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Developing a patient-derived model of cholangiocarcinoma using Precision Cut Tissue Slices (PCTS).

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Background:
Cholangiocarcinoma (CCA) is an aggressive malignancy with increasing incidence and persistently poor prognosis. None surgical treatments options for these patients are limited and a preponderance to chemoresistance means the benefits from chemotherapy are modest. The development of new targeted therapeutic strategies that prolong survival and reduce the risk of recurrence post-surgery are required. Accurate models that recapitulate tumour biology are essential to test and identify these novel therapies. Precision-cut tissue slices (PCTS) are patient-derived whole tissue explants that can be cultured ex-vivo. By retaining all aspects of the tumour micro-environment, they recapitulate critical aspects of cancer biology, a significant advantage over 3D organoid culture. Our aim is to establish and validate the use of the PCTS technique in creating a patient-derived model of cholangiocarcinoma for use as a drug discovery platform.

Methods:
CCA tissue samples and matched healthy liver tissue were collected from the operating theatre during surgical resection from fully consented patients in agreement with UoL / HTA policies and procedures. Multiple tissue slices (250µm, 5mm diameter) were prepared using a Krumdiek tissue slicer. Tissue slices are randomly incubated in triplicate and cultured for 0-15 days in 24 well plates. Tissue perfusion and air-liquid interface was maintained using Millipore well inserts. Tissue viability was determined at timepoints utilising MTS and ATP viability assays along with histological markers of proliferation (Ki-67) and apoptosis (cleaved-caspase 3). Maintenance of tumour morphology and phenotype during culture was confirmed on histological H&E staining and staining of tumour specific markers (e.g CK19) on IHC and Western Blot.

Results:
Thus far n=4 CCA patients have had PCTS generated (3 = pCCA 1=iCCA) from their tumour along with matched healthy liver. Following optimisation of the experimental protocol viability of the matched normal and tumour tissue slices can be maintained for upto 10 days as assessed via in situ MTS assay. Histological assessment of tissue slices harvested at day 0 1,3,7 of culture shows the morphological and phenotypic structures of the source tumour are maintained during ex-vivo culture. Viability of the tumour cells was additionally confirmed through the absence of cleave caspase 3 markers of apoptosis on IHC staining.

Conclusion:
This initial work has shown it is feasible to generate PCTS of CCA from resected surgical specimens with good viability. Further work is still required to ensure that our model remains a robust recapitulation of the in-vivo tumour including examination of the gene-expression profile. However, these initial results provide encouragement for the use of PCTS as a patient derived model for drug discovery in CCA