Integration of liver tumor organoids and *in silico* methods to address drug resistance: LIVERtera

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Introduction

- Organoid technology have recently revolutionized research in hepatic oncology. Approaching drug resistance is essential to tackle such challenges, particularly in hepatocellular carcinoma (HCC) and metastatic colorectal cancer (mCRC).
- Standard-of-care (SoC) treatments are sorafenib for HCC and cetuximab for mCRC. Although most of patients respond well, treatment resistance arises in most of the cases, leaving patients without therapeutical alternatives:
- EGFR mutations impacting the extracellular domain such as S492R, G465R, G465E and S464L have been clinically reported but there is limited experimental evidence about their impact in cetuximab resistance.
- *In silico* technologies such as molecular dynamics (MD) and molecular docking allow a detailed analysis of atomic interactions between a drug and its target.



Figure 1. Three-dimensional structure of the EGFR extracellular domain (green) in complex with cetuximab (purple-orange). PDB ID: 6ARU.

Aims of the study

- To characterize the impact of cetuximab resistance-conferring EGFR mutations using chemoinformatic tools in the context of mCRC.
- To determine the ADME properties of sorafenib for HCC.
- To establish patient-derived organoids from HCC and mCRC patients that recapitulate the malignant phenotype.
- To identify compounds that overcome the traits of the interactions that confer resistance and test them in patient-derived organoids.

Methodology

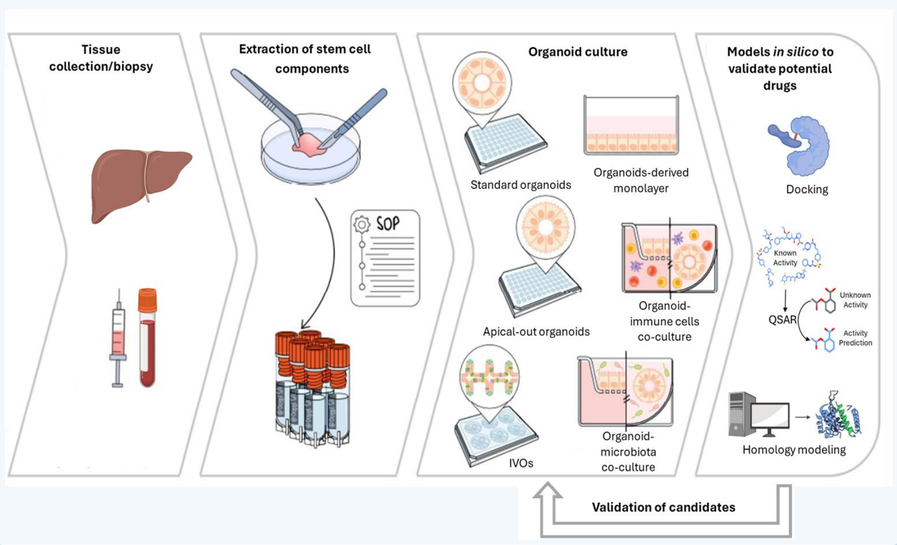
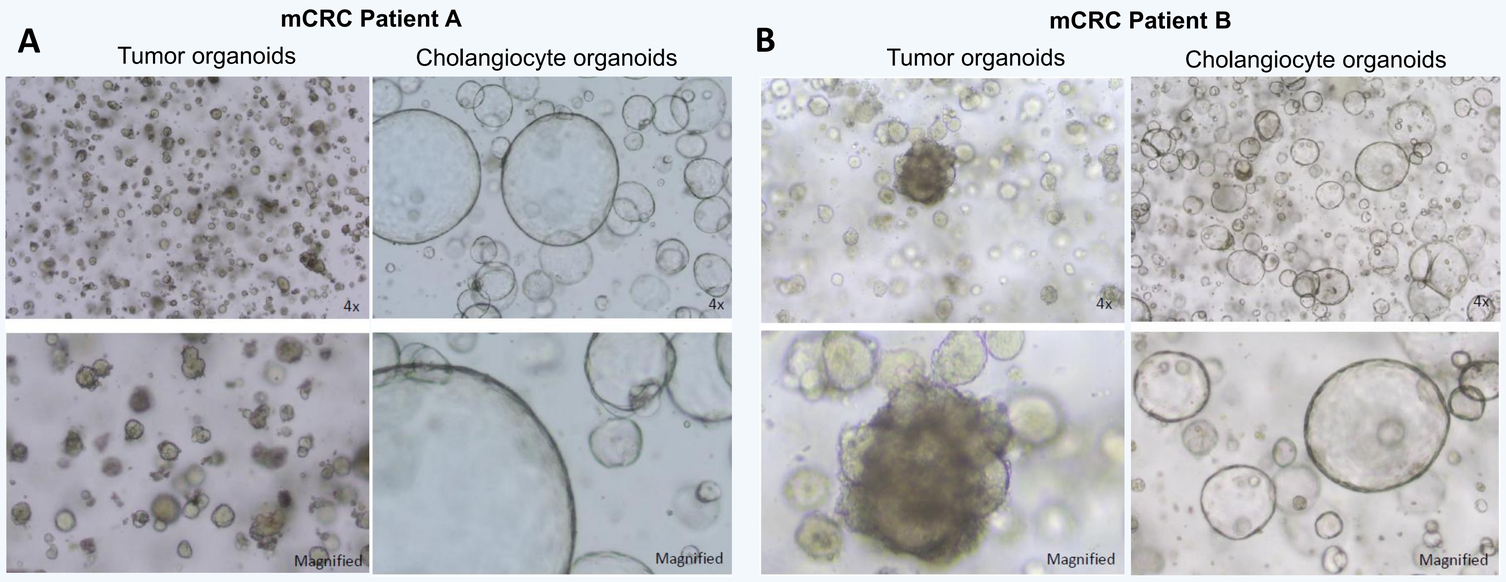


Figure 2. From the operating room to computational models: LIVERtera integrates the co-culture of patient-derived tumor organoids with peripheral blood mononuclear cells (PBMCs) to test computationally identified repurposed compounds that show potent anti-tumor activity in heavily pre-treated cases.

Results



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System	Structure1	Structure2	Interface_area_A^2	ΔG_kcal_mol
WT	EGFR	LC	665.1	-1.2
	HC	EGFR	577.4	-1
S468R	EGFR	HC	567	0.9
	EGFR	LC	391.1	-1.4
G441R	EGFR	HC	526	-0.5
	EGFR	LC	361.6	-1.7
G441E	EGFR	HC	524.7	-0.1
	EGFR	LC	364.3	-1.8
S440L	EGFR	HC	565.2	0.4
	EGFR	LC	391.2	-1.4

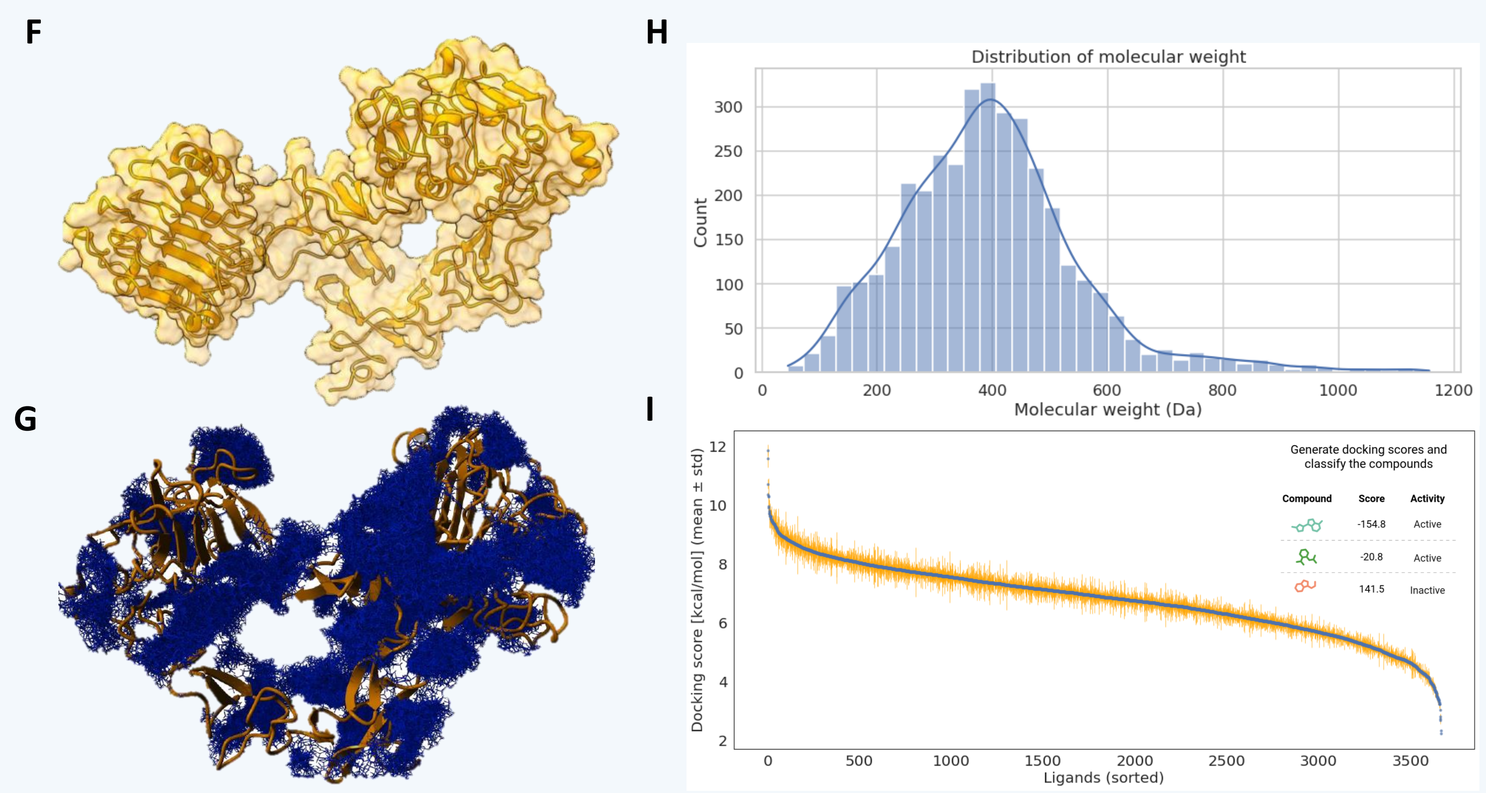


Figure 3. **A** Liver metastatic colorectal cancer (mCRC) and cholangiocyte patient-derived organoids from patient A and **B** from patient B. **C** Immunohistochemistry for human cytokeratin-20 performed on normal adjacent (NAT) liver tissue and **D** tumor tissue. **E** Interface area of contact between EGFR (wild type and mutant systems) and cetuximab. **F** Wild type EGFR system structural preparation prior computational simulations. **G** Docking of the 3669 compounds (blue) of the MolPort Database with wild type EGFR **H** Distribution of the molecular weight of the compounds used for the screening with EGFR. **I** Compound ranking based on their docking score, expressed in kcal/mol.

Conclusions

- Patient-derived tumor and cholangiocyte organoids (PDOs) were successfully established.
- Computational modelling confirmed the destabilizing effect of clinically reported EGFR variants via reduction of interface contact area with cetuximab.
- A set of compounds have been ranked with promising EGFR binding activity, which will be tested in PDOs.

Acknowledgements