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Perspectives in Cancer Research

Priorities in Experimental Therapeutics—A Surfeit of Riches*

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Abstract—*Experimental therapeutics plays a key role in determining which areas of basic biological research are developed to see if they can be of use in cancer medicine. The recent expansion in our basic knowledge about biology and cancer makes difficult the choice of what areas should be developed. Six such areas are reviewed and assessed. Several hold the promise of improving the management of cancer. More important, developmental therapeutics itself adds to our basic knowledge of cancer and this in turn may indicate those areas which, if developed, will lead to cure of this disease.*

INTRODUCTION

EXPERIMENTAL THERAPEUTICS stands between basic biological research and clinical oncology, and it plays a key role in determining which areas of research eventually become part of medical practice. The many areas of biological research present the experimental therapist with multiple and often difficult choices. We believe somewhere in the huge database of molecular biology, basic immunology and other research areas there is information that can be successfully exploited, developed and applied to prevent and cure cancer.

Choices are difficult but funding bodies and more recently government committees require them. What guidelines can we use? This review looks critically at current directions in experimental therapeutics and argues that the following approaches may be helpful in establishing priorities. It seems obvious that we should choose directions which will lead to a certain cure for cancer. However, this is not possible in practice as the basic cause of cancer has not been identified and a rational approach to correcting this cause cannot yet be made. Reasonable guidelines to establish priorities in experimental therapeutics would therefore be (1) to select research with the potential to tell us something new and important about cancer biology; (2) to identify research that is unlikely to lead to a cure for cancer and which has less chance of discovering key biological information so these areas can be given a lower priority; (3) to be aware of novel and exciting discoveries in different fields of biology and to see the potential of these discoveries with respect to the experimental therapeutics of cancer.

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EXPERIMENTAL THERAPEUTICS IN CANCER RESEARCH

If papers presented at the last annual meeting of the American Association for Cancer Research (AACR) reflect research funding which in turn reflects priorities in cancer research, experimental therapeutics, including preclinical pharmacology, was the largest component, with 28 sessions compared to 17 on carcinogenesis, 14 on immunology, and 13 on biology. As well, the area of new therapeutic procedures is fifth of six designated research areas in the comprehensive 10 year strategy for cancer control from the Foundation for Cancer Research, National Cancer Centre of Japan. However, although experimental therapeutics forms an important part of cancer research, it still deals mainly with existing drugs. Of the 17 different topics in the experimental therapeutics sessions at

the 1988 AACR meeting, eight dealt with existing drugs, five with drug resistance, two with new drugs and two with other assorted topics. This again emphasizes the need to look critically at the areas of research included in experimental therapeutics.

LEADING TOPICS IN EXPERIMENTAL THERAPEUTICS

The choice and assessment of six leading research areas which follow is in part arbitrary and in part based on research papers, reviews and recent texts. Each research area has a certain rationale, some have achieved limited success and many have problems in their continuing development.

New drugs and related therapies

Novel compounds, synthetic and natural. The synthesis or acquisition of novel anticancer compounds has been a priority in experimental therapeutics since the clinical use of nitrogen mustard in 1949 [1]. Since then, approximately 30 drugs have been introduced into the clinic. The success of this approach has been the cure of a percentage of eight cancers in which treatment by chemotherapy plays a major role (Table 1). Fourteen of the 30 drugs in current use are used to cure these cancers, either alone or in combination therapy. Some drugs are used more often than others and this allows them to be ranked, with vincristine number 1 (Table 2). If we consider the date when the anticancer activity of each drug was first reported and the date when the same drug was approved for clinical use (Fig. 1B), we see an increasing delay from 1963 to the present. As well, no curative agent has been identified since the antitumour activity of VP-16 was identified in 1972, the drug being first used clinically in 1982 (Fig. 1A). Agents with antitumour activity in experimental systems reported since 1972 are also shown in Fig. 1B. None of these have shown curative potential in the clinic. Perhaps the curable cancers have been cured. The eight cancers that can be cured have common features (Table 3) which may be necessary for cure at least by drugs of the types under discussion. The majority of cancers, lacking such properties, may not be curable by compounds which are synthesized empirically, semi-empirically or even rationally and which are selected for antitumour activity against animal tumour screens. Concern that these animal tumours might not select drugs active against solid tumours has led to changes in drug screening. The original panel of the NCI's murine tumours first had human xenografts added and now has been replaced by cell lines cultured from human tumours [2]. These latest screening procedures represent a large scale experiment whose results are not yet known.

Table 1. Cancers in which chemotherapy can play a major curative role

1.	Leukaemias and lymphomas:
	Acute lymphoblastic leukaemia in children
	Acute myeloid leukaemia
	Lymphomas such as Hodgkin's disease and diffuse histocytic lymphoma
2.	Childhood cancers
	Wilm's tumour
	Rhabdomyosarcoma
	Ewing's sarcoma
3.	Other cancers:
	Choriocarcinomas
	Germ cell tumours of the testis and ovary

Table 2. Curative anticancer drugs. The number of cancers cured by each drug has been estimated by totalling the number of times the drug was used alone or in combination to cure the cancers in Table 1

Drug	Number of cancers cured
Vincristine	7
Cyclophosphamide	6
Actinomycin D, Adriamycin®	4*
Methotrexate, vinblastine, bleomycin	2*
Mechlorethamine, procarbazine, cytosine arabinoside, daunorubicin, *cis*-platinum, VP-16-213	1*
(Prednisone	3)

*For each drug.

Targeted cytotoxics. With few exceptions, cytotoxic agents do not show absolute tumour specificity. The idea of coupling cytotoxic drugs [3], toxins [4], and radioisotopes [5] to antibodies prepared against tumour antigens is sound in theory. However, the therapeutic effect of such treatments in man has been small. Imaging tumours with antigens coupled to radioisotopes [5] may prove more useful. The problems remain those of specificity of the target antigen and of the antibody raised against it and the non-specific uptake of coupled materials by a very efficient reticulo-endothelial system. We don't know the fate of such conjugates in the cell cytoplasm. Do those with coupled alkylating agents enter the nucleus and, if so, in what form? Better tumour specificity might be obtained by comparing

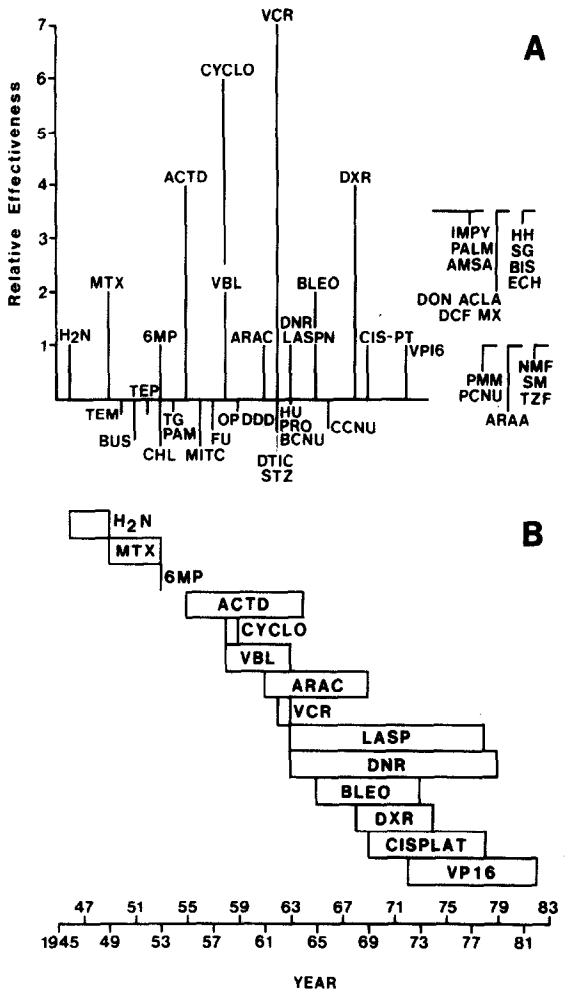

Fig. 1. Dates of discovery and use of curative anticancer drugs. A. Relative effectiveness is shown by the number of cancers cured by each drug, alone or in combination. Drugs below the lines, including newer agents shown on the right, have not been shown to be curative. B. Time between published cytotoxic or antitumour activity and introduction of curative drugs into the clinic. Abbreviations: ACLA, aclacinomycin; ACTD, actinomycin D; AMSA, m-AMSA; ARAA, adenosine–arabinoside; ARAC, cytosine arabinoside; ASPN, L-asparaginase; BCNU, BCNU; BIS, bisbenzinidazole; BLEO, bleomycin; BUS, busulphan; CCNU, CCNU; CHL, chlorambucil; CPT, cis-platinum; CYCLO, cyclophosphamide; DCF, deoxycoformycin; DNR, daunorubicin; DON, DON; DXR, doxorubicin; DTIC, DTIC; ECH, echinomycin; FU, 5-fluorouracil; H_2N, nitrogen mustard; HH, hexamethylhexylamine; HU, hydroxyurea; IMPY, IMPY; MITC, mitomycin C; 6MP, 6-mercaptopurine; MTX, methotrexate; MX, mitoxantrone; NMF, N-methylformamide; OPDDD, OP-DDD; PALM, palmo-AraC; PAM, L-phenylalanine mustard; PCNU, PCNU; PMM, pentamethyl-melamine; PRO, Procarbazine; SG, spirogermanium; SM, spirohydantoin mustard; STZ, streptozotocin; TEM, triethylmelamine; TEP, thiotepa; TG, thioguanine; TZF, tiazofurin; VBL, vinblastine; VCR, vincristine; VP16, VP-16-213. Abbreviations for chemical structures are not written out.

the signal sequences needed to transport macromolecules into the nuclei of tumour cells compared to normal cells since proteins such as nucleoplasmin when coupled to other proteins or to gold particles readily transport these into the nucleus. The synthetic seven amino acid signal peptide of SV40-T antigen coupled to a variety of non-nuclear proteins

Table 3. Characteristics of curable cancers

1.	Short doubling time; high growth fraction
2.	Embryonal type tumours in young patients or of lymphoid origin
3.	Unusual sensitivity to vincristine or vinblastine (all cases) or to corticosteroids (some cases)

transports these readily into the nucleus. Evidence suggests a family of signals exist in various cells (see Ref. [6] for review of the above topics) so a difference may exist between tumour and normal cells. The problem then is whether such molecules can cross the cytoplasmic membrane but here specificity would not matter, since this would occur at the level of the nuclear pore.

Modulation of cancer treatment. Here the rationale is to enhance the anti-cancer effect or to reduce toxicity of treatment. The toxicity of methotrexate has been reversed by folinic acid [7], of *cis*-platinum by sodium thiosulfate [8], of ifosphamide by mesna [9], of drug-induced bone marrow suppression induced by drugs by colony stimulating factors such as rh G-CSF [10] and rh GM-CSF [11]. Enhancement of cytotoxicity has been less well defined, especially in comparing effects on tumour versus normal cells. Examples include 5-fluorouracil (5FU) and PALA [12], 5FU and folinic acid [13], 5FU and methotrexate [14] and alkylating agents, *cis*-platinum and radiation combined with agents depleting intracellular thiols [15].

Increased response rates and survival time have been reported in patients with advanced colorectal cancer treated with 5FU and folinic acid [16] but unexpected GI toxicity of a cholera-like syndrome has been of concern [17]. Much has been learned of the biochemical effects and interactions produced by these drugs and their modulators. Reducing toxicity in certain situations can allow treatment with a conventional dose of chemotherapy in patients at risk of serious toxicity due to such problems as reduced renal or hepatic clearance of drugs. Beyond this, the rationale for preventing toxic death when large and very large doses of chemotherapy are given is that such large doses can eradicate the cancer. Few dose–response curves for cancers are known and the assumption that such curves remain linear into the high dose range has been questioned [18, 19].

Drug resistance. Cancers are often naturally resistant to drugs or acquire resistance during treatment. The question of natural resistance to drugs has not often been studied although it is a major problem with colon and non-small cell lung cancers.

Acquired resistance has received much more attention partly because it is more easily studied under *in vitro* conditions of cell culture. The rationale is that were the mechanisms of resistance understood, they could be manipulated or drugs modified to overcome the resistance. Many types of mechanisms have been identified and success achieved in overcoming some types of drug resistance in experimental situations, e.g. by the use of the MTX analogue trimetrexate in cells resistant to MTX because their carrier-mediated transport of MTX is decreased [20]; lowering intracellular levels of reduced glutathione, high levels being associated with resistance to *cis*-platinum in some cells [15]; altering drug transport with verapamil in cells showing the multidrug resistant phenotype [15]. Studies on the latter mechanism have provided important basic information about how well cells such as those in the GI tract which are in contact with the external environment can protect themselves against the effects of external chemicals and toxins [21]. The positive result is effective protection against carcinogens but, once cancerous, these cells can deal with anticancer drugs in a similar way to carcinogens and so are drug resistant.

Problems with studies on drug resistance are whether the mechanisms identified in animal tumours are the same as in patients; are multiple mechanisms involved, e.g. in resistance to *cis*-platinum; and, given the relationship between tumour heterogeneity and resistance, how can we deal with resistance in subclinical disease? The latter seems unlikely to be successful, since subclinical disease can rarely be diagnosed and genetic manipulation of tumours is not possible in man. Each mechanism of resistance that has been biochemically identified will need individual biochemical manipulation to overcome drug resistance.

Growth factors, oncogenes and suppressor genes. A growth area in biological research comes from the application of DNA technology to the cloning and the identification of factors which regulate cell growth and differentiation, probably by cascades of signal transduction. Many such factors and many c-oncogenes await definition of their true *in vivo* roles [22]. In cancer research much has been learned. *Ras* has an important role in oncogenesis [23] and perhaps in drug and radiation resistance [24, 25] and many other oncogenes are under investigation, being used as prognostic factors in clinical disease [26]. Basic information about c-oncogenes has come from such research. Another example of basic information arising from this type of mission-oriented research is that of leukaemia inhibitory factor (LIF), which causes differentiation of pre-myeloid leukaemia cells *in vitro* [27] and has been shown to be a hormone necessary to maintain the undifferentiated state of normal embryonal cells [28]. Just as aberrant stimulation of cells plays a role in malignancy, suppressor genes play a counter-balancing role in preventing aberrant growth [29]. When these gene products and their regulatory pathways are known, hopes are that cancer control may be possible by using these products or manipulating the pathways to inhibit the abnormal growth of malignant cells. G-CSF and GM-CSF are growth factors used to modulate drug treatment by decreasing toxicity (see above) rather than by changing the biology of the cancer cell. There remains hope of finding factors which will cause differentiation of the malignant cell *in vivo*. The problems are those of specificity of action as different cancers may have various responses to growth factors or to the products produced *in vivo* by them; a possible cascade effect of such products affecting normal tissue; and how to introduce regulatory products such as the products of suppressor genes into cancer cells and not normal cells. It is clear that attempts at solving such problems will provide essential basic information about these products that is necessary for their further development.

Mechanisms of cell death. There is little detailed information about how and why cells die. A major step was the description of shrinkage necrosis [30], later called apoptosis with the subsequent findings of endonuclease-mediated activation [31]. It is associated not only with normal cell turnover but with drug and tumour necrosis factor-related cell death [32]. Cytotoxic drugs have varied actions but many, including the antimetabolites, methotrexate (MTX) and 5-fluorouracil (5FU) damage DNA [33, 34]. The molecular events causing cell death in these situations are unknown, but may relate to cell cycle progression in the presence of DNA damage, as in the case if radiation damage to DNA. If these mechanisms were better understood, it might be possible to enhance the cell killing action of cytotoxic agents. As well, genes controlling programmed cell death [35] and cell cycle arrest which allows repair of damaged DNA [36] require further investigation.

Biological response modifiers (BRM). These agents are similar to growth factors in many ways. They have been successful in producing responses in human cancers but not in producing cure. Problems relate to the unrealistic expectations held for the older agents such as the interferons at a time when little was known about their biological effects. The interleukins are reenacting the history of the interferons, but knowledge of their biological effects is better keeping pace with their clinical use. Some interleukins stimulate the growth of several tumour cell lines and their cascade effect *in vivo* can be

striking. Future work on modification of the modifiers as in the synthesis of a consensus interferon [37], the synthesis of hydrid protein such as between interferon-γ and tumour necrosis factor [38] and combinations of BRMs with cytotoxic drugs and growth factors holds promise.

Tumour complexity. It is fair to say that in the past we have underestimated the complexity of tumour biology, especially of solid tumours. This is clear from studies on cell spheroids [39], the vascularization of tumours [40], the process of tumour metastases [41] and tumour heterogeneity [42]. Given the variable drug distribution seen within tumours of drug-treated animals and the unknown regulating and protecting factors in the extracellular fluid of the tumour, it is surprising any tumour responds to drug treatment. Dealing with this problem appears a difficult task but may be solved as the regulatory and genetic properties of the cancer cell are identified. In the meantime, implications for the design of novel anticancer agents have been presented [43], which take into account some of the above factors.

BASIC SCIENTIFIC KNOWLEDGE OF CANCER

Birch has recently classified research into three inter-related groups, *fundamental uncommitted* research (basic), *strategic* research (basic but mission-oriented), and *tactical* research (converting difficulties into defined problems and solving them within the paradigm) [44]. Birch points out that the first two are technically identical but differ in the reasons they are undertaken and also that, in strategic research, the scientist is not able to follow up exciting leads that aren't related to the goal of the research. Basic knowledge accrues from both. Experimental therapeutics is strategic research and with fundamental uncommitted biological research has contributed much to our basic scientific knowledge of cancer. Much more however needs to be known. Important questions still to be answered are the true *in vivo* role of growth factors and proto-oncogenes; the function of suppressor genes and their products; the mechanism and regulation of cell death; and how heterogeneity is established in solid tumours.

Tactical research in experimental therapeutics

Several important problems need urgent solutions.

Oligodeoxynucleotides (ODN). Results with these molecules are exciting and show great potential for the manipulation of gene expression (see review, Ref. [45]). Synthetic ODNs can reduce 5-fold the synthesis of dihydrofolate reductase in a cell-free system [46]. This enzyme is the target for MTX and is increased in some MTX resistant cells. Also anti-*myc* ODNs inhibited the mitogen induced c-*myc* protein expression in T-lymphocytes and prevented them entering S-phase [47] while anti-*myb* ODNs inhibited normal human haematopoiesis *in vitro* [48]. Stein and Cohen [45] suggest how ODNs might be used as site-specific endonucleases but naturally occurring endonucleases called 'ribozymes' have been found recently to cleave specific mRNAs.

Can 'molecular scissors' modify gene expression in cancer cells?

'Molecular scissors' or 'ribozymes' are small RNA molecules which specifically cleave cytoplasmic mRNA at GU(X) sequences [49]. One such ribozyme occurs naturally as 'satellite RNA' accompanying tobacco ringspot virus and prevents replication of the virus in the plant. The synthetic gene for this ribozyme has been used to produce transgenic plants resistant to the virus [49]. Similar constructs could selectively destroy mRNA species such as those transcribed from abnormal oncogenes. If oligodeoxynucleotides can enter cells and survive there (see above), it is possible that ribozymes can also.

Monitoring basic biological research

This is important since such research may provide the 'breakthrough' knowledge that we need to cure cancer. This monitoring requires a broad scientific view and imagination and is closed to the non-scientist. Research reports of potential importance have come from basic mission-oriented research into plant viruses (see above). Basic uncommitted research into genes controlling the cell cycle of yeast has shown that one gene (RAD 9) allows the cell to repair radiation damaged DNA by prolonging the cell cycle. Mutants lacking these gene go through the cycle without DNA repair and die [36]. The implications for cancer therapy are obvious.

Predicting the future is never easy. Tiresias could do this but at the price of not seeing the present. General Haig believed in the great future for equestrian warfare because 'bullets had little stopping power against the horse'. Nevertheless, some predictions do come true and we hope that one of these is that cancer will be cured. If so, such a cure will be based on basic knowledge accumulated from many diverse areas of uncommitted and mission-oriented research.

REFERENCES

1. Gilman A. The initial clinical trial of nitrogen mustard. *Am J Surg* 1963, **105**, 574–578.
2. Boyd MR, Shoemaker RH, McLemore TL *et al.* In: Roth JA, Ruckdeschel JC, Weisenberger

TS, eds. *New Drug Development in Thoracic Oncology*. New York, WB Saunders, 1986, Ch. 51.
3. Ghose T, Blair AH, Vadia P *et al.* Antibodies as carriers of cancer chemotherapeutic agents. *Ann NY Acad Sci* 1985, **446**, 213–227.
4. Vitetta ES, Uhr JW. The use of immunotoxins containing Ricin A or B chains to kill neoplastic B cells. *Ann NY Acad Sci* 1985, **446**, 228–236.
5. Goldenberg DM. Targeting of cancers with radiolabelled antibodies. *Arch Pathol Lab Med* 1988, **112**, 580–586.
6. Newport JW, Forber DJ. The nucleus: structure, function and dynamics. *Ann Rev Biochem* 1987, **56**, 535–586.
7. Hryniuk WM, Bertino JR. Treatment of leukaemia with large doses of methotrexate and folinic acid: clinical–biochemical correlates. *J Clin Invest* 1969, **48**, 2140–2155.
8. Howell SB, Pfeifle CL, Wung WE *et al.* Intraperitoneal cisplatin with systemic thiosulfate protection. *Ann Intern Med* 1982, **97**, 845–851.
9. Brock N, Pohl J, Stekar S. Studies on the urotoxicity of oxazaphosphorine cytostatics and its prevention. 2. Comparative study of uroprotective efficacy of thiols and other sulfur compounds. *Eur J Cancer Clin Oncol* 1981, **17**, 1155–1163.
10. Gabrilove JL, Jakubowski A, Scher H *et al.* Effect of granulocyte colony-stimulating factor on neutropenia and associated morbidity due to chemotherapy for transitional-cell carcinoma of the urothelium. *N Engl J Med* 1988, **318**, 1414–1422.
11. Antman K, Griffin JD, Elias A *et al.* Effect of recombinant human granulocyte–macrophage colony stimulating factor on chemotherapy-induced myelosuppresion. *N Engl J Med* 1988, **319**, 593–598.
12. O'Connell MJ, Moertel CG, Rubin J, Hahn RG, Kvols LK, Schutt AJ. Clinical trials of sequential *N*-phosphonacetyl-L-asparate, thymidine and 5-fluorouracil in advanced colorectal carcinoma. *J Clin Oncol* 1984, **2**, 1133–1138.
13. Evans RM, Laskin JD, Hakala MT. Effect of excess folates and deoxyinosine on the activity and site of action of 5-fluorouracil. *Cancer Res* 1981, **41**, 3288–3295.
14. Bertino JR, Mini E, Fernandes DJ. Sequential methotrexate and 5-fluorouracil: mechanisms of synergy. *Sem Oncol* 1983, **10**, 2–5.
15. Ozols RF, Cowan K. New aspects of clinical drug resistance: the role of gene amplification and the reversal of resistance in drug refractory cancer. In: de Vita VT, Hellman S, Rosenberg SA, eds. *Import Adv in Oncol*. Philadelphia, JB Lippincott, 1987, 129–157.
16. Erlichman C, Fine S, Wong A, Elhakim T. A randomised trial of fluorouracil and folinic acid in patients with metastatic colorectal carcinoma. *J Clin Oncol* 1988, **6**, 469–475.
17. Petrelli N, Stablein D, Bruckner H, Megibow A, Mayer R, Douglas H. A prospective randomised phase III trial of 5-fluorouracil, leucovorin (LDCF) in patients (Pts) with metastatic colorectal adenocarcinoma: a report of the Gastrointestinal Tumour Study Group. *Proc Am Soc Clin Oncol* 1988, **7**, 94 (357).
18. Henderson IC, Hayes DF, Gelman R. Dose–response in the treatment of breast cancer. *J Clin Oncol* 1988, **6**, 1501–1515.
19. Canellos GP. The dose dilemma. Editorial. *J Clin Oncol* 1988, **6**, 1363–1364.
20. Ohnoshi T, Ohnuma T, Takahashi I, Scanlon K, Kamen BA, Holland JF. Establishment of methotrexate resistant human acute lymphoblastic leukemia cells in culture and effects of folate antagonists. *Cancer Res* 1982, **42**, 1655–1600.
21. Klohs WD, Steinkampf RW. Possible link between the intrinsic drug resistance of colon tumours and a detoxification mechanism of intestinal cells. *Cancer Res* 1988, **48**, 3025–3030.
22. Sehgal PB, May LT, Tam I, Vilcek J. Human β2-interferon and B-cell differentiation factor BSF-2 are identical. *Science* 1987, **235**, 731–732.
23. Weinberg R. The action of oncogenes in the cytoplasm and nucleus. *Science* 1985, **230**, 770–776.
24. Sklar MD. Increased resistance to *cis*-diamminedichloroplatinum(II) in NIH 3T3 cells transformed by *ras* oncogenes. *Cancer Res* 1988, **48**, 793–797.
25. Sklar MD. The *ras*-oncogenes increase the intrinsic resistance of NIH 3T3 cells to ionising radiation. *Science* 1988, **239**, 645–647.
26. Slamon DJ, Clark GM, Wong SG, Levin WJ, Ullrich A, McGuire WL. Human breast cancer: correlation of relapse and survival with amplification of the HER-2/neu oncogene. *Science* 1987, **235**, 177–182.
27. Metcalf D, Hilton DJ, Nicola NA. Clonal analysis of the actions of the murine leukemia inhibitory factor on leukemic and normal murine hemopoeitic cells. *Leukemia* 1988, **2**, 216–221.
28. Williams RL, Hilton DJ, Pease F *et al.* Myeloid leukemia inhibitory factor maintains the developmental potential of embryonal stem cell. *Nature* 1988, **336**, 684–687.
29. Sager R. Genetic suppression of tumour formation: a new frontier in cancer research. *Cancer Res* 1986, **46**, 1573–1580.
30. Kerr JFR. A histochemical study of hypertrophy and ischemic injury of rat liver with special reference to changes in liposomes. *J Pathol Bacteriol* 1965, **90**, 419–435.
31. Williamson R. Properties of rapidly labelled deoxyribonucleic acid fragments isolated from the cytoplasm of primary cultures of embryonic mouse liver cells. *J Mol Biol* 1970, **51**, 157–168.

32. Kyprianou N, Isaacs JT. Endonuclease-mediated DNA fragmentation. *Proc AACR* 1988, **29**, 502 (1997).
33. Li J, Kaminskas E. Accumulation of DNA strand breaks and methotrexate toxicity. *Proc Natl Acad Sci USA* 1984, **81**, 5694–5698.
34. Lonn V, Lonn S. DNA lesions in human neoplastic cells and cytotoxicity of 5-fluoropyrimidines. *Cancer Res* 1986, **46**, 3866–3870.
35. Pereira-Smith O. Are ageing and death programmed in our genes? Report from the XVIth International Genetics Congress, Toronto. *Science* 1988, **242**, 33.
36. Weinert TA, Hartwell LH. The RAD 9 gene controls the cell cycle response to DNA damage in *Saccharomyces cerevisiae*. *Science* 1988, **241**, 317–322.
37. AmGen Biologicals Product Information. The design of IFN-α Con_1.
38. Feng G-S, Gray PW, Shepard HM, Taylor MW. Antiproliferative activity of a hybrid protein between interferon-γ and tumour necrosis factor-β. *Science* 1988, **241**, 1501–1503.
39. Sutherland RM. Cell and environmental interactions in tumour microregions: the multicell spheroid model. *Science* 1988, **240**, 177–184.
40. Jain RK. Determinants of tumour blood flow: a review. *Cancer Res* 1988, **48**, 2641–2658.
41. Nicolson GL, Luka M, eds. *Cancer Invasion and Metastasis: Biologic and Therapeutic Aspects*. New York, Raven Press, 1984.
42. Leith JT, Dexter DL. *Mammalian Tumour Cell Heterogeneity*. Boca Raton, CRC Press, 1986.
43. Denny WA. New directions in the design and evaluation of anti-cancer drugs. In: *Drug Design and Delivery*. Harwood Academic Publishers, 1988.
44. Birch AJ. Relevance in research: a question of terminology. *ANU Reporter* 1988, **19**, 2.
45. Stein CA, Cohen JS. Oligodeoxynucleotides as inhibitors of gene expression: a review. *Cancer Res* 1988, **48**, 2659–2668.
46. Maher L, Dolnick B. Specific hybridisation arrest of dihydrofolate reductase mRNA *in vitro* using antisense RNA or antisense oligonucleotides. *Arch Biochem Biophys* 1987, **253**, 214–220.
47. Heikkila R, Schwab G, Wickstrom E *et al.* A *c-myc* antisense oligodeoxynucleotide inhibits entry into S phase but not progress from G_0 to G_1. *Nature* 1987, **328**, 445–449.
48. Gewirtz AM, Calabretta B. A *c-myb* anti-sense oligodeoxynucleotide inhibits normal human hematopoeisis *in vitro*. *Science* 1988, **242**, 1303–1306.
49. Haseloff J, Gerlach WL. Simple RNA enzymes with new and highly specific endoribonuclease activity. *Nature* 1988, **334**, 585–591.