Autologous tumor cell immunotherapeutic platform induces stress-correlated immunogenic cell death leading to immune activation within the draining lymph nodes

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BACKGROUND

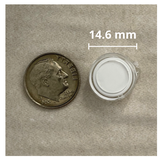
Imvax, Inc. is developing a novel personalized immunotherapeutic platform combining irradiated patient-derived tumor cells and insulin-like growth factor type-1 receptor antisense oligonucleotide (IMV-001) in biodiffusion chambers (BDCs; 0.1-micron pores). The glioblastoma (GBM) drug-device combination product, IGV-001, was evaluated in a newly diagnosed GBM phase 1b clinical trial[1]. Median overall survival of highest exposure IGV-001-treated 'Stupp-eligible'[2] patients (n=10) was 38.2 months compared with 16.2 months in recent standard-of-care-treated patients (P=0.044)[1] [NCT02507583]. Imvax also reported anti-tumor activity of IGV-001 in the GL261-Luc intracranial GBM murine model[3]. Here, we show that *m*IGV-001 (prepared with murine GL261 cells) is associated with activation of stress-related pathways and the release of immunogenic cell death (ICD)[4] molecules capable of stimulating myeloid and T cell subsets with potential anti-tumor activity in the draining lymph nodes (dLNs) proximal to the BDC.

METHODOLOGY

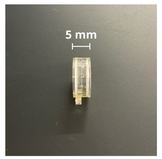
*m*IGV-001 was incubated at 37°C and 5% CO_2 for 48 h as shown in **Figure 1**. BDC contents were analyzed for extracellular ATP (eATP) using a luciferase bioluminescent system and high mobility group box 1 (HMGB1) protein via ELISA, as indicators of ICD. Reactive oxygen species (ROS) accumulation was determined by flow cytometry as well as cell viability via Annexin V and 7-AAD analysis. Changes in stress-related apoptotic signaling pathways were analyzed by immunoblotting. dLNs from mice receiving *m*IGV-001 and s.c. tumor-challenge with GL261-luc cells were isolated for immunophenotyping.

FIGURE 1

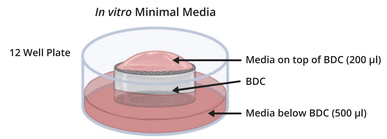
Front View



Side View



In vitro BDC Culture Assay

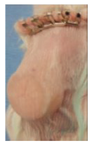


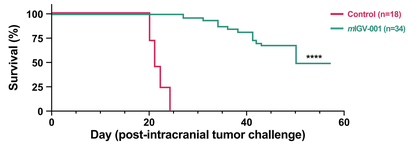
RESULTS

- *m*IGV-001 treated mice exhibit substantially longer median survival time compared to control animals in the GL261-Luc intracranial GBM model (fig. 2).
- Phenotyping analyses showed increased CD45+CD11b+CD11c+MHCII+ DCs in the dLNs compared to the contralateral control site without BDC. Likewise, the percentage of effector and effector memory in CD4+ and CD8+ T cells was also significantly higher in the dLNs (fig. 3), as were CD8+TIM3+, CD8+PD1+, and CD4+PD1+ T cells (data not shown).
- Cells in *m*IGV-001 released eATP after formulation and eATP was detectable within the BDCs over 48 h (fig. 4).
- HMGB1 was released from dying tumor cells in *m*IGV-001 (fig. 4), while surface calreticulin was undetectable (data not shown).
- Similar levels of cell death and HMGB1 release were observed *in vivo* and *in vitro* (fig. 4).
- Immunoblot analysis showed induction of the integrated stress response (ISR) pathway via eif2α activation and upregulation of the ATF4-CHOP axis (fig. 5).
- ROS levels were elevated after 24 h with subsequent activation of the JNK pathway, downregulation of the anti-apoptotic marker BCL-2, and increased activity of the apoptotic effector caspase-3/7 (fig. 5).

FIGURE 2

*m*IGV-001 extends the survival of GBM-bearing mice in the GL261-Luc intracranial GBM murine model

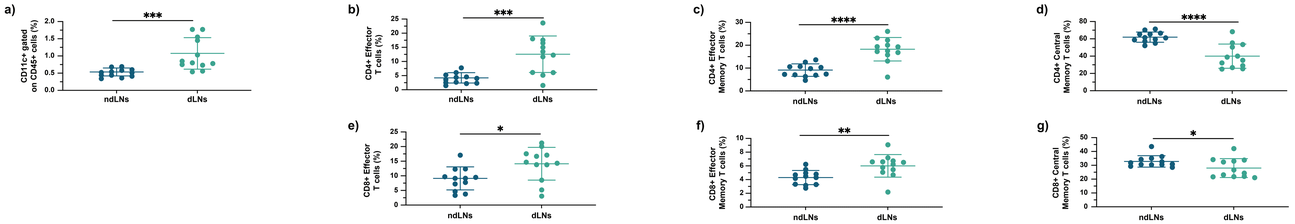




Mice were treated with *m*IGV-001 BDC or PBS-loaded BDC (i.e., Control BDC) for 48 h prior to explantation. Mice received tumor challenge 28 days later and monitored for disease progression and survival for a further 60 days. **a)** Kaplan-Meier survival curves, data combined from 2 separate experiments. Log-Rank test, **** P<0.0001.

FIGURE 3

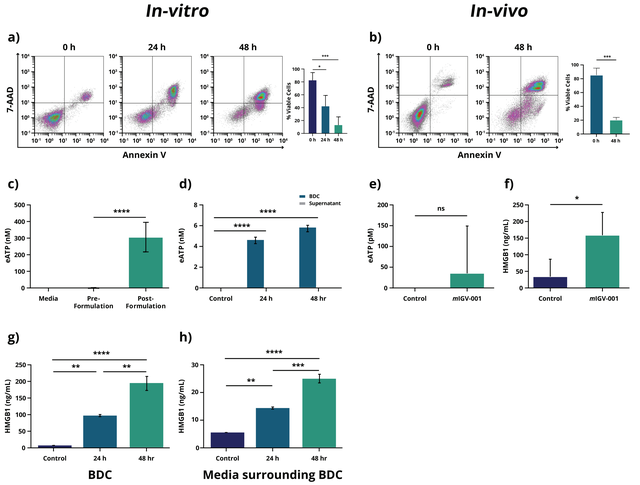
*m*IGV-001 promotes the expansion of myeloid and T cell subsets in the dLNs proximal to the DBC



BDC's dLN and non-draining LN (ndLN) were isolated from 12 mice and analyzed by flow cytometric analysis to compare the distribution of myeloid and T cell subsets. **a)** Percentage of CD11c+ cells. **b)** Percentage of CD4+CD62L-CD44- effector T cells. **c)** Percentage of CD4+CD62L-CD44+ effector memory T cells. **d)** CD4+CD62L+CD44+ central memory T cells. **e)** Percentage of CD8+CD62L-CD44- effector T cells. **f)** Percentage of CD8+CD62L-CD44+ effector memory T cells. **g)** Percentage of CD8+CD62L+CD44+ central memory T cells. Statistical analysis was performed using unpaired t-test, **** P<0.0001, *** P<0.001, ** P=0.0061, * P<0.05.

FIGURE 4

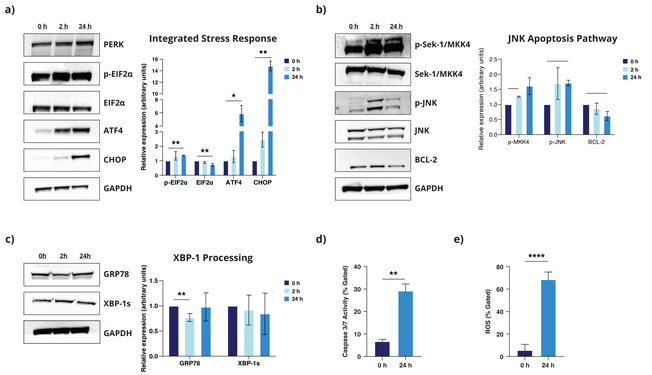
Cell death in, and DAMP release from, *m*IGV-001



Cell viability in BDCs cultured **a)** *in vitro* or **b)** from *in vivo* explanted BDCs. eATP measured **c)** pre- and post-formulation, **d)** in BDC supernatants and released into the culture medium over time *in vitro*, and **e)** within *in vivo* explanted BDC supernatant. HMGB1 measured **f)** in explanted BDC supernatant or **g-h)** HMGB1 measured in BDCs cultured *in vitro* and released into the culture medium over time. Data is representative of 2-3 independent *in vitro* experiments and 3-12 mice for *in vivo* data. Control = PBS-filled BDC. *In vivo* HMGB1 data was normalized to surgical controls. Statistical analysis was performed using One-way Anova with multiple comparisons or unpaired t-test, * P<0.05, ** P<0.01, *** P<0.001, **** P<0.0001.

FIGURE 5

Cell death in cultured *m*IGV-001 involves integrated stress response induction and ROS related apoptosis



Western blot analyses of *m*IGV-001 showing **a)** ISR pathway, **b)** JNK-apoptosis, and **c)** GRP78 and XBP-1s expression as surrogates for non-ISR ER stress pathways. **d)** Caspase-3/7 activity in *m*IGV-001. **e)** ROS accumulation in *m*IGV-001. 0 h time point = immediately after formulation. Data is representative of 2-3 independent experiments. Statistical analysis was performed using either a standard unpaired or Welch's corrected t-test, * P<0.05, ** P<0.01, **** P<0.0001.

CONCLUSIONS

- Death in *m*IGV-001 cells correlated both *in vitro* and *in vivo* with the detection of ICD damage-associated molecular patterns (eATP and HMGB1).
- The *m*IGV-001 mechanism of action involves stress-mediated activation of pathways consistent with apoptosis, and induced immune responses specifically detected in the dLNs vs non-draining nodes.
- These data suggest a potential mechanism of action of IGV-001 in GBM via ICD stimulation of an antitumor immune response.

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