Predictive Biomarkers for Endocrine Therapy

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Document Version
Peer reviewed version

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SUPPLEMENTARY MATERIALS

SUPPLEMENTARY METHODS

Patients and study design

The TEAM trial inclusion and exclusion criteria differed slightly by participating country. Eligible patients were postmenopausal women with HRec-positive breast cancer (defined locally), with histologically or cytologically confirmed T1-3 N0-2 M0 adenocarcinoma and adequate local therapy (surgical resection +/- radiotherapy) and/or chemotherapy. The United Kingdom/Ireland (1053/1275; 82.5%), the Netherlands (2710/2753; 98.4%), Belgium (113/414; 27.3%), Germany (670/1471; 45.5%), and Greece (85/207; 41.1%) provided eligible tumour samples from surgical resections for tissue microarray (TMA; n=4631) construction with linked clinicopathological data. This study hypothesised that low expression of our biomarkers would predict response to exemestane alone.

Procedures

Antigen retrieval for IKKα and p44/42 MAPK$^{202/204}$ used citrate buffer pH6 at 96ºC, and for ER^{118}, ER^{167}, p65^{536}, Raf-1^{338}, and N-Ras used TE buffer pH8 at 96ºC. 2% normal donkey serum (ER^{118}/ER^{167}/IKKα/p65^{536}/N-Ras/Raf-1^{338}) or 10% Casein (p44/42 MAPK$^{202/204}$) was the blocking solution. TMA sections were incubated at 37ºC with primary ER^{118} (1:500, Cell Signaling Ltd, #2511), ER^{167} (1:200, Cell Signaling Ltd, #5587), IKKα (1:800, Geneway, #GTW662250), p65^{536} (1:50, Abcam, #ab28856), Raf-1^{338} (1:300, Millipore, #05-538), and p44/42 MAPK$^{202/204}$ (1:600, Cell Signaling Ltd, #4376) or at 42ºC with primary N-Ras (1:30, Santa Cruz, #SC-51). HRP-multispecies secondary antibody

(Roche) followed by DAB substrate for color development. Slides were counterstained with hematoxylin.

Stained TMA sections were scanned using a Hamamatsu NanoZoomer at x20 magnification and visualized on Slidepath Digital Image Hub (Leica Biosystems). The weighted histoscore is calculated as follows: 0x% not stained + 1x% weakly stained + 2x% moderately stained + 3x% strongly stained and gives a range from 0 to 300.

Supplementary Table 1. Biomarker associations with common clinicopathological factors

Clinicopathological factors	ER[167] (n=4133)			IKK alpha (n=3024)			Raf-1[338] (n=4030)			p44/42 MAPK[202/204] (n=4055)		
	Negative (%) (n=1150)	Positive (%) (n=2983)	p value[*]	Negative (%) (n=1579)	Positive (%) (n=1445)	p value[*]	Negative (%) (n=2797)	Positive (%) (n=1233)	p value[*]	Negative (%) (n=2249)	Positive (%) (n=1806)	p value[*]
Age			0.27			0.75			0.15			0.001
<50	28 (2.4)	76 (2.5)		39 (2.5)	42 (2.9)		60 (2.1)	47 (3.8)		48 (2.1)	62 (3.4)	
50-59	337 (29.3)	901 (30.2)		465 (29.4)	420 (29.1)		866 (31.0)	335 (27.2)		659 (29.3)	546 (30.2)	
60-69	428 (37.2)	1142 (38.2)		604 (38.3)	559 (38.7)		1016 (36.3)	530 (43.0)		833 (37.0)	723 (40.0)	
>70	357 (31.1)	864 (29.1)		471 (29.8)	424 (29.3)		855 (30.6)	321 (26.0)		709 (31.6)	475 (26.4)	
Grade			0.001			0.02			<0.001			<0.001
1	126 (11.0)	363 (12.1)		178 (11.3)	172 (11.9)		406 (14.5)	64 (5.2)		258 (11.5)	212 (11.7)	
2	552 (48.0)	1554 (52.1)		792 (50.2)	752 (52.0)		1267 (45.3)	780 (63.3)		1055 (46.9)	1013 (56.1)	
3-4	412 (35.8)	879 (29.4)		529 (33.5)	430 (29.8)		940 (33.6)	297 (24.1)		766 (34.1)	484 (26.8)	
unknown	60 (5.2)	187 (6.4)		80 (5.0)	91 (6.3)		184 (6.6)	92 (7.4)		170 (7.5)	97 (5.4)	
Nodal Status			0.55			<0.001			<0.001			<0.001
absent	298 (25.9)	746 (25.0)		338 (21.4)	445 (30.7)		351 (12.5)	625 (50.7)		449 (20.0)	537 (29.7)	
present	852 (74.1)	2237 (75.0)		1241 (78.6)	1000 (69.3)		2446 (87.5)	608 (49.3)		1800 (80.0)	1269 (70.3)	
Size			0.04			0.06			<0.001			<0.001
<20mm	531(46.2)	1463 (49.0)		765 (48.4)	716 (49.6)		1265 (45.3)	670 (54.3)		971 (43.2)	972 (53.8)	
>20mm	600 (52.2)	1428 (47.9)		799 (50.6)	651 (45.1)		1449 (51.8)	471 (38.2)		1203 (53.5)	783 (43.4)	
unknown	19 (1.6)	92 (3.1)		15 (1.0)	78 (5.3)		83 (2.9)	92 (7.5)		75 (3.3)	51 (2.8)	
PR status			0.13			0.02			<0.001			0.23
absent	227 (19.7)	548 (18.4)		335 (21.2)	259 (17.9)		610 (21.8)	183 (14.7)		454 (20.2)	342 (18.9)	
present	757 (65.8)	2041 (68.4)		1118 (70.8)	1006 (69.6)		2010 (71.9)	824 (66.7)		1563 (69.5)	1289 (71.4)	
not performed	116 (14.5)	394 (13.2)		126 (8.0)	180 (12.5)		177 (6.3)	229 (18.6)		232 (10.3)	175 (9.7)	
HER2 status			0.05			<0.001			<0.001			<0.001
absent	406 (35.3)	1218 (40.8)		630 (39.9)	675 (46.7)		933 (33.4)	723 (58.6)		729 (32.4)	952 (52.7)	
present	454 (39.5)	1167 (39.1)		759 (48.1)	423 (29.3)		1653 (59.1)	76 (6.2)		1096 (48.7)	632 (35.0)	
not performed	290 (25.2)	598 (20.1)		210 (12.0)	347 (24.0)		211 (7.5)	434 (35.2)		424 (18.9)	222 (12.3)	
Ki67 status			0.99			0.008			0.004			0.52
low (<15)	607 (52.8)	1602 (53.7)		898 (56.9)	839 (58.1)		1434 (51.3)	635 (51.5)		1176 (52.3)	907 (50.2)	
high (>15)	274 (23.8)	722 (24.2)		374 (23.7)	409 (28.3)		601 (21.5)	337 (27.3)		521 (23.2)	423 (23.4)	
unknown	269 (23.4)	659 (22.1)		307 (19.4)	197 (13.6)		762 (27.2)	261 (21.2)		552 (24.5)	476 (26.4)	
Treatment			0.34			0.44			0.18			0.05
Exemestane	594 (51.6)	1491 (50.0)		791 (50.1)	744 (51.4)		1390 (49.7)	641 (52.0)		1103 (49.0)	942 (52.2)	
Tamoxifen followed by exemestane	556 (48.4)	1492 (50.0)		788 (49.9)	701 (48.6)		1407 (50.3)	592 (48.0)		1146 (51.0)	864 (47.8)	
Lymphovascular Invasion			0.34			0.54			0.95			<0.001
no	978 (85.0)	2509 (84.1)		1327 (84.0)	1209 (83.7)		2345 (83.8)	1043 (84.6)		1885 (83.8)	1557 (86.2)	
yes	112 (9.7)	256 (8.6)		139 (8.8)	137 (9.4)		231 (8.3)	102 (8.3)		221 (9.8)	112 (6.2)	
unknown	60 (5.3)	218 (7.3)		113 (7.2)	99 (6.9)		221 (7.9)	88 (7.1)		143 (6.4)	137 (7.6)	

[*] P values were calculated using a two-sided Chi-square test.