A Tumor-Targeting c-Met ADC

**ABBV-399, a c-Met Antibody Drug Conjugate that Targets Both MET Amplified and c-Met Overexpressing Tumors, Irrespective of MET Pathway Dependence**

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Running Title
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Abstract

Purpose: Despite the importance of the MET oncogene in many malignancies, clinical strategies targeting c-Met have benefitted only small subsets of patients with tumors driven by signaling through the c-Met pathway, thereby necessitating selection of patients with MET amplification and/or c-Met activation most likely to respond. An antibody drug conjugate (ADC) targeting c-Met could overcome these limitations with potential as a broad acting therapeutic.

Experimental Design: ADC ABBV-399 was generated with the c-Met targeting antibody, ABT-700. Anti-tumor activity was evaluated in cancer cells with overexpressed c-Met or amplified MET and in xenografts including patient-derived xenograft (PDX) models and those refractory
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to other c-Met inhibitors. The correlation between c-Met expression and sensitivity to ABBV-399 in tumor and normal cell lines was assessed to evaluate risk of on target toxicity.

**Results:** A threshold level of c-Met expressed by sensitive tumor but not normal cells is required for significant ABBV-399-mediated killing of tumor cells. Activity extends to c-Met or amplified MET cell line and PDX models where significant tumor growth inhibition and regressions are observed. ABBV-399 inhibits growth of xenograft tumors refractory to other c-Met inhibitors and provides significant therapeutic benefit in combination with standard of care chemotherapy.

**Conclusion:** ABBV-399 represents a novel therapeutic strategy to deliver a potent cytotoxin to c-Met-overexpressing tumor cells enabling cell killing regardless of reliance on MET signaling. ABBV-399 has progressed to a Phase 1 study where it has been well tolerated and has produced objective responses in c-Met expressing non small cell lung cancer (NSCLC) patients.

**Translational Relevance**

The anti-tumor activity of c-Met inhibitors is generally limited to tumors that are MET-activated and driven predominately by c-Met signaling. ABBV-399, a c-Met targeting ADC, represents a novel therapeutic delivering a potent cytotoxin to c-Met-overexpressing tumor cells enabling cell killing regardless of reliance on MET signaling. ABBV-399 treatment, alone and in combination with standard of care chemotherapy, induces significant tumor growth inhibition and regressions in tumor cell line and PDX models with overexpressed c-Met or amplified MET including tumors refractory to other c-Met inhibitors. We demonstrate ABBV-399 killing requires a threshold level of c-Met, expressed by sensitive tumor but not normal cells. These data indicate that ABBV-399 may be an effective broad acting c-Met targeting therapeutic that can overcome limitations associated with other c-Met inhibitors. ABBV-399 has progressed to a Phase 1 study where it has been well tolerated and has produced objective responses in c-Met expressing NSCLC patients.
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Introduction

The c-Met receptor tyrosine kinase is the cell surface receptor for hepatocyte growth factor (HGF) encoded by the MET proto-oncogene (1). The c-Met/HGF axis is aberrantly activated in multiple cancers through MET genomic amplification, transcriptional up-regulation and ligand-dependent mechanisms, thereby contributing to tumor progression, angiogenesis, invasive growth, metastasis, and resistance to therapies (1). The development of c-Met/HGF axis inhibitors, both antibodies and small molecules, has been an active area of cancer research (2-4). The development of c-Met-directed therapeutic antibodies has been hampered by the induction of agonistic activity (5,6). The resulting approach to c-Met-directed therapeutic antibody development has, therefore, focused on the “one-armed” antibody (Onartuzumab; Roche) or antibodies to the HGF ligand (Rilotumumab; Amgen) (7-9). Small molecule inhibitors of c-Met signaling have also been developed (e.g., cabozatinib, Exelixis; crizotinib, Pfizer, tivantinib, ArQule) although many of these are non-selective broad spectrum kinase inhibitors or may not directly target c-Met (10-13).

Although several of these inhibitors have advanced into clinical trials, results with this drug class did not induce demonstrable survival benefit (14,15). Scrutiny of these trial outcomes suggests that c-Met expression on tumors by itself is not a sufficient predictor for activity. Instead patient selection strategies to identify those tumors in which MET is constitutively activated through gene amplification, mutation or ligand-dependent activation may be necessary to predict sensitivity to many of these inhibitors (14,15). Consistent with reliance on MET activation for activity, ABT-700, a c-Met targeting antibody without the agonist activity associated with many c-Met antibodies, was well tolerated in a Phase 1 trial and demonstrated anti-tumor activity in select patients with MET amplified solid tumors (16,17). Although increased frequencies of MET amplification may be associated with relapsed/refractory tumors including EGFR activating mutation in non-small cell lung cancer (NSCLC), primary MET genomic amplification is a low frequency event in most tumors (1%-5%), thereby limiting the patient population where inhibitors that block MET signaling may be effective (18-21).
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An antibody drug conjugate (ADC) targeting c-Met represents an attractive therapeutic strategy that does not depend on downstream signaling for efficacy but rather on target expression. If successful, this approach could expand the breadth of treatment beyond that attainable with other c-Met inhibitors since c-Met overexpression occurs in 30 - 50% of solid tumors, including NSCLC, colorectal cancer (CRC) and advanced gastroesophageal cancer (22-24). ABBV-399 is an ADC comprised of the ABT-700 antibody conjugated to the clinically validated cytotoxic microtubule inhibitor monomethylauristatin E (MMAE) via a cleavable valine-citruline (VC) linker (25,26). Although a c-Met targeting ADC presents the risk of on-target toxicity based on c-Met normal tissue expression, c-Met expression is significantly higher in many cancers compared to normal tissues (27-31) suggesting that a therapeutic window may exist for a selectively targeting ADC. We investigated the preclinical characteristics of ABBV-399 including its anti-tumor activity against a variety of c-Met overexpressed, MET amplified and c-Met-inhibitor refractory tumor models. Collectively these results provided the basis for advancing ABBV-399 to Phase 1 studies in patients with c-Met overexpression where objective responses have been observed (32).

Material and Methods

Antibodies and Reagents

ABT-700, an anti-human c-Met targeting antibody derived from the mAb 224G11 was produced from a stable transfected stable CHO cell line as described previously (12). ABBV-399 was generated from the conjugation of valine-citruline (vc) MMAE to interchain disulfide bonds in ABT-700 after mild reduction to the sulfhydryl group (25). The average drug:antibody ratio of ABBV-399 was approximately 3.1. Recombinant human c-Met extracellular domain with a His tag (rh-c-Met ECD-6His) was expressed in and purified from HEK293 cells. HGF was purchased from R&D Systems. 5-Fluorouracil (APP Pharmaceuticals) and irinotecan (Hospira) were...
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obtained as solutions and diluted with 0.9% NaCl for injection (USP), and leucovorin calcium (Fluka Chemical) was obtained as a salt and reconstituted with saline before dosing.

Cell Culture

The tumor cell lines A549, Hs 746T, SW48, HT-29, MDA-MB-231, MCF-7, U-87MG, and IM-95 were obtained from ATCC and maintained in DMEM (Life Technologies) supplemented with 10% fetal bovine serum (FBS) (HyClone). All cells were cultured in a humidified, 5% CO₂ environment; EBC-1 was obtained from the JCRB Cell Bank, Japan; IM-95 was supplemented with 10 mg/L insulin (Sigma). NCI-H1573, NCI-H820, NCI-H441, NCI-H1650, SNU-620, and SNU-5 were obtained from ATCC and maintained in RPMI (Life Technologies) supplemented with 10% FBS. The NCI-H1573, EBC-1, Hs 746T, SNU-620 and SNU-5 tumor cell lines were previously shown to harbor MET gene amplification (17). KP4 cells were obtained from RIKEN (Riken BioResource Center) and cultured in DMEM and 10% FBS. Non-transformed cell lines NHBE (CC-3170), HUVEC (CC-3162, EGM-2), HMEC (CC-3150), PrEC (CC-3166) and NHDF (CC-3132) were obtained from LONZA and cultured using manufacturer recommended conditions.

All cell lines were expanded in culture upon receipt and cryopreserved to provide cells at a similar stage passage for all subsequent experiments. All cell lines were authenticated, however for cell lines not authenticated in the 6 months before use, their c-Met expression levels were confirmed by FACS analysis.

Binding ELISA and Fluorescence-Activated Cell Sorting (FACS) Analysis

Binding ELISA was performed as previously described (17). For cellular c-Met binding studies, cells were harvested from flasks when approximately 80% confluent using Cell Dissociation Buffer (Life Sciences). Cells were washed once in PBS/1% FBS (FACS buffer), re-suspended at 1.5–2 x 10⁶ cells/mL in FACS buffer and transferred to a round bottom 96-well plate Corning Life Sciences) at 100 μL/well. Ten μL of a 10x concentration of ABT-700, ABBV-399, or controls was
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added and plates were incubated at 4°C for 2 hours. Wells were washed twice with FACS buffer and re-suspended in 50 μL of 1:500 anti-human IgG Ab (AlexaFluor 488, Invitrogen #11013) diluted in FACS buffer. Plates were incubated at 4°C for 1 hour, washed twice with FACS buffer. Cells were re-suspended in 100 μL of PBS/1% formaldehyde and analyzed on a Becton Dickinson LSRII flow cytometer.

**Determination of Receptor Density**

c-Met cell surface density (antigen binding capacity per cell) was determined by indirect immunofluorescence staining of cell surface antigens on cultured cells using QIFIKIT (Dako). Briefly, cells were harvested from a culture flask as described above for FACS analysis, added to a round bottom 96-well plate at 100 μL/well and incubated at 4°C with 3 μg/mL c-Met antibody m224G11 (the murine parent antibody of ABT-700). Wells treated with an irrelevant mouse monoclonal antibody of the same isotype mlgG1 at 3 μg/mL were included as controls. Following a 1 hour incubation with primary antibody, cells were centrifuged for 3 minutes at 300 x g, washed twice with FACS buffer, and incubated for 1 hour at 4°C with 100 μL of the QIFIT-provided FITC conjugated antibody diluted 1:50 in FACS buffer. Cells were centrifuged for 3 minutes at 300 x g, washed twice with FACS buffer, and fixed with 100 μL/well of 1% formaldehyde in PBS. Indirect immunofluorescence staining of the QIFIKIT beads was carried out according to the manufacturer’s instructions and data was acquired on a Becton Dickinson LSRII flow cytometer. The standard curve was used to assign ABC (Antibody Binding Capacity) or number of receptors for each cell line.

**Immunohistochemistry**

Immunohistochemical staining was performed on 4 μm sections using the CONFIRM anti-c-Met (SP44) rabbit monoclonal primary antibody (Ventana Medical Systems) on the Ventana Benchmark Ultra Autostainer according to the manufacturer’s protocol. Briefly, antigen retrieval was performed by placing unstained slides in Ventana Ultra CC1 buffer (Tris-
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EDTA/EGTA, pH 9) at 64 °C for 95 min followed by incubation of tissues with the primary antibody (SP44) at 36 °C for 16 min. Antigen–antibody reaction was visualized using Ultraview® Universal DAB Detection Kit. Hematoxylin staining for 8 min followed by a bluing reagent (Ventana Medical Systems, Tucson, AZ) for 4 min was used as a counter stain. To ensure antibody specificity, isotype controls were performed as above except primary antibodies were replaced with rabbit IgG. Immunohistochemical staining was evaluated semi-quantitatively for both per cent positivity and intensity and an H-score ranging from 0-300 was derived based on the percentage of cells stained multiplied by the intensity (0-3) of staining.

Cytotoxicity Assay

Cells were plated at 2000-5000 cells/well in 180 µL growth medium containing 10% FBS in 96-well plates, and cultured at 37°C in a humidified incubator with 5% CO₂. The following day, titrations of antibodies or ADCs in 20 µL were added and cells were incubated for 6 days. Cell viability was determined using a CellTiter-Glo Luminescent Cell Viability Assay (Promega) according to the manufacturer’s instructions. A non-binding, irrelevant negative control ADC conjugated to MMAE was also included in all assays to confirm that cell killing was antigen dependent.

In Vivo Studies

Female SCID (SW-48), Male SCID (Hs 746T), and SCID-Beige (NCI-H441) mice were obtained from Charles River (Wilmington, MA) and housed at ten mice per cage. The body weight upon arrival was 20-22 g. Food and water were available ad libitum. Mice were acclimated to the animal facilities for a period of at least one week prior to commencement of experiments. Animals were tested in the light phase of a 12-hr light: 12-hr dark schedule (lights on at 06:00 hours). All experiments were conducted in compliance with AbbVie's Institutional Animal Care and Use Committee and the National Institutes of Health Guide for Care and Use of Laboratory Animals Guidelines in a facility accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care.
To generate xenografts, a suspension of viable tumors cells (SW-48: 5 x 10^6, NCI-H441: 5 x 10^6, and Hs746T: 2 x 10^6) mixed with an equal amount of Matrigel (BD Biosciences) was injected subcutaneously into the flank of 6- to 8-week old mice. The injection volume was 0.1 mL composed of a 1:1 mixture of S-MEM and Matrigel (BD Biosciences). Tumors were size matched at approximately 200-250 mm^3 unless otherwise indicated. Therapy began the day of or 24 h after size matching the tumors. Each experimental group included 8-10 animals. Tumors were measured two to three times weekly. Measurements of the length (L) and width (W) of the tumor were obtained via electronic calipers and the volume was calculated according to the following equation: \( V = L \times W^2/2 \). Mice were euthanized when tumor volume reached a maximum of 3,000 mm^3 or upon presentation of skin ulcerations or other morbidities, whichever occurred first. For the LG0703 and LG1049 patient-derived xenograft (PDX) models (The Jackson Laboratory, Sacramento, CA), tumor fragments of 3 to 5 mm^3 at passage 3 (P3) were implanted subcutaneously in the right rear flank of NOD scid gamma (NSG) mice (The Jackson Laboratory) with a trochar. For all groups, tumor volumes were plotted only for the duration that allowed the full set of animal to remain on study. If animals had to be taken off study, the remaining animals were monitored for tumor growth until they reached defined end-points. Maximal tumor growth inhibition (TGI_{max}), expressed as a percentage, indicates the maximal divergence between the mean tumor volume of the test article-treated group and the control group treated with drug vehicle or isotype-matched non-binding antibody. Tumor growth delay (TGD), expressed as a percentage, is the difference of the median time of the test article treated group tumors to reach 1 cm^3 as compared to the control group. Complete responses (CR) were defined by tumor volume \( \leq 25 \) mm^3 for at least 3 consecutive measurements. Standard of care agents 5-fluorouracil (50 mg/kg), and irinotecan (30 mg/kg) were administered intravenously and leucovorin (25 mg/kg) was administered orally on Q7Dx5 regimen (FOLFIRI). IgG control, control Ig MMAE, ABBV-399, and ABT-700 were administered intraperitoneally as indicated. The carrier moiety of the control ADC represents an isotype-
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matched human IgG recognizing tetanus toxoid antigen that is displayed neither by the xenograft nor by murine tissue antigens.

**Statistical analysis**

IC$_{50}$ and EC$_{50}$ values were determined by nonlinear regression analysis of concentration response curves using GraphPad Prism 6.0 (GraphPad Software, La Jolla, CA). Data from experiments *in vivo* were analyzed using the two-way ANOVA with post-hoc Bonferroni correction for TGI$_{\text{max}}$, and the Mantel-Cox log-rank test for TGD (GraphPad Prism).

**Results**

**Binding properties of ABBV-399 for c-Met**

ABT-399 was generated by the conjugation of MMAE to the interchain cysteines of ABT-700 via a valine/citruline linker with an average drug-antibody ratio of ~3.1 (25). Upon binding to its target antigen on the surface of tumor cells, ABBV-399 is internalized by the cell (Supplementary Fig. S1). To confirm that the binding characteristics of the parental ABT-700 were not altered by conjugation to MMAE, both ELISA and FACS-based assays were performed. As determined by an ELISA binding assay to the recombinant c-Met ECD, ABBV-399 has an apparent EC$_{50}$ of 0.30 nM, comparable to that of the parent molecule, ABT-700 (Table 1). The binding affinities of ABBV-399 to surface c-Met on a panel of human cancer cells are similar to those of ABT-700 (0.2 to 1.5 nM) by fluorescence-assisted cell sorting (FACS) (Tables 1). These results indicate that conjugation of ABT-700 to vcMMAE does not alter the binding properties of the parental antibody.

**In vitro potency of ABBV-399 against tumor cell lines and correlation with c-Met expression**

To determine if there is a correlation between c-Met expression level and sensitivity to ABBV-399, a panel of additional tumor and normal cell lines were assessed for c-Met expression and...
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response to ABBV-399. These included NSCLC, gastro-esophageal, CRC, breast, pancreatic, and glioblastoma cancer lines. FACS analysis demonstrated that these cell lines possess a range of c-Met expression levels as quantified via c-Met antibody binding capacity representing the number of cell surface c-Met molecules (Table 2). Sensitivity to ABBV-399 in the cell proliferation assay was quantified as maximal killing and IC50 (Table 2). The results indicated that an approximate threshold of c-Met cell surface molecules >100,000 was required for sensitivity to ABBV-399-mediated killing. Exceptions to this were the tumor cell lines known to have an autocrine HGF loop including IM-95, KP4, and U-87 MG, in which lower c-Met expression levels were sufficient for ABBV-399 to exert significant cytotoxicity (17). The differential response of some cell lines to ABBV-399 may also reflect the sensitivity of different tumor types to the auristatin payload.

ABBV-399 inhibited the proliferation of cancer cells that overexpress c-Met, including the MET-amplified cell lines SNU-620, SNU-5 and Hs 746T gastric cancer cells and the c-Met overexpressed NCI-H441, EBC-1 and NCI-H820 NSCLC cell lines (Table 2). As a comparison, ABT-700 inhibited proliferation of cells with MET amplification but not cell lines without MET amplification, i.e., the NCI-H820 and NCI-H441 (data not shown and 17). It is possible that ABBV-399 mediated c-Met signaling inhibition may contribute to its mechanism of action as both ABT-700 and ABBV-399 can inhibit phospho-and total c-Met and downstream signaling molecules (Supplementary Fig. S2). However, since ABBV-399 is significantly more potent than ABT-700 at lower doses and many tumor cell lines are sensitive to ABBV-399, but not to ABT-700-mediated killing, it is unlikely that signaling inhibition is a major component of ABBV-399 anti-tumor activity. A panel of five normal cell lines including epithelial, endothelial and fibroblast derived cell lines had lower levels of c-Met expression compared to the sensitive tumor cell lines and these normal cells lines were largely insensitive to ABBV-399 mediated killing (Table 2). These data suggest that the levels of c-Met expression on normal cell lines
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may fall below the threshold level of c-Met expression required for significant killing by ABBV-399.

**ABV-399 in vivo efficacy in MET amplified and c-Met overexpressed tumor models**

Inhibition of tumor growth by ABBV-399 was evaluated in multiple human xenograft models derived from a variety of tumor types (Table 3). Efficacy of the ADC was quantified by assessing maximal tumor growth inhibition and delayed outgrowth of tumors (tumor growth delay) following therapy in mice treated with ABBV-399 when compared to treatment with a vehicle control. Selectivity of this response was determined by comparing efficacy of a control ADC with ABBV-399.

ABBV-399 activity was compared to the parental ABT-700 antibody in the Hs 746T gastric xenograft model with amplified MET. One or 3 mg/kg of ABBV-399 administered once every four days for a total of six doses (Q4D×6) induced complete and durable tumor regression (Figure 1A, Table 3). At the 3 mg/kg dose, 100% CRs were achieved, whereas treatment with 1 mg/kg yielded 40% CRs. ABBV-399 at 3 mg/kg was more effective than ABT-700 dosed at 10 mg/kg (Figure 1A).

ABBV-399 also inhibited growth of NCI-H441 xenografts, a papillary lung adenocarcinoma with c-Met overexpression not caused by gene amplification (Figure 1B, Table 3). NCI-H441 xenografts were eliminated (100% CR) after treatment with 1 mg/kg (Q4D×6) or higher of ABBV-399. At the same dose, ABT-700 did not significantly inhibit tumor growth. At an equivalent dose and regimen, the control IgG ADC also demonstrated no significant activity. Activity of ABBV-399 in all human xenograft models tested is summarized in Table 3 together with immunohistochemistry (IHC) results. These results show that ABBV-399 is most effective against models with high levels of c-Met expression.
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**ABBV-399 combination with chemotherapy**

Since clinical application of targeted therapeutics often leverage combination approaches to enhance efficacy, ABBV-399 in combination with other chemotherapies was evaluated. For these studies, SW-48 xenograft tumors derived from colorectal carcinoma were used since it had lower levels of c-Met and showed only a modest response to ABBV-399 monotherapy, providing the potential to observe combination effects (Table 2-3). FOLFIRI (5-fluorouracil (5-FU), leucovorin, and irinotecan) is the standard second line treatment for metastatic colorectal cancer (33). As shown in Figure 1C, ABBV-399 combined with FOLFIRI had improved potency compared to either ABBV-399 or FOLFIRI alone.

**Efficacy of ABBV-399 on tumors refractory to ABT-700 therapy**

Efficacy of ABBV-399 was evaluated in a gastric carcinoma model (Hs 746T) that was made refractory to ABT-700 by repeated exposure to the antibody *in vivo* (Hs 746T ABT-700R). Initially, treatment of the parental Hs 746T xenografts with ABT-700 resulted in tumor stasis followed by relapse (Figure 2A; blue line). Treatment of these relapsed tumors with ABBV-399 led to regression (Figure 2A, red line). In contrast, Hs 746T ABT-700R xenografts were refractory to ABT-700 treatment with quick tumor outgrowth on therapy (Figure 2B; blue line). When these refractory tumors reached a mean cohort size of approximately 1,000 mm³, treatment with ABBV-399 resulted in tumor regression (Figure 2B; red line) followed by eventual outgrowth. Treatment of Hs 746T ABT-700R of approximately 200 mm³ with ABBV-399 resulted in complete tumor regression (Figure 2B). Similar results were observed subsequent to treatment of the ABT-700-resistant NSCLC cell line EBC-1 ABT-700R with ABT-700 followed by ABBV-399 (Figure 2C). These results demonstrate that tumors that are no longer sensitive to c-Met pathway inhibition remain sensitive to targeted delivery of a cytotoxin with the activity of ABBV-399 independent of response to ABT-700.
Efficacy of ABBV-399 in primary patient-derived xenograft (PDX) tumors

The anti-tumor activity of ABBV-399 was also evaluated in primary PDX tumors from NSCLC, hepatocellular and ovarian carcinoma. PDX models consist of tumor fragments implanted directly from patients into immune-compromised mice and may represent a more complex model with higher heterogeneity and a closer approximation to human tumors. Because of the variable growth rate of implanted PDX tumors, these studies were performed with an accrual design with data presented as a Kaplan-Meier plot. ABBV-399 was efficacious in both lung adenocarcinoma models (LG703, LG1049) which express moderate to high levels of c-Met expression by immunohistochemistry (IHC) (Figure 3A, Table 3). In both models the control IgG-MMAE was also active, but generally at higher doses, likely resulting from the enhanced permeability and retention effect from a combination of MMAE sensitivity and antibody accumulation in the tumor rather than the recognition of a tumor-associated antigen (34,35). The LG1049 tumor model with the higher c-Met expression (H-score of 170 versus H-score of 85 for LG0703) responded better to ABBV-399 treatment (Figure 3C-D, Table 3). In the hepatocellular (LI0752) and ovarian (OV250) cancer models, both showing lower c-Met expression than the LG1049 lung model, there was a minor but statistically significant delay in tumor growth and inhibition in response to ABBV-399 treatment (Table 3).

Discussion

Development of therapeutic strategies that target c-Met activity have met with limited success. While the reasons for this are undoubtedly complex, emerging results suggest that both small molecule and antibody c-Met inhibitors are likely to be effective primarily in those tumors that are MET-activated and driven predominantly by c-Met signaling (14,15). One such example is the c-Met targeting antibody ABT-700 which demonstrated anti-tumor activity only in patients with MET-amplified tumors (16,17). The low frequency of MET amplification may limit the population responsive to this class of inhibitors. Beyond gene amplification, difficulty in
identifying MET-activated tumors presents challenging biomarker strategies for patient selection. We describe here properties of ABBV-399 that indicate it is dependent on c-Met target expression for activity. The mechanism of action for ABBV-399 is distinct from previous c-Met inhibitors and suggests that ABBV-399 can overcome the limitations associated with inhibitors that are only active in MET dependent tumors. ABBV-399 induces complete regressions of xenografts derived from c-Met overexpressing or MET-amplified tumor cells. ABBV-399 is also effective in tumor models that are refractory to ABT-700. Extensive preclinical mouse and cynomolgus monkey pharmacokinetic studies indicate that the stability and serum clearance of ABBV-399 is comparable to the unconjugated antibody and support a once every three week dosing regimen in humans similar to that of other MMAE ADCs (26). These properties provide a sound rationale for the development of ABBV-399 as a therapeutic with the potential to be active beyond the small subgroup of patients where tumor growth is driven by c-Met signaling. In fact, in a Phase 1 open label study in patients with advanced solid tumors, ABBV-399 monotherapy has demonstrated durable tumor shrinkage in patients that were selected based on c-Met overexpression as determined by IHC (32). The utility of IHC as a relevant companion diagnostic to identify patients most likely to respond to ABBV-399 therapy is based on the preclinical results demonstrating a strong correlation between ABBV-399 antitumor efficacy and c-met expression levels.

A c-Met targeting ADC presents the risk of on-target toxicity based on expression of c-Met on normal tissues including epithelial cells and hepatocytes (27) so the choice of targeting c-Met with an ADC was counterintuitive. The anti-tumor efficacy of ABBV-399, both in vitro and in vivo, correlated well with c-Met expression levels with tumor growth inhibition observed in both tumor cell line and primary patient derived xenografts with high c-Met expression but much less so in individual xenograft models with low c-Met expression. Consistent with the correlation of c-Met expression and sensitivity to ABBV-399, minimal inhibitory effects were observed on several c-Met-expressing normal endothelial, epithelial and fibroblast cells.
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Primary toxicities observed following repeated dosing of ABBV-399 in cynomolgus monkeys were either non-adverse or reversible, consistent with those observed with other MMAE conjugates and supportive of initiation of investigational trials with this compound in humans. It is also possible that the unique properties of the parental antibody, ABT-700 targeting the immunoglobulin-like domain of the c-Met receptor may contribute to its properties as the tumor targeting component of an ADC (36).

In a Phase 1 clinical trial in patients with advanced solid tumors, the unconjugated ABT-700 was well tolerated at the recommended dose of 15 mg/kg (16). These clinical results together with the preclinical attributes of ABBV-399 suggest that a therapeutic window may be attainable with ABBV-399 despite the normal tissue expression profile of c-Met. This premise is supported by results of the ABBV-399 Phase 1 expansion cohort indicating that ABBV-399 is well tolerated at a dose of 2.7 mg/kg (32). As a point of reference, the recommended clinical dose for Adcetris (brentuximab vedotin), an FDA approved antibody drug conjugated with MMAE, is 1.8 mg/kg (30 min IV, q3w) (26).

The tolerability of ABBV-399 monotherapy in clinical studies coupled with preclinical results showing improved potency in combination with chemotherapy also suggests the potential for ABBV-399 combination therapy. Combination treatments may enhance efficacy and forestall or prevent the emergence of drug resistance. The precedence for ADC combination with chemotherapy is increasingly being established as a viable treatment option (37). Strategies combining ABBV-399 with immunotherapy agents that activate the immune system could also represent a promising treatment option. There exists a strong rationale for this combination since microtubule inhibitor ADCs based ADCs have been shown to induce dendritic cell homing to tumor draining lymph nodes and augment host immunity in preclinical models (38).

In summary, ABBV-399 is a novel c-Met targeted therapy that may overcome limitations that have adversely influenced clinical development of other c-Met inhibitors. Continued assessment of ABBV-399, both as monotherapy and in combination across a broad range of c-Met expressing tumors is warranted.
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References


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Table 1. Binding affinity of ABBV-399 to recombinant and cellular c-Met

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<thead>
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<th>ABBV-399 (EC_{50} nmol/L)</th>
<th>ABT-700 (EC_{50} nmol/L)</th>
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<tr>
<td>c-Met ECD(^a) by ELISA(^b)</td>
<td>0.30</td>
<td>0.22</td>
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<tr>
<td>Cellular c-Met by FACS(^c)</td>
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<tr>
<td>Hs 746T</td>
<td>0.4 +/- 0.1</td>
<td>0.4 +/- 0.1</td>
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<tr>
<td>SNU-5</td>
<td>1.4 +/- 0.4</td>
<td>1.6 +/- 1.1</td>
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<td>IM-95</td>
<td>1.5 +/- 0.9</td>
<td>1.8 +/- 0.4</td>
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<td>0.2 +/- 0.1</td>
<td>0.3 +/- 0.2</td>
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<tr>
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<td>1.0 +/- 0.6</td>
<td>1.1 +/- 1.1</td>
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<td>0.4 +/- 0.2</td>
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</table>

\(^a\) Extracellular domain (residues 25-932 of c-Met)

\(^b\) EC\(_{50}\) values derived from ELISA in which c-Met ECD was captured on the plate via a His tag. Values are the average of six experiments, +/- SD.

\(^c\) EC\(_{50}\) values derived from FACS analysis of ABBV-399 on cancer cell lines. Values are the average of at least two experiments, +/- SD.
Table 2. c-Met expression on tumor cells in vitro and sensitivity to ABBV-399

<table>
<thead>
<tr>
<th>Cell Line</th>
<th>c-Met Expression</th>
<th>Maximal Killing</th>
<th>ABBV-399 IC\textsubscript{50} +/- SD</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Lung Cancer</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NCI-H1650</td>
<td>4,500</td>
<td>13%</td>
<td>47.9 +/- 8.5</td>
</tr>
<tr>
<td>A549</td>
<td>43,000</td>
<td>22%</td>
<td>1.6 +/- 1.1</td>
</tr>
<tr>
<td>NCI-H1573\textd</td>
<td>116,000</td>
<td>18%</td>
<td>18 +/- 14</td>
</tr>
<tr>
<td>NCI-H441\textd</td>
<td>197,000</td>
<td>56%</td>
<td>0.06 +/- 0.05</td>
</tr>
<tr>
<td>EBC-1\textd</td>
<td>233,000</td>
<td>96%</td>
<td>0.06 +/- 0.03</td>
</tr>
<tr>
<td>NCI-H820\textd</td>
<td>320,000</td>
<td>87%</td>
<td>0.20 +/- 0.07</td>
</tr>
<tr>
<td><strong>Gastric Cancer</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IM-95</td>
<td>22,000</td>
<td>53%</td>
<td>1.7 +/- 0.9</td>
</tr>
<tr>
<td>SNU-620\textd</td>
<td>230,000</td>
<td>80%</td>
<td>0.17 +/- 0.08</td>
</tr>
<tr>
<td>SNU-5\textd</td>
<td>291,000</td>
<td>85%</td>
<td>0.28 +/- 0.07</td>
</tr>
<tr>
<td>Hs 746T\textd</td>
<td>350,000</td>
<td>87%</td>
<td>0.11 +/- 0.06</td>
</tr>
<tr>
<td><strong>Colorectal Cancer</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SW48</td>
<td>26,000</td>
<td>0%</td>
<td>NA\textsuperscript{e}</td>
</tr>
<tr>
<td>HT-29</td>
<td>161,000</td>
<td>70%</td>
<td>9.0 +/- 1.4</td>
</tr>
<tr>
<td><strong>Breast Cancer</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MCF-7</td>
<td>8,000</td>
<td>0%</td>
<td>NA</td>
</tr>
<tr>
<td>MDA-MB-231</td>
<td>30,000</td>
<td>0%</td>
<td>NA</td>
</tr>
<tr>
<td><strong>Pancreatic Cancer</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>KP4</td>
<td>15,000</td>
<td>53%</td>
<td>2.9 +/- 1.9</td>
</tr>
<tr>
<td><strong>Glioblastoma</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>U-87MG</td>
<td>22,000</td>
<td>30%</td>
<td>1.9 +/- 0.1</td>
</tr>
<tr>
<td><strong>Non-tumor Cell Lines</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NHBE (bronchial epithelial)</td>
<td>40,000</td>
<td>10%</td>
<td>NA</td>
</tr>
<tr>
<td>HUVEC (vascular endothelial)</td>
<td>16,000</td>
<td>6%</td>
<td>NA</td>
</tr>
<tr>
<td>HMEC (mammary epithelial)</td>
<td>ND</td>
<td>0%</td>
<td>NA</td>
</tr>
<tr>
<td>PrEC (prostate epithelial)</td>
<td>65,000</td>
<td>0%</td>
<td>NA</td>
</tr>
<tr>
<td>NHDF (dermal fibroblasts)</td>
<td>1,600</td>
<td>0%</td>
<td>NA</td>
</tr>
</tbody>
</table>
A Tumor-Targeting c-Met ADC

Approximate number of c-Met molecules on cell surface determined by FACS analysis as antibody binding capacity for m224G11 (the murine parent of ABT-700) binding at 10 μg/mL.

Relative to untreated control at < 1 μg/mL in a six day proliferation

IC₅₀ values (nmol/L) for anti-proliferative activity of ABBV-399 or ABT-700 in 6-day proliferation assay. Values are average of ≥2 experiments, +/- the standard deviation.

MET amplified cell lines (17)

Not available
A Tumor-Targeting c-Met ADC

Table 3. Activity of ABBV-399 and c-Met Expression in Xenograft Models

<table>
<thead>
<tr>
<th>Xenograft</th>
<th>Membrane H-Score&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Dose (mg/kg)</th>
<th>TGI&lt;sub&gt;max&lt;/sub&gt; (%)&lt;sup&gt;d&lt;/sup&gt;</th>
<th>TGD (%)&lt;sup&gt;e&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hs 746T</td>
<td>300</td>
<td>3</td>
<td>95</td>
<td>&gt;629</td>
</tr>
<tr>
<td>NCI-H441</td>
<td>280</td>
<td>3</td>
<td>96</td>
<td>&gt;250</td>
</tr>
<tr>
<td>EBC-1</td>
<td>280</td>
<td>3</td>
<td>100</td>
<td>&gt;329</td>
</tr>
<tr>
<td>SW 48</td>
<td>40</td>
<td>3</td>
<td>65</td>
<td>162</td>
</tr>
<tr>
<td>LG1049&lt;sup&gt;b&lt;/sup&gt;</td>
<td>170</td>
<td>3</td>
<td>NA&lt;sup&gt;c&lt;/sup&gt;</td>
<td>&gt;110</td>
</tr>
<tr>
<td>LG0703&lt;sup&gt;b&lt;/sup&gt;</td>
<td>85</td>
<td>3</td>
<td>NA&lt;sup&gt;c&lt;/sup&gt;</td>
<td>&gt;53</td>
</tr>
<tr>
<td>LI0752&lt;sup&gt;b&lt;/sup&gt;</td>
<td>130</td>
<td>6</td>
<td>57</td>
<td>129</td>
</tr>
<tr>
<td>OV250&lt;sup&gt;b&lt;/sup&gt;</td>
<td>125</td>
<td>3</td>
<td>29</td>
<td>17</td>
</tr>
</tbody>
</table>

<sup>a</sup> H-score is a reflection of staining intensity in conjunction with percentage of cells staining positively.

<sup>b</sup>PDX models

<sup>c</sup> Not available; Cannot be calculated due to an accrual trial

<sup>d</sup> Maximal tumor growth inhibition (TGI<sub>max</sub>), expressed as a percentage, indicates the maximal divergence between the mean tumor volume of the test article-treated group and the control group treated with drug vehicle or isotype-matched non-binding antibody.

<sup>e</sup> Tumor growth delay (TGD), expressed as a percentage, is the difference of the median time of the test article treated group tumors to reach 1 cm<sup>3</sup> as compared to the control group. Complete responses (CR) were defined by tumor volume ≤ 25 mm<sup>3</sup> for at least 3 consecutive measurements.
A Tumor-Targeting c-Met ADC

**Figure Legends**

**Figure 1. ABBV-399 efficacy as monotherapy and in combination against human tumor xenograft models.** The *in vivo* efficacy of ABBV-399 was evaluated in mice transplanted with (A) Hs 746T and (B) NCI-H441 cells. For Hs 746T, ABT-700 was administered every 7 days while ABBV-399 was administered every 4 days. For NCI-H441 xenografts, both ABT-700 and ABBV-399 were administered every 4 days for a total of six doses. (C) Combination efficacy of ABBV-399 and FOLFIRI was determined using SW-48 xenografts. IgG MMAE was administered as a non-targeting control agent for ABBV-399. All agents were administered every 7 days. Numbers in parentheses represent dose administered in mg/kg and arrows indicate days of administration. Administration and regimen of agents in FOLFIRI are indicated in “Materials and Methods”. Tumor volumes are shown as mean ± S.E.M.

**Figure 2. ABBV-399 efficacy against human tumor xenograft models refractory to ABT-700.**

ABBV-399 efficacy was evaluated in mice transplanted with (A) parental Hs 746T following relapse upon treatment with ABT-700, (B) Hs 746T ABT-700R as monotherapy alone or following treatment with ABT-700 and (C) EBC-1 ABT-700R following treatment with ABT-700. Numbers in parentheses represent dose administered in mg/kg and arrows indicate days of administration. Tumor volumes are shown as mean ± S.E.M.

**Figure 3. ABBV-399 is active in patient derived xenograft models**

Efficacy of ABBV-399 was determined in xenografts derived from NSCLC patients. Efficacy is depicted on a Kaplan-Meier plot for (A) LG0703 and (B) LG1049 models as fractions reaching the indicated tumor volumes following therapy. (C) and (D) represent Immunohistochemical staining for c-Met for LG0703 and LG1049 models, respectively. In both models ABBV-399 and control agents were administered every 4 days for a total of six doses. In the LG1049 model, ABT-700 was administered every 7 days for a total of six doses. Numbers in parentheses represent dose administered in mg/kg.
Figure 1. ABBV-399 efficacy, alone and in combination, against human tumor xenograft models
Figure 2. ABBV-399 efficacy against human tumor xenograft models refractory to ABT-700
Figure 3. ABBV-399 is active on patient derived xenograft models
ABBV-399, a c-Met Antibody Drug Conjugate that Targets Both MET Amplified and c-Met Overexpressing Tumors, Irrespective of MET Pathway Dependence

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