**SUPPLEMENTARY DATA**

Supplementary Table 1: List of Antibodies used for Western and IHC

Supplementary Table 2: Plating efficiency (clonogenic assay)

Supplementary Table 3: Statistical analysis of tumor growth rates in xenograft experiments (linear mixed effects model with animal-specific random effects)

Supplementary Figure 1: CLR127 effect on cell viability (MTT Assay)

Supplementary Figure 2: Effect of CLR127 (CLR) and radiation on DNA damage repair by western blot

Supplementary Figure 3: Tumor growth rates of flank xenografts after treatment with radiation, CLR127 or combination therapy

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Specificity** | **Source** | **Company** | **Catalog no.** | **Dilutions** |
| Phospho-BRCA1 (Ser1524) | Rabbit polyclonal | Cell Signaling Technology | 9009 | 1:1,000 |
| γ-H2AX | Mouse monoclonal | Millipore | 05-636 | 1:1,000 |
| XLF | Rabbit polyclonal | Cell Signaling Technology | 2854 | 1:1,000 |
| Vinculin | Rabbit monoclonal | Cell Signaling Technology | 13901 | 1:2,000 |
| β-Actin | Rabbit monoclonal | Cell Signaling Technology | 4970 | 1:2,000 |

**Supplementary Table 1.**

Table depicts the detailed information on the antibodies used in this study.

|  |  |  |  |
| --- | --- | --- | --- |
| CLR127 ConcentrationCell Line | 5 µM | 7.5 µM | 10 µM |
| Rh30 | 85.2% (1.6) | 79.8% (4.7) | 62.4% (4.4) |
| PC-3 | 92.6% (4.9) | 59.3% (1.5) | 46.6% (4.6) |
| CHLA-20 | 88.3% (7.6) | 65.1% (5.9) | 61.6% (10) |

**Supplementary Table 2:** In vitro cytotoxicity of CLR127 relative to plating efficiency (PE). Numbers represent average percentage PE of treated cells relative to PE of control (excipient) of 3 independent experiments, where PE of control cells is set to 100%. Standard Deviation shown in parentheses.

|  |  |  |
| --- | --- | --- |
| Xenograft | Comparison | p-value |
| SK-N-AS | Excipient/ -XRT vs. Excipient / +XRT | <0.0001 |
| Excipient/ -XRT vs. Drug / -XRT | 0.7173 |
| Excipient/ -XRT vs. Drug / +XRT | <0.0001 |
| Excipient / +XRT vs. Drug / -XRT | <0.0001 |
| ***Excipient / +XRT vs. Drug / +XRT*** | <0.0001 |
| Drug / -XRT vs. Drug / +XRT | <0.0001 |
| Rh30 | Excipient/ -XRT vs. Excipient / +XRT | <0.0001 |
| Excipient/ -XRT vs. Drug / -XRT | 0.0908 |
| Excipient/ -XRT vs. Drug / +XRT | <0.0001 |
| Excipient / +XRT vs. Drug / -XRT | <0.0001 |
| ***Excipient / +XRT vs. Drug / +XRT*** | *<0.0001* |
| Drug / -XRT vs. Drug / +XRT | <0.0001 |
| TC-71 | Excipient/ -XRT vs. Excipient / +XRT | <0.0001 |
| Excipient/ -XRT vs. Drug / -XRT | 0.0350 |
| Excipient/ -XRT vs. Drug / +XRT | <0.0001 |
| Excipient / +XRT vs. Drug / -XRT | <0.0001 |
| ***Excipient / +XRT vs. Drug / +XRT*** | <0.0001 |
| Drug / -XRT vs. Drug / +XRT | <0.0001 |
| PC-3 | Excipient/ -XRT vs. Excipient / +XRT | <0.0001 |
| Excipient/ -XRT vs. Drug / -XRT | <0.0001 |
| Excipient/ -XRT vs. Drug / +XRT | <0.0001 |
| Excipient / +XRT vs. Drug / -XRT | <0.0001 |
| ***Excipient / +XRT vs. Drug / +XRT*** | <0.0001 |
| Drug / -XRT vs. Drug / +XRT | <0.0001 |

**Supplementary Table 3:**

Statistical significances of the linear mixed effects model with animal-specific random effects that was used to evaluate and compare log tumor growth rates between the four treatment groups. Drug, CLR127.



**Supplementary Figure 1.**

CLR127 effect on cell viability. Cell survival of CLR127-treated cells was examined by MTT assay. 2x10**6** cells/ well were seeded in 96-well plates and treated with 5, 7.5, 10, 15 or 30 μM CLR127 for 20 hours. Excipient was present at the same concentration in all samples. 0.8% formaldehyde was used as positive control of cell death. Percent viability was calculated as live cells equivalents determined from standard curves, and calculated as percentage from 100% cell growth of the wells with excipient alone. Averages ± SE of 3 separate experiments are shown. \*P < 0.05 and \*\*P ≤ 0.005 of CLR127-treated versus excipient-only cells.



**Supplementary Figure 2:** Effect of CLR127 (CLR) and radiation on DNA damage repair in vitro assessed by Western blot and shown as relative expression of γH2AX. Cells were treated with 7.5 μM CLR 127 or excipient for 16 hours, and exposed to 5 Gy radiation. Cells were collected immediately prior to radiation (0) and at 1, 6 and 24 hours (h) after radiation and subjected to Western blotting. γH2AX bands were quantified relative to β-actin loading control using ImageJ software. Left row: representative western blots. Right row: Bar graph showing mean relative γH2AX expression of three independent experiments, small bars depicting standard error. Statistical differences were calculated with Student’s two-tailed t-test (\*p≤0.05; \*\*p≤0.01).



**Supplementary Figure 3:**

Tumor growth rates were analyzed and compared between the four experimental groups by a linear mixed effects model, with the group as the fixed effect and the animal as random effect and using log-transformed tumor volume as the outcome measure. The tumor growth rates of mice that received XRT plus CLR127 were significantly reduced when compared to mice treated with radiotherapy alone. See supplementary table 3 for detailed statistical analysis.