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Non-germline genetically engineered mouse models for translational cancer research

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Abstract

Genetically engineered mouse models (GEMMs) of cancer have affected virtually all areas of cancer research. However, the accelerated discovery of new cancer genes emerging from large-scale cancer genomics and new chemical entities pouring from the drug discovery pipeline have strained the capacity of traditional germline mouse models to provide crucial insights. This Review introduces new approaches to modelling cancer, with emphasis on a growing collection of non-germline GEMMs (nGEMMs). These offer flexibility, speed and uniformity at reduced costs, thus paving the way for much needed throughput and practical preclinical therapeutic testing models.

Genetically engineered mouse models (GEMMs) have been the mainstay of basic cancer biology research for the past two decades. Germline models allow for testing and understanding the mechanisms of oncogenic transformation, as well as for probing the kinetics and therapeutic responses of autochthonous tumours in an intact microenvironment¹. Although most inherited cancer syndromes have been recapitulated with germline GEMMs, mimicking spontaneous tumorigenesis has become possible only in the past decade with the development of sophisticated multi-allelic mouse strains that have been guided by an improved understanding of the genetics of human counterparts.

Both the traditional and more advanced germline GEMMs have greatly contributed to understanding the different phases of tumorigenesis: initiation, maintenance, progression and regression (including minimal residual disease)^{2,3}. Additional improvements include the development of germline inducible models in which a genetic element is activated only in a small proportion of cells in a specified tissue, usually by exogenous chemicals or viruses. Such knock-in systems enable the modelling of the stochastic nature of tumorigenesis^{4,5}.

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Competing interests statement

The authors declare competing financial interests; see Web version for details.

However, even the most advanced germline GEMMs are not easily adapted to high-throughput translational research and drug testing owing to long timelines, difficulties in creating large cohorts in a short time frame, cost and space. In this Review, we discuss the advent and advantages of non-germline GEMMs (nGEMMs) for translational cancer research, with an emphasis on cancer gene validation and preclinical therapeutics.

Useful mouse models for translational research need to replicate the genetics and genomics, the context and the heterogeneity of human tumours. With respect to human relevance and predictability, a model should be driven by signature genetic events, with tumours occurring *de novo* in an immune-competent microenvironmental context, harbouring spontaneously acquired syntenic genomic alterations and variations representative of the heterogeneity, as well as exhibiting relevant clinical behaviour such as disease pathology and sites of metastasis. Furthermore, such models should develop the relevant tumours with high penetrance, reproducibility and synchronous kinetics, and should also be amenable to imaging modalities such as positron emission tomography (PET), magnetic resonance imaging (MRI) and/or computerized tomography (CT), thus offering ease of cohorting for monitoring tumour growth and regression in live animals in multi-arm preclinical trial studies. Finally, particularly with respect to drug response testing, such models should ideally recapitulate the genomic instability characteristics of human cancers.

Traditional germline GEMMs and their limitations

Despite their success in modelling human cancers for biological studies, challenges remain for the widespread and economic use of germline GEMMs for preclinical therapeutic research and development. First, GEMMs have a long lead-time to generation and modification, and require the targeting of embryonic stem cells (ESCs), the generation of chimeras, germline transmission, complex intercrosses and colony expansion, as well as characterization of the phenotypes – a process that typically takes several years. Additionally, not all and in some cases only a minority of the mice generated from breeding will inherit the desired genotypes, owing to the multi-allelic intercrosses required to model the complex genetics of human cancers. This problem can be further exacerbated in cases in which homozygosity is lethal. Second, the vast majority of models have incomplete penetrance, coupled with a non-synchronous and often prolonged latency to tumour emergence, creating logistical and financial barriers for their use in preclinical therapeutic studies. Last, many GEMMs exhibit heterogeneity in their tumour phenotypes, including tissue type and location, which may or may not be desirable, and which increases the colony size that is required to generate statistically meaningful cohorts.

Even in situations in which all of these desirable features are achieved in a GEMM, limitations remain. For example, the adenomatosis polyposis coli (*Apc*)^{Min} mouse accurately models a mutation that initiates 80% of human colon cancer, and the mice exhibit a highly replicable tumour multiplicity with a short (2-month) latency⁶. However, as the mutation is present in all tissues from birth, the *Apc*^{Min} mouse only models tumorigenesis in patients with familial adenomatous polyposis (FAP), which represents less than 5% of all the patients with colorectal cancer. Although a wide variety of drug and supplement studies

have been successfully carried out using the *Apc*^{Min} mouse model⁷, their direct applicability to sporadic human colon cancer is unknown.

Despite these shortcomings, several germline GEMMs have proved useful for improving the understanding of tumour initiation and progression; these include the breakpoint cluster region (BCR)-ABL1 mouse model², which enabled early translational drug studies for chronic myelogenous leukaemia. New concepts and understandings of therapeutic consequences have been elucidated in GEMMs. For example, experiments in a mouse model of pancreatic islet cell tumours have led to a new dosing schedule of standard chemotherapy combined with anti-angiogenic therapy⁸. In addition, the mouse model for mutS homologue 6 (MSH6) deficiency revealed an additional aspect of hereditary non-polyposis colorectal cancer (HNPCC) — namely, replication error-negative, lymphoma-correlated gastrointestinal tumours — that had previously not been captured in the traditional Amsterdam criteria and which is now being used in the clinic⁹. In summary, germline GEMMs of cancer have provided a useful starting point for disease modelling, but improvements are needed to facilitate their use as preclinical models for translational science.

Mosaic or conditional models: enhanced GEMMs

Mosaic models or conditional GEMMs are an extension of germline models in which a latent allele is phenotypically wild type until stimulated in a tissue- and time-specific manner with exogenous chemicals or viruses. These models include the Cre-lox and Flp-Frt systems (FIG. 1). Cre-lox models harbour genetic elements flanked by loxP sites that are modified by Cre recombinase. Traditionally, these alleles become constitutively activated or inactivated in compound transgenic animals by Cre recombinase under the control of a tissue-specific promoter. To bypass this constitutive feature, delivery of Cre can be temporally controlled in two different ways to result in the activation or inactivation of the LoxP alleles. In the first system, Cre can be delivered with a virus directly to an organ — as infectivity of the virus will not be 100%, a genetic mosaic will be created in the target tissue. For example, using adenovirus as the vector, Cre mosaic cancer models for lung and ovary have been established using a *Kras* lox-STOP-lox allele^{10–13} (FIG. 2). These mosaic systems have the advantage of reduced tumour burden, which typically leads to a prolonged lifespan that allows for advanced tumour progression compared with traditional GEMMs — a feature that can be useful for preclinical studies. Indeed, the KRAS-driven lung model has been effectively used to demonstrate the efficacy of the pan-PI3K inhibitor BEZ235 (REF. ¹⁴). However, the disadvantages of such viral delivery approaches include the unpredictability of delivery and efficiency, as well as the limited accessibility of certain target tissues for infection.

A second system makes use of a fusion of oestrogen response elements (EREs) to Cre¹⁵: the injection or application of tamoxifen analogues to the mouse activates Cre in both a tissue-specific and a time-specific manner. For example, a tyrosinase-driven CreERT2 allele excises lox-STOP-lox elements in a melanocyte-dependent manner when tamoxifen is topically administered¹⁶; this has been used to develop mouse models of melanoma^{17,18}. These models illustrate the usefulness of the CreERT2 allele, as widespread tamoxifen

application results in massive tumorigenesis across the whole mouse skin, whereas focal application results in well-defined and predetermined sites of tumour formation. This not only increases the lifespan of the mice for preclinical therapeutic analyses, but also provides biological reproducibility. Finally, the analogous Flp-Frt system allows for the possibility of two different recombinases (Cre and Flp) to be used in the same mouse for targeting different elements by excision in a temporally independent manner. Although this has not yet been extended to the manipulation of multiple genes in the same mouse, this dual system has been used *in vivo* to remove unwanted engineered elements, such as neomycin, from integrated targeting vectors¹⁹.

Another layer of control is provided by the flexible Tet-On and Tet-Off systems²⁰. Each system is composed of two parts: the Tet operon promoter (TetO) that regulates the expression of the gene of interest, and either the transactivator (tTA) or the reverse transactivator (rtTA) transgenes, which are capable of binding to and regulating TetO. In the Tet-Off system, TetO is constitutively bound by tTA, which stimulates the expression of the gene of interest: when a tetracycline analogue (most commonly doxycycline) is introduced, it binds the tTA and prevents its interaction with TetO, thus shutting off expression of the gene. In the Tet-On system, rtTA is unable to bind TetO by itself, leaving the gene switched off. In the presence of doxycycline, rtTA binds to TetO and the gene is expressed. Therefore, the temporal control of gene expression is achieved by choosing when to feed the mouse doxycycline, and tissue specificity is governed by the promoter driving tTA or rtTA expression. The regulated induction of oncogene expression through doxycycline administration to mice that have undergone thymic maturation also mimics the somatic acquisition of genetic alterations that could otherwise be recognized by the immune system as foreign antigens. Unlike the Cre-lox system (which is irreversible after recombination) the Tet systems are reversible by withdrawal of doxycycline. This has also enabled the demonstration of a requirement for tumour maintenance in which, on doxycycline withdrawal, the transcriptional extinction of an oncogene such as HRAS or MYC leads to the almost complete regression of melanoma⁵ and lymphoma²¹, and liver²² or breast cancer²³ (FIG. 2).

A variation on the mosaic models is the replication-competent avian leukosis virus long terminal repeat with splice acceptor (RCAS) avian retrovirus receptor system. Transgenic animals expressing the chicken receptor in a tissue-specific manner using tissue-specific promoters are infected with the RCAS virus that carries the oncogene of interest, sometimes inducible with the Tet system. Using the RCAS system, a series of brain tumour models, including models incorporating sonic hedgehog (SHH)²⁴ or platelet-derived growth factor (PDGF)²⁵, have been produced and used effectively in preclinical studies. For example, a vascular endothelial growth factor receptor (VEGFR) inhibitor²⁵ or perifosine, a dual MAPK and AKT inhibitor, have been used in the PDGF model²⁶. Similarly, lung cancer models that are driven by epidermal growth factor receptor (EGFR) mutations have been generated²⁷, and erlotinib resistance in tumours carrying the EGFR-T790M mutation has been confirmed²⁸. To overcome the limitations of the RCAS viral system, lentiviruses have recently been used for the somatic delivery of genes or switch elements²⁹.

Overall, conditional GEMMs provide improved flexibility, statistical power and accuracy compared with the standard germline model design. However, despite their potential usefulness, a major hurdle for both traditional and conditional GEMMs has been the tremendous breeding efforts that are necessary to establish large cohorts for drug treatments that have enough power for statistical significance. Only a few drug development companies have established the means of producing such large cohorts of GEMMs. Nevertheless, when preclinical trials were carried out in lung cancer GEMMs, the results proved to be comparable to findings from human clinical trials that had parallel designs³⁰, thus validating the use of GEMMs for therapeutic studies.

Non-germline GEMMs

The recent development of nGEMMs of cancer in various systems, including the lung³¹, breast³², liver and haematopoietic organs^{33–35}, has revealed new insights into tumorigenesis. These models have been generated through the establishment of chimeric mice that develop spontaneous tumours in a tissue-specific manner or by the transplantation of cells (TABLE 1). Chimeric models require the implantation of genetically engineered ESCs into pre-implantation embryos, leading to chimeras that carry a mixture of predisposed cells that are derived from the ESCs and from wild-type host cells. In these settings, tumours develop in the context of normal tissue, recapitulating human tumorigenesis. Transplantation models are generated by implanting normal, stem or tumour cells into the respective adult tissue. The transplanted cells can be derived either from genetically engineered donor mice harbouring cancer-predisposing mutations or from mouse or human cells that have first undergone *ex vivo* engineering. Several of these systems have emerged with features that render them particularly useful for translational studies, as discussed below.

Chimeric models

The use of chimeric models is a new approach for improving clinical relevance, in which developing cancer cells are seeded in the context of normal surrounding tissue. This feature overcomes the genetic field effect of expressing an oncogene in the whole tissue of an adult animal, a limitation that is inherent to the GEMMs. The development of chimeric models begins with engineering tetracycline-inducible cancer-relevant alleles and imaging markers exclusively into mouse ESCs. For example, Zhou *et al.*³¹ described a model in which both alleles of the *Cdkn2a* locus (which encodes the tumour suppressors INK4A and ARF) were deleted and a tissue-specific rtTA, tetracycline-dependent oncogenes and a luciferase imaging marker were engineered into the ESCs. Injecting these genetically engineered ESCs into wild-type host blastocysts generated cancer-prone chimeric mice in which specific tumour types developed in the context of normal stroma³¹ – a situation that mirrors cancer development in humans. The importance of stromal influence on tumorigenesis is increasingly recognized^{36,37}, making this an even more attractive feature of a chimeric model. Further tissue and temporal specificity can be achieved by the localized or timed application of induction agents.

As the transgenic alleles are engineered *ex vivo*, the generation of chimeric models does not require large breeding cohorts. They can therefore be produced rapidly and at a reduced cost compared with standard GEMMs. Even after the establishment of a GEMM, multi-allelic

GEMMs often require management of heterozygous and homozygous breeding, leading to laborious genotyping efforts, the necessary generation of unwanted genotypes, considerable time investments and a substantial amount of space requirement, all of which add to the already challenging costs that are associated with mouse studies. It is therefore of no surprise that only a few studies have used GEMM breeding schemes involving four or more modified alleles^{38,39}. By contrast, chimeric models considerably reduce the number of animals generated, as all mutations and allele modifications can be carried out *ex vivo* in ESCs, which can be rapidly expanded as well as readily frozen for storage in a cost-efficient manner. Producing the actual chimeras requires only generating or purchasing the host blastocysts, thawing the ESCs and injecting them into the blastocysts and housing the resulting chimeras. Therefore, large study cohorts can be generated in a time-, labour- and cost-effective manner.

In principle, two major challenges could limit the reliability of chimeric models: the functional validation of individual genetic elements and the maintenance of pluripotency of the ESC clones. Recent studies suggest that such challenges can be overcome if the ESCs are modified in a stepwise manner and functionally tested for the introduced genetic element at each step before selection for their ability to contribute to a host embryo. For example, chimeric models of lung cancer induced by ERBB2-V665E (also known as HER2-V665E), EGFR-L858R or KRAS-G12V gave rise to invasive adenocarcinomas³¹. Importantly, each initiating genetic lesion results in key differences in pathway activation. Overall, this approach satisfies the need for an immunocompetent host and for rapid cohort generation, in which the rate-limiting factor is the generation of ESCs and blastocysts. Importantly, every mouse will be primed for cancer induction, removing the dependence on Mendelian segregation for generating the desired genotypes. Conversely, the usefulness of the chimeric models depends on the capability of ESCs to populate the target organ; although it has not yet been observed, it is possible that there are cell lineages that do not lend themselves for ESC repopulation.

As for drug trials, particularly in epithelial cancers, chimeric nGEMMs can mimic the tumour–stroma interaction better than xenograft models, which should lead to improved understanding of drug–tumour interactions. For example, an allelic series of lung cancer chimeric models containing *ERBB2*^{V659E}, *KRAS*^{G12V} or *EGFR*^{L858R} oncogenes demonstrated that the resulting adenocarcinomas in normal lung tissue exhibited features of advanced malignancies. An experimental therapeutic trial carried out on the EGFR-L858R and KRAS-G12V chimeric models with an EGFR inhibitor (AV-412) accurately reflected the clinical observations³¹: chimeric mice harbouring tumours driven by EGFR-L858R exhibited a dramatic response to AV-412 treatment, with nearly complete tumour elimination and with lung volume returning close to normal values. KRAS-G12V-dependent chimeric lung tumours did not show any appreciable response to the treatment, and clinical symptoms did not improve. Interestingly, some EGFR-L858R tumours did not show a complete response to AV-412 treatment, probably reflecting the early emergence of drug resistance, which is consistent with clinical observations. A breast chimeric model and its application in translational research has also been described⁴⁰.

Mouse in mouse transplantation models

The chimeric models described above are produced by the manipulation of ESCs followed by injection into blastocysts. A related approach involves the transplantation of genetically altered tissue-restricted stem and progenitor cells into primed syngeneic recipient mice, in which the stem or progenitor cells home to the appropriate tissue and the tumours arise surrounded by otherwise normal tissue. Such systems have been widely used in the haematopoietic system, in which haematopoietic stem and progenitor cells can be readily isolated from bone marrow or fetal livers and intravenously transplanted into lethally irradiated recipient mice⁴¹. As occurs following high-dose chemotherapy in human patients before bone marrow transplantation, irradiation creates a niche for the modified haematopoietic stem and progenitor cells to engraft^{42,43}. Importantly, if placed into short-term culture before transplantation, the stem and progenitor cells can be transduced with retroviral vectors expressing oncogenes that recapitulate genetic changes that occur in human leukaemia and lymphoma⁴⁴. Moreover, short hairpin RNAs (shRNAs) encoded from viral vectors can be introduced into precursor cells to mimic tumour suppressor gene loss⁴¹, greatly facilitating the modelling of tumour suppressor genes that would otherwise require laborious knockout gene targeting strategies in GEMMs. Because it is possible to mix and match different retroviral vectors with different target stem and progenitor cell populations, leukaemias and lymphomas harbouring many genotypic combinations can be rapidly produced without extensive allelic intercrosses.

These transplantation systems can be rapidly established and are, despite their simplicity, good models of the human counterparts (as elaborated below). Owing to their flexibility, it has been possible to study many genes, or gene combinations, and to carry out structure–function studies on various oncogenes^{45–47}. For example, a haematopoietic transduction–transplantation system was used to show that tumour-derived MYC mutants from human Burkitt’s lymphoma are more oncogenic than wild-type MYC because they evade the activation of the p53 tumour suppressor pathway⁴⁸. Furthermore, similar systems have been used to rapidly produce mice for studies using conventional (see for example REFS^{49,50}) or targeted therapeutics^{51–53}. Notably, the resulting leukaemias and lymphomas can be readily transplanted into syngeneic recipients, in which they home to the appropriate organs and produce secondary malignancies that are identical to the primary hosts. Indeed, the importance of syngeneic transplantation in preclinical therapeutics has previously been highlighted by the efficacy of studies using donor GEMM primary tumours, such as those that are derived from multiple different models of breast cancer^{54–56}. Therefore, nGEMM systems can be readily propagated *in vivo* and transferred between metastatic sites, even in immunocompetent hosts.

Although the haematopoietic system is perhaps the most tractable tissue that lends itself to the transplantation approach, conceptually similar systems can be adapted to produce cancer types for which progenitor cells can be isolated, transduced and transplanted. To date, such models have been created for various cancer types, including that of breast⁵⁷, brain^{58,59}, ovary⁶⁰ and liver³⁴. By combining transplantation models with other genetic tools – for example, conditional gene expression or RNA interference (RNAi) — it is possible to rapidly study the role of candidate genes in tumour maintenance. Furthermore, by

implanting cells that are engineered with somatic signature mutations into strains of different genetic backgrounds it is possible to explore the role of germline variability on biological phenotypes and/or the influence of tumour microenvironment. In one example, such studies have demonstrated that p53 loss is required for tumour maintenance, and that p53 reactivation leads to tumour regression by a combination of cell autonomous arrest programmes together with a new form of immune surveillance leading to the clearance of the former tumour cells, indicating the importance of a competent stroma⁶¹. It also becomes possible to carry out *in vivo* forward genetic screens (discussed below). However, one potential drawback of this system is that in some models, tumour phenotypes are not always replicated from mouse to mouse (TABLE 1). For example, liver progenitor cells engineered to express HRAS-V12 have a consistent latency and full penetrance, whereas those expressing MYC have a broad latency and ~40% penetrance³⁴. This may be partly due to MYC not being sufficient to induce tumorigenesis alone and therefore it might rely on stochastic genetic changes to bring about full transformation. However, the effect of this potential shortcoming, if present, can be mitigated with a study design that is careful with the timing of treatment events.

Another application of transplantable models is the identification of cells-of-origin for particular malignancies by transducing and transplanting various progenitor cells for a particular phenotype. For example, *Cdkn2a*-null mouse neural stem cells or astrocytes transduced *ex vivo* to express constitutively active EGFR (the EGFRvIII mutant) have been shown to drive the development of high-grade gliomas when transplanted orthotopically into immunodeficient host mice⁵⁸, showing that the neural stem cells would be the cell-of-origin for high-grade gliomas. More recently, neural stem cells isolated from the subventricular zone were further shown to be the cell-of-origin for glioblastoma⁶². Conceptually, many mouse tissues may be amenable to a similar modelling approach. Using a mouse origin for these progenitor cells provides the advantage of using an immunocompetent syngeneic host to ensure that tumour development is subjected to the same types of immune-mediated selective pressure as those seen in standard GEMMs, and as in human patients.

Transplantation nGEMMs offer major advantages in both genetic and drug screens. In germline GEMMs, large-scale genetic screens are carried out using retroviral or transposon mutagenesis and insertion sites are largely random. These screens are useful in constitutive germline models when used with mouse moloney leukaemia virus (MuLV; a common tool for retroviral insertion screens⁶³) or in combination with mosaic models (for example, in colon cancer⁶⁴). However, owing to their simplicity and ease-of-use, transplantation-based mosaic nGEMMs particularly lend themselves to the rapid validation of cancer genes. If one presumes that most of the genes that modulate cancer in humans will do so in mice, it becomes possible to use genomic information from human tumours to focus candidate-testing studies in mouse mosaic models for identifying, validating and characterizing functionally relevant cancer genes.

As one example illustrating the above approach, pools of shRNAs targeting the mouse orthologues of genes recurrently deleted in human hepatocellular carcinomas (HCCs) were tested for their ability to promote tumorigenesis in a mosaic mouse model of HCC⁶⁵. In contrast to randomly selected shRNA pools, many deletion-specific pools accelerated

hepatocarcinogenesis in the mice. Through further analysis, several new tumour suppressor genes were identified and validated, many of which had not been previously linked to cancer. These included the gene encoding the nuclear export protein exportin 4 (*Xpo4*) — which is also lost in other tumour types, including breast carcinoma⁶⁵. In another example, a series of shRNA pools targeting a focused set of cancer-associated genes (a curated list of 1,000 cancer genes informed by multiple studies⁶⁶) were introduced into haematopoietic progenitor cells derived from E μ -*Myc* transgenic mice and screened for their ability to promote lymphomagenesis following engraftment into syngeneic recipients^{67,68}.

Among the new tumour suppressors that were identified, *Rad17*, the product of which is involved in the oncogene-induced replicative stress response, proved to be a haploinsufficient tumour suppressor the complete inactivation of which is otherwise deleterious to embryogenesis⁶⁷. Therefore, in this instance, shRNA screens could identify a tumour suppressor the heterozygous inactivation of which may promote tumorigenesis. Interestingly, although heterozygous deletions encompassing *RAD17* occur in human cancers, it would have been difficult to pinpoint *RAD17* as a relevant tumour suppressor through genomic approaches alone, as it does not particularly stand out using traditional computational methods. Surprisingly, some of the other tumour suppressors identified in this screen may have pro-oncogenic activities in other contexts, such as *MEK1*, a well-known mediator of the oncogenic MAPK pathway; and a surprising number of them encode secreted proteins — functional aspects that were not suspected *a priori*. This emphasizes the importance of tissue specificity, including tumour microenvironments, in determining the pro-cancer or anti-cancer functions of any particular gene.

These results establish the feasibility of *in vivo* genetic screens and illustrate how combining cancer genomics, functional genetic screens and mosaic mouse models can facilitate the functional annotation of the cancer genome. Current large-scale cancer genomics efforts are identifying many candidate genes; although extremely powerful by itself, genomic evidence is not sufficient to prove cancer relevance. Biological activities of these candidates must be functionally validated in various models, which is time intensive and expensive. Through functional genetic screens, it is possible to rapidly filter large numbers of candidate oncogenes and tumour suppressor genes for relevant biological activities to prioritize downstream follow-up studies. The ease of manipulation of mosaic models enables the modelling of the appropriate context for such genetic screens, an important parameter in a screen system given the context specificity of gene function.

This relatively high-throughput approach could be expanded to other mouse models or could include shRNA or open reading frame (ORF) libraries targeting genes that are affected by larger deletions, promoter methylation or point mutations. Furthermore, the concepts described above can be expanded in the future by incorporating more complex genetic models, viral vectors and/or selection schemes. For example, doxycycline-inducible vectors can dissect the role of genes in tumour maintenance versus initiation, for example, a cDNA library that can be turned off once the tumour is already established. Another possibility is carrying out a synthetic lethal screen with a known anti-oncogenic compound, in which the combination of the drug and the suppression of molecular targets can enhance the rational

design of combination therapy. This broad range of possibilities highlights the flexibility of nGEMM systems for both basic and translational research (BOX 1).

Importantly, studies examining the relationship between cancer genotype and sensitivity to conventional chemotherapy suggest that nGEMMs can accurately model therapeutic response. For example, mice transplanted with lymphoma cells from E μ -Myc mice, an nGEMM of human Burkitt's lymphoma, are highly responsive to the chemotherapeutic drug cyclophosphamide, which parallels the situation in the primary transgenic mice and mirrors the situation in human patients³³. Furthermore, disruption of p53 by RNAi produces an attenuated response to therapy and early relapse in these mice, recapitulating the association between p53 mutations and treatment failure found in various haematopoietic malignancies (see REF. 69). More recent work has dissected the roles of the DNA damage response factors in response to conventional chemotherapy in the mouse in a high-throughput manner that was not possible using traditional GEMMs⁷⁰. Insights from such approaches may eventually enable the stratification of patients for the appropriate therapy in the clinic⁷⁰.

In nGEMMs of acute myelogenous leukaemia (AML), mice with leukaemia expressing the runt-related transcription factor 1 (RUNX1; also known as AML1)–RUNX1T1 (also known as ETO) fusion protein, which mimics t(8;21) that occurs in human patients, show robust responses and even cures to frontline chemotherapy. Mice with leukaemia that express mixed-lineage leukaemia (MLL) fusions, which mimic translocations involving 11q23, are refractory to standard therapy, much like their human counterparts⁵⁰.

Expanding beyond conventional chemotherapy, nGEMM mice harbouring myeloproliferative diseases that are triggered by the deletion of the tumour suppressor neurofibromin 1 (Nf1) are insensitive to MEK inhibitors (which target a downstream component of the Ras–MAPK pathway that becomes overactivated by NF1 loss), but full-blown AMLs with the same initiating lesion plus a randomly integrated retrovirus are surprisingly sensitive⁵³. Nevertheless, these AMLs eventually acquire drug resistance that can be traced back to the integrated retroviruses, which have generated subpopulations of AML cells that are sensitive or resistant; MEK inhibition selects for the resistant subpopulations and reveals the role of genes such as RAS guanyl releasing protein 1 (Rasgrp1) in mediating drug resistance. Overall, mouse in mouse transplantation models offer a high degree of *ex vivo* engineering flexibility, the rapid generation of cohorts and the possibility of a fully competent immune environment. However, the direct applicability of mouse tumours to the clinic must be complemented with studies using human tissues.

Human in mouse (HIM) transplantation models

The analysis of GEMMs of cancer and the corresponding human cancers has established that many of the bona fide transforming or tumour suppressor genes and key transformation pathways are conserved between mouse and human cells^{71,72}. At the same time, it is well known that cross-species differences exist on the cellular level, such as telomere dynamics⁷³, or on the macro-environmental level, such as diet and carcinogen exposure. For example, inactivation of p53 (the most commonly inactivated tumour suppressor in humans) in the mouse has failed to recapitulate the epithelial cancer phenotypes: these mice do not develop the same range of tumours as that observed in patients with Li–Fraumeni syndrome.

The reason for this difference became clear when it was appreciated that the mouse has long telomeres and promiscuous expression of telomerase⁷⁴; therefore, mouse epithelial cells do not experience telomere-based crisis (when damaged telomeres require repair to prevent cell death), which is a potent cooperative driver of epithelial carcinogenesis in the setting of p53 deficiency⁷⁵. When telomerase deficiency is introduced by inactivating telomerase reverse transcriptase (*Tert*), p53 inactivation led to the development of a range of epithelial tumours similar to those that occur in human patients with Li–Fraumeni syndrome⁷³. Interestingly, even the knockin mutants of gain-of-function p53 alleles from some patients with Li–Fraumeni syndrome conspicuously failed to generate breast cancers, suggesting subtle species differences⁷⁶. Historically, the solution to this problem has been the use of human tumour-derived cell lines. When transplanted into immune compromised mice, some of these lines have the ability to form tumours. Such xenografts have been a staple of cancer research for decades — and although they have provided a platform for many important discoveries, they have unfortunately failed to predict drug responses¹. This failure might be due to *in vitro* propagation, an environment with different growth factors from those in the *in vivo* environment, as well as the subcutaneous grafting of the cells into non-physiological space.

To circumvent the species differences, recent advances have included the use of human primary, non-cancerous tissue transplanted into mice (as described below) (FIG. 3). In general, these approaches rely on the isolation of differentiated or tissue progenitor cells from donor tissue for *ex vivo* genetic engineering, followed by transplantation into the appropriate mouse tissue of an adult immune compromised mouse. This approach avoids artefacts being introduced during prolonged *in vitro* culturing and allows the experiment to start with euploid, non-transformed cells. One of the first models to use primary, non-cancerous human tissue was that of melanoma and skin models (REFS^{77,78} and reviewed in REF.⁷⁹). Primary cells from human skin were isolated, cultured and transfected with retroviruses. The cells were allowed to form skin using three-dimensional culture *in vitro* and then grafted onto immune compromised mice. Transduction of keratinocytes with SHH resulted in basal cell carcinomas⁷⁷, whereas transduction with HRAS-V12 in combination with cyclin-dependent kinase 4 (CDK4) or nuclear factor-κB inhibitor-α (IKBα) resulted in squamous cell carcinomas⁸⁰. Transduction of melanocytes with an oncogenic mutant of NRAS in combination with the overexpression of TERT, dominant-negative p53 and an activating CDK4 mutant resulted in melanomas⁷⁸. Another model incorporates human newborn foreskin grafts onto recombination activating gene 1 (*Rag1*)-deficient mice, inducing melanoma formation by a combination of treatment with DMBA and ultraviolet B (UVB) radiation⁸¹. Therefore, the modelling of skin tumours using human donor tissue was able to recapitulate all three major forms of skin cancer.

Others have reported a leukaemia model using cord blood as donor tissue⁸². Umbilical cord cells were enriched for stem cells, transduced with a retrovirus containing the *MLL-MLLT1* (also known as *ENL*) fusion gene and transplanted into irradiated mice, shortly after which acute lymphoblastic leukaemia formed within 140 days and featured hallmarks of the human disease. Transducing the cells with a retrovirus encoding the *MLL-MLLT3* (also known as *AF9*) fusion gene resulted in various AMLs, as occurs in patients. This model allowed the

authors to identify and characterize MLL leukaemia-initiating cells (LICs), which retained both myeloid and lymphoid lineage potential and remained responsive to microenvironmental cues. The properties of these cells provide a biological basis for several clinical hallmarks of *MLL* leukaemias⁸².

More recently, Kuperwasser and colleagues³² reported a breast cancer model in which primary human mammary epithelial cells were isolated from donor tissue as organoids, which are cells that retain some of the original three-dimensional context, that is, they are not a single cell suspension. These organoids were transplanted into the cleared mammary fat pad of immune compromised mice and formed structures that are indistinguishable from human mammary gland structures⁸³. When the organoids are transfected with lentivirus harbouring *KRAS*^{G12V} or *ERBB2*^{V665E} in combination with simian virus 40 (SV40) large T antigen *in vitro* without culture and transplanted into the mouse cleared fat pad, human mammary gland structures formed and developed tumours from these glandular structures, just as occurs in humans³². Tumorigenesis goes through stages identified in patients, such as ductal carcinoma *in situ* (DCIS). Interestingly, in these tumours, overexpression of TERT is not necessary, although this has not been observed following *in vitro* manipulation of human mammary epithelial cells. The developing tumours show hallmarks of basal-like breast cancer, a tumour with few treatment options. Therefore, this model allows the study of the development and therapeutic sensitivities of this important tumour type for the first time.

Although these human in mouse (HIM) model systems are clearly powerful in many ways, disadvantages remain, including the absence of a memory-based immune system and the reliance on virally introduced transgenes rather than endogenous genetic aberrations, as well as the potential of ligand–receptor mismatch owing to species-specific variation. A well-recognized example is the fact that human MET (the hepatocyte growth factor (HGF) receptor) does not bind mouse HGF⁸⁴. How such differences might influence therapy responses in preclinical studies remains to be determined.

Finally, inherent genomic instability characteristics of human cancers may modulate therapeutic response and resistance; therefore, nGEMMs with similar genomic instability would probably be more predictive preclinical therapeutic models. The HIM tumours can circumvent this particular issue by using telomerase-deficient cells. Treating human tumours originating in the mouse, in a non-xenograft cell line, will give rise to new insights that are difficult to obtain using mouse tumours only. Wu and colleagues³² have demonstrated that using the breast HIM model, treatment with trastuzumab is efficacious. This will enable better characterization and understanding of trastuzumab responses in primary human tumours, which has not been possible so far in GEMMs owing to the inability of trastuzumab to bind to mouse ERBB2. Although more work is needed, the successful use of these models in preclinical studies could dramatically reduce the time and cost of drug development and so warrant further investigation. This will become even more important as we embark on the exploration of combination strategies.

Archived tumours in translational research

A major limitation of xenograft transplantation models using established human tumour cell lines is the inability to model the heterogeneity of a patient population. The replication of such heterogeneity is a key advantage of GEMMs and nGEMMs, as such models are typically engineered with one or more initiating genetic events that are not sufficient for full transformation. As reflected by the latency and incomplete penetrance of these models, and consistent with recent hallmark reviews of cancer biology^{85,86} that postulate the requirement for mutations in 8–12 key pathways, tumours that emerge *de novo* from these GEMMs and nGEMMs have additional spontaneously acquired mutations, which lead to molecular and biological variations that mirror the heterogeneity of a patient population. Such heterogeneity can be exploited, as exemplified by drug studies in germline non-small-cell lung cancer (NSCLC) and pancreatic ductal adenocarcinoma (PDAC) KRAS models using EGFR (erlotinib) and VEGF (anti-mouse VEGFA antibody mimicking bevacizumab) inhibitors³⁰. However, a challenge introduced by this genetic diversity in tumours that emerge from GEMMs and nGEMMs is the difficulty of obtaining matched pairs of pre-treatment and post-treatment tumour tissues for comparative analyses. Therefore, tumour archives have become popular as a way of preserving this feature of heterogeneity in a tumour population while enabling access to pre-treatment tumour materials. Watters et al.⁴⁰ exploited this concept by establishing a well-characterized archive of 107 propagated tumours from a breast ERBB2 chimeric model. Testing the archive with a γ -secretase inhibitor responder–non-responder analysis revealed a set of biomarkers that predicted response to the compound. Additionally, archives from chimeric tumours retain the characteristics of the primary tumours, as has been demonstrated for a lung NSCLC KRAS archive (Y. Zhou, A. Bressel, T. Zi, D. Potz, Z. Cai, I. Chiu, M. Robinson and J. H., unpublished observations). Archives from HIM transplantation models can also be established (M. Wu, K. Clark, Z. Cai, N. Deng, I. Chiu, M. Robinson and J. H., unpublished observations). Similarly, Jimeno *et al.*⁸ have developed an archive of primary patient explants for pancreatic tumours. These tumour archives are another platform for studies of drug response and resistance that is complementary to GEMMs and nGEMMs.

Conclusion and perspective

One of the most exciting aspects of nGEMMs is their applicability to preclinical drug development. The essential interaction of normal stroma with the tumour cells allows for testing drugs that not only interfere with tumour cell growth but that also target the tumour–stroma interaction. Moreover, the ability to establish cohorts of *de novo* tumours enables population-based empirical testing of drug responses. Finally, the use of HIM models using cells from human donors now enables the generation of tumours arising from primary human cells at orthotopic sites, thus avoiding many of the problems that are associated with prolonged cell culture and xenografts. However, it is worth remembering that each nGEMM has its limitations. For example, humanized nGEMMs are limited by the availability of donors, the species incompatibility of ligands and the absence of a memory-based immune system. Mouse nGEMMs still require improvement in the reproducibility of tumour phenotype and in the more widespread use of immunocompetent hosts. Nonetheless, nGEMMs of cancer provide valuable systems for designing and testing new therapeutics,

including rapid cohort generation, reproducibility of phenotypes and improvements in modelling the tumour microenvironment.

In summary, no single model is likely to recapitulate all aspects of the complex genetics and biology of human cancers; therefore, understanding the strength and limitation of each model is necessary to maximally leverage these complimentary engineered model systems to facilitate the development of drugs and drug combinations in the future.

Glossary

Germline model	Mouse model that carries genetic modifications in its germline and which is maintained through breeding
Inducible model	Mouse model that activates the expression of a transgene through a transactivator transgene that is, tTA or rtTA
Non-germline GEMM (nGEMM)	Mouse model that carries genetic modifications in some of its somatic cells but not in the germline cells. Each model has to be individually generated through, for example, transplantation and injection
Mosaic model	Germline model that acquires modifications of the germline genetic modification in some of its somatic cells
Conditional GEMM	Model that acquires an activation or inactivation of the original genetic modification in somatic cells through the temporal or spatial expression of a modifier such as Cre
Chimeric model	Mouse model that has been generated by ESC manipulation followed by the injection of these cells into a pre-implantation embryo. The resulting chimeric animal is the model animal
Transplantation model	Mouse model in which part of a tissue is modified by transplanting tissue stem cells that carry genetic modifications
Human in mouse (HIM) model	Transplantation model in which the transplanted cells are human tissue stem cells

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At a glance

- Genetically engineered mouse models (GEMMs) have been invaluable in advancing our knowledge of tumour biology. However, accelerated cancer gene discovery through large-scale cancer genomics and an increasing desire to use GEMMs for preclinical therapeutic studies have strained the capacity of germline GEMMs.
- Non-germline genetic engineering approaches allow for accelerated and flexible genetic manipulation of models.
- Chimeric models develop tumours in the context of normal stroma, with reduced timelines and mouse housing cost.
- Transplantation models allow flexible and speedy manipulation of tissue stem and/or progenitor cells with multiple genetic tools (such as knock out, transgenes and RNA interference).
- Human donor tissue models (or human in mouse models) allow the *de novo* development of primary human tumours in mouse stroma by manipulating primary human cells.
- Therapeutic studies *in vivo* benefit from the wealth of complex GEMM and non-GEMM models to guide drug discovery.

Box 1**Genetic screens, and gene and drug discovery in models****Types of screens**

Genetic modifier screen

- Straightforward screen of cDNA or short hairpin RNA, small interfering RNA or microRNA libraries for genes that enhance or suppress tumour initiation, growth and/or progression.

Drug screen

- Similar to genetic modifier screens, but using a library of usually unknown, but rationally designed, chemical compounds.

Second site suppressor screen

- Screen that seeks to find genes that, after withdrawal of the initiating oncogenic signal (such as Ras or MYC), can prevent regression of the tumour.

Synthetic lethal screen

- Screen that looks for genetic elements or chemical compounds that singly have no effect on tumour viability, but together induce potent lethality.

Molecular target screen

- Instead of looking for broad tumour phenotypes, the readout of this screen is the modulation of particular molecular targets.

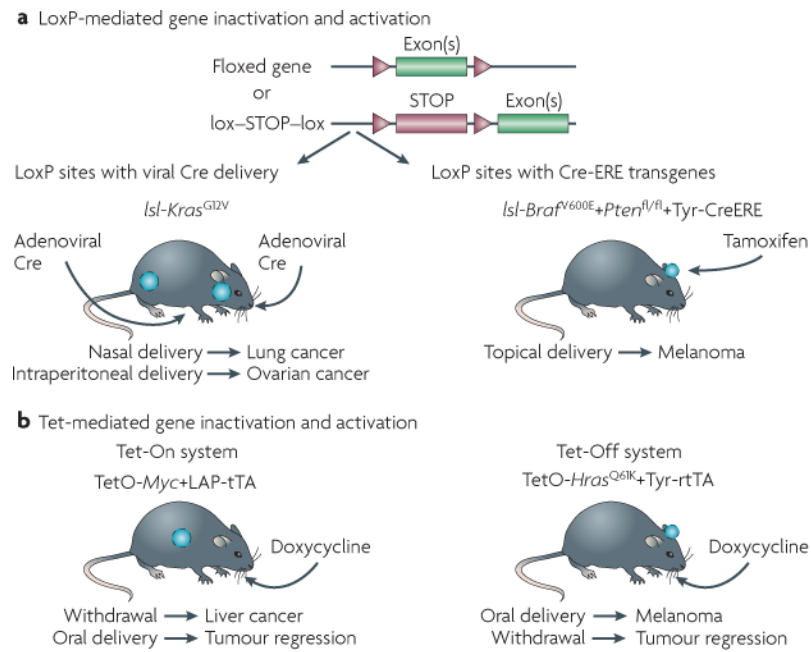


Figure 1. Conditional genetically engineered mouse models (GEMMs)

a | Activating Cre in a tissue-specific manner, by either viral delivery or Cre-oestrogen response element (ERE)-mediated tamoxifen administration. Examples are shown for lung cancer⁷ and ovarian cancer¹², and melanoma¹¹. **b** | Tetracycline (Tet)-mediated transgene inactivation or activation by doxycycline administration or withdrawal using the Tet-On or Tet-Off systems. Examples are shown for liver cancer²² and melanoma⁵. fl, flox; lsl, lox-STOP-lox; rtTA, reverse transactivator; tTA, transactivator.

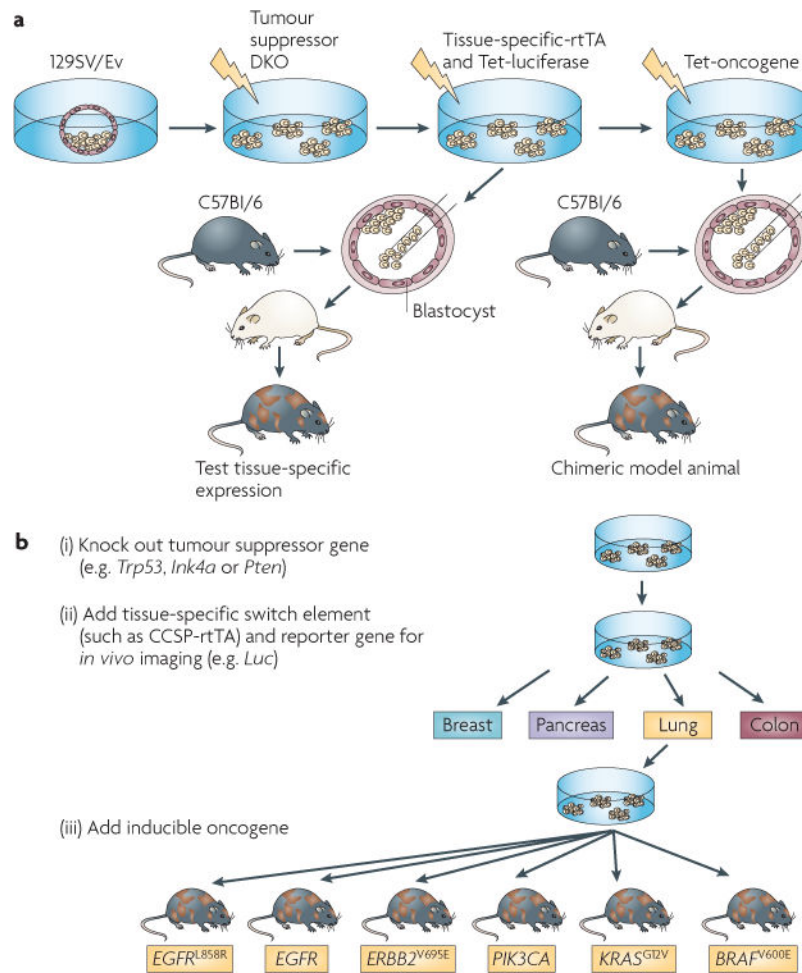


Figure 2. Chimeric model generation

a | Genetically modified embryonic stem cells are transduced *ex vivo* with conditional oncogenic transgenes, injected into blastocysts and implanted into pseudopregnant mice to generate chimeras. **b** | By activating the respective transgenes in a tissue-specific manner, multiple tumour types can be induced in these chimeras in the context of normal stromal tissue derived from the wild-type blastocysts. Image is modified, with permission, from *Nature Biotechnology* REF. ³¹ © (2010) Macmillan Publishers Ltd. All rights reserved. DKO, double knockout; EGFR, epidermal growth factor receptor; Luc, luciferase; rtTA, reverse transactivator; Tet, tetracycline.

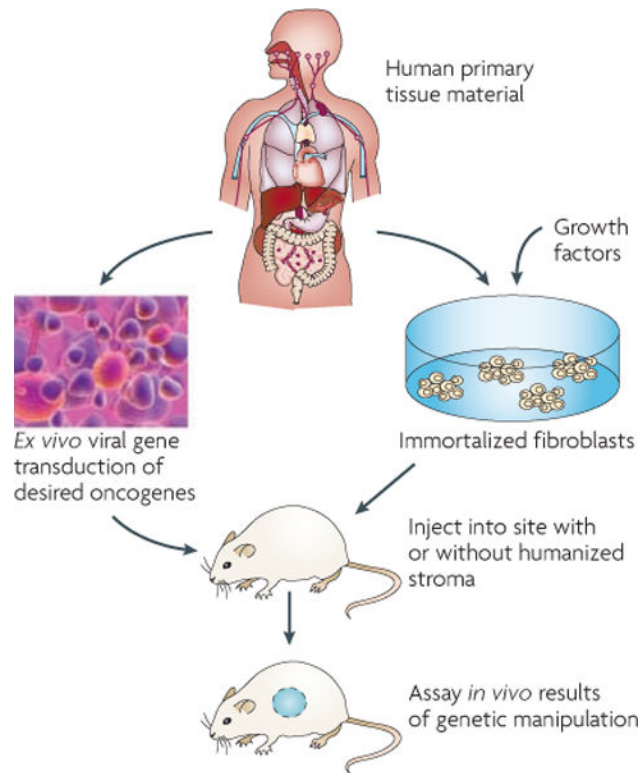


Figure 3. Human in mouse transplantation models

Pluripotent human stem cells are grown *ex vivo* on feeder cells and transduced with the oncogenic elements of interest. Without long culture times, the cells are injected into orthotopic sites on the mice with or without humanized stromal cells. Here, the tumour types of interest will develop from human cells within the context of the relevant mouse or human normal stroma.

Table 1

Comparison of the key features of GEMMs and nGEMMs

Characteristics	Traditional GEMM	Conditional GEMM	Chimeric models	Mouse in mouse	Classic xenograft	Human in mouse
Penetrance*	Model-dependent	Model-dependent	Usually high	Usually high	Usually high	Low
Synchronicity [‡]	Model-dependent, usually poor	Model-dependent, can be high	Model-dependent, can be high	High	High owing to cohosting	Poor
Reproducibility [§]	Model-dependent	Model-dependent, can be high	High	High	Often depends on passaging conditions	Model-dependent
Immunocompetent host ^{//}	Yes	Yes	Yes	Either	No	No
Rapid cohort generation [¶]	Model-dependent, usually no	Model-dependent, usually no	Yes	Yes	Yes	Dependent on human tissue donations
Relevant genetic aberrations	Can be engineered	Can be engineered	Can be engineered	Can be engineered	Confounded by culturing artefacts	Can be engineered
Relevant microenvironment [#]	Yes	Yes	Yes	Yes or partial	Possible by addition of stroma	Possible by addition of stroma
Familial or spontaneous model ^{**}	Familial	Spontaneous	Spontaneous	Spontaneous	Neither	Spontaneous
Genome instability ^{‡‡}	Generally no, except when engineered specifically with mutations that cause genomic instability	Generally no, except when engineered specifically with mutations that cause genomic instability	Likely	Generally no, except when engineered specifically with mutations that cause genomic instability	Yes, but can be confounded with culturing artefacts	Yes

GEMM, genetically engineered mouse model; nGEMM, non-germline GEMM.

* Penetrance is the number of mice developing a relevant tumour.

[‡] Synchronicity is low variance in tumour latency.

[§] Reproducibility is similarity in tumour phenotypes within a cohort.

^{//} Immunocompetent host is whether the host has an intact immune system.

[¶] Rapid cohort generation is the generation of tumour-bearing mice within a short time period.

[#] Relevant microenvironment is the similarity of the surrounding stromal tissue to that in a human tumour.

^{**} Familial or spontaneous model is the type of human cancer being modelled.

^{‡‡} Genome instability is the presence or absence of genomic instability in the tumours.