The road towards conquering DCIS overtreatment

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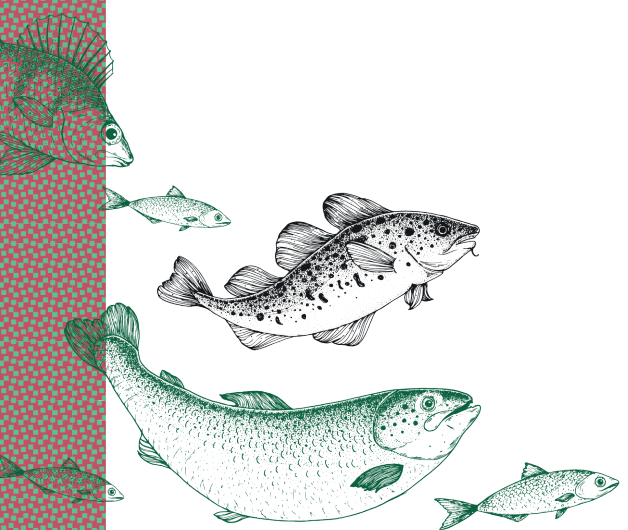

Chapter 1

Finding the balance between over- and under-treatment of ductal carcinoma in situ (DCIS)

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Abstract

With the widespread adoption of population-based breast cancer screening, ductal carcinoma in situ (DCIS) has come to represent 20-25% of all breast neoplastic lesions diagnosed. Current treatment aims at preventing invasive breast cancer, but the majority of DCIS lesions will never progress to invasive disease. Still, DCIS is treated by surgical excision, followed by radiotherapy as part of breast conserving treatment, and/or endocrine therapy. This implies over-treatment of the majority of DCIS, as less than 1% of DCIS patients will go on to develop invasive breast cancer annually. If we are able to identify which DCIS is likely to progress or recur as invasive breast cancer and which DCIS would remain indolent, we can treat the first group intensively, while sparing the second group from such unnecessary treatment (surgery, radiotherapy, endocrine therapy) preserving the quality of life of these women. This review summarizes our current knowledge on DCIS and the risks involved regarding progression into invasive breast cancer. It also shows current knowledge gaps, areas where profound research is highly necessary for women with DCIS to prevent their over-treatment in case of a harmless DCIS, but provide optimal treatment for potentially hazardous DCIS.

Highlights

- DCIS incidence is highly increased since population-based breast cancer screening.
- There is strong evidence that breast cancer screening results in overdiagnosis of DCIS.
- We are unable to predict the individual risk of DCIS progression into invasive carcinoma.
- Distinguishing harmless from potentially hazardous DCIS is essential to offer customized therapy in the future.

Introduction

Since the introduction of population-based breast cancer screening and digital mammography, the incidence of precursor lesions has substantially increased in the Western world, without a decline in invasive breast cancer incidence. This suggests that overdiagnosis of such lesions exists. Most precursor lesions are Ductal Carcinoma In Situ (DCIS) cases. DCIS of the breast represents a heterogeneous group of neoplastic lesions confined to the breast ducts and lobules that differ in histologic appearance and biological potential.

The major gap in our current understanding of DCIS is, that we do not know yet which DCIS lesions will develop into invasive breast cancer and which will not. As a consequence, women with low risk DCIS face being harmed by intensive treatment without any benefit. If such overtreatment can be avoided without compromising the excellent outcomes presently achieved, this will safely spare many women with low risk DCIS intensive treatment and so preserve their quality of life.

Here, we summarize our current understanding of DCIS and the challenges that lie ahead of us to find the balance between DCIS over- and under-treatment.

DCIS incidence has increased over time

In the United States (US), the incidence of DCIS markedly increased from 5.8 per 100,000 women in the 1970s to 32.5 per 100,000 women in 2004 and then reached a plateau [1]. Approximately 25% of breast neoplastic lesions diagnosed in the US are DCIS, i.e. over 51,000 women in the US alone in 2015 [2]. In the Netherlands and the UK, similar rates apply (www.cijfersoverkanker.nl; www.cancerresearchuk.org/). This increase is attributed primarily to the widespread adoption of mammographic screening in the United States, Europe and other high-income countries that has dramatically increased the number of DCIS cases, as more than 90% of all cases of DCIS are detected only on imaging studies [3].

DCIS is less common than invasive breast cancer. Like invasive breast cancer, the risk increases with age. DCIS is uncommon in women younger than 30. In the US, the rate of DCIS increases with age from 0.6 per 1000 screening examinations in women aged 40-49 years to 1.3 per 1000 screening examinations in women aged 70-84 years [4]. Risk of development of metastases and/or death in a patient diagnosed with pure DCIS is very low (<1%) [5].

The risk factors for DCIS and invasive breast cancer are similar, and include family history of breast cancer, increased breast density, obesity, and nulliparity or late age at first birth [6-9]. DCIS is also a component of the inherited breast-ovarian cancer syndrome defined by deleterious mutations in BRCA1 and BRCA2 genes; mutation rates, i.e. up to 5%, are similar to those for invasive breast cancer [9].

Over-treatment of DCIS exists

Increasing DCIS incidence is due mostly to introduction and uptake of population-based breast cancer screening [1,10-12] and use of digital mammography. The latter detecting significantly more DCIS lesions [13,14]. In the Netherlands, the incidence of in situ lesions has increased 5.6-fold between 1989 and 2011 (www.cijfersoverkanker.nl). Higher screening sensitivity also labels more women as having disease, many of whom may never develop invasive cancer [15,16]. However, the incidence of advanced breast cancer has not decreased, despite screening [13,17]. In addition, there is strong evidence that treatment of DCIS in most women has no clear effect on mortality reduction [18].

This suggests overdiagnosis and hence overtreatment exists of DCIS in general, and of low-grade DCIS in particular. The implication is that we could manage a subgroup of women with low-grade DCIS using active surveillance only [11,19,20]. The number of women eligible for this management strategy would be high, since 80% of all in situ carcinomas are DCIS lesions, and about 20% of all DCIS lesions is low grade [21,22]. Fig. 1 illustrates the heterogeneous course of cancer, including its preliminary stages.

Remarkably, a lesion with a similar risk of progression to invasive breast cancer is classic lobular carcinoma in situ [23,24]. If LCIS is the only finding, active surveillance is frequently offered. Somewhat incongruously, this risk is acceptable for both patients and clinicians.

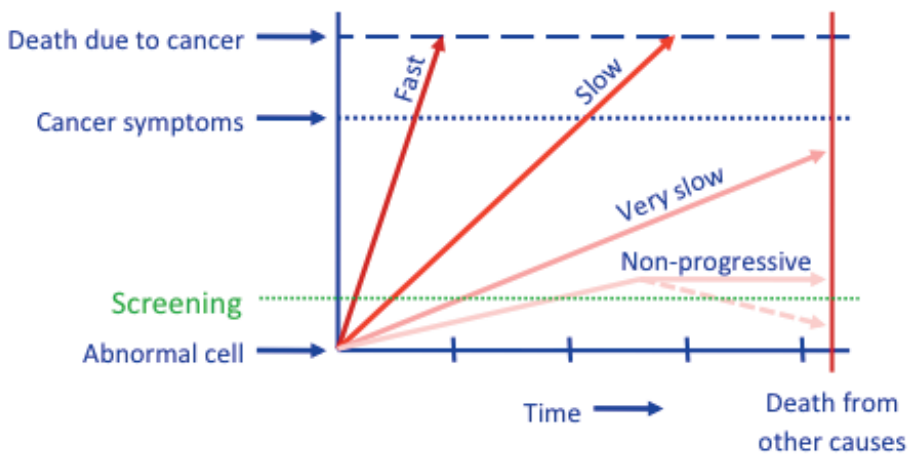

Fig. 1 Heterogeneity of cancer progression in general. Fast growing cancers are likely to lead to symptoms, and even death, after a relatively short period of time, whereas slow growing cancers may lead to symptoms, and maybe death, after many years. The very slowly proliferating lesions most likely never lead to symptoms, implying that patients with such a lesion are likely to die due to other causes. This is also true for non-progressive lesions that might even regress. Adapted from Welch and Black [16].

Most DCIS lesions go undetected

Only 10% of DCIS cases is detected due to symptoms, such as nipple discharge, Paget's disease of the nipple, or a palpable mass [1]. As pointed out above, the majority of DCIS lesions found are detected by screening, as many DCIS lesions do not come with symptoms, but do contain calcifications that can be seen upon mammography. Obviously, DCIS lesions may be occult by mammography or the diameter of the area containing calcifications underestimates the extent of DCIS [25,26]. This is also illustrated by the much higher prevalence of DCIS (7-39%) found in autopsy studies concerning the age group for which population-based screening programs are in place, whereas in screening and clinical practice breast cancer was diagnosed in only 1% of women within a similar age range [21,27].

The natural course of DCIS is poorly understood

A multitude of factors have been implicated in the risk of developing an in situ or invasive recurrence [28]. It has been suggested that paracrine regulation is crucial for malignantly transformed luminal cells to become invasive [29]. By analysing stromal expression signatures in DCIS, it was shown that the microenvironment plays a role in the transition from pre-invasive to invasive growth [30,31]. The myoepithelium is considered as a factor preventing invasive growth by regulating luminal cell polarity, ductal morphogenesis, and basement membrane deposition. In DCIS, the myoepithelium shows decreased expression of e.g. thrombospondin, laminin, and oxytocin, promoting proliferation, migration, invasion, and angiogenesis [32,33]. It is uncertain whether changes in stroma and/or myoepithelium precede invasive growth or that the luminal DCIS cells can induce stromal and/or myoepithelial changes, and thereby paving the way for their own invasion.

The pathology of DCIS provides limited prognostic value

The pathology of DCIS aims to assess subtype and grade. Additionally, pathology will report on extent and margin status in case of surgical resection of DCIS. These aspects provide important prognostic information about the 'aggressiveness' of a particular DCIS lesion. DCIS is morphologically described by growth pattern, i.e. arrangement of the ductal cells, such as cribriform, solid, micropapillary, etc., cytoplasmic features, degree of nuclear pleomorphism, and degree of mitotic activity. Grading systems for DCIS are based on these cytonuclear features resulting in low (1), intermediate (2), or high (3) grade [34]. However, the accuracy of DCIS grading has some limitations, as diagnostic criteria are not always clear. Furthermore, poor to modest interobserver agreement exists, as has been reported in subgroups of in situ lesions, which is mainly due to differences in morphological interpretation and field selection in the often heterogeneous intraductal lesions [35-39]. Obviously, it is of utmost importance to classify the primary lesion reliably to be able to evaluate the natural course of DCIS or to interpret follow-up after treatment. Reliability studies are hard to compare as they often differ in study design. Also they are limited due to: mostly examining a small number of highly selected cases

[35,40-45]; being assessed by expert breast pathologists only and; often after being giving instructions or tutorials beforehand [35,41,42,45,46]. Translation of these findings into daily practice is therefore questionable and, so far, has not reduced inter-observer variability. In addition, the interpretation of results and evaluation of potential bias is complicated by often inadequate reporting and missing information on important issues in reliability testing. In 2011, guidelines for reporting reliability and agreement studies were developed, highlighting key methodological issues that should be carefully thought through when reporting on reliability and agreement studies [47].

A recent study used exactly these guidelines to construct their study design [48]. In this study 115 pathologists each classified a set of 60 cases as either benign without atypia, atypia, DCIS, or invasive carcinoma. They found an overall concordance rate of 75%, and concordance rates of 48% and 84% for atypia and DCIS respectively, when compared with expert reference diagnoses. The concordance rate for invasive breast cancer was excellent (96%). Test cases were randomly selected, oversampling atypia and DCIS cases, and the participating pathologists had different geographic and clinical setting backgrounds leaving little room for selection bias. Unfortunately, as 'gold standard' they used consensus-derived expert diagnoses without any information on follow up. Whether such results on concordance are biologically relevant, therefore remain unanswered.

Although inter-observer variability may lead to overtreatment of DCIS, even with perfect (i.e. biologically relevant) definitions and classification systems, a 100% agreement will never be reached, as histological examination is not an absolute science. Hopefully the integration of various clinical, radiological, histological, and molecular markers will improve our ability to reliably distinguish between low- and high-risk lesions.

A DCIS diagnosis comes with a chance missing invasive breast cancer

Invasive breast cancer has been found in 8-43% of resection specimens from patients who were primarily diagnosed with DCIS based on a preoperative biopsy [49-58]. At least some of these highly variable numbers can be explained by differences in the size and quantity of biopsies taken as well as by the use of different imaging techniques. In addition, it is essential to be informed about why the biopsy was taken. This is illustrated by the fact that the risk of upgrade will be higher when mass lesions or architectural distortions are found on imaging compared to calcifications only [49,51,53,55,57,58]. Most studies also agree that larger lesions - based on the effect of size on imaging diagnosis - carry a higher upgrade risk than smaller ones [49-52,55,59]. Paradoxically, the upgrade risk for smaller tumours is higher, because the sensitivity of mammographically detecting tumours of only 0,5 cm is low (<30%) and high (>90%) for tumours of 1,0 cm [60]. In some studies a higher grade of DCIS was a significant predictor of upgrade with an upgrade occurring in only 6-7% of patients versus 13-31% for low and intermediate/high grade respectively [52-54,58]. Others found grade not to be predictive [49-51,53,55].

Uncertainties about DCIS risks exist

Among health care providers as well as among women with DCIS, considerable uncertainties exist regarding the degree of risk involved for developing invasive breast cancer. In general, DCIS has a relative risk (RR) of 8-11 for subsequent development of invasive carcinoma [22,34]. DCIS in itself has an excellent long-term breast cancer-specific survival exceeding 98% after 10 years of follow-up [1,61,62]. Strikingly, grade was not significantly associated with the risk of local recurrence.

Factors associated with DCIS progression to invasive breast carcinoma remain poorly defined, because most patients are treated in order to completely eradicate the lesion [63]. Several studies have shown that high-grade DCIS has a higher probability of ipsilateral invasive breast cancer than low-grade DCIS. One of the largest studies is that conducted by The Eastern Cooperative Oncology Group (ECOG). This non-randomized prospective study included 670 patients with either low/intermediate grade DCIS or high-grade DCIS who underwent complete excision. At a median follow-up of 6.7 years, the low-intermediate group had a 10.5% risk of local relapse, whereas the high-grade group had a 18% recurrence rate, of which 35% were invasive breast cancers [13].

Our group analysed an unbiased, large population-based, nation-wide cohort, comprising 10,090 women with a primary diagnosis of DCIS between 1989 and 2004 (Elshof et al., submitted). In total, 5.8% developed ipsilateral invasive recurrence after treatment for DCIS (breast sparing or mastectomy) after a median follow-up of 11.6 years (Elshof et al, submitted). Narod and co-workers analysed the SEER database and showed that women younger than 35 and women of African-American descent have a higher risk of invasive recurrence and death [18]. A meta-analysis of four randomized clinical trials to investigate the role of radiotherapy in BCT for DCIS after a complete local excision of the lesion showed a 50% reduction in the risk of local recurrence with half of these recurrences being invasive, but has no effect on breast cancer-specific mortality [15].

Taken together, these studies provide a generalized estimation of how large the risk is that DCIS progresses into invasive breast cancer, but without allowing individualized prediction.

Current DCIS management is at the safe side

In DCIS, prognosis is based on the risk of (invasive) local recurrence, although such risk estimations are far from precise as described above. If the lesion is not too extensive, breast-conserving treatment for DCIS is frequently recommended, resulting in 60-70% of women being suitable for this therapy [64]. If the lesion is too extensive, a mastectomy with or without immediate reconstruction is generally advised. Radiotherapy after surgery is nowadays standard treatment for DCIS, as randomized controlled trials have demonstrated a 50% reduction in ipsilateral breast cancer risk [15]. For tamoxifen use there is no consensus if there is any absolute survival benefit that outweighs the harm of long term endocrine treatment [65,66].

According to Dutch, English, and American guidelines and based on higher upgrade risks, indications

for a sentinel lymph node biopsy (SLNB) in DCIS patients planning to undergo breast sparing surgery include a palpable mass, age below 55 years, intermediate or high grade DCIS, and a solid mass or a lesion larger than 25 mm or extensive calcifications on imaging (see e.g. www.oncoline.nl). As a SLNB is less reliable after mastectomy, it is also recommended for all patients treated by mastectomy.

Strikingly, there is a tendency towards minimizing axiliary surgery for invasive breast cancer [67,68]. There are now even trials investigating whether a SLNB can be left out of treatment of clinically node negative invasive breast cancer patients [69]. To date, no comparable trials have been undertaken for patients with 'only' DCIS, while logically, risks seem even lower. It has been shown that even a positive SLNB in DCIS patients does not affect survival, although some patients did receive systemic treatment [70-72]. We need to await more definitive results indicating that omitting a SLNB for women with pure DCIS patients is likely to be safe.

Distinguishing harmless from potentially hazardous DCIS is challenging

Evidently, overtreatment of harmless DCIS should be prevented, without compromising the excellent outcomes presently achieved in DCIS management. This means being able to reliably distinguish harmless from potentially hazardous DCIS. Therefore, on-going research aims to find and validate much more accurate prognostic biomarkers, applying e.g. immunohistochemistry and genomic techniques, pursuing the Holy Grail in prediction will be described below.

A multitude of markers have been implicated in identifying subgroups of DCIS by immunohistochemistry (IHC; see for a brief overview Table 1). The most commonly used markers are ER, PR, HER2, and Ki67. As in invasive breast cancer, they are sometimes used to determine the subtype and 'aggressiveness' of DCIS. Expression of the hormone receptors, a low-grade, and a low percentage of Ki67-postive cells in DCIS are related to a lower rate of invasive recurrence and/or lower grade [4,73-75]. In general, overexpression of HER2 is associated with higher recurrence rates [74]. Besides the usual markers, expression of p16 and p53 is related to a higher local recurrence rate [4,76-79]. COX-2 is related to proliferation and as such risk on local recurrence [4,79]. Annexin A1 (ANXA1) might play dualistic roles being involved in variable mechanisms related to cancer development and progression. Loss of ANXA1 expression, as observed in the majority of breast cancers, seems to be related to early events of malignant transformation. However, overexpression was shown to be associated with poor relapse free survival [80,81]. Interestingly, intra-individual DCIS heterogeneity (high Ki67, mutant p53, and low p16) is associated with more aggressive DCIS [77]. This is relevant for the interpretation of further genomic profiling of DCIS.

However, the impact of most of these studies is limited, as they involve small patient series relate to series with an adjacent invasive component and are therefore not 'pure DCIS', and information on follow up is also often lacking [82,83].

In recent years, several studies have also focused on finding molecular markers associated

with aggressiveness in DCIS [28]. The use of laser capture microdissection to harvest defined cell populations has proven essential for the study of DCIS. Studies on DCIS and an adjacent invasive component have shown that molecular characteristics associated with invasiveness are already present in the DCIS lesion [84,85]. Petridis and co-workers showed that shared genetic susceptibility exists for DCIS and invasive ductal carcinoma (IDC) and that studies with larger numbers are needed to determine if IDC or DCIS specific loci exists [86]. Gene expression analysis has shown that pre-invasive lesions and invasive breast cancer display remarkable similar gene expression patterns [85]. Carraro et al. summarized differently expressed genes associated with aggressive behaviour of DCIS lesions [87]. Genes belonging to cell signalling (i.e. CDH1), cellular movement (MMPs), growth and proliferation are involved. Other studies focus on specific copy number alterations. 16q loss is found in the majority of low-grade DCIS lesions, while more complex karyotypes are observed in high-grade lesions. Specific copy number aberrations reported to be associated with DCIS are amplifications of MYC, FGFR1 and CCND1 [88]. Complicating factors in the studies employed so far are the low numbers of samples studied and the heterogeneity between lesions and within the lesions [89,90].

The Oncotype DX DCIS score is the first multi-gene assay that has been claimed to be validated in an independent study [91]. This score predicts both the risk of an in situ and invasive recurrence but still assumes that every DCIS should be treated by surgery, as the assay merely indicates patients having benefit from radiotherapy. Prospective validation of this assay has not been done yet.

Taken together, a conclusive set of biomarkers suitable for implementation in routine clinical practice has not been identified yet. Campbell et al. therefore argued for the development of a “Pre-Cancer Genome Atlas” to gain insight in the earliest molecular and cellular events associated with cancer initiation which eventually will enable us to find biomarkers for risk stratification.

Table 1 IHC marker selection to determine DCIS to estimate DCIS aggressiveness

Antigens	No. of cases	Finding(s)	Reference
ER/PR	119 DCIS	Presence of PR expression is associated with expression of ER and lack of comedo-necrosis in DCIS. Increasing tumor grade correlated with decrease in ER and PR positivity. Comedo-necrosis is associated with ER and PR negativity.	[107]
	118 pure DCIS, 100 IBC	Invasion is associated with a significant increase in Ki67 expression and decreases in ER, PR and Her-2 expression.	[73]
	95 DCIS	A direct positive relationship is observed for the expression of ER, PR and Bcl-2 negativity for the clinical recurrence of DCIS.	[75]
HER2	180 DCIS	HER2neu is regarded as an important prognostic and predictive marker, with its overexpression predicting local recurrence.	[74]
	118 pure DCIS, 100 IBC	Invasion is associated with a significant increase in Ki67 expression and decreases in ER, PR and Her-2 expression.	[73]]
	130 DCIS, 159 DCIS+IBC	No significant differences between the gene amplification status of DCIS and invasive breast cancer concerning HER2, ESR1, CCND1, and MYC. Data suggest an early role of all analyzed gene amplifications in breast cancer development but not in the initiation of invasive tumor growth.	[108]
	226 DCIS cases	Data suggests loss of RB can contribute to the function of ErbB2 (HER2) in driving disease progression. ErbB2 (HER2) alone is not sufficient to drive invasion into the surrounding matrix. RB deficiency potentially cooperates with ErbB2 loss and drive the phenotype towards EMT.	[109]
AR		Findings suggest that decreases in AR and androgen-metabolising enzymes (17βHSD5 and 5αR1) may be involved in the increased biological aggressiveness in triple-negative breast cancer. Also relating to triple-neg DCIS.	[110]
Ki-67	324 initial DCIS	p16+ COX-2+ and Ki67+ in DCIS is prognostic for recurrence/ invasive cancer and suggests that the biological correlation between COX-2 levels and proliferation may be significant.	[4]
	36 DCIS+IBC	Multiple DCIS lesions from the same patient frequently exhibit heterogeneity in the expression of clinically relevant markers: PR, HER2, Ki-67, and p16. Individuals with a heterogeneous DCIS cell population combined with high levels of Ki-67, increased mutant p53 and low p16 should be clinically managed more aggressively.	[77]
p53	118 pure DCIS, 100 IBC	Invasion is associated with a significant increase in Ki67 expression and decreases in ER, PR and Her-2 expression. P53 more frequent in high-grade DCIS.	[73]
	103 DCIS	Expression of mutant p53 is associated with high expression of VEGF and correlates with biological aggressiveness of DCIS lesions.	[111]
	36 DCIS+IBC	Multiple DCIS lesions from the same patient frequently exhibit heterogeneity in the expression of clinically relevant markers: PR, HER2, Ki-67, and p16. Individuals with a heterogeneous DCIS cell population combined with high levels of Ki-67, increased mutant p53 and low p16 should be clinically managed more aggressively.	[77]

Table 1 continued.

Antigens	No. of cases	Finding(s)	Reference
p16	324 initial DCIS	p16+ COX-2+ and Ki67+ in DCIS is prognostic for recurrence of DCIS and/or invasive cancer.	[4]
	50 DCIS, 50 IDC, 50 benign	Luminal lesions of DCIS with high p16 are more likely to develop into aggressive breast cancer. p16 expression in luminal A breast cancer is associated with progression from DCIS to IDC.	[78]
	40 UDH, 20 FEA, 40 ADH, 40 DCIS	p16INK4a methylation is associated with DCIS, plays an important role in the initiation and progression of premalignant lesions and carcinomas and may be a crucial event in cell transformation.	[112]
	36 DCIS+IBC	Multiple DCIS lesions from the same patient frequently exhibit heterogeneity in the expression of clinically relevant markers: PR, HER2, Ki-67, and p16. Individuals with a heterogeneous DCIS cell population combined with high levels of Ki-67, increased mutant p53 and low p16 should be clinically managed more aggressively.	[77]
MYC	141 DCIS, 18 DCIS+IBC	High expression of c-myc in DCIS did not predict local recurrence, but still is of interest. Has to be confirmed in a larger trial.	[113]
	130 DCIS, 159 DCIS+IBC	No significant differences between the gene amplification status of DCIS and invasive breast cancer concerning HER2, ESR1, CCND1, and MYC. Data suggest an early role of all analyzed gene amplifications in breast cancer development but not in the initiation of invasive tumor growth.	[108]
COX-2	58 pure DCIS	Findings suggest that COX-2 may be a predictive marker of early relapse in with DCIS	[79]
	324 initial DCIS	P16+ COX-2+ and Ki67+ in DCIS is prognostic for recurrence/ invasive cancer and suggests that the biological correlation between COX-2 levels and proliferation may be significant.	[4]
ALDH1	236 DCIS	Combination of EZH2 with ALDH1 within the DCIS epithelial compartment is associated with the prognosis for ipsilateral breast event and invasive progression.	[114]
EZH2	236 DCIS	Combination of EZH2 with ALDH1 within the DCIS epithelial compartment is associated with the prognosis for ipsilateral breast event and invasive progression.	[114]
ANXA	82 IBC+LN metastasis and 21 DCIS+IBC	Lack of ANXA1 expression in breast cancer and early loss of ANXA1 in DCIS, suggests a possible role for ANXA1 in early events of malignant transformation.	[80]
	182 cases	Significant loss of ANXA1 in DCIS and IBC as compared to normal. ANXA1 overexpression was correlated with poor RFS.	[81]

Selected antigens reported to be related with 'aggressiveness' of DCIS based on: (1) differential expression of the antigen between DCIS and IDC; (2) multivariable significance; (3) confirmation in more than 1 research paper.

Solving the DCIS dilemma requires integrated and novel approaches

Current pathology has limited additional value for more nuanced clinical practice when dealing with DCIS, its diagnosis and consequences for the women involved. We need to more seriously consider opportunities for integrated and novel approaches. To prioritize DCIS research, the US Patient-Centred Outcomes Research Institute commissioned a study to do so [92]. Stakeholders prioritized evidence gaps related to incorporation of patient-centred outcomes into future studies on DCIS, development of better methods to predict risk for invasive cancer, evaluation of a strategy of active surveillance, and testing of decision-making tools.

First, individualized risk prediction should be optimized using well annotated retrospective data sets, enabling integration of clinical, morphological and molecular features. Strikingly, such an integrative approach is not available yet. Ultimately, such tools should be able to distinguish harmless from potentially hazardous screen-detected DCIS and help clinicians and women with DCIS to decide between management using active surveillance or more intensive treatment. For this, data from population-based screening, hospital records, cancer registries, pathology, current and upcoming molecular and biological techniques should be integrated in a stepwise manner:

1. *Compile representative DCIS patient cohorts and collect all necessary data and material.* Better methods to predict DCIS risk rely on large series of clinical data and tissue blocks for histopathologic and molecular analysis. Such studies have started in the Netherlands with the collection of a large nationwide, population-based, retrospective study (n = 10,090) (Elshof et al., submitted). Clinical, radiological and molecular data will be integrated and compared between women with DCIS who may or may not have developed an ipsilateral invasive recurrence after breast-conserving treatment, during a follow up period of more than 10 years. The excellent registration in the Netherlands at the Dutch Cancer Registry (NKR), the breast cancer screening and PALGA (Pathology National Automated Archive) is unique in the world and makes reliable and complete data collection possible. Another huge effort is the Sloane Project, a UK wide prospective audit of screen detected non-invasive carcinoma and atypical hyperplasia of the breast. All UK NHS Breast Screening Units are invited to participate. It is a multi-disciplinary project involving radiologists, pathologists, surgeons and oncologists. Detailed follow up data of all DCIS detected by the NHS Breast Screening Program will be collected such as information on local recurrence, contralateral cancer, metastases, and death, as well as data on screening and treatment, and most importantly for biomarker research, tissue blocks will be collected, enabling molecular pathology studies (www.sloaneproject.co.uk).
2. *Find and validate molecular markers related to outcome.* To obtain reliable, detailed results, DCIS should be analysed applying immunohistochemistry and genomic analysis on resection specimens, as the size of the biopsies is too small for these analyses. For these analyses, laser microdissection or alternative strategies should be used to capture the cells and tissue regions of interest at high specificity. Comprehensive genomic characterisation has to be done to understand the biological properties of DCIS that contribute to the evolution and 'aggressiveness' of DCIS. This includes complete description of all drivers and mutation signatures in DCIS, exploring intralesional heterogeneity in DCIS, and finding putative associations between mutation signatures (see [93]) and the risk of progression into invasive

breast cancer. By this means, clonal evolution, evolutionary pathways, and rare events in DCIS related to outcome (recurrence, progression to invasive disease) can be characterized. In addition, we can also test if genetic and microenvironmental diversity, including immune responses [94], provide universal biomarkers, helping to predict progression to invasive disease. This innovative approach could yield a universally applicable construct for understanding interactions between precancerous lesions and their environments.

3. *Apply innovative molecular imaging technologies to understand the transition of DCIS into invasive breast cancer.* The missing link in the full molecular picture can be obtained by analysing sub-regions of a DCIS lesion, e.g. by applying Mass Spectrometry Imaging (MSI), as this technique can be successfully applied on formalin-fixed, paraffin-embedded tissue [95]. Our preliminary evidence shows substantial intralesional heterogeneity of putative genomic markers in DCIS. Perhaps only a small part of the DCIS lesion has invasive potential, which means our tools need to be able to detect molecular differences within the lesion. Most likely, MSI has vital additional value in combination with advanced bioinformatics and statistical analysis, to characterize intralesional heterogeneity to determine phenotypes based on specific molecular signatures at different levels (e.g. metabolomics, lipidomics, and peptidomics).
4. *Integrate clinical, morphological, and molecular data to build a robust risk stratification tool*. Associations between clinical, morphological, and molecular data should be analysed to build a model accurately predicting subsequent risk for developing ipsilateral invasive breast cancer. Candidate risk stratification tools should then be thoroughly validated in independent retrospective DCIS series and prospective clinical trials. In order to communicate such a risk prediction model to patients and doctors, risk calculator software should be developed in analogy to existing calculators such as adjuvant online (www.adjuvantonline.com) and the breast cancer risk assessment tool (http://www.cancer.gov/bcrisktool/). These online tools have proven themselves to be very helpful and easy to use, which is essential when incorporated into daily practice.

This ultimately will provide holistic integrative profiles per patient and an innovative multifactorial algorithm able to identify patients with very low-risk for invasive recurrence, i.e. indolent DCIS, that can be managed safely by active surveillance only. This can save many women from the potential physical and psychological harm of invasive treatment. Evidently, such an approach will only be successful if international collaboration between experienced dedicated researchers, clinicians, and patient partners are well established.

Second, prospective studies on active surveillance should be conducted to deliver final proof that active surveillance is safe for DCIS already known to be low-risk. For example, the international LORD trial (LOw Risk Dcis), which will start to recruit women with low grade DCIS in Europe in 2016 under the

auspices of the European Organisation for Research and Treatment of Cancer (EORTC). In this study, women with 'pure' low-grade DCIS detected at screening based on calcifications are randomized to either an 'active surveillance' policy or standard therapy [19]. After inclusion, women will be followed for 10 years and main outcome measure is the risk of developing invasive breast cancer. If a relapse occurs, breast-conserving therapy with radiation therapy will still be an option. By contrast, when a recurrence develops after standard treatment for DCIS, an ablation is usually the only choice. Similar studies are the LORIS trial in the UK [96], the COMET trial in the USA (http://www.pcori.org/research-results/2016/comparison-operative-versus-medical-endocrine-therapy-low-risk-dcis-comet), and the Australian LARRIKIN trial for which no detailed information is available yet (see Table 2).

In moving forward, the following considerations are of paramount importance. First, low-grade hormone receptor-positive invasive breast cancer grows only a few millimetres per year and a delay in detection will not affect the excellent prognosis inherent to these tumours [97]. Second, there is convincing evidence that low grade invasive breast tumours originate from low grade precursor lesions [84,98-103]. Third, women with low-grade lesions who meet these criteria for inclusion in the LORIS trial did not show any upgrade to invasive cancer [104]. This underlines again that active surveillance for women with low-grade screen-detected DCIS is likely to be a safe option, sparing these women the harms of ineffective treatment, preserving their quality of life.

Table 2 Comparison of the designed and initiated prospective, randomised, open-label, phase III, non-inferiority trials to test whether less intensive treatment of low risk DCIS is safe. The information provided is based on literature for the LORIS and LORD trial [19,96] and on personal communication for the COMET and LARRIKIN trial

Trial name	LORD	LORIS	COMET	LARRIKIN
Clarification acronym/trial name	**LO**w **R**isk **D**CIS	**LO**w **R**isk DC**IS**	**C**omparison of **O**perative versus **M**edical **E**ndocrine **T**herapy for Low Risk DCIS	The Australian slang word 'larrikin' is associated with the Australian identity: a bloke who refuses to stand on ceremony.
Trial status	Recruitment will start in 2016	Recruiting from July 2014	Not yet recruiting	Funding request submitted
Setting and locations	Mainland Europe (n>30)	United Kingdom (n>20)	United States (n=100)	Australia and New Zealand (n≥12)
Inclusion criteria	Women ≥ 45 years with asymptomatic, pure low-grade DCIS based on representative vacuum-assisted biopsies (at least 6) of unilateral, calcifications only of any size detected by population-based or opportunistic screening mammography.	Women ≥ 46 years with asymptomatic pure, non-high grade DCIS (e.g. low grade DCIS and intermediate grade DCIS with low grade features) based on vacuum assisted core biopsies of screen-detected or incidental calcifications only of any size (uni-/bilateral).	Women ≥ 40 years with pure, non-mass forming low-risk DCIS, e.g. ER + and/or PR + and HER-2 receptor-negative grade I or II DCIS based on a core biopsy without evidence of other breast disease on physical examination and breast imaging within 6 months of registration.	Women ≥ 55 years with pure, asymptomatic and low risk DCIS (low and intermediate grade) based on either a core biopsy and/or vacuum-assisted biopsy or open diagnostic surgical biopsy of screen detected or incidental calcifications (uni/bilateral but unifocal) ≤ 20 mm.
Exclusion criteria	No prior history of DCIS or invasive breast cancer, a BRCA 1/2 gene mutation present in family, no bilateral DCIS, synchronous contralateral invasive breast cancer, lobular carcinoma in situ, Paget's disease, or invasive breast disease on cytology/histology	No prior history or current diagnosis of invasive breast cancer or ipsilateral DCIS and no high risk group for developing breast cancer	Not known.	No previous or current diagnosis of invasive cancer, previous ipsilateral DCIS, Paget's disease or LCIS, pregnancy/lactation or a known BRCA1/2 mutation
Central review	No central review of pathology.	Real time central review of histological slides by expert DCIS pathologists.	Not known.	No central review planned.

Table 2 continued.

Trial name	LORD	LORIS	COMET	LARRIKIN
Interventions	Randomisation between standard treatment according to local policy (wide local excision with/without radiotherapy, mastectomy and possibly hormonal therapy by Tamoxifen) and active surveillance. Both study arms will be monitored with annual digital mammography for 10 years.	Randomisation between standard surgical and adjuvant treatment according to local policy and active surveillance, with specific notification that patients in the latter group should not receive anti-estrogen treatment. Both study arms will be monitored with annual mammography for 10 years. Anti-oestrogen treatment is not allowed in the active surveillance arm.	Randomisation between standard treatment including surgery and radiation and active surveillance. Patients in both groups are free to decide whether to choose endocrine therapy. Both study arms will be carefully monitored with mammograms and physical exams every 6 months for 5 years.	Randomisation between standard treatment according to physician and patient choice (surgery with/ without radiotherapy) and active surveillance. Patients in both groups are free to decide whether to opt for endocrine therapy for 5 years. Both groups will be monitored with annual mammography for at least 10 years and regular clinical examinations or at patient's request for 5 years then annually.
Randomisation	Allocation ratio 1:1	Allocation ratio 1:1	Allocation ratio 1:1	Allocation ratio 1:1
Primary end-points	Safety will be measured by ipsilateral invasive breast cancer-free percentage at 5 and 10 years.	Safety will be measured by ipsilateral invasive breast cancer-free survival time at 5 and 10 years.	Safety will be measured by assessing the invasive cancer rate in the affected breast at 2 and 5 years.	Safety will be measured by ipsilateral breast cancer free survival at 5 and 10 years.
Secondary end-points	-Rate of invasive disease or DCIS grade 2/3 at final pathology specimen -Time to ipsilateral grade II or III DCIS and time to contralateral DCIS -Cumulative incidence of contralateral invasive breast cancer -Ipsilateral mastectomy rate -Biopsy rate during follow-up -Time to failure of active surveillance strategy -Distant metastases free interval -Overall survival -Central collection of imaging data and biosamples for translational research purposes -Patient reported outcomes -Cost-effectiveness	-Time to development of ipsilateral, contralateral and any invasive breast cancer -Overall survival -Time to mastectomy or surgery -Quality of Life -Quality-adjusted life years -Translational exploratory assessment of predictive biomarkers -Patient reported outcomes -Cost-effectiveness	-Mastectomy and breast conservation rate -Contralateral invasive cancer rate -Overall and disease specific survival -Breast MRI rate -Breast biopsy rate -Radiation rate -Chemotherapy rate -Psychosocial outcomes -Decision quality -Financial burden/employment	-Rate of invasive disease and higher grade DCIS in final pathology specimen -Time to development of ipsilateral and any invasive breast cancer -Ipsilateral mastectomy rate at 5 years -Biopsy rate during follow-up -Overall survival -Time to failure of active surveillance strategy -Quality of Life -Cost Effectiveness
Sample size needed	1240 patients	932 patients	1189 patients	550 patients

Adequate communication about DCIS risks involved is key

In general, improving communication about the diagnosis and prognosis of DCIS patients will likely deliver the most essential improvements in the management of DCIS. This because there is much uncertainty about the long-term implications of the diagnosis of DCIS (including the risk of invasive breast cancer, therapeutic efficacy and safety), making it difficult for patients and health care providers to make well-informed decisions on treatment options. For a woman, it is difficult to understand that on the one hand DCIS is a breast cancer precursor but not yet an invasive disease, and on the other hand that intensive treatment is necessary. It is essential to better assess the risks involved and put these into perspective, taking into account the quality of life and competitive factors in terms of morbidity and mortality. Educating health care providers and developing a risk prediction model will contribute to this better understanding. It has been shown for prostate cancer, that such a strategy is well-accepted [105,106].

Conclusion

The incidence of DCIS has increased substantially. The rationale of DCIS treatment is mortality reduction as a result of invasive breast carcinoma. However, 'pure' DCIS (without any invasive component) usually shows no symptoms and does not cause mortality. We know that a significant proportion of the DCIS lesions will never lead to invasive breast cancer. But right now we don't know which DCIS lesions will progress and which will not. The result of this knowledge gap is that every DCIS lesion is treated similar to invasive breast cancer. Risk stratification is therefore essential for making better-informed treatment decisions. In addition, large randomized clinical trials are necessary to investigate if active surveillance is an option for low grade DCIS. Last but not least, communicating in a correct and nuanced manner about the implications of the diagnosis of DCIS is essential for a realistic risk perception and optimal decision-making by the patient and the health care professionals involved.

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Conflict of interest

None of the authors have a conflict of interest.

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