Humanized 3D tumor models that are mutationally aligned with AACR GENIE patients predict IMM-1-104 activity in RAS-addicted tumors

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Introduction

IMM-1-104, with Universal-RAS activity through Deep Cyclic Inhibition (DCI) of MEK, was evaluated in humanized 3D preclinical tumor models displaying diverse MAPK pathway activation events. Based on drug sensitivity and resistance profiles, a biomarker signature for IMM-1-104 was developed in order to project potential therapeutic response of cancer patients found in the AACR Project GENIE database. Modeling assumptions are supported by initial pharmacokinetics (PK) and pharmacodynamics (PD) data from an ongoing phase 1/2a trial (NCT05585320).

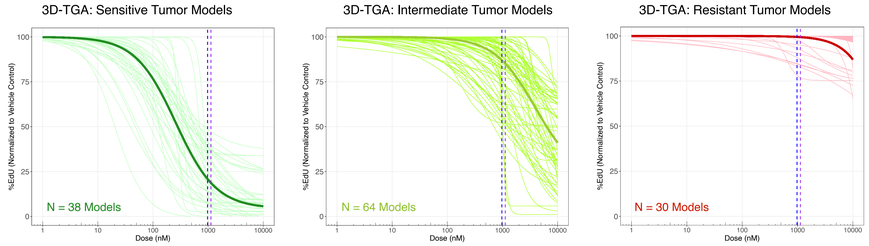
Experimental Procedures

Humanized 3D preclinical models better predict *in vivo* tumor responses versus 2D culture and more accurately replicate biology of human tumors[1,2]. Therefore, the antitumor activity of IMM-1-104 was evaluated in over 130 tumor models spanning 12 distinct tumor types in the humanized 3D tumor growth assay (3D-TGA) (**Fig. 1**). Cell-based whole exome sequencing (WES) readouts were combined with 3D-TGA results to build a pharmacogenomic response algorithm. When applied to the GENIE patient database[3], resultant tumor-specific response landscapes helped to inform an early Universal-RAS clinical trial design for IMM-1-104 (**Fig. 2**). Initial findings from an ongoing phase 1/2a trial show favorable top line safety, PK and PD readouts (**Table 1 & Fig. 3**).

Benchmarking 3D-TGA Response to IMM-1-104

A machine learning model was developed to predict IMM-1-104 sensitivity using response-associated genes and signaling networks that were identified using 3D-TGA pharmacogenomics data. This model was used to estimate GENIE patient IMM-1-104 response profiles across key solid tumor indications. In addition, mutation constellations from GENIE were compared with those observed in cell lines to identify preclinical models that best resemble real-world patients. This effort was designed to further enrich the translational fidelity of specific tumor models with the goal of translationally identifying patient populations most likely to benefit from IMM-1-104 treatment[4].

Figure 1: 3D-TGA IMM-1-104 Dose Responses (N = 132 Models; 12 Tumor Indications)

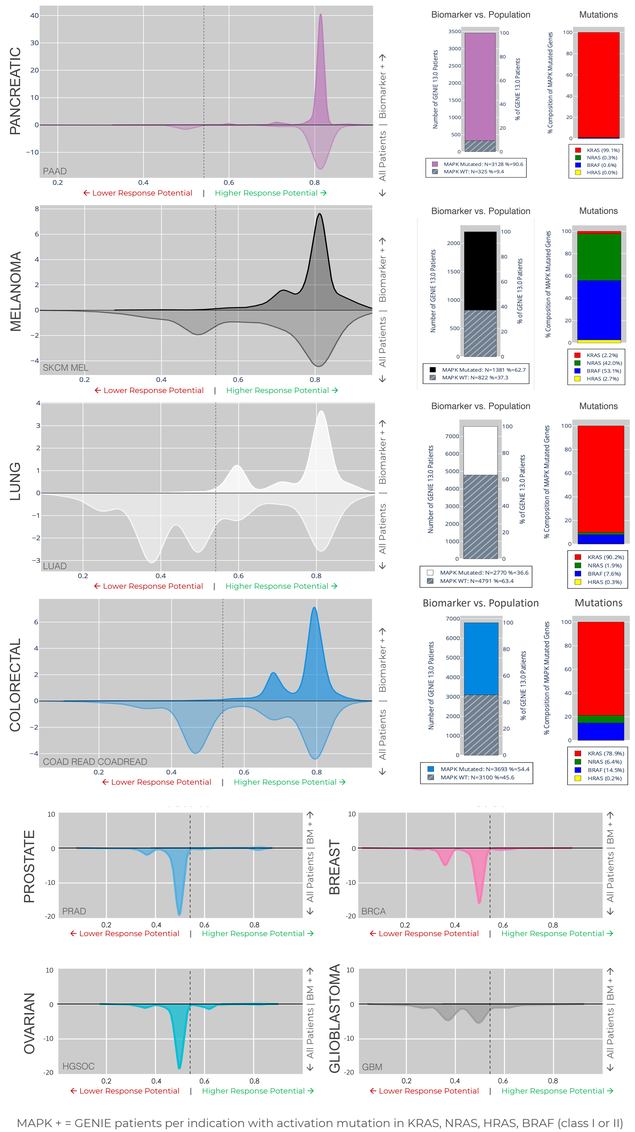


Cell lines tested in 3D-TGA (N=132) were assigned response of sensitive (IC50 < 1uM), intermediate (IC50 ≥ 1 and >25% reduction at 10uM), and resistant otherwise
The dark line on each plot represents the median of the individual curves; Dotted vertical lines match Cmax IMM-1-104 drug free-fractions attained in patients 3 and 4 at 160 mg QD p.o.

Projecting Biomarker Sensitive Populations: 3D-TGA Models → GENIE Patients

For 122 of the 3D-TGA cell lines where in-house WES data were also available, the upper plateau-to-1 uM difference was used to delineate response from non-response, and thus train a logistic regression model with 10-fold cross validation. There were 28 features used, one comprised of a consolidated MAPK mutations status (mutations in K/H/NRAS, GNAQ/11, and BRAF class I/II), and 27 other genes representing key pathways. With an AUC of 0.84 on the training cohort and 0.72 on test cohort, the model was then applied to GENIE cohorts (**Fig. 2**) where all features were included on the testing panel. For each indication of interest, a kernel density plot of the probability of predicted response for the entire cohort (projected onto the negative y-axis), and the MAPK(+) subset (positive y-axis) shows enrichment for higher probability of response with restriction to MAPK alteration status.

Figure 2. Mapping Biomarker Sensitive Patient Populations (GENIE®)



MAPK + = GENIE patients per indication with activation mutation in KRAS, NRAS, HRAS, BRAF (class I or II)

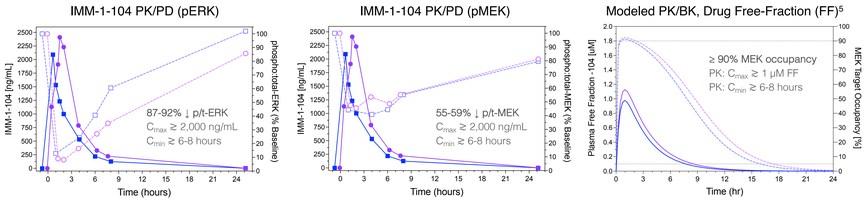
Early Phase 1/2a Dose Escalation IMM-1-104 Clinical Trial Summary

Table 1. Patient Status Summary for IMM-1-104 Phase 1 Dose Escalation

#	Patient	RAS Mutation	DL	Dose	C1D1 ($t_{1/2}$)	C1D15 ($t_{1/2}$)	Mean ($t_{1/2}$)	IMM-1-104 Status
1.	PANCREATIC	KRAS-G12D	I	40 mg QD p.o.	1.82 hours	2.10 hours	**1.96 hours**	Off Treatment
2.	COLORECTAL	KRAS-G12V	II	80 mg QD p.o.	1.41 hours	1.43 hours	**1.42 hours**	Off Treatment
3.	COLORECTAL	NRAS-Q61L	III	160 mg QD p.o.	2.04 hours	1.83 hours	**1.94 hours**	Off Treatment
4.	COLORECTAL	NRAS-Q61K	III	160 mg QD p.o.	1.91 hours	1.97 hours	**1.94 hours**	On Treatment
5.	PANCREATIC	KRAS-G12D	IV	320 mg QD p.o.	t.b.d.	t.b.d.	t.b.d.	On Treatment

- Clinical data timeline reported through April 10th, 2023 (i.e., 20 weeks since first patient dosed)
- Actively enrolling patients at 320 mg QD p.o. with 2 additional patients already consented (KRAS-G12V Pancreatic and KRAS-G12S Colorectal)
- No dose limiting toxicities (DLTs) or serious adverse events (SAEs); early PK data are approximately dose linear with no drug accumulation
- No drug-related adverse events beyond grade 1 have been reported in dose levels III or IV

Figure 3. Pharmacokinetics (PK), Pharmacodynamics (PD), and Binding Kinetics (BK)



- Dose Level III: Cycle 1 Day 1 (C1D1) PK (solid), BK (dotted), and PD (dotted) for patient 3 (purple) and 4 (blue), both at 160 mg QD p.o.
- PK plasma: pre-dose (0), 0.5, 1, 1.5, 2*, 4, 6, 8, 24 hours; PD plasma: PK-matched without 0.5 or 1.5 hours (*poor sample quality for Pt.4 at 2 hour)
- PD method: A549 ($KRAS^{G12S}$) cells were exposed to patient plasma for 2-hours before quantifying phosphorylated (p) and total (t) ERK and MEK

Conclusions

The depth of response to IMM-1-104 was evaluated across a panel of diverse 3D-TGA tumor models and led to identification of a biomarker signature for therapeutically addressable MAPK pathway addiction. To translate these findings into a relevant clinical application, a response algorithm was developed and applied to the GENIE database, which has cataloged the molecular profiles of over 100,000 cancer patients. Mutational landscapes of patients within GENIE helped identify preclinical models that better represent patient profiles likely to be encountered in the clinic. This approach could, as a general principle, be applied as a tool for improving biomarker discovery and clinical translation of oncology drugs. Immuneering has pioneered the concept of targeting MAPK pathway addiction through Deep Cyclic Inhibition (DCI). Initial phase 1/2a clinical data (NCT05585320) demonstrate favorable initial safety along with PK and PD profiles that are consistent with preclinical modeling of DCI, including C_{max} levels over 2,000 ng/mL (~1 uM drug FF), a median plasma $t_{1/2}$ of 1.94 hours, and ~90% pharmacodynamic inhibition of pERK.

References

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