

ONCOGENE -BASINGSTOKE-
ONCOGENE
ONCOGENE



6256.782000

VOL 19 NUMB 56

AC

JCP

*ETOC

SS

1/1

LOAN BAN EXPIRES

~~13 AUG 2001~~

Reviews

New Drug Targets and Therapies for Cancer

Guest Editor S. Sebti

Volume 19 • Number 56 • 27 December 2000 • Review Issue 6

DOCKET
ALARM

Find authenticated court documents without watermarks at docketalarm.com.

ONCOGENE

Reviews

New Drug Targets and Therapies for Cancer

Guest Editor **Saïd Sebti**

- 6549 **Guest Editor**
Saïd M Sebti
- 6550 **The EGF receptor family as targets for cancer therapy**
John Mendelsohn and Jose Baselga
- 6566 **Design of growth factor antagonists with antiangiogenic and antitumor properties**
Saïd M Sebti and Andrew D Hamilton
- 6574 **From oncogene to drug: development of small molecule tyrosine kinase inhibitors as anti-tumor and anti-angiogenic agents**
Michael J Morin
- 6584 **Farnesyltransferase and geranylgeranyltransferase I inhibitors and cancer therapy: Lessons from mechanism and bench-to-bedside translational studies**
Saïd M Sebti and Andrew D Hamilton
- 6594 **Development of anticancer drugs targeting the MAP kinase pathway**
Judith S Sebolt-Leopold
- 6600 **Small molecule modulators of cyclin-dependent kinases for cancer therapy**
Adrian M Senderowicz
- 6607 **Small molecule inhibitors of dual specificity protein phosphatases**
Katharine E Pestell, Alexander P Ducruet, Peter Wipf and John S Lazo
- 6613 **STAT proteins: novel molecular targets for cancer drug discovery**
James Turkson and Richard Jove
- 6627 **Bcl-2 family proteins as targets for anticancer drug design**
Ziwei Huang
- 6632 **Telomere maintenance mechanisms as a target for drug development**
David J Bearss, Laurence H Hurley and Daniel D Von Hoff
- 6642 **Critical appraisal of the use of matrix metalloproteinase inhibitors in cancer treatment**
Stanley Zucker, Jian Cao and Wen-Tien Chen
- 6651 **Potential roles of antisense technology in cancer chemotherapy**
Stanley T Crooke
- 6660 **Replication-selective oncolytic adenoviruses: virotherapy aimed at genetic targets in cancer**
David Kirn
- 6670 **ONYX-015 selectivity and the p14ARF pathway**
Frank McCormick
- 6673 **Dendritic cell vaccination for cancer therapy**
Frank O Nestle
- 6680 **The rapamycin-sensitive signal transduction pathway as a target for cancer therapy**
Manuel Hidalgo and Eric K Rowinsky
- 6687 **New agents in cancer clinical trials**
Julian Adams and Peter J Elliott

Copyright © 2000 Nature Publishing Group

Subscribing organisations are encouraged to copy and distribute this table of contents for internal, non-commercial purposes

This issue is now available at:
www.nature.com/onc



Oncogene, including *Oncogene Reviews*, is published by Nature Publishing Group, a division of Macmillan Publishers Ltd.

Scope The 2001 volume will include 50 regular issues and 8 issues of *Oncogene Reviews*. The Editors will consider for publication all papers and communications on all aspects of cellular growth control and the molecular mechanisms underlying malignant change.

This journal is covered by Current Contents, EMBASE Excerpta Medica and Index Veterinarius.

Editorial Manuscripts and all editorial correspondence should be sent to: John Jenkins, The Oncogene Editorial Offices, Eden House, Enterprise Way, Edenbridge, Kent, TN8 6HF, UK. Tel: +44 1732 860111 Fax: +44 1732 860222 E-mail: oncogene@globalnet.co.uk; or E Premkumar Reddy, The Fels Institute for Cancer Research & Molecular Biology, Medical Research Building, 3307 North Broad St., Philadelphia, PA 19140, USA. Tel: +1 215 707 4307 Fax: +1 215 707 1454.

Publisher All business correspondence and enquiries about supplement publication and sponsorship opportunities should be addressed to *Oncogene*, Nature Publishing Group, Houndmills, Basingstoke, Hampshire RG21 6XS, UK. Tel: +44 1256 329242 Fax: +44 1256 810526.
Publishing Manager: Sue Deeley
Production Controller: Debbie Cole

Oncogene, including *Oncogene Reviews*, is online at www.nature.com/onc

Visit the journal's home pages for details of the aims and scope, readership, instructions to authors and how to contact the Editors and publishing staff. Use the website to order a subscription, reprints, a sample copy or individual articles.

Free to all readers: tables of contents and abstracts for all articles published since 1997 and the complete text of the January 2001 issue. Register to receive the table of contents by e-mail as each issue is published.

Subscribers to the 2001 online version of the journal have access to PDF and HTML files with the full text of all articles published since 1997.

Subscriptions – 2001 subscription rates INSTITUTIONAL SUBSCRIPTIONS

Combined (electronic online & print)	
EU	£2310
Rest of World	£2420/US\$3872
Electronic online only	£2100/US\$3360
Print (hard copy) only	
EU	£2100
Rest of World	£2200/US\$3520

Site licences and institutional online access – for information on multi-user or multi-site access to Nature Publishing Group products please contact s.archer@macmillan.co.uk or telephone +44 20 7843 6426. For other enquiries please contact sjsupport@nature.com or telephone +44 20 7843 4759.

PERSONAL SUBSCRIPTIONS

Combined (electronic online & print)	
EU	£633
Rest of World	£633/US\$1013
Electronic online only	£576/US\$922
Print (hard copy) only	
EU	£576
Rest of World	£576/US\$922
Prices for airmail delivery on application	

Typeset by Elite Typesetting Techniques, Eastleigh, Hants and printed in Great Britain by The Friary Press, Dorchester
Printed on acid-free paper, effective with Volume 9, Issue 1, 1994

be made payable to Nature Publishing Group and sent to: The Subscription Department, Nature Publishing Group, Houndmills, Basingstoke, Hampshire RG21 6XS, UK. E-mail: subscriptions@nature.com. Where appropriate, subscribers may make payments into UK Post Office Giro Account No: 519 2455. Full details must accompany the payment.

Subscriptions – USA

USA subscribers can call toll free: 1 800 747 3187. Please send check/money order/credit card details to: The Subscription Department, Nature Publishing Group, Houndmills, Basingstoke, Hampshire RG21 6XS, UK.
E-mail: subscriptions@nature.com

Prices are set in UK Sterling. Dollar prices are converted from UK Sterling at the current exchange rate. Accordingly, your credit card charge may vary slightly from the Dollar rate shown. To obtain the exact Dollar rate shown, please remit by check. All prices, specifications and details are subject to change without prior notification. Single issues of *Oncogene Reviews* are available. For information, please contact Marketing Dept, Nature Publishing Group, Houndmills, Basingstoke, Hampshire, RG21 6XS, UK. Tel: +44 1256 351898 Fax: +44 1256 328339.

Oncogene, including *Oncogene Reviews* (ISSN 0950-9232) is published 58 times a year by Nature Publishing Group, c/o Mercury Airfreight International Ltd, 365 Blair Road, Avenel, New Jersey, NJ 07001, USA. Subscription price for institutions is \$3520 per annum. Periodicals postage is paid at Rahway NJ. Postmaster: send address corrections to *Oncogene*, Nature Publishing Group, c/o Mercury Airfreight International Ltd, 365 Blair Road, Avenel, NJ 07001.

Advertisements Enquiries concerning advertisements should be addressed to: Robert Sloan, Advertisement Manager, 84 Arnos Grove, Southgate, London, N14 7AR, UK. Tel: +44 20 8882 7199 Fax: +44 20 8882 7299. E-mail: rsloan@rsa2.demon.co.uk

Reprints and permissions For reprints of any article in this journal or reproduction rights, please contact Tracé Noel (t.noel@nature.com) at the publisher's address (above).

Copyright © 2000 Nature Publishing Group
ISSN 0950-9232

All rights of reproduction are reserved in respect of all papers, articles, illustrations, etc., published in this journal in all countries of the world.

All material published in this journal is protected by copyright, which covers exclusive rights to reproduce and distribute the material. No material published in this journal may be reproduced or stored on microfilm or in electronic, optical or magnetic form without the written authorisation of the Publisher.

Authorisation to photocopy items for internal or personal use of specific clients, is granted by Nature Publishing Group, for libraries and other users registered with the Copyright Clearance Centre (CCC) Transaction Reporting Service, provided that the base fee of \$15.00 per copy is paid directly to CCC, 222 Rosewood Drive, Danvers, USA 0950-9232/00 \$15.00 + \$0.00

Apart from any fair dealing for the purposes of research or private study, or criticism or review, as permitted under the Copyright, Designs and Patent Act 1988, this publication may be reproduced, stored or transmitted, in any form or by any means, only with the prior permission in writing of the publishers, or in the case of reprographic reproduction, in accordance with the terms of licences issued by the Copyright Licensing Agency.

The rapamycin-sensitive signal transduction pathway as a target for cancer therapy

Manuel Hidalgo^{*,1} and Eric K Rowinsky¹

¹The University of Texas Health Science Center at San Antonio, The Institute for Drug Development, Cancer Therapy and Research Center, San Antonio, Texas, USA

The high frequency of mutations in cancer cells which result in altered cell cycle regulation and growth signal transduction, conferring a proliferative advantage, indicates that many of these aberrant mechanisms may be strategic targets for cancer therapy. The macrolide fungicide rapamycin, a natural product with potent antimicrobial, immunosuppressant, and anti-tumor properties, inhibits the translation of key mRNAs of proteins required for cell cycle progression from G₁ to S phase. Rapamycin binds intracellularly to the immunophilin FK506 binding protein 12 (FKBP12), and the resultant complex inhibits the protein kinase activity of a protein kinase termed mammalian target of rapamycin (mTOR). The inhibition of mTOR, in turn, blocks signals to two separate downstream pathways which control the translation of specific mRNAs required for cell cycle traverse from G₁ to S phase. Blocking mTOR affects the activity of the 40S ribosomal protein S6 kinase (p70^{s6k}) and the function of the eukaryotic initiation factor 4E-binding protein-1 (4E-BP1), leading to growth arrest in the G₁ phase of the cell cycle. In addition to its actions on p70^{s6k} and 4E-BP1, rapamycin prevents cyclin-dependent kinase activation, inhibits retinoblastoma protein (pRb) phosphorylation, and accelerates the turnover of cyclin D1 that leads to a deficiency of active cdk4/cyclin D1 complexes, all of which can inhibit cell cycle traverse at the G₁/S phase transition. Both rapamycin and CCI-779, an ester analog of rapamycin with improved pharmaceutical properties and aqueous solubility, have demonstrated impressive activity against a broad range of human cancers growing in tissue culture and in human tumor xenograft models, which has supported the development of compounds targeting rapamycin-sensitive signal-transduction pathways. CCI-779 has completed several phase I clinical evaluations and is currently undergoing broad disease-directed efficacy studies. The agent appears to be well tolerated at doses that have resulted in impressive anti-tumor activity in several types of refractory neoplasms. Important challenges during clinical development include the definition of a recommended dose range associated with optimal biological activity and maximal therapeutic indices, as well as the ability to predict which tumors will be sensitive or resistant to CCI-779. *Oncogene* (2000) 19, 6680–6686.

Keywords: rapamycin; CCI-779; signal transduction; clinical development

*Correspondence: M Hidalgo, Department of Medicine, Division of Medical Oncology, The University of Texas Health Science Center at San Antonio, 7703 Floyd Curl Dr. Mail code 7884. San Antonio, Texas, TX, 78229, USA

Cell proliferation is a complex multifaceted process that requires the synthesis of essential regulatory proteins involved in the transduction of extracellular and autocrine proliferative stimuli. Since several of these highly regulated processes are aberrant in many types of cancers, conferring a proliferative advantage, they are potential strategic targets for therapeutic development against cancer (Sherr, 2000). Indeed, several novel classes of therapeutics that interfere with discrete essential elements of aberrant signal transduction and cell cycle regulation, such as inhibitors of various receptor tyrosine kinases, oncogenes, critical proteins involved in signal transduction (e.g. Ras, Raf), and cyclin-dependent kinases, are being developed as anti-cancer agents (Rowinsky *et al.*, 1999, Senderowicz and Sausville 2000). One such agent, rapamycin (sirolimus; Rapamune[®]; Wyeth-Ayerst, PA, USA), a macrolide fungicide isolated from the bacteria *Streptomyces hygroscopicus*, possesses potent antimicrobial, immunosuppressant, and antitumor properties (Baker *et al.*, 1978; Sehgal *et al.*, 1975; Vezina *et al.*, 1975). Because of its profound immunosuppressive actions, rapamycin was initially developed and received regulatory approval for the indication of prevention of allograft rejection following organ transplantation (Sehgal, 1995). The antiproliferative actions of rapamycin have been demonstrated to be due to its ability to modulate critical signal transduction pathways that link mitogenic stimuli to the synthesis of proteins required for cell cycle traverse from G₁ to S (Wiederrecht *et al.*, 1995). Impressive antiproliferative activity has been demonstrated following treatment of a diverse types of experimental tumors with rapamycin (Eng *et al.*, 1984, Muthukkumar *et al.*, 1995; Seufferlein and Rozengurt, 1996). However, the poor aqueous solubility and chemical stability of rapamycin precluded its clinical development as an anti-cancer agent. Recently, a series of rapamycin analogs with improved aqueous solubility and stability have been synthesized and evaluated. CCI-779 (Wyeth Ayerst, PA, USA), a soluble ester analog of rapamycin, was selected for development as an anti-cancer agent based on its prominent anti-tumor profile and favorable pharmaceutical and toxicological characteristics in preclinical studies (Gibbons *et al.*, 2000). Several phase I studies of CCI-779 have been completed and disease-directed efficacy evaluations in a number of tumor types are being performed (Raymond *et al.*, 2000; Hidalgo *et al.*, 2000). This review will summarize the principal mechanisms of anti-tumor action of rapamycin, specifically its effect on rapamycin-sensitive signal transduction pathways, and will discuss the preliminary results of experimental and clinical studies with this novel class of anti-cancer agents.

Mechanism of action of rapamycin and rapamycin analogs

Rapamycin, and its ester analog, CCI-779, uniquely interfere with cell cycle progression from G₁ to S phase in response to proliferative stimuli by blocking the translation of mRNAs of essential cell cycle proteins (Wiederrecht *et al.*, 1995). The principal mechanisms responsible for these actions, which have been elucidated only over the last several years, are graphically depicted in Figures 1 and 2.

Upstream actions and the target of rapamycin

Rapamycin binds intracellularly to members of the immunophilin family of FK506 binding proteins (FKBPs), inhibiting their enzymatic activity as prolyl isomerases (Heitman *et al.*, 1991; Koltin *et al.*, 1991; Fruman *et al.*, 1995). Although there are many members of the FKBP family, a large body of biochemical and genetic studies suggest that FKBP12 is the most important binding protein with respect to the rapamycin-sensitive signal transduction pathway (Heitman *et al.*, 1991; Koltin *et al.*, 1991; Fruman *et al.*, 1995). The resultant rapamycin-FKBP12 complex interacts with and inhibits the activity of a 290 kd kinase, termed mammalian target of rapamycin (mTOR) (also known as FRAP, RAFT1, and RAP1) (Figure 1) (Sabatini *et al.*, 1994; Sabers *et al.*, 1995; Brown *et al.*, 1994; Chiu *et al.*, 1994). mTOR is a member of a recently identified family of protein kinases termed phosphoinositide 3-kinase related kinases (PIKKs), which are involved in many critical regulatory cellular functions pertaining to cell cycle progression, cell cycle checkpoints that govern cellular responses to DNA damage, DNA repair, and DNA recombination (Sarkaria *et al.*, 1998).

In response to growth stimuli, quiescent cells increase the translation of a subset of mRNAs whose protein products are required for traverse through the G₁ phase of the cell cycle. mTOR regulates essential signal transduction pathways and is involved in the

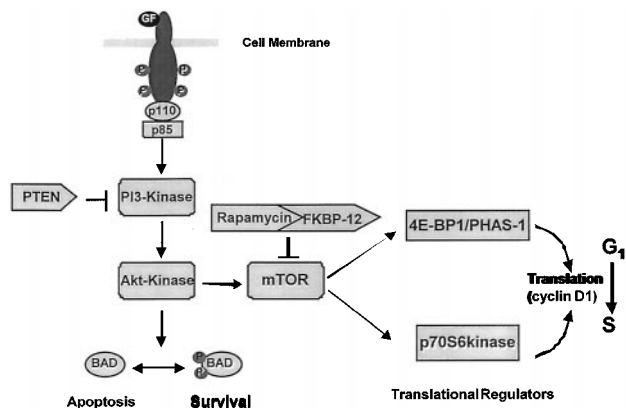


Figure 1 Rapamycin-sensitive signal transduction pathway. Rapamycin and CCI-779 bind to the immunophilin FK506 binding protein-12 (FKBP-12). The rapamycin-FKBP12 complex blocks the kinase activity of the mammalian target of rapamycin (mTOR). The inhibition of mTOR kinase activity inhibits the downstream translational regulators 4E-BP1/PHAS and p70^{S6k}. The inhibition of 4E-BP1/PHAS and p70^{S6k} decrease the translation of mRNA of specific proteins essential for cell cycle progression from G₁ to S phase.

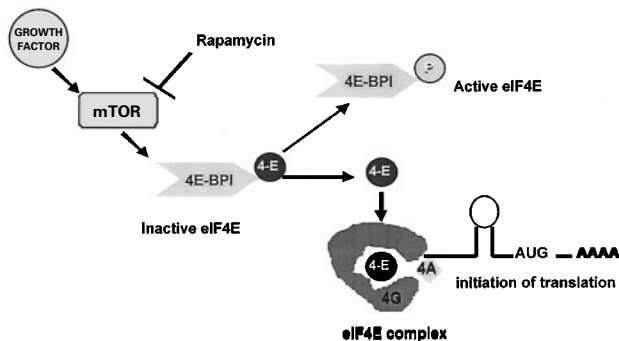


Figure 2 Rapamycin and CCI-779 inhibits the phosphorylation of 4E-BP1/PHAS, preventing the release of the eIF-4E and the activation of the eIF4F complex

coupling of growth stimuli with cell cycle progression. Phosphatidylinositol 3-kinase (PI3K)/protein kinase B (Akt) (PI3K/Akt) appears to be the key modulatory factor in the upstream pathway by which growth factor-growth factor receptor interactions affect the phosphorylation state of mTOR (Figure 1) (Downward 1998; Scott *et al.*, 1998; Nave *et al.*, 1999). PI3K plays a central role in cellular proliferation, motility, neovascularization, viability, and senescence and is upregulated in cancer cells (Shayesteh *et al.*, 1999; Cantley *et al.*, 1991). Its main physiological function is the phosphorylation of the D3 portion of membrane phosphoinositols (Cantley *et al.*, 1991; Carpenter *et al.*, 1990). Although the role of PI3K and its lipid products in signal transduction processes is not clear, the activity of this enzyme on tyrosine kinases induces mitogenesis, cellular growth, and cellular transformation (Carpenter *et al.*, 1990; Varticovski *et al.*, 1994; Hu *et al.*, 1995). Recently, several studies have investigated the role of small molecule-inhibitors of PI3K as potential tumor suppressor agents. For example, the flavonoid derivative, LY294002 (Eli Lilly, Indianapolis, IN, USA), a potent PI3K inhibitor, is a competitive, reversible inhibitor of the ATP binding site of the enzyme (Vlahos *et al.*, 1994; Hu *et al.*, 2000). The agent induces G₁ arrest in proliferating cells, leading to almost complete inhibition of melanoma cell proliferation, partial inhibition of MG-63 osteosarcoma cell growth, and inhibitor of OVCAR-3 ovarian carcinoma inducing prominent apoptotic effects (Hu *et al.*, 2000; Casagrande *et al.*, 1998; Thomas *et al.*, 1997). The inhibitor also completely inhibits the retinoblastoma protein (pRb) hyperphosphorylation that normally occurs during G₁ progression and induces up-regulation of the cyclin-dependent kinase inhibitor p27 (Casagrande *et al.*, 1998).

There are ample experimental data indicating that mTOR functions downstream of the PI3K/Akt pathway and is phosphorylated in responses to stimuli that activate the PI3K/Akt pathway (Scott *et al.*, 1998; Nave *et al.*, 1999; Hu *et al.*, 1995; Sekulic *et al.*, 2000). PI3K and Akt are considered proto-oncogenes, and the pathway is inhibited by the tumor suppressor gene *PTEN* (Wu *et al.*, 1998). There are other signaling pathways that are activated downstream of PI3K, but the Akt pathway is of particular interest because of its role in inhibiting apoptosis and promoting cell proliferation by affecting the phosphorylation status

Explore Litigation Insights

Docket Alarm provides insights to develop a more informed litigation strategy and the peace of mind of knowing you're on top of things.

Real-Time Litigation Alerts



Keep your litigation team up-to-date with **real-time alerts** and advanced team management tools built for the enterprise, all while greatly reducing PACER spend.

Our comprehensive service means we can handle Federal, State, and Administrative courts across the country.

Advanced Docket Research



With over 230 million records, Docket Alarm's cloud-native docket research platform finds what other services can't. Coverage includes Federal, State, plus PTAB, TTAB, ITC and NLRB decisions, all in one place.

Identify arguments that have been successful in the past with full text, pinpoint searching. Link to case law cited within any court document via Fastcase.

Analytics At Your Fingertips



Learn what happened the last time a particular judge, opposing counsel or company faced cases similar to yours.

Advanced out-of-the-box PTAB and TTAB analytics are always at your fingertips.

API

Docket Alarm offers a powerful API (application programming interface) to developers that want to integrate case filings into their apps.

LAW FIRMS

Build custom dashboards for your attorneys and clients with live data direct from the court.

Automate many repetitive legal tasks like conflict checks, document management, and marketing.

FINANCIAL INSTITUTIONS

Litigation and bankruptcy checks for companies and debtors.

E-DISCOVERY AND LEGAL VENDORS

Sync your system to PACER to automate legal marketing.