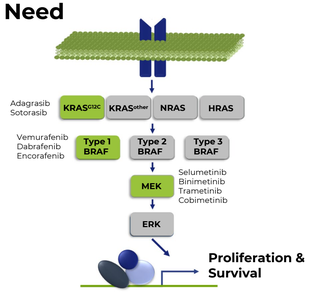
The class I Selective, Oral HDAC Inhibitor Bocodepsin Enhances the Response to MAPK Pathway Inhibitors in Multiple Tumor Types with Mutations in MAPK Pathway Signaling Proteins

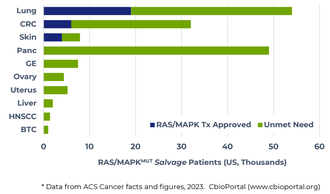
Abstract 33415

Rich Woessner, Maria Hoh and Duncan Walker
OnKure, Inc., 6707 Winchester Circle, Suite 400, Boulder, CO 80301

The RAS-Pathway in Cancer: Still a Significant Unmet Need

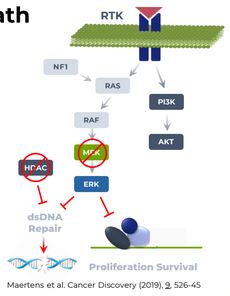


- RAS pathway targeting is not effective in many indications
- Even in "sensitive" settings, many patients do not benefit from single agent RAS pathway inhibition

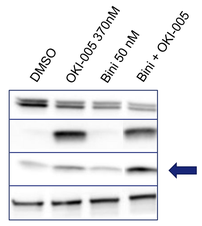


* Data from ACS Cancer facts and figures, 2023. CbioPortal (www.cbioportal.org)

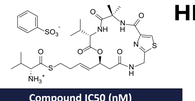
HDACi + MEKi leads to accumulated dsDNA breaks and cell death



Maertens et al. Cancer Discovery (2019), 9, 526-45



Bocodepsin is a novel, clinical-stage Class I selective HDAC inhibitor



Compound IC50 (nM)				
Class	HDAC	OKI-006*	Red-FK228**	Vorinostat
I	1	1.2	0.8	75.5
I	2	2.4	1.0	362
I	3	2.0	1.3	57.4
I	8	49.0	>1000	1069
IIa	4	>1000	647.0	>1000
IIa	5	>1000	>1000	163
IIa	7	>1000	>1000	>1000
IIa	9	>1000	>1000	78.1
IIb	6	47.0	226.0	27.1
IIb	10	2.8	0.9	88.4
IV	11	2.3	0.3	109.0

* active metabolite of bocodepsin, ** active metabolite of romidepsin

- Orally-bioavailable prodrug, rapidly converting to the active parent, OKI-006 in vivo.
- Completed Ph1 clinical trials as a single agent.
- Good PK and PD activity at the proposed RP2D with a well-tolerated safety profile
- OKI-005 is a research tool compound that also converts to OKI-006 in vitro.
- OKI-006 shows low nM activity on Class I HDACs, with high-selectivity relative to Class IIa
- **Bocodepsin is in a Ph1b/2 trial in combination with binimetinib in NRAS^{MUT} melanoma (NAUTILUS trial)**

In vitro and *in vivo* assays

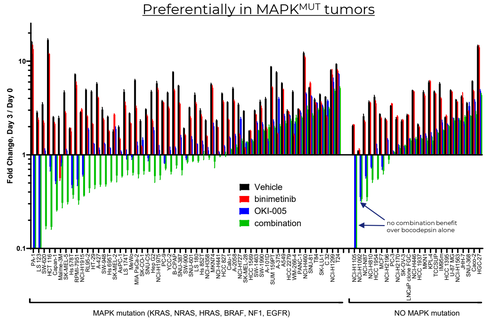
***In vitro* assays:** cell proliferation assays were performed at Crown Bioscience. Single agent and combination treatments were evaluated in a 6x6 combination matrix format after 72H of treatment, using the Cell Titer Glo assay. Data reported are at clinically relevant concentrations of MEK inhibitor binimetinib (123 nM) and OKI-005 (370 - 1,110 nM), 1,100 nM pan RAF inhibitor TAK-632 and 1,100 nM ERK inhibitor GDC-0994. Western blot assays were performed at OnKure (HCT-116, NCI-H358, using antibodies to pERK (CST#4370), total ERK (CST#9102), α-actinin (SantaCruz#17829), β-tubulin (CST#5346), acetylated histone H3K9 (Millipore#06942), cleaved PARP (CST#9541), pH2X-ser139 (CST#9718), GAPDH (CST#2118), Ku70 (CST#4588) and Ku70-acetyl K549 (ABM#Y407701), or at Crown Bioscience (SK-MEL-2), using antibodies to pERK (CST#4370), GAPDH (KANGCHEN#KC-5G4), acetylated histone H3K9 (CST#9649), cleaved PARP (CST#9542) and pH2X-ser139 (CST#2577).

***In vivo* assays:** tumor xenograft experiments were performed at Pharmaron. SK-MEL-2 tumors were grown subcutaneously in female NOD-SCID mice, NCI-H358 tumors in female BALB/c nude mice. Mice were randomized into treatment groups and treatment initiated at an average tumor volume of 170 mm^3 (SK-MEL-2) or 190 mm^3 (NCI-H358) for efficacy experiments, and 575 mm^3 (SK-MEL-2) for pharmacodynamic experiments. Western blot analyses used antibodies to pERK (CST#4370), acetylated histone H3K9 (CST#9649), acetylated histone H3K27 (CST#8173) and GAPDH (CST#97166).

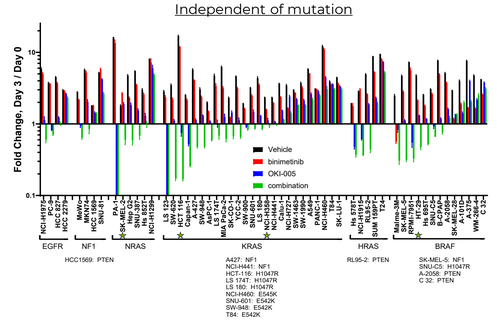
Bocodepsin + MEK inhibitor binimetinib enhances net cell killing

Day 3 / day 0 fold change <1 indicates net cell killing, >1 indicates net cell growth

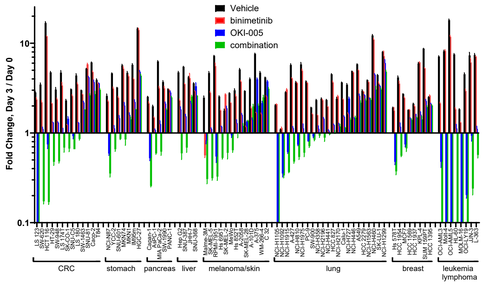
Preferentially in MAPK^{MUT} tumors



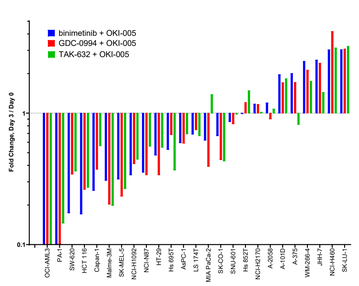
Independent of mutation



Independent of tumor type

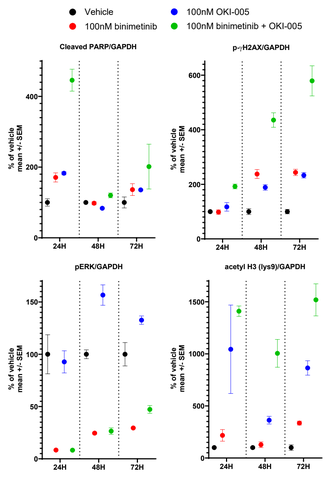


In combination with ERK or RAF inhibitors

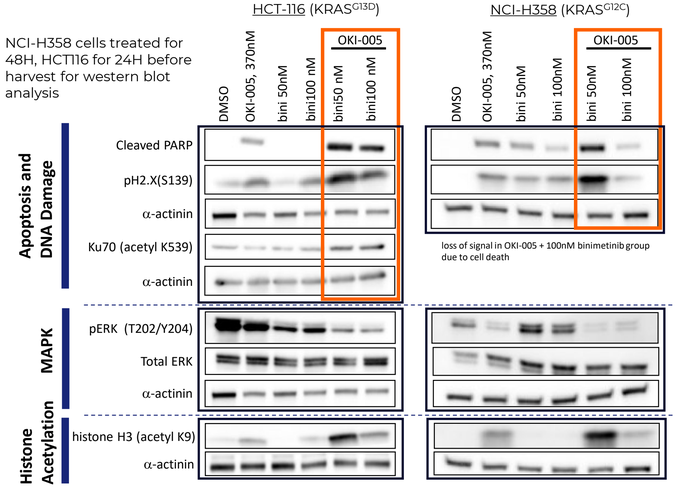


Binimetinib + OKI-005 combination Induces Apoptosis and DNA Damage in MAPK Pathway Mutant Tumor Cell Lines

SK-MEL-2 (NRAS^{Q61R})

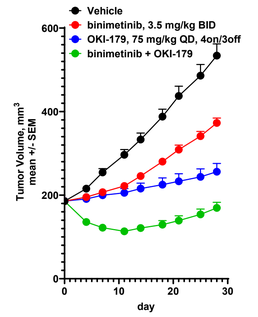


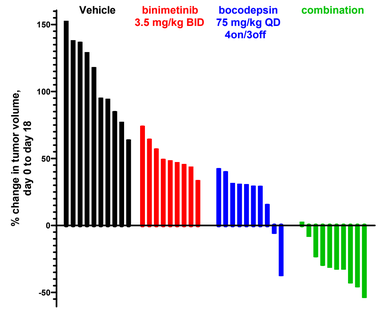
NCI-H358 cells treated for 48H, HCT116 for 24H before harvest for western blot analysis



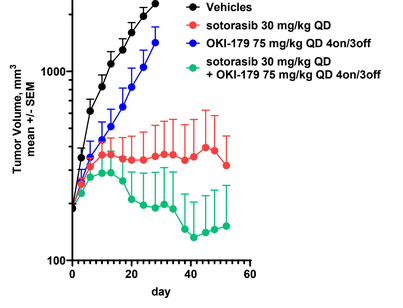
Combination leads to robust tumor regressions in xenograft models

SK-MEL-2 (NRAS^{Q61R} melanoma)
regressions induced in combination with binimetinib

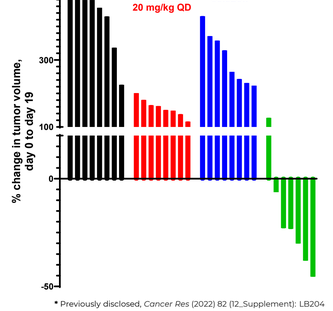




NCI-H358 (KRAS^{G12C} NSCLC)
deeper regressions with combination



HT-29 (BRAF^{V600E} CRC)
regressions induced in combination with binimetinib *



* Previously disclosed, *Cancer Res* (2022) 82 (12_Supplement): LB204

Summary

In cells, bocodepsin + MAPK inhibitors leads to cell death / apoptosis:
- with any RAS pathway mutation
- regardless of tumor type
- regardless of which RAS pathway inhibitor is used

This novel anti-tumor biology translates into multiple tumor xenograft models, three of which are shown here:
- bocodepsin + binimetinib leads to tumor regressions in NRAS^{MUT} melanoma
- bocodepsin + sotorasib leads to enhanced regressions in KRAS^{MUT} NSCLC
- bocodepsin + binimetinib/encorafenib leads to tumor regressions in BRAF^{MUT} CRC

The findings suggest broad application for bocodepsin combined with any MAPK pathway inhibitor in any cancer with a RAS pathway mutation

- **Bocodepsin is in a Ph1b/2 trial in combination with binimetinib in NRAS^{MUT} melanoma (NAUTILUS trial, NCT05340621). The phase 2 portion of the trial is open**
- **Preliminary efficacy and safety data are presented at this meeting (Spotlight on Proffered Papers 2 Friday AM and poster LB-B25 Friday PM)**