Prolonged cell cycle arrest by the CDK4/6 antagonist narazaciclib restores ibrutinib response in preclinical models of BTKi-resistant mantle cell lymphoma

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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.

AIMS

To evaluate the **activity** and **mechanism of action** of the CDK4/6i, narazaciclib, as single agent and or combined with BTK inhibitors in preclinical models of mantle cell lymphoma with distinct sensitivities to the first-in-class and FDA-approved BTKi, ibrutinib.

METHODS

We compared the efficacy and safety of narazaciclib vs other approved CDKi, in association with various BTKi, in a panel of 10 MCL cell lines with distinct sensitivities to ibrutinib.

Effects of the combinations were determined by CTG-proliferation assay, FACS-mediated quantification of cell cycle RNA sequencing and phospho-proteomics, followed by GSEA, RT-PCR and WB.

Efficacy and safety of narazaciclib/BTKi combo was evaluated *in vivo* in chicken embryo chorioallantoic membrane (CAM) xenograft models of MCL.

RESULTS

NARAZACICLIB EXHIBITS AN ANTITUMOR AND SYNERGISTIC ACTIVITY WITH BTKi

A

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

B

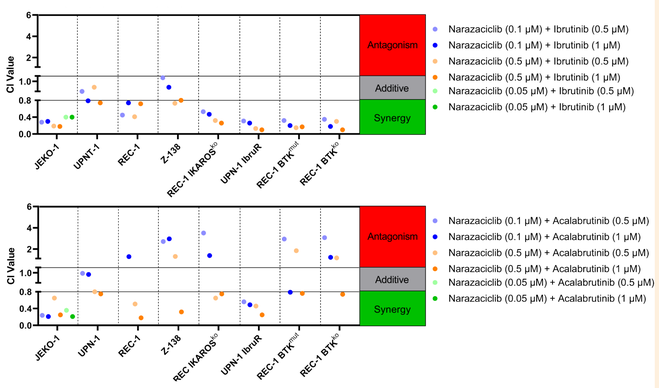


Figure 1. A. IC50 values at 72h for four different CDK inhibitors (narazaciclib, abemaciclib, palbociclib and ribociclib). **B.** Combination index (CI) of narazaciclib combined with BTKis (ibrutinib and acalabrutinib.

NARAZACICLIB AND BTKi TREATMENTS CONVERGE TO G1 CELL CYCLE ARREST

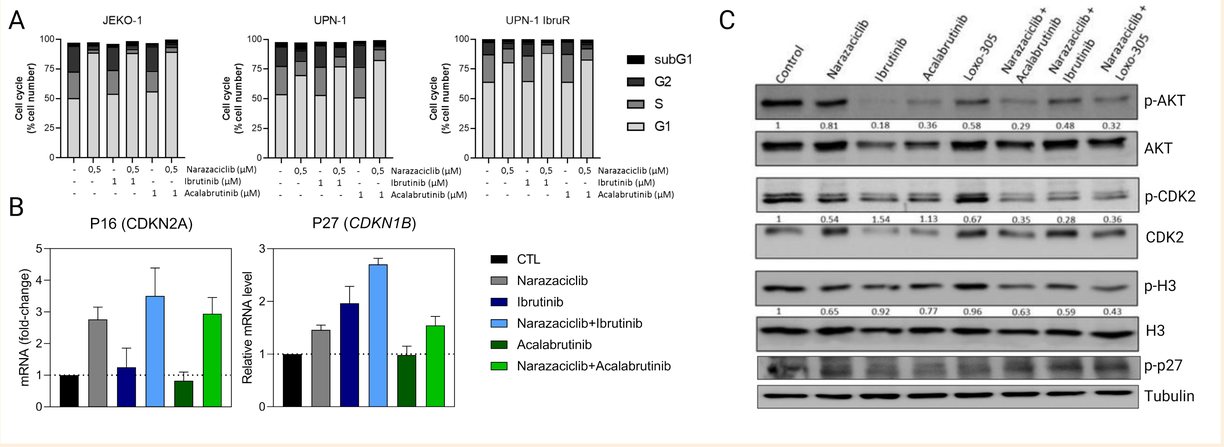


Figure 2. A. Cell cycle analysis after treatment for 24h. **B.** qRT-PCR quantification and **C.** Western Blot validation of cell-cycle related transcripts.

NARAZACICLIB/IBRUTINIB COMBO DOWNREGULATES CELL CYCLE CHECKPOINTS

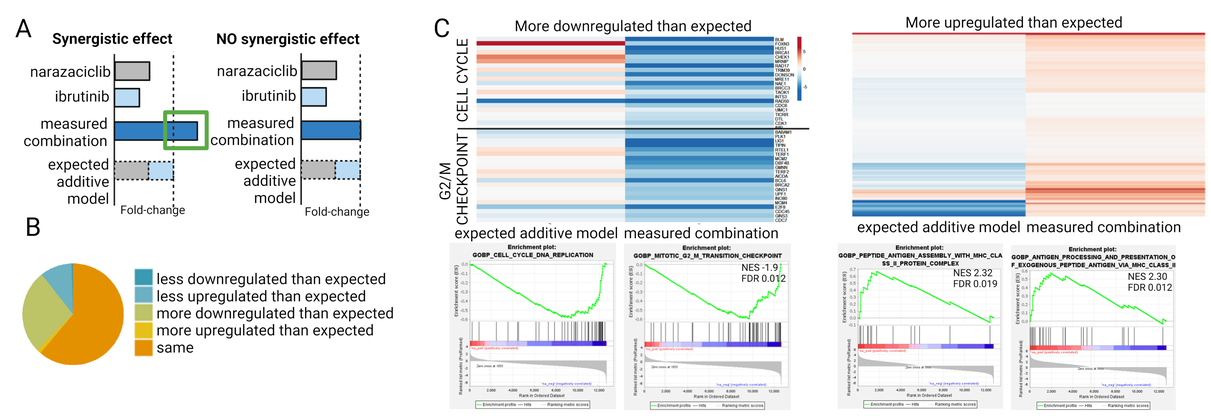


Figure 3. A. Scheme of the differential expression analysis of the synergistic effect of the combination narazaciclib/ibrutinib in the UPN and UPN IbruR cell lines. **B.** Pie chart and **C.** GSEA and heatmaps of the more upregulated or downregulated genes in the combination compared to the expected additive model.

NARAZACICLIB EXHIBITS A SIGNIFICANT ANTITUMOR ACTIVITY IN I*N VIVO MODELS*

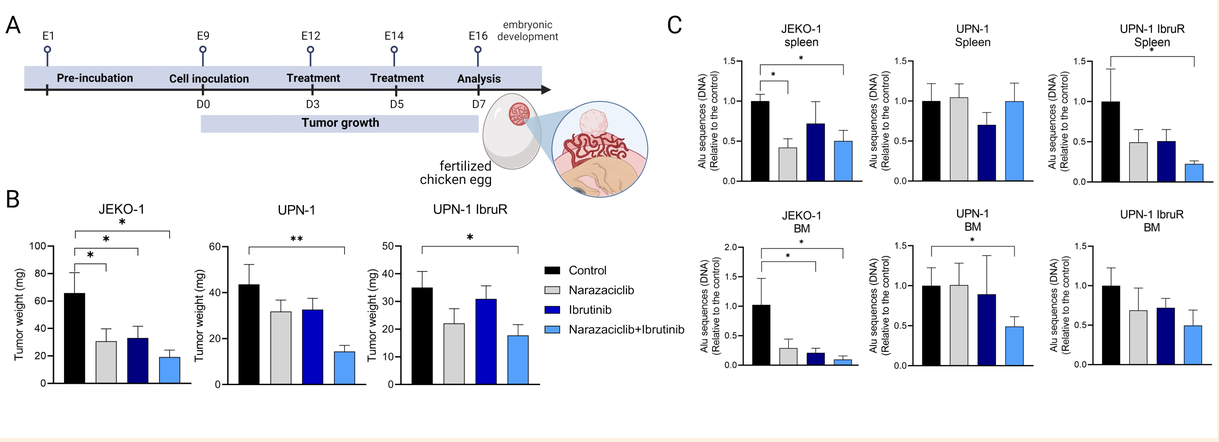
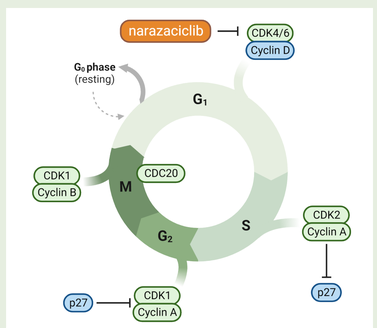


Figure 4. A. Timeline of the CAM assay. **B.** Tumor weight. **C.** MCL infiltration properties by qPCR-mediated relative determination of human Alu sequences in spleen and bone marrow (BM).

CONCLUSIONS

Due to its complete distinct MoA from BTKi involving the direct modulation of cell cycle, nazaraciclib, but not abemaciclib or palbociclib, can achieve significant synergistic activity in vitro and in vivo in combination with ibrutinib, especially in BTKi-resistant MCL cases.



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