

Abstract #7815: Endogenous Retrovirus–Derived Neoantigens Enable a Personalized Cancer Vaccine Strategy for Glioblastoma

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Introduction

- **Glioblastoma (GBM)** is the most common and most aggressive primary malignant brain tumor
- **Standard of care:** surgical resection followed by chemoradiation
- **Outcomes remain poor:** median overall survival ~15 months; 5-year survival <10%
- **Key challenge:** profound immunosuppression and low tumor mutational burden limit traditional mutation-derived neoantigen discovery and reduce responsiveness to immunotherapy
- **Hypothesis:** computational/AI-predicted, personalized GBM-specific Endogenous retroviral elements (ERVs) and neoantigens can be combined to enable a novel personalized cancer vaccine strategy
- **Purpose:** to demonstrate vaccine design feasibility and *in vitro* antigen proof-of-concept
- **Approach:** AI-informed computational antigen predictions derived from tumor DNA- and RNA-sequencing data (Tumor and matched normal tissues from 24 GBM patients 17 long-term survivors >5 years; 7 with <18-month survival)

Antigen sources in GBM: ERVs and Neoantigens

- Neoantigens are novel protein fragments that arise from somatic mutations in tumor cells
- ERVs are genomic remnants of ancient viral insertions, which are epigenetically silenced, but can become aberrantly reactivated in cancer
- ERVs serve as alternative tumor-specific antigens (TSA), alongside somatic mutation-derived neoantigens
- Our computational approach using Evaxion's AI-Immunology™ platform identifies ERV-derived antigens in most patient GBM samples, which are used as complimentary source in vaccine strategy

Key Conclusions

- For all 24 GBM tumors a personalized ERV/neoantigen vaccine could be designed using Evaxion's AI-Immunology™ platform
- 21 out of 24 designs included both antigen types, whereas 2 designs only contained neoantigens and 1 with only ERV antigens
- Functional *in vitro* validation studies demonstrated IFN-γ responses in 3 tested donors with different HLAs

Personalized DNA Vaccine Strategy

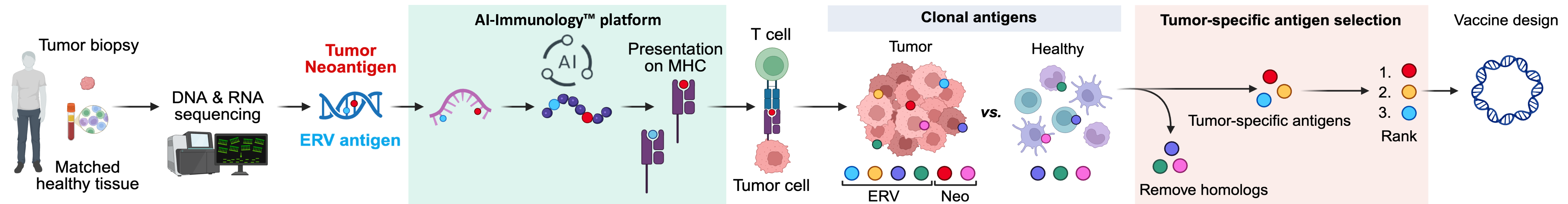


Figure 1. AI-informed, computationally directed, personalized DNA-based vaccine strategy. Paired tumor and matched normal samples (PBMCs) from each patient underwent DNA and RNA sequencing and were analyzed using two proprietary pipelines from Evaxion's AI-Immunology™ platform: PIONEER™, identifying mutation-derived neoantigens, and ObsERV™, identifying ERV-derived antigens. Neoantigen candidates were defined by tumor-specific DNA variants with RNA expression, while ERV tumor specificity was defined by dysregulated tumor expression with ERVs expressed in healthy tissues (GTEX) removed as a blacklist. Resulting tumor-specific antigens were ranked by expression, predicted MHC class I and II presentation, clonality, and immunogenicity, synthesized as pooled peptides, and functionally validated *in vitro* by ELISpot.

Antigen Burden in Glioblastoma and Personalized Vaccine Designs

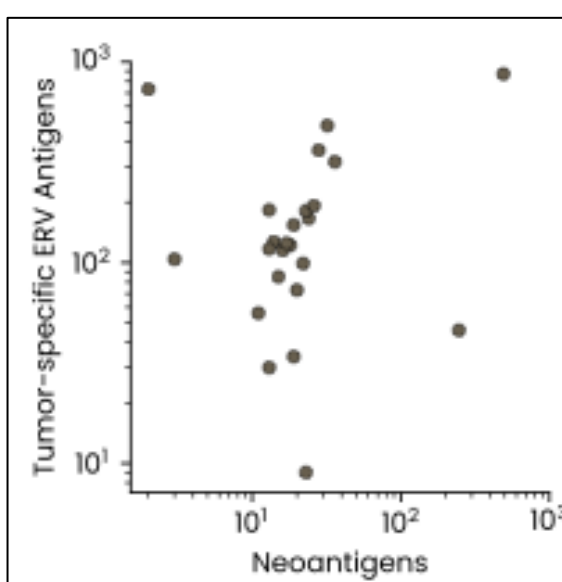


Figure 2. Scatterplot diagram comparing number of identified neoantigens (X-axis) to number of tumor-specific ERV antigens (Y-axis) across compiled patients. One dot = one subject. N=24.

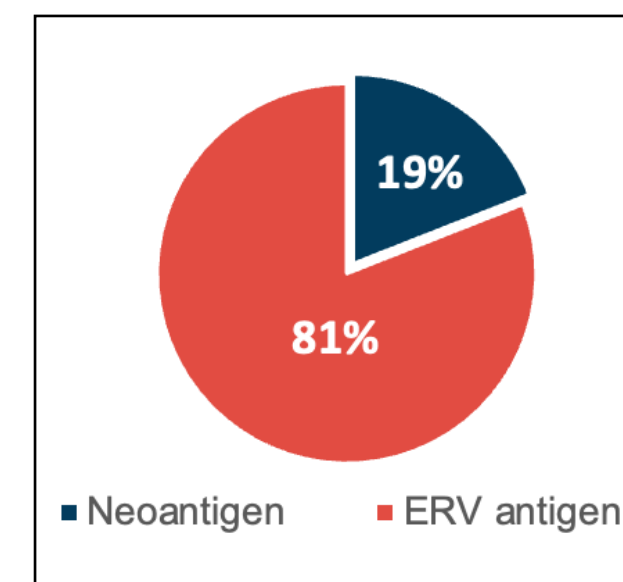


Figure 3. TSA abundance by source (neoantigen vs ERV antigen). Number of observations for each antigen class summed up across all patients. N=24. Neoantigens=blue, ERV antigens=red.

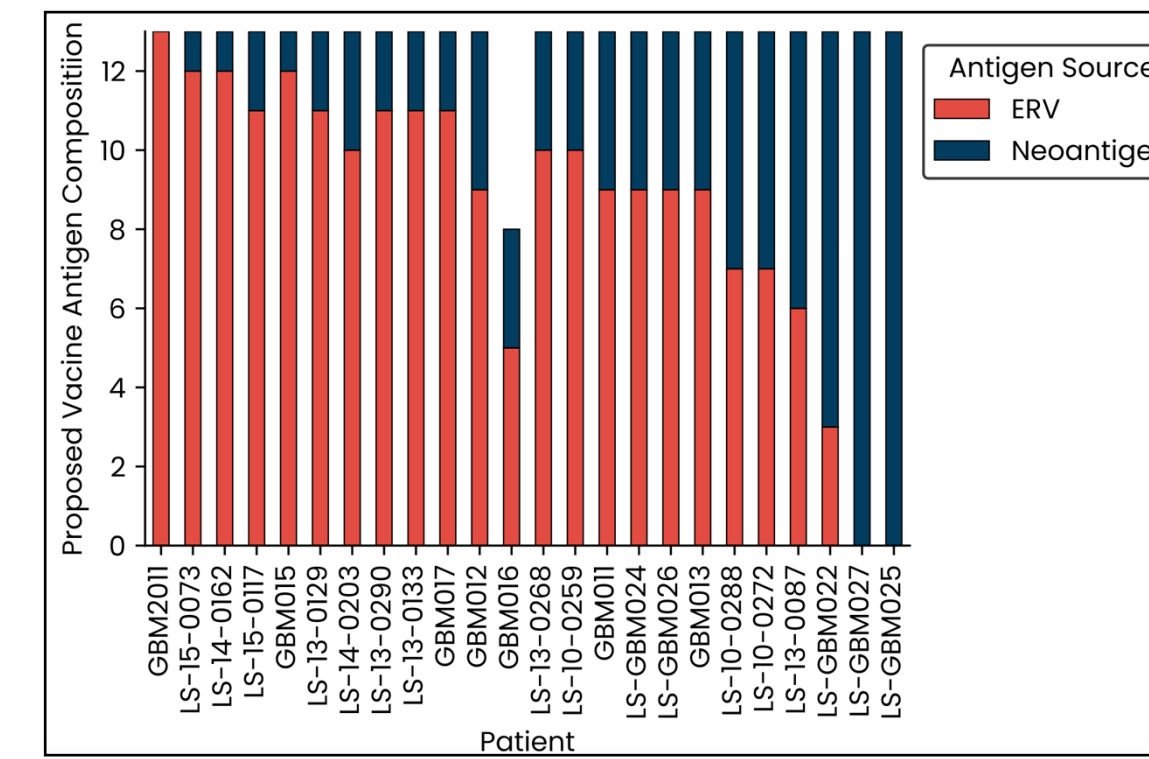


Figure 4. Personalized vaccine designs for the 24 GBM patients. The bar plot illustrates the antigen composition of the individual design: red color marks ERV-derived antigens, blue neoantigens. On the Y-axis the number of antigens included in design (max. 13). Neoantigens=blue, ERV antigens=red.

In Vitro Functional Assessments

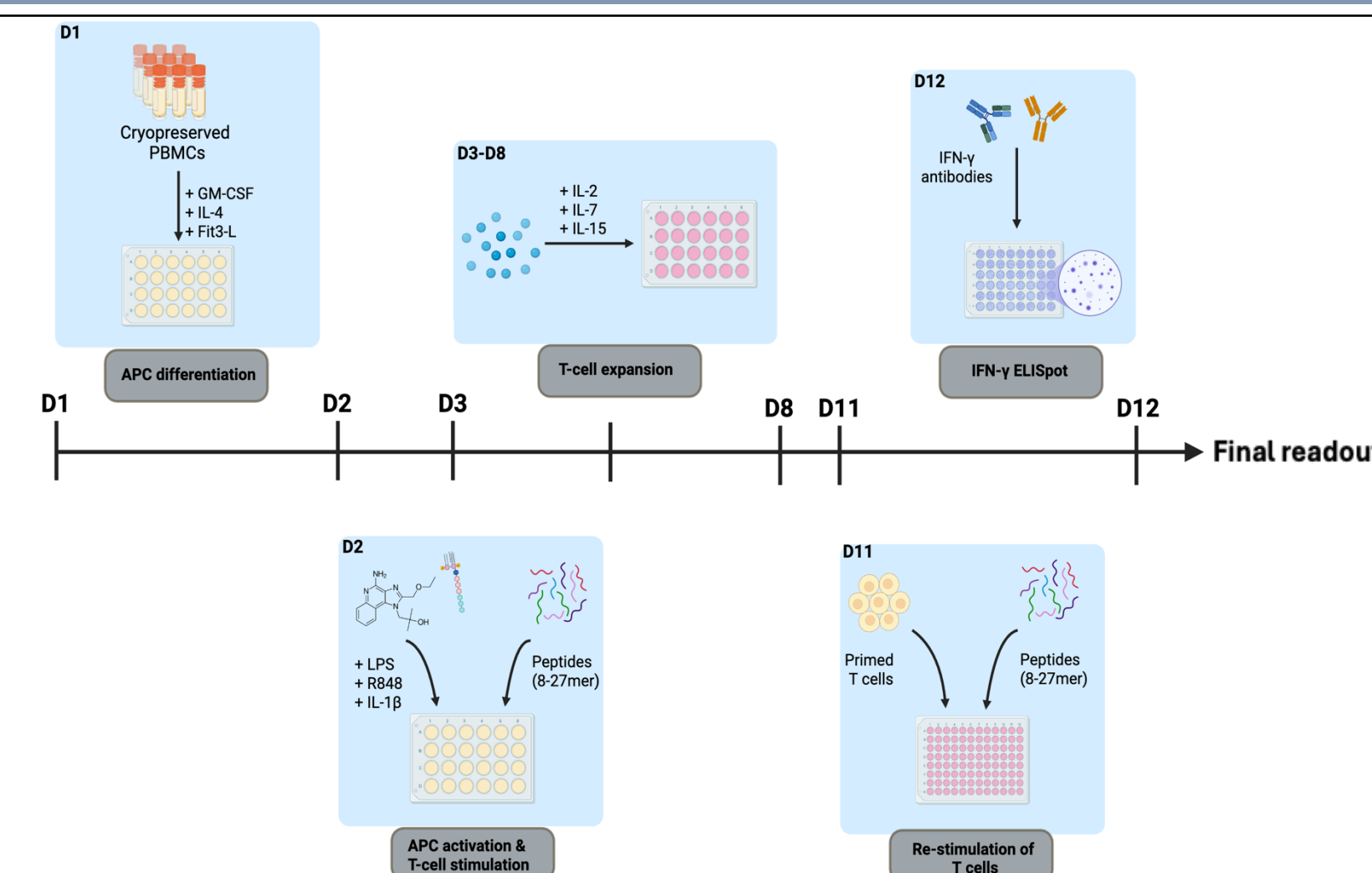


Figure 5. *In vitro* stimulation of human peripheral blood mononuclear cells (PBMCs) to prime antigen-specific T cells (adapted from Bozkus et al. 2021). PBMCs were cultured with cytokines for antigen-presenting cell (APC) maturation (24h), then incubated with peptides and adjuvants for T-cell priming (24h), followed by cytokine-mediated T-cell expansion (10 days). Immunogenicity was assessed by IFN-γ ELISpot.

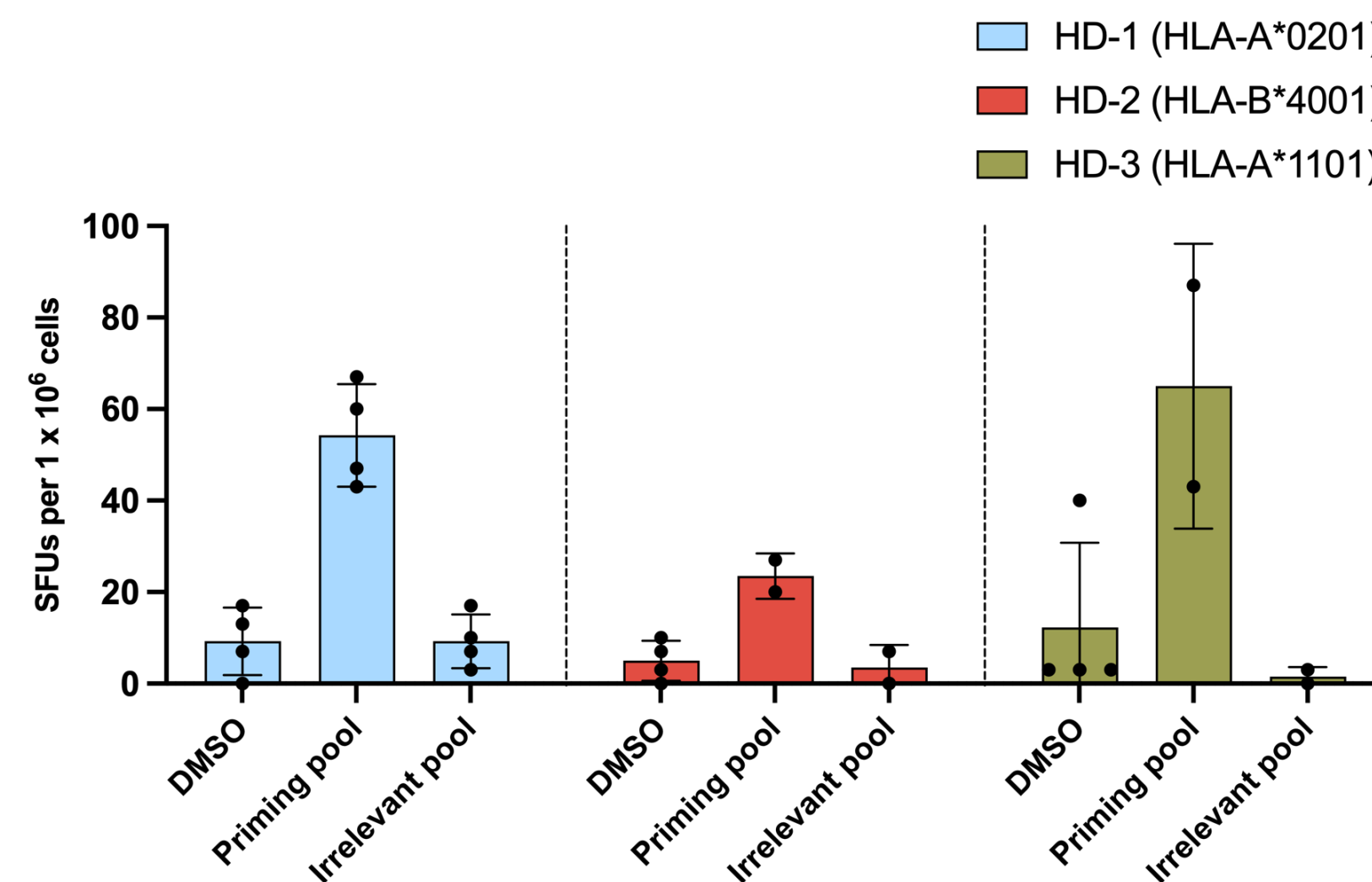
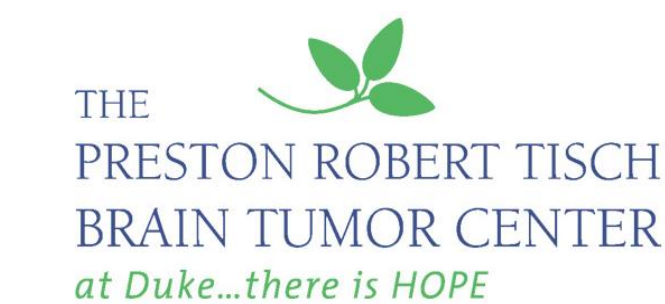


Figure 6. IFN-γ ELISpot on *in vitro* primed peripheral blood mononuclear cells (PBMCs). For each healthy donor (HD), the primed PBMCs were re-stimulated with the priming pool of HLA-matching minimal peptides as well as DMSO and an irrelevant pool of minimal peptides as negative controls. Data are reported as means with standard deviation (SD) and individual values (2-4 replicates per condition).

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