

SEMINAR

Molecular Events as Targets of Anticancer Drug Therapy

Adorján ASZALÓS,¹ Sándor ECKHARDT²

¹Food and Drug Administration, Washington DC, USA; ²National Institute of Oncology, Budapest, Hungary

The aim of this review is to introduce some molecular targets for cancer chemotherapy, with comments on their mode of action, preclinical and clinical results. The representatives of the following groups are covered: phosphorylation inhibitors,

protein kinase modulators, receptor antagonists, immunomodulators, differentiating agents, multidrug resistance modulation, telomerase inhibitors, and bioreductive agents. (Pathology Oncology Research Vol 3, No 2, 147-158, 1997)

Key words: cancer, molecular targets, chemotherapy

Introduction

Search for specific inhibitors of cancer cells is a complex and elusive approach. These "specific" drugs would target cellular events related more to cancerous cell growth than to normal cells, therefore, they would be less toxic to the host. In this short review, examples of potential anti-cancer drugs acting on e.g. gene expression, phosphorylation of certain proteins, modulation of the immune system, cell differentiation, will be discussed with an emphasis on those, which already showed promising clinical results. (No attempt was made to incorporate biological agents, gene therapy and some other modalities such as angiogenesis inhibitors.)

1 Phosphorylation inhibitors

Signals received at the surface of a cell are transduced via a complex cascade of biochemical reactions to the nucleus involving mostly phosphorylation and dephosphorylation of specific proteins. They are usually phosphorylated at serine/threonine or at tyrosine sites and multiple signals are generated at the same time. The cell integrates the different signals and decides to choose among proliferation, death, expression of receptors or just

stay quiescent.¹ Signals that allow a cell to become cancerous also involve phosphorylation cascades. Interruption of this unwanted actions at specific sites, related to unwanted cell proliferation, is the aim of using drugs to block phosphorylation.

Somatostatin

Somatostatin is a cyclic peptide hormone exhibiting several activities, including inhibition of growth hormone and gut hormone secretion². Because of its short half life, analogs with longer half lives were prepared. Sandostatin (Octreotide) is in the clinic for treating e.g. carcinoid syndrome, acromegaly³ and diarrhea⁴. Other studies revealed that many tumor cells have receptors for somatostatin and its analogs⁵ and that these drugs could be useful in tumor-growth inhibition.⁶⁻⁸

As a mode of action it has been shown that epidermal growth factor (EGF) initiated tyrosine phosphorylation was blocked by somatostatin analogs.⁹ Also, somatostatin receptor-somatostatin complex was found to act as tyrosine phosphatase and this effect was related to growth inhibition.¹⁰ A schematic presentation of signal initiation and transduction through the EGF receptor is shown in (Fig.1.)¹¹. Other activities of somatostatin and its analogues are related to antisecretory functions on pancreatic, gastric and intestinal hormones. By this action, secretion of gastrin, secretin and cholecystokinin are suppressed. These hormones are likely to stimulate growth of pancreatic cells.⁷ Also, EGF stimulates pancreatic cancer cells in an autocrine fashion, involving centrosomal separation.

Received: Jan 25, 1997; *accepted:* May 28, 1997

Correspondence: Adorján ASZALÓS PhD.; Department of Health, Education and Welfare, Food and Drug Administration; 200 C Street SW, Washington DC 20204, USA. Tel: (202) 205-4177 Fax: (202) 205-4758

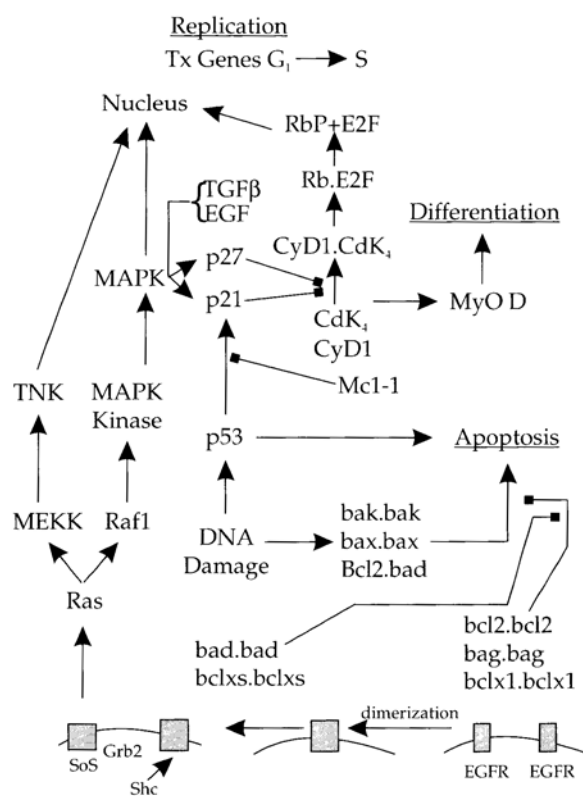


Figure 1. Parts of the signal transduction pathways that can lead to either replication, differentiation or apoptosis. Some of the proteins involved in these signal transduction pathways are explored as diagnostic markers (p53, bcl2, Rb) or as possible targets of cancer chemotherapeutic agents (several phosphorylases and phosphatase enzymes, CyD1, Cdk1, Ras).

Production of EGF is reduced by somatostatin and in turn reduces cell growth, perhaps through the microfilament system or through Ca^{2+} homeostasis.^{12,13}

It was speculated that growth factors, such as IGF-1 and TGF- β may also be involved in tumor growth regulation, since some tumors express receptors for these factors.^{14,15} Somatostatin analogues can reduce the serum concentration of these factors and thereby interfere with the growth of tumors which express receptors for endocrine factors.^{16,17} The binding of analogues to tumor cells was used to detect and localize certain tumor types: ¹²⁵I-tyr3-octreotide for neuroblastoma,¹⁸ and ¹¹¹In-DTPA-D-octreotide for carcinoids and gastrinomas.¹⁹

Encouraged by the positive preclinical results,^{20,25} clinical trials were started to cure cancers, such as colorectal, small cell lung and pancreatic cancers in addition to cancers known to bear somatostatin receptors or are under the influence of growth hormones. Investigations on the effect of octreotide on different hormones in humans revealed that this drug acts mostly on growth hormones and TSH levels, besides gastroenteropancreatic hormones. Octreotide is distributed rapidly after iv. or sc. administration

with a half life of about 1.5 h. The parent hormone, somatostatin has a half life of minutes only.

Recent clinical trials showed some success. Buzzati et al. treated patients with growth hormone producing densely granulated pituitary adenoma with 300 mg/day octreotide. They found that growth hormone level was reduced to about 30%, to the same extent as by surgery. Combined modality reduced further growth hormone secretion.²⁶ Studies with leukemias was also encouraging. Cells from patients with ALL (n=7), AML (n=21) and CLL (n=2) were treated ex vivo with somatostatin and octreotide. Spontaneous growth of cells was arrested by both drugs in about one-third the cases.²⁷ The use of octreotide in the clinical management of acromegaly was summarized recently.²⁸

Flavopiridol

Inhibition of tyrosine phosphorylation can be achieved by induction of phosphatases as with some somatostatins (see above) or by direct inhibition of tyrosine kinase activity. One agent in clinical trial with tyrosine kinase inhibitory activity is flavopiridol.

Flavopiridol [(-)-cis-5,7-dihydroxy-2-(2-chlorophenyl)-8-[4-(3-hydroxy-1-methyl) piperidinyl]-4H-1-benzopyran-4-one], was found to be inhibitory to breast, lung and prostate carcinoma cells, and 60 and 400 times more potent than other tested phosphorylation inhibitors, quercetin or genistein, respectively.²⁹ Flavopiridol, *in vitro*, was not cytotoxic to stationary-phase cells but inhibited exponentially growing cells. In other studies flavopiridol blocked histone H1 kinase activity affected by p34/cdc2.³⁰ It was also found that phosphorylation of p34/cdc2 kinase was blocked at tyrosine and threonine residues and that this blocking was specific and that flavopiridol did not inhibit the expression of p34/cdc2. It was shown recently³¹ that substitution of chlorine with brom or fluor increased selectivity of the analog towards cdc2. Other analogs exhibited selectivity for other cyclin dependent kinases.

In vivo flavopiridol exhibits moderate retardation of xenografted colorectal and prostate tumors in mice with considerable bone marrow toxicity and weight loss. However, dividing the daily dose for multiple daily doses results in arrest of tumor growth with minimal weight loss. Ongoing clinical trials will determine the usefulness of this agent.

2 Modulation of Protein Kinase Activity

Cell signals are translated from the cell surface to the nucleus through second messengers and interacting enzyme systems. Best studied second messengers are cAMP, Ca^{2+} and the inositol phosphates. One of the interacting type of enzymes are the protein kinases (PK). Research with the aim to modulate the function of PKC

and its isoenzymes was hoped to yield compounds able to interrupt signals of cancer cells. Similar results are expected from PKA modulators.

Bryostatin-1

Modulation of PKC was target of many investigations for the purpose of cancer chemotherapy.³² A natural substance, Bryostatin-1 was shown to activate PKC without being a tumor promoter.³³ The activation followed by release of TNF- α in MONO-MAC-6 cells.³⁴ In this activity it synergized with lipopolysaccharide. Blockade of PKC or the receptor for lipopolysaccharide, CD14, resulted in decreased TNF- α release, indicating two signal transduction pathways for this cytokine. Bryostatin-1 was found to induce apoptosis and inhibit the growth of WSU-DLC2 cells.³⁵ It was found to synergize with vincristin in these activities. Also these two drugs acted synergistically in the oncogenic signal transduction pathway, namely, suppressed bcl-2 and increased p53 expression. The same was found in large cell lymphoma cells upon treatment with 200 nM bryostatin-1.³⁶ Furthermore, bryostatin inhibits the growth of U937 cells, induces p21^{Waf1}, inhibitor of cdk2 activity, and this event was found to be followed by dephosphorylation of cdk2 (on threonine 160).³⁷ Besides its antitumor activity, bryostatin was also shown to induce IL-2 receptors on CD4 and CD8 human lymphocytes,³⁸ to express mRNA for granulocyte-macrophage colony stimulating factor³⁹ and to differentiate chronic lymphocytic leukemia⁴⁰ and non-Hodgkin lymphoma cells.⁴¹ Preclinical toxicological studies in mice revealed that high toxic doses of bryostatin, 75 mg/kg, causes kidney and lung tissue necrosis. At lower, tolerated doses the initial toxic symptoms, lethargy, weight loss and reduced hematocrit returned to normal in the recovery period. Phase I study with 35 patients bearing various tumors defined a usable schedule: 25 mg/m², iv, within 1 h, once a week for 3 weeks.⁴² The dose limiting factor was myalgia. Other symptoms were headache and phlebitis. The data concerning the effect of bryostatin on IL-6 and TNF- α are controversial. At present further clinical trials are being conducted with lymphocytic leukemia and lymphoma patients.

Interestingly, bryostatin-1 was shown to block the MDR1 gene product, P-glycoprotein, function, independent of its ability to modulate PKC activity. In resistant cancer cells, depleted from PKC by TPA treatment, bryostatin-1 still modulated P-glycoprotein activity.⁴³

8-Cloroadenosine 3'5'-cyclic monophosphate (8-Cl-cAMP)

Cyclic adenosin monophosphate (cAMP) is a second messenger signaling through cAMP-dependent protein kinases. The ratio of expression of the two types of these enzyme, type I and type II, is different in normal, differ-

entiated and in malignant cells. cAMP and its analogs can modulate the expression of these two types of enzymes and can cause for example differentiation of malignant cells. North et al.⁴⁴ showed that 8-Cl-cAMP, one of the most investigated cAMP analog, inhibits the expression of RI alpha, the cAMP-binding regulatory subunit of PKAI, associated with malignant transformation. It also induces the regulatory subunit of PKAII, found in normal cells and thereby causes cell growth arrest. Activity of PKAI seems to interfere with Topo II nuclear enzyme-related events. In other studies Vintermyr et al.⁴⁵ showed that 8-substituted cAMP analogs i.e. 8-Cl-cAMP and 8-NHZ-cAMP can induce irreversible growth arrest after these cAMP analogs are metabolized in carcinoma MCF-7 cells. When metabolism is blocked by inhibiting the activity of phosphodiesterases the ability of these drugs to cause irreversible growth arrest is also inhibited. Also in MCF7 cells, 8-Cl-cAMP metabolite, possible 8-Cl-adenosine, induced apoptosis-like cell death, without DNA fragmentation and without activation of cAMP kinase.⁴⁶ It is possible, that 8-Cl-cAMP induction of apoptosis is linked to a silent bcl-x_L gene and not to bcl-2.⁴⁷

The mechanism, that 8-Cl-cAMP is associated with the change in the ratio of PKA isoenzymes was also shown in vivo.⁴⁸ It was found that 8-Cl-cAMP treated animals had slower tumor growth for both tumor types; cAMP binding protein content of tumors in 8-Cl-cAMP treated animals was reduced; treated xenografts displayed reduced ratio of RI/RII regulatory subunits of PKA.

In vivo 8-Cl-cAMP may have dual effects. The effect of 8-Cl-cAMP was found to be different in vivo in hormone (estrogen, progesteron) dependent and hormone independent mammary tumor cells.⁴⁹ In hormone dependent cells 8-Cl-cAMP additively stimulated cell proliferation with estrogen receptor stimulation. In hormone independent cells 8-Cl-cAMP inhibited cell proliferation. Both studied cell types expressed estrogen and progesteron receptors. This finding indicates complex cellular regulation mechanisms by the different cell signals and may limit the use of 8-Cl-cAMP as protein kinase modulator in some (hormon dependent) tumors.

3 Receptor antagonists

Trifluoro-5-methyl-4-isoxazol carboxy-p-toluid

This receptor antagonist, coded SU101, blocks signals transmitted through platelet-derived growth factor (PDGF) receptor and through vascular endothelial growth factor (VEGF) receptor.⁵³⁻⁵⁵ SU101 and its metabolite, (SU20), inhibits cell growth of several established ovarian, prostate and leukemia cell lines at micromolar concentrations, where the responding cell lines express PDGF and/or VEGF receptors.

In vivo, the growth of human glioma or glioblastoma cells implanted into BALB/c nude mice was inhibited by doses 15 to 20 mg/kg/d, ip., if administered daily.⁵³ In the clinic, patients with recurrent glioma were infused during 24 h period/week for 4 weeks.⁵⁴ Complete elimination of SU20 was approximately 320 h, and was dose dependent between 15 and 40 mg/m². The long clearance time indicates the possibility of infrequent maintenance schedule.

4 Modulation of the immune system

Stimulation of the immune system to combat neoplastic diseases is an old concept. In recent days, nonspecific active immunotherapy is being changed to more specific immunization. Namely, it was observed that tumor bearing host is immunosuppressed and was postulated that active stimulation of the immune system may have advantages. Administration of recombinant cytokines or drugs inducing cytokine generation in the host was tried in many clinical trials. This discussion is restricted to a drug, that is an example of cytokine inducing agents.

Bropirimine

One of the most successful potential interferon (IFN) inducer drug is Bropirimine. This is a nucleoside analogue and was shown to induce IFN- α and other lymphokines to stimulate the cellular immune mechanism and to suppress tumor growth.⁵⁵⁻⁵⁶ The same mechanism of action renders this compound antiviral.⁵⁸⁻⁵⁷ The in vivo antitumor effect of bropirimine was evaluated in renal cell carcinoma bearing euthymic hairy and athymic nude BALB/c mice.⁵⁹ Renal carcinoma is known to be resistant to chemotherapy, probably due to expression of the multidrug resistant pump(s). For this reason adaptive transfer of immune cells (LAK, TIL) were tried in the past.⁶⁰ Bropirimine was thought to have good potential in this type of cancer. In the study of Fujioka et al⁵⁹ mice was inoculated with adenocarcinoma cells and then treated with bropirimine, 100-2000 mg/kg, on day 1 or 6, po. Survival time increased to 38 days in treated animals as compared to the controls of 28 days. The effect of bropirimine on suppression of tumor growth could be reversed with simultaneous introduction of anti-asialo GM1 serum, which serum can eliminate NK and T cell activity in the presence of rabbit complement. This in vivo finding was supported by in vitro experiments. It could be shown by the ⁵¹Cr-release assay that lymphocytes isolated from the lung and spleen of 1000 mg/kg bropirimine-treated athymic mice were significantly more cytotoxic against Renca and Yac-1 cells than lymphocytes from untreated mice. These investigators also showed that serum alpha interferon level of treated mice was 9 times higher at 3h time and about 6 times higher at 6 h time after drug

administration. These experiments clearly showed the immunomodulatory effect of this drug.

Early clinical trials were encouraging. In one trial⁶¹ with 34 patients with measurable superficial transitional cell cancer of the bladder, 26 could be evaluated. Doses of 500 to 1750 mg, 20 to 70 mg/kg/day, were introduced po. 3 times/day with 2 h pauses, 3 days per week for 12 weeks. All patients with in situ carcinoma showed dose-dependent response. Complete response was seen with 1 patient at low doses, but 4 out of 6 at high doses. Only 1 patient showed complete remission with in situ plus papillary tumor and only at the highest dose level. Beside the fact that 31% overall response rate could be achieved, two other significant observations could be made. First, out of 7 patients who had previous BCG treatment without improvement, 3 had complete response to bropirimine. Second, the toxicity of this drug is possible related to its IFN inducing ability. However, IFN induction was not observed at all times with curative effects. Generally, only minimal toxicity was observed during this clinical trial.

5 Farnesyl-protein transferase inhibitors

Attachment of farnesyl residues to proteins involved in cell signal transduction is an important step in the cell proliferation process. For example, p21^{ras} protein is farnesylated after ligand binding to receptors, such as T-cell, IL-2, EGF and PDGF receptors. The farnesylated p21^{ras} anchored to membranes can interact with other proteins more effectively than without anchoring. Therefore, interruption of farnesylation, the function of enzymes involved in this process can block signal transduction. The effectiveness of inhibition of expression of the beta subunit of farnesyltransferase in blocking tumor growth was demonstrated in nude mice. Stable insertion of antisense gene into human lung carcinoma cells and use of these cells in nude mice resulted in decreased farnesyltransferase activity, ras processing, MAP kinase activity and tumor growth.⁶² It should be mentioned, that inhibition of K-ras 4B, the most prevalent ras oncoprotein in human cancers is inhibited more selectively by geranylgeranyltransferase inhibitors and by palmytoiltransferase inhibitors than by farnesyltransferase inhibitors.⁶³ Strong industrial and academic interest exist to develop inhibitors for these enzymes with the hope to control cancer cell growth in a specific way.

Farnesyltransferase can be inhibited by different type of compounds. Structural studies for the isoprenoid phosphonic acid type established that trans:trans isomer is active (IC₅₀ 30 nM) and that the isoprenoid chain length is important for the specific inhibition of this enzyme in vitro and in vivo.⁶⁴ A pentapeptide, (CBZ-L-his-tyr(OBzI)-ser(OBenz)-trp-D-ala-NHZ) was found to inhibit farnesyltransferase with an IC₅₀ of 20 nM, but is not cell permeable. However an analog of this peptide was cell perme-

able, selective against ras farnesyltransferase in vitro and in vivo.⁶⁵ The potential of ras protein farnesylation inhibitors for cancer chemotherapy was summarized by Gibbs et al.⁶⁶

In vivo studies proved the effectiveness of farnesyltransferase inhibitors. For example, S,R-N-(-L-(N-(2-amino-3-mercaptopropyl)-L-tert-leucyl)-1,2,3,4-tetrahydro-3-isoquinoliny)-carboxyl)-L-glutamine in athymic mouse implanted ip. With H-ras transformed rat-1 tumor cells showed good activity. The T/C value was 145 if the compound was injected with a dose of 45 mg/kg twice/day without detectable toxicity.⁶⁷ In other studies, the growth of human lung carcinoma, with K-ras mutation and deletion of p53 gene implanted into nude mice could be inhibited by a farnesyltransferase inhibitor, FTI-276, mimetic of the carboxy terminus of K-ras 4B.⁶⁸

Ex vivo studies of patients bearing basal cell carcinoma revealed that the expression of the alpha and beta subunits of farnesyltransferase and the membrane bound was suppressed in the cells. This finding suggests an association between this enzyme and the processing of p21^{ras} in humans.⁶⁹

6 Differentiating agents

Cancer can be regarded as disorder of cell differentiation. A cancer cell may have increased sensitivity to growth factors, decreased sensitivity to differentiation factors and may produce great amount of endogenous growth factors. A logical approach to cancer chemotherapy is to develop differentiating agents which could work specifically in cancer cells.⁷⁰ All-trans retinoic acid and butyric acid had some early success in this respect.

Butyric acid

Butyric acid is a natural substance, it occurs in healthy people, mostly in the colon. Its physiological role in humans is well summarized by Clausen.⁷¹ It was tested against several tumor cell lines and found to exhibit growth arrest and induction of morphological changes.⁷²

Mode of action studies indicate modulation of gene expression. In a recent study Buguet et al⁷³ showed that the hyperphosphorylation of retinoblastoma protein, Rb, is blocked by butyric acid. This block can be prevented by actinomycin D. The accumulation of cyclic-dependent kinase inhibitory protein mRNA, Waf1/CIP1 mRNA, in butyric acid treated BP-A31 cells and suppression of cyclin D1/PRAD1 protein in carcinoma cells provided further evidences on the involvement of regulatory proteins in butyric acid action.⁷⁴ Other studies found that in smooth muscle cells butyric acid inhibited PDGF AA, AB and BB induced proliferation probably via the suppression of the expression of c-fos, c-jun and c-myc.⁷⁵ Modulation of c-

fos and c-jun mRNA expression was also shown to occur in the U937 promonocytic leukemia cells upon treatment with 0.75 mM butyrate.⁷⁶ Simultaneous increase of heat shock protein 70 and 27 were also demonstrated.

The first clinical success with butyric acid was reported in 1983 by Novogrodsky et al.⁷⁷ However, pharmacokinetic studies indicated that in leukemia patients butyric acid is rapidly metabolized with a half life of about 6 min and with complete clearance in 1 h.⁷⁸ Because of the short half life, and fast clearance of butyric acid, derivatives with better pharmacokinetic parameters were investigated.⁷⁹ Several derivatives were synthesized and (pivaloyloxy) methyl butyrate (PMbutyrate) exhibited the best biological activities suppressing the proliferation of HL-60 leukemic cells and B16FO melanoma cells. Superior biological activity of PMbutyrate than the parent compound can be attributed to its good lipophilicity, penetration through the cell membrane. It was found⁸⁰ that the above effects are elicited significantly only by butyric acid⁸¹ or its derivatives and not by other small carboxylic acids. Other derivatives of butyric acid were also promising, e.g. triglycerin tributyrin given per os to mice and rats prolonged the life of tumor bearing animals and showed synergism with retinoic acid.

Pharmacotoxicity studies indicated that a bolus injection of 150 mg/kg PMbutyrate cause death of mice. The toxicity is related to C_{max} values and no or very little side effects can be seen with low dose continuous infusion. Dose escalation studies in baboons indicated that 4 g/kg/day, continuous infusion for 2-3 weeks results in minimal toxicity and with considerable efficacy.⁸² Continuous infusion of the butyric acid prodrug, arginine butyrate was used in patients with sickle cell anemia to increase the expression of fetal-globin gene.⁸³ For this treatment 20 to 80 g/m²/d drug was infused for 7 to 21 days depending on the results with the individual patient. Highest blood level was 0.05 mmol/l and butyric acid cleared completely in 15 min after discontinuation of infusion. No significant side effect was detected during this treatment.

An other butyric acid prodrug was shown to increase acetylation of histone H4, to induce p21Waf1 independent of p53 modulation in TSU prostate cancer cell line.⁸⁴ Phenyl butyrate is being tried in the clinic in phase I studies. With doses of 150, 225 and 285 mg/kg/day iv. injection through 120 h, each 21 days, only minimal toxicity was noted. The C_{max} was 224-479 mM/l in 2-4 h. It seems that higher doses than recently used will be necessary to achieve pharmacologically effective drug levels in patients.⁸⁵

Retinoids

Retinoids are vitamin A type compounds. In nature, vitamin A is metabolized to all-trans retinoic acid, which is the active form in regulating cell differentiation.⁸⁶ It

affects the growth and differentiation of many cell types in vitro and in vivo.⁸⁷⁻⁹⁰

The effect of retinoic acid is mediated by receptors. The first group of receptors are the nuclear receptors RAR alpha, beta and gamma. First a complex of nuclear proteins is formed and such complex binds to retinoic acid response element in the promoter region of the inducible genes, causing alteration in the rate of transcription.⁹¹ Another set of receptors are the RXR alpha, beta and gamma receptors, which bind 9-cis retinoic acid, a metabolite of retinoic acid. For chemotherapeutic purposes ligands of the RAR receptors are important.

From all the extensively tested retinoids all-trans retinoic acid was the most successful in patients with acute promyelocytic leukemia (APL). Part of the success can be attributed to the fact that an indicator of the disease, a fusion protein of PML and RAR- α genes was characterized and associated with the disease progression in APL patients. Development of a RT-PCR for the mRNA of this fused genes was then applied to follow treatment efficacy.⁹² All patients with the aberrant RAR- α mRNA responded to retinoic acid treatment, whereas patients with normal pattern of RAR mRNA did not. In a trial with 22 patients with APL 63% responded with complete remission to the treatment with 45 mg/m²/day all-trans retinoic acid for three months. The longest remission was for 13 months.⁹³ Unfortunately, most patients relapse if further treatment is discontinued. This is because resistance to retinoid treatment develops in most patients. The reason for the resistance is mostly due to poor drug distribution after initial treatment possibly due to development of malabsorption, increased metabolism or/and adsorption to intracellular proteins.⁹³

Retinoid treatment was also investigated in prevention and treatment of skin cancers. Dose dependent response rate and toxicity was demonstrated in patients with xeroderma pigmentosum using all-trans retinoic acid and with doses between 0.5-2.0 mg/kg/day.⁹⁴ A tumor reduction rate of 63% could be achieved in 2 years. In squamous cell carcinoma a 47% response rate could be obtained.⁹⁵ While successes in skin cancer and in acute promyelocytic leukemia is significant with single retinoid treatment, toxicity and development of resistance points in the direction of combination therapy, for example with INF- α .⁹⁶ Also, retinoids are promising agents in prevention of cancer.⁹⁷

7 Modulation of multidrug resistance

One of the most common and most investigated cause of resistance of cancer cells to chemotherapy is the overexpression of the MDR1 gene, and its product, a glycoprotein, p170, which is inserted into the plasma membrane and can pump out chemotherapeutic agents from cancer cells.⁹⁸ Prevention of this pumping activity is one objective

of recent cancer chemotherapy.⁹⁹ However, blocking this pumping activity is not a simple matter, since p170 is expressed in normal cells, therefore, blocking p170 function may result in host toxicity. Another problem with concomitantly administered p170 blocker is that it changes the pharmacokinetics of the cancer chemotherapeutic drug(s). This is because higher cellular and blood levels of the chemotherapeutic drug is achieved than if doses are maintained at the level what was used without blockers.¹⁰⁰ The use of blockers can increase cell and host toxicities therefore. It was discovered recently that another gene, Multidrug Resistance Associated Protein gene can also be expressed in resistant cancer cells.¹⁰¹ This gene product, multidrug resistance associated protein (MRP) also can pump out chemotherapeutic drugs from cells, albeit by a different mechanism than p170.¹⁰² It may be expressed on cell membranes alone or together with p170. MRP is expressed at low level in most of cells in humans, complicating potential treatment with blockers with potential host toxicity. Recently, besides p170 and MRP, plasma membrane efflux pumps were also discovered.¹⁰³

8-Cl-cAMP

One way to deal with p170 associated multidrug resistance is to suppress the expression of the MDR1 gene. It was shown recently that 8-Cl-cAMP can reverse MDR1 gene expression in multidrug resistant cancer cells.¹⁰⁴ 8-Cl-cAMP is in clinical trials as a cancer chemotherapeutic drug, as was discussed above. Multidrug resistance is associated with overexpression of this gene and this overexpression can be suppressed by the treatment with the differentiating agent 8-Cl-cAMP or its active metabolite, 8-Cl-adenosine. At 5 μ M concentration 8-Cl-cAMP suppresses the MDR1 gene expression in MCF-7^{T11} human breast cancer cells within 48 h.¹⁰⁵

The mode of action of 8-Cl-cAMP was associated with the inhibition of expression of RI alpha, the cAMP-binding regulatory subunit of protein kinase A I, which enzyme was associated with malignancy.^{106,107} 8-Cl-cAMP induces the regulatory subunit PKAII, found in normal cells and causes cell growth arrest. On the molecular level, 8-Cl-cAMP exerts its effect by binding to the regulatory subunit of PKA, releasing the catalytic subunit, resulting in down-regulation of RI alpha.^{108,109}

Different Pgp (P 170) blocking agents, Cremophor EL and PSC833

Search for an effective and non-toxic p170 blocker began in the 1980's. There are a good number of blockers in clinical trials.¹¹⁰

One interesting example of p170 blocker is *Cremophor EL* (Polyoxyethylene Castor Oil, POCO). Interesting,

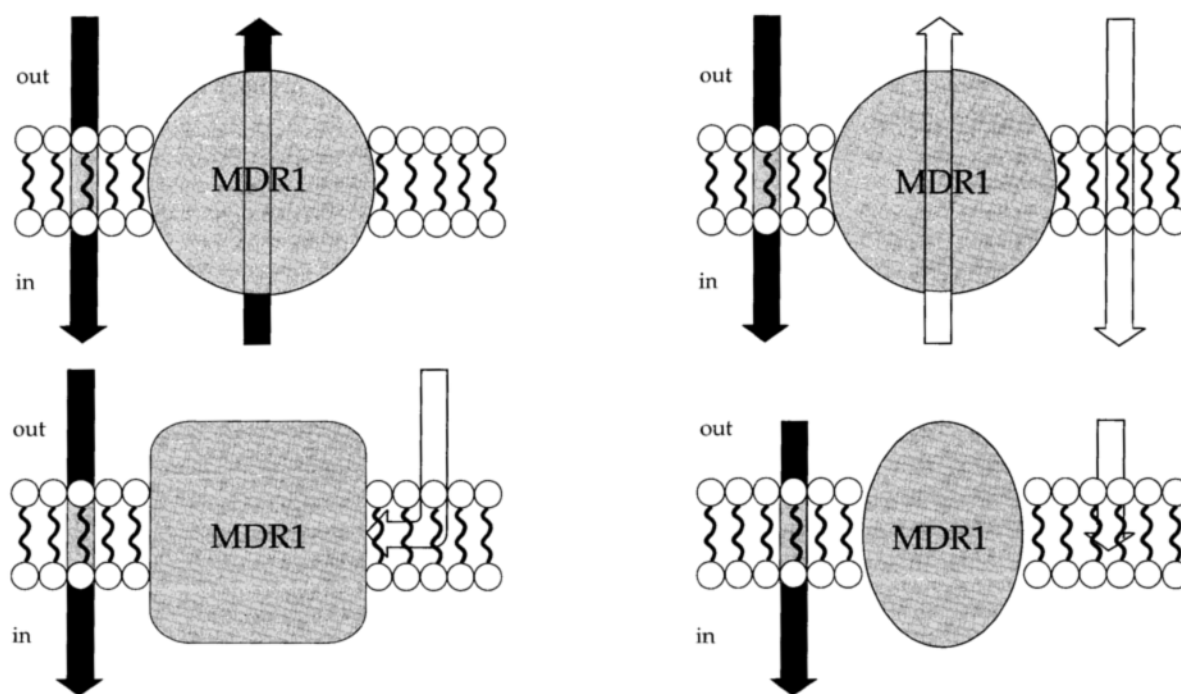


Figure 2. Schematic representation of the possible mode of action of P-glycoprotein blockers. Top left: no blocker function, substrates pumped out; top right: function of a competitive substrate; bottom left: function of a competitive substrate with additional indirect effect through the plasma membrane, inducing conformational changes in the P-glycoprotein; bottom right: function of a blocker affecting P-glycoprotein indirectly through the plasma membrane, inducing conformational changes that makes the pump non-functional.

because POCO was and is used as a formulating agent, for⁸ example for taxol, before it was discovered its p170 blocking ability. In fact it was demonstrated that after 3 h infusion of the formulated taxol, 135 to 175 mg/m² the blood level of POCO reaches about 0.1% v/v in patients.¹¹⁰ This level of POCO is known to block p170 completely. POCO is a mixture of chemical compounds and the most active inhibitor of p170 function was shown to be a molecule with two 18 carbon fatty acids linked by a polyethylene linker. In vitro POCO, together with other surfactants,¹¹¹ was shown to block the efflux of different chemotherapeutic agents from p170 expressing multidrug resistant cancer cells.¹¹² POCO inhibits [³H] azidopine labeling of Pgp in cells at a concentration of 0.003% v/v. Consistent with the in vitro findings, simultaneous use of POCO with doxorubicin in vivo, increased the survival time of mice bearing P388/ADR tumor as compared with doxorubicin alone.¹¹²

Clinical application of POCO was based on experience with taxol chemotherapy. Doses of 130 to 170 mg/m²/day taxol introduces a minimum of about 20 g POCO/patient/day. This amount of POCO yields plasma concentration (about 0.1 % v/v) sufficient to reverse Pgp function in vitro.¹¹³ Clinical trials are being conducted with initial doses of 12 g/person/day, infused through 4 to

6 h periods for several days. In experimental animals 85% of POCO is eliminated within 24 h. This pharmacokinetics indicate that no POCO accumulation can be expected when POCO is applied in several consecutive days.

Another example of p170 blocker which is in extensive clinical trials is PSC833, a compound related to the immunosuppressive drug cyclosporin A,¹¹⁴ but PSC833 has minimal immunosuppressive activity. Clinical trials were conducted with PSC833 in combination with single chemotherapeutic drugs, doxorubicin, azetoposide¹¹⁵ and with combination of chemotherapeutic drugs. Serum levels of PSC833 necessary to block p170 activity was found to be 0.4 to 0.8 mM.¹¹⁶

To avoid additional toxicities to the anticancer drugs and to minimize pharmacokinetic alterations (liver, kidney) occurring with the use of optimal doses of a single blocker, combination of different blockers at suboptimal doses was thought to be advantageous. This idea was advanced on the following bases: the different p170 blockers have different types of toxicities and they exert their effect on Pgp by different biophysical ways.¹¹⁷ E.g. verapamil blocks by being a substrate of p170 while POCO alters membrane "fluidity" and blocks p170 function indirectly through membrane effects (Fig.2.). The toxicity of these two agents are also different: verapamil (R or S) has car-

diotoxicity while POCO alters blood flow dynamics. The combination of these two agents and also of PSC833 at suboptimal doses was proven to be effective p170 blocker.¹¹⁸ This approach awaits clinical trials.

8 Telomerase Inhibitors

Recently ample evidence has been accumulated that telomerase activity plays an important role in maintaining cellular immortality. This enzyme a terminal ribonuclein-transferase of a unique structure, is responsible for maintaining telomeric repeats in germ cells.^{119,120} Telomerase may not be detected in the majority of somatic cells.¹²¹ In contrast to this finding, cancer cells in tissue culture, express telomerase activity. Kim has demonstrated that 98 of 100 immortal, but none of 22 mortal cell population contain telomerase.¹²² Thus, telomerase activity appears to be repressed in somatic cells while a reactivation occurs in immortal cells.

Indeed, by a highly sensitive PCR-based assay called Telomeric Repeat Amplification Protocol (TRAP) it was possible to detect telomerase activity in several human malignancies.¹²³ Telomerase activity was expressed in 94% of neuroblastomas,¹²⁴ in 85% of gastric¹²⁵ and in 85% of hepatocellular cancer.¹²⁶ In another study telomerase activity could not be detected in normal or benign proliferative myometrium but was present in leiomyosarcomas.¹²³ Similarly, Hiyama found telomerase activity in 130 out of 140 breast cancer patients (93%) while only 2 out of 55 samples taken from normal adjacent tissues expressed telomerase.¹²⁷ By the detailed analysis of his data he was able to demonstrate that advanced cancer was more likely to be telomerase positive (95%) than the localized (68-81%). Accordingly, he proposed that the potential prognostic value of telomerase should be explored. These observations suggest that telomerase activity is a potential target for specific inhibitory molecules which are candidate chemotherapeutic agents. Since several authors found, that the formation of G-quarter structure in telomeric DNA is required to the normal functioning of telomerase, it is obvious, that substances capable to inhibit this structural change might block cell division. Subsequently, such compounds were synthesized. Among them, 7-deaza-dATP and 7-deaza-dGTP are potent inhibitors.¹²⁸ More recently, AZT triphosphate (3'-azido-2'3'-dideoxythymidine), a well known drug used for the treatment of AIDS, entered into clinical trials.¹²⁹ Several derivatives of AZT might follow this compound, especially azido-AZT (AZTTP).¹³⁰

9 Bioreductive Agents

Tirapazamine (3-amino-1,2,4,-benzotriazine-1,4-dioxide) is the first clinically investigated drug among a new

class of antitumour agents, the benzotriazine-di-N-oxides. These molecules are bioreductive compounds characterized by their preferential cytotoxicity for hypoxic cells. When cytotoxicity was assessed in exponentially growing cells under hypoxic and aerobic conditions the result was a 4-5 times more frequent DNA damage in an oxygen deprived environment. This finding may be explained by the unique chemical structure of these bioreductive substances. In hypoxic conditions they undergo reduction yielding a highly reactive anion which is responsible for the DNA strand breaks. Thus, tirapazamine is a potent cell division inhibitor, as demonstrated in several testing systems.^{131,132}

Preclinical data show a synergism between tirapazamine and several cytostatic agents. Durand found that the synergistic effect of the combination of tirapazamine with platinum derivatives, etoposid, 5-FU, bleomycin or vinblastin was raised under hypoxic conditions while the increase was not so apparent in the presence of oxygen.¹³³ This "chemosensitizing" effect was also observed when cisplatin-resistant cells were tested. Similarly, tirapazamine proved to be a potential "radiosensitizing" agent.¹³⁴ Based on this preclinical studies¹³⁴ the drug currently entered phase I-II trials. Rodriguez reported on a 39% overall response rate at a dose level of 390 mg/m² in heavily pretreated NSCLC patients.¹³⁵ These results have been partly confirmed by others^{136,137} in administering tirapazamine with cisplatin. This combination (390 mg/m² T + 75 mg/m² DDP) had a well tolerable toxicity. Non-hematologic toxicity was manifested in muscle crampings, nausea, fatigue, skin rashes and acute hearing loss. Phase I and II studies are performed in melanoma and breast cancer as well.^{138,139} According to the limited data collected so far in these malignancies chemotherapy naive patients respond better to this combination than pre-treated individuals.

Summary

We have summarized efforts, preclinical and clinical, directed toward chemotherapy with specificity to cancer cells. As stated in the introduction, our discussion was limited to chemotherapy with drugs, excluded biologics, and only to drugs with some clinical experience.

We recognize that efforts with drugs other than discussed above are under way. For example drug developments are under way to target cyclin dependent kinases, the p53 gene and its products and elements of the metastatic process. We are not in the position to describe preclinical or clinical results on these fields yet. However, we do hope that the above summary will orient scientists and clinicians about the ongoing efforts so that they may utilize it in their own studies and practice.

References

1. Nishizuka Y: Signal transduction: crosstalk. *Trends Biochem. Sci.* 17:367-443, 1992.
2. Reichlin S: Somatostatin. *New Engl J Med* 309:1495-1501, 1983.
3. Bernard L, Grantham W, Lamberton P et al: Treatment of resistant acromegaly with a long-acting somatostatin analogue (SMS 201-995). *Ann Int Med* 105:8556-8861, 1986.
4. Katz M, Erstad B, Rose C: Treatment of severe cryptosporidium-related diarrhea with octreotide in a patient with AIDS. *Drug Intel. Clin. Pharm.* 22:134-136, 1988.
5. Lamberts SWJ, Krenning EP, Reubi JC: The role of somatostatin and its analogs in the diagnosis and treatment of tumors. *Endocrine Reviews* 12:450-482, 1991.
6. Mascardo RN, Sherline P: Somatostatin inhibits rapid centrosomal separation and cell proliferation induced by epidermal growth factor. *Endocrinology* 111: 1394-1396, 1982.
7. Schally AV: Oncological applications of somatostatin analogues. *Cancer Res.* 48:6977-6985, 1988.
8. Lamberts S W, van der Lely AJ, de Herder WW, Hofland LJ: Octreotide. *N Engl J Med* 334:246-254, 1996.
9. Liebow C, Reilly C, Serrano M, Schally AV: Somatostatin analogues inhibit growth of pancreatic cancer by stimulating tyrosine phosphatase. *Proc Natl Acad Sci US* 86:2003-2007, 1989.
10. Liebow C, Hierowski M, DuSapin K: Hormonal control of pancreatic cancer growth. *Pancreas* 1:44-48, 1986.
11. Lemmon MA, Schlessinger J: Regulation of signal transduction and signal diversity by receptor oligomerization. *Trends-Biochem Sci* 19:459-463, 1994.
12. Lamberts SWJ, Koper JW, Reubi JC: Potential role of somatostatin analogues in the treatment of cancer. *Eur J Clin Invest* 17:281-287, 1987.
13. Schally AV, Redding TW: Somatostatin analogues as adjuncts to agonists of luteinizing hormone-releasing hormone in the treatment of experimental prostate cancer. *Proc Natl. Acad Sci* 84:7275-7279, 1987.
14. Malarkey WB, Kennedy WB, Allred LE et al: Physiological concentrations of prolactin can promote the growth of human breast tumor cells in culture. *J Clin Endocrinol Metab* 56: 673-677, 1983.
15. Pollak MN, Polychronakos C, Guyda H: Somatostatin analogue SMS 201-995 reduces serum IGF-I levels in patients with neoplasms potentially dependent on IGF-I. *Anticancer Res* 9:889-892, 1989.
16. Lamberts SWJ, Uitterlinde P, DelPozo E: Sandostatin (SMS 201-995) induces a continuous further decline in circulating growth hormone and somatomedin-C levels during therapy of acromegalic patients for over two years. *J Clin Endocrinol Metab* 65:703-710, 1987.
17. Pollak MN, Polychronakos C, Guyda H: Somatostatin analogue SMS 201-995 reduces serum IGF-I levels in patients with neoplasms potentially dependent on IGF-I. *Anticancer Res* 9:889-892, 1989.
18. Martinez DA, O'Doriso MS, O'Doriso JM et al: Intraoperative detection and resection of occult neuroblastoma: a technique exploiting somatostatin receptor expression. *J Pediatr Surg* 30: 1580-1589, 1995.
19. Yamamoto K, Ishii Y, Furudate M et al: Phase 3 multicenter clinical study of ¹¹¹In-DTPA-D-octreotide (MP-1727) in patients with gastrointestinal hormone producing tumors. *Kagu Ikagu* 32:1269-1280, 1995.
20. Radulovic S et al: Somatostatin analogue RC-160 and LH-RH antagonist SB-75 inhibit growth of MIA Pa Ca-2 xenografts in nude mice. *Pancreas* 8:88-97, 1993.
21. Hajri A, Bruns C, Marbach P et al: Inhibition of the growth of transplanted rat pancreatic acinar carcinoma with the long-acting somatostatin analogue SMS 201-995. *Eur J Cancer* 27:1247-1252, 1991.
22. Upp JR, Olson Djr, Poston GJ et al: Inhibition of growth of two human pancreatic adenocarcinomas in vivo by somatostatin analogue SMS 201-995. *Am J Surg* 155:29-35, 1988.
23. Hung H, Pollak M: Synergistic reduction of expression of the IGF-I gene by the combination of somatostatin and tamoxifen. *Proc Am Soc Clin Oncol* 12:283-289, 1993.
24. Weber C, Merriam L, Koschitzky T et al: Inhibition of growth of human breast carcinomas in vivo by somatostatin analogue, SMS#201-995, treatment of nude mouse xenografts. *Surgery*, 106:416-422, 1989.
25. Davies N, Kynaston H, Yates J et al: Effect of octreotide in fusion on hepatic and tumor blood flow in two experimental models of liver metastasis. *Eur J Gastroenterol Hepatol* 7:971-974, 1995.
26. Buzzat S, Kontogeorgos G, Redelmeier DA et al: In vivo responsiveness of morphological variants of growth hormone-producing pituitary adenomas to octreotide. *Eur J Endocrinol* 133: 686-690, 1995.
27. Santini V, Lamberts SW, Krenning EP et al: Somatostatin and its cyclic octapeptide analogue SMS 201-995 as inhibitors of proliferation of human acute lymphoblastic and acute myeloid leukemia. *Leuk. Res.* 19: 707-712, 1995.
28. Melmed S, Ho K, Klibauski A et al: Clinical review 75: recent advances in pathogenesis, diagnosis and management of acromegaly. *J Clin Endocrinol* 80:3395-3402, 1995.
29. Kaur G, Stetler-Stevenson M, Sebers S et al: Growth inhibition with reversible cell cycle arrest of carcinoma cells by flavone L86-8275. *J Natl Cancer Inst* 84:1736-1740, 1992.
30. Worland PJ, Kaur G, Stetler-Stevenson M et al: Alteration of the phosphorylation state of p34cdc2 kinase by the flavone L86-8275 in breast carcinoma cells. Correlation with decreased H1 kinase activity. *Biochem Pharmacol* 46:1831-1840, 1993.
31. Carlson BA, Pearlstein RA, Naik RG et al: Inhibition of CDK2, CDK4 and CDK7 by flavopiridol and structural analogs. *Proc Am Assoc Cancer Res* 37:424, 1996.
32. Philip PA, Harris AL: Potential for protein kinase C inhibitors in cancer chemotherapy *Cancer Treatment Res* 78:271-278, 1995.
33. Pettit GR: The bryostatins. In *Progress in the chemistry of organic natural products* (Herz W, KirbyGW, Steglich W, Tamm CH, eds) Springer, Vienna, 1991, pp 153-195.
34. Steube KG, Drexler HG: The protein kinase activator bryostatin-1 induces the rapid release of TNF alpha from MONO-MAC-6 cells. *Biochem Biophys Res Commun* 214:1197-2003, 1995.
35. Mohammed RM, Diwakaran H, Maki A et al: Bryostatin induces apoptosis and augments inhibitory effects of vincristine in human diffuse large cells. *Leuk Res* 19:667-673, 1995.
36. Maki A, Diwakaran H, Redman B et al: The bcl-2 and p53 oncoproteins can be modulated by bryostatin 1 and dolastatins in human diffuse large cell lymphoma. *Anticancer Drugs* 6:392-397, 1995.
37. Asiedu C, Biggs J, Lilly M, Kraft AS: Inhibition of leukemic cell growth by the protein kinase C activator bryostatin 1 correlates with the dephosphorylation of cyclin-dependent kinase 2. *Cancer Res* 55:3716-3720, 1995.
38. Hess AD, Silankis MK, Esa AH et al: Activation of human T lymphocytes by bryostatin. *J Immunol* 141:3263-3269, 1988.
39. Sharkis SJ, Jones RJ, Bellis ML: The action of bryostatin on normal human hematopoietic progenitor is mediated by accessory cell release of growth factors. *Blood* 76: 716-720, 1990.
40. Drexler HG, Gignac SM, Jones RA et al: Bryostatin 1 induces differentiation of B-chronic lymphocytic leukemia cells. *Blood* 74:1747-1757, 1989.

41. Al-Katib A, Mohammad RM, Mohamed AN *et al*: Conversion of a high grade lymphoma tumor cell line to intermediate grade with TPA and bryostatin as determined by polypeptide analysis on 2D-gel electrophoresis. *Hematol Oncol* 8:81-89, 1990.
42. Philip PA, Rea D, Thavasu P *et al*: Phase I study of bryostatin 1: Assessment of interleukin 6 and tumor necrosis factor alpha induction in vivo. *J Natl Cancer Inst* 85:1812-1818, 1993.
43. Utz I, Hofman J, Grunicke H: Bryostatin 1 regulates multidrug resistance by PKC independent mechanism. *Eur J Cancer* 31A:513, 1995.
44. North PS, Davies SL, Ciardiello F *et al*: Overexpression of the Rialpha subunit of protein kinase A confers hypersensitivity to topoisomerase II inhibitors and 8-Cl-cyclic adenosine 3',5'-monophosphate in chinese hamster ovary cells. *Cancer Res* 54:4123-4128, 1994.
45. Vintermyr OK, Boe R, Brustugun OT *et al*: Cyclic adenosine monophosphate (cAMP) analog 8-Cl- and 8-NH₂-cAMP induce cell death independently of cAMP kinase-mediated inhibition of the G_i/S transition in mammary carcinoma cells (MCF-7). *Endocrinology* 136:1523-2520, 1995.
46. Boe R, Grejtsen BT, Doskeland SO, Vintermyr OK: 8-Cl-cAMP induces apoptotic cell death in a human mammary carcinoma cell (MCF-7) line. *Br J Cancer* 72: 1151-1159, 1995.
47. Han Z, Chatterjee D, Early J *et al*: Isolation and characterization of an apoptosis resistant variant of human leukemia HL-60 cells that has switched expression from Bcl-2 to Bcl-XL. *Cancer Res* 56:1621-1628, 1996.
48. Ramage AD, Langdon SP, Ritchie AA *et al*: Growth inhibition by 8-Cloro-cyclic AMP of human HT29 colorectal and ZR-75-1 breast carcinoma xenografts is associated with selective modulation of protein kinase A isoenzymes. *Europ J Cancer* 31A: 969-973, 1995.
49. Actis AM, Caruso SP, Levin E: Opposite effect of a cAMP analogue on tumor growth related to hormone dependence of a murine mammary tumor. *Cancer Lett* 96:81-85, 1995.
50. Harsh GR, Keating MT, Escobedo JA, Williams LT: Platelet derived growth factor (PDGF) autocrine components in human tumor cell lines. *J Neuro-Oncology* 8: 1012, 1990.
51. Antoniadis HN, Galanopoulos T, Neville-Golden J, O'Hara CJ: Malignant epithelial cells in primary human lung carcinomas coexpress in vivo platelet-derived growth factor (PDGF) and PDGF receptor mRNA and their protein products. *Proc Natl Acad Sci* 89: 3942-3946, 1992.
52. Yoshida HN, Kuniyasu H, Yasui W *et al*: Expression of growth factors and their receptors in human esophageal carcinomas: regulation of expression by epidermal growth factor and transforming growth factor alpha. *J Cancer Res Clin Oncol* 19: 401-407, 1993.
53. Cropp G, Hannah A, Mason W: Personal communication.
54. Mason W, Malkin M, Lieberman F *et al*: Pharmacokinetics of SU101, a novel signal transduction inhibitor, in patients with recurrent malignant glioma. *Proc Am Assoc Cancer Res* 37:166, 1996.
55. Stringfellow DA, Vanderberg HC, Weed SD: Interferon induction by 5-halo-6-phenyl pyrimidinones. *J Interferon Res* 1 :1-14, 1980.
56. Lotzova E, Savary CA, Stringfellow DA: 5-halo-6-phenyl pyrimidinones: new molecules with cancer therapeutic potential and interferon-inducing capacity are strong inducer of murine natural killer cells. *J Immunol* 130:965-969, 1983.
57. Wierenga W: Antiviral and other bioactivities of pyrimidinones. *Pharm. Ther.* 30:67-89, 1985.
58. Warren RP, Morrey JP, Burger RA *et al*: Murine retroviral disease enhancing effects of a pyrimidone immunomodulator. *Antiviral Res* 19:233-245, 1992.
59. Fujioka T, Ishikura K, Hasegawa M *et al*: Antitumor effect of oral administration of an interferon-inducing pyrimidinone, bropirimine, on murine renal-cell carcinoma. *Cancer Chemother. Pharmacol.* 36:7-12, 1995.
60. Rosenberg SA: Clinical immunotherapy studies in the surgery branch of the US National Cancer Institute: brief review. *Cancer Treatment* 16:115-121, 1989.
61. Sarosdy MF, Lamm DL, Williams RD *et al*: Phase I trial of oral bropirimine in superficial bladder cancer. *J. Urology* 147:31-33, 1992.
62. Sun J, Pei Z, Sebt SM: Stable expression of a 5' 400 bp antisense of the beta subunit of farnesyltransferase in human lung carcinoma blocks oncogenic signaling in vitro and in vivo. *Proceedings Am Assoc Cancer Res* 37:419, 1996.
63. Sun J, Qian Y, Hamilton AD, Sebt SM: The growth in nude mice of H-ras and K-ras 4B transformed cells is suppressed by farnesyltransferase and geranylgeranyltransferase I inhibitors, respectively. *Pro Am Assoc Cancer Res* 37:419, 1996.
64. Hohl RJ, Lewis-Tisebar K, Pogatchik DM, Wiemer DF: Structural requirement for inhibition of farnesyl protein transferase by isoprenoid phosphonic acid. *Proc Am Assoc Cancer Res* 37:426, 1996.
65. Sebold-Leopold JS, Gowan RC, Gibbs BS *et al*: Biological evaluation of cell permeable inhibitors of ras farnesyl transferase. *Proc Am Assoc Cancer Res* 37:423, 1996.
66. Gibbs JB, Oliff A, Kohl NE: Farnesyltransferase inhibitors: ras research yields a potential cancer therapeutic. *Cell* 77: 175-178, 1994.
67. Leftheris K, Kline T, Vite GD *et al*: Development of highly potent inhibitors of Ras farnesyltransferase possessing cellular and in vivo activity. *J. Med. Chem.* 39:224-236, 1996.
68. Sun J, Qian Y, Hamilton AD, Sebt SM: Ras CAAX peptidomimetic FTI-276 selectively blocks tumor growth in nude mice of a human lung carcinoma with K-ras mutation and p53 deletion. *Cancer Res* 55:4243-4247, 1995.
69. Khan SG, Dummer R, Siddigni J *et al*: Farnesyltransferase activity and mRNA expression in human skin basal cell carcinoma. *Biochem Biophys Res Commun* 220:795-801, 1996.
70. Bloch A: Induced cell differentiation in cancer therapy. *Cancer Treat Rep* 68:199-205, 1984.
71. Clausen MR: Butyrate and colorectal cancer in animals and in humans. *Eur J Cancer Prevention* 4:483-490, 1995.
72. Prasad NK: Butyric acid: a small fatty acid with diverse biological function. *Life Sci* 27:1351-1358, 1980.
73. Buguet-Fagot C, Lallemand F, Charollais RH, Mester J: Sodium butyrate inhibits the phosphorylation of the retinoblastoma gene product in mouse fibroblasts by a transcription dependent mechanism. *J Cell Physiol* 166:631-636, 1996.
74. Krupitza G, Crill S, Harant H *et al*: Genes related to growth and invasiveness are repressed by sodium butyrate in ovarian carcinoma cells. *Br J Cancer* 73:433-438, 1996.
75. Ranganna K, Joshi T, Yatsu FM: Sodium butyrate inhibits platelet-derived growth factor induced proliferation of vascular smooth muscle cells. *Arterioscler Thromb Vasc Biol* 15:2273-2283, 1995.
76. Garcia-Berjevo L, Vilaboa NE, Perez C *et al*: Modulation of H5P70 and H5P27 gene expression by the differentiation inducer sodium butyrate in U937 human promonocytic leukemia cells. *Leuk Res* 19:713-718, 1995.
77. Novogrodsky A, Dvir A, Ravid A *et al*: Effect of polar organic compounds on myeloid cells: butyrate induced partial remission of acute myelogenous leukemia in a child. *Cancer* 51:9-14, 1983.
78. Miller AA, Kurschel E, Osieka R, Schmidt CG: Clinical pharmacology of sodium butyrate in patients with acute leukemia. *Eur J Clin Oncol* 23:1283-1287, 1987.

79. Nudelman A, Ruse M, Aviram A *et al*: Novel anticancer prodrug of butyric acid. *J Med Chem* 35:687-694, 1992.
80. Raphaeli A, Rabizadeh E, Aviram A *et al*: Derivatives of butyric acid as potential anti-neoplastic agents. *Int J Cancer* 49: 66-72, 1991.
81. Newmark HL, Young CW: Butyrate and phenylacetate as differentiating agents: practical problems and opportunities. *J Cell Biochem Suppl* 11:247-253, 1995.
82. Blau CA, Constantoulakis P, Shaw CM, Stamatoyannopoulos G: Fetal hemoglobin induction with butyric acid: efficacy and toxicity. *Blood* 81:529-537, 1993.
83. Perrine SP, Ginder GD, Faller DV *et al*: A short-term trial of butyrate to stimulate fetal-globin-gene expression in the 3 globin disorders. *N Eng J Med* 328:81-86, 1993.
84. Tong KP, DeWeese TL, Mansfield EP, Carducci MA: New insights into phenylbutyrate bioactivity in human prostate cancer. *Proc Am Assoc Cancer Res* 37:361, 1996.
85. Carducci M, Bowling M, Eisenberger M *et al*: Phenylbutyrate (PB) for refractory solid tumors: A phase I clinical and pharmacological evaluation. *Proc Am Assoc Cancer Res* vol: p, 1996.
86. Napoli JL: The biogenesis of retinoic acid: a physiologically significant promoter of differentiation. In: *Chemistry and biology of retinoic acid*. Dawson MI, Okamura H eds., CRC Press, Boca Raton, FL, 1990, pp 229-249.
87. Sherman MI: ed. *Retinoids and cell differentiation*. CRC Press, Boca Raton, FL, 1984.
88. Jetten AM: Multi stage program of differentiation in human epidermal keratinocytes: regulation by retinoids. *J Invest Dermatol* 95:44-46, 1990.
89. Amos B, Lotan R: Retinoid-sensitive cells and cell lines. *Methods Enzymol* 190:217-225, 1991.
90. DeLuca LM: Retinoids and their receptors in differentiation, embryogenesis and neoplasia. *FASEB J* 5:2924-2933, 1991.
91. de The H, Vivanco-Ruiz M, Tiollais P *et al*: Identification of a retinoic acid responsive element in the retinoic acid receptor beta gene. *Nature* 343:177-180, 1990.
92. Rambaldi A, Biondi A, Pandolfi PP *et al*: Molecular monitoring of the myl/RAR-alpha fusion gene in acute promyelocytic leukemia by polymerase chain reaction. *Blood* 78:336a, 1991.
93. Muindi J, Frankel SR, Miller WH *et al*: Continuous treatment with all-trans retinoic acid causes a progressive reduction in plasma drug concentrations: implication for relapse and retinoid resistance in patients with acute promyelocytic leukemia. *Blood* 79:299-303, 1992.
94. Kraemer KH, DiGiovanna JJ, Moshell AN *et al*: Prevention of skin cancer in xeroderma pigmentosum with the use of oral isotretinoin. *N Engl J Med* 318:1633-1637, 1988.
95. Lippman SM, Clark LC, Parkinson D *et al*: Pharmacologic prevention and therapy of skin cancer. In: *Chemo and immuno prevention of cancer*. Pastorino U, Hong WK, eds. George Thieme Verlag, Stuttgart 1991, pp 177-187.
96. Lippman SM, Parkinson Dr, Itri L *et al*: 13-cis retinoic acid plus interferon alpha: effective therapy for advanced squamous cell carcinoma of the skin. *J Natl Cancer Inst* 84:235-241, 1992.
97. Hong WK, Lotan R: eds. *Retenoids in Oncology*. Marcel Dekker, NY, 1993.
98. Gottesman MM, Pastan I: Biochemistry of multidrug resistance mediated by the multidrug transporter. *Annu Rev Biochem* 62:385-427, 1993.
99. Raderer M, Scheithauer W: Clinical trials of agents that reverse multidrug resistance. *Cancer* 72:3553-3563, 1993.
100. Ehrlichman C, Moore M, Thiessen J *et al*: A phase I trial of doxorubicin and PSC833, a modulator of multidrug resistance (MDR). *Proc Am Soc Clin Oncol* 13:A326, 1994.
101. Cole SPC, Bhardway G, Gerlach JH *et al*: Over-expression of a transporter gene in a multidrug-resistant lung cancer line. *Science* 258:1650-1654, 1992.
102. Schneider E, Yamazaki H, Sinka BK, Cowan KH: Buthionine sulfoximine-mediated sensitisation of etoposide-resistant human breast cancer MCF7 cells overexpressing the multidrug resistance-associated protein involved increased drug accumulation. *Br J Cancer* 71:738-743, 1995.
103. Slovak ML, Ho JP, Cole SPC *et al*: The LRP gene encoding a major vault protein associated with drug resistance maps proximal to MRP on chromosome 16: Evidence that chromosomal breakage plays a key role in MRP or LRP gene amplification. *Cancer Res* 55:4214-4219, 1995.
104. Cho-Chung YS, Clair T, Tortora G, Yokozaki H: Role of site-selective cAMP analogs on control and reversal of malignancy. *Pharmacol Ther* 50:1-33, 1991.
105. Scala S, Budillon A, Zhan Z *et al*: Downregulation of mdrl expression by 8-Cl-cAMP in multidrug resistant MCF-7 human breast cancer cells *J Clin Invest* 96:1026-1034, 1995.
106. Beebe SJ, Carbin JD: Cyclic nucleotide-dependent protein kinases. In: *The Enzymes: Control by phosphorylation*. Part A Vol 17 Boyer PD, Kerbes EG, eds Academic, NY, 1986 pp 43-111.
107. North PS, Davies SL, Ciardiello F *et al*: Overexpression of the RI alpha subunit of protein kinase A confers hypersensitivity to topoisomerase II inhibitor and 8-Chloro-cyclic adenosin 3',5'-monophosphate in chinese hamster ovary cells. *Cancer Research* 54:4123-4128, 1994.
108. Rohlf C, Clair T, Cho-Chung YS: 8-Cl-cAMP induces truncation and down-regulation of the RI alpha subunit and up-regulation of the RI beta subunit of cAMP-dependent protein kinase leading to type II holoenzyme-dependent growth inhibition and differentiation of HL-60 leukemic cells. *J Biol Chem* 268:5774-5782, 1993.
109. Rohlf C, Glazer RI: Regulation of multidrug resistance through the cAMP and EGF signaling pathways. *Cell-Signal* 7:431-443, 1995.
110. Ferry DR, Traunecker H, Kerr DJ: Clinical trials of P. Glycoprotein reversal in solid tumors. *E J Cancer* 32A:, 1996.
111. Al-Khalidi AHE, Vasanthakumar G, Balasubramanian M *et al*: Fractionation of Cremophor EL to increase potency for reversing multidrug resistance. *Proc Am Assoc Canc Res* 36:371, 1995.
112. Friche E, Jensen PB, Schested M *et al*: The solvent Cremophor EL and Tween 80 modulate daunorubicin resistance in the multidrug resistant Ehrlich ascites tumor. *Cancer Comm* 2: 297-303, 1990.
113. Bowers VD, Locker S, Ames S *et al*: The hemodynamic effects of Cremophor EL. *Transplantation* 51:847-890, 1991.
114. Twentyman PR, Bleehen NM: Resistance modification by PSC-833, a novel nonimmunosuppressive cyclosporin. *Eur J Cancer* 27:1639-1642, 1991.
115. Duran GE, Gosland MP, Ho AL, Sikic BI: In vitro modulation of multidrug resistance (MDR) using human patient serum from EP-1: a phase I clinical trial of etoposide (VP-16) and SDZ PSC833. *Proc Am Assoc Cancer Res* 35:A2093, 1994.
116. Sonneveld P, Marie JP, Laburte C, Schoester M: Phase I study of SDZ PSC833, a multidrug resistance modulating agent, in refractory multiple myeloma. *Proc Am Assoc. Cancer Res* 35:A2141, 1994.
117. Weaver JL, Szabo G Jr., Pine PS *et al*: The effect of ion channel blockers, immunosuppressive agents and other drugs on the activity of the multi-drug transporter. *Intl J Cancer* 54:456-461, 1993.
118. Hwang M, Ahn CH, Pine PS *et al*: Effect of combination of suboptimal concentrations of P-glycoprotein blockers on the prolifer-

- ation of MDRI gene expressing cells. *Int J Cancer* 65:389-397, 1996.
119. Blackburn, EH: Telomerase. *Ann Rev Biochem* 61: 113-129, 1992.
 120. Harley CB, Kim NW, Prowse K *et al*: Telomerase, cell immortality and cancer. *Quant Biol* 59: 307-315, 1994.
 121. Fen J, Funk WD, Wang SS *et al*: The RNA component of human telomerase: *Science* 269: 1236-1241, 1995.
 122. Kim NW, Piatyszek MA, Prowse KR *et al*: Specific association of human telomerase activity with immortal cells and cancer. *Science* 266: 2011-2015, 1994.
 123. Piatyszek, MA, Kim, N W, Weinrich, S *et al*: Detection of telomerase activity in human cells and tumors by a telomeric repeat amplification protocol (TRAP). *Methods Cell Sci* 17: 1-15, 1995.
 124. Hiyama E, Hiyama R, Yokoiama T *et al*: Correlating telomerase activity levels with human neuroblastoma outcomes. *Nature Med* 1: 249-255, 1995.
 125. Hiyama, E., Yokoyama, T., Tatsumoto, N *et al*: Telomerase activity in Gastric Cancer. *Cancer Res* 55: 3258-3262, 1995.
 126. Piatyszek MA, Word RA, Shay J: Late activation of telomerase activity in human tumors. *Proc Am Assoc Canc Res* 36:554, 1995.
 127. Hiyama E, Gollahon L, Kataoka T *et al*: Telomerase activity in human breast tumors. *J Natl Cancer Inst* 88:116-122, 1996.
 128. Fletcher TM, Chen SF: The effect of 7-deaza-2'-deoxyguanosine-5-triphosphate and 7-deaza-2'-deoxyadenosine-5-triphosphate on telomerase activity. *Ann Oncol* 7 (suppl 1):71, 1996.
 129. Chen SF, Maine JP, Windle B: Effect of 3'-acidothymidine triphosphate and 3'-azido-thymidine on telomerase activity and telomere function. *Proc Am Assoc Canc Res* 36:554, 1996.
 130. Maie JP, Windle B, Chen SF: In vitro investigation of processing and nonprocessive telomerase activities. *Proc Am Assoc Canc Res* 36:554, 1996.
 131. Hinch RCA, Siemann DW: Cytotoxicity of bioreductive agents tirapazamine (R 4233) and mitomycin C (MMC) in human ovarian carcinoma cell lines. *Proc Amer Ass Cancer Res* 36: 306, 1995.
 132. Lewis AD, Walker C, Morecroft, I *et al*: Role of human NADPH: cytochrome P-450 CYPB2 gene family in the metabolism of the anticancer bioreductive drug Tirapazamine (WIN 59075, SR 4233). *Proc Am Ass Cancer Res* 36: 602, 1995.
 133. Durand R: Chemosensitization with Tirapazamine. *Proc Am Assoc Cancer Res* 36: 297, 1995.
 134. Elsaid AA, Menke D, Dorie MJ, Brown JM: Study comparing the effect of fractionated irradiation and tirapazamine with and without carbogen and nicotinamide on human tumor xenografts. *Proc Am Soc Clin Oncol* 15: 516, 1996.
 135. Rodriguez GI, Waldvieso M, Von Hoff DD *et al*: A phase I/PI trial of the combination of Tirapazamine and Cisplatin in patients with NSCLC. *Proc Amer Soc Clin Oncol* 15: 382, 1996.
 136. Treat J, Greenbergi R, Rodriguez R *et al*: Tirapazamine with Cisplatin: phase II in advanced stage NSCLC. *Proc Am Soc Clin Oncol* 15: 400, 1996.
 137. Aghajanian C, Fennelly D, Shapiro F *et al*: Phase I trial of tirapazamine (WIN 59075, SR 4233) plus Cisplatin in cervical cancer. *Proc Am Soc Clin Oncol* 15:298, 1996.
 138. Bedikian AY, Legha SS, Buzaid AC *et al*: Phase II trial of tirapazamine plus cisplatin in patients with advanced melanoma. *Proc Amer Soc Clin Oncol* 15: 434, 1996.
 139. Marshall HL, von Roemelinai RW, Trul J, Haakins MJ: A phase I study of tirapazamine plus cisplatin in patients with breast cancer and other platinum resistant tumors. *Annals Oncol* 7 (suppl. 1): 135, 1996.