Novel orally bioavailable macrocycles that target cyclin A and B elicit antitumor activity in breast cancer patient-derived xenograft models

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Background

Cyclin/cyclin-dependent kinase (CDK) complexes regulate the activity of RB1 and E2F to drive cell cycle progression while ensuring fidelity of DNA replication. The interaction between cyclins and a critical subset of their substrates, including RB1 and E2F, is mediated by a conserved short linear RxL motif. RxL peptides have been shown to inhibit substrate phosphorylation by CDK2/cyclin A (AACR 2022 poster #5379).

Previously, we have shown that macrocycles that selectively inhibit RxL-mediated binding to cyclins A and B (RxL inhibitors) (Figure 1) lead to growth inhibitory activity in multiple cancer cell lines, and result in tumor regression in small cell lung cancer (SCLC) and ovarian cancer xenograft models (AACR 2023 poster #1560).

Here, we test the antitumor activity of a cyclin A/B inhibitor CID-016 (CIR7-7321) in patient-derived xenograft (PDX) models from breast cancer. Although the focus of this work is on the effects of this compound in preclinical breast cancer models, cyclin A/B inhibitors have potential therapeutic application in a broader range of indications (Figure 2).

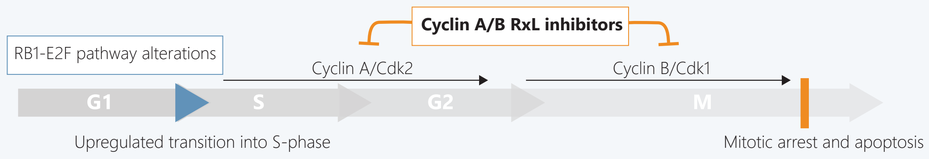


Figure 1 - Synthetic lethality model between cancer cells with RB1-E2F pathway dysregulation and cyclin A/B inhibition. RB1 and E2Fs are frequently altered in cancer. Cyclin/CDK complexes regulate the activity of RB1 and E2F to drive cell cycle progression while ensuring fidelity of DNA replication. RXL peptides have been shown to inhibit E2F recruitment and phosphorylation by CDK2/cyclin A, and to be synthetically lethal in cancer cell lines with RB1-E2F pathway dysregulation (Chen et al, 1999; Mendoza et al, 2003).

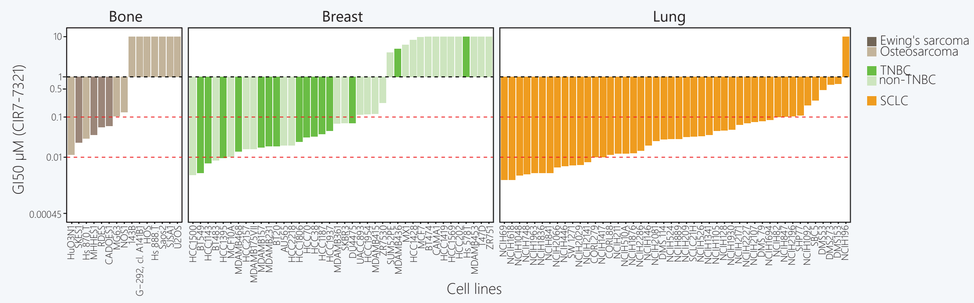
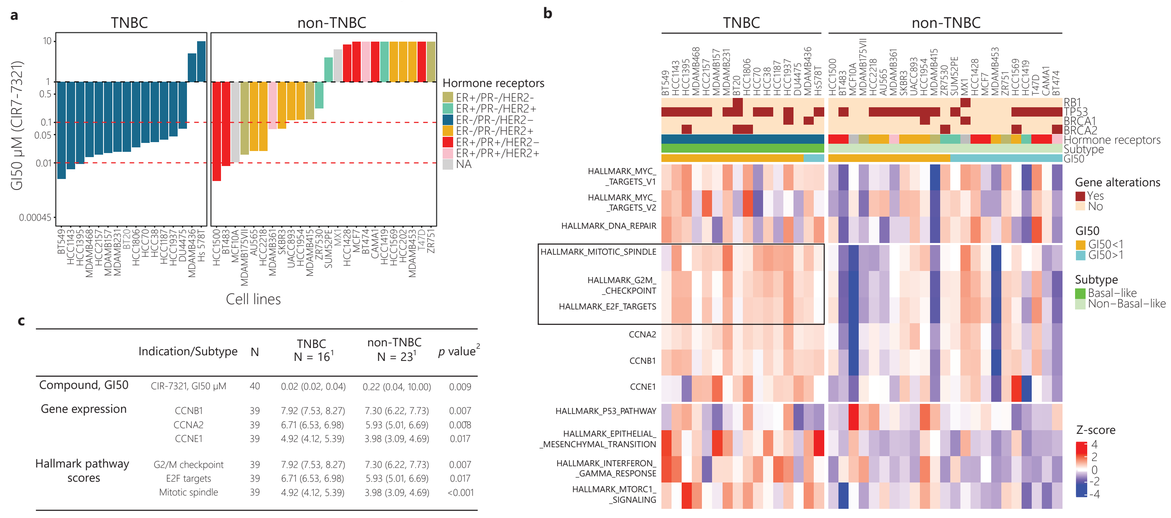


Figure 2 - Waterfall plot of *in vitro* response to CIR7-7321 (assessed by GI50) in a cell line panel representing breast, lung and osterosarcoma (including Ewing's sarcoma). Cell lines were exposed to CIR7-7321 for 4-8 days depending on length of time required for at least two cell doublings to occur. Cell growth inhibition was determined by Cell Titer Glo assay.

Results

1. Treatment of a breast cancer cell line panel with CIR-7321 demonstrated sensitivity in association with selected Hallmark pathway scores



c

	Indication/Subtype	N	TNBC N = 16[1]	non-TNBC N = 23[1]	*p* value[2]
Compound, GI50	CIR-7321, GI50 µM	40	0.02 (0.02, 0.04)	0.22 (0.04, 10.00)	0.009
Gene expression	CCNB1	39	7.92 (7.53, 8.27)	7.30 (6.22, 7.73)	0.007
	CCNA2	39	6.71 (6.53, 6.98)	5.93 (5.01, 6.69)	0.008
	CCNE1	39	4.92 (4.12, 5.39)	3.98 (3.09, 4.69)	0.017
Hallmark pathway scores	G2/M checkpoint	39	7.92 (7.53, 8.27)	7.30 (6.22, 7.73)	0.007
	E2F targets	39	6.71 (6.53, 6.98)	5.93 (5.01, 6.69)	0.017
	Mitotic spindle	39	4.92 (4.12, 5.39)	3.98 (3.09, 4.69)	<0.001

Figure 3 - **a.** Waterfall plot of *in vitro* response to CIR7-7321 in a panel of 40 breast cancer cell lines. Cell lines were exposed to CIR7-7321 for 4-8 days depending on length of time required for at least two cell doublings to occur. Cell growth inhibition was determined by Cell Titer Glo assay. **b.** Heatmap of selected Hallmark pathway scores in a panel of 39 breast cancer cell lines. RNA sequencing (RNAseq) and whole exome sequencing (WES) data was used to identify biomarker profiles that associate with sensitivity to CIR-7321. Hallmark pathway scores were calculated by Gene Set Variation Analysis (GSVA) method using the MSigDb Hallmark collection (Hänzelmann et al, 2013; Liberzon et al, 2015). **c.** Table: Significant association of genes and Hallmark pathway scores with sensitivity to CIR7-7321. Summary table of GI50, baseline mRNA gene expression levels and target hallmark scores between TNBC and non-TNBC cell lines. [1] Median (IQR); [2] Wilcoxon rank sum test.

2. Hallmark pathway scores of breast cancer PDX models

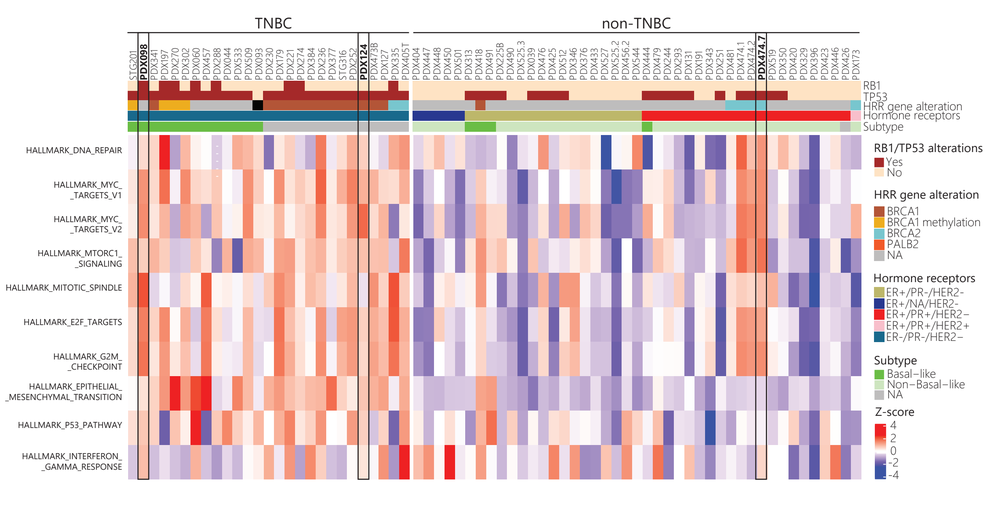


Figure 4 - Heatmap of selected Hallmark pathway scores in a panel of 70 breast cancer patient-derived xenograft (PDX) models. Hallmark pathway scores were calculated by GSVA method using MSigDb Hallmark collection (Hänzelmann et al, 2013; Liberzon et al, 2015). Based on the calculated hallmark pathway score, PDX98, PDX124 and PDX474.7 were selected (highlighted) to test the anti-tumor activity of CIR7-7321 (Figure 5).

3. Oral treatment with CIR7-7321 resulted in tumor regression in breast cancer PDX models and was well tolerated with no significant body weight changes

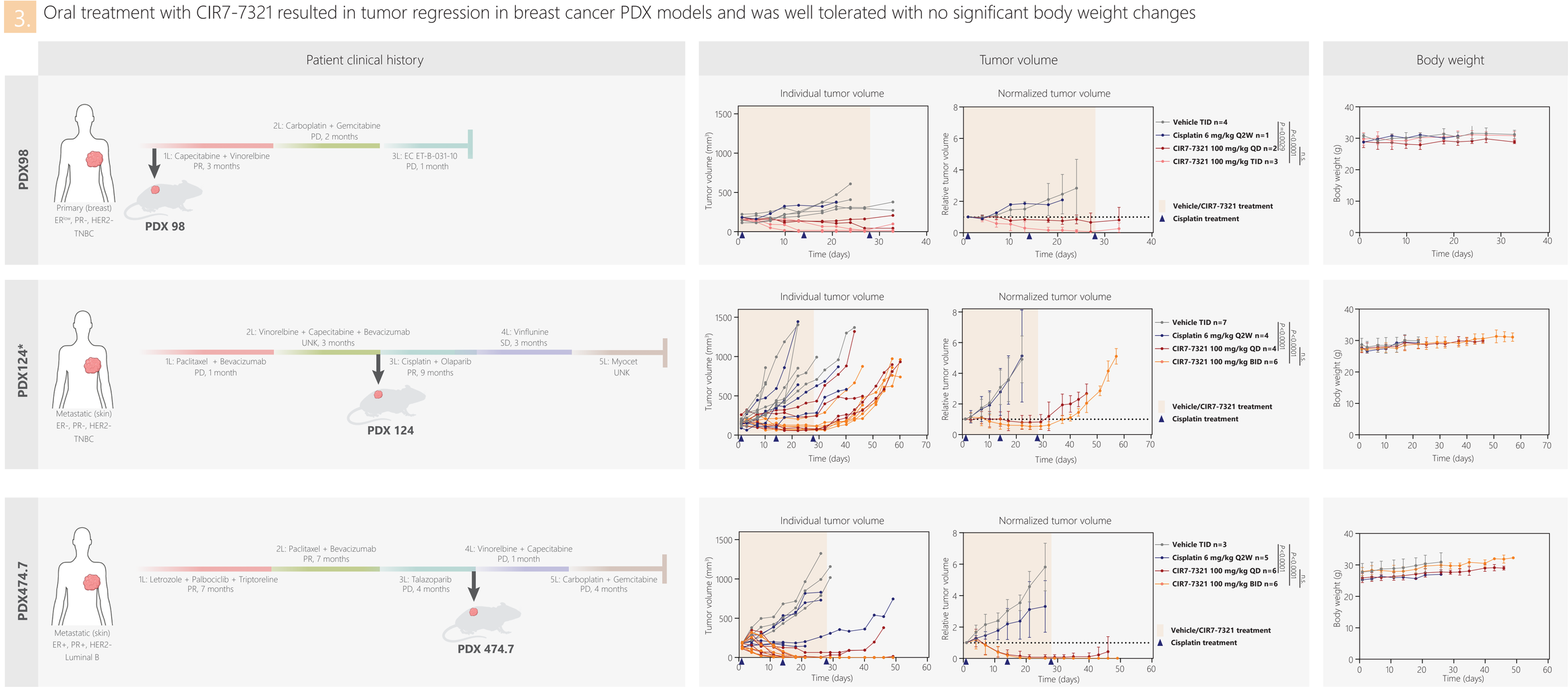


Figure 5 - Patient's clinical history (left), mice tumor volume (middle) and mice body weight (right) of 2 TNBC and 1 ER+ breast cancer PDX models treated with CIR7-7321. NMRI-*Foxn1*$^{nu/nu}$ mice were implanted with tumor fragments in the lower flank. Upon tumor outgrowth (150-250mm3), mice were treated either with vehicle, CIR7-7321 oral formulation of 50-100 mg/kg three times a day (TID), twice a day (BID) or once a day (QD), as indicated, or with 6mg/kg cisplatin every two weeks (Q2W), for 28 days. Cisplatin was included as a standard-of-care control for comparison. Tumor volume and body weight were measured biweekly. Efficacy of CIR7-7321 is beeing tested in 6 additional TNBC and ER+ breast cancer PDX models. Complete tumor regression was observed in PDX124 with a related compound. Complete response was obtained for PDX 474.7 at QD and BID. Response evaluation criteria in solid tumors (RECIST): PD, progressive disease, SD, stable disease, PR, partial response, UNK, unknown response.
Criteria in Solid Tumors (RECIST)

4. PD analysis of luminal breast cancer model (PDX 474.7) tumor tissue shows reduction of Ki67 expression after 5 days of treatment with CIR7-7321

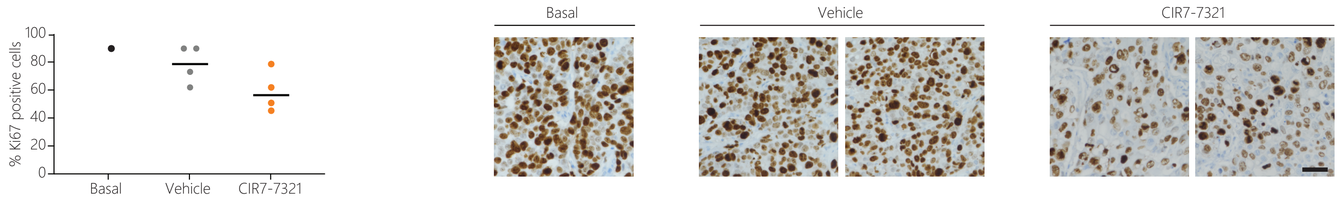


Figure 6 - IHC analysis of Ki67 in tumor tissue of PDX474.7 at the time of tumor implantation (basal), and after 5 days of treatment with vehicle or with CIR7-7321. NMRI-*Foxn1*$^{nu/nu}$ mice were implanted with tumor fragments in the lower flank. Upon tumor outgrowth (150-250mm^3), mice were treated either with vehicle or CIR7-7321 oral formulation of 100mg/kg twice a day (BID), for 5 days. Mice were sacrificed 4h post-treatment. Note: Further evaluation at later time points in progress. Scale bar: 100µm

Summary & Conclusions

- Biomarker profiling of a breast cancer cell line panel reveals association of E2F and G2M Hallmark pathway targets with sensitivity to CIR7-7321.
- CIR7-7321 antitumor activity was observed in two PDX TNBC and one luminal B breast cancer (HR+/HER2-) models which were selected for elevated E2F and G2M Hallmark pathway targets.
- Ki67 expression levels show decrement at early timepoints in PDX474.7 tumors treated with CIR7-7321, consistent with the proposed antiproliferative effects of the drug.
- The data for CIR7-7321 are an example of the antitumor activity for the first-in-class, orally bioavailable cyclin A/B RxL inhibitor drug candidate which is advancing to clinical trial.

References:
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Liberzon et al (2015) Cell Syst 1(6), 417-425
Chen et al. (1999) PNAS96, 4325–4329
Mendoza et al. (2003) Cancer Res. 63, 1020–1024

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