

# A Cancer Research UK First Time in Human Phase I Trial of IMA950 (Novel Multi-peptide Therapeutic Vaccine) in Patients with Newly Diagnosed Glioblastoma

Roy Rampling<sup>1</sup>, Sharon Peoples<sup>2</sup>, Paul J. Mulholland<sup>3</sup>, Allan James<sup>1</sup>, Omar Al-Salih<sup>4</sup>, Christopher J. Twelves<sup>5</sup>, Catherine McBain<sup>6</sup>, Sarah Jefferies<sup>7</sup>, Alan Jackson<sup>8</sup>, Willie Stewart<sup>9</sup>, Juha Lindner<sup>10</sup>, Sarah Kutscher<sup>10</sup>, Norbert Hilf<sup>10</sup>, Lesley McGuigan<sup>11</sup>, Jane Peters<sup>11</sup>, Karen Hill<sup>11</sup>, Oliver Schoor<sup>10</sup>, Harpreet Singh-Jasuja<sup>10</sup>, Sarah E. Halford<sup>11</sup>, and James W.A. Ritchie<sup>11</sup>

## Abstract

**Purpose:** To perform a two-cohort, phase I safety and immunogenicity study of IMA950 in addition to standard chemoradiotherapy and adjuvant temozolomide in patients with newly diagnosed glioblastoma. IMA950 is a novel glioblastoma-specific therapeutic vaccine containing 11 tumor-associated peptides (TUMAP), identified on human leukocyte antigen (HLA) surface receptors in primary human glioblastoma tissue.

**Experimental Design:** Patients were HLA-A\*02-positive and had undergone tumor resection. Vaccination comprised 11 intradermal injections with IMA950 plus granulocyte macrophage colony-stimulating factor (GM-CSF) over a 24-week period, beginning 7 to 14 days prior to initiation of chemoradiotherapy (Cohort 1) or 7 days after chemoradiotherapy (Cohort 2). Safety was assessed according to NCI CTCAE Version 4.0 and TUMAP-specific T-cell immune responses determined. Secondary observations included progression-free survival (PFS), pretreatment regulatory T cell (T<sub>reg</sub>) levels, and the effect of steroids on T-cell responses.

**Results:** Forty-five patients were recruited. Related adverse events included minor injection site reactions, rash, pruritus, fatigue, neutropenia and single cases of allergic reaction, anemia and anaphylaxis. Two patients experienced grade 3 dose-limiting toxicity of fatigue and anaphylaxis. Of 40 evaluable patients, 36 were TUMAP responders and 20 were multi-TUMAP responders, with no important differences between cohorts. No effect of pretreatment Treg levels on IMA950 immunogenicity was observed, and steroids did not affect TUMAP responses. PFS rates were 74% at 6 months and 31% at 9 months.

**Conclusions:** IMA950 plus GM-CSF was well-tolerated with the primary immunogenicity endpoint of observing multi-TUMAP responses in at least 30% of patients exceeded. Further development of IMA950 is encouraged. *Clin Cancer Res*; 22(19):4776–85. ©2016 AACR.

See related commentary by Lowenstein and Castro, p. 4760

## Introduction

Glioblastoma, the most aggressive central nervous system tumor, develops from glial tissue of the brain and spinal cord (1). Newly diagnosed glioblastoma is an orphan disease with 100% mortality rate and a median overall survival (OS) of only 14.6 months (2). Standard first-line therapy comprises maximal

safe tumor resection, followed by concomitant chemoradiotherapy (radiotherapy plus daily temozolomide) and six 28-day cycles of adjuvant temozolomide (2). Although the incidence is relatively low, around 3 to 4 cases per 100,000 population (3), glioblastoma affects patients of all ages, and there is a large unmet medical need for improved first-line therapy. Furthermore, there

<sup>1</sup>University of Glasgow, Beatson West of Scotland Cancer Centre, Glasgow, United Kingdom. <sup>2</sup>Edinburgh Centre for Neuro-Oncology, Western General Hospital, Edinburgh, United Kingdom. <sup>3</sup>Department of Oncology, University College London Hospitals, London, United Kingdom. <sup>4</sup>Adult Neuro-Oncology, Southampton University Hospitals NHS Trust, Southampton, United Kingdom. <sup>5</sup>Cancer Research UK Clinical Centre, St James's University Hospital, Leeds, United Kingdom. <sup>6</sup>The Christie NHS Foundation Trust, Withington, Manchester, United Kingdom. <sup>7</sup>Cambridge Cancer Trials Centre, Oncology Clinical Trials, Addensbrooke's Hospital, Cambridge, United Kingdom. <sup>8</sup>Wolfson Molecular Imaging Centre, University of Manchester, Manchester, United Kingdom. <sup>9</sup>Department of Neuropathology, The Queen Elizabeth University Hospital, Glasgow, United Kingdom. <sup>10</sup>Immatics Biotechnologies GmbH, Tübingen, Germany. <sup>11</sup>Cancer Research UK Centre for Drug Development, London, United Kingdom.

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

Prior presentation: Presented in abstract form at the European Society for Medical Oncology 2012 Congress, Vienna, Austria, September 28 to October 2, 2012; and European Society for Medical Oncology 2014 Congress, Madrid, Spain, September 26 to 30, 2014.

**Corresponding Author:** James W.A. Ritchie, Cancer Research UK Centre for Drug Development, Angel Building, 407 St John Street, London EC1V 4AD, United Kingdom. Phone: 44-1367-240373; Fax: 44-20-3357-3182; E-mail: james.ritchie@cancer.org.uk

**doi:** 10.1158/1078-0432.CCR-16-0506

©2016 American Association for Cancer Research.

### Translational Relevance

Survival rates for patients with glioblastoma are abysmal, with median overall survival of approximately 15 months. Immunotherapy of glioblastoma is a promising area of investigation, although challenges around identification of novel and immunogenic target antigens exist. IMA950 is a glioblastoma-specific vaccine comprising 11 tumor-associated peptides (TUMAP) developed to address this challenge. We have performed a phase I safety and immunogenicity study in newly diagnosed patients with glioblastoma using IMA950 plus granulocyte macrophage colony-stimulating factor (GM-CSF) alongside standard-of-care chemoradiotherapy. Our results demonstrate that IMA950 is well tolerated with 90% of patients having a CD8<sup>+</sup> T-cell immune response to at least one TUMAP, with 50% responding to two or more TUMAPs. No effect of pretreatment regulatory T cell levels on IMA950 immunogenicity was found, and steroids did not appear to affect immune responses to the TUMAPs. These data provide evidence to support further development and optimization of IMA950 together with other immunotherapies for glioblastoma.

is evidence to suggest that the overall incidence of glioblastoma is increasing over time and will continue to increase in an ageing population (4, 5).

IMA950 is an immunotherapeutic multiple-peptide vaccine specifically developed to treat glioblastoma (6). It contains 11 tumor-associated peptides (TUMAP) that are presented by a majority of glioblastoma tumors on human leukocyte antigen (HLA) surface receptors. IMA950 is designed to trigger the immune system by activation of TUMAP-specific cytotoxic T cells. Once activated, these cells are postulated to find and destroy malignant tumor cells presenting the cognate TUMAPs. By vaccinating with 11 TUMAPs simultaneously, there is an increased probability that a multiclonal, broad yet highly specific T-cell response can be mounted against tumor cells, thus hindering potential tumor escape mechanisms.

The primary objectives of this first time in human study were to assess the safety, tolerability, and immunogenicity of IMA950 plus granulocyte macrophage colony-stimulating factor (GM-CSF) given alongside standard therapy in newly diagnosed patients with glioblastoma.

## Patients and Methods

### Patients

Eligible patients had histologically or cytologically proven glioblastoma, an operable tumor which had already been maximally resected, were at least 18 years of age, human leukocyte antigen (HLA) A\*02-positive and hepatitis B core antigen seronegative; had a WHO performance status 0 or 1, a life expectancy of at least 30 weeks, and were expected to complete standard chemoradiotherapy and six 28-day cycles of adjuvant temozolomide. Key exclusion criteria included receipt of any prior glioblastoma treatment apart from surgery, vaccination within 2 weeks or having taken dexamethasone at a dose >4 mg/d within 7 days prior to the first IMA950 plus GM-CSF vaccination, a history of serious cardiac or autoimmune disease or any condition

which might interfere with the patient's ability to generate an immune response. This study was conducted in accordance with the principles of International Conference on Harmonisation (ICH) Good Clinical Practice (GCP), the requirements of the UK Clinical Trials regulations (SI 2004/1031 and SI 2006/1928), and the Declaration of Helsinki. The study protocol, patient information sheet, and informed consent form were approved by the Sponsor's Central Institutional Review Board and the appropriate Research Ethics Committee prior to study conduct. After a full explanation of the study protocol, written informed consent was obtained from all patients before being enrolled. (Clinical Trial registration ID: NCT01222221.)

### IMA950 vaccine

IMA950 is a novel multi-peptide glioblastoma-specific vaccine comprising 11 HLA-binding TUMAPs and one viral marker peptide, identified on HLA surface receptors in primary human glioblastoma tissue, as described previously (6). Supplementary Table S1 gives an overview of the TUMAP source antigens and their respective expression levels found in primary glioblastoma tumor samples. Selected TUMAPs are designed to activate TUMAP-specific CD8<sup>+</sup> cytotoxic and CD4<sup>+</sup> helper T lymphocytes, which then recognize cognate TUMAPs presented by glioblastoma tumor cells and effect a targeted immune response. Nine of the 11 TUMAPs were selected on the basis of their functional relevance, association with the human leukocyte antigen HLA-A\*02, overexpression in glioblastoma, and proven immunogenicity using *in vitro* T-cell assays. The other 2 TUMAPs contained in IMA950 are both HLA class II-binding peptides designated IMA-BIR-002 and IMA-MET-005. IMA-BIR-002 has the capacity to activate CD4<sup>+</sup> helper T cells (7) and potentially cytotoxic T lymphocytes (CTL). IMA-MET-005 contains a known HLA class I epitope, which was elongated on the basis of the natural sequence of c-Met [known oncogene and potential marker of glioblastoma stem cells (8), with the capacity to activate helper T cells (9) and, after processing, CTLs]. An additional non-TUMAP (IMA-HBV-001) was included in IMA950 derived from Hepatitis B virus (HBV) core antigen, to act as a positive control from a "non-self" antigen in cases where no vaccine-induced T-cell responses to TUMAPs from "self" antigens are observed.

### Study design and treatment

Vaccination comprised fixed doses of recombinant GM-CSF (75 µg), a commonly used immunomodulator (10), followed by IMA950 (4.96 mg, 413 µg each peptide) injected intradermally (i.d.) at 11 time points over a 24-week period. All patients received the same vaccination schedule comprising an "Induction Phase" (VIP) of 6 intensive vaccinations (V1–V6), followed by a "Maintenance Phase" (VMP) of 5 vaccinations (V7–V11) over a longer period. Forty-five patients with newly diagnosed glioblastoma were entered into 1 of 2 cohorts that differed by virtue of the first vaccination given at different time points alongside standard therapy (rationale for recruiting at least 20 patients per cohort is given in Supplementary Table S2). In Cohort 1, the VIP started 7 to 14 days before the scheduled onset of chemoradiotherapy to ensure that at least the first 3 vaccinations (days 1–3) were administered prior to the start of chemoradiotherapy. In Cohort 2, the VIP started a minimum of 7 days after the final dose of chemoradiotherapy and 28 days (+7 days) prior to the first scheduled dose of adjuvant temozolomide. This ensured that all

6 vaccinations in the VIP were administered at least a week after the end of immunosuppressive chemoradiotherapy and completed a week prior to the start of adjuvant temozolomide. Three safety observation periods of 21 days were included after 1, 3, and 6 patients had completed 21 days of treatment before opening to general recruitment. Chemoradiotherapy comprised 54 to 60 Gy in 30 daily fractions over 6 weeks with concomitant daily temozolomide, 75 mg/m<sup>2</sup> throughout. Adjuvant temozolomide, 150 to 200 mg/m<sup>2</sup> for 5 days began 35 ( $\pm$ 7 days) following the last fraction of radiotherapy and repeated every 28 days for a total of 6 cycles. See Supplementary Fig. S1 for a detailed overview of the treatment and assessment schedule.

### Patient monitoring and assessment

The primary study endpoint of safety and tolerability was assessed according to National Cancer Institute Common Terminology Criteria for Adverse Events (NCI CTCAE) version 4.0. Disease was assessed using MacDonald criteria (11) with the secondary endpoint of progression-free survival (PFS) evaluated at 6 (PFS-6) and 9 months (PFS-9) from date of surgery. Any clinical complete (CR) or partial response (PR) to therapy was confirmed by an independent neuro-oncologist and radiologist. Although not an endpoint of the study, survival data were collected for 2 years after the final patient had received their first vaccination. The cutoff date for analysis was February 18, 2015.

### Pharmacodynamic analysis

A co-primary endpoint was determining the number of patients showing patient individual T-cell responses directed against TUMAPs contained in IMA950 at one or more post-vaccination time points, as determined by HLA multimer analysis (12, 13). Individual patient peripheral blood mononuclear cell (PBMC) samples were pooled to ensure sufficient viable PBMCs for multimer analysis as follows: "pre-vaccination" (PBMC samples 1 and 2), "post-vaccination 1" (PBMC samples 3 and 4), "post-vaccination 2" (PBMC samples 5 and 6), and "post-vaccination 3" (samples 7 and 8). See Supplementary Fig. S1 for further details. Tetramer staining for each TUMAP and control antigen was performed after an *in vitro* sensitization as described previously (13). Exemplary gating is shown in Supplementary Fig. S2. A positive vaccine-induced multimer CD8 T-cell response for any specific post-vaccination time point of a given antigen and patient was assigned if the following criteria were met: an above threshold immune response (assessed by 5 independent, trained, and blinded jurors and according to Association for Cancer Immunotherapy recommendations; ref. 14) and an at least 4-fold higher frequency of multimer-positive CD8 T cells (normalized to total CD8 T cells) compared with the respective pre-vaccination time point. On the basis of prior clinical experience, at the time of study inception, with similar multi-peptide vaccines (13), study success criteria were defined as either  $\geq$ 30% multi-TUMAP response or  $>$ 60% single TUMAP response in the study population. Further development would be recommended if either criterion was met. Secondary outcome measures included Treg levels (defined as CD3<sup>+</sup>CD4<sup>+</sup>CD8<sup>-</sup>CD25<sup>high</sup>CD127<sup>low</sup>Foxp3<sup>+</sup> lymphocytes; ref. 15) pre- and post-vaccination and correlation of steroid dose with observed T-cell responses. Research analysis examined the kinetics of TUMAP immunogenicity, effect of O<sup>6</sup>-methylguanine DNA methyltransferase (MGMT) promoter methylation status on PFS and exploring the possible effects of vaccination on observed disease pseudo-progression and pseudo-regression measured

using a standardized diffusion-weighted (DWI) and perfusion-weighted (PWI) MRI protocol. Pseudo-progression was defined as an apparent increase in the enhancing tumor ( $>$ 25%) on an early reference scan followed by a reduction in subsequent scans (assessed at week 25 onwards), with no associated clinical deterioration. Pseudo-regression was assessed using an inverse definition. It was recommended that patients continue on therapy until the true clinical diagnosis was clarified. Although this design pre-dated that of recently published guidance, suggesting that patients continue the immunotherapy regimen for 3 months prior to progressive disease (PD) confirmation (16), it is generally in line with these recommendations.

### Statistical analysis

For the pharmacodynamic analysis, several different methods were used to calculate statistical significance depending on the type of data being examined. All statistical analysis was performed using Prism version 6.02 software (GraphPad Software Inc.). Two-tailed nonparametric Mann-Whitney test was used to determine differences between independent groups under examination. This included, for example, the number of vaccine-induced TUMAP responses per patient between cohorts and frequency of Treg as a percentage of total lymphocytes for a given patient compared between cohorts. Fisher exact test was used to analyze contingency tables. This included a comparison of the proportion of patients showing a TUMAP response between cohorts. Nonparametric Spearman rank correlation test was used to analyze dependence between 2 variables such as immune responses and regulatory T-cell levels.

The Kaplan-Meier method was used to generate survival curves and estimate OS rates. Log-rank test was used to compare the survival distributions between groups of patients that included censored data.

Statistical analysis of imaging parameters was performed using a one-way ANOVA analysis with *post hoc* intergroup analysis using Tukey test, due to a significant number of datasets being unavailable for analysis.

## Results

### Patient demographics

Table 1 provides an overview of patient demographics. Of 138 patients screened, 53% were HLA-A\*02-positive, which is in the expected range for a United Kingdom population (17). Reasons for non-entry of 26 HLA-A\*02 patients is given in Supplementary Table S3. Forty-five patients were recruited into the study; 22 in Cohort 1 and 23 in Cohort 2. Forty patients were immune evaluable, with 39 evaluable for clinical activity assessment. This discrepancy is a result of 2 patients being lost for follow-up between blood sample 6 and week 25 scan (see Supplementary Fig. S1), including one patient that was immune evaluable. The overall median age was 53 years (range, 20–75 years) with no meaningful difference between cohorts. All patients had WHO performance status (PS) 0 or 1 at recruitment. A larger proportion of patients in Cohort 2 (65%) had a PS of 1 compared with Cohort 1 (27%), most likely due to Cohort 2 patients having undergone treatment with chemoradiotherapy. As expected, the lymphocyte count on patient entry was lower in Cohort 2 ( $0.80 \times 10^9/L$ ) compared with Cohort 1 ( $1.49 \times 10^9/L$ ) reflecting the effect of concomitant temozolomide in the former. Of the 38 patients evaluable for MGMT promoter methylation testing, 11 (29%)

**Table 1.** Patients' baseline characteristics

Characteristic	Cohort 1	Cohort 2	Overall
Age, y			
Median	54	49	53
Range	21-75	20-68	20-75
Sex, n (%)			
Male	15 (68%)	15 (65%)	30 (67%)
Female	7 (32%)	8 (35%)	15 (33%)
Total	22	23	45
WHO performance status, n (%)			
0	16 (73%)	8 (35%)	24 (53%)
1	6 (27%)	15 (65%)	21 (47%)
MGMT methylation status, <sup>a</sup> n (%)			
Methylated	8 (42%)	3 (16%)	11 (29%)
Unmethylated	11 (58%)	16 (84%)	27 (71%)
Unavailable	3	4	7
Lymphocyte count, ×10 <sup>9</sup> /L			
Median	1.49 <sup>b</sup>	0.80 <sup>b</sup>	1.12
Range	0.88-2.50	0.35-1.91	0.35-2.50
Concomitant steroid use, n (%)			
Yes	16 (73%)	17 (74%)	43 (73%)
Entry concomitant steroid dose, mg			
Median	2.0	1.5	2.0
Range	0-4.0	0-4.0	0-4.0

<sup>a</sup>Percentages calculated excluding those patients whose MGMT methylation status was unavailable.

<sup>b</sup>Significantly different lymphocyte counts between the two cohorts; *P* < 0.0001, two-tailed Mann-Whitney test.

were positive for methylated promoter, 8 of 19 (42%) in Cohort 1 compared with 3 of 19 (16%) in Cohort 2.

**Safety**

All patients received at least one vaccination and were evaluated for safety [see Table 2 for the most commonly reported adverse events (AE), regardless of causality]. Injection site reaction (ISR) was the most frequent AE, and most common study drug-related AE with 81 instances reported in 26 patients (12 of 22 patients in Cohort 1 and 14 of 23 patients in Cohort 2). The majority of ISRs were grade 1 (24 of 26 patients) with only 2 instances of grade 2 events. Thirty-one patients experienced at least one serious adverse event (SAE), one of which was a death unrelated to the study drug. The most frequently reported SAEs were seizure in 8 patients followed by thromboembolic events in 6 patients, none was study drug-related. Investigators considered 4 SAEs to be related to the study drug including 2 cases of grade 4 neutrophil count decrease and 1 case each of grade 3 fatigue and anaphylaxis. The related SAEs of anaphylaxis and fatigue were both considered dose-limiting toxicities. There were no unexpected differences in the safety profiles observed in the 2 cohorts.

**Pharmacodynamics**

Thirty-six of 40 immune evaluable patients (90%) were TUMAP responders, with 20 (50%) responding to more than one TUMAP (Fig. 1A). The predefined primary immunologic endpoint for recommending further development (≥60% single or ≥30% multi-TUMAP responders) was therefore reached for the total immune evaluable study population and each of the 2 individual study cohorts. In Cohort 1, 9 of 19 (47%) evaluable

patients responded to multiple TUMAPs, with a further 9 (47%) responding to a single TUMAP. Similarly, in Cohort 2, 11 of 21 (52%) evaluable patients had multiple TUMAP responses and a further 7 (33%) had a single response. Although the number of vaccine-induced responses per patient in Cohort 2 appeared to be greater than in Cohort 1 (an arithmetic mean of 2.2 in Cohort 2 vs. 1.6 in Cohort 1), this was not statistically significant (*P* = 0.3; Mann-Whitney test; Fig. 1B). Immune response kinetics showed a predominant onset of vaccine-induced TUMAP responses in the post-vaccination 1 sample PBMC pool, with 47 (61%) being detected at this time point (Fig. 2A). This was also true for each cohort. In addition, 24 of 77 (31%) of vaccine-induced TUMAP responses were already detectable pre-vaccination and were boosted at least 4-fold by administration of IMA950 plus GM-CSF (data not shown). The majority of vaccine-induced TUMAP responses were detectable at one post-vaccination assay time point only (61%, 52 of 77 immune responses; Fig. 2A) and were of relatively low magnitude (see Supplementary Fig. S3 for exemplary data). The proportion of vaccine-induced TUMAP responses detected at only one post-vaccination assay time point was significantly higher (*P* = 0.025; Fisher exact test) in Cohort 1 (25 of 30 immune responses; 83%) than in Cohort 2 (27 of 47 immune responses; 57%; Fig. 2B). No apparent differences in TUMAP responses were noted between patients who were and were not receiving concomitant steroid treatment (data not shown).

Twenty-five immune evaluable patients (63%) responded to the "non-self" viral antigen, IMA-HBV-001 (13) and was by trend, associated with the number of vaccine-induced TUMAP responses (*P* = 0.117 by Wilcoxon test; data not shown). There was also a trend for the proportion of IMA-HBV-001 responders to be enriched within the multi-TUMAP responder fraction of patients (*P* = 0.191 by Fisher exact test; data not shown).

There was no correlation between pretreatment Treg levels and number of vaccine-induced TUMAP responses overall (Fig. 3A) or within either cohort of patients (Fig. 3B and C). A comparative analysis of study cohorts revealed that pretreatment Treg levels normalized to lymphocytes were significantly increased (*P* = 0.0003 by Wilcoxon test) in Cohort 2 compared with Cohort 1 (Fig. 3D).

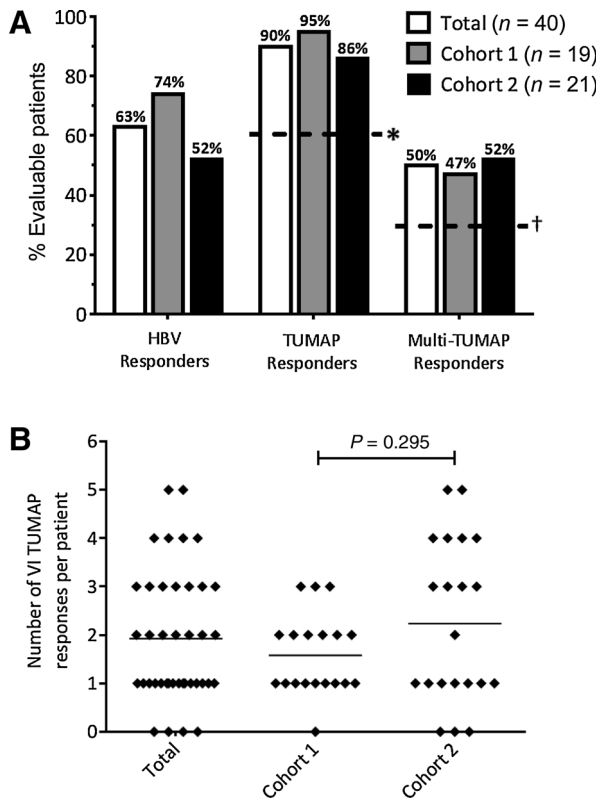
To explore possible effects of vaccination on observed pseudo-progression and pseudo-regression of disease, DWI and PWI were performed alongside standard gadolinium MRI scans. Cohort 1 patients showed increases in apparent diffusional coefficient (*P* < 0.05), following chemoradiotherapy (see Supplementary Fig. S4). Over the same period, PWI parameters showed a trend (albeit not statistically significance) toward increased T1 values, contrast transfer coefficient (*k*<sup>trans</sup>) and

**Table 2.** Most common AEs occurring in >20% patients (regardless of causality)

Symptoms <sup>a</sup>	Grade, n				Total n (% of patients)
	1	2	3	4	
Nausea	21	6	0	0	27 (60%)
ISR	24	2	0	0	26 (58%)
Fatigue	16	5	4	0	25 (56%)
Headache	20	2	0	0	22 (49%)
Vomiting	16	4	1	0	21 (47%)
Alopecia	8	8	0	0	16 (36%)
Dizziness	11	3	0	0	14 (31%)
Seizure	4	4	3	2	13 (29%)
Cough	9	2	0	0	11 (24%)

<sup>a</sup>Patients may have experienced multiple AEs of the same type.

Downloaded from http://aacrjournals.org/clinccancerres/article-pdf/22/19/4776/1929158/4776.pdf by guest on 14 February 2025



**Figure 1.** Primary immune response summary. **A**, further development is based on:  $>60\%$  of patients being single or  $>30\%$  of patients being multi-TUMAP responders. **B**, number of vaccine-induced TUMAP responses is shown for the overall immune evaluable patient population ( $n = 40$ ) as well as for study cohorts. Black lines indicate mean values. For statistical analysis, the Mann-Whitney test was used. Abbreviation: HBV, hepatitis B virus-derived vaccinated marker peptide.

total enhancing volume ( $v_e$ ) with an associated decrease in plasma volume ( $v_p$ ) between scans 1 and 2 (data not shown).

**Clinical activity**

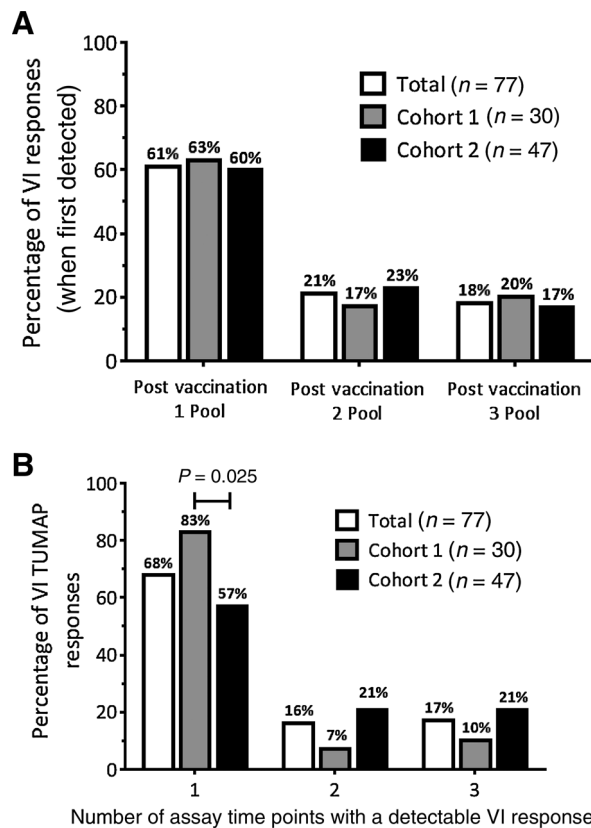
Twenty-nine of 39 evaluable patients were progression-free at 6 months (PFS-6 of 74.4%) with 12 continuing to be progression-free at 9 months (PFS-9 of 30.8%). Stable disease (SD) was confirmed for 11 evaluable patients (28.2%) at week 40. One patient with residual disease at baseline had a PR at week 40, with tumor size decreasing from 357 mm<sup>2</sup> at baseline to 25 mm<sup>2</sup> at week 17, being maintained until they went off-study. Four patients with SD and the patient with PR at week 40 had MGMT promoter methylation (5 of 11 patients with a methylated MGMT promoter; 45.5%). Five other patients with SD at week 40 had unmethylated MGMT promoters (5 of 27 patients with an unmethylated MGMT promoter; 18.5%). Eleven patients of an evaluable 38 (29%) had a methylated MGMT promoter, which conferred a significant survival advantage (28.3 vs. 14.8 months;  $P = 0.025$  using log-rank test; data not shown).

As of the cut-off date (February 18, 2015), median OS for the study was 15.3 months (Fig. 4A) with no significant differences between the cohorts or those patients that responded to multiple TUMAPs compared with those that did not respond or to one TUMAP only (Fig. 4B). Interestingly, patients experiencing one

or more ISRs had a significantly improved ( $P = 0.0001$ ; HR, 0.33) median OS of 26.7 months compared with 13.2 months for those who did not (Fig. 4C). The median age of patients in the ISR group was significantly lower than that of the non-ISR (47 vs. 57 years respectively;  $P = 0.023$  by Mann-Whitney test). Imaging parameters in patients displaying ISRs showed no significant difference. However, in Cohort 2, ISR was associated with lower  $K^{trans}$  ( $P < 0.05$ ),  $v_p$  ( $P < 0.01$ ),  $v_e$  ( $P < 0.05$ ), and rate constant  $K_{ep}$  ( $P < 0.05$ ) values at baseline.

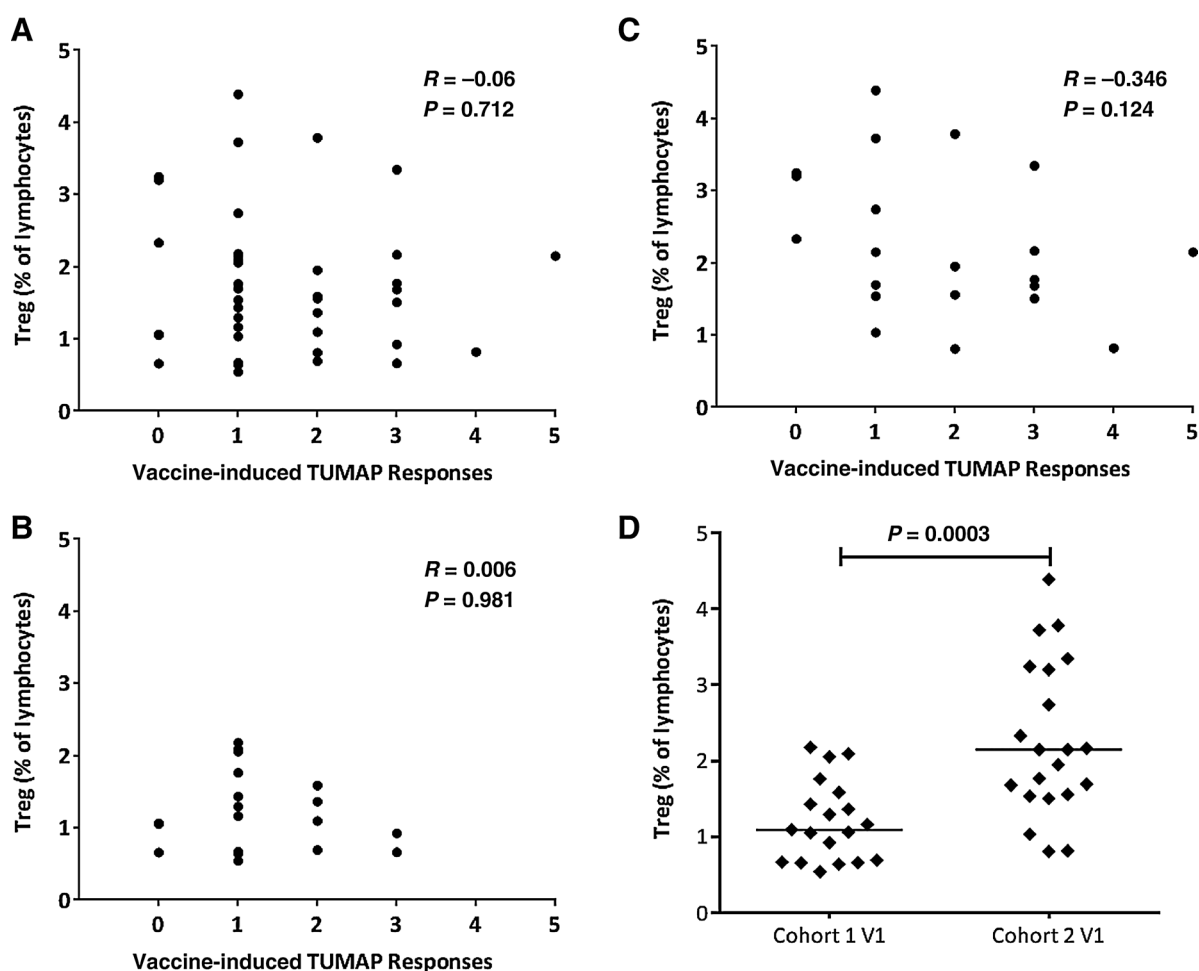
**Discussion**

In the majority of treated patients with glioblastoma, IMA950 produced antigen-specific peripheral CD8<sup>+</sup> T-cell immune responses to the TUMAPs contained within the vaccine, with a relatively benign drug-related toxicity profile comprising mainly minor injection site reactions. The two-cohort study design was used to help define the most biologically effective and clinically feasible administration schedule of IMA950 for subsequent development as determined by the level of vaccine-induced TUMAP-specific immune responses for each schedule. However, it does not allow direct comparison of clinical efficacy between cohorts, as recruitment was not randomized, nor was the trial



**Figure 2.** Onset and sustainability of vaccine induced immune responses. **A**, onset (first appearance) of vaccine-induced immune responses to IMA950 TUMAPs ( $n = 77$  total detected vaccine-induced responses in  $n = 40$  immune evaluable patients). **B**, percentage of vaccine-induced responses to IMA950 TUMAPs with detection at 1, 2, or 3 post-vaccination assay time points.  $P$  values were calculated using the Fisher exact test (only significant results are shown).

Downloaded from http://aacrjournals.org/clinccancerres/article-pdf/22/19/4776/1929158/4776.pdf by guest on 14 February 2025



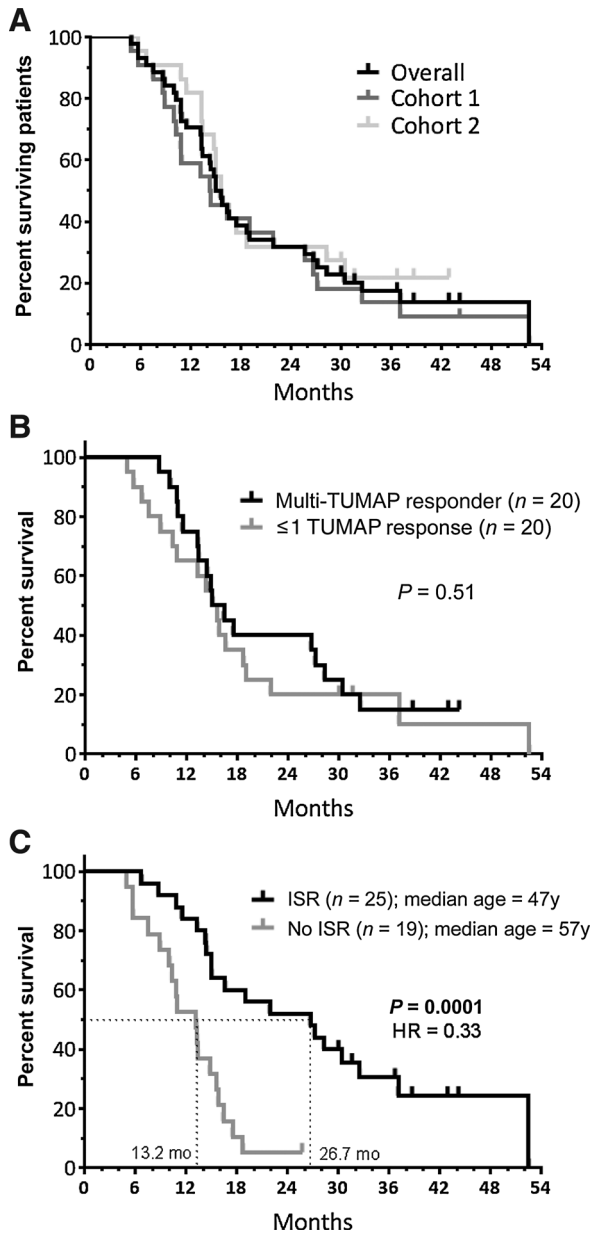
**Figure 3.** Correlation of pretreatment levels of Treg cells with vaccine-induced immune responses to IMA950 TUMAPs. Treg (CD4<sup>+</sup>/CD25<sup>hi</sup>/CD127<sup>lo</sup>/FoxP3<sup>+</sup>) levels, normalized to lymphocytes, at V1 were analyzed in correlation with vaccine-induced CD8 T-cell responses to IMA950 TUMAPs in (A) all immune evaluable patients with *n* = 40, (B) study Cohort 1 with *n* = 19, and (C) study Cohort 2 with *n* = 21. Correlation coefficients and *P* values, calculated using Spearman correlation, are indicated on each graph. D, cohort comparison of pretreatment Treg levels on the first vaccination day for immune evaluable patients. For statistical analysis, the Mann-Whitney test was used.

prospectively powered to make such a comparison. Both cohorts presented challenges that had the potential to interfere with successful vaccination and the mounting of a measurable TUMAP-specific immune response. In Cohort 1, there was a risk that chemoradiotherapy could be immunosuppressive (18, 19) and interfere with the induction and maintenance of TUMAP-specific CD8<sup>+</sup> T cells, whereas in Cohort 2, there was the possibility that following completion of chemoradiotherapy, patient lymphocyte counts would be depleted and have lost the ability to mount a detectable immune response to IMA950. Indeed, immune data showed that Cohort 1 patients had a decreased detection rate of vaccine-induced TUMAP responses at later time points (Fig. 2), suggesting that chemoradiotherapy may interfere with the induction and maintenance of antigen-specific CD8<sup>+</sup> T cells. The greater number and improved durability of TUMAP responses in Cohort 2 suggests that lymphocyte depletion caused by chemoradiotherapy is either insufficient to hinder induction of

antigen-specific CD8<sup>+</sup> T cells or can be recovered sufficiently rapidly to support their expansion.

Tregs are a potent immunosuppressive cell population (20) that may interfere with the immunogenicity of cancer vaccines (21). Given this, an additional key biologic endpoint of this study was to explore the effect of pretreatment Treg levels on the immunogenicity of IMA950. There was no correlation between pretreatment Treg levels (relative to the overall lymphocyte population) and the number of vaccine-induced TUMAP responses for the overall group of immune evaluable study patients. This result is similar to previous reports in other glioblastoma vaccine studies (22, 23). There was a significant increase in the Treg levels at the start of vaccinations in Cohort 2 compared with Cohort 1, likely indicating a relative increase of Tregs compared with other lymphocyte subpopulations as a result of the preceding chemoradiotherapy (24). The importance of this finding is unclear given that there were more vaccine-induced immune responses in Cohort 2.

The overall number of immune evaluable patients responding to multiple TUMAPs in this study (50%) exceeded that demonstrated for other similar vaccine products (13) such as IMA901,



**Figure 4.** OS from date of surgery for different patient subsets. **A**, median OS was 15.3 months for all patients ( $n = 44$ ), 14.4 months for patients in Cohort 1 ( $n = 22$ ), and 15.7 months for patients in Cohort 2 ( $n = 22$ ). There was no significant difference between each of the cohorts ( $P = 0.63$ , log-rank test); one patient was lost for follow-up in Cohort 2 and excluded from survival analysis. **B**, relationship between survival and TUMAP response. Only patients that were immune evaluable were included in the analysis. Log-rank test was used to calculate significance between the 2 different patient populations. **C**, relationship between OS and injection site reaction. One patient was lost to survival follow-up and is excluded from the analysis. Log-rank test was used to calculate significance and HR. Median age of patients in the ISR group was significantly lower than that of the non-ISR (47 vs. 57 years respectively;  $P = 0.023$  by Mann-Whitney test).

which had a multi-TUMAP response rate of 26%. In contrast to that found with IMA901, there was no apparent correlation between the number of TUMAP responses and improved survival (Fig. 4B). However, there are key differences between this study and that of IMA901. IMA901 comprises different TUMAPs, selected specifically for the treatment of patients with renal cell carcinoma (RCC), and the IMA901 study was conducted in the absence of potentially confounding standard-of-care therapy. Low-dose cyclophosphamide [shown to decrease the number and function of Treg (refs. 25, 26)] was also used alongside GM-CSF to further enhance immune response potential. In addition, RCC is known to be an immune-responsive tumor type (27), whereas immunotherapy for glioblastoma is still in its infancy. Indeed, cancer vaccine immunotherapy strategies for patients with glioblastoma require considerable refinement due to the challenges posed by immune resistance and suppression in this tumor type (28). Multiple immunosuppressive mechanisms are likely to be important in glioblastoma, including enhanced secretion of immunosuppressive factors after exposure to standard therapy (29), induction of tumor-infiltrating lymphocytes and Treg activity (30) as well as immune checkpoint pathways such as PD-1/PD-L1 and CTLA-4 (31, 32).

The aim of administering adjuvant(s) alongside therapeutic vaccines is to attempt to augment immune response and overcome immunosuppression by either moving the immune response toward  $T_H1$  or  $T_H2$  immunity, activating innate immunity or to serve as a local repository for prolonged antigen release and protection from degradation. In this study, we utilized GM-CSF as an adjuvant on the basis of the principle that it should enhance effective priming of T-cell responses (33, 34), and the fact that it had been successfully applied in late-stage clinical trials (35). There is evidence to suggest that in some circumstances, at least, GM-CSF may not significantly enhance immune responses and may even be detrimental (36). Even so, an earlier meta-analysis of published trials suggests that low-dose GM-CSF (40–80  $\mu\text{g}$  for 1–5 days) given s.c. or i.d. at the site of vaccination enhances the cellular immune response, whereas high-dose, systemic treatment ( $\geq 100 \mu\text{g}$ ) does not increase the efficacy of a peptide vaccine due to expansion of immune-inhibiting myeloid-derived suppressor cells (MDSC; ref. 10). On the basis of this evidence, we opted for a fixed dose of 75  $\mu\text{g}$  GM-CSF given i.d. prior to vaccination with IMA950. In light of the relatively low magnitude and transient immune responses, enhancement of the vaccination regimen, including selection of the most effective adjuvant partner(s), is necessary; for example, by using alternate or additional adjuvants such as locally applied poly-ICLC (37), imiquimod (38), or systemically administering CD40 ligand (39) or cyclophosphamide (40). Combining cancer vaccines such as IMA950 with immune checkpoint inhibitors such as anti-PD1/PD-L1 or anti-CTLA4 antibodies should also be expected to enhance antitumor immune responses. This is based on the rationale that overcoming local immunosuppression and T-cell anergy by checkpoint blockade can be limited by the specificity/size of the pre-existing T-cell population and the fact that some tumors are relatively nonimmunogenic. Indeed, preclinical and clinical data are beginning to emerge demonstrating that the antitumor activity of immune checkpoint blockade can be enhanced by vaccination (41, 42).

The observation that patients experiencing one or more ISRs had improved survival and were generally of younger age

suggests that ISR may be a prognostic marker for a patient population with an inherently healthier immune system (43). This is supported by the significantly different imaging features in Cohort 2 patients experiencing ISRs whose tumors showed less vascularity and reduced angiogenesis-associated vascular permeability. Although this was an unplanned and retrospective analysis, a contribution of the vaccine to patient survival for those with a more vigorous immune system cannot be ruled out and could be investigated in future randomized studies that might include a nonspecific immunogen. In addition, methylation of MGMT promoter conferred a survival advantage for patients with glioblastoma, as previously reported (44).

A key factor that will need to be considered during the future development of IMA950 and therapeutic cancer vaccines more generally is the need to continue vaccination even after the disease appears to be progressing. Unlike conventional cancer chemotherapy, the effect of cancer immunotherapies is not directly on the disease but rather on the immune system which leads to a cellular immune response followed by tumoricidal biologic activity and potentially improved patient survival (45). This can lead to nontypical patient survival curves and misinterpretation of study results. Given this, chronic vaccination beyond disease progression, and potentially during subsequent therapy, will need to be carefully planned as part of future positioning alongside other therapy for the treatment of glioblastoma.

IMA-HBV-001 was also included in the IMA950 vaccine to act as a positive control in cases where no vaccine-induced T-cell responses to TUMAPs from "self" antigens are observed. There was a trend (albeit not reaching statistical significance) for patients mounting an immune response toward IMA-HBV-001 also to respond to one or more TUMAP, supporting its use as a general immunogenicity marker. However, these findings also suggest that IMA-HBV-001 has limited use as an independent control peptide for association analysis.

Successful development of effective therapeutic vaccines for cancer has proven to be particularly challenging. In the context of glioblastoma, the most advanced therapeutic vaccine approach was that of rindopepimut (CDX-110) which consists a single 14-mer peptide derived from EGF receptor variant III deletion mutation (EGFRvIII; ref. 46). Results from a phase II single-arm study of rindopepimut, given to newly diagnosed EGFRvIII<sup>+</sup> patients with glioblastoma post-chemoradiotherapy in combination with adjuvant temozolomide, demonstrated a median OS of 21.8 months, an increase in anti-EGFRvIII antibody titer and clearance of EGFRvIII from the majority of analyzed posttreatment tumors (47). Even so, the resulting pivotal, double-blind, randomized phase III trial using the same schedule and setting was terminated at a planned interim analysis due to emergent data indicating that the study would not reach statistical significance for the primary OS endpoint (48). It is currently unclear as to why the study failed to meet the primary endpoint, albeit a median OS of 21.1 months was reported for the placebo-treated group (vs. 20.4 months for vaccinated), well above the expected median of approximately 16 months, which may have confounded the data. A previous report suggests that patients with glioblastoma taking part in US-based phase II trials have significantly longer survival compared with historical data (49). The authors speculate that this may be due to the novel

agent being tested or advances in standard of care. If the latter is correct, the apparent improvement in survival found in the phase II rindopepimut study may have led to an overly optimistic prediction of clinical benefit and subsequent failure of the phase III trial. It is also possible that the reported loss of EGFRvIII from tumors during the vaccination period may have led to escape from immunosurveillance, an issue that the IMA950 vaccine attempts to address by simultaneous targeting of 11 different antigens (TUMAPs). Nevertheless, even though the study reported here clearly met predefined immune response success criteria, further clinical optimization should precede transition of IMA950 into the next phase of clinical development. This should include selection of the most appropriate adjuvant(s) and gaining a deeper understanding of how best to combine IMA950 with other immunotherapies, such as immune checkpoint inhibitors, to maximize the magnitude of immune response, as well as gaining a better understanding as to the optimal position and schedule of the vaccine relative to the current standard of care.

### Disclosure of Potential Conflicts of Interest

C. McBain is a consultant/advisory board member for Bristol-Myers Squibb. H. Singh-Jasuja has ownership interest (including patents) in Immatics Biotechnologies. No potential conflicts of interest were disclosed by the other authors.

### Authors' Contributions

**Conception and design:** R. Rampling, J. Lindner, N. Hilf, J. Peters, O. Schoor, H. Singh-Jasuja, S. Halford, J.W.A. Ritchie

**Development of methodology:** R. Rampling, P.J. Mulholland, A. Jackson, J. Lindner, S. Kutscher, J. Peters, H. Singh-Jasuja, S. Halford

**Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.):** S. Peoples, P.J. Mulholland, A. James, C.J. Twelves, C. McBain, S. Jefferies, A. Jackson, W. Stewart, J. Lindner, S. Kutscher, S. Halford  
**Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis):** R. Rampling, C.J. Twelves, A. Jackson, J. Lindner, S. Kutscher, N. Hilf, L. McGuigan, K. Hill, H. Singh-Jasuja, S. Halford, J.W.A. Ritchie

**Writing, review, and/or revision of the manuscript:** R. Rampling, P.J. Mulholland, A. James, C.J. Twelves, C. McBain, S. Jefferies, A. Jackson, W. Stewart, J. Lindner, S. Kutscher, N. Hilf, L. McGuigan, O. Schoor, H. Singh-Jasuja, S. Halford, J.W.A. Ritchie

**Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases):** A. Jackson, J. Lindner, L. McGuigan, O. Schoor, J.W.A. Ritchie

**Study supervision:** R. Rampling, L. McGuigan, J. Peters, K. Hill, H. Singh-Jasuja, S. Halford

**Other (contributed patients to study):** O. Al-Salihi

**Other (clinical study manager):** K. Hill

### Acknowledgments

Immatics Biotechnologies provided pharmacodynamic assay support and supplied IMA950 for this study.

### Grant Support

This work was supported by grant C222/A11422 from Cancer Research UK. This work was also managed and sponsored by the Cancer Research UK Centre for Drug Development.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Received February 24, 2016; revised May 6, 2016; accepted May 11, 2016; published OnlineFirst May 25, 2016.



## References

- Schwartzbaum JA, Fisher JL, Aldape KD, Wrensch M. Epidemiology and molecular pathology of glioma. *Nat Clin Pract Neurol* 2006;2:494–503.
- Stupp R, Mason WP, van den Bent MJ, Weller M, Fisher B, Taphoorn MJ, et al. Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma. *N Engl J Med* 2005;352:987–96.
- Dolecek TA, Propp JM, Stroup NE, Kruchko C. CBTRUS statistical report: primary brain and central nervous system tumors diagnosed in the United States in 2005–2009. *Neuro-oncology* 2012;14Suppl 5:v1–49.
- Dobes M, Khurana VG, Shadbolt B, Jain S, Smith SF, Smee R, et al. Increasing incidence of glioblastoma multiforme and meningioma, and decreasing incidence of Schwannoma (2000–2008): findings of a multi-center Australian study. *Surg Neurol Int* 2011;2:176.
- Johnson DR. Rising incidence of glioblastoma and meningioma in the United States: projections through 2050. *J Clin Oncol* 30, 2012(suppl; abstr 2065).
- Dutoit V, Herold-Mende C, Hilf N, Schoor O, Beckhove P, Bucher J, et al. Exploiting the glioblastoma peptidome to discover novel tumour-associated antigens for immunotherapy. *Brain* 2012;135:1042–54.
- Widenmeyer M, Griesemann H, Stevanovic S, Feyerabend S, Klein R, Attig S, et al. Promiscuous survivin peptide induces robust CD4+ T-cell responses in the majority of vaccinated cancer patients. *Int J Cancer* 2012;131:140–9.
- Li Y, Li A, Glas M, Lal B, Ying M, Sang Y, et al. c-Met signaling induces a reprogramming network and supports the glioblastoma stem-like phenotype. *Proc Natl Acad Sci U S A* 2011;108:9951–6.
- Melief CJ, van der Burg SH. Immunotherapy of established (pre)malignant disease by synthetic long peptide vaccines. *Nat Rev Cancer* 2008;8:351–60.
- Parmiani G, Castelli C, Pilla L, Santinami M, Colombo MP, Rivoltini L. Opposite immune functions of GM-CSF administered as vaccine adjuvant in cancer patients. *Ann Oncol* 2007;18:226–32.
- Macdonald DR, Cascino TL, Schold SC Jr, Cairncross JG. Response criteria for phase II studies of supratentorial malignant glioma. *J Clin Oncol* 1990;8:1277–80.
- Chudley L, McCann KJ, Coleman A, Cazaly AM, Bidmon N, Britten CM, et al. Harmonisation of short-term in vitro culture for the expansion of antigen-specific CD8(+) T cells with detection by ELISPOT and HLA-multimer staining. *Cancer Immunol Immunother* 2014;63:1199–211.
- Walter S, Weinschenk T, Stenzl A, Zdrojowy R, Pluzanska A, Szczylik C, et al. Multipptide immune response to cancer vaccine IMA901 after single-dose cyclophosphamide associates with longer patient survival. *Nat Med* 2012;18:1254–61.
- Britten CM, Janetzki S, Ben-Porat L, Clay TM, Kalos M, Maecker H, et al. Harmonization guidelines for HLA-peptide multimer assays derived from results of a large scale international proficiency panel of the Cancer Vaccine Consortium. *Cancer Immunol Immunother* 2009;58:1701–13.
- Seddiki N, Santner-Nanan B, Martinson J, Zaunders J, Sasson S, Landay A, et al. Expression of interleukin (IL)-2 and IL-7 receptors discriminates between human regulatory and activated T cells. *J Exp Med* 2006;203:1693–700.
- Okada H, Weller M, Huang R, Finocchiaro G, Gilbert MR, Wick W, et al. Immunotherapy response assessment in neuro-oncology: a report of the RANO working group. *Lancet Oncol* 2015;16:e534–42.
- Winney B, Boumertit A, Day T, Davison D, Echeta C, Evseeva I, et al. People of the British Isles: preliminary analysis of genotypes and surnames in a UK-control population. *Eur J Hum Genet* 2012;20:203–10.
- Kempuraj D, Devi RS, Madhappan B, Conti P, Nazer MY, Christodoulou S, et al. T lymphocyte subsets and immunoglobulins in intracranial tumor patients before and after treatment, and based on histological type of tumors. *Int J Immunopathol Pharmacol* 2004;17:57–64.
- Kocher M, Kunze S, Eich HT, Semrau R, Muller RP. Efficacy and toxicity of postoperative temozolomide radiochemotherapy in malignant glioma. *Strahlenther Onkol* 2005;181:157–63.
- Campbell DJ, Koch MA. Phenotypical and functional specialization of FOXP3+ regulatory T cells. *Nat Rev Immunol* 2011;11:119–30.
- Butt AQ, Mills KH. Immunosuppressive networks and checkpoints controlling antitumor immunity and their blockade in the development of cancer immunotherapeutics and vaccines. *Oncogene* 2014;33:4623–31.
- Chiba Y, Hashimoto N, Tsuboi A, Oka Y, Murao A, Kinoshita M, et al. Effects of concomitant temozolomide and radiation therapies on WT1-specific T-cells in malignant glioma. *Jpn J Clin Oncol* 2010;40:395–403.
- Sampson JH, Aldape KD, Archer GE, Coan A, Desjardins A, Friedman AH, et al. Greater chemotherapy-induced lymphopenia enhances tumor-specific immune responses that eliminate EGFRvIII-expressing tumor cells in patients with glioblastoma. *Neuro-oncology* 2011;13:324–33.
- Fadul CE, Fisher JL, Gui J, Hampton TH, Cote AL, Ernstoff MS. Immune modulation effects of concomitant temozolomide and radiation therapy on peripheral blood mononuclear cells in patients with glioblastoma multiforme. *Neuro-oncology* 2011;13:393–400.
- Ghiringhelli F, Menard C, Puig PE, Ladoire S, Roux S, Martin F, et al. Metronomic cyclophosphamide regimen selectively depletes CD4+CD25+ regulatory T cells and restores T and NK effector functions in end stage cancer patients. *Cancer Immunol Immunother* 2007;56:641–8.
- Lutsiak ME, Semnani RT, DePascalis R, Kashmiri SV, Schlom J, Sabzevari H. Inhibition of CD4(+)25+ T regulatory cell function implicated in enhanced immune response by low-dose cyclophosphamide. *Blood* 2005;105:2862–8.
- Inamoto T, Azuma H. Immunotherapy of genitourinary malignancies. *J Oncol* 2012;2012:397267.
- Nduom EK, Weller M, Heimberger AB. Immunosuppressive mechanisms in glioblastoma. *Neuro-oncology* 2015;17Suppl 7:vii9–vii14.
- Authier A, Farrand KJ, Broadley KW, Ancelet LR, Hunn MK, Stone S, et al. Enhanced immunosuppression by therapy-exposed glioblastoma multiforme tumor cells. *Int J Cancer* 2015;136:2566–78.
- Wainwright DA, Dey M, Chang A, Lesniak MS. Targeting tregs in malignant brain cancer: overcoming IDO. *Front Immunol* 2013;4:116.
- Berghoff AS, Kiesel B, Widhalm G, Rajky O, Ricken G, Wohrer A, et al. Programmed death ligand 1 expression and tumor-infiltrating lymphocytes in glioblastoma. *Neuro-oncology* 2015;17:1064–75.
- Wainwright DA, Chang AL, Dey M, Balyasnikova IV, Kim CK, Tobias A, et al. Durable therapeutic efficacy utilizing combinatorial blockade against IDO, CTLA-4, and PD-L1 in mice with brain tumors. *Clin Cancer Res* 2014;20:5290–301.
- Dranoff G, Jaffee E, Lazenby A, Golumbek P, Levitsky H, Brose K, et al. Vaccination with irradiated tumor cells engineered to secrete murine granulocyte-macrophage colony-stimulating factor stimulates potent, specific, and long-lasting anti-tumor immunity. *Proc Natl Acad Sci U S A* 1993;90:3539–43.
- Ahlers JD, Dunlop N, Alling DW, Nara PL, Berzofsky JA. Cytokine-adjunct steering of the immune response phenotype to HIV-1 vaccine constructs: granulocyte-macrophage colony-stimulating factor and TNF-alpha synergize with IL-12 to enhance induction of cytotoxic T lymphocytes. *J Immunol* 1997;158:3947–58.
- Hege KM, Jooss K, Pardoll D. GM-CSF gene-modified cancer cell immunotherapies: of mice and men. *Int Rev Immunol* 2006;25:321–52.
- Kaufman HL, Ruby CE, Hughes T, Slingluff CL Jr. Current status of granulocyte-macrophage colony-stimulating factor in the immunotherapy of melanoma. *J Immunother Cancer* 2014;2:11.
- Martins KA, Bavari S, Salazar AM. Vaccine adjuvant uses of poly-IC and derivatives. *Expert Rev Vaccines* 2015;14:447–59.
- Fehres CM, Bruijns SC, van Beelen AJ, Kalay H, Ambrosini M, Hooijberg E, et al. Topical rather than intradermal application of the TLR7 ligand imiquimod leads to human dermal dendritic cell maturation and CD8+ T-cell cross-priming. *Eur J Immunol* 2014;44:2415–24.
- Gupta S, Termini JM, Kanagavelu S, Stone GW. Design of vaccine adjuvants incorporating TNF superfamily ligands and TNF superfamily molecular mimics. *Immunol Res* 2013;57:303–10.
- Madondo MT, Quinn M, Plebanski M. Low dose cyclophosphamide: mechanisms of T cell modulation. *Cancer Treat Rev* 2016;42:3–9.
- Fu J, Malm JJ, Kadayakkara DK, Levitsky H, Pardoll D, Kim YJ. Preclinical evidence that PD1 blockade cooperates with cancer vaccine TEGVAX to elicit regression of established tumors. *Cancer Res* 2014;74:4042–52.
- Le DT, Lutz E, Uram JN, Sugar EA, Onners B, Solt S, et al. Evaluation of ipilimumab in combination with allogeneic pancreatic tumor cells transfected with a GM-CSF gene in previously treated pancreatic cancer. *J Immunother* 2013;36:382–9.
- Aruga A, Takeshita N, Kotera Y, Okuyama R, Matsushita N, Ohta T, et al. Long-term vaccination with multiple peptides derived from cancer-testis antigens can maintain a specific T-cell response and achieve

- disease stability in advanced biliary tract cancer. *Clin Cancer Res* 2013;19:2224–31.
44. Fukushima T, Takeshima H, Kataoka H. Anti-glioma therapy with temozolomide and status of the DNA-repair gene MGMT. *Anticancer Res* 2009;29:4845–54.
  45. Hoos A. Evolution of end points for cancer immunotherapy trials. *Ann Oncol* 2012;23Suppl 8:viii47–52.
  46. Del Vecchio CA, Wong AJ. Rindopepimut, a 14-mer injectable peptide vaccine against EGFRvIII for the potential treatment of glioblastoma multiforme. *Curr Opin Mol Ther* 2010;12:741–54.
  47. Schuster J, Lai RK, Recht LD, Reardon DA, Paleologos NA, Groves MD, et al. A phase II, multicenter trial of rindopepimut (CDX-110) in newly diagnosed glioblastoma: the ACT III study. *Neuro-oncology* 2015;17:854–61.
  48. Celldex Therapeutics. Data Safety and Monitoring Board recommends Celldex's phase 3 study of RINTEGA® (rindopepimut) in newly diagnosed glioblastoma be discontinued as it is unlikely to meet primary overall survival endpoint in patients with minimal residual disease 2016. [cited 2016 01 April]; Available from: <http://ir.celldex.com/releasedetail.cfm?ReleaseID=959021>.
  49. Grossman SA, Ye X, Piantadosi S, Desideri S, Nabors LB, Rosenfeld M, et al. Survival of patients with newly diagnosed glioblastoma treated with radiation and temozolomide in research studies in the United States. *Clin Cancer Res* 2010;16:2443–9.