GDC-4198, a next-generation CDK4/2 inhibitor, induces durable cell cycle arrest and overcomes CDK2-driven adaptation to CDK4 inhibition

PS2-11-28

Marc Hafner[1] , Steffan Vartanian[1], Luca Gerosa[1], Nont Kosaisawe[1], Michael Hwang[1], Eva Lin[1], Linus Wang[1], Gino Prasad[1], Andrew Goetz[1], Jason Oeh[1], Karen Samy[1], Udi Segal[1], John Moffat[1], Daniel Zingg[1], Ann E Collier[1], Ioannis Sanidas[2], Julie Xie[3], Seth Wander[2]

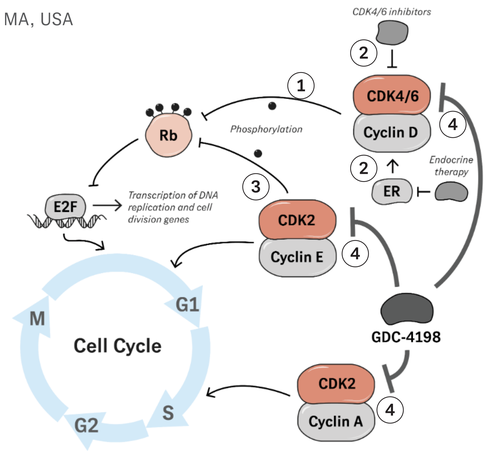
[1]Genentech, Inc., South San Francisco, CA, USA
[2]Massachusetts General Hospital, Harvard Medical School, Boston, MA, USA
[3]Regor Pharmaceuticals Inc., Cambridge, MA, USA

Introduction

While CDK4/6 inhibitors (CDK4/6i) have changed the therapeutic landscape of hormone receptor-positive (HR+) breast cancer (BC), resistance to these therapies is a major challenge limiting their clinical benefit.

Recent studies have shown that CDK2 activity is a key mechanism of resistance to CDK4/6i[1-5]. Overexpression of cyclin E1/E2 and other mechanisms can lead to increased CDK2 activity which phosphorylates Rb and bypasses CDK4/6i-induced G1 arrest.

It has been proposed that targeting CDK2, in addition to CDK4, can lead to more durable cell cycle arrest in HR+ BC cells. In this study, we evaluated the preclinical activity of GDC-4198, a next-generation CDK4/2 inhibitor currently being tested in clinical trials, and its potential to address CDK2-driven resistance to CDK4 inhibition.

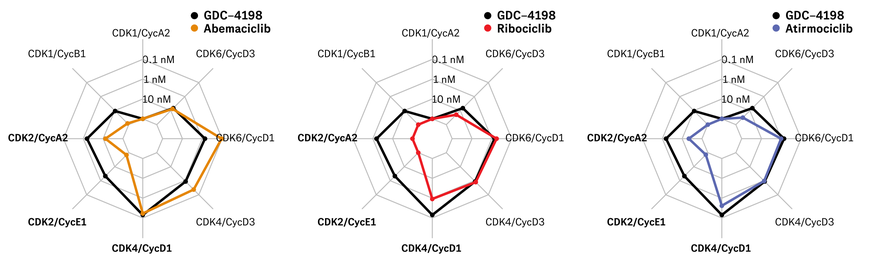


Cell cycle and effect of CDK inhibitors. (1) CDK4/6/CycD phosphorylates Rb to initiate G1 progression and expression of cell cycle genes[6,7]. (2) CDK4/6 inhibitors are combined with endocrine therapy (ET) to block CDK4/6/CycD activity and achieve cell cycle arrest. (3) High CDK2 activity (due to CyclinE1/2 overexpression or other mechanisms) phosphorylates Rb and leads to resistance to CDK4/6 inhibitors. (4) GDC-4198 targets both CDK4/6 and CDK2 to suppress Rb phosphorylation.

References: 1. Wander SA, et al. Cancer Discov. 2020;10:11174-1193. 2. Goel S, et al. Nat Rev Cancer. 2022;22(6):356-372. 3. Guarducci C, et al. NPJ Breast Cancer. 2018;4:38. 4. Hafner M, et al. Cell Chem Biol. 2019;26:1067-1080. 5. Turner NC, et al. J Clin Oncol. 2019;37(14):1169-1178. 6. Matthews HK, et al. Nat Rev Mol Cell Biol. 2022;23(1):74-88. 7. Malumbres M. Genome Biol. 2014;15(6):122.

GDC-4198 is a highly potent CDK4 inhibitor with substantial CDK2 activity

In biochemical kinase activity assays, GDC-4198 exhibits sub-nanomolar potency against CDK4 and is a more potent inhibitor of CDK2/CycA and CDK2/CycE compared to CDK6/CycD3. In contrast, approved CDK4/6is and next-gen CDK4-targeted inhibitor have limited inhibitory activity on CDK2. GDC-4198 has high selectivity for CDK4 and CDK2 over other kinases associated with off-target toxicities, including CDK1, CDK9, and GSK3β.

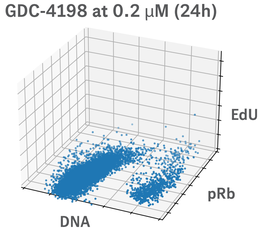


Comparison of Ki values (biochemical kinase inhibition) for different CDK/cyclin complexes for GDC-4198 and CDK4(/6) inhibitors. Ki values above 100 nM are reported at the center of the radar plot.

GDC-4198 blocks cell cycle and completely suppresses S phase entry in T-47D cells

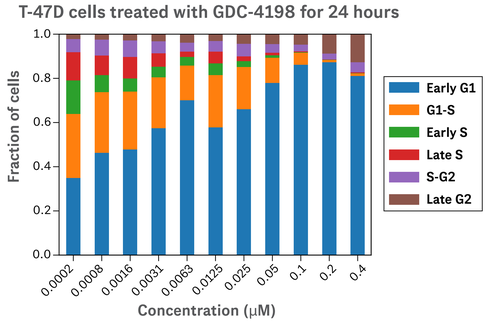
Immunofluorescence (IF) experiment at different doses of GDC-4198 shows cell cycle arrest in a dose-dependent manner at 24 hours with complete blockade of S phase entry (EdU positivity) at 0.2 μM.

GDC-4198 at 0.2 μM (24h)



IF data from T-47D cells treated with GDC-4198 at 200 nM for 24 hours. Left axis represents DNA content (G1 vs. G2 phases), right axis pRb, and z-axis EdU incorporation (S phase).

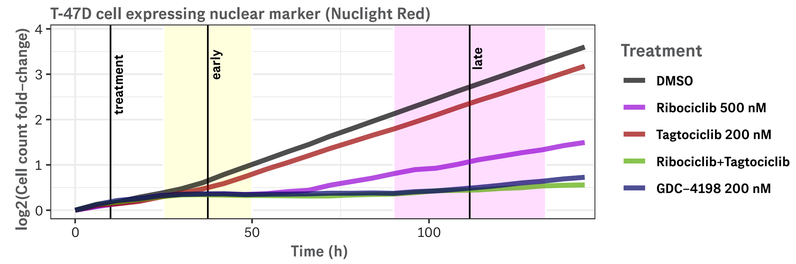
T-47D cells treated with GDC-4198 for 24 hours



Distriution of T-47D cells in the cell cycle after treatment with different doses of GDC-4198. Assignment of cells is based on DNA and EdU values measured by IF.

GDC-4198 shows durable growth suppression in HR+ BC cell lines, contrasting with CDK4/6 inhibitors

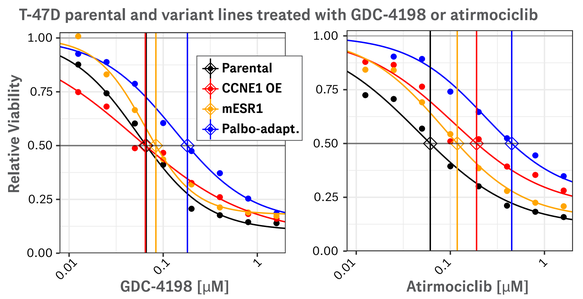
While HR+ BC T-47D cells stopped proliferating when treated with the CDK4/6i ribociclib (early response), they adapted and started proliferating again after four days (late response). GDC-4198 showed more sustained *in vitro* cell growth inhibition than ribociclib. Efficacy of GDC-4198 was similar to the activity observed with the combination of ribociclib with the CDK2 inhibitor tagtociclib. This result suggests that inhibition of CDK2 by GDC-4198 may limit the adaptation to CDK4 inhibition observed with current standard-of-care.



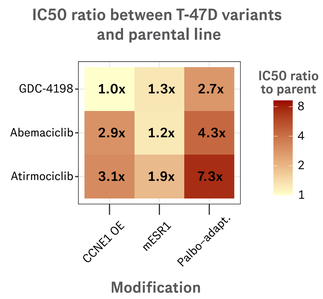
In vitro potency of GDC-4198 is minimally impacted by adaptation to CDK4/6 inhibitors, the introduction of known resistance mechanisms to CDK4 inhibition, or *PIK3CA* status

GDC-4198 showed no shift in potency when *CCNE1* overexpression or an *ESR1* mutation are engineered in HR+ BC T-47D cells. In contrast, response to the CDK4 inhibitor atirmociclib is shifted 3.1 and 1.9 fold respectively. For T-47D cells adapted to grow in 300 nM of palbociclib, IC50 for GDC-4198 is shifted only 2.7 fold, whereas it is shifted 7.3 fold for atirmociclib. The CDK4/6i abemaciclib showed similar results as atirmociclib across T-47D variants.

T-47D parental and variant lines treated with GDC-4198 or atirmociclib

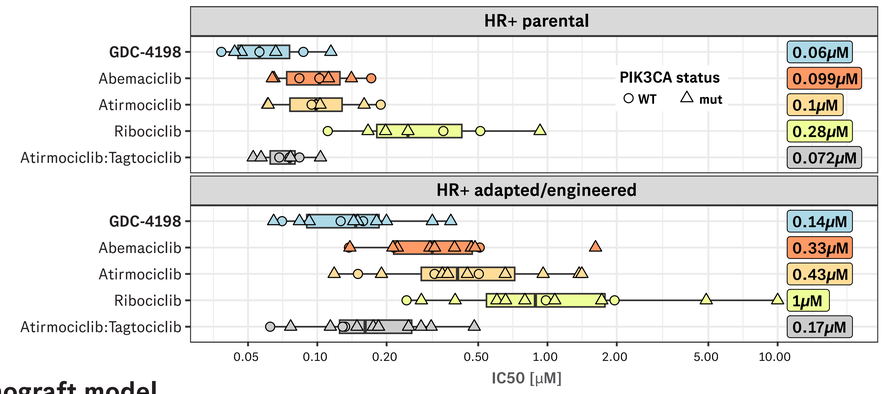


IC50 ratio between T-47D variants and parental line



When expanding to a larger panel of HR+ BC cell lines, GDC-4198 is more potent in the parental lines (geometric mean of 0.06 μM, independent of *PIK3CA* status) than approved CDK4/6 inhibitors and next-gen CDK4-targeted inhibitor.

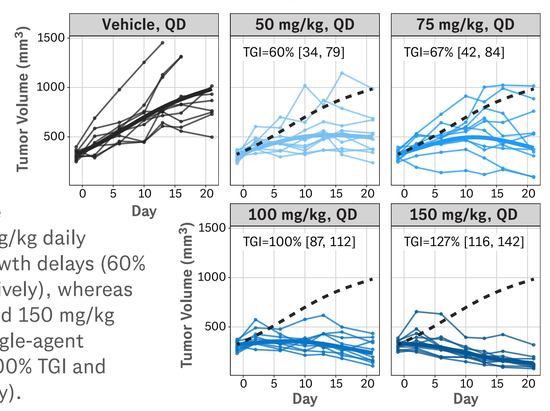
In cell lines engineered with either *CCNE1/2* overexpression, *TP53* knock-out, or *ESR1* mutations, as well as cell lines adapted to either palbociclib or atirmociclib, GDC-4198 is more active (geometric mean of 0.14 μM) than other CDK4/6is. GDC-4198 is most similar to the combination of atirmociclib and tagtociclib (1:1 mixture).



Efficacy of GDC-4198 in MCF-7-Ser xenograft model

GDC-4198 inhibition of tumor growth was investigated in an HR+ MCF-7-Ser BC xenograft model.

Administration of GDC-4198 as a single agent at 50 and 75 mg/kg daily resulted in tumor growth delays (60% and 67% TGI, respectively), whereas daily doses of 100 and 150 mg/kg resulted in strong single-agent antitumor activity (100% TGI and 127% TGI, respectively).



Notes: MCF-7-Ser is an MCF-7 variant optimized for xenograft. TGI=tumor growth inhibition; PO=oral; QD=once daily (21 days)

Conclusion

Taken together, these preclinical findings demonstrate that GDC-4198 can induce more durable cell cycle arrest than approved CDK4/6 inhibitors and next-gen CDK4-targeted inhibitors by overcoming CDK2-driven resistance to CDK4 inhibition. These results suggest that GDC-4198 is a promising investigational agent with the potential to prolong benefit in earlier disease settings and provide benefit to those who have progressed on approved CDK4/6 inhibitors.

In the clinic, GDC-4198 monotherapy has demonstrated preliminary evidence of disease control in the post-CDK4/6i setting with sustained responses in a heavily pre-treated patient population. Further dose escalation with GDC-4198 as a single agent is ongoing and evaluation of GDC-4198 in combination with giredestrant in a Phase 1b/2 study has been initiated (NCT07100106; see poster PS1-11-07).