tion of an acquired neutrophil chemotaxis defect and differential suppression of interleukin-2 associated side effects. *Blood* 1990, **76**, 1933–1940.
81. Fraker DL, Langstein HN, Norton JA. Passive immunization against tumor necrosis factor partially abrogates interleukin-2 toxicity. *J Exp Med* 1989, **170**, 1015–1020.
82. Vachino G, Gelfand JA, Atkins MB, Tamerius JD, Demchak P, Mier JW. Complement activation in cancer patients undergoing immunotherapy with interleukin-2 (IL-2): binding of complement and C-reactive protein by IL-2-activated lymphocytes. *Blood* 1991, **78**, 2505–2513.
83. Rabinovici R, Sofronski MD, Borboruglu P, *et al.* Interleukin-2-induced lung injury: the role of complement. *Circulation Res* 1994, **74**, 329–335.
84. Fleischmann JD, Shingleton WB, Gallagher C, Ratnoff OD, Chahine A. Fibrinolysis, thrombocytopenia and coagulation abnormalities complicating high-dose interleukin-2 immunotherapy. *J Lab Clin Med* 1991, **117**, 76–82.
85. Strack van Schijndel RJM, Voerman HJ, Golding RP, Wagstaff J. Interleukin-2 therapy and blockage of double-lumen catheters. *Lancet* 1989, **1**, 962.
86. van der Poll T, Levi M, Büller HR, *et al.* Fibrinolytic response to tumor necrosis factor in healthy subjects. *J Exp Med* 1991, **174**, 729–732.
87. Moncada S, Higgs A. The L-arginine-nitric oxide pathway. *New Engl J Med* 1993, **329**, 2002–2012.
88. Kilbourn RG, Belloni P. Endothelial production of nitrogen oxides in response to interferon-gamma in combination with tumor necrosis factor, interleukin-1, or endotoxin. *J Natl Cancer Inst* 1990, **82**, 772–776.
89. Hibbs JB, Westenfelder C, Taintor R, *et al.* Evidence after cytokine-inducible nitric oxide synthesis from L-arginine in patients receiving interleukin-2 therapy. *J Clin Invest* 1992, **89**, 867–877.
90. Kilbourn RG, Griffith OW. Overproduction of nitric oxide in cytokine-mediated and septic shock. *J Natl Cancer Inst* 1992, **84**, 827–831.
91. Petros A, Bennett D, Vallance P. Effect of nitric oxide synthase inhibitors on hypotension in patients with septic shock. *Lancet* 1991, **338**, 1557–1558.
92. Kilbourn RG, Gross SS, Jubran A, *et al.* N^G- Methyl-L-arginine inhibits tumor necrosis factor-induced hypotension: implications for the involvement of nitric oxide. *Proc Natl Acad Sci USA* 1990, **87**, 3629–3632.
93. Baars JW, Wolbink GJ, Hart MHL, *et al.* Release of interleukin-8 during intravenous bolus treatment with interleukin-2. *Ann Oncol* 1994, 5, in press.
94. Baars JW, Hack CE, Wagstaff J, *et al.* The activation of polymorphonuclear neutrophils and the complement system during immunotherapy with recombinant interleukin-2. *Br J Cancer* 1992, **65**, 96–101.
95. Thijs LG, Hack CE, Strack van Schijndel RJM, *et al.* Activation of the complement system during immunotherapy with interleukin-2. Relation to the development of side effects. *J Immunol* 1990, **144**, 2419–2424.
96. Hack CE, Ogilvie AC, Eisele B, Eerenberg AJM, Wagstaff J, Thijs LG. C1-inhibitor substitution therapy in septic shock and in the vascular leak syndrome induced by high doses of interleukin-2. *Intensive Care Med* 1993, **19**, S19–S23.
97. Ogilvie AC, Baars JW, Eerenberg AJM, *et al.* Pilot study to evaluate the effects of C1 esterase inhibitor on the toxicity of high-dose interleukin-2. *Br J Cancer* 1994, **69**, 596–598.
98. Hack CE, Wagstaff J, Strack van Schijndel RJM, *et al.* Studies on the contact system of coagulation during therapy with high doses of recombinant IL-2: implications for septic shock. *Thromb Haemost* 1991, **65**, 497–504.
99. Baars J, Boer JP. de, Wagstaff J, *et al.* Interleukin-2 induces activation coagulation and fibrinolysis: resemblance to the changes seen during experimental endotoxaemia. *Br J Haemat* 1992, **82**, 295–301.
100. Olthof CG, Baars JW, Wagstaff J, Donker AJM, Schneider H, de Vries PMJM. Determination of capillary leakage due to recombinant interleukin-2 by means of non invasive conductivity measurements. *Eur J Appl Physiol* 1993, **67**, 168–173.
101. Raijmakers PGHM, Groeneveld ABJ, Schneider AJ, *et al.* Transvascular transport ^{67}Ga in the lung after cardiopulmonary bypass surgery. *Chest* 1993, **104**, 1825–1832.
102. Daemen-Gubbels CRGM, Groeneveld PHP, Groeneveld ABJ, van Kamp GJ, Bronsveld W, Thijs LG. Methylene blue increases myocardial performance in septic shock. *Intensive Care Med* 1995, in press.

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Revisions of General Guidelines for the Preclinical Toxicology of New Cytotoxic Anticancer Agents in Europe

S.S. Burtles, D.R. Newell, R.E.C. Henrar and T.A. Connors on behalf of the Cancer Research Campaign (CRC) Phase I/II Clinical Trials Committee and the European Organization for Research and Treatment of Cancer (EORTC) New Drug Development Office

INTRODUCTION

IN 1980, the Cancer Research Campaign (CRC) defined minimal toxicology requirements for the testing of novel anticancer drugs prior to Phase I clinical trials. These protocols were adopted soon afterwards by the European Organization for Research and Treatment of Cancer (EORTC). Through experience of working

to these guidelines, the CRC/EORTC have modified and refined them a number of times with the aim of recommending the minimum preclinical toxicology that needs to be carried out to ensure adequate safety evaluation of new agents. The guidelines are an attempt to produce generally accepted European standards for toxicology in anticancer drug development which offer patient safety and high quality data with the minimum delay, use of animals and expense.

The CRC/EORTC guidelines [1], first published in 1990, are further refined here for a number of reasons. First, it was recognised that the schedule of drug administration used in toxicology studies should reflect the proposed regimen for the clinical trial. It was also felt that little useful additional information was provided by carrying out intraperitoneal as well as intravenous dosing, and that on occasion misleading data were generated because of local toxicity, e.g. peritonitis. Second, it was noted that detailed histopathology on all organs from all animals did not provide results of greater utility than histopathology of selected organs from specific groups. Third, it was found that little contribution was made by only dosing rats at one-tenth of the equivalent dose which is lethal to 10% of mice (LD_{10}), and that it was more important to show evidence of drug effects in both species. Finally, it was recommended that pharmacokinetics should be included.

AIMS

The aims of the studies contained in this protocol, based on the use of mice and rats, are:

(i) to provide a safe starting dose for clinical studies in humans;
(ii) to define the most likely targets of toxicity;
(iii) to determine the extent to which cumulative toxicity occurs with repeated administration, e.g. daily × 5 or weekly, if the drug is to be administered on a multiple dose schedule;
(iv) to investigate the reversibility of toxicity.

If possible, the investigations will be extended to include a limited preclinical pharmacokinetic study where the area under the plasma drug concentration versus time curve (AUC) is measured in mice dosed at the maximum tolerated dose (MTD) and at doses below the MTD. The results of such a study may be used to facilitate dose escalation during subsequent Phase I studies.

The protocol is intended for the preclinical toxicology of new chemotherapeutic agents for cancer treatment, before the commencement of Phase I clinical trials. However, if the new drug is an analogue of an existing clinically used agent, additional studies on the specific toxicities associated with that drug class may be needed. Additional studies will also be required in cases where unpredicted toxicity is found for a novel structure or if there are mechanistic data to suggest that effects in rodents may not be representative of those likely to be encountered in humans.

Correspondence to Dr S. Burtles at the Cancer Research Campaign, 10 Cambridge Terrace, London, NW1 4JL, U.K.
D.R. Newell is at the Cancer Research Unit, Medical School, The University, Newcastle Upon Tyne, NE2 4H, U.K.; R.E.C. Henrar is at the EORTC New Drug Development Office, Free University Hospital, Amsterdam, The Netherlands; T.A. Connors is at the School of Pharmacy, Brunswick Square, London, U.K.
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The starting dose for the Phase I clinical trial is calculated from the mouse MTD (or previously, the LD_{10}) and is usually one-tenth of the mouse MTD expressed in mg/m^2 [1]. For all species body surface area is calculated from the following equation:

$$\text{Body surface area (m}^2\text{)} = (\text{Body weight (kg)})^{0.66} \times 0.1.$$

STUDY DESIGN AND REQUIREMENTS

Responsibility

The responsibility for the design of the study rests with the sponsor, i.e. the EORTC New Drug Development Office of the CRC Phase I/II Clinical Trials Committee. The sponsor is responsible for the supply and quality control of the drug material.

Good laboratory practice (GLP)

All tests and procedures will be carried out and be quality controlled in conformity with the EC guidelines for GLP.

Dosing solutions

Testing will be done with the clinical formulation of the drug to ensure that any excipients or special solvents are investigated for possible toxicity. Samples of stock solutions and dosing solutions (or suspensions) should be stored under appropriate conditions for subsequent analyses of drug content. Relevant information will be supplied by the sponsor. Accuracy of preparation of dosing solutions will be checked by the toxicology laboratory.

Animals

The studies will be carried out with male animals, unless there is evidence for differences in sensitivity to toxic effects of the drug between male and female animals, in which case the most sensitive sex will be utilised. Females should be nulliparous and non-pregnant, and will be used for compounds not intended for clinical use in males.

Barrier-reared young MF1 mice and Wistar or Sprague Dawley rats will be obtained from regular animal suppliers, and will be transferred to the testing laboratory 7–14 days before they are required for the study. All animals will be housed under the standard conditions of the testing laboratory, and will have free access to standard laboratory diet and tap water.

PROCEDURES

Preliminary studies

Single dose intravenous study in mice. For all drugs, a preliminary dose finding study with 2 mice per group will be performed, with drug administered as a single intravenous dose in order to establish an approximate MTD. This will be followed by intravenous dosing of groups of 4 mice to determine more accurately the maximum tolerated dose of the test material. Surviving animals, i.e. those not showing clinical effects or body weight loss which necessitate killing, will be killed without further examination 14 days after treatment.

If the compound proves to be non-toxic, the maximum dose that can be given to mice within the limitations of the formulation

concentration and the volume which can be administered, the maximum administered dose (MAD), will be established.

Single dose oral study in mice If the test article is to be administered orally in the clinic, the MTD or MAD should also be determined, as above, with oral administration of the test article to groups of 4 mice. Surviving animals will be killed without further examination 14 days after treatment.

Main studies

Detailed toxicology study in mice. The dosing schedule for the detailed toxicology studies should be as similar to the proposed clinical schedule as is reasonable in mice. The drug is likely to be administered either intravenously or orally, and the most common schedules are: single, daily over a number of days, or weekly for a number of weeks. For repeat dose schedules, the MTD or MAD for this multiple dose intravenous or oral schedule will be determined in groups of 4 mice.

In the main study, to determine the toxic effects induced by the test article and whether they are reversible, groups of 20 mice will be dosed intravenously or orally with the test article on the above schedule at the predetermined MTD (or MAD), half the MTD (or MAD) or with the control article (vehicle alone). The first 10 mice in each group will be brought to necropsy 1–3 days after the last treatment, as appropriate, to assess the toxic effects of the drug, and the second group of 10 will be brought to necropsy 28 days later to determine whether the effects of the compound are reversible.

Throughout the study, animals will be weighed twice weekly. Blood samples will be taken from 10 animals at each dose level on days 3 and 14 during the study (or on other days if more appropriate for the particular drug), and at scheduled necropsy for full haematological examination. The samples taken at necropsy will also be subject to clinical chemistry analysis, i.e. urea, creatinine, alkaline phosphatase, alanine and aspartate transaminases. Selected tissues (brain, caecum, colon, heart, ileum, kidneys, liver, lung, lymph node, skin, spleen, stomach, testes, thymus and sternum for marrow examination) from the control and MTD (or MAD) groups killed at the first scheduled necropsy day will be subject to histopathological examination. At the second necropsy, tissues identified as showing an effect of the treatment at the first necropsy, or macroscopically abnormal at the second necropsy, will be histologically examined from the MTD (or MAD) and control groups. Tissues from the other groups will be preserved, but not sectioned unless required.

Rat study

The MTD or MAD will be determined in rats by dosing groups of 4 rats with the proposed clinical schedule and route of administration. The first dose will be determined from the mouse data described above, and then increased or decreased accordingly to determine the MTD.

The main study will be repeated in three groups of 20 rats as described for the mouse main study. Haematology and clinical chemistry will be carried out as described. The tissues from the rats will be preserved, but will not be processed or examined unless there is evidence of clinical toxicity in rats which is different from that caused in mice.

CONCLUSIONS

The CRC and the EORTC have defined minimum preclinical toxicology requirements for the testing of novel anticancer agents prior to Phase I clinical trials. With experience of working to these guidelines, they have been revised a number of times over the years with the latest revisions being summarised here. Over the 10 years that these guidelines have been used, more than 30 novel anticancer agents have been put into Phase I clinical trials and this has demonstrated clearly that acute rodent toxicology, as described, is sufficient to take such compounds safely and rapidly into clinical trials [2, 3].

1. Joint Steering Committee of the EORTC and CRC. General guidelines for the preclinical toxicology of new cytotoxic anti-cancer agents in Europe. *Eur J Cancer* 1990, **26**, 411–414.
2. Henrar REC, Koier IJ, Kalakun L, Hornstra HW, Pinedo HM, Schwartsmann G. Predictive value of animal toxicology for new anticancer agents: the experience of the EORTC New Drug Development Office/NDDO (1986-1991). *Proc AACR* 1992, **33**, 3281.
3. Secher DS, Burtles SS, Newell DR, Connors TA. Phase I/II clinical trials of new cancer treatments — toxicological review of the Cancer Research Campaign experience. *Proc AACR* 1994, **35**, 2744.