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## Meeting report

# First international conference on new molecular targets for anticancer therapy, Naples, 22–23 June 1998

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**The First International Conference on New Molecular Targets for Anticancer Therapy was held in Naples on 22–23 June 1998, and represented an excellent occasion for discussing ongoing preclinical and clinical studies in the field of angiogenesis, signal trasduction and antisense. In two general lectures, early clinical trials which are ongoing at EORTC and NCI, Bethesda, were summarized. Neoangiogenesis has been considered as a central pathogenic step in the process of tumor growth, invasion and metastasis. This complex process involves multiple steps and pathways dependent on the local balance between positive and negative regulatory factors, as well as interaction between the tumor, its vasculature and the surrounding extracellular matrix. Therapeutic agents and strategies are currently being devised to block one or more of the pathogenic steps involved in the process of tumor neovascularization or to directly target and destroy tumor vasculature. Since aberrant cell signaling plays a key role in the initiation, growth and progression of many tumors, signal trasduction inhibitors may have a role as cytostatic agents. In addition, cancer sensitivity/resistance to conventional chemo/radiotherapy is largely dependent on cell signaling; hence its inhibition may induce a fundamental shift towards sensitivity. Thus, signal transduction inhibitors may play an important role also as modulators of conventional therapies, by shifting the balance towards pro-apoptotic signaling. Modulation of gene expression using oligonucleotides is currently an area of intense preclinical and clinical investigation. The effectiveness of antisense oligonucleotides as therapeutic agents depends, in addition to biological activity, on pharmacokinetics, tissue disposition, *in vivo* metabolic activity, elimination and safety profile. Probably the most clever way of using antisense oligonucleotides is to combine them with conventional chemotherapy, exploiting the different and possibly complementary mechanisms of action of the two treatment modalities.** [© **1999 Lippincott Williams & Wilkins.**]

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## Introduction

The search for new anticancer drugs was stimulated some 30 years ago by findings that certain analogs of anticancer agents might have a better therapeutic index (doxorubicin having, for example, a much wider spectrum of antitumor activity over daunorubicin and carboplatin a lower toxicity with respect to cisplatin).

In spite of major efforts, investment in the synthesis and development of analogs has not resulted in more antitumor agents showing consistent advantages.[1] However, in recent years, the molecular biology of cancer cell growth has been explored in greater depth and a number of new targets for anticancer drug action have been provided.[2]

The First International Conference on New Molecular Targets for Anticancer Therapy was held in Naples, Italy, on 22-23 June 1998, and focused on angiogenesis, antisense, signal trasduction and cell cycle, highlighting novel approaches and strategies that could result in enhanced effectiveness and selectivity of therapeutic tumor targeting.

Two general lectures were given by Pierre Fumoleau (Centre Rene Gauducheau, Nantes) and Percy Ivy (NCI, Bethesda). Dr Fumoleau summarized the studies which are currently ongoing at EORTC—Early Clinical Studies Group. He emphasized particularly the phase II trials with new antimetabolites, such as S1, which is

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currently in phase II evaluation in gastric cancer and colorectal cancer, and UFT, which is undergoing a phase II randomized cross-over study with 5-fluorouracil (5-FU) plus folinic acid. Among phase I studies, Fumoleau particularly emphasized the ongoing trial with ET-743 (a marine compound which targets the DNA minor groove), CGP 48664 (a selective inhibitor of a critical enzyme in the polyamine biosynthetic pathway), and the studies with antisense oligonuleotides ISIS 3521 and ISIS 5132 targeting protein kinase C (PKC)-$\alpha$ and *raf*-kinase, respectively.

Dr Ivy reviewed the drugs under development at NCI. She focused on drugs which act through down-regulation of kinases and consequent interference with intracellular signal trasduction. In particular, she focused on bryostatin 1, which exerts many of its biologic effects through modulation of PKC, besides having immunomodulatory activities. The drug, whose main toxic effect is myalgia, has now entered phase II clinical trials in non-Hodgkin's lymphoma, melanoma and renal cell carcinoma with different schedules. Flavopiridol blocks cell cycle progression at either $G_1$ or $G_2$ as a consequence of the inhibition of cyclin-dependent kinases. Phase I clinical trials of 72-h continous infusion of flavopiridol have been completed and GI tract toxicity is the main toxic effect. UCN-01 is a staurosporine analog in phase II trial evaluation, which blocks transition of the cell cycle at the $G_1$ phase mainly acting as a potent and selective antagonist of PKC isoenzymes $\alpha$, $\beta$, and $\gamma$. Among the DNA-interacting agents, some emphasis was given to Bizelesin, which is a minor-groove binder and potent inhibitor of DNA synthesis. The clinical studies with this drug confirmed preclinical evidence of substantial myelosuppression.

## Angiogenesis and cellular matrix

Angiogenesis and tumor-associated neovascularization have been considered as central pathogenic events in the process of tumor growth, invasion and metastasis. These complex processes involve multiple steps and pathways dependent on the local balance between positive and negative regulatory factors, as well as interaction among the tumor, its vasculature and the surrounding extracellular tissue matrix.[3] Therapeutic agents and strategies are being devised either to interrupt or inhibit one or more of the pathogenic steps involved in the process of tumor neovascularization, or to directly target and destroy the tumor vasculature as pointed out by James Pluda (NCI, Bethesda) who chaired the 'Angiogenesis and Cellular Matrix' section. While there is evidence that drugs can block angiogenesis by interfering with vascular endothelial growth factor (VEGF) release from the primary tumor [interferon (IFN)-$\alpha$] or with its binding to one of its receptors upon endothelial cells (SU5416), the majority of the drugs which have undergone preclinical and clinical evaluation act by directly inhibiting the proliferation of endothelial cells (TNP-470, platelet factor-4, Thalidomide, etc.). This mechanism of action is common also to angiostatin and endostatin, which have given extremely encouraging results in mice, both alone and in combination, and are to undergo phase I evaluation in USA by the end of the current year. Antiangiogenic activity can be obtained also through matrix metalloproteinases (MMP) inhibition. An overview of the ongoing clinical trials with this class of compounds was presented by Filippo De Braud (IEO, Milan). MMP are a family of more than 15 enzymes responsible for the degradation of extracellular matrix and connective tissue remodeling during the angiogenic process. Currently, the following MMP inhibitors have entered phase I/II studies: BB2516 (Marimastat), BAY 12-9566, CGS 27023A, AG 3340 and R031-9790. Preclinical studies indicate single-agent activity in reducing tumor growth and the capability to synergize with chemotherapeutic agents. In clinical studies, the major side effect for all of them but BAY 12.9566 has been a dose-related reversible joint pain. Marimastat has been the most widely studied compound with over 400 patients accrued in six studies, i.e. in colorectal cancer ($n$=2), ovarian cancer ($n$=2), prostate cancer ($n$=1) and pancreatic cancer ($n$=1). Tumor marker reduction was used as biological endpoint in these studies. The combination of Marimastat or AG 3340 with chemotherapy has been reported to be feasible and phase III studies are ongoing, mainly exploring the possibility of an improvement of time to progression by adding a MMP inhibitor to standard chemotherapy.

Adriana Albini's (IST, Genoa) talk focused on Kaposi's sarcoma (KS), a multifocal lesion developing with high incidence in AIDS patients, which can be considered as a model to study antiangiogenic therapy. An imbalance between angiogenesis inducers and inhibitors may play a role in the pathogenesis of KS. Vascular endothelial growth factor (VEGF), one of the major angiogenesis inducers, is overexpressed in KS, both in the neoplasm and *in vitro*. Other important molecules in KS vascularization are $\beta$-fibroblast growth factor (bFGF) and heparin binding factors. HIV-tat, a potential cofactor in epidemic KS, is a new heparin binding growth factor and is angiogenic at picomolar concentrations *in vivo* and *in vitro*. HIV-tat uses the VEGF receptor flk-1/KDR to stimulate endothelial cells. KS-conditioned medium (CM) supernatants, KS-

immortal cells and purified HIV-tat have been used to stimulate endothelial cells *in vitro* and to induce angiogenesis *in vivo*, in order to screen for antiangiogenic compounds. The tissue inhibitor of metalloproteases 2 (TIMP-2), a natural inhibitor of MMP mostly involved in the prevention of basement membrane digestion, is able to inhibit KS-stimulated endothelial cell invasion *in vitro and angiogenesis in vivo*, and is a potential candidate for gene therapy of angiogenesis. Other anti-angiogenic agents efficacious in the KS model are: interferon class I, particularly in combination with retinoids, platelet thrombospondin and interleukin (IL)-12. Tyrosine kinase receptors for heparin binding growth factors are also good candidates for the study of experimental antiangiogenic protocols in KS. A factor of still debated nature, present in urinary preparations of human chorionic gonadotropin (hCG), is another promising anti-KS and antiangiogenic molecule. Finally, since KS are infiltrated with mononuclear cells as well, one future step will be to inhibit monocyte/macrophages as important inflammation and neovascularization mediators in KS reactive lesions.

The cellular matrix has been considered another potential target for anticancer therapeutic approaches that affect tumor cell growth indirectly.[4] In this regard Patrizia Stoppelli (IIGB, Naples) described potential molecular antagonists to urokinase receptor-dependent tumor cell motility. Urokinase-dependent proteolytic modulation of cell–matrix adhesive interactions is required for cell migration and tumor metastasis. Recent findings indicate that urokinase proteolytic activator (uPA) promotes pericellular proteolysis and modulates cell motility and matrix attachment through a specific GPI-anchored uPA receptor (uPAR). Urokinase induces motility in a catalytic-independent manner, through uPAR-dependent signal transduction. In this context, a naturally occurring regulatory mechanism of uPA signaling has been identified. Human carcinoma A431 cells overexpress uPA which is phosphorylated on two serine residues (Ser138 and Ser303): this post-translational modification dramatically impairs the ability of pro-uPA to stimulate cell migration of a large variety of tumor cells (A431, HeLa, MCF-7, HT1080, LB6-uPAR and HepG2). However, receptor binding is not affected by serine phosphorylation and therefore phosphorylated uPA is indeed a competitive antagonist of uPAR function. In particular, substitution of serines with glutamic residues as well as the naturally occurring phosphoserines at positions 138 and 303 abolishes pro-uPA ability to elicit cell migration, while the exclusive presence of Glu138 reduces pro-uPA chemotactic ability by 70–80%, indicating that a critical residue has been altered. Surprisingly, the phosphorylation-like variants were able to inhibit random cell migration and to prevent the chemotactic effect by unrelated chemoattractants, such as fibronectin and placenta-derived growth factor (PlGF). These data suggest that these variants alter a general mechanism required for cell migration.

## Signal transduction and cell cycle

Since aberrant cell signaling plays a key role in the initiation, growth and progression of many tumors, signal transduction inhibitors may have a role as cytostatic agents[5] as reviewed by Stan Kaye (University of Glasgow, UK). In this regard, a widely studied signal transduction pathway is the mitogen activated *ras*-dependent MAP kinase cascade which transduces signals from the growth factor receptor tyrosine kinases to the nucleus, initiating proliferation.[6,7] In addition, cancer sensitivity/resistance to conventional chemotherapy/radiotherapy is largely dependent on cell signaling; hence its inhibition may induce a fundamental shift towards sensitivity.[8,9] Thus, signal transduction inhibitors may play an equally or even more important role as modulators of existing therapy, by shifting the balance towards pro-apoptotic signaling, and away from signals leading to cell cycle arrest and increasing time for damage repair. Agents to which these arguments apply include: (i) monoclonal antibodies to specific receptors, e.g. epidermal growth factor receptor (EGFr) and HER 2-neu, (ii) antisense oligonucleotides, e.g. anti-PKC $\alpha$, and (iii) natural or semi-synthetic products, e.g. bryostatin, flavopiridol and staurosporine analogs. Great emphasis was given in Kaye's talk to PKC as a target for anticancer drugs. In fact PKC is a serine-threonine kinase, which phosphorylates proteins which are crucial for the transmission of intracellular signals. In this regard, Francesco Caponigro's (INT Pascale, Naples) presentation described the results of a phase I and pharmacokinetic study with CGP 41 251, an inhibitor of PKC. CGP 41251 is a staurosporine analog with good oral bioavailability and more selective PKC inhibitory activity with respect to its parent compound. The phase I study was undertaken in Glasgow and in Oxford in order to evaluate its safety profile, antitumor activity and effect on markers of PKC signaling in patients with advanced solid tumors. Thirty-two patients were treated at seven dose levels. Main toxicities were fatigue at doses less than mg/m$^2$/day and nausea/vomiting at higher doses. No significant myelosuppression was observed. Antitumor activity was observed in one patient with cholangiocarcinoma.

Pharmacokinetics showed a linear relationship between CGP 41251 dose and both day 1 AUC and $C_{max}$, but with a quite marked interpatient variability. Inhibitory effects on the PKC signaling pathway *in vivo* were assessed by measuring cytokine release from phytohemagglutinin-stimulated fresh whole blood cells obtained from patients at various time points during treatment. The release of tumor necrosis factor (TNF) and IL-6 were both significantly inhibited in a time- and dose-dependent manner ($p < 0.001$). This represents probably the most interesting finding of the study, since it points to biochemical endpoints which can be used to monitor biologic activity of the drug, as an extension of a classical phase I study. Since CGP 41251 is supposed to have MDR-modulating properties and antiangiogenic activity as well, it looks a suitable agent both for phase II studies and for phase I combination studies, which are currently being planned.

Members of the EGFR family of tyrosine kinases which include EGFR, ErbB2, ErbB3 and ErbB4, as pointed out before, have frequently been implicated in human neoplasia by overexpression and gene amplification and therefore have been considered as specific targets for new therapeutical anticancer approaches.[10] Mathias Kraus (IEO, Milan) reviewed recent advances in the understanding of the pleiotropism of biological responses controlled by these receptors including growth proliferation, transformation and differentiation. In part, this capacity is determined by distinct, yet complementary, mechanistic properties of the four ErbB family receptors regarding ligand binding specificity, intrinsic tyrosine kinase activity, receptor half-life and activation of cytoplasmic signal transduction pathways. Their ability of associating in 10 different homodimeric and heterodimeric combinations have projected enormous potential of diversification in intracellular signaling by these four receptors. In particular, in different cellular model systems, heterodimers involving ErbB2 have generally been found more active in promoting growth proliferation or transformation. Under conditions in which neither ErbB2 nor ErbB3 gave rise to neoplastic transformation, coexpression of both receptors induced primary transformation of NIH3T3 cells. At high ErbB2 expression levels causing transformation, ErbB3 enhanced primary focus formation by one order of magnitude. Evidence for constitutive signaling by ErbB3-ErbB2 heterodimer in certain mammary carcinoma cell lines indicated relevance of active erbB receptor heterodimers for human neoplasia. Moreover, biological synergy between ErbB2 and EGFR or ErbB4 has been obseved as well. Based upon functional evidence and observations in human tumor cell lines, it is conceivable that ErbB2 containing heterodimers with the other three ErbB receptors may provide improved targets for diagnostic or therapeutic objectives.

Jim Woodburn's (Zeneca Pharmaceuticals, Macclesfield) lecture focused mainly on the properties of Zeneca's ZD 1839, a low molecular weight molecule, which potently inhibits EGFR tyrosine kinase (0.02 micromolar) and the growth of EGF-stimulated KB oral carcinoma cells (0.08 micromolar). Autophosphorylation of EGFR was inhibited in several tumor cell lines (KB oral squamous, A 549 lung, DU 145 prostate and HT29 colorectal; $IC_{100}$= 0.16-0.8 mM). ZD 1839 showed good oral bioavailability and demonstrated significant antitumor activity in a broad range of solid human tumor xenografts implanted in nude mice in the dose range 12.5-200 mg/kg once per day orally. Antitumor effects ranged from slowed growth to stasis, with marked regression in some tumors. Therapy for 3-4 months was well tolerated. Following the completion of preclinical studies, two placebo-controlled clinical trials have been performed in 29 healthy male volunteers. The first study looked at the tolerability and pharmacokinetics of single raising doses of ZD 1839 up to 75 mg. The second study looked at the tolerability and PK of three daily doses of 100 mg of ZD 1839. In both studies ZD 1839 was well tolerated. Adverse events were similar in the ZD 1839 and placebo groups. Dose-proportional oral bioavailability was confirmed. The terminal half-life was 27-41 h, compatible with daily dosing. Dr Woodburn concluded that ZD 1839 is a low toxicity compound with potential benefits in a range of solid tumors.

Ahmdal Awada (Institute Jules Bordet, Brussels) reviewed the use of farnesyl trasferase inhibitors as anticancer drugs. Since Ras is constitutively activated in a wide percentage of human cancers and Ras farnesylation is required for its cancer-causing activity, there is a great surge of interest in farnesyl trasferase as a novel target for anticancer therapy.[2] Furthermore, since Ras is geranylgeranylated when farnesyltrasferase is inhibited, and geranylgeranylated proteins are involved in $G_1$/S phase transition of the cell cycle as well as in trasformation, interest has been gained also by GGTase inhibitors. For the time being, two farnesyl trasferase inhibitors have entered clinical trials. The study design and the very preliminary results of the phase I study with SCH 66366, that is ongoing in Brussels and Rotterdam within the EORTC—Early Clinical Studies Group, was descibed.

The activated serine-threonine protein kinases collectively referred to as mitogen-activated protein kinases (MAPKs) constitute a superfamily of proteins that includes the extracellular-signal-regulated kinases

(ERKs), c-Jun amino-terminal kinases (JNKs) and p38/RK MAP kinases.

These are uniquely identified by the Thr-Xaa-Tyr dual-phosphorylation motif, were Xaa is Glu, Pro and Gly for the ERKs, JNKs and P38 kinases, respectively. Phosphorylation of both threonine and tyrosine residues is essential for full kinase activiy of the MAPKs.[6]

As discussed by Roger Davis (Howard Hughes Medical Institute, Worcester), the JNK group of MAP kinases has been identified in mammals and insects. JNK is activated by exposure of cells to cytokines (TNF, IL-1) or environmental stress (UV light, chemical toxins, osmotic shock) indicating that this signaling pathway may contribute to inflammatory responses. Genetic and biochemical studies demonstrate that this signaling pathway also regulates cellular proliferation, oncogenic transformation, apoptosis and tissue morphogenesis. A functional role for JNK is therefore established in both the cellular response to stress and in many normal physiological processes. Whereas ERKs are strongly activated by receptor tyrosine kinases, such as EGF, platelet-derived growth factor (PDGF), FGF, etc., the JNKs are potentially activated by heterotrimeric-G$\alpha\beta\gamma$-protein-coupled receptors, such as the muscarinic acethylcholine receptor, in a Ras- and PKC-independent manner that also does not involve the ERKs. Targets of the JNK signal transduction pathway include the transcription factors c-Jun, activating transcription factor-2 (ATF2) and ELK-1. Davis *et al.* characterized and cloned a murine cytoplasmic protein that binds specifically to JNK, the JNK interacting protein-1 (JIP-1) that caused cytoplasmic retention of JNK and inhibition of JNK-regulated gene expression. In addition, JIP-1 suppressed the effects of the JNK signaling pathway on cellular proliferation, including pre-B cell transformation by the *bcr-Abl* oncogene. This analysis identifies JIP-1 as a specific inhibitor of the JNK signal transduction pathway and establishes protein targeting as a mechanism that regulates signaling by stress-activated MAP kinases. In particular these data implicating JNK pathway in pre-B cell transformation indicated JNK and JIP-1 as candidate targets for the design of therapeutic strategies for the treatment of chronic myeloid leukemia. Furthermore, more recent data have demonstrated that JIP-1 appears to function as a mammalian scaffold protein for the JNK signaling pathway. In particular, in mammalian normal cells JIP-1 binds multiple components of a JNK signaling module, specifically mixed lineage kinase (MLK)-MAP kinase kinase 7 (MKK7)-JNK, and facilitates signal trasduction mediated by the bound proteins.

The stress-activated kinase pathway is also activated by treatment with IFN-$\alpha$ as shown by Pierosandro Tagliaferri (University of Naples). The enhanced activity of the amino-terminal Jun kinase-1 and p38 kinase, induced by IFN-$\alpha$, has been shown to be paralleled by the induction of apoptosis by this agent . These effects were accompanied in the treated cells by the increased expression of EGFR and the increased activation of all the component of the *ras*-dependent MAPK pathway. The latter findings and the antagonization of the IFN-induced effects by EGF have been proposed as protective responses of tumor cells to the antiproliferative action of IFN-$\alpha$. In addition, the selective inhibition of the *ras*-dependent MAPK pathway by transfection of the dominant-negative *ras* plasmid RASN17 induced a strong potentiation of apoptosis caused by IFN-$\alpha$. Therefore, the selective target of the *ras*-dependent MAPK signaling appears an interesting approach in order to enhance the antitumor activity of IFN-$\alpha$.

Alfredo Budillon (INT Pascale, Naples) demonstrated that 8-Cl-cAMP, a specific antagonist of type I cAMP-dependent protein kinase which has recently undergone phase I clinical studies as an antitumor agent,[11] antagonizes the mitogenic effect of EGF on human epidermoid cancer KB cells by blocking EGF-induced activation of MAPKs ERK-1 and ERK-2. Conversely, 8-Cl-cAMP induced a dose- and time-dependent increase of EGFR expression and EGFR tyrosine phosphorylation while Raf-1 and MEK-1 protein kinases, the upstream MAPK activators in the phosphorylation cascade induced by EGF, are normally activated in treated cells.

These results add new insights into the mechanism of antitumor action of 8-Cl-cAMP demonstrating that this agent can specifically inhibit the deregulated pathways of EGF-EGFR in human cancer cells. Since 8-Cl-cAMP underwent clinical evaluation as an anticancer agent in a recent phase I study, the identification of the specific molecular targets of 8-Cl-cAMP may allow the design of selective approaches in order to enhance the antitumor activity of this compound.

The variety of external signals transduced by the signal transduction pathway interferes with the cell cycle machinery. Progression through the cell cycle is a complex process characterized by checkpoints at which cells make sure that the previous steps have been completed before embarking on subsequent steps.[12] Breakdown of any of the steps in this complex process, that is regulated at many levels by several proteins, can lead to cancer. Among these regulators, great interest has been recently placed on the cyclin-dependent kinase (cdk) inhibitory proteins as potential target for anticancer treatment.[13] In this regard,

evidence has also been obtained that 8-Cl-cAMP increased the half-life of $p27^{Kip1}$ cdk inhibitor by inhibiting the proteosome-mediated degradation of p27 protein. The latter finding may explain the progressive accumulation of KB cells in late S and $G_2$/M phases of the cell cycle after 24–96 h of treatment with 8-Cl-cAMP. Conversely, Giuseppe Viglietto (INT Pascale, Naples) discussed the distinct role of the cdk inhibitors p27 and p21 cdk inhibitory proteins in retinoic acid (RA)-induced growth arrest and neuronal differentiation of embryonal carcinoma. RA induces the embryonal carcinoma cell line NTERA-2 clone D1 (NT2/D1) to arrest growth in $G_1$ and to terminally differentiate along the neuronal pathway. Neuronal differentiation of NT2/D1 cells by RA involves coordinate expression of the cdk inhibitors p27 and p21, enhanced association with cdk2 and cdk4, and suppression of their kinase activity, in neuronal differentiation of embryonal carcinoma cells. Two antisense clones which have lost RA inducibility were generated to address the role of p27 or of p21 (NT2-p27AS or NT2-p21AS, respectively). RA-treated NT2-p27AS cells continued to synthesize DNA and were unable to differentiate properly, as demonstrated by different morphological features, lack of neurite outgrowth and altered expression of differentiative markers ($SSEA\text{-}3^+/A2B5^-$). Conversely, although RA-treated NT2-p21AS cells showed an increased proliferation rate, they retained the ability to terminally differentiate. However, expression of neuronal-specific antigens (i.e. A2B5) was obtained only when p27 and p21 were expressed simultaneously. These results demonstrated that p27 and p21 have distinct roles in the process of neuronal differentiation induced by RA. In fact, both p27 and p21 are involved in RA-induced growth arrest. However, p27, but not p21, is required for morphologycal or biochemical differentiation, although cooperation with p21 enhances terminal differentiation along the neuronal pathway.

The selection of specific cell models for investigating drugs acting on the cell cycle and apoptosis was emphasised by Maurizio D'Incalci (Institute Mario Negri, Milan). Nine human ovarian cancer cell lines that express wild-type or mutated p53 were used to evaluate the cytotoxicity induced by paclitaxel and cisplatin. The presence of p53 is not a determinant for the cytotoxicity induced by paclitaxel or cisplatin in human ovarian cancer cell lines. Differences in the activation of p53 downstream genes could be observed in wild-type versus mutated p53-expressing cells, but this does not account either for a differential induction of apoptosis or for a change in cytotoxicity induced by paclitaxel. The p53-negative subclone obtained (A2780/E6) upon transfection with the product of the E6 gene of the human papilloma virus HPV16 was approximately 50-fold more sensitive to paclitaxel than wild-type p53-expressing A2780 cells. This increased sensitivity was related to the ability of paclitaxel to induce a strong arrest of cells in the $G_2$/M phase of the cell cycle in A2780/E6 but not in A2780 cells. This different cell cycle arrest was accompanied by increased frequency of paclitaxel-induced p53-independent apoptosis. Initial studies on proteases activation tend to exclude a direct role of IL-1$\beta$-converting enzyme (ICE) and the more recently described member of the ICE family, CPP32, in the induction of apoptosis in these cells. On the other hand, these studies showed a paclitaxel-dependent increase in the levels of FADD-like ICE (FLICE), whose biological relevance is, however, currently not defined.

## Antisense

Modulation of gene expression using oligonucleotides is currently an area of intense preclinical and clinical investigation.[14–16] Sudhir Agrawal (Hybridon, Cambridge, MA), who chaired the section on antisense, showed that the effectiveness of antisense oligonucleotides as therapeutic agents depends, in addition to demonstrable biological activity, on their pharmacokinetics, tissue disposition, *in vivo* metabolic stability, elimination and safety profile. Phosphorothioate oligonucleotides are the first antisense analogs that have been studied extensively and are currently in clinical trial evaluation. In an effort to improve the antisense properties of these compounds, mixed-backbone oligonucleotides (MBOs) incorporating different chemical modifications have been synthesized. In particular, the end-modified MBOs have pharmacokinetic profiles similar to those of the parent phosphorothioate oligonucleotides, but they are significantly more stable *in vivo* and can be administered orally. Dr Agrawal showed also some preliminary data on an antisense oligodeoxynucleotide directed against the *mdm2* oncogene which encodes an inhibitor of the p53 tumor suppressor protein whose amplification and overexpression occurs in several types of tumors. Antisense inhibition of *mdm2* is associated with a decrease in *mdm2*-p53 complex formation, increase in p53-inducible gene expression, increase in p53 transcriptional activity and apoptosis. Significantly, inhibition of *mdm2* expression enhances the activation of p53 by a DNA-damaging cancer chemotherapy agent in a synergistic fashion.

While specific antisense activity has been demonstrated *in vitro* and in preclinical animal models, the

use of these antisense compounds in humans has been limited thus far. Alan Yuen (Stanford) presented a review on some clinical studies of antisense oligonucleotides in cancer. Studies at Stanford University Medical Center have focused on antisense phosphorothioate oligonucleotides targeted to PKC and developed by Isis Pharmaceuticals. Because of the preclinical evidence supporting antitumor activity and a favorable safety profile, ISIS 3521 was selected for clinical development. A continuous i.v. infusion schedule was selected for this study based on the preclinical data suggesting that continuous exposure to ISIS 3521 may be necessary to maintain inhibition of PKC-$\alpha$ expression. Patients with incurable malignancies received antisense oligonucleotides over 21 days by continuous i.v. infusion followed by a 7 day rest period. Doses were increased from 0.5 to 3.0 mg/kg/day among five cohorts of 21 patients. The maximum tolerated dose of antisense oligonucleotide to PKC-$\alpha$ for future studies is 2.0 mg/kg/day when given as a 21-day continuous infusion. The dose-limiting toxicities were thrombocytopenia and clinically significant bleeding at a dose of 3.0 mg/kg/day while pharmacokinetic measurements showed rapid plasma clearance and dose-dependent steady-state concentrations of antisense oligonucleotides. Evidence of tumor response lasting up to 11 months was observed in three out of four patients with ovarian cancer.[17] Ongoing studies of antisense to PKC-$\alpha$ include a phase II study in ovarian cancer and a phase I study of PKC-$\alpha$ antisense in combination with carboplatin and paclitaxel. Other phase II studies of antisense to PKC-$\alpha$ are planned in prostate, colon, breast, glioblastoma, melanoma and non-small cell lung cancer. In parallel with the above studies, Isis Pharmaceuticals has completed a study with an antisense targeted to c-*raf* in which 34 patients were treated up to a dose of 5.0 mg/kg/day, but no dose-limiting toxicity was reached. The phase I study showed that the antisense to c-*raf* could be given safely with minimal toxic effects at the doses which were studied (unpublished data). Phase II studies with antisense directed against c-*raf* are about to start in prostate, colon, breast, ovary, pancreas, and small cell and non-small cell lung cancers.

Nicola Normanno (INT Pascale, Naples) described novel therapeutic approaches of breast carcinoma targeting proteins of the EGF family. A majority of human primary breast carcinomas and human breast carcinoma cell lines coexpress the EGF-like peptides CRIPTO (CR), amphiregulin (AR) and transforming growth factor (TGF-$\alpha$). Antisense phosphorothioate oligodeoxynucleotides (AS S-Oligos) directed against either CR, AR or TGF-$\alpha$ were able to inhibit the proliferation of MDA-MB-468 breast carcinoma cells. A 40–50% growth inhibition was observed at a 2 mM concentration of each AS S-Oligo, while no significant growth inhibition was observed when breast carcinoma cells were treated with a missense S-Oligo. The AS S-Oligos were able to inhibit the expression of AR, CR or TGF-$\alpha$ proteins and mRNAs, as assessed by immunocytochemistry and semi-quantitative reverse transcriptase-polymerase chain reaction (RT-PCR). A more significant growth inhibition was observed when MDA-MB-468 cells were treated with a combination of EGF-related AS S-Oligos. Finally, treatment of MDA-MB-468 cells with either a combination of the three AS S-Oligos, or with a combination of an EGF receptor blocking antibody (MAb 225) and either CR, AR or TGFa AS S-Oligos, resulted in a significant increase in DNA fragmentation. Taken together, these data suggest that the EGF-related peptides are involved in the proliferation and survival of breast carcinoma cells, and may represent suitable targets for experimental therapy approaches of breast carcinoma.

Probably the most clever way of using antisense oligodeoxynucleotides is to combine them with conventional therapy, as pointed out by Gabriella Zupi (INT Regina Elena, Rome). She presented a study designed to assess the efficacy of a new antimelanoma therapeutic strategy that relies on the use of a c-*myc* antisense 15mer phosphorothioate oligodeoxynucleotide ([S]ODN), in combination with cisplatin. Proliferation and colony formation of melanoma cells were both inhibited by the cisplatin/c-*myc* antisense [S]ODN combination to a greater extent than that observed with either agent alone. Inhibition was most effective when cisplatin was followed by c-*myc* antisense [S]ODNs. The combination induced also a higher percentage of apoptosis, evident at day 3 from the start of treatment, that correlated with a marked reduction in Bcl-2 expression. Mice bearing human melanoma xenografts and treated sequentially with cisplatin and c-*myc* antisense [S]ODNs showed a higher inhibition of tumor growth, reduction in the number of lung metastases and increase in life span compared with those treated with either agent alone. Together, these data lend support to the development of anticancer therapies involving oncogene-targeted antisense ODNs and conventional antineoplastic drugs.

Protein kinase A type I has been recently considered an important target for antitumor selective therapy since it plays a key role in neoplastic transformation, conveying mitogenic signals of different growth

factors and oncogenes. Giampaolo Tortora (University of Naples) showed that inhibition of protein kinase A type I by antisense oligonucleotides targeting its RI-α regulatory subunit results in cancer cell growth inhibition *in vitro* and *in vivo*. A novel mixed backbone oligonucleotide HYB 190 and its mismatched control HYB 239 were tested on soft agar growth and on xenografts of several human cancer cell types. HYB 190 demonstrated a dose-dependent inhibition of colony formation in all cell lines tested, whereas the HYB 239 at the same doses caused a modest or no growth inhibition. A synergistic growth inhibition, which correlated with increased apoptosis, was observed when HYB 190 at a non-inhibitory dose was added to cancer cells treated with taxanes, platinum-based compounds and topoisomerase II-selective drugs. Combination of HYB 190 and paclitaxel resulted in an accumulation of cells in late S-$G_2$ phases of the cell cycle and marked induction of apoptosis. A cooperative effect of HYB 190 and paclitaxel was also obtained *in vivo* in nude mice bearing human GEO colon cancer xenografts. This study represents another example of a cooperative growth inhibitory effect obtained in a variety of human cancer cell lines by antisense oligonucleotides and specific cytotoxic drugs.

An antisense-based therapeutical approach was also used by Stefania Scala (University of Naples) to evaluate the feasibility of a cancer gene therapy based on the inhibition of high mobility group I (Y) (HMGI) protein synthesis. The chromatinic HMGI proteins are abundantly expressed in several human malignant tumors, while the expression is low or undetectable in the respective normal tissues. It was shown that adenovirus-mediated gene transfer of a HMGI (Y) antisense sequence induced apoptosis in human thyroid, colon and lung carcinoma cell lines, while the growth of normal rat and human thyroid cells was unaffected. In addition, injection of the Ad-Yas virus in tumors induced in athymic mice by a human anaplastic thyroid carcinoma cell line (ARO) caused a drastic reduction in tumor size. Therefore, the suppression of the HMGI (Y) protein synthesis by a HMGI (Y) antisense adenoviral vector may represent a valid tool in the therapy of multiple human malignant neoplasias in which HMGI (Y) gene overexpression occurs quite widely.

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