Clinical aspects of immunotherapy and targeted therapy of advanced melanoma

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Appendices

ENGLISH SUMMARY

This thesis focused on different aspects of melanoma treatment with immunotherapy and targeted therapy. **Chapter 1** provides an introduction into (metastatic) melanoma. Furthermore, the rationale and outline of this thesis were described. The first part of this thesis focused on biomarker discovery. Hereby making it possible to select patients upfront that should, or should not, be treated with immunotherapy. In **chapter 2** we search for biomarkers in a large retrospective multicenter study using routine blood parameters combined with flow cytometry. In a discovery cohort consisting of 105 patients from five different sites, biomarkers that were significantly correlated with overall survival were identified. These biomarkers were then validated in another cohort of 104 patients from three different sites. Five different parameters were significantly correlated with overall survival in both cohorts. Using these five parameters (lactate dehydrogenase; LDH, absolute monocyte counts; AMC, myeloid-derived suppressor cell frequencies; MDSCs, absolute eosinophil counts; AEC and relative lymphocyte counts; RLC) a model was created. However, the best discriminatory ability of this model was achieved when regulatory T-cells were also considered, despite this factor having no significant independent impact on survival according to Cox regression analysis. A nomogram-based linear predictor measure was calculated for each patient considering the relative impact of single factors according to Cox regression analyses. Patients could be divided into three groups; a risk score of 0 (low), a risk score ≤ 130 (intermediate) and a risk score > 130 (high). Using this biomarker model the 2-year survival rate for patients (n = 60) with all favorable parameters (risk score = 0) was 40.8%. On the other hand, no patients (n = 38) were alive after 15 months with a risk score of > 130. We also found a statistical significant correlation between this model and best overall response rate (the percentage of patients with a complete or partial response). Patients with all favorable parameters (risk score = 0) had a best overall response rate of 31% compared to only 3% for patients with a risk score of > 130. Another (easier) model was developed in which only the routine blood parameters were used (LDH, AMC, AEC and RLC). In this model the number of favorable parameters would be counted. Using this model the 2-year survival probability for patients (n = 141) with all favorable parameters was 43.1%, compared to 2.5% for patients (n = 109) with only 0 – 2 favorable parameters. Similarly to the first model there was also a statistical significant correlation with best overall response rate. Patients with all favorable parameters had a best overall response rate of 31% compared to 8% in the group of patients with 0-2 favorable parameters.

As already briefly discussed in chapter 1 all patients treated with immunotherapy are at risk for serious adverse events. In **chapter 3** we described a cohort of 92 patients treated with immunotherapy for either metastatic melanoma or non-small cell lung cancer. All

of these patients developed diarrhea as an adverse event for which they were treated with corticosteroids and/or underwent an endoscopy. Of all patients endoscopy images, together with pathology slides, were re-assessed. Management of immune-related diarrhea is based upon treatment algorithms that have been developed for immunotherapeutics. Immune-related diarrhea is scored according to the common terminology criteria for adverse events (CTCAE). An increase in stools per day over baseline indicates the grade of immune-related diarrhea. The treatment algorithms are based upon the grade of diarrhea according to CTCAE. According to these algorithms, the higher the grade the more aggressive therapy is indicated (e.g. symptomatic treatment for grade 1, addition of prednisone for grade 2 and the possible addition of infliximab for grade ≥ 3). We discovered that there was absolutely no statistical significant correlation between the grade of diarrhea at presentation and the severity of colitis as seen during endoscopy and quantified according to the endoscopic Mayo score. A score commonly used to assess severity of inflammatory bowel disease; ρ 0.12; $p = 0.28$. Another interesting discovery was the fact that patients in which ulcers were seen during endoscopy needed infliximab significantly more frequently than patients that did not have ulcers ($p = 0.002$).

In **chapters 4 and 5** we studied a subpopulation of patients with metastatic melanoma, namely those with brain metastases and/or leptomeningeal metastases. In **chapter 4** we retrospectively described a cohort of 146 patients with brain metastases from melanoma treated with the BRAF-inhibitors vemurafenib or dabrafenib, or with the combination of a BRAF-inhibitor with a MEK-inhibitor. In this cohort 85 patients were treated with vemurafenib, 31 with dabrafenib and 30 with the combination of dabrafenib + trametinib. We showed that median overall survival is 5.7 months for patients treated with vemurafenib, 8.8 months for patients treated with dabrafenib and 11.2 months for patients treated with the combination of dabrafenib + trametinib. The difference in median overall survival between vemurafenib and the combination of dabrafenib + trametinib was statistically significant (hazard ratio for death, 0.52; 95%, 0.30 – 0.89; $p = 0.02$). A possible explanation for this better overall survival may lie in the fact that dabrafenib has shown to penetrate the blood brain barrier to a higher extent than vemurafenib. Furthermore, the addition of the MEK inhibitor has been shown to delay BRAF-inhibitor resistance often caused by the recovery of phospho-ERK signaling. Another key aspect of **chapter 4** was to analyze the potential improvement in neurological symptoms (such as nausea, vomiting and headache) upon treatment. We showed that in 46% of symptomatic patients an improvement of neurological symptoms was seen and in 21% neurological symptoms remained stable. This is of great palliative significance. In **chapter 5** we looked into a cohort of 39 patients with leptomeningeal metastases from melanoma treated with immunotherapy or targeted therapy. Historically median overall survival has been dismal for this patient population with a median survival of only two months despite treatment with chemotherapy and/or

radiotherapy. Median overall survival for our entire population was 6.9 weeks (95% CI 0.9 – 12.8). In our cohort we showed that there is a statistically significant difference in median overall survival between treated and untreated patients (16.9 weeks versus 2.9 weeks). Especially patients treated with ipilimumab in combination with radiotherapy seemed to be doing better with a median overall survival of 47 weeks. As previously described in **chapter 2** serum LDH was also a predictive biomarker for overall survival in this cohort. Patients with a LDH higher than the upper limit of normal had a median overall survival of only 3.1 weeks, compared to 18.9 weeks for patients with a normal LDH.

Vemurafenib was the first approved BRAF-inhibitor in the treatment of patients with metastatic melanoma. Unfortunately a large percentage of patients will eventually develop progression of disease on this therapy. In **chapter 6** we described a cohort of 70 patients with metastatic melanoma treated with vemurafenib. In patients treated with chemotherapy treatment is usually stopped at progression of disease. However, in the clinic we saw that after stopping vemurafenib progression of disease would oftentimes be accelerated, quickly followed by death of the patient. We therefore retrospectively analyzed a cohort of 35 patients that stopped vemurafenib at disease progression and another cohort of 35 patients that continued vemurafenib treatment despite progression of disease. Median overall survival in the group of patients that continued vemurafenib despite progression of disease was 5.2 months (95% CI 3.8 – 7.4) versus 1.4 months (95% CI 0.6 – 3.4) for patients that stopped vemurafenib at disease progression ($p = 0.002$).

Another potent therapy against cancer is the adoptive transfer of cells, particularly of lymphocytes. In **chapter 7** we reviewed the past, present and future of patients with different kinds of cancer treated with tumor-infiltrating lymphocytes. Finally in **chapter 8** the results presented in this thesis were discussed and future perspectives are outlined.