Targeted immunotherapy with SV-BR-1-GM: Mechanism of action and companion diagnostic development

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AACR 2018
#5632

ABSTRACT

SV-BR-1-GM is a GM-CSF-engineered whole-cell targeted immunotherapy derived from a breast cancer cell line (SV-BR-1). We are currently assessing SV-BR-1-GM in a phase I/IIa clinical trial in metastatic and locally recurrent breast cancer (ClinicalTrials.gov identifier NCT03066947). Subjects who fail to respond with tumor regression are offered participation in a "roll-over" study in which an immune checkpoint inhibitor (pembrolizumab or ipilimumab) is added to the SV-BR-1-GM regimen (ClinicalTrials.gov identifier NCT03328026). To identify patients likely to respond to SV-BR-1-GM we are co-developing a companion diagnostic.

In a pilot phase I study with four evaluable patients, one subject (A002), with metastatic breast cancer, experienced systemic tumor regression including in soft tissue, lung, and brain lesions. In contrast to the three nonresponders, A002 matched with SV-BR-1-GM at both a class I (HLA-A) and a class II (HLA-DRB3) HLA allele. Strikingly, SV-BR-1-GM cells also express HLA class II "accessory" factors such as *HLA-DMA* and *-DMB*, *HLA-DRA*, and *CD74* (encoding invariant chain and CLIP), and immunostimulators such as *IL6*, *Il18*, and *KITLG*, and the damage-associated molecular pattern (DAMP) protein HMGB1. SV-BR-1-GM cells do not express the T cell co-stimulatory ligands CD80 or CD86. These findings are consistent with a potential to activate previously primed but not naïve CD4+ T cells. In agreement, peptide-pulsed SV-BR-1-GM cells selectively activated peptide-HLA specific, pre-primed T cells.

Given this functional data and the expression of the "immune signature", comprised of factors including those mentioned above, we hypothesized that SV-BR-1-GM cells can act as antigen-presenting cells (APCs) and present tumor-associated antigens (TAAs) directly to HLA-matched T cells, which in turn would induce an immune response directed to the patients' tumors. To evaluate this hypothesis, we have begun developing bioassays for assessing antibody and T cell responses to SV-BR-1-GM.
Here, we present data on SV-BR-1-GM's molecular makeup and development of anti-SV-BR-1-GM humoral and cellular immune responses. Our findings support SV-BR-1-GM acting as antigen-presenting cells for HLA class I and class II restricted immune responses.

ABBREVIATIONS

APC:	Antigen-Presenting Cell	**MoA:**	Mechanism of Action
DAMP:	Damage-Associated Molecular Pattern	**SN:**	Culture Supernatant
HLA:	Human Leukocyte Antigen	**SD:**	Standard Deviation
HMGB1:	High Mobility Group Box 1	**TAA:**	Tumor-Associated Antigen

Background

- SV-BR-1-GM is a whole-cell, GM-CSF expressing vaccine prepared from a breast cancer cell line with an unusual variety of cytogenetic abnormalities (Wiseman and Kharazi, 2006 and 2010).
- In a small, **initial Phase I** clinical trial, one "Special Responder" (subject A002) experienced prompt, widespread regression at multiple sites of metastatic breast cancer (Wiseman and Kharazi, 2006).
- In an **ongoing Phase I/IIa** clinical trial for advanced breast cancer (ClinicalTrials.gov NCT03066947) with thus far 6 subjects treated with the SV-BR-1-GM regimen, tumor regression was observed in 1 subject. See additional AACR 2018 poster (Abstract Control Number: 10970).
- We analyzed gene expression profiles of SV-BR-1-GM using Illumina BeadChip microarray and other technologies and concluded that histocompatibility (HLA) allele match(es) between SV-BR-1-GM and patients may increase the probability that the patient responds with tumor regression(s), assuming a mechanism of action in which patient T cells are activated via cancer antigens co-expressed in SV-BR-1-GM and patient tumors and displayed on SV-BR-1-GM MHCs (accepted for publication in *Frontiers in Immunology*).

METHODS AND PATIENTS (OF WHOM DATA SHOWN)

Patient Treatment

SV-BR-1-GM is grown in simple tissue culture media under GMP conditions (University of California, Davis, GMP facility). Prior to inoculation, the cells are serum starved for 24 hours and then irradiated (20,000 cGy) prior to inoculation. The cells are shipped at 4 ºC to the site and injected intradermally within 24 hours. The regimen includes

Pre-dose cyclophosphamide (300 mg/m2) 2-3 days prior to SV-BR-1-GM inoculation;

20 million irradiated SV-BR-1-GM cells inoculated intradermally split into 4 inoculations (x2 in the thighs and x2 in the upper back);

Interferon-α2b intradermally (10,000 IU per inoculation site) ~2 and ~4 days following SV-BR-1-GM inoculation.

Treatment is performed every 2 weeks for the first month and then every month with evaluation every 8-12 weeks.

Subject ID	Tumor Regression?	HLA-DRB3 match
A001	No	No
A002	**Yes (mets in multiple organs)**	**Yes**
01-002	**Yes (lung but not liver mets)**	**Yes**
01-003	No	No
02-003	No	No
02-004	Withdrew prior imaging	**Yes**

RESULTS

Tumor Response in Subject A002

Systemic tumor response was observed in subject A002 including in lungs and brain. Details published in Wiseman and Kharazi, *Breast J.* 2006 Sep-Oct;12(5):475-80.

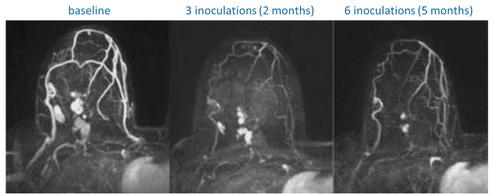

Figure 1. Tumor regression in breast after 3 and 6 cycles. See Wiseman and Kharazi, 2006.

HLA Allele Matching

Out of 4 evaluable subjects (A001, A002, A003, B001; Table 1) in the initial Phase I clinical trial, only A002 responded to the SV-BR-1-GM regimen with tumor regression. While some patients matched at an MHC I (HLA-A) allele with SV-BR-1-GM, only A002 had an MHC II (HLA-DRB3) allele match.

Subject ID (primary cancer)	Survival (mts)	Tumor regression	HLA-A		HLA-B		HLA-DRB3	
A001 (breast)	40.7	No	02:01	24:02	13:02	41:01	03:01	-
A002 (breast)	33.7	Yes	02:01	11:01	18:03	44:02	02:02	-
A003 (ovarian)	35.6	No	02:01	03:01	07:02	13:02	Neg.	-
B001 (breast)	7.0	No	11:01	-	35:01	40:01	Neg.	-
SV-BR-1-GM	N/A	N/A	11:01	24:02	35:08	55:01	01:01	02:02

Table 1. SV-BR-1-GM and patient HLA alleles.

Mechanism of Action

We recently identified an Immune Signature expressed in SV-BR-1-GM cells and demonstrated that SV-BR-1-GM cells can directly activate pre-primed CD4+ T cells (accepted for publication in *Frontiers in Immunology* (doi: 10.3389/fimmu.2018.00776). A model of SV-BR-1-GM's proposed mechanism of action (MoA) is illustrated in **Figure 2**.

SV-BR-1-GM cells (Bria-IMT™) directly activates CD4+ and CD8+ T cells

SV-BR-1-GM expresses breast cancer antigens which are taken up by dendritic cells and presented to CD4+ and CD8+ T cells which thereafter may induce a tumor-directed immune response.

SV-BR-1-GM also secretes GM-CSF which supports antigen presentation by dendritic cells

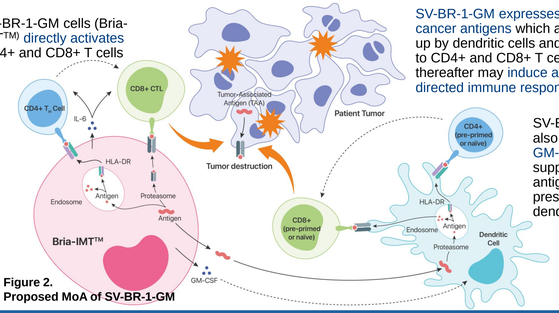

Figure 2. Proposed MoA of SV-BR-1-GM

Anti-SV-BR-1 Antibodies in Patient Sera

To assess whether the postulated role of SV-BR-1-GM as antigen-presenting cells for CD4+ T cells extends to B cell responses, we have developed an assay to measure anti-SV-BR-1 antibody titers in sera from subjects enrolled in the original Phase I and our current Phase I/IIa clinical trial (ClinicalTrials.gov NCT03066947) to unpack potential correlations with clinical response. As demonstrated in **Figure 3**, several patients experienced an increase in their anti-SV-BR-1 IgG antibody titers following inoculation with SV-BR-1-GM. For A001, however, a *decrease* was observed. It remains to be seen whether or not this finding reflects a technical artifact without a clinical consequence, or whether it, for instance, indicates a desensitization to the SV-BR-1-GM regimen which may, potentially, explain the lack tumor regression in this patient.

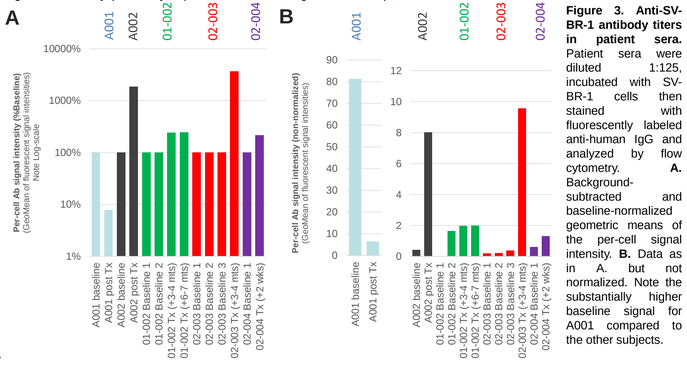

Figure 3. Anti-SV-BR-1 antibody titers in patient sera. Patient sera were diluted 1:125, incubated with SV-BR-1 cells then stained with fluorescently labeled anti-human IgG and analyzed by flow cytometry. **A.** Background-subtracted and baseline-normalized geometric means of the per-cell signal intensity. **B.** Data as in A. but not normalized. Note the substantially higher baseline signal for A001 compared to the other subjects.

Damage-Associated Molecular Pattern Factor Release

To expand our knowledge on SV-BR-1-GM's potential immunostimulatory repertoire, we measured the levels of the DAMP factor HMGB1 in the supernatants of formulated (irradiated) SV-BR-1-GM cells cultured for 1 and 3 days. **Figure 5** demonstrates that such cultured cells release high levels of HMGB1 (~50-60 ng per 1 million cells / 24 h). This raises the question of whether SV-BR-1-GM, through DAMPs such as HMGB1, also promotes an inflammatory response via Toll-like receptors.

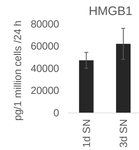

Figure 5. HMGB1 release by irradiated SV-BR-1-GM. See text for details. 1d SN, supernatant from 1-day culture; 3d SN, culture SN from 3-day culture.

Direct Activation of CD4+ T cells

MHC (HLA) class II gene expression *per se* does not ensure functionality. To assess whether functional MHC II complexes involving HLA-DRβ3 (encoded by *HLA-DRB3*) are formed on SV-BR-1-GM cells, irradiated SV-BR-1-GM cells, as well as irradiated HLA-DRB3+ and HLA-DRB3- PBMCs as controls (reference), were loaded with yellow fever virus (YFV) envelope peptides then co-cultured with a CD4+ T cell clone known to recognize YFV-DRβ3 (*01:01) MHCs. **Figure 6** demonstrates that SV-BR-1-GM cells indeed can directly activate peptide-specific CD4+ T cells

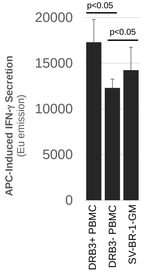

Figure 5. Direct activation of CD4+ T cells by SV-BR-1-GM cells. See text for details. Error bars indicate SDs. p-values refer to 1-tailed *t*-Tests.

Cytokine Responses

In the context of our efforts to develop a companion diagnostic (BriaDX™) for SV-BR-1-GM we have assessed several cytokines in serum samples of patients enrolled in our Phase I/IIa clinical trial for advanced breast cancer (ClinicalTrials.gov NCT03066947). As demonstrated in **Figure 4**, IL-8 levels rose only rose in the two subjects with an HLA-DRB3 allele match, namely in subjects 01-002 and 02-004.

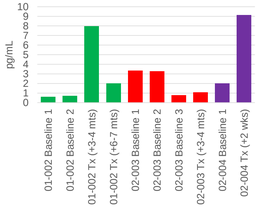

Figure 4. IL-8 in patient sera. Sera from subjects enrolled in our Phase I/IIa clinical trial were assessed for cytokine presence using the Human Key Cytokines 17-plex Immunoassay Panel (ab229791) (Abcam), as a service by Abcam. Among the cytokines assessed (TNF alpha , IFN gamma, IL-1 beta, IL-2 , IL-4, IL-5, IL-6, IL-8, IL-9, IL-10, IL-12p70, IL-13, IL-17A, GM-CSF, MCP1, MIP1a, MIP1b), IL-8 demonstated the most compelling profile as its levels rose with treatment in subjects matching at HLA-DRB3 with SV-BR-1-GM.

SUMMARY & DISCUSSION

- **Tumor regressions were observed, especially in breast cancer patients matching at MHC II (HLA-DRB3) with SV-BR-1-GM.**
- **Anti-SV-BR-1 antibody titer changes upon SV-BR-1-GM inoculation**
- **Interleukin (IL)-8 increase in HLA-DRB3 matched subjects**
- **Limitations: very small sample size.**

REFERENCES

- Wiseman and Kharazi, Breast J. 2006;12(5):475-80.
- Wiseman and Kharazi, The Open Breast Cancer Journal 02/2010; 2(1):4-11.

ACKNOWLEDGEMENTS

We are grateful to Gerhard Bauer, Brian Fury, Emily Lynn Fledderman, Tye Daniel Petrie, Dane P. Coleal-Bergum, and Tia M. Hackett from the UC Davis GMP facility (Sacramento, CA), and Drs. Don Healey and Lauren Collison at KBI Biopharma, Inc., for their SV-BR-1-GM manufacturing efforts. Special thank you to the clinical investigators and patients.