Androgen Receptors in Breast Cancer, What Pathologists Should Know

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No conflicts of interest with this presentation.
1. Androgen receptor (structure, function, biologic interactions)
2. Recommend AR testing in all TN and Apocrine IDCas?
3. Prostate carcinoma as a model
4. Targeted therapies
   - Aberaterone acetate
   - Bicalutamide
   - Enzalutamide
5. Optimization of IHC assays
6. DCIS definitions, natural history, treatment options
7. AR in DCIS
8. Future directions?
Methods
We searched Medline, Embase, Web of Science, and Cochrane Library for articles written in English and published before December 2015. Search terms were androgen receptor, androgen, and anti-androgen alone or in combination with search strings connected to the topics of interest—for example, breast cancer, agonist, or antagonist. Furthermore, references cited in the retrieved articles were screened for additional articles. In addition, abstracts from annual meetings from 2011 to 2015 of the American Society of Clinical Oncology, American Association for Cancer Research, European Society of Medical Oncology, and San Antonio Breast Cancer Symposium were screened. ClinicalTrials.gov was searched for ongoing clinical trials. From these data bases, 74 articles and abstracts and 9 clinical trials were included.
Androgen receptors function as nuclear transcription factors and consist of 4 domains: N-terminal domain, DNA-binding domain, a hinge, and Ligand-binding domain.

In the absence of ligand, AR attaches to heat shock proteins and is located mainly in the cytoplasm.

In the presence of ligand, the ligand-binding domain is unbound from heat shock protein and AR translocates into the nucleus, where the DNA-binding domain binds to androgen responsive elements in DNA and recruits additional coactivators, corepressors, and transcriptional modulators.

Androgen receptor has been investigated extensively in prostate cancer, and these studies have shown cross-talk of the AR pathway with several other key signaling pathways, including the PI3K/Akt/mTOR and MAPK pathways, and with several key proteins, including Forkhead box protein A1 (FOXA1), phosphatase and tensin homologue deleted on chromosome 10 (PTEN), PI3K, and receptor tyrosine kinases, including ERBB2 (formerly HER2 or HER2/neu) and ERBB3.
Approximately 70% to 90% of ER-positive tumors are also AR positive, depending on the definition of AR positivity.

In ER+ AR+ cell lines, ligand-bound AR binds to estrogen-related elements in the nucleus, which leads to cell apoptosis, whereas in ER- AR+ cell lines, AR binds to androgen-related elements in the nucleus, leading to cell proliferation.

Several clinical studies confirmed this preclinical finding, showing that among postmenopausal women, AR expression was a more favorable prognostic factor in women with ER positive breast cancer than in women with ER negative breast cancer.
Non–Tissue-Selective Androgens:
Drugs such as fluoxymesterone or danazol, which were used for treatment of metastatic breast cancer in the 1960s, fell out of use because of their adverse effects, such as hirsutism, hoarseness, or alopecia.

AR Agonists
Selective AR modulators are a potential treatment option for breast cancer. In the cell line MDA-MB-231, TNBC cells stably expressing wild-type AR, treatment with the selective AR modulator enobosarm showed inhibition of metastasis-promoting paracrine factors such as interleukin 6 and matrix metalloproteinase 13 and subsequent migration and invasion. Clinical trials of 2 selective AR modulators, enobosarm and GSK2849466, are ongoing.

PI3K Pathway Inhibitors Combined with AR Therapies
The PIK3CA mutation rate is higher in AR-positive breast cancer than in AR-negative breast cancer (40% vs 4%). Our group previously reported that breast cancers with kinase domain PIK3CA hot spot mutations expressed significantly higher levels of AR than did breast cancers with helical domain PIK3CA mutations or wild-type PIK3CA (P = .02 and P < .001, respectively).

AR Antagonists
The AR antagonists, which are among the therapeutic agents most commonly used to treat prostate cancer, include several categories of drugs, for example, AR inhibitors and CYP17A inhibitors. Many AR antagonists are currently being investigated in breast cancer in preclinical and clinical studies.

Immunotherapy
Data regarding association between AR and immune response are limited. Patients with non-AR-driven enzalutamide-resistant prostate cancer had significantly more programmed death-ligand (PDL1)-positive and PD-L2-positive dendritic cells in blood than did patients who responded to the drug or had never been treated with it, which suggests that enzalutamide resistance that is independent of AR reactivation may be overcome by anti–programmed death-1 therapy.
AR is the most widely expressed nuclear hormone receptor in invasive breast cancers:  
85-95% of invasive ER positive cancers  
40-70% of invasive ER negative cancers

Majority of AR negative invasive carcinomas are grade 3. Majority of AR positive invasive carcinomas are grade 1&2.

One unique feature of invasive apocrine carcinomas is that they express AR.
Conclusions. The AR is expressed in normal breast tissue, and expression decreases with advancement to DCIS and invasive cancer. AR-positive TNBC was more common in older patients and had a higher propensity for LN metastases. AR-positive TNBC may represent a breast cancer subtype with unique features that may be amenable to treatment with alternative targeted therapies.

Conclusion. The relatively high proportion of AR+ tumors (36%) among the 50 triple negative carcinomas is an important finding in support of routine assessment of AR in at least all TNBCs and apocrine carcinomas as a potential target for therapy.
2002  55F R Br lump / skin dimpling
pT2, pN1 Grade 2 Inv Duct Ca, Triple Negative Carcinoma with apocrine features
Partial mastectomy negative margins
Axillary dissection (2 positive nodes)
Adjuvant combination chemotherapy
Adjuvant radiotherapy

2009 Recurrence in R Br and L axilla
Morphologically similar and Triple Negative
Palliative chemotherapy
R mastectomy and left axillary dissection.
7 cm tumor and multifocal lvi, margins negative
3 positive left axillary nodes

2010 Disease progression with
Masses in left breast and involvement of right chest skin
Additional excisions and radiation

2012 bilateral chest skin involvement with confluent nodules

Bicalutamide started (AR antagonist).
Complete clinical response, > 12 months.

AR IHC
100% immunoreactivity
strong intensity
An overwhelming number of publications demonstrate that AR is a favorable prognostic marker in invasive breast cancers, regardless of subtype. These studies also suggest that AR expression is directly proportional to DFS, PFS, and OS.
<table>
<thead>
<tr>
<th>Reference</th>
<th>Ref</th>
<th>Summary</th>
</tr>
</thead>
</table>
| Hermann and Adair, 1947, 1946 | [71,84] | - Treatment of patients with breast cancer with testosterone propionate showed significant regression of cancer and disappearance of metastases.  
- Four out of 11 breast cancer patients treated with testosterone propionate exhibited favorable response. |
| Bines et al., 2014 | [88] | - Clinical trial with Megestrol acetate, a synthetic progestin that also has AR agonistic activity was conducted in ER-positive breast cancer patients.  
- Clinical benefit rate of 40% was achieved with a duration of clinical benefit of 10 months. |
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| Bines et al., 2014 | [88] | - Clinical trial with Megestrol acetate, a synthetic progestin that also has AR agonistic activity was conducted in ER-positive breast cancer patients.  
- Clinical benefit rate of 40% was achieved with a duration of clinical benefit of 10 months. |
| Tormey et al., 1983 | [90] | - Combination of halotestin and tamoxifen was tested in a clinical trial conducted in ER-positive breast cancer patients.  
- Combination was more effective with 38% partial and complete remission rates, while tamoxifen had only 15%.  
- The duration of response was also longer in the combination group than in the tamoxifen group. |
| Guocalp et al., 2013 | [101] | - Clinical trial with an AR antagonist, bicalutamide, was performed in ER-negative breast cancer patients.  
- The 6 month clinical benefit rate was 19% and the median PFS was 12 weeks.  
- The drug was well tolerated. |
- The patient showed a complete response and the response was also durable for over a year. |
| Bonnefoi et al., 2016 | [104] | - A clinical trial with abiraterone+prednisone in 30 AR-positive TNBC patients was performed.  
- A clinical benefit rate of 20% was observed in this trial with an overall response rate of 6.7%. |
| O’Shaughnessy et al., 2016 | [99] | - Abiraterone acetate was tested alone or in combination with exemestane in patients with ER-positive breast cancer.  
- There was no significant difference in the PFS in the combination arm compared to the exemestane arm. |

Based partially on the modest success achieved with bicalutamide, clinical trials in TNBC and ER-positive breast cancer were initiated with a second generation AR antagonist, enzalutamide. Enzalutamide has a unique mechanism of action where it blocks AR nuclear translocation and is more potent than bicalutamide [103]. Although no publications have come out on the trial, data presented in San Antonio breast cancer conference in 2014 and 2015 and in American Society for Clinical oncology (ASCO) 2015 annual meeting indicated a favorable response, including partial and complete responses, in approximately 40% of the patients. Details will emerge when the data are published.
Enzalutamide for the Treatment of Androgen Receptor–Expressing Triple-Negative Breast Cancer

Tiffany A. Traina, Kathy Miller, Denise A. Yardley, Janice Eakle, Lee S. Schwartzberg, Joyce O’Shaughnessy, William Gradishar, Peter Schmid, Eric Winer, Catherine Kelly, Rita Nanda, Ayca Gucalp, Ahmad Awada, Laura Garcia-Estevez, Maureen E. Trudeau, Joyce Steinberg, Hirdesh Uppal, Iulia Cristina Tudor, Amy Peterson, and Javier Cortes

MDV3100-11: a single-arm, open-label, phase II trial to evaluate the efficacy and safety of enzalutamide in patients with locally advanced or metastatic TNBC. (ClinicalTrials.gov identifier: NCT01889238).

Purpose
Studies suggest that a subset of patients with triple-negative breast cancer (TNBC) have tumors that express the androgen receptor (AR) and may benefit from an AR inhibitor. This phase II study evaluated the antitumor activity and safety of enzalutamide in patients with locally advanced or metastatic AR-positive TNBC.

Patients and Methods
Tumors were tested for AR with an immunohistochemistry assay optimized for breast cancer nuclear AR staining > 0% was considered positive. Patients received enzalutamide 160 mg once per day until disease progression. The primary end point was clinical benefit rate (CBR) at 16 weeks. Secondary end points included CBR at 24 weeks, progression-free survival, and safety. End points were analyzed in all enrolled patients (the intent-to-treat [ITT] population) and in patients with one or more postbaseline assessment whose tumor expressed ≥ 10% nuclear AR (the evaluable subgroup).

Results
Of 118 patients enrolled, 78 were evaluable. CBR at 16 weeks was 25% (95% CI, 17% to 33%) in the ITT population and 33% (95% CI, 23% to 45%) in the evaluable subgroup. Median progression-free survival was 2.9 months (95% CI, 1.9 to 3.7 months) in the ITT population and 3.3 months (95% CI, 1.9 to 4.1 months) in the evaluable subgroup. Median overall survival was 12.7 months (95% CI, 8.5 months to not yet reached) in the ITT population and 17.6 months (95% CI, 11.6 months to not yet reached) in the evaluable subgroup. Fatigue was the only treatment-related grade 3 or higher adverse event with an incidence of > 2%.

Conclusion
Enzalutamide demonstrated clinical activity and was well tolerated in patients with advanced AR-positive TNBC. Adverse events related to enzalutamide were consistent with its known safety profile. This study supports additional development of enzalutamide in advanced TNBC.
Triple-negative breast cancer (TNBC) carries the worst prognosis among all breast cancer subtypes, with a median overall survival (OS) rarely extending beyond 12 to 18 months in advanced disease.

Enzalutamide is a potent AR inhibitor that acts on multiple steps in the AR signaling pathway and is currently approved for the treatment of patients with metastatic castration-resistant prostate cancer in >50 countries in the chemotherapy-naive setting and >75 countries in the postchemotherapy setting on the basis of significant survival advantages demonstrated in two large, randomized, placebo controlled, phase III studies.
Enzalutamide in Metastatic Prostate Cancer before Chemotherapy


CONCLUSIONS

Enzalutamide significantly decreased the risk of radiographic progression and death and delayed the initiation of chemotherapy in men with metastatic prostate cancer. (Funded by Medivation and Astellas Pharma; PREVAIL ClinicalTrials.gov number, NCT01212991.)

Increased Survival with Enzalutamide in Prostate Cancer after Chemotherapy

Howard I. Scher, M.D., Karim Fizazi, M.D., Ph.D., Fred Saad, M.D., Marc-Frédéric Tabar, M.D., Carl N. Sternberg, M.D., Kurt Miller, M.D., Ronald de Wit, M.D., Peter Scher, M.D., Andrew J. Armstrong, M.D., Thomas W. Flanagan, M.D., Mark Fleming, M.D., John D. Hainsworth, M.D., and Johann S. de Bono, M.B., B.Chir.

CONCLUSIONS

Enzalutamide significantly prolonged the survival of men with metastatic castration-resistant prostate cancer after chemotherapy. (Funded by Medivation and Astellas Pharma Global Development; AFFIRM ClinicalTrials.gov number, NCT00974311.)
Submission of tumor tissue for AR screening was allowed at any time in a patient’s disease course and before treatment consent.

IHC assays using two AR antibodies, AR441 (Dako, Carpinteria, CA) and SP107 (Ventana, Tucson, AZ), were optimized for testing breast cancer tissue.

IHC results using SP107 determined eligibility and were used to report study-related outcomes. Investigators were informed only that a patient was AR positive or negative.

If available, RNA and DNA were extracted from remaining tumor tissue from all enrolled and treated patients and also from a subset of patients who submitted tissue through prescreening but did not receive enzalutamide (some of whom tested AR 0%) to perform next-generation sequencing.

Enrolled patients received enzalutamide 160 mg once per day until disease progression.
AR expression >0% was observed in 80% of tumors and AR expression >10% was observed in 55% of tumors.

Two other prospective clinical studies of AR inhibitors in TNBC have been recently published:

1) Translation Breast Cancer Research Consortium TBCRC 011 trial, bicalutamide:
   Of 424 patients, 12% AR positive (using AR441)

2) French Breast Cancer Intergroup UCBG 12-1 trial, abiraterone acetate:
   Of 138 patients, 38% AR positive (using AR441).

AR IHC assays have been largely developed for prostate cancers that express high amounts of AR with little to no dynamic range of AR expression.
Androgen Receptor Immunohistochemistry as a Companion Diagnostic Approach to Predict Clinical Response to Enzalutamide in Triple-Negative Breast Cancer

Purpose The androgen receptor (AR) is increasingly recognized as a potential biomarker for identifying a subset of patients with possible hormonally driven triple-negative breast cancer (TNBC). However, its performance as a companion diagnostic remains elusive. Thus, we evaluated AR expression by immunohistochemistry in patients with advanced TNBC before treatment with the AR inhibitor enzalutamide.

Methods We optimized and validated immunohistochemistry assays in breast and prostate cancer cell lines and tissues using two commercial AR monoclonal antibodies (SP107 and AR441). AR expression was then examined in patients with advanced TNBC enrolled in a phase II study of enzalutamide (ClinicalTrials.gov identifier: NCT01889238) on archived or fresh tissue before treatment. Association with clinical response was assessed by sensitivity, specificity, positive predictive value (PPV), drop-out rate, and survival.

Results AR expression was detected in 80% and 63% of breast cancer tissue using SP107 and AR441, respectively. SP107 was selected for additional analyses because of its higher sensitivity and robustness. Total AR nuclear staining demonstrated the best accuracy in predicting clinical response (area under receiver operating characteristic curve, 0.72; \(P = .0001\)). At a threshold of 10%, 74.6% of patients were AR positive, leading to 30% PPV, 90% sensitivity, and 30% specificity. These patients showed a significantly higher median progression-free survival (hazard ratio, 0.56; 95% CI, 0.36 to 0.88; \(P = .011\)) and overall survival (hazard ratio, 0.54; 95% CI, 0.32 to 0.91; \(P = .019\)) compared with those with AR-negative (< 10%) TNBC.

Conclusion At a threshold of ≥ 10% nuclear expression, the AR was associated with TNBC response to enzalutamide. However, the modest PPV may restrict its clinical application, and additional diagnostic tools may be helpful for improved patient selection.

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RESULTS

IHC Optimization: Two commercial antibodies, SP107 and AR441, were evaluated for IHC optimization and validation.

For SP107, antigen retrieval was optimized for 64 minutes at standard high pH, and primary antibody incubation was optimized without dilution for 24 minutes.

For AR441, antigen retrieval was optimized for 20 minutes at high pH at room temperature. The primary antibody was incubated at a 1:800 dilution for 30 minutes.

The optimized IHC conditions were then verified on breast carcinoma tissue, normal breast tissue, and two breast carcinoma cell lines known to express the AR (T-47D and MCF7). The staining patterns clearly indicated that SP107 displayed more intense AR expression than did AR441.

<table>
<thead>
<tr>
<th>Tissue Type</th>
<th>AR+ ≥ 10% of Cells</th>
<th>AR411 (Dako)</th>
<th>Tissue Type</th>
<th>AR+ ≥ 10% of Cells</th>
<th>AR411 (Dako)</th>
<th>Tissue Type</th>
<th>AR+ ≥ 10% of Cells</th>
<th>AR411 (Dako)</th>
<th>Tissue Type</th>
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<th>Tissue Type</th>
<th>AR+ ≥ 10% of Cells</th>
<th>AR411 (Dako)</th>
<th>Tissue Type</th>
<th>AR+ ≥ 10% of Cells</th>
<th>AR411 (Dako)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TNBC</td>
<td>9 of 26 (35)</td>
<td>6 of 15 (40)</td>
<td>AR+ Clone (source)</td>
<td>AR411 (Thermo)</td>
<td>14</td>
<td>First Author</td>
<td>Park</td>
<td>11</td>
<td>ER+ and/or PgR+ breast carcinoma</td>
<td>50 of 50 (100)</td>
<td>19 of 25 (76)</td>
<td>AR+ Clone (source)</td>
<td>AR411 (Thermo)</td>
<td>69</td>
<td>First Author</td>
<td>Park</td>
<td>11</td>
</tr>
<tr>
<td>ER+ and/or PgR+ breast carcinoma</td>
<td>50 of 50 (100)</td>
<td>19 of 25 (76)</td>
<td>AR411 (Dako)</td>
<td>AR411 (Novocastra)</td>
<td>30</td>
<td>Micello</td>
<td></td>
<td>9</td>
<td>ER+ and/or PgR+ breast carcinoma</td>
<td>50 of 50 (100)</td>
<td>19 of 25 (76)</td>
<td>AR411 (Dako)</td>
<td>AR411 (Novocastra)</td>
<td>30</td>
<td>Micello</td>
<td></td>
<td>9</td>
</tr>
<tr>
<td>HER2+ breast carcinoma</td>
<td>15 of 17 (88)</td>
<td>10 of 16 (63)</td>
<td>AR411 (Dako)</td>
<td>AR411 (Dako)</td>
<td>77</td>
<td>Collins</td>
<td></td>
<td>10</td>
<td>HER2+ breast carcinoma</td>
<td>15 of 17 (88)</td>
<td>10 of 16 (63)</td>
<td>AR411 (Dako)</td>
<td>AR411 (Dako)</td>
<td>77</td>
<td>Collins</td>
<td></td>
<td>10</td>
</tr>
<tr>
<td>Breast carcinoma, any subtype</td>
<td>74 of 93 (80)</td>
<td>35 of 56 (63)</td>
<td>AR411 (Dako)</td>
<td>AR411 (Dako)</td>
<td>69</td>
<td>Ogawa</td>
<td></td>
<td>20</td>
<td>Breast carcinoma, any subtype</td>
<td>74 of 93 (80)</td>
<td>35 of 56 (63)</td>
<td>AR411 (Dako)</td>
<td>AR411 (Dako)</td>
<td>69</td>
<td>Ogawa</td>
<td></td>
<td>20</td>
</tr>
<tr>
<td>Prostate carcinoma</td>
<td>8 of 10 (80)</td>
<td>4 of 9 (44)</td>
<td>AR411 (Dako)</td>
<td>AR411 (Dako)</td>
<td>90</td>
<td>Qiu</td>
<td></td>
<td>21</td>
<td>Prostate carcinoma</td>
<td>8 of 10 (80)</td>
<td>4 of 9 (44)</td>
<td>AR411 (Dako)</td>
<td>AR411 (Dako)</td>
<td>90</td>
<td>Qiu</td>
<td></td>
<td>21</td>
</tr>
</tbody>
</table>

Abbreviations: AR, androgen receptor; AR+ androgen receptor positive; ER+, estrogen receptor positive; HER2+, human epidermal growth factor receptor 2 positive; PgR+, progesterone receptor positive; TNBC, triple-negative breast cancer.
Optimization of the IHC assays in this study was intended to lead to increased sensitivity and a potential for a higher prevalence of AR expression.

However, the performance characteristics of IHC as a treatment-associated and potentially predictive assay were suboptimal. With a threshold of $>10\%$ nuclear expression, the positive predictive value of AR IHC was a modest 30%, which may restrict its clinical application.

This is a critical consideration in the development of a treatment-associated assay, because one would not want to exclude patients from receiving a potentially beneficial and well-tolerated treatment.
Clinical Benefit Rates at 24 weeks:
19% in TBCRC 011 (Bicalutamide)
20% in UCBG 12-1 (Aberaterone acetate)
28% in MDV3100-11 (Enzalutamide)

Study authors state:
Higher clinical benefit rates with enzalutamide could be explained by different mechanisms of action between agents. Unlike bicalutamide, enzalutamide has no known AR agonist activity and was superior to bicalutamide in two large randomized phase II studies in patients with prostate cancer.

Prednisone, a requisite concomitant medication for abiraterone acetate, stimulates the glucocorticoid receptor, which is expressed in approximately 25% of TNBCs. It is possible that glucocorticoid receptor stimulation from prednisone results in tumor growth, limiting the efficacy of abiraterone acetate. Furthermore, abiraterone acetate treatment results in appreciable increases in progesterone, potentially stimulating the PgR, albeit at low levels given in TNBC.

I wonder:
Could AR IHC optimization in part explain differences?
Definition of DCIS:

A neoplastic proliferation of epithelial cells confined to the mammary ductal-lobular system and characterized by subtle to marked cytological atypia and an inherent but not necessarily obligate tendency for progression to invasive breast cancer.

Epidemiology of DCIS:

Infrequent prior to mammography (3% of palpable breast cancers).

Now accounts for 20-25% of newly diagnosed breast cancers in USA.

In women aged >/= 50 yrs of age, 500% increase in DCIS observed from 1983 to 2003.

Associated with increasing age, personal history of breast disease (such as ADH), family history, nulliparity, late age at birth of first child, late menopause, elevated BMI after menopause, high mammographic breast density, genetic mutations (BRCA1 & BRCA2).

Mean Age between 50 and 59 years.
Most often unilateral, although can be bilateral in in 20%.
DCIS is a segmental disease, originating in the TDLU and progressing within the duct system along adjacent branches which may extend to the nipple.

Clinical presentations variable:
85% asymptomatic / mammographic findings only
5% incidental in biopsies for other reasons
(10%) remainder with symptoms:
  Nipple discharge
  Paget’s disease
  Palpable mass

Breast cancer specific mortality among women with DCIS is extremely low, varying from 1% to 2.5% at 10 year interval (all deaths attributed to occult invasion and/or invasion with recurrence of DCIS).
Chapter 5
Intraductal Proliferative Lesions

ADH
Simpson et al
p. 89
2012

Table 5.01 Morphological characteristics useful in distinguishing ADH from UDH and from low-grade DCIS.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>UDH</th>
<th>ADH</th>
<th>DCIS (low grade)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Architecture</td>
<td>Cellular swirling and streaming; stretched or twisted epithelial bridges; peripheral, irregular, and slit-like fenestrations.</td>
<td>Rigid cellular bars; bulbous micropapillae; round, punched out spaces.</td>
<td>Rigid cellular bars; bulbous micropapillae; round, punched out spaces.</td>
</tr>
<tr>
<td>Cytology</td>
<td>Multiple cell types; uneven distribution and overlapping of cells and nuclei; indistinct cell borders.</td>
<td>Cellular uniformity; even cell placement; distinct cell borders; residual normally polarized cells.</td>
<td>Cellular uniformity; even cell placement; distinct cell borders; no residual normally polarized cells.</td>
</tr>
<tr>
<td>Extent</td>
<td>Variable, ranging from one to multiple TDLUs.</td>
<td>Partial involvement of multiple spaces; complete involvement of &lt; 2 spaces or ≤ 2 mm in extent (see text).</td>
<td>Complete involvement of ≥ 2 spaces or &gt; 2 mm in extent (see text).</td>
</tr>
<tr>
<td>Risk of developing breast cancer; laterality of risk</td>
<td>Slight risk; generalized bilateral risk.</td>
<td>Moderate risk; generalized bilateral risk.</td>
<td>High risk; regional ipsilateral risk.</td>
</tr>
</tbody>
</table>

ADH, atypical ductal hyperplasia; DCIS, ductal carcinoma in situ; TDLU, terminal-duct lobular unit; UDH, usual ductal hyperplasia.
The natural history of ductal carcinoma in situ of the breast: a review

Bircan Erbas¹, Elena Provenzano², Jane Armes³-⁴, and Dorota Gertig¹

¹Centre for Molecular, Environmental, Genetic and Analytic Epidemiology, School of Population Health, The University of Melbourne, Carlton, Victoria, Australia; ²Department of Anatomical Pathology, Austin Hospital, Melbourne, Victoria, Australia; ³Molecular Pathology, Victorian Breast Cancer Research Consortium, Department of Pathology, The University of Melbourne, Carlton, Victoria, Australia; ⁴Department of Anatomical Pathology, Mater Adult Hospital, South Brisbane, Queensland, Australia

Abstract

Background. Ductal carcinoma in situ represents about 20% of all tumours diagnosed within mammographic screening programs. The natural history of DCIS is poorly understood, as it cannot be observed directly. Estimates of the proportion of DCIS that progress to invasive cancer, as well as factors that may influence progression, are important for clinical management. Here we review various sources of evidence regarding the natural history of DCIS.

Methods. We identified relevant publications of studies on: follow-up studies of DCIS initially misdiagnosed as benign, studies of recurrence of DCIS as invasive cancer, autopsy studies, studies of risk factors for DCIS, animal studies and studies that used mathematical models to study growth of DCIS and invasive cancer. Data sources included the MEDLINE data base, searches of articles cited in key reviews and editorials.

Results. The most direct evidence regarding the progression of DCIS to invasive cancer comes from studies where DCIS was initially misdiagnosed as benign and treated by biopsy alone. These studies suggest that between 14–53% of DCIS may progress to invasive cancer over a period of 10 or more years. The reported prevalence of undiagnosed DCIS in autopsy studies, of approximately 9%, has been used to suggest a larger reservoir of DCIS may exist in the population. All types of study designs reviewed had limitations that may bias the estimate of progression in either direction.

Conclusion. The available evidence suggests not all DCIS will progress to invasive cancer in the medium term but precise estimates of progression are not possible given the limitations of the data. Mathematical modelling of various scenarios of progression and studies of genetic factors involved in progression may shed further light on the natural history of DCIS.
At a Glance

• The purpose of this study was to estimate the mortality from breast cancer following a diagnosis of ductal carcinoma in situ (DCIS) and to identify risk factors for death from breast cancer.

• The 20-year breast cancer–specific mortality rate following a diagnosis of DCIS was 3.3%.

• Young age at diagnosis and black ethnicity were significant predictors of breast cancer mortality.

• Prevention of invasive in-breast recurrence with either radiotherapy or mastectomy did not prevent death from breast cancer.

• The clinical course of women with DCIS is similar to that of women with small invasive breast cancers.
Ductal Carcinoma In Situ, Complexities and Challenges

 Gregory D. Leonard, Sandra M. Swain

Table 2. Axillary lymph node metastases in selected studies involving DCIS and DCISM*

<table>
<thead>
<tr>
<th>Author and reference</th>
<th>No. of ALND</th>
<th>No. of positive ALND</th>
</tr>
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<tbody>
<tr>
<td><strong>DCIS</strong></td>
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<td>Amichetti et al. (35)</td>
<td>97</td>
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<td>Ashikari et al. (36)</td>
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<td>Carter et al. (38)</td>
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<td>Lagios et al. (34)</td>
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<td>2</td>
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<td>Parker et al. (42)</td>
<td>104</td>
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<tr>
<td>Patchefsky et al. (43)</td>
<td>13</td>
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<td>Roehl et al. (44)</td>
<td>210</td>
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<td>Rosner et al. (45)</td>
<td>52</td>
<td>1</td>
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<td>Schuh et al. (46)</td>
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<td>1</td>
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<tr>
<td>Silverstein et al. (21)</td>
<td>86</td>
<td>0</td>
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<tr>
<td>Simon et al. (47)</td>
<td>61</td>
<td>0</td>
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<td>Sunshine et al. (48)</td>
<td>6</td>
<td>0</td>
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<tr>
<td>von Rueden et al. (49)</td>
<td>32</td>
<td>0</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>1621</td>
<td>22 (1.4%)</td>
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</tbody>
</table>

| **DCISM**            |            |                      |
| Akhtar et al. (30)   | 25         | 0                    |
| Kimne et al. (31)    | 42         | 4                    |
| Jimenez et al. (32)  | 69         | 5                    |
| Le Besace et al. (33)| 80         | 3                    |
| Pohore et al. (29)   | 59         | 0                    |
| Patchefsky et al. (43)| 16       | 2                    |
| Prasad et al. (54)   | 15         | 2                    |
| Rosner et al. (33)   | 34         | 1                    |
| Schmitt et al. (33)  | 38         | 1                    |
| Schuh et al. (46)    | 30         | 6                    |
| Schwarz et al. (36)  | 257        | 0                    |
| Silver et al. (30)   | 38         | 0                    |
| Silverstein et al. (97)| 30     | 0                    |
| Simpson et al. (37)  | 55         | 2                    |
| Solin et al. (38)    | 39         | 1                    |
| Wong et al. (39)     | 33         | 1                    |
| Zavosky et al. (60)  | 14         | 0                    |
| **Total**            | 547        | 28 (5.1%)            |

Microinvasion: stromal invasion <= 1 mm greatest dimension
Treatment Options for DCIS

- Observation with clinical and imaging surveillance.

- Complete surgical excision with negative margins.
  Standard of care.

- Adjuvant radiotherapy
  Decreased risk of local recurrence when breast conserving surgery used.

- Adjuvant endocrine therapy
  Antiestrogen (tamoxifen) / aromatase inhibitor (naloxone).
  Risk reduction of subsequent invasive cancers (chemoprevention).
  Feasible in 70% to 80% of patients (those shown to be ER positive).
An increasing proportion of patients with DCIS choose breast-conserving therapy rather than mastectomy.

Four randomized clinical trials comparing excision of DCIS only to excision of DCIS followed by radiation therapy have shown that the addition of radiation reduces the risk of local recurrence by approximately 50% (older women benefiting more substantially). Radiation of DCIS patients has not been shown to reduce the risk of metastasis or mortality from breast cancer.
Adjuvant tamoxifen significantly reduces the ipsilateral risk of DCIS recurrence and/or progression to invasive cancer by about 50% in patients treated with lumpectomy and radiation, and that benefit is restricted to patients with ER positive DCIS.
More than 50,000 women in the U.S. each year (1 of 33 women over lifetime) will be diagnosed with DCIS. Almost all of these patients are asymptomatic.


Meta-analysis by Erbas et al 2006 suggested that up to 22% of DCIS progresses to cancer without treatment.

Many (if not most) women treated for DCIS may not directly benefit from treatment.
Currently three randomized controlled trials for low-risk DCIS underway in Europe and the United States.

All designed to test safety, efficacy, and trade-offs of active surveillance versus usual interventions.

**LORIS** (UK study)  **Low Risk Ductal Carcinoma in Situ**  
A Phase III Trial of Surgery versus Active Monitoring

**LORD** (European study – EORTC) Management of **Low Risk DCIS**  
A Phase III trial of Surveillance versus Conventional Treatments.

**COMET** (U.S. PCORI cooperative trial) **Comparison of Operative versus Medical Endocrine Therapy for Low Risk DCIS**  
70 month trial (2016-2021); project budget of $13 million  

Algorithms combining imaging features, demographics, clinical setting and pathology data may allow for safe surveillance in some patients.

De-escalation of therapy can be a frightening concept, and doing away with treatments is always more challenging than adding additional therapies.

Important to emphasize endpoints of mortality and quality of life.
Women with Low-Risk DCIS Eligible for the LORIS Trial After Complete Surgical Excision: How Low Is Their Risk After Standard Therapy?

Melissa Pilewskie, MD1, Cristina Oleese, BS1, Sujata Patil, PhD2, and Kimberly J. Van Zee, MS, MD1

1Breast Service, Department of Surgery, Memorial Sloan Kettering Cancer Center, New York, NY; 2Department of Epidemiology and Biostatistics, Memorial Sloan Kettering Cancer Center, New York, NY

Background. Identifying DCIS patients at low risk for disease progression could obviate need for standard therapy. The LORIS (surgery versus active monitoring for low-risk DCIS) trial is studying the safety of monitoring low-risk DCIS, although ipsilateral breast tumor recurrence (IBTR) rates in patients meeting enrollment criteria after complete surgical excision are unknown.

Methods. Women with pure DCIS treated with breast-conserving surgery (BCS) with/without radiation therapy (RT) from 1/1996–1/2011 were included from a prospectively maintained database. IBTR rates were compared between those who did and did not meet LORIS eligibility criteria (age ≥ 46 years, screen-detected calcifications, nipple discharge absence, minimal family history, non-high-grade DCIS) after complete surgical excision.

Results. A total of 2394 women were identified: 401 met LORIS criteria. Median follow-up was 5.9 years; 431 had ≥10 years follow-up. LORIS cohort median age was 61 years (range 46–86 years); 207 (52%) underwent RT, 79 (20%) received endocrine therapy. Of 401 patients, 24 experienced an IBTR. Overall 10-year IBTR rates were 10.3% (LORIS) versus 15.4% (non-LORIS) (p = 0.08): without RT, 12.1 versus 21.4%, respectively (p = 0.06). The 10-year invasive-IBTR rates for women meeting LORIS criteria were: 5.3% BCS overall, 6.0% without RT.

Conclusions. Women meeting LORIS criteria (after complete surgical excision) are at somewhat lower risk for IBTR. Among such women undergoing excision without RT, the 10-year invasive-IBTR rate was 6%. Given that approximately 20% of women with core biopsy-proven non-high-grade DCIS have invasive cancer at excision, women managed without excision would be expected to incur higher invasive cancer rates. Additional criteria are needed to identify women not requiring intervention for DCIS.
Androgen Receptor

Member of the nuclear hormone receptor family of ligand-activated transcription factors
Located on X chromosome at q11

Activated by binding of androgenic hormones (testosterone and dihydrotestosterone)

Eight exons encoding:
N terminus domain (NTD) [contains activation function domain AF-1, drives most activity]
DNA binding domain (DBD) [2 zinc fingers anchor AR to recognized sequences]
Hinge region (HR) [responsible for nucleo-cytoplasmic shuffling]
Ligand binding domain (LBD) [houses AF-2 domain]

Unliganded AR maintained in inactive complex by HSP-70 & HSP-90
Upon ligand binding HSPs dissociate from AR enabling translocation into nucleus
AR binds DNA
AR recruits coactivators and general transcription factors to alter transcription and translation of target genes.

Agonists recruit coactivators
Antagonists act by one of three mechanisms:
Recruit corepressors, prevent coactivators from associating with AR, retain AR in cytoplasm
RNA-Seq of Human Breast Ductal Carcinoma In Situ Models Reveals Aldehyde Dehydrogenase Isoform 5A1 as a Novel Potential Target

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¹ Department of Pharmacology, Wayne State University School of Medicine, Detroit, Michigan, United States of America, ² Center for Molecular Medicine and Genetics, Department of Obstetrics and Gynecology, Wayne State University School of Medicine, Detroit, Michigan, United States of America, ³ Barbara Ann Karmanos Cancer Institute, Wayne State University, Detroit, Michigan, United States of America

Abstract

Breast ductal carcinoma in situ (DCIS) is being found in great numbers of women due to the widespread use of mammography. To increase knowledge of DCIS, we determined the expression changes that are common among three DCIS models (MCF10.DCIS, SUM102 and SUM225) compared to the MCF10A model of non-tumorigenic mammary epithelial cells in three dimensional (3D) overlay culture with reconstituted basement membrane (rBM). Extracted mRNA was subjected to 76 cycles of deep sequencing (RNA-Seq) using Illumina Genome Analyzer GAIx. Analysis of RNA-Seq results showed 295 consistently differentially expressed transcripts in the DCIS models. These differentially expressed genes encode proteins that are associated with a number of signaling pathways such as integrin, fibroblast growth factor and TGFβ signaling, show association with cell-cell signaling, cell-cell adhesion and cell proliferation, and have a notable bias toward localization in the extracellular and plasma membrane compartments. RNA-Seq data was validated by quantitative real-time PCR of selected differentially expressed genes. Aldehyde dehydrogenase 5A1 (ALDH5A1), which is an enzyme that is involved in mitochondrial glutamate metabolism, was over-expressed in all three DCIS models at both the mRNA and protein levels. Disulfiram and valproic acid are known to inhibit ALDH5A1 and are safe for chronic use in humans for other disorders. Both of these drugs significantly inhibited net proliferation of the DCIS 3D rBM overlay models, but had minimal effect on MCF10A 3D rBM overlay models. These results suggest that ALDH5A1 may play an important role in DCIS and potentially serve as a novel molecular therapeutic target.
Mechanistic Theories for Myoepithelial Cell loss

1. Genetic changes in tumor epithelial cells may select for a clone with invasive properties that can escape from the duct, spread into the stroma, and subsequently expand. However, countering this hypothesis are studies demonstrating that the tumor epithelial cells do not undergo significant gene expression changes in the transition from DCIS to IDC.

2. Phenotypic changes in DCIS myoepithelial cells, together with accumulation of stromal inflammatory cells and myofibroblasts, lead to breakdown of the ducts and release of tumor epithelial cells into the surrounding stroma. Many of the genes specific for normal myoepithelial cells, such as CTK14, CTK17, OXTR, and EGFR, have been shown to be absent or dramatically down-regulated in the myoepithelial cells of DCIS lesions. Not only do the DCIS myoepithelial cells lack their normal tumor suppressive abilities, they may instead possess a reversed functions, with the ability to promote tumor progression.
In transition from normal epithelium to DCIS, cells undergo changes in miRNA expression and methylation patterns of various genes.

Genome-wide methylation screen approach:
Set of genes such as APC, CACNA1A, CDH1, FOXC1, HOXA10, MGMT, SFPR1, TFAP2A, and TWIST1 that exhibit differences in either frequency or density of methylation between DCIS and invasive carcinoma.
In agreement with previous studies, most of the aberrations in the epigenetic profile were observed already in the preinvasive stage.

Farazi et al profiled miRNAs from normal breast tissues, DCIS, invasive breast carcinomas, and 6 cell lines by Solexa sequencing and showed that normal breast samples were separated from most noninvasive DCIS and invasive carcinomas by increased miR-21 (the most abundant miRNA in carcinomas). The study confirmed that most miRNA changes in IDC were already apparent in DCIS samples.
Similarly, Volinia et al created miRNA profiles for normal breast, DCIS, and invasive carcinomas to study the global changes of the miRNA repertoire along the transition defining breast carcinoma progression. The miRNA profile established for the normal breast to DCIS transition was largely maintained in the in situ to IDC progression.
Androgen receptor expression in ductal carcinoma in situ of the breast: relation to oestrogen and progesterone receptors

A-G A Selim, G El-Ayat, C A Wells

Aims: Ductal carcinoma in situ (DCIS) of the breast has been diagnosed increasingly since the advent of mammographic screening. In contrast to the situation in invasive breast carcinoma, there are no reports on androgen receptor (AR) status in DCIS and few reports on oestrogen (ER) and progesterone (PR) receptors.

Methods: AR expression was examined in 57 cases of DCIS of the breast and correlated to the degree of differentiation and ER/PR status using immunohistochemical methods.

Results: AR positivity was noted in 19 of the cases, whereas the other 38 cases were negative. There was no significant association between AR expression and the degree of differentiation of DCIS; three of the 13 well differentiated DCIS cases, 10 of the 19 intermediately differentiated cases, and six of the 25 poorly differentiated cases were positive (p = 0.093). However, a strong association was shown between the expression of ER (p < 0.0001) and PR (p = 0.002) and the degree of differentiation of DCIS. In addition, no significant association was found between the expression of AR and the expression of ER (p = 0.26) or PR (p = 0.57) in DCIS of the breast.

Conclusions: A large number of cases of DCIS of the breast express AR and this may be associated with apocrine differentiation, which may impact on accurate typing of DCIS. Moreover, the expression of AR (but not ER or PR) in DCIS does not appear to be associated with the degree of differentiation.

Assessment

Nuclear staining was taken as positive, with cytoplasmic staining being ignored. The Quick Score method was used for semiquantitation of AR, ER, and PR status as follows:

1. Intensity of staining. Slides were assessed for the average degree of staining on low power (×10) and the following scores were allocated: weak (1), moderate (2), or strong (3).

2. The percentage of cells with positive nuclei was counted on high power (×40) and the following scores were allocated: < 25% (1), 25–< 50% (2), 50–< 75% (3), > 75% (4).

The scores from (1) and (2) were added together to give a final score ranging from 0 to 7, designated as negative or positive as follows: score of 0–3, negative; score of 4–7, positive.

Table 3: Association between AR expression and ER and PR expression in DCIS

<table>
<thead>
<tr>
<th></th>
<th>AR (+)</th>
<th>AR (–)</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>ER</td>
<td>8</td>
<td>23</td>
<td>0.26</td>
</tr>
<tr>
<td>PR</td>
<td>11</td>
<td>15</td>
<td>0.002</td>
</tr>
</tbody>
</table>

AR, androgen receptor; DCIS, ductal carcinoma in situ; ER, oestrogen receptor; PR, progesterone receptor.

Take home messages

- Many ductal carcinoma in situ (DCIS) cases are positive for the androgen receptor (AR) but negative for oestrogen (ER) and progesterone (PR) receptors
- There was no association between AR expression and the degree of differentiation in DCIS of the breast
- There was no association between AR expression and the expression of ER and PR in DCIS of the breast
Lack of AR expression was associated with HGIDC and with basal subtypes of HG-IDC, suggesting AR may play an important role in preventing the invasive transformation in this subgroup of breast carcinoma.

HG-IDC and HG-DCIS more frequently expressed AR than ER (55%-93% for AR and 18%-30% for ER) and were frequently AR+/ER− (63% for HG-DCIS and 39% for HG-IDC), which made AR a possible therapeutic target.

One third of HG-IDC was negative for AR, ER, PR, and HER-2, suggesting that further studies are needed to identify other key molecules for targeted therapy. We purpose that AR should be routinely measured for breast cancer.
Retrospective case series, 2005-2013.
221 non-consecutive women, mean age 60.1 years (25-91 years range).
All patients underwent definitive lumpectomy or mastectomy.
All patients with “pure” DCIS.

Exclusion criteria including:
Invasive or microinvasive carcinoma
Metastatic disease (lymph nodes or other sites)
Paget’s disease of skin or nipple
Neoadjuvant therapy
Intracystic papillary carcinomas
DCIS involving papillomas
The following were recorded:

Case numbers
Specimen type and size (ex. Mastectomy, 13 cm)
Patient demographics
DCIS grade
DCIS histologic subtypes
Presence of apocrine features
ER status

Case review performed by CDS / OO / LN, and a representative DCIS-rich block was selected for IHC.

AR IHC, Ventana Benchmark Ultra, Tucson, AZ, 4 micron sections
Mouse monoclonal AR441, Dako, Carpenteria, CA, prediluted 1:100, 32 minutes, no heat, VMS OptiView DAB detection kit

AR IHC slides interpreted by CDS & OO

Cases with ≥ 10% nuclear immunoreactivity scored as positive regardless of intensity
DCIS AR 10% + IHC

DCIS AR 30% + IHC

DCIS AR 70% + IHC

DCIS AR 90% + IHC
Normal duct H&E
Normal duct AR IHC
DCIS H&E

51F

DCIS AR 60% + IHC
Normal duct H&E

DCIS H&E

Normal duct AR IHC

DCIS AR 80% + IHC

66F
Results:
Mean age all patients 60 years, Mean age AR pos patients 62 years

AR reactivity present in non-neoplastic breast epithelium in 51% (113/221) of patients.
33% (72/221) of DCIS cases positive [THRESHOLD DEPENDENT].
AR positivity identified in all grades of DCIS.
Of 72 AR pos cases, 22 (31%) were ER neg, corresponding to 10% (22/221) of all patients.
Conclusions / Discussion

AR immunoreactivity at or above a 10% threshold can be identified in all grades of DCIS.

In our study, 10% (22/221) of resected DCIS patients were found to be ER negative and AR positive.

The majority of AR positive / ER negative DCIS cases were high grade (p <0.0001) with solid growth and apocrine features (p = 0.61).
For the Future

Could AR targeted chemopreventive endocrine therapy benefit DCIS patients with ER negative and AR positive disease?

AR ICH triage could be considered in resected, ER negative DCIS patients in the context of a clinical trial to assess proof of principle.
My pleasure to speak with you today!!!

Comments?
Critiques?
Insights?
Questions?
Feedback?
Cleveland Clinic

Every life deserves world class care.