Antitumor Activity of Amivantamab (JNJ-61186372), an EGFR-cMet Bispecific Antibody, in Diverse Models of EGFR Exon 20 Insertion-Driven NSCLC

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CONFLICT OF INTEREST STATEMENT

None declared.

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ABSTRACT

EGFR exon 20 insertion driver mutations (Exon20ins) in NSCLC are insensitive to EGFR-TKIs. Amivantamab (JNJ-61186372), a bispecific antibody targeting EGFR/cMet, has shown preclinical activity in TKI-sensitive EGFR-mutated NSCLC models and in an ongoing first-in-human study in advanced NSCLC patients. However, the activity of amivantamab in Exon20ins-driven tumors has not yet been described. Ba/F3 cells and patient-derived cells/organoids/xenograft models harboring diverse Exon20ins were used to characterize the antitumor mechanism of amivantamab. Amivantamab inhibited proliferation by effectively downmodulating EGFR/cMet levels and inducing immune-directed antitumor activity with increased IFN-γ secretion in various models. Importantly, in vivo efficacy of amivantamab was superior to cetuximab or poziotinib, an experimental Exon20ins targeted-TKI. Amivantamab produced robust tumor responses in two Exon20ins patients, highlighting the important translational nature of this preclinical work. These findings provide mechanistic insight into the activity of amivantamab and support its continued clinical development in Exon20ins patients, an area of high unmet medical need.

SIGNIFICANCE

Presently, there are no approved targeted therapies for EGFR Exon20ins-driven NSCLC. Preclinical data shown here, together with promising clinical activity in an ongoing Phase I study, strongly supports further clinical investigation of amivantamab in EGFR Exon20ins-driven NSCLC.
INTRODUCTION

Molecular segmentation of advanced non-small cell lung cancer (NSCLC) based on oncogenic driver mutations has improved the overall survival and quality of life for patients with actionable driver mutations, and solidified solid tumor target therapy. Mutations in the epidermal growth factor receptor (EGFR) (1,2) gene constitutively activate downstream growth and survival signaling pathways leading to dependency on the EGFR pathway for tumor growth. Nearly 20% of Caucasians and up to 50% of Asians with lung adenocarcinomas harbor mutations in EGFR (3,4).

EGFR activating mutations have been reported in the first four exons (18 through 21) of its tyrosine kinase domain. NSCLCs that harbor “classical” EGFR mutations in exons 18, 19 and 21, e.g. Exon 19 deletions or L858R, are sensitive to treatment with first-, second- and third-generation EGFR tyrosine kinase inhibitors (TKIs) such as erlotinib, afatinib and osimertinib (5,6). In contrast, the EGFR exon 20 mutations encompass nucleotides that translate into amino acids at position 762-823, and include a C-helix (762–766) followed by a loop (767–775) (7). The insertion mutations of one to seven amino acids in exon 20 form a wedge at the end of the C-helix in EGFR that promotes active kinase conformation. EGFR Exon20 insertion driver mutations (Exon20ins), a distinct and highly heterogeneous subset of NSCLCs, represent 4%-12% of all EGFR mutations (8,9). These Exon20ins mutations are generally insensitive to approved EGFR-TKIs and are associated with poor prognosis; thus representing an area of high unmet medical needs (10,11).

Recently, poziotinib and TAK-788 have been undergoing clinical evaluation in patients whose tumors carry EGFR Exon20ins mutations (12,13). Despite initial promising efficacy, the Zenith 20 trial demonstrated that poziotinib had a low response rate (~14%) in NSCLC patients with the EGFR Exon20ins mutation. Furthermore, both poziotinib and TAK-788 have high rates of EGFR wild-type driven toxicity due to the lack of selectivity upon Exon20ins as compared to EGFR wild-type and other kinases, limiting their clinical utility (10).

Amivantamab (JNJ-61186372, Fig. 1A) is an EGFR-cMet bispecific antibody with immune cell-directing activity that targets activating and resistance EGFR mutations and cMet mutations and amplifications. Ongoing first-in-human studies in patients with advanced, refractory EGFR mutant
NSCLC have demonstrated preliminary clinical activity of amivantamab in patients with diverse EGFR mutations (14,15). Of note, amivantamab showed promising efficacy (30% response rate) with a manageable safety profile in heavily pretreated EGFR Exon20ins NSCLC patients. While amivantamab has been reported to harbor activity in preclinical tumor models driven by EGFR mutations sensitive to approved TKIs (e.g. L858R and Exon 19 deletions) (16), its activity has not yet been explored in the context of EGFR Exon20ins.

Here, we comprehensively evaluated the antitumor activity and mechanisms of action of amivantamab in multiple EGFR Exon20ins models, including engineered cell lines, patient-derived cells (PDCs), and patient-derived xenografts (PDXs). We also present evidence of clinical activity in two case studies of EGFR Exon20ins NSCLC patients treated with amivantamab from an ongoing Phase I clinical trial, highlighting the important translational nature of this work.

RESULTS

Amivantamab inhibits proliferation of Ba/F3 cells harboring diverse EGFR Exon20ins mutations.

In order to demonstrate the antitumor activity of amivantamab in the context of Exon20ins, multiple Exon20ins were stably expressed in Ba/F3 cells. Five distinct Exon20ins were introduced (Fig. 1B), all of which have been observed in NSCLC patients (V769_D770insASV, D770delinsGY, H773_V774insH, Y764_V765insHH and D770_N771insSVD) (17,18). In Ba/F3 cells treated with amivantamab ranging from 0.05 to 1 mg/mL, a significant and dose-dependent decrease in Ba/F3 cell viability ($P < 0.0001$) was observed in all five EGFR Exon20ins mutations (Fig. 1C). In contrast, treatment with the first and third-generation irreversible EGFR-TKI, gefitinib and osimertinib, respectively, showed limited antiproliferative activity compared to amivantamab (Fig. 1C), confirming the well-known resistance of Exon20ins to EGFR-TKIs. No effect on cell viability was observed when IgG1 control antibodies were used in the same Ba/F3 cell lines (Supplementary Fig. S1A). In tumor models driven by TKI-sensitive EGFR mutations such as L858R or Exon 19 deletions, amivantamab
has several proposed mechanisms of action (MOAs) including blocking ligand binding, receptor downmodulation, downstream signaling inhibition and triggering immune-directed antitumor activity (19). To determine if these MOAs are also observed in the context of Exon20ins and contribute to the observed anti-proliferative activity in Fig. 1C, immunoblot analysis was performed in Ba/F3 cells overexpressing the EGFR D770delinsGY and H773_V774insH Exon20ins mutations. The total EGFR levels were reduced following treatment with amivantamab, compared to those of untreated cells (Fig. 1D and Supplementary Fig. S1B) or cells treated with the IgG1 control antibody (Supplementary Fig. S1C). Consistent with the reduction in EGFR expression levels, the EGFR downstream signaling pathways phospho-EGFR (p-EGFR), phospho-AKT (p-AKT), phospho-ERK (p-ERK), and phospho-S6 (p-S6) were also significantly reduced following amivantamab treatment (Fig. 1D), suggesting that amivantamab targeted EGFR and inhibited EGFR-related downstream signaling cascades. Similar results were observed in Ba/F3 cells expressing the V769insASV, Y764 insHH and D770_N771insSVD Exon20ins mutations (Supplementary Fig. S1B). Although 100 nM of gefitinib and osimertinib reduced p-EGFR in Ba/F3 cells overexpressing D770delinsGY and H773_V774insH, downstream EGFR signaling pathway components were not significantly inhibited, which correlated with the lack of TKI effects on cell viability (Fig. 1E and Supplementary Fig. S1D). In recent studies, poziotinib has shown antitumor activity in EGFR Exon20ins NSCLC (20,21). We further assessed the cell viability test for poziotinib in Ba/F3 overexpressing EGFR Exon20ins (Supplementary Table S1). Consistent with a previous report (20), poziotinib strongly inhibited the cell viability in the mutant EGFR Exon20ins cells (IC\textsubscript{50} ranging from 0.8 to 10.9 nM). As reported in a previous study (22), poziotinib also potently suppressed proliferation of Ba/F3 cells harboring WT EGFR (IC\textsubscript{50} = 0.8 nM). To present the selectivity for Exon20ins mutation in a more balanced manner, we compared antiproliferative potency between amivantamab and poziotinib in EGFR Exon20ins mutants over WT EGFR. Poziotinib exhibited lower EGFR Exon20ins mutant selectivity over WT EGFR, compared to amivantamab, suggesting that poziotinib may adversely affect normal tissues, thereby producing substantial toxicities, such skin rash and diarrhea (23). To better understand the mechanisms involved in amivantamab-mediated cellular cytotoxicity, we assessed the effect of amivantamab treatment on cell cycle progression and programmed cell death. In Ba/F3 cells expressing the EGFR D770delinsGY and H773_V774insH Exon20ins mutations, an accumulation of cells in G1 phase was
observed in amivantamab-treated cells compared to vehicle-treated cells (Fig. 1F). As EGFR-TKIs have been reported to drive apoptosis in NSCSEL cells harboring sensitizing EGFR mutations (24,25), we investigated whether treatment with amivantamab resulted in engagement of the apoptotic machinery. Amivantamab treatment resulted in the induction of pro-apoptotic proteins, including BIM and cleaved caspase 3 (Fig. 1G), suggesting that amivantamab, in addition to inhibition of downstream EGFR signaling cascade, also induced apoptosis in a BIM- and caspase-dependent manner.

**Amivantamab displays antitumor activity in PDCs and organoids**

To extend our findings from Ba/F3 cells engineered to express the exogenous EGFR Exon20ins mutations, we evaluated the activity of amivantamab in several PDCs harboring the Exon20ins. The antitumor activity of amivantamab and associated mechanistic endpoints were evaluated in PDCs generated from patients harboring P772ins_H773insPNP (DFCI-127), H773_V774insNPH (DFCI-58), and S768_D770dup (YU-1163) Exon20ins mutations (Supplementary Figs. S2A-S2C and Supplementary Table S2). In both DFCI-127 and DFCI-58 cells, amivantamab treatment resulted in decreased expression of total EGFR and cMet levels as well as inhibition of p-EGFR, p-cMet, p-AKT, p-ERK, and p-S6 (Fig. 2A), consistent with the results observed in Ba/F3 cell lines harboring EGFR Exon20ins mutations. Analysis of cell viability and colony formation revealed that amivantamab dose-dependently inhibited the cell growth and proliferation of PDCs, compared to IgG1 controls (Figs. 2B and 2C). In contrast to the significant reduction in EGFR, cMet, p-EGFR, p-cMet, p-AKT, and p-S6 in DFCI-127 and DFCI-58 cells, YU-1163 treated with amivantamab unexpectedly revealed an induction of p-ERK (Fig. 2A). Consistent with this result, the growth of YU-1163 was not inhibited after amivantamab treatment for 72 hours or following long term treatment (Figs. 2B and 2C). From the whole exome sequencing data of YU-1163, we observed a co-occurring mutation in the TP53 gene (R280T; 96% of mutant allele frequency) (Supplementary Figs. S2C and S2D). According to recent studies, mutations in TP53 commonly occurred with EGFR mutations in NSCLC. Particularly, TP53 mutations in exon 8 in NSCLC patients with EGFR mutations show lower responsiveness to EGFR-TKIs and worse prognosis than the patients with WT TP53 (26,27). Indeed, accumulated studies have revealed that the R280T mutation in TP53 plays crucial roles in the
proliferation and survival of cancer cells and knockdown of the mutant TP53 causes G2 arrest and apoptosis in bladder cancer cells (28,29). As shown in Supplementary Fig. S2E, depletion of mutant TP53 by three different TP53-directed siRNAs significantly inhibited the cell proliferation with a reduction in activated ERK in YU-1163 pretreated with 1 mg/mL amivantamab. Given that mutant TP53 is associated with EGFR-TKI resistance (30) and the depleted mutant TP53 restored the sensitivity of amivantamab by downregulation of p-ERK, induction of p-ERK following amivantamab treatment in YU-1163 cells might be a key regulator of cell survival potentially through the crosstalk between mutant TP53 and ERK signaling cascade (31,32). Additionally, we generated two PDO models from plural effusion of patients who had A767_V769dup (YUO-036) and S768_D770dup (YUO-029) to recapitulate the phenotypic and molecular landscape of the original NSCLC with EGFR Exon20ins (Supplementary Figs. S2F-S2G and Supplementary Table S2). YUO-029 was derived from the same patient from whom YU-1163 PDC (S768_D770dup) was derived. As shown in Fig. 2D, YUO-036 was sensitive to amivantamab in a dose dependent manner, whereas YUO-029 derived from the same patient with YU-1163 showed no significant decrease in cell viability following amivantamab treatment compared to IgG1 control (Fig. 2E). Taken together, these results indicate that amivantamab has potent antitumor activity in NSCLC patient-derived cancer cells with EGFR Exon20ins mutations by downmodulation of EGFR and cMet signaling pathways.

**EGFR and cMet are internalized in response to amivantamab.**

Treatment with amivantamab results in downmodulation of EGFR and cMet, as observed in Ba/F3 cells (Fig. 1) and PDCs (Fig. 2). According to many studies, anti-EGFR mAb induces internalization of EGFR leading to downregulation of its expression on the cell surface (33,34). To investigate whether amivantamab directly binds to EGFR on cells with EGFR Exon20ins mutation, Ba/F3 cells overexpressing D770delinsGY or H773_V774insH were incubated with 0.1 mg/mL IgG1 control and 0.1 mg/mL amivantamab. Fluorescence-activated cell sorting (FACS) was used to measure the level of plasma membrane-bound EGFR. EGFR expression on the plasma membrane began to dwindle by almost two-fold 30 min after amivantamab treatment. The % changes in median fluorescence intensity (MFI) of EGFR relative to IgG1 control treated cells at 30 min were 56 % and 68 % in D770delinsGY and H773_V774insH, respectively, and subsequently remained at 40% EGFR.
expression relative to IgG1 control-treated cells 72 hours after amivantamab treatment (Fig. 3A). To explore the internalization of cMet as well as EGFR on PDCs harboring EGFR Exon20ins, DFCI-127 and DFCI-58 PDCs were treated with 0.1 mg/mL amivantamab and the plasma membrane-bound cMet and EGFR were measured 72 hours after amivantamab treatment (Figs. 3B and 3C). The results showed that amivantamab reduced EGFR and cMet on PDCs compared to IgG1 control. Immunofluorescence (IF) staining was used to visualize the internalization of EGFR and cMet following amivantamab treatment. Treatment with 0.1 mg/mL amivantamab for 72 hours led to the redistribution of EGFR and cMet receptors into internal compartments whereas IgG-treated cells showed no change in the staining pattern for EGFR or cMet (Fig. 3D and Supplementary Fig. S3). Internalization and subsequent downregulation of EGFR and cMet receptors by lysosomes could account for the decreased EGFR and cMet protein levels observed in the immunoblot, FACS and IF assays following amivantamab treatment. To determine if lysosomal degradation was involved in downregulating EGFR protein levels, Ba/F3 cells overexpressing D770delinsGY and H773_V774insH were treated with amivantamab in the absence and presence of the autophagy inhibitor bafilomycin. Bafilomycin treatment inhibited the degradation of EGFR (Fig. 3E), suggesting that downmodulation of the total EGFR protein level following amivantamab treatment may involve lysosomal degradation of internalized cell surface receptors. Taken together, these results suggest that treatment with amivantamab induces receptor internalization and may contribute to the observed antiproliferative effects of amivantamab by inhibiting EGFR and cMet-mediated signaling.

Amivantamab inhibits EGFR Exon20ins mutation-driven growth of Ba/F3 and PDC models in vivo

To determine if amivantamab is active against EGFR Exon20ins-derived tumors in vivo, xenograft models were generated using Ba/F3 cells overexpressing EGFR D770delinsGY and H773_V774insH Exon20ins mutations and PDCs (DFCI-127 and YU-1163) harboring P772insPNP and S768_D770dup EGFR Exon20ins mutations, respectively. Mice were treated with amivantamab, IgG1 control, or vehicle at 30 mg/kg twice per week i.p. Amivantamab-treated mice showed reduced tumor volumes compared to vehicle or IgG1 control-treated mice in the Ba/F3 cells-bearing NOG mice models (Figs. 4A-4B and Supplementary Figs. S4A-S4B). Inhibition of tumor growth occurred early
and was sustained 15 days following treatment. As shown in Ba/F3 and PDC cells in vitro (Figs. 1D and 2A), protein expression of EGFR, cMet, p-EGFR, and p-cMet were significantly reduced following amivantamab treatment in the Ba/F3-bearing NOG mice models (Fig. 4C and Supplementary Fig. S4C). Similarly, in the PDC xenograft models, amivantamab-treated mice showed a reduction in tumor volume compared to vehicle-treated mice (Figs. 4D-4E and 4F-4G), as well as a reduction in EGFR, cMet, p-EGFR, and p-cMet protein levels (Figs. 4H and 4I). Intriguingly, although amivantamab could not inhibit the proliferation of YU-1163 PDC in vitro (Fig. 2), a dramatic tumor regression was observed in YU-1163-bearing BALB/c nude mice after amivantamab treatment (Fig. 4F), suggesting that additional factors might contribute to the in vivo antitumor effect of amivantamab. As mentioned above, poziotinib is a targeted agent that has shown preliminary clinical activity in EGFR Exon20ins disease (20,21). We compared the antitumor activity and safety of poziotinib with those of amivantamab in YU-1163 (S768_D770dup)-bearing BALB/c nude mice and Ba/F3 cells overexpressing D770_N771insSVD-bearing NOG mice (Supplementary Figs. S4D and S4E). Using the previously reported dosing regimen of 5 mg/kg poziotinib, Q.D. (20), sudden death occurred within 6 days of treatment. Skin toxicity analyses with poziotinib and amivantamab revealed that poziotinib-treated mice showed severe skin toxicities on the face, abdomen, and back at dose of 5 mg/kg and 10 mg/kg, while 30 mg/kg amivantamab showed only minimal keratosis on the face (Supplementary Figs. S4F and S4G). In addition to skin toxicity, a dramatic loss of body weight was observed in poziotinib-treated mice compared to amivantamab treated mice (Supplementary Fig. S4H). The favorable toxicity profiles with amivantamab were consistent with those shown in an ongoing Phase I study (15).

Amivantamab induces antibody-dependent cell-mediated cytotoxicity (ADCC) in Exon20ins models

The process of ADCC is known to be initiated when both the target cell antigen and an activated Fcy receptor (FcyR) are engaged respectively by the Fab and Fc portions of an antibody. The effector cells, mainly natural killer (NK) cells, trigger degranulation and subsequent cytokine production, resulting in the elimination of the target cells (35). To determine whether ADCC plays a role in amivantamab-mediated antitumor activity, ADCC assays were performed using PDCs (DFCI-127 and YU-1163) expressing EGFR Exon20ins mutations co-cultured with peripheral blood
mononuclear cells (PBMCs) as effector cells (E:T = 50:1). Treatment with amivantamab resulted in cytotoxicity in both PDCs in a dose-dependent manner and to a greater extent than cetuximab, a monoclonal antibody targeting EGFR (Figs. 5A-5C). By extension, cetuximab treatment led to a less pronounced reduction in tumor volume in YU-1163-bearing BALB/c nude mice models relative to that observed with amivantamab (Supplementary Fig. S5A). Amivantamab-mediated cellular cytotoxicity shown in Fig. 5A was significantly impaired by incubation with an Fc receptor (FcR) blocker in DFCI-127 and YU-1163 PDCs (Fig. 5D), suggesting that the amivantamab-mediated ADCC effect requires the interaction with FcRs on PBMCs. Similarly, the antitumor effect of amivantamab was abrogated in vivo when amivantamab was co-treated with anti-mouse CD16/CD32 antibodies to block FcRγIII/FcRγII on monocytes/macrophages and NK cells in YU-1163-bearing BALB/c nude mice (Supplementary Fig. S5A). It is known that inflammatory cytokines such as IFN-γ and TNFα are secreted from infected monocytes and activated NK cells during ADCC, encouraging antigen presentation and adaptive immune responses (36,37). To explore the correlation between amivantamab-dependent ADCC and secreted IFN-γ levels, we measured the level of IFN-γ in medium co-cultured with PDCs and PBMC after amivantamab treatment. Consistent with the degree of the ADCC effect, IFN-γ levels were significantly increased with amivantamab treatment compared to cetuximab treatment (Fig. 5E). Treatment with a FcR blocker reduced IFN-γ secretion, indicating that IFN-γ secretion was dependent on the interaction between the Fc domain of amivantamab and the FcR on immune cells (Fig. 5F). Induced inflammatory cytokines including IFN-γ secreted from NK cells activated by amivantamab bound to EGFR and cMet on EGFR Exon20ins-driven tumors may lead to the recruitment and activation of adjacent immune cells to tumor cells in vivo. To explore this, we analyzed the infiltration of macrophages and NK cells into the tumor in a PDX model (YHIM-1029)-, which was generated from a patient-derived tumor harboring the D770_N771insG Exon20ins mutation (Supplementary Table S2), and YU-1163-bearing BALB/c nude mice models treated with amivantamab at 10 mg/kg and 30 mg/kg dose, respectively. mF4/80 and mNKp46, markers of macrophages and NK cells in BALB/c nude mice, respectively, were elevated in tumors following treatment with amivantamab, suggesting that the mechanistic components of ADCC observed in vitro may translate to recruitment of key effector cells in tumors in vivo (Fig. 5G and Supplementary Fig. SSB). Additionally, these results suggest that amivantamab has greater ADCC and antitumor activity.
than cetuximab in the context of EGFR Exon20ins and that ADCC is an important mechanism in mediating the cytotoxic effects of amivantamab.

**Amivantamab demonstrates antitumor activity in a PDX model harboring the D770_N771insG Exon20ins mutation.**

Treatment with amivantamab in YHIM-1029 PDX model with D770_N771insG (Fig. 6A) resulted in a robust decrease in tumor volume, indicating that the antitumor activity observed in Ba/F3 and PDC models was preserved in a PDX model (Fig. 6B). In contrast, treatment with cetuximab (10 mg/kg) or poziotinib (1 mg/kg) only modestly reduced tumor volume. The dose of poziotinib was reduced to 1 mg/kg for this experiment due to the toxicity of poziotinib described above (Supplementary Figs. S4D-S4H). Pharmacodynamic analysis showed that amivantamab treatment resulted in EGFR and cMet downmodulation, inhibition of the downstream signaling pathways p-AKT, p-ERK, and p-S6, and increased markers of apoptosis (Fig. 6C). In contrast, tumors from mice treated with cetuximab or poziotinib maintained EGFR downstream signaling components p-ERK and p-S6 (Fig. 6D), which was consistent with the modest effects observed on tumor growth. Histopathological examination of tumor sections obtained following amivantamab or vehicle treatment using hematoxylin and eosin (H&E) staining, and immunohistochemical staining for EGFR, cMet, Ki-67, and TUNEL staining, further confirmed receptor inhibition and engagement of apoptotic machinery in EGFR Exon20ins-driven tumors *in vivo* (Fig. 6E). To verify whether the antitumor effect of amivantamab was affected by innate immunity in the *in vivo* models, we blocked the mouse CD16/CD32 via administration of anti-CD16/CD32 antibodies. The antitumor effect of amivantamab shown in Fig. 6B was abrogated when the amivantamab-treated PDX bearing BALB/c nude mice were co-treated with anti-CD16/CD32 antibodies, indicating that the antitumor effects of amivantamab were partially mediated by immune cells in this condition (Supplementary Fig. S6).

**Antitumor activity of amivantamab in patients with EGFR Exon20ins disease**

In an ongoing first-in-human study of amivantamab in patients with advanced NSCLC (NCT02609776), promising clinical activity has been observed in patients with *EGFR* Exon20ins disease (15). A 58-year-old patient harboring the *EGFR* H773delinsNPY Exon20ins mutation achieved
a partial response with a 65% tumor reduction (Fig. 7A), and a 48-year-old patient with the EGFR S768_D770dup Exon20ins mutation achieved a partial response with a 38.9% tumor reduction (Fig. 7B). These patients were progression-free for 92 and 32 weeks, respectively, on amivantamab with manageable toxicities.

DISCUSSION

In our study, we characterized the antitumor activity of amivantamab, a novel EGFR/cMet bispecific antibody, in multiple preclinical models harboring EGFR Exon20ins mutations. In several Ba/F3 and PDC models expressing diverse EGFR Exon20ins mutations, amivantamab treatment resulted in EGFR and cMet internalization, inhibition of downstream signaling cascades, engagement of apoptotic machinery, and subsequent inhibition of tumor cell proliferation. Importantly, these diverse action mechanisms of amivantamab were preserved in vivo as evidenced by pharmacodynamic analyses of tumors from cell line xenografts and PDX models treated with amivantamab (Fig. 7C). Furthermore, to the best of our knowledge, we first presented evidence of clinical activity of amivantamab in two case studies of EGFR Exon20ins NSCLC patients from an ongoing Phase I trial, highlighting the important translational nature of our preclinical work.

Presently, there are no targeted therapies approved for EGFR Exon20ins-positive advanced NSCLC. Owing to its small size and flexibility, poziotinib, an oral pan-HER inhibitor, has demonstrated greater activity than approved EGFR-TKIs in vitro and in PDX models of EGFR Exon20ins mutant NSCLC (20). In a single center Phase II trial, poziotinib showed a 43% confirmed response rate (RR) in heavily pretreated advanced EGFR Exon20ins mutant NSCLC (23). However, in a subsequent pivotal Phase II trial (NCT03318939), poziotinib yielded only 14.8% RR in a similar patient population (https://www.precisiononcologynews.com/drug-discovery-development/spectrums-poziotinib-failed-meet-primary-phase-ii-trial-endpoint#.XjJWkWgzaUk). TAK-788 has also shown preclinical activity against activating EGFR and HER2 mutations including EGFR Exon20ins. Although preliminary, in a Phase I/II study (NCT02716116), TAK-788 produced preliminary clinical activity in a small subset of EGFR Exon20ins mutant NSCLC (13). Importantly, treatment with poziotinib or TAK-788 was associated with a high incidence of EGFR WT driven toxicity such as diarrhea and rashes, further
limiting their clinical utility. Therefore, there is a substantial clinical need to identify new therapies for patients with EGFR Exon20ins.

In our study, amivantamab was clearly superior to poziotinib or cetuximab in terms of efficacy and tolerability in xenografts. Multiple facets of the MOA of amivantamab may contribute to the superior antitumor activity of amivantamab in the context of Exon20ins. In addition to the different MOA described in the various Exon20ins models described above, the ability of amivantamab to simultaneously bind two distinct epitopes of EGFR and cMet may result in the concurrent interference with highly interconnected signaling pathways. To this end, amivantamab has shown to have higher efficacy in decreasing tumor growth in the H1975-HGF model compared to the combination of anti-EGFR and anti-cMet monovalent antibodies (38). This finding can be partly explained by an “avidity effect” whereby tumor cells expressing both targets, i.e., EGFR and cMet, bind to both arms of amivantamab with higher affinity than do cells that express only one target or engage a single Fab arm. A previous study has described correlations between binding affinity, receptor density, and receptor phosphorylation with amivantamab (39). Overall, bispecific antibodies show greater antitumor efficacy compared to a combination of monospecific monoclonal antibodies via potential synergistic effects, and they increase selectivity by simultaneous targeting of both receptors, favoring overexpressing cells as a consequence of avidity effects (40).

Amivantamab, produced by an engineered cell line defective for protein fucosylation, has a low-level core fucosylation. The human FcyRIIIa, critical for ADCC, binds antibodies with low core fucosylation with higher affinity and consequently mediates more potent and effective NK cell mediated killing of cancer cells (14). In this study, amivantamab demonstrated more robust ADCC in EGFR Exon20ins mutation models than to cetuximab, an EGFR-directed antibody that has not shown robust utility in NSCLC (41,42). This ADCC activity was correlated with secreted IFN-γ levels. It is possible that the IFN-γ secreted from amivantamab mediated active immune cells, including NK cells and macrophages, may re-stimulate and recruit surrounding immune cells to the tumor, although additional studies are required to investigate this hypothesis. Additionally, immunocytokines secreted from NK cells lead to up-regulation of ICAM-1 on target cells, rendering them more susceptible to
target cell cytolysis (43). Indeed, pharmacodynamic analyses of tumors from mice treated with amivantamab revealed increased tumor levels of NK cells and macrophages. In contrast to many other therapeutic antibodies used in the clinical setting, amivantamab was designed and engineered with a low fucose backbone, which enhances its binding to FcγRIIIa (19), which is present on NK cells, monocytes and macrophages. The human FcγRIIIa, critical for ADCC, binds antibodies with low-level core fucosylation more tightly and consequently mediates more potent and effective ADCC killing of cancer cells (44). Thus, the enhanced binding of amivantamab to FcγRIIIa may lead to increased induction of Fc effector functions in comparison to other (normal fucose) hIgG1 antibodies such as cetuximab.

In the study, we used two different xenograft models NOD.Cg-Prkdcscid Il2rgtm1Sug/Jic (NOG mice) and BALB/c nude mice for in vivo study. As demonstrated in many studies, NOG mice have impaired innate immunity and extremely low NK cell activity, whereas BALB/c nude mice have intact innate immunity and active NK cells (45,46). For this reason, more potent ADCC activity was expected in BALB/c nude mice than NOG mice. Therefore, a minimal amivantamab-mediated ADCC activity resulted in the modest efficacy with amivantamab in Ba/F3 and DFCI-127-bearing NOG mice (Figs. 4A, 4D, and Supplementary Fig. S4A). On the other hand, the significant tumor regression was observed with amivantamab in YU-1163-bearing and YHIM-1029-bearing BALB/c nude mice (Figs. 4F and 6B), which resulted from multiple MOA of amivantamab to block EGFR and cMet downstream signaling pathway and elicit ADCC.

It has been observed that EGFR and tumor suppressor TP53 genes are commonly mutated in patients with NSCLC with independent prognostic implications. Furthermore, in patients with concomitant mutations in EGFR and TP53, there have been reports of decreased responsiveness to EGFR-TKIs (47). A similar effect was observed in our study when the YU-1163 PDC and YUO-029 PDO following treatment with amivantamab. On the other hand, amivantamab exhibited a potent in vivo activity in YU-1163-bearing BALB/c nude mice, suggesting that ADCC activity of amivantamab shown in Figs. 5A-5G was believed to be involved in the in vivo antitumor activity. These results suggest that the combination of effector cell-dependent and -independent MOAs elicited by...
amivantamab (Fig. 7C) may result in antitumor activity in tumors harboring a coalescence of intractable mutations, for example in EGFR Exon20ins disease, and concomitant deleterious mutations such as the TP53 mutation present in our preclinical model and reported in the broader patient population.

In our study, amivantamab demonstrated lower skin toxicity than poziotinib. Amivantamab-treated BALB/c nude mice appeared phenotypically normal with only minimal signs of keratosis on the face, indicating that amivantamab was well tolerated in this preclinical model. In contrast, treatment with poziotinib resulted in severe keratosis, significant weight loss and even sudden death (Supplementary Figs. S4D-S4H).

In conclusion, our data demonstrated that amivantamab functions through multiple MOA to elicit antitumor activity in multiple preclinical models of EGFR Exon20ins disease. Consequently, amivantamab warrants further clinical investigation, as evidenced by clinical results from two EGFR Exon20ins NSCLC patients who have been treated with amivantamab in the clinical setting. This represents important progress toward the identification of an effective therapeutic option for NSCLC patients with EGFR Exon20ins, an area of high unmet medical need.

METHODS

Ba/F3 cell lines and drug compounds

All mutant Ba/F3 cell lines were purchased from the German Collection of Microorganisms and Cell Cultures and were obtained from the Dana-Farber Cancer Institute, Harvard University, USA. All cells were maintained in RPMI 1640 medium supplemented with 10% fetal bovine serum (FBS) and puromycin in a humidified incubator with 5% CO2. Amivantamab and IgG1 controls were provided by Janssen. Gefitinib, osimertinib, cetuximab, and poziotinib were purchased from SelleckChem (Houston, TX, USA).

Antibodies
Primary antibodies specific for p-EGFR (2234), EGFR (4267), p-cMet (3077), cMet (8198), p-ERK (4370), ERK (9107), p-AKT (9271), AKT (9272), p-S6 (4858), S6 (2217), p27 (2252), cleaved PARP (5625S), cleaved caspase 3 (9661), and BIM (2933) were purchased from Cell Signaling Technologies; p21(sc-817) and p53 (sc-126) were purchased from Santa Cruz Biotechnology, Inc.. GAPDH (PAB13195) purchased from Abnova (Taipei, Taiwan). For the IHC assay, mF4/80 (#70076) and mNKp46 (AF2225) were purchased from Cell Signaling Technologies and R&D systems, respectively.

Patient-derived cells

YU-1163 (S768_D770dup) cell lines were derived from malignant effusions from patients with NSCLC and cultured on collagen-coated plates in ACL-4 medium supplemented with 5% FBS. The cells maintained the driver oncogenes that were observed in the patients. Cells were enriched in an epithelial cell adhesion molecule (EpCAM)-positive cell population with a purity of over 95% before they were subjected to further assays. DFCI-58 (H773_V774insNPH) and DFCI-127 (P772_H773insPNP) cell lines were obtained from the Dana-Farber Cancer Institute, Harvard University, USA, and were cultured in ACL-4 medium and RPMI medium with 10% FBS, respectively. All patient samples were collected after written informed consent from the patients was obtained. The study protocols were approved by the respective institutional review boards.

Patient-derived organoid culture

Patient-derived organoids (YUO-029 and YUO-036) were established as previously described (48). Briefly, malignant effusions from two patients with NSCLC were collected, centrifuged, and the cell pellets were mixed with growth-factor reduced Matrigel (Corning) and seeded into 48-well plates. Solidified gels were overlaid with advanced DMEM/F12 (Invitrogen) containing 1X Glutamax (Invitrogen), 10 mM HEPES (Invitrogen), 1X antibiotic-antimycotic (Invitrogen), 1X B-27 (Invitrogen), 20% R-spondin conditioned medium, 5 mM nicotinamide (Sigma), 1.25 mM N-acetylcysteine (Sigma), 500 nM SB-202190 (Sigma), 500 nM A83-01 (Tocris), 100 ng/mL mouse noggin (Peprotech), 100
ng/mL human FGF10 (Peprotech), 25 ng/mL human FGF7 (Peprotech), 50 µg/mL primocin (Invivogen), and 10 µM Y-27632 (Enzo). R-spondin-conditioned medium was produced from HA-R-Spondin1-Fc 293T cells (Amsbio, Abingdon, United Kingdom). For passaging, organoids were collected, mechanically sheared with a 25-gauge needle, and washed with cold PBS before the organoid pellets were resuspended in the Matrigel and seeded into 24-well plates at ratios of 1:2 to 1:4. The culture medium was replenished at least twice a week. Cell viability test were performed as previously described (49). Briefly, organoids were trypsinized into single cells and cultured for 5 to 10 days. Then, the organoids were collected, resuspended in the medium containing 5% matrigel, and plated in a 96-well plate (Corning) at a concentration of 2,000 organoids/µL. The medium with the IgG1 control or Amivantamab at diverse concentrations were added and incubated for 72 h. Cell viability was measured using CellTiter-Glo 3D culture reagent (Promega) on a microplate luminometer according to the manufacturer’s instructions.

Patient-derived xenograft models

PDXs were created using 6–8-week old female severe combined immunodeficient (NOG) and nude (nu/nu) mice obtained from OrientBio (Seoul, Korea). All methods complied with the guidelines of our Institutional Animal Research Committee (Yonsei University College of Medicine) and were approved by the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC). After removal of the necrotic and supporting tissues from core biopsy specimens, small specimens of the tumor tissue (3 mm × 3 mm × 3 mm) from each patient were implanted subcutaneously in 1–2 mice. After the tumor reached 1.5 cm in diameter, it was excised, dissected into small specimens (3 mm × 3 mm × 3 mm), and re-implanted into nude mice.

In vivo xenograft studies

Female athymic BALB-c/nu mice were obtained from Orient Bio at 5–6 weeks of age. All mice were handled in accordance with the Animal Research Committee’s Guidelines at Yonsei University College of Medicine, and all facilities were approved by AAALAC. Ba/F3 cells and PDCs (1x10^7 cells)
were injected subcutaneously into the NOG and BALB-c/nu mice, respectively, and growth was measured twice weekly; after establishment of palpable lesions, mice were assigned to testing. Once the tumor volume reached approximately 150–200 mm$^3$, mice were randomly allocated into groups of five animals to receive either vehicle, IgG1 control, or Amivantamab. The tumor size was measured every 2 days using calipers. The average tumor volume in each group was expressed in mm$^3$ and calculated according to the equation for a prolate spheroid: tumor volume = 0.523 × (large diameter) × (small diameter)$^2$.

**Anti-proliferation assay**

Ba/F3 cells or PDCs expressing EGFR Exon20ins mutations were seeded onto 96-well plates in 100 µL. After treatment with IgG1 control, amivantamab, gefitinib, or osimertinib for 72 hours, cell viability was measured by quantifying the total amount of ATP using the CellTiter-Glo® 2.0 assay kit (Promega) according to the manufacturer’s instructions.

**Colony formation assay**

Cells were seeded onto 6-well culture plates and incubated for 12 days at 37°C with amivantamab (0, 0.1, or 1 mg/mL). Cells were washed with phosphate-buffered saline (PBS), fixed, and stained with 4% paraformaldehyde in 5% crystal violet for 10 mins. Colonies were eluted with 1% sodium dodecyl sulfate, and the optical density value was determined using ELISA at 470 nm.

**Antibody-dependent cellular cytotoxicity assays**

The ADCC assay was conducted using the Lactase Dehydrogenase (LDH) Cytotoxicity Detection Kit (Roche) in accordance with manufacturer's instructions. Human PBMC obtained from healthy volunteers were used as the effector cells. ADCC was conducted using an effector : target (E:T) cell ratio ranging from 50:1 to 5:1 and incubated for 4 to 24 hours at 37 °C in 5% CO$_2$. Amivantamab concentrations of 100 µg/mL to 0.01 µg/mL were tested. The lactate dehydrogenase
activity of the cell culture supernatants was measured, and the percentage cytotoxicity was calculated as described in the manufacturer's protocol.

**Immunofluorescence analysis**

PDCs were seeded on 0.01% poly-L-lysine (Sigma-Aldrich) coated coverslips. The following day, cells were treated with IgG1 control or Amivantamab at 0.1 mg/mL. After 72 hours, the coverslips were fixed in 4% formaldehyde for 15 minutes, permeabilized with 0.5% Triton X-100 for 5 minutes and incubated with primary antibody for 1 hour at room temperature. The primary antibodies used in the study were rabbit monoclonal anti-EGFR and anti-cMet (Santa Cruz Biotechnology) and ab992 (Millipore) at a dilution of 1:100. The coverslips were rinsed twice with PBS, followed by incubation with the appropriate fluorophore-conjugated secondary antibody (Invitrogen) for 1 hour at room temperature. The cells were counterstained with 4′,6-diamidino-2-phenylindole (DAPI; 300 nmol/L; Invitrogen), and the coverslips were mounted on slides using Faramount aqueous mounting medium (DAKO).

**Immunohistochemistry**

Immunohistochemistry was performed using the automated staining system (BOND Rx, Leica Biosystems). Briefly, 4-mm paraffin-embedded tumor sections were deparaffinized and rehydrated. Slides then underwent heat-induced epitope retrieval with citrate buffer at 100°C for 20 min. Antibodies were used at 1:100 dilution and hematoxylin solution was used for counterstaining. Stained slides were visualized with a Vectra Polaris and the Phenochart program.

**In vivo pharmacodynamic study**

Mice bearing tumor tissues were treated with vehicle, IgG1 control, or Amivantamab (10 or 30 mg/kg) twice per week intraperitoneally (i.p), or cetuximab (10 mg/kg), poziotinib (1 mg/kg) once daily. The tumor samples were collected 48 hours after 15 days of treatment, and EGFR and cMet
downstream signaling was evaluated by immunoblotting.

Patients

The clinical study (NCT02609776) was approved by appropriate Institutional Review Boards at each participating site and conducted in accordance with Good Clinical Practice guidelines and the ethical principles of the Declaration of Helsinki. All patients provided written informed consent.

Statistical analysis

Data were collected from three independent experiments and presented either descriptively or analyzed by one-way ANOVA, followed by the Dunnett’s test or Student’s t-test. Dose-response curves were prepared using the GraphPad Prism (Ver. 5, GraphPad Software Inc.).

Disclosures

JY, SYK, SYJ, JHK, KHP, CWP, SGH, and SL are employees of Severance Biomedical Science Institute, Korea. SHL and MRY are employees of JE-UK. Institute for Cancer Research, Republic of Korea. SML, MHH, HRK, and BCC are employees of Yonsei University College of Medicine, Korea. MT, JCC, REK, MVL, and AR are employees of Janssen research and development, USA and may hold company stocks.

Author contributions

JY, BCC, and SHL were involved in the conception and design of this study. JY and BCC were involved in the design of the study and data analyses. SYK, SYJ, JHK, KHP, CWP, SGH, MRY, and SL were involved in the data analyses. SML, MHH, HRK, MT, JCC, REK, MVL, and AR were involved in data interpretation, manuscript development and critical evaluation of the manuscript. All authors approved the final manuscript for submission, had full access to all the data in this study and take final responsibility for the decision to submit it for publication.
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Conflict of interest statement

None declared.

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FIGURE LEGENDS

Fig. 1. Amivantamab shows antitumor activity and suppresses EGFR and cMet signaling pathways in Ba/F3 cells with EGFR Exon20ins mutations

A, Schematic of the structure of amivantamab, an EGFR and cMet bispecific antibody. B, Schematic of EGFR Exon20 insertions in stable Ba/F3 cells, PDC, PDO, and PDX models. C, The viability of Ba/F3 cells was determined via CellTiter-Glo. Amivantamab, gefitinib, or osimertinib were treated for 72 hours. Data are presented as averages ± SD of triplicate independent experiments. *P < 0.0001, **P < 0.001; Student's t-test. D, Ba/F3 cells overexpressing the indicated EGFR Exon20ins mutations were treated with amivantamab for 72 hours at the indicated concentrations. E, Ba/F3 cells overexpressing the indicated EGFR Exon20ins mutations were treated with osimertinib or gefitinib for 6 hours at the indicated concentrations. Immunoblot analysis was performed for EGFR, cMet, AKT, ERK, and S6 expression after amivantamab treatment. Amivantamab inhibited the cell cycle and induces synergistic apoptosis in Ba/F3 cells overexpressing the EGFR Exon20ins mutations. F, Amivantamab induced G1 arrest after amivantamab treatment for 72 hours in Ba/F3 cells overexpressing the indicated types of EGFR Exon20ins mutations. Cell cycles were analyzed using PI staining and FACS analysis. Data are presented as averages ± SD of triplicate independent experiments. *P < 0.0001; Student's t-test. G, BIM- and caspase-dependent apoptosis were induced during amivantamab treatment. The expression of BIM and cleaved caspase 3 were detected by Western blotting.

Fig. 2. Amivantamab has antitumoral activity and suppresses EGFR and cMet signaling pathways in Patient-Derived Cells (PDCs) and Organoids (PDOs) harboring EGFR Exon20ins mutations

A, PDCs with the indicated EGFR Exon20ins mutations were treated with amivantamab for 72 hours at the indicated concentrations. Immunoblot analysis was performed for EGFR, cMet, AKT, ERK, and S6 expression after amivantamab treatment. B, The viability of PDCs was determined via CellTiter-Glo. Amivantamab was treated for 72 hours. Data are presented as averages ± SD of triplicate independent experiments. *P < 0.0001, **P < 0.001; Student's t-test. C, Effects of amivantamab on the colony formation and cell proliferation of PDCs. Representative images and quantitative analysis of the colony formation assay. Data are presented as averages ± SD of triplicate independent experiments. *P < 0.0001, **P < 0.001; Student's t-test. D-E, Dose-response curves of D, YUO-036 (A767_V769dup) and E, YUO-029 (S768_D770dup) PDOs treated with IgG1 control or amivantamab. Cell viability was measured using CellTiter-Glo 3D cell viability reagent 72 hours after drug treatment. Representative images of PDOs treated with amivantamab for 72 hours at the indicated concentrations. Data are presented as averages ± SD of triplicate independent experiments. *P < 0.0001; Student's t-test.
Fig. 3. Amivantamab strongly promotes internalization of EGFR and cMet in Ba/F3 and PDC cells expressing EGFR Exon20ins mutations.

The expression of EGFR and cMet on the cell surface were determined by FACS analysis. A, EGFR expression on the plasma membrane was detected in Ba/F3 cells overexpressing D770delinsGY and H773_V774insH at the indicated time. After 0.1 mg/mL IgG1 control or 0.1 mg/mL amivantamab treatment for 72 hours, PE-EGFR and FITC-cMet expression on the plasma membrane was detected in B, DFCI-127 (P772_H773insPNP) and C, DFCI-58 (H773_V774insNPH) cells. D, Amivantamab-induced redistribution of EGFR and cMet in DFCI-127 PDCs. Immunofluorescence staining for EGFR (green) and cMet (red) in a panel of DFCI-127 treated with 0.1 mg/mL IgG1 control or 0.1 mg/mL amivantamab for 72 hours. E, Pre-treatment with the autophagy inhibitor bafilomycin (100 nM) for 30 min rescued the decreased EGFR expression in 1 mg/mL amivantamab treated Ba/F3 cell lines overexpressing D770delinsGY or H773_V774insH

Fig. 4. Amivantamab reduces tumor burden in Ba/F3 cells and PDCs with EGFR Exon20ins xenograft models

Antitumor effects of amivantamab in (A-C) Ba/F3 cells overexpressing D770delinsGY- or H773_V774insH-bearing NOG mice and (D-I) DFCI-127- or YU-1163-bearing NOG or BALB/c nude mice, respectively. Mice were treated with vehicle, IgG1 control, or amivantamab twice per week i.p. injections dosing with 30 mg/kg. Data represent the mean ± SEM (n = 5/group). *P < 0.0001 vs. vehicle or IgG1 control; B, E, and G, A waterfall representation of the response of each tumor taken on the last day of treatment in the xenograft mice. C, H, and I, Tumor lysates of vehicle- or amivantamab-treated Ba/F3 cells or PDCs xenograft mice were harvested and subjected to immunoblotting for p-EGFR (Y1068), EGFR, p-cMet, and cMet.

Fig. 5. Amivantamab has superior ADCC activity compared to cetuximab

A, Amivantamab-mediated ADCC activity against NSCLC PDCs expressing EGFR Exon20ins mutations using PBMC, E : T (50 : 1) ratio. ADCC assays were performed using DFCI-127 and YU-1163 PDCs as targets in the presence of IgG1 control, amivantamab, or cetuximab at various concentrations. PBMCs were co-cultured for 4 hours with PDCs. B, Amivantamab-mediated cytotoxicity against DFCI-127 and YU-1163 PDCs. PDCs were treated with IgG1, amivantamab (10 µg/ml) or cetuximab (10 µg/ml) for 24 hours in the presence or absence of PBMC, E : T (5 : 1) ratio. C, Quantitative analysis of the cells shown in the representative images. (n = 3). *P < 0.0001, **P < 0.001. D, Pre-treatment with Fc receptor blocker with PBMC (E : T ratio = 50 : 1) reduced the amivantamab (10 µg/ml)-mediated ADCC effects. *P < 0.0001. Data are presented as averages ± SD of triplicate independent experiments. E, IFN-γ (pg/ml) levels in the cell culture media were detected by ELISA. The PDCs were co-cultured with PBMCs in the presence of IgG1, amivantamab, or cetuximab at 1 µg/ml for 4 hours and the culture medium was used for detection of IFN-γ, *P < 0.0001 vs. cetuximab at the same concentration. F, PBMCs pretreated with Fc receptor blocker reduced the
IFN-γ level in the culture medium in the presence of amivantamab (10 µg/ml), *P<0.0001, **P<0.001. Data are presented as averages ± SD of triplicate independent experiments. G, Immunohistochemical staining for mF4/80 (macrophages) and mNKp46 (NK cells) of tumor sections in YHIM-1029 PDX-bearing BALB/c nude mice following 10 mg/kg IgG1 or 10 mg/kg amivantamab treatment.

Fig. 6. Amivantamab reduces tumors in a PDX model with D770_N771insG EGFR mutation. A, Sanger sequencing data depicting the D770_N771 insG mutations of the EGFR gene in a PDX model. B, Patient-derived tumors implanted in BALB/c nude mice were treated with vehicle, amivantamab (10 mg/kg), cetuximab (10 mg/kg), twice per week, i.p. injections or poziotinib (1 mg/kg), Q.D. Data represent the mean ± SEM (n = 7/group). *P < 0.0001. Western blot for the downstream signaling pathways of EGFR, cMet, and apoptosis markers in tumors obtained from YHIM-1029 PDX models treated with C, 10 mg/kg amivantamab, D, 10 mg/kg cetuximab, or 1 mg/kg poziotinib. E, Histopathological examination of tumor sections obtained from the PDX models following 10 mg/kg amivantamab or vehicle treatment. H&E staining and immune histochemical staining for EGFR, p-EGFR, cMet, p-cMet, Ki-67, and TUNEL.

Fig. 7. Amivantamab reduces tumors in NSCLC patients with EGFR Exon20ins mutations
Radiologic response following amivantamab 1050 mg treatment in A, a 58-year old patient with the EGFR H773delinsNPY mutation and B, a 48-year old patient with the EGFR S768_D770dup mutation. C, A proposed model of diverse antitumor mechanisms of amivantamab in NSCLC with EGFR Exon20ins.
**Figure 2**

A. Western blot analysis of various cell lines (DFCI-127, DFCI-58, YU-1163) showing expression levels of EGFR, p-EGFR, cMET, p-cMET, AKT, p-AKT, ERK, p-ERK, S6, p-S6, with GAPDH as a loading control.

B. Graph illustrating cell viability (%) as a function of IgG1 and Amivantamab concentrations for DFCI-127, DFCI-58, YU-1163.

C. Colony formation assay showing the effect of Amivantamab on cell proliferation for DFCI-127, DFCI-58, YU-1163.

D. Cell viability (%) assay for YUO-036 (A767_V769dup) treated with IgG1 control and Amivantamab.

E. Cell viability (%) assay for YUO-029 (S768_D770dup) treated with IgG1 control and Amivantamab.
Figure 3

A

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*% MFI (Median Fluorescence intensity)

B

DFCI-127 (P772_H773insPNP)

C

DFCI-58 (H773_V774insNPH)

D

E

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Amivantamab (1 mg/ml)

Bafilomycin (nM)

EGFR

GAPDH
Figure 6

A

YHIM-1029 (D770_N771insG)

B

YHIM-1029

C

YHIM-1029 (D770_N771insG)

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D

YHIM-1029 (D770_N771insG)

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E

YHIM-1029

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</table>
Figure 7

A

CASE #1

Pre  Amivantamab  Post

EGFR H773delinsNPY
ORR PR (-65% tumor reduction)

B

CASE #2

Pre  Amivantamab  Post

EGFR S768_D770dup
ORR PR (-38.9% tumor reduction)

C

NK cells

③ Direct inhibition of tumor growth mediated by ADCC and ADCP

NSCLC with EGFR Exon20ins

Amivantamab

④ Blockade of EGFR and cMET downstream signaling pathways by internalization of EGFR and cMET

Osimertinib  Gefitinib
Antitumor Activity of Amivantamab (JNJ-61186372), an EGFR-cMet Bispecific Antibody, in Diverse Models of EGFR Exon 20 Insertion-Driven NSCLC

Jiyeon Yun, Soo-Hwan Lee, Seok-Young Kim, et al.

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