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Retrospective response analysis of BAP1 expression to predict the clinical activity of systemic cytotoxic chemotherapy in mesothelioma

Author Listing

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Conflicts of interest

The authors do not have any conflicts of interest to declare.

Abstract

Introduction: BRCA1 associated protein-1 (BAP1) is a key tumor driver in mesothelioma and a potential biomarker predicting response to several targeted therapies in clinical testing. Whether it also modulates response to cytotoxic chemotherapy is undetermined. This study used retrospective response analysis of BAP1 expression in archival tumor biopsies taken from patients in the MS01 trial (NCT00075699). We aimed to determine if BAP1 expression correlated with overall survival within the three treatment arms in this trial, namely active symptom control (ASC); ASC plus mitomycin, vinblastine and cisplatin (MVP); and ASC plus vinorelbine.

Materials and Methods: We used immunohistochemical analysis of tumor samples from the MS01 trial to identify subgroups with and without nuclear BAP1 expression. We performed correlative analysis of clinical characteristics (age at diagnosis, sex and histological subtype) and overall survival within treatment arms with nuclear BAP1 expression.

Results: 89 tumor samples from the 409 patients originally in the trial were available for analysis. Of these, 60 samples harbored a positive internal control, in the form of positive staining of inflammatory cells for BAP1, and were carried forward for analysis. Correlative analysis suggested no significant association between loss of nuclear BAP1 expression and age at diagnosis, sex and histological subtype. Kaplan Meier survival analysis revealed a small, though non-significant, overall survival disadvantage associated with BAP1 expression in tumors from patients treated with vinorelbine.

Discussion: This exploratory analysis suggests BAP1 expression may modify response to vinorelbine in MPM, possibly due to prevention of mitotic microtubule formation. We suggest ongoing and planned clinical studies of vinorelbine in MPM assess BAP1 expression as a predictive biomarker of response.

Keywords

Mesothelioma, BRCA-1 associated protein 1, vinorelbine, biomarker

Text Body

Introduction

Malignant pleural mesothelioma (MPM) remains a devastating disease with an increasing worldwide incidence. Prognosis is poor; median overall survival is only 9-18 months. Few patients are suitable for surgery and the mainstay of therapy remains systemic cytotoxic chemotherapy. Combination cisplatin/pemetrexed is the standard of care first line regimen, offering survival benefit of a few months. Patients invariably progress, and while there is currently no established second line regimen, single agent vinorelbine is used on the basis of activity in small trials.

Recently, large-scale genomic analyses of MPM have identified recurrent mutations in several genes that offer promise as novel therapeutic targets and biomarkers. In particular, BRCA1 associated protein-1 (BAP1) offers significant therapeutic potential. Comprehensive integrated molecular studies reveal *BAP1* mutations in approximately two thirds of MPM, almost all of which are loss-of-function, implicating it as a key tumor driver [1]. BAP1 is a nuclear deubiquitinase with roles in several cellular processes including the DNA damage response and the cell cycle, where it regulates microtubule organization at mitosis [2, 3]. As an epigenetic transcriptional regulator, it also modulates multiple cellular pathways. Loss of function has been identified as a predictive biomarker of response to novel therapeutic agents that target these pathways including EZH2 inhibitors, PARP inhibitors and death receptor agonists in preclinical studies [4-6]. Phase II trials of the EZH2 inhibitor Tazemetostat (NCT02860286) and the PARP inhibitor Niraparib (NCT03207347) with BAP1 expression as a stratification factor are underway to assess its utility as a predictive biomarker of response in the clinical setting. Immunohistochemistry is a rapid and cost effective method to stratify tumors by *BAP1* status as loss-of-function mutations correlate with loss of nuclear staining with a sensitivity and specificity of 88% and 97% respectively [7].

In view of the diverse cellular effects of BAP1 it is prudent to also consider if BAP1 activity contributes to the observed heterogeneous response to systemic cytotoxic chemotherapy.

Several agents, including platinum-based drugs, interfere with the DNA damage response, while others, such as vinca alkaloids and taxanes, induce mitotic arrest, both cellular processes modulated by BAP1 [2, 3]. Furthermore, evidence supports BAP1 regulates apoptosis, the endpoint induced by chemotherapy [6, 8]. Retrospective response analysis of existing clinical trial data is an efficient method by which to initially explore this question. We therefore analyzed data from the MS01 trial, a phase III, three-arm randomized controlled trial in which active symptom control (ASC) was compared to ASC plus combination mitomycin, vinblastine and cisplatin (MVP) and ASC plus single agent vinorelbine [9]. The two chemotherapy arms were combined in view of slow accrual and survival analysis revealed a small, non-significant benefit for ASC plus chemotherapy versus ASC alone in 409 patients, driven by a small, non-significant survival benefit in the ASC plus vinorelbine arm. Using the prospectively collected data in this trial, we aimed to determine if stratification by tumor BAP1 expression within each treatment arm revealed a differential response, implicating BAP1 as a potential predictive biomarker for these chemotherapy regimens.

Materials and Methods

MS01 tumor samples

409 patients were originally enrolled into the trial from 76 centers in the UK and two in Australia between Sept 17, 2001 and July 31, 2006. From September 2003 tumor samples were sent to an independent reference histopathologist and stored at the National Institute for Health Research Respiratory Biomedical Unit Biobank, Royal Brompton Hospital. These tumor samples were used for this study. Samples from 89 of the 409 patients were stored; 32 as formalin fixed paraffin embedded (FFPE) blocks and 57 as unstained mounted sections. Clinical data from the trial corresponding to these samples was provided by the MRC trials unit.

Immunohistochemistry

Automated staining on a Leica Bond III staining platform was used. Slides were incubated with BAP1 primary antibody (Santa Cruz Biotechnology Cat# sc-28383, 1:150) for 15 min at room temperature. Epitope retrieval was completed using HIER using Leica Bond ER2 (high pH) for 30 min and a Leica Bond Polymer Refine with DAB chromogen detections system.

Sections were assessed for presence (BAP1 positive) or absence (BAP1 negative) of nuclear BAP1 expression independently by two consultant histopathologists. Those sections that did not contain a positive internal control in the form of inflammatory cells with positive staining for BAP1 were not carried further for analysis.

Statistics

Chi square analysis was used to assess the relationship between BAP1 expression and gender and histological subtype and a two-sided t-test for the relationship with age at diagnosis. Overall survival (OS) data was estimated using the Kaplan-Meier method and the Log-rank (Mantel Cox) test used to compare differences in survival between groups.

Results

Tumor samples from 89 of the 409 patients included in the MS01 trial were available, of which 60 harbored a positive internal control and were carried forward for analysis. Table 1 shows the clinical characteristics of the 60 patients with tumors assessed for BAP1 immunohistochemistry, revealing no significant difference in gender, age or histological subtype when stratified by tumor BAP1 expression. Table 2 shows the median OS and associated hazard ratios of the 60 patients assessed for BAP1 immunohistochemistry stratified by treatment arm and tumor BAP1 expression. For both patients with tumors with and without nuclear BAP1 expression (BAP1 positive and BAP1 negative respectively) there was no statistically significant difference in OS between any of the treatment arms. There was also no significant difference in OS within the ASC and the ASC plus MVP arms between those patients with BAP1 positive or negative tumors. However there is a non-significant trend towards decreased OS in patients with BAP1 positive tumors compared with those with BAP1 negative tumors in the ASC plus vinorelbine arm (p = 0.06; HR 4.87 (0.94 – 25.16).

Discussion

BAP1 is an important tumor driver in MPM and through modulation of multiple cellular pathways a potential biomarker of response to novel targeted agents. Our study aimed to investigate if BAP1 determines response to systemic cytotoxic chemotherapy through retrospective response analysis of survival data from a subset of patients from the MS01 trial.

Irrespective of tumor BAP1 expression, treatment with neither MVP nor vinorelbine offers a significant survival benefit above treatment with ASC in this cohort. This is consistent with results from the original trial, although the non-significant trend towards a survival benefit in the vinorelbine arm is not seen. Analysis within treatment arms suggests no effect of BAP1 expression for treatment with ASC alone or ASC plus MVP. The non-significant trend towards decreased OS in patients with BAP1 positive tumors seen in the ASC plus vinorelbine arm is however suggestive of a potential modulatory effect of BAP1 on vinorelbine response. Closer observation of the data suggests treatment with vinorelbine in patients with BAP1 positive tumors may be detrimental. Vinorelbine offers no significant survival benefit over ASC in patients with BAP1 negative tumors and the data also suggests BAP1 expression does not affect response to ASC. Indeed, the median OS of patients with BAP1 positive tumors treated with vinorelbine is almost half that of those treated with ASC, however the limited sample size precludes statistical significance. Limited evidence suggests *BAP1* mutant tumours are associated with prolonged survival independent of treatment [10]. The observation in our data however that there is no effect of BAP1 expression on survival for those patients treated with ASC alone suggests no independent effect of BAP1 activity on survival.

Single agent vinorelbine remains a viable second line regimen for MPM. Indeed a prospective, placebo-controlled, randomized phase II trial of vinorelbine in the second-line setting with BRCA1 expression as a stratification factor is ongoing (NCT02139904). Notably, BRCA1 and BAP1 cooperatively regulate microtubule organization and mitotic progression, and loss of either results in chromosomal instability [2, 11]. The BRCA1/BARD1 heterodimer ubiquitinates γ-tubulin, a tubulin isoform that regulates microtubule nucleation and seals the minus ends of the α/β-tubulin polymers, while the deubiquitinase activity of BAP1 opposes this. Loss of BRCA1 expression is associated with resistance to vinorelbine in *in vitro* studies [12]. While the precise mechanism by which BRCA1 expression modulates response to anti-microtubule agents is undetermined, the suggestion in our study that loss of BAP1 expression might modulate response to vinorelbine suggests a common mechanism, possibly centered on γ-tubulin and microtubule organization. It would be of significant interest, and eminently

feasible, to also stratify by BAP1 expression in ongoing trials. Interestingly, patients with tumors with loss of BAP1 expression treated with ASC plus MVP, which contains the anti-microtubule agent vinblastine, did not demonstrate a survival difference compared to patients with tumors that retained BAP1 expression. It may be that the additional agents mask any effect of BAP1 on response to vinblastine or alternatively, the association may be specific to vinorelbine. The original MS01 trial revealed no survival benefit of MVP over ASC alone and this regimen is no longer used for MPM and rarely used outside the palliative setting for any cancer [9].

This analysis has some limitations. The sample size taken from the MS01 trial was not powered for the subgroup analyses conducted and the results are exploratory in nature only. The FFPE tumor samples had been stored since 2003-4, however confounding from sample degradation was minimized by including only those tumor samples with a positive internal control for BAP1 immunohistochemistry in the analysis.

This exploratory study suggests that BAP1 expression does not modify response to systemic cytotoxic chemotherapy in the form of combination MVP but may modify response to vinorelbine, a drug used as a second line agent for MPM. The role for BAP1 and BRCA1 in microtubule organization and γ-tubulin ubiquitination as an underlying mechanism warrants further study. More immediately however, we would encourage ongoing and planned clinical studies of vinorelbine in MPM assess BAP1 in addition to other biomarkers as a predictor of response.

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Table 1: Clinical features of 60 patients from the MS01 trial with tissue assessed for BAP1 immunohistochemistry stratified by nuclear BAP1 expression

	Nuclear BAP1 IHC + (n=13)	**Nuclear BAP1 IHC –** (n=47)	***p***
Gender (M = male)	M: 100% (n=13)	M: 91% (n=43)	0.22
Median age at diagnosis (years)	69 (n=13)	66 (n=47)	0.80
Histological subtype			0.21
Epitheloid	77% (n=10)	89% (n=42)	
Biphasic/mixed	15.0% (n=2)	9.0% (n=4)	
Sarcomatoid/other	0% (n=0)	2% (n=1)	
Not reported	8% (n=1)	0% (n=0)	

Table 2: Median overall survival (months) of 60 patients from the MS01 trial with tissue assessed for BAP1 immunohistochemistry stratified by nuclear BAP1 expression and treatment arm

	Nuclear BAP1 IHC + (n=13)	**Nuclear BAP1 IHC –** (n=47)	***p***	**HR BAP1 + / BAP1 - (95% CI)**
ASC	10.4 (n=4)	11.6 (n=19)	0.29	0.58 (0.15 – 2.15)
ASC + vinorelbine	5.5 (n=4)	11.5 (n=12)	0.06	4.87 (0.94 – 25.16)
ASC + MVP	6.7 (n=5)	8.3 (n=16)	0.55	0.75 (0.25 – 2.22)
p	0.30	0.22		
HR ASC + vinorelbine / ASC (95% CI)	2.14 (0.48 – 9.49)	1.23 (0.59 – 2.83)		
HR ASC + MVP / ASC (95% CI)	1.65 (0.44 – 6.16)	1.73 (0.83 – 3.59)		

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