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Human Tumor Xenografts: The Good, the Bad, and the Ugly

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There has been a robust discussion for many years now on the utility, or lack thereof, of mouse tumor xenograft models in the study of human cell and gene therapies.[1–4] Of course, the reality is that the value of a model depends on what the modeler is trying to accomplish. A good use of human tumor xenograft models would be to support an experimental hypothesis, a bad use would be to present animal data that add little to the value of *in vitro* data, and an ugly use of tumor xenografts would be to facilitate publication of a manuscript or give a false sense of safety or efficacy.

The first use of inbred mouse strains for the propagation of human tumors was reported 50 years ago with the transplantation of human tumors into athymic "nude" mice.[5,6] This was followed a decade later by the adoption of the severe combined immunodeficiency (SCID) mouse as the mainstay of tumor xenotransplantation work.[7,8] Because the *scid* mutation (now called *Prkdc*) is leaky and does not negate the development of cells of the innate immune system, particularly natural killer cells, the SCID mouse cannot sustain complex xenografts involving human hematopoietic cells. In 1995, Shultz and colleagues[8] utilized a cross of SCID mice with nonobese diabetic (NOD) mice to generate animals with defects in both the adaptive and innate immune systems; this facilitated hematopoietic cell engraftment, but these animals have some residual innate immune cell reactivity. Currently, many investigators use third-generation immunodeficient mice that combine the NOD-SCID mice with animals that have additional defects in the interleukin-2 receptor γ-chain gene (*IL2rg*), also called the common γ-chain gene (γc), and these animals are often referred to as NSG (NOD-*scid*-γc) mice (see ref. 4 for a review of the development of these mice).

The successful clinical application of adoptive cell therapy for cancer,[9–11] along with the US Food and Drug Administration approval of the cellular vaccine sipuleucel-T and the immune checkpoint–blocking antibody ipilimumab, has led to an increased interest in immune-based anticancer therapies. In the context of human tumor xenotransplantation, when the innate and adaptive immune systems of the mouse have been eliminated, cell-based anticancer therapies can be thought of as add-back experiments. Two successful applications for which these mice have been used are in studying homeostatic expansion of various human T-cell subsets and in the development of "humanized" mice containing multiple cells and tissues of the human immune system. Human T cells come in multiple subtypes that are beyond the scope of this Commentary, and immunodeficient mice have been valuable for studies in which purified subsets are transplanted into these animals and the survival of various populations followed over time. For example, in a recent article by Gattinoni and colleagues,[12] a distinct subset of human $CD8^+$ T cells with self-renewal and memory properties (stem cell–like memory T cells, T_{SCM} cells) was demonstrated to reconstitute NSG mice with multiple effector cell lineages and could also be effectively used to transfer antitumor activity.

It is also possible to add back multiple components of the human immune system to immunodeficient mice. Sometimes called BLT (bone marrow, liver, and thymus) mice, these animals are regenerated with fetal tissues and $CD34^+$ hematopoietic progenitor cells to incorporate multiple components of the human immune system.[13–15] Although BLT mice are difficult to establish, they have been used with success in studies of pathogens that infect cells of the human immune system (e.g., HIV), in which it has been demonstrated that these animals can support the development of anti-HIV human cytotoxic T cells.

Although the mouse has been the paradigm for understanding human immunology, as Barrett and Melenhorst pointed out in a Commentary in *Molecular Therapy* last year, human cell and gene therapy researchers need to be careful not to get caught in a "mousetrap."[1] Any study in immunodeficient mice must consider several factors when attempting to extrapolate findings to humans. First and foremost is that the study subject (e.g., an NSG mouse) is a genetically uniform organism that is born and raised in a sterile environment. The clean environment should not be viewed as an irrelevant consequence of animal-handling requirements, because it can have dramatic consequences on experimental outcomes. For example, Paulos and colleagues[16] demonstrated that microbial translocation from the gut augments the function of adoptively transferred T cells, suggesting that differences in the extent to which a given mouse room is "clean" could have an impact on experimental results.

The limitations of the utility of tumor xenograft models go beyond the tumor itself and are restricted by the biology of the mouse. Not to belabor a point that has been made many times in other reviews, I will only briefly mention that tumor xenograft models utilize a cancer-treatment model that is based on an animal that, in comparison to humans, is significantly smaller, has a much higher metabolic rate, is inbred, and has a short life span. These differences cannot be overcome. In addition, whereas most human tumors take years to grow, tumor xenografts are transplantable tumors designed to grow to treatment size in weeks, not years. The tumor line chosen to be transplanted is probably one of the most significant reasons for failure of xenotransplantation data to be translated to human clinical results. Monogenic human tumor cell lines are almost exclusively used, and it is now well established that tumor cell lines can bear little resemblance to primary cancers. For an example,

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one can look at the work in glioblastoma—detailed analysis of genomic stability and gene expression changes revealed considerable differences when primary tumors were compared with established cell lines.[17]

What is potentially most concerning is that we may simply never be able to mimic the *in vivo* heterogeneity of human tumors. In an illuminating study recently presented in the *New England Journal of Medicine*, Gerlinger and colleagues[18] analyzed intratumoral heterogeneity in four patients with renal carcinomas. They reported that an individual tumor could have distinct differences in gene expression and combinations of genetic mutations depending on where the biopsy specimen is taken. Even if a patients' tumor is homogeneous, the treatment received will probably be extremely variable. For example, one patient recently enrolled in a protocol at the National Cancer Institute's Surgery Branch that used antitumor antigen gene–engineered T cells had been extensively pretreated with the following chemotherapy agents within the previous two years, including (in order) rituximab, cyclophosphamide, vincristine, prednisone, rituximab, cyclophosphamide, hydroxydoxorubicin, vincristine, prednisone, and two more cycles of rituximab. Personalized medicine must face the reality that not only may every tumor have unique spatially distinct combinations of mutations but also many patients will have been heavily pretreated with different combinations of chemotherapy agents.

Even if one could transplant a reasonable sample of a primary human tumor, the environment in which it propagates is vastly different from that of the original human host. The most important difference is probably the tumor stroma. Because the tumor stroma will be of murine origin, all of its constituents will be murine. These cell types include endothelial cells, pericytes, fibroblasts, tumor-associated macrophages, and myeloid-derived suppressor cells, to name a few.[19] The stromal cells are not merely a scaffold on which tumor cells grow. Rather, they participate actively in tumor formation, progression, and metastasis; produce numerous unique combinations of cytokines/chemokines; and manufacture an extracellular matrix with a variety of adhesion molecules.

Good translation research should aim at the development of novel clinical therapies that can be tested in a given patient population to attempt to treat disease with minimal or manageable side effects. Several recent studies have sought to make more realistic tumor xenograft models by using primary tumor samples or modeling metastasis by first implanting a primary tumor followed by surgical removal and then treating the resulting metastasis.[20–22] Although these models may be more representative than subcutaneous injection of an established tumor cell line, they still present many of the challenges mentioned above.

Bad translational-research designs use tumor xenografts in an unrealistic treatment model. An experimental design that aims to prevent the formation of micrometastases based on short-term (a few days) growth of implanted or injected tumors has little bearing on the situation that most cancer patients face and clinicians are trying to treat, yet you will encounter this experimental design in nearly any issue of your favorite cancer journal. One should also question the relevance of the direct intratumoral injection of effector cells, which is very frequently reported but has limited clinical application. Another example of the *bad* use of tumor xenografts concerns the targeting of tumor-specific mutations. For example, one common variant of epidermal growth factor receptor (*EGFR*) found in about 30% of glioblastoma and head and neck cancers is EGFR variant III (EGFRvIII).[23] EGFRvIII is not known to be expressed in any normal tissue, and this rearrangement is lost when glioma cells are propagated in culture. Is there truly any valuable information to be gained by setting up an artificial situation in which a cell line engineered to overexpress a tumor-specific mutation such as EGFRvIII is implanted subcutaneously into an immune-deficient mouse, followed by a cell or gene therapy product that you know kills these cells in a dish? Would you expect the same cell or gene therapy product to react in the xenograft situation as it would in an immune-competent human with glioblastoma? The realities of the vagaries of clinical research are such that it is difficult to predict the outcome of first-in-human trials of any cell and gene therapy product with great certainty. Ultimately, good clinical trial design is the only way to answer questions concerning efficacy and safety of cell and gene therapy products.

The *ugly* use of tumor xenograft models is to use the mouse as a simple walking test tube, and the more dangerous application is to interpret a lack of toxicity as an indicator of patient safety. One example of the walking test tube, I suggest, is testing oncolytic viruses in tumor xenografts where the host range of that virus differs substantially between the mouse and human (e.g., adenovirus). Another example from cell therapy research entails mixing the tumor target cell with the antitumor T cells and simultaneously injecting these into a mouse. I believe that this use of an animal's blood as a surrogate for an *in vitro* cell lysate assay is also unethical. Finally, regulatory agencies need to take responsibility and stop asking for mouse safety studies that have little chance of identifying potential adverse events in humans.

Beyond the scientific and ethical concerns of the appropriate use of tumor xenograft models, there are practical issues. An NSG mouse can cost upwards of $100 per animal, with a single experiment costing thousands of dollars and a research project adding up to a postdoctoral salary. It is economically unsustainable for many investigators to pursue this avenue of research, and we may thus be missing out on potentially interesting research results because a reviewer and editor think that a given report lacks an *in vivo* experiment to confirm interesting *in vitro* findings.

I propose the following criteria for the use of tumor xenografts in cell and gene therapy research. Contributors, reviewers, and editors must take collective responsibility for the misuse of tumor xenograft models in cell and gene therapy research. Misuse is defined as an experiment that does not provide any value to the translation of the work to the clinical disease model under study. A contributor who feels that a specific tumor xenograft model is relevant should state clearly why he or she believes this is the case.

For a case in which a contributor states the rationale for not presenting data using a tumor xenograft model and a reviewer disagrees, the reviewer must justify why he or she feels the model is essential. The editor must then consider whether the animal model is essential for the particular study and state his or her views explicitly in the reply letter.

Molecular Therapy has the responsibility to disseminate outstanding research from the extremely diverse group of investigators who study cell and gene therapy. It is time that we all consider the extent to which it is appropriate to continue to utilize tumor

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xenograft models in situations where their use adds little to our understanding of tumor biology and to the potential development of cell and gene-based therapies for cancer patients.

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Is the Induction of Tumor Cell Senescence the Key to a Good Irradiated Tumor Vaccine?

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Along with surgery and chemotherapy, radiation therapy (RT) is a mainstay of both curative and palliative anticancer treatment. Recent US Food and Drug Administration approval of two immunotherapeutic agents brings immunotherapy into this clinical armamentarium. There is strong emerging rationale for a clinical partnership between RT and immunotherapy in cancer treatment, based primarily on advances in our understanding of the molecular basis of the activation of the innate immune system. In this context, in this issue of *Molecular Therapy*, Meng *et al.* present work that further refines our understanding of the immunological mechanisms underlying the systemic effects of RT.[1] The new work shows that RT-induced senescence is the key event driving antitumor immunity in their model system. The report corroborates other studies in preclinical animal models that demonstrate that ionizing radiation (IR) markedly alters the tumor microenvironment.[2,3] IR increases tumor cell immunogenicity by improving antigen processing and presentation as well as by engendering immunogenic tumor cell death, which, together with the production of intratumoral proinflammatory cytokines and chemokines, links innate immune system activation to the development of a broadening adaptive cellular immune response that is directed toward tumor cells outside the radiation field.[2–4]

RT combined with radiosensitizing chemotherapy is the standard of care for inoperable, locally advanced cancers, and it is curative in about half of cases. Because cure implies eradication of both locoregional and distant disease, the curative potential of combined-modality therapy is believed to result indirectly from improved locoregional control rates, although chemotherapy delivered at radiosensitizing doses may act directly against systemic micrometastases.[5,6] As was previously shown in human tumor cells,[7,8] the new study by Meng *et al.* shows that combined treatment of B16SIY murine melanoma cells with the poly(ADP–ribose) polymerase (PARP) inhibitor, veliparib, and ionizing radiation (V+IR) induced hallmarks of cumulative DNA damage, persistent cell cycle arrest, and accelerated senescence *in vitro* and *in vivo* that were significantly greater than either treatment alone. Moreover, the combined treatment significantly slowed tumor growth in an immunocompetent syngeneic murine host, similar to what had been previously observed with human tumor cells in immunodeficient murine hosts.[1,7,8] It seems that delayed tumor growth resulted from paracrine factors from senescent tumor cells (STCs), because tumor growth slowed progressively when seeded with an increasing number of admixed purified STCs.

Gene expression analyses of V+IR-treated tumor cells confirmed the senescence phenotype and demonstrated significantly increased expression of cytokine and chemokine genes. This expression pattern differed from that reported as the senescence-associated secretory phenotype,[9] because interleukin-6 expression was reduced and expression of interferon-β (IFN-β) and its inducible chemokine, CXCL11, which attracts activated T cells, was significantly increased. Furthermore, intratumoral STCs were shown to express IFN-β as well as various chemokines. In

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