Technology Transfer Grant (TTG) Report

The effect of PARPi on therapy resistance of cervical cancer stem cells

Host Institute: *OncoRay, Dresden, Germany*

Date of visit: *11 – 30 November 2019*

Primary objective of visit and duration of stay

The aim of my three-week visit to Professor Anna Dubrovska's laboratory at the National Center for Radiation Research in Oncology (OncoRay), Dresden, Germany, was to learn a new technique to identify cancer stem cells (CSCs).

Background

I am a Newton International Fellow at Newcastle University, UK, where I work with Professor Nicola Curtin. In the Newcastle lab, I test different approaches in vitro and in vivo to see whether rucaparib, an inhibitor of poly-adenosine di-phosphate ribose polymerase (PARP) can be used in cervical cancer treatment. PARP inhibitors (PARPi) that target cancer cells with homologous recombination repair deficiency (HRD) are well known, but the chemo-radio-sensitising properties of PARPi have widened their application. Therefore, application of PARPi is not restricted only to HRD cancers; they are now being investigated in other cancer types in which HRD is not well known (e.g. cervical cancer). So far, I have had some success, as I have observed that radio-sensitisation with rucaparib (1.0 μM) + 2 Gy radiation (IR) was ~1.5 fold in C33a and HeLa and ~1.2 fold in SiHa cervical cancer cell lines. Similar patterns of chemo-sensitisation with rucaparib (1.0 μM) + cisplatin (0.1 μM) was ~2.0 fold in C33a and HeLa and ~1.5 fold in SiHa cells (data unpublished). With these preliminary data, I wanted to test any other possible approach that could be beneficial for the treatment or prevention of chemo/radioresistant tumours. The aim of my research visit to Professor Dubrovska's lab was to learn the ALDEFLUOR assay, which is not performed in the Newcastle lab. Professor Dubrovska is the leader of the Biomarkers for Individualised Radiotherapy research group at OncoRay and she is an expert in CSC research.

Principle of ALDEFLUOR assay and method in brief

High aldehyde dehydrogenase (ALDH) activity is observed in CSCs of multiple cancer types and is often used to isolate and functionally characterise CSCs. The CSC population was identified in HeLa cells in terms of measuring ALDH+ cells by use of the ALDEFLUOR™ Kit (STEMCELL Technologies) according to the manufacturer's instructions and standard operating procedures that are followed at Professor Dubrovska's lab. Cells were suspended in assay buffer that contained ALDH substrate (BAAA). In the presence of ALDH, the substrate converted to a green fluorescent product that was retained within the cell due to its negative charge. These ALDH-bright (ALDH+) cells were analysed by BD FACS Celesta flow cytometry (BD Bioscience). A specific ALDH inhibitor, DEAB, was used as a negative control and propidium iodide staining was performed to access cell viability. At least 50,000 cells from each sample were measured. Instrument-generated data were retrieved. Raw data were re-analysed using FlowJo software (TRIAL version 10.6.2). After optimisation of the gating parameters in the HeLa cell line, which I had never tested before I visited Professor Dubrovska's group, two biological repeats were performed successfully within the short tenure.

Results

Here the data of N=2 are presented. Since only two biological repeats have been performed so far, I am not making any conclusive statement regarding the efficacy of the drugs. Overall data suggest that the ALDH+ population (CSC population) was identified within HeLa cells in normoxia and hypoxia (Figures 1 and 2).

Figure 1

(A) **(B)**

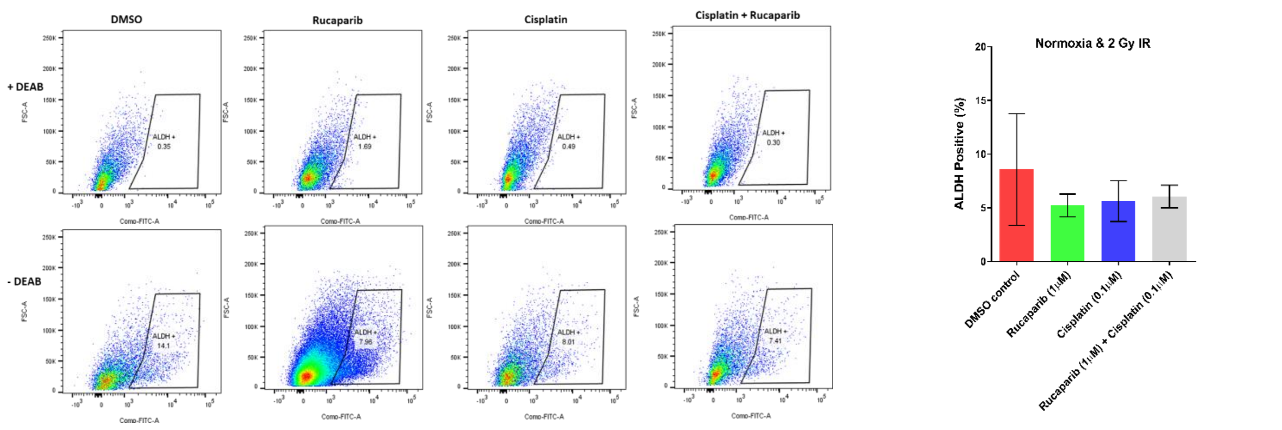

Figure 1: (A) Representative flow cytometry dot plots are shown for HeLa cells in normoxia study. Percentages of ALDH+ cells among total cells counted (≥50,000) are presented within the ALDH+ gating. HeLa cells were transferred from the Newcastle lab to the OncoRay lab. Cell-line authentication was performed in the Newcastle lab. Before starting the experiment, mycoplasma testing was performed in the OncoRay lab. Cells were maintained at Professor Dubrovska's lab in a humidified 37°C incubator supplemented with 5% CO2. For the ALDEFLUOR assay, cells were plated in 90 mm dishes. Overnight or 24 hr after plating, cells were treated with drugs (rucaparib 1.0 μM and cisplatin 0.1 μM) followed by 2 Gy IR with X-rays (Yxlon Y.TU 320; 200 kV X-rays, dose rate 1.3 Gy/min at 20 mA) filtered with 0.5 mm Cu. For the normoxia study, drug/IR treated dishes were incubated in a humidified 37°C incubator supplemented with 5% CO2 for 24 hr. (B) Bar diagram showing the percentage of ALDH+ cells in the normoxia study. Mean values of percentages of ALDH+ cells after treatment of drugs/IR from two experiment sets are: DMSO control (8.5), rucaparib (5.2), cisplatin (5.6), cisplatin and rucaparib combination (6.1).

Figure 2

(A) **(B)**

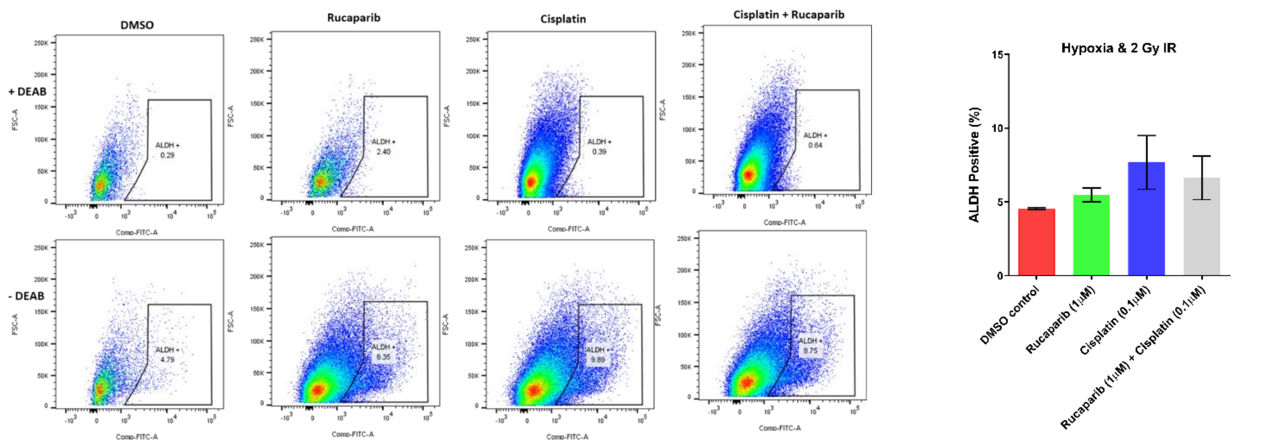

Figure 2: (A) Representative flow cytometry dot plots are shown for HeLa cells in the hypoxia study. Percentages of ALDH+ cells among total cells counted (≥50,000) are presented within the ALDH+ gating. For the hypoxia study, after plating in Professor Dubrovska's lab, the cells were taken to Professor Gelinsk's lab (MTZ) under protected conditions within a cell locker and maintained overnight or for 24 hr in a hypoxic incubator (1% O2) before treatment. During treatment, cells were returned to Professor Dubrovska's lab and, after drug/IR treatment, cells were transferred back to the hypoxic incubator (1% O2) for 24-hr incubation. The ALDEFLUOR assay was done at Professor Dubrovska's lab. (B) Bar diagram shows percentage of ALDH+ cells in hypoxia study. Mean values of percentages of ALDH+ cells after treatment of drugs/IR from two experiment sets are: DMSO control (4.5), rucaparib (5.5), cisplatin (7.7), cisplatin and rucaparib combination (6.6).

Benefits of my visit

In addition to learning a new technique, Professor Dubrovska introduced me to her colleagues, Professor Leoni Kunz-Schughart and Dr Annette Linge. Through this international scientific network (collaboration), I am looking forward to application for international grant(s).

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With Professor Dubrovska's team during my visit. I (front row, centre) wish to thank Professor Dubrovska (back row, centre) and her group for their welcome and a wonderful, friendly working atmosphere that made me feel very comfortable in their lab and helped to make my research visit successful.