NExT project: Establishing *in vitro* and *in vivo* models of pancreatic neuroendocrine tumors for translational research.

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Background and motivation: Pancreatic Neuroendocrine Tumours (PNET) have a poor prognosis due to late detection and limited response to treatment. Furthermore, there is a lack of preclinical models to test novel therapies and to characterize mechanisms of drug resistance. *The international ERA-NET project NExT is building a tissue bank of genetically characterized PNET tumours and developing patient-derived xenografts (PDXs) and 3D organoids to use as models in translational research. Furthermore, this project will develop PNET-specific biomarkers and a microfluidic device liquid biopsy for the early detection of PNETs using the liquid biopsy.*

General information

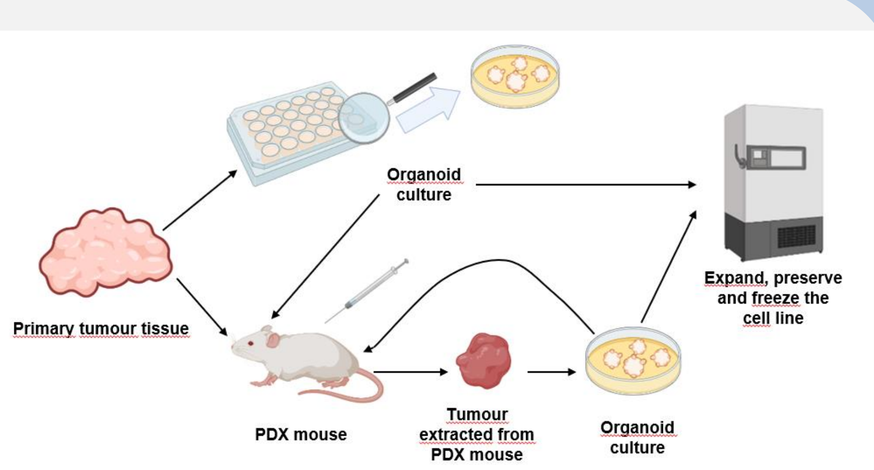
Consortium: 6 partners
5 countries
(1 university, 4 RTO, 1 SME)
Duration: 01.09.2019 – 31.08.2022
Coordination: Fraunhofer IBMT, Germany
Website: https://www.next-project.eu

Scientific Aims

1. *Identification & characterization of etiopathogenetic determinants responsible for the development of PNET*
2. *Establishment and development of new 3D in vitro (organoids) and in vivo models (PDX) to understand the biology of pancreatic tumors*
3. *Minimally-invasive early detection and follow-up of PNET patients by means of an advanced microfluidic device made possible because of previous tumor characterization*
4. *Optimize advanced cultures protocols for expand the establishment of 3D models to other types of tumor not tested yet (such as glioblastoma).*

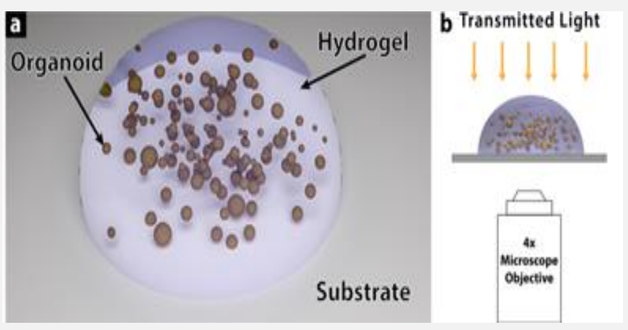
Material and methods

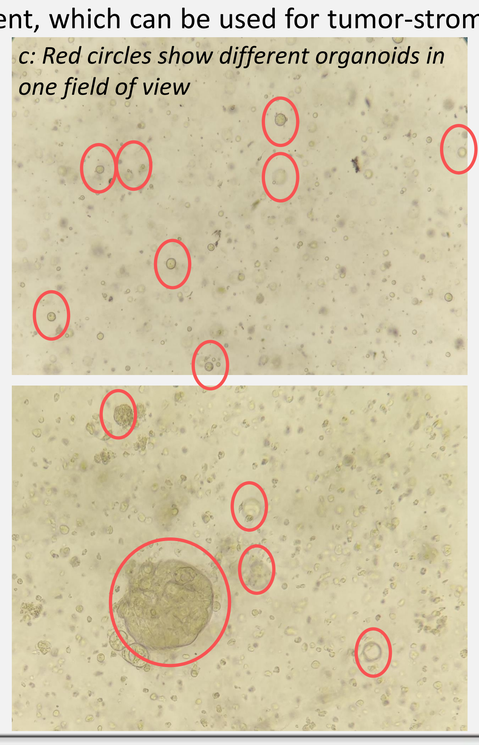
3D *in vitro* models (organoids) are established from fresh tumor tissue (which have a size near to 5 mm^3) and embedded in Matrigel© with liquid media (DMEM-F12) supplemented with 5% FBS, 1% L-Glutamine and 1% Antibiotics (PenStrep-Amphotericin B). This media contains different growing factors as well (such as PlGF, IGF-1, FGF-β, EGF, etc.) and ROCK Inhibitor (to protect the cells from the stress caused by the protocol). The process to establish the organoids is very difficult and contains several steps, working always at low temperatures to avoid the apoptosis during the management of the sample and processing it inmediately after the surgery. The protocol starts with the complete digestion of the tissue (adding media with Collagenase IV, Tripsine and DNAse to the sample and leaving it during 1 hour in a GentleMACS © Dissociator). Another key to increase the success of the protocol is to repeatedly wash the cells (by numerous centrifugations) with different reagents (for example DNAse dissolved in Accutase and ACK Lysis Buffer). Organoids take up to 30 days to form.



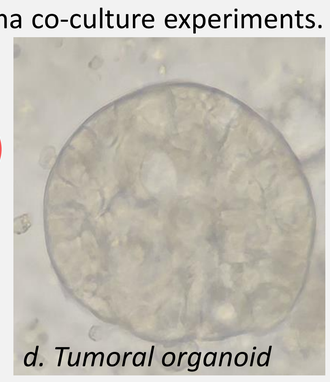
Results

Organoids are embedded within matrigel (**a**) and can be visualized by focusing a the microscope at different levels under low power (**b and c)** at different levels within the matrigel dome. Organoid cultures have been successfully established (with a succes ratio of around 50%) and maintained from PNET and PDAC primary tumors and various organoids can be seen in one field of view (**c**) with organoids from tumor (**d**) and non-tumor cells (**e**). Furthermore, other relevant cells within the tumor, such as fibroblasts, are also isolated and maintained for experiments related with the microenvironment, which can be used for tumor-stroma co-culture experiments. In (**f**) we can see the progression of the culture during time.



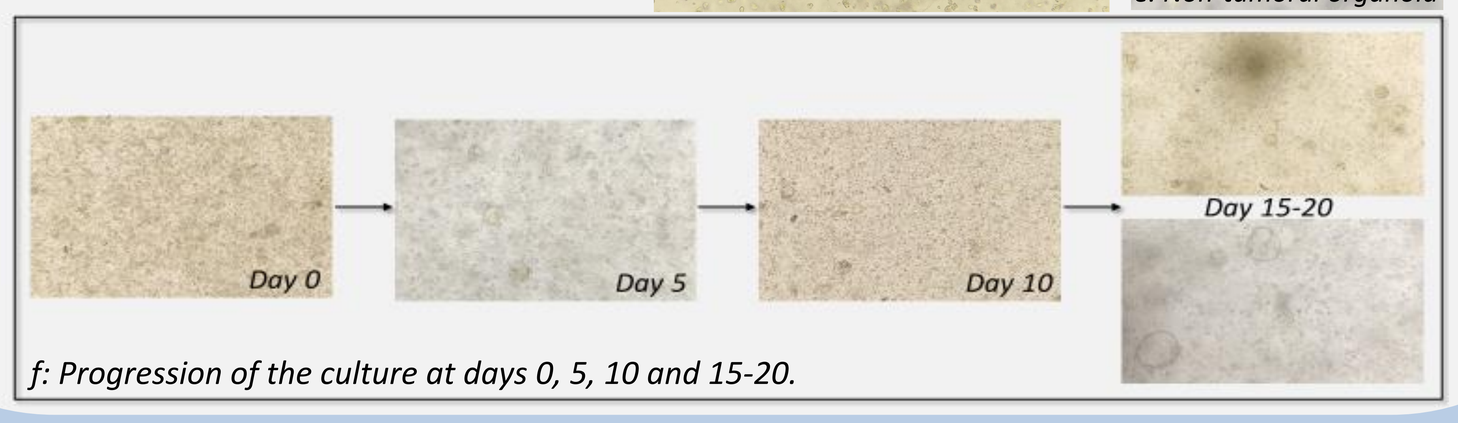


c: Red circles show different organoids in one field of view



d. Tumoral organoid

e. Non-tumoral organoid



f: Progression of the culture at days 0, 5, 10 and 15-20.

Conclusions

An important unmet clinical need to study biological characteristics, testing novel therapies and characterize drug resistance mechanisms in pancreatic neuroendocrine primary tumor is the development of faithfully preclinical models. Organoids have enormous potential applications and will allow us to make a more personalized treatment strategy in the clinic.

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