Optimizing immunotherapy in locoregional and metastatic urothelial cancer

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Summary
Samenvatting

SUMMARY

Immunotherapy has shown promising response rates and survival benefit in urothelial cancer. In this thesis, various studies on immunotherapy in the metastatic and preoperative setting are described, as well as biomarkers studies that may inform response and clinical outcome.

Part I of this thesis is focused on the clinical outcome of patients that received immunotherapy in the metastatic setting. In **Chapter 2**, we retrospectively studied whether chemotherapy is still effective after progression to immune checkpoint inhibitors in metastatic UC. We found that patients that receive chemotherapy after progressing to frontline immune checkpoint inhibitors (ICIs) maintain a high response rate to chemotherapy, which suggests a lack of cross resistance between ICI and chemotherapy. Similar findings were observed for the cohort that received chemotherapy after previous exposure to both chemotherapy and ICIs. Together these results suggest that chemotherapy responses are maintained irrespective of previous exposure to ICIs in metastatic UC. **Chapter 3** describes the survival outcome and efficacy of subsequent systemic treatment (SST) after discontinuation of anti–PD-1/PD-L1 treatment in first-line and second-line UC using patient data from various patient (study)cohorts. Findings from our study indicate that many metastatic UC patients that progress to ICIs do not get subsequent systemic treatment, including 43% of patients that were treated with frontline ICIs. Patients treated with first-line ICIs are at risk of early death, depriving them of the potential clinical benefits of chemotherapy. Clinical outcomes of platinum-refractory patients were consistent with historical data. Our results on first-line ICIs are concerning and justify limiting first-line immunotherapy to patients at low risk of clinical deterioration in the first few months of treatment.

In **part II** of this thesis we proceed from the metastatic setting to the preoperative setting, involving patients without distant metastasis. In **chapter 4**, we reported the results of a phase Ib single-arm trial (NABUCCO). In this trial, we studied whether the addition of anti-CTLA-4 to PD-1 blockade is feasible as preoperative treatment strategy in patients having locoregionally advanced (stage III) UC. Furthermore, we performed exploratory analysis to study associations with baseline $CD8^+$ T cell immunity and B cell presence with response, whereas correlations between response and TLS dynamics were also explored. The primary endpoint was the feasibility of resection within 12 weeks of starting treatment. All patients could undergo resection, 23 (96%) within 12 weeks. A total of 11 patients (46%) showed a pathological complete response (pCR), whereas 14 patients (58%) demonstrated no remaining invasive disease (pCR or pTisN0/pTaN0) in the resection specimen. In contrast to studies testing single-agent anti-PD1/PD-L1,

complete response to ipilimumab plus nivolumab was irrespective of baseline $CD8^+$ T-cell abundance or T-cell effector signatures. The presence of tertiary lymphoid structures increased upon treatment in patients responding to ipilimumab plus nivolumab. This study indicates that CTLA-4 plus PD-1 combination treatment may provide an effective preoperative treatment approach in locoregionally advanced UC, independent of pre-treatment $CD8^+$ T cell activity. In **Chapter 5**, we retrospectively compared the efficacy of anti-CTLA-4 plus anti-PD-1 (NABUCCO) to neoadjuvant/induction platinum-based combination chemotherapy in cohorts of locoregionally-advanced UC patients. Results from our study demonstrated a superior survival in stage III UC patients that were pre-treated with anti-CTLA-4 plus anti-PD-1 when compared to the cohort of stage-matched patients treated with preoperative platinum-based combination chemotherapy. These results provide a strong rationale for testing anti-CTLA-4 plus anti-PD-1 for locally advanced UC in a phase-3 randomized-controlled trial.

In **part III** of this thesis we proceed to biomarkers that may inform clinical outcome in urothelial cancer. In **Chapter 6**, we describe the tumor immune landscape in resectable UC slides by using (immunofluorescence)staining and subsequent computational image analysis to better understand the complex interplay between immune cells and tertiary lymphoid structures (TLS), given the importance for biomarker discovery. We focused on the preoperative setting, as this limited effects from bias. Tumors that lacked response to anti-PD-1 plus anti-CTLA-4 immunotherapy showed an increase of a FoxP3+ T-cell-low TLS cluster following therapy. In addition, cluster 5 (macrophage low) TLS were more abundant after pre-operative immunotherapy when compared to untreated UC. Our study showed that superficial TLS demonstrated higher presence of T-helper cells and enrichment of early TLS when compared to TLS observed in deeper tissue. Additionally, superficial TLS showed a lower fraction of secondary-follicle like TLS when compared to TLS in deeper tissue. Results from our study provide a detailed overview of the tumor immune contexture in UC, which could serve as a basis for further research. In **Chapter 7**, we proposed a novel framework for UC (UC immunogram), based on translational and clinical data. The UC immunogram describes various tumor and host-specific parameters that are necessary for immunotherapy to be effective. These seven parameters include tumor foreignness, immune cell infiltration, absence of inhibitory checkpoints, general performance and immune status, absence of soluble inhibitors, absence of inhibitory tumor metabolism, and tumor sensitivity to immune effectors. The UC immunogram can be used to better understand the complexity of cancer immune responses in UC and to help prioritize biomarkers for prospective testing in clinical trials, ultimately leading to multifactorial models to predict immunotherapy responses in UC.

Altogether, this thesis points towards an exciting future for (personalized) immunotherapy in urothelial cancer, strengthened by our findings in the preoperative setting.