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ARGX-111 depletes MET-expressing circulating tumor cells via enhanced ADCC, resulting in inhibition of metastasis

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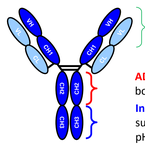
ABSTRACT

Several lines of experimental evidence suggest that Hepatocyte Growth Factor (HGF) and its receptor MET play an important role in breast cancer progression and drug resistance. To date, targeted MET inhibitors in clinical development have primarily shown cytostatic rather than cytotoxic effects. Development of a cytotoxic MET inhibitor would serve to complement standard breast cancer therapy, especially when administered in the adjuvant/neo-adjuvant setting. We have developed ARGX-111, a human antibody antagonist of MET function. ARGX-111 blocks both HGF-dependent and -independent signaling, down-regulates tumor cell surface expression of MET and kills MET-overexpressing cells by enhanced antibody-dependent cellular cytotoxicity (ADCC). ARGX-111 was shown to be more efficacious than an ADCC-inactive control antibody in both HGF-dependent and -independent tumor xenograft models. ADCC reporter assays confirmed the cytotoxic effects of ARGX-111 in patient-derived primary tumor specimens, including MET-expressing breast cancer stem-like cells. In an orthotopic mouse model of metastatic mammary carcinoma (MDA-MB-231), adjuvant or neo-adjuvant treatment with ARGX-111 was dramatically more effective in depleting circulating tumor cells (CTCs) and suppressing the development of bone and lung metastases than the ADCC-inactive control. These results provide a rationale for clinical investigation of ARGX-111 in the early breast cancer setting. An ongoing Phase 1 study (NCT02055066) is examining the effects of ARGX-111 on CTCs, alongside the assessment of its safety and efficacy.

REFERENCES

Hultberg *et al*., 2015, Cancer Research **15**;75(16):3373-83

ARGX-111: an ADCC-enhanced anti-MET antibody



ARGX-111

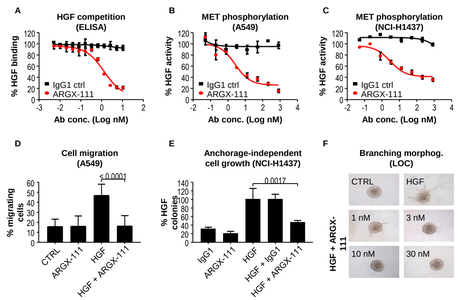
Germlining to 95% human identity
8 aa subst. in the VL framework region
9 aa subst. in the VH framework region

ADCC enhancement: defucosylation increases affinity for both V158 FcγRIIIa and F158 FcγRIIIa

Increased stability and transport: H433K/N434F subst. in the CH3 region increase affinity for FcRn at pH 6.0 thus enhancing recycling from the endosome

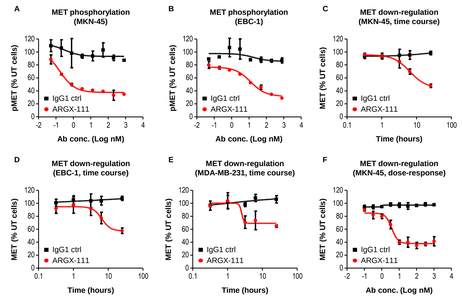
ARGX-111 was generated by genetic engineering of WT52, a chimeric llama-human HGF-competing antibody that displays 88% average human identity. WT52 was germlined to 95% human identity by substituting key residues in the VH and VL regions (G52). G52 was further engineered by introducing the H433K/N434F substitutions in the CH3 region to increase binding to human FcRn at acidic pH while not affecting its affinity at neutral pH, thus enhancing antibody recycling from the sorting endosome (G52-HN). G52-HN was produced in a GS-CHO cell line lacking alpha-1,6-fucosyltransferase, thus obtaining a defucosylated G52-HN antibody (ARGX-111). Antibody defucosylation is known to increase affinity for human FcγRIIIa, thus resulting in enhanced ADCC.

ARGX-111 inhibits HGF-dependent MET activity by competing with HGF for binding to MET



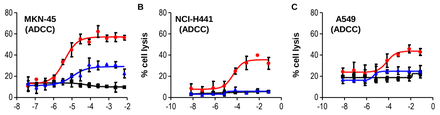
ARGX-111 competes with HGF for the binding to MET, thus impairing HGF-dependent activities. (A) The competition between HGF and ARGX-111 for binding to MET was determined by ELISA. Data are expressed as % HGF binding in the absence of antibody. **(B)** The ability of ARGX-111 to inhibit HGF-induced MET auto-phosphorylation was determined using A549 cells. Data are expressed as % phospho-MET levels relative to cells stimulated with HGF alone. **(C)** Same as in B but using NCI-H1437 cells. **(D)** Boyden chamber migration assay using A549 cells. Data are expressed as % migrating cells relative to total cell number. **(E)** Anchorage-independent assay using NCI-H1437 cells. Colony number is expressed as % colonies relative to cells stimulated with HGF alone. **(F)** LOC cell spheroids were seeded in collagen and then stimulated with a fixed dose of recombinant HGF in the presence of increasing concentrations of ARGX-111. Branching morphogenesis was assessed by microscopy.

ARGX-111 inhibits HGF-independent MET activity by promoting receptor downregulation



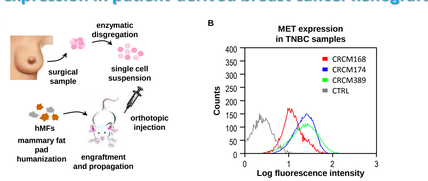
ARGX-111 promotes MET down-regulation from the plasma membrane resulting in inhibition of HGF-independent MET activity. The ability of ARGX-111 to inhibit HGF-independent MET auto-phosphorylation was determined using MKN-45 **(A)** and EBC-1 **(B)** cells. Data are expressed as % phospho-MET levels compared to untreated (UT) cells. Flow cytometry analysis of ARGX-111-mediated MET downregulation in MKN-45 **(C)**, EBC-1 **(D)** and MDA-MB-231 **(E)** cells. Data are expressed as % MET levels compared to untreated cells. **(F)** Dose-response analysis of ARGX-111-mediated MET down-regulation in MKN-45 cells.

ARGX-111 kills MET-expressing cancer cells by ADCC



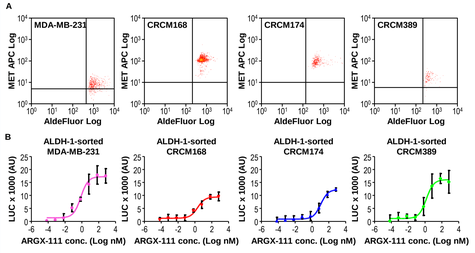
ARGX-111 kills MET-expressing cancer cells by enhanced ADCC. The ability of ARGX-111 to kill cancer cells by ADCC was analyzed using the MKN-45 **(A)**, NCI-H441 **(B)** and A549 **(C)** human tumor cell lines. Cells were incubated with increasing concentrations of ARGX-111 (red line) in the presence of NK cells, and tumor cell lysis was determined using a standard 51Cr-release assay. An irrelevant IgG1 (black line) and the fucosylated G52 (blue line) antibody were used as controls.

MET expression in patient-derived breast cancer xenografts



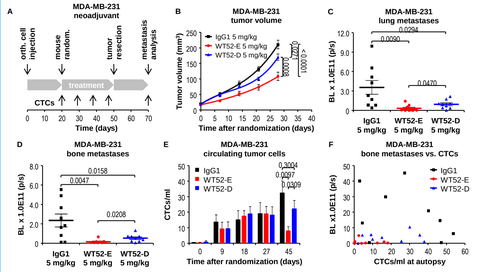
Patient-derived breast cancer xenografts express MET. (A) Tumor cell suspensions obtained by enzymatic digestion of patient-derived mammary carcinoma samples are injected orthotopically into the mammary fat pad of a NOG mouse that has been humanized with human mammary fibroblasts (hMFS). Once engrafted, tumors are propagated from mouse to mouse using the same technique. **(B)** MET expression analysis of cells derived from the vital xenograft library described in A.

ARGX-111 kills MET-expressing breast cancer stem cells



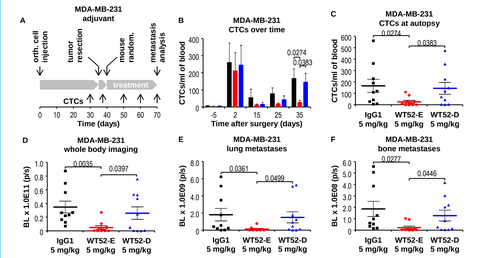
ARGX-111 kills breast cancer stem cells expressing Met. (A) Flow cytometry analysis of MET expression in Aldefluor+ mammary carcinoma cells. **(B)** Met-targeted ADCC kills mammary carcinoma stem cells *in vitro*. ALDH+ sorted cells from MDA-MB-231, CRCM168, CRCM174, CRCM389 were incubated with increasing ARGX-111 concentrations (0-667 nM) in the presence of bioluminescent ADCC reporter cells, and ADCC was determined by analyzing luciferase activity.

ARGX-111 inhibits tumor growth and metastasis in a neoadjuvant orthotopic mammary carcinoma model



Met-targeted ADCC impairs tumor growth and metastasis in a neo-adjuvant orthotopic breast cancer model. (A) Schematic protocol representation. MDA-MB-231 cells expressing luciferase (MDA-MB-231-luc) were injected into the mammary fat pad of SCID along with HGF-secreting, immortalized human mammary fibroblasts. Tumor-bearing mice were randomly assigned to 3 treatment arms (IgG1; WT52-E; WT52-D), and antibodies were administered at a dose of 5 mg/kg. After 4 weeks of treatment, tumors were surgically removed, and neo-adjuvant therapy was interrupted. Two weeks after surgery, mice were injected with luciferin, sacrificed, and subjected to autopsy. Metastatic dissemination was determined by bioluminescence analysis of isolated lungs and femurs. **(B)** Analysis of tumor volume over time. Statistical significance was calculated by a Student *t test*. **(C)** Pulmonary metastases as assessed by lung bioluminescence (BL). **(D)** Bone metastases as assessed by femur bioluminescence. **(E)** CTCs over time as assessed by blood luciferase activity. **(F)** At autopsy, CTC number correlated directly with the extent of metastatic dissemination and inversely with anti-MET treatment.

Adjuvant ARGX-111 therapy suppresses metastasis in an orthotopic model of breast cancer by eliminating CTCs



Adjuvant ARGX-11 treatment of breast cancer effectively reduces metastasis dissemination by eliminating CTCs. (A) Schematic protocol representation. MDA-MB-231-luc cells and HGF-secreting human fibroblasts were injected orthotopically into the mammary fat pad of SCID mice, and CTCs were determined at regular time intervals. Primary tumors were removed by surgery 5 weeks after cell injection. Two days after surgery, mice were randomized based on CTC number and assigned to 3 treatment arms as above (IgG1; WT52-E; WT52-D; 5 mg/kg). After 4 weeks of treatment, mice were sacrificed and metastatic dissemination was assessed by bioluminescence analysis of whole body and isolated organs. **(B)** Analysis of CTC number over time. Statistical significance was calculated by a Student's T-test. **(C)** CTC number at autopsy. **(D)** Whole body bioluminescence (BL) at autopsy. **(E)** Lung bioluminescence at autopsy. **(F)** Femur bioluminescenceat autopsy.

ARGX-111 Phase 1 clinical trial in advanced solid tumors (NCT02055066)

Trial objectives

Primary:
- To determine the MTD and recommended Phase 2 dose (RP2D) of ARGX-111

Secondary:
- Characterize safety profile of ARGX 111
- Characterize pharmacokinetics (PK) and immunogenicity of ARGX 111
- Characterize biomarkers of ARGX-111 activity (PD)
- Document preliminary evidence of antitumor efficacy

Inclusion criteria

- Age ≥18 years
- Written informed consent
- Histological/cytological diagnosis of cancer
- Cancer relapsing after or refractory to standard therapy
- Over-expression of c-Met (> 50% of cells; 2+/3+ intensity)
- ECOG performance status of 0 or 1
- Adequate haematological hepatic and renal function
- At least one tumor lesion > 2 cm on 18F-FDG PET/CT

Patients characteristics (n=18)

Gender M:F	11:7
Median age	56 (28-71)
Prior therapy	Chemo (89%)
	Targeted (TKI) (28%)
	Biological (mAbs) (33%)
Histologies	4 gastric/esophagus
	3 RCC
	3 pancreas
	2 NSCLC
	2 cervix
	1 breast
	3 others

Drug exposure and safety profile

Dose escalation: 0,3- 1- 3- 10 mg/kg (N= 2-2- 11- 3 respectively) and received a total of 57 cycles (median = 2; range 1-9)

Two dose-limiting events were observed in the 10 mg/kg cohort. MTD is established at 3 mg/kg IV q3 weeks. Most common drug-related adverse events are: Infusion Related Reactions (IRR) (70%), fatigue (29%), nausea (24%), back pain (18%), arthralgia (18%) and myalgia (18%). Most common drug related grade 3 are IRR, back pain and pain in extremities.

Mixed metabolic response in gastric patient with *MET* amplification

50 year old; metastatic gastric cancer with metastasis to bone and lymph nodes

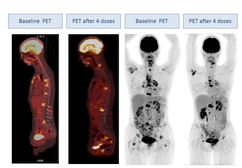
Only patient with c-Met amplification on study (1/16)

2 prior lines of triplet chemotherapy

Patient treated with 0,3 mg/kg ARGX-111 (escalated to 1 mg/kg after 2 cycles)

CTCs reduced by 75%

ECOG performance status maintained



CONCLUSIONS

- ARGX-111 is an antagonistic mAb that (i) potently competes with HGF for binding to MET, (ii) promotes MET down-regulation, (iii) engages NK cells to kill MET-expressing cancer cells
- ADCC enhancement results in improved inhibition of tumor growth and metastasis compared to plain HGF/MET blockade in both HGF-dependent and -independent tumor models
- Triple negative breast cancer stem cells express MET and can be killed by MET-targeted ADCC
- MET-targeted ADCC is a valid therapeutic strategy to eradicate circulating metastasis-initiating cells in either the neoadjuvant or adjuvant setting
- Early signs of activity observed in patient with gastric c-Met amplified cancer by PET/CT scan and CTC reduction. This confirms the clinical mechanism of action of ARGX-111