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Meeting Highlight

European Organization for Research and Treatment of Cancer (EORTC) Laboratory Research Division workshop on the role of *in vivo* pre-clinical models in the development of contemporary cancer therapeutics, Verona, Italy, 3 February 2001

EORTC Laboratory Research Division*

Cancer Research Unit, University of Newcastle, Newcastle NE2 4HH, UK

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1. Background

The development of new cancer treatments and their initial clinical evaluation requires clear justification, for both scientific and ethical reasons, of likely patient benefit. Activity in a pre-clinical *in vivo* antitumour model is seen as part of this justification, and *in vivo* studies are a component of current guidance from the European Medicines Evaluation Agency [1].

Initially, *in vivo* antitumour studies were performed using rodent tumours grown in mice or rats. Subsequently, with the development of immuno-incompetent hosts (i.e. athymic and severe combined immunodeficient mice) it has been possible to use human tumour xenografts. Activity in human tumour xenograft models, i.e. tumour growth delay, tumour regression and in many cases cures, can be demonstrated with conventional cytotoxic drugs and this has encouraged the widespread use of the model in drug development programmes. However, the development of the new generation of cancer treatments designed to exploit our evolving understanding of the molecular and cellular pathology of cancer requires a re-evaluation of pre-clinical *in vivo* antitumour models. Specifically, the models developed and validated using cytotoxic agents may not be relevant to the development of novel mechanism-based agents. Furthermore, endpoints such as tumour regression and cure may not be appropriate.

The Laboratory Research Division of the European Organization for Research and Treatment of Cancer (EORTC) has extensive experience of the use of *in vivo* pre-clinical models in drug development, pharmacokinetic and pharmacodynamic studies. Recognising the need to review preclinical models and their utility in contemporary developmental therapeutics, the EORTC Laboratory Research Division held a Workshop during the Winter 2001 meeting of the Screening and Pharmacology, and Pharmacology and Molecular Mechanisms, groups in Verona, Italy. Specifically, the utility of pre-clinical *in vivo* models in the development of signal transduction modifiers, anti-angiogenic agents and chemopreventive agents was addressed. Lastly, the roles of preclinical *in vivo* studies in the development of drug combinations and in the Developmental Therapeutics Programme of the National Cancer Institute, USA, were reviewed.

2. Signal transduction modifiers

Contemporary approaches to drug development are target-based, with the target being one implicated in the molecular or cellular pathology of cancer. Initial compound identification involves cell-free or whole cell screens, with appropriate positive and negative controls. Once a compound with the required target selectivity and potency has been identified, the locus of action in whole cells must be confirmed and the structure modified in a manner predicted to give satisfactory pharmacokinetic and pharmaceutical properties. At this stage, a compound represents a pharmacological probe, which can be used in *in vivo* studies to address the biological effects of the new agent on tumour and normal tissues. Prior to initiating *in vivo* studies, it is essential that the concentration/time profile needed for *in vitro* activity is defined in detail as this should be the primary endpoint

* Tel.: +44-191-222-5712; fax: +44-191-222-7556.
E-mail address: herbie.newell@newcastle.ac.uk (D.R. Newell).

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of *in vivo* studies. It is no longer acceptable to embark directly on efficacy (antitumour) experiments without establishing that at tolerated doses the compound achieves and maintains plasma, and preferably tumour, levels associated with activity *in vitro*. In projects where a number of structural classes are under consideration, simultaneous administration of sets of compounds (cassette dosing) may be appropriate with limited pharmacokinetic analysis, for example plasma concentrations at fixed time points and half life determinations.

Once it has been shown that potentially active drug levels can be achieved and maintained for the required period at a tolerated dose, an *in vivo* efficacy study can be initiated. Human tumour xenografts have been widely used with signal transduction modifiers, and in initial studies the tumours used should be those which were sensitive to the agent *in vitro*. For example, the A431 human vulval carcinoma, which has high levels of the epidermal growth factor (EGF) receptor, has been extensively used in the development of receptor tyrosine kinase inhibitors. Studies with a variety of signal transduction inhibitors, including cyclin-dependent kinase inhibitors, farnesyl transferase inhibitors, receptor tyrosine kinase inhibitors and the heat shock protein 90 antagonist 17-allylaminogeldanamycin have shown that these agents can produce growth inhibition in human tumour xenograft models. To produce growth inhibition, treatment is initiated when tumours are relatively small (ca. 0.5×0.5 cm). However, the consensus is that tumour regression is rarely observed and hence clinical responses with these agents using standard tumour regression criteria should not be anticipated, although they have been observed (e.g. with receptor tyrosine kinase and farnesyl transferase inhibitors). Consistent with these clinical observations, pre-clinical studies with established (ca. 1×1 cm) tumours can also show marked regressions with both vascular and tumour mitogen growth factor receptor tyrosine kinase inhibitors, suggesting an interplay between tumour biology and target interaction that requires further investigation. A second feature of the *in vivo* preclinical activity of signal transduction modifiers is the rapid re-growth of tumours on cessation of therapy, consistent with a cytostatic as opposed to cytotoxic mechanism of action. Chronic dosing may therefore be needed to reveal fully the activity of signal transduction modifiers and oral administration is frequently used. Whilst prolonged oral dosing is technically feasible in mice, the impact on animal welfare must be carefully monitored.

Given that the ultimate clinical impact of signal transduction modifiers may only be apparent in phase III studies with survival endpoints, it is essential that direct or indirect (surrogate) pharmacodynamic markers are developed and validated during pre-clinical *in vitro* and *in vivo* studies. There are now multiple examples of such markers for signal transduction modifiers, for example, retinoblastoma protein phosphorylation (cyclin dependent kinase inhibitors), prelamin A induction (farnesyl transferase inhibitors), receptor autophosphorylation (receptor tyrosine kinase inhibitors) and downregulation of oncogenes (HSP90 inhibitors, e.g. as in Ref. [2]).

Although antimitogenic, anti-angiogenic and antimetastatic signal transduction modifiers are still in their therapeutic infancy, antihormonal agents are well established as cancer therapeutics. Experience with anti-endocrine agents, exemplified by studies with tamoxifen, have shown that in preclinical models inhibition of oestrogen-stimulated tumour growth is the primary effect [3], although tumour regressions and cures can be obtained in appropriate xenografts following anti-oestrogen administration for an extended period of time. As a paradigm for the resistance that is widely predicted for signal transduction inhibitors, human breast tumour xenografts have been developed that mirror clinical resistance [4], and similar models should be developed for signal transduction modifiers. In general, experience with anti-oestrogens suggests that the growth delays observed in preclinical models are a prelude to clinically meaningful activity. Whilst the clinical evaluation of signal transduction modifiers is still at an early stage, initial results with some compounds are encouraging. On the basis of these early results, it would appear that the human tumour xenograft is an appropriate model for the development of signal transduction modifiers, as it has been in the development of anti-endocrine drugs. However, it is already clear that growth delay and not marked tumour regression or cure can be the maximum therapeutic effect produced.

3. Anti-angiogenic and antivascular agents

The development of anti-angiogenic and antivascular therapies raises the specific challenge of modelling the interplay between host- and tumour-derived cells in the biology of the tumour and its response to therapy. Whilst *in vitro* (e.g. human umbilical vein endothelial cells) and *ex vivo* (e.g. the aortic ring assay) models are available, *in vivo* assays are needed at an early stage in the development process to provide a meaningful experimental model of the clinical situation. A large number of anti-angiogenic and antivascular agents are under preclinical or clinical development and these include agents targeted at: endothelial cell mitogenic signalling (e.g. the vascular endothelial cell growth (VEGF) receptor), endothelial cell integrity (e.g. endothelial cell tubulin binding agents), tumour vessel physiology (e.g. the enhanced permeability of 'leaky' tumour blood vessel endothelium) and enzymes involved in degrading the extracellular matrix (e.g. matrix metalloproteinases) [5].

In vivo models used in the development of anti-angiogenic/vascular therapies can be divided into those which involve tumours, and those which use normal vascular tissues as surrogates for tumour blood vessels (e.g. Matrigel plug, corneal pocket, skin pouch and chick allantoic membrane models) [6]. Whilst normal vascular tissues may be useful in studies of blood vessel biology, and hence in proof-of-principle mechanistic studies, effects in a tumour-based model are needed prior to clinical trials, and both rodent tumours and human tumour xenografts have been used. Rodent models have the advantage that both the tumour and vascular/stromal cells are from the same species, but the limitation that the treatment target, if tumour-based, is non-human. Conversely, with human tumour xenograft models, artefacts due to rodent vascular–human tumour interactions may arise. Furthermore, with both rodent and human models, in comparison with the clinical setting, tumour growth rates are often very rapid with poorly understood implications for the physiology of the vasculature that develops and its relevance as a therapeutic model. The need for vasculature to be established before a model can be used to test an anti-vascular, as opposed to anti-angiogenic, therapy can also raise animal welfare considerations as the time between initiation of therapy and the development of an unacceptably large tumour may be limited.

Anti-angiogenic/vascular agents as a class, like the signal transduction modifiers, produce tumour growth inhibition, but not regression in many models, as exemplified by the effects of VEGF receptor tyrosine kinase inhibitors and anti-VEGF receptor antibodies. However, despite the lack of tumour regression in most models, microscopic examination of the tumour can reveal pronounced changes. For example, a number of tubulin binding agents can induce vascular shutdown as a result of endothelial cell shape changes which leads to extensive and acute haemorrhagic necrosis, but not to tumour shrinkage. The lead endothelial cell tubulin binding molecule is combretastatin A4 phosphate, and clinical data will shortly become available for comparison with preclinical results. In general, given the primarily cytostatic effects of anti-angiogenic/vascular agents, there is a clear need for surrogate and direct pharmacodynamic markers to be developed and validated in preclinical studies.

In the absence of anti-angiogenic/vascular agents with proven clinical activity, pre-clinical models remain unvalidated and results obtained with such models must be interpreted with caution. In particular, it would be extremely unwise to use the currently available models as screens for agents in the absence of a mechanistic hypothesis for the proposed anti-angiogenic/vascular effect and, as important, a pharmacodynamic assay to demonstrate that the proposed mechanism is operative. For example, studies with VEGF receptor tyrosine kinase inhibitors are instructive as activity can most clearly be demonstrated in tumours which express VEGF, consistent with the proposed anti-angiogenic mechanism of action. Lastly, when negative clinical data are obtained, preclinical models should be 'revisited' to address why they were not predictive of the eventual clinical properties of the drug. For example, clinical experience with the first generation of matrix metalloproteinase inhibitors has not, to date, been encouraging despite the fact that biological effects can be clearly demonstrated with these agents in a number of commonly used preclinical invasion, angiogenesis and metastasis models [7,8]. In such cases, reasons for the discrepancy between the preclinical and clinical data, i.e. preclinical model deficiency, lack of appropriate clinical pharmacokinetics or pharmacodynamics, and/or poor clinical trial design, urgently need to be identified.

4. Chemopreventive agents

The increasing understanding of genetic and environmental factors that predispose individuals to developing cancer has emphasised the importance of chemoprevention as an approach to cancer management. Preclinical models used in the identification of chemopreventive agents include both carcinogen-induced tumours and spontaneous tumours in mice with defined genetic lesions (e.g. the multiple intestinal neoplasia or MIN mouse model due to germline adenomatous polyposis coli (*APC*) mutation). However, these models have a number of potential limitations [9]. For example, both carcinogen- and genetically-induced tumours often have a long latent period and, in the case of carcinogen-induced tumours, the agent used is not always a proven human carcinogen. Furthermore, the disease phenotype in rodents may fail to match that seen in patients. For example, carcinogen-induced tumours are rarely metastatic in rodents and in the MIN mouse model, adenomas, but not carcinomas are the predominant lesion. As a result, caution must be exercised in the use of such models as screens to select novel chemopreventive agents as the carcinogenic events represented in the model may not be pertinent to the clinical setting in either type, number and/or sequence.

Confidence in the use of preclinical models for the development of chemopreventive agents would be greatly strengthened by positive data from clinical trials of chemo-prevention. However, with the notable and mechanistically well understood exception of the use of tamoxifen for the prevention of breast cancer, clinical trials with chemopreventive agents have, for the most part, been disappointing [10]. Given that many of the agents that have been used in clinical trials (i.e. various vitamins, retinoids, N-acetylcysteine and polyphenols derived from plants) often show clear evidence of activ-

ity in preclinical models of chemoprevention, the lack of clinical activity with many of these agents is again a cause for concern. As with anti-angiogenic/vascular agents, lack of clinical chemopreventive activity may be due to poor pharmacokinetics (failure to achieve and maintain adequate target tissue concentrations), pharmacodynamics (lack of drug–target interaction), trial design (e.g. selection of an inappropriate 'at risk' population), or a reflection of the inadequacy of the preclinical models used to justify the clinical trial. In attempting to resolve these various issues, the use of biomarkers that reflect drug–target interactions, as well as a clear and testable mechanistic basis for the trial, is now mandatory. Given that the majority of clinical chemoprevention trials have yielded negative results, current pre-clinical models may be of limited value and future studies should use tumours with defined and clinically relevant genetic lesions, either germline or human carcinogen induced. Furthermore, prior to undertaking clinical studies, the mechanism of action of the chemopreventive agents must be known, and appropriate biomarkers developed and validated (e.g. as in Ref. [11]).

5. Preclinical models for the development of drug combinations

The most impressive clinical results with cytotoxic drugs have been obtained with combination therapy, and new cytotoxic drug combinations continue to be evaluated in clinical trials. In addition, cytotoxic drugs have been combined with modulators (e.g. drug resistance modifiers) and more recently with agents directed at novel targets (e.g. signal transduction modifiers, anti-angiogenic agents, etc.). Combination studies are either performed empirically in clinical phase I/II trials, or are based on preclinical data. However, even in one *in vivo* tumour model, which may or may not have any clinical relevance, a simple two-drug combination study with the variables of relative dose, timing and schedule, can rapidly escalate to large and unmanageable proportions. Furthermore, where both agents have activity when given alone, therapeutic synergism should be confirmed and it can be very difficult to distinguish between *in vivo* effects that are synergistic and additive [12]. Taken together, these issues make the empirical use of preclinical *in vivo* models for the selection of drug combinations both impractical and, because of the large number of animals needed, unethical. Instead, preclinical studies should be based on a clear hypothesis to explain the expected improvement in activity, and supported by *in vitro* data showing synergism. An *in vivo* model may be of help in confirming that the combination is at least no less active and no more toxic than either component alone, and for the development and validation of surrogate or direct pharmacodynamic assays for use in clinical trials. Access to such pharmacodynamic assays is particularly important in the development of combinations involving cytostatic agents, where tumour response may not be a relevant endpoint, and robust time to progression or survival data may not be available prior to phase III studies.

6. Recent experience in the Developmental Therapeutics Programme of the National Cancer Institute

The Development Therapeutics Programme (DTP) of the National Cancer Institute (NCI), USA, has for many decades been a key resource for cancer pharmacologists worldwide. Historically, the DTP offered a screening service based on the use of *in vivo* rodent tumour models, *in vivo* human tumour xenografts and, more recently, human tumour cell lines grown *in vitro* and *in vivo* in the hollow fibre assay. For cytotoxic drugs, the ability of human tumour xenograft models, the hollow fibre assay and the *in vitro* cell line screen to predict clinical activity has recently been reviewed [13]. Cytotoxic drug activity in human tumour xenografts, which could to some extent be predicted by activity in the hollow fibre model and *in vitro*, was associated with subsequent clinical activity, emphasising the importance of the xenograft model in cytotoxic drug development.

In keeping with the advent of target-based drug discovery, the DTP has recently moved to drug discovery based on known genetic lesions in human tumours [14], and 40 000 unique sequences have been identified by the Cancer Genome Anatomy Project (http://cgap.nci.nih.gov). Once a target is identified, it is validated or 'credentialed' using a combination of clinical correlative, molecular genetic and pharmacological approaches. Following target validation, contemporary developmental therapeutic approaches are used, and the relationship between drug activity and molecular target levels in a large panel of cell lines (ca. 60—http://dtp.nci.nih.gov) can be valuable in confirming the locus of action *in vitro* [15]. Subsequent studies in *in vivo* models again seek to demonstrate that drug-target interactions can be obtained at tolerated doses and, in addition to established human tumours, genetically modified tumours may be used. Finally, *in vivo* tumour models are used to develop and validate surrogate and direct pharmacodynamic markers for use in subsequent clinical trials.

7. Conclusions

Whilst *in vivo* antitumour models have played a key role in identifying and developing the cytotoxic therapies in use today, the future role of such models will be

very different. Examples of the use of *in vivo* models in the development of signal transduction modifiers, tumour angiogenesis inhibitors and chemopreventive agents have been discussed above. Specifically, *in vivo* models should no longer be used to 'screen' compounds. Instead, once pharmacokinetic studies have shown that potentially active plasma and tumour concentrations can be achieved, *in vivo* models should be used to demonstrate that the required drug–target interaction takes place, and that the interaction leads to the desired downstream biological effects. Increasingly, such biological effects will not be measured as tumour regression. An additional key role of *in vivo* models is in the development and validation of clinically practicable surrogate and direct pharmacodynamic assays, and clinical trials on agents which lack suitable pharmacodynamic assays are becoming increasingly difficult to justify. Lastly, but importantly, the redefined role of *in vivo* tumour models, in particular the move away from *in vivo* screening, has significant animal welfare benefits in terms of the numbers of experimental animals used.

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