Poster No. 1335P

CTC biomarker assessment to aid dosing schedule of E6201, a potential MEK1 inhibitor for treatment of BRAF-mutated melanoma

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1 ABSTRACT

Background: E6201 has in vitro anticancer activity in BRAF-mutated tumors such as melanoma. In tumor xenografts, E6201 had a short half-life but prolonged effect on the RAS/RAF/MEK pathway. In the escalation stage of a Phase I trial with weekly dosing, mean $t_{½}$ was 3–6 h. As serial assessments of patient tumors were impossible, we evaluated if dynamic monitoring of MEK1-dependent PD biomarkers in melanoma CTCs could aid selection of an E6201 dosing regimen.

Methods: CTCs from 15 patients in hospice care were immunomagnetically isolated from 30 mL whole blood. All patients harbored a BRAF V600E mutation (CTC genotyping via allele-specific PCR). Blood was treated ex vivo with E6201 at 18 μM (C_{max} at Phase I MTD) for 0, 4 and 30 h. Baseline changes in pERK/ERK (extracellular signal-related kinase) and p-pRB1/pRB1 (retinoblastoma protein) ratios as well as % apoptosis (TUNEL) in individual CTCs were determined by laser scanning cytometry. CTCs evaluated in a pre-defined slide area numbered 11–819 (median 51) per patient. CTC mRNA was used for qRT-PCR assessment of gene expression (ETV1, MIA, SERPINE2, PRMT2 and MAGED2) previously associated with BRAF-mutant melanoma.

Results: E6201 produced a time-dependent decrease in pERK/ERK ratio in 5/7 paired samples after 4 h. There was a similar decrease in p-pRB1/pRB1 ratio in 4/7 evaluable paired samples and increase in TUNEL staining in 3/6 paired samples after 4 h. Gene expression was decreased, on average between 30 and 45%, in the paired samples treated with E6201 ex vivo for 4 h. Such trends continued in the paired samples evaluated at 30 h.

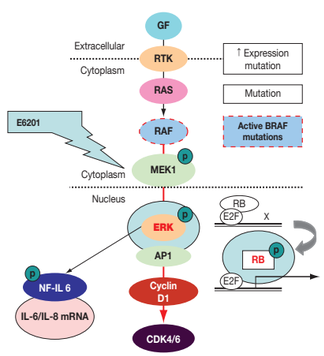
Conclusions: PD biomarkers reflective of MEK1 inhibition by E6201 were measurable and affected in isolated CTCs. Significantly more CTCs could have been evaluated if a larger slide area had been examined. Larger numbers of CTCs will be evaluated in the clinical trial in order to assess a 25% reduction from baseline in the parameters with 80% power. The degree and duration of biomarker change will be utilized in an adaptive trial designed to ascertain if the current weekly schedule needs to be modified to include more frequent drug administration.

Abbreviations

AP, activator protein; BRAF, B-type RAF kinase; CDK, cyclin-dependent kinase; C-Src, cellular src; CTC, circulating tumor cells; EDTA, ethylenediaminetetraacetic acid; ERK, extracellular signal-regulated kinase; ETV1, ETS variant gene; Flt-3, fms-related tyrosine kinase; GF, growth factor; HMW, high molecular weight; IC_{50}, half maximal inhibitory concentration; IL, interleukin; Lck, leucocyte-specific protein tyrosine kinase; LOX, lysyl oxidase; LSC, laser scanning cytometry; Lyn, v-yes-1 Yamaguchi sarcoma viral-related oncogene homolog; MAA, melanoma-associated antigen; MAGED2, melanoma antigen family D, 2; MEK, mitogen-activated protein; MFI, mean fluorescence intensity; MIA, melanoma inhibitory activity gene; NF, nuclear factor; pERK1/2, phosphorylated extracellular signal-related kinase half-life; p-pRB, phosphorylated-retinoblastoma protein; PRMT2, proteinarginine methyltransferase 2; qPCR, quantitative polymerase chain reaction; RB, retinoblastoma; RNA, ribonucleic acid; RTK, receptor tyrosine kinase; SEM, standard error of the mean; SERPINE2, serine- or cysteine-peptidase inhibitor, clad E, member 2; TrkB, tyrosine kinase B; Yes, v-yes-1 Yamaguchi sarcoma viral oncogene homolog

2 E6201 PROPERTIES

The RAS/RAF/MEK signaling pathway



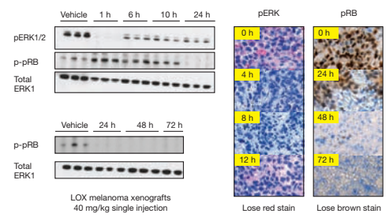
E6201 inhibits selected cancer-relevant kinases from an 85 kinase panel

Kinase	Classification	E6201 IC_{50} nmol/L)	SEM
MEK1	MAPKK	46	2.9
TrkB	GFRTK	23	1.9
Flt-3	GFRTK	35	3.5
Abl	TK	133	15.6
C-Src	Src TK family	35	6.4
Fyn	Src TK family	29	6.1
Lyn	Src TK family	15	3.8
Lck	Src TK family	25	5.9
Yes	Src TK family	55	3.3

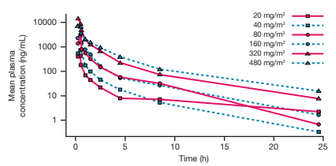
E6201, a synthetic analog of a fungal natural product, has activity against both MEK1 and the Src kinase, which is unique. In both in vitro and xenograft models, E6201 demonstrated potent activity against BRAF-mutated tumors such as melanoma.

3 PK/PD DISCREPANCY

Effect of E6201 on tumor xenografts

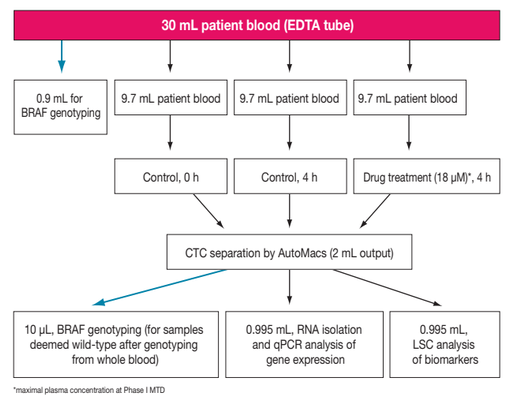


Pharmacokinetic profile of E6201 in patients



In tumor xenografts, E6201 had a short half-life ($t_{½}$ approximately 1 h) but a prolonged effect on the RAS/RAF/MEK pathway. The $t_{½}$ of E6201 in humans is consistent with the xenograft data

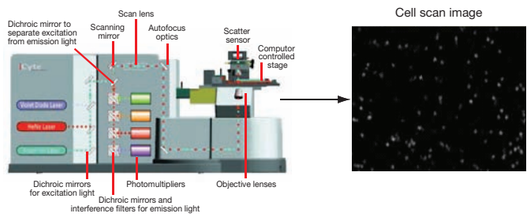
4 CTC ISOLATION PROTOCOL

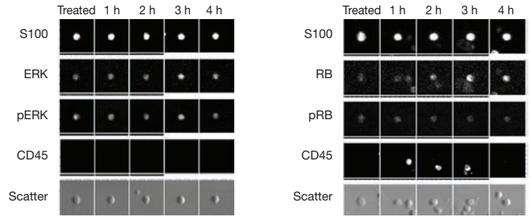


Initial studies were carried out using melanoma cells spiked into whole blood and then involved hospice patients with end-stage melanoma who harbored the BRAF V600E mutation. CTCs were isolated by immunomagnetic capture of CD45+/HMW-MAA+/CD146+ cells

5 SCANNING CYTOMETRY

Circulating tumor cell analysis by laser scanning cytometry





Baseline changes in the following biomarkers of the signaling pathway were monitored in S100+ CTCs in the enriched population by laser scanning cytometry: phosphorylated extracellular signal-related kinase (p-ERK)/ERK and phosphorylated retinoblastoma protein (p-pRB1)/pRB1 ratios

6 CYTOMETRY RESULTS

Laser scanning cytometry biomarker data: E6201 4 h versus control 4 h (percentage change)

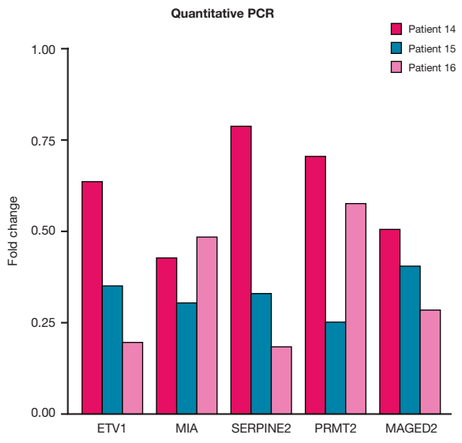
Patient	Percentage change (E6201 4 h versus control 4 h) ERK-MFI	pERK-MFI	Ratio (pERK/ERK)	RB-MFI	pRB-MFI	Ratio (pRB/RB)	TUNEL+
1	5.85	-15.08	-19.78	-32.79	1.38	50.85	44.84
2	-12.95	-24.16	-12.88	57.63	-43.43	-64.11	1.24
6	51.30	8.01	-28.61	20.55	185.15	136.55	40.44
7	-16.97	-16.63	0.41	-23.85	-28.40	-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

Negative and positive values indicate a post-treatment decrease or increase, respectively

PD biomarkers reflective of MEK1 inhibition by E6201 were measurable and affected in isolated CTCs

7 GENE EXPRESSION RESULTS

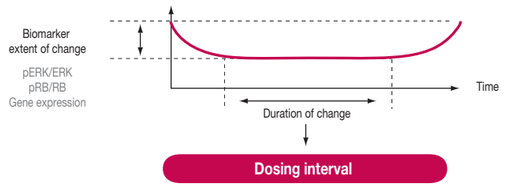
E6201 inhibits gene expression in circulating melanoma cells isolated from patients with the BRAF V600E mutation



CTC mRNA was used for quantitative real-time PCR assessment of gene expression in a number of genes previously associated with BRAF-mutant melanoma. E6201 inhibited gene expression in circulating melanoma cells isolated from patients with the BRAF V600E mutation

8 CONCLUSIONS

Confirmation of dosing interval



- **This pilot study indicated that cells with features of melanoma CTCs (CD45-/HMW MAA+/CD146+/S100+) could be enriched from whole blood taken from patients with melanoma**
- **Biomarkers of E6201 activity could be evaluated by laser scanning cytometry and quantitative real-time PCR. These biomarkers demonstrated expected changes from baseline in response to *ex vivo* E6201 treatment**
- **Sufficient CTCs could be recovered and evaluated to assess whether PD effects persist long enough to warrant once-weekly dosing in the clinic**

Acknowlegments

Editorial support was provided by Emma Robinson at Complete Medical Communications and was funded by Eisai Inc.

References

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Poster presented at the European Society for Medical Oncology, Milan, Italy, 8-12 October, 2010