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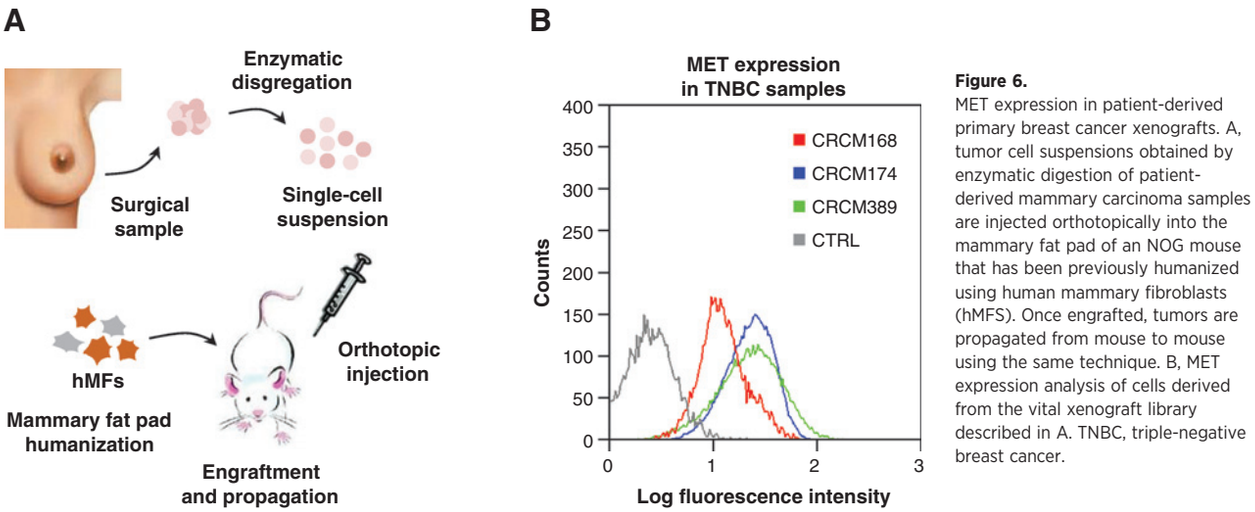

Figure 6.
MET expression in patient-derived primary breast cancer xenografts. A, tumor cell suspensions obtained by enzymatic digestion of patient-derived mammary carcinoma samples are injected orthotopically into the mammary fat pad of an NOG mouse that has been previously humanized using human mammary 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. TNBC, triple-negative breast cancer.

JNJ-38877605 used in these experiments achieved complete MET kinase blockade in all cells (Supplementary Fig S6C).

ARGX-111 kills MET-expressing cancer cells in the presence of NK cells

We investigated whether ARGX-111 could kill cancer cells by ADCC using a representative panel of human tumor cell lines expressing different MET levels. Cells were incubated with increasing concentrations of ARGX-111 (0-1,000 nmol/L) in the presence of NK cells, and tumor cell lysis was determined by a standard ^{51}Cr-release assay. An irrelevant IgG1 and the fucosylated G52 antibody were used as controls. Each assay was repeated using effector cells derived from three different healthy donors (Supplementary Table S6). This analysis revealed that ARGX-111 induces dose-dependent lysis of all tumor cell lines tested with an efficacy directly proportional to MET protein expression levels. The fucosylated G52 antibody displayed only a modest ADCC activity on MKN-45 cells, which express very high MET levels, but did not have any effect on the other cell lines tested (Supplementary Fig. S7A). We also analyzed ARGX-111-induced antibody-dependent cellular phagocytosis (ADCP) using MKN-45 cells or 786-O human renal cell carcinoma cells and human monocyte-derived macrophages. Consistent with our finding that afucosylation does not significantly change antibody affinity for FcγRI (Supplementary Table S4), ARGX-111 did not display increased ADCP compared with G52 in any condition tested (Supplementary Fig. S7B).

MET-targeted ADCC kills mammary carcinoma stem cells

The results obtained in the orthotopic mammary carcinoma models indicate that MET-targeted ADCC impairs the ability of CTCs to colonize distant organs and to give rise to metastases. Because only tumor cells with stem-like characteristics are competent to originate secondary colonies, we hypothesized that the mammary carcinoma stem cell population may express MET. To test this hypothesis, we analyzed coexpression of MET with established breast cancer stem cell markers (33) in MDA-MB-231 tumors as well as in a vital library of patient-derived breast xenografts (CRCM tumors; ref. 25) generated by implantation of primary mammary carcinomas into immunodeficient mice (Fig. 6A). Interestingly, all triple-negative tumors from this library expressed MET (Fig. 6B). Flow-cytometry analysis of CD24, CD44 and MET unveiled that the majority of cells in the CD44^{+}/CD24^{lo} population express MET in both the MDA-MB-231 cell line (99%) and the primary breast carcinoma samples (78%–98%; Supplementary Table S7). Similarly, dual staining with AldeFluor reaction and anti-MET antibodies revealed that ALDH-1–positive tumor cells express MET in all samples analyzed (Fig. 7A). Prompted by these data, we determined whether MET-targeted ADCC could kill mammary carcinoma stem cells *in vitro*. To this end, cells derived from MDA-MB-231, CRCM168, CRCM174, and CRCM389 tumors were stained with AldeFluor and then sorted by FACS. Sorted cells were incubated with increasing ARGX-111 concentrations (0–667 nmol/L) in the presence of bioluminescent ADCC reporter cells (34), and ADCC was determined by analyzing luciferase activity. Remarkably, ARGX-111 promoted dose-dependent ADCC of all stem cell populations analyzed (Fig. 7B). We conclude that MET-targeted ADCC may be an effective method to eliminate breast cancer stem cells.

Discussion

HGF and its receptor MET have been attracting increasing interest in the last two decades as appealing targets for cancer therapy. This interest has led to the development of numerous targeted drugs, some of which have reached the clinic. The initial enthusiasm has been partially mitigated, however, by the realization that anti-HGF/MET drugs display cytostatic rather than cytotoxic activity in most systems, including cell-based assays, mouse models of cancer, and oncologic patients. In this study, we report the engineering of an antagonistic anti-MET antibody that, in addition to inhibiting HGF/MET signaling, kills MET-expressing tumor cells by enhanced ADCC. The data presented here suggest that depleting MET-positive cancer cells is therapeutically more effective than simply inhibiting HGF/MET signaling.

These results must be interpreted at the light of the role played by MET in cancer biology. It has been shown that tumors take advantage of HGF/MET signaling in two different situations, determined by the number of c-*MET* gene copies contained in their genome (22). Tumors bearing normal c-*MET* gene copy-number exploit paracrine HGF stimulation to proliferate in adverse

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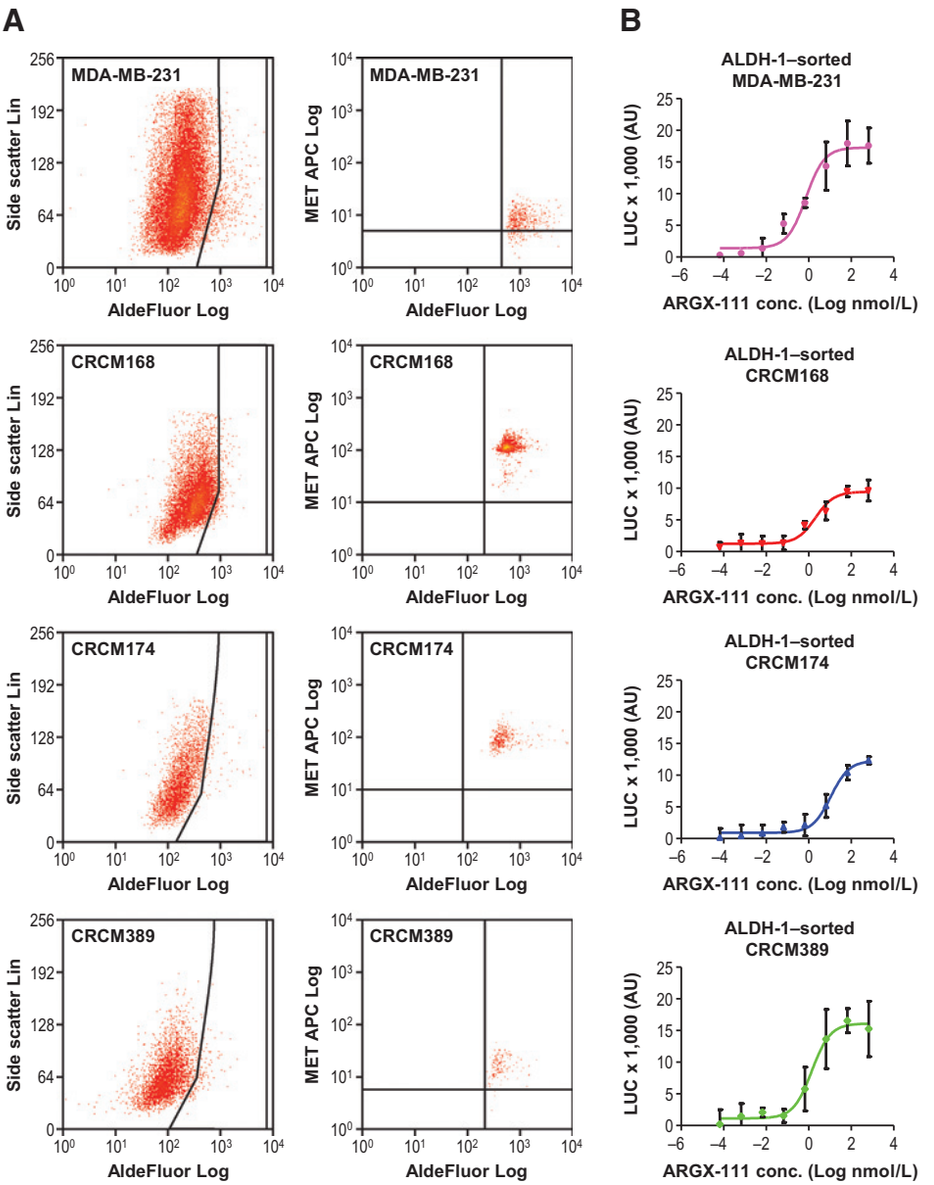

Figure 7.
MET-targeted ADCC kills mammary carcinoma stem cells. A, flow-cytometry analysis of MET expression in ALDH-1$^+$ mammary carcinoma cells. B, ADCC assay using ALDH-1–sorted cells derived from enzymatic digestion of the tumors described in A. ADCC was determined using bioluminescent ADCC reporter cells. LUC, luciferase; AU, arbitrary units.

environmental conditions and to invade adjacent tissues, but do not strictly depend on HGF/MET signaling for their own survival. In this setting, inhibition of HGF/MET signaling attenuates proliferation and invasion, but does not eliminate MET-expressing cancer cells per se. On the other hand, tumors displaying high-grade focal c-*MET* amplification totally rely on constitutive HGF-independent MET signaling, resulting from MET protein overexpression, to sustain their proliferation and EMT phenotype (35, 36). In these tumors, MET inhibition leads to G_1 arrest and to transient reversion of EMT; however, both proliferation and EMT can be resumed upon removal of MET blockade (20). Therefore, whether we are hindering HGF-mediated oncogenic "expedience" in tumors bearing normal c-*MET* gene copy-number or MET-dependent oncogenic "addiction" in c-*MET*–amplified lesions, we are temporarily preventing tumor cells to carry out their malignant program, but we are not eradicating them.

This notion acquires high therapeutic relevance in light of the emerging evidence that MET is expressed and functionally required in the stem cell compartment of several tumor types, including head and neck carcinoma (37), colorectal carcinoma (38), prostate cancer (39), breast cancer (40), and glioblastoma multiforme (41). In analogy with normal tissue stem cells, cancer stem cells represent a multipotent tumor cell subpopulation that possesses the ability to replicate indefinitely and to give rise to secondary tumor colonies with the full differentiation spectrum of the primary lesion. Circulating cancer cells with stem-like characteristics are the only cell type that can give rise to metastases and are often drug resistant (42). Following therapy interruption, residual cancer stem cells that survived treatment may resume their proliferative cycle, reconquer space in the host organism, and give rise to malignant lesions, resulting in new metastases or tumor recurrence (43). From a therapeutic viewpoint, killing

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rather than simply inhibiting the proliferation of cancer stem cells may be associated with a greater patient benefit.

The possibility to intercept MET-positive CTCs with stem-like properties generates a new paradigm for the treatment of MET-positive tumors. So far, MET-targeted therapy has been envisioned either for causing the regression of established HGF/MET-dependent lesions or for overcoming HGF/MET-driven resistance to a second anticancer drug (44). The data presented here suggest that these strategies may not exploit the full therapeutic potential of an ADCC-enhanced anti-MET antibody, and point at the neoadjuvant and adjuvant settings as valid alternative approaches. Indeed, CTCs are ultimately responsible for tumor recurrence or metastasis onset following surgery, independently of how well the original lesion has responded to a previous therapy. On the basis of the results obtained in our neoadjuvant and adjuvant orthotopic breast cancer models, it would be reasonable to expect that eradication of CTCs by MET-targeted ADCC is associated with prolonged disease-free survival. Importantly, this opportunity is unique to an ADCC-enhanced anti-MET antibody. In fact, inhibition of HGF/MET signaling using a conventional HGF/MET–targeted drug, whether an antibody or a small molecule, would probably be able to prevent the escape of cancer cells from a primary lesion, but would have no effect on the survival of metastasis-initiating cells once they are in circulation, especially if these cells have become resistant to anoikis during their journey from the home epithelium to the breakout vessel.

Although MET plays its most relevant physiologic role during embryo development and remains mostly silent during the adult stage, it is widely expressed in epithelial and endothelial tissues of virtually all organs. This broad expression pattern could raise the concern that MET-targeted ADCC may cause damage to normal tissues, resulting in systemic toxicity. However, toxicologic studies conducted in cynomolgus monkeys (*Macaca fascicularis*) indicate that this is not the case. In fact, although ARGX-111 does not cross-react with mouse MET, it binds with high affinity to simian MET and to simian FcγRIIIa (Supplementary Table S4). In a large study involving 40 adult animals, the no-observed adverse event level of ARGX-111 corresponded to a dose of 30 mg/kg administered weekly (data on file). These results suggest that MET-targeted ADCC may be well tolerated by normal tissues expressing MET and may, therefore, not interfere with housekeeping physiologic functions at doses that are active against MET-dependent tumors. More definitive data on ARGX-111 safety will emerge from the phase I study that has started in January 2014 (NCT02055066).

Disclosure of Potential Conflicts of Interest

A. Hultberg is a senior scientist and has ownership interest (including patents) in arGEN-X. C. Blanchetot is an employee in arGEN-X. M. Saunders is a senior director in arGEN-X. A. Thibault has ownership interest (including patents) in arGEN-X. H. De Haard is a CSO in arGEN-X BVBA. P. Michieli is a consultant/advisory board member in arGEN-X BVBA. No potential conflicts of interest were disclosed by the other authors.

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