

Retargeted oncolytic viruses engineered to remodel the tumor microenvironment for glioblastoma immunotherapy

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Supplementary Information

Supplementary Tables:

Supplementary Table 1. HSV-1 Receptor Expression in GBM Tumor Cores

Antigen	Tumor Cores with biomarker $\geq 1\%$ ^a	Average % of biomarker positive cells	Average H-Score ^b
NECTIN-1	1	23	39.6
EGFR	35	62	161
HVEM	9	8.35	10.07

^aTotal number of tumor cores = 41

^bH-score is a semi-quantitative score system that integrates the intensity of staining with the percentage of cells stained¹. Scores fall in a range of 0-300.

Supplementary Table 2. Configuration of miR-T Cassettes in ONCR-2169 and derivatives

Cassette ID	HSV-1 Gene ^a	Gene Class	miR Targets
T3060	ICP4	a/Immediate Early	miR-122-5p miR-124-3p miR-128T-M miR-137-3p
T9919	ICP8	b/Early	miR-34c-5p miR-124-3p miR-129-2-3p miR-132-3p
T3012	ICP27	a/Immediate Early	miR-34c-5p miR-124-3p miR-128T-M miR-137-3p
T3096	UL8	b/Early	miR-34b-3p miR-34c-5p miR-132-3p miR-145-5p

^aICP, Infected cell protein; UL8, HSV-1 helicase-primase subunit. miR response elements were inserted in the 3'-UTR of the indicated genes.

Supplementary Table 3. *In vivo* anti-tumor activity in GL261-N of ONCR-142 expressing candidate payload in combination with ONCR-142_{IL12+aPD-1} vs. ONCR-142_{IL12+aPD-1} monotherapy

Candidate ^a	Candidate mOS	ONCR-142 _{IL12+aPD-1} mOS	p Value ^b	Number of mice
mHPGD	59	31	0.059	5

CTX-BiTE	53	31	0.251	5
Anti-TREM2	> 140	26	0.254	7
Anti-TGFb	53	31	0.128	4
Anti-CD47	41	31	0.426	5
Anti-IFNg	45	31	0.437	4
mCCL21	> 46	45	0.219	5
hADA2 + hSPAM1	26	44	0.262	5
CHIPS + anti-CD47	36	44	0.275	5

^a mHPGD, mouse 15-hydroxyprostaglandin dehydrogenase; CTX, chlorotoxin; BiTE, bi-specific T cell engager; TREM2, triggering receptor expressed on myeloid cells 2; TGFb, transforming growth factor b; CD47, cluster of differentiation 47; IFNg, interferon g; CCL21, CC motif chemokine ligand 21; hADA2, human adenosine deaminase 2; hSPAM1, human sperm adhesion molecule 1 (also hyaluronidase PH-20); CHIPS, Chemotaxis inhibitory protein of Staphylococcus aureus.

^bP-values of survival comparison to PBS treatment using a two-sided Mantel–Cox log-rank test.

Supplementary Table 4. Efficacy score, or differential survival in GL261-N of ONCR-142 expressing candidate payload in combination with ONCR-142_{IL12+aPD-1} vs. ONCR-142_{IL12+aPD-1} monotherapy

Candidate	Efficacy score D mOS ^a
mHPGD	1.90
CTX-BiTE	1.71
Anti-TREM2	> 5.38
Anti-TGFb	1.71
Anti-CD47	1.32
Anti-IFNg	1.45
mCCL21	1.02
hADA2 + hSPAM1	0.59
CHIPS + anti-CD47	0.82

^a(mOS of candidate + ONCR-142_{IL12+aPD-1})/(mOS of ONCR-142_{IL12+aPD-1})

Supplementary Table 5. Summary of *in vivo* efficacy of candidate GBM Viruses

Virus	GL261-N			GL261-E			CT2A-N			CT2A-E		
	mOS ^a	P ^b	n	mOS	P	n	mOS	P	n	mOS	P	n
PBS	22		12				19.5		12			
KPiONCR-2178	28	0.0011	12				23.5	< 0.0001	12			
KPiONCR-2178 _{IL12} +aPD-1+HPGD+aTREM2	40.5	< 0.0001	12				27	< 0.0001	12			
KPiONCR-2178 _{IL12} +aPD-1+HPGD+aTREM2+CTX	34	< 0.0001	12				30.5	< 0.0001	12			
PBS	24.5		10	25.5		8	23.5		10	23		11
VEONCR-2178				27.5	0.9819	8				28	0.0026	11
VEONCR-2178 _{IL12} +aPD-1+HPGD+aTREM2	31	0.0003	10	32.5	0.0167	8	35	0.0001	10	31	<0.0001	11
VEONCR-2178 _{IL12} +aPD-1+HPGD+aTREM2+CTX	40	<0.0001	15	29.5	0.2390	8	37	<0.0001	10	31	<0.0001	11

^amOS, median overall survival.

^bP-values of survival comparison to PBS treatment using a two-sided Mantel–Cox log-rank test.

Supplementary Table 6. Antibodies used in the study.

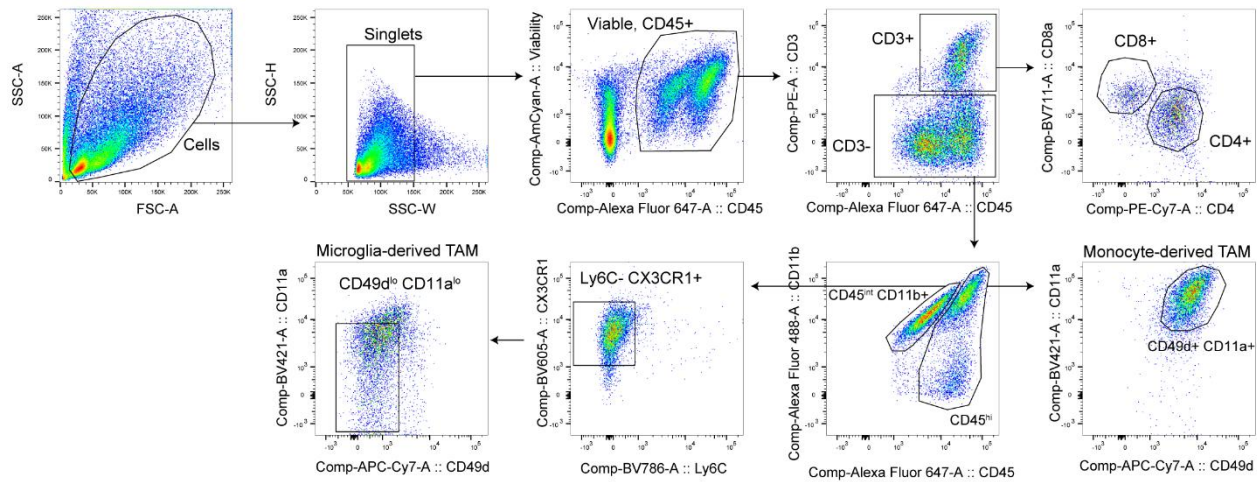
Antibody	Source	Catalog number	Dilution
FITC anti-CD11b	eBioscience	11-0112-85	1:100
APC anti-CD45	eBioscience	17-0451-83	1:100
BV421 Anti-CD11a	BD Biosciences	740020	1:100
BV605 anti-CX3CR1	Biolegend	149027	1:100
BV711 anti-CD8a	BD Biosciences	752634	1:100
PE anti-CD3	BioLegend	100206	1:100
PE/Cy7 anti-CD4	BioLegend	100422	1:100
APC/Cy7 anti-CD49d	Biolegend	103635	1:100
FITC anti-CD19	BioLegend	152404	1:100
BV421 anti-NK1.1	BD Biosciences	562921	1:100
BUV661 anti-CD45	BD Biosciences	612975	1:100
BUV737 anti-CD11b	BD Biosciences	612800	1:100
BV650 anti-CD3	BD Biosciences	740530	1:100
BV750 anti-mouse TNF	BD Biosciences	566365	1:100

FITC anti-CD8a	Biolegend	100804	1:100
PE/Cy7 anti-CD4	BioLegend	100422	1:100
APC-Cy7 Rat anti-IFN- γ	BD Biosciences	561479	1:100
Rabbit anti-phospho-STAT3	Cell Signaling Technology	9145T	1:50
Rat anti-CD68	Bio-Rad	MCA1957T	1:100
Donkey anti-Rabbit-AF568	Thermo Fisher Scientific	A10042	1:1000
Goat anti-Rat-AF647	Thermo Fisher Scientific	A21247	1:1000
Rabbit anti-Cleaved Caspase-3 (Asp175)	Cell Signaling Technology	9661T	1:400
Mouse anti-NeuN	EMD Millipore	MAB377	1:500
Donkey anti-Rabbit-AF647	Thermo Fisher Scientific	A31573	1:1000
Donkey anti-Mouse-AF594	Thermo Fisher Scientific	A21203	1:1000
Rabbit anti-HSV-1 polyclonal antibody	Thermo Fisher Scientific	RBP3-1800-P0	1:200
Donkey anti-Rabbit-AF488	Thermo Fisher Scientific	A21206	1:1000

Supplementary Table 7. Primers used in the study.

Primer	Sequence
US6 fwd	CCCGCTGGA ACTACTATGACA
US6 rev	GCATCAGGAACCCCAGGTT
US6 probe	TTCAGCGCCGTCAGCGAGGA
VP5 fwd	TCGTATTGCAACACCCTGTC
VP5 rev	TGGTGGACCTCGAACTGTA
VP5 probe	TGTGTACCAAGTTTCCGGAGCTGG
aPD1 fwd	AGTCCACGATGATGTTCCGC
aPD1 rev	CGCGACA ACTCGAAGAACAC
aPD1 probe	TAGACCGCGGTGTCCTCTGC
mIL12 fwd	CGCTGGATCCTCACGAAGAA
mIL12 rev	CGCTGAAGAACTCCCAGGTC
mIL12 probe	AGTAGCTGTGCGGCGTCGAC
aTREM2 fwd	ACCTCATGCACTGGGTCAAG
aTREM2 rev	TGGA ACTTCTGCGCTACTT
aTREM2 probe	ATCGGCTGGATCGACCCGGA
HPGD fwd	ACGGCCTCATCAACCTGATC
HPGD rev	GGGCTGATGTCGTAGTCCTG
HPGD probe	AGATCACCGCGTCTGAAGGGC
CTXBiTE fwd	ATGCACATACTACCGCCTCC
CTXBiTE rev	ACA ACTACCCCTGGACGTTT
CTXBiTE probe	ATCTCCAGCTTCGTGCCGGG

Supplementary Figures:



Supplementary Figure 1: Flow cytometry gating strategy for T cell and TAM analysis.

Representative gating strategy is shown for analysis of T cells (CD8⁺ gate for CD8⁺ T cells, CD4⁺ gate for CD4⁺ T cells) and TAMs (CD49d^{low} CD11a^{low} gate for microglia-derived TAMs, CD49d⁺ CD11a⁺ gate for monocyte-derived TAMs) in **Figs. 4 and 6**.

References

1. Wen, Z. et al. Deep Learning-Based H-Score Quantification of Immunohistochemistry-Stained Images. *Mod Pathol* **37**, 100398 (2024).