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

Valeria Gonzalez-Nicolini, Sylvia Herter, Sabine Lang, Inja Waldhauer, Marina Bacac, Michaela Roemmle, Esther Bommer, Olivier Freytag, Erwin van Puijlenbroek, Pablo Umaña, and Christian A. Gerdes

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.

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 FcγRIIa 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). 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 iniximab (9–11). It is therefore important to establish whether coadministration of premedication drugs might potentially interfere with the mode of action of novel...
Translational Relevance

Therapeutic mAbs are frequently coadministered with chemotherapy agents and premedication drugs. These drugs are often immunosuppressive; therefore, it is important to establish whether the activity of novel mAbs is negatively affected by these agents. This is particularly important for mAbs which recruit immune effector cells, such as the anti-EGFR mAb, imgatuzumab. We demonstrate that commonly used premedication and cancer drugs have little effect on the degree of imgatuzumab-mediated antibody-dependent cellular cytotoxicity in vitro. Furthermore, coadministration of chemotherapy agents with imgatuzumab achieves greater efficacy than either agent alone in orthotopic xenograft models. These data indicate that imgatuzumab–chemotherapy regimens should not directly diminish the enhanced innate immune effector function of the mode of action of imgatuzumab. Our findings should facilitate the design of clinical trials of combination therapy in patients with solid tumors, which may further improve on the efficacy achieved with single-agent imgatuzumab.

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 in vitro complement-dependent cytotoxicity activity of rituximab and has a minimal effect on ADCC (13).

Treatment protocols for imgatuzumab 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 (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 imgatuzumab. 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. Paclitaxel is broadly immunosuppressive at standard doses and inhibits cell recruitment and function. 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 × 10^6 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 doxorubicin, or 1.5 to 0.3 μg/mL oxaliplatin. Drug cocktails consisted of mix 1 (1 μg/mL cisplatin, 63.8 ng/mL 5-FU, 100 ng/mL dexamethasone, 1.6 μg/mL hydrocortisone, 10 μg/mL doxorubicin, 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 aperipant, 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 carboplatin) and with imogumub 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 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 imogumub. The production and maintenance of these models has been described previously (3). Briefly, female SCID-beige (Taconics) or SCID-huCD16 transgenic (tg) mice (Roche-Glycánt) 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 monocytoid macrophages that express FcγRIV, the murine homolog of human FcγRIIA. Murine NK cells do not express FcγRIV, therefore the SCID/beige mouse model can only demonstrate the antitumoral effect of ADCC (and/or FcγRIV-triggered antibody-dependent cellular phagocytosis and cytokine release) mediated by monocytoid 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 7 days or 14 days after tumor cell inoculation). All treatments were administered intravenously and nontoxic effects were observed (7 days or 14 days after tumor cell inoculation). All treatments were administered intravenously and nontoxic effects were observed (7 days or 14 days after tumor cell inoculation). All treatments were administered intravenously and nontoxic effects were observed (7 days or 14 days after tumor cell inoculation).

Results

Common cancer premedications do not negatively affect imogumub-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 imogumub-mediated cell killing. Incubation of human PBMC effector cells with 8 µmol/L hydrocortisone for 23 hours prior to performing ADCC experiments had no effect on imogumub-mediated killing of A549 lung adenocarcinoma cells (Fig. 1A). The level of imogumub-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 imogumub in a human lung adenocarcinoma model (SCID-beige mice inoculated with A549 cells). The median OS achieved by imogumub was identical when administered as a 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 imogumub (P = 0.36; Fig. 1B). Both single-agent imogumub and imogumub 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 imogumub-mediated ADCC
The effect of chemotherapy agents on the in vitro ADCC activity and in vivo efficacy of imogumub was investigated using gemcitabine, cisplatin, carboplatin, paclitaxel, and irinotecan. These compounds are currently used clinically to treat solid tumor types in which imogumub is likely to be indicated (colorectal cancer, lung cancer, breast cancer, and head and neck cancer) and are therefore potential partners for imogumub in future combination chemotherapy regimens.

Preincubation of PBMC effector cells in 1 µg/mL gemcitabine had no effect on imogumub- 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 imogumub 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 imogumub-mediated ADCC of KRAS wild-type HT29 colorectal cancer cells but reduced the degree of cell killing mediated by imgatuzumab.
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 EC50 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 EC50 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 medium to high concentrations 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 vitro 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 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).

Combining imatuzumab with irinotecan also improved median OS compared with either agent alone in the colorectal cancer SCID/huCD16 tg xenograft models. In mice inoculated with KRAS wild-type HT29 cells, median OS was 79 days when treated with irinotecan plus 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 tg xenograft models. In mice inoculated with KRAS wild-type HT29 cells, median OS was 79 days when treated with irinotecan plus 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 vitro, improved efficacy was observed when chemotherapeutic agents were combined with imatuzumab compared with mAb therapy.
Figure 2.
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 28 μg/mL carboplatin preincubation, 98.4%, and 99.1%. 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>Concentration used</th>
<th>C_{50} reported</th>
<th>Imgatuzumab EC_{50} values (ng/mL)</th>
<th>Maximal killing (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Untreated</td>
<td>Treated</td>
</tr>
<tr>
<td>Hydrocortisone</td>
<td>8 μmol/L</td>
<td>8 μmol/L</td>
<td>0.28</td>
</tr>
<tr>
<td>Dexamethasone</td>
<td>0.1 μg/mL</td>
<td>n.c.</td>
<td>0.16</td>
</tr>
<tr>
<td>Prednisolone</td>
<td>0.65 μg/mL</td>
<td>0.65 μg/mL</td>
<td>0.28</td>
</tr>
<tr>
<td>Gemcitabine</td>
<td>10 μg/mL</td>
<td>10 μg/mL</td>
<td>0.56</td>
</tr>
<tr>
<td>Cisplatin</td>
<td>2 μg/mL</td>
<td>n.c.</td>
<td>0.16</td>
</tr>
<tr>
<td>Carboplatin</td>
<td>28 μg/mL</td>
<td>28 μg/mL</td>
<td>0.57</td>
</tr>
<tr>
<td>Paclitaxel</td>
<td>5–25 μg/mL</td>
<td>5 μg/mL</td>
<td>1.83</td>
</tr>
<tr>
<td>SN-38 (metabolite of irinotecan)</td>
<td>0.062 μg/mL</td>
<td>0.062 μg/mL</td>
<td>1.07</td>
</tr>
<tr>
<td>Doxycycline</td>
<td>10 μg/mL</td>
<td>7 μg/mL</td>
<td>0.81</td>
</tr>
<tr>
<td>Oxaliplatin</td>
<td>1.5–0.3 μg/mL</td>
<td>1.5–0.3 μg/mL</td>
<td>4.01</td>
</tr>
</tbody>
</table>

NOTE: PBMCs were preincubated at concentrations shown for approximately 24 hours after which ADCC was performed using a range of imgatuzumab concentrations (0.01–1,000 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 pretreated 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 imgatuzumab. These data indicate that strategies to reduce the risk and severity of IRRs in the clinic can be potentially achieved with hydrocortisone without compromising the efficacy of imgatuzumab-based treatment regimens. Future combination regimens will need to be given cautiously due to the presence of the innate immune system but in the presence of the innate immune effector cells that mediate ADCC.

Two chemotherapy agents reduced the ADCC potential of human effector cells mediated by imgatuzumab. Preincubation of PBMCs in paclitaxel reduced the percentage of maximal cell mediated by imgatuzumab 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 imgatuzumab 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 imgatuzumab (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 imgatuzumab in the presence of SN-38 was seen, which may indicate that imgatuzumab–irinotecan combinations can further improve outcomes in previously treated patients compared with cetuximab–irinotecan.

Cell killing mediated by imgatuzumab 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.

Figure 3.
Preincubation of PBMC effector cells with a cocktail of cisplatin (1 μg/mL), granisetron hydrochloride (63.8 ng/mL), dexamethasone (100 ng/mL), aprepitant (1.6 μg/mL), pemetrexed (10 μg/mL), pantoprazole (1.25 μg/mL), vitamin B12 (600 pg/mL), and folic acid (15 ng/mL; mix 1) had no effect on imgatuzumab-mediated antibody-dependent killing (4-hour LDH release assay using A549 target cells at an E:T ratio of 25:1). A slight reduction in ADCC was seen at higher imgatuzumab concentrations after preincubation of PBMCs in cisplatin (1 μg/mL), granisetron hydrochloride (63.8 ng/mL), dexamethasone (100 ng/mL), aprepitant (1.6 μg/mL), gemcitabine (10 μg/mL), and ranitidine hydrochloride (94 ng/mL; mix 2). In contrast, both drug cocktails reduced cetuximab-mediated ADCC. PBMC viability measured as follows: before mix 1 and mix 2 preincubation, 99.9%; after mix 1 and mix 2 preincubation, 96.4% and 97.9%, respectively. N = 3.
for anti-EGFR mAb-mediated cell killing (low EGFR expression and mutant KRAS), and cetuximab is known to be relatively ineffective againstKRAS-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 carboplatin in SCID-beige mice and with paclitaxel, carboplatin, and irinotecan in SCID-huCD16 tg 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

![Graphs showing percentage of surviving cells over time for different treatments.](image)

Figure 4. SCID-beige mice (A and B) or SCID-huCD16 tg mice (C-F; n = 10 per group) were inoculated with A549 cells (A–D), KRAS wild-type HT29 cells (E) or KRAS-mutant LS174T cells (F) on day 0. Animals were treated with either saline (vehicle control), single-agent imgatuzumab (25 mg/kg), single-agent chemotherapy (gemcitabine 150 mg/kg, cisplatin 2 mg/kg, paclitaxel 20 mg/kg, carboplatin 60 mg/kg, or irinotecan 30 mg/kg), imgatuzumab plus chemotherapy, or cetuximab (25 mg/kg) plus chemotherapy. All therapies began on day 7 and were given once weekly for 3 weeks (6 weeks for cisplatin). Median OS with imgatuzumab combined with chemotherapy agents was superior to both single-agent imgatuzumab and single-agent chemotherapy in all models investigated. Imgatuzumab combined with chemotherapy was also superior to cetuximab combined with chemotherapy.
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 imtgatuzumab 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 imtgatuzumab compared with cetuximab appears to be unaffected by common cancer premedication drugs and chemotherapy agents when tested in models bearing a functional innate immune system only. Building on these preclinical data, several imtgatuzumab combination chemotherapy regimens are under investigation. In NSCLC, the combinations of 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 imtgatuzumab 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.

References

11. Roche Registration Ltd. MabThera® summary of product characteristics. Weheygen Garden City, United Kingdom, 2012.
12. Genzyme Europe BV. MabCampath® Summary of product characteristics. Naarden, the Netherlands; 2012.


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

Valeria Gonzalez-Nicolini, Sylvia Herter, Sabine Lang, et al.