Biochemical Pharmacology, Vol. 37, No. 2, pp. 149–154, 1988.
Printed in Great Britain.

0006-2952/88 $3.00 + 0.00

COMMENTARY

CAN ANTICANCER THERAPY BE IMPROVED BY SEQUENTIAL USE OF CYTOTOXIC AND CYTOSTATIC (DIFFERENTIATING OR IMMUNOMODULATING) AGENTS TO SUPPRESS TUMOR CELL PHENOTYPIC DIVERSIFICATION?

REUBEN LOTAN* and GARTH L. NICOLSON

Department of Tumor Biology, The University of Texas - M.D. Anderson Hospital and Tumor Institute, Houston, TX 77030, U.S.A.

The inherently heterogeneous characteristics of most tumor cells and their abilities to undergo rapid phenotypic change represent two of the most formidable obstacles to effective antineoplastic therapies. Most neoplasms, by the time they are diagnosed, are comprised of heterogeneous cell subpopulations expressing a wide range of different properties [1–4], including many of those once thought to be uniformly expressed among different neoplastic cells [5]. Such heterogeneity (and the possible rapid appearance of tumor cell clones exhibiting significant differences in cellular phenotype) also extends to therapeutic sensitivities and malignant properties. Thus, tumors that exhibit an initial sensitivity to a particular therapeutic agent can become highly resistant to it and to a variety of other agents, including those that act by mechanisms distinct from the initial treatment. Such tumors can also become more malignant. When this occurs, completely successful therapeutic interventions are rare. The dynamic emergence of tumor cells with diverse phenotypes ensures that some cells will arise in the tumor cell population with enhanced metastatic potential and resistance to a variety of physical and chemical agents.

Another important aspect of antineoplastic therapy is that the treatments themselves can modify tumor cell phenotype and cellular heterogeneity and the rates at which variant cell subpopulations are generated. This is due partly to the cytotoxic and mutagenic natures of most antineoplastic therapies and the selection of therapy-resistant tumor cell subpopulations, but it is also attributable to nonmutagenic changes, such as gene amplification and recombination [6], and to the high rates of diversification of surviving cells that often possess unstable phenotypes [4, 7, 8]. In the latter example, *in vitro* studies with malignant animal tumors have demonstrated that restricting the diversity of tumor cell subpopulations by treatment with cytotoxic agents under conditions that allow some cell survival leads to enhanced generation of new variant tumor cells, including those that are much more metastatic than the original neoplastic cells [9]. In heterogeneous tumor cell populations such cellular diversity appears to be modulated or controlled by complex cell–cell and cell–stromal interactions among different cell subpopulations, which can influence a wide variety of tumor properties [7–11].

Most clinically useful therapeutic agents are cytotoxic to both normal and tumor cells, so they are often administered in repeated cycles interrupted by agent-free periods to allow recovery from toxic side effects. We believe that the intervening periods between cytotoxic cycles could be better exploited because these intervening periods might compromise the success of the therapy by allowing the diversification of surviving neoplastic cells and the emergence of highly malignant and drug-resistant tumor cell subpopulations.

In this commentary we will focus on some of the factors controlling tumor cell heterogeneity, drug resistance and the generation of phenotypic diversity, and will propose the application of sequential therapies that use cytotoxic agents to kill tumor cells plus cytostatic agents to prevent the diversification of surviving tumor cells. The cytostatic agents, including differentiation inducers and immunostimulatory factors, could be used to suppress tumor cell diversification during therapeutic recovery periods between cycles of cytotoxic therapy and thus improve the outcome of treatment.

PHENOTYPIC DIVERSIFICATION AND GENERATION OF TUMOR CELL HETEROGENEITY

Spontaneous and induced neoplasms usually develop from the progeny of single transformed cells, and evidence for their clonal origin exists in well-advanced tumors [1, 12]. Nonetheless, such neoplasms are almost always heterogeneous in their cellular characteristics. Tumor heterogeneity is usually seen in terms of cell morphology, surface antigens and adhesive molecules, surface receptors for hormones and growth factors, susceptibility to host nonimmune and immune responses, invasiveness and metastatic potential, and sensitivity to a variety of physical and chemical agents [1–5, 8–11].

Tumor heterogeneity has its origins in the relative instability of tumor cells. As a tumor grows, its cell progeny diversify into numerous subpopulations by a complex process of reversible and irreversible steps that are modulated by tumor and host cell inter-

* Corresponding author: Dr. Reuben Lotan, Department of Tumor Biology, Box 108, The University of Texas - M.D. Anderson Hospital and Tumor Institute, 1515 Holcombe Blvd., Houston, TX 77030.

actions until certain cellular characteristics become dominant. This phenomenon, called tumor progression, usually results in the loss of normal differentiated cell properties and more commonly in the acquisition of properties that allow more autonomous growth and escape from host control mechanisms [12]. A variety of mechanisms have been proposed for generating increased instability of tumor cells [8, 12, 13]: chromosomal alterations; gene mutations, deletions, rearrangements and amplifications; inherited defects in DNA repair and maintenance genes; acquired defects in "mutator" genes or genes regulating DNA synthesis; integrated virus sequences; alterations in expression of oncogenes or protooncogenes; and other changes [see Ref. 8].

The rates of formation of malignant cell variants that differ quantitatively in their expression of various enzymes, antigens, cell surface glycoproteins, and other components as well as in their sensitivities to radiation, chemotherapeutic drugs, or hyperthermia are often much higher than the rates of spontaneous mutations and other genotypic changes [8, 14, 15]. To explain this, it has been proposed that, in addition to the genotypic instability of tumor cells, there must also be phenomena that lead to phenotypic instability, a term that encompasses all of the epigenetic and microenvironmental influences on tumor cell phenotype and heterogeneity. Phenotypic instability appears to be governed by quantitative rather than qualitative changes in cellular properties, so it is reasonable that the observed rates of phenotypic change are orders of magnitude higher than those found for genotypic changes [8].

CONSEQUENCES OF TUMOR CELL INSTABILITY AND HETEROGENEITY

Two major consequences of genetically unstable, heterogeneous neoplasms are the generation of highly malignant (metastatic) cell variants and drug-resistant cell subpopulations prior to and during antineoplastic therapy. Evidence suggests, for example, that random and spontaneous genetic changes can lead to the acquisition of drug resistance and metastatic potential [6, 8, 16]. Drug resistance can be stably inherited in the absence of selection pressure, and it is apparently generated at rates similar to spontaneous mutation rates found in normal cell populations. Moreover, mutagens can increase the frequency with which drug-resistant cell phenotypes are formed. Modified gene products can be demonstrated in treated cells resistant to certain drugs, and altered genes can be demonstrated on specific chromosomes [16–18].

As tumors progress and undergo phenotypic diversification, they become less likely to succumb to antitumor therapy because of the prevalence of drug-resistant variants and the dissemination of metastatic cells to distant organs [7, 8]. Metastases can exhibit drug sensitivity profiles that are different from those of the primary tumor or other metastases [19, 20]. In addition, treatment with chemotherapeutic agents has been shown to induce the metastatic phenotype in animal tumor cells [21, 22]. Highly metastatic and drug-resistant tumor cells are most difficult to eradicate clinically, and their appearance during tumor progression usually indicates a poor prognosis, even with aggressive therapy.

The phenotypic diversification of malignant cells during tumor progression may be similar to that seen in normal cells during development [8]. As normal cells mature and acquire a differentiated state, however, they become more, not less, phenotypically stable. In contrast, the characteristics of tumor cells that are not phenotypically modulated or restricted by normal tissue and cell regulatory mechanism tend to become more diverse [3–8].

Differences between normal and malignant cells are particularly striking in the generation of drug-resistant tumor cells. Whereas neoplastic cell populations can spontaneously generate a wide range of cells exhibiting varying resistances to virtually any type of currently available cytotoxic anticancer therapeutic agent, normal cells do not give rise to drug-resistant progeny, even after repeated exposure to such cytotoxic agents [16]. Although treatment with cytotoxic agents usually eradicates the bulk of tumor cells that are sensitive, it can also select a few drug-resistant cell subpopulations that survive repeated cycles of therapy [23, 24]. Some of the most commonly used cytotoxic drugs, such as the alkylating agents melphalan, cyclophosphamide and nitrosoureas, can cross-link DNA and alkylate nucleotides and nucleic acids. Consequently, these drugs are very effective at killing sensitive cells, but in the surviving cells they can also increase the frequency of genetic and chromosomal changes, including those that render such surviving cells more resistant to different drugs [6, 25].

An important aspect of tumor heterogeneity is the ability of one subpopulation of cells in a malignant tumor to modify the properties of other subpopulations with respect to cell proliferative, immunological, biochemical, and metastatic properties [7–9, 26, 27]. For example, the interactions between mammary tumor cell subpopulations affect their sensitivity to antineoplastic agents [28]. Although new cell phenotypes are thought to be emerging continuously during tumor progression, the complex interactions among different cell subpopulations in advanced tumors can lead to a stabilization of the rate at which new variants are formed [4, 7–11, 26]. However, conditions such as poor blood supply, assaults by nonimmune or immune host cells, or elimination of some or most of a tumor's cell subpopulations by surgery, radiation, or chemotherapy can induce rapid clonal diversification due to the loss of regulatory interactions between surviving cell clones. In mouse melanomas, the cell clones surviving drug treatment were found to be phenotypically unstable, and they generated variants with altered metastatic properties at very high rates [9, 26]. Thus, some therapeutic agents may stimulate phenotypic diversification by directly modifying tumor cells or by eliminating particular cell subpopulations within a tumor and rendering the surviving clones phenotypically unstable.

Epigenetic mechanisms can also modify tumor cell phenotypic diversification [8, 21]. For example, DNA hypomethylation resulting from tumor cell exposure to 5-azacytidine or other agents can tran-

siently modulate gene expression and alter tumorigenic and metastatic properties [21, 29, 30]. Similarly, low concentrations of carcinogens and chemotherapeutic agents that cause DNA hypomethylation can result in phenotypic changes that may also include increases in cellular diversification [30–32].

CYTOSTATIC AND DIFFERENTIATING AGENTS AS SUPPRESSORS OF TUMOR CELL GROWTH AND DIVERSIFICATION

Physiologic and pharmacologic modulators of cell proliferation and differentiation can inhibit the growth of certain tumor cells, suppress the malignant cell phenotype, and enhance cell differentiation *in vitro* and *in vivo* [33–38]. When tumor cells differentiate, they can become subject to normal growth regulatory mechanisms that prevent cellular diversification. Alternatively, such agents can prevent tumor cell diversification by inhibiting cell proliferation and thereby suppressing clonal expansion, with or without inducing differentiation. The search for therapeutically effective cytostatic and differentiation-inducing agents was prompted by the shortcomings of cytotoxic antineoplastic therapy, especially the side effects resulting from toxicity to normal cells and the failure of cytotoxic agents to increase long-term survival in patients with many of the most common cancers.

The development of physiologic, naturally-occurring, and synthetic antineoplastic agents has followed from their abilities to inhibit the growth and enhance the differentiation of various cultured tumor cells, including leukemias, carcinomas, teratocarcinomas, neuroblastomas and melanomas [33–38]. These agents include: (a) *hematopoietic regulatory proteins* (cytokines), such as granulocyte-macrophage colony stimulating factors (GM-CSF, G-CSF), that are able to enhance the proliferation and differentiation of granulocyte-macrophage precursors and induce the differentiation of cultured leukemia cells and suppress their clonogenicity [35, 36, 39]; (b) *interferons*, or other regulatory molecules in the hemopoietic system, that can exert growth inhibitory effects on various tumor cells and can enhance the differentiation of certain malignant cells [40]; (c) *tumor necrosis factors*, which exhibit cytostatic and cytotoxic effects on different tumor cells and induce differentiation of leukemia cells [41, 42]; (d) *hormones*, including glucocorticoids, hydrocortisone, dexamethasone and prostaglandins, that have been reported to inhibit growth and enhance differentiation of various tumor cells [36]; (e) *vitamins* and their metabolites or synthetic analogs, such as vitamins A, D, and E, that are capable of modulating the growth and differentiation of many cultured neoplastic cells and some tumors *in vivo* [33–38, 43, 44]; (f) *polar-planar compounds*, such as dimethyl sulfoxide (DMSO), hexamethylene bisacetamide (HMBA) and *N*-methylformamide (NMF), that are potent inducers of differentiation of a number of different tumor cells *in vitro* and *in vivo* [35–37, 45–47]; and (g) *differentiation-inducing agents*, including 5-azacytidine, butyrate, dibutyryl cyclic adenosine monophosphate, phosphodiesterase inhibitors, and some cytotoxic agents (cytosine arabinoside, doxorubicin) when used at low doses [36]. Some of the many agents described above are currently under evaluation in clinical trials [35, 37]. These include: aclacinomycin, 5-azacytidine, β-cytosine arabinoside, butyric acid, vitamin D_3, vitamin A analogs (retinoids), interferons, DMSO, NMF, and HMBA [37].

Even with the advances in the development of cytostatic and differentiating agents for antineoplastic therapy, it is difficult to envisage how these might succeed in eradicating advanced tumors when used as single treatment modalities. Such agents are more likely to be beneficial in adjuvant therapy after a "debulking" of the tumor mass has occurred, or in combination with cytoreductive drugs [36]. Indeed, studies on the effects of retinoids [48] or NMF [49] on tumor-bearing mice indicated successful antitumor activity only against small tumors.

Combinations of cytostatic and cytotoxic drugs have been suggested as potentially useful. For example, NMF has been suggested as a potential candidate for combination chemotherapy [50, 51]. Similarly, retinoids have been shown to exert additive antitumor effects in combination with tamoxifen [52] and synergistic effects in combination with low-dose cytosine arabinoside [53], interferon [54], dexamethasone, or prostaglandins [55]. *In vivo* studies with experimental animals have demonstrated that retinoids can enhance the antitumor effects of nitrosourea, cyclophosphamide [56], and 5-fluorouracil [57]. Combining a retinoid with tamoxifen was better than each agent along in suppressing mammary cancer development [58], and combinations of retinoids with 5-fluorouracil and irradiation in the treatment of head and neck cancers have shown some degree of improvement over cytotoxic agents alone [59]. In other studies, oral administration of retinoids in combination with bleomycin, cyclophosphamide, prednisone, and transfer factor improved the treatment of mycosis fungoides [60]. In contrast, a retinoid failed to augment therapy of advanced squamous cell lung carcinoma in combination with lomustine and bleomycin, but the doses of these cytotoxic agents may have been too low [61].

TREATMENT STRATEGIES TO OVERCOME TUMOR HETEROGENEITY AND PHENOTYPIC DIVERSIFICATION

As discussed above, tumor instability and heterogeneity ensure that neoplasms contain some drug-resistant and malignant (metastatic) cells. Obviously, initiation of antineoplastic treatment when a tumor is small and presumably less heterogeneous would be more likely than treatment of advanced cancer to result in a cure. Unfortunately, by the time cancer is first diagnosed, many patients have substantial tumor burdens and evidence of metastatic disease. Ideally, an oncologist looks for treatment strategies that will overcome tumor heterogeneity and metastatic disease, such as combinations of surgery, radiation, or cytotoxic chemotherapeutic agents that do not share the same resistance mechanisms for use in different schedules and doses [62, 63]. Combination chemotherapy is considered to be the most successful strategy against clinically evident metastatic disease

[17]. This approach is limited, however, by the overlapping toxicities of different drugs and the presence of multiple drug-resistant mutants. In addition, current antineoplastic treatment strategies make no provision for overcoming the increased phenotypic instability of tumor cell clones that survive combination therapy [14]. This problem is demonstrated in Fig. 1a. After removal of the bulk of a malignant tumor by surgery, radiation, or both, surviving cell subpopulations begin to expand and diversify. This occult process continues until the patient is deemed suitably recovered from the initial treatment and capable of tolerating several cycles of cytotoxic chemotherapy. If relatively successful, such treatment will eradicate the majority of the tumor cells. However, some surviving drug-resistant cells can continue to proliferate and undergo phenotypic diversification.

Because most cytotoxic drugs cause side effects such as myelosuppression, patients usually need a treatment-free period for recovery before another cycle of cytotoxic therapy can be administered. These intervening periods between sequential therapies can allow the surviving cells to proliferate and diversify. It has been suggested that this problem can be overcome by shortening the time interval between successive treatments with different agents [4, 7, 27]. This strategy, termed FAST (fast accelerated staging of therapy) [7], is presented schematically in Fig. 1b. The main factors in this strategy are an increase in the frequency of treatments (a shorter cycle time) and the use of varied agents with different mechanisms of action designed to kill tumor cell subpopulations as soon as they arise [7]. This strategy, however, presents several potential problems, the most prominent of which is the limited amount of toxicity a patient can tolerate. So that the time intervals between successive treatments could be decreased, patients would be subject to a new cytotoxic treatment before recovering from the previous one. Such a strategy would not be practical for most cancer patients.

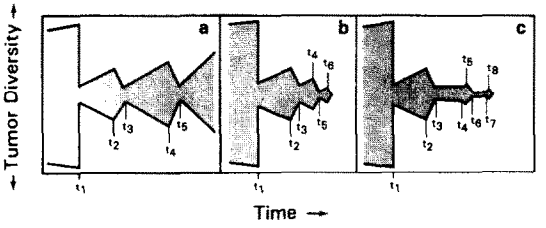

Fig. 1. Schematic illustration of the possible effects of surgery and adjuvant therapy on the diversity of cell subpopulations in a malignant tumor. (a) After surgery (t_1), undetectable, residual tumor cells proliferate and diversify in their phenotypic characteristics. When the tumor grows to a detectable size (t_2), cytotoxic therapy is initiated, resulting in the destruction of most tumor cells and restriction of tumor subpopulation diversity (t_3). However, surviving tumor cells proliferate and diversify to form a new heterogeneous population of malignant cells. When the tumor is detected (t_4), a new course of the same or different cytotoxic therapy is initiated, resulting in the eradication of most tumor cells and restriction of diversity (t_5). After treatment is stopped, the residual tumor cells proliferate and diversify again. This process can continue until the tumor cells are refractory to subsequent treatments. (b) Fast accelerated staging of therapy (FAST) is a strategy whereby the intervening periods (t_2–t_3, t_3–t_4, etc.) between sequential therapies are shortened in an attempt to wipe out as many different tumor cell subpopulations as possible before they have a chance to proliferate and diversify. (c) Sequential use of cytotoxic and cytostatic agents to prevent tumor cell diversification. As in (a), surgery (t_1) and the initial cytotoxic therapy (t_2) result in tumor cell death and restriction of tumor subpopulation diversity (t_3). However, instead of a "recovery" period, cytostatic therapy is used to suppress tumor cell proliferation and diversification. When the cytostatic therapy is terminated (t_4), tumor growth is briefly stimulated by a hormone or a growth factor prior to the beginning of a new course of cytotoxic therapy (t_5). After this cytotoxic treatment is stopped (t_6), cytostatic therapy is again used to limit tumor cell growth and diversification. After the cycle of cytostatic therapy is terminated (t_7), tumor growth is briefly stimulated until the next cytotoxic therapy is initiated (t_8). Several cycles of cytotoxic–cytostatic therapy could eventually result in a "cure" by limiting tumor cell diversification and evolution of therapy-resistant cell subpopulations.

We propose, instead, to combine cytotoxic and cytostatic agents in a treatment strategy directed at suppressing the diversification of tumor cells that survive cytotoxic treatment [64]. Several clinical trials have used combinations of cytotoxic and cytostatic agents, but these agents were usually administered together. We believe that this approach is suboptimal, because many of the cytostatic agents that also stimulate the host immune responses cannot exert their full potential if their normal target cells are killed by the cytotoxic agents. In addition, some of the cytostatic agents also have side effects and overlapping toxicities with cytotoxic agents. This can limit the doses at which such agents can be administered. Therefore, we propose to confine the use of cytostatic, differentiating, or immunostimulatory agents to the intervals between successive cytotoxic cycles (Fig. 1c). In this scheme, the treatment is modulated so that the recovery periods between cytotoxic therapies might not be characterized by extensive tumor cell proliferation and diversification. Thus, there would be less chance of generating highly malignant and drug-resistant variants. For such protocols, cytostatic or differentiating agents would be administered immediately after cytotoxic therapy and continued until a new cycle of cytotoxic therapy is initiated. Because most cytotoxic drugs act predominantly on cycling cells whereas cytostatic agents inhibit cell proliferation, we propose to add a brief period, after the cytostatic agent is withdrawn, for a mitogenic stimulus just prior to the new cycle of cytotoxic treatment. This would alleviate the possible long-term problems that could occur if non-cycling or dormant tumor cells were present during the cytotoxic cycle. The mitogens that can be used in this fashion are those that have already been shown to augment cytotoxic drug action such as estrogen [65–67], epidermal growth factor, hydrocortisone or insulin [67]. In particular cancers, the use of a specific growth factor such as bombesin in small cell lung cancer [68] would seem to be the most suitable method for brief mitogenic stimulation. Such agents could be used to synchronize and initiate tumor cell division for maximal response to a subsequent cytotoxic cycle. The sequence of cytotoxic agent/

cytostatic-differentiation agent/mitogenic agent would have to be repeated several times with different therapeutic agents to be effective in the long-term management of highly malignant and unstable cancers (Fig. 1c).

Finally, there are other problems associated with antineoplastic therapy that have not been considered here, such as the development of better agents and tumor delivery systems, agent inactivation and augmentation, host antitumor responses, and tumor sensitivity screening. These factors will also be important considerations in the development of more effective anticancer treatments.

REFERENCES

1. I. R. Hart and I. J. Fidler, *Biochim. biophys. Acta* **651**, 37 (1981).
2. G. H. Heppner, *Cancer Res.* **44**, 2259 (1984).
3. G. L. Nicolson and G. Poste, *Curr. Probl. Cancer* **7**, 1 (1982).
4. G. L. Nicolson and G. Poste, *Curr. Probl. Cancer* **7**, 1 (1983).
5. S. M. North and G. L. Nicolson, in *Immune Responses to Metastases* (Eds. R. B. Herberman, R. H. Wiltrout and E. Gorelik), p. 1. CRC Press, Boca Raton (1987).
6. R. T. Schimke, S. W. Sherwood, A. B. Hill and R. N. Johnston, *Proc. natn. Acad. Sci. U.S.A.* **83**, 2157 (1986).
7. G. Poste, *Cancer Treat. Rep.* **70**, 183 (1986).
8. G. L. Nicolson, *Cancer Res.* **47**, 1473 (1987).
9. G. Poste, J. Doll and I. J. Fidler, *Proc. natn. Acad. Sci. U.S.A* **78**, 6226 (1981).
10. K. M. Miner, T. Kawaguchi, G. W. Uba and G. L. Nicolson, *Cancer Res.* **42**, 6431 (1982).
11. G. H. Heppner, B. E. Miller and F. R. Miller, *Biochim. biophys. Acta* **695**, 215 (1984).
12. P. C. Nowell, *Cancer Res.* **46**, 2203 (1986).
13. M. Chorazy, *J. Cancer Res. clin. Oncol.* **109**, 159 (1985).
14. J. Peterson, *J. theoret. Biol.* **102**, 41 (1983).
15. D. R. Welch, D. P. Evans, S. P. Tomasovic, L. Milas and G. L. Nicolson, *Clin. expl. Metastasis* **2**, 357 (1984).
16. J. H. Goldie and A. J. Coldman, *Semin. Oncol.* **12**, 222 (1985).
17. S. K. Carter, in *Cancer Invasion and Metastasis: Biological and Therapeutic Aspects* (Eds. G. L. Nicolson and L. Milas), p. 397. Raven Press, New York (1984).
18. R. T. Schimke, in *Accomplishments in Cancer Research* (Eds. J. G. Fortner and J. E. Rhoads), p. 75. J. B. Lippincott, Philadelphia (1986).
19. N. Tanigawa, Y. Mizuno and T. Hasimura, *Cancer Res.* **44**, 2309 (1984).
20. R. S. Kerbel and A. J. S. Davies, *Lancet* **2**, 977 (1982).
21. R. S. Kerbel, P. Frost, R. Lietplo, D. Carlow and B. E. Elliott, *J. cell. Physiol.* suppl. **3**, 87 (1984).
22. T. J. McMillan, J. Rao and I. R. Hart, *Int. J. Cancer* **38**, 61 (1986).
23. H. E. Skipper, F. M. Schable and H. H. Loyd, *Semin. Hemat.* **15**, 207 (1978).
24. T. J. McMillan, T. C. Stephens and G. G. Steel, *Br. J. Cancer* **52**, 823 (1985).
25. J. R. Bertino, M. D. Carman, H. L. Weiner, A. Cashmore, B. A. Moroson, S. Srimatkandada, J. H. Schornagel, W. D. Medina and S. K. Dube, *Cancer Treat. Rep.* **67**, 901 (1983).
26. G. Poste, R. Greig, J. Tzeng, T. Kestler and S. Corwin, in *Cancer Invasion and Metastasis: Biological and Therapeutic Aspects* (Eds. G. L. Nicolson and L. Milas), p. 223. Raven Press, New York (1984).
27. I. J. Fidler and G. Poste, *Semin. Oncol.* **12**, 207 (1985).
28. B. E. Miller, F. R. Miller and G. Heppner, *Cancer Res.* **41**, 4379 (1981).
29. P. D. Jones, *Cancer Res.* **46**, 461 (1986).
30. L. Olsson and J. Forchhammer, *Proc. natn. Acad. Sci. U.S.A.* **81**, 3389 (1984).
31. D. L. Trainer, T. Kline, F. Mallon, R. Greig and G. Poste, *Cancer Res.* **46**, 6124 (1985).
32. V. L. Wilson and P. A. Jones, *Cell* **32**, 239 (1983).
33. R. Lotan, R. Thein and D. Lotan, in *The Modulation and Mediation of Cancer by Vitamins* (Eds. F. L. Meyskens and K. Prasad), p. 211. S. Karger, Basel (1983).
34. A. Bloch, *Cancer Treat. Rep.* **68**, 199 (1984).
35. J. C. Gabrilove, *Semin. Oncol.* **13**, 228 (1986).
36. M. Reiss, C. Gamba-Vitalo and A. C. Sartorelli, *Cancer Treat. Rep.* **70**, 201 (1986).
37. B. D. Cheson, D. M. Jasperse, H. G. Chun and M. A. Friedman, *Cancer Treat. Rev.* **13**, 129, (1986).
38. M. I. Sherman (Ed.), *Retinoids and Cell Differentiation.* CRC Press, Boca Raton (1986).
39. C. G. Bigley, D. Metcalf and N. A. Nicola, *Int. J. Cancer* **39**, 99 (1987).
40. G. Trinchieri and B. Perussia, *Immun. Today* **6**, 131 (1985).
41. B. J. Sugarman, B. B. Agrawal, P. E. Hass, I. S. Figari, M. A. Paladino and H. M. Shepard, *Science* **230**, 943 (1985).
42. G. Trinchieri, M. Kobayashi, M. Rosen, R. Loudon, M. Murphy and B. Perussia, *J. exp. Med.* **164**, 1206 (1986).
43. R. Lotan, *Biochim. biophys. Acta* **605**, 33 (1980).
44. R. C. Moon and L. M. Itri, in *The Retinoids* (Eds. M. B. Sporn, A. B. Roberts and D. S. Goodman), Vol. 2, p. 327. Academic Press, Orlando (1984).
45. E. N. Spermulli and D. L. Dexter, *Clin. Oncol.* **2**, 227 (1984).
46. P. A. Marks, M. Sheffery and R. A. Rifkind, *Cancer Res.* **47**, 659 (1987).
47. M. Iwakawa, P. J. Tofilon, N. Hunter, L. C. Stephen and L. Milas, *Clin. expl. Metastasis*, in press.
48. R. Lotan, G. Neumann and D. Lotan, *Ann. N.Y. Acad. Sci.* **359**, 150 (1981).
49. A. Gescher, N. W. Gibson, J. A. Hickman, S. P. Langdon, D. Ross and G. Atassi, *Br. J. Cancer* **45**, 843 (1982).
50. S. P. Langdon, J. A. Hickman, A. Gescher, M. F. G. Stevens, D. Chubb and L. M. Vickers, *Eur. J. Cancer clin. Oncol.* **21**, 745 (1985).
51. P. J. Tofilon, C. M. Vines and L. Milas, *Cancer Chemother. Pharmac.* **17**, 269 (1986).
52. N. T. Wetherall and C. M. Taylor, *Eur. J. Cancer clin. Oncol.* **22**, 53 (1986).
53. H. W. Findley, Jr., D. L. Williams, C. P. Steuber and A. H. Ragab, *Proc. Am. Ass. Cancer Res.* **26**, 333 (1985).
54. C. Marth, G. Daxenbichler and O. Dapunt, *J. natn. Cancer Inst.* **77**, 1197 (1986).
55. M. D. Bregman, E. Peters, D. Sanden and F. L. Meyskens, Jr., *J. natn. Cancer Inst.* **71**, 929 (1983).
56. M. H. Cohen and P. P. Carbone, *J. natn. Cancer Inst.* **48**, 921 (1972).
57. Y. Tomita, K. Himeno, K. Nomoto, H. Endo and T. Hirohata, *J. natn. Cancer Inst.* **68**, 823 (1982).
58. D. L. McCormick and R. C. Moon, *Carcinogenesis* **7**, 193 (1986).
59. F. Takaku, *J. natn. Cancer Inst.* **73**, 1483 (1984).
60. H. Zachariae, E. Grunnet, K. Thestrup-Pedersen, L. Malin, H. Schmidt, F. Starfelt and K. Thomsen, *Acta derm.-vener., Stockh.* **62**, 162 (1981).
61. W. Weber and J. P. Obrecht, *Cancer Treat. Rep.* **67**, 847 (1983).
62. J. H. Goldie and A. J. Coldman, *Cancer Res.* **44**, 3643 (1984).
63. A. Goldin, *Cancer Res.* **54**, 1155 (1984).

64. G. L. Nicolson and R. Lotan, *Clin. expl. Metastasis* **4**, 231 (1986).
65. B. M. Markaverich, D. Medina and J. H. Clark, *Cancer Res.* **43**, 3208 (1983).
66. M. E. Lippman, J. Cassidy, M. Wesley and R. C. Young, *J. clin. Oncol.* **2**, 28 (1984).
67. V. Hug, D. Johnston, M. Finders and G. Hortobagyi, *Cancer Res.* **40**, 147 (1986).
68. D. N. Carney, F. Cuttitta, T. W. Moody and J. D. Minna, *Cancer Res.* **47**, 821 (1987).