# Cyclic disruption of the mitogen-activated protein kinase (MAPK) pathway by the Dual MEK inhibitor, IMM-6-415, enhances PD-1 and CTLA-4 checkpoint blockade in RAS mutant tumors

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## Introduction

KRAS is the most frequently altered RAS gene (~85%) and is often mutated in pancreatic ductal adenocarcinoma (PDAC; 95%), non-small cell lung cancer (NSCLC; 40%) and colorectal cancer (CRC; 45%)[1]. KRAS-G12C inhibitors (sotorasib/adagrasib) have demonstrated single-agent activity in all three tumor types. However, acquired resistance and limited biomarker positive patients (e.g., only 1-3% of PDAC and CRC) limit broader access and overall response to G12C inhibitors, prompting evaluation of combination partners including immune therapies. In contrast to G12C-mutant focused KRAS inhibitors, MEK inhibitors could broaden the potential for immune therapy in RAS-mutant tumors, but they have been largely ineffective in this setting as monotherapy. Here, we demonstrate that the short-lived Dual-MEK inhibitor, IMM-6-415, is active across multiple MAPK-driven tumor models both as a single agent and in combination with checkpoint inhibitors (CPI).

## Methods

IMM-6-415 is a novel, third-generation Dual MEK inhibitor that reduces both pMEK and pERK in RAS- and RAF-mutant tumor models at sub-100 nM potencies. IMM-6-415 was evaluated in a series of preclinical *in vitro* and *in vivo* models enriched for activation mutations that increase MAPK pathway signaling. Cell-based 2D biochemical and 3D pharmacologic assays were performed along with multiple *in vivo* studies in RAS mutant and wildtype models: (1.) Colon 26, a KRAS$^{G12D}$ CRC syngeneic model, (2.) A549, a KRAS$^{G12S}$ NSCLC xenograft model, (3.) CT-26, a KRAS$^{G12D}$ syngeneic model and (4.) MC38, a RAS wild-type syngeneic model. CT-26 (BALB/c) and MC38 (C57BL/6) *in vivo* studies evaluated single-agent IMM-6-415, PD-1 and CTLA-4 versus IMM-6-415 + CPI combinations.

## Results

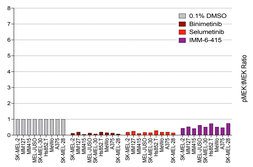
IMM-6-415 reduced pERK and pMEK across all RAS mutant models tested. Humanized 3D tumor models revealed a promising sensitivity profile for IMM-6-415 in RAF- and RAS-mutant models. The maximum tolerated dose (MTD) for BID dosing of IMM-6-415 was 175 to 180 mg/kg BID PO based on Colon 26 (96.4% TGI) and A549 (93.9% TGI) studies, yet enhanced MEKio + CPI combinations were identified at only 120 mg/kg BID PO IMM-6-415. At 28 days treatment, 33% (4/12) CT-26 mice remained on study in the (10 mg/kg BIW IP) anti-PD-1 or anti-CTLA-4 alone treated groups, whereas 58% (7/12) mice remained in the IMM-6-415 treatment arm at 120 mg/kg BID PO. However, 92% (11/12) and 83% (10/12) mice remained in the IMM-6-415 plus anti-PD-1 or anti-CTLA-4 combination at the same doses.

### Figure 1. IMM-6-415: a Dual-MEK Inhibitor in RAS and RAF Mutant Tumors

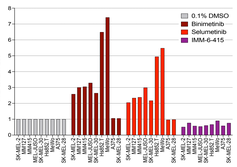
Key Mutations & 3D-TGA Response
[Melanoma Tumor Models]

| Model | HRAS | NRAS | BRAF | NF1 | 3D-TGA |
|---|---|---|---|---|---|
| SK-MEL-2 | | p.Q61R | | | |
| MM127 | | p.G13R | p.G464E | | n.t. |
| MM415 | | p.Q61L | | | n.t. |
| MEL-JUSO | p.G13D | p.Q61L | | | n.t. |
| SK-MEL-30 | | p.Q61K | | | n.t. |
| Hs852T | | p.G12V | | | n.t. |
| MeWo | | | | LoF | n.t. |
| A375 | | | p.V600E | | |
| SK-MEL-28 | | | p.V600E | | |

pERK to Total-ERK Ratio
[100nM Drug]

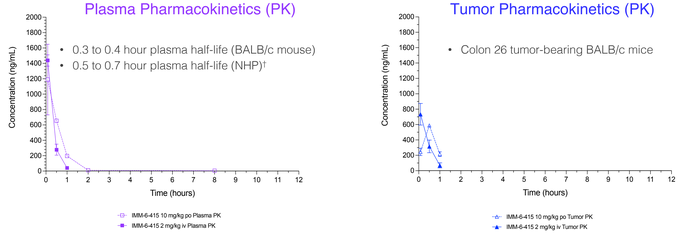



pMEK to Total-MEK Ratio
[100nM Drug]




Tumor cell lines were acquired from ATCC, ECACC and DSMZ. 3D-Tumor Growth Assay (3D-TGA) sensitivity (green) defined as $IC_{50}$ < 10uM in 72-hour ECM-based assay with %EdU readout, and $IC_{50}$ ≥10uM considered resistant. Cell-based 2D *in vitro* molecular assays were performed to assess cellular levels of phosphorylated and total ERK and MEK across 9 melanoma models (100 nM drug for 2-hours followed by quantitative Western blot analysis for pERK, total ERK, pMEK and total MEK); Binimetinib & selumetinib were commercially purchased; n.t. = not yet tested

### Figure 2. IMM-6-415 Displays a Short Plasma & Tumor PK Half Life *In Vivo*

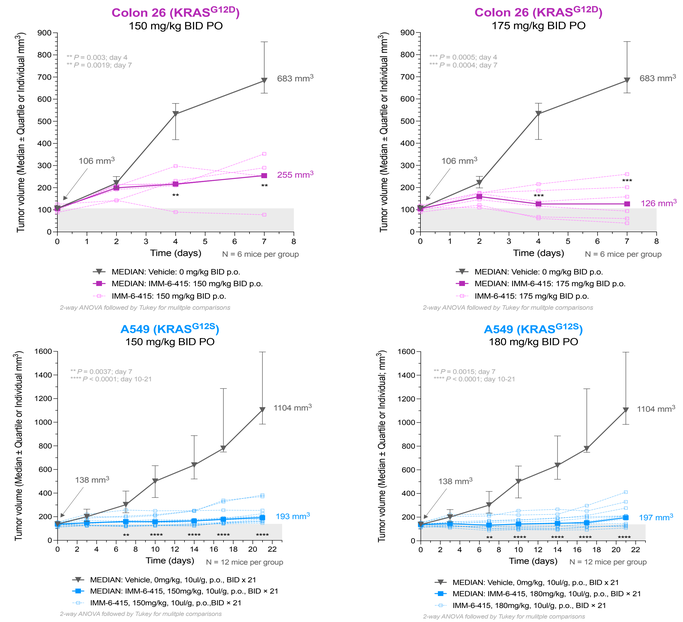



Colon 26 tumor-bearing syngeneic BALB/c mice Pharmacokinetics (PK) of IMM-6-415 in Colon 26 tumor bearing syngeneic BALB/c mice (timepoints: 0.083, 0.5, 1, 2, 8 hours after 10 mg/kg p.o. or 2 mg/kg i.v. dose); Standard Deviation (n=3 per timepoint); † non-human primate (NHP) dosed at 5 and 25 mg/kg p.o. and 1 mg/kg i.v. for PK assessment (*PK data not shown*)

### Table 1. Differentiating Characteristics of 1st, 2nd, 3rd Generation MEK Inhibitors

| MEKi Generation | pMEK Response (RAS$^{mut}$ Models) | $C_{max}$ or Drug Trough | Chronic or Cyclic | Example Drugs |
|---|---|---|---|---|
| 1st | ↑ pMEK | Drug Trough | Chronic Inhibition | binimetinib, selumetinib |
| 2nd | ↓ pMEK | Drug Trough | Chronic Inhibition | VS-6766 [2] |
| 3rd | ↓ pMEK | $C_{max}$ | Cyclic Inhibition | IMM-1-104, IMM-6-415 |

### Figure 3. Drug Pharmacology and Maximum Effective Dose (MED) in Mice




Top Row: Colon 26 (KRAS$^{G12D}$) syngeneic colorectal tumor model in immune competent BALB/c mice; Bottom Row: A549 (KRAS$^{G12S}$) human NSCLC xenograft tumor model in athymic nude BALB/c mice; Tumor Growth Inhibition (TGI) % = [1 – (Ti – T0)/(Ci – C0)]x100%; Maximum Antitumor Effective Dose Range for IMM-6-415 in mice is 150 mg/kg to 175-180 mg/kg BID p.o.

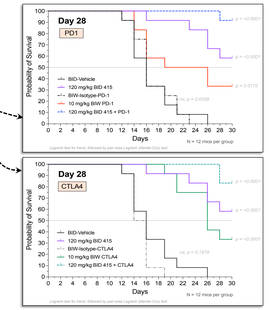
### Figure 4. MEKi, α-PD-1, α-CTLA-4 Alone and Combinations in CT-26 / MC38

IMM-6-415 +/- Checkpoint Inhibitor in **KRAS$^{G12D}$ CT-26**

| Schedule | Dose (mg/kg) | Veh | αPD-1 (BIW) | αCTLA-4 (BIW) | IMM-6-415 | IMM-6-415 + PD-1 | IMM-6-415 + CTLA-4 |
|---|---|---|---|---|---|---|---|
| BID | 120 | 0/12 | | | 7/12 | 11/12 | 10/12 |
| BID | 60 | 0/12 | | | 0/12 | 8/12 | 8/12 |
| BID | 30 | 0/12 | | | 0/12 | 5/12 | 5/12 |
| QD | 120 | 0/12 | | | 0/12 | 7/12 | 7/12 |
| QD | 60 | 0/12 | | | 1/12 | 4/12 | 8/12 |
| QD | 30 | 0/12 | | | 0/12 | 6/12 | 7/12 |
| BIW | 10 | 0/12 | 4/12 | | | | |
| BIW | 10 | 0/12 | | 4/12 | | | |

• Number of BALB/c mice (out of 12) with tumors through Day 28 with volumes lower than 2,000 mm$^3$

Monotherapy Treated Alive at Day 28
Combination ≥ 3 Advantage

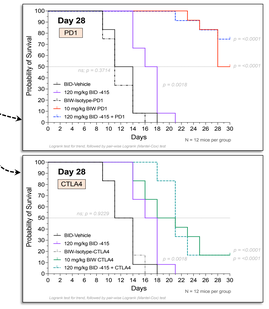



IMM-6-415 +/- Checkpoint Inhibitor in **RAS$^{wild\text{-}type}$ MC38**

| Schedule | Dose (mg/kg) | Veh | αPD-1 (BIW) | αCTLA-4 (BIW) | IMM-6-415 | IMM-6-415 + PD-1 | IMM-6-415 + CTLA-4 |
|---|---|---|---|---|---|---|---|
| BID | 120 | 0/12 | | | 0/12 | 9/12 | 2/12 |
| BID | 60 | 0/12 | | | 0/12 | 5/12 | 0/12 |
| BID | 30 | 0/12 | | | 1/12 | 6/12 | 0/12 |
| QD | 120 | 0/12 | | | 0/12 | 4/12 | 1/12 |
| QD | 60 | 0/12 | | | 0/12 | 4/12 | 2/12 |
| QD | 30 | 0/12 | | | 0/12 | 5/12 | 2/12 |
| BIW | 10 | 0/12 | 6/12 | | | | |
| BIW | 10 | 0/12 | | 2/12 | | | |

• Number of C57BL/6 mice (out of 12) with tumors through Day 28 with volumes lower than 2,000 mm$^3$

Monotherapy Treated Alive at Day 28
Combination ≥ 3 Advantage




Top Panels: CT-26 (KRAS$^{G12D}$) syngeneic colorectal tumor model in immune competent BALB/c mice (note: monotherapy and combinations were inactive in athymic nude CT-26 model – *data not shown*)
Bottom Panels: MC38 (RAS$^{wild\text{-}type}$) syngeneic colorectal tumor model in immune competent C57BL/6 mice (note: immune compromised model not evaluated)

## Conclusions

Elevated MAPK pathway signaling can promote deleterious effects on antitumor immunity, which has prompted multiple MEKi plus CPI combination trials[3]. However, MEKi class effect toxicities have limited clinical utility of MEKi combinations[4]. Instead of chronic MAPK pathway ablation, IMM-6-415 was designed to drive short bursts of $C_{max}$ driven inhibition of MEK. IMM-6-415 displayed activity across multiple RAS- and RAF-mutant tumor models, and when combined with PD-1 or CTLA-4 checkpoint inhibitors at well-tolerated, sub-MED dose levels, significant survival benefit was observed (p-values: <0.05 to <0.0001; CT-26). These data suggest that moderated, cyclic inhibition of MEK in combination with CPIs may improve survival times versus monotherapy in MAPK-activated tumors. Antitumor responses with IMM-6-415 +/- CPIs in an immune compromised CT-26 model at the same doses, combinations and schedules were not observed, suggesting that moderated, cyclic disruption of the MAPK pathway can enhance CPI-dependent adaptive antitumor immunity and improve overall MEKio combination tolerability.

## References

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Presented at SITC, November 2022 (Abstract Number 449)

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