Response to a modified whole tumor cell targeted immunotherapy in patients with advanced breast cancer correlates with tumor grade

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ABSTRACT

Background: SV-BR-1-GM is a GM-CSF transfected breast cancer cell line, exceptional for having antigen presenting capability and expressing both HLA I and II. The parent cell line, SV-BR-1, was derived from a patient with grade II (moderately differentiated) breast cancer. We report molecular characterization of SV-BR-1-GM, noting it retains features of a grade II tumor, and report enhanced disease control in patients with grade I or II breast cancer.

Methods: SV-BR-1 and SV-BR-1-GM were characterized molecularly using RNAseq and proteomic analyses. We treated 23 evaluable patients with recurrent and/or metastatic breast cancer refractory to standard therapy. The SV-BR-1-GM regimen included cyclophosphamide 300 mg/m^2 2-3d prior to intradermal injection of SV-BR-1-GM (20-40x10^6 cells divided into 4 sites) and IFNα into the inoculation sites (10,000 IU/site) about 48 and 96 hours subsequently. Cycles were q2 weeks x3 then qmo x 3 (clinical trial NCT03066947). Eleven patients were treated with the above regimen in combination with a PD-1 inhibitor (pembrolizumab or INCMGA00012) (clinical trial NCT03328026). Disease response was evaluated radiographically q3 mo and as clinically indicated.

Results: To estimate the tumor grade represented by the SV-BR-1-GM cell line, we developed a score we refer to as Relative Molecular Grade (RMG). SV-BR-1-GM is most similar to the MDA-MB-468 cell line (RMG of 58.5; corrected after submission of Abstract), which was classified as Basal A phenotype. Basal A cancers are less aggressive than Basal B but more aggressive than Luminal, suggesting that SV-BR-1-GM may have retained features of a grade II breast cancer. We also noted that SV-BR-1-GM expresses both Class I (HLA-A, B & C) and Class II (HLA-DR and -DP) molecules, and that the HLA-DR expression is enhanced by treatment with IFNγ. SV-BR-1-GM expressed 31 genes which are overexpressed in breast cancer, 8 cancer-testis antigens and 3 genes expressed in breast tissue. In 30 patients treated with the SV-BR-1-GM regimen (19 with the SV-BR-1-GM regimen alone, 4 who began on the SV-BR-1-GM regimen and transitioned to combination with a PD-1i, and 7 with combination therapy alone) there were 7 with grade II breast cancer and 1 with grade I breast cancer (Table). These patients were heavily pre-treated with an average of 10 prior regimens. While only one patient with grade III cancer showed disease control, 75% of the patients with grade I or II tumors showed disease control. Patients remained on study for up to 259 days.

Conclusions: SV-BR-1-GM appears to retain characteristics of a moderately differentiated breast cancer, expresses multiple potential tumor antigens, and can elicit disease control especially in patients with grade I and II breast cancer.

BACKGROUND AND OBJECTIVES

- SV-BR-1-GM is a breast cancer cell line with features of antigen-presenting cells including expression of HLA class II molecules (Lacher et al., Front Immunol. 2018 May 15;9:776)
- SV-BR-1-GM was derived from a Grade II (moderately differentiated) breast cancer biopsy tumor. SV-BR-1-GM was used in 2 clinical studies:
- **"Monotherapy" Study (WRI-GEV-007):** The SV-BR-1-GM regimen includes: low dose cyclophosphamide to reduce immune suppression (300 mg/m^2 2-3 days prior to inoculation); 20-40 million irradiated SV-BR-1-GM cells intradermally split into 4 sites; and interferon-α2b (10,000 IU x 4) into the inoculation sites ~2 & ~4 days later with cycles every 2 weeks x3 then monthly. Prior to SV-BR-1-GM inoculation, a skin test for immediate hypersensitivity is conducted using irradiated SV-BR-1 parent cells or to SV-BR-1-GM (1±0.2 million cells into the forearm).
- **Combination Therapy Study (BRI-ROL-001):** pembrolizumab or INCMGA00012 (200 mg IV) in combination with the regimen from the Monotherapy study with cycles every 3 weeks
- Here we characterize the SV-BR-1-GM cell line molecularly and evaluate the clinical response in patients with Grade I or Grade II tumors.

RESULTS

Molecular Characterization of SV-BR-1-GM

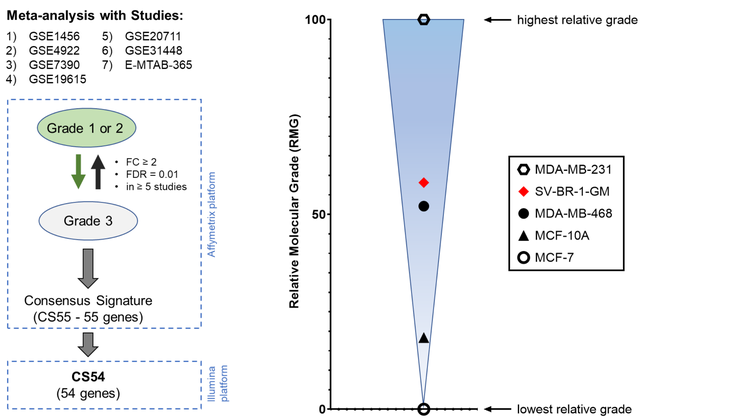

Figure 1. Molecular signature distinguishing grade 1 and 2 from grade 3 breast tumors. A) Meta-analysis to identify a gene signature distinguishing grade I and II from grade III breast tumors. Using GENEVESTIGATOR® (Nebion AG, Switzerland), seven breast cancer studies run on Affymetrix platforms with grading information were identified then individually queried for genes expressed either ≥2 times higher or ≥2 times lower (fold-change (FC) ≥ 2) in grade 3 tumors compared to grade 1 or 2 tumors. Genes present in the signatures of at least 5 out of the 7 studies were included in the "consensus signature" CS55. From this 55-gene consensus signature, 54 genes (CS54 signature) were represented in a normalized data set used previously. B) To estimate the tumor grade represented by SV-BR-1-GM cells, we developed a score referred to as Relative Molecular Grade (RMG) taking the expression levels of each of the CS54 signature genes into account, weighted according to the fold-changes in the meta-analysis. With a similar RMG, SV-BR-1-GM cells most closely resemble the triple-negative MDA-MB-468 cell line, representing the Basal A subtype. **Conclusion: SV-BR-1-GM has a molecular signature most closely related to other breast cancer cell lines derived from grade I & II breast cancers.**

	Patient Characteristics – Grade I/II Patients		
Study	MonoRx (n=8)	ComboRx (n=4)	All (n=11)*
Grade I/II Tumors	1/7	0/4	1/10
Age	63 ± 7	63 ± 10	62 ± 7
Median Prior Systemic Regimens	6 (range 1-12)	9 (range 8-10)	7 (range 1-12)
Median Prior Hormonal Regimens	1 (range 0-2)	3 (range 0-5)	2 (range 0-5)
% ER/PR +	75%	100%	82%
% Her2/neu +	13%	50%	27%
% Triple Negative	13%	0%	9%
% HLA Matched (1+ matches/2+ matches)	75%/38%	75%/50%	82%/45%
Disease Control (SD, PR or CR)	5/8 (63%)	3/4 (75%)	7/11 (64%)
DTH Response	7/8 (88%)	4/4 (100%)	10/11 (91%)
Disease Control in DTH Responders	5/7 (71%)	3/4 (75%)	7/10 (70%)

*Note that one patient participated in both the monotherapy and combination therapy studies

RESULTS CONTINUED

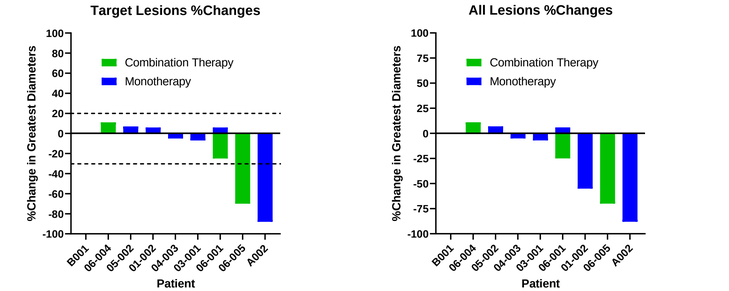

Figure 2. Lesion Size Changes. There were 9 patients with grade I/II tumors who had imaging done both before and after treatment. A) Target lesion % change of the sum of greatest diameters for the best response in each patient. B) All lesion % change of the sum of greatest diameters for the best response in each patient.
Conclusion: Of the 11 patients with grade I/II tumors, there were 9 with evaluable lesions including 6 with stable disease and 2 partial responses according to RECIST criteria. One patient with stable disease had marked reduction in numerous non-target lesions.

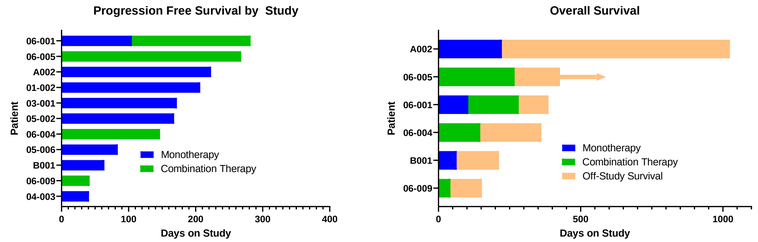

Figure 3. Survival Data. Progression-free survival (PFS) was collected on all patients, and overall survival (OS) on 6 patients.
Conclusion: Of the 11 patients with grade I/II tumors, median PFS for monotherapy was 170 days, for combination therapy was 208 days, and for all patients was 168 days. The median OS for the combined monotherapy and combination therapy was 375 days

CONCLUSIONS AND HYPOTHESES

- **SV-BR-1-GM has a molecular signature most closely related to grade I/II tumors.**
- **The patients with grade I/II tumors in our studies were very heavily pre-treated with a median of 7 prior systemic therapy regimens (including chemotherapy, biological and "targeted" therapy).**
- **The SV-BR-1-GM regimen +/- PD1 inhibitor can induce an effective immune response and tumor regression in heavily pre-treated advanced breast cancer, especially patients with grade I/II tumors.**
- **PFS and OS in this heavily pre-treated group compares well with 3rd line or later metastatic breast cancer[1].**
- **PD1 inhibition to the SV-BR-1-GM regimen appears to provide additional clinical benefit in this group.**

References:
[1]Kazmi S et al, 2020 Breast Cancer Res Treat. 2020 Aug 17. doi: 10.1007/s10549-020-05867-0; Manso L et al, Breast J. 2019 Mar;25(2):219-225; Jacot W et al, Int J Cancer. 2019 Dec 15;145(12):3359-3369; Leo S et al, Oncologist. 2019 Jun;24(6):e232-e240; Varella L et al, Breast Cancer Res Treat. 2019 Jul;176(2):429-434; Maeda S et al, Breast. 2017 Apr;32:66-72. – see for PFS and OS in 3rd line or later metastatic breast cancer
Wiseman CL and Kharazi A. Breast J. 2006 Sep-Oct;12(5):475-80 – see for Patient A002 prior report
Lacher MD et al, Front Immunol. 2018 May 15;9:776 – see for prior characterization of SV-BR-1-GM