Angiogenesis is a critical step in tumor progression and is redundantly present in highly vascularized tumor types including renal cell cancer (RCC). Multiple kinases are involved in angiogenesis, including receptor tyrosine kinases such as the vascular endothelial growth factor receptor (VEGFR) and platelet-derived growth factor receptor (PDGFR). Inhibition of angiogenic tyrosine kinases has been developed as a systemic treatment strategy for cancer. Several antiangiogenic tyrosine kinase inhibitors (TKIs), including sunitinib, sorafenib, pazopanib and axitinib, have been approved for treatment of patients with advanced cancer (RCC, gastro-intestinal stromal tumors, hepatocellular cancer, pancreatic neuroendocrine tumors). Despite the clinical benefits achieved with antiangiogenic TKIs, they also cause significant toxicities and inevitably induce resistance. Pre-existing non-responsiveness (intrinsic resistance) as well as the development of acquired drug resistance is a major clinical problem, but the underlying mechanisms to fully explain and overcome resistance remain unclear. Most studies focused on potential angiogenic-factor-mediated mechanisms of resistance and the microenvironment of the host, while relatively little attention has been paid to the contribution of tumor-cell-related mechanisms of resistance to antiangiogenic TKIs. In this thesis, we have studied the potential mechanisms of resistance to antiangiogenic TKIs, especially sunitinib, by evaluating direct activity of TKIs on tumor cells, in order to improve their clinical use.

In chapter 2, an overview of the design and development of antiangiogenic TKIs is given. We described their molecular structure and classification, their mechanism of action and their inhibitory activity against specific kinase signaling pathways. In addition, we provided insight to what extent selective targeting of angiogenic kinases by TKIs may contribute to the clinically observed anti-tumor activity, resistance and toxicity.

Sunitinib has been developed as an antiangiogenic agent, targeting endothelial and perivascular cells through its high affinity binding to VEGFR2 and PDGFR, but in addition it inhibits many other kinases. We investigated whether an alternative mechanism of action may play a role in the antitumor activity of sunitinib rather than solely its antiangiogenic activity. In chapter 3, we explored the effect of sunitinib on tumor cells in vitro and found that sunitinib directly inhibited tumor cells at clinically relevant intratumoral drug concentrations. In addition, continuous exposure to sunitinib resulted in resistance of 786-O renal and HT-29 colorectal cancer cells. In these sunitinib resistant cells, intracellular drug concentrations were significantly higher compared to parental cells. Based on the chemical properties of sunitinib –
Summary

sunitinib is a hydrophobic weak base (logP = 5.2; pKa = 8.95) – we hypothesized that sunitinib preferably accumulates in specific organelles. Subcellular distribution of sunitinib was analyzed with fluorescence microscopy and showed that sunitinib is co-localized with acidic lysosomes. Lysosomal sequestration of sunitinib was higher in resistant cells compared to parental cells, without affecting intracellular signaling, providing a new resistance mechanism for this TKI.

To what extent acquired resistance to sunitinib is determined by microenvironmental host-factors or by tumor cells directly is unknown. In chapter 4, we studied whether the in vitro induced resistance of tumor cells determines in vivo resistance to sunitinib. In severe combined immunodeficient mice, tumors were established from HT-29 parental colon cancer cells (HT-29PAR) or the in vitro induced sunitinib resistant HT-29 cells (HT-29SUN), as described in chapter 3. Treatment with sunitinib inhibited tumor growth of HT-29PAR tumors while no inhibition of HT-29SUN tumor growth was observed. In parallel, tumor cell proliferation was reduced in HT-29PAR tumors but unaffected in HT-29SUN tumors, upon sunitinib treatment. In addition, the lysosomal capacity reflected by LAMP-1 and -2 expression was higher in HT-29SUN compared to HT-29PAR tumors. Reduction in microvessel density was similar in sensitive and resistant tumors. In this model acquired resistance to sunitinib depends on tumor cells rather than on host- and angiogenesis-mediated factors.

When during cancer treatment resistance to a TKI occurs, switching to another TKI is often considered as a reasonable treatment option. Currently, detailed information is lacking on patterns of resistance or cross-resistance of tumor cells after long-term exposure to multi-targeted TKIs. In chapter 5, we studied the effect of several multi-targeted TKIs and the mTOR inhibitor everolimus on the development of (cross-) resistance. We found that the sunitinib-resistant (SUN) 786-O renal and HT-29 colorectal cancer cells, as described in chapter 3, were cross-resistant to pazopanib, erlotinib and lapatinib, but not to sorafenib. Upon continuous exposure of tumor cells, resistance could be induced to some - but not all - TKIs, comparable to the cross-resistance findings. Such (cross-)resistance includes increased intracellular drug accumulation accompanied by increased lysosomal storage. No cross-resistance to the mTOR inhibitor everolimus was detected.

The research described in chapters 3-5 may give new treatment options. Sunitinib resistance in vitro and in vivo, as well as in vitro resistance to some other TKIs, was accompanied by an increased lysosomal storage capacity. Lysosomal drug sequestration can be modulated by interference with lysosomal function, for example by bafilomycin in vitro (chapter 3) or chloroquine in vivo (chapter 4). It
warrants clinical evaluation whether targeting lysosomal function will overcome resistance to sunitinib and other TKIs.

No cross-resistance of sunitinib resistant tumor cells to the TKI sorafenib or the mTOR inhibitor everolimus was detected (chapter 5), indicating that, upon sunitinib resistance in patients, switching to one of these drugs may be a (new) therapeutic opportunity.

In addition, we found that sunitinib resistance (chapter 3), as well as pazopanib resistance (chapter 5), was reversible upon removal of the drug within several weeks. Therefore, this transient form of resistance may be an adaptation to (partial) inhibition of multiple kinases and/or to a partly disturbed lysosomal function, rather than a stable, genetic form of resistance. This indicates that rechallenging the patient with sunitinib/ pazopanib after treatment switching or interruption is a promising strategy.

Antiangiogenic TKIs are only effective in a subgroup of cancer patients. Patients with metastasized RCC have clinical benefit upon treatment with sunitinib, but a subgroup will have no benefit of this treatment at all. One of the major efforts of current clinical practice is to adequately define upfront which patient will benefit from targeted treatments, so-called ‘personalized’ or ‘precision’ medicine. However, currently there is no reliable test to predict TKI response in patients. In chapter 6 we evaluated a protein tyrosine kinase (PTK) microarray to determine its potential for clinical use. Multiple technical conditions were evaluated, including protein and ATP concentrations, background reduction, lysis efficiency, substrate specificity, reproducibility, samples of different biological origin, inhibition profiles, and other factors. Specific activity of recombinant kinases could be adequately measured on the PTK microarray, but for its use with more complex biological samples containing multiple kinases, such as tumor lysates, the array peptides need further optimization for specificity.

The research described in this thesis supports a direct role of tumor cells in resistance to sunitinib and other antiangiogenic TKIs. These findings may give new treatment opportunities, such as combining sunitinib with compounds that interfere with lysosomal drug sequestration, switching to another TKI to which no cross-resistance was observed, and/or rechallenging the tumor with the same drug after a period of treatment interruption. These (new) treatment strategies might prevent or overcome resistance to antiangiogenic TKIs. Ultimately, this will result in more effective treatment opportunities for patients with cancer.