Chapter 1

General introduction

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Background

Molecular biology has rapidly evolved and resulted in detailed knowledge of inter- and intracellular signaling pathways. Identification of key pathways that are differentially activated in tumors compared to normal tissue, and drive tumor initiation and progression, facilitated development of targeted treatment for cancer patients. Monoclonal antibodies and small molecules that are engineered to block essential molecules in signaling pathways can inhibit tumor growth. Today, the selection of patients who are most likely to benefit from targeted therapy is a major challenge in medical oncology. A prerequisite for this personalized treatment is availability of reliable predictive biomarkers. Whereas a prognostic biomarker provides information on disease outcome regardless of treatment, a predictive biomarker foretells efficacy of a therapeutic intervention. Two classes of predictive biomarkers can be distinguished: upfront and early on treatment predictive biomarkers. Upfront predictive biomarkers can be used for selection whereas early predictive biomarkers can serve as surrogate endpoints and guide decisions on continuation or cessation of therapy.

Molecular imaging is defined as the visualization, characterization and measurement of biological processes at the molecular and cellular levels in humans and other living systems.[1] Part of standard clinical care is the well known ^{18}F-fluorodeoxyglucose positron emission tomography (^{18}F-FDG PET), an imaging technique for visualization and quantification of glucose metabolism in tumors and normal tissues. Over the last decade, a range of small molecules and monoclonal antibodies have been labeled with radionuclides for targeted imaging with PET or single photon emission computed tomography (SPECT). Molecular imaging can provide non-invasive whole body information on metabolic activity and distribution of drugs and drug targets in a time series in tumors as well as in normal tissues of patients. This technique can serve as a predictive or prognostic biomarker. One of the diseases of particular interest for imaging biomarker development is gastrointestinal stromal tumor (GIST). This was the first solid tumor in which a targeted drug, blocking the constitutively activated c-KIT receptor was demonstrated to have robust activity in the majority of the patients.[2] Furthermore, Von Hippel-Lindau (VHL) disease and hereditary hemorrhagic telangiectasia (HHT) are autosomal dominant inherited disorders where germline mutations result in switching on angiogenesis. These diseases can serve as a model to better understand biology of sporadic cancer in which angiogenesis is of paramount importance and target for treatment, including renal cell carcinoma (RCC) and neuroendocrine tumors (NET).

GISTs are mesenchymal tumors characterized by activating mutations in tyrosine kinase receptors c-KIT or platelet derived growth factor receptor alpha. Tumor growth can be effectively blocked in the majority of the patients with the tyrosine kinase inhibitor imatinib.[2] In patients with GIST, mutation analysis predicts outcome of imatinib treatment

to some extent but primary imatinib resistance cannot reliably be assessed.[3] Imatinib can rapidly inhibit glucose uptake by tumor cells as can be assessed with ^{18}F-fluorodeoxyglucose PET.

VHL disease is an autosomal dominant inherited tumor syndrome caused by an inactivating germline mutation in the VHL tumor suppressor gene. Acquired somatic mutation or silencing of the normal allele results in disease manifestations such as retinal angiomas, hemangioblastomas, RCC and pancreatic neuroendocrine tumors. An important function of VHL protein is recruitment of hypoxia inducible factor 1 alpha (HIF-1α) for degradation. During hypoxia, or in absence of functional VHL protein, accumulation of HIF-1α results in activation of cellular survival strategies including switch on of angiogenesis via production of pro-angiogenic growth factors. Vascular endothelial growth factor A (VEGF-A) is the best studied and probably most important pro-angiogenic factor. VHL patients often have multiple manifestations of their disease and participate in stringent screening and surveillance programs with the intent to early identify progressive lesions. Surgery is reserved for progressive disease manifestations such as expanding hemangioblastomas and growing solid renal masses, trying to avoid harm from the disease manifestations while minimizing iatrogenic damage. VHL patients have shorter life expectancy than the general population and the leading cause of death is metastatic RCC. A prognostic biomarker that can predict the behavior of disease manifestations in the course of VHL disease is currently not available.

The majority of sporadic clear cell RCCs also lack functional VHL protein due to somatic mutations or hypermethylation resulting in activation of angiogenesis. Angiogenesis inhibitors are the mainstay of treatment for patients with metastatic RCC. Bevacizumab is a monoclonal antibody against VEGF-A that prevents receptor activation. Bevacizumab in combination with interferon alpha (INF-α) increased median progression free survival in metastatic clear cell RCC from 5.4 to 10.2 months compared to single agent INF-α.[4] Sunitinib is one of the tyrosine kinase inhibitors that block VEGF receptors at the intracellular level. Sunitinib also increased progression free survival compared to INF-α from 5 to 11 months but has not been directly compared to bevacizumab plus INF-α.[5] Both treatment strategies are available as first line treatment options for patients with metastatic RCC. Mammalian target of rapamycin (mTOR) is a threonine kinase that regulates protein synthesis and controls intracellular HIF-1α levels. One of the mechanisms of action of mTOR inhibitors is reduction of HIF-1α levels and subsequent reduced production of pro-angiogenic factors, thereby indirectly inhibiting angiogenesis. Temsirolimus, an intravenously administered mTOR inhibitor, increases overall survival in poor prognosis RCC patients from 7.3 months to 10.9 months compared to INF-α.[6] Everolimus, an orally administered mTOR inhibitor, has antitumor activity as second line therapy after tyrosine kinase inhibitors in patients with advanced RCC, improving progression free survival from 1.9 to 4.0 months.[7]

Well-differentiated NETs share with RCC abundant vascularization and responsiveness to angiogenesis inhibitors and mTOR inhibitors. In patients with advanced well-differentiated

pancreatic NET, sunitinib as well as everolimus approximately doubled progression free survival compared to placebo.[7,8] The role of VEGF signaling in NET is not completely understood but the mTOR pathway is frequently activated in pancreatic NET because of down regulation of the tumor suppressor genes tuberous sclerosis 2 (TSC-2) and phosphatase and tensin homolog (PTEN).[10] However, not all RCC and NET patients benefit of angiogenesis and mTOR inhibitors which can have burdensome side effects and are all very costly to administer for a prolonged period of time. Validated predictive biomarkers for outcome of treatment with these drugs are not available despite extensive research. We developed the radioactive SPECT tracer ^{111}In-bevacizumab and the PET tracer ^{89}Zr-bevacizumab for VEGF-A imaging.[11-13] Insight in bevacizumab distribution in tumors and normal tissue might provide prognostic information for disease outcome in VHL and predictive information about efficacy of anti-angiogenic therapy in patients with solid tumors.
The aim of this thesis is to investigate the role of molecular PET imaging as biomarker to guide clinical decisions on targeted treatment of solid tumors, with an emphasis on GIST and highly vascular tumor types.

Outline of this thesis

Chapter 2 is an overview of the progress in the development of biomarkers in solid tumors. We elaborated the concept of predictive versus prognostic biomarkers and illustrated this with examples from the whole field of oncology. Reasons for difficult elucidation as well as future perspectives for biomarker development are discussed.

In **chapter 3** we report on a study investigating the predictive value of an early change in tumor ^{18}F-FDG uptake for primary imatinib resistance in GIST patients. Approximately 15% of GIST patients have primary imatinib resistant disease, defined as progressive disease on CT after 8 weeks. Patients with metastatic or locally advanced GIST underwent a ^{18}F-FDG PET scan before and 1 week after start of imatinib. Relationship between ^{18}F-FDG PET response and CT response after 2 months of treatment was analyzed. For evaluation of ^{18}F-FDG PET response the criteria developed by the European Organization for Research and Treatment of Cancer (EORTC) were used as well as cut off values described for GIST, and for CT the response criteria in solid tumors (RECIST version 1.0) and the Choi criteria were used.

In **chapter 4**, the effect of sunitinib treatment and treatment withdrawal was studied in two xenograft models of human tumors. Mice were treated daily with sunitinib for 14 days or for 7 days followed by a stop week. Animal-PET scans were done at baseline, day 7 and day 14. For imaging the PET tracers ^{18}F-FDG, ^{15}O-water and ^{89}Zr-ranibizumab were used and imaging results were related with tumor growth, immunohistochemistry results and plasma VEGF levels. ^{15}O-water PET is an established method for perfusion measurement. Ranibizumab is a monoclonal antibody fragment with high affinity for VEGF-A, but with a shorter half life than bevacizumab. This allows a shorter interval between scans which is an advantage for preclinical studies.

In **chapter 5** we investigated ^{89}Zr-bevacizumab PET imaging in VHL patients. The aim was to assess whether VHL manifestations could be visualized with this technique and to explore whether ^{89}Zr-bevacizumab PET can differentiate progressive lesions from non-progressive lesions. Adult VHL patients with ≥ 1 measurable hemangioblastoma were eligible. PET scans were performed 1 hour, 2 days and 4 days after injection of 37 MBq ^{89}Zr-bevacizumab in the first three patients, in the remaining patients only after 4 days. Maximum standardized uptake values (SUVmax) were calculated. PET scans were fused with routine MRI of the central nervous system and abdominal MRI or CT. Progressive lesions were defined as new lesions, lesions that became symptomatic and lesions ≥ 10 mm that increased ≥ 10% and ≥ 4 mm on repeat anatomic imaging < 12 months.

Hereditary hemorrhagic telangiectasia (HHT) is an autosomal dominant inherited disease characterized by epistaxis, gastrointestinal bleeding, mucocutaneous telangiectasias and arteriovenous malformations (AVM) in the vasculature of the lungs, liver and central nervous system. Single mutations in several genes that are involved in the transforming growth factor beta (TGF-β) pathway can cause HHT. Patients with HHT have elevated VEGF levels but the exact role of VEGF in the development of this vascular disorder is unknown.[14] Several case reports have highlighted the therapeutic effect of bevacizumab on epistaxis, reducing the need for transfusions and iron infusions, but also alleviation of liver failure and reversal of high cardiac output as a result from arteriovenous shunting has been described.[15-17] In **Chapter 5a** we report a patient with HHT with recurrent episodes of pancreatitis caused by pancreatic AVM. We performed ^{111}In-bevacizumab SPECT imaging and subsequently treated this patient with bevacizumab.

In **chapter 6** we report a feasibility study with serial ^{89}Zr-bevacizumab PET imaging in renal cancer patients who were treated with either bevacizumab 10 mg/kg intravenously every 2 weeks plus interferon alpha (IFNα) 3 times per week subcutaneously (n=11), or sunitinib 50 mg orally, daily for 4 out of 6 weeks (n=11). PET scans were performed 4 days after injection of 37 MBq ^{89}Zr-bevacizumab at baseline, day 15 and day 43. SUVs were compared with plasma VEGF-A levels and time to disease progression.

In **Chapter 7** serial ^{89}Zr-bevacizumab PET imaging in NET patients treated with the orally administered mTOR inhibitor everolimus is investigated. The aim of the study was to investigate the effect of everolimus on tumor uptake of ^{89}Zr-bevacizumab. PET scans were performed before start of treatment and at 2 and 12 weeks during treatment. ^{89}Zr-bevacizumab uptake was quantified with SUVmax. Tumor response and % change in the sum of target lesion diameters was determined according to RECIST version 1.1 on CT every 3 months.

Chapter 8 summarizes the results of the studies described in this thesis and in **chapter 9** we discuss the new insights gained, with emphasis on tumor heterogeneity and recommend directions for future research.

References

1. Mankoff DA. A definition of molecular imaging. J Nucl Med 2007;48:18N-21N.
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. 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.
4. Escudier B, Pluzanska A, Koralewski P, et al. Bevacizumab plus interferon alfa-2a for treatment of metastatic renal cell carcinoma: a randomised, double-blind phase III trial. Lancet 2007;370:2103–11.
5. Motzer RJ, Hutson TE, Tomczak P, et al. Sunitinib versus interferon alfa in metastatic renal-cell carcinoma. N Engl J Med 2007;356:115–24.
6. Hudes G, Carducci M, Tomczak P et al. Temsirolimus, interferon alfa, or both for advanced renal-cell carcinoma. N Engl J Med 2007; 356:2271-81.
7. Motzer RJ, Escudier B, Oudard S et al. Efficacy of everolimus in advanced renal cell carcinoma: a double-blind, randomised, placebo-controlled phase III trial. Lancet 2008; 372:449-56.
8. Raymond E, Dahan L, Raoul JL, et al. Sunitinib malate for the treatment of pancreatic neuroendocrine tumors. N Engl J Med 2011;364:501-13.
9. Yao JC, Shah MH, Ito T, et al. Everolimus for advanced pancreatic neuroendocrine tumors. N Engl J Med 2011;364:514-23.
10. Missiaglia E, Dalai I, Barbi S, et al. Pancreatic endocrine tumors: expression profiling evidences a role for AKT-mTOR pathway. J Clin Oncol 2010;28:245-55.
11. Nagengast WB, de Vries EG, Hospers GA, et al. In vivo VEGF imaging with radiolabeled bevacizumab in a human ovarian tumor xenograft. J Nucl Med 2007;48:1313-9.
12. Nagengast WB, Hooge MN, van Straten EM, et al. VEGF-SPECT with ^{111}In-bevacizumab in stage III/IV melanoma patients. Eur J Cancer 2011;47:1595-602.
13. Gaykema SB, Brouwers AH, Lub-de Hooge MN, et al. ^{89}Zr-bevacizumab PET imaging in primary breast cancer. J Nucl Med 2013;54:1014-8.
14. Cirulli A, Liso A, D'Ovidio F, et al. Vascular endothelial growth factor serum levels are elevated in patients with hereditary hemorrhagic telangiectasia. Acta Haematol 2003;110:29-32.
15. Flieger D, Hainke S and Fischbach W. Dramatic improvement in hereditary hemorrhagic telangiectasia after treatment with the vascular endothelial growth factor (VEGF) antagonist bevacizumab. Ann Hematol 2006;85:631-2.
16. Mitchell A, Adams LA, MacQuillan G, Tibbals J, Vanden Driesen R and Delriviere L. Bevacizumab reverses the need for liver transplantation in hereditary hemorrhagic telangiectasia. Liver Transpl 2008;14:210-3.

17. Dupuis-Girod S, Ginon I, Saurin JC, et al. Bevacizumab in patients with hereditary hemorrhagic telangiectasia and severe hepatic vascular malformations and high cardiac output. JAMA 2012;307:948-55.