Preclinical Phase II Studies in Human Tumor Lines: a European Multicenter Study

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Abstract—*In an attempt to increase the predictability and to extend the differential capacity of the anticancer drug development program the American National Cancer Institute has recently proposed the introduction of a screening system consisting of human tumor cell lines to select drugs in a disease-oriented fashion rather than by the previously applied drug-oriented strategy. Although this new approach offers great advantages, assay limitations can be identified in testing unknown compounds for antitumor activity in vitro. Human tumor xenografts grown in nude mice may play an additional role in the prediction of clinical activity and the assessment of the spectrum of activity of potential anticancer drugs, because they have a better relationship with the clinical situation of cancer treatment.*

In a European multicenter collaboration it has been proposed to use panels of human tumor lines from solid tumor types to study:

- the antitumor activity of three different drugs per tumor type;*
- the reliability of 'preclinical' phase II studies by comparison of the obtained data with results of phase II clinical trials;*
- the feasibility of this joint project, such as the methodology, the reproducibility of experimental data and the introduction of uniform activity criteria.*

If preclinical phase II studies in human tumor lines generate reliable results, this in vivo screening system will create a unique possibility to better identify promising clinical candidate compounds or analogs of conventional cytostatic agents as well as those tumor types likely to respond to the selected investigational drugs.

INTRODUCTION

A NEW drug is considered a candidate for clinical evaluation of anticancer activity if it has a certain efficacy in a preclinical screening system. The first selection of active compounds from a usually large number of products is carried out through relatively simple test models and often by the use of only one model: the primary screen or prescreen. Drugs active in the primary screen are then subjected to further analysis of their activity in a secondary screen, generally consisting of a series of more sophisticated test models.

From 1975 until 1985 the screening system for new anticancer drugs employed by the American National Cancer Institute (NCI) has consisted of the *in vivo* P388 murine leukemia prescreen, because it appeared to be the most sensitive single test model

to detect clinically useful anticancer drugs [1-3]. Compounds without minimal activity in P388 were usually rejected from further testing, unless they showed activity in other relevant biological or biochemical test models ('bypass' compounds). Compounds with reproducible activity in P388 proceeded to the secondary screen formed by a panel of five to six murine tumor models (lung cancer, colon cancer, breast cancer, leukemia, melanoma) and three human tumor xenografts grown in nude mice (lung cancer, colon cancer, breast cancer). In view of the clinical results for compounds selected by this drug screening system, it has recently been concluded that this screen had a low predictive capacity and had defined a few new anticancer drugs [3-6]. In addition, most of the clinically effective drugs were active against selected tumor types, mainly leukemias and lymphomas [7]. As a result of the emergence of a variety of compounds proven inactive in phase II clinical trials many

patients have been 'treated' with inactive agents.

Obviously, there is a need for a preclinical screening system with a higher predictive value in the development of new anticancer drugs. New screens will have to relate better to the clinical situation of cancer treatment, and they should be disease-oriented rather than drug-oriented [8]. In contrast with the murine tumor models the incorporation of assays based on human tumor tissue may not only improve the correlation between preclinical and clinical results, but also detect new compounds which would have been missed by the P388 pre-screen. The NCI is presently in the process of changing its screening policy by the introduction of a human tumor cell-culture screening system. The clinical predictive potential of this new approach has been suggested by experimental data obtained from the colony-forming assay [9–12]. In the new primary screen, each tumor type will be represented by a large panel of *in vitro* human malignant cell lines [8]. As for any screening system assay limitations can be identified, such as compounds requiring systemic metabolic activation will be inactive *in vitro*, components of the culture medium may reverse the activity of certain antimetabolites and compounds without *in vivo* therapeutic efficacy may be active in the assay. Human tumor xenografts grown in nude mice may relate better to the clinical situation of cancer treatment and therefore, in addition to *in vitro* screening, play an important role in the prediction of clinical activity and in the assessment of the spectrum of activity of potential anticancer drugs [13].

HUMAN TUMOR LINES

We have recently reviewed the value of human tumor xenografts grown in nude mice as test models in anticancer drug development [14]. In general, human tumor lines retain not only the histological, biochemical and antigenic characteristics, but most importantly the chemosensitivity pattern of the tumor tissue of the donating patient [14–19]. This latter property emphasizes the potential value of a panel of lines as an advanced model to predict the efficacy of novel agents in a particular malignancy. The model may also identify active analogs or analogs with a different spectrum of activity by comparative efficacy studies with parent conventional cytostatic agents. Subcutaneously implanted tumor tissue fragments offer a good visualization of tumor growth and repeated measurements will easily indicate volume progression or regression. Similarly to a phase II trial in patients, for drug screening the model should contain a variety of lines per tumor type, each differing in histology, growth rate and chemosensitivity. To validate this new screening system a positive correlation has to be demonstrated between experimental data and

results of phase II clinical trials, both for clinically active and inactive drugs.

In Europe, several investigators have gained experience in the establishment of human tumor lines in nude mice, their characterization and the assessment of their sensitivity to conventional and new cytostatic drugs [16, 19–34]. Collaboration between these centers would obviously facilitate and accelerate experiments in order to evaluate the potential and to define the specific role of human tumor xenografts in the screening of new compounds in specific malignancies. The reliability of this experimental approach will be determined in panels of human tumor lines from several tumor types present in the participating institutes by studying:

- the antitumor activity of three different drugs per tumor type;
- the reliability of 'preclinical' phase II studies by comparison of the data obtained with results of phase II clinical trials;
- the feasibility of this joint project, such as the methodology, the exchange of data and the introduction of uniform activity criteria.

If the study is successful, this multicenter collaboration will create a unique possibility to better select promising clinical candidate compounds and indicate those tumor types likely to respond to the investigational drugs selected by this human tumor tissue-based *in vivo* screening system.

PRECLINICAL PHASE II STUDIES: OBJECTIVES

The purpose of the experimental approach is a European multicenter collaboration to determine the predictive value and the feasibility of preclinical phase II studies in human tumor lines. Participants are indicated in Table 1.

1. Tumor types to be studied are: lung cancer (small cell and non-small cell), colorectal cancer, ovarian cancer, melanoma, breast cancer, head and neck cancer, and soft tissue sarcoma. Each tumor type will be represented by eight human tumor lines grown in nude mice and differing in histological subtype, growth rate and sensitivity to cytostatic drugs.
2. The activity of three different drugs, adriamycin, m-AMSA and DuP 785, will be assessed with the use of one treatment schedule for each agent.
3. Preclinical activity criteria have to be evaluated and discussed in order to be able to compare experimental data with clinical findings of phase II evaluation of these drugs in the respective tumor types. In parallel a variety of activity measurements, such as 'specific growth delay' and 'optimal growth inhibition' will be calculated and compared. A choice of the most appropriate evaluation criterium should be possible at the end of these studies.

Table 1. Participants in the phase II preclinical European multicenter project

Name	Center, city and country	Tumor types
G. Atassi*	Institut Jules Bordet, Brussels, Belgium	
E. Boven*	Free University Hospital, Amsterdam, The Netherlands	Ovarian cancer, soft tissue sarcoma
B.J.M. Braakhuis	Free University Hospital, Amsterdam, The Netherlands	Head and neck cancer
V. Budach	Strahlenklinik, Essen, Federal Republic Germany	Soft tissue sarcoma
H.H. Fiebig*	Albert-Ludwigs University, Freiburg, Federal Republic Germany	Lung cancer, breast cancer, colon cancer, ovarian cancer, melanoma
Ø. Fodstad*	Norwegian Radium Hospital, Oslo, Norway	Melanoma, soft tissue sarcoma, lung cancer
S.B. Kaye	University of Glasgow, Glasgow, United Kingdom	Lung cancer
J.F. Smyth	Western General Hospital, Edinburgh, United Kingdom	Lung cancer
M. Spang-Thomsen	University of Copenhagen, Copenhagen, Denmark	Breast cancer, lung cancer

*Participants contributing to the pilot study.

4. Assessment of the feasibility of this joint project will be carried out considering the influence of various aspects related to the institutes, such as laboratory facilities, differences in nude mouse strains (drug pharmacology), characteristics of tumor lines, methods of treatment and evaluation, and exchange and reproducibility of experimental data.

PLAN OF INVESTIGATION

Animals

Participants will use the nude mouse strain available in their laboratories. The animals are maintained in isolation under sterile conditions. Animal handling is carried out in a laminar flow hood.

Tumor lines

At first, a pilot study will be carried out by four participants (Table 1) in a series of four lines derived from lung cancer (small cell and non-small cell), melanoma, ovarian cancer, colorectal cancer and breast cancer in order to adjust experimental methods. In the plan described for each tumor type, eight tumor lines will be chosen for representative characteristics based on histological subtype, tumor doubling time and chemosensitivity pattern. Chemotherapy experiments will be divided evenly among participants for which is necessary that a number of lines are transferred to other institutes. Human tumor lines will be studied as subcutaneous bilateral implants (fragments 2–3 mm in diameter) in the flanks of the nude mice.

Drugs, doses and schedules

Drugs to be studied are adriamycin (clinically active in various tumor types), m-AMSA (inactive in solid tumor types), and DuP 785 (antimetabolite, presently in early clinical evaluation) (Fig. 1). Schedules have been designed dependent on the cytotoxic properties of the respective compounds. Doses will be administered according to the maximum tolerated dose (MTD) for tumor-bearing nude mice. At this MTD the mice should have a mean weight loss of 10% in the week following the first injection. Drugs are administered i.v. unless hampered by a high volume of injection (>0.25 ml) or by repeated injections (>3). Death occurring within 2 weeks after the final injection will be considered a toxic death and the animal will be excluded from the evaluation. The fact that investigators will use their strain of nude mice adjustments of the proposed dose (Table 2) may be necessary in order to administer the MTD in their animals.

Drug information

Adriamycin (doxorubicin, NSC 123127) is an anticancer agent used in the standard chemotherapy regimens of most hematological and many solid tumor types [7, 35]. It is a glycoside antibiotic originally isolated from the fungus *Streptomyces peuce-tius*. The drug was chosen for clinical evaluation because of antitumor activity in the murine tumor models B16 melanoma, CD8F₁ mammary tumor, L1210 leukemia, and in LX-1 human lung xeno-graft and MX-1 human mammary xenograft [2].

adriamycin

m-AMSA

DuP 785

Fig. 1. Structural formulas of adriamycin, m-AMSA and DuP 785.

Three major mechanisms of action resulting in cytotoxicity have been postulated: DNA intercalation, membrane binding, and free-radical formation [36]. Doses of 60–75 mg/m² i.v. as a bolus are usually administered if the drug is used as a

single agent every 3 weeks in patients not heavily pretreated. Drug elimination occurs mainly through the biliary system.

m-AMSA (4'-(9-acridinylamino)-methanesulfon-m-anisidide, amsacrine, NSC 249992) is a synthetic aminoacridine derivative and has been introduced into clinical trials in 1977. Preclinically, significant activity for m-AMSA was demonstrated in the P388 and L1210 leukemias and B16 melanoma. Moderate activity was observed against the CD8F₁ mammary tumor, and the murine colon 26 and colon 38 tumors [37, 38]. In phase II trials m-AMSA appeared to be inactive in solid tumor types, whereas a variety of the hematological malignancies responded to the drug [7]. Presently, the drug is under investigation in combination chemotherapy in the treatment of leukemias. m-AMSA acts by binding to DNA through intercalation. The recommended phase II dose for solid tumors when given as an i.v. bolus injection was 120 mg/m² for good risk patients [38]. Pharmacokinetic studies of radio-labelled m-AMSA showed a decreased urinary excretion of total radioactivity in patients with severe liver dysfunction, whereas in patients with severe renal disease excretion was only slightly affected [39].

DuP 785 (6-fluoro-2-(2'-fluoro-1,1'-biphenyl-4-yl)-3-methyl-4-quinoline carboxylic acid sodium salt, NSC 339786) is a substituted 4-quinolinecarboxylic acid and is currently under investigation in phase I clinical trials [40–42]. Antitumor activity in mouse tumor models was shown in P388 and L1210 leukemia, and colon 38. Marginal activity was observed in B16 melanoma [43]. Repeated daily doses appeared more effective than the every 4 days schedule against i.p. L1210 leukemia, if similar cumulative doses of the drug had been administered. DuP 785 showed activity also in the LX-1 human lung, MX-1 human mammary and CX-1 human colon xenografts in the subrenal capsule assay in nude mice. The drug is an antimetabolite and acts through a potent inhibition of the mitochondrial enzyme dihydroorotic acid dehydrogenase involved in the pyrimidine synthesis [44]. Because of the observed schedule-dependent preclinical antitumor activity of DuP 785, it was

Table 2. Treatment schedules (MTD) designed in tumor-bearing female NMRI/cpb nude mice, age 10–12 weeks, weight 26–32 g*

Drug	Company	Solution	Dose (mg/kg)	Route	Days of treatment
Adriamycin	Farmitalia	2 mg/ml	8	i.v.	0,7
m-AMSA	Parke-Davis†	1 mg/ml	8	i.v.	0,7
DuP 785	Du Pont de Nemours†	10 mg/ml	50	i.p.	0,1,2,3,4

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†Generous gift.

Fig. 2. Diagrams of tumor volume response to treatment, indicating the measurement of specific growth delay (a) and optimal growth inhibition (b).

decided to administer the drug at a daily base in the proposed study.

EVALUATION OF TREATMENT

Tumor growth will be measured weekly in two dimensions with calipers (twice weekly in case of tumor lines with a volume doubling time less than 5 days). Volume of nodules is calculated according to the formula $0.5 \times \text{length} \times \text{width}^2$. Treatment is started when tumors have reached a mean diameter of 6 mm (4–8 mm). The first treatment day is indicated as day 0. The number of mice required will be such that at the time of evaluation there are at least five mice per treatment/control group and 6–10 tumors in each group. Experiments will be finished 5 weeks after the last injection. In case of complete remissions the mice have to be observed for at least another 4 weeks to check on regrowth of the tumors.

For all individual tumors the volume changes from the start of treatment (V_0) will be expressed as V_t/V_0 at each day of measurement (V_t). The mean of the relative volumes V_t/V_0 for all tumors of the control group and that of the treatment group ($\pm$ S.E.) is used for drawing growth curves. Complete remissions will be mentioned separately.

Several criteria for evaluation will be applied in parallel, such as specific growth delay (SGD, Fig. 2a) and optimal growth inhibition ($T/C\%$, Fig. 2b). To calculate SGD [18] the end-point of evaluation is taken at a relative tumor volume of twice the treatment size from day 0 for control and

treated tumors. In the case of rapidly growing tumor lines (tumor doubling time less than 5 days) SGD will also be calculated over two doubling times. The following formula is used:

$$\text{SGD} = \frac{T_d \text{ treated} - T_d \text{ control}}{T_d \text{ control}}$$

and $\text{SGD} \leq 1$ is expressed as $-$, >1 as $+$, >2 as $++$ and complete remissions as $+++$.

For the $T/C\%$ calculations [21] the following formula will be applied at each day of tumor measurements:

$$T/C\% = \frac{\text{mean } V_t/V_0 \text{ treated}}{\text{mean } V_t/V_0 \text{ control}} \times 100\%$$

with an optimal $T/C\%$ within 5 weeks after the last injection $\geq 50\%$ designated as $-$, $<50\%$ as $+$, $<25\%$ as $++$ and complete remissions as $+++$. Both formulas for SGD and $T/C\%$ have disadvantages if various drug effects have to be compared in tumor lines varying in growth rate. In the course of the study methods have to be explored to circumvent these limitations such as the design of evaluation criteria independent of tumor growth rate.

Another point of discussion will be the comparison of experimental data with results of phase II trials in patients. In the clinic response rates are expressed as the number of patients with tumor reductions over 50% and with complete remissions. The criteria for drug activity in human tumor xenografts outlined above are arbitrary and may be subjected to adjustments.

CONCLUDING REMARKS

The European data collected over the past few years indicate that human tumor lines in nude mice represent a promising model of human cancer and may be useful in drug activity screening. A multi-center European collaborative effort is ongoing to analyze the predictive value of the model and establish the role of this advanced system in anticancer drug development. To guarantee the high quality of the experiments laboratory facilities and personnel will fulfil the requirements [14]. Tumor lines have to be monitored at regular time intervals to detect phenotypic changes or alterations in chemosensitivity.

With regard to chemotherapy experiments in nude mice and in patients, several pharmacological differences may be anticipated. Such limitations may interfere with the correlation between experimental and clinical data and will be elucidated partially by our initiative. If panels of human tumor lines in the nude mouse appear to be predictive for the clinical response of a drug, this approach will assist in reducing the number of phase II clinical trials with negative results and rapidly identify sensitivity tumor types for a new anticancer drug.

REFERENCES

1. Goldin A, Venditti JM, MacDonald JS, Muggia FM, Henney JE, DeVita VT. Current results of the screening program at the Division of Cancer Treatment, National Cancer Institute. *Eur J Cancer Clin Oncol* 1981, **17**, 129–142.
2. Venditti JM. Preclinical drug development: rationale and methods. *Sem Oncol* 1981, **8**, 349–361.
3. Staquet MJ, Byar DP, Green SB, Rozenzweig M. Clinical predictivity and transplantable tumor systems in the selection of new drugs for solid tumors: rationale for a three-stage strategy. *Cancer Treat Rep* 1983, **67**, 753–765.
4. Venditti JM. The National Cancer Institute antitumor drug discovery program, current and future perspectives: a commentary. *Cancer Treat Rep* 1983, **67**, 767–772.
5. Atassi G, Staquet M. The clinical predictive value of the mouse screening method. In: Hilgard P, Hellman K, eds. *Anticancer Drug Development*. Barcelona, Prous, 1983, 27–34.
6. Corbett TH, Valeriote FA, Baker LH. Is the P388 murine tumor no longer adequate as a drug discovery model? *Invest New Drugs* 1987, **5**, 3–20.
7. Marsoni S, Hoth D, Simon R, Leyland-Jones B, De Rosa M, Wittes RE. Clinical drug development: an analysis of phase II trials, 1970–1985. *Cancer Treat Rep* 1987, **71**, 71–80.
8. Boyd MR, Shoemaker RH, McLemore TL et al. New drug development. In: Roth JA, Ruckdeschel JC, Weisenburger TH, eds. *Thoracic Oncology*. Philadelphia, WB Saunders, in press.
9. Von Hoff DD, Clark GM, Stogdill BJ et al. Prospective clinical trial of a human tumor cloning system. *Cancer Res* 1983, **43**, 1926–1931.
10. Shoemaker RH, Wolpert-DeFilippes MK, Kern DH et al. Application of a human tumor colony-forming assay to new drug screening. *Cancer Res* 1985, **45**, 2145–2153.
11. Staquet M, Brown BW, Rozenzweig M, Van Muylder E, Dodion P, Sanders C. Validation of the clinical predictive values of the *in vitro* phase II clonogenic assay in cancer of the breast and of the ovary. *Am J Clin Oncol* (in press).
12. Fiebig HH, Schmid JR, Bieser W, Henss H, Löhr GW. Colony assay with human tumor xenografts, murine tumors and human bone marrow. Potential for anticancer drug development. *Eur J Cancer Clin Oncol* 1987, **23**, 937–948.
13. Pinedo HM. Development of new anti-cancer drugs. *Med Oncol Tumor Pharmacother* 1986, **3**, 63–69.
14. Winograd B, Boven E, Lobbezoo MW, Pinedo HM. Human tumor xenografts in the nude mouse and their value as test models in anticancer drug development. *In Vivo* 1987, **1**, 1–14.
15. Bailey MJ, Gazet JC, Smith IE, Steel GG. Chemotherapy of human breast-carcinoma xenografts. *Br J Cancer* 1980, **42**, 530–536.
16. Shorthouse AJ, Smyth JF, Steel GG, Ellison M, Mills J, Peckham MJ. The human tumour-xenograft—a valid model in experimental chemotherapy? *Br J Surg* 1980, **67**, 715–722.
17. Giovanella BC, Stehlin JS, Shepard RC, Williams LJ. Correlation between response to chemotherapy of human tumors in patients and in nude mice. *Cancer* 1983, **52**, 1146–1152.
18. Steel GG, Courtenay VD, Peckham MJ. The response to chemotherapy of a variety of human tumour xenografts. *Br J Cancer* 1983, **47**, 1–13.
19. Fiebig HH, Schuchhardt C, Henss H, Fiedler L, Löhr GW. Comparison of tumor response in nude mice and in the patients. *Behring Inst Mitt* 1984, **74**, 343–352.
20. Atassi G, Dumont P, Fournier J. New methods for determining tumor sensitivity. *Eur J Cancer Clin Oncol* 1985, **21**, 1299–1301.
21. Boven E, Van der Vijgh WJF, Nauta MM, Schlüper HMM, Pinedo HM. Comparative activity and distribution studies of five platinum analogues in nude mice bearing human ovarian carcinoma xenografts. *Cancer Res* 1985, **48**, 86–90.
22. Boven E, Nauta MM, Schlüper HMM, Elferink F, Van der Vijgh WJF, Pinedo HM. Secondary screening of platinum compounds in human ovarian cancer xenografts in nude mice. *Eur J Cancer Clin Oncol* 1985 **10**, 1253–1260.
23. Boven E, Nauta MM, Schlüper HMM, Erkelens CAM, Pinedo HM. Superior efficacy of trimelamol to hexamethylmelamine in human ovarian cancer xenografts. *Cancer Chemother Pharmacol* 1986, **18**, 124–128.
24. Braakhuis BHM, Schoevers EJ, Heinerma ECM, Sneeuwloper G, Snow GB. Chemotherapy of human head and neck cancer xenografts with three clinically active drugs: cis-platinum, bleomycin and methotrexate. *Br J Cancer* 1983, **48**, 711–716.
25. Braakhuis BJM, Leyva A, Schoevers EJ, Boerrigter GH, Schornagel JH, Snow GB. Lack of effect of methotrexate on human head and neck tumours transplanted in athymic nude mice. *Acta Otolaryngol* 1985, **99**, 208–213.
26. Budach V, Bamberg M, Scheulen M, Niederle N, Krause U. Preclinical chemosensitivity studies with human soft tissue sarcomas in nude mice. *J Cancer Res Clin Oncol* 1986, abstr. Ifo 16.
27. Donhuijsen K, Van Beuningen D, Budach V, Schmidt U, Streffer C. Do heterotransplanted soft tissue sarcomas retain their primary morphologic and flow cytometric features? *J Cancer Res Clin Oncol* 1986, abstr. Sof 01.
28. Fiebig HH, Neumann HA, Henss H, Koch H, Kaiser D, Arnold H. Development of three human small cell lung cancer models in nude mice. *Recent Res Cancer Res* 1985, **97**, 77–86.

29. Fiebig HH, Widmer KH, Fiedler L, Wittekind C, Löhr GW. Development and characterization of 51 human tumor models for large bowel, stomach and esophageal cancers. *Dig Surg* 1984, **1**, 225–235.
30. Fodstad Ø, Olsnes S, Pihl A. Inhibitory effect of abrin and ricin on the growth of transplantable murine tumors and of abrin in human cancers in nude mice. *Cancer Res* 1977, **37**, 4559–4567.
31. Fodstad Ø, Aass N, Pihl A. Response to chemotherapy of human malignant xenografts in athymic, nude mice. *Int J Cancer* 1980, **25**, 453–458.
32. Fodstad Ø, Aamdal, Pihl A, Boyd MR. Activity of mitozolomide (NSC 353451), a new imidazoltetrazine, against human lung-, melanoma-, sarcoma-, and colon tumor xenografts. *Cancer Res* 1985, **45**, 1776–1786.
33. Merry S, Cunningham D, Courtney ER, Hamilton T, Kaye SB, Freshney RI. Circumvention of inherent resistance with verapamil in a human tumor xenograft. In: Winograd B, Peckham MJ, Pinedo HM, eds. *Human Tumor Xenografts in Anticancer Drug Development*. Heidelberg, Springer, in press.
34. Brünnner N, Spang-Thomsen M, Vindeløv LL, Wolff J, Engelholm SA. Effect of tamoxifen on the receptor-positive T61 and the receptor-negative T60 human breast carcinoma grown in nude mice. *Eur J Cancer Clin Oncol* 1985, **21**, 1349–1354.
35. Blum RH, Carter SK. Adriamycin: a new anticancer drug with significant clinical activity. *Ann Intern Med* 1974, **80**, 249–259.
36. Young RC, Ozols RF, Myers CE. The anthracycline antineoplastic drugs. *N Engl J Med* 1981, **305**, 139–153.
37. Legha SS, Gutterman JU, Hall SW *et al.* Phase I clinical investigation of 4'-(9-acridinylamino)methanesulfon-m-anisidine (NSC 249992), a new acridine derivative. *Cancer Res* 1978, **38**, 3712–3716.
38. Von Hoff DD, Howser D, Gormley P *et al.* Phase I study of methanesulfonamide, N-[4-(9-acridinylamino)-3-methoxyphenyl]-(m-AMSA) using a single-dose schedule. *Cancer Treat Rep* 1978, **62**, 1421–1426.
39. Hall SW, Friedman J, Legha SS, Benjamin RS, Gutterman JU, Li Loo T. Human pharmacokinetics of a new acridine derivative, 4'-(9-acridinylamino)methanesulfon-m-anisidine (NSC 249992). *Cancer Res* 1983, **43**, 3422–3426.
40. Schwartzmann G, Ten Bokkel Huinink WW, Dodion P *et al.* Phase I study of DuP 785 (NSC 368390) in solid tumors. *Proc Am Soc Clin Oncol* 1987, **6**, 41.
41. Bork E, Hansen HH. A phase I clinical trial of DuP 785 (NSC 368390) 4-quinolinecarboxylic acid using a weekly schedule. *Proc Am Assoc Cancer Res* 1987, **28**, 223.
42. Armand JP, Fontana X, De Forni M *et al.* A phase I study of DuP-785 (NSC 368390) using a 5 daily i.v. schedule. *Proc Am Soc Clin Oncol* 1987, **6**, 46.
43. Ardeckey RJ, Dexter DL, Dusak BA *et al.* 4-Quinolinecarboxylic acids as novel chemotherapeutic agents. *Proc Am Assoc Cancer Res* 1985, **26**, 251.
44. Peters GJ, Sharma SL, Laurensse E, Pinedo HM. Inhibition of pyrimidine *de novo* synthesis by DuP-785 (NSC 368390). *Invest New Drugs* 1987, **5**, 235–244.