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Gender differences in molecular-guided therapy recommendations for metastatic malignant mesothelioma

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Abstract

Background: Malignant mesothelioma is an aggressive cancer and has a poor prognosis. Here, we analyzed the feasibility, molecular and gender aspects of targeted therapy recommendations for malignant mesothelioma based on the individual molecular tumor profile.

Methods: In this single-center, real-world retrospective analysis of our platform for precision medicine, we evaluated the molecular profiling of malignant mesothelioma in 14 patients, including nine men and five women. Tumor samples of the patients were examined with a 50 gene next-generation sequencing (NGS) panel, immunohistochemistry, and fluorescence in situ hybridization, to detect possible molecular aberrations which may be targeted by off-label therapy custom-tailored to the individual patient.

Results: In total, we identified 11 mutations in six of the 14 patients, including *BAP1*, *FANCA*, *NF1*, *NF2*, *PD-L1*, *RAD52D*, *SETD2*, *SRC*, and *TP53*. No mutation was detected in eight of the 14 patients. Targeted therapy was recommended for 11 out of the 14 patients. All recommendations were mainly based on the molecular characteristics determined by immunohistochemistry. Targeted therapy recommendations were significantly more often for men than women due to gender-specific differences in PDGFRα expression. Eventually, four patients received the targeted therapy, of whom one patient subsequently achieved stable disease.

Conclusions: Our observations suggest that a molecular-guided treatment approach is feasible for the management of advanced malignant mesothelioma. Our analysis revealed gender specific differences in PDGFRα expression that should be further evaluated in clinical trials.

Introduction

Malignant mesothelioma (MM) is a relatively rare and aggressive malignancy of the mesothelium. It develops most commonly in the visceral and parietal pleura and less frequently in the peritoneum. The single most important risk factor is asbestos exposure.[1–3] It develops in about one to two persons per million of the general population.[4] Despite its rarity, in comparison with other malignant diseases, MM causes a disproportionate amount of morbidity, including respiratory complications, and mortality.[5,6]

Management of MM poses a great challenge to physicians and requires an experienced multidisciplinary team and dedicated centers. During the early stages of MM, a trimodal treatment approach is followed, consisting of surgery, radiation therapy, and chemotherapy. At stage IV, MM has a dismal prognosis of 12 month median survival despite intense therapeutic efforts. In this palliative setting,

systemic chemotherapy with cisplatin and pemetrexed in the first-line of the treatment is still the mainstay of MM treatment.[1–4,7–9] Unfavorably, there is no standard treatment once the first-line therapy fails and there are no established biomarkers for disease classification and for prediction of therapy response. In contrast, in other well studied more frequent solid tumors, including colorectal cancer the use of predictive markers such as BRAF, KRAS and MSI-status are integrated into daily clinical routine and are of major clinical relevance.[10] Similarly, the hormone receptor status in breast cancer is of utmost importance in therapy decision.[11]

In recent years, there have been efforts to progressively individualize therapy options in specific cancer types. In a few particular types, treatment with custom-tailored tyrosine kinase inhibitors or immunotherapeutic agents has become possible, such as trastuzumab in human epidermal growth factor receptor 2 (HER2+) breast cancer or gastric cancer, imatinib in Ph + chronic myelogenous leukemia (CML) or in KIT+ gastrointestinal stromal tumor (GIST), pazopanib and sunitinib in advanced renal cell carcinoma (RCC), and B-rapidly accelerated fibrosarcoma (BRAF)-directed therapy with vemurafenib or dabrafenib/trametinib in melanoma.[12–14]

Emerging techniques provide new potentials for effective therapies. For instance, profiling molecular alterations and mutations in tumors allows identification of the molecular targets suitable for specific treatments and subsequent development of drug treatments specific to an individual patient. This approach is known as precision medicine.[15,16]

The goal of precision medicine is to achieve a more durable and deep response than conventional treatments sparing healthy cells and tissues as recently demonstrated in our EXACT trial.[17]

In this study, we conducted a retrospective subgroup analysis of all the 14 patients with MM that had been enrolled and profiled in our special platform of molecular oncological diagnostics and therapy (MONDTI) of the Comprehensive Cancer Centre of the Medical University of Vienna (CCC-MUV). We sought to map the molecular profiles of advanced, pretreated, and mainly relapsed MM to identify and target specific molecular alterations.

Methods

Patients and design of the precision medicine platform

Patients with metastasized pleural or peritoneal MM who were refractory to all standard treatment options were eligible for inclusion in MONDTI, provided archival tissue samples were available. Patients had to have an Eastern Cooperative Oncology Group (ECOG) performance status of 0 or 1. MONDTI is not a clinical trial, but intends to provide the possibility of a targeted therapy to patients where no standard antitumoral treatment is available. Patients had to provide informed consent before inclusion in MONDTI. Furthermore, the Institutional Ethics Committee of the Medical University of Vienna also approved this subanalysis (Nr. 1039/2017).

Tissue samples

Formalin-fixed, paraffin-embedded tissue from patients with advanced MM who were refractory to all available standard treatment lines were sent to, or retrieved from, the archive of the Department of Pathology, Medical University Vienna, Vienna, Austria.

Cancer gene panel sequencing

DNA was extracted from paraffin-embedded tissue blocks with a QIAamp Tissue Kit (Qiagen, Hilden, Germany), and 10 ng DNA per tissue sample was provided for sequencing. The DNA library was created by multiplex polymerase chain reaction with the Ion AmpliSeq Cancer Hotspot Panel v2 (Thermo Fisher Scientific, Waltham, MA, USA) that covers mutation hotspots of 50 genes. The panel includes driver mutations, oncogenes and tumor suppressor genes. By mid-2018, the gene panel was expanded using the 161-gene next-generation sequencing (NGS) panel of oncomine comprehensive assay v3 (Thermo Fisher Scientific, Waltham, MA, USA). The oncomine comprehensive assay v3 was optimized for sequencing on an Ion Personal Genome Machine System (Thermo Fisher Scientific, Waltham, MA, USA). The generated sequencing data were afterwards analyzed with the help of the Ion Reporter Software (Thermo Scientific Fisher). We referred to BRCA Exchange, ClinVar, COSMIC, dbSNP, OMIM and 1000 genomes for variant calling and classification. The variants introduced by the American College of Medical Genetics and Genomics were classified according to a five tier system comprising of the modifiers pathogenic, likely pathogenic, uncertain significance, likely benign, or benign. The variants pathogenic and likely pathogenic were taken into consideration for the recommendation of targeted therapy.

Immunohistochemistry

IHC was performed using 2 μm thin tissue sections read by a Ventana Benchmark Ultra stainer (Ventana, Tucson, Arizona, USA). The following antibodies were applied:

anaplastic lymphoma kinase (ALK) (clone 1A4; Zytomed, Berlin, Germany), CD20 (clone L26; Dako), CD30 (clone BerH2; Agilent Technologies, Vienna,

Austria), epidermal growth factor receptor (EGFR) (clone 3C6; Ventana), estrogen receptor (clone SP1; Ventana), human epidermal growth factor receptor 2 (HER2) (clone 4B5; Ventana), HER3 (clone SP71; Abcam, Cambridge, UK), C-kit receptor (KIT) (clone 9.7; Ventana), MET (clone SP44; Ventana), NTRK (clone EPR17341, Abcam), phosphorylated mammalian target of rapamycin (p-mTOR) (clone 49F9; Cell Signaling Technology, Danvers, Massachusetts, USA), platelet-derived growth factor alpha (PDGFRα) (rabbit polyclonal; Thermo Fisher Scientific), PDGFRβ (clone 28E1, Cell Signaling Technology), programmed death-ligand 1 (PD-L1) (clone E1L3N; Cell Signaling Technology), progesteron receptor (clone 1E2; Ventana), phosphatase and tensin homolog (PTEN) (clone Y184; Abcam) and ROS1 (clone D4D6; Cell Signaling Technology).

To assess the immunostaining intensity for the antigens EGFR, p-mTOR, PDGFRα, PDGFRβ and PTEN, a combinative semiquantitative score for immunohistochemistry was used. The immunostaining intensity was graded from 0 to 3 (0 = negative, 1 = weak, 2 = moderate, 3 = strong). To calculate the score, the intensity grade was multiplied by the percentage of corresponding positive cells: (maximum 300) = (% negative × 0) + (% weak × 1) + (% moderate × 2) + (% strong × 3).

The immunohistochemical staining intensity for HER2 was scored from 0 to 3+ (0 = negative, 1+ = negative, 2 + = positive, 3+ = positive) pursuant to the scoring guidelines of the Dako HercepTestR from the company Agilent Technologies (Agilent Technologies, Vienna, Austria). In case of HER2 2+, a further test with HER2 in situ hybridization was performed to verify the HER2 gene amplification.

Estrogen receptor and progesterone receptor stainings were graded according to the Allred scoring system from 0 to 8. MET staining was scored from 0 to 3 (0 = negative, 1 = weak, 2 = moderate, 3 = strong).

For PD-L1, the tumor proportion score (TPS) was calculated which is the percentage of viable malignant cells showing membrane staining.

ALK, CD30, CD20 and ROS1 staining were classified positive or negative based on the percentage of reactive tumor cells, however without graduation of the staining intensity. In ALK or ROS1 positive cases, the presence of a possible gene translocation was evaluated by fluorescence in situ hybridization (FISH).

The status of MSI was analyzed by the MSI Analysis System, Version 1.1 (Promega Corporation, Madison, Wisconsin, USA).

Fluorescence in situ hybridization (FISH)

FISH was performed with 4 μm thick formalin-fixed, paraffin-embedded tissue sections. The following FISH probes were used: Anaplastic lymphoma kinase (ALK) (2p23.1; Abbott, Abbott Park, IL, USA), rearranged during transfection (RET) (10q11; Kreatech, Berlin, Germany), PTEN (10q23.31)/Centromere 10, and ROS1 (ZytoVision, Bremerhaven, Germany). A total of 200 cell nuclei per tumor were evaluated. The cutoff level for an aberrant ALK, RET, and ROS1 FISH was ≥15% of cells with a split-apart signal. The PTEN FISH was considered positive for PTEN gene loss with ≥30% of cells with only one or no PTEN signals.

Multidisciplinary boards (MTB)

After thorough examination of the molecular profile of each tumor sample by a qualified and competent molecular pathologist, the results and findings were reviewed in a multidisciplinary tumor board (MTB) that was held every other week.

Members of the board included molecular pathologists, radiologists, cardiothoracic surgeon, clinical oncologists, biostatisticians, and basic scientists. The MTB recommended the targeted therapy based on the specific molecular profile of each patient. The targeted therapies included tyrosine kinase inhibitors, checkpoint inhibitors (eg, anti- PD-L1 monoclonal antibodies), and growth factor receptor antibodies with or without endocrine therapy. The treatment recommendations by the MTB were prioritized dependent on the level of evidence from high to low according to phase III to phase I trials.

Study design and statistical analysis

This study was a retrospective exploratory single center cohort analysis of 14 patients with therapy-refractory metastatic malignant mesothelioma. Other rare tumor types with less than 10 patients per tumor type were excluded. We also used the method of frequency distribution to delineate the characteristics of the cancer patients. This study was designed as an exploratory, hypothesis-generating research work.

To explore possible gender-specific differences the Chi-squared test $\chi 2$ was applied.

For the statistical analysis the software package IBM SPSS Statistics Version 26 was used.

Results

From June 2013 to January 2019, a total of 14 patients diagnosed with pleural or peritoneal MM were included in this subgroup analysis from the cohort of our platform MONDTI that has so far profiled 570 patients with various advanced and therapy-refractory cancer types. In this analysis, all the patients were Caucasians including nine men

and five women. Of these, 11 and three patients were diagnosed with pleural and peritoneal MM, respectively. The median age at first diagnosis was 56.2 years, ranging from 33 to 71 years, and the median age at the time when the molecular profiling was performed was 57.8 years, ranging from 34 to 74 (Table 1). The tumor tissue was obtained by biopsy or during the surgical treatment. All the patients were diagnosed with the epithelioid subtype of MM, except for one patient, who had a biphasic subtype.

At the time of molecular profiling, all the patients had an advanced and therapy-refractory MM at stage IV. The pleural MM patients who had been treated in the multimodality protocol (n = 5) also received neoadjuvant chemotherapy and underwent extrapleural pneumonectomy, and hemithoracic intensity modulated radiation therapy (IMRT). In total, eight of the patients had undergone surgical treatment within a multimodality protocol. All the patients had received systemic chemotherapy with cisplatin and pemetrexed. There were 7 patients who received three cycles, two patients received four cycles and five patients were given six cycles of cisplatin and pemetrexed, respectively before experiencing progressive disease.

After the failure of cisplatin and pemetrexed, four patients received 1–2 lines of further therapy including irinotecan, vinorelbine, gemcitabine, nab-paclitaxel, pembrolizumab, and cetuximab.

Of the 14 tissue samples, two were from the metastatic sites and 12 were from the primary sites. In total, we identified 11 molecular aberrations in six patients; two mutations were identified in *NF2* and *TP53* genes each, and *BAP1*, *FANCA*, *NF1*, *PD-L1 (CD274)*, *RAD51D*, *SETD2*, and *SRC* genes each had one mutation. No mutation was detected in eight patients.

None of the patients had copy number alterations or MSI high status. IHC or FISH could not be performed for one patient due to insufficient tumor material.

Table 1 Patient characteristics (n = 14)

Patient Characteristics	Number
Median age at first diagnosis	54
Median age at molecular profiling	56.4
Men	9
Women	5
Caucasian	14
Epitheloid malignant mesothelioma	13
Biphasic malignant mesothelioma	1
Pleural malignant mesothelioma	11
Peritoneal malignant mesothelioma	3
Relapsed disease	8
Systemic chemotherapy with cisplatin and pemetrexed	14
Prior chemotherapy regimens	1–3
Prior radiotherapy	5
Prior surgical treatment	8

IHC demonstrated elevated expression levels of EGFR, p-mTOR, and PTEN in 12 patients. The median score of EGFR and p-mTOR expression among the patients was 250 and 143, respectively. Additionally, elevated expression levels of PD-L1 and MET were each observed in four patients.

Furthermore, PDGFRα and PDGFRβ levels were elevated in six and four patients, respectively.

Remarkably, the Chi-squared test χ^2 revealed that male patients had significantly more often PDGFRα expression than women (6/9 men vs. 0/5 women; P = 0.016).

For 11 of the 14 patients (79%), a targeted therapy was suggested based on their individual molecular profile. All recommendations were mainly based on the molecular characteristics determined by immunohistochemistry.

The gender specific differences in the PDGFRα expression are reflected by the type of the recommended targeted agents. The multitargeted tyrosine kinase inhibitors sunitinib (n = 2), dasatinib, and nintedanib were only recommended for male patients.

Cetuximab and pembrolizumab each were recommended for three patients each. Everolimus was considered for one patient. Tables 2 and 3 describe the rationale for the recommended targeted therapy approaches. Eventually, four of the 11 patients (36%) received the targeted therapy; however, three of them died due to disease progression before restaging could be performed. A male peritoneal MM patient was treated with 200 mg nintedanib tablets twice per day at 12 hours intervals for 21 days. He achieved stable disease for three months and the therapy was tolerated well with only grade I fatigue. There were 7 patients who did not receive the offered targeted therapy. Reasons for not applying the recommended targeted agent included the following: deterioration of performance status, death of patients, the treating oncologist favored another treatment regimen due to the clinical overall situation of the patients or refusal of any further treatment, including targeted therapy options.

Discussion

To our knowledge, this is the first time that the information about individual molecular aberrations in patients with metastasized MM who are refractory to the standard treatment has been translated into specific therapeutic recommendations. The analysis presented in this study shows that molecular profiling from tumor samples of patients with advanced MM and subsequent identification of the therapeutic options appears feasible and safe. Further, we provide the first evidence of gender specific differences in MM patients.

In this retrospective exploratory single-center analysis, we presented the molecular profiles of 14 MM patients

Table 2 Rationale for therapy recommendations

Therapeutic agent (trading name) and number of recommendations	Targets	Overview of current FDA approval in different entities	Overview of current EMA approval in different entities
Cetuximab (Erbitux) *N* = 3	EGFR expression	CRC, HNSCC	CRC, HNSCC
Pembrolizumab (Keytruda) *N* = 3	PD-1, hypermutability	Melanoma, NSCLC, HNSCC, HL, urothelial carcinoma, microsatellite instability-high cancer, gastric cancer, cervical cancer	Melanoma, NSCLC, HNSCC, HL, urothelial carcinoma
Sunitinib (Sutent) *N* = 2	PDGFR, KIT, VEGFR, RET, FLT3	RCC, PDAC, GIST	RCC, PDAC, GIST
Dasatinib (Sprycel) *N* = 1	BCR/ABL, Src family, PDGFR	Ph + CML, Ph + ALL	Ph + CML, Ph + ALL
Nintedanib (Vargatef, Ofev) *N* = 1	PDGFR, FLT3, FGFR, VEGFR	Idiopathic pulmonary fibrosis	NSCLC
Everolimus (Afinitor) *N* = 1	mTOR expression	Breast cancer, PNET, RCC, renal angiomyolipoma,	Breast cancer, RCC, neuroendocrine tumors of pancreatic, gastrointestinal or lung origin

ABL, Abelson murine leukemia viral oncogene homolog 1; ALL, acute lymphatic leukemia; BCR, breakpoint cluster region; CML, chronic myeloid leukemia; EGFR epidermal growth factor receptor; EMA, European Medicines Agency; FDA, Food and Drug Administration; FLT3, fms like tyrosine kinase 3; GIST, gastrointestinal stromal tumor; HL, Hodgkin's lymphoma; HNSCC, head and neck squamous cell carcinoma; NSCLC, non-small cell lung carcinoma; PD-1, programmed cell death protein 1; PDAC, pancreatic ductal adenocarcinoma; PDGFR, platelet-derived growth factor receptor; Ph+, Philadelphia chromosome positive; p-mTOR, phosphorylated mammalian target of rapamycin; RCC, renal cell carcinoma; RET, rearranged during transfection; TP53, tumor protein 53; VEGFR, vascular endothelial growth factor.

from the MONDTI cohort. Their disease was therapy-refractory and advanced. Tumor tissue was obtained from all the patients and characterized for their molecular profiles. Subsequently, the molecular alterations in these patients were discussed in an MTB for precision medicine to evaluate the possibility of a molecular-based treatment independent of the tumor's histological classification (tissue-agnostic treatment).

The unique feature of the MONDTI platform and thus the added value of this analysis is that apart from the genomic sequencing also RNA sequencing, IHC and cytogenetics were performed to create a comprehensive molecular profile. Together these combined techniques formed a solid base for the recommendation of molecular-guided targeted agents for the patients. Another important characteristic of the MONDTI platform is that it is an open platform that enrolls all patients with solid tumors with no further standard treatment options. Thus, unlike a clinical trial, MONDTI provides real-life data that are relatively unbiased.

A treatment recommendation was derived for 11 patients from the MTB. All recommendations were mainly based on the molecular targets determined by immunohistochemistry. Thus, our study underscores the major clinical relevance of immunohistochemistry in precision medicine.

The recommended targeted agents were carefully selected for individualized treatment, taking into account the patient's clinical and treatment history, performance status, comorbidities, and previous and concomitant therapies. Eventually, four patients received the recommended therapies. However, three patients died because the disease progressed before restaging was performed. The surviving patient with peritoneal MM was given nintedanib and achieved stable disease for three months. Although this analysis shows that precision medicine is implementable in daily clinical routine, only one patient had a clinical benefit from this therapeutic approach. One reason may be the turnaround time; a shorter turnaround time may allow the therapy to start earlier and control the cancer disease. Liquid biopsy may be a viable option to reduce the turnaround time, monitor the disease, and assess the therapy response. Another reason may be the complexity of MM.

Due to the extreme rarity of peritoneal MM, clinical trials and research are conducted in the pleural variant and the data are extrapolated for peritoneal MM.[1]

With the exception of NF2 and TP53, mutations in all other genes were detected only once in the current study. This finding is consistent with the well-described extreme and complex intratumoral heterogeneity in MM occurring within the same tumor tissue; vascularization amount, proliferation rate, and subclone characteristics are all known to be highly variable. The patterns of genetic and epigenetic aberrations change both spatially and temporally. The tumor biology at metastatic sites is different from the

Table 3 Detailed characteristics of the MM patients (n = 14)

Patients	Histological subtype and previous treatment	Stage, Site, Side	Age at molecular profiling and gender	Detected mutations by NGS	IHC	FiSH	Therapy recommendation and response
1	Epitheloid multimodality treatment	IV°, Pleura, Left	34 years, Female	No mutation detected	Not done (due to insufficient tissue material)	Not done (due to insufficient tissue material)	No recommendation
2	Epitheloid multimodality treatment	IV°, Pleura, Right	74 years, Male	TP53 (exon 5)	EGFR 3+, MET 3+, PDGFRa 1+, PDGFRb 1+, TPS PD-L1 ≥ 50%, p-mTOR 1+, PTEN 1+	No alteration	Pembrolizumab
3	Epitheloid multimodality treatment	IV°, Pleura, Right	49 years, Male	SRC (exon 9)	EGFR 3+, MET 3+, PDGFRa 1+, PTEN 1+, p-mTOR 2+	No alteration	Dasatinib
4	Epitheloid	IV°, Pleura, Left	48 years, Male	No mutation detected	EGFR 3+, MET 1+, PDGFRa 1+, PTEN 1+, p-mTOR 1+	No alteration	Cetuximab
5	Epitheloid	IV°, Pleura, Right	41 years, Male	No mutation detected	EGFR 2+, MET 2+, TPS PD-L1 ≥ 50%, p-mTOR 2+, PTEN 1+	No alteration	Pembrolizumab
6	Epitheloid	IV°, Pleura, Left	59 years, Male	No mutation detected	EGFR 1+, PDGFRa 1+, PDGFRb 1+, p-mTOR 1+, PTEN 1+	No alteration	Sunitinib
7	Epitheloid	IV°, Pleura, Right	59 years, Male	No mutation detected	EGFR 2+, PDGFRa 1+, PDGFRb 2+, PTEN 1+	No alteration	Sunitinib
8	Epitheloid multimodality treatment	IV°, Pleura, Left	58 years, Male	No mutation detected	EGFR 3+, MET 3+, PDGFRa 2+, PTEN 1+, p-mTOR 2+	No alteration	Cetuximab
9	Epitheloid	IV°,	56 years,	No mutation detected	EGFR 3+,	No alteration	Cetuximab

Table 3 Continued

Patients	Histological subtype and previous treatment	Stage, Site, Side	Age at molecular profiling and gender	Detected mutations by NGS	IHC	FiSH	Therapy recommendation and response
		Pleura, Right	Female		PDGFRb 1+, p-mTOR 2+, PTEN 2+		
10	Epitheloid multimodality treatment	IV°, Pleura, Right	57 years, Male	NF2 (exon 4), PD-L1 (exon 5)	EGFR 2+, TPS PD-L1 ≥ 50%, p-mTOR 1+	No alteration	Pembrolizumab
11	Epitheloid	IV°, Pleura, Right	37 years, Female	FANCA (exon 40), NF1 (exon 17), RAD51D (exon 5)	EGFR 3+, p-mTOR 2+, PTEN 2+	No alteration	No recommendation
12	Epitheloid	IV° Peritoneum, Right	66 years, Male	No mutation detected	EGFR 3+, MET 2+, p-mTOR 3+, PTEN+2	No alteration	Nintedanib Patient achieved stable disease for 3 months
13	Epitheloid	IV° Peritoneum, Right	48 years, Female	TP53 (exon 5)	MET 2+, p-mTOR 2+, Loss of PTEN	Loss of PTEN	Everolimus
14	Biphasic	IV° Peritoneum, Right	48 years, Female	BAP1 (exon 14), SETD2 (exon 3), NF2 (exon 6)	EGFR 3+, p-mTOR 3+, PTEN 2+	No alteration	No recommendation

BAP1, BRCA1 associated protein-1; EGFR, epidermal growth factor receptor; FANCA, Fanconi anemia, complementation group A; FiSH: fluorescence in situ hybridization; NF, neurofibromin; PDGFR, platelet-derived growth factor receptor; PD-1, programmed cell death protein 1; PD-L1, programmed death-ligand 1; p-mTOR, phosphorylated mammalian target of rapamycin; PTEN, phosphatase and tensin homolog; TP53, tumor protein 53; IHC, immunohistochemistry; NGS, next-generation sequencing; SETD2, SET domain containing 2, TPS, tumor proportion score.

primary sites and differs at the time point of relapse. In addition, it is known that the therapy itself can influence and inform the clonal tumor evolution by creating new driver mutations in subclones that become insensitive to drugs.[18]

Bueno *et al.* performed a comprehensive genomic analysis of pleural MM and identified frequent mutations in *BAP1*, *NF2*, *TP53*, *SETD2*, *DDX3X*, *ULK2*, *RYR2*, *CFAP45*, *SETDB1*, and *DDX51*. They found that recurrent gene fusions and splice alterations were frequently the underlying reasons for the inactivation of *NF2*, *BAP1*, and *SETD2*.[19] Particularly, inactivation of the tumor suppressor genes *NF2* and *BAP1* is frequently observed in MM, possibly playing a pivotal role in the tumorigenesis. BAP1 is a ubiquitin C-terminal hydrolase (de-ubiquitinase), and it can reverse the ubiquitin linkages formed by E3 ubiquitin ligases. NF2 is a negative regulator of the E3 ubiquitin ligase CRL4DCAF1.[20]

The detected mutations and IHC scores observed in MM in this analysis are in line with previous studies[21–25]

Most importantly, we detected significant gender specific differences regarding the PDGFRα expression that had an impact on the type of recommended agents and led to a significant difference in targeted therapy recommendations between male and female patients. Until now, only an Italian research group has described gender-specific differences in MM. Marinaccio *et al.* observed based on long-term epidemiological surveillance of MM incidence (ReNaM) that approximately 28% of mesotheliomas in Italy occurred among women with an overall F/M ratio equal to 0.40 which was almost steady over the incidence period (1993–2012).

Due to the significant prevalence of PDGFRα expression among male patients, the multitargeted tyrosine kinase inhibitors sunitinib (n = 2), dasatinib, and nintedanib were only recommended for male patients.

Sunitinib was offered for two male patients. Sunitinib has been shown to be well tolerated with remarkable antitumor activity in a phase II trial performed with 53 patients who had pretreated progressive pleural MM. It achieved a confirmed radiological partial response in six patients (12%) and stable disease in 34 patients (65%).[26]

In our analysis, a male patient with peritoneal MM had received three lines of therapy, including cisplatin and pemetrexed, irinotecan and cetuximab, and gemcitabine and nab-paclitaxel. He was refractory to all these agents. This patient harbored an expression of EGFR and MET and therefore was recommended nintedanib as an experimental treatment after multiple lines of therapy.

In a large phase III LUME-Meso Trial, a combinatorial therapy with nintedanib, cisplatin, and pemetrexed was tested as the first-line therapy for >400 patients with epithelioid pleural MM. However, this treatment option failed to improve progression-free survival (PFS) or overall survival (OS).[27]

In our MTB, cetuximab was recommended in three cases. De Paepe *et al.* tested the combination of cetuximab in combination with cisplatin and pemetrexed as the first-line regimen in a phase II trial (NCT00996567) with 18 epithelioid pleural MM patients. They reported a partial response in eight of the patients; however, the PFS rate after 18 weeks as the primary endpoint was not reached.[28]

Everolimus was recommended for one patient with loss of PTEN and high p-mTOR expression.

For three patients, pembrolizumab was considered as targeted therapy approach. An important trial (KEYNOTE-028, NCT02054806) reported by Alley *et al.* investigated the efficacy and safety of pembrolizumab in 25 patients. Of these, five (20%) patients had a partial response and 13 (52%) had stable disease. Responses were durable with a median response duration of 12.0 months.[29] Based on these encouraging results, a phase II trial was initiated by Kindler *et al.* to examine the antitumor activity of pembrolizumab in 35 MM patients, including 30 patients diagnosed with pleural MM, and five patients with peritoneal MM. The interim analysis showed that pembrolizumab achieved a median progression-free survival of 6.2 months. The median overall survival has not been reached yet. Additionally, seven patients had a partial response and 19 patients achieved stable disease.[30]

Generally, for the establishment of new standard of care therapy strategies in oncology, large number of cancer patients with the same tumor entity are included in a large randomized clinical trial to test the efficacy of a new treatment.

However, the conduction of these trials are hardly feasible in rare tumor entities, including MM, due to the rarity and heterogeneity of these cancer diseases. Thus, other trial designs, namely basket trial and umbrella trial, have been established in precision medicine that have been proven to be more effective in studying new agents in different tumor entities, including rare tumor types.

Basket trial contains one specific molecular alteration as the common denominator across the included various tumor types that is targeted by a targeted therapy agent. Basket trials are often planned as single-arm, phase II trials, exploratory proof of concept trials. In contrast to basket trials, umbrella trials examine several targeted therapies assigned to different molecular markers within the same tumor entity that forms the "umbrella" for the substudies.[31–33]

This study had several limitations. The sample size was small and patients had a good ECOG status (0 and 1). However, the novelty of this analysis is that it shows for the first time gender specific differences in MM patients. Further, it demonstrated the feasibility of molecular driven treatment approaches for MM patients.

In conclusion, the rarity, the complex tumor biology in combination with the spatial and temporal heterogeneity of MM genetics pose unique challenges for the management of MM. Based on our experience, precision medicine is feasible and implementable in clinical routine. Our exploratory analysis highlighted the clinical relevance of immunohistochemistry and revealed gender specific differences in PDGFRα expression that should be further evaluated in clinical trials. In our study, the clinical benefit of precision medicine for patients with therapy-refractory MM was limited. The concept of molecular guided-therapy strategies is a relatively new concept and further research is warranted to develop it further.

Disclosure

The authors declare that they have no competing interests.

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