Review

Application of highly immunocompromised mice for establishment of Patient-Derived Xenograft (PDX) model.

Seiji Okada [1,2*], Kulthida Vaeteewootthacharn [1,3,4] , Ryusho Kariya [1]

[1] Division of Hematopoiesis, Joint Research Center for Human Retrovirus Infection, Kumamoto University, Kumamoto 860-0811, Japan; okadas@kumamoto-u.ac.jp(S.O.); kulthidava@kku.ac.th (K.V.); ryushokariya@gmail.com (R.K.)
[2] Graduate School of Medical Sciences, Kumamoto University, Kumamoto 860-0811, Japan
[3] Department of Biochemistry, Khon Kaen University, Khon Kaen 40002, Thailand
[4] Cholangiocarcinoma Research Institute, Khon Kaen University, Khon Kaen 40002, Thailand
* Correspondence: okadas@kumamoto-u.ac.jp; Tel.: +81-9-6373-6522 (S.O.)

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Abstract: Patient-derived xenograft (PDX) models are created by engraftment of patients' tumor tissues into immunocompetent mice. Since PDX model keep the characteristics of primary patient's tumor such as gene expression profiles and drug sensitivity, it now becomes most reliable *in vivo* human cancer model. The engraftment rate are increased with the introduction of NOD/Scid based immunocompromised mice, especially, NK cell defective NOD strains such as NOD/Scid/IL2Rγ^{nu} (NOG/ NSG) mice and NOD/Scid/Jak3^{null} (NOJ) mice. Success ratio differs from the origin of tumor: Gastrointestinal tumors tend to higher success rate and breast cancer is lower. Subcutaneous transplantation is most popular method to establish PDX, but some tumor needs orthotropic or renal capsule transplantation, and human hormone treatment is needed to establish hormone dependent cancers such as prostate and breast cancer. PDX library with patient's clinical data, gene-expression patterns, mutational status, drug responsiveness and tumor architecture will be the powerful tool for developing specific biomarker and novel individualized therapy and establishing precision cancer medicine.

Keywords: patient-derived xenograft; immunocompromised mice; precision medicine; drug screening; cancer; cell line

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1. Introduction

The preclinical study using animal model is essential for drug development. However, even preclinical trial is successful, fewer than 10% of drug candidates was approved for market[1]. Success rate of oncology field drug development has been ~5%, worst of all of field [2]. It is explained that there is not appropriate animal model of human cancers. Mice tumors and human cell line transplanted animal models are not always reflected the human cancer pathogenesis and drug response [3], because mice and humans are considerably different [4] and human cancer cell lines lost the character of original tumor[5]. National Cancer Institute (NCI, USA) recently decided to retire NCI-60, a panel of 60 human cell lines from its drug screening, and use Patient-derived xenograft (PDX) with these reasons [3]. PDX is established with direct engraftment of patient's tumors into immunocompromised mice and maintained *in vivo*, which have emerged as important tool for preclinical and translational research, especially to investigate the nature of tumor and drug development. With the introduction of highly immunocompromised mice as recipients, PDX models are now widely spread and are becoming standard "Avatar" models for cancer research.

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2. Establishment of immunocompromised mice

2.1. Nude mice

In 1962, .first known immunocompromised mice, namely Nude mice, were discovered by Dr. Norman. R. Grist. Since the coat hair is lacking in this mice, the "Nude" nickname was given for the mice. Flanagan SP showed that nude mice also lacked thymus and Y lymphocytes are lacking in these mice[6]. Therefore they are lacking adaptive immune response including T cell mediated immune responses and antibody formation that requires helper T cells. Nude mice have been used as the recipient of human tumor xenografts since then, however, the there are limitations on transplantable human tumor cells due to intact (or rather activated) innate immunity [7].

2.2. SCID mice

In 1983, Bosma GC (Fox Chase Cancer Institute) first described severe combined immunodeficient (SCID) mice lacking both functional T and B lymphocytes[8]. Since Prkdc (Protein kinase, DNA activated, catalytic polypeptide: DNK-PKCs)) is lacking, V(D)J recombination does not occur and B and T lymphocytes fail to mature. The engraftment efficiency of human tumor is higher in SCID mice than nude mice [9]. SCID mice were first used as recipient of human hematopoietic stem cells (HSCs) and peripheral blood mononuclear cell (PBMC) transplantation [10,11]. However, the transplantation efficiency of human blood cells and tumor cells were not high enough, which was considered that remaining NK cells inhibited homing and maintenance of human cells. To overcome the effects of NK cells, Scid/Beige mice were established by crossbreeding SCID mice and Beige mice. The taking rate of human tumor cells are increased in Scid/Beige mice compared with Scid mice as expected. However, the engraft rate of human HSCs are not clearly increased [12].

2.3. NOD/Scid mice

In 1980, Non-obese diabetic (NOD) mice were discovered by Makino S, which develop diabetes by the infiltration of T lymphocytes into the pancreatic islets [13]. It is also showed that NOD mice multiple immune abnormalities including loss of complement, impaired NK, macrophage and dendritic cell function [14]. NOD/Scid mice were established by crossing NOD and Scid mice, which do not develop diabetes due to loss of functional T lymphocytes. NOD/Scid mice were shown to have multiple defects in innate and adaptive immunity, which provided an excellent recipient of human hematopoietic stem cell transplantation [15] and human solid tumors. Several trials were performed to suppress the residual NK activity using anti-IL-2 receptor antibody or asialoGM1 or cross with β2 microgloblin or perforin deficient mice, and improved the efficacy of transplantation. Finally NOD/Scid mice with complete loss of NK cells were established by crossing NOD/Scid mice with IL-2 receptor deficient (NOD/Scid/IL2Rγ^{nul}:NOG[16], NOD/Scid/IL2Rγ^{nul}:NSG[17])or Jak3 deficient mice (NOD/Scid/Jak3^{null} :NOJ[18])(Table 1). Recently, Signal regulatory protein alpha (SIRPα)-CD47 signaling, so called "Don't eat me" signal was shown to play an important role in tumor and graft rejection by macrophages[19], and polymorphism of SIRPα in the NOD mice strain contributes the efficient human cell engraftment into NOD strain (Figure 1) [20,21]. BALB/c mice strain also have SIRPα polymorphism with affinity to human CD47, and in fact, BALB/c strain immunocompromised mice such as BALB/c Rag-2^{null}/IL2Rγ^{null}[22]and Rag-2^{null}/Jak3^{null} mice[23] are also useful recipient mice for human cell and tissue transplantation[24,25]. Other genetic background of the mice such as C57/BL6 mice were shown to have lower efficacy to accepting human normal and malignant cells [23,26]. Since SCID mutation has several disadvantages such as high radiation and drug sensitivity and leakage of T lymphocytes, Rag-1/Rag-2 knock out mice are also using for eliminating mature lymphocytes (Table 2) [22,27].

Table 1. NOD/Scid based severe immunocompromised mice

strain	NOD/Scid	NOG	NSG	NOJ
strain	NOD.Cg-$Prkdc^{scid}$	NOD.Cg-$Prkdc^{scid}Il2rg^{tm1Sug}/Jic$	NOD.Cg-$Prkdc^{scid}Il2rg^{tm1Wjl}/SzJ$	NOD.Cg-$Prkdc^{scid}Jak3^{tm1card}$
Genetic defects	Scid	Scid, IL-2γPartial deficiency	Scid,IL-2RγComplete deficiency	Scid,Jak3 deficiency
Developer	CIEA[1], Jackson Laboratory	CIEA[1]	Jackson Laboratory	Kumamoto Univ.
Supplier	Japan Clea Charles River	Japan Clea	Charles River	Kumamoto Univ.
Reference		***Blood*** 100:3175, 2002	***J Immunol*** 174:6477, 2005	***Int J Hematol*** 88:476, 2008
NK cells	NK cell dysfunction	Complete loss of NK cells		
	Loss of mature B, T, NKT cells, Loss of complement			

[1] Central Institute for Experimental Animals (CIEA)

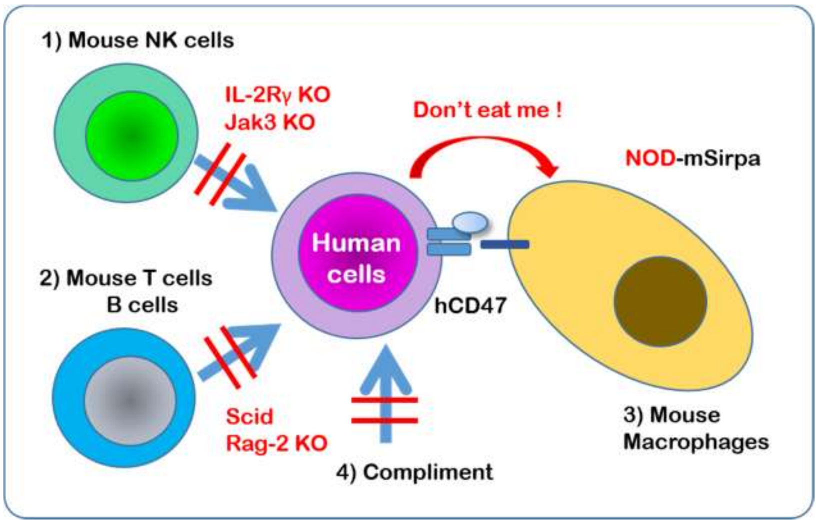

Figure 1. NOG, NSG, and NOJ mice support engraftment of human cells with multiple immune deficiencies. 1) Loss of NK cells, 2) Loss of acquired immunity by T and B lymphocytes deficiency, 3) "Don't eat me" signal by NOD-Sirpα, 4) Loss of Compliment

Table 2. Comparison of SCID and Rag-1/Rag-2 mutation

	ScCID mice	**Rag-1/Rag-2 knock out mice**
Chromosome	Chr.16	Chr.11 p13
Mutated gene	Prkdc	Recombination-activation gene-1/-2
Mutation	Natural mutant	Homologous reconbination
Repair		
Immunological phenotype	Deficiency of Mature B and T lymphocytes NK cells are normal	Deficiency of Mature B and T lymphocytes NK cells are normal
Radiation sensitivity	Sensitive (Lethal dose <3Gy)	Normal (Lethal dose 9 Gy)
Leakage	Leaky	None

3. Establishment of Nude/Hairless immunocompromised mice

Although more combined immunocompromised mice have been developed, Nude mice were still used in human tumor engraftment due to the benefit of hairless phenotype. It is easy to detect subcutaneous tumors and its application for *in vivo* imaging. We crossed Nude mice with Rag-2^{null} and Jak3^{null} mice with a BALB/c background and established BALB/c Nude Rag-2/Jak3 double deficient (Nude RJ) mice[28,29]. Nude RJ mice has no B and T lymphocytes with Rag-2 deficiency, no NK cells with Jak3 deficiency, and had "Don't eat me signal" with BALB/c background. Nude RJ mice keep the advantages of no coat hair and higher immunocompromised level than Nude mice, and consequently, optimized for *in vivo* imaging (Figure 2). The mice expressing fluorescent protein are powerful tool in cancer research to visualize the tumor-host interaction[30], and several types of fluorescence expressing immunocompromised mice are established and utilized for human cancer research [31-33]. These mice are useful to analyze the relation with human tumor and tumor microenvironment such as tumor vessel, tumor associated macrophages (TAM) and cancer associated fibroblasts (CAF) [34]. There exists another type of no coat hair mice, hairless mice, without major immunodeficiency [35,36]. SCID hairless (SHO) mice (Charles River) and Hairless NOD/Scid mice (Envigo) were established backcrossing with Hairless mice and also using *in vivo* imaging (Table 3) [37,38]. However, expected engraftment efficiency is lower than NK deficient strains.

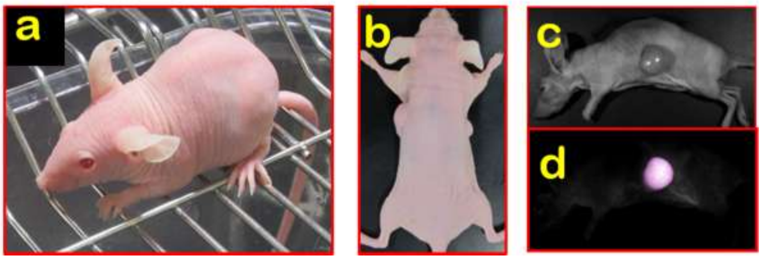

Figure 2. Nude RJ mice. Nude RJ mice keep no coat hair phenotype (a), easy to observe subcutaneous tumors (b), and optimized for *in vivo* fluorescent imaging (c, d).

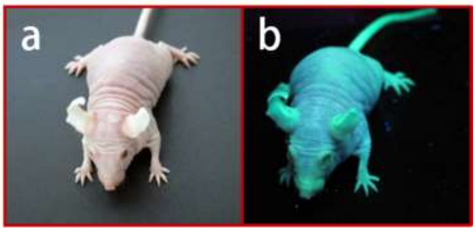

Figure 3. GFP Nude RJ mice. Transgenic Nude mice with ubiquitous green fluorescent protein (GFP) expression (β-actin promoter) (a) fluoresced very bright green with UV light (b). [33]

Table 3. Comparison of hairless immunocompromised mice

mice		Hairless	Nude	SCID Hairless	Nude-R/J
Strain		Balb/c	Balb/c	CB17.Cg/ICR	Balb/c
Gene abnormality		Hairless	FOXN1	Hairless, SCID	FOXN1, Rag-2, Jak3
Immune system	T cells	+	-	-	-
	B cells	+	+	-	-
	NK cells	+	+	+	-
Hair coat		None	None	None	None

3. Establishment of PDX model using various immunocompromised mice

PDX models are generated with engraftment of patient tumor samples into immunocompromised mice (Figure 4). An important advantage of PDX model is that they retain key characteristics of patient's tumor, such as gene expression profile, heterogeneity of tumor cells. Currently, PDX models are most clinically relevant in vivo cancer models, and represent highly predictive drug response platform [39] US National Cancer Institute (NCI) decided to retire NCI-60, a panel of 60 human cell lines from its drug screening, and use PDX model [3]. PDX is now expected as the most useful "Avatars" for individualized medicine. The duration of first tumor growth in mice differs and it usually takes a few months to observe the tumor growth (F0). The duration of tumor growth is going to stably approximately 2 months with the serial transplantation [40]. PDX samples can be stored with patient's clinical data, gene-expression patterns, mutational status, drug responsiveness and pathological analysis to make PDX library.

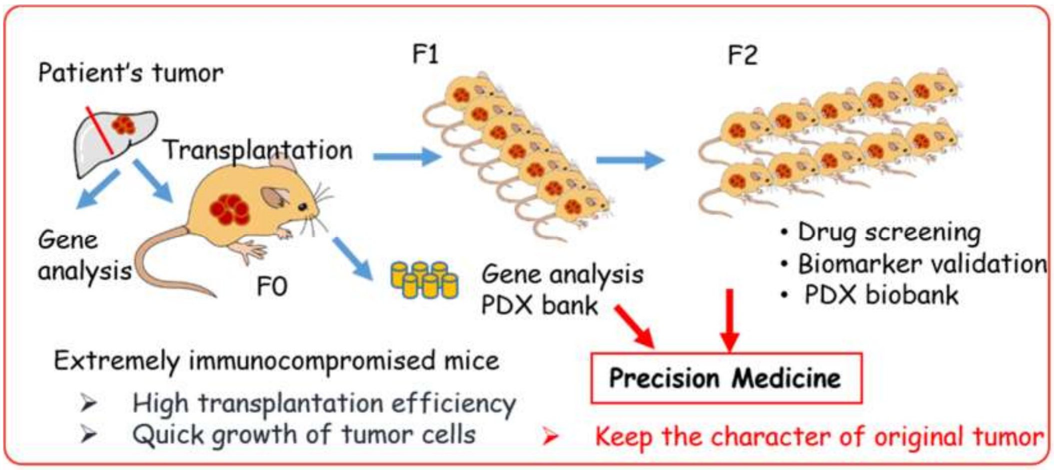

Figure 4. Patient-derived xenograft (PDX) model

Nude mice have been used to generate PDX models with reasonable efficacy and continuously used as standard recipient (Table 5). In fact, the engraft efficiency of gastrointestinal tumors are relatively high, however; establishment of hematological tumor PDX is almost impossible with Nude mice. Introduction of Scid and NOD/Scid mice increased the success ratio [41]. As NOD/Scid mice is known to has relatively short life span and develop thymoma [15], recipient of PDX is now shifting to more immunocompetent NOG/NSG mice [42-44]. Success ratio of PDX varies between tumor origin, aggressiveness, relapsed or not, primary tumor or metastatic tumor. Gastrointestinal cancers such as colon and pancreatic cancer tends to high engraft ratio compared with hematological malignancies. Orthotropic or renal capsule engraftment is needed some tumors [24]. Human hormone replacement supports hormone dependent tumors such as breast and prostate cancers [45,46].

4. Generation of PDX derived cell lines

Tumor cell line can be generated from PDX tissue sample [40,47,48]. It is hard to establish tumor cell lines from primary tissue, because fibroblasts are predominantly developed during in vitro culture in most of the cases. Human fibroblasts are replaced to murine fibroblasts in the PDX tissue, and these fibroblasts are regenerated during *in vitro* culture. It is of interest that male derived tumor cells keep Y chromosome in PDX tissues but lose it during developing cell lines, indicating that at least one more hit is needed to establish cell lined from PDX. PDX derived tumor cell lines can use for the drug screening as they still keep the character of primary tumors.

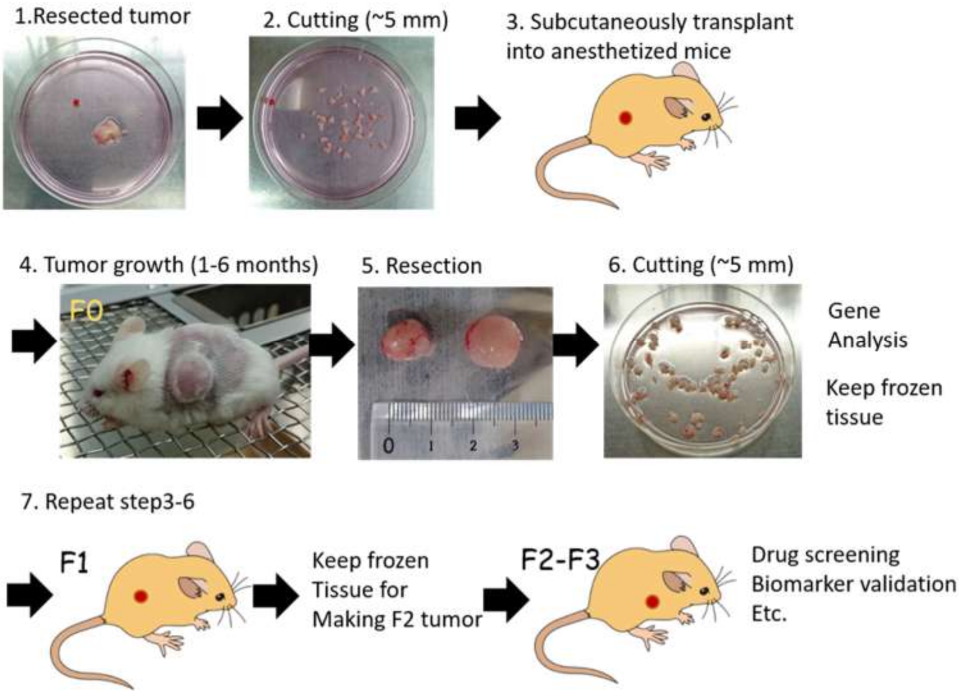

Figure 5. Generation process of PDX

Surgical specimen from patient's tumor (1) are divided into small pieces (2) and transplanted into anesthetized immunocompromised mice (3). It takes 1-6 months for tumor growth (4). When tumors are grown in F0 mice, xenograft are resected (5) and cut into small pieces (6). Tumor cells are analyzed for characterization such as whole exome sequencing (WES), RNA sequence (RNA-seq), and copy number alteration (CAN) analysis. Tumor cells are also preserved in liquid nitrogen tank. Tumor cells are further transplanted into immunocompetent mice (7), and expanded tumor xenografts (F2-F3) are used for drug screening, validation of biomarkers, characterization of tumor, etc.

Table 5. Engraft rates of PDX in different mice

Tumor type	Mice strain	Implantation site	Engraftment ratio	References
Cholangiocarcinoma	Scid	s.c. *	34.5%	Ojima, 2010 [49]
	NOD/Scid	s.c.	5.8%	Cavalloni, 2016 [50]
	BALB/c RJ	s.c.	75%	Vaeteewoottacharn, 2019 [40]
Colorectal cancer	Nude	s.c.	63.5%	Julien S, 2012 [51]
	NOD/Scid	s.c.	87%	Bertolini, 2011 [52]
	NSG	s.c	54%	Chou, 2013 [53]
Pancreatic cancer	Nude	s.c.	61%	Garrido-Laguna, 2011 [54]
	SCID	s.c.	67%	Mattie, 2013 [55]
	NSG	s.c	71.1%	Guo, 2019 [56]
Gastric cancer	Nude	s.c.	73.7%	Wang, 2017 [57]
	NOD/Scid	s.c.	34.1%	Zhu, 2015 [58]
	Nude/SCID	s.c	16.9%/26.9%	Zhang, 2015 [59]
	Nude/NOG	s.c	24.2%	Choi, 2016 [60]
Head & Neck cancer	Nude	s.c.	54%	Keysar, 2013 [61]
	NSG	s.c	85%	Kimple, 2013 [62]
Breast cancer	Nude	s.c.	13%	Marangoni, 2007 [63]
	NOD/Scid	breast	27%	DeRose , 2011 [64]
	Scid/beige/ NSG	breast	19%/21%	Zhang, 2013 [65]

* s.c. subcutaneous

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5. Perspective

PDX models have emerged as important tools for cancer research with the promise of enabling a more personalized approach together with gene-expression and drug sensitivity profiles. However, PDX requires long time for establishment (several months to 2 years) and success rate is not 100% (10-90%). So it is difficult to restore the data for the patient of tumor source. Therefore, many institutions and organizations are focus on creating large stock of PDXs and PDX libraries. European institutions established EurOPDX, a consortium to store PDXs and have already accumulated more than 1,500 samples in a PDX bank [66,67]. Jackson Laboratory provides more than 450 samples to researchers [43]. Mega Pharmacies are also establishing their own PDX libraries, and Novartis recently published data on drug screening using 1,000 PDXs [68]. These PDX banks are very useful source for precision cancer medicine. As current source of PDX is biased in USA and European countries and common cancers, it is necessary to establish PDX in Asian countries and rare cancers.

Developments of xenograft technology and highly immunocompromised mice such as NSG mice enable us for broadening the application of the PDX platform. However, we need more effort to establish clinically relevant PDX. For example, Humanized mice with PDX are expected to function as a novel platform for examining immunotherapy [69]. Several attempts have been made to establish more humanized microenvironments in immunocompromised mice [70].

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References

1. Alteri, E.; Guizzaro, L. Be open about drug failures to speed up research. *Nature* **2018**, *563*, 317-319, doi:10.1038/d41586-018-07352-7.
2. DiMasi, J.A.; Reichert, J.M.; Feldman, L.; Malins, A. Clinical approval success rates for investigational cancer drugs. *Clinical pharmacology and therapeutics* **2013**, *94*, 329-335, doi:10.1038/clpt.2013.117.
3. Ledford, H. US cancer institute overhauls cell lines. Veteran cells to be replaced by human tumours grown in mice. *Nature* **2016**, *530*, 391.
4. Mestas, J.; Hughes, C.C. Of mice and not men: differences between mouse and human immunology. *J Immunol* **2004**, *172*, 2731-2738.
5. Kojima, Y.; Hayakawa, F.; Morishita, T.; Sugimoto, K.; Minamikawa, Y.; Iwase, M.; Yamamoto, H.; Hirano, D.; Imoto, N.; Shimada, K., et al. YM155 induces apoptosis through proteasome-dependent degradation of MCL-1 in primary effusion lymphoma. *Pharmacological research* **2017**, *120*, 242-251, doi:10.1016/j.phrs.2017.04.006.
6. Flanagan, S.P. 'Nude', a new hairless gene with pleiotropic effects in the mouse. *Genet Res* **1966**, *8*, 295-309.
7. Budzynski, W.; Radzikowski, C. Cytotoxic cells in immunodeficient athymic mice. *Immunopharmacology and immunotoxicology* **1994**, *16*, 319-346, doi:10.3109/08923979409007097.
8. Bosma, G.C.; Custer, R.P.; Bosma, M.J. A severe combined immunodeficiency mutation in the mouse. *Nature* **1983**, *301*, 527-530.
9. Taghian, A.; Budach, W.; Zietman, A.; Freeman, J.; Gioioso, D.; Ruka, W.; Suit, H.D. Quantitative comparison between the transplantability of human and murine tumors into the subcutaneous tissue

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of NCr/Sed-nu/nu nude and severe combined immunodeficient mice. *Cancer research* **1993**, *53*, 5012-5017.

10. McCune, J.M.; Namikawa, R.; Kaneshima, H.; Shultz, L.D.; Lieberman, M.; Weissman, I.L. The SCID-hu mouse: murine model for the analysis of human hematolymphoid differentiation and function. *Science* **1988**, *241*, 1632-1639.
11. Mosier, D.E.; Gulizia, R.J.; Baird, S.M.; Wilson, D.B. Transfer of a functional human immune system to mice with severe combined immunodeficiency. *Nature* **1988**, *335*, 256-259, doi:10.1038/335256a0.
12. Thomsen, M.; Galvani, S.; Canivet, C.; Kamar, N.; Bohler, T. Reconstitution of immunodeficient SCID/beige mice with human cells: applications in preclinical studies. *Toxicology* **2008**, *246*, 18-23, doi:10.1016/j.tox.2007.10.017.
13. Makino, S.; Kunimoto, K.; Muraoka, Y.; Mizushima, Y.; Katagiri, K.; Tochino, Y. Breeding of a non-obese, diabetic strain of mice. *Jikken dobutsu. Experimental animals* **1980**, *29*, 1-13.
14. Kikutani, H.; Makino, S. The murine autoimmune diabetes model: NOD and related strains. *Adv Immunol* **1992**, *51*, 285-322.
15. Shultz, L.D.; Schweitzer, P.A.; Christianson, S.W.; Gott, B.; Schweitzer, I.B.; Tennent, B.; McKenna, S.; Mobraaten, L.; Rajan, T.V.; Greiner, D.L., et al. Multiple defects in innate and adaptive immunologic function in NOD/LtSz-scid mice. *J Immunol* **1995**, *154*, 180-191.
16. Ito, M.; Hiramatsu, H.; Kobayashi, K.; Suzue, K.; Kawahata, M.; Hioki, K.; Ueyama, Y.; Koyanagi, Y.; Sugamura, K.; Tsuji, K., et al. NOD/SCID/gamma(c)(null) mouse: an excellent recipient mouse model for engraftment of human cells. *Blood* **2002**, *100*, 3175-3182, doi:10.1182/blood-2001-12-0207.
17. Shultz, L.D.; Lyons, B.L.; Burzenski, L.M.; Gott, B.; Chen, X.; Chaleff, S.; Kotb, M.; Gillies, S.D.; King, M.; Mangada, J., et al. Human lymphoid and myeloid cell development in NOD/LtSz-scid IL2R gamma null mice engrafted with mobilized human hemopoietic stem cells. *J Immunol* **2005**, *174*, 6477-6489, doi:174/10/6477 [pii].
18. Okada, S.; Harada, H.; Ito, T.; Saito, T.; Suzu, S. Early development of human hematopoietic and acquired immune systems in new born NOD/Scid/Jak3null mice intrahepatic engrafted with cord blood-derived CD34 + cells. *International journal of hematology* **2008**, *88*, 476-482, doi:10.1007/s12185-008-0215-z.
19. Navarro-Alvarez, N.; Yang, Y.G. CD47: a new player in phagocytosis and xenograft rejection. *Cellular & molecular immunology* **2011**, *8*, 285-288, doi:10.1038/cmi.2010.83.
20. Takenaka, K.; Prasolava, T.K.; Wang, J.C.; Mortin-Toth, S.M.; Khalouei, S.; Gan, O.I.; Dick, J.E.; Danska, J.S. Polymorphism in Sirpa modulates engraftment of human hematopoietic stem cells. *Nat Immunol* **2007**, *8*, 1313-1323, doi:10.1038/ni1527.
21. Yamauchi, T.; Takenaka, K.; Urata, S.; Shima, T.; Kikushige, Y.; Tokuyama, T.; Iwamoto, C.; Nishihara, M.; Iwasaki, H.; Miyamoto, T., et al. Polymorphic Sirpa is the genetic determinant for NOD-based mouse lines to achieve efficient human cell engraftment. *Blood* **2013**, *121*, 1316-1325, doi:10.1182/blood-2012-06-440354.
22. Traggiai, E.; Chicha, L.; Mazzucchelli, L.; Bronz, L.; Piffaretti, J.C.; Lanzavecchia, A.; Manz, M.G. Development of a human adaptive immune system in cord blood cell-transplanted mice. *Science* **2004**, *304*, 104-107, doi:10.1126/science.1093933.
23. Ono, A.; Hattori, S.; Kariya, R.; Iwanaga, S.; Taura, M.; Harada, H.; Suzu, S.; Okada, S. Comparative study of human hematopoietic cell engraftment into BALB/c and C57BL/6 strain of rag-2/jak3 double-deficient mice. *J Biomed Biotechnol* **2011**, *2011*, 539748, doi:10.1155/2011/539748.
24. Okada, S.; Vaeteewoottacharn, K.; Kariya, R. Establishment of a Patient-Derived Tumor Xenograft Model and Application for Precision Cancer Medicine. *Chemical & pharmaceutical bulletin* **2018**, *66*, 225-230, doi:10.1248/cpb.c17-00789.
25. Iwamoto, C.; Takenaka, K.; Urata, S.; Yamauchi, T.; Shima, T.; Kuriyama, T.; Daitoku, S.; Saito, Y.; Miyamoto, T.; Iwasaki, H., et al. The BALB/c-specific polymorphic SIRPA enhances its affinity for human CD47, inhibiting phagocytosis against human cells to promote xenogeneic engraftment. *Experimental hematology* **2014**, *42*, 163-171 e161, doi:10.1016/j.exphem.2013.11.005.
26. Goto, H.; Kariya, R.; Matsuda, K.; Kudo, E.; Katano, H.; Okada, S. A potential role of the NOD genetic background in mouse peritoneal macrophages for the development of primary effusion lymphoma. *Leuk Res* **2016**, *42*, 37-42, doi:10.1016/j.leukres.2016.01.011.
27. Goto, H.; Kojima, Y.; Matsuda, K.; Kariya, R.; Taura, M.; Kuwahara, K.; Nagai, H.; Katano, H.; Okada, S. Efficacy of anti-CD47 antibody-mediated phagocytosis with macrophages against primary effusion lymphoma. *Eur J Cancer* **2014**, *50*, 1836-1846, doi:10.1016/j.ejca.2014.03.004.

28. Kariya, R.; Matsuda, K.; Gotoh, K.; Vaeteewoottacharn, K.; Hattori, S.; Okada, S. Establishment of Nude Mice with Complete Loss of Lymphocytes and NK Cells and Application for In Vivo Bio-imaging. *In Vivo* **2014**, *28*, 779-784.
29. Tanaka, A.; Takeda, S.; Kariya, R.; Matsuda, K.; Urano, E.; Okada, S.; Komano, J. A novel therapeutic molecule against HTLV-1 infection targeting provirus. *Leukemia* **2013**, *27*, 1621-1627, doi:10.1038/leu.2013.46.
30. Hoffman, R. Green fluorescent protein imaging of tumour growth, metastasis, and angiogenesis in mouse models. *The Lancet. Oncology* **2002**, *3*, 546-556.
31. Yang, M.; Reynoso, J.; Jiang, P.; Li, L.; Moossa, A.R.; Hoffman, R.M. Transgenic nude mouse with ubiquitous green fluorescent protein expression as a host for human tumors. *Cancer research* **2004**, *64*, 8651-8656, doi:10.1158/0008-5472.CAN-04-3118.
32. Niclou, S.P.; Danzeisen, C.; Eikesdal, H.P.; Wiig, H.; Brons, N.H.; Poli, A.M.; Svendsen, A.; Torsvik, A.; Enger, P.O.; Terzis, J.A., et al. A novel eGFP-expressing immunodeficient mouse model to study tumor-host interactions. *FASEB journal : official publication of the Federation of American Societies for Experimental Biology* **2008**, *22*, 3120-3128, doi:10.1096/fj.08-109611.
33. Gotoh, K.; Kariya, R.; Matsuda, K.; Hattori, S.; Vaeteewoottacharn, K.; Okada, S. A novel EGFP-expressing nude mice with complete loss of lymphocytes and NK cells to study tumor-host interactions. *Biosci Trends* **2014**, *8*, 202-205.
34. Vaeteewoottacharn, K.; Kariya, R.; Dana, P.; Fujikawa, S.; Matsuda, K.; Ohkuma, K.; Kudo, E.; Kraiklang, R.; Wongkham, C.; Wongkham, S., et al. Inhibition of carbonic anhydrase potentiates bevacizumab treatment in cholangiocarcinoma. *Tumour biology : the journal of the International Society for Oncodevelopmental Biology and Medicine* **2016**, *37*, 9023-9035, doi:10.1007/s13277-016-4785-8.
35. Benavides, F.; Oberyszyn, T.M.; VanBuskirk, A.M.; Reeve, V.E.; Kusewitt, D.F. The hairless mouse in skin research. *Journal of dermatological science* **2009**, *53*, 10-18, doi:10.1016/j.jdermsci.2008.08.012.
36. Heiniger, H.J.; Meier, H.; Kaliss, N.; Cherry, M.; Chen, H.W.; Stoner, R.D. Hereditary immunodeficiency and leukemogenesis in HRS-J mice. *Cancer research* **1974**, *34*, 201-211.
37. Crottes, D.; Rapetti-Mauss, R.; Alcaraz-Perez, F.; Tichet, M.; Gariano, G.; Martial, S.; Guizouarn, H.; Pellissier, B.; Loubat, A.; Popa, A., et al. SIGMAR1 Regulates Membrane Electrical Activity in Response to Extracellular Matrix Stimulation to Drive Cancer Cell Invasiveness. *Cancer research* **2016**, *76*, 607-618, doi:10.1158/0008-5472.CAN-15-1465.
38. Smee, D.F.; Dagley, A.; Downs, B.; Hagloch, J.; Tarbet, E.B. Enhanced efficacy of cidofovir combined with vaccinia immune globulin in treating progressive cutaneous vaccinia virus infections in immunosuppressed hairless mice. *Antimicrobial agents and chemotherapy* **2015**, *59*, 520-526, doi:10.1128/AAC.04289-14.
39. Tentler, J.J.; Tan, A.C.; Weekes, C.D.; Jimeno, A.; Leong, S.; Pitts, T.M.; Arcaroli, J.J.; Messersmith, W.A.; Eckhardt, S.G. Patient-derived tumour xenografts as models for oncology drug development. *Nat Rev Clin Oncol* **2012**, *9*, 338-350, doi:10.1038/nrclinonc.2012.61.
40. Vaeteewoottacharn, K.; Pairojkul, C.; Kariya, R.; Muisuk, K.; Imtawil, K.; Chamgramol, Y.; Bhudhisawasdi, V.; Khuntikeo, N.; Pugkhem, A.; Saeseow, O.T., et al. Establishment of Highly Transplantable Cholangiocarcinoma Cell Lines from a Patient-Derived Xenograft Mouse Model. *Cells* **2019**, *8*, doi:10.3390/cells8050496.
41. Jin, K.; Teng, L.; Shen, Y.; He, K.; Xu, Z.; Li, G. Patient-derived human tumour tissue xenografts in immunodeficient mice: a systematic review. *Clinical & translational oncology : official publication of the Federation of Spanish Oncology Societies and of the National Cancer Institute of Mexico* **2010**, *12*, 473-480, doi:10.1007/s12094-010-0540-6.
42. Chijiwa, T.; Kawai, K.; Noguchi, A.; Sato, H.; Hayashi, A.; Cho, H.; Shiozawa, M.; Kishida, T.; Morinaga, S.; Yokose, T., et al. Establishment of patient-derived cancer xenografts in immunodeficient NOG mice. *International journal of oncology* **2015**, *47*, 61-70, doi:10.3892/ijo.2015.2997.
43. Shultz, L.D.; Goodwin, N.; Ishikawa, F.; Hosur, V.; Lyons, B.L.; Greiner, D.L. Human cancer growth and therapy in immunodeficient mouse models. *Cold Spring Harb Protoc* **2014**, *2014*, 694-708, doi:10.1101/pdb.top073585.
44. Brown, K.M.; Xue, A.; Mittal, A.; Samra, J.S.; Smith, R.; Hugh, T.J. Patient-derived xenograft models of colorectal cancer in pre-clinical research: a systematic review. *Oncotarget* **2016**, *7*, 66212-66225, doi:10.18632/oncotarget.11184.
45. Cho, S.Y.; Kang, W.; Han, J.Y.; Min, S.; Kang, J.; Lee, A.; Kwon, J.Y.; Lee, C.; Park, H. An Integrative Approach to Precision Cancer Medicine Using Patient-Derived Xenografts. *Mol Cells* **2016**, *39*, 77-86, doi:10.14348/molcells.2016.2350.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 3 June 2019 doi:10.20944/preprints201906.0002.v1

46. Whittle, J.R.; Lewis, M.T.; Lindeman, G.J.; Visvader, J.E. Patient-derived xenograft models of breast cancer and their predictive power. *Breast cancer research : BCR* **2015**, *17*, 17, doi:10.1186/s13058-015-0523-1.
47. Oyama, R.; Takahashi, M.; Yoshida, A.; Sakumoto, M.; Takai, Y.; Kito, F.; Shiozawa, K.; Qiao, Z.; Arai, Y.; Shibata, T., et al. Generation of novel patient-derived CIC- DUX4 sarcoma xenografts and cell lines. *Scientific reports* **2017**, *7*, 4712, doi:10.1038/s41598-017-04967-0.
48. Borodovsky, A.; McQuiston, T.J.; Stetson, D.; Ahmed, A.; Whitston, D.; Zhang, J.; Grondine, M.; Lawson, D.; Challberg, S.S.; Zinda, M., et al. Generation of stable PDX derived cell lines using conditional reprogramming. *Molecular cancer* **2017**, *16*, 177, doi:10.1186/s12943-017-0745-1.
49. Ojima, H.; Yoshikawa, D.; Ino, Y.; Shimizu, H.; Miyamoto, M.; Kokubu, A.; Hiraoka, N.; Morofuji, N.; Kondo, T.; Onaya, H., et al. Establishment of six new human biliary tract carcinoma cell lines and identification of MAGEH1 as a candidate biomarker for predicting the efficacy of gemcitabine treatment. *Cancer science* **2010**, *101*, 882-888, doi:10.1111/j.1349-7006.2009.01462.x.
50. Cavalloni, G.; Peraldo-Neia, C.; Sassi, F.; Chiorino, G.; Sarotto, I.; Aglietta, M.; Leone, F. Establishment of a patient-derived intrahepatic cholangiocarcinoma xenograft model with KRAS mutation. *BMC cancer* **2016**, *16*, 90, doi:10.1186/s12885-016-2136-1.
51. Julien, S.; Merino-Trigo, A.; Lacroix, L.; Pocard, M.; Goere, D.; Mariani, P.; Landron, S.; Bigot, L.; Nemati, F.; Dartigues, P., et al. Characterization of a large panel of patient-derived tumor xenografts representing the clinical heterogeneity of human colorectal cancer. *Clinical cancer research : an official journal of the American Association for Cancer Research* **2012**, *18*, 5314-5328, doi:10.1158/1078-0432.CCR-12-0372.
52. Bertotti, A.; Migliardi, G.; Galimi, F.; Sassi, F.; Torti, D.; Isella, C.; Cora, D.; Di Nicolantonio, F.; Buscarino, M.; Petti, C., et al. A molecularly annotated platform of patient-derived xenografts ("xenopatients") identifies HER2 as an effective therapeutic target in cetuximab-resistant colorectal cancer. *Cancer discovery* **2011**, *1*, 508-523, doi:10.1158/2159-8290.CD-11-0109.
53. Chou, J.; Fitzgibbon, M.P.; Mortales, C.L.; Towlerton, A.M.; Upton, M.P.; Yeung, R.S.; McIntosh, M.W.; Warren, E.H. Phenotypic and transcriptional fidelity of patient-derived colon cancer xenografts in immune-deficient mice. *PloS one* **2013**, *8*, e79874, doi:10.1371/journal.pone.0079874.
54. Garrido-Laguna, I.; Uson, M.; Rajeshkumar, N.V.; Tan, A.C.; de Oliveira, E.; Karikari, C.; Villaroel, M.C.; Salomon, A.; Taylor, G.; Sharma, R., et al. Tumor engraftment in nude mice and enrichment in stroma-related gene pathways predict poor survival and resistance to gemcitabine in patients with pancreatic cancer. *Clinical cancer research : an official journal of the American Association for Cancer Research* **2011**, *17*, 5793-5800, doi:10.1158/1078-0432.CCR-11-0341.
55. Mattie, M.; Christensen, A.; Chang, M.S.; Yeh, W.; Said, S.; Shostak, Y.; Capo, L.; Verlinsky, A.; An, Z.; Joseph, I., et al. Molecular characterization of patient-derived human pancreatic tumor xenograft models for preclinical and translational development of cancer therapeutics. *Neoplasia* **2013**, *15*, 1138-1150.
56. Guo, S.; Gao, S.; Liu, R.; Shen, J.; Shi, X.; Bai, S.; Wang, H.; Zheng, K.; Shao, Z.; Liang, C., et al. Oncological and genetic factors impacting PDX model construction with NSG mice in pancreatic cancer. *FASEB journal : official publication of the Federation of American Societies for Experimental Biology* **2019**, *33*, 873-884, doi:10.1096/fj.201800617R.
57. Wang, H.; Lu, J.; Tang, J.; Chen, S.; He, K.; Jiang, X.; Jiang, W.; Teng, L. Establishment of patient-derived gastric cancer xenografts: a useful tool for preclinical evaluation of targeted therapies involving alterations in HER-2, MET and FGFR2 signaling pathways. *BMC cancer* **2017**, *17*, 191, doi:10.1186/s12885-017-3177-9.
58. Zhu, Y.; Tian, T.; Li, Z.; Tang, Z.; Wang, L.; Wu, J.; Li, Y.; Dong, B.; Li, N.; Zou, J., et al. Establishment and characterization of patient-derived tumor xenograft using gastroscopic biopsies in gastric cancer. *Scientific reports* **2015**, *5*, 8542, doi:10.1038/srep08542.
59. Zhang, T.; Zhang, L.; Fan, S.; Zhang, M.; Fu, H.; Liu, Y.; Yin, X.; Chen, H.; Xie, L.; Zhang, J., et al. Patient-Derived Gastric Carcinoma Xenograft Mouse Models Faithfully Represent Human Tumor Molecular Diversity. *PloS one* **2015**, *10*, e0134493, doi:10.1371/journal.pone.0134493.
60. Choi, Y.Y.; Lee, J.E.; Kim, H.; Sim, M.H.; Kim, K.K.; Lee, G.; Kim, H.I.; An, J.Y.; Hyung, W.J.; Kim, C.B., et al. Establishment and characterisation of patient-derived xenografts as paraclinical models for gastric cancer. *Scientific reports* **2016**, *6*, 22172, doi:10.1038/srep22172.
61. Keysar, S.B.; Astling, D.P.; Anderson, R.T.; Vogler, B.W.; Bowles, D.W.; Morton, J.J.; Paylor, J.J.; Glogowska, M.J.; Le, P.N.; Eagles-Soukup, J.R., et al. A patient tumor transplant model of squamous cell cancer identifies PI3K inhibitors as candidate therapeutics in defined molecular bins. *Molecular oncology* **2013**, *7*, 776-790, doi:10.1016/j.molonc.2013.03.004.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 3 June 2019 doi:10.20944/preprints201906.0002.v1

62. Kimple, R.J.; Harari, P.M.; Torres, A.D.; Yang, R.Z.; Soriano, B.J.; Yu, M.; Armstrong, E.A.; Blitzer, G.C.; Smith, M.A.; Lorenz, L.D., et al. Development and characterization of HPV-positive and HPV-negative head and neck squamous cell carcinoma tumorgrafts. *Clinical cancer research : an official journal of the American Association for Cancer Research* **2013**, *19*, 855-864, doi:10.1158/1078-0432.CCR-12-2746.
63. Marangoni, E.; Vincent-Salomon, A.; Auger, N.; Degeorges, A.; Assayag, F.; de Cremoux, P.; de Plater, L.; Guyader, C.; De Pinieux, G.; Judde, J.G., et al. A new model of patient tumor-derived breast cancer xenografts for preclinical assays. *Clinical cancer research : an official journal of the American Association for Cancer Research* **2007**, *13*, 3989-3998, doi:10.1158/1078-0432.CCR-07-0078.
64. DeRose, Y.S.; Wang, G.; Lin, Y.C.; Bernard, P.S.; Buys, S.S.; Ebbert, M.T.; Factor, R.; Matsen, C.; Milash, B.A.; Nelson, E., et al. Tumor grafts derived from women with breast cancer authentically reflect tumor pathology, growth, metastasis and disease outcomes. *Nature medicine* **2011**, *17*, 1514-1520, doi:10.1038/nm.2454.
65. Zhang, X.; Claerhout, S.; Prat, A.; Dobrolecki, L.E.; Petrovic, I.; Lai, Q.; Landis, M.D.; Wiechmann, L.; Schiff, R.; Giuliano, M., et al. A renewable tissue resource of phenotypically stable, biologically and ethnically diverse, patient-derived human breast cancer xenograft models. *Cancer research* **2013**, *73*, 4885-4897, doi:10.1158/0008-5472.CAN-12-4081.
66. Byrne, A.T.; Alferez, D.G.; Amant, F.; Annibali, D.; Arribas, J.; Biankin, A.V.; Bruna, A.; Budinska, E.; Caldas, C.; Chang, D.K., et al. Interrogating open issues in cancer precision medicine with patient-derived xenografts. *Nat Rev Cancer* **2017**, *17*, 254-268, doi:10.1038/nrc.2016.140.
67. Hidalgo, M.; Amant, F.; Biankin, A.V.; Budinska, E.; Byrne, A.T.; Caldas, C.; Clarke, R.B.; de Jong, S.; Jonkers, J.; Maelandsmo, G.M., et al. Patient-derived xenograft models: an emerging platform for translational cancer research. *Cancer discovery* **2014**, *4*, 998-1013, doi:10.1158/2159-8290.CD-14-0001.
68. Gao, H.; Korn, J.M.; Ferretti, S.; Monahan, J.E.; Wang, Y.; Singh, M.; Zhang, C.; Schnell, C.; Yang, G.; Zhang, Y., et al. High-throughput screening using patient-derived tumor xenografts to predict clinical trial drug response. *Nature medicine* **2015**, *21*, 1318-1325, doi:10.1038/nm.3954.
69. Choi, Y.; Lee, S.; Kim, K.; Kim, S.H.; Chung, Y.J.; Lee, C. Studying cancer immunotherapy using patient-derived xenografts (PDXs) in humanized mice. *Experimental & molecular medicine* **2018**, *50*, 99, doi:10.1038/s12276-018-0115-0.
70. Theocharides, A.P.; Rongvaux, A.; Fritsch, K.; Flavell, R.A.; Manz, M.G. Humanized hemato-lymphoid system mice. *Haematologica* **2016**, *101*, 5-19, doi:10.3324/haematol.2014.115212.