MSS/KRAS^{G12X} CRC Depends on CBM Signalosome for Survival - A Discovery that May Transform KRAS^{G12X} CRC Therapy

Fang Wang[1], Yi Yao[1], Kevin Ling[1], Huili Xia[1], Yilin Qi[1], Huadong Sun[1], Sharon Wu[2], Wei Liu[1], Gerry Harriman[1]

HotSpot Therapeutics, Inc, One Design Center Place, Suite 19-600, Boston, MA 02210, USA; . Caris Life Science, 750 W John Carpenter Fwy, Suite 800, Irving, TX 75039, USA

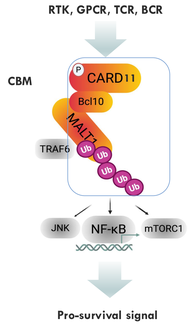
Introduction

KRAS activation is a prominent genetic feature of colorectal cancer (CRC), however, CRC patients did not demonstrate deep or durable responses when treated with KRAS inhibitors.

We and others have observed that KRAS inhibitors, while potently inhibiting cancer proliferation, do not induce strong apoptosis *in vitro*, *in vivo* or in patients with CRC. This has led to limited and non-durable clinical response of KRASi, because tumor cells remain alive and soon relapse or develop resistance.

We discovered that MSS/KRAS^{G12} CRC depends on CBM signalosome for survival. Inhibition of CBM selectively induces selective and potent apoptosis in KRAS^{G12} CRC.

CBM Signalosome, the Pro-Survival Hub for Cancer Progression

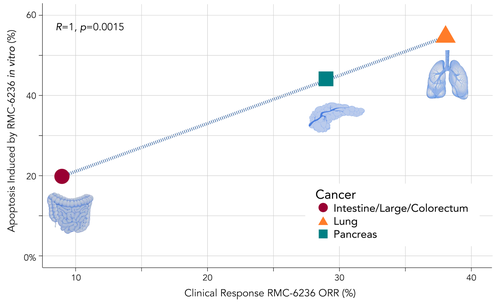


CBM signalosome: CARD11-BCL10-MALT1 complex.

CBM transduces upstream growth & survival signals via a series of phosphorylation and ubiquitination reactions, and activates multiple pro-survival pathways, including canonical NFκB, mTORC1 and JNK.

In cancer, upstream GoFs (BTK, PKC, etc), CARD11 GoF mutations, or CARD11 overexpression activate CBM signalosome, promoting cancer progression.

Lack of Potent Apoptosis Could Explain the Limited and Non-Durable Response of KRASi in CRC



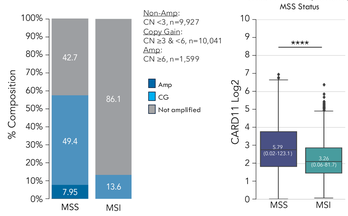
In vitro data was from Revolution Medicine and HotSpot's data; the rate represents the percentage of cell lines responding to KRASi induced anti-proliferation or apoptosis in a cell panel screen.

Results

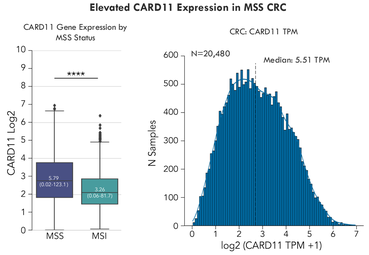
Figure 1. In CRC, High CARD11 CRC had Highest KRAS Activity and Shorter KRASi Time-on-Treatment

A Study of > 20,000 CRC Patients Database Revealed CARD11 CNA or Amp in >50% of MSS CRC

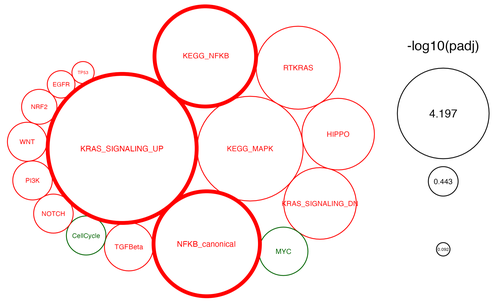
CARD11 CNA or Amp were Discovered in >50% of MSS CRC Patients



Elevated CARD11 Expression in MSS CRC



Same Database Revealed High CARD11 CRC has the Highest KRAS Activity Among Oncogenic Pathways (P< 0.0001)



Top 25% expressing CARD11 CRC vs low 25% CARD11 expressing CRC.

CARD11 CRC had Shorter Time-on-Treatment of KRAS^{G12C} Inhibitor

Adagrasib/Sotorasib Time on Treatment (TOT)

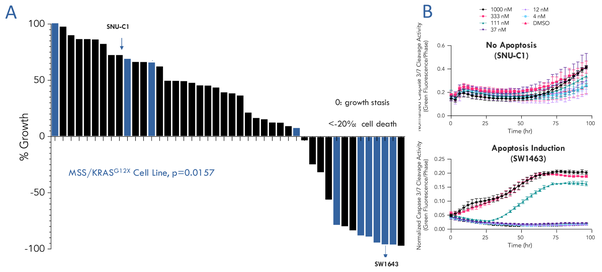
- N=50 treated with Sotorasib (N=46 treated with only Sotorasib)
- N=35 treated with Adagrasib (N=31 treated with only Adagrasib)
- N=4 treated with both Sotorasib and Adagrasib

Statistics in Months	MSS	MSS, KRAS G12C	MSS, CARD11 Q1*	MSS, CARD11 Q4**
N#	62	53	15	14
Min	0	3	3	5
25%	18	26	24	21.5
Median	62	73	93	58.5
75%	124	134	136	88
Max	430	430	430	188

patients who received Sotorasib or Adgrasib in the group of interest
* Q1: Lowest 25% CARD11 expression
** Q4: Highest 25% CARD11 expression
^ Chi-square analyses on the table

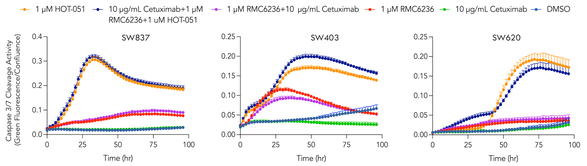
Figure 2. CBM Inhibitor Selectively Induced Apoptosis in MSS/KRAS^{G12X} CRC

CBM Inhibitor Selectively Induced Apoptosis in MSS/KRAS^{G12X} CRC



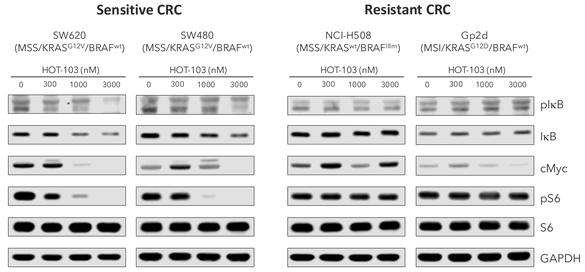
(A) A panel of CRC lines (including KRAS WT and KRAS mutant) were treated with CBM inhibitor (HOT-051) in dose and time-dependent manner. The plot was graphed based on 3 μM of HOT-051 (maximum inhibition) after 96hr treatment. 0-100: growth inhibition; 0: growth stasis; <-20%: cell death. (B) Representative cell lines for HOT-051 induced apoptosis. Dose and time-dependent caspase 3/7 cleavage was monitored using Incucyte.

Figure 3. CBM Inhibitor Induced Potent Apoptosis in MSS/KRAS^{G12X} CRC, Superior to KRASi Plus EGFR Antibody



Apoptosis was measured by caspase 3/7 cleavage using Incucyte.

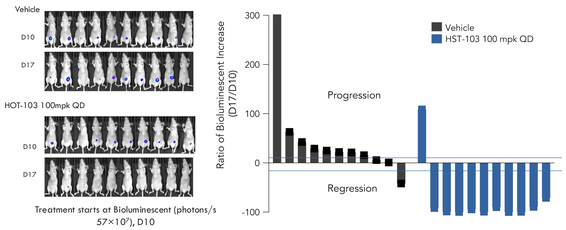
Figure 4. CBM Inhibitors Blocked Multiple Oncogenic Survival Signals in Sensitive KRAS^{G12X} CRC



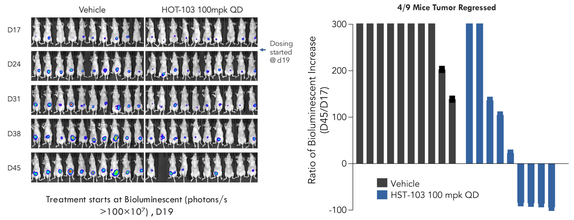
Cells were treated with HOT-103 (CBMi) for 24hr, followed by Western blot analysis of protein expression.

Figure 5. CBM Inhibitor Blocked Tumor Growth in Orthotopic SW620 (KRAS^{G12V}) CRC Model

Part 1: Tumor Regression After 7-day Treatment

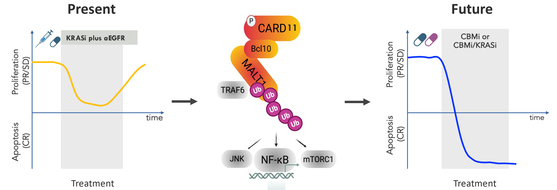


Part 2: CBM Inhibitor Blocked Progression of High-Burden Tumors



Conclusions

- KRAS inhibitors do not induce strong apoptosis in vitro, in vivo, or in patients, especially for CRC, resulting in limited and non-durable response of KRASi.
- We discovered that KRAS^{G12X} CRC depends on the CBM signalosome for survival. Inhibition of CBM signalosome induced profound apoptosis/cell death of KRAS^{G12X} CRC in *in vitro* and *in vivo* preclinical models.
- CBMi plus KRASi may provide deep and durable responses by targeting two cancer hallmarks: proliferation & survival.



Complete inhibition of MAPK to have strong anti-proliferation effect

Pro-survival signals for KRAS^{G12X} CRC

- Complete inhibition of MAPK and multiple oncogenic signals (NFκB, mTORC1 and C-MYC)
- Anti-proliferation+ apoptosis effect for deep & durable responses