Anti-Cancer Drugs 1994, **5**, pp. 379–393

Review paper

Pharmacological modulation of peptide growth factor receptor expression on tumor cells as a basis for cancer therapy

Pierosandro Tagliaferri,[1] Michele Caraglia,[1] Raffaella Muraro,[2] Antonio Pinto,[3] Alfredo Budillon,[4] Vittorina Zagonel[3] and Angelo Raffaele Bianco[1]

[1]Cattedra di Oncologia Medica, Facoltà di Medicina, Università 'Federico II' di Napoli, via S Pansini, 5 80131 Naples, Italy. Tel: (+39) 81 746 2064; Fax: (+39) 81 746 2066. [2]Istituto di Patologia Umana e Medicina Sociale, Facoltà di Medicina Università 'G D'Annunzio' Via dei Vestini, Chieti, Italy. [3]Unità Operativa Leucemie and Divisione di Oncologia Medica, Centro di Riferimento Oncologico, Istituto Nazionale di Ricovero e Cura a Carattere Scientifico, 33081 Aviano, Italy. [4]Laboratory of Tumor Immunology and Biology, National Cancer Institute, Bethesda, MD 20892, USA.

Membrane receptors for peptide growth factor receptors (PGF-R) play a crucial role in the regulation of cancer cell proliferation and may behave as tumor associated antigens (TAA), which are currently regarded as specific targets for immunodetection and immunotherapy of human cancer. PGF-R are often more expressed by tumor cells than by normal counterparts and, by analogy to TAA, their surface expression may be regulated by cytokines. Moreover, the biological functions and specific ligands of most PGF-R are presently well elucidated as opposed to the great majority of TAA. PGF-R may, therefore, represent ideal cellular targets for at least two different therapeutic approaches: (i) naked or conjugated monoclonal antibodies and (ii) genetically engineered fusion proteins composed of PGF-R physiological ligands linked to genetically modified bacterial toxins. To date, clinical studies based on targeting of receptors for epidermal growth factor and interleukin-2 on tumor cells have been performed. Information from such studies suggests that PGF-R as well as TAA targeting strategies are clinically feasible, but that they still have to be optimized. A variety of host and tumor factors which affect targeting of neoplastic cells have been recently identified. For instance, it has been demonstrated that the antigenic density of the targeted molecule at the tumor cell surface is an important factor. In this view upregulation of PGF-R on cancer cells could be of major clinical advantage in immunotargeting. It has been reported that several cytokines and chemical compounds can induce PGF-R modulation on tumor cells. This paper reviews therapeutic opportunities related to the pharmacologic modulation of PGF-R expression. In addition a mechanistic hypothesis regarding PGF-R upregulation induced by cytostatic drugs and cytokines is proposed.

Key words: Cytokines, drugs, EGF-R, IL-2-R, peptide growth factor receptors, targeting modulation.

Introduction

Peptide growth factor receptors on human tumor cells

Cell proliferation is mediated by proteic or glycoproteic molecules, peptide growth factors (PGFs), which interact with specific cell surface receptors (PGFRs). It has been demonstrated that genetic aberrations in growth factor signaling pathways are linked to developmental abnormalities and to a variety of diseases, including cancer.[1] In fact, many cancer cells show alterations in growth factor signal transduction or changes in the structure of their receptors.[2–4] Tumor cells can promote their own proliferation by secreting PGFs which act on the adjacent cancer cells ('paracrine secretion') or on the same tumor cell producing PGFs ('autocrine secretion').[5–8] Interaction between PGFs and their receptors may also occur while the PGFs are still anchored to the plasma membranes of producer cells, as in the case of transforming growth factor receptors (TGF)-α. They may also bind their PGF-R, e.g. epidermal growth factor (EGFRs), expressed by the adjacent cells ('juxtacrine interaction'),[9,10] in which case a tight interaction of producer cells with receptor expressing target cells is often required.

MC is a recipient of a fellowship from 'Angelo Marinelli Foundation'. Studies carried out in the laboratories of PT and AP were supported by the Associazione Italiana per la Ricerca sul Cancro and by Consiglio Nazionale Ricerche, project ACRO (Grants nos 92.02274.PF39 and 92.02364PF39).

Correspondence to P Tagliaferri

This type of cell–cell interaction is mediated by a number of cell adhesion molecules (CAMs),[11] which represent in turn important 'co-factors' regulating the growth factor receptor 'loop' on both normal and neoplastic cells.[12] Proliferation of v-*sis*-transformed cells has been reported to require the interaction of platelet derived growth factor (PDGF) with its receptor in exocytosis vesicles ('intracrine interaction').[13] PGFs appear involved in the control of tumor cell proliferation not only *in vitro* but also in the natural history of tumors *in vivo*.[3,4] In fact, studies on tumor biopsies indicated that PGF-Rs are often overexpressed on tumors compared with the normal tissues from which they are derived. The receptors for EGF and for transferrin (TRF-R) are in fact highly expressed on several human tumors.[14–19] Furthermore, the expression of EGF-R is associated with a poor prognosis in breast, bladder, kidney and squamous cell esophageal tumors while that of TRF-R is associated with aggressive phenotype of breast cancers.[20–22] The receptor for interleukin-2 (IL-2-R) is highly expressed on Hodgkin (HL) and non-Hodgkin lymphoma (NHL), on chronic lymphocytic leukemia (CLL) cells and has been detected on adult T cell leukemia/lymphoma (ATL) cells at a higher cell density than on activated normal T lymphocytes.[23,24] On the basis of their differential expression on normal versus neoplastic cells, PGF-Rs may be considered as true tumor associated antigens (TAAs) and have been proposed as cellular targets for anti-tumor strategies based on anti-PGF-R monoclonal antibodies (mAbs).[23,25,26] In fact, anti-PGF-R, as well as anti-TAA mAbs, would preferentially recognize neoplastic tissues and induce specific tumor cell killing either by complement-dependent cytolysis and antibody-dependent cell cytotoxicity (ADCC) or by direct carrying at the tumor site of cytotoxic drugs, toxins or radioisotopes.[27,28] A further advantage of PGF-R targeting is the accessibility of such antigens on the cell surface. In fact, it would be much more difficult to interfere with cytoplasmic or nuclear molecules (e.g. oncogene products) than with membrane structures. Moreover, since PGF-R structure, function and ligands have been characterized, in contrast to those of commonly defined TAAs,[29–31] anti-PGF-R mAbs may exert their tumoricidal effects not only through the interaction with the host immune system, but also by interferring with the transductional pathways regulating cancer cell proliferation.[27] In fact, PGF-Rs do not merely represent inactive anchors for mAbs at the tumor cell surface, but they also behave as *active targets*.[27] Several anti-PGF-R mAbs, which block the receptor function, have been generated in the last decades.[32–37] The mechanisms of receptor blockade by mAbs are represented by the competitive or uncompetitive displacement of physiological ligands from their binding sites on PGF-Rs, or by activation of the receptor internalization process, with subsequent PGF-R downregulation and tumor cell desensitization to the growth promoting activities exerted by the specific ligands.[32,38,39]

Targeting stategies

PGF-R targeting strategies are currently based on the use of mAbs or other biotechnological products, as genetically engineered fusion proteins. Anti-PGF-R mAbs can exert anticancer activities not only by blocking of receptor functions or activating physiological anti-tumor host immune responses, but they can also effectively deliver drugs, plant or bacterial toxins and radioisotopes directly at the tumor sites (Table 1).[40]

Anti-PGF-R mAbs have been successfully conjugated with cytotoxic drugs.[27] Problems with drug-conjugated mAbs are the lack of stability of the mAb–drug conjugates, the broad spectrum cellular drug resistance and the efficient delivery of adequate amounts of mAb–drug conjugates.[27,41,42] To overcome this last shortcoming, mAbs have been conjugated with drug-activating enzymes, so that large amounts of inactive and non-toxic pro-drugs can be systemically administred to achieve their effective diffusion within tumor tissues. Such pro-drugs can be subsequently activated *in situ* by the specific enzymes delivered to the cancer cell surface by mAbs.[43,44]

mAbs linked to toxins (immunotoxins) have been also generated and clinically tested.[45] Toxins, if efficiently targeted, can specifically kill tumor cells.[46] However, the development of host immune responses directed against both immunoglobulin and toxin antigenic determinants has turned out to be a major problem in therapeutic approaches based on immunotoxin use.[40,45]

A more recent approach for PGF-R targeting relies on the use of recombinant fusion proteins derived from the physiological ligands of PGF-R or single chain immunoglobulin fragment (Fv) and a recombinant toxin modified for non-specific binding capacity.[47,48] These products are less immunogenic than classical immunotoxins (mAb + toxin) and their smaller size permits improved tumor tissue diffusion.[40,45] Since PGF/PGF-R interaction induces the PGF-R internalization process, a further ad-

Table 1. Modes of targeted therapy

Naked mAbs	The antitumor effect is immunologically mediated by means of ADCC and complement-dependent cytotoxicity.[27,28] This effect should be specifically exerted by IgG2b mAbs. In the case of anti-PGF-R mAbs, the antiproliferative effects could be also determined by direct functional receptor blockade (receptor antagonism, receptor downregulation, inhibition of receptor internalization).[32,38,39]
Drug- or toxin-conjugated mAbs	The antibody acts as a selective carrier of drugs or toxins to the tumor cells.[27,41,42,45] mAb internalization is required for toxin cytotoxicity,[50]
Radioisotope-conjugated mAbs	The antibody acts as a carrier of radioisotopes to the tumor cells. mAb internalization is not required. The cytotoxic effect is exerted also on neighborhood cells and could be, therefore, extended also to TAA-negative tumor cells. The antitumor activity is strictly dependent on the radiosensitivity of the targeted tumor tissues.
Enzyme-conjugated mAbs	The antibody delivers prodrug-activating enzymes to the tumor tissues. This approach should theoretically enhance the therapeutic index of selected antitumor drugs.[43,44]
Recombinant fusion proteins	Genetically engineered constructs, consisting of a recombinant toxin which has been fused to a targeting component as single chain antibody fragments or a natural ligand (growth factor).[45,46] In the latter case, the cytotoxic action of the targeted toxin takes advantage from the physiological internalization process of the bound receptor.[46,47] Moreover, they are less immunogenic and have a better tumor diffusion.[47,48]

vantage of fusion proteins is the fail-safe internalization of the toxin, which is necessary to achieve efficient antitumor effects.[48–50]

Moreover, altered expression of PGF (e.g. v-*sis* product) or of their receptors (e.g. v-*erb*-b product) is a frequent event in tumor cells, and antisense RNAs have been shown to inhibit oncogene expression and tumorigenicity of cancer cells.[4,51] Since PGF-R and their ligands are involved in the acquisition of the tumorigenic phenotype by cancer cells, it appears possible to target neoplastic cells with antisense RNAs raised against normal or mutated PGF-R RNA.[52]

Basic research has, therefore, provided a pleiotropic series of biotechnological products which can be applied in therapeutic strategies involving tumor cell targeting. Here we review results from the major clinical studies aimed at PGF-R targeting.

Therapeutical approaches based on PGF-R targeting

Preliminary results of clinical studies with anti-PGF-R targeting agents

IL-2-R targeting. Early clinical studies on IL-2-R targeting agents were performed in patients affected by ATL in which tumor cells express increased levels of high affinity IL-2-R.[23] The efficacy of the unmodified anti-Tac murine mAb recognizing the 50 kDa α chain of IL-2-R (CD25) expressed at a high antigenic density on ATL cells and, in smaller amounts, at the surface of activated T lymphocytes was evaluated. Nine patients were accrued in the study and received a single dose of anti-Tac mAb.[37] One out of nine patients showed a complete response (CR) while another two patients had transient and mixed responses lasting from 1 to more than 8 months.[37] To improve the effectiveness of IL-2-R-directed therapy, the anti-Tac antibody was modified by linking with pseudomonas exotoxin (PE).[54] This early PE–anti-Tac conjugate was, however, highly hepatotoxic due to the non-specific binding of PE to galactose receptors (GRs) expressed on hepatocyte surfaces.[23] In a second generation of immunoconjugates, the I domain of PE, responsible for non-specific binding to GR and of the subsequent hepatotoxicity, was deleted,[55] so generating a genetically modified PE40 toxin.[56] This toxin preserved its intrinsic cytotoxic capacity, but it was no longer able to bind GR aspecifically. PE40–anti-Tac mAb immunoconjugates and the recombinant construct anti-Tac Fv fragment/PE40 both recognize and inhibit protein synthesis only on CD25 expressing T cells.[56] Phase I/II trials employing these agents are currently in progress.[23] Further modifications have been introduced on the PE40 COOH terminus by the insertion of a novel amino acid sequence (lysine, aspartate, glutamate, leucine).[57] Native proteins harboring this sequence are efficiently retained in the endoplasmic reticulum and could diffuse to the cytosol, which is the specific site of toxin action.[58] The derivative

Table 2. Biotechnological products raised against IL-2-R

IL-2-R targeting	Characteristics	Reference
mAbs		
anti-Tac	mouse-IgG1 blocking	37
Immunoconjugates		
anti-Tac–PE	anti-Tac/unmodified PE	24
anti-Tac–PE40	anti-Tac/modified PE	55
^{212}Bi–anti-Tac	anti-Tac/α-emitting radionuclide	69
^{90}Y–anti-Tac	anti-Tac/β-emitting radionuclide	70
Recombinant fusion proteins		
PE40–IL-2	IL-2/modified PE	61
DAB_{389}–IL-2	IL-2/modified DT	65
DAB_{486}–IL-2	IL-2/modified DT	62

genetic construct anti-Tac Fv fragment/PE40KDEL has been proved to be a more potent inhibitor of protein synthesis *in vitro* than other anti-Tac conjugates in leukemic cells of patients affected by CLL.[59]

In HL, NHL and CLL the neoplastic cells often express large amounts of the 75 kDa β chain of IL-2-R (CD122), which is not recognized by anti-Tac mabs.[60–62] IL-2 and its recombinant derivatives, however, bind both to α and β chains of IL-2-R.[63] IL-2 has been, therefore, genetically linked to PE40 or to other similarly modified diphteria toxins (DAB_{389} and DAB_{486}), with the aim of increasing the spectrum of targetable malignancies which express the IL-2-R in the absence of the 50 kDa α chain.[64] DAB_{486}–IL-2 (0.2 mg/kg/day) has been administred over 90 min × 5 days at 21 day intervals for up to four courses to 12 patients affected by cutaneous T cell lymphoma (CTCL). One patient achieved a CR, while five had minor responses (MRs). The expression of α and β chain of IL-2-R was evaluated on cutaneous lesions of patients affected by cutaneous lymphoma prior to treatment, and it was found that the concurrent expression of both α and β IL-2-R chains positively correlated with clinical response.[65] DAB_{389}–IL-2 was also able to induce a partial response (PR) in a patient affected by CLL.[66] DAB_{389}–IL-2 is presently under evaluation on phase I/II clinical trials on lymphoma patients. This latter toxin displays a higher anti-tumor activity *in vitro* than DAB_{486}–IL-2,[67] it is 2-fold less toxic and shows a much slower *in vivo* clearance than DAB_{486}–IL-2. In early clinical studies, DAB_{486}–IL-2 was administered to 69 highly pretreated lymphoma patients. Three patients achieved a CR, while eight had PR.[67] Moreover, one CR and two PRs induced by DAB_{486}–IL-2 in chemoresistant CTLC patients have been recently reported by Hesketh *et al.*[68] Table 2 lists main biotechnological products able to target IL-2-R.

EGF-R targeting. In an early phase I clinical trial, the anti-EGF-R 225 mAb was administered to 19 patients with advanced squamous cell lung carcinoma. The efficacy of a ^{111}In-conjugated 225 mAb in tumor imaging was also assessed in the same study. mAb concentrations up to 300 mg (total dose) could be delivered in the absence of toxicity and the uptake of labeled mAb was dose-dependent on the amount of administred mAb.[71] Anti-EGF-R mAb RG83852 has been recently administered to 13 patients with non-small cell lung cancer (NSCLC) and head and neck cancer, in a phase I clinical trial at the MD Anderson Cancer Center. EGF-R saturation has been evaluated by image analysis and significant tumor localization was observed at mAb doses higher than 400 mg/m^2. Moreover, mAb RG83852 appeared also to regulate EGF-R expression.[72] Combined therapeutical approaches might provide increased efficacy.

Anti-EGF-R mAbs (mAb 528 and mAb 225) appear to synergize with cisplatin in the suppression of subcutaneously established A431 cell tumors.[73]

CD30 and CD40 targeting. CD30 and CD40 are surface receptors belonging to the nerve growth factor/tumor necrosis factor family (NGF-R/TNF-R).[74,75] This family includes at least nine different transmembrane proteins such as APO-1/Fas antigen, the human homolog of the OX40 molecule, CD27, NGF-R, TNF-R, 4-1BB, CD30 and CD40.[74–77] Specific ligands for several of such receptors have been cloned so that a parallel family of peptide ligands showing homology to TNF and

often expressed in a transmembrane form has emerged.[78] CD30 is a 120 kDa glycoprotein[73] recognized by mAbs such as Ki-1 and Ber-H2,[79] which is strongly expressed at the surface of RS cells of HL, on neoplastic cells of ALCL, on a restricted number of non-ALCL NHL, some carcinomas and on small subsets of activated normal cells.[74,79] Accordingly, anti-CD30 mAbs are currently employed for the routine immunophenotypic identification of RS cells and ALCL cells for diagnostic purposes.[79–87] The specific CD30-L has been recently cloned and identified as an extensively glycosylated peptide whose C-terminal receptor binding portion shows sequence homology with TNF-α, TNF-β, CD40-L and CD27-L.[82] After binding, its specific receptor, CD30-L, is capable of stimulating growth of the human HL cell line HDLM-2 and of exerting a cytotoxic activity on the human ALCL cell line Karpas 299.[82] CD30 and CD30-L can be therefore regarded as a receptor–ligand pair regulating cell growth of a restricted subset of human lymphomas. Due to its restricted expression on normal tissues and its strong antigenic density on tumor cells of HL and ALCL, the CD30 receptor represents a candidate molecule for immunotargeting strategies in these lymphomas. Preclinical studies have shown that anti-CD30 immunoconjugates (ricin A or saporin) exert a powerful killing activity on HL cell lines *in vitro*.[83,84] Anti-CD30/toxin immunoconjugates also showed significant antitumor activity when tested *in vivo* on mice subcutaneously transplanted with human HL cell lines.[85] In a preliminary clinical study, increasing quantities of cold anti-CD30 mAbs Ber-H2 with a small dose of ^{131}I-labeled Ber-H2 were administred to six patients with advanced HL.[86] Immunoscintigraphy studies performed at 48–72 h after mAb administration showed that about 50% of tumor sites previously documented to be involved by HL were detected. Immunohistochemistry studies with rabbit anti-mouse Ig and the APAAP technique on tissue biopsies taken 24–72 h following mAb injection indicated that RS cells in all tumor sites, including those not detected by immunoscintigraphy, were stained by the injected mAb. *In vivo* targeting of RS cells and of their mononuclear variants was specific and highly efficient following a single dose (35–50 mg) of mAb Ber-H2, as shown by the strong stain of tumor cells on post-treatment tissue biopsies. mAb administration did not result in clinical toxicity and, even though antitumor effects were not observed after a single dose of cold Ber-H2 mAb, the highly efficient tumor cell targeting achieved *in vivo*, suggests that anti-CD30 mAbs constitute optimal reagents for HL therapy with immunoconjugates.[86] Due to the radiosensitivity of HL, the ^{131}I– or ^{90}Y–Ber-H2 immunoconjugates may turn out to represent ideal candidates for radioimmunotherapy of this lymphoma and possibly also for eradication of residual disease following chemotherapy. Based on the good results achieved *in vitro* with anti-CD30 toxin immunoconjugates, a clinical study with Ber-H2–saporin was recently undertaken.[87] Nine patients with refractory HL were treated with a total dose of Ber-H2–saporin ranging from 0.4 to 0.8 mg/kg. In such patients the circulating immunoconjugates displayed a half-life of 20–25 h, and appeared to be immunologically and biologically active, as indicated by the ability of patients' serum to bind autologous RS cells (on pre-treatment frozen sections) and to kill HL cell lines *in vitro*. A striking reduction in tumor size (50 to more than 75%) was documented by computed tomography scanning performed 7 and 30 days after infusion of Ber-H2–saporin in five out of nine patients. Response duration ranged from 2 to 4 months and therapy was overall well tolerated. Toxicity consisted of fever, capillary leak syndrome in a mild form in half of the patients, abnormalities of liver enzyme levels and mild thrombocytopenia in three patients. Other approaches under current evaluation for CD30 targeting in HD include the generation of anti-CD30 mAbs complexes with pro-drug activating enzymes such as AP-1,[85,88] and the development of bispecific monoclonal antibodies (BimAbs) recognizing CD30 and CD3 or CD28 determinants on T cells.[89] A construct constituted by bispecific anti-CD30 mAb HRS-3/AP-1 was able to render HL cell lines about 100 times more sensitive to the cytotoxic effects of MOP pro-drug.[85,88] Interestingly, such constructs, although unable to increase MOP-mediated killing of CD30$^-$ cells alone, resulted in enhanced cytotoxicity of MOP on CD30$^-$ tumor cells when these latter were admixed to CD30$^+$ L540 HL cells.[85,88] These data indicate that the HRS-3/AP-1 construct may be advantageous in eradicating CD30$^-$ tumor cells subclones within CD30$^+$ tissues of HL. BimAbs OKT3/HRS-3 (anti-CD3/anti-CD30) and 15E8/HRS-3 (anti-CD28/anti-CD30) were able to induce an antigen-dependent cytotoxicity on CD30$^+$ cell targets by antigen presenting cell (APC)-depleted peripheral blood lymphocytes.[89] IL-2 production by T cell lines co-cultivated with CD30$^+$ tumor cells was also specifically activated in the presence of the cross-linking BimAb 15E8/HRS-3.[89] Similarly anti-CD16/anti-CD30 BimAbs were able to induce lysis of HL cell lines *in vitro* by unstimulated natural killer cells.[90] These results

overall indicate that *in vivo* targeting of the CD30 receptor is specific and clinically feasible in HL patients. In addition, significant antitumor responses have been observed in patients with refractory HL, suggesting that RS cell targeting via CD30 may represent a useful tool for eradicating residual disease in such patients. The tumor cell killing ability of anti-CD30–saporin conjugates has also been recently tested on ALCL xenografts in a SCID mouse system.[91] Results of these studies indicated that Ber-H2–saporin complexes at doses corresponding to 50% of LD_{50} were able to induce a complete tumor eradication in six out of 21 xenotransplanted mice and significantly delayed tumor growth in the others.[91] Biological studies aimed at evaluating the regulation of CD30 receptor expression on RS and ALCL cells are currently ongoing.[92] Cytokines and other biological agents able to upregulate the expression of CD30 on lymphoma cells will constitute a useful complement for CD30 targeting in a clinical setting.

CD40 is a surface receptor mainly expressed on normal and neoplastic B lymphocytes and on certain human carcinoma cells.[75,93,94] Expression of CD40 appears to be regulated by cytokines on several cell types including B cells and carcinoma cells.[95,96] CD40 acts as a receptor for a recently identified protein (CD40-L) which shows homology to ligands for other receptors of the NGF-R/TNF-R family.[97,98] The biological activities of CD40 include stimulation of B cells,[99,100] modulation of Ig switching,[96,100] regulation of the apoptotic process in the follicular centers[96,101] and modulation of B cell interactions with T lymphocytes which express a membrane form of CD40-L.[98,102] Among neoplastic proliferation of B cells CD40 is expressed on most (more than 80%) of B-NHL, B-CLL and hairy cell leukemias, and on 30–40% of B lineage acute lymphoblastic leukemias (ALL).[103,104] More interestingly, it has been shown that clonogenic precursors in B-NHL and B-lineage ALL express CD40. Sorting experiments have demonstrated that $CD40^+$ cell fractions obtained from B cell neoplasms contain precursor able to form *in vitro* colonies upon addition of low molecular weight B cell growth factor.[104] Conversely $CD40^-$ fractions in B lineage ALL and NHL either do not contain clonogenic precursors or these latter are present at a very low frequency.[104] On the basis of these results the anti-CD40 mAb G28.5 has been conjugated to the plant toxin pokeweed antiviral protein (PAP). G28.5–PAP immunoconjugates proved to be highly effective in inhibiting clonogenic growth (up to 99–100%) of neoplastic precursors in B lineage ALL and NHL cell samples.[104] The strong killing effects of G28.5–PAP complexes *in vitro* on the clonogenic cell fraction of B cell neoplasms suggest that anti-CD40 toxin conjugates may represent an important tool for clinical targeting of ALL and NHL. Again, cytokine-induced upregulation of CD40 on clonogenic cells in these malignancies is an additional approach to be explored for optimizing CD40 targeting in humans. Finally, the fact that CD40 is expressed only in a relatively small fraction of $CD34^+$ hemopoietic progenitors[94,104] renders CD40 targeting an appealing new strategy for selective immunotherapy of B cell neoplasms including eradication of residual disease and bone marrow purging. CD30 and CD40 represent, therefore, surface receptors for a new emerging class of ligands involved in the biology of important types of human lymphomas.[105] Targeting of such molecules by appropriate immunoconjugates will hopefully lead to a further improvement of selective therapeutic strategies for patients affected by HL, $CD30^+$ ALCL and subsets of NHL and B lineage ALL.

Challenges in PGF-R targeting strategies. PGF-R targeting strategies, although promising are still in a preliminary clinical stage (Tables 3 and 4 list the currently employed biotechnologic products for targeting of EGF-R and TRF-R). At present, it is not possible to predict their future impact in the therapy of human cancer. However, the results so far suggest that substantial clinical advantages will be achieved only on the basis of the above described technical and theoretical considerations.

The obstacles in the PGF-R targeting are similar to the well known restraints of TAA targeted therapies (Table 5).[40] In this regard, the generation of novel genetically engineered fusion proteins appears to exploit the major chances of improvement both in selectivity and cytotoxic potential.[47,48] An additional improvement will be provided by the generation of humanized chimeric anti-PGF-R mAbs, which theoretically could allow better immune system reactivity (ADCC) and avoid the generation of human anti-murine antibodies (HAMA) in treated patients.[123]

Pharmacological modulation of PGF-R expression

Rationale for TAA and PGF-R modulation: TAA density is one of the major limiting factors of targeting

The *in vivo* binding of mAbs to tumor targets is strongly influenced by antigen density at the cell

Table 3. Biotechnological products raised against EGF-R and related receptors

EGF-R targeting	Characteristics	Reference
mAbs		
425	EGF-competing	106
425.3	EGF-competing	107
225	EGF-competing	71
528	EGF-competing	108
RG83852	EGF-non-competing	72
108.1	EGF-non-competing	109
108.4	EGF-non-competing	110
CH225	chimeric mouse/human EGF-competing	111
Immunoconjugates		
108.4–Dx	108.4/doxorubicin	110
Recombinant fusion proteins		
TP40	TGF-α/modified Pseudomonas exotoxin	112
DAB_{486}–EGF	EGF/modified DT	113
DAB_{389}–EGF	EGF/modified DT	113
$p185^{erbB2}$ targeting		
mAbs		
4D5	cytotoxic	114
TAb250	cytotoxic	115

Table 4. Biotechnological products raised against TRF-R

TRF-R targeting	Characteristics	References
mAbs		
R17 208	rat IgM multimeric	114
REM 17.2	rat IgM multimeric	115
42/6	mouse IgA	116
B3/25	mouse IgG1 blocking	117
D65.30	mouse IgG1 blocking	118
D86.13	mouse IgG1 non blocking	119
Immunoconjugates		
45 4A12 rRA		120
anti-TRF-R CRM 107		121
Recombinant fusion proteins		
Fv anti-TRF PE40		120

Table 5. Problems of targeted therapies

Binding site barrier
Non-homogeneous antigen expression
Low TAA density
Large size of targeting molecules
(a) Selectivity
TAA expression not absolutely restricted to tumor tissues
(b) Pharmacokinetics of targeting agent
non-specific tissue and organ binding
trapping by circulating shedded antigens (CEA, Tac)
unfavorable plasma clearance
(c) Tumor tissue distribution
poor tissue vascularization and necrosis
(d) Immunological pitfals
human anti-mouse antibodies
anti-toxin antibodies

surface. In fact, several studies have pointed out this limiting factor in the efficient targeting of tumors with anti-TAA mAbs.[124,125] The efficiency of *in vivo* targeting with ^{111}In-labeled anti-EGF-R mAbs is strictly correlated with the number of membrane EGF receptor sites expressed by the cells.[124] Moreover, Sung *et al.* have reported that two mAbs with a similar affinity, recognizing antigens expressed at a different density, were rather different in their relative efficiency of *in vivo* tumor targeting. A logarithmic increase of tumor uptake was observed with the mAb specific for the TAA expressed with a 2- to 3-fold higher *in vivo* antigen density. These latter results were obtained at mAb doses high enough to approach saturating conditions, which represent the most typical therapeutic application.[125] Furthermore, Fujimori *et al.* have recently

proposed a mathematical model accounting for the tissue distribution of the radioconjugated mAbs: better tumor tissue percolation could be achieved for antibodies raised against antigens highly expressed at the tumor cell surface, while the use of mAbs which have high affinity for less expressed antigens could lead to heterogeneous microdistribution and to a relatively higher mAb radiolocalization at the surface of tumor nodules (binding site barrier).[126] A more efficient tumor cell targeting could perhaps be accomplished by complementing the use of mAbs with agents able to increase the expression of TAAs at the cancer cell surface, provided that *in vitro* and *in vivo* antigenic profiles are similar, and the enhanced expression of TAA does not concurrently result in an increased shedding of the targeted antigens.[127–129]

PGF-R modulating agents

Interferons (IFNs) have been shown to act as powerful modulators of antigenic expression on tumor cells and appear also capable of increasing the immunotargeting of tumor tissues *in vivo*.[130–134] We have recently reported that cellular receptors for EGF could be upregulated by IFN-α on human epidermoidal carcinoma cells.[135] We found that a major upregulation could be achieved at IFN-α concentrations capable of inducing 50% growth inhibition on such cells.[135] Moreover, IFN-α and IFN-β have been reported by others to inhibit proliferation and induce an increased expression of EGF-R transcripts in squamous carcinoma cells.[136] These latter effects were paralleled by the induction of terminal differentiation in epidermoidal cancer cells and, therefore, by slowing cell growth.[137] IFN-γ can also increase the expression of EGF-R both at the mRNA and protein level in human breast carcinoma cell lines.[138] On the other hand, IFN-α has been also shown to decrease EGF binding in other tumor cell systems. Interstingly, Zoon *et al.* have shown that IFN-α was able to induce EGF-R downregulation on bovine MDBK untransformed cells, occurring between 8 and 20 h after the beginning of treatment. Normal bovine cells have, however, been used in this study: a diverse effect of IFN-α on EGF-R expression in normal cells as compared with cancer cells could be therefore possible.[139] This apparent discrepancy may suggest a novel and selective way of interfering with tumor cell growth. In fact, selective EGF-R upregulation on tumor cells may significantly improve the specific targeting of cancer tissues, protecting normal cells from the cytotoxic effects of delivered agents. An opposite effect of IFN-α on EGF-R expression by human tumor cells has been also described by Eisenkraft *et al.*, who reported that IFN-α can inhibit EGF-R synthesis on EGF-sensitive kidney cancer cells.[140] Nevertheless, in this study IFN-α has been used at cytotoxic concentrations and it is conceivable that overall protein synthesis could have been depressed, hampering the specificity of IFN-α effects on EGF-R synthesis. Anti-tumor agents (including cytokines), when used *in vitro* at cytotoxic concentrations, kill the majority of the tumor cell population and, under these conditions, it is unlikely that one can detect any specific tumor cell response different from the cytolytic effect itself. Like IFN-α, retinoic acid has been reported to induce divergent effects on EGF-R expression: upregulation on epithelial tumor cells, normal and transformed fibroblastic cell lines and mammary gland cells,[141,142] and decreased receptor synthesis on the human epidermoidal ME200 cancer cell line.[143] These differences could be partially explained by the heterogeneous lineage and differentiation stage of the tumor cell lines employed as targets of such experiments. PGFs can in fact determine dissimilar cellular responses: they can merely promote cell proliferation or can induce phenotypical changes as part of a differentiation program triggered by their action, depending on the transductional pathways activated on different target cells.[144–146] Moreover, the EGF-R downregulating effects reported by Zheng *et al.*[143] occur after short-term exposure to retinoids (8–24 h), while EGF-R upregulation is generally induced by a longer exposure to retinoids (72 h), and is paralleled by tumor cell growth inhibition. However, no data about retinoid effects on ME200 cell proliferation are reported in the study of Zheng *et al.*[143] Other studies have shown that TNF-α also increases the expression of EGF-R and enhances the binding of anti-EGF-R mAbs at the surface of human glioma cells.[147] Conventional anticancer drugs at a low concentration are also capable of modulating PGF-R expression by tumor cells. Zuckier *et al.*[148] have in fact demonstrated that adriamycin, at a low concentration, increases EGF binding to human HeLa and mouse 3T3 cells along with a cytostatic effect. In addition, Baselga *et al.*[149] have recently shown an increased expression of EGF-R and TGF-α mRNA in epidermoidal cancer cell lines which have been exposed to the same drug. We have moreover found that cytosine arabinoside (ara-C) and 5aza2′deoxycytidine (5azaCdR) were both able to induce EGF-R and TRF-R upregulation

on human epidermoidal and lung cancer cells. These effects were paralleled by the induction of cell growth inhibition and by the increased cell binding of anti-EGF-R and anti-TRF-R mAbs.[150–152]

Receptor modulation and antiproliferative activity: a mechanistic hypothesis.

All the previously reported observations suggest that modulation of PGF-R expression is a common event in tumor cells where cytostatic effects have been induced by cytotoxic drugs at low doses or by biological agents.

We propose that PGF-R upregulation induced by drugs or cytokines could be part of a homeostatic cell response to antiproliferative stimuli, since this phenomenon is generally paralleled by tumor cell growth retardation. According to this hypothesis, the enhanced expression of PGF-R and the secretion of autocrine factors by the tumor cells could represent an attempt to provide an upshot to the growth retardation induced by cytostatic agents or cytokines. In this way, viable but growth-inhibited tumor cells could activate metabolic pathways involved in growth promotion. Moreover, PGF-R overexpression can sustain tumor cell spreading and tissue colonization, allowing the adaption to microenvironments propitious to tumor cell seeding and proliferation.[153]

Drug-resistance could also be determined, in some instances, by induction of a homeostatic response in the tumor cells. In fact, it has been shown that alkylating agents and heavy metals can induce high levels of metallothionein which confer drug resistance and also cause increased synthesis of stress proteins in tumor cells.[154–155] From all these considerations we can hypothesize that an important cause of the failure of conventional chemotherapy, together with the escape from the host immune surveillance of residual valuable cells, is the tumor cell response to stress induced by antitumor agents. Such a response may consist of: (i) the blockade of tumoricidal effects of antitumor drugs by detoxication and DNA repair mechanisms,[156,157] or (ii) the superactivation of metabolic pathways involved in tumor cell proliferation (e.g. PGF and PGF-R upregulation). After conventional chemotherapy, an apparently drug-sensitive tumor mass could, therefore, consist of: (i) abundant necrotic areas due to the killing of drug-sensitive tumor cells, or (ii) minimal residual viable tumor composed of constitutively drug-resistant cells (kinetically resting or genetically resistant) or by a 'sensitive' tumor cell population where drug exposure has induced a transient 'homeostatic' protective response. This latter cell compartment is often responsible for the regrowth of tumors, which become poorly responsive to the tumoricidal effects of the previously used agent and may account for a more aggressive biological behavior (PGF-R upregulation) of residual disease.[158] We propose, therefore, that the homeostatic cancer cell response, which leads to a defensive reaction in order to escape from drug cytotoxicity, could, conversely, allow a better tumor uptake of targeted therapeutic agents. A further advantage for therapeutic strategies taking advantage of such a tumor cell response could be based on its theoretical specificity. As the 'homeostatic response' is a reaction to antiproliferative stimuli, it might occur only in proliferating cells and, therefore, take place more efficiently in tumor cells than in normal counterparts. This hypothesis is partially supported by the data reported by Zoon *et al.*[139] describing downregulation of EGF-R expression and of an EGF proliferative response induced by IFN on normal cells, and suggests that PGF-R upregulation may selectively occur in the tumor cells.

Therapeutic strategies

Since TAA density is an important limiting factor for efficient tumor targeting, it appears evident that upregulation of PGF-R expression at the tumor cell surface by pharmacologic means could clearly improve targeting of PGF-R *in vivo*.[125,126] We propose, therefore, the sequential administration of selected conventional antineoplastic drugs at cytostatic doses or cytokines in order to induce PGF-R upregulation in tumor cells, followed by PGF-R-targeted therapeutic agents.

It should be also considered that cytotoxic agents (at conventional doses) often induce extensive necrosis in the tumor mass which limits the vascularization, consequently hampering the diffusion at the tumor site of targeting agents and of immune effector cells, both necessary for complete eradication of residual neoplastic cells.[27,40] The use of these drugs at low doses, however effective in regulating PGF-R, may overcome these detrimental effects at the tumor site and, therefore, create optimal conditions for tumor cell targeting. Notably, some cytotoxic drugs, when used at low doses, can in some instances also improve the host immune antitumor response.[158,159] Conversely, it is well known that conventional doses of cytotoxic drugs usually result in severe immunosuppression, which is clearly unfavorable for neoplasm eradication.[160] Further-

more, all the available information on drug-induced modulation of PGF-R and our recent findings of a concomitant upregulation of TRF-R and EGF-R, as induced by IFN-α and ara-C, suggest two additional important conclusions: (i) PGF-R modulation is a broad phenomenon, because it is induced by different agents and may involve different types of PGF-Rs at the same time, and (ii) PGF-R upregulation can overcome two important mechanisms of tumor cell resistance to targeting agents: heterogeneous expression of TAAs and their down-regulation or 'modulation' by the neoplastic cells.[37,40,132,161] It can be predicted that the combined use of anti-EGF-R and anti-TRF-R targeting agents could theoretically accomplish the complete destruction of the whole cancer cell population in neoplasms expressing such PGF-Rs.

A further advantage for therapeutic strategies based on the pharmacologic enhancement of PGF-R expression is provided by an additional important finding: neither alterations of PGF-R affinity for specific ligands or modifications of their internalization processes have been detected as a consequence of pharmacologically induced PGF-R upregulation.[135,141,143,150–152] The unmodified receptor affinity for PGFs could in fact allow optimal binding of fusion proteins to the receptor, while the preserved internalization capability of this latter could endorse the intracytoplasmic cytotoxic action of engineered toxins or of drug- and toxin-conjugated mAbs.[27,45,47,48,50] An additional therapeutic advantage provided by PGF-R modulation is cell cycle synchronization of tumor cells after growth stimulation by PGFs, which may allow the subsequent delivery of S phase cytotoxic drugs, alone or conjugated to anti-PGF-R targeting agents. Interestingly, ara-C is able to induce in human tumor cells an increase of [^{3}H]thymidine uptake and a concomitant upregulation of EGF-R and TRF-R even in the absence of PGFs stimulation.[150,158] On the basis of these results, ara-C appears capable of acting at the same time as a modulator of PGF-R expression and a cell cycle synchronizer.

Promising preclinical results have been obtained by Baselga *et al.*[162] and Zan *et al.*[163] which have been used blocking anti-EGF-R mAbs in combination with chemotherapic agents. In fact, anti-EGF-R 528 and 225 mAbs increase the antitumor effects of doxorubicin by 32–42% on human cell lines MDA-MB468 and A431. The same treatment determines 40–100% eradication of A431 and MDA-MB468 xenografts in athymic mice, whereas doxorubicin or anti-EGF-R mAbs alone temporarily inhibit growth.[162] Similar results are determined by combination of anti-EGF-R mAbs and cisplatin.[163] At the present clinical trials with anti-EGF-R mAbs are being conducted and trials with anti-EGF-R mAbs combined with cytotoxic drugs are planned.[162]

Conclusions

Pharmacological modulation of PGF-R represents an important tool for the optimization of targeted therapy of human cancer. While targeting strategies still hold a great potential, their therapeutic results are still far from satisfactory, although almost a century has past since Ehrlich's dream formulation of a 'magic bullet'.[164] Regulation of PGF-R on tumor cells by pharmacologic means may be regarded as a further step towards exploiting the therapeutic potential of neoplastic cell targeting in a clinical setting.

References

1. Aaronson SA. Growth factors and cancer. *Science* 1991; **254**: 1143–53.
2. Bishop JM. Molecular themes in oncogenesis. *Cell* 1991; **64**: 235–48.
3. Cross M, Dexter, TM. Growth factors in development, transformation, and tumorigenesis. *Cell* 1991; **64**: 271–80.
4. Stoscheck CM, King LE. Role of epidermal growth factor in carcinogenesis. *Cancer Res* 1986; **46**: 1030–7.
5. De Larco JE, Todaro GJ. Growth factor from murine sarcoma virus-transformed cells. *Proc Natl Acad Sci USA* 1978; **75**: 4001–5.
6. Sporn MB, Todaro GJ. Autocrine secretion and malignant transformation of cells. *N Engl J Med* 1980; **308**: 878–80.
7. Sporn MB, Todaro GJ. Autocrine growth factors and cancer *Nature.* 1985; **313**: 747–51.
8. Goustin AS, Leof EB, Shipley GD, *et al.* Growth factors and cancer. *Cancer Res* 1986; **46**: 1015–29.
9. Wong ST, Winchell LF, McCune BK, *et al.* The TGF-α precursor expressed on the cell surface binds to the EGF receptor on adjacent cells, leading to signal transduction. *Cell* 1989; **56**: 495–506.
10. Anklesaria P, Teixido J, Laiho M, *et al.* Cell–cell adhesion mediated by binding of membrane anchored transforming growth factor-α to epidermal growth factor receptors promotes cell proliferation. *Proc Natl Acad Sci USA* 1990; **87**: 3289–93.
11. Spinger TA. Adhesion receptors of the immune system. *Nature* 1990; **346**: 425–33.
12. Long MW. Blood cell cytoadhesion molecules. *Exp Hematol* 1992; **20**: 288–301.
13. Keating MT, Williams LT. Autocrine stimulation of intracellular PDGF receptors in v-SIS-transformed cells. *Science* 1988; **239**: 914–6.

14. Yamamoto T, Kamata N, Kawano H, *et al.* High incidence of amplification of epidermal growth factor receptor gene in human squamous carcinoma cell lines. *Cancer Res* 1986; **46**: 414–6.
15. Gullick WJ, Marsden JJ, Whittle N, *et al.* Expression of epidermal growth factor receptors on human cervical, ovarian and vulval carcinomas. *Cancer Res* 1986; **46**: 285–92.
16. Jones NR, Rossi ML, Gregoriou M, *et al.* Epidermal growth factor receptor expression in 72 meningiomas. *Cancer* 1990; **66**: 152–5.
17. Gatter KC, Brown G, Trowbridge IS, *et al.* Transferrin receptors in human tissues: their distribution and possible clinical relevance. *J Clin Pathol* 1983; **36**: 539–45.
18. Faulk WP, Hsi BL, Stevens PJ. Transferrin and trasferrin receptors in carcinoma of the breast. *Lancet* 1980; **23**: 390–2.
19. Libermann TA, Nusbaum HR, Razon N, *et al.* Amplification, enhanced expression and possible rearrangement of EGF receptor gene in primary human brain tumors of glial origin. *Nature* 1985; **313**: 144–7.
20. Toi M, Nakamura T, Mukaida H, Wada T, *et al.* Relationship between epidermal growth factor status and various prognostic factors in human breast cancer. *Cancer* 1990; **65**: 1980–4.
21. Neal DE, Sharples L, Smith K, *et al.* The epidermal growth factor receptor and the prognosis of bladder cancer. *Cancer* 1990; **65**: 1619–25.
22. Yano H, Shiozaki H, Kobayashi K, *et al.* Immunohistologic detection of the epidermal growth factor receptor in human esophageal squamous cell carcinoma. *Cancer* 1991; **67**: 91–8.
23. Waldmann TA. The interleukin-2 receptor on malignat cell: a target for diagnosis and therapy. *Cell Immunol* 1986; **99**: 53–60.
24. Waldmann TA. The multichain interleukin 2 receptor: a target for immunotherapy in lymphoma, autoimmune disorders, and organ allografts. *J Am Med Ass* 1990; **263**: 272–4.
25. Mendelsohn J. Growth factor receptors as targets for antitumor therapy with monoclonal antibodies. *Prog Allergy* 1988; **45**: 147–60.
26. Trowbridge IS. Transferrin receptor as a potential therapeutic target. *Prog Allergy* 1988; **45**: 128–46.
27. Biddle WC, Foon KA. Monoclonal antibodies in therapy, alone or conjugated with drugs. In: Mitchell MS, ed. *Biological approaches to cancer treatment*. New York: McGraw-Hill 1993: 209–51.
28. Fanger MW, Shen L, Graziano RF, *et al.* Cytotoxicity mediated by human Fc receptors for IgG. *Immunol Today* 1989; **10**: 92–9.
29. Schlessinger J. Allosteric regulation of epidermal growth factor receptor kinase. *J Cell Biol* 1986; **103**: 2067–72.
30. Schneider C, Sutherland R, Newmann R, *et al.* Structural features of the cell surface receptor for transferrin that is recognized by the monoclonal antibody OKT9. *J Biol Chem* 1982; **257**: 8516–20.
31. Kohler G, Milstein C. Continous cultures of fused cells secreting antibodies of predefined specificity. *Nature* 1975; **256**: 495–8.
32. Masui H, Kawamoto T, Sato J-D, *et al.* Growth inhibition of human tumor cells in athymic mice by anti-epidermal growth factor receptor monoclonal antibodies. *Cancer Res* 1984; **44**: 1002–7.
33. Sato J-D, Kawamoto T, Le AD, *et al.* Biological effects *in vitro* of monoclonal antibodies to human epidermal growth factor receptors. *Mol Biol Med* 1983; **1**: 511–29.
34. Taetle R, Honeysett JM. Effects of monoclonal antibodies on *in vitro* growth of human solid tumor cells. *Cancer Res* 1987; **47**: 2040–4.
35. Sauvage CA, Mendelsohn JF Trowbridge IS. Effects of monoclonal antibodies that block transferrin receptor function on the *in vivo* growth of a syngeneic murine leukemia. *Cancer Res* 1987; **47**: 747–53.
36. Trowbridge IS. Therapeutic potential of monoclonal antibodies that block biological function. In Boss BD, Langmann R, Trowbridge I, *et al.*, eds. *Monoclonal antibodies and Cancer*. New York: Academic Press 1983: 53–61.
37. Waldman TA, Goldman CK, Bongiovanni K F. Therapy of patients with human T-cell lymphotrophic virus-I-induced adult T-cell leukemia with anti-Tac, a monoclonal antibody to the receptor for interleukin-2. *Blood* 1988; **72**: 2005–10.
38. Lesley J, Schulte R, Woods J. Modulation of transferrin receptor expression and function by anti-transferrin receptor antibodies and antibody fragments. *Exp Cell Res* 1989; **202**: 215–33.
39. Taetle R, Castagnola J, Mendelsohn J. Mechanisms of growth inhibition by anti-transferrin receptor monoclonal antibodies. *Cancer Res* 1986; **46**: 1759–63.
40. Foon AK. Biological response modifiers: the new immunotherapy. *Cancer Res* 1989; **49**: 1621–39.
41. Dillman RO, Shawler DL, Johnson DE, *et al.* Preclinical trials with combinations and conjugates of T101 monoclonal antibody and doxorubicin. *Cancer Res* 1986; **46**: 4886–91.
42. Matsui M, Nakanishi T, Nogucki T, *et al.* Synergistic in *in vitro* and *in vivo* antitumor effect of daunomycin-anti-96kDa melanoma associated antigen monoclonal antibody CL207 conjugate and recombinant IFN-γ. *J Immunol* 1988; **141**: 1410–7.
43. Senter PD, Saulnier MG, Schreiber GJ, *et al.* Anti-tumor effects of antibody-alkaline phosphatase conjugates in combination with etoposide phosphatase. *Proc Natl Acad Sci USA* 1988; **85**: 4842–6.
44. Senter PD, Schreiber GJ, Hirschberg DL, *et al.* Enhancement of the *in vitro* and *in vivo* antitumor activities of phosphorylated mitomycin C and etoposide derivatives by monoclonal antibody-alkaline phosphatase conjugates. *Cancer Res* 1989; **49**: 5789–95.
45. Baldwin RW, Byers VS. Immunotoxins in cancer treatment. In: Mitchell MS, ed. *Biological approaches to cancer treatment*. New York: McGraw-Hill 1993: 252–76.
46. Yamaizumi M, Mekada E, Uchida T, *et al.* One molecule of diphtheria toxin fragment A introduced into a cell can kill the cell. *Cell* 1978; **15**: 245–50.
47. Pastan I, Fitzgerald DJ. Recombinant toxins for cancer treatment. *Science* 1991; **254**: 1173–77.
48. Pastan I, Chaudhary V, Fitzgerald DJ. Recombinant toxins as novel therapeutic agents. *Annu Rev Biochem* 1992; **61**: 331–54.
49. Kondo T, Fitzgerald D, Chaudhary VK. Activity of immunotoxins constructed with modified Pseudomonas

exotoxin A lacking the cell recognition domain. *J Biol Chem* 1988; **80:** 937–42.
50. Byers VS, Pawluczyk IZA, Hooi DS, *et al.* Endocytosis of immunotoxin-791T/3-RTA by tumor cells in relation to its cytotoxic action. *Cancer Res* 1991; **51:** 1990–5.
51. Mukhopadhyav T, Tainsky M, Cavender A-C, *et al.* Specific inhibition of K-ras expression and tumorigenicity of lung cancer cells by anti-sense RNA. *Cancer Res* 1991; **51:** 1744–8.
52. Moroni MC, Willingham MC, Beguinot L. EGF-R antisense RNA blocks expression of the epidermal growth factor receptor and suppresses the transforming phenotype of a human carcinoma cell line. *J Biol Chem* 1992; **267:** 2714–22.
53. Waldmann TA. The multichain interleukin receptor. *Annu Rev Biochem* 1989; **58:** 875–911.
54. Fitzgerald DJP, Waldmann TA, Willingham MC, Pastan I. Pseudomonas exotoxin-anti-Tac: cell specific immunotoxin active against cells expressing the human T-cell growth factor receptor. *J Clin Invest* 1984; **74:** 966–71.
55. Hwang J, FitzGerald DJP, Adya S, *et al.* Functional domains of Pseudomonas exotoxin identified by delection analysis of the gene expressed in *E. coli*. *Cell* 1987; **48:** 129–36.
56. Kreitman RJ, Chaudhary VK, Waldmann T, *et al.* The recombinant immunotoxin anti-Tac(Fv)–Pseudomonas exotoxin 40 is cytotoxic toward peripheral blood malignant cells from patients with adult T-cell leukemia. *Proc Natl Acad Sci USA* 1990; **87:** 8291–95.
57. Seetharam S, Chaudhary VK, Fitzgerald D, *et al.* Increased cytotoxic activity of Pseudomonas exotoxin and two chimeric toxins ending in KDEL. *J Biol Chem* 1991; **266:** 17376–81.
58. Munro S, Pelham HR. A C-terminal signal prevents secretion of luminal ER proteins. *Cell* 1987; **48:** 899–907.
59. Kreitman RJ, Chaudhary VK, Kozak RW, *et al.* Recombinant toxins containing the variable domains of the anti-Tac monoclonal antibody to the interleukin-2 receptor kill malignant cells from patients with chronic lymphocitic leukemia. *Blood* 1992; **9:** 2344–52.
60. Tsudo M, Kozak RW, Goldman CK, Waldmann TA. Demonstration of a new (non-Tac) peptide that binds interleukin-2: a potential participant in a multichain inteleukin-2-receptor complex. *Proc Natl Acad Sci USA* 1986; **83:** 9694–8.
61. Tsudo M, Karasuyama H, Kitamura F. The IL-2 receptor β-chain (p70). Ligand binding ability of the cDNA-encoding membrane and secreted forms. *J Immunol* 1990; **145:** 599–606.
62. Rosolen A, Nakanishi M, Poplack DG, *et al.* Expression of interleukin-2 receptor β subunit in hematopoietic malignancies. *Blood* 1989; **73:** 1968–72.
63. Lorberboum-Galski H, Kozak R, Waldmann T, *et al.* IL-2-PE40 is cytotoxic to cells displaying either the p55 or p75 subunit of the IL-2 receptor. *J Biol Chem* 1988; **263:** 20650–6.
64. Williams DP, Wen Z, Watson RS, *et al.* Cellular processing of the interleukin-2 fusion toxin DAB_{486}IL-2 and efficient delivery of diphteria fragment A to the cytosol of target cells requires Arg194. *J Biol Chem* 1990; **265:** 20673–7.
65. Foss F, Borkowski T, Tompkins A, *et al.* Pilot phase II study of a DAB_{486}IL-2 fusion toxin in cutaneous T-cell lymphoma. *Proc Am Ass Cancer Res* 1993; **34:** 1236.
66. Le Maistre CF, Rosemblum MG, Reuben JM, *et al.* Therapeutic effects of genetically engineered toxin (DAB_{486}IL-2) in patient with chronic lymphocitic leukemia. *Lancet* 1991; **337:** 1124–5.
67. Bacha P, Parker K, Nylen P, *et al.* DAB_{389}IL-2 fusion toxin evaluation in lymphoma clinical trials. *Proc Am Ass Cancer Res* 1993; **34:** 1237.
68. Hesketh P, Caguioa P, Koh H, *et al.* Clinical activity of a cytotoxic fusion protein in the treatment of cutaneous T-cell lymphoma. *J Clin Oncol* 1993; **11:** 1682–90.
69. Kozak RW, Acther RW, Gansow OA. Bismuth-212-labeled anti-Tac monoclonal antibody: α-particle-emitting radionuclide as modalities for radioimmuno-therapy. *Proc Natl Acad Sci USA* 1986; **83:** 474–8.
70. Kozak RW, Raubitschek A, Mirzadeh S. Nature of the bifunctional chelating agent used in radioimmunotherapy with yttrium-90 monoclonal antibodies: critical factors in determining *in vivo* survival and organ toxicity. *Cancer Res* 1988; **49:** 2639–44.
71. Divgi CR, Welt S, Kris M, *et al.* Phase I and imaging trial of indium 111-labeled anti-epidermal growth factor receptor monoclonal antibody 225 in patients with squamous cell lung carcinoma. *J Natl Cancer Inst* 1988; **83:** 97–106.
72. Shin DM, Perez-soler R, Donato NJ, *et al.* Tumor epidermal growth factor receptor (EGF-R) saturation and proliferating cell nuclear antigen (PCNA) expression in patients treated with anti-EGF-R monoclonal antibody (MoAb) RG83852 (in phase I clinical study). *Proc Am Ass Clin Oncol* 1993; **12:** 940.
73. Fan Z, Masui H, Baselga J, *et al.* Antitumor effect of anti EGF receptor monoclonal antibodies is enhanced by combination treatment with cisplatinum. *Proc Am Ass Cancer Res* 1993; **34:** 2037.
74. Durkop H, Latza U, Hummel M, *et al.* Molecular cloning and expression of a new member of the nerve growth factor receptor family that is characteristic of Hodgkin's disease. *Cell* 1992; **68:** 421–7.
75. Clark EA. CD40 a cytokine receptor in search of a ligand. *Tissue Antigens* 1990; **35:** 33–6.
76. Smith CA, Davis T, Anderson D, *et al.* A receptor for tumor necrosis factor defines an unusual family of cellular and viral proteins. *Science* 1990; **248:** 1019–23.
77. Smith CA, Davis T, Wignall J, *et al.* T2 open reading frame from the shope fibroma virus encodes a soluble form of the TNF receptor. *Biochem Biophys Res Commun* 1991; **176:** 335–42.
78. Farrah T, Smith C. An emerging cytokine family. *Nature* 1992; **358:** 26.
79. Stein H, Mason DY, Gerdes J, *et al.* The expression of the Hodgkin's disease associated antigen Ki-1 in reactive and neoplastic lymphoid tissue: evidence that Reed–Sternberg cells and histiocytic malignancies are derived from activated lymphoid cells. *Blood* 1985; **66:** 848–58.
80. Chittal SM, Caverivière P, Schwartzing R. Monoclonbal antibodies in the diagnosis of Hodgkin's disease: the search of a rationale panel. *Am J Surg Pathol* 1988; **12:** 9–21.
81. Carbone A, Gloghini A, Volpe R. Paraffin section immunohistochemistry in the diagnosis of Hodgkin's

disease and anaplastic large cell ($CD30^+$) lymphomas. *Virchows Archiv Pathol Anat* 1992; **420**: 527–32.
82. Smith CA, Gruss HJ, Davis T, *et al.* CD30 antigen, a marker for Hodgkin's lymphoma, is a receptor whose ligand defines an emerging family of cytokines with homology to TNF. *Cell* 1993; **73**: 1349–60.
83. Tazzari PL, Bolognesi A, De Todero D, *et al.* Ber-H2 (anti-CD30)-saporin immunotoxin: a new tool for the treatment of Hodgkin's disease and $CD30^+$. *In vitro* evaluation. *Br J Haematol* 1992; **81**: 203–11.
84. Engert A, Burrows F, Jung W, *et al.* Evaluation of ricin A chain-containing immunotoxins directed against the CD30 antigen as potential reagents for the treatment of Hodgkin's disease. *Cancer Res* 1990; **50**: 84–8.
85. Engert A, Pohl C, Diehl V. Experimental therapy in Hodgkin's disease. *Ann Oncol* 1992; **3** (Suppl 4): 97–100.
86. Falini B, Flenghi L, Fedeli L, *et al. In vivo* targeting of Hodgkin and Reed–Sternberg cells of Hodgkin's disease with monoclonal antibody Ber-H2 (CD30): immunohistological evidence. *Br J Haematol* 1992; **82**: 38–45.
87. Falini B, Flenghi L, Pasqualucci L, *et al.* Therapy of refractory Hodgkin's disease with anti-Cd30/saporin immunotoxin. *Tissue Antigens* 1993; **42**: 243 (Abs).
88. Sahin U, Hartman FW, Senter P, *et al.* Specific activation of the prodrug mitomycin-C phosphate on Hodgkin's derived cells by a bispecific anti CD30-anti alkaline phosphatase monoclonal antibody. *Cancer Res* 1990; **50**: 6944–8.
89. Pohl C, Denfeld R, Renner C, *et al.* CD30-antigen-specific targeting and activation of T cells via murine bispecific monoclonal antibodies against CD30 and CD28: potential use for the treatment of Hodgkin's lymphoma. *Int J Cancer* 1993; **54**: 820–7.
90. Hombach A, Jung W, Pohl C, *et al.* A CD16/CD30 bispecific monoclonal antibody induces lysis of Hodgkin cells by unstimulated natural killer cells. *Int J Cancer*, in press.
91. Pasqualucci I, Wasik M, Teicher B, *et al. In vivo* antitumor activity of BER-H2 (CD30)/saporin immunotoxin against human Ki-1 anaplastic large cell lymphoma in SCID mice. *Blood* 1993; **82** (Suppl 1): 137a (524).
92. Gruss HJ, Da Silva N, Hu ZB, *et al.* Expression and modulation of CD30-ligand and CD30 in 102 human lymphoma/leukemia cell lines. *Blood* 1993; **82** (Suppl 1): 428a (1913).
93. Paulie S, Rosen A, Ehlin-Henriksson B, Braesch-Anderson S, *et al.* The human B lymphocyte and carcinoma antigen. CDw40, is a phosphoprotein involved in growth signal transduction. *J Immunol* 1989; **142**: 590–5.
94. Banchereau J, Rousset F. Human B lymphocytes: phenotype, proliferation, and differentiation. *Adv Immunol* 1992; **52**: 125–261.
95. Stamenkovic I, Clark EA, Seed B. A B-lymphocyte activation molecule related to the nerve growth factor receptor and induced by cytokines in carcinomas. *EMBO J* 1989; **8**: 1403–10.
96. Lederman S, Yellin MJ, Covey LR, *et al.* Non-antigen signals for B-cell growth and differentiation to antibody secretion. *Curr Opinion Immunol* 1993; **5**: 439–44.
97. Armitage RJ, Fanslow WC, Strockbine L, *et al.* Molecular and biological characterization of a murine ligand for CD40. *Nature* 1992; **357**: 80–2.
98. Hollembaugh D, Grosmarie LS, Kullas CD, *et al.* The human T cell antigen gp39, a member of the TNF gene family, is a ligand for the CD40 receptor: expression of a soluble form of gp39 with B cell co-stimulatory activity. *EMBO J* 1992; **11**: 4313–21.
99. Banchereau J, de Paoli P, Valle A, *et al.* Long term human B cell lines dependent on interleukin-4 and antibody to CD40. *Science* 1991; **251**: 70–2.
100. Spriggs MK, Armitage RJ, Strockbine L, *et al.* Recombinant human CD40 ligand stimulates B cell proliferation and immunoglobulin E secretion. *J Exp Med* 1992; **176**: 1543–50.
101. Liu YJ, Mason DY, Johnson GD, *et al.* Germinal center cells express Bcl-2 protein after activation by signals which prevent the entry into apoptosis. *Eur J Immunol* 1991; **21**: 1905–10.
102. Lederman S, Yellin MJ, Inghirami G, *et al.* Molecular interactions mediating T-B lymphocytes collaboration in human lymphoid follicles: roles of T-B activating molecule (5c8 antigen) and CD40 in contact dependent help. *J Immunol* 1992; **149**: 3817–26.
103. Law CL, Wormann B, Le Bien TW. Analysis of expression and function of CD40 on normal and leukemic human B cell precursors. *Leukemia* 1990; **4**: 732–8.
104. Uckun FM, Gajl-Peczalska K, Myers DE, *et al.* Temporal association of CD40 antigen expression with discrete stages of human B-cell ontogeny and the efficacy of anti-CD40 immunotoxins against clonogenic B lineage acute lymphoblastic leukemia as well as B-lineage non Hodgkin's lymphoma cells. *Blood* 1990; **76**: 2449–56.
105. Saeland S, Duvert V, Caux C, *et al.* Distribution of surface membrane molecules on bone marrow and cord blood $CD34^+$ hematopoietic cells. *Exp Hematol* 1992; **20**: 24–33.
106. Rodeck U, Herlyn M, Herlyn D, *et al.* Tumor growth modulation by a monoclonal antibody to the epidermal growth factor receptor: immunologically mediated and effector cell-independent effects. *Cancer Res* 1983; **47**: 3692–6.
107. Mueller BM, Romerdahl CA, Trent JM, *et al.* Suppression of spontaneous melanoma metastasis in SCID mice with an antibody to the epidermal growth factor receptor. *Cancer Res* 1991; **51**: 2193–8.
108. Kawamoto T, Sato TD, Le AD, *et al.* Growth stimulation of A431 cells by epidermal growth factor: identification of high-affinity receptors for epidermal growth factor by an anti-receptor monoclonal antibody. *Proc Natl Acad Sci USA* 1983; **80**: 1337–41.
109. Aboud-Pirak AE, Hurwitz E, Pirak ME, *et al.* Efficacy of antibodies to epidermal growth factor receptor against KB carcinoma in vitro and in nude mice. *J Natl Cancer Inst* 1988; **80**: 1605–11.
110. Aboud-Pirak E, Hurwitz E, Bellot F. Inhibition of human tumor growth in nude mice by a conjugate of doxorubicin with monoclonal antibodies to epidermal growth factor receptor. *Proc Natl Acad Sci USA* 1989; **86**: 3778–81.
111. Mueller BM, Reisfeld RA, Mendelsohn J, *et al.* Effect of a chimeric anti-EGF receptor antibody on melanoma metastasis. *Proc Am Ass Cancer Res* 1992; **33**: 2026.

112. Pai LH, Gallo MG, Fitzgerald DJ, *et al.* Antitumor activity of a transforming growth factor α–*Pseudomonas* exotoxin fusion protein (TGF-α–PE40). *Cancer Res* 1991; **51**: 2808–12.
113. Shaw JP, Akiyoshi DE, Arrigo DA, *et al.* Cytotoxic properties of $DAB_{486}EGF$ and $DAB_{389}EGF$, epidermal growth factor (EGF) receptor-targeted fusion toxins. *J Biol Chem* 1991; **266**: 21120–5.
114. Drebin J, Link V, Stern D, *et al.* Down-modulation of an oncogene protein product and reversion of the transformed phenotype by monoclonal antibodies. *Cell* 1985; **41**: 695–706.
115. Hancock MC, Langton BC, Chan T, *et al.* A monoclonal antibody against the c-*erbB-2* protein enhances the cytotoxicity of *cis*-diamminedichloroplatinum against human breast and ovarian tumor cell lines. *Cancer Res* 1991; **51**: 4575–80.
116. Trowbridge IS, Lesley JF, Schulte R. Murine cell surface transferrin receptor: studies with an anti-receptor monoclonal antibody. *J Cell Physiol* 1982; **112**: 403–10.
117. Lesley JF, Schulte RJ. Selection of cell lines resistant to anti-transferrin receptor antibody: evidence for a mutation in transferrin receptor. *Mol Cell Biol* 1984; **4**: 1675–81.
118. Trowbridge IS, Lopez F. Monoclonal antibody to transferrin receptor blocks transferrin binding and inhibits human tumor cell growth *in vitro*. *Proc Natl Acad Sci USA* 1982; **79**: 1175–9.
119. Trowbridge IS, Omary MB. Human cell surface glycoprotein related to cell proliferation is the receptor for transferrin. *Proc Natl Acad Sci USA* 1981; **78**: 3039–43.
120. White S, Taetle R, Seligman PA, *et al.* Combinations of anti-trasferrin receptor monoclonal antibodies inhibit human tumor cell growth *in vitro* and *in vivo*: evidence for synergistic antiproliferative effects. *Cancer Res* 1990; **50**: 6295–301.
121. Martell LA, Muraszko KM, Agrawal A, *et al.* Efficacy of transferrin receptor-targeted immunotoxins in brain tumors. *Proc Am Ass Cancer Res* 1993; **34**: 2851.
122. Debinski W, Fitzgerald D, Pastan I. An active monovalent immunotoxin composed of *Pseudomonas* exotoxin coupled to the Fab' fragment of an antibody to the transferrin receptor. *Proc Am Ass Cancer Res* 1992; **33**: 2049.
123. Glassy RC, Dillmann RO. Molecular biotherapy with human monoclonal antibodies. *Mol Biother* 1988; **1**: 7–13.
124. Goldenberg A, Masui H, Divgi C, *et al.* EGF receptor overexpression and localization of nude mouse xenografts using an indium-111-labeled anti-EGF receptor monoclonal antibody. *J Natl Cancer Inst* 1989; **81**: 1616–25.
125. Sung C, Shockley TR, Morrison PF, *et al.* Predicted and observed effects of antibody affinity and antigen density on monoclonal antibody uptake in solid tumors. *Cancer Res* 1992; **52**: 377–84.
126. Fujimori K, Fisher DR, Winstein JN. Integrated microscopic–macroscopic pharmacology of monoclonal antibody radioconjugates: the radiation dose distribution. *Cancer Res* 1991; **51**: 4821–7.
127. Boerman O, Massuger L, Makkink K, *et al.* Comparative *in vitro* binding characteristics and biodistribution in tumor-bearing athymic mice of anti-ovarian carcinoma monoclonal antibodies. *Anticancer Res* 1990; **10**: 1289–96.
128. Matzku S, Bruggen J, Brocker E-B, *et al.* Criteria for selecting monoclonal antibodies with respect to accumulation in melanoma tissue. *Cancer Immunol Immunother* 1987; **24**: 151–7.
129. Philben VJ, Jakowatz JG, Beatty BG, *et al.* The effect of tumor CEA content and tumor sites on tissue uptake of Indium 111-labeled anti-CEA monoclonal antibody. *Cancer* 1986; **57**: 571–6.
130. Greiner JW, Horan Hand P, Noguchi P, *et al.* Enhanced expression of surface tumor-associated antigens on human breast and colon tumor cells after recombinant human leukocyte α-interferon treatment. *Cancer Res* 1984; **44**: 3208–14.
131. Guadagni F, Schlom J, Johnston WW, *et al.* Selective interferon-induced enhancement of tumor–associated antigens on a spectrum of freshly isolated human adenocarcinoma cells. *J Natl Cancer Inst* 1989; **81**: 502–12.
132. Greiner JW, Guadagni F, Noguchi P, *et al.* Recombinant interferon enhances monoclonal antibody-targeting of carcinoma lesions *in vivo*. *Science* 1987; **235**: 895–8.
133. Rosemblum MG, Lamki LM, Murray JL, *et al.* Interferon-induced changes in pharmacokinetics and tumor uptake of ^{111}In-labelled antimelanoma antibody 96.5 in melanoma patients. *J Natl Cancer Inst* 1988; **80**: 160–5.
134. Khun JA, Wong JYC, Beatty BG, *et al.* γ-interferon enhancement of carcinoembrionic antigen expression in human colon carcinoma xenografts. *J Immunother* 1992; **11**: 257–66.
135. Budillon A, Tagliaferri P, Caraglia M, *et al.* Upregulation of epidermal growth factor receptor induced by α-interferon in human epidermoid cancer cells. *Cancer Res* 1991; **51**: 1294–9.
136. Bernstein W, Zou Z-Q, Black RJ, *et al.* Association of interferon-γ induced growth inhibition and modulation of epidermal growth factor receptor gene expression in squamous cell carcinoma cell lines. *J Biol Regul Homeost Agents* 1988; **2**: 206–12.
137. Chang EH, Ridge J, Black R, *et al.* Interferon-γ induces altered oncogene expression and terminal differentiation in A431 cells. *Proc Soc Exp Biol Med* 1987; **206**: 319–26.
138. Hamburger AW, Pinnamaneni GD. Increased epidermal growth factor receptor gene expression in a human breast carcinoma cell line. *Br J Cancer* 1991; **64**: 64–8.
139. Zoon KC, Karasaki Y, zur Nedden DL, *et al.* Modulation of epidermal growth factor receptors by human α-interferon. *Proc Natl Acad Sci USA* 1986; **83**: 8226–30.
140. Eisenkraft BL, Nanus DM, Albino AP, *et al.* α-interferon downregulates epidermal growth factor receptors on renal carcinoma cells: relation to cellular responsiveness to the antiproliferative action of α-interferon. *Cancer Res* 1991; **51**: 5881–7.
141. Jetten AM. Retinoids specifically enhance the number of epidermal growth factor receptors. *Nature* 1980; **284**: 626–9.
142. Komura H, Wakimoto H, Chu-Fung C, *et al.* Retinoic acid enhances cell responses to epidermal growth factor in mouse mammary gland in culture. *Endocrinology* 1986; **120**: 1530–6.
143. Zheng Z-S, Goldsmith LA. Modulation of epidermal growth factor receptors by retinoic acids in ME200 cells. *Cancer Res* 1990; **50**: 1201–5.

144. Sporn MB, Roberts AB. Peptide growth factors are multifunctional. *Nature* 1988; **332**: 217–9.
145. Roberts AB, Flanders KC, Heine UI, *et al.* Transforming growth factor β: multifunctional regulator of differentiation and development. *Phil Trans Roy Soc (London)* 1990; **327**: 145–54.
146. Baird A. Editorial: fibroblast growth factor: what's in a name? *Endocrinology* 1993; **132**: 487–8.
147. Adachi K, Belser P, Bender H, *et al.* Enhancement of epidermal growth factor receptor expression on glioma cells by recombinant tumor necrosis factor α. *Cancer Immunol Immunother* 1992; **34**: 370–6.
148. Zuckier G, Tritton TR. Adriamycin causes up regulation of epidermal growth factor receptors in actively growing cells. *Exp Cell Res* 1983; **148**: 155–61.
149. Baselga J, Miller W, Norton L, *et al.* Modulation of epidermal growth factor receptor/transforming growth factor-α (TGF-α) pathway by adriamycin. *Proc Am Ass Cancer Res* 1992; **33**: 2947.
150. Caraglia M, Tagliaferri P, Correale, *et al.* Cytosine arabinoside increases the binding of ^{125}I-labelled epidermal growth factor and ^{125}I-transferrin and enhances the *in vitro* targeting of human tumor cells with anti-(growth factor receptor) mAb. *Cancer Immunol Immunother* 1993; **37**: 150–6.
151. Caraglia M, Pinto A, Correale P, *et al.* 5-aza-2′-deoxycytidine induces growth inhibition and upregulation of epidermal growth factor receptor on human epithelial cancer cells. *Ann Oncol* 1994; **5**: 269–76.
152. Caraglia M, Correale P, Genua G, *et al.* Epidermal growth factor (EGFR) and transferrin (TRFR) receptor upregulation is induced by arabinoside cytosine (ARA-C) on human tumor cells. *Cancer Detect Prevent* 1993; **17**: 337–8.
153. Liotta LA, Stetler-Stevenson WG. Principles of molecular cell biology: Cancer metastasis. In: De Vita TV jr, Hellman S, Rosenberg SA, eds. *Cancer: principles and practice of oncology*. Philadelphia: JB Lippincott 1993: 134–49.
154. Kelley SL, Basu A, Teicher BA, *et al.* Overexpression of metallothionein confers resistance to anticancer drugs. *Science* 1988; **24**: 2013–5.
155. Levinson W, Oppermann H, Jackson J. Transition series metals and sulfhydryl reagents induce the synthesis of four proteins in eukaryotic cells. *Biochim Biophys Acta* 1980; **606**: 170–80.
156. Warr JR, Atkinson GF. Genetic aspects of resistance to anticancer drugs. *Physiol Rev* 1988; **68**: 1–25.
157. Slovak ML, Hoeltge GA, Dalton WS, *et al.* Pharmacological and biological evidence for differing mechanism of doxorubicin resistance in two human tumor cell lines. *Cancer Res* 1988; **48**: 2793–7.
158. Perkins AS, Vande Woude GF. Principles of molecular cell biology: Oncogenes. In: De Vita TV Jr, Hellman S, Rosenberg SA, eds. *Cancer: principles and practice of oncology*. Philadelphia: JB Lippincott 1993: 35–59.
159. Mitchell MS. Preface. In: Mitchell MS, ed. *Biological approaches to cancer treatment*. New York: McGraw-Hill 1993: xii.
160. Khazaeli M, Lo Buglio AF, Wheeler R, *et al.* The effects of immunosuppressive regimens on human immune response to murine monoclonal anti-melanoma antibody-ricin A chain. *Proc Am Ass Clin Oncol* 1988; **29**: 420.
161. Heppner GH, Miller BE. Therapeutic implications of tumor heterogeneity. *Sem Oncol* 1989; **16**: 9–15.
162. Baselga J, Norton L, Masui H, *et al.* Antitumor effects of doxorubicin in combination with anti-epidermal growth factor receptor monoclonal antibodies. *J Natl Cancer Inst* 1993; **85**: 1327–33.
163. Fan Z, Baselga J, Masui H, *et al.* Antitumor effect of anti-epidermal growth factor receptor monoclonal antibodies plus *cis*-diamminedichloroplatinum on well established A431 cell xenografts. *Cancer Res* 1993; **53**: 4637–42.
164. Erlich P. Uber den jetzigen stand der karzinomforschung. In: Himmelweit F, ed. *The collected papers of Paul Erlich*. Oxford: Peragamon Press 1957: 550.

(Received 3 February 1994; received in revised form 11 April 1994; accepted 12 May 1994)