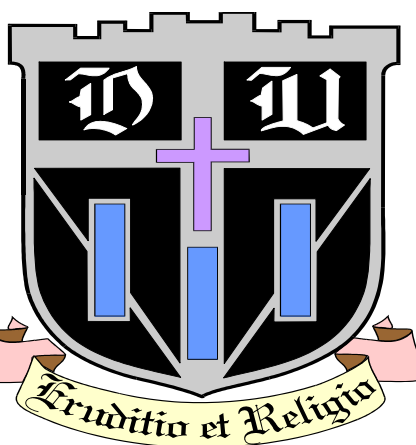




# Epidermal growth factor receptor variant III (EGFRvIII)-targeted vaccine (CDX-110/PF-04948568) in GBM

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## ABSTRACT

**Background:** Unlike conventional therapies for GBM, immunologic targeting of tumor-specific gene mutations allows precise eradication of neoplastic cells with reduced toxicity. EGFRvIII is a constitutively activated and immunogenic mutation not expressed in normal tissues, but widely expressed in GBM and other neoplasms. The cancer vaccine CDX-110 (PF-04948568) is comprised of an EGFRvIII-specific peptide sequence linked to keyhole limpet hemocyanin (KLH). **Methods:** A phase II multi-center trial assessed the immunogenicity and efficacy of CDX-110 in patients with newly-diagnosed, EGFRvIII+ GBM. After resection and radiation / TMZ, patients received CDX-110 vaccinations biweekly x 3, then monthly until tumor progression. Sequential cohorts received CDX-110 alone [ACTIVATE (n = 18)] or in combination with TMZ (200 mg/m<sup>2</sup> x 5/28 days [ACT II A (n = 12)] or (100 mg/m<sup>2</sup> x 21/28 days [ACT II B (n=10)]). **Results:** Reversible systemic drug hypersensitivity reactions were seen in 1 ACTIVATE and 4 ACT II patients. Two patients had non-specific changes on MRI which were possibly due to the vaccine but which resolved. Despite grade 2 or 3 lymphopenia in all ACT II patients, EGFRvIII-specific immune responses were generated in all ACT II patients, and all immune responses were sustained or enhanced during subsequent TMZ cycles. Although ACT II B patients had more severe TMZ-induced lymphopenia, they developed greater EGFRvIII-specific immune responses (p = 0.028) when compared to ACT II A. EGFRvIII-specific IgG1 also increased in avidity with vaccination (K<sub>a</sub>>>2x10<sup>9</sup>M<sup>-1</sup>) in a randomly selected subset of 4 patients (p = 0.000068). Of the 23 recurrent tumors studied, 18 lost EGFRvIII expression (p = 0.001). There are no significant differences between ACT II A and B in estimated median TTP (18.5 vs. 14.9 months, p = 0.31) and OS (23.6 vs. 19.9 months, p = 0.75). ACTIVATE TTP (14.2 months) and OS (26.0 months) and ACT II TTP (15.2 months) and OS (23.6 months) compare favorably to a TMZ-treated, matched historical control group (TTP: 6.3 months; OS: 15.0 months). **Conclusions:** CDX-110 vaccination in patients with GBM appears very promising. TMZ enhances immune responses despite lymphodepletion. CDX-110 with simultaneous TMZ is under further investigation in a larger phase II trial.

## INTRODUCTION

Immunologic targeting of tumor-specific gene mutations may allow more precise eradication of neoplastic cells. Most well-characterized tumor antigens, however, are over expressed normal proteins that have triggered immunologic tolerance to some degree. This compromises their effectiveness as tumor rejection antigens and poses a risk of autoimmunity if these normal proteins are effectively targeted.

The epidermal growth factor receptor variant III (EGFRvIII) is a consistent and tumor-specific mutation widely expressed in GBMs and other neoplasms. This mutation encodes a constitutively active tyrosine kinase that enhances tumorigenicity and tumor cell migration and confers radiation and chemotherapeutic resistance to tumor cells. The new glycine inserted at the fusion junction of normally distant parts of the extracellular domain also results in a tumor-specific epitope not found in any normal adult tissues. Thus, several factors make EGFRvIII an ideal target for antitumor immunotherapy.

A substantial barrier to the activation of antitumor immune responses in patients with GBM is their well-documented impairment of T- and B-cell immunity manifest by a CD4 count <200 /μL and a substantially increased level of T<sub>Regs</sub>.

Temozolomide (TMZ) is an alkylating chemotherapeutic that provides a survival benefit in patients with GBM and has become part of the standard regimen used to treat these patients. Mild to moderate myelosuppression is the only major dose limiting toxicity with nadirs occurring 21-28 days after drug administration. In animal studies the activity of TMZ has been shown to be schedule dependent, which is likely related to increased depletion of O<sup>6</sup>-alkylguanine-DNA-alkyltransferase (MGMT) which otherwise can recover within 24 hours. Based on this rationale, dose-intensified regimens, for example treating for 21 days of a 28 day cycle, are currently being tested in humans (RTOG-0525). These prolonged schedules are also limited by myelosuppression which is most severe in the lymphoid compartment with the majority of patients experiencing Grade 3 or 4 lymphopenia. Unfortunately, this profound lymphopenia would be expected to limit further any vaccine-induced anti-tumor immunotherapy.

## METHODS

Two sequential phase II, multi-center trials were undertaken to assess the immunogenicity and potential efficacy of an EGFRvIII-targeted peptide vaccine in patients with newly-diagnosed, EGFRvIII-expressing GBM with minimal residual disease. Intradermal vaccinations were given until toxicity or tumor progression was observed. Sample size was calculated to differentiate between progression-free rates of 20% and 40% six months after vaccination. Historical cohorts of unvaccinated patients matched for certain eligibility criteria, prognostic factors, and temozolomide (TMZ) treatment were used for comparison.

The historical cohort selected for TTP and OS comparisons were all treated after 1993 and were obtained from the University of Texas M. D. Anderson Cancer Center. In the historical control cohort (n=17) all patients were adults, had EGFRvIII-expressing primary GBMs, a KPS ≥80%, a resection of >95% of the original tumor volume and had been treated with radiation and TMZ. Patients with tumor progression during or within 4 weeks of completing radiation therapy were also excluded from this historical control cohort.

PEPvIII (LEEKKGNYVVDHC) is a 13-amino-acid peptide with an additional terminal cysteine that spans the EGFRvIII mutation. The peptide preparation was >95% pure as assessed by high-pressure liquid chromatography and was conjugated to keyhole limpet hemocyanin (KLH; Biosyn Corporation, Carlsbad, CA) at a 1:1 ratio (w/w) (PEPvIII-KLH) using the heterobifunctional cross-linker sulfo-succinimidyl 6-[3'-(2-pyridyldithio)-propionamido]hexanoate (Pierce, Rockford, IL).

The ACTII Trial was similar to the ACTIVATE trial except:

- After the first 3 vaccines the patients received their CDX-110 vaccine in concert with monthly cycles of TMZ
- There were two monthly regimens of TMZ:

**ACTII A:** 200 mg/m<sup>2</sup> for 5/28 day cycle

**ACTII B:** 100 mg/m<sup>2</sup> for 21/28 day cycle

### Inclusion Criteria For ACTIVATE and ACT II

- Newly diagnosed Glioblastoma Multiforme with expression of EGFRvIII.
- Patients must have a 95% or greater volumetric resection.
- Patients must have a Karnofsky Performance Status of > 80% and a Curran Group Status of I-IV.
- Patients must have no evidence of progression on MRI following chemo/RT.
- Patients in ACTII Trial must be able to tolerate temozolomide

### Exclusion Criteria for ACTIVATE and ACT II

- Patients must not be pregnant or breast-feeding during the study period.
- Patients must not be on corticosteroids above physiologic levels, defined as < 2 mg of dexamethasone per day.
- Patients must not have leptomeningeal disease.
- Patients with an active infection requiring treatment or having an unexplained febrile illness (T<sub>max</sub> > 101.5 F).
- Patients with inflammatory bowel disease, lupus erythematosus, rheumatoid arthritis or other autoimmune disease.
- Patients with known immunosuppressive disease or known human immunodeficiency virus infection.
- Patients with unstable or severe intercurrent medical conditions such as severe heart and lung disease or active hepatitis.

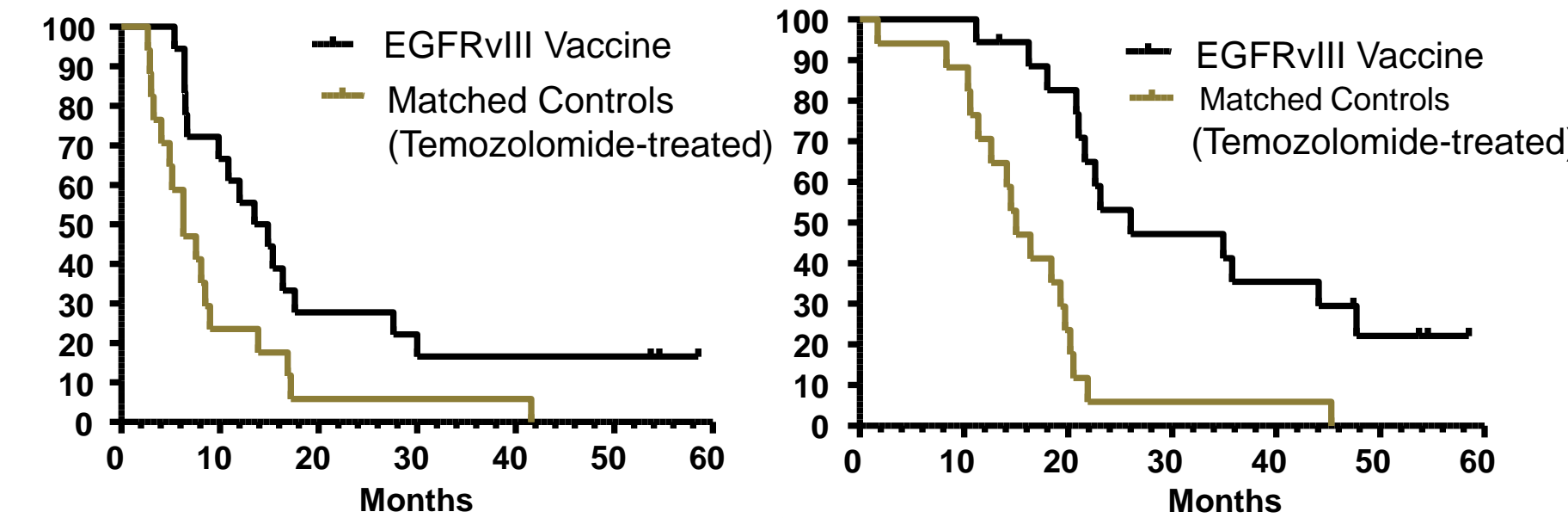
### ACTIVATE

XRT ~2 Gy per fraction ~60 Gy Total Daily TMZ at 75 mg/m <sup>2</sup> /day	Day 0 PEPvIII-KLH Vaccine (Vaccine 1)	Day 14 PEPvIII-KLH Vaccine (Vaccine 2)	Day 28 PEPvIII-KLH Vaccine (Vaccine 3)	Day 58 PEPvIII-KLH Vaccine (Vaccine 4) Repeat Monthly Until Progression
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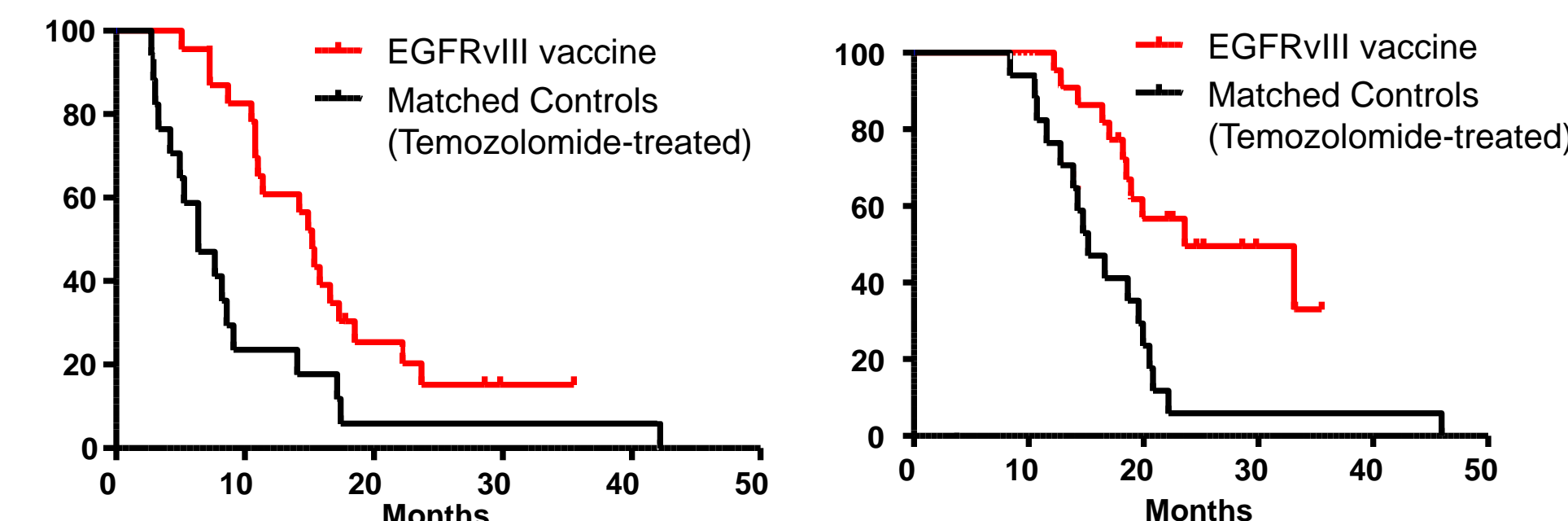
### ACTII

XRT ~2 Gy per fraction ~60 Gy Total Daily TMZ at 75 mg/m <sup>2</sup> /day	Day 0 PEPvIII-KLH Vaccine (Vaccine 1)	Day 14 PEPvIII-KLH Vaccine (Vaccine 2)	Day 28 PEPvIII-KLH Vaccine (Vaccine 3)	↑↑↑↑↑ TMZ Day 35 ACTII A: 200mg/m <sup>2</sup> (5/28 days) or ACTII B: 100 mg/m <sup>2</sup> (21/28 days)	Day 58 PEPvIII-KLH Vaccine (Vaccine 4) Repeat Monthly Until Progression
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**Figure 1: ACTIVATE Time to Progression (TTP) Overall Survival (OS)**



**ACT II Time to Progression (TTP) Overall Survival (OS)**



**Table 1: TTP and OS in ACTIVATE and ACT II**

	ACTIVATE / Comparison to Control <sup>1</sup> (n=18)	ACTII / Comparison to Control <sup>1</sup> (n=22)	Matched Historical Control (n=17)
Median TTP (months[CI95])	14.2 (9.9, 17.6) p=0.041 HR=2.2 (1.0, 4.8)	15.2 (11, 18.5) p=0.0237 HR=0.35 (0.14, 0.87)	6.3 (4.1, 9.0)
progression-free at 6 months (%[CI95])	94% (67%, 99%)	95.5% (71.9%, 99.3%)	59% (33%, 78%)
Median OS (months[CI95])	26.0 (21.0, 47.7) p=0.001 HR=5.1 (1.9, 13.9)	23.6* (18.5, 33.1) p=0.0194 HR=0.23 (0.07, 0.79)	15.0 (11.4, 19.7)

All analysis are from time of surgery. For ACT II, median TTP from vaccination was 11.8 months (81, 15.6) and OS from vaccination was 19.3 months (15.6, 30.7).

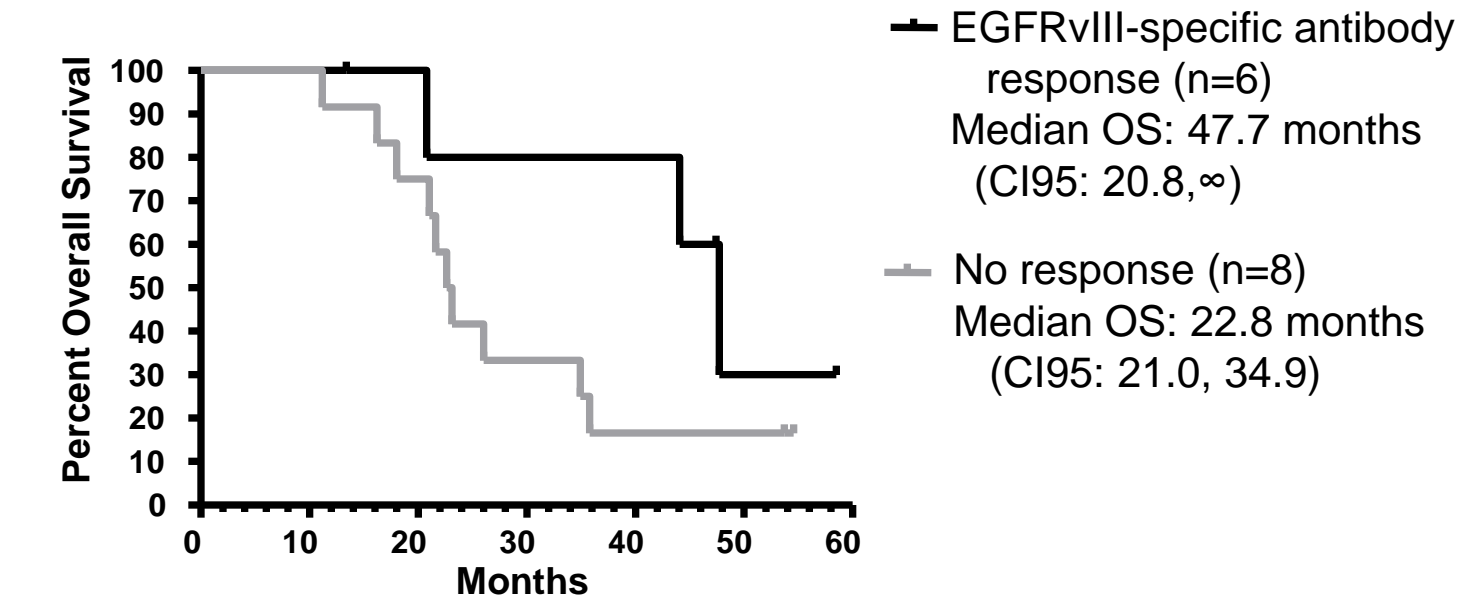
- Analysis adjusted for age, Karnofsky performance status and TMZ usage.

\* ACT II OS is not yet final

**Table 2: No difference in TTP or OS between ACT II A (standard TMZ) and ACT II B (dose-intensified TMZ)**

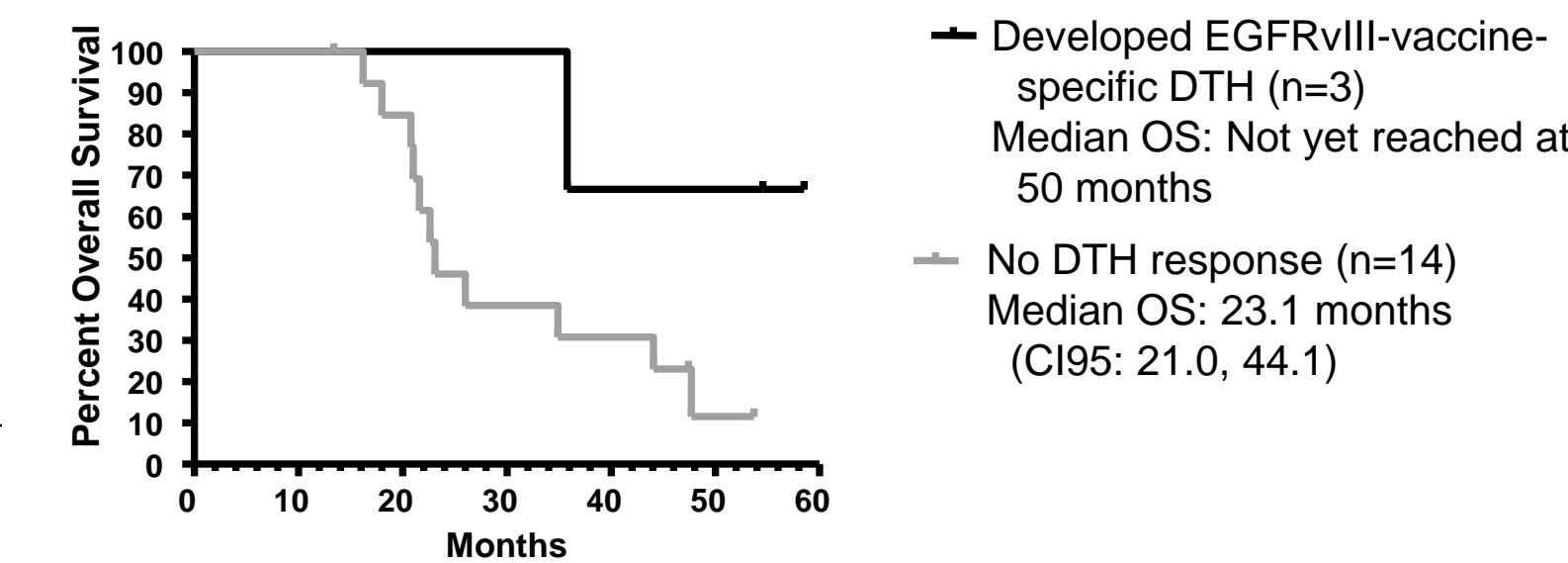
	ACT II A (n=12)	ACT II B (n=10)	ACT II A vs. B		
			univariate analysis	adjusted for age and KPS	adjusted for MGMT
Progression-free at 6 months (%[CI95])					
post-surgery	100%	90% (47%, 99%)	p=0.5018	p=0.5828	p=0.9239
from vaccination	75% (41%, 91%)	90% (47%, 98%)	p=0.5848	p=0.6604	p=0.8425
12-month OS (%[CI95])					
post-surgery	100%	100%	p=0.5028	p=0.3850	p=0.4353
from vaccination	83% (48%, 96%)	90% (47%, 98%)	p=0.3969	p=0.2825	p=0.3670

**Figure 2: Overall survival correlates with humoral responses and delayed-type hypersensitivity responses (ACTIVATE)**



After adjustment for age, KPS, and MGMT methylation, the OS was found to be greater for patients that developed antibody responses (p=0.049; HR=0.09 [CI95: 0.008, 0.99]).

Analysis completed for ACTIVATE patients with serum available to test for EGFRvIII-specific antibody titers (n=14). The same analysis could not be done for ACT II as all patients had measurable immune responses

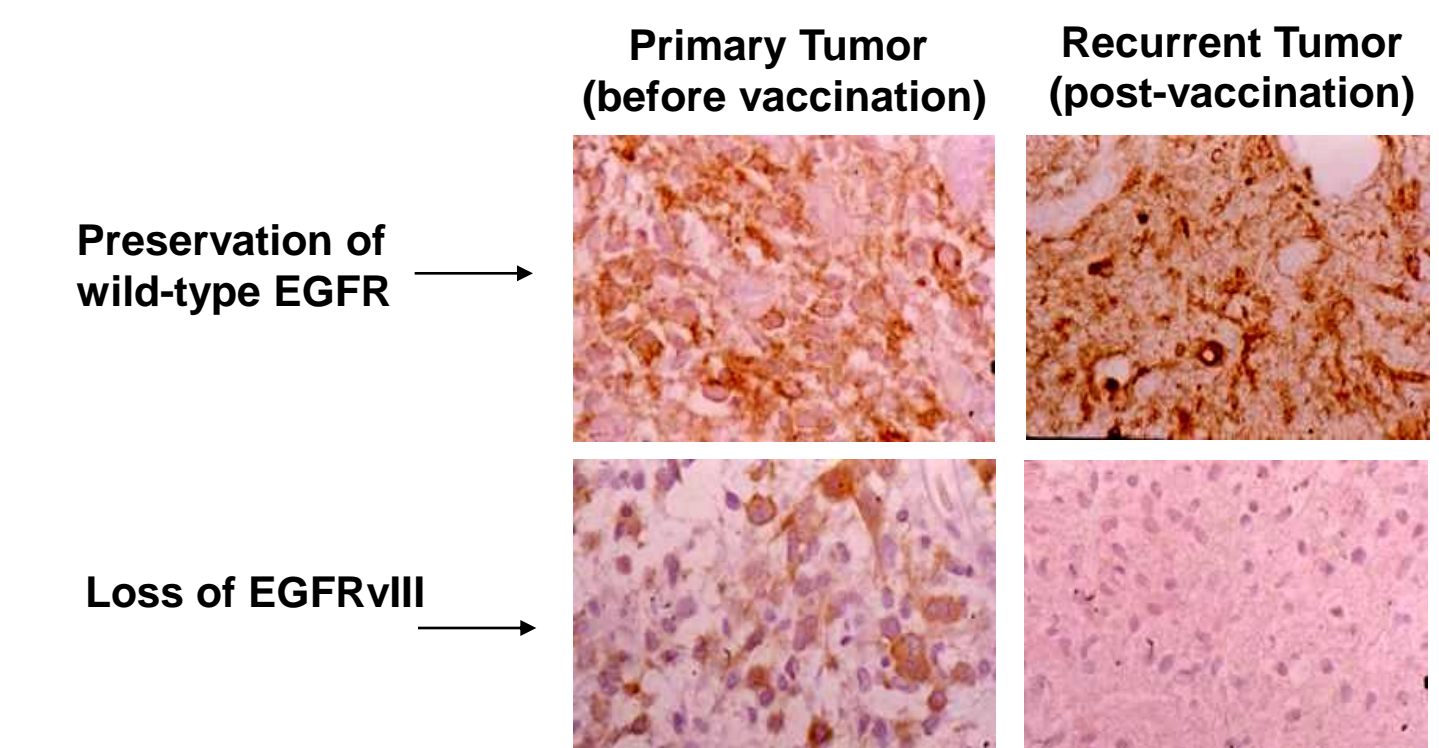


Patients developing PEPvIII DTH responses have a significantly longer OS (p=0.03).

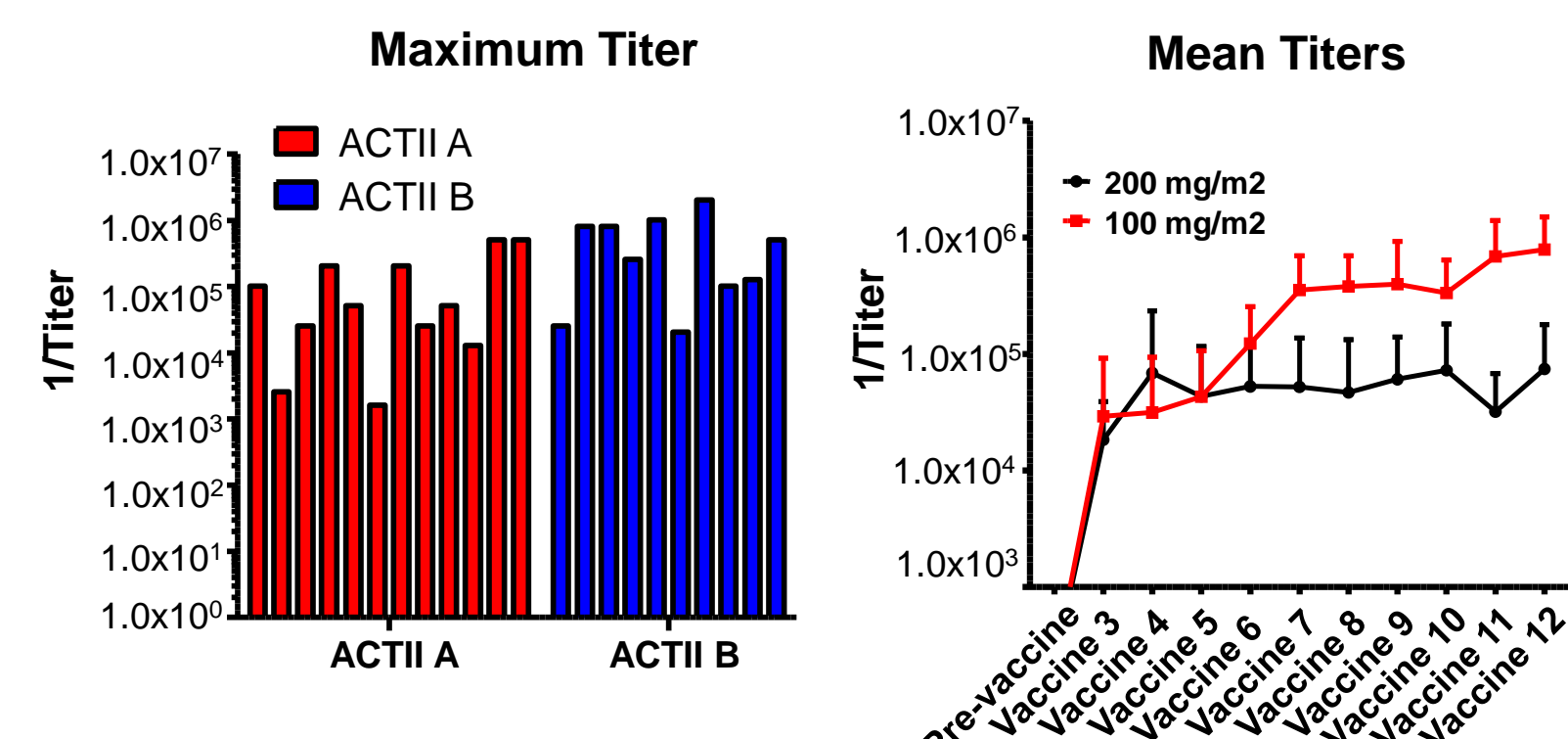
**Table 3: Loss of EGFRvIII Expression in Recurrent Tumors (Immunohistochemical Analysis)**

	ACTIVATE (n=11)	ACT II (n=13)
Patients with loss of EGFRvIII expression by IHC (%[CI95])	82% (48%, 97%) <sup>1</sup> p<0.0001	92% (64.0%, 99.8%) <sup>2</sup> p<0.001

- One patient with <1% of cells staining
- Positive IHC defined as 10% of cells staining for EGFRvIII

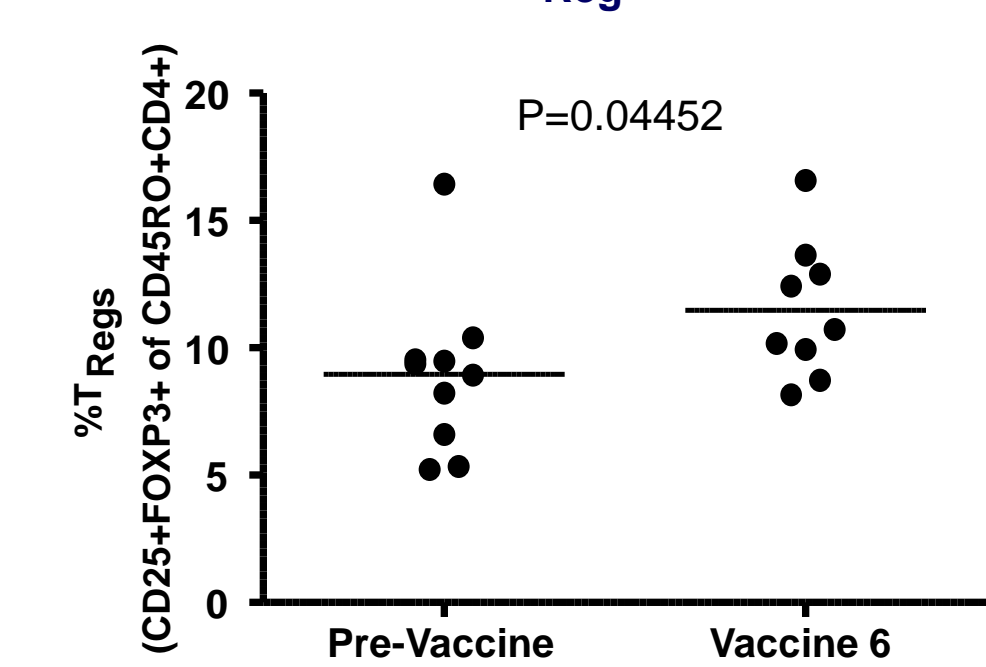


**Figure 3: Humoral responses (ACT II)**



Serum titers were measured to the EGFRvIII peptide by ELISA before the first vaccine, after vaccine 3 and before each successive vaccine. No patients had a humoral response before vaccination and all vaccinated patients had a response post 3 vaccinations.

**Figure 4: T<sub>Reg</sub> Levels (ACT II B)**



There is a statistically significant increase in T<sub>Reg</sub> levels by vaccine 6 in patients treated with TMZ, suggesting that the increased temozolomide dosage is not influencing the increased humoral response in ACTII B through decreasing T<sub>Reg</sub> levels.

## DISCUSSION

Despite all of our patients having tumors that expressed EGFRvIII, none had developed immunity to EGFRvIII prior to vaccination. However, despite endogenous immunosuppression and profound TMZ-induced lymphopenia, vaccination with EGFRvIII-KLH generated de novo potent and highly specific immune responses against this tumor specific antigen in all ACT II patients without evidence of autoimmunity. All patients experienced tolerable local reactions and four patients experienced reversible systemic allergic reactions.

The induced EGFRvIII-specific antibodies were capable of recognizing full-length EGFRvIII on the surface of tumor cells and were also capable of eliciting antibody dependent cell mediated cytotoxicity. This is consistent with our previous work showing that unarmored EGFRvIII-specific antibodies or passive transfer of EGFRvIII immune serum can mediate tumor elimination or protect against tumor challenge in murine models.

In patients receiving dose-intensified TMZ, who all experienced a Grade III lymphopenia, vaccine induced humoral and cell-mediated immune responses were dramatically enhanced when compared to less myelosuppressive TMZ doses and increased with serial TMZ-induced lymphodepletion. In these patients, EGFRvIII-specific vaccine titers exceeded 1:2,000,000 and delayed type hypersensitivity reactions exceeded 20 cm<sup>2</sup>. This was in contradistinction to pre-existing recall responses, for example to Candida, which were rapidly abolished during TMZ cycles in these patients. These results highlight vaccination during hematopoietic recovery from serial TMZ as a novel strategy for engendering potent and broad humoral and cellular antitumor immunity. In the context of serial TMZ-induced lymphodepletion, these vaccines were sufficiently potent to translate into significant antibody responses while leveraging the myelosuppressive side effects of this efficacious chemotherapeutic.