連載

21世紀の外科的癌治療指針

第①回

乳癌治療

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はじめに

膨大な臨床データの積みかさねとその詳細な解析とによって，乳癌治療における標準化は飛躍的にすすんでいる．手術療法，薬物療法，放射線療法などの集学的治療体系のうち，ここではtrastuzumabを用いた抗体療法の占める役割の可能性や，化学療法，ホルモン療法といった薬物療法における「標準」について考察する．これら「標準」は，実地診療において患者へ正しくインフォームされるべきであり，最新の知見をふまえたうえで絶えず進化し続けている．

I. Trastuzumab—併用療法と単独療法

Trastuzumabが，HER-2過剰発現を有する乳癌に対してのみ選択的に抗腫瘍効果をもたらすことはよく知られている．昨年，『The New England Journal of Medicine』に掲載された再発進行乳癌を対象にした通常の化学療法(アントラサイクリンor paclitaxel)との併用比較試験では，いずれの抗癌薬においてもtrastuzumab併用群が非併用群に比し，抗腫瘍効果，生存率ともに優れていた[1]．生存期間延長効果を標準的な数値として示すことが可能である．加えて，化学療法単独群の相当数の患者が後にtrastuzumab治療を受けていた．これらのことからtrastuzumabはHER-2陽性患者におけるfirst line治療と考えられるようになった．再発乳癌に対する治療のアルゴリズムとしては，Hortobagyiのチャートが現在も標準的であるが，HER-2陽性乳癌に関してはtrastuzumabを含む治療がfirst line治療となりつつある(図1，表1)[2]．

Trastuzumabは併用(taxane)療法で開始するべきか，あるいは単独療法で開始してもよいのか，議論のあるところである．単独療法で開始した場合に，仮に併用療法で開始する場合と同等の予後が得られるなら，QOLが良好なぶんだけ単独療法で開始するほうがよいことになる．最近，Vogelらによりtrastuzumab単独療法の成績が報告された[3]．114例の再発乳癌患者を対象にfirst

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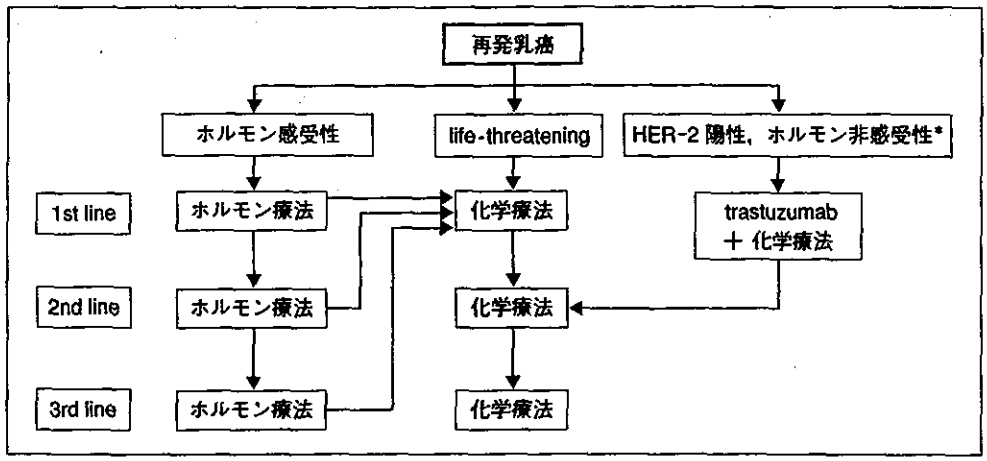

図1．再発乳癌治療アルゴリズム(Hortobagyiモデル改変)
*：ホルモン感受性，non-life-threateningの場合はホルモン療法を先行する．

表1．再発治療アルゴリズム

1) Hortobagyiのアルゴリズムは標準的に用いられている
2) ホルモン療法奏効例では有意の予後延長が認められる
3) 予後延長効果が証明されている化学療法レジメンは少ない
4) HER-2陽性乳癌の第1次治療にはtrastuzumab＋タキサンが広く用いられている
5) Trastuzumabの適応は免疫染色HER-2(+++)，あるいはFISH陽性が一般的である

line治療におけるtrastuzumab単独療法の有用性が検討されたが，奏効率は26%であった．免疫組織学的(IHC)HER-2(+++)における奏効率は35%，IHC(++)は0%，fluorescence *in situ* hybridisation(FISH)による層別ではFISH(+)，FISH(−)それぞれ34%，7%の奏効率であった．奏効例の約半数では1年以上にわたって病勢の増悪が認められていない．毒性に関しては2%の症例で心毒性が認められたが，いずれも既往歴のある症例であった．Trastuzumab単独療法により，HER-2発現乳癌のおよそ15%の症例において良好なQOLを保ちながら長期の病勢コントロールが可能であることを示している．これは重要な成績であるが，first line治療におけるタキサン併用療法の奏効率と比較すればtrastuzumab単独療法の奏効率は明らかに下回り，trastuzumab単独療法における治療奏効亜群をより正確に予測することはまだ困難である．したがって，現時点ではtrastuzumab単独療法を標準的というにはまだデータが不十分であり，"investigational"な治療法であろう．無論，なんらかの理由によりtaxane投与ができない場合には，trastuzumab単独療法は重要な選択肢である．トライアルとして，trastuzumab単独療法で始め，病勢の増悪をみればtaxaneなどを併用する治療法と，最初からタキサン併用療法を比較することはたいへん興味深い．

奏効例におけるtrastuzumabの投与期間をどうするか，あるいは抗癌薬との併用の場合どの時点で抗癌薬をオフにするか，さらには抗癌薬併用trastuzumab療法に耐性になった場合，その後の治療の中でtrastuzumab投与を継続するか否か，

表2. 乳癌術前後の化学療法

1) 原発性乳癌において多剤併用術後補助化学療法は有意に再発を抑制する
2) St. Gallenの再発リスク評価，補助療法指針は世界的に受け入れられている
3) 多剤併用療法のほうが単剤療法に比べ効果が高い
4) その効果は年齢依存性である
5) アントラサイクリンの効果には用量依存性が認められる
6) 投与期間は4～8ヵ月が妥当である
7) タキサンの有用性に関する評価はまだ確立されていない
8) 術前治療におけるpCR例の予後は良好である

表3. Epirubicin-based(E-base) vs CMF

レジメン	閉経後患者の割合(%)	5年無再発生存率%		リスクリダクション(%)
		E-base	CMF	
CEF 120×6 vs CMF	—	63	53	29
CEF 60×9 vs CMF	27	63	58	27
CE 100×8 vs CMF	42	67	66	4
アントラサイクリン併用多剤化学療法 vs CMF		57	54	11

いずれもまだ標準的な手法，コンセンサスは確立されていない．トライアルを必要とするテーマと考えられる．

II. 術後補助化学療法における アントラサイクリンの投与量

乳癌の術後補助化学療法においてアントラサイクリンが中心的役割を演じていることには議論の余地がない(表2)．しかし，その対象，投与量については依然として議論があり，多くの研究が現在も行われている[4]．Oxford meta-analysis(2000年)の結果では，overallとしてアントラサイクリンを含む多剤併用療法はCMF(CPA，methotrexate，5-FU)療法に比較し有意に高い再発抑制効果を示すことが証明されている．ただし，詳細をみれば，その効果はアントラサイクリンの投与量，投与対象に依存する可能性が高いことがわかる．多くの試験においてAC(adriamycin 60 mg/m^2，cyclophosphamide：CPA 600 mg/m^2) 4サイクルCMF(CPA，methotrexate，5-FU) 6サイクルと同等である．Epirubicinに関しては，CEF(CPA，epirubicin：Epi，5-FU)とCMFの比較においていくつかの大きなトライアルが行われている(表3)．もっとも代表的なものはNCIC，CTG，MA5トライアルで，CEF：CPA 75 mg/m^2 1～14日；Epi 60 mg/m^2 1日，8日；5-FU 500 mg/m^2 1日，8日を6サイクル，CMF：CPA 100 mg/m^2 1～14日；methotrexate 40 mg/m^2 1日，8日；5-FU 600 mg/m^2 1日，8日を6サイクルの比較である．対象は閉経前，腋窩リンパ節転移陽性乳癌である．結果はCEFがannual reduction in odds of recurrence(AROR)で29%の差をもってCMFに対して優位であった．CEF(E：60 mg/m^2×9)とCMFを比較したDanishトライアルの成績もほぼ同様でCEFが上まわっている．さらにepirubicinの用量依存性に

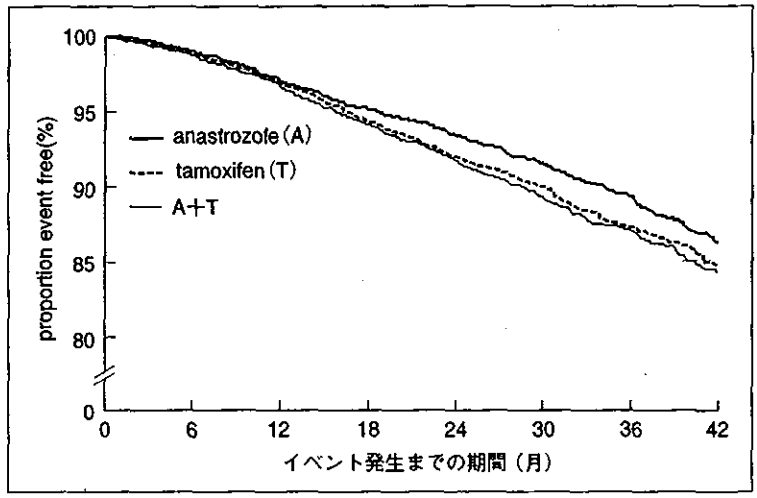

図2．術後補助療法における tamoxifen，anastrozole，両者併用の比較試験の中間解析結果(9,366例)

関してはFASG trialがよく知られている．High-dose CEF：CPA 500 mg/m², Epi 100 mg/m², 5-FU 500 mg/m² vs low-dose CEF：CPA 500 mg/m², Epi 50 mg/m², 5-FU 500 mg/m²，それぞれ1日のみ投与6サイクルが比較され，high-dose CEFがlow-dose CEFに対してARORで32%上回った．リンパ節転移を有する閉経前症例では，CEF療法はCMF療法にまさり，epirubicinの推奨用量は90～100 mg/m²/3週を6サイクルである．ところが，最近low-CE(CPA 500 mg/m², Epi 60 mg/m²を8サイクル)，high-CE(CPA 830 mg/m², Epi 100 mg/m²を8サイクル)，CMF(CPA 100 mg/m² 1～14日，methotrexate 40 mg/m² 1日，8日，5-FU 600 mg/m² 1日)の比較試験の成績が報告された．対象は70歳以下の閉経後症例を含むリンパ節転移陽性乳癌である．結果は，high-CEは当初の予想通りlow-CEより優れた再発抑制効果を示したが，high-CEとCMFの比較では予想とは異なり，両者は再発抑制に関してまったく同等であった[5]．異なるランダム化比較試験を直接比べることはできないが，high-CEとCMFのあいだで差が認められなかった理由として，閉経後症例が多く含まれていること，5-FUがレジメンに含まれていないことなどがあげられる．標準的治療という観点からすれば，閉経前リンパ節転移陽性乳癌に対するCEF療法は，推奨用量が用いられた場合，ある一定の標準的再発抑制効果(AROR)が期待される標準的治療といえる．それ以外の対象患者，あるいは推奨用量が用いられない場合，CEFがCMFに比べどれほど優れているかに関する標準値はまだ明確でないように思われる．

III．術後補助ホルモン療法における アロマターゼ阻害薬

ホルモン療法における最近のトピックスの一つは，術後補助療法におけるアロマターゼ阻害薬の有用性である．9,000例以上の閉経後乳癌症例を集積して行われたtamoxifen，anastrozole，両者併用の比較試験(anastrozole or tamoxifen alone or in combination：ATACトライアル)の中間解析では，anastrozole群が他の2治療群に対して有意に良好な無病生存期間を示した[6]．アロマターゼ阻害薬の臨床的有用性を評価するうえで，きわめて重要な結果である．この成績を受けて日米の認可当局は，閉経後原発性乳癌に対するanastrozoleの術後補助療法としての使用を認めた．ホルモン依存性原発性乳癌の治療選択肢は確

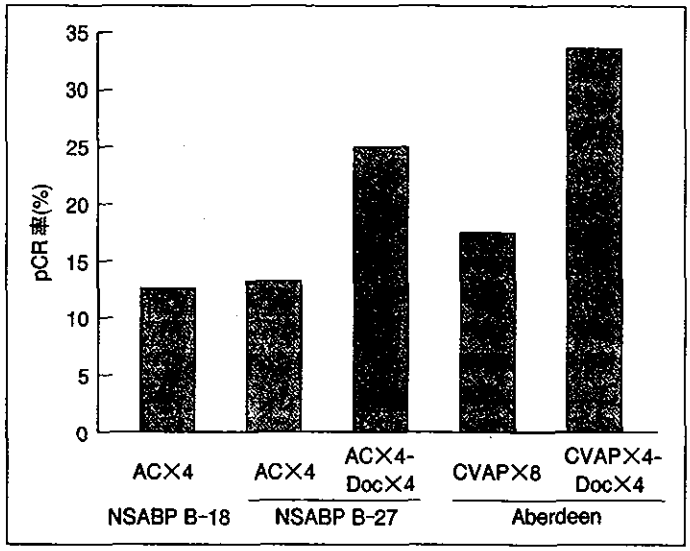

図3. NSABP B-18, B-27, Aberdeenトライアルにおける pCR率

実に一つ増加した．しかしながら，標準的治療という観点からすれば，ATACトライアルの結果は中間解析であり，薬剤を投与中の症例も多く長期毒性は不明であることなどから，まだそのメリットを標準的な数値として示すことはむずかしいように思う．もう少し経過観察が必要である．最近『Journal of Clinical Oncology』に発表されたASCOコンセンサスパネルの見解では“A 5-year course of adjuvant tamoxifen remains the standard therapy for women with hormone receptor-positive breast cancer. The panel recommends that physicians discuss the available information with patients, and, in making a decision, acknowledge that treatment approaches can change over time”と結んでいる[7]．

IV. 術前化学療法

術前化学療法は，化学療法の領域のみならず，原発性乳癌の治療システム全体における最大のトピックスである．図3は最近の術前化学療法における主要な成績を示している．術前化学療法において，その意義をどのような指標で評価するか，指標の尺度をどうするか，まだ必ずしも固まっていない．現時点でおそらく「確からしい」といえるのは，①術前化学療法はある一定の割合で乳房温存手術の頻度を高めることができる，②術前治療による局所治療の遅れが長期予後にデメリットをもたらすことは少ない，③病理学的にCR(pCR)になった症例の予後は非pCR症例の予後に比べ有意に良好であることであろう[8]．したがって，乳房温存手術の割合，あるいは腋窩郭清省略の割合，そしてpCR率が術前化学療法を評価するうえでの指標になると考えられる．図3に最近の主要なトライアルのpCR率を示す．NSABP B-18, B-27は1,000例を超す大きな臨床試験である．AC 4サイクル，AC→docetaxel 100 mg/m^2 4サイクルのpCR率はそれぞれ13%, 26%である．これらの値は術前化学療法における各レジメンの標準値といってもいいかもしれない．

おわりに

標準的治療には厳密な意味の定義がない．評価指標における標準値は存在しても，そしてその標準値を上回る治療を標準的治療と仮定しても，実際の臨床では症例個々のheterogeneityを考慮し始めたとたん，「標準」がみえなくなることはし

ばしばである．その対処として，欧米では最近，症例ベースのミニコンセンサスミーティングがしばしば行われている．会の大小を問わず，いろいろなところで，グループ内，施設内，地域内で頻繁に催されている．絶えず，微妙に(ときには激しく)変化する治療の標準，あるいは標準値をup-to-dateするために優れた方法なのであろう．さらに，エビデンスをつくる作業，トライアルに携わることの重要性もよく指摘される．前者では意識的に，後者ではどちらかといえば無意識に「標準」にかかわることになる．Trastuzumabの例をみると，個別化医療の時代になっても，個々の治療，診断ごとに標準は存在するのであろう．治療の「標準」は曖昧で相対的であるが，確実に進化している気がする．

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Decision tree and paradigms of primary breast cancer: changes elicited by preoperative therapy

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Summary

A small difference in decision-making causes a big impact on the long-term outcome in cancer treatment. Novel methods such as preoperative systemic treatment or sentinel node biopsy (SNB) may alter the decision-trees in primary breast cancer management. Recent data showed that preoperative chemotherapy drives pathological complete response (pCR) that implies long-term relapse-free, for about 25% to 30% of early breast cancer patients. In fact, preoperative systemic therapy has come to be used regardless of the tumor stage. From the point of the clinical trial, pCR can be recognized as a new endpoint, which promises to speed up new therapy development. Therefore, it is crucial to consider new paradigms including preoperative therapy in the decision-tree. There are several criticisms regarding the preoperative therapy, such as a possibility of over-treatment, however these issues might be resolved by changing the concept or procedures slightly. For instance, if SNB is conducted before the treatment, the over-treatment issue can be eliminated. In this article, we will discuss the changes in decision-tree and paradigms for primary breast cancer patients.

key words: **decision-tree • breast cancer • preoperative therapy • sentinel node biopsy**

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DECISION-TREE OF PRIMARY BREAST CANCER MANAGEMENT

Multidisciplinary treatment has brought significant advances in primary breast cancer management. It is evident that postoperative adjuvant chemotherapy (Post-CT) and/or hormone therapy (Post-HT) improves long-term survival rate remarkably, while radiotherapy reduces local relapse significantly, and breast-conserving treatment (BCT) or breast reconstruction surgery provides good quality of life (QOL) without affecting survival rates [1–5]. In addition, various novel diagnostic or treatment options have been added. For instance, sentinel node biopsy (SNB) provides the chance to avoid surgical clearance of axillary nodes [6] (Figure 1). Preoperative systemic therapy increases the chance of BCT as well as presenting prognostic information. Thereby, treatment options are becoming more diverse, and decision-making processes can be more complicated. At present, in general, the decision-tree of primary breast cancer consists of three major parts including the diagnostic phase by clinical, radiological and pathological examinations, the initial treatment phase by surgery, and the second treatment phase by systemic therapy or radiotherapy. Postoperative systemic therapy is chosen based upon the data obtained from pathological, biochemical and molecular analysis of the primary tumors or of the nodes [7,8]. For example, at least five prognostic or predictive markers including age, tumor size, histologic nodal status, histologic grade and hormone receptor status are recommended criteria scheduling the systemic treatment. Surgical margin or numbers of node involvement is a key issue in deciding the indication and dosage of radiotherapy [5,9,10]. This type of decision-making process has been established over the past several decades.

POSTOPERATIVE SYSTEMIC THERAPY

The current status of Post-CT for primary breast cancer would be summarized as follows. Post-CT by polychemotherapy improves the postoperative survival significantly [3]. Its effect on survival is age-dependent. Younger patients are likely to have a bigger survival advantage compared with older patients. The survival benefit driven by an anthracycline-containing regimen is dose-dependent. Appropriate duration of polychemotherapy would be 4 to 6 months involving 4 to 8 courses of treatment [11–13]. It is still not easy to predict the efficacy of polychemotherapy on survival by analyzing the molecular or biological profile of the tumor. Therefore, there is a universal trend for primary breast cancer patients who have any significant risk of recurrence to be treated by Post CT. Post-HT by 5-year antiestrogen has been established as a gold standard for both premenopausal and postmenopausal patients [2]. The annual reduction in relapse of 5-year tamoxifen treatment reaches 40% for hormone receptor (HR) positive subgroup, whereas no survival benefit is provided for the hormone receptor negative subgroup.

Figure 1. Immunostaining of a sentinel node by anti-cytokeratin antibodies. *Positive signals indicate the presence of micro*-metastasis of cancer cell.

PREOPERATIVE SYSTEMIC THERAPY

In the 1980' or before, preoperative chemotherapy (Pre-CT) was regarded as a tool to reduce tumor volume from inoperable to operable stage [14], and in the 90's, Pre-CT was a method to increase the chance of BCS from total mastectomy [15–19]. Nevertheless, the purpose of Pre-CT now may be being changed radically from the reduction of tumor volume to complete remission of tumor, as well as to increase the ratio of BCS (Table 1). Recent studies have documented remarkably high pathological complete response (pCR) rates and extremely favorable prognosis of those pCR patients [20].

The NSABP B-18 trial, which examined the primary treatment effect of combination chemotherapy of 4-cycles of AC, doxorubicin and cyclophosphamide, in patients with Stage I to Stage III primary breast cancer, showed that pCR was achievable for about 13%, and the prognosis of the pCR patients was remarkably favorable compared to that of non-pCR patients. The eight-year survival rate of the pCR patients reached 85% or more regardless of initial tumour stage [16]. Several other clinical trials, including smaller-sized studies than NSABP B-18, have also confirmed that the prognosis of pCR patients is favorable, ranging from 80% to 89% for 5-year disease-free survival (DFS) [21]. Furthermore, according to the recent presentation on the NSABP B-

Table 1. The benefit and problem of primary breast cancer patients from preoperative systemic treatment.

Benefit
• Better QOL (Minimal operation, well monitored toxicity etc)
• Multiple choices of treatment
• To know the survival benefit
• To know the treatment response
• To know the tumor kinetics and characteristics
• To participate in the new therapy development
Problem
• Over-treatment
• Delay of local control
• Decrease in the accuracy of sentinel node biopsy
• Uncertainty of nodal status before treatment
• Lack of diagnostic tool or surrogate marker of pCR except surgical confirmation
• Possible increase in the risk of local recurrence after breast-conserving treatment

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27 trial, it was reported in December of 2001 that the pCR rate was further improved to about 26% by the addition of 4-cycles of docetaxel to 4-cycle of AC sequentially [22]. Approximately, two-thirds of pCR cases showed total disappearance of tumor cells from the breast and one-third showed disappearance of the invasive component of the invasive tumor. An Aberdeen group also documented that, from a preoperative randomized study comparing 4-cycles of docetaxel and 4-cycles of CVAP, cyclophosphamide, doxorubicin, vincristine, and prednisolone in primary breast cancer patients who responded to an initial 4-cycles of CVAP, the pCR rate for the docetaxel group was 34%, whereas that of 8-cycle CVAP group was 16% [23]. These two studies, NSABP B-27 and Aberdeen, indicate that the sequential use of core non-cross resistant agents and response to both core agents are crucial to increase the pCR rate. As in the NSABP B-18 study, the Aberdeen study confirmed that pCR is a potent and independent indicator of favorable prognosis. The frequency of BCS was increased significantly in the Pre-CT arm patients compared with the reference arm patients in the NSABP B-18 trial, whereas no additional increase by docetaxel treatment has been reported in the NSABP B-27 trial. Taken together, it is suggested that pathological complete tumor remission has been revealed to be a potent indicator for favorable prognosis, and Pre-CT might be a novel method to identify patients who can drive the survival benefits from the treatment within a short period. There was no significant difference in OS between Pre-CT and Post-CT, however pCR seems to clarify the benefit at an individual level. It is also important to know that the prognostic significance of pCR for long-term outcome has been confirmed in other types of cancers such as lung, ovarian, head and neck.

Preoperative hormone therapy (Pre-HT) using antiestrogens or aromatase inhibitors has been also applied to patients, particularly elderly patients, with HR positive status [24–26]. Pre-HT focuses upon characterizing hormone sensitivity rather than achieving pCR. Recent data using a 3rd generation of aromatase inhibitors showed that Pre-HT is able to induce a remarkable shrinkage of the primary tumor in about 60% of the patients, who would have a higher chance of receiving BCS compared with the tamoxifen control, and also provides crucial information on the behavior of biological markers such as progesterone receptor (PgR) and Her-2 and on those values as predictive markers. In fact, PgR tended to be down-regulated by an aromatase inhibitor, letrozole, whereas tamoxifen up-regulates PgR. In addition, it was also found that Her-2 positive patients were more likely to respond to the aromatase inhibition, compared with Her-2 negative patients. It is impossible to obtain such information from situations other than preoperative treatment. Since it seems unlikely that Pre-HT drives pCR as frequently as Pre-CT, it is interesting whether the responders to Pre-HT are compatible with subgroup patients who obtain survival advantage from long-term exposure to hormone therapy or not. Though enough clinical evidences have not been accumulated yet, several studies have already dealt with this issue and described promising result preliminarily [27].

PROBLEMS OF PREOPERATIVE SYSTEMIC TREATMENT

Two major concerns, over-treatment due to the uncertainty of pre-treatment status of the disease and delay of regional control due to resistance to the primary systemic treatment, might be raised for preoperative systemic treatment (Table 1). In fact, two major factors including histologic nodal status and histologic grade, out of five prognostic or predictive markers recommended by consensus meetings, remain unknown before starting preoperative therapy. In particular, the uncertainty of nodal status might be a big issue because half of the node-negative patients are classified as having minimal-risk of relapse in many international guidelines. As to the delay of local control, a possible disadvantage was pointed out based on an analysis on the biostatistical model [28]. Nevertheless, no report has demonstrated that the resistance to primary systemic therapy results in disadvantage for survival [17]. The only concern would be that local recurrence might increase slightly in patients who underwent BCT after Pre-CT. This slight increase was recognized in premenopausal patients, whereas no increase was seen in postmenopausal patients. The risk factors of local relapse were totally identical between Pre-CT and Post-CT patients. Other concerns such as induction of resistance to the treatments and the increase of complications during postoperative radiotherapy are also raised, however, to the best of our knowledge, no clear evidence of this has been shown to date.

NODAL STATUS IN AXILLA

Since the presence of histologic metastasis in the axilla is the most reliable marker of systemic spread of the disease, and it is uncommon to change the policy of systemic adjuvant therapy due to the number of involved nodes, it is critical to identify the absence or presence of metastasis in the nodes. For the purpose of deciding the schedule of adjuvant therapy, it is widely accepted that either information from fully dissected nodes or biopsied sentinel nodes is available [29]. After Pre-CT, the positive rate of histologic nodal metastasis decreases significantly in pCR patients as compared to non-pCR patients. In the NSABP B-18 trial, the positive rate of pCR patients was 13%, whereas that of non-pCR patients was 44% [16]. A possible explanation for this data may be that Pre-CT diminished metastatic cells in the nodes completely, however it is unclear if pCR might be more likely to occur in originally node-negative cases rather than originally node-positive cases. Sentinel node biopsy (SNB) has come to be used very frequently in practice [30]. Even though it has not been proven yet whether radiotherapy to the axilla is an alternative to full dissection for sentinel node negative cases or not, a number of patients with small-size tumors choose radiotherapy to avoid surgical clearance [6]. This decision is supported by high true negative rate between SN examination and entire regional nodes examination. It is true that nodal status, the number of involved nodes, determined after Pre-CT, provides significant prognostic value similar to conventional implication of the nodal status. However, as described earlier, the real nodal status is unclear in the

most clinical trials investigating Pre-CT. In addition, there is another concern that nodal assessment by sentinel nodes after the preoperative systemic therapy might not be as accurate as that conducted before the treatment because the lymphatic route is altered occasionally by the treatment. Recent publications or reports have shown that the false negative rates range from 6% to 14% in Pre-CT cases. This may affect chances to avoid surgical clearance [31–33].

Novel Decision-Tree and Paradigm

A simple resolution to avoid over-treatment by Pre-CT would be to conduct SNB prior to starting any type of treatment. Although the limitation of several factors such as suspicious palpable node, multicentric tumor and history of surgery in the axilla mimicking the reliability of SNB still remains, it would reduce the risk of over-treatment for patients with minimal-risk of recurrence remarkably. In addition, it would help to understand the activity of primary systemic chemotherapy for node-negative cases. Patients have to stand for small surgical biopsy, but the merits out-weigh the demerit.

In addition, we need to consider more seriously the indication of Pre-HT. Pre-HT is extremely beneficial for patients with hormone-dependent tumors because of minimal toxicity. At present, however, very limited data is available to compare the benefits between starting with Pre-CT followed by unselected Post-HT or starting with Pre-HT followed by selected Post-HT according to the response to the Pre-HT, particularly for patients with highly hormone-dependent phenotype, such as post-menopausal status and both ER and PgR positive status.

Recently, we designed a novel decision-tree and paradigm (Figure 2). The core concept of this tree is to confirm pre-treatment tumor stage more precisely and to realize more individualized treatment. From January 2002 we preliminarily used this decision tree for consecutive 177 primary breast cancer patients {Age: 31yrs–67yrs(mean: 50yrs), ER positive: 70.1%, Tumor size: 1.5 cm–8.5 cm (mean: 2.7 cm)}. Fifteen percent of clinical N0 patients were categorized to minimal-risk of relapse in St. Gallen criteria by pretreatment staging using SNB and CNB [5]. Since negative HR status, high histologic grade and younger age have been characterized as predictive markers for pCR by Pre-CT, the patients with those phenotypes would be a particular candidate for Pre-CT [34].

Clinical Trials

The real impact derived from the NSABP B-18 and B-27 studies would be that pCR might become a novel endpoint of chemotherapy. In conventional trials, improvement of disease free survival (DFS) or overall survival (OS) was only the endpoint of clinical trials for primary breast cancer. Thereby, clinical trials required long-term follow-up to prove the efficacy of new treatment modalities. However, if pCR would be recognized as a surrogate endpoint, the time for assessing would be markedly shortened. Though we have to maintain the survival course of the pCR patients in the NSABP B-27 study, it seems highly likely that pCR is a potent indicator of favorable prognosis. DFS and OS are possible core primary endpoints in future trials and a long-term toxicity profile has to be monitored carefully, however there is no reason to exclude pCR from primary endpoints according to the recent data. In the new paradigm, as shown in Figure 3, the increase in the ratio of pCR would be the first point in assessing the efficacy of new treatment or new concepts. The trials that fail to demonstrate certain pCR rates would end immediately, making the adjuvant trial system more efficient. For non-pCR cases, it is necessary to analyze the details of resistance mechanisms to determine the new target of treatment. Translational research by various methods including modern technologies will help to investigate the mechanism and to explore the novel therapeutic approach. At present, it seems very experimental with respect to the salvage treatment for non-pCR patients, except endocrine therapy. Considering the necessity of immediate evaluation for many new promising cancer drugs or therapies, the new concept and new paradigm are needed.

Future Perspectives

Pre- and post-treatment molecular profiling of the tumor and host would be key issues in realizing a more individualized therapy. For example, Her-2 status, determined either by fluorescence *in situ* hybridization (FISH) or immunohistochemistry, has been involved in the decision-making process of systemic adjuvant therapy, because Her-2 status is predictive for the response to anthracycline and herceptin [35–38]. Combined determination of Her-2 and topoisomerase gene amplifications might be useful predictor of response to anthracycline

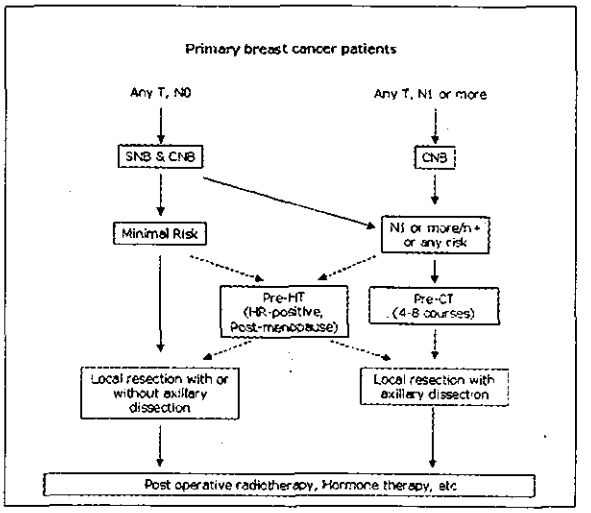
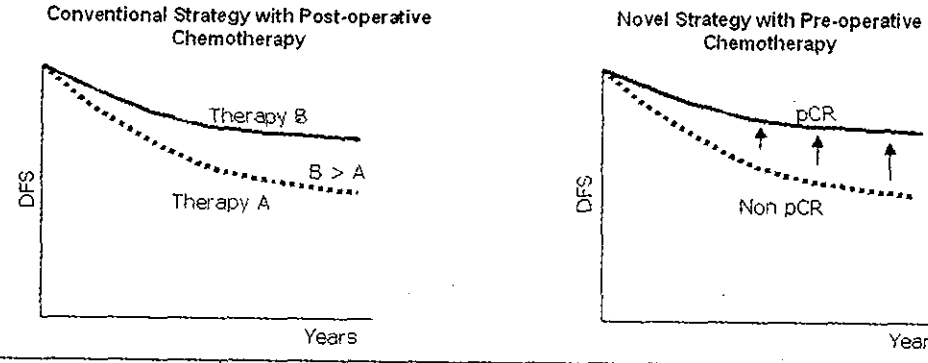


Figure 2. A novel decision tree in primary breast cancer.
T – tumor size; N – nodal status (TNM classification, UICC);
SNB – sentinel lymph node biopsy; CNB – core needle biopsy;
n+ – nodal involvement confirmed by pathological examination; Pre-HT – preoperative hormone therapy;
HR – hormone receptor; Pre-CT – preoperative chemotherapy.



	Conventional Strategy with Post-operative Chemotherapy	Novel Strategy with Pre-operative Chemotherapy
Required No. of Patients	Many (Randomized 2 or More Arms)	Relatively Few (Even One-Arm)
Major Endpoints	DFS / OS	Rate of pCR and DFS/OS
Major Indicator	Decrease in Risk of Recurrence	Increase in Rate of pCR
Required Time for Evaluation	More than 5 years	Even a few years
Cost	High	Relatively Low

Figure 3. Strategies for new therapy development in primary breast cancer. DFS – disease free survival; OS – overall survival; pCR – pathological complete response.

[39]. Herceptin use is limited for metastatic breast cancer at present, however, the accumulated administered dosage of anthracyclines does link to the cardiac toxicity of Herceptin. Tumour kinetics should also be analyzed at pre- and post-treatment. The sequential analysis enables us to determine the changes in tumor behavior, for instance, an increase of apoptosis index, by preoperative treatment quantitatively [40]. Other various alterations in molecular profiles, such as p53 mutation, the bcl-2 family, and in angiogenesis are also useful markers to be monitored [41–43]. From the point of drug resistance, pCR might be categorized as chemo-sensitive but other responses might be grouped as chemo-resistant, because only pCR links to survival benefit. Recent technologies including microarrays and proteomics allow precise assess meant of prognostic and predictive values [44,45]. According to a recent study, it was documented that a custom array analysis focusing on about 80 genes may be able to select patients who do not require any type of adjuvant systemic therapy. Furthermore, in order to realize these paradigms, it is important to be familiar with the handling of biomaterials, especially small samples obtained by CNB, because inappropriate handling results in unreliable data in these sensitive technologies. This point may not differe from the decision-tree, but it is crucial for working with new paradigms.

Conclusion

Still we need maturation of results on the preoperative therapy before taking the novel decision-tree as a standard for all stages of primary breast cancer. SNB is not readily available and not everyone has expertise in doing it. Nevertheless, it is also true that a small change such as the discovery of new diagnostic device or new therapeutic agent elicits a big impact on the tree. The decision-tree has to be biology-based, individualized and multidisciplinary in this regard. Overview analyses clearly demonstrated that survival benefits from antiestrogens treatment are only derived for estrogen receptor positive patients. Herceptin showed survival impact on Her-2 positive tumors. SNB is useful to identify node-negative cases. Primary preoperative systemic therapy is an option for primary breast cancer patients with invasive carcinoma at any stage. Pathological CR can be a novel surrogate marker of clinical trial, which promises an efficient new therapy development.

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Modulation of thymidine phosphorylase by neoadjuvant chemotherapy in primary breast cancer

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The combination effect of docetaxel and capecitabine on tumour response rate and survival was demonstrated recently in metastatic breast cancer patients. This combination was based on an experimental hypothesis that taxane can increase tumour sensitivity to the effect of capecitabine through the upregulation of thymidine phosphorylase (TP), which is responsible for the metabolism of 5-fluorouracil (5-FU) and its derivatives, including capecitabine. To examine the alteration in TP expression before and after neoadjuvant chemotherapy, 92 patients with primary breast cancer (T2-4N0-1M0) were enrolled in this study; 14 were treated with adriamycin and cyclophosphamide (AC) or epirubicin and cyclophosphamide (EC); 58 with 5-FU, adriamycin, and cyclophosphamide (FAC) or 5-FU, epirubicin, and cyclophosphamide (FEC); and 20 with FEC followed by docetaxel/taxotere (TXT-containing regimen). Thymidine phosphorylase upregulation was seen in 54.4% and 32.6% of patients in tumour cells and stromal cells, respectively. Increases in TP expression were found only in the AC/EC and TXT-containing regimen groups. In conclusion, it was strongly suggested that unlike 5-FU-containing regimens, the taxane and AC combination therapies upregulate TP expression in primary breast cancer. Thymidine phosphorylase upregulation by several anticancer drugs implies the importance of individualised strategies for sensitisation of tumour tissues to 5-FU and its derivatives.

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Thymidine phosphorylase (TP) is an enzyme that is responsible for nucleoside metabolism, antiapoptosis activity, and promotion of angiogenesis. Thymidine phosphorylase acts mainly in the salvage cascade of DNA metabolism in response to various types of stresses. Thymidine phosphorylase functions in the prevention of hypoxia-induced apoptosis according to recent experimental analyses (Ikeda *et al*, 2003). In addition, it has been documented that a metabolite of thymidine generated by TP, 2-deoxy-D-ribose (2-DDR), acts as a potent chemotactic factor on the endothelium, which results in the promotion of neovascularisation (Haraguchi *et al*, 1994). In fact, in a variety of tumour tissues, overexpression of TP was found to correlate significantly with an increase in neovascularisation (Toi *et al*, 1995; Tanigawa *et al*, 1996; Matsuura *et al*, 1999) and poor prognosis (Maeda *et al*, 1996; Takebayashi *et al*, 1996; Koukourakis *et al*, 1998; Toi *et al*, 1999).

The regulation of TP has been also studied from various points of view. Generally, TP is upregulated by stress such as hypoxia (Griffiths *et al*, 1997), radiation (Sawada *et al*, 1999), and chemotherapeutic damage (Sawada *et al*, 1998; Endo *et al*, 1999). Several types of cytokines such as interleukin (IL)-1, tumour necrosis factor (TNF)-α, and interferon (IFN)-γ also upregulate the expression of TP in both nonmalignant and malignant cells (Eda *et al*, 1993). Therefore, it is likely that these factors have important functions in stress-induced TP upregulation.

Thymidine phosphorylase has also been studied as a key enzyme involved in nucleoside metabolism. In particular, TP is known to be essential for the activation of capecitabine from the intermediate form 5′-deoxy-5-fluorouridine (5′-DFUR) to the active form 5-fluorouracil (5-FU). Experimental studies showed that 5′-DFUR is much more active in TP-transfected cells than in mock-transfected cells (Patterson *et al*, 1995; Evrard *et al*, 1999). It is also true that 5′-DFUR is more effective for TP-overexpressing tumour xenografts than for tumour xenografts expressing normal or low levels of TP (Morita *et al*, 2001; Ishikawa *et al*, 1998). Furthermore, several preliminary studies also confirmed that TP expression in tumour cells was a predictive factor for favourable prognosis in cancer patients treated with 5′-DFUR (Yamamoto *et al*, 1996; Ishii *et al*, 1996; Koizumi *et al*, 1999; Nishimura *et al*, 2002). In primary breast cancer, an analysis of the relationship between TP expression and the therapeutic effect of 5′-DFUR as a retrospective study in a prospective clinical randomised study has recently been reported, where patients who received no systemic adjuvant treatment were compared with those who received treatment with 5′-DFUR alone. It concluded that TP is a promising marker for predicting the survival benefit from 5′-DFUR treatment in early breast cancer patients (Tominaga *et al*, 2002).

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On the other hand, a hypothesis that TP modulation could enhance the therapeutic activity of 5′-DFUR/capecitabine has been tested at the experimental level. In various types of tumour xenograft models, the combination of capecitabine and various TP modulating chemotherapeutic agents achieved synergistic effects (Sawada *et al*, 1998; Fujimoto-Ouchi *et al*, 2002). Differences in the duration between the induction chemotherapy, with respect to TP modulation, and capecitabine treatment elicited different tumour responses, indicating that TP modulation is time dependent (Fujimoto-Ouchi *et al*, 2001) and that the timing of capecitabine treatment after the initial chemotherapy is important. In a clinical situation, it was demonstrated that therapy with capecitabine plus TXT achieved a significantly higher response and longer time to progression (TTP) than TXT therapy alone in the first-line treatment of metastatic breast cancer patients (O'Shaughnessy *et al*, 2002). This clinical finding would reflect on the basic hypothesis that TXT sensitises tumours to the effect of capecitabine. This suggests the importance of considering TP modulation from the point of sensitising breast cancer tumours to 5-FU derivatives such as capecitabine and 5′-DFUR, because the likelihood of their efficacy might be increased for TP upregulated tumours.

Issues related to TP modulation in human tumour tissues, however, are still largely unknown. Very few papers have touched on this crucial question. Thus, in the present study, we examined TP expression prior to and after the administration of chemotherapy in a neoadjuvant setting of primary breast cancer treatment. We will demonstrate that TP expression is modulated significantly by certain chemotherapies in a defined patient population.

MATERIALS AND METHODS

Patient characteristics

Between January 1, 1998 and December 30, 2002, women at the Tokyo Metropolitan Komagome Hospital and the National Kyushu Cancer Hospital who had primary, palpable, operable breast cancer (T2-4N0-1M0, according to the tumour, node, metastasis staging system) were included in this study. All patients were diagnosed by core needle biopsy or excisional biopsy prior to starting chemotherapy, and all patients were informed about the investigational nature of the study, which had been approved by the institutional review board. Written informed consent was obtained from each woman before entering her into the trial. All patients received either partial mastectomy or modified radical mastectomy with full dissection of axillary nodes after the treatment by neoadjuvant chemotherapy. Both biopsied and surgically resected samples were sufficient for accurate histological diagnosis and measurement of biomarkers.

Treatment regimens

Patients were treated with anthracycline-containing regimens or a taxane-containing regimen. The anthracycline-containing regimens consisted of adriamycin (ADR) and cyclophosphamide (CPA), (AC); epirubicin (EPI) and CPA (EC) or 5-FU, ADR, and CPA (FAC); and 5-FU, EPI, and CPA (FEC). Patients were given chemotherapy every 21 days with either the AC (ADR 50 mg m^{-2} and CPA 500 mg m^{-2}), EC (EPI 75 mg m^{-2} and CPA 600 mg m^{-2}), FAC (5-FU 500 mg m^{-2}, ADR 50 mg m^{-2}, and CPA 500 mg m^{-2}), and FEC (5-FU 500 mg m^{-2}, EPI 100 mg m^{-2}, and CPA 500 mg m^{-2}) or the TXT-containing regimen (FEC followed by TXT 75 mg m^{-2} or TXT 60 mg m^{-2}).

Efficacy assessment

Responses of the primary tumours to each chemotherapy regimen were evaluated according to the criteria established by the Japanese Breast Cancer Society (The Japanese Breast Cancer Society, 2000), which are essentially the same as those of the World Health Organization. A complete response (CR) is defined as the disappearance of tumour; partial response (PR) refers to a decrease in tumour size of 50% or more; no change (NC) indicates a decrease in tumour size of 50% or less or an increase of tumour size by less than 25%; and progressive disease (PD) indicates an increase in tumour size of 25% or more.

The grading of the pathological efficiency of chemotherapy, which was evaluated microscopically by a skilled pathologist, was also categorised according to the criteria established by the Japanese Breast Cancer Society (The Japanese Breast Cancer Society, 2000). The three grades are defined as follows: Grade 3 is the complete disappearance of variable cancer cells on the examined specimens; Grade 2, the apparent degeneration of two out of three or more of the population of observed cancer cells; Grade 1, the presence of degenerated cells in less than two out of three of examined tumour cells; and Grade 0, the presence of no degenerative cancer cells on specimens.

Immunohistochemical assessment

All samples were retrospectively processed with haematoxylin–eosin staining, negative control staining, and immunostaining for TP in our laboratory. Thymidine phosphorylase antibody was obtained from Roche Diagnostics (Basel, Switzerland), and the method for immunohistochemistry followed the protocol given in the immunohistochemistry kit 'Anti-TP Antibody, Formalin-Grade' (Roche Diagnostics Corporation, USA). The TP-stained slides were assessed for tumour cells and stromal cells according to the criteria defined in the kit. Staining intensities were scored as one of the four grades 0, 1+, 2+, and 3+, and staining patterns were scored as one of the five grades 0, 1+, 2+, 3+, and 4+.

Oestrogen receptor (ER) status progesterone receptor (PR) was also determined by an immunohistochemical method as described previously (Saji *et al*, 2002). Tumours containing 10% or more receptor-positive cells were scored as being receptor-positive.

Statistical methods

All patients with tissue staining data were included in the analysis. The statistical analyses for the TP-immunostained preparations were conducted as follows. The four grades of staining intensities were scored as 0, 1, 2, and 3. Similarly, the five grades of staining patterns were scored as 0, 1, 2, 3, and 4. Thymidine phosphorylase up- or down-regulation was evaluated as the difference between the sample score after chemotherapy minus the sample score prior to chemotherapy for each patient. Samples with score differences greater than 1 were evaluated as 'upregulated', and less than −1 as 'downregulated.' Score differences in the range between −1 and 1 were evaluated as 'no change.' Scores of staining intensities and staining patterns were analysed, and the summation of staining intensity and pattern scores were also analysed. After checking the distribution of the score differences, the *t*-test was used to compare the means.

For the contingency tables, Fisher's exact test was used to assess the potential different distribution. To relate the score differences with the treatment groups, we used the Mantel–Haenszel test for contingency tables and the *t*-test to compare the means. Since the known prognostic factors such as tumour size were distributed differently in each treatment group, tumour size was used as a stratified factor for both the Mantel–Haenszel and *t*-test. Bonferroni's correction was applied to adjust the *P*-values of the pairwise comparisons between each treatment group.

All analyses were carried out by using SAS 8.2, and alpha was set at 0.05.

RESULTS

Patient characteristics

A total of 92 patients were enrolled in this study. All the 92 patients were eligible and provided tissue staining results. The patient characteristics are shown in Table 1. Imbalances were observed for tumour size and number of patients, *n*, between the treatment groups, which would not affect the results of the present study, because no correlation was observed with TP regulation as reported below. At initial diagnosis, the average age of the women in this study was 51 years (range, 28–74 years). With respect to tumour size, those of 11 patients were less than 3.0 cm and those of 81 patients were greater than 3.1 cm. In all, 79% of patients had positive nodal status and 64.1% of patients had oestrogen-receptor-positive tumours.

Among the patients, 14 were treated with AC or EC, 58 were treated with FAC or FEC, and 20 were treated with the TXT-containing regimen.

Table 1 Patients' characteristics and overall response rate

		Regimen (%)			
Characteristics	***n***	**AC/EC**	**FAC/FEC**	**TXT**	***P*-value***
Menopausal status					
Pre	46	5 (10.9)	27 (58.7)	14 (30.4)	
Post	46	9 (19.6)	31 (67.4)	6 (13.0)	0.107
Tumour size					
−3.0 cm	11	0 (0.0)	2 (18.2)	9 (81.8)	
3.1 cm-	81	14 (17.3)	56 (69.1)	11 (13.6)	<0.001
Number of nodes involved					
0	19	4 (21.1)	5 (26.3)	10 (52.6)	
1–3	18	2 (11.1)	9 (50.0)	7 (38.9)	
4–	55	8 (14.6)	44 (80.0)	3 (5.4)	<0.001
Oestrogen receptor					
+	59	9 (15.3)	35 (59.3)	15 (25.4)	
−	33	5 (15.2)	23 (69.7)	5 (15.1)	0.571
Progesterone receptor					
+	39	6 (15.4)	26 (66.7)	7 (17.9)	
−	53	8 (15.1)	32 (60.4)	13 (24.5)	0.789
Cycle (median)	92	2–4 (4.0)	2–6 (3.0)	7–8 (8.0)	—
Response rate	92	50.0%	41.4%	70.0%	—
(95% CI)		(23.0–77.0)	(28.6–55.1)	(45.7–88.1)	—

AC = adriamycin (ADR) and cyclophosphamide (CPA); EC = epirubicin (EPI) and CPA; FAC = 5-fluorouracil (5-FU), ADR, and CPA; FEC = 5-FU, EPI, and CPA; TXT = docetaxel-containing regimen, CI = confidence interval, *Fisher's exact test.

Thymidine phosphorylase immunohistochemistry

We used the difference in each patient's tissue staining scores before and after chemotherapy to assess TP up- or down-regulation (Figure 1). Thymidine phosphorylase scores, staining intensities, and staining patterns from both tumour cells and stromal cells were available. No correlations were observed between the tumour and stromal scores. TP changes were seen in response to chemotherapy; TP levels in tumour and stromal cells were upregulated in 50 patients (54.4%) and 30 patients (32.6%), and downregulated in 15 patients (16.3%) and 29 patients (31.5%), respectively.

Table 2 shows the correlation between TP changes and patients' characteristics (Table 2A: tumour, 2B: stroma, respectively). An association between them was seen only in tumour size for stromal TP ($P = 0.020$). On the other hand, there were no significant differences for relationships for the number of nodes involved, ER status, or menopausal status.

Table 3 shows the relation between TP changes and treatment groups. TP changes were lowest in the FAC/FEC group and highest in the AC/EC group. Adjusted *P*-values of pairwise comparisons by Bonferroni's correction suggest that the TP score changes in the FAC/FEC group are significantly different from those in the AC/EC group (tumour: $P = 0.0001$, stromal: $P = 0.0001$). Nevertheless, no association was observed between scores of tumour and stroma, and the association with treatment regimen was similar for both tumour and stroma.

In the AC or EC group, TP was upregulated in the tumour and stromal cells of 92.9 and 85.7% of patients, respectively; however,

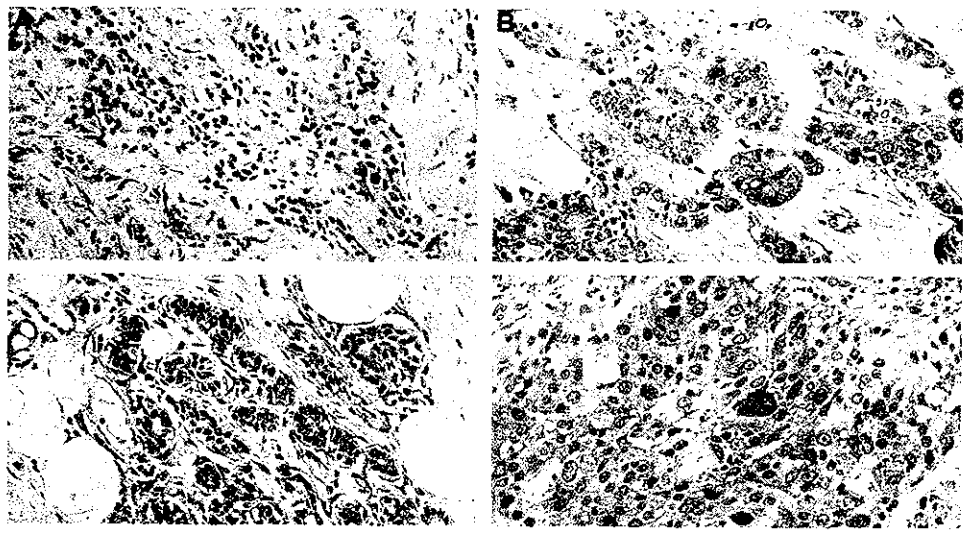

Figure 1 TP expression status of pre- and post-treatment. (**A**) An invasive ductal carcinoma: TP expression was upregulated remarkably by the treatment with FEC (5-FU, epirubicin, and cyclophosphamide) followed by docetaxel. Tumour TP score: pretreatment; 0 (upper), post-treatment; 7 (bottom), yielding a score difference of 7. The treatment achieved PR. (**B**) An invasive ductal carcinoma; TP expression was not changed remarkably by the treatment with FEC followed by docetaxel. Tumour TP score: pretreatment; 6 (upper), post-treatment; 5 (bottom), yielding a score difference of −1. The treatment achieved PR.

Table 2 Association of (**A**) tumour TP changes and (**B**) stromal TP changes with patients' characteristics

(A) Tumour TP changes

	Tumour TP			
	Up (%)	NC (%)	Down (%)	P-value*
Menopausal status				
Pre	29 (63.0)	9 (19.6)	8 (17.4)	
Post	21 (45.7)	18 (39.1)	7 (15.2)	0.122
Tumour size				
Median (range)	6.2 (1.5–18.0)			
−3.0 cm	6 (54.6)	2 (18.2)	3 (27.3)	
3.1 cm −	44 (54.3)	25 (30.9)	12 (14.8)	0.456
Number of nodes involved				
0	13 (68.4)	5 (26.3)	1 (5.3)	
1–3	9 (50.0)	5 (27.8)	4 (22.2)	
4−	28 (50.9)	17 (30.9)	10 (18.2)	0.578
Oestrogen receptor				
Positive	36 (61.0)	16 (27.1)	7 (11.9)	
Negative	14 (42.4)	11 (33.3)	8 (24.2)	0.157

(B) Stromal TP changes

	Stromal TP			
	Up (%)	NC (%)	Down (%)	P-value*
Menopausal status				
Pre	15 (32.6)	16 (34.8)	15 (32.6)	
Post	15 (32.6)	17 (37.0)	14 (30.4)	1.000
Tumour size				
Median (range)	6.2 (1.5–18.0)			
−3.0 cm	0 (0.0)	7 (63.6)	4 (36.4)	
3.1 cm−	30 (37.0)	26 (32.1)	25 (30.9)	0.020
Number of nodes involved				
0	10 (52.6)	3 (15.8)	6 (31.6)	
1–3	4 (22.2)	9 (50.0)	5 (27.8)	
4−	16 (29.1)	21 (38.2)	18 (32.7)	0.173
Oestrogen receptor				
Positive	21 (35.6)	20 (33.9)	18 (30.5)	
Negative	9 (27.3)	13 (39.4)	11 (33.3)	0.736

TP = thymidine phosphorylase; Up = upregulated; NC = no change; Down = down-downregulated; *Fisher's exact test.

TP was not downregulated in any patient. In the FAC or FEC group, tumour TP was upregulated in 41.4% of patients and downregulated in 20.7%. In the TXT-containing regimen, tumour TP was upregulated in 65.0% of patients and downregulated in 15.0%.

Clinical response rates

Of the 92 patients available for analysis, an overall response rate (ORR) of of 50.0%. (95% confidence interval (CI): 23.0–77.0%) was achieved by patients who were treated with AC or EC, an ORR of 41.4% (95% CI: 28.6–55.1%) by the patients treated with FAC or FEC, and an ORR of 70.0% (95% CI: 45.7–88.1%) by those patients given the TXT-containing regimen, as shown in Table 1.

The relationship between ORR and TP status is shown in Table 4. There was no correlation observed between clinical response and TP status, for either tumour or stromal cells ($P = 0.383$ and $P = 0.461$, respectively).

Table 3 Tumour TP changes by each regimen

Regimen	n	Gain in TP score (mean)	Up (%)	NC (%)	Down (%)
AC/EC					
Tumour	14	4.3	13 (92.9)	1 (7.1)	0 (0.0)
Stroma	14	3.6	12 (85.7)	2 (14.3)	0 (0.0)
FAC/FEC					
Tumour	58	0.7	24 (41.4)	22 (37.9)	12 (20.7)
Stroma	58	−0.9	10 (17.2)	25 (43.1)	23 (39.7)
TXT-containing regimen					
Tumour	20	1.8	13 (65.0)	4 (20.0)	3 (15.0)
Stroma	20	0.0	8 (40.0)	6 (30.0)	6 (30.0)
Total					
Tumour	92	—	50 (54.4)	27 (29.3)	15 (16.3)
Stroma	92	—	30 (32.6)	33 (35.9)	29 (31.5)

Regimen compared	Tumour		Stroma	
	t-test	M–H	t-test	M–H
AC/EC vs FAC/FEC	<0.0001	0.0114	<0.0001	<0.0001
FAC/FEC vs TXT	0.2287	0.5700	0.0580	0.0021
AC/EC vs TXT	0.1527	0.5616	0.0339	0.7773

Up = upregulated; NC = no change; Down = downregulated; AC = adriamycin (ADR) and cyclophosphamide (CPA); EC = epirubicin (EPI) and CPA; FAC = 5-fluorouracil (5-FU), ADR, and CPA; FEC = 5-FU, EPI, and CPA; P-values with Bonferroni's correction, adjusted by tumour size; M–H = Mantel–Haenszel test.

Table 4 Relationship between TP changes and response

	n	Up (%)	NC (%)	Down (%)	P-value*
Tumour					
Responder	45	24 (53.3)	16 (35.6)	5 (11.1)	
Nonresponder	47	26 (55.3)	11 (23.4)	10 (21.3)	0.383
Stroma					
Responder	45	14 (31.1)	14 (31.1)	17 (37.8)	
Nonresponder	47	16 (34.0)	19 (40.5)	12 (25.5)	0.461

Up = upregulated; NC = no change; Down = downregulated; *Mantel–Haenszel test adjusted by tumour size.

Pathological response rate

Of the 87 patients available for analysis, a grade 2 response was achieved by 14.3%. of patients who were treated with AC or EC (95% CI: 1.78–42.8%), 12.1% of those treated with FAC or FEC (95% CI: 4.99–23.3%), and 6.7% of those treated with the TXT-containing regimen (95% CI: 0.17–32.0%). Overall, a grade 2 response of 11.5% (95% CI: 5.65–20.1%) was seen in this study. There was no significant correlation between the pathological responses of grade 2 and TP changes in both tumour and stromal cells ($P = 0.600$ and $P = 0.273$, respectively).

DISCUSSION

Although the predictive value of TP expression in tumour tissues has been studied extensively for 5-FU or 5-FU-containing treatments, there is still little known about changes in TP levels in human tumours in response to chemotherapy. In this study, we showed that TP expression is often enhanced in primary breast

tumours in response to neoadjuvant chemotherapy. In particular, we found that TP was frequently upregulated in response to treatment by an EC/AC- or TXT-containing regimen. These results seem to be compatible with the data for human cancer xenograft experiments where taxanes, CPA, and mitomycin-C showed the potent ability to upregulate TP (Sawada *et al*, 1998; Endo *et al*, 1999). TXT also caused TP upregulation as a neoadjuvant in advanced breast cancer patients (Kurosumi *et al*, 2000), a result that also seems to be compatible with the clinical data. Thymidine phosphorylase in tumour cells tended to be co-upregulated with TP in tumour-associated stromal cells such as macrophages, indicating a possible role for microenvironmental factors in this response. In previous studies looking at correlations between TP and various immune mediators in the human breast tumour microenvironment, TP expression was associated significantly with expressions of TNF-α (Leek *et al*, 1998), IL-1α (Eda *et al*, 1993), and monocyte chemoattractant protein (MCP)-1 (Saji *et al*, 2002). From the molecular profile, it is known that multiple copies of potential Sp-1 binding sites are clustered upstream of the start site for the initiation of TP transcription (Zhu *et al*, 2002). Therefore, it is possible that TP upregulation would be triggered by increases in the intratumoural concentrations of these immune mediators in response to chemotherapy. As chemotherapy causes massive damage in tumour cells, the immune cells, especially macrophages, are activated to eliminate the damaged cells. In this process, it is estimated that large amounts of chemical immune mediators are produced by tumour-associated macrophages in the tumour microenvironment. Since hypoxia and hypoglucose are also characterised as stimuli of TP expression (Griffiths *et al*, 1997), these physical factors might help to enhance TP expression in association with local hyper-cytokinaemia.

For those patients treated with FAC or FEC, the 5-FU-containing regimens, we found no increased frequency of TP upregulation after chemotherapy. There are at least two possible explanations for this phenomenon. Firstly, the high concentration of 5-FU might downregulate TP expression. It is known that high concentrations of pyrimidine substrate change or downregulate the expression levels of nucleoside metabolism enzymes. There are few reports investigating the effect of high concentrations of 5-FU on TP; however, this mechanism is likely to be involved.

Secondarily, 5-FU might selectively kill or suppress TP-over-expressing cells. Many basic and clinical studies have indicated that 5-FU-contaning regimens are more effective for TP-over-expressing tumour cells as compared with TP-less-expressing tumour cells (Fox *et al*, 1997; Evrard *et al*, 1999; Gasparini *et al*, 1999; Morita *et al*, 2001; Yang *et al*, 2002). Therefore, these two scenarios should be further studied. Thymidine phosphorylase is stress-induced and, basically, TP is shown to be an enzyme that contributes to cell survival, because 2-DDR, a metabolite of thymidine via TP, induces neovascularisation and contributes to antiapoptosis (Haraguchi *et al*, 1994). After exposure to chemotherapy, TP might also function as mechanism for survival by the tumour cells. Based upon this hypothesis, a sequential treatment consisting of TP-upregulating chemotherapy followed by TP-dependent chemotherapy, such as by capecitabine, might be a reasonable therapeutic approach. In fact, the combination treatment with taxane and capecitabine showed a synergistic effect in animal experiments (Sawada *et al*, 1998) and induced a significant improvement in the survival of metastatic breast cancer patients (O'Shaughnessy *et al*, 2002). Therefore, the examination of TP expression in detail might provide various ideas to consider about optimal combinations in dosage and timing between capecitabine and other chemotherapeutic drugs. For example, a TC or TAC regimen might be promising to induce maximal TP expression. Furthermore, in cases where TP is not upregulated after the initial chemotherapy, the subsequent capecitabine monotherapy might not be effective.

In conclusion, TP is frequently up- or down-regulated after EC/AC- or taxane-containing chemotherapy in primary breast tumour tissues. The upregulated levels of TP are less for 5-FU-containing regimens. Thymidine phosphorylase is indeed upregulated by several anticancer drugs in human breast cancer cells, including both tumour and stromal cells; however, there are variations in the level. Thus, it is important to consider an individual strategy for sensitisation of breast tumour tissues to 5-FU by chemotherapy through TP.

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A close association between alteration in growth kinetics by neoadjuvant chemotherapy and survival outcome in primary breast cancer

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Abstract. Few surrogate markers are available for predicting the survival benefit from chemotherapy in primary breast cancer. We examined tumor growth kinetics by assessing cytokeratin 18 neo-epitope (CK18NE), an apoptosis marker detected by M30 antibody and Ki-67 antigen, a proliferation marker detected by MIB-1 antibody in 72 primary breast cancer patients who underwent pre-operative anthracycline-based chemotherapy. Increase in M30 index and decrease in MIB-1 index after the exposure of 2 to 4 cycles of chemotherapy correlated significantly with pathological tumor response. Univariate survival analysis, conducted in the subgroup of 42 patients who underwent CAF (cyclophosphamide, adriamycin and 5-FU) therapy alone, showed that the patients with the high levels of M30 index (>35 counts/1000 tumor cells) and the low levels of MIB-1 index (<140 counts/1000 tumor cells) after chemotherapy had a remarkably favorable prognosis as compared with patients in other categories. In addition, the alteration in growth kinetics by the treatment showed a significant prognostic value. Multivariate analysis also confirmed that the post-treatment growth kinetics was an independent prognostic indicator. These findings suggest that the alteration in growth kinetics revealed by CK18NE and MIB-1 might be a surrogate marker for predicting the survival benefit from chemotherapy in primary breast cancer.

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Abbreviations: ER, estrogen receptor; PgR, progesterone receptor; 5-FU, 5-fluorouracil; ADR, adriamycin; Epi, epirubicin; CPA, cyclophosphamide, pre-CT, pre-operative chemotherapy

Key words: cytokeratin 18, M30, preoperative chemotherapy, apoptosis, breast cancer

Introduction

Chemotherapy cures a certain population of cancer patients (1). For instance, post-operative adjuvant poly-chemotherapy provides a significant annual reduction in chances of recurrence for patients with primary breast cancer. Nevertheless, it is still difficult to predict the survival benefit from chemotherapy individually without long-term follow-up. Although several molecules such as the p53 mutation have been raised as a surrogate of chemotherapeutic effect, most of them were characterized as resistance markers rather than sensitive markers for chemotherapy (2,3).

Recently, the NSABP B-18 trial, investigating the clinical impact of pre-operative chemotherapy (pre-CT) using 4-cycles of adriamycin (ADR) plus cyclophosphamide (CPA) for primary breast cancer patients, showed that pathological complete response (pCR) was a potent prognostic indicator for favorable prognosis (4,5). Clinical response, even clinical CR, did not show any significant prognostic value for long-term outcome. Since other pre-CT studies using different types of chemotherapy regimens have also confirmed the prognostic significance of pCR, pCR is regarded as a surrogate marker for identifying the chemo-sensitive subpopulation in primary breast cancer (6).

In addition to pCR, various markers are tested currently to explore the surrogate value for long-term therapeutic benefit from chemotherapy, because it is estimated that a chemotherapy-sensitive subpopulation might exist in non-pCR group. We have studied the changes of tumor growth kinetics elicited by chemotherapy for a similar purpose. Among apoptosis markers, we focused on the neo-epitope of cytokeratin 18 (CK18NE), which often emerges with chemotherapy. It is known that structural changes of cells occur during apoptosis mediated by proteases such as those of

the caspase family, which cleave a number of intracellular substrates. One of these substrates, CK18 is a major component of intermediate filaments of simple epithelial cells and tumor cells derived from such cells, and it is cleaved into proteolytic fragments by caspases during apoptosis. Recently, a monoclonal antibody, M30 was generated to identify CK18NE, mapping to amino acids 387-396, exposed after caspase cleavage during apoptosis (7-9). The previous immunohistochemical studies confirmed that M30 recognizes apoptotic cells that show cytoplasmic staining, while it does not recognize viable and necrotic cells (7,10,11).

In the present study, we analyzed tumor growth kinetics using CK18NE and cell proliferation antigen Ki-67, detected by MIB-1 antibody, and examined its changes by pre-operative anthracycline-based chemotherapy in primary breast cancer patients. The results will indicate that the alteration in tumor growth kinetics by pre-operative chemotherapy is useful for assessing long-term therapeutic benefit from the chemotherapy in primary breast cancer.

Materials and methods

In vitro study. In order to confirm the induction of M30 by chemotherapy, we used MDA-MB-231, a hormone-independent human breast carcinoma cell line. The cells were grown in 96-well plates in 200 μl DMEM 10% FCS overnight, the medium changed, and then doxorubicin was added to 1 μg/ml. NP40 was added to the medium (final 0.5%) at the times indicated to assay total accumulated CK18-Asp396 (in living cells still attached + in dead cells + released activity in medium), and 25 μl was assayed by M30-Apoptosense™ enzyme-linked immunosorbent assay (ELISA) kit (PEVIVA, Bromma, Sweden). ELISA was conducted according to the manufacturer's instruction.

Patients and tumor materials. Seventy-two women receiving pre-CT for untreated breast cancer in the National Kyushu Cancer Hospital and the Komagome Metropolitan Hospital between December 1992 and December 1999 were enrolled. Clinico-pathological features of the patients enrolled are shown in Table I. Pairs of breast cancer samples were obtained before and after pre-CT by excisional biopsy and radical operation. Informed consent was obtained according to the procedures of each institute. Both biopsied and surgical resected samples were sufficient for accurate histological diagnosis, measurement of apoptosis, proliferation and biomarkers. Out of 72 patients, 49 were treated with FAC regimen, adriamycin (ADR) 40 mg/m^2 at day 1 and cyclophosphamide (CPA) 400 mg/m^2 and 5-FU 400 mg/m^2 at day 1 and day 8 repeated every 3 weeks (q3), 9 with CEF, CPA 600 mg/m^2, epirubicin (Epi) 60 mg/m^2, 5-FU 600 mg/m^2 q3, 7 with AC, ADR 60 mg/m^2 and CPA 600 mg/m^2 q3, and 7 with EC, Epi 75 mg/m^2 and CPA 600 mg/m^2 q3. All the patients were without distant metastasis at the time of diagnosis and received operations with axillary dissection. Clinico-pathological data and response to pre-CT are summarized in Table II. Response to pre-CT was established by clinical assessment of breast mass according to the criteria of International Union Against Cancer. Survival analysis was performed in a subgroup of 42 patients who underwent FAC therapy alone pre- and post-operatively. These

Table I. Clinico-pathological data and response to pre-operative chemotherapy.

No. of patients	72
Median age (years)	51 (28-74)
Menopause	
Pre-menopausal	33
Post-menopausal	39
Pre-tumor size	
T1, T2	9
T3	26
T4	37
Pre-nodal status	
N0	16
N1	29
N2	16
N3	10
Unknown	1
Histlogical subtype	
Invasive ductal carcinoma	66
Invasive lobular carcinoma	1
Unknown	5
Estrogen receptor	
Positive	44
Negative	28
Progesterone receptor	
Positive	32
Negative	40
Chemotherapy regimen	
FAC	49
CEF	9
AC	7
EC	7
No. of cycles of preoperative chemotherapy	
2 cycles	29
3 cycles	26
4 cycles	14
≥5 cycles	3
Clinical response to chemotherapy	
CR	2
PR	29
NC	39
PD	2
Post-chemotherapy size	
t1, t2	24
t3	17
t4	30
Unknown	1
Pathological response to chemotherapy	
0	18
1a	34
1b	12
2	7
3	1

FAC, 5-FU, adriamycin (Adr) and cyclophosphamide (CPA); CEF, CPA, epirubicin (Epi) and AC, Adr and CPA; EC, Epi and CPA. CR, complete response; PR, partial response defined as a >50% reduction rate; NC, no significant change; PD, progressive disease.