Profiling of human CD34+ donors in a myeloid-supportive HIS model provides increased predictability and facilitates study design for evaluation of immuno-oncology therapeutics

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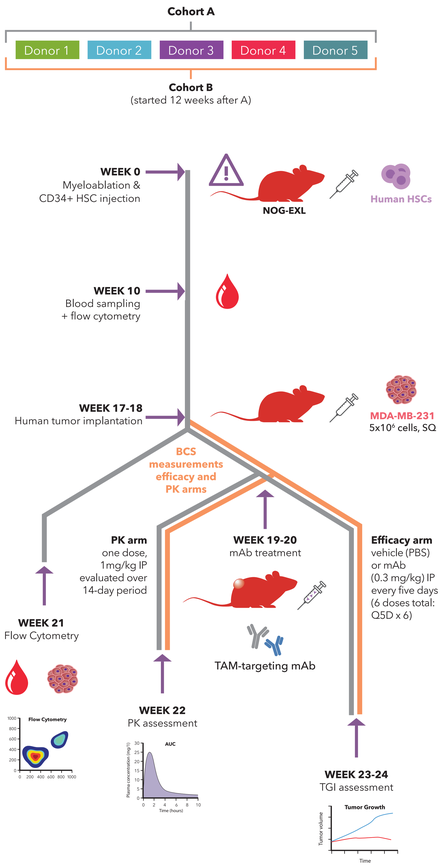
Abstract 5337

Background

Humanized immune system (HIS) mice are critical immuno-oncology tools for *in vivo* evaluation of therapeutic target engagement and efficacy. HIS mice require a human donor source of CD34+ hematopoietic stem cells to provide the human immune cell system. However, variance across donors complicates study design and hypothesis testing[1]. To determine if parameters critical to drug discovery are consistent across batches of HIS mice from the same donor, we evaluated two independent cohorts of human tumor xenograft-bearing mice made using a common set of five different CD34+ cell donors. Our study included evaluation of tumor growth kinetics, human immune reconstitution and phenotype, and the pharmacokinetics and anti-tumor efficacy of a therapeutic monoclonal antibody directed against a human target expressed on tumor-associated macrophages (TAMs).

Methods



Results

Health outcomes are consistent by donor across cohorts

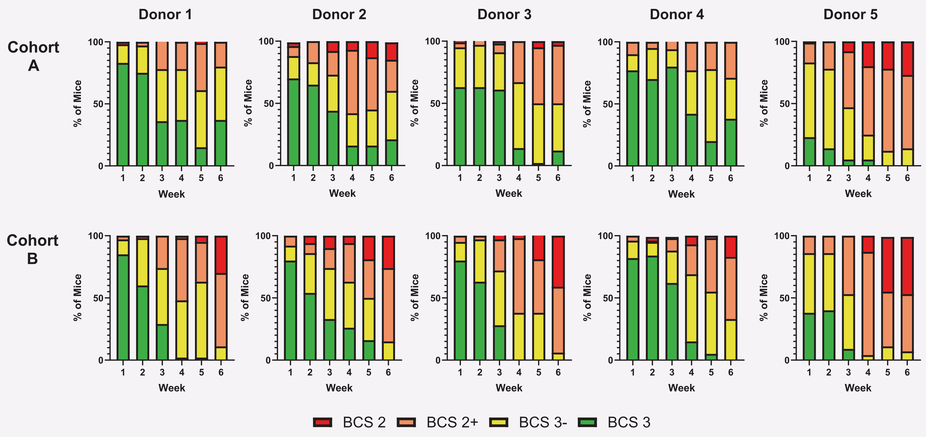


Fig 2. Body condition score (BCS) was evaluated weekly for all mice on study for a total of 6 weeks, starting around the time of tumor implantation (~17-18 WPE). BCS ranges from 1-5 with a score of 3 considered optimal and lower scores indicating poorer health status[2]. Data are from an expanded set of PK and efficacy studies and include both control and experimental groups.

Human immune cell reconstitution and kinetics in periphery are reproducible across cohorts

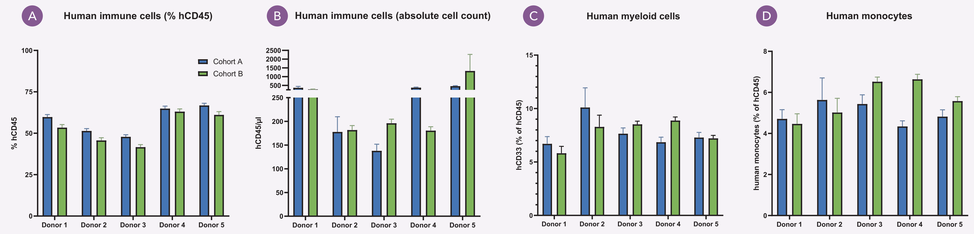


Fig 3. Mean human immune cell reconstitution in peripheral blood was analyzed by flow cytometry prior to tumor implantation (10 weeks post HSC engraftment). Mean total human CD45+ cells and myeloid subsets (hCD33+ cells and monocytes) were similar across cohorts, with no significant difference between cohorts within a single donor (p≤0.01, two-way ANOVA, Sidak's multiple comparisons test).

Pharmacokinetics of a TAM-targeting mAb vary within donors but show similar patterns across cohorts

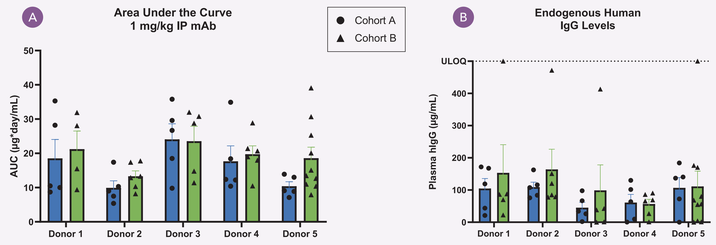


Fig 4. Pharmacokinetics of a human TAM-targeting mAb following a single dose (1 mg/kg IP) were evaluated in tumor-bearing mice over a 14-day period. Endogenous levels of human IgG were measured in pre-dose plasma samples. No significant difference between cohorts within a single donor (p≤0.01, two-way ANOVA, Sidak's multiple comparisons test).

Total human immune infiltration in tumors is similar across cohorts but the immune composition varies

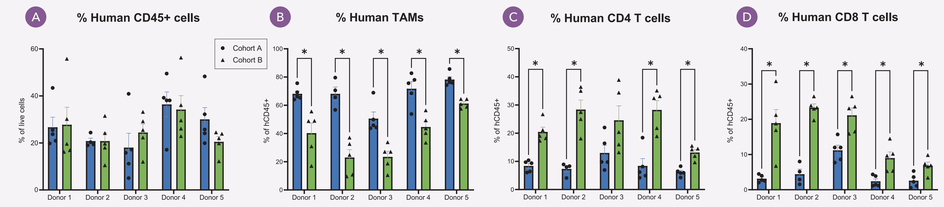


Fig 5. The human immune cell infiltrate in tumors was analyzed by flow cytometry at 3.5-4 weeks post tumor implantation. (A) Total human immune cells (hCD45+) as a % of all live cells. Frequencies of human (B) macrophages, (C) CD4+ T cells, and (D) CD8+ T cells among human immune cells. Multiple unpaired t tests, * = q≤0.01.

Macrophage polarization markers show similar expression patterns across cohorts

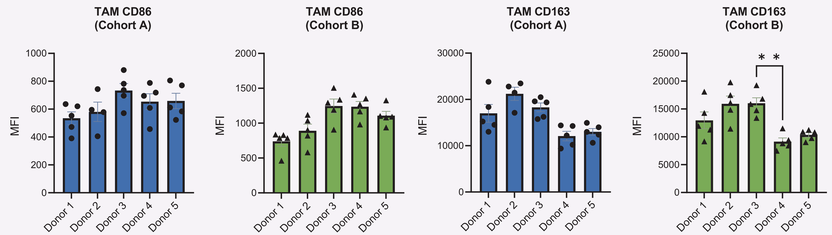
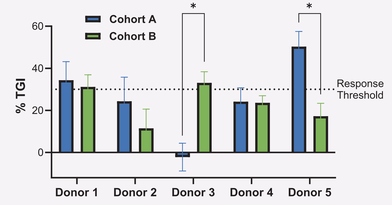


Fig 6. Markers of macrophage polarization were assessed on tumor-associated macrophages by flow cytometry. CD163 is a marker associated with anti-inflammatory M2-like macrophages, while CD86 is associated with pro-inflammatory M1-like macrophages. Median fluorescence intensity (MFI) values are shown. One-way ANOVA, multiple comparisons, Brown-Forsythe and Welch, p≤0.01.

Anti-tumor efficacy with a TAM-targeting mAb is consistent across cohorts for donors with better health outcomes (Donors 1 & 4)

Fig 7. Mice were dosed with a TAM-targeting mAb (0.3 mg/kg IP) or vehicle control every 5 days for a total of 5-6 doses (n=3-5 mice per treatment group per donor). Efficacy was assessed as % tumor growth inhibition (TGI) of individual tumors from mAb-treated group relative to mean tumor volume of vehicle control group at end of study (28-32 days post initial treatment). Groups showing TGIs ≥30% were considered treatment responders. Statistics were calculated by multiple unpaired t tests with multiple comparisons correction. *, p<0.01.



Conclusions

- The health trajectory, peripheral human immune cell engraftment, and PK profiles were similar across experiments for a given donor
- Consistency of efficacy was impacted by health outcomes for a given donor, with consistent efficacy in donors with good health outcomes (1 and 4) versus inconsistent in donors with poor health outcomes (3 and 5)
- Total human immune cell infiltration in tumors was similar across experiments, but immune cell composition shifted
  - Cohort A showed a dominant TAM phenotype within the tumor (~>50% of all hCD45+ cells)
  - Cohort B had reduced TAMs and corresponding increases in several T cell subsets

Our study demonstrates that a return-to-donor approach can provide information that is reproducible across cohorts to help build donor profiles for greater predictability and to facilitate study design for drug discovery applications

REFERENCES

1. Kenkel, et al. Dectin-2 agonist antibodies reprogram tumor-associated macrophages to drive anti-tumor immunity. Poster presented at AACR 2022.
2. Ullman-Cullerè MH, Foltz CJ. Body condition scoring: a rapid and accurate method for assessing health status in mice. Lab Anim Sci. 1999 Jun;49(3):319-23.

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