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.

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PLENARY SESSION 6

Proffered papers

2LB LATE BREAKING ORAL

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

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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