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ZenoFishDb v1.1: A Database for Xenotransplantation Studies in Zebrafish

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Abstract

Rapidly accumulating literature has proven feasibility of the zebrafish xenograft models in cancer research. Nevertheless, online databases for searching the current zebrafish xenograft literature are in great demand. Herein, we have developed a manually curated database, called ZenoFishDb v1.1 (https://konulab.shinyapps.io/zenofishdb), based on R Shiny platform aiming to provide searchable information on ever increasing collection of zebrafish studies for cancer cell line transplantation and patient-derived xenografts (PDXs). ZenoFishDb v1.1 user interface contains four modules: *DataTable*, *Visualization*, *PDX Details*, and *PDX Charts*. The *DataTable* and *Visualization* pages represent xenograft study details, including injected cell lines, PDX injections, molecular modifications of cell lines, zebrafish strains, as well as technical aspects of the xenotransplantation procedures in table, bar, and/or pie chart formats. The *PDX Details* module provides comprehensive information on the patient details in table format and can be searched and visualized. Overall, ZenoFishDb v1.1 enables researchers to effectively search, list, and visualize different technical and biological attributes of zebrafish xenotransplantation studies particularly focusing on the new trends that make use of reporters, RNA interference, overexpression, or mutant gene constructs of transplanted cancer cells, stem cells, and PDXs, as well as distinguished host modifications.

Keywords: zebrafish, xenograft, cancer, database, R shiny, patient-derived xenograft

Introduction

Tumor xenograft models, particularly of rodents, have long been used in scientific research.[1–4] Today's state-of-the-art technologies allow use of transgenic rodent models in cancer research through cell line-derived xenotransplantation[5] and transplantation of patient-derived xenografts (PDXs).[5,6] Innumerable xenograft studies performed in rodents have resulted in great demand for established bibliotheca where information from them could be entered and updated collectively providing easy access. Accordingly, several databases or tools exhibiting collection of rodent xenotransplantation studies have been developed, and they mainly focus on PDX studies in mouse models.[7–10] For example, MTB (Mouse Tumor Biology)[7] provides information on tumor, strain, genetic architecture, pathology images, and gene expression datasets, as well as providing a link to The Jackson Laboratory and EMBL-EBI joint project, PDX Finder.[11] In addition, organ specific xenograft databases of mouse models are also present,[9] while a commercial xenograft cell line database by Taconic Biosciences, Inc.,[12] provides another platform for cell-line specific transplantations.

Zebrafish is a valuable vertebrate model organism that has more recently emerged in the xenograft field.[13] The use of zebrafish embryos in xenotransplantation has generated novel avenues for researchers to explore different aspects of basic and applied sciences, including cancer biology as reviewed in the literature.[14–16] Moreover, xenograft studies in zebrafish offer enormous benefits and a broad range of applications since effects of transient or stable modifications in immortalized or primary cell lines can be tested during embryogenesis/organogenesis. In particular, the modifications introduced by overexpression vectors,[17–19] as well as RNA interference technologies,[20,21] help identify gene- and/or mutation-specific effects on tumor characteristics *in vivo* in zebrafish. However, the increasing number of zebrafish

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xenograft studies in cancer biology has made systematic analysis and curation necessary.

Herein, first ever zebrafish-specific xenograft database, ZenoFishDb v1.1, has been generated using Shiny package[22] in the R programming environment[23] with a particular focus on zebrafish transplantation studies of molecularly modified cells, PDXs, and cancer stem cells (CSCs), as well as those performed on modified hosts.

Materials and Methods

Contents of ZenoFishDb v1.1

We have reviewed and manually curated the literature regarding zebrafish xenograft studies, particularly focusing on molecular- and strain-specific modifications; and an updatable excel spread sheet containing different attributes from the selected studies has been generated. Accordingly, the data used in ZenoFishDb v1.1 include different individual research elements/fields extracted from full texts, including the type of cancer, injected cell line or cell type, taxonomic species of the injected cell line, type of the molecular modification (e.g., overexpression, short hairpin RNA [shRNA], small interfering RNA [siRNA]), official name of the modified gene, number of cells injected, injection site and time, developmental stage of the fish, name of the injected zebrafish line, fluorescence source (or reporter), biological assessment (e.g., invasion, angiogenesis, tumor size), type of host strain modifications (e.g., transgenes and mutations), and references, including PubMed IDs. The excel spread sheet has been imported into the R environment before parsing and processing for downstream analyses and visualization processes.

Development of ZenoFishDb v1.1 using R Shiny

ZenoFishDb v1.1 is an interactive web application developed using the Shiny framework in R.[22,23] The database features four main components: *DataTable, Visualization, PDX Details*, and *PDX Charts*.

The *DataTable* provides sorting, pagination, and filtering while containing comprehensive information about xenograft studies in ZenoFishDb v1.1 using the *DT package,* an R interface of JavaScript library *DataTables.*[24] In addition to the intrinsic filtering operations done by *DataTables* library, other filtering options are presented to the user upon selection of attributes of interest and respective subselections based on *dplyr* package.[25]

ZenoFishDb v1.1 *Visualization* page allows for the statistical analysis of selected data. This component of the database operations works upon selection of a column of interest from the uploaded excel file to display pie and/or bar chart of the proportional distribution of the selected data using *Plotly,* an open source R graphing library.[26]

PDX Details and *PDX Charts* utilize the same R packages for tabular data manipulation and visualization as the previously aforementioned components of the application, while expanding on the PDX study details specifically. ZenoFishDb v1.1 is hosted and maintained online at *shinyapps.io* servers. Updates are planned biannually and will be performed upon collection and manual curation of new publications as they arise in the zebrafish xenograft research field.

Results

ZenoFishDb v1.1: DataTable, Visualization, PDX Details, PDX chart modules

ZenoFishDb v1.1 enables a thorough search for existing zebrafish xenograft studies in the literature focusing on those with molecular interventions and/or involving use of stem cells and PDXs. With this intention, the literature has been mined for ''zebrafish xenograft,'' ''zebrafish xenotransplant,'' ''zebrafish xenotransplantation,'' ''zebrafish patient derived xenograft,'' ''zebrafish xenograft microenvironment,'' ''zebrafish xenograft morpholino,'' ''zebrafish xenograft crispr,'' ''zebrafish xenograft mutation,'' ''zebrafish xenograft primary cell,'' and similar keywords through NCBI PubMed search page. A total number of 211 studies focusing on the application of molecularly modified cell, PDX, and/or stem cell transplantations, as well as studies with distinct host modifications and microenvironments, have been incorporated into the current version of ZenoFishDb v1.1 manually. Accordingly, the reviewed literature and curated data have been projected onto four compartments and described in detail as follows.

The *DataTable* provides information on the technical and biological details of research articles in a table format. The origin of transplanted cancer cells and/or tissue, their abbreviations, species of the injected cell lines, injected cell lines and cell lines subjected to molecular modifications, modified genes, available PDX studies, stem cell properties of injected cells, treatments applied to xenografts, injection sites, original and categorized injected cell numbers, developmental stage, injection time, zebrafish strains, host modifications and their details, cell tracking sources, biological assessments, tumor assessment end points, references, and PubMed hyperlinks are included in the *DataTable*. A fine-tuned search is also available through the ''Attributes'' and the ''Subselections'' tabs on the *DataTable* (Fig. 1A).

The *Visualization* webpage is designed to deliver graphical and statistical data for the information displayed through the *DataTable.* Herein, an attribute could be selected through the ''Columns'' tab, and the schematic representation could be accessed through the ''Bar Chart'' and ''Pie Chart'' options. The information provided through the page includes the number of total variables, unique variables, and percentage of the selected attribute. Visualization and generation of figures can be manually adjusted through ''Chart height,'' ''Legend font size,'' ''Inside text font size,'' and ''Barplot label size'' options, and images can be downloaded as .png files. In addition, information represented on histograms can be downloaded in the table format. A screenshot displaying all the features of the *Visualization* module has been provided with an example attribute, that is, ''cancer/tissue of origin'' (Fig. 1B and Supplementary Table S1).

The *PDX Details* module (Fig. 2A) is designated to deliver cumulative information on the PDX studies incorporated to ZenoFishDb v1.1. Herein, the data on patients and/or tumors, including age/sex/ethnicity, disease name, primary site, metastasis or recurrence status, treatment status, clinical information, cytogenetic information, karyotype analysis, and other relevant data, are provided. In addition, details about the engraftment have also been incorporated for each case. These features include type of injection (patient-derived tissue engraftment [PDX-tissue] or tissue-derived cell line engraftment

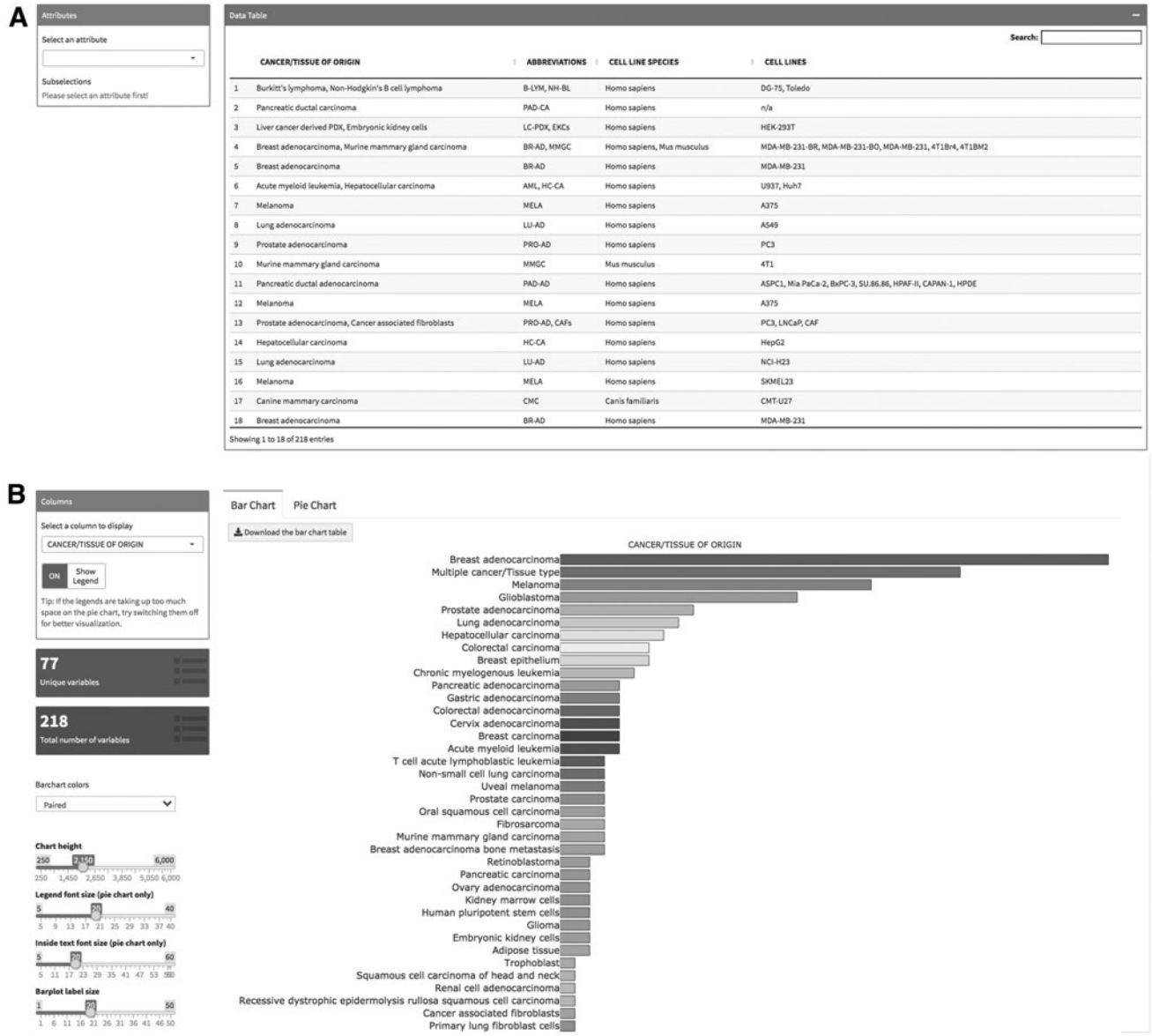


FIG. 1. *DataTable* and *Visualization* modules of ZenoFishDb v1.1. **(A)** Screenshot of *DataTable* displaying the list of the reviewed and manually curated data in a table format. Selected articles are displayed in descending order according to their release dates as default. Selection and subselection tabs enable fine-tuned search categories providing detailed information for the selected items. **(B)** Screenshot of introductory *Visualization* page displaying the overview of this module with an example of descriptive statistics of the cancer types/tissue of origin entitled with full names. Figures are available in greater detail online.

[PDX-cell line]), injected cell numbers, injection site, fish strain, and injection period together with relevant PMID ID.

The *PDX Charts* module (Fig. 2B) has been integrated to display the bar chart of the most frequently mentioned attributes of the *PDX Details* module. These attributes include detailed nature of disease, sex, primary/metastatic/recurrent status, zebrafish line, injection period, PDX-cell line/PDX-tissue information, and cell numbers.

Types of cancers studied using zebrafish xenograft models

The feasibility of transplantation of immortalized cells, PDXs, primary cells or stem cells into zebrafish embryos, juvenile,[27] and/or adult fish[28] offers an exquisite opportunity for assessing various aspects of tumor biology.[29–36] Searching the current version of ZenoFishDb v1.1, we have identified that breast adenocarcinoma (14.74%) is the most studied cancer followed by multiple cancer/tissue types (MULTIPLE) (10.76%), melanoma (8.37%), and glioblastoma (6.38%) (Fig. 1B and Supplementary Table S1). Expectedly, a cell line of breast adenocarcinoma origin, MDA-MB-231 (8.71%), accounts for the most investigated cell line, whereas majority of the cancer types or tissue of origins are represented by a single cell line (Supplementary Table S2). The injected cell lines belong to human (80.51%), mouse (14.41%), zebrafish (2.97%), rat (1.27%), goldfish (0.42%), and dog (0.42%).

FIG. 2. *PDX Details* and *PDX Charts* modules of ZenoFishDb v1.1. **(A)** Screenshot of the *PDX Details* page displaying patient details and detailed nature of disease. **(B)** Screenshot of the *PDX Charts* displaying the graphical representation for detailed disease nature of the transplanted tissue or primary cell line. PDX, patient-derived xenograft. Figures are available in greater detail online.

The nature of zebrafish xenografts: molecularly modified cells, PDXs, and stem cells

ZenoFishDb v1.1 prioritizes the molecularly modified cell transplantations that have been useful for establishing gene functionality in tumorigenesis[37,38] and associated events such as proliferation,[39,40] invasion,[41,42] angiogenesis,[43,44] metastasis,[18,45] apoptosis,[36] and cytotoxicity.[46] Our thoroughly systematized data curation emphasizes the molecular modifications (e.g., cells accommodating transient and/or stable overexpression vectors,[17,47,48] interfering RNAs[41,49] and/or Crispr-Cas9/TALEN/ZFN/Cre-LoxP[44,50,51] technologies) performed in cells used for transplantation.

A ZenoFishDb v1.1 search shows that these molecular modifications predominantly include siRNA (9.81%), shRNA (10.94%), expression vectors (12.45%), CRISPR/Cas9 (0.38%), and tag expression vectors (37.36%) for tracking purposes (Fig. 3A and Supplementary Table S3). In addition, the cell lines subjected to molecular modifications have been also separately attributed as ''modified cell lines'' and can be displayed through the *Visualization* page and are now provided in the table format (Supplementary Table S4). Among different molecularly-modified cell lines, MDA-MB-231 (7.19%), MCF7 (2.40%), U-87MG (2.74%), and PDXs (2.06%) represent the commonly modified cells in zebrafish xenograft studies incorporated into our database.

Another highlight of ZenoFishDb v1.1 is the inclusion of PDXs along with their clinical and genetic details when available. Patient-derived xenografting is achieved through direct transplantation of patient derived tissues[52] or primary

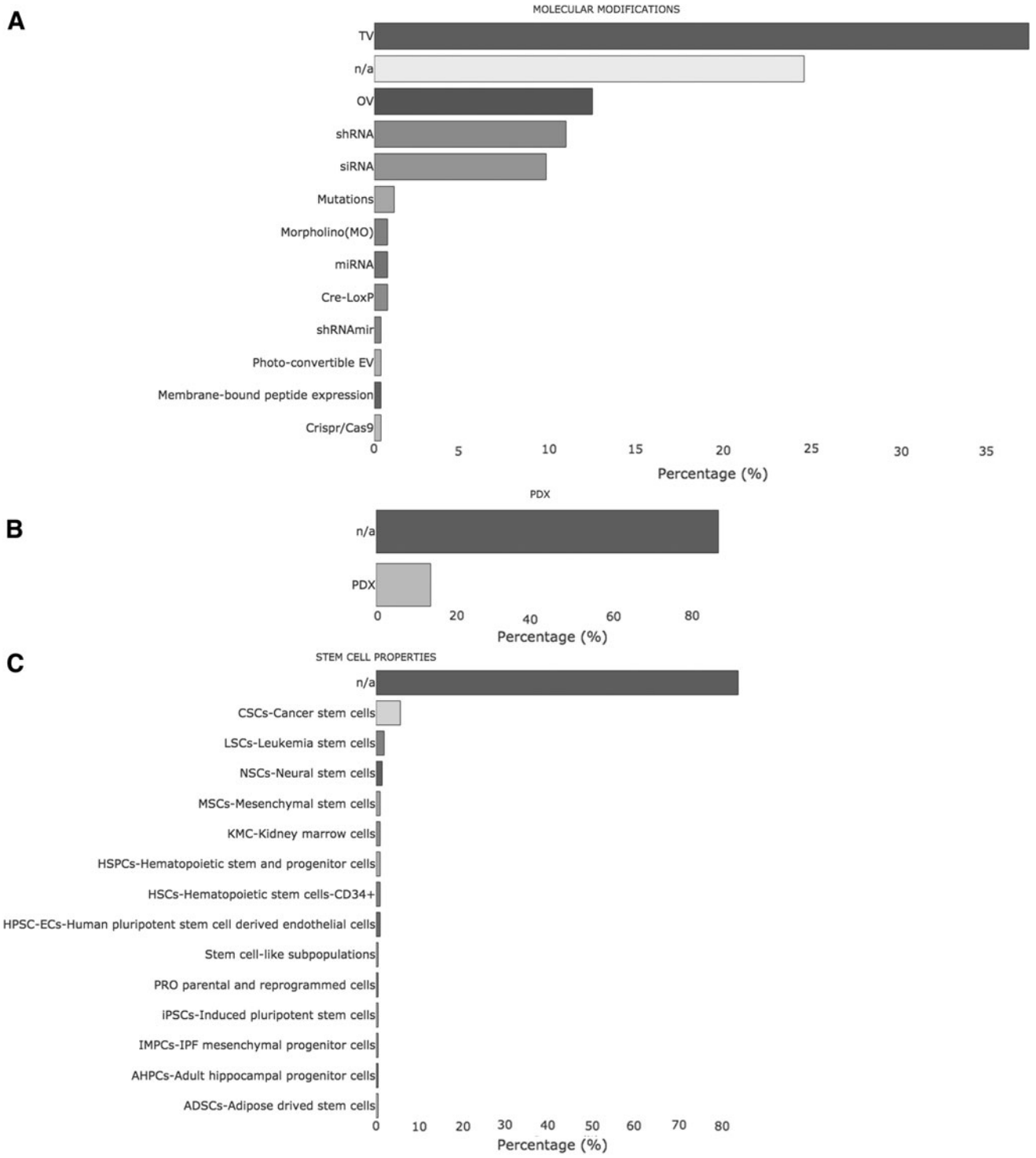


FIG. 3. The nature of xenograft studies represented on the ZenoFishDb v1.1: Molecular modifications, modified cell lines, PDXs, and stem cells. **(A)** Molecular modifications; **(B)** PDXs; **(C)** stem and cancer stem cell studies. Figures are available in greater detail online.

cell cultures with minimal passage numbers[53] and is ideal for mirroring the true nature of carcinogenesis. In fact, implantation of PDXs from cancerous tissues in comparison to immortalized cell lines better represents patient's genomic status and the tumor heterogeneity.[54,55] Altogether, the advantageous features of PDXs allow drug screening and development of personalized therapy both in rodents and zebrafish.[55–57] Hence, zebrafish PDX models have also been incorporated into ZenoFishDb v1.1 through PubMed search using a keyword query of ''zebrafish patient derived xenograft'' or ''zebrafish xenograft primary cells'' keywords. This has revealed the various types of cancers used in such studies, including breast cancer bone metastasis,[58] colorectal cancer,[59] multiple myeloma,[34] T cell acute lymphoblastic

leukemia,[60] gastric cancer,[35] neuroendocrine tumors,[61] adenoid cystic carcinoma,[33] glioblastoma,[62] as well as primary cells/tissues.[63] Curated PDX studies represent 13.74% of the studies incorporated into ZenoFishDb v1.1 (Fig. 3B).

Current version of the database also houses in-detail information on the PDXs accessible through the *PDX Details* and *PDX Charts* pages as explained above (Fig. 2A, B). Most frequently provided elements/attributes of the PDX details hence can be analyzed through *PDX Charts*. For instance, the number of glioblastoma patients recorded accounts for the highest number/percentage followed by the liver and colorectal cancer patients among many others, including prostate cancer, pancreatic ductal adenocarcinoma, melanoma, and acute leukemia (Fig. 2B). ZenoFishDb v1.1, therefore, is the first database accommodating detailed and searchable information from PDX studies in the zebrafish model.

ZenoFishDb v1.1 also houses the xenograft studies using stem cells (SCs) obtained from normal tissue or cancer tissue of origin. Xenografting of CSCs of blood cancers[64] and solid tumors of different origins[65] to rodents has paved the way for understanding behavior of CSCs in cancer development and therapy assessments. Zebrafish model organism serving as host for CSC transplantation also enables, for example, the assessment of metastatic behavior and drug screening in prostate cancer,[66] migratory behavior in breast cancer,[67] and proliferative behavior in leukemia stem cells.[68]

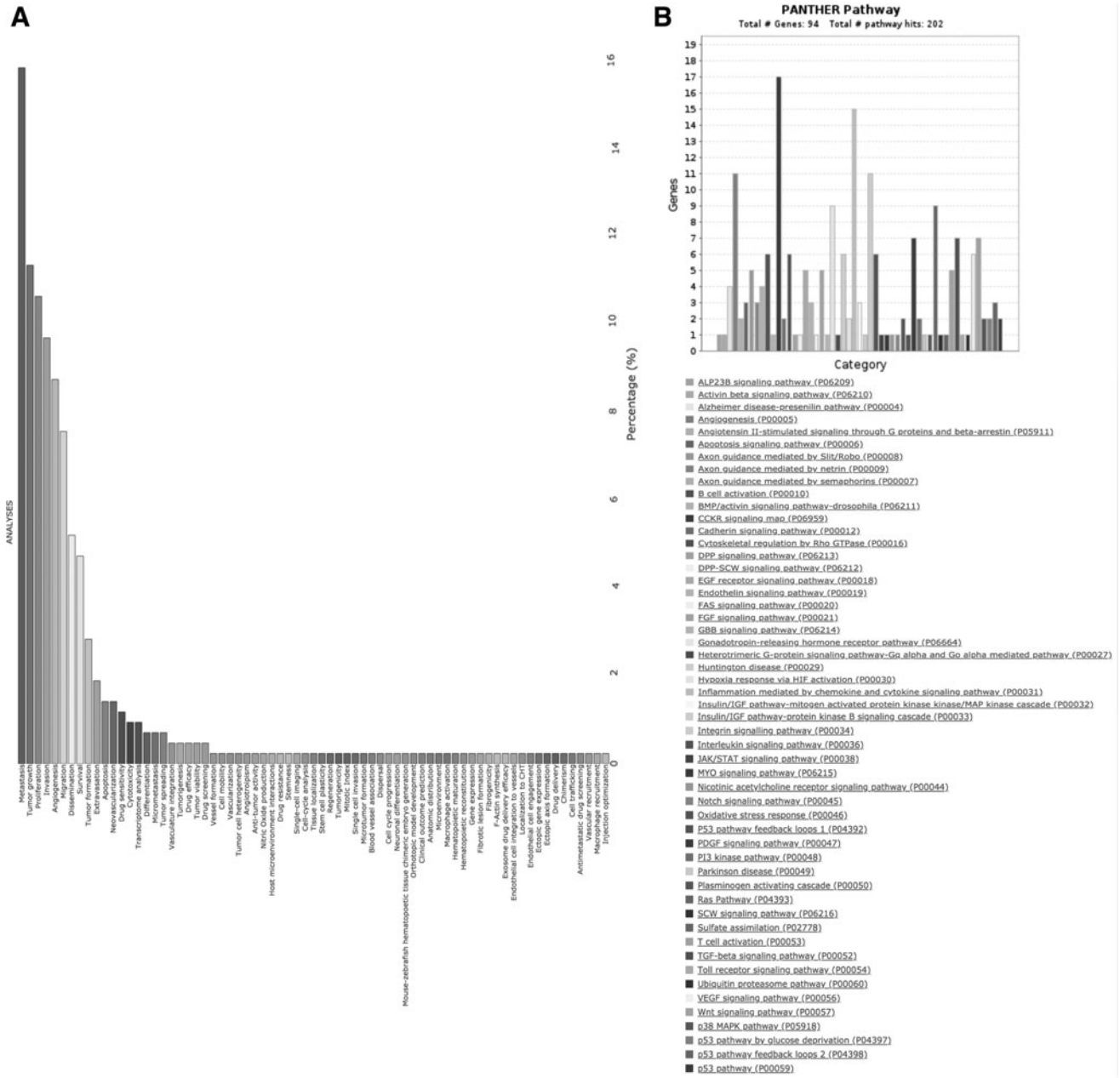


FIG. 4. Types of biological assessments performed on zebrafish xenograft models. **(A)** Biological analyses performed on zebrafish xenograft models through molecularly modified cell, PDX, and SC injections revealing major attributes studied in the field. **(B)** Representative bar chart of GO Panther pathway enrichment analysis on the modified genes revealing the more profoundly studied pathways. Figures are available in greater detail online.

Transplantation of induced pluripotent stem cell (iPSC)-driven differentiated cells,[69] hematopoietic stem cells,[70,71] and mesenchymal stem cells (from adipose tissue)[72] are among those studied in zebrafish xenograft models. SC studies account for the 16.36% of all curated xenograft studies in ZenoFishDb v1.1 with incorporated details of the origin of SC and CSCs transplanted into zebrafish embryos (Fig. 3C and Supplementary Table S5).

Biological assessments on zebrafish xenograft models

Searches performed with ZenoFishDb v1.1 reveal a broad range of tumor-biology associated attributes in zebrafish xenograft studies, including tumor growth (11.32%), proliferation (10.61%), invasion (9.67%), extravasation (1.89%), migration (7.55%), metastasis (15.80%), angiogenesis (8.73%), cytotoxicity (0.94%), apoptosis (1.42%), and drug sensitivity (1.18%) (Fig. 4A and Supplementary Table S6). In addition, the list of modified genes (Supplementary Table S7) gathered from these articles has been subjected to an in-depth pathway analysis using GOPANTHER.[73] The outcome of the pathway analysis (Fig. 4B) has revealed a total of 94 genes leading to 202 pathways out of which 18 major pathways are represented with at least 5 or more genes as visualized by the bar chart. Most frequently studied pathways include CCKR signaling, inflammation mediated by chemokine and cytokine signaling, integrin signaling, gonadotropin-releasing hormone receptor, angiogenesis, and Ras pathways (Fig. 4B).

In addition to these enriched pathways, we have also gathered information on the end point of biological assessments of each publication in our repertoire as hours postinjection (hpi) for embryos and as hpi or weeks postinjection (wpi) for adults. Forty-eight and 72 hpi are among the most analyzed time points after injection, while other time points uniformly included are 24, 96, 120, and 144 hpi in xenografted embryos (Supplementary Fig. S1).

Although not a drastic percentage difference has been detected in the majority of the end points, other parameters such as tissue of origin, cancer cell type, injected number of cells, or location could also affect the experimental course and selection of end time point. For instance, Mercatali *et al.*, studied metastases of breast cancer cell lines of different invasive capacity of MCF7 (hormone receptor positive, noninvasive) and MDA-MB-231 (triple negative breast cancer, invasive) together with a patient-derived breast cancer bone metastasis primary cell line. Herein, at 120 hpi, only MDA-MB-231 cells and primary cells survived, disseminated, and colonized in other parts of the fish implying the importance of choice of cell line and type of assessments to be performed at a specific time point.[58]

Moshal *et al.* studied angiogenic capacity of human and mice lung tumor cell lines, H1299 (nonsmall cell lung carcinoma) and CL13 (lung adenocarcinoma), respectively. Both of these cell lines and a nontumorigenic 3T3-L1 cell line were injected to Tg(*flk1*:eGFP) fish at 24 hours postfertilization (hpf), and angiogenic capacity was assessed at 48 hpi testing alkaline phosphatase activity. In addition, significant increases in the number and length of ectopic vessels were detected in tumorigenic cell lines confirming presence of angiogenesis at 48 hpi.[74] Hence, when metastasis-related events such as extravasation, migration, invasion, and angiogenesis were considered together, a relatively homogenous distribution emerges for scoring xenografts at 48 or 72 hpi.

Based on data housed in ZenoFishDb v1.1, tumor growth and proliferation although generally not assessed solely are also collected frequently at 48 and 72 hpi. However, assessment-specific prolonged end points are also observed in xenotransplantation studies in embryos, for example, with respect to survival[62] and immunohistochemical[75] measurements. Xenotransplantation in adult fish on the other hand is scarce yet assessments are recorded by means of hpi,[76,77] as well as wpi,[28,78] onto our database (Supplementary Fig. S1 and Supplementary Table S8).

These findings altogether highlight the importance of variability in spatial and temporal characteristics of xenotransplantation studies that should be taken into consideration while addressing different biological assessments, as well as the choice of cell lines, PDXs, and injection sites. ZenoFishDb v1.1 allows for evaluation of such parameters readily helping users to plan and execute their experiments.

Zebrafish xenograft model as a tool for drug screening

Zebrafish has been long used for drug screening as thoroughly revived by different authors in the field.[15,79] Yet, availability of zebrafish xenograft models further enhanced the applications of drug screening on human-derived tumor bearing fish. In fact, models such as ZeOncoTest have been used to refine and automate use of zebrafish xenotransplantation for cancer drug discovery.[80] Using ZenoFishDb v1.1 one can identify individual studies harboring different routes of drug administration such as those given before transplantation,[81–83] as well as those in which drugs are directly added to the fish water.[84] More than 200 different drugs have been identified and incorporated into the current version of the database. Dasatinib,[48,85] SU5416,[17,86] and Doxorubicin[87] are among the most commonly used drugs, while use of nanoparticles[88] and exosomes[89] has been also recorded in the list of zebrafish xenograft drug studies (Supplementary Fig. S2 and Supplementary Table S9). Hence, ZenoFishDb v1.1 provides a platform for the feasible search, cataloging, and comparison of drug applications performed on zebrafish xenograft models.

Zebrafish host modifications for xenotransplantation

The availability of *in vivo* imaging of vascular development by Tg(*fli1*:EGFP) zebrafish embryos[90] provides great ease for visualization across embryonic development. In fact, a majority of the xenograft studies harboring angiogenesis, invasion, and metastasis assays[49,91] benefits from Tg(*fli1*:EGFP) line where the *fli1* promoter, the earliest-known endothelial marker,[92] is used for driving the green fluorescent protein (GFP) expression. Similarly, Tg(*flk1*:EGFP)^{s843} zebrafish line[93] generating green vasculature under *flk1* is widely used to investigate invasive and metastatic capacity of tumor cells.[19,94] The use of transparent *casper*, as well as albino fish, has further improved visualization of transplanted cell behavior in zebrafish.[95] Using ZenoFishDb v1.1, one can obtain a listing of all studies that contain zebrafish modified/mutant strains used with transplantation of cells with molecular modifications and PDX or SC xenografts.

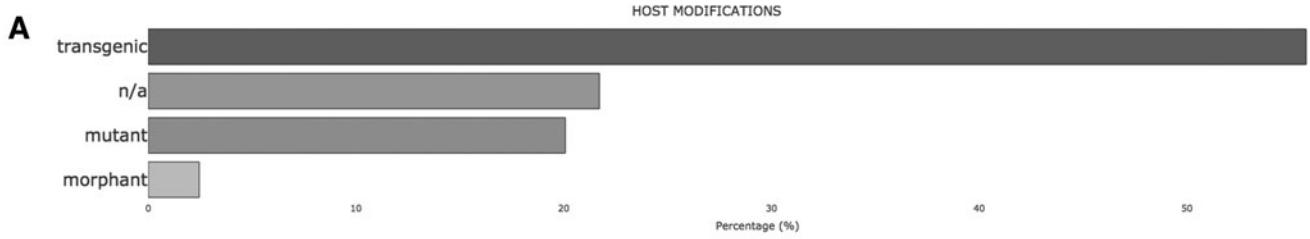
Other mutant and knockout/knockdown zebrafish strains are becoming central for understanding the effects of microenvironment in tumorigenesis. For instance, acetylcholinesterase mutant *ache*, harboring excess acetylcholine, is a model to test the role of *ache* deficiency of the host on size of the liver tumors.[96] Similarly, *cloche* mutant fish is lacking nearly all blood cells and, therefore, functional circulation, and vasculature *(cloche*$^{-/-}$*)* allows for testing whether metastasis and tumor growth require host vasculature.[63,97] In addition, morpholinos (MO) that are widely applicable for discovery of gene function can be used in xenotransplantation to modify host microenvironment. For example, transplantation of retinoblastoma cells into zebrafish embryos microinjected with MO against *vegf-aa* lowered levels of metastasis compared to control MO-treated embryos.[98] In another example, the injection of HCT116 cells into Tg(*fli1*:EGFP) protein kinase D1 morphant abolished tumor angiogenesis.[99]

A search using ZenoFishDb v1.1 *Visualization* page, upon selecting the ''host strain'' column, shows the presence of transgenic (55.74%), mutant (20.08%), and/or morphant (2.46%) strains used as modified host microenvironments (Fig. 5A and Supplementary Table S10). Accordingly, a representative image of the subselected mutant ''host details'' and corresponding ''host detail modifications'' has been provided using the *DataTable* pages (Fig. 5B).

These studies demonstrate the undeniable power of using morphant, mutant, and transgenic zebrafish embryos and larvae to understand the role of microenvironment in human tumor growth, angiogenesis, and metastasis. ZenoFishDb v1.1 database thus can be useful in keeping up with the ever-increasing studies in the xenotransplantation field in which zebrafish host is often genetically and/or epigenetically modified.

Zebrafish xenograft models from a technical point of view

ZenoFishDb v1.1 can also be used to search the zebrafish literature for differences in the technical aspects of xenotransplantation, such as the site and timing of injection, number of cells injected, and types of tracking dyes used. Precise location of the injection site is crucial for the type of biological analysis to be performed in xenograft studies. In fact, yolk sac injections are ideally used for testing initiation



B

Attributes

Select an attribute

HOST MODIFICATIONS

Subselections

mutant

morphant, transgenic
mutant, transgenic
n/a
transgenic
transgenic, morphant
transgenic, mutant

Data Table

Search:

ZEBRAFISH LINES	HOST MODIFICATIONS	HOST MODIFICATIONS DETAILS	CELL TRACKING SOURCES
Casper	mutant	Transparent embryo	GFP
Casper	mutant	Transparent embryo	n/a
Casper	mutant	Transparent embryo	CMTPX-Red
prkdc-/-;il2rga-/-;casper	mutant	Transparent embryo, Lacks T, B and NK cells	GFP, CFSE, DiI
Casper	mutant	Transparent embryo	CM-DiI
Wild type, ache-sb55 mutant	mutant	achesb55 mutants (homozygous mutant: paralyzed at 3dpf,hererozygous mutant: normal development)	DiO, DiI, GFP
Casper	mutant	Transparent embryo	GFP
Casper	mutant	Casper (roy-/-; mitfa-/-)	GFP
Casper	mutant	Transparent embryo	CM-DiI
Casper	mutant	Transparent embryo	GFP
Casper	mutant	Transparent embryo	GFP
Casper	mutant	Transparent embryo	CMTMR
Wild type, Casper	mutant	Transparent embryo	GFP
Casper	mutant	Transparent embryo	CM-DiI
Casper	mutant	Transparent embryo	CM-DiI
prkdc-/-, prkdc+/-	mutant	Prkdc-Null severe combined immunodeficient (SCID) zebrafish	tRFP
Casper	mutant	Transparent embryo	GFP, mCherry, CFSE
Casper	mutant	Transparent embryo	CM-DiI, GFP

Showing 1 to 18 of 22 entries

FIG. 5. ZenoFishDb v1.1 reveals distinct host modifications and microenvironment studies used in xenograft studies. **(A)** Graphical representation of host modifications obtained from the *Visualization* page. **(B)** Screenshot of *DataTable* page with ''host modifications'' attribute, and subselection choice of ''mutant'' attribute. Figures are available in greater detail online.

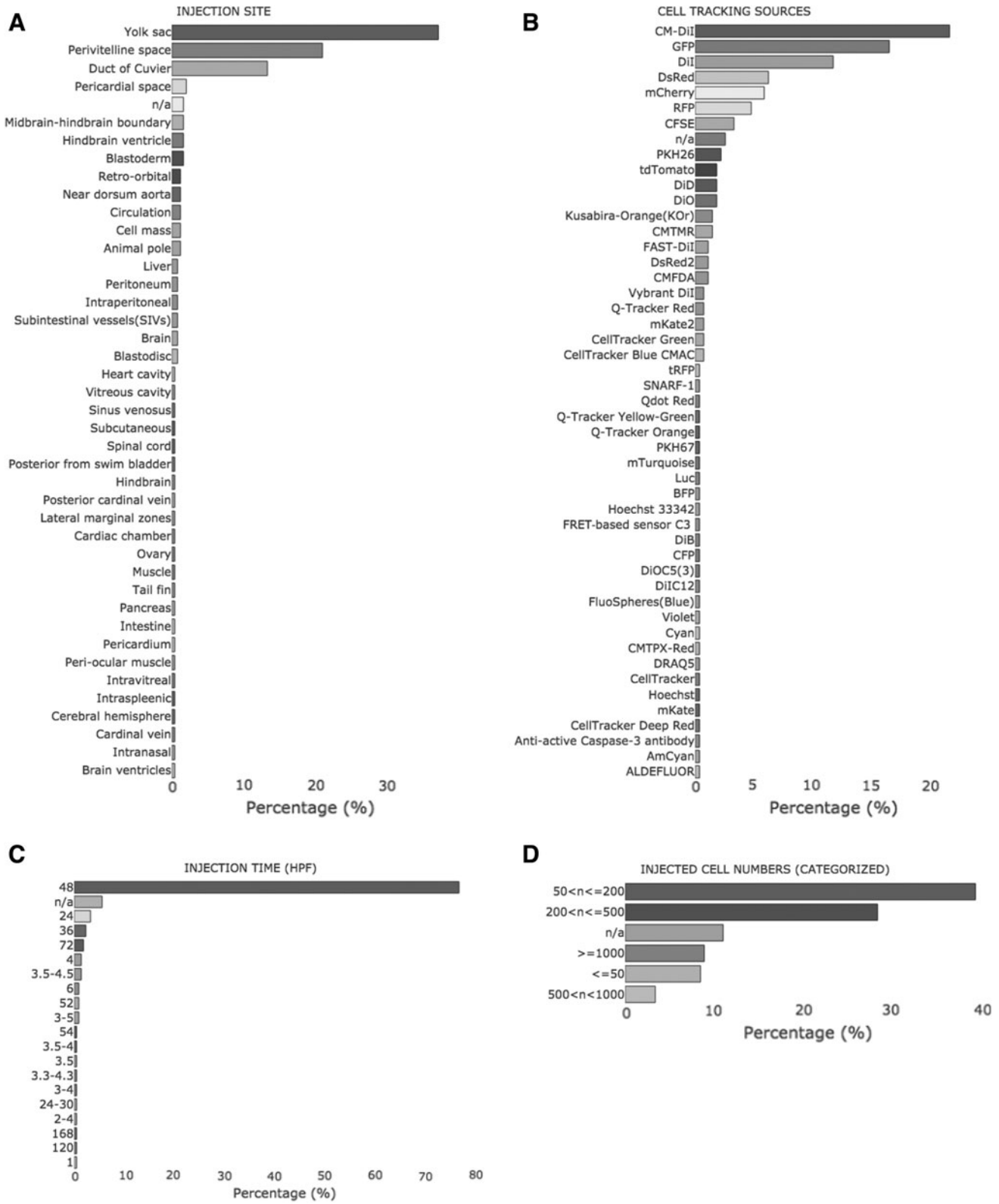


FIG. 6. ZenoFishDb v1.1 reveals statistical data on technical prospects of xenografting in zebrafish. **(A)** Injection sites; **(B)** cell tracking systems; **(C)** time of injection; **(D)** injected cell numbers-categorized. Figures are available in greater detail online.

of tumor formation, tumor growth, or proliferation,[39,40] whereas duct of Cuvier opens to the sinus venosus of the heart and allows analysis of circulating injected cells and hence cellular migration[70] and metastasis to tail fin.[100] Injection into the perivitelline space of the zebrafish embryo has been initially used for an angiogenesis assay by Nicoli and Presta[101] and similarly by other groups where the ectopic SIV-sprouting has been tested.[99] Although these exemplify common examples of injection sites for specific biological assessments, there are other possibilities. Statistical representation of injection sites using ZenoFishDb v1.1 reveals the yolk sac (37.10%) as the most preferred injection site followed by perivitelline space (20.97%) and duct of Cuvier (13.31%) (Fig. 6A and Supplementary Table S11).

Transparent zebrafish embryos are enabling precise tracking of the location and migration of the fluorescently labeled transplanted cells. In fact, solid tumors inside the yolk[96,102,103] or brain[104–107] and migrating cells in the veins[103,108] can be detected readily by fluorescence microscopy. Cell lines transplanted in zebrafish are often stained by fluorescent protein vectors such as GFP,[29] mCherry,[100] DsRed,[106] and live dyes, among which visualization by CM-DiI,[103] DiI,[96,102] DiD,[109] CFSE,[110] and CMTMR[37] is the most frequently used based on a ZenoFishDb v1.1 search (Fig. 6B and Supplementary Table S12).

Another technical aspect highlights the timing of the injection to be performed at different injection points in zebrafish embryos (92.66%) and/or adult fish (6.42%), which holds great importance for the strategic decision-making for assessments to be performed.[111] Great majority of the embryos (76.85%) have undergone the injection during the first 48 hpf (Fig. 6C and Supplementary Table S13).

In addition, we have also reviewed the differences in the number of cells injected. In the literature, studies testing different number of cells in different biological concepts exist; among these Fior *et al.*,[59] for example, injected 500 and 1000 colorectal cancer primary cells into the perivitelline space for testing early and late metastatic events, respectively. However, another study assessing tumor size used 50–100 cells for cell lines and 500 cells for patient samples when injecting into the yolk sac.[60] Using ZenoFishDb v1.1, we show the percentage of studies with different number of cells injected, for example, $50 < n \leq 200$ cells (39.57%) or $200 < n \leq 500$ cells (28.51%), where n represents the number of cells. Injections harboring cells $n \leq 50$, $500 < n < 1000$, and $n \geq 1000$ also exist, yet they are sparser (Fig. 6D and Supplementary Table S14). ZenoFishDb v1.1, hence, covers technical aspects of zebrafish xenografting models pinpointing the specifics of experimental design.

Conclusions and Future Perspectives

ZenoFishDb v1.1 offers an easy access to zebrafish xenograft studies with a specific focus on PDXs and the molecular modifications in the transplanted cells, as well as on host microenvironment. In addition, our findings address recent and novel perspectives in the literature, such as use of SCs and CSCs, along with therapeutic approaches that can be useful in translational medicine. Future inclusions of zebrafish xenotransplantation studies that use unmodified cells or hosts and drug screens with different time intervals and dosing are also planned in the upcoming versions of ZenoFishDb v1.1. Moreover, keywords used for searching literature will be diversified and generalized to be more comprehensive in case ''xenograft'' or ''xenotransplant'' is not included in the study abstract. In conclusion, ZenoFishDb v1.1 incorporates a thorough and systematic review of 211 transplantation studies highlighting the extent of xenografting molecularly modified cells in wild-type/transgenic/knockout/morphant/mutant zebrafish (reviewed until November 29, 2019) and shows that the emerging applications of *in vivo* cancer and personalized medicine in the zebrafish xenograft field complement the studies performed in mice and other organisms.

Authors' Contributions

O.K. conceptualized the ZenoFishDb v1.1 and supervised the study; S.T., O.K., M.E.A. identified criteria to be curated; S.T. curated the data and drafted the data table; T.K. developed and tested the database; S.T., M.E.A., D.G., A.G.K. performed literature search; S.T., O.K., and M.E.A. wrote the article, and S.T. made the figures; M.E.A., D.G., A.G.K., T.K. helped with data curation; and all authors tested the database and read, revised, and authorized the article.

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Disclosure Statement

No competing financial interests exist.

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Supplementary Material

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