Intratumoral *Fusobacterium* as prognostic factor in early stage colorectal cancer: results of the FUSOMAP study.

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BACKGROUND

The prevalence, geographical distribution, and prognostic significance of intratumoral *Fusobacterium* (iFUSO) infection in early stage colorectal cancer (CRC) is largely unknown. Here, we present the first results of a large national retrospective-prospective multicenter study (FUSOMAP) which aims to enhance our understanding on the pathophysiology of the CRC microbiome for the development of microbiota-based diagnostic and prognostic methods.

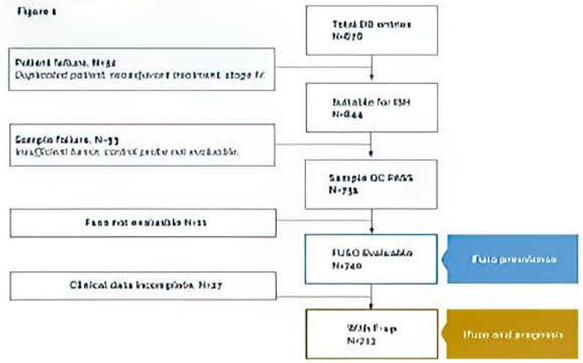
METHODS

Patients from different geographic and socioeconomic populations diagnosed at 9 hospitals across Spain as part of regional CRC screening programs were included in the present retrospective analysis. Formalin-fixed paraffin-embedded primary tumor tissue samples were retrieved for iFUSO quantification and visualization using an in-house developed RNA in situ hybridization assay. Positivity was defined as tumors with density values ≥4 bacteria/mm2. Cox proportional hazards model of disease-free survival (DFS) was used to compute hazard ratio (HR) controlling for potential confounders including gender, age, site, stage, grade, vascular invasion and adjuvant treatment.

RESULTS

1. Fusomap study patient cohort

Figure 1



2. Intratumoral *Fusobacterium* (iFuso) prevalence

iFUSO was found in 21% of 740 evaluable CRC patients. The bacterium predominantly accumulates in the luminal surface in correspondence of the tumor (Figure 2A). The absolute amount of iFUSO varied significantly according to geography of primary tumor origin even after adjusting for confounding covariates (Figure 2 B).

Figure 2A

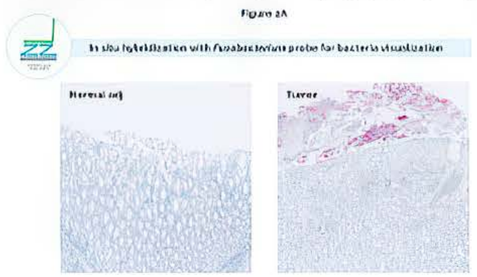
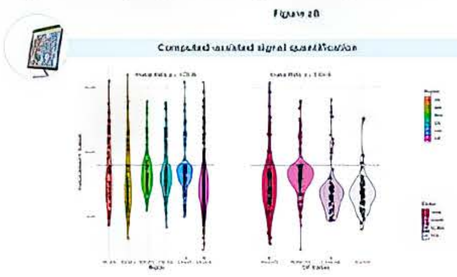


Figure 2B



When classifying tumors according to iFuso status, positivity rates ranged from 15.3% to 30.7% (from 5.9 to 22.3% within Catalunya, Figure 3).

Figure 3

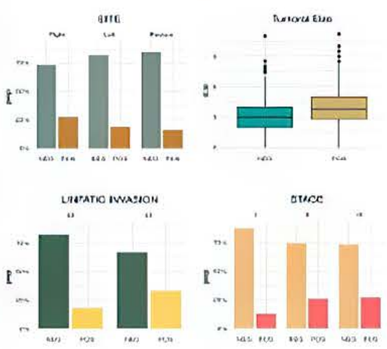
3. Association between Intratumoral *Fusobacterium* (iFuso) and clinicopathological characteristics

iFUSO positivity increased with tumor stage (Stage I 13%; II 26%; III 27%), tumor size, presence of vascular invasion, and decreased from right colon (27%) to rectum (16%) location (Table 1, Figure 4).

Table 1

		iFUSO		
		Negative	Positive	p-val
Age		70 (63-76)	69 (62-75)	0.333
Sex	Male	342 (79.4%)	89 (20.6%)	0.887
	Female	84 (79.2%)	22 (20.8%)	
Side	Right	236 (73.1%)	87 (26.9%)	0.007
	Left	157 (83.5%)	31 (16.5%)	
	Rectum	128 (83.7%)	25 (16.3%)	
Histology	ADK	402 (80.4%)	98 (19.6%)	0.117
	ADK + mucin	20 (76.9%)	6 (23.1%)	
	ADK + other	19 (95.0%)	1 (5.0%)	
Histo Grade	G1	59 (91.5%)	14 (18.5%)	0.103
	G2	328 (79.0%)	78 (21.0%)	
	G3	39 (76.5%)	18 (23.5%)	
Size		4.1 (3.1-5.3)	4.5 (3.7-5.8)	<0.001
Venous Inv	V0	443 (80.8%)	105 (19.2%)	0.001
	V1	78 (70.9%)	32 (29.1%)	
Lymph Inv	L0	479 (82.2%)	104 (17.8%)	<0.001
	L1	47 (59.5%)	32 (40.5%)	
Perineural Inv	Pn0	464 (80.3%)	114 (19.7%)	0.133
	Pn1	65 (74.7%)	22 (25.3%)	
WHO	Reg	393 (82.1%)	86 (17.9%)	0.003
	Pos	120 (73.6%)	43 (26.4%)	
	Cachexia	16 (53.3%)	14 (46.7%)	0.003
Stage	I	253 (87.2%)	37 (12.8%)	
	II	144 (74.2%)	50 (25.8%)	<0.001
	III	74 (72.5%)	28 (27.5%)	
Relapse	No	312 (81.3%)	72 (18.8%)	0.001
	Yes	69 (64.5%)	38 (35.5%)	

Figure 4



4. Association between Intratumoral *Fusobacterium* (iFuso) and disease-free survival in stage I-III CRC

With a median follow-up of 3.70 years, patients with iFUSO-positive tumors had a significantly worse DFS compared to iFUSO-negative tumors (HR=2.03, 95% CI=1.34-3.08, p=0.0071) (Figure 5).

In multivariable analysis, iFuso remained significantly independently associated with DFS (p=0.04) when adjusted for age, sex, stage, grade, site, adjuvant chemotherapy and venous invasion. 3-years and 5-years DFS HRs (95% CI) for iFUSO-positive vs negative tumors were 1.58 (1.00-2.50) and 1.58 (1.02-2.4), respectively (Figure 6).

Figure 5

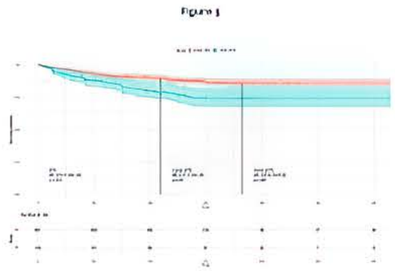
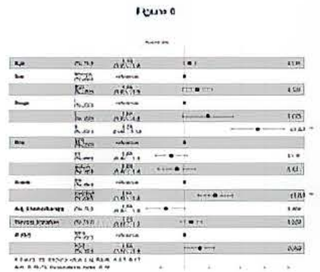


Figure 6



5. Association between Intratumoral *Fusobacterium* (iFuso) and disease-free survival in patients with stages II-III CRC treated with adjuvant 5-FU-based chemotherapy.

For chemo-treated patients (39.4 % of the cohort), a statistically significant interaction was observed for the predictive value of iFUSO in term of DFS. iFUSO positivity significantly predicted worse outcome after standard-of-care 5-FU chemotherapy (HR=1.85, CI=1.11-3.07, p-value=0.018, Figure 7A). Conversely, no significant difference in DFS were observed according to iFuso status in untreated patients (Figure 7B).

Figure 7A

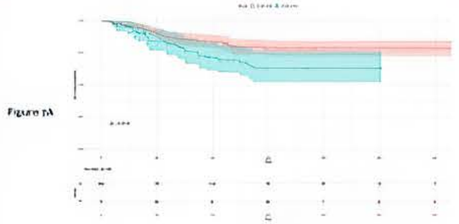
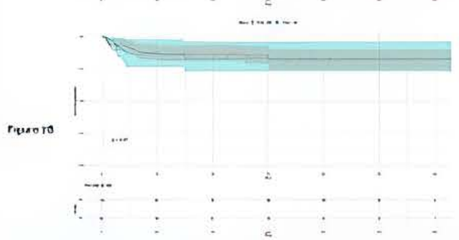
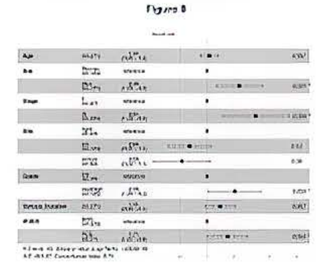


Figure 7B



In stratified Cox multivariable analysis for DFS, iFuso association to outcome after adjuvant treatment was independent of the patient's age, sidedness, gender, stage, tumor grade and venous invasion (Figure 8).

Figure 8



CONCLUSIONS

1. iFUSO was found in 21% of stages I-III CRC patients.
2. The absolute amount of iFUSO varied significantly according to geography of primary tumor origin even after adjusting for confounding covariates.
3. iFUSO positivity increased with tumor stage, tumor size, presence of vascular invasion, and decreased from right colon to rectum location.
4. iFUSO was an independent negative prognostic factor in the overall population (stages I-III) and in stages II-III patients treated with 5-FU adjuvant chemotherapy.

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