Immune checkpoint inhibitors in mesothelioma

Disselhorst, M.J.

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Chapter 11

Summary

Malignant pleural mesothelioma is an aggressive tumor originating from the mesothelial cells of the pleural cavity. It has a causal relation with (occupational) asbestos exposure. The latency time between asbestos exposure and diagnosis of mesothelioma is 20 to 50 years. Despite the fact that the use of asbestos has been banned for almost 30 years, approximately 500 patients in the Netherlands are diagnosed with mesothelioma every year. Patients are typically men and older than 65 years. The tumor spreads along the pleura and can result in pleural thickening and fluid in the pleural cavity. Leading to symptoms of shortness of breath, chest pain, night sweats, fatigue and weight loss. Upon diagnosis, usually there is no treatment with curative intent, and systemic treatment is given.

This dissertation focuses on the treatment of malignant mesothelioma, with the aim of improving patient outcomes.

Chapter 1 provides a general introduction to mesothelioma, focusing on treatment options, tumor microenvironment, and possible biomarkers.

Part I – Treatment of mesothelioma

This part of the thesis focuses on what is already known about treatment of mesothelioma.

In *Chapters 2 and 3* we provide an overview of systemic treatment of malignant pleural mesothelioma. Standard of care has long been platinum containing chemotherapy plus an antifolate, leading to a median survival of 12 to 16 months. Several trials have been published on the addition of an anti-angiogenesis inhibitor to chemotherapy. Bevacizumab gives a survival gain compared to the control group, but this has not led to a change in the standard approach, especially given the side effects. Other angiogenesis inhibitors do not give any gain over chemotherapy.

Maintenance therapy with pemetrexed, thalidomide or defactinib did not show benefit. In the NVALT19 trial, better progression-free survival is seen with switch maintenance gemcitabine. No single chemotherapy regimen did prove clinical benefit as a second- or third-line systemic treatment over best supportive care.

Other potential treatment options in mesothelioma are mesothelin-targeted therapy. Mesothelin is a tumor antigen that is strongly expressed in mesothelioma. Several agents, for example anti-body drug conjugates, anti-mesothelin immunotoxins and chimeric antigen receptor T cell therapies are being tested in clinical trials, with varying degrees of success.

BRCA1-associated protein-1 (BAP1) has a role in DNA repair. It is inactivated in around 25% of tumors and could be a potential target. This is also being tested in clinical trials.

Immune checkpoint inhibitor treatment, or immunotherapy for short, has emerged as an effective treatment for certain types of cancer. In normal cells, immune checkpoints ensure immune tolerance and prevent autoimmune diseases. In tumor cells, the expression of these inhibitory checkpoints can be dysregulated, causing the cells to become immune resistant and not recognized by the immune system. Binding of, for example, inhibitory programmed death-ligand 1 (PD-L1) on tumor cells to programmed death 1 (PD-1) on immune cells activates an inhibitory signal. Immune cells are thereby inactivated and the tumor can evade the immune system. Immune checkpoint inhibitors can block these inhibitory checkpoints, thereby restoring immune system function and evoking an anti-cancer immune response. In mesotheliomas PD-L1 is expressed in about 25%, and as in other tumor types, is associated with worse survival.

Another inhibitory checkpoint is Cytotoxic T-Lymphocyte-associated protein 4 (CTLA-4). Anti CTLA-4 antibodies impact the lymphoid compartment, increasing the immune T cell response.

In *Chapter 4* a part of the "ESMO handbook immuno-oncology" is published, namely the chapter mesothelioma. It describes several promising phase I and II trials that test PD-(L)1 inhibitors in mesothelioma. The disease control rate (complete response plus partial response plus stable disease, i.e. that is no growth of the tumor) in these trials ranged from 50 to 76%. The overall response rate ranged from 9 to 21%. CTLA-4 inhibitor tremelimumab failed to show a survival benefit in a phase IIB trial.

Combining PD-(L)1 and CTLA-4 inhibitors has been shown to induce synergistic effects in preclinical and clinical trials.

Part II – Clinical research

Chapter 5 describes the INITIATE study, in which this combination is given to investigate whether this is also effective in mesothelioma. It is a prospective phase 2 study with one arm, conducted in the NKI-AvL. Patients should have been treated with platinum-containing chemotherapy at least earlier, and then had progression. They received intravenous nivolumab (anti-PD-1) every two weeks plus intravenous ipilimumab (anti-CTLA-4) every six weeks, the latter being given up to 4 times. Treatment was continued for as long as it was effective, or until serious side effects occurred, and for up to 2 years.

Primary endpoint was disease control rate at 12 weeks, as measured by modified response evaluation criteria in solid tumors (RECIST) for mesothelioma.

35 patients were included in the safety analysis and 34 were evaluable for response assessment at 12 weeks. Of these, ten (29%) patients had a partial response and 13 (38%) patients had stable disease, so disease control was achieved in 23 (68%) of the 34 patients. Safety was similar to known data on this combination treatment, with the exception of infusion-related reactions, which were more common in this study (49%).

In conclusion, the study shows that treatment with combination immunotherapy appears to be effective and well tolerated, that toxicity is largely reversible and considered manageable.

Part III – translational research

In the INITIATE study, all patients did give permission to take biopsies of the pleura prior to treatment and during treatment (after 6 weeks), extra blood was also taken at these times and lung function tests have been performed. Various translational studies have been carried out with all these materials and data.

Some of these studies also used material from the Nivomes study. This is also a prospective phase 2 single arm study, conducted in the NKI-AvL, with the same inclusion and exclusion criteria as the INITIATE study. Patients in this study received intravenous nivolumab only every two weeks, without ipilimumab.

The success of immune checkpoint inhibitors depends on presence and activation of tumor-specific T cells. In *Chapter 6* comprehensive immune cell profiling was performed on pre-treatment and on-treatment peripheral blood samples of patients treated with nivolumab monotherapy (from the Nivomes trial) and patients treated with combination of nivolumab plus ipilimumab (from the INITIATE trial). Characteristics and quantities of the different immune cells can be assessed with this. Combination immunotherapy has been shown to induce a profound increase in proliferation and activation of T cells, which is not seen in nivolumab monotherapy. In addition, in patients that responded to combination treatment had low frequencies of naive CD8 T cells and high frequencies of effector memory CD8 T cells that re-expressed RA (TEMRA) in the pre-treatment blood samples. These TEMRAs also produce cytokines more often. These TEMRAs probably comprise tumor-specific T cells, and need blocking of both PD-1 and CTLA-4 to be reactivated.

In *Chapter 7*, exhaled breath is analyzed. This is a non-invasive and easy-to-use technology for diagnosing and phenotyping a wide range of diseases, for example asthma, lung cancer and mesothelioma. Exhaled breath consists of up to thousands of volatile organic compounds (VOCs) that are produced by (patho)physiological processes of the body.

The electronic nose (eNose) technology can be used for pattern recognition of the mixture of VOCs. Combined sensor signals produce a characteristic breath profile. It is shown that an electronic nose can be used to discriminate upfront between responders and non-responders to pembrolizumab or nivolumab in patients with stage IV NSCLC with an accuracy of 90%.

Here, the eNose (in this case the SpiroNose) is used to predict the response to nivolumab plus ipilimumab. For 31 patients of the INITIATE trial eNose data were available, for 16 responders (including complete response, partial response and stable disease for 6 months) and 15 non-responders (progressive disease and stable disease for less than 6 months). At baseline, the breath profiles differed significantly between responders and non-responders to treatment. The eNose could become a tool for prediction of response to immune checkpoint inhibitors, although this needs to be evaluated in larger trials.

We also assessed changes in breath profiles during the first 6 weeks of treatment with nivolumab plus ipilimumab. We observed a significant change in sensor values from baseline in patients with partial response and progressive disease, though in opposite directions. This suggests the eNose may be used as a monitoring tool to asses prognosis or effect of therapy in mesothelioma. Although this also should to be evaluated in larger trials.

Chapter 8 describes translational data from the biopsies in patients treated with nivolumab from the Nivomes study and nivolumab plus ipilimumab from the INITIATE study. Staining has been done with different markers to be able to characterize the cells. The marker-positive cells are counted using software, the cell density is defined as the amount of positive cells per mm2. Prior to combination treatment, in patients with a partial response at 24 weeks, there are higher cell densities of CD4+, CD8+, FoxP3+ and PD-1+ cells, compared to patients with progressive disease after 24 weeks of treatment. This difference is not seen in patients receiving nivolumab monotherapy.

After six weeks of treatment, there are no significant changes compared to baseline biopsies. Not in number and not in type of marker.

A single marker may not be specific enough to be able to say anything about the tumor microenvironment and thus the effect of treatment with immunotherapy.

Chapter 9 describes the sequencing data on the biopsies of the Nivomes and INTIATE study. Genetic analysis was performed on the freshly frozen samples using RNA and whole genome sequencing. In mesothelioma, structural chromosomal changes are found, which can result in neoantigens. These junctions alone are not predictive of survival after immunotherapy. Different gene sets were also looked at and the gene set 'regulation of antigen processing and presentation of peptide antigen' showed an interaction with the amount of junctions and the combination is predictive of survival in patients treated with immunotherapy.

Finally, *Chapter 10* provides a brief summary of recent developments in the systemic treatment of malignant pleural mesothelioma.