Analysis of Antibody Response to SV-BR-1-GM Therapeutic Vaccine in Breast Cancer Patients Using Human Protein Microarrays: Potential Correlations with Therapy Response

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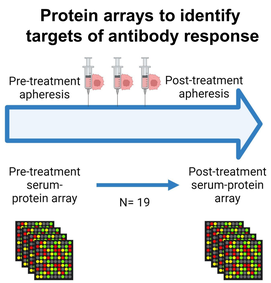
BACKGROUND AND OBJECTIVES

Therapeutic cancer vaccines aims to stimulate the immune system by utilizing tumor antigens to trigger an antitumor response. In our clinical trials, we have focused on evaluating the efficacy of the breast cancer cell line secreting GM-CSF, SV-BR-1-GM as a therapeutic vaccine, and we have observed encouraging clinical outcomes. The SV-BR-1-GM regimen has been used alone ("monotherapy", ClinicalTrials.gov NCT03066947 – study completed) and in combination with checkpoint inhibitors ("combination", ClinicalTrials.gov NCT03328026 – study ongoing). To assess the efficacy of the treatment, identify biomarkers and overall optimize therapy for future clinical trials we have initiated immuno-monitoring studies. It has been demonstrated that cancer vaccines can generate both humoral and cellular immune responses. However, predicting immune responses to cancer vaccines, especially when whole cells are employed as immunogens, presents significant challenges. We present here a preliminary analysis of the antibody response to SV-BR-1-GM in breast cancer patients using antigen arrays.

METHODS

Large-scale protein arrays are versatile and sensitive platforms for antibody specificity evaluation. The HuProt™ Human Proteome Microarray (CDI Laboratories, Inc., Baltimore, Maryland, United States) provides the largest number of unique, full-length, individually purified human proteins on a single microscope slide. This allows thousands of interactions to be profiled in a high-throughput manner. We utilized here an unbiased human protein microarray platform encompassing >21,000 proteins and isoforms from ~19,000 unique genes to identify IgG and IgM responses against self-antigens elicited by treatment. Serum samples from SV-BR-1-GM-treated patients were analyzed, comparing pre- and post-treatment. The human serum samples were probed at 1:1000 dilution. After sample processing and data collection the raw signal intensities on all arrays were quantile normalized using CDI software. Heatmaps were generated using MetaboAnalyst 5.0, which utilizes the R heatmap package (version 0.7.7). For paired analysis, fold changes (FCs) were calculated by determining the ratio between paired pre- and post-treatment samples, resulting in one FC per pair. The means of these FCs (pair means) were then computed. ANOVA tests were performed using the RStatix R package, which automatically determines the appropriate Type I, II, or III errors for the analysis.



RESULTS

Subject ID	disease control	regimen
01-002	yes	Monotherapy Study
03-001	yes	Monotherapy Study
04-001	no	Monotherapy Study
04-005	no	Monotherapy Study
04-006	yes	Monotherapy Study
05-002	yes	Monotherapy Study
06-001	yes	Monotherapy Study
01-009	yes	Combination study
01-010	no	Combination study
04-008	no	Combination study
06-001	yes	Combination study
06-005	yes	Combination study
06-006	no	Combination study
06-007	yes	Combination study
06-009	no	Combination study
07-001	yes	Combination study
07-004	no	Combination study
09-001	no	Combination study
11-003	yes	Combination study

***Table 1. Clinical data. Combination study**: an ongoing prospective, phase 1/2 with a randomized phase 2 cohort (NCT03328026; 2018-present) using SVBR-1-GM with a PD-1 inhibitor (pembrolizumab or retifanlimab) with cycles every 3 weeks (30 patients dosed to date). **Monotherapy study**: SV-BR-1-GM "monotherapy" (NCT03066947; 2013-8), a completed prospective phase 1-2 study of the SV-BR1-GM regimen every 2 weeks x 2 then monthly. Both regimens included cyclophosphamide 300 mg/m2 i.v. 48-72 hours prior to SV-BR-1-GM (~20 x 106 cells) intradermally followed by interferon-alpha at the SV-BR-1-GM inoculation sites 2 days afterwards. We analyzed plasma from 19 patients, comprising 7 from the monotherapy group and 12 from the combination therapy group. Disease control 2 partial response (PR) and 9 stable disease (SD).*

RESULTS

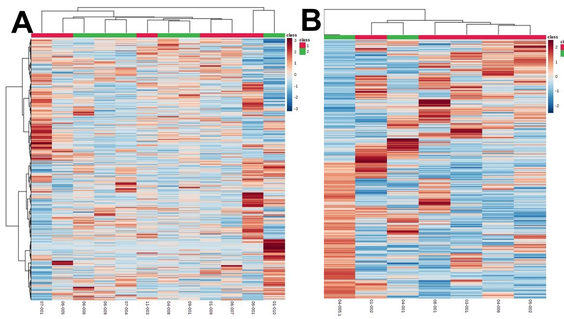


***Fig. 1. Hierarchical clustering analysis of patient response to therapy:** Samples data was processed and analyzed using R heatmap (version 0.7.7) **A)** Combination study **B)** Monotherapy study.*
Responders
Non-responders

Conclusion: modest association between drug response and global antibody responses.

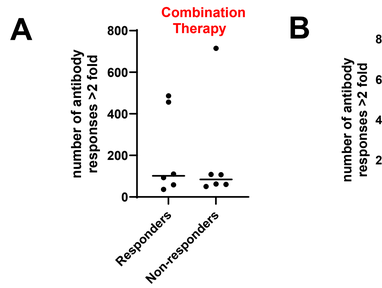


***Fig. 2. The distinction between clinical responders and non-responders lies not in the quantity of antibody responses but in their quality** Samples data was processed and analyzed using R heatmap (version 0.7.7) **A)** In the combination study, the number of antibody responses is not significantly different between responders and non-responders, and there is no overlap between the two groups **B)** In the monotherapy study, the number of antibody responses is not significantly different between responders and non-responders, and only 3 responses overlap between the two groups.*

Conclusion: the discerning factor between clinical responders and non-responders did not hinge on the sheer quantity of antibody responses but rather on their qualitative aspects

A

Gene ID	Fold change	p.value	FDR
Lupus La	4.2849	0.049848	0.99998
TNFSF11	3.8636	0.080603	0.99998
N4BP2L2	2.6668	0.14787	0.99998
CDC45	1.9371	0.0017181	0.99998
RXRA	1.8592	0.023896	0.99998
ILF2	1.8393	0.0034926	0.99998
UROD	1.808	0.022338	0.99998
CTRL	1.7029	0.54763	0.99998
NRG3	1.6856	0.73198	0.99998
LAT	1.6838	0.37966	0.99998
PMEPA	1.6526	0.089946	0.99998
ANXA11	1.6241	0.92405	0.99998
LGALS1	1.592	0.092879	0.99998

B

Gene ID	Fold Change	p.value	FDR
LGALS1	3.8481	0.063842	0.99997
LGALS8	3.1857	0.098843	0.99997
LGALS8	3.0982	0.1181	0.99997
OR13J1	2.423	0.047161	0.99997
LGALS9	2.277	0.1361	0.99997
SPN	2.2443	0.65218	0.99997
LGALS3	2.0217	0.13554	0.99997
GBP6	1.9386	0.083499	0.99997
ZFYVE21	1.8633	0.0057085	0.99997
SLC25A29	1.8451	0.045024	0.99997
TPO	1.8442	0.20933	0.99997
TRPM8	1.8051	0.038066	0.99997
NAV1	1.7828	0.091152	0.99997

***Table 2. Paired fold change analysis:** For paired analysis, fold Changes are calculated by computing the ratio between paired samples (i.e. one FC per pair), and then compute their means (i.e. pair means). To calculate statistical significance a paired t-test was used. Benjamini-Hochberg procedure was used to calculate the false discovery rate (FDR). **A)** Combination study **B)** Monotherapy study.*

The vaccine monotherapy and its combination with an anti-PD1 antibody exhibited largely non-overlapping patterns in antibody profiles

RESULTS

A

Gen ID	treated (F.val)	treated (F.val)	treated (adj.p)	disease control(F.val)	disease control(raw.p)	disease control(adj.p)	Interaction (F.val)	Interaction (raw.p)	Interaction (adj.p)
NRG1	0.435	0.517	1	1.431	0.246	0.38295	1.827	0.192	1
NFIB/2	0.917	0.35	1	1.634	0.216	0.38095	1.428	0.246	1
LDOC1	0.949	0.342	1	3.507	0.076	0.38095	1.088	0.309	1
IRF2BP2	0.808	0.379	1	2.082	0.165	0.38095	1.034	0.321	1
ARL2BP	0.932	0.346	1	0.935	0.345	0.44204	1.009	0.327	1
TCEAL6	0.618	0.441	1	0.432	0.518	0.57989	0.99	0.332	1
LARP7	0.9	0.354	1	0.669	0.423	0.50357	0.986	0.333	1
MTUS1	0.912	0.351	1	1.419	0.247	0.38295	0.984	0.333	1
TCEAL6	0.898	0.355	1	0.299	0.591	0.63209	0.952	0.341	1
MDP1	1.022	0.324	1	0.855	0.366	0.46038	0.936	0.345	1
PACSIN	0.707	0.41	1	0.365	0.553	0.60437	0.927	0.347	1
KBTBD7	0.806	0.38	1	1.466	0.24	0.38095	0.901	0.354	1
DAB2	0.8	0.382	1	0.18	0.676	0.70785	0.853	0.367	1
NOL3	0.083	0.764	1	0.795	0.383	0.47561	0.827	0.374	1
LSM1	0.917	0.35	1	0.864	0.364	0.46038	0.824	0.375	1
EMC8	0.734	0.402	1	1.472	0.239	0.38095	0.804	0.38	1
SUDS3	1.115	0.304	1	0.562	0.462	0.53103	0.746	0.398	1
DACT3	0.723	0.405	1	1.464	0.24	0.38095	0.732	0.402	1
YARS	0.738	0.401	1	1.944	0.179	0.38095	0.709	0.41	1
TNFSF11	3.216	0.088	1	0.398	0.535	0.59116	0.697	0.414	1

B

Gene ID	Treatment (F.val)	Treatment (raw.p)	Treatment (adj.p)	disease control (F.val)	disease control (raw.p)	disease control (adj.p)	Interaction (F.val)	Interaction (raw.p)	Interaction (adj.p)
STAM	1.714	0.22	1	6.329	0.031	0.31	5.337	0.043	0.999
SMRP1	1.317	0.278	1	4.594	0.058	0.41429	3.852	0.078	0.999
CATIP	1.262	0.288	1	13.82	0.004	0.31	3.678	0.084	0.999
GNB2	1.454	0.256	1	4.039	0.072	0.46452	3.582	0.088	0.999
MTUS1	1.153	0.308	1	4.4	0.062	0.42759	3.001	0.114	0.999
NXN	0.93	0.358	1	6.737	0.027	0.31	2.426	0.15	0.999
LIN28B	0.681	0.428	1	4.784	0.054	0.4	2.139	0.174	0.999
ZNF783	0.816	0.388	1	9.907	0.01	0.31	1.929	0.195	0.999
LGALS1	4.234	0.067	1	0.783	0.397	0.61039	1.408	0.263	0.999
ASB9	0.518	0.488	1	8.335	0.016	0.31	1.266	0.287	0.999
LGALS8	3.114	0.108	1	0.63	0.446	0.61039	1.043	0.331	0.999
LGALS9/1	2.39	0.153	1	0.271	0.614	0.67473	0.964	0.349	0.999
LGALS8/3	2.726	0.13	1	0.638	0.443	0.61039	0.906	0.364	0.999

***Table 3. Analysis of the impact of treatment and disease control on antibody responses using two-way ANOVA** Analysis of the impact of treatment and disease control on antibody responses was conducted using a two-way ANOVA. To identify if potential antibody responses correlated with treatment and disease control, we applied a two-way ANOVA, treating antibody responses as the dependent variable and considering treatment and disease status as the independent variables. The F-values associated with treatment, disease status, and their interaction effect on antibody responses were analyzed. Antibody responses were subsequently ranked based on the interaction effect in two separate studies:. **A)** Combination study **B)** Monotherapy study.*

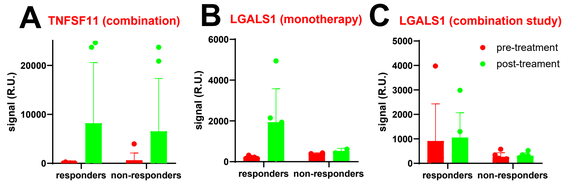


Fig. 3. Examples of specific target of antibody responses in monotherapy and combination studies:

DISCUSSIONS AND CLONCLUSSIONS

- By using protein arrays, we were able to evaluate a broader range of antigens compared to previous investigations.
- The changes in antibody profiles was limited to a restricted number of antigens.
- The distinguishing factor between clinical responders and non-responders did not hinge on the sheer quantity of antibody responses but rather on their qualitative aspects.
- Both the vaccine monotherapy and its combination with an anti-PD1 antibody exhibited largely non-overlapping patterns in antibody profiles.
- Potential correlations between specific antibody responses patient survival were identified.