Humanized mouse models for preclinical evaluation of anti-cancer treatment

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Background

The preclinical evaluation of novel immune therapies demands humanized mouse models with functional human immune cells. In previous studies we have established a humanized immune system in immunodeficient mice by either transfer of hematopoietic stem cells (HSCs), PBMCs or NK cells. With the development of 2nd generation NOG mice a lineage specific differentiation of immune sub-population of interest can be supported. By transplantation of cell-line-derived (CDX) or patient-derived (PDX) tumor xenografts on humanized mice, we successfully generated a full human tumor-immune-cell model for different tumor entities. Finally, we validated the functionality of these models using checkpoint inhibitors or anti-cancer antibodies.

Methods

HSC-humanized mice were generated by single i.v. transplantation of CD34+ stem cells to immunodeficient NOG mice. 1st and 2nd generation NOG mouse strains were used: NOG, NOG-EXL, NOG-IL15, NOG-IL2, NOG-IL6 and FcResolv NOG were compared for their lineage specific differentiation to each other using single donors or a mixed HSC donor pool. Engraftment of immune cells was monitored by FACS analysis of blood samples. Partially HLA-matched PBMC were used to humanize tumor-bearing mice followed by treatment with checkpoint inhibitor. NK-cell humanization was done in hIL-15 NOG and FcResolv™ hIL-15 NOG mice. Here, a lung cancer PDX model was treated with cetuximab to analyse the effect of antibody-dependent cellular cytotoxicity.

Conclusions

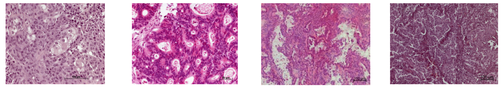
Next-generation NOG mouse strains are characterized by a lineage-specific differentiation of immune cells depending on integrated human cytokines. We established human tumor-immune-cell models of different entities using CDX or PDX in combination with different donor derived immune cell subsets as effector cells. Our human tumor-immune-cell models allow preclinical, translational studies on tumor immune biology as well as evaluation of new therapies, drug combinations and biomarker identification and validation.

Available PDX models at EPO

Evaluation of treatments with:

- Cell-based therapies
- Antibody-based therapies
- Oncolytic microorganisms
- Immune modulators

Head & Neck Ca PDX | Colon Ca PDX | Lung Ca PDX | Breast Ca PDX



PDX	n
Breast	39
Gastrointestinal	
Colon	142
Gastric	14
Oesophagus	4
Pancreatic	46
Pediatric Brain tumors*	46
HG Glioma, Ependymoma	
Medulloblastoma	65

PDX	n
Gynecologic	
Endometrial	8
Cervical	4
Ovarian	27
Haematological	
ALL	8
AML	8
Lymphoma (B & T cell)	23
pediatric ALL*	13

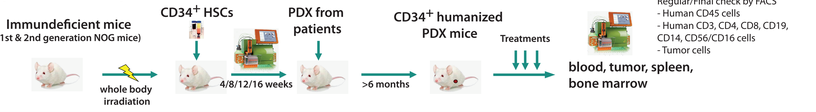
PDX	n
Head & Neck	90
Lung	62
NSCLC	59
SCLC	3
Melanoma	16
Mesothelioma	8
Neuroblastoma	3
Pediatric Neuroblastoma*	33
Neurendocrine	5

PDX	n
Sarcoma	22
Pediatric Sarcoma*	25
Pediatric STS*	
Ewing Sarcoma	26
Osteosarcoma	31
Rhabdomyosarcoma	34
Urological	
Bladder, Prostate	4
Renal	35

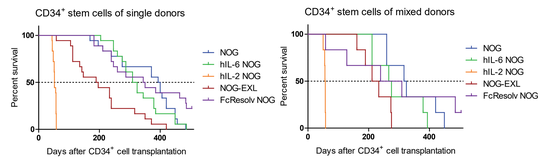
* PDX available through the IMI ITCC P4 platform

Humanization with CD34+ stem cells

Experimental set-up



Survival of CD34+ humanized 2nd generation NOG mice

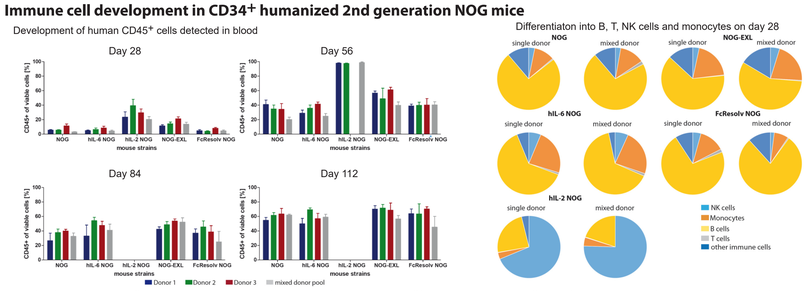


Mouse strain	CD34+ cell donor	n	Median survival (days)
NOG	single	18	397
NOG	mixed	6	320
hIL-6 NOG	single	18	317
hIL-6 NOG	mixed	6	271
hIL-2 NOG	single	18	52
hIL-2 NOG	mixed	6	57
NOG-EXL	single	18	193
NOG-EXL	mixed	6	222
FcResolv™ NOG	single	14	347
FcResolv™ NOG	mixed	5	274

Mouse strains were transplanted with 5 x 10^4 CD34+ stem cells isolated from the cord blood of three different donors, with 6 mice injected per donor. Additionally, 6 mice received a mixed injection of CD34+ stem cells from all three donors. Overall survival data is presented as a Kaplan-Meier curve and median survival was calculated.

- After CD34+ stem cell-humanization the hIL-2 NOG mice showed the shortest survival of >60 days, followed by NOG-EXL mice with 183-222 days of survival
- NOG, hIL-6 NOG and FcResolv NOG mice showed a much longer survival which makes these models suitable for treatment of slowly growing tumors

Immune cell development in CD34+ humanized 2nd generation NOG mice

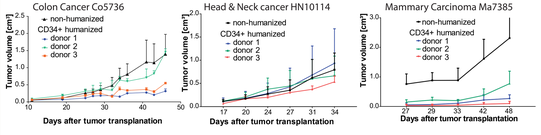


Blood samples were collected every 4 weeks: on day 28, 56, 84 and 112 post-stem cell humanization and analyzed by FACS for viable human CD45+ cells.

CD45+ cells were further categorized into B cells (CD19+), T cells (CD3+), NK cells (CD16/CD56+) and monocytes (CD14+).

- hIL-2 NOG mice exhibit the earliest appearance of human CD45+ cells in the blood, reaching nearly 100% by day 56.
- NOG-EXL mice display higher levels of CD45 cells during the first 84 days, with a slightly higher percentage of CD45+ cells compared to other mouse strains on day 112.
- hIL-6 NOG mice and FcResolv NOG mice show similar levels of CD45+ cells as the NOG mice.
- The main population of immune cells on day 28 are B cells except for hIL-2 NOG mice which have a much higher population of NK cells.
- The percentage of monocytes are higher in NOG-EXL and hIL-6 NOG mice compared to NOG and FcResolv NOG mice.
- The T cell population is at very low percentage at this time point and only increases by day 112 (data not shown).

PDX tumor models on CD34+ humanized mice

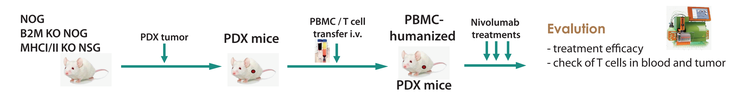


NOG mice humanized with CD34+ stem cells from cord blood of three different donors were transplanted with different PDX tumors. Tumor growth was measured over time.

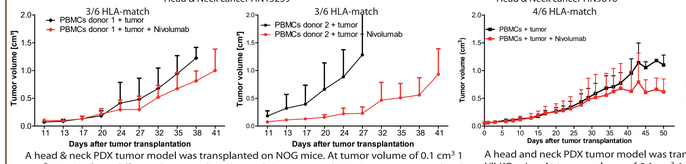
- PDX tumor engraftment can be influenced by CD34+ stem cell donor.

Immune cell subset Humanization

PBMC-humanization



Check point inhibitor treatment in partially HLA-matched PBMC-humanized mice

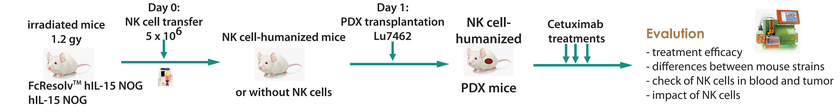


- Nivolumab can lead to tumor growth inhibition in partially HLA-matched PBMC-humanized mice

A head & neck PDX tumor model was transplanted on NOG mice. At tumor volume of 0.1 cm^3 1 x 10^7 PBMCs of two different donors with a 3/6 match (HLA-A, B and DRB1) were i.v. injected and i.p. treatment with 3 mg/kg Nivolumab was started in a biweekly schedule.

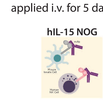
A head and neck PDX tumor model was transplanted on NSG MHC-I/II KO mice. At tumor volume of 0.1 cm^3 1 x 10^7 PBMCs of a donor with a 4/6 match (HLA-A, B and DRB1) were i.v. injected.

NK cell-humanization

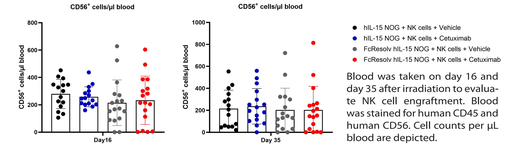


Treatment of PDX tumor on NK cell-humanized FcResolv™ hIL-15 NOG mouse model to analyze the effect of antibody-dependent cellular cytotoxicity of cetuximab

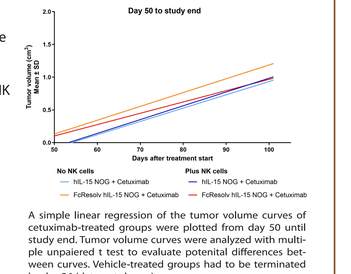
FcResolv™ hIL-15 NOG (n=49) and hIL-15 NOG mice (n=42) were irradiated with a myeloablative dose of 1.2 gy. 24 hours later, 5 x 10^6 NK cells ($CD56^+$ MACS-sorted from PBMCs of healthy blood donors) were injected i.v. in FcResolv hIL-15 NOG (n=34) and hIL-15 NOG mice (n=30) . The next day, tumor fragments on Lu7462 were transplanted on all mice. On day 19, a stratified randomization of tumor volumes was performed and treatment started. Treatment with cetuximab (50 mg/kg) was applied i.v. for 5 days and than weekly for two weeks.



- No difference detected in treatment effect in the lung cancer treated with cetuximab in NK cell-humanized hIL-15 NOG compared to NK cell-humanized FcResolv™ hIL-15 NOG mice and no NK



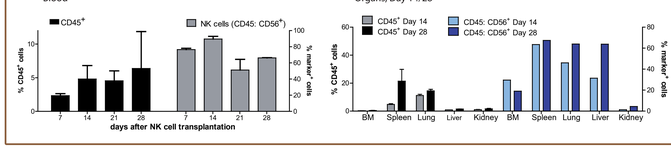
Blood was taken on day 16 and day 35 after irradiation to evaluate NK cell engraftment. Blood was stained for human CD45 and human CD56. Cell counts per µL blood are depicted.



A simple linear regression of the tumor volume curves of cetuximab-treated groups were plotted from day 50 until study end. Tumor volume curves were analyzed with multiple unpaired t test to evaluate potential differences between curves. Vehicle-treated groups had to be terminated by day 26 (data not shown).

- NK cells engraft well in PDX-bearing FcResolv™ hIL-15 NOG and hIL-15 NOG mice.

NK cell engraftment of in vitro-expanded NK cells in hIL15-NOG mice



- NK cells engraft well in hIL15-NOG mice with a proliferation peak on day 14
- NK cells engraft mainly in spleen and lung

SCAN ME

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