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Integrating pharmacology and *in vivo* cancer models in preclinical and clinical drug development

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Abstract

Historically, cancer drug development has been a roller coaster. Numerous agents have shown exciting activity in preclinical models and yet have had minimal activity clinically. These disappointments have led to reasonable scepticism about the true value of both syngeneic and xenograft rodent tumour models in accurately identifying agents that will have important clinical utility. Whereas the development of newer techniques, including transgenic mouse models of cancer, offers the potential to develop more predictive models, the role of such mice in cancer drug development is not yet validated. To advance in our understanding of predictive model systems it may be wise to analyse both the successes and the failures of conventional models in order to understand some of their limitations and perhaps to avoid making the same mistakes in the future. Here we review the value and limitations of xenograft models, and the role of integrating preclinical pharmacology in developing new treatments for solid tumours of childhood.

Keywords: Paediatric cancer; Pharmacokinetics; Pharmacodynamics; Molecular targets; Xenografts

1. Introduction

The evaluation of antitumour agents in immune-deficient mice (athymic nude or severe combined immunodeficient (*scid*) mice) transplanted with human tumours is the major model system for drug development. In its most simple iteration, tumours are grown subcutaneously, and the model allows rapid and quantifiable assessment of antitumour activity relative to mouse toxicity. Logically, precedence should be given to those agents that show the greatest antitumour activity in the preclinical setting, assuming the preclinical data are predictive of drug activity in human studies. The challenge lies in being able to extrapolate these results to the clinic. Indeed, can this ever be done with any degree of confidence? The extensive screening for over 10 years by the National Cancer Institute (NCI) suggests only a moderate predictive value for their xenograft models, and even less concordance between *in vitro* testing data and clinical utility [1]. In this analysis, xenograft tumours derived from a particular cancer type did not predict for activity in the respective clinical disease; rather broad-spectrum activity in the preclinical models was associated with greater clinical activity. Interestingly, these results recapitulate those from syngeneic rodent tumour models used in the NCI screening programme before 1985, where clinical activity was associated with a high response rate in five of eight unrelated solid tumour models. The deficiency in all of these studies has been an inability or failure to relate tumour-response data to clinically achievable drug systemic exposures (i.e. studies on pharmacokinetics were not undertaken).

There are many reasons why preclinical results do not predict human efficacy. Here we will focus on differences in interspecies pharmacology. However, it is clear that the design of therapeutic clinical trials often fails to build upon the strong preclinical leads that may guide aspects of clinical trials' design, such as scheduling of drug administration. Conversely, criteria used to advance an agent in preclinical trials may not be as stringent as those used to evaluate response rates in the clinical setting. For example, 58% inhibition of tumour growth, a criterion used by NCI for assessing the activity of a drug against xenograft tumour models, represents progressive disease in a clinical trial. Our data suggest that if certain aspects of the study design are

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given careful consideration, it may be valid to predict clinical results derived from preclinical work with the xenograft model. These aspects include (a) the development of early-passage models of the appropriate human cancer, rather than the use of 'ancient' cell lines that have been in culture for decades; (b) the use of clinically relevant response criteria to evaluate a new entity; (c) the assessment of tumour responsiveness relative to drug systemic exposure; and (d) a rational consideration of the major/minor strengths and weaknesses of the model (i.e. all models have certain limitations). Here we review our experience using models of childhood cancers in drug development.

2. Retrospective studies: validation of tumour models

Xenograft tumour models, in which a human cancer is transplanted into immune-deficient mice, have been explored since the mid 1960s, but became more frequently used after the identification of the athymic nude mutant mouse which is deficient in T cells [2,3]. The more recent discovery of other immune-deficient mouse strains has further expanded the options for host transplantation. For example, the non-obese diabetic (NOD) *scid* mouse has proved useful for the propagation and testing of agents against acute lymphocytic leukaemias established from children [4]. It has been well recognised that when human cancers are transplanted into mice they retain many characteristics of the original tumour (histology, chromosomal abnormalities, surface antigen expression). Although subcutaneous tumours metastasise infrequently this rate is increased when transplanted to orthotopic (natural) sites. However, from the perspective of drug sensitivity, at least to the conventional cytotoxic agents that comprise most of our current experience, the subcutaneous models appear relatively predictive. That is, agents known to be active in a clinical disease can be identified as active in the models. Examples are shown in Table 1, and summarise the preclinical and clinical activities of vincristine, cyclophosphamide, actinomycin D and doxorubicin against a panel of childhood rhabdomyosarcomas [5,6], and also colon adenocarcinomas (unpublished data). When one uses objective response (i.e. partial response or $\geqslant 50\%$ volume regression) as the criterion for 'activity' the results are rather interesting. The rhabdomyosarcoma 'model' identifies agents known to be active in this disease, whereas the same agents have little activity against colon carcinoma xenografts. This, at least, indicates that tumour responsiveness is not merely a consequence of heterografting tumour into the mouse. We have now established comprehensive panels of different childhood solid tumours and where there are adequate data to make reasonable judgments, similar correlations have been found. For example, atypical teratoid rhabdoid tumours (ATRT) of the central nervous system or kidney are essentially resistant to all agents tested in the model, consistent with their known clinical sensitivity. In contrast, xenografts of histologically favourable Wilms' tumours (nephroblastoma) established from patients before treatment are exquisitely sensitive to vincristine, actinomycin D and cyclophosphamide (J. Dome, P. Houghton, data not shown) agents that are used in the curative treatment of this cancer. Wilms' tumours established as xenografts from patients at relapse are far less sensitive to these agents, consistent with previous observations with models of rhabdomyosarcoma and acute lymphocytic leukaemia [4,5]. Poor-prognosis Wilms' tumours having diffuse anaplastic histology are also poorly responsive as xenografts to conventional chemotherapy agents. Thus, there seems to be a reasonable correlation between drug activity in these preclinical models and their known activity against the same clinical disease. So, if there is such a good *retro*-predictive correlation, what goes wrong when we try to extrapolate from preclinical to clinical activity?

Table 1
Sensitivity of rhabdomyosarcoma and colon adenocarcinoma xenografts to conventional cytotoxic agents

Agent/tumour type	Objective response rate (%) in the model	Objective response rate (%) in the clinic
Rhabdomyosarcoma[a]		
Vincristine	78	59
Cyclophosphamide	44	54
Actinomycin D	11	24
Doxorubicin	19	31
Colon carcinoma		
Vincristine	0	<10
Cyclophosphamide	0	<10
Actinomycin D	0	<10
Doxorubicin	0	<10
5-fluorouracil	17	15–20
Methyl CCNU	17	15–20

[a] References [5,6] (and references therein).

3. Prospective use of xenograft models

The first prospective use of xenograft data followed perhaps our observation that melphalan had very significant activity against the 'diagnosis' panel of rhabdomyosarcomas [7]. In a phase II clinical trial in 13 patients who had failed standard chemotherapy, melphalan demonstrated marginal activity (one partial response). However, pharmacokinetic analysis showed that exposure to this agent in children was essentially similar to that in mice at doses inducing tumour regression. Consequently, we extended the trial to include children at diagnosis with very advanced disease and a dismal long-term outcome [8]. Against disease at diagnosis melphalan demonstrated very significant activity

(10 objective responses in 13 patients). This study was important for two reasons. It taught us that the preclinical model used to select an agent for clinical trial should closely mimic that patient population against which it will be tested (i.e. diagnosis models may not predict for relapse), and that comparative drug exposures may be one metric that should be considered in predicting clinical antitumour activity. Some examples of drugs that showed activity in preclinical childhood tumours, and their success or failure in clinical trials, are summarised in Table 2. DMP840, Carzelesin, and Sulophenur each showed activity in the preclinical model [9–11], but failed to demonstrate significant activity in either phase I or II clinical trials. Examination of the systemic exposure (area under the concentration-time curve; AUC) to each agent at the maximum tolerated dose (MTD) in mice and that achieved at the MTD in phase I testing is shown in the table, as is the range of drug doses (relative to the MTD in mice) over which the agents caused objective tumour regressions. For these agents it would be predicted that adequate systemic exposure in patients would not be attained to achieve significant antitumour activity. For example, even at the minimum dose of DMP840 causing tumour regressions in the mouse (approximately 30% of the MTD) the systemic exposure is still 5-fold higher in rodents than can be achieved in patients. In contrast, it would be anticipated that exposures to melphalan, topotecan and irinotecan (in this case the active metabolite SN-38) would be adequate to anticipate clinical antitumour activity. Indeed, as with melphalan, both topotecan and irinotecan are highly active drugs in the treatment of rhabdomyosarcoma [12–14]. This approach has been extended to the design of clinical trials [15] in which a retrospective analysis of the exposure to topotecan required to induce objective regressions in most neuroblastoma xenografts [16] was used to define an optimal target for drug exposure in children. Preliminary results indicate that the doses in children can be adjusted to achieve the exposures that caused regressions in this model of relapsed neuroblastoma [15]. Further, clinical responses in this trial are consistent with the predictive value of the model. In a subsequent phase II clinical trial, targeting the daily topotecan lactone exposure to achieve 100 ng/h per ml yielded a high response rate (58%) in newly diagnosed patients with advanced neuroblastoma, consistent with that predicted by the neuroblastoma xenograft models [17].

The evaluation of the illudin S derivative Irofulven (MGI-114) serves to illustrate how pharmacokinetic studies may be valuable in making informed decisions on clinical development. As shown in Table 3, Irofulven shows rather dramatic activity against a panel of 20 tumour xenografts derived from various types of brain tumour, neuroblastoma and rhabdomyosarcoma [18]. Indeed at the MTD (approximately 4.6 mg/kg), objective regressions in 14 or 18 models were obtained. However, at 1.3 mg/kg this agent demonstrated poor activity, causing regression in only one of 14 tumour models examined. At dosages in mice ranging from 1.3 to 7.0 mg/kg (4.0–22 mg/m^2), the daily systemic exposure to Irofulven ranged from 214 to 1152 ng/h per ml. This is compared with the systemic exposure associated with the maximally tolerated dosage from the adult phase I clinical trial of Eckhardt and colleagues [19] (Fig. 1). For the clinical trial the mean (±SD) daily AUC for Irofulven at the MTD were approximately

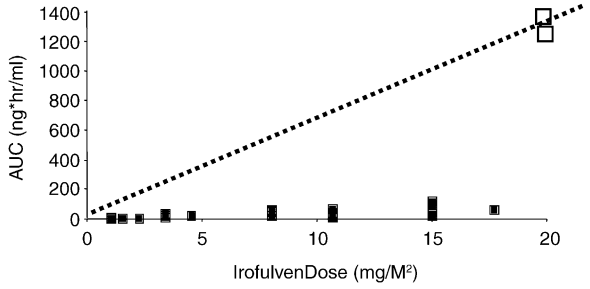

Fig. 1. Relation between Irofulven systemic exposure (area under the curve; AUC) in man and mouse. Data, replotted from Eckhardt and colleagues [18], show the daily AUC in individual patients enrolled in a phase I clinical trial of Irofulven (closed symbols). Shown also are AUC determined in mice following administration of Irofulven (21 mg/m^2) to mice not bearing tumours (open symbols). Data are from Leggas *et al*. [17]. The dashed line shows the approximate AUC in the mouse over a dose range similar to that in patients (assuming linear pharmacokinetics). At the minimum effective dose in mice (4 mg/m^2) the systemic exposure still significantly exceeds that in patients at the maximum tolerated dose (MTD) (15 mg/m^2).

Table 2
Retrospective analysis of drug response-exposure correlations in childhood tumour models

	Agent	AUC at mouse MTD AUC at human MTD	Effective dose range from the mouse MTD[a]
'Clinical failures'	DMP840	15–20	approximately 2–3
	Carzelesin	Approx. 80	<2
	Sulophenur	Approx. 8	Approx. 3
'Clinically active'	Melphalan	1	Approx. 3–4
	Topotecan	Approx. 3	>10
	Irinotecan	Approx. 16	>100

[a] Effective range is defined as the minimal drug dose causing 50% tumour regression relative to the MTD in mice.

33 ± 15 ng/ml per h and 50 ± 18 ng/l per h on days 1 and 5, respectively. In Fig. 1 the systemic exposure achieved in mice (at 7 mg/kg, equivalent to approximately 22 mg/M^2) is superimposed upon clinical results obtained in the phase I trial of Irofulven. An important consideration when comparing systemic exposures for Irofulven across species is the contribution of protein binding. The *in vitro* serum protein binding shows that Irofulven is 80–82% bound in mice and 51–52% bound in man (over a concentration range from 500 to 1000 ng/ml). Although a slight difference in protein binding exists between species, it cannot account for the greater than 6-fold difference in plasma systemic exposure between that required for even minimal antitumour effect and that tolerated in the adult phase I study. These results help in understanding the 'disconnect' often observed between results from preclinical testing and clinical activity. Comparing those systemic exposures that yield significant antitumour activity against human cancers in mice with systemic drug exposure at the MTD in patients may identify, at a relatively early stage in development, those agents that will ultimately fail in the clinic. While such data are not available before testing in man, such 'retrotranslation'[1] can be rather useful in making informed decisions about advancing an agent to phase II testing.

3.1. Predicting human pharmacology

As described above, perhaps the greatest challenge in achieving relative uniformity between the research conditions present in the preclinical and clinical settings is accounting for the pharmacokinetic differences between mouse and man. The conventional approach is to evaluate the absorption, distribution, metabolism and excretion (ADME) properties to select compounds that have acceptable pharmacokinetic properties. Because of the complexity of factors involved in ADME, several approaches have been proposed to develop accurate methods for predicting the human pharmacology of an agent. This prediction is particularly important for agents that demonstrate marked interspecies differences in their pharmacokinetics. Conventional allometric scaling (*CAS*) is the most commonly used technique for predicting human pharmacokinetic variables [20]. Nomura and colleagues have applied these principles to calculating the clinically effective doses (CED) of anticancer agents in the context of human xenograft experiments [21]. An example of their studies using mitomycin C is shown in Fig. 2. Based upon rates of drug clearance and the toxic doses in several species, they calculated the CED. Allometric scaling has provided useful information for a number of compounds

Table 3
Antitumour activity of Irofulven against childhood tumour xenografts

Tumour	Dose (mg/kg)				
	7.0	4.6	3.0	2.0	1.3[a]
DAOY	+ + + + + +	+ + + + + +	+ + + + + +	+ + + + +	+ +
D283	+ + + + + +	+ + + + + +	+ + + + + +	+ + +	+[a]
SJ-BT12	ND	+ + + +	+/−[a]	−[a]	−[a]
SJ-BT16	+ + +	+ + + +	+ + +	+ +	+[a]
SJ-BT27	ND	+ + + + + +	+ + + + + +	+ + +	+
SJ-BT29	+ + + + + +	+ + +	+ + +[a]	ND	ND
SJ-BT33	−[a]	−[a]	−[a]	−[a]	−[a]
SJ-BT34	+ + + + + +	+ + + + + +	+ + + + + +	ND	ND
SJ-BT36	+ + + + + +	+ + + + + +	+ + + +	+ + +	+ + +[a]
SJ-BT37	+ + + + + +	+ + + + +	+ + +	+[a]	−[a]
SJ-BT40	+ + + +	+ + + +	+ +[a]	+[a]	−[a]
SJ-GBM2	+ + + + + +	+ + + + + +	+[a]	ND	ND
NB-1771	ND	ND	+	−[a]	−[a]
NB-1382	+ + + +	+ + + +	+ + +[a]	ND	ND
NB-1643	+ + + + + +	+ + + + + +	+ + + +	ND	ND
NB-1691	+ + + + +	+ + +	+ +	+[a]	ND
Rh18	+ + + + + +	+ + + +	+ + + +	+ + +	+ +
Rh28	+ + + + + +	+ + + + + +	+ + + + + +	+ + + + +	+ + +
Rh30	ND	ND	ND	+ + + + + +	+ + + + + +
Rh36	+ + + +	+ + +	+ +	+[a]	−[a]
CR + PR/Total	14/16	14/18	8/19	3/16	1/14

Response criteria: −, no growth inhibition; +, growth inhibition equals one tumour-volume doubling time; + +, growth inhibition equals two tumor-volume doubling times; + + +, growth stasis; + + + +, partial response (≥0% regression; PR); + + + + +, complete response (CR) with regrowth by week 12; + + + + + +, CR maintained at week 12 (no regrowth by week 12); ND, not determined.

[a] Growth inhibition not significant ($P > 0.05$); all other results were significantly different from controls ($P < 0.05$).

[1] A termed coined by Scott Kaufmann at Mayo Clinic to describe such studies.

that are metabolised and eliminated via the kidney [22]. The method is less robust for agents whose elimination is due to active transport in the liver or kidney. Such agents generally show large variations in interspecies pharmacokinetic properties. Newer methods incorporating additional scaling factors such as time normalisation, protein binding, brain weight and liver conjugation activity have been proposed [23]. For drugs eliminated by liver metabolism alone or by metabolism and elimination in the bile, other factors such as bile flow rates and *in vitro* microsome or hepatocyte metabolism have been used to normalise the *in vivo* clearance and have improved the prediction of their *in vivo* human clearances. An alternative approach to modelling, termed physiologically based pharmacokinetics (PBPK), has been proposed recently by Poulin and Thiele [24]. This model allows prediction of disposition profiles based on the physicochemical and biochemical properties of the drug combined with the species-specific physiological characteristics. The value of PBPK prediction is highly dependent on input variables such as tissue:plasma partition coefficients and *in vivo* blood clearance. A comparison of the predictive value of PBPK using the antimicrobial diaminopyrimidine, epiroprim, an agent that has marked species differences in its elimination pathways, has recently been reported [23]. The best prediction of human pharmacokinetics was made by using the tissue composition model to predict tissue:plasma partition coefficients, and allometric scaling of the animal's intrinsic *in vivo* blood clearance normalised by the ratios of animal:human intrinsic clearances determined *in vitro* with hepatocytes. We are unaware of similar studies with anticancer drugs, but it may be of interest to test the predictive value of the models using some of the 'failed' antitumour agents discussed above.

3.2. Molecular-target inhibition

The discussion above on the role of pharmacokinetic modelling, and the 'disconnect' between preclinical and clinical results, is based largely on our experiences with classical cytotoxic agents. However, there is an increasing focus on developing novel agents that target specific molecules that drive the transformed phenotype. The now 'classic' example is Imatinib, a 2-phenylaminopyrimidine that inhibits the Bcr/Abl kinase in chronic myelogenous leukaemia, and c-*kit* kinase in gastrointestinal stromal tumours. In developing agents against very specific targets it is a reasonable concern whether conventional xenograft models will be valuable for identifying and prioritising such compounds for clinical development, or whether this is the role of genetically engineered mouse models? Clearly, where a tumour is 'driven' by a single genetic change (i.e. overexpression of a receptor as in *HER2* amplification, or mutation leading to constitutive activation of a tyrosine kinase as in the case of Bcr/Abl) there should be an absolute correlation between target inhibition and biological response. For example when induced by doxycycline, bitransgenic MMTV-rtTA/TetO-NeuNT mice develop multiple invasive mammary carcinomas that regress completely following abrogation of Neu expression [25]. In other cases, such as overexpression of *ERBB1*, there is less 'coupling' between target inhibition and the biological response of human tumours (e.g. the effect of Gefitinib). Thus, although genetically engineered models may be very useful for testing the ability of a

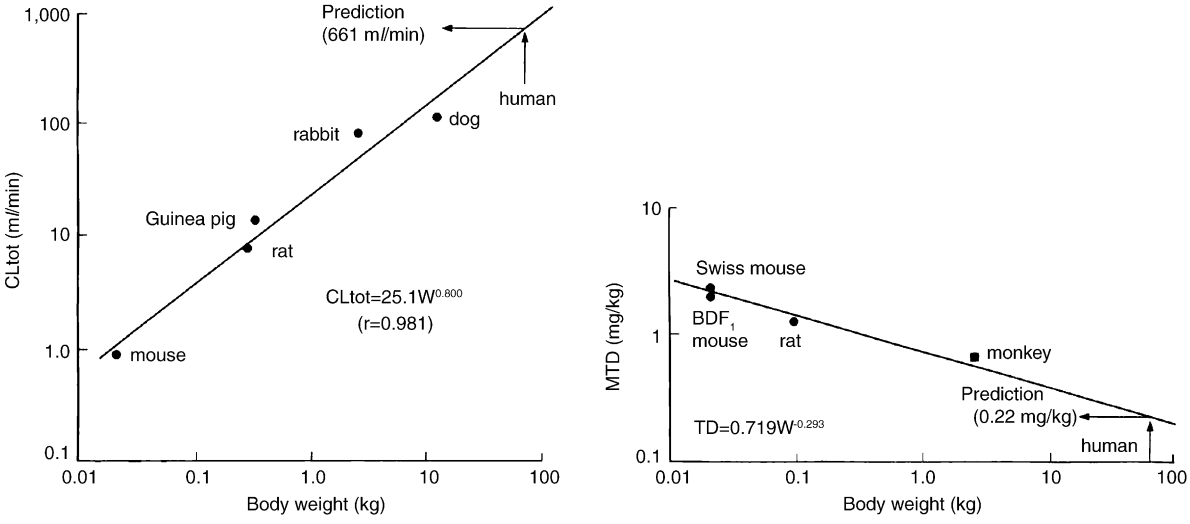

Fig. 2. Use of allometric scaling to estimate the clinically effective dose (CED) for mitomycin C. The CED is calculated as the human AUC×mouse CL_{tot}. The left panel demonstrates clearance against body weight for various species. The right panel plots the maximum tolerated dose (MTD) as a function of body weight in different species. (From Nomura *et al.* [21]. Figure originally published in [31], Mordenti J. Dosage regimen design for pharmaceutical studies conducted in animals. *J Pharm Sci* 1986, **75**, 852–857. Reproduced with permission of John Wiley & Sons, Inc. Copyright ©1986 Wiley-Liss, Inc., A Wiley Company. URL: http://www3.interscience.wiley.com/cgi-bin/jabout/68503813/ProductInformation.htm).

compound to inhibit its putative molecular target, they do not necessarily predict that the target itself will be coupled to the biological 'read-out' in real human cancers. Indeed, the value of the xenograft model might be that such 'coupling' information could be obtained at an early stage in preclinical development. One can extend the principles of relating pharmacokinetics to tumour response to encompass target inhibition in the model system. These experiments, which require sampling of tumour tissue at multiple time points, are used to construct a pharmacodynamic model that relates the amount and duration of target inhibition to the tumour response. This approach has been used extensively in the development of the MEK1 inhibitor PD184352 [26]. Such studies can be extended to establish a correlation between target inhibition and drug systemic exposure in the mouse model, and potentially allow for predicting both dose and frequency of drug administration in patients. Of essence, however, is that the models used should accurately parallel the metabolic characteristics of human cancers with respect to target expression and dependence. Whether the models commonly used for drug development meet these criteria is less well established. Most commonly used models in drug development have been grown in cell culture for many years and may have deviated significantly from the original human cancer. Our experience with rhabdomyosarcoma cell lines suggests that significant changes occur with serial passaging *in vitro*, and indeed tumours established from such *in vitro* cultured lines have very different drug sensitivities from those of the 'parental' tumours established directly from biopsies and maintained in mice. In general our studies have limited the use of tumours to the first 30 serial passages in mice in an attempt to limit such 'drift'. However, it should now be possible to determine more accurately whether the model is representative of the original tumour, and how long it can be passaged in mice before it deviates significantly. A recent initiative by the NCI/ Cancer Treatment Evaluation Program (CTEP) and the Children's Oncology Group aims to characterise the available models of childhood tumours through gene-expression and proteomics profiling (Pediatric oncology preclinical protein tissue array project; POPP-TAP). These data will be of value in establishing whether tumours grown as xenografts maintain molecular characteristics that will be essential for their use in developing so-called molecularly targeted therapies. Where we have access to the original tumour we have started to compare expression profiles between the biopsy and early-passage xenograft tumours. Initial results (J. Dome, P. Houghton, data not shown) with Wilms' tumours look promising in that there is a very high correlation between primary tumour and xenograft, particularly for more highly expressed genes. This technique may be very useful for monitoring changes in gene expression with continuous passage of tumour in mice, and for setting criteria that allow us to determine the number of passages over which a particular model remains valid.

3.3. *Application of preclinical* in vivo *models to childhood cancer*

Developing new therapies for childhood solid tumours has certain constraints that are seldom encountered with the neoplastic diseases of adults. Childhood tumours are rare, with about 12 500 new cases in patients less than 21 years old each year in the United States; hence, the numbers of children with a particular diagnosis at any one institution are usually not adequate for large-scale drug evaluation or randomised clinical trials. For example, of the new phase I agents evaluated in adult malignancies, less than 30% receive adequate evaluation in children. Furthermore, the NCI drug-screening strategy focuses on the selection of new anticancer agents with specific activity against adult neoplastic diseases (e.g. colon, lung, breast, prostate etc.), so that agents with specific activity against childhood malignancies may not be identified. Many common solid tumours of childhood respond to drugs of established efficacy, resulting in cure for a substantial number of patients. This ethically precludes the use of 'experimental' agents in many untreated cases. However, over the last decade, survival rates for patients with disseminated tumours at diagnosis have improved only slightly, if at all. This lack of progress must be attributed in part to the slow rate at which new active compounds reach the clinic, and the failure to integrate laboratory and clinical efforts in a way that will generate a steady flow of promising experimental leads that can be used in the design of productive approaches to treatment. The results obtained using the xenograft models of childhood tumours suggest that the integration of preclinical pharmacology and the rational use of such tumour models could provide a system for identifying, and prioritising, novel agents for clinical trials in children. However, a systematic approach has yet to be established.

To address this problem, NCI/CTEP initiated a series of meetings that resulted in a consensus document [27] detailing the available models of childhood tumours. These models included human xenografts, syngeneic rodent tumours and genetically engineered (knockout and transgenic) mice that would be available for a national effort to screen new agents. A tentative plan for evaluating new agents that incorporates the principles of integrating pharmacokinetic and pharmacodynamic studies discussed above is presented in Fig. 3. In this schema, drugs that are close to entering clinical trials, or are in early-stage clinical development (phase I in adults) will be screened against panels of childhood cancers including the more frequently occurring solid

tumours and acute lymphocytic leukaemia. In this proposal, new agents are screened initially at the MTD in each panel comprising 6–10 independently derived xenografts representing a tumour type (e.g. neuroblastoma). Where available a transgenic model will be included. For example the targeted overexpression of the human *MYCN* proto-oncogene to the neural crest of C57B6/J×Balb/c or 129X1/SvJ mice predisposes the animals to develop neuroblastoma with high penetrance [28]. Similarly, models of rhabdomyosarcoma [29] and medulloblastoma [30] have been developed and may be utilised in the screening. Where the agent demonstrates significant activity (i.e. regression for an agent expected to be cytotoxic, and $<25\%$ tumour growth for a cytostatic), a full dose–response correlation together with pharmacokinetic and, where appropriate, pharmacodynamic studies will be undertaken. For agents that inhibit signal transduction, pharmacodynamic studies may be necessary even in the absence of significant antitumour activity, as it will be important to establish that drug exposures in the mouse are indeed adequate to inhibit the drug target. The approach presented in Fig. 3 reflects much of the uncertainty over the development of 'molecularly targeted' agents. That is, should we evaluate the agent only against tumours expressing the target, or do we 'assume' that the agent may have numerous targets (e.g. most tyrosine kinase inhibitors). Hopefully, the POPP-TAP initiative will ultimately catalogue 'molecular targets' in these tumours and have the potential to identify activated pathways that would allow 'matching' of drug to particular tumour models. Should we test such agents at a dose that gives a biological response (i.e. target inhibition) or at the MTD? The simplest approach is to test at the MTD, irrespective of the drug mechanism. The 'up side' of this is that it reduces the number of false-negative results, but potentially will increase the number of false positives. For example, the mouse may be highly tolerant of an agent, so allowing high systemic exposure to the drug and revealing antitumour activities independent of the primary molecular target. If such exposures cannot be achieved in man, then it is likely that any antitumour activity determined in the models will not be reproduced in the clinic.

In summary, when antitumour activity in preclinical *in vivo* models of childhood cancer is integrated with pharmacological studies, there appears reason to be optimistic about the predictive value of such experiments. While one may have reason to be optimistic, one should also be cautious and not extrapolate beyond the real use of any model system. The project outlined in Fig. 3, if it is initiated, will be an interesting experiment

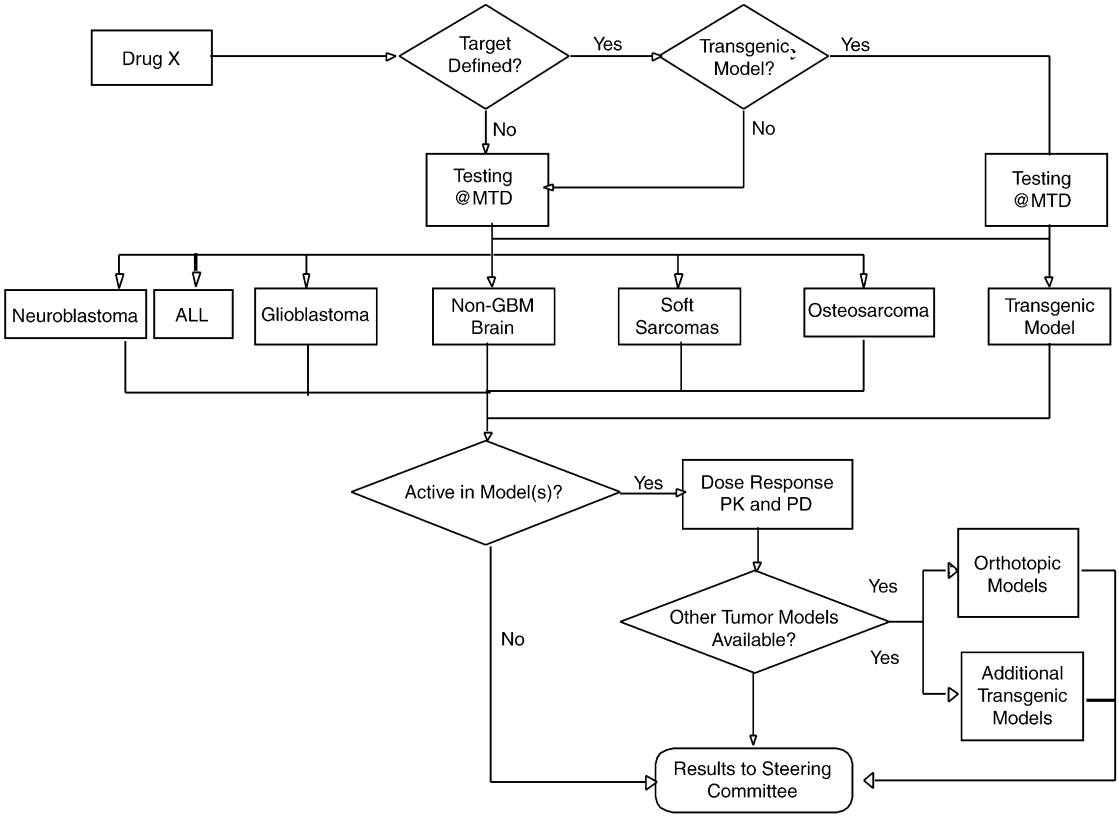

Fig. 3. Schematic representation of a potential preclinical testing programme (redrawn from Houghton and colleagues [27], and used with permission from the American Association for Cancer Research Inc). ALL, acute lymphocytic leukaemia; MTD, maximum tolerated dose; PD, Pharmacodynamic; PK pharmacokinetic.

that will test the value of integrating pharmacokinetic and pharmacodynamic studies with antitumour testing as a paradigm for drug development, particularly for rare cancers.

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