Immune checkpoint inhibitors in mesothelioma

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Chapter 1

General introduction and outline of thesis

Malignant pleural mesothelioma (MPM) is an aggressive tumor originating from the mesothelial cells of the pleural cavity. It has a causal relation with (occupational) asbestos exposure (1).

Asbestos is a group of 6 different mineral fibers naturally occurring throughout the world; all are composed of long and thin fibrous crystals. Two large subgroups are known as the serpentine and amphibole subgroup. Chrysotile (white asbestos) is a serpentine mineral, of which the fibers are relatively large and curly and it is the most commonly used type of asbestos. Amphibole minerals are needle-like and members of this class are amosite (brown asbestos), crocidolite (blue asbestos), tremolite, actinolite and anthophyllite. Asbestos is being used since prehistoric times due to its fire-resistant properties (2). In the last century it has been used extensively in buildings and ship-building, because of its strength, fire-resistance and isolating properties. Furthermore it is cheap. All types of asbestos fibers can cause mesothelioma.

Asbestos is banned from most countries in the world, but it is estimated that approximately 43,000 people will die from this disease worldwide (3). The survival is poor, with a 5 year survival rate in Europe of 7% (4). In the Netherlands, spray asbestos was banned in 1978 and complete use of asbestos in 1993. Unfortunately, exposure is still possible since it is incorporated in many buildings and sheds. With a latency time between asbestos exposure and diagnosis of mesothelioma of 20 to 50 years (1, 5) we are still confronted with 600 patients per year in the Netherlands.

The carcinogenic mechanism of how asbestos can cause MPM is not completely understood. Chronic inflammation may predispose individuals to develop this malignancy as is concluded from microscopic examinations. In the tumor microenvironment (TME) inflammation promotes proliferation and survival of malignant cells (6). Asbestos can cause an influx of mononuclear phagocytic cells into the tumor that internalize asbestos fibers. These phagocytic cells will release proinflammatory cytokines. In combination with chronic inflammation, oxygen radical release and DNA damage, these processes promote malignant transformation. In combination with the immunosuppressive environment, this promotes cancer growth. It has been shown that CXCR3 (the chemokine receptor on the surface of T helper cells) and the production of interferon gamma (IFN-γ) were reduced in peripheral CD4+ cells of asbestos-exposed patients, thereby showing the decreased antitumor immunity of asbestos (7).

Only a minority of asbestos exposed people develop mesothelioma. This might for some cases be explained by genetic susceptibility. Germline mutations in (BRCA1) associated protein-1 (BAP1) tumor suppressor gene cause the BAP1 tumor predisposition syndrome. Carriers have an increased risk of developing

mesothelioma, (uveal) melanoma, renal cell, basal cell and hepatocellular carcinoma. It is thought that loss of BAP1 may predispose to mesothelioma after asbestos exposure. Homozygous deletion of CDKN2A, loss of NF2 or germline PALB2 deletions may also favor the development of MPM (8, 9). Genetic susceptibility can predispose to MPM via chronic exposition.

MPM is classified in 3 histological subtypes, epithelioid, biphasic and sarcomatoid. The sarcomatoid subtype is composed of malignant spindle cells and occurs in 10-15% of MPM, is chemotherapy-resistant and has the worst survival. The epithelioid subtype is the most common variant. It accounts for 50-70% of all mesotheliomas, and is composed of epithelioid polygonal cells. The biphasic subtype has features of both epithelioid and sarcomatoid subtype, larger biopsies are needed to demonstrate both components. Examination of both tumor and surrounding stroma has revealed that features such as inflammation, cellular diversity and vacuolization within the stroma all have a prognostic effect, besides the histopathological findings (10).

Diagnosis

First step in diagnostic process is usually a contrast-enhanced computed tomography (CT) of chest or a positive-emission tomography (PET) with CT, showing pleural enlargement, pleural fluid and sometimes thoracic wall invasion.

A cytological diagnosis of mesothelioma is often difficult when thoracocentesis is used to obtain the pleural fluid. This material provides a diagnosis in 20-50% of patients and only in epithelioid subtype, but it can often exclude other diagnoses. Histological biopsies by thoracoscopy or ultrasound or CT-guided have a high diagnostic accuracy. Immunohistochemistry markers usually include calretinine, cytokeratin 5/6, Wilms Tumor 1 antigen (WT1), those should be positive. Markers for adenocarcinoma should be negative (TTF-1, CEA, Ber-EP4). The sensitivity for sarcomatoid subtype is poor. Absence of BAP1 expression could be an important extra tool, it is lost in up to 60% of cases, most often in epithelioid subtype (11, 12).

In the Netherlands, nearly all mesothelioma diagnoses (and possible diagnoses) are centrally reviewed by an expert pathology board, the "Nederlands Mesotheliomen Panel" because of the rareness of the disease and the difficulty of the diagnosis.

Comprehensive genomic and transcriptomic sequencing of MPM revealed large heterogeneity between patients. Most mutations found inactivation of tumor suppressor genes (f.e. BAP1, CDKN2A, NF2, TP53, SETD2) (13-15). Heterogeneity has been reported

within the tumor location in the chest cavity. Kiyotani examined biopsies of patients at 3 different sites and showed intratumoral heterogeneity in somatic mutations and unique TCRβ clonotypes of TILs (16).

Clinical

Patients with MPM are typically men and older than 65. Symptoms are gradually worsening and include dyspnea, chest pain, cough, night sweats, fatigue and weight loss. Tumor spreads throughout the pleural cavity, and can result in pleural effusions. Metastases are rare, but can involve the lungs, bone, liver and CNS. Most patients present with advanced disease, which is incurable.

Treatment

Surgical treatment for MPM remains controversial in many parts of the world, since it is always incomplete. Whether cytoreductive surgery prolongs overall survival is unclear, studies did not provide a clear positive outcome that outweighs the risk, with high morbidity for surgery. This is beyond the scope of this thesis, which is focused on systemic treatment.

For almost 20 years, platinum containing chemotherapy combined with an antifolate has been the standard of care for patients. Leading to a median overall survival of about 12 to 16 months. Unfortunately, the mean progression free survival (PFS) is only 6 months (17, 18).

The MAPS trial (Mesothelioma Avastin plus Pemetrexed-Cisplatin) showed that standard of care chemotherapy combined with bevacizumab (a monoclonal antibody targeting vascular endothelial growth factor), improved survival over chemotherapy alone (18.8 vs 16.1 months). Although there is a survival improvement, there is also an increased adverse event profile for bevacizumab. So it failed to be approved as standard treatment (19). Other anti-angiogenetic drugs also failed to show benefit (20).

In the past it has been observed that installation of BCG (Bacillus Calmette-Guérin) vaccine immunotherapy could have an improved survival rate for MPM (21).

This led to the idea that the immune system could play an important role in the biology and treatment of MPM. Cancer immunotherapy makes use of the host system to induce

or enhance an effective immune response against cancer cells. Different types of immunotherapy use different parts of the immune system to evoke effect on tumor cells.

Immune checkpoint proteins are crucial for maintenance of self-tolerance. Expression of these proteins is dysregulated in tumor cells, thereby making the tumor cell immune resistant. Immune checkpoint inhibitors (ICI) can block inhibitory checkpoints, thereby restoring immune system function and evoking an anti-cancer immune response.

Anti-Cytotoxic T-Lymphocyte-associated protein 4 (CTLA-4) antibodies impact the lymphoid compartment; increasing the number and broadening the tumor antigen reactive T cells; stimulating priming of naive T cells and enhancing antigen presentation. PD-L1 checkpoints are mainly expressed in activated lymphocytes and exhausted T cells. Anti-PD-(L)1 antibodies can promote T cell activation during the effector phase and can restore exhausted T cell functionality, mainly in the tumor microenvironment.

These immune checkpoint inhibitors are the most widely used agents of cancer immunotherapy and completely changed treatment of many cancer types over the last decade. In 2011, ipilimumab was the first checkpoint inhibitor approved by the FDA for treatment of melanoma (22). Ipilimumab blocks immune checkpoint molecule CTLA-4. After that PD-1 (nivolumab, pembrolizumab, cemiplimab), and PD-L1 (atezolizumab, durvalumab, avelumab) checkpoint inhibitors are approved for many cancer types.

For mesothelioma some promising data on ICI treatment have been reported in the second or later lines, mostly in single arm trials. Single agent PD-1 ICI have consistent objective response rates of about 20%, and disease control rates (DCR) between 48 and 72% in mainly phase II trials (23-28). The single agent CTLA-4 checkpoint inhibitor tremelimumab however, did not show any benefit compared to placebo (29).

The first randomized trial of pembrolizumab (PD-1 antibody) failed to improve PFS or OS over single agent chemotherapy (vinorelbine or gemcitabine) in later lines. Although pembrolizumab did have a higher overall response rate (ORR), 22% versus 6% (P=0.004) (30).

The second phase III trial of monotherapy of anti-PD-1 (nivolumab)showed a survival benefit of nivolumab over best supportive care in relapsed MPM, mOS was 10.2 months (95% CI 8.5-12.1) in the nivolumab group versus 6.9 months (5.0-8.0) in the placebo group (adjusted HR 0.69 [95% CI 0.52-0.91]; p=0.0090). Placebo was used for the comparator arm since no approved second line therapy exists (31).

Combining aPD-(L)1 and aCTLA-4 therapy has been shown to induce synergistic effects in preclinical and clinical trials (32, 33). Combining them can induce a more potent antitumor immune response (34).

This led to setting up a clinical trial in MPM with combination therapy, the INITIATE trial, which is described in chapter in this thesis (35).

For combination treatment with anti-PD-1 plus anti-CTLA-4, the ORR is around 27% and mPFS 6 months in single arm phase II trials, in recurrent disease (27, 35, 36).

In 2021, the Checkmate 743 trial was published. This international randomized phase III trial compared standard of care chemotherapy with combined nivolumab plus ipilimumab. ICI treatment significantly increased overall survival compared to chemotherapy by 4 months (mOS 18.1 months [95% CI 16.8 – 21.4] versus 14.1 months [95% CI 12.4-16.2], HR 0.74 [p=0.0020]). This lead to approval of nivolumab plus ipilimumab as first line therapy for MPM by the FDA and EMA. The benefit is most prominent in the non-epithelioid subgroup, as revealed by a post-hoc subgroup analysis, epithelioid subgroup HR 0.86 (95% CI 0.69–1.08) and non-epithelioid subgroup HR 0.46 (95% CI 0.31–0.68) (37).

Tumor microenvironment

The mesothelioma tumor microenvironment (TME) is composed of heterogeneous stromal, endothelial and immune cells.

The TME in MPM is known to be highly immunosuppressive, with large numbers of tumor associated macrophages (TAMs), myeloid-derived suppressor cells (MDSC) and regulatory T cells (Tregs) (38-41).

Macrophages are plentiful present in MPM, with large heterogeneity, in both the epithelial and non-epithelial subtype. Mesothelial cells produce cytokines, which give chemotactic and stimulatory signals to immune cells of the myeloid lineage and recruit monocytes. In the tumor mass the monocytes differentiate into macrophages. Interleukins such as IL-1, IL-4, and IL-10 produced by tumor infiltrating lymphocytes (TILs) promote differentiation of macrophages towards a certain phenotype. This phenotype is pro-tumorgenic and promotes tumor growth by production of multiple cytokines. Higher percentages of macrophages are negatively correlated with overall survival and are positively correlated to the number of Tregs in tumor microenvironment (42-45).

MDSCs are immature myeloid cells and have immunosuppressive properties. They induce Tregs and produce nitric oxide and arginase, which leads to loss of function of CD4+ and CD8+ T cells (46).

T-Lymphocytes play an important role in the immune defense in cancer. These immune cells may influence tumor growth, but also mediate response to therapy. Twenty to 42% of the cells in the immune infiltrate consist of CD3+ T-lymphocytes. Besides the CD8+ T-lymphocytes, regulatory CD4+ FoxP3+ T-cells are frequently observed (39, 47, 48). Some studies suggest that higher levels of CD8+ T-cells have a favorable prognostic impact, while others found that high CD4+ and CD20+ and low FoxP3+ cells are linked to a better outcome (47-49).

The composition of the TME is different between subtypes, between individuals and within individuals (16, 40).

Biomarkers

Although a number of patients with cancer benefit from ICI treatment, many patients do not. Different mechanisms are proposed to explain these (non)responses to ICI treatment in cancers in general and in mesothelioma specifically.

The number of non-synonymous single nucleotide variants, referred to as the tumor mutation burden (TMB) may affect the odds of generating immunogenic peptides and thereby influence ICI response. In different tumor types the response to ICI treatment is positively correlated to TMB; a higher TMB resulting in a higher overall response rate (50). However, some tumor types respond better (51) and some worse (52) than would be expected based on TMB alone. And even within a specific tumor type some patients respond better than others. So although the association between TMB and ICI response is pretty robust, other factors are involved. MPM shows a rather low mutation rate, so TMB alone does not explain the response rates (13, 14, 16, 53).

It is hypothesized by Mansfield et al. that the number of alterations actually targeted by T cells, may have a stronger association with ICI response than does TMB (54). This includes immunogenic translocations or insertions/deletions, called chromoplexy and chromothrypsis.

A strong expression of the immune-checkpoint gene VISTA was found on tumor cells in epithelioid subtype. VISTA is a negative checkpoint regulator, possibly it avoids

an antitumor immune response (15, 55). The immunoregulatory impact needs to be elucidated.

In MPM, PD-L1 expression on tumor cells is observed in about 40% and is frequently associated with non-epithelioid subtype. PD-L1 expression is a prognostic marker and associated with worse outcome, when used in patients that are not treated with IO agents (56-61).

In NSCLC, PD-L1 expression is predictive of response to PD-1 checkpoint inhibitors. Tumors with higher PD-L1 expression usually respond better to IO treatment. But responses occur even in PD-L1 negative tumors and not all patient with high PD-L1 expression respond to treatment. In different other tumor types PD-L1 expression on tumor cells is not associated with response, whereas PD-L1 expression on tumor-infiltrating immune cells is (62-64).

In some phase II trials with ICI treatment for MPM, a (poor) correlation of PD-L1 expression with objective response rate and/or survival is shown. But in most other trials no correlation was found. Data are inconsistent (24-27, 31).

The predictive role of PD-L1 expression for dual agent ICI treatment has not been established either.

In the Checkmate 743 trial a relatively large amount of patients had PD-L1 positive tumors (77%), PD-L1 expression did not correlate with outcome. However survival with chemotherapy was better in patients with PD-L1 expression of less than 1% than in those with expression higher than 1%, this is probably more prognostic than predictive (35, 37).

In several tumor-types it is shown that density of tumor-infiltrating lymphocytes (TIL) is a positive prognostic indicator (regardless of ICI treatment) (65). In melanoma it is shown that pre-treatment TIL-density at the invasive margin is associated with response to anti-PD-1 treatment (62). Standardization is difficult, especially in MPM, which does not even have a distinct invasive margin.

Not only density of TILs impacts ICI outcomes, but also the type of immune cells. In melanoma, response to ICI treatment relies on pre-treatment infiltration of activated CD8 T-effector cells (62). In many more different cancers the association of infiltrating CD8+ cytotoxic T cells with longer disease free survival and/or overall survival has been demonstrated (66). In NSCLC, CD8 cell infiltration was positively correlated with ORR and PFS in patients treated with PD-1 blockade (67). A positive correlation of CD8+ T cells with overall survival has been reported, but not in all studies. One study even described opposite negative correlation (47-49, 68). This might be caused by sampling bias due to a

heterogeneous distribution in tumors, more advanced stage or from functional variability. The CD8+ cells could be exhausted cytotoxic T cells, with relatively high expression of multiple inhibitory receptors.

One study reported more CD8+ cells in PD-L1+ tumors versus PD-L1- tumors (69). Another study showed a higher ratio of cytotoxic T cells to malignant cells in the sarcomatoid subtype (70). Furthermore, CD8+ cells increased after administration of platinum plus pemetrexed, examined in paired biopsies (71).

For further analyses of mechanism of effect of ICI treatment, longitudinal tumor biopsies are needed. However these are not always possible to obtain, since in patients having a complete or partial response it is no longer possible to biopsy.

Peripheral blood T cells can provide insight of understanding immunological responses induced by ICI treatment. It also can provide biomarkers to monitor or predict response to ICI treatment. In lung cancer, it is shown that an increase in Ki-67+ PD-1+ CD8+ T cells is seen after ICI treatment in most patients. This may indicate activation of tumor-specific CD8+ T cells. These cells co-expressed CTLA-4 after PD-1 antibody treatment (72). In melanoma, presence of neoantigen specific T cells in peripheral blood is shown. Mainly in CD8+ PD-1+ T cells, which account for < 5% of all peripheral blood lymphocytes, patient specific neoantigens that target mutant and/or shared tumor neoantigens in all the melanoma patients (73).

Other blood biomarkers have been a focus in biomarker research, since they are easily accessible, are independent from intra- and inter-tumor heterogeneity, and reflect multiple factors (e.g. tumor cells, tumor-microenvironment and patient's immune system). Inflammation is a mechanism of immune-resistance in patients with cancer, promoting cancer growth and dissemination, based on activating oncogenic signaling pathways. Proposed inflammatory biomarkers that might be prognostic or predictive include LDH, CRP, white blood cells, absolute neutrophil count, neutrophil to lymphocyte ratio (NLR) derived neutrophil to lymphocyte ratio (dNLR; absolute neutrophil count/(white blood cell concentration – absolute neutrophil)). In melanoma a pro-inflammatory status is correlated with poor outcomes in patients treated with ICIs (74, 75). In NSCLC pretreatment Lung Immune Prognostic Index (LIPI), combining dNLR greater than 3 and LDH greater than ULN was correlated with worse outcome for ICI, but not for chemotherapy (76).

Exhaled breath analysis has shown potential as a non-invasive and easy-to-use technology for diagnosis and phenotyping of a wide range of diseases including mesothelioma and lung cancer. Electronic nose (eNose) technology can be used for this breath analysis (77-81). This eNose could be used for immunotherapy response in lung cancer. In lung cancer

it has been shown that exhaled breath analysis before start of treatment could identify patients that show progressive disease to anti-PD-1 therapy, thereby ICI treatment could possibly be with-held (82). In addition, it can identify patients with an objective response to anti-PD-1 therapy early during treatment (83).

Outline of thesis

This thesis aims to contribute to a better treatment of malignant pleural mesothelioma, and is specifically evaluating dual checkpoint inhibitor treatment. Besides the clinical effect of ICI treatment also the search for an explanation for the effect of this treatment, thereby aiming to predict response to treatment.

Part I summarizes what is known about treatment of mesothelioma

Chapter 2 is a review that discusses optimal systemic therapy for patients with advanced MPM. Including first-line, maintenance and second-line therapy, as well as antibody drug conjugates and targeted agents.

Chapter 3 is a review that focusses more in detail on novel treatment options in MPM, including immune checkpoint inhibitors.

Chapter 4 is a chapter from the ESMO handbook Immuno-Oncology on mesothelioma, describing what is known about immune checkpoint inhibition in MPM.

Part II is the clinical part of this thesis.

Chapter 5 describes the single center, single arm, phase 2 clinical INITIATE trial of nivolumab plus ipilimumab. This combination of checkpoint inhibitors shows marked efficacy in MPM, with no new safety concerns.

Part III is the translational research part of the thesis.

In chapter 6 immune cell profiling was performed on screening and on treatment peripheral blood samples of MPM patients treated with nivolumab (anti PD-1 antibody) monotherapy or a combination of nivolumab and ipilimumab (anti-CTLA-4 antibody). High proportions of effector memory CD8 T cells that re-expressed RA (TEMRA) and cytokine production by TEMRAs before treatment was associated with a better clinical outcome.

In chapter 7 exhaled breath analysis of volatile organic compounds by electronic technology (eNose) is performed in patients treated with nivolumab plus ipilimumab. An eNose is able to discriminate between responders and non-responders to treatment at baseline.

In chapter 8 immunohistochemistry analysis was performed on baseline and on-treatment biopsies from INITIATE trial and from a clinical trial using nivolumab in MPM. Cell density of CD4+, CD8+, and FoxP3+ cells is higher in patients having a response to nivolumab plus ipilimumab compared to patients having progressive disease at 24 weeks.

In chapter 9 RNA and whole genome sequencing was performed on the same biopsies as described above. A particular gene set demonstrated an interaction with tumor junction burden and was predictive of overall survival. Thus, analysis of structural variants and gene expression may facilitate patient selection for immune checkpoint inhibitors.

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