Poster# 2935

Defining Variation within a Next Gen Humanized Immune System Mouse Model of Human Lung Cancer in the Context of a Checkpoint Inhibitor Efficacy Study

Janell R. Richardson, Megan MacBride, Esther Andino, Marla Wilwol, SueEllen Bethmann, Taylor Knapp, Emily Shako, Paul Volden
Taconic Biosciences, Inc., Rensselaer NY

TACONIC
Models For **Life.**

INTRODUCTION

Humanized immune system mice are critical immuno-oncology tools, and next gen models using human cytokine-expressing mice, such as the NOG-EXL expressing hGM-CSF and hIL-3, drive differentiation of specific cell types. The huNOG-EXL (engrafted with human CD34+ hematopoietic stem cells) is widely used as it develops both myeloid and lymphoid lineages. Our goal was to determine the degree of variance between stem cell donors, tumor types, and immune cell reconstitution (tissue, phenotype, and/or kinetics) within a huNOG-EXL lung cancer model. We studied tumor growth kinetics, human immune reconstitution, and tumor infiltrating leukocytes (TIL) in a human cell-derived model of non-small cell lung cancer (NSCLC; A549) and a patient-derived model of small cell lung cancer (SCLC; LU5173).

Our study provides a comprehensive look at a HIS mouse that reconstitutes multiple human lineages in the context of a human lung cancer model. The significant power and large-scale FACS analysis in multiple tissues demonstrates the impact that tumor type, mouse strain, timeline, and donor can impart on an *in vivo* efficacy study. Proper power analysis accounting for these variables is required for successful immuno-oncology studies.

OBJECTIVE

How does factor X impact the reconstitution and potential IO utility of the model?

Experimental Hypothesis: Factor X significantly effects the reconstitution (chimerism/phenotype/TILs), kinetics, and/or tumor (CDX or PDX) growth profile.

Null Hypothesis: There is no significant difference between animals with factor X and those without.

METHODS

Study design

3 different arms (Factor X, X+2, null) x 2 tumors x 2 treatment arms

Limit for study design sample size = the titer of CD34+ cells of a given donor

- This study: total n=180, n= 60/donor x 3 donors; sample size n=20 (calculated n=13 + 54% attrition factor)
- Provides adequate power for essential question (Factor X), less desirable for 2' variable (tumor) and not adequate for 3' variable (treatment)
- Overage for study constrained to 50%, but we recommend (especially for PDXs) 100%; chimerism SD=20, humanization failure rate <10%

Mice: huNOG-EXL mice were generated via i.v. tail vein injection of human CD34+ hematopoietic stem cells into irradiated, juvenile female NOG-EXL mice (Taconic).

Tumors: SCLC PDX (LU5173) Caucasian, male, 68 yo, right upper lobe, naïve, P5. Cryopreserved tumor cells were SQ inoculated into NSG to generate warm tumors harvested at a volume of 600–1000 mm^3 and cut into 3 mm^3 chunks. These were then SQ inoculated into huNOG-EXL for the pilot tumor growth study. The pilot study mice then provided the 3 mm^3 tumor pieces that were SQ inoculated into the main study huNOG-EXL animals.

NSCLC CDX (A549; ATCC CCL-185) Caucasian, male, 58 yo, P11, *in vitro* expansion of cells. Inoculum of 5x10^6 cells were SQ injected into pilot and main study huNOG-EXL animals.

Pilot tumor growth study: huNOG-EXL were inoculated (n=5 x 2 donors x tumor) with A549 (expanded *in vitro* to 5x10^6 cells per inoculum) or LU5173 (P5, warm tumors 600-1000 mm^3 total size derived 3 mm^3 per inoculum) in the flank SQ. Tumor growth and body weight were measured for 80+ days.

Main study: n=20 huNOG-EXL per donor across three different donors and both tumors for a total n=120. Mice were randomized at a tumor volume of 80–120 mm^3 (A549) or 150-250 mm^3 (LU5173) into an isotype control (human IgG4) or test article group (Pembrolizumab; humanized anti-PD1 antibody). 24 h later, mice were given (BIW x 7) drug or vehicle (10 mg/kg; 5 ml/kg; i.p.). 24 h after final dose, animals were sacrificed and blood, spleen, and tumor collected for a 2-panel 16 color FACS analysis.

Generation of huNOG-EXL

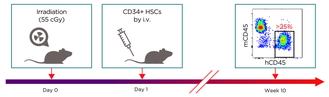


Figure 1: Humanized NOG-EXL mice were generated via i.v. tail vein injection of human CD34+ hematopoietic stem cells into irradiated juvenile female mice.

Graphical representation of study design

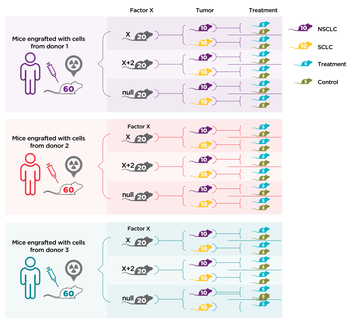


Figure 2: Mice were randomized into the main tumor study based on tumor size and stable bodyweight. Power analysis + overage, n=20 (G Power).

Details of flow cytometry analysis

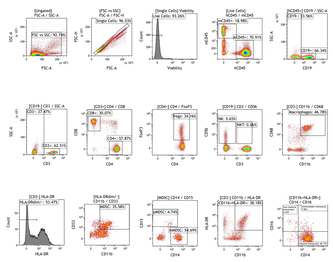
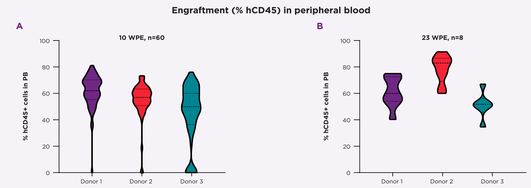


Figure 3: Representative image showing the flow cytometry gating strategy used for this experiment. Cell populations were isolated based on forward scatter, side scatter, and marker expression (list is provided on the left). The single histogram and two parameter density plots are shown in sequential order starting from the top left and finishing at the bottom right.

RESULTS

Human chimerism was stable in huNOG-EXL



	10 WPE	23 WPE
% hCD45 in peripheral blood	Mean (SD)	Mean (SD)
Donor 1	61.4 (12.8)	61.0 (11.8)
Donor 2	55.1 (13.8)	78.5 (10.7)
Donor 3	45.0 (22.1)	51.2 (10.3)

Donors Significantly DIFFERENT
One-way ANOVA p<0.0001

Figure 4: Engraftment varied significantly between donors at A) 10 weeks post-engraftment (no tumor), B) 23 weeks-post engraftment (tumor on-board). Violin plot shown, with median as thicker dashed line and interquartiles as thinner dashed lines.

Pilot study tumor growth

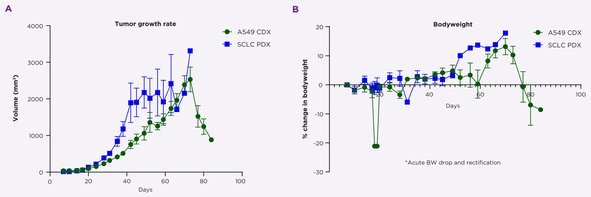
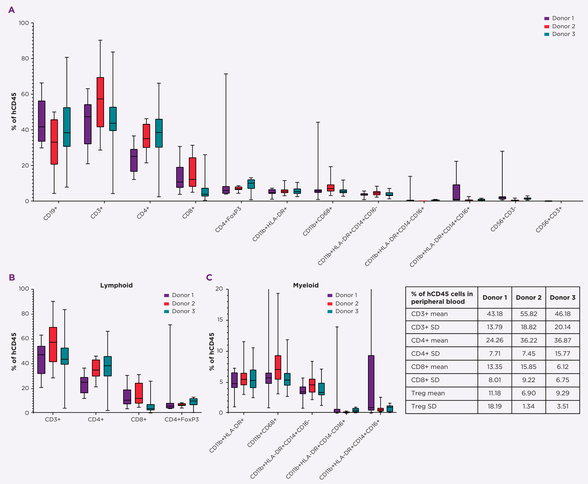


Figure 5: Both the NSCLC cell line and SCLC PDX successfully engrafted in huNOG-EXL mice, n=10/tumor. A) Tumor growth, B) bodyweight. The NSCLC group demonstrated an acute bodyweight loss that was rectified quickly without intervention.

Human T and B cells predominate, but myeloid cells were present at 23 WPE in peripheral blood



% of hCD45 cells in peripheral blood	Donor 1	Donor 2	Donor 3
CD3+ mean	43.18	55.82	46.18
CD3+ SD	13.79	18.82	20.14
CD4+ mean	24.26	36.22	36.87
CD4+ SD	7.71	7.45	15.77
CD8+ mean	13.35	15.85	6.12
CD8+ SD	8.01	9.22	6.75
Treg mean	11.18	6.90	9.29
Treg SD	18.19	1.34	3.51

Figure 6: A) Flow cytometric analysis of human cells in peripheral blood in tumor-engrafted mice (both tumor types), isotype control group, n=12/donor. B) B cells (CD19+) and T cells (CD3+) comprised the majority of human cells in huNOG-EXL, with CD4+ and CD8+ T cells and CD4+ FoxP3+ Tregs present. Classical (CD11b+ HLA-DR+ CD14+ CD16-), intermediate (CD11b+ HLA-DR+ CD14+ CD16+) and non-classical (CD11b+ HLA-DR+ CD14- CD16+) monocytes and macrophages (CD11b+ CD68+) were present in lower levels. Cell distribution in spleen was similar to that in peripheral blood (data not shown).

Analysis of tumor infiltrates: A549 NSCLC CDX

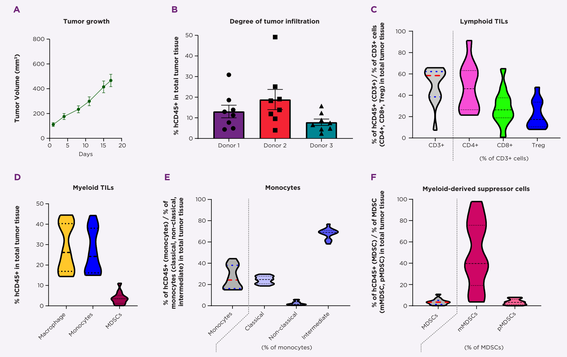


Figure 7: A) A549 NSCLC successfully engrafted in humanized mice, n=23. B) Infiltration of human leukocytes into tumors in isotype control mice (n=8/donor) varied by donor. Human lymphoid cells (C) and myeloid cells (D) infiltrated tumors, including monocytes (E) and myeloid-derived suppressor cells (MDSCs, F), n=12.

Analysis of tumor infiltrates: SCLC PDX

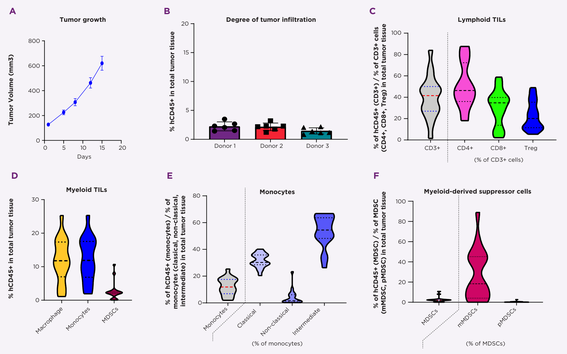


Figure 8: A) A SCLC PDX successfully engrafted in humanized mice, n=33. B) Infiltration of human leukocytes into tumors in isotype control mice (n=6/donor) varied by donor. Human lymphoid cells (C) and myeloid cells (D) infiltrated tumors, including monocytes (E) and myeloid-derived suppressor cells (MDSCs, F), n=18.

Not able to detect a treatment effect due to power limits

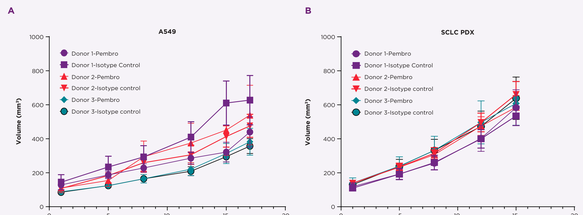


Figure 9: As CD34+ cells per donor limited overall study size, the power was not sufficient to determine a treatment effect with either the A) CDX or B) PDX tumor. This was also impacted by COVID-related scheduling issues, with study duration limited due to late implantation of tumors relative to immune system engraftment. Timing of tumor engraftment in humanized mice is critical to experimental success.

SUMMARY

- Humanized immune engraftment was stable over time in huNOG-EXL mice:
  - Engraftment characteristics varied by donor (inter- and intra-variance important to power analysis).
  - Human T and B cells predominated, but myeloid cells, including monocytes and macrophages, and NK cells were also present.
  - Donor differences were seen in the overall reconstitution and distribution across the 16 cell types measured.
- huNOG-EXL mice successfully engrafted the A549 NSCLC cell line and a SCLC PDX. A549 had accelerated growth but at late time points (day 70) tumor volume and body weight declined for both donors. LU5173 had delayed growth but larger sustained volume sizes, with no adverse affects on animal health or tumor volume reduction in both donors in the pilot study.
- There was a significant difference in the extent of human immune cell infiltration between tumor types, but both lymphoid and myeloid TILs, including MDSCs and TAMs were present. A549 tumors had significantly more hCD45+ cells than LU5173 tumors.
- The tumor growth inhibition did not significantly differ between treatment groups — impact of limited power and study scheduling issues resulted in advanced age prior to dosing.
- Accepted the null hypothesis and rejected the experimental (1' question, Factor X).
- Study power can easily be diluted based on the # of questions: n=60 to n=20 to n=4.
  - Unable to determine treatment effect due to insufficient power for 3' question (too many variables).

CONCLUSIONS

- Unless the effect is predicted to be large, a sufficient n-value must account for both variance of the introduced variables and that imparted by the donor
- The limit to the power (n-value) of a HIS study design is the # of CD34+ cells of a donor
- For drug efficacy studies, n-values must be sufficiently powered to account for animal health, donor variance, and variance of effect
- Tumor growth and infiltration can vary based on tumor type, HIS donor, tumor donor, passage #, strain, orthotopic vs. SQ, etc.
- Study duration vs. model kinetics/health outcomes must align
- Recapitulating checkpoint inhibitor efficacy studies published in humanized immune system mice requires maintenance of methodology, vision of variables, and adequate powering of an experiment accounting for both

For humanized mice, the methods matter!

- Level of chimerism
- Type of human cells present
- Duration of study window
- Expected variance
- Overall utility

Even if we could use the same donor: the results produced with one model may NOT carry over to another.