Wednesday, 17 November 2010 14:45–16:15

PLENARY SESSION 2

Proffered papers

1LB LATE BREAKING ORAL

Development and validation of robust immunohistochemical assays for phospho-histone-H3 and Eg5 as pharmacodynamic biomarkers to support Eg5 inhibitor (LY2523355) clinical trials in patients with advanced malignancies

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Background: The ATP-dependent kinesin motor protein Eg5 plays an essential role in the formation of bipolar mitotic spindle and segregation of chromosomes. Inhibitors of Eg5 block ATPase activity and cause mitotic arrest and cell death *in vitro*. The Eg5 inhibitor, LY 2523355, is a novel anticancer therapeutic in clinical development with a need for optimal biomarker strategy.
Materials and Methods: We used well-characterized cultured human colorectal carcinoma cells (HCT-116), HCT-116 mouse xenografts and mouse skin to develop and analytically validate chromogenic immunohistochemical assays for phospho-histone H3 (pHH3) and Eg5. We used a rabbit polyclonal antibody specific for histone H3 phosphorylated at Serine 10, and a purified mouse monoclonal antibody for Eg5. Standard technical protocols were used to optimize the immunohistochemical assay conditions in our laboratory. Semi-quantitative assay results were evaluated manually by experienced pathologists and confirmed by matched immunoblot analyses. Prospectively collected human skin biopsies were used as an optimal surrogate tissue to demonstrate the pharmacodynamic effect of Eg5 inhibitor therapy in patients with advanced malignancies including breast, lung and ovary.
Results: Chromogenic immunohistochemistry assays demonstrated that the number of pHH3-labeled cell nuclei increased in cultured cells, xenograft cells and basal keratinocytes of mouse skin samples as the concentration of LY2523355 was increased. Similarly, we demonstrated a dose-dependent increase in accumulation of the Eg5 in both *in vitro* and *in vivo* experiments. Using control cell lines, xenografts and donated human skin samples, these chromogenic IHC assays were validated for accuracy, precision, linearity and range in a CAP/CLIA certified anatomic pathology laboratory. In the vast majority of human skin samples stained, there was a significant increase in pHH3 and Eg5 levels following administration of LY 2523355.
Conclusion: Using well-characterized *in vitro* and *in vivo* models, we have developed and analytically validated immunohistochemical assays for pHH3 and Eg5 as pharmacodynamic biomarkers for the Eg5 inhibitor (LY2523355) trials. We have also translated these assays to human skin as an optimal surrogate tissue for pharmacodynamic evaluation of these biomarkers. Our data support future in-study and advanced clinical validation of these promising immunohistochemical biomarkers to further substantiate their clinical utility.

Thursday, 18 November 2010 14:45–16:15

PLENARY SESSION 6

Proffered papers

2LB LATE BREAKING ORAL

Anti-tumor activity of anti-RON antibodies and biomarker of response

M. Han[1], K. Whalen[1], J. Gifford[1], A. Boudrow[1], K. Meetze[1], Q. Liu[2], B. Feng[3], W. Winston[1], S. Weiler[1], J. Gyuris[1]. *[1]AVEO Pharmaceuticals, Antibody Drug Discovery, Cambridge, USA; [2]AVEO Pharmaceuticals, Translational Research, Cambridge, USA; [3]AVEO Pharmaceuticals, Bioinformatics, Cambridge, USA*

Background: RON (Recepteur d'Origine Nanatais, or MST1R) receptor tyrosine kinase is a member of the c-Met RTK family. Macrophage stimulating protein (MSP or MST1) is its only known activating ligand. Over-expression of RON has been demonstrated in multiple solid tumor types and correlates with disease progression. A potentially oncogenic splicing variant has also been observed in colorectal cancer. The over-expression of RON in lung and breast epithelial cells has been shown to induce tumor development and metastasis in animal models. Inhibition of RON kinase activity via dominant negative receptor, small-molecule inhibitor, and antibodies leads to tumor growth inhibition in several preclinical models. Investigating the anti-tumor therapeutic potential of an anti-RON antibody with a predictive biomarker to guide the therapeutic development is warranted.
Material and Methods: Hybridomas producing anti-RON murine antibodies were generated by mouse immunization using the full extracellular domain of human RON. The ability of the antibodies to inhibit MSP induced RON signaling, cell proliferation, cell migration and invasion were assessed by Western blot, BrdU ELISA, and transwell assays, respectively. Binding affinities were assessed by surface plasmon resonance (Biacore) or by FACS. Inhibition of MSP binding to RON was tested using electrochemiluminescent (ECL) binding assays. Receptor internalization and degradation was also characterized using FACS and Western blot. The *in vivo* anti-tumor activity of the antibodies was assessed by xenograft studies.
Results: A panel of functional anti-RON antibodies with high binding affinity were isolated from murine hybridomas and extensively characterized. Several antagonistic antibodies were identified by their ability to inhibit MSP induced cell signaling, cell proliferation, migration, and invasion. Some of these antibodies can induce receptor internalization and degradation. The antibodies were also able to inhibit xenograft tumor growth driven by wild type RON or the RON delta 160 variant. Humanized versions of lead murine antibodies 29B06 and 07F01 have comparable potency to the parental antibodies.
A multi-gene biomarker capable of identifying tumor lines with potentially activated RON pathway was identified, and it is being validated by a panel of *in vivo* tumor models.
Conclusions: Anti-RON antibodies with potent anti-tumor activity have been described. These antibodies can inhibit the function of both wild type RON and the RON delta160 variant, in a ligand−dependent and independent manner. The lead antibodies have been humanized for therapeutic development. A potential multigene biomarker has also been discovered with preliminary validation by *in vivo* tumor models, which may help predict which tumor types or subtypes are more likely to respond to anti-RON antibody treatment.

3LB LATE BREAKING ORAL

MEDI-573, a dual IGF-1/-2 neutralizing antibody, blocks IGF-1R and IR-A signaling and maintains glucose homeostasis in a Phase 1 study for advanced solid tumors

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Background: MEDI-573, a dual-targeting human antibody that neutralizes the IGF-1/-2 ligands, represents a novel mechanism for inhibiting IGF-1R and insulin receptor-A (IR-A) signaling pathways, which play a significant role in carcinogenesis. Dual inhibition of these pathways is

anticipated to achieve broad antitumor efficacy without perturbing insulin signaling/glucose homeostasis.
Methods: Sequential cohorts of three to six subjects with advanced solid tumors received MEDI-573 as an IV infusion with consecutive dose escalations. Subjects had KPS ⩾60 and adequate hematologic, renal and hepatic function. Study (NCT00816361, sponsor: MedImmune, LLC) objectives included determinations of MTD, safety characteristics, pharmacokinetics (PK), pharmacodynamics (PD), and tumor response.
Results: 17 subjects (7M/10F), median age 58 yrs were treated (15 evaluable for DLT). No DLTs, drug-related serious adverse events or toxicity patterns have been reported to date. CTC grade 1−2 adverse events considered treatment-related have included decreased appetite and fatigue (24% each), anemia and diarrhea (18% each), leukopenia, nausea, and vomiting (12% each), thrombocytopenia, abdominal pain, systolic hypertension, pyrexia, back pain, and exertional dyspnea (6% each). No significant changes in plasma glucose, insulin, or growth hormone levels have been reported. MEDI-573 exhibited a dose-proportional increase in exposure over the range of doses tested and achieved the anticipated pharmacodynamic effects (including free IGF-1 and IGF-2 suppression). Stable disease spanning 12 weeks or more was seen in 5/13 subjects (range 12−30+ weeks), including 1 subject each with Ewing's sarcoma (18w) and liposarcoma (30w+). To date, anti-drug antibodies have not been reported. An expansion cohort (bladder cancer) biomarker focused phase is ongoing at doses that completely suppressed IGF ligands in the circulation.
Conclusions: In this study of 17 subjects, MEDI-573 has shown acceptable safety and favorable PK profiles. It did not directly or through compensatory mechanisms induce changes in glucose, insulin or growth hormone, which are endocrine liabilities observed with other IGF targeting strategies. MEDI-573 appears to have antitumor activity in some multiply refractory subjects. Continued clinical development is being pursued at doses that deplete IGF-1/-2 in the peripheral circulation.

16, 17 and 18 November 2010

POSTER SESSION

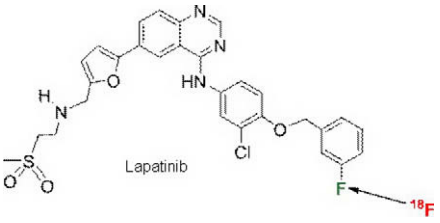
Late breaking posters

4LB LATE BREAKING POSTER

A first synthesis of [18F]-lapatinib: a new agent for positron emission tomographic studies of kinase receptors

G.L. Griffiths[1], F. Basuli[1], H. Wu[1], C. Li[1], J.L. Tatum[2], J.H. Doroshow[2]. [1]*NHLBI, IPDC, Rockville MD, USA;* [2]*NCI, NIH, Bethesda, USA*

Lapatinib ditosylate (Tykerb®) is a new drug from GlaxoSmithKline approved for treatment of advanced metastatic breast cancer in combination chemotherapy in patients who have failed Herceptin® and other initial therapies, and is also under investigation in other tumor indications. It is an epidermal growth factor receptor (EGFR) and ErbB-2 (Her2/neu) tyrosine kinase inhibitor. Lapatinib contains a fluorine atom in a *meta*-position on one benzyl ring, and ideally, if an ^{18}F atom could be placed here it would result in a chemically identical radiofluorinated analog of the approved drug. The total synthesis of an ^{18}F-lapatinib analog represents a significant overall radiochemical challenge while the generation of the required 3-[^{18}F] fluorobenzylbromide intermediate, itself, has not yet been reported. We based our approach to [^{18}F]-lapatinib on ether bond formation between 3-[^{18}F]fluorobenzylbromide and a *Boc*-protected Lapatinib fragment, followed by deprotection of the N-*Boc* secondary amino protecting group with TFA. In order to synthesize 3-[^{18}F]fluorobenzylbromide a series of aryliodonium salts of 3-formylbenzene with different counter-ions [PhIPhCHO]X (where X = Cl, Br, OTs, OTf) were prepared and radiolabeling of the precursors with ^{18}F was investigated using different bases, different temperatures, and in presence of the free radical scavenger, TEMPO. The best conversion (~80%) was obtained using $CsHCO_3$ at a reaction temperature of 110ºC. 3-[^{18}F] Fluorobenzaldehyde thus obtained was then converted to 3-[^{18}F] Fluorobenzylbromide and treatment with *Boc*-protected lapatinib precursor fragment in the presence of K_2CO_3 at 100 °C for 10 minutes in a microwave followed by *Boc* deprotection afforded [^{18}F]lapatinib (**1**) (65%, uncorrected, isolated). The overall radiochemical yield of the reaction starting from the radiofluorination of the iodonium salt was 5−10% (uncorrected) in a 130 minute synthesis time. The availability of the [^{18}F]-lapatinib, which represented a heretofore complex and unresolved radiochemical challenge will enable future PET imaging studies related to lapatinib uses.



5LB LATE BREAKING POSTER

Anti-tumor activity of MPC-9528, GMX1778 and APO866: Nampt inhibitors of three different structural classes

J.J. Boniface[1], V.R. Baichwal[1], L. DeMie[1], J.P. Green[1], W.R. Judd[1], J.W. Lockman[1], D.I. Papac[1], R.T. Terry-Lorenzo[1], J.A. Willardsen[1], R.O. Carlson[1]. [1]*Myrexis Inc., Research, Salt Lake City, USA*

Background: Nicotinamide phosphoribosyltransferase (Nampt) catalyzes the rate limiting step in the recycling of nicotinamide to NAD. Increased metabolic demands and higher activity of NAD consuming enzymes, such as PARPs, make cancer cells rely more upon Nampt. We compare MPC-9528, GMX1778 and APO866, which represent three structural classes of selective Nampt inhibitors.
Material and Methods: In vitro and cellular Nampt activity was measured using a coupled biochemical reaction based on fluorescent resorufin. Viability was measured by cellular ATP levels. Cellular Nampt activity was measured by NAD levels and detection of nuclear poly(ADP-ribose) (PAR). Xenografts were performed in nu/nu mice.
Results: Nampt enzyme was potently inhibited in vitro with IC_{50} values of 0.06, 0.1 and 0.4 nM for MPC-9528, GMX1778 and APO866, respectively. Cellular IC_{50} values were 0.2, 3.0 and 0.4 nM by measure of NAD and 0.1, 0.6 and 0.4 nM by measure of PAR for MPC-9528, GMX1778 and APO866, respectively. In 72 hour viability assays in HCT116 cells, MPC-9528, GMX1778 and APO866 exhibited IC_{50} values of 0.4, 2.0 and 1.5 nM, respectively. However, in an HCT116 xenograft model, oral administration of a maximally tolerated dose (MTD) of 75 mg/kg of MPC-9528 on days 1 and 10 gave comparable tumor regression as weekly oral dosing of GMX1778 at 250 mg/kg. Pharmacokinetic (PK) studies indicated that the Cmax and AUC were approximately 1.5 and 4 fold greater for GMX1778 than MPC-9528. In vivo anti-tumor activity was not observed for GMX1778 at 100 mg/kg despite a comparable Cmax and 3 fold greater AUC than was obtained for MPC-9528 at 75 mg/kg. The in vivo activities of MPC-9528 and APO866 were also compared in the HCT116 xenograft model. Administration of MPC-9528 orally at its daily MTD of 10 mg/kg for 21 days induced tumor regression, while APO866 dosed IP at its reported MTD of 20 mg/kg twice daily for 4 consecutive days per week for 3 weeks resulted in only tumor growth inhibition, but no regression. PK studies indicated that Cmax and AUC were approximately 15 and 4-fold greater, respectively, for APO866 than MPC-9528.
Conclusions: This study demonstrates that Nampt inhibitors with comparable in vitro activities, but different structures, display widely different anti-tumor activity in vivo.

6LB LATE BREAKING POSTER

The Nampt inhibitor MPC-9528 and the PARP inhibitor olaparib synergize in killing a BRCA-deficient cancer cell line

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Background: MPC-9528, a tumoridal agent with picomolar potency, is an inhibitor of nicotinamide phosphoribosyltransferase (Nampt). Nampt catalyzes the first step in NAD synthesis from nicotinamide, and when Nampt is inhibited, cellular NAD levels are depleted. NAD is an essential substrate for the enzyme poly(ADP-ribose) polymerase (PARP), and PARP activity is diminished following Nampt inhibition. PARP inhibitors such as olaparib selectively kill cells with impaired homologous recombination due to BRCA deficiency. Because MPC-9528 and olaparib inhibit PARP by different mechanisms, we hypothesized that, when combined, these two agents would synergize in killing BRCA-deficient cells dependent upon PARP for survival.
Material and Methods: Cellular NAD was measured using a coupled enzymatic assay. PARP activity was assessed using an imaging-based assay for detection of nuclear poly(ADP ribose) deposition. Cell viability was assessed by measuring ATP levels. In drug combination experiments, synergy, antagonism, or additivity was assessed using the MacSynergy program.