SV-BR-1-GM, a whole-cell targeted immunotherapy for advanced breast cancer: Pharmacodynamic markers of response

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ABSTRACT

BACKGROUND:

SV-BR-1-GM is a GM-CSF-engineered breast cancer cell line employed – after irradiation – as a targeted immunotherapy for advanced breast cancer. Tumor regressions at metastatic sites have been observed, most notably in patients with HLA allele matches to the cell line. We are assessing SV-BR-1-GM in the Phase IIa portion of a Phase I/IIa clinical trial in metastatic and locally recurrent breast cancer (ClinicalTrials.gov identifier NCT03066947). Additionally, we are co-developing a companion diagnostic (BriaDX™) to identify patients likely to respond to SV-BR-1-GM. Currently, BriaDX™ consists of HLA typing; however, we have begun assessing biomarkers in sera, lymphocyte characteristics, circulating tumor cells (CTCs), and cancer-associated macrophage-like cells (CAMLs) from patients collected at baseline and after inoculation of SV-BR-1-GM to layer in additional components to improve accuracy, with the number/subtyping of CTCs and CAMLs being prognostic indicators.

METHODS:

Subjects are pre-treated with low dose cyclophosphamide to reduce immune suppression. SV-BR-1-GM is inoculated intradermally with follow-up local injections of IFNα2. Cycles are every 2 weeks x 3 then monthly. HLA typing was conducted via LabType R-SSO Kits (One Lambda). Cytokines were measured via single- or multiplex assays. Anti-SV-BR-1 antibodies were determined by incubation of SV-BR-1 cells with diluted patient sera followed by staining with a fluorescently-labeled anti-IgG antibody and detection by flow cytometry. CTCs and CAMLs were evaluated by CellSieve™ at Creatv MicroTech.

RESULTS:

To date, 16 clinical trial subjects have been inoculated with the SV-BR-1-GM regimen as rescue immunotherapy. All were treatment refractory and had received a median of 4.5 prior chemo/biological therapy regimens (range 1-13). Two of the 16 patients remained on study for ≥3 months (5 cycles) with 4 patients currently on study not having reached the 3-month evaluation time point. Objective regression of tumor was seen in 2 subjects. One subject had virtually complete regression of 20 of 20 lung metastases noted at 3 and 6 months (but with progressive bone and liver metastases). Another subject had improvement of chest wall metastases and quality of life but expired due to nontreatment related causes. Response appeared to correlate with HLA allele-matching to SV-BR-1-GM. Anti-SV-BR-1 antibody titers increased in several patients. Among the cytokines assessed, interleukin (IL)-8 levels increased in HLA-DRB3 allele-matched subjects after SV-BR-1-GM inoculation. Of 15 patients evaluated, CTCs were present in 6 patients at baseline while CAMLs were present in all 15. Five of 5 patients evaluated for PD-L1 expression had mostly low-to-medium expression of PD-L1 on their CTCs/CAMLs. In the patient who had regression of lung metastases but progression of liver metastases, PD-L1 expression and maximum CAML size increased, but the number of CAMLs decreased during treatment. CAML number also decreased in a patient who reached the 3-month evaluation visit without progression and in a patient with inflammatory breast cancer who dropped out due to worsening inflammation.

CONCLUSIONS:

In addition to the patients' HLA types, several pharmacodynamic parameters correlated with tumor regression and/or HLA matching status. CTCs or CAMLs are frequently detectable in this population, and PD-L1 expression appears common on these cells. Both CAML number and size appear to correlate with response; though, larger studies are needed. Future steps include evaluation of SV-BR-1-GM with checkpoint inhibitors

Background

- SV-BR-1-GM is a whole-cell targeted immunotherapy prepared from a breast cancer cell line with an unusual variety of cytogenetic abnormalities and transfected with the *CSF2* gene to produce GM-CSF (Wiseman and Kharazi, 2006; Lacher et al., 2018).
- In a small initial clinical trial, one subject (A002) experienced prompt, widespread, and replicable regression at multiple sites of metastatic breast cancer (Wiseman and Kharazi, 2006).
- We analyzed gene expression profiles of SV-BR-1-GM using Illumina BeadChip microarray and other technologies and concluded that histocompatibility allele match(es) between SV-BR-1-GM and patients may be a requirement for therapy efficacy, supporting 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 (**Figure 1** and Lacher et al., 2018).
- SV-BR-1-GM has been evaluated in a clinical trial of patients with advanced breast cancer (NCT03066947 in ClinicalTrials.gov). This study, similar to the original study, uses low-dose cyclophosphamide pre-dose to reduce immune suppression (Day -2 or -3), inoculation of ~20 million irradiated SV-BR-1-GM cells intradermally and follow-up injections of IFN-α2b ~2 & 4 days later to boost the response. Cycles are every 2 weeks for the first month and then monthly. Pharmacodynamic data is presented here from both the small original study and the more recent study.

RESULTS

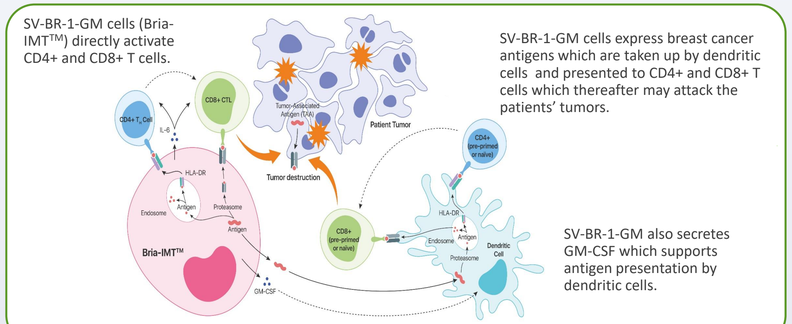

Figure 1. Proposed Mechanism of Action of SV-BR-1-GM

Subject IDs	Tumor Regression?	HLA-A	HLA-B	HLA-DRB3
A001	No	A*02:01 A*24:02	B*13:02 B*41:01	DRB3*03:01
A002	Yes (multiple sites)	A*02:01 *A*11:01(*)*	B*18:03 B*44:02	DRB3*02:02
01-002	Yes (lungs)	A*03:01 A*24:02	B*15:01 (B62) B*51:01	DRB3*02:02
02-003	No	A*01:01 A*02:05	B*49*:01 B*53:01	DRB3*03:01
02-004	Lost to Follow-Up (biol. resp.: # CAML ↓)	A*03:01 *A*11:01(*)*	B*07:02 B*55:01	DRB3*02:02
04-001	No	A*02:01 A*25:01	B*40:02 B*40:02	DRB3*02:02
04-005	No	A*02:01 A*68:01	B*40:01 B*44:02	No HLA-DRB3
04-006	No	A*02:01 A*33:01	B*14:01 B*35:01	DRB3*02:01
SV-BR-1-GM	N/A	*A*11:01(*)* A*24:02	B*35:08 B*55:01	DRB3*01:01 DRB3*02:02

Table 1. HLA-A/-B/-DRB3 Alleles of Select Clinical Trial Subjects. A002 was enrolled in a previous Phase I study (Wiseman and Kharazi, 2006), the other subjects in WRI-GEV-007 (ClinicalTrials.gov Identifier: NCT03066947). Allele matches to SV-BR-1-GM are highlighted in **green** while allele group matches are highlighted in gray.
*(*): HLA-A*11:01 RNA was not found to be expressed in SV-BR-1-GM cells. (**): see* ***Figure 5****.*

Anti-SV-BR-1 Antibody (IgG) titers in patient sera

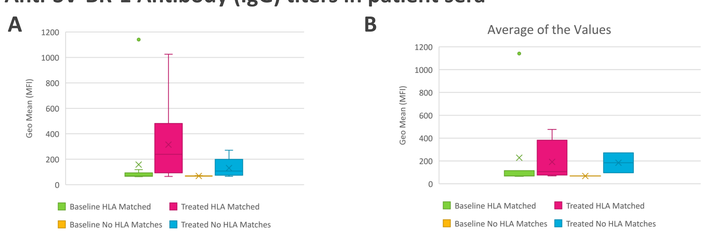

Figure 2. Anti-SV-BR-1 antibody titers in patient sera.
SV-BR-1 cells were incubated with 1:10 diluted patient sera then stained with fluorescently labeled anti-human IgG and analyzed by flow cytometry. **A.** Anti-SV-BR-1 antibodies in all patient's sera samples. **B.** Data as in A. but samples segregated either as HLA matched (≥ 1 allele) or non-HLA matched. Baseline: before treatment with first dose of SV-BR-1-GM.
Conclusion: The average anti-SV-BR-1 IgG level increase following SV-BR-1-GM inoculation is higher in HLA matched than in non-HLA matched subjects.

Cytokine responses to SV-BR-1-GM

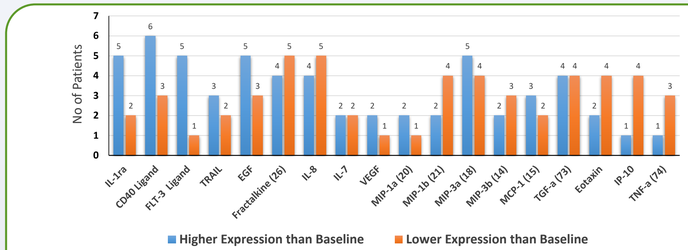

Figure 3. Cytokine Response in 9 SV-BR-1-GM Treated Patients. Luminex-based multiplex cytokine assay with a panel of 39 analytes in sera from 9 evaluable patients treated with SV-BR-1-GM. Each analyte was assessed for increased vs. decreased serum levels relative to pre-treatment baseline, irrespective of HLA type or clinical response. For each cytokine, samples from subjects not responding with cytokine level changes were not included.
Conclusions: Multiple cytokines respond to SV-BR-1-GM with increased or decreased serum levels. A possible correlation with HLA matching and treatment respond was seen for IL-1RA (see **Figure 4**).

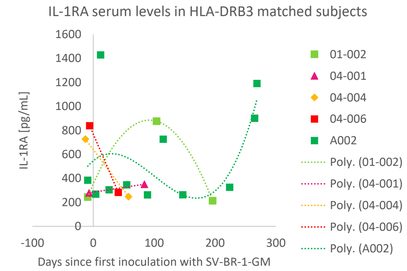

Figure 4. IL-1RA as a prognostic biomarker. Among HLA-DRB3-matched subjects, serum IL-1RA rose substantially in responding subjects A002 and 01-002 but not in non-responding subjects (04-001, 04-004, 04-006). A002 underwent 2 treatment series. 01-002's initial increase of IL-1RA may reflect the tumor regression seen in the lungs (liver lesions were stable at 3 months), and the drop at approximately Day 200 after the first dose of SV-BR-1 may reflect the progressive disease in the liver.
Conclusion: Early increase in serum IL-1RA may indicate tumor regression in patients *HLA-DRB3* allele-matching with SV-BR-1-GM.

Circulating Cancer-Associated Macrophage-Like Cells (CAMLs)

CAMLs are giant macrophage-like cells associated with patient tumors and found in the circulation of cancer patients from a variety of cancer types. The presence of tumor markers in CAMLs suggests that CAMLs phagocytose tumor material (Adams et al., 2014). Reduction in CAML frequency and max. CAML size following treatment may indicate a favorable prognosis. **Figure 5A** indicates that subjects with at least an *HLA-DRB3* allele match to SV-BR-1-GM responded with a reduction in their CAML numbers. CAML numbers were also reduced for 02-003, who had no HLA match to SV-BR-1-GM and did not respond with tumor regression. **Figure 5B** suggests that the max. CAML size has less of a predictive value than CAML numbers in our very small study.

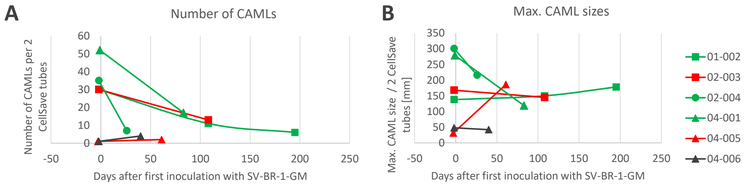

Figure 5. CAMLs. See text for details. Marker/line colors: **Green**: HLA allele-matched, **Black:** HLA allele group-matched. **Red:** no HLA match. **Conclusion:** Our data is consistent with the hypothesis that reduction of CAML number is restricted to HLA-DRB3-matched subjects or to HLA-mismatched subjects without tumor regression.

SUMMARY

Compared to HLA-mismatched subjects, HLA-matched subjects tend to respond to SV-BR-1-GM treatment with:

- **Increase in anti-SV-BR-1 IgG titers**
- **Increase in IL-1RA serum levels**
- **Reduction of CAML number**

REFERENCES

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