REVIEW

DEVELOPMENT OF NEW ANTI-CANCER DRUGS

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In the first award lecture of the European Society of Medical Oncology (ESMO) the topic of new drug development and the role of the European Organization for Research and Treatment of Cancer (EORTC) was highlighted. New aspects in each of the drug development steps are discussed: acquisition, screening, formulation, toxicology and phase I studies. In the search for new compounds to treat human solid tumors it is proposed to use human tumors as xenografts in primary screening. Phenomena related to doxorubicin resistance are presented together with a new approach to circumvent this in the clinic. The value of biochemical modulation is discussed, exemplified by the combination of 5-fluorouracil and uridine. The complexity of the biological response modifiers and the importance of evaluating them adequately in the clinic is stressed. The EORTC has recently decided on requirements for the minimum toxicology for phase I trials of a new cytostatic drug in order to ensure safe and rapid evaluation of new anti-cancer compounds. The therapeutic intents of phase I studies are questionable and therefore the main goals of these studies to be reached quickly; possibly supported by a pharmacokinetic rational.

Key words: Development, Anti-cancer drugs.

INTRODUCTION

During the past few years Europe has experienced an outburst of activity in the area of drug development of anti-cancer agents. Many cancer research institutes and clinical centers cooperating in the EORTC, the European Organization for Research and Treatment of Cancer, have contributed to this development. This is reflected by the fact that in 1985 nine new compounds were evaluated in phase I studies within the framework of the EORTC. New drug development has been coordinated through the New Drug Development and Coordinating Committee of the EORTC Board. These increased activities have culminated in the establishment of the EORTC New Drug Development Office, the NDDO, which is situated at the Free University Hospital in Amsterdam.

The NDDO aims to:

1. accelerate drug development within the EORTC;
2. reduce the time lag between drug synthesis and introduction into the clinic;
3. solve bottlenecks in drug development in Europe, such as formulation and toxicology; and
4. ensure high standards of drug development and phase I studies.

Besides the EORTC several national leagues, e.g. the Netherlands Cancer Foundation and the British Cancer Research Campaign, have increasingly supported drug development. The NCI Liaison Office in Brussels, headed by Dr. Omar Yoder, has played a major role in European drug development.

Drug development may be divided into the five steps shown in Table 1. The aim of this paper is not to review the entire field of anti-cancer drug development, but to highlight certain important aspects of drug development and indicate new directions.

<table>
<thead>
<tr>
<th>Table 1. Steps in cancer drug development</th>
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<tr>
<td>Acquisition</td>
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<td>Screening</td>
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<td>Formulation</td>
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<td>Toxicology</td>
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<td>Phase I studies</td>
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63
SCREENING

The U.S.A. National Cancer Institute (NCI) is the largest institute applying random screening for anti-tumor activity on new compounds. This drug-oriented screening program is based on a single pre-screen, the mouse leukemia L1210, and from 1974 the mouse leukemia P388. This pre-screening was followed by a secondary screen in a panel of different murine tumors. Thereafter 1-3 human xenografts in the nude mouse were added to this panel.¹ In an interim analysis of the NCI screening panel the following conclusions were reached:² high and broad spectrum activity of a compound in a variety of tumors in the panel appears to increase the predictability of at least minimal clinical activity for one or more human tumors. Human tumors in xenografts appear to be relatively resistant to therapy. Only about 50% of the clinical active compounds show activity in the MX-1 mammary xenograft (subcutaneous or subrenal capsule assay). On the other hand, 96% and 92% of the clinical compounds are active in the P388 and L1210 leukemia respectively. This is not surprising because by definition of this screening model compounds selected for clinical studies must be active in the pre-screen and they are sensitive models of rapidly proliferating cells. The concern about false negatives, compounds that are inactive in the screen but which would have been active if tested in the clinic, has always existed.² This concern is growing as more compounds bypass the pre-screen, showing activity in the clinic: hexamethylmelamine (ovarian cancer), deoxycoformycin (mycosis fungoides), quinazoline (breast, ovarian cancer and hepatoma).

The disaffection with the NCI screening panel has been growing because few clinically active agents have been identified and compounds have been active almost exclusively in the treatment of leukemias and lymphomas. Several reasons for this low predictive value have been mentioned, such as a different biology and doubling time of murine leukemias (P388 and L1210) and human solid tumors or the difference in measuring response criteria in murine tumors and human tumors.

There is a great need to develop new screening strategies with a higher predictive value in order to detect compounds which give positive results in treating human solid tumors. The NCI therefore decided to embark on a tumor-oriented screening project, based on a panel of human tumor cell lines of the main tumor types as a pre-screen. Hereby 'compounds with highly specific anti-tumor activity' might be detected as well as 'compounds with non-specific anti-tumor activity'.³ Non-specific compounds will be developed in a similar way to that used at present, however, priority will be given to the development of compounds with a specific anti-tumor activity which can be evaluated in 'disease orientated phase I/II trials'. This project is challenging in its simplicity, the number of human tumors it encompasses and its low cost. In a recent paper the value of applying human tumor material in vitro as a pre-screen has been demonstrated.⁴ Fourteen out of 79 compounds showed activity in a human tumor colony-forming assay, compounds which have been designated previously as negative in the current P388 pre-screen. Those 14 compounds are structurally unrelated to compounds already in development.

However, there are several drawbacks to in vitro screening: an artificial environment of cells may alter chemo-sensitivity and culture conditions may cause artificial changes in tumor cell biology; the effects of drug distribution, metabolism and excretion in the in vitro situation are not taken into account, and neither is normal tissue toxicity.⁵ For these reasons in the initial phase this new NCI screening project will run parallel to the P388 pre-screen in order that the two systems may be compared.⁶

Another strategy is to use human tumor xenografts in immune-deprived mice for upfront drug screening. This strategy is based on the excellent predictive value of xenografts for individual clinical response between mouse and man (Table 2); and on the prediction of clinically active drugs per tumor type, once a few xenografts of the same tumor type are introduced as a test. The cumulative data in Table 2 show 87 experiments presently available for this comparison⁷-⁹ performed with the nude mouse subcutaneous assay only. The first two columns show human tumors which responded similarly in the mouse and the subject; there were only 4 false positive experiments and one false negative test. These data indicate that xenografts in immune-

<table>
<thead>
<tr>
<th>Tumor type</th>
<th>Total</th>
<th>R/R</th>
<th>P/P</th>
<th>R/P</th>
<th>P/R</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colorectal</td>
<td>27</td>
<td>3</td>
<td>23</td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Stomach</td>
<td>9</td>
<td>4</td>
<td>5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SCLC</td>
<td>9</td>
<td>7</td>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NSCLC</td>
<td>16</td>
<td></td>
<td>16</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Melanoma</td>
<td>23</td>
<td>6</td>
<td>13</td>
<td>4</td>
<td></td>
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<td>2</td>
<td>2</td>
<td></td>
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<td></td>
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<tr>
<td>Ovary</td>
<td>1</td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>87</td>
<td>22</td>
<td>60</td>
<td>4</td>
<td>1</td>
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</table>

*R = remission; P = progression.
deprived mice are excellent predictors of individual responses to chemotherapy.

Retrospective studies have shown that a series of xenografts of the same tumor type select the clinically effective drugs in this tumor type. Those studies have been done in the subcutaneous assay for small cell carcinoma of the lung,\textsuperscript{10} breast carcinoma,\textsuperscript{11} colorectal cancer,\textsuperscript{12} head and neck tumors\textsuperscript{13} and ovarian carcinoma;\textsuperscript{14} and in the subrenal capsule assay for melanoma, colorectal cancer and breast carcinoma.\textsuperscript{15} The spectrum of sensitivity of a number of xenografts of the same tumor type agrees with the clinical findings. This has stimulated researchers to initiate phase II trials in the nude mice in order to compare the results with clinical phase II trials.

Considering these interesting observations on xenografts, it appears reasonable to embark on a study to delineate the potential role of xenografts in pre-screening. This would offer supplementary data to the new plans of the NCI to investigate the value of \textit{in vitro} screening on human cell lines. My proposal for such a pre-screen in human xenograft in nude mice is outlined as follows:

1. Selection of two to three human tumor xenografts each from tumors with a broad spectrum of sensitivity to chemotherapy, i.e. Wilms tumor, Ewing sarcoma, breast cancer, teratoma and/or small cell lung carcinoma (Group A);
2. Selection of two to three xenografts each from tumors resistant to chemotherapy, i.e. colon carcinoma, non-small cell lung cancer and melanoma, as even xenografts which are sensitive to conventional types of drugs may not be representative for the category of agents for which we are looking to eradicate resistant tumor types (Group B);
3. Determination of the spectrum of activity of 20 clinically active compounds and 10–20 clinically non-active compounds in groups A and B. Depending on the results of steps 1–3 one may select a definitive pre-screen with:
4. Two to three xenografts which are sensitive to as many clinically active compounds as possible (from group A) and
5. Two resistant xenografts (from group B).

Human tumor xenografts are an excellent tool in the screening of analogs of cisplatin in two ovarian carcinoma xenografts, a sensitive (MRI) and a resistant (PE) line.\textsuperscript{16} The results are summarized in Table 3 and show the exact percentage of growth inhibition of each experiment. The resistant line PE responded best to cisplatin and carboplatin, and least to TNO-6. The spectrum of sensitivity of this xenograft appears to correlate with that of ovarian cancer in the clinic. In the search for third generation analogs of cisplatin, which should be more potent than cisplatin, the ovarian carcinoma line PE will function as one parameter in the selection of such a compound.

**ACQUISITION OF ANTIMEOPLASTIC AGENTS**

At present one may distinguish five subclasses of antineoplastic agents as outlined in Table 4. As a result of experience in early clinical trials with new cytostatic drugs (a) and analogs of these compounds (b) it is now common practice to study these compounds with different schedules in phase I trials in order to determine the optimal dose schedule for phase II studies. A great deal of knowledge is now accumulating on the mechanisms of drug resistance (c), biochemical modulation (d) and the mechanisms of action of biological response modifiers (e). Experience in early clinical trials with the latter three groups of compounds is still minimal, but in the near future clinical researchers will be increasingly involved in trials with these categories of compounds. In order to perform comparable and meaningful clinical studies with non-cytostatic compounds one might advocate designing master protocols for early clinical trials with these new groups of compounds. The EORTC Early Clinical Trials Group has initiated this activity.

Although several mechanisms of drug resistance have been elucidated, attempts to overcome drug resistance clinically have been initiated only recently. Findings related to resistance to anthracyclines have been identified as:

1. Decreased levels of free radicals in resistant cells, an observation which may be related to either a decreased formation of free radicals or a better protection against free radicals;
2. Impaired drug retention in resistant cells due to a higher efflux, which appears to be related to amplification of certain membrane glycoproteins, which in their turn appear to be related to amplified genes. In particular the glycoprotein P170 is to some extent related to pleotropic drug resistance, i.e. cross-resistance to a number of natural products including the anthracyclines.

Recent experiments in our department show that the concentration of radio-labeled doxorubicin is significantly lower in the resistant CHO line \((\text{CHO}_{R})\) than in its sensitive parent line \((\text{CHO}_{S})\) - Fig. 1). This phenomenon is due to an increased efflux of doxorubicin from the resistant cells. Resistance has been reversed \textit{in vitro} and in the mouse by calcium channel blockers such as verapamil, which
Table 3. Comparative activity of platinum compounds in the human ovarian cancer xenografts MRI-H-207 and ov.PE in nude mice.

<table>
<thead>
<tr>
<th>Analogs</th>
<th>MRI-H-207</th>
<th>Ov.PE</th>
<th>%*</th>
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<tr>
<td>Cisplatin</td>
<td></td>
<td></td>
<td>(100-67)</td>
</tr>
<tr>
<td>Carboplatin</td>
<td></td>
<td></td>
<td>(100-63)</td>
</tr>
<tr>
<td>Iproplatin</td>
<td></td>
<td></td>
<td>(100-34)</td>
</tr>
<tr>
<td>JM-40</td>
<td></td>
<td>Ⅰ</td>
<td>(100-24)</td>
</tr>
<tr>
<td>TNO-6</td>
<td></td>
<td>Ⅰ</td>
<td>(96-0)</td>
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*Growth inhibition (%)  

Table 4. Classes of antineoplastic agents

New anti-cancer drugs  
Analogs of anti-cancer drugs  
Drugs which reverse resistance  
Biochemical modulators  
Biologicals and biological response modifiers

Fig. 1. Retention of [14C]adriamycin (ADM) in a sensitive (AuxB₁) and a resistant (CH₃C₆) chinese hamster ovarian (CHO) cell line. Cells were incubated at 37°C with 2μM of [14C]ADM.

blocks the efflux of doxorubicin. However, verapamil does not appear to be clinically effective, probably because the concentration that is effective in vitro appears to affect the heart function in man. We are presently experimenting with another compound, which appears to enhance doxorubicin cytotoxicity not only to the resistant cell line but also to the sensitive cell line of Fig. 1. The concentration of this compound which is needed to modulate the cytostatic effect of doxorubicin in vitro can indeed be achieved clinically. This is a crucial question to be taken into consideration in this type of experiment. A clinical study is now being initiated with this compound in subjects who become resistant to doxorubicin treatment.

Biochemical modulators form a separate group of agents which are being evaluated clinically. A well known example of a modulator is that of leucovorin which reduces methotrexate toxicity. Peters et al. observed a similar effect in mice bearing the colon-26 tumor. Mice treated with 5-fluorouracil (5-FU) plus uridine are rescued from 5-FU toxicity, as is shown by a prolonged survival rate compared to both non-treated mice and mice treated with 5-FU alone. These observations confirm the earlier findings of Martin. Rescue of the tumor by uridine was negligible (Fig. 2). Phase I and II trials with biochemical modulators are complicated and require extensive pharmacological monitoring of both the antineoplastic agents and the modulator, besides the evaluation of the effect on the tumor and normal

Fig. 2. The effect of 5-fluorouracil (5-FU at 300 mg kg⁻¹ per dose) on the murine colon-26 tumor given with or without uridine (URD at 3500 mg kg⁻¹) 2 and 20 h after each 5-FU dose.
Development of new anti-cancer drugs

At present we are investigating clinically the effect of uridine on 5-FU toxicity. This study required a more sensitive assay of 5-FU than the one which has been available up to now. For this purpose Kok and Lankelma developed an assay applying HPLC plus mass spectrometry, which is one hundred-fold more sensitive than the conventional gas-liquid chromatography assay. Applying this assay we found that 5-FU is present for a prolonged time following a standard bolus injection of 5-FU. Thus 5-FU appears to be slowly released from the nucleotide pool, resulting in a plateau of which the concentration is probably related to the biological activity of the drug. In our clinical study on 5-FU plus uridine, 5-FU is administered weekly as a bolus and our recent observation is that uridine indeed rescues the white blood cells and the platelets, even with continued weekly exposure to 5-FU. We are evaluating the time period over which this effect of a single administration of uridine is maintained, and will continue experimenting with increasing doses of 5-FU. As mentioned above, plasma levels of uridine and 5-FU are monitored during this study, reflecting the complicated investigations required in clinical studies with biochemical modulators.

The fifth class of antineoplastic agents, the biologicals and/or biological response modifiers, consist of immunomodulators, lymphokines and cytokines, growth and maturation factors, effector cells, tumor-associated antigens and monoclonal antibodies. Several tests have been developed in order to screen for the in vitro and/or in vivo activity of these compounds. A complicating factor with the biological response modifiers is their species specificity. It is apparent that, due to lack of clinical evaluation, the selection of predictive pre-clinical assays for biological activity and efficacy is difficult. Interleukin 2 (IL-2), a lymphokine, is just beginning to enter clinical trials. In this context it is important to be aware of the following observations which may be relevant for the clinical application of IL-2:

1. it may restore T-cell function, if this is related to deficient endogenous production of IL-2;
2. chemotherapy may inhibit IL-2 production and coadministration of IL-2 together with chemotherapeutics circumvent this effect;
3. IL-2 can increase the activities of cytotoxic effector cells resulting in considerable anti-tumor effects;
4. simultaneous administration of IL-2 and cultured effector cells lead to a greater anti-tumor effect than when either of them is administered alone.

Other interesting compounds among the biologicals are the growth factors. Growth factor research originally stems from tissue culture studies. Figure 3 shows the cloning efficiency of the human ovarian cancer cell line OVCAR in soft agar with medium containing either fetal calf serum (FCS) or cell-free ascites (CFA), both with or without the addition of epithelial growth factor (EGF). From the findings in Fig. 3 it is apparent that the CFA contain more potent proliferation-inducing factors than FCS. With the combination of CFA and EGF the cloning efficiency of these ovarian cancer cells was greatest. Our group is at present working on the isolation of the growth factors present in these ascites. Exploitation of growth factors as a target of cancer therapy involves a number of approaches: attempts are being made to develop analogs of the growth factors themselves; antibodies to growth factor receptors are being developed as are antibodies to growth factor themselves; the effects of interference with the steps of growth factor action, such as protein kinase, are being studied.

As a last exciting example of a biological agent the monoclonal antibody must be mentioned. It is critical to demonstrate that monoclonal antibodies, when given intravenously, reach the tumor and show a degree of specificity to tumor cells, as has been shown by Colcher et al. While radio-isotopically-labeled antibodies can be used to image the tumor, sensitive immunologic techniques provide evidence for localization of the antibody on the tumor cell membrane. The first reports suggest that anti-idiotypic antibodies may be of therapeutic benefit,

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Fig. 3. Cloning efficiency of the human ovarian cell line OVCAR-3 in dependence of the biological growth factors: fetal calf serum (FCS), cell-free ascites (CFA) and epithelial growth factor (EGF). $\Delta = \text{CFA} + \text{EGF}; \Delta = \text{CFA} - \text{EGF}; \bullet = \text{FCS} + \text{EGF}; \circ = \text{FCS} - \text{EGF}.$
however, recently published data suggest that those antibodies may exert a strong selective force against tumor cells expressing the idotype determinant. 23

Again, clinical trials with these biologicals are very difficult and should consist of two steps. The first step concerns the determination of potential toxicity with standard phase I procedures in patients with advanced cancer, while the second step should assess the effects of the biologicals on the immunologic systems of the patients. The latter implies that studies should be performed in patients with minimal or no detectable disease, while immunologic parameters must be determined throughout the study.

TOXICOLOGY

The EORTC has prepared a document on the 'requirement for the minimum toxicology for phase I trials of a new cytostatic', which calls for pre-clinical safety studies with a single dose and repeated doses in the mouse. For reasons of safety the starting dose for phase I trials, the mouse equivalent dose LD10, should be tested in a second species, i.e. the rat. With this document the EORTC aims to eliminate unpredictable and over-predictive toxicology tests, 24 accelerate drug development and introduce into the clinic new and promising compounds for cancer therapy.

However, for the development of analogues more detailed pre-clinical safety studies are indicated. During the development of analogues of cisplatin we performed comparative renal toxicity studies in the dog. Although the doses tolerated by the dog are not entirely comparable to those in man, extreme proteinuria, caused by TNO-6 in man was reliably predicted by the dog. 25

PHASE I STUDIES

Finally, to end this discussion of new anti-cancer drug development, a few remarks concerning phase I studies should be made.

Phase I studies have no therapeutic intent, and therefore the main aims of these studies must be achieved as quickly as possible; that is, to define the maximum tolerable doses (MTD) and dose-limiting toxicity and to define a safe starting dose for phase II studies. Many phase I studies take a long time and too many subjects have to volunteer for a single study. This may be because the starting dose chosen is too low, or the escalation scheme is too slow, following the modified Fibonacci sequence. Therefore, changing the escalation scheme, as proposed by Collins, 26 might be a good approach towards minimizing the number of patients needed to volunteer for these studies. According to Collins such a dose escalation scheme might be guided pharmacologically rather than being based on a fixed sequence like the Fibonacci one. His proposition makes use of the assumption that the area under the plasma concentration times time curve at the mouse LD10 is equal to the area under the plasma concentration times time curve at the human MTD. If this method proves to be valid, more new concepts of cancer therapy may be tested in patients volunteering for the necessary phase I studies.

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