Clinical aspects of immunotherapy and targeted therapy of advanced melanoma

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Chapter 1

General introduction

Melanoma is an aggressive form of skin cancer developing from melanocytes, which can affect men and women of all ages. Melanoma typically occurs in the skin, but it may also occur on mucosal surfaces such as intestines, vulva, nasopharynx, sinuses and mouth. Rarely melanoma is found in the eye [1]. Of all types of skin cancer, melanoma causes the most skin cancer related deaths.

INCIDENCE AND SURVIVAL

Melanoma was diagnosed in nearly 6000 patients in the Netherlands in 2015 and as can be seen in Figure 1. the incidence of melanoma in the Netherlands is steadily increasing. In 2015 more than 800 patients died due to melanoma in the Netherlands. Survival from melanoma is mainly dependent on the stage of the disease at diagnosis (Figure 2). Stage of melanoma is based on the staging system as defined by the American Joint Committee on Cancer (AJCC) [2]. This staging system focusses on tumor thickness, mitotic rate, ulcerations, the presence of nodal metastases and distant metastases. Patients without distant metastases are classified as stage I-III, while patients with distant metastases are classified as stage IV. The focus of this thesis lies on stage IV melanoma.

IMMUNOTHERAPY FOR THE TREATMENT OF METASTATIC MELANOMA

In 2013 the editors of *Science* chose cancer immunotherapy as the breakthrough of the year, hereby showing the importance of the immune system to combat tumors. Already in 1863 Rudolf Virchow described the presence of lymphoid cells in cancerous tissue and hypothesized a connection between inflammation and cancer [3]. For decades it is now known that these lymphocytic infiltrates play a crucial role in patients' clinical outcome in not only melanoma, but in the majority of cancers [4-8]. Pioneering work in this field of research has been performed by Dr. Steven Rosenberg from the Surgery Branch (SB) of the National Institutes of Health (NIH), Bethesda, Maryland. Work from Rosenberg et al. showed that harvesting tumor-infiltrating lymphocytes (TIL), expanding them *ex-vivo* and reinfusing them into patients with metastatic cancers could induce clinical responses [9]. A process called adoptive cell transfer, or ACT. However, these positive effects are mainly limited to metastatic melanoma. The discovery of T-cell checkpoint molecules such as Cytotoxic T-Lymphocyte-Associated protein 4 (CTLA-4) and Programmed Death receptor 1 (PD-1) paved the way for a new form of immunotherapy [10, 11]. Several years after this discovery antibodies directed against these molecules were manufactured. Prior to 2010 the chemotherapeutic dacarbazine, and in some countries high-dose IL-2, were the only

registered treatments against metastatic melanoma. Median overall survival of patients treated with dacarbazine was only 6-9 months [12, 13]. In 2010 the fully human monoclonal antibody ipilimumab, targeting CTLA-4 on the activated T-cell showed, for the first time, a survival benefit in patients with metastatic melanoma [14, 15].

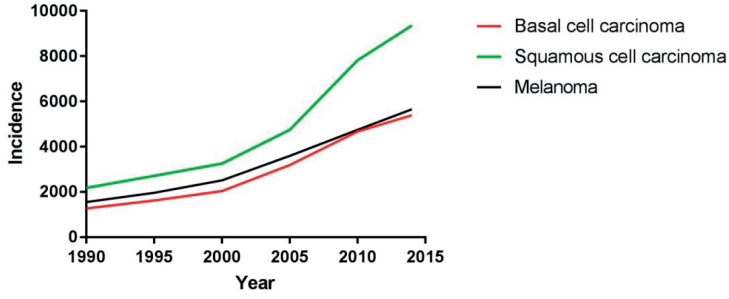

Figure 1. Incidence of skin cancer over the last 25 years (*Netherlands Cancer Registration*)

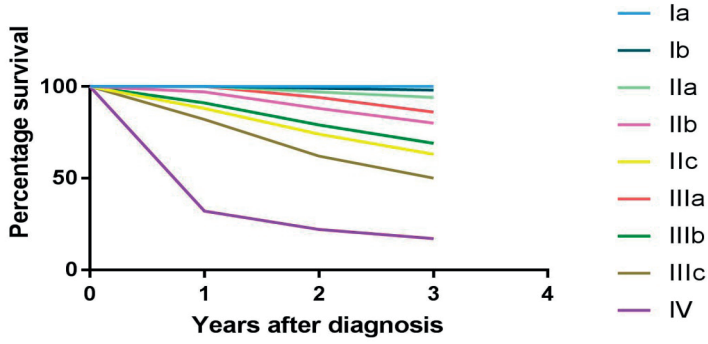

Figure 2. Mortality after diagnosis according to the AJCC staging system (*Netherlands Cancer Registration*)

Stage Ia: T1a, No, Mo; stage Ib: T1b/T2a, No, Mo; stage IIa: T2b/T3a, No, Mo; stage IIb: T3b/T4a, No, Mo; stage IIc: T4b, No, Mo; stage IIIa: T1-4a, N1a/N2a, Mo; stage IIIb: T1-4a/T1-4b, N1a/N2a/N1b/N2b/N2c, Mo; stage IIIc: T1-4b, N1b, N2b, N2c, N3, Mo; stage IV: all T, all N, M1.

This led to regulatory approval of ipilimumab for the treatment of metastatic melanoma. Roughly four years later pembrolizumab and nivolumab, both antibodies targeting PD-1, either as monotherapy or in combination with anti-CTLA-4 antibodies, showed even more impressive clinical results [16-18]. Median overall survival for patients with metastatic melanoma has since increased from 6-9 months with dacarbazine, to 10-20 months with ipilimumab to more than two years with anti-PD-1 antibodies as monotherapy or the com-

bination with an anti-CTLA-4 antibody. Despite these promising results, treating patients with these new antibodies has serious financial implications. The cost of treating a patient with four cycles of ipilimumab equals to about €90,000 (250 mg flat dose, for four cycles), while costs can run as high as €150,000 (240 mg flat dose, once every 2 weeks, for up to two years) for nivolumab and €260,000 (200 mg flat dose, once every 3 weeks for up to two years) for pembrolizumab [19]. Besides the financial aspects, some patients treated with these antibodies are at risk of serious, sometimes life-threatening adverse events (AEs), which are often immune-related (irAEs). For example, treatment related AEs of any grade in patients treated with ipilimumab can be seen in 89% of patients [14-16]. Although the majority of AEs was only grade I or II (the lower grades of AEs), 23% of patients had grade III or IV AEs (the higher grades of AEs). For patients treated with anti-PD-1 antibodies grade III/IV treatment related AEs are seen in up to 20% of patients, while patients treated with the combination of anti-PD1-antibodies and anti-CTLA4-antibodies grade III/IV treatment related AEs are seen in up to 59% [17, 18, 20-25]. Being able to select patients who will benefit the most from a certain treatment upfront remains one of the goals in cancer immunotherapy. Not only to reduce health-care costs, but mainly to steer patients into the right treatment, and thereby not treating patients with a certain immunotherapeutic agent that they are likely not to respond to. Until this date, several biomarkers have been discovered, but no biomarker (or combinations of biomarkers) has been incorporated into daily routine clinical practice. An example is serum lactate dehydrogenase (LDH). Three years ago, Kelderman et al. retrospectively showed that patients with a high LDH are less likely to respond to anti-CTLA-4 treatment. However, even at a serum LDH value of > 2 times the upper limit of normal a minority of patients still responded to this treatment [26]. Recently Blank et al. hypothesized a framework (the "cancer immunogram", Figure 3) consisting of seven parameters which could be crucial in anti-tumor response [27]. These seven parameters consist of: tumor foreignness, immune cell infiltration, absence of checkpoints, absence of soluble inhibitors, absence of inhibitory tumor metabolism, tumor sensitivity to immune effectors and general immune status. These parameters by themselves are all associated with response, or lack thereof, to immunotherapy. But what the cancer immunogram tries to show the treating physician is that it probably will not be just one single biomarker, but a combination of biomarkers which will make it possible to select patients upfront.

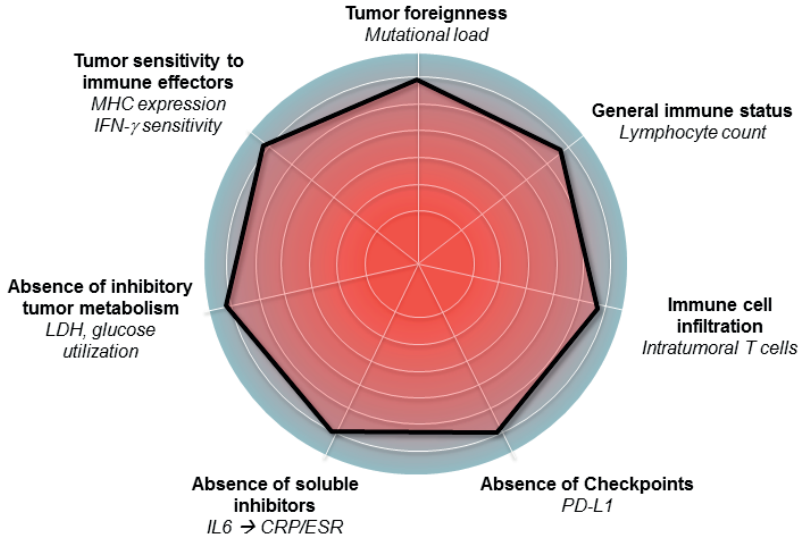

Figure 3. The "cancer immunogram"

BRAF INHIBITORS FOR THE TREATMENT OF METASTATIC MELANOMA

Approximately 40-60% of cutaneous melanoma harbor a mutation in the gene encoding *BRAF* [28, 29]. This mutation leads to constitutive activation of downstream signaling through the Mitogen-Activated Protein Kinase (MAPK) pathway. In approximately 80% of cases this mutation results in the substitution of valine by glutamic acid at codon 600 (V600E) [28, 29]. Other gene mutations, such as V600K and V600R are also known, but these mutations occur less frequently. Vemurafenib and dabrafenib are potent inhibitors of the mutated BRAF protein. Both have shown impressive objective response rates and improve progression free survival and overall survival when randomly compared to the chemotherapeutic dacarbazine in randomized phase III trials [30, 31]. Double targeting the MAPK pathway by combining BRAF inhibitors with MEK 1/2 inhibitors has clearly shown an improvement in not only efficacy, but also tolerability compared to BRAF inhibitor monotherapy [32-35].

CONCLUSION AND OUTLINE OF THE THESIS

Throughout melanoma history, significant progress has been made in treating patients with metastatic melanoma. This thesis will focus on different aspects of melanoma treatment with immunotherapy and targeted therapy.

In **chapter 2** we search for the perfect biomarker (or combination of biomarkers) to predict response to ipilimumab treatment. Here we look into different routine blood parameters, but also certain immune cell populations analyzed by flow cytometry. Identified parameters were first assessed in a discovery cohort and later validated in a validation cohort.

As previously mentioned a selection of patients treated with immunotherapeutics is at risk of developing adverse events, some of which can be life-threatening. One of those commonly seen adverse events is diarrhea. In **chapter 3** we retrospectively analyzed a cohort of 93 patients treated with immunotherapy for metastatic melanoma or non-small cell lung cancer. All patients underwent an endoscopy and/or were treated with high-dose corticosteroids for immune-related diarrhea. We describe the correlation between symptoms, endoscopic features, histological features and response to management.

In **chapters 4 and 5** we look into a select group of patients with metastatic melanoma. Namely, those with brain metastases and/or leptomeningeal metastases. The incidence of brain metastases ranges from 10% up to 73% based on clinical and post-mortem research [36-41]. Brain metastases from melanoma carry a poor prognosis with a median overall survival not exceeding five months [42]. In **chapter 4** we retrospectively analyzed a cohort of 146 patients with brain metastases from melanoma with a BRAF mutation. We describe the overall survival, progression free survival, clinical response and radiological response to BRAF inhibitors with or without the addition of a MEK inhibitor. In **chapter 5** we study patients with leptomeningeal metastases from metastatic melanoma. Literature has shown that patients with untreated leptomeningeal metastases from solid tumors have an even worse median overall survival of only 4 to 6 weeks [43]. In our retrospective analysis we identified a cohort of 39 patients with leptomeningeal metastases from melanoma and describe the effects of targeted therapy and immunotherapy on this disease.

BRAF inhibitors have proven to be an effective treatment against metastatic melanoma for patients harboring a BRAF mutation. However, a large proportion of patients treated with BRAF inhibitors will eventually relapse. In the clinical setting stopping the BRAF inhibitor after progression of disease oftentimes lead to an accelerated growth of the metastases, quickly followed by death of the patient.

In **chapter 6** we analyze two groups of 35 patients treated with the BRAF inhibitor vemurafenib. One group of patients continues with the BRAF inhibitor, despite documented progression of disease. The other group discontinues the BRAF inhibitor at documented progression. Here we describe the results of this analysis.

At the Netherlands Cancer Institute a phase III trial is in progress for patients with metastatic melanoma, comparing treatment with the adoptive transfer of TIL to ipilimumab. Patients receiving TIL are pre-treated with high-dose chemotherapy and receive high-dose bolus IL-2 after the infusion of the TIL. In **chapter 7** we review the past, present and future of treating patients with melanoma and other types of cancer with TIL.

Finally, in **chapter 8** the results obtained in this thesis are discussed and implications for further research are presented.

REFERENCES

1. Melanoma Treatment - Health Professional Version. Accessed online at https://www.cancer.gov/types/skin/hp/melanoma-treatment-pdq. 2017.
2. Balch CM, Gershenwald JE, Soong SJ et al. Final version of 2009 AJCC melanoma staging and classification. J Clin Oncol 2009; 27: 6199-6206.
3. Virchow R. Cellular Pathology. Philadelphia,1863.
4. Clemente CG, Mihm MC, Jr., Bufalino R et al. Prognostic value of tumor infiltrating lymphocytes in the vertical growth phase of primary cutaneous melanoma. Cancer 1996; 77: 1303-1310.
5. Tuthill RJ, Unger JM, Liu PY et al. Risk assessment in localized primary cutaneous melanoma: a Southwest Oncology Group study evaluating nine factors and a test of the Clark logistic regression prediction model. Am J Clin Pathol 2002; 118: 504-511.
6. Santoiemma PP, Powell DJ, Jr. Tumor Infiltrating Lymphocytes in Ovarian Cancer. Cancer Biol Ther 2015; 0.
7. Zhang L, Conejo-Garcia JR, Katsaros D et al. Intratumoral T cells, recurrence, and survival in epithelial ovarian cancer. N Engl J Med 2003; 348: 203-213.
8. Pages F, Galon J, Dieu-Nosjean MC et al. Immune infiltration in human tumors: a prognostic factor that should not be ignored. Oncogene 2010; 29: 1093-1102.
9. Topalian SL, Solomon D, Avis FP et al. Immunotherapy of patients with advanced cancer using tumor-infiltrating lymphocytes and recombinant interleukin-2: a pilot study. J Clin Oncol 1988; 6: 839-853.
10. Brunet JF, Denizot F, Luciani MF et al. A new member of the immunoglobulin superfamily--CTLA-4. Nature 1987; 328: 267-270.
11. Linsley PS, Brady W, Grosmaire L et al. Binding of the B cell activation antigen B7 to CD28 costimulates T cell proliferation and interleukin 2 mRNA accumulation. J Exp Med 1991; 173: 721-730.
12. Chapman PB, Einhorn LH, Meyers ML et al. Phase III multicenter randomized trial of the Dartmouth regimen versus dacarbazine in patients with metastatic melanoma. J Clin Oncol 1999; 17: 2745-2751.
13. Patel PM, Suciu S, Mortier L et al. Extended schedule, escalated dose temozolomide versus dacarbazine in stage IV melanoma: final results of a randomised phase III study (EORTC 18032). Eur J Cancer 2011; 47: 1476-1483.
14. Hodi FS, O'Day SJ, McDermott DF et al. Improved survival with ipilimumab in patients with metastatic melanoma. N Engl J Med 2010; 363: 711-723.
15. Robert C, Thomas L, Bondarenko I et al. Ipilimumab plus dacarbazine for previously untreated metastatic melanoma. N Engl J Med 2011; 364: 2517-2526.
16. Robert C, Schachter J, Long GV et al. Pembrolizumab versus Ipilimumab in Advanced Melanoma. N Engl J Med 2015; 372: 2521-2532.
17. Robert C, Long GV, Brady B et al. Nivolumab in previously untreated melanoma without BRAF mutation. N Engl J Med 2015; 372: 320-330.
18. Larkin J, Chiarion-Sileni V, Gonzalez R et al. Combined Nivolumab and Ipilimumab or Monotherapy in Untreated Melanoma. N Engl J Med 2015; 373: 23-34.
19. Medicijnkosten. Medicijnkosten ipilimumab, nivolumab en pembrolizumab. In. 2017.
20. Hamid O, Robert C, Daud A et al. Safety and tumor responses with lambrolizumab (anti-PD-1) in melanoma. N Engl J Med 2013; 369: 134-144.

21. Robert C, Ribas A, Wolchok JD et al. Anti-programmed-death-receptor-1 treatment with pembrolizumab in ipilimumab-refractory advanced melanoma: a randomised dose-comparison cohort of a phase 1 trial. Lancet 2014; 384: 1109-1117.
22. Ribas A, Puzanov I, Dummer R et al. Pembrolizumab versus investigator-choice chemotherapy for ipilimumab-refractory melanoma (KEYNOTE-002): a randomised, controlled, phase 2 trial. Lancet Oncol 2015; 16: 908-918.
23. Garon EB, Rizvi NA, Hui R et al. Pembrolizumab for the treatment of non-small-cell lung cancer. N Engl J Med 2015; 372: 2018-2028.
24. Topalian SL, Hodi FS, Brahmer JR et al. Safety, activity, and immune correlates of anti-PD-1 antibody in cancer. N Engl J Med 2012; 366: 2443-2454.
25. Postow MA, Chesney J, Pavlick AC et al. Nivolumab and Ipilimumab versus Ipilimumab in Untreated Melanoma. N Engl J Med 2015.
26. Kelderman S, Heemskerk B, van Tinteren H et al. Lactate dehydrogenase as a selection criterion for ipilimumab treatment in metastatic melanoma. Cancer Immunol Immunother 2014; 63: 449-458.
27. Blank CU, Haanen JB, Ribas A, Schumacher TN. Cancer Immunology. The "cancer immunogram". Science 2016; 352: 658-660.
28. Long GV, Menzies AM, Nagrial AM et al. Prognostic and clinicopathologic associations of oncogenic BRAF in metastatic melanoma. J Clin Oncol 2011; 29: 1239-1246.
29. Menzies AM, Haydu LE, Visintin L et al. Distinguishing clinicopathologic features of patients with V600E and V600K BRAF-mutant metastatic melanoma. Clin Cancer Res 2012; 18: 3242-3249.
30. Hauschild A, Grob JJ, Demidov LV et al. Dabrafenib in BRAF-mutated metastatic melanoma: a multicentre, open-label, phase 3 randomised controlled trial. Lancet 2012; 380: 358-365.
31. Chapman PB, Hauschild A, Robert C et al. Improved survival with vemurafenib in melanoma with BRAF V600E mutation. N Engl J Med 2011; 364: 2507-2516.
32. Flaherty KT, Infante JR, Daud A et al. Combined BRAF and MEK inhibition in melanoma with BRAF V600 mutations. N Engl J Med 2012; 367: 1694-1703.
33. Long GV, Stroyakovskiy D, Gogas H et al. Combined BRAF and MEK inhibition versus BRAF inhibition alone in melanoma. N Engl J Med 2014; 371: 1877-1888.
34. Larkin J, Ascierto PA, Dreno B et al. Combined vemurafenib and cobimetinib in BRAF-mutated melanoma. N Engl J Med 2014; 371: 1867-1876.
35. Dummer R, Ascierto PA, Gogas HJ et al. Results of COLUMBUS Part 1: A Phase 3 Trial of Encorafenib (ENCO) Plus Binimetinib (BINI) Versus Vemurafenib (VEM) or ENCO in BRAF-Mutant Melanoma. SMR 2016.
36. Nayak L, Lee EQ, Wen PY. Epidemiology of brain metastases. Curr Oncol Rep 2012; 14: 48-54.
37. Dasgupta T, Brasfield R. Metastatic Melanoma. A Clinicopathological Study. Cancer 1964; 17: 1323-1339.
38. Patel JK, Didolkar MS, Pickren JW, Moore RH. Metastatic pattern of malignant melanoma. A study of 216 autopsy cases. Am J Surg 1978; 135: 807-810.
39. de la Monte SM, Moore GW, Hutchins GM. Patterned distribution of metastases from malignant melanoma in humans. Cancer Res 1983; 43: 3427-3433.
40. Sampson JH, Carter JH, Jr., Friedman AH, Seigler HF. Demographics, prognosis, and therapy in 702 patients with brain metastases from malignant melanoma. J Neurosurg 1998; 88: 11-20.

41. Zakrzewski J, Geraghty LN, Rose AE et al. Clinical variables and primary tumor characteristics predictive of the development of melanoma brain metastases and post-brain metastases survival. Cancer 2011; 117: 1711-1720.
42. Staudt M, Lasithiotakis K, Leiter U et al. Determinants of survival in patients with brain metastases from cutaneous melanoma. Br J Cancer 2010; 102: 1213-1218.
43. Wasserstrom WR, Glass JP, Posner JB. Diagnosis and treatment of leptomeningeal metastases from solid tumors: experience with 90 patients. Cancer 1982; 49: 759-772.