Biomarker Analysis to Inform Dosing Schedule of a MEK1 Inhibitor for Treatment of BRAF-Mutated Melanoma

APOCELL
molecular profiling & diagnostics

Clinical Application: Pharmacodynamic monitoring in CTCs to inform patient dosing for a novel treatment in melanoma

Key Words: protein expression, gene expression, assay development, CTCs

Background: E6201 is a novel MEK1-inhibitor that has in vitro anticancer activity in BRAF-mutated cancers such as melanoma and exhibits potent inhibition of the RAS-RAF-MEK pathway. Pharmacokinetic results from a Phase I trial administering E6201 once a week indicated that the mean half-life was 3-6 hours. Serial assessment of patient tumors is challenging and prompts the need for identification of appropriate surrogate tissues. ApoCell was contracted to evaluate the feasibility of using circulating tumor cells (CTCs) as surrogate tissue for monitoring pharmacodynamic effects of E6201 to inform the selection of an appropriate dosing schedule for the ongoing trial.

Methods: Three tubes of blood were collected from fifteen melanoma patients in hospice care. Approximately 1ml of blood was taken for BRAF genotyping prior to drug treatment. Because melanoma cells do not typically express EpCAM, the industry standard at the time for capturing CTCs, alternative methods needed to be explored. ApoCell developed a system of triple immunomagnetic isolation, capturing cells with a phenotype of CD45-/CD146+/HMW-MAA+, using Miltenyi's AutoMACS® technology. One tube of blood underwent this triple immunomagnetic isolation at 0h, as the baseline untreated control. A second tube of blood was untreated and placed at 37°C for four hours, as the untreated 4h control. The final tube of blood was treated *ex vivo* with E6201 and placed at 37°C for four hours, to serve as the 4h treated sample. Tubes two and three underwent the triple immunomagnetic separation simultaneously after the four hour treatment window. The resulting enriched cells were then split in half; one set was cytospun and stained with S100 and the downstream markers of interest (ERK/pERK, Rb/pRb). Laser Scanning Cytometry (LSC) analysis was then performed on these cells, which then underwent the TUNEL assay. The second half of the enriched cells went through RNA isolation and qPCR for the melanoma genomic markers of interest (ETV1, MIA, SERPINE2, PRMT2, MAGED2).

Results:

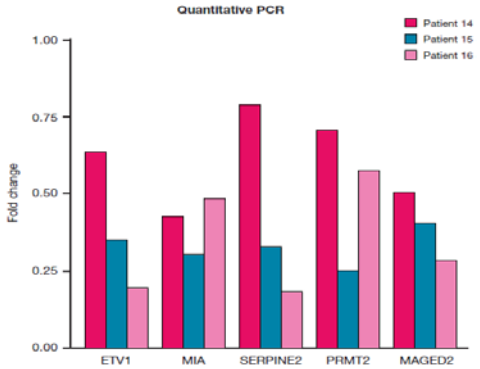

Figure 1. qPCR results for melanoma markers. Gene expression was examined and compared in the 4h E6201-treated sample and the 4h untreated control. Treatment with E6201 inhibited gene expression in each of the markers of interest in patients with the BRAF-V600E mutation.

Patient	ERK-MFI	pERK-MFI	Ratio (pERK/ERK)	Rb-MFI	pRb-MFI	Ratio (pRb/Rb)	TUNEL+
1	5.85	-15.08	-19.78	-32.79	1.38	50.85	44.84
2	-12.95	-24.16	-12.88	57.63	-43.43	-64.11	1.24
6	51.30	8.01	-28.61	20.55	185.15	136.55	40.44
7	-16.97	-16.63	0.41	-23.85	28.40	-5.98	N/A
8	48.30	5.96	-26.32	20.59	62.88	35.07	N/A
9	-18.17	-22.38	-5.14	42.41	-13.07	-38.95	N/A
10	-4.57	17.70	23.35	29.81	21.03	-6.76	40.27

Figure 2. Percentage changes in protein biomarkers. Protein biomarker expression levels were compared in the 4h untreated controls and the 4h E6201-treated samples. The values above show the percentage change in the treated samples as compared to the untreated samples. The results indicate pharmacodynamic effects reflective of MEK1 inhibition by E6201, particularly in ERK/pERK.

Impact: This pilot study indicated the feasibility of using a triple-immunomagnetic separation protocol to isolate sufficient numbers of melanoma CTCs to monitor the pharmacodynamic effects of treatment with E6201 to evaluate if the effects persist long enough to warrant once-weekly dosing. The assay was incorporated into the ongoing clinical trial.

References: 1. Eisen, A., et al. "CTC biomarker assessment to aid dosing schedule of E6201, a potential MEK1 inhibitor for treatment of BRAF-mutated melanoma." ESMO, 2010.

ApoCell, Inc.
2575 West Bellfort, Suite 190 | Houston, TX 77054
Office: (713) 440-6070 | Fax: (713) 440-6074
biomarkers@apocell.com | www.apocell.com