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Volitinib, a potent and highly selective c-Met inhibitor, effectively blocks c-Met signaling and growth in *c-MET* amplified gastric cancer patient-derived tumor xenograft models☆

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ABSTRACT

Purpose: To investigate the incidence of *cMET* gene copy number changes and protein overexpression in Chinese gastric cancer (GC) and to preclinically test the hypothesis that the novel, potent and selective cMET small-molecule inhibitor volitinib, will deliver potent anti-tumor activity in cMET-dysregulated GC patient-derived tumor xenograft (PDX) models.

Experimental design: A range of assays were used and included; *in vitro* cell line panel screening and pharmacodynamic (PD) analysis, *cMET* fluorescence *in-situ* hybridization (FISH) and immunohistochemical (IHC) tissue microarray (TMA) analysis of Chinese GC ($n = 170$), and anti-tumor efficacy testing and PD analysis of gastric PDX models using volitinib.

Results: The incidence of *cMET* gene amplification and protein overexpression within Chinese patient GC tumors was 6% and 13%, respectively. Volitinib displayed a highly selective profile across a gastric cell line panel, potently inhibiting cell growth only in those lines with dysregulated cMET (EC_{50} values 0.6 nM/L–12.5 nM/L). Volitinib treatment led to pharmacodynamic modulation of cMET signaling and potent tumor stasis in 3/3 cMET-dysregulated GC PDX models, but had negligible activity in a GC control model.

Conclusions: This study provides an assessment of tumor *cMET* gene copy number changes and protein overexpression incidence in a cohort of Chinese GC patients. To our knowledge, this is the first study to demonstrate anti-tumor efficacy in a panel of cMET-dysregulated gastric cancer PDX models, using a novel selective cMET-inhibitor (volitinib). Thus, the translational science presented here provides strong rationale for the

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investigation of volitinib as a therapeutic option for patients with GC tumors harboring amplified *cMET*.

1. Introduction

Located on the 7q31 locus, the MET proto-oncogene encodes a receptor tyrosine kinase which exhibits specificity for a single known high affinity ligand, hepatocyte growth factor (HGF) (Nakamura et al., 1989; Park et al., 1987; Stoker et al., 1987). HGF binding to MET leads to receptor dimerization and autophosphorylation on multiple tyrosine residues within the intracellular kinase domain, resulting in subsequent phosphorylation of the juxtamembrane domain and C-terminal docking sites (Gherardi et al., 2012). These phosphorylation events enable a striking diversity of cellular responses through activation of multiple downstream effector proteins (such as the adaptor proteins Grb2 and Gab1) (Birchmeier et al., 2003; Weidner et al., 1996), leading to activation of the PI-3-kinase, Ras/RAF/MEK/ERK, PLCγ, STAT and FAK signaling pathways. Such signaling allows MET to regulate cell growth, migration, invasion, proliferation and angiogenesis (Humphrey et al., 1995; Matsumoto and Nakamura, 1996).

In normal tissues, MET expression is tightly regulated in cells of epithelial origin (Prat et al., 1991) however, dysregulation of the MET signaling pathway occurs in a wide range of human epithelial cancers including lung, colorectal, breast, pancreatic, ovarian, hepatic and gastric cancers (Di Renzo et al., 1995; Edakuni et al., 2001; Fujita and Sugano, 1997; Humphrey et al., 1995; Inoue et al., 2004; Tsuta et al., 2012). Molecular mechanisms which contribute to this dysregulation are varied and include germline or somatic MET mutation, gene rearrangement, amplification, protein overexpression or changes in ligand-induced autocrine or paracrine signaling. Indeed, the occurrence of such dysregulating mechanisms often correlates with poor prognosis, as demonstrated for several types of cancer, including gastric (Go et al., 2010; Ichimura et al., 1996; Miyata et al., 2009; Nakajima et al., 1999).

Despite improvements in early diagnosis, surgical techniques and more recently, the uptake of targeted therapies including trastuzumab (Bang et al., 2010), advanced gastric cancer remains the second most common cause of global cancer-related death with high incidence, relatively poor prognosis and limited treatment options (Jemal et al., 2011). Eastern Asia in particular (notably China, Japan and Korea), suffers from a high incidence of gastric cancer due in part to dietary factors, smoking and the high prevalence of *Helicobacter pylori* infection (Naylor et al., 2006; Parkin, 2006). Taken together, these data provide a compelling rationale for targeting of the HGF/MET signaling pathway as a therapeutic strategy in multiple tumor types, and especially in gastric cancer of Asian origin.

A number of strategies are being explored to therapeutically inhibit c-Met activity, including c-Met or HGF-specific antibodies and small molecule tyrosine kinase inhibitors. In the latter category, a major challenge to the development of selective ATP-competitive inhibitors has been the high degree of sequence similarity within the ATP-binding pockets of canonical protein kinases, and indeed, many current c-Met targeted agents have relatively promiscuous, mixed pharmacology profiles (recently reviewed in (Scagliotti et al., 2013)). Volitinib represents a novel, potent and highly selective c-Met small molecule tyrosine kinase inhibitor with favorable preclinical pharmacokinetic and tolerance profiles (Cui et al., 2013; Gu et al., 2013). Volitinib is currently in Phase I clinical trials in China and Australia. A further challenge facing the development of novel agents targeting the cMET signaling pathway concerns the definition of appropriate and accurate biomarker criteria to enable prospective selection of patients. Within gastric cancer specifically, a number of early phase trials have been conducted using cMET tyrosine kinase inhibitors and cMET or HGF-binding antibodies and unfortunately, despite evidence of clinical responses, none have yet definitively identified robust prospective biomarkers of response (Catenacci et al., 2011; Lennerz et al., 2011; Oliner et al., 2012; Shah et al., 2013). Clinical responses to some of these agents have been documented in patients with tumors harboring *cMET* gene amplification or cMET protein 'overexpression', but consistent data linking scoring criteria to response, or the relationship between *cMET* gene amplification and protein overexpression, is limited.

In this study we performed a detailed analysis of *cMET* gene copy number and protein overexpression in a cohort of Chinese gastric cancer patients. We describe one of the first reports of the novel, potent and selective cMET tyrosine kinase inhibitor, volitinib, which was screened across a panel of gastric cancer cell lines and displayed potent antiproliferative activity only in cell lines harboring aberrant cMet signaling. More importantly, we established translational significance by demonstrating volitinib anti-tumor efficacy and pharmacodynamic activity in a panel of cMET-dysregulated gastric patient-derived tumor xenograft (PDX) models. In doing so, we provide insight into the relationship between *cMET* gene amplification and protein expression in gastric cancer and highlight expression thresholds required for preclinical response to volitinib.

2. Materials and methods

2.1. Volitinib

For *in vitro* studies, volitinib was prepared as a 10 mM DMSO stock solution and diluted in the relevant assay media. For *in vivo* studies, volitinib was formulated in a 0.5% (v/v) solution of carboxymethylcellulose-sodium. Animals were given volitinib or vehicle control once daily (qd) by oral gavage.