Development and Mechanism of Action of a Novel Cellular Immunotherapeutic Platform for the Treatment of Advanced Solid Tumors

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BACKGROUND

Therapeutic cancer vaccines are designed to program a patient's own immune system to recognize and eliminate tumor cells. We sought to harness gene-modified tumor cells as a vaccine platform and developed cancer vaccines composed of breast cancer cells expressing GM-CSF (SV-BR-1-GM). We have recently reported favorable clinical outcomes in patient populations that match SV-BR-1-GM at one or more HLA alleles. Mechanistically, SV-BR-1-GM cells can directly activate CD4+ T-cells in an antigen-specific HLA-restricted manner, as demonstrated by an in vitro antigen presentation assay (1). Building upon these observations, we hypothesized that tumor cells, in addition to providing tumor antigens, can also directly stimulate the immune system.

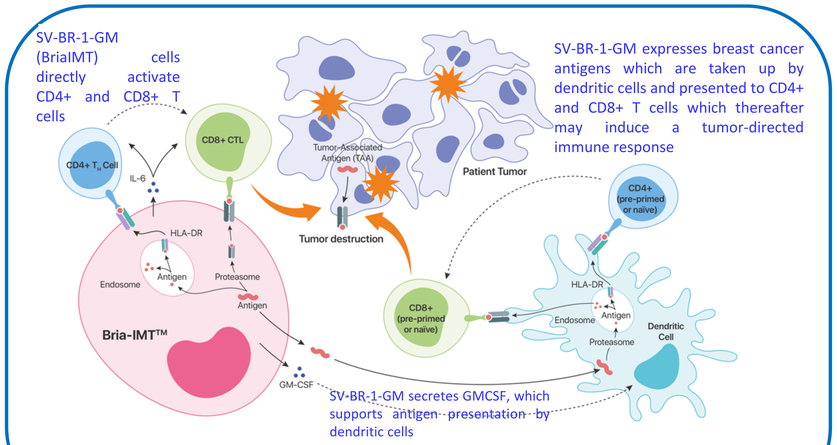

Fig. 1 Proposed mechanism of action (MoA). *SV-BR-1-GM acts as an antigen-presenting cell for primed T cells (Lacher et al., Front Immunol. 2018 May 15;9:776).*

METHODS

- We engineered Bria-OTS cell lines from various cancer types, including breast cancer (SV-BR-1), prostate cancer (PC3), melanoma (SK-MEL-24), and lung cancer (NCI-H2228). These selections were based on their expression of a distinct 22-gene immune signature, initially characterized in SV-BR-1 cells.
- To enhance these tumor cells' antigen presentation capabilities and immune system stimulation, we genetically modified them to express co-stimulatory molecules and immunomodulatory cytokines. These modified cells were named antigen presenting tumor cells (APTC) (see Fig. 3).
- To create a semi-allogeneic cell therapy with broad applicability, we expanded the repertoire of HLA alleles expressed by SV-BR-1. Population analysis suggests that four cell lines, each carrying two HLA-A and two HLA-DRB3/4/5 alleles, should offer at least a single match in 99% of the population. This includes a 92% match at Class I HLA-A alleles and a 98% match at Class II HLA-DRB3/4/5 alleles (see Fig.3).
- Functional Validation:
  1. Modified mixed lymphocyte reaction assay (mMLRA)

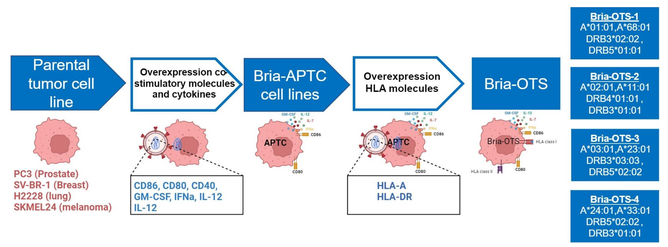

Fig. 3. Experimental strategy to develop Bria- OTS cell lines. *First, GM-CSF and other cytokines and co stimulatory molecules are overexpressed by sequential lentiviral transduction of 4 unique constructs to generate Bria-APTC cells. In addition, two HLA-A and two HLA-DRB alleles are overexpressed by lentiviral transduction, to generate a collection of 4 cell lines (Bria OTS 1 2 3 4)*

RESULTS

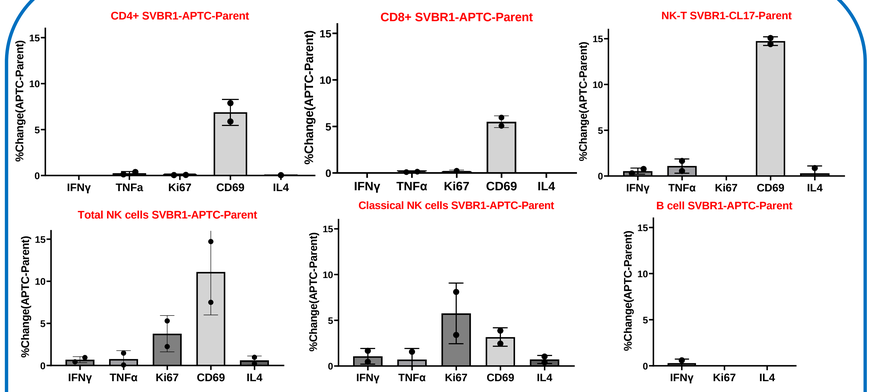

Fig.6. SV-BR-1-APTC can activate a range of immune cells including T- cells and NK-cells*. SV-BR-1, and SV-BR-1-APTC (7000 cells) were plated individually and allowed to attach overnight. Next day, 2 different donor PBMCs (E:T =10:1) were added to the cancer cells. After 48 hours of co-culture, cells were analyzed by flow cytometry. Results are presented as %change = %positive for the PBMCs+SV-BR-1-APTCs (Minus) %positive for the PBMCs+SV-BR-1*

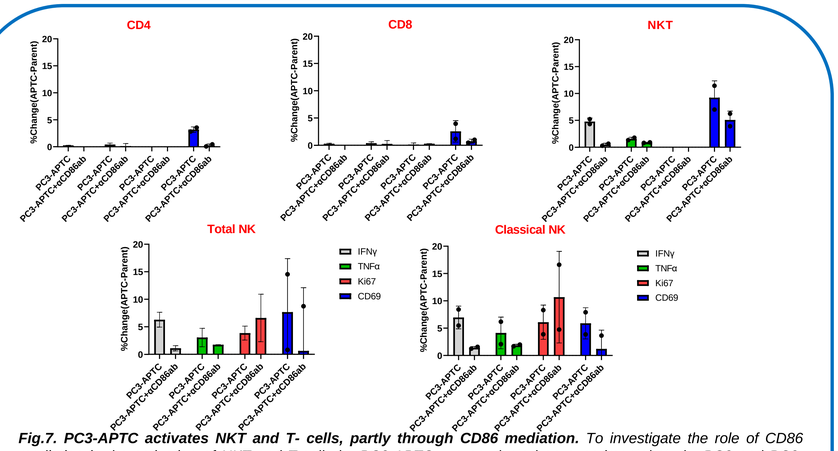

Fig.7. PC3-APTC activates NKT and T- cells, partly through CD86 mediation. *To investigate the role of CD86 mediation in the activation of NKT and T-cells by PC3-APTC, we conducted an experimental study. PC3 and PC3-APTC (7000 cells) were individually plated and allowed to attach overnight. The following day, two different donor PBMCs (E:T = 10:1) were added to the cancer cells with or w/o anti-CD86 blocking antibody. After 48 hours of co-culture, the cells were subjected to analysis via flow cytometry. The results are presented as follows: % Change = (% Positive for PBMCs + PC3-APTC) Minus (% Positive for PBMCs + PC3)*

OBJECTIVES

In response to the demand for innovative immunotherapeutic strategies for advanced solid tumors, BriaCell Therapeutics is pioneering the development of a groundbreaking whole tumor cell therapeutic vaccine. This vaccine is designed to operate through two synergistic mechanisms: 1) facilitating the cross-presentation of cancer cell antigens by host immune cells, and 2) directly activating the immune system. To achieve this objective, we will create a series of breast, prostate, melanoma, and lung cell lines with heightened immunogenicity through the following genetic modifications:

1. Expression of costimulatory molecules (CD80, CD86, 4-1BB-L,CD40) and immunomodulatory cytokines (GM-CSF, IFNa, IL-12 and IL-7). This modification aims to improve the cell's ability to present antigens and activate the host immune system.
2. Expression of an extended repertoire of HLA alleles to generate semi-allogeneic cell lines that will match the whole population at least at one HLA allele. This approach is intended to stimulate the host immune response to recognize tumor-associated antigens within the context of syngeneic MHC-I or II molecules with other allo-HLA molecules serving as a molecular adjuvant by activating the immune system (Fig. 2).

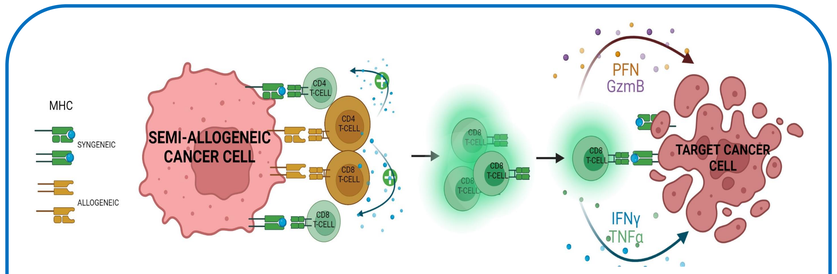

Fig.2. *Direct CD4+ T-cell allorecognition for provision of T-cell help for the generation of self-MHC restricted T-cell responses to tumor peptides*.

RESULTS

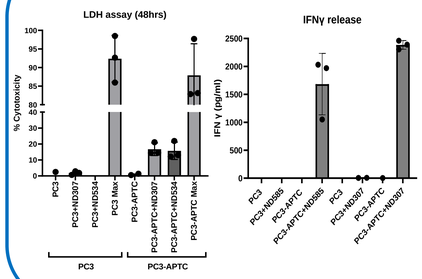

Fig.4. PC3 cells, engineered to express co-stimulatory molecules and immuno-modulatory cytokines, exhibit an enhanced ability to induce PBMCs cytotoxicity. *PC3, and PC3-APTC (5000/well) were plated individually in a 96-well plate and allowed to attach overnight. The next day, PBMCs from two different healthy donors (ND307 or ND585 at E:T =10:1) were added to the tumor cells. After incubation (48 hours for LDH and 72 hours for IFNγ release), the plates were centrifuged, and the supernatant was collected and analyzed for LDH release (Promega) or IFNγ production (R&D system). LDH max (maximum) was calculated by treating tumor cells with cell lysis buffer. Results are presented as average ± SD.*

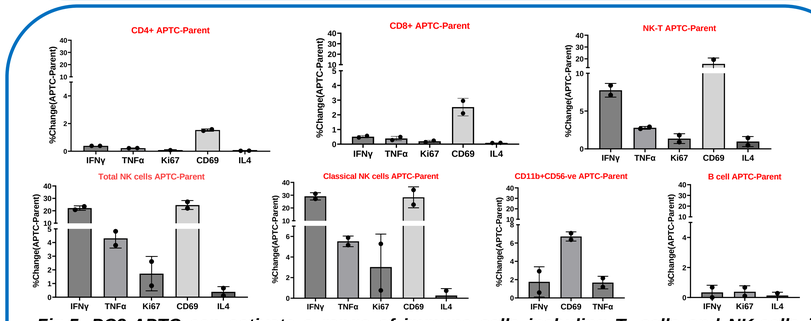

Fig.5. PC3-APTC can activate a range of immune cells including T- cells and NK-cells in a mMLRA. *PC3, and PC3-APTC (7000 cells) were plated individually and allowed to attach overnight. Next day, 2 different donor PBMCs (E:T=10:1) were added to the cancer cells. After 48 hours of co-culture, cells were analyzed by flow cytometry. Results are presented as %change = %positive for the PBMCs+PC3-APTCs (Minus) %positive for the PBMCs+PC3.*

DISCUSSION AND CONCLUSIONS

We are in the process of creating 'off-the-shelf' personalized cell-based therapeutic cancer vaccines designed to elicit potent immune responses against various solid tumors. The Bria-OTS cell line collection will offer the following key features:

1. Multimodal Mechanisms of Action: Bria-OTS cell lines have been engineered to trigger robust immune responses and deliver clinical efficacy through diverse mechanisms. These mechanisms include adaptive responses involving T-cells and innate responses involving dendritic cells and NK cells.
2. Precision Therapy: Bria-OTS cell lines will be precisely matched to individual patients based on their HLA antigens, encompassing over 99% of the U.S. population. This personalized approach ensures optimal compatibility and effectiveness.
3. Enhanced Safety: In comparison to current chemotherapeutic and hormone-based treatments, which are often associated with severe and potentially life-threatening adverse events, Bria-OTS offers improved safety profiles.
4. Rapid, Cost-Effective Treatment: Our 'off-the-shelf' cell lines eliminate the need for personalized manufacturing, allowing for immediate administration following patient HLA genotyping. This streamlined process offers efficient and cost-effective treatment options.

References

1. Lacher MD et al, Front Immunol. 2018 May 15:9:776