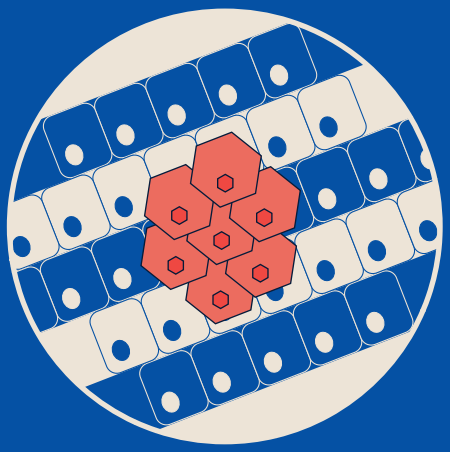
Preclinical targeting of the tumor microenvironment

Arjaans, Marlous



Chapter 1

General introduction

Background

Cancer is the leading cause of death worldwide. The prime cause of cancer related deaths is the development of metastatic disease, which accounts for ~90% of these deaths (1, 2). This underscores the need for new treatment options for patients with metastatic disease. Current treatment options for cancer patients consist of radiotherapy, systemic therapy with chemotherapy, hormonal treatment, targeted agents or immunotherapy and surgery. There is a continuous search for new treatment strategies that may increase the effect of systemic therapy. Currently, the importance of the tumor microenvironment as a contributor to tumor growth and metastatic disease has become more evident (3). Furthermore, the tumor microenvironment is an important regulator of cancer cell related drug sensitivity (4). This provides the rational for targeting not only the cancer cells, but also the tumor microenvironment to improve treatment options for cancer patients. The research described in this thesis focuses on potential targets for therapy present in the tumor microenvironment namely, transforming growth factor (TGF)-β and vascular endothelial growth factor (VEGF).

TGF-β is involved in maintaining the balance of normal tissue homeostasis and suppression of tumor progression in healthy and premalignant tissues. In the tumor microenvironment, TGF-β prevents malignant progression (5). However, in many tumor types the tumor-suppressive responses to TGF-β are lost in the malignant phase. TGF-β then functions to promote epithelial-to-mesenchymal transition, angiogenesis, migration, invasion and immune suppression. TGF-β in that setting contributes to a more invasive and metastatic tumor phenotype (6).

Angiogenesis, the formation of new blood vessels in the tumor, is one of the hallmarks of cancer and is thus far one of the most studied components of the tumor microenvironment (4). VEGF is one of the key players in the process of tumor angiogenesis. The transcription of VEGF is regulated by hypoxia inducible factor 1α (HIF-1α). When hypoxia arises in the tumor, this will lead to an increase in the expression of HIF-1α, followed by an increased production of VEGF. The process of angiogenesis enables tumor growth and can eventually lead to metastatic disease.

Early evaluation of drug effects is of major interest to support drug development. Molecular imaging can be used as one of the tools to evaluate new treatment regimens targeted at the tumor microenvironment. ImmunoPET (7), PET imaging with radiolabeled antibodies, can non-invasively provide information about the presence of specific targets in the tumor and microenvironment of a patient. In addition, this information

can potentially be used for patient selection and as a biomarker for treatment response.

This thesis aims to describe the development of microenvironmental factors as potential markers of tumor response and their use for molecular imaging. In preclinical co-culture models, drug effects on tumor cell - microenvironment interactions are studied for TGF-β. In addition, the development of a new tracer based on an existing antibody is described for TGF-β. For VEGF, the effects of VEGF targeted therapy on the tumor microenvironment and tumor drug uptake is studied in preclinical animal imaging models with already more established tracers.

Outline of the thesis

In **chapter 2** a literature review is performed concerning the mechanisms of TGF-β production and activation as potential targets for TGF-β modulation. Sources used to identify data for this review were PubMed and ClinicalTrials.gov. Articles published in English between 1985 and 2011 were included. Furthermore, an overview of published as well as preliminary results from clinical trials targeting TGF-β is presented by mode of action: modulating TGF-β production, activation or signaling. The opposing role of TGF-β under physiological circumstances makes the window of effective TGF-β targeting evidently small. This poses a clear challenge in selecting the right patients at the right time. Therefore, methods for patient selection and evaluation of the different modulation strategies are also summarized in this review.

The tumor microenvironment as a new potential target, subsequently leads to new treatment strategies focusing on microenvironmental factors. However, to assess treatment efficacy of this new approach most *in vitro* models fall short. An *in vivo* model in which the direct interaction between human tumor cells and human stromal cells can be studied would be of great additional value. Such a model should mimic the human situation, by consisting of both human cancer and human stromal components (8). The use of traditional mouse models would be insufficient, since host stromal infiltration into the human tumors occurs to a high extent in xenografts from cell lines as well as patient material (9,10). The chorioallantoic membrane (CAM) model potentially offers a solution because it allows evaluation of the direct interactions between human tumor cells and human stromal cells in an immune deprived *in vivo* setting. Bisphosphonates, currently used as supportive treatment in breast cancer patients with bone metastases, have a potential anti-cancer effect via microenvironmenal cells. Most research has been done with focus on the biphosphanate zoledronic acid (11). In **chapter 3** we aimed to assess the mechanism behind the anti-cancer effect of modulating the microenvironment with

zoledronic acid. In an *in vitro* co-culture model, we assessed the effect of zoledronic acid on tumor cell survival and excretion of TGF-β in cell lines of different breast cancer subtypes cultured alone or in combination with human stromal cells. The *in vitro* validated cell lines were inoculated in the *in vivo* CAM model and the effect of zoledronic acid treatment on tumor growth with and without stromal cells was evaluated.

Fresolimumab is a fully human IgG4 κ-monoclonal antibody capable of neutralizing all mammalian isoforms of active TGF-β. A phase I study with fresolimumab in 22 patients with advanced melanoma and renal cell carcinoma showed stable disease in one patient, a partial tumor response in one patient, a mixed tumor response in three patients, and no dose-limiting toxicity (12). For further clinical development of fresolimumab and in order to identify the patients most likely to benefit, it will be helpful to know whether TGF-β is being overexpressed and activated in the tumor and whether fresolimumab reaches the target. In **chapter 4** we describe the development and preclinical validation of labeling fresolimumab with the long-lived positron emitter Zirconium-89 (^{89}Zr) for non-invasive PET imaging of TGF-β. ^{89}Zr-fresolimumab tumor uptake and organ distribution was assessed using three protein doses and compared with ^{111}In-IgG. These experiments were performed in different human tumor bearing mouse models; two models transfected to produce different amounts of human TGF-β and a model of human triple negative metastatic breast cancer.

In solid tumors, angiogenesis leads to a defective vasculature and impaired lymphatic drainage. This is associated with increased vascular permeability and enhanced tumor permeability. Several preclinical and clinical studies indicate that antiangiogenic drugs, including the anti-VEGF-A antibody bevacizumab, lead to vessel normalization in addition to their antivascular effect. Characteristics of vessel normalization include reduced number and size of immature vessels, increased vessel pericyte coverage and reduced interstitial fluid pressure (13). In the process of vessel normalization, the architecture of the remaining vasculature is largely restored, leading to reduced vessel permeability and thereby improving tumor blood flow and tumor oxygenation (14). The effects of vessel normalization can have a marked influence on intratumoral delivery of macromolecular drugs, such as antibodies (15). These effects might have implications for therapies, when bevacizumab is combined with other monoclonal antibodies. In **chapter 5A** we aimed to investigate the effect of blood vessel normalization in tumors by the antiangiogenic drug bevacizumab on antibody uptake in these tumors. In human tumor bearing mice models of ovarian and esophageal cancer we evaluated antibody uptake in tumors and organ distribution by PET imaging of ^{89}Zr-trastuzumab, ^{89}Zr-bevacizumab

and ^{89}Zr-IgG before and after treatment with bevacizumab. In addition we tracked the localization of IgG in the tumors with *ex vivo* fluorescent labeling and performed immunohistochemistry to assess the effects of bevacizumab treatment on tumor vessel density and vessel normalization. **Chapter 5B** further contemplates in a reply letter on the dose dependency of vessel normalization after antiangiogenic therapy. To date, small molecule tyrosine kinase inhibitors (TKI) targeting the VEGF receptors (VEGFR), an antibody directly targeting VEGF and an antibody targeting VEGFR2 are VEGF pathway targeting drugs that are registered. In **chapter 6** results of clinical trials combining these agents with chemotherapeutics or other targeted therapies is discussed. Furthermore, a comprehensive overview of preclinical and clinical studies illustrating the effect of vessel normalization after VEGF targeted therapy on tumor drug uptake is given.

Finally, all findings presented in this thesis are summarized in **chapter 7**. This summary is followed by a discussion on the interpretation of these findings and prospects for future studies. In **chapter 8** a summary of the thesis in Dutch is given.

References

1. Ferlay J, Soerjomataram I, Ervik M, Dikshit R, Eser S, Mathers C *et al*. GLOBOCAN 2012 v1.0, cancer incidence and mortality worldwide: IARC CancerBase No. 11. Lyon, France: International Agency for Research on Cancer;2013. Available from: http://globocan.iarc.fr, accessed on 04/11/2014.
2. Sleeman J, Steeg PS. Cancer metastasis as a therapeutic target. Eur J Cancer 2010;46:1177-1180.
3. Hanahan D, Coussens LM. Accessories to the crime: functions of cells recruited to the tumor microenvironment. Cancer Cell. 2012; 21:309-322.
4. Östman A. The tumor microenvironment controls drug sensitivity. Nat. Med. 2012; 18:1332-1334.
5. Massagué J, Blain SW, Lo RR. TGFβ signaling in growth control, cancer, and heritable disorders. Cell 2000;103:295-309.
6. Massagué J. TGFβ in cancer. Cell 2008;134:215-230.
7. van Dongen GA, Visser GW, Lub-de Hooge MN, de Vries EG, Perk LR. Immuno-PET: a navigator in monoclonal antibody development and applications. Oncologist 2007;12:1379-1389.
8. Wang J, Xia TS, Liu XA, Ding Q, Du Q, Yin H, et al. A novel orthotopic and metastatic mouse model of breast cancer in human mammary microenvironment. Breast Cancer Res Treat. 2010; 120:337-344.
9. Sausville EA, Burger AM. Contributions of human tumor xenografts to anticancer drug development. Cancer Res. 2006; 66:3351-3354.
10. Lin MT, Tseng LH, Kamiyama H, Kamiyama M, Lim P, Hidalgo M, et al. Quantifying the relative amount of mouse and human DNA in cancer xenografts using species-specific variation in gene length. Biotechniques. 2010; 48:211-218.
11. Gnant M. Adjuvant bisphosphonates: a new standard of care? Curr Opin Oncol 2012;24:635-642.
12. Morris JC, Tan AR, Olencki TE, Shapiro GI, Dezube BJ, Reiss M *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.
13. Carmeliet P, Jain RK. Principles and mechanisms of vessel normalization for cancer and other angiogenic diseases. Nat Rev Drug Discov 2011;10:417-427.
14. Yuan F, Chen Y, Dellian M, Safabakhsh N, Ferrara N, Jain RK. Time-dependent vascular regression and permeability changes in established human tumor xenografts induced by an anti-vascular endothelial growth factor/vascular permeability factor antibody. Proc Natl Acad Sci USA 1996;93:14765-14770.
15. Nakahara T, Norberg SM, Shalinsky DR, Hu-Lowe DD, McDonald DM. Effect of inhibition of vascular endothelial growth factor signaling on distribution of extravasated antibodies in tumors. Cancer Res. 2006;66:1434-1445.