INX-315, a potent and selective CDK2 inhibitor, demonstrates robust antitumor activity in CCNE1-amplified cancers

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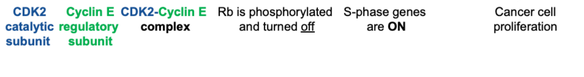
Introduction

- Cyclin-dependent kinases (CDK) are a family of serine/threonine kinases that heterodimerize with regulatory subunits called cyclins to drive cell cycle progression
- Uncontrolled cellular proliferation is a hallmark of cancer commonly driven by dysregulated kinase activity of specific CDK family members, including cyclin-dependent kinase 2 (CDK2)
- Cyclin E1 and E2 are critical cell cycle regulators, that bind to CDK2 to drive transition from G1 to S phase of the cell cycle (Figure 1)
- Amplification/overexpression of CCNE (cyclin E1 and E2) is the most frequent mechanism of aberrant CDK2 activity and occurs across a broad range of solid tumors including gynecological, breast, bladder, lung, gastric, and others[2,3]
- Aberrant activation of CDK2 is a known mechanism of resistance that allows hormone receptor-positive (HR+) and HER2-negative (HER2-) breast cancer to escape CDK4/6 inhibition[1]
- Selective inhibition of CDK2 is thus a compelling therapeutic approach to regain cell cycle control in tumor cells with amplification or overexpression of CCNE
- We report preclinical data supporting the development of INX-315 for patients with CDK2-dependent cancer

Figure 1. Aberrant CDK2/Cyclin E activity drives G1 to S phase transition leading to uncontrolled cellular proliferation



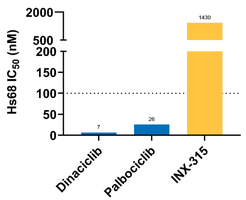
Results

Table 1. INX-315 is a selective and potent CDK2 inhibitor

		INX-315						Kinome S (10)[c]
		CDK1/B	CDK2/A	CDK2/E	CDK4/D1	CDK6/D3	CDK9/T	
Biochemical IC_{50} (nM)[a]	IC_{50} (nM)	30	2.4	**0.6**	133	338	73	0.012
	Fold vs CDK2/E	*55*	*4*	*1*	*241*	*615*	*132*	
NanoBRET (nM)[b]	IC_{50} (nM)	374	71.3	**2.3**	ND	ND	2950	
	Fold vs CDK2/E	*163*	*31*	*1*	-	-	*1282*	

[a]The biochemical IC_{50} of INX-315 was assessed against selected CDKs and their canonical cyclin binding partners in 12-point dose response format at the Km for ATP (Nanosyn). Results showed INX-315 is a picomolar inhibitor of CDK2/cyclin E1 with selectivity over other CDKs. [b]The intracellular IC_{50} was determined using the NanoBRET displacement assay (Reaction Bio) and showed INX-315 is cell permeable and its selectivity and potency is maintained in a cellular environment. [c]A kinome screen was performed to assess selectivity against the broader kinase family (Thermo) and showed 1.2% of kinases had greater that 90% inhibition at 100 nM INX-315. Hits were followed up individually and based on the results, it is unlikely that INX-315 will produce adverse effects due to off-target kinase inhibition. (ATP, adenosine triphosphate; CDK, cyclin-dependent kinase; IC_{50}, half-maximal inhibitory concentration; nM, nanomolar)

Figure 2. Normal cells are insensitive to INX-315

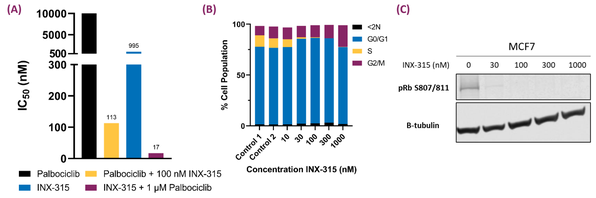


INX-315 does not inhibit proliferation of normal cells. Hs68 human fibroblasts were treated with dinaciclib (pan-CDK inhibitor), palbociclib (selective CDK4/6i), and INX-315 (selective CDK2 inhibitor) in a 10-point dose response format to determine the IC_{50} using the CTG assay from Promega. Hs68 cells were sensitive to dinaciclib and palbociclib and insensitive to INX-315.

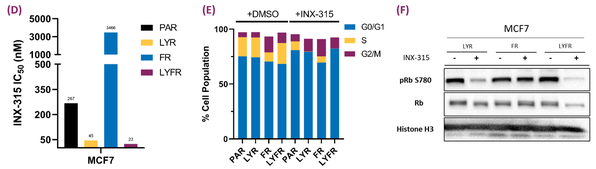
(CTG, Celltiter-Glo; CDK4/6i, CDK4 and CDK6 dual inhibitor)

Figure 3. INX-315 restores sensitivity to CDK4/6i in HR+ breast cancer

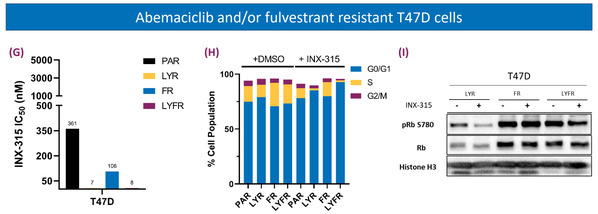
Palbociclib resistant MCF7 cells



Abemaciclib and/or fulvestrant resistant MCF7 cells



Abemaciclib and/or fulvestrant resistant T47D cells



(A) INX-315 restores sensitivity to palbociclib resistant cells. MCF7 cells resistant to palbociclib were treated with a dose curve of palbociclib or INX-315 with or without the other inhibitor present at a fixed concentration. INX-315 re-sensitizes the cells to palbociclib, reducing the IC_{50} from over 10 µM to 113 nM.

(B) INX-315 arrests palbociclib resistant cells in G1. MCF7 cells resistant to palbociclib were treated with a dose curve of INX-315 and 1 µM palbociclib. Cell cycle was assessed using EdU and DNA labeling analyzed by flow cytometry.

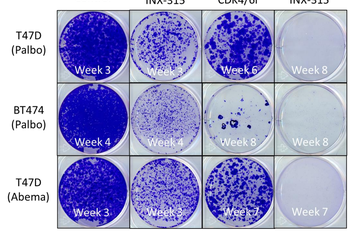
(C, F, I) INX-315 inhibits Rb phosphorylation. Treatment of palbociclib or abemaciclib resistant breast cancer cells with a combination of CDK4/6i and INX-315 shows a reduction in pRb. Resistant cells were co-treated with their resistant drug(s).

(D, G) T47D and MCF7 cell lines with resistance to abemaciclib and/or fulvestrant show sensitivity to INX-315. Cells with resistance to fulvestrant, abemaciclib, or both were treated with their corresponding resistant drug and a dose curve of INX-315. INX-315 has low, nanomolar IC_{50}s in cells with resistance to CDK4/6 inhibitors.

(E, H) INX-315 induces a cell cycle arrest in cells resistant to abemaciclib. INX-315 treatment with the corresponding resistant drug eliminates cells in S phase and primarily arrests cells in G1. INX-315 concentration was 100 or 300 nM for T47D and MCF7.

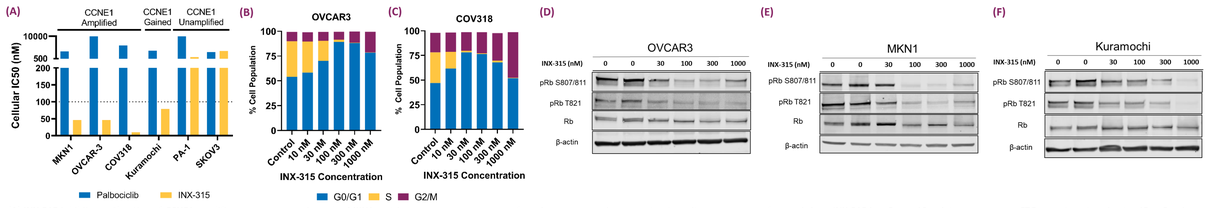
(Rb, retinoblastoma protein; pRb, phosphorylated Rb; PAR: parental line; LYR: cell line resistant to abemaciclib; FR: cell line resistant to fulvestrant; LYFR: cell line resistant to abemaciclib and fulvestrant)

Figure 5. INX-315 delays time to CDK4/6i resistance in HR+ breast cancer



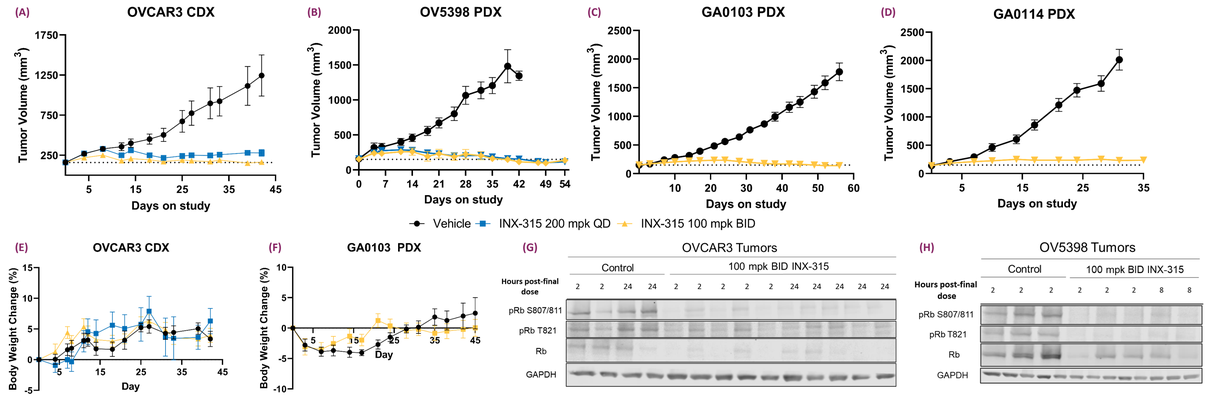
INX-315 delays the time to CDK4/6i resistance in HR+ breast cancer. HR+ breast cancer cells (not resistant to CDK4/6i) were treated with a CDK4/6i (palbociclib or abemaciclib) and/or INX-315 for up to 8 weeks and monitored for growth using crystal violet colony assay. INX-315 alone reduced growth but not as effectively as the CDK4/6 inhibitors. While palbociclib and abemaciclib slowed growth, cells were ultimately able to overcome the treatment. Cells treated with both INX-315 and a CDK4/6i resulted in no noticeable growth after 8 weeks.

Figure 6. *CCNE1*-amplified cells are sensitive to INX-315 and arrest in G1



(A) INX-315 inhibits proliferation of *CCNE1*-amplified or over expressing cells. MKN1 gastric cancer cells and a panel of ovarian cancer cell lines were treated with a dose curve of palbociclib or INX-315 in a 6-day, 10-point dose response CTG assay to determine the IC_{50}. Cell lines with increased *CCNE1* are sensitive to INX-315 treatment but not palbociclib, while cancer cells without increased *CCNE1* were insensitive to INX-315 treatment. Collectively, these data demonstrate the selectivity of INX-315 for cancer cells that are CDK2-dependent.
(B-C) *CCNE1*-amplified cells are arrested in G1 by INX-315. *CCNE1*-amplified ovarian cancer cells were treated for 24 hours at the indicated concentrations of INX-315, which induced a dose-dependent G1 cell cycle arrest.
(D-F) INX-315 inhibits Rb phosphorylation. INX-315 treatment at 30 to 100 nM for 24 hours results in decreased phosphorylation of Rb at multiple sites.

Figure 7. INX-315 displays single agent efficacy in *CCNE1*-amplified ovarian and gastric tumor models



(A-D) INX-315 demonstrates robust single agent activity across *CCNE1*-amplified tumor models.
(A) OVCAR3 ovarian CDX mouse model: Mice were treated for 42 days and showed tumor stasis with 100 BID treatment or 89% TGI with 200 QD treatment. n = 10, no body weights loss was >5 % at any point.
(B) OV5398 ovarian PDX mouse model: Mice were treated for 56 days and showed tumor regression in both treatment groups. n = 10, no body weights loss was >5 % at any point.
(C) GA0103 gastric PDX mouse model: Mice were treated for 56 days and showed tumor stasis with 100 BID treatment. n = 8, no body weights loss was >5 % at any point.
(D) GA0114 gastric PDX mouse mode: Mice were treated for 35 days and showed 95% TGI with 100 BID treatment. n = 8, no body weights loss was >5 % at any point
(E-F) INX-315 is well tolerated in mouse models. Mice were monitored for the duration of each study and body weight measurements were taken twice per week. Figures are representative of body weights from the studies presented in A-D.
(G-H) INX-315 treatment reduces Rb phosphorylation. Tumors lysates from experiments in panels A and B were probed for phosphorylation and protein changes at the indicated times after the last dose.
(CDX, cell line-derived xenograft; PDX, patient-derived xenograft; QD, once per day; BID, twice per day; mpk, milligrams of drug per kilogram of mouse weight)

Conclusions

- INX-315 is a highly potent and selective CDK2 inhibitor
- In HR+/HER2- breast cancer models:
  - INX-315 restores CDK4/6 inhibitor sensitivity in HR+/HER2- breast cancer cell lines with acquired CDK4/6i resistance
  - INX-315 effectively delays acquired resistance to CDK4/6 inhibitors in HR+/HER2- breast cancer cell lines
- In *CCNE1*-amplifed cancer models:
  - INX-315 potently blocks Rb phosphorylation, arrests cells in the G1 phase of the cell cycle, and inhibits cell proliferation
  - INX-315 displays robust single agent *in vivo* efficacy
- INX-315 is currently enrolling INX-315-01: a first-in-human, Phase 1/2, open-label, dose escalation and dose-expansion study to evaluate the safety, PK, and preliminary antitumor activity of INX-315 in patients with advanced/metastatic cancers (NCT05735080)

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References

1. Tadesse et al. Drug Discov Today. 2020;25(2):406-13.
2. cbioportal.org (Cerami et al. 2012, Gao et al. 2013)
3. TCGA Research Network: https://www.cancer.gov/tcga2