Key steps for effective breast cancer prevention

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Abstract

Despite decades of laboratory, epidemiological and clinical research, breast cancer incidence continues to rise. Breast cancer remains the leading cancer –related cause of disease burden for women, affecting 1 in 20 globally and as many as 1 in 8 in high income countries [1] reducing breast cancer incidence will likely require both a population-based approach of reducing exposure to modifiable risk factors, and a precision-prevention approach of identifying women at increased risk and targeting them for specific interventions, such as risk-reducing medication. We already have the capacity to estimate an individual woman's breast cancer risk using validated risk assessment models, and the accuracy of these is likely to continue to improve over time, particularly with inclusion of newer risk factors, such as polygenic risk and mammographic density. Evidence-based risk-reducing medications are cheap, widely available and recommended by professional health bodies however, widespread implementation of these has proven challenging. The barriers to uptake of, and adherence to, current medications will need to be considered as we deepen our understanding of breast cancer initiation and begin developing and testing novel preventatives.

Introduction

In high income countries, breast cancer (BC) mortality is decreasing, largely owing to improved treatments[2]. Conversely, incidence has been steadily increasing [3-8] due in part to an increase in diagnosis as a result of the implementation of mammographic screening, but also perhaps implying a failure of existing BC prevention strategies[2]. BC will affect as many as 1 in 8 in high income countries by age 85 and remains the leading cancer –related cause of disease burden for [1]. Prevention potentially offers the most cost-effective strategy for cancer control and would reduce the social impact of BC.

Clinically, specific subtypes of BC are defined by their histopathological appearance and expression of hormone receptors and growth factors (namely the estrogen receptor (ER), the progesterone receptor (PR) and human epidermal growth factor receptor 2 (HER2, also known as ERBB2)). Yet, it is mostly ER positive BC that is increasing in incidence[5,6,9].

Both genetic and non-genetic risk factors influence BC development. Genetic factors include pathogenic mutations in high and moderate risk cancer predisposition genes (e.g. *BRCA1* or *BRCA2* and checkpoint kinase 2 (*CHEK2*) respectively) and BC-associated common single nucleotide polymorphisms (SNPs)[10]. Non-genetic risk factors include increasing age, personal history of breast pathologies such as atypical hyperplasia and lobular carcinoma in situ), high mammographic density (MD), exposure to therapeutic chest radiation (e.g. for treatment of Hodgkins disease), high body mass index (BMI), exogenous female hormone use (e.g. menopausal hormone therapy (MHT) and hormonal contraceptives), alcohol, inadequate physical activity, and reproductive factors (early menarche, low parity, shorter breastfeeding periods and late menopause). The population frequency of some of these genetic and non-genetic factors, and their associations with BC risk are shown in Figure 1. The distinction between genetic and non-genetic risk factors is not absolute, as many of the 'non-genetic' risk factors may have a genetic component that is yet to be fully elucidated[11-13].

This review discusses the evidence for the role of risk factors in driving BC incidence and their integration into tools to estimate BC risk for an individual woman - the first essential step towards precision prevention. Furthermore, it evaluates existing medications to reduce BC risk and their associated challenges, as well as outlines the search to find better alternatives. Lastly, learning from the uptake and adherence issues of available medications, it also discusses the priorities that need to be considered when developing and implementing alternatives.

Genetic risk factors

A high incidence of BC in certain families was first noted in 1866[14]; however the most common BC susceptibility genes, *BRCA1* and *BRCA2*, were not discovered until the mid 1990s[15,16]. *BRCA1* and *BRCA2* are involved in the repair of DNA double strand breaks through homologous recombination. Inherited mutations in these genes account for about 2.5% of all BCs, are responsible for only a minority of BCs in women with a strong family history of the disease[17] and result, on average, in about a 70% risk of BC by age 80 years [18]. That average high risk is modified up or down for an individual mutation carrier by her family history of BC, site of mutation, and other genetic and non-

genetic factors[18]. Other high and moderate penetrance BC predisposition genes include cadherin-1 (*CDH1*; which encodes E-cadherin), *PTEN*, serine/threonine protein kinase 11 (*STK11*; also known as *LKB1*), *TP53*, *CHEK2*, ataxia telangiectasia mutated (*ATM*), nibrin (*NBN*) and partner and localizer of BRCA2 (*PALB2*), but germline mutations in all of these are rare[19]. However, they are still included on many genetic risk gene testing panels, and additional screening, preventive options and genetic counselling are offered to mutation carriers[20].

Other much more common low penetrance SNPs also affect BC risk. While they confer small risks individually, their combined effect, when summarised as a polygenic risk score (PRS), can be substantial[21-23]. SNP-based PRS can also be combined with other risk factors in risk prediction models such as breast and ovarian analysis of disease incidence and carrier estimation algorithm (BOADICEA) and IBIS, which incorporate family history, age, genetic and other risk factors[24]. A SNP-based PRS also improves risk prediction in women with pathogenic mutations in rare high and moderate penetrance genes[25,26]. Despite the PRS not being routinely used in clinics, there are large cohorts currently being assessed to see how SNP-based PRS might affect BC risk management in various settings including the WISDOM (Women Informed to Screen Depending On Measures of risk) study[27-29]. Additionally, studies to assess chromatin organization are ongoing to identify the actual genes affected by the BC-associated SNPs, which are often not located (in the nucleotide sequence) close to the genes they most strongly influence[30].

Non-genetic risk factors

Whilst obesity and alcohol use both contribute, the increased incidence of ER positive BC is driven to a large extent by changes in reproductive patterns[31-35].

Age of menarche and menopause

Since the mid-19th century the average menarcheal age has decreased from 17 to 12 years of age[32,36,37]. The relative risk (RR) of BC increases by 5% for each year younger a women is at menarche[38]. Factors known to affect age at menarche include gestational exposure to cigarette smoke,

diet, psychological state, maternal weight gain and BMI[39-45]. Moreover, the inverse association between BMI and menarche timing is particularly strong[34]. In one sequencing study, 30 new genetic loci encoding proteins involved in lipid metabolism and cell growth were shown to be associated with menarche timing [46]. Additionally, separate studies have shown that increased gestational weight gain is associated with a greater chance of obesity in adolescent offspring and excessive maternal weight gain has been shown to lower the age at menarche in daughters [34,47,48].

Older age at menopause is associated with an increased RR of BC of 2.9% per year of delay when compared to the mean age of natural menopause[32,38,49-51]. The average age of menopause has increased from approximately 49 in 1908[52] to 51.4 now [53,54]. This 2 year increase in age at menopause would instil a moderate 6% increased RR of BC. Menopause timing is affected by socioeconomic status, parity, use of the oral contraceptive pill (OCP) and smoking[55]. In addition, through mother–daughter and twin studies, it has been demonstrated that 44–63% of the timing can be accounted for by heritability [34]. Polymorphisms within the ER signaling pathway have also been found, but more work is required to determine what this means for the level of ER signaling [56,57]. Further implicating hormones in menopause timing, women with a later menopause have longer menstrual cycles and the latter is suggested to be related to hormone levels in the follicular phase [58]. Research in pre-clinical models and women where possible should focus on determining why the breast is particularly sensitive to cancer risk if there are changes in hormonal exposure at both the beginning and end of reproductive cycling [34].

Childbearing

Women are having fewer children (and often later in life) which also increases BC risk, an association identified in the 18th century when nuns were found to have an increased risk of BC[59]. Childbearing prior to 35 years of age provides longer term protection against BC with the age of first birth being particularly important. If aged < 20 years, the longer term RR is reduced by 70% compared with nulliparous women. As the age at first full term birth increases, the longer-term protection from parity is progressively lost[35] and for those women who begin childbearing after age 35, the risk of BC is

higher than for nulliparous women[35,60]. This parity associated protection has been shown to be specific for ER positive BC[61-63] but the data related to molecular subtypes of BC is mixed [64,65].

In Australia, as in other high income countries[66-68], fertility rates have dropped to an average of 1.7 children per woman (compared to 3.5 in 1960 and 5 earlier in the 20th century), almost a quarter of women will remain nulliparous[69,70] and over 60% of parous women delay childbearing until after age 30, which provides little or no BC protection[71]. Older age at first birth is most common among highly educated women[69] (average age of first birth in the USA in 2017 was 3.5 years older for college-educated women[72]). These changes in reproductive behaviors and increase in BC risk are observed globally [49,73,74].

The protection afforded by pregnancy is not immediate; first there is a period of increased risk as the breast undergoes a post-partum involution process to return to its pre-pregnant state. This takes on average 10 years [75]. Older age of first-time childbearing means that this transient increased RR of BC after birth is more important because baseline BC risk increases with age and also the transient increase is more prolonged in older first-time mothers[75].

The mechanisms that underlie the protection from BC following childbirth have not been defined. A reduction in the number of mammary stem cells (MaSCs)[76] and reduced sensitivity to estrogens[77] have been postulated. MaSCs are thought to be the cells of origin for carcinogenic transformation[78,79], and therefore, reduced levels of them would leave the breast less susceptible to tumorigenesis[80]. In support of this, the RR of BC owing to radiation exposure is highest in young women, whom, it is proposed, acquire radiation-induced mutations (environmental exposure or for treatment of other cancer types) in long lived MaSCs[81,82]. Moreover, rat mammary glands are most sensitive to dimethybenz-(a)-anthracene (DMBA) induced carcinogenesis in puberty, when terminal end buds (believed to serve as niches for MaSCs) are most abundant[79]. However, mouse studies directly assessing the role of MaSCs in parity protection have provided conflicting results [83-85] with one study in particular showing that MaSCs are not in fact localized in terminal end buds[86].

Our group has recently provided some insight into this controversy by demonstrating that whilst cellular repopulating activity is reduced by parity, it is not due to the classically defined MaSCs (Britt and colleagues unpublished data). Additionally we have also shown that the number of ER positive epithelial cells are decreased by parity leaving the breast less sensitive to the pro-proliferative effects of estrogen[77]. In line with this, Jindal and colleagues have also shown that breast tissue of parous women has reduced proliferation [87].

The immune microenvironment may also contribute to parity induced protection. However, the relationship is complicated by the fact that protection occurs only after women pass through an increased risk period immediately following the pregnancy as the breast undergoes post-partum involution. During the involution process (first five years post pregnancy in women, and first weeks in mice) there are increased myeloid cells which can dampen the adaptive immune response and lead to a pro-tumorigenic environment [75,88,89]. However, once involution completes parous women are afforded long-term protection against BC. The immune changes that occur in the resting parous women are long-term changes to the breast and may mediate the decreased BC risk in parous women. Resting parous breast has an enrichment of genes involved in immune-surveillance (*SARM1*, T cell receptor β (*TCRβ*), human leukocyte antigen-A24 (*HLA-A24*) and interleukin-22 receptor subunit α2 (*IL22RA2*)) when compared to nulliparous postmenopausal glands [89,90]. These genes are instrumental in triggering innate immune responses, activating T cells, eliciting cytotoxic T cell anti-tumor immunity, and promoting apoptosis of tumor cells. Further work is needed to align these gene expression changes to the specific protective changes in the immune microenvironment. Understanding these may allow us to begin assessing the potential of therapeutically instilling a protective immune microenvironment.

Breastfeeding

For every 12 months of breastfeeding, there is a RR reduction for BC of ~4%[31,73,91,92]. Importantly, the protection conferred by breastfeeding is not limited to ER positive BC [61,93,94]. The mechanisms of breastfeeding-induced protection are largely unknown; however, glycoproteins stanniocalcin-1

(STC1) and STC2 are increased during lactation and these in turn inhibit protease pappalysin-1 (also known as PAPP-A), an oncogene that is increased during pregnancy, which along with insulin-like growth factor-binding protein 5 (IGFBP5) stimulates tumour formation[95].

Current breastfeeding rates are much lower than the recommendation of the World Health Organization (WHO), which calls for breastfeeding only for the first 6 months of life, with continued breastfeeding and complementary foods up until two years of age or beyond[96]. In Australia and the UK respectively, 90% and 69% of women initiate exclusive breastfeeding; however 50% and 23% of these have ceased by 6-8 weeks[97-99]. Moreover, Victora and colleagues[100] found that in low-income and middle-income countries, only 37% of children younger than 6 months of age were exclusively breastfed. Breastfeeding rates and duration could potentially be rapidly increased by scaling up known interventions, policies and programs, such as lactation support programs, reinforcing a breastfeeding culture (e.g. by removing actual and perceived restrictions on breastfeeding in public), adequate paid parental leave, flexible working arrangements and prohibition of aggressive and inappropriate marketing of breastmilk substitutes[101].

Mammographic density

MD is the extent of white or radio-opaque tissue (dense area) on a mammogram, and the term percent MD (PMD) is used to represent this dense area as a proportion of the total tissue area of the breast on a mammogram. There are multiple ways to measure MD and controversy exists over the measure that best correlates with BC risk. The Breast Imaging Reporting and Data System (BI-RADS) is the most commonly used tool clinically and includes 4 categories (almost entirely fat, scattered density, heterogeneously dense, and extremely dense)[102]. Limitations of the BI-RAD assessment include that it provides crude categorical estimates of density (rather than a continuous measure) and is reader dependent. There have been 5 BI-RAD editions with the 2017 release including clarification of previous terms to assist with risk stratification[103].

Many studies have demonstrated that, after adjustment for age and BMI, MD is an independent risk factor for BC, with a RR ranging from 1.8 to 6.0 in women with high MD (HMD) when compared to those with low MD (LMD)[104]. A systematic review and meta-analysis of 42 studies found that the RRs for BC were 2.92 and 4.64 for women with heterogeneously dense or extremely dense breasts respectively, compared to women with almost entirely fatty breasts[104]. Hopper and colleagues showed that measures of MD may explain more variation in risk across the population than known genetic variants, when adjusted for other risk factors, in particular age and BMI[105,106].

HMD is an important BC risk factor, not only because of the magnitude of the risk with which it is associated, but because it is highly prevalent; 43% of women in high income countries aged 40-74 have extremely or heterogeneously dense breasts[107]. In the United States (US), this corresponds to more than 27.6 million women. The US and the state of Western Australia are the only places where standardized mammographic reporting includes a MD measure, largely resulting from consumer advocacy campaigns. The lack of routine MD reporting globally may be owing to controversy over which density measure best correlates with risk and a lack of clear clinical pathways for management of women with HMD.

Although generally considered a non-genetic risk factor, twin studies have demonstrated that about 60% of the variation in MD is explained by genetic factors[13]. The pathobiology underlying HMD is not well understood but recently has been correlated with increased levels of stroma and epithelium[108] as well as immune cells[109] compared with LMD (Figure 2).

Lastly, MD is also emerging as a potential biomarker for prevention. A reduction of MD greater than 10% following treatment with a selective ER modulator (SERM), tamoxifen has been associated with a 63% BC risk reduction (odds ratio (OR): 0.37) [110]. However, the case for aromatase inhibitors (which reduce post-menopausal estrogen synthesis) is not as strong [111]. The reasons why MD is appealing as a predictive biomarker are that it is strongly associated with endocrine exposure, is non-invasively measured and can be incorporated into routine patient management. Nevertheless before it is introduced we need to determine the change threshold in MD that best predicts improved outcome,

the most accurate predictive parameter of MD i.e. percent density vs. absolute measures or categorical density (BI-RAD, Boyd or Wolfe) [112,113] and how we should interpret MD i.e. visual vs. computer assisted vs. fully automated methods.

Overweight and obesity

High BMI in the post-menopausal years is associated with a significant increase in BC risk, although it appears protective in premenopausal women. Specifically, in an international meta-analysis of 10 studies from 9 prospective cohorts and 22 case control studies, postmenopausal women in the highest body weight categories had an 82% increased RR for ER positive BC compared with those in the lowest body weight categories; there was no association with the other BC subtypes[114]. Conversely, pre-menopausal women in the highest body weight category had a 20% lower risk of developing ER positive BCs (similarly, there was no association with the other BC subtypes). Several mechanisms have been proposed to explain the link between increased BMI and cancer risk including increased conversion of androgens to estrogens, insulin and insulin-like growth factor (IGF) signalling, adipokine pathophysiology and chronic inflammation[115]. For BC specifically, the case for hormonal stimulation is supported by in vitro and in vivo experimental data [116] and the fact that male BC risk factors (obesity, Klinefelter syndrome and gynaecomastia) are associated with increased estrogen levels[117].

The Iowa Women's Health and Nurses' Health studies showed that women who maintained or lost weight as they got older had a reduced RR of post-menopausal BC [118,119] . This is supported by earlier epidemiological studies showing >10kg weight loss between 22-44 years of age was associated with an OR of 0.6[120]. Meta-analyses have also confirmed adult weight gain is associated with increased post-menopausal, but not pre-menopausal BC risk [121]. However, it is only those women with BMI of <23.4 kg/m^2 at age 20 years who had their BC risk influenced by adult weight gain [122]. It is not clear why the BMI at age 20 impacts postmenopausal BC risk, but it is postulated to be due to hormonal differences in adolescent girls with high BMI [122].

Physical Inactivity

Independent of BMI - mediated risk reduction, moderate to vigorous physical activity is associated with about a 20% reduced RR of BC when comparing the most to least physically active women [123-126]. Informed by these findings, the World Cancer Research Fund has concluded that physical activity probably protects against BC [127]. Independent of changes in adiposity, mechanisms that may account for this protection include physical activity effects on estrogen metabolism, insulin sensitivity, chronic low-level inflammation, oxidative stress, and immune function[124,126]. Physical activity-induced transcriptional changes are also possible[128,129]. Experimental studies have also directly addressed why exercise is beneficial. For example, the colony forming ability of non-small-cell lung cancer (NSCLC) cells is reduced by 80% after pre-incubation with conditioned serum from exercised individuals[130] and tumour incidence in mice is halved[131,132]. Work is underway to define the molecular signals underlying this. Whilst the optimal level of physical activity necessary for BC prevention is not clear with more than half the population in high income countries (including Australia, UK and the US) not meeting the recommended physical activity guidelines[133], there are opportunities for improvement.

Alcohol

Data from the Nurses' Health Study showed that women consuming 5-10 grams of alcohol per day (i.e. 3-6 glasses of wine per week) were 15% more likely (RR 1.15) to develop BC than non-drinkers, and those consuming at least 30 grams per day (i.e. at least 2 drinks per day) were 50% (RR 1.50) more likely[134]. Similar results were found in the Million Women Study[135]. A large prospective pooled Australian cohort, Arriaga et al., 2019[136] have recently shown that regular alcohol consumption is the leading modifiable cause of BC burden for premenopausal women, explaining 12.6% of BCs.

The mechanism by which alcohol (now considered a class I carcinogen by the international agency for research on cancer (IARC)) increases BC risk is an active area of study. Ethanol is known to stimulate cell proliferation and the transcriptional activity of ligand activated ER, which in turn increases levels of circulating estrogen levels[137,138]. Ethanol metabolism takes place mainly in the liver

where it is oxidized to acetaldehyde by the alcohol dehydrogenase (ADH) enzymes; however, ADH enzymes are also expressed in the breast [139]. Acetaldehyde can induce DNA strand deletions, chromosome aberrations and DNA adducts and is considered mutagenic and carcinogenic[140]. Furthermore, some experimental work has been performed in mice looking at the effects of alcohol on the immune response to cancer [141]. They found that CD8 cytotoxic T cells (which are capable of killing tumour cells) were decreased, in particular the CD8 memory T cells, which allow an efficient anti-tumour response should re-infection occur. Myeloid derived suppressor cells were also increased, which supress T cell responses and an increase in CD3+ invariant NKT cells that had a pro-tumorigenic expression profile[141]. Overall this suggests that alcohol supresses the ability to the immune system to respond to cancer.

The World Cancer Research Fund and the American Institute for Cancer Research (AICR) report recommends that if alcoholic drinks are to be consumed, that this is limited to no more than two drinks a day for men and one drink a day for women[142]. Although earlier research supported potential health benefits for low to moderate alcohol intake[143], more recent, methodologically robust research has concluded that the safest level of alcohol intake is none[144]. Alcohol is an ingrained aspect of the culture in many parts of the world. Reducing population intake of alcohol will require government commitments to developing and implementing policies similar to those that have reduced smoking rates in many jurisdictions, such as increased alcohol taxation, control of the physical availability of alcohol and hours of sale and banning alcohol advertising and implementing plain packaging.

Lifestyle

It is important to note that the benefits of a healthy lifestyle in terms of reducing BC risk are particularly important, in absolute terms, in women at high familial risk of the disease. We have shown that the RR for associations between BC risk factors such as BMI and physical activity are similar regardless of the underlying familial risk; this means that the absolute risk associated with higher BMI or lower physical activity is much greater for women at high familial risk compared with those at population risk [145,146]. Therefore, it is crucial that the larger potential benefits for lifestyle

changes are explained to women at increased risk who may otherwise feel that the familial factors are so overwhelming that there is little to be gained by lifestyle adjustment. Unfortunately, there is very limited interventional trial data on lifestyle changes. However, one study, the women's health initiative (WHI) dietary modification trial, showed that reduced fat intake and increased consumption of vegetables, fruits and grains led to a 5% reduction in BC risk (Hazards ratio (HR): 0.95) at the long-term follow-up (19.6 years) [147]. Further well designed lifestyle intervention trials assessing impacts on BC risk are needed and will surely help to convince those at risk of the impact these changes could have on their personal risk.

BC risk in diverse populations

The National Cancer Institute (NCI)'s Surveillance, Epidemiology, and End Results (SEER) program showed that in the U.S. the age-adjusted BC incidence for ethnic minorities was lower than those for white women, with 141 cases per 100 000 in white women, 122 in African Americans, 97 in Asian and Pacific Islanders, 90 in Hispanics, and 58 in American Indians and Alaskan Natives [148]. The difference in risk factors across the ethnicities and the use of screening mammography could explain some of the differences, but BC incidence was still significantly lower in African Americans than whites when adjusted for these differences[148]. Despite the lower overall incidence, African American women are more likely to be diagnosed with advanced and largely ER negative BC compared with white women [149]. Whilst the reason for these differences is not fully understood, it may involve the known associations between certain risk factors and disease subtypes. For example, multiparity and early first pregnancy protect against ER+ luminal BC, but do not protect against the development of basal-like breast cancer [62,150]

Heritability analyses show that BC is a highly polygenic disease [151]. In addition to the rare, high risk alleles, there are common variants with a small effect on risk (Figure 1). The use of a PRS assessing the effects of these variants on risk has only been thoroughly validated in European populations. Only the Breast Cancer Risk Assessment Tool (BCRAT) from the national institutes of health (NIH) has been validated for use in black or African American women, Hispanic women and Asian and Pacific

Islander women [152-154]. Genome wide association studies (GWAS) in multiple ethnicities such as the National Cancer Institute led Confluence project (300,000 BC cases and 300,000 controls) will drive a better understanding of the etiology of BC and allow us to improve risk stratification across ancestry groups.

[H1] Predicting BC risk

A key component of optimal precision prevention is the capacity to accurately estimate a woman's BC risk. This facilitates the use of evidence-based prevention interventions appropriate to the woman's personal risk level. It also enables calculation of the absolute risk-reduction from preventive interventions, thus assisting informed decision making.

BC risk estimation models now exist which attempt to quantify the combined effect of many of the BC risk factors discussed above[155]. Many of these have not undergone independent validation in study populations other than those used in their development and will not be considered further here. The independently validated models vary regarding the risk factors they utilise. The risk factor inputs for some of the main models are shown in Table 1[156-169].

Of the validated models, most[156,157,159,166,170-174], but not all[160,167-169,175], incorporate non-familial risk factors to varying degrees. The IBIS model encompasses the most comprehensive list of risk factors and performs well in comparative validation studies[158,176-179]. Polygenic risk to SNPs has been shown to predict BC risk almost independently of other factors, including MD[179], and the IBIS model is the only validated, widely available model that currently incorporates polygenic risk[179,180].

The performance of risk prediction models is often measured based on their discriminatory accuracy and calibration. The performance of the various BC risk prediction models varies, with discriminatory accuracy ranging from 0.56 to 0.71 (poor to good)[181] and calibration ranging from 0.85 to 1.52 according to a recent systematic review[182]. Work is ongoing to improve the accuracy of these risk

prediction models. For example, common risk prediction models do not currently include some modifiable risk factors such as alcohol, hormonal contraception use, physical activity or time since last pregnancy. It will also be important to determine if additional, more novel risk factors, such as steroid hormone levels (e.g. estradiol and testosterone)[183], epigenetic markers[184] and double-strand DNA repair phenotype[185], will give maximal improvement to the models. Incorporation of new risk factors into existing models will require consideration of potential interactions with existing risk factors and extensive validation, preferably using prospective data.

The current models have other limitations besides their limited discriminatory accuracy. Firstly, the models tend to have different performance characteristics depending on the subset of women they are applied to, but many clinicians are not skilled in choosing the most appropriate risk model, nor do clear guidelines exist[186]. A related issue is that the models have been developed and validated largely in populations of European descent, so their accuracy in estimating BC risk for women of other ethnicities is uncertain. Importantly, none of the major validated risk estimation models couple the risk estimation to comprehensive, personalised BC prevention and screening advice, nor estimation of the absolute risk reduction that can be achieved. And lastly, most have user interfaces that are difficult for women and less experienced clinicians to use. We have recently developed iPrevent[187] to overcome these issues and to facilitate collaborative decision making about BC risk management, between women and their clinicians. Women can complete the tool online at home and print the output for discussion with their clinician. It has been independently validated, is well calibrated and has good discriminatory accuracy (0.70 overall and 0.74 for women under age 50) [188]. It has good acceptability and usability for both women and clinicians and seems to improve the accuracy of risk perception without adversely affecting anxiety[189].

To date, all of these BC risk models have generally been used on an ad hoc basis and to our knowledge, there has been little consideration of population-based risk assessment followed by targeted risk reduction, despite the potential of precision prevention to reduce BC incidence.

Targeted risk reduction might include modifying specific risk factors (such as alcohol intake, use of MHT and hormonal contraceptives, physical inactivity and obesity) contributing to each woman's personal risk and, for some women at higher risk, consideration of risk-reducing medication. It is known that consumers find the constant information about BC risk factors in the media and other sources confusing, and are often uncertain how it pertains to them as individuals, with many having expressed a preference for more targeted information[190]. In Australia, formally assessing BC risk at the time of (free, government-funded) breast screening in order to risk stratify women for different screening approaches is currently being considered. However, given that breast screening usually starts at age 50, such an approach would provide no opportunity to prevent the approximately 20% of BCs that occur before that age **[191].** We suggest that consideration should be given to routine risk assessment of women in the general population in early adulthood (and at regular intervals thereafter, given that risk factors change over time). Nevertheless it will be important to 1) identify a risk assessment tool that is accurate and easy to use, 2) show that such risk assessment results in behavioural change and uptake of risk-reducing medication that will reduce BC risk without increasing anxiety beyond acceptable thresholds, and 3) determine the cost, suitability and feasibility of such an approach in different healthcare systems and among different subgroups (e.g. by ethnicity, age and socioeconomic status).

Currently Available Preventive Options

Women at increased risk of BC have several options to reduce their risk, including surgery, medication and lifestyle options (the last is also relevant to women at moderate risk). Table 2 summarises the major US and UK guidelines[192-194].

Risk-Reducing Bilateral Mastectomy

The most effective measure for reducing BC risk is bilateral mastectomy, although guidelines recommend limiting this to women at substantially increased risk. There are no randomised trials of this intervention, but observational studies show it is associated with a 90% reduction in risk[195,196]. Immediate breast reconstruction is usually offered, although it is associated with much higher rates of

unanticipated reoperations. Most women are satisfied with their decision to have bilateral risk-reducing mastectomy and have a significant reduction in worry-associated with getting BC, but there is less satisfaction with cosmetic results, body image, and sexual feelings[197]. Risk-reducing mastectomy that spares the nipple has better cosmetic outcomes than simple or skin-sparing mastectomy, and limited data suggests it confers similar risk reduction[198]. Uptake of risk-reducing bilateral mastectomy in high risk women is highly variable, with high uptake rates in the US, UK, Netherlands and Norway and low rates in Poland and France[199].

Bilateral Salpingo-oophorectomy

Bilateral salpingo-oophorectomy is effective at reducing the risk of cancers of the ovary and fallopian tube. *BRCA1* and *BRCA*2 mutation carriers are generally counselled to consider this procedure by the age at which their ovarian and fallopian tube cancer risk increases above that of the general population, that is by late 30s (for *BRCA1* carriers) and late 40s (for *BRCA2* carriers)[18]. Historically these women have also been counselled to consider the procedure at an earlier age (after childbearing) in order to reduce BC risk. Randomised trial data on the efficacy of bilateral salpingo-oophorectomy in reducing BC risk are not available. Earlier studies suggested a halving of BC risk for mutation carriers who underwent risk-reducing salpingo-oophorectomy (RRSO)[200]; however issues related to the methodology used in this study have been raised[201]. Furthermore, recent prospective cohort studies have found no convincing overall association between RRSO and BC risk in *BRCA1* or *BRCA2* mutation carriers[202-204].

Lifestyle Modification

Modification of non-genetic risk factors, such as obesity, alcohol use and lack of physical activity is an important component of BC prevention. In general, these non-genetic risk factors confer similar RRs of BC in high risk women as for those in the general population[205]. Unfortunately, lifestyle modification can be difficult to achieve and sustain. Therefore, focus on the development of efficacious interventions for behavioural change as well as government policies, as already discussed, to support healthy lifestyles will be essential.

Clinically Available Risk Reducing Medication

Risk reducing medication is an important prevention option for women at increased risk of BC who do not wish to undergo (or who wish to postpone) risk-reducing mastectomy or whose risk is increased but not elevated enough for surgery to be considered appropriate. The risk-reducing medications recommended in international guidelines are the selective ER modulators (SERMs), tamoxifen and raloxifene, and the aromatase inhibitors, exemestane and anastrozole (see Table 2). None of these have been shown to reduce BC mortality and all of them are only able to reduce risk of ER positive BC. Nevertheless, ER positive BC is the most common type and avoiding a BC diagnosis and subsequent treatment, even if that BC was not going to result in premature mortality seems a worthwhile goal in terms of reducing burden on the healthcare system, women and their families.

Tamoxifen is the best studied risk-reducing medication and is the only preventative agent that has been demonstrated to be effective in pre- and post-menopausal women. It reduces ER-positive BC risk by 33%[206], with the risk reduction seen not only during the 5 years whilst taking the medication, but also for at least 15 years after cessation[207]. Reductions in MD in tamoxifen users correlate with its preventive efficacy[110]. However, side-effects of tamoxifen can include menopausal symptoms, such as hot flushes, and a doubling of the risk of thrombosis, although the absolute risk remains low, particularly in younger women[208]. Tamoxifen also doubles the risk of endometrial cancer in post-menopausal women, although again the absolute risk is small[208]. Another major impediment to uptake of tamoxifen by pre-menopausal women for a 5 year period is the inability to prescribe it safely in women who are trying to conceive, who are pregnant or who are lactating and the fact that women need to use a non-hormonal form of contraception[209].

Another SERM, raloxifene has only undergone trials in postmenopausal women. Raloxifene (60mg daily for 5 years) was compared directly with tamoxifen (20mg daily for 5 years) in the STAR trial and at the 81 month median follow-up raloxifene was only 76% as effective at reducing ER-positive BCs compared with tamoxifen, but without the increased endometrial cancer risk seen with tamoxifen and with fewer thromboembolic events[210]. Risks and benefits of treatment with raloxifene or

tamoxifen in post-menopausal women depend on age, ethnicity, BC risk, and hysterectomy status. Tables have been published for both tamoxifen and raloxifene that can help identify groups of women for whom the benefits of these risk-reducing medications outweigh the risks[211].

Randomised controlled trials of the aromatase inhibitors exemestane and anastrozole have also shown that these medications can reduce BC risk by 60% at median 2.5 years follow-up and 49% at median 10.9 years follow-up, respectively[212-214]. These medications can only be used in post-menopausal women as they are ineffective in women with functioning ovaries.

Despite the clear benefits of risk-reducing medication, uptake is low among women at increased risk[215,216]. The reasons are complex and both clinician and patient-related. There is lack of clarity over the most appropriate type of clinician to initiate discussions about risk reducing medications[186,215], in addition to clinicians having difficulty using the existing risk assessment models[215,217], and preferring to have a tool that links risk assessment with risk management [217]. Furthermore, clinicians often lack deep knowledge about prevention medications[218-220] and are concerned over the lack of surrogate markers for the effectiveness of preventive medications as well as the overall lack of commercial interest in prevention[215]. The latter concern comes about because all current prevention medications were off-patent by the time their role in prevention was proven. Thus, unlike newer patented drugs, where companies have a commercial incentive and spend considerable proportions of their budget educating clinicians about their drug, there is no investment to educate clinicians about implementing these older generic prevention medicines into their practice. Additionally, in some countries/regions in Europe and in Australia, there is lack of a clear pathway for regulatory approval of repurposed, off-patent drugs. Tamoxifen was shown to reduce BC risk in 1998 [221], and was promptly approved by the US food and drug administration (FDA) for primary prevention but in Australia regulatory approval was not sought until 2016 and only then after substantial advocacy by clinicians and consumer groups. Lack of regulatory approval in Australia before 2016 was a factor in the low rate of tamoxifen prescriptions[218].

The major patient factor contributing to low uptake of risk-reducing medications is said to be fear of side-effects[222,223]. However, in the main prevention trials, fewer than 5% of women ceased treatment because of side-effects [213,214,221,224]. Clinician recommendation and the way clinicians frame information about side-effects is important. For example, regarding the risk for endometrial cancer for post-menopausal women, it may be better to frame the risk as "approximately 996 in every thousand women can take tamoxifen for 5 years without getting endometrial cancer", rather than "your risk is doubled". Few online tools are available currently to help clinicians balance absolute benefits against absolute risks for individual women[187]. Clinicians should also be sure to convey not only potentially adverse side-effects, but also beneficial ones, such as, for example, the potential for decreased breast tenderness, lighter menstrual periods, better bone density and lower cholesterol for women considering tamoxifen use. Clinicians should also consider offering women a short trial of 6-8 weeks of risk-reducing medication to assess their tolerance and so that women do not feel they are committing to a 5 year course with no knowledge of how well they, as an individual, will tolerate the drug. If such a short trial also had a biomarker of effectiveness, it may assist women in drug adherence.

Other patient factors that limit uptake of tamoxifen for ER-positive BC risk-reduction include the fact that it is a cancer drug, the experience of others (usually those with cancer), and the tablet being a daily reminder of their increased cancer risk; although the latter can presumably also work in reverse, with some women reassured by the daily tablet that they are actively reducing their BC risk[222]. Importantly it has also been shown that women often confuse tamoxifen with chemotherapy, and this has led to recommendations that the word 'chemoprevention' should be avoided[222,225] with 'risk-reducing medication' seemingly a more appropriate term.

Developing novel preventive agents

The 'perfect' risk-reducing medication would be highly efficacious, have minimal adverse side effects but potentially several beneficial ones, and be able to be used even if on hormonal contraception or during pregnancy or when lactating. It could potentially be a long-acting depot preparation, avoiding

the need for a daily tablet, and would not be associated in the public mind with a cancer drug. It would be inexpensive and preferably developed in a way that facilitated rapid regulatory approval and engagement of the pharmaceutical industry in implementation. Ongoing trials of BC prevention medications are summarised in Table 3.

One tactic to provide a new approach to risk-reducing medication that has fewer adverse side-effects than current agents is to modify the dose and delivery system of available agents. Tamoxifen is largely a pro-drug that is metabolised to its active metabolites, including endoxifen, by hepatic enzymes (e.g. cytochrome P450 2D6 (CYP2D6)) [226]. Biomarker studies have suggested that 5mg per day is equivalent to the usual 20mg per day dose in inhibiting BC proliferation[227], suggesting that low dose tamoxifen might be efficacious for prevention. Furthermore, a recent multicentre, randomised trial suggested that a lower dose and duration of tamoxifen (5mg daily for 3 years) might have similar BC prevention efficacy as the usual 20mg daily for 5 years dose, with fewer side-effects. Unfortunately, these 2 tamoxifen regimens were not compared against one another but, based on this trial[228], 5mg of daily tamoxifen for 3 years is now a reasonable BC prevention option for women who do not tolerate dosing at 20mg. It will be important to assess whether this smaller dose for a shorter duration provides the same long-term risk-reduction as 20mg daily for 5 years and whether CYP2D6 status affects the efficacy of the smaller dose. Another approach to potentially reduce the side-effects of tamoxifen is transdermal therapy which can result in high drug concentrations in the breast, but low systemic exposure. A window of opportunity trial in patients with ER positive ductal carcinoma in situ (DCIS) (NCT00952731)[229] showed oral and transdermal delivery both decreased (by 50-60%) expression of the proliferation marker Ki67. Atossa Genetics recently announced the results of a phase II study of daily topical endoxifen applied to the breasts, which showed reductions in MD in women using the transdermal medication, with no difference in menopausal side-effects between the topical endoxifen and placebo groups, although the duration of treatment was limited by skin rash.

Metformin is a drug that is commonly used to treat type 2 diabetes [230]. Metformin users have a decreased incidence of cancer, and more long-term use (≥ 5 years) is associated with a reduced, adjusted OR of 0.63 for developing BC [231]. This, and promising pre-clinical work has led to the phase

III randomized control trial (the PLOTINA study EudraCT Number 2009-009921-28) [232], comparing metformin versus placebo in post-menopausal women at high risk of type 2 diabetes.

Bisphosphonates, originally used as a treatment for osteoporosis, have been shown in pre-clinical studies to inhibit BC proliferation and metastasis and have been proposed as BC preventives [233-236]. They are currently used in patients with metastatic BC to reduce skeletal-related events, and their use in the adjuvant setting in post-menopausal women reduces mortality[237] and is recommended in North American and European guidelines[238,239]. Women who take bisphosphonates for bone density have reduced BC incidence (20-47% lower depending on the study)[234,240] suggesting a possible role in BC prevention. Conversely, they do not reduce contralateral BC risk when given adjuvantly[237]. An interventional prevention trial is underway (NCT02781805)[229] assessing the effects of the bisphosphonate, alendronate, on mammary epithelial cell differentiation and immune cells in high risk women.

Retinoids are another class of drugs that are currently in BC prevention trials (EudraCT Number 2009-010260-41 and NCT03323658) [229,241]. Retinoids are anti-proliferative, cyto-differentiating and apoptotic through their activation of the nuclear hormone retinoic acid receptor α (RARα), RARβ and RARγ. Strong data in pre-clinical models using the retinoid fenretinide[242] led to a phase III prevention trial in the late 1980s. Fenretinide showed a trend for reducing the incidence of second primary BCs in premenopausal women (HR: 0.66 and HR: 0.65 for contralateral and ipsilateral BC respectively), which was maintained at 15-year follow-up[243]. This drug has a very low toxicity profile (mainly reversible skin dryness and rashes as well as difficulties adapting to darkness) which are often overcome by a monthly weekend suspension of the drug. However, it is not safe for pregnant women and so has similar reproductive contraindications in pre-menopausal women as tamoxifen. Yet, the results of these novel BC preventative trials are eagerly awaited.

Medical prevention of BCs in *BRCA1* mutation carriers has been controversial. These women usually develop ER negative BC, and existing prevention agents have not reduced ER negative BC in clinical

trials, although observational data in the secondary prevention setting[244,245] show that tamoxifen is associated with reduced contralateral BC risk. There is growing evidence suggesting that receptor activator of nuclear factor-κB (RANK; also known as TNFRSF11A) and its ligand (RANKL) play a pivotal role in the development of *BRCA1*mutant-associated tumors. $RANK^+$ luminal progenitors are increased in pre-neoplastic tissue of *BRCA1* mutation carriers compared with non-mutation carriers[246]. Moreover, these cells have been identified as the cell of origin for the basal-like BC that develop in *BRCA1* mutation carriers. Pre-clinical studies in *Brca1*-deficient mice targeting these cells with the RANKL inhibitor (and osteoporosis drug) denosumab successfully inhibited tumour development[247]. Preliminary data from a preclinical window study to evaluate the biological effects of the denosumab on breast tissue biopsies from *BRCA1* mutation carriers showed proliferation was markedly reduced[246]. An international phase III randomised trial of denosumab is testing whether administering denosumab once every 6 months for 5 years will reduce BC incidence in *BRCA1* mutation carriers (EudraCT Number**:** 2017-002505-35)[248].

There is also interest in anti-progestins (synthetic progestogens) for BC prevention. Treatment of *BRCA1* deficient mice with the progesterone antagonist mifepristone inhibits tumorigenesis[249]. Mifepristone is considered too toxic to move into the prevention setting, but other less toxic progesterone receptor modulators are under investigation (NCT02408770)[250]. Aspirin, other non-steroidal anti-inflammatory drugs (NSAIDs), and the statins are inexpensive, widely available and relatively safe drugs, making their potential repurposing for BC prevention an attractive strategy. Whilst mature, randomised trial data are not available for any of these agents in the BC primary prevention setting, at a dose of ≥2 times per week aspirin use for 5 years was associated with reduced BC risk (RR: 0.86), with decreasing risk with longer duration (RR: 0.73 for 10 years and RR: 0.54 for 20 years)[251]. Similar results are observed with another type of NSAID, the cyclooxygenase 2 (COX2) inhibitors[252]. Recent work assessing these associations in a cohort enriched in women with a strong family history showed regular aspirin was associated with a 37-39% reduction in BC risk, whilst for COX2 inhibitors it was 61-71%[253]. Some studies have found that aspirin and COX2 inhibitors reduce both ER positive and ER negative BC[253] whilst in others only ER positive BC was reduced[254-256].

Large scale, randomised controlled trials with both population risk women and those at higher risk are needed to define the true benefits of long-term aspirin use in the preventative setting.

For all of these preventatives, a major task will be determining the best timing of preventative therapy. It is possible that preventive therapy may be delivered immediately prior to the age dependent increase in risk of hormonal BCs or the likely age of onset for familial cancers. This would drive high protection levels during the most crucial time. The 96-month follow-up of the randomized IBIS-I trial showed that 5years of taxoxifen prevention provided better protection in late premenopausal women (35-50 years old) rather than women 50 years or older (RR: 0.65 vs RR: 0.79) [257]. Instead, aromatase inhibitors offer an attractive alternative for postmenopausal women. However, as the time between puberty and first pregnancy is known to be a window of risk [34], ongoing work should determine why this period is so important and if it is also the most effective time to deliver long-lasting preventative therapy. Prevention trials are, by their very nature, quite lengthy and thus clinical trials assessing the impact of new therapies on early breast lesions (such as, hyperplasia and in-situ carcinoma) can be informative when assessing efficacy.

A deeper understanding of the earliest steps in BC development will aid in our quest to develop novel preventatives. The normal breast epithelium contains numerous cell types and is imbedded within a dense stromal and immune microenvironment [258,259]. Epithelial changes occur in *BRCA1* mutation carriers at risk of BC [246] and it is possible that other epithelial cell subtypes may be increased under alternate risk conditions (Figure 3). Additionally, the stromal and immune microenvironments play a significant role in the growth and progression of pre-invasive and invasive BC[258,260] and can stimulate tumour development in the normal post-partum breast[88]. The microenvironment of early lesions and breasts at risk of cancer should be studied in order to determine whether these cells can be targeted for BC prevention (Figure 3).

Conclusions and perspective

To date, BC prevention in most parts of the world, has largely focused on untargeted, population-based educational interventions (such as, increasing physical activity and reducing BMI and alcohol intake). This will remain an appropriate component of BC prevention, as these interventions also reduce risk of other important causes of morbidity. However, we are moving towards the ability to augment this approach with systematic targeting, or precision prevention. Precision BC prevention will mean delivering the right risk-reducing intervention, at the right time, to the right woman. A vital starting point will be to have a systematic and accurate method of assessing each individual woman's BC risk. Risk assessment models currently exist and their accuracy will continue to improve. Developing better risk assessment algorithms for specific BC subtypes that are validated in ethnically diverse populations is a high priority. Having a user friendly interface that enables women and clinicians to identify and manage risk will be important in implementing risk management. Implementation researchers and policy-makers should consider how models can be applied to populations in order to ensure that women at increased risk are identified at an early age while there is still time to effectively reduce their risk with existing proven interventions. To efficiently deliver this we will need to define which treatments can be given at which ages for maximal protection. Pre-clinical studies will be informative in determining such dosing regimens. The treatments will also need to be well tolerated as they are being used in otherwise healthy individuals. Ultimately it is hoped that risk assessment models might one day predict not only whether a woman will or will not develop BC but at what age, so that risk-reducing interventions can be applied in the most appropriate timeframe. The perfect intervention may target all molecular subtypes of BC, but given their different etiologies, this is unlikely, so models that predict subtype and thus enable the application of future medications that target particular subtypes would be optimal.

As we move to find preventative therapies that do not rely on disrupting estrogen activity, we need to understand more about what drives increased BC risk. Assessing the pre-neoplastic breast tissue of women at increased risk of basal-like BC (*BRCA1* mutation carriers) led to the identification of the cell of origin and the first cell-specific potential BC preventative (Figure 3). Now we need to ask how *BRCA2* mutation status alters the breast epithelial hierarchy and if this can be targeted for

preventative therapies. Furthermore, how do BMI, age and reproductive factors alter breast epithelial cells. If we find that aberrant control of distinct populations of breast epithelial cells are responsible for the generation of the different BC subtypes (such as for $RANK^+$ luminal progenitors and basal-like BC), the development of new preventives may need to be subtype specific.

In the 20th century, the eradication or control of many deadly communicable diseases transformed human health [261]. It is not impossible to imagine that, with the augmentation of our existing BC prevention toolbox with future discoveries, we could achieve the same for BC in the 21st century. By focusing on the risk factors for BC and their incorporation into effective risk estimation tools we will identify those women at increased risk. Research into the mechanisms underlying risks will be instrumental in driving the development of therapies to effectively counter or manage those risks and prevent BC where we can. It is unlikely we can reverse the reproductive choices that are driving hormonally responsive BC; however, public health awareness and preventative therapies will be important, as will a focus on the development of improved hormonal therapies (MHT and OCP) that deliver symptom control and contraceptive benefits, but without increasing BC risk. Whilst this may begin in high income countries a global move to prioritize women's health is required.

Table 1: **Comparison of model inputs for major breast cancer risk estimation models**

Model input	Risk Estimation Model				
	BCRAT[159,164,166]	**IBIS**[156,158,165]	**BRCAPRO**[160,162,167]	**BCSC**[157,161]	**BOADICEA**[163,168,169]
Individual factors					
Age	≥35	✓	✓	✓	✓
Race or ethnicity	✓	✓	✓	✓	✓
Age at menarche	✓	✓	NA	NA	NA
Age at menopause	NA	✓	NA	NA	NA
Age at 1st birth	✓	✓	NA	NA	NA
Parity	NA	✓	NA	NA	NA
BMI	NA	✓	NA	NA	NA
Hormonal contraception use	NA	NA	NA	NA	NA
MHT use	NA	✓	NA	NA	NA
Alcohol use	NA	NA	NA	NA	NA
Breast-related factors					
No. of prior breast biopsies	✓	✓	NA	✓	NA
Atypical hyperplasia	✓	✓	NA	NA	NA
LCIS	NA	✓	NA	NA	NA
Other benign pathology	NA	✓	NA	NA	NA
Mammographic density	NA	✓	NA	✓	NA
Therapeutic irradiation*	NA	NA	NA	NA	NA
Genetic testing					
BRCA1 or *BRCA2*	NA	✓	✓	NA	✓
Other high risk genes	NA	NA	NA	NA	✓
SNPs or polygenic risk score**	NA	✓	NA	NA	*
FHx factors* **					
Cancer status of 1st degree relatives	✓	✓	✓	✓	✓
Cancer status of 2nd degree relatives	NA	✓	✓	NA	✓**
Age at BC diagnosis	NA	✓	✓	NA	✓
Pathology of BC	NA	NA	✓	NA	✓
Bilateral BC	NA	✓	✓	NA	✓
Male BC	NA	✓	✓	NA	✓
Ovarian cancer	NA	✓	✓	NA	✓
Pancreatic and prostate cancer	NA	NA	NA	NA	✓

Genetic testing	NA	✓	✓	NA	✓
Mastectomy status	NA	NA	✓	NA	NA
Oophorectomy status	NA	NA	✓	NA	NA

BC, breast cancer; BCRAT, breast cancer risk assessment tool; BCSC, breast cancer surveillance consortium; BMI, body mass index; BOADICEA, breast and ovarian analysis of disease incidence and carrier estimation algorithm; FHx, family history; MHT, menopausal hormone therapy; NA, not applicable; LCIS, lobular carcinoma in situ; SNPs, single nucleotide polymorphisms

* eg mantle radiation for Hodgkins disease

**newest version of BOADICEA, V5, includes SNPs.

***includes family history of breast, ovarian, pancreatic and prostate cancer in 1st, 2nd and 3rd degree relatives

Table 2: **UK and US breast cancer prevention guidelines for women at increased risk**

	RRBM	RRSO	Medication #	Lifestyle Factors
NCCN [193]	Consider for: • high risk BC gene mutation • compelling FHx • prior thoracic RT below the age of 30	Controversy over whether RRSO reduces BC risk for BRCA mutation carriers but, based on OC risk, recommend for: • *BRCA1* – between 35-40yrs • *BRCA2* – between 40-45 years • Exercise caution in prescribing HRT post RRSO	Offer if • ≥35 years old with 5 year BC risk ≥1.7% • have LCIS Premenopausal: Tamoxifen Postmenopausal: Tamoxifen, raloxifene, exemestane or anastrozole	• MHT (consider associated BC risk) • Alcohol (limit consumption) • Exercise (premenopausal: vigorous; postmenopausal: moderate to vigorous) • Healthy weight • Breastfeeding
NICE [194]	Consider for: • lifetime risk ≥ 30%	Consider for: • lifetime risk ≥ 30% • offer MHT up until	Consider if: • lifetime risk ≥17% Premenopausal:	• OCP- (if >35 years old inform of increased risk of BC. For *BRCA1* mutation carriers discuss potential increased risk of BC before

		age of natural menopause– oestrogen alone if prior hysterectomy, combined MHT otherwise	Tamoxifen Postmenopausal: Anastrozole (unless severe osteoporosis) or tamoxifen (if severe osteoporosis or if the individual does not want to take anastrozole) or raloxifene (if the individual does not want to take tamoxifen)	age 40) • Breastfeeding • MHT (advise of increased BC risk; tailor use to individual circumstances; use lowest dose for shortest time possible (generally not after age 50); prescribe estrogen without progesterone if hysterectomy) • Alcohol (advise of increased BC risk) • Smoking (advise cessation) • Healthy weight • Exercise
ASCO [192]	NA	NA	Consider if: ≥35 years old with 5 year risk ≥1.66 or have LCIS Premenopausal: Tamoxifen Postmenopausal: Raloxifene, exemestane or anastrozole	NA

ACSO, American society of clinical oncology; BC, breast cancer; FHx, family history; HRT, hormone replacement therapy; LCIS, lobular carcinoma in situ; MHT, menopausal hormone therapy; NA, not applicable; NCCN, (US) national comprehensive cancer network; NICE, (UK) national institute for health and care excellence; OC, ovarian cancer; OCP, oral contraceptive pill; RRBM, risk-reducing bilateral mastectomy; RRSO, risk-reducing bilateral salpingo-oophorectomy; RT, radiotherapy.

5 year course; no guideline currently recommends a 3 year lower dose course as tested in DeCensi 2019 [228], although ASCO guidelines suggest women who stop tamoxifen after 3 years will likely still derive benefit and that for women with intraepithelial neoplasia the low 5mg per day dose of tamoxifen may be an alternative if there are concerns over adverse events with the higher dose.

Table 3: **Ongoing registered clinical trials of pharmacological interventions for breast cancer prevention**

Clinical trial identifier	Short study name	Sponsor	Phase	Intervention	Study design	Study population	Primary outcome	Secondary outcomes
ENDOCRINE AGENTS								
NCT02408770 [250]	BC-APPS1	Manchester University	II	• **Ulipristal acetate** 5mg oral daily for 3 months	Single arm	• Premenopausal • >17% lifetime BC risk	Change in Ki67 staining of breast epitheliu m	• % luminal, basal and mixed colonies • MRI background parenchyma enhancement • side-effects
NCT00078832 [262]	IBIS-II	Queen Mary University of London	III	• **Anastrozole** 1mg oral daily for 5 years • Placebo	Randomized, double-blind, placebo-controlled	• Postmenopausal • 40 – 70 years old • Increased risk of BC	BC incidence (invasive and non-invasive)	BC mortality
NCT03063619 [263]	Afimoxifene in reducing the risk of BC in women with mammographically dense breasts	M.D. Anderson Cancer Center	II	• **Afimoxifene gel** 4mg topically to each breast daily for up to 52 weeks • Placebo	Randomized, Double-Blind, Placebo-Controlled	• 40-69 years old, or less than 40 years if 5-year BCRAT risk is ≥ 1.66% BIRADS score 3 or 4	Percentage change in mammographic density (using Cumulus software)	• Other breast density measures and measurement methods • Breast tissue biomarkers • Hormone-mediated cellular activity • Inflammatory response • Markers

								of tamoxifen exposure • Toxicity • Pharmacogenomics
EudraCT Number 2016-001087-11 [264]	CIBRAC	Belfast Health and Social Care Trust	N/A	• **Tamoxifen** 20mg oral daily • **Anastrazole** 1mg oral daily with **Goserelin** 3.6mg s/c every 28 days	Randomised, open label, crossover	• *BRCA1* mutation • Premenopausal • > 18 years old	Feasibility – recruitment and compliance	Tolerability – QOL, AEs
RETINOIDS								
NCT03323658 [263]	Bexarotene in preventing BC in patients at high risk for BC	NCI	I	**Bexarotene** topically to one breast	Single arm, dose escalation	• Hx of BC and ≥5years since diagnosis, or • Hx of LCIS, ADH or ALH or • *BRCA 1* or *BRCA2* mutation carrier or • BC risk ≥ 1.7% in 5 years or lifetime risk ≥ 20%	Incidence of AEs	• Systemic toxicity • Bexarotene concentration • Tissue markers

						• ≥ 18 years old		
EudraCT Number 2009-010260-41 [241]	BC prevention with fenretinide in young women at genetic and familial risk.	Istituto European Di Oncologia	III	• **Retinamide** 200 mg daily oral for 5 years • Placebo	Double blind, randomised, placebo-controlled	• *BRCA 1* or *BRCA2* mutation or 20% chance of mutation • 25-44 years old	BC incidence (invasive and DCIS)	Incidence of LCIS, atypical hyperplasia, ovarian cancer and other cancers
BISPHOSPHONATES								
NCT02781805 [265]	Pilot study of bisphosphonates for BC	University of Wisconsin	I	**Alendronate** 10 mg daily for 1-3 weeks before breast surgery	Single arm, window study	• Women ≥18 years old • Referred for risk reducing mastectomy • Premenopausal	Percentage change in γδ T cells in breast tissue	• % change in mammary epithelial basal cells • % change in mammary luminal cells
RANKL INHIBITORS								
ACTRN12614000694617 [266]	BRCA-D	Melbourne Health	N/A	**Denosumab** 120 mg s/c monthly for 3 months	Single arm, window study	• *BRCA 1* or *BRCA2* mutation carrier • Premenopausal • 18-50 years old	Change in Ki67 expression in breast epithelium	• Safety and tolerability • Change in RANK and RANKL expression in epithelial and stromal breast cells • Change in ER and PR levels

								• Change in c-KIT, ALDH1 and RANK immunostaining • Change in luminal cell expression • Change in MRI breast parenchymal enhancement
EudraCT Number 2017-002505-35 [248]	BRCA-P	ABCSG	III	• **Denosumab** 70mg, s/c 6 monthly for 5 years • Placebo	Double-blind, randomised, placebo-controlled	• *BRCA1* mutation • Age ≥ 25 years and ≤ 55 years	BC incidence (invasive or DCIS)	• Incidence of invasive BC, invasive TNBC, ovarian, fallopian and peritoneal cancer, other cancers, breast biopsies and benign lesions, and clinical fractures
METFORMIN								
EudraCT Number 2009-009921-28 [232]	PLOTINA	Istituti Fisioterapici Ospitalieri	III	• **Metformin** 850mg oral, twice daily • Placebo	Double-blind, randomized, placebo controlled	• Postmenopausal • Central obesity • Another component of metabolic syndrome	BC incidence	CVD incidence

ABCSG = Austrian Breast & Colorectal Cancer Study Group; ADH, atypical ductal hyperplasia; ALDH1, aldehyde dehydrogenase 1; AE, adverse events; ALH, atypical lobular hyperplasia; BC, breast cancer; BCRAT, breast cancer risk assessment tool; BIRADS, breast imaging reporting and data system; CVD, cardiovascular disease; DCIS, ductal carcinoma in situ; ER, estrogen receptor; FHx, family history; LCIS, lobular carcinoma in situ; MRI, magnetic resonance imaging; NCI, national cancer institute; N/A, not applicable; PR, progesterone receptor; QOL, quality of life; RANK, receptor activator of nuclear factor-κB; RANKL, RANK ligand; s/c, subcutaneous; TNBC, triple negative breast cancer.

Figure legends

Figure 1:| **Breast cancer risk modifiers and population frequency**. The population frequency (horizontal x-axis) of genetic and non-genetic breast cancer risk modifiers are shown with their effects on, or associations with, relative risk of breast cancer (vertical y-axis). Rare, high risk alleles are shown as are rare, moderate risk alleles considered to have sufficient evidence to support their association. Examples of common low penetrance variants, of which there are now several hundred, are also listed[18,19,21-23]. For menopausal hormone therapy (MHT) and oral contraceptive pill (OCP) use, combined estrogen and progestogen therapy is assumed and dark blue denotes risk for current, long term users, mid blue denotes shorter periods of use and light blue past users[33,267,268]. * refers only to postmenopausal obesity[269]. # refers to 2 glasses of alcohol per day, which is the average consumption level in the 72% of the high socio-demographic index population who are drinkers[134,144]. Exercise refers to most active compared to least physically active[124]. The relative risk reduction associated with breastfeeding is for 12 months of cumulative breastfeeding[31]. Parity refers to a first full term childbirth prior to 25 years of age[35]. The RR of breast cancer in women with moderate to high mammographic density (>25% to >75% density) is 1.8 to 6.0 compared to women with low mammographic density[104]. Currently 50% of the female population are considered to have moderate (25-50%) to high (>75%) breast density [107]. Yellow refers to genetic risk factors, blue to reproductive and orange to lifestyle. *APOBEC3*, apolipoprotein B mRNA editing enzyme catalytic polypeptide-like; *ATM*, ataxia telangiectasia mutated; cadherin-1 (*CDH1*; which encodes E-cadherin), *CASP8*, caspase 8; *CHEK2*, checkpoint kinase 2; *FGFR2*, fibroblast growth factor receptor 2; *NBN*, nibrin; *PALB2*, partner and localizer of BRCA2; *STK11*, serine/threonine protein kinase 11.

Figure 2**The biological differences between high and low mammographic density.**
Breast tissue with high mammographic density (HMD) has been shown to have increased levels of stroma and epithelium compared with areas with low mammographic density (LMD)[108]. Note that within the epithelium however, an increase in stem or progenitor cells has not yet been shown. Tissue with HMD also has an increased amount of structured collagen. BCs are often localized in areas of dense collagen or are stimulated to grow when the breast has increased stromal collagen[270]. Additionally, the collagen-binding proteoglycans, lumican, decorin, fibromodulin, and biglycan are also associated with HMD[271]. Lumican can induce initiation and progression of BC by increasing angiogenesis, epithelial cell growth, migration, and invasion[272]. The increased stiffness resulting from these extracellular matrix (ECM) changes may drive cancer formation through higher mechanical force and resistance to contractility on the epithelial cells (via focal adhesions and the RHO GTPase signalling pathway) driving proliferation [273]. Stromal fibroblasts in areas of HMD have also been shown to exhibit gene expression signatures associated with cancer stimulating pathways such as stress response, inflammation, stemness, and signal transduction[274]. BCs with immune infiltration are known to have better prognosis and may respond to chemotherapeutics and be responsive to immune based therapies [275,276]. However, less is known about immune infiltration in the normal breast and early, pre-invasive lesions. Tissue with HMD has been shown to have a pro-tumorigenic immune microenvironment including increased innate (macrophages and dendritic cells), adaptive (T and B cells) and increased interleukin 6 (IL-6), which may aid escape from immune regulation for early tumor cell variants[109]. Furthermore, the ECM has been shown to modulate activation, fate determination, and chemotaxis of immune cells[277-279] indicating that the changes may be interrelated.

Figure 3: **Developing novel preventatives based on a deeper understanding of the early events in breast cancer development**. Schematic of the normal breast, pre-neoplastic changes and invasive breast cancer (BC) showing the alterations that occur and the drugs currently used in prevention and treatment. One of the earliest stages of cancer development is the transformation of a single cell within the epithelial layer. In the normal breast, the oncogene expressing cells are ejected from the epithelium by surrounding normal cells in a process called oncogenic extrusion [280]. Work is

undergoing to assess oncogenic extrusion in early tumour development and the factors that control it. The selective estrogen receptor modulator (SERM), tamoxifen is used to prevent BC in women with normal breast tissue and also in those women at high risk of BC owing to pathogenic mutations. Recently, denosumab, a receptor activator of nuclear factor-κB ligand (RANKL) inhibitor has shown promising results in BC prevention in *BRCA1* mutation carriers by targeting the $RANK^+$ luminal progenitor population that is increased in these women. Future work should determine whether *BRCA2* mutation status also alters the breast epithelial hierarchy and how this can be exploited to develop therapies for *BRCA2* mutation carriers. In terms of the non-genetic risk factors it is important that we define how body mass index (BMI), age and reproductive factors alter the breast epithelial cells. The epithelial cells sit embedded in a stromal and immune microenvironment, which is emerging as having a significant role in the growth and progression of pre-invasive and invasive BC [258,260]. In the stroma of pre-neoplastic lesions fibroblasts become activated and the macrophage and T cell populations are altered. Currently immune modulating therapies are not used this early in tumour development but as we define how the immune system changes at this time, it is possible they can also be used to prevent or delay tumour formation. In invasive BC, the luminal epithelial cells have transformed, extrusion does not occur and the basement membrane is breached. The epithelial cells can be targeted in invasive BC with, tamoxifen (for estrogen receptor $(ER)^+$BCs) and trastuzumab (for human epidermal growth factor receptor 2 $(HER2)^+$ BCs). There are also additional changes to the stromal fibroblasts and immune cells (for example, fewer T cells and repolarisation of macrophages), which can further stimulate cancer growth. Immune-based therapies, such as pembrolizumab, the programmed cell death protein 1 (PD1) inhibitor, inhibitors of granulocyte colony-stimulating factor receptor (GCSFR) and colony stimulating factor 1 receptor (CSF1R) are currently being explored in cancer treatment. Similarly, cancer associated fibroblast (CAF)-targeting therapies, such as fibroblast activation protein (FAP) antibodies conjugated to cytotoxic drugs (FAP5–DM1) are being investigated. As we begin to understand more about the changes occurring in the pre-neoplastic breast, it can be envisioned that the use of additional epithelial and stromal or immune-targeted therapies will be explored also at this early timepoint.

The role of exogenous hormones in breast cancer risk

A major change in reproductive behaviours in the last century has been the introduction and widespread use of exogenous estrogens in the form of hormonal contraceptives and menopausal hormone therapy (MHT), [33,267,281-283] which increase ER positive breast cancer (BC) risk[33,268,282-284]. The oral contraceptive pill (OCP) is now the most popular form of contraception with a quarter of women of childbearing age in high income countries using it at any one time[285].

In the late 1990s, a meta-analysis of individual data from over 150,000 women demonstrated that current users of OCPs had a 24% increased relative risk (RR) of BC. The increased risk attenuates after cessation and is no longer evident 10 years post-cessation[267]. A more recent, large Danish study supports these findings and also showed an increased risk associated with use of progestogen-containing intrauterine devices[268]. For OCPs, the RR of BC is higher in current users who commenced use prior to 20 years of age, but because the baseline risk of BC at such a young age is very low, for any given duration of use, early commencement of OCP does not contribute to more BC being diagnosed in younger women than in those who start later in life[267]. Duration of use also impacts on risk; 5 years of use is associated with at least a 5% increased RR whilst 10 and 13 years is associated with a 12% and 18% increase, respectively[267,268].

A key point is that the increase in absolute risk of BC associated with hormonal contraception is low when the underlying risk is low (e.g. in young women at average lifetime risk of the disease), but when the underlying risk is higher (e.g. older premenopausal women with a strong family history of the disease) the increase in absolute risk is likely to be of more importance. In fact, it has been estimated that 7% of BC burden for premenopausal women is owing to the use of hormonal contraceptives for 5 or more years[136]. US statistics indicate that as much as 10% of OCP users are older premenopausal women aged 40-49[286]. Therefore, when estimating the risk–benefit ratio for an individual woman, her underlying BC risk at her current age is an important consideration.

The Collaborative Group on Hormonal Factors in Breast Cancer recently published their individual participant meta-analysis of the worldwide epidemiological evidence related to MHT and BC risk[33]. They estimated that about 1 million of the approximately 20 million BCs diagnosed in Western countries since 1990 were due to MHT use[33]. Every MHT type, except vaginal estrogens increased BC risk (compared with non-users), which steadily rose with duration of use and were greater for oestrogen and progestogen preparations (combined MHT) than oestrogen only ones. Specifically, for combined MHT use (1-4 years), there was a 60% increase in risk of BC (RR: 1·60), and for estrogen only MHT, a 17% increase (RR: 1·17). Risk was greater for longer durations of use: for example, for 5-14 years of combined MHT use, the risk was more than doubled (RR: 2·08) and it was 33% higher for estrogen-only MHT (RR: 1·33). Furthermore, the RRs during years 5–14 were much greater for ER positive tumours than for ER negative tumours. After ceasing MHT, some excess risk persisted for more than 10 years but its magnitude was dependent on the duration of previous use [33]. These findings are consistent with other large studies[284,287-289], although the women's health initiative (WHI) randomised trial showed a protective effect of estrogen only MHT for BC, resulting in ongoing controversy over the risks and benefits of estrogen only MHT [290].

The relationship between estrogen and BC risk is complex and it is hoped that pre-clinical work assessing the effects of estrogen alone and estrogen – progestogen therapies on breast tissue may reveal how these therapies alter the breast to impact on cancer risk.

Glossary

ADIPOKINE: cell signalling protein secreted by adipose (fat) cells

Basal-like breast cancer: breast cancer subtype that is more prevalent in African-American women, and are characterised by high histological grade, high mitotic indices and lack of ER and PR abd HER2 protein overexpression.

BC RISK ESTIMATION MODELS: Tools that estimate a person's likelihood of developing breast cancer within a specific timeframe.

BILATERAL SALPINGO-OOPHORECTOMY: A surgical procedure to remove both ovaries and fallopian tubes.

CALIBRATION: the ratio of the observed number of breast cancer cases to the expected number; values of 1 indicate optimal calibration

DISCRIMINATORY ACCURACY: the ability of a risk model to separate individuals who will get breast cancer from those who will not. A value of 1.0 represents perfect discrimination, a value of 0.5 means that the model performance is no better than chance alone, values of 0.6-0.7 are considered good and 0.5-0.6 sufficient

GYNAECOMASTIA: excessive enlargement of the male breast. May be unilateral (one side) or bilateral (both)

HAZARDS RATIO (HR): a measure of how often a particular event happens in one group compared to another group, over time. A HR=1.0 means that there is no difference in survival between the two groups. A HR > 1.0 or <1.0 means that survival was better in one of the groups.

HOMOLOGOUS RECOMBINATION: the exchange of nucleotide sequences between two similar or identical molecules of DNA. It is used by cells to accurately repair damage that occurs on both strands of DNA, such as double-strand breaks or interstrand DNA crosslinks.

KLINEFELTER SYNDROME: A genetic condition, affecting about 1 in every 550 men, in which a male is born with an extra copy of the X chromosome. This results in higher levels of female hormones.

LUMINAL PROGENITORS: A type of luminal epithelial cell within the mammary epithelium that has both luminal differentiation markers and progenitor activity (colony forming and repopulating activity in vivo).

MAMMARY STEM CELLS (MASCS): Cells within the mammary gland that have the capacity to form a new mammary tree when transplanted into a cleared mammary fat pad. Reside within the basal/myoepithelial compartment and can be identified with CD24/EpCAM and either CD29 or CD49f.

Mammographic density: Mammographic density (MD) describes the extent of white or radio-opaque tissue (dense area) on a mammogram, and percent MD is used to represent this dense area as a proportion of the total tissue area of the breast on a mammogram.

MENARCHE: The time in a girl's life when her first menstrual bleeding or period begins.

MENOPAUSAL HORMONE THERAPY: sex hormones given to treat symptoms or prevent long-term morbidities associated with female menopause Also known as hormone replacement therapy (HRT)

ODDS RATIO (OR): a statistic that quantifies the strength of the association between an exposure and an outcome. An OR=1 means the exposure does not affect odds of outcome, an OR>1 means the exposure is associated with higher odds of outcome and that an OR<1 means the exposure is associated with lower odds of outcome.

ORAL CONTRACEPTIVE PILL: (OCP). A birth control pill taken orally. Most contain estrogen and progesterone which when given at certain times in the mentrual cycle at defined doses can prevent the ovary from releasing the egg for fertilization.

PARITY: The state of having borne offspring (liveborn or stillborn). Also used to indicate the number of pregnancies reaching viable gestational age (liveborn or stillborn – pregnancies resulting in multiple births, such as twins, count as 1)

POST-PARTUM INVOLUTION PROCESS: A cell-death–mediated process by which the lactating breast returns to the pre-pregnant state after weaning (or after childbirth if lactation is not initiated). It is characterized by robust tissue remodeling.

RELATIVE RISK (RR): a ratio of the probability of an event occurring in the group exposed to the modifier of interest versus the probability of the event occurring in the non-exposed group. A relative risk of 1.5 means people exposed to the risk modifier, on average have a 50% higher risk than those not exposed.

ABSOLUTE RISK: The absolute risk of a disease is the risk of developing the disease over a time period, e.g. a person may have a 1 in 10 risk (i.e a 10% risk) of a certain disease in their life. Absolute risk is one of the most understandable ways of communicating health risks to the general public.

BILATERAL MASTECTOMY: removal of as much breast tissue as possible to reduce breast cancer risk

TRANSDERMAL THERAPY: a route of drug administration wherein the drug is delivered across the skin, via patches or creams, for systemic distribution.

Polygenic disease: A genetic **disorder** that is caused by the combined action of more than one gene

Author contributions

K.L.B and K.A.P researched data for the article, made substantial contributions to discussions of the content and wrote the article. J.C. provided vital input to the article and insight in to preventatives that are currently being trialled. All authors reviewed and/or edited the manuscript before submission.

Competing interests

The authors wish to disclose that CRUK licences the IBIS (Tyrer-Cuzick) model for commercial use and J.C. receives some benefit. K.A.P has a patent, System and Process of Cancer Risk Estimation (Australian Innovation Patent) issued regarding iPrevent. K.L.B has no competing interests.

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Related Links

https://tools.bcsc-scc.org/BC5yearRisk/intro.htm
https://projects.iq.harvard.edu/bayesmendel/bayesmendel-r-package
https://ccge.medschl.cam.ac.uk/boadicea/
https://bcrisktool.cancer.gov/calculator.html
http://www.ems-trials.org/riskevaluator/
https://dceg.cancer.gov/research/cancer-types/breast-cancer/confluence-project

Table of contents summary

This review presents the evidence for the role of risk factors in breast cancer incidence and their inclusion in risk estimation tools as a step towards precision prevention to specifically target those women at increased risk for appropriate risk-reducing interventions.

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