Pan-RAS IMM-1-104 Activity in Humanized 3D Tumor Models is Independent of Specific Amino Acid Substitution

Sarah Kolitz, Praveen Nair, Mai Johnson, Jason Funt, Peter J King, Kevin D Fowler, Anna Travesa, Frank Wang, John Brothers II, Amy Axel, Scott Barrett, Benjamin J Zeskind, Brett Hall

Immuneering Corporation, Cambridge, MA, San Diego, CA, and New York, NY USA

Introduction

IMM-1-104, a novel dual-MEK inhibitor, is under clinical investigation for use in patients with advanced, RAS mutated solid tumors. Approved KRAS G12C inhibitors are available but cover a limited subset of high unmet need patients. For example, the KRAS G12C substitution occurs in only ~ 1% of pancreatic cancers where ~ 90% of patients are KRAS mutant. We evaluated IMM-1-104 responses across a large number of RAS mutant preclinical tumor models to examine IMM-1-104 responsive mutation profiles.

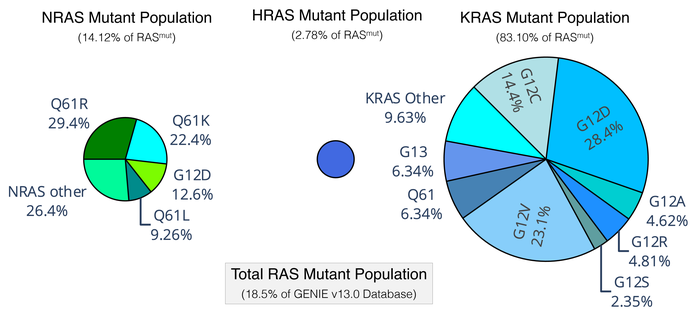
Experimental Procedures

Response to IMM-1-104 was measured in the humanized 3D tumor growth assay (3D-TGA) across 132 tumor models[1,2]. Seventy-five (57%) of these models have previously reported a RAS mutation, and all models are being verified by whole exome sequencing, with the majority (~ 85%) completed to date. The pan-RAS-mutant tumor panel spans 12 tissue types and includes a subset of 30 confirmed KRAS G12 mutated tumor cell lines drawn from three major tumor types: 12 pancreatic, 11 lung, and 7 colorectal cancer models. Based on the 3D-TGA assay, cell lines were classified into responsive (i.e., sensitive or intermediate) or non-responsive (i.e., resistant) to IMM-1-104. The distribution of responses was then assessed across RAS paralogs, mutation position and specific amino acid substitutions.

Results

Across all RAS-mutant models, at least one model displayed response to IMM-1-104 for each observed mutation in K/N/HRAS. That is, no particular mutation position or amino acid substitution was exclusively found to confer resistance to drug exposure.

Figure 1. Distribution of RAS^{mut} Across Patients in the GENIE (v13.0) Database[3]



Association of IMM-1-104 response with amino acid identity was further evaluated in a subset of models, based on that status of KRAS, G12. A distribution of responses was observed for each amino acid substitution, and there were at least four matched substitutions in each G12 subgroup tested: G12C (8 lines), D (5 lines), R (4 lines), and V (11 lines). Across cell lines for each of these substitutions, multiple response categories were observed. In each case, half or more lines fell into the intermediate response category with the rest falling into sensitive or resistance response categories. For example, out of the 8 KRAS G12C lines, 6 showed intermediate response, 1 showed resistance, and 1 showed sensitivity. Examining these distributions together, no significant statistical relationship was seen between the amino acid substitution and response categories by Fisher's exact test (p-value = 0.434).

Figure 2. Mutation Profiles Across Indications in GENIE (v13.0) Patient Database[3]

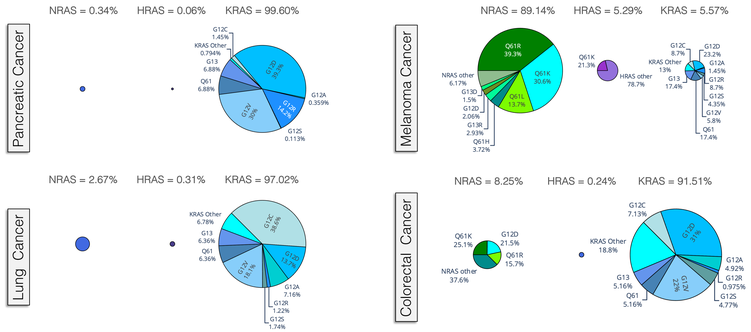
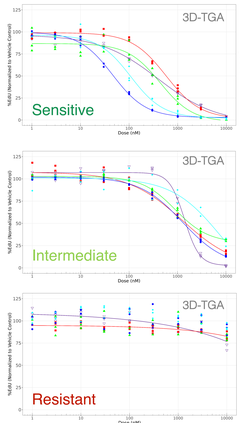


Figure 3: Representative Subset of IMM-1-104 Dose Responses (132 3D-TGAs)



	Tissue	Tumor Cell Line	RAS Mutation
1.	Lung	SW1271	NRAS Q61R
2.	Melanoma	MEL-JUSO	HRAS G13D NRAS Q61L
3.	Pancreatic	KP-2	KRAS G12R
4.	Colorectal	NCI-H747	KRAS G13D
5.	Thyroid	KMH-2	NRAS Q61R

	Tissue	Tumor Cell line	RAS Mutation
1.	Melanoma	SK-MEL-30	NRAS Q61K
2.	Pancreatic	PA-TU-8902	KRAS G12V
3.	Liver	HEPG2	NRAS Q61L
4.	Lung	NCI-H2087	NRAS Q61K
5.	Colorectal	LS1034	KRAS A146T

	Tissue	Tumor Cell line	RAS Mutation
1.	Lung	NCI-H1581	none
2.	Gastric	NCI-N87	none
3.	Breast	HCC38	none
4.	Ovarian	OVCAR-3	none
5.	Prostate	LNCaP	none

Cell lines tested in 3D-TGA were assigned response of sensitive (IC50 < 1uM), intermediate (IC50 ≥ 1 and >25% reduction at 10uM), and resistant otherwise (3D-TGA).

Table 1. IMM-1-104 Responses in 3D-TGA: Patient-aligned Model Subsets

	Depth of RESPONSE		Non-RESP	Total RESP	Depth of RESPONSE		Non-RESP	Total RESP
	Sensitive	Intermediate	Resistant	Overall Response	Sensitive	Intermediate	Resistant	Overall Response
RAS mutant	23 (30.7%)	41 (54.7%)	11 (14.7%)	**85%**	13 (27.1%)	28 (58.3%)	7 (14.5%)	**85%**
MAPK normal	2 (6.2%)	15 (46.9%)	15 (46.9%)	**53%**	1 (6.3%)	8 (50%)	7 (43.7%)	**56%**
Total	25	56	26	**107**	14	36	14	**64**
Patient Alignment	All Tumor Models (with low GENIE v13.0 Alignment)				Translationally-aligned Tumor Models (GENIE v13.0)			
	Fisher's exact p-value 0.0004				Fisher's exact p-value 0.0430			

Subset (N = 107) of 132 models, where 'RAS Mutant' includes H/N/K isoforms; 'MAPK Normal' additionally excludes models with BRAF (class I/II) & GNAQ/GNA11 mutations
'Patient-aligned Tumor Models' represent models where mutational profile mapped to most frequent 95% of GENIE v13.0 patients of the same indication

Table 2. Humanized 3D-TGA Response: Tumor Tissue and RAS Mutation Status

Tissue	Sensitive #	Intermediate #	Resistant
Pancreatic	6	11	2
Melanoma	14	8	0
CRC	2	18	5
Lung	3	16	6
Thyroid	5	1	1
Soft Tissue	1	1	1
Breast	1	1	6
Gastric	1	3	2
Ovary	3	0	2
Prostate	1	0	2
Fibrosarcoma	1	0	0
Liver	0	4	2
Neuroblastoma	0	1	1
Response I Non-Resp	**102** (77.3%)		**30** (22.7%)

Together, project as Responsive to IMM-1-104, based on 3D-TGA and *in vivo* studies (parallel efforts are focused on projecting patient-aligned molecular profiles, 'Targetability')

RAS or RAF mutation	Sensitive	Intermediate	Resistant
NRAS G12	1	1	0
NRAS G13	1	0	0
NRAS Q61	11	6	2
KRAS A146	0	1	0
KRAS G12	7	29	8
KRAS G13 ^	1	2	1
KRAS Q61	1	2	0
HRAS G13 *	1	0	0
BRAF (Class I or II)	13	8	4
Response I Non-Resp	**85** (85.0%)		**15** (15.0%)

RAS or RAF mutation	Sensitive	Intermediate	Resistant
None	2	15	15
Response I Non-Resp	**17** (53.1%)		**15** (46.9%)

^ 1 model also bearing KRAS Q61
* 1 model also bearing NRAS Q61

Cell lines tested in 3D-TGA were assigned response of sensitive (IC50 < 1uM), intermediate (IC50 ≥ 1 and >25% reduction at 10uM), and resistant otherwise (3D-TGA).

Table 3. KRAS G12 Variant and Associated 3D-TGA Response Category

Tumor Tissue	Sensitive	Intermediate	Resistant
Pancreatic	3	7	2
Colorectal	0	5	2
Lung	0	10	1
Response I Non-Resp	**25** (83.3%)		**5** (16.7%)

G12 Amino Acid	Sensitive	Intermediate	Resistant
A	0	1	0
C	1	6	1
D	0	3	2
R	2	2	0
S	0	1	0
V	0	9	2
Response I Non-Resp	**25** (83.3%)		**5** (16.7%)

Cell lines tested in 3D-TGA were assigned response of sensitive (IC50 < 1uM), intermediate (IC50 ≥ 1 and >25% reduction at 10uM), and resistant otherwise (3D-TGA).

Conclusions

Across all RAS-mutated tumor models tested, at least one model with a given mutation position or amino acid substitution was associated with response to IMM-1-104. When examining the frequently altered position at G12 in KRAS, across 30 KRAS mutated cell lines that spanned three tumor types, no preference was observed with respect to IMM-1-104 response based on a particular amino acid at G12, nor did we observe a lack of activity for any specific activation mutation in RAS. These observations suggest IMM-1-104 therapy may benefit a broad, RAS-mutant patient population or 'Universal-RAS'. Our past[2] and ongoing translational efforts are focused on better defining RAS/MAPK pathway addiction and utilization within the backdrop of certain types of resistance mechanisms to better identify key determinants of MAPK pathway addiction that may ultimately help inform optimal response to IMM-1-104.

References

1. D. Onion, et al. 2016 Mol Cancer Ther 15(4):753-763
2. B. Hall, et al. 2022 J Clin Oncol 40 (suppl 16; abstr e15084)
3. GENIE v13.0: The AACR Project GENIE Consortium. AACR Project GENIE: Powering Precision Medicine Through An International Consortium, Cancer Discov. 2017 Aug;7(8):818-831

The authors would like to acknowledge the American Association for Cancer Research and its financial and material support in the development of the AACR Project GENIE registry, as well as members of the consortium for their commitment to data sharing. Interpretations are the responsibility of study authors.

Contact: Brett Hall (bhall@immuneering.com). All authors are employees and/or stockholders of Immuneering Corporation.

Presented at AACR Special Conference: Targeting RAS (March 5-8, 2023)