# PARP inhibitors Talazoparib and Niraparib sensitize melanoma cells to ionizing radiation

Stephanie Jonuscheit[1]*, Tina Jost[1]*, Fritzi Gajdošová[1], Maximilian Wrobel[1], Markus Hecht[1], Rainer Fietkau[1], Luitpold Distel[1]

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*These authors contributed equally to this work.

## Supplementary Material

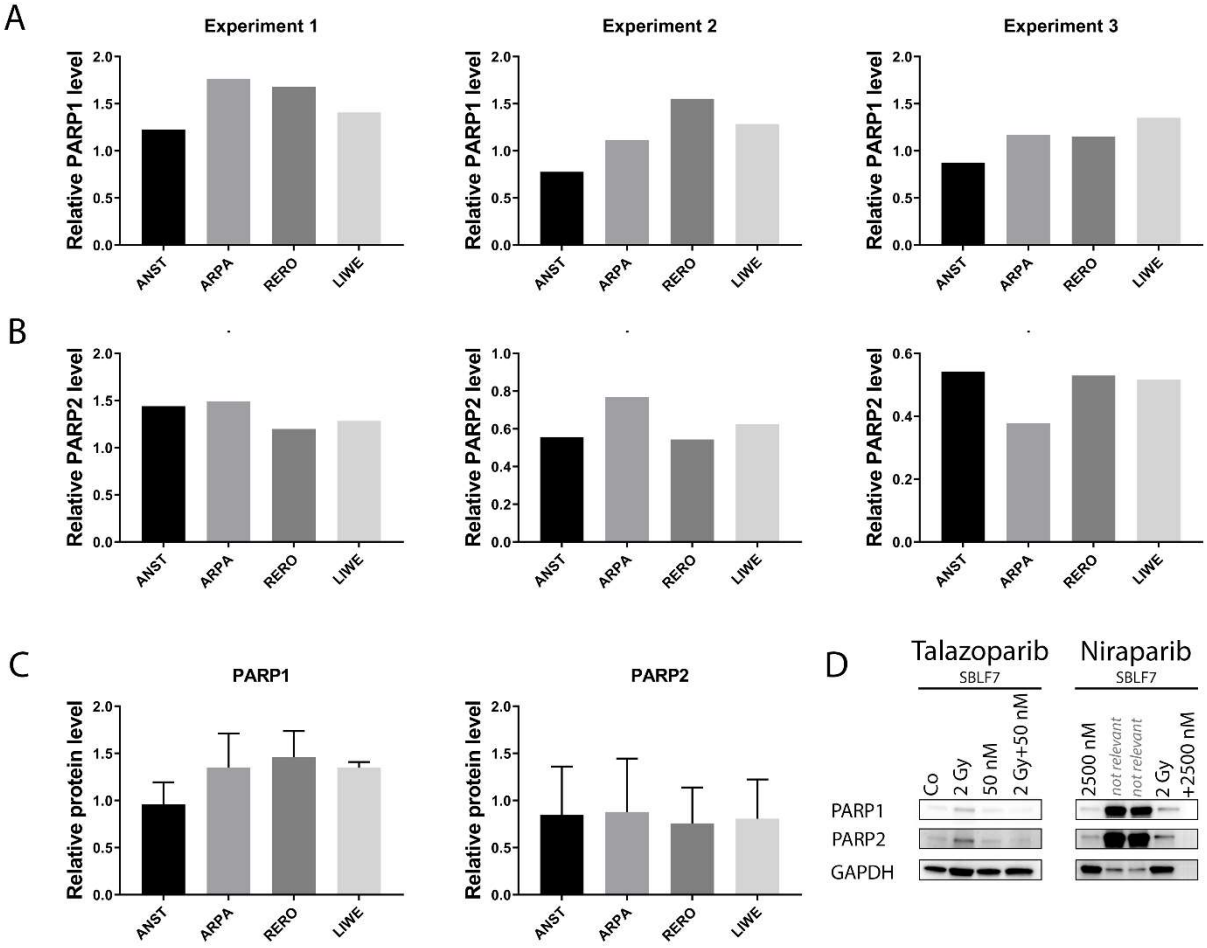


**Figure S1**: Western blot analysis of three individual experiments of PARP1 and PARP2 expression in four skin cancer cell lines. A) Relative expression level of PARP1 in untreated ANST, ARPA, RERO and LIWE cells in three independent experiments. B) Relative expression level of PARP2 in untreated ANST, ARPA, RERO and LIWE cells in three independent experiments. C) Relative protein level of PARP1 and PARP2 in four skin cancer cell lines. Values represent three independent experiments (mean ± SD). Statistical significance was calculated by Mann-Whitney-U test and $p \leq 0.05$ (*) was set as significant. D) PARP1 and PARP2 expression in healthy fibroblasts SBLF7 in an untreated control, after 2 Gy IR, monotherapy of Talazoparib (50 nM), Niraparib (2500 nM) and combination therapy of KI + IR.

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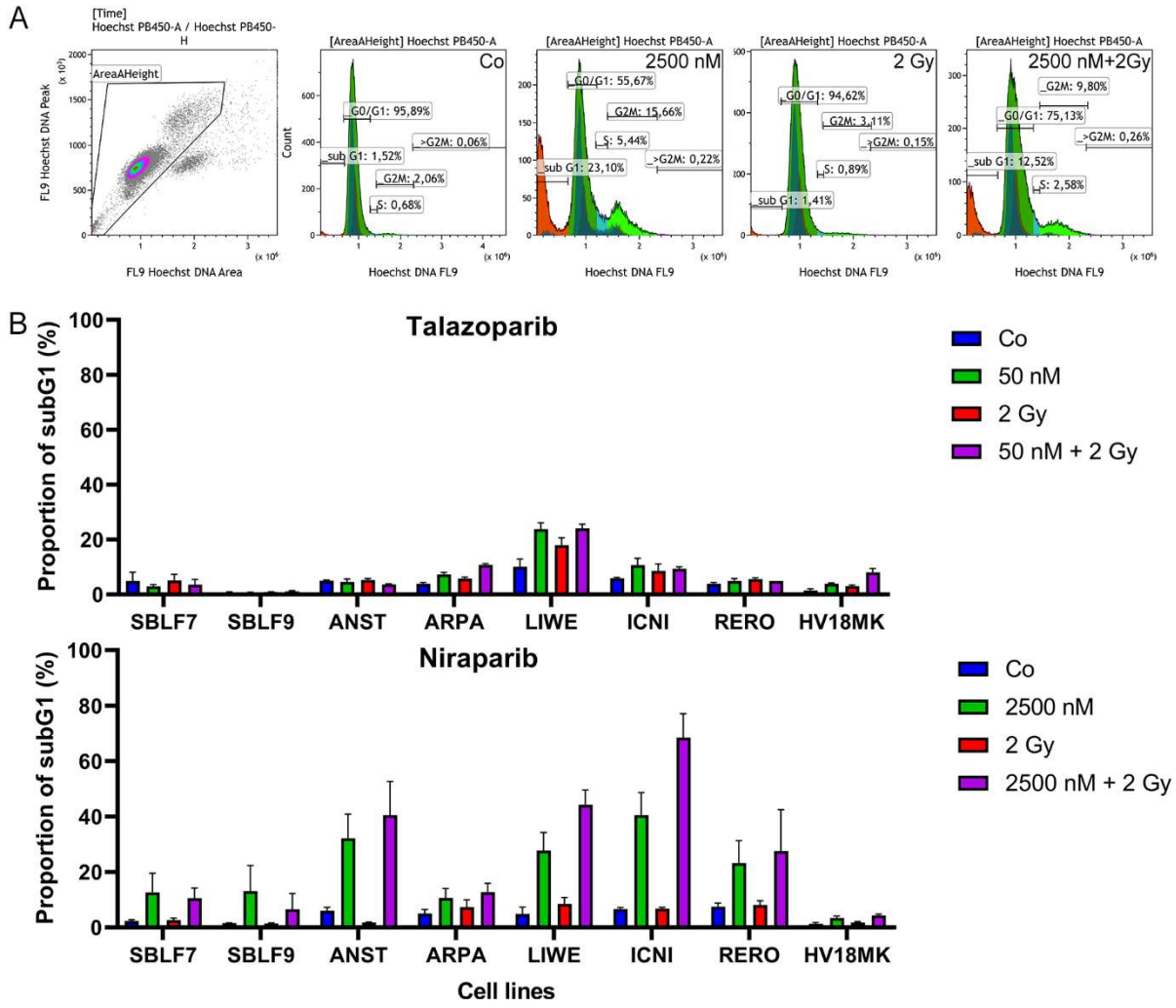


**Figure S2**: Proportion of six skin cancer cell lines in subG1 phase (ANST, ARPA, LIWE, ICNI, RERO and HV18MK) and of two healthy donor fibroblasts (SBLF7, SBLF9). A) Gating strategy to exclude cell duplets. Representative histograms of Hoechst-staining of SBLF9 fibroblasts under niraparib, irradiation or combination therapy. Distribution of cell cycles phases and gating of subG1 phase as indicator for apoptotic bodies (apoptosis). B) Proportion of six skin cancer cell lines and healthy fibroblasts in subG1 phase as indicator for apoptosis. Cells were treated with Talazoparib (50 nM), Niraparib (2500 nM), irradiation (2 Gy) or a combination therapy for 48 h. Values represent mean ± SD (n = 3).

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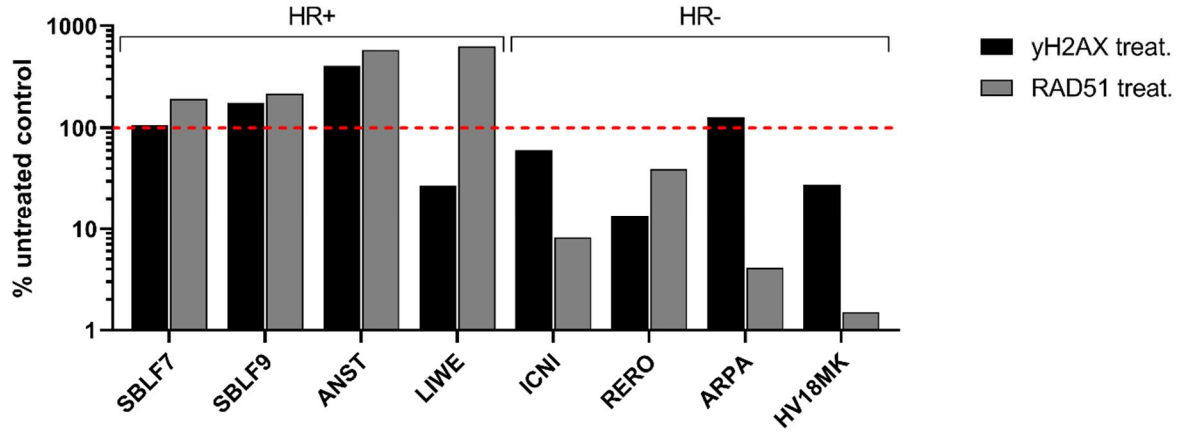


**Figure S3**: Efficiency of homologous recombination, a double strand break repair pathway, in the skin cancer panel and both healthy controls. Analysis of γH2AX and Rad51 foci after a dose of 10 Gy IR and treatment with the DNA-PK inhibitor CC-115, an inhibitor of the non-homologous end joining pathway. Compared to the untreated control, Rad51 foci increased upon IR in SBLF7, SBLF9, ANST and LIWE. Therefore, these four cell lines are considered as HR-proficient. Rad51 foci decreased in skin cancer cell lines ICNI, RERO, ARPA and HV18MK which indicates a HR-deficiency.

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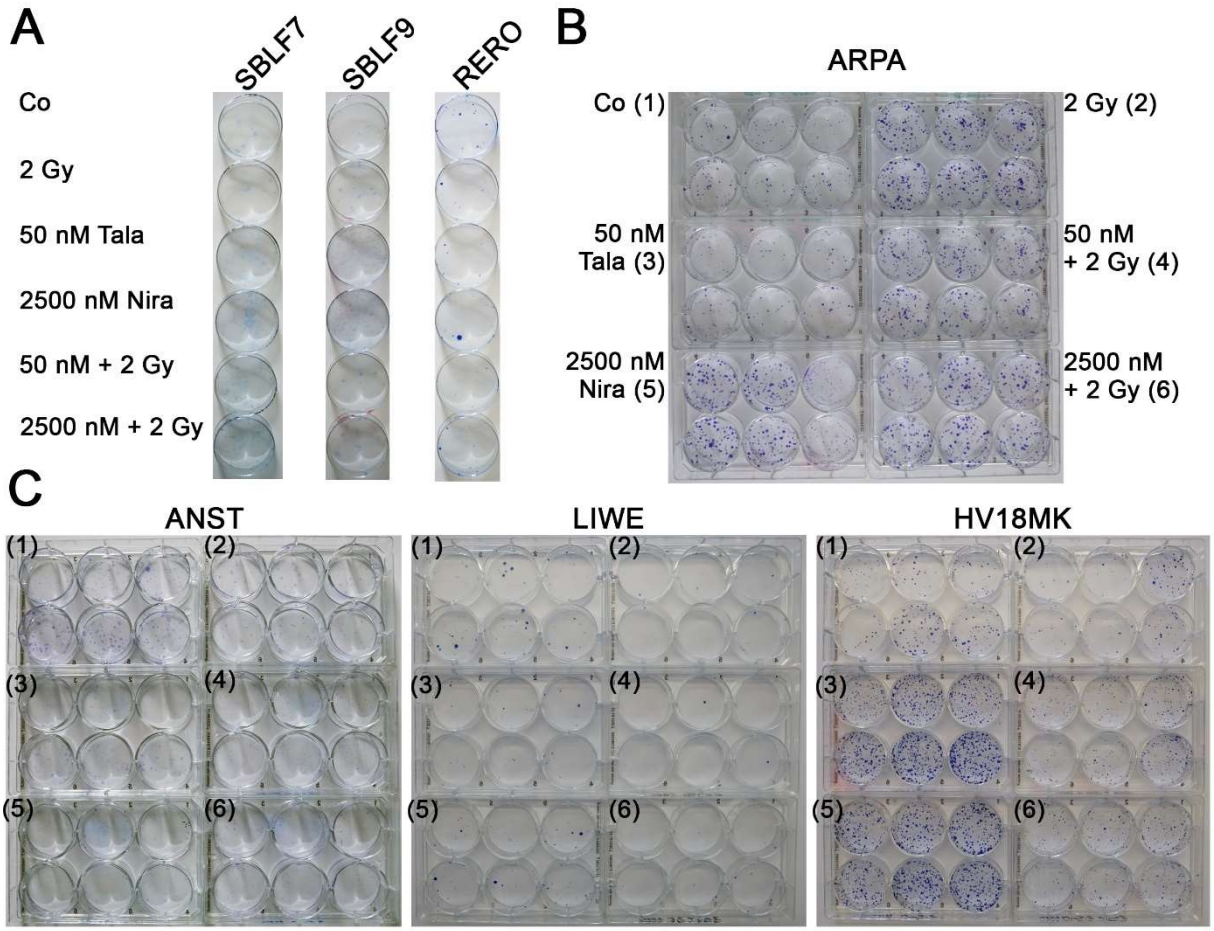


**Figure S4:** Representative images of colony forming assays of two healthy fibroblasts and four skin cancer cell lines. A) SBLF7, SLBF9 and RERO seeded in petri-dishes. Without treatment (Co), with irradiation (2 Gy), with kinase inhibitor Talazoparib (50 nM) and Niraparib (2500 nM) and a combination of both Tala+IR and Nira+IR. B) Skin cancer cells ARPA seeded in 6-well-plates. Without treatment (Co), with irradiation (2 Gy), with kinase inhibitor Talazoparib (50 nM) and Niraparib (2500 nM) and a combination of both Tala+IR and Nira+IR. C) Skin cancer cells ANST, LIWE and HV18MK seeded in 6-well-plates and treated according to cell line ARPA (1: Co, 2: 2 Gy IR, 3: 50 nM Talazoparib, 4: 50 nM Talazoparib + 2 Gy IR, 5: 2500 nM Niraparib, 6: 2500 nM Niraparib + 2 Gy IR. Images B) and C) represent 6 technical replicates.

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# Analysis of the flow cytometry experiments

## Cell death experiments

Table S1: Cell death in %.

| | Control | IR | Talazoparib | Talazoparib + IR | Niraparib | Niraparib + IR |
|---|---|---|---|---|---|---|
| SBLF7 | 1.2 | 0.9 | 1.3 | 1.6 | 7.0 | 3.8 |
| SBLF9 | 1.2 | 1.3 | 1.0 | 1.2 | 22.3 | 11.8 |
| ANST | 1.7 | 2.3 | 2.3 | 2.3 | 35.0 | 22.8 |
| ARPA | 1.1 | 1.7 | 3.1 | 4.4 | 5.4 | 8.1 |
| RERO | 1.7 | 1.9 | 1.8 | 1.2 | 11.0 | 18.0 |
| LIWE | 3.0 | 7.6 | 16.1 | 20.8 | 27.6 | 47.8 |
| ICNI | 3.1 | 4.4 | 5.4 | 4.8 | 26.5 | 68.4 |
| HV18MK | 1.1 | 1.6 | 4.3 | 5.6 | 3.9 | 4.0 |

Table S2: Cell death: significant change to Control in %.

| | IR | Talazoparib | Talazoparib + IR | Niraparib | Niraparib + IR |
|---|---|---|---|---|---|
| SBLF7 | - | - | - | 5.8<br>p = 0.0357 | 2.6<br>p = 0.0357 |
| SBLF9 | - | - | - | 21.1<br>p = 0.0238 | 10.6<br>p = 0.0476 |
| ANST | - | - | - | 33.3<br>p = 0.0238 | 21.1<br>p = 0.0238 |
| ARPA | 0.6<br>p = 0.0043 | 2.0<br>p = 0.0238 | 3.3<br>p = 0.0238 | 4.3<br>p = 0.0238 | 7.0<br>p = 0.0238 |
| RERO | - | - | -0.5<br>p = 0.0357 | 9.3<br>p = 0.0238 | 16.3<br>p = 0.0238 |
| LIWE | 4.6<br>p = 0.0022 | 13.1<br>p = 0.0238 | 17.8<br>p = 0.0238 | 24.6<br>p = 0.0238 | 44.8<br>p = 0.0238 |
| ICNI | 1.3<br>p = 0.0087 | 2.3<br>p = 0.0476 | 1.7<br>p = 0.0476 | 23.4<br>p = 0.0238 | 65.3<br>p = 0.0238 |
| HV18MK | - | 3.2%<br>p = 0.0238 | 4.5%<br>p = 0.0238 | 2.8%<br>p = 0.0238 | 2.9%<br>p = 0.0238 |

Table S3: Cell death: significant change to IR in %.

| | Talazoparib + IR | | Niraparib + IR | |
|---|---|---|---|---|
| SBLF7 | - | | 2.9 | p = 0.0238 |
| SBLF9 | - | | 10.5 | p = 0.0476 |
| ANST | - | | 20.5 | p = 0.0238 |
| ARPA | 2.7 | p = 0.0238 | 6.4 | p = 0.0238 |
| RERO | -0.7 | p = 0.0238 | 16.1 | p = 0.0238 |
| LIWE | 13.2 | p = 0.0238 | 40.2 | p = 0.0238 |
| ICNI | - | | 64.0 | p = 0.0238 |
| HV18MK | 4.0 | p = 0.0238 | 2.4 | p = 0.0238 |

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# Cell Cycle experiments

Table S4: Cell fraction in G2/M phase in %.

| | Control | IR | Talazoparib | Talazoparib + IR | Niraparib | Niraparib + IR |
|---|---|---|---|---|---|---|
| SBLF7 | 3.0 | 7.5 | 4.0 | 4.5 | 14.4 | 11.8 |
| SBLF9 | 3.4 | 8.4 | 8.3 | 17.0 | 16.3 | 6.9 |
| ANST | 17.0 | 21.1 | 28.3 | 27.8 | 30.3 | 28.6 |
| ARPA | 12.4 | 13.6 | 15.4 | 25.5 | 16.8 | 22.0 |
| RERO | 20.2 | 24.5 | 48.0 | 52.6 | 43.6 | 48.1 |
| LIWE | 12.3 | 12.4 | 34.0 | 31.7 | 37.9 | 39.2 |
| ICNI | 23.2 | 27.8 | 46.1 | 61.4 | 44.3 | 43.6 |
| HV18MK | 17.4 | 18.7 | 33.6 | 44.0 | 22.9 | 31.4 |

Table S5: Cell fraction in G2/M phase: significant change to Control in %.

| | IR | Talazoparib | Talazoparib + IR | Niraparib | Niraparib + IR |
|---|---|---|---|---|---|
| SBLF7 | 4.5<br>p = 0.0455 | - | 1.5<br>p = 0.0238 | 11.4<br>p = 0.0238 | 8.8<br>p = 0.0238 |
| SBLF9 | - | 4.9<br>p = 0.0238 | 13.6<br>p = 0.0238 | 12.9<br>p = 0.0238 | - |
| ANST | 4.1<br>p = 0.0022 | 11.3<br>p = 0.0238 | 10.8<br>p = 0.0238 | 13.3<br>p = 0.0238 | 11.6<br>p = 0.0238 |
| ARPA | - | 3.0<br>p = 0.0242 | 13.1<br>p = 0.0121 | 4.4<br>p = 0.0295 | 7.6<br>p = 0.0016 |
| RERO | 4.3<br>p = 0.0022 | 27.8<br>p = 0.0238 | 32.4<br>p = 0.0238 | 23.4<br>p = 0.0238 | 27.9<br>p = 0.0238 |
| LIWE | - | 21.7<br>p = 0.0121 | 19.4<br>p = 0.0121 | 25.6<br>p = 0.0016 | 26.9<br>p = 0.0016 |
| ICNI | 4.6<br>p = 0.0043 | 22.9<br>p = 0.0238 | 38.2<br>p = 0.0238 | 21.1<br>p = 0.0238 | 20.4<br>p = 0.0238 |
| HV18MK | - | 16.2<br>p = 0.0238 | 26.6<br>p = 0.0238 | 5.5<br>p = 0.0238 | 14.0<br>p = 0.0238 |

Table S6: Cell fraction in G2/M phase: significant change to IR in %.

| | Talazoparib + IR | | Niraparib + IR | |
|---|---|---|---|---|
| SBLF7 | - | | - | |
| SBLF9 | 8.6 | p = 0.0238 | - | |
| ANST | 6.7 | p = 0.0238 | 7.5 | p = 0.0238 |
| ARPA | 11.9 | p = 0.0121 | 8.4 | p = 0.0016 |
| RERO | 28.1 | p = 0.0238 | 23.6 | p = 0.0238 |
| LIWE | 19.3 | p =0.0121 | 26.8 | p = 0.0016 |
| ICNI | 33.6 | p = 0.0357 | 15.8 | p = 0.0357 |
| HV18MK | 25.3 | p = 0.0238 | 12.7 | p = 0.0238 |