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Mice humanized for the immune system as validated tool for therapeutic antibody development

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Abstract

Therapeutic antibodies modulating the immune response are frequently specific for human proteins but fail to cross-react with the corresponding rodent homologs. Hence, their preclinical development requires suitable "humanized" mouse models, i.e. mice bearing a human immune system, so that immunotherapies can be evaluated in a more relevant immune context *in vivo*. Such "humanized" mice are developed by reconstituting the immune system of immunodeficient animals with human immune cells from 2 possible origins: mature peripheral blood mononuclear cells prepared from buffy coats (huPBMC mice) or CD34⁺ hematopoietic stem cells (huCD34⁺ mice) isolated from cord blood. According to the compound to be tested and the goal of the research, one model may be preferred over the other.

We established humanized mice models from peripheral blood mononuclear cells or cord blood CD34⁺ hematopoietic stem cells for immune-oncology studies evaluating new therapeutic agents.

Choosing the right model according to the compound to be tested and the goal of the research

HuPBMC mice

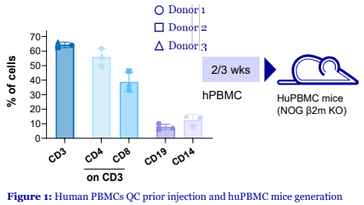


Figure 1: Human PBMCs QC prior injection and huPBMC mice generation

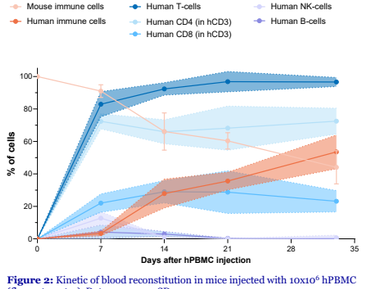


Figure 2: Kinetic of blood reconstitution in mice injected with 10x10⁶ hPBMC (flow cytometry). Data as mean ± SD.

HuCD34⁺ mice

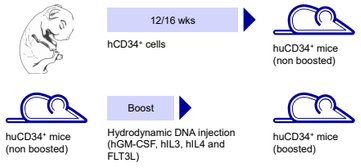


Figure 3: Schematic illustration of huCD34⁺ mice generation

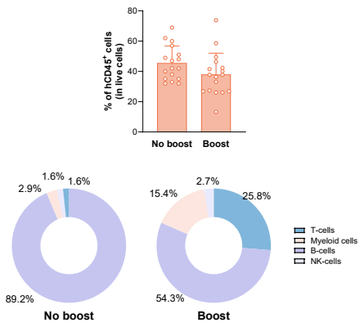


Figure 4 & 5: Blood reconstitution in non-boosted and boosted CD34⁺ mice (flow cytometry). Human immune cells (Fig. 4). Data as mean ± SD. Main human immune cell subtypes (Fig. 5).

Mice humanized from PBMCs (huPBMC) exhibit exclusively T-cell lineage whereas mice humanized from CD34⁺ hematopoietic stem cells (huCD34⁺) exhibit multilineage immune population

HuPBMC mice: Human T-cell engraftment is PBMC cell-dose dependent (no outgrowth of other human immune cells)

hPBMC engraftment in naive mice

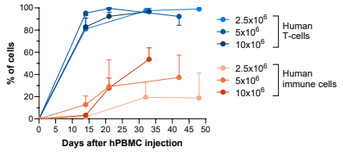


Figure 6: Kinetic of blood reconstitution in mice injected with 2.5, 5 or 10 x10⁶ hPBMC. Data as mean ± SD. NOG β2m KO female mice were injected i.v. with 2.5, 5 or 10 x10⁶ hPBMC from 3 donors. Blood was collected qwk to assess humanization by flow cytometry.

HuPBMC mice model shows tumour infiltration of human T-cells and is resistant to GvHD permitting a long therapeutic window

hPBMC engraftment in tumour-bearing mice

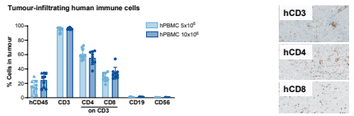


Figure 7 & 8: Human immune cells tumour infiltration (Fig. 7 flow cytometry; Fig. 8 IHC) assessed from huPBMC mice bearing subcutaneous A375 tumours. PBMC were injected i.v. once the tumour volume reached ~100mm³ and tumours were collected 2 weeks post hPBMC injection. Data as mean ± SD.

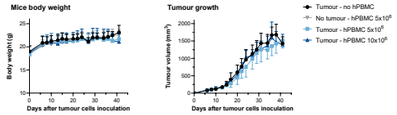
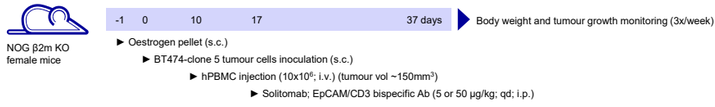


Figure 9 & 10: Body weight and tumour growth monitoring in huPBMC mice bearing subcutaneous BT474 clone 5 tumours. PBMC were injected i.v. once the tumour volume reached ~150mm³. Data as mean ± SD.

HuPBMC mice a valuable model to evaluate T-cell engagers



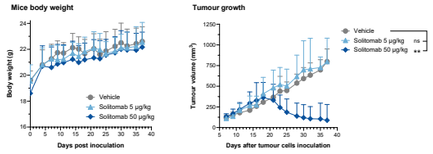


Figure 11 & 12: Body weight and tumour growth. Data as mean ± SD; Statistics: Mixed-effects model with Dunnett's multiple comparisons test vs Vehicle (ns p>0.05 and ** p<0.01).

With fast T-cell engraftment at a high rate the huPBMC model is well suited to test therapeutics requiring effector T-cell function

HuCD34⁺ mice: Human T-cells are functional and can be activated *in vivo*

Expression of activation markers on human CD4 T-cells

Blood levels of human pro-inflammatory cytokines

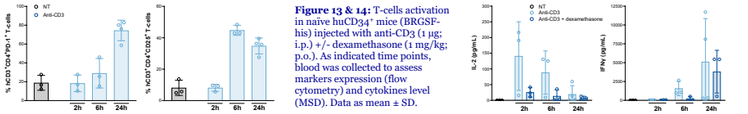
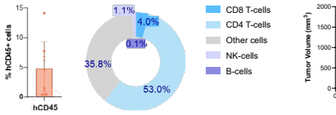


Figure 13 & 14: T-cells activation in naïve huCD34⁺ mice (BRGSF-his) injected with anti-CD3 (1 µg; i.p.) +/- dexamethasone (1 mg/kg; p.o.). As indicated time points, blood was collected to assess markers expression (flow cytometry) and cytokines level (MSD). Data as mean ± SD.

HuCD34⁺ mice: "Hot" versus "cold" tumours

"Hot" tumours: presence of tumour infiltrated immune cell – slow tumour growth

Tumour-infiltrating human immune cells



Tumour growth

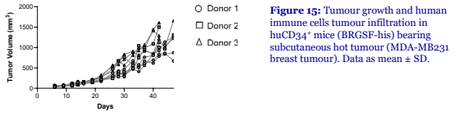
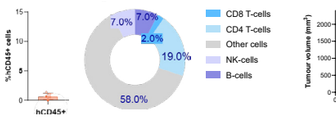


Figure 15: Tumour growth and human immune cells tumour infiltration in huCD34⁺ mice (BRGSF-his) bearing subcutaneous hot tumour (MDA-MB231 breast tumour). Data as mean ± SD.

"Cold" tumours: few tumour infiltrated immune cells – fast tumour growth

Tumour-infiltrating human immune cells



Tumour growth

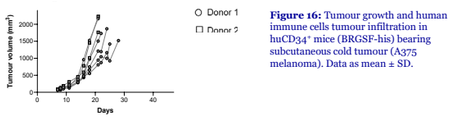
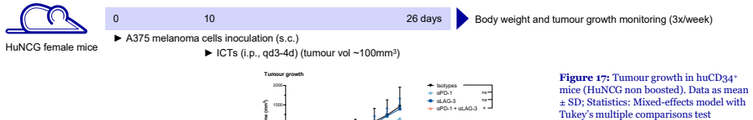


Figure 16: Tumour growth and human immune cells tumour infiltration in huCD34⁺ mice (BRGSF-his) bearing subcutaneous cold tumour (A375 melanoma). Data as mean ± SD.

HuCD34⁺ model and immune checkpoint therapies: model recapitulating human clinical observations



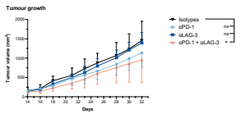


Figure 17: Tumour growth in huCD34⁺ mice (HuNCG non boosted). Data as mean ± SD; Statistics: Mixed-effects model with Tukey's multiple comparisons test between all groups on log transformed data (ns p>0.05 and * p<0.05).

HuCD34⁺ mice model is applicable to assess immunomodulatory agents which require multilineage immunity

Conclusion

Mice with a humanized immune system exhibiting either multiple lineages (CD34⁺ huNCG) or just the T-cell lineage (huPBMC) have proven to be suitable tools for assessing non-mouse cross-reactive anti-tumor immunotherapy assets. Expertise in handling such models and deep knowledge of their benefits and limitations are required for selecting the most valuable model to perform preclinical studies predictive of human therapeutic response.

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