Clinically-relevant *in vitro* and *in vivo* Oncology Models for Drug Discovery

PEER REVIEWED

Introduction

The success rate in predicting clinical efficacy of anti-cancer modalities using current xenograft models has been reported to be only 30-40%[1,2]. Standard xenograft models use cell lines that are maintained in plastic and have adapted to grow independently of the tumour microenvironment, resulting in models with genetic and phenotypic characteristics distinct from those seen in the clinic[3]. In an attempt to reduce drug attrition and improve clinical predictivity, patient-derived xenograft tumours (PDX) are being used to improve and refine preclinical modelling. These models provide a more relevant heterogeneous system, in which human tumour and stromal cells are in close co-operation within a unique microenvironment, thereby testing a candidate's therapeutic agent in the most relevant manner. Maintaining the human microenvironment in models also sustains molecular, genetic and histological heterogeneity of the original tumours, which are important features to ensure authentic responses to current targeted agents or chemotherapeutics. This article will describe how patient-derived preclinical *in vitro* and *in vivo* models can be established, characterised and used to enhance the drug discovery process.

PDX Models in Drug Discovery

Within oncology drug discovery today there are major challenges in identifying novel efficacious and tolerable targeted small molecule and antibody agents suitable for clinical evaluation. To address this challenge, pharmaceutical and biotechnology companies are seeking more relevant and accurate preclinical models to underpin their drug discovery work. The benefits offered by patient-derived tumour models reside predominantly in their ability to replicate a more authentic tumour microenvironment; this has significant appeal to companies at the forefront of cutting edge drug discovery and oncology research. Such models allow researchers to obtain deeper and more accurate molecular, genetic, SNP, epigenetic and proteomic knowledge relevant to the clinic in order to develop a greater understanding of the disease state. By providing a more clinically relevant heterogeneous microenvironment, it is anticipated that these PDX models will be superior for the discovery and characterisation of novel therapeutics, biomarkers and translational research readouts such as responder/non-responder profiles which can be tested further in the clinic.

The key to these models being relevant and useful as a preclinical tool is that a PDX panel for a chosen cancer type should encompass all of the relevant histological and genetic features which make up very specific subsets. It is becoming increasingly clear that the response in the clinic of many targeted agents and some chemotherapeutics is critically dependent upon using these only in the correct tumour subset. For example, lung cancer, which is an area of high unmet need and claims approximately 160,000 deaths/year in the USA, and has a poor five-year survival rate, is composed of several defined histological (especially adenocarcinoma and squamous cell carcinoma) and mutational subsets[4]. Over the past decade, it has also become evident that subsets of non small cell lung cancer (NSCLC) can be defined at the molecular level by recurrent 'driver' mutations/fusions that occur in multiple oncogenes, including EGFR, KRAS, ALK, FGFR, BRAF, HER2, AKT1, MEK1, MET, NRAS, PIK3CA, KIF5B-RET and ROS1. For many of these, there are targeted agents, either already registered or currently undergoing clinical evaluation.

In some clinical settings, for example the mutant EGFR and EML4-Alk subsets of adenocarcinoma (ADC) NSCLC, significant benefits in terms of progression-free and overall survival have been seen in patients treated with targeted agents. This is a fast-growing area with new targets being identified e.g. recently, researchers using whole-transcriptome sequencing identified in-frame fusion transcripts of KIF5B (the kinesin family 5B gene) and the RET oncogene, which were present in 1–2% of lung adenocarcinomas from people in Japan and the US[5]. At present, there are several therapeutic agents that include potent inhibition of RET kinase in their profile and warrant clinical evaluation. In addition, lung squamous cell carcinoma (SCC), just like adenocarcinoma, is now starting to split into defined driver subsets, and there is currently major interest in deriving and evaluating targeted agents against the FGFR (mutations and amplifications) and DDR2 (mutations) subsets.

A new era in lung cancer is emerging, R&D where defined genetic and histological patient subsets will be selected and evaluated with new targeted agents. It is therefore crucial that representative preclinical *in vitro* and *in vivo* models for these defined lung cancer subsets are used to discover, optimise and evaluate new targeted agents and biomarkers suitable for clinical testing. Patient-derived lung tumour models are used to establish unique histological and mutational models representing

the key subsets of interest for drug discovery. For example, adenocarcinoma/mutant EGFR, adenocarcinoma/mutant KRAS, and squamous/mutant/amplified FGFR isolates, are all models currently running within ongoing *in vitro* and *in vivo* studies.

Establishment of PDX Models

In addition to lung models, patient-derived xenografts from numerous different cancer types, including colorectal, pancreas, oesophageal, gastric and liver metastases, have been established, which are used for therapeutic evaluation; further expansion of this PDX portfolio will include examples of prostate, breast, haematopoietic and brain tumour models. All samples are collected from the clinic with informed consent following ethical review and are then stored and used for research purposes under the Human Tissue Authority (HTA) license. Associated anonymised patient information, treatment history and histopathological reports are also available. Tracking the patient post-surgery, especially when treatment is pursued, is also critical for validating models as well as reporting back to the clinic on potential second-line treatment options for a specific patient.

Tumour samples are implanted subcutaneously in immunocompromised mice, which allows monitoring of tumour establishment and growth by calliper measurement. Typically a lag time precedes exponential growth and this lag time is generally longer in earlier passages, which is likely due to replacement of human stroma by murine stroma. There are also differences in successful establishment across tumour types, and in some cases subsets, as well as dependency on the strain of mouse used. For example, prostate samples are more fastidious than colorectal samples, SCC NSCLC are slightly more amenable to transplantation than ADC NSCLC. Other sites of implantation which improve establishment include orthotopic sites relevant to the cancer type or niche environments such as

the kidney capsule or testes, which are rich in vasculature. In addition, supplementation with matrigel and human stromal cells, such as mesenchymal stem cells (MSCs) or cancer associated fibroblasts (CAFs), which supports retention of the human stroma, may improve tumour take rate. For some models where the paracrine signalling is paramount, stromal components are selected to optimally model the targeted pathway; for example the c-Met/HGF (receptor/ligand) axis is supported through supplementation with MRC5 stromal cells[6].

Serial propagation of transplanted tumour material in immunocompromised mice allows sufficient material to be available for therapeutic efficacy evaluation at subsequent passages. Depending on the rate of implant of the tumour, this approach could generate rapid and more clinically relevant data for different drugs and different combinations in a broad set of tumour types and subsets which represent different clinical settings. For this there must be a high correlation between the efficacy readouts of these preclinical models and the clinic to allow accurate predictions of novel therapeutic strategies. Increasing evidence to support the observations that patient-derived tumours in mice can predict the clinical response have been demonstrated in a number of different laboratories[7,8].

Establishment of tumour growth rate, stromal retention, doubling rate and generation of donor material between passages are all important factors to catalogue. In addition histopathological characterisation, genetic profiling and response to standards of

care are all key parameters that should be ascertained as soon as tumour establishment has been confirmed. Subset panels can then be clearly identified and used in the most informative way to select for appropriate targeted therapies or strategies for combination. For NSCLC we have identified a panel of SCC and ADC examples and identified responder and non-responder subsets to a panel of standard of care treatments such as paclitaxel/carboplatin & Iressa. Within each of these subsets there are specific mutations or amplifications that are valuable for therapeutic assessment and understanding resistant mechanisms. A mutant EGFR adenocarcinoma PDX model (Figure 1) harbouring the EGFR mutation L858R shows exquisite sensitivity to EGFR inhibitors such as Tarceva and Iressa, whereas an FGFR amplified SCC model has shown sensitivity to FGFR inhibitors. Other mutations identified include K-Ras, p53 and LKB1.

Other areas where patient-derived tumour models have added value include: (a) the derivation of chemoresistant isolates to accurately mirror the clinical situation in which new agents are evaluated, (b) to fast-track resistance to new targeted agents and analyse the mechanisms involved and (c) rationally design and evaluate in a high-throughput manner combination studies to delay/ overcome the resistance issues and generate evidence-based hypotheses suitable for testing in clinical trials. Implantation of PDX models at orthotopic sites e.g. intra-thoracic lung injection of PDX cells for NSCLC models (Figure 2), implantation of breast tissue into the mammary fat

Figure 1: ADC NSCLC PDX model with activating EGFR mutation showing exquisite sensitivity to the EGFR inhibitor Erlotinib (Tarceva)
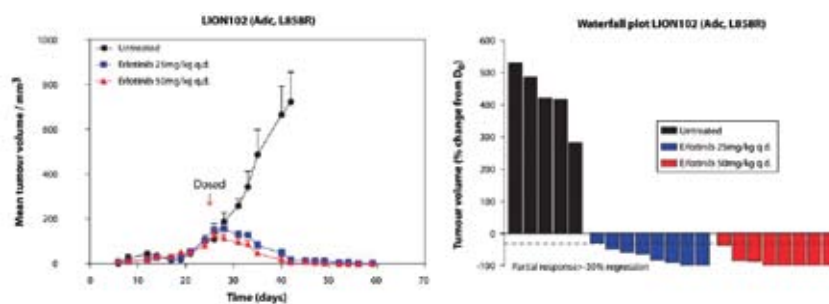
pad or direct injection of models into the prostate. These sites provide the necessary microenvironment to promote metastasis, epithelial:mesenchymal transition (EMT) and chemoresistance.

Three-dimensional Tumour Growth Assay (3D-TGA)

Establishment of a well-characterised panel of tumour types is time-consuming and costly and together with limited access to original tumour material presents some challenges to deriving PDX models. *In vitro* establishment of cell lines derived from patient tumour material is very poor, and introducing cells to plastic presents a number of disadvantages to this approach. As an alternative, a 3D-TGA can be established using patient-derived tumour samples which have been disaggregated and admixed with stroma and matrix membrane in 3D to recapitulate the tumour microenvironment as closely as possible in 96- or 384-well format. This is in contrast to the evaluation using monolayer single cell cultures, which are standards

within the pharma and biotech industries, and whilst predictive to some degree of the biology and mechanism-of-action, are poorly predictive of clinical efficacy. The 3D-TGA allows for a rapid non-invasive *in vitro* measurement of cancer cell expansion in the presence of multiple tumour-associated cell types or soluble factors and facilitates the medium-throughput screening of test agents. The main advantages of the 3D-TGA include (a) the fact that experimental tumour cultures are established in complex mixtures of tumour-derived factors and (b) in comparison to traditional 2D tissue culture systems, both physical and soluble matrix interactions are permitted and spatial limitations are greatly reduced, and therefore *in vitro* 3D tumour cell cultures are able to more accurately reflect the complex *in vivo* microenvironment, thus providing a more relevant screening system.

Proprietary TGAs can be used to enable detailed pharmacological mapping of multiple agents in isolation, or importantly in

combinations, in clinically-relevant tumour models. Thus, novel compounds in 3D-TGAs, with or without human stroma, are assessed against multiple standards of care, targeted agents or in combination therapies, and can be used to benchmark against competitor agents. The aim is to develop an informative response "heat-map" of the lead therapeutic agent against and/or in combination with the landscape of existing treatment regimens and develop rational combination strategies in resistant profiles. This allows screening and identification of a more focussed set of agents/combinations suitable for *in vivo* and ultimately clinical evaluation. This combination approach can also be used to evaluate means to delay or overcome resistance that arises against the targeted agents in specific PDX subsets.

Optical Imaging

Innovative new approaches are being applied to these PDX *in vitro* and *in vivo* models. Through the development of bio-imaging techniques it is possible to monitor in real time the individual tumour and stroma components of the complex microenvironment and how this responds to therapeutic agents. This would allow optimal timing of drug administration to be determined based on the micro-environmental signals measured. This new approach is facilitated by the development of innovative new technology which involves bioluminescent/fluorescent biological reporters. These are engineered to be expressed in human cancer cells so that they emit light or fluorescence in a constitutive manner. Optical imaging also enables monitoring of internal tumours transplanted in mice e.g. orthotopic site such as the lung for NSCLC models or disease progression and metastases. The challenge is to ensure transduction of patient-derived tumour material has minimal impact on the phenotypic and genetic properties of the model. Transduction of the stroma can be achieved through culturing of cells *in vitro*, however, the patient-derived material may be limited in supply or susceptibility to transduction. As

Figure 2: Intra-thoracic implantation of NSCLC PDX
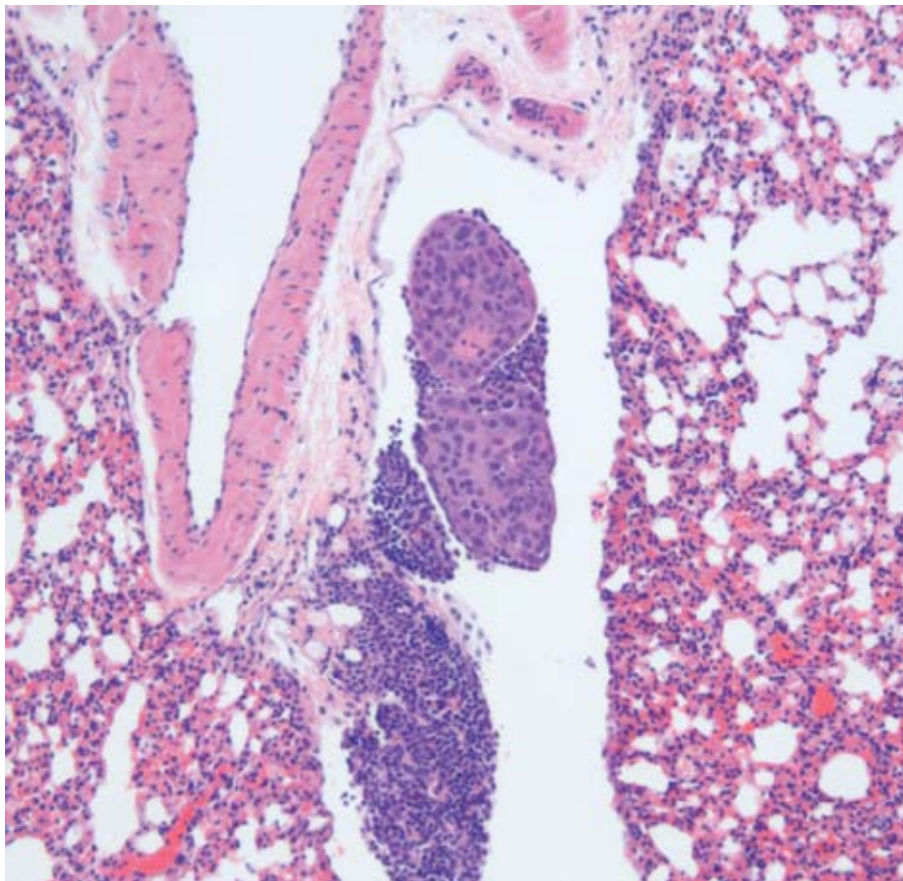
an alternative, fluorescent-tagged probes can be used to assess internal tumours and also measure a number of different biological responses. For example a caspase-cleavable probe can be used to measure apoptosis in an EGFR mutant NSCLC *in vivo* model following treatment with Erlotinib and the fluorescent signal can be captured using the Spectrum/CT imaging system.

Concluding Remarks

Pre-clinical models that closely recapitulate the human tumour heterogeneity are imperative in the optimisation of novel cancer therapeutics, strategies and patient selection. A comprehensive collection of well-characterised and subset-specific clinically-derived tumour models provides a powerful screening platform to support the rapid pace of novel target identification/validation, biomarker and drug evaluation studies. In addition these models can be used to better understand the tumour microenvironment, EMT, resistance and metastases and through innovative approaches, such as supplementation with human stroma, optical imaging and 3D modelling, allow these models to be more efficient for oncology drug discovery.

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