Evaluation of checkpoint inhibitor efficacy in a humanized immune system mouse model lacking murine Fc gamma receptors

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Abstract 5338

Introduction

- Humanized immune system (HIS) mice are critical tools for evaluating antibody-based therapeutic target engagement and efficacy in a human-like context[1]
- Therapeutic mAbs have an IgG backbone and will engage Fc gamma receptors
- In the context of HIS mouse studies, therapeutics with Fc domains can engage not only human Fc receptors, but may also interact with residual murine innate cells which express murine Fc gamma receptors such as neutrophils and monocytes/macrophages,[2,4] leading to confounding effects

Experimental Aim

To determine whether knockout of murine Fc gamma receptors in a super immunodeficient mouse model would alter an IgG4 mAb checkpoint inhibitor (anti-PD1) efficacy compared to the parent strain in a lung adenocarcinoma model, we studied tumor growth kinetics, human reconstitution, and tumor infiltrating leukocytes (TILs) via flow cytometry in each strain engrafted with human HCC872 tumor cells treated with pembrolizumab or vehicle.

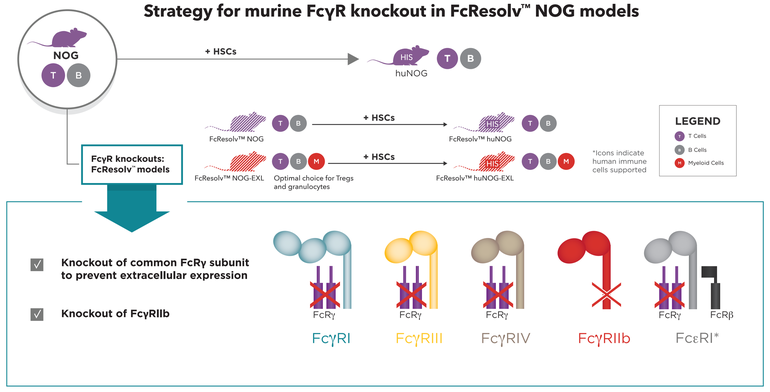


Figure 1. *Functional deletion of murine Fc gamma receptors is designed to alleviate confounding effects and improve accuracy*

Methods

Methods: Expt A. HIS NOG (huNOG) or HIS FcγR knockout NOG mice (available as the FcResolv™ huNOG mouse) were created using identical protocols with CD34+ cells from three human donors (A-C) shared across both strains and animals evaluated for human chimerism at 12 weeks post engraftment (WPE) via peripheral blood (inclusion criteria ≥ 25% hCD45 and > 3500 live single cell count). Animals were shipped to study site, acclimated and baseline reconstitution (prior to study start) was evaluated in a cohort of naïve animals (n=5-6/strain/donor) via flow cytometry at 17 WPE (study start D0; blood, spleen, and bone marrow samples were evaluated) to confirm human T cells (hCD3) were present. HCC827 (10x10^6 cells in 200 µl RMPI 1640 via subcutaneous injection) cells were inoculated in remaining animals (n=58 huNOG, n=79 FcResolv™ huNOG). Animals were randomized on day 7 (D7; criteria = individual tumor volume and % hCD45 at 12 WPE) post-tumor implantation into 12 groups (n=9-14 x 3 donors x treatment/vehicle). Daily clinical observations and twice weekly body weights and tumor growth were measured (length x width via calipers) and recorded. Mice received treatment (pembrolizumab 10 mg/kg IP, 10 ml/kg) or vehicle (0.9% NaCl IP, 10 ml/kg) from D7, dosed twice weekly for four weeks, and were then euthanized for FACS analysis (utilization of a human and murine marker panel) collecting blood, spleen, and tumor samples. Expt B. HIS NOG-EXL (huNOG-EXL) or HIS FcγR knockout NOG-EXL (available as the FcResolv™ huNOG-EXL mouse) were created using identical protocols with CD34+ cells from three human donors (D-F) shared across both strains. Animals were evaluated for chimerism at 10 WPE.

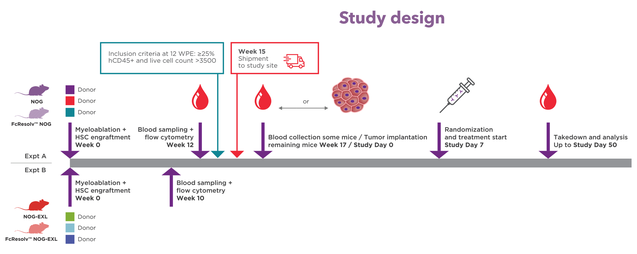


Fig 2. NOG and FcResolv™ NOG (Expt A) and NOG-EXL and FcResolv™ NOG-EXL (Expt B) mice were humanized using identical protocols with three different donors per experiment. Engraftments for a given donor occurred on the same date for both strains. For Expt A, humanized mice (those that met the inclusion criteria) were shipped from the humanization lab to a study site where tumor implantation, randomization, and treatment occurred.

Results

FcResolv™ NOG strains humanize similarly to parent NOG strains

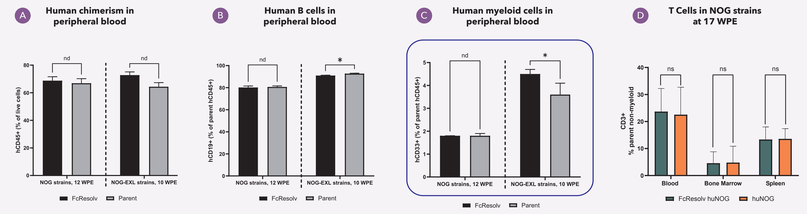


Fig 3. No significant differences were seen in A) overall human immune reconstitution between the parent strain and the FcResolv™ version for NOG (at 12 WPE) and NOG-EXL (at 10 WPE). B) However, there were significant differences in both B) human B cells and C) human myeloid cells ONLY in the FcResolv™ NOG-EXL compared to parent. The FcResolv™ NOG showed no differences in B or C compared to the parent. Multiple unpaired t tests, * q<0.01. D) No significant differences were seen in human T cells in peripheral blood, bone marrow, or spleen of naïve engrafted animals between FcResolv™ NOG (green) and NOG (orange) at either 12 (not shown) or 17 WPE. Human T cells were present at study start (D0).

Donor C responds to pembrolizumab in FcResolv™ NOG but not in NOG

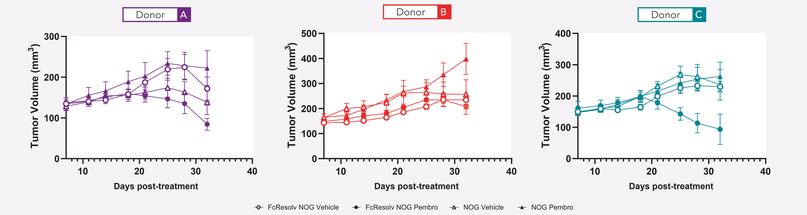


Fig 4. Pembrolizumab treatment showed significant tumor growth inhibition in one donor (Donor C; teal) in FcResolv™ huNOG, but not in donor-matched huNOG mice. Donor B (red) did not show a response to treatment in either strain, whereas in Donor A (purple) the trend was present in FcResolv™ huNOG but did not reach significance due to spontaneous regression in the vehicle arm post D28.

Pembro-treated FcResolv™ huNOG mice had significantly higher human immune cell infiltration into the tumor

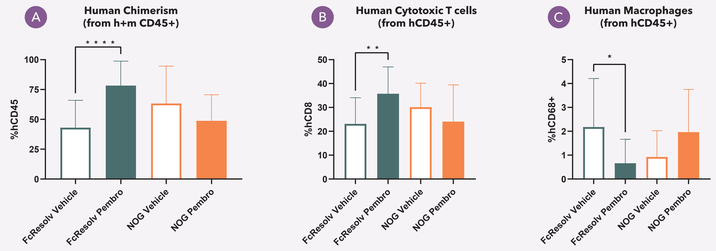


Fig 5. Evaluation of human TILs in pembrolizumab-treated animals showed significant differences between the strains across all donors (A-C; represented as single bar per treatment x strain), with FcResolv™ huNOG mice (green) showing overall higher human infiltration (hCD45+) (panel A), hCD8+ T cells (panel B) and decreased tumor-associated macrophages (panel C) compared to vehicle-treated mice. In contrast, there were no significant differences intratumorally between vehicle and pembrolizumab-treated huNOG (orange).

Human TIL changes in pembro-treated FcResolv huNOG are more reflective of clinical results compared to huNOG

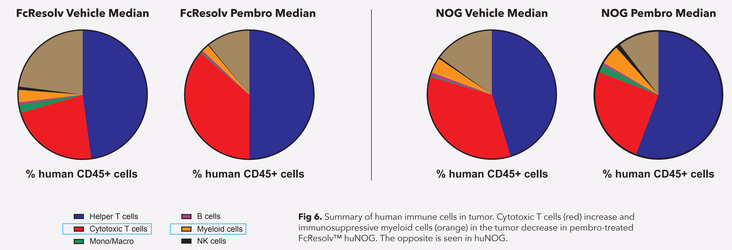
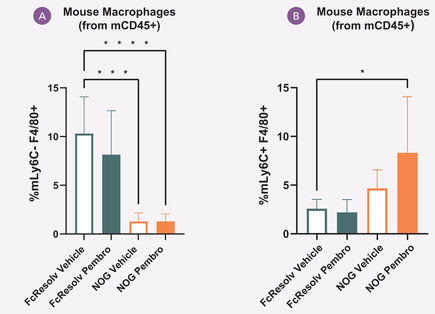


Fig 6. Summary of human immune cells in tumor. Cytotoxic T cells (red) increase and immunosuppressive myeloid cells (orange) in the tumor decrease in pembro-treated FcResolv™ huNOG. The opposite is seen in huNOG.

Ly6C^{lo} dominant macrophage population in tumor of FcResolv™ huNOG mice

Fig 7. Evaluation of murine TILs revealed all the cells present in the tumor were of myeloid origin (data not shown). There were significant differences in the murine macrophage intratumoral populations, regardless of treatment or donor, with a F4/80+Ly6C^{lo} dominant phenotype in FcResolv™ huNOG (panel A; green) compared to the F4/80+Ly6C^{hi} phenotype in the huNOG (panel B; orange). In the presence of pembrolizumab, the murine myeloid population within the tumor, showed a decreased trend in FcResolv™ huNOG but it did not reach significance. In contrast, the huNOG pembro-treated animals showed an increased trend in the intratumoral murine myeloid population compared to vehicle but like the FcResolv™ huNOG, did not reach significance.



Human and mouse immune cells in the spleen show significant differences in the FcResolv™ huNOG but not in the huNOG when treated with pembro

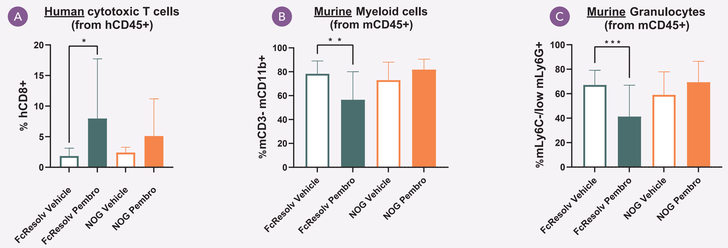


Fig 8. In the spleen, hCD8+ cells (panel A) were increased and murine myeloid (panel B) and granulocytes (panel C) were significantly decreased in the presence of pembrolizumab in the FcResolv™ huNOG (green) compared to vehicle. There were no differences in the spleen with either human or murine immune cells composition between vehicle and treated huNOG animals (orange).

Conclusions

The FcResolv™ NOG and FcResolv™ NOG-EXL strains have similar humanization performance compared to the relevant parent strain, NOG or NOG-EXL

In a tumor-bearing context:

- Pembro-treated FcResolv™ huNOG mice showed an increase in human T cells and decrease in human and murine immunosuppressive myeloid cells as compared to controls
- When treated with anti-PD1, FcResolv™ huNOG mice show expected pharmacodynamic changes and donor-dependent efficacy
- Despite the same donors and identical engraftment parameters, pembro-treated huNOG mice did not show efficacy or expected pharmacodynamic changes
- **These differences are due to the FcγR knockout**, despite pembro being an Fc-silenced IgG4 anti-PD1
- Cannot fully accept the hypothesis (Donor A spontaneous tumor regression), but conclusions support it

Murine innate cells are not inert in the context of human mAbs, and their interactions with Fc domains of antibody-based therapies can introduce confounding factors. FcResolv™ NOG strains represent a cleaner system for efficacy studies of antibody-based therapies which remove potential confounding variables due to interaction with murine Fc gamma receptors.

REFERENCES

1. Katano I, et al. Development of a novel humanized mouse model for improved evaluation of in vivo anti-cancer effects of anti-PD-1 antibody. Sci Rep. 2021 Oct 26;11(1):21087.
2. Dekkers G, et al. Affinity of human IgG subclasses to mouse Fc gamma receptors. MAbs. 2017 Jul;9(5):767-773.
3. Nimmerjahn F, Ravetch JV. Fcgamma receptors as regulators of immune responses. Nat Rev Immunol. 2008 Jan;8(1):34-47.
4. Bruhns P. Properties of mouse and human IgG receptors and their contribution to disease models. Blood. 2012 Jun 14;119(24):5640-9.
5. Bruhns P, et al. Specificity and affinity of human Fcgamma receptors and their polymorphic variants for human IgG subclasses. Blood. 2009 Apr 16;113(16):3716-25.
6. Arlauckas SP, et al. In vivo imaging reveals a tumor-associated macrophage-mediated resistance pathway in anti-PD-1 therapy. Sci Transl Med. 2017 May 10;9(389):eaal3604.
7. Dahan R, et al. FcγRs Modulate the Anti-tumor Activity of Antibodies Targeting the PD-1/PD-L1 Axis. Cancer Cell. 2015 Sep 14;28(3):285-95.
8. Chen X, et al. FcγR-Binding Is an Important Functional Attribute for Immune Checkpoint Antibodies in Cancer Immunotherapy. Front Immunol. 2019 Feb 26;10:292.
9. Li YH, et al. Occurrences and Functions of Ly6C^{hi} and Ly6C^{lo} Macrophages in Health and Disease. Front Immunol. 2022 May 30;13:901672.
10. Zhang T, et al. The binding of an anti-PD-1 antibody to FcγRI has a profound impact on its biological functions. Cancer Immunol Immunother. 2018 Jul;67(7):1079-1090.

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