Background

Imvax 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 (BDC; 0.1-micron pores). The combination product IMV-001 was recently evaluated in a newly diagnosed glioblastoma (GBM) phase 1b clinical trial. Median overall survival of highest exposure IMV-001-treated ‘Stupp-eligible’ patients (n=10) was 38.2 months compared with 16.2 months in recent standard-of-care-treated patients (P=0.044). Imvax also reported anti-tumor activity of IMV-001 in the GL261-luc GBM murine model. Here, we show that IMV-001 is associated with activation of stress-related pathways and the release of immunogenic cell death (ICD) molecules capable of stimulating myeloid and T cell subsets with potential anti-tumor activity in the draining lymph nodes (DLN) proximal to the BDCs.

Methods

GL261-IGV-001 was formulated and BDCs were incubated at 37°C and 5% CO2 for 48h. Supernatants were analyzed for extracellular ATP (eATP) and high mobility group box 1 (HMGB1) protein as indicators of ICD, along with flow cytometric analysis of viability, surface calreticulin exposure, and reactive oxygen species (ROS). Stress-related pathways were analyzed by immunoblotting. DLN from mice receiving GL261-IGV-001 and s.c. tumor-challenge were isolated for immunophenotyping.

Results

GL261-IGV-001 cells in BDCs showed significant increase in cell death in vitro (>80%, P<0.001) after 48h incubation. eATP was present after cell preparation. HMGB1 was present in BDCs containing dying cells, while surface calreticulin was undetectable. Immunoblot analysis showed induction of the integrated stress response pathway via eif2α activation and upregulation of the ATF4-CHOP axis. ROS levels were elevated after 24h with subsequent activation of the JNK pathway and downregulation of the anti-apoptotic marker BCL-2. Importantly, similar levels of cell death were observed in vivo implanted BDCs. Phenotyping analyses showed increased CD45+CD11b+CD11c+MHCI+ DCs in the DLN compared to the contralateral control site without BDC (P<0.001). Likewise, the percentage of effector (P<0.001) and effector memory (P<0.0001) in CD4+ T cells and effector memory CD8+ T cells (P=0.006) were also higher in the DLNs, as were CD8+Tim3+, CD8+PD1+, and CD4+PD1+ T cells (P=0.03, P=0.0024, P<0.0001, respectively).

Conclusions

Cell death in IMV-001 correlated with the detection of ICD damage-associated molecular patterns (eATP and HMGB1), was characterized by stress-mediated activation of apoptosis pathways, and induced immune responses detected in the DLNs. These data suggest a potential mechanism of action of IMV-001 in GBM via ICD.

REFERENCES


