The Development and Characterization of a Humanized Xenograft Murine Model for Osteosarcoma

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Introduction

- Osteosarcoma (OS) therapy has stagnated for nearly 40 years.
- There is a tremendous need for the development of novel therapeutic approaches such as immunotherapy.
- However, evaluation of immunotherapy for OS is challenging within current pre-clinical models, which generally utilize immunocompromised animals.
- A humanized model would more accurately recapitulate the human scenario and permit a more accurate assessment of novel therapies such as immunotherapy.

Purpose

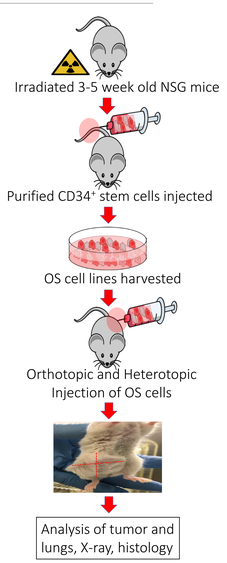
A. To characterize OS growth within a humanized xenograft murine model
B. To create a cost-effective and reproducible process for scaling this model

Research Questions

1. Can human osteosarcoma grow within a humanized mouse model?
2. What is the lymphocyte infiltration pattern associated with tumor growth within this model?
3. What is the origin of vasculature within the tumor?

Methods

- NSG mice were irradiated with 250 cGY using a Cs^{137} source irradiator.
- CD34+ lymphocyte progenitor stem cells were isolated from human umbilical cord blood using density gradient centrifugation.
- CD34+ cells were purified using Magnetic-activated cell sorting (MACS®) and injected into mice.
- Peripheral blood was used to validate engraftment using a BD LSR II flow cytometer.
- Multiple standard and patient-derived xenograft tumors were cultured and implanted.
- Mice were euthanized when tumors palpable.
- Tumor analyzed radiographically and histologically.
- Tumor infiltrating lymphocytes identified using immunohistochemistry.



Results

Figure 1: 30 % of Mice Humanized by Week 10

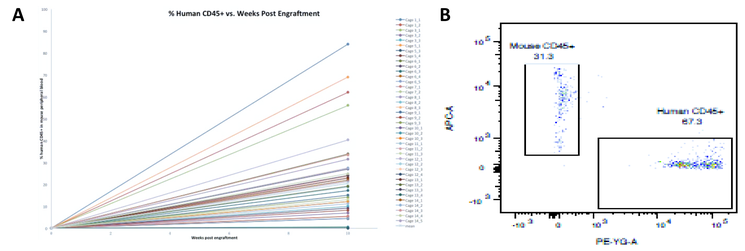


Figure 1. (A). Line plot representation of % human engraftment at weeks 10, n=40 **(B).** Flow cytometry analysis demonstrating distinct leukocyte populations (human CD45+, x axis) and (mouse CD45+, y axis)

Figure 2: Human OS Grows in Humanized Mouse

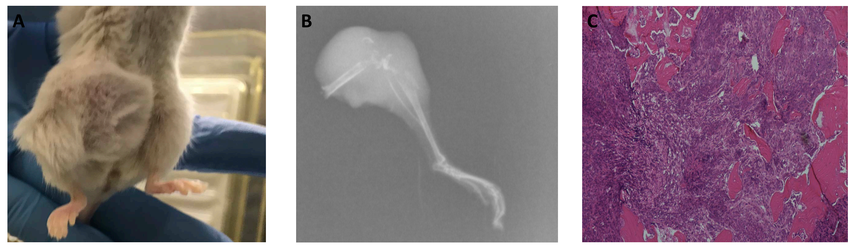


Figure 2. (A). Tibial growth of 143B osteosarcoma tumor **(B).** Lateral radiograph of mouse tibia with OS33 tumor demonstrating bone destruction **(C).** H&E stain of 143B osteosarcoma tumor within humanized mouse tibia

Figure 3: Human Lymphocytes are Present in Medullary Cavity

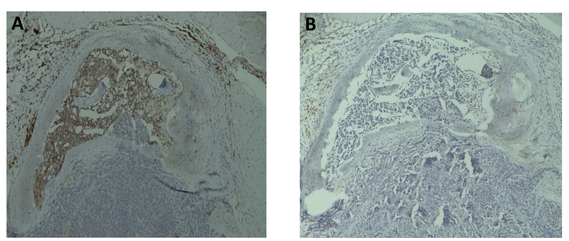


Figure 3. (A). Illustrates positive staining for human CD45+ in humanized mouse tibial medullary space indicating leukocytes are of human origin **(B).** Illustrates negative staining for mouse CD45+ within humanized mouse tibial medullary space, confirming leukocytes are of human origin

Figure 4: Human Lymphocytes Infiltrate Both Primary and Metastatic Lesions

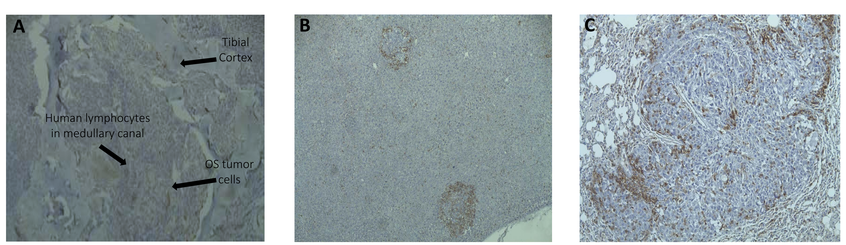


Figure 4. (A). Illustrates positive staining for human CD45+ in OS33 primary tumor lesion **(B).** Illustrates positive staining for human CD45+ in an OS liver metastatic lesion **(C).** Illustrates positive staining for human CD45+ in an OS pulmonary metastatic lesion.

Figure 4: Endothelial Cells are of Mixed Origin

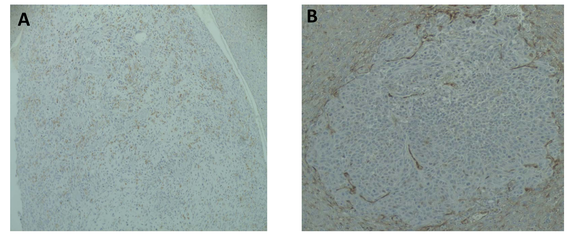


Figure 4 (A). Illustrates positive staining for human CD31+ within a metastatic pulmonary nodule, indicating that vasculature contains human endothelial cells **(B).** Illustrates positive staining for human CD31+within a metastatic lesion, indicating that vasculature contains mouse endothelial cells

Figure 5: Lymphocytes include CD3+ and CD8+

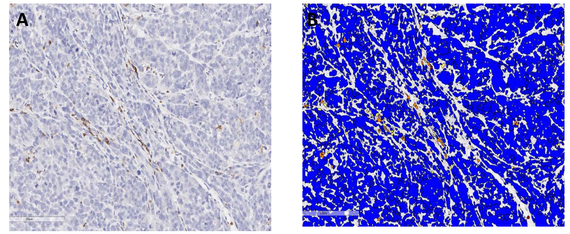


Figure 5. (A). Illustrates positive straining for human CD3+, confirming the presence of infiltrating T-cells within OS tumor (brown) (Similar CD8+ staining not shown) **(B).** Image analysis algorithm, used to quantify immune infiltrate, counted the number of labeling cells (labeled as red, yellow and orange) verse non-labeling cells (blue)

Conclusions & Future Research

- Humanized model permits for reliable tumor implantation and growth.
- Human T-cells are the predominant tumor-infiltrating cells.
- Tumor vasculature appears to derive from both host and tumor origin.
- Infiltrating lymphocytes include CD3+ and CD8+ cells.
- Future work will focus on defining the humanization timeline, the infiltrative CD45+ leukocyte subpopulation, and the effect of various therapies on tumor within this model.