1   **TPOAb and thyroid function are not associated with breast cancer outcome; evidence**

2   **from a large-scale study using data from the Taxotere as Adjuvant Chemotherapy Trial**

3   **(TACT, CRUK01/001)**

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ABSTRACT

Background: Small-scale studies correlated the presence of thyroid autoimmunity with both improved or worsened breast cancer outcome.

Objectives: We aimed to clarify this association in a large cohort using the phase-III randomized controlled "Taxotere as Adjuvant Chemotherapy Trial" (TACT, CRUK01/001).

Methods: TACT women >18-years-old with node-positive or high risk node-negative early breast cancer (pT1-3a,pN0-1,M0), with stored plasma (n=1974), taken 15.5 [7.0-24.0] months (median [IQR]) after breast surgery were studied. Patients had also received chemotherapy (100%), radiotherapy (1745/1974 [88.4%]), hormonal therapy (1378/1974 [69.8%]), or trastuzumab (48/1974 [2.4%]). History of thyroid diseases and/or related treatments was not available.

The prognostic significance of autoantibodies to thyroid peroxidase (TPOAb; positive ≥ 6 kIU/L), free-thyroxine and thyrotropin (combined: euthyroid, hypothyroid, hyperthyroid) was evaluated for disease-free survival (DFS), overall-survival (OS), time-to-recurrence (TTR), with Cox regression models in univariate and multivariable analyses. The extended median follow-up was 97.5 months.

Results: No difference in DFS was found by TPOAb status (unadjusted-hazard ratio [HR]: 0.97, 95%CI: 0.78-1.19, P=0.75) and/or thyroid function (unadjusted-HR [hypothyroid versus euthyroid]: 1.15, 95%CI: 0.79-1.68, P=0.46; unadjusted-HR [hyperthyroid versus euthyroid]: 1.14, 95%CI: 0.82-1.61, P=0.44). Similar results were obtained for OS, TTR, multivariable analyses, when TPOAb titre by tertiles was considered and in a subgroup of 123 patients with plasma collected before adjuvant treatments.

Conclusions: No evidence for a prognostic role of TPOAb and/or thyroid function in moderate-high risk early breast cancer was found in the largest and longest observational study to date.

3

INTRODUCTION

An association between breast cancer (BC) and benign thyroid disorders has been debated for decades, reported in several [1,2], but not all [3] studies; the most recent meta-analyses and reviews reached contrasting conclusions [1,4-6]. Hypothyroidism was found to correlate with both an increased [7,8] or reduced [9-11] risk of developing BC, whilst other authors did not report a significant correlation [12,13]. BC has been particularly associated with thyroid autoimmunity (TA); a higher prevalence of anti-thyroid peroxidase (TPO) autoantibodies (TPOAb) was found among BC patients, compared with healthy controls [8,14]. Furthermore, a better BC outcome has been reported in TPOAb positive (TPOAb+) versus TPOAb negative (TPOAb-) patients in some [15-18], but not all [19] studies.

Currently no validated major blood prognostic markers for BC are available; carcinoembryonic antigen and cancer antigen 15.3 are the most used, but have low specificity and sensitivity [20]. Circulating tumour DNA and tumour cells seem very promising markers, however further studies are needed to validate them in routine clinical practice [21]. It would therefore be valuable if TPOAb could be confirmed as a blood BC prognostic marker.

Two studies evaluated 5-year outcomes in 142 [15] and 47 [16] BC women: Smyth *et al.* [15] reporting TPOAb- as a poor prognostic factor for disease-free survival (DFS) and overall survival (OS), and Fiore *et al.* [16] reporting 6.7% mortality in patients positive for anti-thyroid autoantibodies (TAb), mainly TPOAb+, compared with 46.9% in TAb negative patients. Farahati *et al.* evaluated 314 newly diagnosed BC patients and found no distant metastases among TPOAb+ patients compared with 6.6% among TPOAb- patients [17]. In contrast, Jiskra et al. followed 84 BC patients for 136 months (median), finding no impact of TPOAb on DFS or OS [19].

The aim of the present study was to clarify the impact of TPOAb on BC prognosis in a large, well powered patient cohort with long-term follow-up, according to the "REporting recommendations for tumour MARKer prognostic studies (REMARK)" guidelines [22]. The

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81 "Taxotere as Adjuvant Chemotherapy Trial (TACT)" recruited 4162 women diagnosed with

82 moderate-high risk early BC, evaluating whether sequential docetaxel (Taxotere) after

83 anthracycline therapy would improve patient outcome compared with standard anthracycline

84 chemotherapy: analyses were conducted at 62 months [23] and 97.5 months [24] follow-up,

85 both showing no evidence of a difference between the two chemotherapy regimens. Of

86 relevance, stored plasma was available in a significant number of these patients.

87 Furthermore, TPO is expressed in BC tissue [25], providing a possible mechanistic link:

88 a thyroid/breast shared autoimmune response might target tumour cells and improve BC

89 outcome. If TPOAb+ was confirmed as associated with a better BC outcome, new BC

90 therapeutic approaches based on antigen-specific immunotherapies targeting TPO could be

91 explored.

92

93 **MATERIALS AND METHODS**

94 **Patients**

95 The TACT study [23] was a multicentre, open-label, phase-III, randomised controlled

96 trial of women aged >18 years diagnosed with operable early BC (pT1-3a, pN0-1, M0), with

97 indication for adjuvant chemotherapy, including both lymph-node positive (node+) patients

98 and lymph-node negative (node-) but high risk (e.g., tumour grade 3, hormonal-receptor

99 expression negative, or lymphovascular invasion) patients.

100 Between February 2001 and June 2003, 4162 women were enrolled across 103 UK

101 and one Belgian centres. All subjects underwent surgery, mastectomy or wide-local-excision

102 (WLE), and were randomized (1:1 ratio) to the experimental regimen FEC-D (n=2073;

103 fluorouracil, epirubicin, cyclophosphamide [FEC] followed by docetaxel) or centre's choice of

104 control chemotherapy, either FEC (n=1265) or E-CMF (n=824; epirubicin followed by CMF

105 [cyclophosphamide, methotrexate, and fluorouracil]). Adjuvant radiotherapy was mandatory

106 after WLE or used after mastectomy according to local guidelines. Endocrine treatments

5

107 (tamoxifen or aromatase-inhibitor monotherapy, tamoxifen followed by aromatase-inhibitor)

108 were administered to patients with oestrogen receptor (ER) positive expression (ER+). Patients

109 with human epidermal growth factor receptor-2 (HER2) positive expression (HER2+) were

110 allowed to enter clinical trials assessing trastuzumab. All subjects have given their informed

111 consent and the study protocol has been approved by the institute's committee on human

112 research.

113

114 **Laboratory measurements**

115   Following a protocol amendment (November 2002), blood was taken for future

116 translational research at the time of randomization, or at their next follow-up visit. Plasma

117 samples were stored at -20°C for 6.5-13 years (range) at The Institute of Cancer Research

118 (London, UK), and transferred to the Thyroid Research Group (Cardiff, UK) for TPOAb,

119 thyrotropin (TSH) and free-thyroxine (FT4) analyses (October 2014) using an ADVIA Centaur

120 automated immunoassay analyser (Bayer plc, UK) and Chemiluminescent Microparticle

121 Immunoassay methods by the ARCHITECT® System (ABBOTT Laboratories, USA).

122 According to the assay cut-off, TPOAb values were dichotomized as ≥6 kIU/L (positive:

123 TPOAb+) versus <6 kIU/L (negative: TPOAb-); TPOAb+ were also categorized into tertiles.

124 FT4 and TSH normal ranges were 9.0–19.1 pmol/L and 0.30–4.40 mIU/L, respectively; they

125 were also combined in a thyroid function status variable: euthyroid (FT4 and TSH within the

126 normal ranges), hypothyroid (FT4 <9.0 pmol/L and/or TSH >4.40 mIU/L); hyperthyroid (FT4

127 >19.1 pmol/L and/or TSH <0.3 mIU/L).

128

129 **Statistical analysis**

130   According to TPOAb prevalence in age-matched females of general population [26,27],

131 20% of BC individuals were expected to be TPOAb+. Power calculations indicated 1158 and

132 1430 samples required to provide respectively 80% and 90% power to detect a 81% 5-year

DFS in TPOAb+ versus 73% in TPOAb- subjects (HR, 0.64; two-sided log-rank test with a 0.05 probability of a type I error), consistent with a 74.9% 5-year DFS rate in the whole TACT cohort [23].

Baseline characteristics, BC treatments and DFS-related characteristics were compared between TACT patients included or not in this study, and presented by dichotomized TPOAb and thyroid function status. Correlations between thyroid biomarkers were assessed using the Spearman rank method.

The primary outcome was to assess TPOAb prognostic significance in relation to DFS; secondary outcomes were TPOAb prognostic significance in relation to OS and time-to-recurrence (TTR), and thyroid function in relation to DFS, OS and TTR.

For DFS, OS and TTR, Kaplan-Meier curves were plotted and biomarkers compared with the log-rank test, and assessed firstly in a univariate Cox proportional hazards regression model stratified by centre's choice of control chemotherapy regimen and ER status, and subsequently included in a multivariable Cox model along with known BC prognostic factors: age, HER2 status, nodal involvement, tumour size and tumour grade. Additional variables, i.e. trial treatment (experimental versus control), type of surgery, trastuzumab use, radiotherapy and menopausal status, were included if, by stepwise selection (P<0.05), shown to add value. TPOAb, TSH and FT4 were subsequently considered for inclusion if providing independent prognostic information. Interaction tests were used to explore differential effects within subgroups. HR with 95% CI were obtained, with HR<1 indicating a better BC prognosis.

All patients with a biomarker value available were included in the analysis, as per an intention-to-treat analysis. All analyses were conducted using Stata version 13.1 (STATACORP, TX) [23,24].

RESULTS

All available TACT plasma samples (N=2000) were analysed for thyroid biomarkers, and 1974 samples were considered for the statistical analyses ("analysis population"; **Supplemental Fig. 1**). The median (IQR; range) blood collection time was 15.5 (7.0-24.0; 0.5–57.2) months after surgery.

Supplemental Table 1 reports analysis population's characteristics; the median (IQR; range) follow-up was 96.7 (87.4-106.3; 3.4-126.4) months. Overall 5-year estimates for DFS, OS and TTR were 79.5% (95% CI, 77.6-81.2), 87.4% (95% CI, 85.9-88.8) and 81.1% (95% CI, 79.3-82.8), respectively.

Distribution of TPOAb and thyroid function

TPOAb+ was detected in 406/1974 (20.6%) patients, distributed in the following tertiles: 137 (6.9%) 6-40 kIU/L (T1), 134 (6.7%) 41-238 kIU/L (T2), 135 (6.8%) 240-2000 kIU/L (T3). Baseline characteristics were largely comparable between TPOAb+ and TPOAb- patients (**Table 1**), apart from age, with TPOAb+ patients slightly older than TPOAb- patients (mean [SD] age, 50.2 [7.7] years versus 48.8 [8.5] years, respectively; P=0.005).

Plasma material was sufficient to determine FT4 and TSH values in 1974/1974 (100%) and 1971/1974 (99.8%) samples respectively. Among the 1974 patients, 1760 (89.2%) were euthyroid, 96 (4.9%) hypothyroid and 118 (6.0%) hyperthyroid; all 3 subgroups had similar baseline characteristics (**Table 1**), apart from age, with hypothyroid and hyperthyroid patients slightly older than euthyroid patients (mean [SD] age, respectively 50.5 [6.6] years and 50.7 [7.6] years, versus 48.9 [8.5] years; P=0.03).

As shown in **Supplemental Fig. 2**, FT4 and TSH were inversely correlated (Spearman rank, -0.23; P<0.001) and TPOAb was positively associated with TSH (Spearman rank, 0.24; P<0.001). The inverse correlation between TPOAb and FT4 was weak (Spearman rank, -0.04;

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182   P=0.09). TPOAb+ cases were more prevalent among hypothyroid and hyperthyroid patients

183   compared with the euthyroid group (73/96 [76.0%] hypothyroid; 45/118 [38.1%] hyperthyroid;

184   288/1760 [16.4%] euthyroid; P<0.001).

185

186   **TPOAb and BC prognosis**

187   The majority of DFS events were related to distant recurrence in both TPOAb+ and

188   TPOAb- groups (**Supplemental Table 2**). There was no evidence of a difference in DFS

189   between TPOAb+ and TPOAb- patients (unadjusted-HR: 0.97, 95% CI: 0.78-1.19, P=0.75,

190   **Fig. 1A**; adjusted-HR: 1.00, 95% CI: 0.81-1.24, P=0.98, **Table 2**). Subgroup analyses showed

191   no evidence of any significant interaction effects (**Fig. 2**). Similarly, there was no evidence of

192   a difference by TPOAb status on OS (unadjusted-HR: 0.86, 95% CI: 0.66-1.11, P=0.24, **Fig.**

193   **1B**; adjusted-HR: 0.89, 95% CI: 0.69-1.14, P=0.35, not shown) and TTR (unadjusted-HR: 0.97,

194   95% CI: 0.78-1.21, P=0.80, **Fig. 1C**; adjusted-HR: 1.02, 95% CI: 0.81-1.27, P=0.89, not

195   shown). TPOAb+ tertiles showed no evidence of a prognostic effect in both univariate (**Fig. 3**)

196   and multivariable (data not shown) analyses for DFS, OS and TTR.

197   Two sensitivity analyses included 126 node+ patients not treated with radiotherapy,

198   similar to Fiore *et al.* cohort [16], and 123 patients with blood taken before any adjuvant

199   therapy. The median (IQR; range) time of blood collection after surgery was 12.4 (4.9-21.6;

200   0.7–47.2) months and 1.1 (0.9-1.4; 0.5-5.9) months, respectively. There was no evidence of a

201   significant impact on DFS by TPOAb status in either of the two analyses, with unadjusted-HRs

202   of 1.48 (95% CI, 0.68-3.25; P=0.32) and 0.83 (95% CI, 0.35-2.03; P=0.69) respectively.

203

204   **Thyroid function and BC prognosis**

205   There was no evidence of a significant difference for DFS, OS and TTR by thyroid

206   function status in either univariate (**Fig. 4**) or multivariable (data not shown) analyses, and

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207 when considering FT4 and TSH separately (DFS, **Supplemental Table 3**; OS and TTR, not

208 shown).

209

210 **DISCUSSION**

211   In this large cohort of moderate-high risk early BC patients receiving adjuvant systemic

212 treatments we found that neither the presence nor the titre of plasma TPOAb, assessed after

213 BC diagnosis and measured with standard assays, had a substantial impact on long-term

214 recurrence or mortality; similar findings were observed for thyroid status. These results

215 confirm one previous finding [19], but contrast with two other studies [15,16]. We believe that

216 our study is reliable, considering that our patient cohort is the largest to date, with one of the

217 longest follow-ups, and focused on a well-defined BC population. Previous studies used

218 smaller patient cohorts with shorter follow-ups [15,16,19], mixed different BC stages [19], or

219 provided no information about BC stage [15], histological [15,19] and molecular subtypes

220 [15,16,19], and adjuvant treatments received [15,19]; they may be susceptible to bias and

221 random findings. In addition, the BC population analysed in this study is very similar to that

222 of Fiore *et al.,* who recruited non-metastatic aggressive BC all treated with chemotherapy [16].

223   The long survival of our patient cohort could obscure a minor prognostic effect of

224 TPOAb and/or thyroid function on BC, hypothetically detectable only among patients not

225 suitable for standard treatments (e.g. medical contraindications) and targeted therapies (e.g.

226 triple negative BC). This is possible but unlikely, since our exploratory analysis conducted

227 among different BC subtypes confirmed our negative results. Furthermore, the multivariable

228 analyses confirmed nodal status and tumour size as the two most important BC prognostic

229 factors [28], proving that the cohort used was appropriate for the research question, and the

230 model reasonably sensitive. Similarly, the better BC prognosis characterizing the intermediate

231 age group (50-59 years) is consistent with the results of a recent large cohort study [29].

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232   Our study cannot exclude a role of different TA parameters on BC prognosis, i.e. the

233   presence of goitre [15] or incidental TA-related ^{18}F-FDG PET/CT uptake [18]. Furthermore,

234   differences in the alternative splicing of TPO in the breast as compared to the thyroid have

235   been described [25], therefore this might also result in different TPO epitopes being targeted.

236   TPOAb prevalence in our cohort, similar to our *a priori* predicted value, reflects

237   TPOAb prevalence among women of general population [26,30], increasing with age [26,31].

238   It remains possible that TPOAb+ rates are higher in the BC population, as our study was not

239   designed to compare TPOAb prevalence among BC patients and the general population.

240   The principal limitations of the present study are the lack of clinical history for thyroid

241   diseases or medications and that, similarly to previous studies [15,19], blood was mainly

242   collected during/after adjuvant BC therapy. The first limitation might influence the prognostic

243   role of thyroid function, but marginally of TPOAb, since they should exert an effect when

244   either pre-existing, or appearing at a later time [32]; however, the evidence that thyroid function

245   influences BC outcome is weak [6]. The finding of more cases of hyper- (6.0%) than hypo-

246   thyroidism (4.9%) may reflect over-treatment with levothyroxine in some individuals.

247   Regarding BC adjuvant treatments, an increased risk of hypothyroidism after

248   chemotherapy [33,34] or radiotherapy [35,36] for BC has been suggested in a few small

249   studies, but not confirmed by others [37]. Tamoxifen can exert a modulation of thyroid

250   function, mainly via an anti-thyroid effect [38,39] and the stress related to the surgical

251   procedure itself has been suggested to cause immunomodulation [40]. However no clear large-

252   scale effects of adjuvant treatments for BC, including trastuzumab, on thyroid function and

253   immunity have been described, and our sensitivity analysis in a subgroup of 123 patients in

254   whom blood was collected before BC adjuvant therapy showed no evidence of TPOAb

255   prognostic ability, even if the wide 95% CI suggests a lack of statistical power.

256   To draw definitive conclusions, a prospective study collecting blood before cancer

257   treatments would be ideal, but difficult to realise because of the large patient number required,

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258 as shown by our *a priori* power calculation. Furthermore, this study analysed moderate-high

259 risk early BC only. BC is a heterogeneous disease, with many subtypes characterised by

260 different clinical behaviour and prognosis; it could be possible that TPOAb and/or thyroid

261 function affect the prognosis of certain specific BC subtypes and stages only, therefore they

262 should be all investigated separately, with a much higher total patient numbers required to

263 reach significant and definitive results.

264   In conclusion, the present study is to our knowledge the largest currently available

265 investigating the impact of blood TPOAb and thyroid function on BC prognosis, providing a

266 detailed description of the BC population analysed, and therefore representing a key-work to

267 clarify this debate over decades. We found that TPOAb and thyroid function, both measured

268 with standard assays and after BC diagnosis, appear not to influence substantially the long-

269 term recurrence and mortality of moderate-high risk early BC in the modern era. Major

270 confounding in this conclusion due to BC treatments seems unlikely. Future studies might

271 explore different BC stages and/or specific subtypes, also searching for non-conventional or

272 breast-specific immune responses to particular TPO epitopes, to determine whether aspects of

273 TA other than standard TPOAb and thyroid function may be relevant to BC outcome.

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414

TABLES

Table 1: Baseline characteristics and treatments for breast cancer by autoantibodies to thyroid peroxidase (TPOAb) and thyroid function status

	TPOAb- N = 1568	TPOAb+ N = 406	P value	Hypothyroid N = 96	Euthyroid N = 1760	Hyperthyroid N = 118	P value
Age (years): mean (SD)	48.8 (8.5)	50.2 (7.7)	0.005[a]	50.5 (6.6)	48.9 (8.5)	50.7 (7.6)	0.03[d]
Age group (years): n (%)							
<40	257 (16.4)	49 (12.1)		8 (8.3)	287 (16.3)	11 (9.3)	
40-49	575 (36.7)	151 (37.2)	0.08[b]	36 (37.5)	647 (36.8)	43 (36.4)	0.62[b]
50-59	590 (37.6)	167 (41.1)		45 (46.9)	657 (37.3)	55 (46.6)	
≥60	146 (9.3)	39 (9.6)		7 (7.3)	169 (9.6)	9 (7.6)	
Nodal status: n (%)							
Node negative	314 (20.0)	93 (22.9)		18 (18.8)	367 (20.9)	22 (18.6)	
1-3 positive nodes	719 (45.9)	171 (42.1)	0.62[b]	33 (34.4)	808 (45.9)	49 (41.5)	0.61[b]
≥4 positive nodes	535 (34.1)	142 (35.0)		45 (46.9)	585 (33.2)	47 (39.8)	
Tumour grade: n (%)							
Grade 1	77 (4.9)	23 (5.7)		4 (4.2)	88 (5.0)	8 (6.8)	
Grade 2	603 (38.5)	155 (38.2)	0.74[b]	35 (36.5)	681 (38.7)	42 (35.6)	0.72[b]
Grade 3	883 (56.3)	228 (56.2)		57 (59.4)	986 (56.0)	68 (57.6)	
Unknown	5 (0.3)	0 (0.0)		0 (0.0)	5 (0.3)	0 (0.0)	
Tumour size (cm): n (%)							
≤2	578 (36.9)	147 (36.2)		25 (26.0)	659 (37.4)	41 (34.8)	
>2 and ≤5	857 (54.7)	220 (54.2)	0.59[b]	61 (63.5)	952 (54.1)	64 (54.2)	0.38[b]
>5	132 (8.4)	39 (9.6)		10 (10.4)	148 (8.4)	13 (11.0)	
Unknown	1 (0.1)	0 (0.0)		0 (0.0)	1 (0.1)	0 (0.0)	
ER & HER2 status: n (%)							
ER+	1107 (70.6)	289 (71.2)		69 (71.9)	1248 (70.9)	79 (67.0)	
& HER2+	198 (12.6)	49 (12.1)		13 (13.5)	220 (12.5)	14 (11.9)	
& HER2-	772 (49.2)	201 (49.5)		46 (47.9)	873 (49.6)	54 (45.8)	
& HER2 unknown	137 (8.7)	39 (9.6)	0.85[c] (ER) 0.45[c] (HER2)	10 (10.4)	155 (8.8)	11 (9.3)	0.62[c] (ER) 0.84[c] (HER2)
ER-	461 (29.4)	117 (28.8)		27 (28.1)	512 (29.1)	39 (33.1)	
& HER2+	118 (7.5)	43 (10.6)		8 (8.3)	141 (8.0)	12 (10.2)	
& HER2-	289 (18.4)	61 (15.0)		15 (15.6)	313 (17.8)	22 (18.6)	
& HER2 unknown	54 (3.4)	13 (3.2)		4 (4.2)	58 (3.3)	5 (4.2)	
Molecular subgroup: n (%)							
ER+/HER2-[1]	784 (50.0)	203 (50.0)		47 (49.0)	885 (50.3)	55 (46.6)	
HER2+	316 (20.2)	92 (22.7)	0.40[c]	21 (21.9)	361 (20.5)	26 (22.0)	0.94[c]
Triple negative	277 (17.7)	59 (14.5)		14 (14.6)	301 (17.1)	21 (17.8)	

	TPOAb- N = 1568	TPOAb+ N = 406	P value	Hypothyroid N = 96	Euthyroid N = 1760	Hyperthyroid N = 118	P value
Type of surgery and radiotherapy use: n (%)			0.74[c] (surgery) 0.61[c] (radiotherapy)				0.99[c] (surgery) 0.33[c] (radiotherapy)
Mastectomy	854 (54.5)	225 (55.4)		53 (55.2)	962 (54.7)	64 (54.2)	
with radiotherapy ^	688 (80.6)	177 (78.7)		47 (88.7)	772 (80.2)	46 (71.9)	
Wide local excision	714 (45.5)	181 (44.6)		43 (44.8)	798 (45.3)	54 (45.8)	
with radiotherapy #	704 (98.6)	176 (97.2)		41 (95.3)	787 (98.6)	52 (96.3)	
Endocrine treatment in ER+ patients: n (%)*							
Tamoxifen monotherapy	696 (62.9)	167 (57.8)	0.13[c]	43 (62.3)	772 (61.9)	48 (60.8)	0.09[c]
Tamoxifen followed by AI	354 (32.0)	100 (34.6)		20 (29.0)	409 (32.8)	25 (31.7)	
AI monotherapy	46 (4.2)	15 (5.2)		6 (8.7)	53 (4.3)	2 (2.5)	
No endocrine treatment/unknown	11 (1.0)	7 (2.4)		0 (0.0)	14 (1.1)	4 (5.1)	
Trastuzumab in HER2+ patients: n (%)**			0.36[c]				0.71[c]
Yes	40 (12.7)	8 (8.7)		1 (4.8)	44 (12.2)	3 (11.5)	
No/Not known	276 (87.3)	84 (91.3)		20 (95.2)	317 (87.8)	23 (88.5)	
Chemotherapy: n (%)							
Control (FEC)	498 (31.8)	128 (31.5)	0.52[c]	27 (28.1)	568 (32.3)	31 (26.3)	0.90[c]
Control (E-CMF)	271 (17.3)	61 (15.0)		16 (16.7)	301 (17.1)	15 (12.7)	
FEC-D	799 (51.0)	217 (53.4)		53 (55.2)	891 (50.6)	52 (44.1)	

417   [1] includes ER-, PgR+, HER2-
418   ^ denominators calculated using patients treated with mastectomy
419   # denominators calculated using patients treated with wide local excision
420   * denominators calculated using ER+ patients
421   ** denominators calculated using HER2+ patients
422   [a] t-test
423   [b] trend test; note "unknowns" excluded from the test
424   [c] Fisher's exact test
425   [d] ANOVA
426
427   AI, aromatase-inhibitors; ER+, positive estrogen receptor (ER); ER-, negative ER; E-CMF, epirubicin 100 mg/m^2 for 4 cycles followed by CMF
428   (cyclophosphamide 600 mg/m^2, methotrexate 40 mg/m^2 and fluorouracil 600 mg/m^2) for 4 cycles; FEC, fluorouracil 600 mg/m^2, epirubicin 60 mg/m^2 and
429   cyclophosphamide 600 mg/m^2 for 8 cycles; FEC-D, FEC for 4 cycles followed by docetaxel 100 mg/m^2 for 4 cycles; HER2+, positive human epidermal growth
430   factor receptor-2 (HER2); HER2-, negative HER2; PgR+, positive progesterone receptor (PgR); SD, standard deviation; TPOAb+, positive TPOAb; TPOAb-,
431   negative TPOAb; triple negative, negative HER2, ER and PgR.

432 **Table 2: Multivariable analysis for disease-free survival by dichotomized autoantibodies to**

433 **thyroid peroxidase (TPOAb)**

434

		HR	95% CI	P value
TPOAb status	negative (n=1568)	1.00	-	-
	positive (n=406)	1.00	0.81-1.24	0.98
Nodal status	positive (n=1567)	1.00	-	-
	negative (n=407)	0.49	0.37-0.64	< 0.001
HER2 status	negative (n=1323)	1.00	-	-
	positive (n=408)	1.19	0.97-1.46	0.09
	unknown (n=243)	0.93	0.71-1.23	0.63
Age group (years)	<40 (n=306)	1.00	-	-
	40-49 (n=726)	0.78	0.61-1.00	0.05
	50-59 (n=757)	0.75	0.59-0.96	0.02
	≥60 (n=185)	0.95	0.69-1.31	0.76
Tumour grade	Grade 1 (n=100)	1.00	-	-
	Grade 2 (n=758)	1.15	0.74-1.78	0.55
	Grade 3 (n=1111)	1.39	0.89-2.17	0.14
	unknown (n=5)	0.77	0.10-5.75	0.80
Tumour size (cm) *	≤2 (n=725)	1.00	-	-
	>2 and ≤5 (n=1077)	1.37	1.12-1.66	0.002
	>5 (n=171)	1.88	1.41-2.52	< 0.001
Type of surgery	Mastectomy (n=1079)	1.00	-	-
	WLE (n=895)	0.79	0.66-0.95	0.01

435
436 HER2, human epidermal growth factor receptor-2; HR, hazard ratio (HR <1 indicates a favorable
437 breast cancer outcome); WLE, wide local excision; 95% CI, 95% confidence interval.
438 * The patient with unknown tumour size (n=1) has not been considered for this analysis.
439

22

440 **FIGURES**

441

442 **Fig. 1: Univariate analyses by dichotomized autoantibodies to thyroid peroxidase**

443 **(TPOAb)**
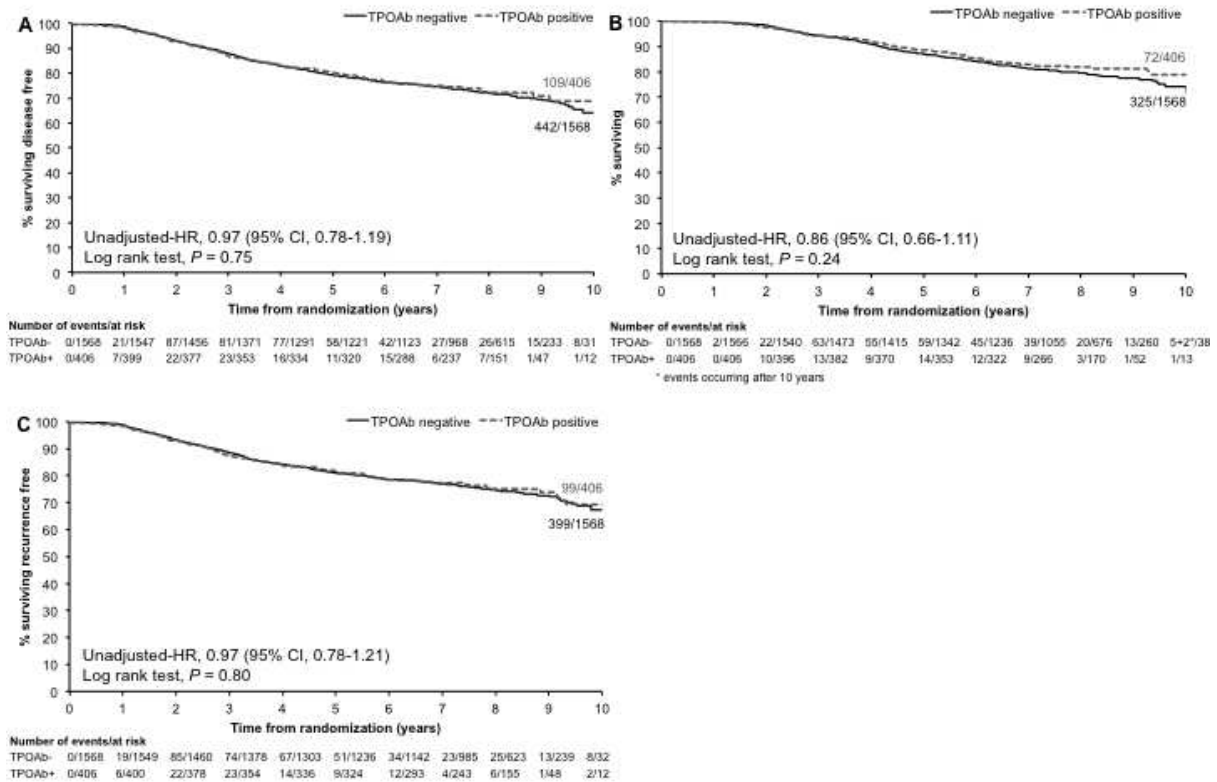
444

445 Kaplan-Meier curves relative to breast cancer (BC) outcome (median follow-up 96.7 months)

446 in patients positive (≥6 kIU/L) and negative (<6 kIU/L) for TPOAb. HR, hazard ratio (HR <1

447 indicates a favorable BC outcome); 95% CI, 95% confidence interval. Panel A: disease-free

448 survival (DFS). Panel B: overall survival (OS). Panel C: time to recurrence (TTR).

449

Fig. 2: Exploratory subgroup analyses for disease-free survival by dichotomized autoantibodies to thyroid peroxidase (TPOAb)
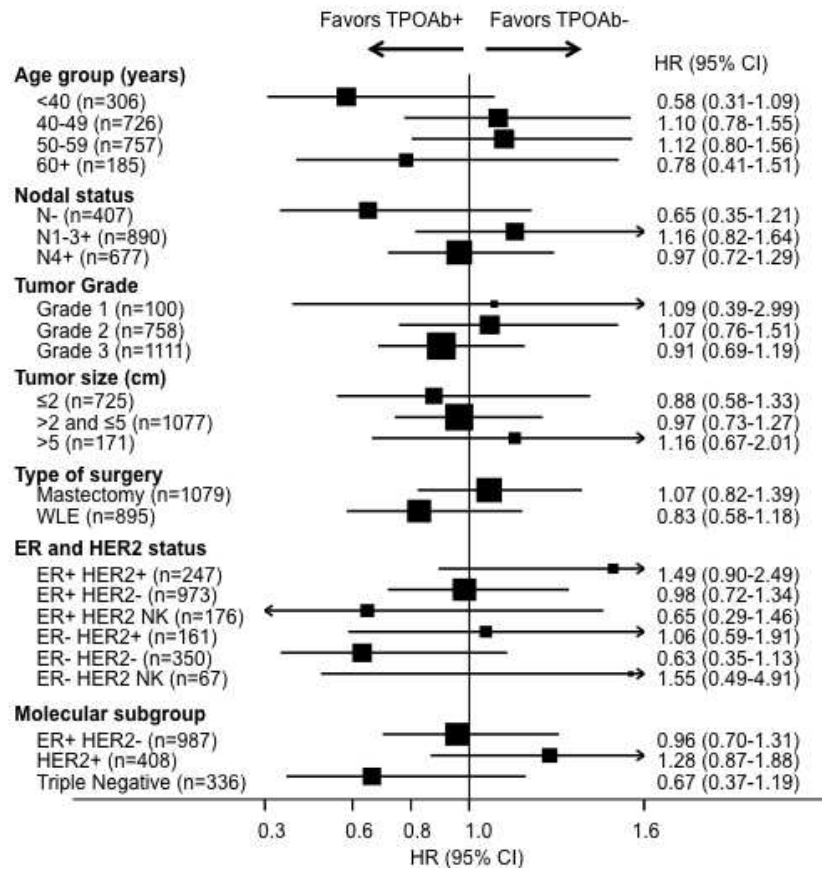
ER+, positive estrogen receptor (ER); ER-, negative ER; HER2+, positive human epidermal growth factor receptor-2 (HER2); HER2-, negative HER2; NK, not known; N-, lymph-node negative; N1-3+, 1-3 lymph-nodes positive, N4+, 4 or more lymph-nodes positive; TPOAb+, positive TPOAb; TPOAb-, negative TPOAb; triple negative, negative HER2, ER and progesterone receptor; WLE, wide local excision; 95% CI, 95% confidence interval.

24

Fig. 3: Univariate analyses by autoantibodies to thyroid peroxidase (TPOAb) categorized

460 **into tertiles**

Panel A data table:

	Unadjusted HR	95%CI	P value
TPOAb negative (Neg)	1.00	-	-
TPOAb positive (T1)	0.97	0.69 - 1.36	0.86
TPOAb positive (T2)	1.06	0.76 - 1.48	0.73
TPOAb positive (T3)	0.88	0.62 - 1.25	0.47

Panel B data table:

	Unadjusted HR	95%CI	P value
TPOAb negative (Neg)	1.00	-	-
TPOAb positive (T1)	0.90	0.60 - 1.35	0.61
TPOAb positive (T2)	1.03	0.70 - 1.53	0.87
TPOAb positive (T3)	0.67	0.42 - 1.05	0.08

Panel C data table:

	Unadjusted HR	95%CI	P value
TPOAb negative (Neg)	1.00	-	-
TPOAb positive (T1)	0.96	0.68 - 1.37	0.84
TPOAb positive (T2)	1.15	0.82 - 1.61	0.43
TPOAb positive (T3)	0.82	0.56 - 1.20	0.31

461

462 Kaplan-Meier curves relative to breast cancer (BC) outcome (median follow-up 96.7 months)

463 in patients negative (<6 kIU/L) and positive for TPOAb categorized into tertiles: 6-40 kIU/L

464 (T1), 41-238 kIU/L (T2), 240-2000 kIU/L (T3). HR, hazard ratio (HR <1 indicates a favorable

465 BC outcome); 95% CI, 95% confidence interval. Panel A: disease-free survival (DFS). Panel

466 B: overall survival (OS). Panel C: time to recurrence (TTR).

467

Fig. 4: Univariate analyses by thyroid function status

A	Unadjusted HR	95% CI	P value
Hypothyroid	1.15	0.79 - 1.68	0.46
Euthyroid	1.00	-	-
Hyperthyroid	1.14	0.82 - 1.61	0.44

B	Unadjusted HR	95% CI	P value
Hypothyroid	1.13	0.73 - 1.76	0.58
Euthyroid	1.00	-	-
Hyperthyroid	1.17	0.79 - 1.74	0.43

C	Unadjusted HR	95% CI	P value
Hypothyroid	1.24	0.85 - 1.82	0.27
Euthyroid	1.00	-	-
Hyperthyroid	1.18	0.82 - 1.67	0.37
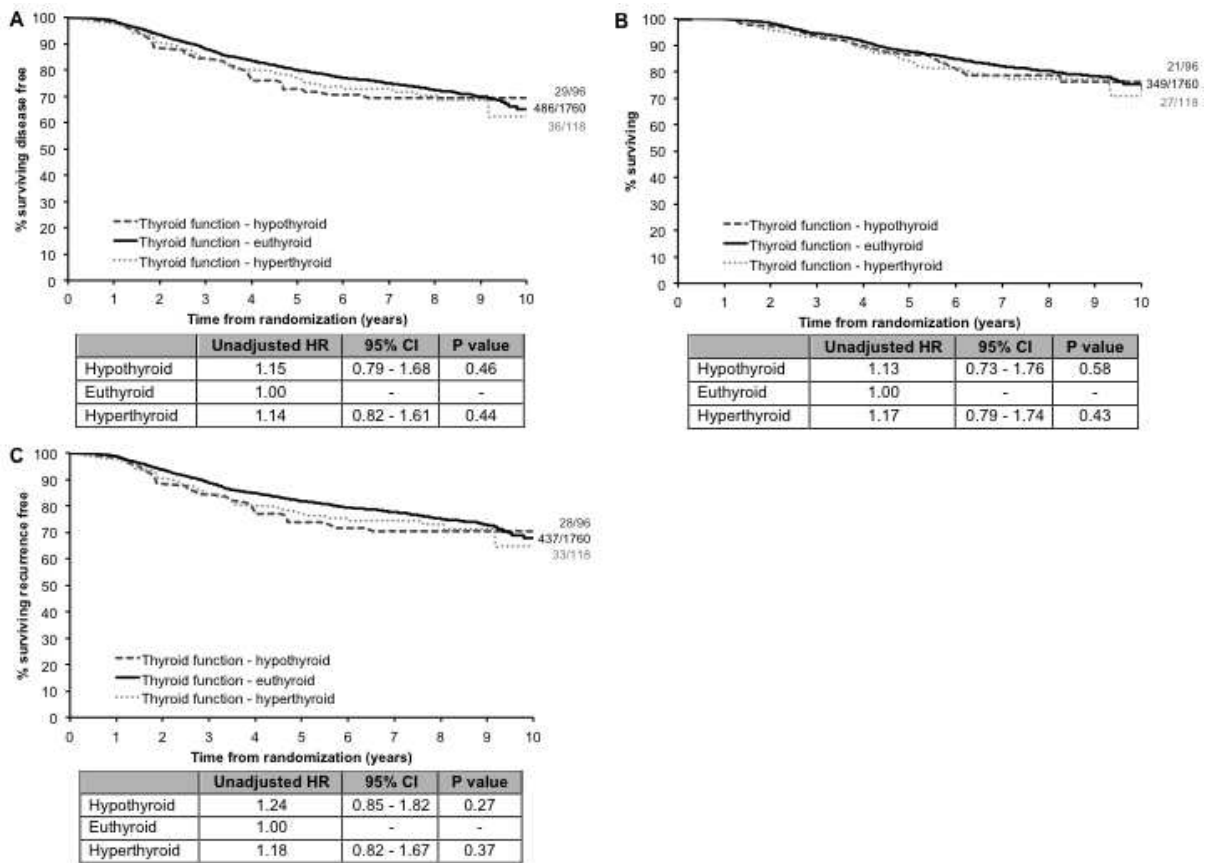
469

470 Kaplan-Meier curves relative to breast cancer (BC) outcome (median follow-up 96.7 months)

471 according to thyroid function status. Euthyroid, free-thyroxine (FT4) 9.0–19.1 pmol/L and

472 thyrotropin (TSH) 0.30–4.40 mIU/L; hyperthyroid, FT4 >19.1 pmol/L and/or TSH <0.3

473 mIU/L; hypothyroid, FT4 <9.0 pmol/L and/or TSH >4.40 mIU/L. HR, hazard ratio (HR <1

474 indicates a favorable BC outcome); 95% CI, 95% confidence interval. Panel A: disease-free

475 survival (DFS). Panel B: overall survival (OS). Panel C: time to recurrence (TTR).

476

SUPPLEMENTAL MATERIAL

478

479 **Supplemental Table 1: Baseline characteristics, treatments for breast cancer and disease-free**

480 **survival (DFS) related characteristics**

481

	Analysis population N = 1974	Not included patients N = 2188	All TACT trial patients N = 4162
Age (years), mean (SD)	49.1 (8.4)	48.2 (8.6)	48.6 (8.5)
Age group (years), n (%)			
<40	306 (15.5)	412 (18.8)	718 (17.3)
40-49	726 (36.8)	841 (38.4)	1567 (37.7)
50-59	757 (38.4)	730 (33.4)	1487 (35.7)
≥60	185 (9.4)	205 (9.4)	390 (9.4)
Nodal status, n (%)			
Node negative	407 (20.6)	428 (19.6)	835 (20.1)
1-3 positive nodes	890 (45.1)	949 (43.4)	1839 (44.2)
≥4 positive nodes	677 (34.3)	811 (37.1)	1488 (35.8)
Tumor grade, n (%)			
Grade 1	100 (5.1)	129 (5.9)	229 (5.5)
Grade 2	758 (38.4)	778 (35.6)	1536 (36.9)
Grade 3	1111 (56.3)	1271 (58.1)	2382 (57.2)
Unknown	5 (0.3)	10 (0.5)	15 (0.4)
Tumor size (cm), n (%)			
≤2	725 (36.7)	711 (32.5)	1436 (34.5)
>2 and ≤5	1077 (54.6)	1253 (57.3)	2330 (56.0)
>5	171 (8.7)	221 (10.1)	392 (9.4)
Unknown	1 (0.1)	3 (0.1)	4 (0.1)
ER & HER2 status, n (%)			
ER+	1396 (70.7)	1479 (67.6)	2875 (69.1)
& HER2+	247 (12.5)	247 (11.3)	494 (11.9)
& HER2-	973 (49.3)	990 (45.2)	1963 (47.2)
& HER2 unknown	176 (8.9)	242 (11.1)	418 (10.0)
ER-	578 (29.3)	709 (32.4)	1287 (30.9)
& HER2+	161 (8.2)	194 (8.9)	355 (8.5)
& HER2-	350 (17.7)	411 (18.8)	761 (18.3)
& HER2 unknown	67 (3.4)	104 (4.8)	171 (4.1)
Molecular subgroup, n (%)			
ER+/HER2-*	987 (50.0)	1014 (46.3)	2001 (48.1)
HER2+	408 (20.7)	441 (20.2)	849 (20.4)
Triple negative	336 (17.0)	387 (17.7)	723 (17.4)
Type of surgery and radiotherapy, n (%)			
Mastectomy	1079 (54.7)	1186 (54.2)	2265 (54.4)
with radiotherapy	865 (43.8)	949 (43.4)	1814 (43.6)
breast	159 (8.1)	254 (11.6)	413 (9.9)
chest wall	709 (35.9)	693 (31.7)	1402 (33.7)
supraclavicular fossa	480 (24.3)	500 (22.9)	980 (23.5)
axilla	85 (4.3)	103 (4.7)	188 (4.5)
Wide local excision	895 (45.3)	1002 (45.8)	1897 (45.6)
with radiotherapy	880 (44.6)	961 (43.9)	1841 (44.2)
breast	856 (43.4)	921 (42.1)	1777 (42.7)
chest wall	31 (1.6)	47 (2.1)	78 (1.9)
supraclavicular fossa	291 (14.7)	283 (12.9)	574 (13.8)
axilla	103 (5.2)	70 (3.2)	173 (4.2)
Endocrine treatment in ER+ patients, n (%)			
Tamoxifen monotherapy	863 (61.8)	927 (62.7)	1790 (62.3)

	Analysis population N = 1974	Not included patients N = 2188	All TACT trial patients N = 4162
Tamoxifen followed by AI	454 (32.5)	439 (29.7)	893 (31.1)
AI monotherapy	61 (4.4)	76 (5.1)	137 (4.8)
No endocrine treatment/unknown	18 (1.3)	37 (2.5)	55 (1.9)
Trastuzumab in HER2+ patients, n (%)			
Yes	48 (11.8)	28 (6.4)	76 (9.0)
No/Not known	360 (88.2)	413 (93.7)	773 (91.0)
Chemotherapy, n (%)			
Control (FEC)	626 (31.7)	639 (29.2)	1265 (30.4)
Control (E-CMF)	332 (16.8)	492 (22.5)	824 (19.8)
FEC-D	1016 (51.5)	1057 (48.3)	2073 (49.5)
Number of patients with event contributing to DFS analysis	**551 (27.9)**	**778 (35.6)**	**1329 (31.9)**
Local recurrence	76 (3.8)	107 (4.9)	183 (4.4)
Distant recurrence	405 (20.5)	572 (26.1)	977 (23.5)
New breast disease	43 (2.2)	44 (2.0)	91 (2.2)
Death from other cause (no recurrence)	27 (1.4)	51 (2.3)	78 (1.9)
Distant relapse ever reported	**462 (23.4)**	**655 (29.9)**	**1117 (26.8)**
New breast disease ever reported	**57 (2.9)**	**67 (3.1)**	**124 (3.0)**
All non-breast cancer second primary	**52 (2.6)**	**54 (2.5)**	**106 (2.5)**
All deaths	**397 (20.1)**	**620 (28.3)**	**1017 (24.4)**
Breast cancer	369 (18.7)	568 (26.0)	937 (22.5)
Death from other causes	28 (1.4)	52 (2.4)	80 (1.9)
Cancer (non-breast)	15 (0.8)	21 (1.0)	36 (0.9)
Treatment toxicity	0	5 (0.2)	5 (0.1)
Other	13 (0.7)	26 (1.2)	39 (0.9)

482
483   * includes ER-, PgR+, HER2-
484
485   AI, aromatase-inhibitors; ER+, positive estrogen receptor (ER); ER-, negative ER; E-CMF, epirubicin
486   100 mg/m^2 for 4 cycles followed by CMF (cyclophosphamide 600 mg/m^2, methotrexate 40 mg/m^2 and
487   fluorouracil 600 mg/m^2) for 4 cycles; FEC, fluorouracil 600 mg/m^2, epirubicin 60 mg/m^2 and
488   cyclophosphamide 600 mg/m^2 for 8 cycles; FEC-D, FEC for 4 cycles followed by docetaxel 100 mg/m^2
489   for 4 cycles; HER2+, positive human epidermal growth factor receptor-2 (HER2); HER2-, negative
490   HER2; PgR+, positive progesterone receptor (PgR); SD, standard deviation; TACT, "Taxotere as
491   adjuvant chemotherapy trial"; TPOAb, autoantibodies to thyroid peroxidase.
492

493 **Supplemental Table 2: Events contributing to disease-free survival (DFS) and numbers of deaths**

494 **by dichotomized TPOAb status**

495

	TPOAb- (N = 1568) n (%)	TPOAb+ (N = 406) n (%)
Number of patients with event contributing to DFS analysis	**442 (28.2)**	**109 (26.8)**
Local recurrence	59 (3.8)	17 (4.2)
Distant recurrence	327 (20.9)	78 (19.2)
New breast disease	33 (2.1)	10 (2.5)
Death from other cause (no recurrence)	23 (1.5)	4 (1.0)
All deaths	**325 (20.7)**	**72 (17.7)**
Breast cancer	301 (19.2)	68 (16.7)
Death from other causes (without distant recurrence)	24 (1.5)	4 (1.0)
Cancer (non-breast)	14 (0.9)	1 (0.2)
Treatment toxicity	0 (0.0)	0 (0.0)
Other	9 (0.6)	3 (0.7)
Vascular (cardiac)	1 (0.1)	1 (0.2)
Vascular (cerebral)	1 (0.1)	0 (0.0)
Vascular (thromboembolic)	0 (0.0)	0 (0.0)
Respiratory	0 (0.0)	0 (0.0)
Accident, suicide, alcoholism	5 (0.3)	0 (0.0)
Infection (not treatment related)	0 (0.0)	1 (0.2)
Gastrointestinal bleed	0 (0.0)	0 (0.0)
Chronic liver disease	1 (0.1)	0 (0.0)
Unknown	2 (0.1)	1 (0.2)

496
497 TPOAb+, positive autoantibodies to thyroid peroxidase (TPOAb); TPOAb-, negative TPOAb.
498

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499 **Supplemental Table 3: Univariate analyses for disease-free survival by FT4 and TSH**
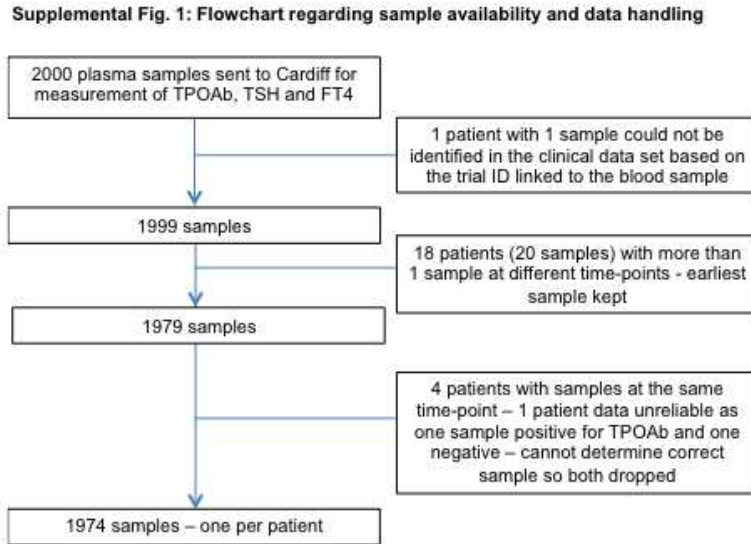
	Variable	Unadjusted HR	95% CI	P value
FT4	Continuous	1.00	0.96-1.04	0.91
	<9.0 pmol/L (Hypothyroid; n=13)	1.61	0.67-3.88	0.29
	9.0–19.1 pmol/L (Euthyroid; n=1917)	1.00	-	-
	>19.1 pmol/L (Hyperthyroid; n=44)	1.08	0.62-1.87	0.79
TSH*	Continuous	1.03	0.94-1.13	0.48
	>4.40 mIU/L (Hypothyroid; n=94)	1.08	0.73-1.59	0.71
	0.3-–4.40 mIU/L (Euthyroid; n=1781)	1.00	-	-
	<0.3 mIU/L (Hyperthyroid; n=96)	1.19	0.82-1.72	0.36

500
501 FT4, free-thyroxine; HR, hazard ratio (HR <1 indicates a favorable breast cancer outcome); TSH,
502 thyrotropin; 95% CI, 95% confidence interval.
503 *TSH value was available in 1971/1974 (99.8%) samples
504

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505   **Supplemental Fig. 1: Flowchart regarding sample availability and data handling**

Supplemental Fig. 1: Flowchart regarding sample availability and data handling

2000 plasma samples sent to Cardiff for measurement of TPOAb, TSH and FT4

1 patient with 1 sample could not be identified in the clinical data set based on the trial ID linked to the blood sample

1999 samples

18 patients (20 samples) with more than 1 sample at different time-points - earliest sample kept

1979 samples

4 patients with samples at the same time-point – 1 patient data unreliable as one sample positive for TPOAb and one negative – cannot determine correct sample so both dropped
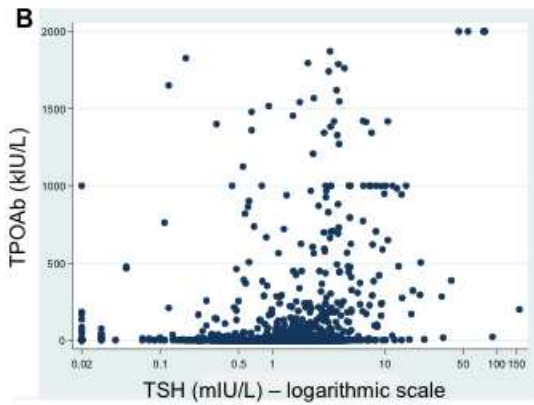
1974 samples – one per patient

506
507   FT4, free-thyroxine; TPOAb, autoantibodies to thyroid peroxidase; TSH, thyrotropin.
508

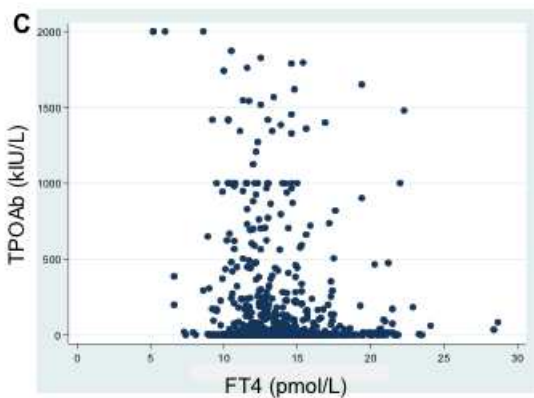509 **Supplemental Fig. 2: Correlation between thyroid markers**

Supplemental Fig. 2: Correlation between thyroid markers
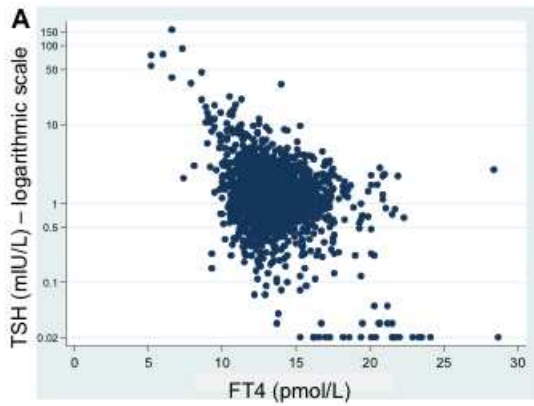
Spearman rank rho, -0.23; $P < 0.001$

Spearman rank rho, 0.24; $P < 0.001$

Spearman rank rho, -0.04; $P = 0.09$

510

511 FT4, free-thyroxine; TPOAb, autoantibodies to thyroid peroxidase; TSH, thyrotropin. Panel A:

512 inverse correlation between TSH (logarithmic scale) and FT4. Panel B: positive correlation

513   between TPOAb and TSH (logarithmic scale). Panel C: inverse correlation between TPOAb

514   and FT4.

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