The ADC payload PNU-159682 is highly active in a wide range of cancer indications by inducing DNA damage and cell death via a distinct mode of action

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Monika Kaiser[1], Ulrike Fiedler[1], Alexandra Popa[2], Daniel Gerlach[2], Norbert Kraut[2] & Lukas Bammert[1]

[1] NBE-Therapeutics AG, Basel, Switzerland; [2] Boehringer Ingelheim RCV GmbH & Co KG, Vienna, Austria

Background

During the last decade antibody-drug-conjugates (ADCs) have become an important and validated treatment modality for cancer patients. Here, we introduce a unique next-generation ADC platform based on Sortase-mediated antibody conjugation (SMAC-Technology™), yielding in very homogenous and stable drug conjugates with limited systemic, but powerful anti-tumor activity. The highly potent payload used is PNU-EDA, a proprietary derivative of the anthracycline PNU-159682. Our PNU-EDA-based ADCs are known to not only induce DNA damage in the target cell, but importantly also trigger immunogenic cell death and thus, stimulating anti-tumor immunity offering untapped combination potential (D'Amico L et al, 2019). ADCs based on the SMAC-Technology™ platform currently undergo clinical development.

In this work, we present functional studies that were performed to further elucidate the mode of action (MoA) of the ADC payload PNU-EDA, and to investigate any potential liabilities with respect to sensitivity, which could limit treatment options for cancer patients. Overall, our screens and mechanistic studies verified that PNU-EDA-based ADCs efficiently kill cancer cells via a particular MoA and thereby, offer highly promising therapeutic options to a diverse and large population of cancer patients.

Induction of DNA damage and S-phase arrest

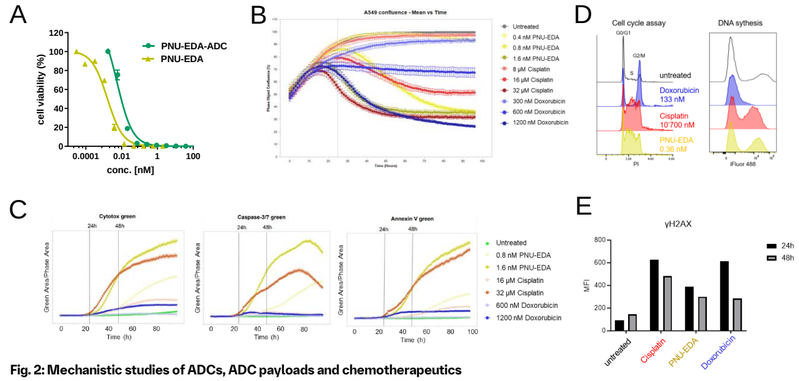

Fig. 2: Mechanistic studies of ADCs, ADC payloads and chemotherapeutics

(A) Induction of cell death in PA1 cells by PNU-EDA and a PNU-EDA-ADC. (B-E) Effect of drugs on A549 cells at drug concentrations as indicated in the figures. (B) Viability over time measured by IncuCyte. (C) Monitoring of apoptosis induction and cell death. (D) Cell cycle and DNA synthesis analysis. (E) Quantification of DNA damage monitored by γH2AX positivity at 1.6 nM PNU-EDA, 1200 nM Doxorubicin and 32 µM Cisplatin.

PNU-EDA's activity is affected by MDR1 and TC-NER*

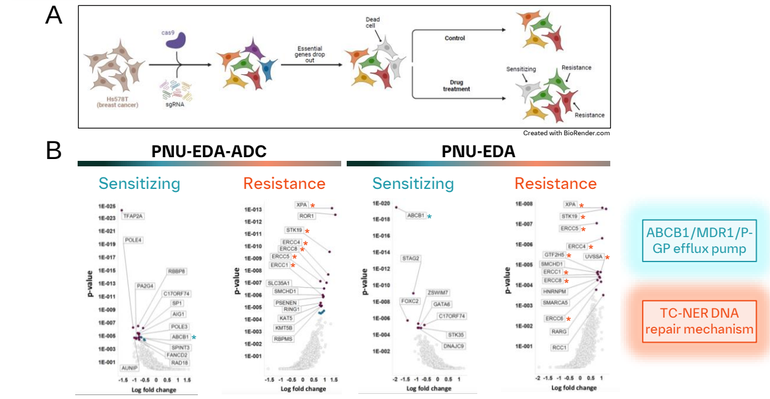

Fig. 4: CRISPRko screen comparing the PNU-EDA-ADC and PNU-EDA

(A) Cartoon outlining the CRISPRko screening strategy. (B) Significant sensitizing(*) and resistance (*) gene hits identified for the PNU-EDA-ADC and PNU-EDA.

* Transcription-Coupled-Nucleotide-Exicion-Repair

Conjugation of PNU-EDA by SMAC-Technology™

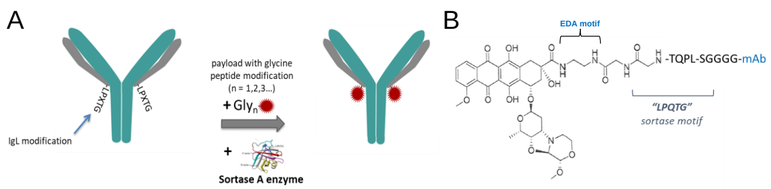

Fig. 1: Sortase-mediated PNU-EDA conjugation

(A) Cartoon describing Sortase-mediated payload conjugation to an antibodies light chain covalently connecting the C-terminal LPXTG tag with the Glycine tag on the payload. (B) Structure of the conjugated PNU-EDA linker payload.

Methods

Cell cycle, proliferation and cell damage were analyzed using standard assays. Briefly; cells were cultured according to manufactures recommendation. Cell viability was measured using CellTiterGlo (Promega) or monitored by measuring cell confluency, viability and cell death by IncuCyte over 4 days using Cytotox Green, Sartorius, 4633, 1:4'000 dilution; Caspase-3/7 Green, Sartorius, 4440, 1:1'000 dilution; Annexin V Green, Sartorius, 4642, 1:200 dilution. DNA damage was quantified after 24 and 48 hours using a Flow-based assay by staining permeabilized cells for γH2AX using a AF488 labeled antibody (BD Bioscience, 560445). Cell cycle was analyzed 24 hours post incubation with toxins by Flow. Cells were stained by the CycleTEST Plus DNA Reagent Kit (BD Bioscience, 340242) or EdU Proliferation Kit (Abcam, ab219801).

PNU-159682 and PNU-EDA compounds were screened in the PRISM assay using an eight-point dose (3-fold dilution) approach with a five-day treatment. More than 880 cell lines have passed the quality control.

PNU-EDA-ADC and PNU-EDA were screened in a whole genome CRISPR knock-out (ko) screen using Hs578T cells. Growth was inhibited by about 20% by repetitive compound treatment over approximately 12 cell population doublings and effect of gene deletion on cell proliferation was measured by NGS. Potential hits were identified based on differential effects on cell survival in the presence of the ADC or PNU-EDA. The screen was conducted at Horizon Discovery.

PNU-EDA but not PNU-159682 is a substrate of MDR1

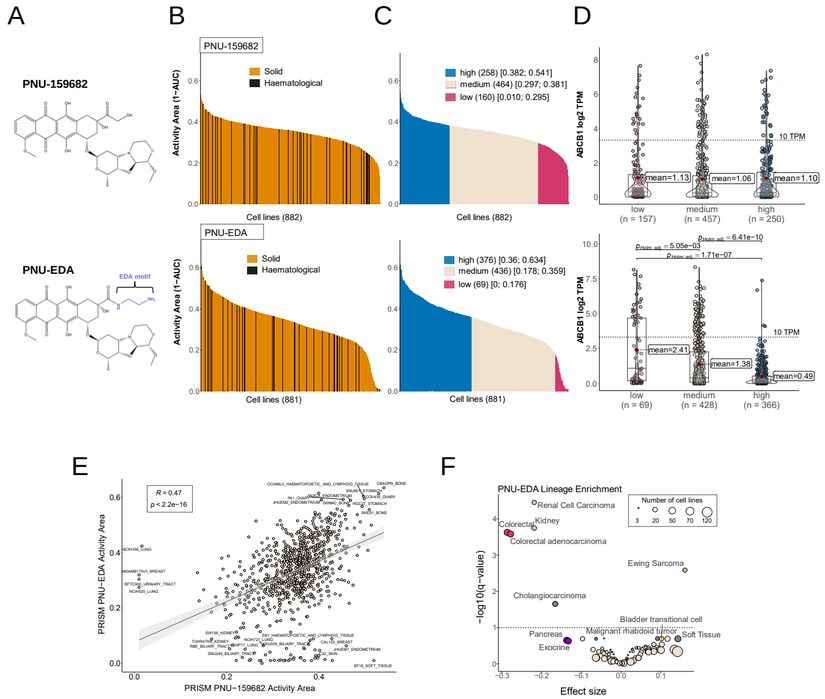

Fig. 3: PRISM screen comparing *in vitro* potency of PNU-159682 and PNU-EDA

(A) Structure of PNU-159682 and its derivative PNU-EDA. (B) Cell line sensitivity (Activity Area) to each compound reflecting solid and hematological tumor indications. (C) Cell line Activity Area to each compound, clustered with K-means algorithm into sensitivity clusters. (D) Violin plots of ABCB1 (MDR1/P-GP) expression across different clusters of sensitivity for each of the two compounds. (E) Overall, there is a good correlation of cell line sensitivity to both compounds, with some cell lines showing specific compound responses. (F) Volcano plot of lineage enrichment for cell line sensitivity response to PNU-EDA.

TC-NER pathway activity is critical for PNU-EDA's MoA

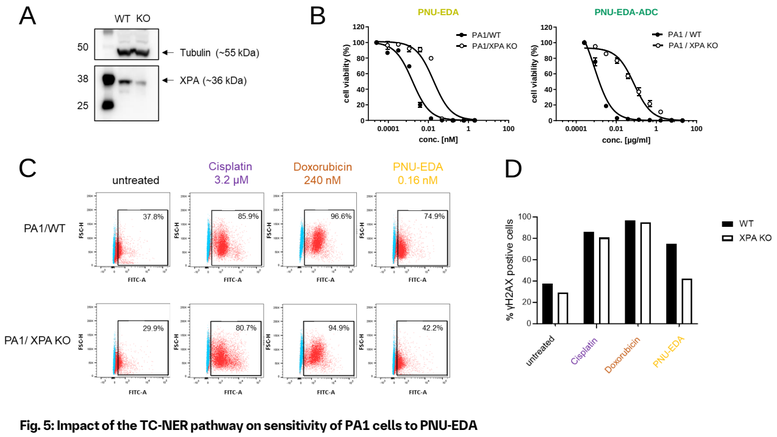

Fig. 5: Impact of the TC-NER pathway on sensitivity of PA1 cells to PNU-EDA

(A) CRISPR XPA knockout in PA1 cells (polyclonal) verified by Western Blot. (B) Efficacy of PNU-EDA and the PNU-EDA-ADC in PA1 cells in dependence on XPA knockout. (C/D) Effect on induction of DNA damage in XPA wildtype and knockout cells.

Key findings

- PNU-EDA is inducing a cell cycle arrest in S-phase in contrast to the anthracycline Doxorubicin
- PNU-EDA is an effective inducer of DNA damage and apoptosis
- PNU-EDA is a substrate of MDR1, while parental PNU-159682 is not a substrate, suggesting that parts of the EDA linker is confounding the MDR1 liability
- PNU-EDA induces DNA damage dependent on the Transcription-Coupled-Nucleotide-Excision-Repair (TC-NER) pathway

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