Supplementary Materialsfor:

Analysis of Molecular Pretreated Tumor Profiles as Predictive Factors of Therapeutic Response and Survival Outcomes After Neoadjuvant Therapy For Rectal Cancer in Moroccan Population

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Table S1:The sequence of primers used in PCR

Sequence of primers F/R : (5'>3')
KRAS gene
Exon 2   *F : GGTGGAGTATTTGATAGTGTA*   *R: GGTCCTGCACCAGTAATATGCA*   **Exon 3**   *F : AGTAAAAGGTGCACTGTAATA*   *R : CTCCTTAATGTCAGCTTATTAT*   **Exon 4**   *F: CTCTGAAGATGTACCTATGGTC*   *R: CAGTGTTACTTACCTGTCTTGT*
NRAS gene
Exon 2   *F : ATTTGCCATAAATAAAGGC*   *R : TAAATAAATTTGCCATA*   **Exon 3**   *F: ACCCTCCACACCCCCAGGATT*   *R: CTGTAGAGGTTAATATCCGCA*   **Exon 4**   *F: CCCGTTTTTAGGGAGCAGATTA*   *R: CCAGAGTTAATCAACTGATGC*
BRAF gene
Exon 15   *F: TGCTTGCTCTGATAGGAAAATG*   *R: GTAACTCAGCAGCATCTCAGGG*

F, Forward. **R**, Reverse. **PCR**, Polymerase chain reaction

Table S2: Association between TRG Dvorak regression and biomarkers

	Dvorak				p value
	1	2	3	4	
Ki 67					
<50	1(5.6)	9(50)	6(33.3)	2(11.1)	0.357
≥50	7(17.9)	11(28.2)	16(41.1)	5(12.8)	
MSI					
Stable	7(15.2)	14(30.4)	20(43.5)	5(18.2)	0.312
unstable	1(9.1)	6(54.5)	2(18.2)	2(10.9)	
KRAS exon 2					
Wild type	6(14.6)	15(36.6)	14(34.1)	6(14.6)	0.673
Mutated	2(12.5)	5(31.3)	8(50)	1(6.3)	
HER 2 scoring					
0	7(16.3)	13(30.2)	17(39.5)	6(14)	0.703
1	0(0)	5(62.5)	2(25)	1(12.5)	
2	1(33.3)	1(33.3)	1(33.3)	0(0)	
3	0(0)	1(33.3)	2(66.7)	0(0)	
P53					
<20	0(0)	3(50)	3(50)	0(0)	0.026
20-50	1(12.5)	2(25)	1(12.5)	4(50)	
≥50	7(16.3)	15(34.9)	18(41.9)	3(7)	

Table S3: Association between the percentage of therapeutic response and biomarkers

	Therapeutic response N=57		P value
	Non responder group <50% of therapeutic response	Responder group ≥50% of therapeutic response	
Ki 67			0.527
<50	7(39.9)	11(61.1)	
≥50	14(35.9)	25(64.1)	
MSI			
Stable	16(34.8)	30(65.2)	0.372
instable	5(45.5)	6(54.5)	
KRAS exon 2			
Wild type	16(39)	25(61)	0.410
Mutated	5(31.3)	11(68.8)	

HER 2 scoring			
0	16(37.2)	27(62.8)	0.998
1	3(37.5)	5(62.5)	
2	1(33.3)	2(66.7)	
3	1(33.3)	2(66.7)	
P53			
<20	3(50)	3(50)	0.628
20-50	2(25)	6(75)	
≥50	16(37.2)	27(62.8)	

TableS4: Association between clinicopathological characteristics and Immunohistochemical biomarkers

	MSI		p	Ki-67		p	HER-2				p	P-53			p
	stable	unstable		Low	high		Score 0	Score 1	Score 2	Score 3		low	moderate	high	
Gender **Female**	26(56.5)	6(54.5)	0.583	11(61.1)	21(53.8)	0.412	22((51.2)	6(75)	3(100)	1(33.3)	0.206	5(83.3)	3(37.5)	24(55.8)	0.231
Male	20(43.5)	5(45.5)		7(38.9)	18(46.2)		21(48.8)	2(25)	0(0)	2(66.7)		1(16.7)	5(62.5)	19(44.2)	
Age **<50**	17(37)	6(54.5)	0.232	9(50)	14(35.9)	0.236	18(41.9)	2(25)	3(100)	0(0)	**0.063**	3(50)	4(50)	16(37.2)	0.698
≥50	29(63)	5(45.5)		9(50)	25(64.1)		25(58.1)	6(75)	0(0)	3(100)		2(50)	4(50)	27(62.8)	
Histologic type **Adenocarcinoma**	43(93.5)	11(100)	0.519	17(94.4)	37(94.9)	0.688	40(93)	8(100)	3(100)	3(100)	0.794	6(100)	8(100)	40(93)	0.597
Mucinous carcinoma/signet ring carcinoma	3(6.5)	0(0)		1(5.6)	2(5.1)		3(7)	0(0)	0(0)	0(0)		0(0)	0(0)	3(7)	
Degrees of differentiation **Well**	24(52.2)	5(45.5)	0.562	9(50)	20(51.3)	0.446	21(48.8)	4(50)	2(66.7)	2(66.7)	0.958	3(50)	3(37.5)	23(53.5)	0.801
Moderate	19(41.3)	6(54.5)		9(50)	16(41)		19(44.2)	4(50)	1(33.3)	1(33.3)		3(50)	4(50)	18(41.9)	
poor/undifferenciated	3(6.5)	0(0)		0(0)	3(7.7)		3(7)	0(0)	0(0)	0(0)		0(0)	1(12.5)	2(4.7)	
Perineural invasion **Yes**	5(10.9)	2(18.2)	0.610	3(16.7)	4(10.3)	0.667	6(14)	1(12.5)	0(0)	0(0)	0.813	0(0)	1(12.5)	6(14)	0.621
No	41(89.1)	9(81.8)		15(83.3)	35(89.7)		37(86)	7(87.5)	3(100)	0(0)		6(100)	7(87.5)	37(86)	
Vascular invasion **Yes**	3(18.2)	2(18.2)	0.244	2(11.1)	3(7.7)	0.646	5(11.6)	0(0)	0(0)	0(0)	0.618	0(0)	0(0)	5(11.6)	0.410
No	43(93.5)	9(81.8)		16(88.9)	36(92.3)		38(88.4)	8(100)	3(100)	3(100)		6(100)	8(100)	38(88.4)	

				p			p					p				p
ypN **N0**		31(67.4)	7(63.6)	0.537	11(61.1)	27(69.2)	0.377	29(67.4)	4(50)	3(100)	2(66.7)	0.473	4(66.7)	7(87.5)	27(62.8)	0.396
N+		15(32.6)	4(36.4)		7(38.9)	12(30.8)		14(32.6)	4(50)	0(0)	1(33?3)		2(33.4)	1(12.5)	16(37.2)	
ypT	**T0**	5(10.9)	2(18.2)	0.086	2(11.1)	5(12.8)	0.515	6(14)	1(12.5)	0(0)	0(0)	0.991	0(0)	4(50)	3(7)	0.091
	T1	2(4.3)	1(9.1)		0(0)	3(7.7)		3(7)	0(0)	0(0)	0(0)		0(0)	0(0)	3(7)	
	T2	20(43.5)	1(9.1)		9(50)	12(30.8)		15(34.9)	3(37.5)	2(66.7)	1(33.3)		3(50)	2(25)	16(37.2)	
	T3	19(41.3)	6(54.5)		7(38.9)	18(46.2)		18(41.9)	4(50)	1(33.3)	2(66.7)		3(50)	2(25)	20(46.5)	
	T4	0(0)	1(0)		0(0)	1(2.6)		1(2.3)	0(0)	0(0)	0(0)		0(0)	0(0)	1(2.3)	
Recurrence	**No**	44(96.7)	1(9.1)	0.481	16(88.9)	38(97.4)	0.232	40(93)	8(100)	3(0)	3((100)	0.794	5(100)	20(95.2)	29(93.5)	0.829
	Yes	2(4.3)	10(90.9)		2(11.1)	1(2.6)		3(7)	0(0)	0(0)	0(0)		0(0)	1(4.8)	2(6.5)	
Metastasis	**No**	29(63)	8(72.7)	0.409	14(77.8)	23(59)	0.139	28(65.1)	7(87.5)	2(66.7)	0(0)	0.062	3(50)	6(75)	28(65.1)	0.624
	Yes	17(37)	3(27.3)		4(22.2)	16(41)		15(34.9)	1(12.5)	1(33.3)	3(100)		3(50)	2(25)	15(34.9)	
Metastasis or/and recurrence **No**		28(60.9)	8(72.7)	0.357	13(72.2)	23(59)	0.254	27(62.8)	7(87.5)	2((66.7)	0(0)	0.066	3(50)	6(75)	27(62.8)	0.628
	Yes	18(39.1)	3(27.3)		5(27.8)	16(41)		16(37.2)	1(33.3)	1(33.3)	3(100)		3(50)	2(25)	16(37.2)	

TableS5: Association between clinicopathological characteristics and KRAS and NRAS mutations

	KRAS status									NRAS status								
	Exon 2 N=57		p	Exon 3 N=50		p	Exon 4 N=51		P	Exon 2 N=47		p	Exon 3 N=57		p	Exon 4 N=44		P
	M	WT		M	WT		M	WT		M	WT		M	WT		M	WT	
Gender			0.189			★			0.383			0.447			0.439			★
Female	7(43.8)	16(39)		0(0)	28(56)		3(75)	25(53.2)		0(0)	26(56.5)		0(0)	32(57.1)		0(0)	23(52.3)	
Male	9(56.3)	25(61)		0(0)	22(44)		1(25)	22(46.8)		1(100)	20(43.5)		1(100)	24(42.9)		0(0)	21(47.7)	
Age			0.285			★			0.161			0.574			0.596			★
<50	5(31.3)	18(43.9)		0(0)	21(42)		3(75)	17(36.2)		0(0)	20(43.5)		0(0)	23(41.1)		0(0)	17(38.6)	
≥50	11(68.8)	23(56.1)		0(0)	29(58)		1(25)	30(63.8)		1(100)	26(56.5)		1(100)	33(58.9)		0(0)	27(61.4)	
Histologic type			0.636			★			0.779			0.936			0.947			★
Adenocarcinoma	15(93.8)	39(95.1)		0(0)	47(94)		4(100)	44(93.6)		1(100)	43(93.5)		1(100)	53(94.6)		0(0)	42(95.5)	
Mucinous carcinoma/signet ring carcinoma	1(6.3)	2(4.9)		0(0)	3(6)		0(0)	3(6.4)		0(0)	3(6.5)		0(0)	3(5.4)		0(0)	2(4.5)	
Degrees of differentiation			0.185			★			0.496			0.587			0.521			★
Well	11(68.8)	18(43.9)		0(0)	24(48)		1(25)	24(51.1)		0(0)	22(47.8)		0(0)	29(51.8)		0(0)	21(47.7)	
Moderate	5(31.3)	20(48.8)		0(0)	24(48)		3(75)	21(44.7)		1(100)	22(47.8)		1(100)	24(42.9)		0(0)	22(50)	
poor/undifferenciated	0(0)	3(7.3)		0(0)	2(4)		0(0)	2(4.3)		0(0)	2(4.3)		0(0)	3(5.4)		0(0)	1(2.3)	
Perineural invasion **Yes**	1(14.6)	6(14.6)	0.660	0(0)	6(12)	★	1(25)	4(8.5)	0.347	1(100)	4(8.7)	0.106	1(100)	6(10.7)	0.123	0(0)	5(11.4)	★
No	15(93.8)	35(85.4)		0(0)	44(88)		3(75)	43(91.5)		0(0)	42(91.3)		0(0)	50(89.3)		0(0)	39(88.6)	
Vascular invasion **Yes**	1(6.3)	4(9.8)	1.000	0(0)	4(8)	★	1(25)	2(4.3)	0.221	1(100)	2(4.3)	0.064	0(0)	5(8.9)	1.000	0(0)	3(6.8)	★
No	15(93.8)	1(6.3)		0(0)	46(92)		3(75)	45(95.7)		0(0)	44(95.7)		1(100)	51(91.1)		0(0)	41(93.2)	

Variable	Category																		
ypN	N0	11(68.8)	27(65.9)	0.548	0(0)	34(68)	★	3(75)	32(68.1)	0.629	1(100)	34(73.9)	0.277	0(0)	37(66.1)	0.667	0(0)	31	★
	N+	5(31.3)	14(34.1)		0(0)	16(32)		1(25)	15(31.9)		0(0)	12(26.1)		1(100)	19(33.9)		0(0)	13	
ypT	T0	1(14.6)	6(14.6)	0.252	0(0)	6(12)	★	0(0)	5(10.6)	0.203	0(0)	5(10.9)	0.884	0	7(12.5)	0.861	0(0)	4(9.1)	★
	T1	0(0)	3(7.3)		0(0)	2(4)		1(25)	1(2.1)		0(0)	2(4.3)		0	3(5.4)		0(0)	1(2.3)	
	T2	5(31.3)	16(39)		0(0)	17(34)		2(50)	18(38.3)		0(0)	17(37)		0	21(37.5)		0(0)	17(38.6)	
	T3	9(56.3)	16(56.3)		0(0)	24(48)		1(25)	22(46.8)		1(100)	21(45.7)		1(100)	24(42.9)		0(0)	21(47.7)	
	T4	1(6.3)	0(0)		0(0)	1(2)		0(0)	1(2.1)		0(0)	1(2.2)		0	1(1.8)		0(0)	1(2.3)	
Recurrence	No	1(2.4)	40(97.6)	0.187	0(0)	47(94)	★	4(100)	44(93.6)	0.779	1(100)	43(93.5)	0.936	1(100)	53(94.6)	0.947	0(0)	41(93.2)	★
	Yes	14(87.5)	1(2.4)		0(0)	3(6)		0(0)	3(6.4)		0(0)	3(6.5)		0(0)	3(5.4)		0(0)	3(6.8)	
Metastasis	No	5(31.3)	26(63.4)	0.478	0(0)	32(64)	★	0(0)	32(68.1)	0.016	1(100)	28(60.9)	0.404	0(0)	37(66.1)	0.351	0(0)	27(61.4)	★
	Yes	15(36.6)	11(68.8)		0(0)	18(36)		4(0)	15(31.9)		0(0)	18(39.1)		1(100)	19(33.9)		0(0)	17(38/6)	
Metastasis or/and recurrence	No	11(68.8)	5(31.3)	0.410	19(38)	31(62)	★	0(0)	31(66)	0.019	0(0)	27(58.7)	0.426	0(0)	36(64.3)	0.368	0(0)	18(40.9)	★
	Yes	5(31.3)	16(39)		0(0)	0(0)		4(100)	16(34)		1(100)	19(41.3)		1(100)	20(35.7)		0(0)	26(59.1)	

★,p was not calculated because the variable is constant