Bv Combined Braf and Mek Inhibition with Dabrafenib and Trametinib in Braf V600 Mutant Colorectal Cancer

Corcoran, et al

METHODOLOGY SUPPLEMENT

Study Design

An Open-Label, Dose-Escalation, Phase I Study to Investigate the Safety, Pharmacokinetics, Pharmacodynamics and Clinical Activity of the BRAF Inhibitor GSK2118436 in Combination with the MEK Inhibitor GSK1120212 in Subjects with BRAF Mutant Metastatic Melanoma opened in November 2009. Part A evaluated for a drug–drug interaction between trametinib and dabrafenib in subjects with melanoma. Part B evaluated the safety, tolerability and pharmacokinetic activity of escalating doses of dabrafenib and trametinib, originally in subjects with melanoma. Amendment 2 (December 8, 2010) added two expansion cohorts to Part B: one for patients with *BRAF* V600-mutant metastatic melanoma who had prior progression on a BRAF inhibitor and one for patients with *BRAF* V600-mutant metastatic colorectal cancer (approximately 25 patients per expansion cohort). Amendment 4 (December 16, 2011) permitted enrollment of approximately 15 additional subjects with *BRAF* V600-mutant metastatic colorectal cancer to the Part B expansion cohort to evaluate effects on pharmacodynamic biomarkers. Part C was a phase 2 study in which patients with *BRAF*-mutant melanoma were randomized to receive dabrafenib plus trametinib or dabrafenib monotherapy. Patients randomized to receive dabrafenib alone were allowed to cross over to receive combination therapy upon progression. Flaherty et al. *NEJM* 2012 [27] reported results in BRAF inhibitor naïve patients with *BRAF*-mutant melanoma; this reference also provides open access to the study protocol and consort diagram.[27] Johnson et al. *J Clin Oncol* 2014 reported results of treatment with dabrafenib plus trametinib in patients with *BRAF*-mutant melanoma in Parts B and C who experienced who had experienced progression with a single agent BRAF inhibitor.[28]

Patient Selection

Inclusion criteria for the study included male or female patients who had given informed consent and were aged 18 years or older with histologically confirmed BRAF V600E or V600K mCRC. BRAF-mutation status was assessed locally. Patients were required to have measurable disease according to the Response Evaluation Criteria in Solid Tumors (RECIST) criteria version 1.1, an Eastern Cooperative Oncology Group (ECOG) performance status of 0 to 1, and adequate organ function. Female patients were required to have a negative pregnancy test and use adequate contraception throughout the study, or be of non-childbearing potential. Patients with unstable brain metastases, more than 1 previous regimen of chemotherapy in a metastatic setting, current or recent anti-cancer surgery or treatment within the previous month, a positive result for HIV or Hepatitis B or C, gastrointestinal illness, lung infections, or a history of central serous retinopathy, retinal vein occlusion, or serious cardiac comorbidities were excluded.

Study Oversight

The study was designed by the academic authors in conjunction with representatives of the sponsor, GlaxoSmithKline, and data were collected by the sponsor and analyzed in collaboration with the authors. The protocol was approved by the Institutional Review Board at each participating center, and complied with country-specific regulatory requirements. All patients provided written informed consent prior to any study procedures. The study was conducted in accordance with the provisions of the Declaration of Helsinki and Good Clinical Practice (GCP) guidelines. All authors vouch for the accuracy and completeness of the data and the fidelity of the study to the protocol. This manuscript was prepared by the first 2 and last author, but all authors contributed to the subsequent drafts. All authors made the decision to

submit the manuscript for publication.

Immunohistochemistry

Formalin-fixed paraffin-embedded tissue was sectioned and stained with pERK Thr202/Tyr204 antibody (clone 20G11, Rabbit monoclonal, Cell Signaling) and pAKT Ser 473 (Clone 14-5, Rabbit monoclonal, Dako) at Mosaic Laboratories (Lake Forest, CA) and graded using H-score as previously described.[28] PTEN and EGFR IHC were conducted at Ventana Medical Systems, Inc. using rabbit monoclonal antibody D4.3 or 5B7 respectively. PTEN loss was defined by H-Score of 50 cytoplasmic expression with maximum intensity of 1+ in the tumor cells.

Reverse Phase Protein Microarray (RPPA)

Laser Capture Microdissection Sample preparation: Eight micron serial sections were cut and mounted on uncharged microscope glass slides. As described previously [29] Laser Capture Microdissection (LCM) (Arcturus Bioscience, Mountain View, CA, USA) was performed to isolate enriched tumor epithelium for RPPA based analysis. For each specimen approximately 5,000–10,000 cells were collected on CapSure Macro LCM caps (Arcturus Bioscience, Mountain View, CA, USA). Caps were lysed using a 1:1 solution of 2x Tris-Glycine SDS Sample buffer (Invitrogen Life Technologies, Carlsbad, CA) and Tissue Protein Extraction Reagent (Pierce, Rockford, IL) supplemented with 2.5% β-mercaptoethanol (Sigma, St. Louis, MO). All samples were boiled for 8 min and stored at -80°C until further analyzed by RPPA.

LCM samples were printed in triplicate onto nitrocellulose coated slides (Grace Bio-labs, Bend, OR) using an Aushon 2470 arrayer (Aushon BioSystems, Billerica, MA). Before proceeding with immunostaining, each array was treated with Reblot antibody stripping solution (Chemicon, Temecula, CA, USA) for 15 minutes and blocked in I-block solution

(Tropix, Bedford, MA) for one hour to reduce non-specific binding. Each array was probed with one primary antibody on an automatic Autostainer (Dako Cytomation, Carpinteria, CA) using the Catalyzed Signal Amplification System kit (CSA; Dako Cytomation). Antibody specificity was tested for single band specificity and ligand induction via western blot analysis. Fluorescent detection was achieved using the streptavidin-conjugated IRDye680 (LI-COR Biosciences, Lincoln, NE) according to the manufacturer's instructions. The total amount of protein contained in each sample was measured by Sypro Ruby Protein Blot Stain (Molecular Probes, Eugene, OR) as previously described.[29] Images were acquired using the PowerScanner (TECAN, Mönnedorf, Switzerland) and spot intensity values were quantified using MicroVigene software Version 5.1.0.0 (Vigenetech, Carlisle, MA) as previously described.[29]

Next Generation Sequencing

Extracted DNA was quantified by Qubit 2.0 Fluorometer (Q32866) (Life Technologies). Targeted sequence enrichment and sequencing was performed by Perkin Elmer (Bradford, CT). Briefly, Agilent SureSelect Target Enrichment Kit (Agilent Technologies Inc. USA) was used for target enrichment. The multiplexed samples were sequenced on the Illumina HiSEQ 2000 platform using 100-bp paired-end reads. On average there were 25 million reads per sample, which translated to 300 fold of coverage. Sequencing data was aligned to human genome hg19 by using Novoalign (Novocraft, Malaysia). PCR duplicates were removed with the samtools and Picard software. The GenomeAnalysis Toolkit (GATK, version 1.4, Broad Institute) was used for variant calling. Variants were annotated based on Ensembl gene transcripts. Identified variants with poor depth or low average base quality scores were filtered out. Germline variants were filtered out by: dnsnp (build 138) with no known clinical significance; the 1000 genomes database (http://www.1000genomes.org/); top variants

identified in the Cancer Genome Atlas (TCGA, http://cancergenome.nih.gov/) colorectal cancer samples. <50.

Patient-derived Xenografts

One additional 1 cm 18 gauge tissue core was collected from each of five consenting UCSF patients at the time of the pre-treatment biopsy. This core was suspended in RPMI medium and couriered on ice to The Jackson Laboratory (JAX) Sacramento, CA, where the tissue was minced and injected into the flanks of JAX NOD *scid* gamma mice (NOD.*Cg-Prkdc*^{scid} *Il2rg*^{tm1Wjl}/Sz, NSG). Tumor tissue from four of five patients successfully established PDX models. Fragments from PDX passage 1 or 3 were shipped to UCSF and MDACC for expansion and drug testing. The initial implantation in animal facilities at UCSF or MDACC was in NSG mice; subsequent expansion and treatment studies occurred in female athymic nude mice under protocols approved by the Institutional Animal Care and Use Committee (IACUC). Mice were randomized into vehicle or dabrafenib plus trametinib groups when tumor volumes reached ~200 mm^3. PDX bearing mice (7-10 per group) were treated with vehicle (0.1% Tween-20 or 0.5% HPMC and 0.2% Tween-80), dabrafenib (30 mg/kg/day) plus trametinib (1 mg/kg/day in the first two models; 0.6 mg/kg/day in the third and fourth model) by oral gavage for 21 days. Mice were monitored for signs of toxicity (e.g. weight loss) and tumor size was evaluated three per week by caliper measurements. The 15% body weight reduction threshold for holding drug was not met.

Appendix Table 2 Reverse Phase Protein Array Targets and Change in Abundance

Target	Average Change (log2)*
Ki67	-1.95
S6 Ribosomal Protein S240/244	-1.63
E-Cadherin	-1.24
S6 Ribosomal Protein S235/236	-1.14
Survivin	-0.91
MEK 1/2 S217/221	-0.88
Src Family Y416	-0.76
4EBP1 T37/46	-0.75
FKHR S256	-0.66
GSK-3a/B S21/9	-0.62
ERK 1/2 T202/Y204	-0.61
RSK3 T356/S360	-0.58
Cleaved PARP	-0.54
FADD S194	-0.54
c-Myc	-0.50
Smad1 (Ser463/465)/ Smad5 (Ser463/465)/ Smad9 (Ser465/4€	-0.50
Beta Actin	-0.49
Jak2 Y1007/1008	-0.47
Bcl-2	-0.46
EGFR	-0.45
Cleaved Caspase 9 D315	-0.43
BAD S112	-0.43
Bak	-0.43
Ron Y1353	-0.42
ErbB3/HER3	-0.41
A-Raf S299	-0.38
Met Y1234/1235	-0.37
HSP27 S82	-0.36
EGFR Y1045	-0.36
Cyclin D1	-0.36
p90RSK S380	-0.34
ErbB2/HER2	-0.33
ErbB3/HER3 Y1289	-0.32
TGF beta	-0.31
EGFR Y845	-0.31
c-Met	-0.30
IGF-1 Rec B Y1131/Insulin Rec beta Y1146	-0.29
p70 S6 T412	-0.29
NF-kappaB p65 S536	-0.26
elF4E S209	-0.25
4EBP1 T70	-0.24
XIAP	-0.23
CREB S133	-0.22
p90RSK T359/S363	-0.21

PAK1 T423/PAK2 T402	-0.19
p70 S6 S371	-0.19
Cyclin D1 T286	-0.19
Bcl-2 S70	-0.18
Cleaved Caspase 3 D175	-0.18
Bcl-xL	-0.15
IL_6	-0.12
mTOR S2448	-0.12
TNF alpha	-0.12
SAPK/JNK T183/Y185	-0.11
SGK1 S78	-0.10
Bcl-2 T56	-0.09
Bax	-0.09
EGFR Y1173	-0.07
p53	-0.05
Smad2 S465/467	-0.04
PAK1 S199/204/ PAK2 S192/197	-0.04
ErbB2/HER2 Y877	-0.04
Bad	-0.04
EGFR Y1148	-0.03
MSK1 S360	-0.03
IGF1 Receptor beta	-0.01
Tubulin alpha/beta	0.01
Raf S259	0.01
CrkL Y207	0.01
elF4G S1108	0.02
PUMA	0.02
Smad2 S245/250/255	0.03
Jak1 Y1022/1023	0.03
IRS1	0.04
c-Raf S338	0.05
EGFR Y1068	0.06
Ret Y905	0.07
Vimentin	0.08
Ras-GRF1 S916	0.08
PDGFR beta Y716	0.09
B-Raf S445	0.10
p70 S6 T389	0.11
PDK1 S241	0.12
p38 MAPK T180/Y182	0.13
AKT T308	0.15
Stat3 S727	0.15
EGFR Y992	0.18
ErbB2/HER2 Y1248	0.21
PTEN	0.37
Src Y527	0.39
AKT S473	0.42

PTEN S380	0.47
Stat3 Y705	0.87
PDGFR Receptor beta	0.95
BIM	1.03
Ras-GRF1	1.05

*Average change in abundance of proteins or phosphoproteins in on-treatment biopsies relative to paired pre-treatment biopsies