Molecular Cancer Therapeutics

Genomic Landscape of Malignant Mesotheliomas

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Abstract

Understanding the genomic landscape of malignant mesothelioma may identify novel molecular drivers of this ultra-rare disease, which can lead to an expanded roster of targeted therapies and clinical trial options for patients with mesothelioma. We examined the molecular profiles of 42 patients with malignant mesothelioma (including pleural, peritoneal, and pericardial) that were referred by clinicians to be tested in a Clinical Laboratory Improvement Amendments (CLIA) laboratory using next-generation sequencing (NGS; 182 or 236 genes). Among 42 patients, there were 116 alterations, with 92 being distinct. The number of genomic alterations per patient ranged from 1 to 5 (median = 3). No two patients had identical molecular portfolios. The most common aberrations were in *BAP1* (*BRCA1*-associated protein 1; 47.6% [20/42]), *NF2* (38.1% [16/42]), and *CDKN2A/B* (loss) (35.7% [15/42]). *BAP1* alterations and *CDKN2A/B* loss were associated with pleural mesothelioma (OR 3.4, $P = 0.059$ [*BAP1*] [trend]; OR 5.8, $P = 0.01$ [*CDKN2A/B*]). All 42 patients had a molecular abnormality that was potentially actionable (median = three actionable alterations per patient; range, 1 to 5), and, in 40 patients (95.2%), a drug approved by the FDA was applicable. In conclusion, each individual with malignant mesothelioma harbored a unique set of genomic aberrations, suggesting that NGS-based profiling of patients will be needed if patients are to be optimally matched to cognate treatments. All 42 patients had at least one alteration that was, in theory, pharmacologically tractable. *Mol Cancer Ther; 15(10); 2498–507. ©2016 AACR.*

Introduction

Malignant mesothelioma is an aggressive, ultra-rare tumor (defined as prevalence of less than 20 per million individuals; ref. 1) arising from mesothelial surfaces. The majority of mesotheliomas derive from pleura (83%) followed by peritoneum (11%; ref. 2). In rare cases, mesotheliomas arise from tunica vaginalis testis and pericardium (1%–2%; ref. 3). Mesotheliomas are associated with poor clinical outcome, with a median survival of 12 to 16 months for advanced stage malignant pleural mesothelioma (4), and 12.5 to 31 months for peritoneal mesothelioma (5). Exposure to asbestos is implicated as a risk factor, and about 50% of patients with malignant pleural mesothelioma were reported to have such exposure (4). Asbestos causes chronic irritation of the mesothelial surface, which leads to local inflammation, scarring, and ultimately development of mesothelioma (6).

For selected patients who can be predicted to achieve complete resection, a combined modality approach with surgery, chemotherapy, and/or radiation therapy has been used, with the literature suggesting clinical improvement when compared to historic controls (7). However, due to the rarity of this cancer type (2) there are no adequately powered trials to evaluate the benefit of combined modality approaches.

For patients with advanced or recurrent disease, chemotherapy with platinum-based doublets has been widely applied. For example, cisplatin plus pemetrexed has been shown to improve clinical outcome in malignant pleural mesothelioma when compared to cisplatin alone, with OS of 12.1 months versus 9.3 months ($P = 0.02$), progression-free survival (PFS) of 5.7 months versus 3.9 months ($P = 0.001$), and a response rate of 41.3% versus 16.7% ($P < 0.0001$; ref. 8). Addition of bevacizumab to cisplatin plus pemetrexed is also associated with better clinical outcome when compared to cisplatin plus pemetrexed alone [OS of 18.8 months versus 16.1 months ($P = 0.0167$), PFS 9.2 months versus 7.3 months ($P < 0.0001$); ref. 9].

Although chemotherapy has shown some salutary effects, prognosis remains poor; thus, targeted therapies such as sunitinib [partial response (PR): 12%; ref. 10], sorafenib (PR: 6%; ref. 11), and imatinib (PR: 0%; ref. 12), have been tried, although with minimal clinical efficacy, perhaps because they were given to patients without genomic selection (13, 14). Because patients with mesothelioma uniformly have high expression of mesothelin (15), clinical trials targeting mesothelin are ongoing [e.g., BAY 94-9343, an anti-mesothelin antibody conjugated to the maytansinoid tubulin inhibitor DM4 (NCT01439152); amatuximab (MORab-009), an anti-mesothelin antibody (NCT02357147); and CRS-207, mesothelin-expressing Listeria cancer vaccine (NCT01675765)]. Immunotherapy approaches are under investigation and early-phase clinical trials in patients who failed standard therapy showed moderate responses with tremelimumab (anti-CTLA4 monoclonal antibody; PR: 7%; ref. 16) and pembrolizumab (anti-PD-1 antibody; PR: 24%; ref. 17).

In several refractory malignancies, such as lung cancer and melanoma, elucidation of the molecular defects and prosecution

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of the tumor with matched targeted therapy has proved effective (18, 19). A deeper understanding of the underlying alterations in various types of mesothelioma may also prove worthwhile. Previous studies show that *BRCA1-associated protein-1* (*BAP1*; 21%–63%; refs. 20–25), *TP53* (57%; ref. 25), *CDKN2A* (45%–75%; refs. 22 and 24), and *NF2* (14%–50%; refs. 20–22, 24, and 25) are frequently abnormal in pleural mesothelioma and that Hippo, mTOR, histone methylation, RNA helicases, and p53 signaling pathways are most often affected (21). Here we examined the genomic portfolios of 42 patients with diverse types of mesothelioma (including pleural, peritoneal, and pericardial) interrogated by clinical-grade NGS, and assessed the resulting implications for potential targeted therapy options.

Materials and Methods

Patients

We investigated the genetic aberration status of 42 patients with mesothelioma (pleural: $n = 23$, peritoneal: $n = 11$, pericardial: $n = 2$, subtype unknown: $n = 6$) referred to Foundation Medicine for NGS from December 2011 through November 2013. Tumor types were provided by the submitting physicians. The database was de-identified. Next-generation sequencing data were collected and interpreted by N-of-One, Inc.

Tissue samples and mutational analysis

We collected sequencing data from 42 mesothelioma patients whose formalin-fixed, paraffin-embedded (FFPE) tumor samples were submitted to a clinical laboratory improvement amendments (CLIA)-certified lab for genetic profiling (Foundation Medicine). Samples required surface area $\geq$25 mm^2, volume $\geq$1 mm^3, nucleated cellularity $\geq$80%, and tumor content $\geq$20% (26). The methods used in this assay have been previously reported and validated (26–28). In short, 50 to 200 ng of genomic DNA was extracted and purified from the submitted FFPE tumor samples. This whole-genome DNA was subjected to shotgun library construction and hybridization-based capture before paired-end sequencing on the Illumina HiSeq2000 platform. Hybridization selection is performed using individually synthesized baits targeting the exons of 182 or 236 cancer-related genes and the introns of 14 or 19 genes frequently rearranged in cancer (29). Sequence data were processed using a customized analysis pipeline (26). Sequencing was performed with an average sequencing depth of coverage greater than $\times$250, with >$\times$100 at >99% of exons. This method of sequencing allows for detection of copy number alterations, gene rearrangements, and somatic mutations with 99% specificity and >99% sensitivity for base substitutions at $\geq$5 mutant allele frequency and >95% sensitivity for copy number alterations. A threshold of $\geq$8 copies for gene amplification with $\geq$6 copies considered equivocal (except for *ERRB2*, which is considered equivocally amplified with $\geq$5 copies) was used. All aberrations were analyzed based on American College of Medical Genetics guidelines to evaluate whether alterations were pathogenic. This study and data analysis was performed in accordance with UCSD IRB guidelines.

Endpoints and statistical methods

Descriptive statistics were used to summarize the baseline patient characteristics. Fisher exact test was used to assess the association between categorical variables in univariate analysis. All tests were two-sided. Statistical analyses were carried out using GraphPad Prism version 6.0.

Results

Genetic aberrations in mesotheliomas

Among all mesotheliomas ($N = 42$), the most common histologic diagnosis was pleural mesothelioma (55% [23/42]) followed by peritoneal mesothelioma (26% [11/42]). Pericardial mesothelioma was the least common subtype (5% [2/42]), and 14% (6/42) of mesothelioma samples had unknown subtype (Figs. 1–4 and Supplementary Tables S1 and S2).

The number of molecular aberrations reported per patient ranged from one to five, with a median of three per patient (Supplementary Fig. S1). The most common genetic aberrations among all mesotheliomas occurred in the *BAP1* gene (47.6% [20/42]), followed by *NF2* (38.1% [16/42]), *CDKN2A/B* loss (35.7% [15/42]), and *TP53* aberrations (16.7% [7/42]; Figs. 1 and 2 and Supplementary Table S1). Among pleural mesothelioma patients ($n = 23$), *BAP1* was the most common gene altered (60.9% [14/23]) followed by *CDKN2A/B* (loss; 52.2% [12/23]), *NF2* (34.8% [8/23]), and *TP53* (17.4% [4/23]; Fig. 3 and Supplementary Table S1). Among peritoneal mesothelioma ($n = 11$), the most common aberration was in *NF2* (36.4% [4/11]), followed by *BAP1* (27.3% [3/11]; Fig. 4 and Supplementary Table S1). *BAP1* aberrations ($n = 20$) consisted of mutation (50% [10/20]), loss (25% [5/20]), rearrangement (5% [1/20]), and cases with multiple alterations (20% [4/20]). Among individual with *NF2* aberrations ($n = 16$), 81.3% (13/16) had a mutation, 12.5% (2/16) had loss, and 6.3% (1/16) had multiple alterations (Fig. 2).

Association between histologic subtypes of mesothelioma and coexisting molecular alterations

Among *BAP1*, *NF2*, *CDKN2A/B*, and *TP53* aberrations, there were no statistically significant associations in terms of coexisting genetic aberrations (Supplementary Tables S3–S5). However, a trend toward less common association between *BAP1* aberration and *TP53* (OR 0.14; $P = 0.096$) or *NF2* aberrations (OR 0.33; $P = 0.12$) was noted (Supplementary Tables S3 and S4). When focusing on the association between histologic diagnosis and molecular aberrations, pleural mesothelioma was significantly associated with loss of *CDKN2A/B* (OR 5.8; $P = 0.01$) and a trend toward association with *BAP1* aberration was noted (OR 3.4; $P = 0.059$). However, there was no association between pleural mesothelioma and *NF2* aberration (OR 0.73; $P = 0.63$; Supplementary Tables S3–S5). However, peritoneal mesothelioma was significantly less associated with *CDKN2A/B* loss (OR 0.12; $P = 0.032$) and a trend suggests less common association with *BAP1* aberration (OR 0.31; $P = 0.12$; Supplementary Tables S3 and S5).

Number of genetic aberrations and possible cognate targeted therapies in patients with mesothelioma

Among 42 mesothelioma cases, a total of 116 aberrations were identified. Among all aberrations, 112 aberrations were potentially actionable either with therapies approved by FDA for other types of malignancies or with therapies currently in clinical trials (112/116 [96.6%]). Among 112 actionable aberrations, 97 (86.6%) were targetable with FDA-approved agents (off label), and an additional 15 (13.4%) were targetable with investigational agents (Table 1 and Supplementary Tables S2 and S6).

Among 116 aberrations, there were 92 distinct alterations. (For example, *BAP1* and *NF2* aberrations were considered distinct; *BAP1* S460* and *BAP1* S63C mutations were also considered to be distinct aberrations. However there were $n = 15$ with *CDKN2A/B* loss and those were counted as a

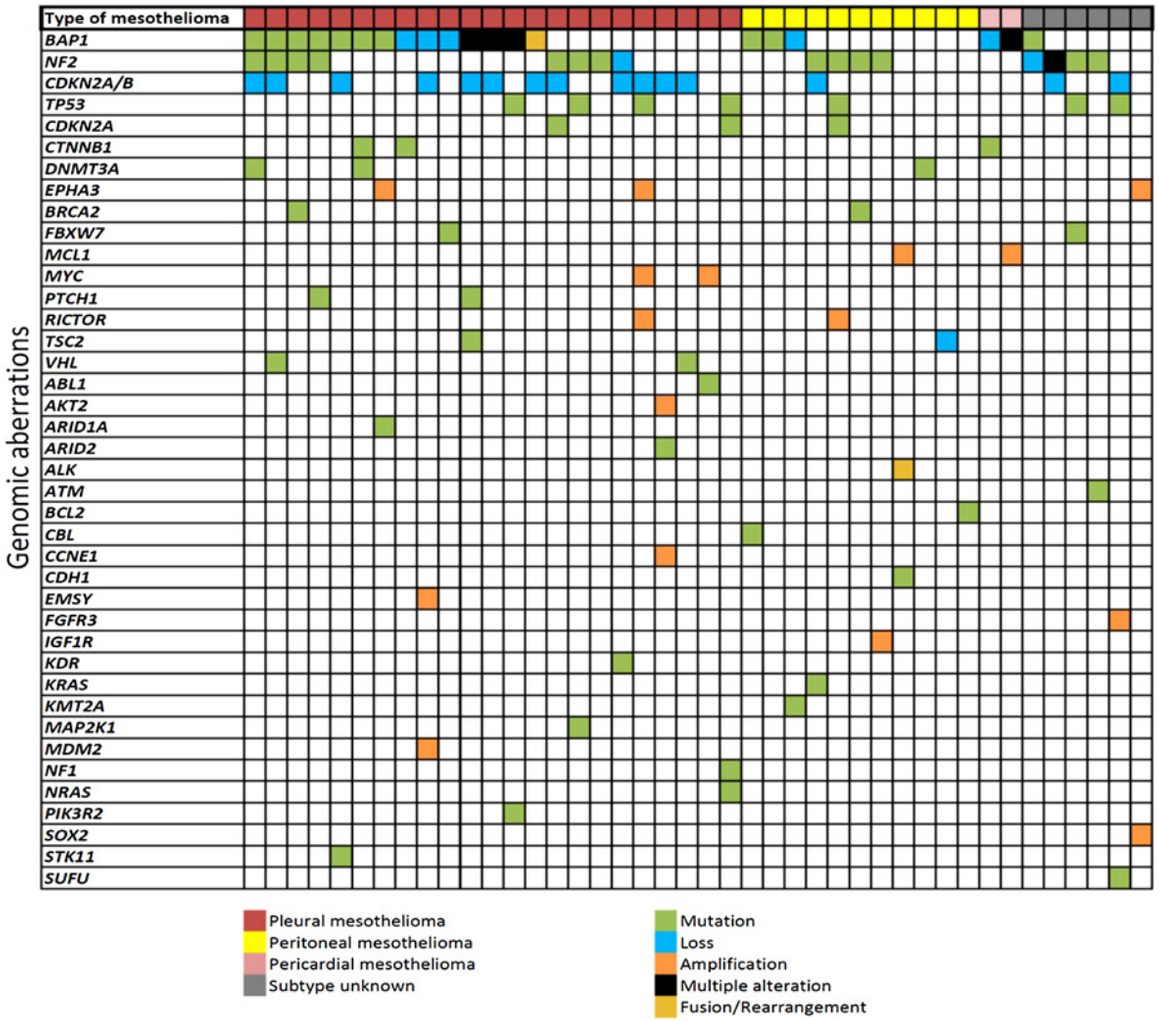

Figure 1.
Overview of aberrations in all patients with mesothelioma ($N = 42$). Genes in order based on the frequency of the genes.

single aberration.) Nearly all of the distinct aberrations (88/92 [95.7%]) were potentially actionable, including 77 (87.5% [77/88]) that were theoretically targetable by an FDA-approved drug. An additional 11 aberrations (12.5% [11/88]) were theoretically targetable by an experimental drug in a clinical trial (Table 1 and Supplementary Tables S2 and 6).

The median number of potentially actionable aberrations per patient was 3 (range, 1 to 5; Supplementary Fig. S1). All 42 patients with mesothelioma had theoretically actionable aberrations. Of the 42 patients, 40 (95.2%) had an aberration targetable by an FDA-approved drug and an additional two (4.8%) had an aberration targetable by an investigational drug in a clinical trial (Table 1 and Supplementary Tables S2 and S6 and Supplementary S1).

Distinctness of the genomic aberrations among 42 mesothelioma patients

As noted, 92 distinct genetic aberrations were detected. Among 42 patients, no two patients had an identical molecular portfolio. If we considered the genetic aberrations at the level of the gene, rather than the specific aberration (for example, different aberrations in same gene would be considered as identical), then there were 40 genetic aberrations and four patients had genomic portfolios identical to at least one other patient. Those include one patient with pleural (*BAP1* loss and *CTNNB1* Q280*) and one with pericardial mesothelioma (*BAP1* loss and *CTNNB1* splice site 1955-2_1955-1ins16) and two patients with pleural mesothelioma (one with a *BAP1* rearrangement and the other with *BAP1* truncation and R610fs*7 mutations. Both also had *CDKN2A/B* loss; Supplementary Table S2).

Discussion

Malignant mesothelioma is an uncommon cancer (2) with limited therapeutic options (8, 10–12) and poor clinical outcomes (4, 5). Thus, we investigated the genomic landscape of this tumor by targeted NGS. In our current study of 42 patients, 55%

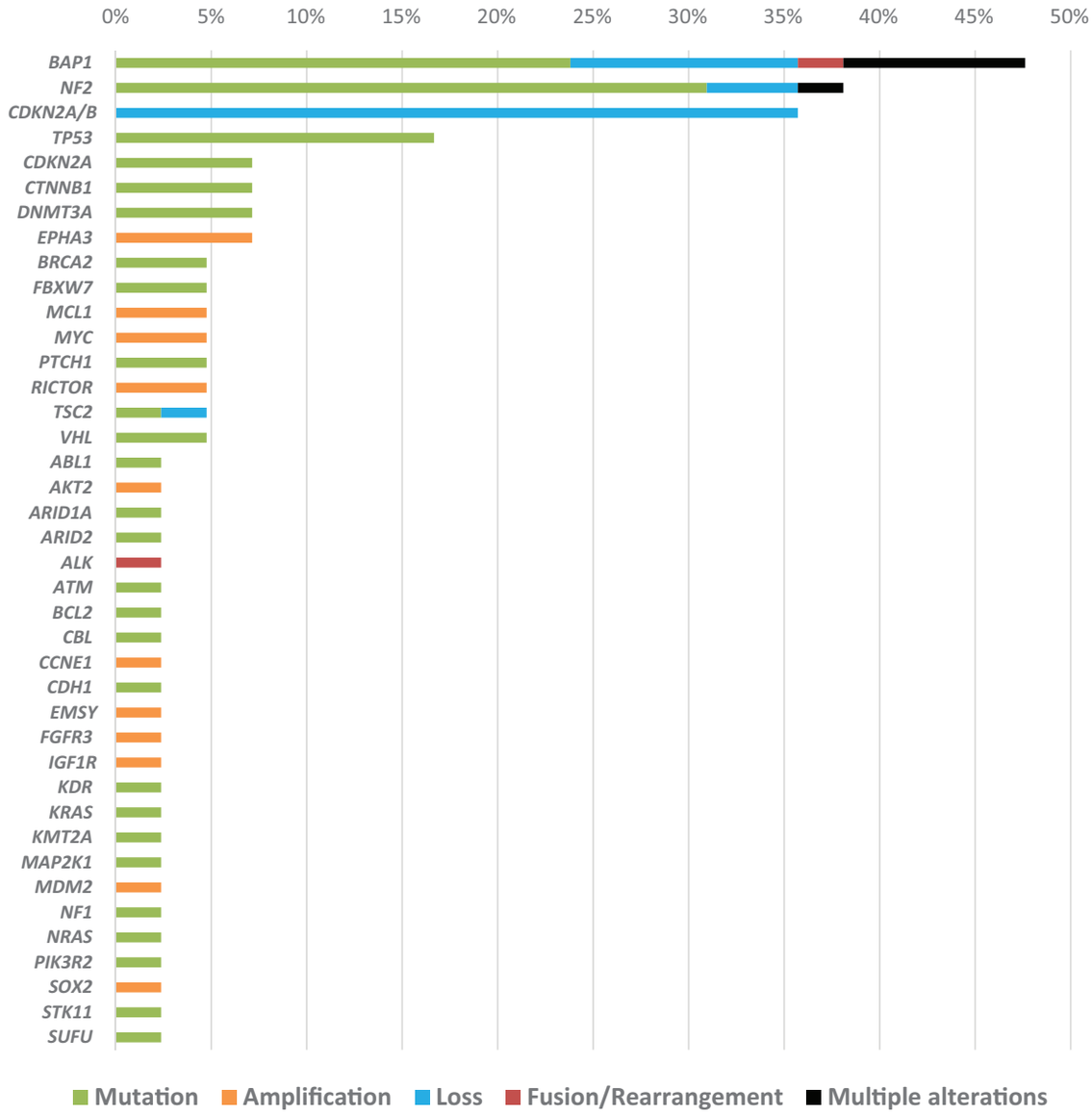

Figure 2.
Frequency and types of genetic aberrations (mutation, amplification, loss, fusion/rearrangement or multiple alteration) among all mesothelioma cases ($N = 42$).

(23/42) had pleural mesothelioma; 26% (11/42), peritoneal mesothelioma; and 5% (2/42), pericardial mesothelioma. Previous literature from the Surveillance Epidemiology and End Results (SEER) database suggests that the majority (83%) of mesothelioma cases are from pleura and 11% are from peritoneum (2).

The most frequent genetic aberrations were in *BAP1* (47.6% [20/42]; Figs. 1 and 2 and Supplementary Table S1). Our observation is in agreement with previous studies demonstrating that 21% to 63% of malignant mesothelioma tumors harbored *BAP1* abnormalities (20–25). *BAP1* aberrations showed a trend to be more commonly associated with pleural mesothelioma (OR 3.4; $P = 0.059$) and less often with peritoneal mesothelioma (OR 0.31; $P = 0.12$). Moreover, *BAP1* aberrations showed a trend to be less likely to be associated with *NF2* (OR 0.33; $P = 0.12$) or *TP53* (OR 0.14; $P = 0.096$) aberrations, but no difference was observed for *CDKN2A/B* loss among patients with or without *BAP1* aberration (OR 0.94; $P = 1.0$; Supplementary Table S3). These associations must, however, be viewed with significant caution, as the total number of patients is small.

BAP1 is a tumor suppressor gene that encodes a deubiquitinating enzyme BAP1 that binds to BRCA1 and regulates key cellular pathways including cellular differentiation, cell cycle, and DNA damage response (30). Because a functional defect in the BRCA1-mediated DNA repair pathway confers synthetic lethality to PARP inhibition (31), the association between *BAP1* mutation and efficacy of PARP inhibitors has been investigated. Pena-Llopis and colleagues showed that clear cell renal cell carcinoma cell

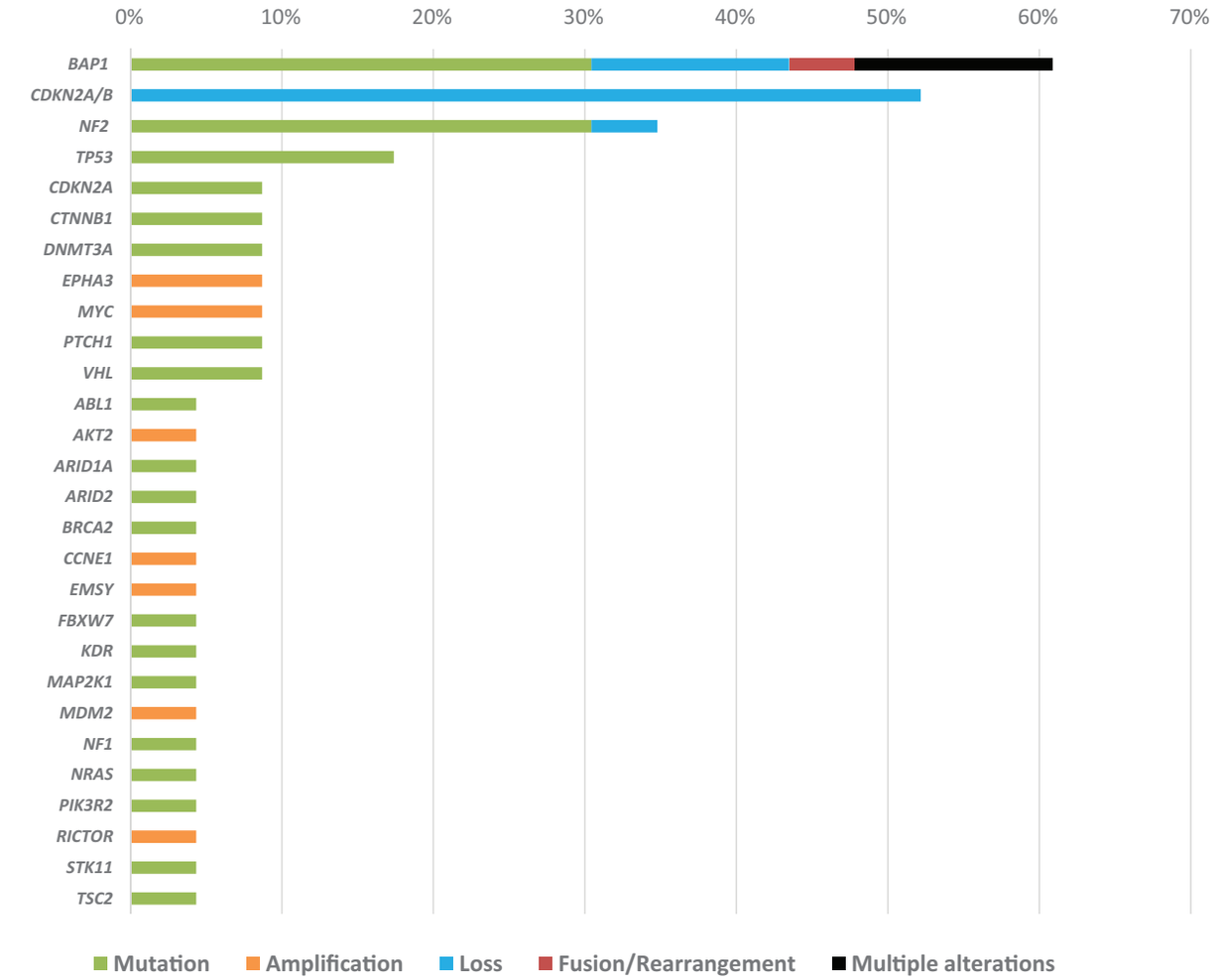

Figure 3.
Frequency and types of genetic aberrations (mutation, amplification, loss, fusion/rearrangement or multiple alteration) among pleural mesothelioma cases ($n = 23$).

lines with *BAP1* loss were associated with higher sensitivity to olaparib (a PARP inhibitor) when compared to cell lines with intact *BAP1* (32). However, another study using mesothelioma cell lines showed no difference between *BAP1*-mutant and wild-type cells in terms of sensitivity to PARP inhibitors (20). *BRCA1* mutation is also associated with increased sensitivity to platinum (33); thus, mesothelioma patients with *BAP1* aberrations may benefit from agents such as cisplatin or carboplatin when compared to patients without these alterations, and this may explain the responses to platinum-based regimens (8, 9). Of note, germline mutations in *BAP1* have been associated with familial cancer syndromes, with an increased risk of malignancies including mesothelioma and uveal melanoma (30). However, *BAP1* germline mutations are rare among sporadic malignant mesothelioma (34). The genomic sequencing performed in this study did not distinguish germline from somatic alterations.

The second most common aberration was in the *NF2* gene (38.1% [16/42]; Figs. 1 and 2 and Supplementary Table S1). Our observation is in agreement with previous studies where 14% to 50% of patients with mesothelioma were found to have aberrations in *NF2* (20–22, 24, 25). As mentioned, *NF2* aberrations trended to be less commonly associated with *BAP1* aberrations (OR 0.33; $P = 0.12$; Supplementary Table S4). *NF2* (*neurofibromin 2*) is a tumor suppressor gene that encodes the protein merlin, which affects multiple signaling pathways (35). Among multiple cancer types, mesothelioma is one of the most common cancers that harbor *NF2* aberrations (35). In a preclinical model with malignant mesothelioma cell lines, inactivation of *NF2* led to enhanced cell spreading and invasion through activation of FAK (36). Interestingly, mouse models with hemizygous *NF2* that were exposed to asbestos had markedly accelerated formation of malignant mesothelioma when compared to asbestos-exposed wild-type mice. In the same study, further molecular profiling of these mesothelioma samples showed frequent deletion of *CDKN2A* and inactivation of *TP53*, suggesting that mesothelioma develops along with the accumulation of additional genetic aberrations (37). Moreover,

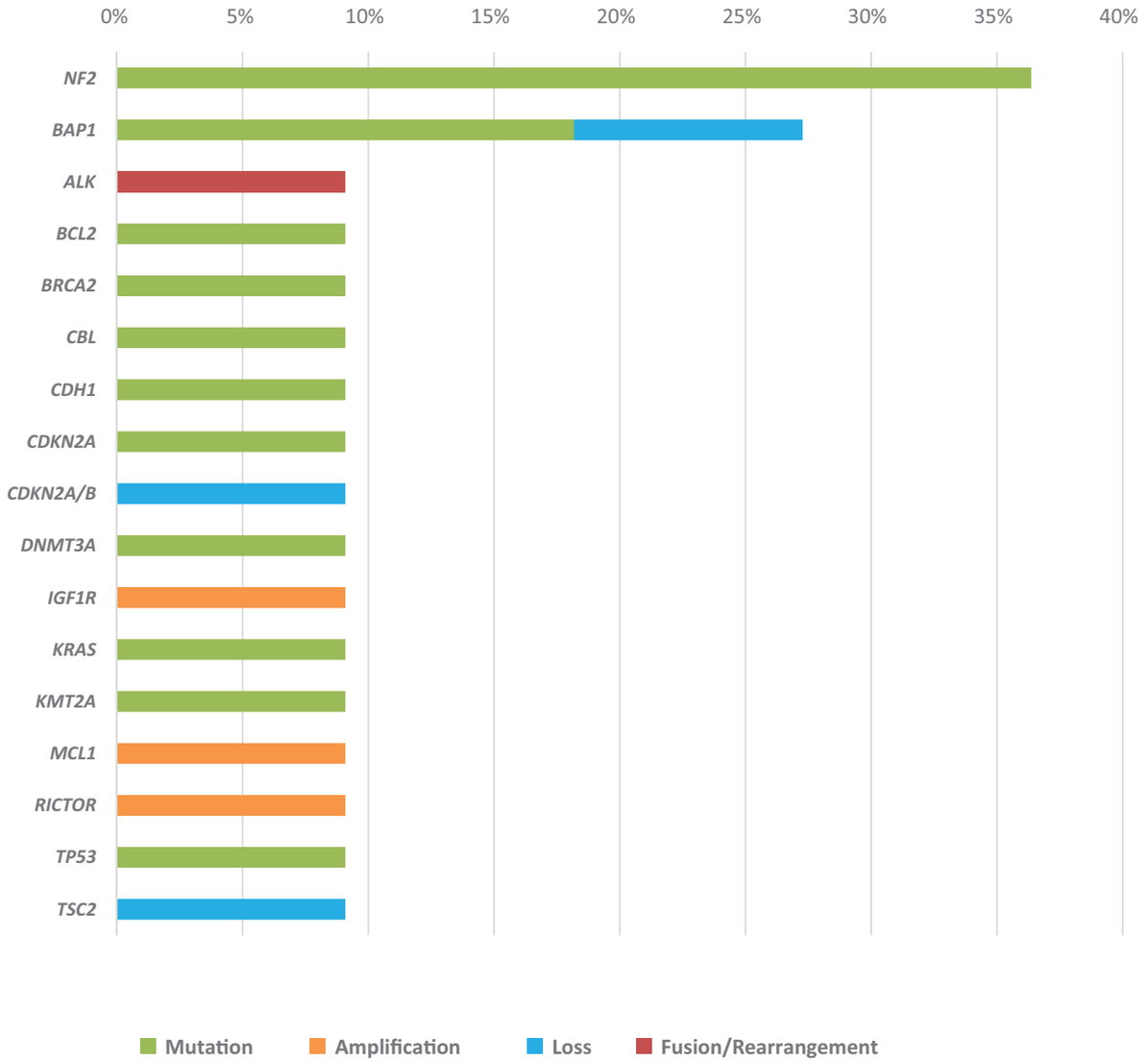

Figure 4.
Frequency and types of genetic aberrations (mutation, amplification, loss, or fusion/rearrangement) among peritoneal mesothelioma cases ($n = 11$).

conditional mouse models showed that loss of all three genes (*NF2*, *CDKN2A*, and *TP53*) was associated with an increased risk of mesothelioma formation and a significant decrease in survival when compared to the mice with loss of two genes (*NF2* and *CDKN2A* or *NF2* and *TP53*), suggesting that aberrations in all three genes enhances tumorigenesis and cancer aggressiveness (38). However, in our current study, only one patient (1/42) was found to have an aberration in all three genes (*NF2*, *CDKN2A*, and *TP53*) (Fig. 1). Of interest in this regard, patients with neurofibromatosis type 2, which is an autosomal-dominant disorder associated with germline mutations in *NF2*, do not have an increased risk of mesothelioma (35). It is unclear why such a dichotomy exists. Because NF2 is a negative regulator of mTOR, it is potentially targetable with mTOR inhibitors, and cases of metaplastic breast cancer and of patients with neurofibromatosis that have achieved response (complete remission for 3+ years for the breast cancer) to a temsirolimus (mTOR inhibitor)-containing regimen have been reported (39, 40). Moreover, in preclinical models, lack of *NF2* was associated with increased sensitivity to FAK inhibitors (41); trials of FAK inhibitors VS-6063 (defactinib) (NCT02004028) and GSK2256098 (NCT01938443) in patients with mesothelioma are ongoing.

Loss of *CDKN2A/B* was the third most common aberration among patients with malignant mesothelioma (35.7% [15/42]; Figs. 1 and 2 and Supplementary Table S1). Interestingly, *CDKN2A/B* loss was significantly more common in pleural mesothelioma (OR 5.8; $P = 0.01$) and less associated with peritoneal histology (OR 0.12; $P = 0.032$; though number of patients are small, suggesting that these correlations require

Table 1. Summary of examples of theoretically matched therapies

Gene aberration	Mechanism of action	Examples of theoretical therapies[a]
ABL1 mutation	ABL1 is a nonreceptor tyrosine kinase that transduces diverse extracellular signaling	ABL kinase inhibitors, such as imatinib, nilotinib or dasatinib (S1)
AKT2 amplification	AKT is downstream of activated tyrosine kinases leading to mTOR signaling	mTOR inhibitors, such as everolimus or temsirolimus (S2, S3)
ALK fusion	*ALK* fusion leads to ligand-independent activation of the tyrosine kinase (S4)	ALK inhibitors, such as crizotinib (S4, S5)
ARID1A mutation	ARID1A is a component of the SWI/SNF chromatin remodeling complex and its aberration is thought to drive tumorigenesis by altering gene expression (S6)	EZH2 inhibitor (EPZ-6438) (NCT01897571)[b]
ARID2 mutation	ARID2 is a subunit of the polybromo- and BRG1-associated factor (PBAF) chromatin remodeling complex, which facilitates transcriptional activation (S7)	Unclear
ATM mutation	*ATM* tumor suppressor gene encodes DNA damage-signaling protein	PARP inhibitors, such as olaparib (S8)
BAP1 aberration	BAP1 (BRCA1 associated protein-1) is a deubiquitinating enzyme (S9)	PARP inhibitors, such as olaparib (S10) Platinum such as cisplatin or carboplatin (S11, S12) EZH2 inhibitor, such as EPZ011989[b] (S13).
BCL2 mutation	*BCL2* encodes protein that regulates cell death and inhibits apoptosis	Bcl-2 inhibitors, such as ABT-199 or ABT-263[b] (S14, S15).
BRCA2 mutation	*BRCA1/2* are important for DNA double-strand break repair by homologous recombination (S16)	PARP inhibitors, such as olaparib (S17) Platinum such as cisplatin or carboplatin (S11, S12)
CBL mutation	CBL (casitas B-lineage lymphoma) is an E3 ubiquitin-protein ligase for tyrosine kinase receptors	Unclear
CCNE1 amplification	CCNE1 (Cyclin E1) forms a complex with cyclin-dependent kinase 2 (CDK2) to regulate G_1–S transition (S18)	Possibly with CKD2 inhibitors such as dinaciclib (CDK1/2/5/9 inhibitor)[b] (S19). Possibly with bortezomib (S20)
CDH1 mutation	*CDH1* (*cadherin 1, type 1, E-cadherin*) is important for cell-cell adhesion and plays fundamental role in the maintenance of cell differentiation and the normal structure of epithelial cells (S21)	Unclear
CDKN2A/B loss and mutation	*CDKN2A/B* are tumor suppressor genes that inhibit cyclin D-cyclin-dependent kinase (CDK) 4/6 complex, which regulates G_1 cell-cycle progression	CDK4/6 inhibitors, such as palbociclib (S22)
CTNNB1 mutation	*CTNNB* (β-catenin) is part of Wnt signaling pathway associated with tumorigenesis (S23)	β-Catenin antagonist[b] (S24)
DNMT3A mutation	*DNMT3A* is tumor suppressor and its mutation disrupts DNA methylation (S25)	DNA methyltransferase inhibitors such as decitabine or azacitidine (S26)
EMSY amplification	EMSY is BRCA2 binding partner and capable of silencing the activity of BRCA2, leading to chromosomal instability (S27)	Possibly with PARP inhibitors such as olaparib. Platinum such as cisplatin or carboplatin (S11, 12).
EPHA3 amplification	*EphA3* is highly expressed on the tumor-initiating cell and involved in maintaining tumor cells in a less differentiated state (S28)	Anti-EphA3 antibody[b]
FBXW7 mutation	*F-box and WD40 repeat domain-containing 7* (*FBXW7*) is involved in ubiquitination and turnover of several oncoproteins (S29)	Possibly with mTOR inhibitors, such as everolimus or temsirolimus (S30)
FGFR3 amplification	FGFRs (fibroblast growth factor receptors) are transmembrane tyrosine kinase receptor (S31)	Tyrosine kinase inhibitors that target FGFR3, such as dovitinib or ponatinib (S4)
IGF-1R amplification	IGF-1R (insulin-like growth factor) signaling is associated with transformation of cells, cancer cell proliferation, and metastasis (S32)	IGF-1R inhibitor[b] (S32, S33)
KDR mutation	KDR (kinase insert domain receptor, also known as VEGFR-2) regulates VEGF-induced endothelial proliferation, survival, and migration	Tyrosine kinase inhibitors that target VEGFR-2, such as cabozantinib (S34)
KMT2A mutation	KMT2 (histone-lysine N-methyltransferase 2) family protein is methyltransferase (S35)	EPZ-5676 (DOT1L inhibitor: NCT02141828)[b] Flavopiridol (NCT00012181)[b]
MAP2K1 mutation	MAP2K1 (mitogen-activated protein kinase kinase 1), also known as MEK1, is involved in MAP kinase signal transduction signaling	MEK inhibitors, such as trametinib
MCL1 amplification	MCL1 is antiapoptotic protein (S36)	Possibly with sorafenib (S37)
MDM2 amplification	MDM2 is E3 ubiquitin protein ligase that suppresses p53 activity (S38)	MDM2 inhibitors (DS-3032b: NCT01877382, RO6839921: NCT02098967)[b] (S38)
MYC amplification	*MYC* is pleiotropic transcription factor (S39)	Possibly with aurora kinase inhibitors such as MLN8237[b] (S40) Possibly with CDK1 inhibitor such as dinaciclib (CDK1/2/5/9 inhibitor)[b] (S41)
NF1 mutation	*NF1* encodes protein neurofibromin that affects RAS activation (S42)	mTOR inhibitors, such as everolimus or temsirolimus (S42, S43) MEK inhibitors, such as trametinib (S44)

(Continued on the following page)

Table 1. Summary of examples of theoretically matched therapies (Cont'd)

Gene aberration	Mechanism of action	Examples of theoretical therapies[a]
NF2 aberration	*Neurofibromin 2* (*NF2*) is a tumor suppressor that affects RAS, Src/FAK, and PI3K pathway (S45)	mTOR inhibitors, such as everolimus or temsirolimus (S45) FAK inhibitor, such as VS-6063 (defactinib) (NCT02004028) or GSK2256098 (NCT01938443) (S46
PIK3R2 mutation	*PIK3R2* aberration leads to the activation PI3K pathway (S47)	mTOR inhibitors, such as everolimus or temsirolimus (S47)
PTCH1 mutation	PTCH1 is receptor for Hedgehog signaling pathway (S48)	SMO inhibitors, such as vismodegib (S48)
RAS mutations	*RAS* mutations lead to constitutive activation of RAS (S49)	MEK inhibitors, such as trametinib (S50)
RICTOR amplification	RICTOR is component of mTORC2 complex, which is required for AKT phosphorylation (S51)	mTORC1/2 inhibitors[b] (AZD2014, MLN0128)
SOX2 amplification	*SOX2* is a transcription factor that is essential for maintaining self-renewal or pluripotency (S52)	Unclear
STK11 mutation	STK11 (serine/threonine-protein kinase 11) inactivates mTORC1 and FAK signaling (S53, S54)	mTORC1 inhibitor, such as everolimus or temsirolimus (S53, S55) FAK inhibitors such as dasatinib (S56) or bosutinib (S57)
SUFU mutation	SUFU (SMO released suppressor of fused) negatively regulates the Hedgehog pathway (S58)	Possibly with arsenic trioxode or bromo and extra C-terminal (BET) inhibitors (GSK525762, NCT01587703).[b] Probably will not respond to hedgehog inhibitor vismodegib because defect is downstream of smoothened receptor (S58).
TP53 mutation	*TP53* is a tumor suppressor gene (S59)	Bevacizumab (pilot retrospective data; S60)
TSC2 aberration	Tuberous sclerosis complex 2 (TSC2) is a negative regulator of mTOR	mTOR inhibitors, such as everolimus or temsirolimus (S61)
VHL mutation	VHL protein functions as ubiquitin ligase which ubiquitylates hypoxia inducible factors (HIF) leading to degradation by proteasome (S62).	VEGF/PDGF receptor inhibitors, such as sunitinib (S63)

NOTE: For references in Table 1, please see supplemental references.

[a]See Supplementary Table S6 for the additional comments for the examples of theoretical therapies.

[b]Therapies currently in clinical trial. All other drugs are FDA approved for other types of cancer treatment.

verification in larger cohorts (Supplementary Table S5). *CDKN2A/B* are tumor suppressor genes that suppress cyclin D–cyclin-dependent kinase (CDK) 4/6 complexes, which regulate G_1 cell-cycle progression. Thus, *CDKN2A/B* loss facilitates G_1 cell-cycle progression, leading to cancer aggressiveness, and has been associated with poor clinical outcome (42). As mentioned earlier, *CDKN2A* aberrations in mouse models increase the risk of the development of mesothelioma along with *NF2* and *TP53* anomalies, and thus, this mutation likely has an important role for tumor initiation (38). Abnormalities in *CDKN2A/B* are potentially targetable with CDK4/6 inhibitors such as palbociclib (42), although some studies suggest that aberrations in the CDK pathway may not be a predictive biomarker for response (43).

Although chromatin-modifying genes including *SETD2* and *SETDB1* aberrations were previously reported in portion of malignant pleural mesothelioma patients (8% and 3%, respectively; ref. 21), we only evaluated *SETD2* aberration, which was negative in current report. Thus, further investigation with larger panel of patients is required.

Among 42 patients with mesothelioma, there were 92 distinct aberrations. Eighty-eight alterations (95.7%) were potentially actionable with either an FDA-approved drug (87.5% [77/88]) or with an experimental drug in a clinical trial (12.5% [11/88]) (Table 1 and Supplementary Tables S2 and S6). Of note, all 42 mesothelioma patients had theoretically actionable alterations; in 40 (95.2%), at least one alteration was potentially targetable by an FDA-approved drug and an additional 2 (4.8%) by an investigational drug in a clinical trial (Table 1 and Supplementary Tables S2 and S6).

Interestingly, among 42 patients, there were no patients who had identical genomic portfolios (Supplementary Table S2). Molecular singletons as the norm are commonly reported in other cancers as well (44–48). Considering that effective therapies for mesothelioma are lacking (8–12), clinical trials for mesothelioma that incorporate molecular profiling for patient selection and appropriately customized therapy are warranted (49).

There are several limitations to current data. First, the dataset was not clinically annotated. Thus, correlation between genomic aberrations and clinical outcomes were not feasible. Second, because we have not evaluated normal tissues, the possibility of underlying germline mutations is not addressed. Third, cancer diagnoses were submitted by referring physicians, which can potentially introduce the sample size bias. Related in this regard, because current data were derived from a de-identified database, we were not able to confirm the histologic subtypes of mesothelioma (epithelioid, biphasic, and sarcomatoid), which are known to have different genomic aberration patterns (e.g., *CDKN2A* aberrations are more often seen in biphasic when compared to epithelioid or sarcomatoid subtypes; ref. 21). In addition, we were not able to review the pathology slide for histological confirmation. Despite these limitations, this study provides comprehensive analysis of genomic landscape of malignant mesothelioma patients using clinical grade NGS.

In conclusion, among 42 patients with mesothelioma, 116 aberrations were identified (median = 3 per patient), 92 of which were distinct. The most common alterations were in *BAP1* (47.6% [20/42]), *NF2* (38.1% [16/42]), and *CDKN2A/B* (loss; 35.7% [15/42]). All patients had at least one aberration that was possibly targetable with either an FDA approved or an investigational drug (Table 1 and Figure 1 and Supplementary Tables S2 and S6). Of interest in this regard, previous studies have shown that targeted drugs are most effective when matched by biomarkers to the tumor, and that use of targeted therapies in unselected patient populations is often ineffective (13, 14). Understanding the landscape of genomic alterations in patients with mesothelioma may therefore assist in informing next generation clinical trials.

Disclosure of Potential Conflicts of Interest

S.K. Elkin has ownership interest (including patents) in N-of-One, Inc. J.L. Carter has ownership interest (including patents) in N-of-One Inc. R. Kurzrock has ownership interest in Novena, Inc. and Curematch, Inc.; reports receiving commercial research grants from Genentech, Merck Serono, Pfizer, Sequenom Foundation Medicine, and Guardant; has ownership interest (including patents) in Novena, Inc. and Curematch, Inc.; and is a consultant/advisory board member for Sequenom, Actuate Therapeutics, and Xbioetch. No potential conflicts of interest were disclosed by the other authors.

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Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): B.N. Tomson
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): S. Kato, T.P.H. Buys, S.K. Elkin, R. Kurzrock
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Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): B.N. Tomson, T.P.H. Buys

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