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**"EXPLORING THE TUMOR
MICROENVIRONMENT WITH
FOCUS ON PLACENTAL GROWTH
FACTOR AND COLORECTAL
CANCER"**

FINAL REPORT ESMO TRANSLATIONAL
RESEARCH FELLOWSHIP

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EXPLORING THE TUMOR MICROENVIRONMENT WITH FOCUS ON PLACENTAL GROWTH FACTOR AND COLORECTAL CANCER

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University of Groningen, University Medical Center Groningen

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Dedication

This work is dedicated to my family and friends, rich source of inspiration and wise support.

Rationale and Aim

This research project is centered on translating know-how from the laboratory to the clinic in the area of tumor microenvironment, with focus on placental growth factor (PIGF) and colorectal cancer.

Drug development in oncology has put much emphasis on inducing DNA damage in fast-proliferating cancer cells. A recently arising alternative is to target the tissue surrounding the tumor – the microenvironment, in view of its increasingly known role in facilitating disease progression (1,2). Sustained and aberrant angiogenesis is a key component of the tumor microenvironment which drives cancer growth and metastases (3,4). Inhibition of angiogenesis, mainly by targeting vascular endothelial growth factor A (VEGF-A) and its corresponding receptors (VEGFRs) has emerged as a valuable strategy to treat cancer (5,6). However, the duration of response is rather short and tumors eventually evade treatment (7-10). Therefore, novel targets are needed to overcome resistance and improve cancer treatment.

A number of factors are of potential interest in this respect. Firstly, PIGF, a VEGF-A homolog, was isolated in 1991 from placenta and found only in very low levels under physiological conditions, but upregulated in pathological circumstances such as wound healing, ischemia and tumor growth (11-13). Preclinical PIGF inhibition restricts growth and metastasis of various tumors, including those resistant to VEGF(R) inhibitors, and enhances the efficacy of chemotherapy and VEGF(R) inhibitors (14-17). These findings led to the clinical development of RO5323441

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(TB-403), a monoclonal antibody against PlGF. The clinical efficacy and safety of RO532344, its pleiotropic and complementary mechanism to VEGF(R) inhibitors and the negligible induction of an angiogenic rescue program suggest that RO5323441 may constitute a novel approach for cancer treatment (18). Since its application did not coincide with toxicity, a more precise definition of the optimal therapeutic dose is required (19,20). Molecular imaging may be an important tool to fill this gap, as previously highlighted (21-24). By incorporating this approach, we could gain an early quantitative insight in the *in vivo* kinetics and tumor uptake of the PlGF antibody (25). Therefore, labeling the RO5323441 with radioactive isotopes followed by imaging its uptake with single-photon emission computed tomography (SPECT) or positron emission tomography (PET) is an interesting option to potentially guide RO5323441-based cancer therapy. To this end, imaging the soluble pro-angiogenic target PlGF with ⁸⁹Zr-RO5323441 PET was the primary research goal addressed during the first year of this ESMO fellowship.

Secondly, chemokine receptors expressed by tumor cells and their corresponding ligands form an important network exploited by cancer cells to interact with their microenvironment. Stromal cell-derived factor-1 (SDF-1/CXCL12) and its chemokine receptor 4 (CXCR4) play a pivotal role in tumor proliferation, angiogenesis, invasive growth and distant spread (26,27). Noteworthy, there is indication that bevacizumab (mAb against VEGF-A) can increase CXCR4 and CXCL12 expression in rectal cancer (28). Activation of this pathway may induce resistance and impede radical treatment. Hence, during the second year of the fellowship, expression of these tumor microenvironment factors in relation to

treatment response and survival was assessed by immunohistochemistry in a well-documented series of rectal cancer patients.

Experimental design

⁸⁹Zr-RO5323441 PET imaging study in human tumor xenografts

In this study we radiolabeled RO5323441 and validated ⁸⁹Zr-RO5323441 as a tool to study drug tumor uptake and organ distribution by PET imaging in human tumor xenograft models with different PIGF expression. ⁸⁹Zr-RO5323441 was tested *in vitro* for stability and immunoreactivity. Subsequently, tracer tumor uptake and organ distribution were evaluated for 10, 50 and 500 µg ⁸⁹Zr-RO5323441 in mice bearing PIGF-expressing human hepatocellular carcinoma xenografts (Huh7) or human renal cell carcinoma xenografts (ACHN) without detectable PIGF expression. ¹¹¹In-IgG served as a control for non-specific tumor uptake and organ distribution assessment. *Ex-vivo* immunofluorescent staining of tumor slides with anti-CD68 antibody labeled with Alexa 488 and RO5323441 labeled with Cy5 were used to detect tumor-associated macrophages and to study the molecular mechanisms behind specific tumor tracer uptake.

⁸⁹Zr-RO5323441 PET imaging study for recurrent glioblastoma patients treated with bevacizumab

A phase 1/2 study of combined bevacizumab and RO5323441 treatment is currently ongoing for patients with recurrent glioblastoma (NCT01308684). However, the amount of RO5323441 to reach the tumor and how uptake is affected by bevacizumab are yet unknown. Molecular imaging could be an innovative approach to answer these questions. Clinical feasibility of immuno-PET to visualize soluble

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factors was already demonstrated for VEGF-A, with ^{89}Zr -bevacizumab and ^{111}In -bevacizumab uptake in renal cell cancer and melanoma lesions exceeding the expectations based on preclinical results (29,30). Therefore, we initiated a phase 2, ^{89}Zr -RO5323441 PET imaging feasibility study for patients with recurrent glioblastoma treated with bevacizumab (NCT01622764; principle investigator: A.M.E. Walenkamp, MD, PhD).

Serial immunohistochemical measurements of tumor microenvironment factors in stage IV rectal cancer patients before and after radiotherapy, bevacizumab and CapeOx treatment

In this study we examined the relation between proteins involved in the CXCR4 & VEGFR signaling pathways and response to treatment and survival in metastatic rectal cancer patients (principle investigator: Prof. G.A.P. Hospers, MD, PhD). Tumors of 50 patients enrolled in a multicenter phase 2 trial were studied (NTR2029). In short, eligible were adults with a rectal adenocarcinoma and synchronous liver or lung metastases at diagnosis. They received short-course radiotherapy followed by preoperative bevacizumab, oxaliplatin and capecitabine (CapeOx) treatment administered every 3 weeks for up to 6 cycles. Surgery (total mesorectal excision, resection or radiofrequency ablation) was performed 6 to 8 weeks after the last bevacizumab dose. Clinicopathological and follow-up data were prospectively acquired. Diagnostic pre-treatment rectal tumor biopsies and post-treatment surgical specimens of the primary rectal cancer, regional lymph nodes, liver and lung metastases were collected from the 7 participating centers in The Netherlands (09/2010–12/2011). Immunohistochemical analysis of formalin-fixed

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and paraffin-embedded (FFPE) paired tumor samples included the evaluation of CXCR4, CXCL12, PIGF and VEGF-A protein expression.

Results, Conclusions and Future Perspectives

⁸⁹Zr-RO5323441 PET is a novel tool for *in vivo* imaging of placental growth factor and provides a real-time assessment of PIGF antibody RO5323441 tumor penetration and biodistribution. Our preclinical validation study showed that the tracer can be produced with high specific activity, a radiochemical purity >95%, stability in human serum and a fully preserved immunoreactivity. ⁸⁹Zr-RO5323441 showed specific tumor uptake. These data support the feasibility of ⁸⁹Zr-RO5323441 PET imaging in further preclinical and clinical studies. The ⁸⁹Zr-RO5323441 PET imaging study for recurrent glioblastoma patients treated with bevacizumab is planned to start in the last quarter of 2012. In addition, a ⁸⁹Zr-RO5323441 PET guided optimal RO5323441 dose-finding phase 1 protocol was fully developed at the FLIMS 13 workshop. The immunohistochemical analysis of rectal cancer microenvironment is almost completed.

I intend to finalize my ESMO research project with a PhD thesis which I hope to defend early 2013. Therefore, I have received a half-year extension of my appointment at the Department of Medical Oncology of University Medical Center Groningen, The Netherlands.

List of Publications Resulting from this Grant

1. T.H. Oude Munnink, **K. Tamas**, M.N. Lub-de Hooge, R.A.J.O. Dierckx, C. Pallaud, M. Bergstrom and E.G.E. de Vries. PIGF-PET with ⁸⁹Zr-RO5323441: Dose

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escalation study in human tumor xenografts. J Nucl Med 2011;52 (Supplement 1):139.

2. T.H. Oude Munnink[#], **K. Tamas[#]**, M.N. Lub-de Hooge, S.R. Vedelaar, H. Timmer-Bosscha, A.M.E. Walenkamp, K.M. Weidner, F. Herting, J. Tessier, E.G.E. de Vries.

[#]Equal contribution. PIGF-PET study with ⁸⁹Zr-RO5323441 in human tumor xenografts (*submitted*).

3. T.H. van Dijk, **K. Tamas**, J. Beukema, G. L. Beets, H. Gelderblom, K.P. de Jong, I. Nagtegaal, H.J. Rutten, C.J.H. van de Velde, T. Wiggers, G.A.P. Hospers, K. Havenga. Evaluation of short-course radiotherapy followed by neoadjuvant bevacizumab, capecitabine, and oxaliplatin and subsequent radical surgical treatment in primary stage IV rectal cancer (*submitted*).

4. **K. Tamas**, et al. Review on the molecular and clinical features of rectal cancer (*manuscript in preparation*).

5. **K. Tamas**, et al. Serial immunohistochemical measurements of tumor microenvironment factors in stage IV rectal cancer patients before and after radiotherapy, bevacizumab and CapeOX treatment (*work in progress; manuscript in preparation*).

Selection of Courses & Workshops Attended During the Fellowship

Molecular mechanisms in cancer. A Cancer Genomics Centre/Centre for Biomedical Genetics meeting. Royal Tropical Institute, Amsterdam, The Netherlands, 11/2012.

Methods in clinical cancer research. FLIMS 13 workshop. Waldhaus Flims, Switzerland, 06/2011.

Grants week. University of Groningen, The Netherlands, 06/2011.

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Translational imaging workshop. Erasmus Molecular Medicine Postgraduate School, Rotterdam, The Netherlands, 01/2011.

Radiation course. University of Groningen, The Netherlands, 09/2010.

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I thank the European Society for Medical Oncology (ESMO) for granting me this experience. In the eclectic research environment at the Medical Oncology department of University Medical Center Groningen, The Netherlands, I have learnt how to design early clinical trials with sound biological background and input from the laboratory. The comprehensive insight I have gained into preclinical and clinical cancer research stimulated me intellectually and strengthened my determination to become a physician scientist, which is my long-term career goal.

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