

Dose-dependent cytotoxicity of arfolitixorin, a direct-acting folate, versus leucovorin with 5-fluorouracil in patient-derived colorectal cancer tumoroids (PDTs)

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BACKGROUND

- Suboptimal treatment outcomes with 5-fluorouracil (5FU)/folate, the backbone chemotherapy agent for colorectal cancer (CRC), have sparked interest in optimizing folate therapy¹
- Arfolitixorin (ARF) is stable and immediately bioactive, bypassing the need for multi-step metabolic activation inherent to currently approved folates such as leucovorin (LEU)^{1,2}
- Despite promising results with ARF in a previous Phase 1/2 study (NCT02244632), in which doses were escalated up to 240 mg/m², the Phase 3 AGENT trial (NCT03750786; ISO-CC-007) failed to demonstrate superiority of ARF over LEU in patients with metastatic CRC, possibly due to suboptimal dose (60+60 mg/m²)^{1,2}

OBJECTIVE

To investigate the Phase 3 suboptimal dose hypothesis, we evaluated the activity and dose-dependent cytotoxic effects of ARF versus LEU with 5FU in cell lines and patient-derived colorectal cancer tumoroids (PDTs)

METHODS

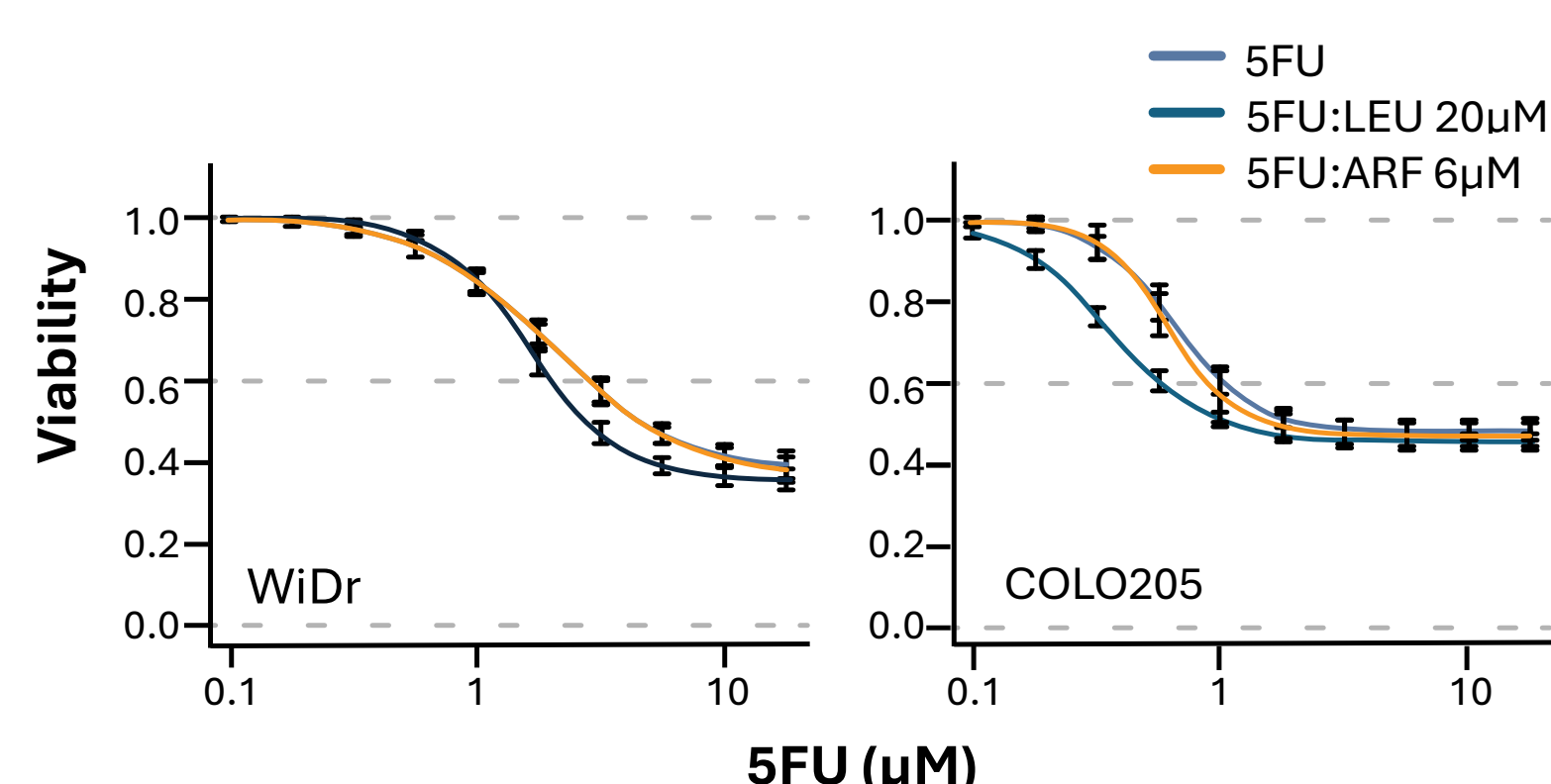
- The activity of 5FU, LEU, leucovorin (LLEU), ARF, and combinations, were evaluated at fixed concentrations, initially in a panel of human cancer cell lines, then in 20 selected PDT models established from resected primary CRCs
- Models were seeded 1,000 cells per well in enriched growth media; test compounds and controls were auto-dispensed; plates were incubated for 120 hours prior to imaging
- Dead cells were assessed via computer vision-based nuclei counting of propidium iodide-stained cells, viability was estimated with CellTiterGlo (Promega), and relative viability was calculated versus controls
- Dose-response models were estimated using the Hill equation, and used to calculate the area over the dose-response curve (AOC, higher values indicate higher activity), EC50 (represents the dose for half-maximum activity), and IC50 (represents the dose for 50% reduction in viability)
- Screening quality was assessed according to industry-standard measures, and statistical significance in AOC differences was determined using Wilcoxon paired-sample tests

RESULTS

ARF activity in cell lines

- A physiologically relevant³ dose range of <2.4 μM 5FU demonstrated strong *in vitro* activity in cell lines, which was potentiated by folates
 - In general, the potentiating effect of LEU 20 μM was indistinguishable from LLEU 10 μM, and was comparable to ARF 20 μM
 - Dose-response curves supported the ARF suboptimal dose hypothesis (Figure 1)

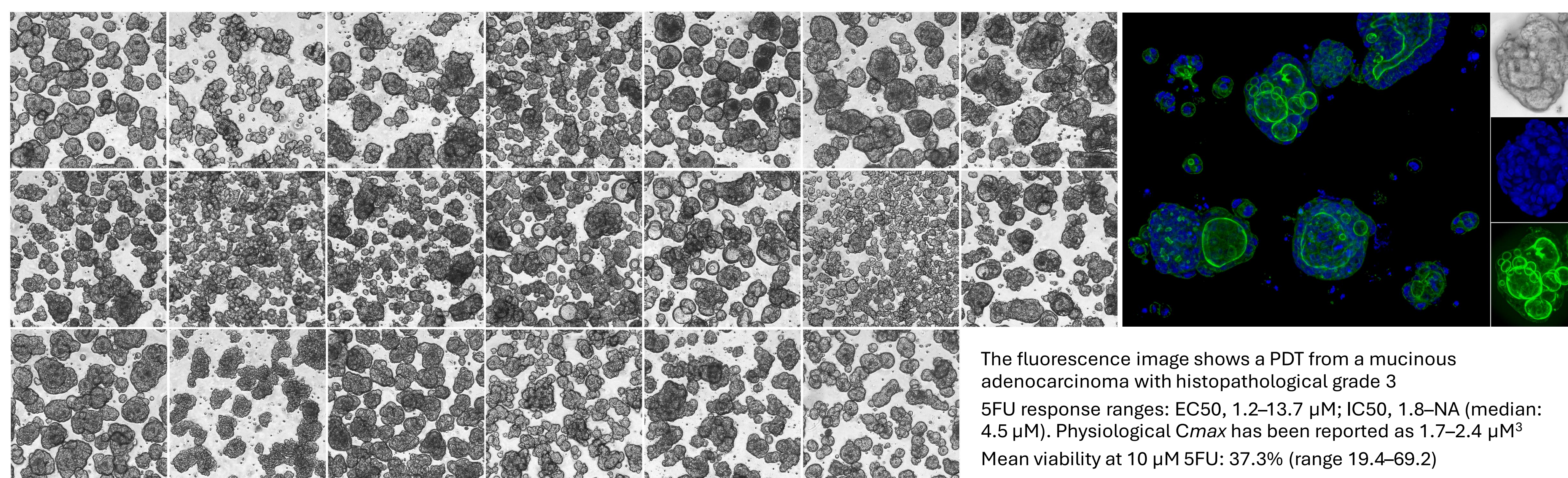
Figure 1: AGENT trial dose (ARF 6 μM) did not potentiate cytotoxic activity of 5FU in cell lines



PDT screening quality, characteristics and 5FU sensitivity

- Screen data included a mean Z'-factor of 0.65 (>0.5 = "excellent") and mean coefficient of variation of negative controls of 11% (acceptable: <20%)
- PDT cultures displayed diverse morphology (Figure 2) and considerable variation in phenotypic characteristics, such as doubling time and size, as well as heterogeneous response to 5FU

Figure 2: PDT biobank represents heterogeneous clinical landscape



ARF activity in PDTs

- ARF 20 μM was more active than LEU 20 μM, and increasing ARF doses led to increased activity (Figure 3)
- The three PDT models most resistant to 5FU alone showed particularly high sensitivity to increasing doses of ARF (Figure 4)
- A partially cytostatic effect of 5FU alone was observed, also when combined with LEU 20 μM or ARF 20 μM
- Cytotoxicity was evident when combined with ARF 40 μM, with relatively higher numbers of cytotoxic cells (Figure 5)

Figure 3: Activity of 5FU is increased when combined with LEU or increasing doses of ARF

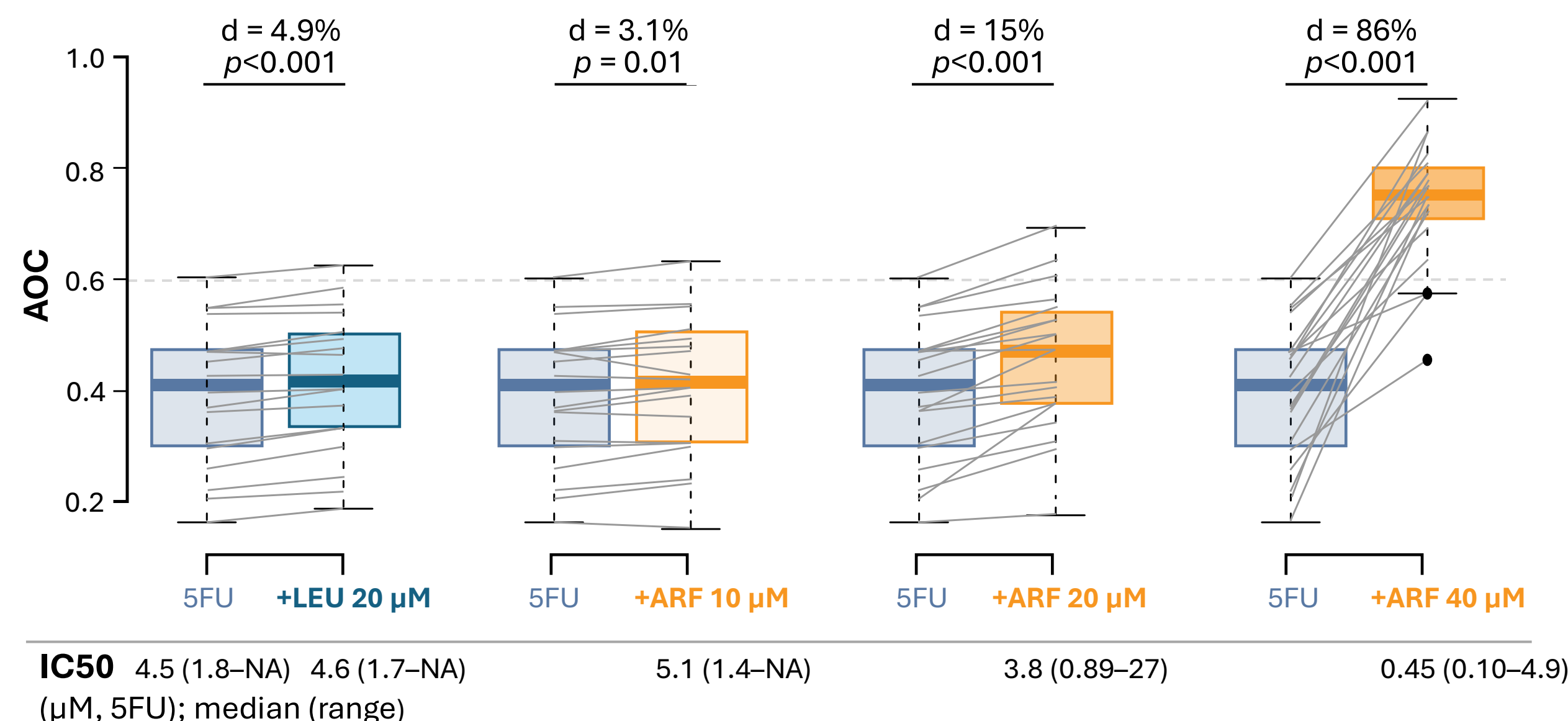


Figure 4: The three most 5FU-resistant PDTs showed the largest decrease in relative viability with increasing ARF dose

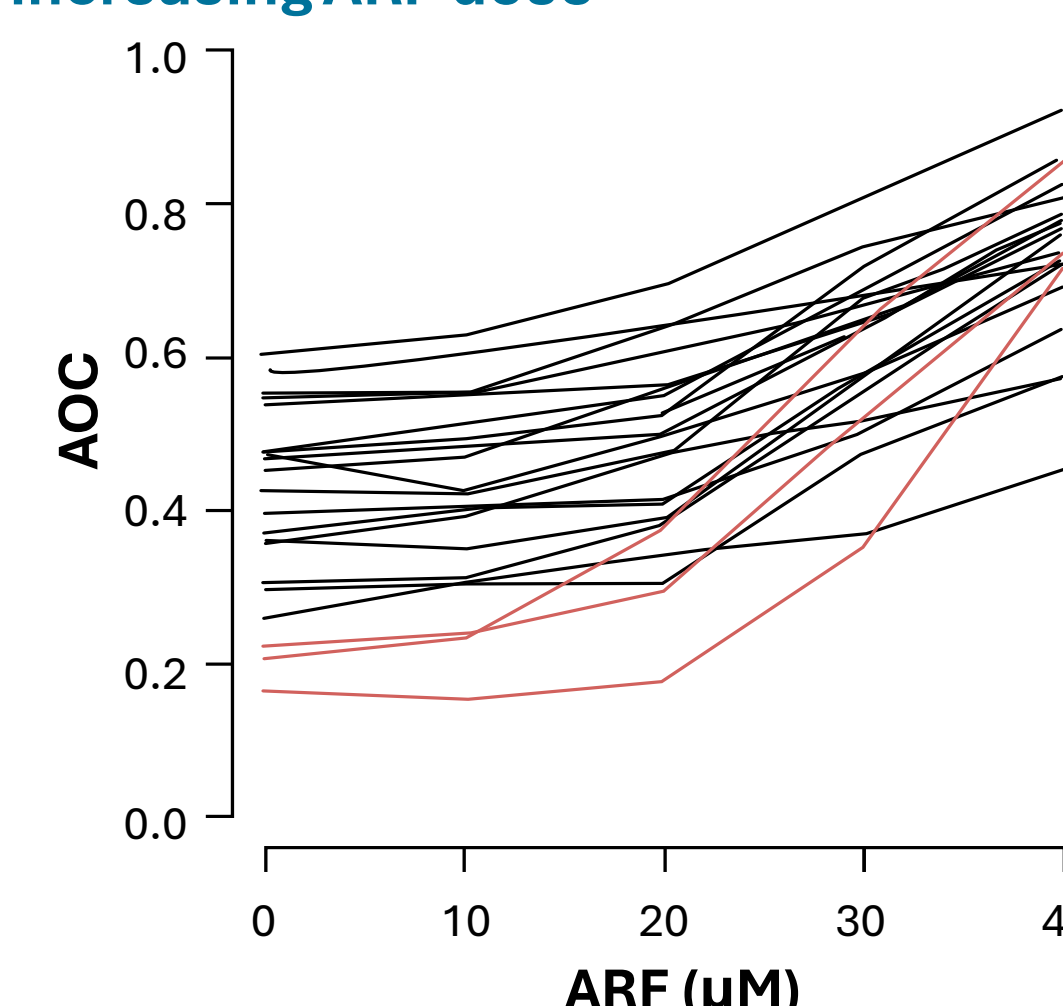
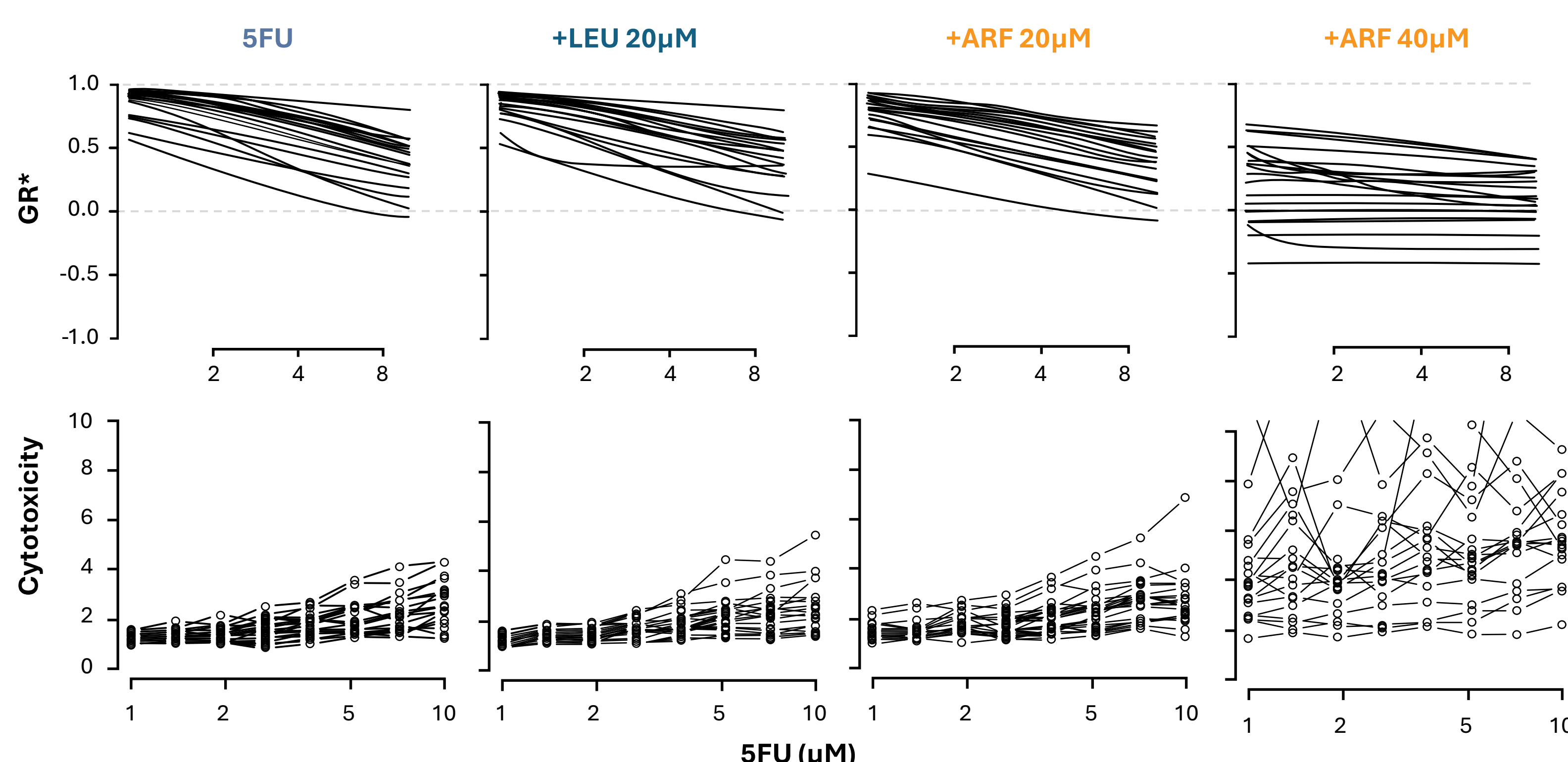


Figure 5: PDTs show decreased viability, and increased cytotoxic activity of 5FU is evident when combined with ARF at higher doses



*GR represents growth-rate inhibition in the presence of a drug, relative to untreated control (1: no growth-rate inhibition; 0: complete cytostatic effect; -1: complete cytotoxic effect)⁴

CONCLUSIONS

- PDTs display a wide range of sensitivity to 5FU, and its modulation by folates, reflecting clinical heterogeneity
- ARF displays a potent, dose-dependent cytotoxic effect and increased activity in 5FU-treated PDTs that, at higher doses, is higher than with LEU, and which was most marked in the PDTs that were most resistant to 5FU
- These findings support that suboptimal ARF dosage in the ISO-CC-007 trial contributed to insufficient efficacy to establish superiority over LEU
- Further clinical development at higher doses could enhance the standard of care for patients, and a Phase 1/2 clinical trial based on this approach is currently being planned in patients with metastatic CRC

References

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