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INTRODUCTION

- Uveal melanoma is the most common primary intraocular malignant tumor in adults.¹
- About 50% of patients develop metastases to the liver within 15 years of their initial diagnosis.²
- There is currently no effective standard of care treatment for patients with metastatic uveal melanoma, with median survival reported to be <6 months.^{2,3}
- Uveal melanoma is characterized by frequent mutations in two genes encoding guanine nucleotide binding protein (G-protein) α -subunits (G α): G-protein Q polypeptide 1 (GNAQ) and G-protein alpha 11 (GNA11). These mediate signaling from G-protein coupled receptors to downstream signaling pathways, including protein kinase C (PKC) (Figure 1).4,5
- Mutations in GNAQ/GNA11 may be the genetic drivers leading to the development of uveal melanoma by causing constitutive activation of pathways involving these genes.4,5
- Here, we conducted genomic profiling of tumor biopsies from patients with metastatic uveal melanoma.

Figure 1. Putative Cellular Functions of Commonly Altered **Proteins in Uveal Melanoma; Activating Mutations in G\alpha q** Subunits GNAQ and GNA11 are Frequent



*Proteins in red are encoded by genes known to be altered in uveal melanoma. ASXL1, additional sex combs-like protein 1: BAP1, BRCA1-associated protein: BRCA1, breast cancer 1, early onset: DAG, diacylglyceraol; EIF1AX, eukaryotic translation initiation factor 1A, X-chromosomal; ERK, extracellularregulated kinase; Ga, G-protein a-subunit; Gag, G-protein ag-subunit; GPCR, G-protein-coupled receptor; H2A, histone 2A; HCF-1, host cell factor 1; MARCKS, myristoylated alanine-rich C-kinase substrate; MEK, mitogen-activated protein kinase/ERK kinase; PKC, protein kinase C; PLC, phosphoinositide-specific phospholipase C; RAF, rapidly accelerated fibrosarcoma; SF3B1, splicing factor 3B subunit 1.

STUDY OBJECTIVES

- AEB071, an oral selective PKC inhibitor.⁵
- paraffin-embedded [FFPE] or archival tumor tissue).

METHODS

Study Design

- of AEB071.⁶
- or withdrawal of consent.⁶
- patient for study enrollment.
- required

Assessments

- Genomic profiling of FFPE biopsies from patients with metastatic uveal melanoma, primarily from liver.
- Genomic profiling was conducted on the baseline pre-treatment biopsy specimens.
- DNA was assayed by massively parallel sequencing, at Foundation Medicine.
- the sample was excluded from further analysis.

Landscape of Genetic Alterations in Uveal Melanoma

• To characterize the genetic landscape of tumors from patients with metastatic uveal melanoma in the Phase I study with

- To determine the frequency and number of baseline gene alterations in samples from metastases (formalin-fixed

• To investigate the potential association of genetic alterations with baseline levels of phosphorylated myristoylated alanine-rich C-kinase substrate (pMARCKS), as an indicator of PKC activity.

 Patients with biopsy-proven metastatic uveal melanoma were enrolled in a Phase I, multicenter, open-label, single-arm study

Patients received oral AEB071 in one of two dosing schedules at total daily doses ranging from 450 mg to 1400 mg, twice daily or three times daily until disease progression, intolerable toxicity

• At baseline, two core needle biopsies were required from each

- If tumor tissue was accessible through a minimally invasive biopsy, then a fresh, pre-dose core needle tumor biopsy was

- If a fresh biopsy could not be collected safely at study entry, an archival tumor sample from a metastatic lesion was required.

• At least one post-treatment core needle biopsy was also obtained, prior to the morning dose of AEB071 on Cycle (C) 1 Day (D) 15.

covering a panel of 288 clinically relevant cancer genes

 Sequencing was performed at high depth (median 611X) to characterize mutations, amplifications (≥ 6 copies), homozygous deletions and loss of heterozygosity (LOH).

- If baseline samples were found to contain insufficient DNA or to have insufficient sequence coverage, the C1D15 sample was analyzed. If this also failed to meet tissue requirements,

- 110 patient samples were collected. Of these, sequencing was successful in 82 samples and results are shown for 76 samples.
- Determination of pMARCKS levels (pharmacodynamic biomarker) in tumor samples.
- Fresh core needle biopsies were collected at baseline and on C1D15.

- The samples were frozen immediately after collection, until analysis.

- Immediately prior to analysis, the samples were thawed, lysed, and then total protein yield was measured.
- Levels of MARCKS and pMARCKS (S152/156) were measured using a custom assay on the MesoScale Discovery platform, and a reference cell lysate was used for calibration.

RESULTS

Genetic Alterations

- Alterations of known or likely functional significance were frequent only in genes previously implicated in uveal melanoma (**Figure 2**).
- These were distinct from the mutational profile of skin melanoma (**Figure 3**).
- Two novel patterns were observed.
- As expected in uveal melanoma biopsies, a large proportion of patients (94%) were found to have activating mutations in either GNAQ (59%; Q209P/L/R or R183Q) or GNA11 (36%; Q209L/H).
- The high prevalence of GNAQ/GNA11 mutations reported here is likely due to the sequencing depth, as evidenced by low allele frequencies in some patients even after correcting for tumor purity
- Among the five samples without a GNAQ or GNA11 mutation, all had very low tumor content ($\leq 20\%$).
- Truncations or splice-site mutations in the gene encoding BRCA1 associated protein-1 (BAP1) were frequently observed (51%), in line with previous reports (**Figure 2**).
- Partial or putatively complete amplifications of chromosome 8q (59%) and recurrent mutations in splicing factor 3B subunit 1 (SF3B1) [23%; R625C/H or V701F] were usually mutually exclusive, which is a pattern previously unreported (Figure 2).
- In addition, *BAP1* mutations were frequent in patients with chromosome 8q amplifications (33/48 [69%]), and rare in patients with SF3B1 mutations (4/19 [21%]) [Figure 4].
- Among the 63 patient samples in which LOH could be evaluated, BAP1-spanning LOH occurred in 45 patients (71%), including 35 of 39 patients (90%) with BAP1 mutations.







- Mutations of known or likely functional impact were otherwise rare.
- Somatic alterations in uveal melanoma patients are shown in Figure 4. Genes with mutations in fewer than two patients are not shown, nor are short variants of unknown functional significance. LOH is only included for BAP1.
- The positions of sequenced genes on chromosome 8 are indicated in Figure 4. No copy number alterations were reported for the four sequenced genes on chromosome 8p.
- Of interest, P53, P16 and SWI/SNF related, matrix associated, actin-dependent regulator of chromatin, subfamily A, member 4 (SMARCA4) mutations were only observed in the tumors of two patients each, and mutations in 25 genes were observed in a single patient (Figure 2).



Figure 4. Somatic Alterations in Uveal Melanoma Patients

Pharmacodynamics

- 52 paired tumor biopsy samples were analyzed to investigate the effect of genetic subtype on baseline levels of pMARCKS.
- Preliminary analyses of the currently available data suggest no obvious relationship between pMARCKS baseline levels with any gene or GNAQ/GNA11 mutant subtypes (Figure 5).

Figure 5. Normalized pMARCKS at Baseline vs Genotype (Full Analysis Set)



CONCLUSIONS

- Genomic profiling has demonstrated that 94% of patients with uveal melanoma had a known activating mutation in either GNAQ or GNA11.
- BAP1 mutations were frequently identified in patients with chromosome 8q amplifications and rarely identified in patients with SF3B1 mutations. This suggests complimentary and redundant consequences of these lesions with BAP1 mutations, respectively.
- High-depth sequencing of clinical patient metastases sheds new light on the interplay among the small group of genetic alterations observed in uveal melanoma.

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