Glioblastoma Lysate-pulsed Dendritic Cell Vaccination Induces Immune Response Against Tumor-specific Mutations

Richard George Everson MD; Joseph Antonios; Alexander M. Tucker MD; Horacio Soto; Namjo Shin; Sue Rathe; Flavia Popescu; David A. Largaespada PhD; Linda M. Liau MD, PhD; Robert M. Prins PhD

Department of Neurosurgery, University of California Los Angeles

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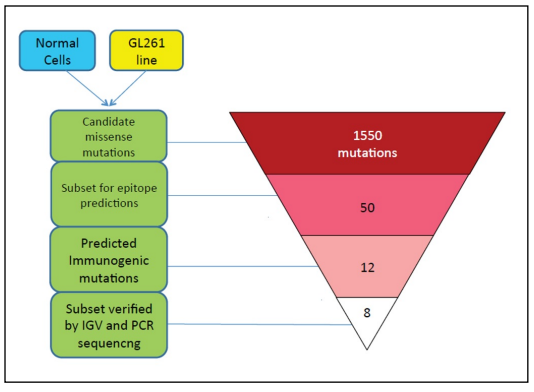
Introduction

Dendritic cell (DC) vaccines currently in phase III trials for the treatment of glioblastoma use autologous tumor lysate (ATL) as an antigen source but it remains unclear what the targets of this personalized therapy are directed against in each patient. We hypothesized that the target antigens are truly tumor-specific, such as mutations and gene fusions found in tumor that create stable peptide-MHC complexes compared with the normal sequences.



Learning Objectives

By the conclusion of this session, participants should be able to:

- Explain the rationale for immunotherapy with autologous tumor lysate dendritic cell vaccination as a treament for glioblastoma
- Discuss the scientific underpinnings of how the treatment might work, namely targeting individual, tumor-specific mutations.

Methods

We performed RNA sequencing on the GL261 mouse glioma cell line. Mutations were identified with the Missense Mutation and Frameshift Location Reporter (MMuFLR) software developed by our group and analyzed by NetMHC3.4 to predict which mutated sequences have enhanced peptide-MHC binding.

- 9 candidate neoantigens were identified and were confirmed by IGV and Sanger sequencing.

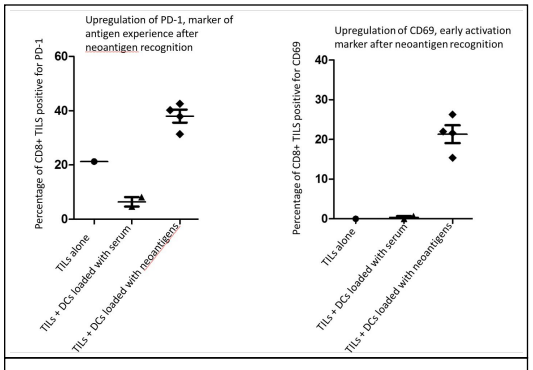
Table 1:

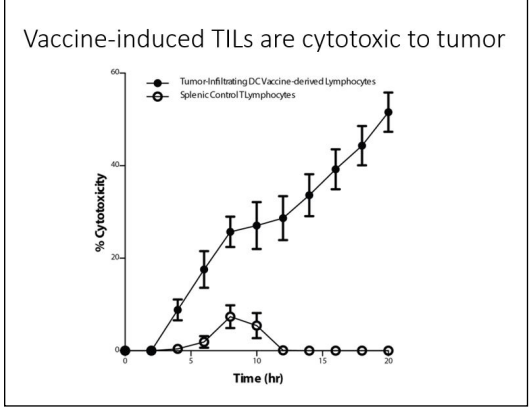
Gene	Variant Position	Reference	Variation	Penetrance	Sequencing Depth	Mutations	Variant	Sequence	epitope	IGV Verification
C1s	chr6:124485405	G	T	0.42	53	22	Q227H	VSLNCPSVSF_H_FASKNQQFG	VSFHFASK	Not valid
Myh14	chr7:51916424	G	T	0.61	56	34	G135V	YYSGLIYTYS_V_LFCVVINPY	LIYTYSYL	Valid candidate
Tmem2	chr19:21910048	G	T	0.42	85	36	I1042N	PQYQPVVMLI_N_GITHWNGP	VMLRNYIT	Valid candidate
Gm5263	chr1:148267697	T	A	0.67	9	6	M45K	EGLTDEFVNL_K_FLSLISVGL	VNLRFLSL	Not valid
Commd7	chr2:153447501	G	A	0.78	9	7	S161N	EKVGSIFLQV_N_LWSVAMGRV	SIFLQVNL	Not valid
Pcf11	chr7:99806156	G	T	0.67	57	38	D1105Y	GPQHQQASRF_Y_IPLGLQGTR	ASRFYIPL	Valid candidate
Sfxn2	chr19:46657467	G	T	0.47	40	19	I111M	LITGFMLQFY_M_TMPAVIFWQ	FMLQFYMT	Valid candidate
Zfr	chr15:12048094	G	C	0.35	20	7	R46S	AAAAAAAQY_S_YSVLWSSMD	AQYSYSYL	Not valid
Pcm1	chr8:42355888	A	T	0.33	120	40	Q352L	LNDLIQRFHN_L_LRDSQPPAV	LLQRFHFL	Valid candidate
Rad54b	chr4:11533390	G	T	0.86	42	36	L550F	LLRSQDVRFC_F_QGLLENSAH	VRFCFQGL	Valid candidate
Gal3st1	chr11:3898707	G	T	0.8	102	82	D304Y	YRRATAWNLL_Y_VRLYRHFNA	RRLLVYRL	Valid candidate
Myo9a	chr9:59651757	G	T	0.61	31	19	R440L	GNISYKKKTY_L_DDSDICNP	ISYRKKTYL	Valid candidate
GARC-1	chr9:56785554	G	A	0.78	60	47	D81N	FRVRASAALL_N_KLYAMGLVPT	AALLNKLYA	Valid candidate

- To evaluate whether ATL DC vaccination induced neoantigen-specific T cell responses, GL261 intracranial tumor-bearing mice were vaccinated with a dendritic cell vaccine loaded with GL261 tumor lysate or control.
- After 7 days, tumor-infiltrating lymphocytes (TILs) were isolated from the brains of the mice by percoll gradient, and counted.
- TILs were co-cultured with DCs pulsed with peptides containing the neoantigen sequences and subjected to flow cytometry evaluation for markers of antigen recognition on the T cells.
- Cytotoxicty of the vaccine generated TILs was measured with the xCELLigence assay

Results

5x10^5-2x10^6 TILs were recovered from vaccine-treated animals, whereas <10^5 were recovered from controls, indicating a T cell response to the vaccine. TILs antitumor activity as measured by the xCELLigence cytotoxicity assay. TILs were co-cultured with DCs pulsed with synthetic peptides containing the neoantigen sequences. 15-25% of tumor-infiltrating CD8+ T cells from tumor lysate-pulsed DC vaccine-treated mice upregulated CD69, an early marker of T cell activation in an antigen-specific manner. These cells also demonstrated elevated expression of PD-1, a marker of antigen-experienced cells.





Conclusions

- Together, these studies indicate that in this murine model, autologous tumor lysate DC vaccines are capable of inducing intratumoral CD8+ T cell responses.
- These TILs are cytotoxic to the tumor and a large percentage of them are reactive to tumor-specific neoantigen mutations.
- Further studies will evaluate these findings in glioblastoma patients treated with DC vaccines.

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