

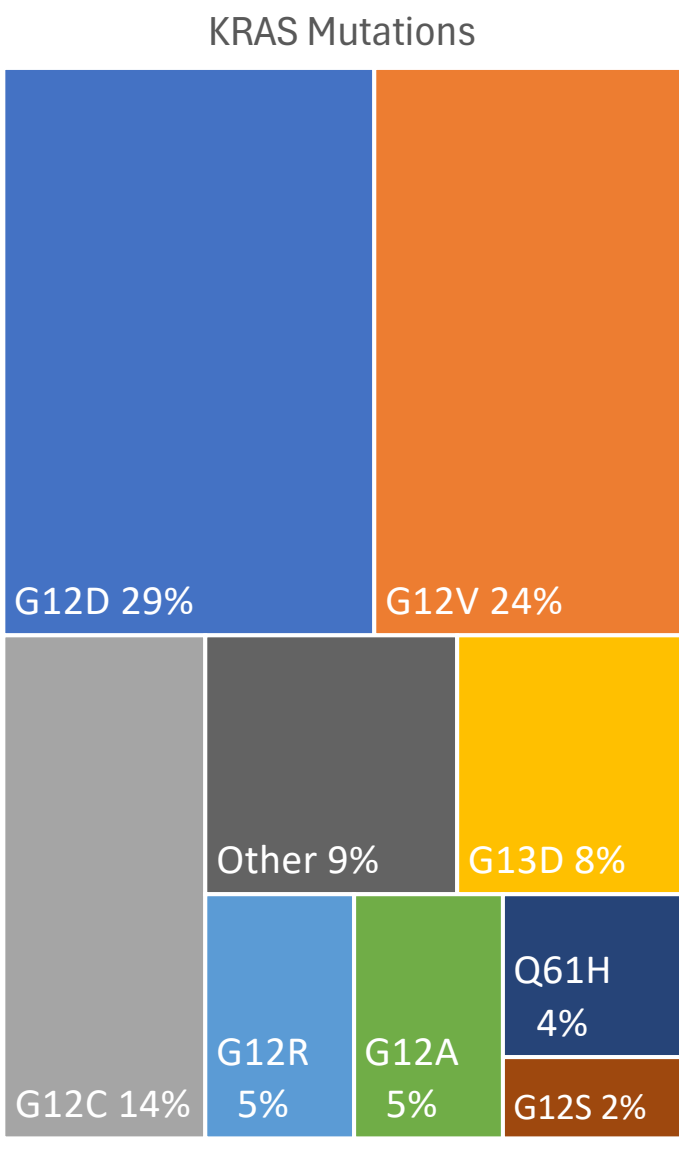
Identification of a Pan KRAS(ON) Inhibitor With Selectivity Over H/NRAS and pM Activity Across Prevalent KRAS Mutations

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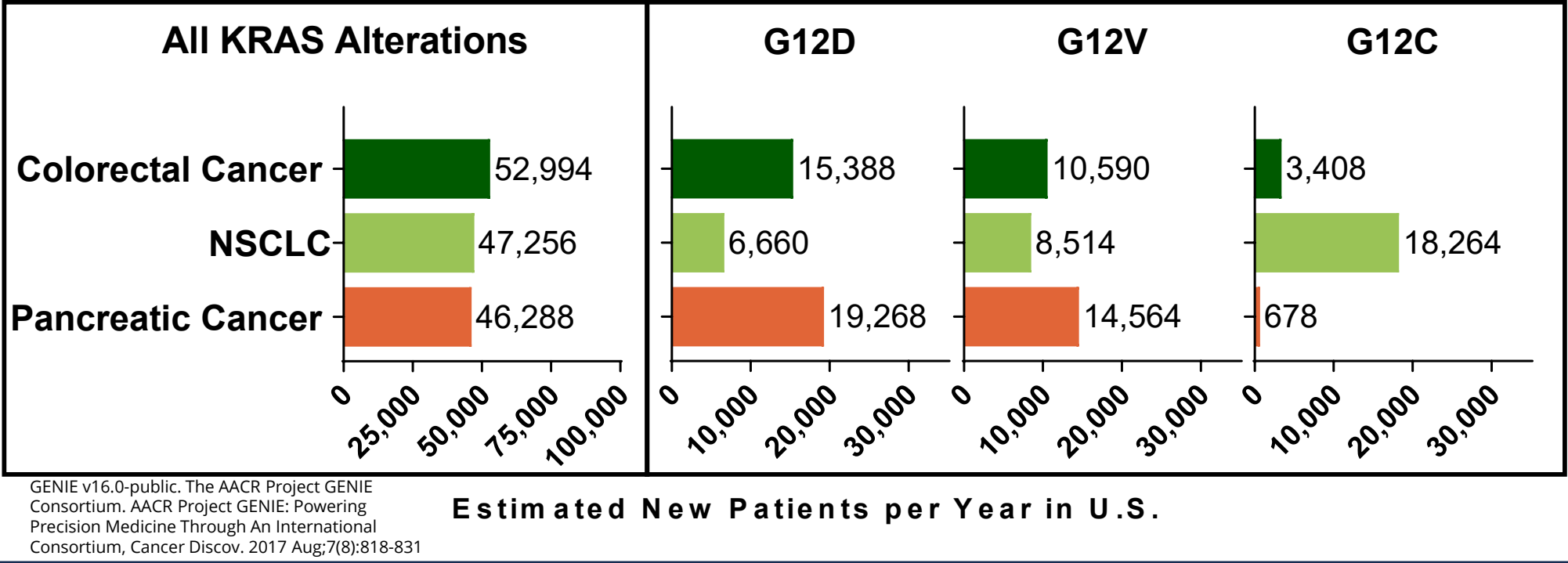
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Introduction

- KRAS mutations are prevalent across multiple tumor types and highest in CRC, NSCLC, and pancreatic cancer^{1,2}
- Annual incidence in the US of new KRAS mutant cancer patients is 175,000
- KRAS mutations primarily occur at codons 12, 13, and 61 with the most common mutations being G12D, G12V, and G12C
- The early lead molecule, CGT6737, is a pan KRAS(ON) small molecule inhibitor, with selectivity over HRAS and NRAS, and pM activity across KRAS mutations
- Robust PK/PD and tumor growth inhibition in mouse xenograft models following oral administration of CGT6737 is presented



Prevalence of KRAS Mutations by Cancer Type



CGT6737 Key Findings

- pM Inhibitor of mutant KRAS:** CGT6737 showed pM inhibitory activity in a panel of mechanistic cell assays from diverse tumor types with KRAS mutations measuring inhibition of pERK
- Binds in the Switch II pocket:** An X-Ray structure of CGT6737 bound to KRAS^{G12D} loaded with GTP analog GMPPCP shows CGT6737 bound in the switch II pocket of KRAS with the gamma phosphate of GMPPCP clearly visible
- SPR confirms active KRAS(ON) binding:** nM-pM Binding was observed by SPR to wild type and mutant KRAS loaded with the GTP stable analog GMPPCP
- Potent inhibitor of active GTP KRAS(ON):** A minimal shift in potency was observed with CGT6737 in a (+/-)EGF challenge cellular assay using AsPC-1 KRAS^{G12D} and SW480 KRAS^{G12V} lines
- Selective for KRAS over HRAS and NRAS:** SPR binding data shows that CGT6737 does not bind to HRAS or NRAS
- 90% PD inhibition in vivo:** Potent inhibition of pERK observed with CGT6737 dosed PO at 30/100 mg/kg in an AsPC-1 KRAS^{G12D} tumor-bearing mouse model
- Robust TGI in KRAS^{G12C} and KRAS^{G12D} models:** CGT6737 demonstrates robust tumor regression when dosed PO BID at 100 mg/kg in 14-day MIA PaCa-2 KRAS^{G12C} and AsPC-1 KRAS^{G12D} tumor bearing mouse TGI models
- Lead optimization of this series is ongoing**

Cogent KRAS(ON) Inhibitor Opportunity and Advantages

KRAS(ON) Inhibitor

Direct inhibition of KRAS signaling may prevent receptor tyrosine kinase amplification resistance mechanism

Differentiated MOA

CGT6737 is a Switch II binder not a tricomplex inhibitor

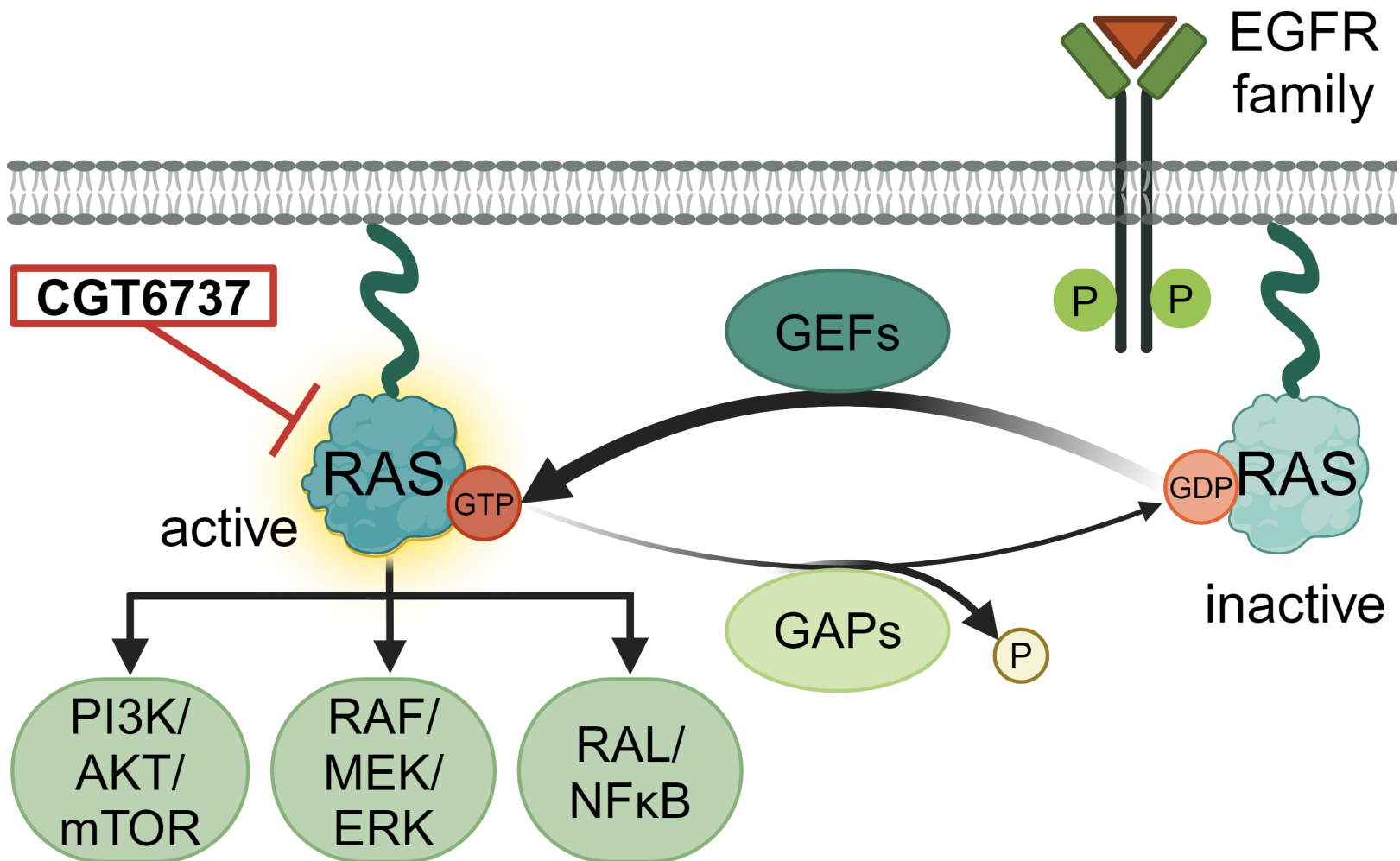
pM Potency

Highly potent against prevalent KRAS mutations for complete target inhibition

Selective over H/NRAS

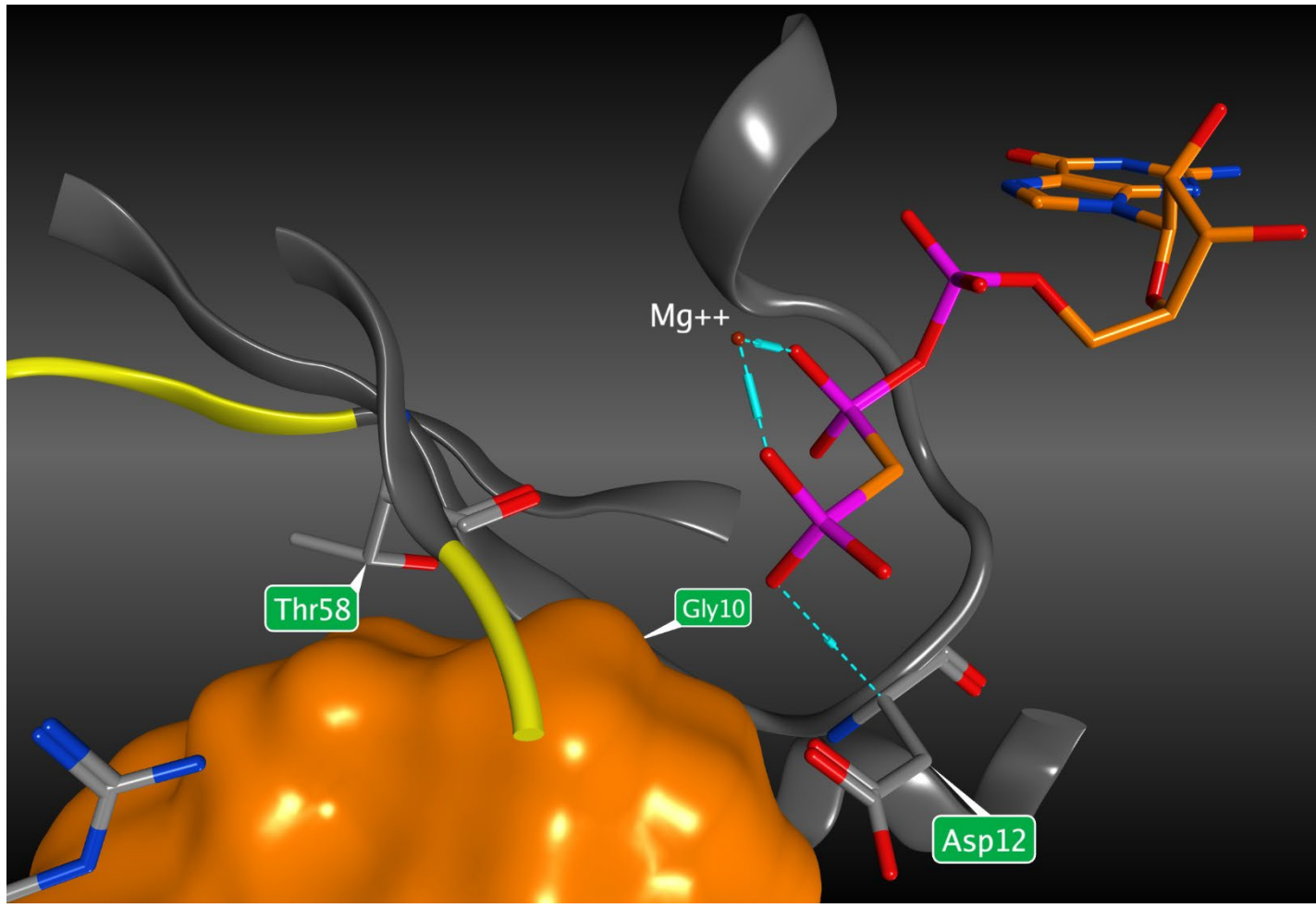
Pan-mutant KRAS inhibitor without the potential liabilities of multi-RAS inhibition

CGT6737 is a KRAS(ON) Inhibitor of Downstream Signaling



- KRAS pathway signaling occurs through the active GTP KRAS(ON) state²
- An equilibrium exists between inactive GDP KRAS and the active GTP KRAS(ON)³
- GEFs catalyze nucleotide exchange of GDP to GTP, GAPs catalyze hydrolysis of GTP to GDP
- KRAS mutations shift the equilibrium toward the active GTP KRAS(ON) state³
- CGT6737 binds and inhibits GTP KRAS(ON) blocking signaling to the PI3K, RAF, and RAL pathways**

CGT6737 Binds In the Switch II Pocket of Active KRAS(ON)



- X-Ray crystal structure of CGT6737 co-crystallized with GMPPCP loaded KRAS^{G12D} Cys-light protein
- 1.9Å resolution with well-defined density around the compound and the GTP nucleotide stable analog
- CGT6737 bound in the switch II pocket is masked with an orange surface
- GMPPCP is clearly visible in the upper right of the image with chelated Mg shown
- CGT6737 is an active GTP KRAS(ON) Switch II binder**

CGT6737 is a Mutant KRAS Inhibitor with pM Activity

Cell Line	Tumor Type	Mutation	CGT6737 Cellular IC ₅₀	RMC-6236 Cellular IC ₅₀
PC-9	Non-small cell lung cancer	EGFR Exon 19 (KRAS ^{WT})	14 nM	2.7 nM
NCI-H2009	Non-small cell lung cancer	KRAS ^{G12A}	0.12 nM	0.75 nM
NCI-H358	Non-small cell lung cancer	KRAS ^{G12C}	0.25 nM	0.27 nM
AsPC-1	Pancreatic cancer	KRAS ^{G12D}	0.64 nM	1.4 nM
A549	Non-small cell lung cancer	KRAS ^{G12S}	0.13 nM	0.59 nM
SW480	Colorectal cancer	KRAS ^{G12V}	0.57 nM	0.28 nM
HCT116	Colorectal cancer	KRAS ^{G13D}	0.66 nM	0.79 nM
NCI-H460	Non-small cell lung cancer	KRAS ^{Q61H}	0.15 nM	0.50 nM

- Mechanistic cellular IC₅₀s measuring inhibition of pERK were determined for CGT6737 and RMC-6236 in a variety of cell lines and tumor types to interrogate inhibition across a range of KRAS mutations
- Both compounds are potent inhibitors of KRAS mutations with IC₅₀s in the pM range for most mutations

CGT6737 Shows nM Binding to KRAS(ON) with Selectivity Over H/NRAS

RAS(ON) SPR Binding Assay K _D								
	KRAS						HRAS	NRAS
	WT	G12V	G12D	G12C	G13D	Q61H	WT	WT
CGT6737	1.3 nM	1.7 nM	0.6 nM	0.3 nM	1.1 nM	1.9 nM	>5000 nM	>5000nM

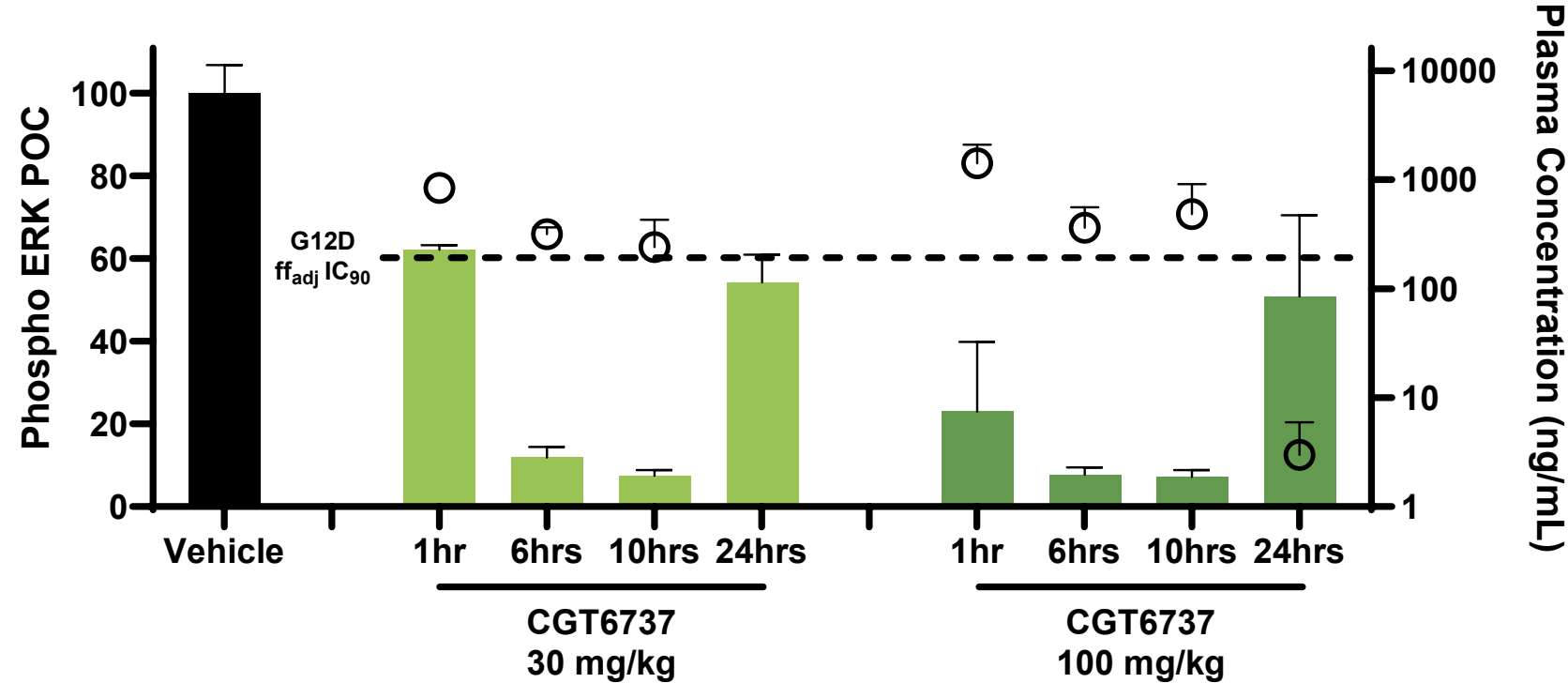
- Kinetic SPR binding assay of CGT6737 to WT and mutant KRAS(ON) loaded with the GTP stable analog GMPPCP
 - Confirms CGT6737 binds to the active form of KRAS(ON) consistent with crystallographic data
- Similar SPR experiment with HRAS and NRAS shows >1000x selectivity for KRAS(ON) over H/NRAS isoforms**
- Selectivity for KRAS over other RAS isoforms may provide tolerability advantages vs. Pan-RAS inhibition**
 - Barbicid et. al. reported that the genetic deletion of H-, N-, and K-RAS in mice stopped proliferation, and induced senescence in keratinocytes, leading to severe hypoproliferation in vivo.⁴

CGT6737 is a nM Inhibitor of Mutant KRAS Cell Lines Forced into the Active GTP KRAS(ON) State

Cellular (+/-) EGF Challenge				
Compound	Cell Line	(-) EGF IC ₅₀	(+) EGF IC ₅₀	Fold Change
CGT6737	AsPC-1 (G12D)	1.3 nM	5.2 nM	4.1X
	SW480 (G12V)	2.6 nM	7.9 nM	3.0x
RMC-6236	AsPC-1 (G12D)	1.6 nM	5.5 nM	3.5x
	SW480 (G12V)	1.0 nM	4.0 nM	4.0x

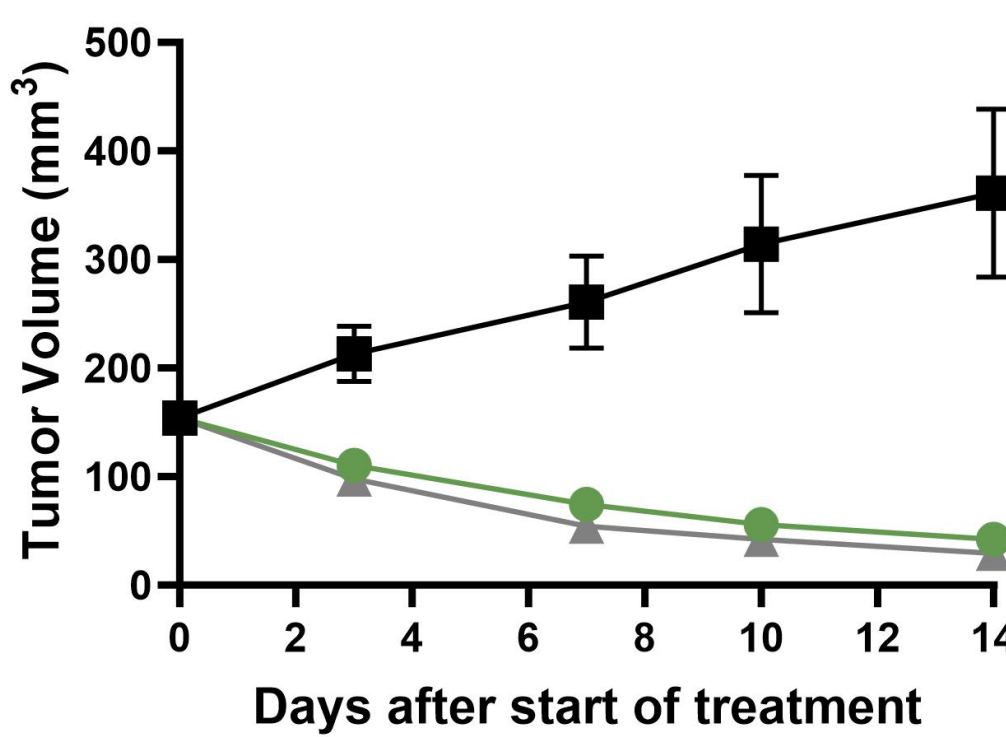
- Mechanistic cellular assay in the presence of EGF forces KRAS into the active GTP KRAS(ON) state and provides a direct measurement of GTP KRAS(ON) inhibition⁵
- Minimal shift in IC₅₀s of CGT6737 (+/-) EGF challenge for both AsPC1(G12D) and SW480(G12V) lines indicates a KRAS(ON) mechanism
- These data for CGT6737 mirror the data of the Multi RAS(ON) inhibitor RMC-6236 both in IC₅₀ potency and fold change (+/-) EGF**

In Vivo CGT6737 Shows >90% PD Effect and Efficacy in TGI Models

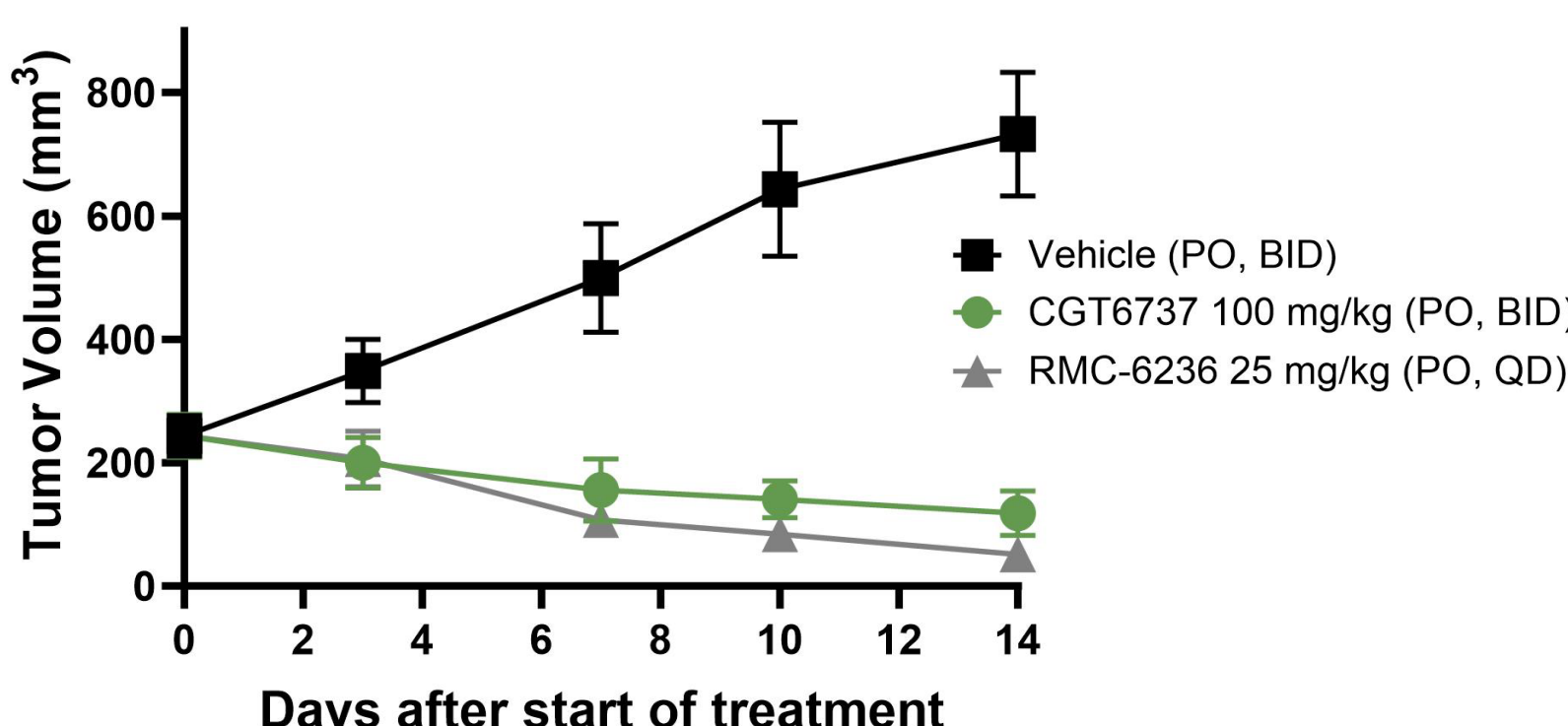


- CGT6737 was dosed PO at 30/100 mg/kg in an AsPC-1 KRAS^{G12D} tumor bearing mouse PK/PD model
- 90% Inhibition of pERK was observed at 10 hr for both the 30 and 100 mg/kg doses
- Promising PK with IC₉₀ coverage from 1-10 hours in both dose groups

MIA PaCa-2 (KRAS^{G12C})



AsPC-1 (KRAS^{G12D})



- CGT6737 was dosed PO BID at 100 mg/kg in MIA PaCa KRAS^{G12C} and AsPC-1 KRAS^{G12D} TGI models
- RMC-6236 was dosed at 25 mg/kg PO QD to match clinically relevant exposures
- Similar efficacy was observed for CGT6737 and RMC-6236 in both models

