

# Supplementary Materials: Genetic Variants of the *TERT* Gene, Telomere Length, and Circulating *TERT* as Prognostic Markers in Rectal Cancer Patients

Enrica Rampazzo, Erika Cecchin, Paola Del Bianco, Chiara Menin, Gaya Spolverato, Silvia Giunco, Sara Lonardi, Sandro Malacrida, Antonino De Paoli, Giuseppe Toffoli, Salvatore Pucciarelli and Anita De Rossi

**Table S1A.** Association between SNP genotypes and RTL in peripheral blood cells after neoadjuvant therapy.

SNP	Genotype	n	RTL median (IQR)	p-value *
<b>rs2736108</b>	TT	8	1.05 (0.95 to 1.14)	<i>Ref</i>
	TC	27	1.17 (1.00 to 1.36)	0.167
	CC	36	1.13 (1.03 to 1.28)	0.238
rs2735940	GG	15	1.09 (1.00 to 1.31)	<i>Ref</i>
	AG	31	1.15 (1.05 to 1.29)	0.526
	AA	13	1.06 (0.96 to 1.16)	0.390
rs2736098	TT	4	1.01 (0.95 to 1.08)	<i>Ref</i>
	TC	19	1.15 (1.00 to 1.26)	0.258
	CC	49	1.15 (1.03 to 1.28)	0.166
rs2736100	CC	19	1.06 (0.95 to 1.21)	<i>Ref</i>
	AC	34	1.15 (1.09 to 1.28)	0.277
	AA	17	1.11 (1.02 to 1.32)	0.530
rs35241335	TT	67	1.14 (0.99 to 1.26)	<i>Ref</i>
	TG	5	1.28 (1.27 to 1.30)	<b>0.039</b>
	GG	1	1.41	0.134
rs11742908	CC	40	1.16 (1.03 to 1.32)	<i>Ref</i>
	CG	23	1.15 (0.95 to 1.26)	0.191
	GG	2	1.11 (1.11 to 1.12)	0.651
rs2736122	GG	45	1.15 (1.05 to 1.30)	<i>Ref</i>
	AG	25	1.10 (0.96 to 1.21)	0.240
	AA	2	1.20 (1.05 to 1.36)	0.733
rs2853690	GG	50	1.14 (1.00 to 1.28)	0.350
	AG	18	1.09 (0.97 to 1.21)	<i>Ref</i>
	AA	2	1.20 (1.05 to 1.36)	0.430

RTL = relative telomere length; IQR= interquartile range; \* adjusted by age.

**Table S1B.** Association between SNP genotypes and RTL changes after neoadjuvant therapy.

SNP	Genotype	n	$\Delta$ RTL (T2-T0) median (IQR)	p-value *
<b>rs2736108</b>	TT	8	-0.35 (-0.65 to 0.20)	<i>Ref</i>
	TC	27	-0.04 (-0.29 to 0.16)	0.171
	CC	36	0.17 (-0.11 to 0.29)	<b>0.020</b>
rs2735940	GG	15	0.17 (-0.05 to 0.32)	<i>Ref</i>
	AG	31	0.02 (-0.12 to 0.33)	0.535
	AA	13	-0.33 (-0.68 to 0.26)	<b>0.008</b>
rs2736098	TT	4	-0.18 (-0.65 to 0.20)	<i>Ref</i>
	TC	19	-0.09 (-0.33 to 0.29)	0.395
	CC	49	0.10 (-0.13 to 0.26)	0.215
rs2736100	CC	19	-0.15 (-0.49 to 0.10)	<i>Ref</i>
	AC	34	0.16 (-0.13 to 0.36)	<b>0.006</b>

	AA	17	0.17 (0.00 to 0.24)	<b>0.007</b>
rs35241335	TT	67	0.00 (-0.22 to 0.26)	<i>Ref</i>
	TG	5	0.26 (0.15 to 0.33)	0.379
	GG	1	0.35	0.381
rs11742908	CC	40	0.13 (-0.14 to 0.26)	<i>Ref</i>
	CG	23	-0.10 (-0.35 to 0.27)	0.276
	GG	2	0.12 (0.003 to 0.25)	0.803
rs2736122	GG	45	0.01 (-0.32 to 0.26)	<i>Ref</i>
	AG	25	0.1 (-0.15 to 0.26)	0.793
	AA	2	-0.04 (-0.07 to -0.02)	0.922
rs2853690	GG	50	0.01 (-0.33 to 0.26)	0.552
	AG	18	0.11 (-0.13 to 0.28)	<i>Ref</i>
	AA	2	-0.04 (-0.07 to -0.02)	0.852

RTL = relative telomere length; IQR= interquartile range; \* adjusted by age.

**Table S2A.** Association between SNP genotypes and *TERT* levels after neoadjuvant therapy.

SNP	Genotype	n	<i>TERT</i> median (IQR)	<i>p</i> -value
rs2736108	TT	12	135.5 (82.5 to 226.2)	<i>Ref</i>
	TC	42	37.5 (0.0 to 142.2)	0.069
	CC	61	40.0 (0.0 to 177.0)	0.088
rs2735940	GG	28	85.0 (0.0 to 184.0)	<i>Ref</i>
	AG	49	30.0 (0.0 to 167.0)	0.468
	AA	26	49.5 (0.0 to 183.0)	0.889
rs2736098	TT	9	122.0 (0.0 to 226.0)	<i>Ref</i>
	TC	31	53.0 (0.0 to 143.0)	0.360
	CC	77	35.0 (0.0 to 177.0)	0.339
rs2736100	CC	30	49.5 (0.0 to 174.5)	<i>Ref</i>
	AC	56	32.5 (0.0 to 152.7)	0.82
	AA	26	85.0 (0.0 to 188.0)	0.643
rs35241335	TT	108	53.5 (0.0 to 177.7)	<i>Ref</i>
	TG	10	0.0 (0.0 to 35.5)	0.230
	GG	1	90.0	0.986
rs11742908	CC	57	40.0 (0.0 to 167.0)	<i>Ref</i>
	CG	53	55.0 (0.0 to 177.0)	0.659
	GG	2	0.0 (0.0 to 0.0)	0.287
rs2736122	GG	71	42.0 (0.0 to 154.0)	<i>Ref</i>
	AG	43	45.0 (0.0 to 218.5)	0.437
	AA	5	0.0 (0.0 to 144.0)	0.672
rs2853690	GG	80	25.0 (0.0 to 134.2)	<b>0.016</b>
	AG	30	131.0 (0.0 to 229.5)	<i>Ref</i>
	AA	4	0.0 (0.0 to 36.0)	0.108

IQR= interquartile range

**Table S2B.** Association between SNP genotypes and *TERT* changes after neoadjuvant therapy.

SNP	Genotype	n	$\Delta$ <i>TERT</i> (T2-T0) median (IQR)	<i>p</i> -value
rs2736108	TT	12	7.5 (-223.5 to 138.7)	<i>Ref</i>
	TC	42	0.0 (-178.5 to 59.2)	0.856
	CC	60	0.0 (-117.7 to 27.0)	0.844
rs2735940	GG	28	0.0 (-96.2 to 72.5)	<i>Ref</i>
	AG	48	0.0 (-157.7 to 20.0)	0.441
	AA	26	-97.5 (-206.2 to 134.7)	0.425
rs2736098	TT	9	-87.0 (-183.0 to 133.0)	<i>Ref</i>
	TC	31	0.0 (-176.0 to 113.0)	0.932
	CC	76	0.0 (-117.7 to 20.5)	0.943
rs2736100	CC	30	-38.0 (-198.0 to 9.0)	<i>Ref</i>
	AC	55	0.0 (-133.0 to 78.0)	0.414
	AA	26	0.0 (-123.2 to 33.7)	0.751

rs35241335	TT	107	0.0 (-128.5 to 56.0)	<i>Ref</i>
	TG	10	-105.0 (-211.7 to -15.5)	0.092
	GG	1	-95.0	0.676
rs11742908	CC	56	0.0 (-207.5 to 23.0)	<i>Ref</i>
	CG	53	0.0 (-126.0 to 101.0)	0.162
	GG	2	0.0 (0.0 to 0.0)	0.602
rs2736122	GG	70	0.0 (-103.7 to 90.2)	<i>Ref</i>
	AG	43	-5.0 (-225.0 to 11.0)	0.126
	AA	5	0.0 (-415.0 to 95.0)	0.683
rs2853690	GG	79	0.0 (-160.5 to 54.0)	0.463
	AG	30	0.0 (-57.5 to 33.7)	<i>Ref</i>
	AA	4	-207.5 (-435.0 to 23.7)	0.066

IQR= interquartile range

**Table S3A.** Univariate logistic regression analysis of clinical and demographical characteristics according to TRG probability in rectal cancer patients.

Characteristics		TRG 1-2 (%) (n = 90)	TRG 3-5 (%) (n = 104)	OR TRG 1-2 vs TRG 3-5 (95% CI)	<i>p</i> -value
Age (years)	cont.			1.03 (1–1.06)	<b>0.043</b>
Gender	M	65 (72.2)	73 (70.2)	1.1 (0.6–2.1)	0.756
	F	25 (27.8)	31 (29.8)	<i>Ref</i>	
Distance from anal verge(cm)	Median (IQR)	5 (3–8)	5 (4–8)	0.95 (0.85–1.06)	0.369
CEA, ng/ml (T0)	Median (IQR)	2.0 (1.0–3.1)	2.2 (1.2–4.0)	1.0 (0.96–1.03)	0.828
Grading	G1	3 (4.5)	9 (10.1)	<i>Ref</i>	
	G2	59 (88.1)	73 (82.0)	2.4 (0.6–9.4)	0.199
	G3	5 (7.5)	7 (7.9)	2.1 (0.4–12.2)	0.390
cTNM	I	5 (5.7)	1 (1.0)	<i>Ref</i>	
	II	11 (12.5)	10 (9.9)	0.2 (0.0–2.2)	0.199
	III	70 (79.5)	89 (88.1)	0.2 (0.0–1.4)	0.095
	IV	2 (2.3)	1 (1.0)	0.4 (0.0–10.0)	0.577
Total RT dose	<50.4 Gy	10 (12.5)	12 (12.2)	<i>Ref</i>	
	50.4 Gy	42 (52.5)	47 (48.0)	1.1 (0.4–2.7)	0.884
	>50.4 Gy	28 (35.0)	39 (39.8)	0.9 (0.3–2.3)	0.763
Fluoropyrimide	Alone	44 (53.7)	49 (48.5)	<i>Ref</i>	
	+ other drugs	36 (43.9)	48 (47.5)	0.8 (0.5–1.5)	0.552
	No	2 (2.4)	4 (4.0)	0.6 (0.1–3.2)	0.511

OR = odds ratio; TRG = tumor regression grade; CI = confidence interval; cont. = continuous variable; CEA = carcinoembryonic antigen; cTNM = clinical Tumor, Node, Metastasis classification; RT= radiotherapy.

**Table S3B.** Univariate logistic regression analysis of covariates of interest according to TRG probability in rectal cancer patients.

		TRG 1-2 (%)	TRG 3-5 (%)	OR TRG 1-2 vs TRG 3-5 (95% CI)	<i>p</i> -value
rs2736108	TT	3 (3.4)	13 (13.3)	<i>Ref</i>	
	TC	29 (33.0)	35 (35.7)	3.6 (0.9–13.8)	0.063
	CC	56 (63.6)	50 (51.0)	4.8 (1.3–18.0)	<b>0.018</b>
rs2735940	AA	13 (16.5)	25 (28.1)	<i>Ref</i>	
	AG	40 (50.6)	44 (49.4)	1.7 (0.8–3.9)	0.169
	GG	26 (32.9)	20 (22.5)	2.5 (1.0–6.1)	<b>0.043</b>
rs2736098	TT	2 (2.2)	10 (10.1)	<i>Ref</i>	
	TC	22 (24.7)	29 (29.3)	3.8 (0.7–19.1)	0.106
	CC	65 (73.0)	60 (60.6)	5.4 (1.1–25.7)	<b>0.034</b>
rs2736100	CC	18 (21.4)	26 (26.5)	<i>Ref</i>	<i>Ref</i>
	AC	44 (52.4)	51 (52.0)	1.2 (0.6–2.6)	0.551

	AA	22 (26.2)	21 (21.4)	1.5 (0.7–3.5)	0.338
rs35241335	TT	80 (88.9)	89 (89.0)	<i>Ref</i>	
	TG	9 (10.0)	11 (11.0)	0.9 (0.4–2.3)	0.843
	GG	1 (1.1)	0 (0)		
rs11742908	CC	49 (57.0)	54 (57.4)	<i>Ref</i>	
	CG	35 (40.7)	38 (40.4)	1.0 (0.6–1.9)	0.961
	GG	2 (2.3)	2 (2.1)	1.1 (0.2–8.1)	0.924
rs2736122	GG	49 (58.3)	61 (60.4)	<i>Ref</i>	
	AG	29 (34.5)	36 (35.6)	1.0 (0.5–1.9)	0.993
	AA	6 (7.1)	4 (4.0)	1.9 (0.5–7.0)	0.354
rs2853690	GG	64 (73.6)	65 (67.0)	1.9 (0.9–3.7)	0.085
	AG	16 (18.4)	30 (30.9)	<i>Ref</i>	
	AA	7 (8.0)	2 (2.1)	6.6 (1.2–35.4)	<b>0.029</b>
RTL (T0)	≤median (1.055)	45 (50.0)	52 (50.0)	<i>Ref</i>	
	>median	45 (50.0)	52 (50.0)	1.0 (0.6–1.8)	1.000
RTL (T2)	≤median (1.14)	14 (43.8)	24 (57.1)	<i>Ref</i>	
	>median	18 (56.2)	18 (42.9)	1.7 (0.7–4.3)	0.255
ΔRTL (T2-T0)	≤0 (stable/decrease)	17 (53.1)	18 (42.9)	<i>Ref</i>	
	>0 (increase)	15 (46.9)	24 (57.1)	0.7 (0.3–1.7)	0.382
<i>TERT</i> (T0)	≤median (122)	29 (50.0)	43 (50.6)	<i>Ref</i>	
	>median	29 (50.0)	42 (49.4)	1.0 (0.5–3.0)	0.945
<i>TERT</i> (T2)	≤median (45)	40 (76.9)	22 (31.0)	7.4 (3.3–16.8)	<b>&lt;0.001</b>
	>median	12 (23.1)	49 (69.0)	<i>Ref</i>	
Δ <i>TERT</i> (T2-T0)	≤0 (stable/decrease)	46 (90.2)	33 (46.5)	10.6 (3.8–29.8)	<b>&lt;0.001</b>
	>0 (increase)	5 (9.8)	38 (53.5)	<i>Ref</i>	

RTL = relative telomere length; TRG = tumor regression grade; OR = odds ratio; CI = confidence interval.

**Table S4A.** Haplotype distribution in responder (TRG 1-2) and non-responders (TRG 3-5) to CRT.

SNP Haplotype id	rs2736108	rs2735940	rs2736098	rs2736100	rs35241335	rs11742908	rs2736122	rs2853690	TRG 1-2 % *	TRG 3-5 % *	OR*		<i>p</i> -value†
											TRG 1-2 vs TRG 3-5 (95% CI)		
1	C	G	C	A	T	C	G	G	0.22	0.15	1	---	
2	C	G	C	A	T	C	A	A	0.11	0.13	0.93 (0.39–2.22)	0.87	
3	T	A	T	C	T	C	G	G	0.07	0.14	0.45 (0.14–1.49)	0.19	
4	C	A	C	C	T	C	G	G	0.09	0.11	0.90 (0.31–2.61)	0.85	
5	C	A	C	C	T	G	G	G	0.05	0.07	0.65 (0.21–2.04)	0.47	
6	T	A	C	C	T	C	G	G	0.06	0.07	1.05 (0.33–3.31)	0.93	
7	C	G	C	A	T	G	G	G	0.05	0.06	1.08 (0.27–4.40)	0.91	
8	C	G	C	A	G	C	G	G	0.05	0.04	1.41 (0.44–4.48)	0.56	
9	C	G	C	C	T	C	G	G	0.03	0.04	1.13 (0.20–6.48)	0.89	
<b>10</b>	<b>C</b>	<b>G</b>	<b>C</b>	<b>A</b>	<b>T</b>	<b>C</b>	<b>A</b>	<b>G</b>	<b>0.04</b>	<b>0.01</b>	<b>6.80 (1.04–44.41)</b>	<b>0.05</b>	
11	T	A	T	C	T	G	G	G	0.06	0.01	1.26 (0.18–8.62)	0.82	
12	C	G	C	C	T	G	G	G	0.03	0.02	10.18 (0.00–20844.24)	0.55	
rare	*	*	*	*	*	*	*	*	NA	NA	0.83 (0.36–1.87)	0.65	

\* frequencies are estimated by SNPStats program; TRG = tumor regression grade; OR = odd ratio; CI= confidence interval; † adjusted by age

**Table S4B.** 5' *TERT* gene haplotype distribution in responders (TRG 1-2) and non-responders (TRG 3-5) to CRT.

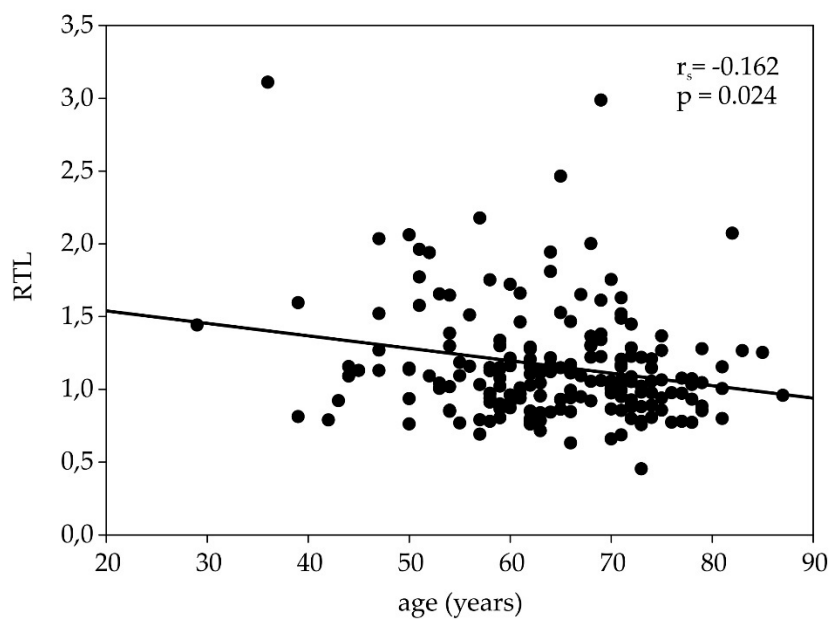
SNP Haplotype id	rs2736108	rs2735940	rs2736098	TRG 1-2* %	TRG 3-5* %	OR* TRG 1-2 vs TRG 3-5 (95% CI)	<i>p</i> -value†
1	C	G	C	0.59	0.46	1	---
2	C	A	C	0.19	0.21	0.69 (0.38–1.27)	0.24
<b>3</b>	<b>T</b>	<b>A</b>	<b>T</b>	<b>0.13</b>	<b>0.24</b>	<b>0.45 (0.26–0.80)</b>	<b>0.007</b>
4	T	A	C	0.07	0.08	0.74 (0.33–1.64)	0.46
rare	*	*	*	NA	NA	1.98 (0.20–19.52)	0.56

\* frequencies are estimated by SNPStats program; TRG= tumor regression grade; OR= odd ratio; CI= confidence interval; † adjusted by age.

Table S5. Univariate Cox regression analysis of EFS.

		E/N	HR (95%CI)	p-value
Age at diagnosis (years)	cont.		1.00 (0.98–1.03)	0.934
Gender	M	45/136	1.0 (0.6–1.7)	0.955
	F	19/56	Ref	
rs2736108	CC	36/104	Ref	
	TC	20/64	0.9 (0.5–1.5)	0.668
	TT	6/16	1.1 (0.5–2.6)	0.806
rs2735940	GG	13/44	Ref	
	AG	33/84	1.3 (0.7–2.4)	0.444
	AA	11/38	0.9 (0.4–2.1)	0.901
rs2736098	CC	43/123	Ref	
	TC	15/51	0.8 (0.5–1.5)	0.524
	TT	3/12	0.7 (0.2–2.4)	0.619
rs2736100	CC	18/44	Ref	
	AC	31/94	0.7 (0.4–1.3)	0.291
	AA	12/42	0.6 (0.3–1.3)	0.236
rs35241335	TT	59/168	Ref	
	TG	3/19	0.4 (0.1–1.3)	0.128
	GG	0/1		
rs11742908	CC	34/103	Ref	
	CG	22/71	0.9 (0.5–1.5)	0.679
	GG	2/4	1.5 (0.4–6.4)	0.554
rs2736122	GG	36/108	Ref	
	AG	22/65	1.0 (0.6–1.6)	0.878
	AA	3/10	0.7 (0.2–2.4)	0.606
rs2853690	GG	36/127	0.7 (0.4–1.1)	0.134
	AG	19/46	Ref	
	AA	3/9	0.7 (0.2–2.5)	0.631
RTL (T0)	≤median (1.055)	29/95	Ref	
	>median	35/97	1.1 (0.7–1.8)	0.670
RTL (T2)	≤median (1.14)	17/38	Ref	
	>median	7/36	0.4 (0.1–0.9)	<b>0.026</b>
ΔRTL (T2-T0)	≤0 (stable/decrease)	16/35	Ref	
	>0 (increase)	8/39	0.4 (0.2–1.0)	<b>0.049</b>
TERT (T0)	≤median (122)	18/70	Ref	
	>median	26/71	1.5 (0.8–2.8)	0.171
TERT (T2)	≤median (45)	12/62	0.4 (0.2–0.8)	<b>0.012</b>
	>median	25/60	Ref	
ΔTERT (T2-T0)	≤0 (stable/decrease)	19/79	0.4 (0.2–0.9)	<b>0.016</b>
	>0 (increase)	18/42	Ref	

EFS = Event –free Survival; HR = hazard ratio; cont. = continuous variable; CI = Confidence Interval; RTL = relative telomere length.



**Figure S1.** Correlation between age and telomere length in peripheral blood cells of rectal cancer patients.



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