PhD Project: Predictive and Functional Evaluation of Patients Derived Tumor Spheroids and Organoids-Investigating the Relationship between Size and Testing Performance

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Abstract

Pancreatic Cancer has proven to be one of the most severe forms of cancer with the worst anticipated prognosis. Mortality due to pancreatic cancer is expected to surpass that of breast and colorectal cancer combined by 2030 due to several reasons including aging, advanced stage when pancreatic cancer is detected in patients, lack of effective treatment strategy particularly in Pancreatic Ductal Adeno Carcinoma PDAC cases [01]. PDAC contributes to more than 7 % of cancer associated deaths world-wide with an overall survival rate 8.5% for 6-11 months only in patients with advanced stages of the Disease [02]. Pathological, genomic and clinical studies on PDAC patients have shown three different progressive forms of the disease Pancreatic Intraepithelial Neoplasia PanINs, Intraductal Mucinous Papillary Neoplas IPMN and Pancreatic Mucinous Cystic Neoplasm MCN which all express different stages of epithelial dysplasia, lobulocentric atrophy and ductal metaplasia [03].

Treatment options for PDAC sounds to be very limited and mostly include combined chemotherapy with gemcitabine/ nab paclitaxel together with surgical interference. Activation of KRAS oncogene via single point mutation in codon 12, inactivation of tumor suppressor genes CDKN2A, TP53, SMAD4 and R1 and RII receptors of TGF-B and activation of certain signaling pathways that include NFKB, STAT3 and SRC were found to be characteristic for PanINs and its progression to PDAC [04, 05]. Patient-derived xenografts PDXs have been validated as an effective tool to study PDAC but it required large amount of tissues and longer time to generate [06, 07]. However, Genetically Engineered Mouse Models GEMMs were found to be more precisely recapitulating the patho-physiological features of human PDA across different stages of the disease progression [08]. Previous studies have shown that both human PDA and GEMMs share common histological features including extensive stromal structure, diminished vasculature infiltration and less neoplastic cellularity content. This makes PDA patients-derived organoids and spheroids more difficult to culture as limited amount of epithelium-derived cancer cells are available to isolate. While 2D culture conditions couldn't support the growth of normal or untransformed non-neoplastic pancreatic cells, 3D culture was found to support both normal and transformed cells but otherwise allowed minimal propagation [09]. In this research, we isolate and culture 3D organoids from normal and malignant murine pancreatic tissues from wild type mice(n=10) and PDA GEMMS (n=20) respectively in order to study and model PDA Pathogenesis in Murine Model. Previous studies have shown success rate of around 70-73%. At the same time, Organoids are also planned to be established from non-PDAC tumors e.g. Pancreatic Acinar Cell Carcinoma and Distal Cholangiocarcinoma as the organoleptic and histopathological features of the lesions may indicate. In addition, some tissue samples are taken from the adjacent normal epithelial layers to the site of tumor in order to allow comparison between normal and cancer tissue cells from the same mouse.

Likewise, 3D organoids are planned to be developed from normal (n=10) and malignant human pancreatic tissues (n=40) either sampled during surgical resection or from endoscopic biopsy samples. Then, orthotopic transplantation of the well-established murine and human PDA organoids will be made in wild mice C57BL/6J in order to assess how pathological lesions reminiscent of Pancreatic Intra-epithelial Neoplasia PanINs can be progressed into varying degrees of invasive PDA and may further develop into metastatic carcinomas in other organs e.g. liver, diaphragm, kidney and peritoneum.

After modeling PDA pathogenesis using patient-derived organoid models in both murine and human, characteristic molecular genes signature in each isolated and in-vitro cultured organoid derived from both models will be further char-

acterized using Whole Genome Sequencing of over 2000 PDA associated genes [03]. This will be further correlating to the whole transcriptomic analysis and comparative genes expression profiling using bulk RNA seq and q-RT PCR in addition to high-throughput proteomics using Immune-blotting and Immune- histochemistry IHC approaches. This can help investigate whether patients-derived organoids may function as an optimal model that recapitulates the characteristics of pancreatic malignancy when comparing histopathological lesions to the corresponding multi-omics data sets of PDAs in human and murine models.

Personalized cancer treatment has proven to be the most effective method as judged by clinicians as some targeted therapies were found to be suitable for only subsets of patients than others who may develop less sensitivity to higher degrees of resistance to the same therapy [03]. In order to investigate how pancreatic organoids may assist clinicians finding the most appropriate target therapy, the isolated 50 PDO organoids including at least 10 organoids derived from normal issues and 40 organoids from tumor tissues will be exposed to a number of candidate anti-cancer compounds belonging to 10 categories of chemotherapy which vary according to their mechanism of action. In order to assess the response of PDA organoids to these drugs, two methods including The Cell Viability Assay developed by Promega Bio Sciences and The Electric Impedance Measurement developed by the Heinz-Nixdorf Lehrustul for Biomedical Electronics at The Technical University of Munich will be comparatively assessed [10-12]. The result will be finally compared to the corresponding patients' clinical data and actual patients' response to medical treatment as suggested by the physician.

Resistance to the most commercially available anti-cancer drugs has been frequently noted by clinicians and it constitutes an actual hurdle towards proceeding with an effective treatment strategy despite being precisely personalized [13]. In order to assess how heterogeneous cells in the patients-derived PDOs may respond differently to anticancer drug compounds, we are going to assess the earliest Drug Tolerant Persisters (DT) and the Extended Drug Tolerant Persisters (EDT) cells isolated from the Murine and Human PDOs before and after exposure to some selected anti-cancer therapeutics using Single Cell RNA- seq called Drop-seq and then we are going to analyze the obtained data using a bioinformatics platform called Uniform Manifold Approximation and Projection (UMAP) Dimensionality Reduction for further characterizing and clustering persistent cells against different increasing drug concentrations over time.

Furthermore, previous studies have shown that resistance mechanisms of PDAC to anti- cancer therapeutics could be more likely associated with the role of epigenetic dysregulation of apoptotic pathways being one of the intrinsic tumor factors rather than being associated with the effectivity of therapeutic agents themselves (14-16). In order to investigate this further, we are going to analyze DT cells and EDT persister cells from both murine and human PDOs using Chromatin Immune-precipitation Technology Chip-Seq with specific antibodies targeted against the histone proteins H3K4me3 and H3K9me3 which were found to activate downstream signaling pathways responsible for attaining the apoptosis-resistance phenotypes of PDAC after treatment.

1. Procedures and Methods

a. Establishing Murine Pancreatic Ductal Organoids expressing only oncogenic Kras (PanINs organoids)
b. Establishing Tumor-derived organoids as a model for malignant murine PDAC Progression (PDAC Organoids)
c. Establishing Human pancreatic organoids model PanIN to PDA progression
d. In-vivo validation of molecular candidates associated with PDA Progression in human and mouse derived organoids
e. Modeling Personalized Drug Screening using pancreatic cancer organoids
f. Assessing Drug Response using
i. Cell Vitality Assay Developed by Promega Bio Sciences
ii. Electric Impedance Spectroscopy

1. 1 Establishing Murine Pancreatic Ductal Organoids expressing only oncogenic Kras (PanINs organoids)

a. Isolate small intralobular duct from pancreatic tissues from wild type mice C57B1/6 (n= 10 called mN) and GEMM carrying conditional KRAS+/LSL-G12D;Pdx1- Cre Mice (n = 10 called mP). We call GEMM mice carrying KRAS allele '' KC'' Mice.
b. Establish organoids from each isolated tissue through collecting the specimen in microbiologically sterile sampling bags or sterile jars containing DMEM/F12 supplemented with 100 ug/ml Primocin, Penicillin-streptomycin, 10 mM Hepes, 1xGlutamax
c. Cut sampled tissues into little fragments of approximately 5-7 mm and store at - 20 0C for DNA isolation or fixed in formalin for histopathology
d. Digest obtained samples in 0.012 % (w/v) collagenase XI (Sigma) and 0.012% (w/v) dispase (Gibco) in DMEM media containing 1% FBS (Gibco), shear fragmented tissue pieces with 5ml pipettes and dilute with 10 ml Plus+++ solution, restrain over 100 um filter, centrifuge at 300Xg, and then re-suspend the sedimented pellet in growth factor-reduced GFR Matrigel (BD) or Cultrex growth factor reduced Basement Membrane Extract BME Type 2.
e. Add 10 µl droplets of re-suspended pellet in a pre-warmed plate in an inverted position until it solidifies and then add pre-warmed organoid media (Complete Media CM). for efficient

culture, 10 µM rho Kinase (ROCK) is added to the media in the first week of culturing

f. Propagate for 20 passages

g. Cryopreserve the established organoids

h. Immunoblotting of Kras, pan-RAS, Tubulin and Kras-GTP by RBD-GST

i. Comparative transcriptional expression analysis by qRT-PCR between ductal (Pdx1, Ck19, Sox9, Hnf6), acinar (Ptf1a, Cpa1, Amy) and endocrine (Ngn3, Chga, Ins2) lineage marker genes in wild- type mice (mN) and KC Mice (mP) organoids.

j. Comparative transcriptional expression analysis by qRT-PCR for the gene's indicative of the PanIN lesion (Muc5ac, Muc6, Tff1, Kif4) in mN and mP organoids

k. Activation of Kras G12D in mP organoids using adenoviral vector Ad-Cre (Kras+/LSL- G12D; R26-YEP) and Adenoviral blank AD-B1 (Kras+/LSL-G12D; R26-LSL-YFP) and confirming the recombination of of the KRAS+/LSL-G12D Allele using PCR.

l. Repeat the previous step (h,i & j) in order to assess whether ductal, acinar and endocrine lineages markers are maintained after KRAS G12D activation in both mN and mP.

m. Orthotopic transplantation of mN and mP organoinds with KRAS+/LSL-G12D Allele in syngeneic mice C57B1/6

n. Orthotopic transplantation of mN and mP organoids with adenoviral activated Kras G12 and Adenoviral blank.

o. Histopathological examination of organoids following transplantation in the steps M&N

1.2 Establishing Tumor-derived organoids as a model for malignant murine PDAC Progression (PDAC Organoids)

a. From two groups of mice, Group (A) carries oncogenic KRAS+/LSL-G12D, Pdx1- Cre Allele, we call this group "KC Mice" and Group (B) has more progressive mPDA traits and carries KRAS+/LSL-G12D, Trp 53+/LSL-R172H, Pdx-Cre, we call this group "KPC" Mice. This KPC Group of Mice has more invasive and malignant pancreatic cancer than the KC mice as it is characterized by metastatic nature. Each group should have at least 10 mice.

b. Prepare pancreatic ductal organoids from primary tumors (mT) and metastatic tumor (mM) from KPC Mice. Organoids prepared from metastatic tumors include Lung, Peritonium, Diaphragm and Liver. Follow the same technique for pancreatic organoids generation which is explained in the previous procedure (Procedure 1)

c. We keep the previous organoids prepared from wild mice in the previous steps as (mN) organoids and mice carrying the KRAS+/LSL-G12D allele that showed PanIN lesions as (mP) organoids

d. Histopathological examination of prepared organoids from primary tumors (mT) organoids and metastatic tumor (mM) organoids directly following isolation in Matrix gel (Day 0) and three days post-isolation (Day 3)

e. Immunoblotting of selected signaling effectors Kras-GTP and Ras GTP by RBD-GST pull-down and Kras, pan-Ras, p-AKT, AKT, p-ERK, ERK, p-S6, S6, Tubulin using Lysates.

f. Activation of the Kras LSL-G12D in mT and mM organoids by recombination using Adnoviral vector Ad-Cre (Kras+/LSL-G12D; R26-YEP) and confirming the result by PCR.

g. Orthotypic transplantation of organoids derived from primary tumors mT and mM organoids in another group of wild mice C57BL/6J and examining the histopathological lesions formed over 1-6 months. It's usually anticipated to making histopathology after one month from transplanting metastatic tumor organoids mM and after 6 months from trans planning primary tumour organoids mT.

h. Examine Loss of Heterozygosity LOH for the wild type Trp53 allele in primary tumor (mT) and metastatic (mM)organoids (Trp53 LOH, unstable karyotype and aneuploidy should be characteristic features of metastatic PDA i.e. mt vs. mM and contributing towards its malignancy nature)

i. Karyotyping for all organoids types wild type (mN)& PanINs (mP)from KC Mice as well as primary tumors (mT) and metastatic type (mM) from KPC Mice to determine the degree of aneupleudy.

j. Targeting tumor suppressor genes TP53 and p16/p17 CDKN2A using short hairpin RNAs (shRNAs) in PanINs (mP) organoids and then orthotopic transplantation in wild-type mice to assess the effect of inhibiting the tumor suppressor gene TP53 on the development of invasive PanINs and its progression into more invasive or metastatic Pancreatic Ductal Adenocarcinoma PDA when combined with the KRAS mutation (within a time period of at least 3 months at least).

k. Comparative genes expression analysis of at least 5 mN organoids whose histopathology showed confirmed normal pancreatic tissues, 5 mP organoids whose histopathology showed confirmed PanINs and mT organoids whose histopathology showed confirmed PDAC using bulk RNA sequencing of 29,000 mouse genes, analysing results using PCA and confirmation of significant genes expression changes using qRT-PCR.

l. Analysing the differential genes expression analysed by RNA Seq using DeSeq statistical program to compare genes expression of mP relative to mN, mT relative to mN and mT relative mP.

m. Repeating step K&I following Ad-Cre adenoviral vector induced expression of oncogenic Kras G12D

n. Differential global proteome analysis of mN, mP and mT organoids using amine-reactive isobaric tags for relative and absolute quantification iTRAQ Mass Spectrometry (more than 6000 unique protein isoforms and proteins encoded by the same gene can be quantified using this method)

o. Linear regression modeling on the normalized intensity peaks of the proteomic data in order to define the differentially expressed proteins among mN, mP and mT organoids.

p. Gene Set Enrichment Analysis GSEA on the RNA-Seq and Proteomic among different organoids which can reflect on which metabolic pathways are upregulated or downregulated during PanINs and its progression to PDAC. Examples of such vital metabolic pathways which are more significantly relevant to assess are:

i. Glutathione Metabolism

ii. Biological Oxidation Reactions

iii. Steroid Biosynthesis& Cholesterol Biosynthesis Pathways

iv. Carbon Pool by Folate

v. Pyrimidine Metabolism Pathway

vi. Fatty Acid Metabolism Pathways

vii. TCA Cycle/Respiratory Electron Transport Pathways

q. Comparative genes expression and proteomic analysis for

the nucleoporin family proteins particularly NUP214, NUP153, NUP1, NUP2, NUP88 in mN, mP and mT organoids by RNA-seq and ITRAQ MS.

1.3 Establishing Human pancreatic organoids model PanIN to PDA progression

a. For samples from human patients, a written consent from physicians for research use needs to be obtained prior to the acquisition of the specimen.

b. Preparation of 10 organoids from normal pancreatic tissues (hN) prepared during surgical resection or islets transplantation as the method described in previous Step 1.

c. Preparation of 15 organoids from resected primary pancreatic tumor (hT) and metastatic tumor (hM) or fine needle aspiration biopsy FNA from metastatic lesion (hFNA)

d. Histopathological examination of the normal and cancerous pancreatic tissues original samples and organoids formed thereof and examination using Brightfield and H&E staining

e. The organoids derived from normal tissues are cultured in duplicates of media one composed of Matrigel containing complete media CM and the other are cultured in the same complete media but lacking each of these elements EGF, Noggin or Rspondin1 called Tumor Media TM.

f. Examine the ability of organoids propagation after several passages for hT and hN

g. Targetting 2,000 cancer associated genes in the hN and hT organoids using NGS particularly mutations in the following PDAC associated genes

h. Characterization of the cell types present in the PDA organoids hN vs. hT to check whether they are derived from ductal, acinar paracrine origins through checking the pancreatic lineage markers ductal (Pdx1, Ck19, Sox9, Hnf6) genes, acinar (Ptf1a, Cpa1, Amy) and endocrine (Ngn3, Chga,

i. Ins2) using qRT-PCR

j. Karyotyping hN, hT and hM organoids and checking for aneuploidy vs. stable diploidy

k. Immunoblotting of PDA-associated CA19-9 (Cancer Associated Antigen 19-9) in hT and mT organoids.

l. Orthotopic Transplantation of normal tissue organoids hN and primary tumor organoids hT organoids in Nu/Nu Mice

m. Histopathological examination of the lesion formed after one month by H&E staining (check epithelium morphology, nuclei size, pleomorphism and hyperchromasia) (Usually disarrangement of epithelial lining, metaplasia, epithelial cells with small nuclei that shows hyperchromasia and lack of pleomorphism are characteristic features of invasive PDA)

n. m-Immunohistochemistry IHC by blotting against apical mucin and CK 19 and detection of mutation or loss of TP53 or SMAD4 Tumor Suppressor genes in hT organoids as compared to hN organoids.

- KRAS oncogene
- MYC Oncogene
- ARID1A
- MLL3
- TP53 Tumor Suppresor Gene
- SMAD Tumor Suppressor Gene
- CDKN2A Tumor Suppressor Gene
- TGFBR2 Tumor Suppressor Gene
- DCC Tumor Suppressor Gene
- CTNB1 Tumor Suppressor Gene
- VHL Tumor Suppressor Gene
- H1F1A Tumor Suppressor Gene
- MAP2K4 Tumor Suppressor Gene

1.4 In-vivo validation of molecular candidates associated with PDA Progression in human and mouse derived organoids

a. A Select THE set of genes upregulated in normal tissues from human and mice
i.e. nH and nM as well as primary tumor organoids from human and mice i.e. mT and hT whose histopathological examinations showed PanIN or PDA in the previous steps (1,2 and 3) then make Immunohistochemistry IHC and immunofluorescence IF using specific antibodies targeting their expressed proteins, enzymes or ligands

b. Validate such results through orthotopic transplantation of the mT and hT organoids into Nu/Nu mice and then make IHC of the transplanted organoids after one month from the transplantation time to indicate the role such molecules play in PDA progression.

1.5 Modeling Personalized Drug Screening using pancreatic cancer organoids

1. Tissues from pancreatic tissues are sampled by biopsy or surgical resection (around 50 samples) 10 samples from normal tissues and 40 samples from tumor tissues

2. For samples from human patients, a written consent from physicians for research use needs to be obtained prior to the acquisition of the specimen.

3. Collect the specimen in sterile sampling bags or sterile jars containing DMEM/F12 supplemented with 100 ug/ml Primocin, Penicillin-streptomycin, 10 mM Hepes, 1xGlutamax

4. Cut sampled tissues into little fragments of approximately 5-7 mm and store at -20 0C for DNA isolation or fixed in formalin for histopathology

5. Digest obtained samples in 0.012 % (w/v) collagenase XI (Sigma) and 0.012% (w/v) dispase (Gibco) in DMEM media containing 1% FBS (Gibco, shear fragmented tissue pieces with 5ml pipettes and dilute with 10 ml Plus+++ solution, restrain over 100 um filter, centrifuge at 300Xg, and then re- suspend the sedimented pellet in growth factor-reduced GFR Matrigel (BD) or Cultrex growth factor reduced Basement Membrane Extract BME Type-2.

6. Add 10 ul droplets of re-suspended pellet in a pre-warmed plate in an inverted position until it solidifies and then add prewarmed organoid media (CM, TM1 or TM2 which are described below). 10uM rho Kinase (ROCK) is added to the media in the first week of culturing

7. Design three types of media, Complete Media (CM) used for normal tissues sample, Tumor Media 1 (TM1) and Tumor Media 2 (TM2) used for tumor- derived organoids.

8. Monitor culture growth in CM, TM1 and TM2 N.B. The aim of having two different media varying in composition first is to assess how Wnt Dependancy may influence GATA6 expression level, a vital gene responsible for pancreatic development and pancreatic epithelium proliferation and differentiation. Second, Nogin and and A83-01 (TGF-B inhibitor) are small molecules cytokines that are essential for cell proliferation and

differentiation. Withdrawal of such molecules leads to induction of BMP Signaling, up-regulation of ID1 and ID3 genes expression and growth cessation after 2 weeks in wild type SMAD4 PDO. However, mutated SMAD PDO can grow well in the media deficient in Nogin and A83-01 and therefore can be further isolated and characarized. Such result can be confirmed using qPCR for the BMP target genes.

Complete Media CM	Tumor Media Type-1 TM-1	Tumor Media Type-2 TM-2
-Wnt3a-conditioned media 50% v/v - Plus+++ containing 1xB27 Supplement - 1,25 mMN-acetyl-l-Cystein - 10mM nicotinamide - 50 ng/ml human EGF -500 nm A83-01 (TGFB-inhibitor) - 100 ng/ml human FGF10 - 500 nm A83-01 -1 uM PGE2 -10 nm gastrin - 4%v/v RSPO - Noggin	Same as CM but without EGF and PGE2	Same as CM but without PGE2; A83-01 and Wnt2a conditioned media

9. Exclude organoids that didn't grow in at least one of the two media TM1 or TM2

10. Histopathological examination of the tissues and their corresponding organoids.

11. IHC staining of the tissues and their corresponding organoids for TP53 and SMAD4 (loss or mutant TP53 and SMAD 4 can be recognized for pancreatic tumor and organoids derived thereof)

12. On the well-formed cultured patients-derived organoids, characterize the type of pancreatic cancer using Whole Genome Sequencing WGS of pancreatic cancer associated genes into its previously classified pathological Categories into Pancreatic Ductal Adeno Carcinoma PDAC, Acinar Cell Carcinoma ACC, Cholangio Carcinoma CC, Intraductal Papillary Mucinous Neoplasm IPMN while a panel of 200 genes (in the appendix) can be target sequenced. Any PDO organoids for which no germ line DNA is available, functionally annotate them using COMSIC.

13. Compare the genetic landscape of different PDOs that could be derived from the same patient and cultured in different growth media. This is to explore how in-vitro culturing selection pressures may influence specific tumor clone's enrichment and therefore influence intra-tumor heterogeneity.

14. Karyotyping of all cultured and grown organoids hN, hT, hM to estimate Copy Number Variations CNV, Genome Ploidy and loss or gains of chromosome arms

15. Unbiased Mutational Signature Analysis on all detected point mutations which can reveal specific mutation signature for each PDO derived organoids and potential biomarker for therapy response

1.6 High throughput Drug Screening using Pancreatic Ductal Orgaboids PDOs

1. A minimal number of 20 organoids hT and hM cultured in Complete Media after confirming 100 % tumor purity representing different mutational signatures identified in the previous stem are used in drug screening. Such organoids used in drug screening should show maintained cell behaviour after being transferred from TM1 and TM2 into CM. This is to avoid the influence of media composition on inducing a biased drug response.

2. Organoid are digested and dissociated by TrypLe and plated in 384 well plate after 4 days to allow cells to self-recover

3. Plate dissociated cells in 384 well plate each in 52 µl CM and 8 µl 50:50 BML (Two set of plates A and B in duplicates experiment)

4. Add test compounds in set A and set B plates along an exposure time of 72 hours using Echo 555 from Labcyte. 50 therapeutic compounds widely used for PDAC can be assessed and be belonging to these 10 categories.

a. Compounds targeting Microtubules e.g. Paclitaxel, Docetaxel, Vinblastine

b. Compounds targeting Aurota Kinas A (AURKA) e.g. Alisertib, c.Tozasertib, ZM447439 Compounds targeting phosphotidylinositol 4,5 bisphophate 3- kinase catalytic subunit alpha (PIK3CA) e.g. Dactoslisib and Pictilisib

d. Compounds targeting Topoisomerase-1 (TOP1) e.g. Irinotecan AND Campatothecin

e. Compounds targeting FGFR e.g. BMS and Linistinib

f. Compounds targeting IGFR e.g. AZD4547

g. Compounds targeting SRC/ACT e.g. Uprosertib and Dasatinib

h. Compounds targeting HDAC e.g. Vorinostat and Entinostat

i. Compounds targeting DNA sensors e.g. Olaparib and Talazoparib

j. Alkylating agents e.g. Carboplatin and Oxaliplatin

5. In Set (A) plates, measure cell viability using Cell Viability Assay (Promega Bio Science) using 15 µl cell titer glue to each well. This assay relies on measuring cell viability through estimating the intracellular Adenosine Triphosphate ATP as it mediates the conversion of Luciferin into Oxyluciferin in the presence of the Ultra-Glu ® luciferase enzyme.

6. In Set (B)plates, measure cell vitality using Electric Impedance E1 Measurement using Trans-Impedance Amplifier e.g. MFLI Lock in Amplifier or Solatron Impedence Analyzer (as indicated in procedures of previous project procedures at The Heinz-Nixdorf Lehrustuhl for Biomedisch Electronik, Transla TUM)

7. Calculate IC50 normalized to z-score for each PDO (Higher value IC50 indicates resistance and lower value IC50 indicates sensitivity).

8. Observe the difference of the organoids PDOs' response to different therapeutic categories

9. Compare PDO's response to therapy to the corresponding actual clinical data of patients' response to therapy

10. Correlate genomic mutational status with PDO Drug Response using Elastic Net Model and Tandem 2 Step Model

11. For PDOs that showed mutational status in MAPK3K1 or PIK3RA, assess the drug response and determine the delta fAUC to the combination therapy of HER2/EGFR Inhibitor e.g. lapatinib together with Gemcitabine which is a commonly used first line of treatment in PDAC patients (high AUC indicate resistance, but lower AUC indicate sensitivity, highest sensitivity mostly noticed in PDOs with EGFR receptor mutation)

12. Similarly, PDOs that expressed mutational status of FGFR1 and CDK2A should be assessed for sensitivity response towards AKT inhibitor MK- 2206

13. Determine MTAP Status of PDOs using targeted DNA or RNA Sequencing. For MTAP+ve & MTAP-ve PDOs, assess their drug response to PRMT5 inhibitor EZP015556 at 14. different concentrations (0.01, 0.1, 10, 100 uM). In MTAP – ve PDOs, Loss of MTAP (a gene commonly lost in pancreatic cancer) is usually associated with higher sensitivity to PRMT5 Inhibition. In order to further explore this anticipated outcome of patient's response to PRMT5 Inhibitors drugs, Wild-Type

MTAP expression is induced in patient-derived MTAP +ve and MTAP –ve PDOs using lentiviral doxycycline inducible MTAP expression vector followed by PRMT5 inhibitor drug exposure e.g. EZP015556.

Measuring Cell Vitality Using Electric Impedance Spectroscopy before and after drug exposure

Design an integrated electric circuit with voltage follower circuit to be attached with the biosensors (working, guarding and counter electrodes) and a Transimpedance Amplifier for measuring electric impedance through the cultured organoids soaked in PBS solution in each well of an ILFA sensor coated PCB as the photos show below. The Circuit Designs and ILFA sensor coated PCB have been developed and validated by colleagues at Heinz Nixdorf- Lehrstuhl für Biomedizinische Elektronik, Transla TUM at the Technical University of Munich.

Cell vitality measured here using Electric Impedance Spectroscopy for each hN and hM organoids will be assessed before and after drug exposure and further correlated to the corresponding Cell Vitality Score measurement using Ultra-glue Luciferase ®developed by Promega Bio Science from the previous step.
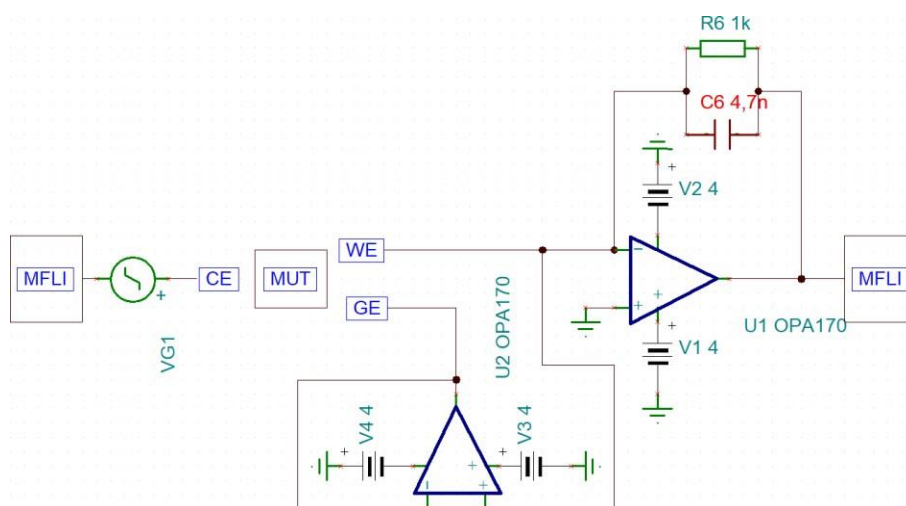

The full measurement circuit depicted from MSc thesis of Eng. Sebastian Siegfried at Transla TUM, it shows the excitation signal is provided to the counter electrode (CE) via the MFLI. The working electrode (WE) is connected to the negative input of the transimpedance amplifier (TIA), amplifying only the current on the working electrode. The guarding electrode (GE) is connected to the output of the voltage follower circuit which is in turn fed by the working electrode. The output of the TIA is fed to the MFLI for a precise voltage measurement.
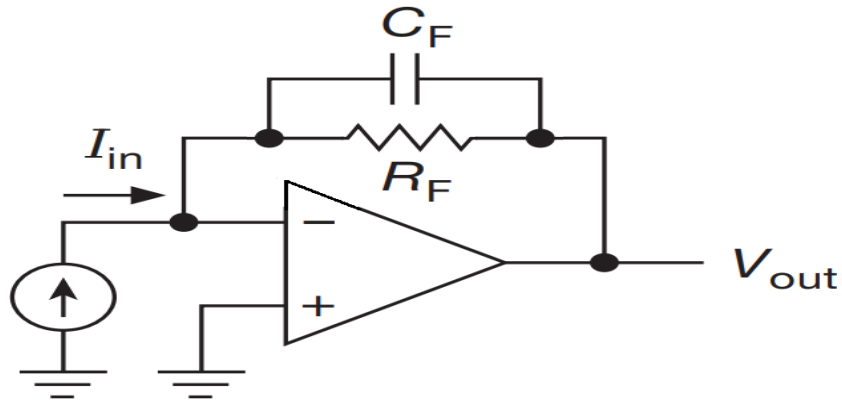
Basic Transimpedance Amplifier Circuit with a feedback capacitor. The output voltage Vout is measured to ground.
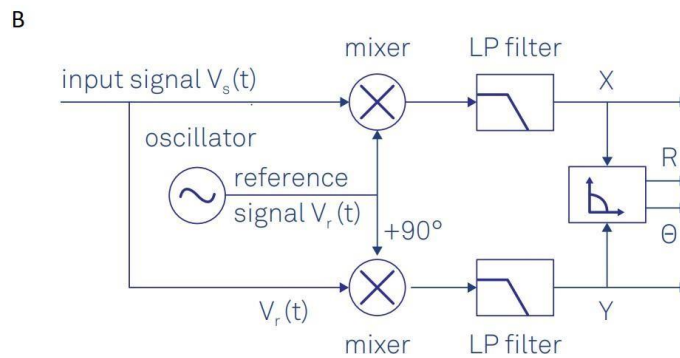
MFLI Lock-In Transimpedence Amplifier developed by Zurich Instruments and used in the measurement of Electric Impedance
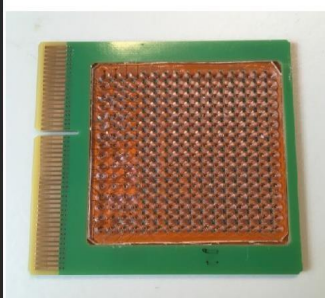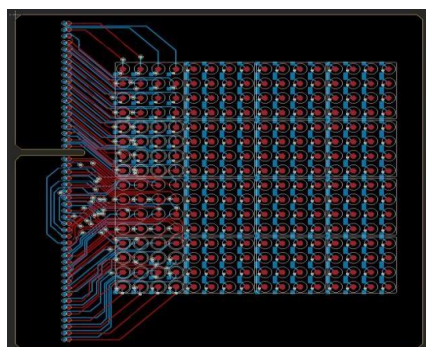
	1	2	3	4	5-16
1	WE: 4 GE: n/a	WE: 2 GE: n/a	WE: 3 GE: n/a	WE: 1 GE: n/a	WE: n/a GE: n/a
2	WE: 8 GE: 10	WE: 6 GE: 9	WE: 7 GE: n/a	WE: 5 GE: n/a	WE: n/a GE: n/a
3	WE: 14 GE: n/a	WE: 12 GE: n/a	WE: 13 GE: n/a	WE: 11 GE: n/a	WE: n/a GE: n/a
4	WE: 18 GE: 20	WE: 16 GE: 19	WE: 17 GE: n/a	WE: 15 GE: n/a	WE: n/a GE: n/a
5	WE: 24 GE: n/a	WE: 22 GE: n/a	WE: 23 GE: n/a	WE: 21 GE: n/a	WE: n/a GE: n/a
6	WE: 28 GE: 30	WE: 26 GE: 29	WE: 27 GE: n/a	WE: 25 GE: n/a	WE: n/a GE: n/a
7	WE: 34 GE: n/a	WE: 32 GE: n/a	WE: 33 GE: n/a	WE: 31 GE: n/a	WE: n/a GE: n/a
8	WE: 38 GE: 40	WE: 36 GE: 39	WE: 37 GE: 42	WE: 35 GE: 44	WE: n/a GE: n/a
9	WE: 61 GE: 46	WE: 63 GE: 48	WE: 65 GE: 55	WE: 67 GE: 53	WE: n/a GE: n/a
10	WE: 62 GE: 52	WE: 64 GE: 50	WE: 66 GE: 47	WE: 68 GE: 45	WE: n/a GE: n/a
11	WE: 71 GE: 56	WE: 73 GE: 54	WE: 75 GE: 51	WE: 77 GE: 49	WE: n/a GE: n/a
12	WE: 72 GE: 59	WE: 74 GE: 57	WE: 76 GE: 60	WE: 78 GE: 58	WE: n/a GE: n/a
13	WE: 81 GE: 70	WE: 83 GE: 69	WE: 85 GE: 55	WE: 87 GE: 53	WE: n/a GE: n/a
14	WE: 82 GE: 79	WE: 84 GE: 80	WE: 86 GE: n/a	WE: 88 GE: n/a	WE: n/a GE: n/a
15	WE: 91 GE: 89	WE: 93 GE: 90	WE: 95 GE: n/a	WE: 97 GE: n/a	WE: n/a GE: n/a
16	WE: 92 GE: n/a	WE: 94 GE: n/a	WE: 96 GE: n/a	WE: 98 GE: n/a	WE: n/a GE: n/a
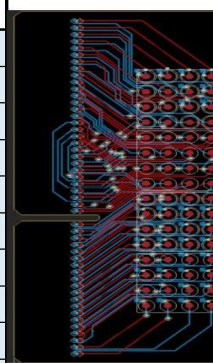
The table shows the numbers of the pin connection for each well.

The wells that have both the working electrode and guarding electrode connected to the socket are marked darker than the cavities with only the working electrode connected.

The layout of the ILFA board design developed by Eng. Sebastian Seyfried. In the first four columns from the left, several working electrodes and their corresponding guarding electrodes are accessible via the 98-Pin-connector.

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