A Single-Chain Immunotoxin against Carcinoembryonic Antigen That Suppresses Growth of Colorectal Carcinoma Cells


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ABSTRACT

We have engineered an anti-carcinoembryonic antigen (CEA) single-chain immunotoxin derived from humanized anti-CEA antibody (hMN14) and a truncated Pseudomonas exotoxin (PE), PE40. The purified anti-CEA immunotoxin (hMN14(Fv)-PE40) was first measured for binding affinity against a CEA-positive colorectal carcinoma cell line and compared with its parental IgG and the monovalent Fab fragment. The $K_d$ of sFv-PE40, Fab, and IgG were $5 \times 10^{-9}$, $6 \times 10^{-9}$, and $3 \times 10^{-9}$ M$^{-1}$, respectively. There was no significant affinity loss by conversion of Fab to the single-chain Fv, but these monovalent forms were 5–6-fold reduced in affinity compared with the parental IgG. In cytotoxicity assays, the hMN14(Fv)-PE40 showed specific growth suppression of CEA-expressing colon cancer cell lines MIP-CEA (high CEA) and LS174T (moderate CEA) with IC$_{50}$s of 12 ng/ml (0.2 nm) and 69 ng/ml (1.1 nm). These IC$_{50}$s correlated inversely with the surface expression of CEA, such that 50% killing was equivalent for each cell type when expressed in toxin molecules bound/cell (3000–5000). The presence of soluble CEA up to 1000 ng/ml did not affect the cytotoxicity against CEA-expressing cells, with 50% suppression only at 4000 ng/ml that correlated with the binding $K_d$ of the single-chain Fv. The stability of the hMN14(Fv)-PE40 molecule at 37°C was confirmed by bioassay and by lack of aggregation. Our hMN14(Fv)-PE40 may be clinically useful for tumors with high CEA expression without affecting normal tissues with low or absent CEA, even in patients with high soluble antigen levels.

INTRODUCTION

CEA$^3$ is a phosphoinositol-linked $M_r$ 180,000–220,000 glycoprotein expressed on a broad range of adenocarcinomas. As an antitumor immunotherapy target, it has particular advantages in terms of tissue expression and specificity, with high expression on tumor cells and a combination of low expression and a protected geometry in its normal tissue distribution (1, 2). Although colorectal carcinoma has been the prototypical malignancy for testing anti-CEA therapies, based on its expression in 60–94% of patients with advanced disease, CEA is also expressed on tumors of ~60% of women with metastatic breast cancer and >30% of patients with cancer of the lung, liver, pancreas, head and neck, bladder, cervix, and prostate (1, 3). Approximately 150,000 people die each year from CEA-positive cancers, with an additional 50,000 eligible for adjuvant therapies who are at high risk for recurrence after initial removal of all macroscopic disease. A new therapeutic option that effectively targets this antigen would have a very high clinical relevance, with potential for major impact on the clinical and financial consequences of cancer in this country.

Monoclonal antibodies specific to CEA have been studied for diagnosis and therapy of CEA-positive human cancers. Several chemical immunoconjugates of anti-CEA whole IgG have also been examined, and their specific cytotoxicities have been shown (4–8). However, chemical conjugation methods can modify antibody with adverse effects on antigen binding. In addition, chemical conjugation yields a heterogeneous mixture of molecules joined via different positions on the antibody and toxin in comparison with the structural uniformity of recombinant immunotoxin. Potent single-chain immunotoxins derived from PE have been made previously, against interleukin-2 receptor (9), transferrin receptor (10), Le$^Y$ family antigen (11), and others, and their specific cytotoxicities have been shown. Several sFv-immunotoxins are presently being evaluated in clinical trials (12).

The objective of the present study was to develop and characterize a single-chain immunotoxin from hMN14, a humanized anti-CEA monoclonal antibody (13), and to make a preliminary evaluation of the potential of this immunotoxin (hMN14(sFv)-PE40) for future colon cancer treatment. To our knowledge, this is the sole example of a recombinant immunotoxin against CEA to be reported to date.

1 The abbreviations used are: CEA, carcinoembryonic antigen; HPLC, high-performance liquid chromatography; PE, Pseudomonas exotoxin; sCEA, soluble CEA; sFv, single-chain Fv.

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MATERIALS AND METHODS

Cell Lines. Human colorectal cancer cell line MIP-101 (14) and MIP-CEA clone 8 (15) were obtained from Dr. P. Thomas, and colon adenocarcinoma cell line LS174T was obtained from American Type Culture Collection (Manassas, VA). All cells were cultured in RPMI 1640 (BioWhittaker, Inc., Walkersville, MD) supplemented with 10% heat-inactivated FCS, 100 units/ml penicillin G, 100 units/ml streptomycin sulfate, and 2 mm L-glutamine.

Cloning of Antibody Fragment of hMN14 Antibody. Cloning experiments and propagation of plasmid were performed in Escherichia coli XL-1 blue (Stratagene, Cambridge, United Kingdom). Total RNA was extracted from 5 × 10⁷ hMN14/7/g9 transfectoma cells (Immunomedics, Inc.) using an RNA isolation kit (Stratagene). Amplification of variable regions was achieved by reverse transcription-PCR using the following primers that also create the sFv linker (restriction sites underlined). VH forward: 5'-GCGGGATCCGGCTCGTGGTGCTCAGATGCTGAGTCGAG-3' incorporates a HindIII site; and VL backward: 5'-GGCGCCGCCATATGGACATCCAGCTGACCCAG-3' incorporates a BamHI site. The single-chain linker, a 15-amino acid long bridge, was modified to TACCACTFGGG-3'.

Preparation of Immunotoxin and Antibody Fragments. The single-chain immunotoxin was obtained by solubilization and refolding of inclusion body proteins from the host E. coli BL21(αDE3), as described (18). Properly refolded proteins were purified by sequential ion exchange chromatography on Q-Sepharose, Mono Q (Pharmacia, Uppsala, Sweden) followed by size exclusion chromatography on a TSK G3000SW (Tosohaas) column on a Dionex 500 HPLC apparatus (Dionex Corp., Sunnyvale, CA). Fab fragment was prepared from hMN14 IgG by papain digestion using a Fab preparation kit (Pierce Chemical Co., Rockford, IL). Fractions containing Fab fragment were concentrated by Centriprep 10 ultrafiltration (Amicon, Inc., Beverly, MA) and dialysed against PBS. Purified proteins were stored until use at −80°C to minimize aggregation and activity loss.

Flow Cytometry Analysis. Cell surface CEA was determined by flow cytometry with hMN14 anti-CEA antibody and a control irrelevant isotype-matched (IgG1,κ) humanized antibody (anti-Tac-H; Ref. 19). Cells (2 × 10⁷) were incubated with antibodies in 50 µl of binding buffer of RPMI 1640 containing 10% horse serum, 50 mM Hepes-NaOH (pH 7.0), and 0.2% sodium azide for 30 min at 4°C with mixing. The cells were then washed with ice-cold PBS twice and incubated under the same conditions with goat-antihuman IgG-γ chain phycocerythrin conjugates (Tago Immunologicals, Burlingame, CA).

RESULTS

Surface CEA Expression of Colon Cancer Cell Lines. We first compared the surface expression of CEA among human colorectal carcinoma cell lines, MIP-101. LS174T, and MIP-CEA with hMN14 antibody. MIP-101 (Fig. 1A) showed no detectable surface CEA expression as previously reported (15), whereas CEA was detected on both LS174T and MIP-CEA cells (Fig. 1, B and C), of which MIP-CEA was the higher expressing.

Preparation of hMN14(sFv)-PE40 Immunotoxin. The initial step was to choose an antibody to CEA. CEA is a member of a family of related proteins, including nonspecific cross-reactive antigen, biliary glycoprotein, and others, among which
Expression of CEA on colon carcinoma cell lines. Surface expression of CEA on MIP-101 (A), LS174T (B), and MIP-CEA (C) was analyzed by flow cytometry. Broken line, control irrelevant antibody; solid line, hMN14 antibody.

anti-CEA antibodies may be cross-reactive, depending on the epitope recognized (2). From the many (>50) antibodies to CEA presently available, MN14 was selected for these studies. MN14 is a Primus class III antibody (i.e., it reacts exclusively with CEA in the family of CEA-related proteins). This was also available in a humanized format, which has advantages in terms of reduced immunogenicity of the antibody moiety of the immunotoxin during human therapies (20).

V\text{L} and V\text{H} antibody segments were cloned and joined with an intervening linker. The canonical (GGGGS)\text{\textsubscript{2}} linker for V\text{L} and V\text{H} joining incorporates a serine residue in the motif to increase hydration and reduce the likelihood of invading and disrupting the native hydrophobic cleft that joins V\text{L} and V\text{H} in the parent antibody. Because a portion of sFv's are unstable (21), we elected to use a linker with even greater hydration, (GGSGS)\text{\textsubscript{3}}, on the possibility that this configuration would favor the sFv stability even more. The product was expressed in E. coli, purified and refolded in a well-behaved manner, with a homogeneous appearance on SDS-PAGE (data not shown) and on non-denaturing HPLC sizing chromatography (see below).

Affinity of hMN14(Fv)-PE40. One of the risks of using sFv is that it may lose affinity for the target antigen (21). This is because these constructs use an artificial bridge (linker) between V\text{L} and V\text{H} in lieu of the C\text{\textsubscript{\text{\textsubscript{1}}}}:C\text{\textsubscript{\text{\textsubscript{1}}}} interaction that normally stabilizes an appropriate V\text{L}:V\text{H} juxtaposition for antigen binding. To assess the affinity of hMN14(sFv), we tested its binding activity against CEA-expressing target cells in comparison with hMN14 Fab and hMN14 whole IgG (Fig. 2). Immunotoxin, Fab, and whole antibody were labeled with \textsuperscript{125}I, and incubated with MIP-CEA cells. The data for the specific binding were analyzed by Scatchard plot. The measured affinity K\textsubscript{a} values of sFv, Fab, and IgG were 5 \times 10^4, 6 \times 10^5, and 3 \times 10^6 M^{-1}, respectively. K\textsubscript{d}s were 21 nM, 16 nM, and 3.4 nM. Although monovalent forms were 5–6-fold reduced in affinity compared with the parental IgG, there was no significant affinity loss between the sFv and Fab. These data indicate that intact hMN14 IgG binds bivalently, with an approximate 2-fold lower B\textsubscript{max} (Fig. 2) and a net affinity enhancement of approximately 2.5–3 fold when normalized to binding sites per antibody molecule (22). Data from this experiment indicate that MIP-CEA expresses \approx 5 \times 10^3 CEA/cell.

Specific Cytotoxicity of hMN14(sFv)-PE40. The cytotoxic activity of immunotoxin was assessed by measuring the suppression of \textsuperscript{3}Hleucine incorporation by human colon cancer cell lines after treatment with serial dilutions of the recombinant protein. The immunotoxin inhibited protein synthesis of all cell lines (Fig. 3, A-C; ●, ▲, and ■). The concentrations that reduced the \textsuperscript{3}Hleucine incorporation by target cells to 50% (IC\textsubscript{50}) were estimated and are shown in Table 1. The susceptibility to anti-CEA immunotoxin paralleled the CEA expression of the tumor cell lines, with MIP-CEA the most sensitive and the CEA-negative MIP-101 the least sensitive. To examine the specificity of the cytotoxicity and the role of antigen expression,
assays were conducted by adding excess competitor hMN14 antibody to block all CEA binding sites for immunotoxin (Fig. 3, A-C; ○, Δ, and □). Cytotoxicity against MIP-CEA and LS174T carcinoma cells was blocked by excess hMN14 but not by nonspecific antibody, thus confirming that the inhibition of protein synthesis by hMN14(sFv)-PE40 is due to specific binding to CEA. On the other hand, the cytotoxicity seen against the CEA-negative MIP-101 cell line at the high-immunotoxin concentrations is caused by nonspecific reaction because it could not be blocked by hMN14 antibody. The nonspecific cytotoxicity was similar for MIP-101 and MIP-CEA to a factor of 2, but a nonspecific threshold was apparent for LS174T over the concentration range studied. This suggests a greater native sensitivity of the MIP cells than the LS174T cells to nonspecific cytotoxicity, by nonspecific internalization of immunotoxin. It is noted that the MIP lines have a common derivation: MIP-CEA was created by CEA gene transfection of the CEA-negative MIP-101 cell line (15).

The Effect of sCEA on Cytotoxicity. Serum CEA up to 1000 ng/ml or more is sometimes observed in patient sera versus the normal level of <5 ng/ml. In the presence of such high sCEA, one could expect that an immunotoxin might be titrated-out before arriving at the target tissue. To examine the effect of high CEA levels on cytotoxic potency, we added free CEA in various concentrations to 100 ng/ml immunotoxin and then assessed the toxicity of the mixture against MIP-CEA. This concentration of immunotoxin provides 77% of maximal suppression of [3H]leucine incorporation (Fig. 3A). Immunotoxin was incubated in growth medium with sCEA for 0 min, 15 min, and 2 h at 37°C before the addition to cells, and then incubated with the cells an additional 24 h before labeling with [3H]leucine. As seen in Fig. 4, no obvious change in protein synthesis suppression was observed for CEA concentrations up to 1000 ng/ml in the media, whereas sCEA at 5000 ng/ml showed ~60% reduction in net killing efficiency for this concentration of immunotoxin, from 77% down to ~30% of maximal cytotoxicity. The duration of preincubation of CEA with immunotoxin to ensure binding equilibrium before addition to the target cells did not affect the toxicity profile. Averaging all curves, the IC50 for sCEA inhibition of immunotoxin activity is
humanized variable region using hMN14 as the parental anti-
of CEA expression (Figs. 1 and 3). Furthermore, the expression
protein, as shown by our data relating cellular sensitivity to level
discrimination between normal and tumorous expression of the
higher than normal colonic mucosa (26). This should enhance
quantitatively much higher levels of CEA, averaging 35-fold
carcinomas (1, 2, 25), and it has accordingly been of interest to
cancers of epithelial cell origin, especially in gastrointestinal
DISCUSSION

corresponding to aggregates even after the 24-h incubation, and
Cytotoxicity was assayed as in Fig. 3. At 100 ng/ml immunotoxin,
\[^{3}H\]leucine incorporation is suppressed by 77% relative to the control.

Estimated at 4000 ng/ml (20 nm) of CEA. The presence of CEA
itself did not affect the rate of protein synthesis (data not shown).

Stability of Immunotoxin. The stability of immunotoxins at 37°C is an important factor in their usefulness as therapeu-
tic agents. Loss of activity by immunotoxin is governed by
its tendency to aggregate at 37°C, which has been documented
previously (21, 23, 24). Prior assays with 2-h preincubation in
media containing 10% serum showed no suggestion of activity
loss relative to unincubated hMN14(sFv)-PE40 (Fig. 4). How-
ever, albumin is typically added to proteins (e.g., enzymes) to
reduce aggregation and improve stability, and serum is 5% by
weight albumin (50 mg/ml). Therefore, we chose to omit serum
in a further assay, as a more stringent test of the tendency of the
immunotoxin to aggregate. In Fig. 5, the thermal stability of
hMN14(Fv)-PE40 was determined by incubating for 8 and 24 h
at 37°C, then measuring the amount of aggregation by HPLC
size analysis and activity loss by bioassay. No peak was detected
Corresponding to aggregates even after the 24-h incubation, and
there was no loss of specific immunotoxin activity by bioassay.

DISCUSSION

CEA is an antigen expressed on the surface of human
cancers of epithelial cell origin, especially in gastrointestinal
carcinomas (1, 2, 25), and it has accordingly been of interest to
target CEA in immunotherapies. Tumor cells typically express
quantitatively much higher levels of CEA, averaging 35-fold
higher than normal colonic mucosa (26). This should enhance
discrimination between normal and tumorous expression of the
protein, as shown by our data relating cellular sensitivity to level
of CEA expression (Figs. 1 and 3). Furthermore, the expression
of CEA on normal cells of the colonic epithelium is on luminal
surfaces that should be less accessible to attack by a blood-borne
immunotoxin (1, 2).

In this study, we engineered an anti-CEA sFv with a
humanized variable region using hMN14 as the parental anti-
body. The therapeutic interval of a particular toxin construct is
usually limited by host immune response against the toxin
moiety, which antibody humanization will not change. How-
ever, antibody humanization in the present setting has the ad-
antage of avoiding concurrent antiglobulin responses, thus
allowing the subsequent use of the hMN14 antibody in other
therapeutic modifications. Our Scatchard analysis indicated that
sFv retains its specific binding activity against CEA with no
obvious affinity loss relative to its two-chain counterpart, Fab
(Fig. 2), and is comparable in affinity to other anti-CEA sFvs
obtained by recombinant phage display technology (27-29).

It is noted that some immunotoxins may lose >70% of
their initial cytotoxic potency due to aggregation during 8-h
incubation at 37°C. To ameliorate this common problem, strat-
egies were devised to include interchain disulfides in some Fv
toxin constructs (21, 23, 24). In contrast, the hMN14(Fv)-PE40
was stable with prolonged incubation at 37°C even without
disulfide-stabilization. The stability of the immunotoxin de-
depends on the structure of the antigen-binding domain, indicating
that the sFv of hMN14 antibody has a suitable character to serve
as a single-chain immunotoxin. An additional possibility is that
the more hydrated linker we applied may foster improved sta-
bility by not invading the hydrophobic cleft that binds V_{H} and
V_{L} to form an appropriate antigen binding site; however, direct
comparisons with the canonical linker were not made from
which to draw a conclusion.

Although membrane-expressed CEA does not internalize
actively, our hMN14(Fv)-PE40 is able to kill target cells. This is
similar to Tac (interleukin-2 receptor α) targeting in which
antigen is not actively internalized, but immunotoxin neverth-
less kills cells in an antigen-dependent manner (9). This mani-
festation of specific cytotoxic activity is thought to be due to the
nonspecific bulk clearing of membrane surface and associated
proteins via on-going cellular endocytic activities, in which
binding of immunotoxin to surface-bound antigen increases the
probability that it will also be internalized. hMN14(Fv)-PE40
showed specific cytotoxicity to MIP-CEA but not to the CEA
nonexpressing parental line, MIP-101 (Fig. 3, A and C). Protein
synthesis in MIP-101 was inhibited at high immunotoxin con-
centrations (~500 ng/ml) that was comparable, to a factor of
two, with the nonspecific killing of MIP-CEA. This residual
killing is presumably a measure of nonspecific cellular uptake
from bulk fluid phase of the medium, which would not be
inhibited by excess hMN14 antibody (Fig. 3C).

Another CEA-expressing target cell, LS174T, was also
specifically killed by hMN14(Fv)-PE40, but not as efficiently as
MIP-CEA (Fig. 3, A and B). This lower activity is plausibly
explained by the approximate 10-fold lower surface CEA on
LS174T versus MIP-CEA (comparing histograms of Fig. 1, B
and C). By our K_{d} measurements, we estimate similar numbers
of toxin molecules bound/cell at the respective IC_{50} values
(5,000 for MIP-CEA and 3,000 for LS174T), thus suggesting a
common final threshold of cell binding to mediate cytotoxicity.
A similar observation correlated the lower sensitivity of Tac-
expressing cell lines to anti-Tac(Fv)-PE40 when they had lower
antigen expression, but which were constant in sensitivity when
normalized to estimated toxin molecules bound/cell (Ref. 9;
R. P. J., calculations not shown). The relation of antigen expres-
sion to specific cytotoxicity suggests that maneuvers to increase
tumorous CEA expression, such as IFN-γ treatment (30, 31), may be productively applied to enhance the toxicity profile against CEA-expressing cancers. But this analysis also suggests a potentially important future direction to improve this agent for therapy: it implies that a higher affinity version of this antibody would be effective at still lower concentrations than seen here—without increasing nonspecific toxicity—thus, significantly enhancing the therapeutic index.

A further possible explanation for the lower sensitivity of LS174T to specific killing that could be considered is the difference in the rate of sCEA production. The CEA shedding rate for LS174T is extremely high, 512 ng/10^6 cells/day, compared with 3.2 ng/10^6 cells/day for MIP-CEA (Table 1). This means 71,000 molecules of sCEA are produced by one cell/hour for LS174T compared with 450 molecules for MIP-CEA. Although total accumulated sCEA in our assays at 24 h (~100 ng/ml) would be far below the inhibitory concentrations in Fig. 4, the immunotoxin bound to LS174T could be thought to have less opportunity to be internalized due to its higher chance to be shed from the cell before endocytosis. However, we consider this explanation less likely. The steady state surface expression should yield the same net internalization for a stochastic endocytosis process, regardless of the synthetic and shedding rates, under certain assumptions. (See Ref. 32 for a more detailed kinetics analysis of protein shedding and expression.) Finally, the sufficiency of the rationale of lower surface CEA expression to explain the lower drug sensitivity of LS174T (see above) would seem to obviate any need to invoke such more complex arguments as involve shedding.

Coincubation with sCEA in the cytotoxicity assay did not affect the activity of the immunotoxin up to 1000 ng/ml, but immunotoxin activity was suppressed approximately 60% at 5000 ng/ml sCEA. sCEA in patient sera only infrequently reaches 1000 ng/ml (5 nM) and it is, therefore, unlikely to be an important factor in the efficacy of this immunotoxin in vivo. In our assay, the CEA on cells (~10–20 fmol) is far less than the CEA in the supernatant (1000 ng/ml = 500 fmol) versus total immunotoxin (100 ng/ml = 200 fmol). The Scatchard plot analysis (Fig. 2) allows us to estimate the surface density of CEA molecules on the MIP-CEA, ~5 × 10^5/cell, which corresponds to a CEA concentration of ~100 μM on the cell surface, and ~10 μM for LS174T relative to sCEA concentrations of 10 nM or less. This might seem to provide a basis for a selective advantage of CEA on cells to acquire anti-CEA immunotoxin and resist sCEA competition. However, this is unlikely to be a suitable explanation. We have previously argued that monovalent antibody binding will normally partition between cellular and soluble antigen in their respective proportion to total antigen present (33). The fact that there is little impact on cellular toxicity under this condition despite the large ratio excess of sCEA suggests a different and more likely rationale, as follows.

Expressed in terms of K_d, the inhibition pattern of sCEA becomes fully understandable. We assume provisionally that sFv affinity for sCEA equals that determined for cellular CEA.
(Fig. 2), sCEA at 1000 ng/ml (5 nm) is still well below the sFv Kₐ (21 nm) and will not appreciably reduce the free sFv toxin that can bind to tumor CEA, and correspondingly has little or no effect on cytotoxicity. At the sCEA IC₅₀ of 4000 ng/ml (20 nm), however, sCEA also equals the Kₐ for the immunotoxin; our Scatchard analysis indicates that the immunotoxin should be half-saturated with sCEA and, correspondingly, the free immunotoxin available for cell CEA binding should be reduced by half. Because MN14(Fv)-PE40 at 100 ng/ml (2 nm) is below the binding Kₐ (21 nm) and in the linear range for immunotoxin activity on MIP-CEA (Fig. 3), this change in free immunotoxin concentration at the sCEA IC₅₀ directly reduces both the cellular binding of toxin and drug potency in parallel. Similarly, cytotoxicity by anti-Tac(Fv)-PE40 against Tac-expressing tumor cell lines was resistant to soluble Tac antigen until exceeding the anti-Tac sFv Kₐ (0.3 nm) and reducing free immunotoxin (34). Finally, this correspondence of expected results with observation ultimately supports our assumption of comparable affinities of sFv for cellular and sCEA.

Logically, as the affinity of immunotoxin for antigen increases, the tumor targeting efficacy increases, but the susceptibility to soluble antigen binding increases also. Ultimately, when soluble antigen greatly exceeds the Kₐ of the sFv-toxin, there will be virtually no free toxin. However, we (33) and others (35) have previously shown that antibody in the setting of saturating antigen still achieves tumor targeting by an exchange partition between cell-bound and soluble antigen. Of particular interest in the present study is the fact that sCEA is a long-lived protein in serum (t½ ~4d; 36), far exceeding the typical half-life of sFv-toxins (t½<1 h; 37). Parallels may be drawn with our prior study of soluble Tac antigen, which has an abbreviated profile in ways that may not be fully predictable a priori, changes its pharmacokinetics, potentially altering its therapeutic systemic toxicity. For hMN14(Fv)-PE40, sCEA binding should be not be a major factor in the drug pharmacokinetics, but this feature will be important to consider in any Phase I anti-CEA studies with sFv-toxins of high affinity, defined as having a Kₐ for CEA that is lower than commonly encountered concentrations of sCEA in vivo (e.g., <1 nm).

By criteria of cytotoxicity, specificity, affinity, and stability, our hMN14(Fv)-PE40 displays a satisfactory in vitro profile. This agent may be clinically useful for tumors with elevated CEA expression without affecting normal tissues with no or low CEA, even for patients with high serum CEA levels.

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References


4 We note that there is an error in the reported unit definition in this reference (34) that underrepresents the mass of soluble Tac antigen by ~10-fold.


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Y Akamatsu, J C Murphy, K F Nolan, et al.