Illustrative guidance for applications to the competent authority to commence an exploratory single dose clinical trial in accordance with guidance in ICH topic M3 (R2) Non-Clinical Safety Studies for the Conduct of Human Clinical Trials and Marketing Authorisation for Pharmaceuticals.

Introduction

It is recognised that in some cases insight on human physiology/ pharmacology, knowledge of drug candidate characteristics and therapeutic target relevance to disease are benefited by earlier access to human data.

Exploratory clinical studies are those intended to be conducted early in Phase 1, involve limited human exposure, have no therapeutic or diagnostic intent, and are not intended to examine maximum tolerated dose. They can be used to investigate a variety of parameters such as pharmacokinetics, pharmacodynamics and other biomarkers, which could include Positron Emission Tomography (PET) receptor binding and displacement.

This illustrative guidance presents a mock application for a fictitious product, for an early exploratory clinical trial. Specifically a microdose approach, where by no more than a total dose of 100 µg can be administered as a single dose or divided doses (≥5 administrations) in any subject in a clinical trial. This type of approach could be useful when investigating target receptor binding or tissue distribution in a PET study or to assess PK with or without the use of an isotopically labelled agent.

This example will specifically illustrate a radiolabeled case for PET scanning. To support a total dose of ≤ 100 µg in a trial, extended single dose toxicity studies in one species (usually rodent) by the intended route of administration with toxicokinetic data, or via the intravenous (iv) route can be used in support of this type of microdose study. For example after a single dose in the rat, reversibility/delayed toxicity could be assessed on Day 14 in order to support such an approach. A maximum dose of 1000-fold the clinical dose on a $mg \cdot kg^{-1}$ basis for i.v and $mg \cdot m^{-2}$ for oral administration should be used.

To support a total cumulative dose of ≤ 500 µg, a maximum of 5 administrations with a washout between doses (6 or more actual or predicted half-lives), with each dose being ≤ 100 µg, multiple dose toxicology studies that involves 1 week repeated dose toxicity studies in one species (usually rodent) by the intended route of administration with toxicokinetic data, or via the i.v route can be used. Haematology, clinical chemistry, necropsy, and histopathology data should be included. A maximum dose of 1000-fold the clinical dose on a mg/kg basis for i.v and mg/m^2 for oral administration should be used.

For both of these types of microdose studies and radiolabeled compounds, genotoxicity studies are not recommended, but any studies or structure-activity relationship assessments conducted should be included in the clinical trial application.

The information presented illustrates the quantity and level of detail expected where it is available. If information for a section is not available this should be clearly stated. In the case of products where certain types of data cannot be provided this should be justified briefly. It should be noted that assessors/reviewers do not need details of methods for standard tests. Tabulated data is and preferred with a brief discussion of the results and any conclusion.

The guidance presents illustrative data relating to non-clinical aspects of an investigational diagnostic product. It is assumed that applicants will derive this information from tests carried out to the current standards of GLP and that they will have followed regulatory guidance on conducting the tests, if available. The application should draw attention to any deviations from these standards and provide a justification. Finally, although this illustrative guidance does not provide information under all headings, it is important to note that all available information relating to the investigational product should be provided at the time of the application.

PHARMACO-TOXICOLOGICAL DATA

INTRODUCTION

This is a single-centre, open-label Positron Emission Tomography (PET) study to evaluate the efficacy and safety of a single intravenous (i.v) dose of ^{18}F (ABC123) injection.

This study will assess the prognostic usefulness of ^{18}F (ABC123) injection for identifying subjects with increased inflammatory cells in the lung due to asthma**.**

The primary objective is to compare and observe the patterns of ^{18}F (ABC123) uptake based on Positron Emission Tomography (PET) scanning.

Up to 12 mildly asthmatic male patients aged 18 to 45 years will participate. All subjects will be given an i.v dose of ^{18}F (ABC123) (approximately 20 µg ABC123). The nominal activity of a single administration of ^{18}F (ABC123) will be 500 MBq (corresponding to an effective dose of approximately 12 mSv). ^{18}F (ABC123) will be given as a bolus injection (less than 40 seconds). PET imaging will be conducted starting approximately 20 minutes after the ^{18}F (ABC123) injection has been administered.

^{18}F (ABC123) is a PET ligand, which may have potential benefit in the diagnosis of inflammation diseases of the lung. ^{18}F (ABC123) binds to XY_2, which is known to be expressed on many of the cells implicated in the allergic process including lymphocytes, eosinophils and basophils.

^{18}F (ABC123) has a relatively short half life of approximately 20 minutes. The half life calculation was based on the radioactive content.

PHARMACOLOGY

Summary

ABC123 has been shown to selectively bind to the XY_2 receptor in diseased lung tissue. Binding has been demonstrated in *in vitro* studies and has been confirmed in a number of in vivo animal models. No significant binding has been demonstrated at 32 other receptor types investigated.

A range of studies have been conducted with XY_2 in non-clinical species. Non-radiolabeled ABC123 were used in these studies. Clear binding using Positron Emission Tomography (PET) PET was demonstrated with XY_2 antagonists in murine models of allergic airway inflammation of the lung.

Primary Pharmacodynamics

In vitro

ABC123 was shown to bind with high affinity to eosinophils cells expressing human recombinant XY_2 receptor with a KD value of 9.6. Binding at rat, mouse, rabbit and dog receptor was similar to that at the human receptor (Table 1).

Table 1: Binding of ABC123 binding to the membranes from eosinophils cells XY_2 receptors from different species.

	KD
Human	**9.6**
Rat	**8.9**
Rabbit	**6.9**
Dog	**8.9**

Cell based studies

The binding of ABC123 has been investigated in a number of cell systems in which response is mediated by the human XY_2 receptor quantified by measuring chemotaxis and basophil morphology (Table 2).

Table 2: Binding of ABC123 for inhibition of cellular responses mediated by the XY_2 receptor

	KD
Human basophils	9.6
Human XY_2	8.4

In vivo

ABC123 binding in antigen induced pulmonary inflammation in the rat

Groups of 3 male rats were given single i.v doses of 0, 5, 10 or 20 $\mu g \cdot kg^{-1}$ and binding was measured as a maker of inflammation, to a control group, with no lung damage. These studies demonstrated consistent binding to the XY_2 using PET in diseased animals (Table 3).

Table 3 levels of electron-positron decay g/ml in rats given i.v doses of ABC123

	0µ	5µg	10µg	20µg
Electron-positron decay	0.2	0.7	0.5	0.4

Effect of i.v administration of ABC123 on tobacco smoke induced lung in the rat.

Groups of 3 male and female SD rats with lung inflammation induced by were tobacco smoke were given i.v doses of 5, 10 or 20 $\mu g \cdot kg^{-1}$ ABC123. Clara cell binding was assessed. It was shown that serum concentrations of Clara cell were increased during lung damaged in the rat caused by inflammation. Binding of ABC123 to Clara cells was dose dependently increased in this study.

Table 4 levels of Clara cell protein (kDa) in rats given oral doses of ABC123

	0µg	5µg	10µg	20µg
Clara cell protein (kDa)	4.1	5.3	6.7	10.54

SAFETY PHARMACOLOGY

Safety pharmacology are not required for microdose studies, but where done should be reported

PHARMACOKINETICS

[Notes to the reader – Try to avoid discussing species separately, unless there are major differences in pharmacokinetics. Discussion of absorption, then distribution, metabolism and excretion is more appropriate. If data is generated as part of the toxicology study (i.e. Toxicokinetic data) this data should be discussed here and not as separate section.]

Summary

Only quantitative biodistribution, excretion and *in vitro* metabolism studied shave been conducted with ^{18} F (ABC123).

Absorption

No absorption studies have been performed.

Distribution

Whole body radiography studies in the rat indicate that the radiolabeled material was rapidly distributed to the lung with very limited central nervous system (CNS) penetration (i.v dose 500 MBq).

Organs that showed the highest levels of radiolabeled material were the lung, followed by small amounts in liver and the adrenals. By 24 hours 0.01% of the radiolabeled material was detected in the lung and no radiolabeled material was detected in the liver and adrenals at 24 hours post dose.

Metabolism

Studies were performed to investigate the in vitro metabolism of ABC123 by incubation with hepatic S9 fraction obtained from monkey, man, dog, mouse and rat. No metabolites were detected.

Excretion

The biodistribution of radiolabeled following the administration of ^{18} F (ABC123) to rats showed that radioactivity is predominantly excreted in faeces (approximately 80% by 2 hours post injection) with minor excretion in urine (approximately 10% by 2 hours post injection).

Pharmacodynamics

No pharmacodynamic or kinetic drug interaction studies have been performed.

TOXICOLOGY

Summary

All studies were performed in accordance with Good laboratory practice (GLP). The batch of compound used in non-clinical studies is the same as that to be used for the proposed clinical trial. A repeat dose study of up to 7 days duration was performed in the rat. Non-radiolabeled ABC123 was used in this study.

Single-dose toxicity studies in animals were not conducted with ABC123. However, toxicity following administration of a single dose was assessed as part of a dose-sighting study (rat). In rats i.v doses up to 20 $mg \cdot kg^{-1}$ were well tolerated.

Single dose data

Toxicity following a single dose was assessed as part of a repeat dose toxicology study in rats on Day 1. SD rats were given a single iv dose (0, 1, 10 and 20 $mg \cdot kg^{-1}$) on Day 1, followed by observations until termination on Day 14. There were no deaths or in-life clinical signs and there were no microscopic effects. Reduced adrenal weight was noted at 20 $mg \cdot kg^{-1}$, however these changes were within historical control range values.

Repeat-dose data.

SD Rats were given i.v doses of 0, 1, 10 and $mg \cdot kg \cdot day^{-1}$ once daily for 7 days, followed by an observation period of 14 days. There were no deaths, in-life clinical signs, body weight, food intake or clinical chemistry effects. Reddening of the injection sites was noted at necropsy, however there were no microscopic correlates.

At 20 $mg \cdot kg \cdot day^{-1}$ a slight increase in white cell parameters were noted in both sexes and males at this dose had slightly higher platelet counts (up 30%) compared with controls.

There was an approximate 20% and 15% increase in absolute and relative lung weights in both sexes at 20 $mg \cdot kg \cdot day^{-1}$. Increased neutrophil, macrophage, dendritic cell and B-lymphocyte cells were noted in the lung at 10 and 20 $mg \cdot kg \cdot day^{-1}$ in both sexes. These increases were within the historical control range at 10 $mg \cdot kg \cdot day^{-1}$, however at 20 $mg \cdot kg \cdot day^{-1}$ increases were beyond historical control range values. There were no other microscopic changes noted at any dose.

The no observed adverse effect level (NOAEL) for this study was 10 $mg \cdot kg \cdot day^{-1}$.

Reproductive Toxicology

No reproductive and development toxicity studies have been conducted. Only males subjects will be included in this trial.

Mutagenicity and Genotoxicity

No genotoxicity or kinetic drug interaction studies have been performed. Structure-activity relationship assessments did not high-light any structural alerts that would be of concern.

Potential genotoxic impurities

Potential impurities that may originate from the synthesis (starting materials, intermediates, etc) have been evaluated for potential genotoxicity. In the batch intended for clinical use levels of potentially genotoxic impurities do not exceed the staged threshold of toxicological concern (TTC) as proposed (EMEA, June 2008). Potential genotoxic impurities that are identified during the development will be controlled according to the staged TTC limits.

Discussion and Conclusion

Overall the package of studies to evaluate the toxicity of ABC123 did not uncover any findings of concern that would preclude appropriate single microdose administration of the

compound in an exploratory study in man. As this is an exploratory trial i.e. a trial that is not intended to identify clinical safety of ABC123, a reduced non-clinical data is presented.

The purpose of the study is to provide an initial clinical pharmacokinetic and binding basis for subsequent studies with ABC1235 with respect to safety and tolerability, and subsequently the potential of ABC123 as a diagnostic agent for inflammatory diseases of the lung via PET scanning.

Results from the general toxicity studies show that single doses of ABC123 up to 20 $mg \cdot kg \cdot day^{-1}$ were well tolerated in the rat.

Data from the 7-day study in rats showed an increased in white cell parameters at 20 $mg \cdot kg \cdot day^{-1}$ in both sexes. Males at 20 $mg \cdot kg \cdot day^{-1}$ had slightly higher platelet counts.

Increased neutrophil, macrophage, dendritic cell and B-lymphocyte cells were noted in the lung at doses of ≥10 mg/kg/day in both sexes. These increased were within the historical control range at 10 mg/kg/day, however this was not the case at 20 $mg \cdot kg \cdot day^{-1}$. There was also an approximate 20% and 15% increase in absolute and relative lung weights in both sexes at 20 $mg \cdot kg^{-1}$

Studies to evaluate genotoxic potential have not been conducted however structure-activity relationship assessments did not high-light any structural alerts that would be of concern.

Justification of the dose:

The NOAEL was 10 $mg \cdot kg.day^{-1}$ (i.v) in the rat, after repeated once-daily dosing for 7 days. The maximum dose of 20 $mg \cdot kg.day^{-1}$ iv used in the 7 day rat toxicology study was 1000-fold the clinical dose (approximately 20 μg). 10 $mg \cdot kg.day^{-1}$ is 500 fold higher than the predicted clinical dose.