41. Growth kinetics and immune cell profiling of BT-474 human breast carcinoma and Raji-Luc human Burkitt lymphoma in four strains of triple immunodeficient mice

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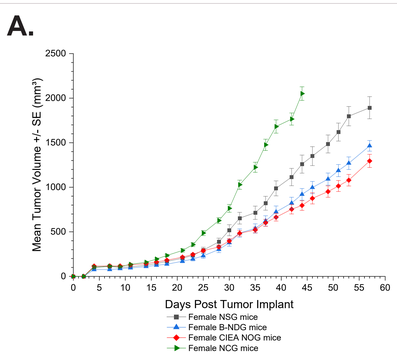
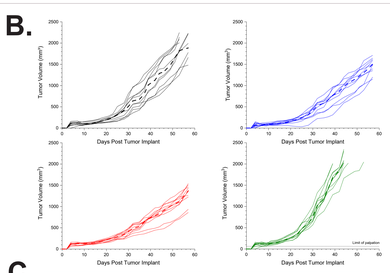
Introduction

- Triple immunodeficient mouse models are often necessary to evaluate xenograft models and adoptive cell therapies using human immune cells.
- Triple immunodeficient mice lack functional T cells, B cells and natural killer (NK) cells.
- Female NOD.Cg-*Prkdc*^{scid} *Il2rg*^{tm1Wjl}/SzJ (NSG) mice, NOD.CB17-*Prkdc*^{scid} *IL2rg*^{tm1}/BcgenHsd (B-NDG) mice, NOD-*Prkdc*^{em26Cd52}*Il2rg*^{em26Cd22}/NjuCrl (NCG) mice and NOD.Cg-*Prkdc*^{scid} *Il2rg*^{tm1Sug}/JicTac (CIEA NOG) mice were implanted with subcutaneous BT-474 or systemic Raji-Luc to evaluate growth kinetics and to collect samples to assess immune cell profiles.
- Disease induction and growth parameters were determined through previous experience and optimization studies with subcutaneous BT-474 and systemic Raji-Luc (data not shown).
- Blood, spleen and tumor samples were collected from mice implanted with BT-474 and blood, spleen and bone marrow (BM) samples were collected from mice implanted with Raji-Luc.
- Immune cell profiles were analyzed via flow cytometry to quantify lymphocyte and myeloid immune cell subsets and to observed differences between the four mouse strains.





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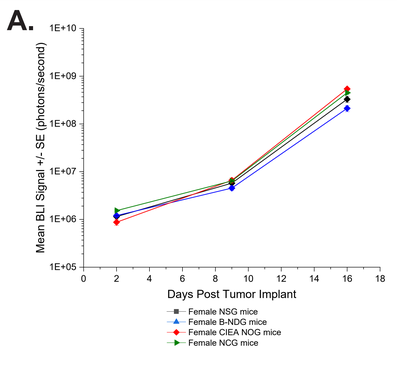
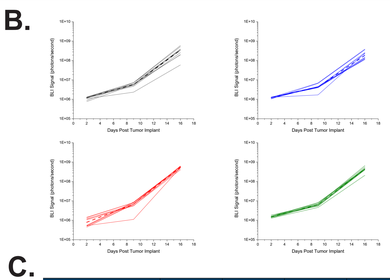
Strain	Mean Body Weight Change (%)	Median Tumor Doubling Time (Days)	Median Time to Evaluation Size 150mm³ (Days)	Median Time to Evaluation Size 1250mm³ (Days)
Female NSG	28.0	7.3	15	44
Female B-NDG	18.8	9.2	18	51
Female CIEA NOG	16.9	12.3	14	55
Female NCG	27.7	6.4	11	35

Figure 1. Growth kinetic evaluation of subcutaneous BT-474 in female NSG, B-NDG, CIEA NOG and NCG mice.
A. Mean tumor volumes + SE
B. Individual tumor plots
C. Group endpoint evaluations





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Treatment(s)	Mean Body Weight Change (%)	BLI Median Tumor Doubling Time (Days)	Median Lifespan (Days)	BLI Median Time to Evaluation Size 1.0E+08 p/s (Days)
Female NSG	-6.6	1.6	18	13
Female B-NDG	-3.0	1.9	18	14
Female CIEA NOG	-9.6	1.4	16	13
Female NCG	-6.6	1.6	16	13

Figure 2. Growth kinetic evaluation of disseminated Raji-Luc in female NSG, B-NDG, CIEA NOG and NCG mice.
A. Mean BLI signal + SE
B. Individual BLI signal plots
C. Group endpoint evaluations

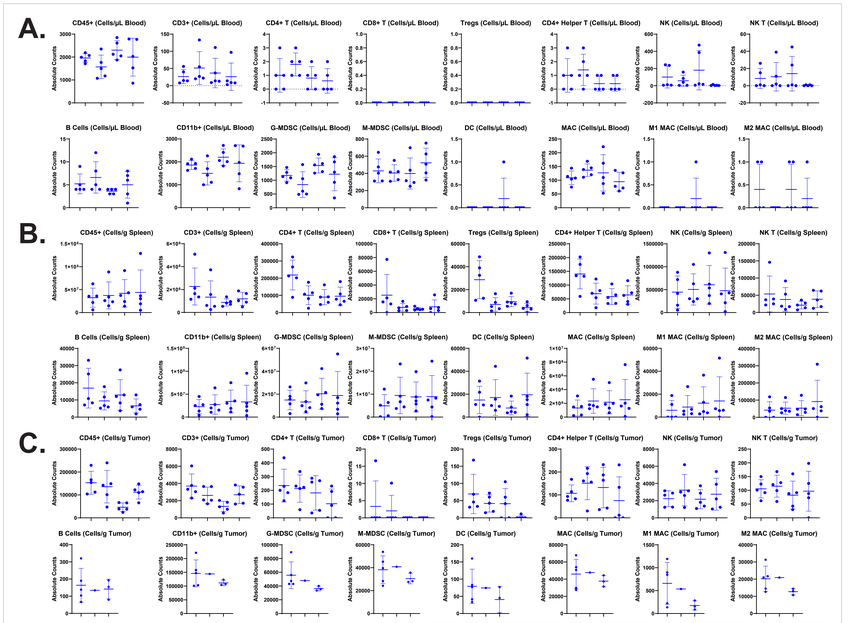

Figure 3. Comprehensive leukocyte immunophenotype assessment for mice implanted with BT-474.
A. Blood
B. Spleen
C. Tumor

Column 1: Female NSG mice
Column 2: Female B-NDG mice
Column 3: Female CIEA NOG mice
Column 4: Female NCG mice

Note: There was not enough tumor material from female CIEA NOG mice to complete a full comprehensive leukocyte flow panel. For the affected groups, female NCG mice are on the third column.

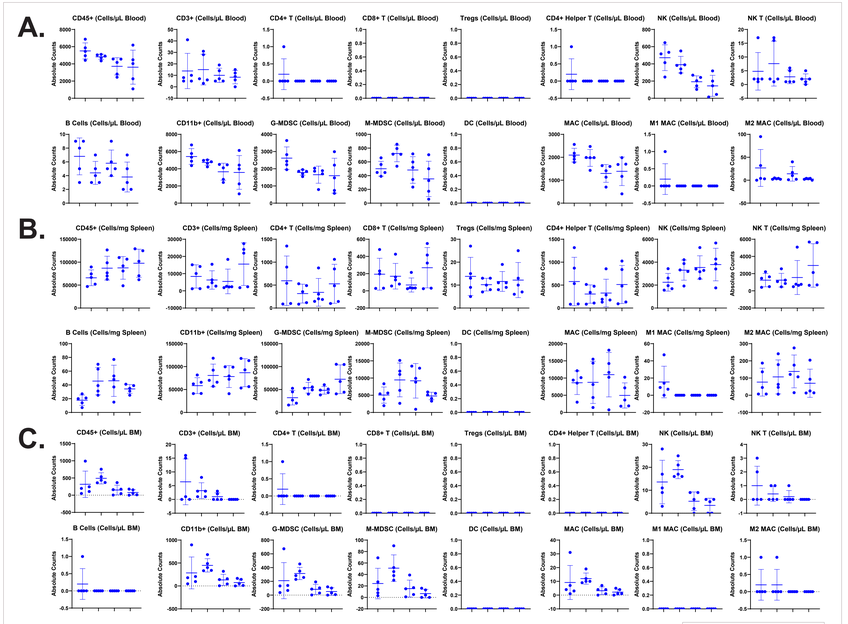

Figure 4. Comprehensive leukocyte immunophenotype assessment for mice implanted with Raji-Luc.
A. Blood
B. Spleen
C.. Bone marrow

Column 1: Female NSG mice
Column 2: Female B-NDG mice
Column 3: Female CIEA NOG mice
Column 4: Female NCG mice

Methods

- Mice were sorted into groups by strain. Body weights were collected prior to cell implant. Tumor growth changes were tracked by digital caliper measurements for subcutaneous tumors and by bioluminescence imaging (BLI) (PerkinElmer IVIS Spectrum, Waltham, MA) for systemic disease.
- BT-474 cells were implanted subcutaneously into the right axilla and Raji-Luc cells were implanted intravenously into the lateral tail vein of female NSG, B-NDG, NCG and CIEA NOG mice.
- Immune cell profiling was performed on samples following disease establishment. For the BT-474 model, whole blood, spleen and tumors were collected when the mean group tumor volumes were ~350-600 mm^3. For the Raji-Luc model, whole blood, spleen and bone marrow was collect on Day 15, when disease is believed to be at late stage, just prior to mice exiting the study. Tumors and spleens were digested to a single cell suspension (Miltenyi, Germany) and bone marrow was filtered through a 70-µm filter for flow cytometry. A comprehensive leukocyte (CompLeukocyte Package™) panel was acquired on an Attune® NxT flow cytometer (Thermo Fisher Scientific) and analyzed with FlowJo® software (FlowJo LLC, Ashland, OR).
- All animal work was performed in an AAALAC-accredited facility, in alignment with applicable animal welfare regulations and with predetermined humane euthanasia criteria on all studies.

Results and Conclusions

- BT-474 and Raji-Luc grew well in all 4 mouse strains, resulting in 100% take rate.
- When B-NDG, CIEA NOG and NCG mice were compared to NSG mice, the median tumor volume doubling times differed by 1-5 days and <1 day, the median time to staging or BLI signal above background (150 mm^3/8.4E+05 p/s) differed by +/- ~4 days and <1 day, and the median time to end of life (or a tumor volume of 1,250 mm^3 for BT-474) differed by +/- ~10 days and +/- 2 days for BT-474 and Raji-Luc, respectively.
- BT-474 did not induce body weight loss in any of the mouse strains and all clinical observations and necropsy findings were similar.
- For subcutaneously implanted BT-474, tumors grew the fastest in female NCG mice and the slowest in female CIEA NOG mice based on tumor doubling times (Td) and time to evaluation size calculated by caliper measurements.
- In the BT-474 model, the absolute counts were similar for all immune cell subsets measured except for regulatory T cells (Tregs). NSG mice had elevated Treg numbers in the spleen and NCG mice had reduced Treg numbers in the tumor.
- Raji-Luc induced the expected body weight loss associated with late-stage disease in all mouse strains and all clinical observations and necropsy findings were similar.
- For disseminated Raji-Luc, disease progressed similarly across all mouse strains. Female CIEA NOG mice exited the study slightly earlier and had the largest BLI signal at the last timepoint and female B-NDG mice exited the study slightly later and had the lowest BLI signal at the last timepoint. This determinations were made based on Td and time to evaluation size/median day of death calculated by BLI.
- In the Raji-Luc model, the absolute counts were similar for all immune cell subsets measured except for NK cells. NSG and B-NDG mice had elevated NK cell numbers in the blood and bone marrow.

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