Toward a Personalized Off-the-Shelf Cellular Immunotherapy for Cancer

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ABSTRACT

Objectives: We have been developing SV-BR-1-GM, a breast cancer cell line with features of an antigen presenting cell which has been stably transfected with the CSF2 gene encoding GM-CSF. SV-BR-1-GM has been in clinical trials in a regimen including low-dose pre-dose cyclophosphamide to reduce immune suppression, and post-dose local interferon alpha to boost the immune response. The SV-BR-1-GM regimen has been administered alone or in combination with PD-1 inhibitors in patients with advanced breast cancer. We have noted that patients that match the SV-BR-1-GM cell line at least at 1 HLA allele are more likely to derive clinical benefit. Therefore, steps were taken to genetically modify the SV-BR-1 cell line to match more patients.

Methodology: We focused on the least polymorphic HLA types in the population: HLA-A (Class I) and HLA-DRB3/4/5 (Class II). The published allele frequencies (Gragert 2013) for HLA-A and HLA-DRB3/4/5 were evaluated for the major demographic groups in the US. The following HLA alleles were selected: A*02:01, A*01:01, A*03:01, A*24:02, A*11:01, A*68:01, A*23:01, A*33:03 for Class I and DRB4*01:01, DRB3*02:02, DRB3*01:01, DRB5*01:01, DRB3*03:01, DRB5*01:02, DRB5*02:02 for HLA-DRB3/4/5. Based on population analysis, this combination of alleles should produce at least a single match in 99% of the population, with a 92% match at Class I HLA-A alleles and a 98% match at Class II HLA-DRB3/4/5 alleles. SV-BR-1 was modified using CRISPR technology deleting expression of the endogenous HLA-A and HLA-DRB3 alleles. Four lentiviral vectors were constructed to express the HLA alleles, along with the CSF2 gene (which encodes GM-CSF), using a 2A self-cleaving multi-gene expression system. Each lentiviral vector expressed 4 HLA types: 2 HLA-A and 2 HLA-DRB3/4/5 types.

Preliminary Data: Following sequential CRISPR treatment, the SV-BR-1 cells were cloned, and one clone selected (clone 17) for further engineering. Lack of expression of HLA-A and HLA-DRB3 was confirmed using flow cytometry and sequencing. Clone 17 was subsequently transduced with the four lentiviral vectors each expressing four HLA genes as well as the CSF2 gene and IFNA2. Following selection and cloning, clones were evaluated by ELISA, flow cytometry and RT-PCR to confirm gene expression. Several clones that secreted GM-CSF and expressed both Class I and Class II HLA alleles have subsequently been transferred to GMP manufacturing.

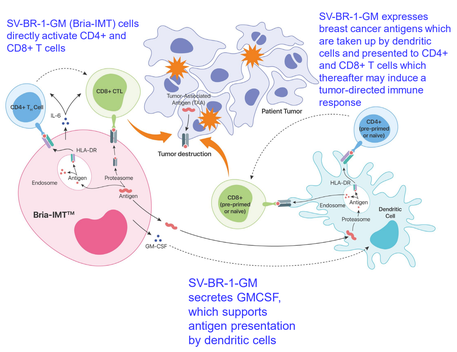
These modified breast cancer cell lines will be used in clinical studies designed to first evaluate the safety subsequentially combined with other agents to augment the immune response. Each patient will be treated with the cell line that matches them at least at one HLA allele.

BACKGROUND

- 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 tumor.
- SV-BR-1-GM was used in 3 clinical studies: In two "Monotherapy" studies the SV-BR-1-GM regimen consisted of 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. For combination therapy, pembrolizumab (200 mg IV) or retifanlimab (375 mg IV) was given in combination with the regimen from the Monotherapy study with cycles every 3 weeks.
- PFS and OS in this group of heavily pretreated metastatic breast cancer patients appears better for those 1+ and 2+ HLA matches with SV-BR-1-GM compared with those with no HLA matches.

Mechanism of Action (MoA)

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



OBJECTIVES

- To modify the SV-BR-1 cell line to express various HLA alleles to permit matching more patients, 8 HLA-A alleles and 7 HLA-DRB3/4/5 alleles were employed in a lentiviral expression system
- To develop cell lines which will be pre-manufactured and will express HLA alleles covering/matching >99% of the overall advanced breast cancer population (double matches in ~90% of the population)

METHODS

In order to generate a cellular immunotherapy for breast cancer matching >99% of the population at least at 1 HLA allele, four cell lines were developed, each carrying two (2) *HLA-A and two (2) HLA-DRB3/4/5* alleles, for a total of eight HLA-A and seven HLA-DRB3/4/5 alleles (Tables 1 and 2). The *HLA-DRB3, HLA-DRB4, and HLA-DRB5* genes occupy essentially the same locus, with the presence of one gene excluding the presence of another. The minimum percentage of patients covered by at least one (1) HLA-match was estimated using published allele frequencies (Tables 1 and 2)[1]. Furthermore, data from the 2010 Census were used to estimate allele matches in different races.

	Alleles in Bria-OTS	African American	White	Asian
US Census 2010 (Frequencies)		12.60%	72.4%	4.8%
			Frequency*	
HLA-A	A*02:01	12.30%	27.60%	14.80%
HLA-A	A*01:01	4.70%	16.50%	1%
HLA-A	A*03:01	8.40%	14%	0.90%
HLA-A	A*24:02	2.50%	8.50%	35.30%
HLA-A	A*11:01	1.40%	6.10%	8.70%
HLA-A	A*68:01	4%	3.20%	0.20%
HLA-A	A*23:01	11%	2%	0.10%
HLA-A	A*33:03	5.20%	0.3%	6.50%
HLA allele frequency	Sum of allele frequencies:	49.40%	78%	67.50%
At least 1 HLA-A match	Per individual (2n):	74.40%	95%	89.40%

Table 1 *HLA allele frequencies by Gragert *et al.*[1], in African American; European Caucasian, and Japanese. Percentages of "At least 1 HLA-A match" are higher per individual than the sum (ΣAFHLA-A) of the allele frequencies (AF) since allele frequencies refer to one chromosome set (1n), with each individual having two chromosome sets (2n). The per-individual (2n) "phenotype frequencies" (PF) indicating the percentage of individuals with at least one HLA-A match with the exogenous HLA-A alleles from the Bria-OTS cell lines were calculated as follows:
PFHLA-A = 1 - (1 - ΣAFHLA-A)2, whereby (1-ΣAFHLA-A)2 is the probability that an individual does **not** carry at least 1 of the HLA-A alleles. Example: for African American, PFHLA-A = 1 - (1 - ΣAFHLA-A)2 = 1 – (1 – 49.4%)2 = 74.4%.

	Alleles in Bria-OTS	African American	White	Asian
US Census 2010 (Frequencies)		12.6%	72.4%	4.8%
			Frequency*	
DRB3/4/5	DRB4*01:01	18.30%	31.20%	38.40%
DRB3/4/5	DRB3*02:02	27.20%	18.20%	10.40%
DRB3/4/5	DRB3*01:01	13.40%	14.90%	6.30%
DRB3/4/5	DRB5*01:01	14.40%	13.50%	8.70%
DRB3/4/5	DRB3*03:01	9.60%	4.90%	7.50%
DRB3/4/5	DRB5*01:02	0.20%	0.70%	9.70%
DRB3/4/5	DRB5*02:02	1.50%	1.60%	0.70%
At least 1 HLA-DRB345 match	Sum of allele frequencies:	84.60%	85%	81.60%
At least 1 HLA-DRB345 match	Per individual (2n):	97.60%	97.80%	96.60%

Table 2. *HLA allele frequencies by Gragert *et al.*[1], African American; European Caucasian, Japanese (lowest HLA-DRB3/4/5 allele frequency among Asians). Allele and phenotype frequencies (2n) were calculated as described for Table 1.

Conclusion: This combination of alleles permits matching ~92% of the total US population for HLA-A (weighted average) and ~98% of the US Population for HLA-DRB3/4/5 with over 99% having at least one match.

METHODS CONTINUED

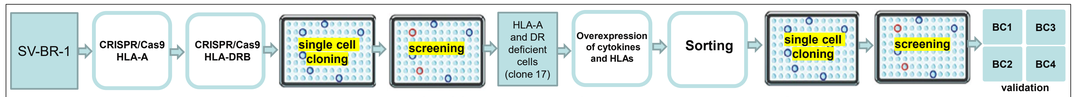


Fig.1. Experimental strategy. The resulting cells express GM-CSF, IFNα and the following HLA combinations **BC1**: *HLA-A*01:01, HLA-A*68:01, HLA-DRB3*02:02, HLA-DRB4*01:01* **BC2**: *HLA-A*02:01, HLA-A*11:01, HLA-DRB4*01:01, HLA-DRB3*03:01* **BC3**: *HLA-A*03:01, HLA-A*23:01, HLA-DRB3*01:01, HLA-DRB5*01:02* **BC4**: *HLA-A*33:03, HLA-DRB5*01:01, HLA-DRB5*02:02*

	EF1 alpha promoter			MNDU3 promoter			
Cell line ID	Cytokine 1	2A cytokine 2		HLA-A Allele 1	2A HLA-A Allele 2	2A HLA-DRB3/4/5 Allele 1	2A HLA-DRB3/4/5 Allele 2
BC1	CFS2	T2A	IFNA2	A*01:01	T2A A*68:01	P2A DRB4*01:01	E2A DRB3*02:02
BC2	CFS2	T2A	IFNA2	A*02:01	T2A A*11:01	P2A DRB4*01:01	E2A DRB3*03:01
BC3	CFS2	T2A	IFNA2	A*03:01	T2A A*23:01	P2A DRB3*01:01	E2A DRB5*01:02
BC4	CFS2	T2A	IFNA2	A*24:02	T2A A*33:01	P2A DRB5*01:01	E2A DRB5*02:02

Fig.2. Lentiviral vectors used to generate Bria-OTS. Polycistronic mRNA were generated by the use of 2A sequences. Vectors carried 2 polycistronic mRNAs

RESULTS

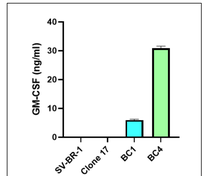


Fig.3. GM-CSF expression on BC1 and BC4 cell lines. Expression of GM-CSF was quantified using an ELISA assay.

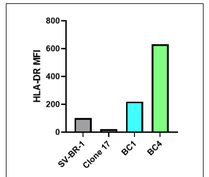


Fig.4. HLA-DR expression on BC1 and BC4. Surface expression was measured by flow cytometry.

Conclusion: Clonal Cell Lines BC1 and BC4 have been developed and express GM-CSF as well as HLA-A and HLA-DRB3/4/5 proteins and mRNA based on cytokine ELISA, flow cytometry and by SYBR-green Q-PCR analyses.

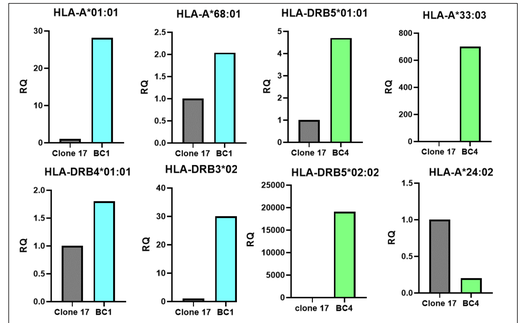


Fig.5. Expression of HLA-A/DR transgenes on BC1 and BC4. Allele specific quantification of HLA-A/DR was done by SYBR-green Q-PCR. Relative quantification is shown. All intended HLA alleles are expressed at higher levels than Clone 17 except HLA-A24.

DISCUSSION AND CONCLUSIONS

SV-BR-1-GM has a unique mechanism of action, acting both as a source of breast cancer antigens and a functional antigen presenting cell, thereby boosting the immune response. This is in part dependent on HLA matching between the patient and the cell line. Based on these observations, SV-BR-1 has been genetically engineered to express 8 Class I and 7 Class II HLA alleles, which will allow a single HLA match with >99% of the population and a double match in ~90%. These cell lines will provide a personalized approach to cancer immunotherapy that is off-the-shelf, eliminating the complex manufacturing logistics of other personalized immunotherapies.

References:

1. Gragert L, Madbouly A, Freeman J, Maiers M. Six-locus high resolution HLA haplotype frequencies derived from mixed-resolution DNA typing for the entire US donor registry. *Hum Immunol.* 2013;74(10):1313-1320
2. Lacher MD et al, Front Immunol. 2018 May 15;9:776