Transplanting human cancer cells into immunodeficient zebrafish

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Pediatric grand round, OUHSC

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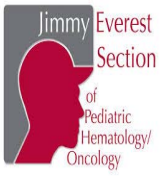


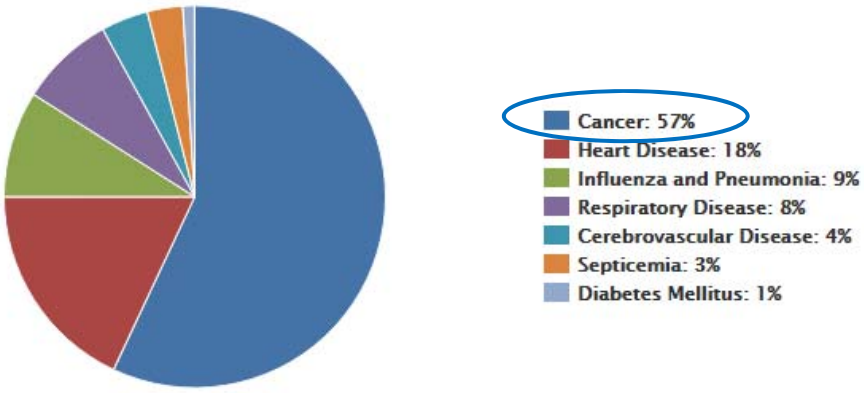
Disclosure

I have no relevant financial relationships or affiliations with commercial interests to disclose

Learning Objectives

- Express that cancer is the leading cause of death in the United Stated pediatric patients
- Explain why there is a need for personalized oncology regimens in pediatric cancer patients
- Discuss how immunodeficient zebrafish can be used as an avatar for personalized oncogenic modeling of pediatric cancers

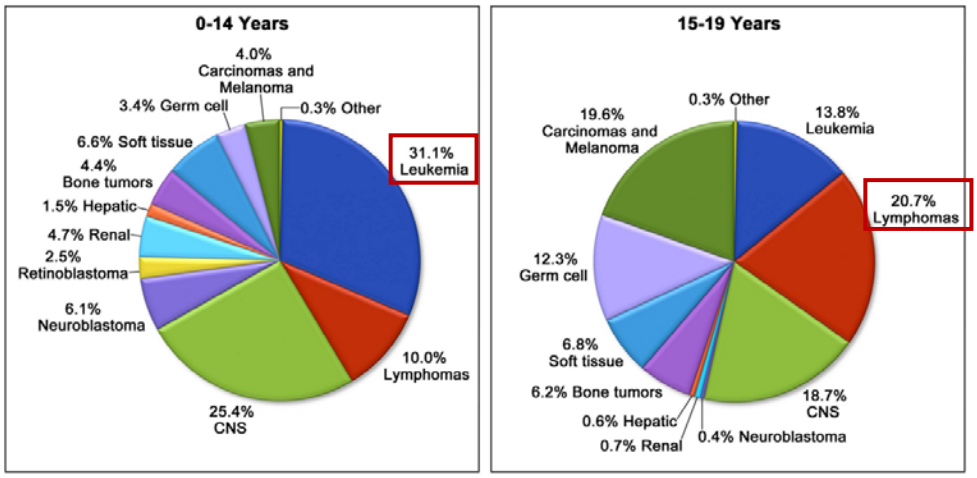
Cancer is the most lethal disease of United States pediatric patients



Number of Childhood Deaths by disease per year
Age 1-19
Total=3,468

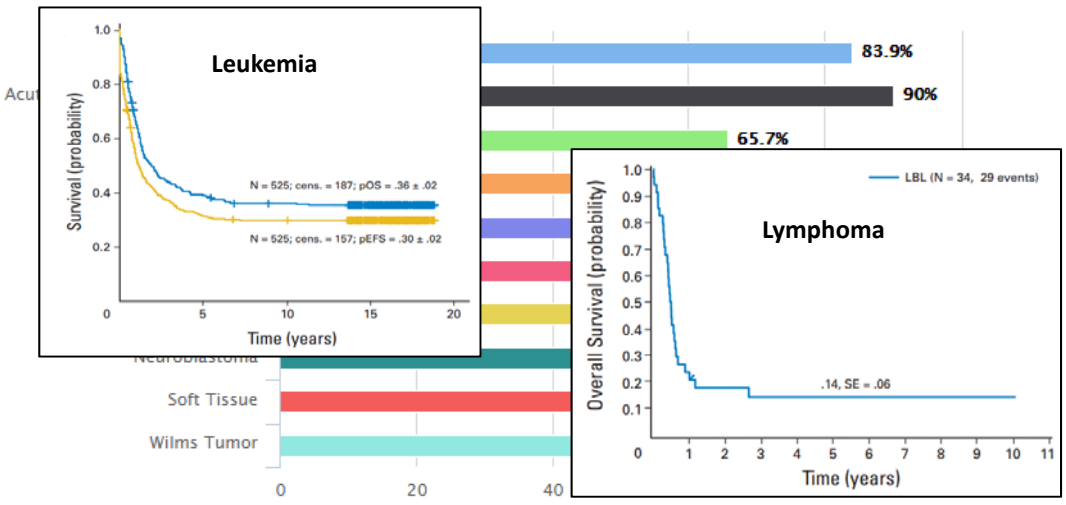
National Vital Statistics Report, 2016 (cdc.gov)

Leukemia most common in 0-14 yrs
Lymphoma most common in 15-19 yrs



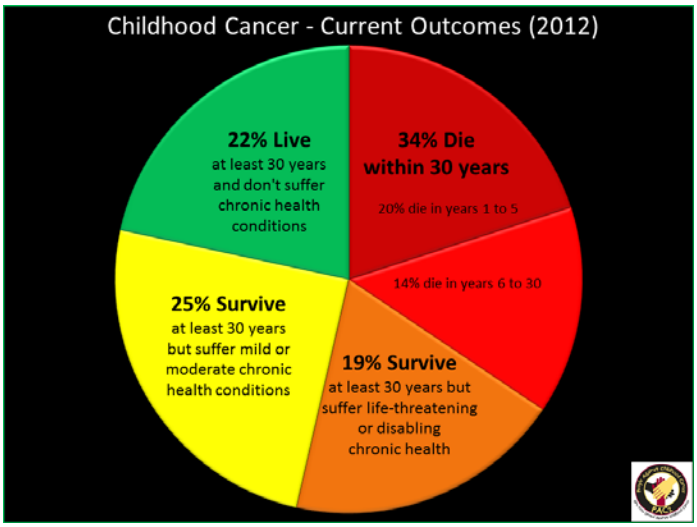
SEER 2005-2009

5-Year Survival Rate, Age 0-19 years



Tallen G et al, J Clin Oncol. 2010
Burkhardt B et al, J Clin Oncol. 2009

Chemotherapies have considerable short and long term toxicity



People against childhood cancer online resources

Need for "Personalized" oncology regimens

To optimize efficacy and minimize toxicity in pediatric ALL and other pediatric cancers, we need strategies to "personalize" oncology regimens

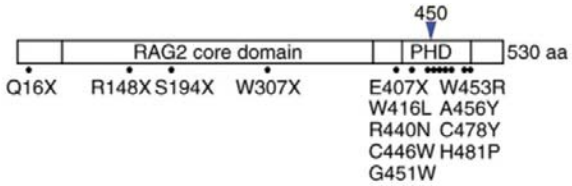
- Personalized oncology regimens are particularly valuable for **relapsed and refractory patients**

Zebrafish (*D. rerio*) as an Oncology Model

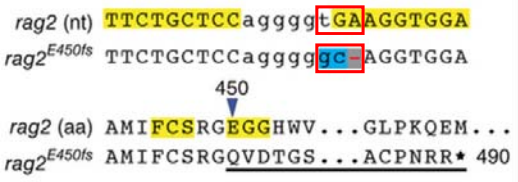
- Easy to mutate or genetically modify
- Small, prolific, develop rapidly, cheap to house & feed
- Mammalian-like innate & adaptive immune systems
- Get near-identical cancers to humans

Immunodeficient (*rag2*^{E450fs} mutant) zebrafish

In vertebrates, mutations in recombination activating gene (RAG2) cause faulty V(D)J recombination, resulting in arrested B- and T-cell development



Langenau lab created *rag2*^{E450fs} mutant zebrafish



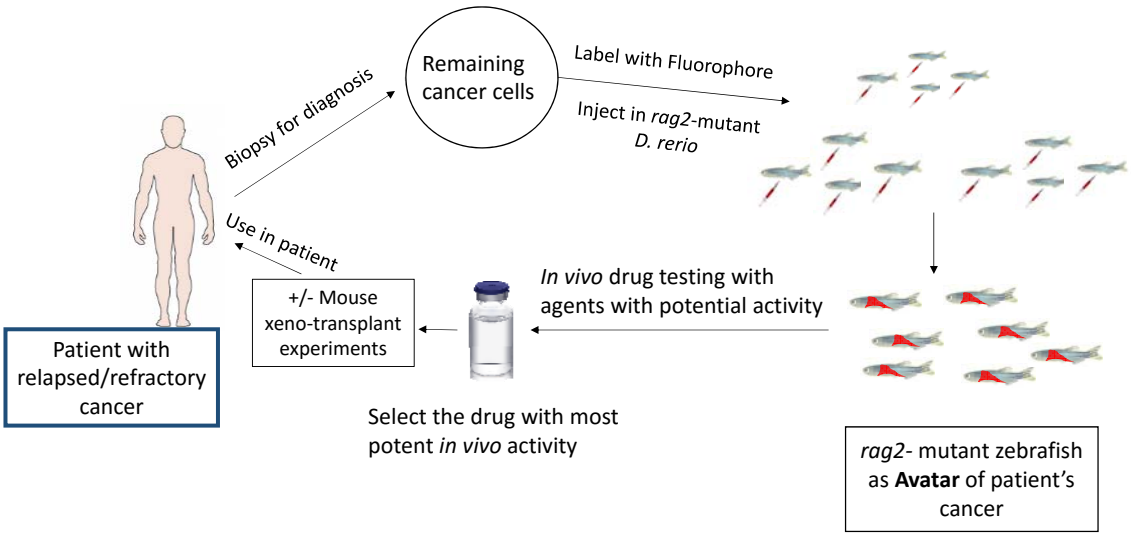
Tang et al, Nat Methods 2014

Developing Allo- and Xeno-transplanted zebrafish as an oncogenic model

Specific aims:

1. Establish T-ALL allografts in *rag2*-mutant *D. rerio*
2. Establish human cancer cell xenografts in *rag2*-mutant *D. rerio*
3. Develop Patient-Derived Xenografts (PDX) in *D. rerio*

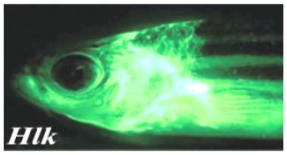
Goal of the project

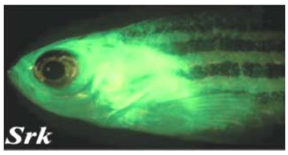


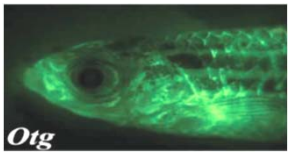
Specific aim:1

T-ALL *D. rerio* donors for allo-tranplant

Mutant zebrafish

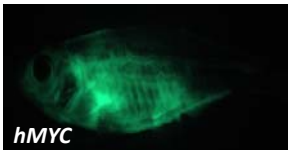






(Frazer et al., Leukemia 2009)

***hMYC* transgenic zebrafish**



(Gutierrez et al., J Exp Med 2011)

Specific aim:1

Experimental design

T-ALL *D. rerio* donor

Single cell suspension, flow cytometric quantification of viable GFP+ cells using PI stain

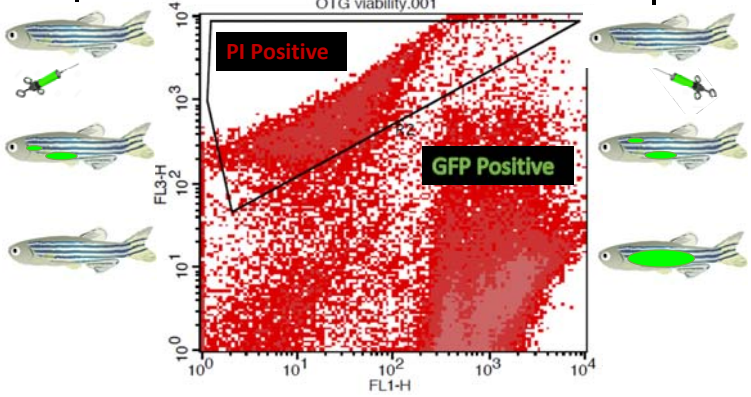
23 Gy

Positive control

Experimental arm

48 hr

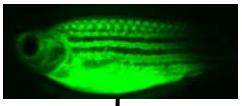
WT *D. rerio*



rag2-mutant *D. rerio*

Specific aim:1

Sample allo-transplant experiment



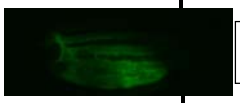
***otg D. rerio* with GFP+ T-ALL**

Single cell suspension, Flow cytometry

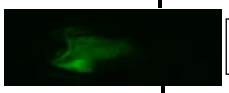
Irradiated WT recipient x 5
~150,000 viable GFP+ cells each

rag2-mutant recipient x 5
~150,000 viable GFP+ cells each

12 *d.p.i*



4/4 with GFP+ cells



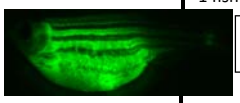
2/4 with GFP+ cells

1 fish deceased due to tumor

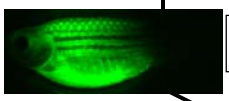
1 fish died due to tumor

1 fish deceased of unknown cause

22 *d.p.i*



3/3 with GFP+ cells



1/1 with GFP+ cells

32 *d.p.i*

2/3: No GFP+ cells
1/3: GFP+

Used for serial transplant on day +25

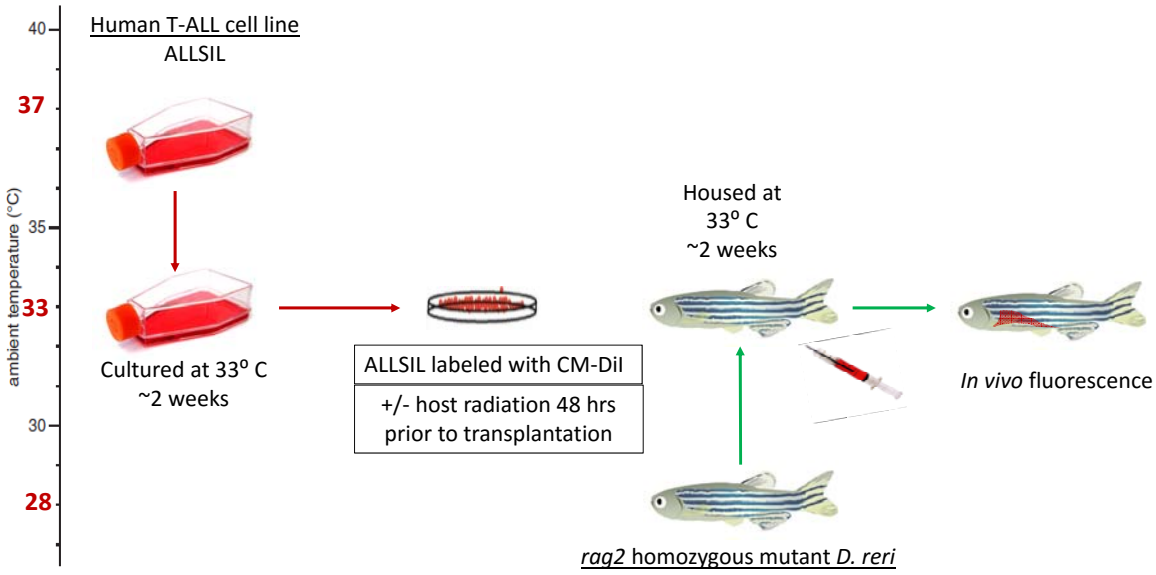
Specific aim:1

Summary

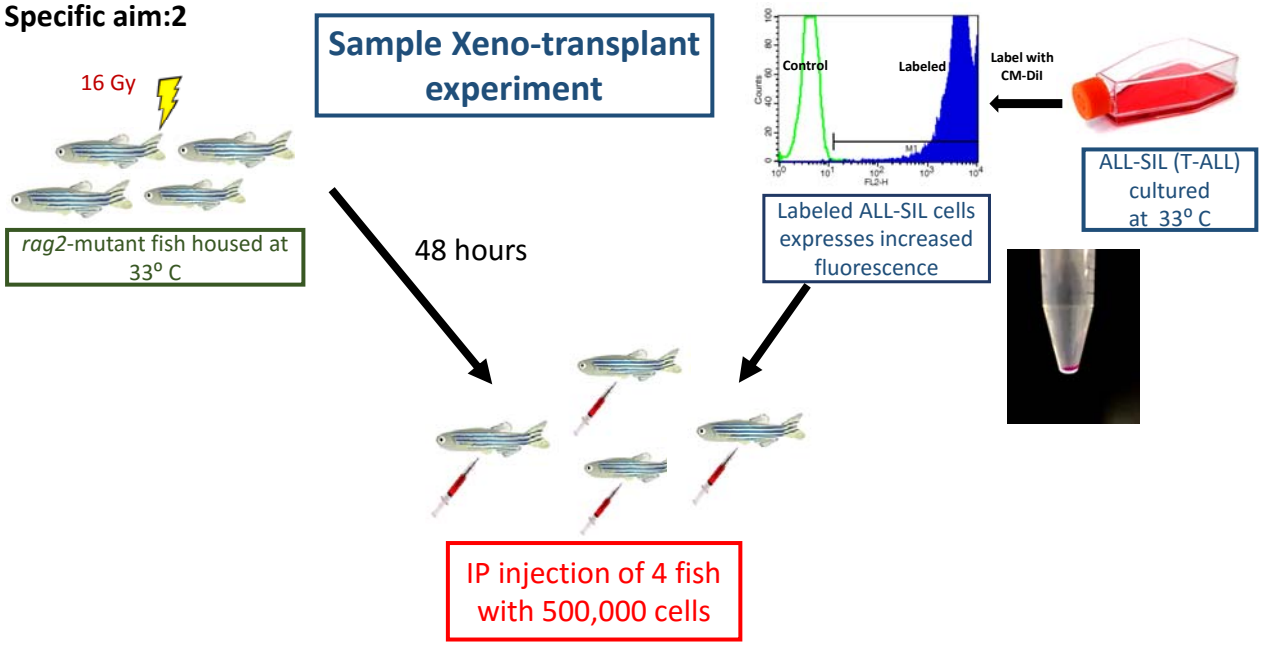
- Transplanted **10** different zebrafish T-ALL in **65** *rag2*-mutant *D. rerio* with engraftment rate of **~15%**
- Sustained primary and serial engraftment in *rag2*-mutant zebrafish was seen
- Remaining recipient T-lymphocytes and Natural killer cells may play part in rejecting the cells, which can be overcome by sub-lethal irradiation of the host

Specific aim:2

Experimental design

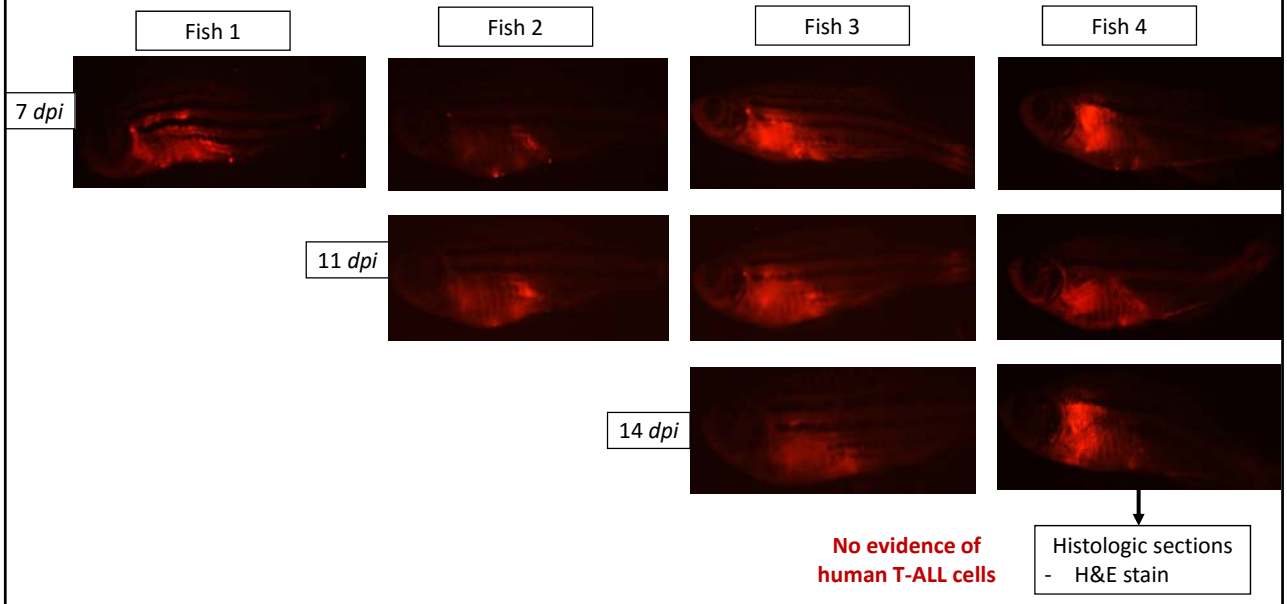


Specific aim:2



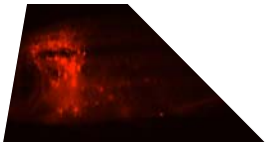
Specific aim:2

Sample Xeno-transplant experiment



Specific aim:2

Detecting human cancer cells in zebrafish using Flow cytometry



Single cell suspension

Stain with
- Anti ***human*** CD-45 (APC)
- Anti ***human*** CD-7 (BB515)
- Anti ***human*** CD-5 (BV414)

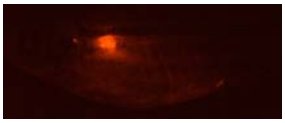
Obtain data using Flow cytometry

Specific aim:2

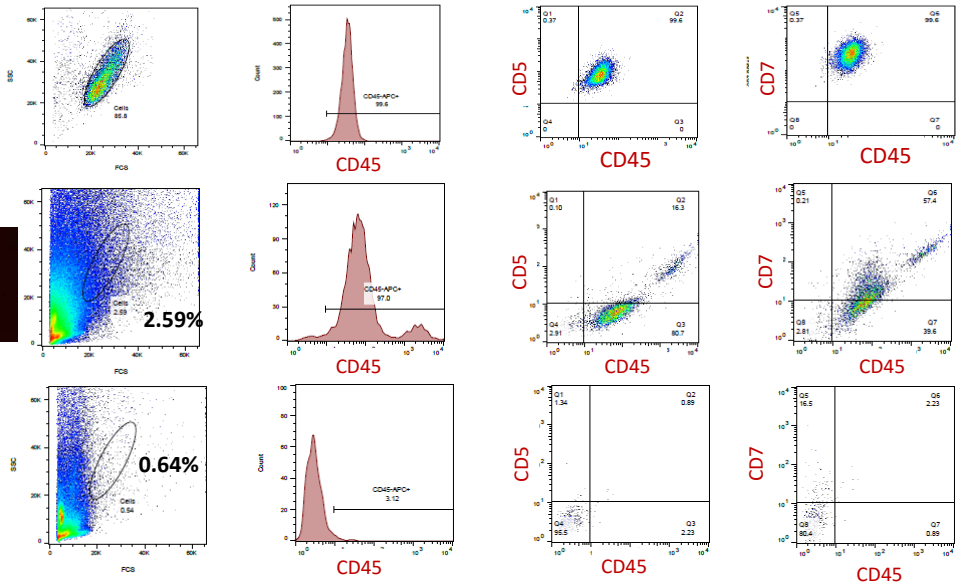
Example of flow cytometry assay

ALL-SIL cells prior to injection

25 *dpi*



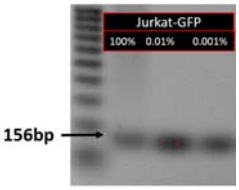
***rag2-mutant* fish not injected with ALL-SIL cells**



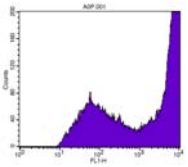
Specific aim:2

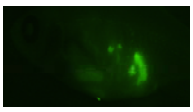
Innovative strategies

- Human gene detection assay designed to detect up to 0.001% of human *TLX1* gene (156bp)



- Human T-ALL cell line (Jurkat) stably transfected with Green Fluorescent Protein (GFP) to allow for stable expression of GFP with cell division





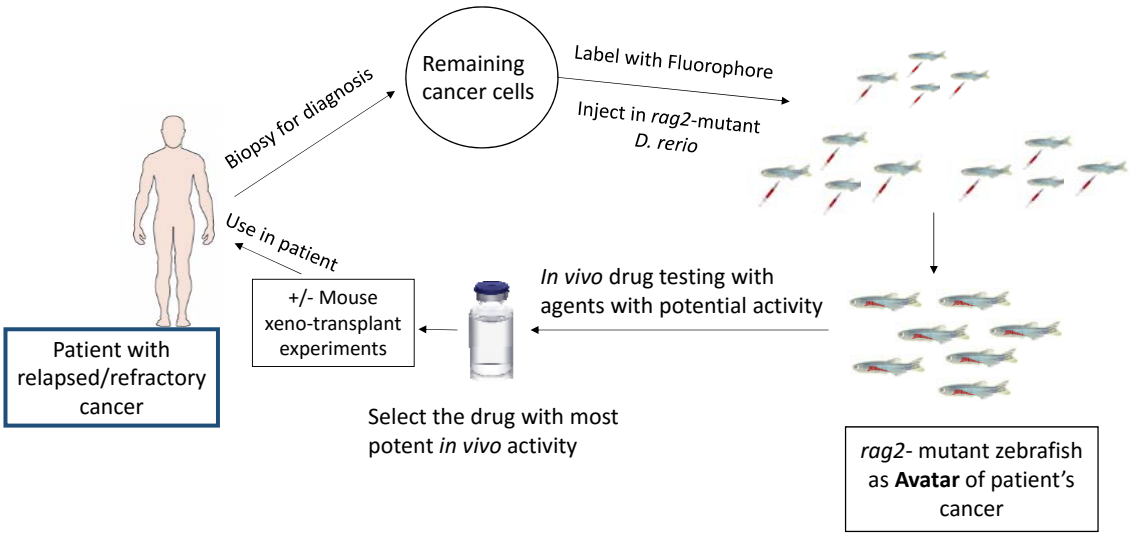
- We have created *rag2*-mutant fish in casper background which would allow direct visualization of engrafted cancer cells

Specific aim:2

Future directions

- Ongoing experiments using Jurkat-GFP cells as donor and *rag2*-mutant zebrafish as recipient
- Use combination of *in vivo* fluorescence, Flow cytometry, PCR-based human gene detection assay and histology to confirm the engraftment
- Perform *in vivo* drug-testing on engrafted zebrafish
- Stable xenotransplant with cell lines (ALLSIL, JurkatGFP) would prove that human cancers can survive in *rag2*-mutant fish, opening the door to patient derived xenografts

Goal of the project

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