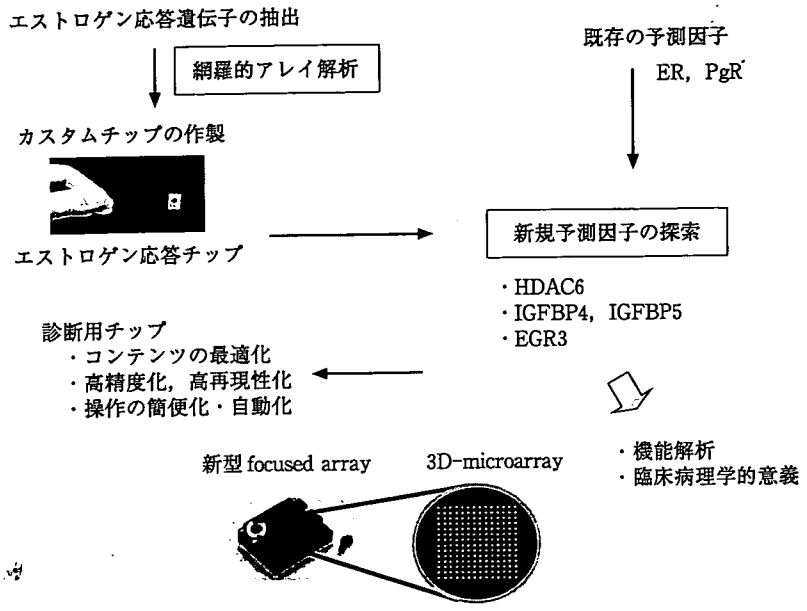

図2 内分泌治療感受性予測を目指した取り組み

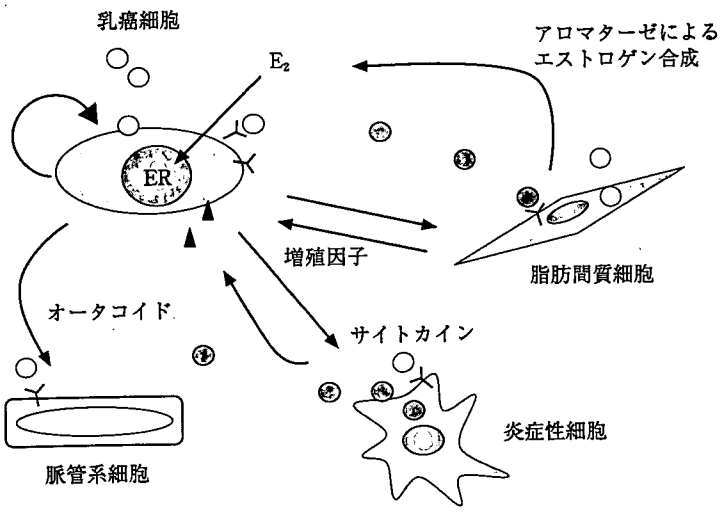

図3 乳癌の生存・増殖を支える局所のエストロゲンシグナルを中心とした微小環境

支えられている(図3). 著者らは実際にERを活性化する間質細胞の能力は症例ごとに異なっていることを最近明らかにした[11]. 高精度な治療奏効性予測にはこれらの癌微小環境の特徴，個性を考慮することは必須であろう. そこで，そのための新しい解析法の開発を進めており，エストロゲンシグナル活性を反映して蛍光を発する細胞やウイルスベクターを使用し，生検から得られた標本を用いて個々の乳癌の微小環境を解析する，あるいはアロマターゼ阻害剤の効果を *in vitro* で評価することが可能となった. 再現性，汎用性を高めることが今後の課題であるが，臨床応用に向けた研究を継続している.

5. 術前内分泌療法の感受性予測

近年，乳癌治療において化学療法を主体に術前治療が広範に行われているが，術前内分泌療法は術前化学療法とは単に用いる薬剤が違うだけでなく，生物学的背景が全く異なるため，その適応に対する考え方や期待される作用など，多くの異なった側面をもつ．ERの阻害，エストロゲン枯渇による治療という現在の内分泌療法は，その作用機序からして，元来，術前治療のような短期間での完全寛解を目指すものではない．しかし，pCRは得られなくても多くの症例で腫瘍の退縮がみられることは事実であり，その有用性は十分考えられ，今後の発展が期待される．現在の術前内分泌療法の効果予測は従来の臨床病理学的因子とホルモン受容体の有無しか確立されたものはないが，今後の発展のためにも新たな効果予測因子が求められる．内分泌療法反応性予測という点では，前述の術後補助内分泌療法の効果予測法ないしは因子と共通であることが期待できるが，術前治療の方が短期で治療効果との相関が検討できることから，研究としては結果を導きやすいと思われる．ただし，現時点では適応例が少なく，今後，質の高い系統的な臨床研究に付随したトランスレーショナル研究が必要である．現在は，術前治療そのものが術後補助療法の薬剤の選択，適応を決めるための試行的治療期間という側面ももっており，その後の補助療法の重要性を考えるとこれも極めて重要と思われる．

一方で，前述のように術前内分泌療法ではpCR率は低くても，総合的な奏効率は高いと思われるが，その治療効果を明瞭に示すことができる客観的判定法はない．現状では触診計測，画像(超音波，マンモグラフィ，MRI)計測によって腫瘍縮小を判定している．そこで，分子生物学的治療効果判定法として治療前の生検標本と治療後の標本におけるエストロゲンカスケードの下流遺伝子群の発現を比較することで，エストロゲンシグナルを標的とした治療(すなわち内分泌療法)が効果的であったかどうかを判定できないだろうか．著者らは数例の術前アロマターゼ阻害剤投与の症例から得られた治療前，治療後の標本を前述のエストロゲン応答性マイクロアレイで解析した結果，治療後に多くのエストロゲン応答遺伝子の発現が強く抑制されている症例とそうでない症例がみられることを観察した．ただし，エストロゲンシグナルが抑えられたからといって腫瘍が退縮するとは限らないという矛盾は，前項で述べたとおり理論的にもあり得ることであり，注意が必要である．この点，今後の更なる検討が必要である．

おわりに

内分泌療法は乳癌の生物学的特性に依存した治療法であるため，その治療効果は個々の腫瘍の生物学的個性に依存する．その個性の把握に個々の腫瘍のエストロゲンシグナル状態を知ることは重要であり，治療奏効性予測のためには必須と思われる．内分泌治療に限ったものではないが，遺伝子発現プロファイルから予後を判定しその結果を治療選択に反映していこうという臨床試験が欧米で行われている．その一つはマイクロアレイ解析研究から抽出した70遺伝子のセットを用いて解析し，予後不良のリスクの高い群，低い群を判定し，治療を選択する試みであり，もう一つは21遺伝子の発現をPCRによって判定(Oncotype DX™)し，同様に治療選択の指標にする試みである[12]．今後このような，ある程度まとまった数の遺伝子の発現をプロファイルという形で捕らえ，スコア化して予後予測や感受性予測の指標とするという手法が一般的に普及してくる可能性は大いにある．このような遺伝子診断の流れは乳癌の診断と治療の領域にパラダイムシフトをもたらすであろう．

大規模な臨床試験の結果に基づいた，より良い標準治療の確立とともに，一方で癌の個性を重視した治療も重要となってくるのは疑いない．すなわち患者ごとの個別化内分泌療法が検討されるべき時期になっているように思われる．今後の新規感受性予測因子の研究と先端技術に基づく診断技術の進展が期待される．

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原　著

3次元マイクロアレイ―乳癌の診断と治療効果予測への臨床応用を目指して

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東北大学医学部保健学科紀要　第16巻　第1号　2007年

東北大医保健学科紀要 16(1)：19～25，2007

原 著

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Three-dimensional Microarray for the Prediction of Response to Hormonal Therapy in Breast Cancer

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Key words: Breast cancer, Estrogen, and Microarray

Estrogen signaling plays crucial roles in genesis and malignant progression of breast cancer. Therefore, it is the most important molecular target for diagnosis and therapy of breast cancer. So far, we investigated the estrogen signaling pathway in breast cancer cells using estrogen-responsive DNA microarray. In this study, we assessed the performance of new microarray technology, 3-dimentinal (3D) microarray, to analyze estrogen signaling in clinical specimens for the future clinical application. 3D-microarray fixes DNA probes on PamChip, the steric microindentation platform. Therefore, 3D microarray is able to fix DNA probes on wide surface area, and thus it has many advantages such as high sensitivity and reproducibility, in comparison with conventional microarray. Furthermore, this system enables short time and automatic analysis from small samples such as biopsy specimens. First we analyzed the estrogen-responsive expression profile in breast cancer tissue using PamChip fixed DNA probes of estrogen-responsive genes, which previously we had identified. The clustering analysis of the results, this array Chip was able to classify the genes distinctly into ER-positive tissue or -negative tissue. Thereafter, we explored to find the minimum amount of the samples in RNA extraction that can keep reproducibility of the result. In

this 3D microarray system, it was proved that the 1×10^4 cells were enough to obtain the reproducibility of the analysis. These results indicate 3D-microarray will be a promising tool which clinically applicable for diagnosis from small amount of biopsy specimens.

はじめに

これまでの基礎研究の知見から，エストロゲンによる乳癌の発生・進展には，エストロゲンによって発現変動した遺伝子群による細胞の増殖促進，浸潤・転移能の獲得，抗アポトーシス作用，血管新生の促進作用[1]などが深く関与すると考えられている。その為，乳癌の治療にはエストロゲンシグナルをブロックするという戦略が有効と考えられ，古くは卵巣摘除，現在では各種ホルモン療法が積極的に行われ，実際に著効を示している。なかでも抗エストロゲン剤であるタモキシフェン（TAM）は二十数年来使用されてきた代表的なホルモン療法剤である[2][3]。また近年，新たに登場した第三世代のアロマターゼ阻害薬（AI）等，より奏効性の高い新規ホルモン療法が実用化されている[3][4]。現在のところ，これらホルモン療法の適応はER発現の有無，およびERの標的遺伝子であるプロゲステロン受容体（PgR）の有無が重要な判断指標となっている。ホルモン療法がERを標的としたものであることから，これはきわめて順当な効果予測因子といえる。しかしながら，実際にはER陽性例の30～40%でこの治療が奏効しないことが知られており[5]，また一方，一部ではあるもののER陰性ながら抗エストロゲン剤が奏効した例があることも知られている。さらに，ヒト乳癌患者の10～30%に過剰発現が認められるヒト上皮細胞増殖因子受容体type 2 (Her 2/neu)の有無によるTAMとAIの有効性に違いがあるといった臨床上の所見は，個々の患者におけるER活性化の多様性や個性の存在を示しており，より有効なホルモン療法の適応，あるいは他の分子標的治療法との使い分けおよび併用といった可能性を示唆している[6]。しかしながら，このような新たな展開に，既存の診断因子・予後因子だけでは適切な対応ができないのは明らかである。そこで，我々は各種ホルモン療法の高精度奏効性予測等の臨床応用と，乳癌におけるエストロゲンシグナル経路の解明を目指して，エストロゲン応答性に着目したマクロアレイ解析を広範に行い，このような目的に最適な遺伝子サブセットを同定し[7]，診断用チップを開発することを目的とした研究を行っている[8][9]。

これまでの，多くの癌研究は特定の遺伝子群の発現を解析することで，患者の層別化治療における奏効性や予後が予測可能であることを示唆し，1回のhybridizationで同時に多種類の遺伝子を解析可能なマイクロアレイは，その臨床応用が期待されている道具の1つである。しかしながら，実際にはhybridizationに時間がかかること（10時間），比較的煩雑な操作のため，実験者の手技が結果の再現性・信頼性に影響を与えがちであるなどの理由から，臨床での応用はまだまだ困難な状況にある。

そこで我々は本研究において新しいタイプのマイクロアレイプラットフォームである3次元(3D)マイクロアレイに臨床応用への可能性を求めた。3Dマイクロアレイシステムの最大の特徴は遺伝子捕捉のためのプローブを固相化する基板に，従来のスライドグラスやシリコンなどの平面基板ではなく，三次元構造を持つフロースルー型多孔質膜を採用していることにある（図1）。この多孔質膜にオリゴDNAプローブをスポットすることで，膜表面だけでなく多孔質膜内にも立体的にプローブ分子が固相化され，検出時にスポット当たりのプローブ分子密度を高めることができる。さらに，この膜にサンプル溶液を強制的に繰り返し透過させることで，hybridization時のサンプルとプローブの会合機会を高め，効率のよいhybridization反応を実現し，従来の1/10程度の反応時間（約100分間）で結果を得ることができる。加えて，3Dマイクロアレイシステムでは，サ

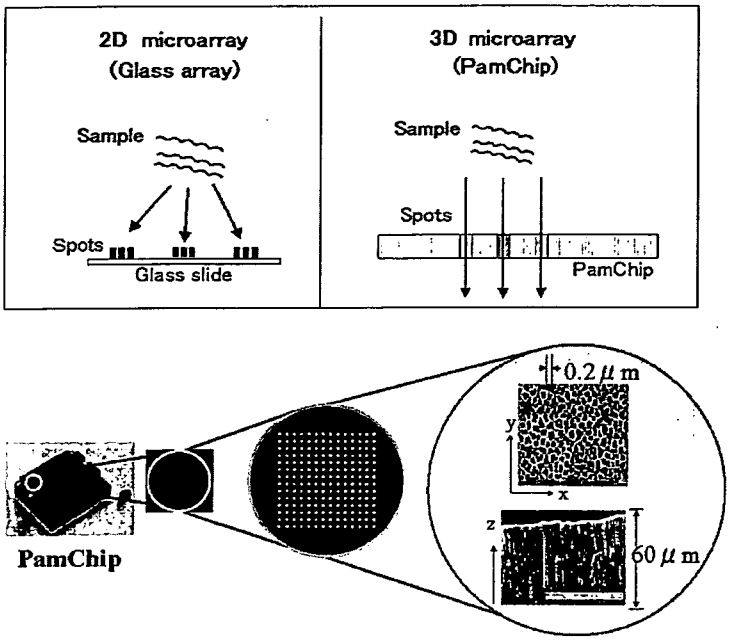

図1. 3Dマイクロアレイの基板構造

ンプル溶液をPamChipに滴下するだけの簡単な操作でhybridizationからシグナル検出・解析までの工程を，あらかじめ設定した反応条件に従って1台の装置でほぼ自動的に実施することができ，簡便な操作性と同時に，実験者の手技の習熟度に囚われない高い再現性を実現している[12]。また，アレイ解析を臨床診断に応用するにあたり，必要サンプル量の微量化は非常に重要である。たとえば針生検サンプルのような微量な検体でも解析可能となれば，術前検査にも応用可能となる。3Dマイクロアレイでは三次元構造基板と工程の自動化のメリットをいかし，hybridization反応後に，アレイ上で酵素による蛍光シグナル増幅を実施することで，従来の1/100～1/1,000程度のサンプル量でも(標識核酸：10～50 ng)，アレイ解析を実施することが可能となった[12]。

しかしながら，アレイ自体の精度は上がったが，total RNA抽出時の必要な組織または細胞といったサンプル量，およびそのサンプル量に起因したアレイの再現性は現在確認されていない。本稿では，total RNA抽出に用いる細胞量が与えるその後のアレイの再現性に注目し，エストロゲン応答遺伝子サブセットを載せたPamChipを用いて，3次元マイクロアレイシステムの臨床応用への可能性を検討した。

材料および方法

1. 細胞と細胞培養

ヒト乳癌細胞MCF-7は10% FCS (Tissue Culture Biologicals, Turale, CA) およびペニシリン/ストレプトマイシン(BIBCO, MD, USA)添加RPMI 1640培地 (GIBCO, NY, USA) で，5% CO_2, 37°Cに調製したCO_2インキュベーターで培養した。

2. 組織からのRNA抽出

−80°Cにて保存された乳癌手術摘出検体を乳鉢に液体窒素と共に入れ，粉々になるまですりつぶした。RNA抽出はISOGEN (Nippon gene, Tokyo, Japan) を用い，後述の通り行った。乳鉢ですりつぶした検体にISOGENを1 ml加え，5分間室温で静置後，0.2 mlのクロロホルムを添加した。よく懸濁後，3分間室温で静置し，高速遠心

(12,000 g, 4°C, 15 分間)をかけた。遠心後，上清を回収し，0.6 ml のクロロホルムを添加，再びよく懸濁後，3 分間室温で静置したのち，高速遠心(12,000 g, 4°C, 15 分間)を行った。上清を回収後，等量のイソプロパノールを加え，高速遠心を行うことで，RNA を濃縮した。また DNA 残渣を除くため DNase I 処理を行った。なお，本研究では乳癌手術検体を用いた実験は全て埼玉県立がんセンターにて行い，乳癌手術検体は，埼玉県立がんセンターにおいてインフォームドコンセント後，同意が得られたもののみを使用し，機関の倫理委員会が定めた手続きに従ったものである。

3. 培養細胞の RNA 抽出

MCF-7 細胞を培養皿に播種後，約 80% コンフルエントになるまで培養した。細胞数は 1% トリプシン/EDTA で細胞を剝がし，トリパンブルーで染色後，血球計算板にて計測し，1×10^1, 1×10^2, 1×10^3, 1×10^4 および 1×10^5 cells 懸濁液を調製した。RNA 抽出は RNeasy キット (QIAGEN, Hilden, Germany) を用いて行った。まず，遠心(300 g, 5 分間)にて細胞を沈渣として得，そこに 350 μl の RLT buffer (含 1% 2-メルカプトエタノール)を加え，1 分間 vortex をかけた。70% エタノールを 350 μl 添加後，全量を RNeasy ミニスピンカラムに添加し，遠心をかけた。RW1 をカラムに 700 μl 添加し，遠心した。さらに RPE buffer を添加後遠心，この作業を 2 回繰り返した後，30 μl の RNase・DNase free 水を添加し，遠心後の flow-through を total RNA 溶液として回収した。また，この時の total RNA 濃度はナノドロップ (NanoDrop Technologies Inc., DE, USA) を用いて測定した。

4. マイクロアレイに用いる RNA の合成 (cDNA の合成・aRNA の合成)

細胞から抽出した (組織；2 μg, 培養細胞 10-1,000 ng) total RNA より，MessageAmp™ aRNA kit (Ambion, Austin, TX) を用い，取扱説明書にしたがって蛍光標識 aRNA の合成を行った。aRNA 溶液は Fragmentation Reagent (Ambion, Austin, TX, USA) を用いて，70°C で 15 分間処理することで断片化した。断片化 aRNA の溶液を Microcon YM-30 (MILLIPORE, MA, USA) を用いて RNase・DNase free 水に置換した後，ディネーチャー処理(95°C, 15 分間)を行った。なお，aRNA 濃度はナノドロップを用いて測定した。

5. 3 次元マイクロアレイとその解析

アレイの基板である PamChip (OLYMPUS, Tokyo, Japan) は，林らが同定した乳癌におけるエストロゲン応答遺伝子群を固着してあるものを使用した[7)10)]。ディネーチャー処理後の 50 ng の aRNA 溶液 (37.5 μl) に 7.5 μl の 20×SSPE (0.2 M sodium phosphate, 3.0 M NaCl, 0.02 M EDTA, pH 7.4) と 5 μl の 10% SDS を添加したものを PamChip に全量 (50 μl) 添加し，hybridization を行った。Hybridization は 3 次元マイクロアレイシステム機器の FD10 (OLYMPUS, Tokyo, Japan) を用いて 40°C で 150 cycle 溶液を駆動しながら行った。また，アレイ画像解析も FD10 によって行われた。クラスター解析は Michael Eisen ら[11)] の cluster と tree view の software によって行われた。

結果および考察

1. 組織検体を用いた 3D マイクロアレイ解析

まず，我々は PamChip に載せたエストロゲン応答遺伝子の動向を調べるため，エストロゲン受容体 (ER) 陽性および陰性検体，各 2 例の乳癌組織から得た RNA を用いて 3D マイクロアレイ解析を行った。約 40 mg の乳癌手術後標品を，乳鉢を用いてすりつぶした後，ISOGEN によって RNA を抽出したところ，total RNA は各約 8.8 μg 回収された。このうち各検体，2 μg の total RNA から aRNA を合成し，3D マイクロアレイ画像解析後，さらに 4 検体の各遺伝子の輝度平均値をコントロールとして用い，その遺伝子発現比をクラスター解析したところ，図 2 に示すように ER の陽性検体では遺伝子発現が高いものが多く，陰性のものでは低いものが多く，2 つの系統に分類された。よって，本アレイシステムは，個々の乳癌患者におけるエストロゲン感受性の把握に役立つかもしれない。しかしながら，これらは各

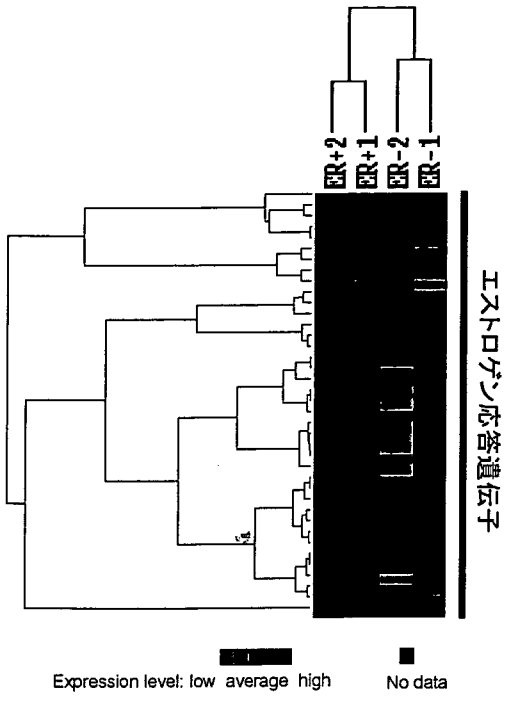

図2. エストロゲン受容体陽性および陰性乳癌組織を用いた3Dマイクロアレイ解析結果に基づくクラスター解析　エストロゲン受容体(ER)陽性および陰性乳癌組織，各2検体からISOGENを用いてRNA抽出後，エストロゲン応答遺伝子を搭載したチップを用いて，3Dマイクロアレイ解析を行った。ER陽性検体と陰性検体は2つのクラスターへと分類された。

2検体のみの解析結果であり，今後更なる検討が必要である。また，今後，針生検といった微量検体におけるマイクロアレイ解析に耐えられるRNA抽出が可能であれば，患者への侵襲を少なくできるだけでなく，患者個々に最適な治療および予後の診断，さらには予防診断といった今後の医療現場における診断法となり得る可能性を持つ。

2. 3Dマイクロアレイ解析限界の検討

そこで，我々は供用検体の少量化を探るため，マイクロアレイにおいて安定した解析結果の得られる細胞量とtotal RNA量を検討した。ISOGENによるRNA抽出は検体が大きな時には良い方法であるが，微小検体からのRNA抽出では，抽出作業の途中でエタノール沈殿法を用いるため，RNAの損失が起こるため適さない。従って，検討におけるRNA抽出は核酸吸着カラムを用いた，RNeasy mini kitによって行った。10倍の段階希釈によって細胞数を1×10^1〜1×10^5個まで設定し，total RNAの抽出を行ったところ，その回収量は当然のことながら細胞数に比例して減少した(表1)。また，一見10個の細胞からもaRNA合成に足るRNAが得られたようにみえるが，その純度(260 nm/280 nm比)は細胞数1×10^4個以下で悪くなった。従って，total RNAの調整は細胞数1×10^4個以上からが望ましいと考えられた。乳癌検査で施行されるCore needle biopsyでの組織採取量はおよそ20 mgであり，FNA (Fine Needle Aspiration)法では数ミリグラムと予想され，今回の検討の際のMCF-7細胞数1×10^4個は0.6 mg以下だったので本法によるRNA抽出量は3Dマイクロアレイ解析に充分耐えうるだろうと思われる。実際，3検体ではあるが，本法によってRNA抽出を行ったところ，0.22〜0.36 μgのtotal RNAが得られたことを確認している。

表1. 細胞数における抽出total RNA量

細胞数	抽出Total RNA量 (μg)	260/280
1×10^1 cells	0.048	3.96
1×10^2 cells	0.277	1.60
1×10^3 cells	0.255	1.56
1×10^4 cells	0.249	2.04
1×10^5 cells	2.421	2.02

また，合成開始時のtotal RNA量が異なるとaRNA増幅率が変わりマイクロアレイ解析結果に再現性が得られなくなってしまうのではないかと考え，次にaRNA合成時に用いる，total RNAの量について検討した。細胞数1×10^5個の細胞から抽出したtotal RNAの10, 100および1,000 ngを開始材料としてaRNA合成を行い，エストロゲン応答遺伝子を載せたPamChipを用いて3Dマイクロアレイシステムによる解析を行った。その結果，100および1,000 ngではaRNAの増幅率に差はほとんどなく，少なくとも100 ng以上1,000

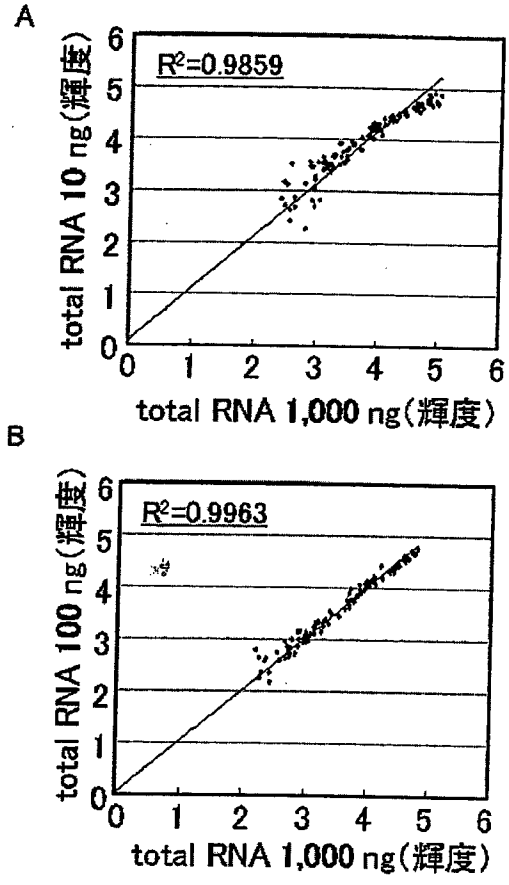

図3. aRNA 合成時に用いる total RNA 量がアレイ結果の再現性に及ぼす影響　MCF-7 細胞数 1×10^5 個から RNeasy mini kit を用いて抽出した total RNA 量を 10～1,000 ng まで 10 倍の段階希釈し，aRNA 合成に用いた。合成した aRNA を 3D マイクロアレイ解析し，(A) 10 ng と 1,000 ng のときの再現性，(B) 100 ng と 1,000 ng のときの再現性を比較した。

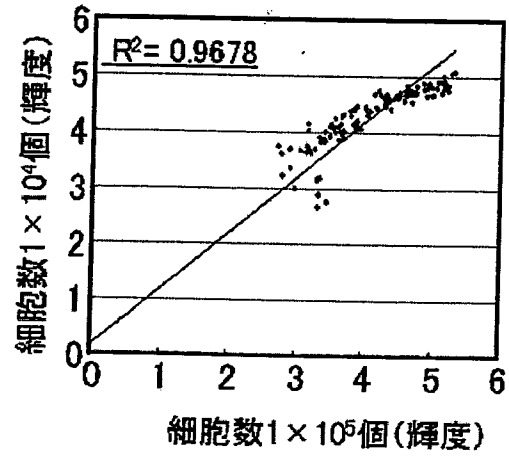

図4. Total RNA 抽出時に用いる細胞量がアレイ結果の再現性に及ぼす影響　MCF-7 細胞の 1×10^4 個と 1×10^5 個から，それぞれ RNeasy mini kit によって RNA 抽出を行った。その後，各検体をもとに 3D マイクロアレイ解析まで行い，RNA 抽出に用いる細胞量によるアレイの再現性への影響をみた。

ng 以下の total RNA から aRNA を合成すれば，3D マイクロアレイ解析結果に確実に再現性を得られることが判明した（図 3)。

そこで，細胞数 1×10^4 個から抽出した total RNA と細胞数 1×10^5 個の細胞から抽出した total RNA の 100 ng からの 3D マイクロアレイ解析を行ったところ，図 4 に示すように，決定係数 0.9678 と高い相関を示したものの，若干の再現性を失った。このことは，マイクロアレイにおける再現性の key point が total RNA の抽出作業にあることを示した。しかしながら，これは厳しく追求した場合であり，実際の臨床応用を含む研究に用いるには充分な再現性が確保された系であるといえる。

本研究から，針生検といった微量な検体からもマイクロアレイ解析に用いることの可能な mRNA の抽出が行えることが示唆され，さらに 3D マイクロアレイシステムによる hybridization 反応以後の自動化によって，結果に高い再現性が得られることが確認された。

近年，ますます発展してきた医療技術や製薬剤の普及のもと，施行される医療は複雑となり，患者個々に対応した治療法が求められている。したがって患者から最小の侵襲でいかに多くの正確で有用な情報を得られるかは今後きわめて重要である。マイクロアレイは一度に多くの遺伝子の発現状況を調べることのできる手法であり，研究レベルでは広範に用いられるようになって来た。ここで示したような 3D マイクロアレイシステムを用いることで微量な細胞からの解析が可能と考えら

れ，組織採取の際の患者への侵襲を少なくできるだけでなく，患者個々に最適な治療選択および予後診断，さらには予防のための検査といった今後の医療現場における新しい検査法となり得る可能性が考えられる。

おわりに

3Dマイクロアレイを用いる際，aRNAの調製に用いるtotal RNA量は100 ng以上1,000 ng以下であることが望ましい。また，RNA抽出時の細胞数は1×10^4 cells以上あることが望ましく，これは乳癌検査で施行されるCore needle biopsyでの組織採取量からも十分に3Dマイクロアレイ解析が許容となるmRNA量が抽出可能であることを示唆した。

マイクロアレイの高感度化，すなわち少量検体からの解析は，検体採取の際の患者への侵襲を少なくできる。本研究の更なる発展によって3Dマイクロアレイ利用による，患者個々の遺伝情報に基づいた疾患の予防，治療への貢献を期待する。

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Breast Cancer Res Treat
DOI 10.1007/s10549-007-9842-y

EPIDEMIOLOGY

Economic evaluation of 21-gene reverse transcriptase-polymerase chain reaction assay in lymph-node-negative, estrogen-receptor-positive, early-stage breast cancer in Japan

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Received: 27 November 2007 / Accepted: 27 November 2007

Abstract The 21-gene reverse transcriptase-polymerase chain reaction assay with a patented algorithm is validated as a good predictor of prognosis and potential benefit from adjuvant chemotherapy for lymph-node-negative, estrogen-receptor-positive, early-stage breast cancer, while its high cost raises concern about how to finance it. Cost-effectiveness analysis comparing prevalent National Comprehensive Cancer Network (NCCN) guideline/St Gallen recommendation-guided treatment with the assay-guided treatment is carried out with budget impact estimation in the context of Japan's health care system. Incremental cost-effectiveness ratios are estimated as 2,997,495 ¥/QALY (26,065 US$/QALY) in the comparison between NCCN guided-treatment vs. the assay-guided treatment, and as 1,239,055 ¥/QALY (10,774 US$/QALY) in the comparison between St Gallen guided-treatment vs. the assay-guided treatment. Budget impact is estimated as ¥2,638 million (US$23 million) to ¥3,225 million (US$28 million) per year. The routine use of the assay is indicated as cost-effective. And the budget impact could be judged as within fundable level.

Keywords Breast cancer · Budget impact · Cost-effectiveness · Gene diagnosis · 21-gene signature · Tailor-made medicine

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Introduction

In recent years, the medical profession as well as the general public have become to have high hopes for the future of "tailor-made medicine", which means individualised treatment according to each patient's pathology, especially using gene diagnoses or biomarkers [1]. And this is the case with cancer care in Japan, as well [2].

Regarding breast cancer care, the role of adjuvant chemotherapy for lymph-node-negative, estrogen-receptor-positive, early-stage breast cancer (LN−, ER+, ESBC) in order to prevent or delay distant recurrence after primary surgery has been debated [3–6], while the use of hormonal therapy with tamoxifen or aromatase inhibitors in those cases is established by several large randomised clinical trials [7, 8]. Efforts to aggregate available evidences have been made in order to best guide the clinical decision of whether to add chemotherapy or not, which result in the development of consensus guidelines, such as National Comprehensive Cancer Network (NCCN) guideline [9, 10] or St Gallen recommendation [5]. These guidelines evaluate patient's risk of recurrence based on factors such as age, tumour size and histology, and then suggest the indication for adjuvant chemotherapy to higher risk patients

based on a judgement that the benefit of survival from chemotherapy overweighs the disbenefit of adverse effects and medical risks [11]. However, the risk classification which underlies this judgement has been considered as not certain nor specific enough, so that it leaves a room for the development of a more accurate and individualised predictor of the risk of recurrence.

A multigene assay of resected breast cancer tumour tissue was implemented in order to realise more informed and individualised decision for adjuvant chemotherapy indication, which resulted in the development of the 21-gene reverse transcriptase-polymerase chain reaction (RT-PCR) assay with a patented algorithm (Oncotype DX® Breast Cancer Assay). It gives an individual case of LN−, ER+, ESBC Recurrence Score (RS) that represents individualised risk of recurrence. The accuracy of RS as criteria in assessing the risk of recurrence was validated by a prospective study of historical clinical trial data from National Surgical Adjuvant Breast Cancer Project (NSABP) B-14 study with the gene assay of preserved tumour tissue [12]. Furthermore, the accuracy of RS in predicting the magnitude of chemotherapy benefit was validated by a similar study including data from NSABP B-20 study with the gene assay [13]. In other words, patients classified as high risk of recurrence by RS criteria are likely to be highly responsive to chemotherapy, which implies that the assay is clinically efficient in identifying those who could benefit from adjuvant chemotherapy.

This development is deemed as a pathway geared towards tailor-made medicine in breast cancer care, which anticipates a similar innovative assay like 70-gene signature (MammaPrint®) [14]. Yet another significant characteristic of the 21-gene RT-PCR assay is its high price, ¥450,000 (US$3,913; US$1 = ¥115), while the reimbursement for a conventional gene diagnosis test of malignant tumour is set at ¥20,000 (US$174) in the social health insurance system of Japan. Needless to say, a valuable innovation of technology deserves patent protection and accompanying financial rewards as its own right. However, from the viewpoint of economics, it is imperative to appraise the "value for money" of such highly priced new technology [15]. The proportion of LN−, ER+ cases among breast cancer is large, 28.7% [16], and the incidence of breast cancer is estimated as 41,494 in 2005 and increasing continuously [17]. Therefore, once the assay becomes a standard procedure within social insurance benefit package, more than 12,000 assays are expected to be implemented in a year. This leads to a concern about its implication for health financing. From the viewpoint of health manager, it is also imperative to appraise the "budget impact" [18], which basically correlates to the product of the price and the quantity of health services provided.

To date, there are two studies that look at economic aspects of the 21-gene RT-PCR assay based on validation studies in the U.S. health system. Hornberger et al. carried out an economic evaluation of the assay, and reported it as cost-saving based on a reclassification of patients' risk using RS criteria, instead of NCCN criteria [19]. Lyman et al. also reported that RS-guided treatment could be cost-saving compared to the treatment with tamoxifen combined with chemotherapy for all patients, and cost-effective compared to the treatment with tamoxifen alone for all patients [20]. There is no report from any other countries nor yet a comparison with St Gallen-guided treatment.

This study aims to evaluate cost-effectiveness and budget impact of the 21-gene RT-PCR assay in Japan's health care system. The results should be useful in considering the diffusion of the assay in Japan, and could inform health care policy in the era of tailor-made medicine in developed countries.

Methods

We conduct a cost-effectiveness analysis with decision trees and Markov modelling based on the validation studies of the 21-gene RT-PCR assay [12, 13, 21], and a costing under Japan's social health insurance system including a sensitivity analysis from societal perspective. We also estimate the budget impact of the assay on Japan's social health insurance system based on our economic model.

Scenarios and comparisons

Both Japanese clinical practice [22] and consensus guidelines [23, 24] are in accordance with NCCN guideline as well as St Gallen recommendation in a mixed way. And changing criteria from NCCN/St Gallen to RS in risk reclassifications with estimated distant recurrence free survival in 10 years ($DRFS_{10}$) were reported in one of the validation studies as shown in Table 1 [21]. (Since $DRFS_{10}$ of patients with intermediate risk according to St Gallen criteria was not yet published, we assume the mid-value of $DRFS_{10}$ between high risk and low risk classified by St Gallen criteria.) Three scenarios are set up in this study: a hypothetical cohort of LN−, ER+, ESBC at the age of 55 undergoes NCCN-guided treatment, St Gallen-guided treatment, and RS-guided treatment. The age of 55 is chosen according to the average age of equivalent patient population in a nationwide cancer registry [16]. The former two scenarios intend to depict the status quo of Japanese practice to some extent. The last scenario intends to illustrate the situation in which the 21-gene RT-PCR assay is applied routinely.

Table 1 Risk reclassification by the 21-gene RT-PCR[a] assay with expected $DRFS_{10}^{b}$

			Recurrence Score criteria		
			High risk	Intermediate risk	Low risk
NCCN[c] criteria	High risk	Probability	29%	22%	49%
		$DRFS_{10}$	0.70	0.86	0.92
		Range tested in sensitivity analyses	Change by ±50%	Change by ±50%	Change by ±50%
		Probability	6%	22%	72%
	Low risk	$DRFS_{10}$	0.57	0.82	1.00
		Range tested in sensitivity analyses	Change by ±50%	Change by ±50%	Change by ±50%
St Gallen criteria	High risk	Probability	36%	22%	42%
		$DRFS_{10}$	0.67	0.82	0.92
		Range tested in sensitivity analyses	Change by ±50%	Change by ±50%	Change by ±50%
		Probability	16%	23%	61%
	Intermediate risk	$DRFS_{10}$	0.62[d]	0.82[d]	0.96[d]
		Range tested in sensitivity analyses	Change by ±50%	Change by ±50%	Change by ±50%
		Probability	6%	22%	72%
	Low risk	$DRFS_{10}$	0.57	0.82	1.00
		Range tested in sensitivity analyses	Change by ±50%	Change by ±50%	Change by ±50%

Source: Reference [21]

[a] Reverse transcriptase-polymerase chain reaction

[b] Distant recurrence free survival in 10 years

[c] National Comprehensive Cancer Network

[d] Assumed as the mid-value of $DRFS_{10}$ between high risk and low risk classified by St Gallen criteria

Regarding the use of adjuvant chemotherapy, 100% of patients classified as high risk by NCCN/St Gallen criteria and 50% of patients classified as intermediate risk by St Gallen criteria are assumed to undergo chemotherapy, while 100% of patients classified as high or intermediate risk by RS criteria are assumed to undergo chemotherapy.

Then, the two pairs of scenarios are compared: NCCN-guided treatment vs. RS-guided treatment, and St Gallen-guided treatment vs. RS-guided treatment. These comparisons intend to depict the diffusion of the assay in Japanese practice. The use of chemotherapy decreases from 92 to 49% under the former comparison, and from 75 to 49% under the latter comparison by the adoption of RS criteria.

Decision tree and Markov model

We construct decision trees with Markov model of clinical courses followed by LN−, ER+, ESBC patients, which is shown in Fig. 1.

The decision tree 1 shows the comparison between NCCN-guided treatment vs. RS-guided treatment; and the decision tree 2 shows the comparison between St Gallen-guided treatment vs. RS-guided treatment. Decision nodes of these trees are as to a decision whether to apply the 21-gene RT-PCR assay or not. Following chance nodes discern the cohort to different adjuvant therapies depending on the risk classification and human epidermal growth factor receptor type2 (HER2) status. Since the use of trastuzumab for HER2 positive (HER2+) cases as adjuvant therapy is about to be included in the social health insurance benefit according to the results of international clinical trials [25, 26], we set up three types of adjuvant therapies: hormonal therapy (HT), HT plus chemotherapy (CT), and HT plus CT plus trastuzumab. Branches with CT lead to subtree B via a chance node, which discern the cohort to different toxicities.

The Markov model shows the clinical course once the adjuvant therapy is completed. Five stages are modelled here: (1) LN−, ER+, ESBC after criteria-guided adjuvant therapy; (2) Distant recurrence with response to treatment; (3) Distant recurrence with no response to treatment; (4) Progression of disease after distant recurrence; and (5) Death. Transitions between the stages are indicated with arrows. Patients follow various courses after recurrence, so conditions other than these five stages and transitions not described with arrows here are possible. However, we model the course in this way based on available reports of prognosis model of metastatic breast cancer, which is calibrated with the results of several randomised trials [19, 27]. Patients with recurrence undergo drug treatment with HT, CT, and/or trastuzumab depending on their status.

The span of each stage is set up at 1 year. Markov process is repeated up to 10 years, since the transitional probabilities of recurrence are calculated from $DRFS_{10}$ and

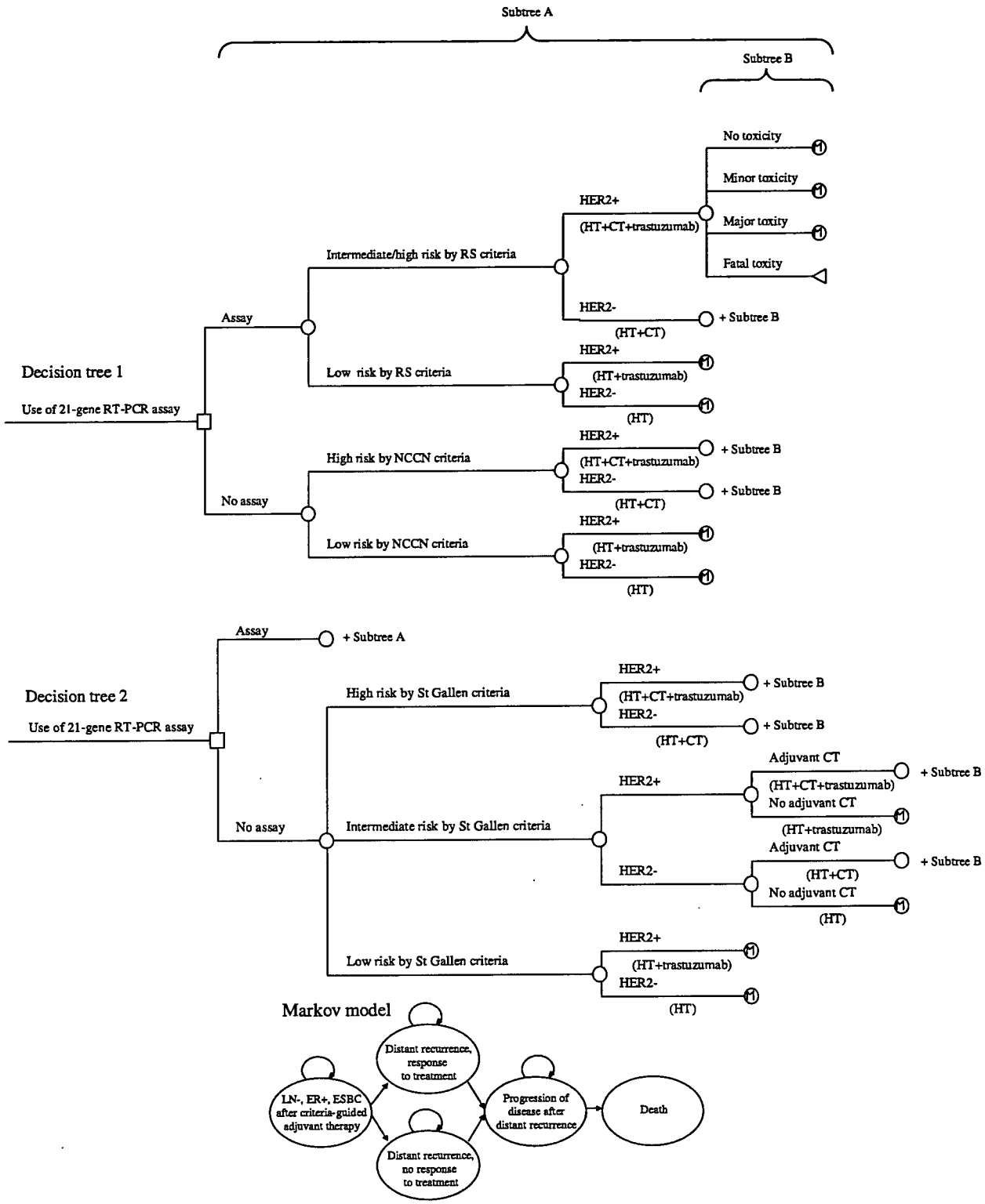

Fig. 1 Decision tree and Markov model. Abbreviations: Reverse transcriptase-polymerase reaction (RT-PCR), recurrence score (RS), human epidermal growth factor receptor type2 (HER2), hormonal therapy (HT), chemotherapy (CT), National Comprehensive Cancer Network (NCCN), lymph-node-negative, estrogen-receptor-positive, early-stage breast cancer (LN−, ER+, ESBC)

most of recurrences are expected to occur within this time horizon. After the 10-year, survived patients without recurrence are assumed to have a life expectancy for Japanese female at age 65 [28], and those with recurrence are to have a life expectancy of 2 years.

Outcome estimation

Outcomes by the scenario in terms of years of life saved (YOLSs) and quality adjusted life years (QALYs) are estimated by assigning probabilities and utility weights to the decision trees and Markov model from the literature.

Probabilities of risk classification, attached to the first chance nodes of each branch, are adopted from one of the validation studies of the 21-gene RT-PCR assay [21] shown in Table 1. Table 2 shows the other probabilities and utility weights used. A probability of HER2+, 9.3%, attached to the second chance nodes, is adopted from a nationwide breast cancer registry [16]. Probabilities of adjuvant chemotherapy toxicity, attached to the chance node in the subtree B, are assumed to be 60% for minor toxicity, 5% for major toxicity and 0.5% for fatal toxicity from a report of efficacy and cost-effectiveness of adjuvant chemotherapy in breast cancer [29].

Regarding the Markov model, transitional probabilities of recurrence with adjuvant HT are calculated from $DRFS_{10}$ in Table 1. The effectiveness of adding adjuvant CT and trastuzumab are incorporated as risk reduction of recurrence. Relative risk reductions resulted from CT among patients classified as high risk and intermediate risk by RS criteria are fixed at 74 and 39%, respectively, which are adopted from one of the validation studies of the 21-gene RT-PCR assay [13]. A relative risk reduction resulted from trastuzumab among HER2+ patients are assumed to be 36% for up to 2 years according to the results of clinical trial [26]. As mentioned earlier, transitional probabilities between stages after recurrence are adopted from prognosis model of metastatic breast cancer [19, 27]. It is assumed that the response to treatment and the prognosis after recurrence differ depending on HER2 status. Probabilities of the response to treatment for recurrence are fixed at 38.0% among HER2− patients and 54.0% among HER2+ patients [27]. Probabilities of the progression of disease after recurrence are also fixed at: 59.7% if HER2− and having responded to treatment, 53.7% if HER2+ and having responded to treatment, 98.3% if HER2− and not having responded to treatment and 88.5% if HER2+ and not having responded to treatment [19]. Probabilities of death after the progression of disease are fixed at 40.0% among HER2− patients and 37.2% among HER2+ patients [19].

In order to estimate the outcome in terms of QALYs, utility weights are chosen for various health statuses during the clinical course which patients follow. A weight for health status after adjuvant therapy without any toxicity or distant recurrence is chosen to be 0.98 [30]. Weights for toxicities are 0.90 for minor toxicity, and 0.80 for major toxicity [29], of which duration is assumed as 6 months. Health status during chemotherapy against the distant recurrence or the progression of disease weighs 0.50 [31], of which duration is assumed as 6 months. Health statuses after the chemotherapy weigh 0.84 if responded, 0.70 if stable and 0.49 if progressive [27].

Outcome is discounted at a rate of 3% [32].

Costing

From societal perspective, costing should cover the opportunity cost borne by various economic entities in the society. In the context of this study, costs borne by social insurers and patients are considered, since these two entities are major payers to health care providers under Japan's social health insurance system. The amount of direct payments by these entities, mostly according to the national medical care fee schedule, are estimated as costs, while costs to sector other than health and productivity losses are left uncounted in this study. This choice of scope in costing allows the following budget impact estimation.

Cost items are identified along the decision trees and Markov model: the 21-gene RT-PCR assay, adjuvant therapies, treatments for toxicity, monitorings, treatments for distant recurrence, and end-of-life treatments as shown in Table 3. As already mentioned, the cost of the assay is ¥450,000 (US$3,913), according to the price offered by Japanese supplier of Oncotype DX® Breast Cancer Assay. Costs of treatments except the end-of-life treatments are estimated by combining a model of breast cancer care and the national medical care fee schedule. The care model is developed based on both a nationwide survey of Japanese expert practice [22] and consensus guidelines [23, 24].

Adjuvant hormonal therapy includes outpatient care with tamoxifen, aromatase inhibitors, and LH–RH analogues depending on patient's status, and is assumed to continue up to 5 years, which costs ¥534,610 (US$4,649) per year. Adjuvant chemotherapy includes various regimens. Anthracycline-based combination chemotherapy is used for about half of the cases, and oral fluorinated pyrimidine and CMF (cyclophosphamide, methotrexate and 5-fluorouracil) therapy are frequently used among other regimens. These cost ¥343,001 (US$2,983). Adjuvant trastuzumab costs ¥3,105,120 (US$27,001) per year, of which administration is assumed to continue for 1 year.

There are three levels of toxicity in the decision tree. However, only the cost of major toxicity is estimated as ¥173,352 (US$1,507), which includes unplanned 1 month

Table 2 Probabilities and utility weights

	Base case value	Range tested in sensitivity analyses	Source
Probabilities			
Patient status			
HER2[a]+	9.3%	Change by ±50%	[16]
Adjuvant chemotherapy toxicity			
Minor	60.0%	Change by ±50%	[29]
Major	5.0%	Change by ±50%	[29]
Fatal	0.5%	Change by ±50%	[29]
Relative risk reduction of distant recurrence			
Chemotherapy			
Intermediate risk classified by RS[b] criteria	39.0%	Change 0–76%	[13]
High risk classified by RS criteria	74.0%	Change 47–87%	[13]
Trastuzumab	36.0%	Change 24–46%	
(Duration)	(2 years)	Change to 5 years	[26]
Response to treatment for distant recurrence			
HER2−	38.0%	Change by ±50%	[27]
HER2+	54.0%	Change by ±50%	[27]
Progression of disease after distant recurrence			
HER2−, response to treatment	59.7%	Change by ±50%	[19, 27]
HER2−, no response to treatment	98.3%	Change by ±50%	[19, 27]
HER2+, response to treatment	53.7%	Change by ±50%	[19, 27]
HER2+, no response to treatment	88.5%	Change by ±50%	[19, 27]
Death after progression of disease			
HER2−	40.0%	Change by ±50%	[19, 27]
HER2+	37.2%	Change by ±50%	[19, 27]
Utility weights			
After adjuvant therapy without distant recurrence	0.98	Change by ±20%	[30]
Toxicity			
Minor	0.90	Change by ±20%	[29]
Major	0.80	Change by ±20%	[29]
Distant recurrence			
Chemotherapy, 6 months only	0.50	Change by ±20%	[31]
Response to treatment	0.84	Change by ±20%	[27]
Stable	0.70	Change by ±20%	[27]
Progression of disease	0.49	Change by ±20%	[27]

[a] Human epidermal growth factor receptor type2

[b] Recurrence Score

hospitalisation in two-fifths of the cases and rescue treatment at outpatient clinic in three-fifths of the cases [33, 34]. The cost of minor toxicity, from which 60% of patients suffer, is included in the cost of adjuvant chemotherapy, since prophylactic use of antiemetic, for example, is applied routinely these days. And the clinical course of fatal toxicity is diverse and not fit to costing by modelling here, so its cost is estimated later coupled with the cost of end-of-life treatment.

Patients who complete adjuvant therapy are assumed to visit a clinic twice a year for the purpose of monitoring, which costs ¥25,340 (US$220) per year.

There are various options of treatments for the distant recurrence depending on regimens used in adjuvant therapy. Yet, we assume crossover hormonal treatments followed by capecitabine within the first year as typical first line and second line therapies for our hypothetical cohort, which cost ¥558,458 (US$4,856) per year. We further assume that this cost is applicable to second year and afterwards. For HER2+ patients, trastuzumab is additionally administered, of which cost is the same as one during the adjuvant therapy.

The end-of-life treatments are diverse in contexts and lack consensus guidelines or survey data. Its practice

Table 3 Costs

	Base case value	Range tested in sensitivity analyses
21-gene RT-PCR[a] assay (Oncotype DX® Breast Cancer Assay)	¥ 450,000	Change by ±50%
Adjuvant therapy		
Hormonal therapy, per year	¥ 534,610	Change by ±50%
Chemotherapy	¥ 343,001	Change by ±50%
Trastuzumab, per year	¥ 3,105,120	Change by ±50%
Treatment for toxicity		
Major	¥ 173,352	Change by ±50%
Monitoring		
After adjuvant therapy without recurrence, per year	¥ 25,340	Change by ±50%
Treatment for distant recurrence		
Hormonal therapy and chemotherapy, per year	¥ 558,458	Change by ±50%
Trastuzumab, per year	¥ 3,105,120	Change by ±50%
End-of-life, per year	¥ 1,315,143	Change by ±50%

[a] Reverse transcriptase-polymerase chain reaction

reflects other factor than medical judgements, for example, patients' and their family's preference. Therefore, we do not try to build care model of these cases but exercise an insurance claim review on 80 recent fatal cases in breast cancer at Tokyo Metropolitan Cancer and Infectious Disease Center Komagome Hospital. This results in ¥1,315,143 (US$11,436) per year, which is also used as the cost of treating fatal toxicity.

Costs are also discounted at a rate of 3% [32].

Comparison of scenarios

Incremental cost-effectiveness ratios (ICER) are calculated for the purpose of comparing the scenarios:

$$\mathrm{ICER} = \frac{\mathrm{Cost}_{\mathrm{RS-guided\_treatment}} - \mathrm{Cost}_{\mathrm{NCCN/St\_Gallen-guided\_treatment}}}{\mathrm{Effect}_{\mathrm{RS-guided\_treatment}} - \mathrm{Effect}_{\mathrm{NCCN/St\_Gallen-guided\_treatment}}}$$

Sensitivity analysis

In order to appraise the stability of ICERs against assumptions and uncertainty of adopted values of probabilities, utility weights, and costs in our economic model, one way sensitivity analyses are performed. The age of cohort is changed to 45 and 65 years old. DFRS_{10}s shown in Table 1 are changed by ±50%, which embrace the relaxation of mid-value assumption of DRFS_{10} of patients with intermediate risk according to St Gallen criteria into both end values. The use of adjuvant chemotherapy in NCCN-guided treatment is changed from 50% of high risk cases only to 100% of high risk cases and 50% of low risk cases; and from 0 to 100% of intermediate risk cases in St Gallen-guided treatment. Propensity to alter treatment among patients classified as intermediate risk by RS criteria reclassification is changed from 100 to 50%. As shown in Table 2, probabilities other than relative risk reductions are changed by ±50%, while the relative risk reductions are changed according to the reported 95% confidence intervals of each value. The effectiveness of adjuvant trastuzumab is extended to 5 years. Utility weights are all changed by ±20%. And as shown in Table 3, costs are all changed by ±50%. Discount rate is also changed from 0 to 5%.

Budget impact estimation

Budget impact is defined as a forecast of rates of use (or changes in rates of use) with their consequent short- and medium-term effects on budgets and other resources to help health service managers [35]. The budget in this study is defined as funds held by social insurers. We estimate the budget impact with our economic model assuming that all new LN−, ER+, ESBC in Japan undergo RS-guided treatment instead of NCCN/St Gallen-guided treatment from 2008 to 2012. The incidence of breast cancer is adopted from a forecast [17], and a share of LN−, ER+, ESBC is fixed at 28.7% [16]. A share of the budget in costs is assumed to be 70% according to the co-payment ratio in Japan's social health insurance system.

Results

Cost-effectiveness

Table 4 shows the result of the cost-effective analysis. The cost of RS-guided treatment, ¥4,135,279 (US$35,959),

Table 4 Result of cost-effectiveness analysis

	Cost (¥)	Incremental cost (¥)	Effect (YOLS)	Incremental effect (YOLS)	Effect (QALY)	Incremental effect (QALY)	Incremental cost-effectiveness ratio	
							(¥/YOLS)	(¥/QALY)
NCCN[a]-guided treatment	3,845,923	–	19.812	–	19.309	–	–	–
vs. RS[b]-guided treatment	4,135,279[c]	289,355	19.895[c]	0.083	19.405[c]	0.097	3,465,713	2,997,495
St Gallen-guided treatment	3,841,580	–	19.679	–	19.173	–	–	–
vs. RS-guided treatment	4,134,791[c]	293,211	19.900[c]	0.221	19.410[c]	0.237	1,328,975	1,239,055

[a] National Comprehensive Cancer Network

[b] Recurrence Score

[c] The cost and effects of RS-guided treatment scenario are slightly different from each other in two comparisons because of the difference in the risk reclassification from counterpart scenarios

exceeds that of NCCN-guided treatment, ¥3,845,923 (US$33,443), which results in a positive incremental cost of ¥289,355 (US$2,516). The effect in YOLSs of RS-guided treatment, 19.895 years, exceeds that of NCCN-guided treatment, 19.812 years, which results in a positive incremental effect of 0.083 year. The effect in QALYs of RS-guided treatment, 19.405 years, exceeds that of NCCN-guided treatment, 19.309 years, which results in a positive incremental effect of 0.097 year.

Similarly, the cost of RS-guided treatment, ¥4,134,791 (US$35,955), exceeds that of St Gallen-guided treatment, ¥3,841,580 (US$33,405), which results in a positive incremental cost of ¥293,211 (US$2,550). The effect in YOLSs of RS-guided treatment, 19.900 years, exceeds that of St Gallen-guided treatment, 19.679 years which results in a positive incremental effect of 0.221 year. The effect in QALYs of RS-guided treatment, 19.410 years, exceeds that of St Gallen-guided treatment, 19.173 years, which results in a positive incremental effect of 0.237 year. The cost and effects of RS-guided treatment scenario in this comparison are slightly different from those in the former comparison because of a difference in the risk reclassification from counterpart scenarios.

In both comparisons, the routine use of the 21-gene RT-PCR assay gains more but costs more at the same time. Incremental cost-effectiveness ratios (ICERs) of the former comparison are 3,465,713 ¥/YOLS (30,137 US$/YOLS) and 2,997,495 ¥/QALY (26,065 US$/QALY), and those of the latter comparison are 1,328,975 ¥/YOLS (11,556 US$/YOLS) and 1,239,055 ¥/QALY (10,774 US$/QALY).

Stability of ICER

Figure 2 shows the results of one way sensitivity analyses. Items are listed in the order of the magnitude of ICER change in terms of yen per QALY, while those change ICER less than 200,000 ¥/QALY (1,739 US$/QALY) are not reported.

Between NCCN-guided treatment vs. RS-guided treatment, ICER is most sensitive to the change of the cost of the 21-gene RT-PCR assay, which ranges from ¥672,402 (US$5,847) to ¥5,322,588 (US$46,283). It is also sensitive to the change of the utility weight for a health status after adjuvant therapy without distant recurrence, which ranges from ¥2,861,163 (US$24,880) to ¥5,725,775 (US$49,789). The changes of ICER by the change of all items fall in a range from ¥672,402 (US$5,847) to ¥5,725,775 (US$49,789). Among the values used in the outcome estimation, $DRFS_{10}$ of patients who are reclassified as intermediate risk by RS criteria from low risk by NCCN criteria, has the largest impact on the result. Among costs of treatments, the cost of adjuvant chemotherapy is most influential to the result.

Between St Gallen-guided treatment and RS-guided treatment, ICER is most sensitive to the change of the assumption on the use of adjuvant chemotherapy among patients classified as intermediate risk by St Gallen criteria, which ranges from ¥788,230 (US$6,854) to ¥2,989,020 (US$25,991). It is also sensitive to the change of the cost of the 21-gene RT-PCR assay, which ranges from ¥290,593 (US$2,527) to ¥2,187,518 (US$19,022). The changes of ICER by the change of all items fall in a range from ¥290,593 (US$2,527) to ¥2,989,020 (US$25,991). Among values used in the outcome estimation, $DRFS_{10}$ of patients who are reclassified as high risk by RS criteria from intermediate risk by St Gallen criteria, has the largest impact on the result. Among costs of treatments, the cost of adjuvant chemotherapy is most influential to the result.

Overall, the change of ICERs by the change of assumptions and values is limited from ¥290,593 (US$2,527) to ¥5,725,775 (US$49,789).

Budget impact

Table 5 shows the result of the budget impact estimation. Annual costs per case by the scenario are calculated from our economic model. RS-guided treatment accompanies high costs in the first year, which probably reflects that the

NCCN-guided treatment vs.RS-guided treatment		Left end value	Base-case value	Right end value
	Item			
1.	Cost of 21-gene RT-PCR assay	¥225,000	¥450,000	¥675,000
2.	Utility weight (without distant recurrence)	1.00	0.98	0.78
3.	Discount rate	0%	3%	5%
4.	$DRFS_{10}$ (NCCN low risk to RS intermediate risk)	0.41	0.82	1.00
5.	Cost of adjuvant chemotherapy	¥514,501	¥343,001	¥171,500
6.	Risk reduction (RS intermediate risk)	76%	39%	0%
7.	Probability of fatal toxicity	0.0075	0.005	0.0025
8.	$DRFS_{10}$ (NCCN low risk to RS high risk)	0.29	0.57	0.86
9.	Utility weight (minor toxicity)	0.72	0.90	1.00
10.	Age of cohort	45	55	65
11.	$DRFS_{10}$ (NCCN high risk to RS high risk)	1.00	0.92	0.46
12.	Risk reduction (RS high risk)	87%	74%	47%
13.	Probability of minor toxicity	0.9	0.6	0.3
14.	Propensity to alter treatment (RS intermediate risk)	-	100%	50%

St Gallen-guided treatment vs. RS-guided treatment		Left end value	Base-case value	Right end value
	Item			
1.	Use of adjuvant chemotherapy (St Gallen intermediate risk)	0%	50%	100%
2.	Cost of 21-gene RT-PCR assay	¥225,000	¥450,000	¥675,000
3.	$DRFS_{10}$ (St Gallen intermediate risk to RS high risk)	0.31	0.62	0.93
4.	Propensity to alter treatment (RS intermediate risk)	-	100%	50%
5.	Discount rate	0%	3%	5%
6.	Risk reduction (RS intermediate risk)	76%	39%	0%
7.	$DRFS_{10}$ (St Gallen intermediate risk to RS intermediate risk)	0.41	0.82	1.00
8.	Risk reduction (RS high risk)	87%	74%	47%
9.	Utility weight (without distant recurrence)	1.00	0.98	0.78
10.	Age of cohort	45	55	65
11.	Cost of adjuvant chemotherapy	¥514,501	¥343,001	¥171,500
12.	$DRFS_{10}$ (St Gallen low risk to RS intermediate risk)	0.41	0.82	1.00
13.	Cost of end-of-life	¥1,972,715	¥1,316,143	¥657,572

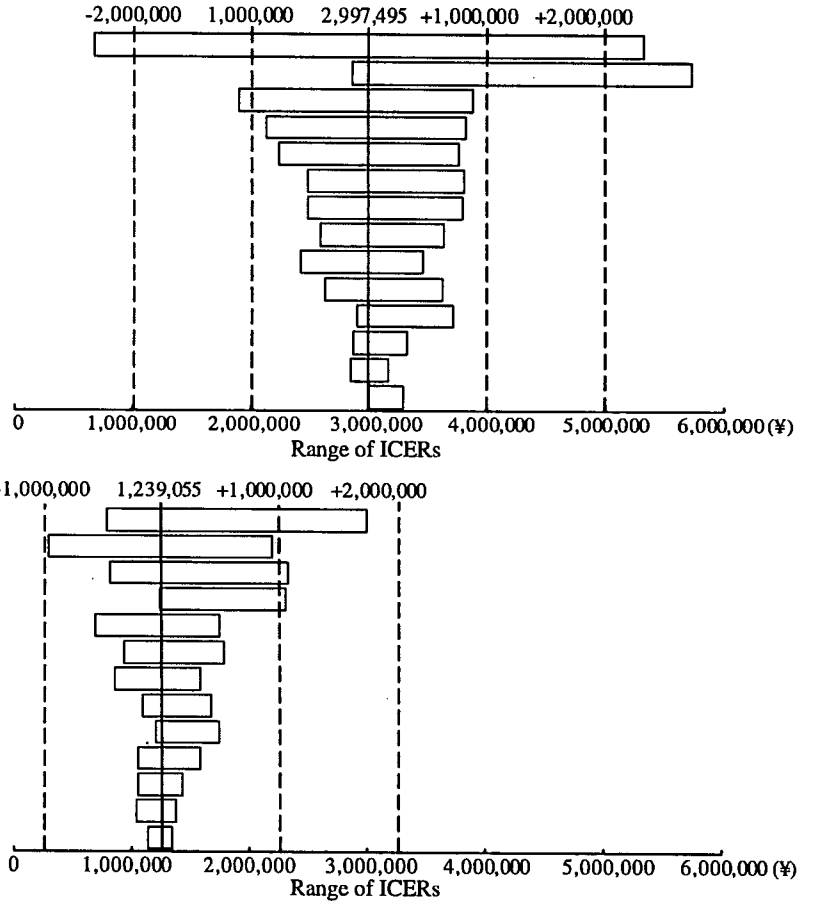

Fig. 2 Results of sensitivity analyses. Abbreviations: National Comprehensive Cancer Network (NCCN), reverse transcriptase-polymerase chain reaction (RT-PCR), recurrence score (RS), distant recurrence free survival in 10 years ($DRFS_{10}$) incremental cost-effectiveness ratio (ICER)