Cancer Treatment Reviews (1990) **17,** 109–117

In vitro and *in vivo* evaluation of US-NCI compounds in human tumor xenografts

Heinz-Herbert Fiebig,* Dietmar P. Berger,* Bernd R. Winterhalter* and Jacqueline Plowman†

**Department of Internal Medicine, University of Freiburg, Hugstetterstrasse 55, D-7800 Freiburg, F.R.G., and †Developmental Therapeutics Program, Division of Cancer Treatment, National Cancer Institute, Bethesda MD 20892, U.S.A.*

Introduction

The search for new drugs with antineoplastic activity or analogs of established cytostatic drugs with increased efficacy and reduced toxicity was the major objective of US-National Cancer Institute screening projects. Between 1975 and 1985 novel agents were tested in a 'compound-oriented' screening system based on initial *in vivo* testing in the mouse leukemia P388 and subsequent studies in a panel of five murine and three human tumor xenografts (10). This screening program was successful primarily in identifying compounds with clinical activity against leukemias and lymphomas (16).

Since 1985, the National Cancer Institute (NCI) has developed a new 'disease-oriented' approach to drug screening, based on human tumor cell line panels representative of particular tumor types (2). The objective of this type of screening is to identify compounds which exert selective effects on particular tumor types and to follow-up these leads *in vivo* utilizing cell lines previously shown to be sensitive.

We have tested 28 compounds which displayed activity in the old or the new NCI primary screen in a combined *in vitro*/*in vivo* secondary screen using human tumor xenografts. First, large scale tests were performed in the clonogenic assay. Only the most sensitive tumors were subsequently studied in nude mice, where the *in vivo* pharmacological behaviour of a drug is considered.

Methods

Our *in vitro* and *in vivo* test procedure has been described recently (6–8). HIuman tumors established in serial passage in nude mice were used for all experiments. The human origin of the tumors was confirmed by isoenzymatic and immunohistochemical methods. Tumor models were selected from a panel of 220 well characterized, regularly growing xenografts (9).

New compounds were studied *in vitro* for anticancer activity in human tumor xenografts, human bone marrow (CFU-GM) and in the leukemia P388 using a modification of the

0305–7372/90/2&3109+09 $03.00/0

clonogenic assay as described by Hamburger & Salmon (11). The most sensitive tumors were subsequently studied *in vivo*. Primary *in vitro* screening was done in four highly sensitive xenografts (small cell and large cell of the lung, breast and stomach), two resistant xenografts as well as the P388. Secondary *in vitro* screening was performed in a total of 14 responsive and six resistant human tumor xenografts and in two to four marrow specimens. Compounds with a greater or similar effect on tumor cells in comparison to human bone marrow were subsequently studied *in vivo* in the two most sensitive xenografts transplanted subcutaneously into nude mice. The comparison of *in vitro*/*in vivo* activity enabled assessment of the relevant *in vitro* dose based on *in vivo* pharmacological behavior of a compound. If remission or at least no change was observed *in vivo*, the new compound undergoes disease-oriented testing usually in 40–60 xenografts. Drugs were applied by continuous exposure until the end of the experiment. A compound was considered active, if it reduced colony formation of treated (T) groups to 30% or less of the control (C) group value.

For *in vivo* experiments 6–8-week-old female athymic nude mice of NMRI genetic background were used. Tumor slices averaging $3 \times 3 \times 0.5$–1 mm in diameter were implanted subcutaneously into both flanks of the animals. Treatment was started after 2–6 weeks when the median tumor diameter was 6–7 mm. The antitumor effect was evaluated following maximal tumor regression, in non-regressing tumors after 3–4 weeks. Data evaluation was performed using specifically designed software. Relative tumor size (RTS) values were calculated for each single tumor by dividing the tumor size day X by the tumor size day 0 at the time of randomization. Median RTS values were used for further evaluation. Tumor doubling time (DT) of test and control groups was defined as the period required to reach a relative tumor size of 200%. The effect of treatment was classified as complete remission (RTS on day 21 or 28 $\leqslant$ 10% of initial value), partial remission (11–50%), minimal regression (51–75%), no change (76–124%) or progression ($\geqslant$ 125%). A tumor was considered to be sensitive, if regression or no change was achieved. Additionally, tumor inhibition was evaluated in comparing the relative tumor size of treated with the control group. The specific growth delay (SGD) was calculated with regard to the tumor doubling time (DT) as described by Steel (17).

Results and discussion

Twenty-eight compounds of interest which have emerged from NCI primary screening were tested *in vitro* and *in vivo*. A summary of the activity in human tumor xenografts in the clonogenic assay and in nude mice is given in Table 1. Results for hepsulfam, 4-ipomeanol, oxanthrazole, penclomedine, pyrazine diazohydroxide and rapamycin are given in detail below.

Hepsulfam (NSC-329680)

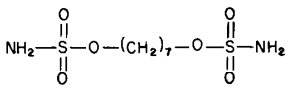
The 1,7-heptanediol-*bis*-sulfamate (Figure 1) was synthesized in an attempt to improve the antitumor efficacy of busulfan through introduction of a more polar leaving group. Hepsulfam showed a broader preclinical activity than busulfan in the NCI *in vivo* screening systems. Schedule dependency studies determined a single i.p. bolus injection as the most effective administration method. Cross-resistance of melphalan and cisplatin-resistant P388-sublines to hepsulfam was observed *in vivo* (18).

Hepsulfam and busulfan were tested simultaneously in human solid tumor xenografts *in*

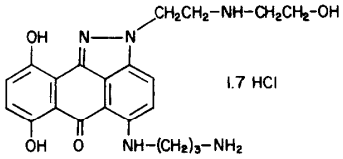
Table 1. Activity of new compounds in human tumor xenografts, human bone marrow (CFU-GM) and the P388 mouse leukemia

Compound	NSC no.	Clonogenic assay: No. of tumors studied	Clonogenic assay: Potency[a]	Clonogenic assay: T/C (%) HBM	Clonogenic assay: T/C (%) P388	Selective for[c]	Nude mice: Active[a] in tumors	Nude mice: Progressi
Aphidicolin glycinate[b]	303812	42	+ + +	5	3	SCLC & TES		3
Batracylin	320846	25	+	4	1			2
Bryostatin 1[c]	339555	13	–	ND	ND		ND	
Carmethizole[b]	602668	30	+ +	ND	ND		1	
Chloroquinoxaline Sulphonamide	339004	23	+	1	0			4
Combretastatin A4[c]	817373	30	+ + + +	ND	ND		1	
Cyanomorpholino-Adriamycin	357704	21	+ + + +	ND	ND			3
Cyclopentenyl cytosine	375575	35	+ + +	4	ND			2
DABIS Maleate[b]	262666	42	+	5	0		1	1
Deoxyspergualin	356894	28	+	1	41			3
Dihydrolenperone	343513	24	+	26	0			3
Geneticin	606702	19	–	98	0		ND	
Hepsulfam	329680	19	+ +	ND	ND		1	
4-Ipomeanol	349438	43	–	100	94	SCLC		3
L-cystein-analog[b]	303861	41	–		0			3
LL-D49194α_1[b]	381856	46	+ + + +	7	0	SCLC & MEL	2	1
Merbarone	336628	24	+	3	0			2
Oxanthrazole	349174	48	+ +	41	0	SCLC	2	5
Pancratistatin	349156	24	+ + + +	13	2			3
Penclomedine	338720	ND					1	1
Phyllanthoside	328426	25	+ + + +	79	116	SCLC		4
Pyrazine diazohydroxide	361456	ND					3	
Pyrazoloacridine	366140	33	+ + +	ND	ND			1
Rapamycin	226080	41	+ + + +	4	1	SCLC & MEL	6	1
Rhizoxin[b]	332598	38	+ + + +	3	0	SCLC	1	1
SRI62-834[c]	614383	32	+	ND	ND	REN	ND	

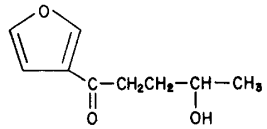
[a] Regressions or no change.
[b] Compounds at present developed by the EORTC.
[c] Compounds developed at present by the Cancer Research Campaign in Great Britain.
[d] + + + +, active (T/C $\leqslant$ 30%) at 0.01 μg/ml in $\geqslant$ 20% of xenografts; + + +, active at 0.1 μg/ml; + +, active at 1.0 μg/ml; +, active at 10.0 μg/ml; –, active at μg/ml.
[e] SCLC, small-cell lung cancer; TES, testicular cancer; MEL, melanoma; REN, renal cancer; HBM, human bone-marrow.
ND, not done.



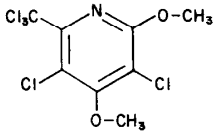
Hepsulfam (NSC-329680)



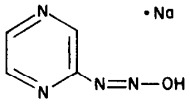
Oxanthrazole (NSC-349174)



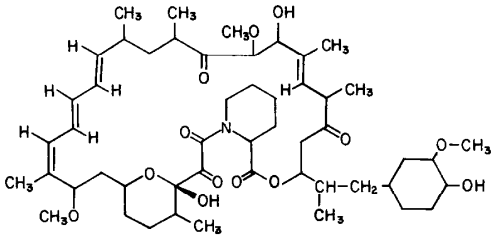
4-Ipomeanol (NSC 349438)



Penclomedine (NSC-338720)



Pyrazine Diazohydroxide
(NSC-361456)



Rapamycin (NSC-226080)

Figure 1. Chemical structures of selected US-NCI compounds.

vitro and *in vivo*. In the clonogenic assay, both compounds showed broad spectrum activity and a similar response profile. However, given the same dose level of 1 μg/ml at continuous drug exposure (Table 2), hepsulfam was active in 6/19 xenografts (32%) whereas busulfan reduced colony growth to a T/C $\leqslant$ 30% in 2/10 tumors (20%).

In vivo both compounds were tested against the large cell lung cancer xenograft LXFL 529. At a dose level of 150 mg/kg/day given day 1 i.p., busulfan therapy resulted in 'no change' on day 21. Hepsulfam-treated tumors regressed completely on day 21 and did not regrow within the observation period of 70 days (Table 4). Further tests will be performed with this compound.

Table 2. *In vitro* effect of hepsulfam (NSC-329680) *vs.* busulfan

Tumor histology	Hepsulfam (μg/ml) 0.1	1.0	10.0	Busulfan (μg/ml) 0.1	1.0	10.0
Xenografts						
Colon	0/3[a]	0/3	0/3	0/2	0/2	0/2
Gastric	0/1	0/1	0/1			
Lung – NSCLC	0/7	3/7	6/7	0/4	1/4	3/4
– SCLC	0/1	1/1	1/1			
Ovarian	0/2	0/2	1/2	0/1	0/1	0/1
Melanoma	0/2	2/2	2/2	0/1	1/1	1/1
Various	0/3	0/3	0/3	0/2	0/2	0/2
Active/total	0/19	*6/19*	10/19	0/10	*2/10*	4/10
	0%	*32%*	53%	0%	*20%*	40%

[a] Responsive (T/C ⩽ 30%)/total.

Table 3. Activity of selected US-NCI compounds in human tumor xenografts in the clonogenic assay *in vitro*

Compound	Dose (μg/ml)	Xenografts Responsive[a]	Total (%)	Bone-marrow median T/C (%)[b]		P388 T/C (%)	
4-Ipomeanol	1.0	0/34	0	112	–	91	–
	10.0	4/41	9	115	–	88	–
	100.0	10/43	26	100	–	94	–
	1000.0	8/8	100	89	–		
Oxanthrazole	0.1	1/32	3	123	–	65	–
	0.3	9/43	21	95	–		
	1.0	20/48	42	41	+	0	+ + +
	3.0	25/36	69	1	+ + +		
	10.0	6/7	85	1	+ + +		
Rapamycin	0.0001	2/31	6	1	+ + +	11	+ + +
	0.0003	4/31	13	2	+ + +	1	+ + +
	0.001	14/41	37	4	+ + +	1	+ + +
	0.003	20/32	63	5	+ + +		
	0.01	23/33	70	3	+ + +		
	0.03	8/11	73	1	+ + +		

[a] T/C ⩽ 30%.
[b] 50% ⩽ T/C, non-toxic; + = 30% ⩽ T/C < 50%, marginally toxic; + + = 10% ⩽ T/C < 30%, toxic; + + + = T/C < 10%, highly toxic.

4-Ipomeanol (NSC-349438)

4-Ipomeanol (Figure 1) is a pulmonary toxin bioactivated through a lung cytochrome P450 pathway. Because of its assumed lung specificity, ipomeanol is being developed for clinical trial by the Lung Cancer Drug Discovery Project of the NCI (5).

Ipomeanol was tested in human tumor xenografts of different histologies in the clonogenic assay. At a dose level of 10 μg/ml ipomeanol was active in 4/41 tumors (9%), namely 4/5 small-cell carcinomas of the lung (Table 3). Bronchogenic carcinomas of large-cell ($n = 4$), squamous-cell ($n = 1$) or adenocarcinoma ($n = 4$) subtypes or tumors of other histologies did not respond to ipomeanol. At the high dose of 100 μg/ml, ipomeanol inhibited colony formation in 10/43 xenografts tested (26%). *In vivo*, ipomeanol was tested in three small-cell lung carcinomas previously shown to be sensitive *in vitro*. The maximally

DOCKET ALARM

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.