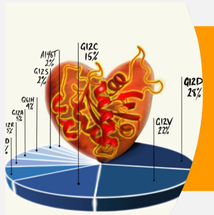

Probing and overcoming KRAS^{G12C} inhibitor resistance by combination with a pan-KRAS SOS1 inhibitor

#2478

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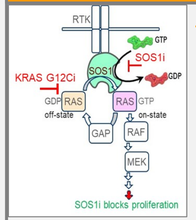
Introduction



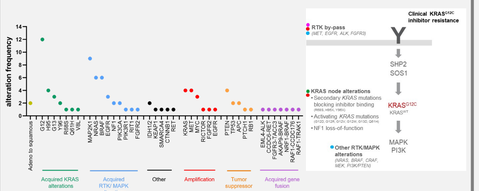
- The recent accelerated approval of the KRAS^{G12C} mutant-selective inhibitor Sotorasib (AMG 510) for the treatment of 2nd line *KRAS*^{G12C} mutation-positive NSCLC patients marks the first approved targeted therapy for tumors with any *KRAS* mutation. While KRAS^{G12C} inhibitors deliver clinical benefit, most patients who achieved an objective response ultimately progressed. Recent insights into clinical KRAS^{G12C} inhibitor resistance identified reactivation of the RAS/MAPK pathway as a common putative driver mechanism of resistance. Multiple ongoing trials seek to augment responses to KRAS^{G12C} inhibitors through rational combination strategies, including the SOS1::KRAS inhibitor BI 1701963.

Here we use different preclinical experimental approaches to interrogate KRAS^{G12C} inhibitor resistance mechanisms with the aim to identify strategies to overcome resistance. To predict on-target resistance,

- Ba/F3 cells were transduced with *KRAS*^{G12C}, ENU-mutagenized and chronically exposed to KRAS^{G12C} inhibitors. Resistant clones were screened for secondary KRAS mutations, highlighting that *KRAS G12C/Y96D* and *Y96S* cis mutations did confer resistance to KRAS^{G12C} inhibition but could be overcome by combining a MEK inhibitor with a SOS1 inhibitor.
- As second strategy a high-complexity single site variant library of *KRAS*^{G12C} encompassing all possible secondary *KRAS* mutations was employed to establish Ba/F3 transgenic cell pools. The response of this *KRAS*^{G12C} Ba/F3 clone library harboring a comprehensive set of secondary mutations was tested following treatment with KRAS^{G12C} inhibitors alone and in combination with a pan-KRAS SOS1 inhibitor. In parallel, acquired KRAS^{G12C} inhibitor resistance was generated in solid cancer cell lines following long-term Adagrasib (MRTX849) treatment. Clones were characterized and their response to KRAS^{G12C} inhibition and combination therapy was analyzed. Both in the Ba/F3 cell pool as well as in KRAS^{G12C} inhibitor resistant solid cancer clones, combining SOS1 inhibition with KRAS^{G12C} inhibition suppressed the incidence of resistant growth.
- Finally, probing resistance *in vivo*, SW837 (CRC) tumor-bearing mice were subjected to long-term treatment with Adagrasib until tumors relapsed after initial regression. Resistant tumors were randomized for second line treatments. In this KRAS^{G12C} inhibitor resistant setting, treatment with Adagrasib plus Cetuximab resulted in tumor stasis while adagrasib plus SOS1i resulted in tumor regressions.

Published data on acquired KRAS^{G12C} resistance

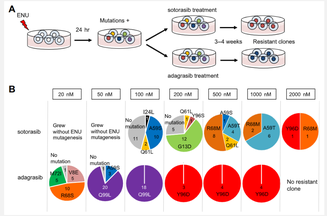
Figure 1: Literatur review. Genetic alterations associated with resistance to Adagrasib or Sotorasib treatment in patients



Based on Awad et al., NEJM 2021 & Zhao et al., Nature 2021

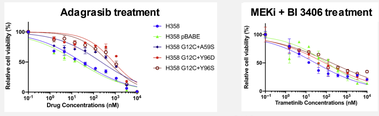
KRAS^{G12C}i resistance by secondary KRAS mutation

Figure 2: Ba/F3 cells were ENU-mutagenized and subjected to long-term KRAS^{G12C}i treatment:



- Resistant clones exhibit secondary KRAS mutations reminiscent of the ones observed in the clinic

Figure 3: NCI-H358 (NSCLC) cells, expressing KRAS secondary mutations, were tested in proliferation assays for drug sensitivity:

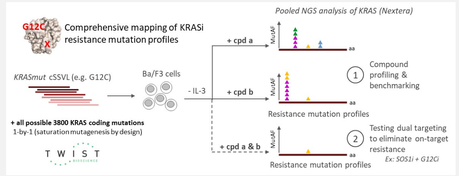


- Secondary KRAS mutations lead to KRAS^{G12C}i resistance in solid cancer cells
- Resistance can be overcome by combined inhibition of SOS1 and MEK

Koga et al., J Thorac Oncol. 2021

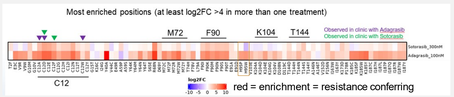
Impact of secondary KRAS mutation on KRASG12C

Figure 4: A Ba/F3 cell pool was generated to express all possible 3800 coding cis-mutations within KRAS^{G12C}. Clones resistant to compound treatment were then sequenced:



On-Target KRAS^{G12C} Inhibitor Resistance Profiling

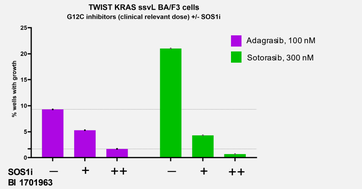
Figure 5: KRAS^{G12C}i resistant Ba/F3 clones were analyzed by NGS to identify resistance-conferring cis-mutations:



- Deep mutational screening allows prediction of clinically relevant mutations
- Pre-clinical comparison of KRAS^{G12C} inhibitors based on their resistance profiles

Influence of SOS1 inhibitor on KRAS^{G12C}i resistance

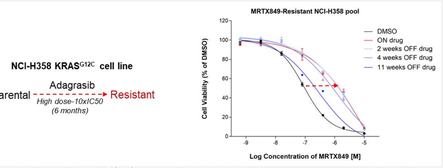
Figure 6: KRAS^{G12C}i resistant Ba/F3 outgrowth was analyzed following SOS1i (BI 1701963) + KRAS^{G12C}i combination treatment:



- Combination of SOS1i with all tested KRAS^{G12C}i suppresses the incidence of resistant growth

Acquired resistance: Long-term KRAS^{G12C}i treatment

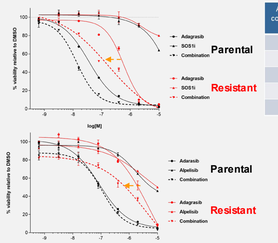
Figure 7: KRAS^{G12C}i resistant NCI-H358 NSCLC cells were generated following long-term Adagrasib treatment:



- Resistance to KRAS^{G12C}i generated *in vitro* by high dose long-term treatment
- Resistance observed in pools has a transient component
- Single clones available, characterization of resistance mechanism(s) ongoing (WGS, RNAseq)

Winning combinations in NCI-H358 resistant pools

Figure 8: KRAS^{G12C}i resistant NCI-H358 cells were used to determine which combination therapy could be successful:

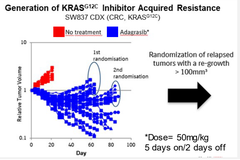


Adagrasib combination partner	Ratio (IC50 Adagrasib/ IC50 Combination)
SHP2	1.3
SOS1	1.7
PI3K	1.8
mTOR	2.8
MEK	3.5

- *In vitro* KRAS^{G12C}i-resistant cells are a great tool to identify adequate combination therapies
- Vertical (e.g. SOS1i or SHP2i + KRAS^{G12C}i) and parallel pathway inhibitors (e.g. PI3Ki or mTOR + KRAS^{G12C}i) appear promising

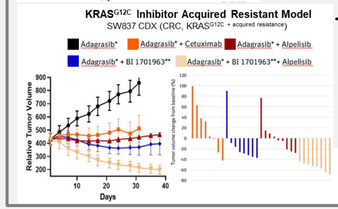
Overcoming KRAS^{G12C} Inhibitor-Induced Resistance

Figure 9: KRAS^{G12C} inhibitor-resistant SW837 (CRC KRAS^{G12C}) tumors were generated in mice following long-term treatment with Adagrasib (50mg/kg, 5 days on/2 days off). Once tumors relapsed on long-term Adagrasib treatment, resistant tumors were randomized and response to several second line treatments was tested:



*Dose= 50mg/kg
5 days on/2 days off

- In the SW837 (CRC) xenograft, Adagrasib (MRTX849)-induced resistance can be overcome by a SOS1i (BI 1701963) + KRAS^{G12C}i (Adagrasib) combination.

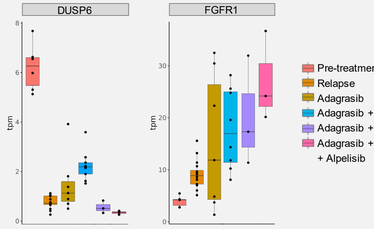


- Supports clinical positioning of BI 1701963 in combination with a KRAS^{G12C} inhibitor in naïve patients and patients with acquired resistance

Adagrasib 50mg/kg, 5 days on/2 days off; BI 1701963 50mg/kg, p.o. bid, Alpelisib 12.5mg/kg, p.o., qd; Cetuximab 20mg/kg, i.p., twice weekly

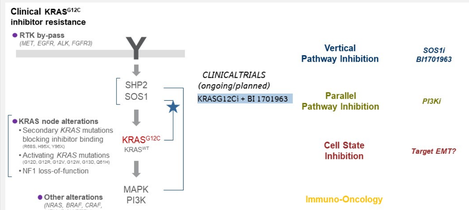
Mapping *in vivo* KRAS^{G12C}i -Induced Resistance

Figure 10: Analysis of SW837 tumors at pre-treatment and after first (Adagrasib) and second line therapy:



- DUSP6 down-regulation is maintained in KRAS^{G12C}i resistant SW837 tumors
- FGFR1 expression is up-regulated in resistant tumors
- More detailed analysis ongoing

Strategy to Overcome KRAS^{G12C}i Resistance



Key findings and conclusions

While more work is currently being undertaken to map the resistance mechanisms in both our *in vitro* and *in vivo* settings, the results highlight the potential of combining a SOS1 inhibitor with a KRAS^{G12C} inhibitor to prevent and/or overcome acquired resistance.

The pan-KRAS SOS1 inhibitor BI 1701963 is the first direct RAS signaling modifier in phase I clinical trials both as a monotherapy as well as in combination with KRAS^{G12C} inhibitors.

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