Bria-OTS+ Immunotherapy Platform: Harnessing Gene-Modified Tumor Cells to Reinvigorate the Cancer Immunity Cycle for Precision Anti-Tumor Responses

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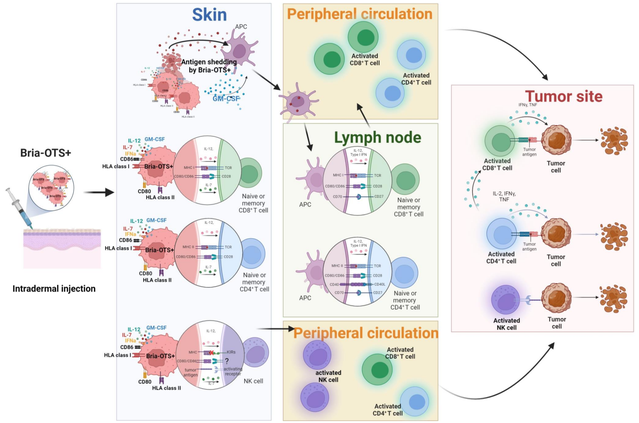
BACKGROUND

For an immune response to lead to an effective anti-tumor response, a series of stepwise events involving a complex network of iterative cellular and molecular interactions must take place. Cancer, however, often derails this immune cycle, prompting contemporary cancer immunotherapy approaches to seek its reinstatement. Despite significant advances, the inherent disruption of the cancer immunity cycle at multiple junctures, coupled with current therapeutic strategies focusing on isolated stages within this intricate process, has left the attainment of curative therapies as an aspirational goal. We believe that targeting multiple immune processes can restore natural anti-cancer immunity and yield sustained responses. To this end, BriaCell is utilizing gene-modified tumor cells as an immunotherapeutic platform. Our initial version, SV-BR-1-GM, a breast cancer cell line expressing GM-CSF, demonstrates promising clinical outcomes by directly activating CD4+ T-cells in an antigen-specific, HLA-restricted manner. Building on our findings, we hypothesize that tumor cells have the potential to not only supply antigens, but also the capability to directly stimulate the immune system.

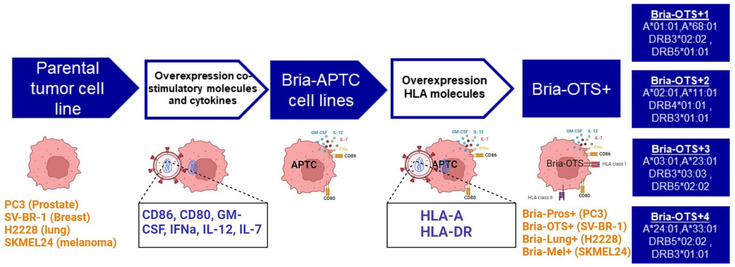
OBJECTIVES

We present our second-generation platform - Bria-OTS+ - in which we enhance antigen cross-presentation and direct immune activation through the expression of immuno-modulatory cytokines (**IL-12, IL-7, GM-CSF, IFNα**), co-stimulatory factors (**CD86, CD80, 4-1BBL**) and an extended repertoire of **HLA alleles**.



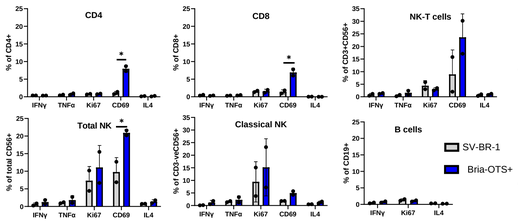
Bria-OTS+ Mechanism of Action: *Bria-OTS, when injected intradermally, directly activates both naive and previously exposed (memory) T-cells, as well as natural killer (NK) cells. Concurrently, professional antigen-presenting cells (APCs) process the Bria-OTS+ antigens. These APCs then migrate to regional lymph nodes, where they prime T-cells against tumor antigens. The activated T-cells and NK cells subsequently travel to the tumor site, where they trigger a robust anti-tumor immune response.*

METHODS

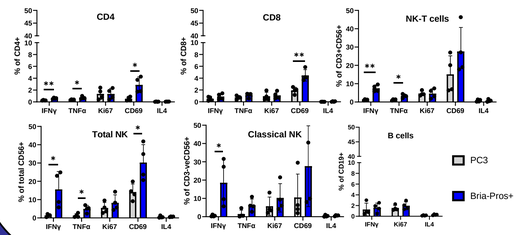


Engineering of Bria-OTS+ Cell Lines: *The Bria-OTS+ cell lines were developed from various types of cancer, including breast cancer (SV-BR-1), prostate cancer (PC3), melanoma (SK-MEL-24), and lung cancer (NCI-H2228), selected for their expression of a unique gene immune signature initially characterized in SV-BR-1 cells. These cell lines were further enhanced to boost their antigen presentation ability and stimulate the immune response by genetically engineering them to express co-stimulatory molecules and immunomodulatory cytokines, resulting in the formation of antigen presenting tumor cells (APTC). Furthermore, to create a semi-allogeneic cell therapy with extensive applicability, the HLA allele expression repertoire of SV-BR-1 was broadened. Population analysis indicated that a configuration of four cell lines, each bearing a combination of two HLA-A and two HLA-DRB3/4/5 alleles, could potentially provide at least one HLA match for 99% of the population. This includes a 92% probability of matching at Class I HLA-A alleles and a 98% likelihood at Class II HLA-DRB3/4/5 alleles.*

RESULTS

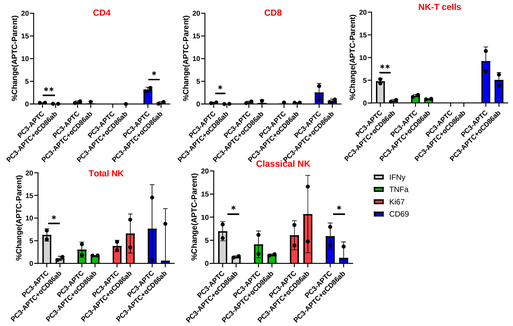


Bria-OTS+ activates T and NK cells in a modified mixed lymphocyte assay. *SV-BR-1, and SV-BR-1-APTC (7000 cells) were plated individually and allowed to attach overnight. The next day, 2 different donor PBMCs (E:T =10:1) were added to the cancer cells. After 48 hours of co-culture, immune cells were analyzed by flow cytometry with a variety of activation markers with the % positive shown. CD4+ & CD8+ T cells, NK-T and Classical NK cells were activated. * = p value< 0.05.*

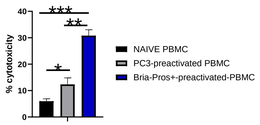


Bria-PROS+ activates T- and NK cells in a modified mixed lymphocyte reaction. *PC3, and Bria-PROS+ (7000 cells) were plated individually and allowed to attach overnight. The next day, 2 different donor PBMCs (E:T=10:1) were added to the cancer cells. After 48 hours of co-culture, immune cells were analyzed by flow cytometry for expression of a variety of activation markers with the %positive shown. CD4+ & CD8+ T cells, NK-T and Classical NK cells were activated. * = p value< 0.05; ** = p value< 0.005.*

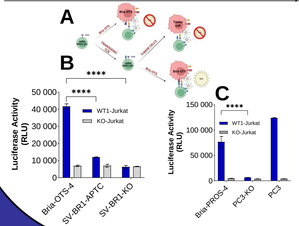
RESULTS



Bria-Pros+ cells activate NK and NKT cells and CD4+ and CD8+ T-cells, partly through CD86. *We explored the role of CD86 by plating 7000 PC3 or PC3-APTC cells overnight. The next day, PBMCs from two donors (E:T = 10:1) were added to the cancer cells, with or without anti-CD86 antibody. Post 48-hour co-culture, flow cytometry analyzed cell activation. Results show the percentage change in activation: (% PBMCs + PC3-APTC positive) - (% PBMCs + PC3 positive)(% Positive for PBMCs + PC3). * = p value< 0.05; ** = p value< 0.005.*



Bria-Pros+ enhances the ability of PBMCs to induce tumor cell cytotoxicity. PBMCs were either left untreated (naïve) or co-cultured with PC3 parental cells or BRIA-PROS+ cells (engineered to express cytokines, co-stimulatory molecules, and HLA alleles) for 24 hours at an effector-to-target (E:T) ratio of 10:1. The PBMCs were then collected and co-cultured with CFSE far-red stained PC3 cells at an E:T ratio of 10:1. After 72 hours, the effect of the PBMCs on tumor cytotoxicity was assessed using annexin V-FITC/propidium iodide staining. *The % cytotoxicity compared with complete lysis with detergent is shown. * = p value< 0.05; ** = p value< 0.005, *** = p value< 0.0005*



Validation of HLA functionality on Bria-OTS+ using a T Cell Activation Bioassay. A) *The assay consists of a genetically engineered Jurkat T cell line with endogenous TCR alpha and beta chains knocked out using CRISPR-Cas9. These cells also express a luciferase reporter driven by a TCR pathway-dependent promoter. Jurkat cells were transduced with a TCR specific for WT1 peptide (HLA-A24 restricted) and incubated with the indicated cell lines and appropriated WT1 peptides. After 6 hours, the bioluminescent signal is quantified using the Bio-Glo-NL™ Luciferase Assay System with luciferase activity shown (B,C).*

CONCLUSIONS

Bria-OTS+ exhibits the following characteristics:

- **Expresses a variety of cancer-related antigens, including Tumor-Associated Antigens (TAAs) and Post-Translational Modifications (PTMs) (data presented previously).**
- **Engages multiple facets of the adaptive immune response.**
- **Activates the innate immune system components.**
- **Counteracts immune escape mechanisms, specifically activating Natural Killer (NK) cells in the case of HLA deletions.**
- **Designed for both personalized and ready-to-use applications, ensuring robust long-term stability.**
- **Simplified administration process.**
- **Anticipated to have a favorable side effect profile, indicating good tolerance.**