# Autologous glioblastoma tumor cells and an antisense oligonucleotide against insulin-like growth factor type 1 receptor protect against tumor challenge and generate T cell anti-tumor responses

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## ABSTRACT

**Background:** *IGV-001 is a novel immunotherapy that combines irradiated, patient-derived glioblastoma tumor cells and an antisense oligonucleotide against insulin-like growth factor type 1 receptor (IMV-001) in biodiffusion chambers (0.1-micron pore size). We recently evaluated IGV-001 in patients with newly diagnosed glioblastoma[1] In a subgroup of IGV-001-treated, Stupp-eligible patients [2] with methylated O6-methylguanine–DNA methyl-transferase (MGMT) promoter, median progression free survival was 38.4 months[1] compared with 8.3 months in historical standard-of-care-treated patients (P=0.0008)[2]. We utilized the GL261-Luciferase (-Luc) glioblastoma orthotopic murine model and conducted in vitro immunological assays using patient-derived GBM tumor cells and matched peripheral blood mononuclear cells (PBMC) to unravel the potential mechanisms associated with the activity of IGV-001.*

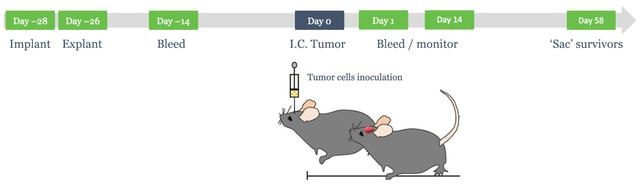
**Methodology:** *Biodiffusion chambers containing phosphate-buffered saline (PBS) alone or IGV-001 prepared with 1x10$^6$ GL261-Luc cells were implanted in the flanks of C57BL/6 albino mice and explanted 48 hours later, as per the clinical protocol. GL261-Luc intracranial tumor challenge was conducted 28 days after chamber implantation. Mice were monitored for survival and tumor growth, as determine by bioluminescence intensity (BLI). For in vitro experiments, IGV-001 prepared with patient tumor cells were co-cultured with patient-derived PBMC to evaluate activated and memory T cell subsets and responses. To elucidate the immunostimulatory underpinnings of IGV-001, ATP release assay was conducted as a surrogate measure of immunogenic cell death.*

**Results:** *59% of IGV-001 treated mice were alive and continued to gain weight at the termination of the study, 58 days post–intracranial tumor challenge. In comparison, there were no survivors in the PBS group by day 24 (p<0.001). In IGV-001 treated mice, serum IL-6 was positively correlated with BLI, meaning that treated mice with lower BLI signal had less circulating IL-6 (P<0.01). Elispot assays demonstrated enhanced T cell IFNγ responses to tumor cell antigens. Tumor co-culture studies showed elevated percentage of activated CD4 and CD8 T cells as well as increased central and effector memory phenotypes in both T cell subsets compared to IMV-001-treated PBMC controls. Lastly, tumor cells treated with IMV-001 released significantly more (p<0.01) ATP than untreated or sense oligonucleotide-treated controls.*

**Conclusions:** *These data support the antitumor activity of IGV-001 in newly diagnosed glioblastoma, as evidenced in the phase 1 study. Th1 anti-tumor T cell activity was demonstrated. The ATP results suggest a possible immunogenic conversion by which IGV-001 stimulates the immune system and suppresses tumor growth, which can be quantified via circulating IL-6.*

## METHODOLOGY

***IN VIVO* MURINE GL261 GBM ORTHOTOPIC MODEL**




**IFNγ ELISPOT ASSAY MURINE GL261 GBM MODEL**

- T cell IFNγ Elispot response to peptide pool of GL261 antigens were normalized to CD3+ T cell populations per well determined by flow cytometry.
- # of spots was calculated as follows: $\frac{(Raw - Background\ counts)}{CD3{+}counts/well} \times 100{,}000\ cells$
- Control wells received T cells from mice implanted with PBS loaded chambers; treatment wells received T cells from mice implanted with IGV-001 + treated tumor chambers; ConA wells received T cells treated with Concanavalin A (10µg/well).

***IN VITRO* CO-CULTURE OF IGV-001 AND PATIENT-MATCHED PBMCS**

IGV-001 prepared with patient GBM cells was co-cultured with matched PBMCs

- Conditions:

(1) PBMCs alone
(2) PBMCs + PHA (Phytohemagglutinin-P)
(4) PBMCs with IMV-001 spike (4 µg/360 µL concentration),
(4) 1:10 Ratio = 1 IGV-001 to 10 PBMCs cells

- IL-2 (5 IU/mL), 200 µL of media per well, ½ replaced every 2-3 days with fresh media and IL-2

***References:***

***[1] Clin Cancer Res. 2021 Apr 1;27(7):1912-1922. doi: 10.1158/1078-0432.CCR-20-3805.***

***[2] N Engl J Med. 2005 Mar 10;352(10):987-96. doi: 10.1056/NEJMoa043330.***

## RESULTS

**Fig 1. Survival (a) and tumor burden quantified using bioluminescence imaging (b).**
Survival data was compared using Log-rank (Mantel-Cox) test

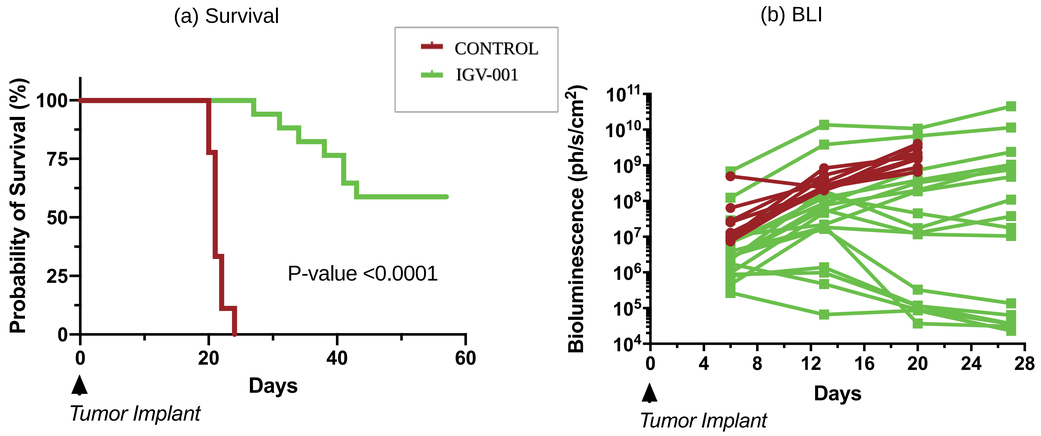



**Fig 2. Correlation between IL-6 and tumor burden in IGV-001-treated mice.** (a) Stable tumors (green) defined as Ln(BLI)<20 and Progressed tumors (red) defined as Ln(BLI)>20. Linear regression of full dataset (stable & progressed) indicates Ln(BLI) and [IL-6] are positively correlated with Pearson correlation coefficient "r"= 0.4159. Data indicates that mice with greater tumor burden had higher serum IL-6, especially for "Progressed" tumors. (b) Average [IL-6] for "Progressed" & "Stable" mouse tumors. Increased [IL-6] for "Progressed" mouse tumors. Unpaired t test with Welch's correction: ** p<0.01

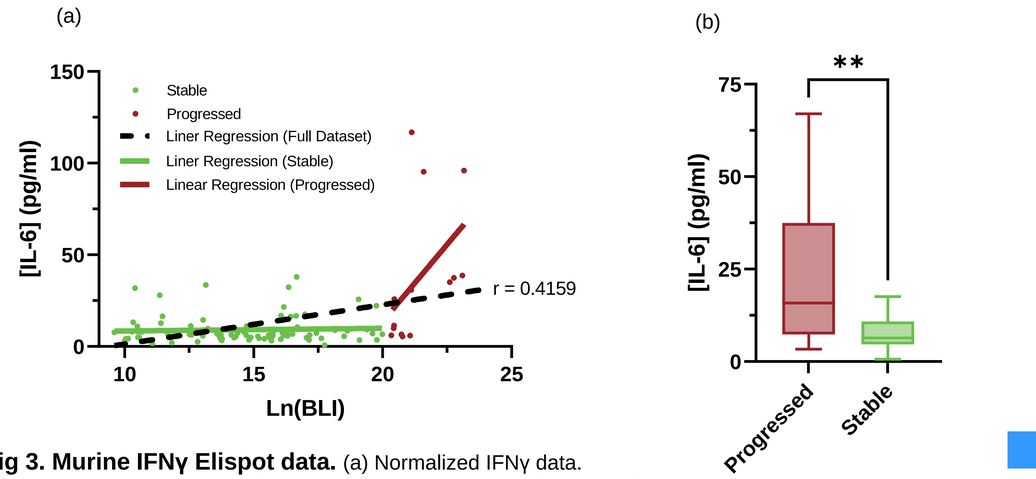



**Fig 3. Murine IFNγ Elispot data.** (a) Normalized IFNγ data. Statistical analysis was by ordinary one-way ANOVA: * p<0.05, ** p<0.01. (b) Representative well images

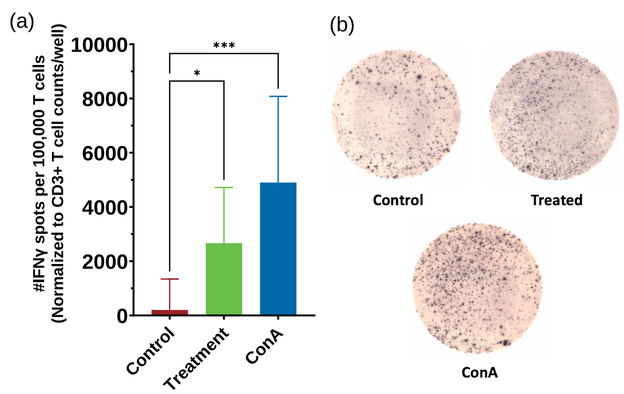



**Fig 4. T cell activation upregulation (a) and memory phenotypes (b) in patient PBMCs co-cultured with IGV-001 prepared from matched patient GBM cells**

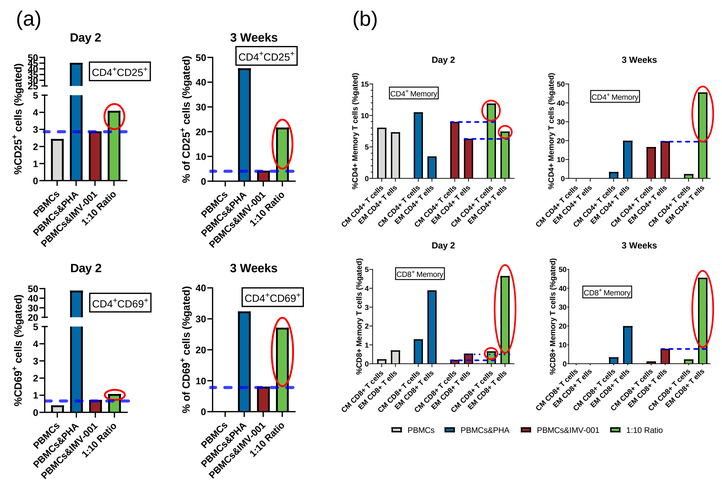
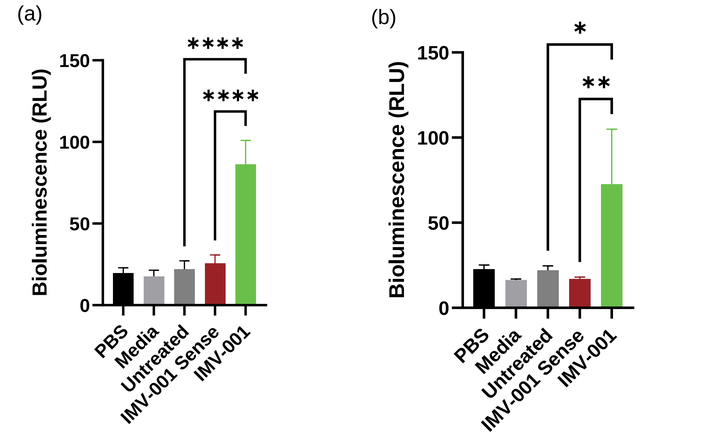



**Fig 5. GL261 extracellular ATP release as a measure of IGV-001 immunogenicity at (a) 16h and (b) 48h**




## CONCLUSIONS

- **IGV-001 educates the immune system and generates strong anti-tumor responses in the GL261 glioblastoma model, supporting clinical findings [1].**
- **The ATP results suggest a possible immunogenic conversion by which IGV-001 could stimulate the immune system.**
- **Th1 anti-tumor T cell activity was demonstrated via IFNγ response against tumor-derived peptide antigens.**
- **Circulating IL-6 correlates with IGV-001 effectiveness and tumor burden.**