Mouse models in oncoimmunology

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Abstract | Fundamental cancer research and the development of efficacious antineoplastic treatments both rely on experimental systems in which the relationship between malignant cells and immune cells can be studied. Mouse models of transplantable, carcinogen-induced or genetically engineered malignancies — each with their specific advantages and difficulties — have laid the foundations of oncoimmunology. These models have guided the immunosurveillance theory that postulates that evasion from immune control is an essential feature of cancer, the concept that the long-term effects of conventional cancer treatments mostly rely on the reinstatement of anticancer immune responses and the preclinical development of immunotherapies, including currently approved immune checkpoint blockers. Specific aspects of pharmacological development, as well as attempts to personalize cancer treatments using patient-derived xenografts, require the development of mouse models in which murine genes and cells are replaced with their human equivalents. Such 'humanized' mouse models are being progressively refined to characterize the leukocyte subpopulations that belong to the innate and acquired arms of the immune system as they infiltrate human cancers that are subjected to experimental therapies. We surmise that the ever-advancing refinement of murine preclinical models will accelerate the pace of therapeutic optimization in patients.

Targeted therapies
Treatments that specifically target proteins to cause the inhibition or modulation of molecular pathways that are crucial for tumour growth and maintenance.

Gastrointestinal stromal tumours
(GISTs). A common form of mesenchymal neoplasm of the gastrointestinal tract, usually driven by mutations in the *KIT* gene.

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Throughout the modern history of cancer research, emphasis has been placed on the characterization of malignant cells that had been thought to invade the host as a passive substratum for their expansion[1,2]. Accordingly, anticancer treatments were conceived either to selectively block the growth of cancer cells (the principle of cytotoxic chemotherapy and radiotherapy) or to specifically target oncogenic pathways (the principle of targeted therapies), but were rarely investigated with respect to their effect on the host, apart from their undesired side effects. Seemingly bolstering this cell-autonomous view of cancer biology and cancer treatment, some cocktails of chemotherapies were optimized to become highly efficient in reducing tumour volumes and achieving complete cure from malignancy, as exemplified by the ever-more successful treatment of childhood leukaemia and adult breast carcinoma[2,3]. Moreover, targeted therapies, exemplified by imatinib mesylate, have achieved long-term remissions from malignancies that express imatinib-targeted tyrosine kinases (such as BCR-ABL in chronic myeloid leukaemia and KIT in gastrointestinal stromal tumours (GISTs)), also supporting the cell-autonomous vision of cancer cure[4,5]. Nonetheless, the treatment of many other cancers, such as melanoma and non-small-cell lung cancer (NSCLC), had been a rather frustrating exercise (with poor, if any, cures and not even long-term remissions attributable to chemotherapy and radiotherapy) before the clinical implementation of immune checkpoint blockers (ICBs) that target either cytotoxic T-lymphocyte-associated protein 4 (CTLA4) or the interaction between programmed death 1 (PD1) and its ligand programmed death ligand 1 (PDL1)[6,7]. The recent success of ICB-based immunotherapy constitutes direct clinical proof that cancer can be treated through the modulation of immunity, a result that was first predicted on the basis of mouse models of cancer[8,9]. Furthermore, studies of the immune repertoire of patients receiving immunotherapies have confirmed the results of mouse model studies of immune evasion and immune editing (BOX 1) that were launched well before immunotherapies were used in patients[10,11]. Similarly, accumulating evidence indicates that the long-term success of breast cancer chemotherapy and imatinib-based treatment of GISTs depends on the capacity of the host immune system to keep residual neoplastic cells in check[3,5]. Therefore, even treatments that were thought a priori to merely target cancer cells may in fact induce therapeutically relevant anticancer immune responses. Furthermore, any kind of cancer therapy (not only immunotherapy but also chemotherapy and radiotherapy) that achieves long-term benefits beyond drug discontinuation may do so

Box 1 | **The cancer immunoediting theory**

It is now widely accepted that the immune system can recognize and eradicate primary developing tumours in the absence of therapeutic intervention, a concept that has existed for more than 100 years. More recently, however, it has also become clear that the immune system may, to some extent, facilitate tumour progression by enabling the selection of cancer cells that are inherently resistant to immune eradication. This dual role of anticancer immunity has urged a refinement of the cancer immunosurveillance theory into one that is known as 'cancer immunoediting'. The so-called 'three Es' form the basis of the immunoediting: elimination, whereby pre-malignant lesions are usually eliminated by effector immune cells; equilibrium, whereby small tumours are in equilibrium with an ongoing anticancer immune response that is steadily becoming ineffective; and escape, whereby neoplastic cells can escape from the anticancer immune response to establish progressive lesions. The cancer immunoediting concept, which was first elucidated in and supported by various and increasingly sophisticated mouse models, has been substantiated through several lines of clinical evidence and the unprecedented successes that have been seen with cancer immunotherapy (for detailed reviews on this subject, see REFS 105,106).

Immunotherapy
Therapy that aims to stimulate or enhance a host immune response against a cause of a disease, resulting in long-term control or eradication of the illness.

Histocompatible
The major histocompatibility locus is shared between cancer cells and their hosts.

C57Bl/6 or BALB/c strains
Standard laboratory strains of mice that are widely used in tumour immunology.

Orthotopically
Engrafting cells into the organ from which they originate. For example, hepatocellular cancer cells are orthortopically injected into the liver.

by mobilizing the host immune system — the 'hidden ally' — against malignant cells[12,13] (BOX 2). As preclinical researchers and clinical oncologists examine and confirm this postulate, it has become an obligation to revise the traditional view of oncology and to investigate cancer cells in the context of the tumour microenvironment, which includes multiple cell types from the host immune system[14,15].

Traditionally, cancer drug developers followed a path that consisted of: first, the discovery of small molecules that inhibit the growth of human cancer cells cultured *in vitro*; second, chemical modification of the inhibitor to achieve higher toxicity for cancer cells coupled with improved pharmacokinetic properties; third, confirmation that the drug can reduce cancer growth *in vivo*, generally in immunodeficient mice bearing xenotransplanted human cancers; fourth, conducting toxicological studies in several mammalian species; and last, assessing drug efficacy and safety in clinical trials[16]. This pipeline of drug development has grossly ignored the possibility (and probably the necessity) that the immune system contributes to the efficacy of anticancer treatments[12,13]. On the basis of the ever-accumulating evidence that the dialogue between the tumour and the immune system determines patient fate[14,15], it seems ever more important to investigate cancer and its treatment in realistic mouse models.

Mouse models have revealed that leukocytes have a dual role in oncogenesis and tumour progression: a pro-tumorigenic role in the context of inflammation and an anti-tumorigenic role in the context of immunosurveillance[14]. In this Review, we discuss the appropriate use of immunocompetent mouse models in the area of oncoimmunology, and focus on immunosurveillance mechanisms. We also discuss the historical utility of mice bearing mouse tumours for research purposes and consider the opportunity of 'humanized' mice for the optimal and personalized development of anticancer treatments.

Transplantable mouse tumours

The most commonly used mouse model in oncoimmunology consists of the inoculation of histocompatible cancer cell lines into immunocompetent inbred mice, generally from the C57Bl/6 or BALB/c strains[17]. In most cases, tumour cells are subcutaneously injected into the flank (a procedure that greatly facilitates tumour monitoring by palpation and visual inspection), but occasionally tumour cells are injected either orthotopically — to mimic their evolution in their 'normal' environment — or systemically (intraperitoneally or intravenously) to monitor their metastatic spread. In the case of profound lesions, this monitoring can be carried out in live mice using X-ray micro-computed tomography (micro-CT) or ultrasound imaging, and increasingly by small animal positron emission tomography (PET), bioluminescence and magnetic resonance imaging (MRI) approaches and then confirmed by autopsy. Alternatively, it is possible to transduce transplantable cancer cells with reporters such as green fluorescent protein (GFP) and luciferase (which are also available as a GFP–luciferase fusion protein) to subsequently monitor fluorescence or bioluminescence signals, respectively. Because GFP and luciferase can be immunogenic, novel transgenic tools have been designed such as glowing head (GH) mice, which have been rendered tolerant to GFP and luciferase owing to their localized transgenic expression in the anterior pituitary gland where GFP and luciferase cause immune tolerance rather than autoimmune responses[18]. GFP (or other fluorescent proteins) expressed by cancer cells can also be used to track the transfer of tumour antigens to myeloid cells during the incipient immune response[19].

Transplantable models have multiple advantages, such as cost, simplicity and the close-to-synchronous growth of tumours. However, they have been accused of being poorly 'realistic' for multiple reasons: first, because they arise from the injection of genetically homogeneous cancer cells that have been propagated *in vitro*, many of which will die shortly after injection, hence causing an initial vaccination effect; second, because they lack the features of the focal multi-step carcinogenesis that occurs in natural circumstances; third, because they grow rapidly without the chronic inflammatory environment that characterizes human tumours; and, last, because they generally arise in an

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Immunogenic cell death (ICD). A cell death modality that is preceded by autophagy and that yields the exposure of immunostimulatory danger signals. ICD of tumour cells, for example, following anthracycline treatment, stimulates immune responses through the activation of antigen-presenting cells.

ectopic (mostly subcutaneous) environment[20,21] (TABLE 1). Notwithstanding these limitations, transplantable mouse tumours have led to three major revolutions in therapy-oriented oncoimmunology: the identification of actionable immune checkpoints that can be targeted by suitable ICBs; the discovery that some chemotherapeutic agents are particularly efficient at stimulating immunogenic cell death (ICD) in cancer cells and thus leading to an anticancer immune response that accounts for the long-term effects of therapy; and the fact that ICD inducers and ICBs can be combined in an advantageous manner (FIG. 1).

CTLA4 blockade with a specific monoclonal antibody (mAb) was first described to inhibit tumour growth in BALB/c mice bearing transplantable mouse colon carcinoma 51BLim1O and in A/JCr mice bearing transplantable SalN fibrosarcoma cells[8]. Similarly, mAb-mediated blockade of PD1 was discovered to control the metastatic spread of intraperitoneally injected B16 melanoma cells into the liver of C57Bl/6 mice and that of intravenously injected CT26 colon cancer cells into the lungs of BALB/c mice[9]. PDL1 blockade was first shown to act against J558L myelomas that evolved on BALB/c mice[22]. These examples demonstrate that the pilot experiments that pioneered the preclinical development of ICBs relied on transplantable tumour models, demonstrating the utility of these tumour models. By sheer extrapolation, it seems possible that other ICBs that are currently showing activity in such mouse models of cancer might be approved for clinical use in the future. One method of identifying ICBs in an unbiased manner consists of transfecting libraries of short hairpin RNAs (shRNAs) into tumour antigen-specific T cells that are then adoptively transferred into tumour-bearing mice and subsequently recovered from the cancer infiltrate with the rationale that the cells that have proliferated *in vivo*, in the immunosuppressive microenvironment of the tumour, must express immunostimulatory shRNAs[23] (see FIG. 1b). It can be anticipated that this and similar approaches (which may be adapted to different methods of genome editing, as well as to different leukocyte subtypes) will lead to the identification of potent ICBs.

Box 2 | **The immune system as a 'hidden ally' for cancer therapies**

It is becoming increasingly clear that many of the most successful anticancer therapies mediate their therapeutic effects by either inducing *de novo* or reactivating existing tumour-specific immune responses. Indications of active antitumour immune responses, and certain polymorphisms in genes encoding immune modulators, have been associated with favourable outcomes to many oncotherapies. Conventional chemotherapeutics and targeted anticancer therapies can each enhance the immunogenic properties of malignant cells, or stimulate immune effectors — these effects are achieved either directly or via the inhibition of the immunosuppressive mechanisms established by developing neoplasms that abrogate antitumour immune responses in cancer patients. A notable example is how certain chemotherapeutics (as well as radiotherapy) can stimulate the immunogenic cell death of cancer cells, a form of cell death that exposes immunostimulatory danger-associated molecules (for example, calreticulin, high mobility group protein B1 (HMGB1) and ATP). This cell death modality activates and matures local antigen-presenting cells (notably dendritic cells), which can take up and present tumour antigens to activate or reactivate the adaptive antitumour immune response (for detailed reviews on this subject, see REFS 12,13,107).

Experiments that involved transplantable tumour models revealed that the mechanism of action of several of the currently used anticancer chemotherapeutics relies on their capacity to stimulate anticancer immune responses through the induction of ICD[24,25]. Thus, transplantable CT26 colon carcinomas respond to chemotherapy with anthracyclines or oxaliplatin much more efficiently when they grow in immunocompetent BALB/c mice than when they develop in athymic mice with the *Foxn1*$^{nu/nu}$ genotype, as these mice lack thymus-dependent T lymphocytes[26,27]. The chemotherapy-induced tumour growth reduction of CT26 colon carcinomas or MCA205 fibrosarcoma is also compromised in mice that lack essential immune subsets (such as α/β and γ/δ T cells)[28], important cytokines (for example, interferon-γ (IFNγ) and interleukin-1β (IL-1β) and IL17A)[29,30], and several pathogen recognition receptors (such as Toll-like receptor 4 (TLR4) and formyl peptide receptor 1 (FPR1))[19,31]. When killed *in vitro* with anthracyclines or oxaliplatin, CT26 colon carcinoma cells and MCA205 fibrosarcoma cells act as a vaccine that, upon subcutaneous injection into immunocompetent mice, elicits a potent immune response that precludes the growth of live tumour cells injected after a latency period of 1 week[28,29]. The capacity of these chemotherapeutics to induce ICD has been amply validated in carcinogen- and oncogene-driven mouse cancer models[32–34], as well as in clinical studies[3,19,35].

Recent studies have been focusing on the efficacy of combination regimens that include ICD-stimulating chemotherapeutics (mostly anthracyclines, cyclophosphamide and oxaliplatin) and ICBs. Dosing and scheduling are major issues in the development of such combination treatments, which are also guided by transplantable models such as MCA205 fibrosarcomas that subcutaneously develop in C57BL/6 mice[32]. Clinical trials that are designed to combine ICD inducers and ICBs have been launched[36].

Together, these findings illustrate the past (and probably the future) utility of transplantable mouse models in preclinical research and drug development.

Carcinogen-induced cancers in mice

Several distinct carcinogens can be used to reproducibly induce oncogenesis in mice. Many pioneering studies have been carried out by painting the skin of mice with chemical carcinogens, thereby inducing the formation of local tumours that can be easily monitored. Thus, the local application of ultraviolet (UV) light induces squamous cell carcinomas (or melanomas in some genetically modified mice) and methylcholanthrene (MCA) induces the formation of fibrosarcomas. A combination of the DNA-damaging agent 7,12-dimethylbenz[a]anthracene (DMBA; known as the 'initiator') and the phorbol ester 12-*O*-tetradecanoylphorbol-13-acetate (TPA; known as the 'promoter') causes the formation of papillomas that may evolve into squamous cell carcinomas, even in immunocompetent mice[37]. Breast cancers, which can also be easily monitored, can be induced by a combination of progesterone receptor stimulation (through the implementation of osmotic pumps that release medroxyprogesterone acetate) and DNA damage inflicted by

Table 1 | **Comparisons between distinct non-humanized mouse models in oncoimmunology**

Category	Description	Advantages	Disadvantages	Refs
Transplantable tumours	The most commonly used mouse model in oncoimmunology. Transplantation of histocompatible cancer cell lines into immunocompetent inbred mice. Cells usually injected s.c. into the flank, but occasionally injected either orthotopically to mimic their evolution in their 'normal' environment or systemically (i.p. or i.v.) to monitor their metastatic spread	• Simple procedure and low cost, reproducible, close-to-synchronous and rapid growth of tumours • Simple tumour monitoring by palpation and visual inspection • In the case of profound (for example, metastatic) lesions, monitoring can be carried out at autopsy, X-ray (micro-CT) or ultrasound imaging	Considered 'poorly realistic' for multiple reasons: tumours arise from genetically homogeneous cancer cells grown *in vitro*; the tumour cell lines used derive from immunocompetent mice and have evaded immune pressure or have been immunologically tolerated; tumour development lacks many features of natural carcinogenesis and tumours grow rapidly without chronic inflammatory environment; and tumours generally arise in an ectopic (subcutaneous) environment	17,20, 21
Carcinogen-induced tumours	Induction of 'natural' oncogenesis in mice following application of distinct carcinogens: local application of UV light or MCA to induce formation of fibrosarcomas with a combination of the DNA-damaging agent DMBA and TPA to induce the formation of papillomas that evolve to squamous cell carcinomas	• Induced neoplasias are more 'realistic': genetically diverse and highly heterogenic with respect to onset, progression, histology and antigenic makeup • Offer the possibility of exploring the contribution of the immune system to the therapeutic effects of conventional anticancer drugs	• May require considerable time to establish tumours and to carry out experiments • Difficulties in continuous tumour monitoring, which may require micro-CT or US imaging • High heterogeneity may add to practical difficulties, and may make data interpretation challenging in smaller group sizes • Absence of defined genetic manipulation	21,33, 34,37, 38
GEMMs	Transgenic expression of oncogenes or the inactivation of tumour suppressor genes by genetic recombination. Such mice have yielded important insights into the relationship between cancer and the immune system, which support the immunosurveillance theory	• Accurately reflect human tumours: highly heterogeneous with respect to their onset, progression, histology and antigenic makeup • Yield useful insights into the dialogue between malignant cells and immune effectors	• Long period of time for tumours to progress • All cells (or, in the case of tissue-specific promoters driving transgenes, all cells of a specific cell type) are concurrently affected in their genome, thus carcinogenesis can occur simultaneously in target cells. GEMMs may thus overwhelm the immune system owing to the multiplicity of transformation events coupled with a relatively reduced load of passenger mutations • Possible difficulties in tumour monitoring	10,52, 53,63, 65,66
nGEMMs	Stochastic activation of oncogenes or inactivation of tumour suppressor genes	More realistic than GEMMs because the cancers arise from a few cells	More difficult to monitor than GEMMs because they are more heterogeneous	65,66

DMBA, 7,12-dimethylbenz[a]-anthracene; GEMMs, genetically engineered mouse models; i.p., intraperitoneally; i.v., intravenously; MCA, methylcholanthrene; Micro-CT, micro-computed tomography; nGEMMs, non-genetically engineered mouse models; s.c, subcutaneously; TPA, 12-O-tetradecanoylphorbol-13-acetate; US, ultrasound; UV, ultraviolet.

DMBA, which is administered through biweekly gavage over 2 months[38]. Moreover, protocols have been developed to reproducibly induce colon cancer (usually through a combination of the pro-inflammatory agent dextran sodium sulfate (DSS) and the DNA-damaging agent azoxymethane (AOM)), lung cancer (usually with urethane) and hepatocellular carcinoma (commonly with *N*-nitrosodiethylamine (DEN)), but these models present difficulties in continuous tumour monitoring, which requires micro-CT or ultrasound imaging. In contrast to transplantable cancers, chemically induced neoplasias are genetically diverse and develop in a highly heterogeneous manner with respect to their onset, progression, histology and antigenic makeup, rendering them more 'realistic' but also more difficult to handle in practical terms.

Carcinogen-induced cancers in immunocompetent versus immunodeficient mice. The comparison of carcinogen-induced oncogenesis in normal and immunodeficient mice revealed that the appearance of detectable cancers, as well as their subsequent growth, was often accelerated in conditions in which the cellular immune response was compromised — for example, in mice lacking the IFNγ receptor 1 subunit (*Ifnar1*$^{-/-}$ mice), which senses type 1 interferons, or recombination activating gene 2 (*Rag2*$^{-/-}$ mice; which fail to generate T lymphocytes, B lymphocytes and invariant natural killer T (iNKT) lymphocytes)[10,11] (FIG. 2a). Systematic analyses of distinct immune defects indicate that many of the cell types that are involved in the cellular immune response (such as α/β T, γ/δ T, natural killer (NK) cells and iNKT cells), cytokines (IFNγ, either of the two IL-12 subunits, tumour necrosis factor (TNF)) and cytokine receptors (such as IFNGR1 and the interferon-α/β receptor subunit 1 (IFNAR1)) contribute to immunosurveillance against MCA-induced cancers[10]. However, DSS plus AOM-induced colon cancers develop more quickly in immunocompetent wild-type mice than in mice that lack T cells or cytotoxic T cell function[39,40], a finding that has been linked to the essential contribution of inflammatory processes to tumour progression[41]. By contrast, DSS plus AOM-induced colon carcinogenesis is accelerated on the removal of IL-15 from the genome of immunocompetent mice[42], supporting the view that some immune functions (for example, IL-15-depedent NK cell cytotoxicity) are involved in immunosurveillance in this model.

Natural killer (NK) cells
Cytotoxic cells of the innate immune system that kill target cells in a nonspecific manner (unlike $CD8^+$ T lymphocytes) using molecular cues on the surface to determine that a target cell is not of a healthy status.

'Regressor' tumours
Cancers that, upon their inoculation into histocompatible, immunocompetent mice, first proliferate and then spontaneously disappear.

Cytotoxic T lymphocytes
T lymphocyte immune cells that kill cancer cells or infected cells after specifically recognizing a foreign (that is, viral) or mutated protein presented on class I major histocompatibility complex molecules.

Ploidy
The content of DNA of cells. A normal ploidy (or euploidy) refers to a normal chromosome content, and aneuploid cells have a higher or lower DNA content.

'Progressor' tumours
Tumours that, upon inoculation into mice, grow inexorably, even in hosts that bear a fully competent immune system.

The theory of immunosurveillance has been refined to a large extent on the basis of using UV- and MCA-induced fibrosarcomas, which revealed for the first time the existence of strongly antigenic 'regressor' tumours that do not grow upon transplantation into syngeneic immunocompetent mice, but progress upon transfer into immunodeficient or immunosuppressed mice[43–45] (FIG. 2b). Careful genetic analysis of MCA-induced regressor tumours revealed that they expressed class I major histocompatibility complex (MHC)-restricted neoantigens resulting from mutations in the tumour genome[46]. Such neoantigens can be recognized by cytotoxic T lymphocytes that control tumour growth[46], especially in conditions of immune checkpoint blockade with anti-CTLA4 and/or anti-PD1 antibodies[47]. Escape variants resulting from the selection of regressor tumours by the hostile immune environment can generate tumours that lack expression of the neoantigens and that have thus undergone immunoselection[46]. In addition, MCA-induced regressor tumours that have been induced in severely immunodeficient mice (such as, *Rag2*$^{-/-}$ γc$^{-/-}$ mice, which lack both RAG2 and the γ-chain of the IL-2 receptor and are hence devoid of T cells, B cells, iNKT cells and NK cells) exhibit enhanced ploidy when compared with 'progressor' tumours that are induced in immunocompetent control mice. They also exhibit an endoplasmic reticulum (ER) stress response and increased calreticulin (CALR) exposure on the cell surface. All these features are lost in escape variants of

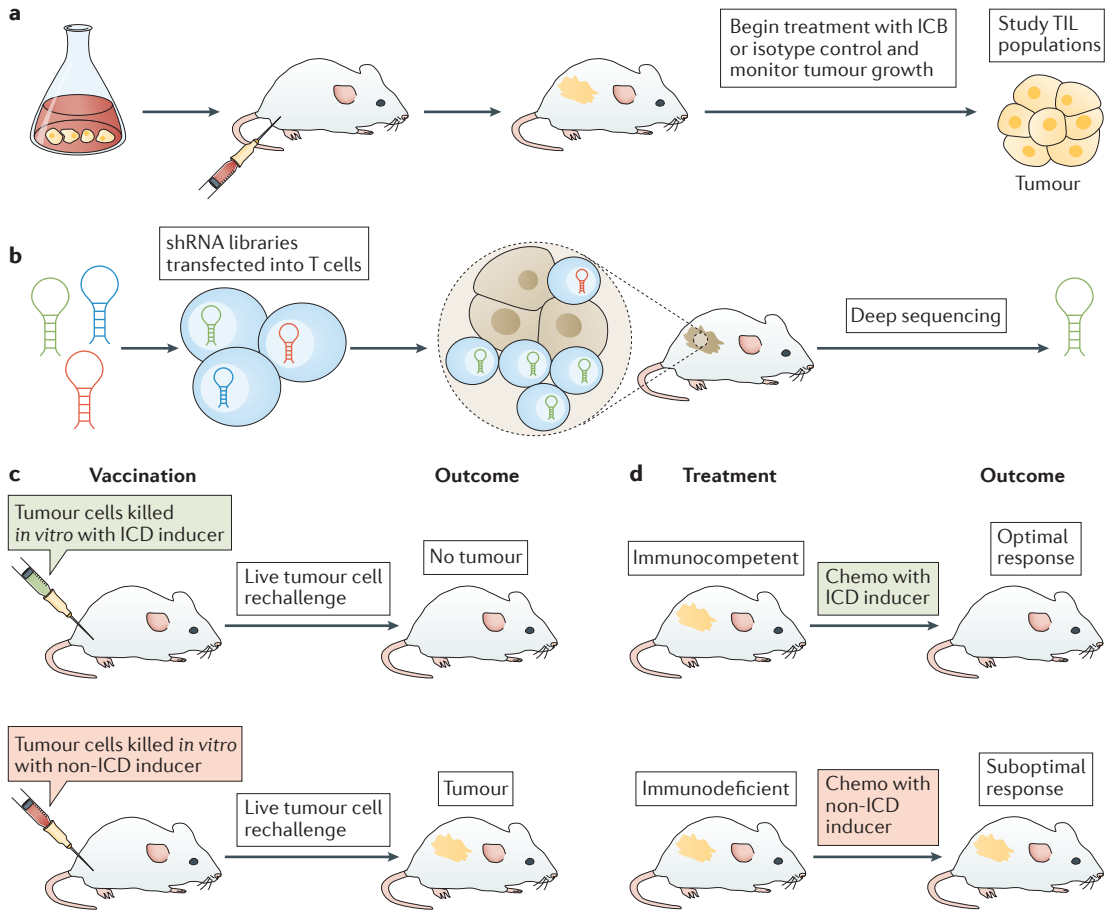

Figure 1 | **Transplantable mouse cancers.** Strategies for developing immune checkpoint blockers (ICBs) (part **a**). In the standard transplantable model, histocompatible cancer cell lines are injected into immunocompetent inbred mice (usually subcutaneously into the flank). After a few days, developing tumours are palpable and experimental immunotherapy with ICBs can commence (for example, every 3 days for 15 days) with regular monitoring of tumour growth. Tumour-infiltrating lymphocyte (TIL) populations may be studied from harvested tumours at the end of the observation period (for example, to investigate treatment-related immune effects). In an innovative approach for discovering new immunotherapy targets (part **b**), T cells infected with short hairpin RNA (shRNA) libraries may be injected into tumour-bearing mice to identify particular shRNAs that enable T cell accumulation in tumours by deep sequencing of the shRNA cassette within purified T cells collected directly from the tumour[23]. Identification of immunogenic cell death (ICD) inducers (parts **c** and **d**). A cancer therapy can be defined as an inducer of ICD if it fulfils two criteria: first, vaccination of the tumour cells killed *in vitro* with the ICD-inducing intervention elicits a protective, tumour antigen-specific immune response in immunocompetent mice in the absence of any adjuvant (part **c**); and, second, chemotherapy with the ICD inducer must exert anticancer effects that are dependent, at least in part, on the immune system (for example, effects that are suboptimal in immunodeficient mice treated in the same manner (part **d**))[24]. Combinations of ICD inducers and ICBs may yield even greater therapeutic responses[32].

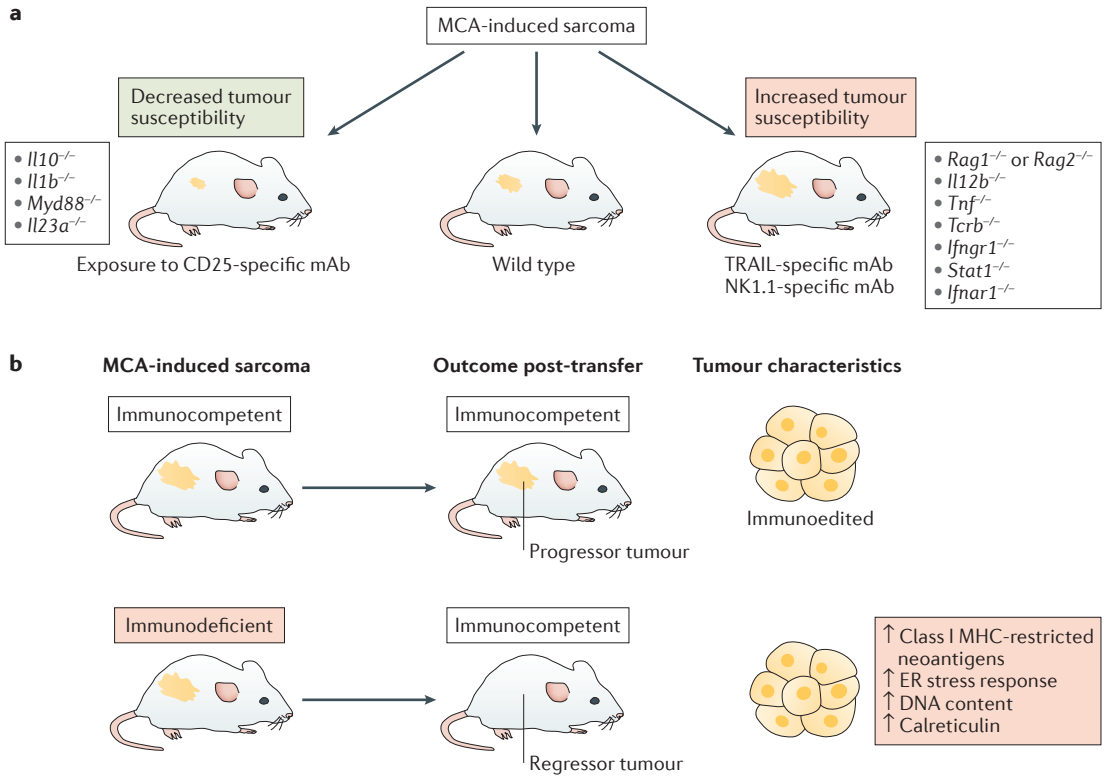

Figure 2 | **Carcinogen-induced mouse models of cancer. a** | The discovery of immunosurveillance by comparing immunocompetent and immunodeficient mice that are exposed to carcinogens. Carcinogen-induced mouse cancer models have been instrumental in defining and supporting the cancer immunoediting theory, as demonstrated by numerous studies comparing methylcholanthrene (MCA)-induced sarcoma in mice that have either genetic deficiencies in or monoclonal antibody (mAb) targeting of immune components with that of wild-type control animals[10,108–110]. **b** | MCA-induced sarcoma from immunocompetent mice can be transplanted into a second immunocompetent host, hence exhibiting a 'progressor' tumour phenotype. By contrast, sarcoma induced in immunodeficient mice (for example, *Rag2*$^{-/-}\gamma c^{-/-}$ mice) usually fails to grow, or regresses shortly after transplantation into immunocompetent recipients, as such tumours have not undergone any immunoselection (owing to the absence of B cells, T cells and natural killer (NK) cells). These findings support the immunoediting theory (BOX 1) [10,48,106]. ER, endoplasmic reticulum; *Ifnar1*, interferon-α/β receptor 1; *Ifngr1*, interferon-γ receptor 1; *Il*, interleukin; MHC, major histocompatibility complex; *Myd88*, myeloid differentiation primary response 88; *Rag*, recombination activating gene; *Stat1*, signal transducer and activator of transcription 1; *Tcrb*, T cell receptor-β; *Tnf*, tumour necrosis factor; TRAIL, TNF-related apoptosis-inducing ligand.

the original regressor tumours that have been passaged in immunocompetent mice[35,48], mimicking the probable effects of immunoselection against enhanced ploidy, ER stress and CALR exposure, which have also been documented in human cancers[35,49].

MCA-induced primary fibrosarcomas have reduced growth in response to anthracycline-based chemotherapies, and this effect is mostly lost upon elimination or neutralization of CD8$^+$ T lymphocytes and essential cytokines such as IFNγ, IL-1β and IL-17A[50]. Primary breast cancers that are induced by medroxyprogesterone and DMBA respond to anthracycline-based chemotherapy, especially in combination with two compounds — cyclophosphamide and hydroxycitrate — that can deplete regulatory T cells (T_{reg} cells)[34]. In this model, tumour growth reduction is lost upon the depletion of CD8$^+$ T lymphocytes or the administration of immunosuppressive cyclosporine H[33,34].

Together, these results underscore the extent to which carcinogen-induced cancer mouse models have been instrumental in delineating the basic principles of the natural capacity of the immune system to control tumour growth and its contribution to the therapeutic effects that are elicited by conventional anticancer drugs.

Regulatory T cells
(T_{reg} cells). Subtypes of CD4$^+$ T lymphocytes that potently suppress immune responses through mechanisms such as the production of immunosuppressive cytokines (for example, interleukin-10). T_{reg} cells are well characterized for their expression of the forkhead box P3 (FOXP3) transcription factor.

Genetically engineered mouse models

The transgenic expression of oncogenes or the inactivation of tumour suppressor genes by genetic recombination yields highly useful mouse models of genetically controlled cancers. Such mice have provided important insights into the relationship between cancer and the immune system when the simultaneous removal of essential immune-related genes (such as those encoding IFNγ, IFNγ receptor and perforin) accelerates the onset of tumour development, supporting the immunosurveillance theory[10]. For example, knockout of the gene coding for perforin in mice (*Pfp*$^{-/-}$ mice) accelerates the development of multiple oncogene-induced cancers, such as lymphomagenesis that is driven by the deletion of *Trp53* (REF. 51), mammary adenocarcinomas driven by the *Erbb2* transgene under the control of the mouse mammary tumour virus (MMTV) promoter, plasmacytomas

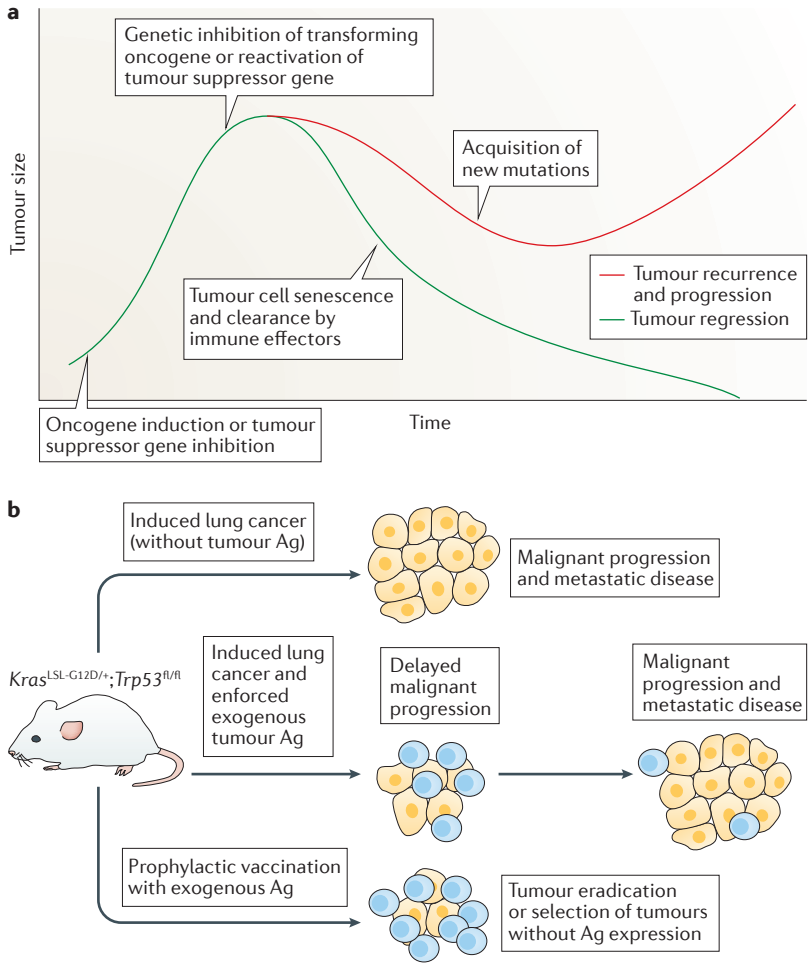

Figure 3 | **Genetically engineered mouse models. a** | The contribution of the immune system to the involution of tumours after the inactivation of oncogenes or the reactivation of tumour suppressor genes. The immune system is required for sustained tumour regression upon the inactivation of oncogenes (for example, *Myc* and *Bcr-Abl*) or the reactivation of tumour suppressor genes (for example, *Trp53*), although tumour cell clones in residual lesions that eventually gain secondary mutations have the potential to generate new immune evasion. **b** | *Kras*-induced lung cancers and immunosurveillance. An instrumental model used to support the immunosurveillance and immunoediting theory has been the $Kras^{LSL\text{-}G12D/+};Trp53^{fl/fl}$ mouse model. In this model, Cre-mediated excision of the *Trp53* gene accelerates lung oncogenesis. Such mice have delayed lung cancers and greater T cell infiltration in which exogenous tumour antigen (Ag) expression is artificially enforced, although the tumours eventually escape immune attack (despite continued Ag expression). Prophylactic vaccination against the autochthonous tumours, however (or transplantation of cell lines derived from such lung tumours), results in either the rapid eradication of tumours, or in a selection of tumours that lose or that lack Ag expression (that is, immunoediting)[66]. The influential T cell responses in this mouse model can be improved by combination therapy of immune checkpoint blockers (ICBs) and immunogenic cell death (ICD) inducers[32].

driven by the *v-Abl* oncogene under the control of the Eμ-associated enhancer from the IgH locus, and follicular lymphomas driven by a *Bcl2* transgene expressed under the control of the *Vav* promoter[10]. These examples illustrate that both solid and haematopoietic cancers are under immunosurveillance. MMTV-*Erbb2* transgenic mice have revealed the possibility of inducing a therapeutic immune response against the aetiological oncogene *Erbb2*, especially if it is recognized by CD4$^+$ T helper type 1 (T_H1) or CD8$^+$ T cells[52,53]. Intriguingly, in patients with *ERBB2*-overexpressing breast cancers, the therapeutic efficacy of the anti-*ERBB2* antibody trastuzumab correlates with the induction of a specific cellular immune response[52], suggesting (although not proving) that mouse and human breast cancers that overexpress *Erbb2* and *ERBB2*, respectively, might be recognized by T cells in a similar way.

Several studies have revealed that even fully transformed, malignant cells can be addicted to oncogenes (or the inactivation of tumour suppressor genes), meaning that genetic inhibition of the transforming oncogene (or reactivation of the tumour suppressor gene) can lead to significant tumour regression[54]. Importantly, such effects may require the contribution of the immunosurveillance system (FIG. 3a). For example, the reactivation of p53 (through the repression of a vector coding for an shRNA that initially reduced p53 expression) in hepatocellular carcinomas causes the senescence of malignant cells that are cleared by innate immune effectors, including macrophages and neutrophil granulocytes[55]. Inactivation of the *Myc* or *Bcr-Abl* oncogenes in mouse models of T cell acute lymphoblastic lymphoma and pro-B cell leukaemia, respectively, results in tumour regression in a CD4$^+$ T lymphocyte-dependent manner, which is required for the induction of tumour cell senescence, as well as for the inhibition of angiogenesis[56]. Indeed, *Myc* binds to the promoters of two immune checkpoint blockers (CD47 and PDL1), meaning that its reduction is compatible with the induction of an anticancer immune response[57]. These examples illustrate the contribution of the immune system to tumour clearance even in conditions in which therapeutic measures are targeted in a purely cell-autonomous manner. Another aspect of tumour immunology that is reflected in oncogene-induced mouse cancers is that the malignant lesion is able to elicit and then locally inactivate tumour antigen-specific T cell responses[58].

Beyond genetically engineered mouse models (GEMMs) in which the germ line of mice is genetically manipulated, so-called non-genetically engineered mouse models (nGEMMs) are also being developed[59]. GEMMs are characterized by the fact that all cells in the organism (or, in the case of tissue-specific promoters driving transgenes, all cells of a specific cell type) are concurrently affected in their genome, meaning that, for example, *Erbb2*-induced carcinogenesis occurs simultaneously in all mammary epithelial cells, resulting in the close-to-synchronous development of multiple neoplastic lesions within a few months after birth[60]. Thus, in contrast to spontaneous or carcinogen-induced tumorigenesis (in which cancers develop one-by-one), GEMMs may overwhelm the immune system owing to the multiplicity of transformation events coupled with a relatively reduced load of passenger mutations (compared with carcinogen-induced oncogenesis). Possibly for this reason, there is no measurable impact of the immune system on the response of *Erbb2*-induced breast cancer to chemotherapy. Thus, breast cancers that are triggered by the MMTV-*Erbb2* transgene reportedly respond to high-dose chemotherapy even in the context of a *Rag2* knockout model, suggesting that adaptive immunity is not

CD4$^+$ T helper type 1 (T_H1). The production of cytokines such as interferon-γ by CD4$^+$ T helper lymphocytes can exert immunostimulatory functions that can direct immune tumour control.

required for such responses[61]. However, this preclinical finding collides with the clinical observation that T lymphocyte infiltration of ERBB2-overexpressing breast cancer predicts favourable responses to chemotherapy plus trastuzumab treatment[62].

To study the immunosurveillance of breast cancer, a GEMM that is based on the use of the mammary tumour virus-polyoma middle T (MMTV-PyMT) transgenic cassette has been modified to include two self-cleaving P2A sequences (downstream of the PyMT cDNA) to produce the mCherry and ovalbumin (OVA) proteins. OVA contains immunogenic T cell epitopes and hence constitutes a potent tumour antigen[63]. In this model, $CD103^+$ dendritic cells constitutively ingest cancer cell-derived proteins and cross-present them to cytotoxic T lymphocytes[64], illustrating a common hierarchy in anticancer immune responses that involves the recognition of tumour cells by innate immune effectors of myeloid origin, followed by the involvement of T cells.

One widely used nGEMM consists of the induction of the oncogenic *Kras*^{G12D} allele, which is provided in the form of a *Lox-Stop-Lox-Kras*^{G12D} transgene, by means of the intrapulmonary instillation of a recombinant adenovirus that produces the Cre recombinase (AdCre). This manipulation, which involves the action of an external agent (in contrast to GEMM models) causes the excision of the Lox-Stop-Lox cassette from type II pneumocytes and their consequent transformation into malignant adenocarcinomas via a stepwise process (through epithelial hyperplasia and benign adenomas)[65]. Although no evidence exists that the Cre recombinase is itself immunogenic, it remains to be explored whether the introduction of such proteins might affect the immunological properties of the system. Cre-recombinase-triggered, *Kras*^{G12D}-induced lung cancers have been thought to completely escape immunosurveillance, and attempts have been made to artificially introduce tumour antigens into such models (FIG. 3b). Thus, *Kras*$^{LSL-G12D/+}$;*Trp53*$^{fl/fl}$ mice (in which Cre-mediated excision of the *Trp53* gene further accelerates lung oncogenesis) develop lung cancers more slowly if they receive a lentivirus that not only expresses Cre but that also artificially introduces novel tumour antigens into pneumocytes[66]. This antigen-induced delay in tumour growth correlates with an enhanced infiltration by B and T lymphocytes and disappears in a *Rag2*$^{-/-}$ background, but is increased upon prophylactic immunization with the tumour antigen[66]. *Kras*^{G12D}-induced p53-negative lung cancers have reduced growth in response to chemotherapy with ICD inducers (oxaliplatin combined with cyclophosphamide) in a T cell-dependent manner, and this therapeutic response can be further improved by simultaneous immune checkpoint blockade (using CTLA4-specific and PD1-specific antibodies)[32]. Therefore, this model of lung cancer oncogenesis may constitute a useful preclinical model. That said, it seems paradoxical that strong anticancer immune responses can be obtained in such a mouse model (in which the mutational load is presumably low), whereas the response of human tumours to immune checkpoint blockade has been related to the frequency of somatic mutation leading to the generation of neoantigens[67]. Further studies are required to determine the relative importance of such mutations across distinct types of neoplasia.

Interestingly, there is a strong tissue-dependent effect of the anticancer immune response. Thus, sarcomas of the hind limb that are induced by the local activation of Cre in *Kras*$^{LSL-G12D/+}$;*Trp53*$^{fl/fl}$ mice with a lentivirus introducing tumour antigens induce a strong anticancer immune response that either blocks tumour formation or forces the loss of the tumour antigen by immunoselection[68]. However, if the same tumorigenic pathway is activated in the lung to cause the formation of adenocarcinomas, no major antigen loss variants develop[66]. This may be explained by the local accumulation of immunosuppressive T_{reg} cells within tumour-associated tertiary lymphoid structures[58]. Tertiary lymphoid structures, which also exist in human NSCLC[69], constitute potential sites for the cross-presentation of tumour antigens by dendritic cells that only become active upon T_{reg} cell depletion[58]. Additional evidence for the local effect of T_{reg} cells in lung cancer has been obtained by modulating autophagy. *Kras*$^{LSL-G12D/+}$;*Atg5*$^{fl/fl}$ mice (in which Cre-mediated excision of the two *Atg5* alleles causes an autophagy defect) develop adenomas more quickly upon AdCre instillation than do *Kras*$^{LSL-G12D/+}$;*Atg5*$^{fl/+}$ control mice (in which Cre only inactivates one copy of the *Atg5* gene), correlating with an enhanced accumulation of T_{reg} cells. Pharmacological induction of autophagy with hydroxycitrate, which depletes T_{reg} cells[34], also delays tumorigenesis in autophagy-sufficient *Kras*$^{LSL-G12D/+}$;*Atg5*$^{fl/+}$mice, but fails to do so in *Kras*$^{LSL-G12D/+}$;*Atg5*$^{fl/fl}$ mice that develop autophagy-incompetent tumours[34,70].

Humanized mice

In spite of the undeniable scientific utility of transplantable, carcinogen-induced and genetically controlled mouse models, specific applications in pharmacological and clinical development have rendered necessary the introduction of human genes and cells into mice, resulting in their 'humanization' (TABLE 2). Although the humanization of mice may be expected to denature the normal function of their immune system, such a strategy is important in two distinct contexts. First, the development of pharmacological agents may need to target human proteins that differ from their endogenous mouse equivalents in their structural or functional characteristics. This applies in particular to vaccination strategies, for which several humanization strategies may be required: for example, the introduction of human tumour cells (such as, for studies of dendritic cell vaccines), a human MHC (as a transgene replacing the endogenous mouse MHC), a human inhibitory receptor for NK cells (for studies probing NK cell biology) and human T lymphocytes into mice that have been rendered immunodeficient (to be able to support the growth of human cancer and immune cells) and that have been manipulated to express human growth factors (to support the survival and expansion of a diverse array of human leukocyte subpopulations). This use of mice expressing human growth factors is a refined degree of humanization that might also be taken advantage of to

Non-genetically engineered mouse models
(nGEMMs). Genetically controllable mouse models in which oncogene activation or inactivation of tumour suppressors is achieved through stochastic effects.

Dendritic cell vaccines
A process in which dendritic cells are removed from a patient, loaded with tumour material or tumour antigens, matured and then re-infused back into the patient to stimulate T cell responses *in vivo*.

Table 2 | **Strategies to humanize mouse models for oncoimmunology**

Category	Examples	Objective	Caveats	Refs
Humanization of genes or loci	• Knock-in mutations replacing mouse proteins and receptors with the equivalent human version (for example, CTLA4 and CD1), yielding mice responsive to clinical antibodies or drugs • TRAMP in which local expression and shedding of soluble human NKG2D ligand MICB mediates the depletion of peripheral NK cells owing to the replacement of the Fc receptor locus • Replacement of mouse MHC class I with human equivalent (often HLA A*02:01), as well as the mouse α/β TCR loci by their human equivalents	Development of pharmacological agents and vaccination strategies against human proteins, study of Fc-related immunostimulatory mode of action of a range of ICB-related monoclonal antibody isotopes	Model remains on a mouse background, so there is the potential for misleading observations owing to poorly compatible downstream or compensatory circuitries. Careful interpretation is required	71–74, 76
Induction of immunodeficiency	• NSG mice engrafted with a variety of sources of human leukocytes • NSG mice expressing HLA alleles, allowing for the functional assessment of $CD8^+$ T cells expressing tumour antigen-specific TCR • Also, mouse MHC class II has been replaced by HLA-DR1 to study the function of human $CD4^+$ T cells *in vivo*	These major immunodeficiencies allow mice to tolerate transplantation with human cells and tumours for the study of therapeutics and antigens	• Engrafting NSG mice with human leukocytes requires careful monitoring (for example, peripheral blood leukocytes cause GVHD) • Purified human $CD34^+$ HSCs do not yield mature T cells owing to the absence of a human thymus • Potential complications in sourcing or deriving patient cells	84–88
Transplantation of human immune cells and their trophic support	• *Rag2*$^{-/-}$γc$^{-/-}$ mice engrafted with human B cell lymphoma, EBV-infected B cell lines or PBMCs that show the effect of human Vγ9Vδ2 T cells • Introduction of human HLA-A*0201$^+$ fetal thymus plus $CD34^+$ fetal liver cells transduced with a lentiviral vector encoding an HLA-A*0201-restricted TCR specific for the melanoma antigen MART1 • Transplantation of MSCs into mice to support human haematopoiesis *in vivo* • Replacement of endogenous mouse cytokines by their human equivalents in *Rag2*$^{-/-}$γc$^{-/-}$ mice that bind to corresponding receptors on human leukocytes (for example, knock in of human M-CSF, IL-3, thrombopoietin and GM-CSF in their respective mouse loci to generate MITRG mice)	• Allows the study of human T cell populations that are absent in the mouse • Allows the generation of mice that express transgenic TCRs on human T cells for tumour antigen vaccination studies • Further optimizes the generation, survival and expansion of human innate effectors	Possible development of severe GVHD: time window for characterization is restricted to a few weeks	80–83, 89–91
Transplantation of cancers	• NSG mice transplanted with PSCA-expressing human pancreatic cancers and PSCA-specific T cells engineered by CAR technology • NSG mice reconstituted with PBMCs and tumours from the same patient ('immuno-avatar' mice) • NSG mice transplanted with primary PDXs, in which the human stroma (including the immune cells) has not yet been replaced by mouse cells • NSG mice first xenotransplanted with human $CD34^+$ haematopoietic stem cells after sublethal irradiation (100–120 Gy) that then receive PDX (meaning that the tumour is allogeneic with respect to the leukocytes previously introduced)	• Demonstration that CAR T cells can mediate anticancer immune responses *in vivo* • Characterization of aspects of the human TME, or of ICB-targeted antibodies that activate human lymphocytes (but that do not crossreact with mouse targets) *in vivo*, or immune-mediated effects of antibodies targeting human cancer cell antigens • Studies of antigenicity (for example, cell type-specific antigens)	• Possible development of severe GVHD: time window for characterization is restricted to a few weeks • Paucity of patient-derived material only allows for the characterization of interactions between tumour cells and pre-existing immune infiltrate, and provides no insights into the chemotactic recruitment of leukocytes by the TME	82,92–96, 99

CAR, chimeric antigen receptor; CTLA4, cytotoxic T-lymphocyte-associated protein 4; EBV, Epstein–Barr virus; GM-CSF, granulocyte–macrophage colony-stimulating factor; GVHD, graft-versus-host disease; HLA, human leukocyte antigen; HSCs, haematopoietic stem cells; ICB, immune checkpoint blocker; IL, interleukin; MART, melanoma antigen recognized by T cells 1; M-CSF, macrophage colony-stimulating factor; MHC, major histocompatibility complex; MICB, MHC class I polypeptide-related sequence B; MSCs, mesenchymal stromal cells; NK, natural killer; PBMCs, peripheral blood mononuclear cells; PDXs, patient-derived xenografts; PSCA, prostate stem cell antigen; TCR, T cell receptor; TME, tumour microenvironment; TRAMP, transgenic adenocarcinoma of the mouse prostate.

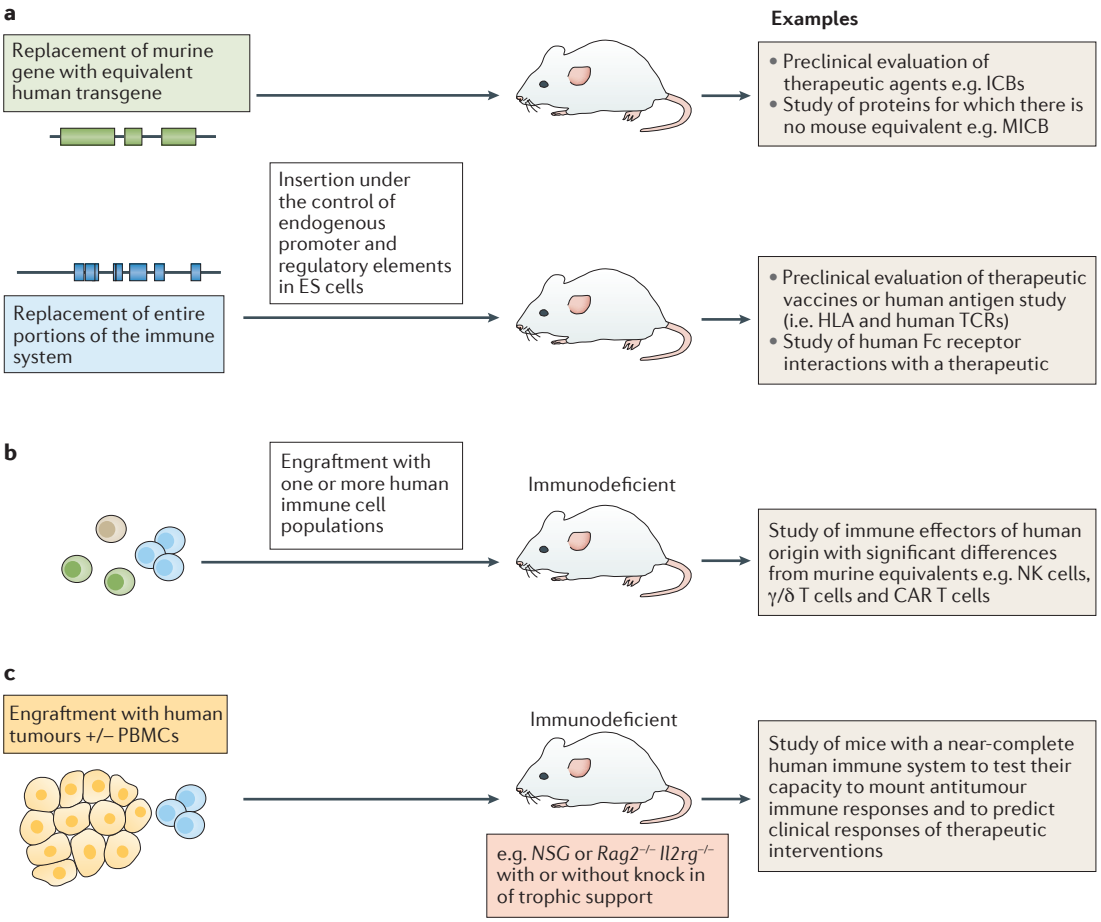

Figure 4 | **Humanized mouse models. a** | Genetic humanization of individual targets or of entire portions of the immune system. To overcome the considerable differences between some of the biological machineries in mice and humans, knock-in mice can be created by replacing a mouse immune gene with the human equivalent. Moreover, mouse immune genetic portions can be deleted and human equivalents (often encoded as transgenes) may be inserted into the mouse genome, resulting in the recapitulation of the unique profile of that human immune portion. **b** | Humanization of individual immune effectors. The specificity and function of several human immune effectors is different from that of rodents (for example, natural killer (NK) cells, as well as certain subsets of γ/δ T cells responding to lipid antigens). Immunodeficient mice (for example, *Rag2*$^{-/-}$*Il2rg*$^{-/-}$ and NSG mice) may be engrafted with such human immune cell populations to study their effects in human cancer models. **c** | Humanization for xenografts. Only mice bearing a major immunodeficiency can tolerate transplantation with human cells (for example, NSG mice). These mouse strains can be further optimized to support human innate immune effectors through the replacement of endogenous mouse cytokines with the human equivalents (for example, interleukin-3 (IL-3), granulocyte–macrophage colony-stimulating factor (GM-CSF) and thrombopoietin). Immunodeficient mice may be reconstituted with peripheral blood mononuclear cells (PBMCs) and tumours, ideally from the same patient, to predict clinical responses to novel therapeutic interventions. CAR, chimeric antigen receptor; ES, embryonic stem; HLA, human leukocyte antigen; ICB, immune checkpoint blocker; MICB, MHC class I polypeptide-related sequence B; TCR, T cell receptor.

transplant mice with patient-derived xenografts (PDXs) to study anticancer immune responses in a 'personalized' manner.

Humanization of individual targets. The preclinical evaluation of therapeutic agents that target human molecules but that fail to interact with the mouse orthologues may require the replacement of the non-crossreactive mouse gene with its human equivalent (FIG. 4a). One prominent example is provided by a knock-in mutation that is designed to replace mouse CTLA4 with human CTLA4, yielding a mouse that responds to treatment with a human CTLA4-specific antibody by developing an autoimmune syndrome[71]. Another example is provided by the knock in of human antigen-presenting glycoprotein CD1D, generating mice that allow more accurate *in vivo* modelling of human iNKT cell responses for future iNKT cell-targeted antitumour therapies[72]. Similarly, it is possible to introduce human genes for which no mouse equivalent is known into tumour models to explore the potential *in vivo* effects of these genes in tumour models. One example is provided by a model of the transgenic adenocarcinoma of the mouse prostate (TRAMP) in which the local

Xenografts
(Also known as xenotransplants). Living cells, tissues or organs that are transplanted from one species to another (such as human haematopoietic cells or tumours to mouse).

Licensing of NK cells
A process in which natural killer (NK) cells are rendered functionally competent to kill target cells.

CRISPR–Cas9
A defence mechanism against foreign genetic elements (for example, plasmids and phages) found in prokaryotes, involving clustered regularly-interspaced short palindromic repeats (CRISPR; that is, segments of prokaryotic DNA containing short repetitions of base sequences) and the DNA nuclease Cas9. It has huge potential applications in the targeted genome editing of humans, animals and other organisms.

Chimeric antigen receptor (CAR) technology
Engineered expression of CARs on the surface of effector T cells to enable the redirection of T cell specificity. T cells removed from a patient may be modified to express CARs that are specific for the particular form of cancer and then adoptively transferred back to the patient to treat the cancer.

expression of human NKG2D ligand MHC class I polypeptide-related sequence B (MICB) mediates the depletion of peripheral NK cells owing to the shedding of soluble MICB into the circulation[73].

Genetic humanization of the murine immune system. For specific applications, it may be interesting to exchange entire parts of the mouse genome with the scope of humanizing them and hence replacing them with their human equivalents. For example, it is possible to replace the locus that encodes Fc receptors (which recognize the common fragments of antibodies that belong to distinct immunoglobulin classes and a non-species-crossreactive) if the mode of action of the mAb bearing the human Fc portion is investigated *in vivo*[74]. This seems particularly important because the immunostimulatory mechanism of action of a range of ICB-related mAbs seems to be related to their isotype (and hence their interaction with Fc receptor)[75]. Similarly, the mouse MHC class I can be replaced with its human equivalent (often the highly prevalent human leukocyte antigen (HLA) class I histocompatibility antigen, A-2 alpha chain (HLA-A*02:01; the class I MHC allele that is expressed in 30–40% of Caucasians in North America and Europe), and the mouse α/β T cell receptor (TCR) loci can be replaced with their human equivalents. Such mice can be efficiently immunized with tumour-associated antigens from human cancers and can elicit highly specific T cell responses because they are not tolerant to such human antigens[76]. Importantly, licensing of NK cells by autologous MHC class I molecules means that the generation of transgenic mice that express a single inhibitory killer-cell immunoglobulin-like receptor (KIR) in the presence of its ligand (HLA) and in the absence of endogenous mouse MHC class I molecules has been instrumental in demonstrating the concept of NK cell education and the efficacy of anti-KIR mAbs (alone or combined with ADCC-mediating mAbs or immunomodulatory drugs) against tumours[77–79].

It can be anticipated that novel genome-editing technologies (and in particular the CRISPR–Cas9 method) will facilitate the genetic humanization of multiple immune-relevant genetic loci from mice, hence rendering such rodents ever more 'human'.

Humanization of individual immune effectors. The specificities and functions of some immune effectors of human origin are quite different from those of rodent origin. This applies to NK cells, as well as to subsets of γ/δ T cells that respond to lipid antigens. *Rag2*$^{-/-}$*γc*$^{-/-}$ mice (which lack T lymphocytes, B lymphocytes and NK lymphocytes) have been engrafted with human B cell lymphoma cell lines to show that human Vγ9Vδ2 T cells can control their growth[80] (FIG. 4b). *Rag2*$^{-/-}$*γc*$^{-/-}$ mice have also been reconstituted with human peripheral blood mononuclear cells (PBMCs) — all types or minus Vγ9Vδ2 T cells — and then with Epstein–Barr virus (EBV)-infected B cell lines to show that the resulting EBV-induced lymphoproliferative disease can be inhibited by injections of the aminobisphosphonate palmidronate in a Vγ9Vδ2 T cell-dependent manner[81]. In a similar way, immunodeficient NSG mice (also known as NOD-Cg-Prkdc^{scid}Il2rg^{tm1Wjl} and NOD-SCID Il2rg^{null} mice; see below) have been transplanted with a combination of human pancreatic cancers cells that express prostate stem cell antigen (PSCA) and PSCA-specific T cells engineered by chimeric antigen receptor (CAR) technology to demonstrate that such T cells can mediate anticancer immune responses *in vivo*[82]. The introduction of human HLA-A*0201^{+} fetal thymus in combination with CD34^{+} fetal liver cells transduced with a lentiviral vector encoding an HLA-A*0201-restricted TCR that is specific for melanoma antigen recognized by T cells 1 (MART1) enabled the generation of mice that express the transgenic TCR on human T cells that respond to vaccination with the MART1-derived peptide and that can control MART1-expressing melanomas *in vivo*[83].

Humanization for xenograft. The supreme (and perhaps utopian) goal of humanization is to generate mice with a fully competent human immune system that can be characterized for its capacity to mount anticancer immune responses, hence enabling the deduction of predictions of the clinical response to therapeutic interventions. To achieve this objective, it is necessary to introduce malignant and immune cells, ideally from the same donor, into mice while creating an environment that guarantees full compatibility between the graft and the host. This means that there should be no rejection of human cells by mouse immune effectors (and no toxic effects of human immune cells on the host) and the unrestricted engraftment of human leukocyte precursors, which must receive full trophic support within the host (FIG. 4c).

Only mice with a major immunodeficiency can tolerate transplantation with human cells. For this purpose, the most widely used NSG strain carries three genetic defects: first, the non-obese-diabetic (NOD) background that is characterized by a haemolytic complement deficiency (to avoid the lysis of human cells by mouse complement) and a unique loss-of-function allele of *Sirpa*, reducing the phagocytosis of human CD47^{+} cells by mouse macrophages; second, the severe combined immunodeficiency (SCID) phenotype that is due to mutations in the *Prkdc* (protein kinase, DNA activated, catalytic polypeptide) gene causing a lack of T lymphocytes and B lymphocytes; and third, the mutation in the IL-2 receptor common γ-chain (*Il2rg*), which is common to the receptor complexes of at least six different interleukin receptors, including IL-15, entailing a profound NK cell deficiency[84,85]. NSG mice can be engrafted with various types of human leukocytes, such as human peripheral blood leukocytes (which, however, cause xenogeneic graft-versus-host disease (GVHD)), purified human CD34^{+} haematopoietic stem cells (HSCs) obtained from bone marrow, umbilical cord blood or fetal liver (which, however, do not yield mature T cells owing to the absence of a human thymus), and simultaneous implantation of bone marrow, fetal liver and thymus from the same donor under the kidney capsule to facilitate the clonal selection of human T cells[86]. NSG mice have been rendered transgenic for human HLA alleles, mostly HLA-A2, thus allowing the functional assessment of CD8^{+} T cells that

Organoids
In vitro cultured three-dimensional organ buds that show a realistic microanatomy. Such culture systems may be used to create cellular models of human disease.

have been genetically engineered to express tumour antigen-specific TCRs[87]. Similarly, the mouse MHC class II has been replaced with HLA-DR1 to study the function of $CD4^+$ T cells *in vivo*[88].

To support human haematopoiesis, mesenchymal stromal cells (MSCs) may need to be additionally introduced into mice[89]. To further optimize the generation, survival and expansion of human innate effectors, mice have been subjected to the replacement of endogenous mouse cytokines with their human equivalents, which bind to their corresponding receptors on human leukocytes. Thus, immunodeficient $Rag2^{-/-}\gamma c^{-/-}$ mice have been manipulated to knock in the genes encoding human macrophage colony-stimulating factor (M-CSF), IL-3, thrombopoietin and granulocyte–macrophage colony-stimulating factor (GM-CSF) in their respective mouse loci to generate MITRG mice[90]. Such mice have been further manipulated to introduce a transgene encoding human *SIRPA* (to reduce the phagocytic removal of human $CD47^+$ cells by mouse macrophages)[91] and hence to generate MISTRG mice[90]. MISTRG mice support human myelogenesis, and human myeloid cells that infiltrate a human melanoma transplanted into such mice exhibit an immunosuppressive M2 phenotype[90].

In the simplest version of humanization, immunodeficient NSG mice are reconstituted with PBMCs and tumours, ideally from the same patient. Such 'immuno-avatar' mice (which eventually develop severe GVHD, meaning that the time window for characterizing them is restricted to a few weeks) have allowed the identification and characterization of ICB-targeted antibodies that activate human lymphocytes (but that do not crossreact with mouse targets) *in vivo*[92,93]. Such a model can also be used to reveal the immune-mediated effects of antibodies that target cancer cell antigens and that result in the infiltration of patient-derived tumours by lymphocytes[94]. Another relatively simple strategy consists of transplanting NSG mice with primary PDX, in which the human stroma (including the immune cells) has not yet been replaced by mouse cells. Such 'immuno-PDX' allowed the characterization of IL-12-mediated anticancer effects in models of primary NSCLC[95] and the characterization of the immunosuppressive microenvironment of patient-derived ovarian cancer that was orthotopically transplanted into the mouse omentum[96]. The major inconvenience of immuno-PDX models is the paucity of the patient-derived material, as well as the fact that they only allow the characterization of the interaction between tumour cells and the pre-existing immune infiltrate, and provide no insights into the chemotactic recruitment of leukocytes by the tumour microenvironment.

A widely used humanized model in oncoimmunology takes advantage of NSG mice that are first xenotransplanted with human $CD34^+$ haematopoietic stem cells after sublethal irradiation (100–120 Gy) and that later receive PDXs (meaning that the tumour is allogeneic with respect to the leukocytes previously introduced). Such models have yielded interesting insights into the mode of action of imiquimod, which is a synthetic Toll-like receptor (TLR7/8) ligand that can be topically applied to the mouse skin and that stimulates the infiltration of melanomas by human plasmacytoid dendritic cells that may have anticancer properties[97]. Nonetheless, this interpretation is at odds with observations in patients and in NOD-SCID $ß2m^{-/-}$ mice reconstituted with $CD34^+$ HPCs and melanoma cells, which suggest that plasmacytoid dendritic cells are actually supporting melanoma growth[98]. Alternatively, NSG mice reconstituted with human fetal liver and/or thymus and autologous $CD34^+$ fetal liver cells have been used to show that different cell types (such as smooth muscle cells versus retinal pigment epithelial cells) that are generated from human induced pluripotent stem cells (hiPSCs) differ in their antigenicity *in vivo*[99]. These studies have led to the identification of immunologically relevant, cell type-specific antigens, suggesting that the reconstitution of the human immune system is sufficiently accurate to yield physiologically relevant information on potential autoimmune reactions against human cells.

Alternatives to mouse models

To respect the current ethical standards regarding animal experimentation (which recommend the 'three Rs': replacement, reduction and refinement), as well as for practical reasons (the reduction of costs and timelines), novel techniques are being developed to study the interaction of cancers and the immune system *in vitro*. One possibility is to develop ever more refined culture technologies in which three-dimensional stem cell-derived organoids from normal versus malignant origin are confronted with multiple haploidentical leukocyte subpopulations to study the interaction among cell types. The development of libraries of organoids and mammospheres from stem cell precursors of human tumours with inherited or somatic genetic defects (such as mutations in *BRCA1*, *BRCA2* and *TP53*), for example, could facilitate the re-creation of the host–tumour interaction. Moreover, several technologies may provide insights into a patient-centred 'personalized' view of the interactions between malignant and human cells.

The 'in sitro' technology. So-called *'in sitro'* technology consists of the *in vitro* and *in situ* culture of oligocellular suspensions of a freshly obtained tumour (which includes malignant and immune cells) upon the addition of chemotherapeutics, immune checkpoint blockers and chemotactic factors or their inhibitors, followed by the measurement of immune functions by cytofluorometry (to determine the proliferation and activation of myeloid and lymphoid cells) or the quantification of the cytokines contained in the culture supernatant. This methodology allows the measurement of hundreds of parameters of immune activation over several days of culture, and can be used to distinguish between cancer patients who respond to different ICBs or immunostimulatory molecules (L.Z., unpublished observations). Although this technique potentially measures the activation or reactivation of immune cells that are already present in the tumour and their associated chemokine release, *in sitro* technology cannot model the recruitment of leukocytes into the tumour bed.

The 'ex vivo' technology. So-called '*ex vivo*' technology consists of the *in vitro* culture of freshly obtained patient-derived tumour slices to which fluorescently labelled autologous leukocytes subtypes (such as T lymphocytes) are added, followed by videomicroscopic observation of the tumour–immune cell interaction in a realistic setting influenced by chemokines and the extracellular matrix[100]. Hence, this technology may be useful for characterizing the mechanisms through which a 'cold' tumour (without tumour-infiltrating lymphocytes) can be converted into a 'hot' tumour (with an active immune infiltrate).

The 'immuno-oncology chip'. The 'immuno-oncology chip' is used with fluorescently labelled malignant cells and leukocytes (such as PBMCs) in a microfluidic device and measures the dynamics of their interaction by video-fluorescence microscopy for several days. This method allows the addition of therapeutic agents, such as chemotactic agonists or antagonists, to study their effect on leukocyte trajectories and function[101]. This technology has led to the discovery of a patient who harboured a genetic defect in a chemotactic receptor — formyl peptide receptor 1 (FPR1) — that affects the capacity of dendritic cells to approach dying cancer cells and to cross-present tumour antigens to T cells[19].

Therefore, it seems plausible that the improvement in organoid culture systems[102] coupled with the sophistication of microfluidic devices[103] will yield novel experimental systems for studying the interactions of a diverse array of parenchymatous and stromal elements from the tumour microenvironment.

Conclusions

Although the prime function of the immune system is to maintain the interior milieu of our body in a sterile state, immune effectors are also able to eliminate cancer cells that express tumour-associated or tumour-specific antigens both in natural circumstances (to avoid oncogenesis) and in conditions of successful antineoplastic treatment. This physiological property of the immune system is conserved from mice to humans, as is the overall organization of the innate and acquired humoural and cellular immune effectors, meaning that results obtained in mice can be extrapolated, to a large extent, to humans. For this reason, cognitive research carried out in immunocompetent mice with mouse cancers — be they transplanted, carcinogen-induced or genetically engineered — will continue to yield useful information. Notwithstanding their intrinsic value for basic research purposes, successful attempts have been made to humanize mouse models so that human-specific pharmacological agents can be explored *in vivo* or the interaction between human (and sometimes patient-derived) cancers and human immune effectors can be investigated in some molecular detail. Given the observation that the gut microbiome has a major modulatory effect on the dialogue between immune and malignant cells to the point that it establishes an effective triangulation[104], a future challenge will be to also humanize mice in their gut microbiome, hence converting them into ever more sophisticated avatars of individual patients that carry their tumours, their immune system and also their gut microflora. Beyond these aspects of rendering mouse models increasingly closer to human reality (and hence to eventual clinical progress), another future challenge consists of simplifying interactions between tumour cells, innate immune cells and effectors of acquired responses to create simplified *in vitro* systems that can be taken advantage of to screen genetic or pharmacological libraries for immunostimulatory effects, and that allow the rapid identification of tumour antigens and their immune receptors. Historically, research has profited from interspersed rounds of reductionism (to discover mechanistic details) and systems analyses (to discover unsuspected general properties); the field of oncoimmunology will continue to profit from the adequate use of mouse models at both these extremes of the scientific spectrum.

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The authors declare no competing interests.