

# Pushing boundaries of preclinical modeling through new type of long-term cultivation of organoids – A case study for cancer drug resistance assessment

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## Introduction

Drug resistance is a common, yet currently unsolved, problem in the treatment of tumor patients with high impact on disease progression<sup>a,b</sup>. Assessing the functional mechanisms that cause tumor relapse has been very challenging so far since there are no reliable *in vitro* models available to physiologically mimic the phenomenon<sup>c</sup>. Using the basis of the 3D CoSeedis *in chip* communication technology<sup>TM</sup> we have created for the first time an *ex vivo* system in which we can identify and analyze micro-tumors showing drug resistance and subsequent relapse. To do so, we overcame two major hurdles that prevented reliable *in vitro* tests so far: the identification of stochastic and rare events of resistance formation and prolonged *ex vivo* culturing to identify the impact of resistance on tumor growth.

By exposing hundreds of physiologically accurate and highly uniform micro-tumors to identical growth and treatment conditions over prolonged culture periods, we are able to identify those few events in which resistance occurs. Consequently, the 3D CoSeedis<sup>TM</sup> *in chip* Relapse Assays provide a unique method to not only identify potential resistance formation but also allow to test for compound adaptations and modifications that minimize such risks and consequently increase the efficacy of cancer treatment.

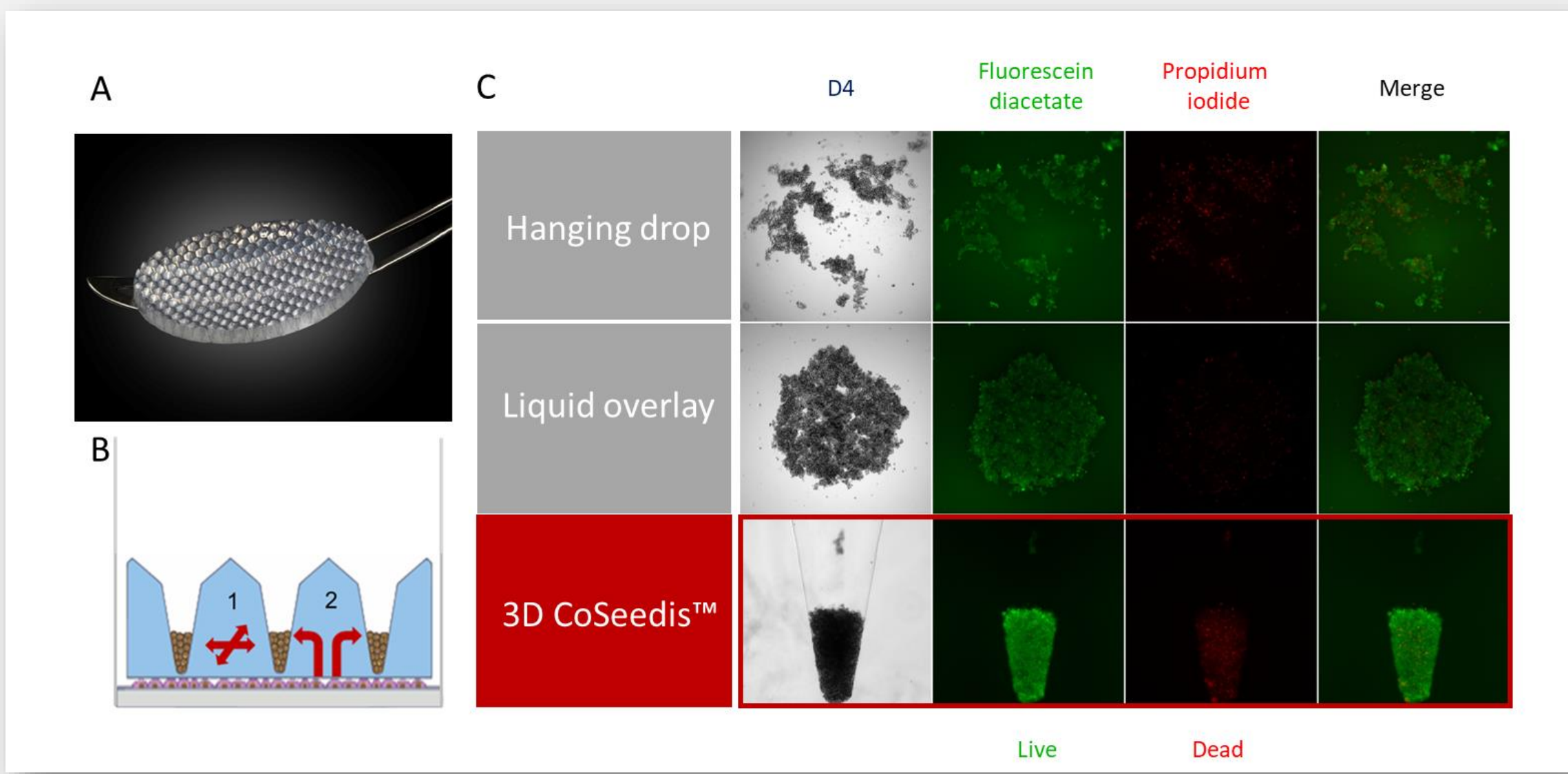


Figure 1: The 3D CoSeedis *in chip* communication technology<sup>TM</sup>

The 3D CoSeedis<sup>TM</sup> chips allows the aggregation and maintenance of hundreds of micro-tissues under the exactly same conditions for a prolonged period of time. Intra-chip communication ensures the formation of a physiological micro-environment that guarantees highly homogenous and uniform organoids/spheroids throughout the chip. A) photograph of a 3D CoSeedis<sup>TM</sup> Chip200 showing the unique topography of the chip. B) Schematic representation of possible intra- and inter-cellular communication pathways. C) The unique features of the 3D CoSeedis<sup>TM</sup> chips allow the formation of 3D aggregates of cells that would normally not do so with alternative technology, such as hanging drop or liquid overlay. Show here are conical 3D micro-tumors of MIAPaCa-2 pancreatic tumor cells.

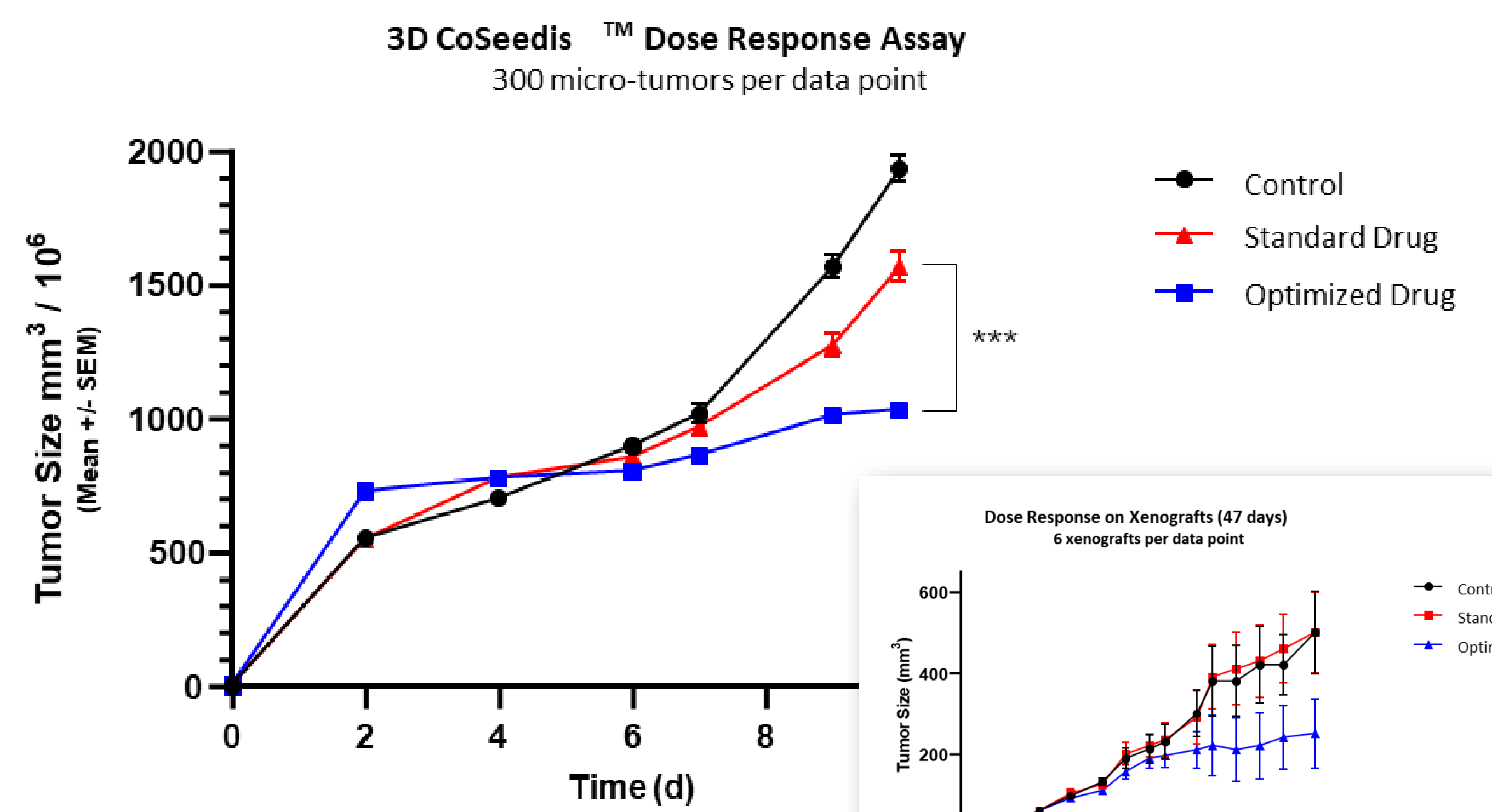
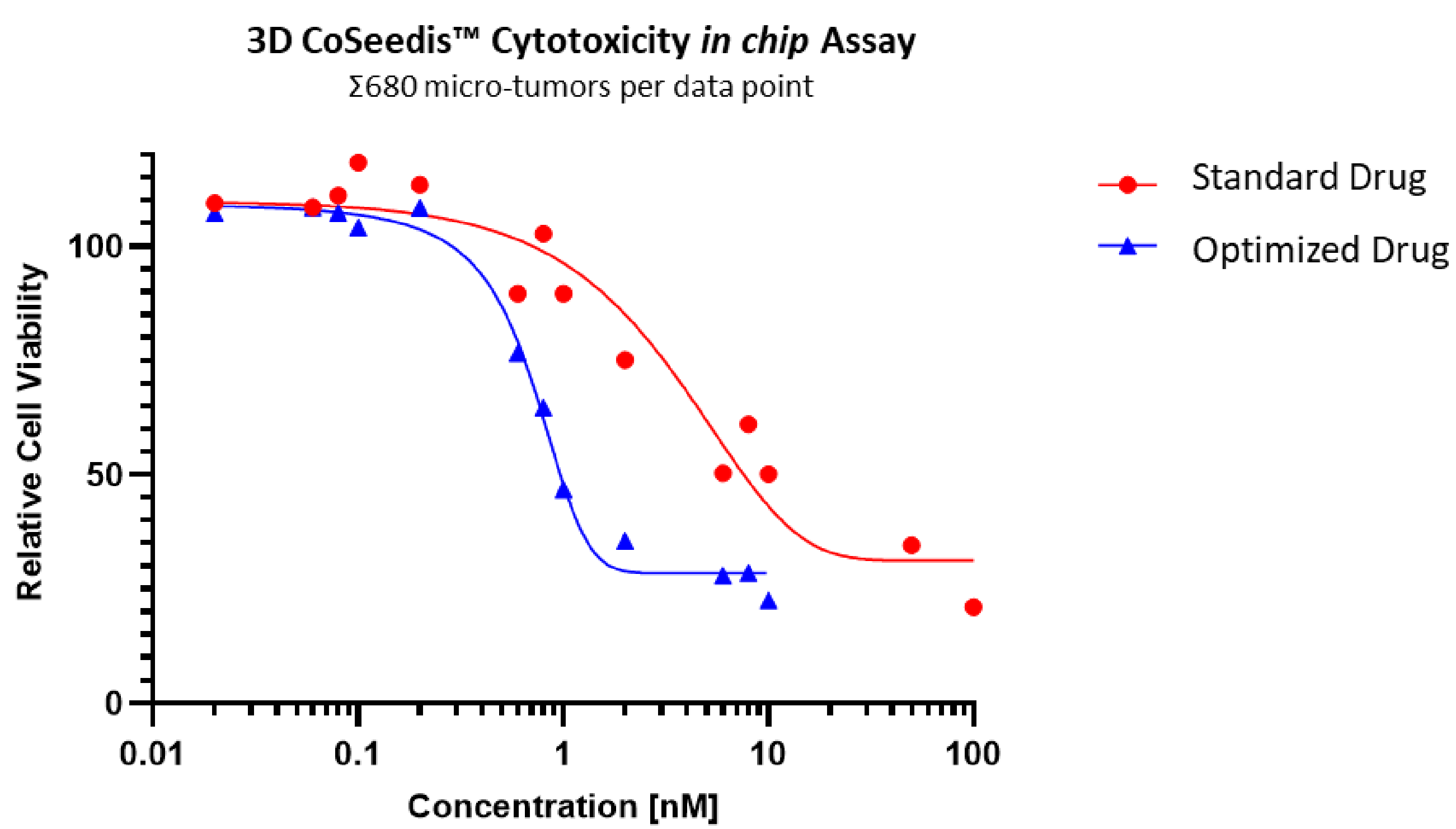


Figure 2: The 3D CoSeedis<sup>TM</sup> *in chip* Assay concept

As a direct consequence of the innovative chip design as well as the resulting 3D CoSeedis *in chip* communication technology<sup>TM</sup>, abc biopply has developed the 3D CoSeedis<sup>TM</sup> *in chip* Assay concept. This specific assay benefits from the high number of biological replicates per chip that are either integrated or analysed individually and consequently deliver unprecedented accuracy and predictability in their respective read-out. Left panel: 3D CoSeedis<sup>TM</sup> Cytotoxicity *in chip* Assay – 680 micro-tumors were integrated to indicate cell viability at each individual data point. Misperforming tumors are therefore neglectable since their relative participation to the overall signal is insignificant. Consequently, the highly physiological conditions lead to more reliable and more predictive results to assess drug efficacy. Right panel: In the 3D CoSeedis<sup>TM</sup> Dose Response Assay, we average the volume of 300 individual micro-tumors per data point. The assay delivers highly accurate dose response data comparable to animal models with an unprecedented sensitivity and therefore allows accurate dosage predictions as early as 7 to 8 days after treatment start. In comparison, current animal-based dose response assessments show much lower sensitivity and take up to 7 times longer from treatment to result (see small insert in right panel).

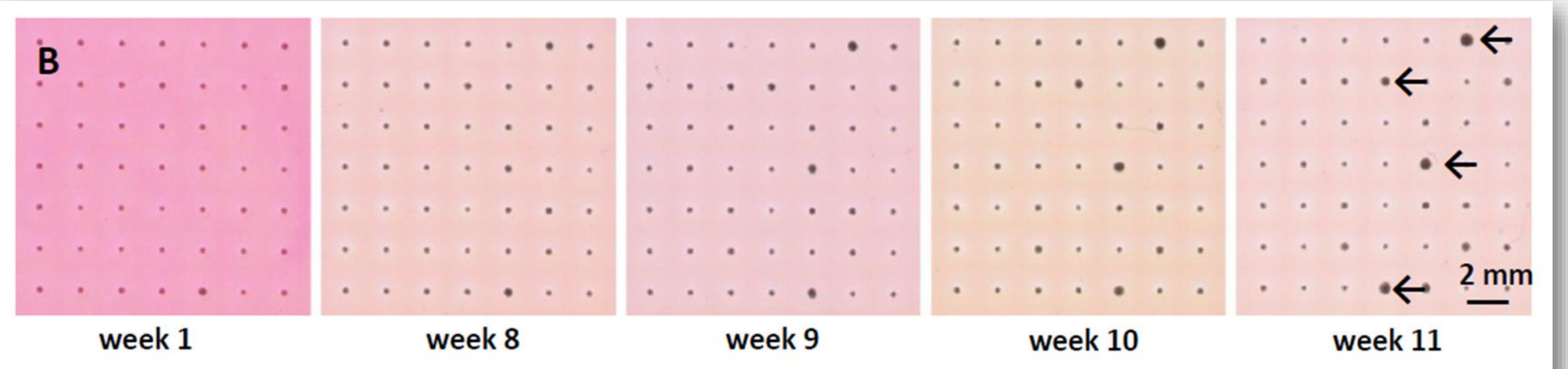
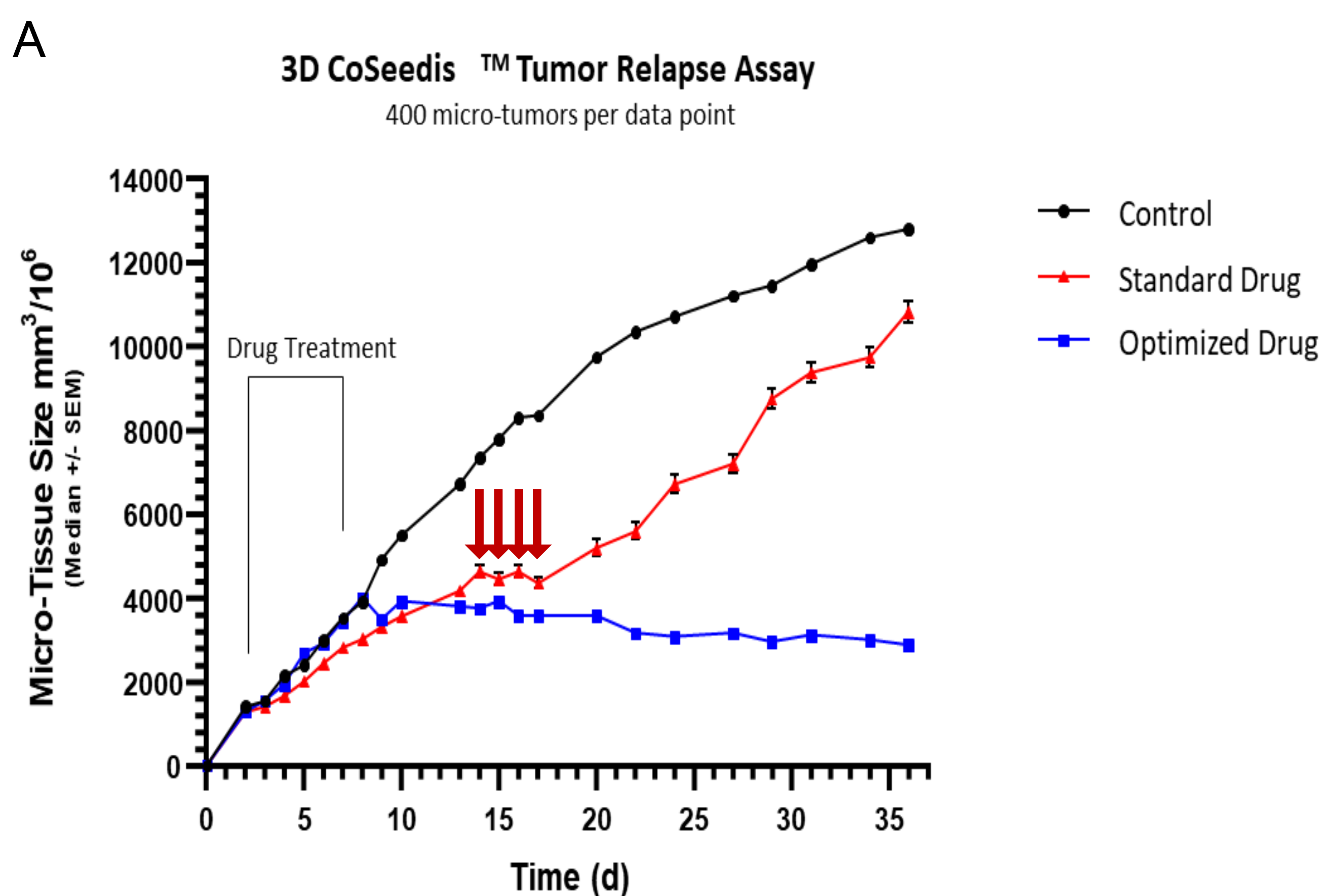


Figure 3: The 3D CoSeedis<sup>TM</sup> Drug Resistance Assay

Based on the specific features of the 3D CoSeedis *in chip* communication technology<sup>TM</sup> and the unique chip design described above, we were able to establish for the first time an *in vitro* assay setup to assess long term drug efficacy, hence identify potential risks of drug resistance formation *ex vivo*. In the case of tumor medicine, the formation of drug resistance is a rather stochastic and rare event. It is therefore only possible to detect if hundreds of biological replicates, all grown and treated under identical conditions, can be assessed accurately. Furthermore, some resistances only occur after prolonged culture time and require conditions that meet this requirement.

A) Red arrows indicate transient growth arrest followed by subsequent, accelerating tumor growth indicative of a potential resistance against the standard drug. In comparison, the optimized compound completely and permanently abolishes tumor growth.

B) Exemplary section of a 3D CoSeedis<sup>TM</sup> Chip880 showing the formation of resistances and tumor relapse in organoids over a period of 11 weeks in culture. Black arrows indicate the stochastic events of resistance formation.

## Conclusion

Due to the 3D CoSeedis *in chip* communication technology<sup>TM</sup>, we do now finally have a tool at hand that allows us to identify and investigate drug resistance formation *ex vivo*. Not only is it possible to positively unmask the risk of certain compounds to cause resistance, but the technology can also be used to improve drug efficacy and ultimately prevent drug resistance. Furthermore, abc biopply's chip technology forms the basis to track and isolate drug resistant micro-tissues and to run comparative studies e.g., assessing co-therapies. They will allow us to pinpoint the molecular background of resistance formation and to include this knowledge in second generation drug development.

a. Sun X, Zhao P, Lin J, Chen K, Shen J. Recent advances in access to overcome cancer drug resistance by nanocarrier drug delivery system. *Cancer Drug Resist.* 2023 Jun 20;6(2):390-415. doi: 10.20517/cdr.2023.16. PMID: 37457134; PMCID: PMC10344729.  
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 c. Rebecca VW, Somasundaram R, Herlyn M. Pre-clinical modeling of cutaneous melanoma. *Nat Commun.* 2020 Jun 5;11(1):2858. doi: 10.1038/s41467-020-15546-9. PMID: 32504051; PMCID: PMC7275051.

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