Cancer Therapy: Preclinical

Premedication and Chemotherapy Agents do not Impair Imgatuzumab (GA201)-Mediated Antibody-Dependent Cellular Cytotoxicity and Combination Therapies Enhance Efficacy

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Abstract

Purpose: Imgatuzumab (GA201) is a novel anti-EGFR mAb that is glycoengineered for enhanced antibody-dependent cellular cytotoxicity (ADCC). Future treatment schedules for imgatuzumab will likely involve the use of potentially immunosuppressive drugs, such as premedication therapies, to mitigate infusion reactions characteristic of mAb therapy and chemotherapy combination partners. Because of the strong immunologic component of mode of action of imgatuzumab, it is important to understand whether these drugs influence imgatuzumab-mediated ADCC and impact efficacy.

Experimental Design: We performed a series of ADCC assays using human peripheral blood mononuclear cells that were first preincubated in physiologically relevant concentrations of commonly used premedication drugs and cancer chemotherapies. The ability of common chemotherapy agents to enhance the efficacy of imgatuzumab in vitro was then examined using orthotopic xenograft models of human cancer.

Results: A majority of premedication and chemotherapy drugs investigated had no significant effect on the ADCC activity of imgatuzumab in vitro. Furthermore, enhanced in vivo efficacy was seen with imgatuzumab combination regimens compared with single-agent imgatuzumab, single-agent chemotherapy, or cetuximab combinations.

Conclusions: These data indicate that medications currently coadministered with anti-EGFR therapies are unlikely to diminish the ADCC capabilities of imgatuzumab. Further studies using syngeneic models with functional adaptive T-cell responses are now required to fully understand how chemotherapy agents will influence a long-term response to imgatuzumab therapy. Thus, this study and future ones can provide a framework for designing imgatuzumab combination regimens with enhanced efficacy for investigation in phase II trials.

Introduction

The EGFR is activated by mutation or overexpression in a variety of epithelial cancers including colorectal cancer, non-small cell lung cancer (NSCLC), and head and neck squamous cell carcinoma (1, 2), making anti-EGFR strategies an attractive therapeutic option for solid tumors. Imgatuzumab (GA201/RG7160) is a novel humanized anti-EGFR monoclonal antibody (mAb) with a dual mode of action. Binding of imgatuzumab to EGFR inhibits signaling pathways that influence proliferation, survival, and apoptosis in a similar manner to other anti-EGFR mAbs such as cetuximab (3) and panitumumab. However, imgatuzumab is specifically glycoengineered to enhance the binding of the Fc portion of the antibody to FcγRIIIa receptors on the surface of immune effector cells. This results in enhanced in vitro antibody-dependent cellular cytotoxicity (ADCC) activity compared with both the wild-type imgatuzumab antibody and with the non-glycoengineered mAb cetuximab (3). This enhanced cell killing in vitro translated into superior in vivo efficacy compared with cetuximab in a series of orthotopic xenograft models (3). Early clinical experience with single-agent imgatuzumab demonstrated promising clinical efficacy in heavily pretreated patients with advanced EGFR-positive tumors, including patients with KRAS-mutant tumors (4).

Infusion-related reactions (IRR) are common with chimeric and humanized mAbs and chemotherapy agents, such as taxanes and platinum analogues, and premedication to reduce the incidence and severity of these reactions is normal practice (5, 6). IRRs were observed in 76% of patients following the first infusion of single-agent imgatuzumab in a dose-escalation trial and have been reported with 7.6% to 33% of cetuximab infusions (4, 7). As a fully human antibody, the incidence of infusion reactions with panitumumab is much lower (0%–4%; ref. 7). Premedication with corticosteroids and/or antihistamines is mandated for the first infusion of cetuximab and recommended for subsequent cycles (8); similar recommendations are made for rituximab, alemtuzumab, and infliximab (9–11). It is therefore important to establish whether coadministration of premedication drugs might potentially interfere with the mode of action of novel
mAbs. Dexamethasone, a potent glucocorticoid frequently used to premedicate patients, reduces the efficacy of trastuzumab in vitro by reversing trastuzumab-induced cell-cycle arrest (12). Conversely, the opposite effect is seen with rituximab, where dexamethasone exhibits a synergistic effect on the complement-dependent cytotoxicity activity of rituximab and has a minimal effect on ADCC (13). Treatment protocols for imatuzumab will likely involve combination with conventional chemotherapeutic agents as well as premedication drugs. The efficacy of most mAbs is potentiated when administered with cytotoxic or cytostatic drugs (14). Initial studies with single-agent cetuximab in previously treated advanced colorectal cancer achieved modest response rates of 8%–11.6% and time-to-progression/progression-free survival (PFS) in the range of 1.4 to 2.6 months (8, 15–19). When combined with first-line irinotecan- or oxaliplatin-based therapy in KRAS wild-type patients, response rates of 57.3% and a median PFS of 9.6 months were achieved (20). Cetuximab has also demonstrated synergistic effects with platinum compounds (21), gemcitabine (22), paclitaxel (23), and topotecan (24).

Selection of appropriate chemotherapy partners is particularly important for mAbs with a strong immunologic component to their mode of action, such as imatuzumab. Effective induction of ADCC requires recruitment and activation of host immune effector cells including natural killer (NK) cells, macrophages, and monocytes; therefore, cytotoxic or cytostatic drugs with minimum immunosuppressive effects would be preferable. Pachtxel is broadly immunosuppressive at standard doses and inhibits cell types such as macrophages, effector T cells, and NK cells (25). Vinblastine, paclitaxel, docetaxel, cladribine, chlorambucil, and bortezomib have been shown to inhibit NK cell–mediated cell killing in vitro, whereas NK cell–mediated killing was unaffected by therapeutic concentrations of asparaginase, bevacizumab, bleomycin, doxorubicin, epirubicin, etoposide, 5-fluorouracil, hydroxyurea, streptozocin, and 6-mercaptopurine (26). Differential effects of various chemotherapy agents on the cell killing potential of cytotoxic T cells have also been demonstrated (27).

Understanding the effect that previously and coadministered compounds may have on the mode of action of novel mAbs will be important in establishing optimal combinations for clinical investigation.

The aim of this study was to investigate the influence of commonly used premedication drugs and potential chemotherapy partners for imatuzumab on the in vitro ADCC activity of imatuzumab and to investigate the in vivo efficacy of imatuzumab combined with chemotherapy in human orthotopic xenograft models.

Materials and Methods

Cell lines, antibodies, and drugs

Human NSCLC (A549) and colorectal (HT29 and LS174T) adenocarcinoma cell lines were obtained from the ATCC. Cells obtained from ATCC were routinely authenticated by karyotyping, short tandem repeat profiling, assessment of cell morphology, and species verification by isoenzymology. Upon receipt, cells were expanded and frozen; cells were not passaged for more than 6 months after resuscitation. No further authentication of cell lines was conducted. A549 cells express low levels of EGFR and, importantly, mutant KRAS; therefore, these cells are likely to be insensitive to inhibition of EGFR signaling but should remain sensitive to ADCC. LS174T cells are also KRAS mutant, whereas HT29 cells are KRAS wild-type. Cells were routinely cultured at 37°C and 5% CO₂ in a humid environment in DMEM (Gibco) supplemented with 10% FCS (PAA laboratories). Commercial-grade cetuximab was obtained from Merck.

Premedication drugs investigated in this study included the immunosuppressive glucocorticoids hydrocortisone (cortisol; Farmamondo), prednisolone (Farmamondo), and dexamethasone (Sigma); the antiemetics granisetron hydrochloride (Kytril; loratadine 5-HT3 receptor antagonists) and aprepitant (Emend; neurokinin 1 receptor antagonist); the antihistamines ranitidine hydrochloride (histamine H₂-receptor antagonist); the proton pump inhibitor pantoprazole (Pantozol); and vitamin B12 and folic acid (all Farmamondo). Chemotherapy agents included gemcitabine (Gemzar, Lilly), cisplatin (Hexal AG), carboplatin (Teva Pharma AG), irinotecan (Campto, Pfizer), SN-38 (the active metabolite of irinotecan, Farmamondo), doxycycline (Farmamondo), oxaliplatin (Sanofi-Aventis), and paclitaxel (Abraxane, Celgene).

ADCC methodology

Peripheral blood mononuclear cells (PBMC) were obtained from healthy volunteers according to standard methods. All PBMCs employed in this study were obtained from individuals who were homozygous for the low-affinity variant of the human IgG1 receptor (FcyRIIA F/F158). PBMCs were pelleted by centrifugation and resuspended in AIM-V media at a concentration of 2 × 10⁷ cells per flask. Cells were then incubated for 17 to 23 hours at 37°C with premedication drugs and chemotherapy agents diluted in AIM-V media at the following concentrations: 8 μmol/L hydrocortisone, 0.65 μg/mL prednisolone, 0.1 μg/mL dexamethasone, 1 to 100 μg/mL gemcitabine, 0.1 to 10 μg/mL cisplatin, 28 μg/mL carboplatin, 5 to 2.5 μg/mL paclitaxel, 61.6 ng/mL SN-38, 10 μg/mL doxycycline, or 1.5 to 0.3 μg/mL oxaliplatin. Drug cocktails consisted of mix 1 (1 μg/mL cisplatin, 63.8 ng/mL granisetron hydrochloride, 100 ng/mL dexamethasone, 1.6 μg/mL aprepitant, 10 μg/mL pemetrexed, 1.25 μg/mL...
pantoprazole, 600 μg/mL vitamin B12, and 15 ng/mL folic acid) and mix 2 (1 μg/mL cisplatin, 63.8 ng/mL granisetron hydrochloride, 100 ng/mL dexamethasone, 1.6 μg/mL aprepitant, 10 μg/mL gemcitabine, and 94 ng/mL ranitidine hydrochloride).

After preincubation with investigational drugs, PBMCs were washed with AIM-V media, checked for viability with a Vi-cell counter (Beckman Coulter), and used as effector cells in a series of ADCC assays. PBMCs (6 × 10^5 cells per well) were seeded in 96-well plates and incubated with A549 target cells (2.4 × 10^4 cells per well) at effector:target (E:T) ratios of 25:1 (22:1 for carboxatin) and with imgatuzumab or cetuximab concentrations ranging from 0.01 ng/mL to 1,000 ng/mL. After 4 hours at 37°C, supernatants were harvested and cell killing was detected using the Roche lactose dehydrogenase (LDH) cytotoxicity detection kit. All ADCC assays were performed in triplicate and the percentage cell-mediated cytotoxicity was calculated as the average absorbance of the triplicates, from which the absorbance of the background controls (untreated PBMCs) was subtracted. Results were presented as mean ± SD. The t test was used to compare cell killing by treated PBMCs with that of untreated control cells.

Mouse xenograft models
Orthotopic xenograft models were used to assess the effect of chemotherapy combinations and the premedication hydrocortisone on the in vivo efficacy of imgatuzumab. The production and maintenance of these models has been described previously (3). Briefly, female SCID-beige (Taconics) or SCID-huCD16 transgenic (tg) mice (Roche-Glycart) were inoculated with 1 or 5 × 10^6 A549 cells injected intravenously. For the colorectal liver metastases models, the colorectal cancer cell lines LS174T or HT29 were injected into the spleen (3 × 10^6 cells). SCID-beige mice contain monocytes and macrophages that express FcγRI, the murine homolog of human FcγRIIIA. Murine NK cells do not express FcγRII, therefore the SCID/beige mouse model can only demonstrate the antitumoral effect of ADCC (and/or FcγRII-triggered antibody-dependent cellular phagocytosis and cytokine release) mediated by monocytes and macrophages as effectors. Mice were maintained under specific pathogen-free conditions with 12-hour light/dark cycle daily according to guidelines (GV-SOLAS; FELASA), and food and water were provided ad libitum. Continuous health monitoring was carried out and the experimental study protocol was reviewed and approved by the Veterinary Department of Kanton, Zurich.

Mice were randomized into different treatment groups (ten mice per group) and therapy started when evidence of tumor growth was visible in the target organ of sacrificed scout animals (7 days or 14 days after tumor cell inoculation). All treatments were administered intravenously and nontoxic efficacious doses were used as follows: imagatuzumab and cetuximab at a dose of 25 mg/kg, gemcitabine at 150 mg/kg, cisplatin at 2 mg/kg, paclitaxel at 20 mg/kg, carboplatin at 60 mg/kg, and irinotecan at 30 mg/kg. Pretreatment with hydrocortisone (20 mg/kg) was performed 1 hour before the first and second imagatuzumab dose. All mAbs and drugs were given once weekly for 3 weeks (6 weeks for cisplatin). The termination criteria for sacrificing animals was sickness with locomotion impairment, and median overall survival (OS) was defined as the experimental day by which 50% of animals had been sacrificed. Kaplan–Meier survival curves and the Pairwise log-rank test were used to compare survival between animals treated with single-agent imagatuzumab and imagatuzumab in combination with chemotherapy.

Results
Common cancer premedications do not negatively affect imagatuzumab-mediated in vitro ADCC or in vivo efficacy in xenograft models
A series of ADCC experiments was performed to investigate whether commonly used premedication therapies affected the immune effector cells responsible for performing imagatuzumab-mediated cell killing. Incubation of human PBMC effector cells with 8 μM hydrocortisone for 24 hours prior to performing ADCC experiments had no effect on imagatuzumab-mediated killing of A549 lung adenocarcinoma cells (Fig. 1A). The level of imagatuzumab-mediated cell killing achieved by hydrocortisone-pretreated PBMCs was the same as that of untreated PBMCs across a range of antibody concentrations. In contrast, ADCC killing of A549 cells in the presence of cetuximab was decreased slightly when PBMCs were preincubated with hydrocortisone. No significant effect on the cell viability of PBMCs was observed after the incubation period with hydrocortisone, before the ADCC assay.

Pretreatment with hydrocortisone also did not affect the in vivo efficacy of imagatuzumab in a human lung adenocarcinoma model (SCID-beige mice inoculated with A549 cells). The median OS achieved by imagatuzumab was identical when administered as single agent (25 mg/kg weekly for 3 weeks) to untreated animals or to animals pretreated with 20 mg/kg hydrocortisone 1 hour before the first and second infusion of imagatuzumab (P = 0.36; Fig. 2B). Both single-agent imagatuzumab and imagatuzumab administered following hydrocortisone pretreatment achieved significantly superior median OS compared with untreated control mice (P = 0.0004 and P = 0.00004, respectively).

Preincubation of ADCC effector cells with chemotherapy agents at physiologically relevant doses does not affect imagatuzumab-mediated ADCC
The effect of chemotherapy agents on the in vitro ADCC activity and in vivo efficacy of imagatuzumab was investigated using gemcitabine, cisplatin, carboplatin, paclitaxel, and irinotecan. These compounds are currently used clinically to treat solid tumor types in which imagatuzumab is likely to be indicated (colorectal cancer, lung cancer, breast cancer, and head and neck cancer) and are therefore potential partners for imagatuzumab in future combination chemotherapy regimens.

Preincubation of PBMC effector cells in 1 μg/mL gemcitabine had no effect on imagatuzumab- or cetuximab-mediated killing of A549 lung cancer cells across the range of mAb concentrations investigated in ADCC assays (Fig. 2A). Cell killing by PBMCs pretreated with 10 μg/mL gemcitabine was reduced at higher imagatuzumab concentrations compared with controls and ADCC was significantly reduced for both anti-EGFR mAbs when PBMCs were preincubated with high concentrations (100 μg/mL) of gemcitabine (~33% relative reduction in maximal cell killing seen for both mAbs). Preincubation of PBMC effector cells with cisplatin (0.1–10 μg/mL; Fig. 2B) and carboplatin (28 μg/mL; Fig. 2C) had no influence on A549 cell killing mediated by either antibody, whereas the ADCC capacity of both anti-EGFR mAbs was reduced following preincubation of PBMCs with paclitaxel (Fig. 2D). Interestingly, SN-38 (the active metabolite of irinotecan) at a single tested concentration of 61.6 ng/mL had no effect on imagatuzumab-mediated ADCC of KRAS wild-type HT29 colorectal cancer cells but reduced the degree of cell killing mediated...
by cetuximab by approximately 40% (Fig. 2E). No significant effect on the cell viability of PBMCs was observed after the incubation period with the different chemotherapeutic agents measured before the respective ADCC assays.

In all ADCC experiments, the degree of cell killing mediated by imatuzumab was higher than with cetuximab, consistent with previous findings (2).

Table 1 summarizes the EC\textsubscript{50} and maximal cell killing values for all premedication drugs/chemotherapies tested as single agents. Significant reductions in the maximal percentage of ADCC were seen following pretreatment of PBMC effector cells with dexamethasone, prednisolone, gemcitabine, and paclitaxel and maximal cell killing increased significantly when effector cells were preincubated in oxaliplatin. Pretreatment with prednisolone and paclitaxel also significantly increased the EC\textsubscript{50} of imatuzumab.

The in vitro ADCC potential of PBMCs mediated by imatuzumab is relatively unaffected by pretreatment with complex drug mixtures. To further investigate the influence of premedication and chemotherapy agents on imatuzumab activity, ADCC assays were performed using PBMCs preincubated for 20 hours in the presence of two different cocktails (mix 1 and mix 2) of multiple drugs currently used in the clinical setting. At a medium to high concentration of imatuzumab (1–1,000 ng/mL), mix 1 had no significant effect on imatuzumab-mediated ADCC activity (Fig. 3). Cell killing was reduced slightly with mix 2 at higher imatuzumab concentrations. In contrast, cetuximab-induced ADCC was reduced following preincubation of immune effector cells with either mix and at all concentrations of antibody. No significant effect on PBMCs cell viability was observed after the incubation period with both cocktails (mix 1 and mix 2) measured before the ADCC assays.

Coadministration of imatuzumab with cytotoxic agents enhances in vivo efficacy. Combining imatuzumab with chemotherapy agents significantly improved efficacy compared with both single-agent imatuzumab and single-agent chemotherapy. This was seen in all combinations tested (Fig. 4). Median OS of SCID-beige mice inoculated with A549 cells was 115 days when treated with gemcitabine plus imatuzumab compared with 95 days for single-agent imatuzumab (P = 0.05) and 69 days for single-agent gemcitabine (P = 0.05; Fig. 4A). Furthermore, OS with imatuzumab plus gemcitabine was significantly longer than for cetuximab plus gemcitabine (98 days; P = 0.05). Similar data were seen when the same xenograft model was treated with imatuzumab in combination with cisplatin (Fig. 4B). Median OS was longer with combination therapy (118 days) compared with single-agent imatuzumab (84 days; P = 0.003) and single-agent cisplatin (70 days; P = 0.001), and a trend towards longer survival over the cetuximab plus cisplatin group (103 days; P = 0.14) was seen. Median OS of SCID/huCD16 \textsuperscript{tg} mice inoculated with A549 cells was 102 days when treated with paclitaxel plus imatuzumab compared with 76 days for single-agent imatuzumab (P = 0.03) and 60 days for single-agent paclitaxel (P = 0.0001; Fig. 4C). Similar data were seen when the same xenograft model was treated with imatuzumab in combination with carboplatin (Fig. 4D). Median OS was longer with combination therapy (112 days) compared with single-agent imatuzumab (75 days; P = 0.003) or single-agent carboplatin (48 days; P = 0.001).

Combining imatuzumab with irinotecan also improved median OS compared with either agent alone in the colorectal cancer SCID/huCD16 \textsuperscript{tg} xenograft models. In mice inoculated with KRAS wild-type HT29 cells, median OS was 79 days when treated with irinotecan plus imatuzumab compared with 59 days for single-agent imatuzumab (P = 0.03) and 49 days for single-agent irinotecan (P = 0.0001; Fig. 4E). In mice inoculated with KRAS-mutant LS174T cells, median OS was longer with combination therapy (60 days) compared with single-agent imatuzumab (45 days; P = 0.04) and single-agent irinotecan (41 days; P = 0.001; Fig. 4F).

Discussion

The novel anti-EGFR mAb imatuzumab is glycoengineered for boosted ADCC (3). In this study, we demonstrated that common chemotherapy agents and medications used to reduce the risk of therapy side effects had no significant negative effect on imatuzumab-mediated ADCC cell killing in vitro. Moreover, in vivo, improved efficacy was observed when chemotherapeutic agents were combined with imatuzumab compared with mAb therapy.
Antibody-dependent killing of A549 cells (A–D) or HT29 cells (E) by PBMCs preincubated for 17–23 hours with increasing concentration of gemcitabine (A) or cisplatin (B) or a single concentration of carboplatin (C), paclitaxel (D), or SN-38, the active metabolite of irinotecan (E). ADCC (4-hour LDH release assay) mediated by either imgatuzumab or cetuximab was not affected by preincubation of effectors cells with gemcitabine at concentrations up to 10 μg/mL. Pretreatment of PBMCs with 28 μg/mL paclitaxel significantly reduced the maximal killing and EC50 for both mAbs compared with untreated PBMCs; whereas cisplatin and carboplatin had no influence on ADCC for both mAbs at all tested concentrations. SN-38 did not affect imgatuzumab-induced ADCC of HT29 cells but reduced the level of cell killing with cetuximab. PBMC viability measured as follows: before gemcitabine preincubation, 98.9%; after 1 μg/mL, 10 μg/mL, and 100 μg/mL gemcitabine preincubation, 96.7%, 96.9%, and 97%, respectively. Before cisplatin preincubation, 99.4%; after 0.1 μg/mL, 1 μg/mL, and 10 μg/mL cisplatin preincubation, 99.8%, 99.6%, and 99.5%, respectively. Before carboplatin preincubation, 98.4%; after carboplatin preincubation, 98.1%. Before paclitaxel preincubation, 99.8%; after paclitaxel preincubation, 99.7%. Before SN-38 preincubation, 99.8%; after SN-38 preincubation, 97.5%. N = 3.
Table 1. Influence of pretreatments and chemotherapies on the ADCC capability of PBMCs

<table>
<thead>
<tr>
<th>Pretreatment</th>
<th>Concentration used</th>
<th>Hydrocortisone</th>
<th>Dexamethasone</th>
<th>Prednisolone</th>
<th>Gemcitabine</th>
<th>Cisplatin</th>
<th>Carboplatin</th>
<th>Paclitaxel</th>
<th>Oxaliplatin</th>
<th>SN-38 (metabolite of irinotecan)</th>
<th>Doxycycline</th>
<th>Diethylstilbestrol (DES)</th>
<th>Vitamin B12</th>
<th>Folic Acid</th>
<th>SN-38 (metabolite of irinotecan)</th>
<th>Concentration reported</th>
<th>Maximal killing (%)</th>
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<td></td>
<td>Cmax reported</td>
<td>Untreated Treated</td>
<td>29.9</td>
<td>31.6</td>
<td>0.0004</td>
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<td>Untreated</td>
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NOTE: PBMCs were preincubated at concentrations shown for approximately 24 hours after which ADCC was performed using a range of imaging values (ng/mL) in the absence of chemotherapy/premedication drugs (4-hour LDH release assay, different target cells used). Bold text indicates significant differences in cell killing between untreated and untreated PBMCs. N = 3.

Abbreviation: n.c., not calculated.

alone. This in vivo effect was observed in xenograft models without an acquired immune system but in the presence of the innate immune effector cells that mediate ADCC.

Pretreatment of human PBMCs and murine xenograft models with hydrocortisone at concentrations similar to those used clinically had no effect on the ADCC activity or efficacy of imogatuzumab. These data indicate that strategies to reduce the risk and severity of IRRs in the clinic can be potentially achieved with imaging and hydrocortisone without compromising the efficacy of imogatuzumab-based treatment regimens. Future combination regimens will be necessary to assess the ADCC activity of imogatuzumab was relatively unaffected by the presence of multiple premedication drugs.

Two chemotherapy agents reduced the ADCC potential of human effector cells mediated by imogatuzumab. Pretreatment of PBMCs in paclitaxel reduced the percentage of maximal cell mediated by imogatuzumab from 53.5% to 36.8%. This was also seen for cetuximab-mediated in vitro cell killing and is consistent with the known immunosuppressive effects of paclitaxel (25). However, despite this reduction in ADCC capability, combining paclitaxel with imogatuzumab still resulted in enhanced efficacy in SCID-beige mice models compared with either agent alone. A slight reduction in cell killing at higher concentrations of gemcitabine was also seen with imogatuzumab (from 92.4% to 87.7%). Interestingly, the ADCC activity of cetuximab was reduced in the presence of the active metabolite of irinotecan (SN-38). The ability of cetuximab to both enhance the efficacy of irinotecan and restore tumor chemosensitivity to irinotecan is well established (15, 29–32). However, no such reduction in the ADCC activity of imogatuzumab in the presence of SN-38 was seen, which may indicate that imogatuzumab–irinotecan combinations can further improve outcomes in previously treated patients compared with cetuximab–irinotecan.

Cell killing mediated by imogatuzumab was superior to cetuximab-mediated cell killing in all ADCC assays performed. This was expected, as the A549 cell line used in the majority of ADCC experiments represented a particularly challenging target.
for anti-EGFR mAb-mediated cell killing (low EGFR expression and mutant KRAS), and cetuximab is known to be relatively ineffective against KRAS-mutant tumors (33). The enhanced cell killing and superior efficacy with imgatuzumab compared with cetuximab demonstrated in this study are consistent with our previous report, which used a variety of human tumor cell line models with different KRAS status and EGFR expression level (3).

All chemotherapy agents tested in vivo significantly increased OS when combined with imgatuzumab. Imgatuzumab demonstrated additive or synergistic efficacy when coadministered with gemcitabine and cisplatin in SCID-beige mice and with paclitaxel, carboplatin, and irinotecan in SCID-huCD16 t g mice. All imgatuzumab-chemotherapy combinations tested to date on xenograft models achieved enhanced efficacy compared with cetuximab plus chemotherapy.

The exact mechanisms by which conventional cytotoxic agents combine with anti-EGFR mAbs for enhanced efficacy are not fully understood; however, there is accumulating evidence that exposure to some chemotherapy agents can upregulate EGFR expression. Membrane EGFR expression was upregulated following exposure of several colorectal cell lines to gemcitabine, irinotecan, levofolinic acid, and 5-fluorouracil, and this resulted in enhanced in vitro cetuximab-mediated ADCC and lymphocyte-assisted killing (34). Furthermore, levels of membrane EGFR expression increased in a series of isogenic colorectal cancer cell lines as cell lines became progressively more oxaliplatin-resistant, and the in vitro sensitivity of the cell lines to cetuximab increased in parallel (35). The sequence of treatments may also be important; platinum and taxane chemotherapy followed by anti-EGFR therapy (both mAb and small-molecule tyrosine kinase inhibitors) strongly enhanced apoptosis and cell-cycle arrest in vitro, whereas...
an antagonistic effect was seen when anti-EGFR therapies were given before chemotherapy (36).

One limitation of this study is that the mouse models used lack a functional adaptive T-cell response; therefore, it was not possible to investigate the imgatuzumab adaptive immune-related mode of action and how the tested agents affect T cells and the influence this has on tumor cell killing. As most chemotherapy agents affect proliferating cells, T-cell response is likely to be affected by some of the drugs if given during previous days or in concomitant administration with the antibody therapy; therefore, further studies using syngeneic models with functional adaptive T-cell responses are now required as well as different schedules of combination therapies.

In conclusion, the enhanced innate immune-mediated ADCC activity of imgatuzumab compared with cetuximab appears to be unaffected by common cancer premedication drugs and chemotherapy agents tested in models bearing a functional innate immune system only. Building on these preclinical data, several imgatuzumab combination chemotherapy regimens are under investigation. In NSCLC, the combinations of imgatuzumab plus cisplatin and gemcitabine (for squamous histology) and imgatuzumab plus cisplatin and pemetrexed (for non-squamous histology) are investigated as first-line treatment (NCT01185847). In colorectal cancer, second-line treatment with imgatuzumab plus FOLFIRI is also under investigation in patients with KRAS-mutant tumors, and imgatuzumab plus FOLFIRI and cetuximab in patients with KRAS wild-type tumors (NCT01326000; ref. 28).

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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