Anti-proliferative effects of the arotinoid Ro 40-8757 on human cancer cell lines in vitro

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Summary A novel arotinoid with a morpholine structure in the polar end group Ro 40-8757 (4-[2-[[p-[(E)-2(5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-2-naphthyl)propenyl]phenoxy]ethyl]-morpholine) was tested for its anti-proliferative activity against nine human cancer cell lines in vitro. The lines included two estrogen receptor positive breast cancer lines (MCF-7 and ZR-75-1), two estrogen receptor negative breast cancer lines (MDA-MB-231 and BT-20), one cervix carcinoma line (KB-3-1), two lung adenocarcinoma lines (A549 and HLC-1), one large cell lung cancer line (LXFL 529) and two colorectal lines (CXF 243 and CXF 280). Proliferation of all the lines, except the two lung adenocarcinoma lines, was inhibited by lower concentrations of Ro 40-8757 than those of all-trans retinoic acid (RA) or 13-cis RA giving the same level of inhibition. The degree of inhibition of Ro 40-8757 was concentration and time dependent. The arotinoid was not cytotoxic and morphological signs by differentiation were not evident in cultures treated with Ro 40-8757 for up to 2 weeks. Because this compound is active on cells such as KB-3-1 that are not inhibited by all-trans RA and because it does not bind to nuclear retinoic acid receptors, it may represent a novel class of anti-proliferative agents.

Materials and methods

Cell lines

The human breast cancer cell lines, ZR-75-1, MDA-MB-231 and MCF-7 were obtained from ATCC and BT-20 was obtained from Prof. Eppenberger (University of Basel, Switzerland). The line KB-3-1 was obtained from Dr M.M. Gottesman (N.C.I., U.S.A.) and originates from the human cervical cancer cell line HeLa. The lung cancer lines HLC-1 and A549 were obtained from ATCC. The large cell lung carcinoma line, LXFL 529, and the two colon carcinoma lines, CXF 243 and CXF 280, were purchased as xenograft lines from Dr H.H. Fiebig (University of Freiburg, Germany). They were adapted for tissue culture in our laboratory and were used between passages 11 and 20 after explantation. The LXFL 529 line grew in vitro with a doubling time of 118 ± 30 h, the CXF 280 line with a 52 ± 14 h doubling time and CXF 243 with a doubling time of 49 ± 27 h.

Retinoids

Stock solutions of all-trans RA and 13-cis RA were prepared under subdued lighting in dimethyl sulfoxide (DMSO) at a concentration of 6 x 10⁻3 M. The arotinoid Ro 40-8757 was dissolved in DMSO to give a stock solution of 2 x 10⁻3 M. These solutions were stored at -80°C in the dark and were diluted in culture medium just before use.

Cell proliferation assay

Cells were cultured in RPMI 1640 nutrient medium (Gibco, UK) supplemented with 10% foetal calf serum (FCS; Gibco). They were seeded into 24 well tissue culture plates (Costar) and incubated for 24 h before drugs were added to ensure attachment of the cells. The culture medium and test substances were refreshed every 2–3 days. The total culture time

It has long been known from many studies that retinoids can inhibit growth in vitro of certain types of cancer cells and can prevent tumour formation in some animal models (Lippman et al., 1987a). Until recently, despite a number of clinical trials in various indications (Lippman et al., 1987b), retinoids have not found widespread usage for treatment of cancer. The demonstration by several groups that all-trans retinoic acid (RA) can induce complete remission in a high proportion of acute promyelocytic leukaemia patients (Huang et al., 1988; Chomienne et al., 1989; Warrell et al., 1991) has renewed clinical interest. It has also been demonstrated that one of the isomers of all-trans RA, 13-cis RA, can significantly inhibit the formation of second cancers in head and neck carcinoma (Hong et al., 1990). Recent clinical reports indicate that the combination of 13-cis RA plus interferon α is highly effective in advanced squamous cell carcinomas of the cervix (Lippman et al., 1992a) and skin (Lippman et al., 1992b).

In searching for a retinoic acid analog that might be devoid of some of the side effects associated with hyper-vitaminosis A syndrome, the arotinoid temarotene (Ro 15-0778), which has an unusual structure because it lacks a polar end group, was shown to be active both in preventing tumour formation in rats given DMBA (Bollag & Hartmann, 1987) and in inducing regression of established mammary carcinomas in these animals (Teelmann & Bollag, 1988). A number of analogs of temarotene have been synthesised to find a more potent compound for therapeutic use. The most active compound identified thus far is Ro 40-8757, an arotinoid containing a morpholine structure in the polar end group (4-[2-[[p-[(E)-2(5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-2-naphthyl)propenyl]phenoxy]ethyl]-morpholine). This compound has considerable anti-tumour activity against established mammary tumours in rats (Eliason et al., 1990b; Hartmann et al., 1992).

We have examined the ability of Ro 40-8757 to inhibit proliferation of human cancer cells in vitro. Nine different cell lines have been used for these studies: two breast cancer lines expressing estrogen receptors (MCF-7 and ZR-75-1), two breast cancer lines that do not express estrogen receptors (BT-20 and MDA-MB-231), two lung adenocarcinoma lines (A549 and HLC-1), one large cell lung cancer line (LXFL 529) and two colorectal cancer lines (CXF 243 and CXF 280). The results have also been compared to those obtained using all-trans RA and 13-cis RA.
was approximately 14 days. Cells were harvested from four replicate wells per group each time the cultures were refed. Viable, trypan blue excluding cells were counted using a hemocytometer.

**MTT assay**

The colorimetric assay for viable cell numbers was performed essentially as described previously (Eliason et al., 1990a). Cells were cultured in EM medium prepared as described (Eliason, 1984; Eliason et al., 1984) and supplemented with 5% FCS. Aliquots of 100 μl of the cell suspensions were plated in 96 well microtiter plates (Falcon-Becton Dickinson, USA) and incubated for 24 h at 37°C in a fully humidified atmosphere of 5% CO₂ in air before addition of drugs. In order to correct for the non-linearity of the MTT assay with respect to cell numbers (Plumb et al., 1989), a control cell titration curve was included in each assay (Eliason et al., 1990a).

The drugs were added in 100 μl of medium containing 0.1% of DMSO to wells in which the highest cell concentration was plated. The optimal concentration for each cell line was determined in preliminary studies. For MCF-7 and ZR-75-1, the highest cell concentration used was 800 cells well⁻¹. For MDA-MB-231, 200 cells well⁻¹ were plated. For BT-20, LXFL 529 and CXF 243, the optimal concentration was 2400 cells well⁻¹ and for CXF 280, 1200 cells well⁻¹ were plated. Every 2 or 3 days, a portion of the medium (100 μl) was removed from the wells and a 2-fold concentrated solution of fresh drug was added in the same volume of medium. After a total of 10 days incubation, 50 μl of a 3 mg ml⁻¹ solution of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was added. The HLC-1 and A549 lines grew much faster, so that the maximum numbers of cells plated were 300 and 200 cells well⁻¹, respectively. The maximum duration of incubation for these two lines was only 4 days and 5 days, respectively.

The cells were incubated with MTT for 6 h at 37°C after which time, 50 μl of a 25% (w/v) solution of sodium dodecyl sulfate (SDS) with a pH of 2.0 was added. The plates were incubated overnight to dissolve the formazan crystals and then the absorbance at 540 nm was measured using a microplate reader (Bio-rad, model 3550).

The relationship between log cell number and log absorbance was determined by least squares regression analysis and this was used to relate the absorbance measured in the drug treated groups to number of cells as has been described (Eliason et al., 1990a). Regression lines for logit percent survival vs log drug concentration were used to calculate the doses of compounds resulting in a 50% reduction in cell numbers compared to control cultures (IC₅₀).

**Cell cycle analysis**

Two treatment protocols were used to examine the effect of Ro 40-8757 on cell cycle parameters. In the first experiment, ZR-75-1 cells were suspended in EM medium containing 5% FCS at a concentration of 1 x 10⁶ cells ml⁻¹ and 5 ml aliquots were seeded in T-25 tissue culture flasks (Falcon-Becton Dickinson, USA). The cells were incubated at 37°C for 5 days, at which time, the medium was removed, and replaced with fresh medium containing 1 x 10⁶ cells ml⁻¹ or with fresh medium alone (controls). The cells were incubated for 1 more day and then harvested using typano-EDTA solution. In the second experiment, the same concentration of cells was plated 1 day before medium removal and treatment with Ro 40-8757.

After harvesting, the cells were stained with propidium iodide according to the instructions supplied with the test kit (CycleTEST DNA Reagent Kit; Becton Dickenson Immunocytochemistry Systems USA). The cell cycle parameters were analysed using an Epics C flow cytometer (Coulter Electronics, Hialeah FL, USA). The proportions of cells in G₀ + G₁, S and G₂ + M were determined by the PARAD program supplied by Coulter.

**Clonogenic assay**

A methylcellulose based medium was used to assay the effect of all-trans RA and Ro 40-8757 on colony formation by HLC-1 and A549 cells (Eliason et al., 1984; 1985). The methylcellulose concentration was 0.9% in EM medium supplemented with 5% FCS. The drugs were added to the plates after 1 day of incubation at 37°C. Fresh drug was added on days 4 and 6. Colonies with greater than approximately 50 cells were counted after a total incubation time of 8 days.

**Statistical evaluation**

Results are expressed as mean of three or four replicates. The standard deviations were typically less than 10% in individual experiments and thus are not depicted. Where error bars are shown, they refer to standard errors for mean results from multiple experiments. Differences between means were tested using Student's t-test with P values less than 0.05 being considered statistically significant.

**Results**

The effect of Ro 40-8757 on cell proliferation has been examined by counting viable cells after various periods of treatment. The ar tinoid did not decrease the number of cells, but did slow down the rate of proliferation. The non-cytotoxic nature of this compound is also reflected by the fact that the proportion of viable cells in all treatment groups in at least 17 experiments was always greater than 90% throughout 14 days of treatment. The highest dose of ar tinoid tested in these experiments was 3 μM. The results of representative experiments with the breast cancer lines BT-20 and ZR-75-1 are shown in Figure 1 (a and b). Inhibition of the rate of proliferation, as indicated by expression of the results as percentage of cell numbers in control cultures, occurred in a dose and time dependent manner. The inhibitory effects of the ar tinoid increased during the first 13–15 days. Ro 40-8757 also inhibited growth of the KB-3-1 line, which proliferates more rapidly then the breast cancer lines and maximal inhibition appeared to be reached by day 9 (Figure 1).

The effect of Ro 40-8757 on cell cycle parameters of ZR-75-1 and MCF-7 cells was examined after 24 or 48 h incubation with the ar tinoid by staining the DNA with propidium iodide and analysing using a flow cytometer. The results are shown in Table I. Both cell lines showed a clear decrease in cells in S-phase following 24 h of treatment. In the ZR-75-1 line, this was accompanied by an increase of cells in G₀ and G₁. After 48 h treatment, the increase was shifted to the G₂ plus M peak. No increase in proportion of cells in the G₀ and G₁-phases were detected with MCF-7 cells at either time point, whereas there was an increase in G₂ plus M-phase cells after 24 h of treatment.

Figure 2 shows a comparison of the dose-response curves for Ro 40-8757 and two retinoic acid isomers, all-trans RA and 13-cis RA, with the three cell lines. The two breast cancer lines show quite different sensitivities to retinoic acids. The estrogen receptor negative line BT-20 was inhibited only at RA concentrations above 3 x 10⁻⁹ M. Growth of the estrogen receptor positive ZR-75-1 line, on the other hand, was inhibited to more than 60% at the lowest dose tested, 1 x 10⁻⁷ M. There was no difference in activity between the two retinoic acid isomers with either of these breast cancer lines. The cervical carcinoma line KB-3-1 was not responsive to treatment with 13-cis RA, whereas its growth appeared to be slightly stimulated by treatment with all-trans RA at concentrations between 3 x 10⁻⁷ and 1 x 10⁻⁶ M. The inhibitory effects of Ro 40-8757 were much greater than those seen with either of the retinoic acids and were approximately the same for all three cell lines, about 40% inhibition at a dose of 3 x 10⁻⁶ M in this experiment.

The Ro 40-8757 IC₅₀ values for three different breast cancer cell lines were determined from dose-response studies
ANTI-PROLIFERATIVE ACTIVITY OF Ro 40-8757

Concentration of Ro 40-875 (>±M)

Figure 1 Time dependency of the anti-proliferative effect of Ro 40-8757 on BT-20 a, and ZR-75-1 b, breast cancer cells and KB-3-1 cervical cancer cells c. The cells were plated at a concentration of 1 x 10^4 cells ml^-1 (panel a and b) or 1 x 10^3 cells ml^-1 (panel c) in the presence of various concentrations of Ro 40-8757. The results are expressed as percent of control (vehicle alone) numbers of viable cells counted on days 3 (open circles), 6 (closed circles), 9 (open triangles), 13 or 14 (a and b; closed triangles) or 15 (open squares).

Table 1 Effect of Ro 40-8757 on cell cycle parameters of human breast cancer cell lines

| Experiment | Cell line | Treatment time (h) | Addition | G1 + G0 | S | G1 + M |
|------------|-----------|-------------------|----------|--------|---|--------|
| 1.         | ZR-75-1   | 24                | medium   | 73     | 26| 1      |
|            |           |                   | Ro 40-8757 | 82   | 15| 3      |
| 2.         | ZR-75-1   | 24                | medium   | 64     | 21| 15     |
|            |           |                   | Ro 40-8757 | 80   | 17| 3      |
|            |           | 48                | medium   | 71     | 27| 2      |
|            |           |                   | Ro 40-8757 | 69   | 19| 12     |
|            | MCF-7     | 24                | medium   | 62     | 34| 4      |
|            |           |                   | Ro 40-8757 | 64   | 16| 20     |
|            |           | 48                | medium   | 57     | 31| 12     |
|            |           |                   | Ro 40-8757 | 63   | 26| 11     |

Figure 2 Comparison of the dose-response curves for Ro 40-8757 (solid bars), all-trans RA (hatched bars) and 13-cis RA (open bars) with BT-20 a, ZR-75-1 b and KB-3-1 c cells. Cells were plated as described in Figure 1. Viable cells were counted on day 12 and results are expressed as a percentage of cell numbers in control wells.

using an indirect colorimetric assay for viable cell numbers (Table II). These values indicate that the MDA-MB-231 line is the most sensitive to inhibition by the arotinoid. The ZR-75-1 line is the least sensitive and MCF-7 has intermediate sensitivity.

The anti-proliferative activities of Ro 40-8757, all-trans RA and 13-cis RA have also been examined using the MTT assay with 3 lung cancer cell lines. Two of the lines, the adenocarcinoma lines A549 and HLC-1, are widely used and have been passage many time in vitro before assay. The third line,
Table II Inhibitory activity of Ro 40-8757 on human breast cancer cell lines in an MTT assay

| Cell line   | IC50 ± s.e.m. | Number of experiments |
|-------------|---------------|-----------------------|
| MDA-MB-231 | 1.2 ± 0.6 x 10^{-7} M | 7                     |
| MCF-7       | 3.8 ± 0.5 x 10^{-7} M | 15                    |
| ZR-75-1     | 9.4 ± 2.8 x 10^{-7} M | 15                    |

Figure 3 Comparison of the IC50 values for all-trans RA, 13-cis RA and Ro 40-8757 obtained with three lung cancer cell lines. The results show the mean values obtained from 2–6 (n) independent experiments using the MTT assay. Solid bars represent results for Ro 40-8757, hatched bars for all-trans RA treated cells, and open bars 13-cis RA. Vertical bars represent standard errors of the mean. Asterisks represent P values <0.05 compared to all-trans RA. Crosses represent P values <0.05 compared to 13-cis RA.

LXFL 529, is derived from a lung large cell carcinoma xenograft line and was recently adapted to growth in vitro. The results showing the mean IC50 values from several independent experiments are shown in Figure 3. The IC50s for the two retinoic acid isomers as well as for Ro 40-8757 were similar for the rapidly growing adenocarcinoma lines. The assay times for these two lines in the MTT assay system were shorter than for the other lines used in these studies because of their rapid growth rate. Therefore they were also assayed in a clonogenic assay employing 7 days of incubation with the compounds. The results are summarised in Table III. The arrotinoid had no effect on colony formation by either cell line at concentrations up to 1 x 10^{-8} M. All-trans RA at the highest dose inhibited colony numbers formed by both cell lines by 20–25%, but these differences were not statistically significant.

The large cell lung line, LXFL 529, had an unusual pattern of reactivity to the retinoic acid isomers. It was significantly less sensitive to 13-cis RA than it was to all-trans RA. The arrotinoid was significantly more active (IC50 = 3.9 ± 0.6 x 10^{-8} M) than either retinoic acid, being 4-fold more potent than all-trans RA and 24-fold more active than 13-cis RA.

The two colorectal cell lines were very sensitive to the growth inhibiting effects of the retinoic acids (Figure 4). The mean IC50 values were between 3 x 10^{-7} M and 5 x 10^{-7} M. There were no significant differences between the two isomers in activity. Both lines were significantly more sensitive to Ro 40-8757 than to all-trans RA or 13-cis RA. The CXF 243 line was the most sensitive, with a mean IC50 of 6 x 10^{-8} M.

Discussion

The arrotinoid Ro 40-8757 was initially identified as a potential anticancer compound because of its activity against chemically induced rat mammary cancers (Eliason et al., 1990b; Hartmann et al., 1992). The results of this study demonstrate that it also has direct anti-proliferative activity in vitro against four different human breast cancer cell lines in vitro as well as against a lung large cell carcinoma line and two colorectal cancer cell lines.

This arrotinoid inhibited cell growth, but was not directly cytotoxic. Furthermore, there appeared to be no specific block in cell cycle in the two breast cancer lines examined. These aspects of the activity of Ro 40-8757 appear to be similar to the results found with many cell lines treated with all-trans RA and other retinoids in vitro (Lippman et al., 1987a). However, the anti-neoplastic effects of all-trans RA in acute promyelocytic leukaemia are the result of inducing the leukaemic cells to differentiate into terminally differentiated mature cells (Castaigne et al., 1990), and we have no evidence that Ro 40-8757 induces cell differentiation in the lines we have examined, at least at the level of observation using light microscopy.

Table III Effect of all-trans RA and Ro 40-8757 on colony formation by A549 and HCL-1 lung cancer cells

| Cell line | Compound  | Control | 10^{-11} M | 10^{-10} M | 10^{-9} M | 10^{-8} M | 10^{-7} M | 10^{-6} M |
|-----------|-----------|---------|------------|------------|-----------|-----------|-----------|-----------|
| A549      | all-trans RA | 126 ± 17 | 163 ± 26 | 170 ± 14 | 179 ± 18 | 149 ± 25 | 149 ± 34 | 93 ± 17 |
| A549      | Ro 40-8757 | 154 ± 12 | 142 ± 11 | 134 ± 16 | 135 ± 15 | 154 ± 16 | 151 ± 15 | 166 ± 22 |
| HLC-1     | all-trans RA | 364 ± 37 | 356 ± 20 | 341 ± 12 | 309 ± 21 | 314 ± 7 | 307 ± 18 | 281 ± 45 |
| HLC-1     | Ro 40-8757 | 282 ± 9 | 309 ± 19 | 264 ± 25 | 366 ± 43 | 351 ± 45 | 325 ± 7 | 369 ± 28 |
The anti-proliferative activity of Ro 40-8757 was fully apparent only after extended periods of incubation up to 1 week or more. Other agents that have similar time courses are often ligands for nuclear receptors such as the retinoids, vitamin D, thyroid hormone, and the steroid hormones. Biological modifiers like the interferons also act in this way. The delay in measurable effect with these agents is due to the fact that they specifically stimulate new gene expression and thereby cause changes in cellular phenotypes.

The basic chemical structure of Ro 40-8757 is related to the arotinoid analogs of retinoic acid. However, because it does not have an acidic group, it does not bind to the nuclear retinoid acid receptors (Crettaz et al., 1990; Eliason et al., 1990b). Furthermore, it does not activate transcription in a trans-activation assay using a reporter gene with a retinoic acid responsive element and it is inactive in many functional assays in which all-trans RA is highly active, such as induction of differentiation by HL-60 leukemia cells and reduction of chemically induced skin papilloma size in mice (Eliason et al., 1990b).

If the growth inhibitory effects of Ro 40-8757 are mediated by a metabolite that binds to and activates the RA receptors, then it would be expected that the parent compound should have a lower activity in vitro than all-trans RA, because few of the synthetic retinoids that have been tested for receptor binding have higher affinities than this physiological ligand (Crettaz et al., 1990; Eliason et al., 1990b). However, in most of the cell lines examined, the arotinoid inhibited proliferation at considerably lower concentrations than did all-trans RA. This difference was greatest in the lines BT-20, CxF 245 and, in particular, KB-3-1. Taken together our results suggest that the anti-proliferative activity of Ro 40-8757 may be mediated through a mechanism that does not involve the RA receptors.

Interestingly, 13-cis RA has a similar anti-proliferative activity to that of all-trans RA in all of the lines except the large cell lung carcinoma line (LXFL 529). Chomienne et al. (1990) have reported that 13-cis RA is also less potent than all-trans RA for inducing differentiation of blast cells from patients in vitro. The affinity of the cis isomer for binding to RA receptors is significantly weaker than that of all-trans RA by about 5- to 10-fold (Crettaz et al., 1990; Eliason et al., 1990b). This implies that all-trans RA should be the more active isomer in most tests as it is with LXFL 529 and acute promyelocytic leukemia cells. However, the fact that the two isomers have nearly identical activities with many cell lines indicates that these cells can isomerise the cis form to the trans configuration and that the few cell types that do not show this equivalence of activity may lack this isomerase activity.

The arotinoid was much less active against the two long established lung adenocarcinoma lines than it was against the other cancer cell lines examined in these studies. This lack of activity was not merely due to the shorter incubation times resulting from the more rapid proliferation rate of these cells, because it was also not active in the clonogenic assay employing a longer treatment time with the drug.

In conclusion, this compound inhibits growth of cells from tumours of epithelial cell origin. The mechanism of action of this growth inhibition is not understood at this time. Interaction with estrogen receptors can be ruled out because it is as effective on breast cancer cell lines, such as BT-20 and MDA-MB-231, that do not have estrogen receptors, as it is on lines that do express functional receptors. Although we cannot rule out the possibility that a metabolite of Ro 40-8757 may bind to either the RA or estrogen nuclear receptors, our current findings suggest that its anti-proliferative activity may be independent of interaction with these receptors. Clearly, more work is required to determine the mode of action of this arotinoid.

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References

BOLLAG, W. & HARTMANN, H.R. (1987). Inhibition of rat mammary carcinogenesis by an arotinoid without a polar end group (Ro 15-0778). Eur. J. Cancer Clin. Oncol., 23, 131–135.

CASTAIGNE, S., CHOMIENNE, C., DANIEL, M.T., BALLERINI, P., BERGER, R., FENAUX, P. & DEGOS, L. (1990). All-trans retinoic acid as a differentiation therapy for acute promyelocytic leukaemia. 1. Clinical results. Blood, 76, 1704–1709.

CHOMIENNE, C., BALLERINI, P., BAILLAND, N., AMAR, M., BERARD, J.F., BOUVIN, P., DANIEL, M.T., BERGER, R., CASTAIGNE, S. & DEGOS, L. (1989). Retinoic acid therapy for promyelocytic leukaemia. Lancet, ii, 746–747.

CHOMIENNE, C., BALLERINI, P., BALITRAND, N., DANIEL, M.T., FENAUX, P., CASTAIGNE, S. & DEGOS, L. (1990). All-trans retinoic acid in acute promyelocytic leukaemias. 2. In vitro studies — structure-function relationship. Blood, 76, 1710–1717.

CRETTAZ, M., BARON, A., SIEGENTHALER, G. & HUNZIKER, W. (1990). Ligand specificities of recombinant retinoic acid receptors RAR-alpha, RAR-beta, RAR-gamma. Biochem. J., 272, 391–397.

ELIASON, J., RAMUZ, H. & KAUFMANN, F. (1990a). Human tumor cells exhibit a high degree of selectivity for steroidsomers of verapamil and quinidine. Int. J. Cancer, 46, 113–117.

ELIASON, J., TEELMANN, K. & CRETZAZ, M. (1990b). New retinoids and the future of retinoids in skin cancer. In Retinoids in Cutaneous Malignancy, Marks, R. (ed) pp 157–170. Blackwell: Oxford.

ELIASON, J.F. (1984). Long-term production of hemopoietic progenitors in cultures containing low levels of serum. Exp. Hematol., 12, 559–567.

ELIASON, J.F., FEKETE, A. & ODARTCHENKO, N. (1984). Improving techniques for clonogenic assays. Recent Results Cancer Res., 94, 267–275.

ELIASON, J.F., AAPRO, M.S., DECREY, D. & BRINK-PETERSEN, M. (1985). Non-linearity of colony formation by human tumour cells from biopsy samples. Br. J. Cancer, 52, 311–318.

HARTMANN, D., TEELMANN, K., ELIASON, J., KAUFMANN, F. & KLAUS, M. (1992). Ro 40-8757, a novel arotinoid with anti-cancer activity. In Retinoids. Progress in Research and Clinical Applications, Livrea, M.A. & Packer, L. (eds) Marcel Dekker, Inc.: New York.

HONG, W.K., LIPPMAN, S.M., ITRI, L.M., KARP, D.D., LEE, J.S., BYERS, R.M., SCHANTZ, S.P., KRAMER, A.M., LOTAN, R., PETERS, L.J., DIMERY, I.W., BROWN, B.W. & GOEFFERT, H. (1990). Prevention of 2nd primary tumours with isotretinoin in squamous-cell carcinoma of the head and neck. N. Engl. J. Med., 323, 795–801.

HUANG, M.-E., YE, Y.-C., CHEN, S., CHAI, J.-R., LU, J.X., ZHOU, L., GU, L.-J. & WANG, Z.-Y. (1988). Use of all-trans retinoic acid in the treatment of acute promyelocytic leukaemia. Blood, 72, 567–572.

LIPPMAN, S.M., KESSLER, J.F. & MEYSKENS, F.L. Jr. (1987a). Retinoids as preventive and therapeutic anticancer agents (Part I). Cancer Treat. Rev., 13, 191–207.

LIPPMAN, S.M., KESSLER, J.F. & MEYSKENS, F.L. Jr. (1987b). Retinoids as preventive and therapeutic anticancer agents (Part II). Cancer Treat. Rev., 13, 493–515.

LIPPMAN, S.M., KAVANAGH, J.J., PAREDES-ESPINOZA, M., DELGADILLO-MADERUENO, F., PAREDES-CASILLAS, P., HONG, W.K., HOLDENER, E. & KRAFOCK, I.H. (1992a). 13-cis-retinoic acid plus interferon alpha-2a — highly active systemic therapy for squamous cell carcinoma of the cervix. J. Natl Cancer Inst., 84, 241–245.

LIPPMAN, S.M., PARKINSON, D.R., ITRI, L.M., WEBER, R.S., SCHANTZ, S.P., OTA, D.M., SCHUSTERMAN, M.A., KRAFOCK, I.H., GUTTERMAN, I.S. & HONG, W.K. (1992b). 13-cis-retinoic acid and interferon alpha-2a — effective combination therapy for advanced squamous cell carcinoma of the skin. J. Natl Cancer Inst., 84, 235–241.
PLUMB, J.A., MILROY, R. & KAYE, S.B. (1989). Effects of the pH dependence of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide-formazan absorption on chemosensitivity determined by a novel tetrazolium-based assay. Cancer Res., 49, 4435–4440.

TEELMANN, K. & BOLLAG, W. (1988). Therapeutic effect of the arotinoid Ro 15-0778 on chemically induced rat mammary carcinoma. Eur. J. Cancer Clin. Oncol., 24, 1205–1209.

WARRELL, R.P., FRANKEL, S.R., MILLER, W.H., SCHEINBERG, D.A., ITRI, L.M., HITTELMAN, W.N., VYAS, R., ANDREEFF, M., TAFURI, A., JAKUBOWSKI, A., GABRILOVE, J., GORDON, M.S. & DMITROVSKY, E. (1991). Differentiation therapy of acute promyelocytic leukemia with Tretinoin (all-trans-retinoic acid). N. Engl. J. Med., 324, 1385–1393.