Alisertib Exhibits Broad Activity And Selectively Synergizes With Romidepsin Through Induction of Cytokinesis Failure in Preclinical Models of T-Cell Lymphoma

Kelly Zullo, MS[1*], Yige Guo, PhD[2], Laurence Cooke, B.A.[3], Xavier Jirau Serrano, BS[1], Michael Mangone, PhD[1], Luigi Scotto, PhD[1*], Jennifer E. Amengual, MD[1], Yinghui Mao, PhD[2], Renu Nandakumar[4], Serge Cremers[2,4], Jimmy Duong ,MPH[5] Daruka Mahadevan, MD, PhD[3] and Owen A. O'Connor, MD, PhD[1]

[1]Center for Lymphoid Malignancies, Columbia University Medical Center, New York, NY; [2] Department of Pathology and Cell Biology, Columbia University Medical Center, NY, NY; [3] University of Tennessee Health Science Center, West Cancer Center, Memphis, TN; [4]Irving Institute for Clinical and Translational Research, Columbia University Medical Center, New York, NY [5] Mailman School of Public Health, Columbia University, New York, NY

Introduction

- Aurora A kinase (AAK), a serine-threonine protein kinase, regulates mitotic entry, spindle formation, and cytokinesis.
- Alisertib (A) is a selective AAK investigational inhibitor with demonstrated clinical activity in acute myeloid leukemia, peripheral T-cell lymphoma (PTCL), DLBCL and other hematologic malignancies.
- Herein we demonstrate the potent cytotoxicity and apoptotic effects of A in a panel of T-cell lymphoma cell-lines (TCL) (CTCL, HTLV+, T-ALL) and B-cell lymphoma cell-lines (DLBCL-ABC, DLBCL-GCB, MCL) alone and in combination with romidepsin (R).

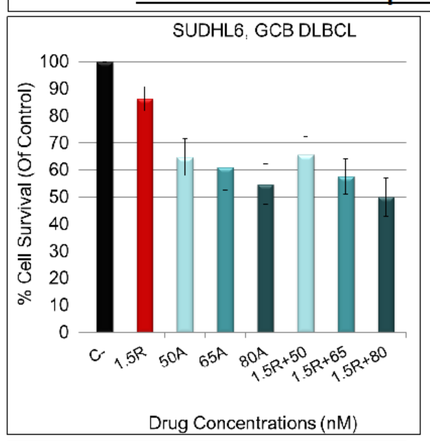
Materials and Methods

- Concentration : Effect cytotoxicity curves were evaluated with Cell Titer Glo Assay
- Apoptosis was quantitated by Alexa Fluor488/Annexin V, FACs flow cytometry was used to measure fluorescence signals.
- Cell Cycle was measured after 24 hours of drug incubation. Cells were suspended in Triton 0.1x containing RNase A and propidium iodide. The fluorescence signal was acquired by FACS flow cytometry.
- All antibodies used are from Cell Signaling
- 5-7 week old female SCID mice were injected subcutaneously with 5 X 10 ^{6} cells/ mL of HH cell line. Mice were randomized into 4 cohorts (control 0.01% DMSO, 1mg/kg R, 20mg/kg A, 1mg/kg R + 20mg/kg A) of 10 mice once tumor volume reached 50 -100 mm^3. The control and R cohorts were treated on days 1,9,16 via I.P. injection. A cohort was treated days 1-21 via oral gavage. Combination cohort was administered the same schedule as each single agent.

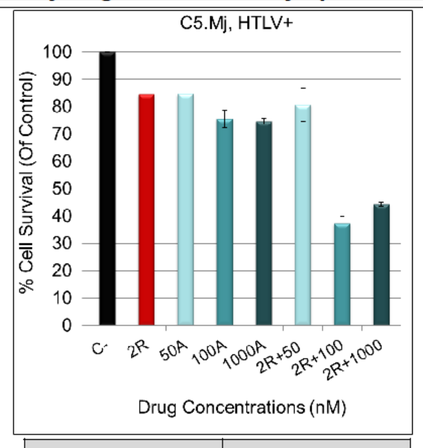
Alisertib Has Median IC50 of 105 nM Across Panel of Lymphoma Cell Lines

Subtype	Cell Line	48 hr	72 hr
ABC DLBCL	OCI-Ly10	268	58
	SU-DHL2	8	10
GCB DLBCL	OCI-LY7	180	81
	SU-DHL6	>1000	482
MCL	Jeko-1	38	29
	JVM-2	30	10
	Rec-1	78	87
	Z-138	22	13
TCL	H9	>1000	600
	HH	>1000	700
T-ALL	DND41	596	100
	CCL119	617	62
	J. Cam 1.6	260	105
	Sup-T1	547	2142
	Tib 152	>1000	800
ATLL- HTLV+	C5MJ	>1000	>1000

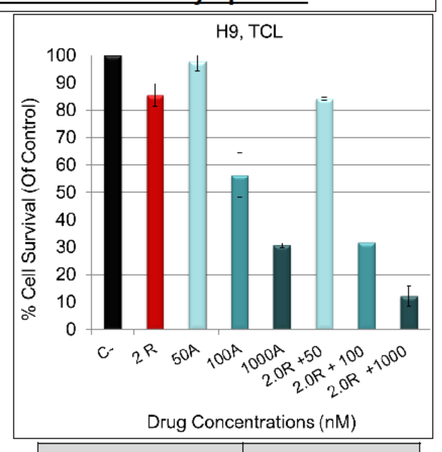
Alisertib Plus Romidepsin is Synergistic in T-Cell Lymphoma But Not in B-Cell Lymphoma



Drug (nM)	EOB
1.5R+50A	-9.70
1.5R+100A	-5.01
1.5R+1000A	-2.68



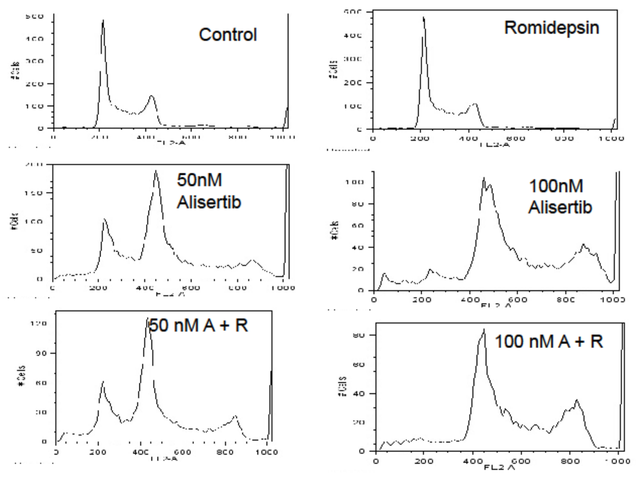
Drug (nM)	EOB
2R+50A	-9.05
2R+100A	26.54
2R+1000A	18.89



Drug (nM)	EOB
2R+50A	-0.61
2R+100A	16.54
2R+1000A	14.07

Alisertib plus Romidepsin Induces Polyploidy in TCL Cell Lines

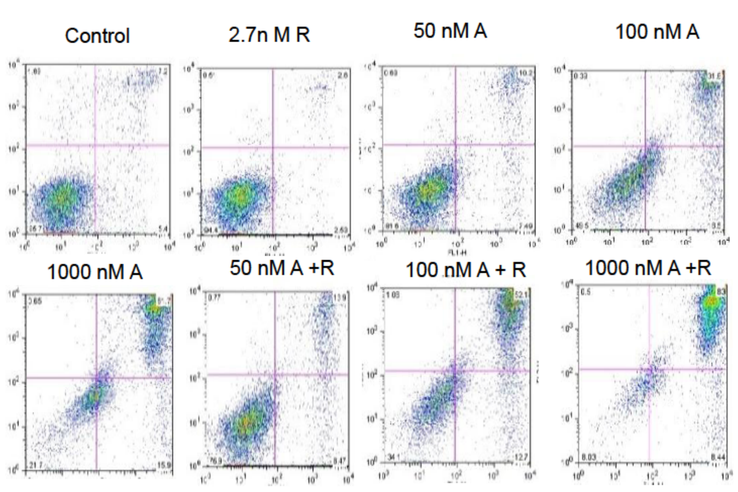
A. H9 (TCL)



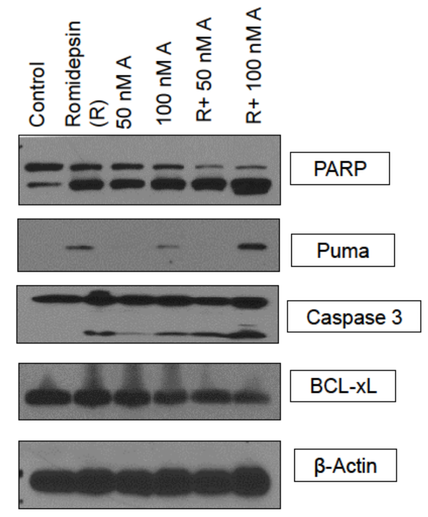
H9(TCL) Treatment Condition	% Of Cells In G1	% Of Cells In S	% Of Cells In G2	% Of Cells In Polyploidy
Control	43	22.6	21	6
Romidepsin (R)	46	23.3	19	4
50nM Alisertib	13	9.78	39	22
100nM Alisertib	4	4	34	47
R+50	13	10	39	25
R+100	2	5	39	49

Alisertib plus Romidepsin Induces Apoptosis

A. DND41 Cell Line at 72h

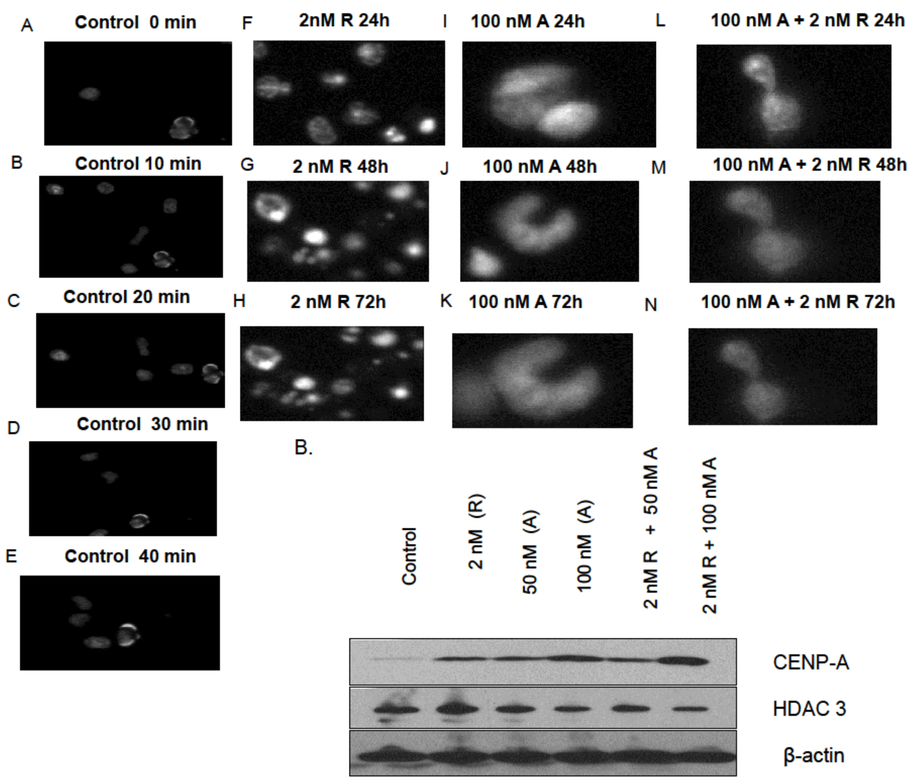


B. H9 (CTCL)



Alisertib plus Romidepsin Leads to Cytokinesis Failure

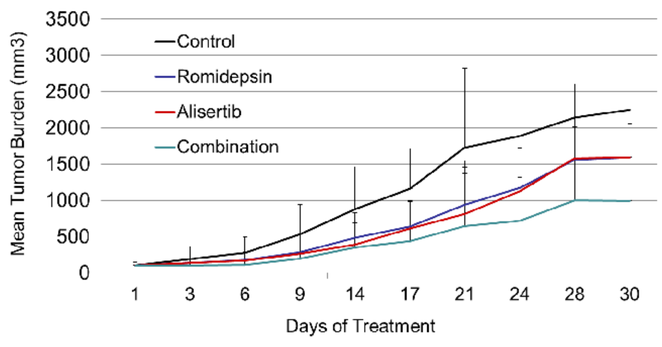
Select still frames from live cell imaging



B.

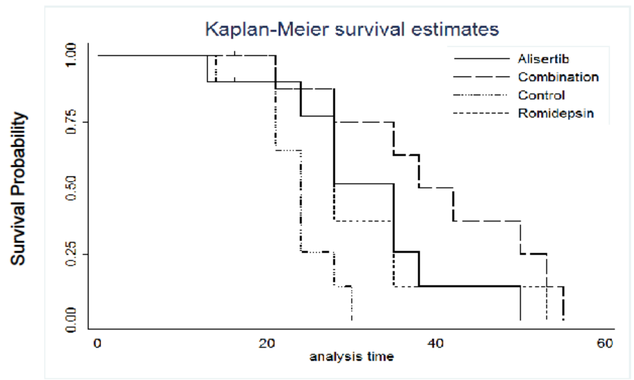
Alisertib Plus Romidepsin Prolongs Overall Survival in a Mouse Model of T-Cell Lymphoma

A.



Comparison Type	Linear Mixed Model	Regression coefficient	Std. Error	*p*-value
Compared to Control intercepts	Intercept	4.56	0.29	<0.0001
	Days of treatment	0.13	0.008	<0.0001
	Romidepsin	-0.07	0.41	0.87
	Alisertib	-0.12	0.41	0.77
	Romidepsin +Alisertib	-0.47	0.41	0.25
Compared to Control Slope over time	Romidepsin **	-0.032	0.009	0.0005
	Alisertib **	-0.036	0.009	0.0001
	Romidepsin + Alisertib **	-0.050	0.009	<0.0001

B.



Survival Curve			
Treatment 1	Treatment 2	Day 30 *p-value*	Day 58 *p-value*
Control	Combination	**0.004213**	**0.0000829**
Alisertib	Combination	**0.053398**	**0.0338372**
Romidepsin	Combination	0.326020	**0.0196656**
Control	Alisertib	0.072655	0.0881032
Control	Romidepsin	**0.040920**	0.0651008
Alisertib	Romidepsin	0.585513	0.8670352

Conclusion

- The mean IC50 of A at 48 hours was 350 nM (range 100-1000 nM) in TLC and 200 nM (range 20-300 nM) in BCL
- Alisertib revealed potent synergy with romidepsin in TCL
- Apoptosis was observed in TCL (DND41 and H9 cell line) following 72 hours of treatment of alisertib plus romidepsin
- Cell cycle analysis confirmed G2-M arrest and polyploidy following 24 hours of treatment with alisertib and romidepsin as a single agent and in combination
- Alisertib plus romidepsin prolonged overrall survival in a TCL mouse model
- This combination is being studied clinically in a phase I trial of refractory lymphoma (NCT01897012)

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