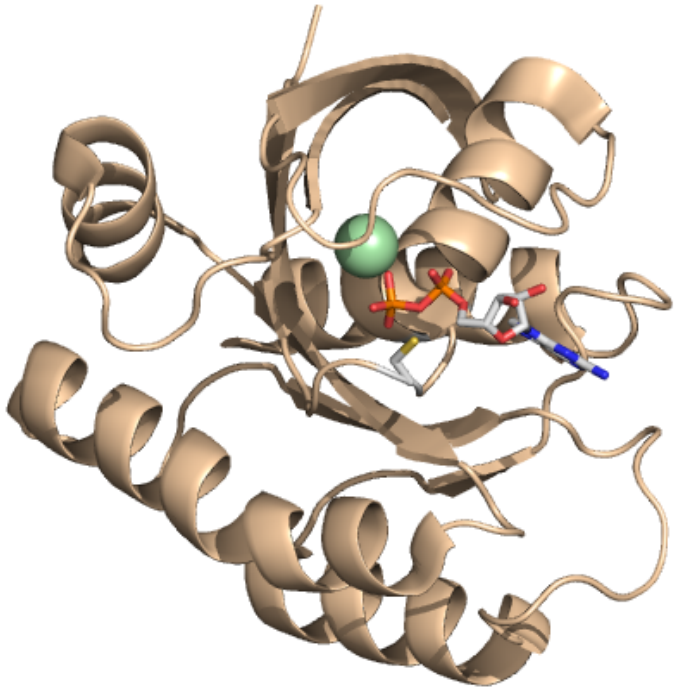
Therapeutic Targeting of Oncogenic K-Ras by a Covalent Catalytic Site Inhibitor

S.M. Lim *et al*. *Angew. Chem. Int. Ed.* **2014**, 53, 199-204.



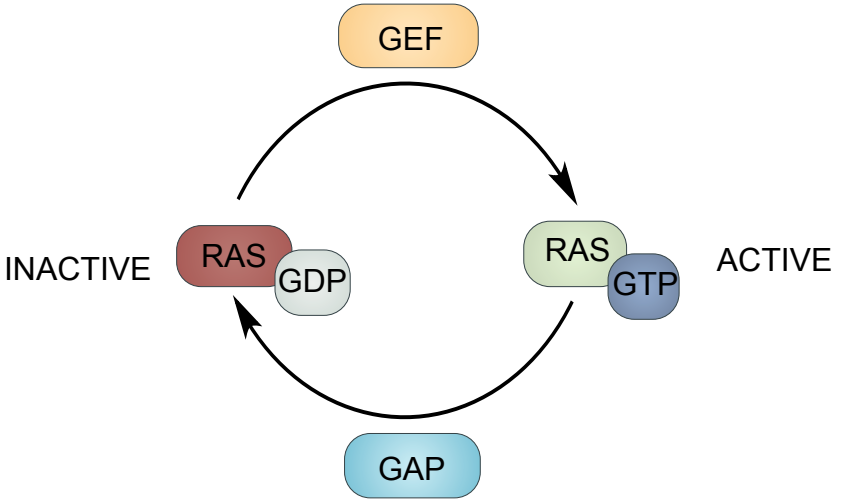
Evan Carder

Wipf Group Current Literature

7 June 2014

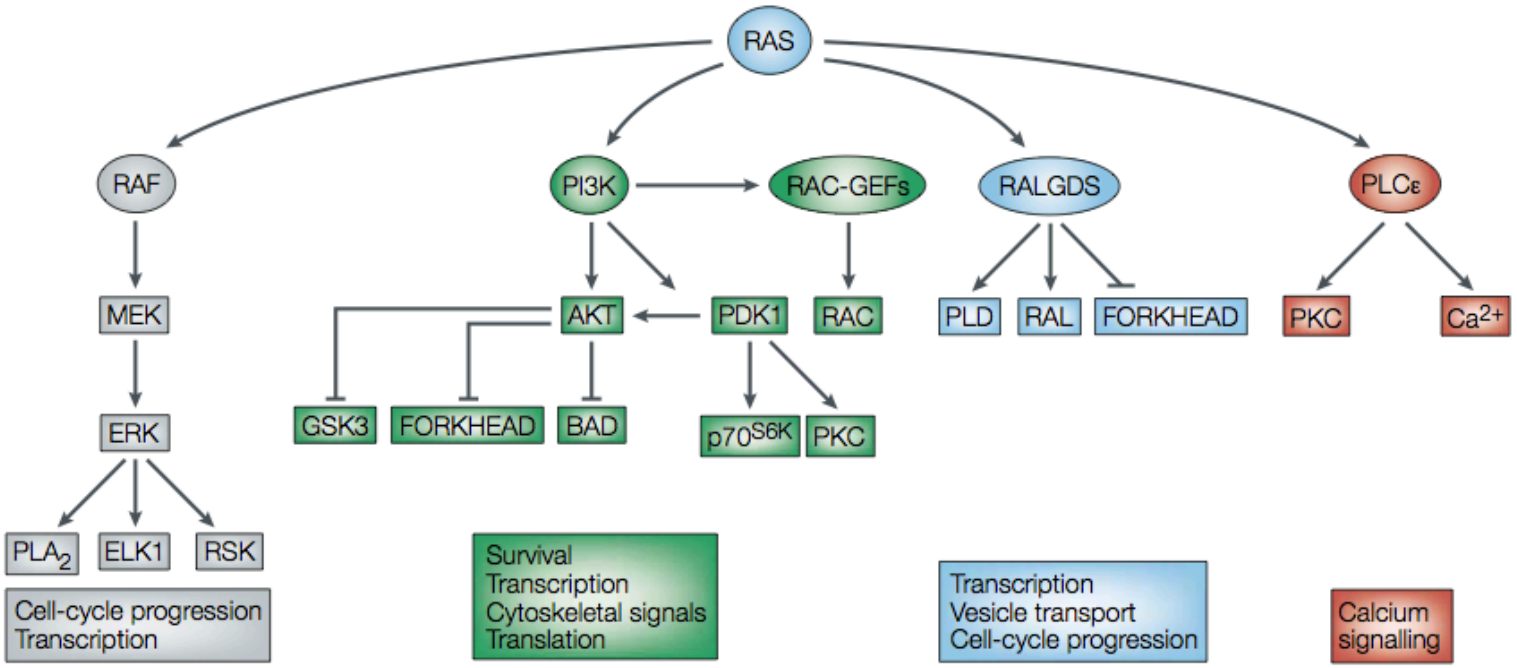
K-Ras

- A small GTPase apart of the Ras protein superfamily
- Most frequently activated driver of human cancer
- Ubiquitously expressed
- Maintains an essential role in regulating important signaling pathways necessary for normal cellular physiology



[1] Y. Pylayeva-Gupta *et. al. Nat. Rev.* **2011**, 11, 761.

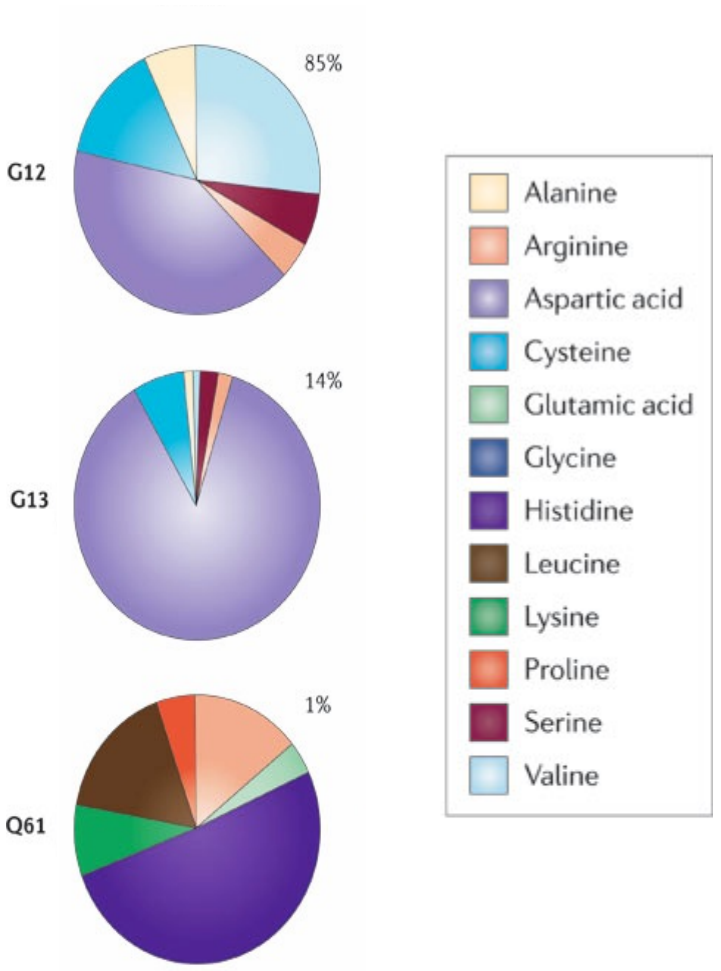
K-Ras



[1] Downward, J. *Nat. Rev.* **2003**, 3, 11-22.

Frequency of K-Ras Mutations in Cancer

Tissue	*KRAS**	Incidence rate‡	Mortality rate‡
Endocrine	0% (670)	0.7	0.3
Biliary tract	31% (1,679)	NA§	NA
Bone	1% (252)	0.9	0.4
Breast	4% (782)	124	24
Central nervous system	1% (1,054)	6.5	4.3
Cervix	7% (637)	8.1	2.4
Endometrium	14% (2,251)	23.9	4.1
Eye	4% (90)	0.8	0.1
Haematopoietic and lymphoid tissue	5% (5,978)	35.2	14.5
Kidney	1% (704)	14.6	4.1
Large intestine	33% (34,013)	47.2	17.6
Liver	5% (461)	7.3	5.2
Lung	17% (16,348)	62	52.5
Oesophagus	4% (375)	4.5	4.4
Ovary	14% (3,181)	12.8	8.6
Pancreas	57% (5,329)	12	10.7
Pleura	0% (45)	NA	NA
Prostate	8% (1,184)	156	24.7
Salivary gland	3% (170)	NA	NA
Skin	3% (1,462)	22.7	3.5
Small intestine	20% (316)	2	0.4
Stomach	6% (2,793)	7.7	3.8
Testis	4% (432)	5.5	0.2
Thymus	2% (186)	NA	NA
Thyroid	2% (5,166)	11	0.5
Upper aerodigestive tract	3% (1,582)	14	3.7
Urinary tract	5% (1,099)	21.1	4.3



[1] Y. Pylayeva-Gupta *et. al. Nat. Rev.* **2011**, 11, 761.

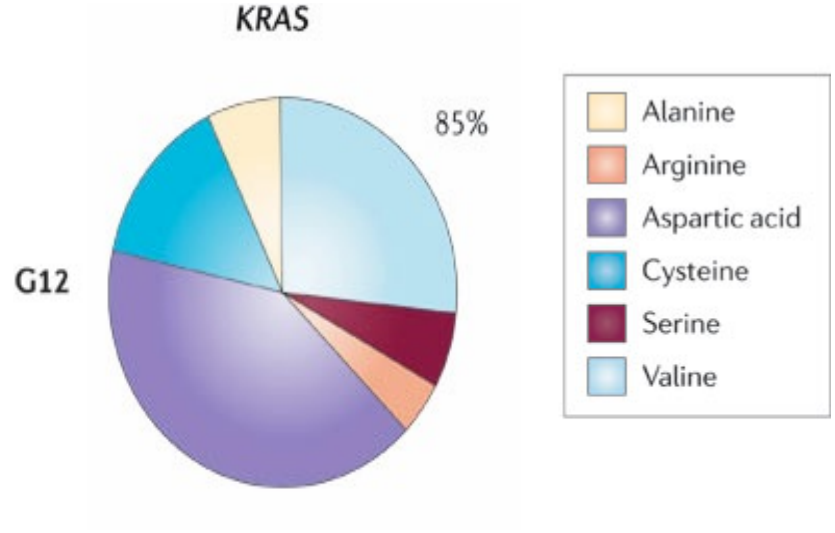
“The Undruggable Target”

- K-Ras activation
  - GEF – SOS inhibitors[1]
- Post-translational modifications
  - Farnesyltransferase inhibitors (FTI)[2]
- Membrane localization
  - PDEδ inhbitors[3]
- Downstream Ras effector proteins
  - RAF, MEK, ERK, PI3K inhibitors[4]

[1] A. Patgiri *et. al. Nat. Chem. Biol.* **2011**, 585-587.
[2] Y. Reiss *et. al. Cell* **1990**, 62, 81-88.
[3] G. Zimmermann *et. al. Nat.* **2013**, 497, 636-642
[4] Downward, J. *Nat. Rev.* **2003**, 3, 11-22.

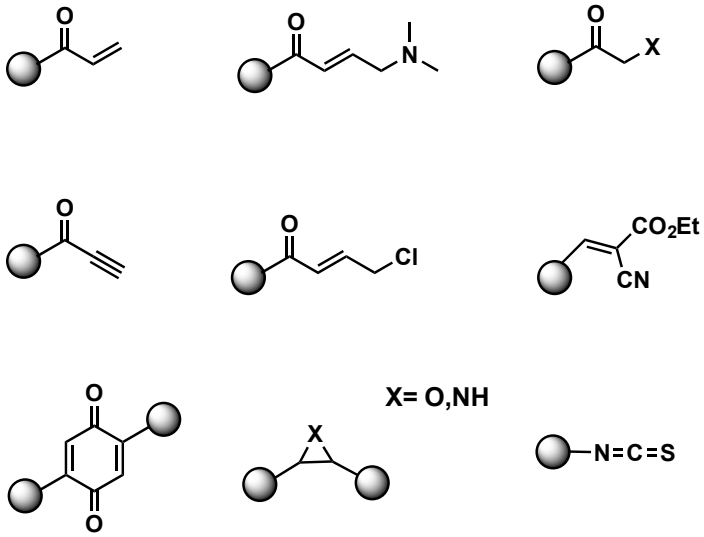
Current Strategy

- All cells utilize Ras signaling pathways to various extents. Therefore, there is great concern that inhibitors will have hazardous effects on normal cells. Is it possible to specifically target mutant K-Ras while having minimal effect on normal Ras signaling?



Irreversible inhibitors

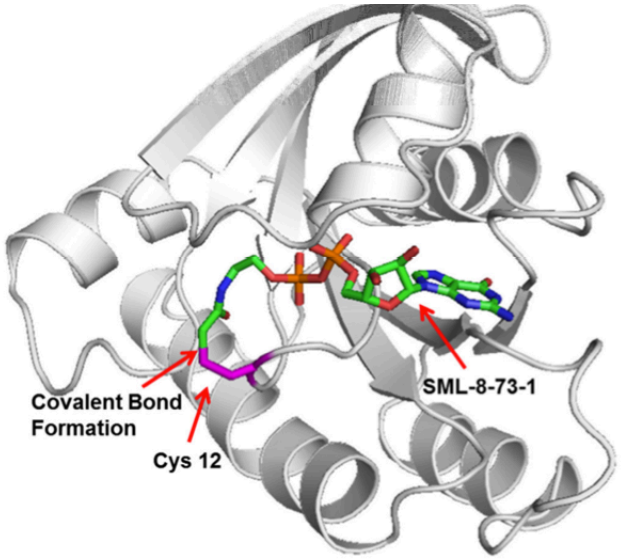
- Many covalent modifiers target nucleophilic cysteine thiolates[1].
- Michael addition as well as nucleophilic displacement reactions are common strategies to achieve irreversible inhibition [1].
- Inhibitors can be developed to react with specific nucleophiles, increasing selectivity among related proteins[2].
- Irreversible inhibitors have prolonged target residence time[2].

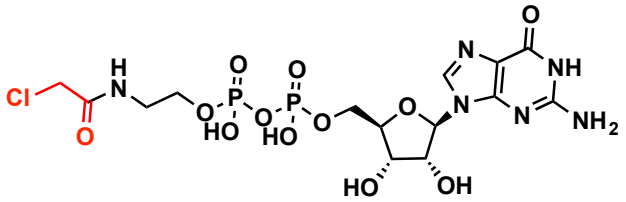


Electrophiles employed in irreversible inhibitors

[1] Q. Liu *et. al. Chem. & Biol.* **2013**, 20, 146-159.
[2] J. Sinh *et. al. Nat. Rev.* **2011**, 10, 307-317.

Targeting the G12C K-Ras mutant





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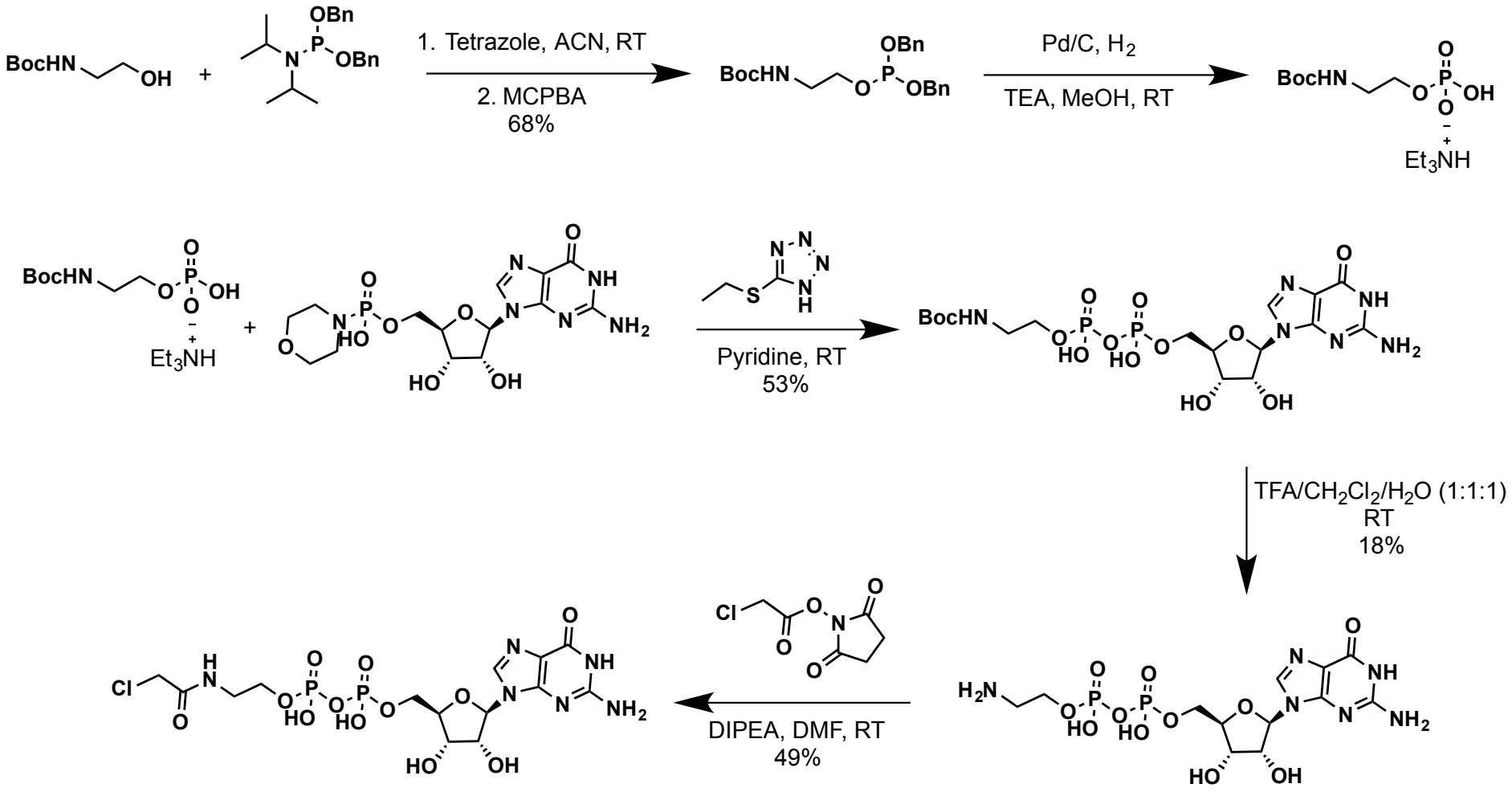
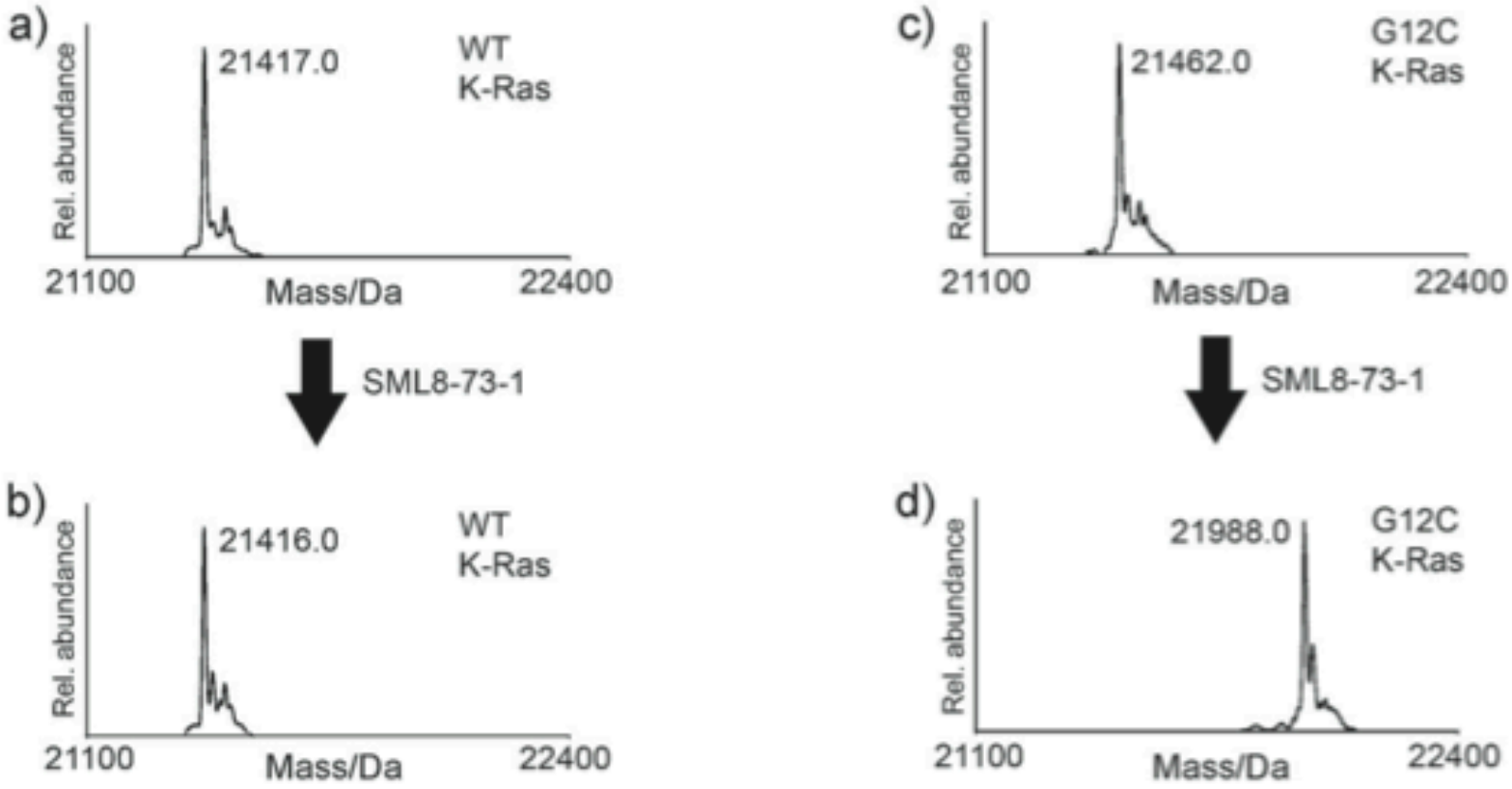
- Electrophilic GDP derivatives targets mutation-selective cancer
  - Theoretically it would exclusively inhibit G12C mutant cells without harming normal cells.

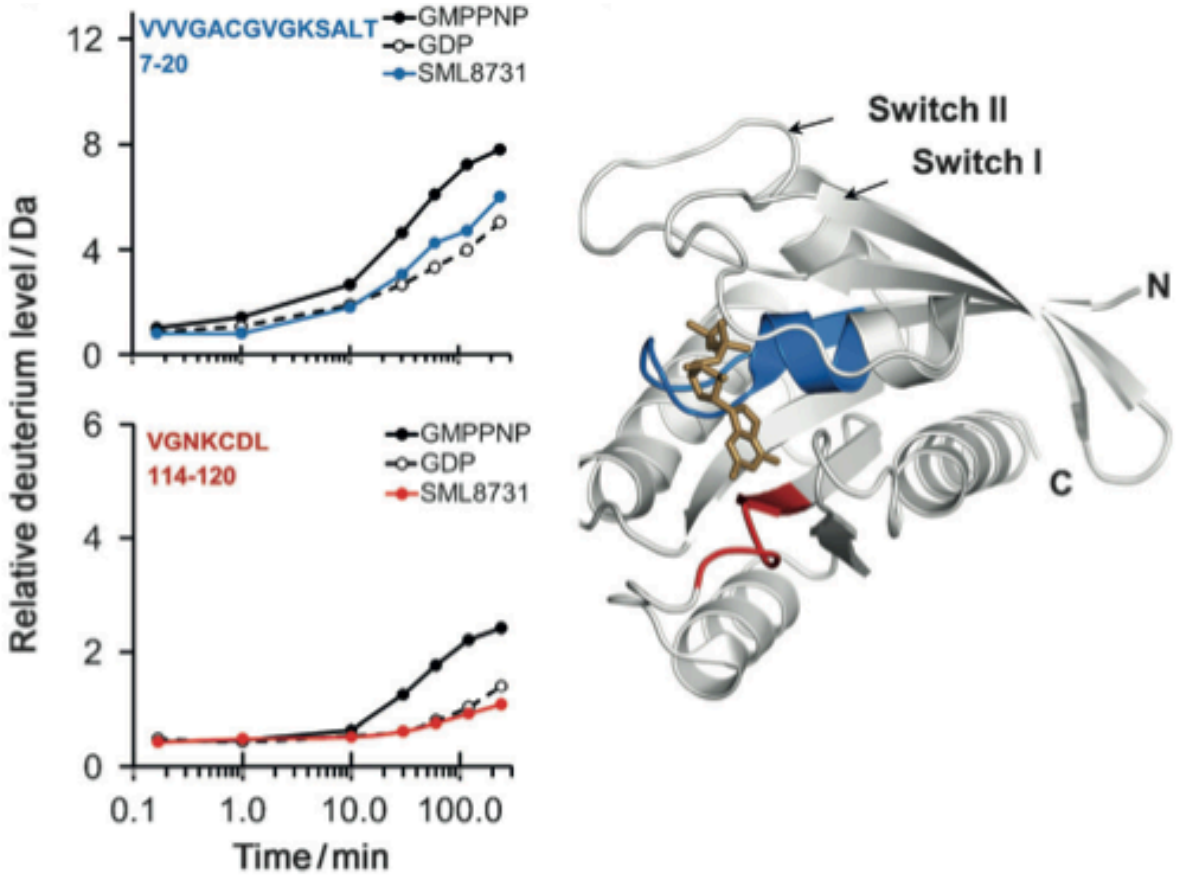
Synthetic scheme



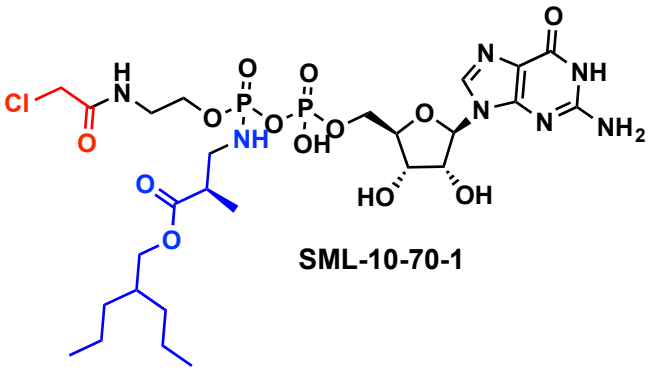
Covalent Modification of K-Ras G12C



Stabilization of the inactive conformation



K-Ras inhibition in H358 cells



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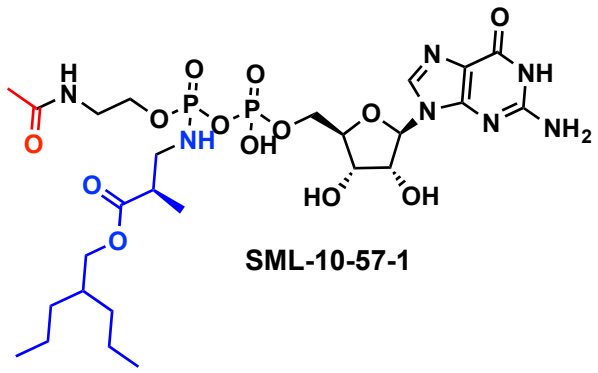

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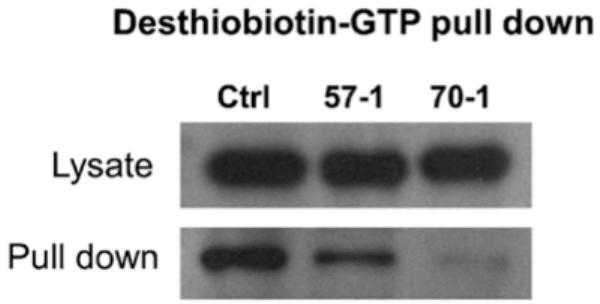

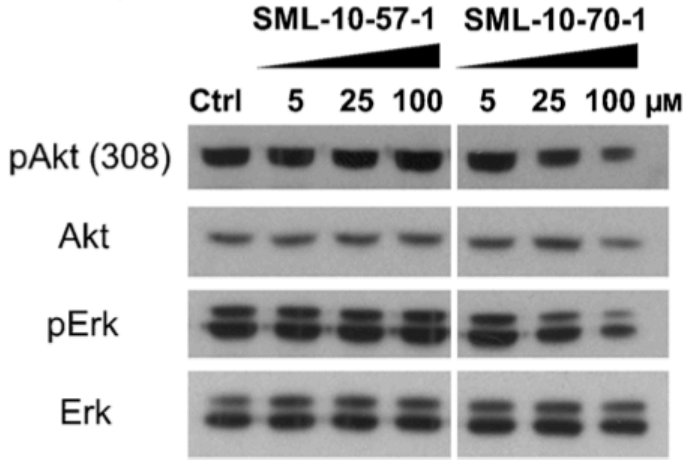

Conclusion

- Showcased a novel approach to selectively target G12C mutant K-Ras.
- Proposed inhibitor has poor cell permeability and requires high concentrations for cellular efficacy. Considerable optimization is required.
- Binds to the GDP-bound, inactive form of K-Ras. Due to a high GTP cellular concentration, newly translated K-Ras will statistically bind to GTP. The rate of hydrolysis for GTP bound mutant Ras is 8-17 hours.