

Supplementary Materials

Prognostic role of hERG1 potassium channels in neuroendocrine tumours of the ileum and pancreas.

Jessica Iorio^{1@}, Lorenzo Antonuzzo^{1,2@}, Emanuela Scarpi³, Massimo D'Amico⁴, Claudia Duranti¹, Luca Messerini¹, Clotilde Sparano⁵, Damiano Caputo^{6,7}, Daniele Lavacchi^{1,2}, Domenico Borzomati^{6,7}, Alice Antonelli^{1,2}, Lorenzo Nibid^{7,8}, Giuseppe Perrone^{7,8}, Alessandro Coppola^{6,7}, Roberto Coppola^{6,7}, Francesco di Costanzo², Elena Lastraioli^{1,9*}, Annarosa Arcangeli^{1,9}

¹Department of Experimental and Clinical Medicine, University of Florence, Florence Italy

²Medical Oncology, Azienda Ospedaliero-Universitaria Careggi, Florence, Italy

³Unit of Biostatistics and Clinical Trials, IRCCS Istituto Romagnolo per lo Studio dei tumori (IRST) "Dino Amadori", Meldola (FC), Italy

⁴DI.V.A.L. Toscana Srl, Sesto Fiorentino (Florence), Italy

⁵Endocrinology Unit, Department of Experimental and Clinical Biomedical Sciences "Mario Serio", University of Florence, Florence Italy

⁶General Surgery, Campus Bio-Medico University, Rome, Italy

⁷Fondazione Policlinico Universitario Campus Bio-Medico, Rome, Italy

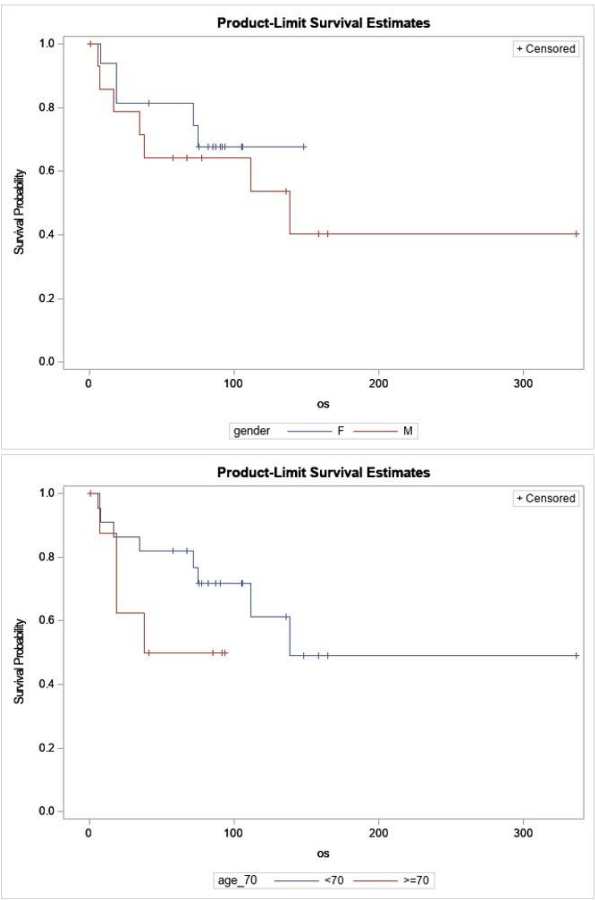
⁸Pathology Unit, Campus Bio-Medico University, Rome, Italy

⁹Complex Dynamics Study Centre (CSDC), University of Florence, Florence Italy

@Equally contributed

*Correspondence: elena.lastraioli@unifi.it; +39 0552751319

Figure S1. Kaplan-Meier plots of OS in ileal NET patients according to gender and age.



The SAS System

Obs	age_70	gender	os	dec
1	<70	F	7.86	1
2	<70	F	71.97	1
3	<70	F	75.10	1
4	<70	F	75.53	0
5	<70	F	81.97	0
6	<70	F	87.11	0
7	<70	F	90.26	0
8	<70	F	105.16	0
9	<70	F	105.46	0
10	<70	F	148.03	0
11	<70	M	5.92	1
12	<70	M	16.94	1
13	<70	M	34.44	1
14	<70	M	57.96	0
15	<70	M	67.04	0
16	<70	M	77.80	0
17	<70	M	111.25	1
18	<70	M	135.72	0
19	<70	M	138.36	1
20	<70	M	157.99	0
21	<70	M	164.47	0
22	<70	M	336.35	0
23	>=70	F	18.36	1
24	>=70	F	18.49	1
25	>=70	F	41.15	0
26	>=70	F	65.16	0
27	>=70	F	91.68	0
28	>=70	F	93.55	0
29	>=70	M	0.63	0
30	>=70	M	6.91	1
31	>=70	M	37.86	1

Table S1. Percentage of *h*ERG1 positive tumour cells in relation to baseline characteristics in ileal NET patients.

	% <i>h</i> ERG1 positive tumor cells	
	Median value (range)	p
Overall	80 (0-100)	
Age		
<70	80 (0-100)	
≥70	90 (0-100)	0.420
Gender		
Female	80 (0-100)	
Male	90 (0-100)	0.496
TNM at diagnosis		
II	45 (0-100)	
III	90 (0-100)	
IV	80 (0-100)	0.289
Metastases at diagnosis		
No	90 (0-100)	
Yes	80 (0-100)	0.233
Radical Surgery		
No	80 (0-100)	
Yes	85 (0-100)	0.549
Ki67 (%)		
<3	80 (0-100)	
3-20	90 (0-100)	
>20	0 (0-0)	0.210
SSA Receptors¹		
No	90 (0-100)	
Yes	80 (0-100)	0.509
PET-FDG²		
No	80 (0-100)	
Yes	90 (0-100)	0.306
Grading		
G1	80 (0-100)	
G2	90 (0-100)	
G3	0 (0-0)	0.210

¹ SSA Receptors: somatostatin analogs receptors.

² PET-FDG: fluorodeoxyglucose (FDG)-positron emission tomography (PET).

Table S2. Univariate analysis of Overall Survival in TNM stage IV iNET. Statistically significant values are reported in bold. nr: not reached

	N. patients	N. deaths	Median OS ³ (months) (95% CI ⁴)	HR ⁵ (95% CI)	P
Overall	15	7	nr	-	-
Age (continuous variable)	15	7	-	1.017 (0.957-1.080)	0.592
<70	12	6	nr	1.00	
≥70	3	1	nr	0.72 (0.08-6.05)	0.761
Gender					
Female	11	4	nr	1.00	
Male	4	3	26 (6-nr)	3.40 (0.74-15.56)	0.114
Metastases at diagnosis					
No	0	-	-	1.00	
Yes	15	7	nr	-	-
Radical Surgery					
No	12	6	75 (8-nr)	1.00	
Yes	3	1	nr	0.63 (0.07-5.23)	0.667
Ki67 (%)					
<3	4	1	nr	1.00	
3-20	9	4	nr	2.40 (0.27-21.58)	
>20	2	2	21 (8-nr)	11.08 (0.87-141.59)	0.137
SSA Receptors⁶					
No	3	2	18 (8-nr)	1.00	
Yes	12	5	nr	0.44 (0.08-2.31)	0.332
PET-FDG⁷					
No	10	5	nr	1.00	
Yes	3	1	nr	0.85 (0.10-7.30)	0.881
% hERG1 positive cells					
Negative (<40)	5	5	34 (6-nr)	1.00	
Positive (≥40)	10	2	nr	0.12 (0.02-0.65)	0.013

³ OS: Overall Survival.

⁴ HR: Hazard Ratio.

⁵ CI: Confidence Interval

⁶ SSA Receptors: somatostatin analogs receptors.

⁷ PET-FDG: fluorodeoxyglucose (FDG)-positron emission tomography (PET).

Table S3. Univariate analysis of Progression Free Survival in TNM I-III ileal NET patients. Statistically significant values are reported in bold.

	N. pts	N. events	% 5-years PFS ⁸ (95% CI ⁹)	P (logrank)	HR ¹⁰ (95% CI)	P (Cox)
Overall	16	10	30 (6-54)		-	-
Age (continuous variable)	16	10	-	-	0.975 (0.928-1.024)	0.312
<70	10	7	30 (2-58)		1.00	
≥70	6	3	27 (0-71)	0.605	0.70 (0.18-2.72)	0.607
Gender						
Female	5	2	53 (5-100)		1.00	
Male	11	8	20 (0-45)	0.198	2.67 (0.56-12.66)	0.215
Metastases at diagnosis						
No	0	-	-		1.00	
Yes	15	9	30 (6-54)	-	-	-
Radical Surgery						
No	1	1	0		1.00	
Yes	15	9	32 (7-58)	0.308	0.34 (0.04-3.02)	0.331
Ki67 (%)						
<3	11	6	35 (4-66)		1.00	
3-20	5	4	20 (0-55)		1.65 (0.46-5.94)	
>20	0	-	-	0.438	-	0.442
SSA Receptors¹¹						
No	8	5	29 (0-62)		1.00	
Yes	8	5	33 (0-69)	0.870	1.11 (0.32-3.86)	0.870
PET-FDG¹²						
No	10	8	13 (0-37)		1.00	
Yes	3	0	100	0.029	ne	-
% hERG1 positive cells						
Negative (<40)	2	0	100		1.00	
Positive (≥40)	14	10	23 (0-46)	0.164	ne	-

⁸ PFS: Progression Free Survival.

⁹ HR: Hazard Ratio.

¹⁰ CI: Confidence Interval

¹¹ SSA Receptors: somatostatin analogs receptors.

¹² PET-FDG: fluorodeoxyglucose (FDG)-positron emission tomography (PET).

Table S4. Univariate analysis of Progression Free Survival in TNM IV ileal NET patients.

	N. pts	N. events	Median PFS ¹³ (months) (95% CI ¹⁴)	P (logrank)	HR ¹⁵ (95% CI)	P (Cox)
Overall	15	14	6 (3-9)	-	-	-
Age (continuous variable)	15	14	-	-	0.977 (0.933-1.023)	0.327
<70	12	12	6 (3-13)		1.00	
≥70	3	2	6 (2-nr)	0.441	0.55 (0.12-2.54)	0.446
Gender						
Female	11	10	7 (3-16)		1.00	
Male	4	4	3 (0-nr)	0.187	2.22 (0.66-7.53)	0.199
Metastases at diagnosis						
No	0	-	-		1.00	
Yes	15	14	6 (3-9)	-	ne	-
Radical Surgery						
No	12	11	6 (2-16)		1.00	
Yes	3	3	5 (3-nr)	0.332	1.94 (0.50-7.61)	0.340
Ki67 (%)						
<3	4	3	12 (5-nr) ²		1.00	
3-20	9	9	3 (0-9)		2.66 (0.70-10.05)	
>20	2	2	9 (5-nr)	0.323	1.81 (0.29-11.26)	0.347
SSA Receptors¹⁶						
No	3	3	5 (2-nr)		1.00	
Yes	12	11	7 (3-16)	0.077	0.28 (0.06-1.26)	0.098
PET-FDG¹⁷						
No	10	9	6 (0-13)		1.00	
Yes	3	3	3 (3-nr)	0.217	2.42 (0.57-10.30)	0.231
% hERG1 positive cells						
Negative (<40)	5	5	9 (3-nr)		1.00	
Positive (≥40)	10	9	5 (0-8)	0.765	1.19 (0.38-3.68)	0.765

¹³ PFS: Progression Free Survival.

¹⁴ HR: Hazard Ratio.

¹⁵ CI: Confidence Interval

¹⁶ SSA Receptors: somatostatin analogs receptors.

¹⁷ PET-FDG: fluorodeoxyglucose (FDG)-positron emission tomography (PET).

Figure S2. Kaplan-Meier plots of PFS in ileal NET patients according to hERG1 expression (cut-off: 40%). Blue curves: hERG1 negative samples (<40%), Red curves: hERG1 positive samples ($\geq 40\%$).

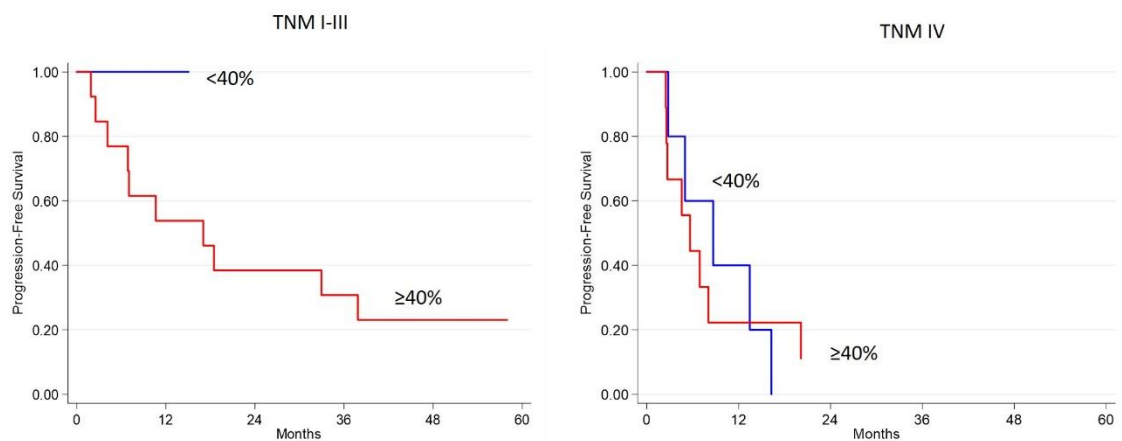


Table S5. Univariate analysis of Progression Free Survival in TNM I-III pancreatic NET patients. Statistically significant values are reported in bold.

	N. pts	N. events	% 5-years PFS ¹⁸ (95% CI ¹⁹)	P (logrank)	HR ²⁰ (95% CI)	P (Cox)
Overall	40	9	76 (63-90)	-	-	-
Age (continuous variable)						
<70	30	8	71 (55-88)		1.00	
≥70	10	1	90 (71-100)	0.240	0.31 (0.04-2.46)	0.267
Gender						
Female	22	4	82 (65-98)		1.00	
Male	18	5	69 (47-92)	0.500	1.57 (0.42-5.84)	0.503
Metastases at diagnosis						
No	40	9	76 (63-90)		1.00	
Yes	0	-	-	-	ne	-
Radical Surgery						
No	0	-	-		1.00	
Yes	40	9	76 (63-90)	-	ne	-
Ki67 (%)						
<3	26	3	88 (76-100)		1.00	
3-20	13	5	55 (26-85)		ne	
>20	1	1	0	<0.0001	ne	-
SSA Receptors²¹						
No	0	-	-		1.00	
Yes	10	6	27 (0-59)	-	ne	-
PET-FDG²²						
No	3	1	67 (13-100)		1.00	
Yes	4	2	50 (1-99)	0.829	1.31 (0.11-15.21)	0.829
% hERG1 positive cells						
Negative (<40)	25	6	73 (55-92)		1.00	
Positive (≥40)	15	3	80 (60-100)	0.734	0.79 (0.20-3.15)	0.734

¹⁸ PFS: Progression Free Survival.

¹⁹ HR: Hazard Ratio.

²⁰ CI: Confidence Interval

²¹ SSA Receptors: somatostatin analogs receptors.

²² PET-FDG: fluorodeoxyglucose (FDG)-positron emission tomography (PET).

Table S6. Univariate analysis of Progression Free Survival in TNM IV pancreatic NET patients. Statistically significant values are reported in bold.

	N. pts	N. events	Median PFS ²³ (months) (95% CI ²⁴)	P (logrank)	HR ²⁵ (95% CI)	P (Cox)
Overall	19	16	9 (4-31)	-	-	-
Age (continuous variable)						
<70	16	14	8 (4-31)		1.00	
≥70	3	2	10 (2-nr)	0.821	0.84 (0.19-3.77)	0.822
Gender						
Female	7	5	74 (2-nr)		1.00	
Male	12	11	8 (2-22)	0.150	2.32 (0.71-7.57)	0.162
Metastases at diagnosis						
No	0	-	-		1.00	
Yes	19	16	9 (4-31)	-	ne	-
Radical Surgery						
No	17	14	8 (2-74)		1.00	
Yes	2	2	16 (10-nr)	0.855	1.15 (0.25-5.30)	0.856
Ki67 (%)						
<3	5	5	22 (5-nr)		1.00	
3-20	8	5	20 (2-nr)		0.95 (0.25-3.56)	
>20	6	6	4 (1-nr)	0.018	4.72 (1.13-19.66)	0.038
SSA Receptors²⁶						
No	1	1	2 (-)		1.00	
Yes	11	9	22 (5-74)	0.0009	ne	-
PET-FDG²⁷						
No	5	3	31 (8-nr)		1.00	
Yes	8	8	5 (2-10)	0.043	3.77 (0.95-14.90)	0.058
% hERG1 positive cells						
Negative (<40)	16	14	10 (5-31)		1.00	
Positive (≥40)	3	2	4 (1-nr)	0.910	1.09 (0.24-4.90)	0.909

²³ PFS: Progression Free Survival.

²⁴ HR: Hazard Ratio.

²⁵ CI: Confidence Interval

²⁶ SSA Receptors: somatostatin analogs receptors

²⁷ PET-FDG: fluorodeoxyglucose (FDG)-positron emission tomography (PET).

Figure S3. Kaplan-Meier plots of PFS in pancreatic NET patients according to hERG1 expression (cut-off: 40%) in TNM I-III (left) and TNM IV patients (right). Blue curves: hERG1 negative samples (<40%), Red curves: hERG1 positive samples ($\geq 40\%$).

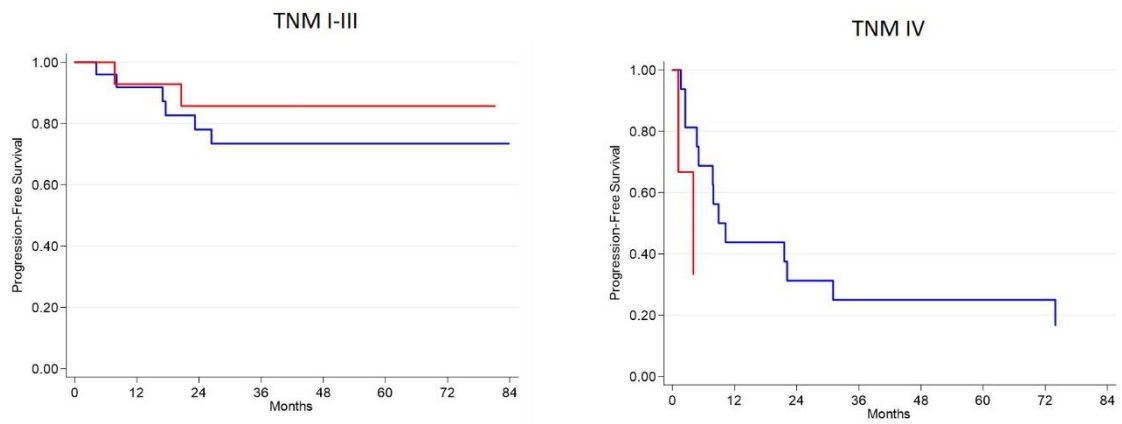


Table S7. *P* values of the comparison between control and E4031- treated INS 1E.

	P value	
CTR vs 0.1 μM	24h	0.002
	48h	0.040
	72h	0.020
	96h	0.004
CTR vs 1 μM	24h	0.013
	48h	0.030
	72h	< 0.001
	96h	< 0.001
CTR vs 10 μM	24h	0.004
	48h	0.010
	72h	< 0.001
	96h	< 0.001
CTR vs 25 μM	24h	0.002
	48h	0.011
	72h	0.001
	96h	< 0.001
CTR vs 50 μM	24h	< 0.001
	48h	0.007
	72h	< 0.001
	96h	< 0.001
CTR vs 100 μM	24h	0.001
	48h	0.006
	72h	< 0.001
	96h	< 0.001
CTR vs 200 μM	24h	0.002
	48h	0.001
	72h	< 0.001
	96h	< 0.001