The search for new treatment strategies for malignant pleural mesothelioma

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Chapter 6

General Discussion

Malignant pleural mesothelioma (MPM) is a malignancy of the mesothelial cells lining the pleura [1-4]. There are three histological subtypes of MPM: epithelioid (60% of the cases), sarcomatoid (20% of the cases) and biphasic (20% of the cases), the later containing both epithelial and sarcomatoid cells [5-7]. The occurrence of MPM is strongly associated with asbestos exposure. Due to the latency period between exposure and development of MPM, ranging from 20 to 50 years, MPM is still diagnosed. The incidence of MPM is slightly increasing over the last years and is not expected to decrease before 2020 [3, 8-10].

The treatment of MPM consist of the chemotherapeutic combination of cisplatin with pemetrexed. This combination showed an overall survival (OS) benefit of 16.1 months versus 9.3 months for patients who only received cisplatin [11, 12]. Since 2003, there are no new treatments licensed, even though there are many clinical trials conducted. An overview of these trials, till 2016, is given in **chapter 1**.

Finding new treatment strategies suitable for MPM is challenging. The mutational load in MPM is low/ intermediate and dominated by mutations in tumor suppressor genes rather than oncogenes. The tumor suppressor genes that are frequently mutated in MPM are cyclin-dependent kinase inhibitor 2A (CDKN2A), neurofibromina 2 (NF2) and BRCA associated protein 1 (BAP1) [13-18]. The absence of drugable molecular targets makes the search for targeted therapy very difficult. Heterogeneity is another explanation why a treatment, suitable for all patients with MPM, is difficult to find. Survival in MPM is associated with histological subtype [19, 20], indicating the impact that inter-patient heterogeneity can have on clinical trial results. It is therefore important to stratify on histological subtype in clinical trials. Recent findings also indicated that MPM could be a polyclonal tumor [21]. Although not a lot is known on this subject, a polyclonal origin would suggest high intra-tumor (genetic) heterogeneity, which is likely to contribute to unresponsiveness of MPM to most treatments.

Personalized treatment

Personalized treatment can be more successful than finding a treatment strategy designed for all patients with MPM. In **chapter 2** we present a method of chemically profiling primary MPM cultures with commonly used anticancer drugs. Patients' own tumor cells, isolated from pleural fluid, were tested for multiple chemotherapeutics to select the best therapeutic option. Because therapy response forms the basis for therapy selection, the biology and the molecular mechanism of the tumor are less relevant and, for the same reason, it is not necessary to select patients with biomarkers.

Unfortunately, this method is not suitable for patients that do not develop pleural fluid.

Thereby, in 50% of the cases it was not possible to successfully screen the primary tumor culture, because of lack of tumor cells. The personalized treatment method is furthermore limited by the fact that it cannot test immuno-oncology drugs, due to the absence of the immune micro-environment.

We showed a strong correlation between the *in vitro* and *in vivo* response in the first ten patients that were treated based on their chemical profile. We foresee that this approach will lead to an improved selection of patients suitable for a specific treatment, especially when the number and classes of compounds is expended and not restricted to commonly used anticancer drugs. In addition, this personalized treatment method may also prevent the use of therapies which are doomed to fail and will only lead to increased toxicity for the patient. However, further validation of this technology is necessary and currently ongoing in a phase II trial (PeRsOnalized treatment fOr patients with pleural eFfusions due to malignant pleural mesothelioma or lung cancer in second or third line (PROOF study)).

Besides personalizing treatment, based on all chemical profiles we could distinguish three groups, so called non-responders, intermediate responders and responders. It is expected that, with more chemical compounds, the intermediate group can be subdivided in two or even more groups. This unique way of classifying MPM, based on drug sensitivity, is not shown before. Transcriptomic analysis of these groups revealed corresponding gene signatures, which made it possible to identify new targets for the treatment of MPM subgroups. Focusing on the non-responder group, the group in which it is most important to find new treatment options, we identified that several genes playing a role in the fibroblast growth factor (FGF) pathway were upregulated. Elaborating on this, we treated non-responder cultures with FGF receptor (FGFR) inhibitors and showed they were highly sensitive. This shows chemical profiling of primary MPM cultures can help identifying new treatments for MPM.

FGFR inhibitors

In a high throughput chemical inhibitor screen we identified that a subset of immortalized and primary cell lines were sensitive for FGFR inhibitors, as described in **chapter 3**. We showed that the sensitive lines were dependent on FGFR3 mediated signaling regulated by BAP1.

Our results are in line with others that showed patients with MPM could benefit from FGFR inhibitors [22-24]. It was published that FGF1 and 2 and FGFR1 were highly expressed in MPM biopsies [22-24]. Treatment of cell lines or mice with MPM tumors with FGFR inhibitors resulted in impairment of proliferation and a reduction of the tumor burden [22, 23]. This

indicates FGFR sensitivity is not only dependent on FGFR3 mediated signaling, but also on FGFR1 mediated signaling.

The only FGFR inhibitor that was tested in clinical trials with MPM was dovitinib. Dovitinib is a tyrosine kinase inhibitor (TKI) that predominantly inhibits vascular endothelial growth factor receptor, but also FGFRs [25]. The phase II study was halted due to minimal activity and poor tolerability [25]. It is possible that dovitinib was not potent enough to inhibit FGFR. However, another explanation in line with our results, is that only a selection of patients is sensitive to FGFR inhibitors. We showed BAP1 protein expression could serve as a biomarker for FGFR inhibitor therapy. Protein expression detected with immunohistochemistry was consistent with mutation data found by sequencing [26-28]. BAP1 is mutated in 47% to 67% of the tumors [13, 26-31] indicating FGFR inhibitors could be useful in a large group of patients with MPM.

BAP1

The group of patients with somatic mutations in BAP1 could also benefit from other therapeutics. As we describe in **chapter 5**, BAP1 is a tumor suppressor gene with many regulatory functions in transcription, cell cycle control, DNA damage repair and cellular differentiation. The many interaction partners or downstream substrates of BAP1, such as histone 2A (H2A), enhancer of zeste homolog 2 (EZH2) and host cell factor 1 (HCF1), may function as attractive drug targets. However, the exact molecular mechanism of BAP1 function is not yet clarified and many interaction partners of BAP1 can also play a role in downstream signaling of NF2 and CDKN2A, two other tumor suppressor genes frequently mutated. It is also described that a subset of patients have mutations in two or three of these genes [14, 15]. This indicates the molecular mechanism of MPM, in which different pathways play a role, can be difficult to unravel.

Therefore, treating MPM is complicated and combining targeted therapies is necessary to optimize survival in MPM. In general, combination therapy is often based on a novel agent combined with an approved drug, or combining two approved drugs [32]. However, with new insights in the molecular pathways it will be more promising to combine two or even more novel agents.

Combining these novel agents will give challenges in which the molecular pharmacology of both drugs plays an important role. What is the optimal dose of each drug? How long should there be dosed and in which schedule? Also toxicity issues make combination therapy challenging. Overlapping toxicities of the individual drugs could lead to accumulation of toxicity and gives a narrow therapeutic window [32]. With a cocktail of therapies, the

pharmacology and toxicity issues become even more challenging. A good design of clinical trials is therefore very important.

Antibody-drug conjugates

In chapter 1 we describe three developments that will improve prospects for patients with MPM: 1. personalized treatment (chapter 2), 2. better understanding of the genetic make-up of MPM (chapter 5) and 3. immunotherapy. One treatment strategy gaining more interest in MPM, was not described in this chapter: antibody-drug conjugates (ADCs). ADCs consist of a drug conjugated to an antibody targeting the tumor cells [33-35]. Anetumab ravtansine, a human anti-mesothelin antibody conjugated to the maytansinoid tubulin inhibitor DM4, was the first ADC clinically tested in MPM. Mesothelin is a cell surface antigen with unknown function that is expressed in normal mesothelial cells and overexpressed in most epithelial MPM tumors, but not in sarcomatoid MPM [36-38]. In preclinical research anetumab ravtansine was cytotoxic for MPM cell lines and showed antitumor activity in mouse models [39]. However, the primary end point, progression free survival, was not met in the phase II trial [40].

In **chapter 4**, we present the effects of 5T4 targeting ADCs. 5T4 is only expressed in tumor cells, making it an excellent candidate for this treatment strategy. We showed that most MPM tumors express 5T4, making it a suitable antigen for ADC targeted therapies in MPM. Subsequently, we showed that the ADC is internalized in MPM cells and enters the lysosomal compartment to release the associated toxin. Unexpectedly, the 5T4 ADCs were only able to kill high 5T4-expressing cells and not the low expressing cells.

To make this treatment strategy suitable for more patients, the minimal expression of 5T4 required to kill the cells (the threshold expression) should be lowered. This is possible by changing the linker and/ or drug of the ADC or by using antibodies with a higher affinity for the target. Each change, however could also lead to unwanted toxicities and should therefore be carefully tested. Another problem of ADC treatment in MPM is lysosomal sequestration of the ADC. Neutralizing the lysosomes by adding chloroquine to the treatment schedule could solve this problem, but this should be further tested in physiologically relevant pre-clinical models.

Before the 5T4 targeting ADCs could be tested in the clinic, they should be further optimized and tested in other relevant models such as mouse models. However, in general, ADC treatment is an elegant strategy by limiting the toxicity to the target cells, minimizing side effects. Because not all biopsies express 5T4 and many MPM tumors progress on treatment at some point, it is important to find more targets specific for MPM tumor cells. Biomarkers

studies could provide more and new options for the ADC treatment strategy.

Other treatment modalities in MPM

Other treatment modalities with positive results on the prognosis of MPM, not studied in this thesis, are the anti-vascular agent bevacizumab and immunotherapy.

First-line treatment

The addition of the anti-vascular agent bevacizumab to standard of care chemotherapy is so far the only progress that was recently established in the first-line treatment of patients with MPM. Bevacizumab is an antibody binding the vascular endothelial growth factor (VEGF). VEGF expression levels are high in most MPM biopsies and VEGF signaling plays a part in MPM cell physiopathology [41, 42]. Addition of bevacizumab to the first-line treatment gave a significant longer survival (18.8 months) compared to cisplatin and pemetrexed (16.1 months) [12]. Because inclusion criteria and study design could have influenced OS, the standard first-line treatment is not yet adjusted.

Immuno-oncology therapeutics

Immunotherapy in MPM is mainly focused on immune checkpoint inhibitors against cytotoxic lymphocyte antigen 4 (CTLA-4), programmed cell death protein 1 (PD-1) and its ligand PD-L1 [43, 44]. Although two single-arm phase II trials with tremelimumab, a selective antibody against CTLA-4 [44, 45], showed promising results [46, 47], the large double-blind placebo-controlled phase IIb trial DETERMINE did not improve OS [45]. Pembrolizumab and nivolumab are molecular antibodies against PD-1 [48, 49] and avelumab and durvalumab block PD-L1 [49]. For both pembrolizumab and nivolumab, response percentages of around 25% are reported [50-53], while avelumab showed a response rate of 14.3% in PD-L1 positive patients [54] and a clinical trial with durvalumab is ongoing (NCT02899195). The promising early results with monotherapy blockers resulted in combining CTLA-4 blockers with PD-1/PD-L1 blockers to enhance T-cell activity in a complementary way. Clinical trials with durvalumab and tremelimumab (NCT02588131, NCT03075527, NCT02592551) or ipilimumab and nivolumab (NCT02716272, NCT02899299) are now recruiting patients.

Response rates in the first clinical trials show that immunotherapy in MPM is promising. However, not all patients will respond to immunotherapy. It is therefore important to find markers that could select patients that will benefit from this therapy. One of the markers that is tested is PD-L1, which is expressed in 20% to 70% of the MPM biopsies [55-59]. Overall, PD-L1 expression was not a good biomarker for PD-1 or PD-L1 blockers. For CTLA-4 blockers there are no predictive biomarkers available [20]. Whether tumor immune infiltrates, like lymphocytes or other tumor molecular features, could be predictive biomarkers should be

further investigated.

Gaps in MPM research

Although our understanding of the molecular and biological behavior of MPM has increased, there are still knowledge gaps in MPM research. As mentioned above, novel biomarkers would strongly facilitate selecting patients for chemotherapy, immunotherapy or targeted treatments, but it can also play a role in finding new targets for ADC treatment.

The insight into MPM genetics provides many opportunities for drug development, however the molecular mechanism behind these genes and the interaction between the mechanisms are not fully understood yet. Insight in the molecular mechanism is very important as it will indicate targetable pathways for which candidate drugs could be explored.

Finally, a rather unexplored research area is heterogeneity in MPM. A better understanding of this topic will undoubtedly strongly improve our insights in how to treat this tumor.

Join forces

To address the major challenge and knowledge gaps in MPM, and to implement this knowledge in the clinic, it is of vital importance to join forces.

Physicians and researchers.
Many drugs evaluated in phase Ib/II clinical trials are tested without a good rational or decent preclinical research. History has proven that this approach has resulted in many failures. To increase the success rate of clinical trials, it is very important that physicians and researchers collaborate. Not only to translate preclinical results to clinical trials, but also to translate clinical problems to concrete research questions. Thereby, high-quality translational research is only possible when clinical trials are conducted in such a way that they facilitate research. With more clinical material available, more research can be performed. Chapter 2 is a strong example of close collaboration between doctors and researchers, which provided the very basis of true personalized therapy in MPM.

Researchers and researchers.
When researchers from different fields work together, more therapies could be developed for patients with MPM. For instance, as described above, biomarker research can help developing new antibody drug conjugates. Furthermore, research on epigenetics in MPM revealed new targetable pathways. Fundamental research further exploring these pathways will give insights in candidate targets. New compounds focusing on these targets could then

be preclinically tested. The EZH2 inhibitor tazemetostat is a prime example, which illustrates that genomic studies in MPM and further exploration of downstream pathways can yield new treatment opportunities in MPM [60]. Undoubtedly, there are more opportunities to find new drugs or combinations of drugs.

Physicians and physicians.

The population of patients with MPM is small. To conduct large clinical trials, it is important that doctors from different centers collaborate. Furthermore, to be able to compare small phase I/II trials with each other, it is critical that trials are conducted in a uniform matter. That is only possible when doctors work together. When patient populations and sample collection are similar between trials, translational research from different trials can be interpreted in a better way. MPM research, and ultimately the patient, will benefit from this. Our understanding of MPM showed a great improvement, which resulted in promising new treatment strategies. However, there are still a lot of opportunities. By joining forces (even more) we will further improve the survival of patients with MPM.

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Addendum