Cooperative Inhibition of Renal Cancer Growth by Anti-Epidermal Growth Factor Receptor Antibody and Protein Kinase A Antisense Oligonucleotide

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Background: The expression of epidermal growth factor receptor (EGFR) and type I cyclic adenosine monophosphate (cAMP)-dependent protein kinase (PKAI) is associated with neoplastic transformation. By use of human renal cancer cell lines (i.e., 769-P, ACHN, A498, and SW839), we investigated the antiproliferative activity and the antitumor activity of an anti-EGFR humanized chimeric mouse monoclonal antibody, MAb C225, and a novel mixed backbone 18-mer antisense oligonucleotide, HYB 190, that targets expression of the R10 regulatory subunit of PKAI. Methods: The antiproliferative activity of MAb C225 and oligonucleotide HYB 190, alone or in combination, on different renal cancer cell lines was determined by monitoring cell growth in soft agar. In addition, the induction of apoptosis by treatment with the anti-EGFR antibody and/or antisense PKAI oligonucleotides was evaluated by flow cytometric analysis of fragmented DNA. The antitumor activity of MAb C225 and oligonucleotide HYB 190 was determined in athymic mice bearing established ACHN tumor xenografts. Cell proliferation and tumor growth data were evaluated for statistical significance using Student’s t test; reported P values are two-sided. Results: MAb C225 and oligonucleotide HYB 190 inhibited colony formation in soft agar in a dose-dependent manner for all renal cancer cell lines tested. We observed a potentiation of growth inhibition and induction of apoptosis when 769-P cells and ACHN cells were treated with both agents. Combination treatment with MAb C225 and oligonucleotide HYB 190 caused regression of ACHN tumor xenografts, whereas single-agent treatment only delayed tumor growth. Conclusion: The combination of anti-EGFR MAb C225 and HYB 190 antisense PKAI oligonucleotides HYB 190 exhibited cooperative antiproliferative effects and cooperative antitumor effects on EGFR and PKAI-expressing human renal cancer cell lines. [J Natl Cancer Inst 1998;90:1087–94]

Renal cell carcinoma is characterized by a lack of specific clinical signs that allow the diagnosis at an early stage (1). Therefore, a high proportion of patients will have metastasis at the time of first diagnosis and cannot be cured, since there is no effective therapy for metastatic renal cancer. Radiotherapy, chemotherapy, or hormonotherapy have little or no effect in this disease (1), although immunomodulating agents, cytokines, or differentiating agents such as retinoids have shown antitumor activity in a small proportion of patients with metastatic renal cancer (2–4). Because of its poor prognosis, there is a need for novel and more selective drugs that are able to interfere with targets directly involved in the process of renal cancer development and progression.

Experimental and clinical studies have provided evidence for...
a transforming growth factor-α (TGF-α)–epidermal growth factor receptor (EGFR)-mediated autocrine growth stimulatory pathway in a variety of human cancers, including renal cancer (5). Overexpression of TGF-α and EGFR has been associated with poor prognosis and metastatic spreading (6–8). Thus, the use of anti-EGFR-blocking monoclonal antibodies (MAbs), of recombinant proteins containing TGF-α or epidermal growth factor (EGF) fused to toxins, or of EGFR-specific tyrosine-kinase inhibitors has been proposed as a potential therapeutic factor (EGF) fused to toxins, or of EGFR-specific tyrosine-kinase inhibitors has been proposed as a potential therapeutic modality (9–16). Several blocking anti-EGFR MAbs that inhibit the in vitro and in vivo growth of human cancer cell lines over-expressing TGF-α and EGFR have been obtained (17,18). Among these, MAb 528 and MAb 225 are two mouse MAbs that have been extensively characterized for their biologic and preclinical properties and represent the first series of anti-EGFR blocking agents that have entered clinical evaluation in cancer patients (9,10,19,20). To allow repeated administrations of these antibodies without the production of human anti-mouse antibodies in cancer patients, a chimeric human-mouse MAb 225 (MAb C225), that contains the human immunoglobulin G1 (IgG1) constant region, has recently been developed and purified for clinical use and is in early clinical trials (21–24).

Protein kinase A (PKA) is an intracellular enzyme involved in the controlling of cell growth and differentiation. Mammalian cells produce two isoforms, type I (PKAI) and type II (PKAII), with identical catalytic subunits but different cyclic adenosine monophosphate (cAMP)-binding regulatory subunits (termed RI in PKAI and RII in PKAII) (25). PKAI mediates mitogenic signals upon receptor ligation of different growth factors, including EGF and TGF-α (26–29). PKAI is also directly involved in cell proliferation and neoplastic transformation (30,31) and is required for the G1–S transition of the cell cycle. PKAI over-exression has been detected in a majority of human cancers and correlates with less favorable clinicopathologic features and poor prognosis in patients with ovarian and breast cancers (25,32,33). In contrast, PKAII is expressed in normal tissues and seems to be involved in cellular differentiation (25,32). The ratio of PKAI levels to PKAII levels in renal cell carcinomas is almost twice that of normal renal cortex (34). For this reason, the inhibition of PKAII activity or synthesis has been an appropriate target for the development of therapeutic agents. The selective down-regulation of PKAI activity by the cAMP analog 8-chloro-cAMP (8-Cl-cAMP) inhibits growth of a wide variety of cancer cell types in vitro and in vivo and is accompanied by decreased expression of different oncogenes and growth factor genes (35–38). In addition, 8-Chl-cAMP can be safely administered to cancer patients (39), justifying further clinical trials. A genetic approach to inhibiting PKAI expression has been recently developed by the use of phosphorothioate antisense oligonucleotides targeting the synthesis of PKAI regulatory subunit RIA. These oligonucleotides inhibit growth of several human cancer cell lines in vitro and in vivo (40–42). However, in vivo toxicity studies conducted with phosphorothioate oligonucleotides have shown nonsequence-specific side effects, which may be due to their polyamionic structure (43). Therefore, to improve the therapeutic potential of the antisense approach, novel antisense oligonucleotides with mixed backbone structure (MBOs) containing both methylphosphonate- and PS-modified residues have been synthesized. The MBOs have better pharmacokinetic and toxicity profiles in vivo (44). We have recently shown that HYB 190, an anti-RIA MBO, inhibits the growth of human colon, ovary, and breast cancer cell lines at submicromolar concentrations and has a cooperative growth inhibitory activity with different classes of cytotoxic drugs, including taxanes, platinum-derived agents, and topoisomerase II-selective drugs (45).

Previously, we provided evidence for a structural and functional basis for neoplastic transformation involving a TGF-α–EGFR autocrine pathway and increased PKAI expression and activation (26,29–31). We also characterized a cooperative antiproliferative effect of anti-EGFR MAbs with the PKAI inhibitor 8-Chl-cAMP on growth of human cancer cell lines in vitro and in vivo (46,47). In the current study, we evaluated the potential antiproliferative and antitumor activity of the anti-EGFR MAb C225 and the RIA HYB 190 antisense MBO separately and in combination on four human renal cancer cell lines.

Materials and Methods

MAbs and MBOs. The biochemical and biological characteristics of MAb C225, a human-mouse chimeric anti-EGFR IgG1, class MAb, have been previously described (21). MAb C225 was provided by H. Waksal (ImClone Systems, New York, NY). Synthesis of antisense 18-mer MBOs targeted to the 5′-terminal 8–13 codons of human RIA regulatory subunit messenger RNA for PKA (45) was previously described (44). The PKAII antisense oligonucleotide, designated HYB 190, had the following sequence: 5′-GCCTGGCCTCCTCACTGGCC-3′. A control PKAI antisense oligonucleotide containing four mismatched nucleotides was designated HYB 239. The mismatched nucleotides in HYB 239 are underlined: 5′-GCTATGCTCCGACACGGC-3′. Both HYB 190 and HYB 239 contain phosphorothioate- and methylphosphonate-internucleotide linkages. These linkages are represented in standard upper case letters (phosphorothioate) and by boldface upper case letters (methylphosphonate) for the flanking nucleotides. The identity of each oligonucleotide was confirmed by 31P nuclear magnetic resonance. Chemical purity of each MBO was determined by capillary gel electrophoresis, hybridization melting temperature, and A269/mass ratio (44).

Cell lines. Human renal cancer 769-P, ACHN, A498, and SW839 cell lines were obtained from the American Type Culture Collection (Manassas, VA). Cells were maintained in Dulbecco’s modified Eagle medium supplemented with 10% heat-inactivated fetal bovine serum, 20 mM HEPES, pH 7.4, penicillin (100 IU/mL), streptomycin (100 μg/mL), and 4 mM glutamine (ICN, Irvine, CA) in a humidified atmosphere of 95% air and 5% CO2 at 37°C.

Protein blot analysis. Samples of total cellular protein (50 μg) were fractionated through 7.5% or 12% sodium dodecyl sulfate–polyacrylamide gels transferred to nitrocellulose filters, incubated with specific MAbs, followed by incubation with horseradish–peroxidase antiserum (Bio-Rad Laboratories, Milan, Italy). Immune-reactive proteins were visualized by enhanced chemiluminescence (Amersham Italia, Milan, Italy), as described previously (31). The following specific antibodies were used: anti-human EGFR mouse MAb (Transduction Laboratories, Lexington, KY); anti-human RIA mouse MAb (Transduction Laboratories). Lysates from MCF-10A cells overexpressing RIA (MCF-10A-RIA) were used as positive controls for RIA expression (25).

Growth in soft agar assay. Cells (105 cells/well) were suspended in 0.5 mL of 0.3% Difco Noble agar (Difco Laboratories, Inc., Detroit, MI) supplemented with 0.1% sodium chloride (see cell lines above). This suspension was layered over 0.5 mL of 0.8% agar-medium base layer in 24 multiwell cluster dishes (Becton Dickinson, Lincoln Park, NJ) and treated with different concentrations of MAb C225 and/or oligonucleotide HYB 190 or oligonucleotide HYB 239 every 2 days, for a total of 4 days. After 12 days, the cells were stained with nitro blue tetrazolium (Sigma Chemical Co., St. Louis, MO) and colonies larger than 0.5 mm were counted as previously described (46).

Apoptosis assay. To evaluate the potential induction of apoptosis, 105 cells were plated in complete medium (see cell lines above) in 60-mm tissue culture dishes (Becton Dickinson, Lincoln Park, NJ) and treated every day for a total of 3 days with different concentrations of MAb C225 and/or oligonucleotide HYB 190. At the indicated time points, both adherent and detached cells were harvested. Flow cytometric analysis of apoptotic cell death was performed on cell
pellet fixed in 70% ethanol, washed in phosphate-buffered saline and mixed with ribonuclease (Sigma Chemical Co.) and propidium iodide (Sigma Chemical Co.) solution as previously reported (48). DNA content was analyzed by a FACScan flow-cytometer (Becton Dickinson, San Jose, CA) coupled with a Hewlett-Packard computer. The percentage of apoptotic cells was calculated by gating the hypodiploid region on the DNA content histogram using the LYSYS software (Becton Dickinson) as previously reported (48).

ACHN xenografts in nude mice. Five- to 6-week-old female BALB/cAnNCrlBR athymic (nu+/nu+) mice were purchased from Charles River Laboratories (Milan, Italy). The research protocol was approved and the mice were maintained in accordance with the institutional guidelines of the University of Naples Animal Care and Use Committee. Mice were acclimated to the University of Naples Medical School Animal Facility for 1 week prior to being injected with cancer cells. Mice were injected subcutaneously with 10^7 ACHN cells that were resuspended in 200 μL of Matrigel (Collaborative Biomedical Products, Bedford, MA). After 10 days, when well-established xenografts were detectable (tumor size approximately 0.30 cm^3), each group of 10 mice received one of the following treatments: 1) intraperitoneal injections of MAb C225 (0.25 mg/dose) twice weekly for 3 weeks; 2) intraperitoneal injections of oligonucleotide HYB 190 (200 μg/dose) for 5 days per week for 3 weeks; or 3) a combination of MAb C225 and oligonucleotide HYB 190 given with the above-described schedule. Tumor size was calculated using the formula \( \pi/6 \times \text{larger diameter} \times (\text{smaller diameter})^2 \) as previously reported (47).

Statistical analysis. Student’s t test was used to evaluate the statistical significance of the results. All P values represent two-sided tests of statistical significance. All analyses were performed with the BMDP New System statistical package version 1.0 for Microsoft Windows (BMDP Statistical Software, Los Angeles, CA).

Results

To determine whether the EGFR and the RIα subunit of PKAI were expressed by renal cancer cell lines, protein blots were performed using cell extracts produced from 769-P, ACHN, A498, and SW839 cells. As shown in Fig. 1, immunoreactive EGFR and RIα bands were detected in all four cell lines, suggesting that these cell lines could be suitable models for examining the effect(s) of agents that block the expression or function of the EGFR and/or the RIα subunit of PKAI. Therefore, we evaluated the effect of MAb C225 or oligonucleotide HYB 190 on the anchorage-independent growth of 769-P, ACHN, A498, and SW839 cells. All of the renal cancer cell lines treated with the anti-EGFR MAb displayed a dose-dependent inhibition of colony formation in soft agar (Fig. 2, A). The IC_{50} (i.e., concentration that causes 50% inhibition of growth) for MAb C225 was between 0.8 and 1.8 μg/mL. Similarly, oligo-

![Fig. 1. Detection of epidermal growth factor receptor (EGFR) (A) and RIα (B) in cell lysates prepared from human renal cancer cell lines by protein blot: 769-P (lane 1), ACHN (lane 2), A498 (lane 3), and SW839 (lane 4). Samples of total cellular protein (50 μg) were fractionated through 7.5% or 12% sodium dodecyl sulfate–polyacrylamide gels, transferred to nitrocellulose filters, and incubated with specific anti-EGFR or anti-RIα monoclonal antibodies, followed by horse-radish-peroxidase antiserum. Immunoreactive proteins were visualized by enhanced chemiluminescence (see “Materials and Methods”).](https://academic.oup.com/jnci/article-abstract/90/14/1087/929818/929818)

![Fig. 2. Dose-dependent growth inhibitory effects of monoclonal antibody (MAb) C225 (A), oligonucleotide HYB 190 (B), or oligonucleotide HYB 239 (C) on the soft agar growth of human renal cancer cell lines. Data represent the average (± standard error) of three different experiments, each performed in triplicate.](https://academic.oup.com/jnci/article-abstract/90/14/1087/929818/929818)
nucleotide HYB 190 inhibited growth in a dose-dependent manner (Fig. 2, B). Growth inhibition was more pronounced in ACHN cells (IC50 of 0.1 μM), while the IC50 values were between 1.2 and 2 μM in the other three cell lines tested. In contrast, the control oligonucleotide, HYB 239, at doses up to 2.5 μM, showed only 5%–15% growth inhibition in the soft agar assay among all of the cell lines tested (Fig. 2, C).

To determine whether the growth inhibitory effect of oligonucleotide HYB 190 correlated with a reduction in R1α protein levels, we performed protein blots on total cell extracts prepared from ACHN cells treated with oligonucleotide HYB 190 or oligonucleotide HYB 239. Compared with untreated ACHN cells, R1α levels were substantially unchanged, even in cells treated with a higher dose of the control oligonucleotide HYB 239 (1 μM). However, dose-dependent inhibition of R1α expression was seen when ACHN cells were treated with oligonucleotide HYB 190 (Fig. 3).

To determine whether a combination of MAb C225 and oligonucleotide HYB 190 could enhance the antiproliferative effect compared with either agent alone, 769-P cells and ACHN cells were treated with different combinations of the two agents. As shown in Fig. 4, a supraadditive effect was observed with both renal cell lines at all doses of MAb C225 and oligonucleotide HYB 190 examined. When lower doses of these agents were used, the growth inhibitory effect was cooperative for either cell line. For example, in 769-P cells treatment with HYB 190, 0.1 μM, determined less than 3% growth inhibition, whereas treatment with MAb C225 (0.25 and 0.5 μg/mL) caused 6% and 35% growth inhibition, respectively. Treatment with oligonucleotide HYB 190 (0.1 μM), plus MAb C225, at a concentration of either 0.25 or 0.5 μg/mL, produced 37% and 62% inhibition of colony formation in soft agar, respectively. For these treatment combinations, the cooperativity quotient, defined as the ratio of the net percent growth-inhibition effect by the agents in combination to the sum of the percent growth inhibition achieved by each agent (35,45), was 4.1 and 1.6, respectively.

Recently, it was shown that treatment of human DiFi colon cancer cells with an anti-EGFR blocking MAb caused the cells to become growth arrested in the G1 phase of the cell cycle, an event that eventually led to apoptotic cell death (49). Therefore, we determined the effect of MAb C225 and/or oligonucleotide HYB 190 on induction of apoptosis in 769-P cells and ACHN cells. Renal cancer cells 769-P and ACHN were treated with MAb C225 (1 μg/mL) and/or oligonucleotide HYB 190 (0.1 μM) for 3 days. The percentage of apoptotic cells was determined on day 4 and day 8 from the beginning of treatment (Fig. 5). Oligonucleotide HYB 190 alone (0.1 μM) did not induce apoptosis in either 769-P or ACHN cells. MAb C225 alone (1

Fig. 3. Protein blot analysis of R1α protein expression in human ACHN cells. MCF-10A-R1α cells (lane 1), untreated ACHN cells (lane 2), ACHN cells treated with oligonucleotide HYB 239, 1 μM (lane 3), or with oligonucleotide HYB 190, at a final concentration of 0.1 μM (lane 4), 0.5 μM (lane 5), and 1 μM (lane 6). See “Materials and Methods” for details on cell culture conditions. Samples of total cellular proteins (50 μg) were fractionated through 12% sodium dodecyl sulfate–polyacrylamide gels, transferred to nitrocellulose filters, and incubated with specific anti-R1α monoclonal antibody, followed by horseradish peroxidase–antiserum. Immunoreactive proteins were visualized by enhanced chemiluminescence (see “Materials and Methods”).
Fig. 5. Induction of programmed cell death by treatment with monoclonal antibody (MAb) C225 and/or oligonucleotide HYB 190 in 769-P (A) or ACHN (B) renal cancer cells. Cells were treated with the indicated concentrations of the two agents for 3 days. Flow cytometric analysis of DNA fragmentation was performed after 4 (○) and 8 (■) days after the beginning of treatment (see "Materials and Methods"). Data represent the average ± standard error of triplicate determinations. Student’s t test was used to compare apoptotic cell death among different treatment groups at days 4 and 8. In panel A, no statistically significant differences in the percent of apoptotic cells were observed between MAb C225-treated 769-P cells and control, nontreated 769-P or between oligonucleotide HYB 190-treated 769-P cells and control, nontreated 769-P cells either at day 4 or day 8. A statistically significant difference in the percent of apoptotic cells was observed between 769-P cells co-treated with MAb C225 and oligonucleotide HYB 190 and control, nontreated 769-P cells (two-sided P = .04 at day 4; two-sided P = .004 at day 8). In panel B, no statistically significant differences in the percent of apoptotic cells were observed between oligonucleotide HYB 190-treated ACHN cells and control, nontreated ACHN cells either at day 4 or at day 8. A statistically significant difference in the percent of apoptotic cells was observed between MAb C225-treated ACHN cells and control, nontreated ACHN cells (two-sided P = .05 at day 4; two-sided P = .05 at day 8). Between MAb C225 and oligonucleotide HYB 190 co-treated ACHN cells and control, untreated ACHN cells (two-sided P = .002 at day 4; two-sided P = .002 at day 8), and between ACHN cells co-treated with MAb C225 and oligonucleotide HYB 190 and ACHN cells treated with only MAb C225 (two-sided P = .01 at day 4; two-sided P = .01 at day 8).

Fig. 6. Antitumor activity of monoclonal antibody (MAb) C225 plus oligonucleotide HYB 190 on established ACHN human renal carcinoma xenografts. Mice were injected subcutaneously in the dorsal flank with 10^6 ACHN cells as described (see "Materials and Methods"). After 10 days (average tumor size, 0.30 cm^3), mice were treated intraperitoneally twice weekly with MAb C225 (0.25 mg/dose) for 3 weeks (▲), or each day for 5 days per week for 3 weeks with oligonucleotide HYB 190 (200 μg/dose) (○), or with the combination of both agents (△), or were untreated (●). Each group consisted of 10 mice. Student’s t test was used to compare tumor sizes among different treatment groups at day 42 following ACHN cell injection. MAb C225 versus control (two-sided P < .0001); oligonucleotide HYB 190 versus control (two-sided P < .0001); MAb C225 plus oligonucleotide HYB 190 versus control (two-sided P < .0001); MAb C225 plus oligonucleotide HYB 190 versus MAb C225 (two-sided P < .0001); MAb C225 plus oligonucleotide HYB 190 versus oligonucleotide HYB 190 (two-sided P < .0001).
of the experiment), the average ACHN cell tumor was reduced to 17% of its original size (day 10 of the experiment) (Fig. 6). In addition, two of 10 mice in the MAB C225 plus oligonucleotide HYB 190 treated group were tumor free from day 28 through day 42 of the experiment. In contrast, tumor size had increased by 377% in the untreated control group and by 198% and 208% in the groups treated with MAB C225 or oligonucleotide HYB 190 alone, respectively (Fig. 6). Both MAB C225 and oligonucleotide HYB 190 treatments alone or in combination were well tolerated by mice: no weight loss or other signs of acute or delayed toxicity were observed.

Discussion

A major approach to treating cancer is based on the use of cytotoxic drugs acting on intracellular targets that are generally common to both cancer and normal cells. Improved treatment efficacy has been obtained by combining two or more drugs and/or modifying their dosage and schedule of administration. However, several human cancer types are still relatively insensitive or have become resistant to cytotoxic drugs. For example, chemotherapeutic agents have little or no effect on advanced-stage renal cell carcinoma (1).

As an alternative cancer therapy, the use of biologic agents acting via different mechanisms may have a direct antitumor effect or may modulate the host immune response during the course of this disease. Examples of such agents are retinoids, the interferons, and interleukin 2. Each of these agents have entered clinical evaluation for the treatment of advanced renal cancer cell lines by blocking EGFR function and inhibiting PKAI expression in a dose-dependent manner. Furthermore, a marked increase in both their in vitro and in vivo antiproliferative activities was observed when the agents were used in combination. In fact, blockade of EGFR function and inhibition of PKAI expression caused an antiproliferative effect that was not simply additive. This effect was specific for the combination of MAB C225 and oligonucleotide HYB 190, because oligonucleotide HYB 239, a four-base mismatched molecule, did not affect renal cancer cell growth when used either alone or in concert with the anti-EGFR MAB C225. A plausible mechanism by which oligonucleotide HYB 190 could exert its antiproliferative effect with MAB C225 is by inducing programmed cell death. In fact, we observed increased numbers of apoptotic 769-P cells and ACHN cells when each was treated with MAB C225 and oligonucleotide HYB 190 in combination. It is likely that the combined blockade of the EGFR and PKAI signal transduction pathways, which are required for cells to traverse from G1 to S phases of the cell cycle, would render cancer cells more susceptible to apoptosis.

We also evaluated whether the cooperative growth inhibitory effect of MAB C225 and oligonucleotide HYB 190 observed in vitro could also be observed in vivo. We found that a 3-week treatment of nude mice bearing established ACHN cell xenografts with MAB C225 or with HYB 190 had a cytostatic growth inhibitory effect with a reduction in the tumor growth rate compared with untreated controls. A dramatic effect on tumor growth was seen in mice treated with both agents for the same period and the same dosage as single agent-treated mice: the induced tumors regressed, with no evidence of any tumor in two of 10 mice. The combined treatment with MAB C225 and oligonucleotide HYB 190 was well tolerated by the mice without signs of acute or delayed toxicity, suggesting that a longer treatment protocol could be given.

In terms of nonspecific toxicity, it should be noted that oligonucleotide HYB 190 does not specifically target mouse RIIα. Therefore, we cannot rule out the possibility of toxicity due to inhibition of endogenous RIIα in mice treated with a specific anti-mouse RIIα antisense oligonucleotide. However, we have recently shown that 8-Cl-cAMP, a cytotoxic compound known to inhibit both mouse PKAI activity and human PKAI activity, can be administered as a single agent or in combination with MAB C225 to nude mice bearing human GEO colon cancer...
xenografts for 5–10 weeks with no signs of toxicity [47]; our unpublished results.

Cancer cell growth can be inhibited by anti-EGFR MAbs or antisense RIs in concert with different classes of cytotoxic drugs, including taxanes, platinum derivatives, and topoisomerase II inhibitors [45,52,53]. In this study, we showed that both anti-EGFR MAbs and antisense RIs MBOs were cooperative inhibitors of renal cancer cell growth. Because anti-EGFR MAbs and antisense RIs MBOs mediate their effects by different mechanisms, it is unlikely that they would also share overlapping side effects or to antagonize the activity of cytotoxic drugs [45,47,52,53]. Therefore, a combination of MAb C225 and oligonucleotide HYB 190 following chemotherapy is an approach worthy of additional investigation in patients with renal cancer.

References

(33) Fossberg TM, Doskeland SO, Ueland PM. Protein kinases in human renal cell carcinoma and renal cortex. A comparison of isoyme distribution and


Notes

Editor’s note: S. Agrawal is an employee of Hybridon, Inc., a biotechnology company conducting clinical research targeting the RIα subunit of PKAI. J. Mendelsohn holds stock options and is on the Board of Directors of IMCLONE SYSTEMS, Inc., a company licensed to produce MAb C225. Supported by a grant from the Associazione Italiana per la Ricerca sul Cancro and from the Consiglio Nazionale delle Ricerche Target Project on Biotechnologies.

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