

## REVIEW

# Ductal carcinoma *in situ* of breast: update 2019

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### Summary

Ductal carcinoma *in situ* is a non-invasive form of breast cancer. Its incidence is increasing due to widespread use of mammographic screening. It presents several diagnostic and management challenges in part due to its relatively indolent behaviour. Most series analysing biomarkers in these lesions are small (<100 patients) and large clinical trials have not been frequent. Herein, we review the recent progress made in understanding the biology of this entity and the tools available for prognostication.

**Key words:** Breast cancer; DCIS; ductal carcinoma *in situ*; pathology; molecular features.

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### INTRODUCTION

The biological basis of micro-calcifications within the breast is poorly defined. In spite of this, it is common to observe micro-calcifications in benign ducts as well as in pre-invasive lesions of the breast. It has long been recognised that breast cancer can be associated with micro-calcifications. Although mammography also detects architectural distortion and masses, the science of mammography is based on the detection of presence and pattern of calcifications. As *in situ* lesions tend to exhibit micro-calcifications more frequently than invasive carcinoma, it is natural that the incidence of detecting pre-invasive lesions has dramatically increased (up to 20%) following the routine use of mammographic screening.<sup>1</sup>

In the pre-mammographic era, ductal carcinoma *in situ* (DCIS) was a palpable lesion accounting for approximately 1–2% of cases.<sup>2</sup> It is believed that palpable DCIS may be biologically different from screen detected DCIS. Although peri-ductal fibrosis is frequently observed in this type of DCIS, its aetiology is far from clear. One possible explanation is that it represents a reaction to ‘early’ invasion either in the form of cells or cellular products. Pathologists should regard this lesion with caution and generous sampling of the tissues to identify small foci of invasion is prudent. On the clinical side, particularly in the United States, many surgeons prefer to perform *a priori* sentinel node biopsies for these lesions. From the biological aspect, these lesions are of great interest for the study of tumour microenvironment and the changes associated with the process of invasion.

There has been significant progress in the last few years in our understanding of DCIS and its management. The focus of this review is to highlight some of these aspects while underlining the large number of unknowns and the need to devise better methods for studying this entity. We presume that the readers of this review are conversant with the basics of DCIS and these issues will not be reviewed in detail.

### DIAGNOSTIC CRITERIA

The morphological changes in DCIS lie within in the spectrum of hyperplasia and invasive cancer. The criteria for differentiation of DCIS from hyperplastic lesions are well described. Stains for high molecular weight keratins (HMWK) can be used to assist the distinction of DCIS from usual hyperplasia. The distinction of DCIS from atypical ductal hyperplasia (ADH) is based on examination of architectural, cytological and nuclear features. Size of the lesion is also an important consideration, with diagnosis of ADH being favoured for smaller lesions. Immunohistochemistry has little role to play in the distinction of DCIS from ADH. However, the strong and uniform expression of oestrogen receptor (ER), indicative of clonal proliferation, can be used to support the diagnosis of DCIS in difficult cases. Stains for basal and myoepithelial markers aid in the identification of invasion. However, some forms of non-invasive lesions such solid papillary carcinoma might not retain myoepithelial staining.

### TERMINOLOGY FOR DCIS

Long-term follow-up studies have documented high overall survival rates (>95% at 10 years) for DCIS; this includes death due to any cause such as road traffic accidents and cardiovascular events. This has raised the important issue of whether DCIS should be considered ‘cancer’? The term ‘cancer’ is associated with considerable emotional baggage including the fear of chemotherapy, and death. The logic of applying such a charged word to something that is indolent and very unlikely to cause significant impediment to life has been questioned. This is particularly true for lobular carcinoma *in situ* (LCIS), which undergoes spontaneous regression in the vast majority of cases. Although it has been suggested that the term carcinoma should be avoided,<sup>3</sup> there is poor consensus on the replacement terminology. The term ‘indolent lesions of epithelial origin (IDLE)’<sup>4</sup> has been suggested but is not yet widely adopted. There are concerns that the term ‘indolent’ might not convey the marked increase in risk for the development of invasive carcinoma and might result in undertreatment of patients.

## ASSESSING THE BIOLOGY OF DCIS

The wide variations in series size and the treatments offered has added to the confusion regarding the prognostic factors in DCIS. Most series of DCIS are small (100–200 patients), limited by follow-up data and have variable endpoints for outcome assessment, i.e., recurrence of DCIS or development of invasive carcinoma or both. Despite this a few generalisations can be made and these are discussed below.

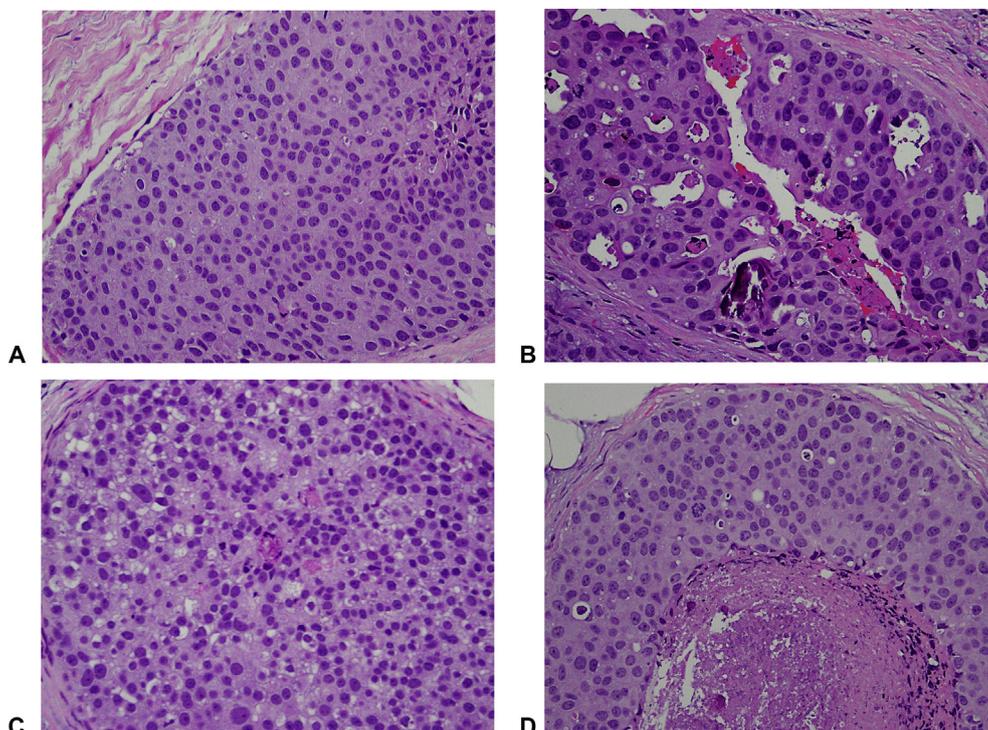
### Clinical and histopathological features

Younger age is associated with increased risk of recurrence of DCIS. This has been observed in almost all the series and clinical trials. The size of DCIS is also a poor prognostic factor with larger size being associated with greater risk of recurrence. The size of DCIS is not always easy to assess and multiple methods for measurement have been described (for review see Lester *et al.*).<sup>5</sup> Accurate assessment of size is greatly aided by a good mammographic correlation.

Margin status is another important consideration. A number of different definitions have been used to define ‘clear’ margins. The NSABP trials have used absence of ‘ink-on-tumour’ for margin negativity, whereas other groups have used 1 mm, 3 mm, 5 mm and even 1 cm margins. The precise definition was quite arbitrary until the recent Society of Surgical Oncology-American Society for Radiation Oncology-American Society of Clinical Oncology (SSO-ASTRO-ASCO) DCIS consensus on margin status.<sup>6</sup> These guidelines recommend adoption of greater than 2 mm as definition of clear margins, and use of clinical judgement for re-excision of lesions with 0–2 mm margin status; these have been successfully implemented within the surgical community.<sup>7</sup>

Architectural features are used to classify DCIS into comedo, solid, cribriform, micropapillary and other subtypes.<sup>8</sup> Multiple architectural patterns can be seen in the same case as illustrated in Fig. 1. Comedo DCIS has been associated with earlier recurrences and micropapillary pattern with extensive/large disease volume. A number of systems have been used to determine histological grade of DCIS.<sup>8</sup> Some of these evaluate cellular features as well as cell polarity, while others are based entirely on nuclear grade. The role of grade in determination of likelihood of recurrence has been controversial. In the subset of patients ( $n=327$ ) from the E5194 clinical trial that was used to develop/validate the DCIS Score (Genomic Health, USA), grade [College of American Pathologists (CAP) grading system] and histological features were not associated with recurrence.<sup>9</sup> In contrast, in the parent trial ( $n=665$ ), high grade was predictive of outcome at 10 and 12 years.<sup>10</sup> The importance of grade was also confirmed in analysis of 57,222 DCIS cases from the SEER database by Sagara *et al.*<sup>11</sup> The weighted 10-year breast cancer-specific survival hazard ratios of low grade DCIS cases were significantly different for intermediate and high grade DCIS [low grade hazard ratio (HR) 0.85, 95% confidence interval (CI) 0.21–3.52; intermediate grade HR 0.23, 95% CI 0.14–0.42; and high grade HR 0.15, 95% CI 0.11–0.23].<sup>11</sup>

Stromal features have also been used for prognostication. The presence of periductal fibrosis has been associated with increased likelihood of recurrence.<sup>12</sup> The mechanisms associated with the development of this feature is uncertain. Some studies have implicated cancer associated fibroblasts (CAFs) as the basis for the altered stroma and for making the tumour microenvironment more conducive for the development of



**Fig. 1** (A–D) Heterogeneity in the morphological patterns of ductal carcinoma *in situ* (DCIS). Images are taken from a needle core biopsy of single patient with DCIS. Note the range of architectural patterns from solid (A) to cribriform (B,C) as well as the variable amounts of necrosis in the ducts (B–D). (H&E; 20x objective using a Olympus DP27 camera and CellSens software.)

invasive cancer.<sup>13,14</sup> Periductal angiogenesis is also often seen in DCIS; the significance of this feature is uncertain. The failure of anti-angiogenic therapies in the treatment of invasive breast cancer has led to a diminishing interest in understanding the molecular basis of this feature.<sup>15</sup>

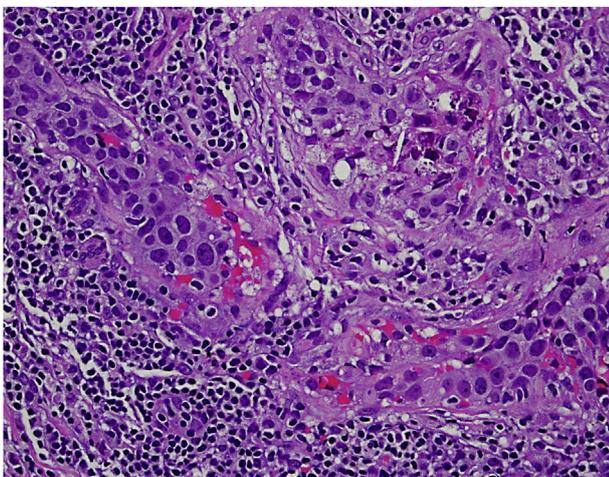
Recognition of the importance of stromal tumour infiltrating lymphocytes (TILs) in breast cancer, particularly triple negative breast cancer,<sup>16–18</sup> has led to a number of studies investigating immune cells in DCIS. DCIS cases harbour stromal TILs, where they are associated with younger age, symptomatic presentation, larger size, higher nuclear grade, comedo necrosis and ER negativity as well as shorter recurrence-free interval.<sup>19</sup> Similarly, high grade DCIS cases are also likely to show expression of FOXP3, CD68, CD4, CD20, HLA-DR<sup>20</sup> and PD-L1.<sup>21</sup> Further parsing of the data is necessary to identify the exact role of TILs in DCIS. The association of immune cells with poor prognosis DCIS could be indicative of a breach of the integrity of the basement membrane, at least at a molecular level. **Figure 2** shows infiltration by immune cells in ductal carcinoma *in situ* (DCIS).

## Biological parameters

### Protein analysis

Immunohistochemistry has been extensively used to identify prognostic factors in DCIS; however, most studies are limited by small sample size and limited follow-up. In spite of this, there are several important parameters that can be used for prognostication. A number of markers related to proliferation, cell cycle regulation, apoptosis, angiogenesis, extracellular matrix proteins (CD10, SPARC) and inflammation including COX2 have been analysed in DCIS. A detailed meta-analysis of the biological markers and risk of recurrence was performed by Lari and Kuerer<sup>22</sup> in 2011; much of the data stands to date and will not be reproduced herein but salient features will be highlighted.

Expression of ER is observed in greater than 80% of cases and in some series 90–95% of cases (E5194 and Toronto cohorts)<sup>23–26</sup> while progesterone receptor (PR) expression is observed in approximately 60% of cases.<sup>22</sup> ER negative DCIS has been associated with increased likelihood of



**Fig. 2** Focal infiltration by immune cells in ductal carcinoma in situ (DCIS) (same patient as **Fig. 1**; H&E, 20x objective using a Nikon DP27 camera and CellSens software).

recurrence. The analysis of expression of ER and PR has become commonplace in the management of DCIS. This in part due to the data from the NSABP B-24 study, which assessed the benefit of tamoxifen in 732 patients treated with surgery and radiation.<sup>27</sup> The patients with ER positive DCIS (76%) had significant benefit from tamoxifen (HR 0.64) at 10 years of follow-up. However, the 22 patients with ER negative DCIS did not obtain any benefit. Although these data have not been further validated, it was deemed practice changing with resultant implementation in clinical practice.

The expression of HER2 has been analysed in a number of cohorts reviewed by Sanati.<sup>26</sup> Expression has been associated with increased likelihood of development of recurrence. Of interest, expression of HER2 is observed in around 40% of cases of DCIS which is a significantly higher rate than in invasive cancer. Theories to explain this finding include loss of HER2 during progression, and invasion arising from a distinct clone with HER2 positive cells acting as ‘enablers’ during the process.

The expression of Ki-67 has been analysed in a number of series. The expression in DCIS, in contrast to invasive cancer, tends to be low. In most series, this results in poor correlation with outcomes. In a large series of patients, Kerlikowske *et al.* demonstrated that the combination of Ki-67 with COX2 and p16 expression could predict development of recurrence.<sup>28</sup> More recently, this set of markers has been expanded to include HER2, PR, Ki-67, COX2, p16/INK4A, FOXA1 and SIAH2 and available as a commercial assay DCISionRT from PreludeDx (discussed later).<sup>29,30</sup>

We have analysed the co-expression of multiple proteins in DCIS using multiplex immunofluorescence on a single 5 µm section. The study revealed marked heterogeneity in expression of oncologically relevant proteins in cells located within the same duct.<sup>31</sup> In addition, there was variability in degree of heterogeneity with some ducts containing predominantly a single subtype of cells and others showing the presence of multiple subpopulations. Further analyses seeking to understand the biological meaning of these findings are ongoing.

### Genomic analysis

Copy number analysis has been used to study the biology of DCIS (reviewed by Rane *et al.*<sup>32</sup>). Array comparative genomic hybridisation (aCGH) analysis has confirmed the presence of low and high grade pathways in DCIS. The low grade pathway is associated with loss of 16q and variable gain of 1q. Deletions of 16q are rare in the high grade pathway, while alterations of 8p, 11q, 13q, 17q and 20q are more frequent. Multiple studies have used fluorescent *in situ* hybridisation (FISH) to study DCIS and have found alteration in copy numbers of *HER2*, *C-MYC*, *CCND1*, *COX2*, *CDH1*, and *TP53*.<sup>33–36</sup>

A number of studies have sought to analyse the mutational patterns in DCIS and compare them with those of invasive carcinomas. Some of these studies have compared concurrent DCIS with invasive lesions from the same patient, while others have compared ‘pure’ DCIS lesions with DCIS associated with invasive carcinoma from different patients or a combination of the two.<sup>37</sup> The pattern of mutations in DCIS appears to be identical to that in invasive carcinomas with high frequency mutations in *PI3K* and *p53*.<sup>38–40</sup> These studies have also noted a significant intra-tumoural

heterogeneity in DCIS. An important study in this area was undertaken by Navin and colleagues wherein single cells from concurrent DCIS and IDC were analysed for their mutation status.<sup>41</sup> They developed and applied topographical single cell sequencing (TSCS) to analyse the genomic copy number profiles of 1,293 cells from 10 patients. The study documented nearly identical mutational patterns in these lesions, suggesting the development of invasion does not need additional mutations. It also suggested a multi-clonal invasion model in which one or more clones assist each other in order to escape the ducts and establish the invasive carcinomas. Epigenetic events in tumour cells might be critical drivers for the progression. A number of studies have analysed DNA methylation, non-coding RNA, histone modification and chromatin remodelling patterns in DCIS and associated these with progression (reviewed by DeVaux and Herschkowitz<sup>42</sup>). Alterations in the character and function of the tumour microenvironment could play a major role.<sup>43–45</sup> There are no data currently available to justify routine genomic analysis of DCIS.

#### RNA expression analysis

RNA expression analyses have compared DCIS with concurrent invasive lesions or pure DCIS with DCIS associated with invasive cancers.<sup>46–50</sup> The patterns of expression have been found to be fairly similar. Hannemann *et al.* analysed a series of 40 *in situ* lesions and compared them with 40 invasive carcinomas using an 18K expression array.<sup>51</sup> They identified 300 differentially expressed genes and developed a 43-gene classifier that could distinguish low grade DCIS from high grade and a 35-gene classifier to distinguish DCIS from invasive carcinoma. Interestingly, many of the genes identified in the classifier belong to the signal transduction and cell growth and maintenance pathways. Muggerud *et al.* studied a set of genes that identified a group that was most similar to invasive cancers.<sup>52</sup> This group was enriched in genes related to re-organisation of the microenvironment. Kristensen *et al.*, applied an integrated approach by modelling mRNA, copy number alterations, microRNAs, and methylation in a pathway context utilising the pathway recognition algorithm using data integration on genomic models (PARADIGM).<sup>53</sup> They found a great degree of similarity in the expression pattern of the five subtypes (PDGM1–5) in DCIS and invasive carcinoma with the exception of PDGM2, which was not identified in any of the 36 cases of (pure) DCIS. They did not find an association between PDGMs and grade, Ki-67 or HER2 staining. Lesurf *et al.*,<sup>39</sup> integrated mRNA, miRNA and copy number analyses to identify features that are unique to each of the intrinsic subtypes of DCIS. Their analysis suggests that there are subtype specific features that determine likelihood of recurrence.

#### Multi-parametric tools including commercial assays

**Nomograms** The need for predictors of likelihood of presence of invasive carcinoma or development of recurrence has resulted in the development of multiple nomograms. The Van Nuys prognostic index (VNPI) is based on patient's age, lesion size and grade, and margin width.<sup>54,55</sup> It stratifies patients into three risk groups and provides treatment guidelines to achieve less than 20% recurrence rate at 12 years. The limitations of this nomogram include lack of robust

independent validation and failure to include molecular characteristics. A more recent modification of this nomogram incorporates Genomic Grade Index (GGI) but needs further validation.<sup>56</sup> The Memorial Sloan Kettering (MSKCC) nomogram<sup>57</sup> is based on age, family history, clinical features at initial presentation, nuclear grade, presence of necrosis, margins, number of excisions, type of surgery, radiation and endocrine therapy. It is widely used and validated in multiple independent studies. However, there are some concerns regarding under-estimation of heterogeneity in DCIS.<sup>58</sup> More recently, the Mayo group has described a nomogram to predict upstaging of DCIS to invasive carcinoma.<sup>59</sup>

**Oncotype Dx DCIS score** The Eastern Co-operative Oncology Group (ECOG) had undertaken a registration (non-randomised) clinical trial of DCIS wherein patients were offered complete surgical excision (with centrally confirmed clean margins) but no radiation therapy.<sup>60</sup> Patients were enrolled onto one of two study cohorts (not randomly assigned): cohort 1, low or intermediate grade DCIS, tumour size  $\leq 2.5$  cm ( $n=561$ ); or cohort 2, high-grade DCIS, tumour size  $\leq 1$  cm ( $n=104$ ). Blocks were obtained from a subset of patients ( $n=327$ ) for Oncotype DX recurrence score and DCIS score. The 21-gene recurrence score was not associated with likelihood of ipsilateral breast event (IBE). In contrast, DCIS score was prognostic for identifying both the likelihood of recurrent DCIS and/or development of invasive disease.<sup>24</sup> These findings have been independently confirmed by Rakovitch's group in a large study from Toronto.<sup>23</sup> One of the limitations of these studies is that the vast majority (>95%) of the DCIS cases were ER positive; the relevance of the signature in ER negative DCIS has not been established.

**DCISionRT (PreludeDx)** DCISionRT from PreludeDx analyses the expression of a set of markers (HER2, PR, Ki-67, COX2, p16/INK4A, FOXA1 and SIAH2) to identify benefit of radiotherapy. It has been validated in multiple independent cohorts from the USA (Kaiser Permanente and UMass), Sweden (Uppsala) and Australia (Melbourne).<sup>30</sup> The test has been recently used in a cohort of 526 patients with DCIS treated with breast conserving surgery with or without radiotherapy (RT). In this cohort, it was prognostic for risk and predicted RT benefit.<sup>29</sup>

## MANAGEMENT ISSUES AND NEWER CLINICAL TRIALS

The management of DCIS has evolved considerably within last few decades. Until the mid-1980s, DCIS was routinely treated with mastectomy.<sup>61</sup> The National Surgical Adjuvant Breast Project (NSABP) B06 clinical trial compared the role of lumpectomy with mastectomy in the treatment of breast cancer.<sup>62</sup> Retrospective analysis of these cases showed that a small percentage of the 2072 patients ( $n=76$ ) had (mostly palpable) DCIS. Twenty-one of these patients had been treated with lumpectomy; of these, nine patients (43%) developed recurrence. The recurrence rate for patients with lumpectomy plus radiation (XRT) was 7%, while that for patients undergoing mastectomy was 0%. The value of addition of radiation to lumpectomy was further confirmed in the NSABP B17 clinical trial in which 81% of the patients had non-palpable DCIS.<sup>61,63</sup> A dramatic decrease in rates of recurrent DCIS (13.4%–8.2%) and development of invasive

disease (13.4%–3.2%) was observed in patients receiving radiation. The compilation of results from multiple clinical trials by the early breast cancer trialists groups has documented a 50% reduction in IBEs after addition of radiation to lumpectomy.<sup>64</sup> Further reduction in recurrence rates also has been observed following the addition of tamoxifen to surgery and radiation (L+ RT+ Tam), making this the standard of care for treatment of DCIS. In a relatively recent series, Palazzo's group studied the outcomes of 141 patients treated with surgery only. Despite negative margins, 60 recurrences (42.5%) were observed after a median follow-up of 122 months.<sup>65</sup> In contrast, Tadros *et al.* reported that for patients with close margins (<2 mm), the addition of radiation was associated with dramatic reduction in recurrence rates at 10 years (30.9% vs 4.8%).<sup>66</sup> There was marginal benefit in adding radiation therapy to patients with greater than 2 mm margins (5.4% and 3.3%, respectively) in this study.

There is a major concern that the standard of care therapy for DCIS (L+ RT+ Tam) might be unnecessarily aggressive for patients with small screen detected DCIS. This has resulted in a number of clinical trials that are addressing the question of reduction of therapy for these patients. The Comparison of Operative to Monitoring and Endocrine Therapy (COMET) trial enrolls patients with non-comedo DCIS for medical (non-surgical) management.<sup>67</sup> Similarly, LORIS (Low Risk DCIS) and LORD (Low Risk DCIS) trials are being conducted around the world to address the issues.<sup>68,69</sup> Trial enrolment is based on the subjective assessment of grade and absence of comedo necrosis. A recent study by Schnitt's group documented the poor correlation amongst pathologists in determining 'comedo necrosis'.<sup>70</sup> In this vein, it is important to remember that a significant number of DCIS lesions, approximately 25%, are upstaged to invasive carcinoma on excision;<sup>71–75</sup> these might be missed in these trials. Based on archival data, it has been estimated that approximately 20% of patients enrolled in the LORIS trial will have invasive cancer.<sup>69</sup> However, a recent analysis found the upstaging rates to invasive disease were only 6% (5/81), 7% (5/74), and 10% (1/10) for the COMET, LORIS, and LORD trials, respectively.<sup>67</sup> It is critical that all conservatively managed patients should be closely monitored.

## CONCLUSION AND FUTURE CHALLENGES

There has been steady, albeit slow, progress in understanding the biology of DCIS. There are multiple lines of evidence to document that DCIS is not a single entity but a collection of biologically heterogeneous lesions. Biologically, the cells in these lesions seem to have acquired all the necessary mutations and are poised to invade. However, the critical event necessary for invasion is not yet identified but could be an epigenetic event involving tumour-stromal interactions.

Although histology, tumour markers such as ER and HER2, and stromal markers such as TILs, provide some clues regarding the likelihood of recurrence, our knowledge regarding the mechanisms associated with progression of DCIS to invasive cancer are still in their infancy. There is a critical need to understand intra-lesional and inter-lesional heterogeneity and their impact on likelihood of recurrence/progression. There is a clear and pressing need for development of well-annotated cohorts with treatment information and follow-up data to understand the molecular basis of

recurrence and progression. This will require a collaborative multi-centre, multi-disciplinary approach, such as the Cancer Research UK Grand Challenge program. It will also be critical to perform integrative analysis (histology, mutations, copy number, mRNA, and protein levels) to predict likelihood of progression and impact of endocrine/radiotherapy. Ultimately, it might become necessary to have large multi-centre trials based on biomarkers of recurrence to convince the breast community regarding the utility of these predictive tools.

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