Simon Leonhard April-Monn[1,2], Renaud Maire[1], Marco Schiavo Lena[3], Mafalda Trippel[1], Francesca Muffatti[4], Valentina Andreasi[4], Claudio Doglioni[3,5], Corina Kim-Fuchs[6], Beat Gloor[6], Stefano Partelli[4,5], Massimo Falconi[4,5], Aurel Perren[1] and Ilaria Marinoni[1]

[1] Institute of Pathology, University of Bern, Bern, Switzerland.

[2] Graduate School for Cellular and Biomedical Sciences University of Bern, Bern, Switzerland.

[3] Unit of Pathology, San Raffaele Scientific Institute, Milan, Italy.

[4] Pancreatic Surgery Unit, Pancreas Translational & Clinical Research Center, San Raffaele Scientific Institute, Milan, Italy.

[5] Università "Vita e Salute", Milan, Italy

[6] Inselspital, University of Bern, Switzerland

A) Background/Significance to NETs

B) Materials and Methods/Experimental Approach

C) Results (key findings to report at this meeting)

D) Conclusions/next steps

A While there are several effective medical treatments for advanced NET, there are controversies about sequencing of the treatments for individual patients. The lack of predictive biomarkers hinders accurate patient stratification and a more personalized treatment selection. Hence, establishing better in vitro preclinical models to find biomarker and to test current and novel pharmacotherapies is an urgent need for more personalized treatments. With this project we aimed at establishing a 3-dimensional (3-D) patient-derived primary PanNET in vitro model that adequately reflects tumor characteristics of specific patients and that allows exploration of personalized in vitro pharmacotyping.

B Tumor cells from 27 cryopreserved PanNET patients were isolated. Cell integrity and viability was quantified using automated multi-parametrical cell counter. Two independent pathologists reconfirmed PanNET specific histomorphology comparing original tumor tissue and micro-cell-blocks in all cultured material at the start- and endpoint. 3-D cultures were kept in ultra-low attachment spheroid microplates combined with moderate scaffold support. Culture medium was supplemented with a PanNET specific growth factors based on growth-factor-receptor expression profiles from 26 low-grade PanNET as well as literature research. Live-cell imaging was performed on an Incucyte platform. Drug sensitivity for first-line treatments were assessed repeatedly in single-well resolution over a time course of 168h and cell viability was monitored using a non-lytic metabolic surrogate. Drug sensitivity profiles were determined calculating classical and parametrized in vitro drug responses.

C We established a PanNET screening platform that allows multi-center sample collection. For each PanNET specimen a first mirror block was formalin-free PAXgene fixated for histomorpholocial and molecular downstream analysis. The mirroring part was cryopreserved and used for 3-D cell culture. Cell isolation and pharmacotyping of PanNET patients was successful in 84% (22/27). To account for more physiological conditions a PanNET-specific culture medium was developed combining literature and human transcriptomic PanNET data from 26 low grade PanNETs. Except of EGFR- the selected target receptors were within the upper expression quintile (<28/151) of all currently available growth factor receptors and related proteins in PanNET patients. Live cell imaging revealed that isolated cells in vitro formed structures similar to non-neoplastic human pancreatic islets. Therefore, we termed these structures Islet-like tumoroids. Patient-derived Islet-like tumoroids retained the histomorphological phenotype of original PanNET including neuroendocrine markers and hormone expression. Notably, we also found a high correlation betweeen patient Ki-67 indices and in vitro metabolic activity. To verify whether patient-derived Islet-like tumoroids can be exploited to monitor drug sensitivity in vitro, specimen from 14 patient were screened using three clinically approved first-line therapies: Patient-derived Islet-like tumoroids displayed varying drug sensitivity to the distinct chemotherapeutics and differed between patient sample. Hierarchical cluster analysis revealed three pharmacotypes potentially reflecting patient responses. These pharmacotypes were stable among the majority of the patients among different time points indicating strong reproducibility of our method. In very few cases differences between short- and long-term treatment were detected, underlying the importance of a prolonged screening window to assess in vitro therapy response and to potentially compare drug sensitivity profiles to clinical patient response.

D Establishing better in vitro preclinical models to define predictive biomarkers and to test existing and novel treatments is an urgent need for PanNET patients. We present a 3-D human primary PanNET screening platform allowing multicenter sample collection and pharmacotyping in human primary PanNET 3-D cultures. These patient-derived Islet-like tumoroids reflects characteristics of an individual tumor and mirror distinctive drug sensitivity profiles. To achieve clinical utility, a comparison of in vitro response with results in patients receiving specific treatments will be needed.