Humanization of NOG mice and next-generation NOG mouse strains to induce lineage specific differentiation of immune cells for assessment of novel immune cell therapies, check point inhibitors, and immune cell engagers for translational immuno-oncology research

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EXPERIMENTAL PHARMACOLOGY
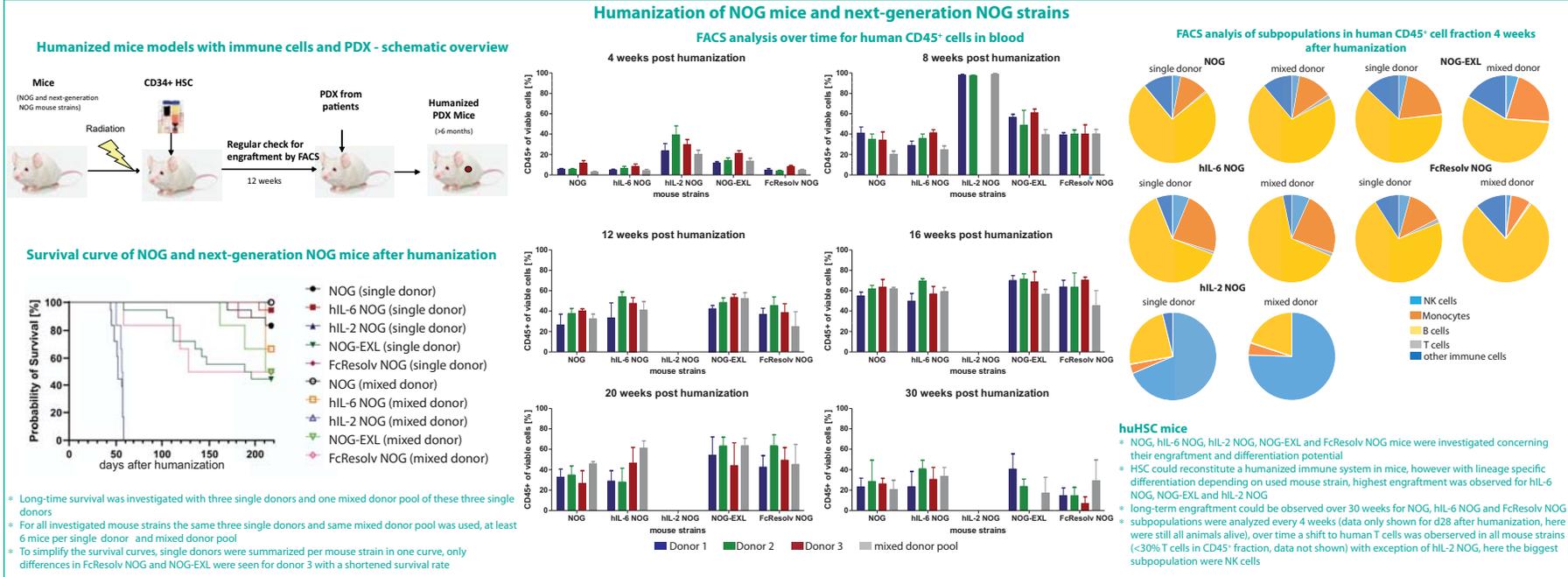& ONCOLOGY BERLIN-BUCH

TACONIC BIOSCIENCES

Background and Aim

* preclinical evaluation of novel immune checkpoint modulators requires models with functional immune cells
* in previous experiments, we have demonstrated that we can use hematopoietic stem cells (HSC), peripheral blood mononuclear cells (PBMCs) or subtypes of PBMCs like T or NK cells to establish a humanized immune system on highly immunodeficient mice with functional T, B or NK cells
* with the development of next-generation NOG mice a lineage-specific differentiation of immune sub-population of interest can be supported
* by co-transplantation of CDX and PDX, we successfully generated a fully human tumor-immune-cell model in mice
* models have been experimentally validated in preclinical studies with checkpoint inhibitors
* humanized models will continuously improved by using new mouse strains or optimized cell numbers

Summary and Outlook

* NOG and next-generation NOG mice are characterized by a lineage specific differentiation of immune cells depending on integrated human cytokines and a differential survival rate. hIL-2 NOG humanized mice showed a significant decreased survival after HSC transplantation compared to the other mouse strains

* NOG-EXL mice are characterized by the highest engraftment rate with a myeloid differentiation of immune cells, also observed in hIL-6 NOG and FcResolv NOG mouse strain

* we successfully established fully humanized mouse models for immuno-oncology by co-transplantation of CDX or PDX and human HSC or immune cells from whole blood (PBMCs, T, or NK cells)

* we observed engraftment of CDX and PDX on most humanized mice, however in some cases it was delayed and seems to be dependent on HLA matching and PD-L1 expression

* we see different therapeutic effects of checkpoint inhibitors like Nivolumab, Pembrolizumab or Ipilimumab with strong to minor responders or non responders

* several CDX and PDX have been investigated in humanized HSC mice, huPBMC, huNK cell and huT cell mice

* we demonstrated in our preclinical studies eligibility of the humanized models for preclinical research in tumor immunology, evaluation of new therapies and combinations, as well as the identification and validation of biomarkers for immune therapy

* combination therapies with radiation and using next-generation mouse strains improving engraftment of HSC (NOG-EXL mice) and immune cells (hIL-15 NOG mice) are continiously improved

* furthermore, these novel models have been successfully used for the preclinical evaluation of new bispecific immune cell engagers (BITE) and cell therapies (CAR T cells)

Humanization of NOG mice and next-generation NOG strains

Humanized mice models with immune cells and PDX - schematic overview

Survival curve of NOG and next-generation NOG mice after humanization

* NOG (single donor)
* hIL-6 NOG (single donor)
* hIL-2 NOG (single donor)
* NOG-EXL (single donor)
* FcResolv NOG (single donor)
* NOG (mixed donor)
* hIL-6 NOG (mixed donor)
* hIL-2 NOG (mixed donor)
* NOG-EXL (mixed donor)
* FcResolv NOG (mixed donor)

* Long-time survival was investigated with three single donors and one mixed donor pool of these three single donors
* For all investigated mouse strains the same three single donors and same mixed donor pool was used, at least 6 mice per single donor and mixed donor pool
* To simplify the survival curves, single donors were summarized per mouse strain in one curve, only differences in FcResolv NOG and NOG-EXL were seen for donor 3 with a shortened survival rate

FACS analysis over time for human CD45+ cells in blood

Donor 1 | Donor 2 | Donor 3 | mixed donor pool

FACS analyis of subpopulations in human CD45+ cell fraction 4 weeks after humanization

NK cells | Monocytes | B cells | T cells | other immune cells

huHSC mice

* NOG, hIL-6 NOG, hIL-2 NOG, NOG-EXL and FcResolv NOG mice were investigated concerning their engraftment and differentiation potential
* HSC could reconstitute a humanized immune system in mice, however with lineage specific differentiation depending on used mouse strain, highest engraftment was observed for hIL-6 NOG, NOG-EXL and hIL-2 NOG
* long-term engraftment could be observed over 30 weeks for NOG, hIL-6 NOG and FcResolv NOG
* subpopulations were analyzed every 4 weeks (data only shown for d28 after humanization, here were still all animals alive), over time a shift to human T cells was oberserved in all mouse strains (<30% T cells in CD45+ fraction, data not shown) with exception of hIL-2 NOG, here the biggest subpopulation were NK cells

Evaluation of immunotherapies in human HSC-CDX and PDX models

* delayed growth of Ovcar3 CDX on humanized HSC mice, distinct efficacy of checkpoint inhibitor Nivolumab could be shown

* comparable growth of DU145 CDX on humanized HSC mice, no effects of checkpoint inhibitor Nivolumab and Pembrolizumab could be shown

* delayed engraftment of glioma PDX on huHSC mice, minor efficacy of ckeckpoint inhibitor Ipilimumab

* delayed engraftment of neuroblastoma PDX on huHSC mice, minor efficacy of checkpoint inhibitor Nivolumab

* efficacy of checkpoint inhibitor Ipilimumab could be shown in Rituximab resistant Lymphoma model

* immune checkpoint inhibitor resistant sarcoma PDX model on huHSC mice

* comparable engraftment of Panc12975 on huHSC and non-humanized mice, efficacy of checkpoint inhibitors Nivolumab and Pembrolizumab could be shown in Panc12975 PDX model

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