Supplementary Methods

Demonstration that the xenografted tumor mass can be assimilated to a sphere.

The demonstration of the tumor mass circularity is carried out acquiring confocal images of xenografted embryos 2 hpi. The embryos are anesthetized with MS 222 0.l6 mg/mL, embedded in a drop of 3% methylcellulose and placed in lumox® dish.

Z-stacks are acquired both on ventral and lateral side for n≥4 embryos from two independent experiment of colon cancer. Then the areas of the tumor from each side are respectively measured, obtaining a ratio of 1.147 ±0.064.

Estimation of the radius at 2hpi and 2dpi

Starting from zPDX area measured at 1dpi and 2dpi, we estimated radius as:

$$r = \sqrt{\frac{Area_{2hpi\ or\ 2dpi}}{\pi}}$$

Apoptotic cells analysis

zPDX were treated with 5-FU and FOLFOXIRI according to Section 2.4 of Materials & Methods and processed for immunofluorescence staining with rabbit anti-human Cleaved Caspase-3 and Hoechst as counterstaining. The quantification of apoptotic cells was performed manually using the Multi point Tool of ImageJ, counting the DiI/Cleaved Caspase double positive with respect to the total number of human cells (DiI-positive cells).

Supplementary Figures

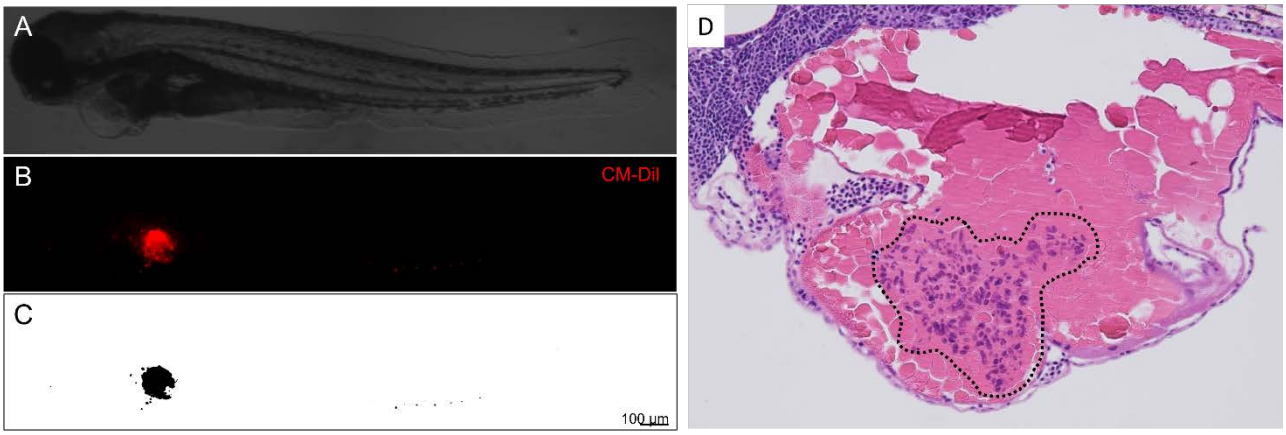

Figure S1 - The tumor tissue is xenografted into the perivitelline space of a zebrafish at 2 dpf. Representative images of a zPDX 2 days post xenotransplantation. (A) Bright-field, (B) fluorescence image of human tumor tissue with cell membranes CM-DiI labeled, (C) 8-bit image converted by Image-J, used for the analysis of the tumor area. (D) Representative Hematoxylin & Eosin stained image of a zPDX with xenografted tumor cells (dashed area). Image acquired at 20x magnification.

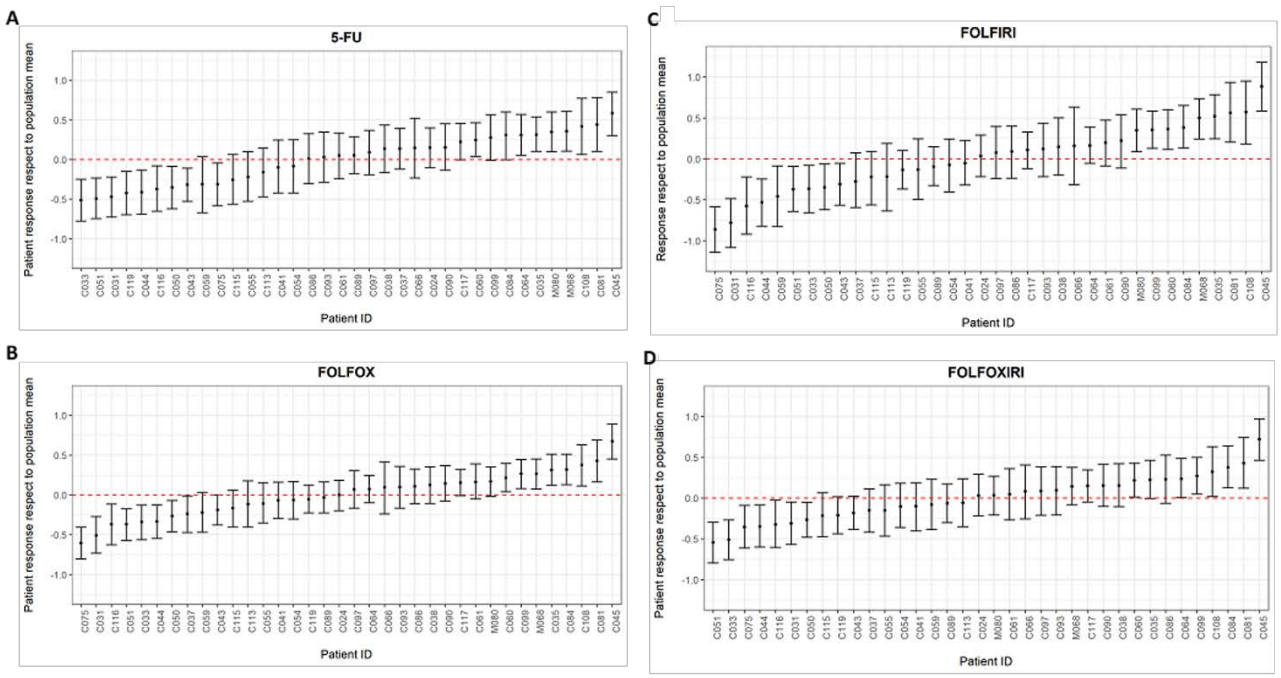

Figure S2. 95% CIs of the difference between fixed (treatments) and random coefficients (zPDXs) estimated by the LMM for 5-FU (A), FOLFOX (B), FOLFIRI (C) and FOLFOXIRI (D) chemotherapy treatments. The dots are the random effect, and the bars represent the 95%CIs of these values. The red dashed line is the mean response to each treatment of the zebrafish embryo populations.

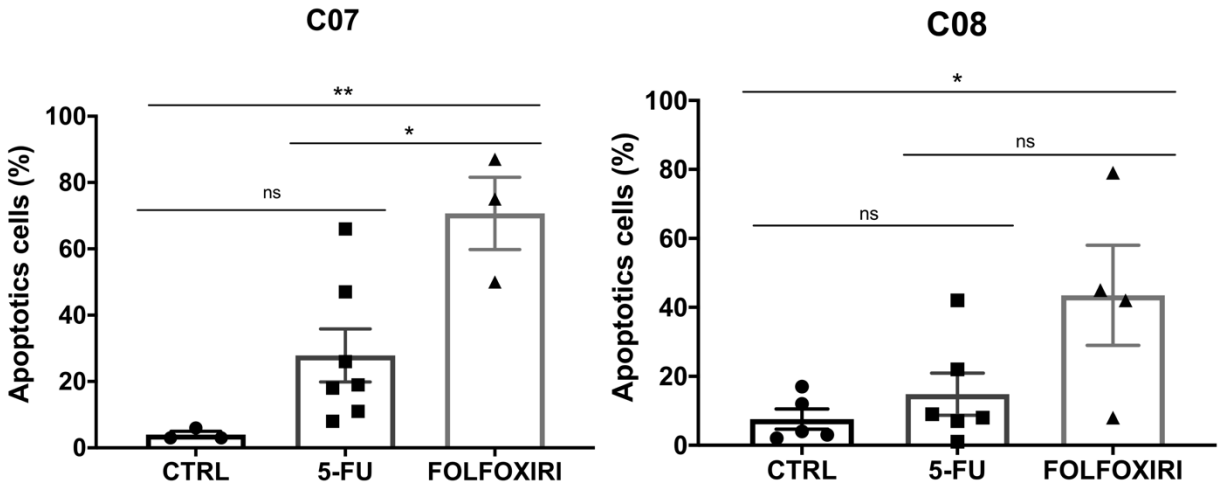

Figure S3. Percentage of human apoptotic cells in zPDX after 5-FU and FOLFOXIRI treatments. Data are expressed as mean ± SEM, n≥3 zPDX for each group. Statistical analysis was performed by Dunnett's multiple comparisons test. *$p < 0.05$, **$p < 0.01$ and ns nonsignificant vs control. C07 and C08 are the patient's identification codes.

Supplementary Table

Table S1. Fixed effects coefficients, their Standard Error (SE) and p-value estimated by LMM.

	Estimate	SE	P-value
Control	4.28	0.15	$<2\times10^{-16}$
5-FU	-0.19	0.06	4.67×10^{-3}
FOLFIRI	-0.28	0.07	4.64×10^{-4}
FOLFOX	-0.15	0.06	2.08×10^{-2}
FOLFOXIRI	-0.25	0.06	2.80×10^{-4}
$\log_{10}(r^3)$	-0.40	0.03	$<2\times10^{-16}$

R packages

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14. *performance* package: Lüdecke. Makowski. Waggoner & Patil (2020). Assessment of Regression Models Performance. CRAN. Available from https://easystats.github.io/performance/

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