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Initial Testing (Stage 1) of TAK-701, a humanized hepatocyte growth factor binding antibody, by the Pediatric Preclinical Testing Program

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Abstract

TAK-701 is a humanized antibody that binds hepatocyte growth factor (HGF), thus suppressing c-Met transduced signaling and c-Met dependent proliferation and migration of tumor cells. Six childhood solid tumor xenografts were selected for evaluating TAK-701 based on immunochemical detection of HGF/c-Met autocrine signaling [i.e., pMet(Tyr1349) and HGF positive]. TAK-701 was tested using a dose of 30 mg/kg administered by the intraperitoneal (IP) route twice weekly for 4 weeks. TAK-701 did not induce significant differences in EFS distribution in treated tumors compared to control tumors. Objective responses were not observed in any of the tested solid tumor xenografts.

Keywords

Preclinical Testing; Developmental Therapeutics; c-Met inhibition

INTRODUCTION

Increased expression of the c-Met receptor or its ligand (hepatocyte growth factor/scatter factor; HGF) occurs frequently in human cancer, and increases with tumor progression in some malignancies [1]. Ligand activation of c-Met results in phosphorylation of GAB1 which, in turn, recruits a number of signaling effectors, including PI3K, SHP2, and PLC- γ [2]. GAB1 phosphorylation by MET results in a sustained signal that mediates most of the downstream signaling pathways including PI3 kinase and Ras. There is evidence that HGF/c-Met may be involved in several childhood cancers. For example, MET has been shown to be highly overexpressed in alveolar rhabdomyosarcoma (ARMS) [3-5], and was detected in

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all of 68 rhabdomyosarcoma specimens. Of these 62 percent of tumors co-expressed HGF, however, neither high MET or HGF expression correlated with metastatic disease [6]. Simultaneous loss of *Ink4a/Arf* function and disruption of c-Met signaling in *Ink4a/Arf*^{-/-} mice transgenic for HGF/SF induces rhabdomyosarcoma with extremely high penetrance and short latency [7]. Neuroblastoma cell lines express HGF, but require exogenous ligand to stimulate c-Met phosphorylation [8], and PHA665752, a small-molecule inhibitor of c-Met, inhibited ligand-induced migration and proliferation of c-Met-positive neuroblastoma cells [9].

Modeling the HGF/MET pathway in mice with xenografts is difficult because murine HGF does not activate human c-Met [10]. One approach to evaluating the role of inhibitors of HGF/MET signaling is use of immunocompromised mice transgenic for human HGF or use of other strategies for expressing human HGF in mice [11,12]. We have taken an alternative approach of identifying a series of pediatric solid tumor xenografts in the Pediatric Preclinical Testing Program (PPTP) panels that exhibit c-Met activation, presumably as a result of autocrine stimulation via secreted HGF. TAK-701 is a humanized monoclonal antibody (MAb) that is derived from the murine MAb L2G7 and that has high affinity for HGF [13-15]. TAK-701 inhibits the binding of HGF to c-Met and inhibits the intra-cellular phosphorylation of c-Met and its downstream factors, Gab1, AKT, and ERK1/2, in a concentration-dependent manner [15]. TAK-701 shows *in vivo* antitumor activity against various adult cancer preclinical models that have autocrine dependence on HGF [14]. We have evaluated TAK-701 in a limited series of pediatric *in vivo* models with autocrine stimulation of HGF/MET signaling.

MATERIALS AND METHODS

In vivo tumor growth inhibition studies

CB17SC *scid*^{-/-} female mice (Taconic Farms, Germantown NY), were used to propagate subcutaneously implanted kidney/rhabdoid tumors, sarcomas (osteosarcoma, rhabdomyosarcoma), and neuroblastoma, while BALB/c nu/nu mice were used for glioma models, as previously described [16,17]. Female mice were used irrespective of the patient gender from which the original tumor was derived. All mice were maintained under barrier conditions and experiments were conducted using protocols and conditions approved by the institutional animal care and use committee of the appropriate consortium member. Ten mice were used in each control or treatment group. Tumor volumes (cm³) for solid tumor xenografts were determined as previously described [16]. Responses were determined using three activity measures as previously described [16]. An in-depth description of the analysis methods is included in the Supplemental Response Definitions section.

Immunohistochemical (IHC) analysis for pMET, MET, and HGF

IHC was performed on paraffin-embedded formalin fixed xenograft tissues. The following antibodies were used: anti-pMet Tyr1349 antibody from Cell Signaling (Danvers, MA); anti-c-Met antibody from Zymed (Carlsbad, CA); anti-HGF antibody from IBL (Minneapolis, MN); mouse IgG isotype control antibody from Dako; and rabbit IgG isotype control antibody from Dako. Stained slides were scanned using an Aperio ScanScope CS system (Vista, CA) to produce whole slide images. Staining was evaluated on a semi-quantitative scale, and the percentage of cancer cells staining at each of the following four levels was recorded: 0 (unstained), 1+ (weak staining), 2+ (moderate staining) and 3+ (strong staining). An H-score was calculated based on the summation of the product of percent of cells stained at each intensity.

Statistical Methods

The exact log-rank test, as implemented using Proc StatXact for SAS®, was used to compare event-free survival distributions between treatment and control groups. P-values were two-sided and were not adjusted for multiple comparisons given the exploratory nature of the studies.

Drugs and Formulation

TAK-701 was provided to the Pediatric Preclinical Testing Program by Millennium Pharmaceuticals, through the Cancer Therapy Evaluation Program (NCI). TAK-701 was diluted in sterile saline and stored at 4°C, protected from light, and was administered intraperitoneally (IP) using a twice-weekly schedule for 4 weeks at a dose of 30 mg/kg. TAK-701 was provided to each consortium investigator in coded vials for blinded testing.

RESULTS

In vivo testing

Tumors were selected for evaluation against TAK-701 based on immunohistochemical detection of activated c-Met(Tyr¹³⁴⁹) and detection of HGF in sections from tumor xenografts (Figure 1 and Supplemental Figure 1). All 6 xenograft models studied were considered evaluable for efficacy. A complete summary of results is provided in Supplemental Table I. TAK-701 administered twice-weekly at 30 mg/kg failed to induce significant differences in EFS distribution compared to control in any of the 6 evaluable solid tumor xenografts, Table I.

DISCUSSION

HGF and the c-Met receptor are overexpressed together in many solid tumors, including some childhood cancers. In humans, HGF can act as both an autocrine and as a paracrine growth factor, inducing signals resulting in increased cancer cell proliferation, migration, invasion, and drug resistance. MET has been shown to be highly overexpressed in alveolar rhabdomyosarcoma (ARMS) [3-5], but while the gene is not mutated or amplified the expression level at the RNA level was found to be significantly higher in patients who died of disease [18]. MET is highly expressed in cell lines derived from ARMS [5], and HGF induces motility and confers drug resistance in rhabdomyosarcoma cells [19].

We selected 6 xenograft models that exhibited robust phosphorylation of c-Met as well as HGF expression by immunohistochemistry. As murine HGF does not activate the human receptor, these data imply autocrine activation, at least under conditions of growth in the mouse. TAK-701 is a humanized monoclonal antibody with high affinity to HGF that blocks various HGF-induced biological activities as well as inhibits tumor growth in an autocrine HGF-MET-driven xenograft model [14,15]. In a previous study [13] it was shown that TAK-701 administered IP biweekly (5 mg/kg) effectively reversed gefitinib resistance in a NSCLC xenograft model engineered to overexpress HGF. In the current experiments TAK-701 was administered IP at 30 mg/kg biweekly for a planned 4 week treatment period but failed to significantly retard growth of any of the PPTP tumor models evaluated. Failure to inhibit growth may have several reasons, although this dose and schedule of TAK-701 effectively stabilized growth of several adult cancer preclinical models including U87MG (glioblastoma), IM95 (gastric), KP4 (pancreatic) and MC-10 (breast cancer) [14]. While several studies have used IV administration of TAK-701, the antibody has been shown effective when administered IP [13], thus it seems unlikely that the route of administration used influenced the negative activity in our study. Our results indicate that while HGF/c-Met signaling may be important for maintaining certain malignant characteristics (e.g., invasion

and metastasis), TAK-701, an antibody known to block HGF binding to its receptor, was not able to significantly inhibit growth of the childhood cancer preclinical models studied.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1.
Photomicrographs (20×) of IHC staining in xenografts.

Table 1Summary of *in Vivo* Activity of TAK-701

Xenograft Line	Histology	Median Time to Event	P-value	EFS T/C	Median Final RTV	T/C	T/C Activity	EFS Activity	Response Activity
Rh18	Embryonal rhabdomyosarcoma	12.7	0.856	1.3	>4	0.88	Low	Low	Low
BT-39	Glioblastoma	14.5	0.421	1.1	>4	0.79	Low	Low	Low
NB-EBc1	Neuroblastoma	4.7	0.603	1.0	>4	0.99	Low	Low	Low
CHLA-79	Neuroblastoma	7.9	0.430	1.1	>4	0.72	Low	Low	Low
OS-1	Osteosarcoma	27.6	0.269	1.1	>4	0.83	Low	Low	Low
OS-2	Osteosarcoma	18.6	0.201	1.1	>4	0.91	Low	Low	Low