Synergistic activity of the CDK4/6 antagonist narazaciclib (ON123300) with irreversible BTK inhibition in ibrutinib-resistant mantle cell lymphoma

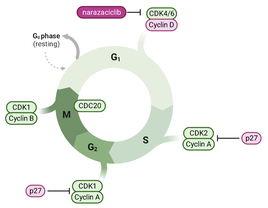
Núria Profitós-Peleja[1], *Marcelo L. Ribeiro*[1], *Jerónimo Parra*[1,2], *Miranda Fernández-Serrano*[1], *Pau Marín-Escudero*[1], *Adar Makovski Silverstein*[2], *Stephen Cosenza*[2], *Manel Esteller*[2,4,5] *and Gaël Roué*[1]

[1] Josep Carreras Leukaemia Research Institute, Badalona, Spain,
[2] Cancer Epigenetics Group, Josep Carreras Leukaemia Research Institute, Badalona, Spain
[3] Onconova Therapeutics, Newtown, PA
[4]Department of Biomedicine, School of Medicine, University of Barcelona, Barcelona, Spain
[5]Centro de Investigación Biomédica en Red de Cáncer (CIBERONC), Instituto de Salud Carlos III, Barcelona, Spain

AACR2023
#5974

INTRODUCTION

Mantle cell lymphoma (MCL) is a rare but aggressive B-cell lymphoma characterized by the chromosomal translocation (11;14) (q13; q32) and constitutive overexpression of cyclin D1 contributing to the uncontrolled growth of the cells. Bruton tyrosine kinase inhibitors (**BTKi**) have transformed the therapeutic landscape of MCL, but despite their efficacy, primary and acquired resistance to these agents is frequently observed in MCL patients. Thus, there is a need for novel therapeutic approaches in clinical use.
Preliminary results have suggested that the second-generation, orally bioavailable and clinical-stage **CDK4/6 inhibitor**, narazaciclib (ON123300), may trigger cell cycle arrest and significant tumor growth inhibition in **preclinical models** of BTKi-resistant MCL.

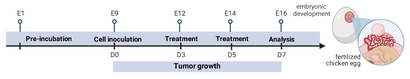


AIMS

To evaluate the **activity** and **mechanism of action** of the CDK4/6 inhibitor, narazaciclib (ON123300), as single agent and/or in combination with BTK inhibitors in preclinical models of MCL with distinct sensitivities to the first-in-class and FDA-approved BTKi ibrutinib.

METHODS

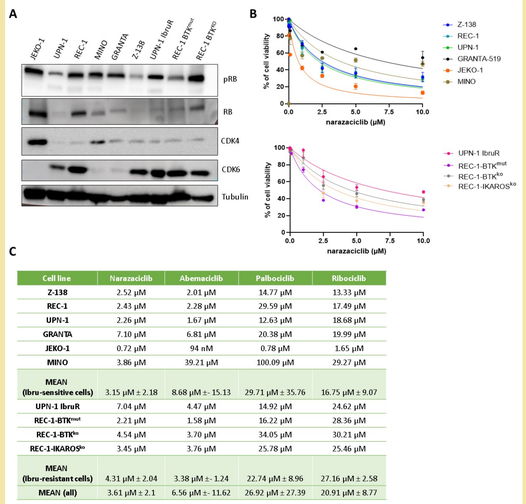
We compared the efficacy and safety of narazaciclib vs the approved CDK inhibitors palbociclib, abemaciclib or ribociclib, in association with various BTKi, in a panel of 10 MCL cell lines with distinct sensitivity to the first-in-class BTKi, ibrutinib, or the second generation acalabrutinib. We evaluated the effects of these combinations by CellTiter-Glo proliferation, FACS-mediated analysis of cell cycle and apoptosis, RT-PCR and WB.
RNA-seq and Gene Set Enrichment Analysis (GSEA) analysis were performed from MCL cells treated with narazaciclib (0.5μM) and Ibrutinib (1μM) for 24h.
Efficacy and safety of narazaciclib *in vivo* was evaluated in both ibrutinib-sensitive (UPN1) and ibrutinib-resistant (UPN-IbruR) chicken embryo chorioallantoic membrane (CAM) xenograft models of MCL.



Timeline of the chicken embryo chorioallantoic membrane (CAM) assay.

RESULTS

NARAZACICLIB EXHIBITS ANTITUMOR ACTIVITY IN MCL CELL LINES REGARDLESS CDK4/6 LEVELS OR BTKI SENSITIVITY



C

Cell line	Narazaciclib	Abemaciclib	Palbociclib	Ribociclib
Z-138	2.52 μM	2.01 μM	14.77 μM	13.33 μM
REC-1	2.43 μM	2.28 μM	29.59 μM	17.49 μM
UPN-1	2.26 μM	1.67 μM	12.63 μM	18.68 μM
GRANTA	7.10 μM	6.81 μM	20.38 μM	19.99 μM
JEKO-1	0.72 μM	94 nM	0.78 μM	1.65 μM
MINO	3.86 μM	39.21 μM	100.09 μM	29.27 μM
MEAN (Ibru-sensitive cells)	3.15 μM ± 2.18	8.68 μM ± 15.13	29.71 μM ± 35.76	16.75 μM ± 9.07
UPN-1 IbruR	7.04 μM	4.47 μM	14.92 μM	24.62 μM
REC-1-BTK^{mut}	2.21 μM	1.58 μM	16.22 μM	28.36 μM
REC-1-BTK^{ko}	4.54 μM	3.70 μM	34.05 μM	30.21 μM
REC-1-IKAROS^{ko}	3.45 μM	3.76 μM	25.78 μM	25.46 μM
MEAN (Ibru-resistant cells)	4.31 μM ± 2.04	3.38 μM ± 1.24	22.74 μM ± 8.96	27.16 μM ± 2.58
MEAN (all)	3.61 μM ± 2.1	6.56 μM ± 11.62	26.92 μM ± 27.39	20.91 μM ± 8.77

Figure 1. A. Western blot (WB) analysis of parental (JEKO, UPN-1, REC-1, MINO, GRANTA-519, Z-138) and BTKi-resistant MCL counterparts (UPN-IbruR, REC-1 BTKmut and REC-1 BTK-KO). **B.** CTG proliferation assay with MCL cell lines treated with increasing doses of narazaciclib for 72h. **C.** IC50 values at 72h for four different CDK inhibitors (narazaciclib, abemaciclib, palbociclib and ribociclib).

SYNERGISTIC EFFECT OF NARAZACICLIB WITH BTKi IN IBRUTINIB-SENSITIVE AND IBRUTINIB-REFRACTORY MCL

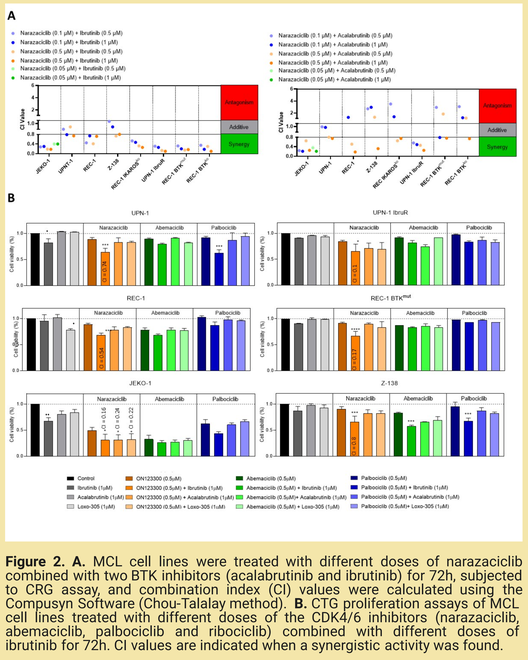


Figure 2. A. MCL cell lines were treated with different doses of narazaciclib combined with two BTK inhibitors (acalabrutinib and ibrutinib) for 72h, subjected to CRG assay, and combination index (CI) values were calculated using the Compusyn Software (Chou-Talalay method). **B.** CTG proliferation assays of MCL cell lines treated with different doses of the CDK4/6 inhibitors (narazaciclib, abemaciclib, palbociclib and ribociclib) combined with different doses of ibrutinib for 72h. CI values are indicated when a synergistic activity was found.

NARAZACICLIB AND BTKI TREATMENTS CONVERGE TO G1 CELL CYCLE ARREST IN MCL

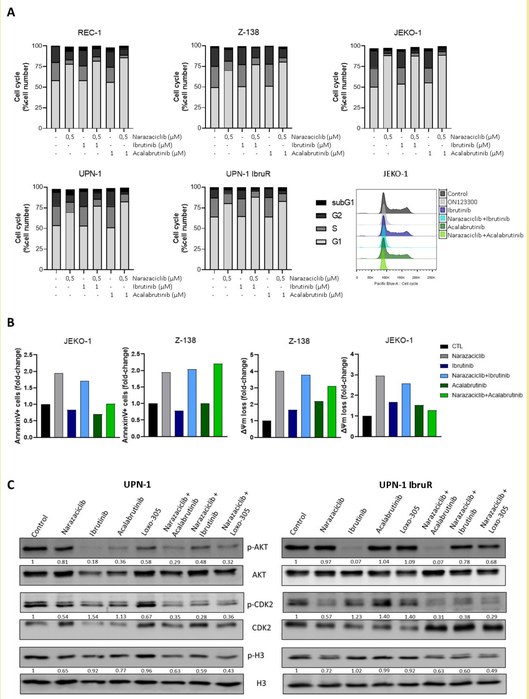
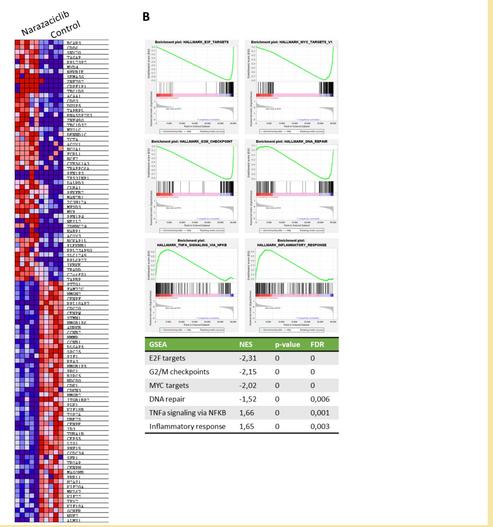


Figure 3. A. Cell cycle analysis from MCL cells treated with narazaciclib (0.5μM) and combined with the BTKi ibrutinib (1μM) or acalabrutinib (1 μM) for 24h. **B.** Apoptosis analysis assessed by AnnexinV+ staining and mitochondrial transmembrane potential (ΔΨm) loss after treatment with narazaciclib (0.5μM) +/- ibrutinib or acalabrutinib (1 μM) for 72h. **C.** Western blot (WB) analysis of UPN-1 and UPN-IbruR after treatment with narazaciclib (0.5μM) +/- ibrutinib (1μM), acalabrutinib (1 μM) or Loxo-305 (1 μM) for 24h. **D.** qRT-PCR quntification of cell-cycle and senescence-related transcripts in JEKO-1 cell line treated with narazaciclib +/- BTKi, for 24h.

NARAZACICLIB TREATMENT MODULATES G2/M CHECKPOINTS, E2F AND MYC TARGET GENES IN MCL



GSEA	NES	p-value	FDR
E2F targets	-2,31	0	0
G2/M checkpoints	-2,15	0	0
MYC targets	-2,02	0	0
DNA repair	-1,52	0	0,006
TNFa signaling via NFKB	1,66	0	0,001
Inflammatory response	1,65	0	0,003

Figure 4. A. Heatmap showing genes differentially regulated upon ON123300 treatment in MCL cell lines (UPN-1, UPN-IbruR, REC-1, JEKO-1 and MINO). **B.** Gene Set Enrichment Analysis (GSEA) after transcriptomic (RNAseq) characterization of n=5 MCL cell lines after a 24h exposure to narazaciclib.

NARAZACICLIB EXHIBITS A SIGNIFICANT ANTITUMOR ACTIVITY THAT IS IMPROVED BY IBRUTINIB THERAPY IN *IN VIVO MODELS OF MCL*

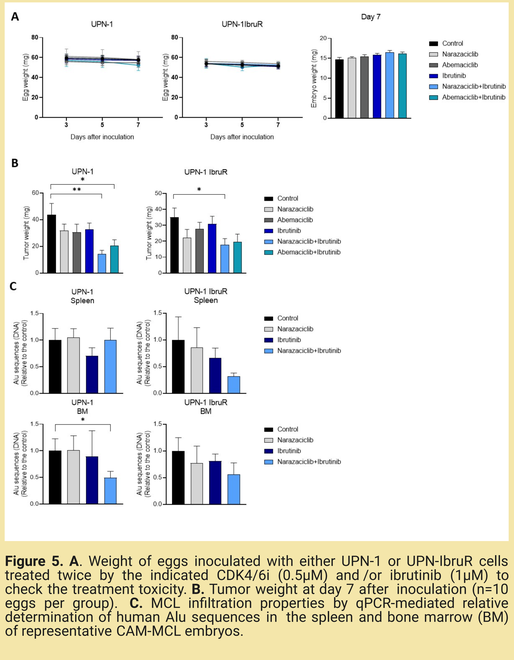


Figure 5. A. Weight of eggs inoculated with either UPN-1 or UPN-IbruR cells treated twice by the indicated CDK4/6i (0.5μM) and /or ibrutinib (1μM) to check the treatment toxicity. **B.** Tumor weight at day 7 after inoculation (n=10 eggs per group). **C.** MCL infiltration properties by qPCR-mediated relative determination of human Alu sequences in the spleen and bone marrow (BM) of representative CAM-MCL embryos.

CONCLUSIONS

Narazaciclib exhibits a significant antitumor activity in MCL cell lines, independently of their sensitivity to the BTK inhibitor, ibrutinib.

In vitro, narazaciclib exhibits a superior activity than palbociclib and ribociclib, while abemaciclib harbored a similar effect.

In vivo, narazaciclib achieved a significant tumor growth inhibition when combined with ibrutinib, with no detectable toxicity in the CAM-MCL model.

Narazaciclib single agent treatment repressed some positive regulators of the G2/M cell cycle phase with the consequent loss of phospho-Histone H3.

Narazaciclib triggered the accumulation of the CDK inhibitors p21, p16, and phospho-p27, leading to CDK2 dephosphorylation.

Accordingly, narazaciclib treatment evokes a G1 cell cycle blockade, which preceded the ignition of mitochondrial apoptosis.

When combined with ibrutinib, rather than with acalabrutinib, narazaciclib showed a synergistic antitumor activity in both BTK-sensitive and BTK-resistant cell lines (Improved G1 blockade, downregulation of p-histone H3/p-CDK2 and upregulation of p-p27/p27/p16)

REFERENCES

1. Roué, G., & Sola, B. (2020). Management of drug resistance in mantle cell lymphoma. *Cancers*, *12*(6), 1565.
2. Hershkovitz-Rokah, O., Pulver, D., Lenz, G., & Shpilberg, O. (2018). Ibrutinib resistance in mantle cell lymphoma: clinical, molecular and treatment aspects. *British journal of haematology* , *181*(3), 306-319.
3. Divakar, S. K. A., Reddy, R., Cosenza, S. C., Baker, S. J., Perumal, D., Antonelli, A. C., ... & Premkumar Reddy, E. (2016). Dual inhibition of CDK4/Rb and PI3K/AKT/mTOR pathways by ON123300 induces synthetic lethality in mantle cell lymphomas. *Leukemia*, *30*(1), 86-93.
4. Jiao, Z., Ke, H., Zhang, F., Li, H., & Wang, J. (2022). HX301 (ON123300) shows broad antitumor activity in preclinical mantle cell lymphoma models, inclusive of those resistant to BTKi. *European Journal of Cancer*, *174*, S18.

GRANTS & ACKNOWLEDGEMENTS

This study was financially supported by Onconova Therapeutics, the Spanish Ministry of Economy and Competitiveness (PID2021-123039OB-C21), and the Catalan Agency for Management of University and Research Grants (2021SGR01535) (to GR). MFS was recipients of a predoctoral fellowship (FI19/00338) from Instituto de Salud Carlos III. This work was carried out under the CERCA Program (Generalitat de Catalunya).

Created with BioRender Poster Builder