Short-term mobility report

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We had the opportunity to be part of this short-term mobility for PhD students between 17th July-7th August 2023 in the project “Strategic interuniversity cooperation to improve research abilities for PhD Students for higher educational quality”. We started the training on the next day of our arrival, where Researcher Deo Prakash Pandey, Professor Arne Klungland and the groups of researchers they were coordinating welcomed us, introduced us to all of the colleagues and presented us the research unit where they work on a daily basis (Sintef) by making a tour. We then started our scientific learning, described in more details below.

1. Cell culture basics

During our first week at Oslo University Hospital, we were trained in cell culture basics, including aseptic techniques, thawing, freezing, subculturing, splitting, and counting adherent cells. After the training, we were provided with our own cells to take care of. We received 2 pancreatic cancer cell line called MiaPaCa-2 and ASPC-3. We practiced how to passage cells, prepare and change medium, identify contamination and other protocols. Media used was DMEM + 10% fetal bovine serum + 1% antibiotics.

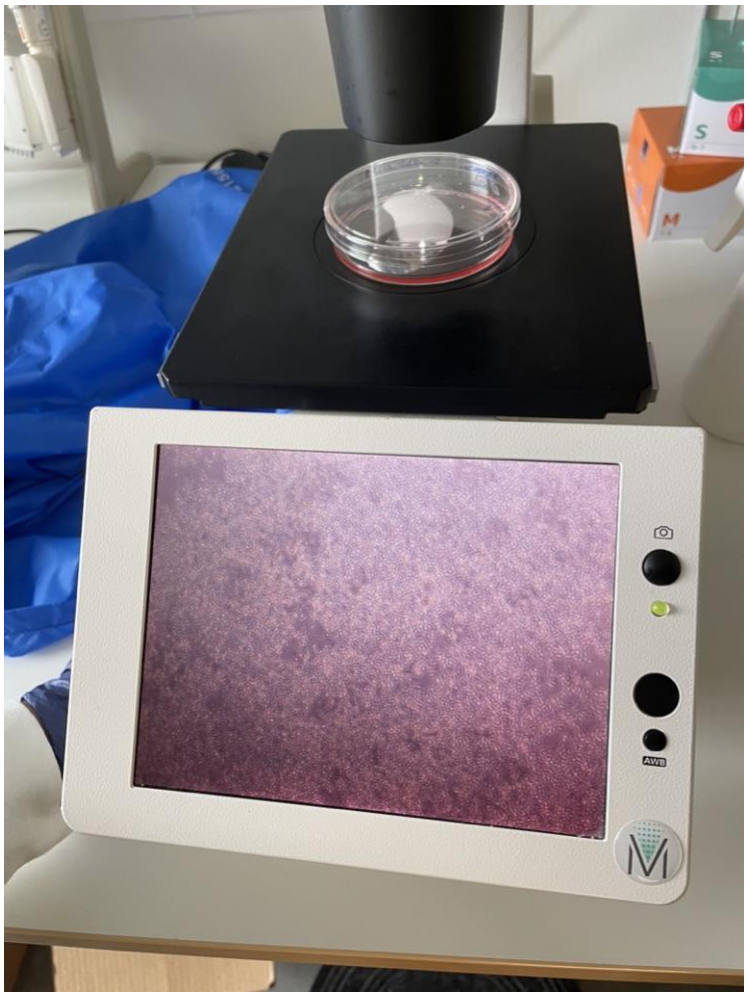


Figure 1. Picture of MiaPaCa-2 cells under optical microscope

2. SLAM sequencing

Our main focus during the next week was on performing SLAM-sequencing. SLAM sequencing (Thiol (SH)-Linked Alkylation for the Metabolic sequencing of RNA, SLAMseq) is a high-sensitivity method for time-resolved measurement of newly synthesized and existing RNA in cultured cells. SLAMseq enables resolution of RNA synthesis and degradation kinetics. SLAMseq enables the identification and quantification of newly synthesized (nascent) and existing RNA from the same sample in parallel, without the need for biochemical isolation.

SLAM-seq workflow:

1) S4U labeling

After being exposed cells to drug treatments for established time, cultured cells are grown in 4-Thiouridine (S4U) to allow S4U nucleotides to be incorporated into newly synthesized RNA.

2) RNA sampling

Cells are sampled at a given timepoints and RNA is extracted under reducing conditions. The isolated total RNA contains both existing (unlabeled) and newly synthesized (labeled) RNA for kinetics experiments.

3) Alkylation

Total RNA is mixed with iodoacetamide (IAA), which modifies the 4-thiol group of S4U-containing nucleotides via the addition of a carboxyamidomethyl group. The RNA is purified by ethanol precipitation prior to proceeding to library preparation.

4) Library Preparation: Nucleotide Conversion

Total RNA is reverse-transcribed to cDNA. For labeled RNA transcripts, reverse transcriptase incorporates a G instead of an A at positions where reduced *S4U-modified nucleotides are encountered. Second-strand cDNA synthesis is then performed to generate a double-stranded SLAMseq RNA-Seq library.

5) Library Amplification and Sequencing

PCR is used to amplify the SLAMseq RNA-Seq library and to add indices and full adapter sequences for next-generation sequencing. Sequencing reads with T>C mutations distinguish labeled from unlabeled transcripts.

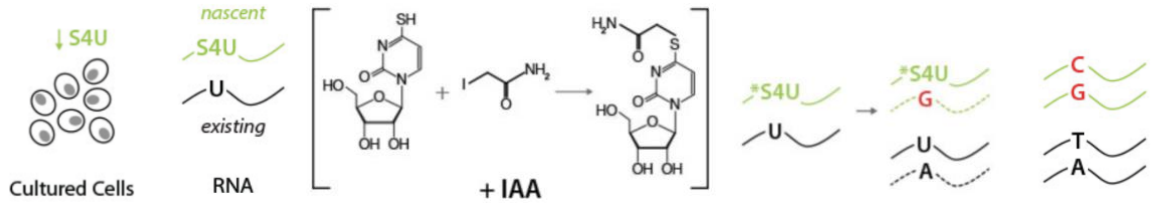


Figure 2. Illustration of SLAM-sequencing workflow

3. Histone expression by qPCR

We evaluated the expression of 7 different histones by qPCR. The histones included: H1H1B, H2AFX, H1H2AC, H1H4A, H1H2BK, H1H2AG and H1H1C. Pancreatic cancer cells were were exposed to different treatments, including DMSO, THZ531 drug (at 2, 4, and 6 hours), SR4835 drug (at 6 hours) and compound 12 drug (at 1, 3, and 10 uM).

After exposure to treatment, cells were labelled with S4U. Cells were then lysed using

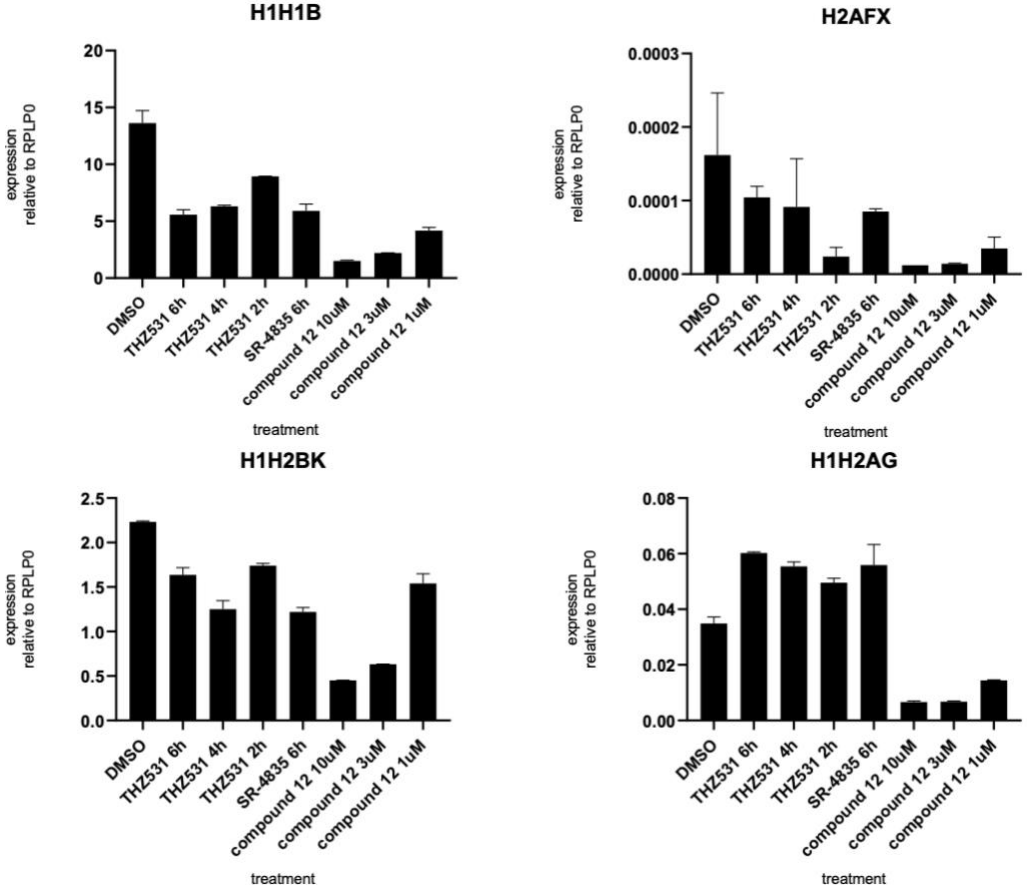
sample	RPO	H1H1B	H2AFX	H1H2AC	H1H4A	H1H2BK	H1H2AG	H1H1C
DMSO	0,47	12,85	0,00	0,11	0,16	2,22	0,03	6,50
	2,12	14,40	0,00	0,09	0,16	2,24	0,04	5,79
THZ531 6h	0,83	5,88	0,00	0,06	0,06	1,58	0,06	4,65
	1,20	5,26	0,00	0,06	0,06	1,69	0,06	4,24
THZ531 4h	0,86	6,38	0,00	0,07	0,06	1,18	0,05	4,88
	1,16	6,23	0,00	0,08	0,07	1,32	0,06	3,82
THZ531 2h	0,77	8,95	0,00	0,09	0,10	1,76	0,05	4,95
	1,29	8,93	0,00	0,09	0,09	1,72	0,05	4,82
SR4835 6h	1,11	5,48	0,00	0,07	0,06	1,18	0,05	4,21
	0,90	6,33	0,00	0,08	0,07	1,26	0,06	4,00
10 uM	0,89	1,46	0,00	0,03	0,01	0,45	0,01	0,95
	1,12	1,55	-	0,02	0,02	0,45	0,01	0,98

mercaptoethanol and harvested. RNA was extracted using different extraction columns, i.e. for the removal of genomic DNA. Total amount of RNA was measured using Nanodrop system. Then, complementary DNA was synthesized using RNA samples and a mix of nucleotides, enzyme, and random primers in buffer. RT-qPCR was performed for ~3 hours and the main results are shown in Table 1 and Figure 3.

Figure 3 shows that compound 12 generally had the highest inhibition on histone expression. In the case of H2AFX histone, THZ531 at 2h had similar inhibition effects.

3 uM	0,89	2,19	0,00	0,04	0,02	0,63	0,01	1,12
	1,13	2,23	0,00	0,04	0,02	0,63	0,01	1,25
1 uM	0,66	4,38	0,00	0,06	0,03	1,46	0,01	2,14
	1,52	3,97	0,00	0,06	0,05	1,62	0,01	2,21
H2O	1,00	-	-	-	-	-	-	1,09
	-	-	-	-	-	-	-	-

Table 1. Histone expression evaluated by qPCR



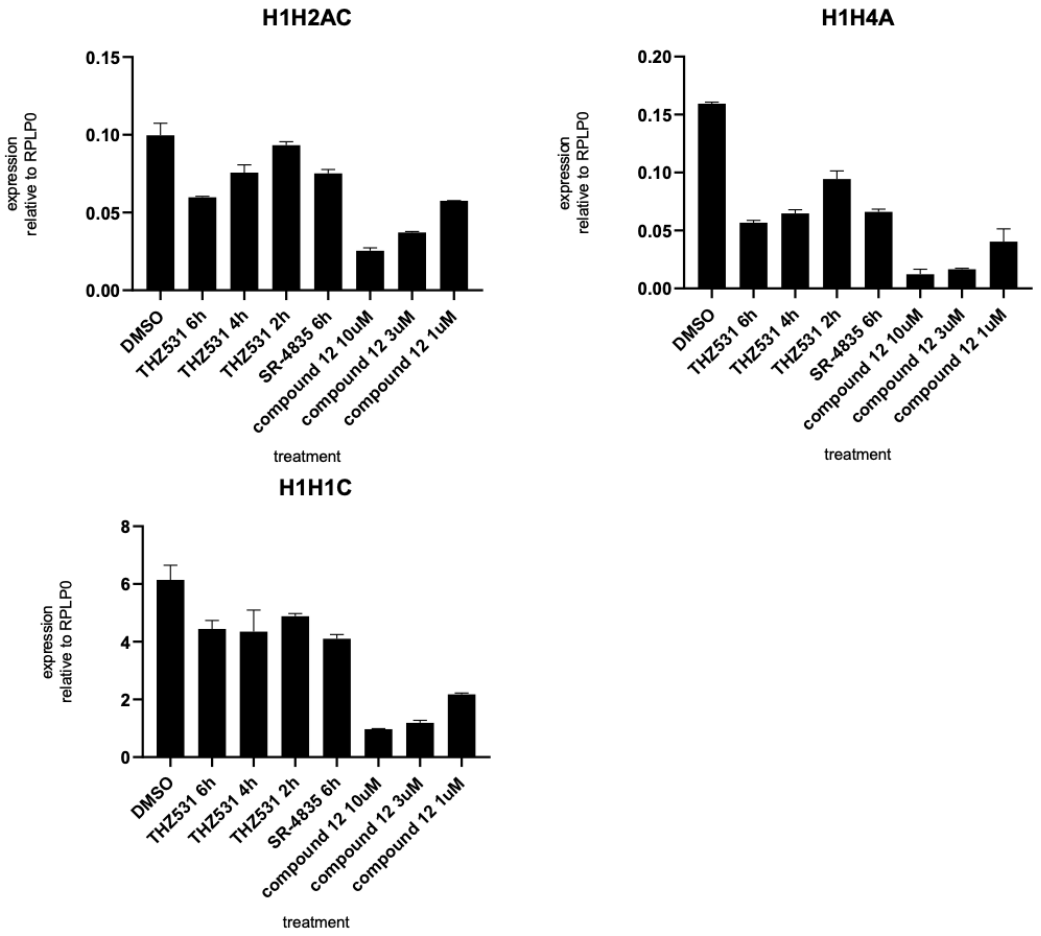


Figure 3. Histone expression evaluated by qPCR

4. Effect of CDK12/CDK13 inhibition on primary pancreatic cancer cells

During the last week, we evaluated the effect of CDK12/CDK13 inhibition on primary pancreatic cancer cells from patients. Two different primary pancreatic cancer cells were used: WM7 PDAC and RA PDAC cells and the effect of three different drugs was tested: THZ521, SR4835 and compound 12.

Figure 4 shows higher IC50 doses on WM7 cells, with 97nM for THZ531, 119nm for SR4835 and 597nm for compound 12. In comparison, lower IC50 doses and thus higher sensitivity was observed on RA cells, with IC50 of 59nM for THZ531 and SR4835, and 344nm for compound 12.
In conclusion, THZ531 seems to be the most efficient drug on WM7 cells, while THZ531 and SR4835 proved to have the same efficacy on RA PDAC cells.

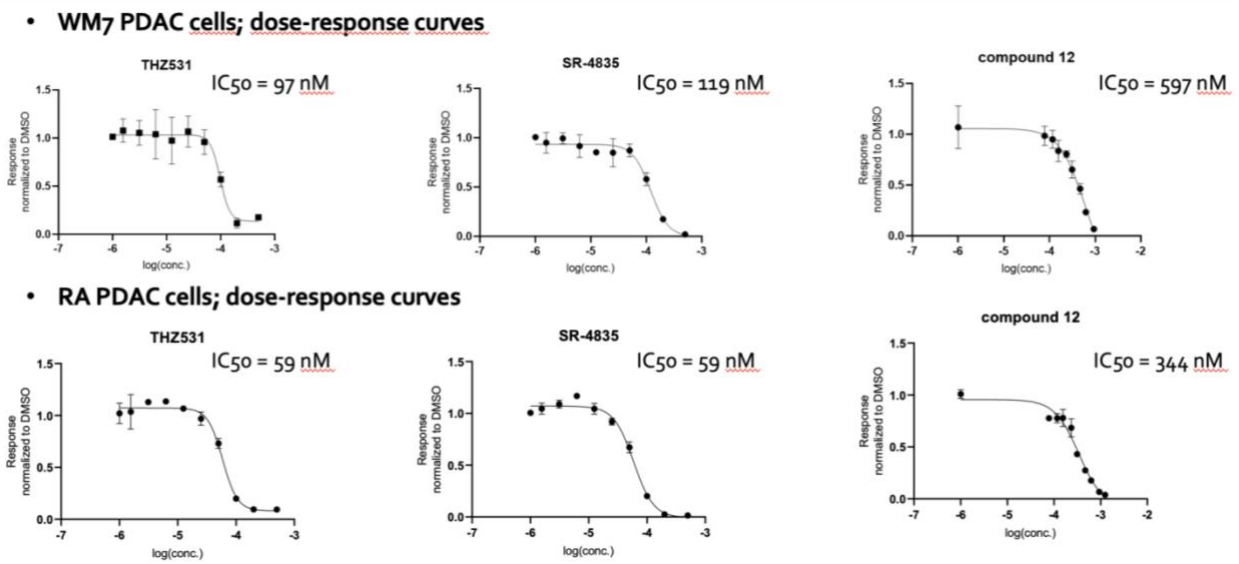


Figure 4. Dose response curves on primary pancreatic cancer cells.

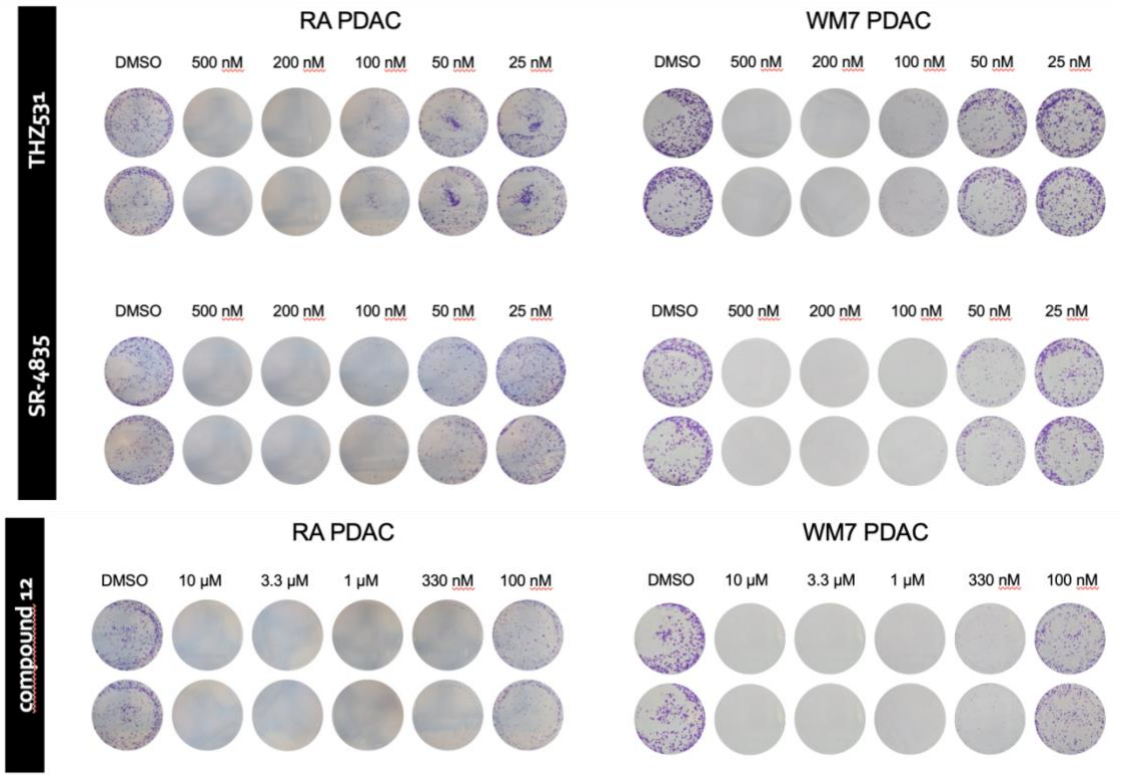


Figure 5. Microscopy images of primary pancreatic cancer cells exposed to drug treatment.

5. Other

We performed WesternBlot on PANC-1 cells, where we learned how to harvest cells, measure proteins using Bradford technique, prepare gels and running buffer, locking primary and secondary antibodies, transfer gels on membranes using Transblot, and data analysis. Moreover, we assisted one flow cytometry study on U2OS osteosarcoma cells, after treatment with THZ drug.

To conclude, we want to thank again for having this extraordinary opportunity, made possible through the collaboration between Oslo University Hospital (represented by Researcher Deo Prakash Pandey and Professor Arne Klungland), and our University, UMF ,,Iuliu Hatieganu" Cluj-Napoca (represented by Professor Ioana Berindan-Neagoe).