TITLE
Choice of non-physiologic HGF concentrations in preclinical evaluations of c-Met targeting agents results in an over-estimation of c-Met inhibitor activity

HYPOTHESIS:
Tumor cells exposed to non-physiologic HGF concentrations will appear sensitive to HGF inhibition when they would otherwise not be.

BACKGROUND/AIMS:
C-Met is a receptor tyrosine kinase critical for embryogenesis and liver repair. In tumors, including breast, prostate, glioblastoma, and thyroid cancer, Met protein levels are often elevated and associated with disease progression and metastasis. The C-Met pathway is activated by the endogenous ligand Hepatocyte Growth Factor (HGF). HGF is produced by mesenchymal cells and binds to the c-Met protein, leading to phosphorylation and upregulation of a variety of downstream signaling pathways that result in increased tumor cell migration, proliferation, survival, and induction of angiogenesis. Consequently, there has been significant interest in the development of c-Met inhibitors as anti-cancer therapeutics. The efficacy of such agents usually initially are determined in vitro utilizing HGF concentrations of 25-50 ng/mL. However, HGF serum levels in humans typically range from 0.4-0.8 ng/mL.

METHODS: To examine the impact of exogenous HGF levels on c-Met inhibitor activity, human prostate (PC-3, DU145) and lung (A549) cancer cell lines were utilized in this study. These tumor cells are characterized as having high levels of total Met, but low levels of phospho-Met. Western blots were performed to assess protein levels and phosphorylation. Migration was assessed with a transwell chamber assay, and invasion was assessed in a similar manner as the migration assay, except with the addition of a Matrigel basement membrane. For tumor studies, 10^6 DU145 cells were inoculated via subcutaneous injection into male NOD SCID Gamma (NSG) mice and measured three times a week using calipers, till the tumors reached 500 mm^3. Mice received BMS-777607 via oral gavage and/or 250 ug/kg HGF via intraperitoneal injection. Tumors were harvested and formalin-fixed and paraffin embedded. Tissue sections were evaluated for Met and phospho-Met staining.

RESULTS & CONCLUSIONS: Phosphorylation of Met is induced by treatment with HGF in a dose-dependent manner in all three cell lines. In the presence of 25-50 ng/mL HGF, these cell lines demonstrated a reduction in migration, invasion, and phosphorylation of Met after treatment with the small molecule c-Met inhibitor BMS-777607. However, such anti-tumor efficacy was absent when these cell lines were treated with BMS-777607 in the presence of physiologically normal concentrations of HGF (0.4-0.8 ng/mL). A human HGF-expressing mouse model has been developed for xenograft studies of human c-Met inhibition, on the basis that mouse HGF does not activate human c-Met. However, we found that mouse HGF was able to induce phosphorylation of Met in the human DU145 cell line. In vivo, DU145 xenografts in NSG mice treated with BMS-777607 showed no reduction in c-Met phosphorylation unless the mice were administered exogenous HGF during tumor growth. Taken together, these findings indicate that the utilization of non-physiologic concentrations of HGF in tissue culture evaluations of c-Met targeting agents may result in an over-estimation of agent activity not likely to be observed in the tumor microenvironment.