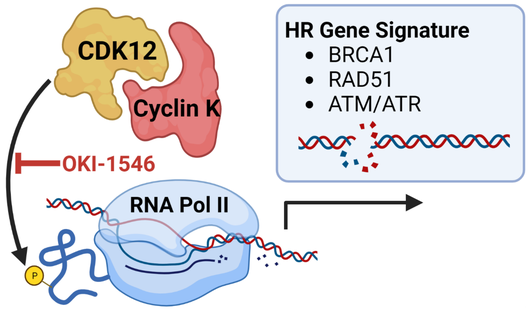
OKI-1546 is a selective, orally bioavailable inhibitor of CDK12 that causes tumor regression in multiple preclinical cancer models

Abstract 1635

Alexander A. Strait,[1] David A. Mareska,[1] Jyothi Mahadevan, Christie A. Schulte, Yevgeniy Izrayelit, Mark L. Boys, and Richard Woessner. OnKure, Inc., 6707 Winchester Circle, Suite 400, Boulder, CO 80301.

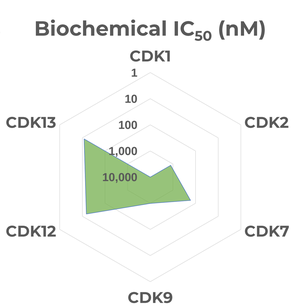
CDK12 regulates the DNA damage response in multiple cancers[2]



- The CDK12-Cyclin K complex phosphorylates the C-terminal domain of RNA Polymerase II (CTD pSer2), resulting in a distinct transcriptional profile responsible for homologous recombination (HR) repair.[3]
- OKI-1546 covalently binds to and inhibits CDK12, resulting in decreased HR proficiency and catastrophic failure of DNA repair in the presence of DNA replication stress.

OKI-1546 is a potent and selective covalent inhibitor of CDK12

A



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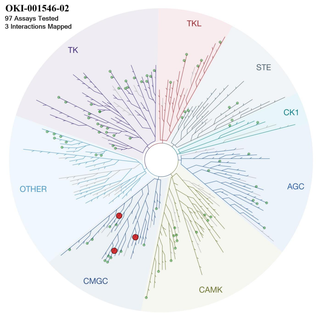
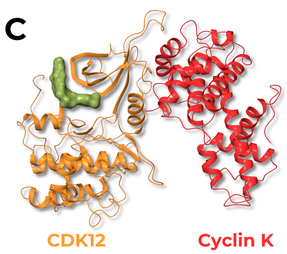


C



D

Assay	IC_{50} (nM)
CTD pSer2	327 nM
MDA-MB-231 proliferation	93 nM
MDA-MB-436 proliferation	80 nM

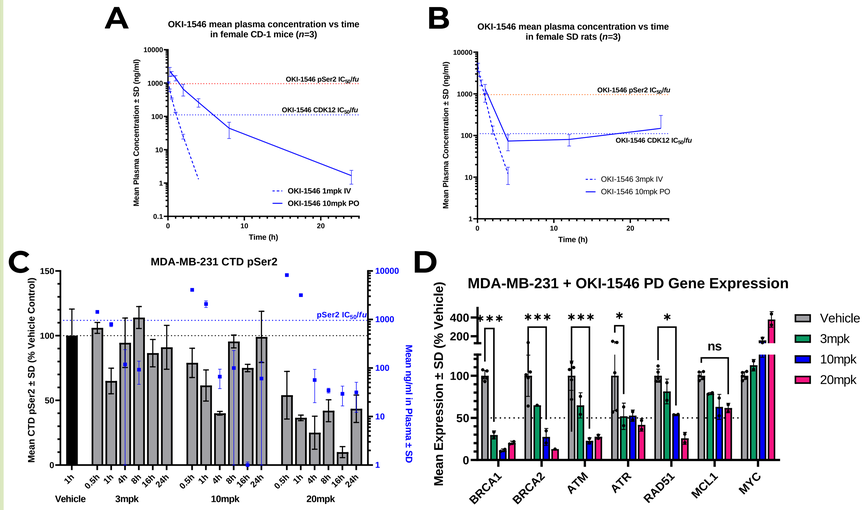
A) OKI-1546 is highly selective against structurally related CDKs in ADP-Glo™ (Promega) biochemical assays.

B) 10 µM OKI-1546 generated 3 hits at <35% of control competitive binding against a 97-kinase panel (KINOMEscan, EuroFins): JNK2, CDK7, and DYRK1B. OKI-1546 K_d is 2 µM for JNK2 and 12 nM for CDK12.

C) The discovery of OKI-1546 (green) was facilitated via structure-based drug design.

D) OKI-1546 demonstrates pharmacodynamic potency against CTD pSer2 in MBA-MB-231 cells (measured by MSD) and inhibits the proliferation (72h CellTiter-Glo) of two triple-negative breast cancer (TNBC) cell lines.

OKI-1546 demonstrates cross-species PK and target engagement in vivo

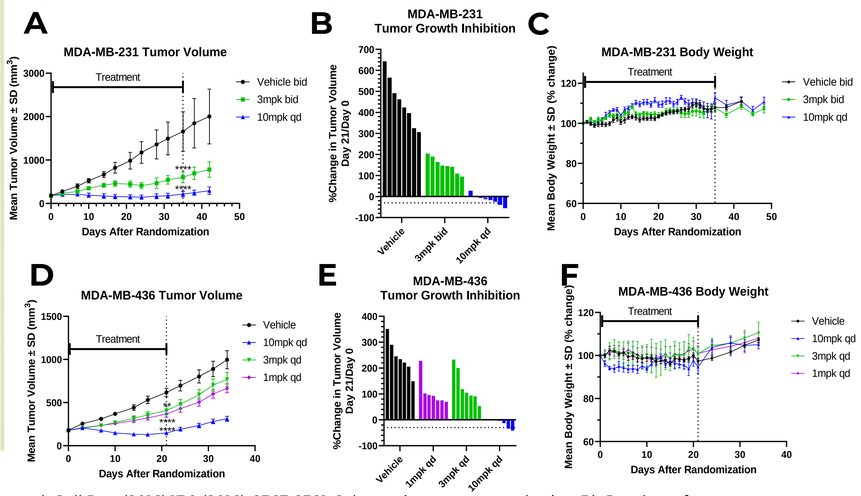


A, B) OKI-1546 demonstrated plasma concentrations at or above the mouse plasma protein binding-adjusted IC_{50} for CDK12 biochemical inhibition CTD pSer2 inhibition in both mouse following a single IV or PO dose.

C) OKI-1546 has plasma exposures that surpass the CTD pSer2 IC_{50} following a single dose (blue squares) and inhibits CTD pSer2 in MDA-MB-231 tumors for a sustained period after the drug is cleared from plasma relative to vehicle control (gray and black bars).

D) qPCR analysis of tumors from **C** shows that OKI-1546 inhibits the expression of components of the HR DNA damage response, including BRCA1, BRCA2, ATR, ATM, and RAD51. There was no significant decrease in MCL1 or MYC expression, indicating that CDK12 inhibition did not affect CDK9 activity. Minimum statistical differences between treatments are indicated after 2-way ANOVA with multiple comparisons.

OKI-1546 demonstrates tumor growth inhibition against TNBC xenograft models at tolerated doses

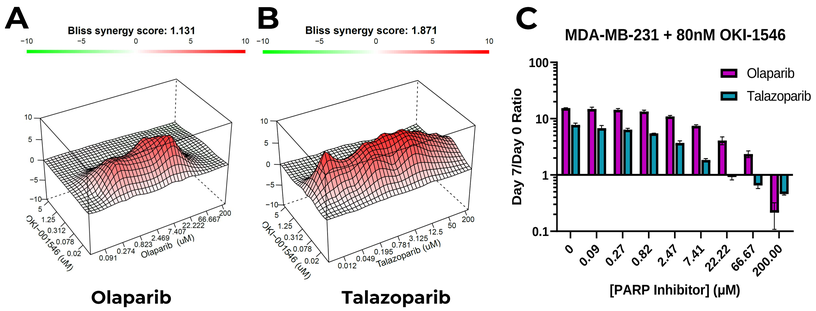


A) Mice bearing MDA-MB-231 tumors were treated with OKI-1546 for 35 days at the indicated daily doses, with net tumor growth inhibition at 21 days shown in (**B**). Body weight was recorded daily (**C**).

D) Mice bearing MDA-MB-436 tumors were treated with OKI-1546 for 21 days at the indicated daily doses, with net tumor growth inhibition at 21 days shown in (**E**). Body weight was recorded daily (**F**).

Statistical differences in mean tumor volume at the end of treatment are shown relative to vehicle control after 2-way ANOVA and multiple comparisons.

OKI-1546 acts in combination with PARP inhibitors to induce cell death in vitro



A, B) OKI-1546 shows synergy in combination with olaparib and talazoparib PARP inhibitors in MDA-MB-231 cells after 7 days at the indicated concentrations; values from 5-10 via the Bliss method indicate synergy.

C) OKI-1546 induces net cell death as indicated by the ratio between CellTiter-Glo™ signal on day 7 and day 0.

Summary

- OKI-1546 demonstrates potency and specificity against CDK12 in vivo and in vitro, resulting in signaling changes and cell death associated with disruption of the DNA damage response.
- OKI-1546 has pharmacokinetic and pharmacodynamic properties appropriate for in vivo applications in multiple species.
- OKI-1546 is an optimal therapeutic to target a wide range of cancers associated with addiction to DNA damage repair, and it can be combined with other therapeutic agents that induce DNA damage or replication stress.

[1] Co-first authors. [2] Krajewska et al. *Nat. Comm.* (2019) 10(1):1757 [3] Liang et al. *Cells* (2020) 9(6):1483. [4] Johnson et al. *Cell Rep.* (2016) 17,9 (2016): 2367-2381. Schematics were created using BioRender software.

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