Clinical aspects of immunotherapy and targeted therapy of advanced melanoma

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Chapter 8

General discussion

Worldwide melanoma incidence has been rising over the past decades. For example in the Netherlands the incidence of melanoma in 1990 was around 1550 patients, while in 2015 nearly 6000 patients had been diagnosed with melanoma [1]. Approximately 10 – 14% of patients diagnosed with melanoma will eventually develop metastases [2, 3]. As previously discussed in **Chapter 1**, approved therapeutic options for metastatic melanoma until 2011 were chemotherapeutic agent dacarbazine and in some countries high dose interleukin-2 (IL-2). Several phase III trials have shown a median overall survival (OS) for dacarbazine of only 6 – 9 months [4, 5]. With the discovery of immune-checkpoints (Cytotoxic T-Lymphocyte-Associated protein 4; CTLA-4, and the Programmed cell Death(-Ligand)-1; PD-1/PD-L1 axis) and the development of monoclonal antibodies blocking these checkpoints, a great improvement in median OS has been achieved [6-10]. In the era of chemotherapy stage IV melanoma was once an almost uniformly deadly disease, with survival at 5 years ranging between 9% and 25% [11]. Now, in the era of immunotherapy, we are seeing 2-year survival rates ranging between 59% and 64%, for patients treated with anti-PD1 monotherapy or the combination of anti-PD1 plus anti CTLA-4, respectively [12]. Furthermore, a proportion of those patients will probably be cured. Nevertheless, these new drugs are costly and can induce serious, sometimes life-threatening adverse events (AEs). The discovery of biomarkers to predict upfront which patients should, and perhaps more importantly, which patients should not be treated with immunotherapeutics remains one of the goals for oncologists and researchers world-wide.

BIOMARKERS AND ADVERSE EVENTS IN IMMUNOTHERAPY

The first part of my thesis focused on the discovery of biomarkers associated with a favorable outcome on ipilimumab treatment, or OS in patients with metastatic melanoma. With the increasing numbers of immunotherapeutics developed by pharmaceutical industries we are in need for biomarkers that are predictive for response upon treatment. In my opinion there are three reasons why biomarker discovery is so important in this era: 1) biomarkers could make it possible to steer patients into their right treatment. This is especially important in patients that would not benefit from the given treatment at all. 2) All treated patients are at risk for developing serious AEs. Even though mortality rates due to treatment with immunotherapy have significantly dropped from for example 2.1% in 2010 to less than 0.2% in the years thereafter, all patients remain at risk for AEs, some of which can severely interfere with quality of life [6, 8, 10, 13]. 3) Lastly, most new treatment options against cancer are prohibitively expensive and put a serious burden on health care costs. Over the past few years many biomarkers (or combinations thereof) have been identified, but so far none have been able to provide a clear cut-off for response

to immunotherapy and targeted therapy (obviously, besides having a BRAF mutation). In **Chapter 2**, we created a model consisting of six different parameters which were gathered through routine blood parameters combined with flow cytometry. Five of these six parameters (lactate dehydrogenase; LDH, absolute monocyte counts; AMC, myeloid-derived suppressor cell frequencies; MDSCs, absolute eosinophil counts; AEC and relative lymphocyte counts; RLC) were significantly associated with OS in patients treated with ipilimumab in a multivariate analysis. Baseline values of regulatory T-cells (Tregs) were not significantly associated with OS in multivariate analysis, however the best discriminatory ability of our model was only achieved when incorporating Tregs into the model. 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 (BORR). Patients with five favorable parameters (risk score = 0) had a BORR of 31% compared to only 3% for patients with a risk score of > 130. All parameters described in our model have already been shown to be either predictive to ipilimumab treatment or prognostic for OS in general. For example the LDH-ratio has been shown to be a strong baseline predictive biomarker and so has an increase in eosinophils after the first cycle of ipilimumab [9, 14, 15]. An interesting parameter in our model are Tregs. In our univariate analysis higher baseline frequencies of Tregs were associated with improved OS. However, this parameter was not significantly associated with OS in multivariate analysis. Nevertheless, adding Tregs to our model did provide the best discriminatory ability. Tregs are direct target cells for ipilimumab due to their constitutive CTLA-4 expression. Therefore, it seems logical that higher frequencies might render patients more susceptible to ipilimumab therapy. Elimination of Tregs due to ipilimumab works probably via antibody-dependent cell-mediated cytotoxicity (ADCC) as a result of binding of ipilimumab to CTLA-4 on the Tregs and FcγRIIIA (CD16A) present on monocytes [16]. Tarhini et al. showed that, although in a neoadjuvant setting, an increase in Tregs between baseline and week 6 was associated with an improvement in progression-free survival (PFS) [17]. On the other hand Simeone et al. showed that a decrease in Tregs, between baseline and week 12, was associated with improved survival and disease control rates [18]. Results like these show exactly how difficult it is to discover a biomarker, or combinations of biomarkers, which can perfectly distinguish responders and non-responders. Furthermore, not only blood-based parameters may have an impact on OS and/or response to ipilimumab treatment. Other known prognostic factors, such as performance status, the presence of brain metastases, or prior systemic therapies may play a crucial role on response to ipilimumab or OS in general. Probably a single biomarker, or even a combination of biomarkers, will not be able to select patients upfront that will benefit from a certain immunotherapeutic agent. It is far more likely that in the near future a biomarker-model will be established which can select patients hardly benefitting from a given treatment

at all. For example, patients in the worst possible category in our study only had a BORR of 3% to ipilimumab and no patients were alive after 15 months. In the Checkmate-067 study where patients were randomized to receive either nivolumab, ipilimumab or the combination of nivolumab + ipilimumab no patients responded to ipilimumab when their LDH was ≥ 2 x the upper limit of normal [19]. Also, in the retrospective analysis by Kelderman et al. the response rate to ipilimumab for patients with a baseline LDH of ≥ 2 x the upper limit of normal was only 7% and only 1 out of 27 patients survived longer than 12 months. For anti-PD1 therapy similar results are seen. In a recently published article Daud et al. pooled data from all 655 patients treated in the KEYNOTE-001 study. A baseline tumor burden below the median, patients with M1b disease, treatment naïve patients and patients with a normal LDH had a significantly higher BORR. Also for patients treated with the combination of nivolumab + ipilimumab a higher BORR was seen in patients with a ≥5% PD-L1 tumor expression [20, 21]. Whether these examples provided here are more prognostic in general or specifically predictive for outcome to ipilimumab treatment remains a difficult question. Recently Blank et al. proposed a framework consisting of seven parameters describing requirements for a sufficient anti-tumor immune response (the "Cancer Immunogram") [22]. We are currently analyzing the Cancer Immunogram in two cohorts of patients treated with either ipilimumab or anti-PD1 (pembrolizumab or nivolumab). Perhaps the Cancer Immunogram can help to identify melanoma patients that will, or will not, respond to immunotherapy. Future research needs to address this.

Every medicine that patients use can elicit an AE. Some of these AEs are more serious than others. This is also true for patients receiving immunotherapeutic drugs. Of these AEs some can be mild (e.g. fatigue, pruritus), some can be severe (arthralgia, vomiting) and some can be potentially life-threatening (colitis, hypophysitis, hepatitis). One of the most common AEs seen during treatment with immunotherapeutic drugs is diarrhea. The incidence of diarrhea as seen during treatment with immunotherapy is 35% for anti-CTLA-4, 20% for anti-PD1 and even 44% for the combination treatment [8, 23]. Most, if not all, studies follow the common terminology criteria for adverse events (CTCAE) to define AEs [24]. From a gastroenterologists point of view the diagnosis of colitis can only be made after inspection of the colon via endoscopy. Recently, the European Society for Medical Oncology has published an article on how to manage toxicities commonly seen during treatment with immunotherapy [25]. For diarrhea the algorithm is based on the grade of diarrhea according to CTCAE. According to these algorithms the higher the grade of diarrhea, the more aggressive therapy is indicated. In **Chapter 3** we retrospectively analyzed a cohort of 92 patients treated with immunotherapy for either metastatic non-small cell lung cancer or melanoma. Immunotherapy-related colitis (IRC) is a particular form of inflammatory bowel disease with both signs of ulcerative colitis (UC) and Crohn's disease (CD) [26]. For this reason we analyzed severity of colitis, as seen during endoscopy, according to two dif-

ferent scoring systems; the endoscopic Mayo score and the 'van der Heide' score [27, 28]. We show that there is no significant correlation between the grade of diarrhea and neither of the two scoring systems used. In the field of IBD, treatment used to be guided solely by symptoms, such as abdominal pain and diarrhea. Symptomatic treatment, however, may not improve long-term outcome or slow disease progression [29]. This is possibly due to the fact that symptoms may not accurately reflect the underlying inflammatory process characterized by ulcers. This is further highlighted in a study by Modigliani et al. in which 142 patients with active CD were included. No significant correlation could be found between clinical severity and nature, surface, or severity of endoscopic lesions. Furthermore, only 29% of patients that went in clinical remission following 1 mg/kg prednisone per day achieved endoscopic remission after 7 weeks of treatment [30]. A biomarker commonly used to assess severity of colitis is fecal calprotectin. Multiple studies have shown a strong correlation between levels of fecal calprotectin and endoscopic disease activity for colitis [31-33]. The correlation between clinical symptoms and fecal calprotectin is however much lower [33, 34]. These examples, and the data presented in **Chapter 3**, could indicate that using diarrhea as a symptom to indicate severity of colitis, and thus treatment approach, might not be optimal. We did, however, find a significant correlation between steroid-refractory colitis and the presence of ulcers and higher endoscopic scores. After failure of high-dose corticosteroids, patients are usually treated with infliximab. Treatment with infliximab in IBD has already shown to result in more clinical responses, mucosal healing, fewer hospital admissions and less surgical interventions [35, 36]. This has led to an earlier introduction of infliximab in many severe cases of IBD [37]. Future research will have to show whether an earlier introduction of infliximab in IRC will also prove to be as efficient.

BRAIN METASTASES AND LEPTOMENINGEAL METASTASES

For a subgroup of patients with metastatic melanoma, namely those with brain metastases and/or leptomeningeal metastases there is still an unmet medical need for improvement of treatment. In most large randomized phase III trials patients with untreated brain metastases were often excluded. Therefore, even in the year 2017, we simply do not know what the best treatment for these patients is. Should we withhold neurosurgery and stereotactic radiotherapy in favor of targeted therapy or immunotherapy in some of these patients? Besides local treatment and immunotherapy another possible treatment option for patients with metastatic melanoma is targeted therapy. In **Chapter 4** we analyzed a cohort of 146 patients with brain metastases from metastatic melanoma. Patients were treated with either vemurafenib, dabrafenib, or the combination of dabrafenib plus trametinib. The difference in median OS between patients treated with the combination of dabrafenib plus trametinib compared to vemurafenib was statistically significant (HR for death, 0.52;

95% CI, 0.30 – 0.89; p = 0.02). The reason that dabrafenib potentially influences OS more than vemurafenib in patients with brain metastases might be due to the fact that dabrafenib passes the blood-brain barrier (BBB) more efficiently than vemurafenib. In the European Medicines Agency (EMA) assessment report of vemurafenib it is described that concentrations of vemurafenib remained below the quantifiable limit in the brain and spinal cord [38]. Furthermore, a study by Elmquist et al. describes that the distribution of vemurafenib in the brain is severely restricted due to active efflux by P-glycoprotein (P-gp) and breast cancer resistance protein (BCRP), two major members of the efflux transporters present on the luminal side of the capillary endothelium of the BBB. Cell lines overexpressing either P-gp or BCRP significantly lowered the accumulation of vemurafenib compared to their wild-type counterpart. The difference in accumulation was abolished after a P-gp or BCRP inhibitor was added, showing that vemurafenib is a substrate for P-gp and BCRP *in vitro*. Also the area under the curve brain (AUC_{brain}) to AUC_{plasma} ratio was 0.004 in FVB (Friend leukemia virus B) wild-type mice, indicating a severely restricted brain distribution of vemurafenib [39]. On the contrary, a study performed by the same group on the distribution of dabrafenib in the central nervous system shows a higher AUC_{brain} to AUC_{plasma} ratio in the same FVB wild-type mice. The ratio for dabrafenib was 0.023, an almost 6 times higher ratio. These data indicate the greater brain penetration for dabrafenib than vemurafenib [40]. Previous data are all based on an intact BBB. However, brain metastases are known to potentially disrupt the BBB and thus making it more permeable for certain drugs [41]. For patients with metastatic melanoma without brain metastases it has already been shown that the combination of a BRAF inhibitor (BRAFi) plus a MEK inhibitor (MEKi) outperforms BRAFi monotherapy in OS and PFS [42-45]. The improved survival in patients treated with the combination regimen as compared to BRAFi monotherapy may be due to prevention of acquisition of BRAFi resistance caused by recovery of phospho-ERK signalling [46]. The recently published prospective phase 2 COMBI-MB trial also showed activity of the combination of dabrafenib plus trametinib in patients with brain metastases from melanoma. Intracranial response for asymptomatic patients without previous local brain therapy was 58% (95% CI, 46 – 69). Median PFS was 5.6 months (95% CI, 5.3 – 7.4) and median OS was 10.8 months (95% CI, 8.7 – 19.6) [47]. In 2012 Long et al. published results from the prospective BREAK-MB study [48]. In this study patients with brain metastases from melanoma were treated with dabrafenib monotherapy. In the cohort of patients that had asymptomatic, previously untreated brain metastases 39% (95% CI, 28 – 51) of patients had an intracranial response. Median PFS was 16.1 weeks (95% CI, 15.7 – 21.9) and median OS was 33.1 weeks (95% CI, 25.6 – NR). Although difficult to compare two separate studies, there appears to be a significant benefit in ORR, OS and PFS in the group of patients treated with the combination of dabrafenib plus trametinib compared to dabrafenib monotherapy. In our retrospective study ORR in the group of patients treated with dabrafenib plus trametinib was 43%, which is slightly

lower than in the study by Davies et al. However, the median intracranial PFS of 5.8 months (95% CI, 3.2 – 8.5) and the median OS of 11.2 months (95% CI, 6.8 – 15.7) are comparable. Interesting results are currently also being discovered in patients with brain metastases treated with immunotherapy. In the Checkmate 204 study patients with asymptomatic brain metastases from melanoma were treated with the combination of nivolumab plus ipilimumab. An ORR of 56% was seen and 19% of treated patients had a complete intracranial response [49]. Also in the phase II anti-PD1 Brain Collaboration study promising results are seen. In the cohort of asymptomatic patients treated with the combination of ipilimumab plus nivolumab an intracranial response rate was seen of 44% (95% CI, 24 – 65) [50]. Despite the promising results found in our study the median duration of response is rather low compared to patients treated with BRAFi +/- MEKi without brain metastases [51, 52]. Loss of the negative regulator phosphatase and tensin homolog (PTEN), resulting in an increased activation of the PI3K-AKT pathway, has been reported in brain metastases from melanoma compared to matched extracranial lesions. This has been associated with resistance to BRAF and MEK inhibitors [53, 54]. Furthermore, the increase in AKT signalling might be due to crosstalk with neighbouring cells such as astrocytes. A study by Niessner et al. has shown that metastatic melanoma cells stimulated by astrocyte-conditioned medium showed higher AKT activation than cells stimulated by fibroblast-conditioned medium [55]. Future research will have to provide us insights on the best treatment and possible combinations with local therapy (e.g. stereotactic radiosurgery, gamma knife and local surgery). Patients with leptomeningeal metastases from melanoma perhaps have the worst possible survival probability. Historical data shows a median survival of untreated patients of about two months [56, 57]. In **Chapter 5** we describe a cohort of 39 patients with leptomeningeal metastases from melanoma. A median OS of 6.9 weeks (95% CI, 0.9 – 12.8) was found for the entire cohort. Patients that received treatment with a targeted agent and/or immunotherapy had a median OS of 21.7 weeks (95% CI, 11.2 – 32.2). In the initial era of immunotherapy, treatment for leptomeningeal metastases of melanoma included intrathecal IL-2. This showed incidental responses, but also marked toxicity [58]. Ipilimumab's mechanism of action is through activation of T-cells. Activated T-cells can cross the BBB, which makes the BBB less relevant for a response within the central nervous system. This has also been shown in a study by Margolin et al. in which patients with asymptomatic intracranial and extracranial metastases from melanoma were treated with ipilimumab at a dose of 10 mg/kg once every 3 weeks. Almost similar disease control rates were seen between intracranial metastases (24%) and extracranial metastases (27%). This is further highlighted in a study in which patients with brain metastases from melanoma were treated with the adoptive transfer of T-cells, either via infusion of autologous ex-vivo expanded tumor-infiltrating lymphocytes (TIL) or autologous peripheral blood lymphocytes retrovirally transduced to express a T-cell receptor (TCR) that recognizes the melanocyte differentiation antigens gp-100 or MART-1. Seven of seven-

teen patients (41%) treated with TIL and two out of nine patients (22%) treated with TCR achieved a complete response of all brain tumor lesions [59]. Nevertheless, recently published data in a small cohort of patients (n = 16) with brain metastases that failed local therapy, were symptomatic and/or had leptomeningeal metastases treated with nivolumab (3 mg/kg q2 weeks) showed an intracranial response rate of only 6% (95% CI 0 – 30) [50]. Treating patients with ipilimumab combined with radiotherapy has shown to increase median OS in our study. We found a median OS of 47 weeks in patients treated with ipilimumab and radiotherapy. This could be partially due to the so-called abscopal effect in which increased release of tumor antigen by radiotherapy can increase antigen presentation to T-cells [60]. Future studies will have to provide us with information on the best combinatorial therapeutic regimens using immunotherapy and radiotherapy and to determine optimal timing and dosage of either treatment in patients with leptomeningeal metastases from melanoma.

ACQUIRED RESISTANCE TO BRAF INHIBITORS AND TREATMENT BEYOND PROGRESSION

Response rates to BRAFi therapy in patients with BRAF mutant metastatic melanoma are high. Response rates for vemurafenib monotherapy range between 40% and 51%, for dabrafenib monotherapy around 50% and for the combination of a BRAFi with a MEKi 63% to 70%. Nevertheless despite these high response rates median PFS is relatively short. This especially true for BRAFi monotherapy. For example, median PFS of vemurafenib is between 5.3 – 7.3 months and dabrafenib 5.1 – 8.8 months. Median PFS for the combination of a BRAFi with a MEKi is longer; 9.3 – 14.9 months [43, 44, 61-66]. Several years ago the BRAFi vemurafenib was the first and only BRAFi available on the market. In the clinic, we observed that stopping vemurafenib treatment due to disease progression would often lead to an accelerated growth of all metastases, followed by quick deterioration of the patient and death. This raised the question whether continuation of vemurafenib despite disease progression (treatment beyond progression, or TBP) could improve OS in these patients. In **Chapter 6** we retrospectively analyzed a cohort of 70 patients with metastatic melanoma treated with vemurafenib who experienced progression of disease after a prior objective response. In this cohort 35 patients stopped vemurafenib at disease progression, whilst the other 35 patients continued vemurafenib treatment despite documented progression. Median OS beyond documented progression of disease was 5.2 months (95% CI, 3.8 – 7.4) for patients that continued vemurafenib, compared to only 1.4 months (95% CI, 0.6 – 3.4) for patients that stopped vemurafenib treatment. This four month benefit in OS in this patient group is an interesting finding. Stopping vemurafenib based on progressive disease results in the growth of both resistant as non-resistant tumor cells. Treatment

beyond progression has been used with success in many different malignancies, including breast cancer, colorectal cancer and non-small cell lung cancer [67-69]. Intra-tumoral and inter-tumoral heterogeneity probably plays a crucial role in why TBP is so effective. The idea is that vemurafenib resistant tumor cells are the reason for progressive disease, while other tumor cells are still responsive to BRAFi therapy. This has been shown in the analyses of tumors progressing on BRAFi therapy in which multiple mechanisms of acquired resistance could be detected in the same tumor biopsy, but also tumors from different metastatic sites [70]. Alternatively, data from single-cell-derived resistant melanoma cells suggest that MAPKi retain some antiproliferative effect, despite signs of progressive disease [71].

CONCLUSIONS AND FUTURE PERSPECTIVES

The past few years have seen a remarkable increase in treatment options not only for patients with metastatic melanoma, but for many patients with different types of cancer. For the treatment of melanoma we are currently in an exciting era. Immunotherapy and targeted therapy have shown to increase PFS, OS and ORR in many patients compared to historical data. New combinations of checkpoint inhibitors, as monotherapy, dual therapy or even triple therapy, are currently being tested in phase 1/2 trials. Nevertheless, despite these promising results there still is a proportion of patients without a durable response upon treatment. This is especially true for patients with brain metastases and/or leptomeningeal metastases. Furthermore, while many of the new combination partners seem very promising, we need to be aware that this will require many patients to be included in future phase 3 studies, all of which may be at risk for serious adverse events. Biomarkers, such as those described in this thesis, might help us select patients in need of these new combination partners, which would allow to design smaller trials. In this thesis I have shown the importance of biomarker discovery, looked at adverse events and its management and have shown that immunotherapy and targeted therapy can have great impact in patients with brain metastases and/or leptomeningeal metastases. Future research will have to be aimed at further biomarker discovery, the discovery of new combination partners for immunotherapy and targeted therapy and better treatment options for patients with brain metastases and/or leptomeningeal metastases.

REFERENCES

1. Mortaliteit melanoom. Accessed online at www.cijfersoverkanker.nl. 2015.
2. Mervic L. Time course and pattern of metastasis of cutaneous melanoma differ between men and women. PLoS One 2012; 7: e32955.
3. Sterfte melanoom. Accessed online at www.cijfersoverkanker.nl. 2015.
4. 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.
5. 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.
6. 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.
7. Schadendorf D, Hodi FS, Robert C et al. Pooled Analysis of Long-Term Survival Data From Phase II and Phase III Trials of Ipilimumab in Unresectable or Metastatic Melanoma. J Clin Oncol 2015.
8. Robert C, Schachter J, Long GV et al. Pembrolizumab versus Ipilimumab in Advanced Melanoma. N Engl J Med 2015; 372: 2521-2532.
9. Robert C, Long GV, Brady B et al. Nivolumab in previously untreated melanoma without BRAF mutation. N Engl J Med 2015; 372: 320-330.
10. 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.
11. Svedman FC, Pillas D, Taylor A et al. Stage-specific survival and recurrence in patients with cutaneous malignant melanoma in Europe - a systematic review of the literature. Clin Epidemiol 2016; 8: 109-122.
12. Larkin J, Chiarion Sileni V, Gonzalez R et al. Overall survival results from a phase III trial of nivolumab combined with ipilimumab in treatment-naïve patients with advanced melanoma (CheckMate-067). AACR Annual Meeting. Washington DC 2017; Abstract CT075.
13. Robert C, Thomas L, Bondarenko I et al. Ipilimumab plus dacarbazine for previously untreated metastatic melanoma. N Engl J Med 2011; 364: 2517-2526.
14. 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.
15. Delyon J, Mateus C, Lefeuvre D et al. Experience in daily practice with ipilimumab for the treatment of patients with metastatic melanoma: an early increase in lymphocyte and eosinophil counts is associated with improved survival. Ann Oncol 2013; 24: 1697-1703.
16. Romano E, Kusio-Kobialka M, Foukas PG et al. Ipilimumab-dependent cell-mediated cytotoxicity of regulatory T cells ex vivo by nonclassical monocytes in melanoma patients. Proc Natl Acad Sci U S A 2015; 112: 6140-6145.
17. Tarhini AA, Edington H, Butterfield LH et al. Immune monitoring of the circulation and the tumor microenvironment in patients with regionally advanced melanoma receiving neoadjuvant ipilimumab. PLoS One 2014; 9: e87705.
18. Simeone E, Gentilcore G, Giannarelli D et al. Immunological and biological changes during ipilimumab treatment and their potential correlation with clinical response and survival in patients with advanced melanoma. Cancer Immunol Immunother 2014; 63: 675-683.

19. Larkin J, Chiarion-Sileni V, Gonzalez R et al. Efficacy and safety in key patient subgroups of nivolumab (NIVO) alone or combined with ipilimumab (IPI) versus IPI alone in treatment-naive patients with advanced melanoma (MEL) (CheckMate 067). European Journal of Cancer 2015; 51: S664-665.
20. Ribas A, Hamid O, Daud A et al. Association of Pembrolizumab With Tumor Response and Survival Among Patients With Advanced Melanoma. JAMA 2016; 315: 1600-1609.
21. Long GV, Larkin J, Ascierto PA et al. PD-L1 expression as a biomarker for nivolumab (NIVO) plus ipilimumab (IPI) and NIVO alone in advanced melanoma (MEL): A pooled analysis. Annals of Oncology 2016; 27: 1112PD-1112PD.
22. Blank CU, Haanen JB, Ribas A, Schumacher TN. Cancer Immunology. The "cancer immunogram". Science 2016; 352: 658-660.
23. Ibrahim N, Berman DM, DePril V et al. Ipilimumab safety profile: Summary of findings from completed trials in advanced melanoma. J Clin Oncol 2011; 29: (suppl; abstr 8583).
24. U.S. Department of Health and Human Services. Common Terminology Criteria for Adverse Events (CTCAE) Version 4.03. Accessed online at https://evs.nci.nih.gov/ftp1/CTCAE/CTCAE_4.03_2010-06-14_QuickReference_8.5x11.pdf. 2010.
25. Haanen JBAG, Carbonnel F, Robert C et al. Management of toxicities from immunotherapy: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up†. Annals of Oncology 2017; 28: iv119-iv142.
26. Boutros C, Tarhini A, Routier E et al. Safety profiles of anti-CTLA-4 and anti-PD-1 antibodies alone and in combination. Nat Rev Clin Oncol 2016; 13: 473-486.
27. Schroeder KW, Tremaine WJ, Ilstrup DM. Coated oral 5-aminosalicylic acid therapy for mildly to moderately active ulcerative colitis. A randomized study. N Engl J Med 1987; 317: 1625-1629.
28. van der Heide H, van den Brandt-Gradel V, Tytgat GN et al. Comparison of beclomethasone dipropionate and prednisolone 21-phosphate enemas in the treatment of ulcerative proctitis. J Clin Gastroenterol 1988; 10: 169-172.
29. Rutgeerts P, Vermeire S, Van Assche G. Mucosal healing in inflammatory bowel disease: impossible ideal or therapeutic target? Gut 2007; 56: 453-455.
30. Modigliani R, Mary JY, Simon JF et al. Clinical, biological, and endoscopic picture of attacks of Crohn's disease. Evolution on prednisolone. Groupe d'Etude Therapeutique des Affections Inflammatoires Digestives. Gastroenterology 1990; 98: 811-818.
31. Canani RB, Terrin G, Rapacciuolo L et al. Faecal calprotectin as reliable non-invasive marker to assess the severity of mucosal inflammation in children with inflammatory bowel disease. Dig Liver Dis 2008; 40: 547-553.
32. D'Haens G, Ferrante M, Vermeire S et al. Fecal calprotectin is a surrogate marker for endoscopic lesions in inflammatory bowel disease. Inflamm Bowel Dis 2012; 18: 2218-2224.
33. Sipponen T, Savilahti E, Kolho KL et al. Crohn's disease activity assessed by fecal calprotectin and lactoferrin: correlation with Crohn's disease activity index and endoscopic findings. Inflamm Bowel Dis 2008; 14: 40-46.
34. Bodelier A, de Boer E, Jonkers D et al. Monitoring disease activity in IBD: correlation between clinical activity indices and biomarkers. Gastroenterology 2011; S423.
35. Hanauer SB, Feagan BG, Lichtenstein GR et al. Maintenance infliximab for Crohn's disease: the ACCENT I randomised trial. Lancet 2002; 359: 1541-1549.
36. Jarnerot G, Hertervig E, Friis-Liby I et al. Infliximab as rescue therapy in severe to moderately severe ulcerative colitis: a randomized, placebo-controlled study. Gastroenterology 2005; 128: 1805-1811.

37. Fan R, Zhong J, Wang ZT et al. Evaluation of "top-down" treatment of early Crohn's disease by double balloon enteroscopy. World J Gastroenterol 2014; 20: 14479-14487.
38. EMA Assessment Report vemurafenib. 2011.
39. Mittapalli RK, Vaidhyanathan S, Sane R, Elmquist WF. Impact of P-glycoprotein (ABCB1) and breast cancer resistance protein (ABCG2) on the brain distribution of a novel BRAF inhibitor: vemurafenib (PLX4032). J Pharmacol Exp Ther 2012; 342: 33-40.
40. Mittapalli RK, Vaidhyanathan S, Dudek AZ, Elmquist WF. Mechanisms limiting distribution of the threonine-protein kinase B-RaF(V600E) inhibitor dabrafenib to the brain: implications for the treatment of melanoma brain metastases. J Pharmacol Exp Ther 2013; 344: 655-664.
41. Gerstner ER, Fine RL. Increased permeability of the blood-brain barrier to chemotherapy in metastatic brain tumors: establishing a treatment paradigm. J Clin Oncol 2007; 25: 2306-2312.
42. 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.
43. 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.
44. Larkin J, Ascierto PA, Dreno B et al. Combined vemurafenib and cobimetinib in BRAF-mutated melanoma. N Engl J Med 2014; 371: 1867-1876.
45. 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.
46. Paraiso KH, Fedorenko IV, Cantini LP et al. Recovery of phospho-ERK activity allows melanoma cells to escape from BRAF inhibitor therapy. Br J Cancer 2010; 102: 1724-1730.
47. Davies MA, Saiag P, Robert C et al. Dabrafenib plus trametinib in patients with BRAFV600-mutant melanoma brain metastases (COMBI-MB): a multicentre, multicohort, open-label, phase 2 trial. Lancet Oncol 2017; 18: 863-873.
48. Long GV, Trefzer U, Davies MA et al. Dabrafenib in patients with Val600Glu or Val600Lys BRAF-mutant melanoma metastatic to the brain (BREAK-MB): a multicentre, open-label, phase 2 trial. Lancet Oncol 2012; 13: 1087-1095.
49. Hussein Abdul-Hassan Tawbi, Peter A. J. Forsyth, Alain Patrick Algazi et al. Efficacy and safety of nivolumab (NIVO) plus ipilimumab (IPI) in patients with melanoma (MEL) metastatic to the brain: Results of the phase II study CheckMate 204. Journal of Clinical Oncology 2017; 35: suppl; abstr 9507.
50. Georgina V. Long, Victoria Atkinson, Alexander M. Menzies et al. A randomized phase II study of nivolumab or nivolumab combined with ipilimumab in patients (pts) with melanoma brain metastases (mets): The Anti-PD1 Brain Collaboration (ABC). Journal of Clinical Oncology 2017; 35: suppl; abstr 9508.
51. Robert C, Karaszewska B, Schachter J et al. Improved overall survival in melanoma with combined dabrafenib and trametinib. N Engl J Med 2015; 372: 30-39.
52. Long GV, Stroyakovskiy D, Gogas H et al. Dabrafenib and trametinib versus dabrafenib and placebo for Val600 BRAF-mutant melanoma: a multicentre, double-blind, phase 3 randomised controlled trial. Lancet 2015; 386: 444-451.
53. Chen G, Chakravarti N, Aardalen K et al. Molecular profiling of patient-matched brain and extracranial melanoma metastases implicates the PI3K pathway as a therapeutic target. Clin Cancer Res 2014; 20: 5537-5546.
54. Amaral T, Sinnberg T, Meier F et al. The mitogen-activated protein kinase pathway in melanoma part I - Activation and primary resistance mechanisms to BRAF inhibition. Eur J Cancer 2017; 73: 85-92.

55. Niessner H, Forschner A, Klumpp B et al. Targeting hyperactivation of the AKT survival pathway to overcome therapy resistance of melanoma brain metastases. Cancer Med 2013; 2: 76-85.
56. Pape E, Desmedt E, Zairi F et al. Leptomeningeal metastasis in melanoma: a prospective clinical study of nine patients. In Vivo 2012; 26: 1079-1086.
57. Harstad L, Hess KR, Groves MD. Prognostic factors and outcomes in patients with leptomeningeal melanomatosis. Neuro Oncol 2008; 10: 1010-1018.
58. Shonka NA, Kessinger AM, Aizenberg MR. Intrathecal interleukin-2 for melanomatous meningitis. J Clin Oncol 2014; 32: e111-113.
59. Hong JJ, Rosenberg SA, Dudley ME et al. Successful treatment of melanoma brain metastases with adoptive cell therapy. Clin Cancer Res 2010; 16: 4892-4898.
60. Grimaldi AM, Simeone E, Giannarelli D et al. Abscopal effects of radiotherapy on advanced melanoma patients who progressed after ipilimumab immunotherapy. Oncoimmunology 2014; 3: e28780.
61. Robert C, Karaszewska B, Schachter J et al. Improved Overall Survival in Melanoma with Combined Dabrafenib and Trametinib. N Engl J Med 2014.
62. McArthur GA, Chapman PB, Robert C et al. Safety and efficacy of vemurafenib in BRAF and BRAF mutation-positive melanoma (BRIM-3): extended follow-up of a phase 3, randomised, open-label study. Lancet Oncol 2014.
63. 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.
64. 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.
65. Ascierto PA, McArthur GA, Dreno B et al. Cobimetinib combined with vemurafenib in advanced BRAF(V600)-mutant melanoma (coBRIM): updated efficacy results from a randomised, double-blind, phase 3 trial. Lancet Oncol 2016; 17: 1248-1260.
66. McArthur G, Dreno B, Atkinson V 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.
67. Cancello G, Montagna E, D'Agostino D et al. Continuing trastuzumab beyond disease progression: outcomes analysis in patients with metastatic breast cancer. Breast Cancer Res 2008; 10: R60.
68. Faehling M, Eckert R, Kamp T et al. EGFR-tyrosine kinase inhibitor treatment beyond progression in long-term Caucasian responders to erlotinib in advanced non-small cell lung cancer: a case-control study of overall survival. Lung Cancer 2013; 80: 306-312.
69. Grothey A, Sugrue MM, Purdie DM et al. Bevacizumab beyond first progression is associated with prolonged overall survival in metastatic colorectal cancer: results from a large observational cohort study (BRiTE). J Clin Oncol 2008; 26: 5326-5334.
70. Shi H, Hugo W, Kong X et al. Acquired resistance and clonal evolution in melanoma during BRAF inhibitor therapy. Cancer Discov 2014; 4: 80-93.
71. Carlino MS, Gowrishankar K, Saunders CA et al. Antiproliferative effects of continued mitogen-activated protein kinase pathway inhibition following acquired resistance to BRAF and/or MEK inhibition in melanoma. Mol Cancer Ther 2013; 12: 1332-1342.