Targeted therapy, molecular imaging and biomarkers in cancer treatment

den Hollander, Martha Willemine

Targeted therapy, molecular imaging and biomarkers in cancer treatment

Getting more personalized

M.W. den Hollander

Targeted therapy, molecular imaging and biomarkers in cancer treatment: getting more personalized

Thesis, University of Groningen, The Netherlands

ISBN 978-94-6259-805-8 (printed version)
ISBN 978-94-6259-820-1 (digital version)

Cover en lay-out: Heleen de Vos, persoonlijkproefschrift.nl
Printing: Ipskamp Drukkers, Amsterdam, The Netherlands

The printing of this thesis was financially supported by Graduate School of Medical Sciences and Stichting Werkgroep Interne Oncologie, the Faculty of Medical Sciences, University of Groningen, and is gratefully acknowledged.

rijksuniversiteit
groningen

Targeted therapy, molecular imaging and biomarkers in cancer treatment

Getting more personalized

Proefschrift

ter verkrijging van de graad van doctor aan de
Rijksuniversiteit Groningen
op gezag van de
rector magnificus prof. dr. E. Sterken
en volgens besluit van het College voor Promoties.

De openbare verdediging zal plaatsvinden op

woensdag 14 oktober 2015 om 12.45 uur

door

Martha Willemine den Hollander

geboren op 16 januari 1985
te Dordrecht

Promotores
Prof. dr. J.A. Gietema
Prof. dr. E.G.E. de Vries

Copromotor
Dr. A.M.E. Walenkamp

Beoordelingscommissie
Prof. dr. M.J. van den Bent
Prof. dr. J.G.W. Kosterink
Prof. dr. M.A.T.M. van Vugt

Paranimfen
Lisanne den Hollander
Irene Roosendaal-Miedema

CONTENTS

Chapter 1

General Introduction

BACKGROUND

Until 2001 chemotherapy and hormonal therapies were the mainstays of systemic treatment of solid tumors. In 2001 it was shown that activation of the mutated c-Kit receptor in gastrointestinal stromal tumors (GIST) could be blocked by imatinib, a c-KIT tyrosine kinase inhibitor. This was one of the first successes of targeted therapy in solid tumors apart from the already long existing anti-hormonal therapy. The objective response rate in metastatic and unresectable GIST went from 0-5% for chemotherapy up to 38% for imatinib [1]. In recent years, more and more drugs specifically targeting diverse tumor characteristics have been developed. Unfortunately, not all targeted agents were as successful and in most cases, the effect of targeted agents is temporarily and often only seen in a subgroup of patients.

A major challenge in oncology is to identify patients that will benefit from various targeted agents. Eventually, this should lead to 'personalized medicine': a specific drug to treat a specific tumor with specific molecular or genetic characteristics in a specifically selected patient. Molecular profiling of tumor tissue is important in this regard, but there may also be a role for molecular imaging in selecting patients and predicting tumor responses. The advantage of molecular imaging is that it is non-invasive and all tumor lesions can be assessed which addresses tumor heterogeneity and circumvents sampling errors.

Another application of molecular imaging may be the (early) assessment of tumor responses. Treatment with targeted drugs may not always lead to a direct volume response of the tumor. Response assessments based on tumor diameter such as RECIST criteria for solid tumors on CT scans may therefore underestimate the anti-tumor activity of targeted agents [2]. In brain tumors, response assessment is especially difficult due to the blood brain barrier. Disruption or normalization of the blood brain barrier by the treatment complicates the evaluation of contrast enhanced MRI imaging [3]. Targeted drugs can block or reactivate pathways in tumors and modify the tumor microenvironment. This can potentially be visualized by molecular imaging with labeled drugs or labeled markers of pathway and microenvironment alterations.

As anticancer treatments are becoming more effective, attention also needs to be paid to side effects of treatment and care for long term cancer survivors. Prognostic markers for toxicity due to anticancer treatments are therefore warranted. Early recognition of susceptibility for toxic effects of anticancer therapy can lead to early treatment adaptations and more specific follow up.

The aim of this thesis is to provide novel insights in targeted drugs and the use of molecular imaging and biomarkers in anticancer treatments.

OUTLINE OF THE THESIS

Currently, the most frequently used way of systemic anticancer treatment is still DNA damage induction via chemotherapy. This DNA damage leads to apoptosis of tumor cells via the intrinsic apoptotic pathway. However, tumor cells often have mutations in this pathway, resulting in resistance to chemotherapy [4]. In addition, chemotherapy does not selectively affect tumor cells, but also induces damage to normal cells. Another way of inducing cell death is by targeting

the extrinsic pathway in tumor cells that induces apoptosis. One of the potentially interesting ways of doing this is via the TRAIL-R1 and TRAIL-R2 death receptors. Agonistic antibodies and recombinant TRAIL (rhTRAIL) can activate these receptors. In preclinical studies, it was found that these drugs can induce apoptosis and can enhance apoptosis in combination with chemotherapy and radiotherapy [5-7]. In **chapter 2**, a literature review was performed concerning the results of the phase 1 and 2 studies with the different agonistic antibodies against the TRAIL receptors and recombinant TRAIL.

When the first studies with imatinib in GIST patients were conducted, a decrease of ^{18}F-fluorodeoxyglucose (FDG) uptake on PET scans was noted within days of starting imatinib therapy. It was suggested that this could be used as an early marker of therapeutic response in these patients [8]. Approximately 15% of GIST patients show primary resistance to imatinib, defined as progressive disease at first CT evaluation after 2 months [9]. In **chapter 3** we retrospectively analyzed FDG-PET scans before start of imatinib and after 1 week of treatment initiation in 36 patients and compared the results with the outcome on CT scan after 2 months of treatment. We investigated whether early changes in tumor FDG uptake can predict primary imatinib resistance.

In brain tumors, the use of FDG-PET scans is hampered by the high uptake of glucose in normal brain tissue. However, accurate assessment of response to standard therapy with MRI imaging in glioblastomas is extremely difficult. Progressive lesions may not represent tumor growth, but rather a treatment effect that subsides in time without a change of therapy. This phenomenon is called pseudoprogression and seen in up to 64% of the patients with progressive disease on MRI directly after radiotherapy [10]. The difficulty to distinguish recurrent tumor growth (true progression) from pseudoprogression complicates the clinical decision making in these patients: in case of pseudoprogression, standard treatment with adjuvant temozolomide should be continued, whereas in case of true tumor progression, other treatment modalities or palliative care would be more appropriate. ^{18}F-fluorothymidine (FLT) is a PET tracer that is taken up by proliferating cells and therefore it may be possible to use FLT-PET scans to discriminate true progressive tumors from pseudoprogression as in the latter less proliferation would be expected. In **chapter 4** we prospectively investigated the capability of FLT-PET scans in discriminating between pseudoprogression and true progression in patients with newly diagnosed glioblastoma treated with radiotherapy and temozolomide. In 30 patients, FLT-PET scans were performed before start and 4 weeks after completion of concomitant radiochemotherapy. MRI scans were performed at these two time points and after 3 cycles of adjuvant TMZ. Pseudoprogression was defined as progressive disease on MRI after radiochemotherapy, with stabilization or improvement of enhancing lesions after 3 cycles of adjuvant TMZ. Changes in FLT uptake were compared between patients with true progression and pseudoprogression.

Another challenge in the management of malignant gliomas is the lack of effective standard therapy for recurrent disease, despite numerous studies with targeted agents and chemotherapeutics conducted in recent years. One reason for this might be that the blood-brain barrier hampers the uptake of targeted agents in brain tumors. An important target in high grade

gliomas is TGF-β, since it was shown that TGF-β functions as a tumor promoter in advanced cancer and is involved in glioma development [11,12]. In **chapter 5**, we describe a clinical study in which patients with recurrent high grade gliomas were treated with fresolimumab, a monoclonal antibody against TGF-β. To investigate whether the antibody actually reached the tumor, patients underwent a PET scan with ^{89}Zirconium labeled fresolimumab before start of treatment.

Testicular cancer patients are mostly treated with a combination of bleomycin, etoposide and cisplatin (BEP) chemotherapy. The cure rate is very high in this patient group. But about 10% of the patients treated with BEP develop bleomycin induced pulmonary toxicity and in up to 3% of the patients this is fatal [13]. TGF-β is involved in many cellular physiological and pathological processes in the body including the immune response, wound healing and fibrosis [14]. In preclinical studies, TGF-β is implicated as an important factor in the development of bleomycin induced pulmonary toxicity [15]. An early marker that can predict which patients will develop this toxicity is not available. In **chapter 6** we investigated the prevalence of abnormalities that were suspect for bleomycin-induced pulmonary changes on post chemotherapy restaging CT scans and whether TGF-β1 and GDF15 (a member of the TGF-β superfamily) and Hs-CRP levels in plasma can be used as biomarkers for the occurrence of these changes in testicular cancer patients treated with bleomycin containing combination chemotherapy. In **chapter 7** the thesis is summarized and future perspectives are given. **Chapter 8** provides a summary of the thesis in Dutch.

REFERENCES

[1] Cohen MH, Farrell A, Justice R, and Pazdur R, Approval summary: Imatinib mesylate in the treatment of metastatic and/or unresectable malignant gastrointestinal stromal tumors, Oncologist. 14 (2009) 174-180.

[2] Eisenhauer EA, Therasse P, Bogaerts J*, et al*, New response evaluation criteria in solid tumours: Revised RECIST guideline (version 1.1), Eur. J. Cancer. 45 (2009) 228-247.

[3] Brandsma D and van den Bent MJ, Pseudoprogression and pseudoresponse in the treatment of gliomas, Curr. Opin. Neurol. 22 (2009) 633-638.

[4] Vousden KH and Lu X, Live or let die: The cell's response to p53, Nat. Rev. Cancer. 2 (2002) 594-604.

[5] Ashkenazi A, Pai RC, Fong S*, et al*, Safety and antitumor activity of recombinant soluble Apo2 ligand, J. Clin. Invest. 104 (1999) 155-162.

[6] Chuntharapai A, Dodge K, Grimmer K, Schroeder K, Marsters SA, Koeppen H, Ashkenazi A, and Kim KJ, Isotype-dependent inhibition of tumor growth in vivo by monoclonal antibodies to death receptor 4, J. Immunol. 166 (2001) 4891-4898.

[7] Ichikawa K, Liu W, Zhao L*, et al*, Tumoricidal activity of a novel anti-human DR5 monoclonal antibody without hepatocyte cytotoxicity, Nat. Med. 7 (2001) 954-960.

[8] Abhyankar SA, Nair N. Highlighting the role of FDG PET scan in early response assessment of gastrointestinal stromal tumor treated with imatinib mesylate., Clin Nucl Med. 33(3) (2008) 213-214.

[9] Demetri GD, von Mehren M, Blanke CD*, et al*, Efficacy and safety of imatinib mesylate in advanced gastrointestinal stromal tumors, N. Engl. J. Med. 347 (2002) 472-480.

[10] Fink J, Born D, and Chamberlain MC, Pseudoprogression: Relevance with respect to treatment of high-grade gliomas, Curr. Treat. Options Oncol. 12 (2011) 240-252.

[11] Massague J, TGFbeta in cancer, Cell. 134 (2008) 215-230.

[12] Kjellman, Olofsson SP, Hansson O, Von Schantz T, Lindvall M, Nilsson I, Salford IG, Sjogren HO, and Widegren B, Expression of TGF-beta isoforms, TGF-beta receptors, and SMAD molecules at different stages of human glioma, Int. J. Cancer. 89 (2000) 251-258.

[13] Simpson AB, Paul J, Graham J, and Kaye SB, Fatal bleomycin pulmonary toxicity in the west of scotland 1991-95: A review of patients with germ cell tumours, Br. J. Cancer. 78 (1998) 1061-1066.

[14] Blobe GC, Schiemann WP, and Lodish HF, Role of transforming growth factor beta in human disease, N. Engl. J. Med. 342 (2000) 1350-1358.

[15] Li M, Krishnaveni MS, Li C*, et al*, Epithelium-specific deletion of TGF-beta receptor type II protects mice from bleomycin-induced pulmonary fibrosis, J. Clin. Invest. 121 (2011) 277-287.

Chapter 2

Translating TRAIL-receptor targeting agents to the clinic

M.W. den Hollander, J.A. Gietema, S. de Jong, A.M.E. Walenkamp, A.K.L. Reyners, C.N.A.M. Oldenhuis, E.G.E. de Vries

Department of Medical Oncology, University of Groningen, University Medical Center Groningen, Groningen, The Netherlands

Cancer Lett. 2013 May 28;332(2):194-201

ABSTRACT

The extrinsic apoptotic pathway can be activated by the endogenous ligand TRAIL (Tumor Necrosis Factor (TNF)-Related Apoptosis-Inducing Ligand) by binding to the death receptors TRAIL-R1 and TRAIL-R2 on the cell surface. This pathway is currently evaluated as an anticancer treatment strategy. Both recombinant human TRAIL and several agonistic antibodies against TRAIL-R1 and R2 have been studied in single agent and combination studies and proved to be safe and well tolerated. In this article, the clinical studies published to date will be reviewed. Also, future perspectives and biomarker studies for selecting patients that will benefit from these agents will be discussed.

INTRODUCTION

There are several different ways of inducing apoptosis in malignant cells as an anticancer treatment strategy. The most frequently exploited ways of treating tumors is to induce DNA damage via chemotherapy and/or radiotherapy, thereby activating the mitochondrial (intrinsic) apoptotic pathway. An important regulator of the intrinsic apoptotic pathway is the tumor suppressor p53, which can induce apoptosis in response to DNA damage inflicted by chemotherapy and radiation. However, p53 function in tumor cells is often lost, resulting in resistance to chemotherapy. In addition, chemotherapy does not selectively affect tumor cells, but also induces damage to normal cells.

Another way of inducing cell death is by stimulation of apoptosis via the extrinsic pathway. The extrinsic pathway is independent of p53 and can be activated by the endogenous ligand TRAIL (Tumor Necrosis Factor (TNF)-Related Apoptosis-Inducing Ligand), a transmembrane protein and a member of the TNF super family [1]. Physiologically, TRAIL is considered to have an anti-inflammatory effect and to play a role in autoimmunity and anti-tumor surveillance [2-4]. TRAIL can induce apoptosis in tumor cells by binding to the death receptors TRAIL-R1 (DR4) and TRAIL-R2 (DR5) on the cell surface. These death receptors are present in a broad range of both normal cells and tumor cells [5]. Interestingly, recombinant human (rh)TRAIL induces cell death only in tumor cells and not in normal cells [6]. What causes this difference is still not elucidated. When death receptors are activated by TRAIL, these receptors undergo homo-trimerization. This trimer forms the death-inducing signaling complex (DISC) together with the Fas-associated death-domain (FADD) and pro-caspases 8 and 10. The activated caspases then activate caspases 3, 6 and 7, eventually resulting in apoptosis. Active caspase 8 also cleaves Bcl-2 interacting domain (Bid) into truncated Bid(tBid), which then triggers the intrinsic apoptotic pathway by activation of caspase 9 and finally caspase 3. Important cellular proteins that inhibit activation of the extrinsic apoptotic pathway are cFLIP, a competitor of caspase 8, and the inhibitor-of-apoptosis proteins (IAPs) that inhibit caspase activity [7]. In preclinical studies, not only rhTRAIL but also the agonistic antibodies against TRAIL-R1 and TRAIL-R2 induced apoptosis in various tumor cell lines, while normal cells were spared [6,8,9]. RhTRAIL and the TRAIL-R antibodies, also called PARAs (pro-apoptotic receptor agonists), in addition enhance the cytotoxic effect of "classic" chemotherapy, targeted therapies and radiotherapy [10-12]. This has led to several studies that are finalized or are ongoing with PARAs as single agent or combined with chemotherapeutic as well as targeted agents. In this review the results of these studies will be summarized and future perspectives and the possible use of biomarkers for selecting eligible patients will be discussed.

CLINICAL STUDIES

In recent years, several phase 1 and 2 single agent and combination studies have been conducted with recombinant human TRAIL (dulanermin, (Amgen/Genentech)) targeting both TRAIL-R1 and TRAIL-R2 and the agonistic monoclonal antibodies to either TRAIL-R1 (mapatumumab (Human Genome Sciences)) or TRAIL-R2 (lexatumumab (Human Genome Sciences), conatumumab (Amgen), drozitumab (Genentech), tigatuzumab (Daiichi-Sankyo) and LBY135

(Novartis)). These trials are summarized in Table 1.

Mapatumumab, lexatumumab, conatumumab and drozitumab are fully human IgG1 antibodies, whereas tigatuzumab is a humanized IgG1 antibody and LBY135 a chimeric (mouse/human) IgG1 antibody.

SINGLE AGENT STUDIES

Dulanermin

In a phase 1 study with dulanermin, 71 patients with advanced cancer received up to 30 mg/kg/day intravenously (iv) for 5 days every 3 weeks. This regime was found to be safe and well tolerated. Two patients with chondrosarcoma achieved a partial tumor response, and were still on treatment after 2.7 and 4.3 years, respectively. Furthermore, in two other sarcoma patients, tumor necrosis was found during surgery after the first cycle of dulanermin therapy, which is possibly an indication of dulanermin induced cell death. The serum half life of dulanermin was found to be 0.5–1 h. No antibodies against dulanermin were detected [13].

Mapatumumab

In two phase 1 trials with mapatumumab up to 10 mg/kg iv every 2 weeks or up to 20 mg/kg iv every 4 weeks, the best responses were stable disease in respectively 19 out of 49 and 12 out of 41 patients with advanced solid tumors. The maximum tolerated doses were not reached [14,15].

In a phase 2 study, patients with colorectal cancer received mapatumumab 10 mg/kg after 2 loading doses of 20 mg/kg iv every 14 days. In a phase 2 study in patients with non-small cell lung cancer (NSCLC), patients received mapatumumab 10 mg/kg every 21 days. In both studies, no objective responses were observed, but respectively 12 out of 38 and 9 out of 32 heavily pretreated patients achieved stable disease [16,17].

In a phase 1b/2 trial in 40 patients with non-Hodgkin's lymphoma (NHL) treated with doses of 3 or 10 mg/kg mapatumumab iv every 21 days, 2 complete responses and 1 partial response were seen in patients with follicular lymphoma and 11 patients achieved stable disease [18].

No anti-mapatumumab antibodies were found in the phase 1 trials. The mean plasma half life value for mapatumumab was found to be 19, 22 and 26 days respectively [14,15,18].

Agonistic TRAIL-R2 antibodies

Lexatumumab was studied as a single agent in two phase 1 studies in patients with advanced solid tumors. The maximum tolerated dose was found to be 10 mg/kg and this dose could be administered every 2 weeks. Dose limiting toxicities, seen in five patients in these 2 studies, consisted of elevations of serum amylase, bilirubin and transaminases. One of these patients developed septicemia and acute renal failure and died 25 days after the lexatumumab administration. Stable disease was achieved in respectively 12 out of 37 and 9 out of 27 patients, while one mixed response was seen in a patient with Hodgkin's lymphoma. In this patient a lung lesion became smaller but other lesions increased in size [19,20]. A phase 1 study with lexatumumab

Table 1: Overview of clinical studies with pro-apoptotic receptor agonists.

Author	Drug	Phase	Tumor type	No. of patients	Combined with	Best response	Results phase 2 randomized studies
Belada 2010 [34]	Dulanermin	2 r.	NHL	48	Arm 1: rituximab + dulanermin Arm 2: rituximab		Arm1/2: ORR: 61.5%/63.6% CR: 4 (1 unconfirmed)/5 PR: 12/9
Fanale 2008 [33]	Dulanermin	1b	Relapsed low grade NHL	12	Rituximab	3 CR, 3 PR	
Herbst 2010 [13]	Dulanermin	1	Advanced cancer	71	-	2 PR, 31 SD Med. PFS 2.3 mo	
Soria 2010 [31]	Dulanermin	1b	Advanced non squamous NSCLC	24	Paclitaxel, carboplatin, bevacizumab	1 CR, 13 PR ORR 58% Med. PFS 7.2 mo	
Soria 2011 [32]	Dulanermin	2 r.	NSCLC	213	Arm 1: paclitaxel (P)+ carboplatin (C) Arm 2: P+C + dulanermin (D) (8 mg/kg/5 days) Arm 3: PC + bevacizumab (B) Arm 4: PCB + D (8 mg/kg/5 days) Arm 5: PCB + D (20 mg/kg/2 days)		Arm 1/2/3/4/5 ORR: 39%/38%/50%/40%/40% PFS: 6.1/5.5/7.3/8.6/9.5 OS: 10.1/9.8/15.1/13.9/14.3
Yee 2009 [35]	Dulanermin	1b	Metastatic colorectal cancer	35	Irinotecan, cetuximab or FOLFIRI	NR	
Greco 2008 [17]	Mapatumumab	2	Advanced NSCLC	32	-	9 SD Med PFS 1.2 mo	
Hotte 2008 [15]	Mapatumumab	1	Advanced solid tumors	41	-	12 SD med PFS 1.7 mo	
Leong 2009 [37]	Mapatumumab	1	Advanced solid tumors	27	Paclitaxel, carboplatin	5 PR 12 SD	

Table 1: continued.

Author	Drug	Phase	Tumor type	No. of patients	Combined with	Best response	Results phase 2 randomized studies
Mom 2009 [36]	Mapatumumab	1	Advanced solid tumors	49	Gemcitabine cisplatin	12 PR 25 SD	
Tolcher 2007 [14]	Mapatumumab	1	Advanced solid tumors	49	-	19 SD	
Trarbach 2010 [16]	Mapatumumab	2	Refractory colorectal cancer	38	-	12 SD med PFS 1.2 mo	
Von Pawel 2010 [38]	Mapatumumab	2 r.	NSCLC	111	Arm 1: paclitaxel + carboplatin (PC) Arm 2: PC + mapatumumab 10 mg/kg Arm 3: PC + mapatumumab 30 mg/kg		Arm 1/2/3 ORR: 30.6%/13.5 %/ 36.1% Med PFS: 4.6/4.6/4.9 Med OS: 10.5/13.6/10.6
Younes 2010 [18]	Mapatumumab	2	Relapsed/refractory NHL	40	-	2CR, 1 PR 11 SD	
Sun 2011 [39]	Mapatumumab	1b	Advanced hepatocellular carcinoma	19	Sorafenib	2 PR, 4 SD	
Merchant 2010 [21]	Lexatumumab	1	Advanced solid tumors (pediatric patients)	24	-	5 SD	
Plummer 2007 [19]	Lexatumumab	1	Advanced solid tumors	37	-	12 SD	
Sikic 2007 [40]	Lexatumumab	1b	Wide range of cancer types	41	Gemcitabine, pemetrexed, doxorubicin, or FOLFIRI	3 PR	
Wakelee 2010 [20]	Lexatumumab	1	Advanced solid tumors	31	-	9 SD 1 mixed response	
Baron 2011 [44]	Drozitumab	1b	Metastatic colorectal cancer	20	Cetuximab + irinotecan or FOLFIRI±bevacizumab	3 PR(1 unconfirmed), 13 SD	

Author	Drug	Phase	Tumor type	No. of patients	Combined with	Best response	Results phase 2 randomized studies
Camidge 2010 [22]	Drozitumab	1	Advanced solid tumors or NHL	50	-	20 SD 3 minor responses	
Karapetis 2010 [41]	Drozitumab	2 r.	NSCLC	62	Arm 1: PCB + Drozitumab Arm 2: PCB + placebo		Arm 1/2: Med PFS: 7.9/7.0 Med OS: 9.9/12.6 ORR: 40%/42%
Rocha Lima 2011 [43]	Drozitumab	1b	Metastatic colorectal cancer	9	FOLFOX + bevacizumab	5 PR (3 unconfirmed), 3 SD	
Wittebol 2010 [42]	Drozitumab	2	NHL	40	Rituximab	2 CR, 18 PR	
Chawla 2010 [49]	Conatumumab	1	Advanced solid tumors	9	AMG 479	3 SD	
Demetri 2012 [51]	Conatumumab	1/2 r.	Metastatic/unresectable soft tissue sarcoma	134	Arm1: conatumumab + doxorubicin Arm 2: placebo + doxorubicin		Arm 1/2: Med PFS: 5.6/6.8 Med OS: 18.2/21.6
Doi 2011 [25]	Conatumumab	1	Advanced solid tumors	18		9 SD	
Herbst 2010 [24]	Conatumumab	1	Advanced solid tumors	37		1 PR, 14 SD	
Kindler 2009 [47]	Conatumumab	1b	Metastatic pancreatic cancer	13	Gemcitabine	4 PR (2 unconfirmed) 38% SD Med PFS 5.3 mo	
Kindler 2010 [50]	Conatumumab	2 r.	Metastatic pancreatic cancer	125	Arm 1: conatumumab + gemcitabine Arm 2: AMG 479 + gemcitabine Arm 3: placebo + gemcitabine		Arm 1: 24/38 SD Med PFS: 3,9 mo Arm 2: 1/38 PR, 19/38 SD Med PFS: 5.1 mo Arm 3: 2/40 PR, 13/40 SD Med PFS: 2.1 mo
Paz Ares 2009 [45]	Conatumumab	1b	Advanced NSCLC	12	Paclitaxel carboplatin	1 CR, 3 PR, 3 SD Med PFS 5.1 mo	

Table 1: continued.

Author	Drug	Phase	Tumor type	No. of patients	Combined with	Best response	Results phase 2 randomized studies
Peeters 2010 [48]	Conatumumab	1b/2	Metastatic colorectal cancer	53	Panitumumab	WT KRAS/MT KRAS 8 SD/4SD Med. PFS 7.3/4.4 mo	
Saltz 2009 [46]	Conatumumab	1b	Metastatic colorectal cancer	12	Modified FOLFOX6 and bevacizumab	5 PR (2 unconfirmed), 6 SD	
Forero-Torres 2010 [23]	Tigatuzumab	1	Relapsed/refractory carcinomas or lymphomas	17	-	7 SD	
Sharma 2008 [26]	LBY135	1	Advanced solid tumors	56	Arm 1: LBY135 Arm 2: LBY135 + capecitabine	Arm 1: 1 minor response Arm 2: 1 PR	
Griffith 2007 [55]	Ad5-TRAIL	1	Prostate cancer	3	-	NR	

NR = not reported, CR = complete response, PR = partial response, SD = stable disease, med PFS = median progression free survival, OS = overall survival, Mo = months, ORR = objective response rate, r = randomized

has also been conducted in 24 pediatric patients with solid tumors. Doses up to 10 mg/kg every 14 days were found to be safe in this population. Five patients achieved stable disease. In one patient stable disease was ongoing at 17 months [21].

Drozitumab was investigated at doses up to 20 mg/kg every 14 days and the maximum tolerated dose was not reached. Twenty out of 41 evaluable patients achieved stable disease. Minor responses were found in 3 patients with colorectal cancer, ovarian cancer and chondrosarcoma with respectively 28%, 23% and 20% reduction in measurable disease [22].

In a phase 1 study evaluating tigatuzumab in doses up to 8 mg/kg iv every week, 7 out of 17 patients experienced stable disease as a best result, with 1 patient having stable disease for over 2 years. The maximum tolerated dose was not reached [23].

Conatumumab was studied in doses up to 20 mg/kg every 2 weeks in 37 patients. The maximum tolerated dose was not reached. One partial response was seen in a patient with NSCLC, who was still on treatment after 4.2 years. One minor response (24% decrease in tumor size) was observed in a patient with colorectal carcinoma and 14 patients achieved stable disease [24]. In another phase 1 study, conatumumab was also well tolerated and 9 out of 18 patients achieved stable disease [25].

LBY135 monotherapy was investigated in doses up to 20 mg/kg every 3 weeks in 32 patients with advanced solid tumors. A minor response in one patient and a decrease in tumor markers in two patients were seen. There were no dose limiting toxicities in patients receiving LBY135 monotherapy [26].

Pharmacokinetic analyses of these antibodies against TRAIL-R2 showed that the serum half life of the antibodies is around 14 days for lexatumumab, 9–19 days for drozitumab and 13–19 days for conatumumab. The plasma half life of tigatuzumab is 6–10 days and preliminary results show a half life value of 10 days for LBY135 [19,20,22-24,26]. In one patient, antibodies against lexatumumab were found before treatment, but this finding was not confirmed in later samples [19]. Antibodies against drozitumab were found in one patient, but because the baseline test of this patient was also positive, this did not seem to be related to treatment [22]. In the study with LBY135, immunogenicity was found in 25% of the patients, which seemed to affect exposure in five patients during later doses [26].

Efficacy of PARAs as single agents

Side-effects in single agent studies were generally mild, with the side-effects seen most frequently being fatigue and nausea. All investigated PARAs were considered safe and well tolerated and a maximum tolerated dose was only found for lexatumumab. From preclinical data there were concerns about a possible toxic effect of PARAs, especially on the liver [27]. However, in the clinical studies so far, this was not confirmed.

Most of the single agent studies had a phase 1 character and were performed in heavily pretreated patients and are therefore not ideal to judge anti-tumor activity. Tumor responses were observed in lymphomas treated with mapatumumab and three partial responses in solid tumors have been reported after treatment with dulanermin and conatunumab. The complete

responses with mapatumumab were achieved at 9 and 11 months, the partial responses with dulanermin at 2 and 8 months and the partial response with conatumumab at 8 months. Interestingly, further decrease in tumor size was seen in some of these patients after about 9 and 11 months (mapatumumab) and 22 months (conatumumab) [13,18,24]. Although these are only individual cases, this indicates that response evaluation with RECIST criteria after the first months of treatment might be underestimating the therapeutic efficacy of PARAs. This underscores the relevance of waterfall plots over time.

The clear difference between the half life values of dulanermin and the agonistic antibodies indicates that dulanermin is only shortly available to bind to death receptors on tumor cells after administration while the antibodies are present for a long time. However, this short availability does not preclude tumor responses. The precise consequences of these differences in half life for trial design are still unknown.

Combination studies

Based on preclinical data there is a strong rationale to combine PARAs with chemotherapy, radiotherapy and other targeted therapies as PARAs enhance their effect [10-12]. These combinations theoretically induce cell death by targeting both the extrinsic and the intrinsic apoptotic pathway. Activation of both the extrinsic and intrinsic apoptotic pathway is amplified by the combination of PARAs with chemotherapy. PARAs amplify signaling of the intrinsic apoptotic pathway via tBid, while chemotherapy augments activation of the extrinsic apoptotic pathway via, among others, TRAIL receptor upregulation at the cell surface and reduction of cellular cFLIP levels. Proteasome inhibition with bortezomib results in pleiotropic effects, but bortezomib treatment is found to induce TRAIL receptor surface expression, reduce FLIP expression, block IAP functionality and prevent proteasomal degradation of p53 and pro-apoptotic Bcl2 family members in cancer cells [28]. Inhibition of the NF-kappaB, Akt or MAPK prosurvival pathways using cetuximab, rituximab or sorafinib synergizes with PARAs targeting the apoptotic pathway. The mechanism of drug interaction can be at the DISC resulting in enhanced DISC formation or more downstream causing reduced expression of anti-apoptotic Bcl-2 family members and IAPs [29,30].

Dulanermin

In a phase 1b study, dulanermin in doses up to 8 mg/kg iv for 5 days or up to 20 mg/kg iv for 2 days every 3 weeks was studied in combination with paclitaxel, carboplatin and bevacizumab. Of the 24 patients with NSCLC included, 1 patient achieved a complete response, 13 a partial response and the median progression free survival was 7.2 months. A maximum tolerated dose was not reached. Combination of dulanermin with these drugs did not significantly affect pharmacokinetics of dulanermin [31]. Results of a randomized phase 2 study with paclitaxel and carboplatin ± bevacizumab ± dulanermin (8 mg/kg iv for 5 days or 20 mg/kg iv for 2 days every 3 weeks) in 213 chemo naïve NSCLC patients showed that this combination is well tolerated. However, this combination did not result in a better objective response rate or progression free survival [32].

Dulanermin in doses up to 8 mg/kg iv for 5 days every 3 weeks was also combined with rituximab during 4 cycles in patients with low-grade NHL. There were 3 complete and 3 partial responses out of 12 patients treated [33]. The preliminary results of a randomized phase 2 study in patients with relapsed follicular NHL did not show a better objective response rate for this combination (61.5% versus 63.6%) [34].

Preliminary results in colorectal cancer patients show that dulanermin (up to 8 mg/kg iv for 5 days every 3 weeks) combined with irinotecan and cetuximab or dulanermin (up to 9 mg/kg iv for 3 days every 2 weeks) with leucovorin, 5-fluorouracil and irinotecan (FOLFIRI) is safe [35].

Mapatumumab

Mapatumumab in doses up to 30 mg/kg iv every 3 weeks was studied in combination with gemcitabine and cisplatin and in doses up to 20 mg/kg iv with paclitaxel and carboplatin in phase 1 studies. The maximum tolerated dose was not reached in either study. Partial responses were observed in respectively 12 out of 49 and 5 out of 27 patients. Combination of mapatumumab with chemotherapy regimens did not seem to influence the pharmacokinetics of any agent [36,37].

Preliminary results from a randomized phase 2 trial of mapatumumab combined with carboplatin and paclitaxel in 111 patients with NSCLC show that this combination does not lead to a better response rate or longer progression free survival [38].

In a phase 1b study, mapatumumab (up to 30 mg/kg every 3 weeks) was combined with sorafenib (400 mg BID) in patients with advanced hepatocellular carcinoma and chronic viral hepatitis. Among 19 patients, a partial response was seen in 2 patients and 4 patients achieved stable disease [39].

Agonistic TRAIL-R2 antibodies

In a phase 1b study, the combination of lexatumumab up to 10 mg/kg every 2 weeks with gemcitabine or FOLFIRI or lexatumumab every 3 weeks with pemetrexed or doxorubicin was studied in 41 patients. Preliminary results show 2 partial responses in colorectal cancer patients in the FOLFIRI arm and 1 partial response in a patient with small cell lung cancer in the doxorubicin arm. Pharmacokinetics of lexatumumab or the chemotherapeutics were not influenced by each other [40].

A randomized phase 2 study in 124 patients was performed comparing drozitumab or placebo plus paclitaxel, carboplatin and bevacizumab in previously untreated patients with NSCLC. The objective response rate did not differ between the two arms (respectively 40% and 42%), nor did the progression free survival [41].

A phase 2 study of drozitumab (10 mg/kg every 3 weeks, after a loading dose of 15 mg/kg) with rituximab in patients with NHL previously treated with rituximab, showed that this combination was well tolerated and 20 out of 40 patients achieved an objective response, consisting of 2 complete responses and 18 partial responses [42].

Two phase 1b studies in metastatic colorectal cancer patients were performed, in which drozitumab was combined with either FOLFOX and bevacizumab or cetuximab and irinotecan

or FOLFIRI ± bevacizumab. All combinations were found to be well tolerated [43, 44].

Conatumumab was studied in combination with several chemotherapy regimens and targeted therapies. In previously untreated patients with advanced NSCLC, the combination of conatumumab (up to 15 mg/kg every 3 weeks) with paclitaxel and carboplatin resulted in 1 complete response and 3 partial responses among 10 evaluable patients. Pharmacokinetics of conatumumab seemed not to be affected by combined treatment with paclitaxel and carboplatin [45].

Combination of conatumumab (up to 10 mg/kg every 2 weeks) with modified FOLFOX6 and bevacizumab in 12 patients with previously untreated colorectal cancer and combination with gemcitabine in 13 previously untreated patients with metastatic pancreatic cancer resulted in partial responses in respectively 5 and 4 patients. Pharmacokinetics showed no differences with those found in single agent studies [46,47].

Conatumumab (10 mg/kg every 2 weeks) in combination with panitumumab in pretreated metastatic colorectal cancer patients appeared to be safe, but did not result in objective responses in either patients with wild-type KRAS tumor status or mutant KRAS tumor status. Stable disease was seen in 8 out of 19 patients with wild-type KRAS and 4 out of 25 patients with KRAS mutant status [48].

Conatumumab (up to 15 mg/kg iv every 3 weeks) was also combined with AMG 479 (an insulin-like growth factor receptor 1 antagonistic antibody) in a phase 1 study. Three out of 9 patients achieved stable disease. Dose limiting toxicities were not observed and no interactions were seen between these agents [49].

In a randomized phase 2 study the combination of gemcitabine with conatumumab (10 mg/kg every 2 weeks) or AMG 479 or placebo in 125 patients with previously untreated pancreatic cancer was studied and showed that these combinations are well tolerated. Although no objective response was seen in the conatumumab arm, stable disease rate, progression free survival and 6 month survival seem to be better in the conatumumab and AMG 479 arms compared to placebo [50].

In a phase 1/2 open-label and double blind study in patients with metastatic or unresectable soft tissue sarcomas, patients were given conatumumab (15 mg/kg every 3 weeks) with doxorubicin or placebo with doxorubicin. Although this combination was safe, the addition of conatumumab did not improve the progression free survival or the response rates [51].

In the phase 1 trial with LBY135, 24 patients received LBY135 in doses up to 20 mg/kg every 3 weeks in combination with capecitabine (2 times daily, 2 weeks on, 1 week off). In these patients, 1 partial response was seen [26].

Efficacy of PARAs in combination studies

Data of 6 randomized phase 2 studies are available. The results indicate that only the combination of conatumumab and gemcitabine shows a trend toward a longer progression free survival and 6 month overall survival [50]. Addition of dulanermin to rituximab in NHL patients

does not seem to improve the objective response rate compared to rituximab alone, nor did the addition of conatumumab to doxorubicin in soft tissue sarcoma patients [34,51].

There were 3 randomized studies in patients with NSCLC. Combination of paclitaxel and carboplatin with mapatumumab does not seem to improve the objective response rate or progression free survival compared to chemotherapy alone. The same was the case for dulanermin and drozitumab with paclitaxel, carboplatin and bevacizumab [32,38, 41]. Therefore regretfully no benefit of the addition of dulanermin, mapatumumab or drozitumab could be shown in these randomized phase 2 studies.

However, we know that the combination of cetuximab or panitumumab with bevacizumab and chemotherapy in colorectal patients performed worse than one of the antibodies separately [52,53]. Therefore the addition of other antibodies to bevacizumab may hide its anti-tumor activity. The reason for this is still unraveled; however bevacizumab effects on tumor vascularization could be involved in this.

Interpretation of the preliminary results of the non-randomized phase 2 combination studies is hampered by the limited size of the studies. In earlier phase 1 combination studies, waterfall plots show anti-tumor effects of treatment with PARAs, although responses often do not meet the formal current RECIST criteria for partial response [31,36,37,54].

FUTURE PERSPECTIVES

Ongoing studies

In Table 2, the diverse ongoing studies with PARAs are shown. Of special interest are the studies that combine PARAs with other targeted therapies, since targeting the apoptosis route on multiple levels might lead to improved effectiveness.

Although PARAs also enhanced the cytotoxic effects of irradiation in preclinical settings, no clinical trials investigating the combination of these agents with radiotherapy have been conducted to date. A phase 1b/2 study combining mapatumumab, radiotherapy and cisplatin in patients with advanced cervical cancer (NCT01088347) and a phase 1/2 study combining conatumumab with gemcitabine, capecitabine and radiation therapy in patients with pancreatic cancer (NCT01017822) have been initiated.

Another approach is direct injection of recombinant adenovirus that encodes for TRAIL in tumor tissue. In a phase 1 trial these injections were given in the prostate of patients with prostate cancer. Preliminary results of the first 3 patients show that the injection was well tolerated [55].

Novel PARAs

In recent years several novel PARAs with improved properties targeting TRAIL receptors have been developed. Fusion of an antibody derivative to TRAIL can result in antibody targeting-dependent activation of TRAIL and other TNF family members that are in their soluble form biologically less active [56]. A number of TRAIL fusion proteins have been constructed, where recombinant soluble TRAIL was genetically linked to a receptor selective antibody fragment

Table 2: Ongoing trials with pro-apoptotic receptor agonists.

Drug	Phase	Tumor type	Combined with/study arms
Dulanermin	1b	Metastatic colorectal cancer	FOLFOX and bevacizumab
Mapatumumab	1b/2	Advanced cervical cancer	Cisplatin and radiotherapy
Mapatumumab	2	Advanced hepatocellular carcinoma	Arm 1: sorafenib + placebo Arm 2: sorafenib + mapatumumab
Mapatumumab	2	Relapsed/refractory multiple myeloma	Arm 1: bortezomib Arm 2: bortezomib + mapatumumab 10 mg/kg Arm 3: bortezomib + mapatumumab 20 mg/kg
Lexatumumab	1	Solid tumors/lymphoma	Recombinant interferon gamma
Conatumumab	1b	Lymphoma	Arms 1, 3, 5, 7: bortezomib + conatumumab Arms 2, 4, 6: vorinostat + conatumumab
Conatumumab	2	KRAS mutant metastatic colorectal cancer	Arm 1: conatumumab + FOLFIRI + AMG 479 placebo Arm 2: AMG 479 + FOLFIRI + conatumumab placebo Arm 3: FOLFIRI + AMG 479 placebo + conatumumab placebo
Conatumumab	1/2	Pancreatic cancer	Gemcitabine hydrochloride, capecitabine and radiation therapy
Conatumumab	1b/2	Metastatic colorectal cancer	Arm 1: conatumumab (low dose) + mFOLFOX + bevacizumab Arm 2: placebo + mFOLFOX + bevacizumab Arm 3: conatumumab (high dose) + mFOLFOX + bevacizumab
Conatumumab	2	Solid tumors/lymphoma	FOLFOX6, ganitumumab, bevacizumab (open label extension study)
Tigatuzumab	2	Pancreatic cancer	Gemcitabine

Drug	Phase	Tumor type	Combined with/study arms
Tigatuzumab	2	NSCLC	Arm 1: carboplatin + paclitaxel + tigatuzumab Arm 2: carboplatin + paclitaxel + placebo
Tigatuzumab	2	Metastatic colorectal caner	Arm 1: irinotecan + tigatuzumab Arm 2: irinotecan
Tigatuzumab	2	Advanced liver cancer	Arm 1: sorafenib + tigatuzumab Arm 2: sorafenib
Tigatuzumab	2	Ovarian cancer	paclitaxel + carboplatin
Tigatuzumab	1	Metastatic colorectal cancer	FOLFIRI
Tigatuzumab	2	Metastatic triple negative breast cancer	Arm 1: paclitaxel protein-bound + tigatuzumab Arm 2: paclitaxel protein-bound

Source: clinicaltrails.gov, August 2011.

based on a single chain variable fragment (scFv). The scFv can be directed against cancer specific targets such as EGFR [57], against AML cells using anti-CD33 scFv and against acute leukemic T-cells using anti-CD7 scFv [58,59].

Another approach to raise more effective PARAs is the computational design of TRAIL variants that bind TRAIL-R1 or TRAIL-R2 with stronger affinity and preferably have reduced affinity to the decoy receptors compared with rhTRAIL [60,61]. A novel antibody based approach is a tetrameric nanobody agonist targeting TRAIL-R2, TAS266 (Novartis) [62].

Biomarkers

Another important subject of intense investigation is the search for biomarkers that can predict the tumor response to these new agents and could thus be used to personalize treatment.

It would be of major interest to know whether apoptosis induction via TRAIL-R1 or TRAIL-R2 is influenced by the expression of these receptors and cellular downstream proteins in the tumors. Just immunohistochemistry of these targets in the tumor tissue may not be enough, since no clear relation between receptor expression and outcome has been shown in clinical studies so far [16-19, 36,37]. Other potential biomarkers for apoptosis are also evaluated in clinical trials [63,64]

In the preclinical setting the rate of O-glycosylation of TRAIL-R1 and TRAIL-R2 appears to be predictive of the sensitivity of tumor cells to dulanermin and drozitumab. In dulanermin and drozitumab sensitive tumor cells higher expression of mRNA encoding enzymes involved in O-glycosylation was found [65,66]. These enzymes can be assessed using immunohistochemistry assays, which are now tested in clinical trials with PARAs [32, 67].

Antibodies that are (radio)labeled could possibly also be used as biomarkers [68-72]. Preliminary results of an imaging study with ^{111}Indium labeled mapatumumab in patients show that mapatumumab is taken up in part of the tumor lesions [73]. An imaging trial with ^{111}Indium labeled CS1008 (tigatuzumab) is currently ongoing (NCT01220999). These imaging techniques could potentially predict availability of the drug at the tumor site and guide future therapy.

CONCLUSION

Based on its property to induce apoptosis in tumor cells while sparing normal cells, PARAs are of interest to explore as a new cancer treatment modality. In clinical studies, the use of both rhTRAIL and antibodies against TRAIL-R1 and TRAIL-R2 appears to be safe and side effects are generally mild. Monotherapy with these agents resulted in some anti-tumor efficacy which could occur after a long treatment period.

Combination of PARAs with other treatments seems to be safe. Although no full phase 3 studies have been performed, all results until now show only modest effects. The maximum tolerated dose with these (combinations of) drugs was mostly not reached. This uncertainty about dosing could partly be addressed by molecular imaging and labeling of the drugs involved. If there is a role for these drugs, it will be in the setting of a rational combination therapy. Ongoing

(randomized) combination studies, including combinations with other targeted therapies and radiotherapy, are awaited. Furthermore, novel PARAs with improved properties targeting TRAIL receptors and new biomarkers and imaging strategies that may help to select patients might lead to higher response rates in future trials.

ACKNOWLEDGEMENTS

Supported by Grants RUG 2005-3361 and RUG 2005-3365 of the Dutch Cancer Society and Grant T3.112 of the Dutch Top Institute Pharma.

References

[1] Wiley SR, Schooley K, Smolak PJ, Din WS, Huang CP, Nicholl JK, et al. Identification and characterization of a new member of the TNF family that induces apoptosis. Immunity. 1995 Dec;3(6):673-82.

[2] Rus V, Nguyen V, Puliaev R, Puliaeva I, Zernetkina V, Luzina I, et al. T cell TRAIL promotes murine lupus by sustaining effector CD4 th cell numbers and by inhibiting CD8 CTL activity. J Immunol. 2007 Mar 15;178(6):3962-72.

[3] Hoffmann O, Priller J, Prozorovski T, Schulze-Topphoff U, Baeva N, Lunemann JD, et al. TRAIL limits excessive host immune responses in bacterial meningitis. J Clin Invest. 2007 Jul;117(7):2004-13.

[4] Finnberg N, Klein-Szanto AJ, El-Deiry WS. TRAIL-R deficiency in mice promotes susceptibility to chronic inflammation and tumorigenesis. J Clin Invest. 2008 Jan;118(1):111-23.

[5] Spierings DC, de Vries EG, Vellenga E, van den Heuvel FA, Koornstra JJ, Wesseling J, et al. Tissue distribution of the death ligand TRAIL and its receptors. J Histochem Cytochem. 2004 Jun;52(6):821-31.

[6] Ashkenazi A, Pai RC, Fong S, Leung S, Lawrence DA, Marsters SA, et al. Safety and antitumor activity of recombinant soluble Apo2 ligand. J Clin Invest. 1999 Jul;104(2):155-62.

[7] Duiker EW, Mom CH, de Jong S, Willemse PH, Gietema JA, van der Zee AG, et al. The clinical trail of TRAIL. Eur J Cancer. 2006 Sep;42(14):2233-40.

[8] Chuntharapai A, Dodge K, Grimmer K, Schroeder K, Marsters SA, Koeppen H, et al. Isotype-dependent inhibition of tumor growth in vivo by monoclonal antibodies to death receptor 4. J Immunol. 2001 Apr 15;166(8):4891-8.

[9] Ichikawa K, Liu W, Zhao L, Wang Z, Liu D, Ohtsuka T, et al. Tumoricidal activity of a novel anti-human DR5 monoclonal antibody without hepatocyte cytotoxicity. Nat Med. 2001 Aug;7(8):954-60.

[10] Jin H, Yang R, Ross J, Fong S, Carano R, Totpal K, et al. Cooperation of the agonistic DR5 antibody apomab with chemotherapy to inhibit orthotopic lung tumor growth and improve survival. Clin Cancer Res. 2008 Dec 1;14(23):7733-40.

[11] Luster TA, Carrell JA, McCormick K, Sun D, Humphreys R. Mapatumumab and lexatumumab induce apoptosis in TRAIL-R1 and TRAIL-R2 antibody-resistant NSCLC cell lines when treated in combination with bortezomib. Mol Cancer Ther. 2009 Feb;8(2):292-302.

[12] Marini P, Junginger D, Stickl S, Budach W, Niyazi M, Belka C. Combined treatment with lexatumumab and irradiation leads to strongly increased long term tumour control under normoxic and hypoxic conditions. Radiat Oncol. 2009 Oct 27;4:49.

[13] Herbst RS, Eckhardt SG, Kurzrock R, Ebbinghaus S, O'Dwyer PJ, Gordon MS, et al. Phase I dose-escalation study of recombinant human Apo2L/TRAIL, a dual proapoptotic receptor agonist, in patients with advanced cancer. J Clin Oncol. 2010 Jun 10;28(17):2839-46.

[14] Tolcher AW, Mita M, Meropol NJ, von Mehren M, Patnaik A, Padavic K, et al. Phase I pharmacokinetic and biologic correlative study of mapatumumab, a fully human monoclonal antibody with agonist activity to tumor necrosis factor-related apoptosis-inducing ligand receptor-1. J Clin Oncol. 2007 Apr 10;25(11):1390-5.

[15] Hotte SJ, Hirte HW, Chen EX, Siu LL, Le LH, Corey A, et al. A phase 1 study of mapatumumab (fully human monoclonal antibody to TRAIL-R1) in patients with advanced solid malignancies. Clin Cancer Res. 2008 Jun 1;14(11):3450-5.

[16] Trarbach T, Moehler M, Heinemann V, Kohne CH, Przyborek M, Schulz C, et al. Phase II trial of mapatumumab, a fully human agonistic monoclonal antibody that targets and activates the tumour necrosis factor apoptosis-inducing ligand receptor-1 (TRAIL-R1), in patients with refractory colorectal cancer. Br J Cancer. 2010 Feb 2;102(3):506-12.

[17] Greco FA, Bonomi P, Crawford J, Kelly K, Oh Y, Halpern W, et al. Phase 2 study of mapatumumab, a fully human agonistic monoclonal antibody which targets and activates the TRAIL receptor-1, in patients with advanced non-small cell lung cancer. Lung Cancer. 2008 Jul;61(1):82-90.

[18] Younes A, Vose JM, Zelenetz AD, Smith MR, Burris HA, Ansell SM, et al. A phase 1b/2 trial of mapatumumab in patients with relapsed/refractory non-hodgkin's lymphoma. Br J Cancer. 2010 Dec 7;103(12):1783-7.

[19] Plummer R, Attard G, Pacey S, Li L, Razak A, Perrett R, et al. Phase 1 and pharmacokinetic study of lexatumumab in patients with advanced cancers. Clin Cancer Res. 2007 Oct 15;13(20):6187-94.

[20] Wakelee HA, Patnaik A, Sikic BI, Mita M, Fox NL, Miceli R, et al. Phase I and pharmacokinetic study of lexatumumab (HGS-ETR2) given every 2 weeks in patients with advanced solid tumors. Ann Oncol. 2010 Feb;21(2):376-81.

[21] Merchant MS, Chou AJ, Price A, Geller JI, Tsokos M, Graham C, et al. Lexatumumab: Results of a phase I trial in pediatric patients with advanced solid tumors. J Clin Oncol (Meeting Abstracts). 2010 May 20;28(15_suppl):9500.

[22] Camidge DR, Herbst RS, Gordon MS, Eckhardt SG, Kurzrock R, Durbin B, et al. A phase I safety and pharmacokinetic study of the death receptor 5 agonistic antibody PRO95780 in patients with advanced malignancies. Clin Cancer Res. 2010 Feb 15;16(4):1256-63.

[23] Forero-Torres A, Shah J, Wood T, Posey J, Carlisle R, Copigneaux C, et al. Phase I trial of weekly tigatuzumab, an agonistic humanized monoclonal antibody targeting death receptor 5 (DR5). Cancer Biother Radiopharm. 2010 Feb;25(1):13-9.

[24] Herbst RS, Kurzrock R, Hong DS, Valdivieso M, Hsu CP, Goyal L, et al. A first-in-human study of conatumumab in adult patients with advanced solid tumors. Clin Cancer Res. 2010 Dec 1;16(23):5883-91.

[25] Doi T, Murakami H, Ohtsu A, Fuse N, Yoshino T, Yamamoto N, et al. Phase 1 study of conatumumab, a pro-apoptotic death receptor 5 agonist antibody, in japanese patients with advanced solid tumors. Cancer Chemother Pharmacol. 2010 Dec 16.

[26] Sharma S, de Vries EG, Infante JR, Oldenhuis C, Chiang L, Bilic S, et al. Phase I trial of LBY135, a monoclonal antibody agonist to DR5, alone and in combination with capecitabine in advanced solid tumors. J Clin Oncol (Meeting Abstracts). 2008 May 20;26(15_suppl):3538.

[27] Jo M, Kim TH, Seol DW, Esplen JE, Dorko K, Billiar TR, et al. Apoptosis induced in normal human hepatocytes by tumor necrosis factor-related apoptosis-inducing ligand. Nat Med. 2000 May;6(5):564-7.

[28] Mahalingam D, Szegezdi E, Keane M, de Jong S, Samali A. TRAIL receptor signalling and modulation: Are we on the right TRAIL? Cancer Treat Rev. 2009 May;35(3):280-8.

[29] Ricci MS, Kim SH, Ogi K, Plastaras JP, Ling J, Wang W, et al. Reduction of TRAIL-induced mcl-1 and cIAP2 by c-myc or sorafenib sensitizes resistant human cancer cells to TRAIL-induced death. Cancer Cell. 2007 Jul;12(1):66-80.

[30] Daniel D, Yang B, Lawrence DA, Totpal K, Balter I, Lee WP, et al. Cooperation of the proapoptotic receptor agonist rhApo2L/TRAIL with the CD20 antibody rituximab against non-hodgkin lymphoma xenografts. Blood. 2007 Dec 1;110(12):4037-46.

[31] Soria JC, Smit E, Khayat D, Besse B, Yang X, Hsu CP, et al. Phase 1b study of dulanermin (recombinant human Apo2L/TRAIL) in combination with paclitaxel, carboplatin, and bevacizumab in patients with advanced non-squamous non-small-cell lung cancer. J Clin Oncol. 2010 Mar 20;28(9):1527-33.

[32} Soria JC, Mark Z, Zatloukal P, Szima B, Albert I, Juhasz E, et al. Randomized phase II study of dulanermin in combination with paclitaxel, carboplatin, and bevacizumab in advanced non-small-cell lung cancer. J Clin Oncol. 2011 Nov 20;29(33):4442-51.

[33] Fanale M, Burris H, Yee L, Lucas J, Dimick K, Goldwasser M, et al. Results of a phase 1B study of recombinant human APO2L/TRAIL with rituximab in patietns with relapsed low-grade NHL. Annals of Oncology. 2008 June;19(suppl 4):161.

[34] Belada D, Mayer J, Czuczman MS, Flinn IW, Durbin-Johnson B, Bray GL. Phase II study of dulanermin plus rituximab in patients with relapsed follicular non-hodgkin's lymphoma (NHL). J Clin Oncol (Meeting Abstracts). 2010 May 20;28(15_suppl):8104.

[35] Yee L, Burris HA, Kozloff M, Wainberg Z, Pao M, Skettino S, et al. Phase ib study of recombinant human Apo2L/TRAIL plus irinotecan and cetuximab or FOLFIRI in metastatic colorectal cancer (mCRC) patients (pts): Preliminary results. J Clin Oncol (Meeting Abstracts). 2009 May 20;27(15S):4129.

[36] Mom CH, Verweij J, Oldenhuis CN, Gietema JA, Fox NL, Miceli R, et al. Mapatumumab, a fully human agonistic monoclonal antibody that targets TRAIL-R1, in combination with gemcitabine and cisplatin: A phase I study. Clin Cancer Res. 2009 Sep 1;15(17):5584-90.

[37] Leong S, Cohen RB, Gustafson DL, Langer CJ, Camidge DR, Padavic K, et al. Mapatumumab, an antibody targeting TRAIL-R1, in combination with paclitaxel and carboplatin in patients with advanced solid malignancies: Results of a phase I and pharmacokinetic study. J Clin Oncol. 2009 Sep 10;27(26):4413-21.

[38] Von Pawel J, Harvey JH, Spigel DR, Dediu M, Reck M, Cebotaru CL, et al. A randomized phase II trial of mapatumumab, a TRAIL-R1 agonist monoclonal antibody, in combination with carboplatin and paclitaxel in patients with advanced NSCLC. J Clin Oncol. 2010;28 suppl(7s).

[39] Sun W, Nelson D, Alberts SR, Poordad F, Leong S, Teitelbaum UR, et al. Phase ib study of mapatumumab in combination with sorafenib in patients with advanced hepatocellular carcinoma (HCC) and chronic viral hepatitis. ASCO Meeting Abstracts. 2011 February 03;29(4_suppl):261.

[40] Sikic BI, Wakelee HA, von Mehren M, Lewis N, Calvert AH, Plummer ER, et al. A phase ib study to assess the safety of lexatumumab, a human monoclonal antibody that activates TRAIL-R2, in combination with gemcitabine, pemetrexed, doxorubicin or FOLFIRI. J Clin Oncol (Meeting Abstracts). 2007 June 20;25(18_suppl):14006.

[41] Karapetis CS, Clingan PR, Leighl NB, Durbin-Johnson B, O'Neill V, Spigel DR. Phase II study of PRO95780 plus paclitaxel, carboplatin, and bevacizumab (PCB) in non-small cell lung cancer (NSCLC). J Clin Oncol (Meeting Abstracts). 2010 May 20;28(15_suppl):7535.

[42] Wittebol S, Ferrant A, Wickham NW, Fehrenbacher L, Durbin-Johnson B, Bray GL. Phase II study of PRO95780 plus rituximab in patients with relapsed follicular non-hodgkin's lymphoma (NHL). J Clin Oncol (Meeting Abstracts). 2010 May 20;28(15_suppl):e18511.

[43] Rocha Lima CS, Baranda JC, Wallmark J, Choi Y, Royer-Joo S, Portera CC. Phase ib study of drozitumab combined with first-line FOLFOX plus bevacizumab (BV) in patients (pts) with metastatic colorectal cancer (mCRC). ASCO Meeting Abstracts. 2011 February 03;29(4_suppl):546.

[44] Baron AD, O'Bryant CL, Choi Y, Ashkenazi A, Royer-Joo S, Portera CC. Phase ib study of drozitumab combined with cetuximab (CET) plus irinotecan (IRI) or with FOLFIRI {+/-} bevacizumab (BV) in previously treated patients (pts) with metastatic colorectal cancer (mCRC). ASCO Meeting Abstracts. 2011 June 09;29(15_suppl):3581.

[45] Paz-Ares L, Sanchez Torres JM, Diaz-Padilla I, Links M, Reguart N, Boyer M, et al. Safety and efficacy of AMG 655 in combination with paclitaxel and carboplatin (PC) in patients with advanced non-small cell lung cancer (NSCLC). J Clin Oncol (Meeting Abstracts). 2009 May 20;27(15S):e19048.

[46] Saltz L, Infante J, Schwartzberg L, Stephenson J, Rocha-Lima C, Galimi F, et al. Safety and efficacy of AMG 655 plus modified FOLFOX6 (mFOLFOX6) and bevacizumab (B) for the first-line treatment of patients (pts) with metastatic colorectal cancer (mCRC). J Clin Oncol (Meeting Abstracts). 2009 May 20;27(15S):4079.

[47] Kindler HL, Garbo L, Stephenson J, Wiezorek J, Sabin T, Hsu M, et al. A phase ib study to evaluate the safety and efficacy of AMG 655 in combination with gemcitabine (G) in patients (pts) with metastatic pancreatic cancer (PC). J Clin Oncol (Meeting Abstracts). 2009 May 20;27(15S):4501.

[48] Peeters M, Infante P, Rougier P:L,J., Uronis HE, Stephenson J, Schwartzberg LS, et al. Phase Ib/II trial of conatumumab and panitumumab (pmab) for the treatment (tx) of metastatic colorectal cancer (mCRC): Safety and efficacy. ASCO Gastrointestinal Cancers Symposium. 2010;abstract 443.

[49] Chawla SP, Tabernero J, Kindler HL, Chiorean EG, LoRusso P, Hsu M, et al. Phase I evaluation of the safety of conatumumab (AMG 655) in combination with AMG 479 in patients (pts) with advanced, refractory solid tumors. J Clin Oncol (Meeting Abstracts). 2010 May 20;28(15_suppl):3102.

[50] Kindler HL, Richards DA, Stephenson J, Garbo LE, Rocha Lima CS, Safran H, et al. A placebo-controlled, randomized phase II study of conatumumab (C) or AMG 479 (A) or placebo (P) plus gemcitabine (G) in patients (pts) with metastatic pancreatic cancer (mPC). J Clin Oncol (Meeting Abstracts). 2010 May 20;28(15_suppl):4035.

[51] Demetri GD, Le Cesne A, Chawla SP, Brodowicz T, Maki RG, Bach BA, et al. First-line treatment of metastatic or locally advanced unresectable soft tissue sarcomas with conatumumab in combination with doxorubicin or doxorubicin alone: A phase I/II open-label and double-blind study. Eur J Cancer. 2012 Mar;48(4):547-63.

[52] Tol J, Koopman M, Cats A, Rodenburg CJ, Creemers GJ, Schrama JG, et al. Chemotherapy, bevacizumab, and cetuximab in metastatic colorectal cancer. N Engl J Med. 2009 Feb 5;360(6):563-72.

[53] Hecht JR, Mitchell E, Chidiac T, Scroggin C, Hagenstad C, Spigel D, et al. A randomized phase IIIB trial of chemotherapy, bevacizumab, and panitumumab compared with chemotherapy and bevacizumab alone for metastatic colorectal cancer. J Clin Oncol. 2009 February 10;27(5):672-80.

[54] Eisenhauer EA, Therasse P, Bogaerts J, Schwartz LH, Sargent D, Ford R, et al. New response evaluation criteria in solid tumours: Revised RECIST guideline (version 1.1). Eur J Cancer. 2009 Jan;45(2):228-47.

[55] Griffith T, Konety B, Joudi F, Aubert H, Cohen M, Ratliff T, et al. Phase I study of Ad5-TRAIL in men with clinically organ confined prostate cancer. AACR Meeting Abstracts. 2007 April 14;2007(1_Annual_Meeting):4870.

[56] Wajant H, Moosmayer D, Wuest T, Bartke T, Gerlach E, Schonherr U, et al. Differential activation of TRAIL-R1 and -2 by soluble and membrane TRAIL allows selective surface antigen-directed activation of TRAIL-R2 by a soluble TRAIL derivative. Oncogene. 2001 Jul 5;20(30):4101-6.

[57] Bremer E, Samplonius DF, van Genne L, Dijkstra MH, Kroesen BJ, de Leij LF, et al. Simultaneous inhibition of epidermal growth factor receptor (EGFR) signaling and enhanced activation of tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) receptor-mediated apoptosis induction by an scFv:STRAIL fusion protein with specificity for human EGFR. J Biol Chem. 2005 Mar 18;280(11):10025-33.

[58] ten Cate B, Bremer E, de Bruyn M, Bijma T, Samplonius D, Schwemmlein M, et al. A novel AML-selective TRAIL fusion protein that is superior to gemtuzumab ozogamicin in terms of in vitro selectivity, activity and stability. Leukemia. 2009 Aug;23(8):1389-97.

[59] Bremer E, Samplonius DF, Peipp M, van Genne L, Kroesen BJ, Fey GH, et al. Target cell-restricted apoptosis induction of acute leukemic T cells by a recombinant tumor necrosis factor-related apoptosis-inducing ligand fusion protein with specificity for human CD7. Cancer Res. 2005 Apr 15;65(8):3380-8.

[60] van der Sloot AM, Tur V, Szegezdi E, Mullally MM, Cool RH, Samali A, et al. Designed tumor necrosis factor-related apoptosis-inducing ligand variants initiating apoptosis exclusively via the DR5 receptor. Proc Natl Acad Sci U S A. 2006 Jun 6;103(23):8634-9.

[61] Reis CR, van der Sloot AM, Natoni A, Szegezdi E, Setroikromo R, Meijer M, et al. Rapid and efficient cancer cell killing mediated by high-affinity death receptor homotrimerizing TRAIL variants. Cell Death Dis. 2010 Oct 21;1:e83.

[62] Huet H, Schuller A, Li J, Johnson J, Dombrecht B, Meerschaert K, Cromie K, Bilic S, Li S, Garner A, Nunes S, Chopra R, Clark K, Yao M, Fawell S, Stover D, Sellers WR, Ettenberg S. TAS266, a novel tetrameric nanobody agonist targeting death receptor 5 (DR5), elicits superior antitumor efficacy than conventional DR5-targeted approaches. AACR Meeting Abstracts. 2012;3853.

[63] Pan Y, Xu R, Peach M, Huang CP, Branstetter D, Novotny W, et al. Evaluation of pharmacodynamic biomarkers in a phase 1a trial of dulanermin (rhApo2L/TRAIL) in patients with advanced tumours. Br J Cancer. 2011 Oct 27.

[64] Zoog SJ, Ma CY, Kaplan-Lefko PJ, Hawkins JM, Moriguchi J, Zhou L, et al. Measurement of conatumumab-induced apoptotic activity in tumors by fine needle aspirate sampling. Cytometry A. 2010 Sep;77(9):849-60.

[65] Wagner KW, Punnoose EA, Januario T, Lawrence DA, Pitti RM, Lancaster K, et al. Death-receptor O-glycosylation controls tumor-cell sensitivity to the proapoptotic ligand Apo2L/TRAIL. Nat Med. 2007 Sep;13(9):1070-7.

[66] Punnoose EA, Wagner K, Amler L, Ashkenazi A. Sensitivity to apomab, an agonistic DR5 specific antibody, is correlated with expression of specific O-glycosyl transferases in tumor-cell lines of both epithelial and non epithelial origin. AACR Annual Meeting. 2009;abstract 687.

[67] Stern HM, Padilla M, Wagner K, Amler L, Ashkenazi A. Development of immunohistochemistry assays to assess GALNT14 and FUT3/6 in clinical trials of dulanermin and drozitumab. Clin Cancer Res. 2010 Mar 1;16(5):1587-96.

[68] Kim H, Chaudhuri TR, Buchsbaum DJ, Wang D, Zinn KR. High-resolution single-photon emission computed tomography and X-ray computed tomography imaging of tc-99m-labeled anti-DR5 antibody in breast tumor xenografts. Mol Cancer Ther. 2007 Mar;6(3):866-75.

[69] Gong J, Yang D, Kohanim S, Humphreys R, Broemeling L, Kurzrock R. Novel in vivo imaging shows up-regulation of death receptors by paclitaxel and correlates with enhanced antitumor effects of receptor agonist antibodies. Mol Cancer Ther. 2006 Dec;5(12):2991-3000.

[70] Zhou L, Dicker D, Wang W, El-Deiry WS. Prediction of pro-apoptotic anti-cancer therapeutic response by visualization of death receptors and specific markers of apoptosis. AACR Annual Meeting. 2009;abstract 4471.

[71] Rossin R, Kohno T, Hagooly A, Sharp T, Gliniak B, Arroll T, et al. Characterization of 64Cu-DOTA-conatumumab: A PET tracer for in vivo imaging of death receptor 5. J Nucl Med. 2011 Jun;52(6):942-9.

[72] Duiker E, Dijkers E, Heerspink HL, de Jong S, van der Zee A, Jager P, et al. Development of radioiodinated rhTRAIL and a radiolabelled agonistic TRAIL receptor antibody for clinical imaging studies. Br J Pharmacol. 2011 Oct 20.

[73] Oldenhuis C, Dijkers EC, Duiker EW, Fox NL, Klein JL, Gietema JA, et al. Development of radiolabeled mapatumumab and imaging in solid tumor patients who are treated with gemcitabine, cisplatin, and mapatumumab. J Clin Oncol (Meeting Abstracts). 2009 May 20;27(15S):e14521.

Chapter 3

^{18}F-FDG-PET response no early predictive marker for primary imatinib resistance in patients with gastrointestinal stromal tumors

S.F. Oosting [1], M.W. den Hollander [1], B. Rikhof [1], D.B. Rouw [2], J.R. de Jong [3], P.L. Jager [4], A.H. Brouwers [3], A.J.H. Suurmeijer [5], W.J. Sluiter [6], W.T.A. van der Graaf [7], E.G.E. de Vries [1], J.A. Gietema [1], A.K.L. Reyners [1]

Departments of [1]Medical Oncology, [2]Radiology, [3]Nuclear Medicine and Molecular Imaging, [5]Pathology, [6]Endocrinology, University of Groningen, University Medical Centre Groningen, Groningen, The Netherlands. [4]Department of Nuclear Medicine, Isala Clinics, Zwolle, The Netherlands. [7]Department of Medical Oncology, Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands.

Submitted

Previously presented at the ASCO Annual Meeting 2011

ABSTRACT

Background: Approximately 15% of gastrointestinal stromal tumor (GIST) patients show primary resistance to imatinib, defined as progressive disease on CT after 8 weeks. We investigated whether early change in tumor ^{18}F-fluorodeoxyglucose uptake on positron emission tomography (FDG-PET) predicts primary imatinib resistance.

Methods: 36 metastatic or locally advanced GIST patients underwent FDG-PET scans before and 1 week after start of imatinib. Relationship between FDG-PET response (EORTC criteria) and CT response after 2 months of treatment (RECIST 1.0 and Choi criteria) was investigated. FDG uptake was measured as Standardized Uptake Value (SUV).

Results: Of the 30 patients evaluable with FDG-PET, 26 experienced a response and 4 had stable disease. Mean tumor SUV_{max} decreased from 7.4 (SD 3.8, range 2.2-18.4) to 3.0 (SD 2.1, range 0.1-11.8) after 1 week imatinib ($P < 0.001$). FDG-PET response had a high positive predictive value for clinical benefit (response or stable disease) according to RECIST 1.0: 92% (95% CI 75-99%) and Choi: 95% (95% CI 76-100%). The false negative rate was respectively 11% (95% CI 2-30%) and 9% (95% CI 1-30%).

Conclusion: While FDG-PET response has a high positive predictive value for clinical benefit of imatinib in GIST patients, it does not predict primary resistance.

INTRODUCTION

Gastrointestinal stromal tumors (GIST) are mesenchymal tumors that arise in the gastrointestinal tract. They are characterized by expression of CD117; the KIT receptor. Approximately 80% of all GISTs have a gain of function mutation in *KIT* resulting in constitutive activation and continuous downstream signaling. Furthermore, 5-10% of GISTs have an activating mutation in the gene encoding platelet derived growth factor receptor α (*PDGFRA*). Imatinib is an oral tyrosine kinase inhibitor that inhibits signaling of both KIT and PDGFRα. The majority of patients with unresectable or metastatic GIST benefits from treatment with imatinib [1]. However, ± 15% of GIST patients have primary imatinib resistant disease, i.e. progressive disease within 3 months after start of treatment [2]. Earlier or upfront identification of primary resistance would spare these patients the side effects of ineffective therapy and allow an earlier switch to alternative treatment. To date, no predictive biomarkers to guide treatment decisions are available. However, it is well appreciated that imatinib can induce a rapid and dramatic decrease in glucose uptake in GIST [3-6].

The objective of this study was to investigate whether metabolic response early after initiation of treatment can be used to predict primary resistance to imatinib in patients with locally advanced or metastatic GIST. In addition, we studied whether the metabolic response correlated with progression free survival (PFS) or specific receptor tyrosine kinase gene mutations.

MATERIALS AND METHODS

Patients and study design

This is a retrospective analysis of consecutive patients with newly diagnosed locally advanced, metastatic or recurrent GIST, who started treatment with imatinib between February 2001 and October 2007 at the University Medical Centre Groningen (UMCG). Imatinib was administered orally at 400 to 800 mg per day. Fourteen patients were part of an earlier study [4].

FDG-PET

FDG-PET scans at baseline and after 1 week imatinib treatment were standard care from February 2001 for patients with advanced GIST in the UMCG. As of October 2007, a different PET scan protocol was used. Therefore, this analysis is restricted to GIST patients who underwent PET scans before October 2007. PET scans were performed on a Siemens ECAT EXACT HR+ scanner in 2D mode. Patients fasted for 6 hours. Ninety min after injection of 5 MBq/kg ^{18}F-FDG, a whole body scan was performed (7-8 bed positions from femur to crown, 8 min per bed position of which 3 min transmission time). The iterative reconstruction algorithm AW-OSEM 2D was used with 2 iterations, 8 subsets and a Gaussian filter of 10 mm.

For each patient, a maximum of 5 target lesions was used for tumor evaluation. Target lesions were defined as the 5 most intense FDG accumulating tumor lesions. The FDG uptake was measured by calculating the Standardized Uptake Value (SUV) as described earlier in regions of interest (ROI) placed over tumor lesions, with Siemens Leonardo software [7]. The maximum SUV (SUV_{max}) and the uptake in the 3-dimensional isocontour at 70% and at 40% of the maximum

pixel value (SUV70 and SUV40) for each target lesion was measured. For all target lesions in a patient the mean SUV_{max}, the mean SUV70 and the mean SUV40 was calculated for both scans. For classification of metabolic tumor response, the EORTC criteria for FDG-PET imaging were used [7]. Also the previously reported thresholds for the single lesion with the most intense uptake at baseline (25% reduction, 40% reduction, <2.5 and <3.4 for SUV_{max} on the second scan) were tested [8] and the definition of metabolic response used by Choi et al (decrease of mean SUV_{max} with ≥70% to less than 2.5) [9].

CT scan

CT scans were performed at baseline, after 8 weeks and every 3 months thereafter. For response classification, Response Evaluation Criteria for Solid Tumors (RECIST) version 1.0 was used as well as the criteria described by Choi et al [9,10].

Mutation analysis

Mutation analysis of *KIT* exons 9, 11, 13 and 17 and *PDGFRA* exons 12, 14 and 18 was performed as described previously [11].

Outcome parameters

Primary resistance is defined as progressive disease after 8 weeks of treatment according to RECIST1.0 or the Choi criteria. Positive predictive value, negative predictive value and false negative rate of FDG-PET for primary resistance were calculated with 95% confidence intervals (CI).

Progression free survival (PFS) was defined as the time from imatinib initiation until disease progression or death, whichever occurred first. For PFS analysis, the occurrence of a new lesion, or an increase in size of pre-existing lesions (as defined by RECIST 1.0), or development of an intra-tumoral nodule and/or an increase in 'solid' tissue, in the background of a hypodense lesion were considered progressive disease according to the ESMO guidelines for GIST [12,13].

Statistics

For comparison of the mean SUV at the first and second FDG-PET scan, the Wilcoxon signed rank test was used. PFS was estimated with the Kaplan-Meier method. Patients were censored at the date of surgery for complete surgical resection and at the date of last follow up for patients alive and progression free at the time of analysis.

RESULTS

Patients

Thirty six patients with a mean age of 62 years (range 23 - 81) were included. Nine patients had locally advanced disease and 27 patients had metastatic or recurrent disease. For characteristics see Table 1. Two patients started with imatinib 400 mg 2 times daily, the others with 400 mg once daily. The median follow-up time was 35 months (range 4 - 87+). In patients who received imatinib with a non-curative intent, median PFS was 23 months (range 2 - 83+ months) and in this subgroup median overall survival was 32 months (range 4 - 87+ months). Twelve patients received subsequent systemic treatment upon disease progression.

Table 1. Patient characteristics.

Characteristics	Total (N = 36)
Age (years)	
Median	62
Range	23-81
Sex, N (%)	
Male	22 (61)
Female	14 (39)
Treatment setting, N (%)	
Neo-adjuvant	7 (19)
Palliative	29 (81)
Primary site, N (%)	
Stomach	13 (36)
Small bowel	14 (39)
Colon	3 (8)
Other	6 (17)
Metastatic sites, N (%)	
Liver	11 (31)
Peritoneal cavity	8 (22)
Liver and peritoneal cavity	6 (17)
Other	2 (6)
Mutation type, N (%)	
KIT exon 11	15 (42)
KIT exon 9	5 (14)
PDGFRA exon 18	3 (8)
Wild typea	2 (6)
Unknown	11 (31)

N: number of patients

a No KIT or PDGFRA mutation

FDG-PET assessment

The baseline FDG-PET scan was performed at a median of 2 days (range 1 - 46) before start of treatment. Four patients had no FDG-avid lesions and therefore did not undergo a second FDG-PET scan. These four patients had normal blood glucose levels and were not on glucose lowering medication. The repeat scan was performed at median 8 days (range, 6-10) after start of imatinib. In two patients, a different FDG-PET imaging protocol was used for the baseline and repeat scan; these patients were therefore excluded from the analysis, resulting in 60 FDG-PET scans of 30 patients available for quantification of metabolic response. Tumor FDG-uptake decreased from baseline with a mean SUV_{max} of 7.4 (SD 3.8, range 2.2 - 18.4) to a mean SUV_{max} of 3.0 (SD 2.1, range 0.1 - 11.8, $P < 0.001$) after 1 week imatinib (Fig. 1).

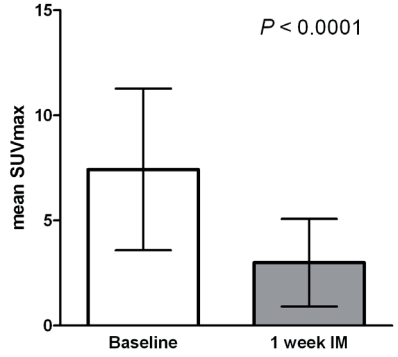
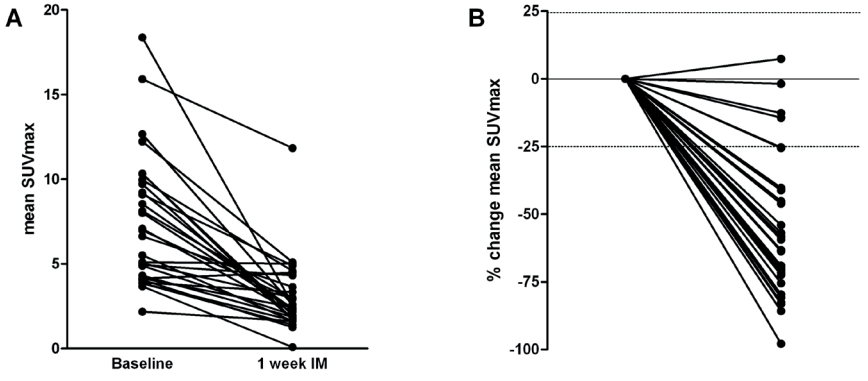


Figure 1. (left) Tumor FDG uptake (in mean SUV_{max} with standard deviation) in 30 GIST patients before start and after 1 week imatinib (IM) treatment.

Figure 2. (below) A. Absolute and B. relative changes in tumor FDG uptake (in mean SUV_{max}) in individual patients (N = 30) in up to 5 tumor lesions between baseline PET scan and PET scan after 1 week imatinib (IM) treatment. Grey dotted lines shows thresholds for response (-25%) and progressive disease (+25%) according to EORTC criteria.



Relative changes in mean SUV_{max} for individual patients ranged from +7.3% to -97.8% (Fig. 2B). Based on change of mean SUV_{max}, 26 patients experienced a metabolic response according to EORTC criteria and four patients had metabolic stable disease (hereafter called non-responders). None of the patients had metabolic progressive disease. Analysis of mean SUV40 and mean SUV70 revealed a similar pattern with 26 responders and four non-responders, although according to mean SUV70, one non-responder had metabolic progressive disease.

Predictive value of metabolic response

One out of 30 patients had non-measurable disease on CT, therefore data of 29 patients were available for response classification according to RECIST. Two patients had progressive disease, i.e. primary imatinib resistance. In six patients Hounsfield Units could not be measured, therefore evaluation according to the Choi criteria could be applied in 23 patients (Table 2). Positive predictive value of a metabolic response (estimated with mean SUV_{max}) for clinical benefit from imatinib is 92% (95% CI 75 - 99%) for RECIST and 95% (95% CI 76 - 100%) for the Choi criteria. As none of the metabolic non-responders in our cohort had primary imatinib resistant disease, a negative predictive value could not be calculated. The false negative rate of FDG-PET for prediction of clinical benefit from imatinib was 11% (95% CI 2-30%) for RECIST and 9% (95% CI

1–30%) for the Choi criteria. Of the four patients with a negative baseline FDG-PET scan, three had clinical benefit (two partial responses and one stable disease) and one had progressive disease according to RECIST. Based on the Choi criteria two out of three derived benefit from imatinib (both partial responses) whereas the third patient had primary resistant disease.

Table 2. FDG PET versus CT response.

	CT scan			
	RECIST (N = 29)		Choi (N = 23)	
	CR/PR/SD[a]	PD	CR/PR/SD[b]	PD
FDG-PET scan				
Response[c]	24	2	20	1
No response	3	0	2	0

FDG-PET response after 1 week and CT response after 8 weeks of imatinib treatment.

N = number of patients, CR = complete response, PR = partial response, SD = stable disease, PD = progressive disease

[a] CR (N = 1), PR (N = 9), SD (N = 17)

[b] CR (N = 1), PR (N = 16), SD (N = 5)

[c] decrease in mean SUVmax ≥ 25% and no new lesions and no visible increase in extent of tumor FDG-uptake >20% in the longest dimension

For mean SUV70 identical predictive values for primary imatinib resistant disease as for mean SUV_{max} were obtained and mean SUV40 performed worse. There was no difference in PFS between metabolic responders, non-responders and patients with non FDG-avid lesions when using mean SUV_{max} according to the EORTC criteria (Fig. 3). This was also the case for mean SUV70, mean SUV40, for the definition of response used by Choi et al and for SUV_{max} of the single lesion with the most intensive uptake at baseline according to the thresholds described by Holdsworth et al [8,9].

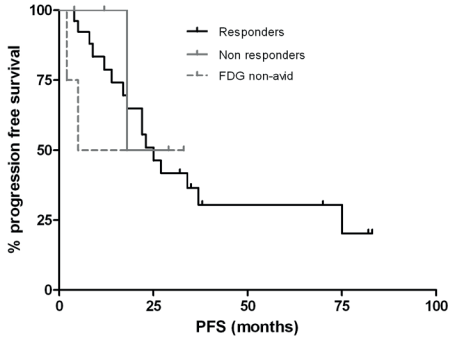


Figure 3. PFS in patients with a metabolic tumor response (***N*** = 26, black line), patients without metabolic response (***N*** = 4, grey solid line) and in patients with non FDG-avid tumor lesions (***N*** = 4, grey dashed line). No difference in PFS was found.

Mutation analysis and metabolic response

In 25 of 36 patients, sufficient tumor tissue was available for mutation analysis. A *KIT* exon 11 mutation was present in 15 cases (60%), a *KIT* exon 9 mutation in five (20%), a *PDGFRA* exon 18 mutation in three (12%), and no mutation was found in either *KIT* or *PDGFRA* in two patients (8%).

One patient with a *KIT* exon 9 mutation and two patients with a *PDGFRA* mutation showed no FDG uptake in tumor lesions at baseline. One patient with a *KIT* exon 11 and one patient with a *KIT* exon 9 mutation were not evaluable for metabolic response. Metabolic response for 20 patients according to mutation is shown in Fig. 4.

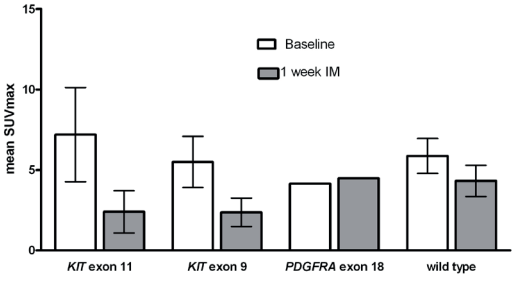


Figure 4. Change in tumor FDG uptake (mean SUV_{max} with standard deviation) after 1 week imatinib treatment according to mutation type: *KIT* exon 11 (*N* = 14), *KIT* exon 9 (*N* = 3), *PDGFRA* (*N* = 1) and in wild type tumors (*N* = 2). IM = imatinib.

DISCUSSION

The results of the current study show that early FDG-PET response cannot be used to identify primary imatinib resistant disease in patients with GIST. Absence of an early metabolic response does not indicate that patients do not benefit from imatinib.

Furthermore, patients with primary resistant disease can have a metabolic response. Although we found high positive predictive values of metabolic response for clinical benefit from imatinib (92% for RECIST and 95% for the Choi criteria) the upfront chance of response or stable disease is 85%, which falls within the 95% CI of the positive predictive value. Therefore, little if any predictive information on treatment outcome is added by early assessing metabolic response.

Stroobants et al. performed FDG-PET scans at baseline and after 1 week of imatinib treatment in 17 GIST patients [6]. From their study a positive predictive value of 92%, a negative predictive value of 75% and a false negative rate of 8% for patients who derive clinical benefit from imatinib (response plus stable disease as best response according to RECIST) can be calculated, which is comparable with our results. Recently FDG-PET results of a study on neoadjuvant imatinib treatment in operable GIST patients were reported [14]. Looking at metabolic responders versus non-responders after 1 week of treatment, a positive predictive value of 100%, a negative predictive value of 14%, and a false negative rate of 16% can be calculated for clinical benefit from imatinib, again in line with our findings. A possible explanation for incidental incongruence between antitumor activity and glucose uptake is given by Tarn et al [15]. They demonstrated in

Table 3. Summary of studies investigating the role of FDG PET in response evaluation of imatinib treated GIST patients.

First author	FDG non avid (N)	Repeat PET (N)	2nd PET	PET analysis	2nd CT	CT analysis	Clinical endpoint	Predictive value PET
Antoch [22]	NR	20	1 mo	For max 5 lesions: - sum of SUV_{max} - EORTC	1 mo	WHO[a] RECIST	NR	NR
Choi [9]	NR	40	2 mo	For all lesions: - mean SUV_{max} - ≥ 70% reduction and < 2.5 = good response	2 mo	RECIST Choi criteria	TTP[d]	Good response predicts TTP
Gayed [23]	NR	49	2 mo	For largest lesion in every organ: - SUV_{max} - adjusted EORTC	2 mo	5% change in longest diameter of largest lesion per organ	NR	NR
Goerres [16]	7	28	NR	For 1 lesion: - visual change - positive vs negative 2nd scan - SUV - adjusted EORTC	NR	RECIST	TTP[e] OS	Negative 2nd PET predicts TTP and OS
Goldstein [17][16]	1	17	2 mo	- visual response vs no response	2 mo	RECIST	NR	For clinical benefit vs PD on 2nd CT: PPV 93%, NPV 100%, FNR 50%

Table 3: continued.

First author	FDG non avid (N)	Repeat PET(N)	2nd PET	PET analysis	2nd CT	CT analysis	Clinical endpoint	Predictive value PET
Holdsworth [8]	NR	63	1 mo	For 1 lesion: - SUV_{max} - 25% reduction - 40% reduction - < 2.5 - < 3.4	1 mo	SWOG[b]	TTF[e]	All PET parameters predict TTF
Jager [4]	NR	14	1 wk	For multiple lesions: - mean SUV_{max} - % change	2 mo	RECIST OR[c]	PFS[e]	- PET response predicts PFS - For CB vs PD on CT 2nd CT: PPV 100%, FNR 21%
Stroobants [6]	2	19	1 wk	For 3 lesions: - SUV_{max} - EORTC	1 mo	RECIST	TTF[e]	- PET response predicts PFS - For CB vs PD as best response: PPV 92%, NPV 75%, FNR 8%
Van den Abbeele [14]	NR	39	1 wk	For target lesions: - background-subtracted SUV_{max} - EORTC	1 mo	RECIST	NR	For CB vs PD as best response: PPV 100% NPV 14% FNR 16%

First author	FDG non avid (N)	Repeat PET(N)	2nd PET	PET analysis	2nd CT	CT analysis	Clinical endpoint	Predictive value PET
Oosting	4	30	1 wk	For max 5 lesions: - mean SUV_{max} - mean SUV40 - mean SUV70 - EORTC - 70% reduction and < 2.5 For 1 lesion: - SUV_{max} - 25% reduction - 40% reduction - < 2.5 - < 3.4	2 mo	RECIST Choi criteria	PD at 2 mo PFS	For CB vs PD on 2nd CT (RECIST): PPV 92% FNR 11% (Choi) PPV 95% FNR 9% - No correlation between PET response and PFS

N: number of patients; SUV = standardized uptake value; NR = not reported; NA not available; mo = months, CB = clinical benefit (complete response + partial response + stable disease); PD = progressive disease; PPV = positive predictive value; NPV = negative predictive value, FNR = false negative rate; TTP = time to progression; OS = overall survival; PFS = progression free survival; TTF = time to treatment failure (failure = progression, death or treatment discontinuation)[a] WHO = World Health Organization guidelines [24].

[b] SWOG = South West Oncology Group criteria [25].

[c] OR = overall treatment response (clinical and radiological parameters and change in the rate of disease progression) [26].

[d] Progression: new lesion on CT, or new intra-tumoral nodule, or increase in size of existing intra-tumoral nodule, or overall increase in size >20% in absence of post treatment hypodense change

[e] No definition of progression

vitro that different intracellular signaling cascades are responsible for imatinib induced down-regulation of Glut4 expression and imatinib induced apoptosis in GIST cells. However, no association between reduction in Glut4 expression and reduction in FDG uptake in tumors of GIST patients treated with imatinib in the neoadjuvant setting was found [14]. Previous studies in GIST patients showed that metabolic response correlated with PFS, time to progression or time to treatment failure (i.e. disease progression or death from any cause whichever occurs first) [4,6,8,9,16].

In Table 3, the current and previous studies are summarized. We did not find a correlation between metabolic response and PFS despite testing different SUV parameters and multiple cut off values for metabolic response. This may be due to the different definitions of metabolic response that are used. EORTC criteria are based on a few small studies in which no GIST patients were included. These criteria should therefore be regarded as consensus recommendations rather than evidence based guidelines [7]. Also, adherence to EORTC FDG-PET criteria does not guarantee similar analysis, as for example the number of lesions to be assessed per patient is not defined. Furthermore, a description of how disease progression is determined, is only provided by Choi et al and differs slightly from the ESMO recommendation that we used [9,13]. Finally, the small size of these studies and the different timing of FDG-PET scans will clearly affect the results.

We found no FDG uptake in tumor lesions before start of treatment in four out of 36 patients. This corresponds with previous findings [6,16,17]. The numbers of patients are too small to draw conclusions on prognostic or predictive value of a negative baseline FDG- PET scan.

We studied metabolic response in GIST patients starting first line treatment, according to mutation. Although small numbers prohibit conclusions, the FDG-PET results for the three patients with a *PDGFRA* exon 18 mutation were striking: two patients showed non FDG-avid lesions and the third patient is the only patient with an increase in FDG uptake after 1 week imatinib treatment. This suggests that in tumors with a *PDGFRA* exon 18 mutation, metabolic response to imatinib differs from *KIT* mutated tumors. Metabolic response was seen in 85% of patients with *KIT* exon 11 mutations (23/27) and in 50% (2/4) of patients without *KIT* or *PDGFRA* mutations after 1 week imatinib [14]. Fuster et al. performed FDG-PET scans in imatinib resistant GIST patients before and after initiation of doxorubicin while continuing imatinib [18]. In 15 patients with mutation analysis available, they demonstrated lower baseline SUV_{max} in patients with wild type *KIT* tumors compared to non-wild type *KIT* tumors. However, in another second line study with sunitinib in imatinib resistant GIST patients, no correlation was found between *KIT* mutational status and metabolic activity or metabolic response in 22 patients [19].

Limitations of our study are the retrospective nature and the relative small size of the cohort. However, the data presented support the conclusion that early assessment of metabolic response with FDG-PET after 1 week of imatinib treatment in GIST patients is not helpful for go-no-go decisions. Primary imatinib resistance cannot be reliably identified with this technique. Therefore absence of progressive disease at 2 and 4 months according to RECIST 1.0 remains the most robust way to identify patients with a survival benefit from imatinib [20]. For second line

treatment with sunitinib also the absence of progressive disease according to RECIST 1.0 at 3 months seems the best way to identify patients benefiting from this treatment [21].

This does not preclude an important role for FDG-PET imaging in staging GIST patients, as FDG-PET can reveal metastases that are missed on CT [4,16,22,23].

In conclusion, the results of our study suggest that repeat FDG-PET imaging early after initiation of imatinib in patients with GIST is not informative for clinical decision making with regard to continuation of imatinib. Imatinib is an extremely effective agent for this disease and should, in the advanced setting, be continued until convincing clinical and/or radiological evidence of progressive disease or unacceptable toxicity.

References

[1] Blanke CD, Demetri GD, von Mehren M et al. Long-term results from a randomized phase II trial of standard- versus higher-dose imatinib mesylate for patients with unresectable or metastatic gastrointestinal stromal tumors expressing KIT. J Clin Oncol 2008;26**:**620-5.

[2] Demetri GD, von Mehren M, Blanke CD et al. Efficacy and safety of imatinib mesylate in advanced gastrointestinal stromal tumors. N Engl J Med 2002;347**:**472-80.

[3] Abhyankar SA, Nair N. Highlighting the role of FDG PET scan in early response assessment of gastrointestinal stromal tumor treated with imatinib mesylate. Clin Nucl Med 2008;33:213-4.

[4] Jager PL, Gietema JA, van der Graaf WTA. Imatinib mesylate for the treatment of gastrointestinal stromal tumours: best monitored with FDG PET. Nucl Med Commun 2004;25:433-8.

[5] Shinto A, Nair N, Dutt A, Baghel NS. Early response assessment in gastrointestinal stromal tumors with FDG PET scan 24 hours after a single dose of imatinib. Clin Nucl Med 2008;33:486-7.

[6] Stroobants S, Goeminne J, Seegers M et al. ^{18}FDG-Positron emission tomography for the early prediction of response in advanced soft tissue sarcoma treated with imatinib mesylate (Glivec). Eur J Cancer 2003;39:2012-20.

[7] Young H, Baum R, Cremerius U et al. Measurement of clinical and subclinical tumour response using [^{18}F]-fluorodeoxyglucose and positron emission tomography: review and 1999 EORTC recommendations. European Organization for Research and Treatment of Cancer (EORTC) PET Study Group. Eur J Cancer 1999;35:1773-82.

[8] Holdsworth CH, Badawi RD, Manola JB et al. CT and PET: early prognostic indicators of response to imatinib mesylate in patients with gastrointestinal stromal tumor. AJR Am J Roentgenol 2007;189:W324-30.

[9] Choi H, Charnsangavej C, Faria SC et al. Correlation of computed tomography and positron emission tomography in patients with metastatic gastrointestinal stromal tumor treated at a single institution with imatinib mesylate: proposal of new computed tomography response criteria. J Clin Oncol 2007;25:1753-9.

[10] Therasse P, Arbuck SG, Eisenhauer EA et al. New guidelines to evaluate the response to treatment in solid tumors. European Organization for Research and Treatment of Cancer, National Cancer Institute of the United States, National Cancer Institute of Canada. J Natl Cancer Inst 2000;92:205-6.

[11] Rikhof B, van Doorn J, Suurmeijer AJ et al. Insulin-like growth factors and insulin-like growth factor-binding proteins in relation to disease status and incidence of hypoglycaemia in patients with a gastrointestinal stromal tumour. Ann Oncol 2009;20:1582-8.

[12] Blay JY, Bonvalot S, Casali P et al. Consensus meeting for the management of gastrointestinal stromal tumors. Report of the GIST Consensus Conference of 20-21 March 2004, under the auspices of ESMO. Ann Oncol 2005;16:566-78.

[13] Mabille M, Vanel D, Albiter M et al. Follow-up of hepatic and peritoneal metastases of gastrointestinal tumors (GIST) under imatinib therapy requires different criteria of radiological evaluation (size is not everything!!!). Eur J Radiol 2009;69:204-8.

[14] Van den Abbeele AD, Gatsonis C, De Vries DJ, et al. ACRIN 6665/RTOG 0132 phase II trial of neoadjuvant imatinib mesylate for operable malignant gastrointestinal stromal tumor: monitoring with [18]F-FDG PET and correlation with genotype and Glut4 expression. J Nucl Med 2012; 53:567-74.

[15] Tarn C, Skorobogatko YV, Taguchi T et al. Therapeutic effect of imatinib in gastrointestinal stromal tumors: AKT signaling dependent and independent mechanisms. Cancer Res 2006;66:5477-86.

[16] Goerres GW, Stupp R, Barghouth G et al. The value of PET, CT and in-line PET/CT in patients with gastrointestinal stromal tumours: long-term outcome of treatment with imatinib mesylate. Eur J Nucl Med Mol Imaging 2005;32:153-62.

[17] Goldstein D, Tan BS, Rossleigh M et al. Gastrointestinal stromal tumours: correlation of [18]F-FDG gamma camera-based coincidence positron emission tomography with CT for the assessment of treatment response - an AGITG study. Oncology 2005;69:326-2.

[18] Fuster D, Ayuso JR, Poveda R et al. Value of FDG-PET for monitoring treatment response in patients with advanced GIST refractory to high-dose imatinib. A multicenter GEIS study. Q J Nucl Med Mol Imaging 2001;55:680-7.

[19] Prior JO, Montemurro M, Orcurto MV et al. Early prediction of response to sunitinib after imatinib failure by [18]F-fluorodeoxyglucose positron emission tomography in patients with gastrointestinal stromal tumor. J Clin Oncol 2009;27:439-45.

[20] Le Cesne A, Van Glabbeke, Verweij J et al. Absence of progression as assessed by response evaluation criteria in solid tumors predicts survival in advanced GI stromal tumors treated with imatinib mesylate: the intergroup EORTC-ISG-AGITG phase III trial. J Clin Oncol 2009;27:3969-74.

[21] Dudeck O, Zelle M, Reichardt P et al. Comparison of RECIST and Choi criteria for computed tomographic response evaluation in patients with advanced gastrointestinal stromal tumor treated with sunitinib. Ann Oncol 2011;22:1828-33.

[22] Antoch G, Kanja J, Bauer S et al. Comparison of PET, CT, and dual-modality PET/CT imaging for monitoring of imatinib (STI571) therapy in patients with gastrointestinal stromal tumors. J Nucl Med 2004;45:357-65.

[23] Gayed I, Vu T, Iyer R et al. The role of ^{18}F-FDG PET in staging and early prediction of response to therapy of recurrent gastrointestinal stromal tumors. J Nucl Med 2004;45:17-21.

[24] World Health Organization. WHO handbook for reporting results of cancer treatment. Offset publication No. 48 Geneva: World Health Organization; 1979.

[25] Green S, Weiss GR. Southwest Oncology Group standard response criteria, endpoint definitions and toxicity criteria. Invest New Drugs 1992;10:239-53.

[26] Therasse P. Measuring the clinical response. What does it mean? Eur J Cancer 2002;38:1817-23.

Chapter 4

Serial FLT-PET scanning does not discriminate between true and pseudoprogression in newly diagnosed glioblastoma patients treated with chemoradiotherapy, a prospective study

M.W. den Hollander[1], R.H. Enting[2], JC de Groot[3], A.M. Solouki[3], W.F.A. den Dunnen[4], W.J. Sluiter[5], M.A.A.M. Heesters[6], M. Wagemakers[7], J.A. Gietema[1], E.G.E. de Vries[1], J.Pruim[8,9], A.M.E. Walenkamp[1]

Departments of [1]Medical Oncology, [2]Neurology, [3]Radiology, [4]Pathology, [5]Internal Medicine, [6]Radiotherapy, [7]Neurosurgery, [8]Nuclear Medicine and Molecular Imaging, University of Groningen, University Medical Center Groningen, Groningen, the Netherlands and [9]Department of Nuclear Medicine, Tygerberg Hospital, Stellenbosch University, Stellenbosch, South-Africa

Submitted

Previously presented at the ASCO Annual Meeting 2014

ABSTRACT

Background: Response evaluation in glioblastoma (GBM) patients after first line radiotherapy and temozolomide (TMZ) is hampered by the occurrence of progressive, contrast-enhancing lesions on MRI not reflecting true tumor progression. ^{18}F-fluorothymidine (FLT) is a Positron Emission Tomography (PET) tracer that is taken up by proliferating cells. The goal of this study was to prospectively assess the value of FLT-PET in discriminating between true and pseudoprogression in patients with primary GBM treated with chemoradiotherapy.

Methods: FLT-PET and MRI scans were performed before start and 4 weeks after chemoradiotherapy. MRI scans were also performed after 3 cycles of adjuvant TMZ. Pseudoprogression was defined as progressive disease on MRI after chemoradiotherapy, with stabilization or reduction of enhancing lesions after 3 cycles of adjuvant TMZ. Changes in maximum standard uptake value (SUV_{max}) and tumor-to-normal brain tissue (T/N) ratios were calculated for FLT uptake and presented as the mean of the SUV_{max} in case of multiple lesions. Ki67 staining in the primary tumor and overall survival were analyzed.

Results: Thirty patients, (28 GBM, two gliosarcoma (WHO grade IV)), were included. Of 24 patients evaluable for pseudoprogression, seven showed pseudoprogression and seven true progression. No difference was found in changes of SUV_{max} and T/N ratios or changes in these parameters between these patient groups. A lower baseline FLT uptake predicted longer overall survival, but baseline FLT uptake did not correlate with Ki67.

Conclusions: FLT-PET scans do not discriminate between true progression and pseudoprogression in GBM patients. Baseline FLT uptake appears to predict overall survival (NTR3680).

INTRODUCTION

Glioblastoma (GBM) is the most common and most aggressive type of primary brain tumors, accounting for more than 50% of all gliomas with an incidence of 3.19 per 100.000 in the United States [1]. After surgery, the addition of temozolomide (TMZ) to standard 60 Gy radiotherapy has improved 2-year survival from 11 to 27% and 5-year survival from 2 to 10%. This is currently the standard of care for newly diagnosed GBM [2]. However, response evaluation of this treatment in these patients is problematic. This is due to the difficulty of distinguishing recurrent tumor (true progression) from pseudoprogression. The latter is defined as the detection of progressive gadolinium-enhanced lesions on an MRI scan immediately after the end of concurrent chemo-radiotherapy, where spontaneous improvement occurs without further treatment other than adjuvant TMZ [3,4]. This is the case in up to 64% of patients with progression on the first MRI scan after radiotherapy [5]. The difficulty of distinguishing true progression from pseudoprogression hampers clinical decision making in these patients. In case of pseudoprogression, standard treatment with adjuvant TMZ should be continued, whereas in case of true tumor progression, other treatment modalities – although scarce – or palliative care might be more appropriate.

^{18}F-fluorothymidine (FLT) is a Positron Emission Tomography (PET) tracer that is taken up by proliferating cells. It is phosphorylated in the cell by thymidine kinase 1, which is involved in DNA synthesis, and subsequently trapped. FLT uptake reflects thymidine kinase 1 activity, and can be used as a measure of cell proliferation. In several tumor types, FLT uptake corresponds with the Ki67 proliferation index and its change correlates with the response to therapy [6,7].

In glioma patients, FLT uptake has been used for tumor grading and was correlated with Ki67 [8,9]. Moreover, FLT-PET performed better in predicting survival and recurrence in glioma patients than FDG-PET and MRI [10,11]. However, no prospective studies have yet been conducted on the efficacy of FLT-PET to discriminate between pseudoprogression and true progression. An effective technique to make this discrimination is urgently needed to improve clinical decision making in these patients. Therefore, the aim of this prospective study in patients with newly diagnosed GBM was to determine whether FLT-PET scans, performed before and after chemora-diotherapy, can discriminate between true progression and pseudoprogression as measured by MRI after 3 courses of adjuvant TMZ.

PATIENTS AND METHODS

Patients and treatment

Patients with newly diagnosed GBM or gliosarcoma (WHO grade IV, hereafter referred to as GBM) who were eligible for standard treatment with radiotherapy and TMZ were prospectively included. After surgical resection or biopsy, patients were treated with radiotherapy consisting of 2 Gy irradiation 5 out of 7 days per week during 6 weeks, for a total dose of 60 Gy. Patients received concomitant TMZ orally in a dose of 75 mg/m^2 daily for 6 weeks. After a treatment break of 4 weeks, patients received up to 6 cycles of adjuvant TMZ (150–200 mg/m^2) for 5 days every 28 days. The use of corticosteroids during treatment was registered. No changes in treatment

were introduced based on the results of the FLT-PET scan. Overall survival was calculated from date of informed consent to date of death or last known date alive, censored at time of analysis (November 2013). All patients gave written informed consent to participate in the study. The protocol was approved by the local ethics committee and registered in the Dutch trial register (NTR3680).

Imaging

Patients underwent standard radiologic follow up with MRI (1.5T using T1, T2 and contrast enhanced 3D T1 Gradient echo sequences) directly after surgery (baseline), 10 weeks after start of treatment (4 weeks after completing chemoradiotherapy), 22 weeks after start of treatment (after the third cycle of adjuvant TMZ or earlier as clinically indicated) and thereafter every 3 months. MRI data for this study were assessed by an independent neuroradiologist and a radiologist in training using the Macdonald criteria for tumor response evaluation [12]. Pseudoprogression was defined as progressive disease on MRI scan at 10 weeks, with stabilization or reduction of enhancing lesions on MRI at 22 weeks. True progression was defined as progressive disease on both the MRI at 10 weeks and the MRI at 22 weeks.

FLT-PET scans were performed after surgery, but before start of radiotherapy (baseline) and 10 weeks after start of treatment (4 weeks after completing chemoradiotherapy). FLT was synthesized as described by Been et al [13]. Patients were instructed to fast for a minimum of 4 hours before tracer injection of 200 MBq FLT intravenously, injected 30 minutes before PET scanning. PET scans were made on either HR+ or mCT PET scanners (Siemens, Knoxville). The maximum Standard Uptake Value (SUV_{max}) was assessed by drawing a region of interest (ROI) around every lesion on a separate reconstruction according to the European Association of Nuclear Medicine Research Ltd [14]. In case of multiple lesions, the mean of the SUV_{max} of the different lesions was calculated. FLT-PET scans were fused with the most recent MRI to differentiate actual tumor from post-surgery effects outside the cerebrum if needed. The SUV_{mean} for normal brain tissue was assessed by drawing a ROI in the contralateral brain tissue. Tumor-to-normal ratios (T/N ratio) were determined by dividing the SUV_{max} of the tumor by the SUV_{mean} of the normal brain tissue. A PET response was defined as a 25% decrease of the SUV_{max} between the first and second FLT-PET scan.

Ki67 immunohistochemical staining

Deparaffinized GBM tissue from primary surgery was used to evaluate the proliferation fraction of tumor cells (4-µm-thick tissue slices). Antigen retrieval was performed using 10 mM Tris/1 mM EDTA (pH 9), in a microwave at 700 W. Endogenous peroxidase and biotin were blocked using routine techniques. The slides were incubated with the primary antibody, Ki67 (Clone MIB-1; Dako, Glostrup, Denmark) at room temperature for 1 hour, followed by application of the secondary antibody peroxidase-conjugated rabbit anti-mouse serum (Dako, Glostrup, Denmark), and the tertiary antibody peroxidase-conjugated goat anti-rabbit serum (Dako, Glostrup, Denmark), for 30 minutes each. The first antibody was diluted 1/100 in 1% bovine serum albumin (BSA)/phosphate buffered saline (PBS). The secondary and tertiary antibodies

were diluted 1/100 in 1% BSA/PBS with 1% AB serum. Color development was performed with 3,3′-diaminobenzidine (Sigma, Zwijndrecht, the Netherlands) for 10 minutes. The slides were scanned for hot spots of proliferative activity. In one high power field (400x magnification) the fraction of Ki67-positive nuclei/total number of nuclei was evaluated.

Statistics

Mann Whitney U tests were used to compare FLT uptake between patients with and without pseudoprogression. To discriminate between true progression and pseudoprogression, Receiver Operating Curves were used to find an optimal cutoff point for FLT uptake and changes in uptake. A Fisher's exact test was used to analyze categorical data. A Kaplan-Meier curve with a log rank test was used to analyze survival. A Pearson correlation test was used to calculate correlations between FLT uptake and proliferation index. A two-sided *P*-value of < .05 was considered significant. For the Fisher's exact test, a one-sided *P* value was given. Statistics were calculated in IBM SPSS statistics 20. Graphs were made using GraphPad Prism version 5.00 for Windows.

RESULTS

Patients

In total, 28 patients with GBM and two with gliosarcoma (WHO grade IV) were included in this study between November 2009 and November 2012. For patient characteristics, see Table 1. All but one patient completed radiotherapy. Seven patients did not complete concomitant TMZ, and of the 27 patients who started adjuvant TMZ, 16 patients did not complete the adjuvant courses. The most frequent reasons for this were progressive disease and thrombocytopenia. One patient with a secondary GBM underwent a short schedule of concomitant radiotherapy (23 x 2 Gy) and TMZ. The median overall survival for all 30 patients was 14 months (range 1-36 months). Five patients were not evaluable for pseudoprogression because of early death, salvage surgery or clinical deterioration that prevented further participation in the study. One patient was not analyzable for the pseudoprogression analysis as only a baseline MRI before tumor resection was available (Fig 1).

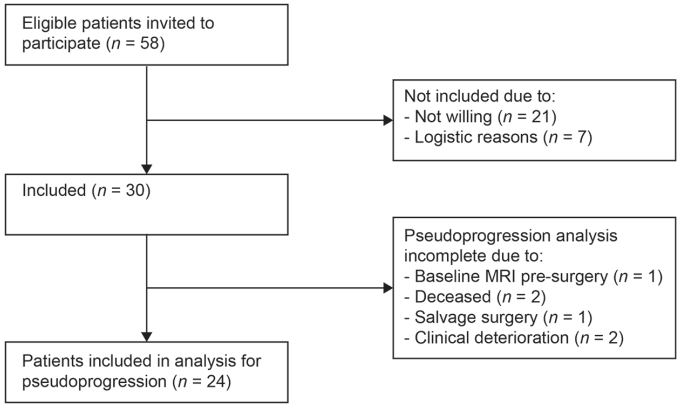


Figure 1. CONSORT diagram.

Table 1: Patient characteristics.

Characteristic	No. of patients (n = 30)	Median	Range
Age, years		58	33 - 68
Sex			
Male	17		
Female	13		
Tumor type			
Glioblastoma	26		
Secondary glioblastoma	2		
Gliosarcoma	2		
Type of intervention			
Biopsy	3		
Surgical resection	27		

Baseline FLT-PET scans were performed 5 days (median) before start of radiotherapy. Two patients had their baseline FLT-PET scan 2 and 4 days after start of radiotherapy for logistic reasons. Follow-up FLT-PET scans were made 27 days (median) after completion of radiotherapy. Three patients had their follow up FLT-PET scan 1 day after the start of adjuvant TMZ. Finally, for logistic reasons two patients had their FLT-PET scan 6 and 22 days, respectively, after the start of adjuvant TMZ.

Pseudoprogression

A total of 24 patients were evaluable for pseudoprogression analysis (Fig 1). Pseudoprogression was seen in seven patients, and true progression in seven other patients (Figures 2 and 3).Ten patients had either stable disease or a complete response on MRI after 10 weeks (Table 2).

We found no difference in or change of SUV_{max} and T/N ratio on FLT-PET scans between patients with pseudoprogression and those with true progression. With 25% reduction of SUV_{max} as a cutoff value, only two of the patients with pseudoprogressivon were identified, while three patients with true progression also showed a decrease in SUV_{max} over 25% (sensitivity 29%, specificity 43%). We also used optimal cutoff points found by others for identifying recurrent tumor of a SUV ≥ 1.34 and T/N ratio of ≥ 4.94 applied to FLT-PET scan at 10 weeks [15,16]. This approach also did not predict all cases correctly. Using a T/N ratio of ≤ 2.95 on the FLT-PET scan at 10 weeks, we identified four out of seven patients with true progression and zero patients with pseudoprogression (sensitivity 100%, specificity 57%, one sided $P = .04$). ROC curves showed no other reasonable cutoff point for any parameter to discriminate between pseudoprogression and true progression.

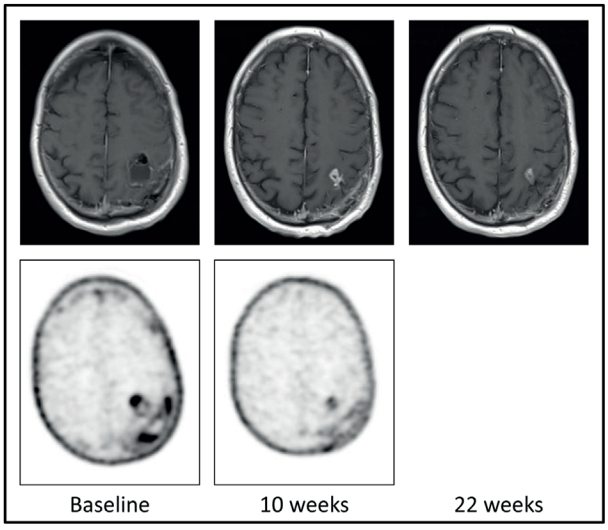


Figure 2. FLT-PET images at baseline (left) and 10 weeks (right) and MRI images at baseline (left), 10 weeks (middle) and 22 weeks (right) of a patient with pseudoprogression. SUV_{max} on baseline FLT-PET was 1.44, SUV_{max} at 10 weeks 0.74.

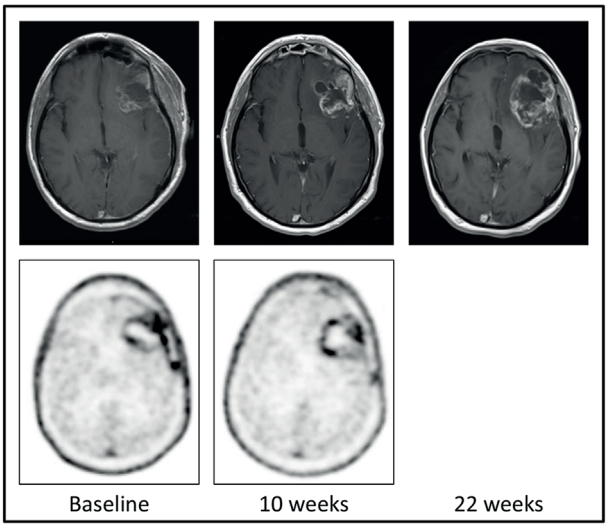


Figure 3. FLT-PET images at baseline (left) and 10 weeks (right) and MRI images at baseline (left), 10 weeks (middle) and 22 weeks (right) of a patient with true progression. SUV_{max} on baseline FLT-PET was 3.70, SUV_{max} at 10 weeks 1.80.

Table 2. Overview of results in all included patients.

Patient No.	SUV_{max} baseline	T/N baseline	SUV_{max} 10 weeks	T/N 10 weeks	Change SUV_{max} %	MRI 10 weeks	MRI 22 weeks	Ki 67 %	Overall survival
1	1.55	3.4	1.33	2.8	14.2	NE	SD	30	29
2	NU	ND	NU	ND	ND	SD	SD	40	36*
3	NU	ND	0.81	3.1	ND	PD	SD	35	32
4	1.73	5.6	1.58	6.3	8.7	PD	SD	35	17
5	1.24	3.1	1.46	3.2	-17.7	SD	PD	40	20
6	1.75	4.4	0.95	2.9	45.7	SD	PD	30	31
7	1.59	7.6	1.34	8.4	15.7	PD	NE	25	10
8	2.18	14.5	0.74	4.4	66.1	PD	PD	50	19
9	2.43	6.2	1.14	2.5	53.1	SD	SD	30	24
10	NU	ND	NU	ND	ND	CR	PD	10	14
11	NU	ND	NU	ND	ND	CR	CR	50	28
12	2.84	9.8	1.64	5.1	42.3	SD	ND	30	4
13	NU	ND	0.96	1.7	ND	PD	PD	25	9
14	1.23	3.0	1.14	3.5	7.3	PD	SD	60	27*
15	1.90	5.3	1.61	4.0	15.3	PD	SD	18	9
16	3.00	5.9	1.26	2.6	58.0	SD	PD	19	10
17	5.02	9.0	ND	ND	ND	ND	ND	50	1
18	4.17	8.7	2.67	3.3	36.0	PD	SD	ND	9
19	1.10	2.0	1.00	1.9	9.1	SD	SD	40	25*
20	1.38	3.9	1.68	2.8	-21.7	PD	PD	30	11
21	1.59	5.9	0.85	2.3	46.5	PD	PD	25	22*
22	0.65	2.0	0.68	2.7	-4.6	PD	PD	20	14
23	0.35	1.5	NU	ND	ND	CR	CR	15	19
24	1.64	6.6	ND	ND	ND	SD	ND	20	9
25	1.61	6.0	1.33	3.9	17.4	PD	SD	50	17*
26	2.85	16.8	0.93	5.5	67.4	SD	PD	60	6
27	3.70	9.5	1.80	5.3	51.4	PD	PD	50	5
28	1.44	7.6	0.74	4.1	48.6	PD	SD	50	13*
29	2.93	8.1	2.23	5.9	23.9	PD	PD	7	7
30	1.42	3.3	ND	ND	ND	SD	SD	ND	10

Abbreviations: PD = Progressive Disease, SD = Stable Disease, ND = Not Done, NE = Non Evaluable, NU = No Uptake, CR = Complete Response.

*Patients censored at date last known alive.

Overall survival

In all 30 patients, a baseline FLT-PET scan was available (Figure 1). In November 2013, 24 patients had died and six were censored at the last known alive date. The median SUV_{max} for all patients on baseline FLT-PET was 1.59. For patients with a $SUV_{max} \leq 1.59$, median overall survival was longer compared to patients with a $SUV_{max} > 1.59$ (20 vs 9 months $P = .01$) (Figure 4).

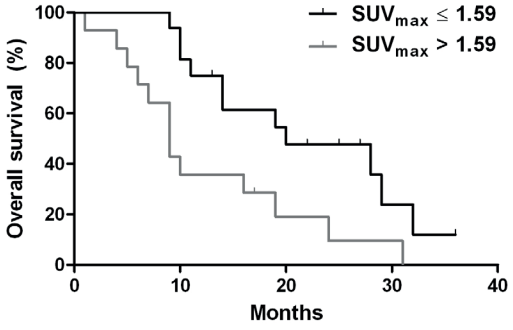


Figure 4. Kaplan-Meier curve for patients with SUV_{max} on first FLT-PET scan ≤ 1.59 and > 1.59. Overall survival is higher for patients with $SUV_{max} \leq 1.59$ ($P = .01$).

Proliferation index

In the 28 patients with available surgical specimens for Ki67 staining, the mean SUV_{max} at baseline FLT-PET did not correlate with the Ki67 index of the tumor tissue before treatment.

DISCUSSION

In this prospective trial we determined that serially measured FLT uptake in GBM patients did not discriminate between true progression and pseudoprogression. Uptake and changes in uptake was measured on FLT-PET scans before (at baseline) and after chemoradiotherapy. Low FLT uptake at baseline was predictive of longer survival, but FLT uptake did not correlate with the Ki-67 index of the primary tumor.

Despite the urgent need to distinguish between true progression and pseudoprogression in GBM patients, until now only retrospective studies have been performed on patients who had a radiological suspicion of recurrent brain tumor at different time points and showed variable results. In one such study, FLT-PET had a low specificity for identifying recurrent tumor from benign lesions in 20 patients [17]. Three other studies were able to discriminate between true progression and radionecrosis in 15, 19 and 21 glioma patients, respectively, using FLT kinetic values and the tumor-to-normal ratio [15,16,18]. A possible explanation for our findings is that FLT uptake in high-grade gliomas reflects not only trapping of FLT in proliferating tumor cells, but also disruption of the blood-brain barrier. As a result, areas with true progression as well as with pseudoprogression would show increased uptake.

To limit the burden of trial participation for the patients in this poor prognosis group, we did not perform kinetic modeling. Instead, we used SUV_{max} for the quantification of FLT uptake. SUV

is easy to obtain, mostly used in clinical practice and has been proven to be robust. However, kinetic analysis might be of interest to distinguish between true FLT uptake due to proliferation and FLT leakage that results from disruption of the blood brain barrier. There are several studies that support this, although others showed a good correlation between FLT kinetic values and SUV [19-24]. Using kinetic analysis has several disadvantages in practice. It is a time-consuming procedure, as the uptake in time needs to be assessed, and it requires collecting multiple arterial blood samples, both of which make the procedure burdensome for patients. Several studies have suggested other parameters for quantification of FLT-PET, such as proliferative volume and parametric response maps [25,26]. Due to the small numbers of patients in the studies performed so far and the different approaches used for quantification, direct comparison of the results is difficult. Consequently, we are unable to draw more definitive conclusions about the usefulness of FLT-PET in glioma. However, the use of SUV_{max} could also have limitations. For instance, the heterogeneity of the FLT uptake is not taken into account by using SUV_{max} only.

Another constraint of the present study is that the optimal time points for serial FLT-PET scanning before and during GBM treatment are difficult to choose. Because the aim of this study was to differentiate between true and pseudoprogression after chemoradiotherapy, we performed the baseline FLT-PET scan after surgery. Scanning before surgery would reveal tumor uptake, but most patients undergo a gross total resection of tumor tissue. However, scanning after surgery may have led to increased uptake of FLT due to increased blood flow and increased proliferation as part of the wound healing process.

A surprising finding was that a T/N ratio ≤ 2.95 on the FLT-PET scan at 10 weeks identified patients with true progression only, as patients with true tumor progression would be expected to have a higher proliferation rate. Because of the small numbers of patients in this analysis, this result should be interpreted with caution; future studies are needed to confirm if this is indeed a clinically relevant finding. Currently, two trials are investigating FLT as an imaging biomarker of early treatment response (NCT01880008, NCT00813566).

In earlier studies, correlations between FLT uptake in brain tumors and the Ki67 index were found [8-10]. However, in these studies FLT-PET scans were often performed before surgery, whereas in the current study post-surgery FLT-PET scans were made. This might explain the lack of correlation between FLT uptake and Ki67 index.

In the current study, pseudoprogression and true progression were determined based on the MRI results at 22 weeks. This time point was chosen due to its clinical relevance. After 3 adjuvant TMZ courses, the diagnosis of true progression results in cessation of TMZ, thus avoiding further side-effects of TMZ and enabling a timely switch to second-line therapy or inclusion in clinical trials. However, the selection of this time point may have resulted in overestimation of the number of patients with pseudoprogression. Taal et al. defined a period with stable disease of 6 months after radiotherapy for the diagnosis pseudoprogression [3]. Applying this criterion would have classified two patients with pseudoprogression as having true progression. However, this did not improve the performance of the FLT-PET scan in discriminating between true progression and pseudoprogression. On the other hand, clinical signs of pseudoprogression can take

longer than 3 months to resolve, so we might also have underestimated the number of patients with pseudoprogression [27].

Fortunately, since the start of this study, several new initiatives have been initiated. The RANO criteria for glioma response evaluation on MRI have been developed, and this reduces the number of patients found with pseudoprogression [28,29]. Also, other imaging modalities such as perfusion MRI and ^{11}C-methionine-PET have shown interesting results, although large prospective studies comparing multiple imaging modalities are still lacking [30,31].

In conclusion, our prospective study suggests that FLT-PET scanning is not useful in for discriminating between pseudoprogression and true progression in GBM patients treated with radiochemotherapy.

ACKNOWLEDGEMENTS

The clinical protocol was drafted at the ECCO-AACR-EORTC-ESMO Workshop Flims, Switzerland 2008 on Methods in Clinical Cancer Research.

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:v1-49 (suppl 5).

[2] Stupp R, Hegi ME, Mason WP, et al. Effects of radiotherapy with concomitant and adjuvant temozolomide versus radiotherapy alone on survival in glioblastoma in a randomised phase III study: 5-year analysis of the EORTC-NCIC trial. *Lancet Oncol.* 2009; 10(5):459-466.

[3] Taal W, Brandsma D, de Bruin HG, et al. Incidence of early pseudo-progression in a cohort of malignant glioma patients treated with chemoirradiation with temozolomide. *Cancer.* 2008; 113(2):405-410.

[4] Brandsma D, Stalpers L, Taal W, Sminia P, van den Bent MJ. Clinical features, mechanisms, and management of pseudoprogression in malignant gliomas. *Lancet Oncol.* 2008; 9(5):453-461.

[5] Fink J, Born D, Chamberlain MC. Pseudoprogression: Relevance with respect to treatment of high-grade gliomas. *Curr Treat Options Oncol.* 2011; 12(3):240-252.

[6] Salskov A, Tammisetti VS, Grierson J, Vesselle H. FLT: Measuring tumor cell proliferation in vivo with positron emission tomography and 3'-deoxy-3'-[18F]fluorothymidine. *Semin Nucl Med.* 2007; 37(6):429-439.

[7] Shields AF, Grierson JR, Dohmen BM, et al. Imaging proliferation in vivo with [F-18]FLT and positron emission tomography. *Nat Med.* 1998; 4(11):1334-1336.

[8] Choi SJ, Kim JS, Kim JH, et al. [18F]3'-deoxy-3'-fluorothymidine PET for the diagnosis and grading of brain tumors. *Eur J Nucl Med Mol Imaging.* 2005; 32(6):653-659.

[9] Saga T, Kawashima H, Araki N, et al. Evaluation of primary brain tumors with FLT-PET: Usefulness and limitations. *Clin Nucl Med.* 2006; 31(12):774-780.

[10] Chen W, Cloughesy T, Kamdar N, et al. Imaging proliferation in brain tumors with 18F-FLT PET: Comparison with 18F-FDG. *J Nucl Med.* 2005; 46(6):945-952.

[11] Chen W, Delaloye S, Silverman DH, et al. Predicting treatment response of malignant gliomas to bevacizumab and irinotecan by imaging proliferation with [18F] fluorothymidine positron emission tomography: A pilot study. *J Clin Oncol.* 2007; 25(30):4714-4721.

[12] Macdonald DR, Cascino TL, Schold SC, Jr, Cairncross JG. Response criteria for phase II studies of supratentorial malignant glioma. *J Clin Oncol.* 1990; 8(7):1277-1280.

[13] Been LB, Hoekstra HJ, Suurmeijer AJ, Jager PL, van der Laan BF, Elsinga PH. [18F]FLT-PET and [18F]FDG-PET in the evaluation of radiotherapy for laryngeal cancer. *Oral Oncol.* 2009; 45(12):e211-e215.

[14] Boellaard R, O'Doherty MJ, Weber WA, et al. FDG PET and PET/CT: EANM procedure guidelines for tumour PET imaging: Version 1.0. *Eur J Nucl Med Mol Imaging.* 2010; 37(1):181-200.

[15] Enslow MS, Zollinger LV, Morton KA, et al. Comparison of F-18 fluorodeoxyglucose and F-18 fluorothymidine PET in differentiating radiation necrosis from recurrent glioma. *Clin Nucl Med.* 2012; 37(9):854-861.

[16] Shishido H, Kawai N, Miyake K, Yamamoto Y, Nishiyama Y, Tamiya T. Diagnostic value of 11C-methionine (MET) and 18F-fluorothymidine (FLT) positron emission tomography in recurrent high-grade gliomas; differentiation from treatment-induced tissue necrosis. *Cancers (Basel).* 2012; 4(1):244-256.

[17] Hong IK, Kim JH, Ra YS, Kwon do H, Oh SJ, Kim JS. Diagnostic usefulness of 3'-deoxy-3'-[18F]fluorothymidine positron emission tomography in recurrent brain tumor. *J Comput Assist Tomogr.* 2011; 35(6):679-684.

[18] Spence AM, Muzi M, Link JM, et al. NCI-sponsored trial for the evaluation of safety and preliminary efficacy of 3'-deoxy-3'-[18F]fluorothymidine (FLT) as a marker of proliferation in patients with recurrent gliomas: Preliminary efficacy studies. *Mol Imaging Biol.* 2009; 11(5):343-355.

[19] Ullrich R, Backes H, Li H, et al. Glioma proliferation as assessed by 3'-fluoro-3'-deoxy-L-thymidine positron emission tomography in patients with newly diagnosed high-grade glioma. *Clin Cancer Res.* 2008; 14(7):2049-2055.

[20] Backes H, Ullrich R, Neumaier B, Kracht L, Wienhard K, Jacobs AH. Noninvasive quantification of 18F-FLT human brain PET for the assessment of tumour proliferation in patients with high-grade glioma. *Eur J Nucl Med Mol Imaging.* 2009; 36(12):1960-1967.

[21] Muzi M, Spence AM, O'Sullivan F, et al. Kinetic analysis of 3'-deoxy-3'-18F-fluorothymidine in patients with gliomas. *J Nucl Med.* 2006; 47(10):1612-1621.

[22] Wardak M, Schiepers C, Dahlbom M, et al. Discriminant analysis of 18F-fluorothymidine kinetic parameters to predict survival in patients with recurrent high-grade glioma. *Clin Cancer Res.* 2011; 17(20):6553-6562.

[23] Price SJ, Fryer TD, Cleij MC, et al. Imaging regional variation of cellular proliferation in gliomas using 3'-deoxy-3'-[18F]fluorothymidine positron-emission tomography: An image-guided biopsy study. *Clin Radiol.* 2009; 64(1):52-63.

[24] Schiepers C, Dahlbom M, Chen W, et al. Kinetics of 3'-deoxy-3'-18F-fluorothymidine during treatment monitoring of recurrent high-grade glioma. *J Nucl Med.* 2010; 51(5):720-727.

[25] Idema AJ, Hoffmann AL, Boogaarts HD, et al. 3'-deoxy-3'-18F-fluorothymidine PET-derived proliferative volume predicts overall survival in high-grade glioma patients. *J Nucl Med.* 2012; 53(12):1904-1910.

[26] Harris RJ, Cloughesy TF, Pope WB, et al. 18F-FDOPA and 18F-FLT positron emission tomography parametric response maps predict response in recurrent malignant gliomas treated with bevacizumab. *Neuro Oncol.* 2012; 14(8):1079-1089.

[27] Brandes AA, Franceschi E, Tosoni A, et al. MGMT promoter methylation status can predict the incidence and outcome of pseudoprogression after concomitant radiochemotherapy in newly diagnosed glioblastoma patients. *J Clin Oncol.* 2008; 26(13):2192-2197.

[28] Wen PY, Macdonald DR, Reardon DA, et al. Updated response assessment criteria for high-grade gliomas: Response assessment in neuro-oncology working group. *J Clin Oncol.* 2010; 28(11):1963-1972.

[29] Linhares P, Carvalho B, Figueiredo R, Reis RM, Vaz R. Early pseudoprogression following chemoradiotherapy in glioblastoma patients: The value of RANO evaluation. *J Oncol*. 2013 [Epub ahead of print].

[30] Dhermain FG, Hau P, Lanfermann H, Jacobs AH, van den Bent MJ. Advanced MRI and PET imaging for assessment of treatment response in patients with gliomas. *Lancet Neurol.* 2010; 9(9):906-920.

[31] Glaudemans AW, Enting RH, Heesters MA, et al. Value of 11C-methionine PET in imaging brain tumours and metastases. *Eur J Nucl Med Mol Imaging*. 2013; 40(4):615-635.

Chapter 5

TGF-β antibody uptake in recurrent high grade glioma imaged with ^{89}Zr-fresolimumab PET

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

Departments of [1]Medical Oncology, [2]Nuclear Medicine and Molecular Imaging, [3]Neurology, [4]Pathology, [5]Radiotherapy, [6]Clinical Pharmacy and Pharmacology, [7]Radiology, University of Groningen, University Medical Center Groningen, Groningen, The Netherlands, [8]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-β (TGF-β) signaling is involved in glioma development. The monoclonal antibody fresolimumab (GC1008) can neutralize all mammalian isoforms of TGF-β and tumor uptake can be visualized and quantified with ^{89}Zr-fresolimumab PET in mice. The aim of this study was to investigate the fresolimumab uptake in recurrent high grade gliomas using ^{89}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}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}Zr-fresolimumab tumor uptake was quantified as maximum standardized uptake value (SUV_{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}Zr-fresolimumab PET scan 4 days after injection. In four patients an additional PET scan was performed on day 2 after injection. SUV_{max} on day 4 in tumor lesions was 4.6 (1.5 - 13.9) versus a median SUV_{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}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-β (TGF-β) acts as a tumor promoter in advanced tumors where it induces proliferation and metastasis and suppresses the immune response [2]. TGF-β and its receptors are overexpressed in GBM and TGF-β signaling is involved in multiple steps of GBM development and invasion [3-5]. Plasma TGF-β 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-β receptor I, compared with glioma patients with low levels of p-SMAD2 [7]. These features make TGF-β a promising target molecule for therapeutic approaches in recurrent glioma and therefore several TGF-β-inhibitors are under investigation in this setting [8].

Fresolimumab (GC1008) is a monoclonal antibody capable of neutralizing all mammalian isoforms of TGF-β (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 (^{89}Zr) fresolimumab PET [13]. Therefore the aim of this study was to visualize and quantify fresolimumab uptake in recurrent high grade glioma using ^{89}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}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}Zr-fresolimumab PET scans were acquired on day 4 after injection. To assess the tumor accumulation of ^{89}Zr-fresolimumab over time, an additional scan was acquired on day 2 after injection in some patients. Normal organ distribution of ^{89}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}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 ^{89}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_2O_2 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 ^{89}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 ^{89}Zr-fresolimumab was seen in tumor lesions (n = 16). The SUV_{max} in tumor lesions on day 4 was 4.6 (1.5 - 13.9), which was higher than the SUV_{mean} of normal brain tissue (0.3 (0.2 - 0.5)) ($P < 0.01$). The SUV_{mean} was 3.0 (2.0 - 6.2) in the blood of the sinus confluens. In patients with a whole body scan the SUV_{mean} 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 ^{89}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.

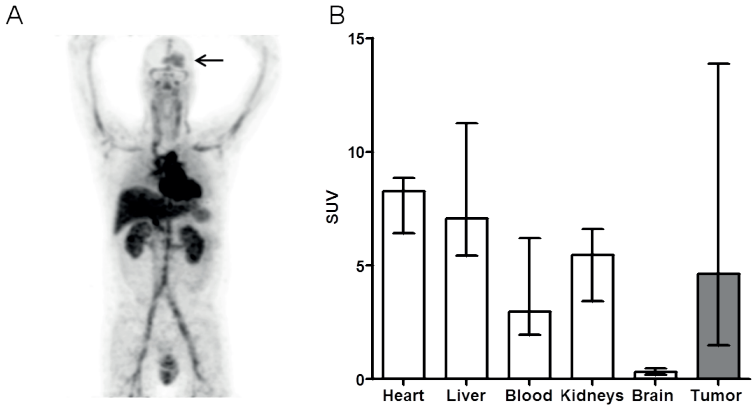


Figure 1. A: Representative example of ^{89}Zr-fresolimumab PET on day 4 and uptake in the brain tumor (arrow). B: Uptake of ^{89}Zr-fresolimumab in different organs (SUV_{mean}) and tumor (SUV_{max}) on day 4 after tracer injection. Blood pool uptake was measured in the sinus confluens. Blood, brain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

Plasma pharmacokinetics and biomarkers

The plasma concentration of ^{89}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_{max}/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_{max} 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_{max} of 4.6 found in the gliomas is comparable to the SUV_{max} of 5.8 (1.7 - 15.1) found in metastatic lesions with ^{89}Zr-bevacizumab PET in patients with neuroendocrine tumors [16]. The $C_{max/}$dose of ^{89}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_{max} with fresolimumab compared to other studies. Three contrast enhancing lesions >10 mm did not take up ^{89}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 ^{89}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

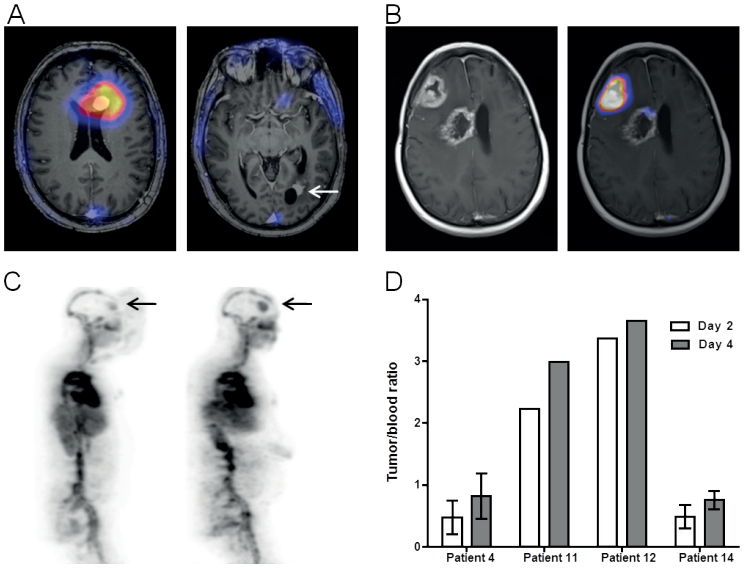


Figure 2. A: Fused MRI and PET scan of a patient with 2 contrast enhancing lesions. High uptake visible in the frontal lesion (left image). No uptake visible on PET scan in occipital lesion (right image, arrow) that was previously irradiated. B: MRI and fused MRI/PET images of a patient with 2 contrast 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 ^{89}Zr-fresolimumab PET in 4 patients on day 2 and day 4 after injection.

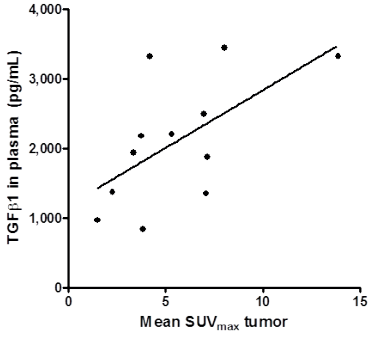
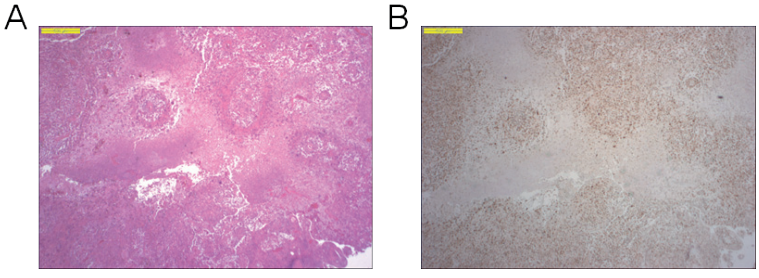


Figure 3. (left) Correlation between TGFβ1 in plasma and mean SUV_{max} of ^{89}Zr-fresilimumab in brain tumor lesions on day 4 after injection ($r = 0.61$, $P = 0.04$).

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



^{89}Zr-fresolimumab and in brain metastases in a clinical study with ^{89}Zr-trastuzumab in metastatic breast cancer patients [13,18]. Taken together these findings suggest that ^{89}Zr-fresolimumab uptake was not only a reflection of antibody leakage due to a damaged blood brain barrier but was tumor specific and TGF-β 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-β 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-β inhibition in glioma patients have been published. Traberdersen is an antisense oligodeoxynucleotide that inhibits TGF-β2. In a randomized 2b study traberdersen 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-β receptor 1 kinase inhibitor) showed confirmed responses in treatment refractory gliomas in three out of 28 patients [24]. TGF-β 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-β 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. TGFβ in cancer. *Cell.* 2008;134:215–230.

[3] Wesolowska A, Kwiatkowska A, Slomnicki L, et al. Microglia-derived TGF-β as an important regulator of glioblastoma invasion-an inhibition of TGF-β-dependent effects by shRNA against human TGF-β type II receptor. *Oncogene.* 2008;27:918–930.

[4] Eichhorn PJ, Rodon L, Gonzalez-Junca A, et al. USP15 stabilizes TGF-β receptor I and promotes oncogenesis through the activation of TGF-β signaling in glioblastoma. *Nat Med.* 2012;18:429–435.

[5] Kjellman C, Olofsson SP, Hansson O, et al. Expression of TGF-β isoforms, TGF-β 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 β1 and β2 in the plasma of patients with glioblastoma. *J Neurooncol.* 2006;79:61–65.

[7] Bruna A, Darken RS, Rojo F, et al. High TGFβ-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)-β 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-β 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 (TGFβ) 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 TGFβ- 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}Zr-labeled transforming growth factor-β 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 ^{89}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-β 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 ^{89}Zr-trastuzumab and PET imaging of HER2-positive lesions in patients with metastatic breast cancer. *Clin Pharmacol Ther.* 2010;87:586–592.

[19] Sigmond 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-β2 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-β receptor I kinase inhibitor LY2157299 in patients with treatment-refractory malignant glioma [abstract]. *J Clin Oncol.* 2011;29(suppl):3011.

Chapter 6

Bleomycin-induced pulmonary changes on restaging CT scans in two thirds of testicular cancer patients: no correlation with fibrosis markers

M.W. den Hollander[1], N.L. Westerink*[1], S. Lubberts*[1], A.H.H. Bongaerts[2], R.F.E. Wolf[2], R. Altena[1], J. Nuver[1], S.F. Oosting[1], E.G.E. de Vries[1], A.M.E. Walenkamp[1], C. Meijer[1], J.A. Gietema[1]

Departments of [1]Medical Oncology and [2]Radiology, University of Groningen, University Medical Center Groningen, Groningen, The Netherlands

*these authors contributed equally

Submitted

Previously presented at the ASCO Annual Meeting 2015

ABSTRACT

Background: Metastatic testicular cancer has a favorable prognosis when treated with BEP (Bleomycin, Etoposide and cisPlatin) chemotherapy. Bleomycin-induced pneumonitis (BIP) is a well known side-effect which can be fatal. In this study, we investigated the prevalence of lesions suspect for bleomycin-induced pulmonary changes on restaging CT scans after treatment, and whether the fibrosis markers Transforming Growth Factor-beta1 (TGF-β1), Growth Differentiation Factor-15 (GDF-15), and hs-CRP were predictive of this.

Methods: Patients between 18 and 50 years of age with metastatic testicular cancer treated with BEP chemotherapy were included in this prospective non-randomized cohort study. Post chemotherapy restaging CT scans were analyzed for abnormalities that were suspect for bleomycin-induced pulmonary changes as judged by two independent radiologists. The radiographic abnormalities were graded as minor, moderate or severe. Plasma samples were collected before, during and after treatment and were quantified for TGF-β1, GDF-15 and hs-CRP.

Results: 66 patients treated with BEP chemotherapy were included. Forty-five (68%) showed signs of bleomycin-induced pulmonary changes on the restaging CT scan, 37 were classified to have minor and eight to have moderate abnormalities. No differences in TGF-β1, GDF-15, or hs-CRP plasma levels were found between these groups.

Conclusion: Bleomycin-induced pulmonary changes are very common on restaging CT scans after BEP chemotherapy for metastatic testicular cancer. TGF-β1, GDF15 and hs-CRP plasma levels before, during and after treatment are not different between patients with and without radiological signs of pulmonary bleomycin-induced toxicity and, therefore, not helpful as early fibrosis biomarkers to predict this.

INTRODUCTION

Metastatic testicular cancer has a favorable prognosis with a 5 year overall survival over 90% when treated with the current standard of BEP (bleomycin, etoposide and cisplatin) chemotherapy. Bleomycin is considered to be an essential component of the regimen [1]. Although well tolerated, in roughly 10% of the patients treated with this regime, bleomycin-induced pulmonary toxicity (BIP) is observed, with clinical symptoms such as dry cough, dyspnea, crackles during auscultation, abnormalities on chest X-ray and fever [2]. BIP is predominantly a fibrotic lung disease and, although its pathogenesis is not resolved, the immune system appears to be involved [3,4].

BIP occurs during bleomycin treatment, but can also develop after a treatment free interval of weeks to months [5]. In 1-3% of the affected patients pulmonary toxicity is fatal [6]. The cumulative bleomycin dose is an important denominator of the risk of BIP, although a safe dose has not been established [7]. Known risk factors are smoking, impaired renal function and higher age, but there is neither a test to predict which patients will develop BIP, nor a therapy to prevent it [8,9]. Standard strategy in case of BIP signs during treatment is to stop bleomycin administration. The change in diffusion capacity (TLCO) is used in several centers to terminate bleomycin administration, although these changes appear not to be specifically caused by bleomycin [10].

In earlier reports, restaging CT scans after completion of BEP chemotherapy for testicular cancer are reported to show signs suggestive for bleomycin-induced pulmonary changes [11-13]. These radiologic changes may be a good surrogate endpoint for the susceptibility for BIP. However, since the bleomycin effect on CT scan is observed only after completion of treatment, an upfront or early biomarker that identifies patients likely to develop BIP would be preferable as these patients could be treated with a non bleomycin containing schedule. An early biomarker change would facilitate a premature halt of the weekly bleomycin administration.

Transforming growth factor-beta 1 (TGF-β1) is a cytokine involved in many physiological and pathological processes, including immune response, cell proliferation, angiogenesis, fibrosis and oncogenesis [14]. TGF-β1 plays an important role in development of BIP and fibrosis in animal models [15-17]. In patients treated with radiotherapy, or patients that underwent stem cell transplantation, a relationship between TGF-β1 levels and treatment-induced pulmonary toxicity was found [18-19]. Growth differentiation factor 15 (GDF-15) (also known as macrophage inhibitory cytokine 1 (MIC-1)) is a member of the TGF-β1 super family. GDF-15 levels are upregulated in many cancer types [20]. GDF-15 expression is induced during fibrosis development and correlates with lung function impairment in systemic sclerosis patients [21]. GDF-15 is a product of activated macrophages and hereby a role player in inflammatory processes [22]. In addition, we evaluated the role of the known inflammation marker high sensitive C-reactive protein (hs-CRP).

In this study, we investigated the prevalence of lesions suspect for bleomycin-induced pulmonary changes found on restaging CT scans after treatment with BEP chemotherapy in patients with metastatic testicular cancer, and whether fibrosis markers TGF-β1 and GDF-15 and the inflammation marker hs-CRP were predictive for these changes.

MATERIAL AND METHODS

Patients

We performed a prospective non-randomized biomarker cohort study for which eligibility criteria were: patients between 18 and 50 years of age with metastatic testicular cancer who were to be treated with BEP chemotherapy in the University Medical Center Groningen. Primary assessments were related to cardiovascular parameters. Exclusion criteria were a medical history of cardiovascular disease or a creatinine clearance < 60 mL/min. An additional analysis was performed on pulmonary parameters. Criteria for this analysis were a minimum of at least two bleomycin administrations (60 USP) and presence of a chest CT scan as part of the restaging investigations after chemotherapy. Patients were treated with a standard regimen of 3 or 4 three weekly BEP chemotherapy courses, depending on International Germ Cell Consensus Classification (IGCCC) prognosis group. During the first six days of each course, patients received daily anti-emetic therapy (dexamethasone and ondansetron). The local ethics committee approved the study. All patients gave written informed consent.

CT scans

Post chemotherapy restaging CT scans were analyzed for abnormalities suspect for bleomycin-induced pulmonary changes as judged by two independent radiologists who were blinded for clinical phenotype. Patients were instructed to inhale during CT scans of the thorax. CT scanning was started 30 seconds after intravenous contrast injection (55 cc Lomeron 350). Scans were made in caudalcranial direction from the deepest costophrenic pleural recesses to above the thorax aperture. For reconstruction the following parameters were used: 3/1.5 mm on Sensation-16 and Symbia T16 and 2/1.5 mm on S-64, Definition and mCT (Kernel B40f).

Radiologic criteria for abnormalities on restaging CT scan that were regarded as bleomycin-induced were that these abnormalities were newly developed since start of BEP chemotherapy and could not readily be explained by other factors such as metastases or infection. When available, the next CT scan (follow-up scan), made as part of the routine follow-up after completion of treatment was assessed to judge whether abnormalities on the restaging CT scan, suspected to be the result of bleomycin-induced pulmonary changes, diminished or resolved. The type of abnormality was described and abnormalities were scored to be unifocal or multifocal with the location and number of lobes involved noted. Extension of radiographic abnormalities were graded as minor (only outer third of the lung involved), moderate (outer and middle third of the lung involved, but not extending across to the mediastinum) or severe abnormalities (whole width of the lung from periphery to mediastinum involved) according to Bellamy et al [11].

Measurement of biomarkers

EDTA plasma samples were collected for all biomarkers before start of chemotherapy (day 1), for TGF-β1 on day 1 of every subsequent chemotherapy course, for GDF-15 and hs-CRP at day 8 of the third course and for all measured biomarkers at follow-up 4 weeks after the end

of chemotherapy. EDTA blood samples were centrifuged at 3000 g at 4°C for 10 minutes and plasma was stored at minus 20°C. In these samples total TGF-β1 levels were analyzed after activation with hydrochloric acid using a human TGF-β1 Quantikine ELISA-kit (R&D systems Abingdon, United Kingdom). GDF-15 levels were measured with the Human GDF-15 Quantikine ELISA-kit (R&D systems). Hs-CRP levels were analyzed using the nephelometer.

Statistics

Differences in biomarker levels were investigated between patients with and without pulmonary changes on CT scan and in patients with and without clinical BIP. We also analyzed differences between the groups based on amount of changes on CT scan (no, minor, moderate or severe abnormalities). Biomarker levels were evaluated as absolute values, relative values (normalized to pre-chemotherapy levels) and absolute increases. Mann Whitney U tests and Kruskal Wallis tests were used to evaluate differences in biomarker levels between groups. Chi square and Fisher exact tests were used to analyze categorical data. A Spearman correlation test was used to test correlations. *P*-values ≤0.05 were considered significant. Statistics were calculated with IBM SPSS statistics 22. Graphs were made using GraphPad Prism version 5.00.

RESULTS

Patients

Between May 2006 and June 2012, 78 patients were included in the biomarker study. Two patients withdrew consent during the study. In total, five patients were excluded from analysis because data were incomplete due to missed measurements. Five patients received ≤ 2 BEP-courses. Data of 66 patients was analyzed (Figure 1, Table 1). Five of these 66 patients did not receive all initially planned bleomycin administrations because of clinical signs of BIP (n = 3), development of bleomycin skin toxicity (n = 1) and development of a pulmonary embolism (n = 1).

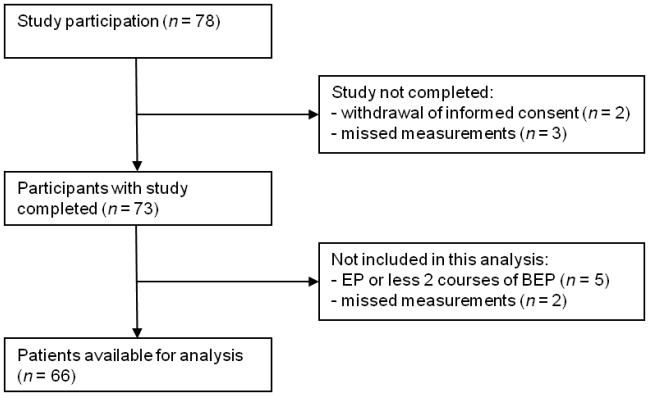


Figure 1. CONSORT diagram.

Table 1: Patient characteristics.

		Median (range)	Number (%)
Total number of patients			66
Age (years)		31 (19-46)	
Diagnosis			
	Non-seminoma		54 (82%)
	Seminoma		12 (18%)
IGCCC prognosis group			
	Good		55 (83%)
	Intermediate		10 (15%)
	Poor		1 (2%)
Cumulative administered bleomycin dose (USP)			
	150		1 (2%)
	180		1 (2%)
	240		2 (3%)
	270		52 (79%)
	330		1 (2%)
	360		9 (14%)
Pretreatment creatinine clearance (mL/min)		77 (53-107)	
Pulmonary metastases			
	Yes		7 (11%)
	No		59 (89%)
Smoking			
	Yes		29 (44%)
	No		26 (39%)
	Ex		10 (15%)
	Unknown		1 (2%)

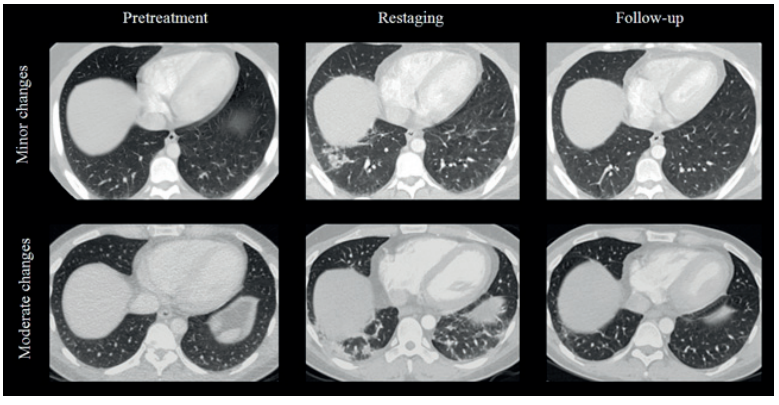


Figure 2. Representative examples of minor graded (upper panel) and moderate graded (lower panel) bleomycin-induced pulmonary changes; pretreatment, restaging and follow-up CT scan (left to right).

Bleomycin-induced pulmonary changes on CT scan

Pretreatment staging CT scans from chest and abdomen were made median 22 (range 1 - 78) days before start of chemotherapy. Restaging CT scans were made median 21 (range 5 - 112) days after the last course of chemotherapy. The first CT scan during follow-up was made median 175 (range 70 - 588) days after the restaging CT scan. In 45 out of 66 (68%) analyzed patients radiological signs suspect for bleomycin induced pulmonary changes were seen on the restaging scan. Of these, 37 were classified to have minor and eight to have moderate abnormalities (Figure 2). None were qualified as severe abnormalities. Most abnormalities were multifocal and found in the basal parts of the lungs.

There was no relation between the cumulative dose of bleomycin and development of lesions suspect for bleomycin toxicity on restaging CT scan. Renal function and smoking frequency were not different between groups (Table 2). On available follow-up scans of patients with suspected bleomycin-induced pulmonary changes (38/45; 84%), these changes diminished in 14 patients and disappeared in 24 patients. No differences were found in patient characteristics between these two groups (Table 3).

In three patients with clinical signs of BIP for which administration of bleomycin was halted, the restaging CT scan showed moderate, minor and no abnormalities respectively.

Biomarker plasma levels

Table 4 shows biomarker plasma levels of all patients at clinical relevant time points. Median plasma levels before start of chemotherapy were 4788 (range 550 - 24369) pg/mL for TGF-β1, 392 (range 187 - 1935) pg/mL for GDF-15 and 1.3 (range 0.2 - 50.4) mg/L for hs-CRP. No correlation was found between TGF-β1, GDF-15, hs-CRP and tumor marker levels (AFP, β-HCG and LDH) before start of treatment in patients with elevated tumor markers (data not shown).

No significant differences in absolute or relative levels of TGF-β1, GDF-15 and hs-CRP at the measured time points were found between patients with no, minor or moderate radiological signs of pulmonary bleomycin toxicity. In addition, there were also no significant differences found in patients with no or minor versus moderate pulmonary bleomycin toxicity (Table 4). However, patients who developed pulmonary abnormalities on CT scan (either minor or moderate) more frequently had an TGF-β1 increase between day one of the first course BEP (before start of chemotherapy) and day one of the second course (86 vs. 60%, p=0.047). Also, GDF-15 levels at the 8th day of the third course were higher in patients with moderate changes on CT scan than in patients with no or minor changes on CT scan, although this difference was not statistically significant (7246 pg/mL vs. 5222 pg/mL, p=0.087). All groups showed the same pattern of hs-CRP decrease during chemotherapy and an increase afterwards. Patients with clinical signs of BIP had no different biomarker levels compared to other patients (Figure 3). In patients with pulmonary abnormalities on follow-up scans that diminished but not disappeared (n=14) TGF-β1, GDF-15 and hs-CRP levels were not different compared to the patient group in which pulmonary abnormalities disappeared (n=24) (Table 3).

Table 2: Characteristics of patients with and without signs of bleomycin-induced pulmonary changes on CT scan.

	Without changes(n = 21)			Minor changes (n = 37)			Moderate changes (n = 8)			
	Median	Range	N (%)	Median	Range	N (%)	Median	Range	N (%)	P-value*
Age (yrs)	32	19-45		31	20-45		28	22-46		0.768
Weight pre-chemotherapy (kg)	88	70-130		88	62-139		87	65-106		0.852
Bleomycin cumulative dose (USP)	270	180-360		270	150-360		270	240-360		0.231
Serum creatinine pre-chemotherapy (mmol/L)	79	63-107		75	52-105		82	64-89		0.549
Serum creatinine 4 weeks after last course of chemotherapy (mmol/L)	82	57-104		78	59-96		82	68-90		0.630
Smoking			8/21 (38%)			15/36 (42%)			3/8 (38%)	0.878

*: P-value based on comparison of patients with moderate bleomycin-induced pulmonary changes on CT scan versus patients with no or minor changes (Mann-Whitney U tests)

Table 3: Characteristics of patients with follow-up CT scans and bleomycin induced pulmonary changes.

		Patients in whom bleomycin induced changes disappeared (n = 24)			Patients in whom bleomycin induced changes diminished (n = 14)			P-value*
	T	Median	Range	N (%)	Median	Range	N (%)	
Age (yrs)		28	20-43		30	26-45		0.215
Weight pre-chemotherapy (kg)		88	62-134		92	72-139		0.150
Bleomycin cumulative dose (USP)		270	150-360		270	270-360		0.075
Serum creatinine pre-chemotherapy (mmol/L)		75	62-105		77	52-93		0.565
Serum creatinine 4 weeks after last course of chemotherapy (mmol/L)		76	62-91		76	59-96		0.693
Smoking				10/24 (42%)			7/13 (54%)	0.484
Moderate changes on CT scan				2/24 (8%)			4/14 (29%)	0.103
TGF-β1 (pg/mL)	1.1	4616	550-16140		5447	2861-15842		0.348
	2.1	7813	909-30130		10364	4156-25887		0.256
	3.1	9818	3582-32057		9533	3329-14513		0.687
	4.1	3546	1768-18911		6602	3664-14108		0.327
	C	4916	595-17636		5909	3212-14207		0.315
GDF-15 (pg/mL)	1.1	396	199-1935		403	187-691		0.882
	3.8	5235	2475-12013		5721	4130-10761		0.366
	C	697	299-1486		1066	421-2601		0.297
hs-CRP (mg/L)	1.1	1.7	0.3-50.4		1.2	0.2-36.1		0.405
	3.8	0.5	0.2-132		0.5	0.2-2.1		0.682
	C	2.3	0.3-29.1		3.0	0.3-26.7		0.445

*: Mann-Whitney U tests. T= time point: course number.day number; C = 4 weeks after last course of chemotherapy.

Table 4: Biomarker levels in relation to presence of bleomycin-induced pulmonary changes on CT scan.

			Whole group (n = 66)		Without changes (n = 21)		Minor changes (n = 37)		Moderate changes (n = 8)		
Biomarker	T	N (%)	Median	Range	Median	Range	Median	Range	Median	Range	P-value*
TGF-β1	1.1	66 (100%)	4788	550-24369	4306	1345-24369	5002	550-16140	5268	2052-13361	0.582
(pg/mL)	2.1	63 (95%)	9570	909-42015	8940	1380-42015	8915	909-30130	10803	4156-23849	0.397
	3.1	59 (89%)	7889	1484-49897	6201	2378-49897	8522	1484-32057	8512	3878-20372	0.765
	4.1	12 (18%)	6774	1768-18911	6774	6232-7316	3912	1768-14108	13977	9043-18911	0.121
	C	64 (97%)	5220	595-18839	5357	1622-18839	5041	595-17636	5116	3223-14207	0.527
GDF-15	1.1	63 (95%)	392	187-1935	388	198-721	366	199-1935	413	187-1876	0.397
(pg/mL)	3.8	58 (88%)	5342	1439-18959	5209	1439-14400	5235	2475-12013	7246	4130-18959	0.087
	C	62 (94%)	880	299-2601	808	350-2023	840	299-1610	1089	442-2601	0.073
hs-CRP	1.1	66 (100%)	1.3	0.2-50.4	1.9	0.2-18.5	1.2	0.2-50.4	2.1	0.3-39.6	0.813
(mg/L)	3.8	61 (92%)	0.5	0.2-132.0	0.5	0.2-25.7	0.5	0.2-132.0	0.7	0.2-7.2	0.626
	C	65 (98%)	2.2	0.3-29.1	2.7	0.4-15.3	2.2	0.3-29.1	1.9	0.3-9.3	0.546

*: P-value based on comparison of patients with moderate bleomycin-induced pulmonary changes on CT scan versus patients with no or minor changes (Mann-Whitney U tests).

T = time point course number.day number; C = 4 weeks after last course of chemotherapy

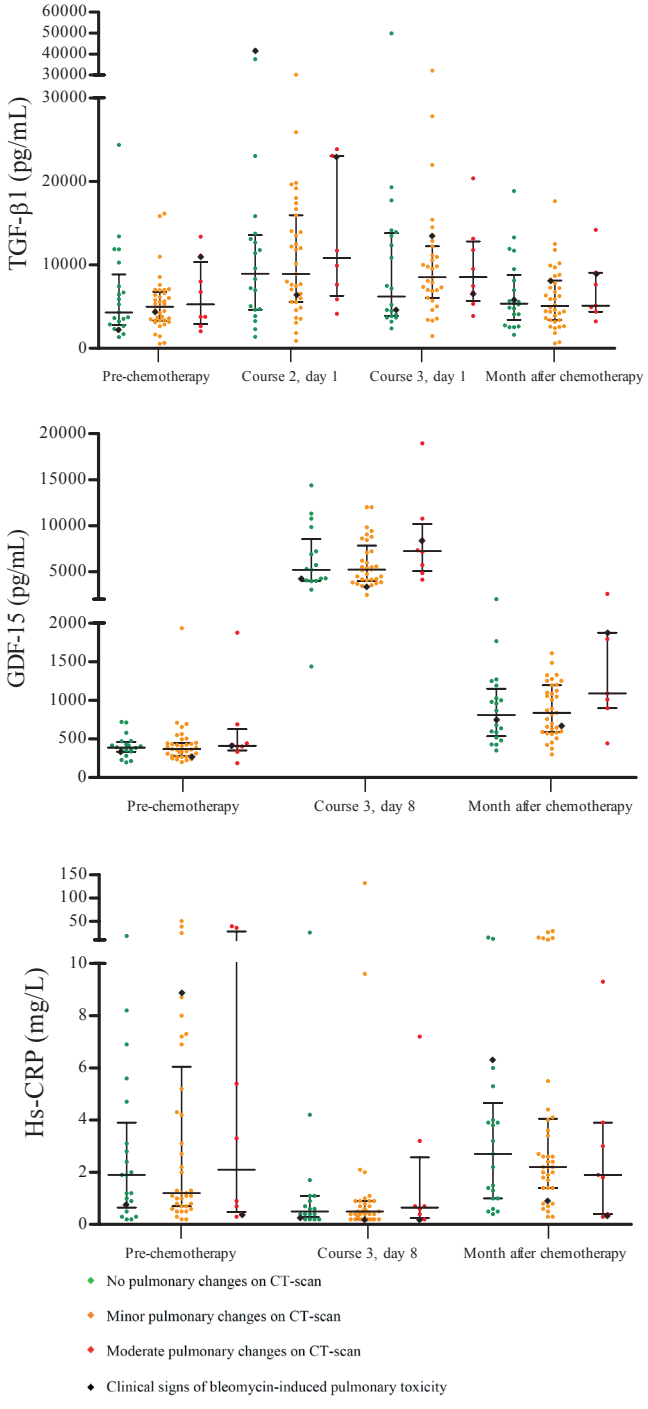


Figure 3. Plasma levels of TGF-β1 (upper graph), GDF-15 (middle graph) and hs-CRP (lower graph) during the chemotherapy courses.

DISCUSSION

In this study, all restaging CT scans of patients with disseminated testicular cancer treated with BEP chemotherapy for signs of bleomycin-induced pulmonary toxicity were evaluated. In 68% of these patients radiological abnormalities on the restaging CT scan interpreted as signs of bleomycin-induced pulmonary changes were observed. Clinical presentation suspect for BIP during treatment, which led to premature termination of bleomycin administration, was seen in only 5% of patients. This discrepancy between clinical presentation of BIP and radiological signs of bleomycin-induced toxicity on CT scans is striking. Earlier studies reported a much higher incidence of abnormalities on restaging CT scans as well, compared to the 10% of the patients who classically develop clinical signs of bleomycin-induced pulmonary toxicity [11-13]. However, these radiological abnormalities are not very well documented and not reported in more recent reports of clinical trials in which bleomycin is a component of the combination regimen for metastatic testicular cancer [23]. No high-resolution CT scans were made in any of the patients precluding statements on the presence of interstitial pneumonitis.

The clinical relevance of radiological signs of bleomycin-induced pulmonary abnormalities on restaging CT scans remains unclear. Most of these abnormalities on CT scans were without accompanying symptoms and resolved spontaneously based on subsequent CT scans several months later during follow-up. We questioned whether post treatment radiological findings were accompanied by biochemical signs of active fibrosis and whether these could be used as early markers of the toxic effect of bleomycin on the lung.

TGF-β1 is a cytokine involved in many processes in the body and plays a pivotal role in the development of lung fibrosis [24]. Various preclinical studies showed a critical role for TGF-β1 in the BIP development. In the current study, we did not find a difference in TGF-β1 levels between patients with and without radiological signs of bleomycin-induced pulmonary changes. However, patients who developed bleomycin-induced pulmonary changes on CT scan more often showed an increase of TGF-β1 levels from pre chemotherapy to day one of the second course. This may indicate involvement of the TGF-β1 pathway in development of pulmonary abnormalities due to bleomycin administration.

The TGF-β1 plasma levels found in our patients at baseline had a broad range. Plasma TGF-β1 levels in healthy volunteers also show a threefold difference with a broad range in other studies [25,26]. Measured TGF-β1 levels in our patient group may also be tumor derived rather than selectively the result of bleomycin-induced pulmonary changes [27]. However, no correlation between known tumor markers in testicular cancer and TGF-β1 levels before treatment was found. An interesting approach to circumvent this would be to assess TGF-β1 levels during bronchoalveolar lavage (BAL) to assess only lung TGF-β1 levels [28]. We collected no platelet poor plasma, while TFG-β1 might have been released from platelets during collection and analyses of the samples [29]. This is a limitation of our study, but does not preclude comparison of TGF-β1 levels within our patient group.

We did not find differences in GDF-15 levels in patients with versus patients without bleomycin-induced pulmonary changes on CT scan. Although the increase of GDF-15 levels seemed

to be larger in patients with moderate pulmonary changes on CT scans, this was not significant. The number of patients with moderate changes (n = 8) might have been too small to detect significant differences. In addition, GDF-15 is also known as apoptosis marker [22]. Therefore, its distinctive value during cancer therapy is probably limited. Nevertheless, it could be worth examining GDF-15 levels in a larger patient group with a moderate degree of pulmonary changes on CT scans after BEP chemotherapy.

Hs-CRP could be an easily accessible biomarker for subclinical inflammation, but levels did not differ in patients with compared to patients without bleomycin-induced pulmonary changes. This concurred with steroid administration as anti-emetic drugs during chemotherapy. Therefore, hs-CRP is probably not an usable biomarker for bleomycin-induced pulmonary changes during chemotherapy.

In conclusion, pulmonary radiological abnormalities suspect for bleomycin induced changes are very common on restaging CT scans after BEP chemotherapy for metastatic testicular cancer, occurring in two third (68%) of the patients. Most of these radiological abnormalities appear to resolve on follow-up CT scans. TGF-β1, GDF-15 and hs-CRP plasma levels before and during treatment were not significantly different between patients with and without radiological signs of pulmonary bleomycin-induced toxicity and therefore do not seem helpful as early predictive biomarkers.

ACKNOWLEDGEMENTS

Nynke Zwart and Gerrie Steursma are acknowledged for their technical and administrative support.

REFERENCES

[1] Loehrer PJS, Johnson D, Elson P, Einhorn LH, Trump D. Importance of bleomycin in favorable-prognosis disseminated germ cell tumors: An eastern cooperative oncology group trial. J Clin Oncol 1995;13:470-6.

[2] Sleijfer S. Bleomycin-induced pneumonitis. Chest 2001;120:617-24.

[3] Saito F, Tasaka S, Inoue K, Miyamoto K, Nakano Y, Ogawa Y, et al. Role of interleukin-6 in bleomycin-induced lung inflammatory changes in mice. Am J Respir Cell Mol Biol 2008;38:566-71.

[4] Froudarakis M, Hatzimichael E, Kyriazopoulou L, Lagos K, Pappas P, Tzakos AG, et al. Revisiting bleomycin from pathophysiology to safe clinical use. Crit Rev Oncol Hematol 2013;87:90-100.

[5] White DA, Stover DE. Severe bleomycin-induced pneumonitis. Clinical features and response to corticosteroids. Chest 1984;86:723-8.

[6] Simpson AB, Paul J, Graham J, Kaye SB. Fatal bleomycin pulmonary toxicity in the west of scotland 1991-95: A review of patients with germ cell tumours. Br J Cancer 1998;78:1061-6.

[7] Collis CH. Lung damage from cytotoxic drugs. Cancer Chemother Pharmacol 1980;4:17-27.

[8] O'Sullivan JM, Huddart RA, Norman AR, Nicholls J, Dearnaley DP, Horwich A. Predicting the risk of bleomycin lung toxicity in patients with germ-cell tumours. Ann Oncol 2003;14:91-6.

[9] Nuver J, Lutke Holzik MF, van Zweeden M, Hoekstra HJ, Meijer C, Suurmeijer AJ, et al. Genetic variation in the bleomycin hydrolase gene and bleomycin-induced pulmonary toxicity in germ cell cancer patients. Pharmacogenet Genomics 2005;15:399-405.

[10] Sleijfer S, van der Mark TW, Schraffordt Koops H, Mulder NH. Decrease in pulmonary function during bleomycin-containing combination chemotherapy for testicular cancer: Not only a bleomycin effect. Br J Cancer 1995;71:120-3.

[11] Bellamy EA, Husband JE, Blaquiere RM, Law MR. Bleomycin-related lung damage: CT evidence. Radiology 1985;156:155-8.

[12] Bellamy EA, Nicholas D, Husband JE. Quantitative assessment of lung damage due to bleomycin using computed tomography. Br J Radiol 1987;60:1205-9.

[13] Rimmer MJ, Dixon AK, Flower CD, Sikora K. Bleomycin lung: Computed tomographic observations. Br J Radiol 1985;58:1041-5.

[14] Blobe GC, Schiemann WP, Lodish HF. Role of transforming growth factor (beta) in human disease. N Engl J Med 2000; 342:1350-8.

[15] Degryse AL, Tanjore H, Xu XC, Polosukhin VV, Jones BR, Boomershine CS, et al. TGFβ signaling in lung epithelium regulates bleomycin-induced alveolar injury and fibroblast recruitment. Am J Physiol Lung Cell Mol Physiol 2011;300: L887-97.

[16] Hoyles RK, Derrett-Smith EC, Khan K, Shiwen X, Howat SL, Wells AU, et al. An essential role for resident fibroblasts in experimental lung fibrosis is defined by lineage-specific deletion of high-affinity type II transforming growth factor β receptor. Am J Respir Crit Care Med 2011;183:249-61.

[17] Li M, Krishnaveni MS, Li C, Zhou B, Xing Y, Banfalvi A, et al. Epithelium-specific deletion of TGF-β receptor type II protects mice from bleomycin-induced pulmonary fibrosis. J Clin Invest 2011;121:277-87.

[18] Boothe DL, Coplowitz S, Greenwood E, Barney CL, Christos PJ, Parashar B, et al. Transforming growth factor beta-1 (TGF-β1) is a serum biomarker of radiation induced fibrosis in patients treated with intracavitary accelerated partial breast irradiation: Preliminary results of a prospective study. Int J Radiat Oncol Biol Phys 2013;87:1030-6.

[19] Anscher MS, Peters WP, Reisenbichler H, Petros WP, Jirtle RL. Transforming growth factor β as a predictor of liver and lung fibrosis after autologous bone marrow transplantation for advanced breast cancer. N Engl J Med 1993;328:1592-8.

[20] Bauskin AR, Brown DA, Kuffner T, Johnen H, Luo XW, Hunter M, et al. Role of macrophage inhibitory cytokine-1 in tumorigenesis and diagnosis of cancer. Cancer Res 2006;66:4983-6.

[21] Lambrecht S, Smith V, De Wilde K, Coudenys J, Decuman S, Deforce D, et al. Growth differentiation factor 15, a marker of lung involvement in systemic sclerosis, is involved in fibrosis development but is not indispensable for fibrosis development. Arthritis Rheumatol 2014;66:418-27.

[22] Breit SN, Johnen H, Cook AD, Tsai VW, Mohammad MG, Kuffner T, et al. The TGF- β superfamily cytokine, MIC-1/GDF15: A pleotrophic cytokine with roles in inflammation, cancer and metabolism. Growth Factors 2011;29:187-95.

[23] de Wit R, Skoneczna I, Daugaard G, De Santis M, Garin A, Aass N, et al. Randomized phase III study comparing paclitaxel-bleomycin, etoposide, and cisplatin (BEP) to standard BEP in intermediate-prognosis germ-cell cancer: Intergroup study EORTC 30983. J Clin Oncol 2012;30:792-9.

[24] Yue X, Shan B, Lasky JA. TGF-beta: Titan of lung fibrogenesis. Curr Enzym Inhib 2010; 6.

[25] van Waarde MA, van Assen AJ, Kampinga HH, Konings AW, Vujaskovic Z. Quantification of transforming growth factor-β in biological material using cells transfected with a plasminogen activator inhibitor-1 promoter-luciferase construct. Anal Biochem 1997;247:45-51.

[26] Schneider T, Sailer M, Ansorge S, Firsching R, Reinhold D. Increased concentrations of transforming growth factor β1 and β2 in the plasma of patients with glioblastoma. J Neurooncol 2006;79:61-5.

[27] Rube CE, Palm J, Erren M, Fleckenstein J, Konig J, Remberger K, et al. Cytokine plasma levels: Reliable predictors for radiation pneumonitis? PLoS One 2008;3:e2898.

[28] Barthelemy-Brichant N, Bosquee L, Cataldo D, Corhay JL, Gustin M, Seidel L, et al. Increased IL-6 and TGF-β1 concentrations in bronchoalveolar lavage fluid associated with thoracic radiotherapy. Int J Radiat Oncol Biol Phys 2004;58:758-67.

[29] Assoian RK, Sporn MB. Type β transforming growth factor in human platelets: Release during platelet degranulation and action on vascular smooth muscle cells. J Cell Biol1986;102:1217-23.

Chapter 7

Summary and future perspectives

SUMMARY

In recent years, great progress has been made in the development of targeted therapy against cancer. However, it is also more recognized that these treatments are often only successful in a subgroup of patients. One of the current challenges in oncology lies in identifying these patients and thereby avoiding treating patients with non-effective treatments and thus avoid side-effects of these drugs and reduce costs. Another challenge in oncology is to minimize the side effects of currently used therapies. Patients are at risk for developing (long term) and even deadly side effects of anticancer treatments and might need adjustments to their treatment schedule and specific follow up long after the end of treatment to timely recognize and address these.

One of the targeted therapies that made its way into clinical research in recent years is the induction of apoptosis by agonistic antibodies or rhTRAIL via the extrinsic pathway. In preclinical studies, these drugs have been shown to induce apoptosis as monotherapy and enhance the effectiveness of chemotherapy and radiotherapy. In **chapter 2**, we reviewed the phase 1 and phase 2 (combination) studies that have been performed with these drugs. Although rhTRAIL and the diverse agonistic antibodies against TRAIL-R1 and TRAIL-R2 all seem safe with only one maximum tolerated dose found for lexatumumab (elevations of serum amylase, bilirubin and transaminases), the effectiveness of these drugs both as monotherapy and in combination with chemotherapy is disappointing. However, we also describe novel approaches with TRAIL linked to a tumor specific antibody that might lead to better results in the clinic. Also, the combination of mapatumumab with chemotherapy and radiotherapy was investigated in a phase 1/2 study (NCT01088347). Results of this study are awaited.

When the targeted drug imatinib entered the clinic in early clinical studies in patients with GIST it was noticed that within one to several days a remarkable change in FDG uptake in the tumors occurred. It was suggested that this change in uptake could serve as a predictive marker for the efficacy of this therapy. Several small studies with FDG-PET in this setting have been conducted since, leading to variable results. However, it has become clear that the majority of patients (85%) have clinical benefit from this treatment. A predictive test that identifies patients that have clinical benefit would therefore not be that informative, but a test that identifies patients that will not respond (primary resistance) is. In **chapter 3** we retrospectively assessed the usefulness of FDG-PET scans made before start of imatinib and 1 week after start of imatinib for identification of non responders. Two out of 29 patients showed progressive disease on CT scan after 8 weeks as defined by the RECIST criteria. These patients also showed a response (defined as at least 25% decrease in standard uptake value (SUV)) on the FDG-PET scan. On the other hand the three patients who did not show a response on the FDG-PET scan all did have clinical benefit of imatinib. We therefore concluded that performing a FDG-PET scan is not useful in identifying patients with primary resistance against imatinib and that the early responses seen on FDG-PET scan might be caused by a change in glucose metabolism that is independent of the tumor response.

In glioblastomas the Macdonalds criteria are used for response assessment and the tumor is measured as the contrast enhancing area found on gadolinium enhanced MRI. Response assessment during and after treatment is hampered by the phenomenon of pseudoprogression: the MRI performed directly after completion of chemoradiotherapy shows increased contrast enhancement compared to the baseline MRI, but this enhancement remains stable or subsides on follow up scans without change of therapy. In **chapter 4** we reported on a study that prospectively assessed FLT-PET scans made before start of treatment (after initial surgery) and 4 weeks after completion of chemoradiotherapy. ^{18}F-FLT is a PET tracer that is taken up by proliferating cells and therefore it might potentially be of interest to use FLT-PET scans to discriminate between true tumor growth and pseudoprogression. In 24 evaluable patients, seven patients showed true progression and seven showed pseudoprogression on MRI. We observed no difference in (change in) FLT uptake between patients classified as true progression and patients classified as pseudoprogression. Therefore FLT-PET scan in this setting cannot be used to discriminate between true disease progression and pseudoprogression in glioblastoma patients treated with chemoradiotherapy.

In high grade gliomas successful targeted drugs are also eagerly awaited, as there is currently no standard therapy for recurrent disease and the median overall survival for the most common subtype (glioblastoma) is only 14 months. Numerous targeted agents have been investigated in recent years, but so far no therapy has improved the overall survival. A key question in systemic treatment of high grade gliomas is if the agents can reach the tumor because of the blood brain barrier. In **chapter 5** we reported on a study in which a monoclonal antibody against TGF-β (fresolimumab) was labeled with Zirkonium-89. After the ^{89}Zr-fresolimumab PET scan, patients were treated with intravenously administered fresolimumab. The goal of this study was to show that the antibody could reach the tumor and to quantify the uptake and correlate this uptake with the outcome of treatment with fresolimumab. In all 12 patients we saw uptake of ^{89}Zr-fresolimumab in tumor lesions, although not in all individual lesions. Of the lesions that were > 1 cm and not visible on the PET scan, 2 out of 3 were previously irradiated and might therefore not represent active tumor tissue with TGF-β signaling. We also found that over time the tumor to blood ratio increased in four patients who underwent a PET scan on both day 2 and day 4 after the tracer injection. These findings suggest that the uptake of ^{89}Zr-fresolimumab in the tumor is specific rather than just a perfusion related phenomenon. Treatment with fresolimumab was well tolerated by all patients, but it did not seem to be beneficial in terms of inducing tumor response as second line treatment. However, we did show that a labeled antibody is able to reach high grade gliomas.

TGF-β is important in many processes in both health and disease. From preclinical research, we know that TGF-β plays an important role in the development of bleomycin induced pulmonary toxicity. This toxicity is a problem in testicular cancer patients that are treated with bleomycin containing chemotherapy regimens. This treatment has a very high cure rate, but in up to 10% of the patients bleomycin induced pulmonary toxicity occurs, which is fatal in 10% of patients experiencing this toxicity. In **chapter 6** we assessed the prevalence of lesions

suspect for bleomycin-induced pulmonary changes on restaging CT scans after treatment, and whether the fibrosis markers TGF-β1 and Growth Differentiation Factor -15 (GDF-15), and hs-CRP were predictive of this. We found signs of bleomycin-induced pulmonary changes on the restaging CT scan in 68% of the patients. Plasma levels of TGF-β1, GDF15 and Hs-CRP were not predictive for the occurrence and severity of CT alterations suspect for bleomycin toxicity or clinical pulmonary toxicity. We concluded that bleomycin-induced pulmonary changes are very common on restaging CT scans after BEP chemotherapy for metastatic testicular cancer, but plasma levels of TGF-β1, GDF15 and Hs-CRP cannot be used as biomarkers for this.

FUTURE PERSPECTIVES

The recent rapid unraveling of tumor characteristics and the use of these insights for the application of targeted therapy has led to hope that cancer will become a chronic disease in the coming years [1]. In spite of this optimism, it has also become clear that new discoveries and developments raise new questions and challenges.

In metastatic and irresectable GIST tumors, the introduction of the targeted drug imatinib spectacularly improved response rates compared to chemotherapy. Unfortunately, many targeted drugs that seemed promising in preclinical research did not live up to the expectations in phase 1 and 2 studies. Agonistic antibodies against TRAIL receptors and rhTRAIL are well tolerated and can be combined with chemotherapy. However, none of the randomized phase 2 studies so far has shown an improvement of progression free survival or overall survival. This underlines the enormous challenges in finding drugable targets, identifying patients that will benefit from treatment with targeted drugs, the need for rational combination therapies and perseverance when clinical results do not directly live up to the expectations found in preclinical research.

In glioblastomas, driver mutations and pathways are still not elucidated and there is no targeted agent available that does improve overall survival [2]. Although TGF-β is an important tumor promoter in these tumors, treatment with fresolimumab did not result in clinical benefit in our small study with pretreated patients. Since many pathways are deregulated in high grade gliomas, better results might be expected from combination treatment targeting multiple relevant pathways. Since we did show in our study that fresolimumab is able to reach brain tumors, other antibody-related drugs such as antibody-drug conjugates are also of potential interest for this indication (NCT01475006).

A key requirement in successful anticancer drug development is to establish the molecular and genetic characteristics of tumors. Studies in which biopsies are taken before and after (investigational) treatment with targeted agents are currently performed and will learn us more about tumor characteristics, patient selection and development of resistance mechanisms against targeted agents. In the future, this will lead to more personalized medicine, in which a patient receives a treatment based on the specific characteristics of the tumor.

However, it is also known that primary tumor lesions and metastatic lesions can have different characteristics and tumor characteristics can change over time [3]. Taking multiple

biopsies at different time points to characterize the heterogeneity will often not be feasible due to patient inconvenience and tumor lesions that cannot be approached safely. Molecular imaging is a more patient friendly approach to assess the characteristics of all different tumor lesions. Diverse studies show promising results with this approach [4,5]. However, we showed that the initial optimism about the use of FDG-PET to predict responses of imatinib in GIST has been premature. Furthermore, response criteria for PET imaging still need to be established and might be different for different PET tracers. More and larger studies establishing this and studies directly comparing PET results with pathology results are therefore urgently needed and are currently performed (NCT01957332).

Since the introduction of targeted agents in the clinic it has also become clear that the current response assessment with CT scans may be suboptimal because targeted agents do not always induce a volume response. Initiatives to define new criteria in which also PET responses might be included are under investigation [6]. In brain tumors the Macdonalds criteria have already been replaced by the RANO criteria, in order decrease the number of pseudoprogression and pseudoresponses found [7]. However, for this indication also a combination of conventional imaging and molecular imaging will be needed to accurately assess responses of current and future therapies. We showed that serial FLT-PET scan was not helpful in discriminating pseudoprogression from true progression in primary glioblastoma patients treated with chemoradiotherapy. However, approaches using other parameters such as kinetic analysis may overcome the difficulty of discriminating between tracer uptake and leakage due to a disrupted blood brain barrier. Also other imaging modalities such as perfusion MRI and ^{11}C-methionine-PET show promising results and are currently under more detailed investigation [8,9].

An interesting development in imaging that circumvents the irradiation burden of PET scans is optical imaging. Its use is currently limited by the tissue penetration of the optical tracers which makes whole body scanning impossible, but it is especially investigated for surgical applications. Since optimal surgical resection is one of the predictive markers for overall survival in glioblastoma, tumor resection using optical imaging with a specific tracer might be a promising tool to discriminate infiltrating tumor from normal brain tissue [10,11].

With the increasing number of cancer patients and improvements in treatment, the number of cancer survivors is also increasing [12]. Long term effects of anticancer treatments are therefore gaining more attention. We know that particular attention needs to be paid to cardiovascular diseases and secondary malignancies in long term cancer survivors [13]. Biomarkers may help to identify patients at risk for long term and harmful side effects. A challenge for the future will be to prevent predicted (long term) toxicity without jeopardizing the efficacy of anticancer treatments resulting in a balanced trade-off.

In conclusion, notwithstanding the enormous challenges ahead, cancer medicine will become more and more personalized: new imaging techniques will be able to reveal tumor characteristics, specific drugs will be able target these, specific patients characteristics will be taken into account and the follow up of patients will be aimed at the specific long term effects based on the therapy and risk factors of the individual patient.

REFERENCES

[1] de Visser E, Waarom we optimistisch mogen zijn over de bestrijding van kanker (of niet), Volkskrant, 30 november 2013.

[2] Tanaka S, Louis DN, Curry WT, Batchelor TT, and Dietrich J., Diagnostic and therapeutic avenues for glioblastoma: No longer a dead end? Nat. Rev. Clin. Oncol. 10 (2013) 14-26.

[3] Amir E, Miller N, Geddie W, *et al*, Prospective study evaluating the impact of tissue confirmation of metastatic disease in patients with breast cancer, J. Clin. Oncol. 30 (2012) 587-592.

[4] van Kruchten M, Glaudemans AW, de Vries EF, Beets-Tan RG, Schroder CP, Dierckx RA, de Vries EG, and Hospers GA, PET imaging of estrogen receptors as a diagnostic tool for breast cancer patients presenting with a clinical dilemma, J. Nucl. Med. 53 (2012) 182-190.

[5] van Asselt SJ, Oosting SF, Brouwers AH*, et al*, Everolimus reduces 89Zr-bevacizumab tumor uptake in patients with neuroendocrine tumors, J. Nucl. Med. 55 (2014) 1087-1092.

[6] Liu Y, Litiere S, de Vries EG, Sargent D, Shankar, Bogaerts J, and Seymour L, The role of response evaluation criteria in solid tumour in anticancer treatment evaluation: Results of a survey in the oncology community, Eur. J. Cancer. 50 (2014) 260-266.

[7] Wen PY, Macdonald DR, Reardon DA*, et al*, Updated response assessment criteria for high-grade gliomas: Response assessment in neuro-oncology working group, Journal of Clinical Oncology. 28 (2010) 1963-1972.

[8] Dhermain FG, Hau P, Lanfermann H, Jacobs AH, and van den Bent MJ, Advanced MRI and PET imaging for assessment of treatment response in patients with gliomas, Lancet Neurol. 9 (2010) 906-920.

[9] Glaudemans AW, Enting RH, Heesters MA, Dierckx RA, van Rheenen RW, Walenkamp AM, and Slart RH, Value of 11C-methionine PET in imaging brain tumours and metastases, Eur. J. Nucl. Med. Mol. Imaging. 40 (2013) 615-635.

[10] Nguyen QT and Tsien RY, Fluorescence-guided surgery with live molecular navigation--a new cutting edge, Nat. Rev. Cancer. 13 (2013) 653-662.

[11] Stummer W, Pichlmeier U, Meinel T, Wiestler OD, Zanella F, Reulen HJ, and ALA-Glioma Study Group, Fluorescence-guided surgery with 5-aminolevulinic acid for resection of malignant glioma: A randomised controlled multicentre phase III trial, Lancet Oncol. 7 (2006) 392-401.

[12] Howlader N, Noone A, Krapcho M*, et al*, SEER cancer statistics review, 1975-2010, national cancer institute. bethesda, MD, (based on November 2012 SEER data submission, posted to the SEER web site, April 2013.) http://seer.cancer.gov/csr/1975_2010/,.

[13] Haugnes HS, Bosl GJ, Boer H, Gietema JA, Brydoy M, Oldenburg J, Dahl AA, Bremnes RM, and Fossa SD, Long-term and late effects of germ cell testicular cancer treatment and implications for follow-up, J. Clin. Oncol. 30 (2012) 3752-3763.

7

Chapter 8

Nederlandse samenvatting

NEDERLANDSE SAMENVATTING

Er is in de afgelopen jaren veel vooruitgang geboekt in de ontwikkeling van 'doelgerichte behandelingen' tegen kanker. Helaas wordt ook steeds duidelijker dat deze behandelingen vaak alleen succesvol zijn in een subgroep van de patiënten. Eén van de belangrijkste uitdagingen in de oncologie op dit moment is het identificeren van deze patiënten, zodat kan worden voorkomen dat patiënten onnodig behandeld worden met voor die patiënt niet werkzame therapieën. Dit voorkomt bij patiënten onnodige bijwerkingen en bespaart kosten. Een andere uitdaging in de oncologie is het minimaliseren van bijwerkingen van huidige behandelingen. Antikankerbehandelingen kunnen ernstige en zelfs dodelijke (lange termijn) bijwerkingen hebben. Aanpassing van het behandelschema en specifieke follow up kunnen nodig zijn om bijwerkingen tijdig te herkennen, te behandelen en voorkomen.

Eén van de doelgerichte antikanker behandelingen die in de afgelopen jaren onderzocht is, is het induceren van apoptose door agonistische antilichamen of rhTRAIL via de extrinsieke route. In preklinische studies is aangetoond dat deze middelen zowel als monotherapie apoptose kunnen veroorzaken, als de effectiviteit van chemotherapie en radiotherapie kunnen verbeteren. In **hoofdstuk 2** hebben we de fase 1 en fase 2 (combinatie) studies die tot dusverre met deze middelen zijn uitgevoerd geanalyseerd. rhTRAIL en de verschillende agonistische antilichamen tegen TRAIL-R1 en TRAIL-R2 lijken veilig te zijn. Alleen voor lexatumumab werd een maximale tolereerbare dosering gevonden. Helaas valt de effectiviteit van deze middelen zowel als monotherapie als in combinatie met chemotherapie en andere doelgerichte medicatie tegen. Nieuwe benaderingen waarbij TRAIL wordt gekoppeld aan een tumor specifiek antilichaam kunnen mogelijk tot betere resultaten leiden. Daarnaast is de combinatie van mapatumumab met chemotherapie en radiotherapie onderzocht in een fase 1/2 studie (NCT01088347).

Toen het medicijn imatinib, een c-Kit-tyrosinekinaseremmer, voor het eerst werd toegepast bij patiënten met een GIST, viel het op dat binnen enkele dagen een verandering zichtbaar was in de hoeveelheid FDG die werd opgenomen door de tumoren. Mogelijk kon deze verandering in opname worden gebruikt als een predictieve marker voor de effectiviteit van de behandeling. Er zijn sindsdien verschillende kleine studies met FDG-PET gedaan in deze setting, met wisselende resultaten. Inmiddels is echter ook duidelijk geworden dat het grootste deel van de patiënten (85%) baat heeft bij behandeling met imatinib. Een predictieve test die patiënten identificeert die baat hebben bij de behandeling is daarom niet zo interessant, maar een test die patiënten identificeert die niet reageren op dit middel en zo primaire resistentie aantoont, wel. In **hoofdstuk 3** beschreven we een retrospectieve studie waarin de bruikbaarheid van FDG-PET scans voor het identificeren van patiënten die niet op imatinib reageren is geanalyseerd. Deze FDG-PET scans werden gemaakt voor start van imatinib en 1 week na start van imatinib. Twee van de 29 patiënten hadden progressieve ziekte op de CT scan na 8 weken volgens de tumor respons RECIST criteria. Deze twee patiënten hadden echter ook een respons (gedefinieerd als tenminste 25% afname in 'standard uptake value' (SUV)) op de FDG-PET scans. Aan de andere kant hadden drie patiënten die geen respons hadden op de FDG-PET scans wel baat bij imatinib. We hebben daarom geconcludeerd dat een FDG-PET-scan niet bruikbaar is

voor het identificeren van patiënten met primaire resistentie tegen imatinib en dat de vroege veranderingen op FDG-PET scan mogelijk veroorzaakt worden door veranderingen in het glucosemetabolisme die niet direct verband houden met een tumorrespons.

In glioblastomen worden de Macdonalds criteria, waarbij het gebied met gadolinium contrast opname op MRI wordt gemeten, gebruikt voor tumorrespons evaluatie. Deze evaluatie wordt echter bemoeilijkt door het fenomeen pseudoprogressie; op de MRI direct na chemoradiotherapie is een toegenomen gebied met contrast opname zichtbaar, maar dit gebied blijft stabiel of neemt af op volgende MRI scans zonder verandering in de behandeling. In **hoofdstuk 4** beschreven we een studie waarbij we prospectief keken naar FLT-PET scans gemaakt voorafgaand aan (na primaire chirurgie) en 4 weken na chemoradiotherapie. ^{18}F-FLT is een PET tracer die wordt opgenomen door prolifererende cellen en de FLT-PET scan kan daarom mogelijk gebruikt worden om onderscheid te maken tussen echte tumorgroei en pseudoprogressie. Van de 24 evalueerbare patiënten hadden er zeven echte progressie en zeven pseudoprogressie. We vonden geen verschil in (verandering van) FLT-opname tussen patiënten met pseudoprogressie en echte progressie. De FLT-PET scan heeft geen directe waarde in het maken van dit onderscheid in deze patiëntengroep.

Voor hooggradige gliomen wordt ook hard gezocht naar succesvolle doelgerichte behandeling. Er is dit moment geen standaardtherapie voor recidief tumoren. De mediane totale overleving voor het meest voorkomende subtype (glioblastoom) is slechts 14 maanden. In de afgelopen jaren zijn verschillende doelgerichte behandelingen onderzocht, maar tot nu toe heeft geen enkele behandeling de totale overleving verbeterd. Een essentiële vraag bij de systemische behandeling van hooggradige gliomen is of de medicatie de tumor wel kan bereiken vanwege de bloedhersenbarrière. In **hoofdstuk 5** beschreven we een studie waarin een monoklonaal antilichaam tegen TGF-β (fresolimumab) gelabeld werd met Zirkonium-89. Na het ondergaan van ^{89}Zr-fresolimumab PET scans werden patiënten behandeld met fresolimumab. Het doel van deze studie was om aan te tonen dat het antilichaam de hersentumor kon bereiken, de opname van deze tracer in de tumor te kwantificeren en deze opname te correleren aan de uitkomst van de behandeling. In alle 12 patiënten zagen we opname van ^{89}Zr-fresolimumab in tumorlaesies. Echter, niet in alle individuele laesies werd opname gezien. Van de 3 laesies die groter waren dan 1 cm en niet zichtbaar op de PET scan, waren er 2 eerder bestraald en doordoor mogelijk radionecrose in plaats van actief tumorweefsel, waardoor er geen ^{89}Zr-fresolimumab opname zichtbaar was. We zagen ook dat de tumor-bloedratio toe nam in de vier patiënten die een PET scan hadden ondergaan op zowel dag 2 en dag 4 na tracerinjectie. Deze bevindingen suggereren dat de tumoropname van ^{89}Zr-fresolimumab specifiek is en niet een aan perfusie gerelateerd fenomeen. Behandeling met fresolimumab werd goed verdragen door patiënten, maar het induceerde geen tumorresponsen. We hebben met deze studie echter wel aangetoond dat een gelabeled antilichaam in staat is om hooggradige gliomen te bereiken.

TGF-β is belangrijk voor diverse fysiologische en pathologische processen in het lichaam. Uit preklinisch onderzoek is bekend dat TGF-β een belangrijke rol speelt in de ontwikkeling van door

bleomycine geïnduceerde longtoxiciteit. Deze toxiciteit vormt een probleem voor patiënten met testiskanker die worden behandeld met chemotherapie waarvan bleomycine een onderdeel is. Deze behandeling is heel vaak curatief, maar in ongeveer 10% van de patiënten ontstaat longtoxiteit door bleomycine, die in 10% van de gevallen fataal is. In **hoofdstuk 6** hebben we de spiegel van TGF-β1, GDF-15 en Hs-CRP in het plasma van testiskankerpatiënten voor, tijdens en na behandeling met bleomycine geanalyseerd. We analyseerden ook de prevalentie van afwijkingen die verdacht waren voor door bleomycine geïnduceerde longtoxiciteit op de restageringsscans. We vonden radiologische veranderingen verdacht voor toxiciteit van bleomycine bij 68% van de patiënten. De hoeveelheid TGF-β1, GDF-15 en Hs-CRP in het plasma was niet voorspellend voor het ontstaan en de ernst van de radiologische veranderingen verdacht voor toxiciteit van bleomycine of voor klinische tekenen van longtoxiciteit. We concludeerden daarom radiologische veranderingen verdacht voor toxiciteit van bleomycine veel voorkomen, maar dat de onderzochte markers niet als biomarkers voor het ontstaan hiervan kunnen worden gebruikt.

Dankwoord

DANKWOORD

In de eerste plaats ben ik dank verschuldigd aan alle patiënten die bereid waren om deel te nemen aan de klinische studies.

Klinisch onderzoek doe je niet alleen. Er zijn vele collega's en afdelingen betrokken geweest bij de uitvoering van de diverse projecten. Hierbij daarom mijn dankwoord aan iedereen die heeft meegewerkt aan de verschillende onderzoeksprojecten en aan de totstandkoming van dit proefschrift.

Mijn promotoren prof. dr. Jourik Gietema, prof. dr. Liesbeth de Vries en dr. Annemiek Walenkamp dank ik voor de mogelijkheid die ze me geboden hebben om in een zo stimulerende (onderzoeks) omgeving te mogen werken.

Beste Jourik, je deur stond altijd open voor overleg en advies, of het nu ging om patiëntenzorg of onderzoek. Dank voor alle begeleiding op de goede, maar juist ook op de moeilijke momenten.

Beste Liesbeth, jouw enorme productiviteit en oog voor detail zijn inspirerend. De tijd die je daarnaast nam voor de patiënten en om mij als jonge dokter en klinisch onderzoeker te begeleiden heb ik zeer gewaardeerd.

Beste Annemiek, de nauwkeurigheid waarmee je werkt is een voorbeeld voor me, vaak wist je bij besprekingen de vinger precies op de zere plek te leggen. Dank voor de aanmoedigingen om in het afgelopen jaar (uiteindelijk) alles af en gesubmit te krijgen.

De leden van de leescommissie, prof. dr. M.J. Van den Bent, prof. dr. J.G.W. Kosterink en prof. dr. M.A.T.M. van Vugt dank ik voor hun beoordeling van dit proefschrift.

Dank aan alle medisch oncologen en fellows van de afdeling medische oncologie voor hun begeleiding en supervisie. Dank aan dr. Sjoukje Oosting, die mij begeleidde tijdens mijn wetenschappelijke stage waaruit uiteindelijk dit proefschrift is voortgekomen en dr. An Reyners met wie ik samen de studie naar cervixcarcinomen mocht uitvoeren.

Ik dank alle medewerkers van het Multidisciplinair Oncologisch Laboratorium voor hun hulp en de gezelligheid op het lab. Dank aan Dr. Coby Meijer en dr. Hetty Timmer voor hun begeleiding. Coby, dank voor de tijd die je hebt willen steken in het uitvoeren en interpreteren van de ELISAs. Thijs Oude Munnink, Linda Pot en Silke Vedelaar dank ik voor alle fresolimumab labelingen en jullie hulp met het verkrijgen en interpreteren van de kinetiek data. Gerry Sieling, dank voor alle hulp en ondersteuning bij het uitvoeren van de fase 1 studies. Vooral voor alle keren dat je spontaan even in kon springen ben ik je zeer dankbaar! Arja Hokke, dank voor de goede samenwerking en gezelligheid bij het uitvoeren van studies in de laatste jaren. Hendrique Huisman, Gerrie Steursma en Greetje de Vries dank ik voor de hulp op allerlei momenten. Dank aan Nynke Zwart voor het uitzoeken van de vele samples. Gretha Beuker, Bianca Smit en Hilda Tooi dank ik voor alle administratieve ondersteuning, ook na mijn vertrek uit Groningen. De verpleegkundigen, secretaresses en poli-assistenten van afdeling D2, het dagcentrum, het oncologisch

centrum en de prikpoli dank ik voor hun hulp bij het uitvoeren van de verschillende studies.

Ik dank alle medeauteurs voor de samenwerking en stimulerende commentaar.

De medewerkers van afdeling Nucleaire Geneeskunde en Moleculaire beeldvorming (prof. dr. Jan Pruim, dr. Andor Glaudemans, dr. Adriënne Brouwers, dr. Walter Noordzij, dr. Johan de Jong, Paul van Snick, Hans ter Veen en Clara Lemstra) dank ik voor het uitvoeren en beoordelen van de verschillende scans.

De afdeling radiologie (drs. Fons Bongaerts, dr. Rienhart Wolf, dr. Jan Cees de Groot, dr. Millad Solouki) dank ik voor hun beoordeling van de MRI en CT scans.

De afdeling gynaecologische oncologie dank ik voor hun hulp bij de cervixstudie (prof. dr. Ate van der Zee, prof. dr. Hans Nijman, dr. Henriëtte Arts, Harry Klip, Wytske Boersma, Roland van Leeuwen en Klaske ten Hoor).

De afdeling pathologie (dr. Wilfred den Dunnen, prof. dr. Harry Hollema) dank ik voor de analyse van de weefsels.

De afdeling klinische farmacie en farmacologie (dr. Marjolijn Lub-de Hooge) dank ik voor het bereiden en valideren van de tracers.

Lieve familie en vrienden, bedankt voor jullie interesse in dit proefschrift dat zo lang 'bijna af' is geweest. In het bijzonder wil ik graag noemen: Marlie en Kjelle, voor de gezelligheid en enorme gastvrijheid tijdens de congressen in Chicago. Namkje, voor de vele momenten waarop we ervaringen konden delen over werk, het schrijven van proefschriften en gelukkig ook heel veel niet werk gerelateerde zaken. Christiaan en Irene, voor alle gezelligheid in Groningen en alle keren dat ik mee mocht genieten van jullie culinaire hoogstandjes. Irene, ik ben heel blij dat je nu ook mijn paranimf wilt zijn!

Papa en Mama, heel veel dank voor alle morele én praktische steun die ik altijd van jullie krijg!

Wouter, ook al promoveren we in verschillende vakgebieden, we konden toch veel van onze ervaringen in de afgelopen jaren delen. Heel veel succes met het afronden van jouw proefschrift!

Lisanne, wat zou ik moeten zonder een zusje om me zo af en toe eens verstandig en motiverend toe te spreken. Ik ben blij dat jij vandaag ook naast me staat!

Ten slotte, dank aan mijn collega's en kamergenoten die er voor hebben gezorgd dat dit promotietraject een onvergetelijke tijd werd. Ik denk met veel plezier terug aan alle (wetenschappelijke) discussies, gezelligheid, pubquiz-avonden, schaatslessen, vrijdagmiddagborrels, weekendjes weg en gezamenlijke congresbezoeken. Corina, Renske, Thijs, Anton, Sophie, Sietske, Michel, Hink, Rob, Titia, Frederike, Hilde, Niek, Sjoukje, Grytsje, Jolien, Elmire, Kees, Tjaart en Lars: bedankt!