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Broad applicability of the Goldspire™ platform for the treatment of solid tumors

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ABSTRACT

Goldspire™ is a personalized immunotherapy platform that combines whole tumor-derived cells with antisense oligonucleotide (IMV-001) against Insulin-Like Growth Factor-1 Receptor (IGF-1R) in biodiffusion chambers (BDCs; 0.1 μm pore). BDCs are exposed to 5–6 Gy and implanted at abdominal sites for ~48 h to deliver an antigenic payload and immunostimulatory factors to train the immune system. Lead product IGV-001 was evaluated in newly diagnosed glioblastoma (ndGBM) patients in Phase 1a and 1b trials (NCT02507583). A Phase 2b study (NCT04485949) recently completed enrollment.

Preventative treatment with tumor-specific products manufactured with Goldspire limited tumor progression and extended overall survival in mice challenged with bladder, pancreatic, ovarian, colorectal, or renal carcinomas. The benefit of this immunotherapy was enhanced with anti-PD-1; combination treatment was superior to either monotherapy in orthotopic GBM and melanoma models. Lastly, Goldspire elicited immune T cell activation and memory phenotypes against patient-derived endometrial tumor-derived products in co-cultures with matching immune cells.

1. Introduction

Conventional therapies for the treatment of solid cancers, including surgical resection, radiotherapy, and chemotherapy, are limited by their side effects and toxicities. The advent of immune checkpoint inhibitors (ICIs), has shown that engaging the immune system can be key to the successful treatment of many cancers. Detailed analysis of T cell responses in those patients that respond to ICIs has revealed therapeutically active tumor-reactive T cells that are sufficient to initiate tumor rejection. Under this umbrella, cancer vaccines have re-emerged as a potential approach to re-educate the patients' immune system towards recognition and attack of tumor antigens [1,2].

Goldspire™ is a personalized immunotherapy platform for the treatment of solid cancers that combines whole tumor-derived cells with an antisense oligonucleotide against insulin-like growth factor 1 receptor (IGF-1R; IMV-001) in proprietary biodiffusion chambers (BDCs; 0.1 μm pore). For patients, the BDCs are irradiated (5–6 Gy) and implanted at abdominal sites for ~48 h to deliver an antigenic payload and immunostimulatory factors that together can induce anti-tumor immune responses. The mechanism of action of Goldspire has been described in detail in the context of IGV-001 [3], the lead product of the platform. IGV-001 was evaluated and showed signals of efficacy in a Phase 1b clinical trial for the treatment of newly diagnosed glioblastoma (ndGBM) [4].

Using both murine and human GBM cell lines, as well as patient-derived GBM cells, Cultrara, et al. [3], demonstrated that the tumor cells within IGV-001 rapidly (<48 h) undergo oxidative and endoplasmic reticulum stress, resulting in their regulated cell death and the release of antigenic material and immunogenic signals, including ATP and High mobility group box 1 protein (HMGB1), from BDCs in support of tumor-targeting immunity. Goldspire is advantageous in that it comprises an unselected population of millions of GBM cancer cells, thereby including a broad antigenic signature of each tumor. Moreover, the BDC pore size allows for the selective release of relatively small subcellular tumor particles, which are known to be taken up efficiently by immunostimulatory dendritic cells (DCs) [5,6], while preventing

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contact between dying cancer cells and macrophages, which generally promote immunosuppression [7,8]. Lastly and also key, the product (i.e., IGV-001) is implanted [4,9] at an anatomical distance from the immunosuppressive GBM tumor microenvironment (TME) and its draining lymph nodes [10,11]. Similarly to cancer vaccines, the Goldspire platform approach has been designed with the intention to engage the immune system, but it does so without the need for predefined tumor antigens [12]. While the effects of reactive oxygen species in anti-tumor immunity can be pleiotropic [13,14], Goldspire harnesses oxidative and other cell stressors as the means to generate a comprehensive tumor-specific antigenic signature, which is delivered away from the constrains of the TME.

A Phase 2b randomized, multicenter, double-blind, placebo-controlled study to assess the safety and efficacy of IGV-001 in patients with ndGBM (NCT04485949) has completed enrollment and results are expected to be available in 2025 [9]. Using various orthotopic and metastatic-like murine cancer models along with in vitro systems with clinical samples, here we provide evidence of the broad applicability of the Goldspire™ platform for the treatment of solid tumors.

2. Materials and methods

2.1. In vivo murine models

In vivo murine studies were designed by Imvax, Inc., and executed by contract research organizations in compliance with the National Institutes of Health and the Animal Care and Use Committee at Accreditation of Laboratory Animal Care International (AAALAC)-accredited animal facilities, under an Institutional Animal Care and Use Committee (IACUC)-approved protocol. The MBT-2 orthotopic model (urothelial cancer) was conducted by Translational Drug Development (Scottsdale, AZ). The PAN02 orthotopic model (pancreatic cancer) was conducted by Charles River Laboratories Discovery Research Services (Morrisville, NC). The GL261-luc2 (GL261) orthotopic model (GBM) and the ID8-luc-mCh-puro (ID8) intraperitoneal (i.p.) model (ovarian cancer) were conducted by Labcorp Drug Development (Ann Arbor, MI). The CT26 and RENCA subcutaneous (s.c.). models (colorectal and renal carcinoma cancers, respectively) were conducted by Champions Oncology (Hackensack, NJ) and the melanoma (Clone M3) orthotopic model (mammary fat pad challenge) was performed by Reaction Biology (Freiburg, Germany).

Mice (strains detailed in figure legends) received either BDCs filled with phosphate buffered saline (PBS) (control mice) or tumor-specific Goldspire products (1×10^6 cells/BDC, one BDC/mouse). BDCs were subcutaneously implanted in the flank for 48 h, followed by explantation and wound closing. For some experimental models (pancreatic, colorectal and renal cell carcinoma cancers), due to limited availability of clinical-grade BDCs, control groups received mock surgery. On day 26 after BDC explantation, mice received tumor challenges, as described in figure legends. Where applicable, tumor burden was evaluated using weekly bioluminescent imaging (BLI) of injected D-luciferin conversion with an IVIS Spectrum imager (Perkin-Elmer, Missouri, USA) or visible tumor caliper measurement. Mice were euthanized before study termination when ethical abortion criteria were reached (supplementary Fig. 1A). Termination guidelines determined by the sites conducting the studies included excessive weight loss, site ulceration, and mean tumor volume of the control group (i.e., Fig. 2), such that the mean tumor volume of the control group reached 1500 mm^3. If this occurred before Day 49 (= 21 days post-tumor challenge), treatment groups and individual mice were measured up to Day 49. If the mean tumor volume of the control group did not reach 1500 mm^3 by Day 49, then the endpoint for all animals was determined as the day when the mean tumor volume of the control group reached 1500mm^3, up to a maximum of Day 60 (=Day 32 post-tumor challenge).

2.2. In vitro co-culture model

Clinical biospecimens were collected by a commercial source (Biotheme Research Solutions; Plantation, FL) with patient consent and shipped in normal saline in temperature-controlled (2–8 °C) containers (tumor samples) or at room temperature (peripheral blood mononuclear cells; PBMC). Dissociated patient-derived tumors and PBMC samples were utilized in a co-culture assay to monitor immune cell response to endometrial IEC-001 biologic product manufactured following published protocols [3]. PBMC were isolated from the patient's blood using Ficoll separation. Co-cultures of isolated PBMC and IEC-001 were established indirectly utilizing 12-well Transwell® insert technology to mimic the BDC membrane separating IEC-001 from the surrounding tissue and cells, as per clinical implantation protocol. The assays were established at a 1:10 ratio of IEC-001 biologic product (150,000) to PBMC (1,500,000). On day 21, co-cultures received a tumor re-challenge where untreated tumor cells (150,000) were added to the wells to restimulate the cultures. Co-cultures were maintained for 28 days, after which PBMC were sampled to quantify levels of T cell activation and T cell memory response. Quantification of PBMC responses to IEC-001 was achieved using various flow cytometry panels containing pertinent extracellular markers for each stage of the immune response investigated. Markers for PBMC activation included: CD4, CD8, CD25, CD69, CD137, and CD107a. Markers for PBMC memory responses included: CD4, CD8, CD197, and CD45RA, with the central memory population defined as $CD197^{+}CD45RA^{-}$ and the effector memory population defined as $CD197^{-}CD45RA^{-}$. Culture media was exchanged 2× per week. A low dose of 5 IU/mL of hIL-2 was added to the media for T cell maintenance. Flow cytometry data was collected utilizing a Cytoflex cytometer (Beckman Coulter) and all flow file analysis was performed using Kaluza software (Beckman Coulter).

2.3. Statistical analyses

Statistical analyses were performed in GraphPad Prism (V.9.4). Survival studies were analyzed using the log- rank test. A $p < 0.05$ was considered statistically significant.

3. Results

3.1. Tumor-specific Goldspire products elicit control of orthotopic urothelial, pancreatic, and i.p. ovarian cancers in murine models

To assess the ability of various tumor-specific Goldspire products at eliciting anti-tumor immunity in orthotopic tumor models, we manufactured mouse (*m*-) versions of the clinical product IGV-001 based on MBT-2 (urothelial), PAN02 (pancreatic), and ID-8 (ovarian) cancer cells and used them in immunogenic cell death (ICD) gold-standard preventative vaccination assays [15]. Specifically, *m*IUC-001, *m*IPC-001, *m*IOC-001 or controls PBS-loaded BDCs were implanted s.c. in the flank of immunocompetent C3H (MBT-2 model) or C57BL/6 (ID-8) mice, left in place for 48 h and then explanted. Twenty-six days later (supplementary Fig. 1A), mice were challenged orthotopically with the respective tumor cell line. Mock surgery was performed in C57BL/6 control mice for the PAN02 model due to a lack of clinical-grade BDCs at the time of experimental execution.

In the MBT-2 urothelial cancer model, the control group had a median overall survival (OS) of 21 days versus 33 days in *m*IUC-001-exposed mice, which also had a 42 % long term-survival group (Fig. 1A) suggesting the induction of protective urothelial cancer-targeting immunity. This observation was further supported by the improvement in hunch postured (Fig. 1B) and a trend in bladder weight reduction experienced by vaccinated mice (Fig. 1C) suggesting better tumor control. Although tumor size was not directly evaluated, a similar, yet more moderate, activity was demonstrated in the PAN02 pancreatic cancer model where the mock surgery group had a median OS of 30 days versus

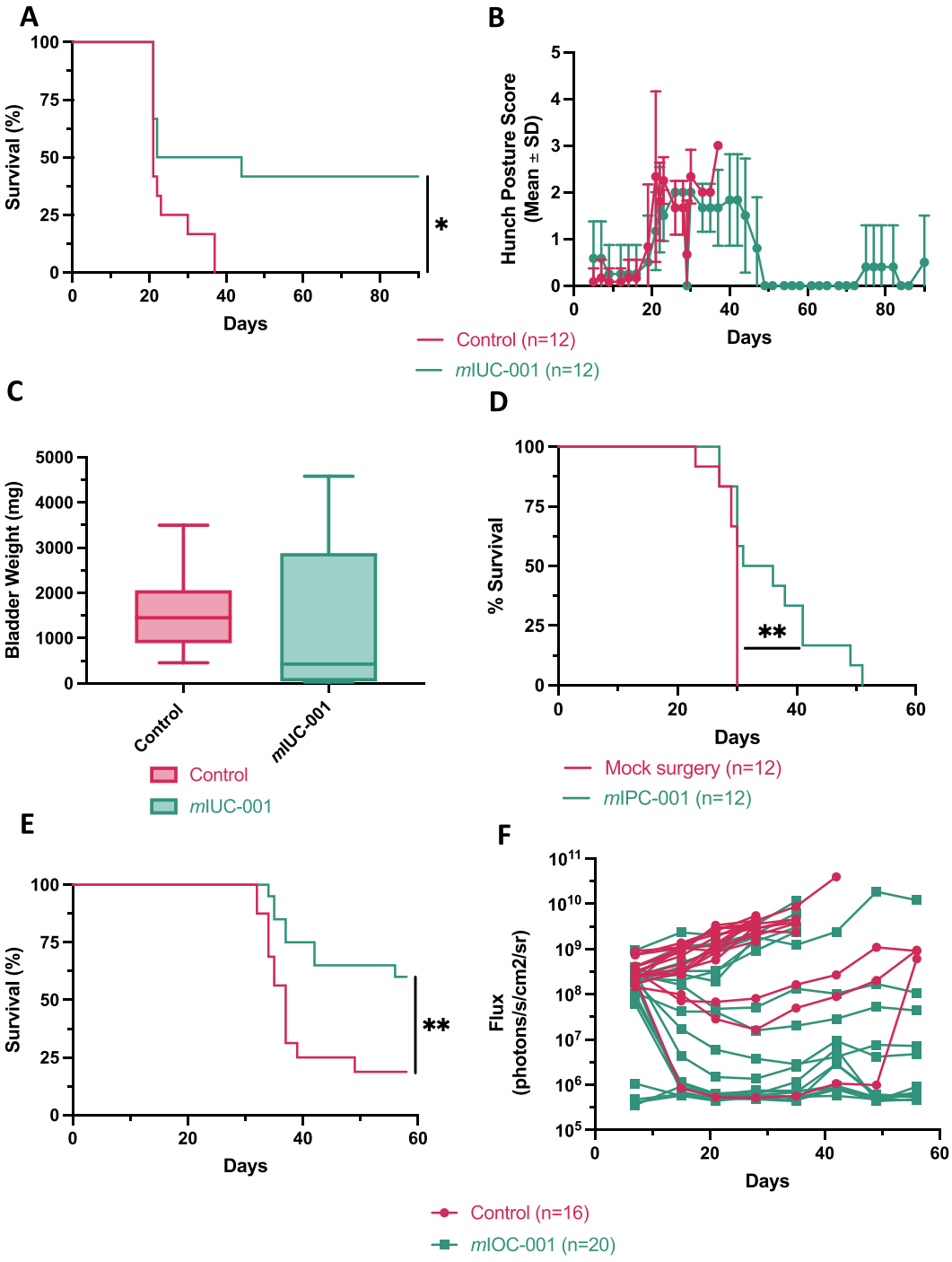

Fig. 1. Mice receiving preventative treatment with products manufactured using the Goldspire platform experienced significantly longer survival after orthotopic (urothelial and pancreatic cancers) or i.p. (ovarian cancer) tumor challenges.

BDCs were manufactured with 1×10^6 tumor cells/BDC or PBS (i.e., control). BDCs were implanted in the flank and left in place for 48 h. Mock surgery was used in cases where clinical-grade BDCs were not available for the studies. Tumor challenges were performed 26 days after BDC explantation or mock surgery. (A) Kaplan-Meier survival curves of C3H/He female mice orthotopically challenged with MBT-2 bladder cancer cells. Median overall survival for control mice = 21 days versus 33 for mice receiving *m*IUC-001. Log-rank test HR of control to *m*IUC-001 = 4.071 (95 % CI 1.3 to 12.8) *$p = 0.0163$. (B) Hunch posture score (range 0–5) of mice pretreated with *m*IUC-001 or PBS-loaded BDCs. (C) Bladder weight at study exit or termination.

(D) Kaplan-Meier survival curves of C57/Bl6 female mice orthotopically challenged with PAN02 pancreatic cancer cells. Median overall survival for control mice subjected to mock surgery = 30 days versus 33.5 for mice receiving *m*IPC-001. Log-rank test HR of control to *m*IPC-001 = 2.1 (95 % CI 0.9 to 4.9) *$p = 0.0086$. (E) Kaplan-Meier survival curves of C57BL/6 albino female mice i.p. challenged with ID8-luc-mCh-puro ovarian cancer cells. Median overall survival for control mice = 37 days versus undefined for mice receiving *m*IOC-001. Log-rank test HR of control to *m*IOC-001 = 3.13 (95 % CI 1.27 to 7.7) *$p = 0.0041$. (F) Individual BLI curves for mice pretreated with *m*IOC-001 or PBS-loaded BDCs. Days = days post-tumor challenge. n = number of mice. HR = hazard ratio.

33.5 days in *m*PC-001 pre-treated mice (Fig. 1D). Lastly, mice receiving *m*IOC-001 had unreached median OS compared to their respective control group (i.e., 37 days; Fig. 1E). Individual bioluminescence images corroborated the development of progressive ovarian cancer in 87.5 % of PBS-loaded BDCs group versus only 35 % of *m*IOC-receiving mice (Fig. 1F). All control animals developed increasing tumor burden, the large majority within a month, the remaining animals later while on study; most treated mice showed baseline or minimal stable controlled tumor during the two-month follow-up (Fig. 1F).

3.2. *Tumor-specific Goldspire products delay progression of s.c. colorectal and renal cell carcinoma cancers in murine models*

The colorectal *m*ICC-001 (Fig. 2A,B) and renal cell carcinoma *m*IRC-001 (Fig. 2C,D) products were prepared with CT-26 and RENCA cells, respectively, and administered as described above. Twenty-six days later, mice were challenged s.c. with the corresponding tumor cell line. In both models, mice vaccinated with tumor-specific Goldspire products exhibited greater tumor control and outperformed their counterparts (i.e., mock surgery-receiving). Statistical analyses are summarized in Table 1, Fig. 2E.

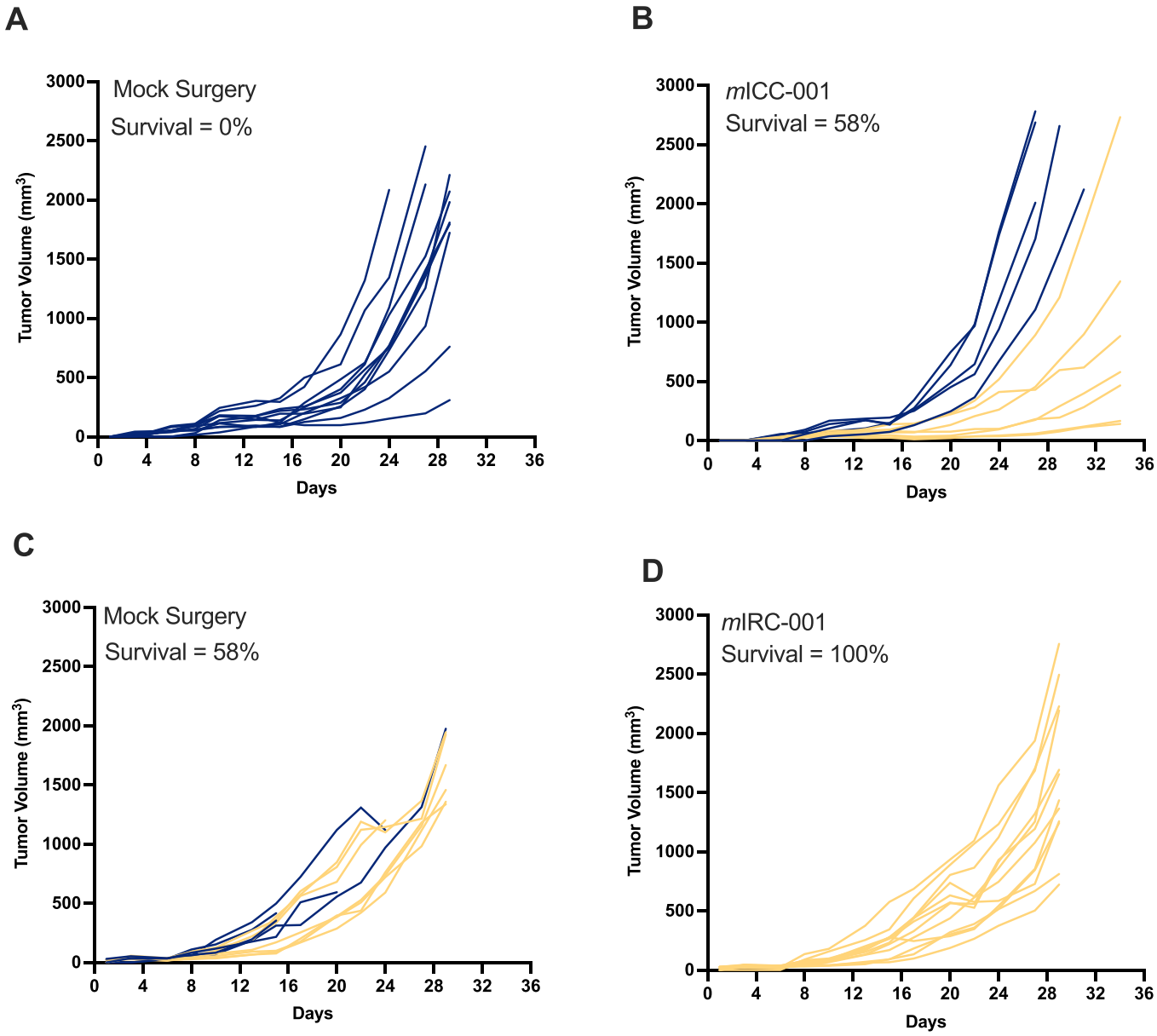

Fig. 2. Mice receiving preventative treatment with products manufactured using the Goldspire platform experienced significantly better tumor control after s.c. tumor challenges of either colorectal or renal carcinoma.
Tumor volume in BALB/c mice given s.c. challenge with CT26 colorectal cancer cells: (A) control mice. (B) *m*ICC-001 treated mice. Tumor volume in BALB/c mice receiving s.c. challenge with RENCA renal adenocarcinoma cells: (C) control mice. (D) *m*IRC-001 treated mice. (E) Summary of statistical evaluations for overall survival comparisons. Survival was taken as the number of days until no tumor volume measurement. When all tumor volume measurements were completed, the animal was censored. When the tumor volume measurements ended prior to that maximum time, then it was uncensored. A 2-sided log-rank test was used to calculate survival. Blue lines = tumor volume in mice that succumbed to tumor, yellow lines = tumor volume in mice that survived until study termination (30 days post tumor-challenge). Days = days post-tumor challenge. n = number of mice.

Table 1
Fig. 2E.

Tumor Type	Control		Treatment		
	% Survival	Dead/ total	% Survival	Dead/ Total	*p* value
Colorectal carcinoma (CT26)	0	11/11	58	5/12	0.021
Renal cell carcinoma (RENCA)	58	5/12	100	0/12	0.014

3.3. Tumor-specific Goldspire products in combination with anti-PD-1 treatment augment GBM and melanoma control in murine models

We have shown that while neither *m*IGV-001 nor a PD-1 blocker alone significantly delayed progression in mice with established GBM, their combination exhibited significant activity [3]. In this study, we tested the addition of anti-PD-1 to tumor-specific Goldspire products used preventatively in GBM and additionally, in melanoma. *m*IGV-001 (prepared with murine GBM GL261-luciferase expressing cells) and *m*IMC-001 (prepared with murine melanoma Clone M3 cells) or PBS-loaded BDCs were administered as described above and 26 days later, mice were challenged with the corresponding tumor cell line. GL261 cells were implanted in the brain, and Clone M3 cells were injected in the mammary fat pad. Three days after tumor challenge, mice in the control (PBS-loaded BDCs) or treated groups received four doses (3–4 days apart) of either anti-PD-1 or isotype mAb, as annotated in the figure legend (Fig. 3). The results of these therapy combination experiments show that mice receiving either *m*IGV-001 (Fig. 3A,B) or *m*IMC-001 (Fig. 3C,D) in combination with anti-PD-1 blockade experienced greater survival and greater tumor control compared to either monotherapy or controls. Long-term survivors in all groups reached baseline tumor levels with no evidence of disease up to 100 days studied (Fig. 3B,D).

3.4. IEC-001 induce dendritic cell maturation and T cell activation and memory phenotypes in in vitro cultures

To quantify immune cell response after exposure to endometrial, patient-specific IEC-001 Goldspire products, an in vitro assay was established using manufactured biological product and patient-matched peripheral blood mononuclear cells (PBMC). This approach is particularly useful to test the efficacy of the Goldspire platform in this cancer type since, to the best of our knowledge, there are no readily available syngeneic orthotopic murine models of endometrial cancer. A schematic of the experimental procedure can be found in supplementary Fig. 1B. Specifically, Dendritic Cell (DC) maturation ($CD11c^{+}HLDR^{+}$), T cell activation (based on surface expression of CD25, CD69, CD107a, and CD137 [also known as 4-1BB] on $CD4^{+}$ and $CD8^{+}$ subsets), and T cell memory (central memory; CM = $CD197^{+}CD45RA^{-}$ and effector memory; EM = $CD197^{-}CD45RA^{-}$) populations were evaluated at different timepoints (days 3, 7, 14, 28) using flow cytometry. Direct co-culture with IMV-001-treated cells was used as positive control. A Transwell® with an identical membrane porosity of 0.1 μm was used to mimic the BDC in in vitro culture settings. In total, matched tumor-PBMC co-cultures from 6 different patients were analyzed.

In panels 4 A-E, each group of 3 bars represents results from an individual patient, 3 representative patients / graph. DC maturation to varying levels was observed by day 3 (Fig. 4A). This was followed by similar increases in T cell activation on days 7 and 14 in both CD4 (Fig. 4E) and CD8 (Fig. 4B-D) T cell subsets. On day 28, 7 days after re-stimulation with freshly thawed tumor cells on day 21, the depicted PBMC patient co-culture (Fig. 4F) underwent upregulation of activation and memory T cell markers, as shown. Of note, DC maturation, T cell activation and memory generation were most notable on experimental setups with Transwell® membrane exclusion for these patients. Gating examples can be found in supplementary Figs. 2–4.

4. Discussion

Despite major advances in the treatment of solid cancers, a large number of patients are still confronted with poor survival outcomes and limited effective treatment options [16,17]. Advances in the field of immunotherapy, particularly with checkpoint inhibitors, have increased the treatment options for these patients, but further strategies must be considered to maximize the efficacy of therapy while controlling or reducing toxicities [18]. In many cases, it is still unclear why only some patients exhibit a clinical response to immunotherapeutic treatments [19], and the immunological mechanisms by which clinically active immunotherapies, including adoptive cell transfer and immune checkpoint inhibitors work, are just emerging [20]. Unlike most other immunotherapies, cancer vaccines typically have minimal side effects, as they rely on the selection of highly immunogenic tumor antigens that are primarily expressed by cancer cells [21,22]. Compared to approaches that use tumor associated antigens, tumor-specific antigens or patient-specific neoantigens to induce tumor-reactive T cells, whole tumor-derived vaccines have the benefit of being truly antigen-inclusive. They most often utilize sizable amounts of resected tumor material, rather than small biopsies, avoiding exclusion of relevant antigens due to tumor heterogeneity or sampling error. They do not depend on empirical antigenic peptide prediction and include post-translationally modified antigens, which can be important drivers of tumor growth but are not encoded in mutations and therefore are not covered by neoantigen-based approaches [11,23]. Goldspire combines whole tumor-derived autologous cells and IMV-001, an IGF1R-directed antisense molecule. The delivery of cellular debris and co-stimulatory molecules through a biodiffusion device with a size exclusion of 0.1 μm makes this the first immunotherapy of its kind and capable of inducing anti-tumor responses via ICD [3,24], while potentially preventing the release and uptake of immunosuppressive cell components [8]. Zemek [1] et al. [25], noted that combination treatments that are given in a time-dependent manner to optimally exploit the interactions between individual therapies are likely to be more effective. As such, Goldspire's administration, which occurs after debulking surgery, but prior to a patient receiving immunosuppressive standard of care also sets this approach apart, because immunization can take place in a relatively intact immune system.

The immunogenicity of IGV-001, the first product of the Goldspire platform for the treatment of ndGBM, has been previously evaluated in murine and in vitro models of GBM [3]. These studies showed that *m*IGV-001 was tolerated and efficacious, mirroring positive signals obtained in a Phase 1b clinical study where the median OS of highest exposure IGV-001-treated Stupp-eligible patients [26] ($n = 10$) was 38.2 mos compared with 16.2 mos in standard-of-care-treated patients ($p = 0.044$; NCT02507583) [4]. The benefits of Goldspire in a murine model of hepatocellular carcinoma, the mechanism of action of Goldspire, and its immunobiology have also been described [3,11]. The findings reported herein, extend previous results and demonstrate that this immunotherapy is conducive for the generation of anti-tumor immunity and extension of OS in mice challenged with various types of solid cancers. Importantly, our findings agree with outcomes from recent clinical trials showing that immune checkpoint blockade enhances the beneficial effects of therapeutic cancer vaccines [27,28]. One of the caveats of this study is its predominant reliance on preventative rather than therapeutic experimental settings. The preventative setting developed here does recapitulate the clinical scenario where a patient's immune system has been exposed to tumor, which is then resected, prior to treatment. Likewise, the mice received product containing tumor cells, hence introducing their immune system to the cancer's antigenic signature at a novel anatomical site and its associated draining lymph nodes. Additionally, our approach largely reflects our focus on the mechanism of action of Goldspire (i.e., bona fide ICD), which can primarily be discerned from regulated cell death-independent immunostimulation in preventative vaccination assays [3].

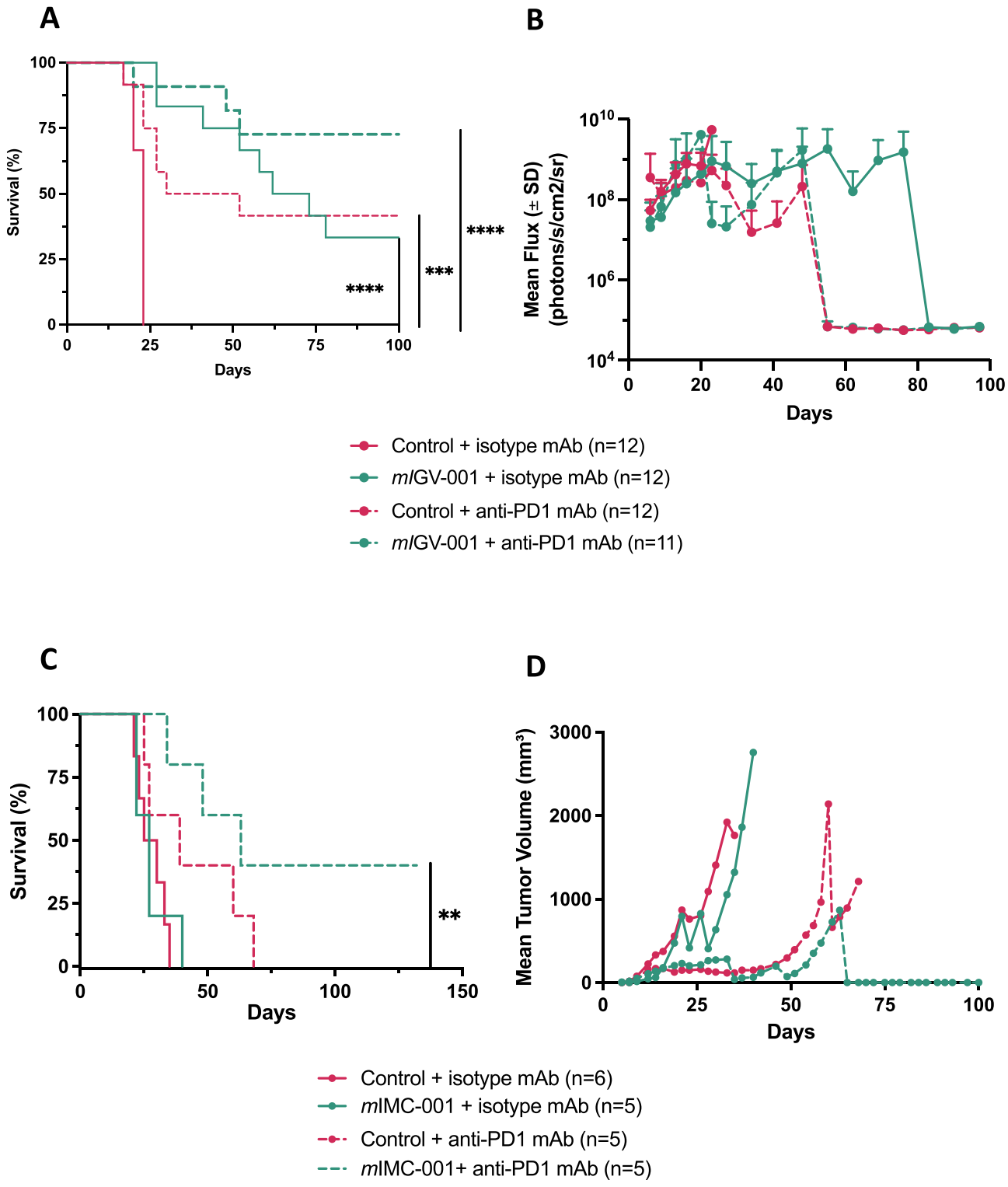

Fig. 3. Mice receiving *m*IGV-001 or *m*IMC-001 in combination with anti-PD-1 antibody experienced significantly longer overall survival after orthotopic tumor challenge.

BDCs were implanted in the flank and left in place for 48 h. Orthotopic tumor challenges were performed 26 days after BDC explantation. Four different groups were evaluated: (1) PBS-loaded BDC (i.e., control) + isotype mAb; (2) treatment (*m*IGV-001 or *m*IMC-001) + isotype mAb; (3) PBS-loaded BDC (i.e., control) + anti-PD-1 mAb; (4) treatment (*m*IGV-001 or *m*IMC-001) + anti-PD-1 mAb. (A) Kaplan-Meier survival curves of C57/BL6 albino female mice intracranially challenged with GL261-Luc2 GBM cells. Median overall survival for Group 1 = 23 days versus 67.5 for mice receiving *m*IGV-001. Log-rank test HR of control to *m*IGV-001 = 4.34 (95 % CI 1.6 to 11.8), ****$p < 0.0001$. Median overall survival for Group 1 = 23 days versus 41 for mice receiving anti-PD-1 mAb (Group 3). Log-rank test HR of Group 1 to Group 3 = 3.14 (95 % CI 1.23 to 8.04), ***$p < 0.001$. Median overall survival for Group 1 = 23 days versus unreached for mice receiving *m*IGV-001 + anti-PD-1 mAb (Group 4). Log-rank test HR of Group 1 to Group 4 = 5.87 (95 % CI 2.1 to 16.5), ****$p < 0.0001$. (B) Mean bioluminescence signal showing greatest reduction in tumor burden in mice receiving anti-PD1 mAb and combination treatment with *m*IGV-001 + anti-PD-1 mAb.

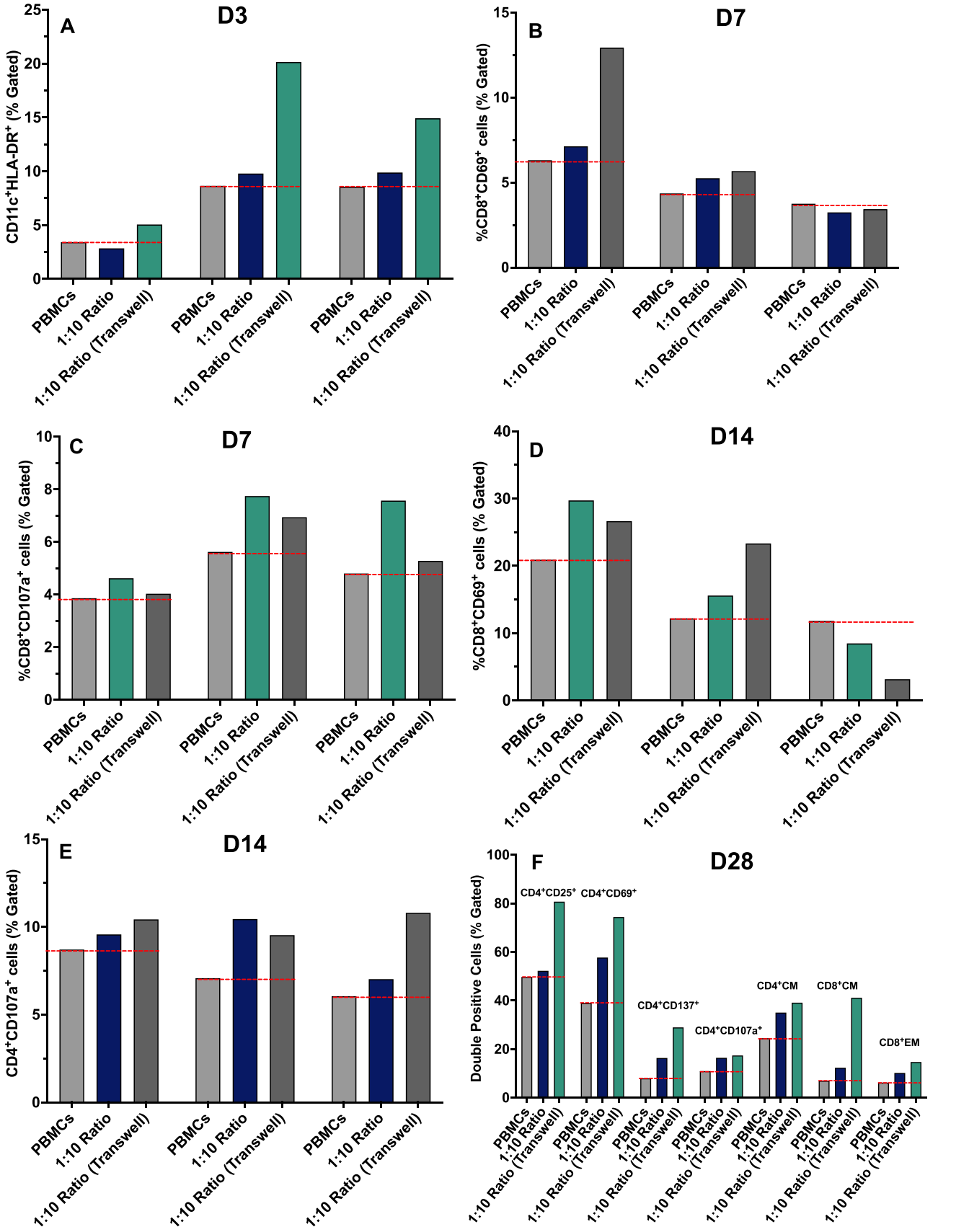

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Fig. 4. Endometrial tumor-derived IEC-001 biological product modulates adaptive immune responses in in vitro co-cultures with autologous PBMCs. Co-cultures of isolated PBMCs and IEC-001 biological product (i.e., dissociated tumor cells from endometrial cancer tumors treated with IMV-001) were established indirectly in 96-well plates using Transwell® well plate technology. The assays were established at a 1:10 ratio of IEC-001 bulk product (25,000) to PBMCs (250,000). Co-cultures were maintained for 28 days during which PBMCs were periodically sampled to quantify levels of DC maturation, T cell activation and T cell memory response. On day 21, co-cultures received tumor re-stimulation, where untreated tumor cells (25,000) were added to the wells to restimulate the cultures. Quantification of in vitro PBMC responses to IEC-001 immunotherapy was achieved using various flow cytometry panels of pertinent surface markers for each stage of the immune response investigated. PBMC samples were sampled on days 3, 7, 14, 21 & 28 (28 days = 7 days post re-stimulation conducted on day 21). Culture media was exchanged 2× per week. A low dose of 5 IU/mL of *h*IL-2 was added to the media for T cell maintenance. Representative percentages of: (A) $CD45^+CD11c^+HLA\text{-}DR^+$ DC cells; day 3 post-culture. (B) $CD8^+CD69^+$ in CD3 T cells; day 7 post-culture. (C) $CD8^+CD107a^+$ in $CD3^+$ T cells; day 7 post-culture. (D) $CD8^+CD69^+$ in $CD3^+$ T cells; day 14 post-culture. (E) $CD8^+CD107a^+$ in $CD3^+$ T cells; day 14 post-culture. (F) $CD4^+CD25^+$, $CD4^+CD69^+$, $CD4^+CD137^+$, $CD4^+CD107a^+$, $CD4^+CD197^+CD45RA^-$, $CD8^+CD197^+CD45RA^-$, and $CD4^+CD197^-CD45RA^-$ in $CD3^+$ T cells; day 28 post-culture. D3 = day 3, D7 = day7, D14 = day 14, D28 = day 28 (=7 days post-re-stimulation on day 21). CM = central memory, EM = effector memory. Six tumor-PBMC matched pairs were evaluated. Data represents results of 1–3 patient samples.

The observation that the biological portion of IEC-001 induced activation and maturation markers on monocytic and T cell subsets of PBMC co-cultured with, but notably with biodiffusion-type membrane separation, supports prior findings and corroborates that Goldspire is capable of inducing T cell activation and central and effector memory phenotypes. Due to a limited amount of sample, the in vitro studies were not run concomitantly with cytotoxic and or cytokine release assays to corroborate that the generated T cells were indeed tumor-killing subpopulations. However, we have shown that the draining lymph nodes to *m*IGV-001 contain both $CD8^+$ and effector memory $CD4^+$ T cells with increased levels of PD-1 expression and that *m*IGV-001 generates cytotoxic lymphocytes directed against GL-261-derived antigenic peptides [3]. Further correlative studies are being conducted as part of Phase 1b NCT04485949 and to continue exploring IGV-001-associated immunity in an ongoing placebo-controlled double-blinded Phase 2b study (NCT02507583). The results of these analyses are expected to shed further light into the immune mechanism and tumor characteristics associated with patient outcomes in ndGBM.

5. Conclusions

The present study expands our previous findings on the use of IGV-001 for the treatment.

of GBM and supports the use of Goldspire, an antigen-inclusive and antigen-agnostic immunotherapy, to generate anti-tumor immunity in multiple solid tumors.

(C) Kaplan-Meier survival curves of DBA/N2 female mice challenged with Clone M3 melanoma cells in the mammary fat pad. Median overall survival for Group 1 = 27.5 days versus 63 for mice receiving *m*IMC-001 + anti-PD-1 mAb. Log-rank test HR of Group 1 to Group 4 = 5.15 (95 % CI 1.2 to 22.1), $^{**}p = 0.0042$. (D) Mean tumor volume showing greatest control and reduction of tumor burden in mice receiving *m*IMC-001 + anti-PD1 mAb. Days = days post-tumor challenge. n = number of mice. mAb = monoclonal antibody. HR = hazard ratio.

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Credit authorship contribution statement

Jenny Zilberberg: Writing – review & editing, Writing – original draft, Project administration, Investigation, Formal analysis, Conceptualization. **Christopher Uhl:** In vitro experiments. Data curation. **Charles B. Scott:** Formal statistical analysis. **Charles B. Scott:** Formal analysis. **David W. Andrews:** Writing – review & editing, Funding acquisition. **Mark A. Exley:** Writing – review & editing, Funding acquisition, Conceptualization.

Declaration of competing interest

JZ, CU, DWA and MAE are/were employed by Imvax, Inc. CBS is a paid consultant of Imvax, Inc.

Data availability

Data will be made available on request.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.clim.2024.110373.

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