Prevalence of *NTRK1/2/3* fusions in dMMR/MSI metastatic colorectal cancer (Poster n°444P)

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Background:

Inhibitors of tropomyosin receptor kinase (TRK) have shown promising activity against neurotrophic TRK (*NTRK*) fusion-driven cancers. *NTRK* gene fusions are observed in less than 1% of colorectal cancers (CRCs). CRCs harboring wild-type *BRAF*, *KRAS* and MisMatch Repair deficiency (dMMR)/MicroSatellite Instability (MSI) due to *MLH1* hypermethylation have been associated with *NTRK* fusions in small cohorts of non-metastatic tumors.

We evaluated the frequency and associated clinical characteristics of *NTRK* fusions among dMMR/MSI metastatic CRCs (mCRCs) patients.

Methods:

Tumor samples of dMMR/MSI mCRC pts were obtained from a French multicenter retrospective cohort and from a single-center cohort of pts treated with immune checkpoint inhibitors (ICI).

Clinico-pathological data including *KRAS* and *BRAF*^{V600E} status, MMR protein and *MLH1* methylation status were available for all pts.

All samples were screened for:

*TRK expression by **immunohistochemistry (IHC)** using a pan-TRK antibody (clone EPR17341, Abcam; positivity: 1% of labeled tumor cells)

NTRK1/2/3* gene rearrangements, by ***fluorescent in situ hybridation* (FISH) (sonde Zytolight SPEC NTRK dual-color Break Apart, Zytovision; threshold of 15% positive nuclei)

***Targeted RNAseq** (FusionPlex® Lung SK0133 ARCHER kit) was performed on IHC or FISH-positive and doubtful cases.

Results:

187 patients with dMMR/MSI CRC (including n=120 patients having received ICI)

- 42 patients with paired primary tumor + metastatic samples
- 152 patients with isolated sample (metastasis or primary tumor
- 1 patient with 2 primary tumors

IHC: 5/10 (cytoplasmic pan-TRK immunostaining) **(Figure 1)**

FISH: 10/187 (including 4 patients with ICI) **(Figure 2)**
NTRK1: 8; NTRK3: 2

Targeted RNA sequencing: 7/10 (1 case with insufficient RNA quality)
TMP3-NTRK1: 3; LMNA-NTRK1: 2; ETV6-NTRK3: 1; EML-NTRK3: 1)

Frequency of *NTRK1/2/3* fusions: 5.3%

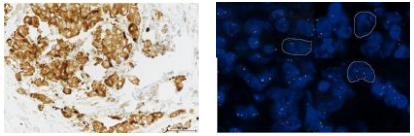

Figure 1: Pan-Trk-positive immunohistochemistry (x400 magnification) in one case displaying strong, diffuse cytoplasmic staining. **Figure 2:** This case harbored *NTRK1* fusion by FISH. Archer assay detected *LMNA-NTRK1* fusion transcript.

N° patient	Lost MMR protein	*MLH1* promoter methylation	*KRAS* status	*BRAF* status	Lynch *versus* sporadic	ICI	IHC anti-pan-TRK	FISH NTRK	Partner gene
1	MLH1/PMS2	1	WT	WT	Sporadic	0	1	*NTRK1*	*TPM3-NTRK1*
2	MLH1/PMS2	1	WT	Mutated	Sporadic	0	0	*NTRK1*	0
3	MLH1/PMS2	1	WT	WT	Sporadic	0	0	*NTRK1*	*TPM3-NTRK1*
4	MSH2/MSH6	NAp	WT	WT	Lynch	0	0	*NTRK1**	0
5	MLH1/PMS2	1	WT	WT	Sporadic	0	0	*NTRK3*	*ETV6-NTRK3*
6	MLH1/PMS2	1	unknown	WT	Sporadic	0	1	*NTRK1*	*LMNA-NTRK1*
7	MLH1/PMS2	1	WT	WT	Sporadic	1	1	*NTRK1*	*LMNA-NTRK1*
8	MLH1/PMS2	1	WT	WT	Sporadic	1	1	*NTRK3*	*EMLA-NTRK3*
9	MSH6	NAp	Mutated	WT	Lynch	1	0	*NTRK1**	Failure (insufficient RNA quality)**
10	MLH1/PMS2	1	WT	WT	Sporadic	1	1	*NTRK1*	*TPM3-NTRK1*

Table 1: **Characteristics of tumors harboring NTRK fusions:** eight tumors were sporadic with *MLH1* hypermethylation and two had germline MMR mutations (lynch syndrome).
Among patients receiving ICI: 3 objective response (iRECIST) (2 complete and one partial responses with 25 to 54 months of follow-up); 1 primary resistance.
* For these two cases, *NTRK1* rearrangement was heterogeneous, with 10 to 15% of nuclei displaying « split-apart signals ».
** New tissue extraction is in progress for this case.

Conclusion:

- Frequency of *NTRK1/2/3* fusions was 5.3% in our dMMR/MSI mCRCs cohort.
- *NTRK* fusions could be not restricted to sporadic cases. *NTRK* fusions in Lynch syndrome's setting need to be further investigated.
- The diagnostic accuracy of pan-TRK IHC was low.
- Optimal testing algorithms for theranostic purpose remain to be defined in this setting.

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Conflicts of interest: none