Zevatars; the future of personalized cancer medicine

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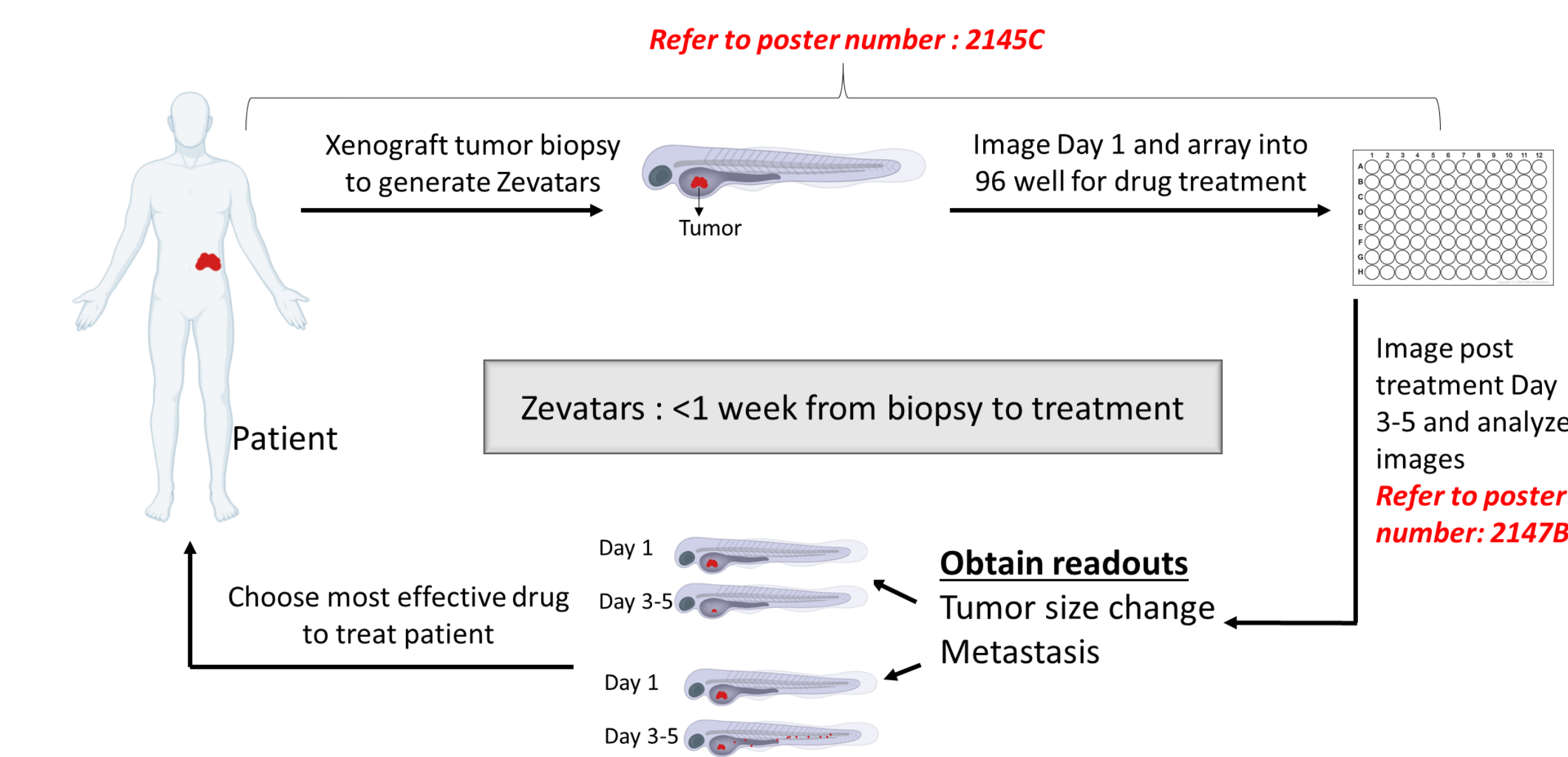MedStar Georgetown University Hospital

Background

Zevatars
- Zevatars involve implanting fresh or cryopreserved patient tumor pieces from biopsy samples into zebrafish and screening for best drug response before treating patients in the clinic
- The main advantage of using zevatars is rapid drug response screening and ability to assess tumor proliferation, angiogenesis, and metastasis by imaging and finally by implanting tumor pieces takes into account the tumor microenvironment as compared to current methods involving injecting dissociated tumor cells
- In this study we describe the application of Zevatars for personalized medicine in pancreatic adenocarcinoma and colorectal cancer.
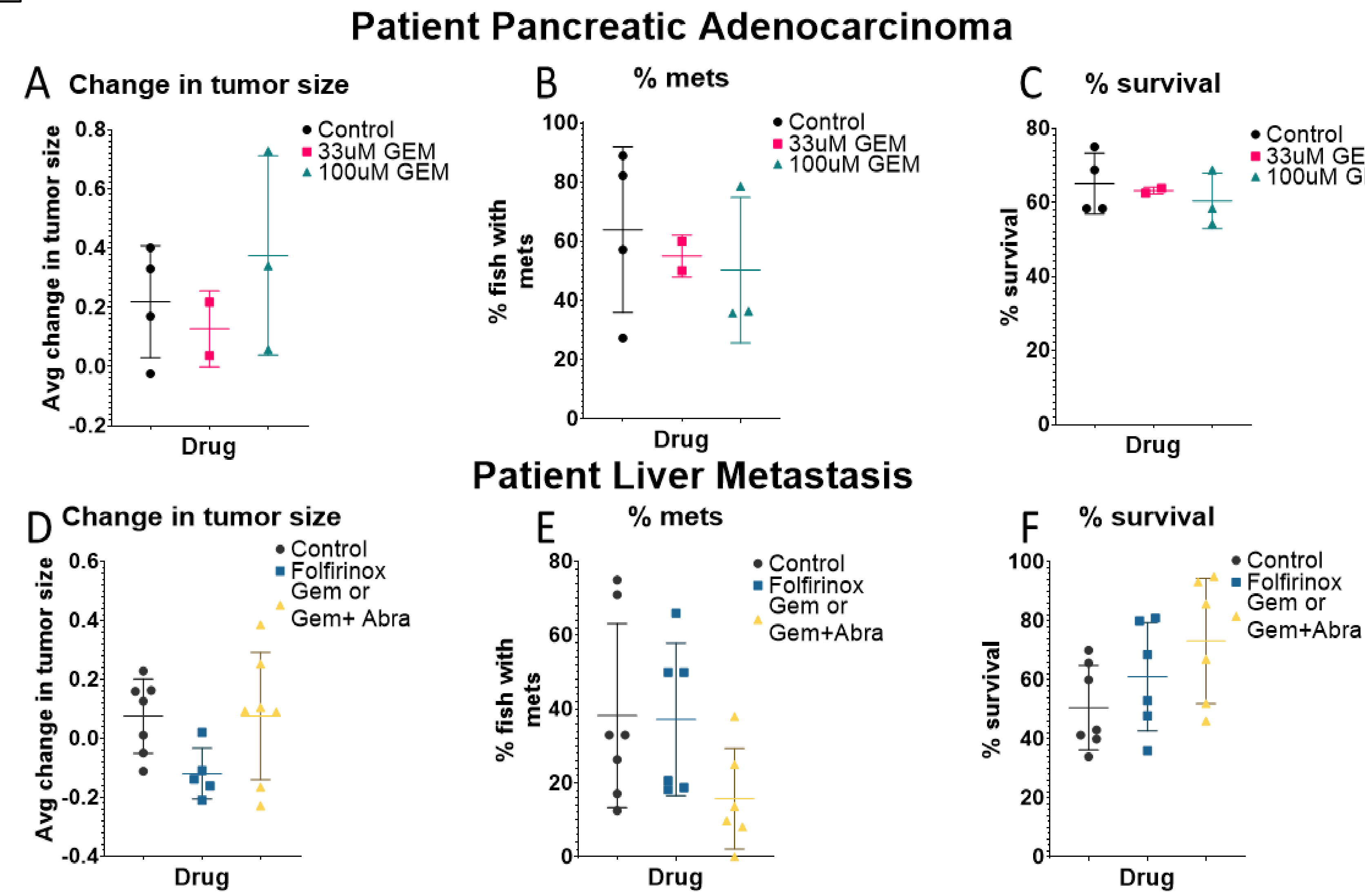
Humanizing zebrafish
- Currently we cannot use Zevatars to test immunotherapy so therefore we aimed to generate humanized fish
- cmyb mutant fish fail to develop definitive hematopoietic cells and therefore provides us a way to completely replace fish system with a human hematopoietic system
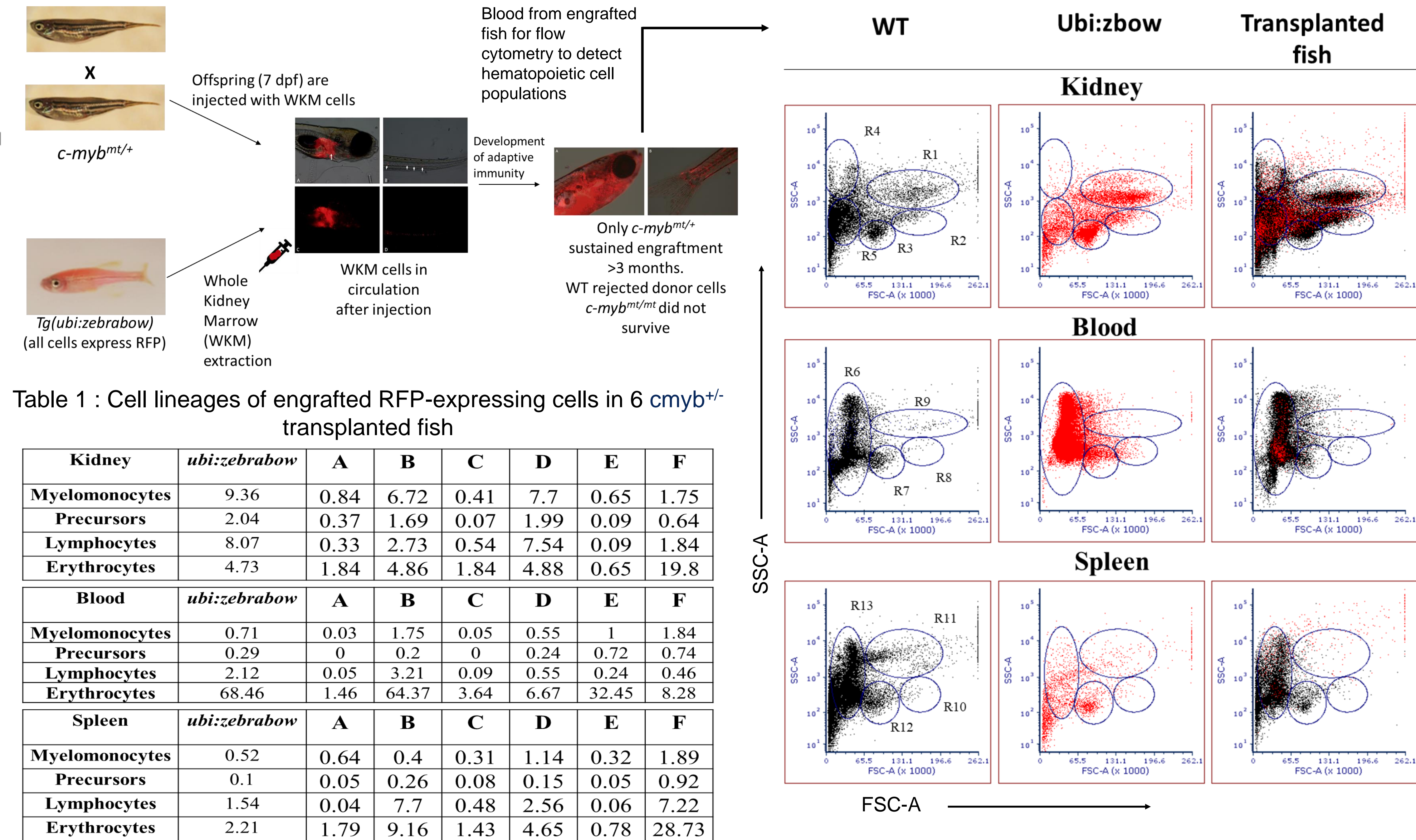
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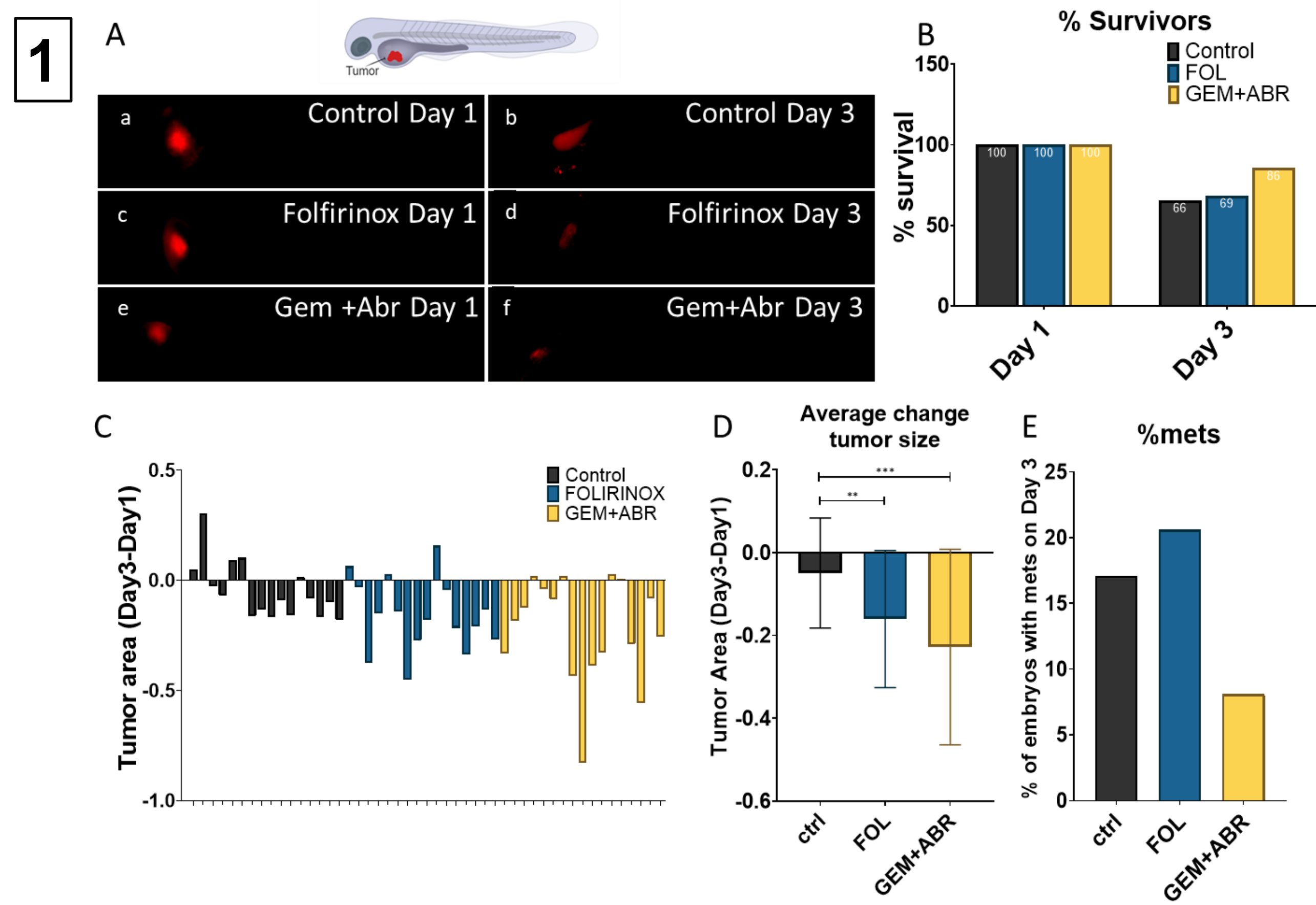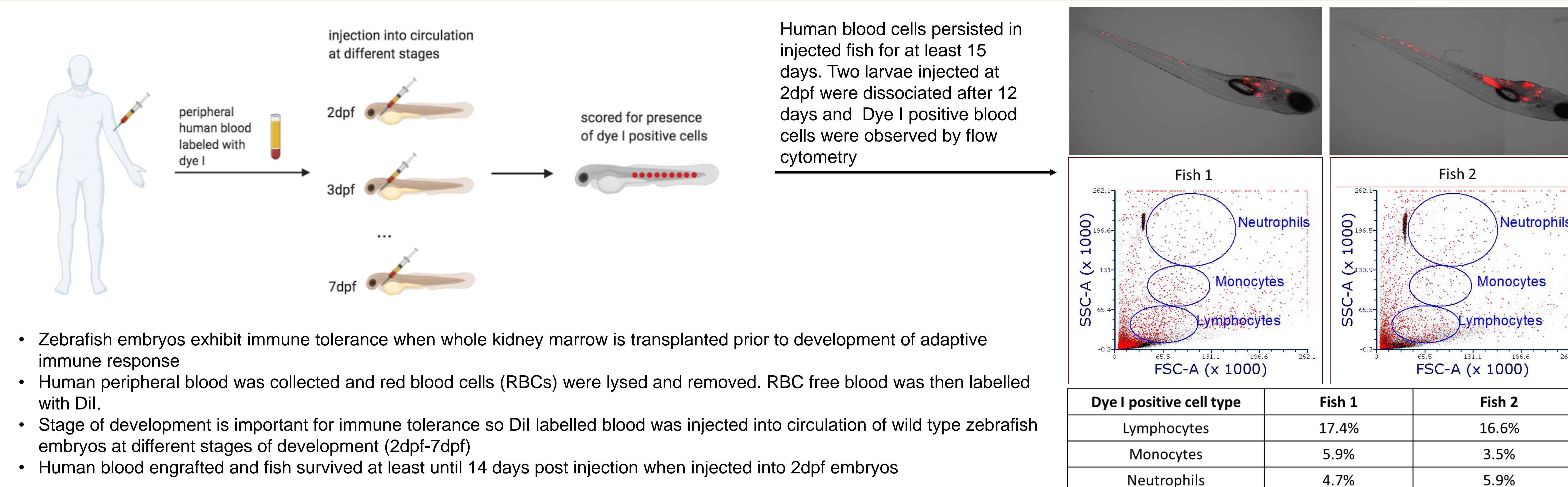### Zevatars determine patient tumor drug response

Figure 1. Differential drug response in cryopreserved liver metastasis biopsy (primary pancreatic cancer): A (a,c,e): Images of implanted tumor (patient ID : 1-01302019-A) on Day 1 of either FOLFIRINOX (4.2mM Fluorouracil, 1mM folinic acid, 0.08mM irinotecan, 0.08mM oxaliplatin) or Gemcitabine (0.1mM) + abraxane (0.005mM) treatment. A(b,d,f): Images of tumors 3 days post treatment. (B) Percentage of fish that survived after drug treatment (C) Change in area of the tumor between Day 1 and Day 3 in each embryo in each group (D) Average change in area between treatment groups. Error bars represent standard deviation (E) Percentage of fish with metastasis after drug treatment.

Recapitulating patient drug responses in the clinic using Zevatars

Figure 2- Summary of patient drug responses; change in tumor size, fish with metastasis and fish survival after 3-5 days of drug treatment: (A-C) Primary PDAC biopsies from 6 patients treated with 2 doses of gemcitabine (D-F) Liver metastasis from 7 patients with either primary pancreatic or colorectal cancer treated with gemcitabine, abraxane or folfirinox. Bars represent maximum-minimum interval range with mean. Interestingly, response varies greatly from one patient biopsy to another, recapitulating patient tumor behavior observed in the clinic

Hematopoietic chimeras and immune tolerance in cmyb+/- zebrafish embryos *(Refer to oral presentation 104)*

Table 1 : Cell lineages of engrafted RFP-expressing cells in 6 cmyb+/- transplanted fish

Kidney	ubi:zebrabow	A	B	C	D	E	F
Myelomonocytes	9.36	0.84	6.72	0.41	7.7	0.65	1.75
Precursors	2.04	0.37	1.69	0.07	1.99	0.09	0.64
Lymphocytes	8.07	0.33	2.73	0.54	7.54	0.09	1.84
Erythrocytes	4.73	1.84	4.86	1.84	4.88	0.65	19.8
Blood	**ubi:zebrabow**	**A**	**B**	**C**	**D**	**E**	**F**
Myelomonocytes	0.71	0.03	1.75	0.05	0.55	1	1.84
Precursors	0.29	0	0.2	0	0.24	0.72	0.74
Lymphocytes	2.12	0.05	3.21	0.09	0.55	0.24	0.46
Erythrocytes	68.46	1.46	64.37	3.64	6.67	32.45	8.28
Spleen	**ubi:zebrabow**	**A**	**B**	**C**	**D**	**E**	**F**
Myelomonocytes	0.52	0.64	0.4	0.31	1.14	0.32	1.89
Precursors	0.1	0.05	0.26	0.08	0.15	0.05	0.92
Lymphocytes	1.54	0.04	7.7	0.48	2.56	0.06	7.22
Erythrocytes	2.21	1.79	9.16	1.43	4.65	0.78	28.73

Humanizing zebrafish embryos

Human blood cells persisted in injected fish for at least 15 days. Two larvae injected at 2dpf were dissociated after 12 days and Dye I positive blood cells were observed by flow cytometry

Dye I positive cell type	Fish 1	Fish 2
Lymphocytes	17.4%	16.6%
Monocytes	5.9%	3.5%
Neutrophils	4.7%	5.9%

- Zebrafish embryos exhibit immune tolerance when whole kidney marrow is transplanted prior to development of adaptive immune response
- Human peripheral blood was collected and red blood cells (RBCs) were lysed and removed. RBC free blood was then labelled with DiI.
- Stage of development is important for immune tolerance so DiI labelled blood was injected into circulation of wild type zebrafish embryos at different stages of development (2dpf-7dpf)
- Human blood engrafted and fish survived at least until 14 days post injection when injected into 2dpf embryos

Conclusions and Future directions

- Zevatars can used to screen for the best treatment and hence applied to patients to improve personalized cancer medicine
- Zebrafish embryos exhibit immune tolerance and we are capable of introducing human hematopoietic cells with successful engraftment even in wild type fish
- The developmental stage at which we introduce the human hematopoietic cells is a crucial consideration when humanizing zebrafish.
- Next steps, we will assess immune interactions between injected human hematopoietic cells and implanted tumor
- In the future we can generate patient Zevatars in which we can test immunotherapy with the patient's own hematopoietic cells and tumor