alternative to current EGFR-TKI, particularly in an optimized combination regimen.

619 POSTER

Combined inhibition of PI3K/AKT and MAPK signaling is required to inhibit translational initiation and to induce apoptosis in human tumors

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Activation of PI3K/AKT signaling via receptor activation, PI3K p110a mutation or mutational inactivation or decreased expression of the PTEN phosphatase is a common event in human tumors, thought to play a role in activating translation, inhibiting apoptosis, and deregulating proliferation. This pathway is thus thought to be an excellent target for therapeutic inhibition. However, we and others have found that both genetic and pharmacologic inhibition of PI3K/AKT has only modest or negligible effects on apoptosis and translation and have only minor antitumor effects in a variety of models. In many tumors, PI3K/AKT activation occurs together with activation of MAPK, which occurs via receptor activation (EGFR in glioblastoma), Ras mutation (colon cancer) or Raf mutation (melanoma). In exemplary tumor models with activation of both Ras/Raf/MAPK and PI3K signaling, we find that inhibition of MAPK signaling with a MEK inhibitor synergizes with pharmacologic inhibition of PI3K/AKT signaling to induce marked apoptosis and antitumor activity in tissue culture and xenograft models. These data suggest the existence of downstream targets or processes that integrate the effects of both pathways. We find that this occurs at the level of regulation of apoptosis and of assembly of the translational preinitiation complex. Activity of either pathway alone is sufficient to prevent activation of the proapoptotic BAD protein and to prevent the binding of the translational inhibitor 4EBP1 to the eIF4E–mRNA complex. The data suggest that the two pathways confer overlapping selective advantages that are integrated by proteins such as BAD and 4EBP1. In tumors in which both pathways are activated, inhibition of both is required to activate BAD and 4EBP1 and induce apoptosis and inhibit translation of capped mRNAs. We have been able to effectively inhibit both pathways *in vivo* and cause significant antitumor activity with limited toxicity to the host. The data therefore suggest that combined inhibition of MAPK and PI3K/AKT signaling may be a useful therapeutic strategy in many tumors.

620 POSTER

Identification of the receptor tyrosine kinase c-Met and its ligand, HGF, as therapeutic targets in clear cell sarcoma

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Background: Clear cell sarcoma (CCS), a tumor of the extremities and aponeuroses of children and young adults, is uniformly fatal once metastatic exhibiting profound resistance to radio- and chemotherapy. Implicated in human cancer, the receptor tyrosine kinase c-Met mediates hepatocyte growth factor (HGF) signaling. Expression of c-Met has recently been found to be transcriptionally regulated by MITF in melanocytes and melanoma. MITF is strongly expressed in CCS, where it has been identified as an oncogenic transcriptional target of EWS-ATF1. We investigated the role of c-Met and HGF in CCS and whether this pathway may constitute a therapeutic target.
Materials and Methods: CCS cells were retrovirally transduced with c-Met-directed shRNA (or control) or were treated with a fully human monoclonal anti-HGF antibody (2.12.1). Viability and proliferation were monitored by propidium iodide exclusion, colony forming assays or WST1 assays. c-Met phosphorylation and signaling pathway status were monitored by immunoblots. HGF expression and secretion were assessed by RT-PCR and ELISA, respectively. Mice bearing xenograft tumors of CCS cells were treated IP with 2.12.1 (or isotype control antibody), and tumor volumes were measured with digital calipers.
Results: Analyses of primary CCS and CCS-derived cell lines demonstrated elevated c-Met expression as compared to other soft tissue sarcomas. c-Met displayed constitutive phosphorylation in CCS cells despite the absence of mutations. In a subset of these tumor cells, HGF secretion and autocrine signaling activated c-Met, resulting in activation of both the MAPK and AKT pathways. Knockdown of c-Met expression by RNAi decreased CCS cell survival/proliferation. In order to block autocrine signaling, CCS cells were treated with a neutralizing monoclonal antibody to HGF, 2.12.1. Treatment with 2.12.1 decreased c-Met activity and intracellular signaling and resulted in growth inhibition in culture. In a murine xenograft model of CCS, anti-HGF treatment significantly decreased tumor development in a minimal residual disease model and inhibited the growth of established tumors.
Conclusion: The receptor tyrosine kinase c-Met is expressed and constitutively activated in a high fraction of CCS. c-Met is critical for CCS viability/proliferation, and in the context of autocrine activation, antibody mediated HGF inhibition significantly suppresses CCS growth. These data suggest the potential for therapeutic targeting of c-Met/HGF in CCS.

621 POSTER

A phase I study of combination therapy with AEE788, a novel multitargeted inhibitor of ErbB and VEGF receptor family tyrosine kinases, and RAD001, a mTOR inhibitor in recurrent GBM patients

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Background: AEE788 (AEE) is a potent oral inhibitor with activity against multiple tyrosine kinases, including EGFR, ErbB2 and VEGFR2. RAD001 (RAD) is an oral inhibitor of mTOR. This study assessed the MTD/DLT, safety, tolerability and pharmacokinetics (PK) of AEE+RAD in recurrent GBM patients (pts) not on CYP3A-inducing anti-convulsants.
Methods: Pts in 1st or 2nd recurrence were enrolled and treated in 28 day (D) cycles (C). A 6 parameter Bayesian logistic regression model using the escalation with overdose control principle was used to guide dose escalation. 24-hr PK was obtained on C1, D1, 15 and 28 and C2 D28. PK parameters of AEE and AQM674 (AQM) were computed by model-independent methods. FLT-PET was performed at baseline (BL) and C1D28 to assess tumor proliferation.
Results: 16 pts (11M/5F), median age 52 yrs (range 28−71), were treated with AEE 200 mg qd/RAD 5 mg qd (cohort 1, n = 2) or AEE 150 mg qd/RAD 5 mg qod (cohort 2, n = 14). 1 pt in cohort 1 had DLT (Grade [Gr] 3 thrombocytopenia); 3 pts in cohort 2 had DLTs (Gr 4 CK, Gr 3 thrombocytopenia and Gr 3 diarrhea). The most common (>15%) adverse events were diarrhea and rash (56% each), fatigue (50%), stomatitis and thrombocytopenia (31% each), hyperglycemia and muscle weakness (25% each), and CK increase (19%). 3 pts in cohort 2 had reversible Gr 3 AST/ALT. PK data indicated AEE increased the exposure of RAD by >2-fold compared to pts who received RAD monotherapy at the same dose in other trials. The PK interaction resulted in Gr 3 thrombocytopenia requiring dose interruption (1 and 3 pts in cohorts 1 and 2 respectively). Administration of RAD 5 mg qod with AEE 150 mg qd increased the exposure of AEE after multiple dosing (AUC values similar to AEE 250 mg qd). Exposure of the main metabolite, AQM, was not altered by RAD. Median time on treatment was 49 days (range 8–224). 7/16 pts had SD at the end of C2. 1 pt (cohort 2) demonstrated a 60% decrease in FLT uptake in 1 of 2 lesions. This pt had SD for 2C.
Conclusion: A drug-drug interaction occurred when AEE and RAD were co-administered, resulting in thrombocytopenia that required interruption of treatment. Thrombocytopenia was not eliminated by dose reduction to 150 mg AEE qd + 5 mg RAD qod. RAD increased the exposure of AEE after multiple dosing, without affecting AQM. 1 pt had response by FLT-PET which correlated with changes in the MRI. The study has been discontinued due to safety data.

622 POSTER

Discovery and characterization of a novel multi-targeted tyrosine kinase inhibitor with activity against c-ret, pdgfr, c-kit and c-src

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Due to the success of multi-targeted agents in the clinic and beyond, interest has risen in compounds with expanded selectivity within the subfamily of protein kinases. Many of the very successful small molecules entering the clinic recently have activity against multiple kinase enzymes, and this appears to be to their benefit. Using our proprietary CLIMB™ drug discovery process, we have endeavored to design and test a novel compound with effective activity against a number of therapeutically relevant protein tyrosine kinases. Through computational modeling and docking with both wild-type and mutant kinase crystal structures or homology models, in silico physicochemical predictions and biochemical and biological assays, we have developed the substituted pyrimido[4,5-*b* indole] compound MP371. MP371 is a very promising drug candidate with expanded selectivity for a number of tyrosine kinases, including mutant forms of c-Kit, found in gastrointestinal stromal tumors, which have been reticent to inhibition by imatinib (opening a niche for this inhibitor) as well as Ret (involved in papillary and medullary thyroid carcinoma, pheochromocytoma and parathyroid cancer), PDGFRa and b (which are implicated in pancreatic carcinoma), and members of the cytoplasmic