Beyond BRAF V600 – Characterizing the genomic landscape of non-BRAF V600 mutations and BRAF fusions in 121,221 in adult patients with cancer.

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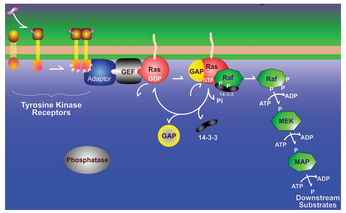
Background

- Atypical BRAF mutations at codons aside from V600 (non-V600) and BRAF fusions activate the MAP kinase/ERK-signaling pathway, however the landscape of these alterations has yet to be well-defined.
- Preclinical work suggests that BRAF non-v600 mutations differ somewhat in their signaling mechanisms, eg.non-v600 BRAF tumors can be categorized into respective classes.[1,2] BRAF non-v600 tumors have been shown to have impaired kinase activity and yet still activate the signaling pathway through dimerization – in contrast BRAF V600E tumors activate as monomers. [2,3]
- While selective inhibitors of the BRAF V600-mutated kinase plus MEK inhibitor combinations are EMA- and FDA-approved for melanoma, non-small cell lung cancer, and anaplastic thyroid cancer, drug development for atypical BRAF alterations has been staggered.
- Here, we present a comprehensive analysis of non-BRAF V600 mutations and BRAF fusions in pan-cancer adult malignancies.

Figure 1. MAPK signaling pathway



Methods

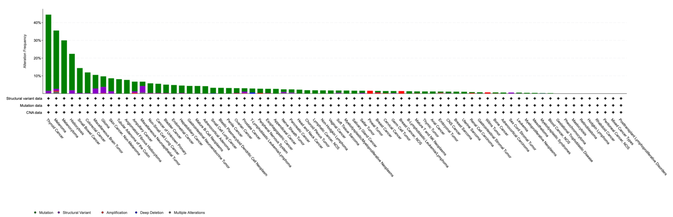
- To define the genomic landscape of non-BRAF V600 BRAF alterations, we analyzed 136,096 samples from 121,221 patients available from AACR Project GENIE v.11 (Cancer Discov. 2017)[4] database for the prevalence of PIK3CA mutations, fusions and copy number alterations in a range of cancer types.
- Mutations and fusions in NSCLC, melanoma and colorectal were analyzed to see if there was an association with other co-altered genes. Analysis of amplification within all tumors was conducted and stratified by copy-number variants.

Results

Pan-cancer frequency of non-v600 BRAF alterations

3624 separate non-V600 BRAF mutations were identified in 3340 samples (2.5%), most frequently in lung (21.3%, 772), melanoma (16.8%, 608), colorectal cancer (12.7%, 461).

Figure 2. Prevalence of BRAF alterations across tumors



Genomic landscape of non-v600 BRAF alterations

- 2938 missense mutations (81%) were identified, most were identified as driver mutations (62%); 1115 missense mutations were variants of uncertain significance (VUS) using OncoKB database (38%).
- Missense mutations occurred across codons, most frequently involving codon 469 (13.8%, 405), 594 (12.7%, 374) 601 (7.9%, 232) 466 (7.5%, 221), 581 (161, 5.5%). 126 truncating mutations (3.5%) were identified, 156 in-frame alterations (4.3%).

Results

Co-mutations of significance

- Co-alterations of significance in AKT-MTOR-PI3K pathway were noted in the presence of non-v600 BRAF mutations in melanoma, colorectal cancer and non-small cell lung cancer **(Table 1)**.
- No co-alterations were deemed significant on analysis of fusions.

Table 1. Co-alterations of significance in genes of AKT-PI3K-MTOR signaling pathway

Cancer subtype	Gene	p value
Melanoma		
	NF1	1.35E-12
	NRAS	5.17E-08
	KRAS	6.68E-05
	HRAS	0.000478
	PIK3CA	0.001181
	AKT1	0.014586
	TSC2	0.020304
	MTOR	0.03033
NSCLC		
	NF1	1.35E-12
Colorectal		
	KRAS	6.6E-15
	MTOR	6.6E-15
	AKT3	0.003417
	AKT2	0.003417
	AKT1	4.06E-11
	NF1	6.04E-10
	MDM2	7.2E-10
	TSC2	7.2E-10
	TSC1	3.79E-07
	PTEN	0.002325
	HRAS	1.58E-14
	SOS1	0.003417

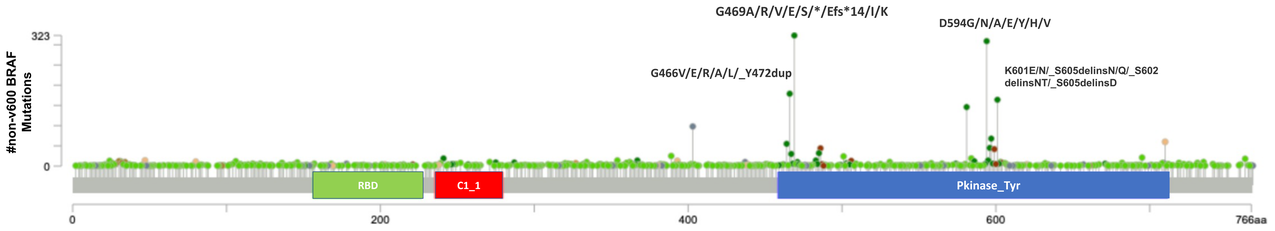


Figure 3. Lollipop plot showing non-v600 BRAF mutations

Results

- BRAF fusions were detected in 0.4% of tumor samples (n=539), most identified as likely oncogenic events (83%).
- Variant fusion partners were diverse (137 identified), most frequently seen in pilocytic astrocytoma (32%, 117), melanoma (11%,39), prostate (10%, 37), lung cancer (6.5%, 24).
- Aside from intragenic fusions, all fusion partners were likely oncogenic. Common fusion gene partners included *KIAA1549, SND1, MKRN1* and *AGK* (34%, 3.9%, 3.5%, 3.3% of 539 samples respectively).
- BRAF amplification occurred in 0.15% of tested samples.

Conclusions

- Atypical non-V600 BRAF mutations and BRAF fusions represent a rare, but distinct cohort across tumor types.
- Most missense mutations and fusion events are described as oncogenic, highlighting the urgent need to develop drugs beyond current BRAF V600 indications.
- Co-occurring alterations may provide insight for the future of non-v600 BRAF inhibition, such as the design rationale combination strategies.
- Further functional characterization of atypical BRAF variants and enrolment on basket trials are warranted.

References

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Acknowledgements

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 respon sibility of study authors.