Prioritization of EGFR/IGF-IR/VEGFR2 Combination Targeted Therapies Utilizing Cancer Models

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Abstract. *Background: Rational strategies utilizing anticancer efficacy and biological principles are needed for the prioritization of specific combination targeted therapy approaches for clinical development, from among the many with experimental support. Materials and Methods: Antibodies targeting epidermal growth factor receptor (EGFR) (cetuximab), insulin-like growth factor-1 receptor (IGF-IR) (IMC-A12) or vascular endothelial growth factor receptor 2 (VEGFR2) (DC101), were dosed alone or in combination, in 11 human tumor xenograft models established in mice. Efficacy readouts included the tumor burden and incidence of metastasis, as well as tumor active hypoxia inducible factor-1 (HIF-1), human VEGF and blood vessel density. Results: Cetuximab and DC101 contributed potent and non-overlapping benefits to the combination approach. Moreover, DC101 prevented escape from IMC-A12 + cetuximab in a colorectal cancer model and cetuximab prevented escape from DC101 therapy in a pancreatic cancer model. Conclusion: Targeting VEGFR2 + EGFR was prioritized over other treatment strategies utilizing EGFR, IGF-IR and VEGFR2 antibodies. The criteria that proved to be valuable were a non-overlapping spectrum of anticancer activity and the prevention of resistance to another therapy in the combination.*

Targeted therapy has become an important treatment modality in cancer. However, cancer is a complex genetic disease, and therefore targeted drugs usually have limited single-agent activity. Hence, there is a major effort to understand the multiple genetic pathways that regulate malignant disease and use this information to design rational combination regimens of targeted agents.

When determining a combination targeted therapy approach to evaluate in patients, the volume of preclinical literature supporting different combinations can be overwhelming. Epidermal growth factor receptor (EGFR) targeted agents for example, have been combined with inhibitors of vascular endothelial growth factor (VEGF) (1-3), insulin-like growth factor-1 receptor (IGF-IR) (4), cyclooxygenase-2 (COX-2) (5), human epidermal growth factor receptor 2 (HER2) (6), and mammalian target of rapamycin (mTOR) (7) to increase antitumor effects. Hence, rational approaches to select the combination(s) likely to achieve the greatest therapeutic benefit in the highest proportion of patients are needed. In this study, in order to select the best combination of antibodies to EGFR, IGF-IR and VEGFR2, the magnitude of the effect on the tumor burden, metastasis and mechanistic biomarkers in preclinical models was utilized to guide the prioritization process.

EGFR and VEGF have important, and in many ways separate roles in cancer pathogenesis. EGFR activation of tumor cells directly supports tumor cell proliferation and survival (8, 9), while the VEGF pathway, mostly through VEGF receptor 2 (VEGFR2) activation on endothelial cells (10), supports the maintenance and growth of the tumor vasculature (11). In preclinical models, combination therapy with agents targeting the EGFR and VEGF pathways has demonstrated increased tumor growth inhibition compared to monotherapies (1, 3, 12). Beyond a simple additive effect, antibodies targeting EGFR and VEGFR2 have been shown to be synergistic with regard to antitumor effects, in that the combination dose necessary to achieve a sub-maximal target level of efficacy was approximately 10 times lower than expected from monotherapy potencies (3).

EGFR and VEGF pathway overlap with regard to hypoxia inducible factor-1 (HIF-1) signaling, may underlie the synergistic interaction between inhibitors of the EGFR and VEGFR2 pathways (3). HIF-1α is stabilized during hypoxia,

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binds to HIF-1β, and this activated HIF-1 complex binds to its DNA response element (HRE) to activate hypoxia responsive genes that can increase angiogenesis and growth factor signaling, to promote tumor cell proliferation and survival (13). The production of VEGF and other proangiogenic factors by tumor cells may be regulated by HIF-1 activity (14), which interestingly is increased by EGFR through effects on HIF-1α stability (3, 14). EGFR inhibition, by blocking HIF-1 pathway activation, may therefore inhibit resistance to VEGFR2 targeted therapy mediated by increased proangiogenic factor expression (15), although this has not been confirmed experimentally.

IGF-IR on tumor cells can also regulate HIF-1 activity and VEGF production (16), indicating that combining both EGFR and IGF-IR targeting agents may augment the ability to inhibit the activity of this pathway and prevent it from acting as a resistance mechanism for VEGF targeted therapy and otherwise contributing towards tumor growth. Moreover, IGF-IR signaling can act as a resistance mechanism for EGFR targeted therapy (17) so targeting IGF-IR may remove a mechanism of resistance to an antibody targeting EGFR such as cetuximab.

Thus the question arises, which strategy utilizing antibodies targeting EGFR, IGF-IR, or VEGFR2 is likely to bring the greatest benefit to patients? Taking the anticancer effects of therapy at high antibody doses as a predictor of potential benefit to patients, multiple preclinical models were utilized to prioritize potential combination and monotherapy approaches with these antibodies.

Materials and Methods

Human cancer cell lines. Human pancreatic cancer line BxPC-3, colorectal cancer line HCT-8, head and neck cancer lines FaDu and Detroit-562, prostate cancer line DU145, and non-small cell lung cancer lines NCI-H460 and NCI-H292 were obtained from the American Type Culture Collection (ATCC, Manassas, VA, USA). GEO human colorectal cancer cells were kindly provided by Dr. Lee Ellis (Department of Surgical Oncology, The University of Texas MD Anderson Cancer Center, Houston, Texas, USA). MDA-MB-435-LM2 cells were derived from the parental MDA-MB-435 from the Division of Cancer Treatment and Diagnosis (DCTD) Tumor Repository of the National Cancer Institute (NCI), by isolating lung metastasis formed from subcutaneous xenografts (ImClone Systems, Branchburg, NJ, USA).

Firefly luciferase expressing HT-29 (parental line from ATCC) colorectal cancer cells (HT-29-LP) were also utilized. The luc gene from pGL3 (Promega, Madison, WI, USA) was subcloned into the retroviral plasmid pLXSN (Clontech, Mountain View, CA, USA). Luciferase containing retrovirus was then collected from the supernatant of pLXSN-luc Lipofectamine 2000 (Invitrogen Corporation, Carlsbad, CA, USA) transfected PT-67 packaging cells (Clontech), and used to infect HT-29 cells. The luciferase expressing HT-29 cell colonies were then isolated following selection with 800 μg/ml G418 (Invitrogen Corporation). The colonies were expanded and tested for luciferase activity, followed by the selection of the HT-29-LP clone through *in vivo* testing.

All the cell lines expressed both EGFR and IGF-IR on the cell surface by fluorescence activated cell sorting analysis with cetuximab and IMC-A12 (anti-IGF-IR) used as primary antibodies, respectively (FACS; not shown), except MDA-MB-435-LM2 which did not express EGFR by FACS. However MDA-MB-435-LM2 cells growing as subcutaneous xenograft tumors did express EGFR by ELISA using the methods described below.

Xenograft models. The animal experiments were performed in accordance with protocols approved by the ImClone Systems Internal Animal Care and Use Committee, and in accordance with current regulations and standards of the United States Department of Agriculture and the National Institute of Health. Subcutaneous xenograft tumors were established in female athymic (nu/nu) mice (Charles River Laboratories, Wilmington, MA, USA) as previously described (3), with the following number of tumor cells injected per mouse ($\times 10^6$): BxPC-3: 3, HCT-8: 5, FaDu: 2, Detroit-562: 5, DU145: 15, NCI-H460: 0.3, NCI-H292: 2, GEO: 10, MDA-MB-435-LM2: 5, HT-29-LP: 5. The tumor volume was calculated as $\pi/2$ ($L \times W^2$), where L is the longest diameter and W is the diameter perpendicular to L. T/C% was calculated as 100* the ratio of relative tumor volume (RTV: mean final tumor volume divided by initial tumor volume) for the experimental group *versus* the saline control group. Nonspecific human IgG and rat IgG at 40 mg/kg, administered Mon-Wed-Fri, were without effect in the utilized models (not shown).

In addition to the subcutaneous models, an orthotopic colorectal cancer model was established with a luciferase expressing HT-29 colorectal cancer cell line (HT-29 LP) as previously described (18), with 1×10^6 cells/mouse implanted in the rectal lining in 50% matrigel in PBS. Treatment was started 2 weeks after cell implantation into the rectal lining. The tumor cell burden was quantified using the IVIS 200 *in vivo* imaging system, by measuring the bioluminescence of injected luciferin, due to luciferase expression by the cancer cells, as per the manufacturer's instructions (Caliper/Xenogen Corporation, Hopkinton, MA, USA). The frequency of metastasis to para-aortic lymph nodes was also evaluated in the HT-29-LP model. The lymph nodes were dissected from mice injected with 150 mg/kg luciferin, and placed in PBS containing 300 μg/ml luciferin in 96 well plates. The exposure time utilized for evaluation of metastasis was 1.5 minutes. Exposure times greater than this picked up background signal, establishing 1.5 minutes exposure as the approximate limit of detection.

Treatments. Cetuximab (IMC-C225), a human chimeric antibody specific for human EGFR, DC101, a rat monoclonal antibody specific for mouse VEGFR2, and IMC-A12, a fully human antibody that recognizes mouse and human IGF-IR (19), were produced at ImClone Systems. Human IgG (BioDesign International, Saco, ME, USA), rat IgG (Equitech-Bio Inc., Kerrville, TX, USA), and/or United States Pharmacopoeia (USP) saline vehicle were used as controls. Monotherapy antibody treatments were administered *i.p.* at 40 mg/kg, Mon-Wed-Fri. Based on the synergism established at a 4:1 dose ratio of DC101:cetuximab (3), antibodies in the Cocktail (DC101 + IMC-A12 + cetuximab) and other combination groups were administered *i.p.* Mon-Wed-Fri at 40 mg/kg DC101, 10 mg/kg IMC-A12 and 10 mg/kg cetuximab. Antibodies in the combination groups were combined in a single dosing solution.

ELISAs with tumor homogenates. The effects of the treatments on HIF-1 activity and human VEGF levels were evaluated in the tumors by ELISA, using three models in which all the monotherapies

showed efficacy. The effects were evaluated through 1 week of treatment, when antitumor and antivascular effects of DC101, with or without cetuximab, have been reported for the BxPC-3 and GEO models (3). The harvested tumors were homogenized in 3 ml radioimmunoprecipitation assay (RIPA) buffer (Santa Cruz Biotechnology, Santa Cruz, CA, USA) per gram frozen tissue. Total protein concentration was measured using bicinchoninic acid (Pierce, Rockford, IL). Active HIF-1 (nuclear HIF-1α binding to the consensus sequences of the hypoxia responsive element (HRE)) was measured by ELISA (Panomics, Fremont, CA, USA) using 20 μg total protein per well. Human VEGF (40 μg) and total EGFR (15 μg) were measured by ELISA (R and D Systems, Minneapolis, MN, USA), using the indicated amount of total protein per well. Since different time-points were evaluated serially in the mechanism of action studies, individual ELISA values were first calculated as a fraction of the total protein, and then expressed as a percentage of the control group mean value at the same time-point.

Evaluation of blood vessel density. Increased proangiogenic factor production during antivascular therapy has recently been demonstrated to contribute towards the development of resistance (15), and it has been proposed that the ability of cetuximab to reduce HIF-1 activity may be utilized to prevent resistance to the antivascular effects of DC101 (3). This hypothesis was tested directly in a model demonstrated to respond to cetuximab by reducing HIF-1 activity, the BxPC-3 subcutaneous xenograft model (see below). The tumors were harvested on Day 7 or Day 36 of treatment and immunostained as previously described (3) for Mouse Endothelial Cell Antigen-32 (Meca-32) (primary antibody from BD Biosciences Pharmingen, San Diego, CA, USA). The quantification of these immunohistological markers was performed in 5 randomly selected fields of view (total area 7.347 mm^2) in the tumor periphery (<1360 microns from the tumor edge) as previously described (3).

Statistical analysis. Repeated measures ANOVA (JMP software, Version 5 from SAS Institute, Cary, NC, USA) was utilized to evaluate the effects of treatment on tumor volume. To evaluate the effects of treatment and time of sampling on active HIF-1α and human VEGF measurements, a standard least squares analysis was utilized. Blood vessel density was compared among the treatment groups at each time-point by One Way ANOVA followed by a Tukey-Kramer posthoc test. The frequency of lymph node metastasis was evaluated by Chi-squared test. For all the comparisons, two-tailed tests are reported using n=number of mice, with $p<0.05$ considered significant.

Results

Monotherapy versus Cocktail efficacy in subcutaneous xenograft models. The antitumor efficacy of the Cocktail treatment was greater than the monotherapy treatments, with T/C% values ranging from 7-38% ($p<0.003$ for all), in all the 10 subcutaneous xenograft models tested (Figures 1 and 2). However in 8 out of the 10 models the benefit of the Cocktail therapy did not reach statistical significance *versus* all three monotherapies. In these 8 models, the antitumor effects of the Cocktail were comparable to the effects of more than one of the antibodies administered as a monotherapy (BxPC-3, DU145, Detroit-562, FaDu, and GEO; Figure 1A), or were

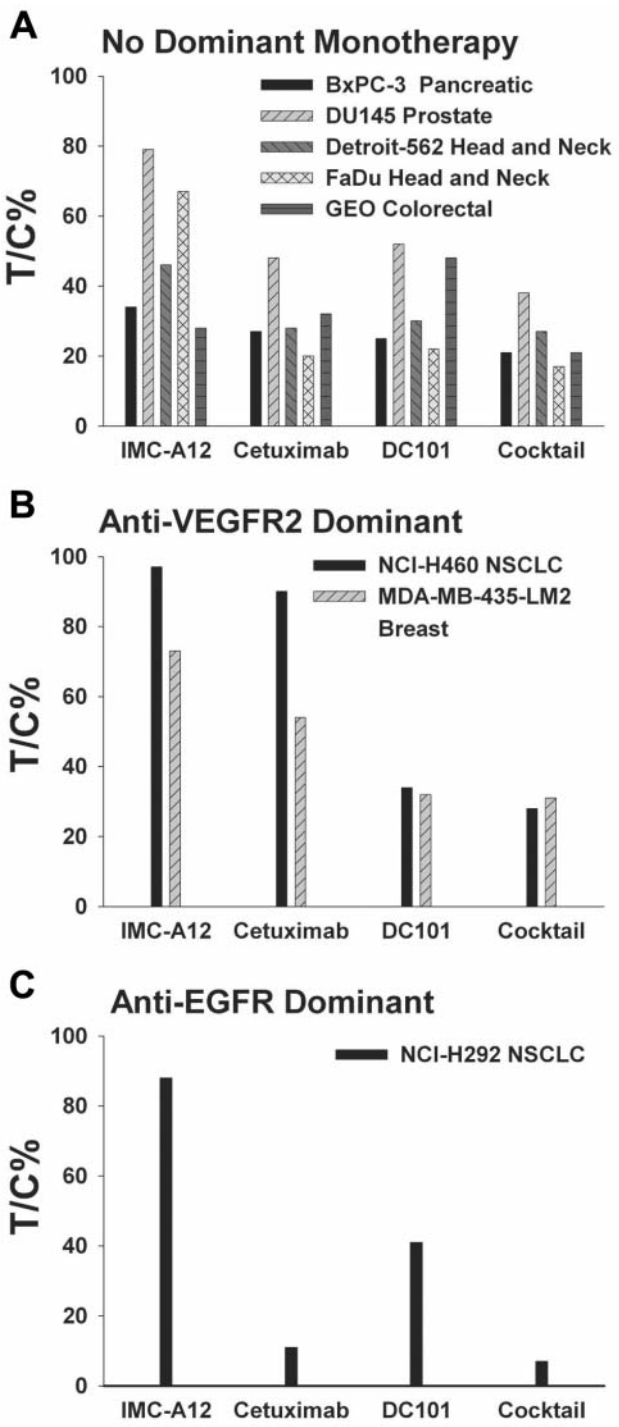

Figure 1. *Subcutaneous xenograft models separated by the dominant monotherapy contributing towards Cocktail potency. (A) no antibody was dominant, (B) DC101 anti-VEGFR2 was dominant, (C) cetuximab anti-EGFR was dominant, with respect to the effects of Cocktail (n=9-10 per treatment group). Treatment duration ranged from 20 to 40 days, guided by the aggressiveness of the model. T/C% : 100 * (ratio of relative tumor volume in experimental group to that in saline control), NSCLC: non-small cell lung cancer, IMC-A12: anti-IGF-IR, cetuximab: anti-EGFR, DC101: anti-VEGFR2, Cocktail: DC101 + IMC-A12 + cetuximab.*

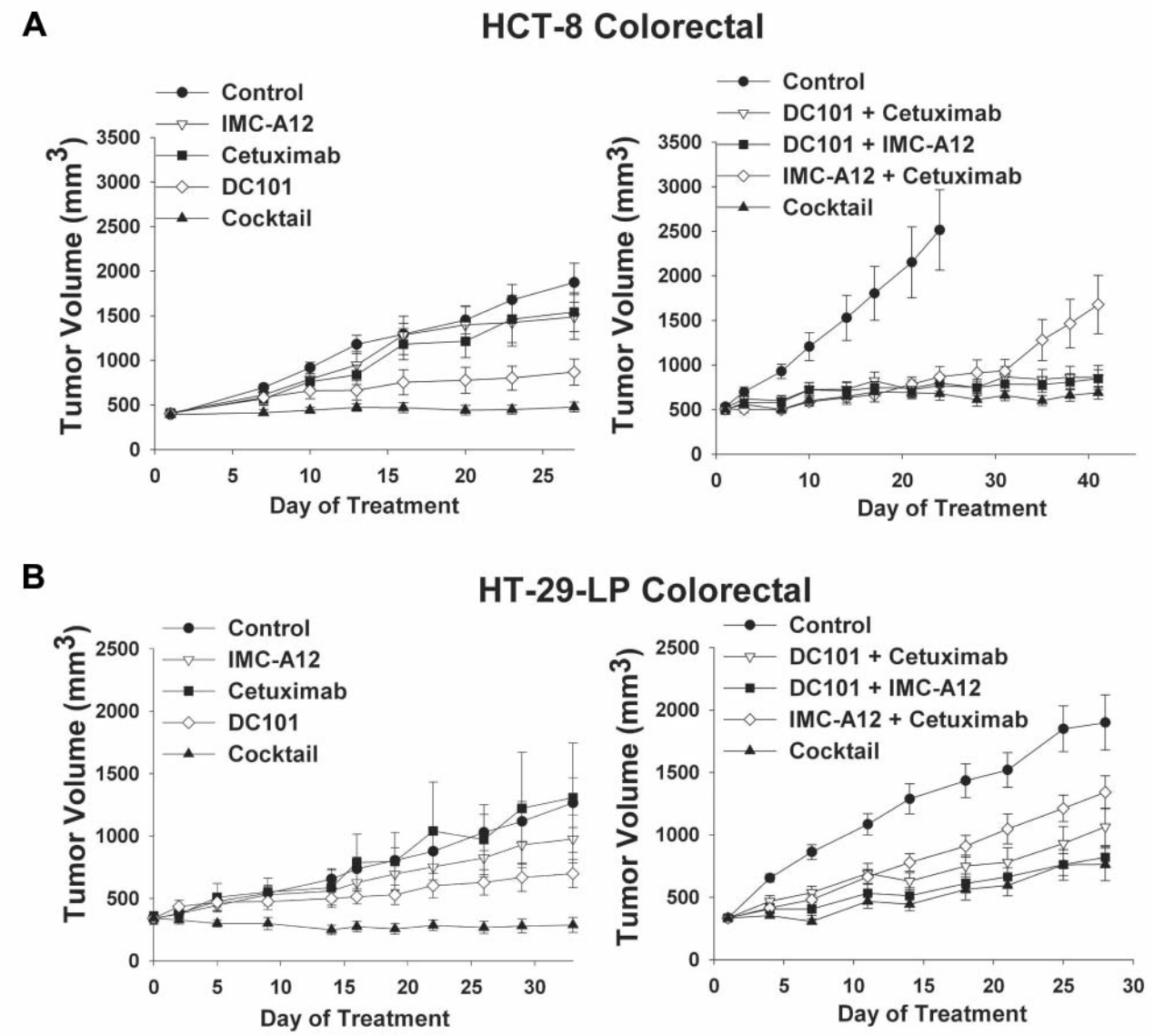

Figure 2. *Subcutaneous xenograft tumor growth in nu/nu athymic mice treated with antibody monotherapy or combinations. (A) HCT-8 and (B) HT-29-LP cell lines. Two separate studies are shown for each model in the left and right panels. Mean±SEM plotted for n=10-12. IMC-A12: anti-IGF-IR, cetuximab: anti-EGFR, DC101: anti-VEGFR2, Cocktail: DC101 + IMC-A12 + cetuximab, Control: saline.*

dominated by the effect of either DC101 (NCI-H460 and MDA-MB-435-LM2; Figure 1B) or cetuximab (NCI-H292; Figure 1C). Interestingly, in all three models responding to IMC-A12 (GEO, BxPC-3 and Detroit-562; $p<0.005$), cetuximab showed comparable efficacy (Figure 1A).

The benefits of the Cocktail treatment *versus* high dose monotherapy reached statistical significance in the HCT-8 (Figure 2A, left) and HT-29 LP (Figure 2B, left) colorectal cancer models ($p<0.04$ *versus* monotherapies). In follow-up studies utilizing both models (Figures 2A and 2B, right), the benefits of the Cocktail were not significantly different than that of cetuximab + DC101 or IMC-A12 + cetuximab. Notably, in the HCT-8 model, while IMC-A12 + cetuximab combination therapy initially achieved the same antitumor effects as the Cocktail, the tumors eventually acquired resistance ($p<0.01$). DC101 prevented this escape from IMC-A12 + cetuximab, and maintained the antitumor effects at the increased level achieved with the Cocktail.

Effect of combination therapy in an orthotopic model of colorectal cancer. In an HT-29-LP orthtopic colorectal cancer model in which cells are implanted in the rectal

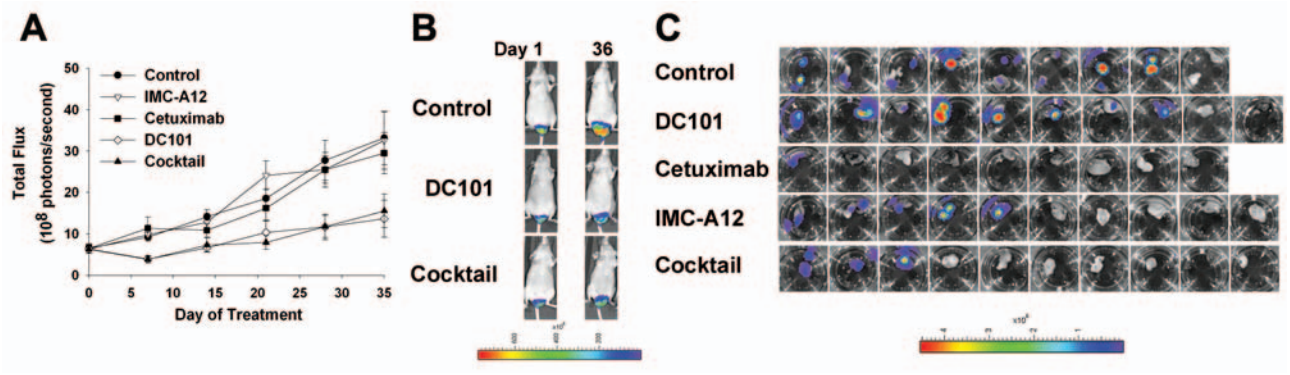

Figure 3. *Effect of antibody treatment in an orthotopic HT-29 luciferase expressing model. (A) bioluminescence of primary tumors in the rectal area (mean±SEM, n=9-10) and (B) representative images of mice (Control = saline). (C) Bioluminescence images for individual dissected para-aortic lymph nodes from all mice, in wells of a 96-well plate, with calibration bars indicating bioluminescence intensity in photons/second. IMC-A12: anti-IGF-IR, cetuximab: anti-EGFR, DC101: anti-VEGFR2, Cocktail: DC101 + IMC-A12 + cetuximab.*

lining, there was a significant effect of treatment on the tumor burden measured as a bioluminescence signal (20) (p=0.04), with DC101 and the Cocktail responsible for this effect (Figure 3A,B). IMC-A12 and cetuximab were without effect on tumor growth, similar to the lack of effect of these antibodies in the subcutaneous xenografts established with the same cell line (Figure 2B, left).

Metastasis of HT-29 LP cells from the rectal lining to the para-aortic lymph nodes was detected in 89% of the saline treated mice and was reduced by treatment (p=0.0006; Figure 3C). Metastasis was detected in only 11, 50 and 30% of the mice treated with cetuximab, IMC-A12 and the Cocktail, respectively, although DC101 was without effect on metastasis (80%). The difference between the Cocktail group and the cetuximab monotherapy group may be related to the lower dose of cetuximab administered in the Cocktail group (10 *versus* 40 mg/kg). In effect, the Cocktail therapy combined the primary tumor growth inhibition of DC101 (Figures 2B and 3A) with the antimetastatic effect of cetuximab (Figure 3C), to achieve a greater overall therapeutic benefit than any monotherapy.

Effect of DC101 and cetuximab on HIF-1 and VEGF induction. Active HIF-1 and human VEGF in the BxPC-3 and Detroit-562 tumors increased over time during DC101 therapy, relative to the control tumor measurements (Figure 4A,B,D,E; p<0.05). In DC101 treated GEO tumors, the measurements of active HIF-1 and VEGF tended to be increased, although the pattern of change was not smooth over the 7 days of treatment (Figure. 4C,F; p=0.04). This different pattern of change may have been related to the significant regional tumor cell apopotosis caused by DC101 within 1 week of treatment in this model (3). Cetuximab reduced active HIF-1 and VEGF in all 3 models, with or without DC101 co-treatment (Figure 4A-F; p<0.05). The effect of combination treatment in this regard, was achieved with a similar magnitude whether or not IMC-A12 was included, in spite of the ability of IMC-A12 to reduce tumor active HIF-1 and VEGF on its own (Figure 4A-F).

Effect of cetuximab on resistance to DC101 therapy. In line with increased HIF-1 activity and VEGF production during DC101 therapy (Figure 4A,D), BxPC-3 tumors began to escape the antivascular effects of DC101 monotherapy after approximately 25 days of treatment (Figure 5A). This escape was prevented by cetuximab, with or without IMC-A12 (p<0.02). Moreover, while the antivascular effects of therapy were similar in all the antibody treatment groups on Day 7 of treatment (p>0.05), by Day 36 of treatment the antivascular effects of DC101 were significantly weaker than in the groups also receiving cetuximab or IMC-A12 + cetuximab (Cocktail) (p<0.05) (Figure 5B). In fact, the only group in which blood vessel density increased from Day 7 to Day 36 of treatment was the DC101 monotherapy group (p<0.05 by Wilcoxon Rank Sums Test).

Discussion

Given the volume of combination approaches to cancer therapy with experimental support, rational methods are clearly necessary for the prioritization of specific regimens for thorough evaluation in the clinic. In the present research, the property that proved most useful in prioritizing the possible combinations of IGF-1R, EGFR, and VEGFR2 antibodies was a non-overlapping spectrum of antitumor activity among the individual antibodies to be included in the selected combination. Moreover, the fortuitous targeting of resistance mechanisms for one therapy, by another therapy in the combination, was of further assistance in the selection process.

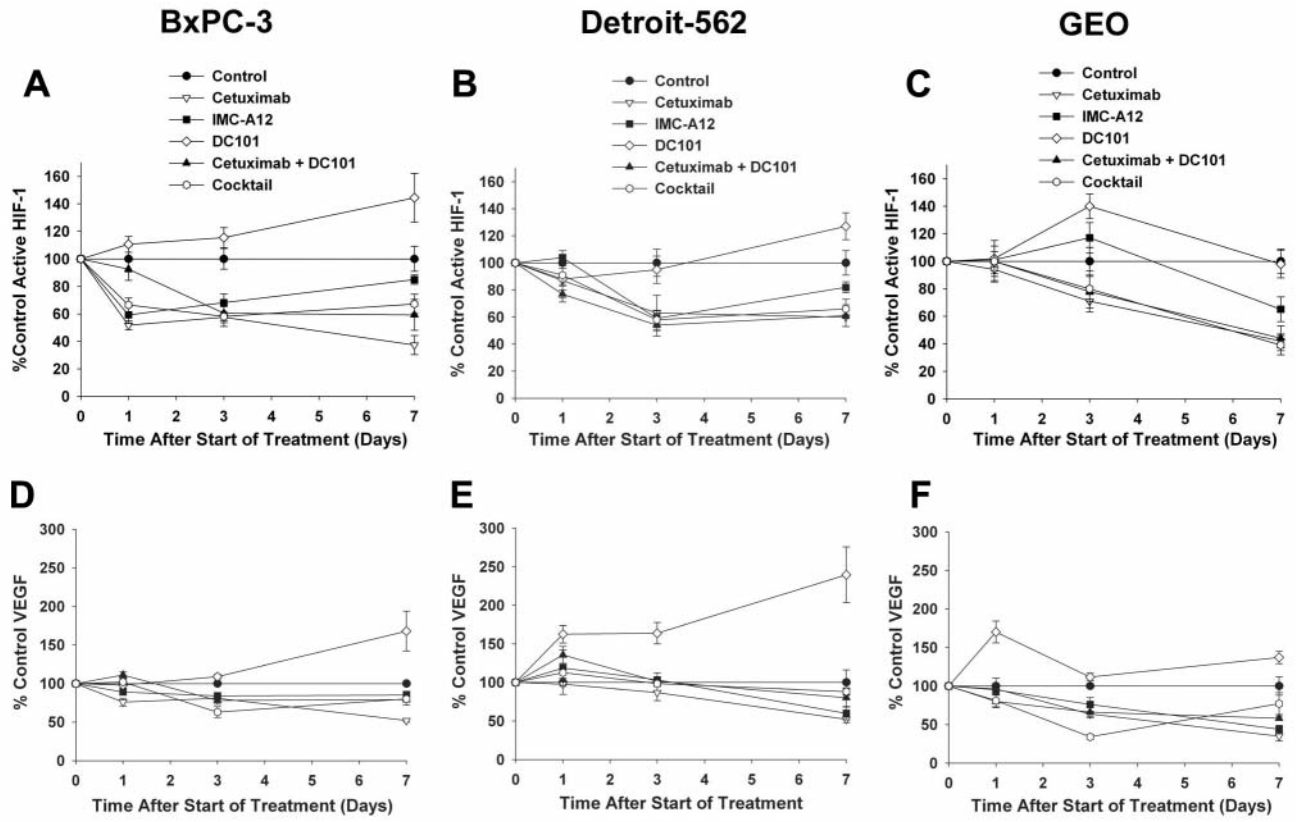

Figure 4. *Effect of DC101 and cetuximab on HIF-1 and VEGF induction. Active HIF-1 (A,B,C) or human VEGF (D,E,F) in BxPC-3 (A,D), Detroit-562 (B,E) and GEO (C,F) tumors, collected 1, 3 or 7 days after the start of treatment. Measurements are expressed as a percentage of the mean control (saline) measurement at the same time-point. Mean±SEM is plotted for n=5-6. IMC-A12: anti-IGF-IR, cetuximab: anti-EGFR, DC101: anti-VEGFR2, Cocktail: DC101 + IMC-A12 + cetuximab.*

The use of specific antibodies in the present studies allowed for the dissection of the benefits offered by targeting different receptors. In eight out of the ten subcutaneous xenograft models the anticancer effects of a cocktail of DC101 + cetuximab + IMC-A12 were very similar to that of one or more of the monotherapies, indicating that the benefits of a combination approach were associated with achieving more consistent antitumor effects, as opposed to increased effects, in 80% of the models. In this regard, a non-overlapping pattern of activity became an important prioritization factor for selecting a combination strategy. Cetuximab showed activity in all the models responding to IMC-A12, and was also efficacious in a model in which IMC-A12 did not have a significant effect. Moreover in this NCI-H292 model, cetuximab had dramatic efficacy significantly greater than that of DC101, and similar to that of the Cocktail therapy. Therefore cetuximab was prioritized to be part of a combination strategy, as was DC101 which dominated the effects of the Cocktail therapy in NCI-H460 and MDA-MB-435-LM2. Further supporting the prioritization of the DC101 + cetuximab combination approach, in an orthotopic colorectal cancer model, the Cocktail effectively combined the potent antitumor and antimetastatic effects of VEGFR2 and EGFR targeting, respectively, to achieve a broader profile with more consistent anticancer effects.

In two of the ten selected subcutaneous xenograft models (HCT-8 and HT-29-LP), the Cocktail therapy had significantly greater efficacy than any of the monotherapies. In these two models, the antitumor benefit of the Cocktail was not different than that achieved by IMC-A12 + DC101 or DC101 + cetuximab. Thus the DC101 + cetuximab combination, selected above based on monotherapy spectrums of activity, would also achieve the benefits of the Cocktail therapy in these two models in which the maximal effect of combination therapy extended beyond that observed with the high dose monotherapies.

Beyond the immediate effects on tumor progression and metastasis, a combination may also be prioritized based on the ability of one treatment in the combination to prevent the development of resistance to another therapy. Mechanistically, one of the means by which individual

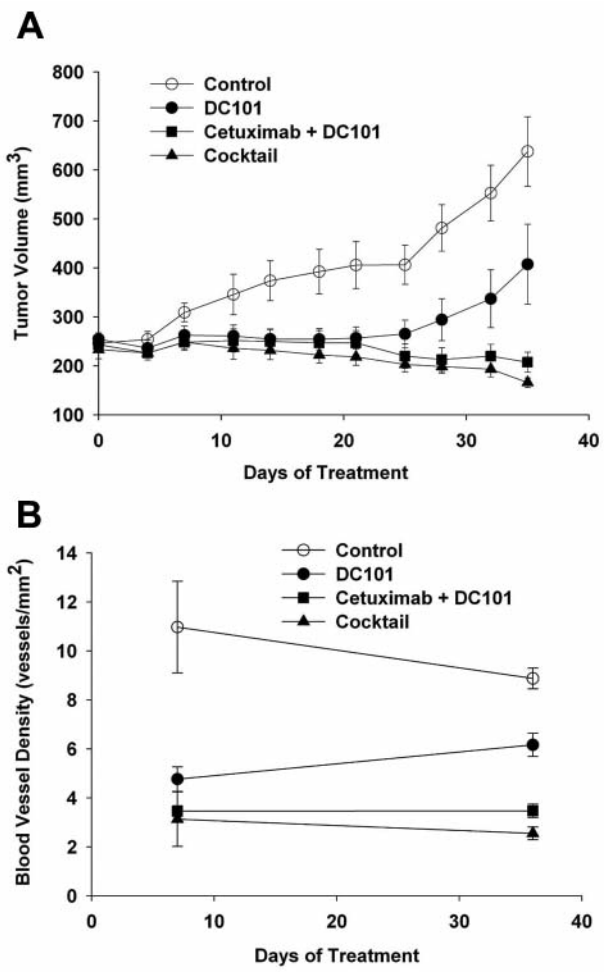

Figure 5. *Effect of cetuximab on resistance to DC101, in association with stabilization of antivascular effects. (A) subcutaneous xenograft tumor growth and (B) Meca-32 positive blood vessel density evaluated in a BxPC-3 model established in nu/nu athymic mice. Mean±SEM plotted for n=9 in (A) and n=6 in (B). IMC-A12: anti-IGF-IR, cetuximab: anti-EGFR, DC101: anti-VEGFR2, Cocktail: DC101 + IMC-A12 + cetuximab, Control: saline.*

agents targeting EGFR, IGF-IR, and VEGFR2 have the potential to affect resistance mechanisms for the other antibodies when given in combination, is through effects on tumor HIF-1 signaling. DC101 mediated inhibition of VEGFR2 signaling increased tumor HIF-1 activity and VEGF production in the present studies, similar to prior reports (15). Tumor cells are therefore responding to the blood vessel loss (3, 21) and ensuing hypoxia (3, 15, 21) induced by DC101, by up-regulating HIF-1 activity, which then increases the expression of VEGF (14, 15). But in addition to VEGF, hypoxia also increases the activity of alternative proangiogenic pathways, such as fibroblast growth factor (FGF), that may overcome the antivascular effects of VEGF pathway inhibition (15). Apart from the ability to regulate proangiogenic pathways, HIF-1 activity in tumor cells can also affect the activity of survival/proliferation pathways (13). Thus, increased HIF-1 signaling in hypoxic conditions during DC101 treatment may support the growth and survival of tumor cells both by increasing the blood vessel supply and through more direct effects on cell signaling.

IMC-A12 and cetuximab both reduced active HIF-1 and human VEGF levels in the tumors, in agreement with previous reports (3, 14, 16). Furthermore, when IMC-A12 and cetuximab were added to DC101 therapy, the HIF-1 activity and VEGF levels were no longer upregulated in response to DC101. The antibodies targeting tumor cells (IMC-A12 and cetuximab) therefore inhibited the numerous potential HIF-1 mediated resistance mechanisms to the antibody targeting the vasculature (DC101). In the BxPC-3 model this ability translated into an inhibition of escape from DC101 therapy by cetuximab, with or without IMC-A12.

With regard to targeting EGFR, increased tumor cell VEGF production in the A431 vulvar squamous carcinoma model (22) and increased VEGF mRNA levels in metastatic colorectal cancer patients (23), are associated with resistance to cetuximab. Resistance to cetuximab may therefore be potentially accomplished through increased VEGF signaling. In the HCT-8 model, DC101 inhibited the development of resistance to IMC-A12 + cetuximab therapy. Given the relationship between cetuximab resistance and VEGF production, this effect of DC101 may be related to the inhibition of VEGF signaling through VEGFR2, although further work is necessary to test this hypothesis. Nevertheless, the data related to resistance in the BxPC-3 and HCT-8 models added further support to the prioritization of DC101 + cetuximab combination therapy, based on the ability of each component of the combination to weaken the factors that negatively impact the efficacy of the other component, or cross-inhibition of resistance mechanisms.

In the selected models the data did not support the need for IMC-A12 in a combination regimen, if antibodies to EGFR and VEGFR2 were already included. While it is certainly true that cancer models probably exist in which IMC-A12 would increase the anticancer effects of EGFR + VEGFR2 inhibition, this did not occur with the selected panel of models in spite of the fact that some of the chosen models responded to IMC-A12 monotherapy. Nevertheless, in patients, IMC-A12 (24), as well as cetuximab (25), may offer benefits not fully modeled in the present studies, such as increasing the immune response against tumors. However based on the present results, combined inhibition of EGFR and VEGFR2 signaling, without inhibition of IGF-IR, was placed at the top of the developmental priority list among the four potential combination approaches and the three potential monotherapy regimens, targeting one or more of these pathways.

In the clinic, combining EGFR + VEGF targeted agents with chemotherapy has not been found to improve efficacy beyond that of the VEGF targeted agent + chemotherapy alone in metastatic colorectal cancer (reviewed in 26). Combined EGFR+VEGF targeted therapy in renal cell cancer, without chemotherapy, was also found to provide no benefits beyond that found with VEGF targeted therapy alone (27). However, encouraging early results with EGFR + VEGF targeted agents have been reported in recurrent non-small cell lung cancer (28) and irinotecan-refractory colorectal cancer patients (29), with regard to safety and treatment response rates, supporting the potential utility of this combination targeted approach in these indications. The specific clinical testing of cetuximab with an antibody inhibitor of VEGFR2, such as IMC-1121B (30), as performed here in preclinical models with DC101, has not yet been performed. The preclinical data however indicate that this combination stands out among the possible combinations of EGFR, VEGFR2 and IGF-IR targeted antibodies, in terms of the potential to achieve a consistent and significant level of anticancer efficacy.

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