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Phase Ib Clinical Trial of IGV-001 for Patients with Newly Diagnosed Glioblastoma

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ABSTRACT

Purpose: Despite standard of care (SOC) established by Stupp, glioblastoma remains a uniformly poor prognosis. We evaluated IGV-001, which combines autologous glioblastoma tumor cells and an antisense oligonucleotide against IGF type 1 receptor (IMV-001), in newly diagnosed glioblastoma.

Patients and Methods: This open-label protocol was approved by the Institutional Review Board at Thomas Jefferson University. Tumor cells collected during resection were treated *ex vivo* with IMV-001, encapsulated in biodiffusion chambers with additional IMV-001, irradiated, then implanted in abdominal acceptor sites. Patients were randomized to four exposure levels, and SOC was initiated 4–6 weeks later. On the basis of clinical improvements, randomization was halted after patient 23, and subsequent patients received only the highest exposure. Safety and tumor progression were primary and secondary objectives, respectively. Time-to-event outcomes were compared with the SOC arms of published studies.

Results: Thirty-three patients were enrolled, and median follow-up was 3.1 years. Six patients had adverse events (grade ≤3) possibly related to IGV-001. Median progression-free survival (PFS) was 9.8 months in the intent-to-treat population (vs. SOC, 6.5 months; $P = 0.0003$). In IGV-001–treated patients who met Stupp-eligible criteria, PFS was 11.6 months overall ($n = 22$; $P = 0.001$) and 17.1 months at the highest exposure ($n = 10$; $P = 0.0025$). The greatest overall survival was observed in Stupp-eligible patients receiving the highest exposure (median, 38.2 months; $P = 0.044$). Stupp-eligible patients with methylated O^6-methylguanine–DNA methyltransferase promoter ($n = 10$) demonstrated median PFS of 38.4 months ($P = 0.0008$). Evidence of immune activation was noted.

Conclusions: IGV-001 was well tolerated, PFS compared favorably with SOC, and evidence suggested an immune-mediated mechanism (ClinicalTrials.gov: NCT02507583).

Introduction

Glioblastoma is classified by the World Health Organization (WHO) as a grade IV astrocytoma (1), and is the most common primary brain malignancy in adults (2). Standard of care (SOC) for suspected glioblastoma begins with maximal safe resection followed by adjuvant radiotherapy and temozolomide, and maintenance temozolomide (3). We evaluated IGV-001, an autologous cell combination product therapy, in adults with newly diagnosed glioblastoma.

IGV-001 consists of autologous glioblastoma tumor cells and an antisense oligodeoxynucleotide against IGF type 1 receptor (IGF-1R) mRNA (IMV-001), irradiated and coadministered via biodiffusion chambers implanted in the abdomen as described previously (4, 5). Together, these components stimulate immunogenic cell death and antigen release (6, 7). In addition, IMV-001 has immunostimulatory properties that are expected to stimulate antigen presentation (8, 9). When mixed with mouse glioma cells, these attributes have been shown to induce strong therapeutic antitumor immunity (8).

IGV-001 was granted Orphan Drug designation by the Office of Orphan Products Development of the FDA in October 2017. This phase Ib study builds on our previous phase Ia study (4).

Patients and Methods

Study design

Patients 18 years and older with radiographically diagnosis of unifocal, multifocal, or bihemispheric glioblastoma were enrolled. A Karnofsky Performance Status (KPS) score of at least 60 or Eastern Cooperative Oncology Group score of 1, 2, or 3 and a positive anergy panel (≥1 antigen) were also required. Delayed type hypersensitivity reaction was read 48 to 72 hours ± 4 hours later. A total of 48 to 72 hours ± 4 hours after application of common antigens (candida, mumps, tetanus, or trichophyton) to the skin, skin reactions were measured. Exclusion criteria included females who are pregnant, an active second primary malignancy under treatment, or a major concomitant medical illness including any autoimmune disorder.

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Note: Supplementary data for this article are available at Clinical Cancer Research Online (http://clincancerres.aacrjournals.org/).

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Translational Relevance

We previously noted a systemic treatment effect of a unique combination product, IGV-001, in recurrent glioblastoma. As an autologous tumor cell treatment, results were postulated to be an immune response. This follow-on phase Ib trial for newly diagnosed glioblastoma was designed to further test this hypothesis with broad entry criteria. While the primary objective remained safety, unexpected clinical and radiographic responses prompted termination of randomization and accrual only to the highest exposure cohort after patient 23. The protocol amendment also elevated analysis of clinical endpoints from exploratory objectives to clinical endpoint assessments joining the established radiographic assessments. The results of this trial reflect compelling improvement in progression-free survival (PFS) in the intent-to-treat population and for PFS, overall survival, and PFS MGMT methylated subgroups who would have met entry criteria for current phase III newly diagnosed glioblastoma trials. These clinical endpoint improvements were accompanied by striking radiographic improvements with sustained meaningful quality of life.

Trial design involved randomization to one of four IGV-001 cohorts: (A) 10 chambers/24 hours; (B) 10 chambers/48 hours; (C) 20 chambers/24 hours; and (D) 20 chambers/48 hours with accrual as outlined in **Fig. 1**.

Surgery and preparation of implantation sites were performed as described previously (4). A Myriad tissue aspirator was utilized to aspirate and comminute the tumor tissue and improve the viability of the tumor sample. After confirmation of glioblastoma, the surgeon created an abdominal acceptor site between the rectus sheath and the rectus abdominis muscle for subsequent implantation of fully formulated 1.4 cm biodiffusion chambers with a 0.1 μ (subcellular) exclusion limit. Harvested tumor cells were treated *ex vivo* with IMV-001 for 6 hours (range, 4–8) then encapsulated in 10 or 20 chambers (depending on randomization), and all cohorts included the addition of 4 μg of IMV-001 per chamber. Encapsulated cells were then irradiated with 5 Gy just prior to implantation. Chambers were implanted in the abdominal acceptor sites within 24 hours of craniotomy under conscious sedation and local anesthesia. Chambers were removed after 24 or 48 hours (depending on randomization), and the abdomen closed. Because previous experience with IMV-001 yielded a higher than expected incidence of deep vein thrombosis (DVT; ref. 5), prophylactic enoxaparin was administered daily for 3 months and patients were monitored for DVT by compression ultrasound twice weekly during initial hospitalization, then monthly for 3 months.

SOC (i.e., radiation and temozolomide) was initiated 4–6 weeks after surgery, lasted 6 weeks, and was followed by maintenance temozolomide (10). In patients with disease progression, investigators proceeded with best clinical judgment. These patients were included in the intent-to-treat (ITT) population but censored for progression-free survival (PFS) if anticancer therapy was initiated prior to disease progression. Four patients with disease progression prior to or during SOC treatment underwent tumor re-resection and retreatment with the combination product at the same exposure as initial treatment.

Cohort D showed the highest levels of IFNγ after therapy ($P < 0.0001$), and these patients also had the most striking clinical and radiographic improvements without an increase in adverse events (AE). As a result, the protocol was amended to stop randomization at patient 23 and assign all subsequent patients to cohort D. The amendment expanded secondary objectives beyond radiographic responses to include clinical endpoints including PFS and overall survival (OS) for the ITT population. The protocol and amendment were approved by Thomas Jefferson University (Philadelphia, PA) Institutional Review Board. All patients provided written informed consent.

Assessments

AEs and serious AEs were recorded from chamber implantation until 30 days post-study exit, for a minimum of 6 weeks after treatment. AEs were categorized and graded according to NCI Common

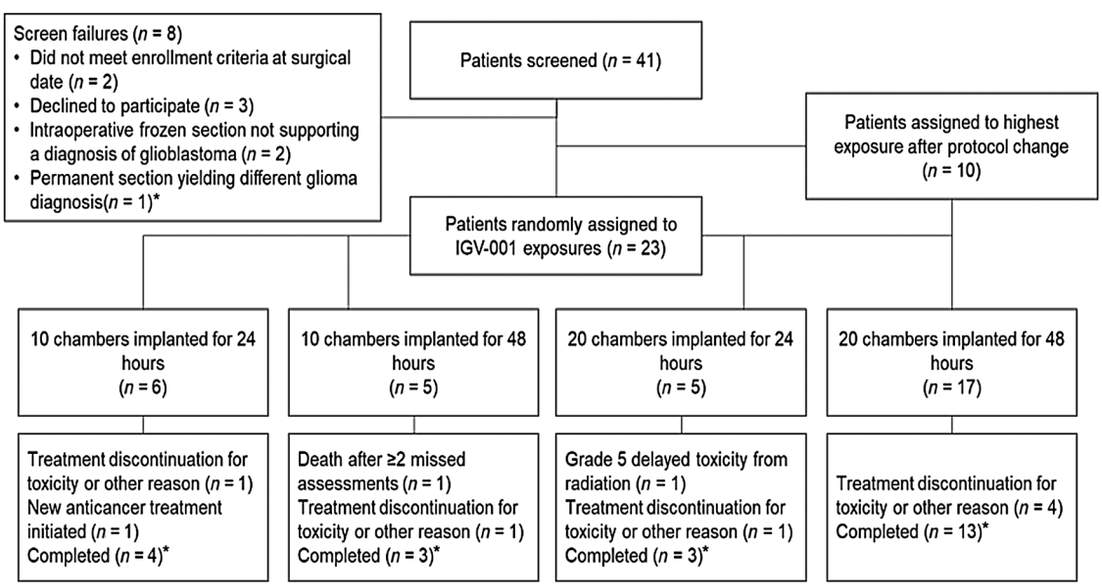

Figure 1.
CONSORT diagram. *Patients with disease progression, 2 years of follow-up, or death.

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Terminology Criteria for Adverse Events Version 4.03. Asymptomatic grade 1–2 laboratory values were not captured as AEs unless determined to be clinically significant by the treating physician. Assessment of relationship of each AE or serious AE to study treatment was performed by the investigator per criteria described in Supplementary Table S1.

MRI

MRI included routine brain MRI with and without contrast (axial T1-weighted, axial T2-weighted, axial fluid-attenuated inversion recovery), axial gradient T2-weighted (T2w-GRE), axial diffusion-weighted imaging, and post-contrast T1-weighted sequence in all three planes including axial three-dimensional T1-weighted sequence, and additional advanced MRI techniques (e.g., dynamic susceptibility-weighted magnetic resonance perfusion and 15-direction diffusion tensor imaging). MRI was performed within 14 days prior to surgery and at postoperative timepoints up to at least 24 months. KPS scores and steroid use were documented at MRI timepoints. Radiographic interpretations of MRI scans were performed by neuroradiologists blinded to patients' clinical status and corticosteroid dosage. Radiographic responses were based on Response Assessment in Neuro-Oncology (RANO; ref. 11) and immunotherapy RANO (iRANO; ref. 12) criteria, as described below. Time to progression was assessed from date of surgery to date of the first observation of objective disease progression measured by MRI. Evidence of disease progression was required to be corroborated by an independent radiology review committee. PFS was measured from date of surgery to progression or censoring. Censoring and determination of progression were limited to scheduled visits. OS was the time elapsed between date of surgery and latest follow-up or death. Patients withdrawn from study were followed for OS.

Assessment of disease progression

Interpretations of MRI scans were based on an index slice from the first post-radiation MRI. To account for potential of pseudoprogression from standard of care (SOC) or inflammation from immunotherapy, assessment for response to treatment incorporated RANO (11) and iRANO (12) criteria. If an MRI within the first 6 months of treatment with IGV-001 showed progression, a follow-up scan was required to be repeated in 3 months to rule out immune-related pseudoprogression. If progression was confirmed on repeat scan, the date of progression was dated back to the original scan when progression was first observed. The appearance of new lesions 6 months or less from the initiation of immunotherapy alone did not define progressive disease. Event scores of progressed disease (uncensored) versus stable or regressed disease (censored) were interpreted according to Table C1 in the FDA Guidance for Industry: Clinical Trial Endpoints (April, 2015). PFS was censored when a patient received anticancer therapy prior to documented disease progression or discontinued therapy due to toxicity.

Exploratory analyses

Serum cytokine levels

Serum cytokines were quantified within 7 days prior to surgery and at postoperative timepoints up to 24 months. Exploratory analyses evaluated the effects of IGV-001 on these serum cytokines.

Luminex bead-based multiplexing immunoassays to assess serum cytokines were performed as described previously (13). Serial serum samples from enrolled patients were analyzed using the Human Cytokine/Chemokine Magnetic Bead Panel (HCYTOMAG-60K, Millipore) on a Luminex FlexMAP 3D for the following analytes: IFNγ, IL2, IL4, IL7, IL10, IL12, IL15, IP-10, monocyte chemoattractant protein-1, macrophage inflammatory protein-1β, and TNFα. Standard curves were generated for each cytokine, and median fluorescent intensities were transformed into concentrations by 5-parameter, nonlinear regression.

Where sufficient data were available, Luminex IL7 data were paired with contemporaneous absolute lymphocyte counts (ALC) data divided into treatment episodes after enrollment. This represented 20 patients. Mean values of each variable derived from each patient during each treatment episode were used as the dataset for linear regression curves.

NanoString PanCancer Immune Profiling pathway analysis of peripheral blood mononuclear cells

Changes in immune parameters were also measured by PanCancer Immune Phenotype (PCIP) pathway analysis of peripheral blood mononuclear cell (PBMC) RNA.

Patient PBMC samples were obtained and stored in liquid nitrogen. RNA was obtained from thawed samples of 5 million live PBMC by Trizol and Qiagen RNeasy mini kit manufacturer's protocol. RNA collected upon isolation was quantified by Nanodrop and stored at −80°C until NanoString analysis. Reporter CodeSet (the barcoded reporter probes), Capture probe set, and hybridization buffer were used from each NanoString kit. The Human PCIP panel used measures over 700 genes. The hybridization reactions contained master mix, 100 ng of sample RNA, and capture probe. Tubes were placed in the preheated thermocycler and incubated at 65°C for 16 hours, as detailed in manufacture's protocol.

Data from the Clinical Laboratory Improvement Amendments–certified Central Laboratories at Thomas Jefferson University

Absolute lymphocyte counts

Absolute lymphocyte counts were obtained from the Thomas Jefferson University Clinical Laboratory Improvement Amendments–certified central laboratory and obtained through the electronic medical record. A value at or below 0.7 was flagged as the threshold of lymphopenia.

MGMT methylation analysis

MGMT promoter methylation analysis was performed on DNA extracted from formalin-fixed paraffin-embedded study subject samples. The DNA was bisulfite treated, and a 62 bp region of MGMT exon 1 [chr10:131,265,494-131,265,556, hg 19; c.15-77 (NM_002412)] that included 12 CpG sequences (potential methylation sites) was amplified using primers that amplify both methylated and unmethylated DNA. The product was then analyzed by high-resolution melting to determine the fraction of methylated DNA. The definition of 100% methylation was that all copies of the MGMT gene in the assessed specimen were all methylated at all 12 CpG islands. The 0% methylation means that all copies of MGMT are not methylated at any of the 12 CpG islands. On the basis of the calibrators (100%, 80%, 40%, 20%, 10%, 0%), specimens were assigned a percentage of methylation. The assessment of methylation of the exon 1 region was a surrogate marker for evaluating the MGMT methylation status. This was a standard practice for clinical research and clinical testing.

At this time, there remains no standard methodology for assessing MGMT methylation status. For this reason, the cut-off value for a positive or negative MGMT result was determined by each performing laboratory. In the Molecular and Genomic Pathology Laboratory at

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Thomas Jefferson University Hospital (TJUH; Philadelphia, PA), a total of 133 brain specimens were evaluated in the assay validation study. On the basis of this validation study, the cut-off threshold for MGMT promoter methylation was and remains 2%. This test was developed, and its performance characteristics determined by the Molecular and Genomic Pathology Laboratory of TJUH (Philadelphia, PA). This test has not been cleared or approved by the FDA. The FDA has determined that such clearance or approval is not necessary. These tests are used for clinical purposes and should not be regarded as investigational or for research. This laboratory is certified under the Clinical Laboratory Improvements Act of 1988 (CLIA-88) as qualified to perform high-complexity clinical testing.

MRI was performed within 14 days prior to surgery and at postoperative timepoints up to at least 24 months. KPS scores and steroid use were documented at MRI timepoints. Radiographic interpretations of MRI scans were performed by neuroradiologists blinded to patients' clinical status and corticosteroid dosage. Radiographic responses were based on RANO (11) and iRANO (12) criteria. Time to progression was assessed from date of surgery to date of the first observation of objective disease progression measured by MRI. Evidence of disease progression was required to be corroborated by an independent radiology review committee. PFS was measured from date of surgery to progression or censoring. OS was the time elapsed between date of surgery and latest follow-up or death. Patients withdrawn from study were followed for OS.

Statistical analysis

The ITT population included all enrolled patients that were not screen failures and was used for evaluation of both safety and clinical outcomes. AEs were summarized descriptively by overall incidence, severity, and association with treatment. PFS and OS were estimated using the product-limit method and graphed with points connected using a step function. Using the method described by Guyot and colleagues, (14) patient-level data from the SOC arms of published studies in patients with newly diagnosed glioblastoma were estimated. OS and PFS for these SOC arms were compared with our IGV-001–treated cohort using a one-sided log-rank test. IGV-001–treated patients who met eligibility criteria for published SOC studies (Stupp-eligible; refs. 10, 15, and 16) were the focus of subgroup analyses. *P* values are provided for context only, and no adjustment was performed for multiple comparisons. SAS version 9.4 (SAS Institute) was used for all analyses.

Table 1. Demographics and baseline disease characteristics.

Characteristic	**IGV-001 (*n* = 33)**
Sex, *n* (%)	
Male	20 (60.6)
Female	13 (39.4)
Age, y	
Mean (SD)	60.2 (10.5)
Median (range)	63 (32–77)
Extent of intracranial disease	
Single lobe	25 (76)
Multiple lobes, unihemispheric	4 (12)
Bihemispheric	4 (12)
Extent of gross resection, *n* (%)	
Total (100%)[a]	10 (30.3)
Near total (95%–99%)	7 (21.2)
Subtotal (>biopsy, <95%)	16 (48.5)
KPS, *n* (%)	
90–100	26 (78.8)
70–80	6 (18.2)
60	1 (3.0)

Dexamethasone administration (time period in days)	**No steroids**	**Increased/ started steroids**	**Decreased steroid dose**	**Stopped steroids**
1–42	12 (41.4%)	7 (24.1%)	4 (13.8%)	6 (20.7%)
43–180	6 (19.4%)	12 (38.7%)	7 (22.6%)	6 (19.4%)
181–365	3 (12.5%)	5 (20.8%)	8 (33.3%)	8 (33.3%)

	N	Mean	SD	Median	Min	Max
Cycles of maintenance TMZ	22	7.5	4.6	8.0	1.0	17.0

MGMT status at diagnosis, *n* (%)	
Methylated	16 (48.5)
Unmethylated	17 (51.5)
IDH-1 mutation	1 (3)

Abbreviations: KPS, Karnofsky Performance Status; MGMT, O^6-methylguanine–DNA methyltransferase; y, years.
[a]Complete removal of the enhancing nidus of the tumor. There is never a complete resection of this infiltrating tumor with a nonenhancing periphery that is unresectable.

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Results

Patients

Thirty-three patients were treated with IGV-001 between September 1, 2015 and March 1, 2018. Enrollment with screen failures are described in **Fig. 1**; none were excluded for unfavorable prognostic variables. The trial had broad inclusion criteria, without an upper age limit or stipulations for extent of resection. Patients with bihemispheric disease were also eligible. Patient characteristics are summarized in **Table 1**. Patients received one of 4 exposures of IGV-001 according to randomization. Four patients were revaccinated, all after discontinuing SOC in the setting of progressive disease. One patient received stereotactic radiotherapy boost prior to documented progression, resulting in a censoring of PFS at that time. The Stupp-eligible analysis set included 22 patients and

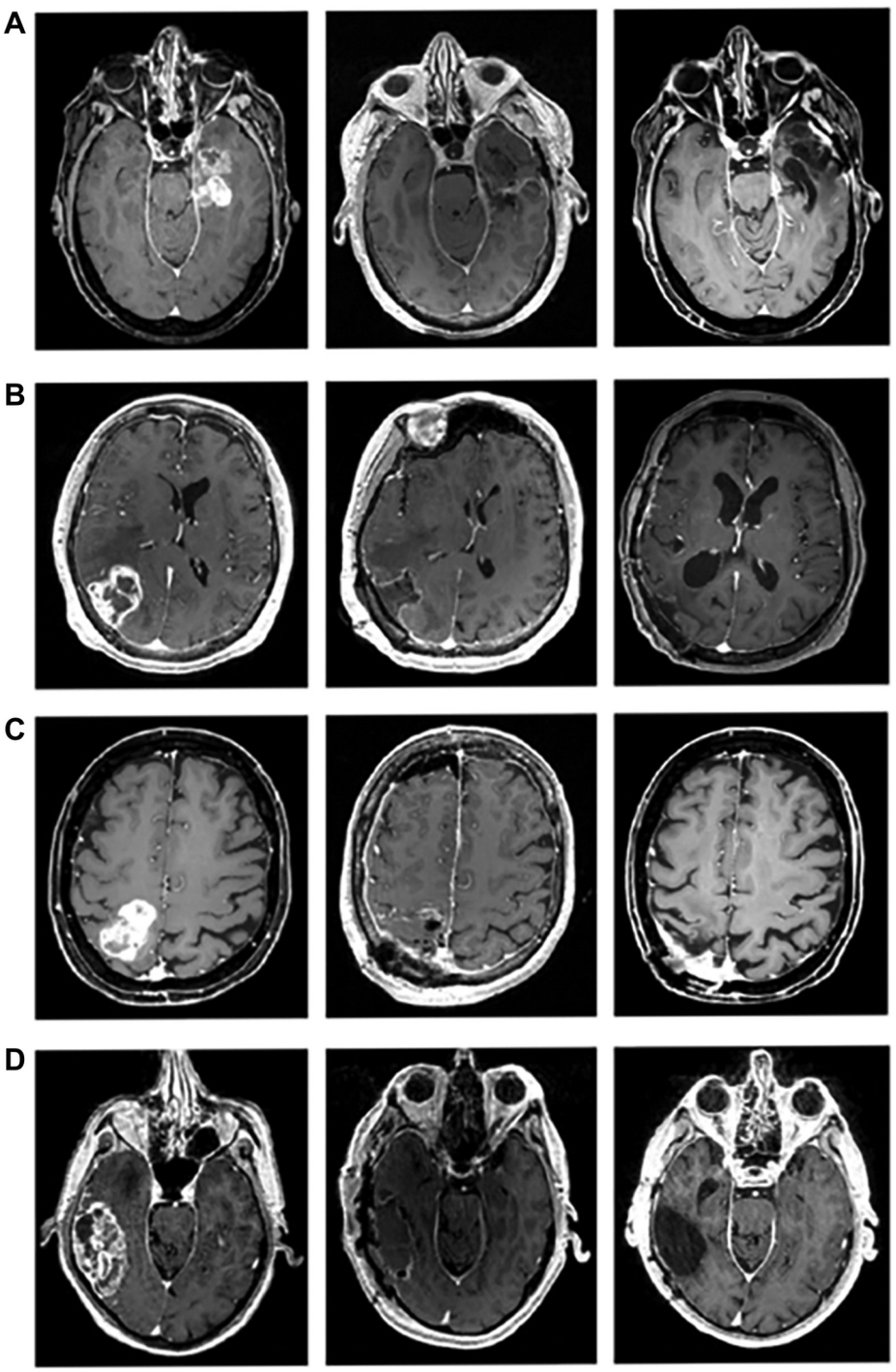

Figure 2.

MRI scans in patients undergoing gross total resection, followed by treatment with IGV-001 (**A–D**). Preoperative, postoperative, and latest scans obtained after trial exit are shown in the left, middle, and right columns, respectively. All scans represent T1-weighted, gadolinium-enhanced axial images obtained on a 3T magnet. Follow-up for each patient at the time of the latest scan was 31 months (**A**), 35 months (**B**), 44 months (**C**), and 24 months (**D**). MRI scans in patients undergoing subtotal resection, followed by treatment with IGV-001 (**E–H**). (*Continued on the following page.*)

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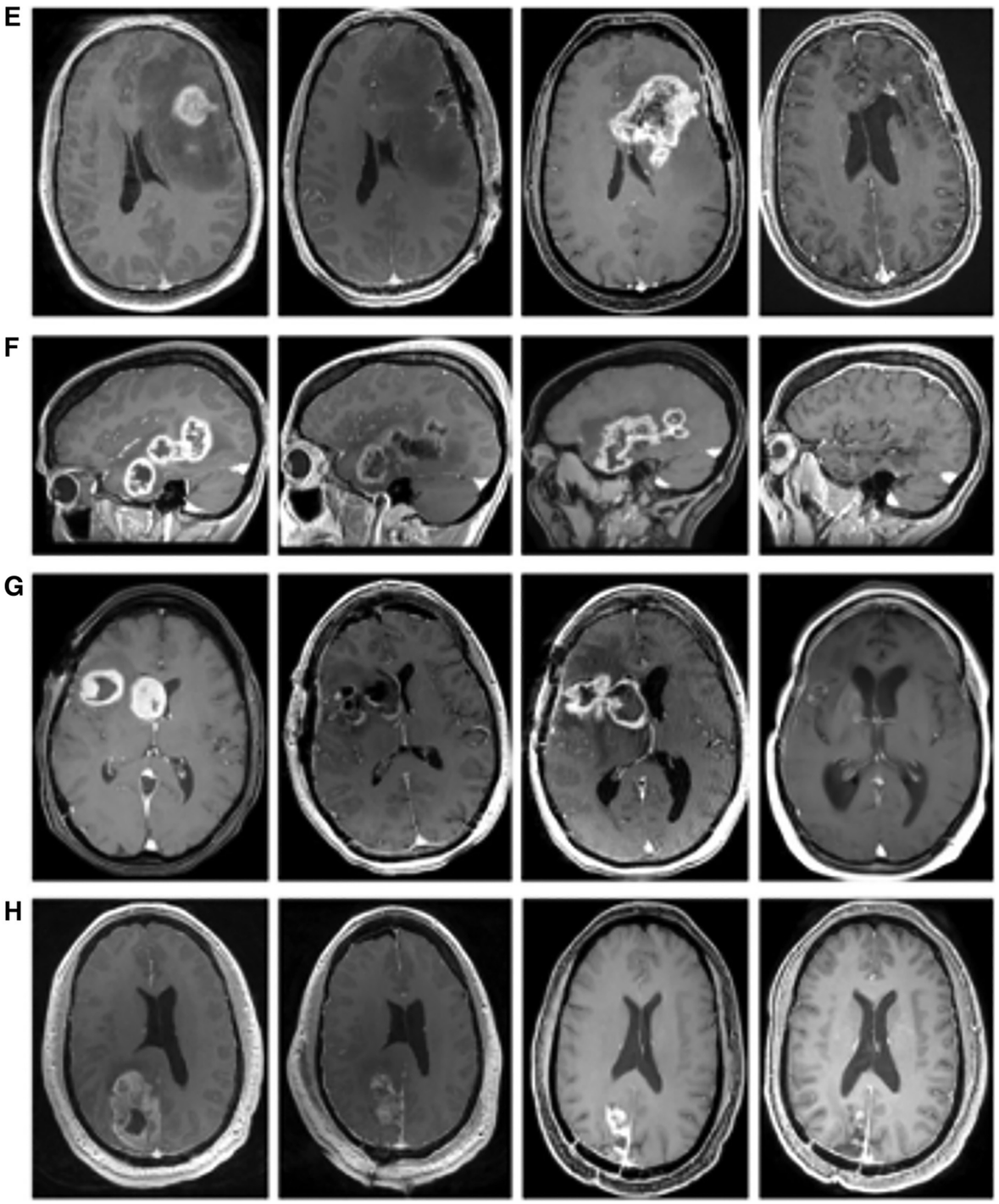

Figure 2.

(*Continued.*) Preoperative, postoperative, 6-month, and latest scans are shown in columns 1, 2, 3, 4, respectively. Scans represent T1-weighted gadolinium-enhanced axial images (**E**, **G**, and **H**) or sagittal images (**F**) obtained on a 3T magnet. Follow-up for each patient at the time of the latest scan was 39 months (**E**), 51 months (**F**), 29 months (**G**), and 24 months (**H**). MRI, magnetic resonance imaging.

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excluded those who would not have been eligible for inclusion in large, published clinical trials of SOC [bihemispheric disease (n = 4), KPS < 70 prior to start of radiotherapy (n = 3), age > 70 years prior to surgery (n = 4; refs. 10, 15, and 16).

Safety

IGV-001 was generally well tolerated. There were five AEs (all grade ≤3) related to abdominal incision: four hematomas and one wound complication. Six patients had treatment-emergent AEs that were possibly related to IGV-001. All were grade ≤3 and were managed conservatively with observation or initiation of standard medical management. Nine of 26 (35%) deaths were not attributed to glioblastoma progression and seven of these deaths occurred within 12 months of treatment with IGV-001.

Radiographic outcomes

As of May 1, 2020, median follow-up for patients receiving IGV-001 was 3.1 years (range, 2.1–4.7 years). Radiographic outcomes after gross total resection included sustained lack of anatomic enhancement at the resection site (**Fig. 2A–D**). Radiographic outcomes after subtotal resection included increases in anatomic tumor volume followed by sustained regression beginning within approximately 6 months of surgery, ruled as true pseudoprogression (**Fig. 2E–H**).

Clinical outcomes

The current SOC was established in 2005 by Stupp and colleagues (3) and demonstrated that adding temozolomide to radiotherapy extended OS over radiotherapy alone (14.6 vs. 12.1 months; ref. 3). The Stupp follow-up publication as well as other recent large, randomized phase III glioblastoma clinical trials have demonstrated remarkable consistency in OS among SOC arms in major peer-reviewed journals (10, 15, 16).

As of May 1, 2020, median PFS in the ITT population receiving IGV-001 was 9.8 months, which compared favorably with SOC arms of published studies (6.5 months; P = 0.0003; **Fig. 3A**; refs. 10, 15, and 16). The 6-month PFS rate among all 33 IGV-001–treated patients was 86% (vs. SOC, 55%). The PFS benefit of IGV-001 was enhanced when the population was limited to the 22 Stupp-eligible patients (median, 11.6 months; P = 0.001; **Fig. 3B**) and further enhanced in the 10 Stupp-eligible patients assigned to cohort D (median, 17.1 months; P = 0.002; **Fig. 3C**).

Methylation of the O^6-methylguanine–DNA methyltransferase (MGMT) promoter increases therapeutic efficacy of the DNA-

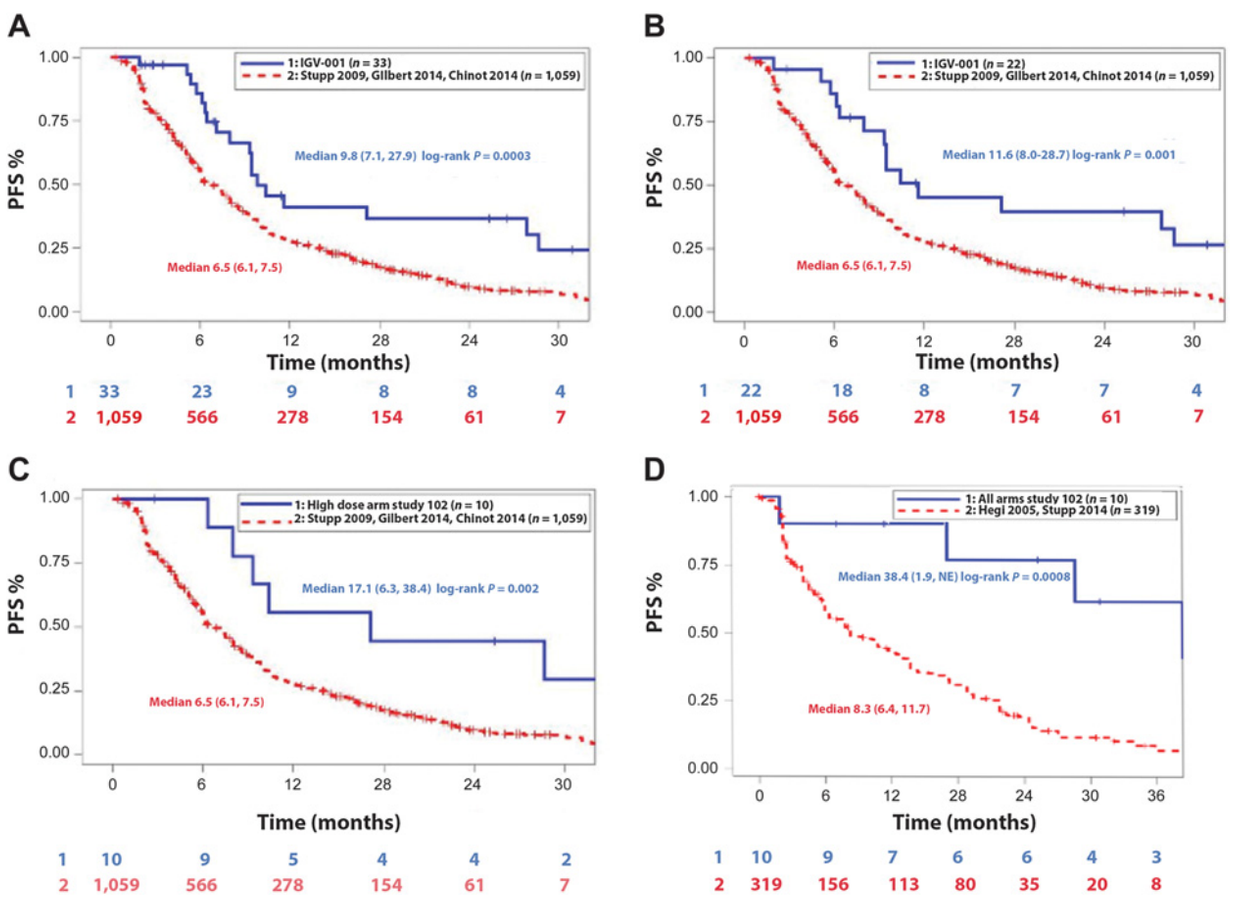

Figure 3.

PFS of patients receiving IGV-001 versus SOC alone. PFS for the ITT population (**A**), the Stupp-eligible population (**B**), and Stupp-eligible population receiving the highest exposure of IGV-001 (**C**) compared with published SOC arms (10, 15, 16). PFS for Stupp-eligible patients with methylated MGMT promoter versus published SOC arms limited to patients with methylated MGMT promoter (**D**; refs. 19 and 20). Tick marks indicate censored data. ITT, intent-to-treat; MGMT, O^6-methylguanine–DNA methyltransferase; NE, not estimable; PFS, progression-free survival; SOC, standard of care.

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alkylating agent temozolomide (17, 18) and conferred a survival advantage in patients with newly diagnosed glioblastoma receiving SOC (19). Stupp-eligible patients with methylated MGMT promoter treated with IGV-001 ($n = 10$) demonstrated a median PFS of 38.4 months, which was significantly greater than published SOC arms limited to patients with methylated MGMT promoter (median, 8.3 months; $P = 0.0008$; **Fig. 3D**; refs. 19 and 20).

Median OS in patients receiving IGV-001 was 17.3 months, which was similar to SOC arms of published studies (16.2 months; $P = 0.30$; **Fig. 4A**; refs. 10, 15, and 16). For Stupp-eligible patients, the OS benefit of IGV-001 approached statistical significance versus SOC (median, 22.3 months; $P = 0.08$; **Fig. 4B**) and was further enhanced in Stupp-eligible patients assigned to cohort D (median, 38.2 months; $P = 0.044$; **Fig. 4C**). Stupp-eligible patients with methylated MGMT promoter showed numerically greater median OS versus SOC (34.3 vs. 26.1 months; $P = 0.24$; **Fig. 4D**).

Exploratory outcomes

IGV-001 is not available for measurement of antigen-specific immunity as tumor material is used in manufacture rather than as assay targets. As a surrogate, we assessed serum cytokines. No changes were found for most cytokines evaluated except peak elevations of proinflammatory IFNγ, IL12p70, and IL2 between 7 and 42 days after treatment, prior to initiation of SOC (**Fig. 5A**). These responses, however, did not necessarily translate to improved outcomes. We attributed this to radiation-induced lymphopenia after SOC. Interestingly, however, PCIP pathway analysis of PBMC RNA showed increasing expression of chemokine and cytokine pathway genes at day 28 after treatment that increased further at day 90 in spite of immunosuppressive SOC (**Fig. 5B** and **C**), whereas regulatory T cell signature reciprocally declined (not shown). As PBMC rather than secreted products, it is perhaps not surprising these were dominated by receptor-related molecules such as CSF2RB, IL1RN (IL1R antagonist), IL6R, CXCR1, and CXCR2 (not shown). TNF and TNF receptor superfamily and macrophage function gene signatures were similarly and progressively elevated (**Fig. 5D** and **E**). Effector immune signatures peaked at day 28, as did "exhausted" T cells. Numerous other immune parameters did not show such changes, nor was methylated status apparently correlated. Most immune parameters declined post-SOC (see Supplementary Fig. S1).

A sustained decrease in ALC was noted during and after radiation (**Fig. 5F**) as did the PCIP cell type scores with the exception of CD45

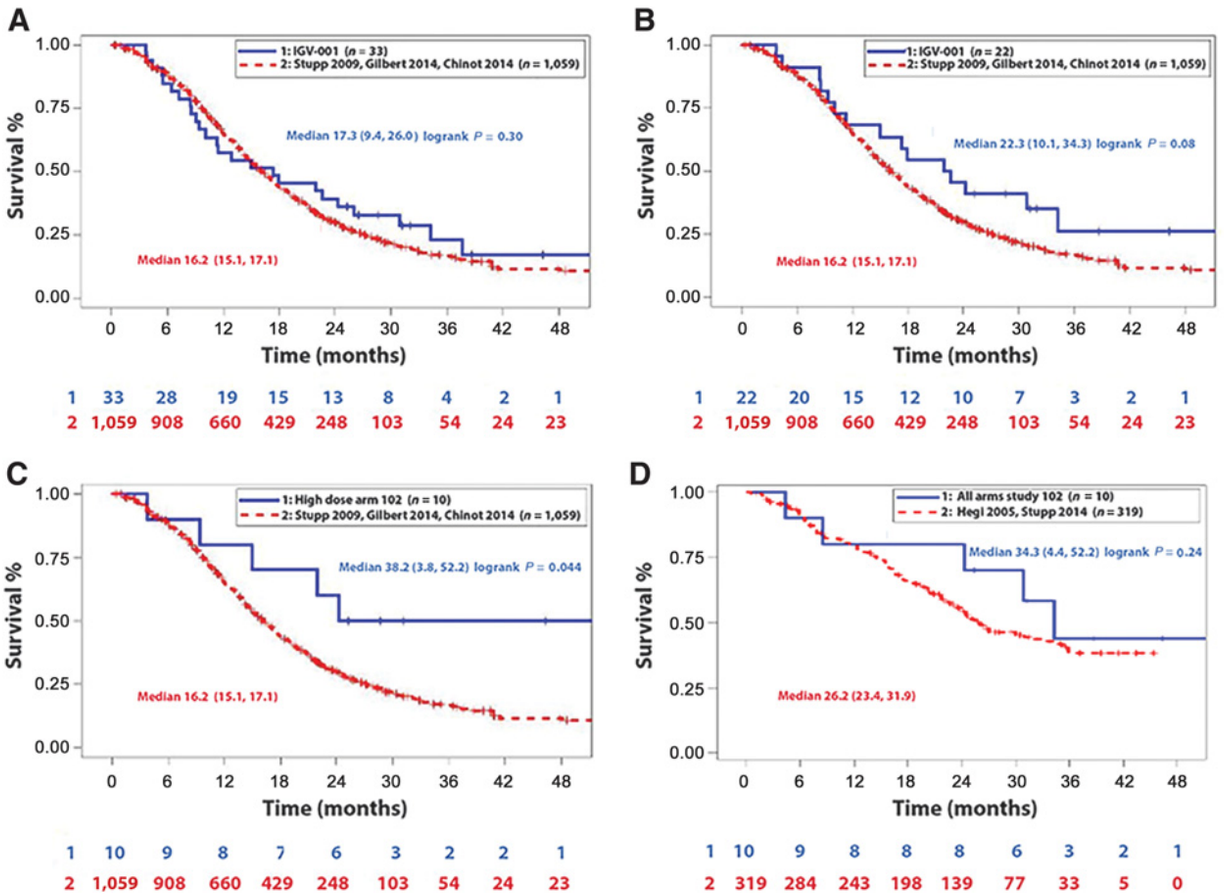

Figure 4.

OS of patients receiving IGV-001 versus SOC alone. OS for the ITT population (**A**), the Stupp-eligible population (**B**), and Stupp-eligible population receiving the highest exposure of IGV-001 (**C**) compared with published SOC arms (10, 15, 16). OS for Stupp-eligible patients with methylated MGMT promoter versus published SOC arms limited to patients with methylated MGMT promoter (**D**; refs. 19 and 20). Tick marks indicate censored data. ITT, intent-to-treat; MGMT, O^6-methylguanine–DNA methyltransferase; OS, overall survival; SOC, standard of care.

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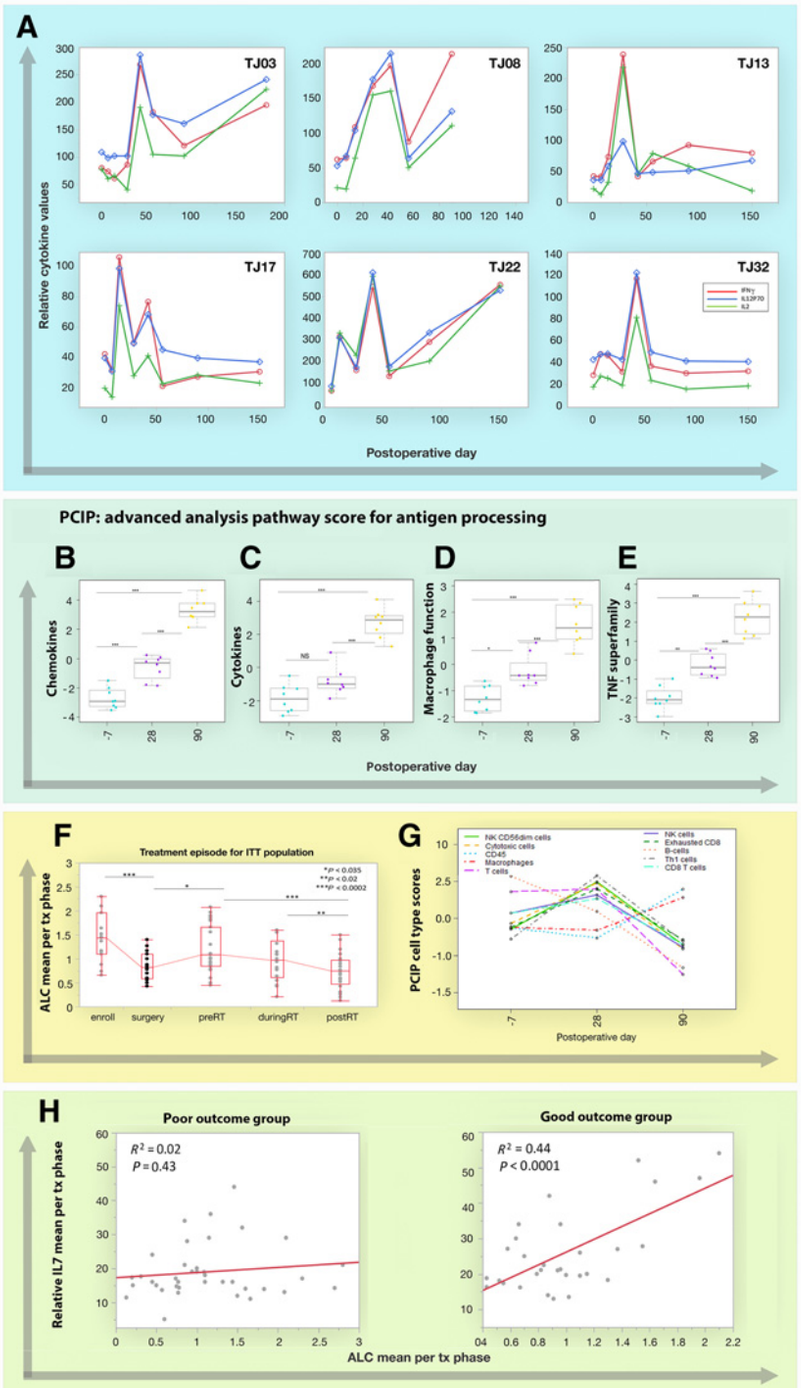

Figure 5.

A, Relative cytokine values (median fluorescence intensity, MFI) for three putative proinflammatory cytokines in 6 patients (TJ03, TJ08, TJ17, TJ32 were in cohort D; TJ22 was in cohort A). Note coincident peak values ranging from 14 to 42 days post-IGV-001 treatment. In some cases, there are two peaks. Figures **B, C, D, E,** and **G** represent advanced analysis pathway scores of samples run with the PCIP Pathway Analysis for 8 patients with total resections [GTR; TJ04, TJ15 (both cohort C) and TJ13 (cohort A)] or subtotal tumor resections [STR; TJ03, TJ11, TJ17 (all cohort D), TJ16 (cohort A)]; showing expression of chemokine (**B**); cytokine (**C**); macrophage function pathway genes (**D**), TNF superfamily (**E**) prior to treatment and at days 28 and 90 after treatment. Immunosuppressive temozolomide and radiotherapy SOC was instituted from approximately day 42–84 with maintenance temozolomide thereafter (***, $P < 0.0001$; **, $P < 0.0005$; *, $P < 0.009$ for **B–E**). **F,** Comparison of means of absolute lymphocyte count for ITT population for each episode of treatment; depression of surgical values attributable to perioperative blood loss and/or hemodilution ***, $P < 0.0002$; **, $P < 0.02$; *, $P < 0.035$. **G,** Immune cell type scores at baseline, before radiation, and after radiation for the 8 patients in the PCIP pathway analysis. **H,** Bivariate correlations of mean values of IL7 and ALC per patient, excluding values at surgery, including pre-radiotherapy through postradiotherapy treatment episodes, by treatment outcome groups. RT, radiotherapy; PCIP, PanCancer Immune Profiling; PBMC, peripheral blood mononuclear cell; SOC, standard of care; TNF, tumor necrosis factor.

and macrophages (**Fig. 5G**). To explore the possibility that this may impact IGV-001 therapy, we assessed serum levels of IL7, a hematopoietic growth factor produced in compensation to declining ALC (21). Excluding the bihemispheric cases, the median OS of 21.9 months was used as the cut-off point between good versus poor outcome. In the course of treatment, serum levels of IL7 correlated significantly with ALC only for the good outcome group (**Fig. 5H**).

Discussion

IGF-1R is widely overexpressed in malignant cells, and its function is crucial for malignant cell survival and tumor progression (22, 23). As a surface receptor, it plays a key role in in the regulation of tumorigenesis, including transformation and antiapoptotic signaling (24, 25). Targeting IGF-1R as a monotherapy has been a uniformly unsuccessful strategy for human cancers (23), perhaps due to redundant downstream IGF-1R activation pathways. In one preclinical study, the only

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drug class successfully stopping all IGF-1R signaling was a siRNA (26). We believe IMV-001, in the siRNA class, serves as the most effective proapoptotic stimulus stressing malignant glioma cells (27) and also eliminating the M2 macrophage phenotype in glioma mouse models (Hooper, manuscript in preparation). We have corroborated our clinical results with other preclinical studies including an orthotopic model (5, 11, 12, 28, 29).

In addition, IMV-001 has an off-target effect that serves as an adjuvant, namely, activation of Toll-like receptor 9 (TLR9) in antigen-presenting cells through its CpG motif (8, 9). Taken together, we hypothesize that IGV-001 creates a local environment at implantation that promotes a proinflammatory innate immune response, trafficking of mature dendritic cells laden with tumor antigens to draining lymph nodes, thereby stimulating an adaptive immune response (8, 9, 30).

The results of this phase Ib clinical trial are encouraging. Phase I studies have typically been skewed to patients with good pretreatment prognostic characteristics. This was not the case in this trial, which was designed with broad entry criteria with respect to extent of disease, functional status, and age (10, 15, 16). The safety profile of IGV-001 in this study was largely consistent with previous experience (4) and the lack of immune-related AEs with IGV-001 compares favorably with serious AEs associated with immune checkpoint inhibition (29) and CAR-T cell therapies (31–33), neither of which have shown clinical efficacy in glioblastoma.

A clinically meaningful prolongation of PFS was observed in the ITT population versus historical controls receiving current SOC (**Fig. 3A**). PFS and OS advantages were conferred by Stupp eligibility, cohort D assignment, and MGMT promoter methylation. Resection subgroups also compared favorably with published literature for newly diagnosed glioblastoma. Among the 10 gross total resections, median PFS has not been reached compared with 8.9 months reported for newly diagnosed patients with tumors considered amenable to gross total resection (34). For subtotal resections with disease progression, excluding patients with bihemispheric disease, median PFS was 9.7 months, comparing favorably with that reported in a similar population (5.9 months; ref. 35). Analyses of cytokines over time supported an immune-mediated mechanism of action of IGV-001. Elevated serum IL7 levels correlated with higher ALC levels and more favorable clinical responses versus those with lower levels, prolonged lymphopenia, and a poor clinical outcome, consistent with a previous report (36). T-cell fitness can be compromised by some solid tumors, and in the case of T cells from patients with pancreatic ductal adenocarcinoma, rendered unresponsive to a clonogenic IL7 stimulus (37). It is possible that subsets of patients with treatment-naïve glioblastoma have similarly compromised T cells less responsive to a proinflammatory stimulus or, more likely, less resilient to the immunocompromising effect of chemotherapy and radiation (38).

This clinical trial confirmed the safety of IGV-001 in adults with newly diagnosed glioblastoma, with clinically meaningful PFS gains over historical controls receiving SOC alone. A phase II, randomized, multicenter, double-blind, placebo-controlled trial in newly diagnosed, grade IV glioma will enroll patients in 2021 (NCT04485949).

Authors' Disclosures

D.W. Andrews reports grants from Albert Stevens Foundation and Imvax during the conduct of the study and other from Imvax outside the submitted work; in addition, D.W. Andrews has a patent for PCT/US2016/026970 issued and licensed to Imvax, a patent for PCT/US2018/021706 issued and licensed to Imvax, a patent for PCT/US2019/014961 issued and licensed to Imvax, a patent for PCT/US2019/059017 pending to Imvax, a patent for PCT/US19/59 pending to Imvax, a patent for PCT/US2019/053102 pending to Imvax, a patent for PCT/US20/025217 pending to Imvax, and a patent for PCT/US2020/pending to Imvax. K.D. Judy reports grants from Imvax, Inc during the conduct of the study. C.B. Scott reports personal fees from Imvax, Inc during the conduct of the study. S. Garcia reports personal fees from Imvax Inc during the conduct of the study; in addition, S. Garcia has a patent for 29/699,152 pending to TJU. K. Talekar reports employment of spouse with GlaxoSmithKline. W. Shi reports consultancy with Brainlab, Varian, Novocure, and Zai Lab and research funding from Brainlab, Novocure, and Regeneron. M.A. Exley reports other from Imvax during the conduct of the study and other from AgenTus Therapeutics Inc. outside the submitted work. K. Pigott reports a patent for Imvax biodiffusion chamber pending. D.C. Hooper reports grants, personal fees, and nonfinancial support from Imvax Inc. during the conduct of the study; in addition, D.C. Hooper has a patent for U.S. Letters Patent No. 10,357,509 issued and licensed to Imvax Inc and is a shareholder of Imvax Inc. No disclosures were reported by the other authors.

Authors' Contributions

D.W. Andrews: Conceptualization, funding acquisition, Investigation, methodology, writing–review and editing. **K.D. Judy:** Supervision, investigation, writing–review and editing. **C.B. Scott:** Formal analysis. **S. Garcia:** Data curation, formal analysis, investigation. **L.A. Harshyne:** Investigation, methodology. **L. Kenyon:** Investigation, writing–review and editing. **K. Talekar:** Formal analysis, writing–review and editing. **A. Flanders:** Supervision, investigation. **K.B. Atsina:** Investigation, writing–review and editing. **L. Kim:** Investigation, writing–review and editing. **N. Martinez:** Investigation, writing–review and editing. **W. Shi:** Investigation, writing–review and editing. **M. Werner-Wasik:** Investigation, writing–review and editing. **H. Liu:** Resources, investigation, writing–review and editing. **M. Prosniak:** Investigation, writing–review and editing. **M. Curtis:** Investigation, writing–review and editing. **R. Kean:** Investigation, writing–review and editing. **D.Y. Ye:** Investigation, writing–review and editing. **E. Bongiorno:** Investigation, writing–review and editing. **S. Sauma:** Investigation, writing–review and editing. **M.A. Exley:** Supervision, writing–review and editing. **K. Pigott:** Data curation, methodology, project administration, writing–review and editing. **D.C. Hooper:** Resources, supervision, investigation, methodology, writing–review and editing.

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