An off-the-shelf personalized cellular approach to immunotherapy for the treatment of advanced solid tumors

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SITC annual meeting– Boston
November 8–12, 2022

BACKGROUND

BriaCell is developing off-the-shelf personalized cellular immunotherapies based on our most advanced lead candidate—SV-BR-1-GM, which is in Phase I/IIa clinical trial in patients with metastatic or locally recurrent breast cancer. SV-BR-1-GM is a breast cancer cell line with features of an antigen presenting cell (APC) (Fig.1) which has been stably transfected with the CSF2 gene encoding GM-CSF (SV-BR1-GM). We have recently reported favorable clinical outcomes in patient populations that match SV-BR-1-GM at one or more HLA alleles. This clinical observation (Fig. 2,3), together with the fact that 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), lead us to hypothesize that SV-BR-1-GM can function as an APC .

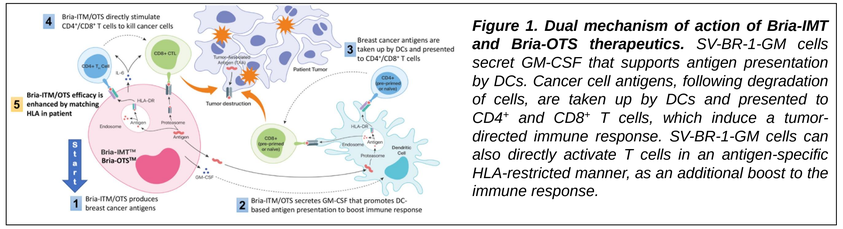


Figure 1. Dual mechanism of action of Bria-IMT and Bria-OTS therapeutics. *SV-BR-1-GM cells secret GM-CSF that supports antigen presentation by DCs. Cancer cell antigens, following degradation of cells, are taken up by DCs and presented to CD4⁺ and CD8⁺ T cells, which induce a tumor-directed immune response. SV-BR-1-GM cells can also directly activate T cells in an antigen-specific HLA-restricted manner, as an additional boost to the immune response.*

Clinical Experience with SV-BR-1-GM

Patients	HLA Match	Disease Control*	Disease Control in Immune Responders**
Monotherapy Studies			
N=6	≥ 2	50%	75%
N=20	≥ 1	25%	33%
N=7	0	29%	29%
PD-1 Inhibitor Combination Study			
N=7	≥2	43%	40%
N=9	≥1	56%	57%
N=5	0	40%	40%

Table 1 Clinical Experience with SV-BR-1-GM. *Patients with refractory advanced breast cancer were treated either with the SV-BR-1-GM regimen alone (pre-dose low dose cyclophosphamide to reduce immune suppression; 20 million irradiated SV-BR-1-GM cells intradermally and local IFNα2 to boost the response) or in combination with a PD-1 inhibitor (either pembrolizumab or retifanlimab) every 2-4 weeks. *Disease control includes 1 partial response (PR) and 7 stable disease (SD) in the monotherapy studies and 2 PR and 5 SD in combination therapy. The combination therapy study is ongoing. **Measured by delayed-type hypersensitivity.*

Conclusion: *Clinical benefit with the SV-BR-1-GM regimen is achievable especially with HLA matching between patients and the cell line*

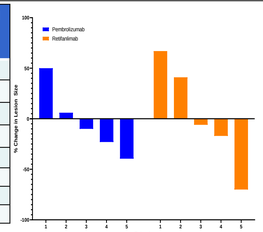


Figure 2 Tumor Size Changes with the SV-BR-1-GM Regimen and PD-1 Inhibitors*. Patients were treated in the combination study of the SV-BR-1-GM regimen with either pembrolizumab (blue) or retifanlimab (orange). The sum of diameters of measurable lesions are shown. Note that patient 3 with pembrolizumab transitioned to the retifanlimab combination (patient 5).*

Conclusion: *Patients with refractory metastatic breast cancer can respond to the SV-BR-1-GM regimen with PD-1 inhibition with marked tumor reductions.*

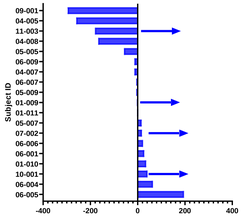


Figure 3 Progression Free Survival (PFS) Compared with Prior Therapy*. The time on study for patients treated in the combination study of the SV-BR-1-GM regimen with a PD-1 inhibitor was compared with the time on study for their last regimen prior to the study. The results shown are days on study minus days on their prior (last) regimen. Several patients remain ongoing (arrows).*

Conclusion: *Patients treated with the SV-BR-1-GM regimen in combination with PD-1 inhibition have PFS in many cases better than their last therapy.*

OBJECTIVES

To address the need for new immunotherapeutic approaches to treat advanced solid tumors, BriaCell Therapeutics is developing a novel whole tumor cell therapeutic vaccine that acts through two complementary mechanisms of action: 1) cross-presentation of cancer cell antigens and 2) direct T cell activation. To this goal we will generate a collection of breast, prostate, melanoma and lung cell lines with enhanced immunogenicity through the following genetic modifications;

1. Expression of costimulatory molecules (CD80, CD86, 4-1BB-L) and immunomodulatory cytokines (GM-CSF, IFNα, IL-12 and IL-7). The purpose of this modification is to enhance the cell's antigen presentation properties.
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. The purpose of this approach is to force the host immune response to recognize tumor-associated antigens in the context of allogeneic MHC-I or II molecules or in proximity of strong non-self antigens.

METHODS

Using CRISPR/Cas9 technology we have inactivated several endogenous *HLA-A* and *HLA-DRB* alleles present in five cancer cell lines (SV-BR-1, PC-3, LNCaP, SK-MEL-24, and NCI-H2228). Cells with inactivated HLA-A/DRB genes were transduced with lentiviral based vectors expressing selected cytokines and costimulatory molecules (GM-CSF, INFα, CD80, CD86, IL-12, IL-7, HLA-DRA, and 4-1BBL) to generate Bria-APTC (Antigen Presenting Tumor Cells) cells. Next, unique combinations of HLA-A and HLA-DRB3/4/5 alleles were transduced into the cells using lentiviral based vectors to generate a collection of cell lines that will match over 99% of the patient population for at least one HLA allele (Fig. 4,5). Expression and functionality of the stimulatory molecules and transgenic HLA alleles was established using flow cytometry and cell-based assays (Fig.6).

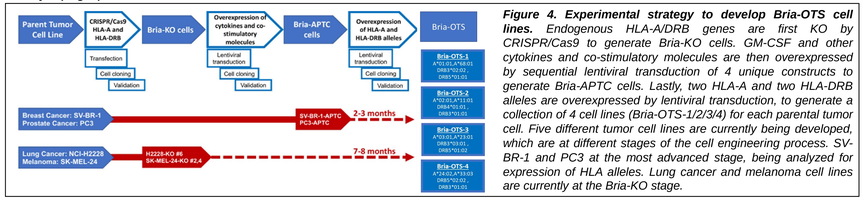
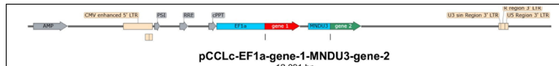


Figure 4. Experimental strategy to develop Bria-OTS cell lines. *Endogenous HLA-A/DRB genes are first KO by CRISPR/Cas9 to generate Bria-KO cells. GM-CSF and other cytokines and co-stimulatory molecules are then overexpressed by sequential lentiviral transduction of 4 unique constructs to generate Bria-APTC cells. Lastly, 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) for each parental tumor cell. Five different tumor cell lines are currently being developed, which are at different stages of the cell engineering process. SV-BR-1 and PC3 at the most advanced stage, being analyzed for expression of HLA alleles. Lung cancer and melanoma cell lines are currently at the Bria-KO stage.*



Cytokines and co-stimulatory molecules		
Lentivirus ID	*EF1a Promoter*	*MNDU3 Promoter*
GM-IFN	CSF2 (GM-CSF)	IFNA2 (IFNα)
CD86-IL12	CD86	IL12
CD80-HLA-DRA	CD80	HLA-DRA
IL7-4-1BBL	IL7	4-1BBL

HLAs		
Lentivirus ID	*EF1a Promoter*	*MNDU3 Promoter*
A2-A11	HLA-A*02:01	HLA-A*11:01
A1-A68	HLA-A*01:01	HLA-A*68:01
A3-A23	HLA-A*03:01	HLA-A*23:01
A24-A33	HLA-A*24:02	HLA-A*33:03
DR401-301	DRB4*01:01	DRB3*01:01
DR302-501	DRB3*02:02	DRB5*01:01
DR303-502	DRB3*03:01	DRB5*01:02
DR301-522	DRB5*02:02	DRB3*01:01

Fig.5. *Lentiviral vectors used to express two different genes from independent promoters. Cytokines and co-stimulatory molecules (Left Table) used to generate Bria-APTC cells. HLA-A and HLA-DRB alleles (Right Table) used to generate final Bria-OTS products.*

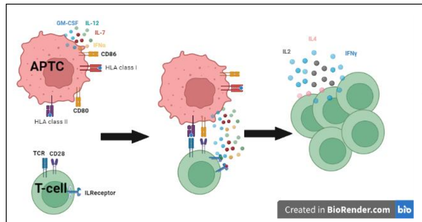


Fig. 6. Modified mixed lymphocyte reaction assay (MLR). *Bria-APTC cells or Bria-KO cells are incubated with PMBCs at different ratios. At days1, 2, 3, and 7, secretion of INFα was measured to assess T cell activation.*

RESULTS

Cell lines that secreted GM-CSF, IFNa, IL12, IL7 and expressed CD80, CD86, 4-1BBL, were derived from SV-BR1 (Fig. 7) and PC3 (data not shown). Using mixed lymphocyte reaction assays we demonstrated that the generated cells stimulate naïve T-cells (Fig. 8).

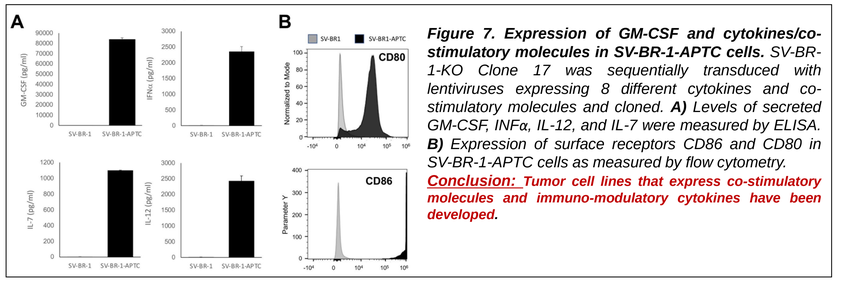


Figure 7. Expression of GM-CSF and cytokines/co-stimulatory molecules in SV-BR-1-APTC cells. *SV-BR-1-KO Clone 17 was sequentially transduced with lentiviruses expressing 8 different cytokines and co-stimulatory molecules and cloned.* ***A)*** *Levels of secreted GM-CSF, INFα, IL-12, and IL-7 were measured by ELISA.* ***B)*** *Expression of surface receptors CD86 and CD80 in SV-BR-1-APTC cells as measured by flow cytometry.*

Conclusion: *Tumor cell lines that express co-stimulatory molecules and immuno-modulatory cytokines have been developed.*

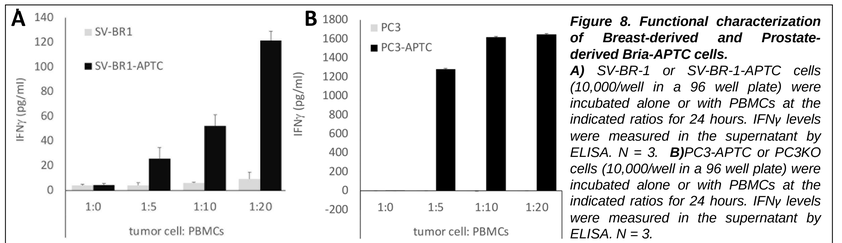


Figure 8. Functional characterization of Breast-derived and Prostate-derived Bria-APTC cells.
A) *SV-BR-1 or SV-BR-1-APTC cells (10,000/well in a 96 well plate) were incubated alone or with PBMCs at the indicated ratios for 24 hours. IFNγ levels were measured in the supernatant by ELISA. N = 3.* ***B)****PC3-APTC or PC3KO cells (10,000/well in a 96 well plate) were incubated alone or with PBMCs at the indicated ratios for 24 hours. IFNγ levels were measured in the supernatant by ELISA. N = 3.*

Conclusion: *Tumor cell lines that express co-stimulatory molecules and immuno-modulatory cytokines are able to activate naïve T-cells*

DISCUSSION AND CONCLUSIONS

We are in the process creating ''off the shelf" personalized cell-based therapeutic cancer vaccines that induce potent T-cell responses for a variety of solid tumors. The Bria-OTS cell line collection will deliver the following features:

- **Dual mechanisms of action to achieve strong immune response and clinical activity**. Bria-OTS cell lines have features of both cancer cells (expressing a myriad of TAAs) and dendritic cells (by presenting TAAs directly to T cells) to enhance the immune response.
- **Precision therapy.** Bria-OTS cell lines will be matched to patients based upon HLA antigens, covering over 99% of the US population .
- **Improved safety.** Relative to current chemotherapeutic and hormone-based therapies that are associated with severe adverse events that can be life threatening.
- **Rapid, cost-effective treatment**. Our "off-the-shelf" cell lines will not require personalized manufacturing and can be administered immediately after patient *HLA* genotyping.

References

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