

University of Groningen

## Targeted therapy, molecular imaging and biomarkers in cancer treatment

den Hollander, Martha Willemine

**IMPORTANT NOTE: You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.**

*Document Version*

Publisher's PDF, also known as Version of record

*Publication date:*

2015

[Link to publication in University of Groningen/UMCG research database](#)

*Citation for published version (APA):*

den Hollander, M. W. (2015). *Targeted therapy, molecular imaging and biomarkers in cancer treatment: Getting more personalized*. University of Groningen.

### Copyright

Other than for strictly personal use, it is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), unless the work is under an open content license (like Creative Commons).

### Take-down policy

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

*Downloaded from the University of Groningen/UMCG research database (Pure): <http://www.rug.nl/research/portal>. For technical reasons the number of authors shown on this cover page is limited to 10 maximum.*

# Chapter 5

## **TGF- $\beta$ antibody uptake in recurrent high grade glioma imaged with $^{89}\text{Zr}$ -fresolimumab PET**

M.W. den Hollander<sup>1</sup>, F. Bensch<sup>1</sup>, A.W.J.M. Glaudemans<sup>2</sup>, T.H. Oude Munnink<sup>1</sup>, R.H. Enting<sup>3</sup>, W.F.A. den Dunnen<sup>4</sup>, M.A.A.M. Heesters<sup>5</sup>, F.A.E. Kruyt<sup>1</sup>, M.N. Lub-de Hooge<sup>2,6</sup>, JC de Groot<sup>7</sup>, J. Pearlberg<sup>8</sup>, J.A. Gietema<sup>1</sup>, E.G.E. de Vries<sup>1</sup>, A.M.E. Walenkamp<sup>1</sup>

Departments of <sup>1</sup>Medical Oncology, <sup>2</sup>Nuclear Medicine and Molecular Imaging, <sup>3</sup>Neurology, <sup>4</sup>Pathology, <sup>5</sup>Radiotherapy, <sup>6</sup>Clinical Pharmacy and Pharmacology, <sup>7</sup>Radiology, University of Groningen, University Medical Center Groningen, Groningen, The Netherlands, <sup>8</sup>Sanofi-Aventis Oncology, Cambridge, MA, USA

J Nucl Med. 2015 Jul 1

*Previously presented at the ASCO Annual Meeting 2013*

## ABSTRACT

**Background:** Transforming growth factor- $\beta$  (TGF- $\beta$ ) signaling is involved in glioma development. The monoclonal antibody fresolimumab (GC1008) can neutralize all mammalian isoforms of TGF- $\beta$  and tumor uptake can be visualized and quantified with  $^{89}\text{Zr}$ -fresolimumab PET in mice. The aim of this study was to investigate the fresolimumab uptake in recurrent high grade gliomas using  $^{89}\text{Zr}$ -fresolimumab PET and to assess treatment outcome in patients with recurrent high grade glioma treated with fresolimumab.

**Methods:** Patients with recurrent glioma were eligible. After intravenous administration of 37 megabecquerel (MBq) (5 mg)  $^{89}\text{Zr}$ -fresolimumab, PET scans were acquired on day 2 and/or day 4 after tracer injection. Thereafter, patients were treated with 5 mg/kg fresolimumab intravenously every 3 weeks.  $^{89}\text{Zr}$ -fresolimumab tumor uptake was quantified as maximum standardized uptake value ( $\text{SUV}_{\text{max}}$ ). MRI scans for response evaluation were performed after 3 infusions or as clinically indicated.

**Results:** Included were 12 recurrent high grade glioma patients: ten glioblastoma, one anaplastic oligodendroglioma and one anaplastic astrocytoma. All patients underwent an  $^{89}\text{Zr}$ -fresolimumab PET scan 4 days after injection. In four patients an additional PET scan was performed on day 2 after injection.  $\text{SUV}_{\text{max}}$  on day 4 in tumor lesions was 4.6 (1.5 - 13.9) versus a median  $\text{SUV}_{\text{mean}}$  of 0.3 (0.2 - 0.5) in normal brain tissue. All patients showed clinical and/or radiological progression after 1-3 infusions fresolimumab. Median progression free survival was 61 days (25-80) and median overall survival 106 days (37-417).

**Conclusion:**  $^{89}\text{Zr}$ -fresolimumab penetrated recurrent high grade gliomas very well, however this did not result in clinical benefit.

## INTRODUCTION

High grade gliomas are rapidly progressive brain tumors that are divided into anaplastic gliomas and glioblastomas based upon their histopathologic features. The 5-year survival rates for anaplastic oligodendroglioma, anaplastic astrocytoma and glioblastoma (GBM) are 49%, 25% and 5%, respectively [1]. Apart from surgery, the standard treatment of gliomas is currently based on tumor cell death induction by radiotherapy and chemotherapy. Given the modest treatment results novel strategies for the treatment of malignant glioma are needed.

Transforming growth factor- $\beta$  (TGF- $\beta$ ) acts as a tumor promoter in advanced tumors where it induces proliferation and metastasis and suppresses the immune response [2]. TGF- $\beta$  and its receptors are overexpressed in GBM and TGF- $\beta$  signaling is involved in multiple steps of GBM development and invasion [3-5]. Plasma TGF- $\beta$  levels are elevated in GBM patients and decrease after surgical tumor resection [6]. In addition, progression-free survival (PFS) and overall survival (OS) are decreased in glioma patients with high levels of phosphorylated SMAD2 (p-SMAD2), the substrate of TGF- $\beta$  receptor I, compared with glioma patients with low levels of p-SMAD2 [7]. These features make TGF- $\beta$  a promising target molecule for therapeutic approaches in recurrent glioma and therefore several TGF- $\beta$ -inhibitors are under investigation in this setting [8].

Fresolimumab (GC1008) is a monoclonal antibody capable of neutralizing all mammalian isoforms of TGF- $\beta$  (i.e., 1, 2, and 3) [9]. In a phase 1 study with fresolimumab in patients with melanoma and renal cell carcinoma, six patients achieved stable disease and one patient had a partial response [10]. In a phase 2 study in 13 mesothelioma patients stable disease was seen in three patients at 3 months [11].

Current standard of care and experimental treatment results in patients with recurrent high grade glioma are disappointing. It is often suggested that this is due to the impermeability of the blood brain barrier which may prevent drugs from reaching the tumor [12]. For therapeutic success in brain tumors, it is essential for a monoclonal antibody like fresolimumab to reach the target site in the brain. In tumor xenograft models, tumor uptake could be visualized and quantified with Zirconium-89 (<sup>89</sup>Zr) fresolimumab PET [13]. Therefore the aim of this study was to visualize and quantify fresolimumab uptake in recurrent high grade glioma using <sup>89</sup>Zr-fresolimumab PET. In addition, we evaluated the effect of treatment with fresolimumab in recurrent high grade glioma patients.

## MATERIAL AND METHODS

### Patients

Adult patients with recurrent glioma with one or more contrast enhancing lesions of at least 20 mm on MRI were eligible. Main other inclusion criteria were: WHO performance score 0 - 2, adequate bone marrow, coagulation, kidney and liver function and negative tests for hepatitis B, C and HIV. Previous surgery, radiotherapy, chemotherapy or investigational agents should have been >4 weeks prior to inclusion (>6 weeks for nitrosourea, or monoclonal antibodies) and patients must have recovered from previous treatment. Main exclusion criteria were: history of

ascites or pleural effusions, active hypercoagulability states or use of anti-coagulants, hypercalcemia, pregnancy or nursing mothers, diagnosis with other malignancies (unless curatively treated), organ transplants, immunosuppressive therapy, active infection, autoimmune disease, and other significant uncontrolled medical illnesses.

This study has been approved by the local medical ethical committee and registered in a clinical trial register (Trial registration ID: NCT01472731). All patients gave written informed consent. A data safety monitoring board reviewed the progress and safety during the study.

### **Treatment**

Patients were treated with 5 mg/kg fresolimumab (provided by Genzyme (Sanofi-Aventis Oncology)) intravenously every 3 weeks until radiological or clinical progression or unacceptable toxicity. Fresolimumab was administered over 90 minutes for the first infusion, thereafter over 60 minutes and finally 30 minutes if no infusion related reactions occurred. Within 30 minutes before infusion, patients received acetaminophen (500 mg) and clemastine (2 mg) as premedication. All adverse events were recorded and graded according to CTCAE version 4. PFS and OS were calculated from date of informed consent to date of disease progression on MRI, clinical progression or death.

### **Imaging**

Conjugation and radio labeling of fresolimumab was performed under good manufacturing conditions (GMP) as previously described [13]. Before start of treatment with fresolimumab, patients were injected with 37 MBq (5 mg)  $^{89}\text{Zr}$ -fresolimumab. The radioactive dose of 37 MBq and the protein dose of 5 mg results in a specific activity of 7.4 MBq/mg. Thereafter, patients were observed for 2 hours for possible infusion related reactions.

$^{89}\text{Zr}$ -fresolimumab PET scans were acquired on day 4 after injection. To assess the tumor accumulation of  $^{89}\text{Zr}$ -fresolimumab over time, an additional scan was acquired on day 2 after injection in some patients. Normal organ distribution of  $^{89}\text{Zr}$ -fresolimumab was assessed by whole body PET scans. The images were acquired using two PET camera systems (ECAT HR+, Siemens Medical Systems, Knoxville, TN; mCT Biograph, Siemens Medical Systems, Knoxville, TN). Acquisition time for the ECAT HR+ PET camera was 10 minutes per bed position on day 2 after injection (of which 20% is transmission time). On day 4 after injection, imaging time was prolonged to 12 minutes per bed position to correct for decay time. For the mCT camera, imaging time was shorter (5 minutes per bed position). All scans were reviewed and analyzed by a nuclear medicine physician (AG) and an investigator (MdH). All attenuation-corrected PET images and MRI series (gadolinium enhanced T1, performed within 4 weeks before start of the study) were retrospectively fused by using a commercially available software program (esoft, 3D fusion, Siemens Medical Solutions) on a Siemens Workstation (Syngo MMWP, Siemens Medical Solutions) to identify tumor lesions. The two datasets were aligned based on mutual information using the anatomical contours of the loaded datasets. Regions Of Interest (ROIs) were drawn around the tumor lesions on the PET scans (MdH). In normal organs ROIs were drawn in the same area of the organs for all patients.  $^{89}\text{Zr}$ -fresolimumab uptake was quantified using

AMIDE Medical Image Data Examiner software (Stanford University, Palo Alto, CA) version 0.9.2 to calculate the standardized uptake value (SUV) [14]. The maximum SUV ( $SUV_{max}$ ) of the tumor lesions and the mean SUV ( $SUV_{mean}$ ) of normal organs including blood (measured in the sinus confluens and iliac artery) was calculated.

Follow-up brain MRI scans (1.5T using T1, T2 and contrast enhanced 3D T1 Gradient echo sequences) were performed after every 3 treatment cycles (every 9 weeks) or as clinically indicated. MRI data for this study were assessed by a neuroradiologist (JCdG) using the Macdonald criteria for tumor response evaluation [15].

### Plasma pharmacokinetics and biomarkers

Heparin plasma samples were collected from patients 1 hour after injection and at the time of PET scanning for <sup>89</sup>Zr-fresolimumab pharmacokinetics. Plasma samples were counted in a gamma counter and the tracer concentration in plasma was calculated using a calibration graph.

Before start of fresolimumab treatment, citrate plasma samples were collected. Blood samples were drawn without tourniquet when possible, immediately placed on ice and centrifuged at 2500 g for 30 minutes at 4 °C without brake. Plasma samples were stored at -70 °C. In these samples, total TGF-β1 was analyzed using a human TGF-β1 immunoassay (Quantikine, R&D Systems Minneapolis, MN).

p-SMAD2 was analyzed as a read out of TGF-β signaling in archival paraffin embedded primary tumor tissue of all patients. Formalin fixed, paraffin embedded 3-μm tissue sections were mounted on microscope slides and dried overnight at 55°C. Tissue sections were dewaxed in xylene, and rehydrated in graded series of ethanol. Sections were subjected to microwave pretreatment with pH 6.0 citrate buffer for staining of p-SMAD2 (# 3101 Cell Signaling Technology, Inc. Danvers, MA). Subsequently sections were treated with 0.3% H<sub>2</sub>O<sub>2</sub> for 30 minutes, blocked for 1 hour with 2% BSA to reduce nonspecific antibody binding and were incubated with primary antibody. All antibody solutions were made in PBS with 1% BSA and 0.1% TritonX-100. Incubation at 4°C overnight was followed by incubation with goat anti-rabbit antibody conjugated to peroxidase (DAKO, Heverlee, Belgium) and subsequently with rabbit anti-goat antibody conjugated to peroxidase (DAKO). Staining was visualized by 3,3'-diaminobenzidine and sections were counterstained with hematoxylin and mounted. As negative control, primary antibody was omitted and incubations were performed as described above.

### Statistical Analysis

In the protocol 2 stopping rules were defined. The study would be terminated i) after inclusion of six patients if no <sup>89</sup>Zr-fresolimumab uptake was seen on the PET scan; and ii) after inclusion of 12 patients if treatment with fresolimumab showed no clinical benefit. If clinical benefit was seen a maximum of 20 patients could be included. Statistical analyses were performed using the Pearson correlation test and the Mann-Whitney U test using IBM SPSS statistics 20. Data are presented as median with range unless stated otherwise. Two-sided *P* - values of 0.05 or less were considered to indicate significance. Graphs were made using GraphPad Prism version 5.00 for Windows.

## RESULTS

### Patients and treatment

Twelve patients with recurrent high grade glioma (nine primary glioblastoma, one secondary glioblastoma (WHO Grade IV), one secondary anaplastic oligodendroglioma and one secondary anaplastic astrocytoma (WHO grade III) were enrolled in this study (Table 1). Patients were previously treated with 2 lines of treatment (1 – 8).

Table 1: Patient characteristics.

Characteristic	No
Number of patients	12
Age, years	
Median	51
Range	32-68
Sex	
Male	6
Female	6
Tumor type	
Glioblastoma	10
Anaplastic astrocytoma	1
Anaplastic oligodendroglioma	1
No of previous treatments	
Median (range)	2 (1-8)
Previous treatment	
Surgery	11
Radiotherapy + temozolomide	9
Radiotherapy	2
Re-resection	5
Re-irradiation	4
Temozolomide	3
Lomustine	2
Bevacizumab	1

Two patients received 1 infusion of fresolimumab, five patients received 2 infusions and five patients received 3 infusions. All patients showed clinical progressive disease during treatment or progressive disease on the first on-treatment MRI scan. PFS was 61 days (25 - 80) and OS 106 days (37 - 417). In the absence of clinical benefit the study was closed after the first 12 patients.

There were no adverse events related to tracer injection. In 12 patients 69 non hematologic adverse events, mostly grade 1 or 2 and mostly related to progression of disease, were observed during the study. Thirteen hematologic grade 1 adverse events were registered. The most common adverse events were neurologic deterioration, headache, skin disorders,

nausea and fatigue (Table 2). Adverse events that were considered as possibly related to fresolimumab were acneiform rash (grade 1, 1 patient), dry skin (grade 1, 1 patient), fatigue (grade 2, 2 patients), thrombocytopenia (grade 1, 1 patient) and epistaxis (grade 1, 1 patient). Four serious adverse events were recorded, of which 3 were neurologic worsening related to progressive disease and one was pain related to an osteoporotic vertebra fracture, all assessed unrelated to fresolimumab.

Table 2: Hematologic adverse events and most common non hematologic adverse events.

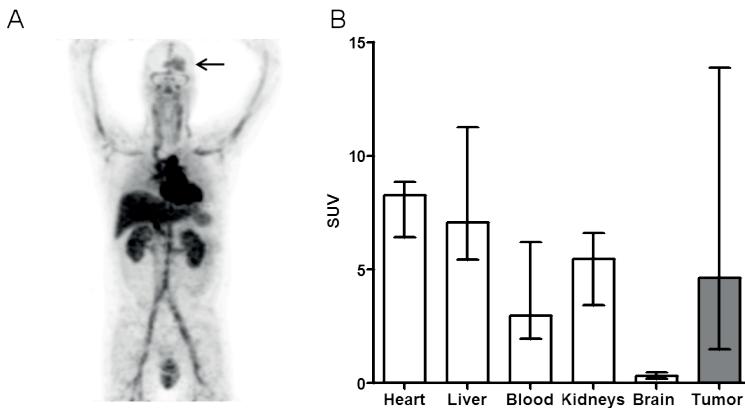
Adverse event	No of cases	Grade 1	Grade 2	Grade 3
Neurologic deterioration	16	3	8	5
Headache	12	7	3	2
Skin disorders	8	7	1	
Nausea	7	3	3	1
Fatigue	6	2	4	
Thrombocytopenia	6	6		
Anemia	5	5		
Leucopenia	2	2		

In four patients no post treatment MRI was made because of clinical deterioration. In two patients, suspected dispersed hemorrhagic spots were seen in the tumor on post treatment MRI. A relationship with fresolimumab could not be excluded, although one of these patients also had a second course of radiotherapy prior to study entry.

### Imaging

All 12 patients underwent at least a “brain only” PET scan on day 4 after injection. Seven patients underwent a whole body scan. Four patients underwent a whole body scan on both day 2 and day 4 after injection. The interval between date of consent en injection of PET tracer was 7 days (0 - 15).

In all patients uptake of <sup>89</sup>Zr-fresolimumab was seen in tumor lesions (n = 16). The SUV<sub>max</sub> in tumor lesions on day 4 was 4.6 (1.5 - 13.9), which was higher than the SUV<sub>mean</sub> of normal brain tissue (0.3 (0.2 - 0.5)) ( $P < 0.01$ ). The SUV<sub>mean</sub> was 3.0 (2.0 - 6.2) in the blood of the sinus confluens. In patients with a whole body scan the SUV<sub>mean</sub> of normal organs was the highest in the heart (8.3 (6.4 - 8.9)) followed by the liver (7.1 (5.4 - 11.2)) and the kidneys (5.5 (3.4 - 6.6)) (Figure 1). In eight patients, uptake of <sup>89</sup>Zr-fresolimumab was not seen in each tumor lesion. Most tumor lesions that did not show uptake were small (< 10 mm on MRI). In three patients no uptake was seen in larger gadolinium enhanced lesions of 13, 18, and 12 mm respectively. The latter 2 lesions were found in previously irradiated areas and 1 of these was not visible on the follow up MRI scan (Figure 2). In all four patients who underwent a PET scan on both day 2 and day 4 after injection, the tumor to blood ratio (measured in the sinus confluens) increased from day 2 to day 4 after injection (Figure 2). There was no correlation between tumor uptake and PFS or OS.



**Figure 1.** A: Representative example of <sup>89</sup>Zr-fresolimumab PET on day 4 and uptake in the brain tumor (arrow). B: Uptake of <sup>89</sup>Zr-fresolimumab in different organs (SUV<sub>mean</sub>) and tumor (SUV<sub>max</sub>) on day 4 after tracer injection. Blood pool uptake was measured in the sinus confluens. Blood, brain and tumor values measured in n = 12, other organs in n = 7 patients.

### Plasma pharmacokinetics and biomarkers

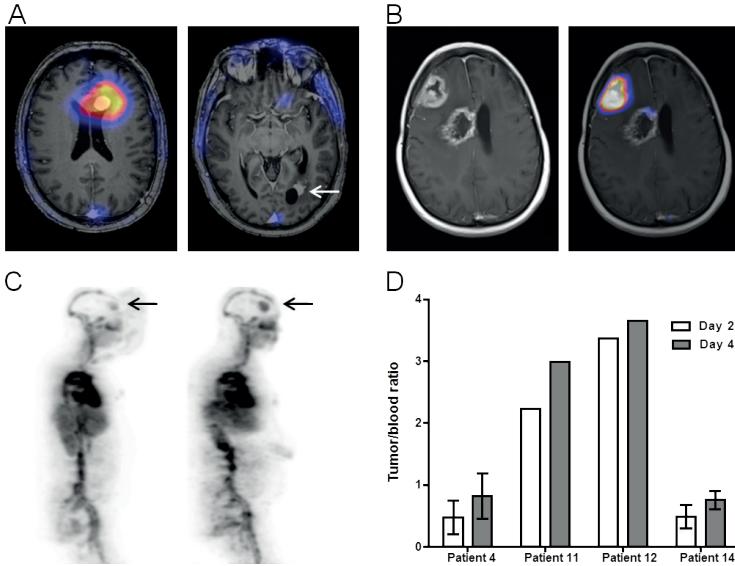
The plasma concentration of <sup>89</sup>Zr-fresolimumab at 1 hour, 2 days and 4 days after injection was 1.87 (1.20 - 2.30), 1.31 (0.96 - 1.76) and 1.06 (0.72 - 1.38) µg/mL respectively. When corrected for the injected dose, the extrapolated C<sub>max</sub>/dose was 0.37 (0.23 - 0.41) µg/mL/mg (n = 10).

Pre-treatment plasma TGFβ1 levels were 2058 pg/mL (837 - 3444) and correlated with mean SUV<sub>max</sub> in the tumor lesions 4 days post injection ( $r = 0.61$ ,  $P = 0.04$ , (Figure 3)). p-SMAD2 staining in primary tumor tissue was positive for all tumors, but also for normal brain tissue (Figure 4).

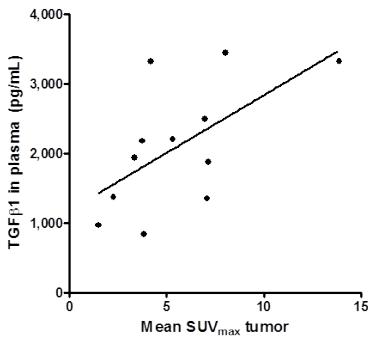
### DISCUSSION

This is the first study that shows tumor uptake of a radiolabeled antibody in recurrent high grade glioma patients, indicating that fresolimumab does reach its target destination within the brain. Unfortunately, mono-therapy with fresolimumab did not result in an antitumor effect.

The median SUV<sub>max</sub> of 4.6 found in the gliomas is comparable to the SUV<sub>max</sub> of 5.8 (1.7 - 15.1) found in metastatic lesions with <sup>89</sup>Zr-bevacizumab PET in patients with neuroendocrine tumors [16]. The C<sub>max</sub>/dose of <sup>89</sup>Zr-fresolimumab 1 hour after injection of 0.37µg/mL/mg is comparable to the pharmacokinetic results of an earlier study with fresolimumab [17]. This indicates that the radio labeled antibody has a similar C<sub>max</sub> with fresolimumab compared to other studies. Three contrast enhancing lesions >10 mm did not take up <sup>89</sup>Zr-fresolimumab. Two were found in previously irradiated areas and one of these disappeared on follow up MRI. These lesions are suspected to represent radionecrosis instead of viable tumor tissue which might be the reason for the lack of TGF-β and uptake of <sup>89</sup>Zr-fresolimumab. In all patients who underwent a whole body PET scan on both day 2 and day 4 after injection, the tumor to blood ratio increased. This increase in ratio supports tumor specific uptake. This pattern of tumor accumulation and increasing tumor to blood ratios over time was also seen in our preclinical study with

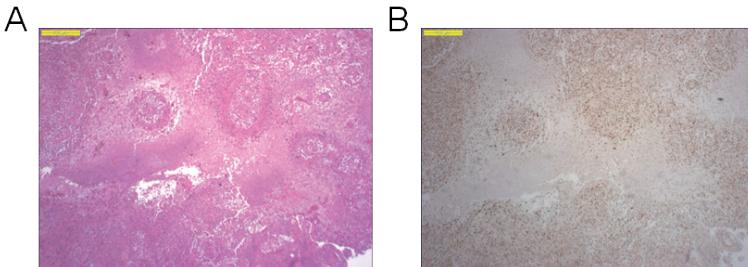


**Figure 2.** A: Fused MRI and PET scan of a patient with 2 contrast enhancing lesions. High uptake visible in the frontal lesion (left image). No uptake visible on PET scan in occipital lesion (right image, arrow) that was previously irradiated. B: MRI and fused MRI/PET images of a patient with 2 contrast enhancing lesions. The  $SUV_{max}$  in the progressive right frontal lesion was 5.5. The  $SUV_{max}$  in the previously irradiated lesion paraventricular right was 2.1. C: Whole body PET scan on day 2 (left) and day 4 (right) with increase of  $SUV_{max}$  in frontal brain lesion (black arrows) from 4.0 to 5.5. Tumor to blood ratio increased from 0.8 to 1.2. D: Tumor to blood ratios on <sup>89</sup>Zr-fresolimumab PET in 4 patients on day 2 and day 4 after injection.



**Figure 3.** (left) Correlation between TGFβ1 in plasma and mean  $SUV_{max}$  of <sup>89</sup>Zr-fresolimumab in brain tumor lesions on day 4 after injection ( $r = 0.61$ ,  $P = 0.04$ ).

**Figure 4.** (below) A: H&E staining of GBM with central necrosis. B: p-SMAD2 staining of the same area.



<sup>89</sup>Zr-fresolimumab and in brain metastases in a clinical study with <sup>89</sup>Zr-trastuzumab in metastatic breast cancer patients [13,18]. Taken together these findings suggest that <sup>89</sup>Zr-fresolimumab uptake was not only a reflection of antibody leakage due to a damaged blood brain barrier but was tumor specific and TGF- $\beta$  driven.

In earlier studies the uptake of gemcitabine and GRN1005 in recurrent glioma patients was shown by analyzing tumor tissue obtained during surgery [19,20]. However, performing tumor biopsies is often not feasible in this patient group and tumor characteristics may change over time. PET scanning can be a non-invasive alternative for exploring potential drugable targets and showing tumor penetration of drugs.

Treatment with fresolimumab was generally well tolerated, without infusion related reactions. Most adverse events were grade 1 or 2 and related to progression of disease. Unfortunately, no clinical benefit was observed in this small and often extensively pretreated patient group in which only one dose of fresolimumab was tested. Possible effects of this treatment in higher doses can therefore not be excluded. The median PFS was 61 days, which is comparable to the PFS of physician choice chemotherapy arm in recurrent glioblastoma in a recently conducted randomized phase 3 trial [21].

In all archival tumor samples, p-SMAD2 was positive, indicating that the TGF- $\beta$  pathway was active in the tumors. In gliomas multiple signaling pathways are activated, and inhibition of just one pathway might be insufficient for a response [22]. Recently, other clinical studies using TGF- $\beta$  inhibition in glioma patients have been published. Trabedersen is an antisense oligodeoxynucleotide that inhibits TGF- $\beta$ 2. In a randomized 2b study trabedersen was administered intra-tumorally by convection-enhanced delivery and compared with standard chemotherapy in patients with recurrent/refractory high-grade glioma. Six-month tumor control rates were not significantly different in the entire study population (anaplastic astrocytoma and GBM). Pre-specified anaplastic astrocytoma subgroup analysis showed a significant benefit regarding the 14-month tumor control rate for trabedersen vs chemotherapy [23]. A phase 1 study with LY2157299 (a TGF- $\beta$  receptor 1 kinase inhibitor) showed confirmed responses in treatment refractory gliomas in three out of 28 patients [24]. TGF- $\beta$  therefore remains a potential interesting target in glioma patients, and more (combination) studies are welcomed.

## CONCLUSION

In this study it was proven that an antibody against TGF- $\beta$  reaches recurrent high grade gliomas. Although no treatment benefit was seen, this finding could be exploited for further development of recurrent high grade glioma treatment with antibodies or antibody-drug conjugates.

## ACKNOWLEDGEMENTS:

This work was supported by Genzyme (Sanofi-Aventis Oncology). Grant of Genzyme (Sanofi-Aventis Oncology) for PET scans to E.G.E. de Vries was made available to the UMCG.

**DISCLOSURE OF POTENTIAL CONFLICTS OF INTEREST:**

J. Pearlberg is employed by Sanofi Aventis Oncology, Cambridge, MA. He is currently working at Infinity Pharmaceuticals, Cambridge, MA. All remaining authors have declared no conflicts of interest.

## REFERENCES

- [1] 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 Oncol.* 2012;14(Suppl 5): v1-49.
- [2] Massague J. TGF $\beta$  in cancer. *Cell.* 2008;134:215-230.
- [3] Wesolowska A, Kwiatkowska A, Slomnicki L, et al. Microglia-derived TGF- $\beta$  as an important regulator of glioblastoma invasion-an inhibition of TGF- $\beta$ -dependent effects by shRNA against human TGF- $\beta$  type II receptor. *Oncogene.* 2008;27:918-930.
- [4] Eichhorn PJ, Rodon L, Gonzalez-Junca A, et al. USP15 stabilizes TGF- $\beta$  receptor I and promotes oncogenesis through the activation of TGF- $\beta$  signaling in glioblastoma. *Nat Med.* 2012;18:429-435.
- [5] Kjellman C, Olofsson SP, Hansson O, et al. Expression of TGF- $\beta$  isoforms, TGF- $\beta$  receptors, and SMAD molecules at different stages of human glioma. *Int J Cancer.* 2000;89:251-258.
- [6] Schneider T, Sailer M, Ansorge S, Firsching R, Reinhold D. Increased concentrations of transforming growth factor  $\beta$ 1 and  $\beta$ 2 in the plasma of patients with glioblastoma. *J Neurooncol.* 2006;79:61-65.
- [7] Bruna A, Darken RS, Rojo F, et al. High TGF $\beta$ -smad activity confers poor prognosis in glioma patients and promotes cell proliferation depending on the methylation of the PDGF- B gene. *Cancer Cell.* 2007;11:147-160.
- [8] Arjaans M, Oude Munnink TH, Timmer-Bosscha H, et al. Transforming growth factor (TGF)- $\beta$  expression and activation mechanisms as potential targets for anti-tumor therapy and tumor imaging. *Pharmacol Ther.* 2012;135:123-132.
- [9] Lonning S, Mannick J, McPherson JM. Antibody targeting of TGF- $\beta$  in cancer patients. *Curr Pharm Biotechnol.* 2011;12:2176-2189.
- [10] Morris JC, Tan AR, Olencki TE, et al. Phase I study of GC1008 (Fresolimumab): A human anti-transforming growth factor-beta (TGF $\beta$ ) monoclonal antibody in patients with advanced malignant melanoma or renal cell carcinoma. *PLoS One.* 2014;9:e90353.
- [11] Stevenson JP, Kindler HL, Papasavvas E, et al. Immunological effects of the TGF $\beta$ - blocking antibody GC1008 in malignant pleural mesothelioma patients. *Oncoimmunology.* 2013;2:e26218.
- [12] Lampson LA. Monoclonal antibodies in neuro-oncology: Getting past the blood-brain barrier. *MAbs.* 2011;3:153-160.
- [13] Oude Munnink TH, Arjaans ME, Timmer-Bosscha H, et al. PET with the  $^{89}\text{Zr}$ -labeled transforming growth factor- $\beta$  antibody fresolimumab in tumor models. *J Nucl Med.* 2011;52:2001-2008.

- [14] Loening AM, Gambhir SS. AMIDE: A free software tool for multimodality medical image analysis. *Mol Imaging*. 2003;2:131–137.
- [15] Macdonald DR, Cascino TL, Schold SC, Jr, Cairncross JG. Response criteria for phase II studies of supratentorial malignant glioma. *J Clin Oncol*. 1990;8:1277–1280.
- [16] Van Asselt SJ, Oosting SF, Brouwers AH, et al. Everolimus reduces <sup>89</sup>Zr-bevacizumab tumor uptake in patients with neuroendocrine tumors. *J Nucl Med*. 2014;55:1087–1092.
- [17] Trachtman H, Fervenza FC, Gipson DS, et al. A phase 1, single-dose study of fresolimumab, an anti-TGF- $\beta$  antibody, in treatment-resistant primary focal segmental glomerulosclerosis. *Kidney Int*. 2011;79:1236–1243.
- [18] Dijkers EC, Oude Munnink TH, Kosterink JG, et al. Biodistribution of <sup>89</sup>Zr-trastuzumab and PET imaging of HER2-positive lesions in patients with metastatic breast cancer. *Clin Pharmacol Ther*. 2010;87:586–592.
- [19] Sigmund J, Honeywell RJ, Postma TJ, et al. Gemcitabine uptake in glioblastoma multiforme: Potential as a radiosensitizer. *Ann Oncol*. 2009;20:182–187.
- [20] Drappatz J, Brenner A, Wong ET, et al. Phase I study of GRN1005 in recurrent malignant glioma. *Clin Cancer Res*. 2013;19:1567–1576.
- [21] Stupp R, Wong ET, Kanner AA, et al. NovoTTF-100A versus physician's choice chemotherapy in recurrent glioblastoma: A randomised phase III trial of a novel treatment modality. *Eur J Cancer*. 2012;48:2192–2202.
- [22] Stommel JM, Kimmelman AC, Ying H, et al. Coactivation of receptor tyrosine kinases affects the response of tumor cells to targeted therapies. *Science*. 2007;318:287–290.
- [23] Bogdahn U, Hau P, Stockhammer G, et al. Targeted therapy for high-grade glioma with the TGF- $\beta$ 2 inhibitor trabedersen: Results of a randomized and controlled phase IIb study. *Neuro Oncol*. 2011;13:132–142.
- [24] Rodon Ahnert J, Baselga J, Calvo E, et al. First human dose (FHD) study of the oral transforming growth factor- $\beta$  receptor I kinase inhibitor LY2157299 in patients with treatment-refractory malignant glioma [abstract]. *J Clin Oncol*. 2011;29(suppl):3011.

