Targeting the mitotic kinesin, KIF18A, in chromosomally unstable cancers

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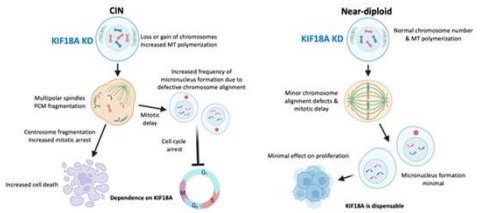
Abstract 4965

INTRODUCTION

Chromosome instability (CIN), characterized by frequent and ongoing loss or gain of chromosome number, is commonly observed in tumor cells. Although long recognized as a potential therapeutic vulnerability, CIN selective therapeutic targets have only recently been discovered. Genetic studies from multiple groups have identified the mitotic kinesin, KIF18A, as selectively essential for proliferation of CIN and aneuploid cells[1-3]. KIF18A, a member of the kinesin-8 family, is an ATP-dependent plus end microtubule motor protein[4]. In normal cells, genetic depletion of *KIF18A* results in minor chromosome alignment defects, elongation of mitotic spindles, and transient activation of the spindle assembly checkpoint. However, in CIN cells, *KIF18A* depletion results in profound chromosome alignment defects, multipolar spindles, chromosome fragmentation and extended spindle checkpoint activation. Thus, unlike inhibitors of the pan-essential mitotic kinesins, Eg5 and CENPE, KIF18A inhibition is predicted to be well tolerated[5,6]. By targeting KIF18A genetically and with novel small molecule inhibitors here we present data supporting KIF18A as a therapeutic target in CIN$^+$ tumors.



Graphical abstract Schematic depicting the effect of KIF18A kncockdown (KD) on cells with CIN$^+$ (left) and near-diploid cells (right). Adapted from *Marquis, et al.* Nat. Commun., 2021. Generated using BioRender.

MATERIALS & METHODS

To explore the therapeutic potential of KIF18A inhibition we evaluated the effects of *KIF18A* genetic depletion and small molecule inhibition in both CIN$^+$ and CIN$^-$ cell lines. Biochemical, cell proliferation, and phenotypic assays were used to characterize the potency and cellular activity of reference (Compound 1[7] and Compound 3[8]) and novel KIF18A small molecule inhibitors (APRN-A and APRN-B). Anti-tumor activity of KIF18A inhibitors was assessed in the CIN$^+$ cell line, OVCAR3.

RESULTS

In triple negative breast and colorectal CIN$^+$ cancer cell lines siRNA mediated *KIF18A* knockdown and small molecule inhibition of the KIF18A ATPase activity both lead to a reduction in proliferation associated with an increase in mitotic index and multi-polar spindles. Consistent with *KIF18A* knockdown, KIF18A inhibition results in increased spindle length, chromosome alignment defects, and inhibition of proliferation (Figures 1-5). Importantly, these effects are not observed with *KIF18A* knockdown or inhibition in non-transformed, near diploid cells. In vivo, treatment of CIN$^+$ xenograft tumors with potent, novel KIF18A inhibitors results in robust anti-tumor activity with minimal impact on body weight (Figure 7).

RESULTS

Treatment of CIN$^+$ cancer cell lines with small molecule inhibitors or siRNA KD of KIF18A inhibits CIN$^+$ cell proliferation

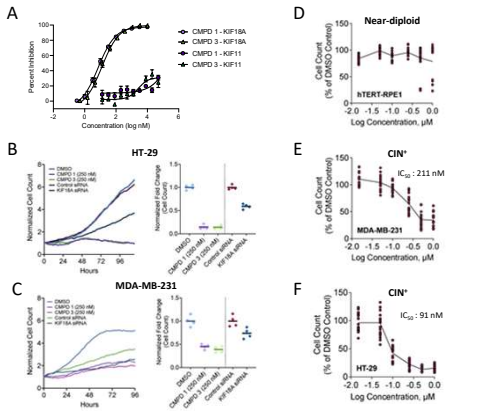


Figure 1. In vitro characterization of Compounds 1 and 3 (A) Human KIF18A or KIF11 motor domain was treated with the indicated concentrations of Compounds 1 or 3 in the presence of microtubules. The ATPase activity was reported as percent inhibition. **(B)** HT-29 (CIN$^+$) and **(C)** MDA-MB-231 (CIN$^+$) cells were treated with 250 nM of Reference Compounds 1 or 3 or the indicated siRNAs for 6 hours prior to the start of the proliferation assay. Cells were imaged every 2 hours to determine Normalized Cell Count. Normalized fold change was calculated by determining the fold change from 0 to final timepoint for each condition and normalizing to control (DMSO or control siRNA). **(D)** hTERT-RPE1 (near-diploid), **(E)** MDA-MB-231, and **(F)** HT-29 cells were treated with the indicated concentrations of Compound 3 for 6 hours before the start of the 4-day proliferation assay. Cells were imaged and quantified to determine the Cell Count as a % of DMSO.

KIF18A inhibition results in an increase in mitotic index and frequency of multipolar spindles in CIN$^+$ cells but not normal cells

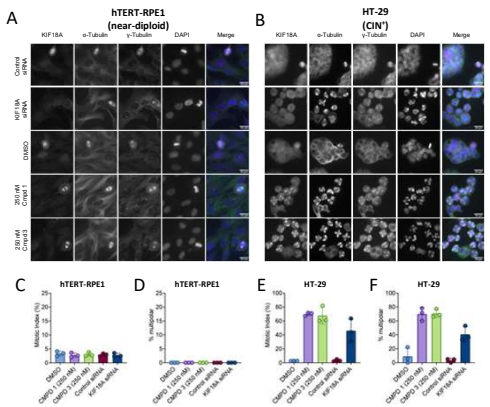


Figure 2. KIF18A inhibition phenocopies *KIF18A* depletion (A) hTERT-RPE1, and **(B)** HT-29 cells were treated with the indicated concentrations of Compound 1 or 3 or with indicated siRNAs for 24 hours prior to fixation and staining for immunofluorescence. Scale bar 20 µm. **(C,E)** Mitotic Index is the percentage of cells observed in mitosis. **(D,F)** Percent multipolar represents the percentage of mitotic cells with greater than 2 spindle poles.

Treatment of MDA-MB-231 (CIN$^+$) cells with KIF18A inhibitors alters chromosome alignment and spindle length

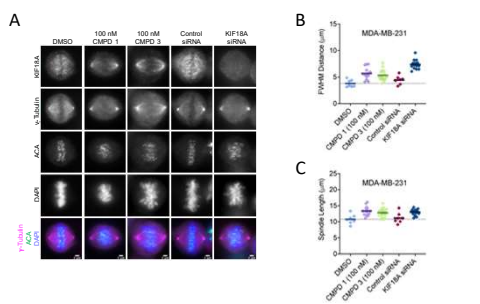


Figure 3. Chromosome alignment and spindle length (A) MDA-MB-231 (CIN$^+$) cells were treated with the indicated concentrations of KIF18A Reference Compound 1 or 3 or with the indicated siRNAs for 24 hours before fixation and immunofluorescence. Scale bar 2 µm. **(B)** Chromosome alignment[9] (FWHM, Full Width at Half Maximum) and **(C)** Spindle Length quantification.

KIF18A inhibition results in rapid relocalization of KIF18A to the spindle poles

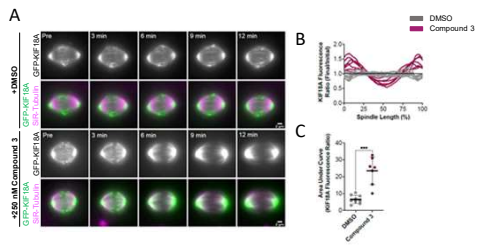


Figure 4. KIF18A Localization (A) Time-lapse imaging of hTERT-RPE1 cells inducibly expressing WT GFP-KIF18A after knock-down of endogenous *KIF18A* at indicated timepoints before and after addition of 250 nM of Compound 3 or DMSO (GFP-KIF18A: green, SiR-Tubulin: magenta). Scale bar 2 µm. (B) Graph of GFP-KIF18A fluorescence signal (final fluorescence values divided by initial fluorescence values capturing the entire spindle) versus percent Spindle Length. Each line represents an individual cell. (C) Quantification of area under the curve from graph in (B). Bars are mean ± standard deviation. Mann-Whitney test, P < 0.001 (***).

Novel compounds selectively inhibit KIF18A ATPase activity resulting in mitotic arrest and reduced proliferation in OVCAR3 (CIN$^+$) cells

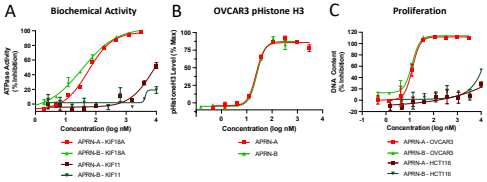


Figure 5. Effects of Apeiron KIF18A inhibitors on ATPase activity, pHistone H3, and proliferation. (A) Human KIF18A or KIF11 ATPase was treated with the indicated Apeiron KIF18A inhibitors (APRN-A, APRN-B) and the biochemical activity was reported as percent inhibition. **(B)** OVCAR3 (CIN$^+$) cells were treated with the indicated compounds for 16 hours prior to the start of the pHistone H3 assay. Total Ser10 phosphorylated Histone H3 was measured by In-Cell Western. **(C)** OVCAR3 (CIN$^+$) or HCT116 (CIN$^-$) cells were treated with the indicated compounds for 72 hours prior to measuring proliferation. Proliferation was determined by monitoring the relative DNA content and is presented as percent inhibition.

CryoEM provides an inhibitor bound structure complex

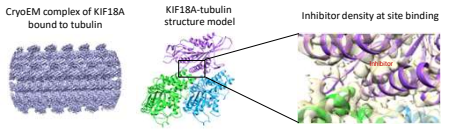


Figure 6. CryoEM structure complex at resolution of 4.1 Å. This structure complex provides insights on allosteric mechanism at the atomic level. Our inhibitor acts as a molecular glue to trap KIF18A in an inactive state bound with tubulin.

Novel KIF18A inhibitors demonstrate potent anti-tumor activity in CIN$^+$ xenograft tumors while sparing body weight

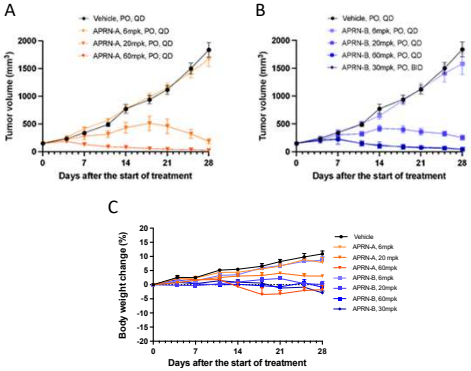


Figure 7. In vivo efficacy of novel KIF18A inhibitors in OVCAR3 xenograft tumor model. Tumor bearing mice were treated with the indicated compounds **(A)** APRN-A or **(B)** APRN-B at the specified doses, routes, and frequencies (n=8 mice/group) and tumor volumes were graphed **(A-B)**. **(C)** Graph of percent body weight change for each treatment group. Dosing, PO: orally, QD: once/day, BID: twice/day.

CONCLUSIONS

Chromosome instability (CIN) is a phenomenon commonly seen in tumor cells. Collectively our data support KIF18A as a therapeutic target and provides rationale for the continued development of potent and selective small molecule KIF18A inhibitors for the treatment of CIN$^+$ cancers. We demonstrate:

- Inhibiting KIF18A with small molecules phenotypically resembles *KIF18A* knockdown in CIN$^+$ cell lines
- Mitotic motor inhibition is a therapeutic modality for CIN$^+$ cancers
- Our novel KIF18A inhibitors are potent, selective, and exhibit in vivo efficacy in a CIN$^+$ xenograft tumor model

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