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Original Research

Malignant pleural mesothelioma: Germline variants in DNA repair genes may steer tailored treatment

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KEYWORDS
Mesothelioma;
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Abstract ***Introduction:*** Malignant pleural mesothelioma (MPM) is a tumour associated with asbestos exposure. Approximately, 10% of patients with MPM carry a germline pathogenic variant (PV), mostly in DNA repair genes, suggesting the occurrence of inherited predispositions.
Aim: This article aimed to 1) search for new predisposing genes and assess the prevalence of PVs in DNA repair genes, by next-generation sequencing (NGS) analysis of germline DNA from 113 unselected patients with MPM and 2) evaluate whether these patients could be sensitive to tailored treatments.
Methods: NGS was performed using a custom panel of 107 cancer-predisposing genes. To investigate the response to selected drugs in conditions of DNA repair insufficiency, we created a three-dimensional-MPM cell model that had a defect in ataxia telangiectasia mutated (ATM), the master regulator of DNA repair.
Results: We identified PVs in approximately 7% of patients with MPM (8/113) and a new PV in *BAP1* in a further patient with familial MPM. Most of these PVs were in genes involved or supposedly involved in DNA repair (*BRCA1, BRIP1, CHEK2, SLX4, FLCN* and *BAP1*). *In vitro* studies showed apoptosis induction in ATM-silenced/inhibited MPM spheroids treated with an enhancer of zeste homologue 2 inhibitor (tazemetostat).
Conclusions: Overall these data suggest that patients with MPM and DNA repair insufficiency may benefit from this treatment, which induces synthetic lethality.

1. Introduction

Malignant pleural mesothelioma (MPM) is a rare and aggressive cancer caused by exposure to a single carcinogen, asbestos [1,2]. MPM incidence is dramatically higher in areas with asbestos pollution, as exemplified by the MPM epidemic in the Northern Italian town of Casale Monferrato, caused by the presence of an asbestos cement factory [3,4]. MPM is typically diagnosed at the late stage of the disease, and the prognosis is poor, with a mean survival from diagnosis between 9 and 17 months [2]. With the current standard of care, a combination of pemetrexed and platinum-based drugs, an increase in survival is observed in only 40% of patients, and that increase is modest (2.8 months) [2]. Recently, the United States Food and Drug Administration approved immunotherapy for first-line treatment of unresectable MPM [5]. Owing to the limited efficacy of available treatments, it is imperative that researchers evaluate new therapeutic approaches and identify subgroups of patients who could benefit from precision medicine. One of these groups could be represented by patients with an inherited cancer syndrome caused by germline pathogenic variants (PVs) in DNA repair genes [6]. The first and most studied syndrome associated with an increased risk of malignant mesothelioma (MM) in individuals exposed to asbestos is the *BAP1*-tumour predisposition syndrome (*BAP1*-TPDS), which is caused by germline PVs in *BAP1*, a gene with many functions, including DNA repair [7]. *BAP1*-TPDS is rare among patients with sporadic MM, but it is found in 6–7% of patients with familial MM [8,9]. Conversely, our group was the first to show that about 10% of patients with MPM carried PVs in other DNA repair genes, and these data were confirmed and extended by other reports [6,10–16]. The DNA repair pathway most commonly affected in these patients was homologous recombination repair (HRR). As a result of gene–environment interaction, it is possible that carriers of germline PVs in DNA repair genes are more sensitive to asbestos-mediated carcinogenesis and require less asbestos exposure to develop MM. Moreover, these patients need to be thoroughly characterised to evaluate whether they could benefit from precision medicine.

In the present article, we aimed to search for new predisposing genes, confirm the prevalence of PVs in DNA repair genes on 113 unselected patients with MPM and evaluate whether these patients could theoretically be sensitive to a treatment based on synthetic lethality, that is, enhancer of zeste homologue 2 (EZH2) inhibitor tazemetostat.

2. Materials and methods

2.1. Patients

The present study included 113 unrelated and unselected patients with MPM from the Piedmont region (Casale Monferrato, Alessandria, Turin and Novara), whose samples were analysed by next-generation sequencing

(NGS). One additional patient with MPM (ID MPM_HO1901) was included because of a peculiar family history of MM that strongly suggested *BAP1*-TPDS, and that sample was analysed only for *BAP1* by Sanger sequencing. All patients signed a written informed consent form, which was approved by the local ethical committee. Additional information is available in Supplementary Methods.

2.2. Next-generation sequencing and variant validation

Blood was collected from all 114 patients, and DNA was extracted using the QIAamp DNA Blood Maxi Kit (QIAGEN Inc., Valencia, CA, USA) as per the manufacturer's protocol. Targeted NGS was performed on genomic DNA, using a custom panel of 107 cancer-predisposing genes (Suppl. Table 1), synthesised by Agilent Technologies (Santa Clara, CA, USA). Detailed protocols for the library preparation, data analyses and variant analysis are described in the Supplementary Methods.

Microsatellite analysis of formalin-fixed paraffin-embedded tumour specimens (when available) was performed to search for loss of heterozygosity (LOH), which supports the role of the variants in carcinogenesis. Sanger sequencing was performed as previously described [9] to analyse *BAP1* in the patient with familial MPM and to confirm the germline PVs identified by NGS.

In addition, we re-evaluated germline variants in 96 healthy controls from our previous study [6] (see Supplementary Methods and Supplementary Table 2).

2.3. Reagents, cell cultures and procedures for functional studies

Reagents, cell cultures and procedures for functional studies reported in Supplementary Methods.

2.4. Multicellular spheroids

Non-adsorbent round-bottomed 96-well plates were coated with a 1:24 dilution of poly(2-hydroxyethyl methacrylate) (120 mg/ml) in 95% ethanol and dried at 37 °C for 24 h. Before use, the plates were sterilised by ultraviolet (UV) light for 30 min. For the generation of multicellular spheroids, 1×10^4 cells were added to each well of the plate. Every 24 h, 50% of the supernatant was replaced with fresh medium ±10 μM EPZ-6438 and/or KU55933.

2.5. Statistical analyses

Cumulative asbestos exposure was compared across our 114 patients with MPM, our 96 controls [6] and 98 patients with MPM from our previous articles [6,9,17,18]. Binary logistic regression and Student's 2-tailed t-test were performed as previously described [6].

3. Results

3.1. Variant analyses

The clinical features of the 114 patients with MPM are reported in Table 1. Eighteen patients with MPM reported a family history of cancer in a first- or second-degree relative; 22 patients were classified as apparently sporadic; for 74 patients, family history was unknown.

NGS analysis in 113 patients with MPM (see Supplementary Results) identified eight heterozygous PVs in the following genes: *BRCA1*, *BRIP1*, *CDKN2A*, *CHEK2*, *FLCN*, *SBDS*, *SLX4* and *VHL* (Table 2). None of these PVs has been previously associated with MM. Detailed information is reported in Supplementary Results. To our knowledge, germline PVs in *BRIP1*, *FLCN* and *SBDS* have never been described in patients with MM, whereas germline PVs in the other five genes have been described by our group and by others [6,10,12]. Supplementary Table 3 reports all the genes found mutated in patients with MM so far, regardless of the pathogenicity of the variant [6,10–16,19]. We evaluated the LOH in the tumour sample by microsatellite analysis for the following genes: *CDKN2A*, *SLX4*, *BRIP1*, *VHL* and *FLCN*. We observed the loss of the second allele in *FLCN* (reduction of 65%) and *BRIP1* (reduction of 95%), as expected for tumour suppressor genes (Suppl. Fig. 1). We did not observe LOH for *CDKN2A*. The microsatellite analysis was inconclusive for *SBDS*, *SLX4* and *VHL*. The tumour sample was not available for the patients with germline variants in *BRCA1* and *CHEK2*.

None of the eight PVs found in patients with MPM was identified in our controls [6]. Data of controls are reported in Supplementary Table 2 and Supplementary Results.

BAP1 was evaluated by Sanger sequencing in a patient with MPM and a family history that strongly suggested a *BAP1*-TPDS (Suppl. Fig. 2). She carried a splice-site PV (c.38-1 G > T) in *BAP1* that has never been reported in the literature or in the population databases we consulted.

3.2. Cumulative asbestos exposure

Of our 114 patients, nine carried germline PVs, and 105 did not. When cumulative asbestos exposure was compared in these groups, patients carrying a germline PV showed lower asbestos exposure, although without statistical significance ($p = 0.23$). After adding the 98 patients with MPM from our previous articles [6,9,17,18] (Suppl. Table 4), altogether, the 23 patients with germline PVs in cancer-predisposing genes showed a statistically significant, lower cumulative asbestos

Table 1
Clinical features of the 114 patients with MPM included in this study.

	Patients with MPM number of subjects = 114 (percentage on the total)	Patients with PVs number of subjects = 9 (percentage on the total)	Patients without PVs number of subjects = 105 (percentage on the total)	OR[a] (95% CI)
Gender				
Male	68 (64.8)	3 (33.3)	71 (62.3)	3.7 (0.9–15.5)
Female	37 (35.2)	6 (66.7)	43 (37.7)	1 (reference)
Histotype				
Epithelioid	81 (71.0)	5 (55.6)	76 (72.4)	0.5 (0.1–2.4)
Biphasic	18 (15.8)	2 (22.2)	16 (15.2)	1 (ref: biphasic and sarcomatoid)
Sarcomatoid	9 (7.9)	1 (11.1)	8 (7.6)	
Unknown	2 (1.8)	0 (0.0)	2 (1.9)	
Not available	4 (3.5)	1 (11.1)	3 (2.9)	
Asbestos exposure				
Occupational	82 (71.9)	5 (55.5)	77 (73.3)	0.5 (0.2–2.2)
Para-occupational	12 (10.5)	2 (22.2)	10 (9.5)	1 (ref: para-occupational, environmental and household)
Environmental	9 (7.9)	1 (11.1)	8 (7.6)	
Household	4 (3.5)	–	4 (3.8)	
Not available	7 (6.1)	1 (11.1)	6 (5.7)	
Age at diagnosis, years				**p (Mann–Whitney test)**
Mean±SD	68.3 ± 9.3	66.0 ± 8.5	68.5 ± 9.4	0.38
Survival				**p (Log rank test)**
1-year (95%CI)	63% (52–72)	76% (33–94)	60% (50–70)	0.16
2-year (95%CI)	33% (24–43)	38% (9–68)	33% (23–43)	
Quantitative asbestos exposure				**p (Student's t-test)**
Mean±SD (f/mL-y)	22.0 ± 74.8[b]	2.9 ± 4.2	23.7 ± 77.8[b]	
Mean±SD (after logarithmic transformation)	1.0 ± 2.1	0.2 ± 1.6	1.0 ± 2.1	0.23

CI, confidence interval; MPM, malignant pleural mesothelioma; OR, odds ratio; PV, pathogenic variant; SD, standard deviation.
[a] Patients with PVs versus patients without PVs.
[b] Not available for 1 patient.

exposure when compared with the 189 patients without germline PVs ($p < 0.0001$) (Fig. 1A).

As expected, patients with MPM had higher asbestos exposure than controls ($p < 0.0001$) (Fig. 1B). Mean quantitative asbestos exposure among controls was 2.7 (standard deviation [SD] 7.9), compared with 21.7 (SD 105.4) among our current and previous patients with MPM [6,9,17,18] combined (Suppl. Table 4). Interestingly, when we compared asbestos exposure in controls (mean 2.7, SD 7.9) and in mutated patients (mean 2.7, SD 6.4), the difference was not statistically significant ($p = 0.57$) (Fig. 1C).

These data support the gene–environment interaction hypothesis: PV carriers have a higher risk of MPM because of the combined effect of germline PVs and asbestos exposure. Moreover, they support the biological impact of the PVs.

3.3. Functional studies

It is well known that defects in the HRR pathway can be exploited for cancer treatment through synthetic lethality, a mechanism by which two, otherwise non-lethal defects, become lethal when simultaneously present in a cell. To test new avenues of synthetic lethality, we set up a three-dimensional-MPM cell model that had a defect in ataxia telangiectasia mutated (ATM), the master regulator of the response to DNA double-strand breaks (Fig. 2). We generated an *ATM*-silenced cell model by transfecting small interfering RNA (siRNA) targeting *ATM* in MSTO-211H cells cultured as multicellular spheroids, because it has been demonstrated that spheroids mimic drug response in MPM tumours more accurately than monolayer cells [20]. Down-regulation of *ATM* expression was confirmed by quantitative reverse transcriptase-polymerase chain reaction (RT-PCR) (Suppl. Fig. 3A). We tested this model with EPZ-6438, a selective inhibitor of EZH2, an enzymatic subunit of the polycomb repressive complex 2. EZH2 plays a key role in cancer initiation and progression and is overexpressed in many tumours, including MPM [21,22].

In MSTO-211H spheroids treated with EPZ-6438, we observed increased expression of the histone γH2AX (gamma-H2A.X variant histone) (Suppl. Fig. 3B), a marker of DNA damage [23] and abolishment of

Table 2
PVs identified in this study.

#	Pz ID	Gene	Ref seq	Mutation	rs	Varsome	ClinVar	Mutation type	Function	Asbestos exposure type	Gene previously reported in mesothelioma
1	MPM_2H	*BRCA1*	NM_007294	c.5212G > A (p.Gly1738Arg)	rs80356937	Pathogenic	Pathogenic	Missense	HRR	Environmental	Betti 2017 Panou 2018
2	MPM_33TO	*SLX4*	NM_032444.4	c.59del (p.Leu20fs)	rs1315905872	Pathogenic	Pathogenic	Frameshift	HRR	Occupational	Betti 2017
3	MPM_29TO	*CDKN2A*	NM_058197.4	c.250A > T (p.Lys84*)	NR	Likely pathogenic	NR	Nonsense	Cell cycle	Occupational	Panou 2018
4	MPM_44TO	*VHL*	NM_000551.3	c.154G > T (p.Glu52*)	rs373068386	Pathogenic	Uncertain significance	Nonsense	Cell cycle, regulation of hypoxia inducible factor expression, protein degradation	Para-occupational	Panou 2018
5	MPM_68TO	*BRIP1*	NM_032043.3	c.1A > G (p.?)	rs764585550	Likely pathogenic	Uncertain significance	Start loss	HRR	Occupational	This article
6	MPM_1108	*FLCN*	NM_144606.7	c.918G > A (p.Trp306*)	rs142934950	Likely pathogenic	Conflicting interpretation	Nonsense	Regulation of amino acid synthesis, HRR[a]	NA	This article
7	MPM_29AL	*CHEK2*	NM_007194.4	c.470T > C (p.Ile157Thr)	rs17879961	Likely pathogenic	Conflicting interpretation	Missense	HRR, cell division	Para-occupational	Panou 2018, Hassan 2019
8	MPM_3TO	*SBDS*	NM_016038.4	c.183_184delinsCT (p.Lys62*)	rs120074160	Pathogenic	Pathogenic/likely pathogenic	Nonsense	Ribosome assembly	Occupational	This article
9	MPM_HO1901	*BAP1*	NM_004656.2	c.38-1G > T	NR	Pathogenic	Likely pathogenic	Splicing	HRR, ubiquitination, cell proliferation, Ca^{++} metabolism	NA	This article

HRR, homologous recombination repair; MPM, malignant pleural mesothelioma; NR, not reported; NA, not available; PV, pathogenic variant.
[a] Gene recently suggested to be involved in HRR (Zhang et al., 2020).

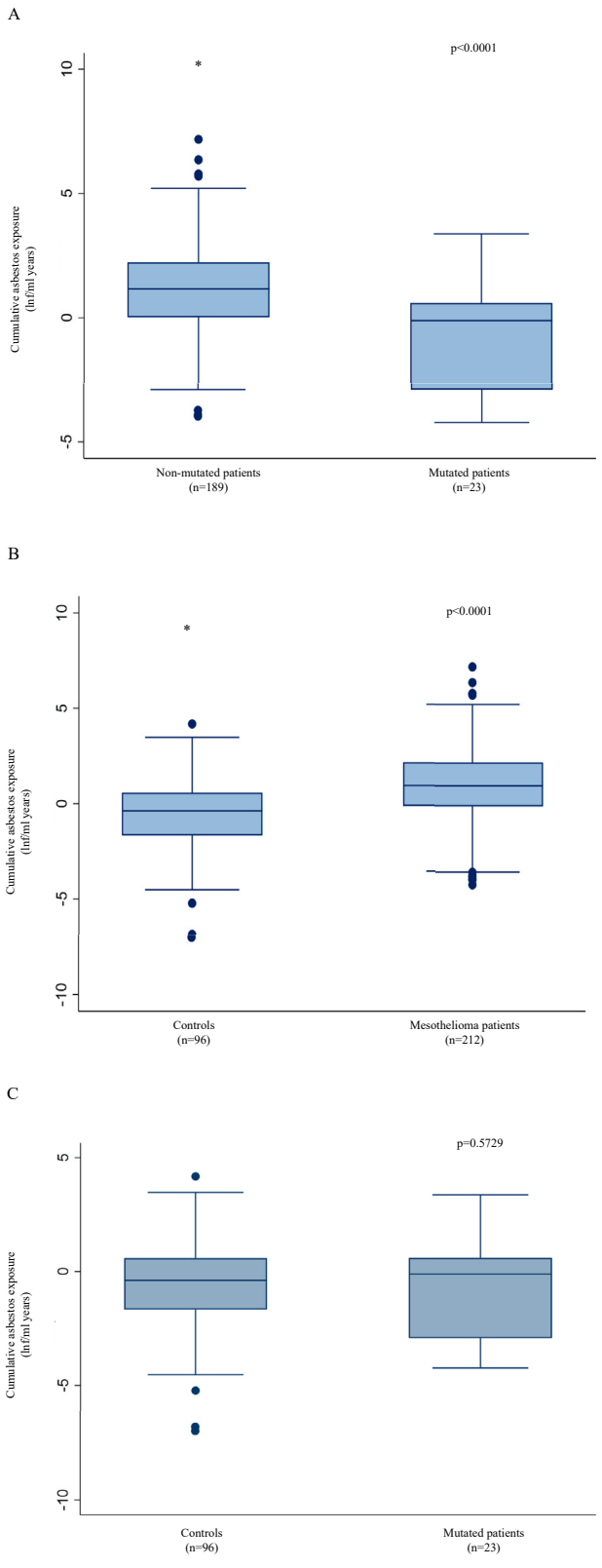

Fig. 1. **Box plots showing the cumulative asbestos exposure in our panel of patients with mesothelioma and controls. (A)**. Mutated patients (n = 23), including those reported in previous studies of ours (Betti et al., 2015; Betti et al., 2016; Betti et al., 2017; Betti et al., 2018), showed a statistically significant lower amount of asbestos exposure ($p < 0.0001$) compared with patients without pathogenic germline variants in cancer-predisposing genes (n = 189). The quantification of the asbestos exposure was missing for two patients with PVs and seven non-mutated patients. **(B)**. Controls (n = 96), previously analysed (Betti et al., 2017) showed a statistically significant lower asbestos exposure ($p < 0.0001$) than patients with mesothelioma (n = 212), including cases from our previous works (Betti et al., 2015; Betti et al., 2016; Betti et al., 2017; Betti et al., 2018) and the present study. **(C)**. Quantitative asbestos exposure of the control group was not different from patients with pathogenic germline variants in cancer-predisposing genes ($p = 0.5729$). PV, pathogenic variant.

H3K27 trimethylation (Suppl. Fig. 3B). EPZ-6438 treatment significantly reduced size and viability of *ATM*-silenced spheroids (Fig. 3A–B). Induction of apoptosis in *ATM*-silenced cells treated with EPZ-6438 was confirmed by poly [ADP-ribose] polymerase-1 (PARP-1) cleavage (Fig. 3C) and increased expression of *BCL2L11* and *PUMA* transcripts (Fig. 3D). The effects of EPZ-6438 on cell viability were reproduced using the selective ATM inhibitor (KU55933) (Suppl. Fig. 3C and D). As shown in Supplementary Fig. 4, similar results were observed using BR95 cells (derived from epithelioid MPM) cultured as spheroids.

4. Discussion

We evaluated the occurrence of germline PVs in a new panel of patients with MPM, who were not selected for their family history for MPM or cancer, and calculated the prevalence of PVs in DNA repair genes. In our sample of 113 patients with MPM, we found eight germline PVs in eight patients with MPM. Five genes were previously reported in patients with MM [6,10,12], whereas the other three have never been associated with a genetic predisposition to MM. We observed *FLCN* and *BRIP1* LOH in patient tumour samples, supporting for the first time an involvement of these genes in MPM carcinogenesis. An additional patient with MPM and a family history of MM carried a PV in *BAP1*.

Several of the genes we found mutated are involved in HRR (*BRCA1, BRIP1, CHEK2, SLX4, FLCN* and *BAP1)*. In particular, *BRCA1*, *SLX4* and *BRIP1* encode for members of the Fanconi anaemia complex that collaborates to repair DNA interstrand crosslinks [24]. CHEK2 is a kinase protein that acts as a tumour suppressor; it regulates not only cell division but also DNA damage response and interacts with ATM in the ATM-CHEK2 cascade [25,26]. BRCA1 Associated Protein 1 (BAP1) is a ubiquitin carboxy-terminal hydrolase implicated in DNA damage repair by directly binding the breast cancer susceptibility protein 1/BRCA1-associated RING domain protein (BRCA1/BARD1) complex [27]. Deficiency of folliculin (FLCN) impairs the integrity of BRCA1 A complex (involved in the

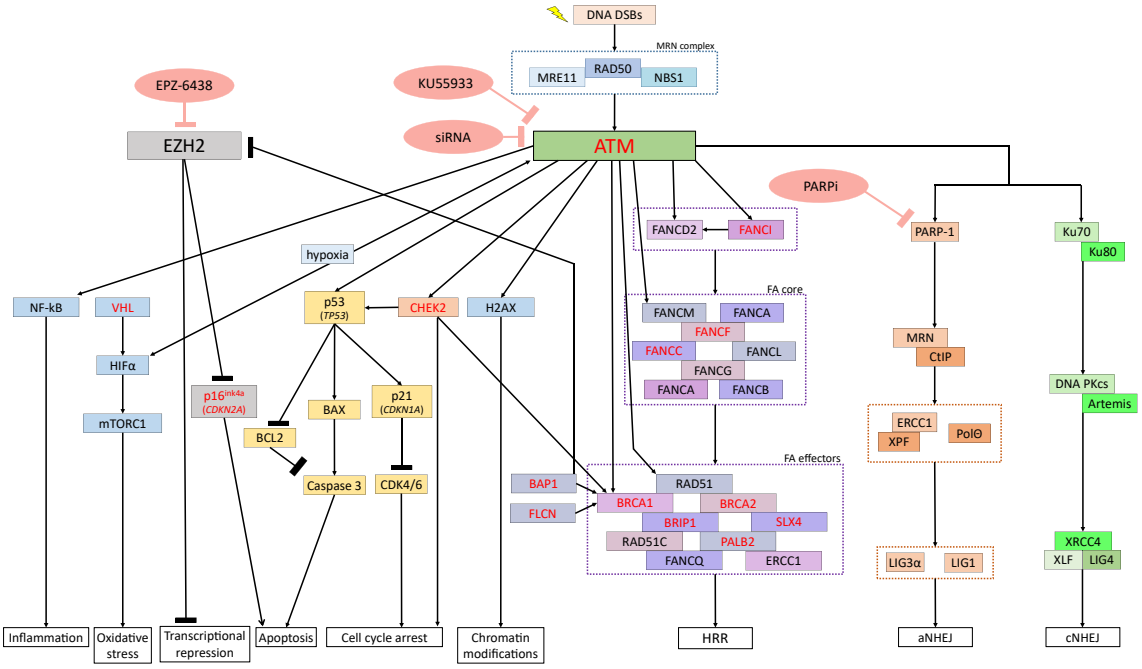

Fig. 2. **Rationale of the *in vitro* experiments**. In our experimental condition, the DNA damage is most probably caused by hypoxia. Loss of EZH2 when the DNA repair master gene ataxia telangiectasia mutated (*ATM*) is inactive causes apoptotic cell death of MPM cells, because the DNA damage cannot be repaired. The pathways modulated by EZH2 and ATM are simplified. Arrows represent protein interaction; truncated lines represent function inhibition. When the names of the gene and the protein are different, the name of the gene is indicated in parentheses. EPZ-6438 favours cell cycle block, because it removes the EZH2-mediated *CDKN2A* inhibition; KU55933 inhibits DNA repair pathways. EZHZ, enhancer of zeste homologue 2; MPM, malignant pleural mesothelioma.

recruitment of BRCA1 to DNA damage sites) and sensitises cells to PARP inhibitor olaparib, suggesting that also FLCN may be involved in the DNA repair [28].

Overall, the prevalence of germline PVs in cancer-predisposing genes in our study was 7.1% (8/113); most of them occurred in genes involved in the HRR pathway (62.5%, 5/8 patients). In a previous study by our group, 9.7% of the 93 patients with MPM carried germline PVs in DNA repair genes, 80% of which affected HRR genes [6]. When we added these data to our present results, the prevalence of germline PVs in cancer-predisposing genes in patients with MPM was 8.25% (17/206), most of them in HRR genes (76.5%, 13/17 patients). These results confirm and extend those of previous studies (Suppl. Table 3) [6,10–14,16]. Riaz et al. [29] observed that 5% of all patients with cancer had a biallelic loss of HRR genes in tumour tissues. Biallelic mutations in HRR genes are more frequent in tumours of individuals with germline PVs than in those without and render the patients sensitive to tailored drugs [29].

It is well known that asbestos fibres induce DNA damage in mesothelial cells both directly and indirectly [30,31], thus carriers of mutations in DNA repair genes are probably less able to correct these lesions. Indeed, our data based on a quantitative assessment of asbestos exposure [6] demonstrate that patients with PVs in DNA repair genes had a statistically significant, lower cumulative asbestos exposure than patients without PVs ($p < 0.0001$). Thus, patients carrying germline PVs in DNA repair genes seem more susceptible to asbestos-induced MPM. It is intriguing to note that the most represented genes were those involved in HRR, the pathway that repairs DNA double-strand breaks, a type of DNA damage caused by asbestos fibres. This suggests that decreased HRR activity may be the cause of the increased sensitivity to asbestos found in these patients. The key role of gene–environment interaction in asbestos-related carcinogenesis is further supported by the similar levels of asbestos exposure we observed between patients with germline PVs and controls from the same area (Fig. 1C).

This scenario may be exploited for the development of tailored treatment. Previous research has proposed that patients with ovarian cancer who carry inherited PVs in two HRR genes, *BRCA1* and *BRCA2*, have increased sensitivity to drugs that induce synthetic lethality and show a better response to PARP-1 inhibitors compared with patients with ovarian cancer without such germline PVs [32,33]. Similarly, patients with MM carrying germline PVs in HRR genes may benefit from drugs that induce synthetic lethality. A phase II trial (ClinicalTrials.gov NCT03654833) to test the effect of rucaparib on patients with MPM and somatic inactivating mutations of *BRCA1*/*BAP1* is ongoing [34]. Moreover, rationale and study design of

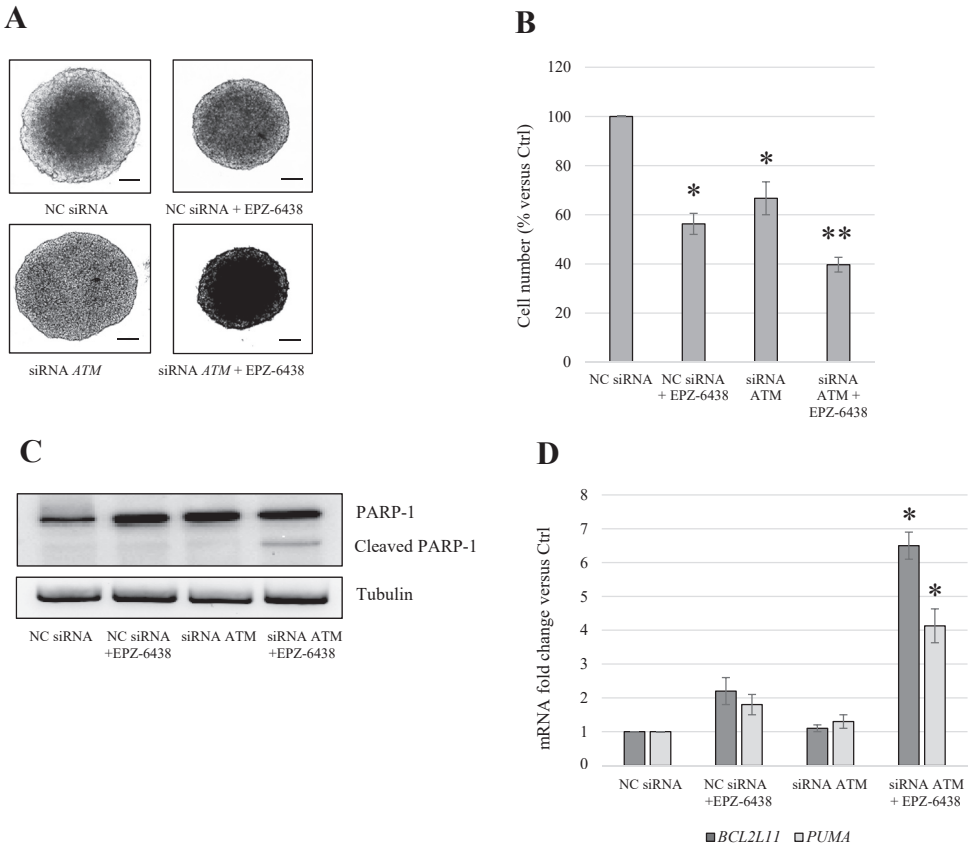

Fig. 3. **Functional studies in an ATM-deficient 3D-MPM cell model. (A)**. Representative phase contrast images of MPM-derived MSTO-211H multicellular spheroids transfected with negative control (NC) siRNAs or *ATM*-specific siRNAs (siRNA ATM) and treated ± with EPZ-6438, 72 h. Scale bar = 100 μM. **(B)**. Bar graph shows the percentage of viable cells in MSTO-211H multicellular spheroids transfected with negative control (NC) siRNAs or *ATM*-specific siRNAs (siRNA ATM) and treated ± EPZ-6438 for 72 h. Each bar represents mean of three independent experiments ±SD, $*p \leq 0.05$, $**p \leq 0.01$. **(C)**. Representative Western blot analysis of PARP-1 and cleaved PARP-1 in MSTO-211H multicellular spheroids transfected with negative control (NC) siRNAs or *ATM*-specific siRNAs (siRNA ATM) treated ± EPZ-6438, 72 h. Tubulin was used as loading control. **(D)**. Quantitative RT-PCR analysis of *BCL2L11* and *PUMA* expression in MSTO-211H multicellular spheroids transfected with negative control (NC) siRNAs or *ATM*-specific siRNAs (siRNA ATM) and treated ± EPZ-6438, 72 h. Each bar represents mean of three independent experiments ±SD, $*p \leq 0.05$. 3D, three-dimensional; MPM, malignant pleural mesothelioma; SD, standard deviation; PARP-1, poly [ADP-ribose] polymerase-1; siRNA, small interfering RNA; RT-PCR, reverse transcriptase-polymerase chain reaction.

the phase II clinical trial on patients with MPM carrying germline and somatic PVs in DNA repair genes and treated with niraparib and dostarlimab have been recently published. The hypothesis is that the presence of PVs in HRR genes increases the tumour mutational burden promoting the expression of neoantigens, which might be targeted by immune checkpoint inhibitors [35].

We decided to investigate the effect of an EZH2 inhibitor on MPM cells with a defect in *ATM*. *ATM* encodes for a protein that represents the principal DNA damage sensor, which acts not only in HRR but also in other DNA repair pathways (Fig. 2) [36]. Germline and somatic PVs in *ATM* have been found in patients with MPM [6,10]. We generated an ATM-deficient MPM cell model to test the effect of the EZH2 inhibitor EPZ-6438 or tazemetostat. EZH2 is a histone-lysine N-methyltransferase enzyme, involved in histone methylation and, ultimately, transcriptional repression. EZH2 has been observed to be overexpressed in MPM [21], and high EZH2 expression is correlated with tumourigenesis, cancer progression and metastasis [22]. Tazemetostat caused an increase in γ-H2AX expression (indicative of increased DNA damage) [23] in MPM spheroids and selectively induced apoptosis in ATM-deficient cells. A likely explanation is that ATM loss causes failure in DNA damage repair, rendering tazemetostat-treated cells prone to apoptosis. These data indicate that loss of ATM in MPM cells is synthetic lethal with EZH2 inhibition, suggesting that patients with loss-of-function mutations in *ATM*, and possibly also those carrying a mutation in other HRR genes, may benefit from this treatment. The efficacy of

tazemetostat in patients with loss of other DNA repair genes is supported by the encouraging results of a phase II clinical trial on BAP1-deficient patients with MPM, where tazemetostat showed promising antitumour activity (ClinicalTrials.gov NCT02860286) [37]. Interestingly, similar results were recently obtained by our group by treating *CDKN2A*-silenced mesothelioma spheroids with EPZ-6438 [38].

In conclusion, our results indicate that the subgroup of patients with germline defects in DNA repair genes, although more prone to asbestos-induced carcinogenesis, may respond to personalised medicine.

Author contribution

Marika Sculco: investigation; methodology; data curation; writing — original draft; visualisation. **Marta La Vecchia:** investigation; methodology; data curation; writing — original draft; visualisation. **Anna Aspesi:** data curation; writing — original draft; visualisation. **Giulia Pinton:** investigation; methodology; writing — review and editing. **Michela Giulia Clavenna:** data curation; writing — review and editing. **Elisabetta Casalone:** writing — review and editing. **Alessandra Allione:** writing — review and editing. **Federica Grosso:** resources; writing — review and editing. **Roberta Libener:** resources; writing — review and editing. **Alberto Muzio:** resources; writing — review and editing. **Ottavio Rena:** resources; writing — review and editing. **Guido Baietto:** resources; writing — review and editing. **Sara Parini:** resources; writing — review and editing. **Renzo Boldorini:** resources; writing — review and editing. **Daniela Giachino:** resources; writing — review and editing. **Mauro Papotti:** resources; writing — review and editing. **Giorgio Vittorio Scagliotti:** resources; writing — review and editing. **Enrica Migliore:** Resources; writing — review and editing. **Dario Mirabelli:** resources; writing — review and editing. **Laura Moro:** methodology; writing — review and editing. **Corrado Magnani:** writing — review and editing; conceptualisation; supervision; funding acquisition. **Daniela Ferrante:** formal analysis; writing — review and editing. **Giuseppe Matullo:** data curation; writing — review and editing; supervision; funding acquisition. **Irma Dianzani:** conceptualisation; data curation; writing — original draft; visualisation; supervision; project administration; funding acquisition.

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Conflict of interest statement

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Grosso reports outside the submitted work personal fees for advisory role, speaker engagements and travel and accommodation expenses from Merck Sharp & Dohme (MSD), Novocure, Bristol Meyer Squibb, Boehringer Ingelheim, PharmaMar and Novartis. Scagliotti reports outside the submitted work personal fees for lectures, presentations, speaker bureaus, article writing and educational events from Eli Lilly, Roche, Pfizer, AstraZeneca, Novartis, MSD, Takeda and Beigene. Scagliotti reports participation on a data safety monitoring board and advisory board from Beigene, Takeda, MSD, Novartis, AstraZeneca, Pfizer, Roche and Eli Lilly outside the submitted work. Mirabelli received payment to discuss court cases with asbestos-related neoplasms from the public prosecution office at the Verbania Court and Turin Court. Magnani received payment for participation in different trials regarding asbestos-related diseases from the public prosecution office and research funding (BRIC Project) from INAIL outside the submitted work. Dianzani has been appointed by the public prosecution office to discuss court cases with asbestos-related neoplasms. The remaining authors declare no conflicts of interest.

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Appendix A. Supplementary data

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