SUNBIOSCIENCE

Assessing combination therapies in human pancreatic cancer organoids using a standardized screening workflow

Roch, A.[1], Kuttler, F.[2], Naret, O.[1], Turcatti, G.[2], Homicsko, K.[3], Brandenberg, N.[1], Hoehnel, S.[1]

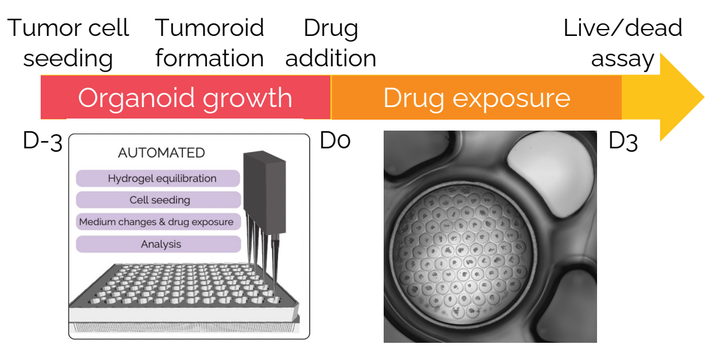
[1]SUN bioscience SA Lausanne, Switzerland. [2]Biomolecular Screening Facility, School of Life Sciences, EPFL, Lausanne, Switzerland. [3]Department of Oncology, University Hospital of Lausanne, and Lausanne Branch, Ludwig Institute for Cancer Research, University of Lausanne, Lausanne, Switzerland.

INTRODUCTION

Cancer remains one of the leading causes of death in the 21st century. Despite the latest advances in oncology, the majority of cancer patients lack tailored therapeutic approaches with lasting benefit[1]. Readily measuring the impact of anticancer compounds and their combinations is possible only on *ex vivo* assays. However, fresh primary cell cultures remain challenging to establish for testing *ex vivo* in a clinically relevant manner both in terms of time and biological relevance[2]. To this end, **patient-derived organoids** (PDOs) have been proposed as viable and efficient alternatives for *ex vivo* testing. PDOs show long-term expansion potential while retaining tumor histopathology as well as cancer gene mutations[3]. However, the translation of PDOs to the industry for screening applications has so far been hampered by the lack of homogeneity, difficult handling and automatability of organoid cultures in solid ECM drops, which is challenging for their analyses. We set up an automated screening workflow using **Gri3D®**, our innovative hydrogel-based ultra-dense U-bottom shaped microcavity array platform[4]. We use a Live/Dead assay to assess drug and drug combination effects on PDOs. Using the SUN bioscience automated analysis platform, acquired images are processed and results are extracted in under one hour, reducing the time until a response is given to the clinics. We demonstrate on human pancreatic cancer organoids (PCOs) how amalgamation of anti-cancer drugs could enhance efficacy compared to mono-therapy approaches.

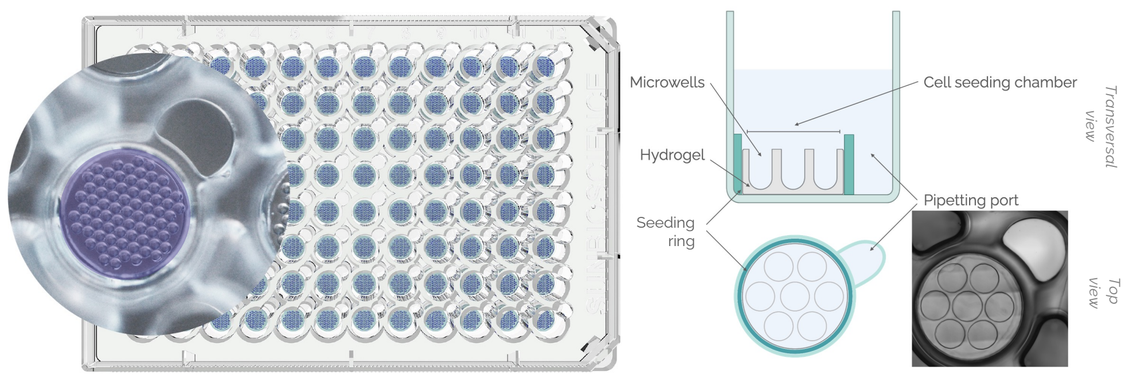
METHODS

Patient-derived PCOs were generated in Gri3D® 96WP imaging-bottom 500 µm microwells and exposed to anti-cancer drugs. All steps of the screen were executed by an automatic liquid-handling system: hydrogel equilibration, cell seeding, medium changes, drug exposure and readout using image-based Live/dead assay. 40 organoids were segmented per well and more than 250 metrics were extracted from each.[4]



Anti-cancer drugs panel:
- Alpelisib (PI3K inhibitor)
- Palbociclib (CD4/6 inhibitor)
- Infigratinib (FGFR inhibitor)

GRI3D® TECHNOLOGY



Gri3D® is a ready-to-use platform for high-throughput and reproducible organoid culture. Based on an array of ultra-dense U-bottom microwells in a hydrogel, single organoids are robustly generated in each microcavity and grown in suspension-like culture without a solid ECM.

RESULTS

A single-drug small-scale screen was first performed on PCOs (N=2, n=2). PI3K inhibitor alpelisib reduced organoid sizes at low doses, whereas infigratinib only showed an effect at doses higher than 10 µM. Palbociclib showed an area reduction only at the highest concentration (50 µM). Moreover, organoids treated with the alpelisib showed significant propidium iodide to calcein AM intensity at doses as small as 5 µM. Alpelisib was therefore tested in combination with the 2 other drugs to assess whether its efficacy could be enhanced. As a result, we could observe that its addition to the FGFR inhibitor, infigratinib, increased toxicity levels compared to the drug alone.

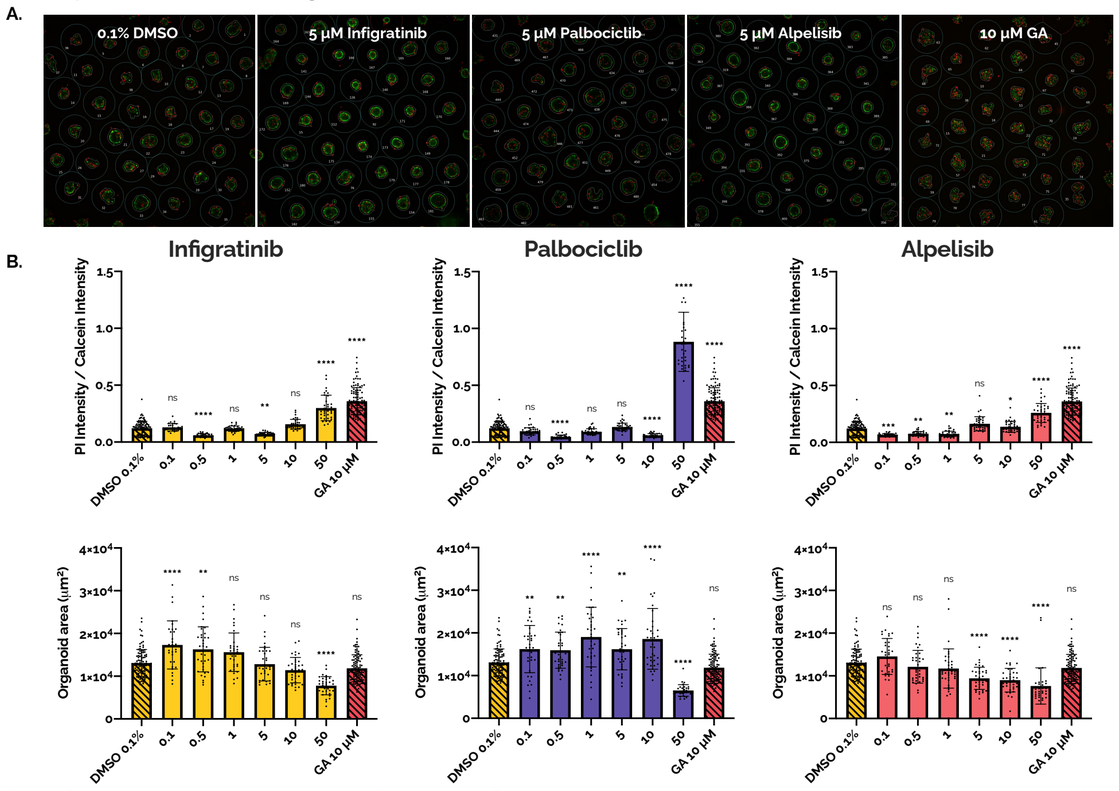


Fig. 1. Results of single-therapy screen. Response of PCOs exposed to different anti-cancer drugs. GA: gambogic acid. **A.** Representative images of Live/Dead assay. PCOs were labelled with Calcein-AM (C-AM, green, live) and propidium iodide (PI, red, dead). Microcavities are shown in cyan, and segmented organoids in white. **B.** Phenotypic response characterized by PI/C-AM and organoids area. One-way ANOVA Dunnett's multiple comparisons, *P < 0.05, **P < 0.01, ***P < 0.001, P**** < 0.0001, ns: non-significant.

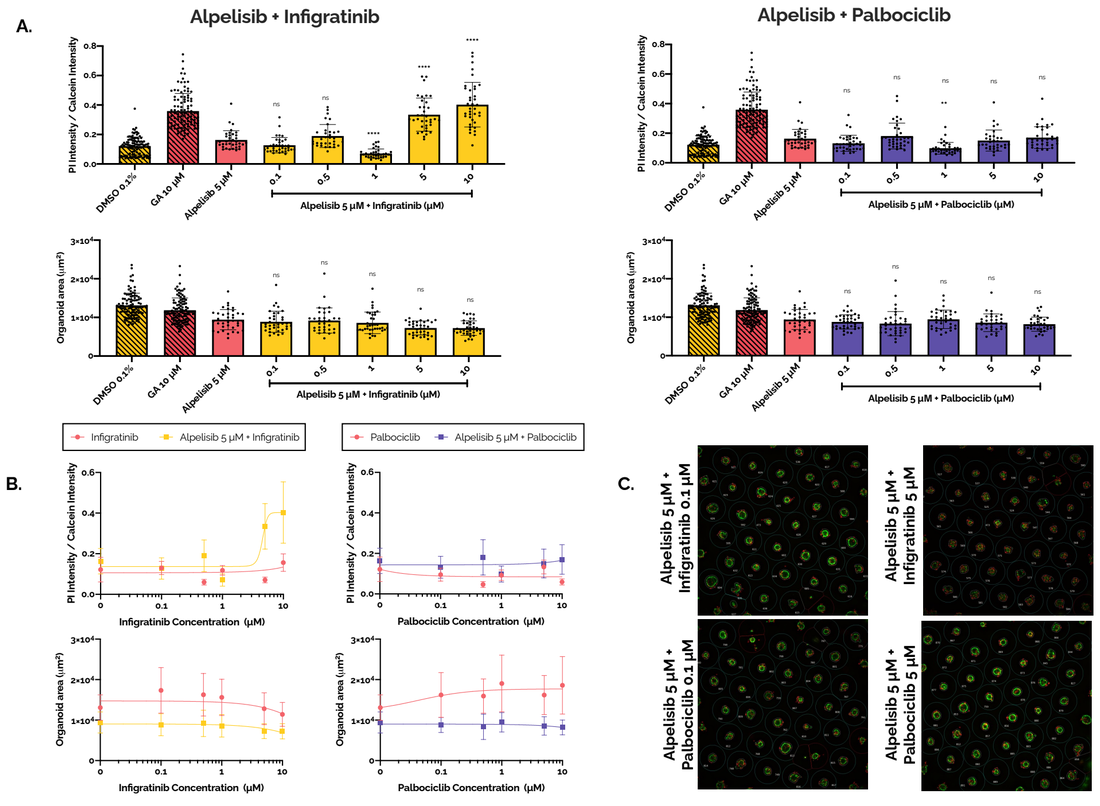


Fig. 2. Results of combination-therapy screen. PCOs were treated with Alpelisib (5 µM) in combination with infigratinib, or palbociclib. **A.** PCOs phenotypic response characterized by organoids area and ratio PI/C-AM. One-way ANOVA Dunnett's multiple comparisons, *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001, ns: non-significant. **B.** Curve fitting of compound dose-response. Exponential or dose-response curves were used. EC50 (alpelisib + infigratinib) = 4.5 µM. **C.** Representative fluorescence images of Live/Dead assay.

CONCLUSIONS

- Gri3D® is a front-to-end solution for high-throughput screening where all steps from seeding to readouts are automated.
- The use of Gri3D® 96 500 µm microwells allows the generation of more than 70 organoids per well, of which 40 are segmented for high-content image analyses in a single field of view.
- Our findings are amongst the first reporting a personalized medicine approach using patient-derived organoids for combination therapies assessment.
- Combination therapies allow a reduction of secondary effects by lowering the doses of the single agents, while avoiding resistance.
- Current developments focus on expanding our organoid patient pool and comparing the organoid results to clinical outcomes.

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Address
EPFL Innovation Park, Bâtiment D, CH-1015 Lausanne