#2836

Assessment of therapeutic antibody efficacy without the interference of murine Fc receptors allows for investigation of human antibody-dependent cellular cytotoxicity mediated by NK cells in the FcResolv™ hIL-15 NOG mouse model

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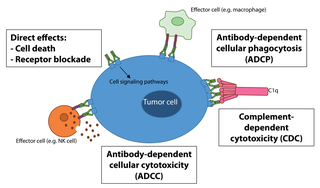
Background

Targeted antibody therapy is applied to treat various cancer types. In addition to the primary mode of action (MOA), which involves direct binding to the tumor antigen, indirect MOA acting through the constant region (Fc) of the antibody can enhance anti-tumor efficacy. Indirect mechanisms engage the innate immune system, mediated by both the complement system (complement-dependent cytotoxicity (CDC)) and immune cells (antibody-dependent cellular phagocytosis (ADCP) and antibody-dependent cellular cytotoxicity (ADCC)). These indirect mechanisms can complicate the evaluation and accurate assessment of antibody-induced ADCC by human NK cells in current mouse models. In immune-deficient mouse strains (e.g. NOG), false positives and/or negatives may occur due to interactions with murine Fc receptors. These can either result in anti-tumor responses via activation of the murine innate immune system or can interfere with the human-targeted therapy's primary MOA. To study the response to anti-cancer antibodies without the interference of these murine Fc receptor interactions and to investigate ADCC mediated by human NK cells, a novel mouse model deficient in Fc receptors and expressing human IL-15 (FcResolv™ hIL-15 NOG) was employed for testing antibody therapies.

Direct and indirect Mode of action (MOA) of antibodies



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Mouse models

FcResolv™ hIL-15 NOG

- immunodeficient mouse model lacking T, B and NK cells
- expressing human human interleukin (IL)-15
- knock out for murine Fc gamma receptors (FcγRs)

hIL-15 NOG

- immunodeficient mouse model lacking T, B and NK cells
- expressing human IL-15

Methods

Patient-derived xenograft (PDX) tumor models were transplanted into hIL-15 NOG and FcResolv™ hIL-15 NOG mice. A human head and neck squamous cell carcinoma and a lung adenocarcinoma PDX model were both treated with cetuximab. Treatment with pertuzumab and trastuzumab was applied in a breast ductal carcinoma PDX model. Based on growth kinetics, the lung cancer PDX model was chosen for further testing of ADCC in the NK cell-humanized FcResolv™ hIL-15 NOG mouse.

A) Experimental set-up for treatment of PDX tumors on FcResolv™ hIL15 NOG mice

Patient-derived tumor xenografts (PDX)

tumor entities tested:
- human head and neck (HN) squamous cell carcinoma
- lung (Lu) adenocarcinoma
- breast ductal carcinoma (Ma)

Antibodies tested

- Cetuximab (anti-EGFR) for HN and Lu PDX
- Pertuzumab (anti-Her2) for Ma PDX
- Trastuzumab (anti-Her2) for Ma PDX

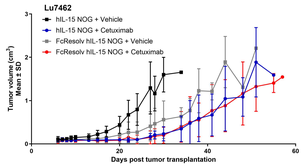
Results

A) Treatment of PDX tumors on FcResolv™ hIL-15 NOG mouse model

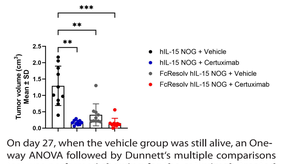
No difference detected in treatment effect in the lung and head and neck cancer treated with cetuximab in hIL-15 NOG compared to FcResolv™ hIL-15 NOG mice.

Lung adenocarcinoma

Tumor fragments of Lu7462 were transplanted on FcResolv hIL-15 NOG and hIL-15 NOG mice and treated with cetuximab (50 mg/kg) or vehicle (n=10) at tumor volume (TV) of 0.1 cm^3. Treatment was started on day 7 and applied i.v. for 5 days and than weekly for two weeks.



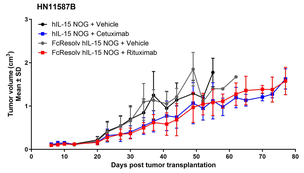
Day 27



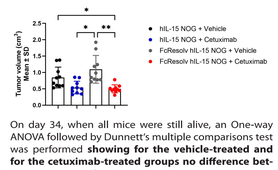
On day 27, when the vehicle group was still alive, an One-way ANOVA followed by Dunnett's multiple comparisons test was performed **showing for the cetuximab-treated groups no difference between mouse strains.**

Head and neck squamous cell carcinoma

Tumor fragments of HN11587B were transplanted on FcResolv hIL-15 NOG and hIL-15 NOG mice and treated with cetuximab (50 mg/kg) or vehicle (n=10) at tumor volume (TV) of 0.1 cm^3. Treatment was started on day 6 and applied i.v. biweekly for 10 weeks.



Day 34

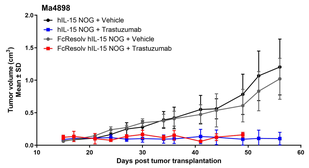


On day 34, when all mice were still alive, an One-way ANOVA followed by Dunnett's multiple comparisons test was performed **showing for the vehicle-treated and for the cetuximab-treated groups no difference between mouse strains.**

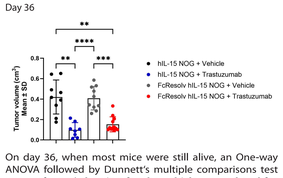
Her2-antibodies, Trastuzumab and Pertuzumab, behave differently in treatment of breast carcinoma in hIL-15 NOG and FcResolv™ hIL-15 NOG mice.

Breast ductal carcinoma

Tumor fragments of Ma4898 were transplanted on FcResolv hIL-15 NOG and hIL-15 NOG mice and treatment with trastuzumab (10 mg/ml), pertuzumab (30 mg/ml) or vehicle of established tumors with a at tumor volume (TV) of 0.1 cm^3 was started on day 1 (n=10). Trastuzumab was applied i.v. biweekly for 6 weeks. Pertuzumab was continued with 15 mg/kg i.p weekly for 8 weeks.

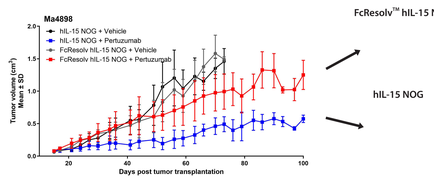


Day 36

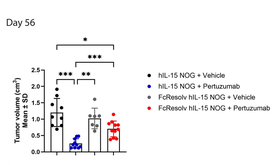


On day 36, when most mice were still alive, an One-way ANOVA followed by Dunnett's multiple comparisons test was performed **showing for the vehicle-treated and for the cetuximab-treated groups no difference between mouse strains.**

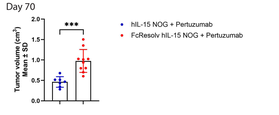
False-positive efficacy of pertuzumab treatment in hIL-15 NOG compared to FcResolv™ hIL-15 NOG mice.



Day 56



Day 70



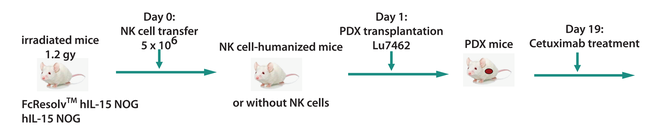
On day 56, when most mice were still alive, an One-way ANOVA followed by Dunnett's multiple comparisons test was performed **showing for the pertuximab-treated groups a statistically significant difference in tumor volumes between mouse strains.**

On day 70, Welch's t test, revealed again a **statistically significant difference in tumor volumes of pertuximab-treated groups.**

Significance code: * p < 0.05; ** p < 0.01; *** p < 0.001; **** p < 0.0001

B) Treatment of PDX tumor on NK cell-humanized FcResolv™ hIL-15 NOG mouse model

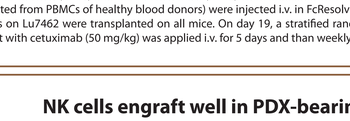
B) Experimental set-up for treatment of PDX tumors on NK cell-humanized FcResolv™ hIL15 NOG mice



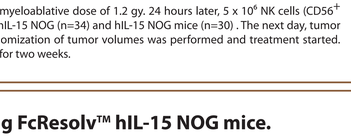
FcResolv hIL-15 NOG (n=49) and hIL-15 NOG mice (n=42) were irradiated with a myeloablative dose of 1.2 gy. 24 hours later, 5 x 10^6 NK cells ($CD56^+$ MACS-sorted from PBMCs of healthy blood donors) were injected i.v. in FcResolv hIL-15 NOG (n=34) and hIL-15 NOG mice (n=30) . The next day, tumor fragments 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.

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

Blood day 16

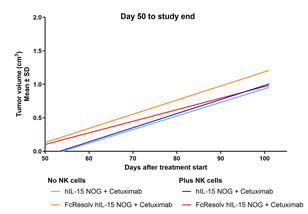


Blood day 35



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. **No difference of NK cell counts in blood was detected between groups** tested by One-way ANOVA and Sidak's multiple comparisons test.

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 cell-dependent treatment effect observed in this tumor model.



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 unpaiered t test to evaluate potenital differences between curves.
No difference in tumor volume curves between cetuximab-treated groups were detected independent on NK cell-humanization.
Vehicle-treated groups had to be terminated by day 26 (data not shown).

Conclusions

There was no difference in percent tumor growth inhibition between the FcResolv™ hIL-15 NOG and hIL-15 NOG mice with regards to cetuximab treatment in the lung and head and neck cancer or for trastuzumab treatment of breast ductal carcinoma. However, pertuzumab treatment revealed a false positive efficacy, with the false positive effect more pronounced in hIL-15 NOG mice than in FcResolv™ hIL-15 NOG mice. These results demonstrate that FcResolv™ hIL-15 NOG mice serve as a suitable mouse model for a more accurate assessment of the therapeutic efficacy of anti-tumor antibodies. Additionally, evaluation of human-mediated ADCC of therapeutic antibodies in NK cell-humanized FcResolv™ hIL-15 NOG allows detection of effects specifically mediated by human NK cells.

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