Diagnosis and Treatment of Patients with early and advanced Breast Cancer

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Pathology

Pathology

- **Versions 2004–2018:**
  Blohmer / Costa / Fehm / Friedrichs / Huober / Kreipe / Lück / Schneeweiss/ Sinn / Thomssen / Schmidt
- **Version 2019:**
  Sinn / Maass

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Preanalytics: Fixation

	Oxford		
	LoE	GR	AGO
▪ Minimize time to fixation (cold ischemia time)	5	D	++
▪ Minimal fixation time of 6 hours for optimal antigen preservation	5	D	++
▪ Optimal fixation time 6 - 72 h for core biopsies	5	D	++
▪ Optimal fixation time for resection specimens: 12 - 72 h	5	D	++
▪ Use of neutral buffered formalin	5	D	++

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Use of Breast Cytology*

	Oxford		
	LoE	GR	AGO
▪ Nipple secretion	5	D	+
▪ Tumor	5	D	-
▪ Cyst	5	D	+/-
▪ Lymph node	5	D	+/-

* Ultrasound-guided core biopsy recommended

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Workup: Core Needle Biopsies (US-guided or stereotactic)

	Oxford		
	LoE	GR	AGO
▪ Routine workup in step sections (14G: 1–3 step sections / 11G, 8G: 6–8 step sections)	5	D	++
▪ Correlation with imaging (density, calcifications), use of B-classification	1b	B	++
▪ Frozen section diagnosis on core biopsies	5	D	--
▪ Routine evaluation of ER/PgR and HER2 status	3b	C	++
▪ Turn-around time < 24 h (histology)	5	D	+

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Workup: Breast-Conserving Specimens

	Oxford LoE	Oxford GR	AGO
▪ Slicing perpendicular to the longitudinal axis (or perpendicular to the nipple-peripheral axis in case of spherical specimens)	5	D	++
▪ Systematic sampling, at least 1 tissue block every 1 cm	5	D	++
▪ Inking of resection margins. Sampling of resection margins	5	D	++
▪ Documentation after slicing using specimen radiography, photo documentation or diagram	5	D	+

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Workup: Mastectomy Specimens

	Oxford		
	LoE	GR	AGO
▪ Margins always to be sampled ▪ Skin close to tumor ▪ Deep margin ▪ Other margins, if close (< 1 cm)	5	D	++
▪ Attention to soft tissue margins in skin sparing mastectomy	5	D	++
▪ Routine sampling of uninvolved quadrants, skin above tumor, and retroareolar region	5	D	++
▪ Systematic sampling in prophylactic mastectomies (BRCA-1/2 pos. patients)	5	D	++

Workup: Sentinel Node Biopsy

	Oxford LoE	Oxford GR	AGO
▪ **Full workup using step sections of ≤ 500 µm on paraffin embedded tissue**	5	D	++
▪ **Cytokeratin immunohistochemistry**			
▪ When suspicious, to detect micrometastases	2b	B	+
▪ For micrometastasis detection after NACT	2b	B	+
▪ As a routine procedure	5	D	+/-
▪ **Frozen section** (compromises paraffin histomorphology)			
▪ If clinical consequence	5	D	+/-
▪ If no clinical consequence from frozen section (e.g. cT1 or cT2 and cN0 and BCT)	5	D	-
▪ **Imprint cytology instead of, or in addition to frozen section**	3b	D	+/-
▪ **RT-PCR for epithelial genes**	4	D	-
▪ OSNA	3b	B	-

Workup: Intraoperative pathologic evaluation and frozen sections

	Oxford LoE	Oxford GR	AGO
▪ Sentinel node biopsy for invasive cancer (compromises final paraffin histomorphology)			
▪ If clinical consequence	5	D	+
▪ No clinical consequence	5	D	-
▪ Closest margin of resection			
▪ If macroscopically < 1 cm	5	D	+
▪ If macroscopically > 1 cm	5	D	-
▪ Lesions ≥ 1 cm, without core biopsy	5	D	+
▪ Non-palpable lesions or lesions < 1 cm	5	D	--
▪ Conservation of fresh tissue (tumor banking)	5	D	+

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Reporting: Histologic Tumor Type

Oxford		
LoE	GR	AGO
3a	C	++

- **Histologic tumor typing according to WHO-Classification, (4th ed., 2012)**
  - **Partial special differentiation:**
    > 50% NST component
    and < 50% special tumor type (minor component)
  - **Mixed differentiation:**
    > 50% special tumor type
    and < 50% NST component
    Example: mucinous breast cancer, mixed type
  - **Pure types:**
    > 90% special tumor type
    Examples: tubular or cribriform Ca.

Reporting: Grade of Malignancy

	Oxford		
	LoE	GR	AGO
▪ Use of Nottingham grading system (Elston & Ellis 1991) for all types of invasive breast cancer	5	D	++
▪ In case of very little tumor tissue, pure nuclear grading or additional criteria, such as Ki-67 proliferation fraction, may be used	5	D	++
▪ Grading of DCIS, e.g. according to WHO-Classification, (4th ed., 2012)	5	D	++
▪ Reporting of tumor grading in numeric form (e.g. G3)	5	D	++

Reporting: Tumor Size and Total Extent of Tumor

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	Oxford		
	LoE	GR	AGO
▪ Reporting of invasive tumor size taking into account macroscopic and histologic findings and clinical imaging results	5	D	++
▪ Additional reporting of total extent of invasive carcinoma in case of satellite nodules or multifocality	5	D	++
▪ Reporting of size of noninvasive component (DCIS or LCIS) when DCIS or LCIS component is extensive (more than 2x invasive Ca)	5	D	++

Reporting: pTNM

Oxford		
LoE	GR	AGO
5	D	++

- **Use of current UICC classification (8th ed.)**

  pT 1-3: Invasive tumor size (largest focus in case of multifocality or multicentricity)

  pT4: Invasion of dermis alone does not qualify as pT4. Criteria for pT4a/b/c/d must be met.

  pT4d: Negative skin biopsy does not rule out pT4d (inflammatory carcinoma).

  pM: pM1 indicates any non-regional disease, except 2nd primary contralateral. Use of MX is not recommended.

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Reporting: Margins of Resection and R-Classification

	Oxford LoE	GR	AGO
▪ Evaluation of distance to all resection margins macro-scopically and close margins histologically (< 1 cm)	5	D	++
▪ Reporting of minimal distance to resection margin and topography thereof	5	D	++
▪ R-Classification	5	D	++

R0: No residual tumor

R1: Microscopic invasive or noninvasive Carcinoma involving resection margin

RX: Presence of residual tumor cannot be assessed (e.g. tumor in multiple specimens)

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Reporting: Lymphovascular Invasion

- **L1: Lymphovascular invasion**
  L0: No lymphovascular invasion
- **IHC for evaluation of lymphovascular invasion**
- **Differentiation of peritumoral and extensive lymphovascular invasion**
- **Reporting of venous invasion (V0/V1) optional, prognostic significance not established**

Oxford		
LoE	GR	AGO
5	D	++
3b	C	-
3b	C	++
5	D	+

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Reporting: Evaluation of Tumor-Infiltrating Lymphocytes (TIL)

	Oxford		
	LoE	GR	AGO
▪ Identification of tumors with predominant lymphocytic infiltrate (> 50%) in tumor stroma (according to Salgado et al.*)	5	D	+/-

Consider only lymphocytic infiltrate in tumor stroma and not at the invasion front

Do not consider central fibrosis and necrotic areas

Report average of lymphocytic infiltrate as percentage

* Salgado, R., Denkert, C., Demaria, S., Sirtaine, N., Klauschen, F., Pruneri, G., et al. (2014). The evaluation of tumor-infiltrating lymphocytes (TILs) in breast cancer: recommendations by an International TILs Working Group 2014. Annals of Oncology

Reporting: Evaluation after Neoadjuvant Chemotherapy

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	Oxford		
	LoE	GR	AGO
▪ Identification of tumor bed, otherwise ypTX	4	D	++
▪ Reporting of tumor size as total extent of tumor bed area involved by infiltrates of residual vital invasive carcinoma	4	D	++
▪ pCR when absence of invasive Ca. and absence of angioinvasion or LN metastases. Presence of ypTis should be recorded	2b	D	+
▪ Use of IHC to identify tumor residues	4	D	+/-
▪ Reporting of ypTN after therapy	5	D	++
▪ Repeat IHC for ER, PgR, and HER2	5	D	+/-

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Special Studies: ER-Testing by IHC

- Immunohistochemical detection on paraffin embedded (FFPE) tissue
- Reporting percentage of pos. tumor nuclei (pos. if ≥ 10%, low pos. if ≥ 1%–9%)
- Only Allred Score (0–8) or Remmele Score (0–12)
- Re-evaluation on excision specimen if uncertain or triple-negative on core biopsy

Oxford		
LoE	GR	AGO
1a	A	++
1a	A	++
4	D	-
5	D	+

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Special Studies: PgR-Testing by IHC

	Oxford		
	LoE	GR	AGO
▪ Immunohistochemical detection on paraffin embedded (FFPE) tissue	1a	A	++
▪ Reporting percentage of pos. tumor nuclei (pos. if ≥ 10%)	1a	A	++
▪ Only Allred Score (0 - 8) or Remmele Score (0 - 12)	4	D	-

Low ER+ (1–10%)

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Sanford AS et al. Cancer 2015	High Incidence of Germline BRCA Mutation in Patients with ER Low-Positive/PR Low-Positive/HER-2 neu Negative Tumors	314 Pat. 1–9% ER, Anteil BRCA mutierter Fälle wie bei ER -
Deyarmin B et al. Ann Surg Oncol (2013) 20:87–93	Effect of ASCO/CAP Guidelines for Determining ER Status on Molecular Subtype	26 Pat. 1–9% ER, Genexpression eher wie TN oder HER2 enr
Prabhu YS et al. 2014; J Cancer 5(2): 156–165.	A Majority of Low (1–10%) ER Positive Breast Cancers Behave Like Hormone Receptor Negative Tumors	21 Pat. 1–9% ER, Genexpression wie ER-, Überleben < ER+
Yi et al. Annals Oncol. 2014	Which threshold for ER positivity? a retrospective study based on 9639 patients	251 Pat. 1–9% ER Überleben = ER-

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Additional Special Studies: Molecular Analysis of ER/PgR Status

	Oxford		
	LoE	GR	AGO
▪ Evaluation of hormone receptors using validated gene expression test kits	3b	A	+/-
▪ Evaluation of hormone receptor by RNA-quantification	5	D	-
▪ Use of molecular receptor analysis for subtyping	3b	A	+

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HER2-Analysis by IHC

	Oxford LoE	Oxford GR	AGO
▪ Reporting of immunohistochemistry (IHC):	1a	A	++
▪ 3+ staining pattern: HER2+ if strong complete circular membrane staining of > 10% invasive cells			
▪ 2+ staining pattern: If > 10% circular but moderate/weak membrane staining or ≤ 10% strong staining, U-shaped staining in micropapillary carcinoma: ISH required (CISH, SISH, FISH)	1a	A	++

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HER2-Analysis by ISH when IHC 2+

	Oxford LoE	Oxford GR	Oxford AGO
▪ **Single-Color In-Situ-Hybridisation (ISH):**	3a	C	++
▪ HER2+ if signal counts ≥ 6 in at least 20 cohesive cells			
▪ negative if signal counts < 4 signals/nucleus			
▪ 2-Color ISH recommended for ≥ 4 and < 6 signals/nucleus			
▪ **Two-Color In-Situ-Hybridisation (ISH):**	3a	D	++
▪ Group 1: Ratio ≥ 2.0 and signals/nucleus ≥ 4.0 -> HER2+			
▪ Group 2: Ratio ≥ 2.0 and signals/nucleus < 4.0 -> HER2- (no benefit of anti-HER2 therapy)			
▪ Group 3: Ratio < 2.0 and signals/nucleus ≥ 6.0 -> HER2+ (but benefit of anti-HER2 therapy not certain)			
▪ Group 4: Ratio < 2.0 and signals/nucleus ≥ 4.0 und < 6 -> HER2- (no benefit of anti-HER2 therapy)			
▪ Group 5: Ratio < 2.0 und signals/nucleus < 4.0 -> HER2-			

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HER2 testing by validated dual-probe ISH assay when IHC = 2+

Batch controls and on-slide controls show appropriate hybridization

HER2/CEP17 ratio ≥ 2.0

- **Group 1**
  Average *HER2* copy number ≥ 4.0 signals/cell
  → HER2 positive
- **Group 2**
  Average *HER2* copy number < 4.0 signals/cell
  → mostly → HER2 negative

HER2/CEP17 ratio < 2.0

- **Group 3**
  Average *HER2* copy number ≥ 6.0 signals/cell
  → mostly → HER2 positive
- **Group 4**
  Average *HER2* copy number ≥ 4.0 - < 6.0 signals/cell
  → mostly → HER2 negative
- **Group 5**
  Average *HER2* copy number <4.0 signals/cell
  → HER2 negative

HER2 Testing on Core Biopsies

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False positive immunohistochemical labeling may occur in core biopsies. Therefore, methods of individual laboratories should be validated by comparison of core biopsies and resection specimens. Background staining should be evaluated by comparison with normal duct epithelium.

Alternatively, all G1 and G2 cases with HER2 3+ in core biopsies may be analyzed by ISH or may be re-evaluated in the resection specimen.
False positivity is likely when HER+ was reported in G1 tumors of the following types: Infiltrating ductal or lobular carcinoma, ER and PgR positive, Tubular (at least 90% pure), Mucinous (at least 90% pure) Cribriform (at least 90% pure), Adenoid cystic carcinoma (90% pure).

In case of discrepancy between core biopsy and specimen, the HER2 overexpressing sample should be re-evaluated by a different method. If still discrepancy – anti-HER2-treatment if amplified in one of both samples. Expected rate of HER2-overexpression: 15% HER2 positive

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Additional Special Studies: Molecular Analysis of HER2 Status

	Oxford		
	LoE	GR	AGO
▪ Therapy decisions should be based on IHC and ISH only	1a	A	++
▪ Evaluation of HER2 using validated gene expression test kits	3b	B	-
▪ Evaluation of HER2-amplification by RNA-sequencing	5	D	-
▪ Use of molecular HER2-testing for subtyping	3b	B	+/-

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Special Studies: Evaluation of Ki-67 Score

	Oxford		
	LoE	GR	AGO
▪ Counting of tumor nuclei at the invasion front	5	D	++
▪ Semiquantitative eyeballing or counting of labelled cells in core needle biopsies	2	A	++
▪ Consideration of weakly stained tumor nuclei	5	D	++
▪ Reporting of Ki-67 positive nuclei as percentage	5	D	++
▪ Establishing of laboratory standards and cut-off values	5	D	++
▪ Use of image analysis for objective Ki-67 evaluation	5	D	+

Intrinsic Breast Cancer Types (Molecular and Immunohistochemical Definitions)

- **Currently there are no generally accepted and proven translation of molecularly defined types (basal, luminal A/B-Typ, HER2) into immunohistochemical counterparts neither with regard to markers nor to thresholds**
- **In terms of practical consequences, re-labeling of clinically established and immunohistochemically defined subgroups might be useful (ER/PR+ for luminal, HER2+ for HER2-type, triple negative for basal type)**
- **The basal type shows an 80% overlap with the triple negative subgroup of ductal invasive breast cancer (ER < 1% & PgR < 1% & HER2 0/1+2+ (non-amplified, ratio < 2))**
- **None of the available markers (Ki-67, grading, recurrence score etc.) can reliably discriminate between luminal A and luminal B type**
- **Although derived from RNA expression studies, RNA measurements are not suited for the definition of intrinsic types for purposes of therapy**

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Prädictive PD-L1 Assay

	Oxford		
	LoE	GR	AGO
▪ **Immunhistochemical assay**	2b	C	
▪ **Prediction of atezolizumab efficacy in triple-negative metastatic breast cancer**			
▪ **Suitable for punch biopsies and resected specimens**			
▪ **Ventana Antibody SP142 with Positive Control (tonsil)**			
▪ **§Cytoplasmic staining of at least 1% of the leucocyte stromal infiltrate (lymphocytes, macrophages, plasma cells, granulocytes outside of abscesses)**			
▪ **No evaluation of tumor staining**			
▪ **Quality assurance**			
▪ **Obligate participation in further education and training measures**			
▪ **Reference pathology in case of not yet completed qualification**	5	D	++

Quality Assurance: Immunohistochemistry

- **Use of automated staining platform**
- **Participation in ring trials**
- **Strict adherence and monitoring of requirements of preanalytics (fixation)**
- **Use of on-slide controls**
- **Plausibility controls (e.g. tumor type, grading)**

Quality Assurance: HER2-Status

- **Continuous documentation of HER2 tests**
- **Quality goal: Rate of HER2-positivity: 15%**
- **Use of standardised and validated HER2 test kits**
- **Participation in ring trials**

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Quality Assurance: Reporting

- **Responsibility of one or two pathologists with special expertise in breast pathology**
- **Regular interdisciplinary conferences with radiologic-pathologic correlation**
- **Participation in quality circles**