

Keywords: prostate cancer; genetic predisposition; predictive model; PSA velocity; *BRCA1*; *BRCA2*

Prostate-specific antigen velocity in a prospective prostate cancer screening study of men with genetic predisposition

Christos Mikropoulos¹ *et al.*

Background: Prostate-specific antigen (PSA) and PSA-velocity (PSAV) have been used to identify men at risk of prostate cancer (PrCa). The IMPACT study is evaluating PSA screening in men with a known genetic predisposition to PrCa due to *BRCA1/2* mutations. This analysis evaluates the utility of PSA and PSAV for identifying PrCa and high-grade disease in this cohort.

Methods: PSAV was calculated using logistic regression to determine if PSA or PSAV predicted the result of prostate biopsy (PB) in men with elevated PSA values. Cox regression was used to determine whether PSA or PSAV predicted PSA elevation in men with low PSAs. Interaction terms were included in the models to determine whether *BRCA* status influenced the predictiveness of PSA or PSAV.

Results: 1634 participants had ≥ 3 PSA readings of whom 174 underwent PB and 45 PrCas diagnosed. In men with PSA $> 3.0 \text{ ng ml}^{-1}$, PSAV was not significantly associated with presence of cancer or high-grade disease. PSAV did not add to PSA for predicting time to an elevated PSA. When comparing *BRCA1/2* carriers to non-carriers, we found a significant interaction between *BRCA* status and last PSA before biopsy ($P = 0.031$) and *BRCA2* status and PSAV ($P = 0.024$). However, PSAV was not predictive of biopsy outcome in *BRCA2* carriers.

Conclusions: PSA is more strongly predictive of PrCa in *BRCA* carriers than non-carriers. We did not find evidence that PSAV aids decision-making for *BRCA* carriers over absolute PSA value alone.

Men with germline mutations in *BRCA2* have an increased risk of prostate cancer (PrCa), estimated at 2.5–8.6 fold increased risk for *BRCA2* mutation carriers (Breast Cancer Linkage Consortium, 1999; van Asperen *et al.*, 2005; Kote-Jarai *et al.*, 2011). There remains debate about whether there is an increased risk of PrCa associated with *BRCA1* mutations, with some studies reporting no increased risk to those reporting a 1.8–3.75 fold increased risk (Thompson *et al.*, 2002; Leongamornlert *et al.*, 2012; Moran *et al.*, 2012). A number of studies have reported that *BRCA2* mutation carriers have more aggressive disease, suggested by their younger age at diagnosis, higher rates of lymph node involvement and distant metastasis at diagnosis, and higher mortality rates compared with non-carriers (Tryggvadóttir *et al.*, 2007; Mitra *et al.*, 2008; Edwards *et al.*, 2010; Gallagher *et al.*, 2010; Thorne *et al.*, 2011; Castro *et al.*, 2013). There is increasing evidence that *BRCA1* mutation carriers may also harbour more aggressive disease (Giusti *et al.*, 2003; Gallagher *et al.*, 2010; Castro *et al.*, 2013). Furthermore,

BRCA2-mutant localised prostate cancer demonstrates increased genomic instability and a mutational profile that more closely resembles metastatic than localised disease, therefore supporting early detection in this at risk patient population (Taylor *et al.*, 2017).

General population prostate specific antigen (PSA) screening remains controversial due to an unclear balance of benefits, in terms of mortality reduction when compared to harms such as overdiagnosis and overtreatment. However, many expert groups continue to recommend PrCa screening with particular attention towards men with risk factors based on family history, genetics and/or race (Roobol *et al.*, 2013; Eeles *et al.*, 2014; Mikropoulos *et al.*, 2014; Murphy *et al.*, 2014).

It has previously been suggested that the rate of PSA change over time, or PSA velocity (PSAV), can be used to assist in differentiating between men with cancer from those with benign disease (Carter *et al.*, 1992; Berger *et al.*, 2005). Monitoring PSA

*Correspondence: Professor RA Eeles; E-mail: rosalind.eeles@icr.ac.uk

Received 17 July 2017; revised 3 November 2017; accepted 6 November 2017; published online 4 January 2018



over time could also improve the sensitivity of screening. It is also possible that PSAV could distinguish between men who might have advanced or aggressive disease that would require definitive treatment thus avoiding overdiagnosis and overtreatment (Carter *et al*, 2006; Carter *et al*, 2007).

However, the utility of PSAV in PrCa decision-making has been called into question. In particular, while PSAV may be predictive of biopsy outcome in univariate analyses, it has not been shown to improve the predictiveness of biopsy outcome over the absolute value of PSA (Roobol *et al*, 2004; Vickers *et al*, 2011; Loughlin, 2014). Although some studies have suggested that PSAV can be used to identify men with aggressive disease, these did not investigate whether calculation of PSAV provided additional information than the most recent PSA value (Carter *et al*, 1992; D'Amico *et al*, 2004; D'Amico *et al*, 2005). PSA and PSAV are highly correlated, and this may explain why PSAV does not add predictive value (Vickers *et al*, 2011). As a result of these considerations, PSAV has been removed from all major guidelines concerning the detection of prostate cancer.

It is currently unknown whether PSAV provides more information, beyond PSA absolute value, among a cohort of men considered to be at increased genetic risk of PrCa and aggressive disease. The IMPACT study (Identification of Men with a genetic predisposition to Prostate Cancer: Targeted screening in men at higher genetic risk and controls; www.impact-study.co.uk) is an international multi-centre study evaluating the role of targeted PSA screening in men with a *BRCA1* or *BRCA2* mutation and was established in 2005 (Bancroft *et al*, 2014). To date, ~3000 men have been recruited from 20 countries across the world. Men are followed up with annual (or biannual in the Dutch cohort) PSA screening for a minimum of 5 years within the study and this has produced a wealth of PSA results and follow-up data over time. The primary end-point of the IMPACT study is to determine the incidence, stage and pathology of screen-detected prostate cancer in the study population; a secondary end-point is to determine a profile of PSA level and its predictive value for the development of prostate cancer in the study population. The objective of the present study was to determine whether PSA values and/or PSAV were associated with PrCa and aggressive tumours among men at increased risk enrolled in the IMPACT trial.

MATERIALS AND METHODS

Patient selection. The design and eligibility criteria for the IMPACT study have been described elsewhere (Mitra *et al*, 2008; Bancroft *et al*, 2014). The protocol was approved by the West-Midlands Research and Ethics Committee in the UK (reference 05/MRE07/25), and subsequently by each participating institution's local committee. Briefly, men aged between 40 and 69 were recruited from families with a known pathogenic germline *BRCA1* or *BRCA2* mutation. Men were invited to enrol if they had tested positive (carriers) or negative for the familial mutation (*BRCA1/2* non-carriers), or if they were at 50% risk of inheriting a mutation but had not yet undergone predictive genetic testing. All participants provide written consent. Men with PrCa or with a prior diagnosis of another cancer with a prognosis of <5 years were excluded. In the Dutch centres, men were also excluded if they had PSA screening prior to study entry.

According to the IMPACT study design, men underwent annual PSA screening and those with a PSA >3.0 ng ml⁻¹ were referred for a 10- or 12-core transrectal ultrasound guided (TRUS) biopsy based on institutional clinic practices. Men with a PSA >3.0 ng/ml and a negative biopsy continue annual screening, with a repeat

biopsy recommended when PSA increased by >50%. Men were also referred for biopsy if they had a PSA ≤3.0 ng ml⁻¹ but clinical suspicion (e.g., abnormal digital rectal examination or clinical symptoms). After 5 years in the study, men at a subset of centres were also offered an elective biopsy.

PSA readings in the study are validated in a central laboratory to exclude inter-site variations. The results found a Spearman's agreement of 0.95 between study sites (Bancroft *et al*, 2014).

Statistical considerations. PSA velocity (PSAV) has been used as a marker to inform decisions about biopsy or about the timing of the next PSA screen. With respect to the former, we considered that a physician had the most recent PSA measurements available. Our study question was therefore whether adding PSAV to this data point improves prediction of presence of PrCa at biopsy. As elevated PSA is the primary indication in routine clinical practice, our main analysis was restricted to men who had any PSA ≥3.0 ng ml⁻¹ prior to biopsy. A sensitivity analysis was conducted including all men who underwent biopsy. We created logistic regression models, adjusted for last PSA measurement and age, for the outcomes of any grade and high-grade cancer. PSAV was calculated using three methods: arithmetic equation of change in PSA over time; linear regression; rate of PSA change using first and last values only. We also used cubic splines with knots at the tertiles to test for non-linearity in PSA and in PSAV.

To investigate whether the effect of PSAV on predicting biopsy outcome differed based on *BRCA* status, we included an interaction term between PSAV and *BRCA* status (*BRCA1* or *BRCA2* carriers vs *BRCA* non-carrier patients, and *BRCA2* carriers vs *BRCA1* and *BRCA* non-carrier patients). Due to a limited number of events, this analysis was performed only for the outcome of any cancer on biopsy. This analysis included 13 cancers diagnosed among 55 *BRCA1* carriers and 23 cancers among 65 *BRCA2* carriers.

To determine whether PSAV could aid decisions about screening frequency, e.g., whether a man with a high PSAV should receive a subsequent PSA test at a shorter interval than a man with low PSAV, we assessed whether PSAV was associated with having a future PSA >3.0 ng ml⁻¹. As a minimum of three PSA measurements are required for accurate estimation of PSAV, we created Cox proportional hazards models for the time from the first PSA measurement to the patient's third PSA measurement >3.0 ng ml⁻¹. Four models were then created: one including the third PSA measurement only, and the others including both the third PSA measurement and each of the three methodologies for calculating PSAV. Men who had a PSA >3.0 ng ml⁻¹ within the first three PSA measurements were excluded from this analysis. A total of 1086 men were included.

We planned to first evaluate the independent statistical significance of PSAV in models that also included absolute PSA level. If significant, we planned to estimate the improvement in concordance index afforded by PSAV after 10-fold cross-validation. All analyses were conducted using Stata 13.0 (Stata Corp., College Station, TX, USA).

RESULTS

Of the 2942 men recruited to the IMPACT study, 1654 men had three or more PSA measurements and appropriate clinical follow-up to be included in the analyses (Figure 1). Table 1 shows the demographic, PSA and biopsy grade characteristics of the analysis cohorts. The cohort of 1654 men consisted of 510 *BRCA1* mutation carriers, 584 *BRCA2* mutation carriers, 260 *BRCA1* non-carriers and 288 *BRCA2* non-carriers. Two men carried both a *BRCA1* and *BRCA2* mutation (included in the *BRCA2* group for genetic sub-analysis) and 10 had not yet had a predictive test for the *BRCA* mutation in their family (excluded from genetic sub-analysis). In

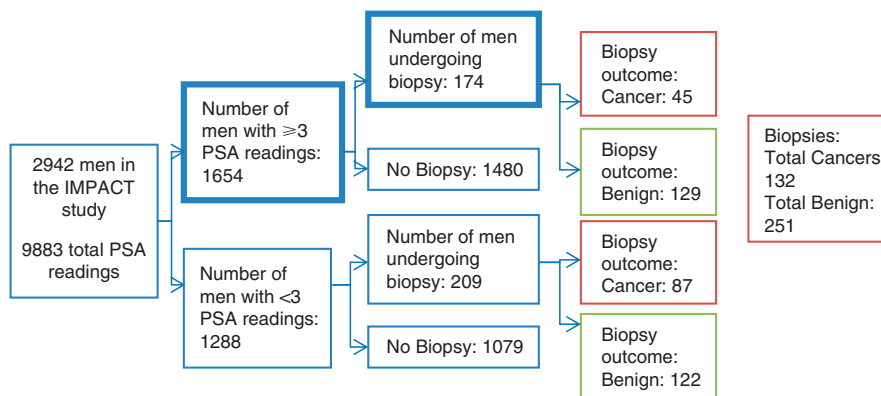


Figure 1. Consort diagram of study population. The two bolded cohorts were included for in-depth analysis, as they had 3 or more PSA values available for analysis and underwent a prostate biopsy.

Table 1. Patient characteristics				
	Total cohort N = 1654	BRCA2 N = 586 ^a	BRCA1 N = 510	Non-carrier controls (BRCA1 and BRCA2 negative) N = 548
Age at first PSA test	53 (46, 60)	51 (45, 59)	53 (46, 60)	54 (48, 61)
Patient underwent biopsy	174 (11%)	65 (11%)	55 (11%)	54 (10%)
Prior negative biopsy	26 (1.6%)	8 (1.4%)	8 (1.6%)	10 (1.8%)
Biopsy Gleason score				
≤6	24 (14%)	11 (17%)	6 (11%)	7 (13%)
7	15 (8.6%)	6 (9.2%)	6 (11%)	3 (5.6%)
>7	6 (3.4%)	5 (7.7%)	0 (0%)	1 (1.9%)
Negative biopsy	129 (74%)	43 (66%)	43 (78%)	43 (80%)
First PSA measurement (ng ml ⁻¹)	0.8 (0.5, 1.3)	0.80 (0.50, 1.20)	0.80 (0.50, 1.30)	0.89 (0.60, 1.40)
Last PSA measurement (ng ml ⁻¹)	0.9 (0.6, 1.6)	0.91 (0.59, 1.50)	0.88 (0.55, 1.70)	1.00 (0.60, 1.70)
Number of PSA tests before biopsy				
3	503 (30%)	163 (28%)	158 (31%)	178 (32%)
4	328 (20%)	123 (21%)	108 (21%)	96 (18%)
5	474 (29%)	160 (27%)	135 (26%)	174 (32%)
6	156 (9.4%)	62 (11%)	50 (10%)	44 (8.0%)
7	108 (6.5%)	36 (6.1%)	38 (7.5%)	34 (6.2%)
8 or more	85 (5.1%)	42 (7.2%)	21 (4.1%)	22 (4.0%)
Data are reported as median (interquartile range) or frequency (%).				
^a Includes 2 men who had both a BRCA1 and a BRCA2 mutation.				

this cohort, 174 men underwent prostate biopsy, with 45 men having any grade cancer of whom 21 having Gleason score 7 or higher (high-grade) cancer. Among men who had any PSA >3.0 ng ml⁻¹, 40 had any grade of whom 20 had high-grade cancer.

The median age at the first PSA of BRCA2 carriers was significantly younger than both BRCA1 carriers and non-carriers (51 vs 53 vs 54 years, respectively, $P < 0.0001$). Overall, BRCA2 and BRCA1 carriers had significantly lower first PSA values than non-carriers (0.80 vs 0.80 vs 0.89 ng ml⁻¹; $P = 0.022$; however, overall there was no statistically significant difference in the median PSAV between the BRCA2, BRCA1 and non-carrier groups ($P = 0.8$).

The median age at first PSA reading of men diagnosed with cancer was higher than that of men without cancer (60 vs 53 years, $U = 22069$, $z = -5.24$, $P < 0.001$). The median most recent PSA (i.e., PSA at diagnosis for cancer cases) was significantly higher for those with cancer compared with those without cancer (3.70 vs 0.90 ng ml⁻¹) $U = 8564$, $z = -9.34$, $P < 0.001$). The median PSAV was significantly higher for those with cancer vs those without cancer (medians: 0.56 vs 0.02 ng/ml/yr, $U = 9641$, $z = -9.012$, $P < 0.001$). Of those diagnosed with cancer, there was no significant difference between the proportion of BRCA2 carriers with a PSAV

(calculated by linear regression) >0.35 ng ml⁻¹ per year compared with BRCA1 carriers and non-carrier controls (78.3 vs 61.5 vs 53.8%, $p < 0.28$).

We next assessed whether adding PSAV to the most recent PSA measurement would improve the ability to determine which men should undergo biopsy. Using cubic splines, we investigated and found no evidence of non-linearity in PSA or in PSAV. Among men with any PSA measurements >3.0 ng/ml, PSAV was not significantly associated with either any grade or high-grade cancer after adjusting for most recent PSA measurement (Table 2). We repeated these analyses including all men who were biopsied, and found that PSAV was not statistically significant in any of the models (Table 3).

Additionally, we assessed whether PSAV affected the prediction of PrCa at biopsy differently based on BRCA status. When comparing BRCA1 and BRCA2 carriers to BRCA1/2 non-carriers, we found a significant interaction between BRCA status and the last PSA before biopsy ($P = 0.031$), however there was no evidence of an interaction between BRCA status and PSAV (Table 4). However, when comparing BRCA2 carriers to BRCA1 carriers and BRCA1/2 non-carriers, we found evidence of interactions between BRCA2 status and last PSA before biopsy ($P = 0.078$) and

Table 2. Models for any grade and high-grade cancers among men with any PSA measurement ≥ 3.0 ng/ml, N = 116

	Any grade cancer			High grade cancer		
	OR	95% CI	P value	OR	95% CI	P value
Age at biopsy	1.05	0.99, 1.12	0.13	1.08	0.99, 1.19	0.073
Last PSA measurement before biopsy	1.05	0.89, 1.23	0.6	1.26	1.04, 1.52	0.017*
Age at biopsy	1.05	0.98, 1.12	0.14	1.09	0.99, 1.19	0.065
Last PSA measurement before biopsy	1.08	0.89, 1.30	0.4	1.20	0.97, 1.49	0.10
PSA velocity (arithmetic equation)	0.91	0.69, 1.21	0.5	1.20	0.81, 1.76	0.4
Age at biopsy	1.06	0.99, 1.13	0.080	1.10	1.00, 1.20	0.047*
Last PSA measurement before biopsy	0.93	0.75, 1.17	0.5	1.12	0.88, 1.44	0.3
PSA velocity (linear regression)	1.92	0.92, 4.00	0.080	2.07	0.93, 4.61	0.076
Age at biopsy	1.06	0.99, 1.13	0.073	1.10	1.00, 1.21	0.042*
Last PSA measurement before biopsy	0.91	0.72, 1.16	0.4	1.09	0.83, 1.42	0.5
PSA velocity (first and last value)	2.01	0.94, 4.29	0.073	2.25	0.96, 5.28	0.063

Abbreviations: CI = confidence interval; PSA = prostate-specific antigen. All models were adjusted for age at biopsy and the last PSA measurement before biopsy. *Statistically significant.

Table 3. Models for any grade and high grade cancers among all men undergoing biopsy, N = 174

	Any grade cancer			High grade cancer		
	OR	95% CI	P value	OR	95% CI	P value
Age at biopsy	1.05	1.00, 1.11	0.051	1.08	1.00, 1.18	0.056
Last PSA measurement before biopsy	1.13	1.00, 1.28	0.058	1.35	1.14, 1.59	0.001*
Age at biopsy	1.05	1.00, 1.10	0.064	1.09	1.00, 1.18	0.050
Last PSA measurement before biopsy	1.17	1.01, 1.36	0.041*	1.29	1.06, 1.57	0.010*
PSA velocity (arithmetic equation)	0.88	0.67, 1.17	0.4	1.19	0.79, 1.78	0.4
Age at biopsy	1.06	1.00, 1.11	0.033*	1.10	1.01, 1.19	0.036*
Last PSA measurement before biopsy	1.01	0.84, 1.22	0.9	1.19	0.95, 1.50	0.14
PSA velocity (linear regression)	1.85	0.92, 3.71	0.085	2.09	0.95, 4.62	0.068
Age at biopsy	1.06	1.01, 1.11	0.030*	1.10	1.01, 1.20	0.033*
Last PSA measurement before biopsy	1.00	0.82, 1.22	>0.9	1.16	0.90, 1.49	0.2
PSA velocity (first and last value)	1.90	0.93, 3.89	0.080	2.24	0.97, 5.20	0.059

Abbreviations: CI = confidence interval; PSA = prostate-specific antigen. All models were adjusted for age at biopsy and the last PSA measurement before biopsy. *Statistically significant.

significant interactions between *BRCA2* status and PSAV calculated using the arithmetic equation and linear regression ($P = 0.024$ and $P = 0.049$ respectively, Table 4).

Based on these interactions, we performed subgroup analyses by *BRCA2* status. All models were adjusted for age at biopsy and last PSA before biopsy. Due to a limited number of events (26 cancers in *BRCA2* non-carriers and 23 in *BRCA2* carriers), these models were somewhat overfit. No evidence of an association between PSA and any grade cancer or PSAV and any grade cancer was seen in *BRCA2* carriers or non-carriers, likely due to the strong correlation between PSA and PSAV (Table 5).

We then investigated whether PSAV was associated with time to PSA ≥ 3.0 ng ml⁻¹. Using Cox proportional hazards models, we found no evidence of an association between PSAV and time from the third PSA measurement to PSA ≥ 3.0 ng ml⁻¹. Out of 1533 men who did not have a PSA ≥ 3.0 ng ml⁻¹ within the first three PSA tests, there were 28 who had a PSA ≥ 3.0 ng ml⁻¹ within 1 year, 50 within 2 years and 62 within 3 years.

DISCUSSION

This is the first study to show that there are differences in PSA values among men with different genetic backgrounds. These PSA differences could be used to identify those men considered to be at high genetic risk of more aggressive disease. However, when evaluated with absolute PSA values, PSAV did not appear to provide additional information for *BRCA1* or *BRCA2* carriers.

A major problem of PSA screening is that, in attempting to detect clinically significant disease, it is inevitable that indolent disease will also be detected leading to overdiagnosis. However, early diagnosis and identification of men with high-risk disease is important to prevent mortality from PrCa. This might be

particularly essential in light of recent publications indicating that men with a *BRCA1* or *BRCA2* mutation are at risk of more aggressive disease (Castro *et al*, 2013; Castro *et al*, 2015), early identification of those with clinically significant disease will be imperative. In view of the controversy about the role of PSAV in prostate screening in the general population, it was important to assess its role in *BRCA1* and *BRCA2* carriers and whether it added to the ability to detect clinically significant disease.

In this analysis of the IMPACT study cohort, we found *BRCA2* carriers on average to be screened at a relatively young age. This may account for lower overall PSA values for *BRCA2* carriers in this analysis compared to non-carrier controls. However, there were no differences in median PSAV between carriers and non-carriers. Given the possibility that higher PSAV may associate with aggressive PrCa (D'Amico *et al*, 2004; D'Amico *et al*, 2005), we would expect *BRCA2* carriers who are at risk of aggressive disease would exhibit higher PSAVs (Castro *et al*, 2013; Castro *et al*, 2015). *BRCA2* carriers in this group may be too young to demonstrate this trend at this point of follow up.

A single PSA reading over 3 ng ml⁻¹ was applied to guide biopsy decisions according to the IMPACT protocol, as well as if there was clinical suspicion on digital rectal examination or clinical symptoms. PSAV was not a good indicator in this analysis for distinguishing between those with any grade cancer and high-grade cancer when men were biopsied for either indication. It is possible that PSAV could be a good predictor of high-grade disease in men who had PSA values ≤ 2 ng ml⁻¹ (Kitagawa *et al*, 2014). However, due to the protocol's 3 ng/ml PSA threshold for prostate biopsy, we were limited in the number of cancers diagnosed when PSA was ≤ 2 ng ml⁻¹. Further follow up will be required to assess when additional cancers are diagnosed. As part of the IMPACT trial, there is an optional end of study biopsy regardless of PSA. This may help delineate PSAV among men diagnosed with PrCa with low PSA values (Carter *et al*, 1992; Kitagawa *et al*, 2014).

Table 4. Models for any grade cancer based on BRCA status (BRCA positive carriers vs BRCA negative non-carriers) and BRCA2 status (BRCA2 positive vs BRCA1 positive and BRCA negative)

	BRCA1 and BRCA2 vs BRCA non-carriers			BRCA2 vs BRCA1 and BRCA non-carriers		
	OR	95% CI	P value	OR	95% CI	P value
Age at biopsy	1.07	1.01, 1.13	0.017*	1.06	1.01, 1.12	0.022*
Last PSA measurement before biopsy	0.87	0.65, 1.16	0.3	0.99	0.81, 1.20	0.9
BRCA1 or BRCA2 Positive	0.40	0.10, 1.67	0.2	0.74	0.21, 2.65	0.6
Interaction between last PSA measurement and BRCA +	1.44	1.03, 2.02	0.031*	1.28	0.97, 1.69	0.078
Age at biopsy	1.06	1.00, 1.12	0.034*	1.06	1.01, 1.12	0.025*
Last PSA measurement before biopsy	1.14	0.97, 1.35	0.10	1.06	0.89, 1.27	0.5
PSA velocity using all PSAs (arithmetic equation)	0.77	0.51, 1.16	0.2	0.72	0.49, 1.08	0.11
BRCA1 or BRCA2 Positive	1.54	0.66, 3.61	0.3	1.07	0.43, 2.67	0.9
Interaction between PSAV and BRCA +	1.31	0.74, 2.34	0.4	2.63	1.13, 6.12	0.024*
Age at biopsy	1.07	1.01, 1.13	0.014*	1.07	1.02, 1.13	0.010*
Last PSA measurement before biopsy	0.97	0.81, 1.18	0.8	0.93	0.75, 1.15	0.5
PSA velocity using all PSAs (linear regression)	1.19	0.49, 2.89	0.7	1.46	0.69, 3.11	0.3
BRCA1 or BRCA2 Positive	0.99	0.37, 2.68	>0.9	0.93	0.34, 2.52	0.9
Interaction between PSAV and BRCA +	2.26	0.74, 6.90	0.2	3.26	1.01, 10.54	0.049*
Age at biopsy	1.07	1.01, 1.13	0.013*	1.07	1.02, 1.13	0.009*
Last PSA measurement before biopsy	0.97	0.79, 1.18	0.7	0.92	0.74, 1.16	0.5
PSA velocity using all PSAs (first and last value)	1.24	0.50, 3.07	0.6	1.48	0.68, 3.18	0.3
BRCA1 or BRCA2 Positive	1.01	0.37, 2.74	>0.9	0.92	0.34, 2.51	0.9
Interaction between PSAV and BRCA +	2.08	0.70, 6.20	0.2	2.96	0.97, 9.02	0.056

Abbreviations: CI = confidence interval; PSA = prostate-specific antigen. All models were adjusted for age at biopsy, last PSA measurement before biopsy, BRCA status and the interaction between PSA or PSA velocity and BRCA status. *Statistically significant.

A strength of this study is the unique patient cohort of men with a genetic predisposition to PrCa, in particular BRCA2 carriers who are predisposed to aggressive PrCas (Mitra *et al*, 2008; Narod *et al*, 2008; Castro *et al*, 2013; Castro *et al*, 2015). Within the group of BRCA2 carriers, PSAV proved predictive of any grade cancer, however, given the low number of cancers diagnosed overall it was not possible to assess whether PSAV was associated with high grade cancer and BRCA2 status. A high PSAV in an individual with a BRCA2 mutation could be used as an indicator of presence of PrCa and therefore as an indication for prostate biopsy. This model

Table 5. Models for any grade cancer by BRCA2 status (BRCA2 carriers vs BRCA1 carriers and BRCA1/2 non-carriers)

	BRCA2 Carriers (N = 65)			BRCA2 Non-carriers (N = 109)		
	OR	95% CI	P value	OR	95% CI	P value
Age at biopsy	1.02	0.95, 1.10	0.6	1.10	1.02, 1.19	0.011*
Last PSA measurement before biopsy	1.27	1.05, 1.54	0.013*	0.97	0.79, 1.19	0.8
Age at biopsy	1.03	0.95, 1.11	0.5	1.10	1.02, 1.19	0.017*
Last PSA measurement before biopsy	1.12	0.81, 1.55	0.5	1.03	0.83, 1.27	0.8
PSA velocity (arithmetic equation)	1.61	0.57, 4.55	0.4	0.74	0.49, 1.11	0.14
Age at biopsy	1.04	0.96, 1.12	0.4	1.11	1.02, 1.19	0.010*
Last PSA measurement before biopsy	1.01	0.71, 1.44	>0.9	0.90	0.68, 1.19	0.4
PSA velocity (linear regression)	3.27	0.65, 16.38	0.15	1.56	0.66, 3.67	0.3
Age at biopsy	1.04	0.96, 1.12	0.4	1.11	1.02, 1.19	0.010*
Last PSA measurement before biopsy	1.00	0.67, 1.49	>0.9	0.89	0.67, 1.19	0.4
PSA velocity (first and last value)	3.08	0.58, 16.42	0.2	1.55	0.66, 3.66	0.3

Abbreviations: CI = confidence interval; OR = odds ratio; PSA = prostate-specific antigen. All models are adjusted for age at biopsy and last PSA measurement before biopsy. *Statistically significant.

could lead to diagnosis of lower grade cancers in BRCA2 carriers. It may lead to better prognosis for men at risk for more aggressive disease and better disease-free survival when treated early. Although there are no definitive treatment recommendations for men with BRCA2 mutations when found to be diagnosed with low grade cancers, their risk for aggressive disease may spur them to follow a more active treatment plan such as radical prostatectomy vs external-beam radiation therapy or active surveillance (Bratt and Loman, 2015; Castro *et al*, 2015).

One major limitation of this analysis is the relatively small number of men in the study who had undergone diagnostic prostate biopsy. End of study biopsies are not mandated and the true incidence of PrCa is unknown in this population. As more men progress through the IMPACT screening study, undergo prostate biopsy and follow-up time increases, the findings from this analysis can be explored and validated. At this point, the results from this analysis did not justify modifying the study algorithm to include a PSAV calculation.

In the general population, PSAV is not part of any major screening guideline. We also did not find PSAV to be an independent prognostic factor in BRCA1 or BRCA2 mutation carriers and therefore for screening an absolute PSA cut-off value should preferably be used.

CONCLUSION

PSA is more strongly predictive of PrCa in BRCA carriers than BRCA non-carriers. We did not find evidence that PSAV aids to decision making for either indicating biopsy or frequency of follow-up testing in BRCA carriers, but further follow-up is required for more definitive conclusions.

ACKNOWLEDGEMENTS

We are indebted to all of the men who are taking part in this study. This research is coordinated by the Institute of Cancer Research, London, UK and is supported by grants from Cancer Research UK (Grant references (C5047/A21332, C5047/A13232 and C5047/A17528) and The Ronald and Rita McAulay Foundation. Mr and Mrs Jack Baker for the study in NorthShore University HealthSystem, Evanston, Illinois and Myriad Genetics Laboratory, Salt Lake City, Utah, for providing research BRCA testing rates for NorthShore University HealthSystem participants. We acknowledge funding from the NIH to the Biomedical Research Center at The Institute of Cancer Research and The Royal Marsden NHS Foundation Trust, at Central Manchester Foundation Trust and the Oxford Biomedical Research Centre Program. We acknowledge that in Australia, this project was co-funded by Cancer Council Tasmania and Cancer Australia, grant number 1006349 (2011–2013), Prostate Cancer Foundation of Australia, grant number PCFA PRO4 (2008) and Cancer Councils of Victoria and South Australia, grant number 400048 (2006–2008), The Victorian Cancer Agency Clinical Trial Capacity CTCB08_14, Cancer Australia & Prostate Cancer Foundation of Australia (2014–2016) grant number 1059423, and Translational grants EO109_50. The Association of International Cancer Research funded data collection in The Netherlands (AICR 10–0596). We acknowledge funding from the Basser Center for BRCA (to S Domchek). We acknowledge funding from the National Cancer Institute [P30-CA008748], the Sidney Kimmel Center for Prostate and Urologic Cancers, and David H. Koch through the Prostate Cancer Foundation, the National Institute for Health Research (NIHR) Oxford Biomedical Research Centre Program in UK, Swedish Cancer Society (Cancerfonden project no. 11–0624), and the Swedish Research Council (VR-MH project no. 2016–02974). We acknowledge funding from the Slovenian Research Agency, Research programme P3–0352. Elena Castro acknowledges funding from a Juan de la Cierva' fellowship from MINIECO (grant reference IJCI- 2014–19129). We acknowledge the support of the Asociación Española Contra el Cáncer (AECC), the Instituto de Salud Carlos III (organismo adscrito al Ministerio de Economía y Competitividad) and 'Fondo Europeo de Desarrollo Regional (FEDER), una manera de hacer Europa' (PI10/01422, PI13/00285, PIE13/00022, PI16/00563 and CIBERONC) and the Institut Català de la Salut and Autonomous Government of Catalonia (2009SGR290, 2014SGR338 and PERIS Project MedPerCan). We acknowledge David Fisas, Consol Lopez and Dr Nuria Calvo for their involvement in the project at Hospital de Sant Pau, Barcelona. We are grateful to the members of the Data and Safety Monitoring Committee: S. Duffy (Chair), P. White (UK NEQAS representative) and J. McGrath (BAUS representative). We acknowledge the contribution of past members of the IMPACT Steering Committee: J. Melia, S. Moss, P. Wilson and G. Mitchell.

CONFLICT OF INTEREST

Hans Lilja holds patents for free PSA, hK2, and intact PSA assays, and is named, along with Andrew J. Vickers, on a patent application for a statistical method to detect prostate cancer. The marker assay patents and the patent application for the statistical model has been licensed and commercialised as the 4Kscore by OPKO Diagnostics. Drs Vickers and Lilja receive royalties from sales of this test. Additionally, Dr Lilja owns stock and Dr Vickers owns stock options in OPKO. Professor Rosalind Eeles—Janssen: provided medical education support to GU ASCO Feb 2013. Succinct Communications: received an honorarium and expenses

for attending and speaking at UK Cancer Convention Oct 2013. The authors have no other conflict of interest to declare.

REFERENCES

- Bancroft EK, Page EC, Castro E, Lilja H, Vickers A, Sjoberg D, Assel M, Foster CS, Mitchell G, Drew K, Mæhle L, Axcrona K, Evans DG, Bulman B, Eccles D, McBride D, van Asperen C, Vasen H, Kiemeny LA, Ringelberg J, Cybulski C, Wokolorczyk D, Selkirk C, Hulick PJ, Bojesen A, Skytte AB, Lam J, Taylor L, Oldenburg R, Cremers R, Verhaegh G, van Zelst-Stams WA, Oosterwijk JC, Blanco I, Salinas M, Cook J, Rosario DJ, Buys S, Conner T, Ausems MG, Ong KR, Hoffman J, Domchek S, Powers J, Teixeira MR, Maia S, Foulkes WD, Taherian N, Ruijs M, Helderma-van den Enden AT, Izatt L, Davidson R, Adank MA, Walker L, Schmutzler R, Tucker K, Kirk J, Hodgson S, Harris M, Douglas F, Lindeman GJ, Zgajnar J, Tischkowitz M, Clowes VE, Susman R, Ramón y Cajal T, Patcher N, Gadea N, Spigelman A, van Os T, Liljegren A, Side L, Brewer C, Brady AF, Donaldson A, Stefansdotter V, Friedman E, Chen-Shtoyerman R, Amor DJ, Copakova L, Barwell J, Giri VN, Murthy V, Nicolai N, Teo SH, Greenhalgh L, Strom S, Henderson A, McGrath J, Gallagher D, Aaronson N, Arderon-Jones A, Bangma C, Dearnaley D, Costello P, Eyfjord J, Rothwell J, Falconer A, Gronberg H, Hamdy FC, Johannsson O, Khoo V, Kote-Jarai Z, Lubinski J, Axcrona U, Melia J, McKinley J, Mitra AV, Moynihan C, Rennett G, Suri M, Wilson P, Killick E. IMPACT Collaborators Moss S, Eeles RA (2014) Targeted prostate cancer screening in BRCA1 and BRCA2 mutation carriers: results from the initial screening round of the IMPACT Study. *Eur Urol* **66**: 489–499.
- Berger AP, Deibl M, Steiner H, Bektic J, Pelzer A, Spranger R, Klocker H, Bartsch G, Horninger W (2005) Longitudinal PSA changes in men with and without prostate cancer: assessment of prostate cancer risk. *Prostate* **64**: 240–245.
- Bratt O, Loman N (2015) Clinical management of prostate cancer in men with BRCA mutations. *Eur Urol* **68**: 194–195.
- Breast Cancer Linkage Consortium (1999) Cancer risks in BRCA2 mutation carriers. *J Natl Cancer Inst* **91**: 1310–1316.
- Carter HB, Pearson JD, Metter EJ, Brant LJ, Chan DW, Andres R, Fozard JL, Walsh PC (1992) Longitudinal evaluation of prostate-specific antigen levels in men with and without prostate disease. *JAMA* **267**: 2215–2220.
- Carter HB, Kettermann A, Ferrucci L, Kettermann A, Landis P, Wright EJ, Epstein JI, Trock BJ, Metter EJ (2006) Detection of life-threatening prostate cancer with prostate-specific antigen velocity during a window of curability. *J Natl Cancer Inst* **98**: 1521–1527.
- Carter HB, Kettermann A, Ferrucci L, Landis P, Metter EJ (2007) Prostate-specific antigen velocity risk count assessment: a new concept for detection of life-threatening prostate cancer during window of curability. *Urol* **70**: 685–690.
- Castro E, Goh C, Olmos D, Saunders E, Leongamornlert D, Tymrakiewicz M, Mahmud N, Dadaev T, Govindasami K, Guy M, Sawyer E, Wilkinson R, Arderon-Jones A, Ellis S, Frost D, Peock S, Evans DG, Tischkowitz M, Cole T, Davidson R, Eccles D, Brewer C, Douglas F, Porteous ME, Donaldson A, Dorkins H, Izatt L, Cook J, Hodgson S, Kennedy MJ, Side LE, Eason J, Murray A, Antoniou AC, Easton DF, Kote-Jarai Z, Eeles R (2013) Germline BRCA mutations are associated with higher risk of nodal involvement, distant metastasis, and poor survival outcomes in prostate cancer. *J Clin Oncol* **31**: 1748–1757.
- Castro E, Goh C, Leongamornlert D, Saunders E, Tymrakiewicz M, Dadaev T, Govindasami K, Guy M, Ellis S, Frost D, Bancroft E, Cole T, Tischkowitz M, Kennedy MJ, Eason J, Brewer C, Evans DG, Davidson R, Eccles D, Porteous ME, Douglas F, Adlard J, Donaldson A, Antoniou AC, Kote-Jarai Z, Easton DF, Olmos D, Eeles R (2015) Effect of BRCA mutations on metastatic relapse and cause-specific survival after radical treatment for localised prostate cancer. *Eur Urol* **68**: 186–193.
- D'Amico AV, Chen MH, Roehl KA, Catalona WJ (2004) Preoperative PSA velocity and the risk of death from prostate cancer after radical prostatectomy. *N Engl J Med* **351**: 125–135.
- D'Amico AV, Renshaw AA, Sussman B, Chen MH (2005) Pretreatment PSA velocity and risk of death from prostate cancer following external beam radiation therapy. *JAMA* **294**: 440–447.
- Edwards SM, Evans DG, Hope Q, Norman AR, Barbachano Y, Bullock S, Kote-Jarai Z, Meitz J, Falconer A, Osin P, Fisher C, Guy M, Jhavar SG, Hall AL, O'Brien LT, Gehr-Swain BN, Wilkinson RA, Forrest MS, Dearnaley DP, Arderon-Jones AT, Page EC, Easton DF, Eeles RA. UK

- Genetic Prostate Cancer Study Collaborators and BAUS Section of Oncology (2010) Prostate cancer in BRCA2 germline mutation carriers is associated with poorer prognosis. *Br J Cancer* **103**: 918–924.
- Eeles R, Goh C, Castro E, Bancroft E, Guy M, Al Olama AA, Easton D, Kote-Jarai Z (2014) The genetic epidemiology of prostate cancer and its clinical implications. *Nat Rev Urol* **11**: 18–31.
- Gallagher DJ, Gaudet MM, Pal P, Kirchoff T, Balistreri L, Vora K, Bhatia J, Stadler Z, Fine SW, Reuter V, Zelefsky M, Morris MJ, Scher HI, Klein RJ, Norton L, Eastham JA, Scardino PT, Robson ME, Offit K (2010) Germline BRCA mutations denote a clinicopathologic subset of prostate cancer. *Clin Cancer Res* **16**: 2115–2121.
- Giusti RM, Rutter JL, Duray PH, Freedman LS, Konichezky M, Fisher-Fischbein J, Greene MH, Maslansky B, Fischbein A, Gruber SB, Rennert G, Ronchetti RD, Hewitt SM, Struewing JP, Iscovich J (2003) A twofold increase in BRCA mutation related prostate cancer among Ashkenazi Israelis is not associated with distinctive histopathology. *J Med Genet* **40**: 787–792.
- Kitagawa Y, Sawada K, Urata S, Izumi K, Ueno S, Kadono Y, Konaka H, Mizokami A, Namiki M (2014) Impact of PSA levels on second-round screening for the development of prostate cancer in men with low baseline PSA levels ($<I = 2.0$ mg/ml). *Anticancer Res* **34**: 6739–6746.
- Kote-Jarai Z, Leongamornlert D, Saunders E, Tymrakiewicz M, Castro E, Mahmud N, Guy M, Edwards S, O'Brien L, Sawyer E, Hall A, Wilkinson R, Dadaev T, Goh C, Easton D. UKGPCS CollaboratorsGoldgar D, Eeles R (2011) BRCA2 is a moderate penetrance gene contributing to young-onset prostate cancer: implications for genetic testing in prostate cancer patients. *Br J Cancer* **105**: 1230–1234.
- Leongamornlert D, Mahmud N, Tymrakiewicz M, Saunders E, Dadaev T, Castro E, Goh C, Govindasami K, Guy M, O'Brien L, Sawyer E, Hall A, Wilkinson R, Easton D. UKGPCS CollaboratorsGoldgar D, Eeles R, Kote-Jarai Z (2012) Germline BRCA1 mutations increase prostate cancer risk. *Br J Cancer* **106**: 1697–1701.
- Loughlin KR (2014) PSA velocity: A systematic review of clinical applications. *Urol Oncol* **32**(8): 1116–1125.
- Mikropoulos C, Goh C, Leongamornlert D, Kote-Jarai Z, Eeles R (2014) Translating genetic risk factors for prostate cancer to the clinic: 2013 and beyond. *Future Oncol* **10**: 1679–1694.
- Mitra A, Fisher C, Foster CS, Jameson C, Barbachanno Y, Bartlett J, Bancroft E, Doherty R, Kote-Jarai Z, Peock S, Easton D. IMPACT and EMBRACE CollaboratorsEeles R (2008) Prostate cancer in male BRCA1 and BRCA2 mutation carriers has a more aggressive phenotype. *Br J Cancer* **98**: 502–507.
- Moran A, O'Hara C, Khan S, Shack L, Woodward E, Maher ER, Lalloo F, Evans DG (2012) Risk of cancer other than breast or ovarian in individuals with BRCA1 and BRCA2 mutations. *Fam Cancer* **11**(2): 235–242.
- Murphy DG, Ahlering T, Catalona WJ, Crowe H, Crowe J, Clarke N, Cooperberg M, Gillatt D, Gleave M, Loeb S, Roobol M, Sartor O, Pickles T, Wooten A, Walsh PC, Costello AJ (2014) The Melbourne Consensus Statement on the early detection of prostate cancer. *BJU Int* **113**(2): 186–188.
- Narod SA, Neuhausen S, Vichodez G, Armel S, Lynch HT, Ghadirian P, Cummings S, Olopade O, Stoppa-Lyonnet D, Couch F, Wagner T, Warner E, Foulkes WD, Saal H, Weitzel J, Tulman A, Poll A, Nam R, Sun P. Hereditary Breast Cancer Study GroupDanquah J, Domchek S, Tung N, Ainsworth P, Horsman D, Kim-Sing C, Maugard C, Eisen A, Daly M, McKinnon W, Wood M, Isaacs C, Gilchrist D, Karlan B, Nedelcu R, Meschino W, Garber J, Pasini B, Manoukian S, Bellati C (2008) Rapid progression of prostate cancer in men with a BRCA2 mutation. *Br J Cancer* **99**: 371–374.
- Roobol MJ, Kranse R, de Koning HJ, Schroder FH (2004) Prostate-specific antigen velocity at low prostate-specific antigen levels as screening tool for prostate cancer: results of second screening round of ERSPC (ROTTERDAM). *Urology* **63**: 309–313discussion 313–315.
- Roobol MJ, Kranse R, Bangma CH, van Leenders AG, Blijenberg BG, van Schaik RH, Kirkels WJ, Otto SJ, van der Kwast TH, de Koning HJ, Schröder FH. ERSPC Rotterdam Study Group (2013) Screening for prostate cancer: results of the Rotterdam section of the european randomized study of screening for prostate cancer. *Eu Urol* **64**: 530–539.
- Taylor RA, Fraser M, Livingstone J, Espiritu SM, Thorne H, Huang V, Lo W, Shiah YJ, Yamaguchi TN, Sliwinski A, Horsburgh S, Meng A, Heisler LE, Yu N, Yousif F, Papargiris M, Lawrence MG, Timms L, Murphy DG, Frydenberg M, Hopkins JF, Bolton D, Clouston D, McPherson JD, van der Kwast T, Boutros PC, Risbridger GP, Bristow RG (2017) Germline BRCA2 mutations drive prostate cancers with distinct evolutionary trajectories. *Nat Commun* **9**(8): 13671.
- Thompson D, Easton DF. Breast Cancer Linkage Consortium (2002) Cancer Incidence in BRCA1 mutation carriers. *J Natl Cancer Inst* **94**: 1358–1365.
- Thorne H, Willems AJ, Niedermayr E, Hoh IM, Li J, Clouston D, Mitchell G, Fox S, Hopper JL. Kathleen Cunningham Consortium for Research in Familial Breast Cancer Consortium, Bolton D (2011) Decreased prostate cancer-specific survival of men with BRCA2 mutations from multiple breast cancer families. *Cancer Prev Res (Phila)* **4**: 1002–1010.
- Tryggvadóttir L, Vidarsdóttir L, Thorgeirsson T, Jonasson JG, Olafsdóttir EJ, Olafsdóttir GH, Rafnar T, Thorlacius S, Jonsson E, Eyfjord JE, Tulinius H (2007) Prostate cancer progression and survival in BRCA2 mutation carriers. *J Natl Cancer Inst* **99**: 929–935.
- van Asperen CJ, Brohet RM, Meijers-Heijboer EJ, Hoogerbrugge N, Verhoef S, Vasen HF, Ausems MG, Menko FH, Gomez Garcia EB, Klijn JG, Hogervorst FB, van Houtwelingen JC, van't Veer LJ, Rookus MA, van Leeuwen FE. Netherlands Collaborative Group on Hereditary Breast Cancer (HEBON) (2005) Cancer risks in BRCA2 families: estimates for sites other than breast and ovary. *J Med Genet* **42**: 711–719.
- Vickers AJ, Till C, Tangen CM, Lilja H, Thompson IM (2011) An empirical evaluation of guidelines on prostate-specific antigen velocity in prostate cancer detection. *J Natl Cancer Inst* **103**(6): 462–469.



This work is licensed under the Creative Commons Attribution-Non-Commercial-Share Alike 4.0 International License. To view a copy of this license, visit <http://creativecommons.org/licenses/by-nc-sa/4.0/>

© The Author(s) named above 2018

APPENDIX 1

The IMPACT Collaborators IMPACT Study Steering Committee

Rosalind Eeles—Institute of Cancer Research, London
Elizabeth Bancroft—Royal Marsden NHS Foundation Trust, London
Elizabeth Page—Institute of Cancer Research, London
Zsofia Kote-Jarai—Institute of Cancer Research, London
Audrey Ardern-Jones—Royal Marsden NHS Foundation Trust, London
Chris Bangma—Erasmus University Medical Center, Rotterdam
Elena Castro—CNIO, Madrid
David Deary—Institute of Cancer Research, London
Alison Falconer—Imperial College Healthcare NHS Trust, London, UK
Christopher Foster—HCA Pathology Laboratories, London
Henrik Grönberg—University Hospital, Umea

Freddie C. Hamdy—University of Oxford, Oxford
Óskar Þór Jóhannsson—Landspítali—National University Hospital of Iceland, Reykjavik
Vincent Khoo—Royal Marsden NHS Foundation Trust, London
Diana Eccles—Wessex Clinical Genetics Service, Southampton
Hans Lilja—MSKCC, New York & University of Oxford, Oxford
Gareth Evans—St Mary's Hospital, Manchester, UK
Jorunn Eyfjord—University of Iceland, Reykjavik
Jan Lubinski—International Hereditary Cancer Center, Szczecin
Lovise Maehle—Norwegian Radium Hospital, Oslo
Christos Mikropoulos—The Institute of Cancer Research
Alan Millner—Royal Marsden NHS Foundation Trust, London
Geoffrey Lindeman—Royal Melbourne Hospital; WEHI; Uni of Melbourne, Melbourne
Anita Mitra—University College London Hospitals, London
Sue Moss—Queen Mary University of London
Clare Moynihan—Institute of Cancer Research, London

Gad Rennert—CHS National Cancer Control Center, Carmel Medical Center, Haifa

Mohnish Suri—Nottingham City Hospital, Nottingham, UK

Penny Wilson—Director, BioZenix, Cheshire

Rosalind Eeles, Elizabeth Bancroft, Elizabeth Page, Sibel Saya, Alex Dias, Natalie Taylor, Kathryn Myhill, Sarah Thomas, Ashton Stroud, Jenny Pope, Anthony Chamberlain, Diana Keating—Coordinating Centre, Institute of Cancer Research, London

Australia (*more than 1 affiliation)

Gillian Mitchell*, Sue Shanley, Kate Richardson, Joanne McKinley, Lara Petelin, Morgan Murphy, Lyon Mascarenhas, Paul James*—Parkville Familial Cancer Centre, Peter MacCallum Cancer Centre, East Melbourne, VIC

Gillian Mitchell*, Paul James*—The Sir Peter MacCallum Department of Oncology, University of Melbourne, VIC

Paul James*—Genetics Medicine, Royal Melbourne Hospital, Melbourne, VIC

Declan Murphy—Department of Urology, Peter MacCallum Cancer Centre, East Melbourne, VIC

Jimmy Lam, Louise Taylor, Cathy Miller, Alan Stapleton, Michael Chong—Department of Urology, Repatriation General Hospital, Daw Park, SA

Graeme Suthers, Nicola Poplawski—SA Clinical Genetics Service, SA Pathology (at Women's & Children's Hospital), North Adelaide, SA

Katherine Tucker*, Lesley Andrews, Jessica Duffy—Hereditary Cancer Clinic, Prince of Wales Hospital, Randwick, NSW

Richard Millard—Department of Urology, Prince of Wales Hospital, Randwick, NSW

Robyn Ward, Rachel Williams—Hereditary Cancer Clinic, Prince of Wales Clinical School, Faculty of Medicine, UNSW, Sydney, NSW

Phillip Stricker—St Vincent's Clinic, Sydney, NSW

Judy Kirk*, Michelle Bowman—Familial Cancer Service, Westmead Hospital, Wentworthville, NSW

Judy Kirk*—Centre for Cancer Research, The Westmead Institute for Medical Research, NSW

Manish Patel—Westmead Hospital, Wentworthville, NSW

Marion Harris, Shona O'Connell, Clare Hunt, Courtney Smyth—Familial Cancer Centre, Monash Health, Clayton, VIC

Mark Frydenberg—Monash Medical Centre, VIC

Geoffrey Lindeman*, Kylie Shackleton, Catherine Morton—Parkville Familial Cancer Centre, The Royal Melbourne Hospital, Grattan St, Parkville, VIC

Geoffrey Lindeman*—Stem Cells and Cancer Division, The Walter and Eliza Hall Institute of Medical Research, Parkville, VIC

Geoffrey Lindeman*—Department of Medicine, The University of Melbourne, Parkville, VIC

Rachel Susman, Julie McGaughan, Melanie Boon—Genetic Health Queensland, Royal Brisbane & Women's Hospital, Herston, QLD

Nicholas Pachter*, Sharron Townshend, Lyn Schofield, Cassandra Nicholls—Genetic Services of WA, King Edward Memorial Hospital, Subiaco, WA

Nicholas Pachter*—Department of Paediatrics, University of Western Australia, Perth, WA

Allan Spigelman*, Margaret Gleeson—Hunter Family Cancer Service, Waratah, NSW

Allan Spigelman*—University of New South Wales, St Vincent's Clinical School, NSW

Allan Spigelman*—Hereditary Cancer Clinic, The Kinghorn Cancer Centre, St Vincent's Hospital, Sydney, NSW

David Amor*, Jo Burke, Briony Patterson—Tasmanian Clinical Genetics Service, Hobart, TAS

David Amor*—Murdoch Childrens Research Institute, Parkville, VIC

Peter Swindle—Mater Private Hospitals, QLD

Rodney Scott—School of Biomedical Sciences and Pharmacy, University of Newcastle, NSW

Victorian Cancer Biobank, Carlton, VIC

Department Gynaecological Oncology Laboratory, Westmead Hospital, Centre for Cancer Research, NSW

PathWest (Clinical Trials Lab), Nedlands, WA

Pathology Queensland (Central Laboratory; Health Support Queensland), QLD Department of Health, Herston, QLD

Canada

William Foulkes, Talia Boshari, Armen Aprikian—McGill University, Montreal

Denmark

Thomas Jensen, Anders Bojeson, Palle Osther, Anne-Bine Skytte, Dorthe Cruger, Majbritt Kure Tøndering—Vejle Hospital, Vejle

Anne-Marie Gerdes—Odense University Hospital, Odense

Germany

Rita Schmutzler, Kerstin Rhiem, Petra Wihler—Center of Familial Breast and Ovarian Cancer, University Hospital of Cologne, Cologne

K Kast, C Griebisch—University Hospital Dresden

Iceland

Oskar Johannsson, Vigdis Stefansdottir—University Hospital of Iceland, Reykjavik

India

Vedang Murthy, Rajiv Sarin, Kasturi Awatagiri, Sujata Ghonge, Pradnya Kowtal, Gouri Mulgund—Tata Memorial Centre, Mumbai

Ireland

David Gallagher, Richard Bambury, Michael Farrell, Fergal Gallagher, Ingrid Kiernan—Mater Private Hospital, Dublin

Israel

Eitan Friedman—Chaim Sheba Medical Center, Tel-Hashomer and the Sackler School of Medicine, Tel-Aviv University, Tel-Aviv

Rakefet Chen-Shtoyerman, Alon Basevitch, Dan Leibovici, Ehud Melzer and Sagi Josefsberg Ben-Yehoshua—The Genetic Institute, the Gastroenterology Institute and the Urology Department, Kaplan Medical Centre, Rehovot

Italy

Nicola Nicolai, Paolo Radice, Riccardo Valdagni, Tiziana Magnani, Simona Gay—Istituto Nazionale dei Tumori, Milan

Malaysia

Soo Hwang Teo, Hui Meng Tan, Sook-Yee Yoon—Cancer Research Initiatives Foundation, Subang Jaya Medical Centre, Selangor Darul Ehsan

Soo Hwang Teo, Meow Keong Thong—University of Malaya, Kuala Lumpur

The Netherlands

STOET—Stichting Opsporing Erfelijke Tumoren, Leiden
Christi van Asperen—Leiden University Medical Centre, Leiden
Bart Kiemeneij, Wendy van Zelst-Stams—Radboud University Nijmegen Medical Centre

Margreet G.E.M. Ausems, Rob B. Van der Luijt—University Medical Center Utrecht

Theo van Os—Academic Medical Center, Amsterdam

Mariëlle W.G. Ruijs—Netherlands Cancer Institute, Amsterdam

Muriel A. Adank—VU University Medical Center, Amsterdam

Rogier A. Oldenburg—Erasmus Medical Center, Rotterdam

A. (Paula) T.J.M. Helderma- van den Enden, B.A.H. Caanen—University Hospital Maastricht

Jan C. Oosterwijk—University Medical Centre Groningen

Norway

Lovise Maehle, Pal Moller, Bjorn Brennhovd, Heidi Medvik, Eldbjørg Hanslien—Norwegian Radium Hospital, Oslo

Poland

Cezary Cybulski, Jan Lubinski, Dominika Wokolorczyk—International Hereditary Cancer Centre, Szczecin

Portugal

Manuel Teixeira, Sofia Maia, Ana Peixoto, Rui Henrique, Jorge Oliveira, Nuno Gonçalves, Luís Araújo, Manuela Seixas, João Paulo Souto, Pedro Nogueira—Portuguese Oncology Institute, Porto

Slovakia

Lucia Copakova—National Cancer Institute, Bratislava

Slovenia

Janez Zgajnar, Mateja Krajc, Alenka Vrecar—Institute of Oncology, Ljubljana

Spain

Gabriel Capellá, Ignacio Blanco—Hereditary Cancer Program, Catalan Institute of Oncology, Barcelona

Teresa Ramón y Cajal, David Fisas, Josefina Mora, Salvador Esquena—Hospital de Sant Pau, Barcelona

Judith Balmaña, Neus Gadea, Juan Morote—Hospital Vall d'Hebron, Barcelona

Sweden

Annelie Liljegren, Marie Hjälms -Eriksson, Karl-Johan Ekdahl, Stefan Carlsson—Karolinska University Hospital, Karolinska Institutet, Stockholm

United Kingdom

Angela George, Zoe Kemp, Jennifer Wiggins, Cathryn Moss, Vincent Khoo, Nicholas Van As, Alan Thompson, Chris Ogden, Christopher Woodhouse, Pardeep Kumar—Royal Marsden NHS Foundation Trust

D Gareth Evans, Barbara Bulman, Jeanette Rothwell, Karen Tricker—Manchester Regional Genetics Service, Manchester

Diana Eccles, Gillian Wise, Catherine Mercer, Donna McBride, Philandra Costello, Allison Pearce, Audrey Torokwa—Wessex Clinical Genetics Service, Southampton.

Marc Tischkowitz, Joan Paterson, Virginia Clowes, Amy Taylor, Barbara Newcombe—East Anglian Regional Genetics Service, Cambridge

Lisa Walker, Dorothy Halliday, Barbara Stayner, D Fleming-Brown—Oxford Regional Genetics Service, Oxford

Katie Snape, Helen Hanson, Shirley Hodgson, Glen Brice, Tessa Homfray, Carrie Hammond, Kelly Kohut, Uruj Anjum, Audrey Dearing, Mark Mencias—South West Thames Regional Genetics Service, London.

Carole Brewer, Alison Potter, Caroline Renton, Anne Searle, Kathryn Hill, Selina Goodman, Lynda Garcia, Gemma Devlin, Sarah Everest, Maria Nadolski—Peninsula Clinical Genetics Service, Exeter.

Alex Henderson, Fiona Douglas, Irene Jobson, Edgar Paez—Northern Clinical Genetics Service, Newcastle.

Alan Donaldson, Sue Tomkins—South West Regional Genetics Service, Bristol

Caroline Langman, Chris Jacobs, Gabriella Pichert, Adam Shaw, Anju Kulkarni, Vishakha Tripathi, Sarah Rose, Cecilia Compton, Michelle Watson and Cherylin Reinholtz—South East Thames Regional Genetics Service, Guys Hospital London

Angela Brady, Virginia Clowes, Huw Dorkins, Athalie Melville, Monika Kosicka-Slawinska, Carole Cummings, Vicki Kiesel, Marion Bartlett, Kashmir Randhawa, Natalie Ellery—North West Thames Regional Genetics Service, Harrow

Lucy Side, Alison Male, Kate Simon, Katie Rees, Cecilia Compton, Lizzie Tidey, Jana Gurasashvili—North East Thames Regional Genetics Service, NE Thames.

Jackie Cook, Louise Nevitt, Stuart Ingram, Alice Howell—North Trent Clinical Genetics Service, Sheffield

Derek Rosario, James Catto, Joanne Howson—Academic Urology Unit, Sheffield

Rachel Hart, Kai-Ren Ong, Cyril Chapman, Trevor Cole, Tricia Heaton, Jonathan Hoffman, Lucy Burgess, Wayne Glover, Camilla Huber—West Midlands Regional Clinical Genetics Service, Birmingham

Rosemarie Davidson, Mark Longmuir, Cathy Watt, Alexis Duncan—West of Scotland Genetics Service, Glasgow

Julian Barwell, Roger Kockelbergh, Shumikazi Mzazi, Amy Dineen, Ayisha Sattar, Beckie Kaemba, Zahirah Sidat, Nafisa Patel, Kas Siguake—Leicester Royal Infirmary

Alex Henderson, Angela Birt, Una Poultney, Nkem Umez-Eronini, Jaswant Mom—North Cumbria University Hospitals Trust

Lynn Greenhalgh, Vivienne Sutton—Cheshire and Mersey Clinical Genetics Service, Liverpool Women's Hospital

Philip Cornford, Nicola Birmingham, Pembe Yesildag, Katy Treherne, Julie Griffiths—Royal Liverpool and Broadgreen Hospital NHS Trust, Liverpool

Carole Brewer, Lyn Cogley, Hannah Gott—Derriford Hospital, Plymouth

United States

Dr Wendy S Rubinstein, Dr Peter Hulick, Dr Michael McGuire, Dr Daniel Shevrin, Dr Karen Kaul, Scott Weissman CGC, Anna Newlin, Kristen Vogel, Shelly Weiss, Nicole Hook—NorthShore University HealthSystem, Evanston

Sandra Buys, David Goldgar, Tom Conner, Vickie Venne, Robert Stephenson, Christopher Dechet—Salt Lake City, Utah

Susan Domchek, Jacquelyn Powers, Neil Rustgi—University of Pennsylvania, Philadelphia

Sara Strom, Banu Arun, John W. Davis, Yuko Yamamura—MD Anderson, Texas

Elias Obeid, Veda Giri, Laura Gross, Linda Okoth—Fox Chase Cancer Center

Kathy Cooney, Elena Stoffel, Linda Oko—University of Michigan

Christos Mikropoulos¹, Christina G Hutten Selkirk^{2,3}, Sibel Saya¹, Elizabeth Bancroft^{1,4}, Emily Vertosick⁵, Tokhir Dadaev¹, Charles Brendler², Elizabeth Page¹, Alexander Dias^{1,4}, D Gareth Evans⁶, Jeanette Rothwell⁶, Lovise Maehle⁷, Karol Axcrona⁸, Kate Richardson^{9,10}, Diana Eccles^{11,12}, Thomas Jensen¹³, Palle J Osther¹³, Christi J van Asperen¹⁴, Hans Vasen¹⁵, Lambertus A Kiemeneij¹⁶, Janneke Ringelberg¹⁵, Cezary Cybulski¹⁷, Dominika Wokolorczyk¹⁷, Rachel Hart¹⁸, Wayne Glover¹⁸, Jimmy Lam¹⁹, Louise Taylor¹⁹, Monica Salinas²⁰, Lidia Feliubadaló²⁰, Rogier Oldenburg²¹, Ruben Cremers¹⁶, Gerald Verhaegh¹⁶, Wendy A van Zelst-Stams¹⁵, Jan C Oosterwijk²², Jackie Cook²³, Derek J Rosario²⁴, Sandra S Buys²⁵, Tom Conner²⁵, Susan Domchek²⁶, Jacquelyn Powers²⁶, Margreet GEM Ausems²⁷, Manuel R Teixeira^{28,29}, Sofia Maia²⁸, Louise Izatt³⁰, Rita Schmutzler³¹, Kerstin Rhiem³¹, William D Foulkes³², Talia Boshari³², Rosemarie Davidson³³, Marielle Ruijs³⁴, Apollonia TJM Helderma-van den Enden³⁵, Lesley Andrews³⁶, Lisa Walker³⁷, Katie Snape³⁸, Alex Henderson³⁹, Irene Jobson³⁹, Geoffrey J Lindeman^{40,41,42}, Annelie Liljegren⁴³, Marion Harris⁴⁴, Muriel A Adank⁴⁵, Judy Kirk^{46,47}, Amy Taylor⁴⁸, Rachel Susman⁴⁹, Rakefet Chen-Shtoyerman⁵⁰, Nicholas Pachter^{51,52}, Allan Spigelman^{53,54,55}, Lucy Side⁵⁶, Janez Zgajnar⁵⁷, Josefina Mora⁵⁸, Carole Brewer^{59,60}, Neus Gadea⁶¹, Angela F Brady⁶², David Gallagher⁶³, Theo van Os⁶⁴, Alan Donaldson⁶⁵, Vigdis Stefansdottir⁶⁶, Julian Barwell^{67,68}, Paul A James^{9,10,69}, Declan Murphy¹⁰, Eitan Friedman^{70,71}, Nicola Nicolai⁷², Lynn Greenhalgh⁷³, Elias Obeid⁷⁴, Vedang Murthy⁷⁵, Lucia Copakova⁷⁶, John McGrath⁶⁰, Soo-Hwang Teo⁷⁷, Sara Strom⁷⁸, Karin Kast^{79,80,81}, Daniel A Leongamornlert¹, Anthony Chamberlain¹, Jenny Pope¹, Anna C Newlin³, Neil Aaronson³⁴, Audrey Ardern-Jones⁴, Chris Bangma²¹, Elena Castro⁸², David Dearnaley^{1,4}, Jorunn Eyfjord⁸³, Alison Falconer⁸⁴, Christopher S Foster⁸⁵, Henrik Gronberg⁸⁶, Freddie C Hamdy^{37,87}, Oskar Johannsson⁶⁶, Vincent Khoo⁴, Jan Lubinski¹⁷, Eli Marie Grindedal⁷, Joanne McKinley⁹, Kylie Shackleton⁴⁰, Anita V Mitra⁸⁸, Clare Moynihan¹, Gad Rennert⁸⁹, Mohnish Suri⁹⁰, Karen Tricker⁶, The IMPACT study collaborators⁹¹, Sue Moss⁹², Zsofia Kote-Jarai¹, Andrew Vickers⁵, Hans Lilja^{87,93}, Brian T Helfand² and Rosalind A Eeles^{*1,4}

¹The Institute of Cancer Research, 15 Cotswold Road, Sutton, Surrey SM2 5NG, UK; ²The John and Carol Walter Center for Urological Health, Department of Surgery, North Shore University Health System, Evanston, IL 60201, USA; ³Center for Medical Genetics, Department of Medicine, NorthShore University HealthSystem, Evanston, IL 60201, USA; ⁴Royal Marsden NHS Foundation Trust, Fulham Rd, London SW3 6JJ, UK; ⁵Department of Epidemiology and Biostatistics, Memorial Sloan Kettering Cancer Center, New York, NY 10065, USA; ⁶Genomic Medicine, Manchester Academic Health Sciences Centre, Division of Evolution and Genomic Sciences, University of Manchester, Central Manchester University Hospitals NHS Foundation Trust, Manchester M13 9WL, UK; ⁷Department of Medical Genetics, Oslo University Hospital, Oslo 0372, Norway; ⁸Akershus University Hospital, Lørenskog 1478, Norway; ⁹Parkville Familial Cancer Centre, Peter MacCallum Cancer Centre, East Melbourne, VIC 3000, Australia; ¹⁰The Sir Peter MacCallum Department of Oncology, University of Melbourne, VIC 3010, Australia; ¹¹Wessex Clinical Genetics Service, Princess Anne Hospital, Southampton SO16 5YA, UK; ¹²Cancer Sciences, Faculty of Medicine, University of Southampton, University Hospital Southampton NHS Foundation Trust, Southampton SO16 6YD, UK; ¹³Department of Clinical Genetics, Vejle Hospital, Vejle 7100, Denmark; ¹⁴Leiden University Medical Center, Department of Clinical Genetics, Leiden, ZA 2333, The Netherlands; ¹⁵Netherlands Foundation for the Detection of Hereditary Tumors, Leiden, ZA 2333, The Netherlands; ¹⁶Radboud University Medical Center, Nijmegen, GA 6525, The Netherlands; ¹⁷International Hereditary Cancer Center, Department of Genetics and Pathology, Pomeranian Medical University, Szczecin 70-204, Poland; ¹⁸Clinical Genetics Unit, Birmingham Women's Hospital, Birmingham B15 2TG, UK; ¹⁹Department of Urology, Repatriation General Hospital, Daw Park, SA 5041, Australia; ²⁰Hereditary Cancer Program, Catalan Institute of Oncology (ICO-IDIBELL, CIBERONC), L'Hospitalet de Llobregat, Barcelona 08908, Spain; ²¹Department of Clinical Genetics, Erasmus Medical Center, Rotterdam 3015 CE, The Netherlands; ²²Department of Genetics, University Medical Center Groningen, University of Groningen, Groningen 9713 GZ, The Netherlands; ²³Sheffield Clinical Genetics Service, Sheffield Children's Hospital, Sheffield S10 2TH, UK; ²⁴Royal Hallamshire Hospital, Sheffield S10 2JF, UK; ²⁵Huntsman Cancer Institute at the University of Utah, Salt Lake City, UT 84103, USA; ²⁶Basser Research Center, University of Pennsylvania, Philadelphia, PA 19104, USA; ²⁷Department of Genetics, University Medical Centre Utrecht, Utrecht, CX, The Netherlands; ²⁸Genetics Department and Research Center, Portuguese Oncology Institute, Porto 4200-072, Portugal; ²⁹Biomedical Sciences Institute (ICBAS), Porto University, Porto 4200-072, Portugal; ³⁰South East Thames Genetics Service, Guy's Hospital, London SE1 9RT, UK; ³¹Center of Familial Breast and Ovarian Cancer, University Hospital of Cologne, Cologne 50937, Germany; ³²McGill Program in Cancer Genetics, Departments of Oncology and Human Genetics, McGill University, Montreal, QC H3A 0G4, Canada; ³³Duncan Guthrie Institute of Medical Genetics, Yorkhill NHS Trust, Glasgow G38SJ, UK; ³⁴The Netherlands Cancer Institute, Amsterdam 1066 CX, The Netherlands; ³⁵Department of Clinical Genetics, Maastricht University Medical Center, Maastricht, HX 6229, The Netherlands; ³⁶Hereditary Cancer Clinic, Prince of Wales Hospital, Randwick, NSW 2031, Australia; ³⁷Churchill Hospital, Headington, Oxford OX3 7LE, UK; ³⁸St George's Hospital, Tooting, London SW17 0QT, UK; ³⁹Northern Genetics Service, Newcastle upon Tyne Hospitals, Newcastle NE1 3BZ, UK; ⁴⁰Parkville Familial Cancer Centre, The Royal

Melbourne Hospital, Grattan St, Parkville, VIC 3050, Australia; ⁴¹Stem Cells and Cancer Division, The Walter and Eliza Hall Institute of Medical Research, Parkville, VIC 3050, Australia; ⁴²Department of Medicine, The University of Melbourne, Parkville, VIC 3050, Australia; ⁴³Karolinska University Hospital and Karolinska Institutet, Solna 171 77, Sweden; ⁴⁴Familial Cancer Centre, Monash Health, Clayton, VIC 3168, Australia; ⁴⁵VU University Medical Center, Amsterdam 1081 HV, The Netherlands; ⁴⁶Familial Cancer Service, Westmead Hospital, Westmead, Sydney, NSW 2145, Australia; ⁴⁷Centre for Cancer Research, The Westmead Institute for Medical Research, Westmead, Sydney, NSW 2155, Australia; ⁴⁸Cambridge University Hospitals NHS Foundation Trust, Cambridge CB2 0QQ, UK; ⁴⁹Genetic Health Queensland, Royal Brisbane & Women's Hospital, Herston, QLD 4029, Australia; ⁵⁰The Genetic Institute, Kaplan Medical Center, Rehovot 76100, Israel; ⁵¹Genetic Services of WA, King Edward Memorial Hospital, Subiaco, WA 6008, Australia; ⁵²School of Medicine and Pharmacology, University of Western Australia, Perth, WA 6009, Australia; ⁵³Hunter Family Cancer Service, Waratah, NSW 2298, Australia; ⁵⁴University of New South Wales, St Vincent's Clinical School, NSW 2052, Australia; ⁵⁵The Kinghorn Cancer Centre, St Vincent's Hospital, Sydney, NSW 2010, Australia; ⁵⁶NE Thames Regional Genetics Service, Great Ormond St Hospital & UCL Institute of Women's Health, London WC1N 3JH, UK; ⁵⁷Institute of Oncology, Ljubljana 1000, Slovenia; ⁵⁸Hospital de Sant Pau, Barcelona 08041, Spain; ⁵⁹Peninsular Genetics, Derriford Hospital, Plymouth PL6 8DH, UK; ⁶⁰Royal Devon and Exeter Hospital, Exeter EX2 5DW, UK; ⁶¹High Risk and Cancer Prevention Clinic, Vall d'Hebron University Hospital, Barcelona 08035, Spain; ⁶²North West Thames Regional Genetics Service, London North West Healthcare NHS Trust, London HA1 3UJ, UK; ⁶³St James' Hospital, Dublin 8, Ireland; ⁶⁴Academic Medical Center, Amsterdam 1105 AZ, The Netherlands; ⁶⁵St Michael's Hospital, Bristol BS2 8EG, UK; ⁶⁶Landspítali—the National University Hospital of Iceland, Reykjavik 101, Iceland; ⁶⁷University of Leicester, Leicester LE1 7RH, UK; ⁶⁸University Hospitals Leicester, Leicester LE1 5WW, UK; ⁶⁹Genetic Medicine, Royal Melbourne Hospital, Melbourne, VIC 3050, Australia; ⁷⁰Chaim Sheba Medical Center, Tel-Hashomer 52621, Israel; ⁷¹Sackler School of Medicine, Tel-Aviv University, Tel-Aviv 6997801, Israel; ⁷²Istituto Nazionale dei Tumori, Milano 20133, Italy; ⁷³Cheshire and Mersey Clinical Genetics Service, Liverpool Women's Hospital, Liverpool L8 7SS, UK; ⁷⁴Fox Chase Cancer Center, Philadelphia, PA 19111, USA; ⁷⁵Tata Memorial Centre, Mumbai, Maharashtra 400012, India; ⁷⁶National Cancer Institute, Bratislava 83310, Slovak Republic; ⁷⁷Cancer Research Initiatives Foundation, Subang Jaya Medical Centre, Subang Jaya, Selangor 47500, Darul Ehsan, Malaysia; ⁷⁸The University of Texas, MD Anderson Cancer Center, Houston, TX 77030, USA; ⁷⁹Department of Gynecology and Obstetrics, Medical Faculty and University Hospital Carl Gustav Carus, Technische Universität Dresden, Dresden 01069, Germany; ⁸⁰National Center for Tumor Diseases (NCT), Partner Site Dresden, Dresden 01307, Germany; ⁸¹German Cancer Consortium (DKTK), Dresden and German Cancer Research Center (DKFZ), Heidelberg 69120, Germany; ⁸²Prostate Cancer Unit, Spanish National Cancer Research Centre, Madrid 28029, Spain; ⁸³Faculty of Medicine, School of Health Sciences, University of Iceland, Reykjavik 101, Iceland; ⁸⁴Imperial College Healthcare NHS Trust, London, London W2 1NY, UK; ⁸⁵HCA Healthcare Laboratories, London WC1E 6JA, UK; ⁸⁶University Hospital, Umea 907 37, Sweden; ⁸⁷Nuffield Department of Surgical Sciences, University of Oxford, Oxford OX1 2JD, UK; ⁸⁸University College London Hospitals NHS Foundation Trust, London NW1 2BU, UK; ⁸⁹CHS National Cancer Control Center, Carmel Medical Center, Haifa 3436212, Israel; ⁹⁰Nottingham City Hospital, Nottingham NG5 1PB, UK; ⁹¹The IMPACT Study Collaborators List see Appendix 1; ⁹²Centre for Cancer Prevention, Queen Mary University of London, London EC1M 6BQ; ⁹³Departments of Laboratory Medicine, Surgery, and Medicine, Memorial Sloan Kettering Cancer Center, New York, NY 10065, USA and ⁹⁴Department of Translational Medicine, Lund University, Malmö 205 02, Sweden