ESRF	**Experiment title:** CYTOTOXICITY OF NEW HEAVY METAL-BASED ANTICANCER DRUGS PLUS SYNCHROTRON RADIATION IN HUMAN CANCER CELL LINES.	**Experiment number**: MD728
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This study was aimed to investigate whether synchrotron radiation (SR) can enhance heavy metal-based anticancer complexes effects in two different human cancer cell lines of non-glial origin (A549 non small-cell lung cancer and IGROV-1 ovarian cancer cells). Cisplatin (CDDP), a widely clinically used chemotherapeutic drug, was used as reference drug.

Material and Methods

A549 and IGROV-1 cells were cultured in complete RPMI medium supplemented with 10% Foetal Bovine Serum (FBS), 2mM L-glutamine, 100 U/ml penicillin, 100 μg/ml streptomycin. For clonogenic assay and nuclear protein extract cells were plated into T25 flasks while for immunofluorescence experiments 6 well plates were used. The day after cells were treated with CDDP, $[Cu(PTA)_4PF_6]$, $[Cu(thp)_4][PF_6]$ or $[Au(thp)_4][PF_6]$ for 24 hours with drug concentrations allowing roughly 90% of cell survival. After treatment drug containing medium was removed and fresh medium was added. Irradiation was then performed at 30 keV (copper based compounds) or 80 keV (gold based compound). CDDP treated cells were irradiated either above (78.8 KeV) and below (78.0 KeV) the Pt K absorption edge. Cell were irradiated in the 0-6 Gy dose-range.

Cells were treated with the following schedule:

1. Untreated control
2. Drug
3. Drug+SR irradiation dose 1Gy
4. Drug+SR irradiation dose 2Gy
5. Drug+SR irradiation dose 4Gy
6. Drug+SR irradiation dose 6Gy
7. SR irradiation dose 1 Gy
8. SR irradiation dose 2 Gy
9. SR irradiation dose 4 Gy
10. SR irradiation dose 6 Gy

Standard clonogenic assay and nuclear protein extract were performed as previously described [1].

In order to assess the effects of the proposed treatment at the molecular level, different parameters linked to HR repair pathways were evaluated:

- BRCA1 nuclear expression evaluated by western blot analysis using an antibody that specifically recognizes phospho BRCA1.
- RAD51-mediated homologous recombination process: cellular localization of RAD51 protein evaluated by immunofluorescence with an anti-RAD51 antibody.

Untreated cells and cells treated with the drugs or the SR alone will be used as control.

Results

Clonogenic assay experiment demonstrated that exposure to SR significantly enhances CDDP activity in both A549 and IGROV-1 cells in the 1-6 Gy dose range at both 78.8 keV (above Pt K-edge) and 78.0 KeV (below Pt K-edge (Fig.1). Similarly pre-treatment with $[Cu(PTA)_4PF_6]$ followed by SR at 30 KeV induced an enhancement in cellular death with respect to drug and irradiation alone (Fig.1) only in IGROV-1 cells. No cell death enhancement was observed with the other drug tested (data not shown).

[1] Bencokova et al., 2008. J Neurooncol, 86: 13-21.

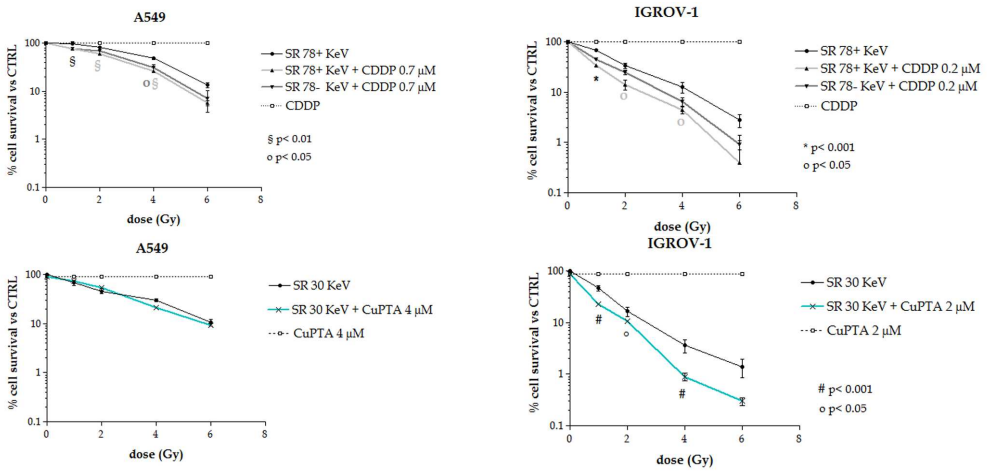

Fig.1. Effect of SR in combination with CDDP or [Cu(PTA)4PF6] evaluated by clonogenic assay. Cells were treated for 24 hours with the drugs (A549: [Cu(PTA)4PF6] 4 µM, CDDP 0.7 µM; IGROV-1: [Cu(PTA)4PF6] 2 µM, CDDP 0.2 µM). At the end of treatment cells were irradiated at 78.8 and 78.8 KeV (above and below Pt K-edge) or 30 KeV (above Cu K-edge) with a total dose of 1, 2, 4 or 6 Gy. Irradiation with a total dose of 1-6 Gy significantly increased cell death with respect to drug or irradiation alone with the exeption of A549 pre-treated with the copper-based compound.

Western blot analysis and immunofluorescence experiments demonstrated that SR in combination with CDDP results in a significative nuclear relocalization of BRCA1 (Fig.2) and RAD51 (Fig.3) proteins with respect to untreated control cells as well as in cells treated with SR or CDDP alone. Similar results were observed in cells pre-treated with $[Cu(PTA)_4PF_6]$. For technical reason no BRCA1 nuclear expression data are available for A549 cells pre-treated with the copper-based compound (Fig.2).

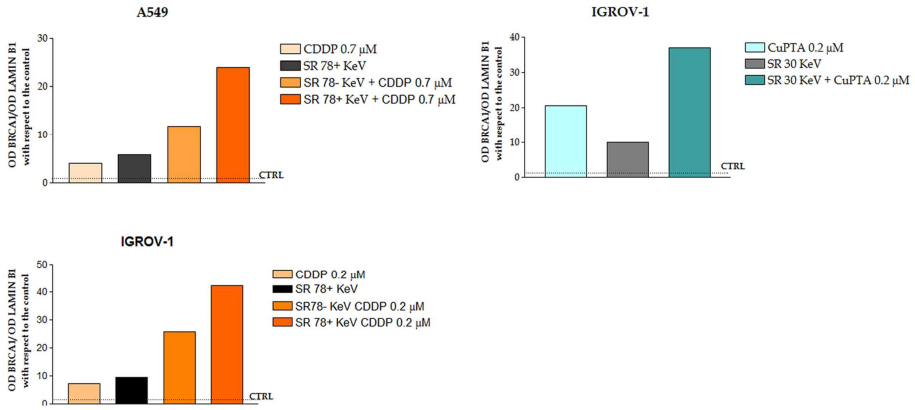

Fig.2. Western blot analysis of BRCA1 nuclear expression. Cells were treated for 24 hours with the drugs (A549: CDDP 0.7 µM; IGROV-1: $[Cu(PTA)_4PF_6]$ 0.4 µM, CDDP 0.2 µM). At the end of treatment cells were irradiated at 78.8 and 78.8 KeV (above and below Pt K-edge) or 30 KeV (above Cu K-edge) with a total dose of 2 Gy. Combinational treatment significantly increased BRCA1 relocalization with respect to drug or SR alone.

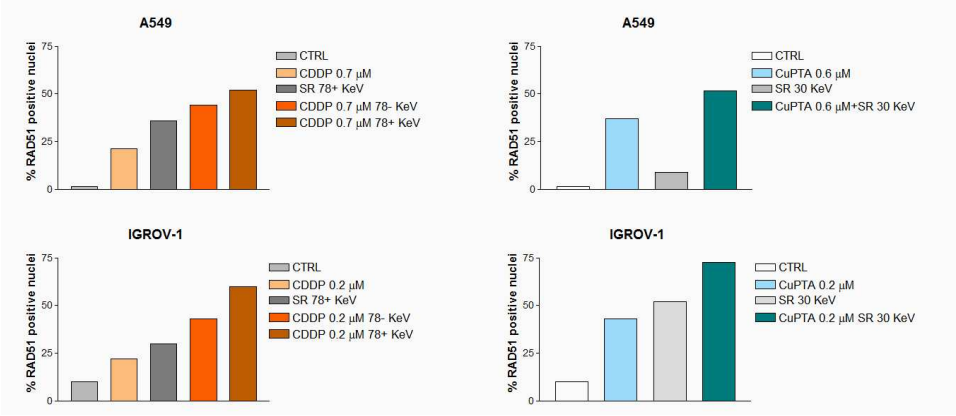

Fig.3. Count of RAD51 positive nuclei. Cells were treated for 24 hours with the drugs (A549: CDDP 0.7 µM, $[Cu(PTA)_4PF_6]$ 0.6 µM; IGROV-1: CDDP 0.2 µM, $[Cu(PTA)_4PF_6]$ 0.4 µM). At the end of treatment cells were irradiated at 78.8 and 78.8 KeV (above and below Pt K-edge) or 30 KeV (above Cu K-edge) with a total dose of 2 Gy. Combinational treatment significantly increased the percentage of cells with RAD51 positive nuclei. RAD51 localization was detected by immunofluorescence experiments.