

In vitro assessment of tumor relapse in a 3D tumor microtissue model

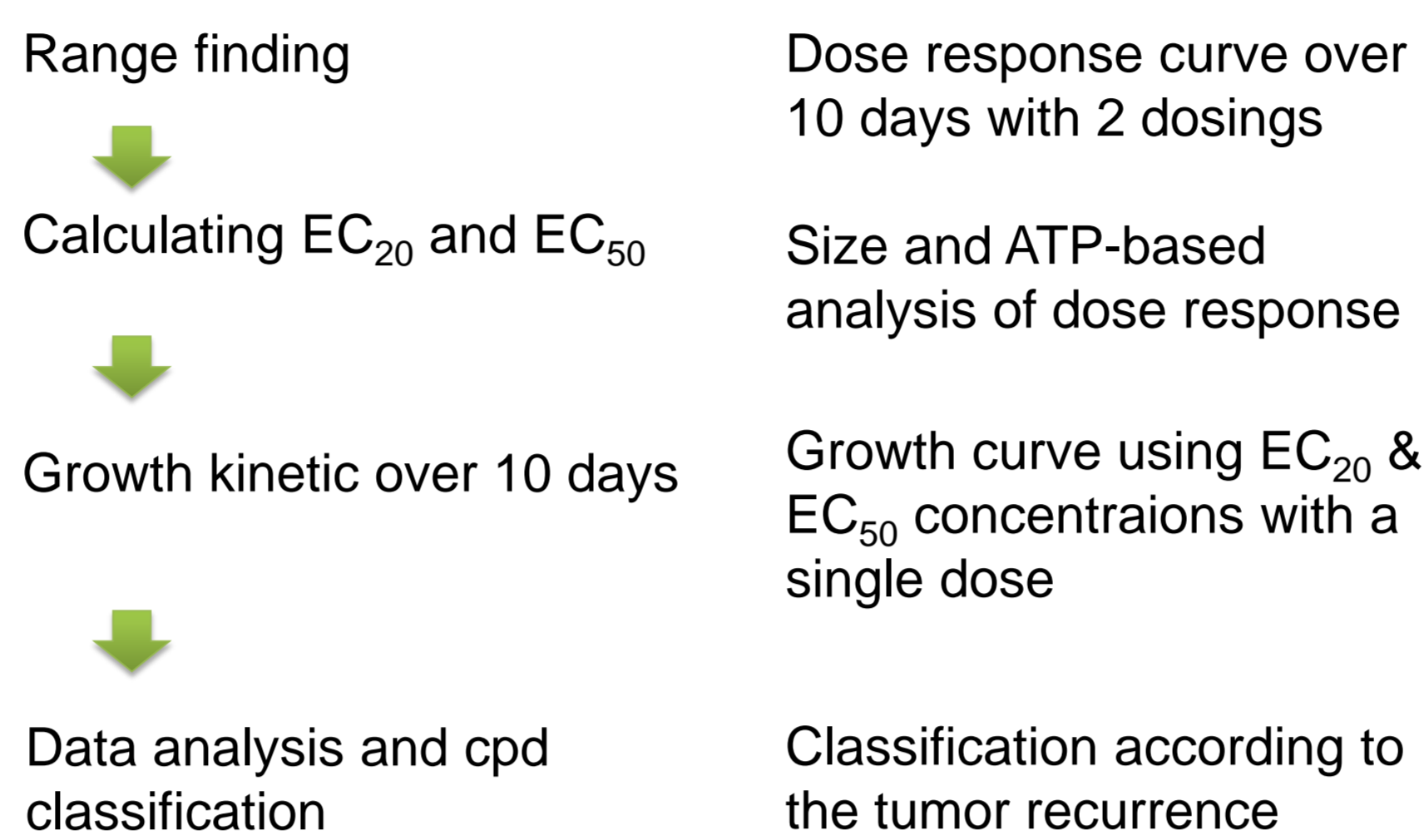
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Introduction

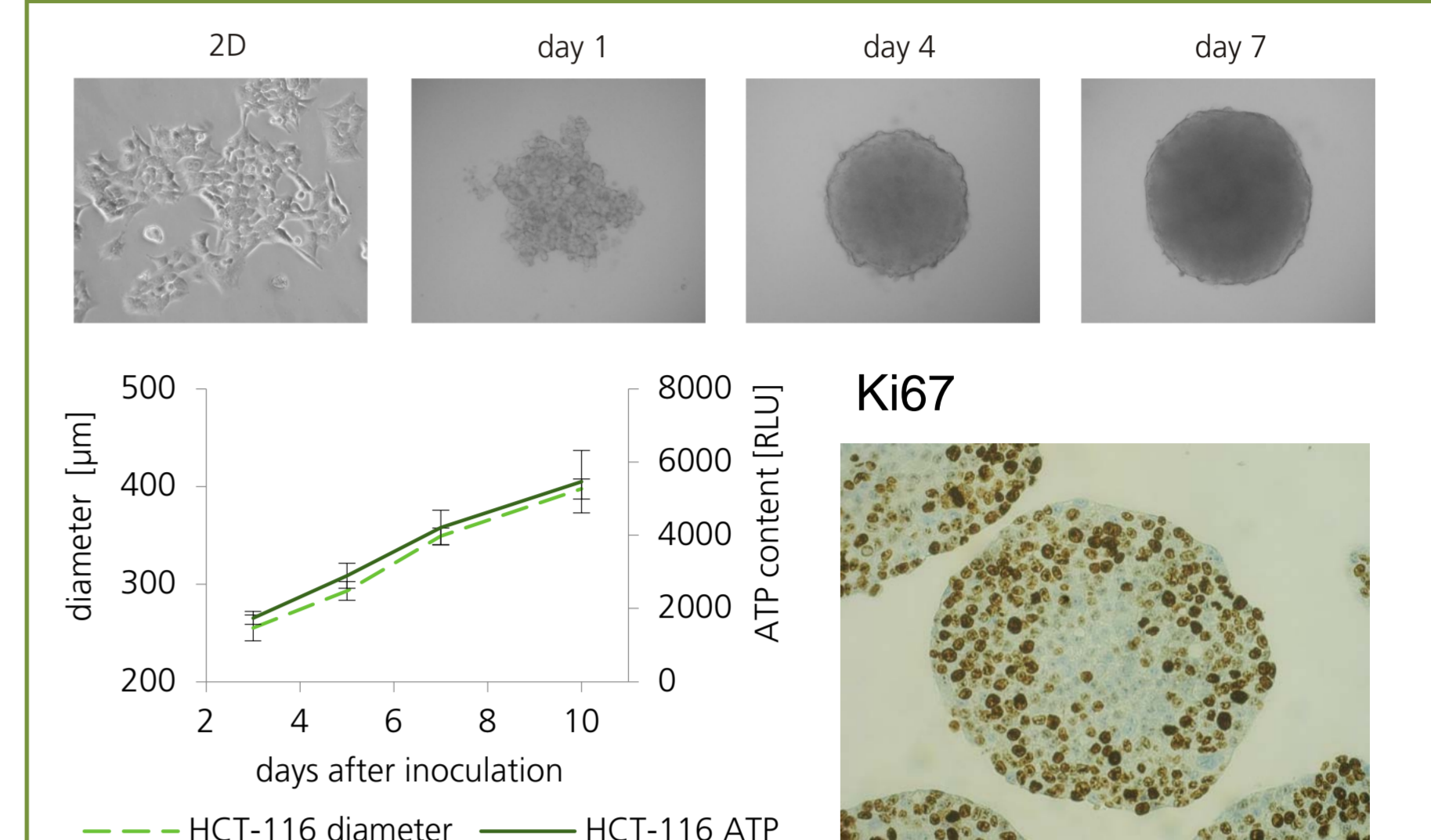
One of the most pressing challenges in the treatment of cancer is tumor relapse. Although many tumors regress in response to neoadjuvant chemotherapy, residual tumor cells are detected in most cancer patients post-treatment. These residual tumor cells are thought to remain dormant for years before resuming growth, resulting in tumor recurrence. Considering that recurrent tumors are most often responsible for patient mortality, there exists an urgent need to study signaling pathways that drive tumor dormancy/recurrence. Current *in vitro* assays based on monolayer cultures do not provide the required time frame to study tumor recurrence.

We have developed a new method to study tumor relapse in a 3D *in vitro* tumor microtissue model. HCT116 colorectal tumor microtissues were produced and dosed with 3 reference compounds, Taxol (TAX, cell division inhibitor), Staurosporine (STA, protein kinase inhibitor) and Doxorubicin (DOX, DNA synthesis inhibitor). Based on an initial range finding to determine EC₂₀ and EC₅₀ values, tumor growth was monitored via size profiling and viability (intra-tissue ATP content) over 10 days. Three different conditions were tested: (i) continuous treated tumor microtissues, (ii) microtissues which were supplemented with the drug for 5 days and (iii) non-treated tumor microtissues.

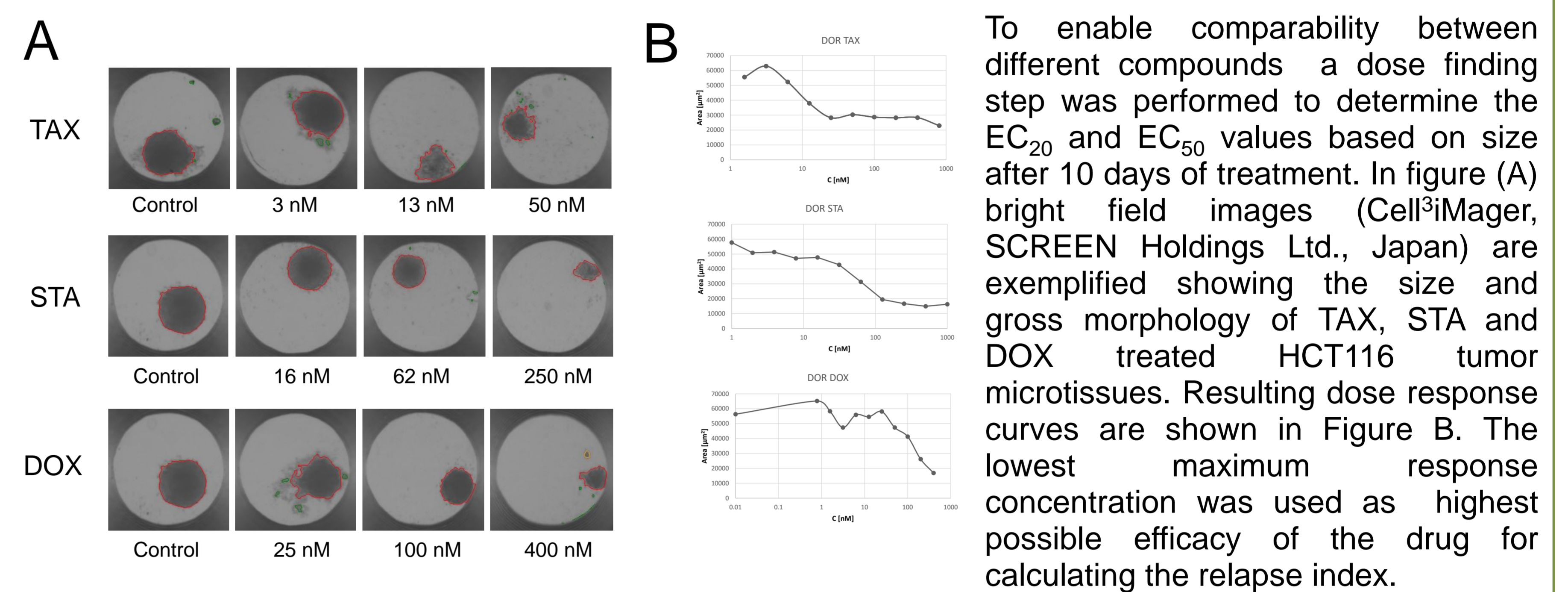
Relapse Testing Concept



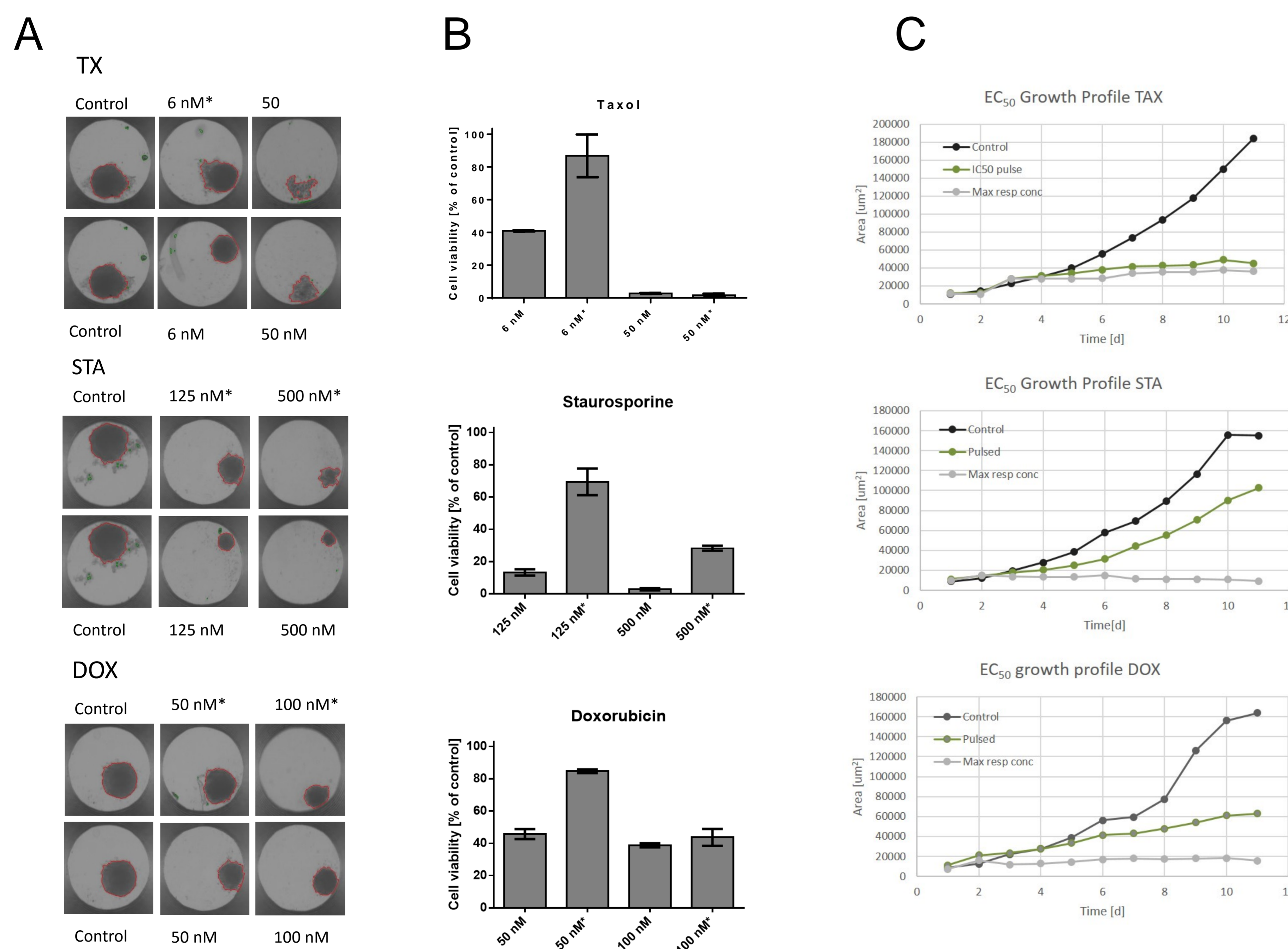
Colorectal Tumor MT



Range Finding

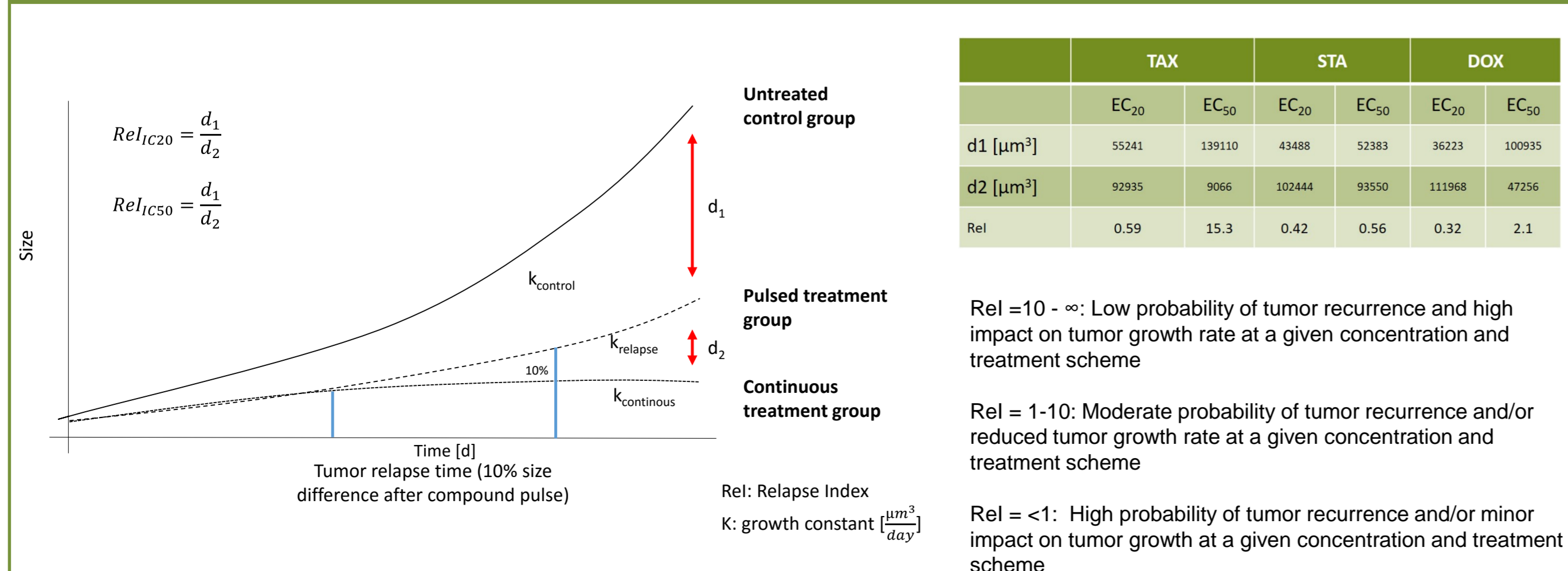


Assessment of Tumor Relapse



Growth differences couldn't be observed comparing EC₂₀ concentrations, however growth profiles significantly varied comparing EC₅₀ concentrations. 5-day treatment with Taxol was observed to lead to a complete growth inhibition after removing the drug, whereas, Staurosporine treated tumor microtissues relapsed at similar growth kinetic as the untreated control group. Pulsed treatment of Doxorubicin was observed to lead to a slower growth kinetic as compared to the control but not to a complete remission. Based on the individual growth profiles relative to the continuously treated and non-treated groups we calculated a relapse index (Rel, *patent pending*) to enable classification of compounds and compound combinations according to their risk for tumor relapse. Figure A exemplifies bright field images of pulsed treated (*) vs continuously treated tumor tissues. ATP-based analysis delineates that across all the compounds tested at lower concentrations the tumor tended to recur. At higher concentrations only Taxol was found to regress the tumor growth completely (Figure B). Figure C shows growth profile of untreated controls, pulsed treated and continuously treated tumor microtissues at the corresponding EC₅₀ values.

Compound Classification



Conclusions

Here we present a novel *in vitro* tumor relapse assay method based on a 3-dimensional tumor microtissue model, to enable compound classification based on their efficiency to prevent tumor relapse. The prolonged life time of tumor microtissues enables the assessment of tumor recurrence. Colorectal tumor microtissue was treated with 3 well known anticancer drugs which resulted in differential relapse characteristics. STA treated cultures relapsed similar to the untreated control, whereas DOX treated microtumors exhibited relapse at least slower than the untreated control. Only TAX lead to a "relapse"-free conditions after a 5-day treatment with the EC₅₀ concentration.