Supplementary Information S1 | Examples of improvements in humanised mouse models for PDX studies

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<th>Pending issues</th>
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<td><strong>Graft versus Host Disease (GvHD)</strong></td>
<td>Engraftment with mature human T cells leads to xenogeneic GvHD due to mismatch between murine MHC and human HLA in engrafted cells or tissues.</td>
<td>• Use of genetically modified mouse strains that develop reduced or no GvHD&lt;br&gt; • GvHD reported to depend on HLA haplotype of HSC donor&lt;sup&gt;1&lt;/sup&gt;</td>
<td>• Immune-deficient strains lacking B2m (NSG-B2m), MHC-I (NSG-(K&lt;sup&gt;D&lt;/sup&gt;)-null) or MHC-II (NSG-(H2-Ab)-null (Jackson Laboratory&lt;sup&gt;2,3&lt;/sup&gt;))&lt;br&gt; • B6RG-Cd47: C57BL/6 mice lacking Rag2 and Il2rg, and deficient for CD47&lt;sup&gt;4&lt;/sup&gt;. Absence of GvHD due to improper “education” and functionality of mouse myeloid cells. However, functionality of human myeloid cells still to be validated.</td>
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<td><strong>HLA restriction of T cells and compatibility with tumours</strong></td>
<td>Absence of human HLA molecules on thymic epithelial cells generates human T cells unable to recognize de novo antigens (eg. Tumor specific antigens) in a HLA-restricted manner in HSC transplanted mice&lt;sup&gt;5&lt;/sup&gt;.</td>
<td>• Transplantation of human thymus tissue (BLT/ Bone Liver Thymus mice)&lt;sup&gt;6&lt;/sup&gt;&lt;br&gt; • Use of human HLA class I and/or class II transgenic immune deficient mice&lt;sup&gt;7,11&lt;/sup&gt;</td>
<td>• E.g. NOG-Dr4 mice (Taconic); NSG-Dr1, NSG-Dr4 or NSG-HLA-A2 mice (Jackson Laboratory).</td>
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<td><strong>Species-specific cytokines and factors</strong></td>
<td>Some human cytokines or factors are species specific, preventing generation or maintenance of specific human immune cell types.</td>
<td>• New mouse strains expressing human cytokines or receptors to obtain a more complete human immune system&lt;sup&gt;12&lt;/sup&gt;&lt;br&gt; • Onset of anaemia described as a limitation for many of the current models with improved myeloid reconstitution. Efforts being made to avoid anaemia for example by introducing human CD47.</td>
<td>Immune deficient mouse strains transgenic for human cytokines to promote myeloid and NK lineage commitment include:&lt;br&gt; • NOG-GM3: NOG mice expressing human GM-CSF and IL-3 (CIEA, Japan)&lt;sup&gt;13&lt;/sup&gt;;&lt;br&gt; • NSG- SGM3: NSG mice expressing human IL-3, GM-CSF and SCF (Jackson Laboratory&lt;sup&gt;14&lt;/sup&gt;);&lt;br&gt; • MiSTRG: BALB/c x 129S4 Rag2;Ii2rg double ko mice expressing human M-CSF, GM-CSF, IL-3, THPO and a human SIRPA allele&lt;sup&gt;15&lt;/sup&gt;;&lt;br&gt; • NOG-hIL6: NOG mice expressing human IL6 (Taconic), featuring increased human monocytes and macrophages (in particular M2 type)&lt;br&gt; • NSG-W41: NSG mice with mutated mouse Kit. Reduced mouse haematopoiesis results in higher human reconstitution levels without the need to...</td>
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### SUPPLEMENTARY INFORMATION

**Remaining mouse innate immunity**

Despite multiple gene modifications to eliminate mouse immune cells, commonly used NSG and NOD mice still have mouse myeloid cells (macrophages, dendritic cells and granulocytes) which can play a role in tumour biology. Models with reduced mouse innate immune cells. These include strains transgenic for human myeloid specific cytokines mentioned earlier. Immune-deficient mice further modified to functionally incapacitate remaining innate cells.

**Impaired humoral immune responses, low Ig levels and impaired Ig class switching**

B cells in humanised NSG or NOG mice do not undergo sufficient maturation to become memory and antibody-producing cells. Development of new mouse strains: e.g. human HLA class II transgenic mice, with improved humoral responses due to increased CD4-mediated help; Mice strains transgenic for human cytokines; Improved lymphoid organ development resulting in increased B cell development and Ig class switching (see below).

**Impaired lymph node development, poorly developed germinal centres**

Defects in cytokine signalling in immune-deficient strains results in poorly developed secondary lymphoid tissues (NSG, NOG and BRG are all Il2rg-deficient). This includes poor germinal centre formation, ineffective class switching of B cells and antigen presentation to naive T cells, impeding robust adaptive immune responses upon humanisation.

**Impaired Immunodeficient strains based on NOD**

Use immune-deficient mice

**NSG precondition mice. Display improved human myeloid reconstitution as compared to NSG mice.**

- **NOGh-IL2**: NOG mice expressing human IL2, featuring higher numbers of human NK cells (Taconic).
- **NSG-Tlr4−/−**: facilitates monitoring of human TLR4 responses only (mentioned in).
- **BRGF**: Rag2-deficient, Il2rg-deficient BALB/c (BRG) mice lacking mouse Flt3, resulting in loss of mouse dendritic cells, increased numbers of human dendritic cells, NK and T cells.
- **B6Rg-Cd47** mice (as above)

**Impaired humoral immune responses, low Ig levels and impaired Ig class switching**

- B cells in humanised NSG or NOG mice do not undergo sufficient maturation to become memory and antibody-producing cells.

**Impaired lymph node development, poorly developed germinal centres**

- Defects in cytokine signalling in immune-deficient strains results in poorly developed secondary lymphoid tissues (NSG, NOG and BRG are all Il2rg-deficient). This includes poor germinal centre formation, ineffective class switching of B cells and antigen presentation to naive T cells, impeding robust adaptive immune responses upon humanisation.

**Impaired Immunodeficient strains based on NOD**

- Use immune-deficient mice

**NSG-C5a**: NSG-C5a mice have the intact Hc gene,
**complement system**

| background lack hemolytic complement due to a mutation in the C5 complement gene, preventing the formation of the C5b-9 membrane attack complex. These mice lack complement dependent cytotoxicity in e.g. antibody dependent therapies. | strains that do not have the NOD background such as SRG mice. • Genetically modified NOD based mice that have a functional C5 gene. | restoring the complement system. |

*B2m*, beta2-microglobulin; DR, Antigen D-related; *Flt3*, Fms-like tyrosine kinase 3; GM-CSF, granulocyte-macrophage colony stimulating factor; GvHD, Graft versus Host Disease; Hc, hemolytic complement; HSC, haematopoietic stem cell; Ig, immunoglobulin; *Il2rg*, interleukin 2 receptor common gamma chain; IL2, interleukin 2; IL3, interleukin 3; IL6, interleukin 6; ko, knockout; M-CSF, macrophage colony-stimulating factor; NK, natural killer; *Rag2*, recombination activating gene 2; SCF, stem cell factor; *SIRPA*, signal-regulatory protein α; TLR4, toll-like receptor 4; THPO, thrombopoietin.

**References:**


