Proteomic analysis of drug synergy between the Hsp90 inhibitor SNX7081 and fludarabine

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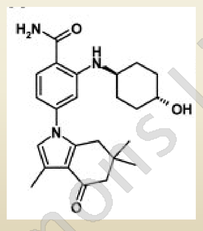
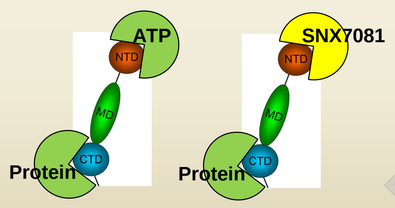
Introduction

- Chronic lymphocytic leukemia (CLL) is the most common leukemia in adults in Western countries.
- MEC1 is a p53-mutated CLL cell line, resistant to fludarabine, the mainstay for first line treatment in CLL.
- The Hsp90 (heat shock protein 90) is a conserved molecular chaperone that participates in stabilizing and activating many oncogenic proteins. Evidence suggests that Hsp90 inhibitors may be highly effective anticancer-agents.
- Hsp90 inhibitor, SNX-7081, competitively binds to the N-terminal ATP binding site of Hsp90, inducing degradation of its client proteins.



SNX-7081

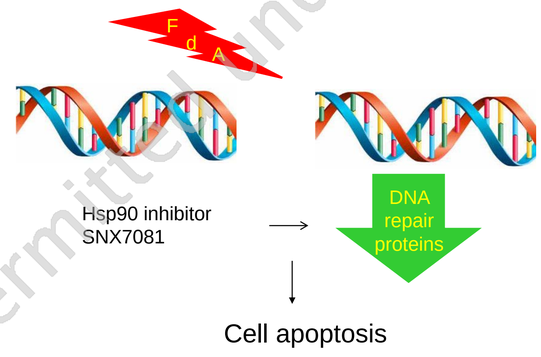


Hsp90 protein

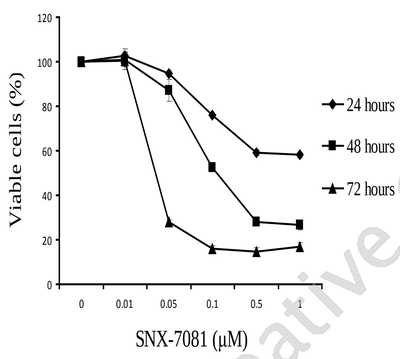
Normal (left), inhibited (right)

SNX-7081 and fludarabine have synergy effect?

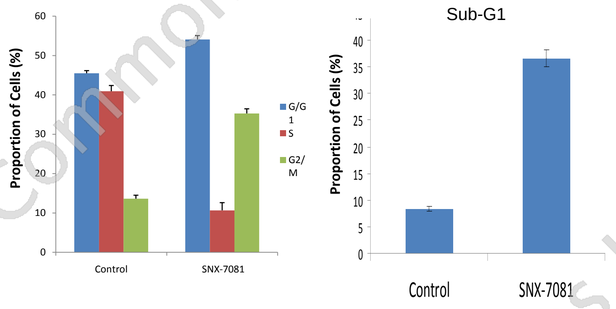
- Our hypothesis is that SNX-7081 might have a synergistic effect with fludarabine (FdA) by down-regulating DNA repair proteins.
- Fludarabine, the mainstay for first line treatment in CLL, is converted to fludarabine triphosphate (FdATP) in cells before being incorporated into elongating nucleic acid chains, leading to the termination of DNA synthesis.



SNX-7081 is cytotoxic to MEC1 cells

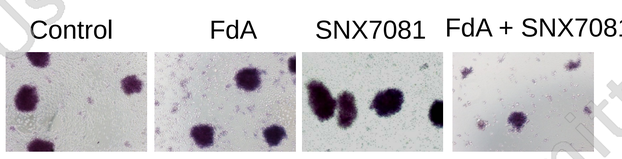


Cell viability by MTT assay

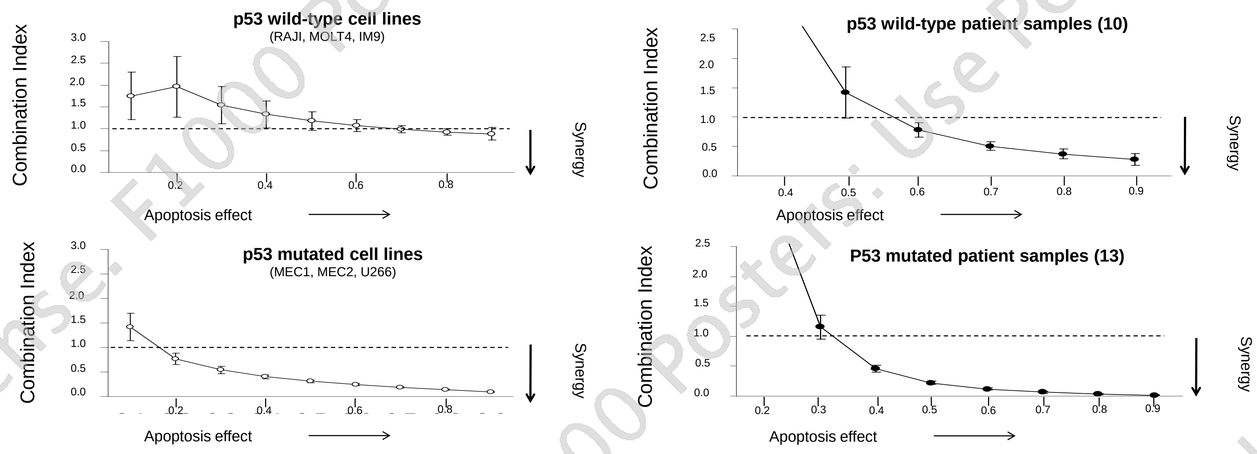


Cell cycle changes induced by SNX-7081(500nM, 24 h), Sub-G1 is where cells contain less DNA than those within the G0/G1 cell cycle phase, providing a measure of the proportion of apoptotic cells.

Drug synergy exist



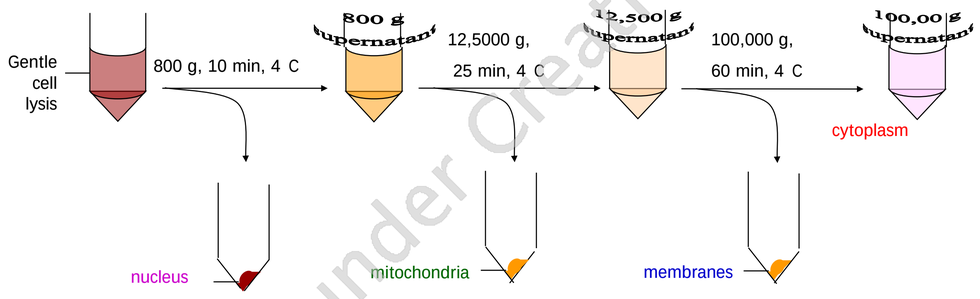
MEC1 cells colony formation by MTT assay



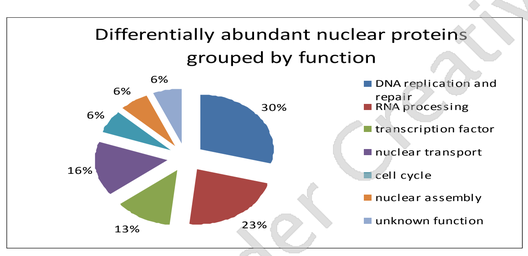
Drug synergy effect by Chou's combination index calculation system, based on viability result by MTT assay. If combination index < 1, there is synergy exist.

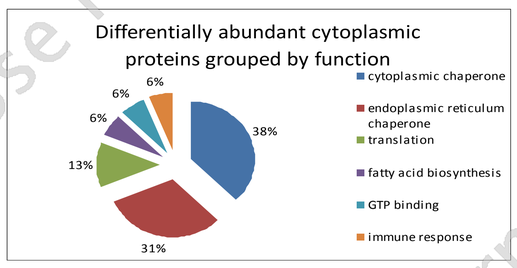
SNX-7081 drug mechanism

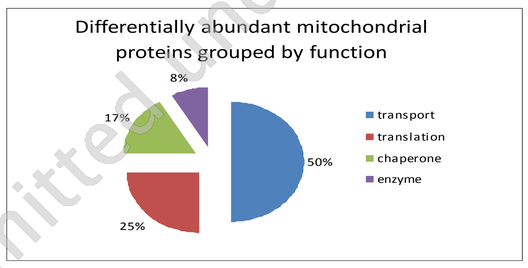
(iTRAQ-Isobaric tags for relative and absolute quantitation)



Treat/Control	Nuclear	Cytoplasm	Mitochondria
p<0.05, fold change>2	1	6	2
p<0.05, fold change<2	30	10	10

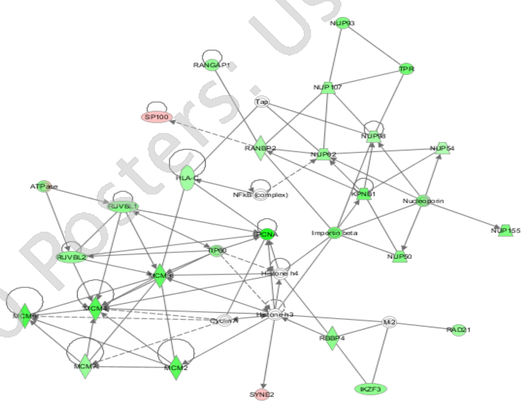


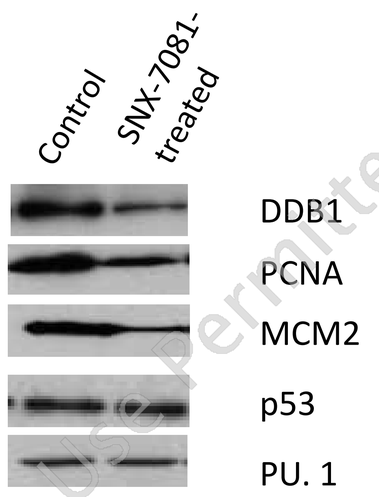




DNA repair network

by Ingenuity Pathway Analysis





Drug synergy mechanism

(label free proteomic analysis)

Sample preparation

Run triplicate sample in 1 D gel
↓
Cut 1D gel in 16 slices
↓
Trypsin in-gel digestion
↓
Run sample in LTQ-XL
↓
Protein ID against GPM database

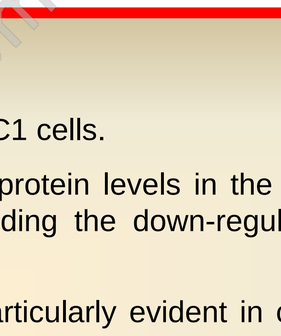
Data analysis

Analyze proteins identified in all three triplicates and a total spectral count of at least 6 in at least 1 condition
↓
NSAF(normalized spectral abundance factors) quantitation was performed based on spectral counts

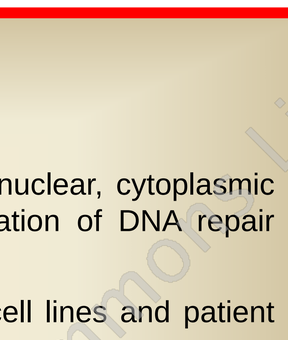
T-test analysis	Down-regulated	Up-regulated	unchanged
Control vs. FdA treated	71	148	1128
Control vs. SNX7081 treated	212	161	930
Control vs. FdA+SNX7081 treated	137	219	1059

Western blot of pH2AX, a DNA damage marker in MEC1 cell

DNA repair proteins down-regulated after SNX7081 treatment



DNA repair proteins down-regulated after FdA+SNX7081 treatment



DNA damage proteins up-regulated after FdA treatment

YWHAQ, YWHAZ, TOP2A, LOC728622/SKP1

DNA damage proteins up-regulated after FdA+SNX7081 treatment

YWHAQ, YWHAB, TOP2B, YWHAZ, TOP2A, LOC728622/SKP1

Summary

- Hsp90 inhibitor SNX-7081 induce apoptosis in MEC1 cells.
- iTRAQ analysis identified significant changes in protein levels in the nuclear, cytoplasmic and mitochondrial fractions of MEC1 cells, including the down-regulation of DNA repair proteins.
- Synergy between SNX7081 and fludarabine is particularly evident in cell lines and patient cells that are resistant to fludarabine.
- Label-free protein analysis identified DNA damage proteins increase in response to fludarabine and DNA repair proteins decrease in response to SNX7081.
- Our data suggest a possible mechanism for the p53-mutated synergy between these drugs, this combination may be effective for CLL patients for whom treatment options are currently very limited.