Severely immunodeficient NOG-EXL mice allow for humanization and development of a human glioblastoma-derived tumor microenvironment

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BACKGROUND

- **Glioblastoma (GBM)** is most common primary brain tumor in adults and is nearly uniformly fatal, with median survival of <2 years.[1,2]
- To date, minimal successes in GBM immunotherapy have translated from the lab to the clinic.[3]
- Traditional *in vivo* model systems are either human xenografts in immunodeficient mice or syngeneic tumors grown in immunocompetent mice.[4]
- Both approaches miss key elements of the tumor microenvironment, potentially contributing to the mismatch between pre-clinical and clinical results in immunotherapy.
- Humanized mice traditionally use healthy donor material for humanization, creating potential immune mismatches with the accompanying tumor model.

OBJECTIVES

1. Demonstrate feasibility of generating an autologous humanized mouse model for GBM.
2. Characterize tumor microenvironment found in humanized mouse model.

MATERIALS & METHODS

- CD34⁺ hematopoietic stem cells were obtained from a GBM patient at the time of tumor resection.
- In parallel, GBM organoids (GBOs) were established from the tumor resection.
- SGM3 and NOG-Exl mice were humanized with injections of either autologous or allogeneic hematopoietic stem cells.
- Humanization was confirmed at ~16 weeks by percentage of CD45 cells that were human in origin.
- Humanized mice received intracranial injections of dissociated GBOs cells.
- Animals were euthanized after 4 weeks of tumor growth and assessed by IHC.

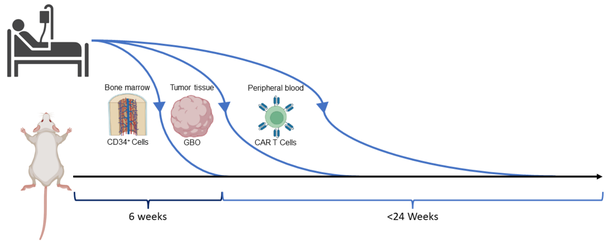


Figure 1. Cartoon schematic of autologous humanized mouse setup.

RESULTS

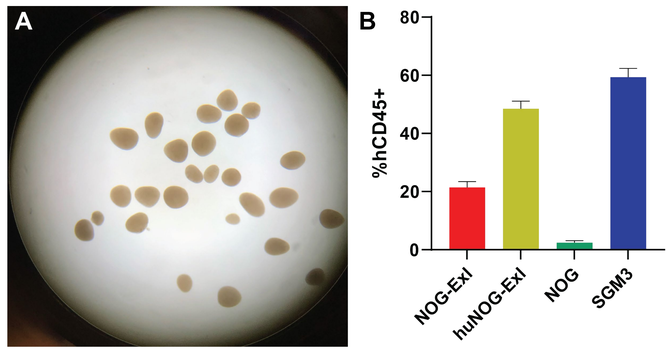
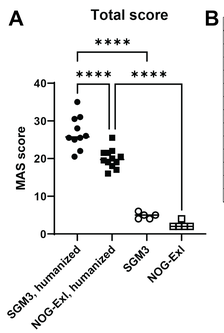


Figure 2. GBO growth and mouse humanization. (A) 2x magnification of GBOs after 14 weeks of culture, highlighting successful growth of tumor model from a recurrent GBM patient. (B) Humanization levels for mice at Week 17, except for SGM3 mice, which were assessed at Week 11 due to early death attributed to macrophage activation syndrome. Human CD45 levels greater than 5% were considered successful humanization.



B

Organ	Evaluation
Liver	Infiltrate (granulomatous/histiocytic, eosinophilic)
Liver	Hepatocellular degeneration/necrosis
Liver	Erythrophagocytosis
Spleen	Erythrophagocytosis
Spleen	Infiltrate (granulomatous/histiocytic)
Pancreas	Infiltrate (mastocytic, eosinophilic)
Salivary glands	Infiltrate (granulomatous/histiocytic, eosinophilic)
Bone marrow	Increased eosinophilopoiesis
Bone marrow	Infiltrate (histiocytic)
Lung	Infiltrate (granulomatous/histiocytic)
Skin	Infiltrates (granulomatous/histiocytic +/- interface dermatitis)
Meninges	Infiltrate (eosinophilic/histiocytic)
Blood	Anemia (Hgb)

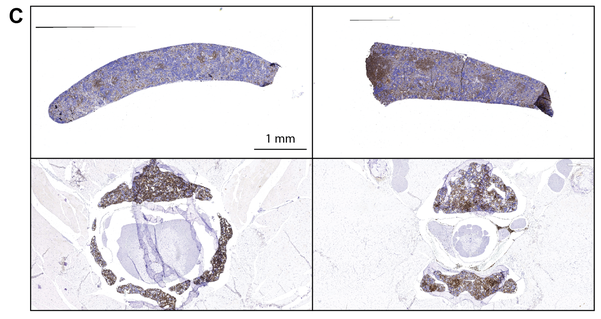
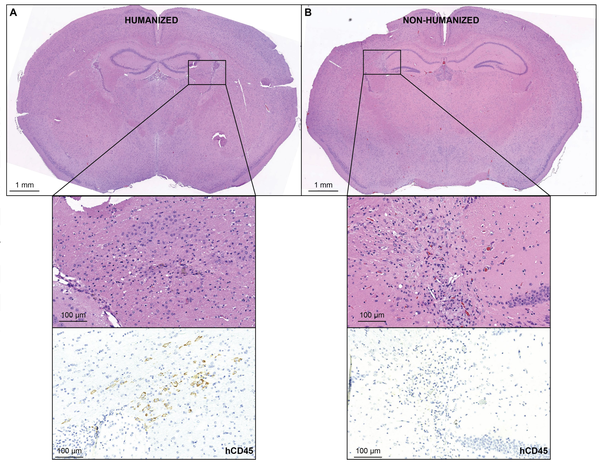


Figure 3. NOG-Exl mice can be humanized by GBM patient hematopoietic stem cells. (A-B) Macrophage activation score (MAS) for SGM3 mice versus NOG-Exl mice (A) and histologic features of MAS (B). NOG-Exl mice did demonstrate features of MAS but to a significantly smaller degree than SGM3 mice. This difference translated into prolonged survival after humanization, allowing for tumor implantation, growth, and tumor microenvironment formation. (C) hCD45 staining for spleens (top) and bone marrow (bottom). Left is from an autologous humanized mouse; right is from an allogeneic humanized mouse. Both autologous and allogeneic sources led to successful engraftment.

Figure 4. Tumor growth in humanized mice. Hematoxylin and Eosin (H&E) and human CD45 staining showing tumor and TME in (A) a humanized mouse with tumor and (B) a non-humanized mouse with tumor. Insets show areas of atypia, increased cell density, and nuclear pleomorphism indicative of tumor growth. Human CD45 stain demonstrates successful humanization and tumor infiltration in the humanized mouse while confirming the lack of human CD45+ cells in the non-humanized mouse.



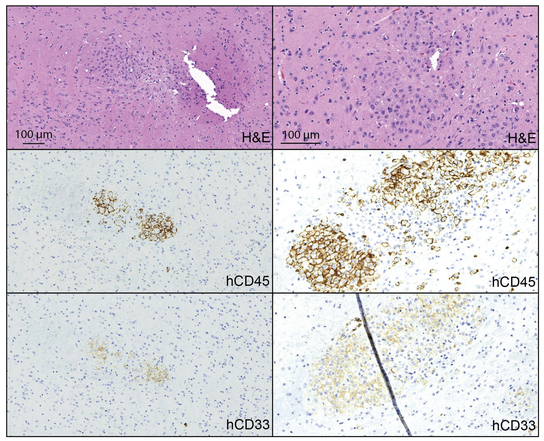


Figure 5. Tumor microenvironment identification in humanized mice. H&E (left), human CD45 (center), and human CD33 (right) staining of both autologous (top) and allogeneic (bottom) humanized mice. The positive CD45 stain confirms the presence of human-derived immune cells in proximity to the tumor. Human CD33, marking myeloid cells, suggests a predominance of macrophages making up the tumor microenvironment, which follows along with GBM pathology.

CONCLUSIONS

- Patient HSCs can be used to successfully humanize mice.
- NOG-Exl mice provide stable humanization levels through 22 weeks of growth.
- Patient-derived GBOs form intracranial tumors in humanized mice.
- CD45⁺ cells of human origin infiltrate intracranial tumors, forming a tumor microenvironment.
- Further work is needed to thoroughly characterize elements of the tumor microenvironment, especially in the context of the originating patient's tumor.

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