Targeted degradation of DNA Ligase IV through a double-stranded DNA-based PROTAC for precision radiosensitization

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Abstract #238

Background & Rationale

DNA Ligase IV (LIG4), the terminal ligase of the non-homologous end-joining (NHEJ) pathway, represents a highly potent yet largely undruggable target for radiosensitization.

- We developed a first-in-class double-stranded DNA-based **PROTAC** (**NHEJ-P**) that couples a 32-bp DNA "bait" to a cereblon E3 ligase ligand to induce selective proteasomal degradation of LIG4
- NHEJ-P achieves near-complete LIG4 loss at **~10 nM**, validated by Western blot and rescued by MG132, confirming proteasome dependence
- Induces sustained γH2AX and 53BP1 foci without affecting RAD51, disrupts end-joining kinetics, and enhances radiation-induced cytotoxicity
- Selectively **sensitizes cancer cells** (U2OS, HeLa) while **sparing normal cells** (RPE1, HEK293T), indicating a favorable therapeutic index
- Suggests genomically unstable cancer cells are uniquely dependent on LIG4 under replication stress

Building on this, we are developing a HER2-targeted antibody–oligonucleotide conjugate (AOC) platform (t-NHEJ-P) for tumor-restricted delivery.

Design of a LIG4-targeting PROTAC (NHEJ-P) and HER2-directed AOC Platform

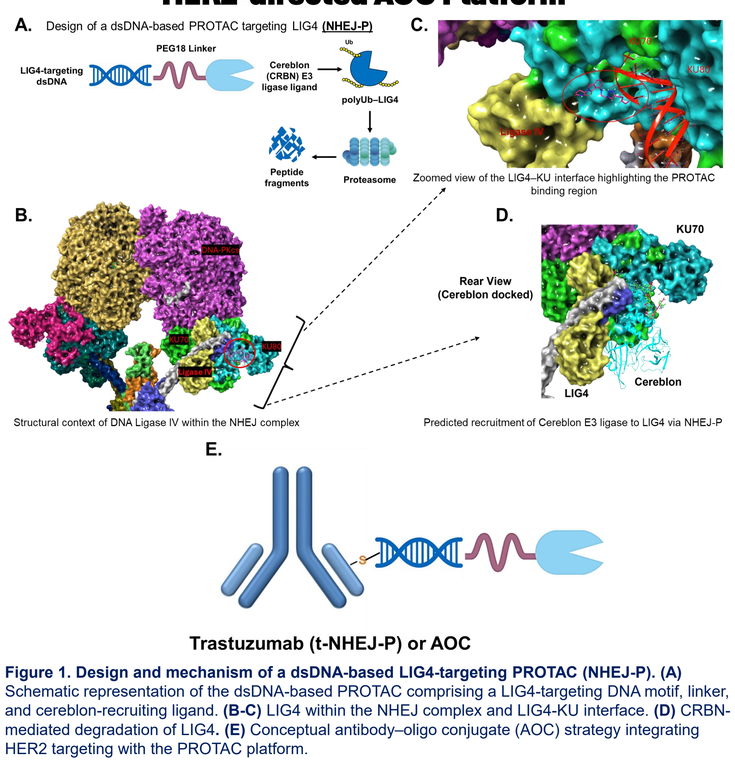

Figure 1. Design and mechanism of a dsDNA-based LIG4-targeting PROTAC (NHEJ-P). (A) Schematic representation of the dsDNA-based PROTAC comprising a LIG4-targeting DNA motif, linker, and cereblon-recruiting ligand. **(B-C)** LIG4 within the NHEJ complex and LIG4-KU interface. **(D)** CRBN-mediated degradation of LIG4. **(E)** Conceptual antibody–oligo conjugate (AOC) strategy integrating HER2 targeting with the PROTAC platform.

Selective degradation of LIG4 by NHEJ-P induces cytotoxicity in cancer cells while sparing normal cells

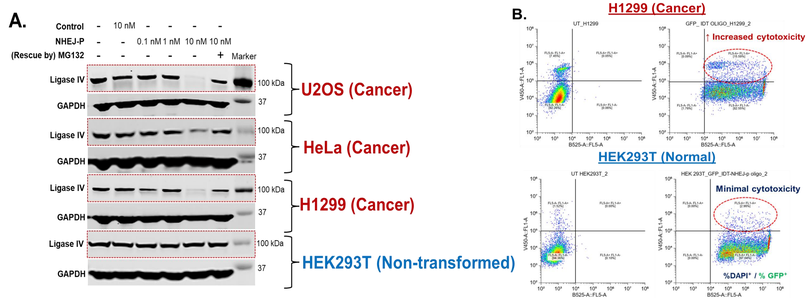

Figure 2. Selective LIG4 degradation by NHEJ-P induces cancer cell cytotoxicity. (A) Immunoblot shows LIG4 reduction by NHEJ-P in cancer cells versus HEK293T. **(B)** Flow cytometry shows increased cytotoxicity in H1299 with minimal effects in HEK293T in presence of NHEJ-P.

NHEJ-P suppresses NHEJ repair activity

NHEJ-P reduces NHEJ activity (~50%)

Alt-EJ does not compensate for NHEJ inhibition

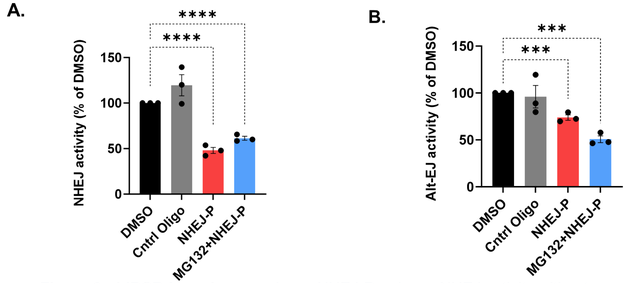

Figure 3. ddPCR-based assay shows NHEJ-P reduces NHEJ activity without compensatory Alt-EJ activation.

NHEJ-P induces DNA damage without activating HR

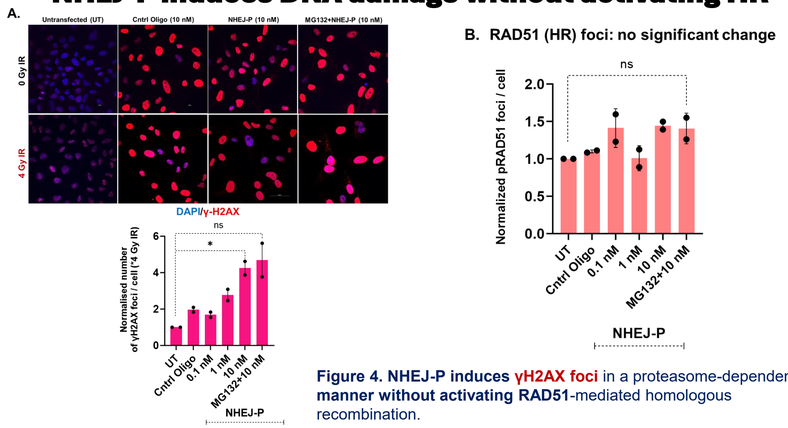

Figure 4. NHEJ-P induces γH2AX foci in a proteasome-dependent **manner without activating RAD51**-mediated homologous recombination.

NHEJ-P enhances radiosensitization and enables HER2-targeted delivery

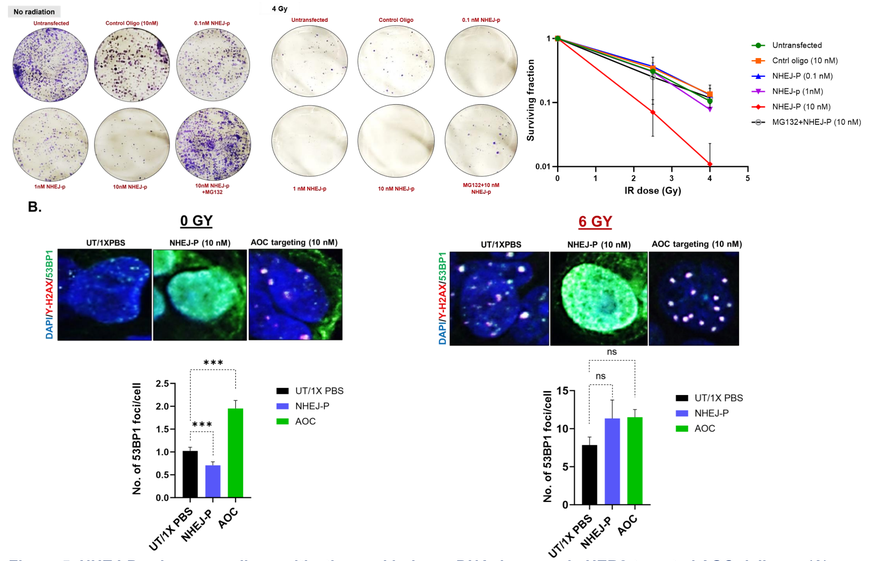

Figure 5. NHEJ-P enhances radiosensitization and induces DNA damage via HER2-targeted AOC delivery. (A) Clonogenic survival assay showing enhanced radiosensitization upon NHEJ-P treatment. **(B)** Representative immunofluorescence images and quantification of **γH2AX**/**53BP1** foci under 0 Gy and 6 Gy irradiation in NCI-N87 HER2+ cells.

Conclusions

- **NHEJ-P induces potent, proteasome-dependent degradation of LIG4**, effectively suppressing NHEJ repair.
- **Selectively enhances radiation-induced DNA damage** without activating homologous recombination (HR).
- **Promotes cancer cell-selective radiosensitization** and can be delivered via a HER2-targeted AOC platform

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