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"Of mice and men": values and liabilities of the athymic nude mouse model in anticancer drug development

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Abstract

Human tumour xenografts implanted subcutaneously (s.c.) into immunosuppressed mice have played a significant role in preclinical anticancer drug development for the past 25 years. Their use as a predictive indicator of probable clinical activity has been validated for cytotoxics. A retrospective analysis for 39 compounds where both extensive xenograft testing and Phase II clinical data were available, performed by the National Cancer Institute (NCI), has shown that 15/33 agents (45%) with activity in more than one-third of xenografts showed clinical activity ($P = 0.04$). However, with the exception of non-small cell lung cancer, activity within a particular histological type of the xenograft generally did not predict for clinical activity in the same tumour. Today, the question (largely unanswered) is how useful is the xenograft model (particularly the traditional s.c. model) in contemporary cancer drug discovery? There are many variables when conducting xenograft experiments which impact on outcome; viz, site of implantation, growth properties of the xenograft and size when treatment is initiated, agent formulation, scheduling, route of administration and dose and the selected endpoint for assessing activity. The xenograft model remains of value in current preclinical cancer drug development, especially when such studies give due consideration to the above variables and are based on sound mechanistic (e.g. status of the selected target in the chosen model) and pharmacological (e.g. use of formulated agent) principles. Dependent upon the drug target, a slowing of xenograft tumour growth (cytostatic effect) rather than tumour shrinkage might be the major observed effect. Human tumour xenografts are also particularly useful in determining pharmacodynamic markers of response for subsequent clinical application. Nevertheless, it needs to be kept in mind that the use of xenografts is relatively time-consuming and expensive, raises animal ethical issues and there are instances where the model is inappropriate as a likely predictor of clinical outcome (e.g. inhibitors of the metastatic process and anti-angiogenic strategies as the vasculature is of murine origin).

Keywords: Athymic nude mouse model; Anti-cancer drug development; Review; Human tumour xenografts; Limitations

1. Introduction

Contemporary anticancer drug development is a multi-million dollar and time-consuming business. Typically, from concept to the completion of Phase III clinical trials and gaining regulatory approval requires in excess of 10 years and as much as 500 million dollars. For the first quarter of a century of modern cancer drug development (circa 1945–1969), thousands of generally randomly produced molecules were tested in mice bearing rapidly growing murine leukaemias (e.g. P388 and L1210) [1]. In 1969, came the first report of the growth of a human tumour in an immunodeficient "nude" (athymic) mouse [2]. Since then, human tumour xenografts grown in nude [3] or in mice with severe combined immunodeficiency (SCID) [4] have covered all of the major tumour types and represented the mainstay of preclinical anticancer drug development testing *in vivo*.

The modern paradigm for anticancer drug discovery, as widely used by drug companies and within some academic groups, comprises a series of carefully constructed steps that are designed to rapidly and efficiently allow "proof of principle", pharmaceutically-tractable, molecules to be tested in Phase I and II clinical trials. Such a cascade (Fig. 1) may be envisaged as a large number of molecules feeding into a series of iterative stop/go tests of increasing biological complexity. The concept of "therapeutic index", that is the demonstration of antitumour efficacy at doses well below those

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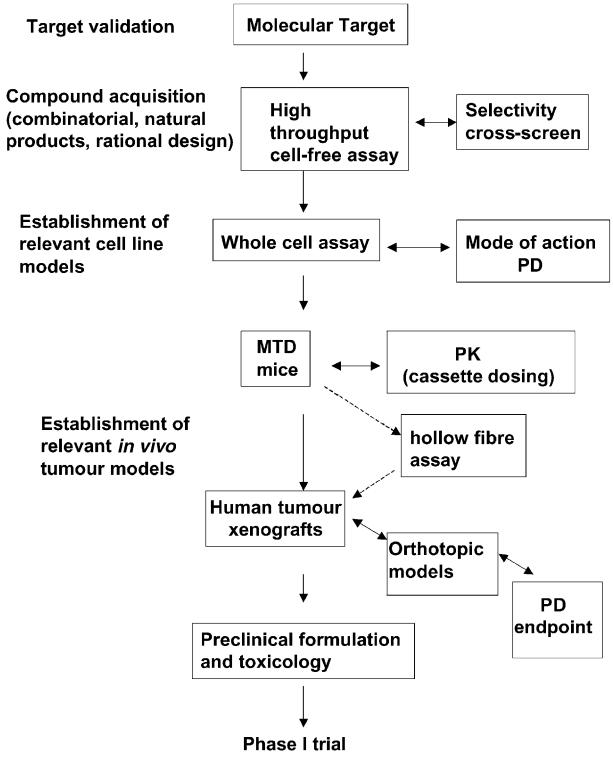

Fig. 1. Generic contemporary drug evaluation cascade. PD, pharmacodynamics; PK, pharmacokinetics; MTD, maximum tolerated dose.

causing severe toxicities, is also a long-established paradigm in preclinical drug discovery [5]. A key part of the preclinical stage of the process, and often representing a significant bottleneck, is the demonstration of antitumour efficacy in a "relevant" tumour model *in vivo*.

However, what is the place for the nude mouse xenograft model in cancer drug development today in this post-genomic era? What are its values? What are its limitations? Is testing *in vivo* even required—could predictive answers be obtained solely using human tumour cells in culture or even using in silico methodology, thus avoiding the increasing ethical issues raised by animal experimentation? As opposed to the previous era of "cytotoxic" cancer drugs, contemporary cancer drug development encompasses a wide variety of approaches generally based on attacking specific molecular targets where often, cytostatic rather than cytotoxic effects may be predicted. Thus, a slowing of tumour growth rather than shrinkage may occur. This may require a re-evaluation of the *in vivo* models developed and validated using cytotoxic drugs when testing such agents. Even within mouse models, is it the case that, in some instances, murine syngeneic models (often dismissed since the advent of human tumour models), transgenic models or orthotopic models may be more appropriate to use than human subcutaneously (s.c.) implanted xenografts in immune-suppressed animals? In some cases, maybe there is no appropriate preclinical model? In an industry where time is paramount, should we dispense with relatively slow and laborious xenograft efficacy determinations in favour of some more rapid, higher-throughput alternative (e.g. the hollow fibre assay [6])?

Hence, this article aims to provide a critical review of the role of human tumour xenografts transplanted in athymic (or SCID) mice in contemporary cancer drug development. Both values and liabilities will be discussed specifically in the context of experience with firstly cytotoxic platinum-based molecules and secondly with small molecules targeted against contemporary cancer targets (e.g. farnesyltransferase, heat shock protein 90, telomerase and tumour vasculature).

2. Human tumour xenografts: a validated model in the development of cytotoxic drugs

Within a few years of the original description of the nude mouse model, the Developmental Therapeutics Program at the National Cancer Institute (NCI) adopted, in 1976, the use of 3 human tumour s.c. xenografts (one representing each of colon, CX-1, breast, MX-1, and lung, LX-1, cancers) into its *in vivo* cancer drug screening programme [7]. Since then, although far greater emphasis has been placed on initial screening using panels of disease-oriented human tumour cell lines [8], the xenograft model has remained important. Early studies by Giovanella and colleagues [3] indicated that higher take rates were obtainable using cultured cells rather than when tumours were transplanted directly from patient biopsies (but see below). Later efforts at the NCI focused on the establishment of s.c. xenograft models from each of the 60 human tumour cell lines used in the *in vitro* screen [7].

In parallel, from the outset of xenografts being established from a variety of tumour types, numerous studies during the 1970s evaluated the predictive utility of the xenograft model in terms of responsiveness to "standard" anticancer drugs in relation to their effectiveness against corresponding tumour types in patients. Steel and colleagues established s.c. xenografts from a variety of tumour types (actually in conventional CBA mice that had been immuno-suppressed by thymectomy, cytosine arabinoside treatment and whole-body irradiation) and recorded that "human tumour xenografts broadly maintain the level of chemotherapeutic responsiveness of the source tumours in man" [9]. For example, xenografts derived from testicular teratomas and small cell lung tumours (both of which are relatively responsive to drugs in man) responded well, whereas xenografts derived from melanomas, colorectal and non-small cell lung tumours responded poorly. Overall, a total of 329 tumours were investigated; 34 (52% success rate) of colorectal xenografts were established, 9

(60%) of melanomas, 11 (23%) of ovarian cancers, 8 (10%) of breast cancers and 14 (30%) of testicular tumours. In addition, it was found that the histological characteristics of the source tumours were generally well maintained by xenografts. Responses to a variety of "standard" drugs available at this time were assessed either *in situ* (by growth delay from calliper measurements) or *ex vivo* by assessment of clonogenic cell survival using a soft-agar based assay [10].

From the early days, human tumour xenografts were established either by direct implantation of patient biopsy material or via inoculation of continuous human tumour cell lines. A particularly important large panel of xenografts, derived directly from biopsies, has been established by Fiebig and colleagues at the University of Freiburg in Germany [11]. More than 1600 tumours have been transplanted s.c. into nude mice and more than 300 xenografts established, representative of all of the major tumour types. A comparison of drug response in the xenograft compared with that in the patient was made in 80 cases in 55 xenografts using either an *in vivo* assay (a comparison of treated versus control tumour volumes) or *ex vivo* using a soft agar clonogenic assay from disaggregated tumours. In accordance with the earlier studies of Steel and colleagues alluded to above, the xenografts predicted correctly for clinical response in 19/21 (90%) of occasions when using the *in vivo* assay (this was reduced a little to 60% using the clonogenic assay) and predicted for resistance in 57/59 (97%) of occasions when using the *in vivo* assay (92% for the clonogenic assay).

In addition, the response pattern of more recently discovered clinically active drugs, paclitaxel, gemcitabine, docetaxel, vindesine and topotecan, was determined in 187 xenografts. Overall, the 5 drugs induced remissions in 24% (45/187) of the xenografts studied, whereas minor regressions or no change occurred in 13% of cases while 63% (117/187) of xenografts progressed on treatment. These findings are similar to the overall response rates recorded for monotherapy clinical trials with these agents. In addition, more responses (37%) were seen in a sub-group of tumours classified by the authors as clinically sensitive (small cell and non-small cell lung, breast, head and neck, leukaemia, melanoma, non-Hodgkin's lymphoma, gastric, testis) in comparison with those designated as resistant (4%; bladder, colon, cervix, central nervous system (CNS), hepatoma, mesothelioma, ovary, pancreas, prostate, renal soft tissue sarcoma). In recent years, this panel has been used to identify new drugs.

3. Use in cytotoxic cancer drug development

In addition, during the 1980s, many groups established disease-specific panels of xenografts from patient biopsies for the purposes of either studying tumour biology, responses to existing therapy (radiotherapy or chemotherapy) or for the discovery of specific classes of new drugs. A particular emphasis at the Institute of Cancer Research, London, was to establish parallel panels of *in vitro* cell lines (as a renewable source for biochemical and molecular studies) and corresponding *in vivo* xenograft counterparts (for pharmacological studies). This was firstly achieved for carcinoma of the cervix where 9 serially transplantable xenograft lines (and 4 parallel continuous cell lines) were established from 23 original biopsies [12,13]. The *in vivo* response to 3 commonly used drugs at the time (cisplatin, etoposide and bleomycin) performed in 3 xenografts showed the models to be relatively unresponsive [13]. A comparison of the *in vitro* and *in vivo* radiation response for 3 of the cell lines/xenografts revealed a general tendency for the *in vivo* results to follow that predicted from the *in vitro* studies [14].

The approach of using disease-specific *in vitro* cell lines and corresponding xenografts for cancer drug discovery was used in a programme designed to discover more effective analogues of cisplatin and carboplatin. To begin with, a panel of 23 serially transplantable ovarian cancer xenografts were established in female nude mice from 42 donor samples (from both previously treated and untreated patients) [15]. Initial "calibration" studies determined the response of 16 of the xenografts to cisplatin and carboplatin and two other platinum-containing agents (iproplatin and tetraplatin which were undergoing clinical testing in the late 1980s). Three distinct patterns of response were observed; comparative responsiveness to all 4 platinum drugs (2 lines), resistance to all 4 drugs (5 lines) and individual drug-specific responses (9 lines). Notably, it was also possible to confirm that in 8/9 lines, the therapeutic response of the xenograft reflected that recorded in the corresponding ovarian cancer patient receiving platinum-based chemotherapy.

Secondly, attempts were made to establish parallel cell line and xenograft counterparts; a total of 8 such paired models were established and sensitivities to cisplatin and carboplatin compared *in vitro* versus *in vivo* [16]. This study highlighted the comparative ease of establishing xenografts from cell lines (6/8 paired models were derived in this way) *versus* the comparative difficulty of establishing cell lines from xenografts. Although this was attempted on numerous occasions over several years for all 16 ovarian models used in the above described platinum calibration studies, cell lines were only derived from two xenografts (HX62 and PXN94). This was predominantly because of the propensity for primary cultures derived from xenografts to be rapidly overrun by murine fibroblasts. By contrast, as described above for cervical carcinoma, a greater success rate was obtained by splitting an original biopsy sample, part for

cell line establishment and part for direct s.c. implantation into nude mice. Notably, despite the presumed very different selection pressures operating with cell line versus xenograft establishment, a high statistically significant positive correlation for *in vitro* sensitivity versus *in vivo* responsiveness was obtained for both cisplatin and carboplatin [16]. This is illustrated in Fig. 2 and led directly to the incorporation of these paired models into a platinum drug discovery evaluation cascade [17].

The ovarian cancer models were further extended in the platinum drug discovery context by the establishment of pairs of platinum-drug sensitive and resistant lines [18]. In contrast to most drug resistance studies, where resistance was generated through repeated exposure of tumour cell lines to increasing concentrations of drug (often in excess of those achievable in plasma *in vivo*) *in vitro*, 7/8 of these platinum-resistant ovarian cancer models were established *in vivo* by repeated treatment of mice bearing xenografts (and repeated passaging into new donors) to cisplatin, carboplatin, iproplatin or tetraplatin. Biochemical studies elucidating the major mechanisms of platinum drug resistance in these acquired resistant cell line (and xenograft) models were then carried out. Paired cisplatin sensitive and acquired or intrinsic resistant cell line and xenograft models were then used over several years to screen in excess of 500 platinum analogues, with the aim of finding new platinum drugs possessing a greater spectrum of activity than cisplatin/carboplatin. Two drugs, the orally bioavailable JM216 (Satraplatin) and AMD473 were introduced into the clinic [19]. While both have demonstrated clinical activity (e.g. in ovarian and hormone-refractory prostate cancer) the activity of AMD473 against acquired cisplatin-resistant ovarian cancer was relatively modest [20]. In many respects, this was reflected in the preclinical xenograft data for this compound as pronounced activity against intrinsic or acquired cisplatin resistant ovarian carcinoma xenografts was limited to one model (CH1cisR) [21].

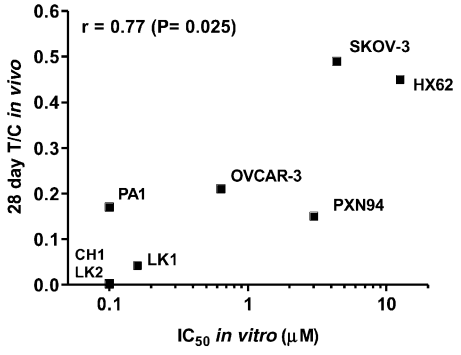

Fig. 2. Positive correlation for cisplatin between *in vivo* response of xenografts versus *in vitro* potency for corresponding cell line, across 8 human ovarian carcinoma models (replotted from Ref. 16). IC_{50}, concentration causing 50% cell growth inhibition; T/C, treated versus control values.

The most compelling evidence for the value of the s.c. xenograft model in cytotoxic cancer drug development comes from an extensive retrospective analysis from the NCI [22]. For 39 compounds, where both xenograft and Phase II data were available, activity (defined as either an increase of mouse survival by more than 25% or by a reduction in tumour weight in treated versus controls to greater than 40%) in at least one-third of tested xenografts, predicted for activity in at least some Phase II trials. Clinical activity (defined as responses in at least two tumour types) was not observed for any of the 6 agents where preclinical activity was seen in fewer than one-third of xenograft models tested. By contrast, 15/33 agents (45%) with activity in more than one-third of xenografts showed clinical activity ($P = 0.04$). Of the 39 agents evaluated, many may be considered as "standard" anticancer agents in use today (such as methotrexate, chlorambucil, 5-fluorouracil, cisplatin, paclitaxel, vinblastine, irinotecan, docetaxel, doxorubicin and cyclophosphamide). These data suggest that, at least for existing cytotoxic anticancer drugs, the s.c. xenograft model is of predictive value. However, activity within a particular histological type of xenograft generally did not predict for clinical activity in the same tumour, with the exception of non-small cell lung cancer.

Interestingly, the NCI analysis also investigated whether correlations exist between activity in xenografts versus activity in a shorter-term (6-day) *in vivo* assay (the hollow fibre assay) or even potency against cell lines *in vitro*. Positive correlations could help in the preselection of compounds for xenograft testing and thereby reduce the number of animals used. The hollow fibre assay is a hybrid *in vitro*/*in vivo* assay whereby particular cell lines from the 60 cell line panel may be grown and compounds rapidly tested *in vivo* [23,6]. In this assay, tumour cells (normally two of each of breast, ovarian, glioma, colon, melanoma and non-small cell lung) are sealed into polyvinylidine fluoride (PVDF) biocompatible hollow fibres and implanted into mice in two anatomically separate sites, intraperitoneally (i.p.) and s.c., and mice dosed i.p. with the test compound at two dose levels for up to 4 days. Two days later, fibres are removed and the numbers of remaining viable cells compared in treated *versus* control groups [Ref.] Decker S, Hollingshead M, Bonomi C, Carter J, Sausville E (2004). The hollow fibre model in cancer during screening: the NCI experience. *Eur J Cancer*, 40, this issue.

Interestingly, increased activity in the hollow fibre assay (especially with i.p. implanted fibres) also predicted for increased xenograft activity. Overall, 35% of 537 compounds tested had activity in at least one xenograft, whereas this increased to 63% for compounds exhibiting a response in more than 10 i.p. fibres [22]. The correlation did not occur with s.c. implanted fibres; this may be because there is insufficient time for angio-

genesis to the fibres to occur in the s.c. setting, thereby potentially limiting drug delivery to the tumour cells [24]. There was also a strong correlation between potency in the 60 cell-line screen and activity in the hollow fibre assay. 56% of compounds with a mean 50% growth inhibition of below 0.032 μM were active in at least six i.p. fibres, whereas only 4% of compounds with a potency of 100 μM were active to the same level.

Overall, taking all of the above into consideration, one may reasonably conclude that, at least for cytotoxic cancer drugs, the human tumour xenograft model, is a good predictor of clinical activity. Therefore, it seems reasonable and valuable, to continue the testing of new cytotoxics using xenografts. This is especially the case when used in combination with sound pharmaceutical and pharmacological principles (see below). Notably, some regulatory authorities (e.g. the European Medicines Evaluation Agency (EMEA) in its guidance notes on the preclinical evaluation of anticancer medicinal products, encourage the use of xenograft studies (http://www.eudra.org/emea.html).

4. A standard operating procedure (SOP) for conducting xenograft experiments? 99 ways to determine efficacy in a xenograft model: values and limitations

Before considering whether xenografts should still be used in contemporary cancer drug discovery (see below), it is critical to appreciate and understand each of the many variables that exist in the use of xenografts in drug testing. The cancer drug discovery literature is overwhelmed with preclinical studies invariably describing "active" new molecules where it is then proposed that these should be tested in the clinic. Commonly, such studies have involved human tumour xenografts, but used in a multitude of ways to assess the activity of molecules, regrettably, often with limited scientific rationale in terms of predictive value and integrating how the corresponding drugs will most likely be used in cancer patients. Hence, one needs to interpret xenograft studies with caution and bearing in mind the many variables that exist. Major variables are listed in Table 1 and include the origin of the tumour (i.e. cell line versus patient biopsy), target/receptor status of the tumour, the site of tumour implantation (e.g. s.c., i.p., orthotopic), the size of tumour at the onset of agent treatment, growth rate and growth characteristics, agent dose, formulation, scheduling and route of administration, and experimental endpoints. In the worst case scenario, many mice have been used in screening experiments of questionable pharmacological relevance and predictive value where poorly formulated agents (e.g. in Tween-based suspensions) have been administered i.p. to mice within 1–2 days of these mice being inoculated, also i.p., with suspensions of tumour cells and early assessments of "activity" made. Such studies add little to *in vitro* findings. Furthermore, to date, xenografts have often been used without characterisation at the molecular level for the particular target being addressed. This has made analyses of the value of the xenograft model in predicting for activity in patients, problematic, and has resulted in a wide-range of views being expressed. In view of the above-described large retrospective analysis by the NCI, proposals such as "activity was seen in the HT29 colon xenograft, therefore clinical trials should be conducted in patients with colon cancer" are naïve, especially in the absence of any consideration of pharmacological and pharmacodynamic principles. Let us consider some of these important variables.

4.1. Origin of the xenograft model

In general, xenografts derived directly from patient biopsies, in contrast to those derived from continuous cell lines, appear to retain better the morphological and molecular marker properties reminiscent of the source tumours in man. By contrast, xenografts derived from cell lines generally show a more homogeneous, undifferentiated histology (and, on occasion, loss of the target receptors/proteins), probably indicative of the higher selection pressure *in vitro* during extensive culturing. However, a disadvantage with lines established

Table 1
Xenograft testing: variables

Variable	Comment
Origin of tumour	Cell line or patient biopsy
Site of tumour implantation	S.c., i.p., orthotopic
Growth properties of tumour	Doubling time, degree of necrosis, stromal compartment
Size of tumour at onset of treatment	'chemoprevention', 'early', 'advanced' stage
Target status of tumour	Receptor/antigen density, presence of target
Agent formulation	Suspension or formulated, correlation with proposed clinical formulation?
Agent scheduling	Bolus, chronic, route of administration (i.v., i.p., intratumoral, oral)
Agent dose	Maximum tolerated? Pharmacokinetic, Pharmacodynamic correlation?
Endpoints	Increase in life-span. T/C, growth delay. When assessed.

s.c., subcutaneously; i.p., intraperitoneally; i.v., intravenously. T/C treated versus control values.

directly from biopsies is the difficulty in establishing cell lines from xenografts (as described above for ovarian cancer). Therefore, it is relatively difficult to establish parallel *in vitro* cell line (valuable as a continued source of pure human tumour cells for biochemical and molecular studies) and corresponding xenograft lines (valuable for pharmacological and pharmacodynamic studies). However, overall it appears that both xenografts derived from cell lines (e.g. the NCI studies) and those derived directly from patient biopsies (e.g. the Frieburg panel) provide some predictive power for selecting cytotoxics with clinical activity.

4.2. Site of xenograft implantation

The site of tumour implantation is another important variable. While most xenograft experiments use s.c. implantation (where it is then relatively easy to assess antitumour effects through the use of callipers to determine tumour diameter/volume), there are many reports of differences in biological behaviour (e.g. ability to metastasise and receptor/target status) when tumours are grown s.c. relative to orthotopically [25]. In addition, one needs to bear in mind the pharmacological limitation (relative to chemotherapy in man) of administering the test compound at the same site as the tumour (e.g. i.p. or intratumorally).

4.3. Growth characteristics of the model

It is also widely recognised that not all xenograft models are usable for compound screening because of their growth properties. Sometimes growth is too slow (over many months-this is often an issue with transgenic models as well), or too inconsistent/erratic/non-linear with time, or they possess cystic or necrotic areas, even at relatively small tumour volumes. The occurrence of cystic or diffuse necrotic areas (even to the edges of some tumours) can often comprise 20–80% of the tumour mass, depending upon the model and number of passages in mice. Although this effect may be less of a problem when SCID mice are used as hosts relative to athymic nudes [26], the issue does require consideration when using new models. For example, from 1600 tumour biopsy samples originally implanted into nude mice by the Frieburg group, 300 xenograft lines were established and 60 of these extensively studied [11]. Second, of 9 cervical carcinoma xenograft lines established, only 3 were applicable to routine use because of either particularly slow growth and or the tendency for possessing diffuse necrosis [13].

4.4. Stage at which the treatment begins

The activity of a test molecule can also be critically dependent upon the stage (size) of the tumour at the onset of treatment. In some instances, (which may be relevant to how this class of agent may be applied to man) agents are administered at the same time as tumour implantation (“chemoprevention” strategy). Herein, a choice of model where the take-rate is reproducibly greater than 90% and with consistent growth properties, is critical. Second, treatments may not begin until tumours are just palpable (approx 5 mm diameter, around 60 mm^3) (“early-stage” strategy). Herein, one needs to be aware of the possibility of the residual immune system of the host (principally natural killer cell activity) also participating in tumour regression or cures. Finally, treatment may not begin until tumours have reached 8–10 mm diameter (“advanced” stage). In many cases, this is more representative of how chemotherapy will be applied in the clinic, but one needs to evaluate the possibility that either target expression may decrease in larger tumours or that drug uptake/penetration may be compromised because of poor vasculature/increased areas of necrosis. In addition, for rapidly-growing tumours, tumour sizes/volumes may exceed those deemed to be ethically acceptable and result in the early termination of experiments before any delayed drug-induced toxicity may be evident (see below). Issues of animal welfare and ethics within cancer research have recently been considered within the United Kingdom (UK) and published as a set of guidelines [27].

4.5. The test compound

Another critical variable concerns the test agent itself, the way it is administered (route, scheduling, dose) and how it is formulated. In my view, xenograft studies should only be conducted where there is also considerable attention paid to these pharmaceutical (formulation) and pharmacological (pharmacokinetic/pharmacodynamic) principles. Having firmly established that a “lead” molecule possesses potent activity against the target in cell-free assays and within the appropriate tumour cell lines *in vitro*, it is now important to maintain a “pharmaceutical perspective” and consider at this point whether a clinically acceptable formulation of the molecule is attainable. Too many mice have been used in the past with compounds given as coarse suspensions in dimethyl sulphoxide (DMSO) or detergents (e.g. Tweens), vehicles which may not be usable in man. Considerable advances in the development of biocompatible formulations for poorly water-soluble drugs have been developed in recent years (e.g. using beta-cyclodextrin-based systems). However, developing a vehicle is still largely empirical. For particular targets, where chronic administration to patients is the likely clinical scenario based on the understanding of the target, an early evaluation of oral bioavailability may also be appropriate. This may initially be achieved using *in*

vitro cell line systems such as the colon cancer cell line CaCo2, or directly in rodents by determining pharmacokinetics following oral versus i.v. administration of the same dose.

Second, from both a scientific and animal ethical standpoint, xenograft antitumour studies conducted in mice should be limited only to those molecules that possess "adequate" pharmacokinetic properties. For closely related analogues, cassette dosing of mice is a useful tool to rapidly select compounds with optimal pharmacokinetic properties. One should also be prepared to have to compromise on potency against the target in favour of selecting an analogue possessing superior pharmacokinetic properties. The random screening of molecules, especially using i.p. drug administration to i.p. implanted tumours, a feature of cancer drug screening prevalent in the past, is no longer acceptable. Therefore, a pharmacological approach should be applied whereby only molecules where plasma (or ideally tumour) drug levels known to be required for activity against the target *in vitro* should be tested, and evaluated in xenografts known to possess the target. An elucidation of the effect of dose (and scheduling) should also be conducted thereby providing information on therapeutic index and how the "biologically effective dose" relates to the maximum tolerated dose (MTD). The NCI has recently adopted such an approach with emphasis on pharmacokinetics, mechanism of action and pharmacodynamics post-testing in the 60 cell-line screen and prior to *in vivo* antitumour xenograft studies [28].

4.6. Experimental endpoints

Finally, the choice of endpoint is a critical variable. The most commonly used are those involving measurements of tumour weight (or volume) in untreated or agent vehicle (control) animals versus treated groups on particular days after the start of treatment (e.g. treated [T] versus control [C] values; T/C). A widely used alternative is that using growth delay (GD), the difference in time for treated versus control groups to reach a preselected increase in volume (often 3–4-fold) normalised to the starting volume. In addition, a consideration of the growth-rate of control tumours may be applied by determination of specific growth delays (SGD). An unfortunately common feature is for assessments to be made at relatively early time-points and, in particular, *before* any drug-induced toxicity (body-weight loss, lethargy, even deaths) is evident. Regardless, in all cases, considerably more information may be provided by the provision of full tumour volume versus time curves. In particular, this may indicate whether cytotoxic (tumour shrinkage) or cytostatic (a slowing of tumour growth, but no shrinkage) effects might be expected in the clinic with the test compound (see example below for the farnesyl transferase inhibitor, R115777).

5. The value (and limitations) of the xenograft model in contemporary cancer drug discovery

Today there is far less focus on the development of further cytotoxics. In addition, there has been a considerable move away from the "black-box" approach to Phase I clinical trials where many agents of unknown mechanisms of action and poorly defined preclinical pharmacokinetics were introduced into the clinic. Does this necessarily mean that the xenograft model is of no further value in contemporary mechanism-directed cancer drug development? I believe that this should be considered on a case-by-case (target-by-target) driven basis. In careful mechanism-based studies, combined with sound pharmacological principles (as described above), then, in my view, the xenograft model remains of great value, both for assisting in the selection of leads for clinical evaluation and for guiding clinical studies (e.g. scheduling, combination strategies).

An example of where xenograft studies have proven useful in selecting and potentially guiding the clinical use of a compound targeting a novel cancer target, is that of G-quadruplex interactive inhibitors of the enzyme telomerase [29]. Herein, the first evidence of *in vivo* activity for such an inhibitor was shown for the trisubstituted acridine BRACO19, when used in combination with paclitaxel in nude mice bearing s.c. A431 epithelial cancer xenografts [30]. In the area of guiding the use of drugs, which *a priori* are not expected to possess significant activity when used alone, but rather will need to be used in combination, our studies with the vascular-targeting drug, 5,6,-dimethylxanthenone acetic acid (DMXAA) have demonstrated the potential clinical utility of combination studies with paclitaxel [31].

Certainly, another area where human tumours transplanted in nude or SCID mice remain of considerable value is in the determination of pharmacodynamic markers of drug efficacy, which may then be applied in the clinic. An example is with xenograft studies with the farnesyl transferase inhibitor R115777 [32]. "Typical" xenograft dose response curves for mice bearing a s.c. breast cancer tumour (MCF7) and treated orally with R115777 are shown in Fig. 3a. A point of note in contrast to responses traditionally observed to cytotoxics (e.g. cisplatin, paclitaxel) (Fig. 3c for paclitaxel against a human ovarian carcinoma xenograft) is that the signal transduction inhibitor induced a slowing (cytostatic) effect on tumour growth rather a cytotoxic, tumour shrinkage effect (as for paclitaxel). In addition, as with R115777, chronic (daily) rather than intermittent (e.g. weekly) dosing was required. As emphasised above, knowledge of both the compound mechanism of action

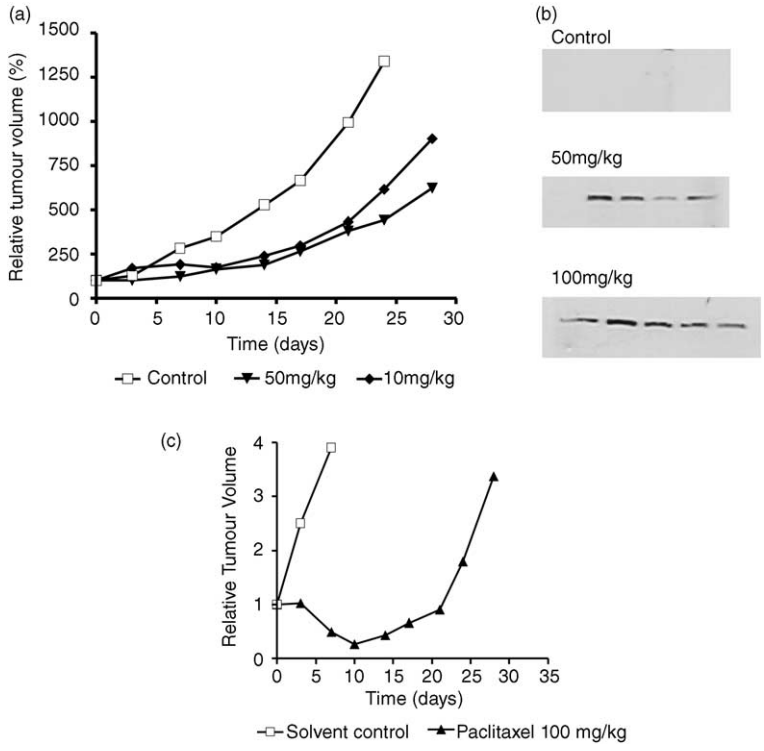

Fig. 3. (a) Tumour dose response curves for the farnesyl transferase inhibitor, R115777, against MCF-7 breast cancer xenografts. Dosing was oral, twice a day for 10 consecutive days. (b) Increase in prelamin A in R115777-treated xenografts (replotted from Ref. 31). (c) Tumour dose response curves for paclitaxel against CH1 ovarian cancer xenografts (dosing was i.p. daily for 5 days).

(molecular target) and pharmacokinetics is essential to guide xenograft experiments and to help predict what effect the compound being tested might be expected to induce. Studies with R115777 and mice bearing MCF-7 breast cancer xenografts showed that the detection of prelamin A may provide a suitable pharmacodynamic marker of response [32] (Fig. 3b).

Another example where xenografts have proven particularly useful in the context of pharmacodynamic markers is in studies of the heat shock 90 inhibitor, 17-allylamino,17-demethoxy geldanamycin (17AAG) [33], now in Phase II clinical trials. For example, it was shown that the detection of an increase in heat shock protein 70 (HSP70) or a decrease in RAF1 in ovarian cancer xenografts (Figs. 4a,b) may represent good pharmacodynamic markers of response in mice or patients receiving 17AAG [34]. In addition, for molecules like 17AAG that possess the potential to disrupt multiple signal transduction pathways, microarray gene expression analyses from treated versus untreated xenografts may also be useful in elucidating mechanisms of action and for identifying potential pharmacodynamic markers of response [35].

Finally, by contrast, it must be borne in mind that there may be instances where the xenograft model is of little or no value. Examples include the use of s.c. xenografts with anti-metastatic strategies as s.c. xenografts rarely metastasise. Herein, orthotopic models may be more appropriate [25]. Second, drugs (or antibodies) that target tumour vasculature or angiogenesis need to keep in mind that the vasculature being targeted in the xenograft is predominantly of mouse origin. This is of particular relevance with the Antisoma antibody muBC1, which targets an epitope found on human (but not mouse or rat) oncofoetal EDB fibronectin [36]. A modification to the xenograft model (at least for prostate cancer) where human vascular endothelium persists in human prostate cancer within implanted human bone fragments [37] may be useful in this context, but is not amenable to large-scale screening. Finally, the use of athymic mice bearing xenografts may be of limited value with naked humanised (or fully human) antibodies. These, such as the Antisoma humanised antibody huHMFG1 (which targets the cell surface antigen, MUC-1) depend upon recruitment of the host's (i.e. mouse) immune response (natural killer cells) to induce antibody-dependent cell-mediated cytotoxicity (ADCC) for activity [38].

6. Summary

During the era of cytotoxic cancer drug discovery, human tumour xenografts s.c. transplanted in athymic

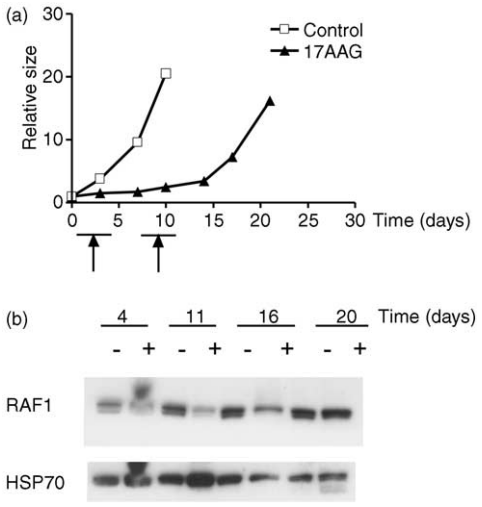

Fig. 4. (a) Tumour growth response curves for the heat shock protein 90 inhibitor, 17-allylamino,17-demethoxy geldanamycin (17AAG) against A2780 ovarian carcinoma xenografts. Dosing was i.p. at 100 mg/kg per day on days 0–4 and 7–11. (b) Immunoblots for HSP70 and RAF1 in treated (+) versus untreated (−) A2780 xenografts on days 4, 11, 16 and 20 after the start of treatment.

mice played a pivotal role in late preclinical agent optimisation and guiding the selection of candidates for Phase I clinical trials. A retrospective review conducted by the NCI for 39 agents for which Phase II activity data were available showed that, where compounds were active in at least one-third of xenograft models tested, there was a statistically significant correlation with activity in at least two tumour types in man [22].

Now, in the post-genomic "molecularly-targeted" era, the case for using human tumour xenografts within a target-driven drug development cascade needs to be justified on a case-by-case basis, keeping in mind both the values and the limitations of the model. These are summarised in Table 2. Limitations include the time and expense (relative to the hollow fibre assay and *in vitro* testing), ethical issues around animal experimentation, a general lack of metastatic spread from primary s.c. implanted xenografts, and the fact that the stromal compartment of xenografts include vascular endothelial cells that are largely of murine origin.

However, where the particular molecular abnormality of cancer being targeted is shown to be present in a particular human tumour xenograft model (and ideally, shown to be important in the proliferation of that tumour) then s.c.-implanted xenografts still remain of significant value in the cancer drug discovery process. This may be in a therapeutic setting, that is, to obtain *in vivo* 'proof of principle' for a particular target and/or to assist in the optimisation of pharmaceutically-tractable molecules. However, the use of mice in *in vivo* anti-tumour studies should be restricted to molecules possessing good pharmaceutical properties where plasma levels above those known to be required for *in vitro* anticancer effects are achievable. At present, in my opinion, it is premature and too much a "leap of faith" to jump directly from *in vitro* activity testing (or even in silico methods) to Phase I clinical trials (via preclinical regulatory toxicology). Furthermore, valuable information assisting guidance of the clinical development of a molecule may be obtained; for example, dose-scheduling studies and sequential combination studies with other anticancer drugs. Additionally, and often in parallel with efficacy determinations, the xenograft model is useful in assessing the agent's pharmacokinetics and pharmacodynamics in that it provides a renewable and readily accessible source of target human tumour cells.

In summary, the human xenograft model in its various guises is here to stay a while longer. Perhaps in another 10 years a similar retrospective analysis as described above by the NCI for cytotoxic cancer drugs, will be applicable to the newer generation "molecularly-targeted" drugs. This *may* then finally settle the debate as to the value (or otherwise) of the xenograft model in contemporary cancer drug discovery.

Table 2
Summary of values and limitations of the xenograft model

Values
• Validated model for the predictive assessment of cytotoxics—uses human cancer cells with 3-dimensional architecture
• To select lead candidates for clinical evaluation and to optimise scheduling and combination with other drugs to guide clinical development (providing conducted with appropriate consideration to pharmacological principles and pharmacokinetics and pharmacodynamics)
• *In vivo* studies of acquired drug resistance and its circumvention
Limitations
• Time (weeks) and expense relative to 'short-term' assays such as hollow fibre or *in vitro* cell line testing (plus animal ethics issue relative to *in vitro* testing)
• The s.c. xenograft model generally does not metastasise: not a good model for studying anti-metastatic strategies
• When studying anti-angiogenic or anti-vascular strategies, these components are of murine not human origin
• When studying humanised/human antibodies (immunotherapy) the effector function (e.g. ADCC) being targeted is mouse

ADCC, antibody-dependent cell-mediated cytotoxicity.

References

1. Harrison P. Perspective on the history of tumor models. In Teicher BA, ed. *Tumor Models in Cancer Research*. Totowa, Humana Press Inc, 2002, 3–19.
2. Rygaard J, Povlsen CO. Heterotransplantation of a human malignant tumour to the mouse mutant "nude". *Acta Pathology Microbiol Scand* 1969, **77**, 758–760.
3. Giovanella BC, Yim SO, Stehlin JS, Williams LJJ. Development of invasive tumors in the "nude" mouse after injection of cultured human melanoma cells. *JNCI* 1972, **48**, 1531–1533.
4. Boxma GC, Custer RP, Bosma MJ. A severe combined immunodeficiency mutation in the mouse. *Nature* 1983, **301**, 527–530.
5. Double JA, Bibby MC. Therapeutic index: a vital component in selection of anticancer agents for clinical trial. *Journal National Cancer Institute* 1989, **81**, 988–994.
6. Hollingshead MG, Alley MC, Camalier RF, *et al*. In vivo cultivation of tumor cells in hollow fibers. *Life Sciences* 1995, **57**, 131–141.
7. Plowman J, Dykes DJ, Hollingshead M, Simpson-Herren L, Alley M.C. Human tumor xenograft models in NCI drug development. In: Teicher B, ed, *Anticancer Drug Development Guide: Preclinical Screening, Clinical Trials and Approval*. Humana Press, Totowa, NJ, 1997, 101–125.
8. Alley MC, Scudiero DA, Monks A, *et al*. Feasibility of drug screening with panels of human tumor cell lines using a microculture tetrazolium assay. *Cancer Research* 1988, **48**, 589–601.
9. Steel GG, Courtenay VD, Peckham MJ. The response to chemotherapy of a variety of human tumour xenografts. *British Journal of Cancer* 1983, **47**, 1–13.
10. Courtenay VD, Mills J. An in vitro colony assay for human tumours grown in immune suppressed mice and treated in vivo with cytotoxic agents. *British Journal of Cancer* 1978, **37**, 261–266.
11. Fiebig H-H, Burger AM. Human tumor xenografts and explants. In Teicher BA, ed. *Tumor Models in Cancer Research*. Totowa, Humana Press Inc, 2002, 113–137.
12. Kelland LR, Burgess L, Steel GG. Characterization of four new cell lines derived from human squamous carcinomas of the uterine cervix. *Cancer Research* 1987, **47**, 4947–4952.
13. Kelland LR, Tonkin KS. Establishment and response to chemotherapy of human cervical carcinoma xenografts. In Winograd B, Peckham MJ, Pinedo HM, eds. *Human Tumour Xenografts in Anticancer Drug Development*. Berlin, Springer-Verlag, 1988, 57–62.
14. Kelland LR, Tonkin KS, Steel GG. A comparison of the in vivo and in vitro radiation response of three human cervix carcinomas. *Radiotherapy & Oncology* 1989, **16**, 55–63.
15. Harrap KR, Jones M, Siracky J, Pollard LA, Kelland LR. The establishment, characterization and calibration of human ovarian carcinoma xenografts for the evaluation of novel platinum anticancer agents. *Annals Oncology* 1990, **1**, 65–76.
16. Kelland LR, Jones M, Abel G, *et al*. Human ovarian carcinoma cell lines and companion xenografts: a disease oriented approach to new platinum anticancer drug development. *Cancer Chemother Pharmacol* 1992, **30**, 43–50.
17. Kelland LR. New platinum antitumor complexes. *Critical Reviews Oncology/Hematology* 1993, **15**, 191–219.
18. Jones M, Siracky J, Kelland LR, Harrap KR. Acquisition of platinum drug resistance and platinum cross resistance patterns in a panel of human ovarian carcinoma xenografts. *British Journal of Cancer* 1993, **67**, 24–29.
19. Kelland LR, Sharp SY, O'Neill CF, *et al*. Mini-review: discovery and development of platinum complexes designed to circumvent cisplatin resistance. *Journal Inorganic Biochemistry* 1999, **77**, 111–115.
20. Gore M, Atkinson RJ, Dirix L, *et al*. ZD0473 phase II monotherapy trial in second-line ovarian cancer. *Proceedings of ASCO* 2001, **20**.
21. Raynaud FI, Boxall FE, Goddard PM, *et al*. Cis-amminedichloro(2-methylpyridine) platinum(II) (AMD473), a novel sterically hindered platinum complex: In vivo activity, toxicology and pharmacokinetics in mice. *Clinical Cancer Research* 1997, **3**, 2063–2074.
22. Johnson JI, Decker S, Zaharevitz D, *et al*. Relationships between drug activity in NCI preclinical in vitro and in vivo models and early clinical trials. *British Journal of Cancer* 2001, **84**, 1424–1431.
23. Casciari JJ, Hollingshead MG, Alley MC, *et al*. Growth and chemotherapeutic response of cells in a hollow-fiber in vitro solid tumor model. *Journal National Cancer Institute* 1994, **86**, 1846–1852.
24. Phillips RM, Pearce J, Loadman PM, *et al*. Angiogenesis in the hollow fiber tumor model influences drug delivery to tumor cells: implications for anticancer drug screening programs. *Cancer Research* 1998, **58**, 5263–5266.
25. Eccles SA. Models for evaluation of targeted therapies of metastatic disease. In Teicher BA, ed. *Tumor Models in Cancer Research*. Humana Press, Totowa, 2002, 293–319.
26. Corbett TH, Polin L, Roberts BJ, *et al*. Transplantable syngeneic rodent tumors. In Teicher BA, ed. *Tumor Models in Cancer Research*. Totowa, Humana Press, 2002, 41–71.
27. Workman P, Twentyman P, Balkwill F, *et al*. United Kingdom Co-ordinating Committee on Cancer Research (UKCCCR) guidelines for the welfare of animals in experimental neoplasia (second edition). *British Journal of Cancer* 1999, **77**, 1–10.
28. Sausville EA, Feigal E. Evolving approaches to cancer drug discovery and development at the National Cancer Institute, USA. *Annals Oncology* 1999, **10**, 1287–1291.
29. Kelland LR. Telomerase inhibitors: targeting the vulnerable end of cancer? *Anticancer Drugs* 2000, **11**, 503–513.
30. Gowan SM, Harrison JR, Patterson L, *et al*. A G-quadruplex-interactive potent small-molecule inhibitor of telomerase exhibiting in vitro and in vivo antitumor activity. *Molecular Pharmacology* 2002, **61**, 1154–1162.
31. Kelland LR, Rowlinson-Busza G, Griffiths-Johnson D, *et al*. Single agent activity and potentiation of the antitumour activity of paclitaxel and gemcitabine with the vascular targeting agent 5,6-dimethylxanthenone acetic acid (DMXAA) in human tumour xenografts. *Proceedings of AACR* 2003, **44**, A3029.
32. Kelland LR, Smith V, Valenti M, *et al*. Preclinical antitumor activity and pharmacodynamic studies with the farnesyl protein transferase inhibitor R115777 in human breast cancer. *Clinical Cancer Research* 2001, **7**, 3544–3550.
33. Kelland LR, Sharp SY, Rogers PM, Myers TG, Workman P. DT-diaphorase expression and tumor cell sensitivity to 17-allylamino,17-demethoxygeldanamycin, an inhibitor of heat shock protein 90. *Journal National Cancer Institute* 1999, **91**, 1940–1949.
34. Banerji U, Walton M, Raynaud F, *et al*. Validation of pharmacodynamic endpoints for the HSP90 molecular chaperone inhibitor 17-allylamino 17-demethoxygeldanamycin (17AAG) in a human tumor xenograft model. *Proceedings of AACR* 2001, **42**.
35. Clarke PA, Hostein I, Banerji U, *et al*. Gene expression profiling of human colon cancer cells following inhibition of signal transduction by 17-allylamino-17-demethoxygeldanamycin, an inhibitor of the hsp90 molecular chaperone. *Oncogene* 2000, **19**, 4125–4133.
36. Midulla M, Verma R, Pignatelli M, *et al*. Source of oncofoetal ED-B containing fibronectin: implications of production by both tumor and endothelial cells. *Cancer Research* 2000, **60**, 164–169.
37. Nemeth JA, Roberts JW, Mullins CM, Cher ML. Persistence of human vascular endothelium in experimental human prostate cancer bone tumors. *Clinical & Experimental Metastasis* 2000, **18**, 231–237.
38. Clynes RA, Towers TL, Presta LG, Ravetch JV. Inhibitory Fc receptors modulate in vivo cytotoxicity against tumor targets. *Nature Medicine* 2000, **6**, 443–446.