Selective Targeting of CDK2 Using Molecular Glue Degraders for the Treatment of HR-Positive/HER2-Negative Breast Cancer

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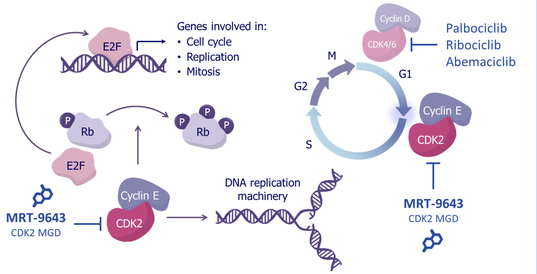
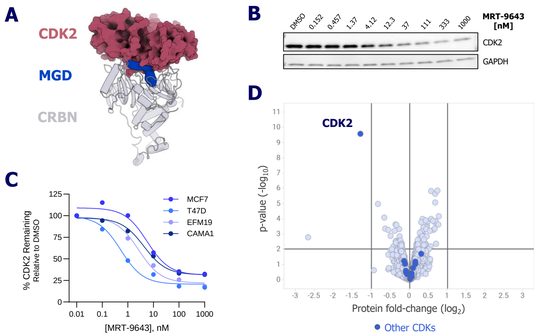
CDK2 is a Key Driver of Cell Cycle Progression in Cancer



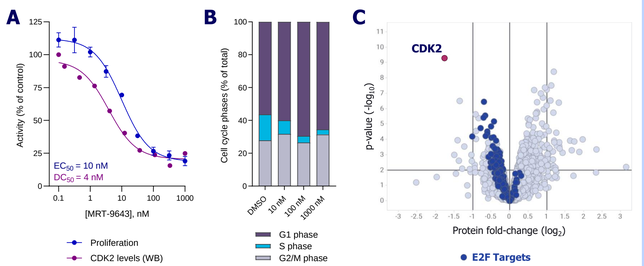
CDK2 phosphorylates RB, freeing E2F transcription factors to modulate gene expression that drives the G1/S progression. CDKs 4 and 6 also impact the G1/S checkpoint and can be targeted with conventional kinase inhibitors in the clinic.

MRT-9643 is a Potent and Highly Selective CDK2-directed Molecular Glue Degrader (MGD)



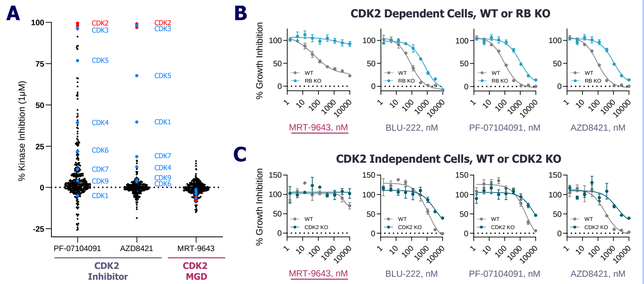
(A) Novel binding mode of MRT-9643, which does not engage a G-loop or the catalytic site. CDK2-MGD-CRBN-DDB1 cryo-EM structure (DDB1 not shown). **(B-C) MRT-9643 is a potent CDK2-directed MGD. (B)** Western blot of CDK2 degradation in MDA-MB-157 cells, with increasing concentration of MRT-9643. **(C)** Degradation of CDK2 in multiple HR-positive breast cell lines, assessed by Western blot. **(D) MRT-9643 induces potent and highly selective CDK2 degradation.** Global protein expression determined with TMT proteomics at 24 hours, with 1μM MRT-9643 treatment in MCF7 cells. Other cyclin-dependent are highlighted in blue.

MRT-9643 Inhibits Proliferation of CDK2-Dependent Cancer Cells



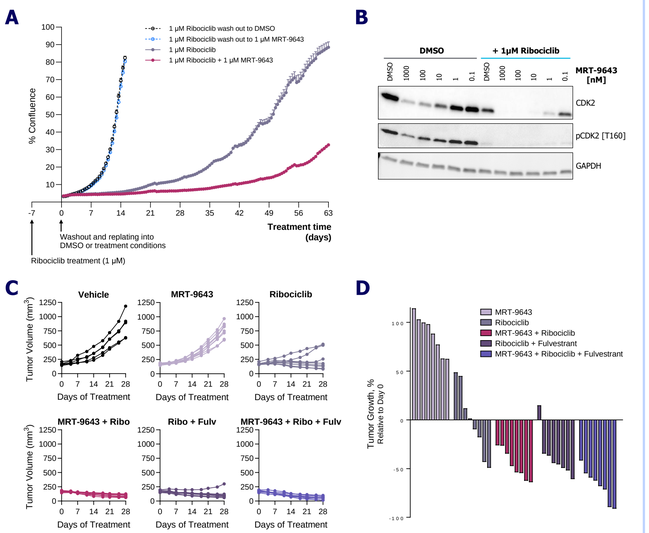
(A) Effect of MRT-9643 on cellular proliferation and CDK2 protein level. Proliferation was assessed by CyQuant Direct after 7 days of treatment in MDA-MB-157 cells. Error bars show SD, N=3, CDK2 protein was determined by western blot. **(B) CDK2 degradation by MRT-9643 arrests CDK2-dependent cells in G1 phase.** Cell cycle phases determined with Click-EdU at 24 hours in MDA-MB-157 cells. Bars show mean, N=3. **(C) CDK2 degradation results in reduction of E2F pathway proteins.** Global protein expression determined with TMT proteomics at 24 hours, with 1μM MRT-9643 treatment in MDA-MB-157 cells. Transcriptional E2F targets are highlighted blue.

MRT-9643 Displays Superior Selectivity Compared to Clinical CDK2 Inhibitors



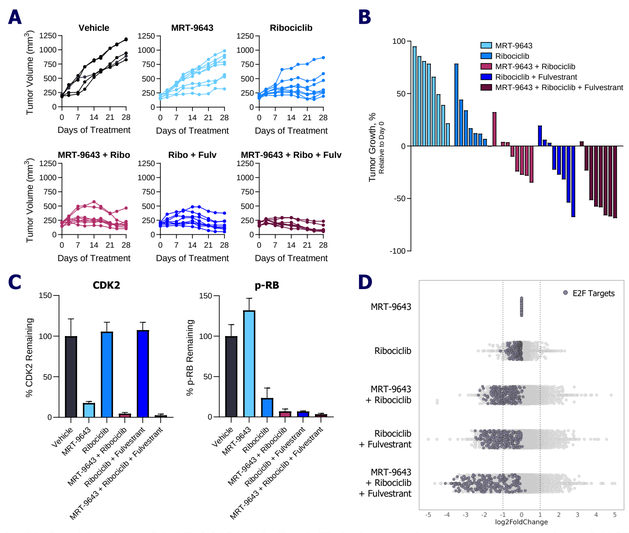
(A) Clinical-stage CDK2 inhibitors show off-target activity in biochemical kinome profiling. Inhibition was determined by Carna Biosciences' mobility shift assay, 1μM CDK2 inhibitor or CDK2 MGD, with 323 human kinases assessed. **(B-C) CDK2 inhibitors, but not a CDK2 MGD, display CDK2-independent activity.** Assessment of growth inhibition of CDK2 MGD MRT-9643 and CDK2 inhibitors in **(B)** MDA-MB-157 cells with wild type or knocked out RB and **(C)** MCF7 cells with wild type or knocked out CDK2. Proliferation was assessed by CyQuant Direct after 7 days of treatment. N=3, with error bars showing SD.

Treatment with MRT-9643 Counteracts Resistance to CDK4/6 Inhibition in HR-Positive Cells



(A) MRT-9643 and ribociclib combination delays resistance onset in MCF7 cells *in vitro*. Growth of MCF7 cells treated with MRT-9643 (1μM), ribociclib (1μM) or combination, and % confluence was measured using Incucyte confluence monitoring. Error bars represent SD, N=2. **(B) Combination of MRT-9643 and ribociclib enhances reduction of CDK2 and pCDK2.** Western blot of CDK2 and pCDK2 [T160] degradation in T47D cells, with or without 1μM ribociclib treatment. **(C-D) Combination of MRT-9643 and ribociclib induces robust tumor regression of T47D xenografts. (C)** T47D cells ($10x10^6$/mouse) were grown subcutaneously in Balb/c nude mice. Tumors of ~200mm^3 were randomized and treated with vehicle, MRT-9643 (30mg/kg PO BID), ribociclib (75mg/kg PO QD), fulvestrant (5mg/mouse SC QD), or combination. **(D)** Waterfall plot of individual tumor growth, relative to vehicle and starting tumor volume.

MRT-9643 Demonstrates Activity and Pathway Suppression in Combination with CDK4/6 Inhibition in HR+ MCF7 CDX



(A-B) Combination of MRT-9643 and ribociclib induces robust tumor regression of MCF7 xenografts. (A) MCF7 cells ($1.5x10^7$/mouse) were grown subcutaneously in Balb/c nude mice. Tumors of ~200mm^3 were randomized and treated with vehicle, MRT-9643 (30mg/kg PO BID), ribociclib (75mg/kg PO QD), fulvestrant (5mg/mouse SC QD), or combination. **(B)** Waterfall plot of individual tumor growth, relative to vehicle and starting tumor volume. **(C-D) MRT-9643 in combination with HR-positive standard of care treatments induce significant pathway suppression. (C)** Relative CDK2 and p-RB [S807/811] protein after indicated treatments in representative xenograft tumors. Samples harvested on Day 28, N=3 tumors per group, error bars represent SEM. **(D)** RNAseq analysis of representative MCF7 xenograft tumors. Samples harvested on Day 28, N=3 tumors per group. Gene expression is shown as fold change relative to vehicle. E2F genes are colored in purple.

Conclusions

- The CDK2-directed molecular glue degrader MRT-9643 exhibits potency, selectivity, and favorable drug-like properties – without engaging the catalytic site or G-loop
- Degradation of CDK2 inhibits CDK2-dependent cancer cell proliferation while displaying superior selectivity compared to clinical CDK2 inhibitors
- CDK2 degradation delays resistance to CDK4/6 inhibition *in vitro* and exhibits activity in combination with CDK4/6 inhibitors *in vivo*
- CDK2 degradation in combination with CDK4/6 inhibition achieves equivalent efficacy compared to anti-CDK4/6 and anti-estrogen standard of care

Want to see what else molecular glues can do?

All authors are current or former employees of Monte Rosa Therapeutics