Narazaciclib, a differentiated CDK4/6 antagonist, prolongs cell cycle arrest and metabolomic reprogramming, enabling restoration of ibrutinib sensitivity in 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 that contributes to the uncontrolled growth of malignant 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 approaches in clinical use.

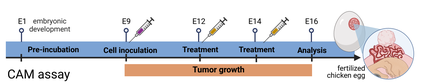
Previous studies have suggested that narazaciclib, a **CDK4/6 inhibitor** (CDKi), may trigger cell cycle arrest and significant tumor growth inhibition in BTKi-resistant MCL.

AIMS

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

METHODS

We compared the efficacy and safety of narazaciclib *vs* FDA-approved CDKi, in association with different covalent (ibrutinib & acalabrutinib) and non-covalent BTKi (ARQ531), in a panel of 10 MCL cell lines with distinct sensitivities to ibrutinib[4]. Effects of the combinations were determined by Cell-Titer-Glo (CTG) proliferation assay, FACS-mediated quantification of cell cycle, RNA sequencing and gene set enrichment analysis (GSEA), Phospho-proteomics analysis followed by qRT-PCR and western blot validation. Efficacy and safety of narazaciclib/BTKi combo was evaluated *in vivo* in an immuno-competent, chicken embryo chorioallantoic membrane (CAM) xenograft model of MCL sensitive and resistant to BTKi.



RESULTS

NARAZACICLIB EXHIBITS A SYNERGISTIC ANTITUMOR ACTIVITY WHEN COMBINED WITH BTKi

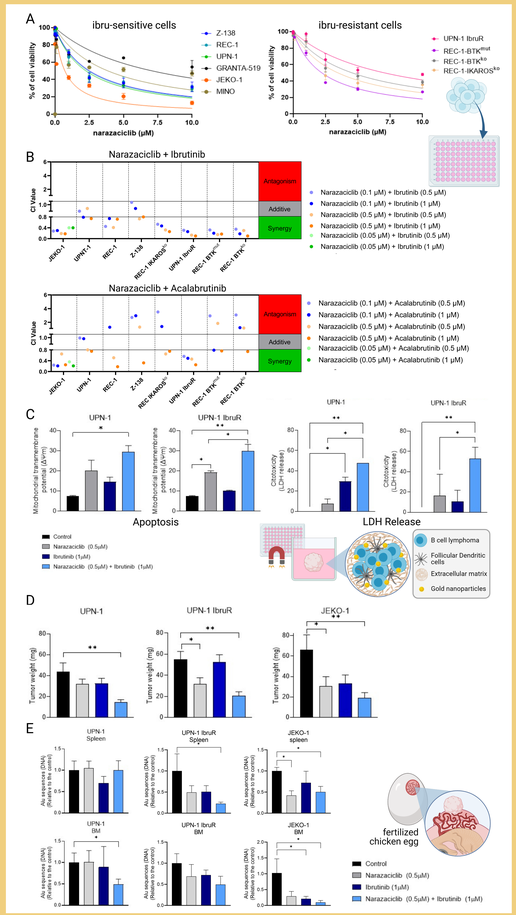
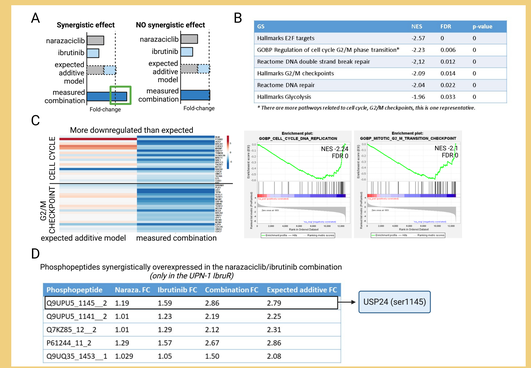


Figure 1. A. CTG of Narazaciclib in MCL cell lines with different sensitivity to ibrutinib **B.** Combination index (CI) of narazaciclib combined with ibrutinib or acalabrutinib. **C.** Apoptosis and LDH release in 3D in cell lines and 2 MCL patients, sensitive to ibrutinib. **D.** Tumor weight measurement in 3 different MCL *in vivo* models dosed with narazaciclib +/- ibrutinib. **E.** Modulation of MCL infiltration properties assessed by qPCR-mediated determination of human Alu sequences in spleen and bone marrow (BM) in representative embryos.

INTEGRATION OF TRANSCRIPTOMICS AND PHOSPHO-PROTEOMICS ANALYSES OF N+I COMBINATION



GS	NES	FDR	p-value
Hallmarks E2F targets	-2.57	0	0
GOBP Regulation of cell cycle G2/M phase transition*	-2.23	0.006	0
Reactome DNA double strand break repair	-2.12	0.012	0
Hallmarks G2/M checkpoints	-2.09	0.014	0
Reactome DNA repair	-2.04	0.022	0
Hallmarks Glycolysis	-1.96	0.033	0

** There are more pathways related to cell cycle, G2/M checkpoints, this is one representative.*

Phosphopeptide	Naraza. FC	Ibrutinib FC	Combination FC	Expected additive FC
Q9UPU5_1145__2	1.19	1.59	2.86	2.79
Q9UPU5_1141__2	1.01	1.23	2.19	2.25
Q7KZ85_12__2	1.01	1.29	2.12	2.31
P61244_11_2	1.29	1.57	2.67	2.86
Q9UQ35_1453__1	1.029	1.05	1.50	2.08

Figure 2. A. Strategy for the determination of the synergistic signature of narazaciclib/ibrutinib, in the transcriptome and phospho-proteome. **B.** Table and **C.** GSEA- mediated identification of the main differentially-expressed pathways in both ibru-sensitive and ibru-resistant. **D.** Table of the top 5 differentially expressed phosphopeptides of the phospho-proteomics analysis to determine the marker in the ibru-resistant cells.

N+I COMBINATION PROMOTES G1 CELL CYCLE BLOCKADE IN MCL

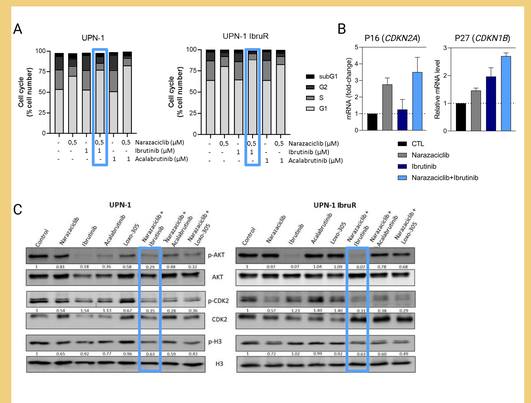


Figure 3. A. Cell cycle analysis of 3 representative MCL cell lines after a 24 hour-exposure to narazaciclib +/- ibrutinib or acalabrutinib. **B.** qRT-PCR- and **C.** western blot-mediated quantification of proliferation and cell cycle-related factors in UPN-1 and UPN-1 IbruR cells treated as in A.

N+I COMBINATION TRIGGERS A METABOLIC REPROGRAMMING IN BTKi-RESISTANT MCL

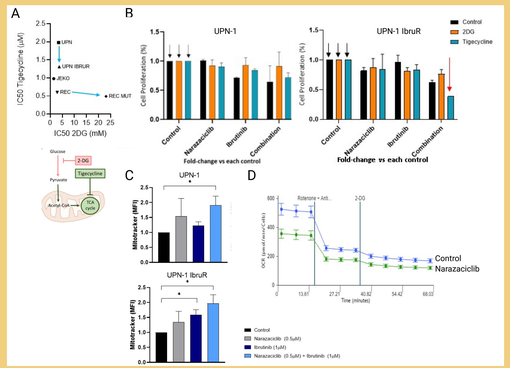


Figure 4. A. CTG of the different MCL cell lines treated with a glycolytic inhibitor (2DG) or OxPhos inhibitor (Tigecycline). **B.** CTG of the MCL cells treated with the metabolic inhibitors + Naracliclib/Ibrutinib for 72h. **C.** Mitochondrial fluorescente probe (Mitotracker) uptake after 72h treated as B. **D.** Oxigen Consumption Rate (OCR) measured with Seahorse of the UPN-1 cells treated with narazaciclib.

N+I COMBINATION TARGETS DNA DAMAGE RESPONSE THROUGH USP24-P53 AXIS IN BTKi-RESISTANT MCL

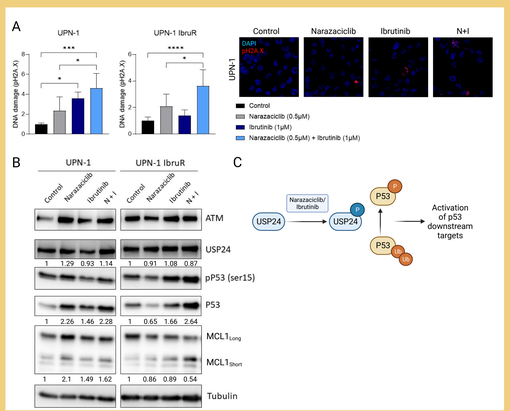


Figure 5. A. DNA damage assesed by an IF of pH2A.X in the MCL cells treated for 72h. Representative IF of pH2A.X in UPN-1 **B.** WB of different downstream markers of the P53 pathway at 24h post-treatment. **C.** Diagram of the mechanism of action of USP24 via p53[7].

CONCLUSION

- Narazaciclib demonstrates both safety and efficacy as a single agent in preclinical models of MCL, including those resistant to BTK inhibitors.
- Its combination with ibrutinib achieved a synergistic antitumoral effect *in vitro* and *in vivo,* particularly in models resistant to ibrutinib, achieving a lower combination index values compared to the sensitive MCL cells.
- This combination induces a superior G1 cell cycle blockade in MCL cells. Notably, in the ibrutinib-resistant cases, the synergistic interaction between narazaciclib and ibrutinib triggers a metabolic reprogramming alongside increased DNA damage, mediated by the USP24 and P53 axis.

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