

IMPLICATIONS OF FISH MODEL IN DRUG DISCOVERY

Abstract

Drug is any chemical that can change the way our body works. They are important to human being because they act as a cure to new diseases or they aid in reduction of previously untreatable conditions. In some cases, it may not be a new drug that is required, but that a previously known drug can be used for a new purpose. Introducing a new drug to market is a complex and time-consuming process that can cost pharmaceutical companies an average \$1.3 billion and ten years of research and development. There are multiple defined stages for this process, each with their own associated challenges, timelines, and costs. Drug development involves extensive testing in animal models to determine if the drug is safe for human trials and if it performs as it should. The reduction of animal testing is one of the goals set out by FDA in ISO10993, where zebrafish can be considered an alternative organism, which is highly efficient to replace rats and other test animals. With the completion of the zebrafish genome project and the establishment of a robust infrastructure for genetic and physiological studies, the zebrafish system sits poised to take on a larger role in the field of drug development. By contributing to target identification and validation, drug lead discovery and toxicology, the zebrafish might provide a shorter route to developing novel therapies for human disease. In the upcoming years, the advancement of contemporary technology may enable the zebrafish to serve as a significant stand-in for other mammalian models used in pharmaceutical discovery.

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Authors

Arya Prabhakaran

Fish Nutrition, Biochemistry and Physiology
ICAR-CIFE, Mumbai, India.

Tincy Varghese

Fish Nutrition, Biochemistry and Physiology
ICAR-CIFE, Mumbai, India.

Subodh Gupta

Fish Nutrition, Biochemistry and Physiology
ICAR-CIFE, Mumbai, India.

Sunil Kumar Nayak

Fish Genetics and Breeding
ICAR-CIFE Powarkheda Centre.

Vasanthakumaran Kumar

Fish Nutrition, Biochemistry and Physiology
ICAR-CIFE, Mumbai, India.

Prabhashlal P

Fisheries Extension Officer
Department of Fisheries
Kerala.

I. INTRODUCTION

Since the beginning of time, illnesses and humans have been engaged in a constant battle. Over the course of human history, attempts to control diseases have been well documented. It cannot be denied that the 20th century was a pharmacological golden age, bringing the majority of the current arsenal of pharmaceuticals at our disposal, despite the fact that many cures had been discovered in the earlier centuries. The rapid advancement of the fields of biology and organic chemistry in science can be partly credited for the pace at which drugs were discovered. Drug discoveries have an impact on all aspects of human life, but perhaps most notably, they have allowed people to live longer and with significantly higher quality of life. While the majority of human diseases have adequate therapeutic options, others have few or no alternatives, and they still place a significant burden on nations and societies. Absolute curative treatments for many disorders are still difficult to find, making the ongoing hunt for novel pharmacological compounds and technological advancements imperative.

II. DRUG DISCOVERY PROCESS AN OVERVIEW

The process of finding new potential medications is called drug discovery. It is highly regulated because of public concern since the final entity will be used in humans for disease treatment. Therefore, each and every step in the drug discovery pipeline is validated by an approved regulatory body to ensure its safety when used in humans. The identification of an illness or disease area with an unmet medical need is the first step in the complex and laborious scientific process of drug discovery and development. The pharmaceutical or biopharmaceutical company begins the pre-discovery phase, which involves identifying the disease's underlying molecular causes and creating suitable animal disease models and test platforms. The next step is to identify potential targets whose chemical manipulation might have a therapeutic effect. The hit-to-lead discovery phase, which follows target identification and validation, involves the systematic modification of primary hit compounds to increase potency, reduce undesirable effects, and improve desirable physicochemical properties. This phase also involves the identification of molecules with the desired pharmacological activity. The outcome of the drug discovery process is a candidate drug that goes through pre-clinical research and later drug development, transforming the molecule into a clinically acceptable medication whose efficacy, safety, dosing, and tolerability are determined through meticulously planned and carried out clinical trials. The drug discovery process is complex, costly, and time-consuming, from the first step of identification to the clinical trial.

Table 1: Overview of Drug Discovery Pipeline

1) Drug Discovery and Development	
Target identification	Defined target gene/receptor/protein/enzyme/ ion channel involved in cause of disease
Target validation	Validate the target effect on disease / druggability
Target selection	Selection of validated target
Lead identification	Selection of Natural or synthetic compound that can bind to the identified target
Lead optimization	Selectivity, metabolic stability, potency on target is optimized

2) Preclinical Trials	Checking the therapeutic and acute side effects of lead for safety- pharmacokinetics, toxicity Preclinical trials are regulated by- CPCSEA and IAEC-Committee for the Purpose of Control and Supervision of Experiments on Animals; Institutional Animal Ethics Committee in India
<i>In vitro</i> / <i>In silico</i>	Monolayer cultures are excellent at detecting tissue-specific toxicity phenotypes cannot provide the level of depth necessary to understand more complex mechanisms arising from interactions between multiple tissues
<i>In vivo</i>	Mice are the eponymous animal model used for preclinical trials
To conduct research on a specific drug, an application must be submitted to the Central Drugs Standard Control Organisation (CDSCO) for approval prior to beginning the clinical study. This application, known as an Investigational New Drug (IND) application, comprises details on the drug, pre-clinical study results, a study protocol or plan, as well as information on the research team that will be in responsible for conducting the trials.	
3) Clinical Trial	In India clinical trials are conducted under the guidance of ICMAR (CEHR)-Central Ethics Committee on Human Research
Phase 0	Pilot study, 10-15 healthy individuals, short period, sub therapeutic dose, drug response to humans.
Phase I	10-100 healthy individuals, safety evaluation, PK/PD, side effects
Phase II	100-300 patients, efficacy evaluation, dosing requirement, long term toxicity.
Phase III	1000+ patients, efficacy and safety, comparative study with a standard.
4) Regulatory Review	NDA(new drug application) to FDA, drug licence and marketing.
5) FDA Review	FDA review team takes 6 to 10 months to decide whether to approve the NDA. Once the NDA is approved “labelling” as well as further remaining issues required to be fixed before the drug to be approved for marketing
6) Post-Market Drug Safety Monitoring-Phase IV	Post marketing surveillances, to evaluate the efficacy, cost effectiveness, and safety of an involvement in real-world settings, risk/benefit ratio

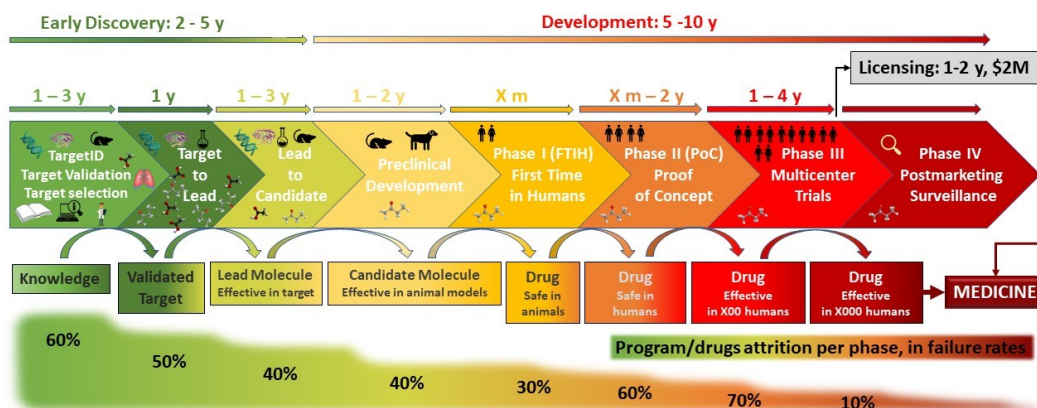


Figure 1: Diagrammatic Representation of Drug Discovery Pipeline

III. CRITICAL WINDOWS IN DRUG DISCOVERY PIPELINE

The development and discovery of drugs is an extremely lengthy and expensive process. Since its inception, it typically takes 12 to 15 years and \$1 billion or more for it to reach the market (Hughes *et al.*, 2011). Ninety eight percent of chemicals tested on animals are eventually abandoned, since they fail at various stages of drug development process. In the vast majority of instances, this is because the substance either lacked adequate therapeutic activity (efficacy) *in vivo* or was deemed dangerous due to its mechanism-based toxicity. Rodents and/or higher mammals are currently used for most of these experiments on animals (Bhusnure *et al.*, 2015). A new drug's market entry expenses range between \$985 million to \$1.3 billion on average (Wouters and Luyten, 2020). Only 11% of drugs actually reach the market despite this significant effort and financial investment; the other 89% are destroyed in the lab. The primary constraints on the pharmaceutical industry are safety issues, drug efficacy problems, or the financial burden of further drug development.

IV. ANIMAL MODELS USED IN DRUG DISCOVERY PROCESS

In order to comprehend the cellular and molecular causes of human disease and to create and test new therapies, biomedical research relies on the use of animal models. Preclinical studies are required following the discovery of a novel scaffold drug. Most testing of new scaffolds is carried out in mice, frogs, and guinea pigs during preclinical studies. Overall, researchers are attempting to develop a treatment that is less dangerous, but it still necessitates many sacrifices. Because of the striking similarity in mammalian genomes and the many similarities in anatomy, cell biology, and physiology, mammalian models, such as the mouse, have been particularly effective at simulating human diseases (Barré and Montagutelli, 2015). When choosing an animal model, a number of other aspects must be taken into account in addition to evolutionary closeness and anatomical similarities.

Table 2: Biomedical Significances and Limitations of Small Animal Models

Animal Models	Significances	Limitations
Rats (<i>Rattus norvegicus</i>) and Mice (<i>Mus musculus</i>)	Easy to breed and handle, less rearing care, easily interchangeable between rats and mice. Since they are primarily inbred, they lack the genetic diversity of humans	Unsuitable model for inflammation study, sequential blood samples from the same animal are challenging
Guinea pig (<i>Cavia porcellus</i>)	Mainly outbred, appropriate for studies on cholesterol metabolism, Alzheimer's research, tuberculosis, asthma research, fetoplacental development, and vaccine development	High phenotypic variability and low viral infectious potential hinder Ebola studies in guinea pigs
Hamster, especially golden hamster (<i>Mesocricetus auratus</i>)	Ease of handling, economical, non-aggressive, Excellent for reproductive study due to stringent progesterone, but not oestrogen, a short gestation time, a unique anatomical feature of a loose subcutaneous region, and significance for microcirculation studies, cancer models, infection models for leptospirosis, and vaccine studies	Fewer genetic reagents and advances in technology available
Rabbit (<i>Oryctolagus cuniculus</i>)	When compared to other large animals, they are inexpensive, easy to handle, widely bred, and readily available. Great model for surgically created osteoarthritis, cardiovascular illness, wound healing, pharmacological study, asthma, cholesterol, and Alzheimer's disease model	Weaker bones, less genetic tools, and a scarcity of reagents
Equids (<i>Equus</i>)	Important for the investigation of articular abnormalities, orthopaedic models, tendinopathies, asthma, and reproductive models	Large size limits the use of radiological imaging equipment. It is costly to purchase and maintain
Cattle (<i>Bos taurus</i>)	Important for research on the female reproductive model, problems around pregnancy, and tuberculosis models	Additional care costs are necessary

Goat (<i>Capra hircus</i>)	Model for female to male XX sex reversal, mechanical circulatory support devices, and potential for orthopaedic investigations, A good fit for antibody work	Expensive and scarce reagent supply
Sheep (<i>Ovis aries</i>)	Ease of handling, convenient for sampling, comparable to people in terms of physiology and anatomy, Great surgical model for bone and wound healing, asthma, and heart pathology model, aid in the development of vaccines	Limited reagent availability, mainly inbred strains, and expensive to procure
Cat (<i>Felis catus</i>)	Important models for asthma, obesity, cerebral palsy, HIV, type-2 diabetes mellitus	Used as pet, involves ethical and societal issues, expensive
Dog (<i>Canis familiaris</i>)	Outbred, large, well-defined in a number of scientific fields, inheritable conditions, musculoskeletal research, cancer and haemophilia B as well as narcolepsy etc	Costly, used as a pet, raises moral and cultural questions
Pig (<i>Sus scrofa</i>)	Important for cardiovascular research, Alzheimer's disease, type 2 diabetes, atherosclerosis, breast cancer, etc. Large litter size, more comparable with human physiology	Costly to maintain, require vascular access port because of deep veins, and shortage of reagents
Minipig	Large, inbred, similar to humans in a number of ways, and appropriate for cutaneous work	Lack of reagents, costly to maintain, and require vascular access port because of deep veins
NHP – Rhesus	Large, comparable to humans, with considerable DNA similarity, a well-defined model, and intricate pedigrees	Pricey, scarce supply, and involves ethical concerns
NHP – Baboon	Similar to rhesus, appropriate for research on alcohol metabolism, lipoproteins, and osteoporosis	Similar to rhesus, challenging to obtain, and scarcely available

(Modified from Mukherjee *et al.*, 2022)

V. FISH MODEL IN DRUG DISCOVERY

The Animals Scientific Procedures Act of 1986 (ASPAs) regulates the procedure for the use of protected animals in research as well as for scientific purposes in United Kingdom

(Herrera, 2023). It provides for the licencing of experimental as well as other scientific procedures performed on any vertebrate animal that may result in suffering, pain, distress, or long-term harm. This law covers all medical procedures on any vertebrate animal, including everything from a quick blood test to extensive surgery. Where as in India animal welfare laws are in accordance with the guidelines of the CPCSEA, [Ministry of Environment & Forests (Animal Welfare Division)].

Furthermore, there is growing demand to restrict the use of animals to absolutely necessary circumstances, such as preclinical toxicity and safety evaluation. Mammalian models of absorption, distribution, metabolism, excretion, and effectiveness are costly, time-consuming, and require significant amounts of valuable compounds. Additionally, one of the objectives outlined by the FDA in ISO 10993 is to reduce the use of test animals, and zebrafish can be used as an alternative organism to rats and other test animals. The three practises defined as the 3Rs—Replace, Reduce, and Refine—are meant to reduce the usage of animals and, when they are used, minimise the suffering they experience (Bhusnure, *et al.*, 2015). Utilising alternate preclinical models, such as *in vitro*, synthetic, or computer-based methods, is necessary to reduce and eventually minimise the use of research on animals. However, even as biotechnology advances, some studies still call for biological organisms. This is particularly valid when it comes to preclinical tests for medicinal substances and toxicological assays. Zebrafish stand out in this setting as an alternate model to bridge the gap between rodent testing and *in vitro* experiments (Sharma and Saneja, 2022). It is a particularly excellent illustration of a cost-efficient alternative model that retains high human homology. Research on embryonic zebrafish offers a great middle ground, giving scientists access to the benefits associated with mammals in a more moral manner that also permits for high-throughput assays.

- 1. Replacement:** The ASPA controls the use of vertebrates in procedures that could result in pain, suffering, distress, or long-term injury. A licence is required to perform regulated procedures on mammals beginning halfway through the gestation period and on fish starting when they can feed themselves independently rather than being dependent on the yolk, which is generally accepted to occur in zebrafish larvae at 5 days post-fertilisation (dpf) (Bhusnure *et al.*, 2015). Zebrafish undergo rapid development to reach an adult-like stage in less than 72 hours as part of their evolutionary strategy in order to escape predation (Dubey *et al.*, 2022). As a result, within 4 days after fertilisation, they are already capable of recognising light and swimming to avoid predation. At early stages, the central nervous system (CNS) is still somewhat undeveloped compared to non-neuronal organs like the heart, which allows for functional tests to be carried out. In fact, sophisticated behaviours like those in reaction to aural and visual stimuli only become apparent after 5 days post-fertilisation, showing that the brain develops more slowly than other organs. As an alternative to using animals at more conscious, licenced phases, zebrafish can be employed at unlicensed phases to generate *in vivo* data for specific organs (Bhusnure *et al.*, 2015). Therefore, by proving that zebra fish larvae are appropriate models for systems through validation studies, zebra fish larvae can be utilised to replace a number of animal toxicity studies.
- 2. Reduction:** Zebra fish larvae can be used as a first-level toxicity model to find potential harmful drugs, enabling safer compounds to be examined in mammalian models and minimising the number of animals needed in testing (Cassar *et al.*, 2019). Zebrafish

larvae are large and transparent, allowing researchers to undertake comparable tests to those conducted on mammals at licenced stages with less invasive techniques (Bauer *et al.*, 2021).

- 3. Refinement:** The drug discovery process involves the use of a large number of animals because compounds that have been found to be active *in vitro* are subsequently investigated in animal models. Because difficulties with absorption, distribution, metabolism, and excretion (ADME) across the entire organism cannot be predicted by cell-based models, *in vitro* results frequently fail to replicate. Zebrafish offer a practical method for bridging the "gap" between *in vitro* and *in vivo* research, lowering the attrition rate and, consequently, the quantity of animals needed in the drug development process (Cornet *et al.*, 2019; Bhusnure *et al.*, 2015). The fact that zebrafish embryos are fertilised externally and are transparent for the first few days of life also makes zebra models of embryos and larvae a benefit over designs that involve animals in study. This makes it possible to observe non-invasive toxicity and have a chance of recuperating.

Fish have been widely used as model organisms for research purposes for more than 200 years, with goldfish (*Carassius auratus*) being among the first species used in toxicity studies. Later, it emerged as a prevalent model in disciplines like growth and development, immunology, behavioural research, and reproduction (Harikumar *et al.*, 2021). The establishment of the medaka (*Oryzias latipes*) as a developmental genetic model organism was spurred by experiments carried out at the beginning of the twentieth century. Similar to zebrafish (*Danio rerio*), medaka are highly useful experimental animals for developmental studies, toxicology, disease modelling, and environmental health sciences due to their complete genome sequencing, adaptation to a wide range of temperatures, high fecundity and transparent embryos. In addition to the zebrafish, the medaka, can be used as a second laboratory fish model. Many cancer bioassays use this kind of tiny fish as their preferred species (Hilgers and Schwarzer, 2019). George Streisinger introduced the zebrafish as a biological model for investigating developmental genetics for the first time in 1960's. It has consistently risen to the top research model position in recent years, with applications in toxicology, physiology, disease modelling, and drug discovery, among many other disciplines. Other fish species, such as rainbow trout and ornamental fish species including *Poecilia* sp., *Rivulus* sp., *Xiphophorus* sp., and *Cyprinodontidae* sp., are currently used as model organisms in experimental pharmacology (Harikumar *et al.*, 2021). One of the most extensively researched fish is the freshwater species known as the rainbow trout (*Oncorhynchus mykiss*). It is one of the fish species that has been extensively investigated in a variety of fields, including physiology, toxicity, disease ecology, comparative immunology, and nutrition (Bunton, 1996). In studies involving the spinal cord, lamprey eels have been used as model organisms. *Clarias gariepinus*, *Channa punctatus*, *Myoxocephalus scorpius*, *Fundulus heteroclitus*, and *O. niloticus* *O. mossambicus*, as well as *S. iredius* and *Salmo trutta* are other fish that have been employed in biomedical research, each having its own merits and demerits (Pandey, 2011). In understanding the skeletal and organ anatomy and colouring of vertebrates, gold fish is a useful model system. Furthermore, molecular components from blood can be easily collected and are helpful for micromanipulation research and developing disease models. Medaka is tougher, less prone to disease, and has clearly defined sex chromosomes than zebrafish (Kondo *et al.*, 2009). Studies on the medaka can also reveal information on additional traits that are helpful for disease

models. Consequently, the medaka has the potential to be a parallel model in genetics and developmental biology. The annual killifish's short life cycle makes it a good model for human ageing (Herrera and Jagadeeswaran, 2004). The most well-known and extensively used vertebrate fish model species in developmental genetics and ecotoxicology is the zebrafish (*Danio rerio*). Zebrafish is a very reliable model for examining human gene activity and medication effects. At present zebrafish is a popular and useful model for studying toxicity, cancer, molecular genetics, developmental biology, and disorders including cancer (Hoo *et al.*, 2016). Fish models, therefore, have the potential to enhance scientific research in the future.

VI. ADVANTAGE OF ZEBRA FISH MODEL OVER OTHER ANIMAL MODELS

Zebrafish are an effective tool for drug discovery due to a number of factors. It is the most studied and recommended species to model humans because its major tissues and organs are analogous to those of humans. This, combined with the orthologous genes of interest and its ability for medium- and high throughput screening, makes zebrafish the most useful model for humans.

First, zebrafish genome sequencing reveals a high degree of conservation, with the human and zebrafish genomes sharing 70% of genes, of which 84% are known to be associated with human diseases, making it a good model for studying the genetics of organogenesis, embryonic development, human physiology, and disease (Katoch and Patial, 2021; Dubey *et al.*, 2022). The ever-expanding libraries of specialised zebrafish lines are simpler and less expensive to maintain than mice as a result of a thorough study of all transcripts and the development of an Expressed Sequence Tag (EST) database.

Second, the high fecundity of zebrafish results in the production of a significant number of embryos. 200–250 eggs can be released by each mature female during mating. Mating occurs throughout the year. Breeding is simple, and more animals may be housed in a compact area for less money (one dollar for a mouse and one cent for a zebrafish) than for mammals (Miyawaki., 2020).

Third, development occurs rapidly, and embryos and larvae can be maintained in 384-well microplates with 50µL of water. By 24-hour post fertilisation (hpf), their complete body was formed, and by 96 hpf, the majority of their internal organs, including the heart, kidneys, liver, and intestines, were fully developed (Cassar *et al.*, 2019). Since their reproductive cycle is measured in days rather than weeks, it is simple to carry out a developmental study in a short amount of time.

Fourth, since the tissues, organs, and cells of larval zebrafish are transparent, they may be observed *in vivo* and studied in real-time. Without dissection, additional morphological monitoring can be carried out at all developmental phases.

Fifth, the zebrafish could potentially be used for a molecular and genetic study by swiftly identifying the temporal and spatial expression of genes, investigating the function of a particular gene through transgenic development, performing antisense gene knockdown, and performing mass mutagenesis (Hsu *et al.*, 2007).

Sixth, because zebrafish embryos are produced via external, live embryos are readily accessible for modification and can be used to screen chemicals in amounts of 50 microliters. When embryos develop the capacity to swallow, substances are put into the water, where they are then absorbed and ingested. Proteins, macromolecules, and substances that are not water-soluble can also be injected directly into the yolk sac, venous sinus, or bloodstream. Additionally, compounds can also be given to adult zebrafish orally or intraperitoneally, and they can take in substances that are soluble in water. Zebrafish trials proved the repeatability of the effects of test substances. In contrast to other animal models, it also lowers husbandry expenses.

Seventh, zebrafish have similar digestive, neurological, and cardiovascular systems to mammals (Hsu *et al.*, 2007).

Eighth, automated fluorescent zebrafish tests will make it possible to screen a lot of chemicals (like 1,000 compounds per day) at medium throughput and high content. The zebra model is an effective method for *in vivo* drug discovery that may be used for high throughput screening to identify leads and optimise novel compounds. It can also be used to quickly and economically conduct pharmacokinetic and toxicological studies on a large number of compounds (Cassar *et al.*, 2019). They can help cut back on the number of sacrificed animals.

Ninth, fish provide versatility in research; if one species proves unsuitable, there is always the option to switch to another species of fish or animal model.

VII. ZEBRAFISH ‘AVATAR MODELS’; AN EMERGING APPROACH IN PRECISION MEDICINE IN ONCOLOGY

It has only recently been discovered, but xenotransplantation is a ground-breaking method for creating tumours in zebra fish that is poised to completely alter cancer research. One of the most important aspects of xenotransplantation is the ability to label or stain malignant cells with a fluorescent dye that readily differentiates implanted cells from normal cells, which facilitates the detection of a positive and substantial tumour's stage of development (Sturtzel *et al.*, 2023). The fact that xenotransplantation is non-invasive is its most crucial feature. For instance, numerous kinds of tumours have been inserted into the bodies of zebrafish to observe how they respond. The most prevalent cancers include pancreatic cancer, lung cancer, ovarian carcinoma, breast cancer, prostate cancer, retinoblastoma, leukaemia, and others. Personalised medicine is a novel and interesting application of zebrafish xenograft models (Xiao *et al.*, 2020). A tumour biopsy could be implanted into several zebrafish eggs in a promising effort to determine a patient's unique chemo sensitivity pattern. Oncologists could use this profile to advise therapy choices and perhaps find alternative chemotherapeutic and targeted drugs. Xenografts of biopsy tissue might make it possible to do comprehensive pharmacological screening to find new, patient-specific, targeted drugs in the worst-case situation when there is no known treatment. Among cancer models for personalised treatment, the zebrafish xenograft assay is unrivalled. New, cutting-edge imaging techniques are revealing cancer cellular activities with previously unprecedented detail in the small, translucent fry, which are ideal for imaging. The zebrafish xenograft model has special attributes that enable patient-specific chemo sensitivity assessments, including imaging capabilities, assay speed (5-7 days), and minimal patient tissue requirements (100-200 cells per animal). The future of zebrafish xenografts aka

zebrafish avatars involves in developments in the identification of novel therapeutic targets, drug discovery, and personalised therapy for oncology studies (Costa *et al.*, 2020).

"Zebrafish avatars" are made by carefully injecting tissue or cells collected from a patient's cancer biopsy onto zebrafish embryos. These tumour-bearing zebrafish embryos are arranged in 96-well plates, where they are imaged and then given a variety of medications chosen as potential chemotherapeutics following molecular profiling, input from the tumour board, and the treating oncologist's opinion. Over the course of three to five days, tumour cell activities such as proliferation, angiogenesis, migration, and metastases are assessed. A chemo sensitivity analysis for each patient's cancer can be created based on how the tested medications or drug combinations affect the chosen behaviour. To guarantee comparable sensitivity to the drug(s), the drug sensitivity assay must first be validated in mouse orthotopic models. In the future, the oncologist will be equipped with information that might be used to inform treatment decisions in less than 7 day studies (Costa *et al.*, 2020).

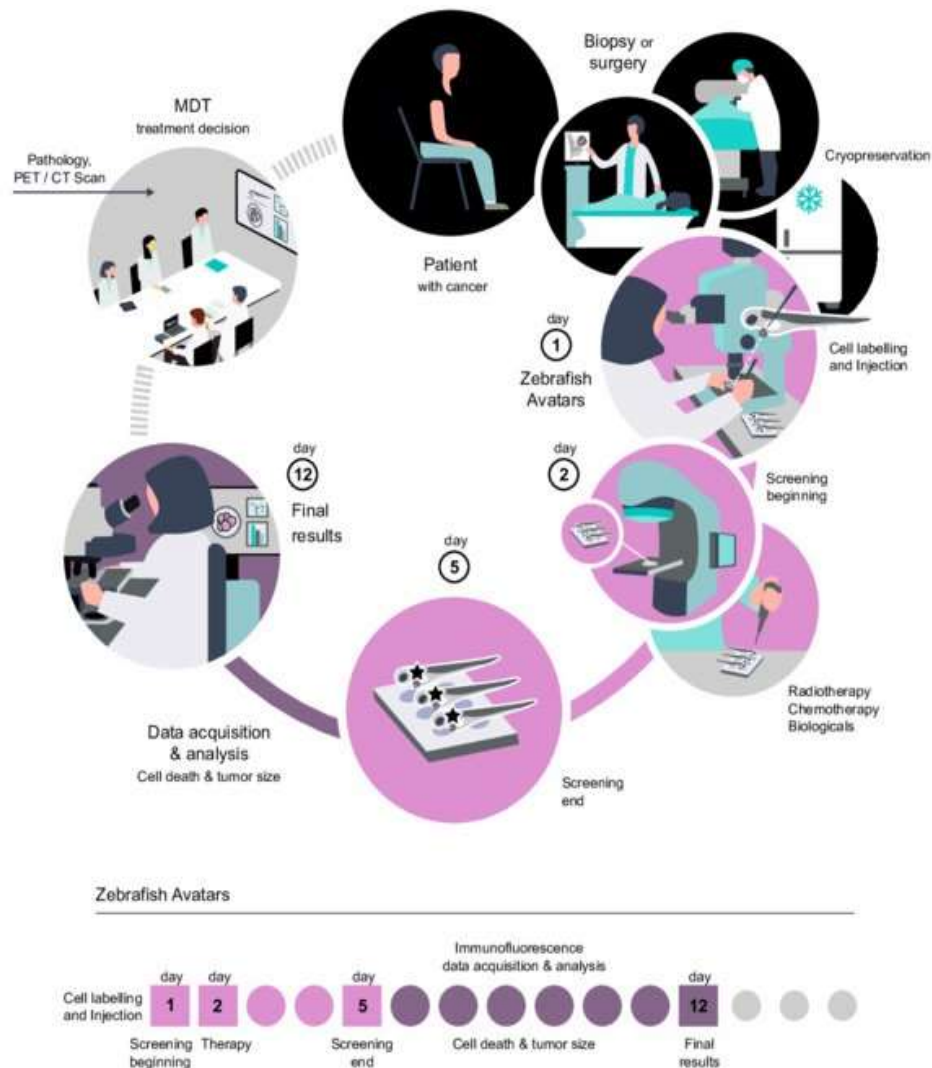


Figure 2: Flow Chart of Zebrafish Avatar Model

Tissue or cells from a tumour biopsy from a patient is collected and precisely injected on to the zebrafish larvae creating “zebrafish avatars”. These tumour-bearing zebrafish embryos are arrayed in 96-well plates where they are imaged and then treated with a battery of drugs selected as potential chemotherapeutics based on molecular profiling, tumour board input, and the judgment of the treating oncologist. Tumour cell behaviours including proliferation, angiogenesis, migration, and metastases are evaluated over a 3–5 days. Based on the effects of the tested drugs, or drug combinations, on the selected behaviours, a chemo sensitivity profile for the tumour of each patient can be developed. The drug sensitivity assay must first be validated in mouse orthotopic models to ensure similar sensitivity to the drug(s). In the future, in less than 7 days, the oncologist will be armed with potentially actionable information to guide treatment decisions.

VIII. LIMITATIONS OF FISH MODEL IN DRUG DISCOVERY STUDIES

In spite of the fact that we can use fish models to get around obstacles in the drug discovery process, each fish model has its own limitations, and fish and humans still differ fundamentally, anatomically and physiologically in that fish lack lungs, prostate glands, and mammary glands. For human diseases caused by genes not found in fish or those affecting a specific tissue or body component not found in fish, a different animal model will be required. Despite the fact that people and fish share a lot of the same genetic makeup, there is a limit. Several zebrafish tissues and organs, including the retina, spinal cord, kidneys, heart, and liver, may regenerate swiftly in contrast to mammalian tissues and organs, which should be taken into consideration while addressing the model. It is challenging to conduct and analyse multi-endpoint assays or pharmacodynamics/pharmacokinetics (PD/PK) from a single animal because there is a restricted quantity of biological samples obtainable, such as blood and tissues, among others. In terms of drug pharmacodynamics and pharmacokinetics, inter-species variations may also be numerous and unpredictable. Fish as a study model may not be as well known to the regulators, who work in the laboratory animal care field. Technical expertise is needed for experimental procedures (administration and blood or tissue samples in adult zebrafish) due to its tiny size (Miyawaki, 2020). The well-being of laboratory fish can also be a problem since, in particular, the intensive use of zebrafish in laboratory research poses ethical questions. If they are housed in the laboratory at the appropriate acclimatisation temperature, acclimatisation is achievable. Humans have a fundamentally different metabolism than fish because we are endotherms, whereas fish are ectotherms. Furthermore, since these fish have temperature-dependent pharmacokinetics and pharmacological activities, the impact of water temperature needs to be carefully considered in this circumstance. Studies can be confounded by the skewed sex ratio in zebrafish cohorts because fish frequently have variable, reversible sex determination, which can be challenging to determine by genetic factors. Ovo-viviparous fish models lack a placenta, which causes direct chemical or drug contact with pertinent organs as opposed to indirect interaction through the placental connection in humans. Numerous more variants of many human genes, such as two copies in zebrafish, may exist in fish. This considerably increases the difficulty of producing knockout strains.

IX. CONCLUSION

Other than the human being, there is currently no other perfect model in existence, and each model animal has its own advantages and disadvantages. In order to address the

biological problems in a particular study, alternative model organisms are therefore developed. Prior to using mouse models in the pre-clinical stage of drug development, zebrafish models can be a very useful screening tool. Zebrafish are being used in place of some mammalian models (such as rodents, dogs, and pigs) at various phases of the drug development process since they are beneficial and less expensive. Both genetic and small-molecule screening are applicable to zebrafish. In every single situation looked at, it is not the ideal model system for people, but the genetic pathways between zebrafish and mammals have been conserved. The functions of the genes within those pathways have not altered, making it an excellent model system if one only takes a look at the specific functions of genes. Examples of this abound, and zebrafish provide a potent experimental and genetic system for the comprehension of vertebrate biology and disease so long as one is ready to "accept the disparities and embrace the parallels" between humans and other animals. Phenotypic and target-based drug discovery techniques work in tandem to find first-in-class molecules while also producing best-in-class follower molecules that are safer, more effective, and more powerful. Zebrafish transgene technology will help pharmaceutical stakeholders in this situation by improving the precision and effectiveness of their nuclear receptor drug screening programmes, decreasing the time and expense of discovery work, and raising the hit-to-lead success rate, which involves advancing the best candidate drug from screening to preclinical research. The final outcome of these will be the faster commercialization of safer and more efficient medications.

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