Vaccination by Homologous Antigenic Loading as Adjuvant Therapy for Glioblastoma: Correlation of Immune Response and Early Efficacy Signal with Vaccine Cell Gene Signature

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ABSTRACT

Background

Glioblastoma is a devastating tumor of the CNS with median survival of 14-18 months despite aggressive intervention. Cellular immunotherapy approaches have previously shown promise in GBM but with often inconclusive results. Dendritic cell (DC) homologous antigenic loading is a technology that leverages p38MAPK and mTORC1 signaling cascades to initiate powerful cDC1-like skewing of monocyte-derived DC, leading to downstream induction of tissue-infiltrating, cytolytic effector memory T-cells.

Methods

This phase I clinical trial evaluated the autologous cell-based vaccine DOC1021 prepared through DC homologous antigenic loading with autologous tumor lysate and amplified tumor mRNA and administered bilaterally near the deep cervical lymph node chains. Three courses of vaccine were administered every two weeks after completion of chemoradiation, adjuvanted contemporaneously with weekly alpha interferon. Four dose levels from $3.5x10^6$ to $3.6x10^7$ total vaccine cells were tested. Patient immune responses were evaluated by flow cytometry of peripheral blood and, in three patients, by spatial transcriptomics. Vaccine cell preparations were analyzed by mRNAseq of vaccine and autologous control cells loaded only with tumor mRNA but not lysate.

Results

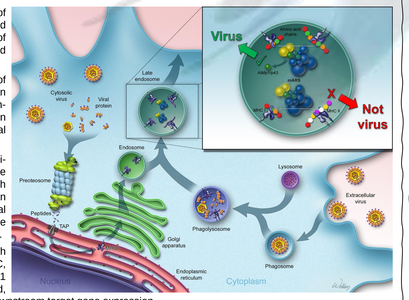
Sixteen newly diagnosed patients completed treatment, median age 63 years, 38% subtotal resection and 94% (15/16) MGMT unmethylated. The most common related AEs were grade 1 injection-site reactions and grade 1-2 fatigue and urticaria. No SAEs nor DLTs were observed. IHC analysis of tumor derived from post-vaccination second resections showed enhanced $CD8^+$ T-cell infiltration and pathology consistent with resolution rather than relapsed GBM in 2/3 patients (not shown). Analysis of post-vaccination PBMC indicated expansion of $CD4^+$ (13/13) and $CD8^+$ (11/13) central memory T-cell compartments (****p<0.00006 and ***p<0.003, respectively) as well as expansion of $CD8^+CD127^+$ MPEC (12/13; ***p<0.002). Augmentation of peripheral immune response was strongly correlated with congruent directional movement of 31 signature genes in patient vaccine cell products (r^2=0.44 for $CD8^+CD127^+$ cells and 0.57 for peripheral tregs). Among three patients analyzed by spatial transcriptomics at second operation, clinical outcomes correlated with postvaccination upregulation of CD141, IL-6, IL-36β, and IL-12Rα and downregulation of IL-1α and TGF-β. Treg infiltration into postvaccination tumor bed was not observed. Cancer-specific median OS of this 15/16 MGMT unmethylated cohort is estimated by Kaplan-Meier analysis to be 19.7 months after 16.0 months median follow-up.

Conclusions

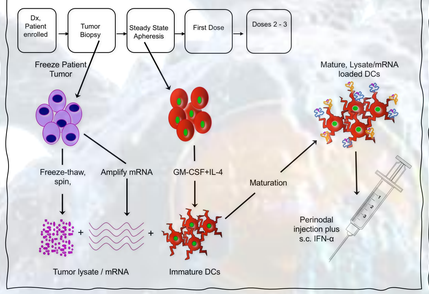
The results indicate that DOC1021 is safe, can be effectively integrated within existing standards of care and appears efficacious in a challenging patient population. Strong correlations between vaccine genetic signature and peripheral immune responses were also observed.

HOMOLOGOUS ANTIGENIC LOADING

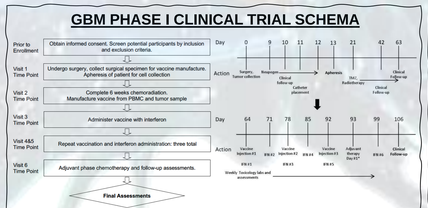
- Defined as the loading of dendritic cell MHC class I and MHC class II with epitopes of overlapping amino acid sequence homology
- Is recognized by elements of innate immunity as an important pathogen-associated molecular pattern (PAMP) associated with viral infection
- Detected by the multi-aminoacyl tRNA synthetase (mARS) com-plex which transmits the information through conformational change that releases the AIMp1 signaling intermediate.
- AIMp1 signals through p38MAPK and mTORC, resulting in altered AP-1 heterodimer composition and, ultimately the specificity of downstream target gene expression.
- Genetic changes that result from homologous antigenic loading generate a cell type with a cDC1-like phenotype, enabling the priming of robust $CD8^+$ T-cell responses with an enhanced cytolytic and memory characteristics coupled with a reduced capacity for exhaustion.
- A cell-based vaccine employing homologous antigenic loading might serve as a powerful, personalized vaccine platform through which to generate durable antitumor responses. MHC class I antigens were delivered ex vivo to patient DC through electroporation of amplified autologous tumor mRNA whereas MHC class II antigens were delivered by subsequent DC incubation with autologous tumor lysate.



VACCINE MANUFACTURING SCHEMA



GBM PHASE I CLINICAL TRIAL SCHEMA



ENROLLED SUBJECTS

Dose Level Cohort	Study ID	Race	Ethnicity	Gender	Age at Diagnosis	Resection Type	MGMTp Status	Newly-Diagnosed	Survival (Months)	October 18 Survival Status
DL1 3.5e6	GBM-MDAC-0001	Caucasian	NOT Hispanic	Female	66	Gross total	Unmethylated	Yes	23.8	Deceased
	GBM-MDAC-0003	Caucasian	NOT Hispanic	Female	64	Subtotal	Unmethylated	Yes	17.2	Deceased
	GBM-MDAC-0006	Caucasian	NOT Hispanic	Male	73	Gross total	Unmethylated	Yes	26.4↔	Alive
DL2 7.0e6	GBM-UT-0008*	Caucasian	NOT Hispanic	Female	47	Subtotal	Unmethylated	No	10.0	Deceased
	GBM-UT-0011	Caucasian	NOT Hispanic	Male	58	Gross total	Unmethylated	Yes	19.7	Deceased
	GBM-UT-0012	Caucasian	Not Reported	Female	67	Gross total	Unmethylated	Yes	14.9	Deceased
	GBM-MDAC-0014	Caucasian	NOT Hispanic	Female	64	Subtotal	Methylated (81%)	Yes	19.6↔	Alive
	GBM-UT-0015	Caucasian	NOT Hispanic	Male	58	Gross total	Unmethylated	Yes	17.4	Deceased
DL3 1.4e7	GBM-UT-0017	Asian	NOT Hispanic	Female	63	Subtotal	Unmethylated	Yes	9.7	Deceased
	GBM-UT-0018	Caucasian	NOT Hispanic	Female	59	Gross total	Unmethylated	Yes	17.6↔	Alive
	GBM-UT-0019	Caucasian	NOT Hispanic	Male	63	Gross total	Unmethylated	Yes	12.9	Deceased
	GBM-UT-0021	Caucasian	NOT Hispanic	Male	51	Gross total	Unmethylated	Yes	16.5↔	Alive
	GBM-UT-0022	Caucasian	NOT Hispanic	Male	59	Gross total	Unmethylated	Yes	14.4	Deceased
DL4 3.6e7	GBM-UT-0023	Caucasian	NOT Hispanic	Male	54	Gross total	Unmethylated	Yes	15.5↔	Alive
	GBM-UT-0024	Asian	NOT Hispanic	Female	58	Gross total	Unmethylated	Yes	15.3↔	Alive
	GBM-UT-0025	Caucasian	NOT Hispanic	Female	73	Gross total	Unmethylated	Yes	15.2↔	Alive
	GBM-MDAC-0027*	Caucasian	NOT Hispanic	Female	66	Subtotal	Unmethylated	No	11.6↔	Alive
	GBM-UT-0028	Caucasian	NOT Hispanic	Male	65	Subtotal	Unmethylated	Yes	8.7	Deceased

SPECIFIC VACCINE CELL GENE SIGNATURE

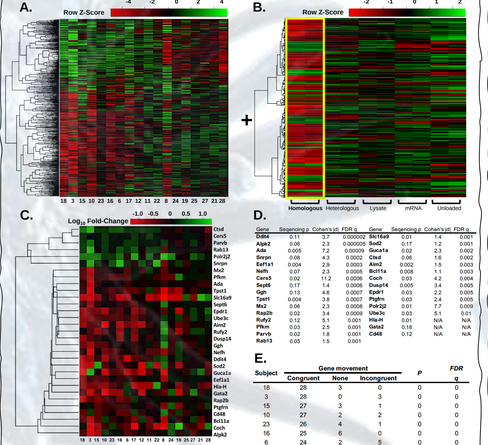


Figure 1. Clinical DC Vaccines Generated by Homologous Antigenic Loading Exhibit a Specific Transcriptional Signature. A. Eighteen patient vaccine products and 18 autologous controls loaded only with amplified tumor mRNA but not with lysate were analyzed by bulk mRNAseq and genes that differed significantly in expression level were identified. **B.** Previous work (Decker et al, 2009) identified gene transcripts that differed between DC loaded in homologous fashion and those loaded by four other methodologies. **C.** The intersection of the two gene sets, partially informed by relevant biology, resulted in the selection of a 31 gene signature outlined in **D. E.** Vaccine preparations were subsequently categorized according to the number of genes that up or downmodulated in congruent fashion. No movement was defined as < +/- 8.5% between vaccine preparation and control preparation (loading with mRNA-only).

POST-VACCINATION PERIPHERAL IMMUNE RESPONSES

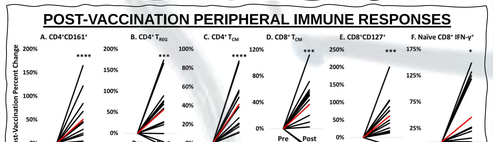


Figure 2. Autologous cell-based vaccines generated through homologous antigenic loading are associated with characteristic peripheral immune responses. Peripheral blood collected from patients at baseline and again at day 106-108 were characterized by flow cytometry. Percent changes in cell populations of individual patients for whom data were available are shown in black with average percent change shown in red **A.** $CD3^+CD4^+CD161^+$ cells, mean +50%. **B.** $CD3^+CD4^+CD25^+$ $Foxp3^+$ T_{REG}, mean +56%. **C.** $CD3^+CD4^+CD45RA^{neg}CD45RO^+CD62L^+CCR7^+$ T_{CM}, mean +41%. **D.** $CD3^+$ $CD8^+CD45RA^{neg}CD45RO^+CD62L^+CCR7^+$ T_{CM}, mean +37%. **E.** $CD3^+CD8^+CD127^+$ MPEC, mean +61%. **F.** Naïve $CD3^+CD8^+CD45RA^+CD45RO^{neg}CD62L^+$ $CCR7^+$ T-cells secreting IFN-γ, mean +46%. *p<0.05, ***p<0.005, ****p<0.001 by paired Student's two-tailed t test.

IMMUNE RESPONSES CORRELATE WITH GENE SIGNATURE AND CELL DOSE

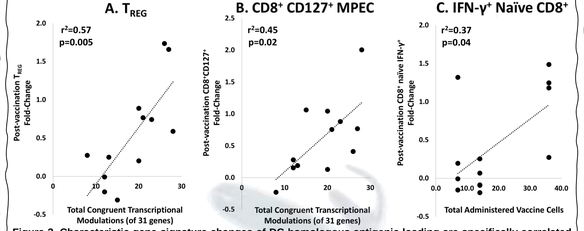


Figure 3. Characteristic gene signature changes of DC homologous antigenic loading are specifically correlated with development of post-vaccination peripheral immune responses. The number of directionally congruent gene alterations (of 31 total in the identified signature) was plotted against fold-change of pre- and post-vaccination cell populations and linear regression analysis was performed to generate the square of the Pearson correlation coefficient. **A.** The number of directionally congruent gene alterations was strongly correlated with postvaccination fold-increase of peripheral blood regulatory T-cells, r^2 = 0.57, Pearson's R = 0.75, ***p = 0.005. **B.** The number of directionally congruent gene alterations was strongly correlated with postvaccination fold-increase of peripheral blood regulatory MPECs, r^2 = 0.45, Pearson's R = 0.67, *p = 0.02. **C.** In addition to correlations between fold-change in peripheral blood immune cell populations, vaccine dose itself was shown to correlate well with fold-increase in IFN-γ-secreting $CD8^+$ T-cell expressing markers of a naïve phenotype ($CD45RA^+CD45RO^{neg}CD62L^+CCR7^+$), r^2 = 0.37, Pearson's R = 0.61, *p = 0.04.

IMMUNE RESPONSES AND GENE SIGNATURE CORRELATE WITH SURVIVAL

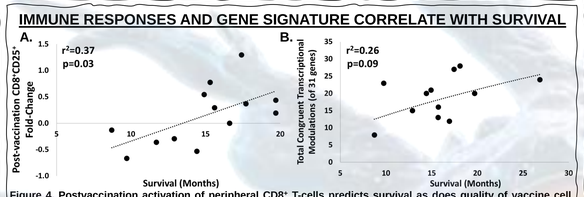


Figure 4. Postvaccination activation of peripheral $CD8^+$ T-cells predicts survival as does quality of vaccine cell gene signature. A. Survival (months) was plotted against fold-change of pre/postvaccination $CD8^+CD25+$ T-cell counts and analyzed by linear regression. R=0.61, *p<0.03. **B.** Cancer-specific survival (months) was plotted against number of congruent gene signature modulations and analyzed by non-linear regression analyses. While these data are still maturing, power regression analysis indicated a strong trend toward statistical significance, R=0.51, p<0.09.

LATE PSEUDOPROGRESSION WITH RESOLUTION IN LONG TERM SURVIVORS

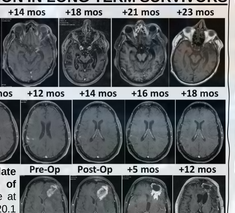


Figure 5. Most newly-diagnosed vaccinated patients exhibited late pseudoprogression that resolved with time in the absence of reoperation. Shown: MGMT unmethylated patient 0006 currently alive at 26.9 months (top) and MGMT methylated patient 0014 currently alive at 20.1 months (middle). Also shown: MGMT unmethylated recurrent patient 0027R currently alive at 12.1 months following reoperation. Pseudoprogression was observed among most vaccinated patients independent of MGMT methylation status but was observed to resolve with time in the absence of reoperation. Reoperation was a significantly negative independent prognostic co-variable.

SURVIVAL

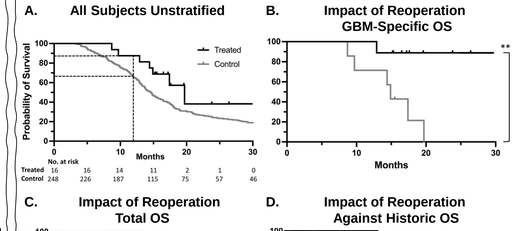


Figure 6. Adjuvant therapeutic vaccination by means of homologous antigenic loading for newly-diagnosed GBM substantially enhances OS in conjunction with Stupp protocol standard of care, particularly in the absence of reoperation. A. At median follow-up of 16.0 months, 12-month cancer-specific OS of the 15/16 MGMT unmethylated newly-diagnosed cohort (modified ITT cohort) was 87.5% vs 65.7% for an age- and treatment-matched contemporary cohort (p<0.07). **B.** Reoperation was a significantly negative independent prognostic co-variable with a GBM-specific OS of patients who did not receive reoperation of 88.9% (8/9) vs 14.3% (1/7) among patients who did (**p<0.006). at 16.0 months median follow-up. **C.** Total OS of 66.6%(6/9) vs 0.0% (0/7) was also very significant (**p<0.01). D. GBM-specific OS among patients who did not receive reoperation was also substantially improved from historic control (**p<0.007); however, among patients who received reoperation, median OS of 14.9 months was statistically identical to that of the control cohort (14.6 months).

CONCLUSIONS

- Adjuvant vaccine immunotherapy with DOC1021, a dendritic cell vaccine platform generated by means of homologous antigenic loading, was both feasible in safe in conjunction with Stupp protocol.
- Successful antigenic loading of DOC1021 was associated with a specific signature of 31 genes, the modulation of which correlated with peripheral postvaccination expansions of both Tregs and MPECs and trended strongly toward correlation with survival.
- Other statistically significant peripheral immune cell expansions were observed after vaccination but were not strongly correlated with the gene signature.
- Pseudoprogression was frequently observed among vaccinated patients including MGMT unmethylated and relapsed/recurrent patients.
- Reoperation was a significantly negative independent prognostic co-variable. Cancer-specific OS of 88.9% (8/9) and total OS of 66.7% (6/9) was substantially better among patients from whom reoperation was withheld at 16.0 months follow-up. Among patients who received reoperation, cancer-specific OS was 14.3% (1/7) and total OS was 0.0% (0/7). Despite the small samples size, these number were statistically significant.
- Among all newly-diagnosed patients, one-year OS of 87.5% was substantially better than that of an age- and treatment-matched contemporary control (65.7%) that included both MGMT unmethylated and IDH mutant patients.
- The results reported here justify continued clinical development of the DOC1021 platform in the setting of randomized, controlled trials.

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