Cyclic disruption of the mitogen-activated protein kinase (MAPK) pathway by the pulsatile MEK inhibitor, atebimetinib, enhances PD-1 checkpoint blockade in RAS mutant tumors

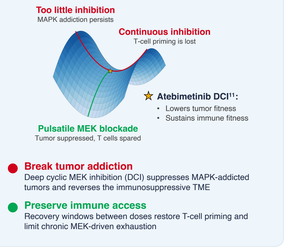
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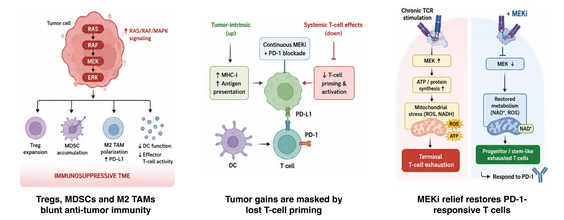
Introduction

Activating RAS/MAPK mutations are common in PDAC, NSCLC, and CRC, and preclinical work shows MEK inhibition (MEKi) can raise tumor immunogenicity by upregulating MHC class I and antigen presentation via JAK/STAT signaling and increasing CD8+ T-cell infiltration. This provides a biologic rationale for combining MAPK pathway inhibitors (MEK or pan-RAS) with immune checkpoint inhibitors (ICIs). Yet these combinations have largely underperformed clinically. A central liability is that continuous MAPK inhibition also blocks the signaling T cells need to activate, directly impairing effector T-cell function and masking the favorable tumor-intrinsic effects. Alternatively, pulsatile MAPK inhibition can instead suppress tumor signaling while allowing T cell recovery, preserving their activation and extending survival with checkpoint blockade. Atebimetinib is a third-generation dual MEK inhibitor engineered for deep cyclic inhibition (DCI), a pulsatile profile suppressing pMEK and pERK at sub-100 nM potency, with tumor growth inhibition across multiple RAS-mutant models and is currently in a Phase 3 PDAC trial in combination with mGnP (MAPKeeper 301; NCT07562152).

How DCI Balances Tumor and Immune Fitness[2,3]



Rationale for DCI + PD-1 Blockade



Methods

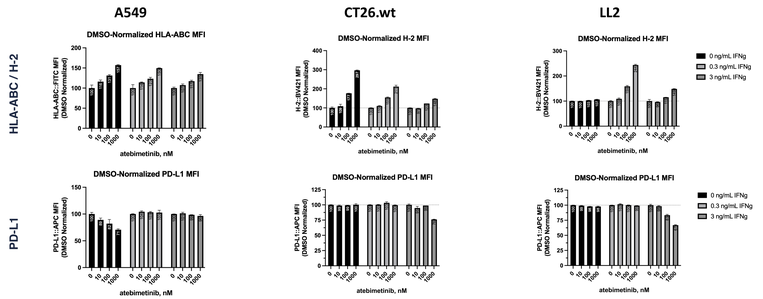
The immunomodulatory effects of atebimetinib were assessed in vitro by measuring surface HLA-ABC and PD-L1 on cell lines by flow cytometry, with and without IFNγ. *In vivo* activity was evaluated in two syngeneic tumor models in immune competent mice. In the CT-26 ($KRAS^{G12D}$) syngeneic colorectal mouse model, atebimetinib was given orally at 75 and 125 mg/kg BID as a single agent and in combination with an anti-PD-1 checkpoint inhibitor. Concurrent dosing of both agents was compared with a one-week PBS lead-in, and survival was followed with treatment stopped at day 90. In the LL2 ($KRAS^{G12C}/NRAS^{Q61H}$) syngeneic lung mouse model, atebimetinib was given orally at 75 and 125 mg/kg BID as a single agent and in combination with an anti-PD-1 checkpoint inhibitor and/or the chemo-doublet (carboplatin and pemetrexed). Blood, spleen and tumor were profiled by flow cytometry on day 12.

Syngeneic Mouse Model Comparisons

	CT26.wt	LL2
Tumor Indication	Colorectal ($KRAS^{G12D}$)	Lung ($KRAS^{G12C}/NRAS^{Q61H}$)
Mouse Strain	BALB/c	C57BL/6
Model Type	Warm	Cold
Immune Phenotype	T-cell exhaustion	Myeloid-suppressive
Baseline Immune Infiltration	High CD8+ CTL	Low TIL High MDSC/TAM
ICI Sensitivity	Partial response to anti-PD-1	No response to anti-PD-1

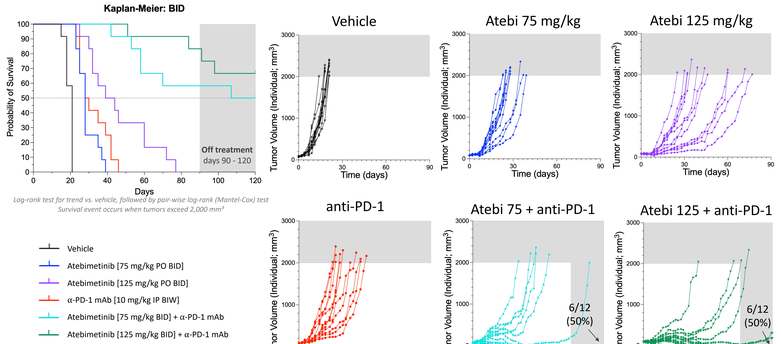
Results: A549, CT26.wt and LL2 *in vitro*

In vitro, IFNγ treatment of A549 and other cell lines for 24 hours increased surface HLA-ABC/H-2 and PD-L1. Atebimetinib produced dose-dependent increases in HLA-ABC with IFNγ and dose-dependent decreases in PD-L1, indicating a favorable immunomodulatory profile.



Cells were treated for 24 h with DMSO or atebimetinib (10 nM, 100 nM, 1 μM) ± IFNγ (0, 10, 20 IU/mL for A549; 0, 0.3, 3.0 ng/mL for CT26.wt and LL2). Surface HLA-ABC/H-2 and PD-L1 were measured by flow cytometry on live single cells (FMO-gated; NovoCyte 3000) and shown as MFI normalized to DMSO at each IFNγ dose.

Warm Tumor: CT-26 colorectal ($KRAS^{G12D}$) *in vivo*



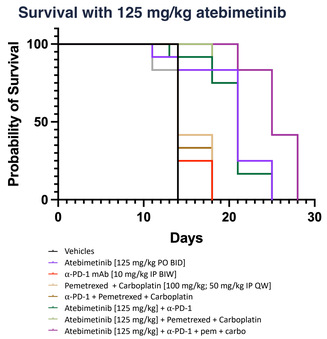
Log-rank test for trend vs. vehicle, followed by pair-wise log-rank (Mantel-Cox) test
Survival event occurs when tumors exceed 2,000 mm³

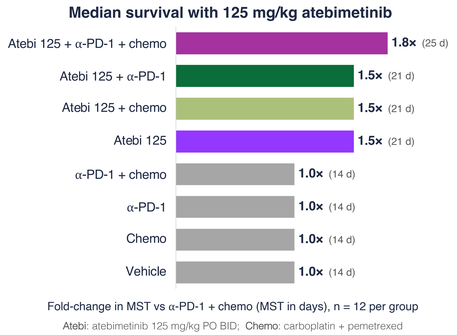
Log-rank test for trend vs. vehicle, followed by pair-wise log-rank (Mantel-Cox) test. Survival event occurs when tumors exceed 2,000 mm³

In the CT-26 ($KRAS^{G12D}$) syngeneic colorectal tumor model (PD-1-warm) in immune competent BALB/c mice, atebimetinib was dosed at 75 and 125 mg/kg BID, alone or with anti-PD-1 mAb. Vehicle controls had a median survival time (MST) of 21 days with no survivors at day 28. Single-agent atebimetinib extended MST dose-dependently (28 to 41.5 days); anti-PD-1 alone gave a modest benefit (MST 29 days). Combination groups did not reach median survival and significantly outperformed both single agents ($p<0.0001$ vs vehicle for nearly all combinations). **Atebimetinib + anti-PD-1 cured 6/12 mice (50%) at both 75 and 125 mg/kg BID (complete regression, no regrowth off-treatment)**. At 125 mg/kg, 8/12 mice were alive at day 120 versus 6/12 at 75 mg/kg (cured mice only). Concurrent dosing outperformed a one-week PBS lead-in (75 mg/kg BID: MST 113.5 vs 44 days; not shown). By comparison, continuous MEKi (trametinib) + anti-PD-1 left 2/10 mice alive at day 68.[1]

50% **of CT-26 mice cured with atebimetinib (75 or 125 mg/kg BID) + anti-PD-1**
6/12 per arm with complete regression and no regrowth after dosing stopped (days 90–120)

Cold Tumor: LL2 lung ($KRAS^{G12C}/NRAS^{Q61H}$) *in vivo*





Atebi: atebimetinib 125 mg/kg PO BID; Chemo: carboplatin + pemetrexed

In the LL2 ($KRAS^{G12C}/NRAS^{Q61H}$) syngeneic lung tumor model, a PD-1-cold model unresponsive to anti-PD-1, in immune competent C57BL/6 mice, atebimetinib was dosed at 75 and 125 mg/kg BID, alone or with anti-PD-1 mAb (10 mg/kg), with or without the chemo doublet (carboplatin, pemetrexed). Vehicle controls had an MST of 14 days. Neither anti-PD-1 nor chemotherapy, alone or combined, extended MST (1.0×), whereas every 125 mg/kg atebimetinib arm extended MST ≥1.5×; 75 mg/kg arms were intermediate (MST 18–21 days, 1.3–1.5×). **Adding atebimetinib 125 mg/kg to anti-PD-1 + chemo extended MST 1.8× (25 vs 14 days) and kept 12/12 mice alive at day 18**, versus 0/12 with anti-PD-1 + chemo alone.

Mechanism: atebimetinib combination reshapes LL2 immunity

LL2 tumor-bearing mice, day 12 of dosing: blood, spleen and tumor sampled **2h (peak)** and **12h (trough)** after atebimetinib. Charts show arm means, % of parent gate. Blood/spleen: atebimetinib 125 mg/kg, α-PD-1 mAb. Tumor: n=4–6/arm (Tregs); n=16–20 (PD-L1).

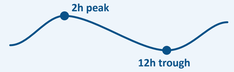
Untreated LL2: myeloid expansion and CD8 loss by day 12

23% → 59% CD11b+ myeloid, % of CD45

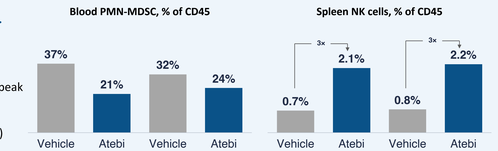
3× blood PMN-MDSC, 12% → 37%

11% → 7% tumor CD8 T cells, % of CD45 (day 0 → day 12 vehicle)

Atebimetinib: pulsatile MEK inhibition lowers PMN-MDSCs and expands NK cells



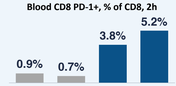
- **Spleen CD8 CD69+** 26% below vehicle at peak
- **Spleen CD8:Treg ratio** up 7–17% (ns)
- **Spleen CD8 central memory** up 5–8% (ns)



Anti-PD-1: engages PD-1+ CD8 T cells

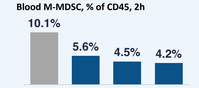
4–7× blood CD8 PD-1+ with α-PD-1 alone vs vehicle; no change with atebi alone

In tumor, α-PD-1 raises CD8 infiltration (7% → 12% of CD45, 2h), mostly $PD\text{-}1^{-}$ cells.



Carbo + pemetrexed: removes M-MDSCs

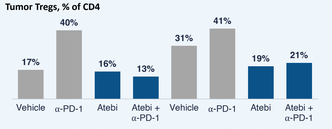
– 45% blood M-MDSC with chemo alone vs vehicle (–42% at trough)



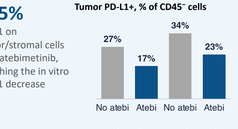
Tumor: atebimetinib lowers Tregs and PD-L1, and blocks anti-PD-1–driven Treg expansion

40% → 13% tumor Tregs, α-PD-1 alone vs atebi + α-PD-1 (2h)

CD8:Treg ratio 7.4 → 10.9 (α-PD-1 alone vs atebi + α-PD-1, 2h)



–35% PD-L1 on tumor/stromal cells with atebimetinib, matching the in vitro PD-L1 decrease



Tregs gated as CD4+FoxP3+CD25+. Treg bars: atebi pools 75 and 125 mg/kg BID, n=4–6 mice per bar. PD-L1 bars pool all arms ± atebimetinib, n=16–20 per bar.

Impact: atebimetinib lowers PMN-MDSCs and expands NK cells systemically; in tumor it lowers Tregs and PD-L1 and prevents anti-PD-1-induced Treg expansion. Chemo removes M-MDSCs; anti-PD-1 engages PD-1+ CD8 T cells.

Result: Atebimetinib + anti-PD-1 + chemo = MST 25 vs 14 days (anti-PD-1 + chemo) and 12/12 alive at day 18 vs 0/12.

Conclusions

Atebimetinib's deep cyclic inhibition (DCI) suppresses tumor pMEK/pERK while sparing T-cell signaling, avoiding the sustained suppression that limited continuous MEKi/ICI combinations. In vitro, it raised MHC-I and lowered PD-L1. In warm CT-26, atebimetinib + anti-PD-1 improved survival ($p<0.0001$) and cured 50% of mice; concurrent dosing beat a one-week lead-in, so the cyclic PK profile itself provides a window for T-cell function. In cold LL2, adding atebimetinib to anti-PD-1 + carboplatin/pemetrexed extended median survival to 25 vs 14 days: atebimetinib lowers PMN-MDSCs, tumor Tregs and PD-L1; chemotherapy removes M-MDSCs; and anti-PD-1 engages PD-1+ CD8 T cells.

These data support evaluation of atebimetinib + anti-PD-1 + platinum doublet in a planned Phase 2 study in front-line (1L) RAS-mutant NSCLC.

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