Personalizing treatment for malignant pleural mesothelioma

Quispel-Janssen, J.M.M.F.

CHAPTER 7

Discussion and Future Perspectives

Mesothelioma research

Research in mesothelioma is notoriously difficult for several reasons. The patient population is small -around 500 new patients a year in the Netherlands- and heterogeneous in presentation. The three main histological types of mesothelioma each have their own disease course in time and response to treatment. Within the epithelial type, there can be large differences in prognosis and responses to therapy. It is likely that genetic variation in the tumor contributes to this heterogeneity. Commonly, the physical condition of patients with mesothelioma is negatively affected by disease symptoms and this reduces the – already small- number of patients eligible for clinical trials and research. The majority of mesothelioma patients has been exposed to asbestos. This material is evidently carcinogenic but it takes a long time to induce cancer; the latency period between asbestos exposure and a diagnosis of mesothelioma is somewhere between 30 and 50 years.

In sophisticated mouse models, the time needed to develop mesothelioma has been reduced significantly [1], but tumor induction still takes several months. Moreover, most mice develop sarcomatoid mesothelioma while in humans, the vast majority has epithelial type, making a mouse model not representative for the bulk of human mesothelioma patients. Cell lines grow faster and are easier to handle than tumors in mice. Long established cell lines however, acquire changes that adapt the cells to life in an artificial medium on plastic. In addition, selection for the fastest growing cell occurs. The longer cells are cultured, the less they resemble the original tumor due to selection pressure. This phenomenon is called genetic drift. We aimed for an *in vitro* model more representative of the original tumor and better reflecting the genetic diversity seen in mesothelioma tumors. Therefore, we developed a short-term primary tumor culture model from tumor cells derived from pleural fluid of patients with mesothelioma.

Mesothelioma short-term primary tumor cultures

The diagnosis of malignant pleural mesothelioma is often complicated. Many different conditions present with pleural fluid and mesothelial cells are shed in this fluid regardless of the underlying condition. On cytological examination, the distinction between reactive and malignant cells cannot be made by hematoxylin eosin (HE) staining. For a definitive diagnosis of mesothelioma, invasive growth on a histologic specimen is required. A pleural fluid sample of a patient diagnosed with mesothelioma contains a mixture of both reactive and malignant mesothelial cells. A known feature of a tumor cell is continuous growth potential. Therefore, one would expect that tumor cells outgrow reactive mesothelial cells, when cultured *in vitro*. However, this does not seem to be the case. We propagated cells derived from pleural fluid and analyzed them by comparative genome hybridization

(CGH). We found that after many passages the CGH patterns normalized and deletions in the genome disappeared, indicating overgrowth of normal mesothelial cells in favor of tumor cells. For this reason, we use our primary tumor cultures only for a short period of time to assure that we have tumor cells in our experiments. Another disadvantage of our model is that we only culture tumor cells from patients that actually have pleural fluid. The sarcomatoid type usually does not produce pleural fluid and in the scarce sarcomatoid cases that do present with pleural fluid, only few tumor cells are shed into this fluid. Therefore, this type is underrepresented in our model. However, the sarcomatoid type represents less than 10% of all mesothelioma [2], so we miss out on only a fraction of patients.

Chemical and pharmacogenomic profiling

Each model is a simplified version of its original and simplification can lead to certain drawbacks. Tumor cells in pleural fluid are easier to extract from the patient than tumor cells that grow in solid tissue. However, cells that have shed into pleural fluid may have different properties than cells that are strongly attached to a solid tumor. The group of Broaddus demonstrated that tumor cells grown in 2 dimensional layers respond differently to certain drugs than 3 dimensional growing tumors; a phenomenon called multicellular resistance [3]. The dual intention of our culture model was 1) to predict the best chemotherapy for an individual patient by testing sensitivity of its tumor cells to a small number of clinically used chemotherapy regimens (chemical profiling to personalize treatment as described in chapter 4) and 2) to expand the number of existing mesothelioma cell lines with several short-term tumor cultures for screening a large number of different drugs and correlating the results with genomic data (pharmacogenomic profiling as described in chapter 5).

Culturing in 3D models is more challenging and time consuming than in 2D models and large-scale drug screening is not possible. Therefore, we accepted the limitations of our 2D model and demonstrated that multicellular resistance was not an issue with the drugs that were found to be effective (FGFR inhibitors) by also testing them in an *in vivo* model. As for the chemical profiling and prediction of the best chemotherapeutic drug(s) for a patient, multicellular resistance is not a problem either since all drugs tested have already proven their value in clinical trials and practice. Several other factors can influence the outcome of our drug sensitivity screens, for example the time of drug exposure and the cut-off levels that were set. Ideally, one would use a test cohort and a validation cohort for determining the cut-off levels but patient numbers were too small for this. That our cut-off levels are indeed well chosen is demonstrated by the RNA sequencing data that demonstrate the 3 groups to be distinctly different.

FGFR inhibition in mesothelioma

Exome sequencing has demonstrated a low mutational load in MPM when compared to other tumor types (Figure 1) [4-6]. The chance of a targetable mutation is highest in tumor types with a high mutational load like non-small cell lung cancer (NSCLC) and melanoma. Several sequencing studies demonstrated loss of tumor suppressor genes as the most common type of mutation in mesothelioma [5-7]. Our pharmacogenomic profiling study confirmed this. Furthermore, we saw increased sensitivity to inhibition of the FGF pathway, both in immortalized cell lines as in short-term cultures. This is previously described in mesothelioma cell lines [8, 9]. FGFR inhibitors so far are mostly 'dirty' drugs targeting not only FGFR but also PDGF and VEGF. Several clinical trials studied the efficacy of FGFR inhibitors in mesothelioma. A study using dovitinib, inhibiting both VEGF and FGFR, was halted prematurely due to lack of activity and poor tolerability [10]. The LUME-meso trial, a large double-blind, randomised, placebo-controlled phase lll study using the multi-RTK inhibitor nintedanib showed no difference in PFS between the study group and the placebo group [11]. A phase Ib trial combining cisplatin and pemetrexed with a FGF ligand trap was recently published and showed a response rate of 44% and PFS of 7,4 months in the group using 15mg weekly. Four out of 36 patients had durable responses lasting over a year [12]. In comparison, the trial by Vogelzang in 2003 setting the standard in mesothelioma treatment, showed an ORR of 41,3% and a time to progression (TTP) of 5,7months [13]. These results show that FGFR inhibition in an unselected population has only minimal activity. However, the durable responses in the trial with the FGF ligand trap suggest that a selected group of patients may benefit from FGF pathway inhibition. The FGF pathway is complex and finding the right biomarker for selecting patients sensitive to FGFR inhibition is challenging. We found a correlation between loss of BAP1 expression and sensitivity to FGFR inhibition but this is not a straight forward biomarker as is, for example, an activating EGFR mutation in NSCLC predicting for sensitivity to EGFR-TKI's. Other, possibly still unknown, factors may play a role in the FGF pathway. Schelch recently described that loss of micro RNA (miR) 15/16 in MPM leads to loss of post-transcriptional control of the FGF-axis [14]. This suggests that combination of miRNA mimics and FGF pathway inhibitors may have synergistic effects but above all, it illustrates the importance of fundamental research to elucidate all aspects of growth and development of cancer cells.

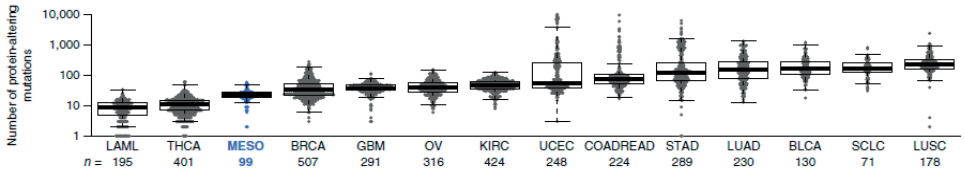

Figure 1. Mutational load for different tumor types.

Bueno *et al*. Nature Genetics 2016

BAP1 in mesothelioma

The most common genomic alterations in mesothelioma are found in the genes *CDKN2A (56%),* NF2 (74%), BAP1 (57%) [5, 6, 15]. The BAP1 protein is a deubiquinating enzyme located in the nucleus. Ubiquitination and deubiquitination are post-translational protein modifications with a number of effects: they can affect protein activity, alter their cellular localization or mark them for degradation. BAP1 protein interacts with several proteins or protein complexes involved in transcription regulation, DNA damage repair, cell differentiation and cell cycle control [16]. Although its function is not fully elucidated, there is clear evidence that loss of BAP1 protein can contribute to cancer development. In mesothelioma, absence of BAP1 protein occurs most commonly through chromosomal deletions of the 3p21.1 region or somatic inactivating mutations of the BAP1 gene [7]. Germline BAP1 mutations give rise to a tumor predisposition syndrome with increased risk of developing melanomas, mesotheliomas and renal cell carcinomas [17-21]. In most cell types BAP1 deficiency causes apoptosis by suppressing expression of prosurvival genes such as bcl2 and mcl1, but not in melanocytes and mesothelial cells explaining the tumor predisposition sites [22]. In our *in vitro* experiments we found a correlation between low BAP1 expression and sensitivity to FGFR inhibition. Although BAP1 loss was not predictive for FGFR inhibitor sensitivity in 100% of cases and the exact mechanism cannot be explained with our current knowledge, the correlation was demonstrated to be plausible by functional assays using BAP1 knock outs and BAP1 constructs by *in vitro* and by *in vivo* experiments. Loss of BAP1 protein expression is easy to assess by immunohistochemistry [23] and thus BAP1 meets one of the requirements of a predictive biomarker. Ideally, a predictive biomarker explains how it predicts for sensitivity. Unfortunately, reality is that biomarkers like activating EGFR mutations in NSCLC where the exact mechanism is known, are extremely rare. Further research to unravel the complexity of the FGF pathway and the multiple functions of BAP1 and validation of BAP1 as a biomarker in a large patient cohort -challenging given the small patient population in mesothelioma- is needed.

Immunotherapy In mesothelioma

Immunotherapy has brought a remarkable improvement in quality of life to those patients that respond to it. In our NivoMes trial, we reported a response rate of 26% which is in line with the response rates in NSCLC and other tumor types [24-27]. Apart from the patients with a significant decrease in tumor volume, there was a group of patients that demonstrated long-term stable disease (>6 months), adding to a total of 39% of patients considered to have clinical benefit from treatment with nivolumab. Compared to the tolerability and response rates of second line cytotoxic therapy in mesothelioma (ranging between 7% and 20% [28, 29]), immunotherapy is a tremendous asset for this disease. But since clinical benefit is still limited to a small group of patients, there is a pressing need for a biomarker that predicts for response, especially given the long median time to response (2,6 months in our trial with one patient reaching response only after 18 weeks) and the phenomenon of pseudoprogression. Several different biomarkers are under investigation. Expression of PD-L1 is amongst the most studied ones. In our NivoMes trial, we detected responses irrespective of PD-L1 expression. High tumor mutational load was reported to predict for response to immune checkpoint inhibition across several tumor types [30]. In mesothelioma however, mutational load is extremely low [5]. Microsatellite instability (MSI) is known to cause a multitude of somatic mutations in tumor cells resulting in a high tumor mutational load, a large lymphocytic infiltrate and increased neoantigen expression, all correlated to response to checkpoint inhibition [31]. Based on these results, the FDA has granted accelerated approval to pembrolizumab in tumor types with MSI. Evidence is emerging that loss of BAP1 expression is correlated to an inflamed tumor microenvironment [32]. In uveal melanoma, CD3 and CD8 positive T cells were more abundantly present in the tumor microenvironment of BAP1 deficient tumors [33]. In peritoneal mesothelioma, BAP1 loss was associated with increased expression of several immune checkpoint molecules [34]. Analysis of 74 pleural mesothelioma samples from The Cancer Genome Atlas (TCGA) revealed upregulation of IRF pathways in BAP1 deficient samples. IRF8 is involved in CD103-positive dendritic cells that have a role as antigen-presenting cells in stimulating cytotoxic T cells in the tumor microenvironment [6]. A gene called VISTA (V-type immunoglobulin domain-containing suppressor of T-cell activation) was recently found to repress activation of T-cells and to be highly expressed in epithelioid mesothelioma. High expression of this gene may thus serve as a negative predictor for immunotherapy [6]. Loss of the gene PBMRI, involved in epigenetic regulation, was recently described to correlate to increased T-cell infiltration and efficacy of checkpoint inhibition [35, 36]. Given the complexity of the immune system and the genomic variation that exists among different cancers, it is likely that we will need sets of biomarkers to predict response to immunotherapy, rather than one biomarker that is applicable in all tumor types. Combinations of several types of immunotherapy and immuno- and chemotherapy hold a strong promise for the future.

A combination of immunotherapy and cytotoxic chemotherapy is currently investigated in mesothelioma in the PreCOG trial (NCT0289919). Results have to be awaited.

Future perspectives

The Netherlands houses a lot of knowledge on mesothelioma. First of all, tumor samples of each patient suspected of having mesothelioma, are validated by a panel of expert pathologists (Nederlands Mesotheliomen Panel NMP) making the diagnoses highly reliable. Secondly, there is an institute for asbestos victims (Instituut Asbest Slachtoffers IAS) that documents the extent of asbestos exposure and performs epidemiological research. Furthermore, there is a national cancer registry (Integraal Kankercentrum Nederland IKNL) including data of all cancer patients in the Netherlands that is very accurate. International acknowledged scientists perform high quality research with international collaborations with several outstanding institutes. In addition, a motivated working group of the Dutch association of pulmonologists (Nederlandse Vereniging voor Artsen voor Longziekten en Tuberculose NVALT) with members across the whole country, form a network to improve quality of care and research by composing guidelines and performing clinical trials. Patients are keen on participating in trials which can be illustrated by the quick accrual of the NivoMes trial for which patients had to have 1 or 2 extra surgical interventions. It would be fantastic to build a large biobank for research by gathering biopsies from all new patients –since the samples are all sent to the NMP for diagnosis, the infrastructure is already in place- together with a sample of blood and basic clinical data. Financial support is usually the limiting factor in propositions like these. Perhaps the Dutch government can provide this to compensate for their past and current omissions, namely 1. installing a ban on the use of asbestos only as late as 1993 while the health threats have been known much earlier and 2. keeping the unethical statute of limitations of 30 years in legal liability cases for a disease that presents commonly only after 30-50 years .

A financial and logistic challenge that our society faces is to get rid of all the asbestos that is used in the Netherlands during the last centuries. The system that is built to asses the extent of asbestos pollution and remove it, has grown to be a complicated industry that keeps prices high by sticking to excessive and incomprehensible rules. As much as eighty percent of all mesothelioma patients have had verifiable asbestos exposure. The risk of getting mesothelioma after extensive exposure is, on the other hand, as low as 5%. This number is calculated from a large cohort (6489 men and 419 women) of heavily exposed asbestos workers who were employed in the asbestos mine or mill in Wittenoom, Australia and were followed for over a period of 50 years [37]. This suggests that additional factors including genetic predisposition may be critical to develop mesothelioma since 95% of asbestos exposed workers did not develop mesothelioma. The previously mentioned BAP1 predisposition syndrome is in line with this hypothesis. It is not reasonable that we fear each

individual asbestos fiber. Instead we would better reform the asbestos renovation industry and screen their workers for this BAP1 tumor predisposition syndrome, and if present, persuade them to abandon this industry and re-educate. A minimum age of 55 years for asbestos renovation workers may also reduce the risk of developing mesothelioma given the long latency period. These propositions can only be introduced after a serious discussion in society.

It is astonishing that while all this effort and money is put into research to treat mesothelioma, asbestos - the main causative agent- is still used and produced in the majority of countries worldwide. Prevention of this disease should be key and this can only be achieved by a complete ban on mining and use of asbestos globally.

References

1. Jongsma, J., et al., *A conditional mouse model for malignant mesothelioma.* Cancer Cell, 2008. **13**(3): p. 261-71.
2. Travis, W.D., et al., *Introduction to The 2015 World Health Organization Classification of Tumors of the Lung, Pleura, Thymus, and Heart.* J Thorac Oncol, 2015. **10**(9): p. 1240-1242.
3. Barbone, D., et al., *Mammalian target of rapamycin contributes to the acquired apoptotic resistance of human mesothelioma multicellular spheroids.* J Biol Chem, 2008. **283**(19): p. 13021-30.
4. Martincorena, I. and P.J. Campbell, *Somatic mutation in cancer and normal cells.* Science, 2015. **349**(6255): p. 1483-9.
5. Bueno, R., et al., *Comprehensive genomic analysis of malignant pleural mesothelioma identifies recurrent mutations, gene fusions and splicing alterations.* Nat Genet, 2016. **48**(4): p. 407-16.
6. Hmeljak, J., et al., *Integrative Molecular Characterization of Malignant Pleural Mesothelioma.* Cancer Discov, 2018. **8**(12): p. 1548-1565.
7. Bott, M., et al., *The nuclear deubiquitinase BAP1 is commonly inactivated by somatic mutations and 3p21.1 losses in malignant pleural mesothelioma.* Nat Genet, 2011. **43**(7): p. 668-72.
8. Schelch, K., et al., *Fibroblast growth factor receptor inhibition is active against mesothelioma and synergizes with radio- and chemotherapy.* Am J Respir Crit Care Med, 2014. **190**(7): p. 763-72.
9. Blackwell, C., et al., *Inhibition of FGF/FGFR autocrine signaling in mesothelioma with the FGF ligand trap, FP-1039/GSK3052230.* Oncotarget, 2016. **7**(26): p. 39861-39871.
10. Laurie, S.A., et al., *A phase II trial of dovitinib in previously-treated advanced pleural mesothelioma: The Ontario Clinical Oncology Group.* Lung Cancer, 2017. **104**: p. 65-69.
11. Scagliotti, G.V., et al., *Nintedanib in combination with pemetrexed and cisplatin for chemotherapy-naive patients with advanced malignant pleural mesothelioma (LUME-Meso): a double-blind, randomised, placebo-controlled phase 3 trial.* Lancet Respir Med, 2019.
12. van Brummelen, E.M.J., et al., *A phase Ib study of GSK3052230, an FGF ligand trap in combination with pemetrexed and cisplatin in patients with malignant pleural mesothelioma.* Invest New Drugs, 2019.
13. Vogelzang, N.J., et al., *Phase III study of pemetrexed in combination with cisplatin versus cisplatin alone in patients with malignant pleural mesothelioma.* J Clin Oncol, 2003. **21**(14): p. 2636-44.
14. Schelch, K., et al., *A link between the fibroblast growth factor axis and the miR-16 family reveals potential new treatment combinations in mesothelioma.* Mol Oncol, 2018. **12**(1): p. 58-73.

15. Nasu, M., et al., *High Incidence of Somatic BAP1 alterations in sporadic malignant mesothelioma.* J Thorac Oncol, 2015. **10**(4): p. 565-76.
16. White, A.E. and J.W. Harper, *Cancer. Emerging anatomy of the BAP1 tumor suppressor system.* Science, 2012. **337**(6101): p. 1463-4.
17. Wiesner, T., et al., *Germline mutations in BAP1 predispose to melanocytic tumors.* Nat Genet, 2011. **43**(10): p. 1018-21.
18. Testa, J.R., et al., *Germline BAP1 mutations predispose to malignant mesothelioma.* Nat Genet, 2011. **43**(10): p. 1022-5.
19. Abdel-Rahman, M.H., et al., *Germline BAP1 mutation predisposes to uveal melanoma, lung adenocarcinoma, meningioma, and other cancers.* J Med Genet, 2011. **48**(12): p. 856-9.
20. Popova, T., et al., *Germline BAP1 mutations predispose to renal cell carcinomas.* Am J Hum Genet, 2013. **92**(6): p. 974-80.
21. Farley, M.N., et al., *A novel germline mutation in BAP1 predisposes to familial clear-cell renal cell carcinoma.* Mol Cancer Res, 2013. **11**(9): p. 1061-1071.
22. He, M., et al., *Intrinsic apoptosis shapes the tumor spectrum linked to inactivation of the deubiquitinase BAP1.* Science, 2019. **364**(6437): p. 283-285.
23. Cigognetti, M., et al., *BAP1 (BRCA1-associated protein 1) is a highly specific marker for differentiating mesothelioma from reactive mesothelial proliferations.* Mod Pathol, 2015. **28**(8): p. 1043-57.
24. Brahmer, J., et al., *Nivolumab versus Docetaxel in Advanced Squamous-Cell Non-Small-Cell Lung Cancer.* N Engl J Med, 2015. **373**(2): p. 123-35.
25. Borghaei, H., et al., *Nivolumab versus Docetaxel in Advanced Nonsquamous Non-Small-Cell Lung Cancer.* N Engl J Med, 2015. **373**(17): p. 1627-39.
26. Herbst, R.S., et al., *Pembrolizumab versus docetaxel for previously treated, PD-L1-positive, advanced non-small-cell lung cancer (KEYNOTE-010): a randomised controlled trial.* Lancet, 2016. **387**(10027): p. 1540-50.
27. Lipson, E.J., et al., *Antagonists of PD-1 and PD-L1 in Cancer Treatment.* Semin Oncol, 2015. **42**(4): p. 587-600.
28. Zucali, P.A., et al., *Second-line chemotherapy in malignant pleural mesothelioma: results of a retrospective multicenter survey.* Lung Cancer, 2012. **75**(3): p. 360-7.
29. Buikhuisen, W.A., et al., *Second line therapy in malignant pleural mesothelioma: A systematic review.* Lung Cancer, 2015. **89**(3): p. 223-31.
30. Samstein, R.M., et al., *Tumor mutational load predicts survival after immunotherapy across multiple cancer types.* Nat Genet, 2019. **51**(2): p. 202-206.
31. Chang, L., et al., *Microsatellite Instability: A Predictive Biomarker for Cancer Immunotherapy.* Appl Immunohistochem Mol Morphol, 2018. **26**(2): p. e15-e21.
32. Ladanyi, M., F. Sanchez Vega, and M. Zauderer, *Loss of BAP1 as a candidate predictive biomarker for immunotherapy of mesothelioma.* Genome Med, 2019. **11**(1): p. 18.
33. Gezgin, G., et al., *Genetic evolution of uveal melanoma guides the development of an inflammatory microenvironment.* Cancer Immunol Immunother, 2017. **66**(7): p. 903-912.

34. Shrestha, R., et al., *BAP1 haploinsufficiency predicts a distinct immunogenic class of malignant peritoneal mesothelioma.* Genome Med, 2019. **11**(1): p. 8.
35. Pan, D., et al., *A major chromatin regulator determines resistance of tumor cells to T cell-mediated killing.* Science, 2018. **359**(6377): p. 770-775.
36. Miao, D., et al., *Genomic correlates of response to immune checkpoint therapies in clear cell renal cell carcinoma.* Science, 2018. **359**(6377): p. 801-806.
37. Berry, G., et al., *Malignant mesotheliomas in former miners and millers of crocidolite at Wittenoom (Western Australia) after more than 50 years follow-up.* Br J Cancer, 2012. **106**(5): p. 1016-20.