AI in Drug Discovery and Development
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Week-01
Lecture-04

Welcome to the course AI in Drug Discovery and Development. Today we will talk about conventional methods for drug discovery. So, by the end of this lecture you will be able to understand experimental drug discovery methods including biochemical and cell-based assays, explore the techniques for target validation. Hit identification and lead optimization and also learn more about pharmacokinetic and pharmacodynamic methods used in the drug discovery. So, let us have a look at some of the key terms for example, IC_{50} which is the half maximal inhibitory concentration. So, it is the concentration of an inhibitor which is required to reduce an enzymes activity or a biological response by 50 percent.

So, if the IC_{50} is having a lower value it means that the compound has a higher inhibitor potency. Another term is K_i, which is called as inhibition constant as well. So, it is a measure of how tightly an inhibitor binds to an enzyme. So, the K_i is on the lower side.

So, it means that it is showing a strong inhibitor binding. So, another term is K_m which is called as Michaelis constant. So, it is the substrate concentration at which the reaction rate is at 50 percent of the V_{max}. So, if there is if the K_m value is lower it means that the substrate has higher affinity. Another term is V_{max} which is the maximum reaction velocity.

So, it is the highest rate at which an enzyme converts substrates to produce to product when fully saturated. So, it is directly proportional to the enzyme concentration. So, if we talk about the inhibition. So, this could be competitive inhibition. So, where the inhibitor bind to the active site competing with the substrate.

So, it is indicated by an increase in the K_m, but the V_{max} remains unchanged and it can be and this kind of inhibition can be overcome by adding excess of the substrate. Another kind of inhibition is called non-competitive inhibition, where the inhibitor binds to an allosteric site and not to the active site. So, in this case it will reduces the V_{max}, but the K_m will remain unchanged. Another kind of inhibition is uncompetitive inhibition. So, where the inhibitor binds only to the enzyme substrate complex preventing product formation and then it there will be decrease in both K_m and V_{max} values.

Some more terms like agonist which is a molecule that binds to a receptor and fully activates it, mimicking the natural ligand. So, what it does is it produces a full biological response. An antagonist is opposite to an agonist where which is a molecule that binds to

a receptor but blocks activation, preventing the natural ligand from exerting its effect. So, it produces no biological response, but prevents the agonist action. Then there is partial agonist which are the molecules that bind to a receptor, but only partially activates it even at full receptor occupancy.

And, they produce a weaker response than a full agonist, but more than an antagonist, but the effect is more than an antagonist. Then, we have inverse agonist which are the molecules that bind to a receptor and decreases its baseline activity producing the opposite effect of an agonist. So, they reduce the receptor activity below its normal level. So, after these key terms, so let us go through what are the target validation experimental methods which are conventionally used in drug discovery and development. So, the target validation ensures that a specific protein is causally linked to a disease confirming its potential as a therapeutic target.

So, some of the methods which are being used for target validation are one of them is siRNA and shRNA knockdown techniques. which reduces the target protein expression in vitro or in vivo and these are used to assess gene function in disease models as well as identification of essential pathways for drug targeting. Then there are CRISPR-Cas9 gene editing tools, which involves creating gene knockouts or precise mutations to study disease mechanism and help in generating loss of function and gain of function models that aid in identifying new therapeutic targets. And then we have antibody-based target validation methods where we use monoclonal antibodies to block or detect proteins in biological systems. So, they help in studying cell signaling and receptor activation as well as developing novel therapeutic antibodies for targeted therapy.

So, once we have validated the target, so the next step is high throughput screening. So, high throughput screening is used to screen large chemical libraries of compounds that modulates biological targets and this is the key starting point where we identify the hit compounds. So, we can say in summary that high throughput screening is being used for identification of hit compounds. So, what are the components of high throughput screening? So, we have to develop an assay and optimize that assay. So, there are different kind of assays for example, you might be using biochemical assays which are enzyme based or you can use cell-based assays where you are determining the viability or receptor activation.

So, this must be optimized for high sensitivity, reproducibility and minimal interference. And then we have recently there has been a lot of upgrade in the automation and robotics. So, one can use the robotic liquid handlers to increase the screening efficiency, which also reduces the human error and enable rapid testing of thousand to millions of compounds. And then plate preparation and compound management. Usually, those screening

compounds are prepared in these multi-well plates.

So, we can use like 384 well or 1536 well plates to maximize the throughput of the assay. And then these compounds are usually stored in DMSO to maintain their chemical stability as well. So, regarding data acquisition and analysis systems, so we use this like luminescence, fluorescence or absorbance-based detection methods for rapid readout. And also, we use the automated hit identification algorithms that can help detect promising compounds in a very small amount of time. So, some of the biochemical assay techniques are as such.

So, these biochemical assays, they analyze enzyme activity, protein ligand interaction and receptor modulation. So, some of the commonly used methods are enzyme inhibition assay, where we used to identify enzyme inhibitors for some diseases like acetylcholinesterase inhibitors in the case of Alzheimer disease. And then we use these kinetic parameters such as IC_{50} value, K_i value, K_m over V_{max} value to determine the inhibitory potency of these molecules. And then we use the receptor binding assays as well like which are used to measure the drug affinity to membrane receptors like GPCRs and tyrosine kinases. So, they use radio labelled ligands for example, tritium labelled or iodine 125 labelled ligands.

And then there are fluorescence-based assay methods as well such as FRET and FP. So, we have FRET is fluorescence resonance energy transfer methods which is very helpful in detecting protein-protein interaction. And then we have fluorescence polarization-based method which measure the ligand binding affinity in small molecule screening. And then we have luminescence-based assay methods like the ATP-dependent luminescence assay, which is used to measure the cell viability. And then there are cell-based assays methods as well.

So, these provide physiological relevance and help assess drug cytotoxicity, efficacy, and mechanism of action. So, for example, the reporter gene assay, which detects gene transcription activation using luciferase or green fluorescent protein. And then we have cell viability and proliferation assays like we use the MTT, XTT and Alamar blue assays which quantify the cell survival. And then there are electrophysiology techniques as well like Patch-clamp assays which measure the ion channel activity. And then there is flow cytometry technique which detect the apoptosis, immune response and protein expression as well.

So, then we come to the phenotypic screening approaches. So, usually there are two approaches which are used for drug discovery. So, one is called as structure-based approach, another one is phenotypic screening approach. So, the phenotypic screening approach, it is an approach where compounds are tested in whole cells or organisms to

observe biological effects without prior knowledge of the target. So, instead of designing molecules for the specific target, here in this case what we do is we are not knowing the target of the molecule, but we are directly interested in knowing the activity of these molecules.

So, it is often contrasted with target-based drug discovery which focuses on specific molecular interactions. So, we identify compounds based on observable changes in the phenotype like cell death, morphology, protein expression rather than direct target binding. So, here what we do is we are, for example, we have those cells in our assay plate and then we add the compound and we see whether that compound is showing any effect or not. And in this case, we are not knowing how this molecule is acting. So, we are not interested in targeting that molecule to that specific receptor or enzyme, but we are interested in knowing the activity.

We treat the cells with some molecules and those molecules are then showing some activity. So, and that activity, the target of activity we are not interested to know. So, in this case, it can identify first in class drugs with novel mechanism of action. So, here we identify compounds based on observable change in the phenotype. For example, cell death, morphology, protein expression rather than direct target binding.

So, in this case, we can discover first-in-class drugs with novel mechanism of action. So, that is one of the major advantages of using phenotypic screening approach. Some of the methods for phenotypic screening are whole organism screening, which involves testing drug candidate in living model organisms to observe systematic effects, toxicity, and efficacy in a biologically relevant environment. So, the example includes using zebrafish, C. elegans, and the fruit fly. And then we have 3D cell culture methods which involves creating tissue like a microenvironment where cells they grow in a more physiologically relevant manner as compared to the 2D monolayers. So, we can use for example spheroids, organoids and the hydrogel-based cultures for using these 3D cell culture-based method in phenotypic screening. And, then recently there have been an upsurge in the use of organ on chip technologies. So, which mimic human organ functions using miniaturized tissue constructs with dynamic perfusion. Example is lung-on-a-chip which simulate alveolar capillary interface.

And then recently researchers have been using high content imaging and analysis where an automated microscopy which is combined with AI driven image analysis to extract quantitative data from cell-based assays. So, the example includes fluorescent imaging and machine learning based image analysis. So, once we have identified the hit compound, so now the next step is hit confirmation and validation. So, it involves a systematic approach to ensure a reliable, selective and drug-like molecule which progresses to the next phase of

drug development. So, these steps help eliminate false positives, optimize lead compounds and improve clinical success rates.

So, some of the methods which are being used for hit confirmation and validation are the dose response study where we determine the potency and efficacy of hits by establishing IC_{50} or EC_{50} and then we do a counter screening for selectivity. So, it ensures that heats are selective for the intended target and are not acting on unrelated proteins or pathways and also, we also identify PAINS, pan assay interference compounds or promiscuous binders or non-specific aggregators in the assay. So, that we can avoid the false positive molecules here. So, then there is another approach called analog testing for SAR development. So, we try to understand how the structural modifications affect the potency, selectivity and toxicity and it guides the lead optimization process to improve efficacy and drug-like properties.

And then we also need to perform orthogonal assay validation as well, which minimizes the false positives from the primary screen by using a different detection method. which is also ensuring that observed activity is not due to assay interference, fluorescence artifacts or compound aggregation. So, after hit validation. So, the next step is lead optimization. So, lead optimization is an important medicinal chemistry driven approach which aims to define hit compounds to improve potency, selectivity, metabolic stability and pharmacokinetic properties.

So, some of the key approaches in lead optimization are the parallel synthesis method where we synthesize multiple analogs simultaneously by altering chemicals group systematically. So, it helps establishing SAR of those molecules. And then we use Microwave-assisted organic synthesis, which uses microwave irradiation to accelerate chemical reactions. So, it reduces reaction times from hours to minutes. And then we have flow chemistry applications as well, where continuous flow reactors enable precise control over reaction conditions.

So, they reduce side effects and increases the yield. And then we have chiral separation techniques where many drugs exist as enantiomers with different biological activity. So, supercritical fluid chromatography and HPLC chiral columns, they help separate those enantiomers. Followed by the lead optimization. So, next step is the in vitro ADMET evaluation.

So, these studies, they help predict how a drug behave in human body before the in vivo testing. So, some of the methods which we use to determine the ADMET properties are like parallel artificial membrane permeability assay which is also known as PAMPA assay which measure the passive drug permeability across synthetic lipid membranes. So, it is

used to establish the blood brain barrier permeability of the drugs in vitro. And then we have microsomal stability assay. So, which uses the liver microsomes to assess metabolism by cytochrome P450 enzymes.

So, it helps identifying fast metabolizing drugs that may require prodrug development. And then we have CACO2 cell permeability studies which where we use the human intestinal epithelial cells to assess oral absorption potential. And it predicts if a drug can cross the intestinal barrier into the main bloodstream. And then we have plasma protein binding experiments where we try to determine the fraction of a drug bound to the albumin and other plasma proteins. So, highly bound drugs such as which are binding to the plasma protein by more than 95 percent.

So, they have lower free drug concentration and thus they will show a reduced activity. So, example is warfarin which is 99 percent bound to plasma protein. So, it affects its doses and interaction. Moving to next, so we use the in vivo pharmacokinetic studies. So, the pharmacokinetic studies, they evaluate how a drug is absorbed, distributed, metabolized and excreted in living organisms.

So, these studies provide critical data on drug bioavailability, clearance, half-life, helping to optimize doses and therapeutic efficacy. So, some of the methodologies which we use are like we use the animal model selection preparation, where the selection of the animal model for doing pharmacokinetic depends on physiological and metabolic similarity to the humans. So commonly used species includes rodents, mice and rats. These are preferred for early-stage PK screening due to genetic and metabolic similarity. So, then we have the non-human primates which are used for studying drug targeting complex systems such as the central nervous systems.

So blood, so regarding the blood sampling, so we measure the systemic drug concentration over time. It can be collected via venous puncture or via catheterization. And then if you wanted to determine the amount of drug in the tissue, so we can determine the drug penetration into the target organ by collecting that tissue and quantifying the drug in that tissue. And urine and fecal collection can also be done to trace the metabolism and elimination of that specific drug. Microdialysis can be performed to continuously sample interstitial fluid to monitor the free drug levels in the tissues.

And once we have collected those samples, we need to quantify the amount of drug or the molecule in those samples and for that we use LCMS/MS for quantification. So, the advantages LCMS/MS offers for quantifying the drug are that it is highly sensitive and specific. And, then simultaneously detection of parent drug and metabolites is possible and it is essential for therapeutic drug monitoring, bioavailability studies and drug-drug

interaction assessment. Once we have quantified the amount of the drug which is available which is present in those fluids.

So, we can do the pharmacokinetic analysis. And, then we can estimate various key PK parameters such as C_{max} which is the maximum concentration, T_{max} time to reach the C_{max}, AUC area under curve, and the half life $t_{1/2}$ of the drug. And, once we have performed the pharmacokinetic experiments. So, the next is pharmacodynamic study techniques. So, the pharmacodynamics describes how a drug affects the body at the molecular, cellular and systemic levels. So, what we, so our objective with the pharmacodynamic studies is to establish mechanism of action, how the drug interacts with its target.

So, our objective with the pharmacodynamic studies is to establish mechanism of action, how the drug interacts with its target. To establish the potency, the concentration which is required to produce a biological effect and to establish the efficacy, the drug's ability to achieve its intended therapeutic response. So, the speedy studies, they help optimize doses, predict therapeutic outcomes, and assess the safety profiles of those molecules being studied. So, to measure the effect of those drugs, we need to identify some biomarkers which can be a marker for the progression of the disease. So, the biomarkers are measurable biological indicators used to track the drug effects.

So, after the pharmacodynamic studies where we have established that the compounds are efficacious and safe to use. So, then we need to have some biomarkers to see whether those molecules are having any effect in the disease or not. So, the biomarkers are measurable biological indicators that are used to track the drug effects. So, there we have several types of biomarkers. For example, we have target and engagement marker which confirms the drug binding and modulation of the target or we have the response biomarkers which indicates whether the physiological or biochemical response has been observed to the treatment or not.

So, the biomarker validation ensures specificity, reproducibility and clinical relevance. So, after all this we can have the ex vivo tissue assays which involves testing drug effects on isolated organs or tissue slices to study local pharmacological responses. So, it help us to assess the receptor binding and signal transduction in a controlled environment. Tissue specific drug metabolism and bioactivity and these are being used in preclinical research to bridge in vitro findings within in-vivo validation. So, then we have in vivo efficacy models where which are the animal models that are being used to study drug response in a living system under physiological condition.

It allows assessment of a dose response relationship and therapeutic windows and also disease progression modulation through drug intervention. So, the translational relevance

of these in vivo efficacy models is quite critical requiring selection of species with similar drug metabolism to humans. So, then we have imaging techniques for pharmacodynamic assessments. So, those non-invasive imaging methods which are used to provide real-time tracking of drug distribution, receptor occupancy and target engagement. Some of the imaging techniques are for example, positron emission tomography which uses a radiolabel tracer to quantify drug target interaction.

Or we have the SPECT which is single photon emission computer tomography which measure the pharmacological effect in the specific tissues. So, these imaging techniques they enables longitudinal studies of drug dynamics without invasive sampling. So, coming to the summary for target identification validation the methods such as CRISPR gene editing, RNA interference, RNAi, knockout, knockdown studies and over expression models are used to confirm the targets role in disease are used. And high throughput screening is performed by automated screening of large compound libraries using biochemical or cell-based assays to identify potential hits. The lead optimization methods, they involve structure activity relationship analysis by chemical modification of leads and pharmacokinetic modelling to improve drug properties like potency, selectivity, and bioavailability.

The in vitro assays in vivo animal models and toxicity studies are performed to assess safety and efficacy of those molecules being studied for development of the drugs. So, then I have a small question for you to think about. So, what are the ethical considerations in using animal models for pharmacokinetic studies? How might emerging technologies they reduce the need of animal testing? These are some of the papers which you can go through for further enhancing your knowledge in this field. With all that, thank you so much.