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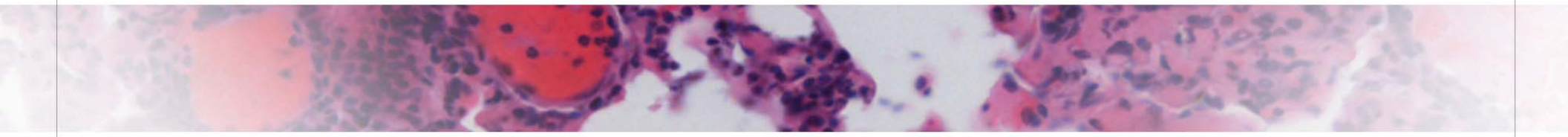

CD19-mediated DNA Damage Boost in Lymphoma Cells Treated with Loncastuximab Tesirine in Combination with PARP Inhibitors

UNIVERSITÀ DEGLI STUDI DI MILANO

IEO Istituto Europeo di Oncologia

Stefania Fusani[1,4], Alessandra Rossi[1], Saveria Mazzara[2], Elena Baiardi[1], Francesca Zammarchi[3], Patrick H. van Berkel[3], Corrado Tarella[2,4], Enrico Derenzini[2,4]

[1]Oncohematology Division, IEO European Institute of Oncology IRCCS, Milan, Italy [2]Hemolymphopathology Division, IEO European Institute of Oncology IRCCS, Milan, Italy
[3]ADC Therapeutics UK (Ltd), London, UK [4]University of Milan, Milan, Italy

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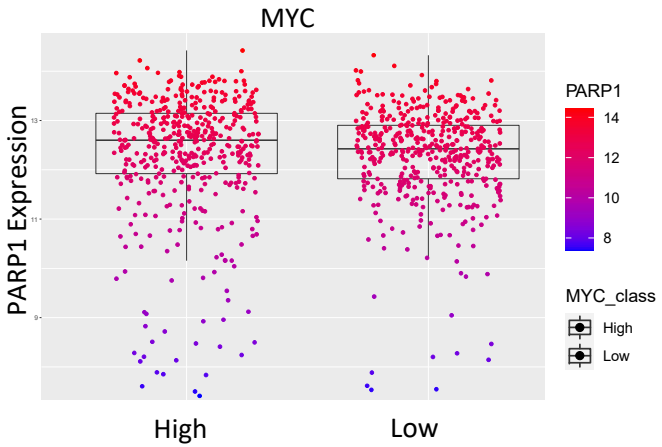
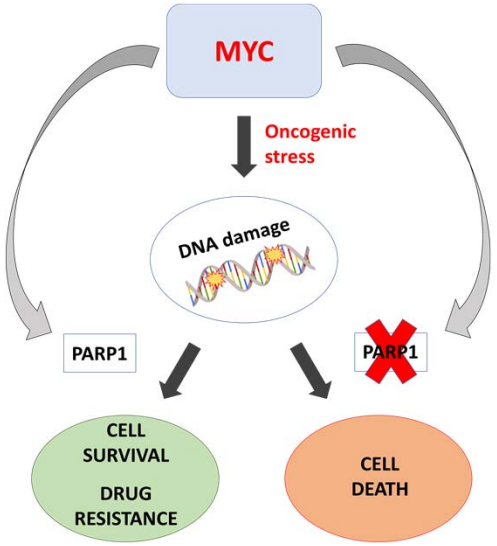
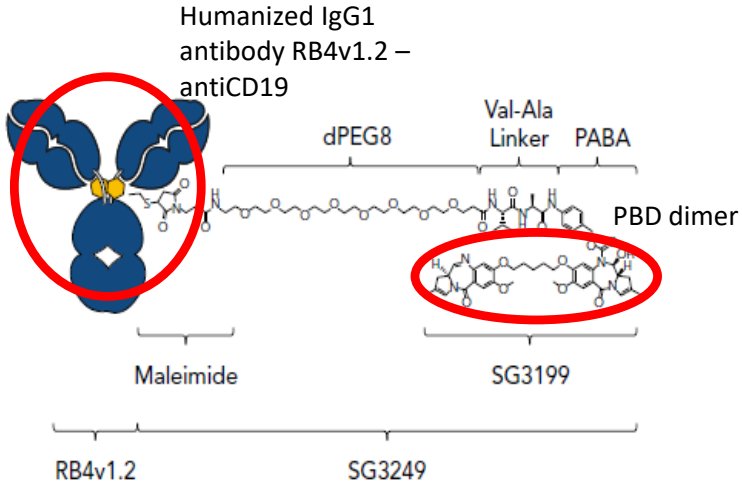
63RD ASH ANNUAL MEETING AND EXPOSITION – DECEMBER 11-14, 2021 – ATLANTA, GA

Targeting PARP in combination with CD19-selective delivery of DNA damage in DLBCL

Caracciolo D, *et al*. Exploiting MYC-induced PARPness to target genomic instability in multiple myeloma. Haematologica. 2021 Jan 1.

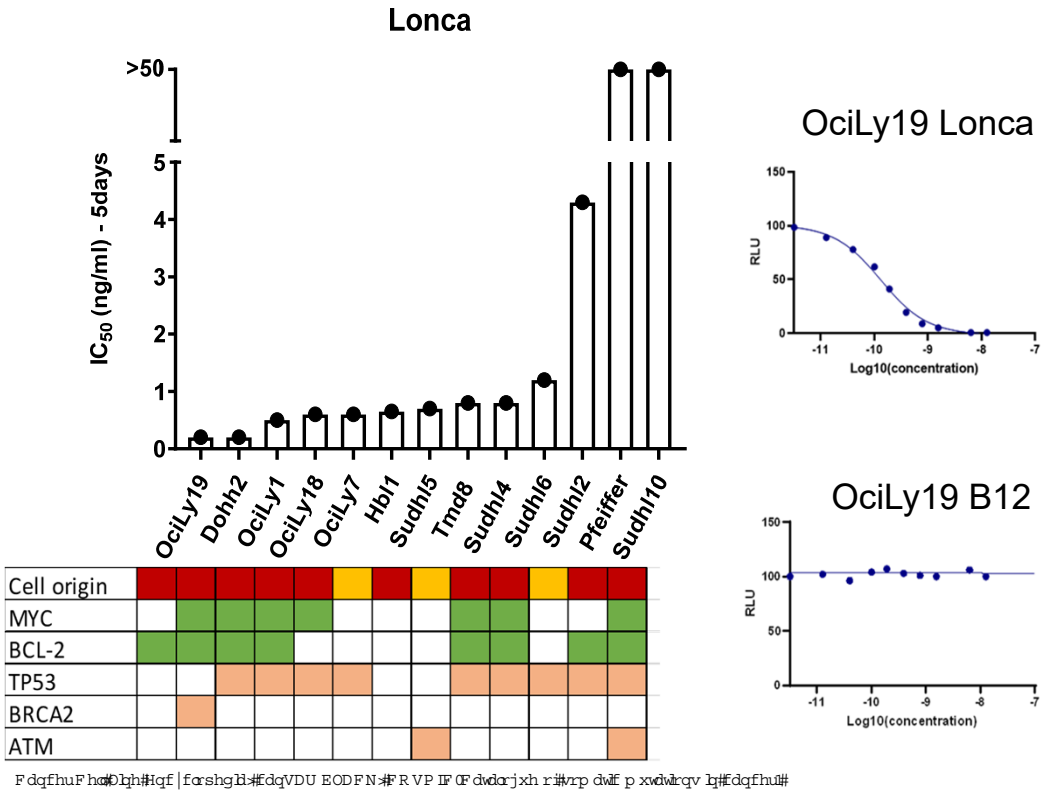


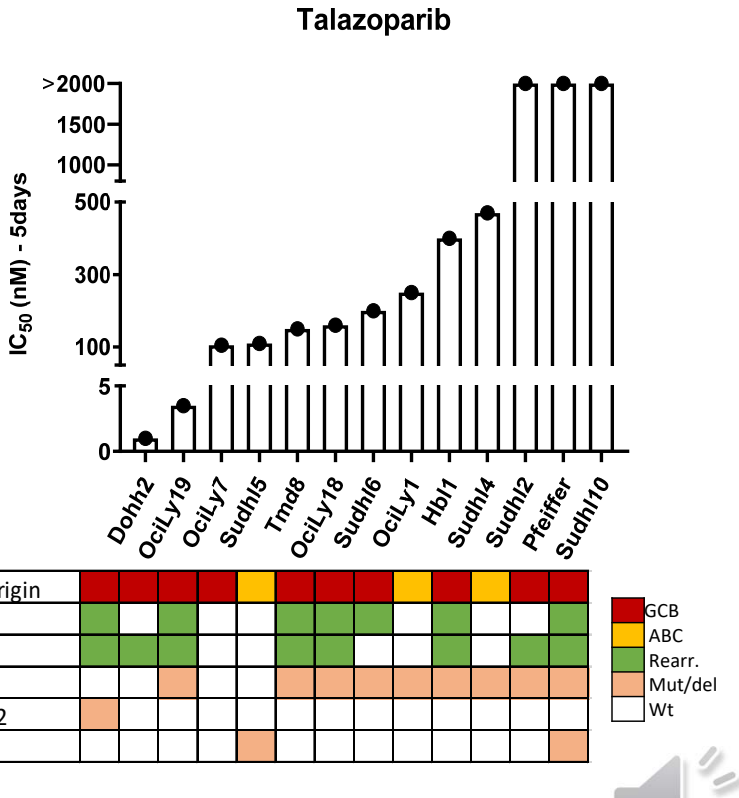




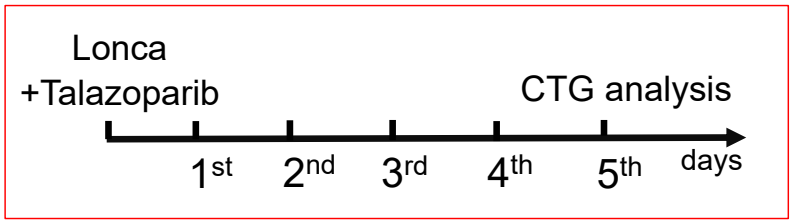
Zammarchi F, *et al*. Blood. 2018 Mar 8;131(10):1094-1105.

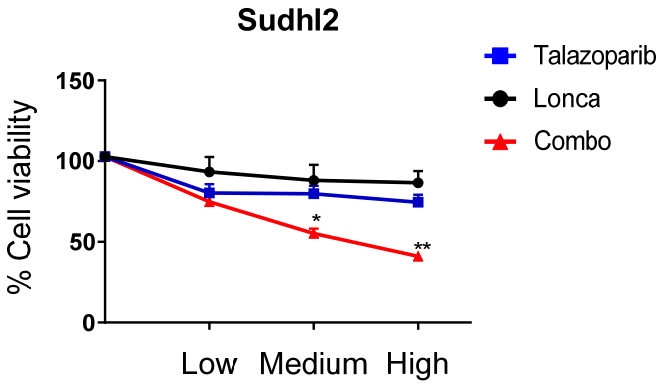
In vitro single agent activity of Loncastuximab tesirine and Talazoparib

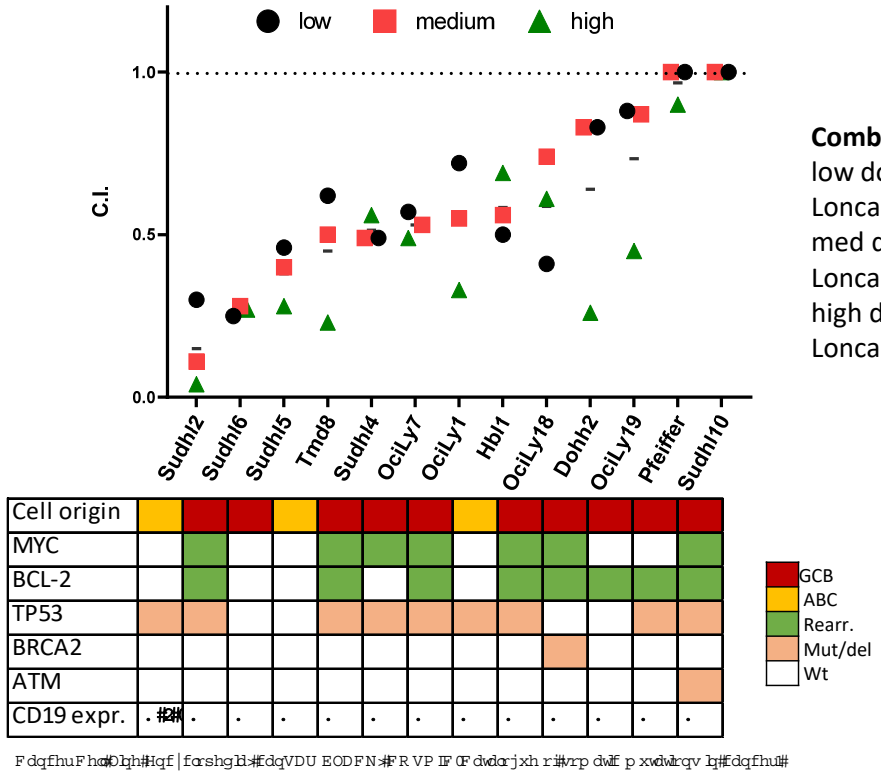




DDR inhibition by Talazoparib enhances the cytotoxic effects of Loncastuximab tesirine in DLBCL cell lines



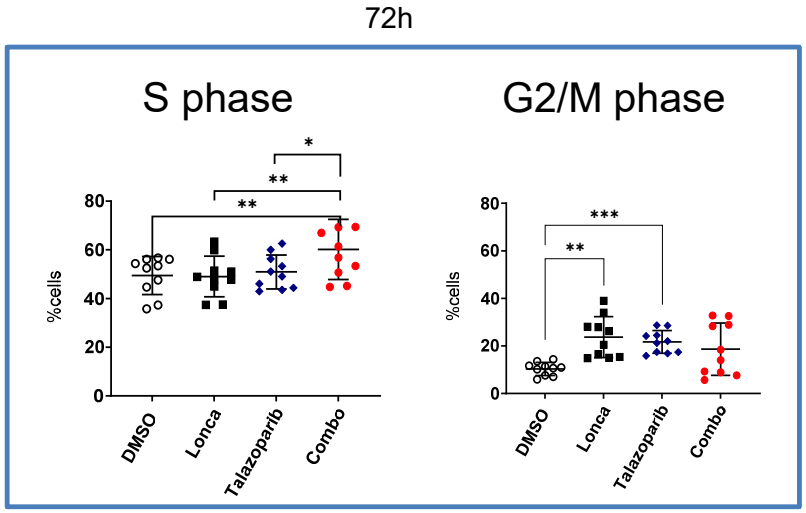




Combination index analysis:
low dose:
Lonca 0.25 ng/ml-Talazo 25nM
med dose:
Lonca 0.5 ng/ml-Talazo 50nM
high dose:
Lonca 1 ng/ml-Talazo 100nM

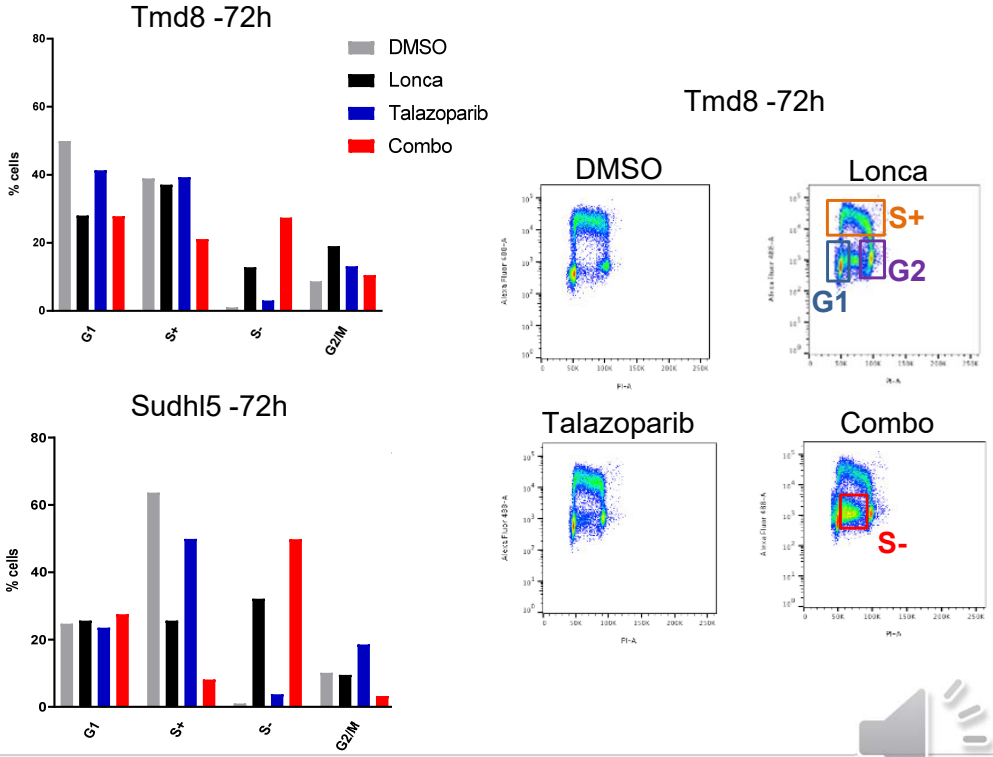
Talazoparib enhances Lonca-mediated S phase cell cycle arrest

1. Cell cycle analysis with Propidium Iodide

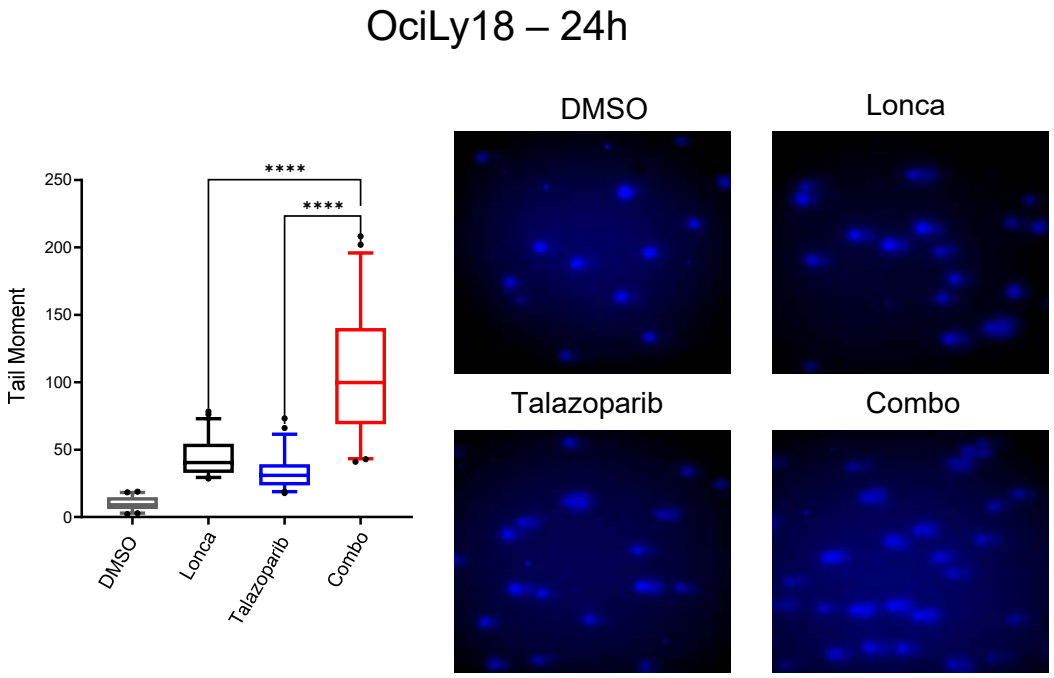


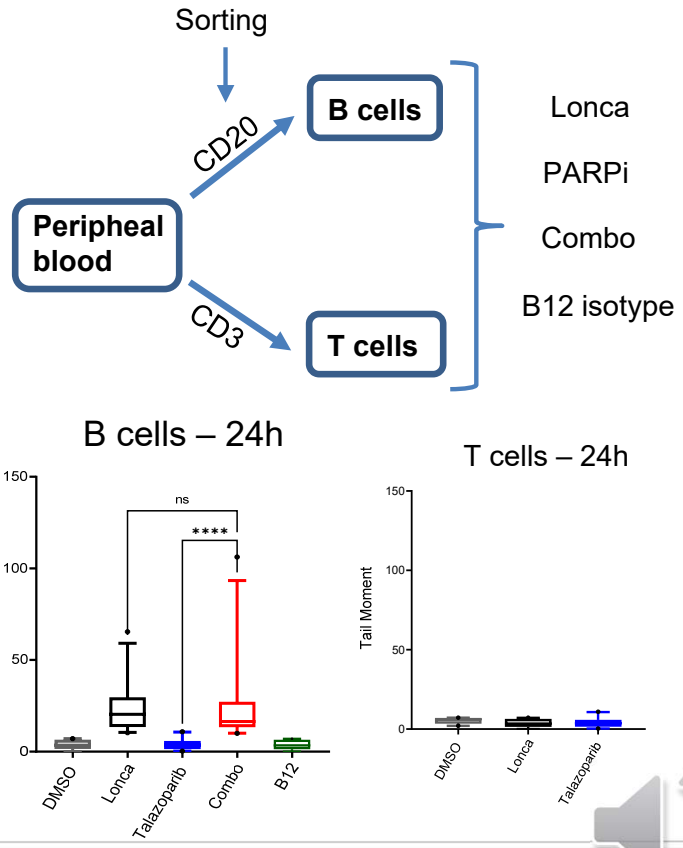
Aggregate data analysis of cell cycle variations after 72h of treatments in 10 representative DLBCL cell lines.

2. Cell cycle analysis with Propidium Iodide and Brdu



DNA damage detection in DLBCL cell lines and healthy donors-derived B and T cells





Conclusions

1. Talazoparib significantly enhances Loncastuximab tesirine anti-proliferative effects in DLBCL cell lines.

2. Combinational treatment induces S-phase cell cycle arrest suggesting a mechanistic interaction between the two drugs

3. DDR inhibition by Talazoparib boosts Lonca-mediated DNA damage in neoplastic B cells.

Disclosures:

Zammarchi: *ADC Therapeutics:* Current Employment, Current equity holder in publicly-traded company, Patents & Royalties.
V**an Berkel:** *ADC Therapeutics:* Current Employment, Current equity holder in publicly-traded company, Patents & Royalties.
Pileri: *NANOSTRING:* Other: ADVISORY BOARD; *ROCHE:* Other: ADVISORY-BOARD; *CELGENE:* Other: ADVISORY BOARD.
Tarella: *ADC-THERAPEUTICS:* Other: ADVISORY BOARD; *Abbvie:* Other: ADVISORY BOARD.
Derenzini: *TG-THERAPEUTICS:* Research Funding; *ASTRA-ZENECA:* Consultancy, Other: ADVISORY-BOARD; *BEIGENE:* Other: ADVISORY BOARD; *TAKEDA:* Research Funding; *ADC-THERAPEUTICS:* Research Funding.

CD19-mediated DNA Damage Boost in Lymphoma Cells Treated with Loncastuximab Tesirine in Combination with PARP Inhibitors

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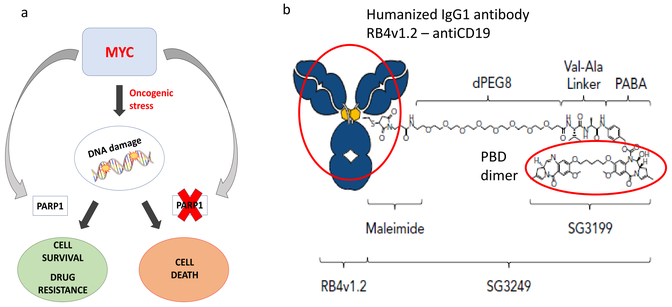
Background

Overexpression of the MYC oncogene is a frequent feature of diffuse large B-cell lymphoma (DLBCL) being associated with poor prognosis following standard R-CHOP (Rituximab, Cyclophosphamide, Doxorubicin, Vincristine, Prednisone) chemoimmunotherapy[1]. Since MYC expression is associated with overactivation of the DNA damage response (DDR), targeting DDR pathways with selective small molecule inhibitors could be a promising strategy to circumvent the inherent resistance to exogenous DNA damage proper of MYC-positive DLBCL[2-3]. Loncastuximab tesirine-lpyl (abbreviated as Lonca) is an antibody-drug conjugate (ADC) composed of a humanized anti-CD19 antibody conjugated to a potent DNA-crosslinking pyrrolobenzodiazepine dimer toxin[4].



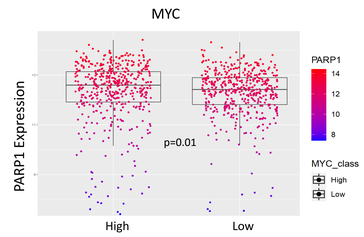
(a) Model describing the interaction between MYC and PARP in B-cell lymphoma[5].
(b) Structure of Loncastuximab tesirine-lypl (Lonca).

Hypothesis and Aim

With the aim of developing a treatment strategy for MYC-positive DLBCL, we hypothesized that DDR inhibition could increase the efficacy of Lonca by selectively enhancing DNA damage induction in DLBCL cells.

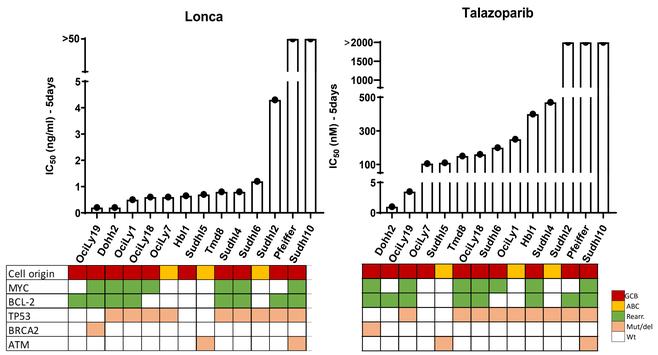
Results

1. CORRELATION BETWEEN MYC AND PARP1 EXPRESSION IN DLBCL



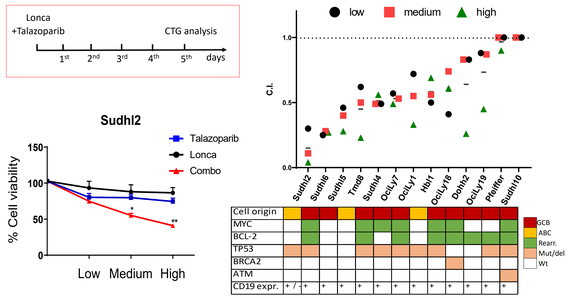
In a preliminary analysis of a publicly available dataset (Sha et al. J Clin Oncol 2019)[6], we found a significant correlation between *MYC* and *PARP1* gene expression levels, with higher *PARP1* levels observed in DLBCL samples characterized by high *MYC* expression.

2. CYTOTOXIC ACTIVITY OF LONCA AND TALAZOPARIB AS SINGLE AGENTS IN DLBCL CELL LINES



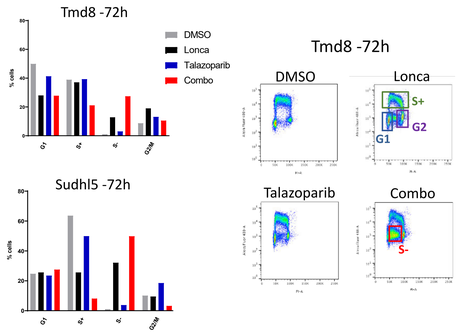
Lonca showed significant cytotoxic activity in 10 of 13 cell lines in the same range of clinically achievable concentrations (Ic50<1ng/ml). Talazoparib showed a strong *in vitro* activity, with IC50 values in the submicromolar range observed in most cell lines. Of note, the *BRCA*-mutated cell line DOHH2 was the most sensitive to PARPi, in line with the known synthetic lethal interaction between *BRCA* mutations and PARP inhibition.

3. TALAZOPARIB ENHANCES THE CYTOTOXIC EFFECTS OF LONCA IN DLBCL CELL LINES



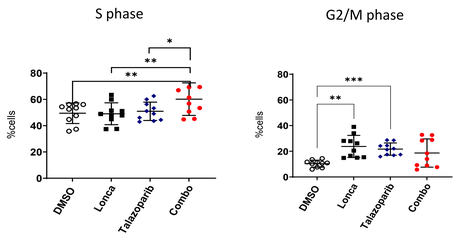
The combination of Talazoparib and Lonca produced enhanced anti-proliferative effects, with strong synergism (evaluated by combination index analysis[7]) observed in most cell lines. Notably, those cell lines resistant to both compounds as single agents became sensitive to the combination (e.g. SUDHL-2). (C.I., combination index)

4. 2D-CELL CYCLE ANALYSIS OF DLBCL CELL LINES EXPOSED TO LONCA/TALAZOPARIB COMBINATION TREATMENT



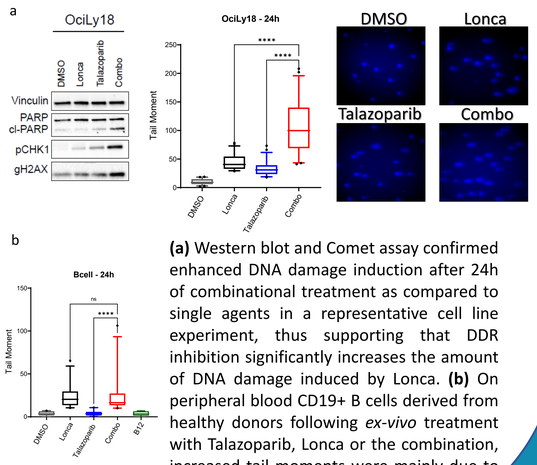
The combination of Talazoparib and Lonca clearly increased the fraction of cells in the BrdU-negative S phase of the cell cycle, (which represents cells that are not actively synthetizing DNA, S-), thus suggesting a mechanistic interaction between the two drugs.

5. AGGREGATE CELL CYCLE ANALYSIS OF DLBCL CELL LINES EXPOSED TO LONCA/TALAZOPARIB COMBINATION



Scatterplot showing S and G2/M phase cell cycle fractions in 10 DLBCL cell lines after 72h of treatment.

6. DDR INHIBITION BY TALAZOPARIB INCREASES DNA DAMAGE INDUCED BY LONCA IN CD19+ LYMPHOMA CELLS BUT NOT IN HEALTHY DONORS-DERIVED B CELLS



(a) Western blot and Comet assay confirmed enhanced DNA damage induction after 24h of combinational treatment as compared to single agents in a representative cell line experiment, thus supporting that DDR inhibition significantly increases the amount of DNA damage induced by Lonca. **(b)** On peripheral blood CD19+ B cells derived from healthy donors following *ex-vivo* treatment with Talazoparib, Lonca or the combination, increased tail moments were mainly due to Lonca activity.

Conclusions

1.DDR inhibition by Talazoparib enhances the cytotoxic activity of Loncastuximab tesirine-lypl in DLBCL cell lines.
2.Combinational treatment induces S phase cell cycle arrest suggesting a mechanistic interaction between Lonca and Talazoparib.
3.DDR inhibition by Talazoparib selectively boosts Lonca-mediated DNA damage in DLBCL cell lines, without enhancing DNA damage in healthy donors-derived B-cells.

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6. Sha, C. *Molecular High-Grade B-Cell Lymphoma: Defining a Poor-Risk Group That Requires Different Approaches to Therapy.* J Clin Oncol., **37**, 202-212 (2019).
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