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Human Cancer Growth and Therapy In NOD/SCID/IL2Rγ^{null} (NSG) Mice

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Abstract

Since the discovery of the “nude” mouse over 40 years ago, investigators have attempted to model human tumor growth in immunodeficient mice. The field has advanced significantly over the ensuing years due to improvements in the murine recipient of human tumors. These improvements include the discovery of the *scid* mutation and development of targeted mutations in the recombination activating genes 1 and 2 (*Rag1*^{null}, *Rag2*^{null}) that severely cripple the adaptive immune response of the murine host. More recently, mice deficient in adaptive immunity have been crossed with mice bearing targeted mutations designed to weaken the innate immune system, ultimately leading to the development of immunodeficient mice bearing a targeted mutation in the IL2 receptor common gamma chain gene (*IL2rγ*^{null}). The *IL2rγ*^{null} mutation has been used to develop several immunodeficient strains of mice, including the NOD-*scid IL2rγ*^{null} (NSG) strain. Using NSG mice as human xenograft recipients, it is now possible to grow almost all types of primary human tumors *in vivo*, including most solid tumors and hematological malignancies that maintain characteristics of the primary tumor in the patient. Programs to optimize patient-specific therapy using patient-derived xenograft (PDX) tumor growth in NSG mice have been established at several institutions, including The Jackson Laboratory. Moreover, NSG mice can be engrafted with functional human immune systems permitting for the first time the potential to study primary human tumors *in vivo* in the presence of a human immune system.

Introduction

The use of human tumor cell lines has led to increased understanding of the molecular events that lead to malignancy and fueled the promise of rapid advances in cancer therapy. However, many of the mechanisms responsible for tumorigenicity are altered during cell culture, and drugs that show efficacy *in vitro* against human tumor cell lines are often ineffective when translated to primary tumors in patients. This can be due to many factors, including genetic alterations that occur through multiple passages of the cell line, lack of

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appropriate human tumor stromal-associated cell populations that are important for tumor growth and maintenance, lack of an immunologic environment in which the tumor is exposed to *in vivo*, and issues associated with vascularization and the three-dimensional structure of the tumor *in vivo* as compared to a two-dimensional cell layer *in vitro*. In attempts to overcome these issues, human tumor cell lines and solid human tumors were first engrafted into nude mice and later into early models of *scid* mice, but these early models have shown limited success for investigating the pathogenesis of tumors and the efficacy of drugs on their growth and survival.

Therefore, a longstanding goal for cancer investigators has been to engraft in an animal model primary patient-derived human tumors (referred to as patient-derived xenografts, or PDX) that maintain the genetic, phenotypic, and functional characteristics of the primary tumor after transplantation. To accomplish this, primary human tumors have been engrafted into numerous strains of immunodeficient mice, but until recently there has been varying and often poor success depending on the nature of the primary tumor and on the immunodeficient recipient used. This lack of success has resulted in a bottleneck in the preclinical evaluation of drugs and in their therapeutic efficacy on primary human tumors *in vivo*, leaving a major knowledge gap on drug efficacy prior to clinical translation. Recently, the development of immunodeficient mice bearing a targeted mutation in the IL2 receptor common gamma chain gene (*IL2rγ*^{null}) has permitted many of the primary human tumor types that previously could not be grown in immunodeficient mice to engraft and maintain the characteristics of the original primary patient tumor, and in certain cases exhibit the ability to metastasize in a manner similar to that seen in the primary tumor in patients.

Here, we present a short historical perspective of immunodeficient mouse models for cancer research and discuss the recent “state of the art” advances in the field. We will focus on NOD-*Prkdc*^{scid}*Il2rg*^{tmiWjl} (NSG) mice as models for PDX tumors and as avatars (representing the individual patient primary tumor) for design and evaluation of patient-specific therapy. Moreover, NSG mice can be engrafted with a functional human immune system, presenting for the first time the promising possibility for studying primary human tumor - immune system interactions *in vivo* and for testing the therapeutic efficacy of immunomodulatory drugs on human tumors without putting patients at risk. Finally, we will describe limitations in the currently available models and opportunities for overcoming these challenges.

Development of immunodeficient mouse models for the *in vivo* investigation of human tumors

Advancements in the use of immunodeficient mice for translational biomedical research in multiple fields of science has been extensively reviewed (Shultz et al. 2007; Shultz et al. 2012), and advancements in using immunodeficient mice for cancer research and therapy has closely paralleled and has been dependent on advancements and improvements in the immunodeficient murine hosts (Table 1).

Athymic *Foxn1*^{nu} (nude) mice

The first pivotal breakthrough in the use of immunodeficient mice in the study of human cancer was the discovery of congenitally athymic nude mice in the 1960s (Table 1). The nude mutation prevents development of functional T cells and provided an early model for engraftment of human cell lines derived from solid tumors (Fogh et al. 1977). However, the presence of an intact humoral adaptive immune system and an intact innate immune system, including high NK cell activity limits engraftment with most primary solid human tumors and prevents engraftment of human normal or malignant hematopoietic cells.

C.B17-*Prkdc*^{scid} (*scid*) mice

The next major breakthrough in the field of PDX modeling was the description of a spontaneous mutation in C.B17 mice termed "*scid*" (*Prkdc*^{scid}, protein kinase DNA activated catalytic polypeptide) (Bosma et al. 1983; Blunt et al. 1996). The *scid* mutation largely prevents the development of mature T and B lymphocytes of the adaptive immune system. The term "SCID" has now been adapted to refer to all severely immunodeficient strains of mice, including those expressing the *Rag1*^{null} or *Rag2*^{null} mutations. Direct comparisons have shown that C.B17-*scid* mice could be engrafted with a larger range of human solid tumors than nude mice (Phillips et al. 1989), and for the first time permitted the engraftment of low levels of human hematopoietic cells as well as certain hematological malignancies that did not grow in nude mice (Table 1).

Although more receptive as hosts for human cells and tissues as compared to nude mice, mature T and B cells develop in some C.B17-*scid* mice upon aging (Shultz et al. 2007). Additionally, C.B17-*scid* mice have an intact innate immune system including moderate NK cell activity that reduces the ability of human hematopoietic cells and PDX tumors to engraft and grow. Furthermore, it has been shown that C.B17-*scid* mouse NK cells are cytotoxic for human HSC and tumor initiating cells (TIC) (Greiner et al. 1998; Sotiropoulou et al. 2006; Tian et al. 2006), further limiting their utility for the study of tumor growth and therapy.

NOD.C.B17-*Prkdc*^{scid} (NOD-*scid*) mice

To reduce NK cell activity and other innate immune components in the murine host, we (and others) backcrossed the *scid* mutation onto several strain backgrounds, including the NOD/Lt strain background (Hesselton et al. 1995; Shultz et al. 1995). The NOD strain background confers intrinsic defects in innate immunity, including lowered NK cell activity, reduced levels of macrophage activation, abnormal dendritic cell development and function, and an absence of hemolytic complement. Combining these innate immune defects with the ablation of adaptive immunity using the *Prkdc*^{scid}, *Rag1*^{null}, or *Rag2*^{null} mutations led to a murine host more receptive for human hematopoietic cells and primary tumors. When compared directly with C.B17-*scid* mice, NOD-*scid* mice exhibit an increased ability to support engraftment with solid human cancers and with transplantable lymphomas and leukemias that either failed to grow or grew only poorly in C.B17-*scid* mice (Williams et al. 1993; Hudson et al. 1998).

One of the major regulators of human cell engraftment in SCID mice, in addition to host adaptive and innate immune systems, is the signal regulatory protein alpha (*Sirpa*) gene

(Takenaka et al. 2007). *Sirpa* is expressed on macrophages and other myeloid cell populations. The ligand for SIRPα is CD47, which is expressed on most human hematopoietic and non-hematopoietic cells. SIRPα binding to CD47 leads to a "do not eat me" signal to the macrophage, which in the absence of appropriate receptor-ligand interaction will lead to phagocytosis of the engrafted human cells by mouse macrophages. The NOD strain has a more human-like polymorphism in the *Sirpα* locus as compared to other mouse strains, enhancing the ability of human solid tumors, hematopoietic cells, and hematological malignancies to engraft in NOD-*scid* mice (Takenaka et al. 2007). However, many cancers and hematological malignancies still fail to engraft efficiently and grow in NOD-*scid* mice, largely due to remaining NK cell activity and other residual innate immune function.

NOD-*Prkdc*^{scid} *IL2rg*^{null} (NSG) mice

A major leap forward in the engraftment of primary human cells, tissues, and tumors was the development of immunodeficient mice bearing a targeted mutation in the IL2-receptor common gamma chain gene (*IL2rg*^{null}). The *IL2rg* gene is responsible for high affinity signaling for the IL2, IL4, IL7, IL9, IL15, and IL21 receptors, and lack of signaling through these receptors cripples both the adaptive and innate immune system (Cao et al. 1995; DiSanto et al. 1995; Ohbo et al. 1996). When combined with the *scid, Rag1*^{null}, or *Rag2*^{null} mutations, a mouse completely deficient in adaptive immunity and severely deficient in innate immunity is generated that is highly receptive to engraftment of human cells, tissues, and primary tumors (Shultz et al. 2007; Shultz et al. 2012).

Three major strains of immunodeficient *IL2rγ*^{null} mice have been developed, and their characteristics have been reviewed (Shultz et al. 2007; Shultz et al. 2012). These immunodeficient *IL2rγ*^{null} mice differ in strain background (NOD *vs.* BALB/c *vs.* BALB/c × 129 mixed background), the mutation used to induce their immunodeficiency in the adaptive immune system (*scid* or *Rag1*^{null} or *Rag2*^{null}), and in the mutation in the *IL2rγ* gene itself (complete null or truncated). These variables result in differences in their ability to be engrafted with human cells and tissues, particularly in engraftment of human hematopoietic cells, immune systems, and primary malignancies (Shultz et al. 2012). NODShi.Cg-*Prkdc*^{scid} *Il2rg*^{tm1Sug} (NOG) mice have also been successfully used as hosts for primary human tumors (Nakamura and Suemizu 2008). The major difference between the NSG and NOG strains is that the *Il2rg* targeted mutation used to develop the NSG strain is a complete null so that no IL2rg is expressed and cytokines cannot bind while the *Il2rg* mutation in the NOG strain produces a protein that is expressed and will bind cytokines but cannot signal. For the purposes of this chapter, we will focus on the use of NSG mice for the study of engrafted human PDX tumors. Direct comparisons revealed that many more human cancers, particularly hematological malignancies, can be engrafted and studied in NSG mice than can be engrafted in NOD-*scid* mice (Agliano et al. 2008).

NSG mice engrafted with human PDX tumors as models for testing drug efficacy

Almost all classes and types of primary solid or hematological human tumors transplanted into NSG mice have grown, providing new *in vivo* models for evaluation of tumorigenicity, identification of tumor initiating cells, and *in vivo* analyses of therapeutic efficacy of drugs on primary human tumors without putting patients at risk.

Solid tumors

A wide variety of solid human tumors can be grown in NSG mice (Table 2). In many cases tumor growth is dependent on the site of implantation (See protocol 1).

The characteristics of the solid tumors grown in NSG mice appear to closely recapitulate the genetic and phenotypic characteristics of the primary tumor in patients. This provides the opportunity to test drug therapy on the primary tumor *in vivo* and to correlate the genetics of the primary tumor with drug efficacy. Aggressive cancers are thought to induce stroma more efficiently and the stroma is considered an important target for drug therapy (Engels et al. 2012). A major advance in the use of NSG mice as recipients of solid PDX tumors is the ability to maintain the stromal cell population following transplantation into the primary recipient. In NSG recipients the stromal component of a subcutaneously transplanted non-small cell lung carcinoma remained intact, and was similar morphologically to the stromal components of the primary tumor (Simpson-Abelson et al. 2008). This included the passenger T cells present in the stromal compartment of the PDX lung tumor. These T cells remained quiescent, as was observed in the primary tumor. Interestingly, when tumor-bearing mice were injected with human recombinant IL12, the human passenger leukocytes (which were predominately $CD3^+$ T cells) became activated, acquired a memory/effector phenotype including production of IFNγ, and migrated from the tumor to the liver, lung, and spleen of the NSG host (Simpson-Abelson et al. 2008).

Hematological tumors

A wide variety of human hematological malignancies can be grown in NSG mice (Table 2). The ability to use engrafted human hematological malignancies for preclinical analyses of drug efficacy is exemplified by the use of an anti-CD47 antibody for the treatment of human acute lymphoblastic leukemia (ALL) (Chao et al. 2011a). Although most ALL patients have a favorable prognosis using standard chemotherapy, a subset of high risk patients express high levels of CD47, resulting in protection from phagocytosis. (Subramanian et al. 2006). Transplantation of ALL from the high CD47 tumor-expressing patients led to robust engraftment of $CD47^+$ tumor cells in NSG mice. Treatment of tumor-bearing mice with a blocking anti-human CD47 antibody led to tumor cell phagocytosis by host mouse macrophages (Chao et al. 2011a). Similar therapeutic effects were observed using a blocking anti-human CD47 antibody for treatment of human acute myelogenous leukemia (AML) (Majeti et al. 2009) and aggressive metastatic leiomyosarcoma tumors (Edris et al. 2012b) engrafted in NSG mice. Anti-human CD47 antibody acted synergistically with anti-human CD20 antibody (Rituximab) in NSG mice engrafted with non-Hodgkin's lymphoma. Interestingly, many human tumors express CD47, including solid tumors such as ovarian,

breast, colon, bladder, glioblastoma, hepatocellular carcinoma, and prostate. Anti-human CD47 antibody has therapeutic effects on the growth of these tumors in NSG mice (Willingham et al. 2012). This latter report illustrates the power that the ability to engraft multiple types of human PDX tumors into NSG mice permits for evaluation of drug efficacy.

Immunodeficient mice engrafted with human PDX tumors as models for immunotherapy

One of the major goals for the use of humanized mice is to test immunotherapeutic approaches on primary human tumors *in vivo*. One of the simplest approaches described was to test the efficacy of IL27 on pediatric AML. Leukemia dissemination was severely hampered by recombinant human IL27 treatment of PDX AML engrafted in NSG mice, and genetic analyses of the tumors revealed a significantly reduced expression of their angiogenic and spreading-related genes (Zorzoli et al. 2012). In an example of cellular therapy, NSG mice were first engrafted with human $CD34^+$ HSC to generate a human hematopoietic and immune system (Liu et al. 2012)., The engrafted mice were injected with human neuroblastoma cells and infused with *ex vivo* expanded NKT cells. NKT cell migration into the tumor was significantly inhibited by neutralization of the chemokine CCL20 secreted by tumor-associated macrophages, which was increased in states of hypoxia at the tumor site. Injection of NKT cells transduced with human IL15 protected the NKT cells from hypoxia, and dramatically enhanced their anti-metastatic activity. These *in vivo* analyses demonstrated a new mechanism of immune escape by tumors that can be reversed by adoptive cellular therapy with IL15-transduced NKT cells (Liu et al. 2012).

In a test of T cell immunotherapy, Provasi *et. al* used zinc finger nucleases to disrupt endogenous TCR β and α chains in primary human CD8 T cells, and then transduced cells with a TCR specific for the Wilms 1 (WT1) tumor antigen expressed by leukemic blasts (Provasi et al. 2012). The CD8 T cells did not cause graft-versus-host reactivity in irradiated recipients but were able to mediate specific anti-tumor activity and lead to long term survival of NSG mice engrafted with $WT1^+$ PDXs. (Provasi et al. 2012). Another approach to engrafting human CD8 T cells having TCR specificity against tumor antigens was to transduce human HSCs with lentivirus encoding a HLA-A2-restricted TCR specific for the MART-1 melanoma antigen (Vatakis et al. 2011). Using a modified human liver/thymus immune system model commonly referred to as the BLT (bone marrow, liver, thymus) humanized mouse model (Shultz et al. 2012), human fetal liver $CD34^+$ cells were transduced with the MART-1-specific TCR and injected into BLT mice engrafted with autologous fetal liver and thymus tissues. This led to the engraftment of human CD8 TCR transgenic-expressing T cells, which cleared human MART-1-expressing melanoma tumor cell lines *in vivo*. The TCR transgenic T cells required proper thymic selection in the HLA-A2 autologous thymus fragment as they could not kill non-HLA-A2 MART-1-expressing melanoma cells (Vatakis et al. 2011).

Although these validations of CD8 TCR-mediated anti-tumor activity provided *in vivo* evidence of the efficacy of these approaches for tumor therapy, the need to provide a transgenic TCR specific for each tumor antigen of interest is a major caveat. A new

approach has been validated in tumor-bearing NSG mice as follows. A chimeric antigen receptor (CAR) was constructed in which a biotin-binding immune receptor consisting of an extracellular avidin is linked to a human CD3 signaling domain to activate T cells (Urbanska et al. 2012). Tumors can then be targeted with, for example, a tumor specific antibody that is conjugated with biotin to target the CD8 T cells transduced with the CAR to the tumor site where they become activated and acquire cytotoxic activity. This CAR approach has been shown to have therapeutic efficacy against human $EpCam^+$ tumor cells (Urbanska et al. 2012). Extending this approach, human T cells were transduced with a CAR directed to the malignant pleural mesothelioma tumor antigen mesothelin (mesoCAR) and the cells were subsequently transduced with the chemokine receptor CCR2b to localize the T cells to the tumor (Moon et al. 2011). The malignant pleural mesotheliomas secreted high levels of CCL2, the chemokine for the CCR2b receptor. Infusion of the transduced CCR2b $mesoCAR^+$ T cells into NSG mice bearing established tumors resulted in targeted T cell infiltration into the tumor and significantly reduced tumor size (Moon et al. 2011).

Finally, to study human tumor immunotherapy in the presence of a complete human immune system, NSG mice must be engrafted with HSCs that can generate a functional human immune system while at the same time support the growth of a human tumor. To begin to establish this system, NSG mice were engrafted with human $CD34^+$ HSC and injected with human breast cancer cell lines (Wege et al. 2011). The human immune system developed and tumor cells were detectable in different lymphoid and non-lymphoid organs. NK cell accumulation and activation was observed in the tumors, and was enhanced by treatment with a recombinant human IL15/IL15-alpha receptor complex. This model combines MHC-mismatched tumor cells with human immune systems for the study of human immune system/tumor interactions.

The *in vivo* analysis of these human-specific tumor therapies was possible because 1) primary human tumors grow efficiently in NSG mice, and 2) NSG mice can be engrafted with functional human immune systems. Continued exploitation of the NSG model for modeling PDX tumor growth and analyses of therapies promises great potential for preclinical evaluation of multiple immunotherapeutic approaches without putting patients at risk.

Immunodeficient mice engrafted with human PDX tumors as models for identification and directed therapy towards tumor initiating cells

Cancers exhibit remarkable heterogeneity, and there are numerous hypotheses that this heterogeneity as well as resistance to therapy is due to the presence of tumor initiating cells (TIC). This cell population, also referred to as cancer stem cells (CSC), is thought to be responsible for propagation of the tumor *in vivo* (Visvader 2011). Immunodeficient mice have been widely used to study human HSC, which are defined functionally by their ability to recapitulate growth of the entire hematopoietic system *in vivo* (Dick 2008). Similarly, immunodeficient mice have been used to identify TIC based on functionality. These cells have been defined as malignant cells that fulfills the classic stem cell criteria (i.e., the ability to undergo self-renewal and the developmental potential to recapitulate all the cell types found in a given tissue).” (Krumbach et al. 2011). This can only be accomplished *in vivo*,

and immunodeficient mice have been used to define human TIC for a number of human malignancies (Table 3). Many human TIC have been defined using NOD-*scid* mice, and more recently NSG mice (Kelly et al. 2007; Quintana et al. 2008; Ishizawa et al. 2010).

Identification of TIC in solid tumors and hematological malignancies has routinely been accomplished using cell surface phenotypic markers that can be used to sort individual cell subsets for adoptive transfer into immunodeficient recipients. For solid tumors, as an example, human brain TIC express CD133. As few as 100 $CD133^+$ brain tumor cells can develop tumors upon adoptive transfer into NOD-*scid* mice whereas adoptive transfer of as many as 1×10^5 CD133-negative brain tumor cells engrafted but failed to form tumors (Singh et al. 2003; Singh et al. 2004). For AML, TIC in the bone marrow were identified as $CD34^+CD38^-CD25^+$ or $CD34^+CD38^-CD133^+CD32^+$ using NSG mice (Saito et al. 2010a).

Although the existence of “true” TIC remains controversial (Dick 2008), the important question is whether specific drug targeting of TIC will provide better outcomes in developing patient-specific and tumor-specific therapies. This may in part be determined by the specific tumor being studied. For example, two extremes for the frequency of TIC in tumors are melanoma and AML. Primary human melanoma can be grown in NSG mice by injection of as few as 100 unfractionated tumor cells, and indeed, 27% of single cell melanoma transplants can form tumors in NSG mice (Quintana et al. 2008). These results demonstrate that the functional TIC frequency in this population is extremely high. In contrast, engraftment of AML in only 38% of sublethally irradiated newborn NSG mice was observed following injection of 4 million T cell-depleted bone marrow cells containing 80-90% blasts (Ishikawa et al. 2007; Ishikawa et al. 2008). These data suggest that essentially all the melanoma cells in the patient must be eradicated for effective therapy of the tumor, whereas fewer TIC are the key targets for treatment of AML. Identification and targeting of this small TIC cell subpopulation in AML may provide an effective therapeutic approach for curing AML in patients.

The frequency of the TIC population can vary widely between each of the tumor types being studied and the immunodeficient host being used, as demonstrated for melanoma, changing the immunodeficient host increased the detection of the frequency of melanoma TIC from 0.0001-0.1% in NOD-*scid* mice to 27% in NSG mice. A similar increase in TIC frequency in NSG mice as compared NOD-*scid* mice for squamous cell lung carcinoma was observed (Ishizawa et al. 2010). In contrast, no differences in TIC frequency in pancreatic adenocarcinoma or head and neck squamous cell carcinoma were observed when limiting numbers of the tumor cells were transplanted into NOD-*scid vs*. NSG mice (Ishizawa et al. 2010). These data suggest that the detection and frequency of functional TIC will increase dramatically in many, but not all tumors analyzed using NSG mice.

The NSG model is particularly useful for testing the efficacy of therapies aimed at eradicating TIC. In solid tumors, the TIC for high-grade glioma cells has been shown to express high levels of IL13 receptor α2 (Brown et al. 2012), representing a potential target for tumor therapy. Similar information has been obtained for hematological malignancies. The TIC for AML has been identified, and when injected into NSG mice, has been found to

engraft in a quiescent form in an osteoblast-rich bone marrow niche that protects it from traditional chemotherapy that reduces the tumor burden, but does not eliminate the TIC in the marrow (Ishikawa et al. 2007). Administration of G-CSF led these TIC to enter the cell cycle and become susceptible to cell cycle-dependent chemotherapy resulting in apoptosis and elimination (Saito et al. 2010b). Although counter-intuitive that a cell cycle inducer in combination with chemotherapy would be important for eliminating AML tumor cells, this study provides preclinical evidence for efficacy of this approach. Furthermore, when AML TIC were defined phenotypically by adoptive transfer into NSG mice and isolated for genomic analyses, a TIC gene signature profile identified novel molecules in the TIC population as targets for potential therapies (Saito et al. 2010a).

Phenotypically identifying TIC can also provide valuable predictive information for clinical outcomes in patients. In breast cancer, two TIC populations have been identified based on their phenotypic characteristics as basal-like ($CD271^+$) or luminal-like (milk $mucin^+$, identified by the M18 antibody that recognizes branched glycans). NSG mice can provide specific information on a patient's tumor that can be used diagnostically, and when combined with genomic analysis of the TIC, provide prognostic information. Identification of TIC can also facilitate new treatment regimens aimed at preventing patient relapse. $CD34^+$ cells were identified as TIC in human chronic myelogenous leukemia (CML) using NSG mice (Zhang et al. 2010). Treatment of patients with a tyrosine kinase inhibitor imatinib mesylate (Gleevec) induces apoptosis in dividing CML cells and drives patients into remission. However, when treatment is withdrawn, there is a significant relapse rate, suggesting that quiescent CML tumor initiating cells survive Gleevec treatment. Using NSG mice, $CD34^+$ CML TIC and progenitor cells were engrafted, and the combination of Gleevec plus a histone deacetylase inhibitor that induces apoptosis in quiescent tumor cells was able to eliminate the tumor in the mice, and no relapse occurred following cessation of treatment (Zhang et al. 2010).

PDX Programs

A number of facilities have established PDX programs for studying patient tumor growth and response to experimental therapy *in vivo* without putting patients at risk. Figure 1 shows a generic diagram of PDX programs for evaluation of solid human tumors. For the engraftment of human leukemias (not shown), young adult or newborn NSG mice are engrafted intravenously, or in the case of adult recipients, cells may be injected directly into the bone marrow cavity (intrafemoral injection). Recipients may be conditioned with sublethal irradiation prior to engraftment.

The PDX program at The Jackson Laboratory

To establish a PDX resource for the global research community, the Jackson Laboratory Primary Human Tumor Consortium has been formed between The Jackson Laboratory, UC-Davis Comprehensive Cancer Center and over 20 additional cancer centers. This high throughput operation enables the study of PDX tumors under standard conditions. Solid and hematological patient tumor specimens are engrafted into NSG mice and the first generation PDX tumors are compared with the primary tumor for fidelity of histopathology, gene

expression and human SNP copy number variance (CNV). The PDX tumors are maintained at low passage so that patient tumor heterogeneity and histology fidelity are maintained and models can be selected for biomarker-driven translational medicine studies. Solid tumor PDX models are passaged as tumor fragments to maintain the tumor and for tissue fragment cryopreservation. By November 2012, the program has engrafted over 600 patient specimens and established 152 PDX models with a 35% tumor model establishment rate across 17 cancer therapeutic areas (N. Goodwin, unpubl.). The top five indications include 34 lung cancer, 26 brain cancer, 15 colorectal cancer, 14 bladder cancer, and 14 pancreatic cancers. The program has the capacity to generate by the 4^{th} in vivo passage generation thousands of PDX tumor-bearing mice for high-throughput drug efficacy studies. Patient tissue sources include solid primary resected tumors, advanced metastases biopsies and pleural effusions, and leukapheresis and bone marrow aspirate specimens from hematological malignancies.

This approach is based on the recognition that tumors, such as non-small cell lung cancer are in fact quite heterogeneous tumors with multiple complex mechanisms underlying tumor development. Moreover, the genomic characterization of PDX tumors in immunodeficient mice has shown that the patient's primary tumor genomic profile is recapitulated in the PDX tumor derived from the explants (Monsma et al. 2012). Combining genomics with targeted cancer therapy is now possible to provide guidance for patient-specific therapies targeted to the particular molecularly-defined pathways that underlie tumor pathogenesis (Gandara et al. 2012).

In a study of human bladder cancer the PDX program at The Jackson Laboratory has teamed up with investigators at the University of California Davis. Bladder cancer is particularly difficult to treat in the clinic. By engrafting PDX-derived bladder cancers both subcutaneously and in the bladder cell wall, solid and invasive tumor models were established in NSG mice. The tumor-bearing mice were treated with nanoparticles coated with an amino acid sequence that recognized a bladder cancer-specific ligand allowing targeting to bladder cancer cells. These nanoparticles could deliver 3-times the systemic toxic dose of the drug with minimal side effects, and demonstrated therapeutic efficacy against both the solid and invasive bladder tumors established in NSG mice, resulting in prolonged overall survival as compared to control treated tumors. Moreover, the specificity of the nanoparticles was demonstrated in NSG mice engrafted with bladder tumors versus H232A lung cancer cells implanted in the contralateral flank. These data led directly to a clinical trial using the newly formulated nanoparticles (Lin et al. 2012).

Avatar mice

Can NSG mice engrafted with PDX tumors truly become Avatar's for primary patient tumors in predicting how the tumor will respond to therapy in the clinic? In fact, this is already happening. In a recent study out of Johns Hopkins, primary solid tumors as well as metastatic tumors from patients with refractory advanced cancers were transplanted into immunodeficient mice. These recipients were used to test various therapeutics to determine the best available approaches for treating individual patients (Hidalgo et al. 2011). In 14 patients in which therapies guided by results in PDX-bearing immunodeficient mice were

developed, treatments were initiated based on the PDX results in 11 of the patients, which proved to have therapeutic efficacy. In 2 patients, no effective treatment in the PDX-bearing immunodeficient mice was identified, and 1 patient died prior to initiation of treatment therapies developed in the PDX-bearing immunodeficient mice. PDX mice have also proved valuable in recent studies of metastasis. Engraftment of primary human stage III cutaneous melanomas into NSG mice has shown that metastasis in the recipient mice correlates with patient-specific clinical outcomes. Human melanomas that metastasized efficiently in the mice eventually progressed to advanced stage IV disease in the patients (Quintana et al. 2012). These findings using PDX mice as avatars suggest that precision medicine using immunodeficient mice as avatars for study of patient tumors may provide personalized medicine for therapeutic decisions and cancer treatment in the clinic.

Remaining challenges and future directions

A note of caution: growth of a tumor following transplantation of the primary tumor does not always mean that the engrafted tumor is representative of the original transplanted tissue. Infections from a number of microorganisms are considered as risk factors for development of cancer in humans and approximately 20% of human cancers have been associated with infection by viruses, bacteria and parasites (Mazza 2010). Infectious agents carried by the primary tumor may induce tumors different than the transplanted primary tumor of human or even mouse origin. In a case of hepatocellular carcinoma, it has been reported that the tumors that grew at the implantation site were in many cases not the primary tumor, but were EBV-induced lymphoblastoid tumors that resulted from the outgrowth of the passenger B cells (Chen et al. 2012).

There are remaining challenges and opportunities. Novel NSG stocks under development transgenically express human HLA class I and class II molecules and microenvironmental factors, lack mouse MHC class I and II molecules, and have additional deficits in innate immunity. These stocks will enable novel approaches such as co-engraftment with PDX tumors and patient T cell populations (Shultz et al. 2012). A number of NSG stocks of mice have been developed that express human growth factors (Shultz et al. 2012). Many of these factors are species specific. For example, many myelomas are dependent on IL6 for their growth *in vivo*. Mouse IL6 will not support the growth of human myeloma cells (Peters et al. 1996), and most primary human myeloma cells fail to engraft in NSG mice. Engraftment with fetal human bone supports the growth of primary human myeloma grafts perhaps through the production of human IL6 (Kim et al. 2012a). Development of a NSG host that transgenically expresses human IL6 may permit better growth of IL6-dependent myelomas. AML engraftment efficiencies were also improved in NSG mice that transgenically expressed human IL3, GM-CSF and SCF (Wunderlich et al. 2010). Similar approaches may be used for enhancement of human breast cancers that require prolactin as mouse prolactin has a low affinity for human prolactin receptors (Utama et al. 2006). Furthermore, ubiquitously expressed GFP in NSG mice is being evaluated as a model for discriminating human-derived tumor stroma from mouse stroma. The maintenance of human tumor stroma is critical as a drug target in cancer (Ahmed et al. 2008) as well as functioning as a mediator in drug resistance (Sebens and Schafer 2012). The role of tumor-stromal interactions is described in a recent study demonstrating that CML stem and progenitor cells are protected

from TKI-mediated inhibition in the presence of BM stromal cells (Zhang et al. 2012a). Finally, patient-derived stem cell populations, such as mesenchymal stem cells, might be able to replace tumor-associated stromal cells that are lost during multiple *in vivo* passages of the tumors (Fig. 1). These emerging stocks of NSG mice are providing novel tools for engraftment of primary human tumors and will contribute to the advancement of patient-specific therapy.

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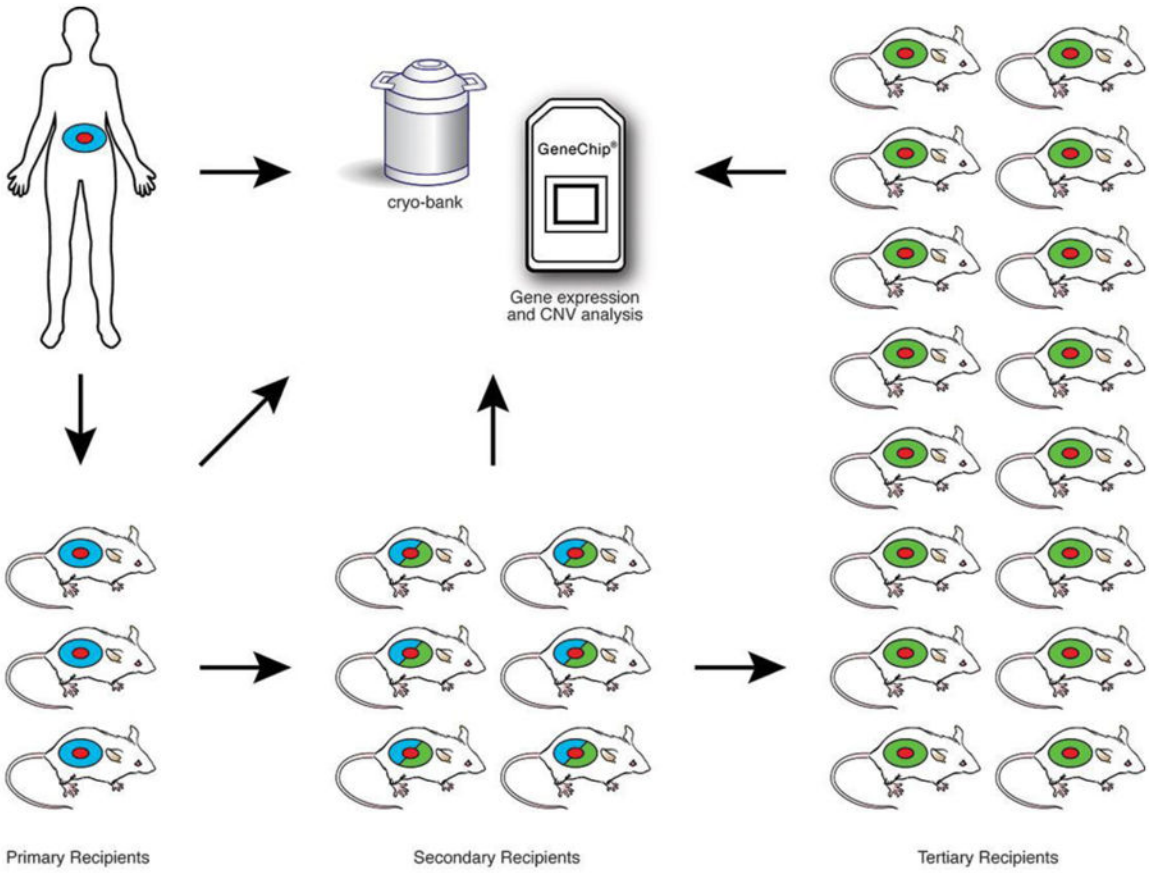

Figure 1.

Engraftment and expansion of patient-derived solid tumor xenografts. Young adult NSG mice are engrafted with solid tumors (shown as red) Human stroma accompanying the tumor is shown in blue. Primary solid tumors are engrafted subcutaneously, orthotopically, or under the renal capsule. Established grafts are excised from primary recipients, a portion is cryopreserved, and a portion is analyzed for gene expression, including copy number variation (CNV). The remainder is expanded through serial transplantation in secondary and tertiary NSG recipients to generate cohorts of sufficient size for therapeutic trials. At each stage tumor samples are cryopreserved and gene expression analyzed to compare with the primary tumor analyzed directly *ex vivo* from the patient. The primary recipients of solid tumors retain human-derived stroma (blue). Tumors in secondary recipients contain a mixture of human-derived and mouse-derived stroma (mixture of blue and green). Tumors in tertiary recipients contain predominantly mouse stroma (green).

Table 1

Engraftment of human tumors and immune systems in strains of immunodeficient mice

Common Strain Name	Mutant gene Nomenclature	Solid Tumor Cell Lines	Primary Solid Tumors	Leukemias	Human Immune Systems	References
Nudea[a]	*Foxn1*^{nu}	+	+/-	-	-	(Fogh et al. 1977; Sharkey and Fogh 1984; Giovanella and Fogh 1985; Price and Zhang 1990)
C.B17-*scid* BALB/c-*scid*	*Prkdc*^{scid}	+	+	+/-	+/-	(Reddy et al. 1987; Kamel-Reid et al. 1989; Charley et al. 1990; Mueller and Reisfeld 1991; Cesano et al. 1992; Taylor et al. 1992; Itoh et al. 1993; Flavell 1996; O'Reilly et al. 1996; Sakakibara et al. 1996; Uckun 1996; Visonneau et al. 1998)
NOD-*scid* NOD-*Rag1*^{null}	*Prkdc*^{scid} *Rag1*^{tm1Mom}	++	++	+	+	(Baersch et al. 1997; Bonnet and Dick 1997; Hudson et al. 1998; Wang et al. 1998; Ailles et al. 1999; Fusetti et al. 2000; Pilarski et al. 2000; Appelbaum et al. 2001; Frost et al. 2004; Huang et al. 2004; Eisterer et al. 2005; Xue et al. 2005; Pearce et al. 2006; Li et al. 2007)
NSG	*Prkdc*^{scid} *Il2rg*^{tm1Wjl}	+++	+++	++	+++	(Shultz et al. 2007; Shultz et al. 2012)

[a]Includes studies of nude mice on non-inbred and a variety of inbred strain background

Table 2

Engraftment of human primary tumors in NSG Mice

Solid Tumors	**References**
Brain	(Silvestre et al. 2011; Brown et al. 2012; Cournoyer et al. 2012)
Breast	(Valdez et al. 2011; Kim et al. 2012b)
Colon	(Dieter et al. 2011)
Fallopian tube carcinoma	(Khabele et al. 2012)
Hepatocellular carcinoma	(Colombo et al. 2011; Chen et al. 2012; Martin-Padura et al. 2012)
Lung	(Simpson-Abelson et al. 2008; Akunuru et al. 2011; Zhang et al. 2012b)
Melanoma	(Quintana et al. 2008; Boonyaratanakornkit et al. 2010; Ma et al. 2010; Quintana et al. 2010; Kupas et al. 2011)
Ovarian	(Bankert et al. 2011; Stewart et al. 2011)
Prostate	(Goldstein et al. 2010)
Sarcoma	(Seitz et al. 2010; Edris et al. 2012a; Ono et al. 2012; Zhang et al. 2012c)
Bladder	(Lin et al. 2012)
Hematopoietic Malignancies	
Acute myeloid leukemia	(Ishikawa et al. 2007; Agliano et al. 2008; Distler et al. 2008; Taussig et al. 2008; Majeti et al. 2009; Sanchez et al. 2009; Saito et al. 2010a; Saito et al. 2010b; Goardon et al. 2011; Sarry et al. 2011; Gerber et al. 2012; Vargaftig et al. 2012)
Acute lymphocytic leukemia	(Kong et al. 2008; Bonapace et al. 2010; Chiu et al. 2010; Medyouf et al. 2010; Morisot et al. 2010; Clappier et al. 2011; Castro Alves et al. 2012)
Chronic lymphocytic leukemia	(Bagnara et al. 2011; Kikushige et al. 2011; Wong et al. 2012)
Chronic myelogenous leukemia	(Zhang et al. 2010)
Non-Hodgkins Lymphoma	(Chao et al. 2010; Chao et al. 2011b)
Blastic NK cell lymphoma	(Agliano et al. 2011)
Myeloma*	(Mirandola et al. 2011; Kim et al. 2012a)

*Includes studies in NRG mice

Table 3

Identification of human tumor initiating cells following engraftment in NOD-*scid* and NOD-*scid IL2rγ*^{null} mice

Solid Tumors	**Timor Initiating Cell Phenotype**	**Reference**
Brain	CD133+	(Singh et al. 2003)
Pancreas	$CD44^+CD24^+ESA^+$	(Li et al. 2007)
Breast	$CD44^+CD24^{-/low}$ & Milk mucin $(MM)^+$ and/or $CD271^+$	(Al-Hajj et al. 2003; Kim et al. 2012c)
Colon	$CD133^+$ $CD133^+$ and $CD133^-$	(O'Brien et al. 2007; Ricci-Vitiani et al. 2007) (Shmelkov et al. 2008)
Glioma	high levels of IL13 receptor α2	(Brown et al. 2012)
Head and Neck Squamous Cell Carcinoma	$CD44^+Lin^-$	(Prince et al. 2007)
Leiomyoma	Leiomyoma-derived side population (LMSP) identified by Hoechst 33342 dye staining	(Ono et al. 2012)
Melanoma	$CD127^+$ or $CD127^-$ CSC phenotypically diverse TIC	(Quintana et al. 2008)
Ovarian	Both $CD133^+$ and $CD133^-$ populations	(Stewart et al. 2011)
Prostate	$CD49f^{hi}Trop2^{hi}$	(Goldstein et al. 2010)
	$CD44^+$	(Patrawala et al. 2006)
Hematological Tumors	**Tumor Initiating Cell Phenotype**	**Reference**
Acute Myelogenous Leukemia	$CD34^+CD38^-$ $CD34^-CD33^+$ (NPM mutated AML)	(Bonnet and Dick 1997); (Ishikawa et al. 2007) (Taussig et al. 2010)
Acute Lymphoblastic Leukemia	$CD34^+CD38^+CD19^+$ & $CD34^+CD38^-CD19^+$	(Kong et al. 2008)
	Multiple phenotypes depending on maturation stage of TIC used $CD34^+CD19^-$, $CD34^+CD19^+$, $CD34^-CD19^+$, (intrafemoral injection)	(le Viseur et al. 2008)
Chronic Myelogenous Leukemia	$CD34^+CD38^-$	(Jiang et al. 2007)
Myeloma	$CD34^+$	(Pilarski and Belch 2002)
	$CD138^+CD38^{hi}CD19^-CD45^{low/-}$	(Kim et al. 2012a)