC, WISSBERG PL, GRAINGER DJ: *Am. J. Physiol.* (1993) **265**(2 Pt 1):C571

55. McCAFFREY TA, FALCONE DJ: *Mol. Biol. Cell.* (1993) **4**: 315

56. NILSEN-HAMILTON M: In: *Growth factors and development. Current Topics in Developmental Biology*, Academic Press, Inc, San Diego, p. 347

57. ISHIKAWA O: *J. Dermatol.* (1992) **19**:657

58. SCHINI VB, DURANTE W, ELIZONDO E, SCOTT-BURDEN T, JUNQUERO DC, SCHAFER AI, VANHOUTTE PM: *Eur. J. Pharmacol.* (1992) **216**:379

59. JUNQUERO DC, SCOTT-BURDEN T, SCHINI VB, VANHOUTTE PM: *J. Physiol. Lond.* (1992) **454**:451

60. JARVELAINEN HT, KINSELLA MG, SANDELL LJ, WIGHT TN: *Arterioscler. Thromb.* (1993) **13**:1026

61. BUTZOW R, FUKUSHIMA D, TWARDZIK DR, RUOSLAHTI E: *J. Cell. Biol.* (1993) **122**:721

62. LOPEZ-CASILLAS F, WRANA JL, MASSAGUE J: *Cell* (1993) **73**:1435

63. ROBERTS AB, SPORN MB: *Growth Factors* (1993) **8**:1
•

Review on the physiological action and clinical applications of TGF-β.

64. MIYAZONO K, ICHIJO H, HELDIN C-H: *Growth Factors* •• (1993) **8**:11
A comprehensive review on the latent form, binding proteins and receptors of TGF-β.

65. WRENN RW, RAEUBER CL, HERMAN LE, WALTON WS, ROSENQUIST TH: *In vitro Cell. Dev. Biol.* (1993) **29A** (1): 73

66. STRENLI CH, SCHMIDHAUSER C, KOBRIN M, BISSELL MJ, DERYNCK K: *J. Cell. Biol.* (1993) **120**:253

67. SHING Y, CHRISOPORI G, HANAHAN D, ONO Y, SASADA R, IGARSHI K, FOLKMAN J: *Science* (1993) **259**:1604

68. SASADA R, ONO Y, TANIYAMA Y, SHING Y, FOLKMAN J, IGARASHI K: *Biochem. Biophys. Res. Comm.* (1993) **190**: 1173

69. ZUKOWSKA-GROJEC Z, PRUSZCZYK P, COLTON C, YAO J, SHEN GH, MYERS AK, WAHLESTEDT C: *Peptides* (1993) **14**:263

70. FOEGH M: *J. Heart Lung Transplant.* (1992) **11** (3 pt 2): 528

71. CONTE JV, FOEGH ML, CALCAGNO D, WALLACE RB, RAMWELL PW: *Transplant. Proc.* (1989) **21**:3686

72. LUNDERGAN C, FOEGH ML, VARGAS R, EUFEMIO M, BORMES GW, KOT PA, RAMWELL PW: *Atherosclerosis* (1989) **80**:49

73. FOEGH ML, KHIRABADI BS, CHAMBERS E, AMAMOO S, RAMWELL PW: *Atherosclerosis* (1989) **78**:229

74. MENNANDER A, RAISANEN A, PAAVONEN T, HAYRY P: *Transplantation* (1993) **55**: 124

75. HAYRY P, RAISANEN A, USTINOV J, MENNANDER A, PAAVONEN T: *FASEB J.* (1993) **7**:1055

76. BADIMON JJ, FUSTER V, CHESEBRO JH, BADIMAON L: • *Circulation* (1993) **87**(3 Suppl.):II3
A review of coronary atherosclerosis .

77. RAKUGI H, JACOB HJ, KRIEGER JE, INGELFINDER JR, PRATT RE: *Circulation* (1993) **87**:283

78. ITOH H, MUKOYAMA M, PRATT RE, GIBBONS GH, DZAU VJ: *J. Clin. Invest.* (1993) **91**:2268

79. BAGBY SP, KIRK EA, MITCHELL LH, O'REILLY MM, HOLDEN WE, STENBERG PE, BAKKE AC: *Am. J. Physiol.* (1993) **265**(2 Pt 2):F239

80. PRESCOTT MF, WEBB RL, REIDY MA: *Am. J. Pathol.* (1991) **139**:1291

81. KAWAMURA M, TERASHITA Z, OKUDA H, IMURA Y, SHINO A, NAKAO M, NISHIKAWA K: *J. Pharmacol. Exp. Ther.* (1993) **266**:1664

82. KAUFFMAN RF, BEAN JS, ZIMMERMAN KM, BROWN RF, STEINBERG MI: *Life Sci.* (1991) **49**:PL223

83. LAM JY, LACOSTE L, BOURASSA MG: *Circulation* (1992) **85**:1542

84. MERCATOR Study Group: *Circulation* (1992) **86**:100

85. ITOH H, PRATT RE, DZAU VJ: *J. Clin. Invest.* (1992) **86**: 1690

86. ASSENDER JW, SOUTHGATE KM, HALLETT MB, NEWBY AC: *Biochem. J.* (1992) **288** (Pt 2):527

87. FURUYA M, TAKEHISA M, MINAMITAKE Y, KITAJIMA Y, HAYASHI Y, OHNUMA N, ISHIHARA T, MINAMINO N, KANGAWA K, MATSUO H: *Biochem. Biophys. Res. Comm.* (1990) **170**:201

88. FURUYA M, TAWARAGI Y, MINAMITAKE Y, KITAJIMA Y, FUCHIMURA K, TANAKA S, MINMINO N, KANGAWA K, MATSUO H: *Biochem. Biophys. Res. Comm.* (1992) **183**: 964

89. ALI S, DAVIS MG, BECKER MW, DORN GW 2nd: *J. Biol. Chem.* (1993) **268**:17397

90. DORN GW, BECKER MW: *Am. J. Physiol.* (1992) **262**(4 Pt 1): C927

91. YANG Z, NOLL G, LUSCHER TF: *Circulation* (1993) **88**: 1190

92. SASO Y, OHTANI A, ODAWARA A, IWASAKI H, TAKASHIMA K, MORITA T: *Cardiovasc. Drugs Ther.* (1993) **7**:257

93. KO YD, SACHINIDIS A, GRAACK GH, APPENHEIMER M, WIECZOREK AJ, DUSING R, VETTER H: *Clin. Investig.* (1992) **70**:113

94. WASTY F, ALAVI MZ, MOORE S: *Diabetologia* (1993) **36**: 316

95. THIE M, HARRACH B, SCHONHERR E, KRESS H, ROBENEK H, RAUTERBERG J: *Arterioscler. Thromb.* (1993) **13**:994

96. WOESSNER JF Jr: *FASEB J.* (1993) **7**:735
•
Introduction to serial reviews on the most intersting and exciting aspects of current studies of the extracellular matrix.

97. YOBKOWITZ R, MANSFIELD PJ, RYAN US, SUCHARD SJ: J. *Cell. Physiol .* (1993) **157**:24

98. PREISSNER KT: *Ann. Rev. Cell Biol.* (1991) **7**:275
•
Review on the structure and properties of vitronectin.

99. CLOWES AW, KARNOVSKY MJ: *Nature* (1977) **265**:625
•
First report on the antiproliferative activity of heparin.

100. KARNOVSKY MJ, WRIGHT TC Jr., CASTELLOT JJ Jr, CHOAY J, LORMEAU JC: *Ann. N. Y. Acad. Sci.* (1989) **556**:268

101. ROSENBERG RD: *Fed. Proc.* (1985) **44**:404

102. CHAN P, MILL S, MULLOY B, KAKKAR V, DEMOLIOU-MASON C: *Int. Angioplasty* (1992) **11**:261

103. GILDERTSON JJ, PETTENGILL OS, CRONENWETT JL: *Ann. Vasc. Surg.* **6**:265

104. AU YP, KENAGY RD, CLOWES MM, CLOWES AW: *Haemo-* • *stasis* (1993) **23**(Suppl 1):177
A study on the mechanisms of inhibition of VSMC proliferation and migration by heparin.

105. LINDNER V, OLSON NE, CLOWES AW, REIDY MA: *J. Clin. Invest.* (1992) **90**:2044

106. HANKE H, OBERHOFF M, HANKE S, HASSENSTEIN S, KAMENZ J, SCHMID KM, BETZ E, KARSCH KR: *Circulation* (1992) **85**:1548

107. ELLIS SG, ROUBIN GS, WILENTZ J, DOUGLAS JS, KING SB: *Am. Heart J.* (1989) **117**:777

108. CHAN P, PATEL M, BETTERIDGE L, MUNRO E, SCHACHTER M, WOLFE J, SEVER P: *Lancet* (1993) **341**(8841):341

109. CONRAD HE: *Annals of N. Y. Acad. of Sci.* (1989) **56**:18
•
Review of non-anticoagulant heparin derivatives.

110. WRIGHT TC Jr, CASTELLOT JJ Jr, PETITOU M, LORMEAU JC, CHOAY J, KARNOVSKY MJ: *J. Biol. Chem.* (1993) **268**: 4684

111. PUKAC LA, HIRSCH GM, LORMEAU J-C, PETITOU M, CHOAY J, KARNOVSKY MJ: *Am. J. Pathol.* (1991) **130**: 1501

112. BERZU T, DESMOULIERE A, HERBERT JM, LEVEL M, HERAULT JP, PETITOU M, LORMEAU JC, GABBIANI G, PASCAL M: *Eur. J. Pharmacol.* (1992) **219**:225

113. TYRRELL DJ, ISHIHARA M, RAO N, HORNE A, KIEFER MC, STANBER GB, LAM LH, STACK RJ: J. *Biol. Chem.* (1993) **268**:1534

114. SALTIS J, AGROTIS A, BOBIK A: *Clin. Exp. Pharmacol. Physiol.* (1992) **19**:396

115. MORRIS R: *J. Heart Lung Transplant* (1992) **11**(pt 2):123

116. GREGORY CR, PRATT RE, HUIE P, SHORTHOUSE R, DZAU VJ: *Transpl. Proc.* (1993) **25**(1 Pt 1):770

117. PAMPLONA R, BELLMUNT MJ, PORTERO M, PRAT J: *Med. Hypotheses* (1993) **40**:174

118. HERMANN HC, OKADA SS, HOZAKOWSKA E, LE VEEN RF, LOLDEN MA, TOMASZEWSKI JE, WEISZ PB, BARNATHAN ES: *Arterioscler. Thromb.* (1993) **13**:924

119. WATSON MH, VENANCE SL, PANG SC, MAK AS: *Circ. Res.* (1993) **142**:1787

120. BARRET JM , ENOULD AP, FERRY G, GENTON A, BOUTIN JA: *Biochem. Pharmacol.* (1993) **46**:439

121. YONEDA T, LYALL RM, ALSINAMM, PERSONS PE, SPADA AP, LEVITZKI A, ZIBERSTEIN A, MUNDY GR: *Cancer Res.* (1991) **51**:4430

122. SECKL M, ROZENGURT E: *J. Biol. Chem.* (1993) **268**: 9548

123. OHMICHI M, PANG L, RIBON V, GAZIT A, LEVITZKI A, SALTIEL AR: *Biochemistry* (1993) **32**:4650

124. ISSHIBI K, NAGANAWA H, TEKEUCHI T, TATSUTA K, UMEZAWA K: *J. Nat. Proc.* (1989) **52**:1252

125. HSU CY, JACOSKI MV, MAGUIRE MP, SPADA AP, ZIBERSTEIN A: *Biochem. Pharmacol.* (1992) **43**:2471

126. SUGATA D, YAMASHITA K, UMEZAWA K: *Biochem. Biophys. Res. Commn.* (1993) **194**:239

127. OIKAWA T, ASHINO H, SHIMAMURA M, HASEGAWA M, MORITA I, MUROTA S, ISHIZUKA M, TAKEUCHI T: *J. Antibiot. Tokyo* (1993) **46**:785

128. FUJIO Y, YAMADA F, TAKAHASHI K, SHIBATA N: *Biochem. Biophys. Res. Commun.* (1993) **195**:79

129. SHARMA RV, BHALLA RC: *Am. J. Physiol.* (1993) **264**(1 Pt 1):C71

130. SASAGURI T, KOSAKA C, HIRATA M, MASUDA J, SHIMOKADO K, FUJISHIMA M, OGATA J: *Exp. Cell Res.* (1993) **208**:311

131. TAKAHASHI S, OIDA K, FUJIWARA R, MAEDA H, HAYASHI S, TAKAI H, TAMAI T, NAKAI T, MIYABO S: *J. Cardiovasc. Pharmacol.* (1992) **20**:900

132. BERENBAUM M, FEENY P: *Science* (1981) **212**:927

133. SONG P-S, OU C-N: *Ann. N.Y. Acad. Sci.* (1980) **346**: 355.
•
A comprehensive review of the biological effects of psoralens.

134. MARCH KL, PATTON BL, WILENSKY RL, HATHAWAY DR: *Circulation* (1993) **87**:184

135. DECKELBAUM LI, SCOTT JJ, STETZ ML, O'BRIEN KM, SUMPIO BE, MADRI JA, BELL L: *Lasers Surg. Med.* (1993) **13**: 4

136. ORTU P, LaMURAGLIA GM, ROBERTS WG, FLOTTE TJ, HASAN T: *Circulation* (1992) **85**:1189

137. SCHWARTZ RS, KOVAL TM, EDWARDS WD, CAMRUD AR, BAILEY KR, BROWN K, VLIETSTRA RE, HOLMES DR Jr: *J. Am. Coll. Cardiol.* (1992) **19**:1109

138. MARCH KL, WILENSKY RL, ROESKE RW, HATHAWAY DR: *Circ. Res.* (1993) **72**:413

139. MILLIGAN JF, MATTEUCCI MD, MARTIN JC: *J. Med. Chem.* (1993) **36**:1923
•
A review article of current concepts of antisense drug design.

140. NABEL EG, PLAUTZ G, BOYCE FM, STANLEY JC, NABEL GJ: *Science* (1989) **244**:1342

141. PALMER H, LIBBY P: *Lab. Invest.* (1992) **66**:715

142. WILENSKY RL, MATCH KL, HATHWAY DR: *Amer. Heart J.* (1991) **122**:1136

143. WOLINSKY *et al.*: *J. Am. Coll. Cardiol.* (1990) **15**:475

144. FLUGELMAN MY, JAKLITSCH MT, NEWMAN KD, CASSCELLS W, BRATTHAUER GL, DICKEK DA: *Circulation* (1992) **85**:1110

145. SIMONS M, ROSENBERG RD: *Circ. Res.* (1992) **70**:835

146. SIMONS M, EDELMAN ER, DEKEYSER JL, LANGER R, ROSENBERG RD: *Nature* (1992) **359**:67

147. SPEIR E, EPSTEIN SE: *Circulation* (1992) **86**:538

148. EBBECKE M, UNTERBERG C, BUCHWALD A, STOHR S, WIEGAND V: *Basic Res. Cardiol.* (1992) **87**:585

149. DZAU VJ, PRATT RE: *J. Vasc. Surg.* (1992) **15**:934

150. GRAINGER DJ, KIRSCHENLOHR HL, METCALFE JC, WEISSBERG PL, WADE DP, LAWN RM: *Science* (1993) **260**: 1655

151. STIKO-RAHM A, HULTGARDH-NILSSON A, REGNSTROM J, HAMSTEN A, NILSSON J: *Arterioscler. Thromb.* (1992) **12**:1099

152. HARRIS-HOOKER S, SANFORD GL, MONTGOMERY V, RIVERS R, EMMETT N: *Cell Biol. Int. Rep.* **16**:433

153. KO Y, HARING R, STIEBLER H, WIECZOREK AJ, VETTER H, SACHINIDIS A: *Atherosclerosis* (1993) **99**:253

154. SIMIONESCU M, SIMIONESCU N: *FASEB J.* (1993) **7**: 1359

155. SCOTT J: *Nature* (1992) **360**:631
•
News and Views

156. MONCADA S, PALMER RM, HIGGS EA: *Pharmacol. Rev.* (1991) **43**:109

157. CALVER A, COLLIER J, VALLANCE P: *Exp. Physiol.* (1993) **78**:303

158. DREXLER H, ZEIHER AM, MEINZER K, JUST H: *Lancet* (1991) **338**:1546

159. FU Y, BLANKENHORN EP: *J. Immunol.* (1992) **148**:2217

160. KRANZHOFER R, SCHIRMER J, SCHOMIG A, VON-HODENBERG E, PESTEL E, METZ J, LANG HJ, KUBLER W: *Circ. Res.* (1993) **73**:264

161. SOUTHGATE KM, DAVIES M, BOOTH RF, NEWBY AC: *Biochem. J.* (1992) **288**(Pt 1):93

162. KANTHOU C, PARRY G, WIJELATH E, KAKKAR VV, DEMOLIOU-MASON C: *FEBS Letts.* (1992) **314**:143

163. TAPPARELLI C, METTERNICH R, EHRHARDT C, COOK NS: *Trends in Prarmacol. Sci.* (1993) **14**:366
•

A review of structure and pharmacological profiles of synthetic low molecular weight, thrombin inhibitors.

164. CRAGG AH, STOLL LL, SMITH TP, BERBAUM K: *J. Vasc. Interv. Radiol.* (1992) 3:273

References to patent literature

201. ZYMOGENETICS INC, EP-487116-A

202. LUDWIC INST FOR CANCER RES, WO9323068

203. DANA-FARBER CANCER INST INC, WO9118001

204. BRITISH BIO-TECHNOLOGY LTD; PFIZER LTD, WO9213073

205. CREATIVE BIOMOLECULES INC, WO9211364

206. FARMITALIA CARLO ERBA SRL, EP-486809-A

207. UPJOHN COMPANY, WO9015816

208. UNIVERSITY OF WASHINGTON, WO9212734

209. COR THERAPEUTICS INC, WO9310805

210. SALK INST; US DEPT OF HEALTH, WO9211872

211. GENENTECH INC, WO9115222

212. BERLIX BIOSCIENCES DIVISION OF BERLIX LABS, WO9321905

213. BRISTOL-MYERS SQUIBB CO, EP-567391-A

214. WHITTIER INST for DIABETES and ENDOCRICOL, WO9319783

215. VICTORIA UNIVERSITY OF MANCHASTER, WO9319769

216. NAKAMURA T, EP-475719-A

217. GENERAL HOSPITAL CORP, WO9319177

218. WHITEHEAD INST for BIOMEDICAL RES, WO9309228

219. SALK INST for BIOLOGICAL STUDIES, WO9220793

220. TAKEDA CHEM IND LTD, EP-555785

221. MAX PLANCK GMBLT, WO9215606

222. SOCIETE D'ETUDE DE PRODUITS CHIMIQUES, WO8912068

223. WARNER-LAMBERT CO, WO9315734

224. F HOFFMANN-LA ROCHE AG, EP-363671

225. JANSSEN PHARMACEUTICAL NV, EP-391462-A

226. SCHERING CORPORATION, EP-393441-A

227. ER SQIBB & SONS INC, GB-2227663

228. SUNTORY LTD, EP-497368-A

229. BOEHRINGER MANHEIM GMBLT, W09205782

230. ADIR ET CO, EP-526342-A

231. THROMBOSIS RES INST, WO9221363

232. HADASSAH MEDICAL ORGANIZATION, EP-354818-A

233. LA JOLLA CANCER RESEARCH FDN, WO9104748

234. GLYCOMED INC, WO9217187

235. GLYCOMED INC, WO9217188

236. GLYCOMED INC, WO9218546

237. AKZO NV; ELF SANOFI, EP-529715-A

238. AKZO NV; SANOFI SA, EP-454220-A

239. AMERICAN HOME PRODUCTS CORP, EP-568310-A

240. CORNELL RES FDN INC, EP-511830-A

241. RHÔNE-POULENC RORER INTERNATIONAL INC, WO9220642

242. HIROYOSHI HIDAKA; TOBISHI YAKUHIN KOGYO KK, GB2248235

243. INDIANA UNIVERSITY FDN, WO9218122

244. GENERAL HOSPITAL CORP, WO9307748

245. RESEARCH CORP TECHNOLOGIES INC, WO9213549

246. MEDEA RES SRL *et al.*, WO9219612

247. THOMAS JEFFERSON UNIVERSITY, WO9006997

248. THOMAS JEFFERSON UNIVERSITY, WO9011734

249. THOMAS JEFFERSON UNIVERSITY, WO9315609

250. MIT, WO9301286

251. TEMPLE UNIVERSITY, WO9219252

252. NISSAN CHEM IND LTD; KOWA CO LTD, EP-535548-A

253. CELL GENESYS INC, WO9110741-A

254. GENENTECH INC, US5192743-A

255. OTSUKA PHARMACEUTICAL CO LTD, EP-393707-A

256. OHIO UNIVERSITY, US5032407-A

257. CELL GENESYS INC, WO9101140-A

258. CELL GENESYS INC *et al*, WO9316177-A

Catherine D Demoliou-Mason, Thrombosis Research Institute, National Heart and Lung Institute, Emmanuel Kaye Building, Manresa Road, London, SW3 6LR, UK.

UNITED STATES DEPARTMENT OF COMMERCE
Patent and Trademark Office
Address: COMMISSIONER OF PATENTS AND TRADEMARKS
Washington, D.C. 20231

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.
09/141,957	12/07/98	WECKBECKER	0 4-100-832/A

EXAMINER
BORIN,M

ART UNIT	PAPER NUMBER
1631	5

DATE MAILED: 02/14/00

091095
THOMAS MOTTE
NOVARTIS CORPORATION
PATENT AND TRADEMARK DEPT
564 MORRIS AVENUE
SUMMIT NJ 07901-1027

HM12/0214

Please find below and/or attached an Office communication concerning this application or proceeding.

Commissioner of Patents and Trademarks

PTO-90C (Rev. 2/95)

2 - Mail Copy

Office Action Summary	Application No. 09/194,957	Applicant(s) Weckbecker
	Examiner M. Borin	Group Art Unit 1631

☐ Responsive to communication(s) filed on _____.

☐ This action is FINAL.

☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11; 453 O.G. 213.

A shortened statutory period for response to this action is set to expire 3 month(s), or thirty days, whichever is longer, from the mailing date of this communication. Failure to respond within the period for response will cause the application to become abandoned. (35 U.S.C. § 133). Extensions of time may be obtained under the provisions of 37 CFR 1.136(a).

Disposition of Claims

☒ Claim(s) *6-15* is/are pending in the application.

Of the above, claim(s) _____ is/are withdrawn from consideration.

☐ Claim(s) _____ is/are allowed.

☒ Claim(s) *6-15* is/are rejected.

☐ Claim(s) _____ is/are objected to.

☐ Claims _____ are subject to restriction or election requirement.

Application Papers

☐ See the attached Notice of Draftsperson's Patent Drawing Review, PTO-948.

☐ The drawing(s) filed on _____ is/are objected to by the Examiner.

☐ The proposed drawing correction, filed on _____ is ☐ approved ☐ disapproved.

☐ The specification is objected to by the Examiner.

☐ The oath or declaration is objected to by the Examiner.

Priority under 35 U.S.C. § 119

☒ Acknowledgement is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d).

☒ All ☐ Some* ☐ None of the CERTIFIED copies of the priority documents have been

☐ received.

☐ received in Application No. (Series Code/Serial Number) _____.

☒ received in this national stage application from the International Bureau (PCT Rule 17.2(a)).

*Certified copies not received: _____.

☐ Acknowledgement is made of a claim for domestic priority under 35 U.S.C. § 119(e).

Attachment(s)

☐ Notice of References Cited, PTO-892

☒ Information Disclosure Statement(s), PTO-1449, Paper No(s). *1*

☐ Interview Summary, PTO-413

☐ Notice of Draftsperson's Patent Drawing Review, PTO-948

☐ Notice of Informal Patent Application, PTO-152

--- SEE OFFICE ACTION ON THE FOLLOWING PAGES ---

DETAILED ACTION

Status of Claims

The Examiner acknowledges preliminary amendments filed 12/7/98 and 05/12/99. Claims 1-5 are canceled . Claims 10-15 are added. Claims 6-15 are pending.

Abstract

This application does not contain an abstract of the disclosure as required by 37 CFR 1.72(b). An abstract on a separate sheet is required.

Objection to specification

The disclosure is objected to under 37 CFR 1.71, as being so incomprehensible as to preclude a reasonable search of the prior art by the examiner. The term "compound B", used on pages 14-18, is not understood.

Applicant is required to submit an amendment which clarifies the disclosure so that the examiner may make a proper comparison of the invention with the prior art.

Applicant should be careful not to introduce any new matter into the disclosure (i.e., matter which is not supported by the disclosure as originally filed).

Claim objections

Claim 9 recites "separate use" of the components of the kit. Does it mean that one component is to be used without a subsequent use of another (If yes, a different set of art rejections can be applied). Cancellation of the term "separate" is suggested.

Claim Rejections - 35 U.S.C. § 112, second paragraph.

The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

Claims 6-15 rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. The rejection is applied for the following reasons:

A. Claims 6, 10, 13 are drawn to composition comprising "a compound of somatostatin class". Specification explains the meaning of other terms such as "somatostatin analog" or "somatostatin derivative" (p. 1-2). The term "a compound of somatostatin class" is not clear, it fails to particularly point out and distinctly define the metes and bounds of the subject matter that will be protected by the patent grant. It is not clear what scope of compounds is encompassed by the claims.

B. The term "a rapamycin macrolide" is not clear. It is not clear whether the claims are drawn to rapamycin, which is a single compound described on p. 10, or any rapamycin derivative (e.g., as described on p. 12).

C. Claims 6-9: Claim 6 recites "instruction for use". It is not clear what kind of use is recited in the instructions enclose in the claimed kit.

Claim Rejections - 35 U.S.C. § 112, first paragraph.

The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

Claims 6,7,9 are rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for synergistically effective amounts of ostreotide and rapamycin, does not reasonably provide enablement for synergistical amounts of any other analogs of somatostatins taken in combination with rapamycin. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to use the invention commensurate in scope with these claims.

Claim 9 is drawn to somatostatin analog and rapamycin used in the amounts that produce synergistic effect. Claim 7 recites a vast variety of compounds of "somatostatin class" with greatly varying structure (and properties). Prior art (e.g. discussed in specification, p. 12-13) teaches use of somatostatins and rapamycin as inhibitors of cell proliferation inhibitors of malignant tumor growth. Prior art does not teach synergism in the effects of somatostatins and rapamycin. The showing of the synergistic effect in the instant specification is limited to demonstration of synergism between 30 mg/kg of ostreotide and 5 mg/kg rapamycin on reduction of tumor caused by injection pancreatic

tumor cells in rats. See Example B, pages 15-17. Other examples in the specifications have not been considered because either they describe unknown "Compound B" (Example A), or do not present any numerical values demonstrating synergistic effect (Example C). The guidance for the dosage range of a somatostatin analog covers four orders of magnitude (10^{-10} - 10^{-6} M); this range is given for *in vitro*, not *in vivo* conditions. Even if considered for *in vitro* use only (which does not guide to amounts to be used *in vivo*), this range does not guide to amounts yielding synergistic effect. Specification fails to identify dosage range for rapamycin (again, "compound B" is not being considered as it is not clear what it is). In view of the above, it is the Examiners position that with the insufficient guidance and working examples and in view of unpredictability of art one skilled in the art could not make and/or use the invention with the claimed breadth without an undue amount of experimentation.

Claims 6-9,13-15 are rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for method of treatment of hyperproliferation of pancreatic cells, does not reasonably provide enablement for prevention of said hyperproliferation, or for treatment of hyperproliferation of other types of cells. The burden of enabling the prevention of a disease (ie. the need for additional testing) would be greater than that of enabling a treatment due to the need to screen those humans susceptible to such diseases and the difficulty of proof that the administration of the drug was the agent that acted to prevent the condition. The specification does not provide guidance as to how one skilled in the art would go about screening those patients susceptible to cell hyperproliferation. Nor is guidance provided as to a specific protocol to be utilized in order to prove

the efficacy of the presently claimed composition in preventing these disease states. Accordingly, undue experimentation is necessary to determine screening and testing protocols to demonstrate the efficacy of the presently claimed invention.

Claim Rejections - 35 U.S.C. § 103.

The following is a quotation of 35 U.S.C. § 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. 103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of 35 U.S.C. 103© and potential 35 U.S.C. 102(f) or (g) prior art under 35 U.S.C. 103(a).

Claims 6-8, 10-15 are rejected under 35 U.S.C. 103(a) as obvious over admitted prior art or GB 2239178 and WO 9311130.

The instant specification discusses knowledge existing in the prior art in regard to use of the components of the claimed composition and demonstrates that both components are known to be used for the same purpose. Thus, somatostatins are well known inhibitors of cell proliferation (e.g., smooth muscle cell proliferation), and have inhibiting effect on malignant tumor growth (e.g., in breast cancer). See p. 13, first paragraph. See also GB 2239178. Rapamycin also has been shown to inhibit smooth muscle cell proliferation and to inhibit cancer growth. See p. 12, last paragraph. See also WO 9311130.

Modification to combine two components known to be useful for the same would have been obvious to one of ordinary skill in the art in view of the fact that the courts have held that "it is *prima facie* obvious to combine two compositions each of which is taught by the prior art to be useful for the same purpose, in order to form a third composition which is to be useful for the very same purpose". See In re Sisi, 169 USPQ 423, 426 (CCPA 1971). Further, because combination therapies for treatment of abnormal cellular hyperproliferation are well-known in the art and because it would have been desirable to use plural therapies in order to maximize the probability that abnormal cellular hyperproliferation is minimized, it would be *prima facie* obvious to one of ordinary skills in the art at the time the invention was made to be motivated to use somatostatins and rapamycin in combination.

It is noted that, first, the instant claims are not drawn to composition having amounts of components rendering synergistic effect, but are rather drawn to any combination of the components. The only claim reciting amounts of components producing synergistic effect is claim 9 (which is

subject to enablement rejection). Second, the specification demonstrates existence of such synergistic effect for a very limited number of species.

In regard to claims 6-8 drawn to a kit, it would be obvious to package the composition of claims 10-12.

Conclusion.

No claims are allowed

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Michael Borin whose telephone number is (703) 305-4506. Dr. Borin can normally be reached between the hours of 8:30 A.M. to 5:00 P.M. EST Monday to Friday. If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Mr. Michael Woodward, can be reached on (703) 308-4028. The fax telephone number for this group is (703) 305-3014.

Any inquiry of a general nature or relating the status of this application should be directed to the Group receptionist whose telephone number is (703) 308-0196.

February 11, 2000

mlb

MICHAEL BORIN, PH.D
PATENT EXAMINER

TO-1449 (85) U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

ATTY. DOCKET NO. 4-100-8322

APPLICATION NO. 09/194,957

APPLICANT Gisbert Weckbecker

35 USC 371 DATE December 7, 1998

Group

INFORMATION DISCLOSURE CITATION

(use several sheets if necessary)

U.S. PATENT DOCUMENTS

EXAMINER INITIAL		DOCUMENT NUMBER	DATE	NAME	CLASS	SUBCLASS	FILING DATE
	AA						
	AB						
	AC						
	AD						
	AE						
	AF						
	AG						
	AH						
	AI						
	AJ						
	AK						
	AL						

FOREIGN PATENT DOCUMENTS

		DOCUMENT NUMBER	DATE	OFFICE	CLASS	SUBCLASS	TRANSLATION YES	TRANSLATION NO
MM	AM	GB 2 239 178 A *	6/26/91	Great Britain			☐	☐
MM	AN	WO 93 11130 A *	6/10/93	PCT			☐	☐
	AO						☐	☐
	AP						☐	☐
	AQ						☐	☐

OTHER DOCUMENTS (Including Author, Title, Date, Pertinent pages, Etc.)

MM	AR	Shi E.A., Cancer Research, Vol. 55, pgs. 1982-19088 (1995). *
	AS	Grant et al., Circulation, Vol. 89, No. 4, pgs 1511-1517 (1994).
MM	AT	Demoliou-Mason, Exp. Opin.Ther. Patents, Vol. 4, No. 7, pgs. 813-829 (1994).

EXAMINER M. Bowers DATE CONSIDERED 02/00

*EXAMINER: Initial of reference considered, whether or not citation is in conformance with MPEP 609: Draw a line through citation if not in conformance and not considered. Include a copy of this form with the next communication to applicant.

GAU: 1631

CASE 4-100-8322

CERTIFICATE OF MAILING

I hereby certify that this paper (along with any paper referred to as being attached or enclosed) is being deposited with the United States Postal Service on the date shown below with sufficient postage as first class mail in an envelope addressed to the: Assistant Commissioner for Patents, Washington, D.C. 20231.

JOSEPH J. BOROVIAN (Type or print name) — Joseph J. Borovian (Signature) — JUNE 9 2000 (Date)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

IN RE PCT NATIONAL STAGE APPLICATION OF
GISBERT WECKBECKER
INTERNATIONAL APPLICATION NO: PCT/EP 97/03036
FILED: 11 JUNE 1997
U.S. APPLICATION NO: 09/194,957
35 USC §371 DATE: 7 DECEMBER 1998
FOR: COMBINATION OF A SOMATOSTATIN ANALOGUE AND A RAPAMYCIN

Art Unit: 1631
Examiner: M. Borin

Assistant Commissioner for Patents
Washington, D.C. 20231

AMENDMENT

Sir:

Responsive to the Official Action dated February 14, 2000, kindly amend the above-identified application as follows:

IN THE CLAIMS

Please amend Claim 6 as follows:

6. (Amended) A kit or package for the treatment or prevention of cell hyperproliferation, said kit or package including a pharmaceutical composition comprising a compound of the somatostatin class selected from naturally occurring somatostatin-14, an analog of somatostatin-14 and a derivative of somatostatin-14 binding to at least the hSST-2 receptor in the nMolar range, in free form or in pharmaceutically acceptable form, and a pharmaceutical composition comprising a rapamycin macrolide selected from rapamycin and 40-O-(2-hydroxyethyl)-rapamycin, said compositions being present in synergistic effective amounts, together with instructions for use.

Claim 9, line 1; after "simultaneous", delete ", separate".

Please amend Claim 10 as follows:

10. (Amended) A pharmaceutical composition comprising a pharmaceutically acceptable carrier and a therapeutically effective amount of: 1) a compound of the somatostatin class selected from naturally occurring somatostatin-14, an analog of somatostatin-14 and a derivative of somatostatin-14 binding to at least the hSST-2 receptor in the nMolar range, in free form or in pharmaceutically acceptable salt form; and 2) a rapamycin macrolide selected from rapamycin and 40-O-(2-hydroxyethyl)-rapamycin, said compound and macrolide being present in synergistic effective amounts.

Please amend Claim 13 as follows:

13. (Amended) A method of preventing or treating cell hyperproliferation comprising administering to a subject in need of such treatment a therapeutically effective amount of: 1) a compound of the somatostatin class selected from naturally occurring somatostatin-14, an analog of somatostatin-14 and a derivative of somatostatin-14 binding to at least the hSST-2 receptor in the nMolar range, in free form or pharmaceutically acceptable salt form; and 2) a rapamycin macrolide selected from rapamycin and 40-O-(2-hydroxyethyl)-rapamycin, said compound and macrolide being present in synergistic effective amounts.

REMARKS

A favorable reconsideration of this application is respectfully requested in view of the foregoing amendments and the following remarks.

Claims 6-15 were presented for examination, and Claims 6-15 are still the only claims present in the case.

Claims 6, 10 and 13 have been amended to limit the "somatostatin" component to a specific Markush group of compounds which bind to at least the hSST-2 receptor in the nMolar range, and the "rapamycin" component to two specific compounds, viz., rapamycin and 40-O-(2-hydroxyethyl)-rapamycin. In addition, Claim 6 now explicitly reflects the fact that the compositions are present in synergistic effective amounts, whereas Claims 10 and 13 now explicitly reflect the fact that the compound and the macrolide are present in synergistic effective amounts.

Claim 9 has been amended to delete the term "separate".

As regards the Examiner's request to provide an Abstract on a separate sheet, enclosed herewith is a copy of a second "original" Page 9 (re-numbered as Page 28), which contains an Abstract of the invention.

With regard to the Examiner's objection to the disclosure under 37 CFR 1.71 on the basis that the term "compound B" is not understood, the Examiner's attention is respectfully directed to Page 12, lines 6 and 7 where the term is clearly defined. In this connection, it should be noted that said compound is one of the "two" compounds to which the rapamycin macrolide component of the instant claims is now limited. Accordingly, the Examiner is respectfully requested to reconsider and withdraw his objection to the specification.

As to the Examiner's objection to the term "separate" in Claim 9, although said claim has been amended to delete the term "separate" as suggested by the Examiner, it should be noted that the term "sequential" embraces separate use. Moreover, it should be noted that the term "simultaneous" does not limit the administration of the "two" drugs in the same form. In other words, it is Applicant's belief that the term "simultaneous" does not exclude the administration of the "two" drugs simultaneously, but in separate forms. In any event, the Examiner is respectfully requested to reconsider and withdraw his objection to Claim 9, as amended.

The Examiner has also rejected Claims 6-15 under the second paragraph of 35 USC 112 as being indefinite for failing to particularly point out and distinctly claim the subject mater of the "alleged" invention. More particularly, the Examiner objects to the presence of certain terms and/or passages which will be commented on in the order that they appear in the Office Action as follows.

A) This portion of the rejection is believed to have been overcome by the limitation of the "somatostatin" component to a specific Markush group of compounds which bind to at least the hSST-2 receptor in the nMolar range.

B) This portion of the rejection is believed to have been overcome by the limitation of the "rapamycin" component to two specific compounds.

C) As regards the Examiner's concern regarding the passage "instructions for use" in Claim 6, it is Applicants belief that one skilled in the art would clearly know what is intended by said passage. In other words, one skilled in the art would recognize that the "claimed" kit would contain instructions advising a patient in need of said treatment when, how often and how much of the two drugs should be administered.

In brief, in view of the foregoing limitations of the "somatostatin" and "rapamycin" components coupled with the foregoing arguments regarding the passage "instructions for use" in Claim 6, the Examiner is respectfully requested to reconsider and withdraw the rejection of Claims 6-15 under the second paragraph of 35 USC 112.

In addition, the Examiner has rejected Claims 6, 7 and 9 under the first paragraph of 35 USC 112 as being based on a specification which is "non-enabling". It is the Examiner's contention that the terms and/or passages "a compound of the somatostatin class" and "a rapamycin macrolide" represent such a wide variety of compounds that the presence of the passage "synergistically effective amounts" in Claim 9 would be virtually meaningless to one skilled in the art. In other words, it is the Examiner's belief that one skilled in the art would be unable to practice the invention without undue experimentation since the instant specification does not provide reasonable assurance that any compound of the somatostatin class and any rapamycin macrolide would act synergistically, especially in view of the paucity of pharmaceutical data present in the case.

Whether or not "original" Claims 6, 7 and 9 were properly rejectable under the first paragraph of 35 USC 112 as being based on a specification which is "non-enabling" is irrelevant. What is relevant is whether Claims 6, 7 and 9, as now amended, are based upon a specification which is "non-enabling" and, therefore, properly rejectable under the first paragraph of 35 USC 112 and, Applicant respectfully submits, they are not.

First of all, by the foregoing amendment to Claim 6, the "somatostatin" component has been limited to a specific Markush group of compounds which bind to at least the hSST-2 receptor in the nMolar range, and the "rapamycin" component has been limited to two specific compounds. In other words, the "broad" scopes of the compositions which comprise the "claimed" kits have been drastically reduced. Secondly, and as regards the Examiner's comment concerning the paucity of test data, there is nothing in the statute or in the case law which requires that test data must be provided in order to meet the "enablement" requirement of 35 USC 112. On the contrary, what is necessary to satisfy the "how-to-use" requirement of section 112 is the disclosure of some activity coupled with knowledge of how to use the compounds for the use disclosed (see, in this connection, In re Bundy, 209 USPQ 48). In the present case, on Page 14, line 18 to Page 15 (including the Table below line 14), there is described an in vitro assay which, when utilized, demonstrates the effectiveness of the instantly claimed compositions in inhibiting the growth of tumor cells. Similarly, on Page 15, line 3 from the bottom of the page to Page 17, line 2, there is described an in vivo assay which, when utilized, demonstrates the effectiveness of the instantly claimed compositions in inhibiting the growth of pancreatic tumor cells. In addition, on Page 17, lines 3-20, a clinical trial is set forth which demonstrates the effectiveness of the instantly claimed compositions in inhibiting breast tumors. Thirdly, and as regards the Examiner's

comment concerning the paucity of "working" examples, the Examiner is respectfully reminded that neither in vivo biological data nor working examples that demonstrate in vivo activity are required by the first paragraph of 35 USC 112. See, in this connection, Nelson v. Bowler, et al., 206 USPQ 881 and Cross, et al. v. Iizuka, et al. 224 USPQ 739.

In view of the foregoing, coupled with the disclosure on Page 17, line 21 to the last line on Page 19, it is clear that the instant specification enables one skilled in the art to practice the invention without undue experimentation. Therefore, withdrawal of the "non-enabling" rejection of Claims 6, 7 and 9, as amended, under the first paragraph of 35 USC 112, is respectfully requested.

Moreover, the Examiner has rejected Claims 6-9 and 13-15 under the first paragraph of 35 USC 112 as being based on a specification which is "non-enabling". It is the Examiner's contention that although the instant specification is enabling for the treatment of hyperproliferation of pancreatic cells, it is non-enabling for the "prevention" of hyperproliferation and for "treating" other than pancreatic cells. In other words, it is the Examiner's belief that one skilled in the art would be unable to practice the invention without undue experimentation since the instant specification is devoid of any guidance as to which subjects would be "susceptible to cell hyperproliferation" and in view of the paucity of pharmaceutical data present in the case.

As with the previous rejection, whether or not "original" Claims 6-9 and 13-15 were properly rejectable under the first paragraph of 35 USC 112 as being based on a specification which is "non-enabling" is irrelevant. What is relevant is whether Claims 6-9 and 13-15, as now amended, are based upon a specification which is "non-enabling" and, therefore, properly rejectable under the first paragraph of 35 USC 112 and, Applicant respectfully submits, they are not.

As indicated above, by the foregoing amendment to Claims 6 and 13, the "somatostatin" component has been limited to a specific Markush group of compounds which bind to at least the hSST-2 receptor in the nMolar range, and the "rapamycin" component has been limited to two specific compounds. In addition, the instant specification discloses an in vitro assay which establishes the effectiveness of the instantly claimed compositions in inhibiting the growth of tumor cells, and an in vivo assay which not only establishes the effectiveness of the instantly claimed compositions in treating and preventing pancreatic tumor growth but, in addition, establishes their effectiveness in preventing the hyperproliferation of malignant pancreatic cells. Since, as indicated above, there is nothing in the statute or in the case law which requires that test data must be provided in order to meet the "enablement" requirement of 35 USC 112, Applicant has, in fact, provided more than is required by the statute.

As to the Examiner's contention that the test data in the instant specification is enabling only for the treatment of pancreatic cells, it should be noted that, more correctly, pancreatic tumor cells were utilized in the in vitro and in vivo assays set forth in the instant specification. In any event, there is nothing in the record nor is Applicant aware of any scientific evidence or reasoning which supports the Examiner's contention. Absent such evidence or reasoning, it is believed that one skilled in the art, from a reading of the instant specification and the test data set forth therein, would conclude that the instantly claimed compositions would be effective in inhibiting the growth of other tumor cells.

In view of the foregoing, the Examiner is respectfully requested to withdraw the "non-enabling" rejection of Claims 6-9 and 13-15, as amended, under the first paragraph of 35 USC 112.

Lastly, the Examiner has rejected Claims 6-8 and 10-15 under 35 USC 103(a) as being obvious over the admitted prior art, British Patent 2,239,178 and WO 93/11130. It is the Examiner's contention that in view of the disclosures in the last paragraph on Page 12 and the first paragraph on Page 13 of the instant specification coupled with the teachings in the British and World patents it would have been obvious for one skilled in the art to use a compound of the somatostatin class and a rapamycin macrolide in combination for treating cell hyperproliferation and to prepare a kit or package comprising said combination. Accordingly, the Examiner concludes that Claims 6-8 and 10-15 are obvious and has cited certain case law to support his position. Although Applicant agrees with the strictures set forth in the case law relied upon by the Examiner, whether or not the teachings of the prior art discussed in the instant specification, British Patent 2,239,178 and WO 93/11130 rendered any of Claims 6-8 and 10-15 obvious prior to the foregoing amendments is irrelevant. What is relevant is whether the teachings of the prior art discussed in the instant specification, British Patent 2,239,178 and WO 93/11130 render any of Claims 6-8 and 10-15, as now amended, obvious and, Applicant respectfully submits, they do not.

As indicated in the instant specification, Applicant has discovered that the administration of a combination of a compound of the somatostatin class and a rapamycin macrolide, i.e., a combination of compounds which belong to different classes and operate by different mechanisms, exhibits synergistic cell hyproliferation inhibition. More particularly, Applicant surprisingly discovered that the administration of a combination of a compound of the somatostatin class which binds to somatostatin receptors (often expressed on tumor cells) and inhibits the growth hormone, and a rapamycin macrolide which binds in the cytoplasm to an immunophilin binding protein called FKBP-12 and the resulting complex, e.g., rapamycin/FKBP-12, binds to a protein named FRAP, inhibit cell hyperproliferation synergistically. Accordingly, in view of their ability to inhibit cell hyperproliferation, said combinations are useful in the prevention or treatment of malignant tumor growth and in the prevention or treatment of proliferative vascular diseases.

As to the relevancy of the teachings of the prior art being relied upon by the Examiner to the instant claims which, by the foregoing amendments, are now limited to a combination of a specific Markush group of somatostatin compounds which bind to at least the hSST-2 receptor in the nMolar range and two specific rapamycin compounds in synergistic effective amounts, there is simply nothing in the prior art being relied upon by the Examiner which would lead one skilled in the art to any of the combinations which characterize the instant claims. This is especially true since it is well known that the two rapamycin compounds of the instant claims are immunosuppresants which block T-cell mitogenesis and were developed to prevent graft rejection. In fact, rapamycin was approved in the U.S. for this use, whereas the other rapamycin compound of the instant claims is in clinical Phase 3 for the same indication. Moreover, neither of the rapamycin compounds of the instant claims was developed as an inhibitor of tumor cells. Accordingly, it is quite surprising that the combinations to which the instant claims are now limited exhibit a synergistic inhibitory effect on the hyperproliferation of malignant cells or vascular cells.

In addition, there is clearly nothing in the prior art being relied upon by the Examiner which would lead one skilled in the art to employ the individual compounds of the instantly claimed combinations in synergistic effective amounts, a conclusion acknowledged by the Examiner since "original" Claim 9 was not included among the rejected claims.

For essentially the reasons discussed above, Applicant does not believe that the teachings of the prior art being relied upon by the Examiner render the pharmaceutical kits of Claims 6-8 prima facie obvious. Applicant submits that it is not obvious to prepare a pharmaceutical kit when the motivation to do so is totally absent from the prior art, as is the case in the instant rejection. It is clear that there is nothing in the prior art relied upon by the Examiner which would provide one skilled in the art with the motivation to prepare any of the claimed pharmaceutical kits. Since the claimed pharmaceutical compositions are novel and unobvious, the claimed pharmaceutical kits are novel and obvious.

Similarly, Applicant does not believe that the teachings of the prior art relied upon by the Examiner render the "method-of-use" of Claims 13-15 prima facie obvious. A process involving the use of a novel and unobvious composition of matter is itself novel and unobvious. In re Kuehl, 177 USPQ 250; In re Schneider, et al., 179 USPQ 46; and In re Wadlinger, et al., 181 USPQ 826.

In view of the foregoing, it is clear that the teachings of the prior art being relied upon by the Examiner do not render any of the instant claims prima facie obvious. Therefore, withdrawal of the 35 USC 103(a) rejection of Claims 6-8 and 10-15, as amended, is respectfully requested.

All of the rejections of record having been overcome, the instant application is deemed to be in condition for allowance, and an early notice to that effect is earnestly solicited.

Since no "new" claims were added by this Amendment, and since the foregoing amendments did not result in either an increase in the total number of claims or an increase in the total number of independent claims, no additional fee is necessitated by the foregoing amendments. However, since this Amendment will be deemed to have been filed more than three months from, but within four months of, the date of the Office Action (i.e, February 14, 2000), it is respectfully requested that the period for filing a response to said Office Action be extended by one month. Please charge the $110 fee required by 37 CFR 1.17(a)(1) for a one month extension of time to Deposit Account No. 19-0134 in the name of Novartis Corporation. In this connection, two additional copies of this page are appended.

Respectfully submitted,

Novartis Pharmaceuticals Corporation
Patent and Trademark Dept.
564 Morris Avenue
Summit, NJ 07901-1027
(908) 522-6921

Joseph J. Borovian
Agent for Applicant
Reg. No. 26,631

JJB:mjl

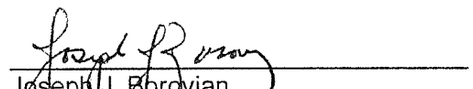
Encls.: Copy of second "original" Page 9
Two additional copies of this page
Postcard

Date: June 9, 2000

All of the rejections of record having been overcome, the instant application is deemed to be in condition for allowance, and an early notice to that effect is earnestly solicited.

Since no "new" claims were added by this Amendment, and since the foregoing amendments did not result in either an increase in the total number of claims or an increase in the total number of independent claims, no additional fee is necessitated by the foregoing amendments. However, since this Amendment will be deemed to have been filed more than three months from, but within four months of, the date of the Office Action (i.e, February 14, 2000), it is respectfully requested that the period for filing a response to said Office Action be extended by one month. Please charge the $110 fee required by 37 CFR 1.17(a)(1) for a one month extension of time to Deposit Account No. 19-0134 in the name of Novartis Corporation. In this connection, two additional copies of this page are appended.

Respectfully submitted,



Joseph J. Borovian
Agent for Applicant
Reg. No. 26,631

Novartis Pharmaceuticals Corporation
Patent and Trademark Dept.
564 Morris Avenue
Summit, NJ 07901-1027
(908) 522-6921

JJB:mjl

Encls.: Copy of second "original" Page 9
Two additional copies of this page
Postcard

Date: June 9, 2000

All of the rejections of record having been overcome, the instant application is deemed to be in condition for allowance, and an early notice to that effect is earnestly solicited.

Since no "new" claims were added by this Amendment, and since the foregoing amendments did not result in either an increase in the total number of claims or an increase in the total number of independent claims, no additional fee is necessitated by the foregoing amendments. However, since this Amendment will be deemed to have been filed more than three months from, but within four months of, the date of the Office Action (i.e, February 14, 2000), it is respectfully requested that the period for filing a response to said Office Action be extended by one month. Please charge the $110 fee required by 37 CFR 1.17(a)(1) for a one month extension of time to Deposit Account No. 19-0134 in the name of Novartis Corporation. In this connection, two additional copies of this page are appended.

Respectfully submitted,

Novartis Pharmaceuticals Corporation
Patent and Trademark Dept.
564 Morris Avenue
Summit, NJ 07901-1027
(908) 522-6921

Joseph J. Borovian
Agent for Applicant
Reg. No. 26,631

JJB:mjl

Encls.: Copy of second "original" Page 9
Two additional copies of this page
Postcard

Date: June 9, 2000

Abstract

A combination of a compound of the somatostatin class and a rapamycin macrolide is useful for the prevention or treatment of cell hyperproliferation.

UNITED STATES DEPARTMENT OF COMMERCE
Patent and Trademark Office
Address: COMMISSIONER OF PATENTS AND TRADEMARKS
Washington, D.C. 20231

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.
09/194,957	12/07/98	WECKBECKER G	4-100-8322/A

001095
THOMAS HOXIE
NOVARTIS CORPORATION
PATENT AND TRADEMARK DEPT
564 MORRIS AVENUE
SUMMIT NJ 07901-1027

HM22/0822

EXAMINER	
BORIN,M	
ART UNIT	PAPER NUMBER
1631	7

DATE MAILED: 08/22/00

Please find below and/or attached an Office communication concerning this application or proceeding.

Commissioner of Patents and Trademarks

Office Action Summary	Application No. 09/194,957	Applic s) Weckbecker
	Examiner M. Borin	Group Art Unit 1631

☒ Responsive to communication(s) filed on *Jun 13, 2000* .

☐ This action is **FINAL.**

☐ Since this application is in condition for allowance except for formal matters, **prosecution as to the merits is closed** in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11; 453 O.G. 213.

A shortened statutory period for response to this action is set to expire *3* month(s), or thirty days, whichever is longer, from the mailing date of this communication. Failure to respond within the period for response will cause the application to become abandoned. (35 U.S.C. § 133). Extensions of time may be obtained under the provisions of 37 CFR 1.136(a).

Disposition of Claims

☒ Claim(s) *6-15* is/are pending in the application.

Of the above, claim(s) _____ is/are withdrawn from consideration.

☐ Claim(s) _____ is/are allowed.

☒ Claim(s) *6-15* is/are rejected.

☐ Claim(s) _____ is/are objected to.

☐ Claims _____ are subject to restriction or election requirement.

Application Papers

☐ See the attached Notice of Draftsperson's Patent Drawing Review, PTO-948.

☐ The drawing(s) filed on _____ is/are objected to by the Examiner.

☐ The proposed drawing correction, filed on _____ is ☐approved ☐disapproved.

☐ The specification is objected to by the Examiner.

☐ The oath or declaration is objected to by the Examiner.

Priority under 35 U.S.C. § 119

☐ Acknowledgement is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d).

☐ All ☐ Some* ☐ None of the CERTIFIED copies of the priority documents have been

☐ received.

☐ received in Application No. (Series Code/Serial Number) _____ .

☐ received in this national stage application from the International Bureau (PCT Rule 17.2(a)).

*Certified copies not received: _____ .

☐ Acknowledgement is made of a claim for domestic priority under 35 U.S.C. § 119(e).

Attachment(s)

☐ Notice of References Cited, PTO-892

☐ Information Disclosure Statement(s), PTO-1449, Paper No(s). _____

☐ Interview Summary, PTO-413

☐ Notice of Draftsperson's Patent Drawing Review, PTO-948

☐ Notice of Informal Patent Application, PTO-152

--- SEE OFFICE ACTION ON THE FOLLOWING PAGES ---

DETAILED ACTION

Status of Claims

1. Amendment filed 06/13/00 is acknowledged. Claims 6,10,13 are amended. Claims 6-15 are pending.

2. Applicants arguments have been considered but are deemed moot in view of the new grounds of rejection. Rejections and/or objections not reiterated from previous Office actions are hereby withdrawn. The following rejections and/or objections are either reiterated or newly applied. They constitute the complete set presently being applied to the instant application.

Claim objections

3. Claim 6,10,13 recite both analogs of somatostatin-14 and derivatives of somatostatin-14. This seems to be redundant as "analogs" are defined as "derivatives" in the specification. See p. 1, lines 17-18.

Claim Rejections - 35 U.S.C. § 112, first paragraph.

The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

4. Claims 6-15 are rejected under 35 U.S.C. 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventors, at the time the application was filed, had possession of the claimed invention.

The presently claimed invention is drawn to pharmaceutical composition comprising synergistic amounts of a compound of somatostatin and a rapamycin macrolide. The compound of somatostatin class binds "to at least hSST-2 receptor in nMolar range" and is defined as a broad genus of compounds of various structure described, in particular, in claims 7,11,14. It does not seem that the inventors had possession of the compositions being present in "synergistic amounts" for the scope of somatostatin analogs claimed.

Unpredictability of synergistic effects is well known in pharmaceutical art. Synergism in *prima facie* unpredictable and need to be demonstrated for any particular combination of biologically active compounds.

The specification describes *in vitro* synergism between only one somatostatin compound, octreotide, and rapamycin or derivative thereof (Compound B). There is no showing of synergism for any other representative of somatostatin compounds. There is no demonstration of "synergistic amounts" for somatostatins other than octreotide, or ways of selecting synergistic dosages them over known dosage ranges for individual compounds. The general guidance for the dosage range of a somatostatin analog *in vitro* covers four orders of magnitude (10^{-10} - 10^{-6} M; see p. 15) out of which

synergism is shown for 1.2 nM (p. 15). For *in vivo* conditions the data in specification are more confusing: p. 18, line 9 from the bottom, guides that the dosage of somatostatin should be below 10 mg/day (for humans), whereas synergistic effect is shown for a much higher dosage of 30 mg/kg for mice (recalculated into mg/day, 30 mg/kg will yield even higher number). Further, there is no demonstration of existence of synergism in *in vivo* conditions (note that claims are drawn to compositions to be used *in vivo).*

With respect to adequate disclosure of the scope of the presently claimed generic, applicant is referred to the discussion in *University of California v. Eli Lilly and Co.* U.S. Court of Appeals Federal Circuit (CA FC) 43 USPQ2d 1398 7/22/1997 Decided July 22, 1997 No. 96-1175 regarding disclosure. For adequate disclosure, like enablement, requires *representative examples* which provide reasonable assurance to one skilled in the art that the compounds falling within the scope, both possess the alleged utility and additionally demonstrate that *applicant had possession of the full scope of the claimed invention.* See In re Riat et al. (CCPA 1964) 327 F2d 685, 140 USPQ 471; In re Barr et al. (CCPA 1971) 444 F 2d 349, 151 USPQ 724 (for enablement) and *University of California v. Eli Lilly and Co* cited above (for disclosure). The more unpredictable the art the greater the showing required (e.g. by "representative examples") for both enablement and adequate disclosure.

Applicant asserts that there is a means of assaying effect of components of composition to identify, if any, their synergism. However, this is not relevant to the disclosure requirement in which the applicant must demonstrate possession of the claimed scope at the time of filing.

Accordingly, it is clear that applicant has not demonstrated possession of the scope of the presently claimed subject matter. Accordingly, applicant is not in possession of the presently claimed invention.

5. Claims 13-15 are rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for method of treatment of hyperproliferation of pancreatic cells, does not reasonably provide enablement for prevention of said hyperproliferation, or for treatment of hyperproliferation of other types of cells. The burden of enabling the prevention of a disease (ie. the need for additional testing) would be greater than that of enabling a treatment due to the need to screen those humans susceptible to such diseases and the difficulty of proof that the administration of the drug was the agent that acted to prevent the condition. The specification does not provide guidance as to how one skilled in the art would go about screening those patients susceptible to cell hyperproliferation. Nor is guidance provided as to a specific protocol to be utilized in order to prove the efficacy of the presently claimed composition in preventing these disease states. Accordingly, undue experimentation is necessary to determine screening and testing protocols to demonstrate the efficacy of the presently claimed invention.

It is noted that applicant provided no response to this rejection.

Claim Rejections - 35 U.S.C. § 103.

6. Claims 6-15 are rejected under 35 U.S.C. 103(a) as obvious over admitted prior art or GB 2239178 and WO 9311130.

The claims are drawn to pharmaceutical compositions comprising synergistic amounts of somatostatin and rapamycin and their derivatives, method of use thereof, and kit comprising said composition.

The instant specification discusses knowledge existing in the prior art in regard to use of the components of the claimed composition and demonstrates that both components are known to be used for the same purpose. Thus, somatostatins are well known inhibitors of cell proliferation (e.g., smooth muscle cell proliferation), and have inhibiting effect on malignant tumor growth (e.g., in breast cancer). See p. 13, first paragraph. See also GB 2239178. Rapamycin also has been shown to inhibit smooth muscle cell proliferation and to inhibit cancer growth. See p. 12, last paragraph. See also WO 9311130. The dosages of somatostatin and rapamycin taught in the prior art are 0.05-1 mg for somatostatin (see GB '178 patent, p. 13, bottom) and 50mg-1g (see WO '130 patent, pages 24-25).

Modification to combine two components known to be useful for the same would have been obvious to one of ordinary skill in the art in view of the fact that the courts have held that "it is *prima facie* obvious to combine two compositions each of which is taught by the prior art to be useful for the same purpose, in order to form a third composition which is to be useful for the very same purpose". See In re Sisi, 169 USPQ 423, 426 (CCPA 1971). Further, because combination therapies for treatment of abnormal cellular hyperproliferation are well-known in the art and because it would

have been desirable to use plural therapies in order to maximize the probability that abnormal cellular hyperproliferation is minimized, it would be *prima facie* obvious to one of ordinary skills in the art at the time the invention was made to be motivated to use somatostatins and rapamycin in combination.

In regard to the "synergistic amounts" the dosages of the components taught in the prior art overlap with the dosage range taught in the instant invention. Compare 0.05-1 mg somatostatin taught in GB '178 patent (p. 13, bottom) with 0.1-10 mg somatostatin taught in the instant specification (p.18); compare 50mg-1000mg rapamycin taught in WO '130 patent (pages 24-25) with 0.5-500 mg rapamycin taught in the instant specification (p. 18). Therefore, the known dosage ranges of the components read on the "synergistic amounts" claimed.

In regard to claims 6-8 drawn to a kit, it would be obvious to package the composition of claims 10-12.

Applicant argues that the synergistic effect of the components is the unexpected beneficial effect. However, there is no specific dosage range identified in the claims, and the dosage range described in the specification overlaps with the dosages commonly used for individual components. Further, the showing of synergistic effect for one somatostatin compound, octreotide (p. 15,16) is not commensurate with the scope of the invention.

Conclusion.

7. No claims are allowed

8. Any inquiry concerning this communication or earlier communications from the examiner should be directed to Michael Borin whose telephone number is (703) 305-4506. Dr. Borin can normally be reached between the hours of 8:30 A.M. to 5:00 P.M. EST Monday to Friday. If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Mr. Michael Woodward, can be reached on (703) 308-4028. The fax telephone number for this group is (703) 305-3014.

Any inquiry of a general nature or relating the status of this application should be directed to the Group receptionist whose telephone number is (703) 308-0196.

August 18, 2000

mlb

MICHAEL BORIN, PH.D
PATENT EXAMINER

GAU 1631 #
#8 Pfaw
8/23/01

CASE 4-100-8322/A/PCT

CERTIFICATE OF MAILING

I hereby certify that this paper (along with any paper referred to as being attached or enclosed) is being deposited with the United States Postal Service on the date shown below with sufficient postage as first class mail in an envelope addressed to the: Assistant Commissioner for Patents, Washington, D.C. 20231.

JOSEPH J. BOROVIAK — Type or print name

Joseph J Borovian — Signature

JANUARY 22 2001 — Date

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

IN RE PCT NATIONAL STAGE APPLICATION OF
GISBERT WECKBECKER
INTERNATIONAL APPLICATION NO: PCT/EP 97/03036
FILED: 11 JUNE 1997
U.S. APPLICATION NO: 09/194,957
35 USC §371 DATE: 7 DECEMBER 1998
FOR: COMBINATION OF A SOMATOSTATIN ANALOGUE AND A RAPAMYCIN

Art Unit: 1631
Examiner: M. Borin

Assistant Commissioner for Patents
Washington, D.C. 20231

AMENDMENT

Sir:

Responsive to the Official Action dated August 22, 2000, kindly amend the above-identified application as follows:

IN THE CLAIMS

Please cancel Claims 7, 11 and 14.

Please amend Claim 6 as follows:

D1

6. (Twice amended) A kit or package for the [treatment or prevention] inhibition of cell hyperproliferation, said kit or package including a pharmaceutical composition comprising [a compound of the somatostatin class selected from naturally occurring somatostatin-14,] an analog of somatostatin-14 [and a derivative of somatostatin-14] binding to at least the hSST-2 receptor in the nMolar range, in

free form or in pharmaceutically acceptable form, and a pharmaceutical composition comprising a rapamycin macrolide selected from rapamycin and 40-O-(2-hydroxyethyl)-rapamycin, said compositions being present in synergistic effective amounts, together with instructions for use.

D1 cont'd

Claim 8, line 1; change "7" to --16--.

Please amend Claim 10 as follows:

10. (Twice amended) A pharmaceutical composition comprising a pharmaceutically acceptable carrier and a therapeutically effective amount of: 1) [a compound of the somatostatin class selected from naturally occurring somatostatin-14,] an analog of somatostatin-14 [and a derivative of somatostatin-14] binding to at least the hSST-2 receptor in the nMolar range, in free form or in pharmaceutically acceptable salt form; and 2) a rapamycin macrolide selected from rapamycin and 40-O-(2-hydroxyethyl)-rapamycin, said compound and macrolide being present in synergistic effective amounts.

D2

Claim 12, line 1; change "11" to --17--.

Please amend Claim 13 as follows:

13. (Twice amended) A method of [preventing or treating] inhibiting cell hyperproliferation comprising administering to a subject in need of such treatment a therapeutically effective amount of: 1) [a compound of the somatostatin class selected from naturally occurring somatostatin-14,] an analog of somatostatin-14 [and a derivative of somatostatin-14] binding to at least the hSST-2 receptor in the nMolar range, in free form or in pharmaceutically acceptable salt form; and 2) a rapamycin macrolide selected from rapamycin and 40-O-(2-hydroxyethyl)-rapamycin, said compound and macrolide being present in synergistic effective amounts.

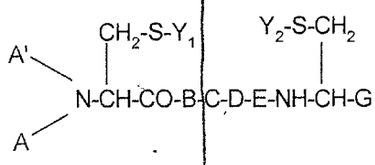
D3

Claim 15, line 1; change "14" to --18--.

Please add the following new claims:

--16. A kit or package according to claim 6, wherein the analog of somatostatin-14 is a compound of formula I

D4



(I)

wherein

A is C_{1-12}alkyl, C_{7-10}phenylalkyl or a group of formula RCO-, where

i) R is hydrogen, C_{1-11}alkyl, phenyl or C_{7-10}phenylalkyl, or

ii) RCO-is

a) a D-phenylalanine residue optionally ring-substituted by halogen, NO_2, NH_2, OH, C_{1-3}alkyl and/or C_{1-3}alkoxy, or

b) the residue of a natural or a synthetic α-amino-acid other than defined under a) above, or of a corresponding D-amino acid, or

c) a dipeptide residue in which the individual amino acid residues are the same or different and are selected from those defined under a) and/or b) above, the α-amino group of amino acid residues a) and b) and the N-terminal amino group of dipeptide residues c) being optionally mono- or di-C_{1-12}alkylated or substituted by C_{1-8}alkanoyl;

A' is hydrogen or C_{1-3}alkyl,

Y_1 and Y_2 represent together a direct bond or each of Y_1 and Y_2 is hydrogen,

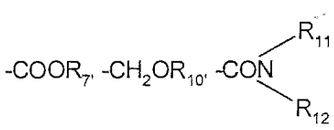
B is - Phe- optionally ring-substituted by halogen, NO_2, NH_2, OH, C_{1-3}alkyl and/or C_{1-3}alkoxy (including pentafluoroalanine), naphthylalanine or pyridylalanine,

C is (L)-Trp- or (D)-Trp- optionally α-N-methylated and optionally benzene-ring-substituted by halogen, NO_2, NH_2, OH, C_{1-3}alkyl and/or C_{1-3}alkoxy,

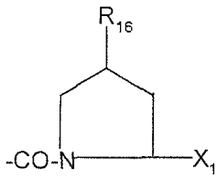
D is Lys, 4-aminocyclohexylAla or 4-aminocyclohexylGly,

E is Thr, Ser, Val, Tyr, Ile, Leu or an aminobutyric or aminoisobutyric acid residue, and

G is a group of formula



or



where

R_7 is hydrogen or C_{1-3}alkyl,

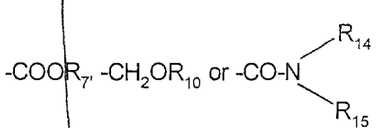
R_{10} is hydrogen or the residue of a physiologically acceptable, physiologically hydrolyzable ester,

R_{11} is hydrogen, C_{1-3}alkyl, phenyl or C_{7-10}phenylalkyl,

R_{12} is hydrogen, C_{1-3}alkyl or a group of formula -CH (R_{13})-X_1, where

R_{13} is CH_2OH, -$(CH_2)_2$-OH, -$(CH_2)_3$-OH, -$CH(CH_3)OH$, isobutyl, butyl, benzyl, naphthyl-methyl or indol-3-yl-methyl, and

X_1 is a group of formula



where

R_7 and R_{10} have the meanings given above,

R_{14} is hydrogen or C_{1-3}alkyl and

R_{15} is hydrogen, C_{1-3}alkyl, phenyl or C_{7-10}phenylalkyl, and

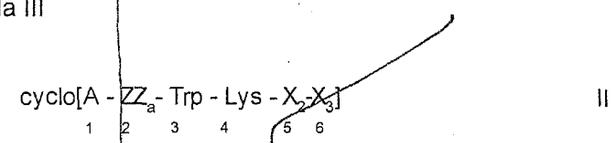
R_{16} is hydrogen or hydroxyl,

with the proviso that

when R_{12} is $-CH(R_{13})-X_1$, then R_{11} is hydrogen or methyl,

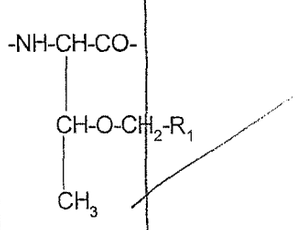
wherein the residues B, D and E have the L-configuration and the residues in the 2- and 7-position each independently have the (L)- or (D)- configuration;

or a compound of formula III



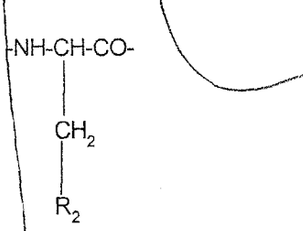
III

wherein X_2 is a radical of formula (a) or (b)



(a)

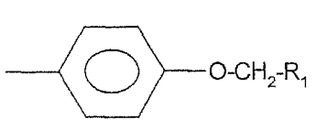
or



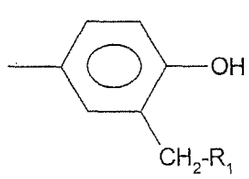
(b)

wherein R_1 is optionally substituted phenyl, and

R_2 is $-Z_1-CH_2-R_1$, $CH_2-CO-O-CH_2-R_1$,



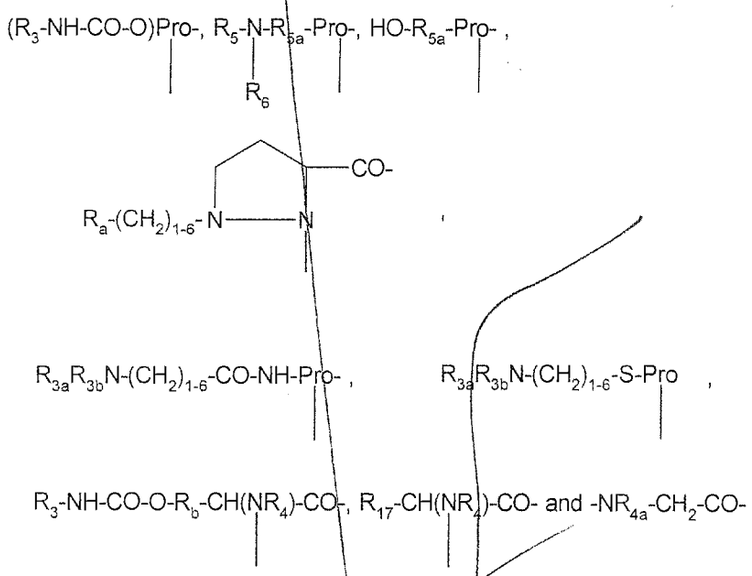
or



wherein Z_1 is O or S,

X_3 is an α-amino acid having an aromatic residue on the C_α side chain, or an amino acid unit selected from Dab, Dpr, Dpm, His, (Bzl)HyPro, thienyl-Ala, cyclohexyl-Ala and t.-butyl-Ala,

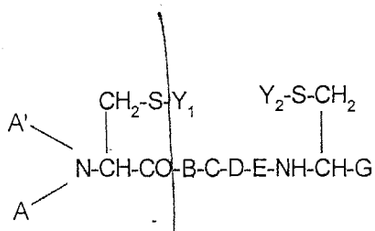
A_1 is a divalent residue selected from Pro,



wherein R_3 is NR_8R_9-C_{2-6}alkylene, guanidino-C_{2-6}alkylene or C_{2-6}alkylene-COOH, R_{3a} is H, C_{1-4}alkyl or has independently one of the significances given for R_3, R_{3b} is H or C_{1-4}alkyl, R_a is OH or NR_5R_6, R_b is $-(CH_2)_{1-3}-$ or $-CH(CH_3)-$, R_4 is H or CH_3, R_{4a} is optionally ring-substituted benzyl, each of R_5 and R_6 independently is H, C_{1-4}alkyl, ω-amino-C_{1-4}alkylene, ω-hydroxy-C_{1-4}alkylene or acyl, R_{5a} is a direct bond or C_{1-6}alkylene, each of R_8 and R_9 independently is H, C_{1-4}alkyl, ω-hydroxy-C_{2-4}alkylene, acyl or CH_2OH-$(CHOH)_c$-CH_2- wherein c is 0, 1, 2, 3 or 4, or R_8 and R_9 form together with the nitrogen atom to which they are attached a heterocyclic group which may comprise a further heteroatom, and R_{17} is optionally ring-substituted benzyl, $-(CH_2)_{1-3}$-OH, $CH_3CH(OH)$- or $-(CH_2)_{1-5}-NR_5R_6$, and

ZZ_a is a natural or unnatural α amino acid unit. --

--17. A pharmaceutical composition according to claim 10 wherein the analog of somatostatin-14 is a compound of formula I



(I)

wherein

A is C_{1-12}alkyl, C_{7-10}phenylalkyl or a group of formula RCO-, where

i) R is hydrogen, C_{1-11}alkyl, phenyl or C_{7-10}phenylalkyl, or

ii) RCO-is

a) a D-phenylalanine residue optionally ring-substituted by halogen, NO_2, NH_2, OH, C_{1-3}alkyl and/or C_{1-3}alkoxy, or

b) the residue of a natural or a synthetic α-amino-acid other than defined under a) above, or of a corresponding D-amino acid, or

c) a dipeptide residue in which the individual amino acid residues are the same or different and are selected from those defined under a) and/or b) above, the α-amino group of amino acid residues a) and b) and the N-terminal amino group of dipeptide residues c) being optionally mono- or di-C_{1-12}alkylated or substituted by C_{1-8}alkanoyl;

A' is hydrogen or C_{1-3}alkyl,

Y_1 and Y_2 represent together a direct bond or each of Y_1 and Y_2 is hydrogen,

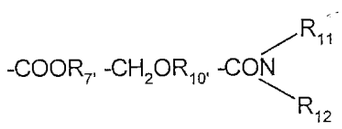
B is -Phe- optionally ring-substituted by halogen, NO_2, NH_2, OH, C_{1-3}alkyl and/or C_{1-3}alkoxy (including pentafluoroalanine), naphthylalanine or pyridylalanine,

C is (L)-Trp- or (D)-Trp- optionally α-N-methylated and optionally benzene-ring-substituted by halogen, NO_2, NH_2, OH, C_{1-3}alkyl and/or C_{1-3}alkoxy,

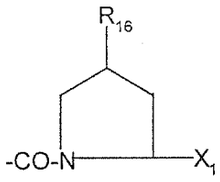
D is Lys, 4-aminocyclohexylAla or 4-aminocyclohexylGly,

E is Thr, Ser, Val, Tyr, Ile, Leu or an aminobutyric or aminoisobutyric acid residue, and

G is a group of formula



or



where

R_7 is hydrogen or C_{1-3}alkyl,

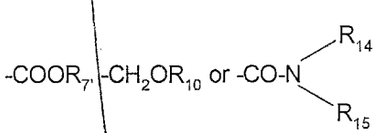
R_{10} is hydrogen or the residue of a physiologically acceptable, physiologically hydrolyzable ester,

R_{11} is hydrogen, C_{1-3}alkyl, phenyl or C_{7-10}phenylalkyl,

R_{12} is hydrogen, C_{1-3}alkyl or a group of formula -CH (R_{13})-X_1, where

R_{13} is CH_2OH, $-(CH_2)_2-OH$, $-(CH_2)_3-OH$, $-CH(CH_3)OH$, isobutyl, butyl, benzyl, naphthyl-methyl or indol-3-yl-methyl, and

X_1 is a group of formula



where

R_7 and R_{10} have the meanings given above,

R_{14} is hydrogen or C_{1-3}alkyl and

R_{15} is hydrogen, C_{1-3}alkyl, phenyl or C_{7-10}phenylalkyl, and

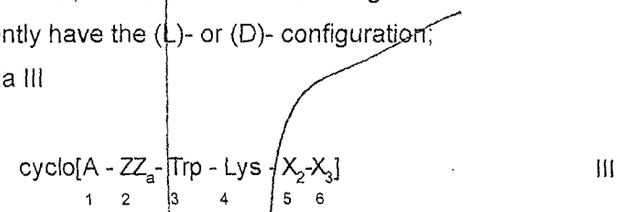
R_{16} is hydrogen or hydroxy,

with the proviso that

when R_{12} is $-CH(R_{13})-X_1$, then R_{11} is hydrogen or methyl,

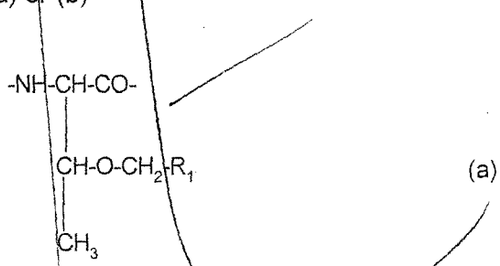
wherein the residues B, D and E have the L-configuration and the residues in the 2- and 7-position each independently have the (L)- or (D)- configuration;

or a compound of formula III



III

wherein X_2 is a radical of formula (a) or (b)



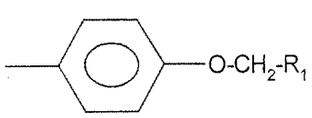
(a)

or

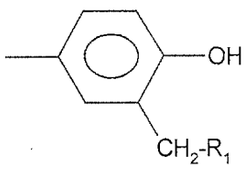
(b)

wherein R_1 is optionally substituted phenyl, and

R_2 is $-Z_1-CH_2-R_1$, $CH_2-CO-O-CH_2-R_1$,



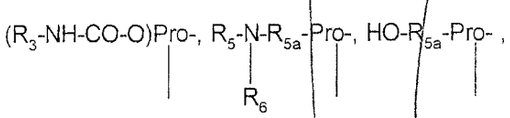
or

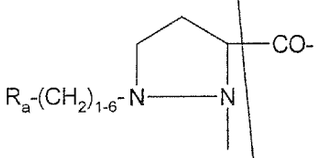


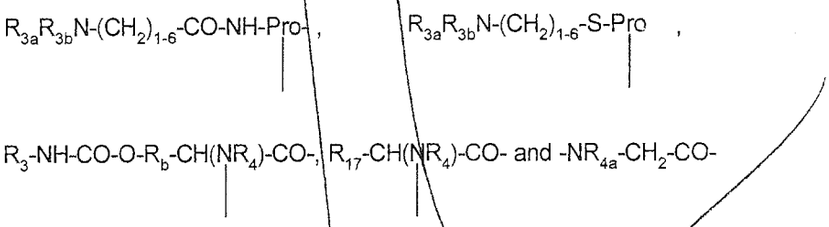
wherein Z_1 is O or S,

X_3 is an α-amino acid having an aromatic residue on the C_α side chain, or an amino acid unit selected from Dab, Dpr, Dpm, His, (Bzl)HyPro, thienyl-Ala, cyclohexyl-Ala and t.-butyl-Ala,

A_1 is a divalent residue selected from Pro,



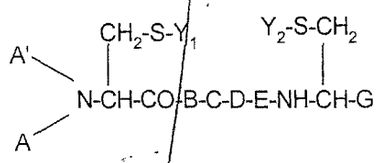




wherein R_3 is NR_8R_9-C_{2-6}alkylene, guanidino-C_{2-6}alkylene or C_{2-6}alkylene-COOH, R_{3a} is H, C_{1-4}alkyl or has independently one of the significances given for R_3, R_{3b} is H or C_{1-4}alkyl, R_a is OH or NR_5R_6, R_b is $-(CH_2)_{1-3}-$ or $-CH(CH_3)-$, R_4 is H or CH_3, R_{4a} is optionally ring-substituted benzyl, each of R_5 and R_6 independently is H, C_{1-4}alkyl, ω-amino-C_{1-4}alkylene, ω-hydroxy-C_{1-4}alkylene or acyl, R_{5a} is a direct bond or C_{1-6}alkylene, each of R_8 and R_9 independently is H, C_{1-4}alkyl, ω-hydroxy-C_{2-4}alkylene, acyl or CH_2OH-$(CHOH)_c$-CH_2- wherein c is 0, 1, 2, 3 or 4, or R_8 and R_9 form together with the nitrogen atom to which they are attached a heterocyclic group which may comprise a further heteroatom, and R_{17} is optionally ring-substituted benzyl, $-(CH_2)_{1-3}-OH$, $CH_3CH(OH)-$ or $-(CH_2)_{1-5}-NR_5R_6$, and

ZZ_a is a natural or unnatural α amino acid unit. --

--18. A method according to claim 13 wherein the analog of somatostatin-14 is a compound of formula I



(I)

wherein

A is C_{1-12}alkyl, C_{7-10}phenylalkyl or a group of formula RCO-, where

i) R is hydrogen, C_{1-11}alkyl, phenyl or C_{7-10}phenylalkyl, or

ii) RCO-is

a) a D-phenylalanine residue optionally ring-substituted by halogen, NO_2, NH_2, OH, C_{1-3}alkyl and/or C_{1-3}alkoxy, or

b) the residue of a natural or a synthetic α-amino-acid other than defined under a) above, or of a corresponding D-amino acid, or

c) a dipeptide residue in which the individual amino acid residues are the same or different and are selected from those defined under a) and/or b) above, the α-amino group of amino acid residues a) and b) and the N-terminal amino group of dipeptide residues c) being optionally mono- or di-C_{1-12}alkylated or substituted by C_{1-8}alkanoyl;

A' is hydrogen or C_{1-3}alkyl,

Y_1 and Y_2 represent together a direct bond or each of Y_1 and Y_2 is hydrogen,

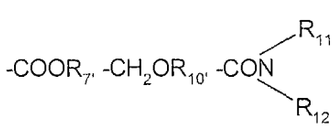
B is -Phe- optionally ring-substituted by halogen, NO_2, NH_2, OH, C_{1-3}alkyl and/or C_{1-3}alkoxy (including pentafluoroalanine), naphthylalanine or pyridylalanine,

C is (L)-Trp- or (D)-Trp- optionally α-N-methylated and optionally benzene-ring-substituted by halogen, NO_2, NH_2, OH, C_{1-3}alkyl and/or C_{1-3}alkoxy,

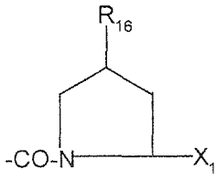
D is Lys, aminocyclohexylAla or 4-aminocyclohexylGly,

E is Thr, Ser, Val, Tyr, Ile, Leu or an aminobutyric or aminoisobutyric acid residue, and

G is a group of formula



or



where

R_7 is hydrogen or C_{1-3}alkyl,

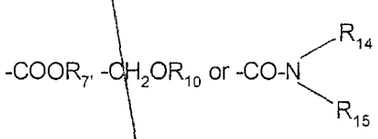
R_{10} is hydrogen or the residue of a physiologically acceptable, physiologically hydrolyzable ester,

R_{11} is hydrogen, C_{1-3}alkyl, phenyl or C_{7-10}phenylalkyl,

R_{12} is hydrogen, C_{1-3}alkyl or a group of formula -CH (R_{13})-X_1, where

R_{13} is CH_2OH, -$(CH_2)_2$-OH, -$(CH_2)_3$-OH, -$CH(CH_3)OH$, isobutyl, butyl, benzyl, naphthyl-methyl or indol-3-yl-methyl, and

X_1 is a group of formula



where

R_7 and R_{10} have the meanings given above,

R_{14} is hydrogen or C_{1-3}alkyl and

R_{15} is hydrogen, C_{1-3}alkyl, phenyl or C_{7-10}phenylalkyl, and

R_{16} is hydrogen or hydroxy,

with the proviso that

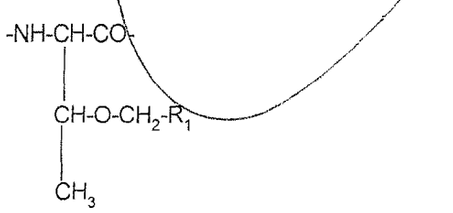
when R_{12} is $-CH(R_{13})-X_1$, then R_{11} is hydrogen or methyl,

wherein the residues B, D and E have the L-configuration and the residues in the 2- and 7-position each independently have the (L)- or (D)- configuration;

or a compound of formula III

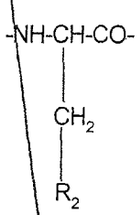
$$\underset{1}{\text{cyclo[A}} - \underset{2}{ZZ_a} - \underset{3}{\text{Trp}} - \underset{4}{\text{Lys}} - \underset{5}{X_2} - \underset{6}{X_3}] \qquad \text{III}$$

wherein X_2 is a radical of formula (a) or (b)



(a)

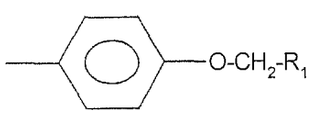
or



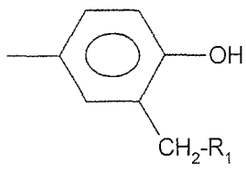
(b)

wherein R_1 is optionally substituted phenyl, and

R_2 is $-Z_1-CH_2-R_1$, $CH_2-CO-O-CH_2-R_1$,



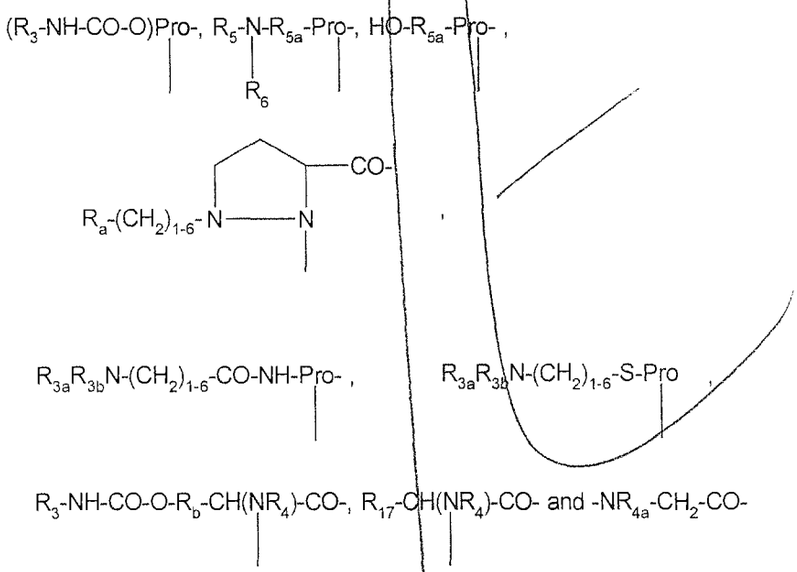
or



wherein Z_1 is O or S,

X_3 is an α-amino acid having an aromatic residue on the C_α side chain, or an amino acid unit selected from Dab, Dpr, Dpm, His, (Bzl)HyPro, thienyl-Ala, cyclohexyl-Ala and t.-butyl-Ala,

A_1 is a divalent residue selected from Pro,



wherein R_3 is NR_8R_9-C_{2-6}alkylene, guanidino-C_{2-6}alkylene or C_{2-6}alkylene-COOH, R_{3a} is H, C_{1-4}alkyl or has independently one of the significances given for R_3, R_{3b} is H or C_{1-4}alkyl, R_a is OH or NR_5R_6, R_b is $-(CH_2)_{1-3}-$ or $-CH(CH_3)-$, R_4 is H or CH_3, R_{4a} is optionally ring-substituted benzyl, each of R_5 and R_6 independently is H, C_{1-4}alkyl, ω-amino-C_{1-4}alkylene, ω-hydroxy-C_{1-4}alkylene or acyl, R_{5a} is a direct bond or C_{1-6}alkylene, each of R_8 and R_9 independently is H, C_{1-4}alkyl, ω-hydroxy-C_{2-4}alkylene, acyl or CH_2OH-$(CHOH)_c$-CH_2- wherein c is 0, 1, 2, 3 or 4, or R_8 and R_9 form together with the nitrogen atom to which they are attached a heterocyclic group which may comprise a further heteroatom, and R_{17} is optionally ring-substituted benzyl, $-(CH_2)_{1-3}$-OH, $CH_3CH(OH)$- or $-(CH_2)_{1-5}$-NR_5R_6, and

04 (mdf)

ZZ_a is a natural or unnatural α amino acid unit. --

REMARKS

A favorable reconsideration of this application is respectfully requested in view of the foregoing amendments and the following remarks.

Claims 6-15 were presented for examination, and Claims 6, 8-10, 12, 13 and 15-18 are now present in the case.

Claims 7, 11 and 14 have been cancelled and replaced by "new" Claims 16-18, respectively. As can be clearly seen, the "alternative" embodiment in said "new" claims has been limited to compounds of formula III.

Claims 6, 10 and 13 have been amended to limit the "somatostatin" component exclusively to an analog of somatostatin-14 which binds to at least the hSST-2 receptor in the nMolar range. In addition, the preamble in Claim 6 has been amended to reflect the fact that the claimed kit or package is useful for the inhibition of cell proliferation, whereas Claim 15 has been amended so that it is now directed to a "method of inhibiting cell hyperproliferation".

Claim 8 has been amended so that it now depends on "new" Claim 16; Claim 12 has been amended so that it now depends on "new" Claim 17; and Claim 15 has been amended so that it now depends on "new" Claim 18.

The Examiner's objection to Claims 6, 10 and 13 is believed to have been overcome by the amending of said claims to limit the "somatostatin" component exclusively to an analog of somatostatin-14 which binds to at least the hSST-2 receptor in the nMolar range.

Claims 6-15 have been rejected under the first paragraph of 35 USC 112 as containing subject matter which is not adequately described in the specification. More particularly, the Examiner is of the opinion that the specification lacks adequate descriptive support for the term "synergistic effective amounts" as applied to the somatostatin analogs. The Examiner is especially troubled that the specification contains "in vitro" synergistic evidence for only one somatostatin compound. In addition, the Examiner deems the "in vivo" data confusing, i.e., it appears to

conflict with the dosage regimen indicated in the "utility statement", and alleges that the specification lacks "in vivo" synergistic evidence.

First of all, since octreotide, as well as all of the other somatostatin analogs of the instant claims are known to inhibit cell proliferation and tumor growth, one skilled in the art would expect that all of the somatostatin analogs would exhibit a synergistic anti-tumor effect when combined with rapamycin.

As regards to the Examiner's allegation that the instant specification lacks in vivo synergistic evidence, it appears that the Examiner has overlooked the results at the top of Page 17 of the instant specification. In this connection, it should be noted that compound C), viz., octreotide pamoate, is administered as a single injection of a slow release form at 30 mg/kg s.c., i.e. , a total amount of 0.6 mg of octreotide pamoate (based on an average weight of a mouse of 20g) is released over the three weeks of treatment in the in vivo assay. In any event, set forth below are the results which appear in the Table bridging Pages 16 and 17 of the instant specification:

Treatment	Volume (mm^3)	Shrinkage over the control
Control	**4020**	
Compound B	**3685**	**8.3%**
Rapamycin	**2748**	**31.7%**
Octreotide C	**2205**	**45%**
B+octreotide C	**130**	**96%**
Rapamycin +octreotide C	**106**	**97.3%**

In brief, the tumors in the "control" animals became so large that the animals had to be killed. In the animals treated with only one of the active components, the tumor growth continued, not as excessive as with the control animals, but could not be stopped. On the other hand, a marked inhibition of the tumor growth occurred in the animals treated with a combination of the active compounds - the tumor inhibition effect was not additive, as would be expected, but synergistic which is quite surprising and unexpected.

Applicant is at a loss to understand the basis of the Examiner's allegation that the in vivo data in the instant application is confusing. As indicated above, octreotide pamoate is administered as a single injection of a slow release form at 30 mg/kg s.c., i.e., a total amount of 0.6 mg of octreotide pamoate is released over the three weeks of treatment in the in vivo assay. The tumor volume is then assessed four weeks later. With regard to the dosage

ranges set forth in the first complete paragraph on Page 18 of the instant specification, the daily dosages for the "somatostatin" component, i.e., the octreotide compound, reflect the amount of the free peptide which is to be administered. This should not be confused with the various salt forms of octreotide, e.g., the pamoate salt form, or administration in slow release form. For purposes of information, Sandostotin LAR® (octreotide in slow release form as used in the in vivo assay on Pages 16 and 17 of the instant specification) is administered in acromegalic humans at a single dose of 20 mg or 30 mg of free peptide every four weeks. RAD (compound B), which is used at a dosage of 5 mg/kg per day in the in vivo assay on Pages 16 and 17 of the instant specification, is administered at a daily dose of 1 mg to 3 mg in transplanted humans. In short, there is nothing confusing between the amounts of the active components utilized in the in vivo assay on Pages 16 and 17 of the instant specification and the dosage ranges set forth in the first complete paragraph on Page 18 of the instant specification.

In view of the foregoing, the Examiner is respectfully requested to reconsider the rejection of Claims 6-15 (now Claims 6, 8-10, 12, 13 and 15-18) under the first paragraph of 35 USC 112 for lack of descriptive support and withdraw it.

The Examiner has also rejected Claims 13-15 under the first paragraph of 35 USC 112 as being based on a specification which is "non-enabling". The Examiner continues to maintain that although the instant specification is enabling for the treatment of hyperproliferation of pancreatic cells, it is non-enabling for the "prevention" of hyperproliferation and for "treating" other than pancreatic cells. In other words, it is the Examiner's belief that one skilled in the art would be unable to practice the intention without undue experimentation since the instant specification is devoid of any guidance as to which subjects would be "susceptible to cell hyperproliferation".

Whether or not Claims 13-15 were properly rejectable under the first paragraph of 35 USC 112 is being based on a specification which is "non-enabling" prior to the foregoing amendments is irrelevant. What is relevant is whether Claim 13 (as amended), "new" Claim 18 (which replaced Claim 14), and Claim 15 are based upon a specification which is "non-enabling" and, therefore, properly rejectable under the first paragraph of 35 USC 112 and, Applicant respectfully submits, they are not.

As indicated above, Claim 13, upon which "new" Claim 18 and Claim 15 depend or ultimately depend, is now directed to a "method of inhibiting cell hyperproliferation", which language is inherently supported by the teachings of the instant specification.

In addition, the instant specification discloses an in vitro assay which establishes the effectiveness of the instantly claimed compositions in inhibiting the growth of tumor cells, and an in vivo assay which not only establishes the effectiveness of the instantly claimed compositions in treating and preventing pancreatic tumor growth but, in addition, establishes their effectiveness in preventing the hyperproliferation of malignant pancreatic cells. Since there is nothing in the statute or in the case law which requires that test data must be provided in order to meet the "enablement" requirement of 35 USC 112, Applicant has, in fact, provided more than is required by the statute.

As to the Examiner's contention that the test data in the instant specification is enabling only for the treatment of pancreatic cells, it should be noted that, more correctly, pancreatic tumor cells were utilized in the in vitro and the in vivo assays set forth in the instant specification. In any event, there is nothing in the record nor is Applicant aware of any scientific evidence or reasoning which supports the Examiner's contention. Absent such evidence or reasoning, it is believed that one skilled in the art, from a reading of the instant specification and the test data set forth therein, would conclude that the instantly claimed compositions would be effective in inhibiting the growth of other tumor cells.

Before concluding, and with regard to the Examiner's contention that Applicant did not address this rejection in the previous response, the Examiner's attention is respectfully directed to Page 5, line 9 to Page 6, line 9 of the Amendment filed June 13, 2000.

In view of the foregoing, the Examiner is respectfully requested to withdraw the "non-enabling" rejection under the first paragraph of 35 USC 112 as it applies to Claim 13, as amended, "new" Claim 18 (which replaced Claim 14) and Claim 15.

Lastly, the Examiner has rejected Claims 6-15 under 35 USC 103(a) as being obvious over the admitted prior art, British Patent 2,239,178 and WO 93/11130. The Examiner again contends that, in view of the disclosures in the last paragraph on Page 12 and the first paragraph on Page 13 of the instant specification, coupled with the teachings in the British and World patents, it would have been obvious for one skilled in the art to use a compound of the somatostatin class and a rapamycin macrolide in combination for treating cell

hyperproliferation and to prepare a kit or package comprising said combination. In addition, the Examiner has noted that the "dosage" ranges" set forth in the specification for the somatostatin and rapamycin compounds overlap with the dosage ranges disclosed in the prior art for said compounds. Accordingly, the Examiner concludes that Claims 6-15 are obvious and has again cited certain case law to support his position. Although Applicant agrees with the strictures set forth in the case law relied upon by the Examiner, whether or not the teachings of the prior art discussed in the instant specification, British Patent 2,239,178 and WO 93/11130 rendered any of Claims 6-15 obvious prior to the foregoing amendments is irrelevant. What is relevant is whether the teachings of the prior art discussed in the instant specification, British Patent 2,239,178 and WO 93/11130 render any of Claims 6 (as amended), 8, 9, 10 (as amended), 12, 13 (as amended), 15 and "new" Claims 16-18 obvious and, Applicant respectfully submits, they do not.

As indicated in the instant specification, Applicant has discovered that the administration of a combination of a compound of the somatostatin class and a rapamycin macrolide, i.e., a combination of compounds which belong to different classes and operate by different mechanisms, exhibits synergistic cell hyperproliferation inhibition. More particularly, Applicant surprisingly discovered that the administration of a combination of a compound of the somatostatin class which binds to somatostatin receptors (often expressed on tumor cells) and inhibits the growth hormone, and a rapamycin macrolide which binds in the cytoplasm to an immunophilin binding protein called FKBP-12 and the resulting complex, e.g., rapamycin/FKBP-12, binds to a protein named FRAP, inhibits cell hyperproliferation synergistically. Accordingly, in view of their ability to inhibit cell hyperproliferation, said combinations are useful in the prevention or treatment of malignant tumor growth and in the prevention or treatment of proliferative vascular diseases.

As to the relevancy of the teachings of the prior art being relied upon by the Examiner to the instant claims which, by the foregoing amendments, are now limited to a combination of an analog of somatostatin-14 which binds to at least the hSST-2 receptor in the nMolar range and two specific rapamycin compounds in synergistic effective amounts, there is simply nothing in the prior art being relied upon by the Examiner which would lead one skilled in the art to any of the combinations which characterize the instant claims. This is especially true since it is well known that the two rapamycin compounds of the instant claims are immunosuppresants which block T-cell mitogenesis and were developed to prevent graft rejection. In fact, rapamycin was approved in the U.S. for this use, whereas the other rapamycin compound of the instant claims is in clinical Phase 3 for the same indication.

Moreover, neither of the rapamycin compounds of the instant claims was developed as an inhibitor of tumor cells. Accordingly, it is quite surprising that the combinations to which the instant claims are now limited exhibit a synergistic inhibitory effect on the hyperproliferation of malignant cells or vascular cells.

In addition, there is clearly nothing in the prior art being relied upon by the Examiner which would lead one skilled in the art to employ the individual compounds of the instantly claimed combinations in synergistic effective amounts. In this connection, it is Applicant's belief that the Examiner's comment concerning the "overlap" in the dosage ranges actually supports patentability since one skilled in the art would expect the claimed combinations to exhibit, at best, an additive effect.

For essentially the reasons discussed above, Applicant does not believe that the teachings of the prior art being relied upon by the Examiner render the pharmaceutical kits of Claims 6, 8, 9 and 16 prima facie obvious. Applicant submits that it is not obvious to prepare a pharmaceutical kit when the motivation to do so is totally absent from the prior art, as is the case in the instant rejection. It is clear that there is nothing in the prior art relied upon by the Examiner which would provide one skilled in the art with the motivation to prepare any of the claimed pharmaceutical kits. Since the claimed pharmaceutical compositions are novel and unobvious, the claimed pharmaceutical kits are novel and obvious.

Similarly, Applicant does not believe that the teachings of the prior art relied upon by the Examiner render the "method-of-use" of Claims 13, 15 and 18 prima facie obvious. A process involving the use of a novel and unobvious composition of matter is itself novel and unobvious. In re Kuehl, 177 USPQ 250; In re Schneider, et al., 179 USPQ 46; and In re Wadlinger, et al., 181 USPQ 826.

In view of the foregoing, it is clear that the teachings of the prior art being relied upon by the Examiner do not render any of the instant claims prima facie obvious. Therefore, withdrawal of the 35 USC 103(a) rejection of Claims 6-15 (now Claims 6, 8-10, 12, 13 and 15-18) is respectfully requested.

All of the rejections of record having been overcome, the instant application is deemed to be in condition for allowance, and an early notice to that effect is earnestly solicited.

Although three "new" dependent claims were added by this Amendment, three dependent claims were also cancelled. Accordingly, no additional fee is necessitated by the foregoing amendments. However, since this Amendment will be deemed to have been filed more than four months from, but within five months of, the date of the Office Action (i.e., August 22, 2000), it is respectfully requested that the period for filing a response to said Office action be extended by two months. Please charge the $390 fee required by 37 CFR 1.17(a)(2) for a two month extension of time to Deposit Account No. 19-0134 in the name of Novartis Corporation. In this connection, two additional copies of this page are appended.

Respectfully submitted,

Novartis Pharmaceuticals Corporation
Patent and Trademark Dept.
564 Morris Avenue
Summit, NJ 07901-1027
(908) 522-6921

Joseph J. Borovian
Agent for Applicant
Reg. No. 26,631

JJB:bks

Encls.: Two additional copies of this page
Postcard

Date: January 22, 2001

Although three "new" dependent claims were added by this Amendment, three dependent claims were also cancelled. Accordingly, no additional fee is necessitated by the foregoing amendments. However, since this Amendment will be deemed to have been filed more than four months from, but within five months of, the date of the Office Action (i.e., August 22, 2000), it is respectfully requested that the period for filing a response to said Office action be extended by two months. Please charge the $390 fee required by 37 CFR 1.17(a)(2) for a two month extension of time to Deposit Account No. 19-0134 in the name of Novartis Corporation. In this connection, two additional copies of this page are appended.

Respectfully submitted,

Novartis Pharmaceuticals Corporation
Patent and Trademark Dept.
564 Morris Avenue
Summit, NJ 07901-1027
(908) 522-6921

Joseph J. Borovian
Agent for Applicant
Reg. No. 26,631

JJB:bks

Encls.: Two additional copies of this page
Postcard

Date: January 22, 2001

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER OF PATENTS AND TRADEMARKS
Washington, D.C. 20231

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.
09/194,957	12/07/98	WECKBECKER	G 4-100-8322/A

EXAMINER	
BORIN, M	
ART UNIT	PAPER NUMBER
1631	9

DATE MAILED: 04/04/01

00095
THOMAS HOXIE
NOVARTIS CORPORATION
PATENT AND TRADEMARK DEPT
564 MORRIS AVENUE
SUMMIT NJ 07901-1027

HM12/0404

Please find below and/or attached an Office communication concerning this application or proceeding.

Commissioner of Patents and Trademarks

PTO-90C (Rev.11/00)

1- File Copy

Office Action Summary

Application No.	Applicant(s)
09/194,957	Weckbecker
Examiner	**Group Art Unit**
Michael Borin	1631

☒ Responsive to communication(s) filed on *Jan 25, 2001* .

☒ This action is **FINAL.**

☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11; 453 O.G. 213.

A shortened statutory period for response to this action is set to expire *3* month(s), or thirty days, whichever is longer, from the mailing date of this communication. Failure to respond within the period for response will cause the application to become abandoned. (35 U.S.C. § 133). Extensions of time may be obtained under the provisions of 37 CFR 1.136(a).

Disposition of Claims

☒ Claim(s) *6, 8-10, 12, 13, and 15-18* is/are pending in the application.

Of the above, claim(s) _____ is/are withdrawn from consideration.

☐ Claim(s) _____ is/are allowed.

☒ Claim(s) *6, 8-10, 12, 13, and 15-18* is/are rejected.

☐ Claim(s) _____ is/are objected to.

☐ Claims _____ are subject to restriction or election requirement.

Application Papers

☐ See the attached Notice of Draftsperson's Patent Drawing Review, PTO-948.

☐ The drawing(s) filed on _____ is/are objected to by the Examiner.

☐ The proposed drawing correction, filed on _____ is ☐ approved ☐ disapproved.

☐ The specification is objected to by the Examiner.

☐ The oath or declaration is objected to by the Examiner.

Priority under 35 U.S.C. § 119

☐ Acknowledgement is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d).

☐ All ☐ Some* ☐ None of the CERTIFIED copies of the priority documents have been

☐ received.

☐ received in Application No. (Series Code/Serial Number) _____ .

☐ received in this national stage application from the International Bureau (PCT Rule 17.2(a)).

*Certified copies not received: _____ .

☐ Acknowledgement is made of a claim for domestic priority under 35 U.S.C. § 119(e).

Attachment(s)

☐ Notice of References Cited, PTO-892

☐ Information Disclosure Statement(s), PTO-1449, Paper No(s). _____

☐ Interview Summary, PTO-413

☐ Notice of Draftsperson's Patent Drawing Review, PTO-948

☐ Notice of Informal Patent Application, PTO-152

--- SEE OFFICE ACTION ON THE FOLLOWING PAGES ---

DETAILED ACTION

Status of Claims

1. Amendment filed 01/25/01 is acknowledged. Claims 6,8,10,12,13,15 are amended. Claims 16-18 are added. Claims 7,11,14 are canceled. Claims 6, 8-10,12, 13, 15-18 are pending.

2. Applicants arguments have been considered and are deemed to be persuasive in part.

3. The objection to claims 6,10,13 is withdrawn in view of the amendment to the claims.

Claim Rejections - 35 U.S.C. § 112, first paragraph.

4. Claims 6, 8-10,12, 13, 15-18 are rejected under 35 U.S.C. 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventors, at the time the application was filed, had possession of the claimed invention. See previous Office action, paragraph #4. The rejection is withdrawn as related to *in vivo* effect but is maintained for the reasons of record in regard to the breadth of the claims.

The only argument presented by applicant is that "one skilled in the art would expect that all of the somatostatin analogs will exhibit a synergistic effect when combined with somatostatin" (p. 16

of the response, first full paragraph). Examiner respectfully disagrees. It does not seem that the inventors had possession of the compositions being present in "synergistic amounts" for the scope of somatostatin analogs claimed. Unpredictability of synergistic effects is well known in pharmaceutical art. Prior art is full of examples that synergism in *prima facie* unpredictable and need to be demonstrated for any particular combination of biologically active compounds. For example, Gilewski et al. (Database Medline, DN 90140745) reviewing anti-cancer treatments concludes that "synergism among active agents is not necessarily assured and quite unexpected". Holmes (Database Caplus, DN 126:54378) emphasizes that synergistic effect is unpredictable because of the unpredictable nature of drug interactions. As another example, Bertrou (Database Caplus, DN 113:112366) demonstrates unpredictability in synergistic effects of antibiotics.

Accordingly, it is clear that applicant is not in possession of the presently claimed invention because the possession of the scope of the presently claimed subject matter is not demonstrated. Limiting the scope of the claims to octreotide would overcome the rejection.

5. Rejection of claims 13-15 under 35 U.S.C. 112, first paragraph, is withdrawn in view of amendment to the claims.

Claim Rejections - 35 U.S.C. § 103.

6. Claims 6, 8-10,12, 13, 15-18 remain rejected under 35 U.S.C. 103(a) as obvious over admitted prior art or GB 2239178 and WO 9311130. The rejection is maintained for the reasons set forth in the previous Office action, paragraph #6.

Applicants asserts that the rejection is irrelevant in view of amendments to the claims. However, Examiner failed to identify changes in the claim language which would make the rejection moot. As stated in the rejection, because combination therapies for treatment of abnormal cellular hyperproliferation are well-known in the art and because it would have been desirable to use plural therapies in order to maximize the probability that abnormal cellular hyperproliferation is minimized, it would be *prima facie* obvious to one of ordinary skills in the art at the time the invention was made to be motivated to use somatostatins and rapamycin in combination. The concentrations of the components, as disclosed in the specification, are within the range known in the art. The claims do not recite any particular dosage range which would distinguish the "synergistic amounts" of the components from those known in the art. The motivation in the prior art to combine references need not be identical to that of the applicant to establish obviousness. In re Kemps, 40 USPQ2d 1309 (Fed. Cir., 1996). Thus, it would be obvious to combine a somatostatin and a rapamycin analog taken in the concentrations known in the art to achieve the antiproliferative effect known for both components. The presence of unexpected results, such as synergistic effect, would obviate the rejection, if the claims are limited to combinations demonstrating such unexpected beneficial effect. Showing of synergistic effect for just one somatostatin compound, however, is not commensurate with the broad scope of somatostatins claimed.

Conclusion.

7. No claims are allowed. Limiting the scope of the claims to combinations of octreotide would overcome the rejections of record.

8. **THIS ACTION IS MADE FINAL.** Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a).

A shortened statutory period for reply to this final action is set to expire THREE MONTHS from the mailing date of this action. In the event a first reply is filed within TWO MONTHS of the mailing date of this final action and the advisory action is not mailed until after the end of the THREE-MONTH shortened statutory period, then the shortened statutory period will expire on the date the advisory action is mailed, and any extension fee pursuant to 37 CFR 1.136(a) will be calculated from the mailing date of the advisory action. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the mailing date of this final action.

9. Any inquiry concerning this communication or earlier communications from the examiner should be directed to Michael Borin whose telephone number is (703) 305-4506. Dr. Borin can normally be reached between the hours of 8:30 A.M. to 5:00 P.M. EST Monday to Friday. If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Mr. Michael Woodward, can be reached on (703) 308-4028. The fax telephone number for this group is (703) 305-3014.

Any inquiry of a general nature or relating the status of this application should be directed to the Group receptionist whose telephone number is (703) 308-0196.

MICHAEL BORIN, PH.D
PRIMARY EXAMINER

07-06-01

AF/ #10/ GP/ 1631 EP 7/11/01 AF/

CASE 4-100-8322/A/PCT

FILING BY "EXPRESS MAIL" UNDER 37 CFR 1.10

EL 820013813 US — Express Mail Label Number

July 5, 2001 — Date of Deposit

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

IN RE PCT NATIONAL STAGE APPLICATION OF
GISBERT WECKBECKER
INTERNATIONAL APPLICATION NO: PCT/EP 97/03036
FILED: 11 JUNE 1997
U.S. APPLICATION NO: 09/194,957
35 USC §371 DATE: 7 DECEMBER 1998
FOR: COMBINATION OF A SOMATOSTATIN ANALOGUE AND A RAPAMYCIN

Art Unit: 1631
Examiner: M. Borin

Assistant Commissioner for Patents
Washington, D.C. 20231

AMENDMENT UNDER 37 CFR 1.116

Sir:

Responsive to the Final Rejection dated April 4, 2001, kindly amend the above-identified application as follows:

IN THE CLAIMS

Please cancel Claims 6, 8-10, 12, 13 and 15-18.

Please add the following new claims:

E1 -- 19. A kit or package for the inhibition of cell hyperproliferation, said kit or package including a pharmaceutical composition comprising an analogue of somatostatin-14 binding to at least the hSST-2 receptor in the nMolar range selected from

a) (D)Phe-Cys-Phe-(D)Trp-Lys-Thr-Cys-Thr-ol

b) (D)Phe-Cys-Tyr-(D)Trp-Lys-Val-Cys-ThrNH_2

c) (D)Phe-Cys-Tyr-(D)Trp-Lys-Val-Cys-TrpNH_2

d) (D)Trp-Cys-Phe-(D)Trp-Lys-Thr-Cys-ThrNH_2

e) (D)Phe-Cys-Phe-(D)Trp-Lys-Thr-Cys-ThrNH_2

f) 3-(2-(Naphthyl)-(D)Ala-Cys-Tyr-(D)Trp-Lys-Val-Cys-ThrNH_2

g) (D)Phe-Cys-Tyr-(D)Trp-Lys-Val-Cys-β-Nal-NH_2

h) 3-(2-naphthyl)Ala-Cys-Tyr-(D)Trp-Lys-Val-Cys-β-Nal-NH_2

i) (D)Phe-Cys-β-Nal-(D)Trp-Lys-Val-Cys-Thr-NH_2

j) (D)Phe-Cys-Tyr-(D)Trp-Lys-Leu-Cys-Thr-NH_2 and

k) (D)Phe-Cys-Tyr-(D)Trp-Lys-Cys-Thr-NH_2,

in free form or in pharmaceutically acceptable salt form, and a pharmaceutical composition comprising a rapamycin macrolide selected from rapamycin and 40-O-(2-hydroxyethyl)-rapamycin, said compositions being present in synergistic effective amounts, together with instructions for use. --

-- 20. A kit or package according to Claim 19 wherein the analogue of somatostatin-14 is selected from

a) (D)Phe-Cys-Phe-(D)Trp-Lys-Thr-Cys-Thr-ol

c) (D)Phe-Cys-Tyr-(D)Trp-Lys-Val-Cys-TrpNH_2 and

f) 3-(2-(Naphthyl)-(D)Ala-Cys-Tyr-(D)Trp-Lys-Val-Cys-ThrNH_2,

in free form or pharmaceutically acceptable salt form. --

21. A kit or package according to Claim 20 wherein the analogue of somatostatin-14 is

(D)Phe-Cys-Phe-(D)Trp-Lys-Thr-Cys-Thr-ol,

in free form or in pamoate salt form. --

-- 22. A kit or package according to Claim 21 wherein the somatastatin-14 analogue is in sustained release form and the rapamycin macrolide is 40-O-(2-hydroxyethyl)-rapamycin. --

-- 23. A kit or package according to Claim 19 for simultaneous or sequential use in synergistically effective amounts. --

-- 24. A pharmaceutical composition comprising a pharmaceutically acceptable carrier and a therapeutically effective amount of: 1) an analogue of somatostatin-14 binding to at least the hSST-2 receptor in the nMolar range selected from

a) (D)Phe-Cys-Phe-(D)Trp-Lys-Thr-Cys-Thr-ol

b) (D)Phe-Cys-Tyr-(D)Trp-Lys-Val-Cys-ThrNH_2

c) (D)Phe-Cys-Tyr-(D)Trp-Lys-Val-Cys-TrpNH_2

d) (D)Trp-Cys-Phe-(D)Trp-Lys-Thr-Cys-ThrNH_2

e) (D)Phe-Cys-Phe-(D)Trp-Lys-Thr-Cys-ThrNH_2

f) 3-(2-(Naphthyl)-(D)Ala-Cys-Tyr-(D)Trp-Lys-Val-Cys-ThrNH_2

g) (D)Phe-Cys-Tyr-(D)Trp-Lys-Val-Cys-β-Nal-NH_2

h) 3-(2-naphthyl)Ala-Cys-Tyr-(D)Trp-Lys-Val-Cys-β-Nal-NH_2

i) (D)Phe-Cys-β-Nal-(D)Trp-Lys-Val-Cys-Thr-NH_2

j) (D)Phe-Cys-Tyr-(D)Trp-Lys-Leu-Cys-Thr-NH_2 and

k) (D)Phe-Cys-Tyr-(D)Trp-Lys-Cys-Thr-NH_2,

in free form or pharmaceutically acceptable salt form; and 2) a rapamycin macrolide selected from rapamycin and 40-O-(2-hydroxyethyl)-rapamycin, said somatastatin-14 analogue and macrolide being present in synergistic effective amounts. --

-- 25. A composition according to Claim 24 wherein the analogue of somatostatin-14 is selected from

a) (D)Phe-Cys-Phe-(D)Trp-Lys-Thr-Cys-Thr-ol

c) (D)Phe-Cys-Tyr-(D)Trp-Lys-Val-Cys-TrpNH_2 and

f) 3-(2-(Naphthyl)-(D)Ala-Cys-Tyr-(D)Trp-Lys-Val-Cys-ThrNH_2 ;

in free form or pharmaceutically acceptable salt form. --

-- 26. A composition according to Claim 25 wherein the analogue of somatastatin-14 is

(D)Phe-Cys-Phe-(D)-Trp-Lys-Thr-Cys-Thr-ol,

in free form or in pamoate salt form. --

-- 27. A composition according to claim 26 wherein the somatostatin-14 analogue is in sustained release form and the rapamycin macrolide is 40-O-(2-hydroxyethyl)-rapamycin. --

-- 28. A method of inhibiting cell hyperproliferation comprising administering to a subject in need of such treatment a therapeutically effective amount of: 1) an analogue of somatostatin-14 binding to at least the hSST-2 receptor in the nMolar range selected from

a) (D)Phe-Cys-Phe-(D)Trp-Lys-Thr-Cys-Thr-ol

b) (D)Phe-Cys-Tyr-(D)Trp-Lys-Val-Cys-ThrNH_2

c) (D)Phe-Cys-Tyr-(D)Trp-Lys-Val-Cys-TrpNH_2

d) (D)Trp-Cys-Phe-(D)Trp-Lys-Thr-Cys-ThrNH_2

e) (D)Phe-Cys-Phe-(D)Trp-Lys-Thr-Cys-ThrNH_2

f) 3-(2-(Naphthyl)-(D)Ala-Cys-Tyr-(D)Trp-Lys-Val-Cys-ThrNH_2

g) (D)Phe-Cys-Tyr-(D)Trp-Lys-Val-Cys-β-Nal-NH_2

h) 3-(2-naphthyl)Ala-Cys-Tyr-(D)Trp-Lys-Val-Cys-β-Nal-NH_2

i) (D)Phe-Cys-β-Nal-(D)Trp-Lys-Val-Cys-Thr-NH_2

j) (D)Phe-Cys-Tyr-(D)Trp-Lys-Leu-Cys-Thr-NH_2 and

k) (D)Phe-Cys-Tyr-(D)Trp-Lys-Cys-Thr-NH_2,

in free form or pharmaceutically acceptable salt form; and 2) a rapamycin macrolide selected from rapamycin and 40-O-(2-hydroxyethyl)-rapamycin, said somatostatin-14 analogue and macrolide being present in synergistic effective amounts. --

-- 29. A method according to Claim 28 wherein the analogue of somatostatin-14 is selected from

a) (D)Phe-Cys-Phe-(D)Trp-Lys-Thr-Cys-Thr-ol,

c) (D)Phe-Cys-Tyr-(D)Trp-Lys-Val-Cys-$TrpNH_2$ and

f) 3-(2-(Naphthyl)-(D)Ala-Cys-Tyr-(D)Trp-Lys-Val-Cys-$ThrNH_2$,

in free form or pharmaceutically acceptable salt form. --

-- 30. A method according to Claim 29 wherein the analogue of somatastatin-14 is

(D)Phe-Cys-Phe-(D)Trp-Lys-Thr-Cys-Thr-ol,

in free form or in pamoate salt form. --

-- 31. A method according to Claim 30 wherein the somatostatin-14 analogue is in sustained release form and the rapamycin macrolide is 40-O-(2-hydroxyethyl)-rapamycin. --

REMARKS

A favorable reconsideration of this application is respectfully requested in view of the foregoing amendments and the following remarks.

Claims 6, 8-10, 12, 13 and 15-18 were presented for examination, and Claims 19-31 are now present in the case.

Claims 6, 8, 9 and 16 have been cancelled and replaced by "new" Claims 19, 20 and 23; Claims 10, 12 and 17 have been cancelled and replaced by "new" Claims 24 and 25; and Claims 13, 15 and 18 have been cancelled and replaced by "new" Claims 28 and 29. As can be clearly

seen, the "somatostatin-14 analogue" in "broad" Claims 19, 24 and 28 has been drastically limited to a Markush group of eleven compounds.

"New" Claims 21 and 22 are directed to preferred embodiments of "new" Claim 19; "new" Claims 26 and 27 are directed to preferred embodiments of "new" Claim 24; and "new" Claims are 30 and 31 are directed to preferred embodiments of "new" Claim 28. Support for said preferred embodiments may be found in the instant specification on Page 5, line 6; Page 12, lines 6 and 7; and the *in vivo* results set forth in the paragraph bridging Pages 16 and 17.

Applicant acknowledges the Examiner's withdrawal of: 1) the objection to Claims 6, 10 and 13; 2) the rejection of Claims 13-15 under the first paragraph of 35 USC 112; and 3) that part of the rejection of Claims 6-15 under the first paragraph of 35 USC 112 based on the contention that the specification not only lacked *in vivo* synergistic evidence, but that said evidence was confusing.

As to the issues which remain, the Examiner has rejected Claims 6, 8-10, 12, 13 and 15-18 under the first paragraph of 35 USC 112 as containing subject matter which is not adequately described in the specification. More particularly, the Examiner is of the opinion that the specification lacks adequate descriptive support regarding the term "synergistic effective amounts" for the scope of the somatostatin analogues claimed and, in this connection, relies on three "new" references as evidence of the unpredictability of synergistic effects in the pharmaceutical area.

As indicated above, the "somatostatin-14 analogue" in "broad" Claims 19, 24 and 28 has been drastically limited to a Markush group of eleven compounds. Since compounds b)-k) in said claims are closely related analogues of compound a), and since the latter compound, as well as the somatostatin analogues of the instant claims are known to inhibit cell proliferation and tumor growth, it is respectfully submitted that <u>all</u> of the somatostatin analogues would exhibit a synergistic anti-tumor effect when combined with the rapamycin macrolide.

In view of the foregoing, the Examiner is respectfully requested to reconsider the rejection of Claims 6, 8-10, 12, 13 and 15-18 (now Claims 19, 20, 23-25, 28 and 29) under the first paragraph of 35 USC 112 for lack of descriptive support and withdraw it.

In addition, the Examiner has rejected Claims 6, 8-10, 12, 13 and 15-18 under 35 USC 103 (a) as being obvious over the admitted prior art, British Patent 2,239,178 and WO 93/11130, for the reasons set forth in the previous Office Action, i.e., the Official Action dated August 22, 2000. The

Examiner continues to maintain that in view of the disclosures in the last paragraph on page 12 and the first paragraph on page 13 of the instant specification coupled with the teachings in the British and World patents, it would have been obvious for one skilled in the art to use a compound of the somatostatin class and rapamycin in combination for treating cell hyperproliferation and to prepare a kit or package comprising said combination. In addition, the Examiner relies on certain case law to support his position and has again noted that the "dosage ranges" set forth in the specification for the somatostatin and rapamycin compounds overlap with the dosage ranges disclosed in the prior art for said compounds. Although Applicant agrees with the strictures set forth in the case law relied upon by the Examiner, Applicant disagrees with the Examiner's conclusion that the teachings of the prior art in the instant specification, British Patent 2,239,178 and WO 93/11130, rendered Claims 6, 8-10, 12, 13 and 15-18 obvious. In addition, Applicant does not believe that the aforementioned prior art prejudices the patentability of any of the instant claims from a 35 USC 103 standpoint.

For purposes of review, Applicant has discovered that the administration of a combination comprising a compound of the somatostatin class and a rapamycin macrolide, i.e., a combination of compounds which belong to different classes and operate by different mechanisms, exhibits synergistic cell hyperproliferation inhibition. More particularly, Applicant surprisingly discovered that the administration of a combination of a compound of the somatostatin class which binds to somatostatin receptors (often expressed on tumor cells) and inhibits the growth hormone, and a rapamycin macrolide which binds in the cytoplasm to an immunophilin binding protein called FKBP-12 and the resulting complex, e.g., rapamycin/FKBP-12, binds to a protein named FRAP, inhibits cell hyperproliferation synergistically. Accordingly, in view of their ability to inhibit cell hyperproliferation, said combinations are useful in the prevention or treatment of malignant tumor growth and in the prevention or treatment of proliferative vascular diseases.

As to the relevancy of the teachings of the prior art being relied upon by the Examiner to the instant claims which, by the foregoing amendments, are now limited to a combination comprising eleven specific analogues of somatostatin-14 which bind to at least the hSST-2 receptor in the nMolar range and two specific rapamycin compounds in synergistic effective amounts, there is simply nothing in the prior art being relied upon by the Examiner which would lead one skilled in the art to any of the combinations which characterize the instant claims. To wit, the rapamycin derivative to which the teachings of WO 93/11130 are limited bears little structural resemblance to the rapamycin macrolides to which the instant claims are limited. In addition, WO 93/11130 contains merely a speculative statement regarding the "anti-cancer" use of the rapamycin derivative

disclosed therein and coupled with the fact that it is devoid of any pharmacological data concerning said use, it is believed that the teachings of WO 93/11130 do not represent an "enabling" disclosure regarding the "anti-cancer" use. Moreover, WO 93/11130 is silent with respect to any teaching that the rapamycin derivative disclosed therein can be combined with any other compound, let alone the specific analogues of somatostatin-14 to which the instant claims are limited.

With regard to British Patent 2,239,178, it involves the discovery that somatostatin analogue-containing compositions can be better tolerated when administered parenterally for treating breast cancer if said compositions contain lactic acid. First of all, there is nothing in British Patent 2,239,178 which points to the specific analogues of somatostatin-14 which characterize the instant claims. In addition, other than the mention that it is preferred that the compositions contain a dopamine agonist, there is nothing within the four corners of British Patent 2,239,178 which suggests that any other compound can be suitably added, let alone either of the two rapamycin macrolides to which the instant claims are limited.

In addition, there is clearly nothing in the prior art being relied upon by the Examiner which would lead one skilled in the art to employ the individual compounds of the instantly claimed combinations in synergistic effective amounts. In this connection, it is Applicant's belief that the Examiner's comment concerning the "overlap" in the dosage ranges actually supports patentability, since one skilled in the art would expect the claimed combinations to exhibit a null or, at best, an additive effect. Accordingly, it is quite surprising that the combinations to which the instant claims are now limited exhibit a synergistic inhibitory effect on the hyperproliferation of malignant cells or vascular cells.

For essentially the reasons discussed above, Applicant does not believe that the teachings of the prior art being relied upon by the Examiner render the pharmaceutical kits of Claims 19-23 *prima facie* obvious. Applicant submits that it is not obvious to prepare a pharmaceutical kit when the motivation to do so is totally absent from the prior art, as is the case in the instant rejection. It is clear that there is nothing in the prior art relied upon by the Examiner which would provide one skilled in the art with the motivation to prepare any of the claimed pharmaceutical kits. Since the claimed pharmaceutical compositions are novel and unobvious, the claimed pharmaceutical kits are novel and obvious.

Similarly, Applicant does not believe that the teachings of the prior art relied upon by the Examiner render the "method-of-use" of Claims 28-31 *prima facie* obvious. A process involving the

use of a novel and unobvious composition of matter is itself novel and unobvious. In re Kuehl, 177 USPQ 250; In re Schneider et al., 179 USPQ 46; and In re Wadlinger et al., 181 USPQ 826.

In view of the foregoing, it is clear that the teachings of the prior art being relied upon by the Examiner do not render any of the instant claims *prima facie* obvious. Therefore, withdrawal of the USC 103(a) rejection of Claims 6, 8-10, 12, 13 and 15-18 (now Claims 19-31) is respectfully requested.

Both of the rejections of record having been overcome, the instant application is deemed to be in condition for allowance, and an early notice to that effect is earnestly solicited. However, in the event that this Amendment under 37 CFR 1.116 is not deemed to place this application in condition for allowance, it is respectfully requested that it be entered for appeal purposes.

Although thirteen "new" claims were added by this Amendment under 37 CFR 1.116, ten claims were cancelled. In any event, since neither the total number of claims nor the total number of independent claims exceed the highest number previously paid for, no additional fee is necessitated by this Amendment under 37 CFR 1.116.

Respectfully submitted,

Novartis Pharmaceuticals Corporation
Patent and Trademark Dept.
564 Morris Avenue
Summit, NJ 07901-1027
(908) 522-6921

Joseph J. Borovian
Agent for Applicant
Reg. No. 26,631

JJB:bks
Encl.: Postcard

Date: July 5, 2001

Interview Summary

Application No.	Applicant(s)
09/194,957	Weckbecker
Examiner	**Group Art Unit**
Michael Borin	1631

All participants (applicant, applicant's representative, PTO personnel):

(1) *Michael Borin* (3) _____

(2) *Joseph J. Borovian* (4) _____

Date of Interview *Jul 16, 2001*

Type: a)☒ Telephonic b)☐ Video Conference
c)☐ Personal [copy is given to 1)☐ applicant 2)☐ applicant's representative]

Exhibit shown or demonstration conducted: d)☐ Yes e)☒ No. If yes, brief description:

Claim(s) discussed: _____

Identification of prior art discussed:

Agreement with respect to the claims f)☐ was reached. g)☐ was not reached. h)☐ N/A.

Substance of Interview including description of the general nature of what was agreed to if an agreement was reached, or any other comments:

Applicant was notified that the response to final rejection has been received and would be acted upon promptly.

(A fuller description, if necessary, and a copy of the amendments which the examiner agreed would render the claims allowable, if available, must be attached. Also, where no copy of the amendments that would render the claims allowable is available, a summary thereof must be attached.)

i)☐ It is not necessary for applicant to provide a separate record of the substance of the interview (if box is checked).

Unless the paragraph above has been checked, THE FORMAL WRITTEN REPLY TO THE LAST OFFICE ACTION MUST INCLUDE THE SUBSTANCE OF THE INTERVIEW. (See MPEP section 713.04). If a reply to the last Office action has already been filed, APPLICANT IS GIVEN ONE MONTH FROM THIS INTERVIEW DATE TO FILE A STATEMENT OF THE SUBSTANCE OF THE INTERVIEW. See Summary of Record of Interview requirements on reverse side or on attached

MICHAEL BORIN
PRIMARY EXAMINER
ART UNIT 1631

Examiner Note: You must sign this form unless it is an Attachment to a signed Office action.

9/5/01 AF 1631

CASE 4-100-8322/A/PCT

CERTIFICATE OF MAILING

I hereby certify that this paper (along with any paper referred to as being attached or enclosed) is being deposited with the United States Postal Service on the date shown below with sufficient postage as first class mail in an envelope addressed to the: Assistant Commissioner for Patents, Washington, D.C. 20231.

Joseph J. Borovian	Joseph J Borovian	August 3, 2001
Type or print name	Signature	Date

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

IN RE PCT NATIONAL STAGE APPLICATION OF
GISBERT WECKBECKER
INTERNATIONAL APPLICATION NO.: PCT/EP 97/03036
FILED: 11 JUNE 1997
U.S. APPLICATION NO: 09/194,957
35 USC §371 DATE: 7 DECEMBER 1998
FOR: COMBINATION OF A SOMATOSTATIN ANALOGUE AND A RAPAMYCIN

Art Unit: 1631
Examiner: M. Bonn

Assistant Commissioner for Patents
Washington, D.C. 20231

NOTICE OF APPEAL

Sir:

Applicant hereby appeals to the Board of Appeals and Interferences from the Office Action dated April 4, 2001 finally rejecting Claims 6, 8-10, 12, 13 and 15-18 (now Claims 19-31 if the Amendment Under 37 CFR 1.116 is entered).

☒ Please charge Deposit Account No. 19-0134 in the name of Novartis Corporation in the amount of $310 for payment of the appeal fee. An additional copy of this paper is here enclosed. The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment, to Account No. 19-0134 in the name of Novartis Corporation.

☐ The appeal fee was paid in a previous appeal herein. The examiner re-opened prosecution prior to any decision by the Board of Patent Appeals and Interferences. No fee is now due.

08/08/2001 HVUONG1 00000008 190134 09194957
01 FC:119 310.00 CH

☒ Enclosed is a Petition for Extension of Time.

Respectfully submitted,

Novartis Pharmaceuticals Corporation
Patent and Trademark Dept.
564 Morris Avenue
Summit, NJ 07901-1027
(908) 522-6921

Joseph J. Borovian
Agent for Applicant
Reg. No. 26,631

JJB/pk

Encls.: An additional copy of this paper
Petition for Extension of Time (2)
Postcard

Date: August 3, 2001

#13 pg
8.5.01

CASE 4-100-8322/A/PCT

CERTIFICATE OF MAILING

I hereby certify that this paper (along with any paper referred to as being attached or enclosed) is being deposited with the United States Postal Service on the date shown below with sufficient postage as first class mail in an envelope addressed to the: Assistant Commissioner for Patents, Washington, D.C. 20231.

Joseph J. Borovian		August 3, 2001
Type or print name	Signature	Date

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

IN RE PCT NATIONAL STAGE APPLICATION OF
GISBERT WECKBECKER
INTERNATIONAL APPLICATION NO: PCT/EP 97/03036
FILED: 11 JUNE 1997
U.S. APPLICATION NO.: 09/194,957
35 USC §371 DATE: 7 DECEMBER 1998
FOR: COMBINATION OF A SOMATOSTATIN ANALOGUE AND A RAPAMYCIN

Art Unit: 1631
Examiner: M. Borin

Assistant Commissioner for Patents
Washington, D.C. 20231

PETITION FOR EXTENSION OF TIME

Sir:

The period for filing a Notice of Appeal has a shortened statutory time set to expire on July 4, 2001. A one-month extension is hereby requested pursuant to 37 CFR §1.136(a).

Please charge Deposit Account No. 19-0134 in the name of Novartis Corporation in the amount of $110 for payment of the extension fee. An additional copy of this paper is here enclosed. The Commissioner is hereby authorized to charge any additional fees under 37 CFR §1.17 which may be required, or credit any overpayment, to Account No. 19-0134 in the name of Novartis Corporation.

08/08/2001 HVUONG1 00000008 190134 09194957
02 FC:115 110.00 CH

Respectfully submitted,

Novartis Pharmaceuticals Corporation
Patent and Trademark Dept.
564 Morris Avenue
Summit, NJ 07901-1027

Date: August 3, 2001

Joseph J. Borovian
Agent for Applicant
Reg. No. 26,631
Phone No. (908) 522-6921

UNITED STATE DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER OF PATENTS AND TRADEMARKS
Washington, D.C. 20231

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.
09/194,957	12/07/98	WECKBECKER	G 4-100-8322/A

001095
THOMAS HOXIE
NOVARTIS CORPORATION
PATENT AND TRADEMARK DEPT
564 MORRIS AVENUE
SUMMIT NJ 07901-1027

HM22/0813

EXAMINER	
BORIN, M	
ART UNIT	PAPER NUMBER
1631	12

DATE MAILED: 08/13/01

Please find below and/or attached an Office communication concerning this application or proceeding.

Commissioner of Patents and Trademarks

PTO-90C (Rev.11/00)

1- File Copy

Advisory Action

Application No.	Applicant(s)
09/194,957	Weckbecker
Examiner	**Art Unit**
Michael Borin	1631

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

THE REPLY FILED *Jul 5, 2001* FAILS TO PLACE THIS APPLICATION IN CONDITION FOR ALLOWANCE. Therefore, further action by the applicant is required to avoid the abandonment of this application. A proper reply to a final rejection under 37 CFR 1.113 may only be either: (1) a timely filed amendment which places the application in condition for allowance; (2) a timely filed Notice of Appeal (with appeal fee); or (3) a timely filed Request for Continued Examination (RCE) in compliance with 37 CFR 1.114.

THE PERIOD FOR REPLY [check only a) or b)]

a) ☒ The period for reply expires *3* months from the mailing date of the final rejection.

b) ☐ In view of the early submission of the proposed reply (within two months as set forth in MPEP § 706.07 (f)), the period for reply expires on the mailing date of this Advisory Action, OR continues to run from the mailing date of the final rejection, whichever is later. In no event, however, will the statutory period for the reply expire later than SIX MONTHS from the mailing date of the final rejection.

Extensions of time may be obtained under 37 CFR 1.136(a). The date on which the petition under 37 CFR 1.136(a) and the appropriate extension fee have been filed is the date for purposes of determining the period of extension and the corresponding amount of the fee. The appropriate extension fee under 37 CFR 1.17(a) is calculated from: (1) the expiration date of the shortened statutory period for reply originally set in the final Office action; or (2) as set forth in (b) above, if checked. Any reply received by the Office later than three months after the mailing date of the final rejection, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

1. ☐ A Notice of Appeal was filed on _____. Appellant's Brief must be filed within the period set forth in 37 CFR 1.192(a), or any extension thereof (37 CFR 1.191(d)), to avoid dismissal of the appeal.

2. ☐ The proposed amendment(s) will be entered upon the timely submission of a Notice of Appeal and Appeal Brief with requisite fees.

3. ☒ The proposed amendment(s) will not be entered because:

   (a) ☒ they raise new issues that would require further consideration and/or search. (See NOTE below);

   (b) ☒ they raise the issue of new matter. (See NOTE below);

   (c) ☐ they are not deemed to place the application in better form for appeal by materially reducing or simplifying the issues for appeal; and/or

   (d) ☐ they present additional claims without cancelling a corresponding number of finally rejected claims.

   NOTE: *New claims 27 and 31 will require enablement and new matter rejections under 112, first paragraph*

4. ☐ Applicant's reply has overcome the following rejection(s):

5. ☐ Newly proposed or amended claim(s) _____ would be allowable if submitted in a separate, timely filed amendment cancelling the non-allowable claim(s).

6. ☒ The a) ☐ affidavit, b) ☐ exhibit, or c) ☒ request for reconsideration has been considered but does NOT place the application in condition for allowance because:
   The issue of non-enablement of synergistic concentrations will remain, except for octreotide, for all somatostatin analogs. 103 rejection is maintained for the pending claims; the rejection will be modified if the proposed new

7. ☐ The affidavit or exhibit will NOT be considered because it is not directed SOLELY to issues which were newly raised by the Examiner in the final rejection.

8. ☒ For purposes of Appeal, the status of the claim(s) is as follows (see attached written explanation, if any):

   Claim(s) allowed: _____

   Claim(s) objected to: _____

   Claim(s) rejected: *6, 8-10, 12, 13, and 15-18*

9. ☐ The proposed drawing correction filed on _____ a) ☐ has b) ☐ has not been approved by the Examiner.

10. ☐ Note the attached Information Disclosure Statement(s) (PTO-1449) Paper No(s). _____.

11. ☐ Other:

MICHAEL BORIN
PRIMARY EXAMINER
ART UNIT 1631

08-29-01

#15 P8 9/5/01
AF/1631

CASE 4-100-8322/A/PCT

FILING BY "EXPRESS MAIL" UNDER 37 CFR 1.10

EL 820013858 US — Express Mail Label Number

August 28, 2001 — Date of Deposit

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

IN RE PCT NATIONAL STAGE APPLICATION OF
GISBERT WECKBECKER
INTERNATIONAL APPLICATION NO: PCT/EP 97/03036
FILED: 11 JUNE 1997
U.S. APPLICATION NO: 09/194,957
35 USC §371 DATE: 7 DECEMBER 1998
FOR: COMBINATION OF A SOMATOSTATIN ANALOGUE AND A RAPAMYCIN

Art Unit: 1631
Examiner: M. Borin

Assistant Commissioner for Patents
Washington, D.C. 20231

REQUEST FOR ENTRY AND RECONSIDERATION OF AMENDMENT UNDER 37 CFR 1.116

Sir:

This is in response to the Advisory Action dated August 13, 2001 denying entry of the Amendment Under 37 CFR 1.116.

According to the aforementioned Advisory Action, the Amendment Under 37 CFR 1.116 was denied entry because "new" Claims 27 and 31 raise: 1) new issues which would require further consideration and/or search; and 2) the issue of "new matter". Quite simply, Applicant is puzzled by the Examiner's contentions since they are clearly devoid of any basis.

As to "new" Claim 27, Applicant respectfully points out that said claim is directed to one of the specific compositions that was tested in the *in vivo* assay, viz., the composition disclosed on line 1 of Page 17, and further support for administering the somatostatin analogues in a "slow release" form may be found in the instant specification on Page 18, lines 4 and 5. With regard to "new" Claim 31, which is directed to the use of the composition of Claim 27, there can be no question that the *in vivo* results on Pages 16 and 17 of the instant specification establish the

efficacy of said composition in treating tumors. It is clear, therefore, that neither "new" Claim 27 nor "new" Claim 31 raises the issue of "new matter".

As to the Examiner's other contention, Applicant respectfully asks: "What new issues are raised by "new" Claims 27 and 31 which would require further consideration and/or search?" Since "new" Claims 27 and 31 do not raise the issue of "new matter", it is Applicant's belief that "new" Claims 27 and 31 do not raise any new issues which require further consideration and/or search. In the latter connection, since "new" Claim 27 ultimately depends from "new" Claim 24 and is embraced thereby, since "new" Claim 24 replaced Claim 10 and the scope of the former is embraced by the scope of the latter, and since the subject matter of Claim 10 was fully searched by the Examiner (see, in this connection, the Official Action dated February 14, 2000), it is clear that "new" Claim 27 does not raise any new issues which require a further search. Similarly, since "new" Claim 31 ultimately depends from "new" Claim 28 and is embraced thereby, since "new" Claim 28 replaced Claim 13 and the scope of the former is embraced by the scope of the latter, and since the subject matter of Claim 13 was fully searched by the Examiner (see again, in this connection, the Official Action dated February 14, 2000), it is clear that "new" Claim 31 also does not raise any issues which require a further search. In brief, it is Applicant's belief that neither "new" Claim 27 nor "new" Claim 31 raises new issues which require further consideration and/or search.

In view of the foregoing, it is clear that neither "new" Claim 27 nor "new" Claim 31 raises the issue of "new matter" or raises new issues which would require further consideration and/or search. Coupled with the fact that the "somatostatin-14 analogue" in "broad" Claims 19, 24 and 28 has been drastically limited to a Markush group of eleven compounds, there can be no question that the amendments effected by the Amendment Under 37 CFR 1.116 (i.e., the cancellation of Claims 6, 8-10, 12, 13 and 15-18 and the presentation of "new" Claims 19-31) place the case in better form for appeal. Accordingly, the Examiner is respectfully requested to reconsider his decision denying entry of the Amendment Under 37 CFR 1.116 and withdraw it.

Moreover, in view of the fact that the "broad" scopes of the claimed kits, compositions and their use have been drastically reduced by the Amendment Under 37 CFR 1.116, it is believed that said amendment has overcome the two remaining issues in the Final Rejection dated April 4, 2001 and placed the instant application in condition for allowance.

More particularly, since the "somatostatin-14 analogue" in "broad" Claims 19, 24 and 28 has been drastically limited to a Markush group of eleven compounds, since compounds b)-k) in said claims are closely related analogues of compound a), and since the latter compound, as well as the other somatostatin analogues of the instant claims are known to inhibit cell proliferation and tumor

growth, it is respectfully submitted that one skilled in the art would expect that all of the somatostatin analogues to which Claims 19, 24 and 28 are limited would exhibit a synergistic anti-tumor effect when combined with the rapamycin macrolide.

In view of the foregoing, the Examiner is again respectfully requested to reconsider the rejection of Claims 6, 8-10, 12, 13, and 15-18 (now Claims 19, 20, 23-25, 28 and 29) under the first paragraph of 35 USC§112 for lack of descriptive support and withdraw it.

Moreover, there is nothing in the prior art being relied upon by the Examiner or in any of the other prior art of which Applicant is aware which would lead one skilled in the art to any of the specific combinations which characterize the instant claims. To wit, the rapamycin derivative to which the teachings of WO 93/11130 are limited bears little structural resemblance to the rapamycin macrolides to which the instant claims are limited. In addition, WO 93/11130 contains merely a speculative statement regarding the "anti-cancer" use of the rapamycin derivative disclosed therein and coupled with the fact that it is devoid of any pharmacological data concerning said use, it is believed that the teachings of WO 93/11130 do not represent an "enabling" disclosure regarding the "anti-cancer" use. Moreover, WO 93/11130 is silent with respect to any teaching that the rapamycin derivative disclosed therein can be combined with any other compound, let alone the specific analogues of somatostatin-14 to which the instant claims are limited.

With regard to British Patent 2,239,178, it involves the discovery that somatostatin analogue-containing compositions can be better tolerated when administered parenterally for treating breast cancer if said compositions contain lactic acid. First of all, there is nothing in British Patent 2,239,178 which points to the specific analogues of somatostatin-14 which characterize the instant claims. In addition, other than the mention that it is preferred that the compositions contain a dopamine agonist, there is nothing within the four corners of British Patent 2,239,178 which suggests that any other compound can be suitably added, let alone either of the two rapamycin macrolides to which the instant claims are limited.

In addition, there is clearly nothing in the prior art being relied upon by the Examiner which would lead one skilled in the art to employ the individual compounds of the instantly claimed combinations in synergistic effective amounts. In this connection, it is Applicant's belief that the Examiner's comment in the Final Rejection dated April 4, 2001 concerning the "overlap" in the dosage ranges actually supports patentability, since one skilled in the art would expect the claimed combinations to exhibit a null or, at best, an additive effect. Accordingly, it is quite surprising that the combinations to which the instant claims are limited exhibit a synergistic inhibitory effect on the hyperproliferation of malignant cells or vascular cells.

For essentially the reasons discussed above, Applicant does not believe that the teachings of the prior art being relied upon by the Examiner render the pharmaceutical kits of Claims 19-23 *prima facie* obvious. Applicant submits that it is not obvious to prepare a pharmaceutical kit when the motivation to do so is totally absent from the prior art, as is the situation in the present case. It is clear that there is nothing in the prior art relied upon by the Examiner which would provide one skilled in the art with the motivation to prepare any of the claimed pharmaceutical kits. Since the claimed pharmaceutical compositions are novel and unobvious, the claimed pharmaceutical kits are novel and obvious.

Similarly, Applicant does not believe that the teachings of the prior art relied upon by the Examiner render the "method-of-use" of Claims 28-31 *prima facie* obvious. A process involving the use of a novel and unobvious composition of matter is itself novel and unobvious. In re Kuehl, 177 USPQ 250; In re Schneider et al., 179 UPSQ 46; and In reWadlinger et al., 181 USPQ 826.

In view of the foregoing, it is clear that the teachings of the prior art being relied upon by the Examiner do not render any of the instant claims prima facie obvious. Therefore, withdrawal of the USC§103(a) rejection of Claims 6, 8-10, 12, 13 and 15-18 (now Claims 19, 20, 23-25, 28 and 29) is respectfully requested.

In summation, for the reasons set forth herein and in the Amendment Under 37 CFR 1.116, both of the rejections of record are believed to have been overcome. Accordingly, the instant application is deemed to be in condition for allowance, and an early notice to that effect is earnestly solicited. However, in the event that the Amendment Under 37 CFR 1.116 is not deemed to place this application in condition for allowance, it is believed that the cancellation of Claims 6, 8-10, 12, 13 and 15-18 and the presentation of "new" Claims 19-31 place the instant application in better form for appeal. Accordingly, it is respectfully requested that it be entered for appeal purposes for the reasons discussed above.

Respectfully submitted,

Joseph J. Borgvian
Agent for Applicant
Reg. No. 26,631

Novartis Pharmaceuticals Corporation
Patent and Trademark Dept.
564 Morris Avenue
Summit, NJ 07901-1027
(908) 522-6921

JJB/ld

Encl.: Postcard

Date: August 28, 2001

Interview Summary

Application No.	Applicant(s)
09/194,957	Weckbecker
Examiner	**Group Art Unit**
Michael Borin	1631

All participants (applicant, applicant's representative, PTO personnel):

(1) *Michael Borin* (3) _____

(2) *J.J. Borovian* (4) _____

Date of Interview *Sep 19, 2001*

Type: a)☒ Telephonic b)☐ Video Conference
c)☐ Personal [copy is given to 1)☐ applicant 2)☐ applicant's representative]

Exhibit shown or demonstration conducted: d)☐ Yes e)☒ No. If yes, brief description:

Claim(s) discussed:

Identification of prior art discussed:
None

Agreement with respect to the claims f)☐ was reached. g)☐ was not reached. h)☐ N/A.

Substance of Interview including description of the general nature of what was agreed to if an agreement was reached, or any other comments:
Applicant was notified that the response to final rejection has been received, and allowability of the claimed subject matter will be revised.

(A fuller description, if necessary, and a copy of the amendments which the examiner agreed would render the claims allowable, if available, must be attached. Also, where no copy of the amendments that would render the claims allowable is available, a summary thereof must be attached.)

i)☐ It is not necessary for applicant to provide a separate record of the substance of the interview (if box is checked).

Unless the paragraph above has been checked, THE FORMAL WRITTEN REPLY TO THE LAST OFFICE ACTION MUST INCLUDE THE SUBSTANCE OF THE INTERVIEW. (See MPEP section 713.04). If a reply to the last Office action has already been filed, APPLICANT IS GIVEN ONE MONTH FROM THIS INTERVIEW DATE TO FILE A STATEMENT OF THE SUBSTANCE OF THE INTERVIEW. See Summary of Record of Interview requirements on reverse side or on attached

MICHAEL BORIN
PRIMARY EXAMINER
ART UNIT 1631

Examiner Note: You must sign this form unless it is an Attachment to a signed Office action.

Interview Summary

Application No.	Applicant(s)
09/194,957	Weckbecker
Examiner	**Group Art Unit**
Michael Borin	1631

All participants (applicant, applicant's representative, PTO personnel):

(1) *Michael Borin* (3) _____

(2) *J.J. Borovian* (4) _____

Date of Interview *Sep 19, 2001*

Type: a)☒ Telephonic b)☐ Video Conference
c)☐ Personal [copy is given to 1)☐ applicant 2)☐ applicant's representative]

Exhibit shown or demonstration conducted: d)☐ Yes e)☒ No. If yes, brief description:

Claim(s) discussed:

Identification of prior art discussed:
None

Agreement with respect to the claims f)☐ was reached. g)☐ was not reached. h)☐ N/A.

Substance of Interview including description of the general nature of what was agreed to if an agreement was reached, or any other comments:
Applicant was notified that the response to final rejection has been received, and allowability of the claimed subject matter will be revised.

(A fuller description, if necessary, and a copy of the amendments which the examiner agreed would render the claims allowable, if available, must be attached. Also, where no copy of the amendments that would render the claims allowable is available, a summary thereof must be attached.)

i)☐ It is not necessary for applicant to provide a separate record of the substance of the interview (if box is checked).

Unless the paragraph above has been checked, THE FORMAL WRITTEN REPLY TO THE LAST OFFICE ACTION MUST INCLUDE THE SUBSTANCE OF THE INTERVIEW. (See MPEP section 713.04). If a reply to the last Office action has already been filed, APPLICANT IS GIVEN ONE MONTH FROM THIS INTERVIEW DATE TO FILE A STATEMENT OF THE SUBSTANCE OF THE INTERVIEW. See Summary of Record of Interview requirements on reverse side or on attached

MICHAEL BORIN
PRIMARY EXAMINER
ART UNIT 1631

Examiner Note: You must sign this form unless it is an Attachment to a signed Office action.

Notice of Allowability	Application No. 09/194,957	Applicant(s) Weckbecker
	Examiner Michael Borin	Art Unit 1631

--The MAILING DATE of this communication appears on the cover sheet with the correspondence address--

All claims being allowable, PROSECUTION ON THE MERITS IS (OR REMAINS) CLOSED in this application. If not included herewith (or previously mailed), a Notice of Allowance and Issue Fee Due or other appropriate communication will be mailed in due course. **THIS NOTICE OF ALLOWABILITY IS NOT A GRANT OF PATENT RIGHTS.** This application is subject to withdrawal from issue at the initiative of the Office or upon petition by the applicant. See 37 CFR 1.313 and MPEP 1308.

1. ☒ This communication is responsive to *communication filed 8/28/01*.

2. ☒ The allowed claim(s) is/are *19-31 renumbered as 1-13.*.

3. ☐ The drawings filed on _____ are acceptable as formal drawings.

4. ☒ Acknowledgement is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d).

a) ☒ All b) ☐ Some* c) ☐ None of the:

1. ☐ Certified copies of the priority documents have been received.

2. ☐ Certified copies of the priority documents have been received in Application No. _____.

3. ☒ Copies of the certified copies of the priority documents have been received in this national stage application from the International Bureau (PCT Rule 17.2(a)).

*Certified copies not received: _____.

5. ☐ Acknowledgement is made of a claim for domestic priority under 35 U.S.C. § 119(e).

Applicant has THREE MONTHS FROM THE "MAILING DATE" of this communication to file a reply complying with the requirements noted below. Failure to timely comply will result in ABANDONMENT of this application. **THIS THREE-MONTH PERIOD IS NOT EXTENDABLE FOR SUBMITTING NEW FORMAL DRAWINGS, OR A SUBSTITUTE OATH OR DECLARATION. This three-month period for complying with the REQUIREMENT FOR THE DEPOSIT OF BIOLOGICAL MATERIAL is extendable under 37 CFR 1.136(a).**

6. ☐ Note the attached EXAMINER'S AMENDMENT or NOTICE OF INFORMAL APPLICATION (PTO-152) which gives reason(s) why the oath or declaration is deficient. A SUBSTITUTE OATH OR DECLARATION IS REQUIRED.

7. ☐ Applicant MUST submit NEW FORMAL DRAWINGS

(a) ☐ including changes required by the Notice of Draftsperson's Patent Drawing Review (PTO-948) attached

1) ☐ hereto or 2) ☐ to Paper No. _____.

(b) ☐ including changes required by the proposed drawing correction filed _____, which has been approved by the examiner.

(c) ☐ including changes required by the attached Examiner's Amendment/Comment or in the Office action of Paper No. _____.

Identifying indicia such as the application number (see 37 CFR 1.84(c)) should be written on the drawings. The drawings should be filed as a separate paper with a transmittal letter addressed to the Official Draftsperson.

8. ☐ Note the attached Examiner's comment regarding REQUIREMENT FOR THE DEPOSIT OF BIOLOGICAL MATERIAL.

Any reply to this letter should include, in the upper right hand corner, the APPLICATION NUMBER (SERIES CODE/SERIAL NUMBER). If applicant has received a Notice of Allowance and Issue Fee Due, the ISSUE BATCH NUMBER and DATE of the NOTICE OF ALLOWANCE should also be included.

Attachment(s)

1 ☐ Notice of References Cited (PTO-892)
2 ☐ Notice of Informal Patent Application (PTO-152)
3 ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
4 ☐ Interview Summary (PTO-413), Paper No. _____.
5 ☐ Information Disclosure Statement(s) (PTO-1449), Paper No(s). _____
6 ☒ Examiner's Amendment/Comment
7 ☐ Examiner's Comment Regarding Requirement for Deposit of Biological Material
8 ☐ Examiner's Statement of Reasons for Allowance
9 ☐ Other

EXAMINER'S COMMENT

Examiner acknowledges that compound C (p. 16, bottom), the somatostatin tested in *in vivo* test, is indeed a sustained release formulation - the fact which was originally overlooked by the Examiner. Consequently, the issues of "new matter" or enablement indicated in the Advisory action are moot.

Pursuant to the amendment filed 07/05/01, all previously pending claims 6,8-10,12,13,15-18 are canceled. Claims 19-31 are added. Claims 19-31 are allowed.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Michael Borin whose telephone number is (703) 305-4506. Dr. Borin can normally be reached between the hours of 8:30 A.M. to 5:00 P.M. EST Monday to Friday. If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor,Mr. Michael Woodward, can be reached on (703) 308-4028. The fax telephone number for this group is (703) 305-3014.

Any inquiry of a general nature or relating the status of this application should be directed to the Group receptionist whose telephone number is (703) 308-0196.

MICHAEL BORIN, PH.D
PRIMARY EXAMINER

October 8, 2001,

mlb

UNITED STATES DEPARTMENT OF COMMERCE
Patent and Trademark Office

NOTICE OF ALLOWANCE AND ISSUE FEE DUE

001695 08/12/1019
THOMAS HOXIE
NOVARTIS CORPORATION
PATENT AND TRADEMARK DEPT
564 MORRIS AVENUE
SUMMIT NJ 07901-1027

APPLICATION NO.	FILING DATE	TOTAL CLAIMS	EXAMINER AND GROUP ART UNIT	DATE MAILED
[illegible]	[illegible]	013	[illegible]	[illegible]

First Named Applicant [illegible] 35 USC 154(b) term (days)

TITLE OF INVENTION [illegible]

ATTY'S DOCKET NO.	CLASS-SUBCLASS	BATCH NO.	APPLN. TYPE	SMALL ENTITY	FEE DUE	DATE DUE
4-100-8022/A	514-016.000	NO3	UTILITY	NO	$1330.00	01/22/02

THE APPLICATION IDENTIFIED ABOVE HAS BEEN EXAMINED AND IS ALLOWED FOR ISSUANCE AS A PATENT. PROSECUTION ON THE MERITS IS CLOSED.

THE ISSUE FEE MUST BE PAID WITHIN THREE MONTHS FROM THE MAILING DATE OF THIS NOTICE OR THIS APPLICATION SHALL BE REGARDED AS ABANDONED. THIS STATUTORY PERIOD CANNOT BE EXTENDED.

HOW TO RESPOND TO THIS NOTICE:

. Review the SMALL ENTITY status shown above.
If the SMALL ENTITY is shown as YES, verify your current SMALL ENTITY status:

A. If the status is changed, pay twice the amount of the FEE DUE shown above and notify the Patent and Trademark Office of the change in status, or
B. If the status is the same, pay the FEE DUE shown above.

. If the SMALL ENTITY is shown as NO:

A. Pay FEE DUE shown above, or

B. File verified statement of Small Entity Status before, or with, payment of 1/2 the FEE DUE shown above.

. Part B-Issue Fee Transmittal should be completed and returned to the Patent and Trademark Office (PTO) with your ISSUE FEE. Even if the ISSUE FEE has already been paid by charge to deposit account, Part B Issue Fee Transmittal should be completed and returned. If you are charging the ISSUE FEE to your deposit account, section "4b" of Part B-Issue Fee Transmittal should be completed and an extra copy of the form should be submitted.

I. All communications regarding this application must give application number and batch number.
Please direct all communications prior to issuance to Box ISSUE FEE unless advised to the contrary.

IMPORTANT REMINDER: Utility patents issuing on applications filed on or after Dec. 12, 1980 may require payment of maintenance fees. It is patentee's responsibility to ensure timely payment of maintenance fees when due.

PATENT AND TRADEMARK OFFICE COPY

OL-85 (REV. 10-95) Approved for use through 06/30/99. (0651-0033)

PART B—ISSUE FEE TRANSMITTAL

Complete and mail this form, together with applicable fees, to: **Box ISSUE FEE**
Assistant Commissioner for Patents
Washington, D.C. 20231

01-17-02

B/$

MAILING INSTRUCTIONS: This form should be used for transmitting the ISSUE FEE. Blocks 1 through 4 should be completed where appropriate. All further correspondence including the Issue Fee Receipt, the Patent, advance orders and notification of maintenance fees will be mailed to the current correspondence address as indicated unless corrected below or directed otherwise in Block 1, by (a) specifying a new correspondence address; and/or (b) indicating a separate "FEE ADDRESS" for maintenance fee notifications.

CURRENT CORRESPONDENCE ADDRESS (Note: Legibly mark-up with any corrections or use Block 1)

001095 HZ12/1019
THOMAS HOXIE
NOVARTIS CORPORATION
PATENT AND TRADEMARK DEPT
564 MORRIS AVENUE
SUMMIT NJ 07901-1027

Note: The certificate of mailing below can only be used for domestic mailings of the Issue Fee Transmittal. This certificate cannot be used for any other accompanying papers. Each additional paper, such as an assignment or formal drawing, must have its own certificate of mailing.

Certificate of Mailing

I hereby certify that this Issue Fee Transmittal is being deposited with the United States Postal Service with sufficient postage for first class mail in an envelope addressed to the Box Issue Fee address above on the date indicated below.

Filing by Express Mail
see stamp below (Depositor's name)

(Signature)

(Date)

APPLICATION NO.	FILING DATE	TOTAL CLAIMS	EXAMINER AND GROUP ART UNIT	DATE MAILED
09/194,957	12/07/98	013	BORIN, M 1631	10/19/01

First Named Applicant: WECKBECKER, 35 USC 154(b) term ext. = 0 Days.

TITLE OF INVENTION: COMBINATION OF A SOMATOSTATIN ANALOGUE AND A RAPAMYCIN

ATTY'S DOCKET NO.	CLASS-SUBCLASS	BATCH NO.	APPLN. TYPE	SMALL ENTITY	FEE DUE	DATE DUE
4-100-8322/A	514-016.000	N63	UTILITY	NO	$1280.00	01/22/02

1. Change of correspondence address or indication of " Fee Address" (37 CFR 1.363). Use of PTO form(s) and Customer Number are recommended, but not required.
   - ☐ Change of correspondence address (or Change of Correspondence Address form PTO/SB/122) attached.
   - ☐ "Fee Address" Indication (or "Fee Address" Indication form PTO/SB/47) attached.

2. For printing on the patent front page, list (1) the names of up to 3 registered patent attorneys or agents OR, alternatively, (2) the name of a single firm (having as a member a registered attorney or agent) and the names of up to 2 registered patent attorneys or agents. If no name is listed, no name will be printed.
   1 Joseph J. Borovian
   2
   3

3. ASSIGNEE NAME AND RESIDENCE DATA TO BE PRINTED ON THE PATENT (print or type)
   PLEASE NOTE: Unless an assignee is identified below, no assignee data will appear on the patent. Inclusion of assignee data is only appropriate when an assignment has been previously submitted to the PTO or is being submitted under separate cover. Completion of this form is NOT a substitute for filing an assignment.
   (A) NAME OF ASSIGNEE: Novartis AG
   (B) RESIDENCE: (CITY & STATE OR COUNTRY): Basel, Switzerland
   Please check the appropriate assignee category indicated below (will not be printed on the patent)
   ☐ Individual ☒ Corporation or other private group entity ☐ government

4a. The following fees are enclosed (make check payable to Commissioner of Patents and Trademarks):
   - ☐ Issue Fee
   - ☐ Advance Order - # of Copies

4b. The following fees or deficiency in these fees should be charged to:
   DEPOSIT ACCOUNT NUMBER 19-0134
   (ENCLOSE AN EXTRA COPY OF THIS FORM)
   - ☒ Issue Fee
   - ☒ Advance Order - # of Copies 10

The COMMISSIONER OF PATENTS AND TRADEMARKS IS requested to apply the Issue Fee to the application identified above.

(Authorized Signature) Joseph J. Borovian / Reg. #26,631 (Date) 1-15-02

NOTE: The Issue Fee will not be accepted from anyone other than the applicant; a registered attorney or agent; or the assignee or other party in interest as shown by the records of the Patent and Trademark Office.

01/23/2002 DNGUYEN2 00000026 190134 09194957
01 FC:142 1280.00 CH
02 FC:561 30.00 CH

FILING BY "EXPRESS MAIL" UNDER 37 CFR 1.10
EL 820012132 US
Express Mail Label Number
01.15.2002
Date of Deposit

Burden Hour Statement: This form is estimated to take 0.2 hours to complete. Time will vary depending on the needs of the individual case. Any comments on the amount of time required to complete this form should be sent to the Chief Information Officer, Patent and Trademark Office, Washington, D.C. 20231. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND FEES AND THIS FORM TO: Box Issue Fee, Assistant Commissioner for Patents, Washington D.C. 20231

Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

TRANSMIT THIS FORM WITH FEE

File History Content Report

The following content is missing from the original file history record obtained from the United States Patent and Trademark Office. No additional information is available.

Document Date - 2002-03-26

Document Title - USPTO Grant

07-16-02

CASE 4-100-8322/A/PCT

FILING BY "EXPRESS MAIL" UNDER 37 CFR 1.10

EL 820010658 US	July 15, 2002
Express Mail Label Number	Date of Deposit

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

IN RE ISSUED PATENT OF WECKBECKER

US PATENT NO. US. 6,362,164 B1 ISSUED: March 26, 2002

ISSUED: March 26, 2002 FILED: JUNE 11, 1997

FOR: COMBINATION OF A SOMATOSTATIN ANALOGUE AND A RAPAMYCIN

Certificate
JUL 2 2 2002
of Correction

Assistant Commissioner for Patents
Washington, D.C. 20231

REQUEST FOR CERTIFICATE OF CORRECTION UNDER 37 CFR §1.322

Sir:

Patentees recently received a copy of the above-issued patent and discovered that there was a clerical error in Column 13, Claim 2, item c) is incorrect and should read -- c) (D)Phe-Cys-Tyr-(D)Trp-Lys-Val-Cys-TrpNH_2, and --. A Request for a Certificate of Correction is hereby submitted pursuant to 37 CFR §1.322.

The above-mentioned error is believed to be attributable to the Patent and Trademark Office as is evident from the table below.

Location and/or Error in Printed Patent	Location of Support in Amendment
Column 13, Claim 2, Item c) is incorrect.	**See Amendment Under 37 CFR 1.116, page 3, dated July 5, 2001.**

Attached in duplicate is Form PTO-1050, with at least one copy being suitable for printing.

23 JUL 2002

As will be noted from the application as filed, this error is not ascribable to the patentees. Therefore, no fee is believed to be payable. However, if a fee is payable, the Commissioner is hereby authorized to charge any additional fees under 37 CFR §1.20(a) to Deposit Account No. 19-0134 in the name of Novartis Corporation.

Please send the Certificate of Correction to the address currently associated with Customer No. 001095, viz.:

Thomas Hoxie
Novartis Corporation
Patent and Trademark Department
564 Morris Avenue
Summit NJ 07901

Respectfully submitted,

Joseph J. Borovian
Agent for Applicant
Reg. No. 26,631
Tel. No. (908) 522-6921

Encls. – PTO-1050 forms (2)
Date: July 15, 2002

Case: 4-100-8322/A/PCT

UNITED STATES PATENT AND TRADEMARK OFFICE

CERTIFICATE OF CORRECTION

PATENT NO : US 6,362,164 B1
DATED: : March 26, 2002
INVENTOR(S) : Gisbert Weckbecker

It is certified that there is an error in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

Column 13, ~~Claim 2,~~ item c) should read:

-- c) (D)Phe-Cys-Tyr-(D)Trp-Lys-Val-Cys-TrpNH_2, and --

MAILING ADDRESS OF SENDER:
Joseph J. Borovian
Novartis Corporation
Patent and Trademark Dept.
564 Morris Avenue
Summit, NJ 07901-1027
(908) 522-6921

PATENT NO. US 6,362,164 B1

FORM PTO-1050

-continued

h) 3-(2-(naphthyl)-Ala-Cys-Tyr-(D)Trp-Lys-Val-Cys-β-Nal-NH_2

i) (D)Phe-Cys-β-Nal-(D)Trp-Lys-Val-Cys-Thr-NH_2

j) (D)Phe-Cys-Tyr-(D)Trp-Lys-Leu-Cys-Thr-NH_2 and

k) (D)Phe-Cys-Tyr-(D)Trp-Lys-Cys-Thr-NH_2,

in free form or in pharmaceutically acceptable salt form, and a pharmaceutical composition comprising a rapamycin macrolide selected from rapamycin and 40-O-(2-hydroxyethyl)-rapamycin, said compositions being present in synergistic effective amounts, together with instructions for use.

2. A kit or package according to claim **1** wherein the analogue of somatostatin-14 is selected from

a) (D)Phe-Cys-Phe-(D)Trp-Lys-Thr-Cys-Thr-ol

c) (D)Phe-Cys-Tyr-(D)Trp-Lys-Val-Cys-$ThrNH_2$, and

f) 3-(2-(Naphthyl)-(D)Ala-Cys-Tyr-(D)Trp-Lys-Val-Cys-$ThrNH_2$,

in free form or pharmaceutically acceptable salt form.

3. A kit or package according to claim **2** wherein the analogue of somatostatin-14 is

(D)Phe-Cys-Phe-(D)Trp-Lys-Thr-Cys-Thr-ol

in free form or in pamoate salt form.

4. A kit or package according to claim **3** wherein the somatastatin-14 analogue is in sustained release form and the rapamycin macrolide is 40-O-(2-hydroxyethyl)-rapamycin.

5. A kit or package according to claim **1** for simultaneous or sequential use in synergistically effective amounts.

6. A pharmaceutical composition comprising a pharmaceutically acceptable carrier and a therapeutically effective amount of: 1) an analogue of somatostatin-14 binding to at least the hSST-2 receptor in the nMolar range selected from

a) (D)Phe-Cys-Phe-(D)Trp-Lys-Thr-Cys-Thr-ol

b) (D)Phe-Cys-Tyr-(D)Trp-Lys-Val-Cys-$ThrNH_2$

c) (D)Phe-Cys-Tyr-(D)Trp-Lys-Val-Cys-$TrpNH_2$

d) (D)Trp-Cys-Phe-(D)Trp-Lys-Thr-Cys-$ThrNH_2$

-continued

e) (D)Phe-Cys-Phe-(D)Trp-Lys-Thr-Cys-$ThrNH_2$

f) 3-(2-(Naphthyl)-(D)Ala-Cys-Try-(D)Trp-Lys-Val-Cys-$ThrNH_2$

g) (D)Phe-Cys-Tyr-(D)Trp-Lys-Val-Cys-β-Nal-NH_2

h) 3-(2-(naphthyl)-Ala-Cys-Tyr-(D)Trp-Lys-Val-Cys-β-Nal-NH_2

i) (D)Phe-Cys-β-Nal-(D)Trp-Lys-Val-Cys-Thr-NH_2

j) (D)Phe-Cys-Tyr-(D)Trp-Lys-Leu-Cys-Thr-NH_2 and

k) (D)Phe-Cys-Tyr-(D)Trp-Lys-Cys-Thr-NH_2,

in free form or pharmaceutically acceptable salt form; and 2) a rapamycin macrolide selected from rapamycin and 40-O-(2-hydroxyethyl)-rapamycin, said somatastatin-14 analogue and macrolide being present in synergistic effective amounts.

7. A composition according to claim **6** wherein the analogue of somatostatin-14 is selected from

a) (D)Phe-Cys-Phe-(D)Trp-Lys-Thr-Cys-Thr-ol

c) (D)Phe-Cys-Tyr-(D)Trp-Lys-Val-Cys-$ThrNH_2$, and

f) 3-(2-(Naphthyl)-(D)Ala-Cys-Tyr-(D)Trp-Lys-Val-Cys-$ThrNH_2$,

in free form or pharmaceutically acceptable salt form.

8. A composition according to claim **7** wherein the analogue of somatastatin-14 is

(D)Phe-Cys-Phe-(D)Trp-Lys-Thr-Cys-Thr-ol

in free form or in pamoate salt form.

9. A composition according to claim **8** wherein the somatostatin-14 analogue is in sustained release form and the rapamycin macrolide is 40-O-(2-hydroxyethyl)-rapamycin.

10. A method of inhibiting cell hyperproliferation comprising administering to a subject in need of such treatment a therapeutically effective amount of: 1) an analogue of somatostatin-14 binding to at least the hSST-2 receptor in the nMolar range selected from

a) (D)Phe-Cys-Phe-(D)Trp-Lys-Thr-Cys-Thr-ol

UNITED STATES PATENT AND TRADEMARK OFFICE

CERTIFICATE OF CORRECTION

PATENT NO. : 6,362,164 B1
DATED : March 26, 2002
INVENTOR(S) : Gisbert Weckbecker

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

Column 13,
Item c) should read:
-- c) (D)Phe-Cys-Tyr-(D)Trp-Lys-Val-Cys-TrpNH_2, and --

Signed and Sealed this

Twenty-sixth Day of November, 2002

Attest:

Attesting Officer

JAMES E. ROGAN
Director of the United States Patent and Trademark Office

File History Content Report

The following content is missing from the original file history record obtained from the United States Patent and Trademark Office. No additional information is available.

Document Date - 2005-09-21

Document Title - Certificate of Correction - Post Issue Communication

PATENT APPLICATION FEE DETERMINATION RECORD

Effective November 10, 1998

Application or Docket Number: 09/194957

CLAIMS AS FILED - PART I

	(Column 1) NUMBER FILED	(Column 2) NUMBER EXTRA
FOR		
BASIC FEE		
TOTAL CLAIMS	9 minus 20=	* —
INDEPENDENT CLAIMS	4 minus 3 =	* 1
MULTIPLE DEPENDENT CLAIM PRESENT		

* If the difference in column 1 is less than zero, enter "0" in column 2

SMALL ENTITY TYPE ☐ RATE	FEE	OR	OTHER THAN SMALL ENTITY RATE	FEE
	380.00	OR		~~760.00~~ 380.00
X$ 9=		OR	X$18=	
X39=		OR	X78=	~~78.00~~
+130=		OR	+260=	
TOTAL		OR	TOTAL	~~918.00~~

CLAIMS AS AMENDED - PART II

AMENDMENT A

	(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA
Total	*	Minus	**	=
Independent	*	Minus	***	=
FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM				

SMALL ENTITY RATE	ADDITIONAL FEE	OR	OTHER THAN SMALL ENTITY RATE	ADDITIONAL FEE
X$ 9=		OR	X$18=	
X39=		OR	X78=	
+130=		OR	+260=	
TOTAL ADDIT. FEE		OR	TOTAL ADDIT. FEE	

AMENDMENT B

	(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA
Total	*	Minus	**	=
Independent	*	Minus	***	=
FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM				

RATE	ADDITIONAL FEE	OR	RATE	ADDITIONAL FEE
X$ 9=		OR	X$18=	
X39=		OR	X78=	
+130=		OR	+260=	
TOTAL ADDIT. FEE		OR	TOTAL ADDIT. FEE	

AMENDMENT C

	(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA
Total	*	Minus	**	=
Independent	*	Minus	***	=
FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM				

RATE	ADDITIONAL FEE	OR	RATE	ADDITIONAL FEE
X$ 9=		OR	X$18=	
X39=		OR	X78=	
+130=		OR	+260=	
TOTAL ADDIT. FEE		OR	TOTAL ADDIT. FEE	

* If the entry in column 1 is less than the entry in column 2, write "0" in column 3.
** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 20, enter "20."
*** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 3, enter "3."
The "Highest Number Previously Paid For" (Total or Independent) is the highest number found in the appropriate box in column 1.

FORM PTO-875
(Rev 6/98)

Patent and Trademark Office, U.S. DEPARTMENT OF COMMERCE

Form PTO 1130
(REV 2/94)

U.S. DEPARTMENT OF COMMERCE
Patent and Trademark Office

PACE DATA ENTRY CODING SHEET

1ST EXAMINER: Young — DATE: 7 Dec 98

2ND EXAMINER: — DATE:

APPLICATION NUMBER	TYPE APPL	FILING DATE (MONTH / DAY / YEAR)	SPECIAL HANDLING	GROUP ART UNIT	CLASS	SHEETS OF DRAWING
	1	12 / 07 / 98	0	1611	540	7

TOTAL CLAIMS	INDEPENDENT CLAIMS	SMALL ENTITY?	FILING FEE	FOREIGN LICENSE	ATTORNEY DOCKET NUMBER
09	14	0	918	Y	4-100-8321A

CONTINUITY DATA

CONT CODE	STATUS CODE	PARENT APPLICATION SERIAL NUMBER	PCT APPLICATION SERIAL NUMBER	PARENT PATENT NUMBER	PARENT FILING DATE (MONTH / DAY / YEAR)
			PCT/ /		
			PCT/ /		
			PCT/ /		
			PCT/ /		
			PCT/ /		

PCT/FOREIGN APPLICATION DATA

FOREIGN PRIORITY CLAIMED	COUNTRY CODE	PCT/FOREIGN APPLICATION SERIAL NUMBER	FOREIGN FILING DATE (MONTH / DAY / YEAR)
Y	GBX	9612/7/1.0	06/01/96
Y	GBX	96193/0.7	09/16/96

MULTIPLE DEPENDENT CLAIM FEE CALCULATION SHEET

(FOR USE WITH FORM PTO-875)

SERIAL NO. 09/194957 FILING DATE 07 DEC 1998

APPLICANT(S) Weckbecker

CLAIMS

	AS FILED		AFTER 1st AMENDMENT		AFTER 2nd AMENDMENT	
	IND.	DEP.	IND.	DEP.	IND.	DEP.
1	1		1			
2	1		1			
3		2		1		
4		2		1		
5	1		1			
6	1		1			
7		1		1		
8		1		1		
9		1		1		
10						
11						
12						
13						
14						
15						
16						
17						
18						
19						
20						
21						
22						
23						
24						
25						
26						
27						
28						
29						
30						
31						
32						
33						
34						
35						
36						
37						
38						
39						
40						
41						
42						
43						
44						
45						
46						
47						
48						
49						
50						
TOTAL IND.	4		4			
TOTAL DEP.	7		5			
TOTAL CLAIMS	11		9			

	*		*		*	
	IND.	DEP.	IND.	DEP.	IND.	DEP.
51						
52						
53						
54						
55						
56						
57						
58						
59						
60						
61						
62						
63						
64						
65						
66						
67						
68						
69						
70						
71						
72						
73						
74						
75						
76						
77						
78						
79						
80						
81						
82						
83						
84						
85						
86						
87						
88						
89						
90						
91						
92						
93						
94						
95						
96						
97						
98						
99						
100						
TOTAL IND.						
TOTAL DEP.						
TOTAL CLAIMS						

PTO-1360 (3-78)

*MAY BE USED FOR ADDITIONAL CLAIMS OR AMENDMENTS

U.S. DEPARTMENT of COMMERCE
Patent and Trademark Office