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Article

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ALK, *ROS1*, and *NTRK* Rearrangements in Metastatic Colorectal Cancer

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Abstract

Background: *ALK*, *ROS1*, and *NTRK* fusions occur in 0.2% to 2.4% of colorectal cancers. Pioneer cases of metastatic colorectal cancer (mCRC) patients bearing rearrangements who benefited from anti-ALK, ROS, and TrkA-B-C therapies have been reported previously. Here we aimed at characterizing the clinical and molecular landscape of *ALK*, *ROS1*, and *NTRK* rearranged mCRC.
Methods: Clinical features and molecular characteristics of 27 mCRC patients bearing *ALK*, *ROS1*, and *NTRK* rearranged tumors were compared with those of a cohort of 319 patients not bearing rearrangements by means of Fisher's exact, $\chi 2$ test, or Mann-Whitney test as appropriate. Overall survival curves were estimated with the Kaplan-Meier method and compared using the log-rank test. A Cox proportional hazard model was adopted in the multivariable analysis. Deep molecular and immunophenotypic characterizations of rearranged cases, including those described in The Cancer Genome Atlas database, were performed. All statistical tests were two-sided.
Results: Closely recalling the "*BRAF* history," *ALK*, *ROS1*, and *NTRK* rearrangements more frequently occurred in elderly patients ($P = .02$) with right-sided tumors ($P < .001$) and node-spreading ($P = .03$), *RAS* wild-type ($P < .001$), and MSI-high ($P < .001$) cancers. All patients bearing *ALK*, *ROS1*, and *NTRK* fusions had shorter overall survival (15.6 months, 95% confidence interval [CI] = 0.0 to 20.4 months) than negative patients (33.7 months, 95% CI = 28.3 to 42.1 months), both in the univariate (hazard ratio [HR] = 2.17, 95% CI = 1.03 to 4.57, $P < .001$) and multivariable models (HR = 2.33, 95% CI = 1.10 to 4.95, $P = .02$). All four evaluable patients with rearrangements showed primary resistance to anti–epidermal growth factor receptor agents. Frequent association with potentially targetable *RNF43* mutations was observed in MSI-high rearranged tumors.

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Conclusions: *ALK*, *ROS1*, and *NTRK* rearrangements define a new rare subtype of mCRC with extremely poor prognosis. Primary tumor site, MSI-high, and *RAS* and *BRAF* wild-type status may help to identify patients bearing these alterations. While sensitivity to available treatments is limited, targeted strategies inhibiting ALK, ROS, and TrkA-B-C provided encouraging results.

Genomic translocations leading to the constitutive activation of receptor tyrosine kinases (RTKs) play a crucial role in tumorigenesis across different malignancies, including colorectal cancer (CRC) (1,2). RTK fusions involving *ALK*, *ROS1*, and *NTRK1-2-3* (*NTRK*) occur in 0.2% to 2.4% of CRCs (3,4) and may represent new targets for therapeutic intervention (5–17). Addiction to kinase suppression or pharmacological inhibition has been reported in CRC preclinical models bearing RTK fusions, including the *TPM3-NTRK1* rearranged KM12 cell line (18), the *ALK* rearranged cell line C10 (19), patient-derived primary cell lines (10) and patient-derived xenografts (20). So far, a single heavily pretreated metastatic CRC (mCRC) patient whose tumor bore an *LMNA-NTRK1* fusion was treated with entrectinib, an oral selective inhibitor of ALK, ROS1, and TrkA-B-C (the protein products of the *NTRK1-2-3* genes, respectively), with clinical benefit (15). Another mCRC patient whose tumor harbored *STRN-ALK* fusion received the oral ALK inhibitor ceritinib and achieved response (16), and a patient with a *CAD-ALK* rearrangement responded to entrectinib (6).

Despite these pioneer case reports, it has not been clearly established whether *ALK*, *ROS1*, or *NTRK* rearranged tumors represent a distinct, although rare, disease subtype that should be detected early in order to adopt a tailored management strategy that may include targeted treatments. Although a few reports have described the occurrence of *ALK*, *ROS1*, and *NTRK* fusions in CRC (2–12), there is still limited knowledge about clinical and pathological characteristics, prognosis, and sensitivity of these tumors to available treatments including anti–epidermal growth factor receptor (EGFR) monoclonal antibodies (MoAbs) such as cetuximab and panitumumab. Similarly, except for some preclinical reports (11,19), comprehensive molecular and functional data to clarify whether these alterations confer oncogene addiction and to suggest perspectives on optimal treatment strategies are not available yet. We therefore carried out a global effort aimed at characterizing the molecular and clinical landscape of *ALK*, *ROS1*, and *NTRK* rearranged mCRCs. Even though a broader list of gene fusions has been described in CRC, including those affecting *RET*, *HER2*, and *BRAF* (2,8,22,23), we specifically focused on mCRC with *ALK*, *ROS1*, and *NTRK* rearrangements because their phylogeny is closely related and they are frequently grouped as targets of newly developed agents such as entrectinib (24).

Methods

Study Design and Participants

In the clinical step (Figure 1), the cohort of 319 *ALK*, *ROS1*, and *NTRK* negative cases included patients screened for Ignyta's phase I program at Samsung Medical Center (SMC), Seoul, South Korea (n = 209); Azienda Ospedaliero-Universitaria Pisana (AOUP), Pisa, Italy (n = 79); and Fondazione IRCCS Istituto Nazionale dei Tumori (INT), Milan, Italy (n = 31). The population of 27 *ALK*, *ROS1*, and *NTRK* rearranged mCRCs included patients collected at Foundation Medicine Inc. (FMI), Cambridge, Massachusetts (n = 12); Samsung Medical Center (SMC), Seoul, South Korea (n = 4); Memorial Sloan Kettering Cancer Center (MSKCC), New York, New York (n = 3); Austin Health, Heidelberg, Australia (n = 3) on behalf of MAX trial Investigators; Fondazione IRCCS Istituto Nazionale dei Tumori (INT), Milan, Italy (n = 2); Niguarda Cancer Center (NCC), Milan, Italy (n = 2); University Hospital Gasthuisberg (UHG), Leuven, Belgium (n = 1). Molecular screening methods are detailed in the Supplementary Methods (available online) and summarized in Figure 1. Study participants signed a written informed consent, and the study was approved by the Institutional Review Board of INT, Milan.

Statistical Analysis

We investigated the association of *ALK*, *ROS1*, and *NTRK* rearrangements with the following variables collected at the diagnosis of mCRC: age, sex, Eastern Cooperative Oncology Group performance status (0, ≥1), primary tumor location (right colon, left colon, rectum), primary tumor resection, mucinous histology, time to metastases (synchronous, metachronous), number of metastatic sites (one, more than one), metastatic sites (lung, lymph nodes, liver, peritoneum), *RAS* and *BRAF* status (mutated, wild-type), mismatch-repair (MMR) status (proficient, deficient). The Fisher's exact test, $\chi 2$ test, or Mann-Whitney tests were used when appropriate to assess the associations of the *ALK*, *ROS1*, *NTRK* rearrangements with investigated characteristics. To provide an estimation of the probability of finding a gene fusion based on an analyzed characteristic, odds ratios (ORs) and relative 95% confidence intervals (CIs) were calculated. Statistical significance was set at a *P* value of .05.

We investigated the impact of *ALK*, *ROS1*, and *NTRK* rearrangements on overall survival (OS), defined as the time from diagnosis of metastatic disease to death or last follow-up for living patients. OS analysis was determined according to the Kaplan-Meier method, and survival curves were compared using the log-rank test. The association of *ALK*, *ROS1*, and *NTRK* status and clinicopathological characteristics with OS was assessed in univariate analysis. In order to minimize the bias of multiple comparisons, according to the false discovery rate correction, statistical significance was set at a *P* value of .009. The Cox proportional hazard model was adopted in the multivariable analysis, including as covariates variables associated with survival with a *P* value of less than .10 in the univariate analyses. Hazards' proportionality was assumed and verified using the goodness-of-fit $\chi 2$ test.

All analyses were carried out by means of Prism 7 for Mac OS X v. 7.0. All statistical tests were two-sided.

Translational Analyses

As shown in Figure 1 and the Supplementary Methods (available online), next-generation sequencing (NGS) data were obtained through three different panels: the FMI panel in 15 cases, Minerva panel (Ignyta Inc.) in 11 cases, and Memorial Sloan Kettering–Integrated Mutation Profiling of Actionable Cancer

	NTRK fusions (n = 13)	ALK fusions (n = 11)	ROS1 fusions (n = 3)
Ignyta's STARTRK-1 phase I study screening program	*LMNA-NTRK1* (n = 1) [7] *TPM3-NTRK1* (n = 3*) [8] *SCYL3-NTRK1* (n = 1*)	*CAD-ALK* (n = 1) [9] *EML4-ALK* (n = 2*) [6]	
MAX trial post hoc analysis		*EML4-ALK* (n = 1) [4]	*Unknown-ROS1* (n = 1) [4] *SLC34A2-ROS1* (n = 1) [4]
Foundation Medicine Inc, Clinical database	*TPM3-NTRK1* (n = 4*) *ETV6-NTRK3* (n = 2) [12] *LMNA-NTRK1* (n = 1*)	*CAD-ALK* (n = 2*) [6] *EML4-ALK* (n = 1) [11] *CENPF-ALK* (n = 1) [11] *PRKAR1B-ALK* (n = 1) [11] *MAPRE3-ALK* (n = 1) [11] *STRN-ALK* (n = 1) [11]	*GOPC-ROS1* (n = 1*)
MSK-IMPACT screening program	*LMNA-NTRK1* (n = 1*)		

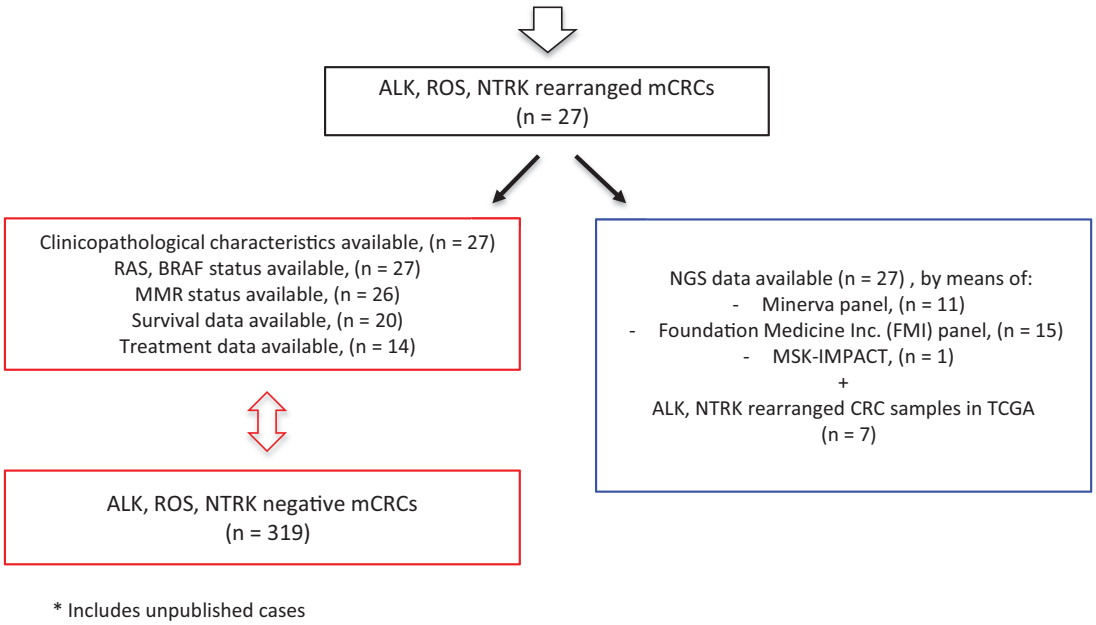

Figure 1. Study flowchart. **A)** A total of 27 metastatic colorectal cancer (mCRC) cases with *ALK* (n = 11), *ROS1* (n = 3), and *NTRK* (n = 13) translocations were collected. Patients were retrieved by Ignyta's phase I screening program in Italy, Belgium, and South Korea; MAX trial's post hoc analysis conducted in Australia; Foundation Medicine Inc. (FMI) data set in the United States; Memorial Sloan Kettering–Integrated Mutation Profiling of Actionable Cancer Targets (MSK-IMPACT) screening program in the United States. **B)** Clinicopathological characteristics, *RAS* and *BRAF* status, mismatch-repair (MMR) status, survival and treatment outcome data in the *ALK*, *ROS1*, and *NTRK* rearranged population (n = 27) were compared with those from a cohort of *ALK*, *ROS1*, and *NTRK* negative mCRC patients (n = 319) included in Ignyta's phase I screening program. **C)** Annotated genetic variants were retrieved from targeted next-generation sequencing analyses of tumor samples (n = 27) from *ALK*, *ROS1*, and *NTRK* rearranged mCRC patients. The number of samples analyzed by different gene panels is shown. Analysis of publicly available RNA sequencing data from The Cancer Genome Atlas (TCGA) COADREAD (colorectal) study allowed the identification of seven additional tumors carrying *ALK* or *NTRK3* translocations. Molecular annotations from TCGA translocated tumors were pooled with those from mCRC patients to increase power of detecting genetic alterations coexisting with *ALK*, *ROS1*, and *NTRK* rearrangements. *Includes unpublished cases. †ClinicalTrials.gov Identifier: NCT02097810. ‡ClinicalTrials.gov Identifier: NCT00294359. mCRC = metastatic colorectal cancer; MMR = mismatch repair; MSK-IMPACT = Memorial Sloan Kettering–Integrated Mutation Profiling of Actionable Cancer Targets; NGS = next-generation sequencing; TCGA = The Cancer Genome Atlas.

Targets (MSK-IMPACT) panel in one case. The association of individual samples with the type of translocation identified and the NGS panel is shown in Supplementary Table 2 (available online). Finally, analysis in silico from The Cancer Genome Atlas (TCGA) data was performed (Supplementary Methods, available online).

Results

Study Population

Based on a systematic literature review, we identified 24 published cases of ALK, ROS1, or NTRK rearranged CRCs

Table 1. Patients' characteristics according to the presence or absence of *ALK*, *ROS1*, and *NTRK* rearrangements, or specifically for presence or absence of *NTRK* and *ALK* rearrangements*

Characteristics	*ALK*, *ROS1*, *NTRK* negative (n = 319) No. (%)	*ALK*, *ROS1*, *NTRK* Rearranged (n = 27) No. (%)	P†	*NTRK* rearranged (n = 13) No. (%)	P‡	*ALK* rearranged (n = 11) No. (%)	P§
Sex							
Female	129 (40.4)	18 (66.7)	.16	9 (69.2)	.04	7 (63.6)	.21
Male	190 (59.6)	9 (33.3)		4 (30.8)		4 (36.4)	
Age, y							
Median	57	64	.02	68	.03	55	.97
Range	15–88	40–62		33–73		40–87	
ECOG performance status							
0	106 (33.4)	9 (64.3)	.25	2 (25.0)	1.00	3 (75.0)	.12
1–2	211 (66.6)	5 (35.7)		6 (75.0)		1 (25.0)	
NA	2	13		5		7	
Primary tumor location							
Right colon	98 (31.0)	20 (80.0)	<.001	10 (90.9)	<.001	8 (72.7)	.01
Left colon	125 (39.6)	3 (12.0)		0		2 (18.2)	
Rectum	93 (29.4)	2 (8.0)		1 (9.1)		1 (9.1)	
NA	3	2		2		0	
Mucinous histology							
Yes	40 (12.7)	1 (5.9)	.71	0	.60	1 (11.1)	1.00
No	276 (87.3)	16 (94.1)		8 (100.0)		8 (88.9)	
NA	3	10		5		2	
Primary tumor resected							
Yes	240 (75.2)	19 (86.4)	.31	8 (72.7)	1.00	0	<.001
No	79 (24.8)	3 (13.6)		3 (27.3)		8 (100.0)	
NA	0	5		2		3	
Time to metastases							
Synchronous	210 (66.2)	11 (64.7)	1.00	5 (62.5)	1.00	6 (75.0)	.72
Metachronous	107 (33.8)	6 (35.3)		3 (37.5)		2 (25.0)	
NA	2	10		5		3	
No. of metastatic sites							
1	161 (50.9)	14 (58.3)	.53	7 (63.6)	.54	6 (54.5)	1.00
>1	155 (49.1)	10 (41.7)		4 (36.4)		5 (45.5)	
NA	3	3		2		0	
Lung metastases							
Yes	129 (40.8)	5 (20.8)	.05	0	1.00	4 (36.4)	1.00
No	187 (59.2)	19 (79.2)		11 (100.0)		7 (63.6)	
NA	3	3		2		0	
Lymph node metastases							
Yes	78 (24.7)	11 (45.8)	.03	7 (63.6)	.008	3 (27.3)	.74
No	238 (75.3)	13 (54.2)		4 (36.4)		8 (72.7)	
NA	3	3		2		0	
Liver metastases							
Yes	207 (65.5)	10 (41.7)	.03	4 (36.4)	.06	5 (45.5)	.20
No	109 (34.5)	14 (58.3)		7 (63.6)		6 (54.5)	
NA	3	3		2		0	
Peritoneal metastases							
Yes	89 (28.2)	8 (33.3)	.64	5 (45.5)	.31	3 (27.3)	1.00
No	227 (71.8)	16 (66.7)		6 (54.5)		8 (72.7)	
NA	3	3		2		0	
RAS status							
Wild-type	155 (51.7)	25 (92.6)	<.001	11 (84.6)	<.001	9 (81.8)	.07
Mutated	145 (48.3)	2 (7.4)		2 (15.4)		2 (18.2)	
NA	19	0		0		0	
BRAF status							
Wild-type	258 (94.2)	26 (96.3)	1.00	13 (100.0)	1.00	11 (100.0)	1.00
V600E mutated	16 (5.8)	1 (3.7)		0		0	
NA	45	0		0		0	

(continued)

Table 1. (continued)

Characteristics	*ALK, ROS1, NTRK* negative (n = 319) No. (%)	*ALK, ROS1, NTRK* Rearranged (n = 27) No. (%)	*P*†	*NTRK* rearranged (n = 13) No. (%)	*P*‡	*ALK* rearranged (n = 11) No. (%)	*P*§
MSI status							
MSS	148 (91.9)	14 (51.9)	<.001	3 (23.1)	<.001	4 (36.4)	<.001
MSI-high	13 (8.1)	13 (48.1)		10 (76.9)		7 (63.6)	
NA	158	0		0		0	

ROS1 rearranged tumors were not separately analyzed because of the small sample size (n = 3). ECOG = Eastern Cooperative Oncology Group; MSI-high = microsatellite instability–high; MSS = microsatellite-stable; NA = not available.

*P values were based on Fisher's exact test, $\chi 2$, or Mann-Whitney tests, whenever appropriate. All statistical tests were two-sided.

†Comparison of *ALK*, *ROS1*, and *NTRK* rearranged vs not rearranged tumors.

‡Comparison of *NTRK* rearranged vs not rearranged tumors.

§Comparison of ALK rearranged vs not rearranged tumors.

(Supplementary Table 1, available online). Nineteen were staged as metastatic according to the 7th edition of the American Joint Committee on Cancer cancer staging manual, and informative medical records were retrieved for 15 of them. Taking advantage of screening programs worldwide, we were able to identify 12 additional cases. Therefore, the final population consisted of 27 ALK, ROS1, and NTRK rearranged mCRCs (Figure 1; Supplementary Table 2, available online), including a newly described SCYL3-NTRK1 fusion (Supplementary Figure 1, available online). We compared the clinical and pathological features of ALK, ROS1, and NTRK rearranged mCRCs with a cohort of ALK, ROS1, and NTRK negative patients (n = 319), screened for phase I studies at three institutions (Figure 1). The overall incidence of ALK, ROS1, or NTRK rearrangements at these institutions was 1.5% (five out of 324 screened samples, data not shown).

Clinical and Pathological Features of ALK, ROS1, and NTRK Rearranged mCRC

As shown in Table 1, rearrangements were more frequent in older patients (median age = 64 years, range = 40–62 years, vs 57 years, range = 15–88 years, $P = .02$) with right-sided tumors (80.0% vs 31.0%, $P < .001$), and spread more frequently to lymph nodes (45.8% vs 24.7%, $P = .03$) and less frequently to the liver (41.7% vs 65.5%, $P = .03$). Additionally, although only 50% of patients in the control group had available information on MSI status, a higher percentage of tumors bearing rearrangements were MSI-high (48.1% vs 8.1%, $P < .001$).

Of note, *RAS* mutations were much less frequent in rearranged than in other tumors (7.4% vs 48.3%, $P < .001$) (Table 1). Only one (3.7%) rearranged sample showed the co-occurrence of *SLC34A2-ROS1* fusion and BRAF V600E mutation. Overall, right-sided primary location, *RAS* wild-type, and MSI-high status, in addition to female sex, were particularly associated with *NTRK* rearrangements. Notably, patients with right-sided, *RAS* and *BRAF* wild-type, MSI-high mCRCs had 54- and 453-fold higher chances of harboring *ALK*, *ROS1*, or *NTRK* rearrangements (OR = 54.00, 95% CI = 13.31 to 219.05, $P < .001$) or specifically *NTRK* rearrangements (OR = 453.00, 95% CI = 67.21 to 3053.38, $P < .001$), respectively (data not shown). These four easy-to-collect characteristics (primary tumor site, MSI, *RAS* and *BRAF* status) enable identification of patients bearing an *ALK*, *ROS1*, or *NTRK* rearrangement with positive and negative predictive values of 75.5% and 95.6%. The positive and negative predictive values with specific regard to *NTRK* rearrangements were 75.5% and 99.0% (data not shown).

Molecular Features of ALK, ROS1, and NTRK Rearranged CRC

Molecular reports from next-generation sequencing DNA analyses performed on rearranged cases were retrieved (Figure 1). Additionally, molecularly annotated genomic variants from seven CRC samples harboring *ALK* or *NTRK3* fusions (Supplementary Figures 2 and 3, available online) in the TCGA database were gathered. First, we focused on the subset of genes previously reported as the most frequently mutated in CRCs (Figure 2A) (23). In line with previous reports regarding MSI-high BRAF mutated CRC (24–26), MSI-high rearranged tumors were enriched for alterations affecting *RNF43* (64.7% vs 5.9%, $P < .001$, Fisher's exact test), most of which were frameshift changes affecting glycine 659, which lies within a mononucleotide repeat (Figure 2A).

A low prevalence of *RAS/BRAF* mutations, also accounting for MSI-high status (Figure 2B), was reported. Only one MSS rearranged tumor displayed a BRAF V600E mutation, while two MSI-high rearranged mCRC samples carried *BRAF* alterations (I371M and K475R) of unknown biological significance and two MSS rearranged CRCs showed a well-established oncogenic variant (G469A) and an alteration (D594H) that impairs BRAF kinase activity but paradoxically activates MEK and ERK through transactivation of CRAF, respectively. The prevalence of *PIK3CA* mutations in CRCs carrying rearrangements did not show a statistically significant difference from what was reported in unselected colorectal tumors (12.1% vs 21.6%, respectively, $P = .27$) (24).

An explorative analysis of selected genes implicated in immune-escape mechanisms (27) was conducted by retrieving the transcriptomic profiles of the seven rearranged samples for which RNA seq data was available from the TCGA, and these were compared with nonrearranged MSI-high CRC samples (n = 92) also from TCGA (Figure 2C). Although the analysis suggested that the presence of rearrangements did not impact the typical MSI-high phenotype represented by the upregulation of immunoinhibitory molecules (27), the small number of samples limits the power of this observation.

Prognostic Impact of ALK, ROS1, and NTRK Rearrangements in mCRC

Finally, we explored the clinical impact of *ALK*, *ROS1*, and *NTRK* rearrangements in the metastatic setting (TCGA samples were excluded from survival analyses because they were mostly found in earlier disease stages and had incomplete follow-up data). When looking at OS results, at a median follow-up of

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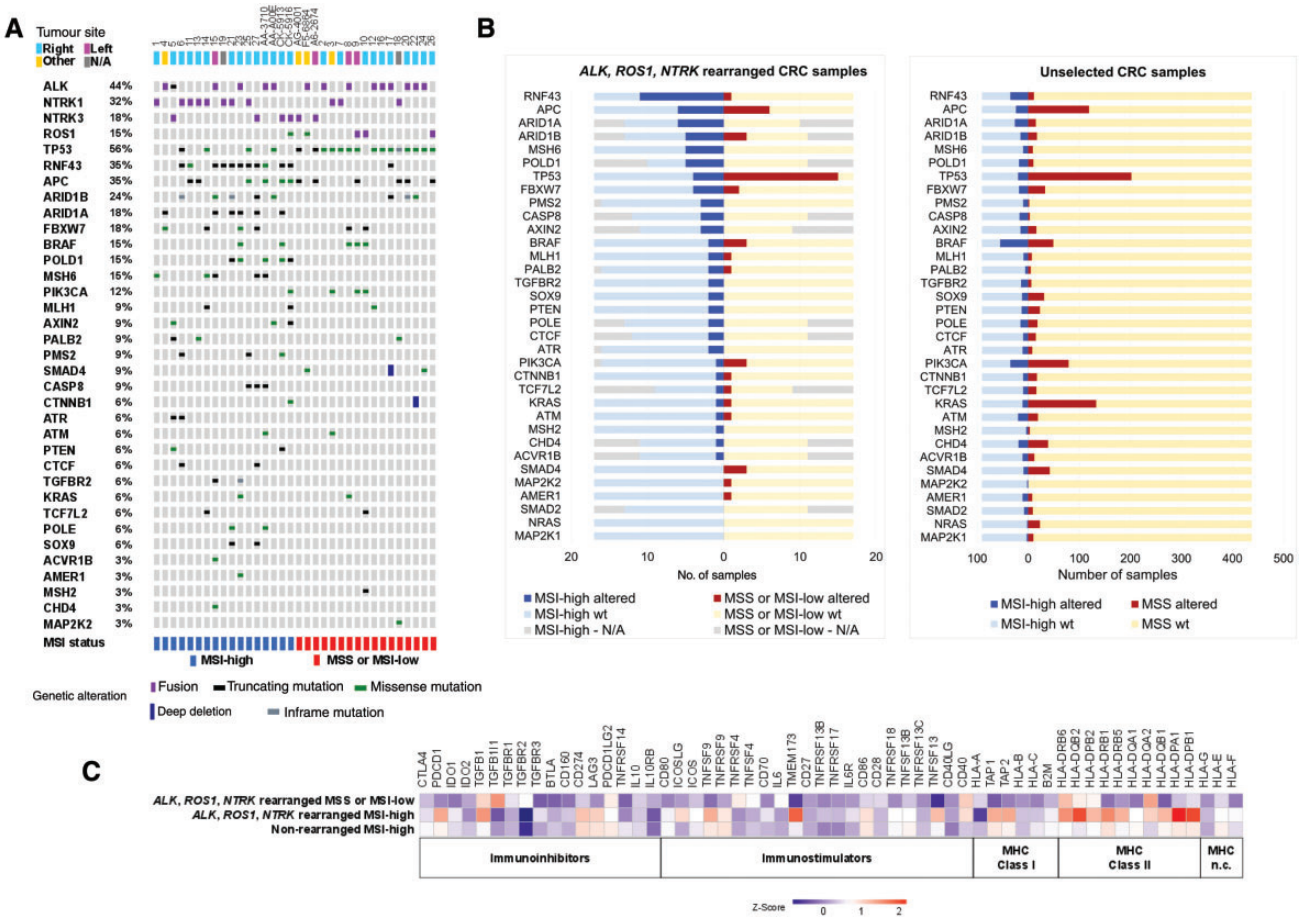

Figure 2. Molecular profile of *ALK, ROS1*, and *NTRK* rearranged colorectal cancer. **A)** OncoPrint map depicting alterations in the top mutated colorectal cancer genes in *ALK, ROS1*, and *NTRK* rearranged cancers (27 cases from this study and seven samples from The Cancer Genome Atlas [TCGA]) (23). Individual sample cases are designated by **columns** (**top**) and grouped by mismatch repair status, while individual genes are presented by **rows**. **B)** Gene mutation profiles, excluding silent mutations, were compared between *ALK, ROS1*, and *NTRK* rearranged cancers (27 cases from this study and seven samples from TCGA) and data previously reported in a large-scale sequencing study of unselected CRC (24). **Gray bars** indicate the number of samples that were not sequenced for the indicated genes. **C)** Expression (RNA sequencing data) of selected genes implicated in immunoevasion (gene list was obtained from [27]) in *ALK* or *NTRK3* rearranged tumors identified in TCGA, grouped based on their MMR status. The average expression of nonrearranged TCGA MSI-high colorectal cancer samples (n = 92) from TCGA is also shown. CRC = colorectal cancer; MHC = major histocompatibility complex; MSI = microsatellite instability; MSS = microsatellite-stable; wt = wild-type.

28.5 months (95% CI = 23.8 to 36.9), patients bearing *ALK, ROS1*, or *NTRK* rearranged tumors had poor prognosis when compared with rearrangement negative tumors (median OS = 15.6 months, 95% CI = 0.0 to 20.4 months, vs median OS = 33.7 months, 95% CI = 28.3 to 42.1 months; HR for death = 2.17, 95% CI = 1.03 to 4.57, $P < .001$) (Figure 3A). When applying the false discovery rate correction, the association of *ALK, ROS1*, and *NTRK* rearrangements with OS was still statistically significant ($P < .005$). In the multivariable model (Table 2) including other covariates associated with OS with a *P* value of less than .10 (age, primary tumor location, primary resection, *BRAF* mutation and MSI status), the presence of gene rearrangements was still associated with shorter OS (HR for death = 2.33, 95% CI = 1.10 to 4.95, $P = .02$). Notably, patients with *ALK, ROS1*, or *NTRK* rearranged tumors had short OS independent from MSI status (Figure 3B). In fact, the median OS was 17.0 months (95% CI = 10.0 to 31.4 months) for patients with MSS rearranged tumors and 15.6 months (95% CI = 10.0 to 20.4 months) for MSI-high ones. Moreover, the poor prognostic impact of gene rearrangements was independent of primary tumor location: both in right- and left-sided tumors, patients bearing rearrangements had shorter OS than those with negative tumors (Supplementary Figure 4, available online).

Therapeutic Implications of ALK, ROS1, and NTRK Rearrangements in mCRC

All the patients with rearranged tumors who were treated with cetuximab or panitumumab (n = 4) experienced disease progression as best response during the treatment with anti-EGFR agents (Supplementary Methods and Supplementary Figure 5, available online). One patient with *EML4-ALK* rearrangement and MSI-high tumor received single-agent anti-PD-1 treatment with nivolumab and achieved a durable response (Supplementary Figure 6, available online). Notably, the immunohistochemistry staining of this tumor revealed intense staining for CD4, CD8, CD68, and especially PDL-1, with an abundant intra- and extratumoral lymphocytic infiltration (Supplementary Figure 6, available online).

Discussion

Here we showed that *ALK, ROS1*, and *NTRK* rearrangements identify an uncommon CRC molecular subtype with specific clinical, pathological, and molecular features. The investigated fusions (and particularly those affecting *NTRK*) were more frequent in elderly females with right-sided tumors, spreading to extraregional

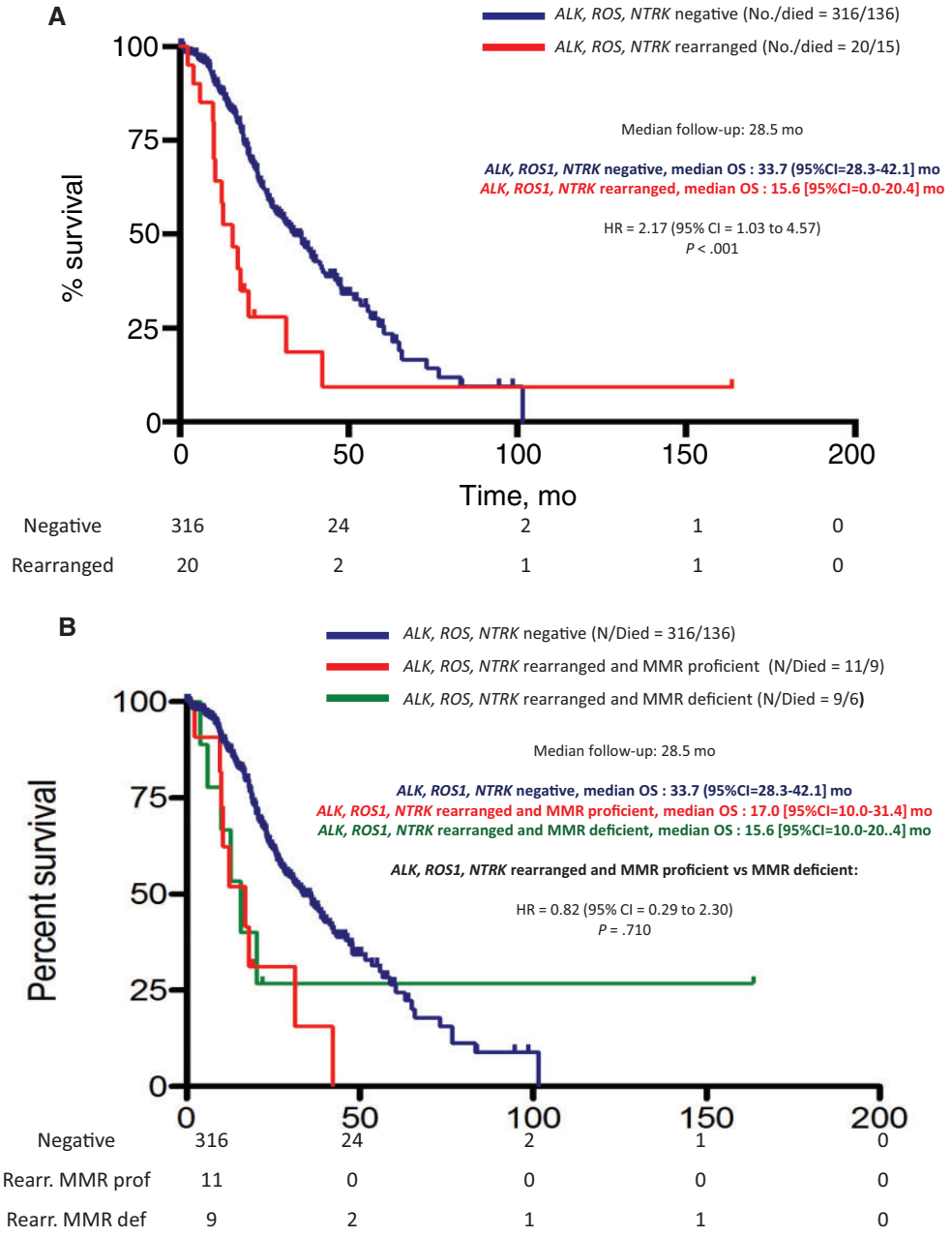

Figure 3. Survival in metastatic colorectal cancer patients carrying *ALK*, *ROS1*, and *NTRK* rearranged tumors. **A)** Kaplan-Meier curves for overall survival (OS) in patients with *ALK*, *ROS1*, and *NTRK* rearrangements as compared with those with *ALK*, *ROS1*, *NTRK* negative tumors. **B)** Kaplan-Meier curves for OS in patients with *ALK*, *ROS1*, and *NTRK* rearrangements and MMR proficient status or patients with *ALK*, *ROS1*, and *NTRK* rearrangements and MMR deficient status as compared with those with *ALK*, *ROS1*, and *NTRK* negative tumors. P values were based on log-rank test and were two-sided. CI = confidence interval; HR = hazard ratio; MMR = mismatch repair; MSI-high = microsatellite instability–high; MSS = microsatellite-stable.

lymph nodes. However, the most clinically relevant association was found with MSI-high and *RAS* wild-type status, which are two relevant and commonly used biomarkers for patient selection for immunotherapy and anti-EGFRs, respectively. These types of clinical and molecular associations resemble very closely what was observed for codon 600 *BRAF* mutations, and, interestingly, *BRAF* V600 mutations and gene fusions were almost invariably mutually exclusive. Because MSI-high status is reported in less than 5% of mCRCs (28), the frequency of MSI-high rearranged tumors is unexpectedly high (48.1%), even considering the right-sided location (29). The frequency of MSI-high status in *ALK*, *ROS1*, and *NTRK* rearranged tumors seems similar or even higher than in BRAF V600E mutated mCRCs, where it reaches 30% to 35% (24,28). While the association between right-sided tumors, MSI-high status, and *BRAF* mutations is well established, we report for the first time a strong association with right-sided tumor location and MSI-high status also for gene fusions. Of note, while frame-shift mutations occurring in MSI-high cancers are heterogeneously represented in tumor subclones (30), gene rearrangements appear as "founder" events because they are present in most, if not all, tumor cells. Nevertheless, because defective

Table 2. Association of *ALK*, *ROS1*, and *NTRK* rearrangements and known prognostic baseline characteristics with overall survival

Characteristics	No.	Univariate analyses			Multivariable model		
		HR	(95% CI)	P*	HR	(95% CI)	P†
ALK, *ROS1*, *NTRK* status							
Negative	316	1.00 (ref.)	–	–	1.00 (ref.)	–	–
Rearranged	20	2.17 (1.03 to 4.57)		<.001	2.33 (1.10 to 4.95)		.02
Age	336	1.04 (1.02 to 1.05)		<.001	1.04 (1.02 to 1.07)		<.001
ECOG performance status							
0	112	1.00 (ref.)	–	–	–	–	–
1–2	216	1.01 (0.72 to 1.42)		.95	–	–	–
Primary tumor site							
Left colon/rectum	221	1.00 (ref.)	–	–	1.00 (ref.)	–	–
Right colon	113	1.41 (1.01 to 2.00)		.04	1.11 (0.62 to 1.98)		.73
Mucinous histology							
No	290	1.00 (ref.)	–	–	–	–	–
Yes	41	0.97 (0.59 to 1.58)		.89	–	–	–
Primary resection							
Yes	257	1.00 (ref.)	–	–	1.00 (ref.)	–	–
No	82	1.51 (1.01 to 2.29)		.02	1.69 (0.94 to 3.05)		.08
Time to metastases							
Metachronous	113	1.00 (ref.)	–	–	–	–	–
Synchronous	220	1.24 (0.88 to 1.74)		.24	–	–	–
No. of metastatic sites							
1	171	1.00 (ref.)	–	–	–	–	–
>1	164	1.28 (0.93 to 1.77)		.13	–	–	–
RAS status							
Wild type	173	1.00 (ref.)	–	–	–	–	–
Mutated	147	1.31 (0.94 to 1.82)		.12	–	–	–
BRAF status							
Wild type	275	1.00 (ref.)	–	–	1.00 (ref.)	–	–
Mutated	17	2.20 (0.97 to 4.95)		.06	0.91 (0.35 to 2.38)		.86
MSI status							
MSS	156	1.00 (ref.)	–	–	1.00 (ref.)	–	–
MSI-high	22	2.28 (1.09 to 4.76)		.005	1.42 (0.63 to 3.21)		.40

*P values were based on log-rank test. CI = confidence interval; ECOG = Eastern Cooperative Oncology Group; HR = hazard ratio; MSI-high = microsatellite instability–high; MSS = microsatellite-stable.
†P values were based on Cox proportional hazard regression analysis.

mismatch repair is also an early event in CRC carcinogenesis, the adenoma-carcinoma sequence should be further elucidated for this rare subtype. Future studies exploring the role of food carcinogens and/or peculiar microbiota components in the right colon are also warranted to clarify the potential link between MSI status and kinase rearrangements.

When compared with negative samples, *ALK*, *ROS1*, and *NTRK* rearranged tumors show a low frequency of *RAS* and *BRAF* oncogenic mutations. A low prevalence of BRAF V600E mutation was reported in the group of negative tumors (5.8%), probably as a consequence of the poor prognosis and rapid progression of *BRAF* mutant tumors, preventing these patients from receiving later lines of therapy and therefore being screened for phase I trials. Therefore, we were unable to identify a statistically significant difference in terms of *BRAF* mutations between rearranged and not rearranged tumors ($P = 1.00$) in the present series. However, the observation that *ALK*, *ROS1*, and *NTRK* rearrangements co-occur rarely with other common driver events in the RTK-RAS pathway, and specifically *RAS* and *BRAF* codon 600 mutations, supports the hypothesis that gene fusions drive oncogene addiction. Indeed, previous reports indicate that *NTRK1* and *ALK* rearranged CRC preclinical models and patients respond to pharmacological blockade of the fusion kinase (6,11,15,19,20). In spite of the relatively low prevalence of gene fusions, the identification of patients with tumors bearing these alterations may be simplified and enriched by the evaluation of four simple and easy-to-collect variables (ie, primary tumor location, *RAS*, *BRAF* and MSI-high status), which are available for the vast majority of patients. Therefore, in an evidence-based perspective of resource sparing, the molecular screening for gene rearrangements should not be denied to patients with *RAS* and *BRAF* wild-type and/or MSI-high mCRC.

A high prevalence of RNF43 frameshift mutations was reported among *ALK*, *ROS1*, and *NTRK* rearranged tumors, though in the absence of concomitant BRAF V600E mutations, thus suggesting that gene rearrangements may act as driver events alternative to *BRAF* in the tumorigenesis of MSI-high right-sided tumors carrying *RNF43* alterations. Because porcupine inhibitors are being developed to suppress paracrine WNT-driven growth of *RNF43* mutant tumors (https://clinicaltrials.gov/ct2/show/NCT02278133), our findings may provide a rationale for cotargeting tyrosine kinase oncogenic fusions as well as the WNT pathway in this rare tumor subset.

Closely recalling the long "*BRAF* history," we found that gene fusions occurring in mCRCs are associated with unfavorable outcome. However, it must be pointed out that patients with MSI-high mCRCs have worse OS independent of the co-occurrence of the *BRAF* V600E mutation (28). Therefore, given

the association of *ALK, ROS1*, or *NTRK* rearrangements with MSI-high status and the mutual exclusivity with codon 600 *BRAF* mutations, our findings may partly explain the aggressive behavior of MSI-high *BRAF* wild-type mCRCs. The same observations are true for the potential contribution of gene fusion to the poor prognosis of some right-sided mCRCs (31).

Again, consistent with previous findings regarding *BRAF* V600E mutations (32), *ALK*, *ROS*, and *NTRK* rearranged tumors seem not to derive benefit from anti-EGFR monoclonal antibodies, thus confirming preclinical observations (19). Given the very low frequency of gene fusions in mCRC, the validation of this finding is quite unrealistic. However, these results are supported by a strong biological rationale and may contribute to explaining—at least in part—the limited activity of anti-EGFRs in right-sided, *RAS*, and *BRAF* wild-type tumors (33). From a clinical perspective, it seems therefore reasonable to offer an intensive firstline regimen, such as the triplet FOLFOXIRI plus bevacizumab, to patients with right-sided, *ALK, ROS1*, and *NTRK* rearranged mCRCs (34), based on their aggressive behavior and in line with current recommendations for *BRAF* V600E mutant tumors.

Our observations argue that the early enrollment of patients with tumors bearing *ALK, ROS1*, and *NTRK* rearrangements in clinical trials with matched targeted agents should be highly encouraged because this subset of patients may in fact be uniquely poised to benefit from targeted strategies. Nevertheless, benefit from targeted strategies against ALK, ROS1, and TrkA-B-C may be transient, and mechanisms of acquired resistance may occur early (17,20). This is quite reasonable, particularly when considering the impressive mutational burden of MSI-high tumors that may promote in these tumors the early emergence of acquired resistance.

The combination of targeted agents and immunotherapy approaches in MSI-high rearranged tumors may be a promising strategy to be further investigated, supported by a strong molecular rationale and by the absence of impact of rearrangements on MSI-high associated immunophenotype. The major limitation of this study is the choice of the control group. Although a wider series of negative cases, especially those analyzed by MSK-IMPACT or FoundationOne tests, would have been more appropriate, both MSK-IMPACT and FoundationOne are DNA-based assays and do not completely cover intronic regions, thus making it possible to miss some gene fusions. Moreover, clinical data were not available for the vast majority of these patients. Therefore, a cohort of well-annotated patients screened at three institutions for a phase I trial and quite representative of the general population of mCRC patients was adopted as control group.

In conclusion, the features of *ALK*, *ROS1*, and *NTRK* rearrangements are somewhat reminiscent of the peculiar traits previously recognized in *BRAF* V600E mutant mCRC. These fusions define a new molecular subtype of mCRC associated with poor prognosis, whose recognition allows a proper tailored management for a new subgroup of patients. The large-scale diffusion of this assessment may be eased by the availability of a multistep procedure for the detection of gene fusions, starting from a simple immunohistochemistry test with high sensitivity or a comprehensive approach able to identify *ALK*, *ROS1*, and *NTRK* rearrangements, as well as other potentially targetable kinase fusions (22). Finally, while the poor prognosis of rearranged tumors may suggest the adoption of upfront intensive treatments when feasible, new targeted strategies are under investigation and the high prevalence of MSI-high status in rearranged tumors opens the way to evaluate new combination approaches, including targeted (ALK, ROS1, TrkA-B-C) and immunotherapy agents.

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