CHAPTER ONE

Mouse Models of Tumor Immunotherapy

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Abstract

Immunotherapy is now evolving into a major therapeutic option for cancer patients. Such clinical advances also promote massive interest in the search for novel immunotherapy targets, and to understand the mechanism of action of current drugs. It is projected that a series of novel immunotherapy agents will be developed and assessed for their therapeutic activity. In light of this, in vivo experimental mouse models that recapitulate human malignancies serve as valuable tools to validate the efficacy and safety profile of immunotherapy agents, before their transition into clinical trials. In this review, we will discuss the major classes of experimental mouse models of cancer commonly used for immunotherapy assessment and provide examples to guide the selection of appropriate models. We present some new data concerning the utility of a carcinogen-induced tumor model for comparing immunotherapies and combining immunotherapy with chemotherapy. We will also highlight some recent advances in experimental modeling of human malignancies in mice that are leading towards personalized therapy in patients.

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1. INTRODUCTION

Immunotherapy has a longstanding history in cancer treatment. Recently, this approach to cancer therapy reinvigorates a massive interest from the academic investigators and pharmaceutical companies. There has been a revolutionizing success of T cell checkpoint inhibitors and chimeric antigen receptor (CAR) T cells in treating various malignancies (Ansell et al., 2015; Hamid et al., 2013; Herbst et al., 2014; Hodi et al., 2010; Maude et al., 2014; Porter, Levine, Kalos, Bagg, & June, 2011; Powles et al., 2014; Robert et al., 2015; Topalian et al., 2012; Wolchok et al., 2013). Beyond these advances in the clinic, it is foreseen that a broad spectrum of promising preclinical immunotherapeutic agents (alone or in combination) will soon be enlisted for clinical trials (Galluzzi et al., 2014). The bench-to-bedside transition of these immunotherapeutic agents has largely stemmed from preclinical assessment in experimental cancer models in mice.

However, the tumor microenvironment (TME) is a dynamic milieu. It is now clear that the interaction between tumor (or early transformed cells) and immune cells plays a significant role in determining disease outcome. Assisted by intrinsic genomic instability and mutations, and multiple dysregulated cellular machineries, tumor cells continue to adapt and survive in their host body (Hanahan & Weinberg, 2011). Human malignancies demonstrate regional heterogeneity in their tumor–immune cellular makeup and variation in the effectiveness of natural tumor immunity. This heterogeneity is perhaps reflected by the absence of an effective clinical immune characterization (immune contexture and/or immunoscore at different tumor stages) for most types of cancer (Fridman, Pagès, Sautès-Fridman, & Galon, 2012). More importantly, for preclinical research, these complexities limit the ideal mimicry of human malignancies by experimental mouse models of cancer. Despite these limitations, in vivo experimental tumor models are valuable tools for the assessment of therapeutic agents before their transition into clinical trials. These experimental studies in mice have provided an understanding of the role for tumor immunity in shaping the TME at different stages of tumor growth, and a proof-of-principle for the therapeutic activity and the associated overt toxicities of numerous immunotherapeutic agents (Gyorki, Callahan, Wolchok, & Ariyan, 2013; Liu, Blake, Smyth, & Teng, 2014). It is not the major purpose of this review to compare the strengths and weaknesses of various experimental mouse tumor models, since this has been done before (Dranoff, 2012;

DuPage & Jacks, 2013; Gould, Junttila, & de Sauvage, 2015), but to highlight some of the advances in experimental mouse tumor models, and to discuss the use of appropriate models to explore antitumor activity of the therapeutic agents of interest.

2. TRANSPLANTABLE TUMOR MODELS

For decades, in vivo mouse tumor cell line transplants have been the major approach to study tumor immunology and immunotherapy. Following injection of in vitro-cultured tumor cells into syngeneic mice, the mice must be sacrificed within weeks due to a large and rapidly growing tumor burden. The relatively reproducible and rapid in vivo tumor growth of transplantable mouse tumor lines when injected subcutaneously or intravenously are major advantages of this experimental model in studies of immunotherapy. It is notable that most of the experimental tumor cell lines are tumor cells that have been derived in immunocompetent mice and thus have evaded immunological pressure and/or been immunologically tolerated (Vesely, Kershaw, Schreiber, & Smyth, 2011). Hence, the tumorigenesis of transplantable tumor models is likely to be different from de novo tumors. The local innate inflammatory response triggered by an injection and presence of a large number of tumor cells may also condition the TME of transplantable tumors (Apetoh et al., 2007; Kroemer, Galluzzi, Kepp, & Zitvogel, 2013; Ladoire et al., 2013; Obeid et al., 2007). To a certain extent, the presence of dead tumor cells might provide a vaccination effect, affecting the therapeutic response of an immunotherapeutic agent (Kroemer et al., 2013; Ladoire et al., 2013). Despite these limitations, transplantable tumor models remain valuable tools for immunotherapy assessment.

An emerging area of importance concerns the epitopes driving tumor-suppressor responses. To track and study tumor-specific T cells, tumor cell lines are often engineered with model antigen, such as ovalbumin or hemagglutinin (Gilboa, 1999). These engineered tumor cells can be subsequently transplanted into transgenic mice expressing T cell receptor specific for the model antigen (eg, OT-I or OT-II transgenic mouse), transgenic mice expressing the model antigen (eg, RIP-OVA mouse), or syngeneic mice; where antigen-specific T cells can be monitored and assessed (Gilboa, 1999). However, it is important for the researcher to note that these modified tumor cells could change the tumor cell immunogenicity and trigger different antitumor immune responses (Schreiber, Old, & Smyth, 2011). In light of this, a careful interpretation of the therapeutic outcome in these

models is required. This is particularly essential in an assessment of antitumor immunity and potential adverse immune responses provoked by immunotherapy agents (John et al., 2013; Klages et al., 2010). In contrast, the assessment of endogenous tumor antigen-specific T cells could provide a less biased readout (Bloom et al., 1997; Overwijk et al., 1999). For example, the detection of melanoma and prostate antigen-specific CD4 and CD8 T cells in B16 melanomas and TRAMP-C1 prostate adenocarcinoma, respectively, can be performed by staining using antigen–tetramer complexes or antibodies to known αβ TCR chains (Hanson et al., 2000; Malchow et al., 2013; Muranski et al., 2008; Overwijk et al., 2003; Savage et al., 2008). As an additional example, in a genomic (exome) analysis of MC38 colon adenocarcinoma, this tumor model was shown to harbor seven mutated antigens, and prophylactic vaccination against these antigens was shown to impair the growth of MC38 subcutaneous tumors (Yadav et al., 2014). Indeed, vaccination against tumor neoantigens has recently been shown to increase the breadth and diversity of antigen-specific T cells in advanced melanoma patients (Carreno et al., 2015). Clinically, better understanding the antigens that drive cell-mediated tumor rejection will lead to the development of tumor-specific, peptide-based vaccination strategies to complement checkpoint inhibition.

To ease the monitoring of tumor growth, tumor cell lines are generally injected subcutaneously in mice. It is however important to understand that unlike de novo formed primary tumors, subcutaneous implanted tumors are not growing within a relevant TME. To mimic the physiological environment of a tumor, some tumor cell lines can be orthotopically injected into the relevant organs (Westwood, Darcy, & Kershaw, 2014). These include intravenous injection for a series of leukemia and lymphoma cell lines (Mattarollo et al., 2012; Minard-Colin et al., 2008; Zhou et al., 2011), mammary fat pad injection for mammary tumor cell lines (Verbrugge et al., 2012), intrapancreatic injection for pancreatic ductal adenocarcinoma lines (Zhu et al., 2014), and intracranial injection for glioma cell lines (Wainwright et al., 2014). This variation in site of tumor formation gives rise to a different tumor–immune cellular makeup, impacting on the progression of tumor and therapeutic outcome of a number of immunotherapy agents (Devaud et al., 2014; DuPage et al., 2011; Knight et al., 2013; Westwood et al., 2014). We have previously shown that Tri-mAb (a combination of monoclonal antibodies targeting DR5, CD40, and CD137) was less effective to treat orthotopically injected Renca renal cell carcinoma, RM-1 prostate carcinoma, and CT26 colon

adenocarcinoma in comparison to their subcutaneously injected counterparts (Devaud et al., 2014). Despite the advantages of mimicking tumor growth in a relevant microenvironment, orthotopic tumor model approaches sometimes require labor-intensive techniques to perform tumor cell implantation (such as surgery techniques) and to monitor/image tumor growth (such as engineered bioluminescence cell lines and imaging technology to monitor visceral tumor sites), limiting its application in less-equipped laboratories.

Metastasis of tumor is the primary obstacle in various cancer treatments. An experimental surrogate of the formation of tumor metastases in mice is commonly performed by intravenous injection of tumor cells. Tumor cells are experimentally disseminated to organs like lung, liver, spleen, or bones (Sathe et al., 2014). Experimental lung metastases models that are relatively well characterized are the B16 melanoma, 3LL Lewis lung carcinoma, and RM-1 prostate carcinoma (Chow, Sceneay, et al., 2012; Teng, von Scheidt, Duret, Towne, & Smyth, 2011). Given the dependency on myeloid and natural killer (NK) cells to control the dissemination of tumor cells (Chow, Sceneay, et al., 2012; Sceneay et al., 2012; Teng et al., 2011), these metastatic tumor models serve as valuable tool for evaluating immunotherapy that could target these immune subsets. In some cases, to model brain metastases, tumor cell lines may also be intracerebral/intracardially injected into mice (Connell et al., 2013; O'Brien et al., 2014). In addition, under specific experimental conditions, a small number of tumor cell lines are capable of forming spontaneous metastases. One of the most commonly used conditions is to surgically remove primary 4T1 or EO771 mammary adenocarcinomas (either subcutaneously or orthotopically injected) for the induction of spontaneous metastases to distant organs (Chow, Sceneay, et al., 2012; Mittal et al., 2014; Stagg et al., 2010). The formation of metastases under these conditions is likely driven by factors like prolonged time span to obtain overt metastases upon primary tumor challenge, and surgery-induced NK cell dysfunction (Tai et al., 2013). Other approaches to model spontaneous metastases include intrapinna injection of B16 melanoma to generate lymph node metastases (Bobek et al., 2010), intracutaneous injection of HCmel12 and HCmel31 melanomas to generate spontaneous lung metastases (Bald et al., 2014), and intrakidney injection of Renca renal cell carcinoma to generate lung and abdominal metastases (Devaud et al., 2014). In light of the different biology of metastatic disease, these tumor models may be utilized to preclinically assesses the ability of immunotherapy to inhibit for metastasis.

3. GENETICALLY ENGINEERED TUMOR MODELS

For most malignancies, the initiation of transforming process is driven by genomic instability and mutations. These genetic changes could lead to the activation of oncogenes (such as *Kras* and *Braf*) and/or suppression of tumor suppressor genes (TSG) (such as *Tp53*), and eventually progress to form cancer (Hanahan & Weinberg, 2000, 2011). Before the recognition of a role for endogenous immune response in affecting tumor progression, most cancer studies were focused on understanding the contribution of cell intrinsic defects to cancer formation (Hanahan & Weinberg, 2000, 2011). This is evidenced by the previous lack of recognition of immunoevasion as a feature characterizing cancer (Hanahan & Weinberg, 2000, 2011), and the massive effort invested in constructing cancer genetic databases and genetically engineered (GE) tumor models for cancer biology studies (DuPage & Jacks, 2013). The development of a diverse collection of GE tumor models allows researchers to study in situ cellular transformation events under defined genetic changes, and to visualize histopathological features at different stages of tumor progression. In contrast to transplantable tumor models, GE tumor models provide a physiological relevant environment for the study of interplay between tumor and immune cells.

GE tumor models are generally developed using transgenic technologies to enforce a systemic or tissue-specific oncogene expression and/or tumor suppressor gene deletion (DuPage & Jacks, 2013; Frese & Tuveson, 2007). These transgenic models can be further categorized into germline GE models and conditional GE models (DuPage & Jacks, 2013; Frese & Tuveson, 2007). For the former, transgenic mice would develop malignancies in a spontaneous manner. As an example, a series of *Tp53*-mutated mice have been shown to induce a wide spectrum of solid and hematological malignancies (Frese & Tuveson, 2007; Zender, Zuber, & Lowe, 2007). As an additional example, an mouse mammary tumor virus-polyoma middle T (MMTV-PyMT) transgenic mouse was shown to closely recapitulate the progression of human breast carcinoma (Fantozzi & Christofori, 2006). A series of mouse models of this class that are useful for preclinical therapy (monotherapy or in combination with targeted therapy) assessment include the MMTV-driven ErbB2/neuT transgenic mouse (mammary adenocarcinoma; anti-Her2 mAb-sensitive) (Guy et al., 1992; Stagg et al., 2008), $Kit^{V558\Delta/+}$ transgenic mouse (gastrointestinal stromal tumor; Imatinib-sensitive) (Balachandran et al., 2011; Sommer et al., 2003), and

$Kras^{LSL\text{-}G12D/+}Trp53^{LSL\text{-}R172H/+}Pdx1\text{-}Cre$ (KPC) (pancreatic ductal adenocarcinoma; anti-CD40 mAb-sensitive) (Beatty et al., 2011). We have used the MMTV-driven ErbB2/neuT transgenic mouse to demonstrate the combination activity of anti-PD-1 and anti-erbB2 therapy (Fig. 1). This study and others with transplanted tumors have led to a phase Ib/II trial evaluating the efficacy of MK-3475 (anti-PD-1) and trastuzumab in patients with trastuzumab-resistant, HER2-positive metastatic breast cancers (International Breast Cancer Study Group—IBCSG 45-13/BIG 4–13). On the other hand, conditional GE models allow spatiotemporal control of the transformation onset. The initiation of tumorigenesis in conditional GE model is generally tissue-specific and triggered by a specific ligand (such as Tet-on/off and tamoxifen-inducible Cre recombinase systems) (DuPage & Jacks, 2013; Frese & Tuveson, 2007). In the context of experimentation, the tumor stages are relatively well controlled in comparison to germline GE model. For example, we and others have recently assessed the efficacy of a series of immunotherapeutic agents in a mouse model of conditionally $Braf^{V600E}$-mutated melanoma (Ho et al., 2014; Hooijkaas et al., 2012; Knight et al., 2013). In addition, a mouse model of conditionally

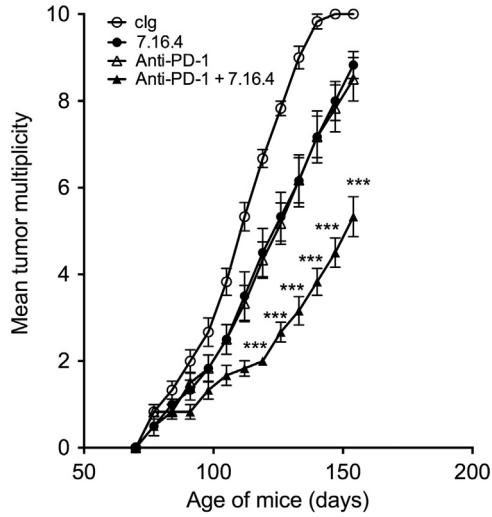

Figure 1 Combined anti-PD-1 and anti-ErbB-2 mAb therapy prolonged the latency of spontaneous mammary carcinomas. Groups of six female ErbB-2/neuT transgenic mice were treated for 6 wk starting at 77 d of age with control Ig (clg, Mac4, 100 μg i.p.) and/or anti-PD-1 (RMP1-14, 100 μg i.p.) and/or anti-erbB2 (7.16.4, 100 μg i.p.). Mean tumor multiplicity ± standard errors of mean versus the age of mice (days). *** $p < 0.001$ combination compared to any single treatment by Mann–Whitney test.

Kras/Tp53-mutated lung carcinoma developed by Tyler Jacks group was shown to be an ideal system to study the role of T cell immunity in controlling lung adenocarcinoma (DuPage et al., 2011).

Gene targeting via homologous recombination in embryonic stem (ES) cells has been a widely used method for genetic modification in mice (Capecchi, 2005). This construction of a GE mouse model of tumor is labor-intensive, time-consuming, and expensive. In many cases, homozygous germline mutations could result in embryonic lethality, off-target developmental defects, and/or nonspecific genetic defects. These flaws limit the study of a gene of interest in adult tissues. More recently, the clustered, regularly interspace, palindromic repeats (CRISPR)-Cas9 (CRISPR-associated protein) technology is being explored to generate GE tumor models in a more efficient manner (Hsu, Lander, & Zhang, 2014; Pelletier, Gingras, & Green, 2015; Sander & Joung, 2014). CRISPR-Cas9 technology utilizes single guide RNA (sgRNA; a small artificial RNA molecule) to direct mutagenesis in zygotes, accelerating the generation of GE models (Jinek et al., 2012). However, similar to ES gene-targeting approach (such as transgenic mouse model), genetic mosaicism is also present in CRISPR-Cas9-generated GE models. Hence, the production of a large cohort of experimental mice requires further intercrossing of the founder mice. Regardless, CRISPR-Cas9 technology is a very efficient new platform to develop GE tumor models. Excitingly, a number of independent laboratories have recently reported the generation of GE mouse models of liver cancer, lung cancer, and intestinal hyperplasia using this technology (Blasco et al., 2014; Dow et al., 2015; Maddalo et al., 2014; Platt et al., 2014; Sanchez-Rivera et al., 2014; Xue et al., 2014). Like conventional GE tumor models, we foresee this class of tumor model will be interrogated in tumor immunology studies in the near future.

However, GE models have limitations. Perhaps the most fundamental of these is the assumption of the order of genetic events in real world tumor development. A specific concern for recapitulation of immune-mediated tumor suppression is initiation of tumor development by inactivation of genes required for induction of senescence—for example, *Trp53*, *Rb1*, and *Cdkn2a*. Senescence is an important tumor-suppressor process that engages the immune system by expression of a slew of signaling molecules—including IL-1, IL-6, and IL-8 (Kansara et al., 2013). Models that abrogate this response ab initio cannot account for a plausible early phase of tumor suppression that eradicates neoplastic clones that sustain an increased mutation burden in other genes. More practically, in stark contrast to transplantable tumor models, most

GE tumor models require a longer period of time for tumors to progress. Perhaps such temporal variation in GE tumor models resembles the chronic inflammatory features of malignancies in humans and allows the development of immune tolerance, immunoediting, and/or immunosuppressive processes (Kim & Ahmed, 2010; Vesely et al., 2011). In concordance, GE tumor models have relatively heterogeneous tumor formation, potentially dictated by the tumor–immune interaction. The constant expression of a transgene may vary in cohorts of transgenic mice, hence producing different levels of immunosurveillance in controlling tumor outgrowth. We now appreciate that certain mutations in cancer may give rise to the development of T cell-sensitive neoantigens (Linnemann et al., 2015; Rizvi et al., 2015; Robbins et al., 2013; Snyder et al., 2014; van Rooij et al., 2013). Of note, the presence of these neoantigens was shown to correlate with immunotherapy outcome (Rizvi et al., 2015; Snyder et al., 2014). In line with this, the assessment of immunotherapy using GE tumor models is particularly important in an examination of antitumor T cell immunity. However, it remains unclear if most of the currently available GE tumor models fulfill such features of cancer (ie, the impact of oncogene/TSG mutation(s) on neoantigen generation). Most of the current GE tumor models demonstrated an inability to fully recapitulate the endogenous T cell response toward tumor antigens. Although there has been effort to construct model antigens in GE tumor models (Cheung, DuPage, Dong, Chen, & Jacks, 2008; DuPage, Mazumdar, Schmidt, Cheung, & Jacks, 2012), it remains challenging to model T cell responses in malignancies. Despite these drawbacks, it is likely that with the appreciation of common ground between tumor geneticists, tumor biologists, and tumor immunologists, more human cancer-like GE models might be developed in the near future to guide preclinical immunotherapy testing.

4. CARCINOGEN-INDUCED TUMOR MODELS

Another class of experimental mouse model that are widely used in the study of tumor immunology is the carcinogen-induced tumor model. A number of carcinogen-induced (CI) tumor models have been used to study tumor immunosurveillance and immunoediting mechanisms (Schreiber et al., 2011; Swann & Smyth, 2007; Vesely et al., 2011). Some of the relatively well-studied CI models include: methylcholanthrene (MCA)-induced fibrosarcomas, 7,12-dimethylbenz[*a*]anthracene (DMBA)/12-*O*-tetradecanoylphorbol-13-acetate (TPA)-induced skin papillomas, azoxymethane/dextran sodium sulfate-induced colon carcinoma, and

ultraviolet B-induced skin cancers (Schreiber et al., 2011; Swann & Smyth, 2007; Vesely et al., 2011). In contrast to the transplantable and GE tumor models, a carcinogen is used to induce de novo tumor formation. Similar to GE tumor models, the in situ induction of genomic instability and mutation using carcinogen allows the natural formation of a tumor–immune interaction in its relevant microenvironment (Hanahan & Weinberg, 2011). For a limited number of tumor types, CI tumor models also provide histopathological features resembling human malignancies (Chow, Tschopp, Moller, & Smyth, 2012; Kansara et al., 2013; Mossman & Churg, 1998). Although both the GE and CI tumor models require a relatively longer period of time for tumor progression, CI tumor models have much complexity in their genetic makeup, depending on the types and doses of carcinogen used, perhaps more accurately reflecting most sporadic carcinogenesis in man. Such variables in carcinogen are potentially generating different levels of immunogenic neoantigens, hence affecting the penetrance and/or latency of disease.

Given the absence of defined genetic manipulation in CI tumor models, it has been technically challenging to effectively track tumor antigen T cell responses in primary tumors, despite the likely presence of a vast number of neoantigens. It is however exciting that in recent studies reported by Schreiber and colleagues, in silico prediction algorithms of high affinity epitopes for major histocompatibility complex class I ($H\text{-}2D^b$ and $H\text{-}2K^b$) presentation and exome analyses were used to identify T cell-reactive neoantigens in a series of MCA-induced fibrosarcoma cell lines (Gubin et al., 2014; Matsushita et al., 2012). In this context, together with the use of DNA sequencing technologies, we may be able to identify and predict tumor-derived peptides for the stimulation of endogenous antitumor T cell response in CI tumor models. Another useful aspect of CI tumor models is their application in most mice strains. The construction of a GE tumor model is a labor-intensive and time-consuming process. Unlike GE tumor models, carcinogens can be applied into a wide range of gene-deficient mice, to help the identification of novel immunotherapy targets. Examples of carcinogen-induced models include radiation-induced osteosarcomas and de novo MCA-induced fibrosarcomas and DMBA/TPA-induced skin papillomas (Kansara et al., 2009, 2013; Teng et al., 2010, 2011). The latter models were used to study the resistance to tumor development in IL-23p19-deficient mice, prompting the use of anti-IL23p19 mAb as an immunotherapy agent (Langowski et al., 2006; Teng et al., 2010, 2011). Similarly, we are currently developing strategies to inhibit CD73 and

CD96 signaling, driven by the tumor formation resistance phenotypes seen in CD73- and CD96-deficient mice (Chan et al., 2014; Stagg et al., 2012), respectively.

The MCA-induced fibrosarcoma model in particular, has also been used as a model to test therapies, given its relative reproducibility and comparatively short latency to palpable tumor size. We first described an important synergy for the Tri-mab (as mentioned above) in collectively triggering tumor cell apoptosis, antigen-presenting cell activation and T cell activation and survival (Uno et al., 2006). More recently, this MCA induction of sarcoma model has been used to demonstrate the role of host immune elements in the efficacy of the chemotherapeutic, doxorubicin (Mattarollo et al., 2011). As with human sarcomas, doxorubicin has limited single agent activity in the MCA model. Compellingly, we show that anti-CD40, anti-CD137, or their combination augments the antitumor efficacy of doxorubicin, preempting clinical scenarios for their use (Fig. 2).

5. HUMANIZED MOUSE TUMOR MODELS

Due to ethical and economical constraints, the use of models more closely related to humans (such as nonhuman primate models) for experimental purposes remains limited. Hence, experimental mouse models are generally recognized as the major surrogate models for biology research. However, it is well known that humans and mice have considerable differences in a number of their biological machineries. Therefore, a complete recapitulation of human physiological or pathological processes in mice is very challenging. In concordance, a huge number of preclinical drug candidates that demonstrated promising outcomes in mice failed to show efficacy when tested in humans (Arrowsmith & Miller, 2013; Kamb, 2005). This issue is particularly obvious when modeling diseases where the hematological system is involved, such as infections, autoimmune conditions, and malignancies. To overcome this problem, there has been a great effort in the field to "humanize" experimental mice for a better modeling of human pathophysiological conditions (Holzapfel, Wagner, Thibaudeau, Levesque, & Hutmacher, 2015; Legrand et al., 2009; Rongvaux et al., 2013; Scheer, Snaith, Wolf, & Seibler, 2013).

One promising approach is the xenotransplantation of functional human tissues (such as immune cells and/or tumor tissues) into immunodeficient mice, for studies of cancer biology. Immunodeficient mouse models, like athymic nude mice, recombination-activating gene (Rag) × common

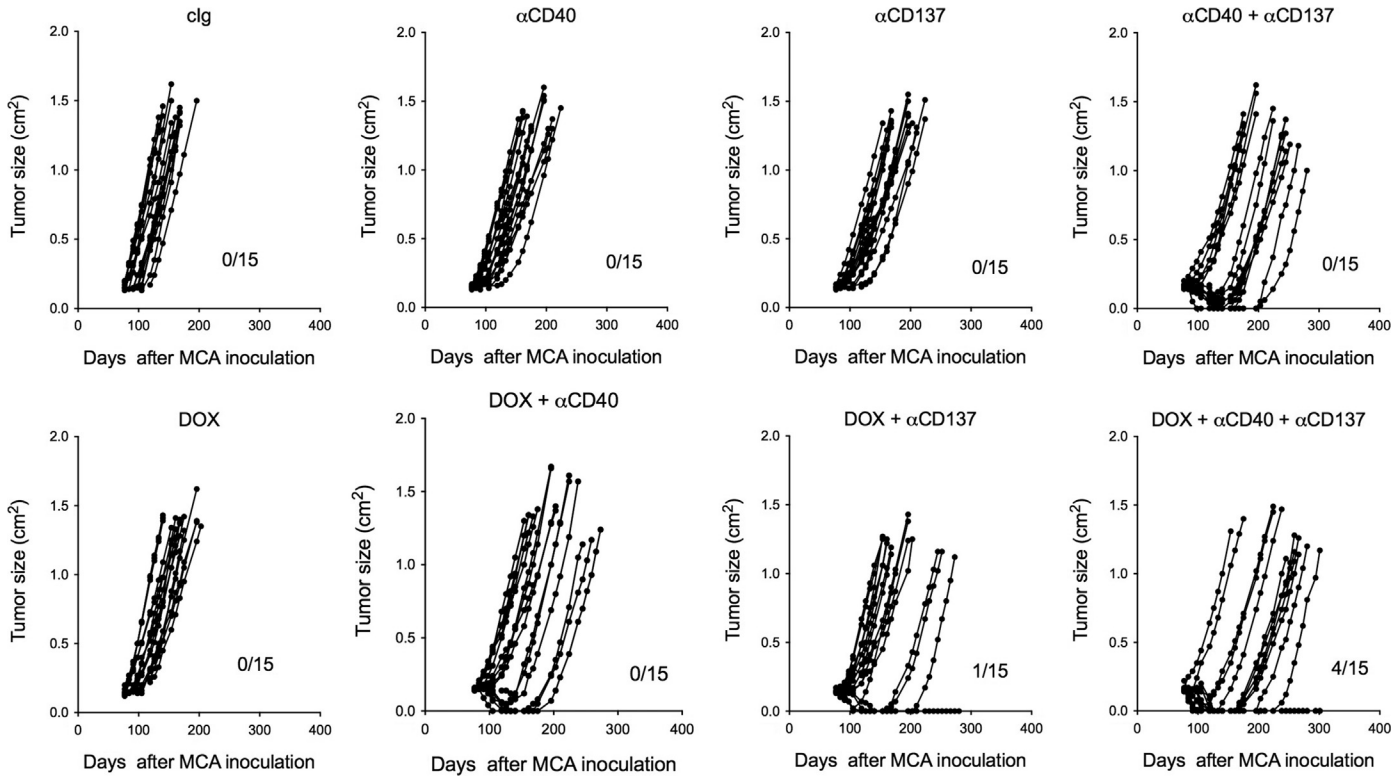

Figure 2 Combination efficacy of doxorubicin (DOX) alone and in combination with mAbs against tumors established de novo in host. Groups of 15 male BALB/c WT mice were inoculated s.c. in the hind flank with 400 μg of MCA in 0.1 mL of corn oil as described in Section 7. Mice were treated with PBS or DOX (2 mg/kg i.v.) from the second palpable tumor measurement (0.1–0.3 cm^2, days 77–119 relative to MCA inoculation). Some groups of mice also received clg (Mac4), anti-αCD40 (FGK45), αCD137 (3H3), or their combination (100 μg i.p., four times/3 d apart) from the first palpable tumor measurement. Mice were then monitored for fibrosarcoma development over 300 d, with measurements made with a caliper square as the product of two perpendicular diameters (cm^2). Data were recorded as tumor size in cm^2 of individual mice with tumor in each group.

gamma chain (γc)-deficient mice, nonobese diabetic (NOD)-severed combined immunodeficiency (SCID)-IL-2 receptor gamma (IL2rg)-deficient mice are commonly used host for xenograft implantation (Aparicio, Hidalgo, & Kung, 2015; Holzapfel et al., 2015; Legrand et al., 2009; Rongvaux et al., 2013). Mouse models xenografted with human tumor tissues are also widely used for immune-based targeted therapy studies, such as anti-VEGF and anti-EGFR mAbs, and adoptive-transferred engineered T cells (Holzapfel et al., 2015; Zhou, Facciponte, Jin, Shen, & Lin, 2014). However, it is notable that an assessment of these classes of therapy is flawed by the lack of intact adaptive immunity in the recipients, including important regulatory T and B cells. For instance, the antibody-dependent cell-mediated cytotoxicity mechanism triggered by a therapeutic mAb might be masked in a host deficient for NK cells and/or lacking the appropriate Fc receptors (Barok et al., 2007; Ito et al., 2009; Kohrt et al., 2012; Park et al., 2010; Pincetic et al., 2014; Weiner, 2010; Zhang et al., 2004).

To overcome this problem, human immune cells are often cotransplanted (prior or concurrently with the tumor xenograft) into recipient mice. The recipient mice can be reconstituted with human hematopoietic progenitor cells (HPCs; generally based on CD34+) or sorted immune subsets (Legrand et al., 2009; Rongvaux et al., 2014). Substantial evidence supports functional properties of the human immune cells in these immunodeficient hosts and tumor–immune interactions were evidenced. Using this approach, Palucka and colleagues demonstrated the role for thymic stromal lymphopoietin (TSLP) in driving OX40L+ dendritic cell (DC)-mediated Th2 inflammatory responses in breast cancer (Pedroza-Gonzalez et al., 2011). More importantly, in this study, the authors also demonstrated suppression of tumor (xenografted Hs578T breast cancer cells) growth in mice treated with anti-OX40L, anti-TSLP, and anti-TSLP receptor (Pedroza-Gonzalez et al., 2011). As an additional example, in human breast cancer (MDA-MB-453) and γδ cotransplanted with Tregs, Ye and colleagues demonstrated that the administration of anti-IP10 (CXCL10) could suppress tumor growth in vivo (Ye et al., 2013). In a more recent study, Rongvaux and colleagues reported a development of two strains of mice, MITRG and MISTRG, which are capable of supporting the development of human innate immune cells (Rongvaux et al., 2014). MITRG were generated in an immunodeficient $Rag2^{-/-}IL2rg^{-/-}$ background, and they additionally encode human M-CSF (macrophage colony-stimulating factor), human IL-3, human GM-CSF (granulocyte macrophage colony-stimulating factor), and human thrombopoietin. A bacterial artificial

chromosome transgene encoding for human SIRPα was inserted into the MITRG strain, to generate the MISTRG strain (Rongvaux et al., 2014). In these models, the administration of Avastin (human vascular endothelial growth factor (VEGF) inhibitor) was shown to suppress the growth of the xenografted melanoma (Me290 melanoma cells) (Rongvaux et al., 2014).

These examples (Pedroza-Gonzalez et al., 2011; Rongvaux et al., 2014; Ye et al., 2013) and others (Aspord, Leccia, Charles, & Plumas, 2013; Bankert et al., 2011; Liddy et al., 2012; Obenaus et al., 2015; Wege et al., 2011), indicate the potential of humanized mouse tumor models in clinical investigations. This is particularly important when assessing a combination therapy involving immunomodulatory agents. However, additional studies are required to interrogate and validate the general translational value of humanized mouse tumor models for such purposes. The origin of immune cells (HPC or sorted immune subsets; patient PBMC or tumor infiltrating immune cells), dynamics of immune cells in the recipient (tumor and periphery), and tumor specimens (regional heterogeneity) are important variables to be considered in the context of personalized therapy. With advances that will further improve humanized mice, we might expect application of this class of tumor model to inform biomedical research.

6. PERSPECTIVES

It is worth noting that many immunotherapy agents target preexisting host immunity. The basal immune system of a tumor-bearing host can be conditioned via multiple tumor intrinsic factors (such as genetics, expression of receptors/ligands, and secretion of soluble factors; Facciabene et al., 2011; Motz et al., 2014; Qian et al., 2011; Ruffell et al., 2013) and extrinsic factors (such as stromal cells, microbiota, and housing environment; Engels, Rowley, & Schreiber, 2012; Grivennikov et al., 2012; Kokolus et al., 2013; Zitvogel et al., 2015). In this light, these variables should be carefully considered in an in vivo experiment. Despite the value of individual experimental mouse tumor models to inform clinical investigation, their findings need to be replicated across multiple tumor models, and ideally more than one strain of mouse. In addition, studies need to be corroborated with the use of appropriate statistical principles, experimental reproducibility, group sizes, endpoint readouts, and careful interpretation, to guide the translation of a new agent into the clinic (Couzin-Frankel, 2014; Kohrt et al., 2012; Peers, South, Ceuppens, Bright, & Pilling, 2014).

Although currently available mouse tumor models may individually recapitulate limited aspects of human cancer, together they allow the functional dissection of phenomena observed in and relevant to human tumor development and therapy. We speculate that with advances in genetic manipulation and imaging technologies, the study of tumor immunology and immunotherapy will eventually tested in more life-like and human-like experimental tumor models. These models should have the capacity to model the physiological environment typical for the tumor's growth, should demonstrate specific cancer-causing genetic changes, should identify relevant neoantigens, and/or should predict immune-related adverse events.

The capacity of tumors to secrete soluble factors (such as cytokines and chemokines) and immunoregulatory receptors/ligands is highly variable between tumor models, hence affecting the recruitment of immune subsets and determining the immunosuppressive mechanisms being deployed. Nonetheless, the immunogenicity of different tumor models might also dictate their tumor–immune contexture (Vesely et al., 2011). Undoubtedly, a better understanding of every tumor model helps the design of therapy. A novel immunotherapeutic agent might first be tested in a series of transplantable tumor models with varied immunogenicity, known immune contexture, and characterized immunosuppressive mechanisms. For example, the B16 melanoma and TRAMP-C1 prostate adenocarcinoma are considered poorly immunogenic compared to MCA-induced fibrosarcoma cell lines or MC38, and so these latter models can be used to validate immunotherapy that targets T cell effector function. Their variations in tumor immunogenicity are likely driven by the presence (and levels) of mutated antigens (ie, neoantigens). By contrast, 4T1 mammary adenocarcinomas have a higher myeloid:T cell ratio compared to CT26 colon adenocarcinoma, and hence these models are useful tools to validate immunotherapy that targets myeloid cell function. These cell lines are also useful tools to assess Treg-targeted therapy, given that CT26 has a higher dependency on Treg number and function to support its growth in vivo, when compared to 4T1 (unpublished data). As an additional example, Rosenberg and colleagues have utilized the pmel transgenic models to uncover a number fundamental principles of adoptive cellular transfer therapy in melanoma (Restifo, Dudley, & Rosenberg, 2012). The efficacy, scheduling and dosing of the immunotherapy of interest might subsequently be assessed in other relevant GI, CI, and/or humanized mouse tumor models.

The in vivo mechanism of action of a therapeutic agent is generally discovered in mouse tumor models appreciably sensitive to the agent

(ie, "a therapeutic window of opportunity"). We suggest that therapy-resistance results in tumor models are less often reported. While substantial effort is seen in assessing novel immunotherapy agents, one should consider to revisit clinically established immunotherapy agents in tumor models of resistance. These studies have the potential to yield key information leading to better clinical practice in patients. For example, by studying mouse melanoma that was resistant to a combination of radiotherapy and anti-CTLA-4 therapy, Twyman-Saint Victor and colleagues reported that the PDL1 upregulation on tumor cells and associated T cell exhaustion are the major drivers of therapy resistance. In concordance, a coblockade of PD-1/PD-L1 pathways using anti-PD-1/PD-L1 mAbs resulted in tumor clearance (Twyman-Saint Victor et al., 2015). This example highlights the importance of mouse tumor models of resistance in the preclinical assessment of immunotherapy agents. Of relevance, with advances in genetic manipulation in experimental tumor models (such as CRISPR), we might foresee a significant interest in the field to further study tumor–immune interactions in therapy-sensitive/resistance models of clinically useful agents (completing the virtuous "bed-to-bench side" cycle). Many future tumor model studies in mice will continue to inspire new investigation in the clinic.

7. MATERIALS AND METHODS

7.1 *BALB/c* MMTV-Her2 Mammary Carcinoma

BALB/c MMTV-ErbB-2/neuT mice were bred and maintained in house. Treatments were scheduled as indicated. Development of mammary tumors was monitored by palpation of mammary glands.

7.2 MCA-Induced Fibrosarcoma

BALB/c wild-type mice were obtained from the Walter and Eliza Hall Institute for Medical Research and were maintained at the Peter MacCallum Cancer Centre. All experiments were approved by the Peter MacCallum Cancer Centre Animal Ethics Committee. Groups of 15–20 male BALB/c WT mice were inoculated s.c. in the hind flank with 400 μg of 3-methylcholanthrene (MCA; Sigma-Aldrich, St. Louis, MO) in 0.1 mL of corn oil as described (Swann et al., 2008). Mice were treated as indicated. Mice were then monitored for fibrosarcoma development over 300 d, with measurements made with a caliper square as the product of two

perpendicular diameters (cm^2). Data were recorded as tumor size in cm^2 of individual mice with tumor in each group.

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