Editorial

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Zebrafish MYC-induced leukemia models: unique *in vivo* systems to study B and T cell acute lymphoblastic leukemia

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"*mMyc*/*hMYC* zebrafish are the first animal models described to develop both T-ALL and B-ALL"

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Over the past two decades, zebrafish (*Danio rerio*) has become an increasingly powerful *in vivo* oncology model system. Technical and practical advantages, such as their low cost, high fecundity and optical translucency, together with the ease of building transgenic zebrafish, all make *D. rerio* excellent for studying pathways altered in cancer and also facilitate their use in high-throughput anticarcinogenic chemical library/drug screens. In particular, both genetically and functionally, oncogenic and developmental hematopoietic pathways are highly conserved between teleost (bony) fish and mammals. These features have allowed the creation of zebrafish models mimicking several human hematopoietic malignancies, such as acute myeloid leukemia (AML) and T-cell lymphoblastic lymphoma (T-LBL) and acute lymphoblastic leukemia (T-ALL) [1,2]. Surprisingly, zebrafish B cell malignancy models lagged behind, with only a single 2006 report of pre-B-cell ALL (pre-B ALL) in a transgenic line expressing human *ETV6-RUNX1* [3]. No subsequent publications using this line exist, likely because it exhibited low incidence and long latency, making it challenging to study.

Recently, the scarcity of zebrafish B-cell leukemia models was addressed by the related but distinct discoveries [4,5] that B-ALL occurs in fish expressing either transgenic murine or human *c-MYC* (*mMyc* and *hMYC*, respectively), controlled by a zebrafish *rag2* promoter, which is active in immature B and T lymphoblasts. Previously, these lines were thought to exclusively develop T-ALL [6,7], although a logical explanation for this exclusivity was lacking. As noted, in zebrafish and in mammals, *rag2* is expressed by both B and T cell progenitors and MYC is known to drive B cell cancers like Burkitt lymphoma and B-ALL [8,9]. These recent papers proved that *mMyc*/*hMYC*-induced B-ALL occurs, but was apparently overlooked until now. In the paper by Garcia *et al.* [4], RNA-seq analysis of a cohort of 12 *rag2:mMyc*-induced monoclonal tumors [10] revealed the presence of B-ALL. Specifically, the authors found nine T-ALL, two pro-B ALL and one bi-phenotypic ALL that expressed both T and B lineage markers. In contrast, Borga *et al.* [5] used double-transgenic *rag2:hMYC;lck:GFP* fish, where differential *lck* expression drives distinct levels of GFP in B- and T- cells, with B cells expressing less GFP than T cells. This study reported data from >50 *hMYC*-driven ALL, proving the existence of highly penetrant B-ALL that resembled human pre-B-ALL. Of note, the authors also found several instances of 'mixed' ALL that was not the bi-phenotypic ALL described by Garcia *et al.*, but rather simultaneous cases of B- and T-ALL arising in the same animal.

Notably, *mMyc*/*hMYC* zebrafish are the first animal models described to develop both T-ALL and B-ALL. This highlights *MYC*'s potency as an oncogene in both lymphocyte lineages, but MYC may use distinct molecular mechanisms and activate unique oncogenic pathways in T- versus B-ALL; these models provide systems to explore these differences. Of note, although *c-MYC* has pathogenic roles in many types of cancer, MYC-mediated molecular mechanisms of tumorigenesis are not fully understood and likely differ by malignancy type. For example, it is known that overexpressing *MYC* is not sufficient for tumorigenesis, with varying cooperative genetic lesions needed to

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develop either T-ALL [11] or Burkitt lymphoma [12]. Similarly, MYC-induced T- or B-ALL probably requires different cooperating genetic events in zebrafish – and in humans.

It can be argued that this hypothesis is bolstered by the finding that even though *MYC* was expressed equally by zebrafish *mMyc/hMYC* T and B malignancies, lower frequencies of leukemia-propagating cells (a surrogate for leukemia stem cells, or 'LSC') were seen in B-ALL than T-ALL [4,5]. These LSC differences may derive from MYC-regulated genes that differ in ALL of the B versus T lymphocyte lineages, distinct contributing genetic events that distinguish B- and T-ALL, a combination of both, or other as-yet-unrecognized factors. Intriguingly, T-ALL has historically been considered more aggressive than B-ALL, requiring intensified therapies; this could parallel the higher LSC numbers in zebrafish *m Myc/h MYC*-driven T- versus B-ALL. Whether true or not, because *mMyc/hMYC*-transgenic fish model both types of human ALL, both transgenic lines offer the significant advantage of studying these diseases in an identical genetic background, or, in the case of *hMYC* fish, the same animal.

Such studies are not limited to LSC biology, but extend to investigations that probe other mechanisms governing leukemogenesis in B and T lymphoblasts, which aim to identify specific molecular targets and, ultimately, more effective treatments. For example, studies with zebrafish monoclonal ALLs, based on the model described by Blackburn *et al.* [10], provide an experimental schema to glean insights relevant to *in vivo* intratumoral heterogeneity and clonal evolution. Using this approach, it is possible to study the clonal composition of MYC-induced T-ALL or B-ALL, characterize clones with salient biologic attributes (e.g., increased *in vivo* aggressiveness, higher LSC activity, greater resistance to a specific therapy, etc.) and identify the pathways and mechanisms responsible for those attributes. To date, such clonal interrogation has only been investigated in *D. rerio mMyc* T-ALL [10], but clearly this tactic is broadly applicable to T-ALL and B-ALL of both MYC models.

It is easy to envision *mMyc/hMYC* B-ALL models' utility in compound library screens, long-standing approaches for *D. rerio* investigators. In fact, zebrafish T-ALL studies have pioneered these strategies. In addition, other work has likewise leveraged the morphologic and genetic similarities between *D. rerio* and human T-ALL [1,2] to identify novel genes and mechanisms that drive T-ALL, which are potential therapeutic targets. For example, studies with zebrafish *mMyc/hMYC* T-ALL have revealed that: different *BCL2* levels in MYC-induced cancers differentiate T-LBL versus T-ALL, showing meaningful differences between two malignancies frequently considered as one disease [13]; repression of the pro-apoptotic *BIM* gene is a key event downstream of MYC and PI3K-AKT in high-risk T-ALL [14]; a key role for dihydrolipoamide S-succinyltransferase (DLST) in the tricarboxylic acid (TCA) cycle metabolism that supports MYC-mediated T-leukemogenesis [15] and MYC-induced overexpression of *UFD1*, which enhances degradation of misfolded proteins in the endoplasmic reticulum (ER), reducing ER stress to promote T-ALL cell survival and aggressiveness [16]. Zebrafish B-ALL models are likewise amenable to the approaches used in these investigations.

Returning to screening, the ability to test hundreds of translucent embryos or adult fish labeled by fluorescent cancer cell markers makes *D. rerio* an excellent system for high-throughput assays rapidly testing large numbers of compounds. *hMYC* fish have previously been used in chemical library screens, which led to the discoveries of lenaldekar [17] and perphenazine as potential drugs for T-ALL treatment [18]. Importantly, both compounds were shown to have direct anticancer effects on primary human leukemias and human cell lines, and perphenazine has since been used to treat canine T-LBL [19]. These efforts demonstrate zebrafish cancer models that can be employed for novel targeted therapeutic discovery. The same *mMyc/hMYC* B-ALL models that were used in the aforementioned T-ALL studies are identically tractable to strategies studying B cell leukemogenesis to find novel anti-B-ALL agents, particularly drugs targeting MYC-mediated oncogenic pathways.

Recently, unexpected differences were found between *mMyc*- and *hMYC*-induced B-ALL [20] indicating these models are not redundant with respect to their utility. This finding was unforeseen, as the two models differ only in terms of their MYC species of origin (murine versus human) and their genetic background. Despite their near-identical composition, gene expression analysis revealed *mMyc* and *hMYC* B-ALL arise in distinct B cell lineages (*ighm*$^+$ and *ighz*$^+$, respectively) and possess highly divergent transcriptional profiles. Because *mMyc* and *hMYC* are not interchangeable and cause different B cell leukemias, this broadens these models' suitability for complementary B-ALL genetic and drug screens. At the same time, they also provide platforms to study leukemogenic molecular mechanisms shared by *mMyc* and *hMYC*, or unique to one but not the other. In the near future, it will be important to resolve whether *mMyc* and *hMYC* B-ALL correspond to defined human B-ALL subtypes, which would enhance their clinical applicability even further. It may be possible to address this via interspecies transcriptional profiling, but an improved understanding of zebrafish B-lymphopoiesis will likely be required to truly determine the human equivalents of the *D. rerio ighz*$^+$ and *ighm*$^+$ B cell lineages.

Together, both MYC transgenic zebrafish lines are outstanding *in vivo* systems that can serve multiple purposes. First, they can be used to identify MYC-activated mechanisms driving T- and B-ALL, including pathways controlling LSC frequency, *in vivo* aggressiveness and clonal competition, differences between ALL and LBL or other biologic features. Second, they provide ideal animal models to seek and test novel therapeutics with antineoplastic effects in B- and/or T-ALL. Third, and surprisingly, they can be used to study oncogenic pathways differentially activated by *mMyc* versus *hMYC*. These, and other ideas not detailed here, will undoubtedly enhance our understanding of both B-ALL, the most common pediatric malignancy, and MYC, arguably the most oncogene in all of human cancer.

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