World Journal of Biology Pharmacy and Health Sciences
eISSN: 2582-5542
Cross Ref DOI: 10.30574/wjbphs
Journal homepage: https://wjbphs.com/

(RESEARCH ARTICLE)

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Zebrafish (*Danio rerio*) in cancer research: A revolutionary model for drug testing, discovery, and precision medicine

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World Journal of Biology Pharmacy and Health Sciences, 2025, 24(02), 329-342

Publication history: Received on 01 October 2025; revised on 08 November 2025; accepted on 10 November 2025

Article DOI: https://doi.org/10.30574/wjbphs.2025.24.2.0972

Abstract

As a useful model in cancer research, zebrafish (Danio rerio) have made substantial contributions to precision medicine, drug testing, and discovery. They are the perfect system for in vivo research of cancer progression and therapy responses because of their fast growth, genetic resemblance to humans, and transparent embryos. This article examines the use of zebrafish in pharmacogenomics, personalised medicine development, and high-throughput cancer medication screening. We also go over the benefits and drawbacks of using zebrafish models in oncological research as well as its possible applications in precision medicine in the future.

Keywords: Zebrafish Model; Cancer Research; Drug Discovery; Precision Medicine; Pharmacogenomics

1. Introduction

Since cancer is still one of the world's top causes of death, new methods to personalised medicine and drug development are required. Zebrafish have become well-known because of their conserved genes linked to cancer, capacity to grow tumours that resemble human cancers, and appropriateness for high-throughput drug screening. Their use in cancer has been further enhanced by the creation of xenografts and transgenic zebrafish models [1].

Zebrafish-based drug discovery has made it possible to quickly and affordably identify new cancer treatments by using their transparent embryos to track the development of tumours in real time [2]. Additionally, zebrafish patient-derived xenografts (PDX) are a promising personalised medicine tool that enhances therapeutic decision-making and enables real-time medication response monitoring [3].

Recent studies have shown that zebrafish may be used to mimic some malignancies, including ovarian and breast cancer, and that zebrafish avatars can be used as a translational platform to evaluate targeted treatments [4]. Furthermore, zebrafish models have been essential to the study of lung cancer, helping to improve therapeutic treatments and drug screening [5].

Because of these benefits, zebrafish are becoming more and more popular as a crucial tool in precision oncology, helping to improve our knowledge of tumour biology and build patient-specific treatment plans [6]. The most recent advancements in precision medicine, drug discovery, and zebrafish-based cancer medication testing will be examined in this review, along with their implications for oncological research in the future. Zebrafish have been widely

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recognized as an essential model for studying human diseases due to their genetic similarity to humans and suitability for high-throughput screening. [7]

1.1. Zebrafish in Cancer Drug Testing

Because of their fast growth, genetic resemblance to humans, and aptitude for high-throughput screening, zebrafish models have become a useful platform for evaluating cancer drugs. These qualities have made it possible for researchers to carry out comprehensive drug testing and therapeutic compound assessment in a timely and economical way [8].

2. Why Zebrafish are Effective in Cancer Drug Testing

Because of their physiological and genetic resemblance to humans and their capacity for extensive, economical research, zebrafish (Danio rerio) have grown in importance in the testing of cancer drugs [89]. Zebrafish are a great model for researching the pathophysiology of cancer since their genomes are almost 70% similar to those of humans, and they contain orthologs for over 84% of the genes linked to human diseases [10]. For cancer medication screening, their transparency in the early phases of development enables real-time visualisation of tumour growth, angiogenesis, and metastasis [5].

2.1. High-throughput Screening

To find anti-cancer drugs, chemical libraries may be quickly screened using zebrafish embryos. Their optical transparency and tiny size allow for real-time tracking of tumour growth and treatment response. Zebrafish were investigated as drug discovery tools by Macrae and Peterson (2015), who focused on their use in phenotype-based screening [8]. The capacity of zebrafish models to simulate genetic and epigenetic changes in cancer was emphasised by Hason and Bartůněk (2019) [9]. While Kirchberger et al. (2017) [11] showed how zebrafish supplement traditional mouse models, Astone et al. (2017) [6] investigated zebrafish xenografts to evaluate precision cancer medications. Zebrafish xenotransplantation was employed by Bentley et al. (2015) [12] to assess leukaemia treatments. Zebrafish-based quantitative and systems pharmacology was created by Tanaka et al. (2018) [1] for high-throughput in vivo screening. [1]

- Dockins et al. (2019) developed standardised procedures for drug testing for leukaemia and breast cancer by optimising human cancer cell xenografts in zebrafish larvae for high-throughput drug screening. [13]
- To find anti-metastatic medications, Nakayama and Makinoshima (2020) examined screening models based on zebrafish [14].
- Gallardo et al. (2015) found new chemicals influencing zebrafish cell migration using phenotype-driven chemical screening. [15]
- Schmitz et al. (2019) investigated high-throughput drug response screening using zebrafish xenograft breast cancer models. [16]
- Mauro et al. (2019) used zebrafish to create automated procedures for the identification of angiogenesis inhibitors. [17]

Table 1 High-throughput Screening in Zebrafish

Year	Key Findings	Reference
2015	Explored zebrafish as tools for drug discovery, emphasizing their role in phenotype-based screening.	[8]
2015	Used zebrafish xenotransplantation to evaluate leukemia therapies.	[12]
2015	Used phenotype-driven chemical screening to discover novel compounds affecting cell migration in zebrafish.	[15]
2017	Studied zebrafish xenografts to test precision oncology drugs.	[6]
2017	Demonstrated how zebrafish complement classic mouse models in cancer drug screening.	[11]
2018	Developed zebrafish-based quantitative and systems pharmacology for high-throughput in vivo screening.	[1]
2019	Optimized human cancer cell xenografts in zebrafish larvae for high-throughput drug screening, establishing standardized protocols for leukemia and breast cancer drug testing.	[13]

2019	Developed automated protocols for angiogenesis inhibitor discovery using zebrafish.	[17]
2019	Explored zebrafish xenograft breast cancer models for high-throughput drug response screening.	[16]
2019	Highlighted zebrafish models' ability to model genetic and epigenetic alterations in cancer.	[9]
2020	Reviewed zebrafish-based screening models for identifying anti-metastatic drugs.	[14]
2023	Studied zebrafish for lung cancer research.	[5]

2.2. In Vivo Drug Efficacy Testing

To evaluate the efficacy of new treatment medicines, zebrafish tumour models offer a physiologically appropriate setting. In vivo zebrafish models take into consideration tumor-microenvironment interactions, which are important in the development of cancer, in contrast to in vitro tests [18]. Zebrafish have shown promise in screening and verifying anti-cancer medications in a number of studies

Table 2 Key Developments in Zebrafish-Based Oncology Models

Year	Key Findings	Reference
2015	Reviewed the translational potential of zebrafish in oncology, demonstrating their role in screening and validating anti-cancer drugs.	[18]
2018	Utilized zebrafish models for pancreatic cancer drug testing.	[1]
2019	Introduced a PCR-based zebrafish model to rapidly test drug efficacy in head and neck cancer.	[19]
2019	Reviewed zebrafish as a biomedical model, highlighting its role in metabolic disorders and oncology.	[20]
2020	Discussed zebrafish avatars as a key tool in precision medicine, enabling patient-specific treatment testing.	[3]
2021	Demonstrated the efficacy of zebrafish models in breast cancer therapy development.	[2]
2023	Studied zebrafish for lung cancer research, highlighting their utility in drug testing and target validation.	[5]
2023	Analyzed zebrafish avatars as a translational platform for predicting patient outcomes in oncology.	[21]
2024	Evaluated zebrafish tumor xenograft models for cancer drug development and high-throughput screening of long non-coding RNAs (lncRNAs).	[22]
2024	Developed an in vivo drug screening protocol in zebrafish to identify anti-metastatic drugs.	[23]

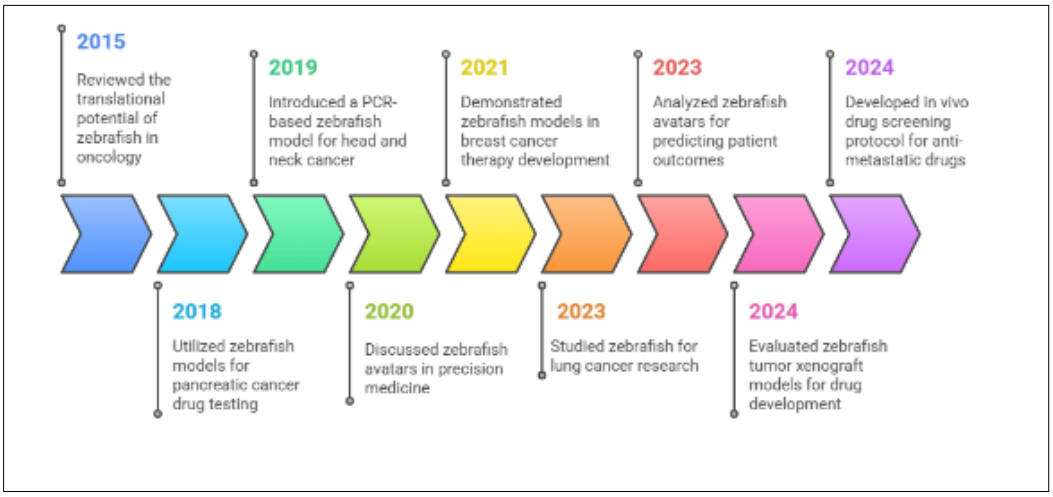

Figure 1 Advancements in Zebrafish Models for Cancer Research

2.3. Toxicity Profiling

Zebrafish lessen the need for mammalian models by facilitating the study of medication toxicity at an early stage. Before moving on to mammalian models, the capacity to detect and measure developmental defects, organ toxicity, and systemic side effects in zebrafish eggs offers a reliable platform for evaluating medication safety. [8] Zebrafish offer a predictive model for evaluating drug-induced toxicity, as shown by McGrath and Li (2008) [24], who also pointed out that the toxicity profiles of zebrafish and mammals are comparable. Their research confirmed that zebrafish are a trustworthy substitute for conventional mouse models in preclinical medication development. This was further supported by He et al. (2014) [25], who demonstrated that zebrafish are very predictive of mammalian reactions to hazardous chemicals, especially in evaluations of developmental and reproductive toxicity.

ZeGlobalTox is a novel screening method created by Cornet et al. (2017) [26] that enables scientists to evaluate drug-induced organ toxicity in zebrafish. Zebrafish are a complete tool for multi-organ toxicity testing since their study included important toxicity endpoints such as cardiotoxicity, neurotoxicity, and hepatotoxicity. In their evaluation of zebrafish as a high-throughput nanotoxicity screening model, Jia et al. (2019) [27] showed how different nanomaterials affect biological systems at varying doses. Zebrafish were highlighted in this study as a useful model for assessing the security of new nanotechnologies.

Applications of zebrafish in preclinical toxicity testing, such as evaluating hepatotoxicity, nephrotoxicity, cardiotoxicity, and teratogenicity, were compiled by Miyawaki (2020) [28]. In a similar vein, Cassar et al. (2020) [29] emphasised zebrafish as a link between in vitro tests and investigations on mammals, showing enhanced prediction for toxicities caused by drugs. According to their findings, zebrafish are a model that can improve translational research while lowering the moral dilemmas associated with testing on mammals.

Zebrafish-based toxicity tests for paediatric cancer medications were examined in recent research by Azzam et al. (2024) [30], which assessed their contribution to guaranteeing drug safety for younger patients. By creating a zebrafish-based high-throughput screening technique for discovering anti-metastatic medications while reducing systemic toxicity, Nakayama et al. (2024) [23] made significant progress in the field.

Together, these results demonstrate the benefits of using zebrafish for medication toxicity testing. They are a vital tool in preclinical drug development and safety pharmacology because of their capacity to anticipate adverse drug responses, offer early-stage toxicity evaluations, and supplement mammalian models.

Table 3 Summary of Key Studies in Zebrafish-Based Toxicity Profiling

Year	Key Findings	Reference
2008	Demonstrated zebrafish as a predictive model for assessing drug-induced toxicity, highlighting similarities between mammalian and zebrafish toxicity profiles.	[24]
2014	Showed zebrafish as a highly predictive model for mammalian responses to toxic substances in developmental and reproductive toxicity assessments.	[25]
2017	Developed ZeGlobalTox, an innovative approach to assessing drug-induced organ toxicity in zebrafish, covering cardio-, neuro-, and hepatotoxicity.	[26]
2019	Reviewed zebrafish as a high-throughput nanotoxicity screening model, evaluating the in vivo toxicological profiles of various nanomaterials.	[27]
2020	Summarized applications of zebrafish in preclinical toxicity testing, including hepatotoxicity, nephrotoxicity, cardiotoxicity, and teratogenicity assessments.	[28]
2020	Highlighted zebrafish as a bridge between in vitro assays and mammalian studies, improving the predictability of drug-induced toxicities.	[29]
2024	Investigated zebrafish-based toxicology assays for pediatric cancer drugs, evaluating their role in drug safety assessments.	[30]
2024	Developed a zebrafish-based high-throughput screening method for identifying anti-metastatic drugs while minimizing systemic toxicity.	[23]

2.4. Personalized Drug Testing

Zebrafish patient-derived xenografts (PDX) provide a potential personalised treatment strategy. Researchers may quickly assess the effectiveness of particular chemotherapy medicines by implanting tumour cells from individual patients into zebrafish eggs. This allows them to customise treatment plans for improved clinical results (Fieuws et al., 2024). [4]
Zebrafish avatars have been shown to be useful in functional precision medicine by Fieuws et al. (2024) [4], allowing for real-time evaluation of tumour response to treatments. Zebrafish models were employed by Fazio et al. (2020) [3] to forecast medication reactions for individual patients, opening the door to customised treatment plans. [3]Zebrafish were used for lung cancer screening by Wu et al. (2023) [5], demonstrating their function in evaluating medication responses in various lung cancer subtypes. [5]

Macrae and Peterson (2015) evaluated the effectiveness of zebrafish in pharmacogenomics, highlighting their capacity to detect medication reactions unique to each patient. [8]. In their investigation of their function in pancreatic cancer therapy, Tanaka et al. (2018) [1] showed that zebrafish PDX models might be employed for quick drug screening and treatment assessment [1]. Zebrafish applications in leukaemia therapy were examined by Astone et al. (2017) [6], who demonstrated how leukaemia xenografts in zebrafish enable customised drug testing [(Astone et al., 2017)]. [6]

Zebrafish PDX models were created by Wang et al. (2022) [31] for the treatment of non-small cell lung cancer, emphasising their application in the screening of immunotherapies and targeted treatments [31].The use of zebrafish patient-derived avatars for intestinal cancer screening was reported by Costa et al. (2022) [32], who also showed how effective these avatars are at evaluating various treatment approaches. [32] Zebrafish leukaemia xenografts were effectively employed by Somasagara et al. (2021) [33] to assess new leukaemia therapy.

Together, these results demonstrate the increasing value of zebrafish as a model for customised drug testing, offering an affordable, scalable, and patient-specific platform for assessing cancer treatments.

Table 4 Summary of Key Studies in Zebrafish-Based Personalized Drug Testing

Year	Study	Key Findings	Reference
2015	Macrae and Peterson	Examined zebrafish efficiency in pharmacogenomics and personalized drug screening.	[8]
2017	Astone et al.	Studied zebrafish applications in leukemia treatment, demonstrating personalized drug testing.	[6]
2018	Tanaka et al.	Explored zebrafish PDX models in pancreatic cancer therapy for rapid treatment evaluation.	[1]
2020	Fazio et al.	Used zebrafish models to predict patient-specific drug responses.	[3]
2021	Somasagara et al.	Applied zebrafish leukemia xenografts to evaluate novel leukemia therapies.	[33]
2022	Wang et al.	Developed zebrafish PDX models for non-small cell lung cancer treatment.	[31]
2022	Costa et al.	Utilized zebrafish patient-derived avatars for digestive cancer therapy screening.	[32]
2023	Wu et al.	Applied zebrafish PDX models for lung cancer screening and drug testing.	[5]
2024	Fieuws et al.	Demonstrated zebrafish avatars in functional precision medicine for rapid drug assessment.	[4]

2.5. Advancements in Zebrafish Research

2.5.1. CRISPR Genome Editing

By enabling precise gene alterations to generate disease models and investigate carcinogenesis, CRISPR/Cas9 technology has completely transformed the study of zebrafish cancer. [34] Researchers have been able to explore new treatment targets in zebrafish, create mutations specific to cancer, and eliminate tumour suppressor genes thanks to this genome-editing technology.

In order to increase accuracy and enable targeted gene alterations in zebrafish cancer models, Liu et al. (2019) extended the CRISPR toolset [35]. Cas12a-mediated gene editing in zebrafish dramatically decreased off-target effects, increasing the precision of cancer model development, as shown by Ouyang et al. (2020).[36]

The translational uses of CRISPR/Cas9 for zebrafish gene editing were reviewed by Sharma et al. (2021), indicating its promise for both basic research and therapeutic discoveries [37]. In order to replicate different human diseases, Chaudhary et al. (2020) investigated the use of CRISPR in creating transgenic zebrafish lines [38].

In ground-breaking research, Ablain et al. (2018) highlighted the utility of zebrafish in functional genomics by identifying PVRL1 as a metastasis suppressor gene in melanoma using a tissue-specific CRISPR strategy [39].

These results highlight how CRISPR/Cas9 technology has revolutionised zebrafish cancer research and is an essential tool for medication development and precision oncology.

2.6. AI-Driven Drug Screening

Because AI speeds up data processing and predictive modelling for cancer treatments, it has greatly improved zebrafish-based drug development. Nguyen et al. (2021) demonstrated the effectiveness of AI in discovering possible therapeutic compounds by streamlining high-throughput drug screening for breast cancer using AI-powered optical imaging methods in zebrafish xenografts. [40]

Grandhi et al. (2021) used AI-driven 3D tumour enrichment tests in zebrafish, which enabled high-throughput real-time monitoring of treatment resistance mutations [41]. Haney et al. (2020) improved the standardisation of engrafted tumour area estimation by automating zebrafish drug screening through the use of AI-assisted fluorescence imaging. [42]

The development of tailored therapies is advanced by AI-based chemical screening techniques that use zebrafish models to quickly identify tiny compounds that inhibit cancer, as noted by Xie et al. (2015) [43].AI-driven phenotyping of zebrafish embryos was proven by Terriente and Pujades (2013), which made it easier to identify anti-cancer drugs through high-throughput screening [44].

An AI-assisted pipeline was shown by Dockins et al. (2019) to standardise zebrafish xenograft procedures for drug testing, improving tumour cell injection and response assessment [13]. Bozhko et al. (2021) showed the potential of AI in pharmacological screens outside of cancer by using AI neural network algorithms to categorise zebrafish behavioural responses to psychoactive substances [45].

2.6.1. 3D Imaging and Optical Screening

Real-time visualisation of tumour growth and medication responses is now possible thanks to recent advancements in 3D imaging technology, revolutionising zebrafish-based cancer research. For the study of zebrafish models, optical projection tomography (OPT) has become a potent 3D imaging method. High-resolution reconstruction of both live and preserved zebrafish specimens is possible with zOPT, an open-source OPT platform designed for zebrafish imaging and created by Zhang et al. (2020) [46].

Nguyen et al. (2021) investigated optical imaging methods in zebrafish xenografts to provide high-throughput drug screening for breast cancer, proving that autofluorescence lifetime imaging could predict treatment response. [40]

To allow single-cell resolution imaging of cancer cell heterogeneity, tumour development, and microenvironment interactions, Tang et al. (2016) used optically transparent, immune-compromised zebrafish [47].Yan et al. (2020) [48] demonstrated the preclinical effectiveness of several chemotherapy combinations by introducing high-resolution dynamic single-cell imaging of human cancer development and treatment responses after engraftment into immunodeficient zebrafish. [47]

Jones matrix optical coherence tomography (JM-OCT) was created by Lichtenegger et al. (2022) to non-destructively assess tumour anomalies in zebrafish models, emphasising its use in preclinical research [49]. In order to provide quantitative optical tomography for cancer progression and vascular development in vivo, Kumar et al. (2016) improved 3D fluorescent imaging in zebrafish [50].

The efficiency, accuracy, and scalability of zebrafish-based cancer research have been greatly enhanced by these developments in CRISPR genome editing, AI-driven drug screening, and 3D imaging, making them a crucial tool in oncology drug discovery.

Table 5 Summary of Key Technological Advancements in Zebrafish Cancer Research

Technology	Key Findings	Reference
CRISPR Genome Editing	Enhanced precision in gene modifications for cancer modeling in zebrafish.	[34]
CRISPR Expansion	Improved targeted gene modifications for disease modeling.	[35]
AI-Driven Drug Screening	AI-powered optical imaging enhances high-throughput screening.	[40]
3D Tumor Enrichment Assays	AI-driven 3D tumor screening identifies drug resistance mutations.	[41]
Live-Cell Imaging	Used zebrafish for melanoma and squamous cell carcinoma studies.	[51]
High-Throughput Imaging	Applied in cardiovascular and cancer research.	[52]
Optical Projection Tomography (OPT)	zOPT platform enables high-resolution reconstruction of zebrafish specimens.	[46]
Autofluorescence Lifetime Imaging	Predicts drug response in zebrafish xenografts for breast cancer screening.	[40]
Immune-Compromised Zebrafish Imaging	Enables single-cell resolution imaging of tumor heterogeneity and evolution.	[47]
Dynamic Single-Cell Imaging	Captures real-time growth and therapy response in zebrafish cancer models.	[48]
Jones Matrix Optical Coherence Tomography (JM-OCT)	Non-destructive tumor characterization in zebrafish models.	[49]
3D Fluorescence Imaging	Enables quantitative optical tomography of cancer progression.	[50]

2.7. Advantages Over Traditional Models

2.7.1. Cost-Effectiveness

An affordable substitute for mammalian models like mice and rats is zebrafish. Because of their compact size, researchers can shelter vast populations in a little amount of area, which drastically lowers facility expenses. Large-scale investigations are also more cost-effective since zebrafish may be kept in high-density aquatic habitats and require less feed.[53]

2.7.2. Rapid Reproduction and Development

In contrast to mice, which take around 20 days to mature, zebrafish embryos grow externally and finish organogenesis 24 to 48 hours after fertilisation. This quick development speeds up the drug discovery process and allows for quicker experimentation.[54]

2.7.3. Genetic Similarity to Humans

Nearly 84% of the genes linked to human diseases have a zebrafish equivalent, and humans and zebrafish share about 70% of their genes [55]. They are a great model for researching cancer and evaluating targeted treatments because of their genetic similarities.

2.7.4. Transparency for Imaging

Real-time, non-invasive visualisation of tumour growth, medication responses, and metastasis is made possible by the optical transparency of zebrafish embryos and larvae. High-resolution imaging methods, which are challenging to accomplish in murine models, can be utilised to follow the growth of tumours using fluorescent markers [56].

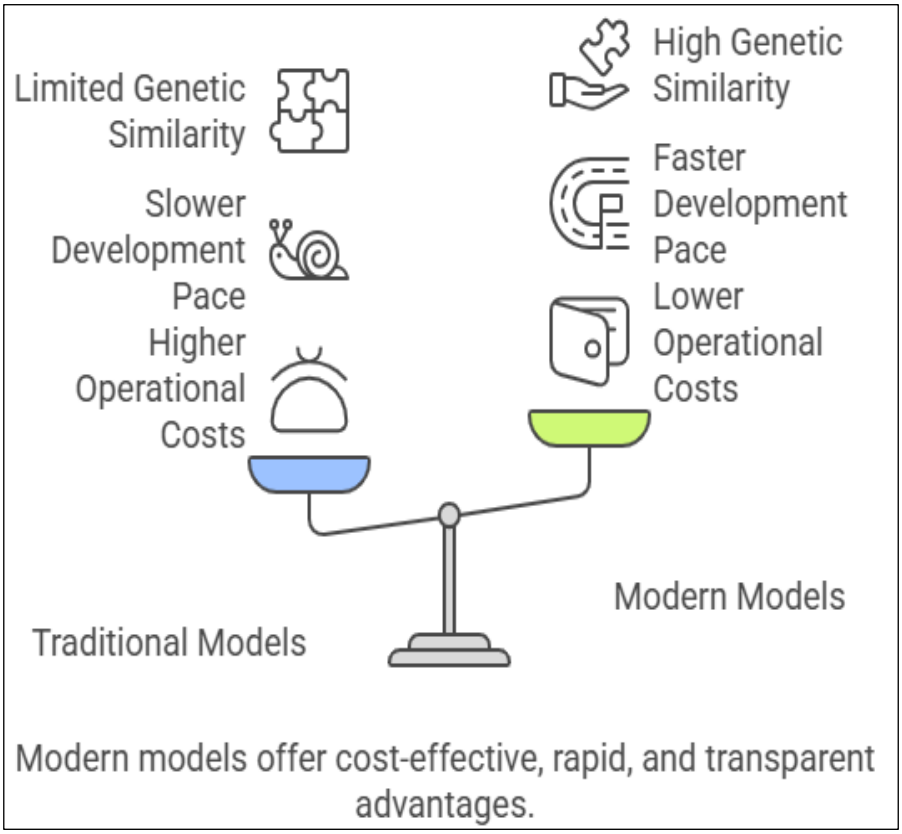

Figure 2 Comparison between Traditional Models vs Modern Models.Modern Models offer cost-effective, rapid and transparent advantages

Future Directions

- *AI and Machine Learning in Drug Screening*

Oncology research is on the brink of a significant revolution, thanks to the integration of artificial intelligence (AI) and machine learning (ML) in zebrafish-based medication screening. By harnessing the power of AI, researchers can utilize advanced picture analysis techniques to swiftly determine how a tumor responds to various medications, eliminating the need for time-consuming human review processes. Moreover, machine learning algorithms have been meticulously trained to analyze and interpret hundreds of zebrafish tumor images, enhancing the efficiency and accuracy of the screening process [40]. This innovative approach holds immense promise for enhancing the effectiveness of oncology research and ultimately improving patient outcomes.

- *Personalized Medicine Applications*

By introducing patient-derived tumour cells into zebrafish embryos, zebrafish xenografts are becoming more potent instruments for personalised treatment. Oncologists may utilise these models to quickly evaluate a variety of medication combinations, assisting them in customising patient care. Zebrafish xenografts have been demonstrated in recent research to reliably predict clinical outcomes in patients with breast and colorectal cancer.[57]

- *CRISPR-Based Cancer Models*

Zebrafish models are increasingly being used to create precise cancer mutations using the CRISPR-Cas9 gene-editing technique. By studying tumour development and progression in real time, these gene-edited animals enable researchers to develop more precisely targeted treatments. It is anticipated that sophisticated CRISPR methods like base editing and prime editing would increase the accuracy of zebrafish cancer models.[58]

- *Integration with Humanized Models*

In order to create "humanised" zebrafish models, human immune cells, tumour microenvironments, and vasculature are being included. These developments will close the gap between preclinical testing and human trials and improve the therapeutic relevance of zebrafish cancer investigations.[48]

3. Comparison of Zebrafish, Mice, and Organoid Models in Cancer Research

Model species and systems that closely mimic human tumour biology, treatment response, and disease progression are used in cancer research. A number of variables, including genetic similarities, tumour microenvironment, drug screening effectiveness, and ethical issues, influence the choice of model, whether it be zebrafish, mice, or organoids. Zebrafish and organoids are becoming strong substitutes with distinct benefits, while mouse models have long been the gold standard.

3.1. Zebrafish (Danio rerio) as a Cancer Model

Because of their approximately 70% genetic closeness to humans and their capacity to form tumours that mimic human cancers, zebrafish have gained popularity as oncology models [9]. Real-time monitoring of tumour development, angiogenesis, and metastasis is made possible by their optically transparent embryos [5]. Because thousands of embryos may be treated at once, zebrafish are also perfect for high-throughput drug screening, which drastically cuts down on time and expense [8].

However, the translational potential of zebrafish models may be limited due to their absence of some essential human physiological characteristics, such as lungs and a fully functional adaptive immune system [10]. In spite of this, zebrafish patient-derived xenografts (PDX) are transforming personalised medicine by allowing quick testing of chemotherapeutic medications on tumours specific to each patient [4].

3.2. Mice Models in Cancer Research

Because of their well-established tumour engraftment techniques, fully developed immune systems, and great genetic resemblance to humans (~85%), mice have been the gold standard in cancer research [59]. In order to comprehend the course of cancer, scientists can investigate certain oncogenes and tumour suppressors using genetically modified mouse models (GEMMs) [46]. Prior to clinical trials, mouse xenograft models (PDX and CDX) provide an in vivo platform for evaluating new cancer medications [60].

- Mice models have drawbacks despite their benefits:
- Expensive and lengthy experimental schedules
- Disparities in human physiology and metabolism that might result in variations in medication metabolism [61].

They are less effective than zebrafish for early-stage drug screening because to their limited high-throughput applications.[11]

3.3. Organoid Models in Cancer Research

Organoids are three-dimensional (3D) cell cultures made from patient tumours that retain the original cancer's genetic and epigenetic features [62]. These models are perfect for drug response testing and personalised treatment because they closely resemble the biology of human tumours [63]. They have been effectively used to lung, breast, and colorectal malignancies, allowing for patient-specific medication screening [31]

However, the absence of essential elements of the tumour microenvironment, including stromal connections, immune cells, and blood arteries, limits the capacity of organoids to simulate immune response and metastasis [64]. Drug penetration issues and culture variability continue to be obstacles in the development of co-culture systems with immune cells [65].

Table 6 Comparison Table: Zebrafish vs. Mice vs. Organoids in Cancer Research

Feature	Zebrafish (Danio rerio)	Mouse Models	Organoid Models	References
Genetic Similarity to Humans	~70% genome homology with humans, 84% of disease-related genes conserved.	~85% genome similarity to humans.	Derived directly from patient cells, retaining human genomic architecture.	[9]; [59]; [62]
Tumor Microenvironment	Supports tumor-microenvironment interactions but lacks adaptive immunity.	Closely mimics human tumor-stroma interactions, including immune system.	Lacks native tumor microenvironment unless co-cultured with stromal/immune cells.	[5][46]; [66]
Drug Screening and High-Throughput Testing	High-throughput, cost-effective screening.	Costly and time-intensive; low-throughput testing.	HTS possible but requires optimized culture conditions.	[8] [11];[65]
Time and Cost Efficiency	Short life cycle; rapid tumor development (weeks), cost-effective.	Long development time (months-years), expensive.	Moderate cost; quicker than mice but longer than zebrafish.	[10]; [61]; [63]
Ethical Considerations	Fewer ethical concerns compared to vertebrate mammals.	Subject to strict ethical guidelines (IACUC, 3Rs principle).	No animal use; considered ethically superior.	[9] [61]; [62]

4. Conclusion

Zebrafish, which provide a special blend of genetic resemblance to humans, quick development, and affordability, have emerged as a ground-breaking model in cancer research. They are an effective tool for researching medication responses and tumour growth because of their transparency and genetic manipulation potential. Zebrafish are set to become increasingly more important in preclinical cancer research as technology develops, especially in the areas of AI-driven screening, CRISPR genome editing, and personalised treatment. To improve their translational relevance, however, issues including immune system responses and drug metabolism that differ from those of mammals must be resolved. This gap will be filled by combining zebrafish with conventional mammalian models and humanised systems, guaranteeing their continued importance in precision medicine and contemporary cancer treatment development.

Compliance with ethical standards

Acknowledgements

The author is thankful for the guidance and support of Dr. Karthickeyan Krishnan, Professor and Head, Ph.D. Guide, Department of Pharmacy Practice, School of Pharmaceutical Sciences, Vels Institute of Science, Technology and Advanced Studies (VISTAS). Also, Principal Dr. Anagha Joshi & Vice-Principal Dr.D.M. Kannur for providing all necessary Facilities at SCES's Indira College of Pharmacy, Pune.

Disclosure of conflict of interest

The authors declare that there is no conflict of interest in this paper.

References

[1] Tanaka, T., Sasagawa, S., Kitahara, E., Okuno, M., Koiwa, J., Takahashi, Y., Sugimura, A., Mikami, I., Isaji, S., and Kuriyama, N. (2018). Zebrafish-Based Drug Discovery and Precision Medicine.https://doi.org/10.1254/jpssuppl.wcp2018.0_po4-10-25.

[2] Corsinovi, D., Usai, A., De Sarlo, M., Giannaccini, M., and Ori, M. (2021). Zebrafish Avatar to Develop Precision Breast Cancer Therapies. Anti-cancer agents in medicinal chemistry. https://doi.org/10.2174/1871520621666210402111634.

[3] Fazio, M., Ablain, J., Chuan, Y., Langenau, D., and Zon, L. (2020). Zebrafish patient avatars in cancer biology and precision cancer therapy. Nature Reviews Cancer, 20, 263 - 273. https://doi.org/10.1038/s41568-020-0252-3.

[4] Fieuws, C., Bek, J., Parton, B., De Neef, E., De Wever, O., Hoorne, M., Estrada, M., Van Dorpe, J., Denys, H., Van De Vijver, K., and Claes, K. (2024). Zebrafish Avatars: Toward Functional Precision Medicine in Low-Grade Serous Ovarian Cancer. Cancers, 16. https://doi.org/10.3390/cancers16101812.

[5] Wu, X., Hua, X., Xu, K., Song, Y., and Lv, T. (2023). Zebrafish in Lung Cancer Research. Cancers, 15. https://doi.org/10.3390/cancers15194721.

[6] Astone, M., Dankert, E., Alam, S., and Hoeppner, L. (2017). Fishing for cures: The alLURE of using zebrafish to develop precision oncology therapies. NPJ Precision Oncology, 1. https://doi.org/10.1038/s41698-017-0043-9.

[7] Sonawane, R., and Krishnan, K. (2023). Zebrafish (Danio rerio) as Biomedical Models in Research. IJBPAS, 12(11), 4956-4965.

[8] Macrae, C., and Peterson, R. (2015). Zebrafish as tools for drug discovery. Nature Reviews Drug Discovery, 14, 721-731. https://doi.org/10.1038/nrd4627.

[9] Hason, M., and Bartůněk, P. (2019). Zebrafish Models of Cancer—New Insights on Modeling Human Cancer in a Non-Mammalian Vertebrate. Genes, 10. https://doi.org/10.3390/genes10110935.

[10] Letrado, P., De Miguel, I., Lamberto, I., Díez-Martínez, R., and Oyarzábal, J. (2018). Zebrafish: Speeding Up the Cancer Drug Discovery Process.. Cancer research, 78 21, 6048-6058 . https://doi.org/10.1158/0008-5472.CAN-18-1029.

[11] Kirchberger, S., Sturtzel, C., Pascoal, S., Distel, M. (2017) Quo natas, Danio?-Recent Progress in Modeling Cancer in Zebrafish.. Frontiers in oncology. 7:186.

[12] Bentley, V.L., Veinotte, C.J., Corkery, D.P., Pinder, J.B., LeBlanc, M.A., Bedard, K., Weng, A.P., Berman, J.N., Dellaire, G. (2015) Focused Chemical Genomics Using Zebrafish Xenotransplantation As A Pre-Clinical Therapeutic Platform For T-Cell Acute Lymphoblastic Leukemia. Haematologica. 100(1):70-6.

[13] Dockins, S., Blackburn, J., Haney, M., and Wilson, B. (2019). Abstract 3697: Optimization of human cancer cell xenografts into zebrafish larvae for high-throughput drug screening. Tumor Biology. https://doi.org/10.1158/1538-7445.AM2019-3697.

[14] Nakayama, J., and Makinoshima, H. (2020). Zebrafish-Based Screening Models for the Identification of Anti-Metastatic Drugs. Molecules, 25. https://doi.org/10.3390/molecules25102407.

[15] Gallardo, V., Varshney, G., Lee, M., Bupp, S., Xu, L., Shinn, P., Crawford, N., Inglese, J., and Burgess, S. (2015). Phenotype-driven chemical screening in zebrafish for compounds that inhibit collective cell migration identifies multiple pathways potentially involved in metastatic invasion. Disease Models and Mechanisms, 8, 565 - 576. https://doi.org/10.1242/dmm.018689.

[16] Schmitz, R., Walsh, A., Tweed, K., Trier, S., Huttenlocher, A., and Skala, M. (2019). Zebrafish xenograft breast cancer models for high-throughput drug response screening. , 10888, 1088804 - 1088804-7. https://doi.org/10.1117/12.2513688.

[17] Mauro, A., Ng, R., Li, J., Guan, R., Wang, Y., Singh, K., and Wen, X. (2019). Protocol development for discovery of angiogenesis inhibitors via automated methods using zebrafish. PLoS ONE. https://doi.org/10.1371/journal.pone.0221796.

[18] Barriuso, J., Nagaraju, R., and Hurlstone, A. (2015). Zebrafish: A New Companion for Translational Research in Oncology. Clinical Cancer Research, 21, 969 - 975. https://doi.org/10.1158/1078-0432.CCR-14-2921.

[19] Al-Samadi, A., Tuomainen, K., Kivimäki, A., Salem, A., Al-Kubati, S., Hyytiäinen, A., Parikka, M., Mesimäki, K., Wilkman, T., Mäkitie, A., Grénman, R., and Salo, T. (2019). PCR-based zebrafish model for personalised medicine in head and neck cancer. Journal of Translational Medicine, 17. https://doi.org/10.1186/s12967-019-1985-1.

[20] Teame T, Zhang Z, Ran C, Zhang H, Yang Y, Ding Q, Xie M, Gao C, Ye Y, Duan M, Zhou Z. The use of zebrafish (Danio rerio) as biomedical models. Anim Front. 2019 Jun 25;9(3):68-77. doi: 10.1093/af/vfz020. PMID: 32002264; PMCID: PMC6951987

[21] Al-Hamaly, M. A., Turner, L. T., Rivera-Martinez, A., Rodriguez, A., and Blackburn, J. S. (2023). Zebrafish Cancer Avatars: A Translational Platform for Analyzing Tumor Heterogeneity and Predicting Patient Outcomes. International journal of molecular sciences, 24(3), 2288. https://doi.org/10.3390/ijms24032288

[22] Hu, C., Sun, L., Chen, J., Lyu, Z., Yuan, C., and Jiang, X. (2024). Advantages of the zebrafish tumor xenograft model: the evaluation of efficacy in cancer therapy and the application to the study of lncRNAs. Frontiers in Immunology, 15. https://doi.org/10.3389/fimmu.2024.1483192.

[23] Nakayama, J., Makinoshima, H., and Gong, Z. (2023). In vivo Drug Screening to Identify Anti-metastatic Drugs in Twist1a-ERT2 Transgenic Zebrafish. Bio-protocol, 13. https://doi.org/10.21769/BioProtoc.4673.

[24] McGrath, P., and Li, C. Q. (2008). Zebrafish: a predictive model for assessing drug-induced toxicity. Drug discovery today, 13(9-10), 394–401. https://doi.org/10.1016/j.drudis.2008.03.002.

[25] He, J. H., Gao, J. M., Huang, C. J., and Li, C. Q. (2014). Zebrafish models for assessing developmental and reproductive toxicity. Neurotoxicology and teratology, 42, 35–42. https://doi.org/10.1016/j.ntt.2014.01.006.

[26] Cornet, C., Calzolari, S., Miñana-Prieto, R., Dyballa, S., van Doornmalen, E., Rutjes, H., Savy, T., D'Amico, D., and Terriente, J. (2017). ZeGlobalTox: An Innovative Approach to Address Organ Drug Toxicity Using Zebrafish. International journal of molecular sciences, 18(4), 864. https://doi.org/10.3390/ijms18040864.

[27] Jia, H., Zhu, Y., Duan, Q., Chen, Z., and Wu, F. (2019). Nanomaterials meet zebrafish: Toxicity evaluation and drug delivery applications.. Journal of controlled release : official journal of the Controlled Release Society. https://doi.org/10.1016/j.jconrel.2019.08.022.

[28] Miyawaki, I. (2020). Application of zebrafish to safety evaluation in drug discovery. Journal of Toxicologic Pathology, 33, 197 - 210. https://doi.org/10.1293/tox.2020-0021.

[29] Cassar, S., Adatto, I., Freeman, J., Gamse, J., Iturria, I., Lawrence, C., Muriana, A., Peterson, R., Van Cruchten, S., and Zon, L. (2020). Use of Zebrafish in Drug Discovery Toxicology.. Chemical research in toxicology. https://doi.org/10.1021/acs.chemrestox.9b00335.

[30] Azzam, N., Fletcher, J., and Stewart, R. (2024). High-risk pediatric cancer models in zebrafish, mouse and short-term culture predict individual patient responses to therapy. Cancer Research, 84(17_Supplement), Abstract B007. September 2024Cancer Research 84(17_Supplement):B007-B007DOI: 10.1158/1538-7445.PEDIATRIC24-B007

[31] Usai, A., Di Franco, G., Piccardi, M., Cateni, P., Pollina, L., Vivaldi, C., Vasile, E., Funel, N., Palmeri, M., Dente, L., Falcone, A., Giunchi, D., Massolo, A., Raffa, V., and Morelli, L. (2021). Zebrafish Patient-Derived Xenografts Identify Chemo-Response in Pancreatic Ductal Adenocarcinoma Patients. Cancers, 13. https://doi.org/10.3390/cancers13164131.

[32] Costa, B., Estrada, M., Barroso, M., and Fior, R. (2022). Zebrafish Patient-Derived Avatars from Digestive Cancers for Anti-cancer Therapy Screening. Current Protocols, 2. https://doi.org/10.1002/cpz1.415.

[33] Somasagara, R., Huang, X., Xu, C., Haider, J., Serody, J., Armistead, P., and Leung, T. (2021). Targeted therapy of human leukemia xenografts in immunodeficient zebrafish. Scientific Reports, 11. https://doi.org/10.1038/s41598-021-85141-5.

[34] Prykhozhij, S., Cáceres, L., and Berman, J. (2018). New Developments in CRISPR/Cas-based Functional Genomics and their Implications for Research Using Zebrafish.. Current gene therapy, 17 4, 286-300 . https://doi.org/10.2174/1566523217666171121164132.

[35] Liu, K., Petree, C., Requena, T., Varshney, P., and Varshney, G. (2019). Expanding the CRISPR Toolbox in Zebrafish for Studying Development and Disease. Frontiers in Cell and Developmental Biology, 7. https://doi.org/10.3389/fcell.2019.00013.

[36] Ouyang, J., Xue, S., Zhou, Q., and Cui, H. (2020). [Research progress and applications of gene editing technology CRISPR/Cas in zebrafish].. Sheng wu gong cheng xue bao = Chinese journal of biotechnology, 36 1, 1-12 . https://doi.org/10.13345/j.cjb.190178.

[37] Sharma, P., Sharma, B., and Verma, R. (2021). CRISPR-based genome editing of zebrafish.. Progress in molecular biology and translational science, 180, 69-84 . https://doi.org/10.1016/bs.pmbts.2021.01.005.

[38] Chaudhary, D., Singh, S., Gohil, N., and Bhattacharjee, G. (2020). Recent progress of CRISPR-Cas9 in zebra fish. , 251-261. https://doi.org/10.1016/b978-0-12-818140-9.00019-2.

[39] Ablain, J., Dang, M., Rothschild, H., and Zon, L. (2018). Abstract 93: Tissue-specific CRISPR in zebrafish identifiesPVRL1as a novel metastasis suppressor gene in melanoma. Tumor Biology. https://doi.org/10.1158/1538-7445.AM2018-93.

[40] Nguyen, U., Webb, J., Schmitz, R., Tweed, K., Huttenlocher, A., Skala, M., and Walsh, A. (2021). Optical imaging of zebrafish xenograft tumors for a high throughput drugs screen. , 11622, 116220E - 116220E-6. https://doi.org/10.1117/12.2577637.

[41] Grandhi, T., Romero, J., Luna, F., Barnes, W., Walker, J., Moran, R., Newlin, R., Miraglia, L., Orth, A., and Horman, S. (2020). High-throughput CRISPR-mediated 3D enrichment platform for functional interrogation of chemotherapeutic resistance. Biotechnology and Bioengineering, 118, 3187 - 3199. https://doi.org/10.1002/bit.27844.

[42] Haney, M., Moore, L., and Blackburn, J. (2020). Drug Screening of Primary Patient Derived Tumor Xenografts in Zebrafish.. Journal of visualized experiments : JoVE, 158. https://doi.org/10.3791/60996

[43] Xie, X., Ross, J., Cowell, J., and Teng, Y. (2015). The promise of zebrafish as a chemical screening tool in cancer therapy.. Future medicinal chemistry, 7 11, 1395-405 . https://doi.org/10.4155/fmc.15.73.

[44] Terriente, J., and Pujades, C. (2013). Use of Zebrafish Embryos for Small Molecule Screening Related to Cancer. Developmental Dynamics, 242. https://doi.org/10.1002/dvdy.23912.

[45] Bozhko, D., Myrov, V., Kolchanova, S., Polovian, A., Galumov, G., Demin, K., Zabegalov, K., Strekalova, T., Abreu, M., Petersen, E., and Kalueff, A. (2021). Artificial intelligence-driven phenotyping of zebrafish psychoactive drug responses. Progress in Neuro-Psychopharmacology and Biological Psychiatry, 112. https://doi.org/10.1016/j.pnpbp.2021.110405.

[46] Zhang, H., Waldmann, L., Manuel, R., Boije, H., Haitina, T., and Allalou, A. (2020). zOPT: an open source optical projection tomography system and methods for rapid 3D zebrafish imaging.. Biomedical optics express, 11 8, 4290-4305 . https://doi.org/10.1364/boe.393519.

[47] Tang, Q., Moore, J., Ignatius, M., Tenente, I., Tenente, I., Hayes, M., Garcia, E., Yordán, N., Bourque, C., He, S., Blackburn, J., Blackburn, J., Look, A., Houvras, Y., and Langenau, D. (2016). Imaging tumour cell heterogeneity following cell transplantation into optically clear immune-deficient zebrafish. Nature Communications, 7. https://doi.org/10.1038/ncomms10358.

[48] Yan, C., Yang, Q., , D., Brunson, D., Iafrate, J., Rawls, J., and Langenau, D. (2020). Abstract PR12: Dynamic single-cell imaging of human cancer growth and therapy responses following engraftment into immunodeficient zebrafish. Oral Presentations - Proffered Abstracts. https://doi.org/10.1158/1538-7445.camodels2020-pr12.

[49] Lichtenegger, A., Mukherjee, P., Zhu, L., Morishita, R., Tomita, K., Oida, D., Leskovar, K., El-Sadek, A., Makita, S., Kirchberger, S., Distel, M., Baumann, B., and Yasuno, Y. (2022). Non-destructive characterization of adult zebrafish models using Jones matrix optical coherence tomography. Biomedical Optics Express, 13, 2202 - 2223. https://doi.org/10.1364/BOE.455876.

[50] Kumar, S., Lockwood, N., Ramel, M., Correia, T., Ellis, M., Alexandrov, Y., Andrews, N., Patel, R., Bugeon, L., Dallman, M., Brandner, S., Arridge, S., Katan, M., McGinty, J., Frankel, P., and French, P. (2016). Quantitative in vivo optical tomography of cancer progression and vasculature development in adult zebrafish. Oncotarget, 7, 43939 - 43948. https://doi.org/10.18632/oncotarget.9756.

[51] Bootorabi, F., Manouchehri, H., Changizi, R., Barker, H., Palazzo, E., Saltari, A., Parikka, M., Pincelli, C., and Aspatwar, A. (2017). Zebrafish as a Model Organism for the Development of Drugs for Skin Cancer. International Journal of Molecular Sciences, 18. https://doi.org/10.3390/ijms18071550.

[52] Pott, A., Rottbauer, W., and Just, S. (2020). Streamlining drug discovery assays for cardiovascular disease using zebrafish. Expert Opinion on Drug Discovery, 15, 27 - 37. https://doi.org/10.1080/17460441.2020.1671351.

[53] Lawrence, C. "The Husbandry of Zebrafish (Danio rerio): A Review." Aquaculture, vol. 269, no. 1-4, 2007, pp. 1-20. https://doi.org/10.1016/j.aquaculture.2007.04.077

[54] Kari, G., Rodeck, U., and Dicker, A. P. (2007). Zebrafish: An emerging model system for human disease and drug discovery. Clinical Pharmacology and Therapeutics, 82(1), 70–80. https://doi.org/10.1038/sj.clpt.6100223.

[55] Howe, K., Clark, M. D., Torroja, C. F., Torrance, J., Berthelot, C., Muffato, M., Collins, J. E., Humphray, S., McLaren, K., Matthews, L., McLaren, S., Sealy, I., Caccamo, M., Churcher, C., Scott, C., Barrett, J. C., Koch, R., Rauch, G. J., White, S., ... Stemple, D. L. (2013). The zebrafish reference genome sequence and its relationship to the human genome. Nature, 496(7446), 498-503. https://doi.org/10.1038/nature12111

[56] White, R. M., Sessa, A., Burke, C., Bowman, T., LeBlanc, J., Ceol, C., Bourque, C., Dovey, M., Goessling, W., Burns, C. E., and Zon, L. I. (2008). Transparent adult zebrafish as a tool for in vivo transplantation analysis. Cell stem cell, 2(2), 183–189. https://doi.org/10.1016/j.stem.2007.11.002

[57] Fior, R., Póvoa, V., Mendes, R. V., Carvalho, T., Gomes, A., Figueiredo, N., and Ferreira, M. G. (2017). Single-cell functional and chemosensitive profiling of combinatorial colorectal therapy in zebrafish xenografts. Proceedings of the National Academy of Sciences of the United States of America, 114(39), E8234–E8243. https://doi.org/10.1073/pnas.1618389114.

[58] Burger, A., Lindsay, H., and Felker, A. (2016). Maximizing mutagenesis with solubilized CRISPR-Cas9 ribonucleoprotein complexes. Development, 143(11), 2025–2037. https://doi.org/10.1242/dev.134775.

[59] Frese, K. K., and Tuveson, D. A. (2007). Maximizing mouse cancer models. Nature Reviews Cancer, 7(9), 645–658. https://doi.org/10.1038/nrc2193.

[60] Hidalgo, M., Amant, F., Biankin, A. V., Budinska, E., Byrne, A. T., Caldas, C., Clarke, R. B., de Jong, S., Jonkers, J., Mælandsmo, G. M., Roman-Roman, S., Seoane, J., Trusolino, L., and Villanueva, A. (2014). Patient-derived xenograft models: An emerging platform for translational cancer research. Cancer Discovery, 4(9), 998–1013. https://doi.org/10.1158/2159-8290.CD-14-0001.

[61] Zhou, J., and Liu, M. (2020). Mouse models for cancer precision medicine: Advantages, limitations, and future perspectives. Nature, 583(7818), 225–230. https://doi.org/10.1038/s41586-020-2256-4

[62] Clevers, H. (2016). Modeling development and disease with organoids. Cell, 165(7), 1586–1597. https://doi.org/10.1016/j.cell.2016.05.082

[63] Fatehullah, A., Tan, S. H., and Barker, N. (2016). Organoids as an in vitro model of human development and disease. Trends in Cell Biology, 26(5), 385–394. https://doi.org/10.1016/j.tcb.2016.02.002

[64] Sachs, N., Papaspyropoulos, A., Zomer-van Ommen, D. D., Heo, I., Böttinger, L., Klay, D., Weeber, F., Huelsz-Prince, G., Iakobachvili, N., Amatngalim, G. D., and Others. (2018). Long-term expanding human airway organoids for disease modeling. The EMBO Journal, 38(4), e100300. https://doi.org/10.1038/s41586-018-0176-2

[65] Drost, J., and Clevers, H. (2018). Organoids in cancer research. Nature Reviews Cancer, 18(7), 407–418. https://doi.org/10.1038/s41568-018-0007-6