

Guidelines for pharmacokinetic and pharmacodynamic studies in early phase clinical trials

Introduction

The Pharmacodynamic/ pharmacokinetic Technologies Advisory Committee (PTAC) of Cancer Research UK has the objective of increasing the extent of mechanistic and hypothesis testing in early phase clinical trials. PTAC reviews all applications submitted to the New Agents Committee (NAC) and with expertise in PET, MRI and MRS as well as many of the other currently emerging technologies, provides advice on the potential use of these techniques to add value to the development of new anticancer agents.

All clinical trials approved by the NAC should include studies to demonstrate whether a drug gets to the tissue of interest, modulates a molecular target or cognate biochemical pathway, and produces the anticipated downstream biological effects [1]. These data can demonstrate proof of principle and provide the rationale for go/no-go decisions, as well as for selecting drug dose and schedule. The PTAC review of an application includes evaluation of useful pharmacokinetic (PK) endpoints, with particular emphasis on in situ measurement and pharmacodynamic (PD) endpoints, including both specific molecular targets and more generic biological endpoints.

The guidelines below apply to all full proposals (Phase I/II) submitted to the Cancer Research UK's NAC and are intended to:

- Improve the quality of the PK and PD sections in NAC submissions.
- Provide guidance to potential applicants on what is required by PTAC for a full review of each proposal to take place, in order to speed up the review process.

Note: PTAC and the NAC also make suggestions of potential pharmacological/ immunological endpoints and appropriate methodologies on preliminary proposals.

Guidelines

The PK/ PD proposals accompanying NAC applications should include:

1) Background and scientific rationale

This section of the proposal should list the PK and PD endpoints and/or techniques that will be used and indicate why measuring the proposed parameters will be important to the trial. A brief background and scientific rationale for using the proposed endpoint or measurement should be indicated. Evidence that the technique measures what it claims to measure should be provided. In some cases this may mean *in vivo* animal data are necessary to show the feasibility of an assay rather than just *in vitro* data. Specific reasons why the endpoint is suitable should be described, e.g., selective over-expression of a target in a tumour type, compelling *in vitro* or *in vivo* (pre-clinical or clinical) studies, etc.

Example: We plan to evaluate the efficacy of drug XXX in patients with tumour-type YYY by measuring changes in glucose uptake by [¹⁸F]fluorodeoxyglucose-positron emission tomography ([¹⁸F]FDG-PET). Glucose uptake is a surrogate early measure of response to anti-cancer therapy [2]. Pre-clinical studies in tumour-type YYY-bearing

mice in our laboratory showed that drug XXX produces a dose dependent decrease in [¹⁸F]FDG standardized uptake within 24 h after treatment (provide reference as evidence). Drug XXX was found to inactivate Glut-1 *in vitro* and *in vivo* consistent with the observed changes in [¹⁸F]FDG-PET.

Additional PK and/or PD data that will be obtained as part of the trial should also be listed and a brief rationale for the inclusion included in the proposal.

Assays used to determine trial endpoints or other trial objectives should be categorised into primary, secondary or tertiary with categorisation reflecting the level of importance to the trial and hence the extent of validation required – see Section 3 (Method validation) for further information.

2. Hypothesis being tested

For each endpoint or measurement the following questions should be addressed:

- a) What is the specific hypothesis being tested?
- b) What is the relationship between the hypothesis and the endpoint or measurement, i.e., is the parameter or change in parameter the appropriate choice for testing the hypothesis?

Example: The Bcr/abl tyrosine kinase is responsible for the oncogenic phenotype observed in Philadelphia chromosome-positive leukaemia. Bcr/abl tyrosine kinase activates the protein p39crk1. Crk1 is constitutively tyrosine phosphorylated in neutrophils from chronic myelogenous leukaemia (CML) patients. Since drug XXX is a selective inhibitor of the bcr/abl tyrosine kinase we hypothesized that it will produce a decrease in p39crk1 phosphorylation in neutrophils from CML patients (greater than 15% at 24 h after treatment). Data supporting the hypothesis and proposed endpoint have been obtained (give brief details).

OR

Protein ZZZ is an angiogenic growth factor that is selectively over-expressed in tumour type YYY. Drug XXX binds to and inactivates protein ZZZ *in vitro* and decreases blood flow within 2-24 h after a single dose in tumour type YYY-bearing mice. We hypothesize that drug XXX will produce a greater than 20% decrease in the K_{trans} parameter in Dynamic Contrast Enhanced Magnetic Resonance Imaging (DCE-MRI) of patients with tumour type YYY treated with a single dose of drug XXX 24 h prior to imaging. Data supporting the hypothesis and proposed endpoint have been obtained (give brief details).

3. Method validation

Method validation ensures that the specific method being used measures what it is supposed to measure, is reproducible, and when quantitative, accurate. Validation is an important component in any PK or PD study and must utilise the analytical equipment that will be used in the clinical trial for laboratory-based assays.

Prioritising Analytical Methods

Analytical methods should be prioritised into primary, secondary or tertiary. Prioritising has two important functions; (a) it enables restricted laboratory analytical resources to be focused to the most important procedures and (b), if asked by Regulatory Authorities it

can be argued that validation efforts have been directed in line with the perceived importance of the analytical procedure to the trial.

Primary assays should have extensive validation and are used to directly support the attainment of primary clinical trial objectives.

Secondary assays support secondary trial objectives, never primary objectives. They should be validated but possibly not to the same level as a primary ranked assay.

Tertiary assays are of lesser importance to the trial outcome and gather what might be described as interesting support data, maybe using new or un-validated techniques. The method should be well documented, preferably by a Standard Operating Procedure.

However, care should be taken to ensure that assays are not dropped into this category just to avoid the validation effort.

Imaging Assays

Pre-clinical validation is considered essential by PTAC for imaging studies and validation in patients is highly desirable. For imaging studies, pre-clinical validation should include establishment of an association between the imaging parameter and an *ex vivo* measure, e.g., the fractional retention of [¹⁸F]fluorothymidine ([¹⁸F]FLT-PET) validated by Ki-67 immunostaining, or choline metabolite profile by ³¹P-MR spectroscopy (³¹P-MRS) validated by measurement of Hsp90 client protein levels [3,4].

It is preferable to conduct the validation studies in the tumour type and with the drug to be studied clinically. Justification should be provided for why a particular method has been chosen, e.g. it should be explained why the obscure and difficult method PPP will be used instead of the well validated method SSS. Equally, clinical validation should also include comparisons with an *ex vivo* measure of target modulation or downstream biological activity. For clinical validation, reproducibility studies should be conducted to define what will be regarded as a 'significant change'. For quantitative methods accuracy and precision of measurement should be validated. If imaging is undertaken at more than one centre then cross validation should be conducted to demonstrate that results from all centres are comparable. The level of validation depends on several factors including signal to noise ratio and also the existence in the literature of related studies using the technique. Examples of generic methods and their levels of validation are shown below (Table 1).

Table 1: A list of generic imaging assays and their level of validation as of 01-09-2003.

Technique	Validation: <i>In vivo</i> /Imaging versus <i>ex vivo</i> comparisons	Validation: Reproducibility (clinical)
Computed tomography or MRI (RESIST criteria [5])	Standard for most clinical trials; well validated	Well documented
[¹⁸ F]FDG-PET for measuring glucose metabolism [2]	Most widely used PET tracer. Well validated. Its use for the Gleevec trial in GIST was pivotal.	A few reproducibility studies have been published
[¹⁸ F]FLT-PET for measuring cellular proliferation [3]	A fairly new radiotracer. Very few validation studies have been performed	Ongoing; None published so far
[¹⁵ O]H ₂ O for measuring perfusion [12]	Well validated. Was used in the Phase I trial of combretastatin A4.	One reproducibility study has been published
[¹²⁴ I]Annexin V for measuring apoptosis [6]	Well validated in mouse models. Binding characteristics & metabolism have not been studied in humans.	None published.
DCE-MRI for measuring blood flow [13]	Well validated. Was used in the Phase I trial of combretastatin A4	One reproducibility study has been published
³¹ P-MRS for measuring phosphocholine [4]	Well validated.	Ongoing; None published so far

Non-imaging assays: Validation is required for non-imaging assays. Examples of such assays are High Performance Liquid Chromatography, ligand binding assays, cell based assays, biomarkers immunohistochemistry, western blots, polymerase chain reaction (PCR), microarray and proteomic assays. These assays may be qualitative or quantitative. There are a number of guidance documents on validation that can be applied to the above techniques [7, 8, 9, 10, 11]. The validation studies required, including selectivity/specificity, precision, accuracy, linearity, limit of detection/quantification, ruggedness and stability are applicable to the validation of all laboratory endpoints. Validation should be undertaken using human matrices as described in the trial proposal wherever possible although cell lines and xenografts may be appropriate in some circumstances.

The stability of samples should be assessed, i.e. is the collection protocol appropriate? Pre-trial stability studies should demonstrate that the proposed sample handling/storage procedures will deliver samples fit for analysis.

Global genomic and proteomic technologies have the potential to be used in early clinical trials. These can be used in an open-ended way (with some aspect of reproducibility, e.g., 2 spot) to determine which genes and proteins may be altered by drug treatment and/or may be potentially involved in response. For use as primary endpoints in clinical trials,

arrays need to be produced to a demonstrably high standard and there should be sufficient reproducibility to assure statistical interpretation of the data.

4. Patient groups and statistical considerations

The primary endpoint, trial design and statistical considerations for the entire trial should be stated in the main submission. The incorporation of PD endpoints in Phase II trials are easier to appreciate and all patients can justifiably be assessed by a particular PD endpoint. PK/PD studies can be valuable for establishing proof of principle in Phase I studies and establishing dose-response relationships and defining the recommended Phase II dose. It is particularly valuable if the data can show whether the molecular and biological desired effect has been achieved. Thus, the PK/PD section should contain a clear description of the number of patients that will be studied. It is not appropriate, for instance, to state that “samples will be taken from patients for analysis where possible”. It may be possible to design the trial in an interactive fashion such that more patients for PK/PD studies are included in the trial as the MTD or biologically effective dose is approached. For instance, plasma PK studies for drug levels could be carried out in all patients; when active PK levels are reached, laboratory PD assays or imaging studies can commence in at least 1 patient per dose level; if a PD effect is seen then the PD endpoint could be incorporated into the protocol for all patients; the top dose could be expanded to have as much information as possible. In trials where a strong hypothesis is being tested, it may be necessary to investigate all patients by laboratory PD methods (pre- and post-treatment biopsies) and/or imaging methods to ensure that the desired biological effect is being achieved and to establish dose-response relationships. Where molecular endpoints require invasive removal of a tissue sample, normal tissues such as peripheral blood lymphocytes, skin or buccal mucosa may be useful and valuable, as kinetic PD data can be obtained from taking multiple samples – generally not feasible for tumours. This will be important for rational design of scheduling of combination studies. However, tumour biopsies should be taken where possible especially at higher, potentially active doses and in certain cases may be mandatory to achieve the trial objectives.

In the statistical section, any previous repeatability studies should be indicated. The statistic used, sample size, statistical powers and levels of significance should be included where applicable. It is also essential to indicate how the results will be used to guide the trial and/or influence subsequent clinical development.

5. Expertise of applicants and operational details

It is necessary to state what expertise the applicant(s) has or which investigator(s) the applicant(s) is collaborating with to carry out the studies. Details on the facilities for doing the assays should be given and whether there is existing expertise in PK/PD studies commensurate with GLP or Good Clinical Laboratory Practice (GCLP) as it is likely to be necessary to confirm that this is the case under the EU Directive. Contacts should be named for each assay proposed. A letter of collaboration from the relevant experts should be included in the application to show that they are willing to take responsibility for the trial.

Preliminary applications should contain a summary of the PK/ PD studies proposed: in full proposals, a detailed protocol for the studies should be provided. This could be attached as an appendix. The protocol should have sufficient details to bestow confidence that the applicant(s) have thought through the investigation in detail and can complete it successfully. The protocol should include the methodology, parameters to be estimated, timing of investigations, etc. Imaging costs and other resources required for the assay should be indicated.

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