

Suppl. Table 1: Colorectal Cancer Panel

The following genes were analyzed for base substitutions, indels (small insertions and deletions) and gene copy number variations (amplifications, deletions) using next generation sequencing:

ACVR1B, AKT1, APC, ATM, ATP6V0D2, AXIN2, BAX, BLM, BMPR1A, BRAF, BRCA1, BRCA2, BUB1B, CASP8, CDC27, CDH1, CDK4, CDKN2A, CHEK2, CTNNA1, CTNNB1, DCC, DMD, EGFR, ENG, EP300, EPCAM, ERBB2, FBXW7, FGFR3, FLCN, FZD3, GALNT12, GPC6, GREM1, KIT, KRAS, MAP2K4, MAP7, MET, MIER3, MLH1, MLH3, MSH2, MSH3, MSH6, MUTYH, MYO1B, NRAS, PALB2, PIK3CA, PIK3R1, PMS1, PMS2, POLD1, POLE, PTEN, PTPN12, RET, RPS20, SCG5, SLC9A9, SMAD2, SMAD4, SRC, STK11, TCERG1, TCF7L2, TGFBR2, TP53, WBSCR17

Suppl. Table 2: Clinical and histopathological baseline characteristics of molecular subgroups in the overall population

BMI: Body Mass Index; BRAF MT: mutated for B-Raf proto-oncogene, serine/threonine kinase; dMMR: deficient mismatch repair; KRAS MT: mutated for Kirsten Rat Sarcoma; MSI-H: microsatellite instability; MSS microsatellite stability; NRAS MT: mutated for Neuroblastoma ras; pMMR: proficient mismatch repair protein; SD: Standard deviation; WT: Wild Type. In this table, 3 patients with missing MSI Status have been included.

Characteristic		AB-WT ₀ (N=1843)		KRAS MT ₀ (N=1946)	NRAS MT ₀ (N=146)	BRAF MT ₀ (N=951)	p-Value
Age (in years)							<0.001
Medium		71		71	70.5	75	
Mean (SD)		69.2 (12.1)		69.4 (11.8)	68 (12.6)	73.7 (10.3)	
Range		25 - 95		27 - 96	26- 92	29 - 100	
Age (categories)							<0.001
< 50		127 (6.9%)		103 (5.3%)	11 (7.5%)	16 (1.7%)	
50 -70		704 (38.2%)		785 (40.3%)	57 (39.0%)	270 (28.4%)	
≥ 70		1012 (54.9%)		1058 (54.4%)	78 (53.4%)	665 (69.9%)	
Sex							<0.001
Female		724 (39.4%)		870 (44.8%)	66 (45.2%)	642 (67.6%)	
Male		1112 (60.6%)		1072 (55.2%)	80 (54.8%)	308 (32.4%)	
Missing		7		4	0	1	
CCI							0.53
< 5		1819 (98.7%)		1924 (98.9%)	146 (100.0%)	938 (98.6%)	
≥ 5		24 (1.3%)		22 (1.1%)	0 (0.0%)	13 (1.4%)	
BMI							0.14
Mean (SD)		26.99 (5.2)		26.7 (5.0)	26.5 (4.8)	26.6 (5.1)	
Range		12.7 - 57.5		14.5 - 54.6	15.9 - 42.2	13.8 - 52.5	
Missing		140		169	8	92	
BMI (categories)							0.50
< 18.5		47 (2.8%)		45 (2.5%)	5 (3.6%)	24 (2.8%)	
18.5 - 25		602 (35.3%)		689 (38.8%)	47 (34.1%)	321 (37.4%)	
≥ 25		1054 (61.9%)		1043 (58.7%)	86 (62.3%)	514 (59.8%)	
Missing		140		169	8	92	
T-Stage							0.45
T 1/2		284 (15.4%)		285 (14.6%)	16 (11.0%)	149 (15.7%)	
T 3/4		1559 (84.6%)		1661 (85.4%)	130 (89.0%)	802 (84.3%)	
N-Stage							0.13
N0		1121 (61.1%)		1128 (58.2%)	82 (56.2%)	539 (57.1%)	
N+		714 (38.9%)		810 (41.8%)	64 (43.8%)	405 (42.9%)	
UICC							0.059
I		202 (11.0%)		182 (9.4%)	7 (4.8%)	100 (10.5%)	
II		918 (49.8%)		951 (48.9%)	76 (52.1%)	438 (46.1%)	
III		722 (39.2%)		813 (41.8%)	63 (43.2%)	413 (43.4%)	
Differential Grade							<0.001
G 1/2		1486 (80.6%)		1603 (82.4%)	122 (83.6%)	535 (56.3%)	
G 3/4		357 (19.4%)		343 (17.6%)	24 (16.4%)	416 (43.7%)	
Histological Subtype							<0.001
Adenocarcinoma		1721 (95.5%)		1806 (94.7%)	138 (95.8%)	824 (87.8%)	
Mucinous adenocarcinoma		44 (2.4%)		77 (4.0%)	6 (4.2%)	80 (8.5%)	
Signet-ring cell carcinoma		7 (0.4%)		0 (0.0%)	0 (0.0%)	4 (0.4%)	
Others		30 (1.7%)		24 (1.3%)	0 (0.0%)	30 (3.2%)	
Missing		41		39	2	13	
Chemotherapy							0.001
Yes		649 (39.5%)		723 (41.8%)	53 (43.1%)	288 (33.9%)	
No		996 (60.5%)		1005 (58.2%)	70 (56.9%)	561 (66.1%)	
Missing		198		218	23	102	
Localization							<0.001
Cecum		150 (8.2%)		411 (21.5%)	22 (15.2%)	206 (21.8%)	
Ascending colon		335 (18.4%)		504 (26.3%)	34 (23.4%)	401 (42.4%)	
Hepatic flexure		114 (6.3%)		110 (5.7%)	5 (3.4%)	100 (10.6%)	
Transverse colon		194 (10.7%)		154 (8.0%)	11 (7.6%)	106 (11.2%)	
Splenic flexure		70 (3.8%)		86 (4.5%)	7 (4.8%)	26 (2.8%)	
Descending colon		138 (7.6%)		97 (5.1%)	11 (7.6%)	35 (3.7%)	
Sigmoid colon		761 (41.8%)		519 (27.1%)	50 (34.5%)	68 (7.2%)	
Rectosigmoid junction		58 (3.2%)		33 (1.7%)	5 (3.4%)	3 (0.3%)	
Missing		23		32	1	6	
Sidedness							<0.001
Right		793 (43.6%)		1179 (61.6%)	72 (49.7%)	813 (86.0%)	
Left		1027 (56.4%)		735 (38.4%)	73 (50.3%)	132 (14.0%)	
Missing		23		32	1	6	
MSI Status							<0.001
MSS-pMMR		1627 (88.3%)		1787 (91.9%)	139 (95.2%)	312 (32.8%)	
MSI-H-dMMR		215 (11.7%)		157 (8.1%)	7 (4.8%)	639 (67.2%)	
Missing		1		2	0	0	

Suppl. Table 3: Clinical and histopathological baseline characteristics of distinct KRAS mutations the MSS and MSI- H cohort

BMI: Body Mass Index; dMMR: deficient mismatch repair; KRAS MT: mutated for Kirsten Rat Sarcoma; MSI-H: microsatellite instability; MSS microsatellite stability; pMMR: proficient mismatch repair protein; SD: Standard deviation; WT: Wild Type

Characteristic	KRAS MT MSS/pMMR							p-Value	KRAS MT MSI/dMMR							p-Value
	p.G12D (N=533)	p.G13D (N=268)	p.G12V (N=373)	p.G12A (N=103)	p.A146T (N=90)	p.G12C (N=106)	Others (N=278)		p.G12D (N=34)	p.G13D (N=44)	p.G12V (N=10)	p.G12A (N=6)	p.A146T (N=9)	p.G12C (N=3)	other (N=46)	
Age (in years)								0.74								0.30
Median	71	70	72	71	72	71.5	72		61.5	65.6	68	69.5	74	77	71.5	
Mean (SD)	69.4 (11.5)	69 (11.6)	70.3 (11.6)	70.4 (11.8)	68.9 (12.9)	70 (9.8)	70 (11.3)		62 (15.1)	64.4 (14.3)	68.5 (13.0)	67.7 (10.5)	69 (15.2)	78.3 (7.1)	68.3 (13.8)	
Range	31 - 93	35 - 93	30 - 96	33 - 96	34 - 92	42 - 87	37 - 96		29 - 85	27 - 90	49 - 94	51 - 78	43 - 90	72 - 86	29 - 87	
Age (categories)								0.29								0.58
< 50	26 (4.9%)	12 (4.5%)	18 (4.8%)	2 (1.9%)	9 (10.0%)	1 (0.9%)	12 (4.3%)		7 (20.6%)	7 (15.9%)	1 (10.0%)	0 (0.0%)	1 (11.1%)	0 (0.0%)	5 (10.9%)	
50-70	220 (41.3%)	115 (42.9%)	138 (37.0%)	42 (40.8%)	32 (35.6%)	44 (41.5%)	114 (41.0%)		12 (35.3%)	20 (45.5%)	5 (50.0%)	3 (50.0%)	2 (22.2%)	0 (0.0%)	15 (32.6%)	
≥ 70	287 (53.8%)	141 (52.6%)	217 (58.2%)	59 (57.3%)	49 (54.4%)	61 (57.5%)	152 (54.7%)		15 (44.1%)	17 (38.6%)	4 (40.0%)	3 (50.0%)	6 (66.7%)	3 (100.0%)	26 (56.5%)	
Sex								0.94								0.048
Female	247 (46.3%)	119 (44.4%)	158 (42.7%)	44 (42.7%)	39 (43.3%)	49 (46.7%)	121 (43.5%)		11 (32.4%)	18 (40.9%)	5 (50.0%)	1 (16.7%)	5 (55.6%)	2 (66.7%)	30 (65.2%)	
Male	286 (53.7%)	149 (55.6%)	212 (57.3%)	59 (57.3%)	51 (56.7%)	56 (53.3%)	157 (56.5%)		23 (67.6%)	26 (59.1%)	5 (50.0%)	5 (83.3%)	4 (44.4%)	1 (33.3%)	16 (34.8%)	
Missing	0	0	3	0	0	1	0									
CCI								0.47								0.87
< 5	526 (98.7%)	267 (99.6%)	367 (98.4%)	102 (99.0%)	90 (100.0%)	106 (100.0%)	273 (98.2%)		34 (100.0%)	43 (97.7%)	10 (100.0%)	6 (100.0%)	9 (100.0%)	3 (100.0%)	46 (100.0%)	
≥ 5	7 (1.3%)	1 (0.4%)	6 (1.6%)	1 (1.0%)	0 (0.0%)	0 (0.0%)	5 (1.8%)		0 (0.0%)	1 (2.3%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	
BMI								0.70								0.51
Mean (SD)	26.674 (5.040)	27 (5.7)	26.5 (4.8)	26.5 (4.2)	25.9 (4.2)	26.6 (5.0)	26.7 (5.0)		25.1 (4.3)	27 (3.9)	25.6 (3.7)	24.5 (4.4)	26.9 (2.5)	27.7 (3.3)	26.9 (5.4)	
Range	15.060 - 53.830	14.5 - 54.2	16.5 - 45.3	15.8 - 35.5	17.7 - 43.5	16.2 - 40.7	15.4 - 47.6		16.7 - 36.6	19.6 - 35.7	20.6 - 30.9	19.5 - 30.4	24.1 - 31.0	24.2 - 30.8	19.8 - 45.2	
Missing	42	24	24	11	6	10	31		4	6	0	1	2	0	6	
BMI (categories)								0.92								0.36
< 18.5	12 (2.4%)	4 (1.6%)	11 (3.2%)	1 (1.1%)	2 (2.4%)	4 (4.2%)	5 (2.0%)		3 (10.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	
18.5 - 25	193 (39.3%)	96 (39.3%)	131 (37.5%)	38 (41.3%)	39 (46.4%)	37 (38.5%)	95 (38.5%)		13 (43.3%)	11 (28.9%)	4 (40.0%)	2 (40.0%)	3 (42.9%)	1 (33.3%)	14 (35.0%)	
≥ 25	286 (58.2%)	144 (59.0%)	207 (59.3%)	53 (57.6%)	43 (51.2%)	55 (57.3%)	147 (59.5%)		14 (46.7%)	27 (71.1%)	6 (60.0%)	3 (60.0%)	4 (57.1%)	2 (66.7%)	26 (65.0%)	
Missing	42	24	24	11	6	10	31		4	6	0	1	2	0	6	
T-Stage								0.26								0.19
T 1/2	76 (14.3%)	50 (18.7%)	49 (13.1%)	11 (10.7%)	14 (15.6%)	20 (18.9%)	36 (12.9%)		2 (5.9%)	4 (9.1%)	3 (30.0%)	0 (0.0%)	0 (0.0%)	1 (33.3%)	5 (10.9%)	
T 3/4	457 (85.7%)	218 (81.3%)	324 (86.9%)	92 (89.3%)	76 (84.4%)	86 (81.1%)	242 (87.1%)		32 (94.1%)	40 (90.9%)	7 (70.0%)	6 (100.0%)	9 (100.0%)	2 (66.7%)	41 (89.1%)	
N-Stage								0.34								0.87
N0	311 (58.3%)	140 (52.8%)	209 (56.3%)	54 (52.9%)	52 (58.4%)	53 (50.0%)	169 (60.8%)		25 (73.5%)	34 (77.3%)	7 (70.0%)	6 (100.0%)	7 (77.8%)	2 (66.7%)	33 (73.3%)	
N+	222 (41.7%)	125 (47.2%)	162 (43.7%)	48 (47.1%)	37 (41.6%)	53 (50.0%)	109 (39.2%)		9 (26.5%)	10 (22.7%)	3 (30.0%)	0 (0.0%)	2 (22.2%)	1 (33.3%)	12 (26.7%)	
UICC								0.063								0.46
I	52 (9.8%)	32 (11.9%)	35 (9.4%)	4 (3.9%)	12 (13.3%)	12 (11.3%)	18 (6.5%)		2 (5.9%)	3 (6.8%)	2 (20.0%)	0 (0.0%)	0 (0.0%)	1 (33.3%)	2 (4.3%)	
II	259 (48.6%)	113 (42.2%)	172 (46.1%)	48 (46.6%)	42 (46.7%)	41 (38.7%)	151 (54.3%)		22 (67.6%)	32 (72.7%)	5 (50.0%)	6 (100.0%)	7 (77.8%)	1 (33.3%)	31 (67.4%)	
III	222 (41.7%)	123 (45.9%)	166 (44.5%)	51 (49.5%)	36 (40.0%)	53 (50.0%)	109 (39.2%)		9 (26.5%)	9 (20.5%)	3 (30.0%)	0 (0.0%)	2 (22.2%)	1 (33.3%)	13 (28.3%)	
Differential Grade								0.79								0.74
G 1/2	441 (82.7%)	229 (85.4%)	315 (84.5%)	84 (81.6%)	72 (80.0%)	86 (81.1%)	236 (84.9%)		23 (67.6%)	30 (68.2%)	8 (80.0%)	5 (83.3%)	5 (55.6%)	3 (100.0%)	30 (65.2%)	
G 3/4	92 (17.3%)	39 (14.6%)	58 (15.5%)	19 (18.4%)	18 (20.0%)	20 (18.9%)	42 (15.1%)		11 (32.4%)	14 (31.8%)	2 (20.0%)	1 (16.7%)	4 (44.4%)	0 (0.0%)	16 (34.8%)	
Histological Subtype								0.12								0.96
Adenocarcinoma	485 (92.9%)	251 (95.1%)	346 (95.8%)	98 (98.0%)	85 (94.4%)	101 (97.1%)	264 (96.0%)		31 (91.2%)	40 (90.9%)	9 (100.0%)	5 (83.3%)	9 (100.0%)	3 (100.0%)	38 (88.4%)	
Mucinous adenocarcinoma	28 (5.4%)	6 (2.3%)	12 (3.3%)	2 (2.0%)	5 (5.6%)	3 (2.9%)	9 (3.3%)		2 (5.9%)	4 (9.1%)	0 (0.0%)	1 (16.7%)	0 (0.0%)	0 (0.0%)	4 (9.3%)	
Others	9 (1.7%)	7 (2.7%)	3 (0.8%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	2 (0.7%)		1 (2.9%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (2.3%)	
Missing	11	4	12	3	0	2	3		0	0	1	0	0	0	3	
Chemotherapy								0.46								0.60
Yes	190 (40.7%)	116 (48.3%)	146 (43.8%)	43 (46.7%)	32 (40.0%)	39 (41.5%)	101 (40.1%)		9 (30.0%)	11 (30.6%)	0 (0.0%)	1 (16.7%)	2 (25.0%)	1 (33.3%)	11 (28.2%)	
No	277 (59.3%)	124 (51.7%)	187 (56.2%)	49 (53.3%)	48 (60.0%)	55 (58.5%)	151 (59.9%)		21 (70.0%)	25 (69.4%)	10 (100.0%)	5 (83.3%)	6 (75.0%)	2 (66.7%)	28 (71.8%)	
Missing	66	28	40	11	10	12	26		4	8	0	0	1	0	7	
Localisation								<0.001								0.18
Cecum	122 (23.2%)	69 (26.2%)	69 (18.9%)	17 (16.7%)	22 (24.7%)	19 (18.3%)	53 (19.4%)		4 (12.5%)	10 (22.7%)	2 (22.2%)	2 (33.3%)	2 (22.2%)	1 (33.3%)	9 (19.6%)	
Ascending colon	156 (29.7%)	59 (22.4%)	77 (21.1%)	20 (19.6%)	29 (32.6%)	27 (26.0%)	76 (27.8%)		6 (18.8%)	12 (27.3%)	3 (33.3%)	1 (16.7%)	2 (22.2%)	2 (66.7%)	22 (47.8%)	
Hepatic flexure	13 (2.5%)	18 (6.8%)	17 (4.7%)	14 (13.7%)	4 (4.5%)	12 (11.5%)	18 (6.6%)		1 (3.1%)	1 (2.3%)	1 (11.1%)	0 (0.0%)	2 (22.2%)	0 (0.0%)	5 (10.9%)	
Transverse colon	50 (9.5%)	18 (6.8%)	24 (6.6%)	10 (9.8%)	10 (11.2%)	9 (8.7%)	15 (5.5%)		5 (15.6%)	4 (9.1%)	0 (0.0%)	1 (16.7%)	1 (22.2%)	0 (0.0%)	3 (6.5%)	
Splenic flexure	19 (3.6%)	10 (3.8%)	20 (5.5%)	3 (2.9%)	7 (7.9%)	4 (3.8%)	12 (4.4%)		4 (12.5%)	4 (9.1%)	0 (0.0%)	1 (16.7%)	0 (0.0%)	0 (0.0%)	1 (2.2%)	
Descending colon	28 (5.3%)	11 (4.2%)	23 (6.3%)	6 (5.9%)	1 (1.1%)	3 (2.9%)	12 (4.4%)		7 (21.9%)	3 (6.8%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (2.2%)	
Sigmoid colon	132 (25.1%)	74 (28.1%)	129 (35.3%)	28 (27.5%)	16 (18.0%)	26 (25.0%)	79 (28.9%)		5 (15.6%)	10 (22.7%)	3 (33.3%)	1 (16.7%)	1 (11.1%)	0 (0.0%)	5 (10.9%)	
Rectosigmoid junction	6 (1.1%)	4 (1.5%)	6 (1.6%)	4 (3.9%)	0 (0.0%)	4 (3.8%)	8 (2.9%)		0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	
Missing	7	5	8	1	1	2	5		2	0	1	0	0	0	0	
Sideliness								<0.001								0.020
Right	341 (64.8%)	164 (62.4%)	187 (51.2%)	61 (59.8%)	65 (73.0%)	67 (64.4%)	162 (59.3%)		16 (50.0%)	27 (61.4%)	6 (66.7%)	4 (66.7%)	8 (88.9%)	3 (100.0%)	39 (84.8%)	
Left	185 (35.2%)	99 (37.6%)	178 (48.8%)	41 (40.2%)	24 (27.0%)	37 (35.6%)	111 (40.7%)		16 (50.0%)	17 (38.6%)	3 (33.3%)	2 (33.3%)	1 (11.1%)	0 (0.0%)	7 (15.2%)	
Missing	7	5	8	1	1	2	5		2	0	1	0	0	0	0	

Suppl. Table 4: Mutations of MMR Genes in the MSI cohort in relation to BRAF V600 MT

WT: Wild Type; MT: mutated

Gene	Level	BRAF V600 WT			BRAF V600 MT		
		N	%	Σ%	N	%	Σ%
MLH1	pathogenic	59	22.6	22.6	30	8.3	8.3
	WT	163	73.4	100	332	91.7	100
MSH2	pathogenic	42	18.9	18.9	23	6.3	6.3
	WT	180	81.1	100	339	93.7	100
PMS2	pathogenic	35	15.8	15.8	35	9.7	9.7
	WT	187	84.2	100	327	90.3	100
MSH6	pathogenic	48	21.6	21.6	64	17.7	17.7
	WT	174	78.4	100	298	82.3	100

Suppl. Table 5 : Mutations of POLE and POLD1 in relation to KRAS and BRAF status

WT: Wild Type; MT: mutated; MSI: microsatellite instability; MSS: microsatellite stable; KRAS MT: mutated for Kirsten Rat
POLE: DNA Polymerase Epsilon; POLD1: DNA polymerase delta

KRAS / BRAF status	Gene	Level	MSI			MSS		
			N	%	Σ%	N	%	Σ%
KRAS MT BRAF WT	POLD1	pathogenic	16	21.9	21.9	38	3.8	3.8
		WT	57	78.1	100	949	96.2	100
	POLE	pathogenic	5	6.8	6.8	11	1.1	1.1
		WT	68	93.2	100	976	98.9	100
KRAS WT BRAF MT	POLD1	pathogenic	97	26.6	26.6	8	3.7	3.7
		WT	267	73.3	100	209	96.3	100
	POLE	pathogenic	23	6.3	6.3	3	1.4	1.4
		WT	341	93.7	100	214	98.6	100
KRAS WT BRAF WT	POLD1	pathogenic	45	31.7	31.7	31	2.8	2.8
		WT	97	68.3	100	1094	97.2	100
	POLE	pathogenic	19	13.4	13.4	13	1.2	1.2
		WT	123	86.6	100	1112	98.8	100