# Inhibition of KIF18A Leads to Mitotic Arrest and Robust Anti-Tumor Activity in Chromosomally Instable Tumors

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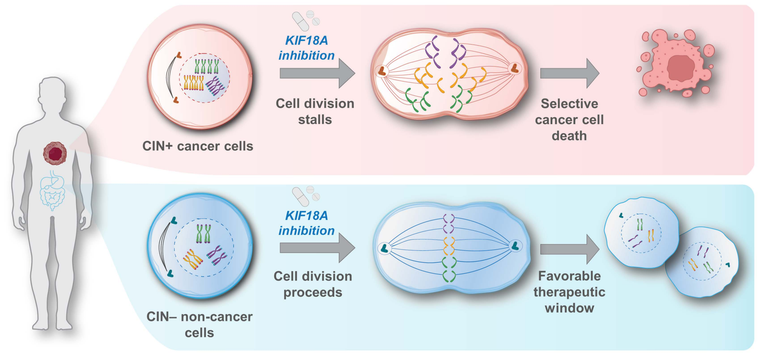
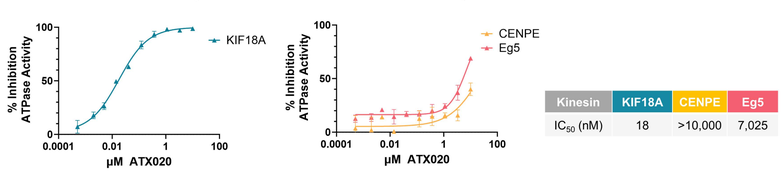
## Mitotic Kinesin KIF18A is a Selective Vulnerability in Chromosomally Instable (CIN) Tumors

- KIF18A is a plus-end directed kinesin that facilitates chromosome alignment and spindle microtubule dynamics during mitosis[1]
- Cells with ongoing chromosomal segregation defects, such as a subset of aneuploid or whole genome doubled cells, are vulnerable to disrupted mitosis when KIF18A is lost; thus, KIF18A is a compelling target for oncology[2,3,4,5]
- A proprietary Accent Therapeutics tool compound, ATX020, is a potent and selective inhibitor of KIF18A
- ATX020 treatment inhibits proliferation in CIN positive ovarian cancer cell lines, while CIN negative cell lines proliferate normally
- ATX020 also leads to mitotic arrest and DNA damage in sensitive cell lines, as exemplified by upregulation of p-HH3 and γH2AX, respectively
- Consistent with these effects and the role of KIF18A in facilitating chromosome positioning, chromosomally instable OVCAR-3 cells exhibit fragmented nuclei and malformed mitotic spindles upon KIF18A inhibition with ATX020, leading to G2M arrest and apoptosis
- Once daily oral dosing of ATX020 leads to robust, dose-dependent regression of OVCAR-3 xenograft tumors; CIN negative OVK18 tumors are unaffected as expected
- Together these results demonstrate that inhibition of KIF18A is a compelling strategy in chromosomally instable tumors such as ovarian cancer



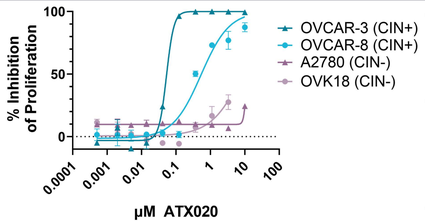

## ATX020 is a Potent and Selective Inhibitor of KIF18A




| Kinesin | KIF18A | CENPE | Eg5 |
|---|---|---|---|
| $IC_{50}$ (nM) | 18 | >10,000 | 7,025 |

- ATX020, a proprietary Accent tool compound, inhibits KIF18A biochemical activity with an $IC_{50}$ of 18 nM
- ATX020 is selective for KIF18A over other mitotic kinesins such as CENPE (>555X) and Eg5 (390X)

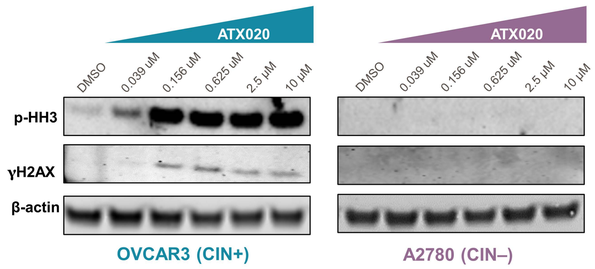
## ATX020 Selectively Inhibits Proliferation of Chromosomally Instable Ovarian Cancer Cell Lines




| Cell Line | OVCAR-3 | OVCAR-8 | A2780 | OVK18 |
|---|---|---|---|---|
| $IC_{50}$ (nM) | 53 | 534 | >10,000 | 8763 |

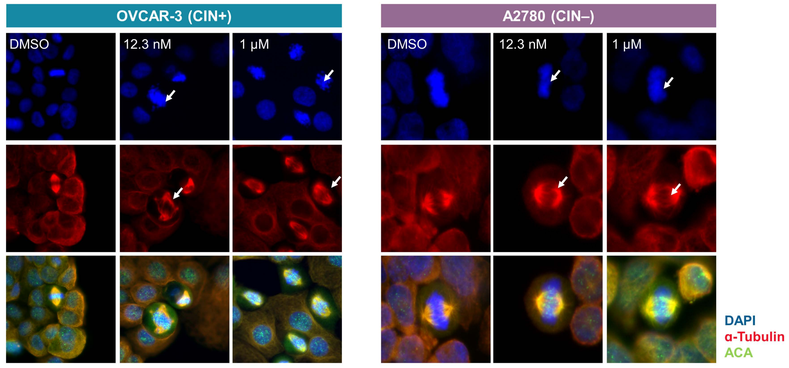
- Ovarian cancer cell lines were treated with ATX020 for 7 days and proliferation was assessed via Cell Titer Glo
- Proliferation of chromosomally instable[6,7] OVCAR-3 and OVCAR-8 cell lines is robustly inhibited by KIF18A inhibition via ATX020
- In contrast, A2780 and OVK18 cell lines, which do not exhibit chromosomal instability[8], proliferate normally upon ATX020 treatment

## KIF18A Inhibition Selectively Induces Mitotic Arrest and DNA Damage in Chromosomally Instable Cell Lines



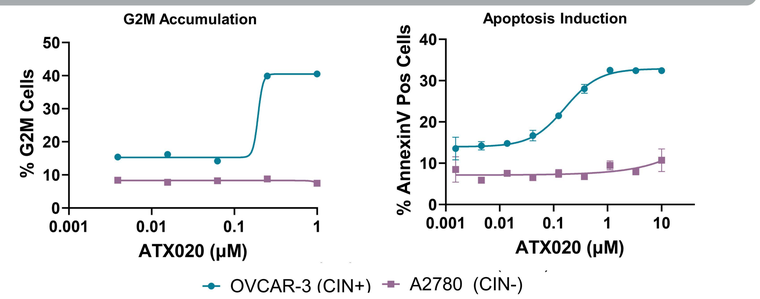

- 24 hours of treatment with ATX020 is sufficient to induce p-HH3 in OVCAR-3 cells, consistent with cells arresting in mitosis due to dependency on KIF18A for M phase progression
- A2780 cells, which are not dependent on KIF18A, do not exhibit elevated p-HH3, consistent with the lack of anti-proliferative effects of ATX020 in this cell line
- γH2AX induction is selectively observed in OVCAR-3 cells, suggesting that inhibition of KIF18A in these cells induces DNA damage, leading to failed progression through M phase

## Aberrant Mitotic Spindle Formation and Fragmented Nuclei in KIF18A Inhibitor-Treated CIN+ Cells



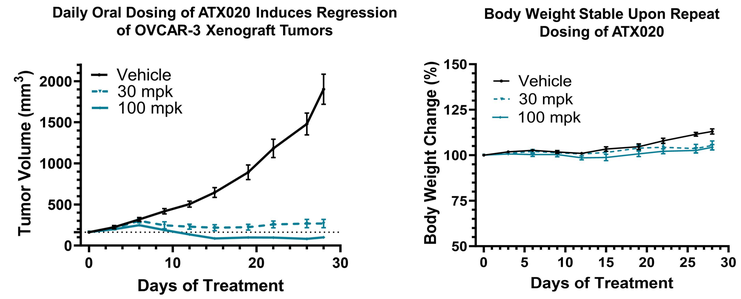

- Treatment of OVCAR-3 cells with ATX020 for 24 hours leads to malformed mitotic spindles and fragmented nuclei; in contrast, mitosis proceeds normally in A2780 cells

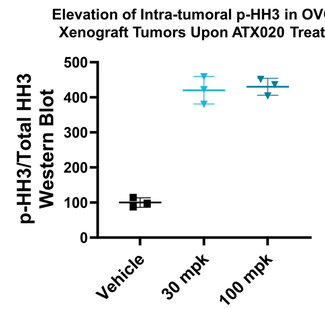
## KIF18A Inhibition Leads to G2M Accumulation and Apoptosis in CIN+ Cells




- Consistent with the above results, OVCAR-3 cells treated with ATX020 arrest in G2M (as assessed by Edu/Far Red 647 DNA, 24 hrs); as expected this is not observed in A2780 cells
- ATX020 treatment also induces dose-dependent and rapid (24 hours) apoptosis in OVCAR-3, but not A2780 cells; together these results demonstrate that KIF18A inhibition leads to catastrophic cell cycle arrest and apoptosis in CIN+ cancer cells

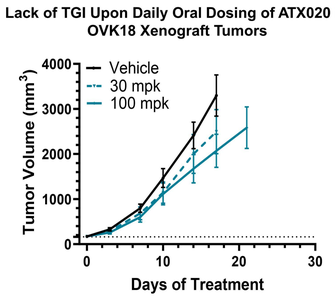
## Once Daily Oral Dosing of ATX020 Leads to Robust Anti-Tumor Activity in OVCAR-3 Xenograft Tumors




- Daily oral dosing of ATX020 for 28 days leads to robust tumor growth at 30 mpk and tumor regression at 100 mpk
- ATX020 treatment is well-tolerated, with no effects on body weight
- Consistent with the mechanism of action of KIF18A inhibition, tumors treated for 7 days exhibit robust and dose-dependent induction of p-HH3 at 6 hours post-final dose

## Anti-Tumor Activity is Selective for CIN+ Tumors; Lack of Tumor Growth Inhibition in OVK18 Xenograft Model




- OVK18 xenograft tumors were dosed for up to 21 days with once daily oral ATX020; all 3 groups were terminated by 21 days due to high tumor volume
- Body weight remained stable (not shown) consistent with the OVCAR-3 BW data above
- As expected for the CIN– OVK18 model, significant TGI was not observed upon ATX020 treatment

## Conclusions

- Accent Therapeutics tool compound ATX020 is a selective KIF18A inhibitor that exhibits robust anti-proliferative effects on CIN+, but not CIN–, cancer cell lines
- ATX020 leads to mitotic arrest and apoptosis due to DNA damage and malformed mitotic spindles in CIN+ cells
- Consistent with these results, ATX020 leads to robust and specific tumor growth inhibition in a CIN+ xenograft model
- Together these results demonstrate the potential for KIF18A inhibition in chromosomally instable tumors such as ovarian cancer

## Acknowledgements & References

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