Epidermal Growth Factor Receptor Blockade by Antibody IMC-C225 Inhibits Growth of a Human Pancreatic Carcinoma Xenograft in Nude Mice

Jay P. Overholser, B.A.
Marie C. Prewett, M.A.
Andrea T. Hooper, B.Sc.
Harlan W. Waksal, M.D.
Daniel J. Hicklin, Ph.D.

Department of Immunology, ImClone Systems Incorporated, New York, New York.

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Address for reprints: Daniel J. Hicklin, Ph.D., Department of Immunology, ImClone Systems Incorporated, 180 Varick Street, New York, NY 10014.,

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BACKGROUND. Pancreatic carcinoma is associated with a poor prognosis, and treatment options for patients with this disease are limited. The epidermal growth factor (EGF) receptor and its ligands are overexpressed in human pancreatic carcinoma and may contribute to the pathophysiology of these tumors.

METHODS. The anti-EGF receptor monoclonal antibody IMC-C225 was used to determine the effects of EGF receptor blockade on the growth of human pancreatic carcinoma BxPC-3 cells in vitro. Athymic mice bearing established (200 mm^3) subcutaneous BxPC-3 xenografts were treated with IMC-C225 (17 or 33 mg/kg every 3 days) alone or in combination with 5-fluorouracil (17 mg/kg twice weekly).

RESULTS. IMC-C225 inhibited exogenous ligand-stimulated tyrosine phosphorylation of the EGF receptor on BxPC-3 tumor cells. Treatment of BxPC-3 cells with IMC-C225 inhibited DNA synthesis (23.8%) and colony formation in soft agar (45.6%). IMC-C225 treatment significantly suppressed the growth of BxPC-3 tumors compared with treatment with vehicle alone ($P = 0.003$). Combination therapy with IMC-C225 and the chemotherapeutic agent 5-fluorouracil enhanced the antitumor effects compared with either agent alone and resulted in regression of pancreatic tumors in several animals. Histologic examination of pancreatic tumors from mice treated with IMC-C225 showed extensive tumor necrosis that coincided with a substantial decrease in tumor cell proliferation and an increase in tumor cell apoptosis.

CONCLUSIONS. These data suggest that IMC-C225 affects the growth of pancreatic tumors by inhibiting EGF receptor–dependent proliferation and survival, and demonstrates the potential for therapeutic application of IMC-C225 antibody in the treatment of human pancreatic carcinoma. ***Cancer*** **2000;89:74–82.**
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Adenocarcinoma of the pancreas ranks fifth among the leading causes of cancer death in the Western world and has an exceedingly poor prognosis. At diagnosis, approximately 80% of patients have lymph node involvement, and half have detectable visceral metastasis with a median survival of 3–6 months.[1] Incidence of pancreatic adenocarcinoma is nearly equal to the mortality rate for this malignancy, which emphasizes the aggressive nature of the disease. Treatment options for pancreatic carcinoma patients with advanced metastatic disease are limited because testing with virtually all approved chemotherapy drugs has been disappointing. Radiotherapy and 5-fluorouracil (5-FU) treatment can decrease locoregional tumor recurrence, but survival remains low because of widespread meta-

static disease.[2] More recently, gemcitabine has been reported to have an improved clinical benefit compared with 5-FU (median survival, 5.7 months compared with 4.4 months) and demonstrated improved relief of symptoms but with a low objective response rate.[3,4] Combination therapy regimens have failed to show improved clinical benefit over any single agent. Novel therapeutic approaches that provide a more effective outcome for a larger number of pancreatic carcinoma patients would have a clear clinical benefit.

Growth and regulation of human pancreatic carcinoma cells involve a variety of growth factors, regulatory peptides, polypeptides, and steroid hormones.[5] In particular, the epidermal growth factor (EGF) receptor pathway appears to play an important role in pancreatic carcinoma.[6] Pancreatic carcinoma cell lines frequently display overexpression of the EGF receptor.[7,8] Many pancreatic tumor cell lines also secrete significant amounts of transforming growth factor-alpha (TGF-α) protein and exogenous TGF-α stimulates the growth and proliferation of these tumor cells.[9–15] EGF receptor is overexpressed in 30–50% of human pancreatic carcinomas.[16–18] Coexpression of EGF receptor and its ligands EGF and TGF-α is also a common occurrence in the majority of pancreatic carcinomas.[18,19] Coexpression of EGF receptor with EGF or TGF-α is associated with tumor size, advanced clinical staging, and decreased patient survival.[18,20] These studies suggest that EGF receptor blockade on pancreatic tumor cells may be a means of limiting disease progression by inhibiting tumor cell growth, inducing terminal differentiation or causing apoptosis.[21–23]

The mouse anti-EGF receptor monoclonal antibody (mAb) 225, and its mouse-human chimeric version IMC-C225, bind the receptor with high affinity, compete for ligand binding, and down-regulate receptor expression on the cell surface.[24–26] Blockade of ligand binding to EGF receptor by mAb 225 inhibits activation of the receptor tyrosine kinase and retards cell cycle progression with accumulations of cells in the G1 phase.[27] Several studies have shown that IMC-C225 is capable of inhibiting growth of EGF receptor–expressing tumor cells in vitro[28–32], and treatment with IMC-C225 results in marked inhibition of tumor growth in nude mice bearing xenografts of human cancer cell lines.[23,33–38] Moreover, treatment with IMC-C225 in combination with the chemotherapeutic drugs or radiotherapy is effective in eradicating well established tumors in nude mice.[39–42] The objective of this study was to determine the potential therapeutic utility of the mAb IMC-C225 to inhibit growth of pancreatic cells in vitro and pancreatic tumor xenografts in a mouse model.

MATERIALS AND METHODS

Animals

Five to 6-week-old female athymic (*nu/nu*) mice were purchased from Harlan Sprague Dawley, Inc. (Indianapolis, IN). Mice were housed under pathogen-free conditions in microisolator cages with laboratory chow and water available ad libitum. All experiments and procedures were performed in accordance with the US Department of Agriculture, Department of Health and Human Services and National Institute of Health policies regarding the humane care and use of laboratory animals.

Cell Line

The pancreatic carcinoma cell line BxPC-3 was obtained from the American Type Culture Collection (Manassas, VA) and maintained in Dulbecco's modification of Eagle's medium (DMEM) (Life Technologies, Gaithersburg, MD) supplemented with 10 % fetal bovine serum (FBS; HyClone, Lenexa, KY) and 2 mM GlutaMAX (Life Technologies). Cells were cultured at 37 °C in a 5 % CO_2 atmosphere, routinely passaged by Trypsin-ethylenediamine tetraacetic acid (Life Technologies) treatment and checked for mycoplasma contamination.

Anti-EGF Receptor Monoclonal Antibody IMC-C225

Clinical grade anti-EGF receptor monoclonal antibody IMC-C225 was produced and supplied by the ImClone Systems Incorporated manufacturing facility (Somerville, NJ).

Phosphorylation Assay

Phosphorylation assays were performed by seeding BxPC-3 cells at 5×10^6/150 mm^2 tissue culture plate in DMEM containing 0.5% FBS in the presence or absence of 5 μg/mL IMC-C225. After culturing for 24 hours, cells then were stimulated with 5 ng/mL EGF or 5 ng/mL TGF-α (Sigma, St. Louis, MO) for 20 minutes at 37 °C. Monolayers then were washed with ice-cold phosphate buffered saline (PBS) containing 1 mM sodium orthovanadate. Cells were lysed, immunoprecipitated with IMC-C225, and subjected to Western blot analysis. The phosphorylation patterns were determined by probing the blots with horseradish peroxidase (HRP)-conjugated antiphosphotyrosine MAb (UBI, Lake Placid, NY) followed by detection using the ECL method (Amersham, Arlington Heights, IL). Loading equivalence of EGF receptor in each lane was verified by stripping and reprobing blots with a polyclonal anti-EGF receptor antibody (Calbiochem, San Diego, CA).

Cell Proliferation Assay

Inhibition of pancreatic carcinoma cell growth in vitro was determined by a ^{3}H-thymidine incorporation assay. Cells were plated in into 96-well tissue culture plates (10^4 cells/well) with IMC-C225 or controls in DMEM containing 2 % FBS. After 24 hours, 0.5 uCi/well ^{3}H-thymidine (New England Nuclear, Boston, MA) was added and cultures incubated an additional 24 hours. Cells then were harvested and the incorporated radioactivity was determined by liquid scintillation counting (Wallac, Gaithersburg, MD). Percent inhibition of cell proliferation was calculated as [1 − (cpm IMC-C225/cpm human (hu) IgG1)] × 100. Data is shown as the mean ± standard deviation (SD) of 6 replicate wells from 3 independent assays.

Colony Formation

Assay BxPC-3 cells (10^4 cells/well) were suspended in 1.0 mL of 0.35 % agar (Sigma) supplemented with complete RPMI-1640 culture medium (Life Technologies). This suspension was layered over 1.0 mL of 0.7 % agar medium base layer in 12-multiwell cluster dishes. After the agar solidified, 1 mL complete RPMI-1640 medium was added. All three layers contained 5 μg/mL IMC-C225 or hu IgG (Jackson ImmunoResearch, West Grove, PA). Medium was changed twice weekly. Three weeks after cell seeding was performed, the immobilized cells were stained with INT solution (*p*-iodonitrotetrazolium violet, 0.1 % w/v; Sigma) overnight (o.n.). Viable colonies larger than 0.05 mm were counted as described previously.[43] Data are shown as the mean ± SD of six wells and representative of three independent experiments.

Treatment of Subcutaneous Pancreatic Carcinoma Xenografts

Subcutaneous pancreatic carcinoma tumors were established by injecting athymic nude mice subcutaneously in the right flank with 2×10^6 BxPC-3 cells mixed in Matrigel (Collaborative Research Biochemicals, Bedford, MA). Tumors were allowed to reach 200 mm^3 in size, and then mice were randomized into groups of 12 animals each. Animals received intraperitoneal injections of IMC-C225 at 17 or 33 mg/kg or saline every 3 days for 10 weeks total treatment. In separate experiments, mice with established BxPC-3 xenografts were randomized into 4 treatment groups receiving: IMC-C225 (33 mg/kg every 3 days), 5-FU (17 mg/kg twice weekly), IMC-C225 and 5-FU in combination, or saline. Pilot experiments determined that this was the maximal tolerated dose and schedule for 5-FU treatment and resulted in the maximal effect on tumor growth. Tumors were measured twice each week with calipers and tumor volumes calculated by the formula $[\pi/6\ (w_1 \times w_2 \times w_2)]$, where w_1 represents the largest tumor diameter and w_2 represents the smallest tumor diameter.[40] Tumor regression was defined as $\geq$ 50% reduction of measured tumor volume compared with tumor volume at the start of treatment.

Histology

Tumors were fixed in 10% neutral buffered formalin, embedded in Paraplast Plus (Shandon Lipshaw, Pittsburgh, PA), and were serially sectioned at 6 μm onto silane (Sigma) coated slides. All tumors were stained with Mayer's hematoxylin (Sigma) and eosin (H & E) for routine histologic examination. After deparaffinization, rehydration, peroxide block, and enzymatic antigen retrieval steps, sections were immunostained using an HRP-labeled mouse anti-human proliferating cell nuclear antigen (PCNA) monoclonal antibody (clone PC10, EPOS Reagent; DAKO, Carpenteria, CA) for 1 hour at room temperature (r.t.). After washing in PBS, immunoreactivity then was developed using a liquid DAB substrate kit (Zymed Laboratories, South San Francisco, CA). Sections were counterstained with Light Green (Biomeda, Foster City, CA), dehydrated, cleared in xylene, and cover slipped. TdT-mediated dUTP nick end labeling (TUNEL) staining was performed using an In Situ Cell Death Detection Kit (Boehringer Mannheim, Indianapolis, IN). Sections were digested for 15 minutes at r.t. with proteinase K (20 μg/mL), permeabilized with 0.1 % sodium citrate buffer containing 0.1 % Triton X-100 for 2 minutes at 4 °C, and blocked for 60 minutes with 1 × Universal Blocker (Biogenex, San Ramon, CA). Sections were incubated with the fluorescein isothiocyanate conjugated TUNEL labeling mix for 1 hour at 37 °C. After washing in PBS, slides were cover slipped. Light and fluorescent images of immunostained tissue were viewed on a Zeiss (Thornwood, NY) Axioskop microscope and were digitized using a SONY (Sony Corp., Irving, TX) camera and Scion (Scion Corp., Frederick, MD) CG-7 framegrabber. All images were captured using Scion Image (version beta 2). PCNA was quantitated by calculating the number of brown pixels in 10 random fields per section from 2 mice in each group by using Corel PhotoPaint (version 8). TUNEL positive events were quantitated by analyzing 10 random fields per section from 2 mice in each group using ImageTool (version 2.0) (University of Texas Health Science Center, San Antonio, TX). TUNEL index was calculated by the formula TE/TV, where TE is the number of TUNEL positive events and TV is the mean tumor volume of the group.

Statistical Analysis

Tumor volumes were analyzed using a Student *t* test. Analyses were computed using the SigmaStat statistical package (version 2.03; Jandel Scientific, San Rafael, CA).

RESULTS

IMC-C225 blockade of ligand-stimulated EGF receptor phosphorylation on BxPC-3 pancreatic tumor cells was evaluated in a receptor phosphorylation assay. BxPC-3 tumor cells express approximately 8×10^5 EGF receptors per cell (M.C. Prewett, unpublished data). Addition of 5 ng/mL exogenous EGF or TGF-α ligand to BxPC-3 cultures increased tyrosine phosphorylation of the EGF receptor (Fig. 1A). Treatment of BxPC-3 cells with 5 μg/mL IMC-C225 significantly inhibited both EGF and TGF-α stimulated EGF receptor tyrosine phosphorylation.

To determine whether IMC-C225 could inhibit the growth of BxPC-3 cells in vitro, we incubated cell lines with various concentrations of IMC-C225 and assessed them for ^{3}H-thymidine incorporation into DNA. IMC-C225 inhibited DNA synthesis of BxPC-3 cells in a concentration-dependent manner (Fig. 1B). Maximal inhibition of DNA synthesis in BxPC-3 cells with IMC-C225 averaged 23.8% compared with control IgG1. The effects of IMC-C225 blockade on pancreatic tumor cell growth also were tested in colony formation assay in soft agar. BxPC-3 cells were incubated with 5 μg/mL IMC-C225 or control IgG and the extent of colony formation was assessed after 21 days. IMC-C225 inhibited the colony formation ability of BxPC-3 cells by 45.6% compared with the hu IgG control (Fig. 1C).

The effects of IMC-C225 on pancreatic tumor growth in vivo were evaluated in a pancreatic subcutaneous xenograft model. Athymic mice with established (200 mm^3) BxPC-3 tumors were treated with IMC-C225 or vehicle alone. IMC-C225 inhibited the growth of subcutaneous BxPC-3 tumors in a dose-dependent manner (Fig. 2A). Mice treated with 17 or 33 mg/kg of IMC-C225 displayed a significant decrease in tumor volume compared with mice treated with vehicle alone ($P = 0.003$ and 0.001, respectively). The effect of IMC-C225 on mean tumor volume appeared to be mainly cytostatic with respect to tumor growth. Treatment did not completely eliminate tumor burden as evidenced by the regrowth of tumors in all mice after Day 38 when treatment was withdrawn.

The effects of IMC-C225 in combination with the chemotherapeutic agent 5-FU also were examined in the BxPC-3 xenograft model. 5-FU was chosen for these studies because pilot in vitro experiments

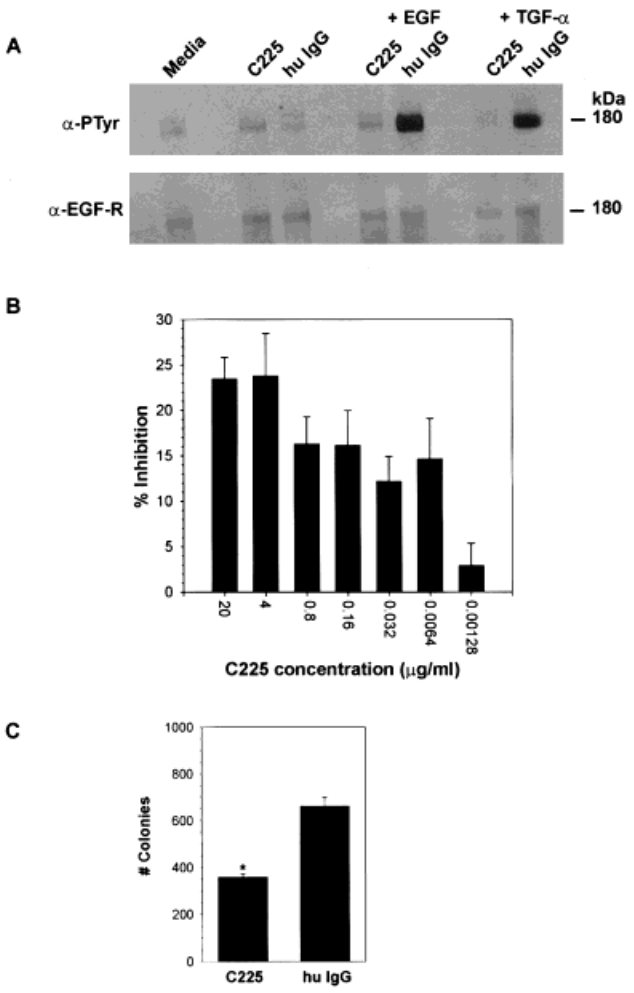

FIGURE 1. (A) Effect of IMC-C225 on EGF receptor tyrosine phosphorylation on human pancreatic BxPC-3 carcinoma cells is shown. (B) IMC-C225 inhibits proliferation of BxPC-3 cells in vitro. Bars indicate percentage of inhibition = [1− (cpm IMC-C225/cpm hu IgG1)] × 100. Error bars indicate SD of representative results from three independent assays. (C) Inhibition of BxPC-3 colony formation by IMC-C225 is shown. Bars indicate the number of BxPC-3 colonies; error bars indicate standard error. Asterisk indicates *P* value < 0.05. EGF: epidermal growth factor; TGF-α: transforming growth factor-alpha; hu IgG: human immunoglobulin; α-PTyr: anti-phosphotyrosine; α-EGF-R: anti-epidermal growth factor receptor; kDa: kilodalton.

showed that BxPC-3 was sensitive to this agent but not sensitive to gemcitabine. Groups of athymic mice bearing established BxPC-3 tumors were treated with IMC-C225 (33 mg/kg), 5-FU (17 mg/kg), and IMC-C225 plus 5-FU, respectively. Treatment with 5-FU alone had no significant (P =0.869, Table 1) effect on BxPC-3 tumor growth compared with vehicle control whereas at IMC-C225 alone significantly inhibited growth of BxPC-3 tumors compared with vehicle and 5-FU ($P = 0.003$ and 0.018, Table 1), respectively (Fig. 2B). IMC-C225 treatment combined with 5-FU

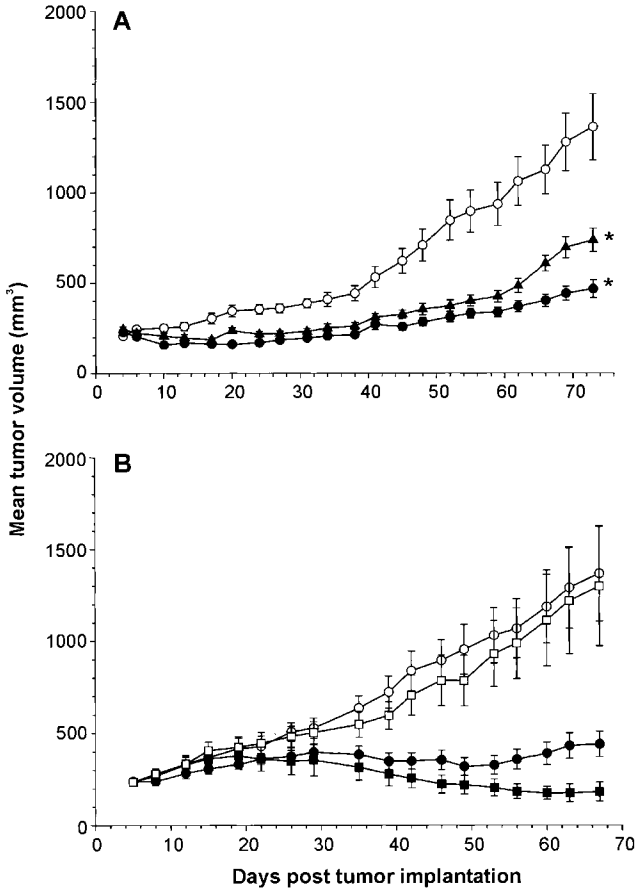

FIGURE 2. Growth inhibition of BxPC-3 xenografts in nude mice by IMC-C225. (A) Dose-dependent effects of IMC-C225 therapy alone on BxPC-3 tumors is shown. Open circles: saline control; triangles: IMC-C225 at 17 mg/kg; filled circles: 33 mg/kg. (B) Effects of IMC-C225 combined with 5-FU on BxPC-3 xenografts. Open circles: saline control; filled circles: IMC-C225; open squares: 5-FU; filled squares: combined IMC-C225 and 5-FU therapy. Error bars indicate standard error.

showed an enhanced antitumor effect compared with IMC-C225 alone ($P = 0.008$, Table 1). Furthermore, combination therapy resulted in tumor regressions in 5 of 10 mice by Day 67. Tumor regression was defined as $\geq$ 50% reduction in measured tumor volume compared with tumor volume at the start of treatment. Only 1 of 10 animals in the single-agent IMC-C225 group met this criteria.

Histologic examination of BxPC-3 xenografts showed dramatic differences in tumors from IMC-C225–treated versus control animals. H & E staining of tumor sections from the IMC-C225–treated group displayed large areas of necrosis and fibrosis, with necrotic regions of tumors replaced by collagen and fibrous tissue (Fig. 3A,B). A marked decrease in tumor cell proliferation also was observed in IMC-C225–treated tumors as measured by anti-PCNA immunostaining (Fig. 3C,D). Quantitation and statistical comparisons of PCNA immunostaining among the treatment groups showed statistically significant lower tumor cell proliferation in the group treated with both IMC-C225 and 5-FU compared with IMC-C225 alone ($P < 0.001$) or 5-FU alone ($P < 0.001$). In addition, increased tumor cell apoptosis was observed in BxPC-3 tumors of IMC-C225–treated animals (Fig. 3E,F). The TUNEL index in the combination therapy tumors was significantly higher than in tumors treated with IMC-C225 alone ($P = 0.033$). A summary of PCNA and TUNEL quantitation is shown in Table 2.

TABLE 1
Summary of Results from BxPC-3 Xenograft Model

Treatment group	Mean tumor volume	SE	*P* value
Saline	1367.52	259.23	—
5-FU	1297.92	323.66	0.869[a]
IMC-C225	439.10	69.27	0.003[a]; 0.018[b]
IMC-C225 + 5-FU	180.92	51.57	0.003[b]; 0.008[c]

SE: standard error; 5-FU: 5-fluorouracil.
[a] Compared with saline control.
[b] Comparison to 5-FU.
[c] Comparison to IMC-C225.

DISCUSSION

Advanced pancreatic carcinoma is not responsive to most chemotherapeutic drugs, and patients with this disease have a poor prognosis. The dismal outlook for these patients underscores the necessity for novel therapeutic approaches for treatment of pancreatic carcinoma. Growth factors and growth factor receptors play an important role in regulating the growth of malignant cells.[44–46] Overexpression of the EGF receptor in 30–50 % of human pancreatic adenocarcinomas suggests that blockade of this receptor may be a useful approach to inhibit pancreatic tumor growth. The current study has demonstrated that blockade of the EGF receptor inhibits the growth of pancreatic carcinoma cells in vitro and growth of pancreatic tumors in athymic mice. In vitro results showed a modest inhibition (23.8%) by IMC-C225 on the growth of cultured pancreatic carcinoma cells as determined in a DNA synthesis assay. The effects of IMC-C225 in vitro were more pronounced in a colony-forming assay, in which the antibody inhibited colony formation of pancreatic tumor cells by 45.6 % compared with control. In vivo, IMC-C225 was very effective in suppressing the growth of BxPC-3 tumors in a subcutaneous tumor model. Significantly, these results were obtained with large established tumors (0.5–1.0% body weight), and regression of these tumors was observed in several

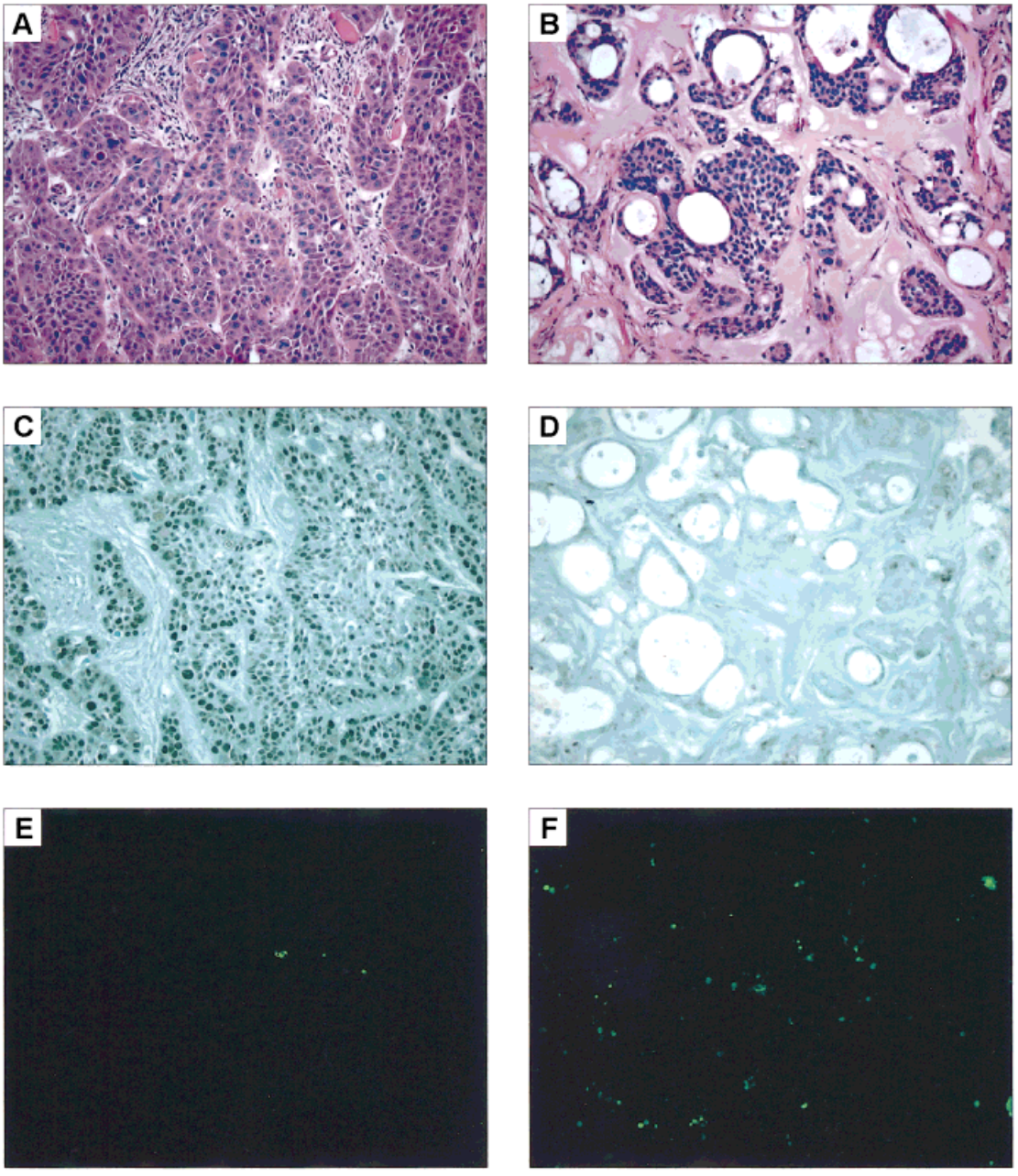

FIGURE 3. Histologic examination of BxPC-3 subcutaneous xenografts. Representative photos from the control group (left panels) and the IMC-C225 group (right panels). (A and B) H & E staining of paraffin sections is shown. (C and D) Sections immunostained for proliferating cell nuclear antigen (brown) is shown. (E and F) Tumor sections were assayed for apoptosis by FITC-labeled TUNEL assay (green). (H & E, original magnification ×200).

IMC-C225–treated animals. These results are consistent with the findings obtained in other tumor models in which IMC-C225 was highly effective at inhibiting tumor growth in vivo with only partial in vitro inhibition of tumor cell proliferation.[35,37,39,41,47] In vivo antitumor effects of IMC-C225 also have been observed in low and moderate EGF receptor–expressing pancreatic xenograft models HPAC (M.C. Prewett, unpublished data) and L3.6pl, respectively.[48]

Combined treatment of IMC-C225 with the antineoplastic drug 5-FU enhanced the antitumor effect against BxPC-3 xenografts and resulted in regression of tumors in 5 of 10 animals. To our knowledge, this is the first study showing that combination of IMC-C225 and 5-FU enhanced antitumor activity, it and suggests that this combination treatment may have efficacy on other EGF receptor positive, 5-FU sensitive tumors as well. Gemcitabine was not used in combination with

TABLE 2
Quantitation of Staining by Immunohistochemical Analysis

Treatment group	PCNA pixels	SE	*P* value	TUNEL index	SE	*P* value
Saline	146,563	7902	—	7.81×10^{-3}	1.58×10^{-3}	—
5-FU	125,972	10,177	0.018[a]	1.40×10^{-2}	1.97×10^{-3}	0.018[a]
IMC-C225	102,526	8284	< 0.001[a]; 0.082[b]	5.02×10^{-2}	8.97×10^{-3}	< 0.001[a]; 0.001[b]
IMC-C225 + 5-FU	53,043	3419	< 0.001[b]; < 0.001[c]	1.83×10^{-1}	5.93×10^{-2}	< 0.001[b]; < 0.033[c]

PCNA: proliferating cell nuclear antigen; SE: standard error; TUNEL: TdT-mediated dUTP nick end labeling; 5-FU: 5-fluorouracil.
[a] Compared with saline control.
[b] Comparison to 5-FU.
[c] Comparison to IMC-C225.

IMC-C225 in these studies because BxPC-3 tumor cells are resistant to this antineoplastic drug (Merriman et al.[49] and M.C. Prewett, unpublished results). However, combination treatment with IMC-C225 and gemcitabine may be active against other pancreatic tumors. In this regard, IMC-C225 has been shown to enhance the antitumor activity of gemcitabine in the orthotopic L3.6pl human pancreatic tumor model.[48] The use of IMC-C225 with appropriate cytotoxic drugs in pancreatic carcinoma will require further testing in a clinical setting to determine the effectiveness of combination therapy.

Histologic examination of subcutaneous pancreatic tumors from IMC-C225–treated animals showed a dramatic decrease in mitotic tumor cells as measured by a decrease in PCNA staining. This decrease was significant between IMC-C225 treated tumors versus controls and between combination therapy versus single-agent IMC-C225. Furthermore, IMC-C225–treated tumors showed evidence of increased tumor cell apoptosis, and combination therapy had a significantly higher TUNEL index compared with single-agent IMC-C225. The decrease in tumor cell proliferation and increase in apoptosis are likely to be responsible for the extensive tumor necrosis observed in IMC-C225–treated tumors. The high incidence of tumor cell apoptosis suggests that the effects of IMC-C225 treatment on pancreatic tumors in vivo are not merely cytostatic but rather affect the growth and survival of these tumors. These findings are consistent with those of previous studies that have demonstrated IMC-C225–induced tumor cell apoptosis in various EGF receptor positive human tumor models in athymic mice.[37,38]

The mechanism of IMC-C225's inhibitory effect on tumor growth has been investigated extensively in other tumor models. Treatment of cells with anti-EGF receptor murine mAb 225 leads to inhibition of EGF receptor downstream signaling and perturbation of cell cycle progression resulting in the accumulation of cells in the G1 phase.[50] A proposed mechanism is cell cycle arrest at the G1 checkpoint, i.e., increased levels of the cell cycle inhibitor p27KIP1 that inactivates CDK2 kinase.[50,51] The extent to which anti-EGF receptor blockade inhibits cell cycle progression appears to be dependent on the particular tumor cell line used in each study. For example, treatment of colon carcinoma cells DiFi with mAb 225 dramatically inhibits the growth of these cells and induces programmed cell death.[34] In other tumor cells, EGF receptor blockade leads to a steady accumulation of cells in G1 but does not induce apoptosis.[52] The results of the current study are compatible with these previous observations in other human tumor models. Treatment of BxPC-3 cells in vitro with IMC-C225 resulted in an accumulation of cells in the G1 phase resulting in decreased proliferation. In vivo analyses demonstrated a decrease in the number of mitotic pancreatic tumor cells in mice treated with IMC-C225. Antiangiogenic mechanisms also have been implicated in the antitumor effects observed in vivo with IMC-C225 treatment. In a study by Petit et al.,[53] IMC-C225 was shown to affect the angiogenic potential of A431 cells by down-regulating VEGF expression by these tumor cells and thus decreasing the angiogenesis associated with growth of A431 tumors in vivo. An antiangiogenic effect of IMC-C225 also was shown in a bladder carcinoma study.[38]

IMC-C225 currently is undergoing testing in Phase III clinical trials in advanced head and neck squamous cell carcinoma. Early clinical trials in patients with head and neck carcinoma have shown promising antitumor effects with IMC-C225 in combination with radiation or cisplatin.[54,55] Advanced pancreatic carcinoma is often refractory to chemotherapy and radiotherapy leading to a poor prognosis for patients with this disease. Novel therapies that would provide effective treatment for a broader number of pancreatic carcinoma patients would have a clear clinical benefit. Results of the current study suggest that blockade of the EGF receptor may be a useful strategy for treat-

ment of EGF receptor positive pancreatic carcinoma. In this respect, a Phase II trial has been initiated to evaluate IMC-C225 as a treatment for human pancreatic carcinoma in a clinical setting.

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