



# Recombinant anti-erbB2 immunotoxins containing *Pseudomonas* exotoxin

(chemotherapy/monoclonal antibodies/growth factor receptors)

JANENDRA K. BATRA\*<sup>†</sup>, PHILIP G. KASPRZYK<sup>‡</sup>, ROBERT E. BIRD<sup>‡</sup>, IRA PASTAN\*, AND C. RICHTER KING<sup>§</sup>

\*Laboratory of Molecular Biology, Division of Cancer Biology, Diagnosis and Centers, National Cancer Institute, National Institutes of Health, 9000 Rockville Pike, 37/4E16, Bethesda, MD 20892; and <sup>‡</sup>Molecular Oncology, Inc., 19 Firstfield Road, Gaithersburg, MD 20878

Contributed by Ira Pastan, March 31, 1992

**ABSTRACT** Immunotoxins were made using five different murine monoclonal antibodies to the human *erbB2* gene product and LysPE40, a 40-kDa recombinant form of *Pseudomonas* exotoxin (PE) lacking its cell-binding domain. All five conjugates were specifically cytotoxic to cancer cell lines overexpressing *erbB2* protein. The most active conjugate was e23-LysPE40, generated by chemical crosslinking of anti-*erbB2* monoclonal antibody e23 to LysPE40. In addition, a recombinant immunotoxin, e23(Fv)PE40, was constructed that consists of the light-chain variable domain of e23 connected through a peptide linker to its heavy-chain variable domain, which in turn is fused to PE40. The recombinant protein was made in *Escherichia coli*, purified to near homogeneity, and shown to selectively kill cells expressing the *erbB2* protooncogene. To improve the cytotoxic activity of e23(Fv)PE40, PE40 was replaced with a variant, PE38KDEL, in which the carboxyl end of PE is changed from Arg-Glu-Asp-Leu-Lys to Lys-Asp-Glu-Leu and amino acids 365–380 of PE are deleted. The e23(Fv)PE38KDEL protein inhibits the growth of tumors formed by the human gastric cancer cell line N87 in immunodeficient mice.

Clinical trials are under way in which monoclonal antibodies are used to carry cytotoxic substances to tumor cells. Immunotoxins made by coupling monoclonal antibodies (mAbs) to toxins have been found to kill cancer cells *in vitro* and also to have antitumor activities in mice bearing human tumor xenografts (1–4). Amplification and overexpression of the *erbB2* gene has been shown to occur in many human cancers, including ≈30% of lung, breast, ovary, and stomach adenocarcinomas (5–11). In breast carcinoma, a correlation has been observed between gene amplification and overexpression of *erbB2* protein and the aggressiveness of the malignancy (7, 8). In cases of gene amplification, there is a resulting 50- to 100-fold increase in *erbB2* mRNA compared with normal cell levels (11). The overexpression of *erbB2* has been directly linked to the malignant conversion of cancer cells (12, 13). This causative role for the *erbB2* protein makes it an excellent target for immunotoxin therapy because cancer cells are unlikely to be able to escape treatment by loss of the antigen.

*Pseudomonas* exotoxin A (PE) and its recombinant forms have been used to make immunotoxins either by conventional chemical coupling methods or by recombinant DNA methods (3, 4, 14–17). PE is made of three structural domains. The N-terminal domain (I) is responsible for the binding of toxin to its receptor on the cells, the middle domain (domain II) has a role in the translocation of toxin across the membrane, and the C-terminal domain (III) has the ADP-ribosylation activity (18). Recently, molecularly defined immunotoxins have been engineered by fusing domains II and

III of PE to the light- and heavy-chain variable regions of specific mAbs. In the resulting single-chain immunotoxin the cell-binding domain of PE is replaced with an antigen combining site in the form of a single-chain antibody, or SC(Fv).

Here, we report the construction of several immunotoxins using various anti-*erbB2* antibodies that have been coupled to native LysPE40, which is a recombinant form of PE devoid of its cell-binding domain. The resulting immunotoxins were specifically cytotoxic to cells expressing *erbB2* with varying degrees of activity. We selected one mAb, e23, to make a single-chain chimeric immunotoxin termed e23(Fv)PE40. In e23(Fv)PE40, the variable domain of the light chain of mAb e23 is attached through a peptide linker to the variable domain of the heavy chain, which in turn is fused to domains II and III of PE. The immunotoxin was found to be specifically cytotoxic to cells expressing *erbB2* and was more active than the chemical conjugate. To increase the activity further, other derivatives of this single-chain immunotoxin were made in which the toxin part of the molecule was altered. One of these molecules, e23(Fv)PE38KDEL, inhibited the human gastric cancer cell line N87 growing as a tumor in immunodeficient mice.

## MATERIALS AND METHODS

**Antibodies and Cell Lines.** e1, e23, e21, e68, and e94 are mouse mAbs against *erbB2* gene product (19). The *erbB2*-expressing cell lines used were BT474 (breast carcinoma), N87 (gastric carcinoma), and SK-OV-3 (ovarian carcinoma). A431 and KB are human epidermoid carcinomas that express low levels of *erbB2*.

**Construction of Chemical Conjugates.** LysPE40 or PE was coupled chemically to mAbs by a thioether linkage using 2-iminothiolane and succinimidyl-4-(*N*-maleimidomethyl)cyclohexane-1-carboxylate to derivatize the antibody and the toxin, respectively (3). Conjugates were purified as described (3).

**Generation of a SC(Fv) from mAb e23.** Poly(A)<sup>+</sup> RNA was extracted from hybridoma cells by oligo(dT) affinity chromatography (Invitrogen, San Diego). cDNA was prepared using random hexanucleotide primer (Boehringer Mannheim). The immunoglobulin light- and heavy-chain clones were isolated using PCR with the following primers: light chain, 5'-CAC-GTC-GAC-ATT-CAG-CTG-ACC-CAC-TCT-CCA-3' and 5'-GAT-GGA-TCC-AGT-TGG-TGC-AGC-ATC-3'; heavy chain, 5'-C-GGA-ATT-TCA-GGT-TCT-GCA-GIA-GTC-WGG-3' and 5'-AGC-GGA-TCC-AGG-GGC-CAG-TGG-ATA-GAC-3' (I, deoxyinosine; W, A

Abbreviations: mAb, monoclonal antibody; PE, *Pseudomonas* exotoxin; SC(Fv), single-chain antibody comprising heavy- and light-chain variable regions.

<sup>†</sup>Present address: National Institute of Immunology, National Institute of Immunology Campus, J.N.U. Complex, Shahid Jeet Singh Marg, New Delhi, 110067, India.

<sup>§</sup>To whom reprint requests should be addressed.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

the individual light- and heavy-chain cDNA clones and four oligonucleotides. The light- and heavy-chain regions were joined by a synthetic linker with amino acid sequence GSTSGSGKSSEGK specified by overlapping oligonucleotides as described. The intact SC(Fv) coding region was inserted in frame with an *ompA* leader sequence under direction of the  $\lambda P_L$  promoter. Induction of protein, and lysis of bacteria, and refolding of protein were as previously described (20). The SC(Fv) was purified as a single peak from CM chromatography and judged to be >70% pure by SDS/PAGE.

**Plasmids.** Plasmid pJB23-40 encodes e23(Fv)PE40 and contains cDNA for light-chain variable region of anti-erbB2 antibody e23 connected through a 42-base-pair linker to its heavy-chain variable region, which in turn is fused to the DNA for PE40 (see Fig. 3). The fusion gene is under the control of a bacteriophage T7 late promoter and the plasmid also contains phage *f1* origin and a terminator. Plasmid pJB23-38 encodes the protein termed e23(Fv)PE38. In pJB23-38, codons for amino acids 365–380 in domain II of PE40 have been deleted. The Fv portion is the same as in pJB23-40. In pJB23-38K, the codons for amino acids REDLK at the 3' end of pJB23-38 have been replaced with those coding for amino acids KDEL (21). The protein encoded by this plasmid is termed e23(Fv)-PE38KDEL. In pJB23-40K, the codons for amino acids REDLK at the 3' end of pJB23-40 have been replaced with those coding for amino acids KDEL (22). The protein encoded by this plasmid is termed e23(Fv)PE40KDEL.

**Expression and Purification of Proteins.** Expression and purification of various fusion proteins in *Escherichia coli* were performed as described (21).

**Characterization of Immunotoxins.** Cytotoxic activities of the chimeric toxins were determined by assaying the inhibition of protein synthesis in various target and nontarget cells (3). Cells were plated in 24-well plates and 24 hr later washed once with medium before addition of the toxins. Results are described as percent of control where no toxin was added. For competition experiments, an excess of antibody (20  $\mu$ g/ml) was added prior to the addition of the toxin.

In binding studies <sup>125</sup>I-labeled e23 Fab was added as a tracer with various concentrations of the competitor (16). Protein was assayed by the Bradford method using Pierce Coomassie blue "plus" reagent. SDS/PAGE was done by the method of Laemmli (23).

For *in vivo* studies N87 tumor cells ( $5 \times 10^6$  per mouse) were subcutaneously injected into the flanks of BNX (beige, nude, xid) mice on day 0. Starting on day 10, six intravenous tail vein treatments (2  $\mu$ g each) were carried out over 3 days. Tumor growth and animal weights were monitored twice a week. Tumor growth is reported as an average relative tumor volume calculated as  $(w^2 \times l)/2$  (mm<sup>3</sup>), where *w* is the width and *l* is the length of the tumor, measured with calipers.

## RESULTS

Previous studies have shown that individual mAbs to a single antigen can result in immunotoxins of widely variable activity (1). To determine which available anti-erbB2 mAb resulted in an immunotoxin of highest activity, we first used chemical crosslinking to produce anti-erbB2 immunotoxins in which the mAbs were coupled to LysPE40 (3, 4, 21). The antibodies used are designated e1, e21, e23, e68, and e94 (19). The activity of the immunotoxins was assessed by measuring their ability to inhibit protein synthesis in target and nontarget cells. BT474, N87, and SK-OV-3 are cell lines that overexpress erbB2, whereas A431 and KB do not. All the conjugates were active on BT474 cells, with e23-LysPE40 being the most active. The same general pattern of activity was observed on

Toxin	ID <sub>50</sub> , ng/ml				
	BT474	N87	SK-OV-3	A431	KB
e23-LysPE40	18	37	180	700	2000
e21-LysPE40	47	38	200	>2000	>2000
e1-LysPE40	36	64	500	ND	ND
e68-LysPE40	180	130	>1000	2000	2000
e94-LysPE40	42	100	600	>2000	>2000
LysPE40	160	ND	>2000	650	>2000

ID<sub>50</sub> values are the concentrations of protein required to inhibit protein synthesis by 50%. ND, not done.

BT474 and N87 cells, but SK-OV-3 cells were less sensitive to these conjugates (Table 1). All of these immunotoxins had little or no toxicity for A431 and KB cells, indicating their specificity for erbB2. Specificity was also demonstrated by showing that excess unconjugated antibodies prevented the inhibition of protein synthesis by the respective immunotoxins (data not shown). Since e23 produced the most active conjugate, it was used for further studies.

To compare the binding activity of e23-LysPE40 with that of the native antibody, competition binding analyses were performed on mouse NIH 3T3 cells engineered to express high levels of human erbB2 protein (12). In these studies we determined the abilities of the two immunotoxins to compete for the binding of <sup>125</sup>I-labeled e23 to transfected NIH 3T3 cells overexpressing human erbB2 protein. e23-LysPE40 and e23-PE were found to compete for binding to the erbB2 antigen very efficiently, with binding affinity slightly lower than that of the native antibody (data not shown).

```

ATGGACCTGCAGCTGACCCAGTCTCCAGCAATCCTGTCTGCATCTCCAGG
MetAspLeuGlnLeuThrGlnSerProAlaIleLeuSerAlaSerProGly

GGAGAAGGTCACAATGACTTGCAGGGCCACCCCAAGTGTAAGTTACATGC
GluLysValThrMetThrCysArgAlaThrProSerValSerTyrMetHis

ACTGGTATCAGCAGAAGCCAGGATCCTCCCAACCTTGGATTATACC
TrpTyrGlnGlnLysProGlySerProLysProTrpIleTyrThr

ACATCCAACCTGGCTTCTGGAGTCCCTGCTCGCTTCAGTGGCGGTGGGTC
ThrSerAsnLeuAlaSerGlyValProAlaArgPheSerGlyGlyGlySer

TGGGACCTCTTACTCTCTCACAGTCAGCAGAGTGGAGGCTGAAGATGCTG
GlyThrSerTyrSerLeuThrValSerArgValGluAlaGluAspAlaAla

CCACTTATTACTGCCAGCAGTGGAGTCCGTAGCCCAACCCAGTTCGGAGGG
ThrTyrTyrCysGlnGlnTrpSerArgSerProProThrPheGlyGly

GGGTCCAAGCTGGAAATAAAAGGTTCTACCTCTGGTTCTGGTAAATCTTC
GlySerLysLeuGluIleLysGlySerThrSerGlySerGlyLysSerSer

TGAAGGTAAAGGTGTGCAGCTGCAGGAGTCAGGACCTGAGGTGGTGAAGC
GluGlyLysGlyValGlnLeuGlnGluSerGlyProGluValValLysPro

CTGGAGGTTCAATGAAGATATCCTGCAGACTTCTGGTTACTCATTCACT
GlyGlySerMetLysIleSerCysLysThrSerGlyTyrSerPheThr

GGCCACACCATGAAGTGGGTGAAGCAGAGCCATGGAAGAACCCTTGAGTG
GlyHisThrMetAsnTrpValLysGlnSerHisGlyLysAsnLeuGluTrp

GATTGGACTTATTAATCCTTACAATGGTGATACTAACAACCCAGAAGT
IleGlyLeuIleAsnProTyrAsnGlyAspThrAsnTyrAsnGlnLysPhe

TCAAGGGCAAGGCCACATTTACTGTAGACAAGTCGTCACAGCACAGCCTAC
LysGlyLysAlaThrPheThrValAspLysSerSerSerThrAlaTyr

ATGGAGCTCCTCAGTCTGACATCTGAGGACTCTGCAGTCTATTACTGTGC
MetGluLeuLeuSerLeuThrSerGluAspSerAlaValTyrTyrCysAla

AAGGAGGGTTACGGACTGGTACTTTCGATGTCTGGGGCGCAGGGACCACGG
ArgArgValThrAspTrpTyrPheAspValTrpGlyAlaGlyThrThrVal

TCACCGTCTCC
ThrValSer

```

FIG. 1. Nucleotide and amino acid sequence of the SC(Fv) for e23. The linker joining the light-chain and heavy-chain variable regions is shown in bold.

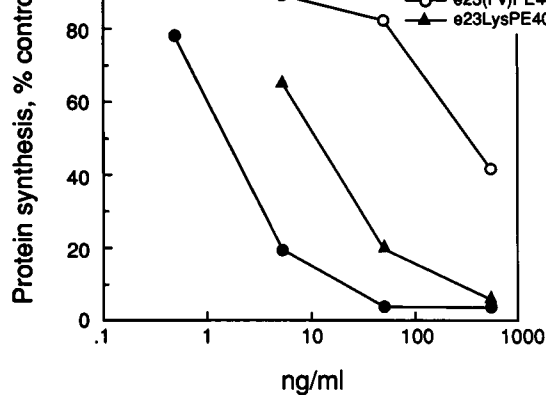


FIG. 2. Cytotoxicity of e23(Fv)PE40 on BT474 cells as shown by inhibition of protein synthesis. Results are shown as percentage of control cells to which no toxin was added. ●, e23(Fv)PE40 alone; ○, e23(Fv)PE40 plus native e23 (20 μg/ml); ▲, e23-LysPE40.

**Construction of Single-Chain Immunotoxin with Anti-erbB2 Antibody e23.** Single-chain immunotoxins made by the fusion of the antigen-binding region (Fv) and PE40 can retain the binding affinity of the native antibody and are often more active than the respective chemical conjugates (15, 16). For this reason, we selected antibody e23 for construction of a first-generation recombinant immunotoxin. First, an intact SC(Fv) for e23(Fv) coding region was generated essentially as previously described. The sequence of the single-chain protein is shown in (Fig. 1). To verify the binding activity of the purified e23(Fv) protein we conducted competition binding using <sup>125</sup>I-labeled e23 Fab (see Fig. 5). The overall structure of our first recombinant immunotoxin is the amino-terminal e23 SC(Fv) domain joined to the translocation (II) and ADP-ribosylating (III) domains of PE. The assembled gene is under control of a bacteriophage T7 promoter. The resulting plasmid, pJB23-40, expresses the variable region of the light chain of e23, a 14-amino acid linker peptide, the variable region of the heavy chain of e23, and amino acids 253–613 of PE. The chimeric protein was expressed in *E. coli* and purified. The resulting protein was >70% pure as judged by SDS/PAGE (data not shown).

**Cytotoxicity of e23(Fv)PE40.** e23(Fv)PE40 was tested on BT474 breast cancer cells and was found to inhibit protein synthesis in a dose-dependent manner with an ID<sub>50</sub> of 1.5 ng/ml (Fig. 2, Table 2). The cytotoxic activity was blocked by competition with excess native e23, demonstrating the specificity of e23(Fv)PE40 for erbB2-containing cells (Fig. 2). Another anti-erbB2 monoclonal antibody, e21, which binds to a different site, had no effect on the toxicity of e23(Fv)PE40 (data not shown). In the same experiment, e23-LysPE40, the chemical conjugate, had an ID<sub>50</sub> of 12 ng/ml (Fig. 2, Table 2). The activity of e23(Fv)PE40 was assayed on several cell lines expressing erbB2 and compared with that of the chemical

Table 2. Comparison of activity of e23(Fv)PE40 and chemical conjugates on various human cell lines

Cells	ID <sub>50</sub> ng/ml (pM)		Relative activity*
	e23(Fv)PE40	e23-LysPE40	
BT474	1.5 (23)	12 (63)	0.37
N87	3.5 (54)	24 (126)	0.43
SK-OV-3	22.0 (338)	180 (947)	0.36
SK-Br-3	32.0 (492)	180 (947)	0.52
A431	170.0 (2615)	>500 (>2631)	NC

\*Ratio of ID<sub>50</sub> (pM) of e23(Fv)PE40 to ID<sub>50</sub> of e23-LysPE40 on the same cell line. NC, not calculated.

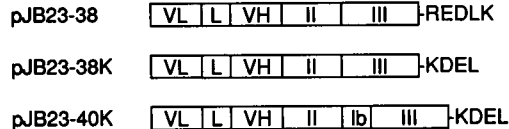


FIG. 3. Various e23(Fv)PE40 derivatives. VH, variable region of heavy chain; VL, variable region of light chain; L, linker; II, domain II of PE; Ib, domain Ib of PE; III, domain III of PE. Carboxyl-terminal amino acid sequences are shown in single-letter code.

conjugate, e23-LysPE40 (Table 2). On all four target cells, e23(Fv)PE40 was active and the ID<sub>50</sub> values on a molar basis were 2- to 3-fold lower than those of e23-LysPE40. Both molecules had very little activity on KB cells, showing their specificity for erbB2-expressing cells.

**Derivatives of e23(Fv)PE40.** The carboxyl terminus of PE ends in the amino acids REDLK. Replacing REDLK with KDEL results in molecules that have 3- to 10-fold higher cytotoxic activities (21). Also, deleting part of domain Ib of PE—i.e., amino acids 365–380, thereby deleting a disulfide bond—does not result in any loss of activity of several fusion proteins including TGFα-PE40, anti-Tac(Fv)-PE40, and B3(Fv)PE40 (17, 24). Since mixed disulfide bonds can form during the renaturation of recombinant proteins, we thought it would be helpful to delete this region. To explore the possibility of making a much more active derivative of e23(Fv)PE40, we made three new constructions: (i) e23(Fv)PE40KDEL, where the carboxyl terminus REDLK is replaced by KDEL in e23(Fv)PE40; (ii) e23(Fv)PE38, in which amino acids 365–380 have been deleted from e23(Fv)PE40 but the carboxyl terminus is still REDLK; and (iii) e23(Fv)PE38KDEL, where REDLK is replaced by KDEL in e23(Fv)PE38. These derivatives are diagrammed in Fig. 3. The chimeric proteins were also purified to >70% purity and tested for cytotoxic activity on target cells. As shown in Fig. 4, all of the new derivatives inhibited the protein synthesis of BT474 cells in a dose-dependent manner, with e23(Fv)PE38KDEL being the most active. Table 3 summarizes the ID<sub>50</sub> values of the e23(Fv)PE40 derivatives on various cell lines. On all three target cell lines, e23(Fv)PE38KDEL, was found to be the most active. e23(Fv)PE38KDEL was 6- to 10-fold more active than e23(Fv)PE40 (Table 3). None of the proteins had any cytotoxicity on KB cells, a cell line that does not overexpress erbB2. In the presence of excess e23, the cytotoxic activity of all derivatives was abolished (data not shown). The binding activity of e23(Fv)PE38KDEL was monitored in a competition bind-

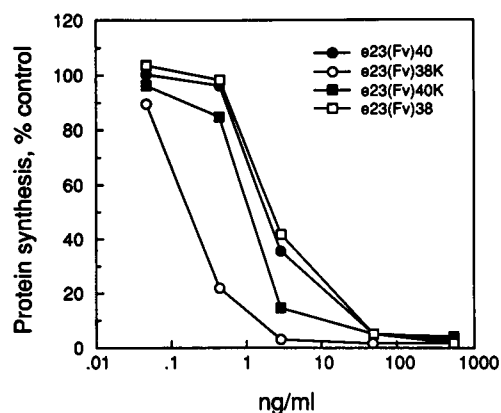


FIG. 4. Comparative cytotoxic activities of various e23(Fv)PE40 derivatives on BT474 cells. ●, e23(Fv)PE40; ○, e23(Fv)PE38KDEL; ■, e23(Fv)PE40KDEL; □, e23(Fv)PE38.

Protein	ID <sub>50</sub> (ng/ml)			KB
	BT474	N87	SK-OV-3	
e23(Fv)PE40	3	8	80	>500
e23(Fv)PE40KDEL	1.6	3.8	22	>500
e23(Fv)PE38	3.6	3.7	62	>500
e23(Fv)PE38KDEL	0.18	1.2	5	>500

ing assay. As shown in Fig. 5, e23(Fv)PE38KDEL was able to compete with homologous e23 Fab for binding, but a higher concentration was required than for e23 Fv. This result is consistent with either a lower overall affinity of e23(Fv)PE38KDEL or the purified protein being a mixture of active and inactive species. Current purification methods for e23(Fv)PE38KDEL do not allow us to separate forms on the basis of binding activity. To verify the binding activity of the e23 Fv, we conducted a similar competition binding assay and found that e23 Fv binds with slightly lower affinity than intact antibody and monomeric Fab produced from e23 (Fig. 5).

**Growth Inhibition of Human Tumors in a Nude Mouse Model.** The selective toxicity of the e23(Fv)PE38KDEL to cells overexpressing erbB2 encouraged us to attempt to treat human tumor cells growing in nude mice. The human gastric cancer cell line N87 has been shown to overexpress erbB2 protein at high levels as a result of gene amplification, and N87 cells grow well as a subcutaneous tumor in immunocompromised mice (19). Injections of  $5 \times 10^6$  cells on day 0 were followed by six intravenous treatments over 3 days, starting on day 10. Immunotoxin treatment inhibited growth of established tumors (Fig. 6). No animal deaths were observed at doses of 2  $\mu$ g. Equivalent amounts of either e23 Fab, e23 SC(Fv) (data not shown), or LysPE38KDEL had no effect on tumor growth. Nonspecific toxicity was assayed by monitoring the animal weight; no weight loss was observed at doses of 2  $\mu$ g.

## DISCUSSION

We examined the activity of immunoconjugates of anti-erbB2 mAbs. All immunotoxins were selectively toxic to cells overexpressing erbB2. Most active were conjugates of mAb e23, which was thus selected for constructing a recombinant

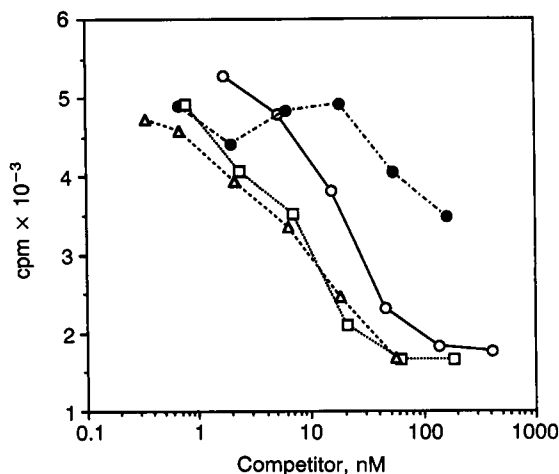


FIG. 5. Binding of SC(Fv) and immunotoxin to erbB2. The ability of purified e23 Fv (○) and e23(Fv)PE38KDEL (●) to inhibit the binding of <sup>125</sup>I-labeled e23 Fab was measured using cells overexpressing erbB2 (N87) as the binding target. Also shown are e23 Fab (□) and intact antibody e23 (Δ).

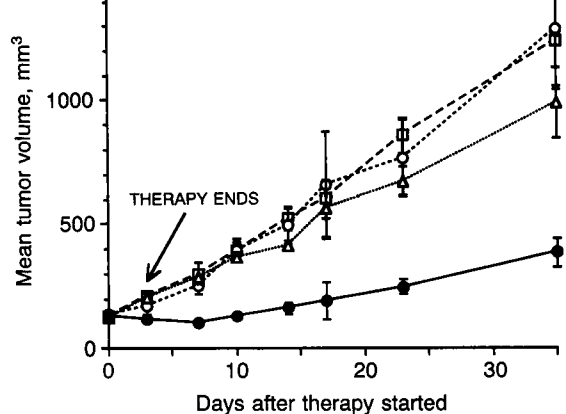


FIG. 6. Therapy of tumors formed in mice by human gastric cancer cell line N87. Tumors were established by injection of  $5 \times 10^6$  N87 cells subcutaneously on the backs of BNX mice. Therapy was initiated 7 days following injection of cells. Treatments were twice daily injections in the tail vein with 2  $\mu$ g of e23(Fv)PE38KDEL (●), LysPE38KDEL (○), or e23 Fab (Δ) or with phosphate-buffered saline (□). Measurements were conducted externally with calipers.

immunotoxin. This recombinant immunotoxin contains a SC(Fv) linked to PE40. As observed previously (17), the recombinant immunotoxin was 2- to 3-fold more active than the analogous chemical conjugate, and, we achieved an additional 6- to 10-fold increase in cytotoxic activity by changing the carboxyl terminus of PE from REDLK to KDEL and by deleting 15 amino acids from domain II of PE. This latter modification is likely to aid in formation of properly folded molecules. Our results indicate that e23(Fv)PE38KDEL is a potent cytotoxic molecule capable of binding specifically to the erbB2 protein. Improved refolding of e23(Fv)PE38KDEL or increasing its binding affinity could significantly improve the effectiveness of this molecule.

The potential clinical application of an immunotoxin such as e23(Fv)PE38KDEL is based on the overexpression of the 185-kDa erbB2 glycoprotein (gp185 erbB2) in about 30% of adenocarcinomas of the breast, stomach, lung, and ovary. Since gp185 erbB2 is expressed on normal cells, therapeutic efficacy will probably require administration of doses in a range sufficient to kill cells that overexpress erbB2, but with limited toxicity to normal cells. Direct evidence for the potential of e23(Fv)PE38KDEL is provided by our results showing inhibition of tumor growth in nude mice. The N87 cells used in these experiments overexpress gp185 erbB2 at high levels. At sublethal doses of e23(Fv)PE38KDEL, tumor growth was significantly reduced. It should be noted that a potentially important form of toxicity was not apparent in this experiment, as we do not expect the e23(Fv)PE38KDEL to bind to murine gp185 *erbB2* (*neu*) gene product. Such toxicity will need to be addressed in animal toxicity experiments where the e23(Fv)PE38KDEL binds to endogenous erbB2 proteins. Our results do suggest that e23(Fv)PE38KDEL may have application in treatment of certain highly malignant tumors, such as adenocarcinoma of the stomach, lung, breast, and ovary.

We thank M. Gallo, E. Lovelace, and T. Clayton for their valuable assistance.

- Pastan, I. & FitzGerald, D. J. P. (1991) *Science* **254**, 1173-1177.
- Vitetta, E. S., Fulton, R. J., May, R. D., Till, M. & Uhr, J. W. (1987) *Science* **238**, 1098-1104.
- Batra, J. K., Jinno, Y., Chaudhary, V. K., Kondo, T., Will-

# Explore Litigation Insights

Docket Alarm provides insights to develop a more informed litigation strategy and the peace of mind of knowing you're on top of things.

## Real-Time Litigation Alerts



Keep your litigation team up-to-date with **real-time alerts** and advanced team management tools built for the enterprise, all while greatly reducing PACER spend.

Our comprehensive service means we can handle Federal, State, and Administrative courts across the country.

## Advanced Docket Research



With over 230 million records, Docket Alarm's cloud-native docket research platform finds what other services can't. Coverage includes Federal, State, plus PTAB, TTAB, ITC and NLRB decisions, all in one place.

Identify arguments that have been successful in the past with full text, pinpoint searching. Link to case law cited within any court document via Fastcase.

## Analytics At Your Fingertips



Learn what happened the last time a particular judge, opposing counsel or company faced cases similar to yours.

Advanced out-of-the-box PTAB and TTAB analytics are always at your fingertips.

## API

Docket Alarm offers a powerful API (application programming interface) to developers that want to integrate case filings into their apps.

## LAW FIRMS

Build custom dashboards for your attorneys and clients with live data direct from the court.

Automate many repetitive legal tasks like conflict checks, document management, and marketing.

## FINANCIAL INSTITUTIONS

Litigation and bankruptcy checks for companies and debtors.

## E-DISCOVERY AND LEGAL VENDORS

Sync your system to PACER to automate legal marketing.