#42P - BAL0891: a novel, small molecule, dual TTK/PLK1 mitotic checkpoint inhibitor (MCI) with potent single agent activity

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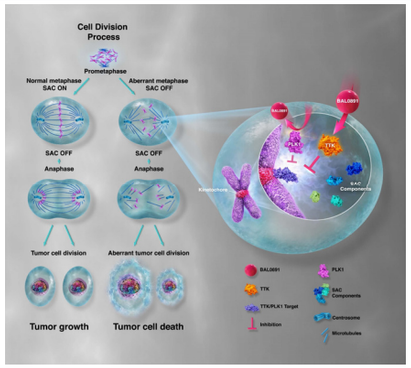
Background: BAL0891 is a dual inhibitor of threonine tyrosine kinase (TTK) and polo-like kinase 1 (PLK1). These kinases collaborate in activating the mitotic spindle assembly checkpoint (SAC) at the kinetochore (KT) ensuring correct chromosome alignment and segregation prior to mitotic exit.

SAC deactivation by BAL0891 causes aberrant tumor cell division

Prolonged inhibition of TTK combined with a transient effect on PLK1 leads to a rapid disruption of the SAC leaving tumor cells without adequate time for correct chromosome segregation.



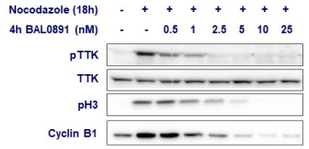
Prolonged TTK and transient PLK1 inhibition associated with aberrant mitotic exit

BAL0891 has dual activity on TTK and PLK1 (a). Reduced expression of mitotic markers cyclin B1 and phospho-histone H3 (pH3) in HT29 tumor cells showed that BAL0891 treatment caused a break of the mitotic block induced by nocodazole (Noc, 25 ng/mL) treatment (b), while the PLK1-specific inhibitor, onvansertib (Onv), maintained cells in mitosis (c). BAL0891 treatment forced HT29 cells blocked in mitosis (with 100 ng/mL Noc or 200 nM Onv, 7h) to exit mitosis leading to increased ploidy (d & e) indicative of a dominant effect on TTK activity.

a

Potency on:	BAL0891
IC50 TTK (nM)	7/0.4*
IC50 PLK1 (nM)	72/46*
TTK target residency	>12 h**
PLK1 target residency	4 min**
Cellular pTTK (38 h drug wash-out)	>38 h

b



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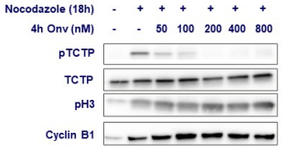

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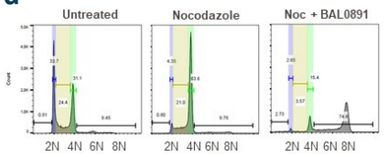

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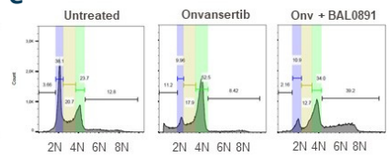
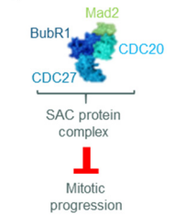


*IC50 calculations using two independent kinase assays
**Target residency by surface plasmon resonance

Dual TTK and PLK1 inhibition by BAL0891 causes a more efficient disruption of the SAC in HT29 tumor cells

TTK and PLK1 kinases collaborate in activating the SAC. SAC complex (a) immunoprecipitation showed BAL0891 treatment of (c) asynchronous HT29 cells or (b) cells blocked in mitosis by nocodazole (25-50 ng/mL) caused breakdown of the SAC complex, which occurred as early as 30' after BAL0891 addition to nocodazole-treated cells (d). Analysis of mitotic markers showed that SAC breakage preceded full mitotic exit (e). SAC integrity at the KT was also analyzed by immunofluorescence (f), which showed that 1h BAL0891 treatment of mitotic HT29 cells resulted in a dramatic reduction in KT-associated SAC which was not observed with the TTK-specific inhibitor CFI-402257 in the same conditions. Moreover, combination of CFI-402257 with onvansertib reproduced the single agent effect of BAL0891.

a



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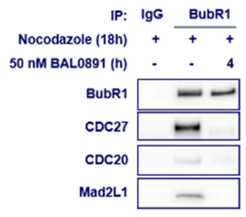

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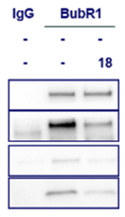

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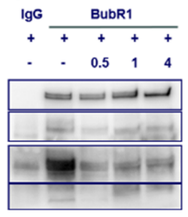

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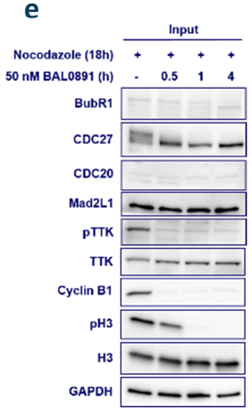

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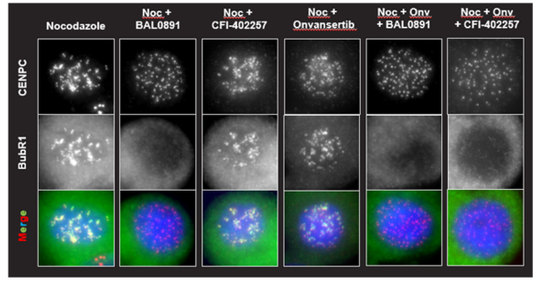
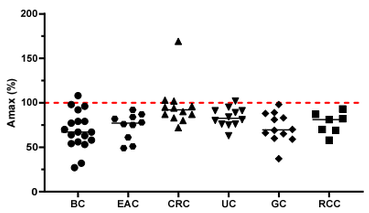


In the merged images the pink stain represents the centrosomal marker CENPC and the green stain the SAC component BubR1. Co-localization is indicated by a white stain. GI50 drug concentrations were used

BAL0891 has a broad anti-cancer potential *in vitro*

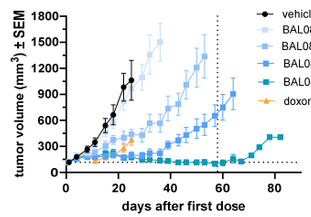
An *in vitro* anti-proliferative screen indicated a broad anti-cancer effect with low nM GI50s observed in most tumor lines and >5uM GI50s on non-immortalized cells. Maximal responses (Amax, shown), were determined from the concentration-response curves.



BAL0891 elicits potent, single agent, dose-dependent anti-tumor activity (with tumor-free animals) *in vivo*

Intermittent 2QW (a) and QW (b) IV-dosing of BAL0891 was well-tolerated with potent anti-tumor activity in the MDA-MB-231 breast xenograft model, associated with regressions and pathological cures; regressions (including pathological cures) were also observed in other models from different tumor types. Tumor TTK drug occupancy for up to one week was consistent with the efficacy of intermittent doses.

a



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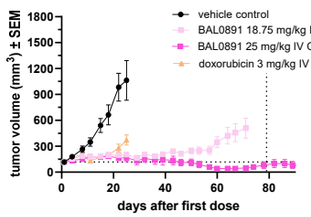

Dashed vertical lines indicate end of treatment

BAL0891	ΔT/C (day 25)	% Regressions (days)	Max % ΔBW ± SEM (on day)	100% TTK occupancy
3 mg/kg, 2QW, IV	0.90	0	12.4 ± 5.9 (32)	24/48 h
6.25 mg/kg, 2QW, IV	0.33	12.5 (29 - 53)	5.8 ± 3.7 (46)	48/72 h
9.4 mg/kg, 2QW, IV	0.08	12.5 (11 - 36)	10.3 ± 3.7 (46)	n.d.
12.5 mg/kg, 2QW, IV	0.07	62.5 (18 - 71)	9.3 ± 4.8 (64)	48/72 h

BAL0891	ΔT/C (day 25)	% Regressions (days)	Tumor-free (%)	Max % ΔBW ± SEM (on day)	100% TTK occupancy
18.75 mg/kg, QW, IV	0.09	12.5 (36 - 71)	-	11.9 ± 4.8 (50)	n.d.
25 mg/kg, QW, IV	0.05	62.5 (22 - 99)	25	-10.1 ± 3.0 (39)	144 h

Target occupancy measured by trypsin digestion & LC MS/MS analysis of isolated drug-unoccupied TTK

Conclusion: BAL0891 is a novel, first-in-class, dual TTK/PLK1 mitotic checkpoint inhibitor. In tumor cells, a prolonged effect on TTK combined with a transient effect on PLK1 contributes to rapid SAC disruption and aberrant mitotic exit, associated with potent single agent activity in mouse models of human cancer. This data warrants further investigation of BAL0891 in the treatment of human cancer, and a Phase 1 clinical trial is planned in 2022.