The title for this talk is enzymes involved in phase I and phase II drug metabolism.

The **learning outcomes** are to have an understanding of enzymes involved in phase I like cytochrome P450 and other phase I enzymes. To understand the relative importance of these phase I enzymes. To have an understanding of enzymes involved in Phase II, such as uridine diphospho glucuronyl transferase, UGT and other phase II enzymes. And to understand the relative importance of these phase II enzymes as well.

Introduction to phase I and phase II metabolism. Phase I or functionalisation reaction exists to produce or uncover functional group for phase II by oxidation, reduction or hydrolysis. it prepares compound for Phase II. Conjugative reaction takes phase I compounds and are conjugated by phase II enzymes like UGT, or sulpho transferase (ST).

They generally make the compound more water soluble and so can be readily excreted via the urine or bile. All of these processes are important in the drug discovery process as they are responsible for metabolising various drugs which may lead to therapeutic or no therapeutic effect.

Metabolism and overall drug clearance. The pie chart to your left shows the overall contribution of metabolism to clearance. You can see that metabolism accounts for 70% of the overall clearance that happens in the body, followed by renal clearance and hepato- biliary clearance. Of the 70% that is due to metabolism, 70% of that is due to cytochrome P450 and the remaining 30% are shared equally between UGT and other enzymes. So you can see from this pie chart that metabolism is very important to the overall drug clearance process. And more importantly, cytochrome P450 is responsible for the lion's share of the overall metabolism that happens in the body.

Cytochrome P450. As already mentioned accounts for 70% of the total metabolism that goes on in the body is due to cytochrome P450. They are membrane-bound enzymes located in the smooth endoplasmic reticulum or the microsomes which are ruptured endoplasmic reticulum.

There are five major isoforms of cytochrome P450. By way of nomenclature if we took cytochrome P450 CYP 3A4, the letter CYP stands for the fact that they are cytochrome containing enzyme, 3 is the family, the A the subfamily and 4 the gene. In CYP 3A4, 3 is Family, A is subfamily and gene is four. So the five major isoforms are CYP 3A4, CYP 2D6, CYP 2C9, CYP 2C19, and CYP 1A2.

And the panel to the right is the conversion of benzene by oxidation to phenol and the demethylation of phenyl methyl ether to phenol. In each of these processes, less water soluble compounds are being made more polar and therefore made more readily excretable by the urine or bile.

Phase I drug metabolism by cytochrome P450. Cytochrome P450 enzymes catalyze thousands of different reactions. They are haem-containing enzyme of the mixed function oxidase system. Which requires both atmospheric oxygen and a reducing system.

So in this panel below, you can see the drug to be oxidised RH, the oxygen from the atmosphere and the reducing equivalents, in the way of the NADPH. The product is oxidized drug, ROH, water and oxidized NADP.

CYP450 can bind carbon monoxide in their reduced form and they absorb UV maximally at 450 nm. Hence their name cytochrome P450.

They can be universally blocked by 1 amino-benzotriazole , 1 ABT, with the exception of CYP 2C9. All inducible by Rifampicin and Dexamethasone with the exception of CYP 2D6. And they can all be inhibited by selective inhibitors as well.

Different isoforms and pharmacogenetics. The pie chart to the left of your slide shows the contribution of different isoforms to the metabolism that happens in the body. CYP 3A4 is responsible for 30% of the overall metabolism that happens in the body, followed by CYP 2D6, at 20%. Together between them, CYP 3A4, 2D6, are responsible for 50% of the overall metabolism that occurs in the body. So these two isoforms takes the lion's share of what isoforms are responsible for which amount of metabolism.

But these isoforms of cytochrome P450 also exhibit some pharmacogenetics, such as CYP 2D6 has between 5-10% of Caucasians who are classified as slow metabolisers and 30% of East Africans as extensive or ultra rapid metabolisers. The consequence of these pharmacogenetics, will be looked at in the next slide. CYP 2C19 has 25% of Asians not expressing it and 3% of Caucasians. In CYP 2C9, 1% Caucasians does not express it. Whilst CYP 3A4 is not polymorphic. It has approximately 40% variability in it's expression level, which means the reaction rate will differ from population to population.

The consequences of pharmacogenetics can be visualised in this slide, where we're looking at the conversion of codeine to morphine by CYP 2D6 O-demethylation. The graph to the right of the slide shows the formation of morphine from codeine as a function of time. In poor metabolisers. If codeine was given to them for controlling pain, they may not have adequate pain control because the level of codeine, convention to morphine is sub-therapeutic. Conversely, in the extensive and ultrarapid metabolisers, the conversion of codeine into morphine is very rapid, such that it might lead to morphe toxicities such as respiratory suppression and constipation. So to mitigate for these two extremes of pharmacogenetics, to control pain in poor metabolisers they are best given morphine directly and in the extensive and ultra rapid metabolisers, they are given indeed CYP 2D6 inhibitor, or lower dose of codeine so that they will not experience morphine toxicity.

Uridine Diphospho glucuronide transferases (UGT). As already mentioned, they are responsible for 15% of the total metabolism that occurs in the body. They are membrane-bound enzymes as well, and they are located in the smooth endoplasmic reticulum. They also exist as isoforms of which UGT 1A1 is the most abundant. They require uridine diphospho glucuronic acid, UDPGA as cofactor. They are inducible by Rifampicin and could be inhibited by diclofenac and other drugs. The active site structure of UGT is shown to the bottom of the slide, which shows it to have 6 beta sheets and 7 alpha helixes, which makes the drug fit perfectly into its active site.

Phase II conjugation reactions. In this example, we'll look at the phase two conjugation of phenol by sulphation to O-sulphate conjugate of phenol or by glucuronide conjugation to O- glucuronide conjugate of phenol or by methylation to all methyl conjugate of phenol. So in these instances, the phenol has been made more water soluble by the conjugation which has happened, with exception of all methyl conjugate, which is not as readily water-soluble as the phenol from which it started from.

There are **other phase I and phase II enzymes.** CYP 450 and UGT are not the only phase I and phase II enzymes.

Other phase I enzymes are, alcohol dehydrogenase, aldehyde oxidase, which are capable of performing oxidative steps. In this example, propanol is being acted upon by alcohol dehydrogenase to give propaldehyde, which is been further oxidised by aldehyde oxidase to propionic acid. By series of one-step oxidation. We have flavin mono oxidase, which can oxidize trimethylamine to its N-oxide, to form trimethylamine N-oxide. We have aldehyde dehydrogenases, ALDH. There is mono amine oxidases MAO, and xanthine oxidases, nitro reductases, which are capable of reducing nitrobenzene to aniline and there is azo reductases. And finally, we have esterases and amidases.

There are **other phase two enzymes** which are Sulpho transferase (ST), Methyl transferase (MT), Catechol O- methyltransferase (COMT), which is capable of methylating catechol, to its methoxy form. As shown in the panel to the right. We have N-acetyltransferases which are capable of N-acetylating aniline to acetanilide. We have Acyl transferases, phosphotransferases, Glutathione Sulpho transferase (GST).

What is common to all these enzyme is they're all functional group selective as they catalyze the oxidation or reduction of such functional groups.

Other phase I and phase II enzyme account for approximately 15% of the total metabolism that occurs in the body.

The **location of other phase 1 and phase 2 metabolic enzymes**. The idea behind this table, is to show the subcellular location of each enzyme and the cofactor that may be required. It's there to give us a guide as to which assay test system we should be using for which particular enzyme. So if we are looking for the involvement of aldehyde oxidase, we will use cytosol as our test system. If we are looking for the involvement of aldehyde dehydrogenase, we could use either cytosol or the mitochondria as our test system. And if we are looking for the involvement of Flavin Mono Oxidase, we will use the Microsomes as our test system for the investigation. So the whole purpose of this table is to indeed be an aide memoir of where the enzymes are located. And so give guidance as to which assay test system to use and whether they require cofactor or not.

In conclusion, we've learned that the most prominent enzymes involved in phase I and phase II drug metabolism, are cytochrome P450 and UGT. There are other less abundant enzymes such as aldehyde oxidase, alcohol dehydrogenase, xanthine oxidase, nitro reductase, and catechol-O-Methyl transferase. All of these enzymes are found in different sub cellular locations, such as the microsomes, which is ruptured, smooth endoplasmic reticulum, the cytosol, and mitochondria. They are all important in the drug discovery process as one or more of them may be responsible for the metabolism of our compound of interests. Thank you for your attention.