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COMPARISON OF AUTOMATED HIGH-CONTENT IMAGE-BASED PHENOTYPIC SCREENING MODELS FOR DRUG DISCOVERY: FROM 2D CULTURES TO 3D SPHEROIDS AND ORGANOIDS

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Abstract

Translation of promising preclinical candidate compounds into clinical drugs is still too often failing, highlighting the persistent need for effective and relevant disease models used in drug discovery, especially for solid tumors. The latest advances in cell-based assay technologies for drug discovery include 3D cultures of patient-derived stem cell-based organoids which are now becoming more standardizable and automatable for routine use at industrial scale.

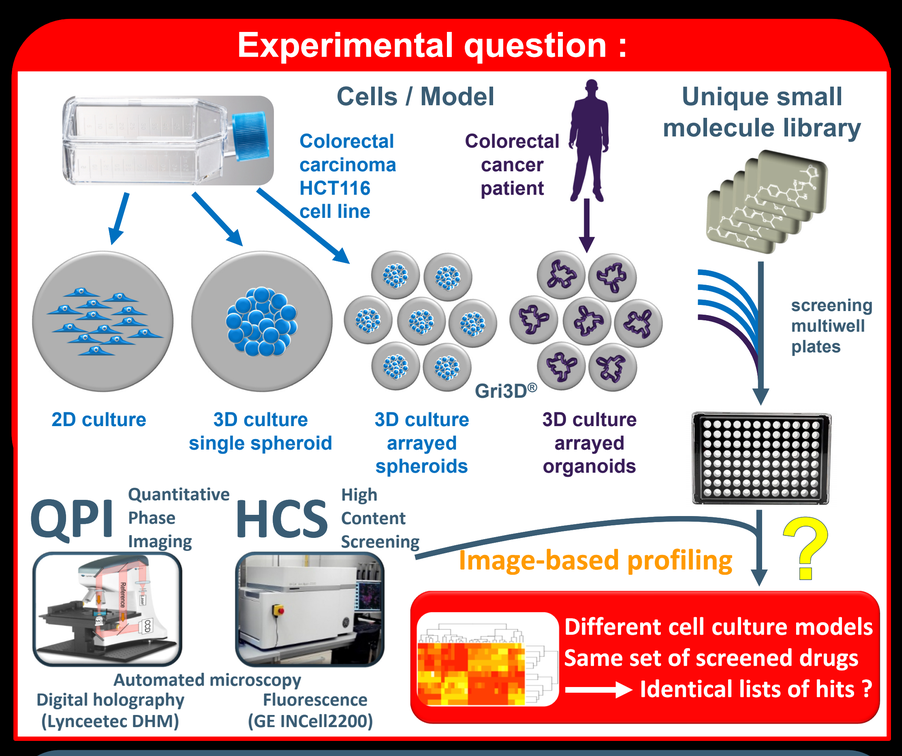
Here we report the direct comparison of screening results of the same set of carefully selected compounds (either FDA-approved or in clinical trials) against different models of colorectal disease. The models range from simple 2D cultures of a standard human colorectal carcinoma cell line (HCT116 cells) to 3D cultures of spheroids generated from the same cell line on agarose layers and up to colorectal cancer spheroids and patient-derived gastrointestinal organoids using an automated culture via stem-cell aggregation in hydrogel-based microcavity arrays (*).

The readouts of screening with the different models are all making use of high-throughput image-based label-free digital holographic microscopy or automated fluorescence microscopy and consist of live/dead assays (either label-free or with the standard Hoechst / Calcein AM / Ethidium Homodimer-1 combination of fluorescent dyes), allowing phenotypic image-based analyses in a toxicity screen context.

For each model, the obtained images are properly analyzed, segmented and quantified, and hundreds of features are extracted from each of them. Standard dimensionality reduction algorithms and multivariate projections are used to integrate the results into simple linear classifications of efficiencies of the screened compounds and compared between the different models, as well as confronted to the literature.

We show not only how phenotypic analyses can reveal insights into mechanisms of drug action depending on the model used, but also how similar and reproducible the results can be between models. However, more subtle differences in effects and efficacy of the tested drugs can be detected only with the state-of-the-art organoid culture derived from patients, in line with the genetic mutation status of specific oncogenes, making this scalable and automatable organoid-culture technology unavoidable in the future of drug discovery through high-content screening of tissue-level function.

Experimental question :



Unique small molecule library screened

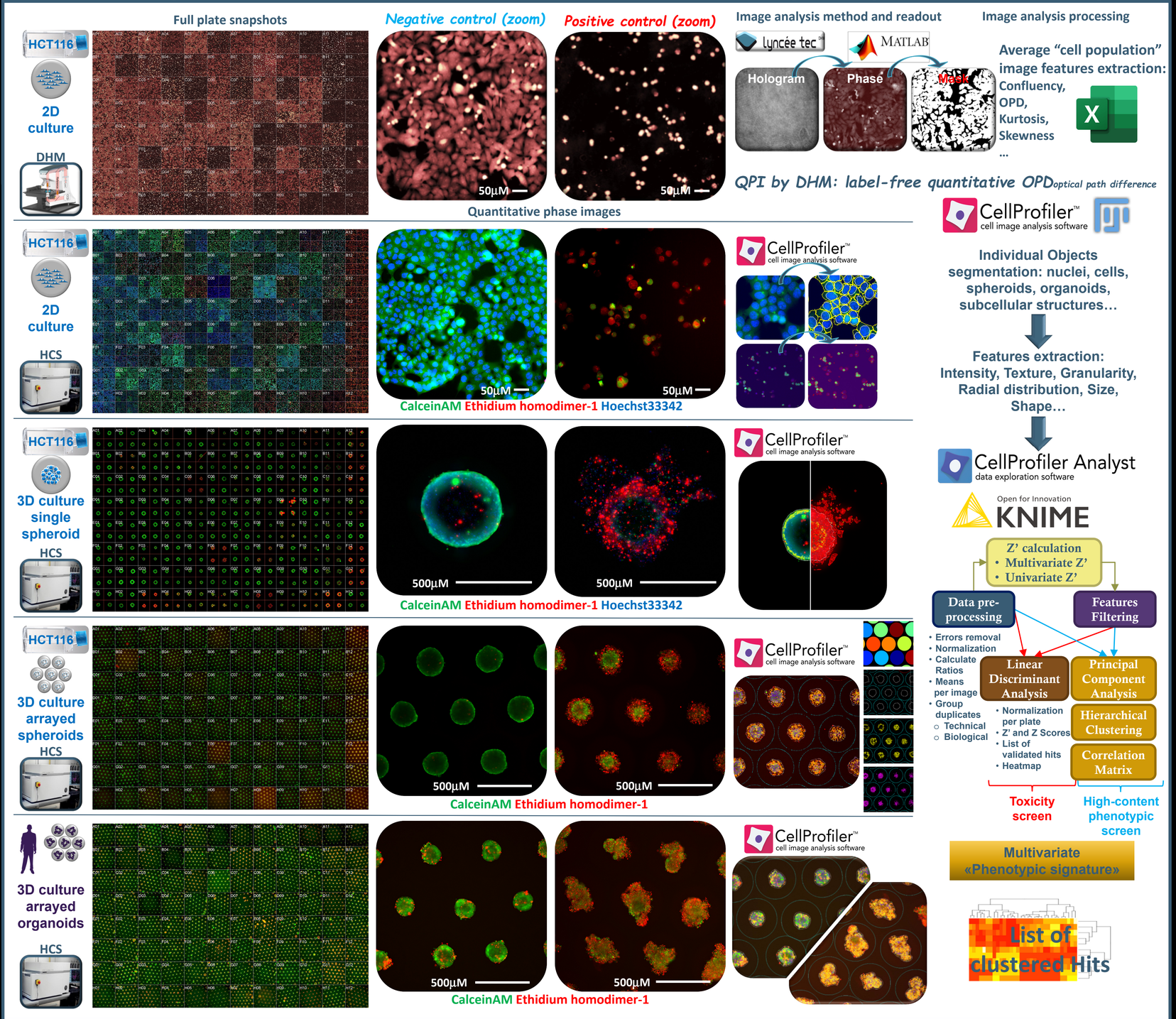
Screen set of 80 compounds for 96-well plates format

	1	2	3	4	5	6	7	8	9	10	11	12
A	DMSO	Capecitabine	Cabozantinib	Ralitrexed	Tofacitinib	VRT752271	Erlotinib	Olaparib	Sunitinib	Camptothecin	Tunicamycin G	Gambogic Acid
B	DMSO	Cobimetinib	Entrectinib	Ganetespib	BYL-719	LEE 011	Bosutinib	Dasatinib	Rapamycin	BAY 43-9006	Podophyllotoxin	Gambogic Acid
C	DMSO	LY2157299	Tivozanib	RG 7388	Pacritinib	Oranitinib	Azacytidine	Lapatinib	Afatinib	Cediranib	Taxol	Gambogic Acid
D	DMSO	CAL-101	Lenvatinib	LDE 225	SAR245409	Varlitinib	Vandetanib	Dovitinib	Enzastaurin	Imiquimod	NT 2 Toxin	Gambogic Acid
E	DMSO	Dacomitinib	BLZ945	Birinapant	INCB 024360	Vismodegib	PLX-4032	Dinaciclib	Foretinib	Regorafenib	RA-XIII	Gambogic Acid
F	DMSO	Batimastat	Ponatinib	XL-147	Afuresertib	SAHA	Lenalidomide	Tivantinib	Pexidartinib	Oxaliplatin	Colcemid	Gambogic Acid
G	DMSO	Apatinib	Defactinib	Neratinib	LY2835219	Nilotinib	Gefitinib	Pozioitinib	Everolimus	Masatinib	Vindesine	Gambogic Acid
H	DMSO	Icotinib	Volasertib	Crizotinib	AZD-9291	Trametinib	Dabrafenib	Belinostat	Fluorouracil	Staurosporine	Aurano fin	Gambogic Acid

Negative control — *Positive control*

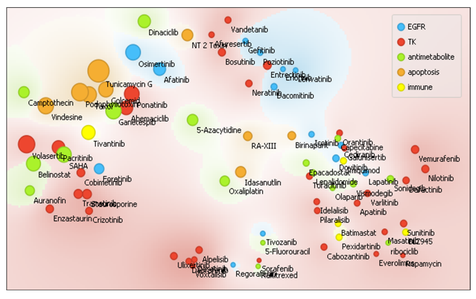
- A set of 80 drugs (FDA-approved or in clinical trials) has been selected based on updated clinical data on colorectal cancer treatment. The set is subdivided according to the known target or mechanism of action (MOA) of the molecules into 5 main categories (antimetabolites, toxins / apoptosis inducers, kinase inhibitors, cytokines or immune cells targeting, and Tyrosine Kinase Receptor inhibitors (EFGR, FGF, VEGFR…)
- The screen was performed on each model at 1 µM and 10 µM final concentration after 2 or 3 days of incubation with the drugs, followed by automated QPI and HCS reading

Screening plate snapshots, example pictures, customized image analysis and processing



t-SNE of all screened compounds per model

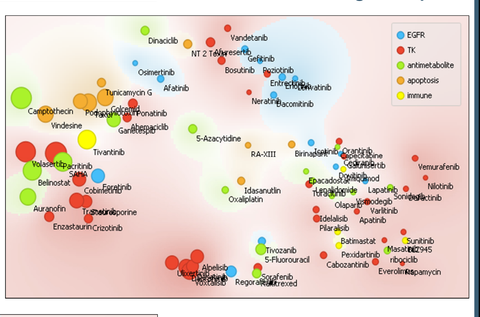
- t-SNE algorithm was applied on all screening data for all models tested and color-coded according to the MOA of compounds.
- The size of the dots reflects the score of each compounds for the specific model tested, as specified for each graph.



2D cells – OPD (DHM)

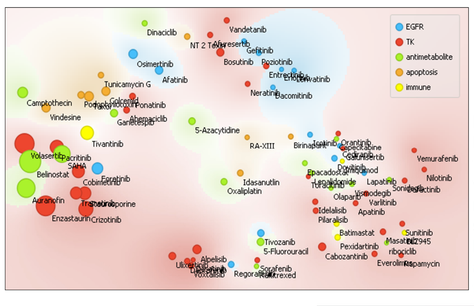
HCT116 cells cultured in 2D, and screened as exemplified with OPD reading (through DHM), show the most intense response with "classic" toxic drugs inducing apoptosis i.e. vindesine, colcemid, podophyllotoxin, or with some specific TK inhibitors, i.e. volasertib or the HSP90-inhibitor ganetespib

Spheroids Gri3D



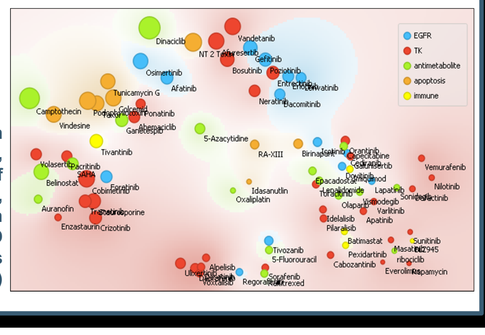
HCT116 cells cultured in 3D in multiple subwells of a Gri3D plate, forming several dozens of homogeneous spheroids per well, show the most intense response with the same set of compounds as in 2D cultures, plus some additional targets i.e. PI3k (alpelisib, voxtalisib)

Spheroids 3D (Agarose)



HCT116 cells cultured in 3D on a layer of agarose, generating the formation of a single spheroid per well, show the most intense response with antimetabolites and some specific TK inhibitiors, i.e. belinostat, volasertib, enzastaurin, auranofin, crizotinib, pacritinib, tivantinib or staurosporine

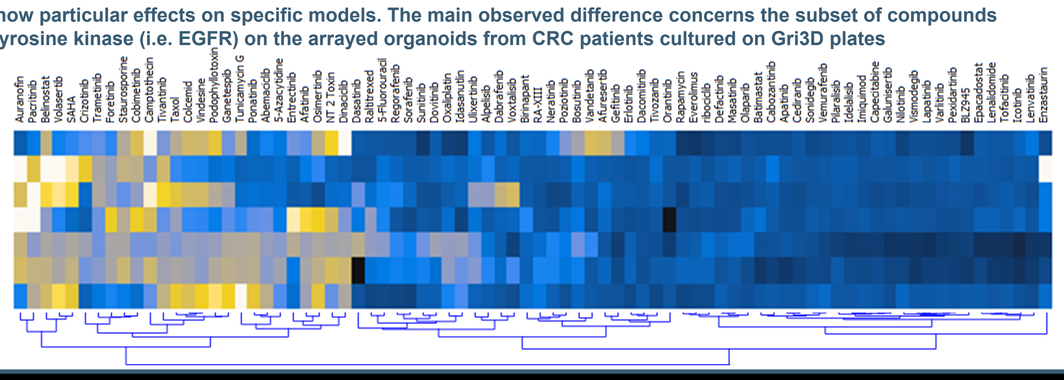
Organoids Gri3D



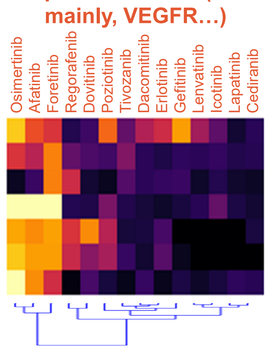
CRC patient cells cultured in 3D in multiple subwells of a Gri3D plate, forming several dozens of homogeneous organoids per well, show the most intense response with the same set of compounds as in 2D cultures, plus some specific targets i.e. EGFR inhibitors (osimertinib…) and other TK inhibitors

Clusterized Heat Map of screening results for the different models tests

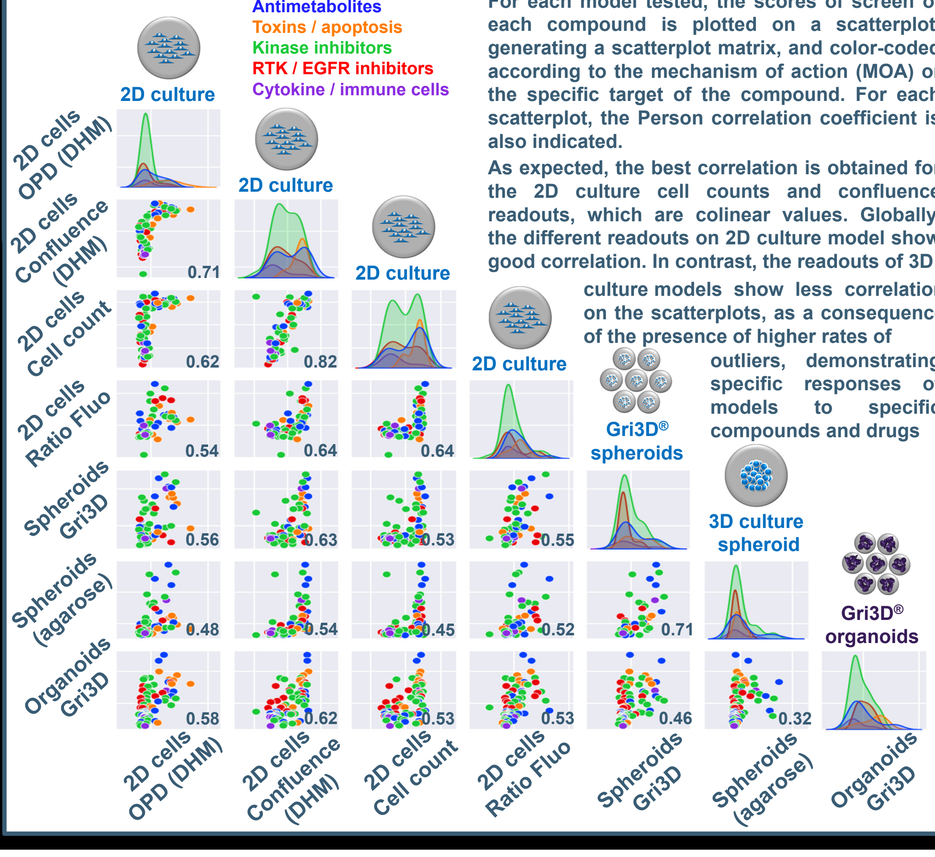
- Hierarchical clustering / Dendrogram for all tested compounds show overall high similarity in responses of the different tested models
- Only few compounds show particular effects on specific models. The main observed difference concerns the subset of compounds targeting the receptor Tyrosine kinase (i.e. EGFR) on the arrayed organoids from CRC patients cultured on Gri3D plates



Receptor Tyr Kinase targeting compounds subset (EGFR mainly, VEGFR…)



Scatterplot matrix of response to drugs for each model



For each model tested, the scores of screen of each compound is plotted on a scatterplot, generating a scatterplot matrix, and color-coded according to the mechanism of action (MOA) or the specific target of the compound. For each scatterplot, the Person correlation coefficient is also indicated.

As expected, the best correlation is obtained for the 2D culture cell counts and confluence readouts, which are colinear values. Globally, the different readouts on 2D culture model show good correlation. In contrast, the readouts of 3D culture models show less correlation on the scatterplots, as a consequence of the presence of higher rates of outliers, demonstrating specific responses of models to specific compounds and drugs

Conclusions

The same set of carefully selected compounds was screened against different models of colorectal cancer, ranging from 2D cultures of HCT116 cells to 3D cultures of spheroids and gastrointestinal organoids of colorectal cancer patient using an automated 3D culture (Gri3D®, SUN Bioscience*).

We show highly similar and reproducible results between the models tested. However, more subtle differences in effects and efficacy of the tested drugs can be detected only with the state-of-the-art organoid culture derived from patients, in line with the genetic mutation status of specific oncogenes, making this scalable and automatable organoid-culture technology unavoidable in the future of drug discovery.

References

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