Potential anti-cachexia properties of novel dual-MEK inhibitor IMM-1-104

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Background

Cancer cachexia is responsible for a significant proportion of cancer morbidity and mortality, affecting a majority of advanced cancer patients[1], yet lacks approved therapies. We previously demonstrated potential anti-cachexia effects of novel dual-MEK inhibitor (MEKi) IMM-1-104 in a cancer cachexia mouse model at a dose (100 mpk) producing significant tumor growth inhibition.[2]

Here, we evaluate a dosage (25 mpk) that does not inhibit tumor growth, allowing assessment of effects of IMM-1-104 relevant for cachexia separately from its anti-tumor properties.

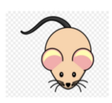
Methods

IMM-1-104 and selumetinib, a first-generation MEKi with reported clinical improvements in lean muscle mass[3], were dosed BID for 18 days in the Colon-26 (C26) mouse model. Tumor growth inhibition (TGI) and body weight were assessed, and transcriptomic effects in tumor and muscle were measured by RNA sequencing as described.

Study Design

BALB/c mouse

C26 Tumor, Muscle (left leg)



BALB/c nude mouse

C26 Tumor, Muscle (left leg)

Day 1 (initial)	Day 18 (chronic)	Day 10 (chronic)
25 mg/kg	25 mg/kg	25 mg/kg
100 mg/kg	100 mg/kg	100 mg/kg
@2h, 12h: IMM-1-104 vs. vehicle	@2h, 12h: IMM-1-104 vs. vehicle selumetinib vs. veh.	@2h, 12h: IMM-1-104 vs. vehicle selumetinib vs. veh.

Mouse Study Methods

Colon-26 (KRAS-G12D) Balb/c syngeneic colorectal tumor model. For BWL data, n=8 mice per group. For transcriptomic data, n=3-4 mice per group. All compounds dosed BID p.o.

RNA Sequencing

RNA was isolated from tumor or muscle samples. Library construction was performed using the Illumina TruSeq Library Preparation Kit and paired-end 150 base pair sequencing was carried out on the NovaSeq 6000 platform.

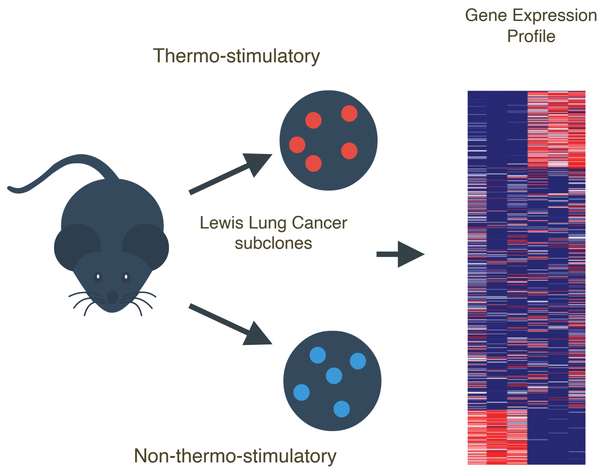
RNAseq Data Analysis

Raw RNA sequencing reads were assessed for quality using fastqc, and adapter trimming performed using bbduk to remove Illumina universal adapters. Reads were quantified using salmon. Differential expression analysis was performed using limma-voom. Multiple hypothesis correction was performed using Benjamini-Hochberg to account for genome-wide assessment. Gene Set Enrichment Analysis (GSEA) was used to assess pathway-level enrichment.

PK/PD Methods

To investigate in vivo modulation of pERK as a pharmacodynamic biomarker, Colon-26 tumor bearing mice were treated with a single oral administration of IMM-1-104, with plasma and tumor harvested at various time points post dose. Samples were extracted and analyzed for IMM-1-104 by LC-MS/MS, and noncompartmental (NCA) pharmacokinetic (PK) parameter estimates of IMM-1-104 were calculated. Pharmacodynamic (PD) readouts of pERK in both isolated PBMC CD3+ cells (FACS analysis) and tumor sections (IHC analysis) were performed.

Gene Expression Signature From Thermo-stimulatory LLC Subclones



As described in [4], a signature of thermo-stimulatory behavior of LLC subclones was derived from dataset GSE57797 and shown to predict prognosis in independent cohorts of cancer patients.

Results

Fig. 1a. PK/PD profile at 25 mpk consistent with DCI

In contrast with traditional MEKi, IMM-1-104 is designed to produce deep cyclic pathway inhibition (DCI) rather than chronic blockade.[5] This dose is lower than the therapeutic dose in mice of 100-150mpk.

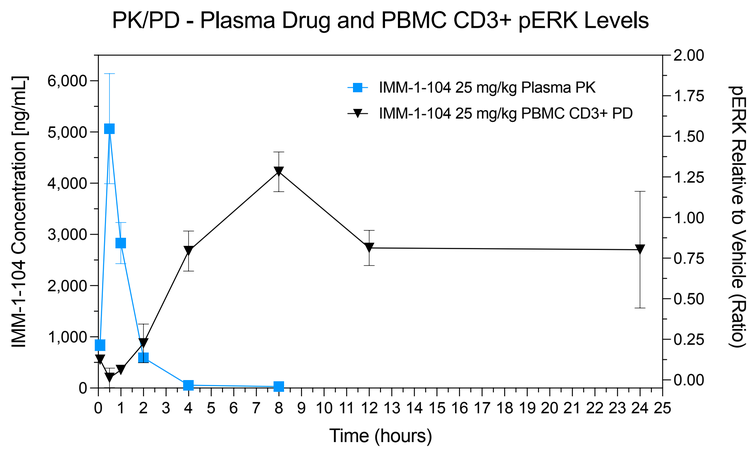
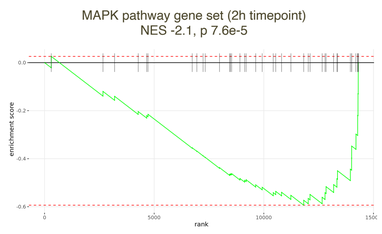


Fig. 1b. Cyclic effect on MAPK pathway observed in muscle: transcriptional output of pathway attenuated by 25 mpk IMM-1-104 at 2h and released at 12h

Enrichment of MAPK pathway transcriptional readout gene signature[6] was observed by GSEA at 2h, but not 12h, in muscle following initial dose of 25 mpk IMM-1-104.

Enrichment of MAPK pathway gene signature (Pratilas et al, PNAS 2009) by GSEA

timepoint	*pval*	*NES*	*size*
2h	7.6E-05	-2.1	39
12h	0.357	1.05	39



The ability to block and release this key pathway in muscle is consistent with the ability to allow resetting of the ERK switch needed to maintain balance for muscle satellite stem cells.[7] The novel mechanism of IMM-1-104 may offer an advantage in addressing cachexia over traditional MEKi that chronically ablate MAPK signaling.

Fig. 2. Molecular effects of 25mpk dose of IMM-1-104 occur in absence of inhibition of tumor growth

In contrast to 100 mpk dose, 25 mpk dose of IMM-1-104 did not significantly inhibit tumor growth (p=0.9998).

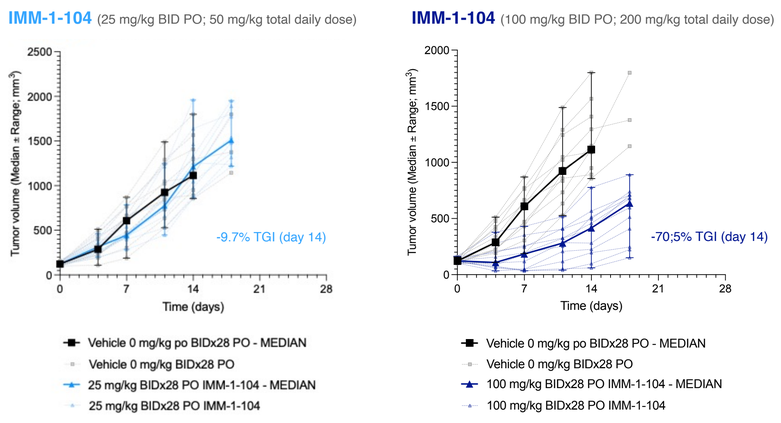


Fig. 3. 25 mpk IMM-1-104 counteracts tumor induction of proteasomal, NFκB pathways in muscle

We previously reported pathways enriched in muscle of tumor-bearing mice versus naïve mice in this system, demonstrating an enrichment in proteolytic and inflammatory pathways including NFκB.[2] Treatment with 25 mpk IMM-1-104 counteracts this effect, showing negative enrichment of these pathways.

Top pathways down in treated muscle (25mpk):

pathway	pval	padj	NES
GO_PROTEASOME_COMPLEX	0.0000198	0.000428	-3.34
GO_ANAPHASE_PROMOTING_COMPLEX_DEPENDENT_CATABOLIC_PROCESS	0.0000198	0.000428	-3.2
GO_ANTIGEN_PROCESSING_AND_PRESENTATION_OF_EXOGENOUS_PEPTIDE_ANTIGEN_VIA_MHC_CLASS_I	0.0000199	0.000428	-3.12
GO_RIBONUCLEOPROTEIN_COMPLEX_BIOGENESIS	0.0000188	0.000428	-3.12
GO_POSITIVE_REGULATION_OF_LIGASE_ACTIVITY	0.0000197	0.000428	-3.09
GO_RIBOSOME_BIOGENESIS	0.000019	0.000428	-3.06
GO_NIK_NF_KAPPAB_SIGNALING	0.0000198	0.000428	-3.06
GO_REGULATION_OF_CELLULAR_AMINO_ACID_METABOLIC_PROCESS	0.0000199	0.000428	-3.05
GO_REGULATION_OF_LIGASE_ACTIVITY	0.0000196	0.000428	-2.98
GO_REGULATION_OF_PROTEIN_UBIQUITINATION_INVOLVED_IN_UBIQUITIN_DEPENDENT_PROTEIN_CATABOLIC_PROCESS	0.0000197	0.000428	-2.95

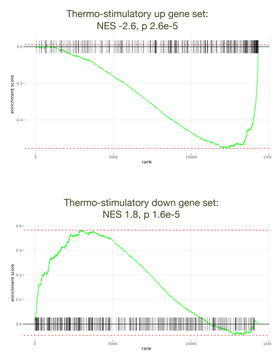
BALB/c chronic, 2h

Pathway enrichment by GSEA using MSigDB v6.1 gene sets[8]

Fig. 4. IMM-1-104 treatment at 25 mpk reversed thermo-stimulatory signature in tumor

Effects of 25 mpk IMM-1-104 on tumor included reversal of signature of thermo-stimulatory behavior, by Gene Set Enrichment Analysis.

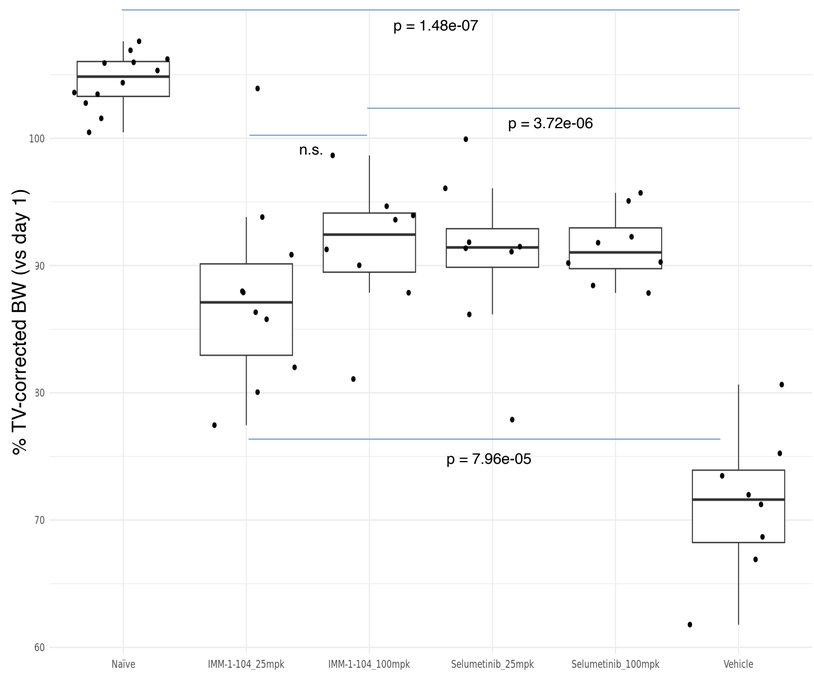
pathway	*pval*	*NES*	*size*
thermo-stimulatory-up	2.6E-05	-2.6	285
thermo-stimulatory-down	1.6E-05	1.8	370



Results (cont'd)

Fig. 5. IMM-1-104 at 25 mpk reduces TV-corrected body weight loss in tumor-bearing animals

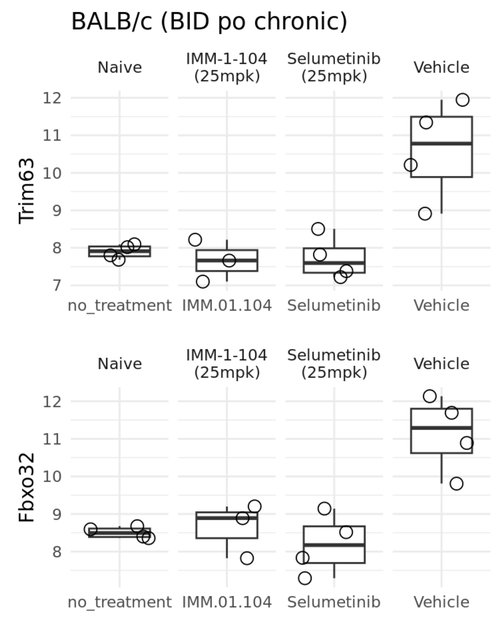
IMM-1-104 preserved body weight similarly to selumetinib. No significant difference was seen between 25 mpk and 100mpk doses.



Data for vehicle and 100 mpk dose from this study as previously reported in [2].

Fig. 6. 25 mpk IMM-1-104 counteracts tumor induction of cachexia-associated E3 ligase transcripts in muscle

25 mpk IMM-1-104 maintains transcripts for MuRF-1 and Atrogin-1 in muscle of tumor-bearing animals at same level as in non-tumor bearing animals. Effect similar to that of selumetinib, a MEKi with reported clinical effects on lean muscle mass.[3]



Trim63: *transcript for MuRF-1*

Fbxo32: *transcript for Atrogin-1*

Naïve: *non-tumor bearing*

Conclusions

In the C26 model, IMM-1-104 dosed at 25 mpk BID mitigated tumor-associated body weight loss, restored normal expression of E3 ubiquitin ligase transcripts MuRF-1 and Atrogin-1 and suppressed pathological activation of inflammatory and proteasomal pathways in muscle, and reversed thermo-stimulatory behavior signatures[4] associated with the tumor. Dosing at 25 mpk did not produce significant reduction in tumor growth.

We previously reported similar body weight and transcriptomic effects for 100 mpk IMM-1-104, a dose that strongly reduced tumor growth. The data reported here, by contrast, allow separating the tumor-reducing effect of IMM-1-104 from its potential anti-cachexia properties. We observed similar cachexia-relevant transcriptomic effects, along with reduction in tumor-associated body weight loss, with 25 mpk as with 100 mpk IMM-1-104. These effects were comparable with those of selumetinib, a compound reported to have clinical anti-cachexia activity.

Clinical evaluation of IMM-1-104 as a tumor-reducing agent in RAS-driven solid tumors is underway in a Phase 1/2a trial [NCT05585320]. The effects reported here observed preclinically at a subtherapeutic dose are consistent with clinical observation of the two patients at subtherapeutic doses of 40 or 80 mg in this trial, one of whom showed reduction in reported Fatigue Score (from 6 to 2) and the other of whom (Fatigue Score 0) had increased stamina to the point of being able to return to the gym for workouts. The preclinical findings reported here support further evaluation of IMM-1-104 for effects against cancer cachexia.

References

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