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EDITORIAL

What value do zebrafish have to anticancer drug discovery?

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1. Introduction

Despite billions of dollars invested into cancer drug development each year, studies have shown that oncological clinical trials had a staggeringly low success rate of 3.5% in 2022 [1]. Drug candidates tested in the commonly used murine models often fail to reproduce a comparable efficacy and safety profile in clinical trials. In addition, failure to meet parameters of ADMET (Absorption, Distribution, Metabolism, Excretion, and Toxicity) results in the filtering out of many drug candidates in the preclinical phase. Such sunken costs call for changes in methodology and policy to increase the success rates of cancer drug development. One of the strategies is to implement additional *in vivo* models to identify suitable hits and filter out inadequate candidates early in the pipeline. From the perspective of clinical application, it is difficult to predict treatment responses due to the genetic predisposition of patients and the heterogenicity of cancer. Patient-derived organoids (PDO) and murine patient-derived xenografts (PDX), the commonly used models to simulate responses, have limitations in practicality and accuracy prediction [2]. This calls for the inclusion of other cancer models to improve the landscape of precision medicine.

The zebrafish was first introduced as a disease model in the 1970s. Owing to its unique strengths, its utilization in cancer research has grown exponentially in recent years. The advantages of zebrafish include 1) high conservation to the human genome and oncologic signaling; 2) high reproduction rates and low cost in animal housing; 3) relative transparency, which enables live-tracking of tumors; and 4) lack of a mature adaptive immune system until four weeks of life, making xenograft studies straightforward. Here, we summarize the current application of zebrafish in the pipeline of cancer drug discovery (Figure 1). Additionally, we provide examples of how zebrafish helped advance therapeutics developments to various stages of clinical trials (Table 1).

2. Target discovery with reduced toxicities

Although *in vitro* cell culture systems are the traditional tools used to screen for primary drug targets, zebrafish offer advantages as a whole organism to filter out those that impact development or induce organ toxicity. Zebrafish are especially useful for studying host-tumor interactions in tumor microenvironment and metastasis. Using tools such as mutagenesis and transgenesis, researchers can manipulate molecular targets transiently or permanently, as well as spatially or temporally. In particular, these techniques have been combined with zebrafish's transparency (e.g. the complete transparent adult fish *Casper*) to fluorescently label and study tumor cells and tissues of interest [3]. Technological advances in imaging also enable noninvasive observation of host-tumor interaction in live zebrafish up to single-cell resolution [4]. Using zebrafish, researchers can also validate drug targets by modulating the expression, structure, or function of the target protein and determining how the intervention impacts tumor development and oncogenic pathways. For example, Vlecken and Bagowski were able to identify and validate LIMK1 and LIMK2 as potential targets for tumor angiogenesis by using RNAi techniques in a zebrafish xenograft model of human pancreatic cancer [3]. In addition, a genetic screen using transgenic zebrafish identified dihydrolipoamide S-succinyltransferase, a TCA-cycle transferase, as a target for MYC-driven tumors [5]. Devimistat, which inhibits the TCA cycle, has been tested in clinical trials for treating both hematological and solid tumors and recently received a fast-track designation from the FDA for treating acute myeloid leukemia (Table 1). Both examples highlight the ability of zebrafish as an *in vivo* model in identifying drug targets with translational value.

3. *In vivo* lead screens

Zebrafish-based screens often utilize transgenic or xenograft models of cancer. The output of administered drugs can be measured in zebrafish models for morphologic, therapeutic, pathway, or behavioral changes [6]. Due to the high fecundity and small size of zebrafish, these drug screens feature high throughput capabilities and reliable statistical outputs. Researchers have also ventured into optimizing the screens by automating steps of the process such as microinjections, embryo sorting, phenotype identification, and image acquisition.

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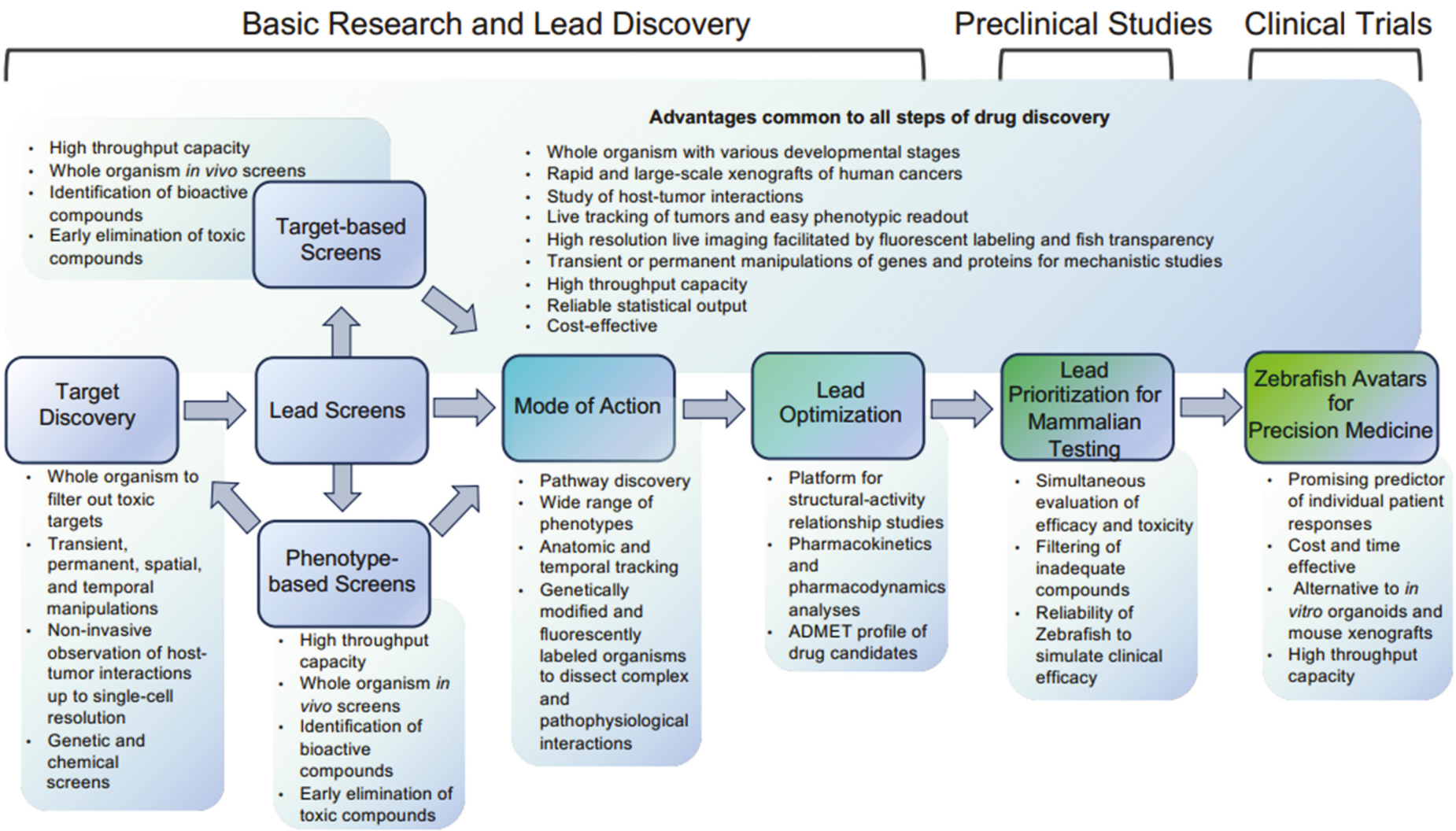

Figure 1. Zebrafish's application in drug discovery and development. The chart outlines the steps of the drug discovery and development pipeline, with the unique strengths of zebrafish described in the shaded boxes.

Table 1. Advances in drug discovery, validation, and personalized medicine using zebrafish.

Drugs	Targets	Modality of Screening	Indications	Stage of Clinical Development/Status	Clinical Trial Identifier	Major References
			Target Identification			
Marizomib	20S proteasome	Systemic angiogenesis assays in zebrafish embryos	Glioblastoma multiforme	Phase III/Active, not recruiting	NCT03345095	Wang and Miao, 2013
ProHema (Chemical derivative of PGE2)	PGE2 receptor	Chemical genetic screens using zebrafish embryos to identify effectors of stem cell induction	Hematologic malignancies	Phase I/Terminated	NCT02354417	North et al. 2007
			Hematologic malignancies	Phase I/Completed	NCT00890500	
			Inherited metabolic disorders	Phase I/Terminated	NCT02354443	
			Hematologic malignancies	Phase II/Terminated	NCT01627314	
Devimistat	Pyruvate dehydrogenase and α-ketoglutarate dehydrogenase complex in the TCA cycle	Genetic screens that identified the TCA cycle enzyme as the drug target based on leukemia-suppressing phenotypes in adult zebrafish	Relapsed or refractory Burkitt Lymphoma/Leukemia or high-grade B-cell lymphoma with rearrangements of MYC and BCL2 and/or BCL6	Phase II/Active, not recruiting	NCT03793140	Anderson et al. 2016
					NCT03504410	
				Phase III/Terminated	NCT04593758	
			Relapsed/refractory acute myeloid leukemia	Phase I/II/Completed	NCT03699319	
			Relapsed or refractory clear cell sarcoma of soft tissue	Phase I/II/Active, not recruiting	NCT04217317	
					NCT03504423	
			Locally advanced pancreatic cancer	Phase II/Recruiting	NCT04203160	
			Relapsed or refractory T-Cell Non-Hodgkin lymphoma	Phase II/Completed	NCT01766219	
				Phase I/II/Recruiting	NCT05854966	
			Metastatic adenocarcinoma of the pancreas	Phase I/II/Completed	NCT02484391	
			Advanced unresectable biliary tract cancer	Phase II/Not yet recruiting	NCT05733000	
			Advanced or metastatic bile duct cancer that cannot be removed by surgery	Phase II/Completed		
				Phase II/Recruiting		
			Relapsed or refractory acute myeloid leukemia			
			Relapsed or refractory acute myeloid leukemia or granulocytic sarcoma			
			Advanced chemorefractory solid tumors			
			Drug Repurposing			
Leflunomide (FDA-approved treatment for Rheumatoid Arthritis)	dihydroorotate dehydrogenase	Chemical genetic screens for compounds impacting neural crest cell development, self-renewal, and lymphatic activity in zebrafish embryos	Previously treated metastatic triple negative breast cancer	Phase I/II/Recruiting	NCT03709446	White et al. 2011
				Phase I/Recruiting	NCT04997993	Santoriello et al. 2020
			PTEN-null advanced solid malignancies	Phase II/Recruiting	NCT04508790	Astin et al. 2014
			Relapsed or refractory multiple myeloma	Phase II/Recruiting	NCT05014646	Seda et al. 2019
			High-risk smoldering myeloma	Cancer Prevention study/Recruiting	NCT05605587	
			Multiple endocrine neoplasia type 1 syndromes			
Rosuvastatin (FDA-approved treatment for hypercholesterolemia)	HMG-CoA reductase	Chemical genetic screens using zebrafish embryos to identify small molecules that inhibit angiogenesis with minimal toxicities	Clonal cytopenia of undetermined significance and myelodysplastic syndromes	Phase II/Not yet recruiting	NCT05483010	Wang et al. 2010
				Phase II/Recruiting	NCT04491643	Kumar et al. 2020
			Early endometrial carcinoma	Phase IV/Completed	NCT04776889	Kumar et al. 2022
			Metastatic prostate cancer	Phase II/Completed	NCT02569645	Han et al. 2022
			Rectal cancer	Phase II/Completed	NCT00966472	
			Advanced solid malignancies with focus on squamous cell carcinomas and NSCL			
All-Trans-Retinoic Acid (FDA-approved treatment for AML, APML, Immune Thrombocytopenia)	Retinoic acid receptor alpha	Chemical genetic screens using pluripotent zebrafish blastomere culture system to identify suppressors of c-myb in adenoid cystic cancers	Advanced adenoid cystic carcinoma	Phase II/Completed	NCT03999684	Mandelbaum et al. 2018
13-cis-Retinoic Acid			Neuroblastoma	Phase II/Completed	NCT00135135	
			Brain cancers	Phase II/Completed	NCT00528437	

(*Continued*)

Table 1. (Continued).

Drugs	Targets	Modality of Screening	Indications	Stage of Clinical Development/Status	Clinical Trial Identifier	Major References
			Cancer Avatars			
Olaparib Temozolomide	Poly(ADP-ribose) polymerase DNA (alkylating agent)	Combination therapies assessed in optically-clear, immunodeficient adult zebrafish engrafted with established human sarcoma cell lines. Validation of zebrafish patient-derived xenografts (zPDXs) using patient-derived cancers	Ewing's sarcoma Rhabdomyosarcoma	Phase I/Recruiting	NCT01858168	Yan et al. 2019
Fluorouracil Lederfolin Oxaliplatin Irinotecan Docetaxel Cisplatin Epirubicin Gemcitabine Nab paclitaxel	Approved chemotherapy drugs	Prediction of patient responses to chemotherapy regimens using embryo zPDXs	Liver cancer Pancreatic cancer Gastric cancer Esophageal cancer Colorectal cancer Gall bladder cancer Biliary tract cancer	Observational/Unknown status	NCT03668418	Di Franco et al. 2020 Usai et al. 2021 Di Franco et al. 2022
Gastric Cancer drugs	Approved chemotherapy drugs	Prediction of patient responses to chemotherapy regimens using zPDXs; fish stage not specified	Gastric cancer	Observational/Not yet recruiting	NCT05616533	Jin, Zhejiang University

3.1. Target-based screens

Zebrafish are suitable for screens to uncover the leads that inhibit well-studied targets from libraries composed of thousands of biochemical compounds. For example, a transgenic zebrafish screen of pharmacologically active compounds identified two antidepressants that target the β-catenin pathway in hepatocellular carcinoma [6]. A zebrafish blastomere screen of 3,840 bioactive small molecules identified retinoid acid agonists with potent anti-cancer properties, which reduce the aberrant *MYB* expression in adenoid cystic carcinoma and leukemia [7]. Retinoid acid is tested in clinical trials for treating multiple types of cancers including advanced adenoid cystic carcinoma, neuroblastoma, and brain cancers (NCT03999684, NCT00135135, and NCT00528437; Table 1). The third example is the discovery of 16,16-dimethyl-PGE2 (dmPGE2; ProHema) through a screen for compounds targeting the prostanoid E receptors [8]. DmPGE2 was found to regulate vertebrate hematopoietic stem cells by increasing their numbers in the aorta-gonad-mesonephros region of zebrafish. Subsequently, dmPGE2 has progressed to a phase II clinical trial for treating hematologic malignancies (NCT00890500; Table 1) [8].

3.2. Phenotype-based screens

Zebrafish are also ideal for screening leads based on phenotypic changes (e.g. cancer-suppressing) when the molecular target is unknown. This phenotype-based screen has several strengths over target-based drug discovery. It can identify entirely new classes of therapeutics and reveal previously unsuspected 'druggable' pathways or molecular targets [9]. Moreover, candidate drugs identified through this method must fulfill parameters of both anticancer efficacy and minimal *in vivo* toxicity. 'Traditional' target-based drug discovery often fails due to *in vivo* toxicities that are typically tested later in the process. Zebrafish phenotypic screens bypass this issue with toxicity assessment as the first filtering criterion, saving both cost and time.

Drugs discovered by this approach include Lenaldekar that was identified from a library of 26,400 molecules [10]. Lenaldekar exhibits selective killing of multiple types of leukemia with potential for clinical utility. Another example is the discovery of perphenazine for killing T-cell leukemia cells through a similar phenotype-based screen of 4,880 FDA-approved drugs or drug-like molecules [11]. Notably, NSC210627 was discovered by screening a 2,000-chemical library based on melanoma-inhibiting phenotypes [12]. Chemoinformatic structural analysis revealed that NSC210627 resembles dihydroorotate dehydrogenase (DHODH) inhibitors and exerts its anti-melanoma effects through DHODH suppression. DHODH was then validated to be a new druggable target for melanoma. Leflunomide, an FDA-approved DHODH inhibitor for treating rheumatoid arthritis, was evaluated in preclinical studies and a phase I trial for melanoma treatment (NCT01611675) [9]. Leflunomide is now tested in clinical trials for treating multiple myeloma and metastatic triple-negative breast cancer (Table 1), demonstrating the ability of zebrafish phenotypic screens to simultaneously identify new targets and therapeutics.

Both target and phenotype-based screens can result in drug repurposing, which is time and cost efficient as the drug is already well characterized. Using zebrafish gastrulation as a readout in a screen, Nakayama et al. identified 20 FDA-approved drugs that can inhibit tumor cell invasion [13]. Their follow-up studies using zebrafish and mouse xenografts led to the identification of pizotifen, an antagonist of serotonin receptor 2C, as a metastasis-suppressing drug. Wang et al. demonstrated that Rosuvastatin, an FDA-approved drug for treating hypercholesterolemia and cardiovascular diseases, impacts endothelial cell function and suppresses prostate tumor growth [14]. Rosuvastatin is now being tested in clinical trials for treating multiple cancers, including metastatic breast cancer, rectal cancer, squamous cell carcinoma, and prostate cancer (Table 1).

4. Mechanistic elucidation of the drug candidates

Zebrafish are optimal for studying complex molecular mechanisms that impact organ and system development. Zhu et al. investigated the spatial and temporal role of an activated anaplastic lymphoma kinase (ALK) mutation to neuroblastoma pathogenesis [15]. Their results demonstrated the prosurvival effects of the ALK F1174L mutation that collaborates with MYCN in tumor development, providing implications for targeted therapy. Additionally, compared to *in-vitro* systems, zebrafish offer a wider range of phenotypes that aid mechanistic studies of drug candidates. For example, fumagillin, an anti-angiogenic natural product that inhibits methionine aminopeptidase type 2 enzyme, induced a gastrulation phenotype in zebrafish embryos like those caused by gene mutations in the noncanonical Wnt5 pathway [16]. This led to further elucidation of the mechanism of action of fumagillin and the discovery of the targetability of the noncanonical Wnt signaling pathway in cancer angiogenesis [16].

5. Lead optimization

Zebrafish are suitable for pharmacokinetics and pharmacodynamics studies using mass spectroscopy and liquid/gas chromatography [6,9]. Therefore, an ADMET profile of selected drugs can be generated using zebrafish. This shows the strong potential of using zebrafish as a platform for structural-activity relationship studies and drug optimization, another vital step in drug discovery [9]. Hence, zebrafish can facilitate the development of the drug prototype into those possessing optimal potency, bioavailability, and minimal toxicity.

6. Lead prioritization for mammalian testing

Mammalian models, particularly mice, are the current gold standard of preclinical testing. However, murine models of cancer are time-consuming to study and are often kept in an artificial sterile environment. Due to cost and regulatory constraints, murine experiments, including toxicity studies, are mostly short-term and predict clinical outcomes inconsistently. On the other hand, the predictability of zebrafish for various toxicity parameters has been validated, with sufficient to good accuracy of 60–100% [17]. Examples of these include cardiotoxicity, developmental toxicity, and seizure liability. The zebrafish is thus suitable for *in vivo* toxicity screens, which should complement and precede mammalian studies to eliminate toxic compounds early on and save costs.

Efficacy is another major reason for drug candidate attrition in the drug discovery process. Indeed, an estimated 40–50% of drug failure is due to a lack of clinical efficacy [18]. As none of the animal models can perfectly simulate human diseases, employing multiple *in vivo* systems in preclinical studies can help increase the confidence of a drug candidate. The zebrafish is suitable for this purpose as its physiology and pathophysiology are highly conserved. It can sometimes simulate drug effects in humans better than mice do, as shown in the study of thalidomide's ability to cause morphological limb defects in zebrafish while lacking any teratogenic effects in mice [9]. Combined with the ease to assess the ADMET profiles of drugs, zebrafish can ascertain and prioritize drug candidates for further testing in mammalian models.

7. Zebrafish avatars to advance precision medicine

Over the past 10 years, precision medicine has emerged to personalize and improve treatment for cancer patients. Cancer avatars, which historically rely on *in vitro* organoids and mice xenografts, serve as a key tool to predict responses and select drugs for individual patients. However, these avatars have substantial limitations including biological fidelity and logistical constraints in cost and time [2,19]. Zebrafish avatars can overcome the limitation of *in vitro* organoid models by simulating complex physiological environments. Additionally, due to their high fecundity and small size, zebrafish cancer avatars can complement murine models with increased statistical outputs, reduced patient tissue usage, and rapid readouts [19]. Currently, zebrafish avatars are often established through transplanting patient-derived tumor samples into zebrafish embryos. High throughput screening with a variety of therapeutic agents is then conducted and readouts are often available within just 4–7 days, enabling the selection of high-efficacy treatments to guide clinical decisions.

Despite being relatively new, the predictability of zebrafish avatars has been validated in several types of cancers, such as lung cancer, breast cancer, colorectal cancer, and leukemia [20]. Recent work demonstrated that zebrafish avatars can faithfully recapitulate radiosensitivity of colorectal cancer and chemosensitivity of both breast cancer and colorectal cancer in patients [20]. Yan et al. discovered the combination therapy of Olaparib and Temozolomide against human rhabdomyosarcoma while validating the biological fidelity of immunodeficient adult zebrafish xenografts [21]. This work subsequently led to the initiation of a clinical trial (NCT01858168). In addition, a trial enrolling 120 cancer patients is currently ongoing to evaluate the ability of zebrafish PDXs to predict individual responses (NCT03668418). This trial is expected to provide valuable insights into the overall utility of zebrafish cancer avatars, highlighting their potential in precision medicine [19].

8. Expert opinion

The zebrafish has demonstrated its unique strengths in cancer research and its suitability to improve the process of drug discovery and development. For instance, it can speed up

the discovery of drug targets and the identification of therapeutic leads through phenotypic screens. With its high reproducibility, mechanistic studies in this versatile organism can dissect complex oncogenic pathways and facilitate the selection of combination treatments in a cost-effective manner. The rapid embryonic development of zebrafish can be leveraged to filter out toxic drug candidates and increase success rates of preclinical and clinical testing. Unlike mice, zebrafish are raised in a non-sterile environment. They also manifest telomere shortening, a feature central to human cancer biology yet lacking in mice. Therefore, zebrafish aptly complement the murine models for preclinical testing to decrease the failure rates of drug candidates in clinical trials. Recent evidence emerging from zebrafish avatars also supports their utility in precision medicine to help stratify patient populations for personalized treatment.

The application of zebrafish in drug discovery and development is still in its early stages, having only been introduced into cancer research in the past two decades. As zebrafish are vertebrates, but not mammals, hesitation in their inclusion in preclinical testing and doubts about their relevance to patients still require additional evidence to be overcome. In addition, even though zebrafish can be used to study ADMET of drugs, their pharmacokinetics may be different from those in humans and should be compared carefully. Despite concerns about potential unphysiological responses in zebrafish avatars, studies show that fish tolerate 32–34°C, a temperature conducive to human tumor cell growth.

Future technological advances, such as the generation of fish suited to live at 37°C, will optimize the utility of zebrafish avatars. Pairing zebrafish with other model systems should hasten the process of drug discovery while increasing its success rates. Enhanced communications among the zebrafish research community, the industry, funding agencies, and policymakers will help steer and prioritize translational research using this model system. We hope that the accumulated scientific evidence collected through collaboration between academia and industry will soon convince policymakers to favor zebrafish as an essential tool for drug discovery and development.

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Declaration of interest

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References

Papers of special note have been highlighted as either of interest (•) or of considerable interest (••) to readers.

1. Aitken M, Kleinrock M, Connely N, et al. Global Oncology Trends-2023. Parsippany, NJ: IQVIA Institute; 2023.
2. Ciucci A, Buttarelli M, Fagotti A, et al. Preclinical models of epithelial ovarian cancer: practical considerations and challenges for a meaningful application. Cell Mol Life Sci. 2022 Jun 16;79(7):364. doi: 10.1007/s00018-022-04395-y
3. Astell KR, Sieger D. Zebrafish in vivo models of cancer and metastasis. Cold Spring Harb Perspect Med. 2020 Aug 3;10(8): a037077. doi: 10.1101/cshperspect.a037077
4. Loveless R, Shay C, Teng Y. Unveiling tumor microenvironment interactions using zebrafish models. Front Mol Biosci. 2020;7:611847. doi: 10.3389/fmolb.2020.611847
5. Anderson NM, Li D, Peng HL, et al. The TCA cycle transferase DLST is important for MYC-mediated leukemogenesis. Leukemia. 2016 Jun;30(6):1365–1374. doi: 10.1038/leu.2016.26
   - **This article showcases the utility of transgenic zebrafish genetic screens for cancer target discovery.**
6. Letrado P, de Miguel I, Lamberto I, et al. Zebrafish: speeding up the cancer drug discovery process. Cancer Res. 2018 Nov 1;78 (21):6048–6058. doi: 10.1158/0008-5472.CAN-18-1029
   - **This article highlights a notable example of zebrafish being used for target-based discovery in treating hepatocellular carcinoma.**
7. Mandelbaum J, Shestopalov IA, Henderson RE, et al. Zebrafish blastomere screen identifies retinoic acid suppression of MYB in adenoid cystic carcinoma. J Exp Med. 2018 Oct 1;215 (10):2673–2685. doi: 10.1084/jem.20180939
   - **This work presents how zebrafish blastomere screens can be utilized for adenoid cystic carcinoma and leukemia drug discovery.**
8. Lee HC, Lin CY, Tsai HJ. Zebrafish, an in vivo platform to screen drugs and proteins for biomedical use. Pharmaceuticals (Basel). 2021 May 24;14(6):500. doi: 10.3390/ph14060500
   - **This article highlights how the zebrafish model helped discover a novel treatment for hematologic malignancies that advanced to clinical trials.**
9. Patton EE, Zon LI, Langenau DM. Zebrafish disease models in drug discovery: from preclinical modelling to clinical trials. Nat Rev Drug Discov. 2021 Aug;20(8):611–628. doi: 10.1038/s41573-021-00210-8
   - •• **This paper describes the ability of zebrafish models to be used for phenotype-based screens and lead optimization. It also showcases a notable example of zebrafish being utilized to discover a treatment for melanoma that advanced to clinical trials.**
10. Ridges S, Heaton WL, Joshi D, et al. Zebrafish screen identifies novel compound with selective toxicity against leukemia. Blood. 2012 Jun 14;119(24):5621–5631. doi: 10.1182/blood-2011-12-398818
11. Gutierrez A, Pan L, Groen RW, et al. Phenothiazines induce PP2A-mediated apoptosis in T cell acute lymphoblastic leukemia. J Clin Invest. 2014 Feb;124(2):644–655.
12. White RM, Cech J, Ratanasirintrawoot S, et al. DHODH modulates transcriptional elongation in the neural crest and melanoma. Nature. 2011 Mar 24;471(7339):518–522. doi: 10.1038/nature09882
13. Nakayama J, Tan L, Li Y, et al. A zebrafish embryo screen utilizing gastrulation identifies the HTR2C inhibitor pizotifen as a suppressor of EMT-mediated metastasis. Elife. 2021 Dec 17;10. doi: 10.7554/eLife.70151
14. Wang C, Tao W, Wang Y, et al. Rosuvastatin, identified from a zebrafish chemical genetic screen for antiangiogenic compounds, suppresses the growth of prostate cancer. Eur Urol. 2010 Sep;58 (3):418–426. doi: 10.1016/j.eururo.2010.05.024
   - **This study showcases a successful example of zebrafish screen-based drug repurposing that led to clinical trials.**

15. Zhu S, Lee JS, Guo F, et al. Activated ALK collaborates with MYCN in neuroblastoma pathogenesis. Cancer Cell. 2012 Mar 20;21 (3):362–373. doi: 10.1016/j.ccr.2012.02.010
16. Zhang Y, Yeh JR, Mara A, et al. A chemical and genetic approach to the mode of action of fumagillin. Chem Biol. 2006 Sep;13(9):1001–1009.
17. Bauer B, Mally A, Liedtke D. Zebrafish embryos and larvae as alternative animal models for toxicity testing. Int J Mol Sci. 2021 Dec 14;22(24):13417. doi: 10.3390/ijms222413417
18. Sun D, Gao W, Hu H, et al. Why 90% of clinical drug development fails and how to improve it? Acta Pharm Sin B. 2022 Jul;12 (7):3049–3062.
19. Fazio M, Ablain J, Chuan Y, et al. Zebrafish patient avatars in cancer biology and precision cancer therapy. Nat Rev Cancer. 2020 May;20 (5):263–273. doi: 10.1038/s41568-020-0252-3

•• **This review highlights the advantages of zebrafish avatar models over other models. It also presents a valuable example of a clinical trial testing the ability of zebrafish PDXs to predict individual responses.**

20. Al-Hamaly MA, Turner LT, Rivera-Martinez A, et al. Zebrafish cancer avatars: a translational platform for analyzing tumor heterogeneity and predicting patient outcomes. Int J Mol Sci. 2023 Jan 24;24 (3):2288. doi: 10.3390/ijms24032288
21. Yan C, Brunson DC, Tang Q, et al. Visualizing engrafted human cancer and therapy responses in immunodeficient zebrafish. Cell. 2019 Jun 13;177(7):1903–1914 e14. doi: 10.1016/j.cell.2019.04.004

• **This article showcases the usefulness of a zebrafish PDX model that allows dynamic tracking of single cancer cells and testing of preclinical therapeutic modalities.**