Somatic alterations of *NF1* in colorectal cancer

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Introduction

- NF1 encodes neurofibromin, which is a key GTPase-activating protein that downregulates RAS activation. Inactivating mutations in NF1 result in sustained activation of RAS signaling, a key driver for development of colorectal cancer (CRC).[1]
- In the TCGA cohort, 9% of lung adenocarcinoma and 10% of melanoma showed *NF1* mutation, exclusively occurring with oncogenic *RAS* and *BRAF* mutations.[2-3] In the TCGA CRC cohort *NF1* mutations were present in 2.8% but one could not assess the relationship to *RAS*/*BRAF* mutations.[4]
- *NF1* mutations have been suggested to be a potential mechanism of resistance to EGFR inhibition in *RAS*-wild type CRC.[5-7]
- We here performed molecular characterization of *NF1* mutated (MT) CRC.

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2. Cancer Genome Atlas Research Network. Nature. 2014. 511:543-50.
3. The Cancer Genome Atlas Network. Cell. 2015. 161:1681-96.
4. Cancer Genome Atlas Network. Nature. 2012. 487:330-7.
5. Woolston A, et al. Cancer Cell. 2019. 36:35-50.
6. Post JB, et al. Oncotarget. 2019. 10:1440-57.
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Method

- Tumor profiles from 8150 CRC patients (pts) with available *NF1* mutation status were retrospectively reviewed.
- NextGen sequencing by a customized 592-gene panel was performed.
- Microsatellite instability (MSI) status was tested with a combination of immunohistochemistry (IHC), fragment analysis and NGS.
- Tumor mutational burden (TMB) was calculated based on somatic nonsynonymous missense mutations.
- PD-L1 was tested by IHC (SP142).
- Molecular profiles between *NF1*-MT and *NF1*-WT pts were compared.
- Student-t test (for mean values) and Wilcoxon rank-sum testing (for median values) were used for comparison of continuous data. Categorical data were analyzed using Fisher's exact test or Chi-square test where appropriate.

Detected mutations in *NF1*

- In total, 176 pts (2.2%) had pathogenic or presumed pathogenic *NF1* mutations.
- 25 cases had >1 *NF1* mutations.
- A total of 204 *NF1* mutations were observed.

Details of observed *NF1* mutations

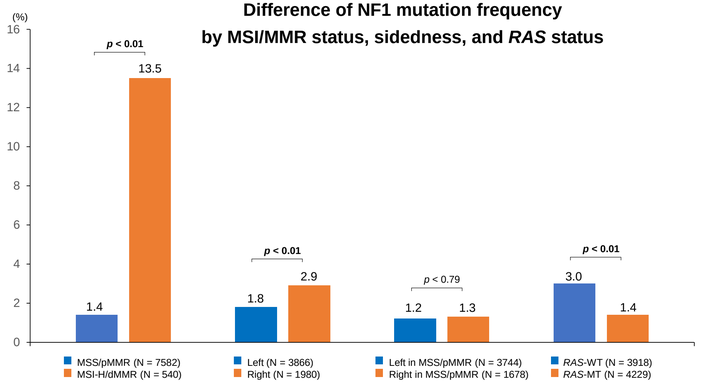
Type	N	% of total	Function
Frameshift	88	43.1%	Truncating mutations are generally presumed loss-of-function.
Nonsense	79	38.7%	
Splicing	24	11.8%	Functional consequence is unclear.
Other	7	3.4%	Functional consequence is unclear.
Missense	5	2.5%	Identified variants are presumed loss-of-function.
UTR	1	0.5%	Functional consequence is unclear.
Total	204	100.0%	Pathogenic/Presumed Pathogenic

Results

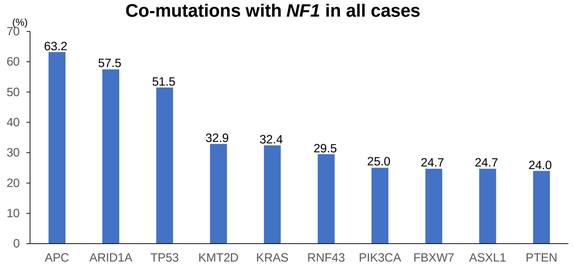
Patient characteristics

	Total	*NF1*-MT	*NF1*-WT	*P*-value (*NF1*-MT vs WT)
Patient number	8150	176 (2.2%)	7974	
Median age (range)	60 (14-90+)	57 (24-89)	60 (14-90+)	**0.04**
Sex				
Male	4404 (54.0%)	103 (58.5%)	4301 (53.9%)	0.28
Female	3746 (46.0%)	73 (41.5%)	3637 (46.1%)	
Primary tumor location				
Left	3866 (47.4%)	69 (39.2%)	3797 (47.6%)	**0.02**
Right	1980 (24.3%)	58 (33.0%)	1922 (24.1%)	
Unclear	2304 (28.3%)	49 (27.8%)	2255 (28.3%)	
MSI/MMR status				
MSI-H/dMMR	540 (6.6%)	73 (41.5%)	467 (5.9%)	**<0.01**
MSS/pMMR	7582 (93.0%)	103 (58.5%)	7479 (93.8%)	
Unclear	28 (0.3%)	0 (0.0%)	28 (0.4%)	

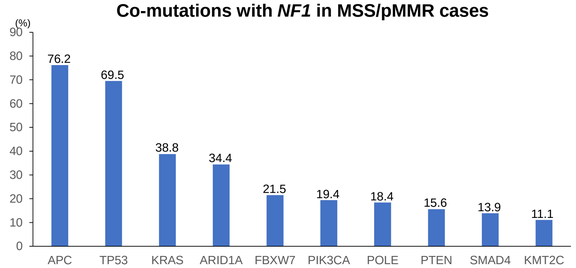
Difference of NF1 mutation frequency by MSI/MMR status, sidedness, and *RAS* status



Co-mutations with *NF1* in all cases



Co-mutations with *NF1* in MSS/pMMR cases



Comparison of NF1-MT and NF1-WT on major gene mutations and immunotherapy-related markers

	All patients (N = 8150)	All			MSS/pMMR		
		NF1-MT (N = 176)	*NF1*-WT (N = 7974)	*P*-value	*NF1*-MT (N = 103)	*NF1*-WT (N = 7479)	*P*-value
APC	73.0%	63.2%	73.2%	**<0.01**	76.2%	75.2%	0.81
TP53	71.6%	51.5%	72.1%	**<0.01**	69.5%	74.4%	0.27
KRAS	48.6%	32.4%	49.0%	**<0.01**	38.8%	50.3%	**0.02**
ARID1A	24.5%	57.5%	23.3%	**<0.01**	34.4%	15.2%	**0.01**
PIK3CA	16.9%	25.0%	16.7%	**<0.01**	19.4%	15.9%	0.34
SMAD4	11.9%	13.2%	11.8%	0.55	13.9%	12.2%	0.65
FBXW7	10.1%	24.7%	9.7%	**<0.01**	21.5%	8.6%	**<0.01**
BRAF	8.9%	16.6%	8.8%	**<0.01**	2.0%	6.7%	0.06
RNF43	6.4%	29.5%	5.9%	**<0.01**	3.9%	2.5%	0.33
AMER1	6.1%	12.1%	5.9%	**<0.01**	8.9%	5.4%	0.13
POLE	0.6%	11.4%	0.4%	**<0.01**	18.4%	0.3%	**<0.01**
HR genes	8.2%	39.8%	7.5%	**<0.01**	17.5%	4.4%	**<0.01**
TMB (mean)		48.9/Mb	10.0/Mb	**<0.01**	48.3/Mb	8.2/Mb	**<0.01**
TMB-H (≥17/Mb)		54.0%	6.3%	**<0.01**	21.4%	1.0%	**<0.001**
PD-L1 ≥5%		12.9%	3.6%	**<0.01**	7.1%	2.6%	**0.02**

Summary

- **Out of 8150 pts, 176 (2.2%) had somatic *NF1* mutations with pathogenic or presumed pathogenic function.**
- **A higher *NF1*-MT frequency was observed in MSI-H/dMMR vs MSS/pMMR (13.5% vs 1.4%, p<0.0001), in right-sided vs left sided (2.9% vs 1.8%, p<0.01), and in *RAS*-WT vs *RAS*-MT (3.0% vs 1.4%, p<0.0001). In MSS/pMMR tumors, no association with sidedness was observed (right: 1.3% vs left: 1.2%, NS).**
- **The most prevalent co-mutations with *NF-1* were *APC* (63.2%), *ARID1A* (57.5%), *TP53* (51.5%), *KMT2D* (32.9%) and *KRAS* (32.4%) in all cases, and *APC* (76.2%), *TP53* (69.5%), *KRAS* (38.8%), *ARID1A* (34.4%) and *FBXW7* (21.5%) in MSS/pMMR cases. *POLE* mutation was observed in 18.4% of *NF1*-MT/MSS/pMMR pts.**
- **Compared to *NF1*-WT pts, *NF1*-MT pts had more frequent mutations in *ARID1A* (All: 57.5% vs 23.3%, p<0.0001; MSS/pMMR: 34.4% vs 15.2%, p<0.05), and less frequent mutations in *KRAS* (All: 32.4% vs 49.0%, p<0.0001; MSS/pMMR: 38.8% vs 50.3%, p<0.05).**
- ***NF1*-MT pts had more frequent alterations in homologous recombination pathway compared to *NF1*-WT pts (All: 39.8% vs 7.5%, p<0.0001; MSS/pMMR: 17.5% vs 4.4%, p<0.0001).**
- **Mean TMB was significantly greater in *NF1*-MT than *NF1*-WT (All: 48.9/Mb vs 10.0/Mb, p<0.0001; MSS/pMMR: 48.3/Mb vs 8.2/Mb, p<0.0001). Also, PD-L1 positivity was higher in *NF1*-MT compared to *NF1*-WT (All: 12.9% vs 3.6%, p<0.0001; MSS/pMMR: 7.1% vs 2.6%, p<0.05).**

Conclusions

- ***NF1* mutations were more frequent in RAS-WT and MSI-H CRC pts.**
- ***NF1*-MT was associated with alterations in chromatin remodeling and DNA damage response pathways, as well as elevated TMB and PD-L1 expression, which may provide alternative therapeutic strategies beyond EGFR inhibition.**

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