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Predictive *in vivo* animal models and translation to clinical trials

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Vast resources are expended during the development of new cancer therapeutics, and selection of optimal *in vivo* models should improve this process. Genetically engineered mouse models (GEMM) of cancer have progressively improved in technical sophistication and, accurately recapitulating the human cognate condition, have had a measureable impact on our knowledge of tumourigenesis. However, the application of GEMMs to facilitate the development of innovative therapeutic and diagnostic approaches has lagged behind. GEMMs that recapitulate human cancer offer an additional opportunity to accelerate drug development, and should complement the role of the widely used engraftment tumour models.

Over many years now there has been a poor correlation between preclinical therapeutic findings and the eventual efficacy of these compounds in clinical trials [1,2]. Two universal approaches have been used in preclinical testing: cell-based *in vitro* systems and *in vivo* animal models. Cultured cells *in vitro* have been used widely in cancer biology, examination of chemotherapeutics and targeted therapeutics; they are certainly responsible for our early progress in cancer research. They have advantages, such as set experimental conditions and environmental factors, and the ability to manipulate almost any target relatively easily and at a low cost [2]. However, they have major drawbacks, such as the inability to replicate the three dimensional tumour structure, the absence of a tumour microenvironment and artificial levels of growth factors and cytokines in cell culture media [3].

In an effort to improve the relevance of the model, cell lines were incorporated into xenografts, in which cells are injected subcutaneously in immunocompromised mice. Murine models have become a main part of research in many laboratories as they are the most accessible animal model. There are extensive reports highlighting the advances we have made in cancer biology by employing these systems [4,5], however, the usefulness of different types of animal models in preclinical compound testing is a much more disputed topic. With an increasing number of new drug

targets and targeted agents available, more advanced ways of testing these compounds are now being developed.

Therapeutic efficacy studies in mice have not typically addressed important factors considered in early stage clinical trials, such as differences in pharmacokinetics (PK) and pharmacodynamics (PD). PK studies address the effect the body has on the drug and encompasses the appropriate drug delivery of therapies. It is influenced by factors, such as absorption, distribution, metabolism and excretion. A PD characterisation examines how drugs affect the body by exploring whether the drug alters its molecular target in tumour and surrogate tissue, and delineates the associated cell biological effects.

The development of antineoplastics is a large investment by the private and public sectors, however, the limited availability of predictive preclinical systems obscures our ability to select the therapeutics that might succeed or fail during clinical investigation. In this article we consider the different types of animals models used to test novel therapeutics and chemotherapies, and discuss the strengths and weaknesses of each in this regard.

Types of animal model used in therapeutic assessment

The most common animal model system currently used in oncology drug development and discovery remain the implantable or engraftment models, in which cultured human (xenografts) or mouse (allograft) cells or tumour tissue explants are grafted into

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TABLE 1

Definitions of different *in vivo* animal models

Type of model	Cell type	Immune status	Site of tumour	Advantages	Disadvantages
Syngeneic or allograft	Mouse	Immunocompetent	SC skin	Low cost Immunocompetent host Reproducible Easily made Large numbers of animals available Wide variety of tumour types can be generated	Rodent tumour cells Poor representation of human disease Unsuitable for testing humanised antibodies Non-natural site of tumour
Xenograft	Human	Immune deficient	SC skin	Low cost Easily made Reproducible Homogenous Large numbers of animals can be made	Immunosuppressed Different representation of human disease Non-natural site of tumour Rodent tumour microenvironment
Orthotopic	Human	Immune deficient	SC organ of interest	Relatively low cost compared to GEMMs Hosts readily available Tumours at natural sites Can mimic human metastases patterns	Immunosuppressed Requires surgical skill Rodent tumour microenvironment Difficult to follow tumour kinetics
GEMM transgenic	Mouse	Immunocompetent	Organ of interest	Resemblance to human cancer Heterogeneous Immunocompetent Investigates the tumour cells and the microenvironment	Expensive Complicated breeding schemes Restricted experience Variable penetrance and tumour latency Heterogeneous Difficult to follow tumour kinetics Gene mutation found in all cells of the body Non-physiological levels of mutated genes
GEMM endo-genous	Mouse	Immunocompetent	Organ of interest	Resemblance to human cancer Heterogeneous Immunocompetent Investigates the tumour cells and the microenvironment	Expensive Complicated breeding schemes Restricted experience Variable penetrance and tumour latency Heterogeneous Difficult to follow tumour kinetics

Abbreviation: SC: subcutaneous.

recipient immunodeficient or immune-competent mice (Table 1). These models have been used extensively in the academic and pharmaceutical industry research settings to prioritise compounds for clinical testing [1,6]. Subcutaneous implantable models offer the ability to rapidly examine large cohorts of relatively uniform tumours whose growth and response to drugs can easily be assessed. Unfortunately, while such models are relatively inexpensive, convenient and easy to use, they generally behave differently than the corresponding human cancer. When used in the drug discovery setting many agents show consistent and compelling anticancer activity in specific implantable model systems, but unfortunately oftentimes fail in later stages of clinical development [7,8].

Xenograft models
The xenograft animal models utilising human cells or tissue fragments require the use of immunocompromised mice to enable engraftment, and have been established for virtually every human cancer to some extent. Xenografts typically make use of only a few human cell lines that grow quickly and are often sensitive to chemotherapy [7]. Xenograft models are a useful approach to evaluate the direct effects of humanised monoclonal antibodies, such as trastuzumab and bevacizumab, although any host dependent immunomodulatory effects are disrupted.

Syngeneic models
A syngeneic model, where murine cell lines are injected subcutaneously in immune-competent murine hosts (Table 1), is a model that avoids the immune-deficiencies found in other xenograft models [2]. However, there is a poor correlation between the therapeutic activity of compounds tested in syngeneics or cell-based assays and their efficacy in humans, potentially owing to innate differences in the biology of human and mouse cells [9].

The basic problem is that neither cell-based studies nor xenograft models accurately reconstruct the complex interactions between tumour and host. Tumours are complex masses composed of neoplastic cells, extracellular matrix, and stromal cells comprising immune, fibroblastic, and vascular compartments. Indeed, in some tumour types, stromal cells outnumber tumour cells [10]. This diversity is diminished and altered in xenograft systems [11]. Other features changed in xenograft models include deranged tumour tissue architecture, lack of normal tissues nearby and the disruption of lymphatic and vascular supply and immune cells [3]. Despite this, they are still used widely in academia and industry because they are relatively inexpensive and easy to use [12,13].

Orthotopic models
A specialised version of the xenograft model has also been produced by transplanting tumour tissue or cancer cells to the orthotopic site (Table 1). Orthotopic models are more technically challenging to generate, however, they offer the advantage of examining effects on the microenvironment (albeit murine microenvironment with human cells and tissue) and the effect on metastatic spread [14]. Although there are instances where subcutaneous and orthotopic models have been compared, a more thorough investigation into the potential advantages of orthotopically transplantable tumours over simple subcutaneous models is required [15]. For example, parameters, such as chemosensitivity and vascularisation are known to be affected by tumour microenvironment [16,17].

Tumour graft models
Many groups have now started to report their experiences with fresh grafting from patient derived tumours into immunocompromised mice as a tool in late preclinical drug development [18]. These patient derived xenograft (PDX) models have been used to screen novel therapeutics, evaluate markers of response and resistance, and could be used to select drugs to treat individual patients. They do have some drawbacks, however, including a variable transplantation failure rate, higher labour costs and, with ongoing passages between mice, a higher mutation rate away from the parent tumour over time. This all leads to overall increased costs compared with normal xenografts. Current trials using PDX models are ongoing [19,20].

Genetically engineered mouse models
The murine model system to be investigated most recently in the therapeutic field is genetically engineered mouse models (GEMMs), where tumour development occurs in situ, in appropriate tissue compartments thus enabling complex processes to be modelled (Table 1). Thus it is reasonable to expect that GEMMs carrying the genetic signature of the native malignancy could recapitulate the biological manifestations of cancer in addition to the clinical behaviour, offering an alternative to traditional preclinical assays [21]. To date, few well characterised GEMMs have been used in preclinical drug evaluation trials.

GEMMs of cancer
GEMMs are the most advanced animal models of human cancer, and many models now exist that closely recapitulate the human disease. The use of transgenic and conditional knockout and/or knockin techniques has enabled many exciting scientific discoveries over the past few decades, including mechanisms of tumour initiation, progression and maintenance [3], in addition to drug resistance [10,22]. Transgenic mice express oncogenes or dominant negative tumour suppressor genes in a non-physiological manner. Endogenous GEMMs use knockout and knockin technology to enable conditional expression of oncogenes from their native promoter, deletion of tumour suppressor genes, or expression of dominant negative versions of tumour suppressor genes. Conditional GEMMs rely on the use of site-specific recombinases, such as the Cre-lox bacterial recombinase system, to control the spatial and temporal control of gene expression in the mouse genome, to further improve the faithfulness of the model [3].

Ideally GEMMs used to model human cancer should harbour similar genetic alterations, and these genetic alterations should be found in the appropriate cell types. In addition, the progression of the cancer in the GEMM should recapitulate the histopathology and molecular abnormalities of the cognate human disease [23]. Furthermore, once the tumour has developed the response to standard clinical treatments should be assessed, in efforts to 'credential' the model [23]. Such an approach has been reported in Kras-driven non-small cell lung carcinoma and pancreatic ductal adenocarcinoma models [10,24]. Comparing these results to corresponding clinical trials indicates these GEMM model human responses well, thereby supporting the utility of certain GEMMs in predicting outcome and interrogating mechanisms of therapeutic response and resistance.

Nonetheless, there are several shortcomings regarding the use of GEMMs that must be considered before use. First, GEMMs often take a long time to develop tumours and might require sophisticated imaging techniques to detect and monitor tumour growth, such as high resolution ultrasound scanning and magnetic resonance imaging [10]. Secondly, the usefulness of any given GEMM is dependent on several further issues including fidelity of the genetic lesions, kinetics of tumour progression, and the ability to detect disease and perform specific interventions. Examples of models that manage to address these factors include a K-ras^{G12D} driven pancreatic cancer model [25] and a B-raf^{V619E} driven melanoma model [26]. Third, GEMMs oftentimes have variable penetrance and require complicated breeding schemes, and the whole process is significantly more expensive than testing drugs in vitro or in xenografts. Finally, some drugs, particularly highly specific monoclonal antibodies, such as trastuzumab and bevacizumab, might only react with human epitopes, precluding an assessment in GEMMs [5]. These limitations notwithstanding, GEMMs have great potential to accelerate assessment of novel therapeutic agents.

Preclinical therapeutic testing
The primary purpose of preclinical therapeutic efficacy testing is to predict whether a particular compound will be successful in the clinic. Despite encouraging preclinical results, unfortunately most drugs are found to be ineffective late in their development, with only a small percentage (5%) of patients in Phase 1 clinical trials responding [27]. Apart from using inaccurate tumour models, there are many other reasons why preclinical studies fail to predict clinical activity. Species-specific PK, in addition to differences in

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PD, drug delivery, and tumour heterogeneity might all contribute to discordant results. Such failures are costly to scientists and drug companies and of great consequence to the patients that optimistically enrol in experimental clinical trials.

Preclinical models should be able to provide information on therapeutic mechanism of action, potential PD biomarkers, including biomarkers for prognostic and diagnostic endpoints,

toxicity, off-target activity and possibly resistance mechanisms. Thus, PK–PD modelling could be used to inform Phase 0 and 1 trial design and therefore should be incorporated in preclinical studies where possible (Fig. 1). However, it remains uncommon to have comparable information relating to tumour PK and how this is related to PD when the drug is evaluated in the clinic. Therefore it is often unknown whether the selected therapy is actually
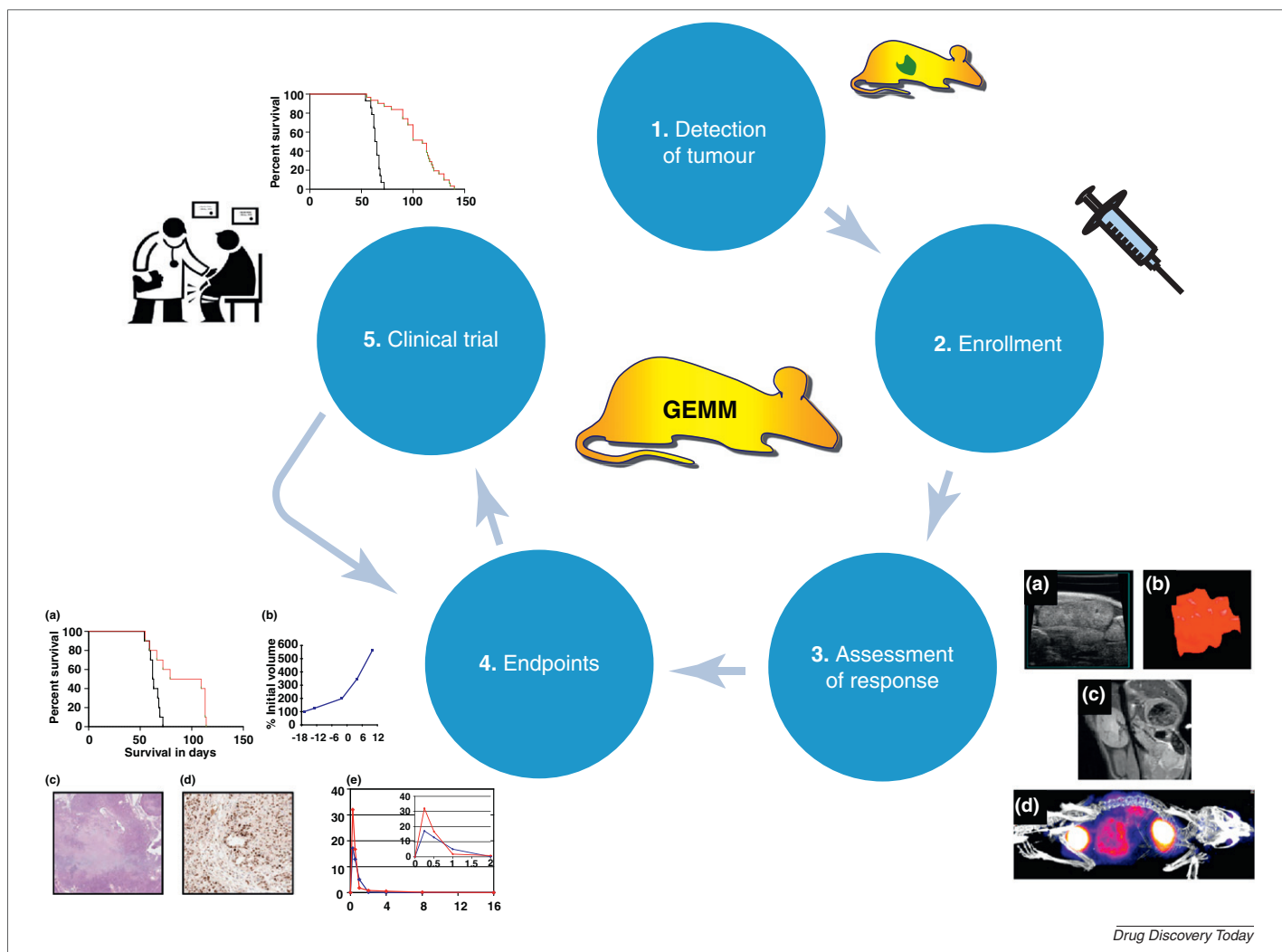
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FIGURE 1

Preclinical trial design. This design incorporates multiple important aspects of early stage clinical trials, which can be directly translated from preclinical studies. **(1)** Detection of tumour. A tumour needs to be detected before enrolment on the trial. This can be performed by regular palpation at the potential site of the tumour, at the hands of skilled technician, or high resolution imaging. For optimal results both should be used. Imaging modalities include ultrasound, magnetic resonance imaging and computerised tomography scans. **(2)** Enrolment. Once a tumour is detected mice will be enrolled. Stratification should be incorporated, similar to that used in a clinical trial, encompassing criteria, such as age, sex, tumour location and health status. Enrolment suitability and stratification should be independently confirmed. The mouse can then be initiated into a variety of different studies. These include (i) Short-term intervention studies: used to establish parameters, such as therapeutic dosing regimen, serum and tumour pharmacokinetic (PK) and pharmacodynamics (PD), and effects on basic tumour cell properties such as proliferation rate and apoptosis. Potential biomarkers of interest should be examined at this point. (ii) Survival studies: used to determine ability of drug to improve overall or progression-free survival. **(3)** Assessment of response. Animals must undergo regular assessments, such as serum biomarkers or serial imaging, to measure response. Reproducible imaging modalities, such as high resolution ultrasound (**(a) and (b)**), dynamic-contrast enhanced magnetic resonance imaging (DCE-MRI) **(c)** and positron emission tomography-computerised tomography PET-CT **(d)** have been used successfully. **(4)** Endpoints. For short term studies there are predetermined timed endpoints with an aim to understand mechanisms of action. In survival studies animals will remain on treatment until they require sacrifice due to pre-established morbidity endpoints. PK and PD effects can also be examined and correlated with the short term study results to examine for potential mechanisms of resistance. Examples shown are (a) Kaplan–Meier survival curve from a preclinical survival study. (b) Tumour volume curve quantification. (c) H & E staining revealing histology from a tumour sample. (d) Immunohistochemistry showing proliferation rates (using a Ki67 antibody) from a tumour. (e) F^{19} nuclear magnetic resonance measurements of drug levels from tumour tissue lysates. **(5)** Clinical trial. Translation to early stage clinical trials should incorporate the preclinical biomarker development, potential novel imaging methods, dosing schedule and endpoints examined. A Kaplan–Meier survival curve is shown. Abbreviation: GEMM: genetically engineered mouse model.

reaching its target, yet alone whether it is inhibiting pathways or impacting tumour cell biology and our clinical trial designs need to be modified to ensure that these data are collected.

In the future, predictive biomarkers are likely to guide therapy, as they already do in patients with breast cancer (trastuzumab) [28] and GIST (c-kit) [29] and when tested using an 'all comer' approach, many novels agents investigated thus far have been found to be ineffective late in their development [27,30]. Clinical trials are now being developed that incorporate predictive molecular biomarkers at an early stage, which would enable potential enrichment for patients most likely to benefit from the drugs [30,31]. Although this approach has advantages, there are multiple cancers for which predictive molecular biomarkers are not yet available. Identification of a validated assay that can measure a biomarker of target activation or target inhibition is often challenging. Animal models offer the promise of being able to identify such biomarkers, thereby accelerating their evaluation in clinical trials.

Predictive *in vivo* models

There are multiple measurements that determine whether an *in vivo* model will accurately predict responses to novel therapeutics. First, the response of the model to standard treatments should be assessed as discussed [23]. Even if a malignancy has limited effective therapeutic options, agents used thus far should still be evaluated for their lack of response. If the model generally responds to therapeutics that fails to have an impact on the corresponding human cancer, it is likely this model will not be effective at predicting responses to new agents. Perhaps this is one reason why xenografts have been poor at predicting responses to novel therapeutics [7]. GEMMs will ideally show reasonable penetrance and tumour latency and recapitulate the histological appearance of the human cancer. If tumour latency is too short the GEMM might not accurately capture the complex interplay between tumour cells, the microenvironment and cooperating genomic changes. By contrast, if the model takes too long to develop tumours it will be impractical for therapeutic assessments. Newer technologies, such as non-germline GEMMs, could be used in the future to overcome some of the limitations posed by traditional GEMMs [32].

There must also be a facile way to assess tumour progression and response to therapeutics. Although serial imaging is commonly performed to assess therapeutic response in human trials, functional imaging in preclinical trials has lagged behind. Many groups are currently investigating different imaging modalities in GEMMs, such as dynamic contrast enhanced magnetic resonance imaging (DCE-MRI), positron emission tomography computer tomography (PET-CT) and high resolution ultrasound, and the best technique will usually vary depending on the model under examination [23]. This rapidly expanding field might also steer the way for superior imaging modalities to permit earlier radiological assessment of therapeutic effects in human cancer [33,34].

A particular difficulty of recent years has been the ability to accurately study metastases in different models. Orthotopic models and GEMMs do metastasise to relevant organs of interest but potentially at a lower rate than the corresponding malignancies [35]. Nevertheless, models have now been established to study the role of spontaneous cancer metastases and examine effects of therapeutic agents in solid tumours [35]. It has recently been shown in a mouse model of pancreatic cancer that tumours metastasise at an early stage and the majority of patients will present with metastatic disease [36,37]. These results have clear implications for the treatment of pancreatic cancer [38].

As no model of cancer is perfect, GEMMs of cancer should be used alongside cell culture-based, xenograft and transplant model systems, in the preclinical evaluation of anticancer targets. The knowledge acquired from each system will aid the understanding of novel therapeutics more so than any system alone. This is

TABLE 2

GEMMs of cancer used to assess therapeutic efficacy preclinically

Tumour type	Model type [Refs]	Therapies tested	Refs
Breast and ovarian	BRCA1 and 2 and p53 deficient models [57]	PARP inhibitors	[60]
	MMTV-Myc and MMTV-Ras models [58,59]	Platinum agents	[61]
		Doxorubicin; paclitaxel	[62]
Lung	Endogenous Kras/PIK3CA or EGFR mutations or p53 mutations [63]	PI3K/AKT inhibitors	[64]
		MEK inhibitors	[40,41]
		EGFR inhibitors	[65]
		HSP90 and rapamycin	[42]
Chronic myeloid leukaemia	BCR-ABL mutation [66]	BCR-ABL TKI	[67]
Pancreatic ductal adenocarcinoma	Endogenous Kras and p53 mutations [54]	Gemcitabine	[10]
		Hedgehog inhibitors	[24]
		EGFR and VEGF inhibitors	
Pancreatic neuroendocrine	RIP-TAg model [47]	Cyclophosphamide	[45]
		Sunitinib (TKI)	[22]
		Everolimus (mTOR)	[47]
Lymphoma	Eμ-myc model [68]	Chemotherapeutics	[69]
APL	PML-RARα and PLZF-RARα models [70]	RA; As$_2$O$_3$	[43,71]
Melanoma	B-Raf model [26]	Rapamycin	[26]
		MEK inhibitor	

Abbreviations: APC: adenomatous polyposis coli; As$_2$O$_3$: arsenic trioxide; MEK: MAP kinase kinase; mTOR: mammalian target of rapamycin; PARP: poly(ADP-ribose) polymerase; PI3K: phosphatidylinositol 3-kinase; RA: retinoic acid; TKI: tyrosine kinase inhibitor.

something that should be encouraged and performed more often by industry and academia.

Preclinical to clinical transition

During recent years there have been many novel therapeutics tested in various GEMMs of cancer. Preclinical efficacy has been shown for many types of drugs, such as receptor tyrosine kinase inhibitors, rapamycin analogues, angiogenesis inhibitors and prostaglandin inhibitors [7]. Although we will not discuss all of these findings, significant recent results are discussed below. Table 2 summarises some of the commonly used GEMMs in the assessment of novel therapeutics.

Epidermal growth factor (EGFR) inhibitors have been applied successfully in the clinic for the treatment of lung adenocarcinoma [39]. Results from GEMMs based on the genetics of lung adenocarcinoma revealed similar successes [40,41]. More recently a KRAS-driven GEMM of lung adenocarcinoma has revealed significant preclinical responses with the combination of a HSP90 inhibitor and rapamycin [42]. These promising results also offer potential for other therapeutic combinations in the treatment of this aggressive cancer, and translation to the clinic is eagerly awaited.

In acute promyelocytic leukaemia (APL), there has been remarkable development and effective new treatments created owing to the accurate GEMMs that have been developed for this disease [43]. APL is now a highly curable condition, and patients are stratified to treatments based on the genetic criteria of their disease. The GEMMs of APL were fundamental in this process, and were used as preclinical predictive engines, with results translated into highly successful clinical trials [44].

Recent significant achievements in the field of pancreatic neuroendocrine tumours (NETs) have also been well documented. Well designed preclinical therapeutic trials investigated the use of sunitinib, and other kinase inhibitors, in the genetically engineered RIP-TAG mouse model [45–47]. The results of these led to the development of Phase 1/2 trials in NET tumours, and eventually successful Phase 3 trials, that are set to change the face of treatment for these rare tumours [48,49]. These are the first Phase 3 therapeutic success stories directly translated from results in GEMMs of cancer.

Despite the multiple success stories there have been some clinical failures that showed efficacy in GEMMs. Perhaps the most renowned story involves inhibitors of farnesyltransferase (FTIs), developed as inhibitors of Ras processing [50]. These drugs showed promise by causing regression of $Hras^{G12V}$-induced mammary tumours [51], but unfortunately these results did not translate to patients whose tumours harboured mutations in the RAS gene [52]. Evaluation of the preclinical studies has permitted further thought into the possible reason for this failure. Patients with KRAS mutations are relatively resistant to the effects of FTIs, unlike those with HRAS mutations. Unfortunately the vast majority of human cancers have mutations in the KRAS oncogene rather than HRAS [53].

Additionally, a recent elegant study has been performed using Kras-driven GEMMs [24]. This study examined the efficacy of chemotherapeutics, EGFR, and vascular endothelial growth factor (VEGF) inhibitors in the treatment of lung and pancreatic adenocarcinoma, clearly showing an excellent correlation between the results in the GEMMs and clinical trial results achieved thus far,

both positive and negative [24]. Although the correlations were analysed retrospectively, there were multiple comprehensive preclinical endpoints and methods used, guiding the way for future therapeutic advances and translation to clinical trials.

Multiple early stage clinical trials are currently underway in different cancer types, designed with the knowledge of successful results from preclinical studies using GEMMs. It is too early to say whether the majority of these will have similar successful outcomes eventually, but results thus far lend confidence to the use of GEMMs as a tool in therapeutic assessments. A thorough understanding of therapeutic mechanisms, preclinical models and early stage clinical trials is required for groups to accomplish successful translation of novel therapeutics. Importantly, if clinical trials fail to show efficacy when GEMMs show response, these should be able to answer the questions as to why the translation has failed. This can only be completed if the trials in question are designed with scientific rationale and biomarker driven endpoints.

Concluding remarks

Selecting the most appropriate in vivo model is essential during the drug development process to enable accurate modelling of therapeutic efficacy. By developing innovative preclinical trials using sophisticated animal models that recapitulate the human malignancies in question, we might be able to advance the field of drug discovery, and improve success rates for potential novel therapeutics in clinical trials. Figure 1 illustrates the approach we have taken in the KPC mouse model of pancreatic cancer [54].

Hurdles remain, however, and no model is going to be able to perfectly recapitulate the human situation. Historically, the majority of Phase 1 trials have admitted patients who were heavily pretreated with multiple different chemotherapeutics and targeted agents. Unfortunately this situation would be difficult to reproduce in the mouse, due to feasibility, time and money, and individualised patient responses to prior treatment. Some laboratories do manage to study therapeutic resistance, but this is only possible in models where initial sensitivity to an agent is dramatic enough to enable the development of acquired resistance [55].

Early stage clinical trials are now being designed with more emphasis on the biological effects of therapeutics, incorporating validated biomarkers as endpoints, and utilising an adaptive approach for analysing information in real time [30]. Window studies and Phase 0 trials are becoming increasingly popular, encouraging further insight into novel therapeutic mechanisms of action at an early stage of development [56].

Pharmaceutical companies have been reluctant to delay any Phase 1/2 trials while awaiting outcome of preclinical trials, potentially taking many years to complete. With recent encouraging Phase 3 results, correlating with earlier GEMM preclinical studies [48,49], the pharmaceutical industry might now decide that it is appropriate to invest additional resources into better designed preclinical trials with predictive animal models. For this to happen close collaborations are required between industry and academia, enabling animal and drug transfers between organisations, and divulging of expert knowledge each possesses. This would ultimately lead to a swifter, hopefully successful, translation to the clinic which, in the long term, would actually be cost-effective compared with the failure of a therapeutic late in its development.

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