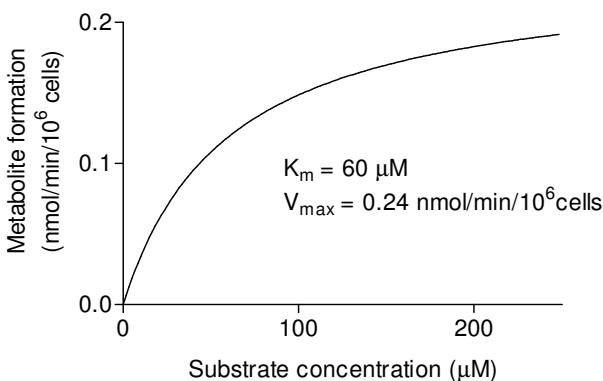


## COMPOUND A

Compound Compound A is a novel anticancer agent. During the development of Compound A the sponsor and CXR Biosciences built up an interactive relationship whereby CXR provided advice and experimental data at all stages of the process. By establishing this relationship the sponsor was able to characterise the bioavailability and metabolism of the compound, avoid problems which could have arisen in clinical trials, and understand interindividual variability in pharmacokinetics and response.

### Metabolism in rodents

At the outset, the metabolism of Compound A was completely uncharacterised. The sponsor did not even know whether it was subject to metabolism in the liver, so the first step was to determine whether hepatocytes from model species could metabolise the compound. Initially, a traditional approach was taken: rodent hepatocytes were incubated with Compound A and a metabolite, Compound A<sup>met</sup>, was identified by mass spectrometry. Non-linear regression analysis enabled us to determine  $K_m$  and  $V_{max}$  in rat hepatocytes *in vitro*.

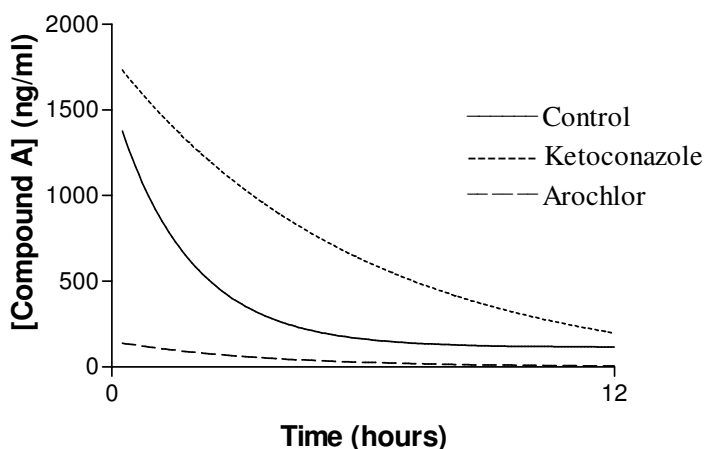


### Effects on hepatic cytochrome P450s (CYPs)

These hepatocyte studies showed that rodent hepatocytes were able to metabolise Compound A *in vitro*. In discussion with the sponsor, we raised the possibility that Compound A might be interacting with the drug metabolising systems of the liver; a further series of studies was commissioned to investigate the effects of Compound A on cytochrome P450s (CYPs) in rats. In males, testosterone metabolism was almost totally inhibited by Compound A and the corresponding enzyme was found to be absent. The data also suggested that the major steroid-inducible CYP of rat liver was being induced by Compound A.

### *In vivo* clearance

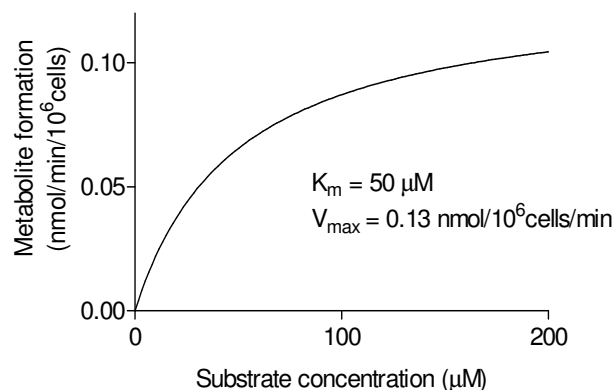
Compounds which induce CYPs are often substrates for the enzymes they have induced, so we next looked at the effects of induction and inhibition of CYP3A1 on the COMPOUND A clearance in the rat. In this study, clearance was studied in rats treated with the CYP inducer Arochlor 1254 or the inhibitor ketoconazole. The clearance of COMPOUND A was dramatically increased in response to induction with Arochlor 1254 and decreased in response to ketoconazole, showing that COMPOUND A undergoes cytochrome CYP-mediated clearance in the rat.



## Metabolism in humans

The first step in understanding the metabolism of Compound A in humans was to replicate the experiment carried out with rat and mouse hepatocytes to see whether the kinetics of the compound were similar in rodent and human cells *in vitro*. The results of this experiment demonstrated that the kinetics were, indeed, similar.

The next step was to identify the CYP isozymes involved in metabolism by human liver microsomes. The results of incubations with microsomes from 12 individuals were analysed by the Spearman Rank Correlation method. In this method, the activities of samples against the test compound are plotted against their precharacterised activities towards model substrates, allowing the determination of a correlation coefficient and calculation of the probability that the same CYP is involved in the metabolism of both compounds. The results indicated that the main CYP involved was CYP3A4, as the rat results had suggested.



## Inhibition of human CYPs

The observation that a candidate compound is metabolised by CYP3A4 can be a cause for concern since this CYP is central to the metabolism of many drugs. Compounds which interact with this isozyme, either as substrates or inhibitors, can generate adverse reactions due to drug-drug interaction. In order to investigate whether this might occur with Compound A, we examined the inhibition of CYP-dependent activities by this compound in comparison with various reference inhibitors.

The IC<sub>50</sub> results showed that Compound A inhibits seven of the nine recombinant human CYPs tested. No inhibition was observed with the other two. We were able to categorise the inhibitions as follows:

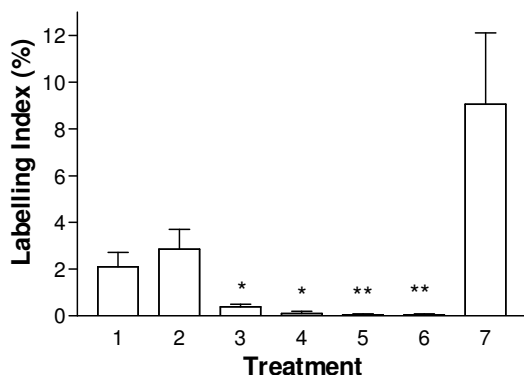
- Strong inhibition of two key enzymes.
- Moderate to strong inhibition of two others, one of which is known to exhibit functional polymorphism

Enzyme inhibition is, by far, the mechanism most often responsible for life-threatening drug interactions. Of the types of CYP inhibition, competitive inhibition with another drug for the enzyme active site is the most common. The inhibition of most isozymes by Compound A exhibited a competitive binding pattern consistent with classical Michaelis-Menten kinetics. In contrast, one of the isozymes which was strongly inhibited exhibited complex kinetics, indicating mixed inhibition or a combined substrate/inhibitor effect.

The sponsor was advised that the use of Compound A for therapy could cause drug-drug interactions or alter the pharmacokinetics of coadministered drugs. The amount of a coadministered drug may have to be reduced or an alternative given to reduce the risk of side effects. By identifying potential drug-drug interactions in advance, we enabled the sponsor to avoid possible problems and establish a safe strategy for clinical trials.

## Liver enlargement and detection of S-phase cells *in vitro* and *in vivo*

During the studies on CYP induction by Compound A in rats there had been some concern about liver enlargement. This can be caused by hypertrophy associated with enzyme induction, but can also be linked to hyperplasia, which is an early event in hepatocarcinogenesis. In order to see whether increased cell



proliferation might be a problem in human hepatocytes, we looked for effects on DNA replication, as determined by incorporation of BrdU. In contrast to the findings in rat liver, significant suppression of cell proliferation was observed in human hepatocytes *in vitro*. This was reassuring, in that it suggested that Compound A was not enhancing hepatocyte proliferation directly, and if anything might be suppressing cell turnover in the liver; however, the results did raise the possibility of hepatotoxicity linked to treatment with Compound A.

## Human hepatotoxicity

The results of *in vitro* analysis of cell turnover raised the possibility of hepatotoxicity. It was important to investigate this because it could compromise the outcome of clinical trials, especially in patients with impaired clearance. In particular, the sponsor wished to know whether hepatotoxicity was due to Compound A itself or its metabolite, Compound A<sup>met</sup>. We characterised cytotoxicity towards human hepatocytes by two methods. Depletion of cellular ATP is a sensitive method that measures biochemical toxicity leading to loss of energy reserves, whereas the MTT assay is based on metabolism by succinate dehydrogenase in the intact mitochondria of viable cells. A decrease in MTT metabolism is associated with cell death and is irreversible.

Determination of cellular ATP content confirmed that Compound A caused ATP depletion in human hepatocytes *in vitro*. Concomitant treatment with ketoconazole had no effect on this, suggesting that the process was not dependent on CYP-mediated metabolism. The metabolite Compound A<sup>met</sup> did not cause marked ATP depletion, demonstrating that it is non-toxic in its own right. Neither Compound A itself, nor Compound A<sup>met</sup>, caused cytotoxicity as determined by the MTT assay.

These data suggest that the toxicity of Compound A towards human hepatocytes in culture occurred via a mechanism which manifested itself in depletion of cellular ATP stores without loss of mitochondrial function. The implication is that Compound A exerts mild hepatotoxicity via a biochemical mechanism which does not involve dramatic loss of mitochondrial function but does lead to depletion of ATP stores; it is unlikely to lead to problems in patients with functional renal excretion mechanisms.

## Drug transporters

The fact that Compound A could itself be toxic towards human hepatocytes led the sponsor to express concern that any exacerbation of uptake or inhibition of excretion could lead to accumulation with the possibility of the drug reaching toxic levels. We therefore undertook to investigate the potential of Compound A and Compound A<sup>met</sup> as substrates or inhibitors of drug transporters using MDCK-II cells, an epithelial cell model of drug permeation. The results indicated that Compound A is a high permeability compound transported across cells by passive diffusion. Compound A<sup>met</sup> is also absorbed and transported passively. Neither compound had a significant inhibitory action on the MRP2-mediated efflux of cellular calcein from the apical side of the cells, indicating that these compounds do not interact with MRP2.

## The future

Compound A is about to enter Phase II clinical trial supported by our data. We are now working closely with the sponsor on follow-up compounds, again adopting an interactive, start-to-finish approach where we are involved from the initial screening of potential hits to the resolution of issues in the run up to clinical trials.