translational research との融合からさらに精度の高い個別化治療の実現をめざしたい。

術前薬物療法後の手術では，その切除範囲やセンチネルリンパ節生検の是非に関してもいろいろな議論が行われているのが現状である。正確な病巣切除には，約6か月にわたる術前治療の間，治療医による適切なモニタリングが必須であろうし，治療後の画像診断には更なる進展を期待したいところである。

4．分子生物学的マーカーを用いたリスク予測

従前の病理学的因子だけでなく，分子生物学的マーカーによるリスク分類の進歩が見られるが，その代表的なものは Oncotype DX と Mamma Print であろう。Oncotype DX は n0，ER 陽性乳癌を対象とし，21 遺伝子の発現パターンから再発スコア（RS）が計算され，化学療法不要群と必須群を見極める。欧米では実用化が始まり，わが国でも希望があれば自費で検査可能ではあるが，高額である。Oncotype DX は，TailoRx Trial，Mamma Print は Mindact Trial として各々臨床応用への検証が，大規模臨床試験で進行中である。

5．外来化学療法システムとチーム医療，副作用マネージメント

近年の薬物療法が進歩した背景には，副作用マネージメントの工夫が進んだことや看護師や薬剤師とのチーム医療が推進したことも忘れてはいけない。癌専門病院の多くは，外来通院型システムを構築し，快適かつ安全な治療環境を整えるべく，設備や人的パワーの投資を行っている。悪心・嘔吐については，ステロイドの適正使用やセロトニン拮抗剤により，かなり改善した。白血球減少に伴う発熱（Febrile neutropenia）は，その多くがシプロキサン内服で対応可能なことを経験し，クール途中での採血検査の省略により，患者負担が減り，さらに予防的 G-CSF 投与の減少により医療経済学的にも改善が進んだ。脱毛については残念ながら得策はまだないが，特に周術期薬物療法の意義を患者が十分に理解することで，脱毛を理由に最適な化学療法を拒否することは以前に比べると激減した。その背景には，薬物療法の必要性を医師のみでなく，医療関係者がチームとして理解し，患者に啓発すること，さらに患者間での正確な情報交換が進んでいることなどが考えられる。

ま と め

乳癌治療に関する考え方はここ10年で大きな変革を遂げ，乳癌を一律に治療する時代は終わった。ER や HER2 を target にする内分泌療法，Herceptin 治療は，個別化治療そのものともいえるが，各薬剤の効果予測因子の探求など Translational Research の益々の進歩，さらには宿主側の薬物代謝の個人差にも注目しつつ，乳癌治療はまさしく「個別化治療」をいかに展開するかという大きな流れの中にある。「乳癌と診断，即，手術」という時代も終焉を迎えそうである。乳癌の診断時には，その病理診断から癌の特性を把握し，色々な治療 modality の意義を理解し，総合的に治療戦略を立てることが重要である。非浸潤癌には原則手術が先行であろう。浸潤癌では，そのタイプに応じて適切な薬物療法が術前に施行され，その効果を評価し，局所療法後の薬物療法が決定されるであろう。夢を追いつつも，今できる「個別化治療」とは何かを考えながら，診断および治療のコーディネーター役として「乳腺専門医」がこの大きな変革の流れに乗って行きたいと考える。

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乳がん治療の現状と展望 －個別化治療をめざして

診断の指針 治療の指針 *Current status and future of breast cancer treatment*

増田 慎三
MASUDA Norikazu

乳癌治療はここ10数年で大きく変遷した．手術は胸筋温存乳房切除から乳房温存手術へ，さらに腋窩リンパ節郭清もセンチネルリンパ節概念の導入により，腋窩温化が図られている．その背景には，浸潤癌は比較的早期の段階から全身転移を起こしやすい性格，つまり全身病の概念が浸透したこと，薬物療法の進歩がある．薬物療法は，内分泌療法，化学療法，分子標的治療に大別されるが，各分野で新薬の開発が進み，海外の大規模臨床試験結果(エビデンス)の導入などにより，治療成績の向上とQOLにも考慮した治療戦略が構築される．近年は「個別化治療」の実地臨床への導入をめざし，分子生物学を基盤とするTranslational researchが注目されている．

1. 手術＝局所療法における個別化治療

乳管内にとどまる非浸潤癌は，確実な切除で100％の救命が保証されることから，局所コントロールがポイントである．一方，全身病の性格を有する浸潤癌の場合は，より薬物療法のウエイトが増す．つまり，診断の際に，非浸潤癌か浸潤癌かの判別が，個別化治療の第1歩である．画像診断で判別可能な場合もあるが，針生検による組織診断が要求されることもある．非浸潤癌の場合，乳房温存手術で断端陰性で完全切除ができればいいが，ある程度の広がりがある時は，温存術を無理に強行せず，乳腺全摘出と乳房再建術のオプションも提示したい．今後，浸潤癌の場合は，術前薬物療法や放射線治療により，その有効例では手術省略の選択も期待される．

腋窩手術にも，センチネルリンパ節の評価による個別化治療が導入された．センチネルリンパ節で転移陰性であれば腋窩郭清省略が実地臨床で広まった．さらにセンチネル陽性であっても，郭清を縮小化する流れもあり，腋窩コントロールに関しては薬物療法の進歩ゆえに今後ますます混沌とすることが予想される．

2. 乳癌の薬物療法

手術や放射線治療による局所コントロールも大切ではあるが，まさしく薬物療法が乳癌治療の中心を担い，その進歩が欧米の乳癌死の減少をもたらしているといっても過言ではない．薬物療法の決定には，従来，リンパ節転移の有無などから評価される「再発リスク分類」に基づく考え方が主流であった．一方，ホルモン感受性(ER)とHER2 statusによるTarget therapyの適応をまず考えること，さらに化学療法についてはその感受性を考慮した決定が，最近は望まれている．

1)内分泌療法

女性ホルモン環境により，閉経前と後では薬剤選択が異なる．閉経前では，卵巣機能抑制(LH-RH analog)とERブロック(Tamoxifen：TAM)が標準である．閉経後では，Aromatase阻害剤(AIs)の開発により，従来の標準とされたTAMから，AIsへシフトした．アリミデックス，フェマーラ，アロマシンの3剤が使用される．これらの至適投与期間は，現在5年間とされるが，ホルモン感受性乳癌の年次別再発リスクと考慮すると，術後2～3年にピークはあるものの，5年以降も一定のリスクが継続することから，内分泌療法の期間は10年の長期を考慮した治療プランが重要となってきた．とくに閉経後の場合，AIsを単に10年にするのか，TAMをいかにその10年の中に組み入れるか，骨や心血管系への影響も考慮しつつ，解決する必要がある．

2)化学療法

基本はAnthracycline系(A)とTaxan系(T)の2本柱である．CMFとの比較から優位性が証明され，A系レジメ(AC/CE/FEC/CAFなど)が標準である．T系薬剤(Paclitaxel：P，Docetaxel：D)の開発により，再発リスクの高い場合はA系との逐次併用が勧められる．一方A系の晩期毒性として心毒性は重要な点で，A系を回避できる症例群の選別が課題である．HER2陽性乳癌はA系感受性が高く，topoⅡ増幅の有無が

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Key words 乳癌 個別化治療 術前薬物療法 分子標的治療 Translational research

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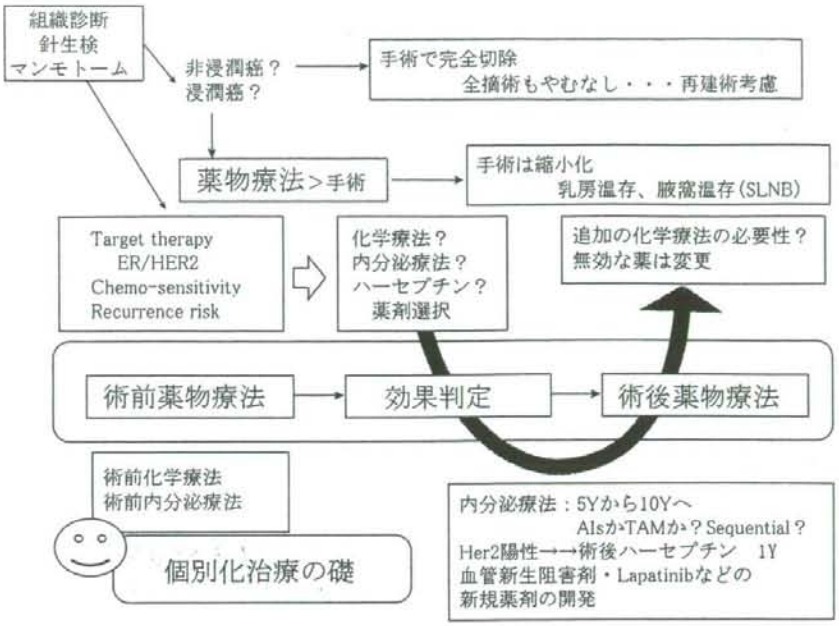

図1 乳癌治療の現状と展望

A系選択の個別化につながるとの報告もあり今後期待される．4コースの比較でDocetaxel＋Cyclophosphamide(DC)がACに勝るとの報告があるが，今後Tベースでどのようなレジメを選択するのが良いか，その解決は急務である．T系の標準投与法に関しては，Dはq3weeks，Pはweekly投与が有効である．他にナベルビン，Xeloda，TS-1，カンプトなどの薬剤が使用可能で，今後も新規抗がん剤として，Ixabepilone，Gemcitabine，Vinflinine，Abraxane(Nab-paclitaxel)などが治験進行中もしくは計画中である．

3)分子標的治療

HER2受容体に対するTrastuzumab(Herceptin：H)は，HER2陽性乳癌の予後を大きく改善し，また乳癌薬物療法に大きな変革を起こした．われわれは術前化学療法の経験から，HER2陽性乳癌はAおよびT系抗がん剤の感受性が高くpCR(癌の完全消失)が得やすいこと，さらにそのTにHを上乗せすることでより高いpCRが期待されることを学んだ．HERA studyなどの大規模臨床試験結果から，周術期における1年間のH上乗せ効果も証明され，今秋には保険認可される予定である．術前の薬物療法で約60～70％の症例でpCRが得られることから，今後の画像診断などの進歩により，手術省略可能例が選別されたい．予後の延長により，HER2乳癌では脳転移へのマネージメントが重要になってきた．

HER2関連をtargetにした分子標的薬の開発も，Lapatinib，Pertuzumabなど進行中である．LapatinibはXelodaとの併用で今春FDAにも認可され，現在最も臨床応用に近い．低分子ゆえに脳転移にも効果が期待される．世界的には周術期においてHとの比較試験が進行中であり，今後，HER2陽性乳癌におけるHerceptin，Lapatinibの位置づけが検討される．

従来予後不良因子とされたHER2発現が，これらの薬剤の開発により，現在のところ最も薬物療法の効果が得られやすいマーカーといっても過言でない．また単にHの選択基準のみならず，A系抗がん剤の選択基準としての意義も深く，この発現を正確に診断することは個別化治療には必須である．

血管新生阻害剤のBevacizumabも注目される．Weekly Pとの併用で有効性が証明された．Bevacizumabの標的は癌の進展に比較的早い段階で関与するVEGFであることから，周術期での臨床応用は期待大である．血管新生阻害剤としてSunitinib，Aixitinibなど多くの薬剤が開発競争されている．

3．術後の薬物療法から術前の薬物療法へ

手術で切除した癌組織の性格診断を元に，術後，再発抑制を目的に薬物療法を決定するのが一般的である．St. Gallenリスク分類，NCCNガイドライン，Adjuvant！Onlineなどが参考になる．しかし，最近，乳房温存やpCR，薬剤感受性把握などを目的に術前化学療法の概念が広まってきた．FEC→Taxanが基

本レジメで，化学療法の効果が期待されやすい症例とそうでない群が徐々に解明され，まさに効果を確認しながら個々にあったよりよい治療法を模索していく礎となるのが術前薬物療法である．同時に gene profiling などの translational research との融合からより精度の高い個別化治療の実現が期待される．

今後は術前内分泌療法の展開も考えられる．術後5年から10年治療の必要な内分泌療法剤もその効果を把握して内服したいものであり，個々の薬剤感受性を知る意味でも，またその治療効果により，術後の化学療法の必要性を検討することも可能であろう．

針生検やマンモトーム生検による癌の性格診断（組織型や悪性度，ER，HER2など）に基づき，術前薬物療法レジメが選定され，その治療効果に応じて，術後の薬物療法レジメが考慮されるという治療体系の構築が，まさしく今すぐできる個別化治療への挑戦である．

4. Translational Research（TR）との融合

従前の病理学的因子だけでなく，分子生物学的マーカーによるリスク分類の進歩が見られるが，その代表的なのは Oncotype DX と MammaPrint であろう．Oncotype DX は n0，ER 陽性乳癌を対象とし，21遺伝子の発現パターンから再発スコア（RS）が計算され，化学療法不要群を見極める．Mamma Print も同様に70遺伝子の発現パターン解析から，再発リスクを予想する．Mindact Trial でそのリスクに応じて，化学療法と内分泌療法の必要性を検証するとともに，その適正レジメも比較検討するデザインで臨床試験が進んでいる．

ま と め

乳癌を一律に治療する時代は終わった．ER による内分泌療法そのものが個別化治療でもあるが，HER2 などの target に対する分子標的薬の開発，各抗がん剤の効果予測因子の探求など TR のますますの進歩により，乳がん治療はまさしく「個別化治療」をいかに展開するかという大きな流れの中にある．「乳癌と確定，即，手術」という時代も終焉を迎えそうである．色々な治療 modality の意義を理解し，癌の性格診断を元に，総合的に治療戦略を立てることが重要で，それは「乳腺専門医」にのみ，成し得る技である．

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CONFERENCE PAPER

Presidential symposium: Individualized diagnosis for tailored treatment of breast cancer

Individualization of breast cancer based on histopathological features and molecular alterations

Hitoshi Tsuda

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Abstract Histopathological findings and molecular alterations well reflect the biological properties of individual primary breast carcinomas. Specifically, pT (size of the invasive component), pN (number of metastatic lymph nodes), histological or nuclear grade, lymphovascular invasion, hormone receptors, and *HER2* (c-erbB-2) gene overexpresison or amplification are known to be effective markers for assessing the risk of operable primary breast carcinoma, albeit incompletely. It is expected that additional molecular markers and novel diagnostic tools will be developed in the future to facilitate a more accurate characterization of higher risk node-negative breast carcinomas.

Keywords Basal-like type · Grade · Lymph node metastasis · Prognostic factor · Tumor size

Abbreviations

CGH	Comparative genomic hybridization
CK	Cytokeratin
DCIS	Ductal carcinoma in situ
EGFR	Epidermal growth factor receptor
ER	Estrogen receptor
HE	Hematoxylin and eosin
IHC	Immunohistochemistry
ITC	Isolated tumor cells
LCIS	Lobular carcinoma in situ
ly	Lymphatic invasion
NCCN	National Comprehensive Cancer Network
pCR	Pathological complete response
PgR	Progesterone receptor
PST	Primary systemic therapies
SLN	Sentinel lymph node
SNNS	Sentinel lymph node navigation surgery
v	Vascular invasion

This article is based on a presentation delivered at Presidential Symposium 1, "Breast cancer: individualized diagnosis for tailored treatment," held on 29 June 2007 at the 15th Annual Meeting of the Japanese Breast Cancer Society in Yokohama.

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Introduction

Breast cancers show considerable variation in terms of their histological features and molecular alterations, and such factors are known to influence patient outcome and tumor clinical behavior. Prognostic factors of breast cancer can be largely categorized into factors related to (1) the extent of (macro- and microscopically visible) tumor spread, (2) biological properties of the cancer cells, and (3) host–tumor relationship (Fig. 1). Factors related to the extent of tumor spread include clinical stage, size of the invasive cancer component, and the status of regional lymph node metastasis and distant metastasis. Those related to the biological properties of cancer cells, which account for differences in prognosis among patients with the same extent of tumor spread, are histological or nuclear grade of the cancer cells, lymphovascular permeation, and *HER2* (HER2/*neu*, c-erbB-2) overexpression and/or gene amplification. Hormone receptor status is used mainly to identify patients eligible for preoperative or postoperative endocrine therapies, but estrogen receptor (ER) and progesterone receptor (PgR) status is also prognostically

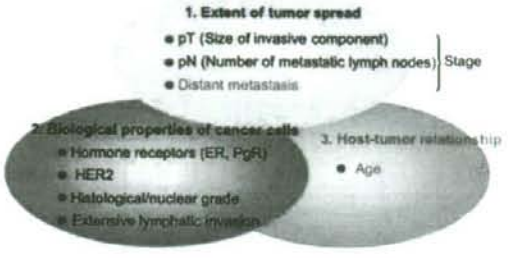

Fig. 1 Prognostic factors of breast cancer. These can be largely categorized into factors of: *1* the extent of tumor spread, *2* biological properties if cancer cells, 3 host-tumor relationship

independent of the extent of tumor spread. Factors related to the host–tumor relationship are patient age and immune status, although these are not well characterized.

In patients with operable breast cancer, these factors are now used routinely for evaluating the prognosis and predicting the response of cancer cells to specific therapeutic drugs. At the St Gallen International Conference in 2007, the use of adjuvant chemotherapies was recommended based on a combination of the above-mentioned factors (Tables 1, 2) [1]. There is a consensus that node-metastasis-positive breast cancers should be treated with systemic adjuvant chemotherapy. In node-metastasis-negative (pN0) breast cancer, risk estimation is based on a combination of age, hormone receptors, size of the invasive tumor component, grade, lymphovascular invasion, and *HER2* expression [1–3]. In the National Comprehensive Cancer Network (NCCN) guidelines, the same factors are utilized for evaluating the risk of primary breast cancer [3].

This article provides an overview of prognostic and predictive factors that are used routinely for designing individual therapies for patients with breast cancer. Because the present classifications of pN0 breast cancers into groups of intermediate and low risk are still insufficient, we also review potentially useful biomarkers or tests that allow more a precise prognostication.

Size of invasive component of primary tumor (pT factor)

In the *TNM Classification of malignant tumours*, 6th edn [4], the size of an invasive primary tumor is classified into pT0, pTis, pT1, pT2, pT3, and pT4 (pT = primary tumor; Table 3). pTis is non-invasive carcinoma and is usually stage 0. According to the histological classification listed in the *General rules for clinical and pathological recording of breast cancer*, 15th edn [5] (abbreviated as "general rules" hereafter), breast carcinomas are classified into 17 histological types, two types of non-invasive carcinoma, three common types of invasive ductal carcinoma, and 11 special types, including Paget's disease. Among these, non-invasive carcinomas and several histological types are of clinical significance. Non-invasive carcinomas, such as non-invasive ductal carcinoma, or ductal carcinoma in situ (DCIS), non-invasive lobular carcinoma or lobular carcinoma in situ (LCIS), and Paget's disease without invasion, are important because the prognosis of patients with these types of breast cancer is excellent. Among the special types, mucinous carcinoma, medullary carcinoma, adenoid cystic carcinoma, and tubular carcinoma are known to show a good clinical outcome.

pT1 is subdivided into pT1mic, pT1a, pT1b, and pT1c when the diameter of the invasive component of the primary tumor is $\leq$ 0.1 cm, >0.1–0.5 cm, >0.5–1.0 cm, and >1.0–2.0 cm, respectively. In the NCCN guidelines, the risk of recurrence is estimated to differ among pT1a, pT1b, and pT1c [3]. In the St Gallen meeting consensus, the size of the invasive tumor is classified into categories of $\leq$ 2.0 and >2.0 cm, although some panel members consider pT1a and pT1b (i.e., pT $\leq$1 cm) tumors that are node-negative to

Table 1 Definition of risk categories for patients with operable breast cancer (reproduced from [1] with modifications)

Risk category	Parameters
Low risk	Node negative AND all of the following features:
	pT $\leq$2 cm, and Grade 1, AND
	Absence of extensive peritumoral vascular invasion, AND
	ER and/or PgR expression, AND
	HER2 gene neither overexpressed nor amplified, AND
	Age $\geq$35 years
Intermediate risk	Node negative AND at least one of the following features:
	pT >2 cm, or Grade 2–3, OR
	Presence of extensive peritumoral vascular invasion, OR
	ER and PgR absent, OR
	HER2 gene overexpressed or amplified, OR
	Age <35 years
	Node positive (1–3 involved nodes) AND
	ER and/or PgR expressed, AND
	HER2 gene neither overexpressed nor amplified
High risk	Node positive (one to three involved nodes) AND
	ER and PgR absent, OR
	HER2 gene overexpressed or amplified
	Node positive (four or more involved nodes)

pT Primary tumor, *PgR* progesterone receptor, *ER* estrogen receptor

Table 2 Choice of treatment modalities 2007 (reproduced from [1] with modifications)

HER2 (c-erbB-2) gene	Highly endocrine responsive	Incompletely endocrine responsive	Endocrine non-responsive
Negative	ET (considering CT according to risk)	ET (considering CT according to risk)	CT
Positive	ET + trastuzumab + CT	ET + trastuzumab + CT	Trastuzumab + CT

ET Endocrine therapy, *CT* chemotherapy

Table 3 The pTNM pathological classification (Reproduced from [4] with modifications)

The pTNM pathological classification system in terms of the primary tomor	Subcategories of the pTNM classification system in terms of the primary tumor
pTX	Primary tumor cannot be assessed
pT0	No evidence of primary tumor
pTis	Carcinoma in situ
	pTis(DCIS): ductal carcinoma in situ (Non-invasive ductal carcinoma)
	pTis(LCIS): lobular carcinoma in situ
	pTis(Paget): Paget disease of the nipple with no tumor[a]
pT1	Tumor 2 cm or less in greatest dimension
	pT1mic: microinvasion 0.1 cm or less in greatest dimension
	pT1a: more than 0.1 cm but not more than 0.5 cm in greatest dimension
	pT1b: more than 0.5 cm but not more than 1 cm in greatest dimension
	pT1c: more than 1 cm but not more than 2 cm in greatest dimension
pT2	Tumor more than 2 cm but not more than 5 cm in greatest dimension
pT3	Tumor more than 5 cm in greatest dimension
pT4	Tumor of any size with direct extension to chest wall or skin only as described in T4a to T4d (chest wall includes ribs, intercostals muscles, and serratus anterior muscle but not pectoral muscle)
	pT4a: extension to chest wall
	pT4b: edema (including peau d'orange), or ulceration of the skin of the breast, or satellite skin nodules confined to the same breast
	pT4c: both 4a and 4b above
	pT4d: inflammatory carcinoma

DCIS Ductal carcinoma in situ, *LCIS* non-invasive lobular carcinoma or lobular carcinoma in situ

[a] Paget disease associated with a tumor is classified according to the size of the tumor

represent a low risk even if they are of higher grade and/or affect younger patients [1, 2].

For the evaluation of the pT factor, it is important to differentiate accurately the invasive component from the non-invasive component (Fig. 2). If there are multiple invasive tumors in a breast, the diameter of the largest invasive tumor should be adopted, and the diameters of multiple invasive tumors should not be added.

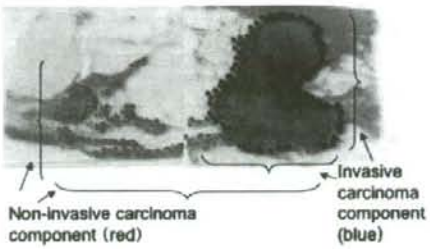

Fig. 2 Measurement of the size of the invasive component in primary breast carcinoma as determined on a stained section of breast tissue. The tumor indicated by *blue dots* is the invasive carcinoma component, and the part indicated by *red dots* is the non-invasive carcinoma component. For risk estimation, the measurement of the invasive component is necessary. H&E stain. ×1

Although the pT does not appear to have been measured very accurately, this measurement is important not only for risk estimation, but also for (1) determining the indication for primary systemic therapies (PST) based on core needle biopsy specimens, (2) assessing the therapeutic response, especially the pathological complete response (pCR), of primary tumors to primary systemic therapies based on an examination of surgically resected specimens, and (3) evaluating HER2 overexpression or gene amplification that is restricted to the invasive component.

Axillary lymph node status (pN factor)

The status of the axillary lymph nodes is the most powerful prognostic indicator in operable primary breast cancer. The outcome of patients becomes worse as the number of lymph nodes with metastasis increases [6]. The status of axillary lymph nodes has recently been classified by histopathological examination into pN0, pN1, pN2, and pN3 in the TNM classification [4]. Currently, parasternal lymph node dissection is usually not performed during breast surgery in Japan. In terms of axillary lymph node status only, pN0, pN1, pN2, and pN3 are defined as no metastasis,

Table 4 The pTNM pathological classification (Reproduced from [4] with modifications)

The pTNM pathological classification system in terms of the regional lymph nodes	Subcategories of the pTNM classification system in terms of the regional lymph nodes
pNX	Regional lymph nodes cannot be assessed (not removed for study or previously removed)
pN0	No regional lymph node metastasis[a]
pNmi	Micrometastasis (larger than 0.2 mm, but none larger than 2 mm in greatest dimension)
pN1	Metastasis in one to three ipsilateral axillary lymph node(s), and/or in ipsilateral internal mammary nodes with microscopic metastasis detected by sentinel lymph node dissection but not clinically apparent.
	pN1a: metastasis in one to three axillary lymph node(s), including at least one larger than 2 mm in greatest dimension
	pN1b: internal mammary lymph nodes with microscopic metastasis detected by sentinel lymph node dissection but not clinically apparent
	pN1c: metastasis in one to three axillary lymph nodes and internal mammary lymph nodes with microscopic metastasis detected by sentinel lymph node dissection but not clinically apparent
pN2	Metastasis in four to nine ipsilateral axillary lymph nodes, or in clinically apparent ipsilateral internal mammary lymph node(s) in the absence of axillary lymph node metastasis
pN3	Metastasis in ten or more ipsilateral axillary lymph nodes; or in ipsilateral infraclavicular lymph nodes; or in clinically apparent ipsilateral internal mammary lymph node(s) in the presence of one or more positive axillary lymph nodes; or in more than three axillary lymph nodes and in internal mammary lymph nodes with clinically negative, microscopic metastasis in internal mammary lymph nodes; or in ipsilateral supraclavicular lymph nodes

pN Regional lymph nodes

[a] Cases with only isolated tumor cells (ITCs) in regional lymph nodes are classified as pN0. ITCs are single tumor cells or small clusters of cells, not more than 0.2 mm in the greatest dimension, which are usually detected by immunohistochemistry or molecular methods but which may be verified by hematoxylin and eosin (HE) staining. ITCs do not typically show evidence of metastatic activity, e.g., proliferation or stromal reaction

metastasis to one to three lymph nodes, metastasis to four to nine lymph nodes, and metastasis to ten or more lymph nodes or to subclavicular lymph nodes, respectively (Table 4) [4].

Sentinel lymph node navigation surgery (SNNS) has become widely used for the surgical treatment of patients with early breast cancer [7]. In Japan, sentinel lymph nodes (SLNs) are identified by radioisotope-labeled colloid and/or dye, and they can be examined by intraoperative histopathology for the presence of metastasis. Axillary lymph node dissection is also carried out if metastasis is detected in SLNs, but it is not used if metastasis is absent.

Based on the diameter of the largest metastatic focus, pN0 and pN1 are sub-classified into pN0, pN0(i+), pN1 mi, and pN1a. pN0(i+) is defined as the presence of isolated tumor cells (ITC), which are tumor cell clusters with a diameter of $\leq$0.2 mm (Fig. 3). If the diameter of a metastatic tumor focus is >0.2–2.0 mm, the case is defined as pN1 mi, i.e., micrometastasis. pN1a is defined as metastasis to one to three lymph nodes with at least one node measuring >2.0 mm in diameter. Detailed cutting of SLNs (at 2-mm intervals) and accurate measurements of metastatic foci are needed for reliable SNNS [4, 7].

In patients with pN1a, the presence of HER2 overexpression/amplification is a feature of the high-risk group, whereas cases of pN1a without HER2 overexpression/amplification are classified as being of intermediate risk. All pN2 and pN3 cases are classified into the high-risk group in the 2007 St Gallen consensus [1].

Hormone receptor status

Endocrine therapies involving the use of effective drugs, such as anti-estrogens, aromatase inhibitors, and luteinizing hormone-releasing hormone (LHRH) analogues, have recently been developed, and the role of endocrine therapy in primary breast cancer is becoming increasingly important [8, 9]. Current approaches now always include the testing of ER and PgR by immunohistochemistry (IHC). If the tumor is ER- and/or PR-positive, the patient is eligible for preoperative or postoperative hormonal therapy.

There are several criteria for evaluating the results of ER and PR tests [1, 2, 10, 11]. In a classification recommended by the Japan Breast Cancer Society, the results are assessed according to the proportion of cells showing positive nuclear staining irrespective of the intensity of stained nuclei [11]: score 0 when there are no positive cells, score 1+ when the proportion of positive cells is <1%, score 2+

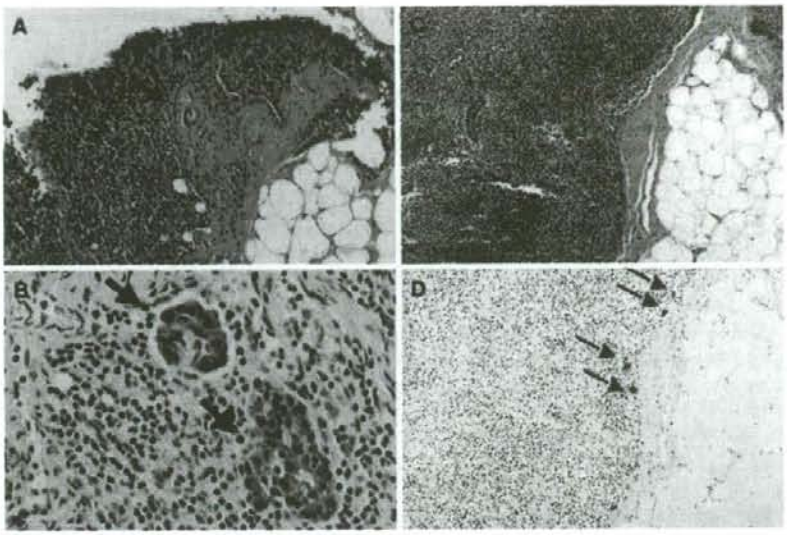

Fig. 3 Detection of micrometastasis of breast cancer cells in sentinel lymph nodes (SLNs). **a**, **b** Micrometastasis. A tumor cell nest is detectable by HE (**a**), and the tumor cells are confirmed to be positive for cytokeratin by immunohistochemistry (IHC) (**b**). *Arrows* indicate tumor cells. **c**, **d** Isolated tumor cells (ITCs). Carcinoma cells are not visible on the HE section (**c**), but single cytokeratin-positive cells (*arrows*) are detectable by IHC. **a**, **c** H&E stain. **b**, **d** Immunoperoxidase stain. **a**, **c**, **d** ×40, **b** ×200

when positive cells account for 1% to <10% of the cells tested, and score 3+ when positive cells account for 10% or more of the cells tested. A score of 3+ corresponds to a positive result, and 1+ and 2+ correspond to equivocal or borderline results. A score of 0 is negative.

The Allred score is another major scoring system that takes both the proportion and intensity of positively stained cells into account (Fig. 4) [11]. The Allred score is the sum (0, 2–8) of the proportional score (0–5) and the intensity score (0–3). In Japan, Allred scores for ER and PR are frequently requested by clinicians because of evidence that the Allred score for ER is correlated with (1) disease-free survival of patients receiving adjuvant endocrine therapies [12] and (2) the response rate of the primary tumor to neoadjuvant endocrine therapy using letrozole (an aromatase inhibitor) or tamoxifen [13].

At the St Gallen meetings, endocrine therapy was recommended for ER- or PR-positive breast cancer (Table 2) [1, 2]. In the report of the 2007 St Gallen meeting, hormone status was classified into highly endocrine responsive, incompletely endocrine responsive, and endocrine nonresponsive; incompletely responsive (previously referred to as endocrine response uncertain) is when there is some expression of steroid hormone receptors but at lower levels, or when either ER or PgR was lacking. For patients with highly endocrine-responsive breast cancer and those with incompletely endocrine-responsive breast cancer, adjuvant

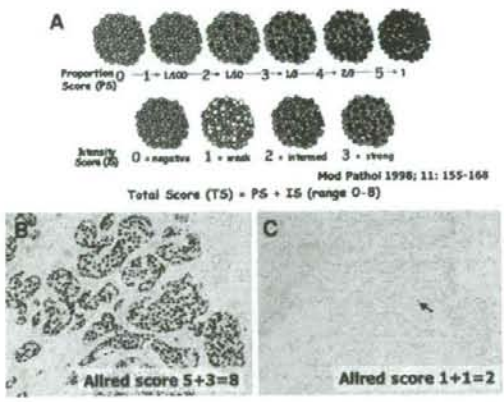

Fig. 4 Allred score system for the evaluation of the estrogen receptor (ER). **a** Schematic presentation of the system. Reproduced from Allred et al. [11]. **b** A case of strongly positive ER. Scores of intensity and proportion are 3 (strong) and 5 (67–100%), respectively, and the Allred score is 8. In the Japanese classification, the score is 3+ (see text). **c** A case of negative ER. *Arrow* Tumor cell. Scores of intensity and proportion are 1 (weak) and 1 (<1%), respectively, and the Allred score is 2. In the Japanese classification, the score is 1+, but in the St Gallen consensus, this case may be incompletely endocrine responsive

endocrine therapy is recommended, with chemotherapy being considered according to the risk presented (see Table 1 [1]).

Grade

The histological grade of the malignancy of breast cancer is usually evaluated as the sum of tubule formation, nuclear pleomorphism, and mitotic count (histological grade), or as the sum of the latter two (nuclear grade), based on an examination of hematoxylin-eosin (HE)-stained histopathological sections. In 1957, Bloom and Richardson reported the important role of histological grade in the prognostication of primary breast cancer, and a modification of their criteria by Elston and Ellis is now widely used [14]. In Japan, a method of nuclear grading is presented in the "general rules" (Table 5, Fig. 5) [15].

Histological or nuclear grading is applied mainly to invasive ductal carcinoma for the purpose of estimating the risk of recurrence and determing the choice of adjuvant therapies [1, 2]. Histological grade and nuclear grade have almost the same prognostic relevance [16]. Histological grade is a powerful prognostic indicator independent of tumor size or lymph nodal status, but it has a strong correlation with HER2 amplification, nuclear p53 immunoreaction (i.e. inactivation of the tumor-suppressor function of p53 protein), hormone receptor negativity, and accumulation of chromosome alterations.

HER2 amplification and overexpression

The *HER2* gene was first cloned as a proto-oncogene homologous to the HER1 [c-erbB-1, or epidermal growth factor receptor (EGFR)] proto-oncogene that encodes tyrosine kinase growth factor receptor localized through cell membrane [17]. The *HER2* gene is located on chromosome arm 17q21.1, and genomic amplification of 17q12-q21.2 containing the *HER2* locus occurs in 10–30% of human breast cancers. *HER2* gene amplification causes overexpression of the HER2 protein and plays a role in the transduction of growth signals to the nucleus [18].

The clinicopathological implications of *HER2* gene amplification and overexpression of its protein are: (1) frequent occurrence in grade 3 carcinomas, comedo-type DCIS, Paget's disease, and inflammatory breast cancer, (2) correlation with poor prognosis independently of tumor size or nodal status, (3) indication for trastuzumab

Table 5 Nuclear grading system (Reproduced from [15] with modifications)

Nuclear grading system			
Nuclear atypia			
Score 1. Nuclei are uniform size and shape. The nuclei are not hyperchromatic or may be hyperchromatic with evenly dispersed chromatin or with finely granular chromatin without clumping			
Score 2. Between scores 1 and 3			
Score 3 Pleomorphic nuclei of varying sized showing hyperchromatism with coarse and irregular distribution often associated with large nucleoli			
Mitotic counts			
After choosing the fields that appear to contain largest number of mitotic figures:			
Score 1: <5 per 10 high-power fields (400×)			
Score 2: 5–10 per 10 high-power fields			
Score 3: ≥ 11 per 10 high-power fields			
Sum or scores in nuclear atypia and mitotic counts[a]			
2, 3: Nuclear grade 1			
4: Nuclear grade 2			
5, 6: Nuclear grade 3			
Visual number	Mitotic counts per 10 high-power fields (400×)		Eyepiece
	Score 1	Score 2	Score 3	
20	0–4	5–10	≥11	WHK 10×
21	0–5	6–11	≥12	CFW 10×, CFWN 10×
22	0–5	6–12	≥13	CFI 10×, WH 10×
25	0–7	8–15	≥16	CFIUW 10×
26.5	0–8	9–17	≥18	SWH 10×, SWHK 10×
27	0–9	10–18	≥19	CFUWN 10×

[a] Adjustment of criteria for mitotic counts according to the properties of eyepieces

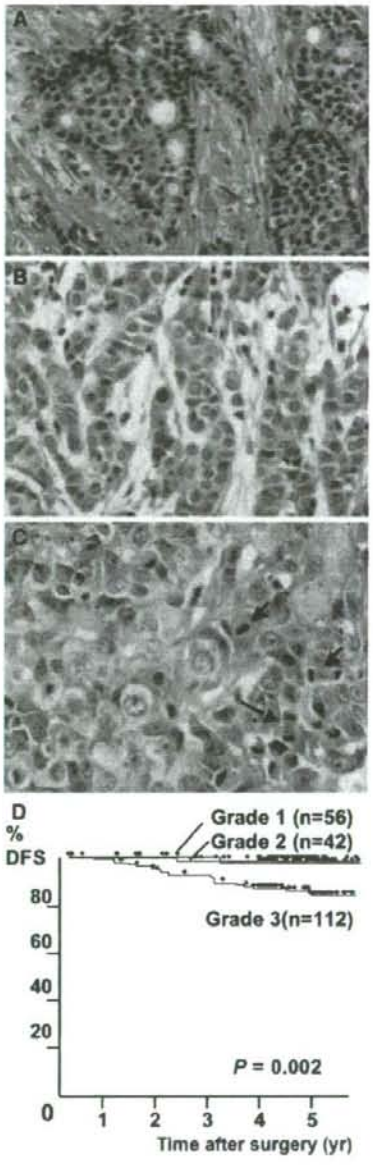

Fig. 5 Nuclear grade of pN0 (regional lymph nodes) invasive ductal carcinoma. Histopathological view of nuclear grade 1 (**a**), grade 2 (**b**), and grade 3 (**c**). **a** Nuclei are uniform, and mitotic figures are not seen. **c** Nuclei are pleomorphic, and there are a lot of mitotic figures (*arrows*). **b** is intermediate between **a** and **c**. H&E stain. ×200. **d** Disease-free survival curves for patients with pN0 breast cancer stratified by nuclear grades. Curves differ significantly ($P = 0.002$). *%DFS* Disease-free survival (%)

(Herceptin) therapy, and (4) high response rate to anthracycline-based chemotherapies [17].

Criteria for *HER2* overexpression and amplification have been established. *HER2* expression status, tested by IHC, is classified as score 0, 1+, 2+, or 3+ (Fig. 1 in [17]). An IHC score of 3+ is assessed to indicate overexpression, or *HER2*-positive; a score of 0 or 1+, as *HER2*-negative; a score of 2+, as equivocal, with a recommendation for retesting by fluorescence in situ hybridization (FISH).

There are two types of FISH tests, single-color FISH and dual-color FISH, but only the latter is approved for diagnostic testing in Japan. Dual-color FISH (PathVysion, Vysis/Abbott) visualizes concurrently red signals of *HER2* on 17q21.1 and green signals of *CEP17* on the centromere of chromosome 17. The sum of signals on 20 nuclei of cancer cells is counted for both *HER2* and *CEP17*, and the *HER2/CEP17* ratio is calculated by dividing the total signal number of *HER2* by that of *CEP17*. If the *HER2/CEP17* ratio is 2.0 or higher, the tumor is assessed to be FISH positive, whereas a ratio of less than 2.0 is taken to indicate that the tumor is FISH negative (Fig. 2 in [17]). *HER2*-positive cases are considered to be eligible for trastuzumab therapy [19].

In 2006, revised criteria for *HER2* gene amplification were recommended by the NCCN HER2 Testing in Breast Cancer Task Force as follows: a tumor with an IHC score of 0 or 1+, or with a *HER2/CEP17* ratio of less than 1.8 by dual-color FISH, is *HER2*-negative; a tumor with an IHC score of 3+, or with a *HER2/CEP17* ratio of more than 2.2 by dual-color FISH, is *HER2*-positive; a tumor with an IHC score of 2+ should be further tested using FISH, with its *HER2* status determined on the basis of the FISH result. Tumor samples with a *HER2/CEP17* ratio of 1.8 to 2.2 are considered to be borderline [20]. In Japan, a cut-off value of 2.0 for the *HER2/CEP17* ratio is still used.

Amplification of the *HER2* gene and overexpression of its protein is correlated with a poorer prognosis of patients with pN0 invasive breast cancer as well as patients with node-positive breast cancer. In a review, Ross et al. stated that *HER2* amplification or overexpression was correlated with poorer prognosis by univariate and/or multivariate analyses in 73 of 81 studies (25,166 of 27,161 patients) published between 1987 and 2003 [21]. Although *HER2* is frequently positive in DCIS of higher grade and with an accumulation of molecular alterations, i.e., comedo-type DCIS and Paget's disease, *HER2* is not a prognostic factor in DCIS.

A correlation between *HER2* overexpression and response to adjuvant or neoadjuvant anthracycline-based chemotherapy has been reported in many studies [22]. In the JBCRG-01 protocol, *HER2* overexpression was

confirmed to be a significant predictive factor of tumor response to anthracycline-based PST [23]. Hayes et al. [24] reported that a node-positive breast cancer showing amplification or overexpression of *HER2* can benefit from the addition of paclitaxel therapy after adjuvant treatment with doxorubicin plus cyclophosphamide, regardless of the ER status. In contrast, they considered that patients with *HER2*-negative, ER-positive, node-positive breast cancer would gain little benefit from the administration of paclitaxel after adjuvant chemotherapy with doxorubicin plus cyclophosphamide.

Lymphovascular involvement

Although lymphatic and vascular invasion (abbreviated as ly and v) is generally considered to be a parameter of aggressiveness of cancers in various organs, the significance of ly or v as a prognostic factor has been the subject of controversy because (1) ly(+) is frequently difficult to differentiate histologically from tumor nests in pseudolymphatic spaces emerging as artifacts, (2) ly(+) or v(+) can be confused with an intraductal component, and (3) pathologists tend to evaluate a cancer as ly(+) unconditionally if lymph node metastasis is present.

Lymphatic and vascular invasion have been excluded from the items routinely described in the "general rules". Nonetheless, obvious lymphatic permeation does exist and is sometimes diffuse in highly aggressive breast cancers, such as inflammatory carcinoma. Yoshimoto et al. [25] defined lymphovascular invasion as being strongly positive if four or more ly or v foci were present, positive if three or fewer ly or v foci were present, and negative if no ly or v focus was evident in all case slides. In their series of cases, the percentages of those that were ly or v strongly positive, positive, and negative were 13, 13, and 74%, respectively, and the corresponding recurrence rates were 60, 40, and 26%, respectively [25].

In the 2007 St Gallen meeting consensus, extensive peritumoral vascular invasion was considered to be one of the parameters for assessing whether pN0 breast cancer is of low risk or intermediate risk. In pN1a breast cancers, however, extensive lymphovascular invasion has been removed from the risk factors [1]. Extensive peritumoral vascular invasion is defined as the presence of neoplastic emboli in two or more blocks, which could be interpreted as both extensive ly(+) and v(+). Intratumoral lymph vessels in invasive breast cancer are suggested to be absent [26], and it is recommended that peritumoral lymphovascular invasion should be taken into account. Recently, several reports have mentioned the utility of D2-40 or vascular endothelial growth factor (VEGF)-C in combination with IHC to visualize lymphatic ducts, and the diagnostic application of these molecules is expected [27, 28].

Future perspectives

According to the recommendation of the St Gallen meetings, more than 70% of pN0 breast cancers are included in the intermediate-risk group and considered eligible for adjuvant chemotherapy, despite the fact that the 10-year disease-free survival rate of patients with pN0 breast cancer in Japan is 10–15% [4]. It is expected that recurrence risk in pN0 breast cancer will be evaluable more accurately based on the properties of the resected tumors. On the basis of estimations from the categories established at the St Gallen consensus meeting, the 10-year recurrence rate of pN0 intermediate-risk cancer would be 13–20%, whereas that of pN0 low-risk cancer would be 5% or less. It is ideal that the pN0 group is classified as representing 33% of the high-risk group, with a recurrence rate of 30–45%, and 67% of the low-risk group with a recurrence rate of 5% or less (Fig 6). To establish a more ideal classification of early breast cancer, various tests are being undertaken that are based on molecular features.

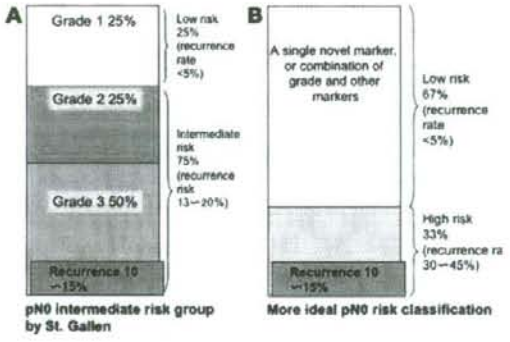

Fig. 6 Comparison between the pN0 intermediate-risk group (**a**) and the ideal pN0 high-risk group (**b**). In **a**, the intermediate-risk group comprises 75% of all patients with pN0 breast cancer and is estimated to show 13–20% of recurrence. Actually, most of pN0 breast cancers have an invasive component that is 2.0 cm or less in size. Lymphovascular invasion and *HER2* overexpression are shown by some researchers to be significant prognostic indicators with a relatively weak impact in pN0. Therefore, the overwhelming risk factor of pN0 breast cancer appears to be histological/nuclear grade. In **b**, the putative higher risk group comprises 33% of all patients with pN0 breast cancer and is estimated to show 30–45% of recurrence. For the concentration of cases, a single novel molecular marker or a combination of grade and other molecular markers would be effective. These molecular markers will be assayed by DNA microarray, Oncotype DX, array CGH, or other tools in the near future

DNA microarray

Using high-throughput microarray analysis, a 70-gene signature has been identified that can accurately select early-stage breast cancer patients who are highly likely to develop distant metastases and who, therefore, may obtain maximal benefit from adjuvant chemotherapy [29]. Validation and feasibility studies of the 70-gene profile (Mammaprint; Agendia) to patients of 60 years or younger with pN0 Stage I, II breast cancer are ongoing in the large adjuvant MINDACT (microarray in node-negative disease may avoid chemotherapy) clinical trial [29].

Oncotype DX

Although patients with pN0 and ER-positive breast cancer have an excellent prognosis, about 15% show relapse after 5 years of endocrine therapy. Clinical trials have provided evidence that these patients gain a significant benefit from chemotherapy; however, it would be significant overtreatment if it were applied to all of them. Strategies for evaluating tumors in a clinical setting have been developed using smaller sets of genes. One such strategy is the 21-gene assay (Oncotype DX; Genomic Health, Redwood City, CA), which is currently in commercial use in the USA. In Oncotype DX, a 21-gene recurrence score (RS) has been developed based on the monitoring of mRNA expression levels of 16 cancer-related genes in relation to five reference genes. One advantage of this test is the use of paraffin-embedded blocks in contrast to previous methods that required fresh frozen tissue. Oncotype DX has been shown to predict 10-year distant recurrence in patients with ER-positive, axillary lymph node-negative breast cancer. This genomic assay has also been shown to predict response to chemotherapy and endocrine therapy. A prospective study—the Trial Assigning Individualized Options for Treatment (Rx) (TAILORx)—to examine whether chemotherapy is required for the intermediate-risk group defined by the RS is accruing in North America [30, 31].

Array CGH (cancer array 800)

Comparative genomic hybridization (CGH) has already made a significant impact on cancer cytogenetics. In array-based CGH, DNAs spotted in a CGH-array contain sequence information directly linked to the genome database, and particular biological aspects of genes that lie within regions involved in copy-number aberrations can be noted. The application of array-based CGH technology for the diagnosis of breast cancer is awaited [32].

Basal-like type

With the use of gene microarrays, different subtypes of breast cancer have been characterized. These subtypes include the basal, ERBB2+, and luminal A, B and C subtypes [33, 34]. Basal-like-type breast cancer was determined by DNA microarray to be a group of tumors that were ER-negative, *HER2*-negative, and positive for myoepithelial/basal markers, such as vimentin, alpha-smooth muscle actin, EGFR, cytokeratin (CK) 5/6, CK14, and/or CK17 [33, 34]. It has also been reported that breast cancers arising in patients with familial breast/ovarian cancer carry germ-line *BRCA1* mutations [35].

There are at least four histologically distinct breast cancer groups with undifferentiated features, and these frequently show bidirectional differentiation toward luminal epithelial and myoepithelial/basal lineages [36, 37]. These groups comprise (1) invasive ductal carcinoma with a large central acellular zone (central acellular carcinoma), (2) atypical medullary carcinoma, (3) matrix-producing carcinoma, and (4) carcinoma with spindle-cell metaplasia (Fig. 7). In these four cancer types, KIT (CD117) expression and EGFR overexpression were detected in 34% and 88% of cancers with frequent expression of myoepithelial/basal markers but a low frequency of *HER2* overexpression or ER/PgR expression (Fig. 8) [37]. For the identification of a basal-like phenotype, confirmation of positivity for basal cytokeratins, i.e., either CK5/6 or CK14, by IHC is recommended [38].

Empirically, cases of node-negative breast cancer showing early recurrence appear to frequently contain the basal-like type [39]. However, Fulford et al. have reported that in node-negative patients, prognosis was similar between basal-like and other Grade 3 invasive ductal carcinomas, whereas basal-like grade 3 invasive ductal carcinoma showed a poorer prognosis than other types in patients who were positive for node metastasis [40].

One important current issue is the so-called triple-negative (i.e., ER/PgR-negative, *HER2*-negative) breast cancer, for which endocrine therapy or trastuzumab is not applicable. Approximately 5–15% of breast cancers are in this category. If systemic chemotherapies are not effective for "triple-negative" breast cancer, there are few treatment choices for this group and, in fact, chemotherapy is frequently not effective. A substantial percentage of "triple-negative" breast cancers appear to be of the basal-like type. The characterization of basal-like breast cancer on the basis of histological characteristics and molecular alterations would be useful for prognostication and treatment selection and also for the identification of targets for molecular therapy. It might be worth investigating whether therapies against activated KIT and/or EGFR are effective for cancers of the above-mentioned four histological types.

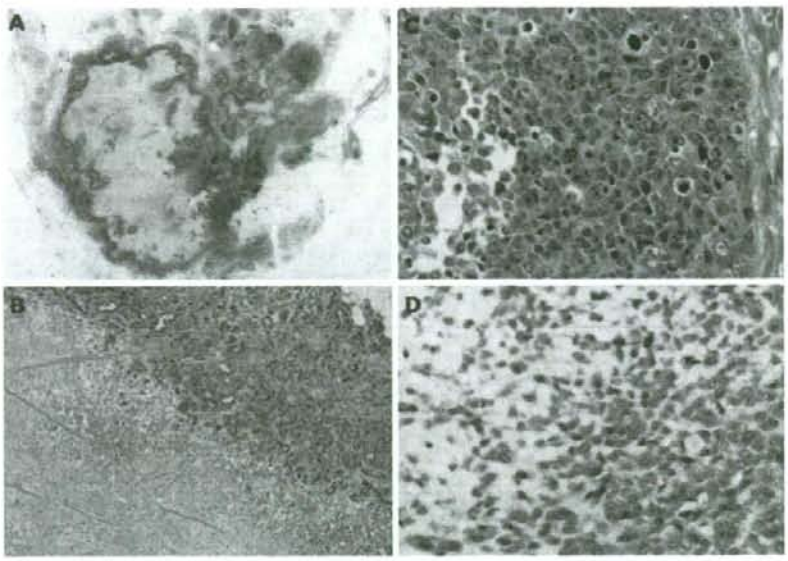

Fig. 7 Distinct breast cancer types of undifferentiated features, that appear to be representative of the basal-like phenotype. **a**, **b** Invasive ductal carcinoma with a large central acellular zone (central acellular carcinoma). **c** atypical medullary carcinoma, **d** matrix-producing carcinoma. Carcinoma with spindle-cell metaplasia is also included in this group. H&E stain. **a** ×1, **b** ×40, **c**, **d** ×200

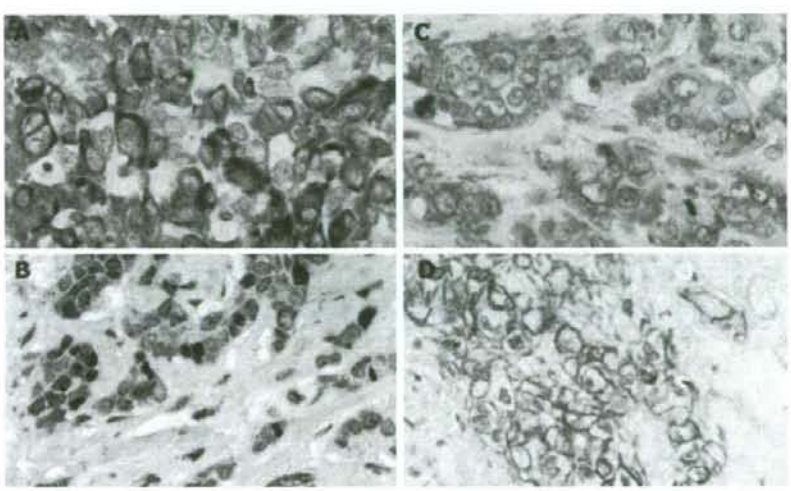

Fig. 8 Expression of myoepithelial/basal markers in undifferentiated-type breast carcinomas. **a** Vimentin, **b** KIT, **c** α-smooth muscle actin (SMA), **d** epidermal growth factor receptor (EGFR). The positive rates of expression of vimentin, α-SMA, KIT (CD117), and EGFR were 88, 41, 34, 88%, respectively, in undifferentiated types, whereas these were 5, 0.7, 5, and 5%, respectively, in other types [37]. Immunoperoxidase stain. ×200

Today, systemic chemotherapy, endocrine therapy, and trastuzumab are very effective, not only for metastatic breast cancer but also operable early breast cancer. Certain chemotherapeutic regimens with or without trastuzumab can achieve a pCR in the primary tumor in a proportion of cases. Nevertheless, these therapies are not perfect; they are not always effective, they may result in adverse events or late complications, and they may induce tumor resistance to the therapy in due course.

To achieve a greater decrease in the incidence of recurrence and death in patients with early breast cancer and those suffering adverse effects from the therapies, it is important to discriminate the higher risk group from the lower risk group more accurately in intermediate-risk

node-negative breast cancer. Furthermore, regardless of nodal status, the establishment of a diagnosis and treatment strategy for "triple-negative" breast cancers, especially the basal-like type, is desirable. To this end, molecular markers and tools, such as Oncotype Dx, Mammaprint, array CGH, and/or IHC markers for the basal-like type, would be effective. In addition, from a pathologist's viewpoint, proposals for the accurate evaluation of pT, pN, grade, ly, v, ER, PgR, and *HER2* have only recently been put forward. Therefore, there are few adequate follow-up data based on accurate descriptions of these pathological parameters. In the future, prospective clinical data as well as the revision of archival cases based on accurate histopathological evaluation might prove to have considerably higher value than expected hitherto, as exemplified by the proverb "it is always dark at the foot of the lighthouse".

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CLINICAL TRIAL

Phase II study of preoperative sequential FEC and docetaxel predicts of pathological response and disease free survival

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Abstract *Purpose* This multicenter phase II study examined the impact of pathological effect on survival after preoperative chemotherapy in Japanese women with early stage breast cancer. *Patients and methods* Prior to surgery, patients received four cycles of FEC (fluorouracil 500 mg/m^2, epirubicin 100 mg/m^2, cyclophosphamide 500 mg/m^2 q3w) followed by four cycles of docetaxel (75 mg/m^2 q3w). Primary endpoint was 3 year disease free survival (DFS) stratified by the absence or presence of Quasi-pCR (QpCR; absence of invasive tumor or only focal residual tumor cells). Secondary endpoints were predictors for QpCR, clinical response, breast conservation rate, and safety. *Results* Between June 2002 and June 2004, 202 women were enrolled. Among 191 assessable patients, 25% achieved QpCR. With 40 months median follow-up, 3 year DFS was estimated at 91% for all patients. 3 year DFS for patients with QpCR was 98% vs. 89% without QpCR (hazard ratio 0.38 [95% Confidence Interval 0.09–0.84], $P = 0.0134$). HER2 status and response to FEC were independent predictors of QpCR. The overall clinical

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response was 75%; 85% of patients achieved breast conservation. Grade 3/4 neutropenia was the most common adverse event, observed in 44% and 35% of patients during FEC and docetaxel, respectively. Treatment related side effects were manageable; there were no treatment related fatalities. *Conclusion* FEC followed by docetaxel is an active and manageable preoperative regimen for women with early stage breast cancer. QpCR following preoperative chemotherapy predicts favorable DFS. HER2 overexpression and clinical response to FEC predict QpCR.

Keywords Clinical trial · Docetaxel · Early stage breast cancer · FEC · Preoperative chemotherapy · Phase II

Introduction

Preoperative systemic chemotherapy has been widely used for patients with operable breast cancer to increase the chance for breast conservation [1–3]. Furthermore, response to preoperative treatment can provide information on long-term survival outcomes. Pathological complete response (pCR) in the breast and axillary lymph nodes predicts a favorable prognosis, whereas non-pCR of the breast or node-positive status does not, which can facilitate tailoring of subsequent treatment [1, 3]. In addition, correlative studies of tumor samples before and after treatment may provide information on markers that could predict response or resistance to treatment [4].

Results from the National Surgical Adjuvant Breast and Bowel Project (NSABP) study B-18 demonstrated the impact of preoperative chemotherapy in patients with operable early stage breast cancer [5]. The protocol-specified anthracycline-containing regimen of four cycles of doxorubicin and cyclophosphamide (AC), resulted in an increased chance of breast-conserving surgery (BCS) compared to no preoperative chemotherapy. The study established pCR as a prognostic marker for long-term disease-free survival and demonstrated that there was no difference in survival whether chemotherapy was administered before or after surgery. Subsequently, studies such as the Aberdeen trial have demonstrated the benefit of the sequential addition of taxanes to preoperative anthracycline regimens [6, 7]. NSABP Protocol B-27 demonstrated that compared to preoperative AC alone, the addition of sequential docetaxel doubled the pCR rate, increased the clinical complete response (cCR) rate, and increased the proportion of patients with negative axillary nodes [3, 7]. Although NSABP B-27 did not show that the addition of docetaxel to AC significantly improved disease free survival (DFS) and overall survival (OS) compared to AC alone, other studies, mainly of patients with node-positive disease, have shown favorable DFS and OS by including a taxane with an anthracycline, either in sequence or combination [8–12]. Multiple neoadjuvant studies demonstrated that patients with pathological complete response to chemotherapy had a good prognosis [1, 2].

Here we conducted a multicenter prospective neoadjuvant trial with four cycles of fluorouracil, epirubicin, and cyclophosphamide (FEC) followed by four cycles of docetaxel in Japanese patients with operable breast cancer to investigate the relationship between pathological effect and survival. The pathological effect was determined using the definitions of Quasi-pCR (QpCR: complete disappearance of invasive carcinoma in the breast or only focal tumor cells remaining in the stroma in the removed breast) [13]. The primary endpoint was to examine 3 year DFS stratified by pathological response (QpCR versus non-QpCR). We also performed a logistic regression analysis to examine which features were associated with QpCR with this regimen. Clinical response, the rate of BCS, and safety were also evaluated.

Methods

Study design and ethics

This multicenter, open-label, single-arm, phase II clinical study was conducted at 13 institutions throughout Japan. This study was conducted in accordance with the Declaration of Helsinki and Good Clinical Practice. The protocol was reviewed and approved by the institutional review board of each participating institution and written informed consent was obtained from all patients prior to the study.

Patients

Women aged 20–59 years of age with histologically proven early stage breast cancer (T1c-3 N0 M0/T1-3 N1 M0)

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were enrolled. No prior chemotherapy, radiotherapy, hormonal therapy, or immunotherapy was allowed. Other inclusion criteria were the following: Eastern Cooperative Oncology Group performance status of 0–1; white blood cell count between 4000/mm^3 and 12000/mm^3; neutrophil count ≥2000/mm^3; platelet count ≥100000/mm^3; hemoglobin ≥9.5 g/dl; serum bilirubin <1.25 times upper normal limit (UNL), creatinine <1.5 times UNL, or AST and ALT <1.5 times UNL. Patients with congestive heart failure or left ventricular ejection fraction ≤60% were excluded. Patients were also excluded if they had confirmed infection; serious concomitant illness such as severe cardiovascular disease, uncontrolled diabetes, malignant hypertension and hemorrhagic disease; active concomitant malignancy; brain metastasis; interstitial pneumonia or lung fibrosis confirmed by chest X-ray or computed tomography; pleural or peritoneal effusion that required treatment; pericardial effusion; motor paralysis, peripheral neuropathy or edema history of severe drug allergy; or had previously received long-term corticosteroid therapy. Pregnant or lactating women were also excluded.

Treatment procedures

Four cycles of FEC (fluorouracil 500 mg/m^2, epirubicin 100 mg/m^2, and cyclophosphamide 500 mg/m^2) administered intravenously (i.v.) on day 1 every 21 days were followed by four cycles of docetaxel i.v. (75 mg/m^2) every 21 days, prior to surgery. The doses of docetaxel and epirubicin selected at the time of this study were higher than the approved doses in Japan (60 mg/m^2 each). Premedication consisted of a 5-HT$_3$ antagonist and dexamethasone i.v. on day 1 with oral dexamethasone on days 2 and 3 with each cycle of FEC and dexamethasone i.v. with or without 5-HT$_3$ antagonist on day 1 with each cycle of docetaxel. Administration of recombinant human granulocyte colony-stimulating factor (rh G-CSF) and antibiotics was left to the judgment of each investigator. If patients prematurely discontinued FEC treatment, they were expected to proceed to four cycles of docetaxel.

Treatment could be postponed for a maximum of 2 weeks for severe toxicity. If toxicity did not improve during this period, chemotherapy was discontinued and surgery was recommended. Dose reductions of epirubicin from 100 mg/m^2 to 75 mg/m^2 and for docetaxel from 75 mg/m^2 to 60 mg/m^2 were permitted in case of febrile neutropenia and grade 3 or 4 non-hematological toxicities except for nausea, vomiting, and fatigue. Following chemotherapy and clinical assessment of response, patients underwent surgery. If the tumor was too large or invasive for breast-conserving surgery, modified radical mastectomy was recommended. Sentinel lymph node biopsy (SNB) was performed to confirm disease stage. Most patients with negative biopsies did not undergo surgical clearance of axillary nodes. Autologous or heterologous reconstructive surgery was performed as needed. All patients who underwent breast-conserving surgery were given standard radiotherapy to the remaining ipsilateral breast tissue after surgical recovery. For patients with node-negative status in the sentinel nodes not requiring axillary dissection, radiotherapy to the axilla was allowed but not required. No recommendations were made for post-study hormone therapy in the protocol.

Assessment

Hormone receptor and HER2 overexpression

Estrogen receptor (ER) status and progesterone receptor (PgR) status were determined by immunohistochemistry at each institute. In general, tumors with >10% positively stained tumor cells were classified positive for ER and PgR. HER2 status was also determined at each institute by immunohistochemistry or by fluorescence in situ hybridization (FISH) analysis. HER2 positive tumors were defined as 3+ on immunohistochemistry staining or as positive by FISH.

Central pathological assessment

Haematoxylin and eosin (H&E) and keratin stained slides were prepared as 5 mm tissue sections from the primary tumor. Pathological breast tumor response was assessed by a central review committee consisting of three pathologists using modified criteria of the Japanese Breast Cancer Society [14]. A blinded central review committee evaluated the pathologic response independently to the local pathologists. In this study, the response of stromal invasion and intraductal component was assessed separately. Cytokeratin immunostaining was performed to confirm residual cancer cells in required cases.

Toxicity and clinical assessment

Toxicity was evaluated according to the National Cancer Institute Common Toxicity Criteria (version 2). Tumor response was assessed using the Response Evaluation Criteria in Solid Tumors (RECIST) guidelines in patients who had measurable lesions. Tumor and toxicity assessments were performed within 4 weeks prior to FEC treatment, after completion of FEC treatment, and before surgery.