Bloody Zebrafish: Novel Methods in Normal and Malignant Hematopoiesis

Emma de Pater* and Eirini Trompouki**

1 Department of Hematology, Erasmus MC, Rotterdam, Netherlands, 2 Department of Cellular and Molecular Immunology, Max Planck Institute of Immunobiology and Epigenetics, Freiburg im Breisgau, Germany

Hematopoiesis is an optimal system for studying stem cell maintenance and lineage differentiation under physiological and pathological conditions. In vertebrate organisms, billions of differentiated hematopoietic cells need to be continuously produced to replenish the blood cell pool. Disruptions in this process have immediate consequences for oxygen transport, responses against pathogens, maintenance of hemostasis and vascular integrity. Zebrafish is a widely used and well-established model for studying the hematopoietic system. Several new hematopoietic regulators were identified in genetic and chemical screens using the zebrafish model. Moreover, zebrafish enables in vivo imaging of hematopoietic stem cell generation and differentiation during embryogenesis, and adulthood. Finally, zebrafish has been used to model hematopoietic diseases. Recent technological advances in single-cell transcriptome analysis, epigenetic regulation, proteomics, metabolomics, and processing of large data sets promise to transform the current understanding of normal, abnormal, and malignant hematopoiesis. In this perspective, we discuss how the zebrafish model has proven beneficial for studying physiological and pathological hematopoiesis and how these novel technologies are transforming the field.

Keywords: zebrafish, hematopoiesis, next generation sequencing, hematopoietic (stem) cells, technology

INTRODUCTION

Over the past three decades, zebrafish has been established as an important model to study various biological processes during development and homeostasis, including hematopoiesis. Many attractive features underpin the success of zebrafish as a model for vertebrate hematopoiesis. Cell-intrinsic and -extrinsic signaling mechanisms in hematopoiesis are well conserved between zebrafish and mammals, with the exception of a few hematopoietic niche components (Liao et al., 1998; Murayama et al., 2006; Bertrand and Traver, 2009; Paik and Zon, 2010; Goessling and North, 2011; Zhang and Liu, 2011; Zhang et al., 2013; Frame et al., 2017; Nik et al., 2017; Gore et al., 2018). Moreover, zebrafish embryos are small and transparent so they are ideal for imaging and easy to manipulate, at low cost. Additionally, genetic manipulation is easy and population studies can be easily performed in zebrafish. Thus, zebrafish have become invaluable vertebrate models for robust large-scale genetic screens (Mullins et al., 1994; Driever et al., 1996; Amsterdam et al., 1999) and, more recently, high-throughput chemical compound screens (North et al., 2007; Yeh et al., 2009). However, there are certain disadvantages in the zebrafish model. For example, zebrafish is not a mammal, but rather a poikilothermic animal in which the development of embryos occurs outside of the animal body and without placenta. That may lead to many metabolic and other differences between zebrafish and mammals, including drug action and utilization. Finally, the
zebrafish genome is duplicated and thus many genes have paralogs and homologs that make the otherwise easy genetic manipulation complicated (Glasauer and Neuhauss, 2014).

Embryonic hematopoiesis in zebrafish is a multistep process occurring in a spatially restricted manner in three distinct waves. During the intraembryonic primitive wave, the medial and anterior lateral mesoderm give rise to erythroid and myeloid cells, respectively. Erythro-myeloid progenitors (EMPs) form in the posterior blood island (PBI) during a transient intermediate wave. Finally, during the definitive wave, hematopoietic stem cells (HSC) with multilineage capacity originate in the aorta-gonad-mesonephros (AGM) region. The HSCs then translocate to and expand in the caudal hematopoietic tissue (CHT), which is followed by the colonization of the kidney and the thymus (Figure 1). Interestingly, it was recently discovered that HSC-independent T-cells can originate from the AGM and PBI during the embryonic and larval stages of development (Tian et al., 2017).

Hematopoietic differentiation in zebrafish is a multistep process involving embryonic hematopoiesis. As most cellular compartments have a certain degree of heterogeneity, with bulk RNA-seq one cannot distinguish between a small transcriptional difference in many cells, and a large transcriptional difference in a few cells. Several insightful reviews describe the different methods used for single-cell RNA-seq (Kolodziejczyk et al., 2015; Ziegenhain et al., 2017; Dal Molin and Di Camillo, 2018).

In zebrafish, one of the first methods used to characterize the transcriptome of single cells was mass parallel qPCR, where up to 96 transcripts could be analyzed in great sequencing depth using the Fluidigm system. This method revealed two distinct sub-populations of HSPCs in the CD41-GFP low-expressing stem cell compartment of the adult kidney marrow. Moreover, by using this technique and genetic ablation of T cells, a previously uncharacterized hematopoietic cytotoxic T/NK cell population in zebrafish was uncovered (Moore et al., 2016).

Recent technological advances in scRNA-seq have enabled analyses without restriction to specific transcripts. A re-examination of the CD41-GFPlow population revealed four HSPC sub-populations with different cellular characteristics and potential novel markers for HSCs were uncovered. Importantly, some cells in these subpopulations expressed the thrombocyte differentiation program long before they would have been characterized as thrombocytes, showing that there is an early lineage bias (Guo et al., 2013; Buenrostro et al., 2015; Paul et al., 2015; Drissen et al., 2016; Grover et al., 2016; Nestorowa et al., 2016; Osloss et al., 2016; Alberti-Servera et al., 2017; Velten et al., 2017; Villani et al., 2017; Buenrostro et al., 2018; Dahlin et al., 2018). In addition, scRNA-seq analyses in various transgenic lines revealed that ribosomal genes and lineage regulators control hematopoietic differentiation (Athanasiadis et al., 2017) and uncovered several novel hematopoietic populations, including two new types of NK cells (Tang et al., 2017). Finally, elegant comparative evolutionary studies on LCK-GFP transgenic zebrafish and mammals showed that membrane proteins are less conserved in NK cells than in T cells (Carmona et al., 2017).
cells promote the disease. This is remarkable as a single transgenic approach was used to initiate leukemogenesis and all leukemia cells overexpress Myc (Moore et al., 2016).

**Lineage Tracing**

Zebrafish has traditionally been utilized to lineage-trace differentiation during embryonic stages by labeling single cells with dyes and following them throughout development, until the dye fades or dilutes. However, the recent development of complex genetic models has removed this time restriction and enabled lineage tracing from the embryo into adulthood. For instance, HSPCs generated from the hemogenic endothelium of the aorta have been lineage-traced by using the multicolor transgenic labeling system "blood bow" (Henninger et al., 2017) in combination with high-end imaging and fluorescence-activated cell sorting (FACS). Additionally, labeling with CRISPR/Cas9 scarring in embryos and tracing of unique hematopoietic clones into adulthood has revealed that the hematopoietic system is only generated from a handful of cells present at dome stage (Alemany et al., 2018). This study claimed that all clones contribute to all blood lineages, a subject that is controversial in mammalian studies (Yamamoto et al., 2013; Notta et al., 2016; Pei et al., 2017).

A different approach for lineage-tracing cells consists of performing high-throughput scRNAseq at various developmental stages and then mapping similarities in transcriptional profiles across a pseudo timescale of differentiation (Macosko et al., 2015). By using this method in early embryogenesis, two independent studies have described gradually divergent differentiation patterns for specific lineages and uncovered signaling networks required for zebrafish development (Farrell et al., 2018; Wagner et al., 2018).

Future studies combining scRNAseq with lineage tracing will be paramount to advance our understanding of the developmental origins of hematopoietic populations. However, this approach has the important caveat that scRNA-seq does not provide topographic information for each individual cell. To overcome this limitation, the Van Oudenaarden and Bakkers laboratories have developed RNA-tomography (TOMOSEQ), a method that combines traditional histological techniques with low-input RNA sequencing and mathematical image reconstruction (Junker et al., 2014).

**IDENTIFICATION OF NOVEL REGULATORY MECHANISMS OF NORMAL AND MALIGNANT HEMATOPOIESIS**

**Chemical Screens to Identify Regulators of Normal and Abnormal Hematopoiesis**

Zebrafish is an ideal vertebrate model system to conduct bio-reactive compound screens (Zon and Peterson, 2005; Cusick et al., 2012; Tamplin et al., 2012; Veinotte et al., 2014; Rennekamp and Peterson, 2015; Deveau et al., 2017). The animals are small-sized and lay hundreds of eggs that develop very rapidly, thereby allowing the monitoring of compound activity and biotoxicity in vivo across development. Such screens have led to the identification of prostaglandin E2 as a compound that increases HSC production (North et al., 2007). Prostaglandin E2 is currently being investigated for HSC expansion applications in human and non-human primates (Goessling et al., 2011; Cutler et al., 2013).

Important insights into the molecular regulation of T-ALL came from zebrafish studies where immature T cells served as models for T-ALL cells. By screening small molecules for an effect on immature T cells using LCK-GFP transgenic zebrafish, a novel compound, 1H-indole-3-carbaldehyde quinolin-8-yl-hydrazone, named Lenaldekar, was identified with the potential to specifically attack T-ALL cells (Ridges et al., 2012). Lenaldekar also has a potential effect against autoimmune diseases such as multiple sclerosis, as they are caused by an off-target activity of T cells (Cusick et al., 2012). Currently there are ongoing clinical trials to study the effectiveness of this promising compound. These examples highlight the power of zebrafish models for screening novel chemical compounds affecting normal, abnormal or malignant hematopoiesis (Shafizadeh et al., 2004; Yeh et al., 2009;
Future studies addressing malignancy heterogeneity may combine chemical screens with scRNAseq to identify therapy-resistant cells and explore the mechanisms underpinning resistance to treatment in individual cells, a fundamental unresolved question in the cancer research field.

Effects of Perturbations in Embryonic HSC Generation and Adult Hematopoiesis

Several acute myeloid leukemia (AML) predisposition syndromes are caused by innate mutations in transcription factors that affect embryonic hematopoiesis, such as Gata2 and Runx1 (Rabushok et al., 2016), suggesting that perturbations in embryonic hematopoiesis affect the adult HSC compartment. As the effects of alterations in embryonic hematopoiesis can be easily monitored in zebrafish throughout development, as well as during adulthood, this is an excellent system to study AML predisposition syndromes. Until the recent development of targeted gene editing, manipulating the zebrafish genome to create specific mutations for making knockout and knockin animals was challenging. Although TILLING (Targeting Induced Local Lesions in Genomes) was a significant advancement, this is a costly method that requires thousands of fish to search for a STOP codon in the gene of interest. Moreover, TILLING is rather limiting as it does not allow to induce specific mutations (Wienholds et al., 2003; Draper et al., 2004). Targeting the zebrafish genome with zinc-finger nucleases was the beginning of a new era in zebrafish biology, as selected genes could finally be specifically targeted for genome editing (Amacher, 2008; Foley et al., 2009). Shortly after this technology was introduced, TALENS (Dahlem et al., 2012; Hwang et al., 2014; Huang et al., 2016; Liu et al., 2016), and more recently, CRISPR/Cas9 (Hruscha et al., 2013; Irion et al., 2014; Shah et al., 2015; Li et al., 2016; Liu et al., 2017) were developed. Whilst it is relatively easy to generate knockouts and large deletions with these gene-targeting techniques, making knockin animals remains challenging. Nevertheless, several laboratories have successfully created knockin animals by using CRISPR/Cas9 and co-injecting a repair template to facilitate homology-directed repair (Hruscha et al., 2013; Auer et al., 2014; Albadri et al., 2017; Kesavan et al., 2017). Additionally, Cre/lox, Flp/FRT, and ΦC31 systems are also currently being used in zebrafish for precise genome editing (Mosimann et al., 2013; Felker and Mosimann, 2016; Carney and Mosimann, 2018). Importantly, tissue-specific expression of Cas9 in the hematopoietic system can be performed in zebrafish to enable conditional manipulation of hematopoietic cells (Abrahmsen et al., 2015). A major caveat in both perturbing the zebrafish genome and comparing the zebrafish with the mammalian transcriptome in the context of clinical translation, is, as previously mentioned, the Teleost genome duplication (Glasauer and Neuhauss, 2014). As a result most genes are present twice with (partially) redundant biological roles. This means that for a complete perturbation of a mammalian gene, the zebrafish counterparts have to be removed both, complicating genetic crossings and analyses.

Epigenetic Regulation of the Hematopoietic System

Chromatin conformation is essential for controlling gene expression, and deregulation of this process may cause malignant transformation (Groschel et al., 2014). Zebrafish is an excellent system to explore the mechanisms underlying chromatin regulation and to evaluate the effects of chromatin-modifying drugs in vivo. Gene regulatory elements can be identified in zebrafish using chromatin immunoprecipitation combined with sequencing (ChiP-seq), however, the technique is limited by the low number of zebrafish-specific antibodies currently available and the large amount of input material required (Havis et al., 2006; Trompouki et al., 2011; Bogdanovic et al., 2013). ChiP-seq has been mostly used in early zebrafish embryos (Paik et al., 2010; Vastenhouw et al., 2010; Bogdanovic et al., 2012; Xu et al., 2012; Winata et al., 2013; Nelson et al., 2017; Meier et al., 2018). Antibodies against histone marks, which are highly conserved between species, have been successfully utilized in zebrafish erythrocytes to describe the potential locus control region (LCR) regulating globin expression (Ganis et al., 2012). Moreover, given the functional conservation of these genes, zebrafish is useful to functionally validate enhancers identified in mouse and/or human models (Tijssen et al., 2011; Chiang et al., 2017).

Other techniques for identifying gene regulatory elements are based on the detection of open chromatin, for instance, assay for transposase-accessible chromatin with high-throughput sequencing (ATAC-seq). ATAC-seq requires much less input material than ChiP-seq, and has even been used successfully with single cells (Fernandez-Minan et al., 2016; Doganli et al., 2017). This method allowed the identification of endothelial enhancers (Quillien et al., 2017) and revealed the role of cohesin in rearranging the genomic architecture during the transition of maternal to zygotic transcription in early embryos (Meier et al., 2018). Combining scRNA-seq with ATAC-seq and immune phenotypic analysis is a powerful approach to integrate our understanding of lineage differentiation with the regulatory elements involved in that process (Buenrostro et al., 2018). DNA methylation studies can also be used to understand chromatin accessibility, although more material is needed in these methods. Methylation experiments have been conducted in zebrafish albeit not specifically in the hematopoietic system (Lee et al., 2015; Kaaij et al., 2016). Since many tissue-specific fluorescent lines exist in zebrafish, future research should aim to identify enhancers and promoters in specific cell types, rather than using whole embryos.

Despite the advantages of ATAC-seq and methylation analyses, these approaches cannot offer the same information as ChiP-seq. Thus, improved ChiP-seq protocols, such as the high sensitivity indexing-first chromatin immunoprecipitation approach (iChIP) developed in Ido Amit’s laboratory, should be adapted to zebrafish (Gury-BenAri et al., 2016). Moreover, it would be important to unravel chromatin interactions in active enhancer and promoter regions during hematopoiesis. However, although chromatin conformation has been studied in early zebrafish embryos (Gomez-Marin et al., 2015; Fernandez-Minan et al., 2016), to date no studies have addressed this question specifically in zebrafish hematopoiesis. It is important to mention...
the combined effort of many groups to collate all available genome-wide data in zebrafish in the DANIO-CODE Data Coordination Center\(^1\) (Tan et al., 2016). This recently launched database will provide an easy access to high-quality genome data to all scientists.

**Proteomics and Metabolomics Studies**

In the era of genome-wide technology, gene expression studies should be complemented with proteomic studies, as transcriptional and translational outcomes can sometimes differ. Additionally, the extension of these analyses to metabolomics may uncover another layer of regulation critical for hematopoiesis. Indeed, it was recently shown that dormant stem cell populations have low metabolic activity, and this is required to maintain the hematopoietic system during aging and periods of intense stress (Cabezas-Wallscheid et al., 2017). Although proteomics and metabolomics methods have not yet been extensively explored in zebrafish, particularly in the hematopoietic system, some studies have reported differences between transcript and protein levels in multiple genes by using proteomic analyses either in whole zebrafish embryos or in specific cell populations during regeneration (Alli Shaik et al., 2014; Baral et al., 2014; Rabinowitz et al., 2017). Metabolomics has proven useful to understand the neurological damage resulting from chemical perturbations in zebrafish embryos (Ong et al., 2009; Rabinowitz et al., 2017; Roy et al., 2017). Finally, as mass spectrometry analyses are constantly improving, the sensitivity of these methods will likely overcome the current problem of heterogeneous and low cell-number populations.

**CONCLUSION**

The zebrafish has become an invaluable model system for understanding how HSCs form and are maintained, and how hematopoietic cell differentiation is regulated during embryogenesis and in adulthood. The unique advantages offered by this model system over traditional mouse models regarding the use in chemical screens and the accessibility during embryonic stages allowing easy manipulation and visualization and tracing into adult stages, in combination with recent new technologies (Figure 2), have opened the way for novel exciting hypotheses on the mechanisms promoting hematopoietic diseases, the role of the niche in normal and malignant hematopoiesis, and the effect of chemical compounds on malignant cells. The high conservation between the zebrafish and human hematopoietic systems means that discoveries in fish may have strong translational potential and important clinical implications for the treatment of hematopoietic diseases.

\(^1\)https://danio-code.zfin.org
AUTHOR CONTRIBUTIONS
Edp and ET conceived and wrote this manuscript.

FUNDING
Edp was supported by EHA junior non-clinical research fellowship and by KWF/Alpe’dHuzes (SK10321). ET was supported by the Max Planck Society, a Marie Curie Career Integration Grant (631432 Bloody Signals), the Deutsche Forschungsgemeinschaft, Research Training Group GRK2344 “MeInBio – BioInMe,” and by The Fritz Thyssen Stiftung (Az 10.17.1.026MN).

ACKNOWLEDGMENTS
We thank Dr. I. P. Touw for careful reading of the manuscript, Dr. van Royen, E. Gioacchino, J. Peulen for graphical contributions and Dr. T. Clapes for producing the second figure.

REFERENCES
Ahlain, J., Durand, E. M., Yang, S., Zhou, Y., and Zon, L. I. (2015). A CRISPR/Cas9 vector system for tissue-specific gene disruption in zebrafish. Dev. Cell 32, 756–764. doi: 10.1016/j.devcel.2015.01.032
Albadri, S., Del Bene, F., and Revenu, C. (2017). Genome editing using CRISPR/Cas9-based knock-in approaches in zebrafish. Methods 121-122, 77–85. doi: 10.1016/j.ymeth.2017.03.005
Alberti-Servera, L., von Muenchow, L., Tsapogas, P., Capoferri, G., Eschbach, K., Beisel, C., et al. (2017). Single-cell RNA sequencing reveals developmental heterogeneity among early lymphoid progenitors. EMBO J. 36, 3619–3633. doi: 10.15252/embj.201797105
Allman, A., Florescu, M., Baron, C. S., Peterson-Maduro, J., and van Oudenaarden, A. (2018). Whole-organism clone tracing using single-cell sequencing. Nature 556, 108–112. doi: 10.1038/nature25969
Alii Shaik, A., Wee, S., Li, R. H., Li, Z., Carney, T. J., Mathavan, S., et al. (2014). Ablain, J., Durand, E. M., Yang, S., Zhou, Y., and Zon, L. I. (2015). A CRISPR/Cas9 vector system for tissue-specific gene disruption in zebrafish. Dev. Cell 32, 756–764. doi: 10.1016/j.devcel.2015.01.032
Albadri, S., Del Bene, F., and Revenu, C. (2017). Genome editing using CRISPR/Cas9-based knock-in approaches in zebrafish. Methods 121-122, 77–85. doi: 10.1016/j.ymeth.2017.03.005
Alberti-Servera, L., von Muenchow, L., Tsapogas, P., Capoferri, G., Eschbach, K., Beisel, C., et al. (2017). Single-cell RNA sequencing reveals developmental heterogeneity among early lymphoid progenitors. EMBO J. 36, 3619–3633. doi: 10.15252/embj.201797105
Allman, A., Florescu, M., Baron, C. S., Peterson-Maduro, J., and van Oudenaarden, A. (2018). Whole-organism clone tracing using single-cell sequencing. Nature 556, 108–112. doi: 10.1038/nature25969
Alii Shaik, A., Wee, S., Li, R. H., Li, Z., Carney, T. J., Mathavan, S., et al. (2014). Functional mapping of the zebrafish early embryo proteome and transcriptome. J. Proteome Res. 13, 5536–5550. doi: 10.1021/pr5005136
Amacher, S. L. (2008). Emerging gene knockout technology in zebrafish: zinc-finger nucleases. Brief. Funct. Genomic. Proteomic. 7, 460–464. doi: 10.1093/bfgp/eln043
Amsterdam, A., Burgess, S., Golling, G., Chen, W., Sun, Z., Townsend, K., et al. (1999). A large-scale insertional mutagenesis screen in zebrafish. Genes Dev. 13, 2713–2724. doi: 10.1101/gad.20.2713
Arrenberg, A. B., Stainier, D. Y., Baier, H., and Hausken, J. (2010). Optogenetic control of cardiac function. Science 330, 971–974. doi: 10.1126/science.1195929
Arulmozhiwarman, G., Stotter, M., Bickle, M., Krater, M., Wobus, M., Ehninger, G., et al. (2016). In vivo chemical screen in zebrafish embryos identifies regulators of hematopoiesis using a semiautomated imaging assay. J. Biomol. Screen. 21, 956–964. doi: 10.1177/108705711664163
Athanasiadis, I. E., Bothof, J. G., Andres, H., Ferreira, L., Lio, P., and Cvejic, A. (2017). Single-cell RNA-sequencing uncovercs transcriptional states and fate decisions in haematopoiesis. Nat. Commun. 8:2045. doi: 10.1038/s41467-017-02305-6
Auer, T. O., Douroue, K., De Cian, A., Concordet, J. P., and Del Bene, F. (2014). Highly efficient CRISPR/Cas9-mediated knock-in in zebrafish by homology-independent DNA repair. Genome Res. 24, 142–153. doi: 10.1101/gr.161638.113
Babushok, D. V., Bessler, M., and Olson, T. S. (2016). Genetic predisposition to myelodysplastic syndrome and acute myeloid leukemia in children and young adults. Leukemia 57, 520–536. doi: 10.1038/leu.2015.115041
Baral, R., Ngounou Wette, A. G., Darie, C. C., and Wallace, K. N. (2014). Mass spectrometry for proteomics-based investigation using the zebrafish vertebrate model system. Adv. Exp. Med. Biol. 806, 331–340. doi: 10.1007/978-3-319-06068-2_15
Bertrand, J. Y., Chi, N. C., Santosto, B., Teng, S., Stainier, D. Y., and Traver, D. (2010). Haematopoietic stem cells derive directly from aortic endothelium during development. Nature 464, 108–111. doi: 10.1038/nature08738
Bertrand, J. Y., and Traver, D. (2009). Haematopoietic cell development in the zebrafish embryo. Curr. Opin. Hematol. 16, 243–248. doi: 10.1097/MOH.0b013e2821de550e
Bogdanovic, O., Fernandez-Minan, A., Tena, J. J., de la Calle-Mustienes, E., and Gomez-Skarmeta, J. L. (2013). The developmental
Travnickova, J., Tran Chau, V., Julien, E., Mateos-Langerak, J., Gonzalez, C., Leleivre, E., et al. (2015). Primitive macrophages control HSCP mobilization and definitive haematopoiesis. Nat. Commun. 6:6227. doi: 10.1038/ncomms7227

Trompouki, E., Bowman, T. V., Dibiase, A., Zhou, Y., and Zon, L. I. (2011). Chromatin immunoprecipitation in adult zebrafish red cells. Methods Cell Biol. 104, 341–352. doi: 10.1016/B978-0-12-374814-0.00019-7

Vastenhouw, N. L., Zhang, Y., Woods, I. G., Imam, F., Regev, A., Liu, X. S., et al. (2010). Chromatin signature of embryonic pluripotency is established during genome activation. Nature 464, 922–926. doi: 10.1038/nature08866

Veinotte, C. J., Dellaire, G., and Berman, J. N. (2014). Hooking the big one: the potential of zebrafish xenotransplantation to reform cancer drug screening in the genomic era. Dis. Model. Mech. 7, 271–281. doi: 10.1038/nbm.2947

Velten, L., Haas, S. F., Raffel, S., Blaszkiewicz, S., Islam, S., Hennig, B. P., et al. (2017). Human haematopoietic stem cell lineage commitment is a continuous process. Nat. Cell Biol. 19, 271–281. doi: 10.1038/ncl3493

Villani, A. C., Satija, R., Reynolds, G., Sarkizova, S., Shekhar, K., Fletcher, I., et al. (2017). Single-cell RNA-seq reveals new types of human blood dendritic cells, monocytes, and progenitors. Science 356:eaah4573. doi: 10.1126/science.aaah4573

Wagner, D. E., Weinreb, C., Collins, Z. M., Briggs, J. A., Megason, S. G., and Klein, A. M. (2018). Single-cell mapping of gene expression landscapes and lineage in the zebrafish embryo. Science 360, 981–987. doi: 10.1126/science.aar4362

White, R. M., Sessa, A., Burke, C., Bowman, T., LeBlanc, J., Ceol, C., et al. (2008). Transparent adult zebrafish as a tool for in vivo transplantation analysis. Cell Stem Cell 2, 183–189. doi: 10.1016/j.stem.2007.11.002

Wienholds, E., van Eeden, F., Kosters, M., Mudde, J., Plasterk, R. H., and Cuppen, E. (2003). Efficient target-selected mutagenesis in zebrafish. Genome Res. 13, 2700–2707. doi: 10.1101/gr.1725103

Winata, C. L., Kondrychyn, I., Kumar, V., Srinivasan, K. G., Orlov, Y., Ravishankar, A., et al. (2013). Genome wide analysis reveals Zic3 interaction with distal regulatory elements of stage specific developmental genes in zebrafish. PLoS Genet. 9:e1003852. doi: 10.1371/journal.pgen.1003852

Xu, C., Fan, Z. P., Muller, P., Fogley, R., DiBiase, A., Trompouki, E., et al. (2012). Nanog-like regulates endoderm formation through the Mxtx2-Nodal pathway. Dev. Cell 22, 625–638. doi: 10.1016/j.devcel.2012.01.003

Yamamoto, R., Morita, Y., Ooehara, J., Hamanaka, S., Onodera, M., Rudolph, K. L., et al. (2013). Clonal analysis unveils self-renewing lineage-restricted progenitors generated directly from hematopoietic stem cells. Cell 154, 1112–1126. doi: 10.1016/j.cell.2013.08.007

Yeh, J. R., Munson, K. M., Elagib, K. E., Goldfarb, A. N., Sweetser, D. A., and Peterson, R. T. (2009). Discovering chemical modifiers of oncogene-regulated hematopoietic differentiation. Nat. Chem. Biol. 5, 236–243. doi: 10.1038/nchembio.147

Zhang, C., Pati, R., and Liu, F. (2013). Hematopoietic stem cell development and regulatory signaling in zebrafish. Biochim. Biophys. Acta 1830, 2370–2374. doi: 10.1016/j.bbagen.2012.06.008

Zhang, P., and Liu, F. (2011). In vivo imaging of hematopoietic stem cell development in the zebrafish. Front. Med. 5:239–247. doi: 10.1007/s11684-011-0123-0

Ziegenhain, C., Vieth, B., Parekh, S., Reinius, B., Guillaume-Adkins, A., Smets, M., et al. (2017). Comparative analysis of single-cell RNA sequencing methods. Mol. Cell. 65, 631–643.e4. doi: 10.1016/j.molcel.2017.01.023

Zon, L. I., and Peterson, R. T. (2005). In vivo drug discovery in the zebrafish. Nat. Rev. Drug Discov. 4, 35–44. doi: 10.1038/nrd1606

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Copyright © 2018 de Pater and Trompouki. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.