A Novel Bispecific Antibody Targeting EGFR and cMet Is Effective against EGFR Inhibitor-Resistant Lung Tumors


Abstract

Non–small cell lung cancers (NSCLC) with activating EGFR mutations become resistant to tyrosine kinase inhibitors (TKI), often through second-site mutations in EGFR (T790M) and/or activation of the cMet pathway. We engineered a bispecific EGFR-cMet antibody (JNJ-61186372) with multiple mechanisms of action to inhibit primary/secondary EGFR mutations and the cMet pathway. JNJ-61186372 blocked ligand-induced phosphorylation of EGFR and cMet and inhibited phosphorylation of EGFR and phospho-AKT more potently than the combination of single receptor–binding antibodies. In NSCLC tumor models driven by EGFR and/or cMet, JNJ-61186372 treatment resulted in tumor regression through inhibition of signaling/receptor downmodulation and Fc-driven effector interactions. Complete and durable regression of human xenograft tumors was observed with the combination of JNJ-61186372 and a third-generation EGFR TKI. Interestingly, treatment of cynomolgus monkeys with JNJ-61186372 resulted in no major toxicities, including absence of skin rash observed with other EGFR-directed agents. These results highlight the differentiated potential of JNJ-61186372 to inhibit the spectrum of mutations driving EGFR TKI resistance in NSCLC.

Introduction

Non–small cell lung cancer (NSCLC) is frequently driven by activating mutations in the kinase domain of EGFR, occurring most commonly as in-frame deletions in exon 19 and L858R exon 21 mutations. Most patients initially respond to first-generation EGFR tyrosine kinase inhibitors (TKI), such as gefitinib and erlotinib, but the clinical benefits are not durable. Drug resistance limits the response to a mean duration of <1 year (1, 2). In addition to the T790M secondary mutation in EGFR (~50% of resistant cases; refs. 1, 3–6) that reduces potency of reversible TKIs (7), resistant tumors may also develop activation of the cMet pathway, through MET gene amplification, increased cMet expression, and/or increased expression of the cMet ligand, hepatocyte growth factor (HGF; refs. 5, 6, 8, 9). Stimulation of the cMet pathway provides an alternate mechanism to bypass the TKI block of EGFR and facilitate the survival of cancer cells. These two mechanisms can also occur simultaneously in EGFR TKI–resistant NSCLC patients (3, 6, 10, 11).

Because of the signaling cross-talk between EGFR and cMet, inhibition of both receptors in combination may limit compensatory pathway activation and improve overall efficacy. A novel bispecific antibody platform was used to produce JNJ-61186372, an antibody that binds EGFR with one Fab arm and cMet with the other Fab arm (12, 13). We have optimized JNJ-61186372 to engage multiple mechanisms of action. First, we demonstrated dual inhibition of both EGFR and cMet signaling by blocking ligand-induced activation and by inducing receptor degradation. In addition, high levels of EGFR and cMet on the surface of tumor cells allow for targeting of these cells for destruction by immune effector cells through Fc-dependent effector mechanisms, such as antibody-dependent cellular cytotoxicity (ADCC). JNJ-61186372 is produced by an engineered Chinese hamster ovary (CHO) cell line defective for protein fucosylation. The human FcRIIIA, critical for ADCC, binds antibodies with low-level core fucosylation more tightly and consequently mediates more potent and effective ADCC killing of cancer cells (14). Thus, the low-level core fucosylation in the JNJ-61186372 molecule translates to an enhanced level of ADCC activity compared with the same fully fucosylated molecule.

JNJ-61186372 was demonstrated to employ multiple mechanisms to inhibit tumors with primary EGFR-activating mutations, tumors with the T790M second-site resistance mutation in EGFR, and tumors with activation of the cMet pathway. Furthermore, the combination of JNJ-61186372 and a third-generation EGFR TKI (AZD9291) resulted in complete and durable regression of tumors. Interestingly, treatment of cynomolgus monkeys with...
INJ-61186372 resulted in no toxicities, including absence of skin rash observed with other EGFR-directed agents. This profile of preclinical data supports the development of INJ-61186372 in patients with lung cancer and other malignancies associated with aberrant EGFR and cMet signaling.

Materials and Methods

Preparation of parental mAbs

The EGFR arm of INJ-61186372 is derived from zalutumumab that has a conformational epitope on EGFR domain III, which overlaps with the EGF ligand–binding site (15) but is different from the epitopes of cetuximab (16) and panitumumab (17). The cMet-binding arm of INJ-61186372 has an epitope that blocks HGF ligand binding but is distinct from the onartuzumab epitope (18). Parental mAbs used to generate INJ-61186372 were prepared from CHO cell lines that generate mAbs with low levels of core fucosylation. Antibody expression and purification is described in detail elsewhere (19).

Controlled Fab-arm exchange to generate bispecific Abs

Bispecific human IgG1 Abs were produced from the two purified bivalent parental antibodies, each with the respective single complementary mutation: K409R or F405L (12). Controlled Fab-arm exchange (cFAE) was performed as described previously (12), with a 5% excess of anti-EGFR-F405L or anti-cMet-F405L, and monovalent EGFR and cMet Abs. All bispecific antibodies were purified using hydrophobic interaction chromatography. The EGFR monovalent antibody consists of an EGFR-binding arm and a nonbinding arm (either anti-HIV-gpl20 or anti-RSV-F, which did not bind the cell lines used); the cMet monovalent antibody consists of a cMet-binding arm and similar aforementioned nonbinding arm.

Competitive ligand binding

EGFR-ECD-Fc (R&D Systems; 200 ng/well) was coated on High Bind Plate [Meso Scale Discovery (MSD)] for 2 hours (all steps performed at room temperature). MSD Blocker A buffer (5%, 150 μL/well) was added and incubated for 2 hours. Plates were washed 3 times with 0.1 mol/L HEPES buffer, pH 7.4, then the mixture of the MSD fluorescence dye–labeled Hu-EGF with different competitors was added. Labeled EGF (50 nmol/L) was incubated with antibody (1 nmol/L–4 μmol/L), then added to wells (25 μL mixture). After 2 hours, plates were washed as above. MSD Read Buffer T was diluted with distilled water (4-fold), dispensed (150 μL/well), and analyzed with a SECTOR Imager 6000.

Polystable mAb050 (R&D Systems; 50 ng/mL) was coated on MSD High Bind Plate for 2 hours. Blocking and washing steps were as above, followed by capturing 30 μL (10 μg/mL) of cMet-ECD-Fc (R&D Systems). After 1 hour, plates were washed as above. After the mixture of biotinylated HGF with different antibodies was added to plates, labeled HGF (10 nmol/L) was incubated with antibodies (0.001–2 μmol/L), then added to wells (25 μL mixture). After 1-hour incubation, plates were washed and then MSD fluorescence dye–labeled streptavidin, 25 μL (10 nmol/L), was added to each well and incubated for 1 hour. Plates were washed, read, and analyzed as above.

Cell culture and HGF-engineered cell line

For these studies, 7 NSCLC tumor cell lines (NCI-H292, HCC827, NCI-H1975, NCI-H3255, HCC4006, NCI-H820, and NCI-H1993) were selected to reflect patient diversity in the mutational status of EGFR and gene copy number of MET. The tumor cell lines were obtained (2011–2013) from the NCI (Bethesda, MD; H3255) or ATCC (all others). Cell lines were authenticated using short tandem repeat assay and either used immediately or banked, with all experiments occurring within 2 months of resuscitation. The mutations in each cell line were confirmed using a custom somatic mutation array (SA Biosciences). Cells were cultured in 150-cm² tissue culture flasks under standard culture conditions (37°C, 5% CO₂, 95% humidity) and grown in RPMI1640 medium + GlutaMAX + 25 mmol/L HEPES (Life Technologies), 10% heat-inactivated FBS (Life Technologies), 0.1 mmol/L Non-Essential Amino Acids (Life Technologies), and 1 mmol/L sodium pyruvate (Life Technologies). Subconfluent cell monolayers were passaged after treatment with 0.25%-w/v trypsin (Life Technologies).

The H1975-HGF cell line was created by transducing H1975 cells with HGF lentivirus (generated using Human HGF Plasmid and Packaging Kit; GeneCopoeia) to express the human HGF gene. H1975 cells were plated at one million cells in a 100 mm dish in RPMI media. After infection, cells were selected using 2 μg/mL puromycin (Invitrogen); 20 pg/mL HGF (MSD assay) was secreted (5,000 cells/well) over 72-hour incubation.

Receptor phosphorylation assays

Receptor phosphorylation assays were performed as described, with cells plated at 6500 to 10,000 cells per well (19). The signals were not corrected for total levels of receptor. Data were plotted as relative enhanced chemiluminescence (ECL; RCL) signal versus the logarithm of antibody concentration. IC₅₀ values were calculated in GraphPad Prism 5 (GraphPad Software, Inc.) using a four-parameter logistic (4PL) model.

ERK and AKT phosphorylation assays

Cells were grown in RPMI growth medium supplemented with 7.5 ng/mL HGF, then lysed after 30-minute (pERK assay) or 1-hour (pAkt assay) treatment with the antibodies. Phospho-ERK (Thr202/Tyr204; Thr185/Tyr187) levels and phospho-AKT (S473) levels were measured using MSD assays. The signals were not corrected for total levels of ERK or Akt. RCL signal was recorded and data plotted as with receptor phosphorylation assays, with statistical analysis using GraphPad Prism 6. Extra sum-of-squares F test was used to compare treatments.

Preparation/analysis of tumor lysates

Mice bearing established H1975-HGF tumors were treated with a single dose of INJ-61186372 (1, 5, or 20 mg/kg) or vehicle control (PBS). 72 hours postdose, tumors were harvested, flash frozen in liquid nitrogen, and lysed in ice-cold RIPA buffer (Thermo Scientific) containing 2x HALT/EDETA protease and phosphatase inhibitor cocktail (Thermo Fisher Scientific), 50 mmol/L NaF, 2 mmol/L sodium orthovanadate (activated), and 1 mmol/L PMSF using a FastPrep-24 homogenizer (MP Biomedicals). Lysates were cleared by centrifugation, and protein concentrations were determined by BCA Protein Assay (Pierce). Protein samples (50 μg) were resolved by SDS-PAGE and transferred to nitrocellulose membranes. Membranes were blocked in Odyssey blocking buffer (LI-COR) for 1 hour at room temperature and incubated with the appropriate primary antibodies overnight at 4°C–anti-EGFR (EGF-R2; Santa Cruz Biotechnology), anti-Met
(L141G3; Cell Signaling Technology), anti-actin (Santa Cruz Biotechnology), and anti-GAPDH (Cell Signaling Technology). Bands were detected with anti-mouse IRDye680 (LI-COR) or anti-rabbit IRDye800 (LI-COR) and imaged using an Odyssey Infrared Imaging System (LI-COR). Average total protein quantitated from Western blots, relative to loading control (GAPDH or actin), was graphed and statistical analysis performed using GraphPad Prism 6. Total cMet data were analyzed using a two-sided, unpaired, equal variance *t* test, and total EGFR data were analyzed using a two-sided, unpaired *t* test with Welch Correction for unequal variance. Tumor lysates were examined using MSD assays for phospho-proteins (p-EGFR, p-cMet, p-ERK, or p-AKT), as described above. Ordinary one-way ANOVA and two-sided, unpaired Dunnett multiple comparisons tests on log-transformed MSD signal values were used to compare treatment groups using GraphPad Prism 6. Multiplicity-adjusted *P* values were reported.

**ADCC assays**

Human peripheral blood mononuclear cells (PBMC) were isolated from normal donor leukopaks (Biological Specialty Corporation) for use as effector cells and were cryopreserved. The PBMCs from donors with FcγRIIIa 158V/F (20) were used in ADCC assays (21, 22).

**In vivo tumor xenograft models**

All of the procedures relating to animal care, handling, and treatment were performed according to the guidelines approved by Institutional Animal Care and Use Committee or under the Animals in Scientific Procedures Act 1986 (Precos). For the H1975 and H1975-HGF models, 5×10⁶ cells were injected subcutaneously into the flanks (0.2 mL/mouse) of female nude mice (CD-1 NU/NU, 4–6 weeks old; Charles River Laboratories). Studies with HCC827 and HCC827-ER1 were performed at Precos. Nude mice (MF1 NU/NU, 5–8 weeks old; Harlan, Teklad) were injected subcutaneously with 1×10⁶ cells (0.2 mL/mouse). The L11235 and L111868 patient-derived xenograft (PDX) models were performed by Crown Biosciences. PDX tumor fragments, harvested from donor mice, were inoculated into female nude mice (BALB/c, 8–10 weeks old; Beijing HFK Bio-Technology Co). Animals were treated with compounds when tumors reached a mean tumor size of 120 to 170 mm³. Percentage tumor growth inhibition (%TGI) was defined as the difference between the control-treated group mean tumor volume (MTV) and the test compound–treated group MTV, expressed as a percentage of the MTV of the control-treated group. The statistical analysis of one H1975-HGF study (Fig. 4) was performed comparing treatment groups with control by two-way ANOVA and Bonferroni multiple comparisons tests (GraphPad Prism). The statistical analysis of HCC827, HCC827-ER1, LU1235, LU1868, H1975, and H1975-HGF (Fig. 7) studies was performed on the log-transformed tumor volume data and a Tukey–Kramer *P* value adjustment for multiplicity (SAS).

**Results**

**Bispecific antibody binding to EGFR and cMet prevents ligand binding**

Parental monospecific bivalent antibodies (F405L for anti-EGFR; K409R for anti-cMet) were expressed separately in cell lines incorporating low levels (<9%) of fucose to enhance ADCC. The bispecific EGFR-cMet Ab, prepared using cFAE with >95% yield (Fig. 1A; refs. 12, 13), was designated JNJ-61186372. After purification with hydrophobic interaction chromatography,
JNJ-61186372 was confirmed to be >99% bispecific Ab with <0.2% parental mAbs.

JNJ-61186372 binding to the extracellular domains (ECD) of EGFR and cMet was confirmed using surface plasmon resonance methods (19). JNJ-61186372 has an affinity ($K_D$) of 1.4 nmol/L for EGFR-ECD and a $K_D = 40$ pmol/L for cMet-ECD. Using similar methods, JNJ-61186372 was shown to bind both EGFR-ECD and cMet-ECD simultaneously (19).

JNJ-61186372 blocked the ligand binding of each receptor in a dose-dependent manner (Fig. 1B). JNJ-61186372 inhibited ruthenium-labeled EGF binding to EGFR-ECD with an IC$_{50}$ = 10 nmol/L and inhibited biotinylated-labeled HGF binding to cMet-ECD with an IC$_{50}$ = 30 nmol/L. These results demonstrated that JNJ-61186372 bound to both EGFR and cMet with high affinity and furthermore inhibited ligand binding to each receptor with similar potency compared with the parental monospecific bivalent antibodies.

JNJ-61186372 inhibits receptor phosphorylation of EGFR and cMet.

Human lung cancer cell lines, representing different EGFR and cMet genotypes that often occur in NSCLC patients, were selected to evaluate the cellular activity of JNJ-61186372 (Supplementary Table S1). These included cell lines with EGFR primary activating mutations, EGFR T790M mutation (confers resistance to EGFR TKIs), either alone or with MET gene amplification, and cell lines with wild-type (WT) EGFR and WT levels of cMet. In these cell lines, JNJ-61186372 inhibited EGF-induced phosphorylation of EGFR and HGF-induced phosphorylation of cMet in a dose-dependent manner (Fig. 2A), with activities (IC$_{50}$) below 100 nmol/L (Fig. 2B). The level of phosphorylated EGFR in the untreated H1975 cells (EGFR mutations L858R; T790M) was higher than that of H292 cells (EGFR WT) due to the activating mutation in EGFR. Addition of EGF to H1975 cells resulted in a smaller induction of phosphorylated EGFR compared with the

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**Figure 2.**

JNJ-61186372 inhibited ligand-induced phosphorylation of both EGFR and cMet in lung cancer cell lines. A, H292 and H1975 cells were serum starved, then stimulated with EGF (left) or HGF (right) in the presence of JNJ-61186372. Levels of phospho-EGFR (left) or phospho-cMet (right) were detected (RCL, relative chemiluminescence), with three biologic replicates at each point. Mean RCL values are plotted ± SEM. B, summary IC$_{50}$ data for a panel of lung cancer cell lines for JNJ-61186372 inhibition of phospho-EGFR (light bars) and phospho-cMet (dark bars) using MSD assays.
magnitude of induction observed with H292 cells. Nevertheless, JNJ-61186372 inhibited phosphorylation of EGFR in the EGF-stimulated H1975 cells, reducing the phosphorylation of EGFR to levels below the unstimulated condition.

Enhanced inhibition of downstream signaling with JNJ-61186372
To determine whether dual inhibition of receptor phosphorylation had downstream signaling consequences, the inhibition of phospho-ERK and phospho-AKT by JNJ-61186372 was assessed in the same panel of cell lines (Fig. 3A). JNJ-61186372 demonstrated potent inhibition of both phospho-ERK and phospho-AKT, with IC50 values in the low or subnanomolar range for most cell lines tested. However, in cell lines with MET gene amplification (H820 and H1993), JNJ-61186372 demonstrated either weak or no inhibition of phospho-ERK or phospho-AKT.

Single-pathway inhibition (EGFR or cMet) by monovalent antibodies was also assessed and compared with that of JNJ-61186372 (Fig. 3B–E). In H292 cells, neither treatment with the monovalent EGFR or monovalent cMet antibody caused complete inhibition of pERK, whereas the combination of the two monovalent antibodies caused complete inhibition of pERK with an IC50 = 35 nmol/L (Fig. 3B). This result demonstrated that inhibition of both EGFR and cMet was necessary for full inhibition of pERK in this cell line. Interestingly in H292 cells, treatment with JNJ-61186372 resulted in complete inhibition of pERK, but with a lower IC50 = 0.64 nmol/L, a 55-fold increase in potency (P < 0.0001) for JNJ-61186372 compared with that of the combination of monovalent antibodies. Also in H292 cells, the monovalent cMet antibody was as effective as the combination of monovalent antibodies at inhibiting pAKT, suggesting that pAKT was more dependent on cMet signaling compared with EGFR signaling in this cell line (Fig. 3C). Nevertheless, JNJ-61186372 was 179-fold more potent for inhibition of pAKT (P < 0.0001) compared with the combination of monovalent antibodies, suggesting that the bispecific molecule had additional biologic effects not present in the combination of monovalent antibodies. Similarly, there was more potent inhibition of pERK and pAKT in H1975 cells using JNJ-61186372 [(65-fold greater inhibition of pERK (P < 0.0001); 75-fold greater inhibition of pAKT (P < 0.0001)] than the combination of monovalent antibodies (Fig. 3D and E).
JNJ-61186372 caused tumor cell lysis through enhanced effector function

We hypothesized that high levels of EGFR and cMet on the surface of tumor cells may allow for selective immune effector cell targeting through ADCC. JNJ-61186372 was engineered to have low levels of core fucose, which allows for tighter binding to human FcγRIIIa on natural killer (NK) cells, thus resulting in a more potent and effective ADCC killing of target cells (14).

The ADCC in vitro activity of JNJ-61186372 was tested using H292 and H1975 cells with human PBMCs as the source of NK cells (Fig. 4A). For comparison, an analogue of JNJ-61186372, but produced with normal fucose levels, and cetuximab (anti-EGFR mAb) were assessed in the same assay. The low fucose JNJ-61186372 was more potent and demonstrated increased levels of tumor cell lysis compared with the EGFR-cMet bispecific antibody with normal fucose levels. Similar results were observed using additional lung cancer cell lines, including those with either WT or mutant EGFR and WT or amplified MET (Supplementary Table S2). The ADCC capacity of the low fucose JNJ-61186372 was similar to the ADCC capacity of cetuximab. However, there were some cases where JNJ-61186372 demonstrated slightly improved ADCC activity in potency (EC50) and/or efficacy (% maximal lysis).

JNJ-61186372 inhibited tumor growth in the H1975-HGF xenograft model

The H1975 cell line carries both an activating mutation (L858R) and a second-site mutation in EGFR (T790M) that confers resistance to small-molecule EGFR TKIs, such as erlotinib. In addition, this line was engineered to express human HGF (H1975-HGF) so the human cMet on the tumor cells would be activated in the mouse host (mouse HGF does not effectively activate human cMet, ref. 23). H1975-HGF cells were implanted into immune-compromised mice to assess in vivo efficacy of JNJ-61186372 on xenograft tumors. In addition, the contribution of effector function to efficacy in vivo was evaluated.

As shown in Fig. 4B, JNJ-61186372 inhibited tumor growth by 80% compared with vehicle-treated animals at day 32 (P < 0.0001). Interestingly, JNJ-61186372-IgG2α antibody (Fc silent version) inhibited tumor growth by 47% (P = 0.0232) but was not as effective as JNJ-61186372 with the FcR-binding framework. These results demonstrate efficacy of JNJ-61186372 in the H1975-HGF model, with EGFR mutations (L858R; T790M) and cMet activation through autocrine expression of HGF. In addition, without effector function, the inhibition of EGFR and cMet alone reduced tumor growth relative to the vehicle control, but the addition of the effector function (IgG1 framework) is necessary for complete JNJ-61186372 in vivo activity.
JNJ-61186372 induced receptor downmodulation in vivo

To further understand the function of JNJ-61186372 in vivo, mice bearing H1975-HGF tumors were treated with either PBS vehicle control or JNJ-61186372 at 1, 5, and 20 mg/kg. Tumor lysates were analyzed for total EGFR and cMet levels by Western blot analysis for total levels of EGFR, cMet, or actin/GAPDH (loading controls). B, average total protein quantitated from Western blots, relative to loading controls. C, mice with H1975-HGF tumors were treated with a single dose of JNJ-61186372 (1, 5, or 20 mg/kg) or vehicle control (PBS). Tumor lysates examined using MSD assays for phospho-EGFR, phospho-cMet, phospho-ERK, or phospho-AKT. These results demonstrated downmodulation of both receptor targets and downstream signaling in the tumor by JNJ-61186372 treatment, thus illustrating an additional antitumor growth mechanism of action.

JNJ-61186372 is effective in EGFR-mutant xenograft models, with or without cMet activation

JNJ-61186372 was evaluated in a pair of human lung adenocarcinoma HCC827 xenograft models, both with a primary EGFR mutation (exon 19 deletion), but one with MET amplification (clone of parental HCC827; originally selected to have resistance to erlotinib; HCC827-ER1). In mice with HCC827 tumors, treatment with either erlotinib or JNJ-61186372 fully suppressed tumor growth during the treatment period (28 days) and to approximately 50 days posttreatment initiation (Fig. 6A). Both JNJ-61186372 and erlotinib inhibited tumor growth by 89% compared with vehicle-treated animals at day 45 (both $P < 0.05$). In mice with HCC827-ER1 tumors, JNJ-61186372 fully suppressed tumor growth, whereas erlotinib was only partially effective during treatment (days 7–31), and mean tumor volume...
increased at a similar rate as vehicle-treated tumors following cessation of treatment (Fig. 6B). However, in animals treated with JNJ-61186372, mean tumor volume slightly increased in 5 weeks following cessation of treatment. At day 37, erlotinib inhibited tumor growth by 63% ($P < 0.05$), while JNJ-61186372 inhibited tumor growth by 92% ($P < 0.05$), compared with vehicle-treated animals. These results suggested that in tumors with both EGFR mutations and MET amplification, dual inhibition of both EGFR and cMet was necessary for complete inhibition of tumor growth.

JNJ-61186372 is effective in PDX models with EGFR mutations

We tested the bispecific EGFR-cMet antibody efficacy in PDX models with EGFR mutations. For these studies, we used a version of JNJ-61186372 prepared with normal fucose levels. LU1235 PDX model is derived from a human lung primary adenocarcinoma tumor with a primary activating EGFR mutation (exon 19 deletion). In these tumors, treatments with erlotinib and JNJ-61186372 were both effective at regressing tumor volumes, but JNJ-61186372 continued to suppress tumor growth following cessation of treatment (Fig. 6C). These results demonstrated that in tumors with a primary activating mutation in EGFR, the bispecific EGFR-cMet antibody led to a more durable response than erlotinib treatment. The human lung adenocarcinoma LU1868 PDX model contains both a primary activating mutation in EGFR (L858R) and the second-site mutation in EGFR (T790M, known to cause resistance to erlotinib treatment). As expected in this model, erlotinib was not effective (Fig. 6D). However, JNJ-61186372 treatment caused regression of the LU1868 tumors in a durable manner for up to 2 months following cessation of treatment. JNJ-61186372 was effective in PDX models with either primary activating mutations in EGFR alone, and with the addition of T790M EGFR TKI resistance mutation.

Combination of JNJ-61186372 and third-generation EGFR TKI resulted in durable tumor regression

Third-generation EGFR TKIs, such as AZD9291 and CO-1686, preferentially inhibit mutant-activated EGFR compared with WT EGFR. To assess whether the combination of a third-generation EGFR TKI and JNJ-61186372 would have benefit, we tested AZD9291 and JNJ-61186372 as single agents and in combination in the parental H1975 model (Fig. 7A) and the engineered H1975-HGF model (Fig. 7B). During the treatment period, AZD9291 inhibited tumor growth in both parental H1975 (TGI = 100%) and H1975-HGF (TGI = 94%) models, $P < 0.05$ for both. However, AZD9291-treated tumors increased in volume after treatment cessation in the H1975-HGF model. During the...
treatment period, JNJ-61186372 was effective in both models (TGI = 79% and 90%, respectively, P < 0.05 for both). The combination treatment with JNJ-61186372 and AZD9291 was more effective than either single treatment in the H1975-HGF model. After 3 weeks of treatment, tumors were monitored for more than a month and no measurable tumors were observed in the combination groups in either tumor model. In a second study using the H1975-HGF model (Fig. 7C–E), tumors were grown to a larger size (average of 500 mm³) before treatment initiation. Again, tumors completely regressed with the combination treatment, and no tumor growth was observed for up to 85 days of treatment. Crizotinib, a small-molecule cMet/ALK inhibitor,
showed no efficacy in either model (Supplementary Fig. S3). Altogether, these results demonstrated that combination of a third-generation EGFR TKI and JNJ-61186372 caused regression of tumors in a sustained and durable manner.

Absence of EGFR-mediated skin rash after repeat administration of JNJ-61186372

Both Good Laboratory Practice (GLP) and non-GLP safety studies were performed in cynomolgus monkeys. Animals were given JNJ-61186372 intravenously at doses up to 120 mg/kg once per week for up to 5 weeks. No toxicities were observed in either study, including none of the skin or gut adverse events that are commonly observed with other anti-EGFR agents (25). Notably, JNJ-61186372 recovered in serum from cynomolgus monkeys treated with JNJ-61186372 was fully functional in inhibition of phospho-EGFR and phospho-Met in vitro using H292 human cancer cell line (Supplementary Fig. S4). Our preliminary data suggested that this lack of skin toxicity may be attributable to a lower single-arm binding affinity for purified EGFR compared with cetuximab [JNJ-61186372 EC50 = 1.4 nmol/L (19); cetuximab EC50 = 0.4 nmol/L in same assay format]. This lower affinity then translates into less potent binding to cells expressing low levels of EGFR and cMet, such as primary human dermal fibroblasts. We found that JNJ-61186372 bound to primary human dermal fibroblasts with an EC50 = 2.0 nmol/L whereas cetuximab bound 20-fold tighter, with an EC50 = 0.1 nmol/L. Binding to normal tissues is also likely enhanced by the bivalent nature of cetuximab, compared with the monovalent EGFR binding of JNJ-61186372.

Discussion

Extensive evidence has demonstrated that the EGFR and cMet signaling pathways are partially compensatory and mediate cross resistance to inhibitors of either pathway (4, 8, 9). Standard-of-care treatment for lung cancer patients with activating mutations in EGFR involves small-molecule EGFR TKIs, such as erlotinib or gefitinib. These therapies, although often initially very effective, ultimately fail due to resistance mediated by secondary-site EGFR mutations, such as T790M, or activation of bypass pathways, such as the cMet pathway (4, 9, 10, 26). Second- and third-generation EGFR TKIs, such as afatinib, AZD9291, and CO-1686, have produced exciting clinical results, but resistance to therapy remains a prominent challenge. Resistance mechanisms to third-generation EGFR TKIs are still being defined in patient samples, but an emerging frequent mechanism is mutation of EGFR (C797S; ref. 27). Evidence of MET amplification in lung tumor cells from a patient treated with AZD9291 has also been reported, suggesting that activation of the cMet pathway is clinically relevant for the third-generation EGFR TKIs (28).

To address these resistance issues, we have created a bispecific antibody, JNJ-61186372, that recognizes both EGFR and cMet. We have demonstrated that JNJ-61186372 inhibits tumor growth and progression by three distinct mechanisms. Two of these mechanisms involve inhibition of EGFR and cMet signaling, first by inhibition of ligand-induced activation via blocking ligand binding to each receptor and second by receptor inactivation via degradation. The third mechanism utilizes Fc effector-mediated killing of EGFR- and cMet-expressing tumor cells by ADCC. Through these mechanisms of action, JNJ-61186372 showed activity in multiple xenograft models containing diverse EGFR mutations (exon 19 deletion, L858R, T790M). Given that JNJ-61186372 recognizes the extracellular region of EGFR, this bispecific antibody has the potential to be active in tumors with C797S mutation (located intracellularly) as well. In addition, JNJ-61186372 was active in models with cMet pathway activation, either by autocrine ligand production or through MET gene amplification. JNJ-61186372 was also active in a model with WT EGFR and autocrine HGF production (Supplementary Fig. S5). Collectively, the unique mechanisms of action of JNJ-61186372 cover the spectrum of EGFR mutations and also inhibit the key pathways driving EGFR TKI–acquired resistances.

Combination therapies may be necessary to fully suppress the EGFR pathway and prevent resistance from occurring through multiple mechanisms. The combination of afatinib and cetuximab was shown to cause near complete tumor regression in mice with L858R/T790M erlotinib-resistant tumors (29) by depleting phosphorylated and total EGFR more completely than either single agent alone. Combining JNJ-61186372 with third-generation EGFR TKIs might have the advantage, in addition to simultaneous inhibition of the cMet pathway, of more complete inhibition of the EGFR pathway through the ligand inhibition and receptor degradation mechanisms. Addition of the immune effector–based mechanisms of JNJ-61186372 may also attack tumor cells resistant due to EGFR- and cMet-independent mechanisms. We have shown here that the combination of JNJ-61186372 and AZD9291 was very effective at inhibiting tumor growth in xenograft models with EGFR mutations and cMet activation. Furthermore, in contrast with monotherapy, treatment with the combination prevented tumor regrowth in all animals tested. In patients, tumor heterogeneity likely contributes to resistance; recent observations suggest that T790M-positive and T790M-WT coexist in some lung cancers with acquired resistance to initial EGFR TKI treatment (28, 30). Combination therapy with JNJ-61186372 may delay or prevent the emergence of resistance, with activity on clonal populations with varying EGFR mutations within the same tumor, more complete suppression of the EGFR pathway, and inhibition of cMet, a key bypass pathway.

Recently, advances in protein engineering have made the production of intact bispecific human antibodies, such as JNJ-61186372, more practical. One potential advantage of the bispecific configuration is the significantly improved inhibition of downstream signaling when both targets are present on the same tumor cell. This property may be explained by an avidity effect, in which cells expressing both targets engage both arms of the bispecific (cross-arm binding) with increased apparent affinity as compared with those expressing only one target or otherwise engaging a single Fab arm. We have described correlations between binding affinity, receptor density, and receptor phosphorylation with JNJ-61186372 (19). Furthermore, we have shown that JNJ-61186372 was more effective than the combination therapy of anti-EGFR and anti-cMet monoclonal antibodies in decreasing tumor growth in the H1975-HGF model (31).

This bispecific structure may also confer unexpected properties on JNJ-61186372. In contrast with other EGFR-targeted agents (25), we did not observe EGFR-mediated skin toxicity with JNJ-61186372 in cynomolgus monkeys, typically a critical limitation of this class of agents, even at doses up to 120 mg/kg. We hypothesize that the lack of skin rash in cynomolgus monkeys treated with JNJ-61186372 is linked to weaker, monovalent binding of JNJ-61186372 to EGFR compared with stronger binding of antibodies interacting bivalently with EGFR.
(e.g., cetuximab, panitumumab) to normal skin and gut cells. Indeed, we found a 20-fold difference in affinity between JNJ-61186372 and cetuximab in binding to normal human dermal fibroblasts. Because normal tissues express low levels of cMet, the cMet-binding arm of JNJ-61186372 is not engaged, thus resulting in overall weaker binding to skin and gut cells, along with lower inhibition of EGFR in these normal tissues. In contrast in tumor cells, where high levels of EGFR and cMet are coexpressed, both arms of JNJ-61186372 are engaged, and stronger binding and functional inhibition is achieved. Alternatively, species differences in binding and activity could contribute to the observed lack of skin toxicity. The potential lower toxicity profile of JNJ-61186372 may also be an advantage in combination therapies, lack of skin toxicity. The potential lower toxicity profiles of JNJ-61186372 are engaged, and stronger binding and functional inhibition is achieved. Alternatively, species differences in binding and activity could contribute to the observed lack of skin toxicity. The potential lower toxicity profile of JNJ-61186372 may also be an advantage in combination therapies, lack of skin toxicity. The potential lower toxicity profile of JNJ-61186372 may also be an advantage in combination therapies, lack of skin toxicity. The potential lower toxicity profile of JNJ-61186372 may also be an advantage in combination therapies, lack of skin toxicity. The potential lower toxicity profile of JNJ-61186372 may also be an advantage in combination therapies, lack of skin toxicity.

We have demonstrated three distinct mechanisms of action for JNJ-61186372 that modulate both EGFR and cMet signaling axes. The unique structure of JNJ-61186372 enables attack of tumor cells with multiple mechanisms in a single agent, resulting in durable antitumor effects in preclinical models resistant to other therapeutic approaches. These results support testing in human clinical trials, as monotherapy in selected patients and in combination with other agents addressing the same pathways, in lung cancer, and other malignancies.

Disclosure of Potential Conflicts of Interest

S.L. Moores is an Associate Director at Janssen R&D. P. Haytko is a Senior Associate Scientist at Janssen Pharmaceuticals. J. Neijssen has ownership interest (including patents) in Genmab. P.W.H.I. Parren is a Professor at Leiden University Medical Center and has ownership interest (including patents) in Genmab. J. Schuurman has ownership interest (including patents) in Genmab and is a consultant/advisory board member for Joint Research Committee. R.M. Attar is a Senior Director at Janssen Pharmaceuticals. G.M. Anderson is a Scientific Director at Janssen R&D. No potential conflicts of interest were disclosed by the other authors.

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A Novel Bispecific Antibody Targeting EGFR and cMet Is Effective against EGFR Inhibitor–Resistant Lung Tumors

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