New Anticancer Drug Development within the EORTC-NDDO

H.R. Hendriks, R.E.C. Henrar, H.M. Pinedo, G. Schwartsmann

EORTC New Drug Development Office, Free University Hospital, Amsterdam, The Netherlands

Introduction

In 1962, the European Organization for Research and Treatment of Cancer (EORTC) was founded by leading European cancer specialists in order to set up a European framework to stimulate and coordinate research into, and treatment of, cancer. Presently, the scientific activities of the EORTC are organized under four branches: education, research, epidemiology, and treatment.

In 1981, the New Drug Development Coordinating Committee (NDGCC) was created as part of the Research Branch of the EORTC to strengthen a comprehensive European anticancer drug development program, covering the stages from drug acquisition up to clinical trials. The NDGCC selects new candidate anticancer agents for preclinical and early clinical development, and has contributed substantially to the setting up of guidelines for the formulation of anticancer agents [1], animal toxicology [2], phase-I and early phase-II clinical trials [3-5]. The NDGCC is composed of representatives from EORTC groups involved in anticancer drug development, such as the Screening and Pharmacology Group, Clonogenic Assay Screening Study Group, Pharmacokinetics and Metabolism Group, Early Clinical Trials Group, Clinical Screening Group, and the disease-oriented groups. In addition, representatives from the National Cancer Institute (NCI) of the USA, and the Cancer Research Campaign (CRC) of the United Kingdom participate in the meetings of the NDGCC.
The tasks of the NDDC are executed by the New Drug Development Office (NDDO). The NDDO was founded in 1984 and is located both at the Free University Hospital and the Netherlands Cancer Institute, in Amsterdam. It coordinates anticancer drug development programs from drug acquisition, in vitro and in vivo screening, drug formulation and synthesis, animal toxicology, to phase-I and early phase-II clinical trials. Compounds entering clinical trials through the NDDO are reviewed by the NDDC. Priority for clinical development is generally given to agents with a novel chemical structure, a unique mechanism of action, and, in case of analogs or pro-drugs of known active agents, a higher therapeutic index than the parent compound in preclinical models.

Since the initial report [6] on the organization and activities of the office, substantial developments have taken place at the NDDO. Comprehensive research programs with other EORTC groups, CRC, NCI, and the pharmaceutical industry, have been established. An outline of these activities is given in the following sections of this paper.

**Drug Development Program**

The NDDO is directly involved in the coordination of all the preclinical and early clinical steps in the development of new anticancer agents: the acquisition of new candidate compounds, in vitro and in vivo drug screening, preparation of a suitable drug formulation for clinical use, production of the drug in sufficient quantities for toxicology and early clinical use, generation of animal toxicology data to establish a safe starting dose in humans, and the planning, data handling and monitoring of phase-I and early phase-II clinical trials.

The first step in the development of new anticancer compounds is acquisition. The selection of candidate compounds is based on the novelty of their chemical structure, their assumed mechanism of action, preliminary data on their antitumor activity, if available, their solubility in an acceptable vehicle, and the patent position. Most compounds acquired by the NDDO come from academic institutions, such as university and research laboratories. A considerable number of new compounds come from the chemical and pharmaceutical industry. In addition, new compounds can be obtained from the NCI and/or CRC, under their collaborative agreement with the EORTC. This agreement, signed in May 1986, aimed to facilitate anticancer drug development through close coopera-
tion and exchange of data on potential new compounds for clinical development.

After acquisition, the strategy of drug development will be based on the knowledge already available on the compound, varying from initial screening tests for antitumor activity up to clinical evaluation in phase-I and early phase-II trials (table 1).

**Drug Screening**

The policy of the NDDO regarding the acquisition and preclinical screening of potential anticancer agents was recently reviewed by a panel of experts. In March 1991, the NDDCC decided to establish a preclinical subcommittee composed of representatives of EORTC preclinical and clinical groups, the NCI and CRC, invited experts, and the NDDO staff involved in preclinical drug testing. This subcommittee will meet several times a year to discuss the ongoing testing of potential new drugs and to identify areas in which action should be taken, e.g., biologicals, hormo-

<table>
<thead>
<tr>
<th>Step 1</th>
<th>Drug acquisition¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Step 2</td>
<td>Screening, pharmacoology</td>
</tr>
<tr>
<td>Step 3</td>
<td>Synthesis on larger scale</td>
</tr>
<tr>
<td>Step 4</td>
<td>Pharmaceutical formulation</td>
</tr>
<tr>
<td>Step 5</td>
<td>Animal toxicology</td>
</tr>
<tr>
<td>Step 6</td>
<td>Preclinical pharmacokinetics</td>
</tr>
<tr>
<td>Step 7</td>
<td>Phase-I clinical studies</td>
</tr>
<tr>
<td>Step 8</td>
<td>Early phase-II clinical studies</td>
</tr>
</tbody>
</table>

¹ Depending on the stage of development at acquisition, new compounds can be directed to a later step in the development program.
nes, etc. This new approach will most probably improve the development of new anticancer agents within the EORTC and in Europe.

The drug discovery program of the NDDO now comprises two strategies: 1) rational drug design, with selection of biochemical and/or molecular targets and the use of appropriate models for evaluation; and 2) random drug screening.

In order to have a fair chance of identifying compounds active against human cancers in random drug screening, a large and complex panel of tumor lines is necessary. The new NCI disease-oriented panel for investigational drug screening, consisting of about 60 human tumor lines in vitro, is an important development towards the achievement of this [7, 8]. Thus, in addition to the in vitro panel of human tumor xenografts developed by Fiebig et al. [9] and used by the NDDO to test compounds for antitumor activity, compounds can also be tested in the new NCI screen. Furthermore, compounds coming from the NCI screen are now offered for in vivo testing in the human tumor xenograft program coordinated by the NDDO as a secondary screen.

Currently, EORTC preclinical groups closely cooperate with the NDDO in the testing of new compounds. The Screening and Pharmacology Group is an example. This group consists of both chemists, synthesizing new compounds, and biologists, evaluating the activity of these compounds in primary and secondary screening in vitro and in vivo. Notably, new agents can also be tested in selected models, depending upon the characteristics of the compound to be evaluated [10].

In Europe, random drug screening of compounds submitted to the NDDO has been carried out using a panel of human tumor xenografts (HTX) [9]. This screen of fully characterized HTXs has the advantage that the activity of a compound can be tested in the same tumor both in vitro and in vivo. The predictive value of the panel for resistance to anticancer agents ("negative predictive value") is about 90% when in vitro clonogenic assay data are compared to the sensitivity of the original patient tumor or to the response of the HTXs in nude mice. The predictive value for sensitivity to clinically established drugs ("positive predictive value") is about 55-60% [11, 12]. The first step in the screen is to test the activity of potential cytostatic or cytotoxic drugs in vitro at three concentrations in a panel of six HTXs. The composition of the panel is based more on the sensitivity of the lines to standard anticancer agents than on ensuring that frequently occurring human tumor types are represented. The panel consists of one very sensitive non-small-cell lung carcinoma
line, two intermediately sensitive lines representing small-cell lung carcinoma and breast adenocarcinoma, and three resistant lines (melanoma, colorectal adenocarcinoma, and ovarian adenocarcinoma). After initial testing, either further tests are done at higher dilutions, or tests are repeated to confirm observed activity. If active, the compound is tested in vivo in the most sensitive tumor or tumors derived from the in vitro results. If sufficient activity is found again, additional HTXs are tested in vitro, and the in vitro sensitivity of human bone-marrow cells (table 2) is also determined.

During the past 6-7 years, the NDDO has screened the activity of over 200 compounds using this panel. So far, most of the compounds were alkylating agents, metal complexes, and natural products, as well as a variety of other compounds such as tubulin inhibitors (rhizoxin and new synthetic Vinca alkaloids) and calmodulin antagonists (fig. 1).

**Synthesis**

For initial evaluation of a new compound in the system, a limited supply is usually sufficient (up to 1000 mg). However, if a compound shows interesting antitumor activity, larger quantities (usually several grams) are needed for subsequent preclinical studies (e.g., formulation, toxicology) and clinical studies. To assist investigators in synthesizing the required amounts of new compounds, technical support can be obtained from the EORTC synthetic laboratory at the Technical University, Twente, The Netherlands.

Table 2. NDDO strategy for random screening of new compounds in human tumor xenografts (HTXs)

<table>
<thead>
<tr>
<th>Acquisition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Screening in a panel of six standardized HTXs in vitro</td>
</tr>
<tr>
<td>Tests in sensitive HTXs in vivo</td>
</tr>
<tr>
<td>Further antitumor activity testing in additional HTXs and human bone marrow cells in vitro</td>
</tr>
<tr>
<td>Additional testing in sensitive HTXs in vivo</td>
</tr>
</tbody>
</table>
Pharmaceutical Formulation and Animal Toxicology

Animal toxicology studies and initial clinical studies are only performed with an adequately formulated compound. Thus, compounds which are poorly water-soluble will need to be provided with a suitable formulation. In addition to its use in rendering the new compound soluble, the vehicle of choice should be nontoxic and, once formulated, stable for longer periods. This step is carried out by the Joint Formulation Working Party of the EORTC, NCI and CRC [1].

The minimum requirements for the testing of new cytostatic agents [2] stipulate that preclinical toxicology studies should be performed initially in two species: the mouse and the rat. The main objectives of these studies are to provide a safe starting dose for clinical phase-I trials, and to gather information about the nature and the reversibility of the toxic effects of the compound. Acute lethality studies are therefore performed in mice to determine the dose lethal to 10% of the animals (LD_{10} value) after a single intravenous or intraperitoneal injection. In subsequent acute
toxicity studies, the approximate LD_{10} is used to define the target organs for toxicity by examining various parameters in blood and histopathology at various time-periods after dosing. Similar studies are carried out with multiple dosing in mice. The starting dose for phase-I clinical trials is based on one-tenth of the LD_{10} value in mice. However, this dose is first administered to rats in acute and chronic toxicity studies, as employed in mice, as a safety check in a second species. If this dose is toxic in the rat, further studies are performed at a lower dose in rats.

*Preclinical Pharmacokinetics*

The NDDO coordinates research on the mechanism of action and pharmacology of new anticancer agents in preclinical models. Knowledge about the fate of new compounds in the organism contributes considerably to an understanding of the effects observed in animal studies, and is of great importance for the choice of proper drug schedules to be studied in the clinic [3].

*Phase-I Clinical Trials*

When a new compound has completed its preclinical evaluation, the first step towards clinical development is the phase-I trial. In these studies, patients with progressive malignancies that are not responding to any form of anticancer therapy are invited to participate in the trial. The main objectives in phase-I trials are to define the toxicity profile, the dose-limiting toxicities, and the maximum tolerated dose of the new agent for a specific schedule of administration [13]. Secondary to these aims, the pharmacokinetic behavior of the drug is studied.

Retrospective analysis [reviewed in 2] has shown that one-tenth of the mouse-equivalent LD_{10} value in mg/m^2 is usually a safe starting dose in phase-I trials. If this dose does not cause significant toxicity, or induces only mild to moderate toxic effects which are reversible before the start of the next drug administration, the dose is increased in further groups of patients [13, 14]. Finally, when the maximum tolerated dose – defined as the highest dose that can safely be administered to patients and that produces significant but reversible toxicity for the schedule used – is reached, a dose for phase-II clinical trials is recommended [15, 16]. This is
usually the dose step immediately before the maximum tolerated dose, or a dose 15-20% lower than the maximum tolerated dose. Examples of new anticancer agents in phase-I clinical trials being coordinated and monitored by the NDDO are discussed elsewhere [17].

**Phase-II Clinical Trials**

The emphasis in phase-I trials is on defining both the toxicity profile of the new agent in man and a recommended dose for further studies. In phase-II studies, however, the evaluation of the antitumor activity of the compound is the main goal. In order to determine the percentage of objective responses to the new agent, groups of patients who meet preestablished eligibility criteria and have the same tumor type are treated at the recommended dose derived from the phase-I study. The design of phase-II studies is such that an estimate of the percentage of objective responses can be derived, on statistical grounds, from a small number of patients. This is done to prevent unnecessary treatment of patients with compounds with a low level of activity, and to give priority to development of other potential anticancer compounds. If the response rates are considered to be above the required percentage (for instance $\geq 20\%$ with a confidence limit of 80%), confirmatory studies are carried out in a larger patient population [18]. The latter studies are generally performed by the EORTC disease-oriented study groups. An overview of the NDDO program for phase-I and phase-II trials with new anticancer agents has been published elsewhere [5].

**Examples of Ongoing Research NDDO Projects**

**Preclinical Phase-II Studies in the HTX Model**

Human tumor xenografts growing in immunodeficient nude mice have become increasingly important in assessing the antitumor activity of potential new anticancer drugs. It has been suggested that this model might reflect the human situation more accurately than autologous tumor models in rodents. A retrospective study undertaken by the NDDO analyzing the value of the HTX model in selecting new anticancer compounds has demonstrated a good correlation between drug efficacy in nude mice
and clinical results in the tumors of donor patients, as well as satisfactory predictability for clinically active agents and the outcome in a panel of human tumor lines for specific tumor types [19]. On the basis of these results and the observation that most newly developed drugs were active in only a few tumor types or were totally inactive in clinical studies, it was proposed to validate the use of panels of HTXs representing various tumor types in nude mice to predict drug efficacy in phase-II clinical trials [20]. If valid, these preclinical phase-II HTX studies could serve to detect the activity of new anticancer agents per tumor type in a panel of well-characterized HTXs using optimal dose and schedule. To reduce the numbers of patients involved in clinical trials with inactive agents, only drugs with at least minimal activity should be evaluated in phase-II clinical trials. As a result, a European multicenter study was initiated to investigate the value of the HTX model in preclinical phase-II studies for predicting clinical efficacy. For this study, a selection of HTXs was made from a large pool available at the participating centers. Four to eight HTXs, differing in histology, growth rate, and chemosensitivity, were chosen to represent each of seven major tumor types (breast, colorectal, head and neck, small-cell and non-small-cell lung, melanoma, ovarian). Four drugs (doxorubicin, amsacrine, and two investigational agents) were tested in this study, each at its maximum tolerated dose [21]. A multicenter study of this sort appeared to be feasible, and the results indicated a good correlation with the data of clinical studies: amsacrine, a clinically inactive drug, was negative in this study, and the results with doxorubicin in the various tumor types corresponded to the clinical situation, with the exception of its activity in non-small-cell lung cancer HTXs [22, 23]. In consequence, a follow-up study was started, in which a clinically active drug (cisplatin), a clinically inactive drug (diaziqione) and two investigational drugs were again tested. Preliminary results confirmed the clinical activity of cisplatin, whereas unexpectedly marked activity was found for diaziqione, which is clinically inactive in most solid tumors [24]. Obviously, these results will be discussed in detail in a future publication as the complete data become available for analysis.

Preclinical Development of the Indoloquinone E09

E09 is a new bioreductive alkylating agent from a series of fully synthetic indoloquinones, which are structurally related to mitomycin C [25]. The chemical structure of E09 is shown in figure 2.
In in-vitro structure-activity studies using R-1 rat rhabdomyosarcoma, L1210 murine leukemia, and the human tumor lines A204 rhabdomyosarcoma, MCF-7 mammary carcinoma, T24 bladder carcinoma, WiDr colon carcinoma, and IgR-37 melanoma, E09 was the most potent compound from the series. Its IC$_{50}$ values ranged from 1-100 ng/ml. This was confirmed in additional in vitro studies using a panel of HTX [26, 27], and in another panel of human small-cell lung cancer lines [28]. The results obtained from the NCI disease-oriented in vitro screen showed an IC$_{50}$ of about 5 ng/ml. Notably, activity was observed against human tumor lines derived from solid tumors, but no activity was found against human leukemia lines.

In vivo experiments corroborated the observations from in vitro studies; E09 was inactive against murine leukemias and two murine colorectal lines (MAC 13, MAC 15A). Activity was found in five out of seven HTXs and in murine colorectal adenocarcinomas MAC 16 and MAC 26 [26, 27]. The antitumor activity of E09 in the well-vascularized MAC 26 could be increased by co-administration of the vasoactive agent hydralazine. Since hydralazine "steals" the blood from the tumor, inducing a hypoxic environment in the tumor, the results indicated that E09 was more active under such conditions. In vitro experiments confirmed the bioreductive potential of E09 [29].

E09 was formulated as a freeze-dried product, using lactose as the excipient. Animal toxicology studies have been carried out with the for-
mulated material. The LD$_{10}$ value in mice after a single intravenous injection was 9 mg/kg, or 27 mg/m$^2$. In the acute toxicity studies, no treatment-related changes have been observed, either macroscopically or microscopically. After multiple intraperitoneal dosing of E09 (1.5 mg/kg) for four weeks, only transient hyperemia in the jejunum was observed. Bone marrow was not affected by the compound. The one-tenth mouse-equiv- alent LD$_{10}$ (0.45 mg/kg) appeared to be safe in rats in acute and chronic toxicity studies [30].

Pharmacokinetic studies of E09 revealed plasma half-lives of two and four minutes in mice and rats, respectively. In dogs, the plasma clearance was biphasic, with a $t_{1/2a}$ of 1-3 min and a $t_{1/2b}$ of 5-14 min. Compared to mitomycin C, E09 seems to have a distinct bioactivation mechanism [31]. The compound is scheduled for phase-I clinical trials in the coming months.

Preclinical and Clinical Studies with Rhizoxin

Rhizoxin is a 16-membered antifungal macrocyclic lactone isolated from the plant pathogenic fungus *Rhizopus chinensis* (fig. 3) [32]. The compound binds to tubulin at the vincristine binding sites, preventing microtubule formation, and inhibits the mitosis of tumor cells. In preliminary studies [33-35], antitumor activity has been demonstrated in murine leukemia and solid tumor models. Rhizoxin was also effective against vincristine- and doxorubicin-resistant leukemia lines [34]. On the basis of these data, rhizoxin was selected by the NCI and the NDDO for further investigation of its preclinical antitumor and toxicity profile.

Moderate to good antitumor activity was found in murine P388 (vincristine-resistant) leukemia and in solid tumor models in vivo, such as B16 melanoma and M5076 fibrosarcoma. Significant cytotoxic activity at very low concentrations (0.1 ng/ml) was observed in HTXs in vitro. Melanoma, small-cell lung cancer and non-small-cell lung cancer were particularly sensitive to rhizoxin. In vivo antitumor activity was also observed in about half of the HTXs tested, e.g., MX-1 breast cancer, LOX melanoma, and small-cell lung cancer LXFS 650. Furthermore, in vitro and in vivo data suggested a schedule dependency.

Animal toxicological studies were performed with rhizoxin solubilized in a formulation for clinical use (propylene glycol 40%, ethanol 10%, water 50%) [36]. Intravenous administration of rhizoxin caused
inflammation and necrosis at the site of injection. This local damage prevented multiple intravenous injections.

The clinical signs of toxicity in rhizoxin-treated mice and rats were sluggishness, piloerection and ataxia, which disappeared several days after rhizoxin administration. The main toxic effects in single-dose and multiple-dose studies consisted of transient changes in erythrocyte and leucocyte numbers. Bone-marrow toxicity was absent. Nonhematological toxicities in acute and subacute studies consisted of decreased testicle weight and spermatogenic arrest.

The LD$_{10}$ value of rhizoxin after a single intravenous injection in mice was 2.8 mg/kg (8.4 mg/m$^2$). The safety of the one-tenth mouse-equivalent LD$_{10}$ (0.84 mg/m$^2$) was tested in rats as a second species, causing no toxic effects [Hendriks HR, Henrar REC, Plowman J, et al., unpublished observations]. At present, rhizoxin is being evaluated in a phase-I clinical trial within the framework of the EORTC Early Clinical Trials Group.

Summary

The New Drug Development Office (NDDO) is the executive office of the EORTC New Drug Development Coordinating Committee (NDDCC), and is involved in the evaluation and development of anticancer agents. New compounds are acquired from research institutes, universities, the pharmaceutical industry, the Cancer Research Campaign (CRC) and the U.S. National Cancer Institute (NCI). Selection for further development is based on the novelty of the chemical structure and the mechanism of action claimed for the drug and/or the quality of the antitumor profile. After acquisition, a comprehensive developmental program is pursued covering all the essential preclinical and clinical steps, until the antitumor activity of a new compound is established in clinical phase-II trials. As an example, the development of the bioreductive indoloquinone E09 and the tubulin binder rhizoxin are discussed. Furthermore, in an attempt to reduce the number of negative phase-II clinical trials, the NDDO is currently investigating the predictive value of the human tumor xenograft model as part of a multicenter preclinical phase-II program.

References


Winograd B, Lobbezoo MW, Pinedo HM: Proposal for the application of xenografts in


Dr. H. R. Hendriks, EORTC New Drug Development Office, Free University Hospital, De Boelelaan 1117, NL-1081 HV Amsterdam (The Netherlands)