BriaCell

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Development of a personalized off-the-shelf whole-cell immunotherapy for breast cancer

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ABSTRACT

BACKGROUND: Whole-cell cancer immunotherapies induce cancer-specific immune responses with the goal of long-term immune surveillance and remission. Non-replicating (irradiated) cancer cells are used to stimulate the immune system to recognize tumor-associated antigens and target tumor cells. Whole-cell immunotherapies have achieved regression of bulky, macroscopic tumors, but clinical trials have shown limited efficacy. SV-BR-1-GM is an HLA class I and II expressing, GM-CSF secreting breast cancer cell line. In a pilot clinical trial, an almost complete response of widely metastatic breast cancer was seen in a patient who allele-matched SV-BR-1-GM at *HLA-DRB3*. A follow-up Phase I/IIa clinical trial is ongoing in subjects with advanced breast cancer.

RESULTS: Extensive in vitro analysis demonstrated that SV-BR-1-GM cells not only have features of breast cancer cells but surprisingly also features of dendritic cells, the latter especially because of the expression of both HLA class I and class II complexes. SV-BR-1-GM cells "loaded" with a peptide known to bind to histocompatibility complexes containing HLA-DRβ3, as allele-encoded by SV-BR-1-GM, induced the activation of a CD4+ T cell clone specific for the peptide-DRβ3 complex, suggesting functionality of SV-BR-1-GM's HLA II machinery. To date, 23 [corrected from submitted abstract stating 24] subjects have been inoculated with the SV-BR-1-GM regimen in a Phase I/IIa trial with no adverse immediate hypersensitivity responses to low-dose inoculations with test cells (SV-BR-1 or SV-BR-1-GM). DTH response was evaluable in 18 patients with 72% developing DTH. The patient with the most pronounced DTH response, 01-002, also had a clinical response with regression of 20 of 20 lung metastases. Two other patients also had evidence of tumor regression. 6 patients were assessed for anti-SV-BR-1 antibodies. Whereas antibodies were found in sera of all patients, higher titers were measured in post-treatment compared to baseline samples. Patients who responded to the SV-BR-1-GM regimen with tumor regression matched SV-BR-1-GM at least at one HLA allele.

CONCLUSIONS AND OUTLOOK: SV-BR-1-GM cells may act as antigen-presenting cells directly activating HLA matching patient T cells. To include more patients predicted to derive clinical benefit from this whole-cell approach, SV-BR-1 cells are being engineered to, among others, overexpress exogenous HLA alleles. The goal is to develop a set of cell lines suitable for personalized off-the-shelf immunotherapy. The strategy will result in cell lines that match ~90% of the US population at 2 or more HLA alleles.

BACKGROUND

- SV-BR-1-GM is a whole-cell, GM-CSF expressing targeted immunotherapy prepared from a breast cancer cell line with features of antigen-presenting cells including HLA class II expression.

- **Table 1: SV-BR-1-GM expresses breast tissue and breast cancer antigens (by RNA-seq)**

ABCA12	AP000322.53	AZIN1	CCL28	CSN3	ELF5	FOXI1	HIST1H4H	KIT	KRTAP21-1	MIA	NQO1	**PGAP3**	SCGB1D2	SLCO1B7	SYNE4	XDH
ABCC11	APCDD1L	BTN1A1	CENPN	CST9	ELOVL3	GJC3	IGFBP5	KRT15	LALBA	**MIEN1**	OBP2B	PIGK	SCGB2A2	SPAG1	TBX15	XPOT
ACSM1	APOD	C10orf90	CEP55	CYP4Z1	EN1	GLRA3	IL17B	KRT17	LGALS7	MMP27	OIP5	PIP	SCGB3A1	SPINK14	TFAP2A	ZNF80
AKR1B15	ARHGAP40	C1orf64	CHIT1	DCAF10	**ERBB2**	GLYATL1P3	IL22RA2	KRT19	LGALS7B	MRGPRX2	OXGR1	PLAC1	SDR16C5	SPINK8	TNPO1	
AKR1C2	ARHGEF38	C2orf82	CLDN8	DCD	ESR1 ??	GLYATL2	INTS7	KRT25	LMX1B	MS4A18	OXTR	PNLIPRP3	SERHL2	ST8SIA6	TRPS1	
ALDH3B2	ARPC5L	C5orf46	CLEC3A	DGAT2L6	FABP7	GPR88	IRX1	KRT27	MAB21L1	MTHFD2	PAK1	**PRAME**	SFRP1	STAC2	TTC6	
ALG8	ATP13A5	C6orf223	CMA1	DHRS2	FABP9	GSTM5	IRX2	KRT28	MAP1LC3C	MUCL1	PAX3	PRSS51	SHB	**STARD3**	UBR5	
ALOX15B	ATP6V1B1	CABYR	COL8A1	DUSP4	FAM180B	GSTT2B	IRX3	KRT71	MATN4	MYB	PBK	PTHLH	SHISA2	STC2	UGT2B11	
ALX4	AWAT2	CARD18	CSN1S1	EFHD1	FAM196B	HIST1H2AE	IRX5	KRT79	MGAT4A	MYEOV	PDCD6	RFC5	SLC28A3	SULT1C3	UGT2B28	
ANKRD30A	AZGP1	CBX2	CSN2	EIF3H	FAM25C	HIST1H2BG	KIF2C	KRT81	MGP	NPY2R	PDRG1	RSF1	SLC35A2	SYCP2	VTCN1	

ERBB2, MIEN1, PGAP3, STARD3: on "HER2 amplicon"

- In an **initial, pilot Phase I** clinical trial with 4 evaluable subjects, one "Special Responder" experienced prompt, widespread regression at multiple sites of metastatic breast cancer (Wiseman and Kharazi, 2006; The Breast Journal, Volume 12 Number 5, 2006 475– 480).

- In a **recently completed Phase I/IIa** clinical trial for advanced breast cancer (ClinicalTrials.gov NCT03066947) with 23 subjects dosed with SV-BR-1-GM, tumor regression was observed in three subjects, all matching with SV-BR-1-GM at least at one HLA allele.

- In a **ongoing Phase I/IIa** clinical trial for advanced breast cancer testing SV-BR-1-GM **in combination with pembrolizumab** (Keytruda) (ClinicalTrials.gov NCT03328026), 6 subjects have thus far been dosed. Tumor regression was observed in one subject and stable disease in another.

BACKGROUND cont'd

Mechanism of Action (MoA)

SV-BR-1-GM acts as antigen-presenting cells for primed T cells (Lacher *et al.*, Front Immunol. 2018 May 15;9:776 and **Figure 1**).

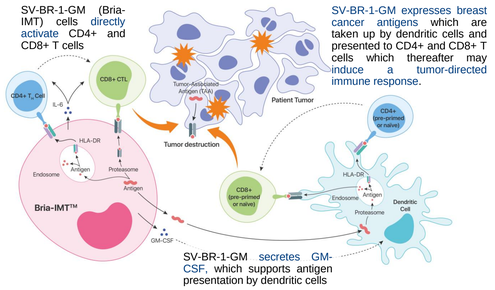

Figure 1. Model of Proposed MoA of SV-BR-1-GM

METHODS

Patient Treatment

- SV-BR-1-GM is grown in simple tissue culture media under GMP conditions. In earlier stages of development (for most patients on NCT03066947), SV-BR-1-GM was formulated for each patient freshly [in essence, cells were serum-starved for 24 hours, irradiated (20,000 cGy), resuspended in Lactated Ringer's solution] then shipped at 2-8 ºC to the clinical sites where it was injected intradermally within 24 hours from completion of the formulation process. Since January 2019, we have been using a frozen formulation (irradiated SV-BR-1-GM cryopreserved in biocompatible freeze medium).
- Regimen:
  - Pre-dose cyclophosphamide (300 mg/m^2) 2-3 days prior to SV-BR-1-GM inoculation.
  - DTH skin test, then inoculation of **~20**-50 million irradiated SV-BR-1-GM cells inoculated intradermally, split into 4 inoculations (x2 upper back, x2 thighs).
  - Interferon-α2b intradermally (10,000 IU in each inoculation site) 2±1 and 4±1 days following SV-BR-1-GM inoculation.
  - **NCT03328026 only: Pembrolizumab** (Keytruda; 200 mg IV) during one of the interferon-α2b visits.
- Cycles:
  - NCT03066947 ("monotherapy"): Treatment is performed every 2 weeks for the first month and then every month.
  - NCT03328026 (with pembrolizumab): Treatment every 3 weeks.

RESULTS

Table 2: HLA Matching Predicts Tumor Shrinkage

Combined Pilot Phase I and Phase I/IIa (NCT03066947), both "monotherapy"

Patients (n)	HLA Match	Tumor Shrinkage
5	≥2	40%
18	≥1	22%
9	0	0%

RESULTS

Phase I/IIa (NCT03066947) ("monotherapy")

Responders with tumor regression have a higher propensity to induce T cell responses

Analysis on subset of patients:

Responders (tumor regression) – subjects 01-002, 05-002; Non-responders – subjects 02-003, 03,001, 04-002, 04-005, 04-006

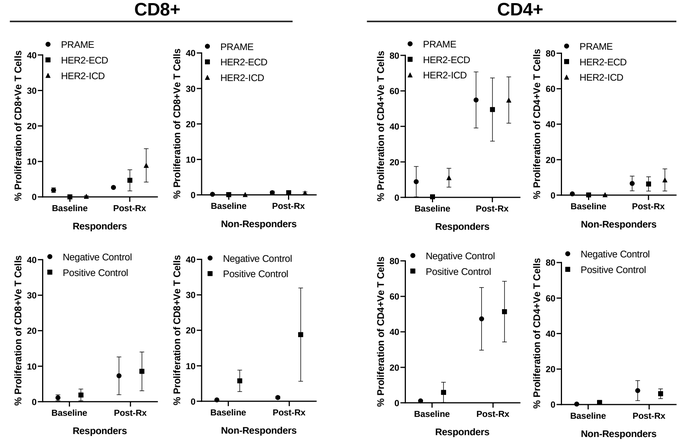

Figure 3. Increased T cell proliferation following inoculation with SV-BR-1-GM in Responders. SV-BR-1-GM expresses the cancer/testis antigen PRAME and HER2 (ERBB2), see **Table 1**. Patient PBMCs were stimulated with overlapping sets of PRAME and HER2 peptides. T cell proliferation was assessed via CellTracer, a fluorescent dye with diminishing fluorescence following cell division. **Substantially higher percentages of PRAME and HER2-specific CD4+ T cells at post-Rx compared to baseline time points for responders (tumor regression) compared to non-responders.** However, PBMCs of responders also demonstrated increased proliferation when stimulated with negative control (actin) and positive control (viral antigens) peptides, **suggesting that responders have a higher tendency to develop T cell responses *per se*** compared to non-responders. Shown are mean values + SDs. ECD, extracellular domain of HER2; ICD, intracellular domain of HER2.

Working Model

Tumor regression requires:

1. HLA matching to SV-BR-1-GM (Table 2)
2. Ability to mount cellular immune response (DTH and *ex vivo* T cell proliferation)

NCT03328026 (pembrolizumab combo)

Table 3: Patient Characteristics and Best Response for the SV-BR-1-GM Regimen + Pembrolizumab

Subject	Age Ethnicity	HLA Allele Matches	Tumor Characteristics			Prior Therapies	Cycles on Monotherapy Study – Best Response on Monotherapy	Cycles on Combo Study – Best Response on Combo
			Her2	ER	PR			
04-005	62 yo WF	0	2+	+	+	4 chemo 2 hormonal	5 - PD	3 – PD
04-007	66 yo WF	1	0	+	+	3 chemo 1 hormonal	3 - PD	2 – Hospice
04-008	63 yo WF	0	0	+	+	2 chemo 1 hormonal	0	3 – PD
05-005	64 yo WF	3	2+	0	0	4 chemo	3 - PD	2 – Hospice
06-004	59 yo WF	1	2+	+	+	3 chemo 3 targeted 3 biol. 5 hormonal	0	7 – SD
06-001	73 yo WF	0	0	+	0	8 chemo, 1 biological	4 - SD	6 – SD*

* A 17% decrease in target lesion diameters was noted for this patient. Bi-dimensional measurements of all lesions showed a 43% decrease.

Table 4: Serum Biomarkers – decrease in responding subject (06-001)

	04-005	04-007	04-008	05-005	06-004	06-001
Baseline CEA	11.6	17.7	2.6	1.3	0.2	**167.8**
Baseline 15-3	47	748	22	16.2	93.4	**164.4**
Initial Eval CEA	*	*	*	*	1.55	**48.15**
Initial Eval 15-3	*	*	*	*	114.4	**114.9**

* not available

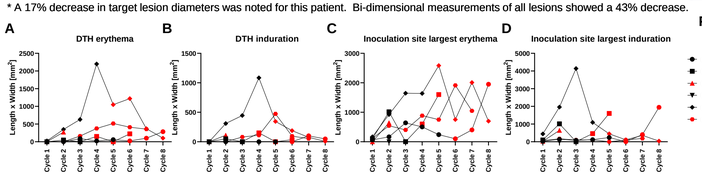

Figure 4. Delayed-Type Hypersensitivity (DTH)

DTH reaction at skin test (1 million irradiated SV-BR-1-GM cells) sites in arm (**A**-erythema, **B**-induration) and at "therapeutic" inoculation sites (2x upper back, 2x thighs) (**C**-largest erythema among all 4 sites; **D**-largest induration among all 4 sites). **Black markers indicate "monotherapy", red markers pembrolizumab combination therapy time points**

Most pronounced reaction in subject 06-001 who experienced tumor regression.

CONCLUSIONS AND HYPOTHESES

- The SV-BR-1-GM regimen +/- pembrolizumab is able to induce an effective immune response and tumor regression in advanced breast cancer patients
- In absence of pembrolizumab, HLA matching and the ability to launch cellular immune response (*ex vivo* and DTH) appear necessary for tumor regression to occur.
- Addition of pembrolizumab can compensate for lack of an HLA match with tumor regression seen in heavily pre-treated metastatic breast cancer.