MEDI-144

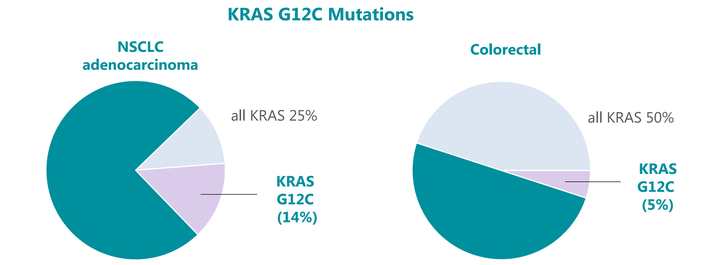
# Structure-Based Drug Discovery of a Selective, Covalent KRAS G12C Inhibitor with Oral Activity in Animal Models of Cancer

*John P. Fischer‡, Matthew A. Marx†, Brian R. Baer‡, Joshua Ballard†, James F. Blake‡, Karyn Bouhana‡, Barbara J. Brandhuber‡, David M. Briere†, Laurence E. Burgess‡, Michael R. Burkard†, Harrah Chiang†, Mark J. Chicarelli‡, James G. Christensen†, Kevin Davidson‡, John J. Gaudino‡, Jill Hallin†, Lauren Hanson‡, Kenneth Hee‡, Erik J. Hicken‡, Ronald J. Hinklin‡, Macedonio J. Mejia‡, Peter Olson†, Pavel Savechenkov‡, Niranjan Sudhakar‡, Tony P. Tang‡, Guy P. Vigers‡, Henry Zecca†, Jay B. Fell‡*

† Mirati Therapeutics, Inc., San Diego, CA, 92121, USA. ‡ Array Biopharma, Inc., Boulder, CO, USA.

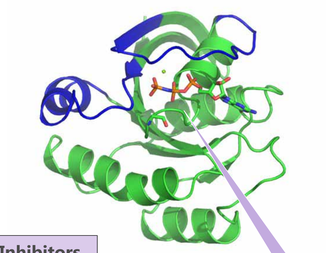
## Introduction

- KRAS is the most frequently mutated driver oncogene in human cancer[1]
- The ability to target and block the function of mutated KRAS has remained elusive despite decades of research
- Findings have demonstrated that directly targeting KRAS G12C with electrophilic molecules that covalently modify the mutated codon 12 cysteine may be feasible[2-6]
- A novel series of potent, irreversible covalent inhibitors of KRAS G12C has been identified
- Displacement of a bound water in KRAS G12 provided a boost in activity
- Inhibitor **11** exhibited dose ascending oral bioavailability and robust in vivo target engagement
- Tumor growth inhibition in a MIA PaCa xenograft was demonstrated




## Historical Challenges in Targeting the KRAS Pathway

**Upstream Inhibitors**
*Blocking Ras Membrane localization*
- Farnesyl transferase inhibitors do not block KRAS localization

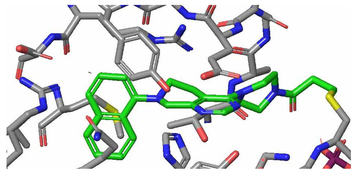


**Reversible Inhibitors**
Targeting KRAS$^{mut}$ is challenging due to small, undefined catalytic site and high affinity for GTP

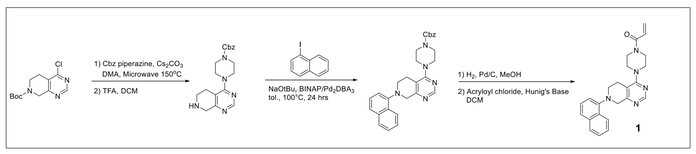
**Downstream Effector Inhibitors**
*Raf / MEK and PI3K / AKT / mTOR*
- Limited effectiveness in KRAS$^{mut}$ tumors
- Incomplete inhibition of KRAS$^{mut}$
- Inhibition of KRAS$^{wt}$ resulting in low therapeutic index

**Covalent Inhibition of KRAS G12C**
- Binding in the switch II pocket of GDP KRAS
- Covalent bond to cysteine 12
- Locked in the inactive conformation

## Tetrahydropyridopyrimidine 1 Modifies KRAS G12C

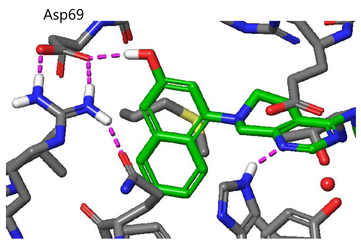


Crystal structure of **1** bound to KRAS G12C

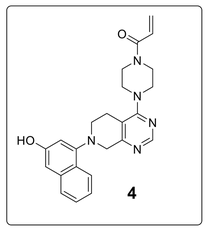



- The prototype tetrahydropyridopyrimidine **1** was synthesized in 5 steps
- **1** showed 13% modification in a 3hr/25 M protein modification assay
- This compound did not inhibit Erk phosphorylation in an H358 cell assay
- The crystal structure of **1** shows the covalent bond between the acrylamide and cysteine 12

## Naphthol Makes a Hydrogen Bond to Asp69



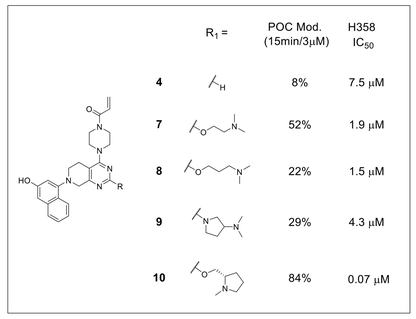

Crystal structure of **4** bound to KRAS G12C




| | $R_1$ = | POC Mod. (3hr/5μM) | H358 $IC_{50}$ μM | | $R_1$ = | POC Mod. (3hr/5μM) | H358 $IC_{50}$ μM |
|---|---|---|---|---|---|---|---|
| **1** |  | 2% | >16 | **4** |  | 99% | 7.6 |
| **2** |  | 22% | >16 | **5** |  | 0% | >16 |
| **3** |  | 2% | >16 | **6** |  | 2% | >16 |

- A series of analogs were synthesized in an attempt to pick up an interaction with Asp69
- Compound **4** exhibited full modification of KRAS and inhibited Erk phosphorylation in a cell, $IC_{50}$ = 7.6 μM
- An X-ray crystal structure confirmed a 2.7Å H-bond between the naphthol OH and Asp69

## C2 Prolinol Side Chain Increases Activity

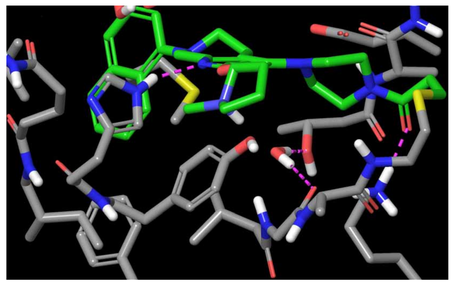


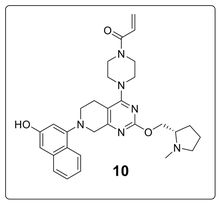
| | $R_1$ = | POC Mod. (15min/3μM) | H358 $IC_{50}$ |
|---|---|---|---|
| **4** |  | 8% | 7.5 μM |
| **7** |  | 52% | 1.9 μM |
| **8** |  | 22% | 1.5 μM |
| **9** |  | 29% | 4.3 μM |
| **10** |  | 84% | 0.07 μM |

| **10** Mouse PK | | | | |
|---|---|---|---|---|
| IV Dose | $AUC_{inf}$ (hr*μg/mL) | $t_{1/2}$ (hr) | Clearance (mL/min/kg) | $V_{ss}$ (L/kg) |
| 3 mpk IV | 1.1 | 0.96 | 46 | 0.53 |
| PO Dose | $AUC_{inf}$ (hr*μg/mL) | Cmax (μg/mL) | $T_{max}$ (hr) | F (%) |
| 100 mpk | 0.88 | 0.59 | 1.0 | 2.4 |

- Substitution at C2 provided compound **10** containing an N-methyl pyrrolidine side chain
- Lipophilic contacts and a hydrogen bond to Glu62 contributed to a 100x potency boost compared to **4**
- This analog inhibited KRAS dependent Erk phosphorylation with a cellular $IC_{50}$ = 0.07 M
- Dosed in mice, **10** showed 51% extraction ratio and oral bioavailability, F= 2.4%

## Pursue Displacement of Bound Water to Increase Activity

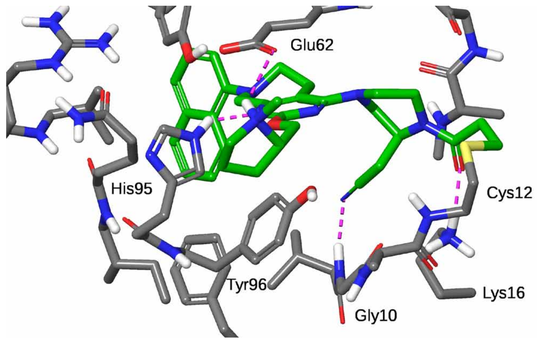


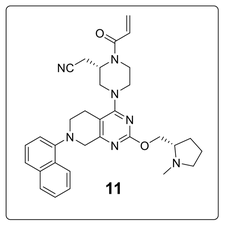



Crystal structure of **10** bound to KRAS G12C

- In the crystal structure of **10**, we observed a bound water complexed to Gly10 and Thr58
- It is possible that displacement of this water could lead to a potency boost[7]
- We hypothesized that removal of the hydroxy with concurrent targeting of the bound water could give a compound with desirable properties

## Nitrile 11 Displaces a Bound Water in KRAS G12C

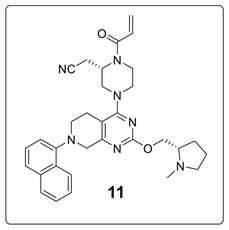



Crystal structure of **11** bound to KRAS G12C

**Key Interactions**
- His95 - H-bond to pyrimidine nitrogen
- Tyr96 - pi-pi interaction to the pyrimidine
- Gly10 - Nitrile H-Bond to backbone N-H
- Lys16 - Acrylamide carbonyl interaction with $NH_3$+
- Cys12 - Covalent bond formed to acrylamide olefin
- Glu62 - C2 Tail salt bridge to the acid

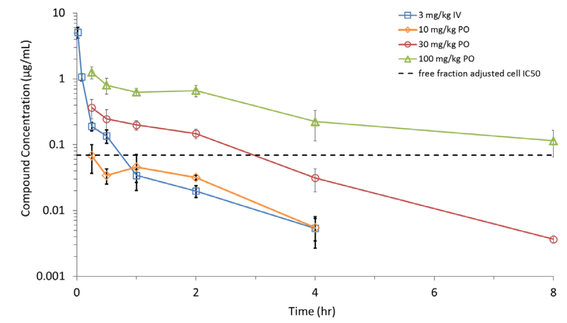
## Compound 11 is an Irreversible Covalent Inhibitor of KRAS G12C




| | |
|---|---|
| MW / ClogP / PSA | 551 / 4.4 / 87 |
| 5min/3 M Protein Modification | 87 % |
| H358 Cell $IC_{50}$ | 5 nM |
| Permeability | Medium/Efflux |
| Mouse Hepatocyte ER | 90% |
| Mouse Microsomal ER | 49% |
| Mouse PPB | 96% |

- The inhibitor **11** showed 87% modification of KRAS G12C in the 5min/3 M modification assay
- Compound **11** inhibited KRAS dependent ERK phosphorylation in the H358 cell assay, $IC_{50}$ = 5 nM
- This compound has high hepatocyte ER with medium permeability and efflux
- Based on 96% protein binding the $ff_{adj}$ cell $IC_{50}$ = 125 nM

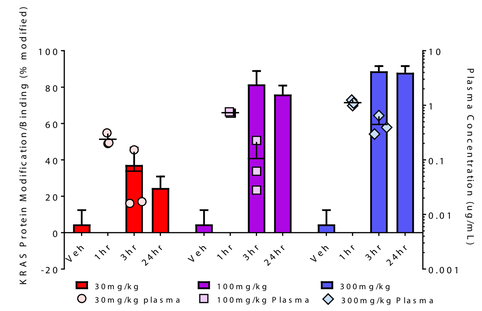
## Inhibitor 11 Demonstrates Dose Ascending Oral PK in Mice




- As predicted by hepatocytes, **11** was a high clearance compound
- PO dosing resulted in super proportional increases in exposure from 10 -100 mg/kg in mice
- The 30 mg/kg dose covered the free fraction adjusted cell $IC_{50}$ for 3 hours

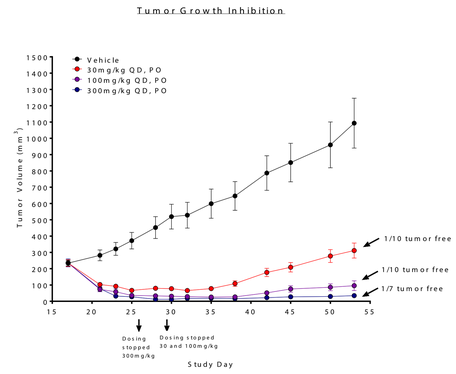
| **11** Mouse PK | | | | |
|---|---|---|---|---|
| IV Dose | $AUC_{inf}$ (hr*μg/mL) | $t_{1/2}$ (hr) | Clearance (mL/min/kg) | $V_{ss}$ (L/kg) |
| 3 mpk | 0.50 | 1.1 | 100 | 2.6 |
| PO Dose | $AUC_{inf}$ (hr*μg/mL) | Cmax (μg/mL) | $T_{max}$ (hr) | F (%) |
| 10 mpk | 0.12 | 0.69 | 0.25 | 7.1 |
| 30 mpk | 0.61 | 0.37 | 0.25 | 12 |
| 100 mpk | 3.3 | 1.3 | 0.25 | 20 |

## Robust Target Engagement Seen in a MIA PaCa Tumor PK/PD




- Inhibitor **11** was dosed at 30, 100, and 300 mg/kg in MIA PaCa tumor bearing mice
- Tumors were collected for processing at the 1, 3, and 24 hour time points
- Target engagement of KRAS G12C + compound was determined by mass spectrometry
- PK/PD experiments showed >80% target engagement at the 100 and 300 mg/kg dose
- This PD effect was maintained for at least 24h in this experiment

## 11 Exhibits Rapid and Sustained Efficacy Against MIA PaCa Xenografts




- **11** was dosed QD PO in a MIA PaCa Antitumor Efficacy Study at 30, 100, and 300 mg/kg
- Rapid tumor growth inhibition was observed in all dose groups
- Dosing was stopped on day 26 for the 300 mg/kg group and on day 29 for the other groups
- Regrowth was observed in the 30 while the 100 and 300 mg/kg groups showed minimal regrowth
- All dose groups had animals that were tumor free at the end of the study

## Summary and Conclusions

- We have identified a novel series of irreversible covalent inhibitors of KRAS G12C
- Interaction with Asp69, by hydroxy substitution of the naphthyl, led to cell activity
- 100x potency boost was gained by substitution at the pyrimidine C2 position
- Introduction of the cyanomethyl group displaced a bound water in KRAS G12C and provided an alternate path forward
- Compound **11** inhibited KRAS dependent ERK phosphorylation in the H358 cell assay with an $IC_{50}$ = 5 nM
- **11** demonstrated super proportional dose ascending oral exposure in mice with 3h target coverage at the 30 mg/kg dose
- In a PK/PD experiment, >80% target engagement was seen in MIA PaCa tumors with an effect lasting for at least 24 hours
- A antitumor efficacy experiment with **11,** dosed orally in mice bearing MIA PaCa tumors, showed rapid growth inhibition and durable efficacy even after dosing was stopped
- Additional compounds will be discussed in future publications

## References and Acknowledgements

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