STANDARD OPERATING PROCEDURE

Title: **Detailed protocol for preliminary toxicological and pharmacokinetic studies with compounds derived from the NCI *in vitro* drug evaluation programme**

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1. Introduction

The in vitro screen of the NCI of the USA was established in an attempt to identify novel agents active against common solid tumours. It employs 60 tumour cell lines derived from 9 human tumour types. The current screening capacity is approximately 10,000 compounds per year. The compounds tested are selected from more than 20,000 chemicals submitted to the screen each year on the basis of structural diversity and novelty. Approximately 20% of the compounds that enter the in vitro screen originate from Europe. A large number of cell type specific agents, compounds with unique mechanisms of action, and new structures related to specific mechanisms of action have been detected and have been selected for in vivo evaluation, many of which are of European origin.

A pharmacological approach to the selection of new anticancer agents has been established within the EORTC Research Division and the CRC, in collaboration with the NCI. The process starts with the review of the available NCI data of European compounds by a panel of CRC/EORTC members. The selection criteria include COMPARE analysis, mean graph and growth curve profiles, potency, molecular target data, and novelty of chemical structure. Compounds then undergo a stepwise development, according to Standard Operating Procedures, starting with pre-formulation, assay development and stability studies. If a compound can be formulated, the seriously toxic dose (STD) in mice is determined using single dose intra-peritoneal administration, followed by plasma pharmacokinetics at various time points using the dose below the STD. If the plasma concentrations approach in vitro GI_{50} values obtained in the NCI in vitro screen, the compounds qualify for further in vitro/in vivo antitumour studies. This Standard Operating Procedure describes the procedures recommended for the toxicological and pharmacokinetic studies.

2. Toxicological studies

A. Species:	Normal adult mice, preferably female Balb/c.
B. Route of administration:	Intra-peritoneal.
C. Formulation:	As recommended for each compound.
D. Volume injected:	Routinely 10 ml/kg but, if necessary, 20 ml/kg.
E. Dose schedule:	Two mice will be treated with a single dose per dose level and observed for 48 hrs and then killed. Laparotomy will be performed on all mice at 48 hrs to look for evidence of drug precipitation or acute peritonitis.
F. Initial dose:	As suggested by NCI in vivo data, where available, or 10 mg/kg if no data are available. If toxicity is encountered at the first dose level the dose should be reduced 10-fold, and 10-fold decrements continued until a non-toxic dose is found.
G. Dose escalation:	If severe clinical toxicity is not observed at the current dose level, the dose should be doubled.
H. Study parameters:	Pre-treatment and post-treatment body weight. Clinical toxicity, i.e. neurological symptoms, respiratory distress, clinical condition, and behavioural changes.
I. Study termination:	Any mouse showing severe clinical toxicity should be immediately killed.
J. Study endpoint:	The dose causing lethal or severe clinical toxicity (STD = seriously toxic dose). The dose to be used for the pharmacokinetic studies (see 2B) is the dose below this level.

3. Pharmacokinetic studies

A. Assay development

HPLC assay methodology with UV/visible detection is recommended as the initial method investigated. Choice of the initial column will depend upon the compound. The wavelength for detection will also be compound dependent but lambda max. values are provided as part of the pre-formulation report.

The assay developed should be able to detect the study compound in the range of the in vitro GI_{50} concentration and should be linear over the defined concentration range (r = >0.98). Quality assurance samples (n=5) for the lowest and highest concentration on the standard curve (at least 5 points) should have coefficients of variation of <20% and precision should be within the range 80-120% for both concentrations. As all the samples will be analysed on the same day, inter-assay reproducibility data are not required. Ideally, the assay should have a lower limit of quantitation in the range 1-10 μM. Values in the range 10-100 μM are, however, still acceptable.

All standards and the quality assurance samples should be prepared in heparinised (10 iu/ml) control mouse plasma or, if not available, human plasma. A sample of the compound in HPLC mobile phase should be included in all assays to assess absolute recovery from plasma.

Extraction conditions for each compound may be different but precipitation of plasma macromolecules with an alcohol is recommended as an initial approach. The solubility of most of the compounds in ethanol will be provided as part of the pre-formulation studies.

B. Murine pharmacokinetic studies

A pilot study should precede the main pharmacokinetic study in order to reduce the number of animals used. In the pilot study 2 mice per time point should be injected intra-peritoneally with the compound vehicle and the study compound at the dose below the STD (see 1.J) (2 mice/per time point) and killed at 15 and 60 min, in order to determine if in vitro GI_{50} levels are approached in the plasma. Blood collection and analysis should be carried out as described below. If the outcome of the pilot study is negative (i.e. the compound is undetectable in plasma or only present at levels well below the GI_{50}), then the pharmacokinetic study is discontinued, otherwise the main study is initiated.

In the main study groups of four mice of the same age, strain, gender as used in the toxicological and pilot pharmacokinetic study should be injected intra-peritoneally with the test compound at the dose below the STD and killed at 15 min, 3 hrs, 6 and 24 hrs. Blood should be collected by cardiac puncture and heparinised plasma (10 iu/ml) prepared by centrifugation. Plasma can be frozen immediately and stored at ≤20°C prior to analysis which should preferably be within one week. Stability at ≤20°C should be confirmed by the analysis of samples stored during the assay development stages.

4 Reporting of the studies

The results of the Preliminary Toxicology and Pharmacokinetic studies should be reported according to the format of the attached Evaluation report on page 5.

Preliminary Preclinical Toxicological and Pharmacokinetic Evaluation Report

Compound:

Pre-formulation report:

Formulation used:

Studies performed by:

Preliminary Toxicological Studies:

Strain and gender of mice:

Volume injected:

Dose level: Mice killed:

.. mg/kg ip

.. mg/kg ip

Preliminary Pharmacokinetic Studies:

Dose:

HPLC Assay:

Results Pilot study:

Results Main study:

Data verified by:

Name:

Date:

Signature: