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REVIEW

Using zebrafish models of leukemia to streamline drug screening and discovery

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Current treatment strategies for acute leukemias largely rely on nonspecific cytotoxic drugs that result in high therapy-related morbidity and mortality. Cost-effective, pertinent animal models are needed to link *in vitro* studies with the development of new therapeutic agents in clinical trials on a high-throughput scale. However, targeted therapies have had limited success moving from bench to clinic, often due to unexpected off-target effects. The zebrafish has emerged as a reliable *in vivo* tool for modeling human leukemia. Zebrafish genetic and xenograft models of acute leukemia provide an unprecedented opportunity to conduct rapid, phenotype-based screens. This allows for the identification of relevant therapies while simultaneously evaluating drug toxicity, thus circumventing the limitations of target-centric approaches.

Need for novel drug discovery platforms for acute leukemia

The completion of the human genome project in 2003 has pushed science into a new era of large-scale genomic, transcriptomic, proteomic, and epigenomic data acquisition and mining. Clinically, this translates into the development of more biologically rational therapeutic strategies for diseases that arise from germline mutations, as well as from somatic lesions such as cancer. New therapies include small chemical inhibitors designed to target specific molecular abnormalities associated with a particular disease, improving therapeutic efficacy and reducing treatment-related toxicity.

Hematological malignancies such as acute myeloid leukemia (AML) and acute lymphoblastic leukemia (ALL) have led the way as a paradigm for targeted therapeutics due to easy access to primary patient-derived material from peripheral blood and bone marrow. Detailed molecular characterization has subsequently resulted in the risk stratification of many leukemia subtypes [1–4]. For instance, large-scale genomic and transcriptomic screening [1] has contributed to more tailored treatment strategies in pediatric ALL such that many subtypes of this disease are now considered curable [1,5]. In contrast, advances in genetic profiling of AML have failed to translate into improved outcomes, in part due to a paucity of therapeutically actionable targets [6,7]. Current overall survival of AML remains less than 60% and cytotoxic chemotherapy agents with substantial morbidity and mortality remain the mainstay of treatment. The plateau in AML treatment highlights the need to rethink chemical screening approaches to better identify promising therapeutic agents.

Phenotype-based therapeutic screening

Before the genomic era, preclinical pharmacological research relied on animal models that permitted the screening of compounds based on their effect on a disease "phenotype," an approach termed "phenotype-based screening" [8]. This approach enabled drug discovery even when disease pathogenesis was poorly characterized and there was no known or validated target. Importantly, using animal models of disease allows for evaluation of prodrugs that require metabolic conversion to an active form, as well as providing

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a whole organism context in which to observe off-target effects, thus simultaneously allowing for toxicity screening. More recently, this approach has been supplanted by a process in which in silico modeling and *in vitro* biochemical and cell-based assays are used to identify "hits" for selected targets, termed "target-centric" drug discovery. Compounds are then evaluated using preclinical animal models, finally making their way through the development pipeline to clinical trials. Although these methods of drug discovery represent a logical and measured strategy of increasing complexity and cost, they are laborious and time consuming (typically taking 12–15 years [9]). Furthermore, only a small percentage of drugs ever make it to the clinic [10], often failing at the level of animal model testing after significant time and financial investment [10,11]. The Tufts Center for the Study of Drug Development has recently estimated the cost of a new drug to arrive to market to be $2.6 billion [11], with approximately 80% of drugs being abandoned at various levels of development. Central to many of these drug candidate failures are the inappropriate and unacceptable drug ADMET (absorption, distribution, metabolism, excretion and toxicity) features only discovered once testing moves *in vivo* [12,13]. Revisiting the use of animal models for phenotype-based screening in the initial stages of drug discovery may circumvent many of the failures associated with an exclusively target-centered drug discovery strategy.

The use of traditional vertebrate animal models to screen candidate drugs is an onerous and cost-prohibitive task. The volume of chemicals and analogs available in various drug libraries is not amenable to murine drug screens. The technical and financial challenges associated with these types of screens may account for the relative scarcity of *in vivo* studies used to identify and verify new treatments. Therefore, most approaches for targeted therapy in myeloid and lymphoid leukemias have relied on candidate drugs for identified mutations. These studies have resulted in limited success often due to unexpected off-target effects from lack of target specificity once the drug is trialed *in vivo*. For example, there is a dose-limiting gastrointestinal toxicity from gamma-secretase inhibitors targeting Notch pathway mutations in T-cell ALL (T-ALL) [14]. In addition, the BCR-ABL1 fusion found in chronic myeloid leukemia can be targeted by tyrosine kinase inhibitors (TKIs) such as imatinib, nilotinib, dasatinib, and bosutinib. However, these TKIs are associated with substantial acute and long-term complications, including cardiovascular, pulmonary, gastrointestinal, and endocrine toxicities, as well as risk of secondary malignancies [15,16]. These off-target effects and the cost associated with traditional vertebrate models illustrate a need to find alternative model systems for further drug discoveries.

The zebrafish (*Danio rerio*) has been established as an effective *in vivo* tool for studying hematopoiesis and modeling human leukemogenesis (recently reviewed by Rasighaemi et al. [17]) and is particularly well-suited for high-throughput drug screening [8]. Zebrafish develop outside of the uterus, allowing for the easy visualization of early embryogenesis and organogenesis. In contrast to classic invertebrate models, zebrafish can be used to study vertebrate-specific processes that affect disease and development due to highly conserved developmental pathways [18–20]. More specifically, many human oncogenes and tumor suppressor genes have zebrafish orthologs, enabling the use of zebrafish in cancer research. There is also a general ease in genetic interrogation through microinjection of RNA, DNA, and protein that enables transiently or permanently altered gene expression and protein translation [21,22]. The optical clarity of zebrafish embryos permits direct visualization of a wide variety of phenotypes and gross morphological changes under standard light microscopy without sacrificing the embryo, facilitating longer-term analyses. Direct observation can be further enhanced through the use of fluorescent transgenic reporter fish that allow for cell-type or pathway-specific visualization [23–25]. These approaches are complemented by the use of zebrafish xenotransplantation (XT), a robust model of human leukemia that facilitates the rapid analysis of tumor proliferation, migration, or drug sensitivity *in vivo* using both human cell lines and primary patient-derived samples (described in Advantages of zebrafish xenotransplantation models) [26–29].

Recent technological advances have automated many techniques such as microinjection, embryo sorting, and fluorescent phenotype identification and image acquisition, allowing for streamlined screening in the zebrafish [30–34]. The zebrafish is poised to fill the missing and critical role of a cost-effective *in vivo* model for high-throughput chemical screening in leukemia. Detailed in this review is the suggestion that the pendulum may be swinging back to phenotype-based drug screens. Therefore, the techniques available to exploit the zebrafish as a preclinical leukemia model are highlighted with reference to relevant reviews in which more detailed descriptions of these technical approaches are included. This review also aims to illustrate how patient data can be used to inform the development of clinically relevant zebrafish leukemia models and how these models can be implemented for high-throughput drug discovery.

Modeling acute leukemia in the zebrafish

Traditionally, zebrafish have been used to model hematopoietic diseases that have known causative underlying genetic aberrations. Using murine *c-Myc* tagged with *enhanced green fluorescent protein* (*EGFP*) under the lymphoid-specific *recombination activating gene 2* (*rag2*) promoter in the zebrafish, Langenau et al. induced T-ALL after a short latency period [20]. Subsequent work included therapeutic testing with cyclophosphamide and vincristine, which demonstrated that T-ALL in the zebrafish recapitulated responses seen in mammalian models, thus validating this model [35]. The first zebrafish AML model employed *t(8;21)(q22;q22)*, resulting in the *RUNX1-ETO* fusion in