Identification of Antigenic Determinants in SV-BR-1 derived Cellular Breast Cancer Vaccines

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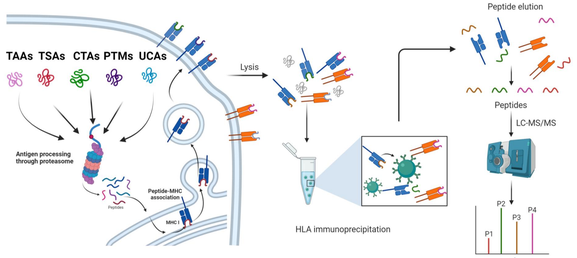
BACKGROUND

Identifying antigenic determinants is crucial for developing effective cancer vaccines. This study focuses on SV-BR-1 derived cellular breast cancer vaccines, aiming to delineate specific antigens that elicit an immune response. These vaccines rely on two key concepts: tumor cells display immunogenic antigens activating T-cells via cross-presentation, where host dendritic cells (DCs) process exogenous tumor antigens and present them on HLA molecules to activate T cells. Additionally, genetic engineering enhances their role as antigen-presenting cells, amplifying immune responses. Bria-IMT, the first version, is a genetically modified tumor cell line engineered to secrete granulocyte-macrophage colony-stimulating factor (GM-CSF). This vaccine has shown encouraging clinical outcomes and is currently being tested in a phase 3 clinical trial, demonstrating its potential in cancer immunotherapy. Bria-OTS+, an advanced version, enhances tumor cells' ability to present antigens by expressing cytokines, co-stimulatory factors, and HLA alleles.

OBJECTIVES

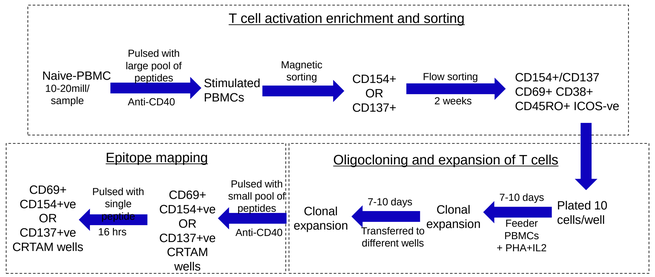
Therapeutic cancer vaccines are designed to stimulate the immune system by leveraging tumor antigens to generate an antitumor response. In our clinical trials, we have been focusing on the SV-BR-1-GM, a breast cancer cell line engineered to secrete GM-CSF, as a therapeutic vaccine. This vaccine has demonstrated encouraging clinical outcomes, both when used as a monotherapy (NCT03066947, completed) and in combination with checkpoint inhibitors (NCT03328026, ongoing). To further enhance the therapeutic efficacy of SV-BR-1-GM, we initiated a study to characterize the patient's immune response to the vaccine. It is well-known that cancer vaccines can induce both humoral and cellular immune responses. However, predicting these responses, especially in the context of whole-cell vaccines like SV-BR-1-GM, presents unique challenges. One of the major difficulties lies in identifying which specific tumor antigens elicit an immune response in patients, as whole-cell vaccines present a wide array of antigens. Here, we present a preliminary analysis of antigen-specific T-cell responses using a T-cell epitope mapping assay in breast cancer patients.

METHODS



Identification of Tumor Antigens with Immunopeptidomics Using SV-BR-1 Cells. *This figure illustrates the workflow for immunopeptidome analysis, detailing the processing and presentation of various categories of tumor antigens, including tumor-associated antigens (TAAs), tumor-specific antigens (TSAs), cancer-testis antigens (CTAs), post-translationally modified (PTM) antigens, and unconventional antigens (UCAs) in the SV-BR-1 cell line.* ***TAAs*** *are proteins that are overexpressed or dysregulated in tumor cells compared to normal tissues.* ***TSAs*** *result from tumor-specific somatic mutations, creating neoantigens unique to cancer cells.* ***CTAs*** *are typically restricted to immune-privileged germline tissues but are aberrantly expressed in tumors.* ***PTM antigens*** *arise from alterations in post-translational modifications, such as phosphorylation or glycosylation, leading to the presentation of novel tumor-specific epitopes.* ***UCAs*** *encompass antigens from non-coding regions, alternative reading frames, or proteins from the dark proteome, providing unique epitopes in tumor cells. These antigens are processed by the proteasome into peptide fragments, which are subsequently loaded onto MHC class I molecules and transported to the cell surface.*
In the immunopeptidomics workflow, the cells are lysed, and MHC-peptide complexes are immunoprecipitated using HLA-specific antibodies MHC I (W6/32), HLA-DR (L243) and MHC II (IVA12). The peptides are then eluted and analyzed by liquid chromatography-tandem mass spectrometry (LC-MS/MS), which identifies them based on their mass-to-charge ratio (m/z). This method provides a comprehensive profile of the tumor immunopeptidome, enabling the identification of antigens for potential therapeutic targeting.

METHODS



Workflow for Class II antigen/epitope mapping. *Class I and II antigen/epitope mapping: CD154-expressing T cells (Class II) and CD137-expressing T cells (Class I) were enriched using magnetic beads and flow sorted with activation and memory markers. The sorted T cells were expanded as oligoclones in 96-well plates with feeder cells, PHA, and IL-2. They were then stimulated with peptide pools, and positive responses were restimulated with individual peptides to identify the specific antigenic peptide.*

Patient ID	Therapy	HLA match with Bria-IMT	PFS (months)	Tumor Regression	RECIST
01-002	Monotherapy	A*24:02	6.9	Yes (Liver,& spleen)	Partial Response
04-001	Monotherapy	DRB3*02:02	2.4		Progressive Disease
06-001	Monotherapy	None	6.1	Yes (Liver)	Partial Response

Patients and clinical data: *The SV-BR-1-GM "monotherapy" (NCT03066947; 2013-8) was a completed prospective phase 1-2 study of the SV-BR-1-GM regimen, administered every two weeks for the first two doses, then monthly. This regimen included a dose of cyclophosphamide (300 mg/m^2) administered intravenously 48–72 hours before each SV-BR-1-GM treatment (~20 x 10^6 cells) delivered intradermally. Interferon-alpha was applied at the SV-BR-1-GM inoculation sites two days after each dose.*

RESULTS

Sample	IP antibody	Total Peptides	Total Intensity
SV-BR-1-GM	L243 (HLA-DR)	308	4.30E+08
SV-BR-1-GM	IVA12 (MHCII)	1885	5.20E+09
SV-BR-1-GM	W6/32 (MHCI)	4123	1.40E+11

MHC Class I and II Profiling of Human Bria-IMT Cells. *A total of 4123 peptides were detected for class I MHC and 2193 for class II at the 1% PSM FDR (based on forward/decoy database searching).*

Identification and Prioritization of Candidate Immunogenic Peptides for immunogenicity testing.

We prioritized peptides from two main sources:

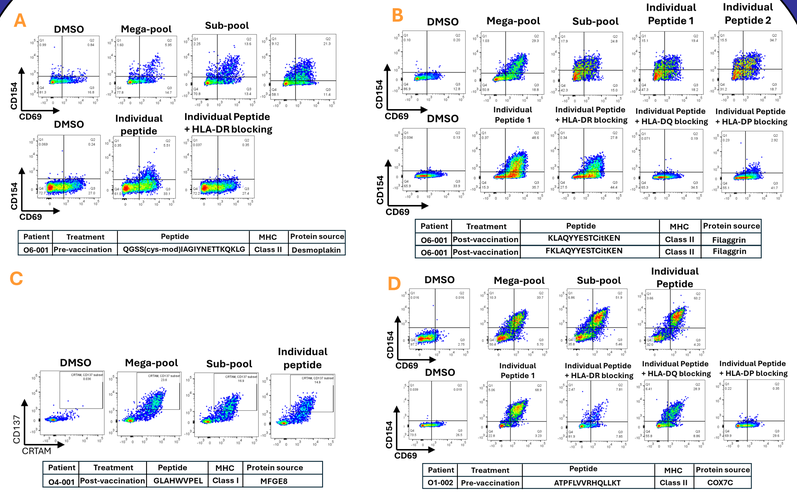
1. Peptides presented by SV-BR-1 cells: These peptides were directly identified in the immunopeptidome analysis of SV-BR-1 cells, indicating natural processing and presentation by the tumor cells used in our vaccine.
2. Expressed peptides with known Immunogenicity: Peptides not detected in our immunopeptidome but confirmed to be expressed in SV-BR-1 cells through RNA-seq analysis. These peptides are known to be presented by breast cancer cells and have demonstrated immunogenicity in public databases such as The Cancer Epitope Database and Analysis Resource (CEDAR) and CaAtlas (https://www.zhang-lab.org/caatlas/).

Selection Strategy:

- MHC Binding Affinity: We used NetMHCpan tools to predict peptides with high binding affinities to prevalent HLA alleles (IC50 ≤ 500 nM for class I, ≤ 1,000 nM for class II).
- Tumor Specificity: Focused on tumor-associated antigens (TAAs) and cancer-testis antigens (CTAs) unique to SV-BR-1 cells.
- Overexpression in Tumors: Prioritized peptides from proteins highly expressed in tumor cells, as confirmed by RNA-seq data.
- Unique PTMs: Peptides containing post-translational modifications specific to tumor cells were included to capture novel epitopes.
- Immunogenicity Evidence: Cross-referenced candidates with immunogenicity databases to select peptides with documented immune responses.
- HLA Matching: Selected peptides relevant to HLA types common in our patient cohort used for the analysis.

80 MHC class I (10 carrying PTM) and 50 class II (18 carrying PTM) were selected for further analysis

RESULTS



Patient	Treatment	Peptide	MHC	Protein source
O6-001	Pre-vaccination	QGSS(cys-mod)IAGIYNETTKQKLG	Class II	Desmoplakin

Patient	Treatment	Peptide	MHC	Protein source
O6-001	Post-vaccination	KLAQYYESTCitKEN	Class II	Filaggrin
O6-001	Post-vaccination	FKLAQYYESTCitKEN	Class II	Filaggrin

Patient	Treatment	Peptide	MHC	Protein source
O4-001	Post-vaccination	GLAHWVPEL	Class I	MFGE8

Patient	Treatment	Peptide	MHC	Protein source
O1-002	Pre-vaccination	ATPFLVVRHQLLKT	Class II	COX7C

Identification of T cell epitopes by CD154 (Class II) or CD1epitope mapping assay. *PBMCs (peripheral blood mononuclear cells) from vaccinated patients, collected both pre- and post-vaccination, were subjected to ex vivo stimulation with various peptide pools to assess immediate T-cell activation. Stimulation conditions included a DMSO control for background measurement, a positive control to confirm cell reactivity, and Class I and Class II peptide megapools designed to stimulate CD8+ (cytotoxic) and CD4+ (helper) T cells, respectively. Following initial ex vivo activation, T cells identified by activation markers—CD137 and CRTAM for CD8+ cells, and CD154 and CD69 for CD4+ cells—were analyzed via flow cytometry to determine peptide-specific responses. Subsequently, each Mega-pool was subdivided into smaller sub-pools, which were tested individually to reduce background noise and improve specificity in identifying immunogenic peptides. Individual peptides were then tested within these sub-pools, allowing for precise identification of specific epitopes that induce T-cell responses. To confirm that CD4+ T-cell responses were MHC Class II-dependent, HLA-DR, HLA-DQ, or HLA-DP blocking antibodies were introduced alongside individual peptides. We identified four peptides that induce an immunogenic response:* ***(A)*** *the pre-vaccination sample responded to a cysteinylated Desmoplakin MHC Class II peptide;* ***(B)*** *the post-vaccination sample responded to a citrullinated Filaggrin MHC Class II peptide; (C) the post-vaccination sample responded to an MFGE8 MHC Class I peptide; and (D) the pre-vaccination sample responded to an MHC Class II peptide from COX7C.*

A total of 1 CD8+ and 3 CD4+ T cell epitopes were identified

CONCLUSIONS

- We successfully identified immunogenic peptides in patients treated with the SV-BR-1-GM cellular vaccine, demonstrating the vaccine's ability to elicit a targeted immune response against tumor antigens.
- Key immunogenic peptides detected include those with **post-translational modifications (PTMs)**, such as citrunilation and cysteinylation.
- This study underscore the advantage of using cellular cancer vaccines over RNA and peptide-based vaccines due to their ability to present a broad and diverse repertoire of antigens, including both conventional and unconventional types.
- Unlike RNA and peptide vaccines, which deliver specific, known antigens, cellular vaccines—such as whole tumor cells or antigen-presenting cells with tumor lysates—present a broad array of naturally occurring tumor antigens. This includes post-translational modifications (PTMs), alternative splicing variants, non-coding sequences, and peptides from pseudogenes. Such antigens often represent unique tumor features absent in healthy cells. Cellular vaccines can also display unknown, patient-specific neoantigens that are hard to predict with RNA or peptide vaccines. This diverse antigen presentation stimulates a robust, polyclonal immune response, engaging both CD8+ and CD4+ T cells against multiple tumor targets, reducing immune escape and potentially leading to more durable clinical outcomes.