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Meeting Highlight

EORTC Early Drug Development Meeting 1995, 21–24 June, Corfu, Greece

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THIS YEAR'S traditional bi-annual joint meeting of the EORTC groups involved in early drug development took place on the beautiful island of Corfu, Greece. The major topics discussed were topoisomerase I inhibitors, minor groove binding agents, apoptosis and cell cycle control, bioreductive agents, tubulin inhibitors, conjugates and prodrug therapy, and the use of haematopoietic growth factors in phase I trials. The goal of this report is to highlight some of the most significant data discussed at the meeting.

The current status of the water-soluble camptothecin derivatives topotecan, irinotecan (CPT-11) and GI 147211 was reviewed by J. Verweij (Rotterdam), J. Robert (Bordeaux), P.J. O'Dwyer (Philadelphia), J. Cassidy (Aberdeen) and J.H. Beijnen (Amsterdam). The dose-limiting toxicity of all these topoisomerase I inhibitors has been granulocytopenia. The reduction in neutrophils can be profound but is usually short-lived and is, therefore, rarely associated with infectious complications. With prolonged administration, thrombocytopenia and diarrhoea become additional dose-limiting factors. For each of the drugs, a close relationship has been found between pharmacokinetics and -dynamics, e.g. AUC (area under curve) and degree of bone marrow toxicities. Phase II population kinetics studies of topotecan showed small intra- but large interpatient variability in the AUC. With either of the currently developed topoisomerase I inhibitors, prolonged exposure seems to enhance antitumour activity. Thus, in most of the phase II studies, these agents have been given at a daily × 5 schedule repeated every 21 days.

Most advanced in its clinical development today is topotecan. Preliminary data suggest topotecan to have similar activity in ovarian cancer as that of paclitaxel. In small cell lung cancer, topotecan achieved response rates of >50%, >30% and <10%, respectively, in chemotherapy-naive patients, so-called "chemotherapy-sensitive" tumours and in refractory cancers. Combination studies with either cisplatin or paclitaxel are in progress in this tumour type. A number of phase I/II studies have also been completed with irinotecan, mainly in Japan, but also in the U.S. and in Europe. For CPT-11 to become active, it must be cleaved by the enzyme carboxylesterase, which is mainly produced in the liver, to the active metabolite SN-38. The main activity has been reported in colorectal cancers, with response rates of around 20–30% in patients refractory to 5-fluorouracil (5-FU). Activity was also seen in ovarian cancer.

Current knowledge on minor groove binders, specifically the cyclopropylpyroloindole compounds carzelesin, adozelesin, bizelesin and the benzoyl mustard derivative of distamycin A, tallimustine, was reviewed by M. D'Incalci (Milan), A. Awada (Brussels) and R. Holland (Puurs). These agents have been found to produce high sequence-specific alkylation, with DNA-binding at AT-rich regions. Compounds which preferentially bind to DNA at GC-rich regions are in preclinical development. The mechanisms through which these drugs kill cells are still unclear. The agents appear to inhibit transcription and to induce cell cycle arrest in G2. Also unclear is how cells repair the particular type of DNA damage caused by these compounds. What is known is that the alkylating moiety is essential for either of these drugs to produce cytotoxic activity.

The dose-limiting toxicity of these agents was found to be granulocytopenia ≥ thrombocytopenia, with nadirs at around days 10 and 20, respectively, and delayed recovery. Of interest, a significant proportion of patients experienced a second granulocyte nadir around day 36. In the phase II studies conducted so far, activity of these compounds has been low. Adozelesin has produced some responses in acute myelocytic leukaemia (AML), but virtually none in solid tumours. Tallimustine was inactive in all trials completed to date.

A class of drugs which has generated much interest in recent years is the bioreductive agents. The lead compound of the bioreductive alkylating indoloquinones, EO9, was discussed by J. Wanders (Amsterdam). EO9, which is structurally related to mitomycin C, is reduced by DT-diaphorase (DTD) to the bioactive species, which produces single-strand DNA breaks. EO9 showed promising preclinical activity, including activity in tumour models which are frequently resistant to cytotoxic drugs. DTD has been found to be elevated in a number of animal and human tumour cell lines, and EO9 was found to be active in tumour cells under hypoxic conditions. All these characteristics indicated that EO9 may be a promising novel cytotoxic drug. The dose-limiting toxicity of EO9 was proteinuria, which usually was fully reversible. Some patients also developed impaired renal function. In phase I, linear pharmacokinetics was observed with wide interpatient variability. In phase II, no significant

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correlation was found between the occurrence of proteinuria and the AUC of EO9. Phase II studies were performed in breast, colorectal, pancreatic, gastric and non-small cell lung cancer, but unfortunately no response was observed in any of these tumour types. Furthermore, the pattern and degree of renal toxicity observed in these studies seems to leave little room for modifications of dose and/or schedule that might result in higher clinical activity of EO9.

The lead compound of the benzotriazine group of bioreductive agents, tirapazamine, was discussed by R. Rampling (Glasgow). In preclinical models, marked synergy was found between this drug and radiation or other cytotoxic agents. Based on this data, tirapazamine is being specifically developed for clinical use in combination with radiation or with other cytotoxic drugs such as cisplatin. The dose-limiting toxicity in phase I was ototoxicity, which was in part severe but usually reversible. At higher doses, all patients had nausea and vomiting, which could be only partially prevented by ondansentron. Furthermore, the majority of patients experienced muscle cramps and fatigue.

The current status of paclitaxel in combination with other agents was reviewed by L. Gianni (Milan). In preclinical models, both synergy and antagonism were found between paclitaxel and other cytotoxic drugs, with schedule and sequence being important factors for the type of drug interaction produced. In the clinic, two extensively tested combinations have been paclitaxel and either platinum or doxorubicin. In phase I/II, paclitaxel followed by platinum has been found to be better tolerated as compared to the alternative sequence. In the European–Canadian ovarian cancer trial, which compared cisplatin-paclitaxel with cisplatin-cyclophosphamide, the paclitaxel combination proved to be significantly more effective in terms of response rate, progression-free and overall survival. This particular combination also proved to be very active in women with anthracycline-refractory breast cancer. A common problem in all studies using paclitaxel-cisplatin was cumulative neurotoxicity, particularly when using paclitaxel at high doses and by 3-h infusion.

Various studies evaluated the combination of paclitaxel and carboplatin. In ovarian cancer, this combination produced substantial activity similar to paclitaxel-cisplatin. In non-small cell lung cancer, paclitaxel-carboplatin achieved response rates in the range of 60%. The main toxicity in the paclitaxel-carboplatin studies was febrile neutropenia and infections. Of interest, the addition of paclitaxel to carboplatin resulted in lower rates of thrombocytopenia than usually seen with carboplatin alone.

The combination of paclitaxel with doxorubicin was mainly tested in breast cancer. Data on schedule-dependency of toxicity of this particular combination differ between studies, with some studies showing lower toxicity when doxorubicin preceded paclitaxel whereas others have found no sequence effects. Of some concern is the 18% rate of myocardiotoxicity observed in Milan with this combination, although most of these patients had received high total cumulative doses of doxorubicin and/or prior radiation of the left chest wall. The response rates reported in the breast cancer trials are as high as >90%. One observation of interest is that paclitaxel seemed to affect the pharmacokinetics of doxorubicin, with a marked increase in the plasma levels of doxorubicin and doxorubicinol. The responsible mechanism may be the presence of the solvent Cremophor EL® in the clinical paclitaxel formulation. Cremophor is known to inhibit P-glycoprotein function and this might lead to interference with doxorubicin excretion.

Interesting data was reported by R. Duncan (London) and J. Cassidy (Aberdeen) on PK1, a N-(2-hydroxypropyl) methacrylamide co-polymer backbone bound to doxorubicin via a peptidyl spacer. This conjugate is engineered to be cleaved intracellularly by lysosomal thiol-dependent proteinases after uptake via endocytosis, thus releasing free doxorubicin inside the cancer cell. In an ongoing phase I study, the dose level of doxorubicin achieved when given as PK1 is equivalent to 320 mg/m^2 of pure doxorubicin. Nonetheless, very little toxicity was observed, particularly no cardiotoxicity. Some responses were observed in patients with non-small cell lung cancer. By comparison, the maximum tolerated dose (MTD) of liposomal doxorubicin has usually been not much higher than that of pure doxorubicin.

The ADEPT (antibody-directed enzyme prodrug therapy) concept was discussed by T.A. Connors (Surrey). It has been shown that the difference in toxicities between the prodrugs that are currently under active investigation and their active metabolites is usually around 200-fold. Accordingly, delivery of a particular enzyme capable of generating the active moiety to tumours via conjugates consisting of this enzyme linked to a particular "tumour-specific" antibody may permit the prodrug, which follows in a second step, to achieve tumour-specific cytotoxicity. In human tumour xenografts, this concept has been found to work, and clinical trials of this strategy are anticipated to start within the next few years.

There has been increasing discussion and some controversy in recent years on the use of haemopoietic growth factors (HGFs) in phase I studies of cytotoxic drugs. This issue was the subject of a controversy session, with D. Parkinson (Bethesda) being announced as pro- and S.B. Kaye (Glasgow) as con-speaker. As it turned out, both speakers arrived at the same conclusion, i.e. that the use of HGFs in phase I is not indicated. Some of the arguments put forward were that HGFs generally permit dose increases of cytotoxic agents by only 1.5- to 2-fold, an order of magnitude which is clinically insignificant for the majority of cancers. In addition, duration of exposure can be more important than dose, as can the total dose achieved as compared to the individual dose; and the requirement for HGFs could be a disincentive to a drug's further development. Some interesting data discussed by S.B. Kaye were that, in the past, most agents which were eventually marketed showed some response in phase I, that such responses were usually seen at doses around the MTD, and that lethality in phase I has been low, around 0.5%. There also seemed to be general agreement that revising phase I methodology is something that should be thought about. Discussed examples included increasing the starting dose (currently usually 10% of the so-called mouse equivalent LD_{10}), decreasing the number of patients per non-toxic dose levels, redefining dose-limiting toxicity and increasing the number of patients entered at toxic dose levels and at the MTD.

Non-EORTC groups represented at this meeting were the U.S. NCI, the NCI Canada, the Cancer Research Campaign in the U.K., early drug development groups from Japan, Australia and Germany, and the SIOP.

D. Parkinson reported that approximately 40 000 compounds have been tested in the past 4 years in the U.S. by the NCI, pharmaceutical and biotechnology companies, and individual investigators. From these agents, usually five per year went into clinical development. An important observation appears that compounds with similar modes of action had similar sensitivity patterns in the NCI cell line screen. Currently, the most important criteria applied by the NCI for selecting particular agents for further development are a different mode of action (so-called COMPARE negativity) and particular structural features.

Thus, an important new endpoint in phase I has become whether agents prove capable of achieving their biochemical goal. Some of the more interesting compounds which have been recently taken into clinical development are: flavopiridol, a cdc2 kinase inhibitor; UCN-01, a PKC inhibitor; CM-101, a compound that induces a strong inflammatory response against neovasculature without having an effect on established blood vessels; and perillyl alcohol, which can inhibit isoprenylation of small G proteins (such as ras).

E. Eisenhauer (Kingston) from the NCI Canada reported that docetaxel had little activity in previously untreated soft tissue sarcomas. Interestingly, 3 of 4 patients with leimyosarcomas had a response. No response was observed with docetaxel in gliomas. Topotecan also had little activity in untreated soft tissue sarcomas and in gliomas. The antigestagene RU 486 achieved a response rate of merely 11% when given as first-line hormone therapy to patients with progesterone receptor positive breast cancer.

The use of enzymes that are critical in cell cycle control as targets for anticancer drugs was discussed by L. Meijer (Roscoff, France), with focus on the G2/M transition, which in all organisms seems to be promoted by the M Phase Promoting Factor (MPF). As for the many other kinases involved in the delicate control of the cell cycle, phosphorylation and dephosphorylation play an essential role in the activation and de-activation of MPF. Using the readily measurable enzyme activity of phosphatases, interaction of a variety of compounds with important cell cycle regulators was analysed. Some drugs which were found to be effective inhibitors are butyrolactone I, fluoropiridol and staurosporine, drugs that are known for other effects such as inhibition of protein or tyrosine kinases.

Apoptosis and apoptosis-associated genes were discussed by A. Clarke (Edinburgh), R. Brown (Glasgow) and J. Hickman (Manchester). Of the many interesting studies and data reported, three appeared of particular significance: (1) in drug-selected resistant cell lines, often a loss in p53 function could be found without mutations of the gene. Hence, analysis in clinical tumour samples should include functional p53 assays rather than solely looking for the presence of p53 mutations; (2) highly sensitive testicular cancer cell lines were found to usually lack *BCL2*, whereas high *BCL2* levels were often expressed in resistant bladder cancer cell lines; (3) in *BCL2* transfectants, drugs caused the same amount of DNA damage as in wild-type cells, yet the transfectants failed to die. This appears to be a potentially dangerous situation. Cells try to repair DNA damage which in turn results in mutations that may lead to a more malignant phenotype. Applied to the clinical scenario, this could imply that cytotoxic treatment of tumours which have increased levels of *BCL2* not only is ineffective but may cause the tumour cells to develop a more malignant phenotype.

As to the future of anticancer drug development, a recurrent message at the meeting was the need to pursue novel strategies. These include mechanism-based screening, the use of biological endpoints in phase I, and close interaction between laboratory and clinic with rapid flow of information. Implementation of such strategies seems to be a prerequisite for identifying novel effective anticancer drugs, and recently the EORTC has been taking some essential steps toward achieving this goal. This report should not end without acknowledging the excellent organisation of the meeting and of all the social events by Dr N. Pavlidis from the University of Ioannina in Greece. The science at the meeting was excellent. However, Nicholas and his team, along with the sunshine, the beautiful scenery and the Greek culture also succeeded in providing the participants with an enjoyable and memorable experience.