Cancer Cell Vulnerability to SMARCA2/4 Degradation by PROTACs

Abstract # 3849

Boehringer Ingelheim

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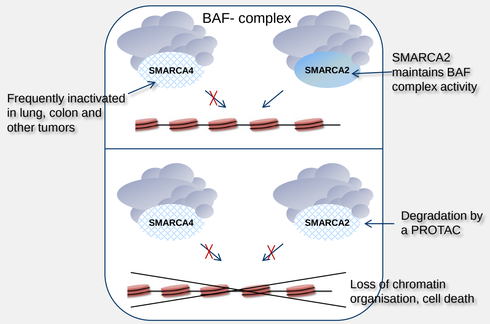
INTRODUCTION

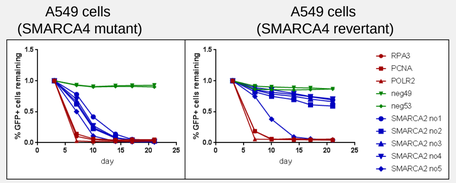
Vulnerability of SMARCA4 mutated cancer cells to loss of SMARCA2



SMARCA2 and SMARCA4 are the two mutually exclusive ATPases of the chromatin remodeling BAF (SWI/SNF) complex. SMARCA4 is frequently inactivated by mutations in a variety of human tumors, including lung and colon. This renders tumor cells dependent on the remaining ATPase, SMARCA2.

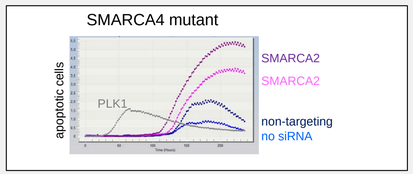
RESULTS

Lack of SMARCA4 Sensitizes A549 Cells to Loss of SMARCA2



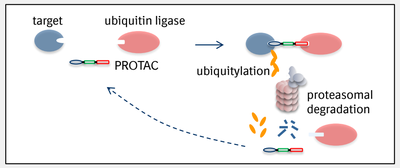
sgRNAs co-expressed with GFP were used to knock out the indicated genes in A549 cells or A549 cells in which SMARCA4 had been reverted to wild-type. Loss of sgRNA expressing cells over time indicates essentiality of the targeted gene.

siRNA Knockdown of SMARCA2 Causes Apoptosis



siRNA targeting SMARCA2 or control siRNA were transfected in SMARCA4 deficient A549 cells and the appearance of cells positive for caspase-3 or -7 was monitored over time.

What Proteolysis Targeting Chimeras (PROTACs) do

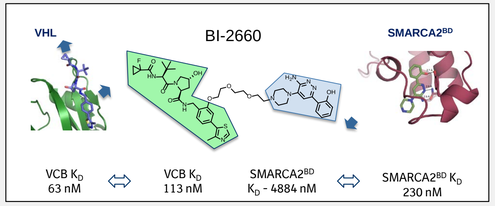


Construction of the First SMARCA2 - PROTAC

Soares et al, J. Med. Chem. 2018, 61, 599

Ternary X-Ray crystal structure of SMARCA2^{BD}-PROTAC-VCB

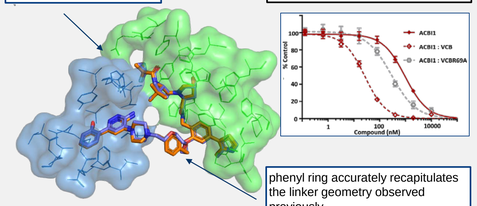
Genentech/Constellation , WO 2016138114



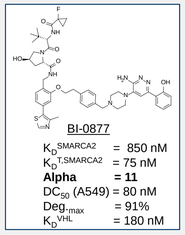
Structure-Guided Drug Design Yields ACBI1

polar interaction R69::F83 important for cooperativity

Triple complex competition assay confirms role of R69

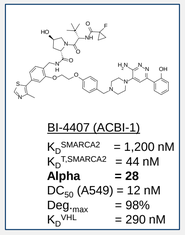


phenyl ring accurately recapitulates the linker geometry observed previously



BI-0877

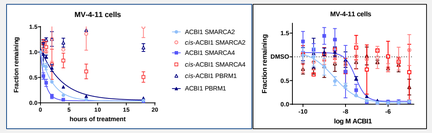
$K_D^{SMARCA2}$ = 850 nM
$K_D^{T:SMARCA2}$ = 75 nM
Alpha = 11
DC_{50} (A549) = 80 nM
Deg_{max} = 91%
K_D^{VHL} = 180 nM



BI-4407 (ACBI-1)

$K_D^{SMARCA2}$ = 1,200 nM
$K_D^{T:SMARCA2}$ = 44 nM
Alpha = 28
DC_{50} (A549) = 12 nM
Deg_{max} = 98%
K_D^{VHL} = 290 nM

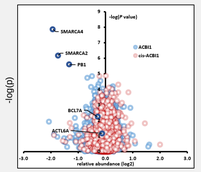
ACBI1 Causes Fast and Complete Degradation of SMARCA2, -4 and PBRM1



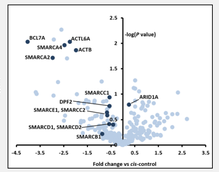
MV-4-11 cells were treated with 1 µM compound (left) for the indicated time or with a dose range for 18 hours (right) before protein levels were measured by capillary electrophoresis. *cis*-ACBI1 is a distomer of ACBI1 with the hydrox-proline of the VHL-binding part in the inactive cis position, which prevents VHL binding.

ACBI1 is Selective towards SMARCA2, -4 and PBRM1

Whole cell

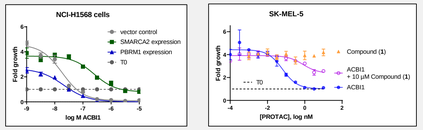


ARID1A immunoprecipitate



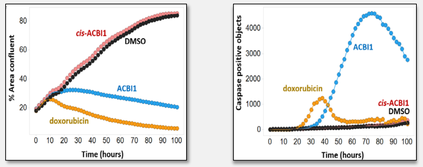
Left, Effects of ACBI1 (blue) and *cis*-ACBI1 (red) at 333 nM for 8 h on the proteome of MV-4-11 cells as detected *via* TMT labelling and mass spectrometry. Right, SWI/SNF complexes were immuno-purified following compound treatment (18h, 1 µM). Abundance of subunits was determined by label free quantitation.

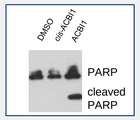
In SMARCA4-Deficient Cells, Degradation of SMARCA2 by ACBI1 Inhibits Proliferation



Left, Rescue experiment: NCI-H1568 cells expressing the indicated constructs were incubated with ACBI1 before cell viability was measured and normalised to day 1 of the treatment. Right, Competition with the bromodomain binder: SK-MEL-5 cells grown in the presence of ACBI1, the non-PROTAC SMARCA2 binder compound (**1**), or both and cell viability was assessed after 3 days.

ACBI1 Induces Apoptosis in SMARCA4-Deficient Cells





Top panels, degradation of SMARCA2 inhibits proliferation and induces apoptosis in SMARCA4-deficient SK-MEL-5 cells (real time microscopy analysis, Incucyte).

Left, ACBI1 treatment induces PARP cleavage in SK-MEL-5 cells.

SUMMARY

Targeting subunits of BAF (SWI/SNF) chromatin remodeling complexes has been proposed as an approach to exploit cancer vulnerabilities. Here we develop PROTAC degraders of the BAF ATPase subunits SMARCA2 and SMARCA4 using a bromodomain ligand and recruitment of the E3 ubiquitin ligase VHL. High-resolution ternary complex crystal structures and biophysical investigation guided rational and efficient optimization towards ACBI1, a potent and cooperative degrader of SMARCA2, SMARCA4 and PBRM1. ACBI1 induced antiproliferative effects and cell death caused by SMARCA2 depletion in SMARCA4 mutant cancer cells. These findings exemplify a successful biophysics- and structure-based PROTAC design approach to degrade high profile drug targets and pave the way towards new therapeutics for the treatment of tumors sensitive to the loss of BAF complex ATPases.

CONCLUSIONS

- **Structure guided drug design leads to potent and efficient PROTACs targeting SMARCA2, -4 and PBRM1.**
- **PROTAC mediated degradation of SMARCA2 and 4 can induce apoptosis in cancer cells.**
- **Selective degradation of BAF complex subunits offers novel opportunities for the development of cancer therapeutics.**

TEAM

AACR Annual Meeting 2019, Atlanta, Georgia, USA; 29 March – 3 April 2019