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 CLINICAL RESEARCH


# zAvatar-test forecasts patient's treatment outcome in colorectal cancer: a co-clinical study towards personalized medicine

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**ABSTRACT:** Cancer patients often undergo rounds of trial-and-error to find the most effective treatment because there is no test in the clinical practice for predicting therapy response. Here, we conducted a co-clinical study to validate the zebrafish patient-derived xenograft model (zAvatar) as a fast predictive platform for personalized treatment in colorectal cancer. zAvatars were generated with patient tumor cells, treated exactly with the same therapy as their corresponding patient and analyzed at single-cell resolution. By individually comparing the clinical responses of 55 patients with their zAvatar-test, we developed a tree decision model integrating tumor stage, zAvatar-apoptosis, and zAvatar-metastatic-potential. This model accurately forecasts patient progression with 91% accuracy. Importantly, patients with a sensitive zAvatar-test exhibited longer progression-free survival compared to those with a resistant test. We propose the zAvatar-test as a rapid approach to guide clinical decisions, optimizing treatment options and improving the survival of cancer patients.

**KEYWORDS:** Colorectal Neoplasms; Disease Models; Animal; Xenograft Model Antitumor Assays; Precision Medicine; Progression-Free Survival

## INTRODUCTION

Colorectal cancer (CRC) remains one of the leading causes of cancer-related mortality worldwide. Although surgical resection is often curative, circulating tumor cells or undetectable micrometastases can be present after surgery. Thus, patients with risk factors for metastatic disease have been shown to greatly benefit from post-surgical systemic therapies to reduce the likelihood of relapse and disease progression, such as FOLFOX or FOLFIRI chemotherapy regimens.

Despite the broad equivalence of these regimens, approximately 50% of patients fail to respond, resulting in unnecessary toxicity and loss of critical clinical time. The absence of reliable predictive biomarkers remains a major limitation to personalized oncology, highlighting the need for functional models capable of anticipating individual tumor responses.

We have been developing a fast *in vivo* functional test with unprecedented cellular resolution: the zebrafish patient-derived xenograft model or zAvatar. This assay relies on the injection of fluorescently labelled patient tumor cells into 2 days post fertilization (dpf) zebrafish embryos. Among its numerous advantages, the most important are the ability to analyze metastatic and angiogenic potentials *in vivo*, and the speed of the test: tumor behavior and response to therapy can be accessed in just 10 days, a time frame compatible with oncological clinical decisions.

Therefore, this study aimed to evaluate the predictive capacity of zAvatars for chemotherapy response in patients with CRC.

## METHODS

Seventy-nine patients undergoing systemic chemotherapy after surgical resection were recruited, and zAvatar-test was technically feasible in 55 patients, representing 70% of the cohort. These included tumors from the colon, rectum, and liver metastases, spanning clinical stages II to IV. Tumor cells were injected into 2dpf zebrafish embryos, which were then treated with exactly the same chemotherapy regimen prescribed to the corresponding patients. Tumor responses in zAvatars were assessed by measuring apoptosis via acti-

vated caspase-3, tumor size, micrometastasis formation, and tumor engraftment. Results from zAvatars were compared in a blinded manner with patient clinical response 12 months after starting chemotherapy.

## MAIN RESULTS

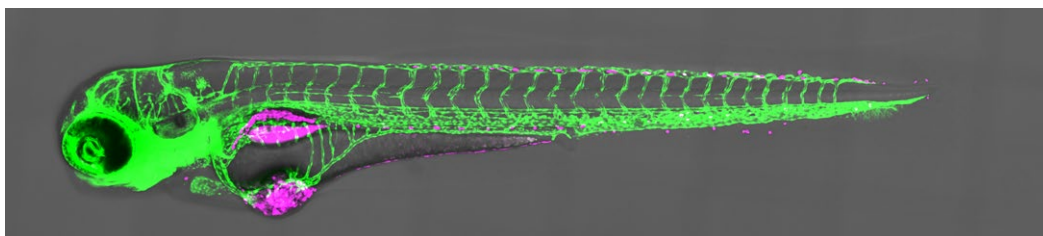
Induction of apoptosis in zAvatars strongly predicted patient clinical response, as tumors from patients who did not experience disease progression exhibited significantly higher apoptosis levels upon treatment ( $p < 0.0001$ ).

Moreover, the presence of micrometastases in untreated zAvatars strongly correlated with clinical staging and actual post-treatment progression, identifying high-risk patients even among those clinically classified as stage II or III. The model also captured intratumoral heterogeneity: in cases with synchronous primary and metastatic surgeries, zAvatars reproduced phenotypic and therapeutic differences between samples from the same patient.

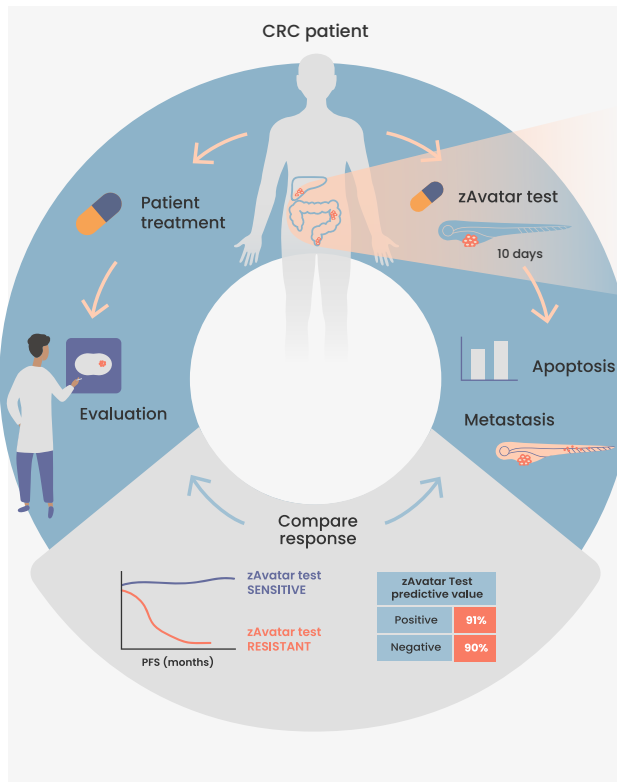
A multivariate decision-tree analysis incorporating patient tumor stage, apoptosis, and metastatic potential in zAvatars achieved an overall accuracy of 91%, correctly predicting outcomes in 50 of 55 patients. Notably, CRC patients whose zAvatar-test show sensitivity to treatment experienced a significantly longer PFS compared to those whose zAvatars were resistant ( $p < 0.0001$ ).

## CONCLUSION

These findings demonstrate that the zAvatar-test, with a timeframe of 10 days, has an exceptional predictive value for personalized medicine (Figure 1).



**Fig 1.** Patient cells (in mangenta) injected in a zebrafish transgenic line with the endothelial vessels depicted in green.



**Fig 2.** Schematic illustration of the workflow of the zAvatar-test and obtained results. Our findings demonstrate that the zAvatar-test is an accurate screening-platform for predicting colorectal cancer treatment outcomes. Illustration by Marta Correia.

Importantly, this test can be expanded to other types of cancers, by optimizing treatment options, improving PFS, preventing unnecessary toxicities and reducing healthcare costs. Furthermore, it serves as a valuable tool for assessing off-label options, particularly in the case of multi-resistant tumors.

In conclusion, zAvatar-test is a promising platform in personalized oncology, serving as a valuable complement to genetic studies. To introduce the zAvatar-test into clinical practice, it is crucial to perform a randomized clinical trial comparing zAvatar-based therapeutic decisions with physician's-choice (standard of care), a step that is already underway.

**ORIGINAL PAPER**

**Costa B, Estrada MF, Gomes A, et al.** Zebrafish Avatar-test forecasts clinical response to chemotherapy in patients with colorectal cancer. *Nat Commun.* 2024;15(1):4771. Published 2024 Jun 5. doi:10.1038/s41467-024-49051-0



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