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FROM THE DEPARTMENT OF CLINICAL ONCOLOGY, ROYAL POSTGRADUATE MEDICAL SCHOOL, HAMMERSMITH HOSPITAL, LONDON, ENGLAND.

NEW THERAPEUTIC MODALITIES FOR CANCER

H. THOMAS and K. SIKORA

Abstract

A review is given on new biological approaches to cancer therapy based on knowledge concerning interferons, interleukins, LAK-cells, tumour-infiltrating lymphocytes, tumour necrosis factor, colony-stimulating factors, monoclonal antibodies and oncogenes. There are many potential permutations for the application of biological therapy for cancer. One of the most important developments has been the increased understanding of the molecular mechanisms of malignancy through which biological manipulation can be tailored to an individual tumour. Although current clinical studies are not demonstrating high response rates they may well be analogous to the advances seen with chemotherapy in its early days. As yet only relatively small numbers of patients have had access to, or been suitable for, treatment. With further refinements in production, administration, and an increase in the specificity of treatment, the possibility of curing metastatic solid tumours may become a reality.

Key words: Cancer, biological therapy, interferons, interleukins, LAK-cells, tumour-infiltrating lymphocytes, tumour necrosis factor, monoclonal antibodies, oncogenes, review.

A potential breakthrough of the 1980s appears to have been the biological approach to cancer therapy. Frustration with the incurable nature of the vast majority of solid tumours, coupled with the commercial pressure on biotechnology companies to produce effective compounds, has resulted in a dramatic increase in the development of 'biologicals'. The exponential expansion in recombinant DNA technology has facilitated large quantities of highly purified biological substances for use in clinical trials. Obtaining the correct balance between toxicity and efficacy has been a significant problem. Cytokines have so far largely been used in patients with widespread metastatic disease in whom other therapies have failed. The tumours in which interleukin-2 has been most successful are renal cell carcinoma and malignant melanoma, both of which are relatively unresponsive to conventional systemic treatments. However, a small proportion of patients do undergo spontaneous remission. An immunological basis for such remissions seems highly likely. We are now beginning to understand the complex 'biological network' and are in a position to combine therapies at immunologically active doses, which may not be pharmacokinetically apparent. Biological agents have effects which have so far been poorly understood. The first group of agents to be used clinically was the interferons. These illustrate the spectrum of diseases, effects and responses and serve as a paradigm for the entire range of biological compounds.

Interferons

The interferons are a family of secreted proteins with similar biological properties, first described by Isaacs and Lindemann in 1957. Their first known property was an ability to induce an antiviral state. More recently they have been found to have potent immunomodulatory and antiproliferative actions. Interferons are classified according to their antigenic type and primary cell of origin—being produced by leukocytes (alpha), fibroblasts (beta) and T-lymphocytes (gamma). Type I (alpha and beta) are produced in response to viral infection, and Type II (gamma) in response to T-lymphocyte antigen stimulation or mitogens (1).

Many studies suggest that interferons play a major role in host defense including increased natural-killer cell activity, macrophage activation and increased expression of MHC class I and II antigens, all of which are likely to facilitate the recognition and lysis of susceptible target cells. The extraction and purification of the various types of interferon from cellular sources was hampered by the fact that IFNs from different sources are not always of

Table 1

Properties of the interferons

alpha	beta	gamma
Leukocyte lymphoblastoid	Fibroblast	Immune
Viral induced	Viral induced	Mitogen / Antigen
Type I	Type I	Type II
pH2 stable	pH2 stable	pH2 unstable
Not glycosylated	Glycosylated	Glycosylated
>20 genes	1 gene	1 gene
No introns	No introns	Introns
Chromosome 9	Chromosome 9	Chromosome 12

one type. Although polyclonal and monoclonal antibodies were raised against specific IFN there is often cross-reaction. IFN cDNA was first cloned in 1980 (2). Most of the work has been performed with alpha IFN whose family of genes is found to be closely linked and arranged in tandem on chromosome 9 (3). Human IFN beta has been cloned and the gene isolated (Table 1).

Mechanism of action

These are very complex and multiple sites of action are likely to be involved. The antiviral state involves temporary inhibition of RNA and protein synthesis and this is mediated by the binding of cell surface receptors. Alpha and beta IFN are thought to occupy the same site and gamma IFN a separate one. IFN is internalised and a rise in cyclic GMP follows with a subsequent rise in cyclic AMP. IN association with these changes the activity of several different enzymes may rise or fall (4). The precise molecular cascade resulting in the physiological effects has not yet been determined.

Mechanism behind antitumour effects

The inhibition of viral replication is achieved by much lower doses than affect the immune system (5). In some systems both tumour necrosis factor (TNF) and interleukin-1 (IL-1) can exhibit antiviral effects. Not only does this illustrate the interplay between these molecules but it suggests overlapping physiological functions. It is believed that the antitumour action of IFN alpha is mediated by the direct inhibition of tumour cell growth or host tumour cell interaction and not the host immune system. One explanation of the efficacy of IFN in hairy-cell leukaemia is defective IFN production whereby exogenous IFN alpha corrects an endogenous defect. IFNs have diverse effects on the immune system and act as communication molecules between cells involved in the immune response (6). The timing of administration is important, which is exemplified by the finding that its presence before antigen administration may reduce antibody production and afterwards it may enhance the same response.

Interferons and cellular proliferation

There is evidence of the relevance of IFNs to cell proliferation in that IFNs act as negative growth factors. The role of cellular proto-oncogenes in regulating the ability of cells to proliferate and the expression of transformed or malignant phenotypes has been investigated. The expression of c-myc RNA in Daudi (Burkitt's lymphoma) cells has shown that the level of transcript is diminished within 24 h of exposure to human beta IFN. This effect is accompanied by the inhibition of cell proliferation. It appears that the decrease in c-myc mRNA concentration is due to decreased processing or stability and not any change in the rate of transcription. The drop in concentration is also seen too rapidly to be explained by the accumulation of Daudi cells in the G0/G1 phase of the cycle. Further investigation has emphasised the multifactorial nature of cell growth regulation and malignant transformation, and it seems unlikely that changes in c-myc expression are the sole cause of the inhibition of cellular growth by IFNs (7).

Toxicity

It was initially thought that many of the side-effects apparently caused by interferon were due to impurities, but they were still seen after the injection of the highly purified recombinant compound (8). The malaise and fever appear to be non-specific, similar to that produced by endotoxins. They often subside rapidly and can be prevented with aspirin and paracetamol. Changes occur in corticosteroid and circulating zinc levels which may influence the course of a patients' disease (9). In high doses there are profound effects on the nervous system whereby drowsiness and disorientation may occur. CNS dysfunction may be the major dose-limiting factor for systemic IFN at high doses such as $100-200 \times 10$ (6) U/m^2. Abnormal liver function tests and altered serum calcium levels also occur, though they do not explain the cerebral changes. There appears to be no correlation between severity of side-effects and serum levels of IFN. Many of the unwanted side-effects are produced in healthy volunteers when injections of purified IFN result in serum levels comparable to those seen in influenza. It seems reasonable to postulate that the mechanism is the same (10).

Clinical trials

Although our understanding of the mechanism of IFNs effects in malignancy is poor many clinical trials have been carried out over the past decade. There has been intense interest in phase I and II clinical evaluation of a spectrum

of malignancies including lymphomas, solid tumours and haematological malignancies. It has important activity in hairy-cell leukaemia, low grade non-Hodgkin's lymphoma, cutaneous T-cell lymphoma, chronic myeloid leukaemia, untreated multiple myeloma, HIV-related Kaposi's sarcoma, superficial bladder cancer, intraperitoneally-treated ovarian carcinoma, renal cell cancer and malignant melanoma.

The interferons can induce antibody formation as part of a generalised immune response. The magnitude of this response, the frequency of antibody occurrence and the type of antibody induced relate to a number of factors. These include the malignancy, the type of IFN, dose, route, schedule and duration as well as assay type and sampling time to determine antibody titre. Different studies have shown conflicting relationships between antibody formation and disease course—it may abrogate or not influence it (11).

The first large-scale clinical trial was performed in 1971 at the Karonlinska Hospital in Stockholm. Relatively impure leukocyte IFN was used as adjuvant treatment in non-randomised patients with operable osteogenic sarcoma. In one study postoperation patients were treated daily with a dose of $3 \times 10(6)$U i.m. for one month. Over the subsequent two years they received the same dose three times a week. Fifty-four patients were compared with a group treated with chemotherapy after operation as well as with two control groups receiving surgical treatment only. There was no major difference in outcome between the groups receiving interferon and chemotherapy respectively, whereas the survival was lower in patients treated by surgery alone. The study is not randomized and therefore hard to evaluate (12). Subsequent prospective trials failed to show any improvement in survival for the IFN treated group (13).

At Stanford patients with good-prognosis histology non-Hodgkin's lymphoma were followed for one year with no other treatment. Response, measured by lymph node diameter, was assessed frequently during this time. Several dramatic regressions were seen again using partially purified leukocyte IFN (14). Extensive data with recombinant interferon confirm these responses but suggest that their duration is short (15).

IFN may reduce the rate of immunoglobulin synthesis by normal and neoplastic B lymphocytes and improvements have certainly been seen in myeloma patients after the administration of IFN (16). Recent data with recombinant preparations suggest a role for IFN in combination with alkylating agents (17).

Melanoma has shown responses to IFNs. Therapy using the conventional modalities alone has been disappointing, although the majority of patients with stage 1 disease can be cured with surgery. Response rates in excess of 10–15% have been reported with regional lymph node disease. The expected 5-year survival in such patients is 20–50%. Patients with metastatic disease have a median survival of 4 to 6 months. Dacarbazine (DTIC) is the most widely used chemotherapeutic agent with response rates, largely in soft tissue disease, of 15%, lasting from 3 to 6 months. With triple-drug combinations these responses have increased to 25–30% and a possible duration of survival about 3 months longer. Nevertheless the activity of biologic agents is not compromised by previous chemotherapy and they have provided useful second-line therapy (18). Responses were first seen with natural human leukocyte IFN and this lead to further work using other species of IFN. The experience with beta and gamma IFN is more limited but nevertheless objective response rates of 10–15% occur. Dose reduction reduces toxicity without affecting efficacy and IFNs in conjunction with chemotherapeutic agents may further augment responses. (19).

In 1978, the American Cancer Society initiated a multicentre trial in which 45 patients at UCLA, Memorial, and Yale received 1, 3 or 9 U daily (i.m.) for 42 days. There was one partial response, 2 minimal responses and no complete responses. Few responses have been seen with intralesional injection of IFN in melanoma (20).

The most promising clinical results have been seen in hairy-cell leukaemia. In 1983, Quesada et al. reported the successful treatment of 10 out of 11 patients with alpha IFN. The results compared favourably with chemotherapy without the toxicity. It is still too early to be sure whether survival has also been improved (21, 22).

There is no curative therapy for metastatic or recurrent renal cell carcinoma—conventional therapy attains a 20% response rate, with 5% of such patients alive at 3 years. Responses to chemotherapy or hormonal therapy are usually brief and not complete, having little impact on survival. Many of the original immunological manipulations carried out were likely to induce IFN but levels were not measured.

At the MD Anderson Hospital using 3 MU daily for 30 days 5 PRs were seen and 2 minor responses from a total of 19 patients (26% and 36% overall). Responses were of median duration just over 4 months and responders had lower nadir granulocyte counts suggesting a greater biologic effect of the IFN. Subsequently the duration of treatment was increased with some studies showing a dose–response relationship (23).

Analysis of a number of published trials shows no dose–response relationship, the highest therapeutic index being seen with a daily dose or 5–10 MU. Time to response is occasionally prolonged and duration is sometimes longer than 12 months. Pulmonary metastases appear to be most responsive. Synergy with several chemotherapeutic agents, in particular vinblastine, has been suggested by some early data (24). Symptomatic patients who respond to interferon undoubtedly experience improved quality of life and prolonged survival.

Interferon is likely to be the treatment of choice in hairy-cell leukaemia and as second or third-line therapy in symptomatic follicular lymphoma. The possibility of delayed and increasing extent of response with duration of treatment must not be ignored along with the effects of combinations of biological therapy where one agent may prime a delayed response in conjunction with another. When used in this way as the 'fourth arm' of cancer therapy the usefulness of the interferons will come into its own. They may be used at pharmacological doses where they are likely to induce enzymes that result in a cytostatic effect in sensitive malignancies, or—as suggested earlier—at physiological doses where immunological and cell membrane effects, such as NK-cell stimulation and tumour-antigen expression, can be modulated. Logically this suggests that high doses may be necessary in combination with chemotherapeutic agents but with other biological agents lower doses may be more effective. Our objective in the forthcoming years must be to establish these optimal doses (25).

From the work on the expression of interferon receptors on lymphocytes (26) it appears that low-dose interferon therapy is just as effective, possibly more so, than higher doses associated with toxic side-effects. Such lower doses may be more effective in augmenting killer cell activity without dose limitation (27).

Table 2 lists results from clinical trials using recombinant interferon in different cancer types (response rates in percentage). The most promising disease is hairy-cell leukaemia although whether survival is indeed prolonged is still in dispute. Other diseases where interferon may play some part in the management include melanoma, renal cell carcinoma, chronic myeloid leukaemia and Kaposi's sarcoma in AIDS patients (28).

Table 2

Type of cancer	CR	PR	LPR	NR
IFN possibly useful				
Hairy cell leukaemia	23	35	12	30
Myeloma	3	16	14	67
Non-Hodgkin's lymphoma	6	38	10	46
Kaposi's sarcoma	18	9	14	59
Renal cell carcinoma	31	43	5	21
Chronic myeloid leukaemia	30	25	9	36
IFN probably not useful				
Lung cancer	0	3	3	94
Breast cancer	0	3	7	90
Ovarian cyst	0	15	8	77
Colon cancer	0	11	16	73
Acute myeloblastic leukaemia	0	1	12	87

CR = clinical remission; PR = partial remission; LPR = minor response and NR = no response

Interferon gamma

Interferon gamma is produced during an immune response by antigen specific T cells, and by natural killer cells recruited by IL-2. It has immunoregulatory effects including macrophage activation, growth enhancement effects on cytolytic T cells and NK cells and the induction of class II MHC antigen on macrophages and other cell types. It also regulates humoral immune responses and potentiates IL-4-induced proliferation of B cells. It does however, inhibit many of the actions of IL-4 in B cells stimulated by lipopolysaccharide which suggests that the two substances work reciprocally to regulate immunoglobulin production and T cell dependent immune responses (29). Although gamma interferon has demonstrated limited antitumour activity, with the possible exception of CML (30), nevertheless interferon gamma does appear to enhance immune responses at intermediate and low doses (31). Interferon gamma is included in Table 3 in view of its functional resemblance to the interleukins.

Interleukins

Interleukins are a family of molecules which are fundamental in generating many types of immunological response. Our knowledge of this family has increased dramatically in the last decade.

Interleukin-1 (IL-1) was first detected in 1972, a macrophage-derived cytokine that activates certain T lymphocytes. Subsequently it stimulates interleukin-2 (IL-2) production. There is much to be said about interleukin-2 in cancer therapy, although we will briefly cover the other factors termed interleukins first.

Interleukin-3 is one of several colony-stimulating factors that regulate haemopoiesis. It is produced by mitogen or antigen-activated T lymphocytes and some cell lines. It regulates aspects of growth and differentiation of haemopoietic progenitor cells, leading to the production of many of the major cell types in bone marrow, including mast cells. Like interleukin-7 it may have therapeutic implications in aplastic anaemia.

Interleukin-4 (IL-4) is a potent mediator of growth and differentiation of cells of several haemopoietic lineages, particularly B cells (32). It can induce resting B cells to increase their expression of class II major histocompatibility (MHC) molecules. It also regulates immunoglobulin isotype production and its induction may be involved in certain parasite infestations.

Interleukin-5 is a lineage-specific haematopoietic growth factor that stimulates the production of eosinophils and eosinophil colonies from normal human bone marrow cells. It has several effects on B cells including the production of IgM and IgA. A small segment of chromosome 5 has been found to contain IL-3, IL-4, IL-5 and GM-CSF. Each of these genes has been found to be deleted in the 5q- chromosome which suggests that loss of function of one or

Table 3

Some characteristics of interleukins

Interleukin	Synonyms	Source	Cell targets	Function
IL-1	Endogenous pyrogen Lymphocyte activating factor (LAF)	Many endothelial, epithelial and haemopoietic cells	Many types lymphoid, other haemopoietic and non-haemopoietic cells	Stimulation of thymocyte proliferation, accessory FG activity for T_H and B cells, lsstimulation of haemopoietic cell growth and differentiation
IL-2	T-cell growth factor (TCGF)	T cells	T cells, thymocytes, NK cells, B cells	GF for mature T cells and thymocytes, induces T lymphocyte cytotoxicity and stimulates NK cell activity
IL-3	Multi-CSF, Haemopoietic growth factor	T cells, myelomonocytic cell line	Haemopoietic cells, pre-B cell line	Regulating growth and differentiation of haemopoietic progenitor cells
IL-4	BCGF 1, BSF-1, BCDF-gamma, MFF, MAF	T cells, mast cells	B cells, T cells, mast cells, other haemopoietic cells	Wide range of activities on B cells-proliferation, class II MHC expression, immunoglobulin regulation
IL-5	TRF, BCGF-II, EDF KHF, Eo-CSF IgA-enhancing factor	T cells thymocytes	B cells eosinophils	Stimulates growth and differentiation of eosinophils in vitro, induces IgM and IgA production by B cells
IL-6	IFN-β2, hybridoma Monocytes, growth factor, BSF-2, BCDF	T cells, fibroblasts epithelial cell types	B cells, fibroblasts, hepatocytes, T cells haemopoietic progenitors	Myeloid GF activity stimulates granulocyte and macrophage colony formation, costimulator of proliferation, induces differentiation of cytotoxic T cells and acute phase protein synthesis
IL-7	Lymphopoietin-1, Pre-B cell growth factor	Stromal cells, thymus thymocytes	Pre-B cells	Involved in development of immature B lymphocytes, induces proliferation in immature and some adult thymocytes.
IL-8	Granulocyte chemotactic peptide	B cells	Granulocytes	Chemotactic for granulocytes
IFN gamma	Macrophage activating factor	T cells NK cells	Macrophages NK cells B cells	Immunoregulatory effects macrophage activation, and growth enhancement of cytolytic T cells and NK cells. Regulates humoral responses and works reciprocally with IL-4

more of these genes may play an important role in the pathogenesis of haematological disorders associated with a del (5q) (32).

Interleukin-6, also known as B cell stimulating-2 (BSF-2) of B cell differentiation factor (BCDF), was identified by its ability to induce antibody secretion by preactivated normal and Epstein Barr virus transformed human B cells without first inducing cellular proliferation (33). In vivo it acts as a stimulus for myelopoiesis and erythropoiesis and causes accompanying peripheral changes in the number of neutrophils, lymphocytes and RBCs (34). The role of IL-6 as an autocrine growth factor for human multiple myeloma raises the possibility that agonists or antibodies to it may be useful to treat the condition. Of the dozens of cytokines released during an immune response, IL-1, tumour necrosis factor alpha (TNF alpha), and IL-6 seem to be the major mediators of intermediary metabolism. These three act together to decrease food intake, increase resting energy expenditure alter metabolism dramatically including changes in circulating insulin, glucagon and corticosterone. Only IL-6 stimulates the synthesis of the full spectrum of acute phase protein synthesis in adult human hepatocytes seen in inflammatory states in humans e.g. C-reactive protein, serum amyloid A, fibrinogen, alpha-1 antitrypsin and haptoglobin are increased while albumen, transferrin and fibronectin are decreased (35, 36). It is also

a costimulator of T cell proliferation, induces differentiation of cytotoxic T cells and thymocyte proliferation.

Interleukin-7 is involved in the development of immature B lymphocytes. It also induces the proliferation of immature thymocytes and some adult thymocytes. Its importance for both B and T lymphocytes, like IL-3, gives it important therapeutic implications in aplastic anaemia (37).

Interleukin-8, which has only recently been described, is structurally related to platelet-derived β thromboglobulin and is chemotactic for granulocytes (38).

Interleukin-2 is a chemically defined lymphokine available as mixed human, partially purified lymphoblastoid or as recombinant human IL-2. Each of these has different actions, varying with the other lymphokines present at the time. It was originally described as a T cell growth factor causing proliferation of activated cells, made competent by an initial activation process to go through the G1 phase of the cell cycle. Subsequently it was shown to cause T cell activation for cytotoxicity and chemotaxis of certain subpopulations of cells, including T cells bearing the CD4 surface marker. IL-2 directly induces the release of other lymphokines, expands activated T cells, activates natural killer cells, lymphokine-activated killer (LAK) cells and cytolytic T-cells (CTL). As a result it influences the maturation of T cells from prothymocytes and immature T cells. IL-2 also induces gamma interferon and activates tumoricidal macrophages. While stable its in vivo half-life is short and its persistence is important for it to induce a response. Antitumour responses are unpredictable in both man and animals, they may be improved by concomitant use of LAK-cells, chemotherapy or other cytoreductive measures and regional or persistent administration (39–41).

Mechanisms of interleukin-2

The actions of IL-2 involve both production of several different lymphokines and the interaction of different component cells of the immune response. At best we have only a model for the events. This currently involves the interaction of an antigen with an antigen-presenting cell—usually a monocyte or a macrophage. Subsequently several factors are released including IL-1, which together with the antigen stimulates several cells including CD4+ T cells to release IL-2; the second signal in lymphocyte mitogenesis. IL-2 interacts with a receptor and causes both up-regulation of IL-2 receptors on T cells and the release of other lymphokines including gamma IFN and tumour necrosis factor (TNF).

Animal studies and phase 1 trials

Arriving at the correct dose regimens, schedules and routes of administration in humans would be time-consuming, potentially dangerous, and expensive. Much of this work has consequently been carried out in animals—particularly mice. Work in mice carrying subcutaneous tranplants of methylcholanthrene-induced sarcomas was performed to compare the tumour-inhibitory effect of highly purified rIL-2 with that of unpurified human and rat lymphoid interleukin-2. The latter was significantly more effective—an indication that other lymphokines may participate in the anti-tumour efficacy of local IL-2.

This work also demonstrated a correlation between tumour sensitivity to the effect of rIL-2 in vivo and susceptibility to the cytolytic effects of rIL-2-activated syngeneic killer spleen (LAK) cells in vitro. This is explained by LAK cells being the effector mechanism for the efficacy of local rIL-2. It may be possible to extrapolate from this to use LAK cells in vitro as a predictor of sensitivity to local rIL-2. Other routes of administration have been used in animal experiments. Intraperitoneal injection of IL-2, followed by intravenous LAK-cell administration and concurrent intraperitoneal cyclophosphamide showed that this form of adoptive immunotherapy mediates regression of micrometastases in a particular organ but is ineffective against intradermal or gross metastatic tumour nodules (42). Unfortunately a number of the soluble factors involved are species specific and many mice tumours, unlike human tumours, are immunogenic and so the relevance of many of these experiments must be questioned. Much of the work in this field has been pioneered by Steven Rosenberg at the National Cancer Institute, Bethesda, Maryland. Using animals he demonstrated that therapy with IL-2 alone or in combination with LAK-cells can decrease the size of established pulmonary and hepatic metastases from experimental tumours. As a results of these studies the use of IL-2 has been extended to patients with advanced disseminated cancer in whom conventional treatment has failed.

Interleukin-2 is the lynchpin of much of the current work involving the 'biologicals'. There has been a plethora of studies—many of which have been combined with other lymphokines. Initially IL-2 was used in high dosage, alone or with LAK cells, as a repeated bolus injection. Administered as a bolus IV it has a distribution half-life of 7–10 min and a clearance half-life of 30–60 min. Of 221 patients treated with this therapy 16 had a complete regression of all known disease and 26 had a partial regression. Treatment with LAK cells entails lymphocytes being harvested from patients by leukapheresis and converted into lymphokine-activated killer cells by incubation with recombinant IL-2. These LAK cells are then reinfused into the patient with intravenous rIL-2 (43, 44).

In patients with malignant ascites the effects on tumour-associated lymphocytes (TALs) and peripheral blood lymphocytes of intraperitoneal IL-2 have been examined. The TALs showed an increase in the proportion of cells expressing IL-2 receptors, and a sharp increase of natural

killer activity and the generation of LAK activity de novo were also observed (45).

Dose

Defining the dose regimen of IL-2 has largely entailed a process of trial and error, balancing the side-effects against an adequate dose to produce a response. One study examined the effects of low-dose rIL-2 in malignant melanoma, preceded by low-dose cyclophosphamide. Six of 24 patients who received more than one 2-week cycle had a response (1 CR and 5 PRs). Mean response duration was 5 months. The significant findings are that all six responders were found to have LAK cell activation and only two of the 24 patients required hospitalisation since toxicity was so moderate (46). The use of IL-2 combined with tumour necrosis factor (TNF) in their usual dose regimens would be both highly toxic and expensive. A study to look at the synergy of these two lymphokines in recombinant form, in mice, was carried out at low doses. Used alone they produced no useful effect but when combined there were significant anti-tumour effects in mice with methylcholanthrene-induced sarcomas. This effect was dependent on the inherent immunity of the mice and did not occur in immunosuppressed mice. The sequence of administration is again important—the prior administration of IL-2 produced more significant tumour regression than when TNF was given first (47).

In essence, the unpredictability and often delayed response to IL-2 might relate to the presence of IL-2, either alone or in conjunction with other lymphokines. The duration and continuity of therapy, and not dosage—or 'area under the curve' as with chemotherapy—may well prove all important as we learn more about the interaction of exogenous and endogenous 'biological' molecules. A better understanding of the IL-2 system and the nature of the signals transduced through it will enable us to manipulate the immune response and thereby obtain a better understanding of immune responsiveness in general.

Toxicity

The use of IL-2 in high dose as a bolus is very toxic—it affects the endothelium causing emigration of lymphoid cells from the peripheral blood and also produces a 'vascular leak syndrome' observed in rodents (48). This appears to occur in humans, as measured by albumen excretion and may explain many of the side-effects associated with altered fluid balance (49). Fluid retention is usually manifest as peripheral, cerebral and pulmonary oedema but myocardial infarctions have also been recorded—first by Rosenberg's group and since by other groups, thought to be due to vascular leakage at the level of the heart muscle (50).

The use of IL-2 in continuous infusion is better tolerated although there is some debate as to whether the response rates are as good (51). Side-effects again include fluid retention—cerebral oedema may produce confusion followed by convulsions. Fevers, nausea, vomiting, eosinophilia and anaemia are also commonly seen with both continuous infusion and bolus therapy. In Rosenbergs original work a high proportion of patients required admission to intensive care units. Subsequent trials have been possible on outpatients but the results are not as encouraging as those seen at the NCI (45). We need further understanding of the mechanisms of toxicity in order to reduce it. Experimental work to ascertain the role of immunocompetent cells in haemopoiesis has thrown some light on the haematological side-effects of IL-2 and LAK cell therapy. A colony-stimulating factor which gave rise to a high number of eosinophil colonies was detected and the presence of LAK cells lead to a decrease in the number of burst-forming units-erythroid. These two findings may explain the eosinophilia and anaemia observed in patients receiving this therapy (52).

Interleukin 2 in minimal residual disease

Most studies of the anti-neoplastic effects if interleukin-2 have been carried out in bulk disease. Logically the biological response modifiers are likely to be more effective in minimal residual disease. The treatment of acute myeloid leukaemia with chemotherapy results in a complete remission rate of around 75% but long-term disease-free survival is only 25%. This implies that the increased survival after autologous and allogeneic bone marrow transplantation is due to the elimination of minimal residual disease. Soon after intensive cytoreductive therapy residual disease is likely to be at its nadir and hence most likely to be susceptible to the effects of biological response modifiers such as IL-2. On this basis a phase I clinical trial was established to ascertain the feasibility of giving IL-2 therapy to patients treated with intensive chemoradiotherapy in doses that produced circulating cells with anti-neoplastic activity. IL-2 was administered to 10 patients in remission of AML and three patients with multiple myeloma 1–4 weeks after ablative chemotherapy or chemotherapy and bone marrow transplantation. In vitro experiments confirmed anti-leukaemic effects with clinically acceptable doses of IL-2 which did not compromise conventional treatment with myelosuppression. Longer term studies are now needed to see if this translates into improved remission duration or cure (53).

LAK cells

Methods

The adoptive transfer of activated lymphocytes into cancer patients is a complicated procedure which is not yet possible on a large scale. Lymphocytes are harvested from

patients by leukapheresis and incubated with rIL-2 so that they become lymphokine-activated killer cells, these are then infused back into the patients, in combination with rIL-2, intravenously.

Lymphokine-activated killer (LAK) cells have been used in conjunction with IL-2 and results suggest that response rates are improved over IL-2 alone. In renal cell carcinoma a randomised trial is being undertaken to resolve the question. It may be that LAK cells are essential to the best response rate but as yet we do not understand the physiological pathway responsible for their regulation in the normal cell or which other lymphokines are involved in their activation. Of 25 patients treated in this way there were 11 responses one of which was complete. Responses were seen in patients with melanoma, renal cell carcinoma and colorectal tumours. The toxicities attributable to IL-2 were dose-limiting (54). Although results using LAK cells are encouraging the increased toxicity and greater likelihood of intensive therapy during treatment make the therapy less practical, particularly when it seems likely that the main gain is in time to response since IL-2 alone ultimately provokes the recruitment and activation of endogenous LAK cells. Where autologous and allogeneic LAK cells have been used alone they appear to be effective in a proportion of patients and without immune side-effects (55).

Production

It has recently become possible to perform long-term culture of cells in rIL-2-containing medium which increases LAK activity on a per cell basis with an average 30–100-fold expansion over 14–21 days. Stimulation of peripheral blood lymphocytes (PBL) with anti-CD3 antibody results in 300–1 000-fold increase in cell number without any loss of LAK activity. Such cells can be further stimulated with other lymphokines to enhance LAK activity with the possibility of large scale 'production' for experimental and therapeutic purposes (56).

Tumour-infiltrating lymphocytes

Experiments in mice have identified tumour-infiltrating lymphocytes (TIL) as tumoricidal cells that are more therapeutic in vivo than LAK cells and less dependent on adjunctive systemically administered IL-2 to mediate the anti-tumour effects. Consequently they are of greater potential use in clinical trials. TILs comprise a subset of lymphocytes found within solid tumours which can be selectively expanded from tumour digests cultured in the presence of IL-2.

Mechanism

The expression of major histocompatibility complex antigens of tumour cells and a subpopulation of TILs were examined in 14 glioma and 13 metastatic brain tumour tissues. In both most of the TILs were T-lymphocytes and both phenotypes of the cytotoxic and helper/inducer T-lymphocyte were found. Examining the MHC antigens revealed beta 2 microglobulin intensely on tumour cells in all cases. HLA-DR (the monomorphic determinant of the human-leukocyte antigen) was shown in 10 glioma and 5 metastatic tumours. Again a correlation was seen between the number of TILs and MHC antigen expression on tumour cells (57). A pilot study was carried out looking at the feasibility of administering IL-2 expanded TILs to humans with metastatic cancer. 12 patients (6 with melanoma, 4 renal cell, 1 breast, 1 colonic cancer) were treated with varying doses and combinations of TIL ($8 \times 10-9$ to $2.3 \times 10-11$ cells per patient), IL-2 10 000–100 000 U/kg $3\times$ daily to dose-limiting toxicity and cyclophosphamide up to 50 mg/kg. Two partial responses were seen—pulmonary and mediastinal masses regressing in one and a lymph node mass in another (melanoma and renal cell carcinoma respectively). Toxicities were comparable to those seen with IL-2, none being directly attributable to TILs. In 5 of the 6 melanoma patients TILs demonstrated lytic activity specific for the autologous tumour targets in short-term chromium release assays, unlike the non-specific activity seen with LAK cells. It would appear that a lymphocyte subset with enhanced tumoricidal capacity has been identified and that further work is needed to elucidate its appropriate administration schedule (58).

In experimental work involving patients sequential tumour biopsies were obtained before, during and after treatment with IL-2, with or without the adoptive transfer of LAK cells. Infiltrating lymphoid and tumour cells were characterised in frozen sections using monoclonal antibodies (MAbs) and the avidin-biotin complex immunoperoxidase technique. In 5 patients there was objective tumour regression (one CR in a follicular lymphoma, 4 PRs in melanoma). Four patients were non-responsive. Responsive tumours showed a pronounced infiltration with T cells after treatment whereas in non-responders there was no significant increase in lymphoid cells after therapy. No difference was noted between groups before therapy. In 4 out of 5 responders tumours were positive for HLA-DR before therapy and in the reamaining responder the tumour became positive during treatment. Non-responders had DR negative tumour cells before and after treatment. It appears that the expression of HLA-DR in tumour cells may play a role in the response to IL-2 with or without LAK cells and that marked infiltration by T-cells accompanies and possibly mediates such a response (59).

Tumour necrosis factor

With the increased availability of recombinant human cytokines analysis of their effects on possible target cells

has become possible. Tumour necrosis factor provides one of the best illustrations that there is little, if any, specificity of cytokine action. One century ago Coley observed that some patients with streptococcal infection had concomitant remission of their malignancies. In 1975 Carswell identified the bacterial lipopolysaccharide more precisely and by 1984 it had been cloned and sequenced (60, 61). Initially it was produced by lipopolysaccharide-induced monocyte lines but can also be produced by activated lymphocytes. It is a protein released by activated macrophages in response to stimulation by endotoxin. In vivo it characteristically produces necrosis of tumours, but is also found to kill tumour cells in vitro, inhibit lipoprotein lipase, stimulate granulocytes and fibroblasts, damage endothelial cells and possess antiviral activity. Despite these effects, however, the ability of TNF to kill tumour cells both in vivo and in vitro may be relatively unimportant both physiologically and therapeutically (62).

The production of recombinant TNF has been possible since 1984, when it was first cloned (63). Its role both physiologically and in cancer is still uncertain, although it has a definite role in the production of endotoxic shock.

It has a role in a number of processes:

1) Inflammation
   TNF is involved in recruitment of the inflammatory process. It activates granulocytes, increases the expression of surface cell adhesion molecules which in turn results in increased adherence to the vascular endothelium. Excessive TNF production appears to be important in Gram-negative sepsis, the cachexia of chronic disease and possibly that of malignancy, although direct evidence of the latter is lacking (64).
2) Immune modulation
   TNF has a direct action on growth and differentiation of T and B cells. Animals deficient in TNF may be predisposed to autoimmune disease.
3) In regulation of tumour cell growth
   a) Direct
   In vitro some tumour cells, namely those expressing TNF receptors, are susceptible to the direct cytotoxic effect of TNF. Although the mechanism is not certain cell death results in association with DNA fragmentation.
   b) Indirect
   TNF appears to destroy tumour cells in vivo even when it has no effect on these particular cells in vitro—this destruction is dependent on tumour vascularisation and may be mediated by the effect of TNF on the vascular endothelium. TNF also kills cells when combined with gamma interferon when they are not susceptible to either agent in isolation. The mechanism has yet to be established but is not related to TNF receptor expression. The combination of these two cytokines may well prove fruitful in the clinical situation.

TNF is a known growth factor for normal T cells, B cells and fibroblasts and it has now been shown to be a growth factor for malignancies of these cell lineages—its role in the treatment of malignancy therefore needs further confirmation and clarification (65).

A study carried out to determine its maximum tolerated dose, pharamocokinetics and anticancer effect on 18 patients with advanced cancer was performed at the Royal Marsden Hospital. Dose levels from $9 \times 10 \sim 3$ units $m \sim 2$ with incremental dose escalations to $1.2 \times 10 \sim 6$ were used. Patients were very carefully monitored and TNF levels measured by an ELISA method. One-third of patients experienced febrile symptoms, which may result in rigors and were not related to dose in incidence or severity. Hypotension was dose-related—appearing in 3/7 patients receiving over $9 \times 10 \sim 5$ u $m \sim 2$. Abnormal liver enzymes were transient but dose-related, as was a neutrophil leukocytosis. Objective disease response was seen in only 3 patients—all three were partial responses in patients with lymphomas. No evidence of elevated endogenous TNF was found in pateints with malignant disease (66). Clinical data from many centres using rTNF alone has been very disappointing with few documented tumour regression (Table 3). There is almost certainly little place for TNF when used as a single agent.

Colony stimulating factors

Colony-stimulating factors were discovered, and purified on the basis of their actions on haemopoietic cells in vitro. Initially the quantities were too small for in vivo work. Recombinant products have now been found to have comparable activity, actions and targets to the corresponding natural product, the main difference being a shorter half-life to the non-glycosylated recombinant product (67). The colony stimulating factors (CSFs) show great promise in the treatment of diseases associated with bone marrow dysfunction (Table 4). These are glycoproteins able to stimulate maturing colonies of neutrophils and monocyte-macrophage precursors. G-CSF is primarily a stimulus for granulocyte formation and M-CSF is a stimulus for monocyte-macrophage formation, while GM-CSF and multi-CSF (interleukin-3) can stimulate formation of both types.

Table 4

Therapeutic applications of colony-stimulating factors

Prevent or mitigate chemotherapy and radiation-induced leukopenia
Improved host defence in immunocompromised patients
Facilitate recovery from bone marrow transplantation
Treat infectious and parasitic diseases
Prevent infection in debilitated, at risk patients
Treat myelodysplasia
Treat aplastic anaemia
Induce anti-tumour activity in vivo

Although they seem unlikely to offer therapeutic efficacy in the treatment of malignancy they may prove a useful adjunct in the context of optimising chemotherapy and other myelotoxic forms of treatment. They also prime mature cells for enhanced chemotaxis, phagocytosis and killing in response to physiologic stimuli. The action of CSFs is mediated by growth factor receptors on precursor and mature effector cells. Studies using granulocyte-macrophage colony-stimulating factors in patients with the acquired immunodeficiency syndrome (AIDS) and myelodysplastic syndrome suggest a possible therapeutic role for CSFs augmenting mechanisms of host defense.

The role of haemopoietic factors in the control of blood cell formation under basal conditions has not been established, but the genes for all nine growth factors have been cloned and active recombinants can now be produced in useful quantities.

G-CSF has not shown any dose-limiting toxicity, symptoms being confined to fevers, rashes and slight bone pain. G-CSF has been evaluated in chemotherapy-induced neutropenia, either as prophylaxis or to accelerate recovery. hG-CSF is a potent stimulus of normal neutrophil proliferation and maturation. Its administration also reduces the haemopoietic and oral toxicity of chemotherapy with agents such as methotrexate (68).

GM-CSF is slightly less effective in stimulating white blood cells, the rise occurring in two distinct phases over 5–7 days. In high doses side-effects are dose-limiting and include oedema, fever, malaise and rash. Both agents were initially given intravenously but subcutaneous injection or infusion is probably more efficient and less toxic. Recombinant human GM-CSF has been used in subhuman primates given total body irradiation and patients with AIDS. In a study of 19 patients with breast cancer or melanoma treated with high-dose chemotherapy and autologous bone marrow support it was found to accelerate myeloid recovery over a range of clinically acceptable doses. Dose limiting side-effects are myalgias and fluid retention (69).

If CSFs are shown to be active in neutropenic, infected patients their potential includes AIDS patients. In addition the ability of CSFs to raise circulating progenitor cell levels may enable the use of peripheral blood cells as an alternative to marrow cells for transplantation (70).

Monoclonal antibodies

Since the development of monoclonal antibodies by Kohler and Milstein in 1975, their use in the diagnosis, investigation and treatment of cancer has become increasingly specific and efficient. They were originally produced after the hybridisation of a malignant myeloma cell line in continuous culture and spleen cells from a mouse immunised against a specific antigen. It is now possible to produce an infinite range of these antibodies in relatively large quantities, inexpensively. This was followed by the discovery of tumour-associated antigens producing large numbers of antibodies against tumour cells and then screening each antibody for its reactivity with a range of tissues. This antibody is then selected and produced in bulk with knowledge limited to the tissue distribution of the target antigen. As yet no highly specific tumour antigens exist, the degree of specificity is only relative. Monoclonal antibodies can be coupled to radioactive isotopes, drugs and toxins, in theory reducing toxicity to normal tissues unreactive with antibody (Table 5).

Table 5

Clinical applications of monoclonal antibodies

Diagnosis	
Tumour markers	
Immunocytochemistry	
Imaging	
Immunoscintigraphy	
Therapy	
Bone marrow 'laundering' and T cell depletion for BMT	
Systemic therapy	
— Antibody alone	
— Antibody conjugates to	— drugs and pro-drugs
	— toxins eg. ricin
	— radionuclides

Table 6

Factors important for MAb tumour localisation

Antibody affinity
Cross-reactivity with normal tissues
Surface membrane antigen distribution
Antigenic modulation
Circulating antigen
Whole immunoglobulin vs fragments
Tumour size
Degree of tumour necrosis
Tumour vasculature

For monoclonal antibodies to be successful there are certain factors which must be addressed with respect to tumour localisation. These are outlined in Table 6.

Diagnosis

In diagnosis many of the important breakthroughs have been in the area of histopathology. Antibodies to intracellular components such as the intermediate filament proteins and to extracellular matrix components found in the vicinity of the tumour are being used to distinguish different tumour types. The cytokeratins form the intermediate filaments of epithelial cells and are found only in epithelial and mesothelial cells. As such they can be effective diagnostic tools in discriminating between carcinomas and lymphomas and for identifying target cells within the tissues. Although the search for a specific tumour marker

has been disappointing, panels of antibodies can be used to separate morphological indistinct tumours. Equally the site of origin can be determined from small biopsy specimens, aspirates and micrometastases. Lymphoma phenotyping has been the most successful area where phenotyping can be used to distinguish grade and cell type (71).

To obtain accurate staging in breast cancer it is important to identify metastatic deposits—both their presence and extent. The detection of micrometastases in histological sections, with the use of MAbs, may be more sensitive than any other methods yet available. The expression of the tumour-associated epithelial antibodies (those directed to human milk-fat globule), may be modulated by the environment, unlike the expression of the cytokeratins, but as yet they are the most reliable breast tumour marker which has been found. Antigens expressed at the cell surface may be of use in the antibody localisation of tumour. Melanomas were among the first neoplasms investigated using MAbs, several antigens have been defined which are preferentially expressed in melanoma. Where other factors are highly suggestive the presence of S100 positivity (an acidic protein widely found in cells of neural crest origin) can help to clinch the diagnosis. Although the expression of some melanoma antigens is 20–1 000-fold greater in melanomas than normal tissues there are no absolute qualitative differences. Three major antigens are detectable in virtually all primary melanomas tested, with varying frequency. This heterogeneity suggests that a combination of antibodies would be advantageous for most clinical purposes (72).

Imaging

Monoclonal antibodies coupled to a suitable radiolabel have been used for tumour imaging. Monoclonal anti-CEA antibody has been used with results that suggest a sensitive technique using emission CT scans which compete with the other radiological diagnostic tests such as CT and MRI. ^{123}I-labelled small fragments of MAb with high affinity for CEA represent a definite improvement over previously published results. Using intact polyclonal CEA Begent et al. (73) reported an increase in the definition of tumour images by removing circulating radiolabelled antibodies using liposome-entrapped second antibodies. Among the different tracers proposed for carcinoma of the colon diagnosis with immunoscintigraphy the authors use the Fab fragment of MAb labelled with ^{123}I. Although this has a short physical half-life the definition of tumour images obtained at 24 h is superior to that obtained with whole antibody.

Treatment

We are now able to produce 'humanised' antibodies, use antibody fragments and have continued to enhance the degree of specificity of antibody to tumour cells. Early work was carried out in leukaemias and lymphomas where there is a source of readily dispersed cells and their products, to which MAbs can be raised. The initial problem encountered was production of anti-mouse immunoglobulin preventing further successful treatment (Table 7). It is now possible for the antigen-binding site of a mouse antibody to be cut out and grafted onto a human immunoglobulin molecule so that it is recognised as human by the immune system (74). Such antibodies have now undergone clinical trials in patients with recurrent non-Hodgkin's lymphoma (75). Recently the discovery that the low molecular weight V_H component of an antibody retains a reasonable affinity for its target antigen will almost certainly stimulate new developments. Such domain antibodies (dAbs) could well revolutionise the entire field providing novel genetically engineered molecules for cancer therapy (76). Other problems included inaccessibility (e.g. brain and testis), the development of resistant mutants by various mechanisms (altered antigenic determinants and sparse or no surface antigen). These problems can be overcome in a number of ways: swamping the tumour with antibody, destroying the antiglobulin clone with cyclophosphamide, substituting murine antibodies with human-mouse chimeric or human antibodies, using fragments of antibody which are less immunogenic or reducing the bulk of tumour with some other agent beforehand. Chronic antigen modulation can also be avoided by a reasonable period between treatments.

Table 7

Limitations to monoclonal antibody therapy

Human antiglobulin response
Therapeutic ratio
Inadequate tumour dose of radiation, drug or toxin

Trials with unlabelled antibodies

An ideal target for antibody therapy would be entirely specific for tumour cells, associated with their neoplastic behaviour and easily accessible. Clinical trials with unlabelled antibodies have yielded only minor responses. The best results were seen with anti-idiotypic antibodies in B-cell lymphoma patients (77) and in melanoma patients treated with an antibody that activates human complement and effector cells (78). Large doses of antibody can be repeatedly given over a 10-day period without unacceptable toxicity and administering 150 mg or more over a 10-day period results in melanoma cells in tumour biopsies being shown to bind antibodies where there was no significant binding to normal stromal cells.

Radioimmunotherapy

Radioimmunotherapy is less well developed than radioimmunodetection but encouraging results have been

seen in some malignancies. The antibodies used exploit quantitative differences in antigen expression between tumour and adjacent normal tissues. An ideal malignancy for the pioneering use of this treatment is carcinoma of the ovary which is usually confined to the peritoneal cavity and with the aid of surgery and chemotherapy is often of small bulk. Twenty-nine patients with resistant ovarian cancer were treated with intraperitoneal ^{131}I-labelled MAbs. Two responses were seen in 15 patients with disease of <2 cm in diameter. Of 6 patients with microscopic disease, 4 were disease-free at follow-up with median duration of 17.5 months. Eight patients with gross disease showed no significant response (79).

Radiolabelled Fab fragments specific for melanoma antigens, can localise in melanoma tissue when injected intravenously into patients. Data on the localisation of ^{131}I-labelled anti p 97 Fab fragments suggests that these can be used to deliver a therapeutic dose of radioactivity to tumour without causing unacceptable damage to normal tissues. Imaging techniques can determine how much of the therapeutic agent is taken up in the tumour before a patient is treated so that uptake can be related to the therapeutic dose necessary. Unfortunately, even the most specific antibodies so far tested localise only 5–10 times better in tumours than in normal tissues.

Toxicity

Treatment with MAbs alone has remarkably few adverse reactions, in spite of large quantities being used. Fever—thought to be due to endogenous pyrogen—is the most common side-effect. Occasionally bronchospasm, urticaria, anaphylaxis and imparied creatinine clearance have been seen.

The major limitation which faces monoclonal antibody therapy is that conjugates with radionuclides, toxins and chemotherapeutic agents are still not able to gain access to the centre of bulky tumour masses. Until this is possible with acceptable toxicity to normal tissues they are useful only as an adjunct to existing therapeutic modalities.

Oncogenes

The discovery of viral oncogenes with counterparts in normal mammalian cells have considerable diagnostic and therapeutic potential. Oncogenes are defined as those sequences of DNA whose altered expression or abnormal product is pivotal to the production or maintenance of the malignant state. Their function appears closely related to the control of gene expression and cellular proliferation. The widespread existence of genetic abnormalities in malignant cells (e.g. Philadelphia chromosome in chronic myeloid leukaemia) along with the high incidence of malignancies in certain inherited disorders are only part of the evidence pointing to a genetic basis for malignancy, i.e. proteins encoding cellular oncogones may be found at the cell surface, in the nucleus or cytoplasm, as GTP-binding proteins, enzymes and growth factors involved in the control of cell replication. It is believed that their alteration is related to the development of the malignant phenotype. By deciphering their function in normal cells it is hoped that their role in control of cellular differentiation and proliferation can be established. Although clinical application is currently limited, oncogene expression has been examined in a wide range of primary tumours and metastases. Released oncogene products may be useful tumour markers for immunocytochemistry purposes, and linked to radionuclides they have enabled accurate tumour localisation (80). In some instances they have proved a useful tool to predict the tumour biology and natural history of a particular patient's disease. There is an important association between prognosis and epidermal growth factor receptor (c-erb-B2) status. This is an important predictor of relapse-free and overall survival in breast cancer (81).

The discovery of oncogenes has clearly given tremendous impetus to the understanding of the biology of cancer. It also provides new targets for developing pharmacological agents. Growth factors, growth factor antagonists and antibodies to the receptors for growth factors are clearly fruitful areas for new drug design (82). Furthermore, nucleoside analogues of guanosine triphosphate may successfully inhibit the *ras* protein even though its function is as yet undefined. Many oncogene products such as tyrosine kinases exert their effects by phosphorylating tyrosine and other proteins. Several agents are available which can block this activity. The most intriguing are suicide peptides containing tyrosine. These molecules mimic the kinases' natural substrate and bind with high affinity to the enzyme thereby irreversibly destroying it. Such peptides are arousing intense interest in the drug industry, but as yet have not entered clinical trials. As more is learnt about the structure and function of oncogene products it is likely that novel selectively toxic agents will be developed with anti-cancer potential.

Corresponding author: Professor Karol Sikora, Department of Clinical Oncology, Royal Postgraduate Medical School, Hammersmith Hospital, Du Cane Road, London W12 0NN, England.

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