Improving perfusion fluid for the next generation of transplants: Identifying agents that lower cellular oxygen consumption and reduce tissue hypoxia

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Introduction: As the need for transplantation increases, the boundaries of what is a marginal organ is being pushed further back. The development of appropriate perfusion fluid has greatly helped in maximising available organs through reducing the effects of unavoidable tissue hypoxia. We believe we can improve this further.
Materials and Methods: Four colorectal cancer cell lines (COLO320DM, DLD1, HCT116 and HT29) and a non-transformed cell line (MRC5) were investigated. Clonogenic and cytotoxicity assays of a range of agents were used to determine sub-lethal concentrations. The oxygen consumption, mitochondrial and glycolytic function of treated cells were assessed with the XF96 Analyser. Flow cytometry, gene array, western blot and high-performance liquid chromatography (HPLC) were performed to delineate mechanisms of action. The most responsive cell lines and promising agents were progressed to spheroids for 3D hypoxia modelling and then advanced to in vivo and radiosensitivity testing. In vivo tissue hypoxia analysis was achieved with the in vivo imaging system (IVIS).
Results: The oxygen consumption of all cell lines were markedly reduced with a number of the agents. DLD1 and MRC5 cell lines showed the greatest balance of toxicity resistance and reduction in oxygen consumption. Hypoxia imaging of the subsequent spheroids further demonstrated a reduction in hypoxia consistent with drug induced decrease in oxygen consumption. This was further replicated in the xenografts resulting in improved radiosensitivity (see Figure 1).

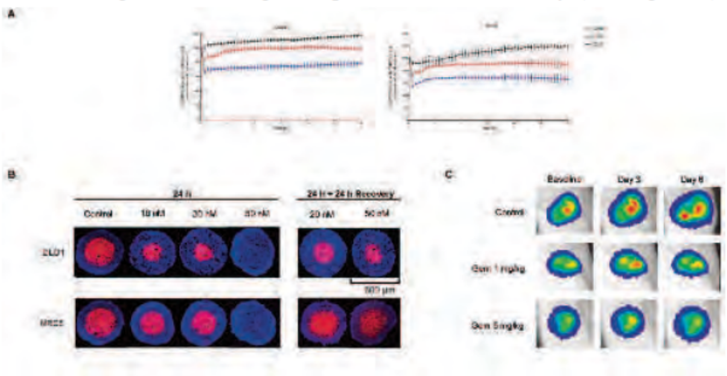

Figure 1. Sub-therapeutic doses of gemcitabine can lower OCR and improve tissue hypoxia in spheroids and *in vivo* as seen on IVIS. A. Oxygen consumption data for gemcitabine in DLD1 and MRC5 human cell lines. B. Hypoxia staining of [illegible] spheroid sections [illegible] with Hoechst as a [illegible]. C. Representative IVIS images of [illegible] DLD1 tumours [illegible] luminescence intensity in control and treated mice. Treatments were administered [illegible] via I.P. injection. Each group had at least [illegible] mice and entered into study once tumour volume reached [illegible]. Error bars represent standard error.

Discussion: A variety of agents originally identified through our studies on improving tumour hypoxia have shown a reduction in oxygen consumption in malignant and non-malignant cells alike, which has been demonstrated to lead to an increase in oxygen availability and hypoxia improvement. This has been shown in monolayer and 3D in vitro models. We hypothesise that by reducing cellular oxygen consumption, cells will be able to withstand hypoxia for a greater period of time and thus lengthen the preservation period. If the data shown in our work in cancer can be replicated in the transplant setting, we can potentially increase the donor pool by enhancing the preservation period of an organ. Ultimately, this will help reduce the waiting time for patients and improve morbidity.
Conclusion: The preservation period of an organ can potentially be increased through using agents that reduce oxygen consumption. This will improve graft function and increase the donor pool.