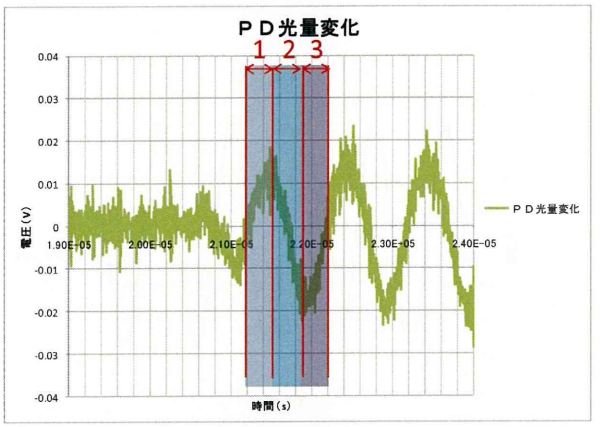
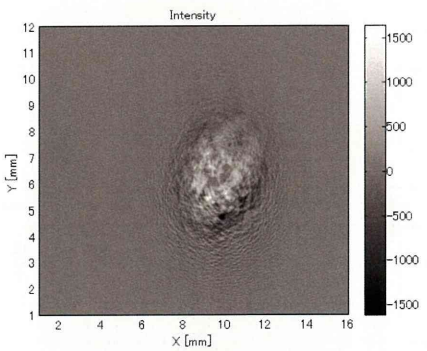
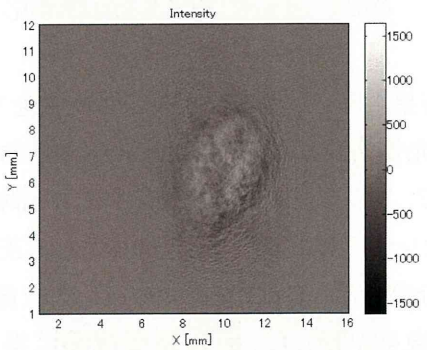


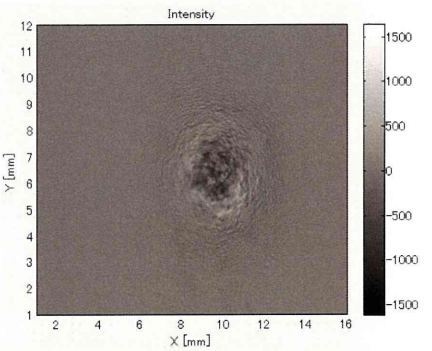
(a) 画像取得のタイミング



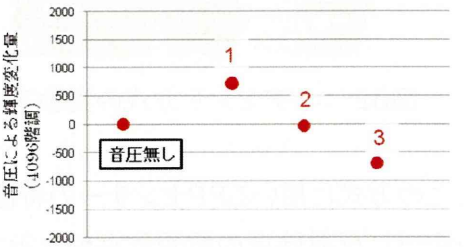
(b) 1のタイミングでの露光時間で取得した画像



(c) 2のタイミングでの露光時間で取得した画像



(d) 3のタイミングでの露光時間で取得した画像



(e) (b)～(d)の各画像の中央近傍での輝度

図35　エリアセンサによる音圧検知結果

図35の結果より、今回の実験によって音圧の有無および音圧の正負によって画像の変化を取得することが可能となった。つまり本研究によって、世界で初めて高速撮像カメラを用いて、超音波像のイメージングを実現することができた。

[3] 実用化に向けた課題

試作当初より、FP 素子用の膜として使用しているパリレン膜の微小粒塊と膜厚分布の問題が明らかになっていた。図36はパリレンの光学顕微鏡写真であり、丸く見える異物のようなものが微小粒塊である。

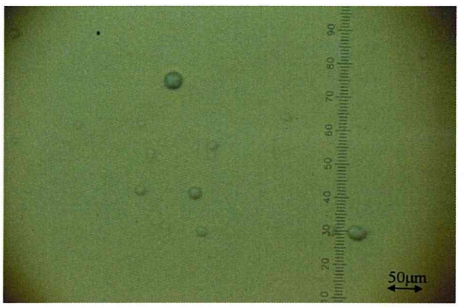
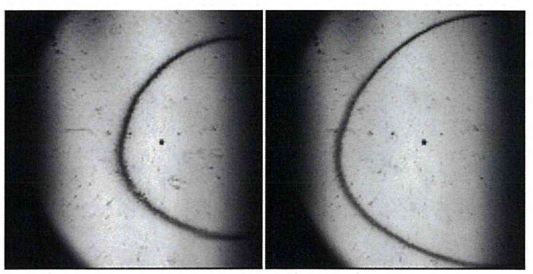


図36　パリレンの微小粒塊

この対策について検討した結果、画像処理によってデータ上で補正を行うことで対応可能であることがわかった。

一方、パリレン膜を成膜する過程において、完全に均一な膜を作ることができない。これによって、基板面内で膜厚の分布が生じる。膜厚に分布が存在するため、以下の様な問題が生じた。

直径 30mm の FP 膜のうち、約 17.4mm 角の領域について、波長を変化させて反射光強度の分布について観測を行った。結果を図37に示す。



(a) 1582nm　　(b) 1585nm

図37　FP 特性の波長依存性

本来膜厚が完全に一定であれば、膜全体が最適となる波長条件が存在するはずであるが、今回の試作ではそれがわずかに幅 1mm 以下の帯状になって現れているのみである。そしてこの帯は、(a)から(b)へと波長を大きくすることによって素子外側に向かって移動することから、パリレン膜は素子の外側ほど厚く成膜されていることがわかった。

本研究の実用化のためにエリアセンサを用いるためには、面内での一括データ取得が必要となる。つまり、すなわち単一波長の光源を用いて1画面分の画像を取得する必要がある。そのためには膜厚分布の課題を解決しなければならない。そこで本年度は数十社のプロセス技術や材料を調査したが、仕様を満足する成膜条件を得ることができなかった。

さらに、新たな課題として、実験系の温度変化に伴って、レーザーの最適波長が変化するという課題が新たに抽出された。つまり実験環境の温度変化がプローブの感度に大きく影響することが明らかとなった。そこで、その温度依存性について調査した。

最適波長の温度依存性を調べるために用いた実験装置を図38に示す。

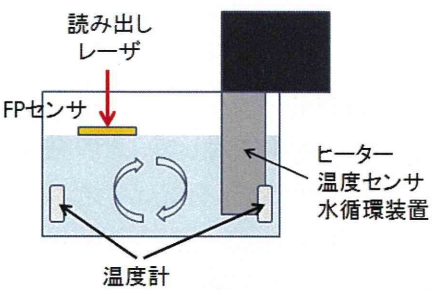


図38　温度可変実験装置の構成図

ヒーターと温度センサーにより装置内の水温を変化させ、その時のFPセンサー面内の最適波長分布を求めた。図39にFPセンサー面の中心点での最適波長の温度依存性を取得した結果を示す。

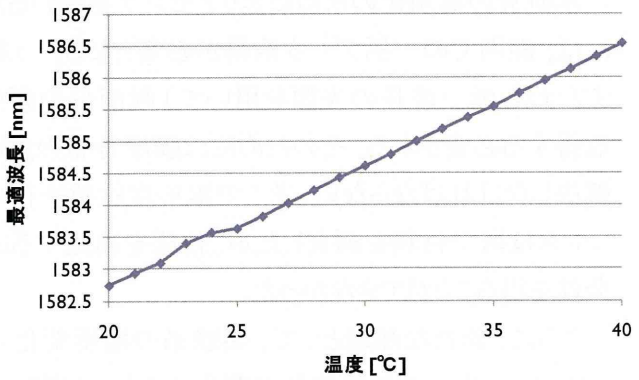


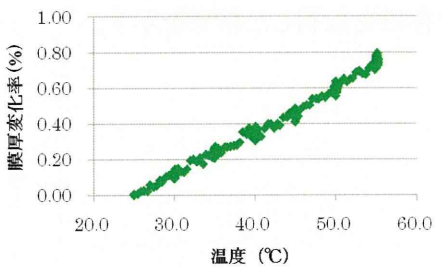
図39　最適波長の温度依存性

この結果より、温度が1℃変化すると、最適波長が約0.2nm 変化するため、環境温度や体温の影響などによって感度が変動してしまうことになる。このため素子の温度依存性についても重要な課題と考えられた。

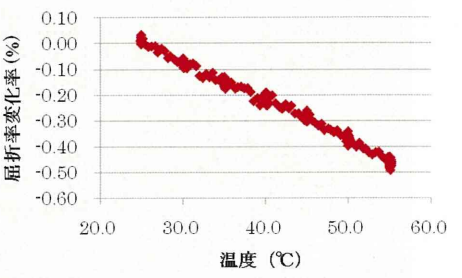
[4] 今後の改善方針

温度依存性の課題に対して、その発生原因について解析を行った。そこで、分光エリプソメトリ M-2000VI (ジェー・エー・ウーラム・ジャパン株式会社製)を用いた光学パラメータ解析を行い、膜厚と屈折率異方性の波長依存性ならびに温度依存性を解析し、評価を行った。ただし、分光エリプソメトリの精度の関係上、パリレン厚を3μmとして評価を行った。

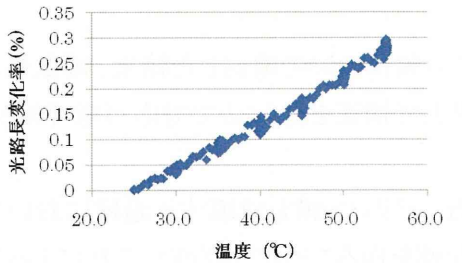
結果を図40(a)～(c)に示す。



(a) 膜厚 d の温度依存性



(b) 基板法線方向の屈折率 n_z の温度依存性



(c) 光路長 ($d \times n_z$) の温度依存性

図40　パリレン膜の温度依存性評価結果

図40(a)の結果より、パリレン膜の線膨張係数は文献値(H. Huang et al., Polymer, Vol. 46, 2005, pp.5949)と合致する値であることが確認された。また、光路長がその変化率よりも小さいのはパリレン膜の基板法線方向の屈折率の温度依存性によって若干キャンセルされていることに起因していることが明らかとなった。

今後こうした温度依存性の問題を解消するためには、膜厚や屈折率の温度依存性がパリレンとは異なる材料を探索し、それらの積である光路長の温度依存性が無いような材料を探索することが解決の指針となる。

一方で上記のような温度依存性の無い膜が実現できるまでは、パリレン膜などの既存材料を使用せざるを得ない。そのため温度依存性の問題は常に生じることから、温度制御を行うなどして、素子面内にて温度分布や温度変化が極力発生しないような構成のセンサー開発を進める方針である。

次いで、膜厚分布の課題について改善の方針を述べる。上述の通り、成膜プロセスの改善によって面内均一化を図ることができなかったため、膜厚分布があ

ってもなお、単一波長光源を利用できる手法を検討した。

上述の通り、FP 基板面内にパリレンの膜厚分布が存在することによって、基板面内にて最適波長に分布を生じる。一方、上記の温度依存性によって、温度が変化すると最適波長が変化してしまう。そこでこれら2つの現象を鑑みて、仮に面内に温度分布を付与することが出来れば、使用する波長を固定することができるのではないかと考えた。

そこで図39の実験の際に得られた最適波長の面内分布の結果を用い、仮に波長を 1585nm に固定したとすれば、基板面内にどのような温度分布を付与すれば最適条件となるかを、計算によって導いた。結果を図41に示す。

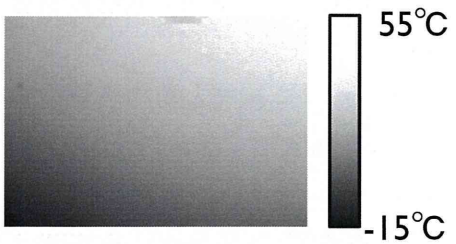


図41　1585nm が最適となる FP 基板面内の温度

つまりこの結果より、温度分布を付与し光路長を素子面内で適宜調整することによって、単一の波長の光源であっても常に 1585nm に最適化されている条件があることが示された。

ここでは基板面内に積極的に温度分布を付与して光路長を制御したが、現実的の素子に温度分布を付与することには限界がある。そこで、電気的に光路長を制御できる手法として、液晶ディスプレイの原理を活用することとした。つまり、液晶素子では電圧を印加することによって分子配向状態を変化させ、光路長を制御することができる性質を利用する。図42に素子の構成図を示す。

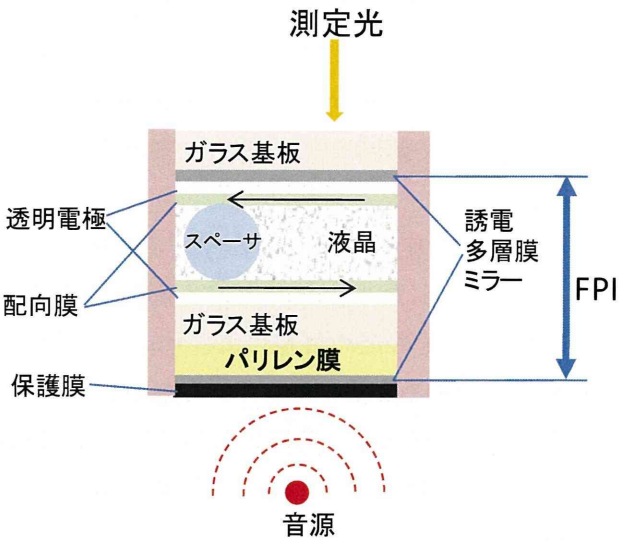


図42　液晶を用いた膜厚分布補償構成

この方式では、液晶層を二枚の誘電多層膜ミラーの間に設け、液晶に電圧を印加することによってFPセンサーの二枚の反射部の間の光路長を制御することでパリレン膜の膜厚ばらつきを補正する。これによって図30の温度分布付与と同等の効果が得られることから、面内の各場所で同一の波長で面内全域の音圧分布測定を行うことができる。さらに液晶素子は数十μm角以下の画素サイズという高精細化が容易に実現できるため、本研究の高精細 PAT プローブにも適用可能である。

本研究期間にて、こうした方針に基づいて開発を開始した。今後この方式の特性評価を行い、有効性の確認を行う予定である。

D. 考察

D－1. 新生血管の病理組織学的解析

今回立ち上げた病理組織の定量化システムは高速に血管の定量化を進めることが可能となるため、有効なツールである。今回の探索的な臨床研究の中で、PAM 画像や MRI 画像のような広い撮像範囲を有する

画像診断方法と、病理による血管解析というミクロな解析結果とを半定量的に対比するには概ね問題ない精度を有していた。そのため、今後も臨床研究の中でデータを蓄積して診断基準の構築に寄与するものと考えられる。

なお一部の症例で CD31 が呈する非特異的な染色によって、こうした半定量比較にも影響を及ぼす場合が存在した。そのため目視によるチェックをまず行い、非特異染色の有無を確認してから進めることとした。若干の手間はかかるが、従来行われていたような手作業での血管カウントよりは、圧倒的に短時間で定量化が可能である。

今後はこうした目視によるチェックを併用した自動定量化技術を高めていくとともに、元のスライドに関しても染色法を改良することで非特異染色を減少させることが重要である。

一方、PAM によって得られる酸素飽和度画像のエビデンス構築のために低酸素マーカーによる染色を行った。これにより血管密度と低酸素状態と血管の成熟度とを同時に解析しうる基盤を構築できたものと考えている。つまり今回の複数マーカーによる画像解析についても半自動化を目指して推進しているものであり、PAM 画像の撮像範囲全体というマクロな画像とミクロな組織状態とを対比しうる検討である。またこの手法を多様な腫瘍に適用することにより、新生血管の発生メカニズム解明に迫る結果につながることが期待でき、今後の医学研究の発展につながるものと考えられる。

D－2. 広帯域 PAT プローブの開発

PA-MS 方式は概ね計画通り、良好な成果が得られている。装置試作を行い高解像な画像を取得することができた。これを改善し計測時間を短縮させた結果、実験効率が高まった。また麻酔下で in vivo マウス測定を可能としたことと合わせて、動物実験においてはマウスに対する負担や苦痛がより少ない形で実験できるようになり、マウスの腫瘍血管の構築の様子が画像化できるようになったのは大きな成果だといえる。

現在のところ、酸素飽和度画像の画質について改善の余地があるが、装置の SN 改善によって今後よりよい画質と定量性を得ることが期待できる。

このように生きたままのマウスを用いて PAT 像を取得できる環境が整うことによって、さまざまな用途に用いることが可能になると考えられる。今後本装置は、広帯域 PAT プローブ製作のリファレンス的な位置づけのみならず、動物実験を通じて各種検討を実施し、PAM の診断基準の構築や、装置の改良に役立てることができる。

また今後、さらなる高速化、さらなる高分解能化を達成させるべく開発を進める計画である。これにより、将来的には分子プローブや各種薬剤が微細血管中を流動する様子を観測することで、体内動態を把握する基礎検討にも使用し、医学研究の発展に寄与することが期待できる。

FP 方式はファントム撮影と動物実験を通して、光音響波を受信して画像化できることを示した。解像度に関して改善の余地がある。

次いで二次試作を行い、高速二次元エリアセンサを用いた撮像システムを構築し、世界で初めて高速シャッター方式によって超音波信号の画像化を実現した。今回用いたエリアセンサの最短露光時間は 300ns であるため、最大約 1.6MHz までの超音波信号受信に限定される。

一方、最新の CMOS エリアセンサの研究では、1Tpixel/s の画素読出しレート性能が実現されており（須川成利、映像情報メディア学会誌 Vol.66, No.3, 2012, p.174）、これが実用化され利用できれば 100×100 の画面構成において1画面あたり10ns で画像取得が可能となる。つまり最大 50MHz までの超音波を受信可能な、高精細高周波二次元プローブを実現することができる。その結果、これと光音響技術とを組み合わせることによって高精細 PAT プローブを実現することが可能となる。本研究はそのフィジビリティを確認し、

基礎的な研究結果と今後の開発方針を示すことができた点で大きな成果であったといえる。

E. 結論

本研究テーマに関して、PAM で得られる画像に対応して、血管の量についての定量化手法に加え、低酸素マーカーと画像解析とを組み合わせた病理検討を開始し、今後の評価に対する実現可能性を示した。これにより、PAM 画像におけるヘモグロビン吸収係数像だけでなく、酸素飽和度像に対してのエビデンスが構築でき、今後の画像診断基準を構築するための見通しが立てられた。

一方、広帯域PATプローブ製作に関して、PA-MS方式については試作と動物実験を推進し in vivo 状態でマウスの微細な血管を描出することが可能となった。酸素飽和度画像の画質改善を推進することによって、本研究のためだけでなく、広く医学研究分野に対して有意義なデータを提供することが可能になる。

FP 方式は論文に記載されていなかった課題が大きかったため、当初計画に対して遅延が生じたが、高速二次元エリアセンサによる世界初の超音波像の画像化を実現するとともに、今後の実用化への方針を示すことができた。この方針にしたがい開発を進め、リアルタイムで微細血管構造を描出することが出来れば画期的な成果であることは間違いない。今後も完成に向けて新技術の導入や装置改良を進めて実用化を目指すことが医学の発展や診断・治療への応用にとって重要である。

F. 健康危険情報

なし。

G. 研究発表

1. 論文発表

1. Toi M, Saeki T, Iwata H, Inoue K, Tokuda Y, Sato Y, Ito Y, Aogi K, Takatsuka Y, Arioka H. A multicenter phase II study of TSU-68, an oral multiple tyrosine kinase inhibitor, in combination with docetaxel in metastatic breast cancer patients with anthracycline resistance. Breast Cancer [Epub ahead of print] 2012
2. Gasparini G,Torino F, Ueno T, Cascinu S, Troiani T, Ballestrero A, Berardi R, Shishido J, Yoshizawa A, Mori Y, Nagayama S, Morosini P, Toi M. A phase II study of neoadjuvant bevacizumab plus capecitabine and concomitant radiotherapy inpatients with locally advanced rectal cancer. Angiogenesis 5(1):141-50, 2012
3. Kitai T, Torii M, Sugie T, Kanao S, Mikami Y, Shiina Y and Toi M. Photoacoustic mammography: initial clinical results. Breast Cancer, DOI 10.1007/s12282-012-0363-0, (2012)
4. Takeda K, Kanao S, Okada T, Kataoka M, Ueno T, Toi M, Ishiguro H, Mikami Y,Togashi K. Assessment of CAD-generated tumor volumes measured using MRI in breast cancers before and after neoadjuvant chemotherapy. Eur J Radiol. 2012 Jan 3.
5. Tang Y, Xu F, Tao K, Qian N, Toi M. Clinical applications of sentinel lymph node biopsy in ductal carcinoma in situ of the breast: a dilemma. Tohoku J Exp Med. 2011;224(1):1-5.
6. Bu S, Liu Z, Shiina, Kondo K, Yamakawa M, Fukutani K, Someda Y, and Asao Y.

Model-Based Reconstruction IntegratedWith Fluence Compensation for Photoacoustic Tomography. IEEE Trans. on Biomedical Engineering, Vol. 59, No. 5, pp.1354-1363, 2012.

7. Taki H, Taki K, Sakamoto T, Yamakawa M, Shiina T, Kudo M, and Sato T. High Range Resolution Ultrasonographic Vascular Imaging Using Frequency Domain Interferometry with the Capon Method. IEEE Trans. on Medical Imaging, Vol. 31, No. 2, pp. 417-429, 2012
8. Taki H, Sakamoto T, Yamakawa M, Shiina T, Nagae K and Sato T. Small calcification depiction in ultrasound B-mode images using decorrelation of echoes caused by forward scattered waves. J. Med. Ultasonics , Vol.38, No.2, pp.73-80, 2011.
9. Kido A, Ascher SM, Kishimoto K, Hahn W, Jha RC, Togashi K. Spies JB.Comparison of uterine peristalsis before and after uterine artery embolization at 3-T MRI. AJR Am J Roentgenol. 2011 Jun;196(6):1431-5. Erratum in: AJR Am J Roentgenol. 2012 Feb;198(2):488.
10. Okada T, Kanao S, Kuhara S, Ninomiya A, Fujimoto K, Kido A, Togashi K. Whole-heart coronary MR angiography under a single breath-hold: a comparative study with respiratory-gated acquisition using a multi-element phased-array coil.Clin Radiol. 2011 Nov;66(11):1060-3.
11. Takeda K, Kanao S, Okada T, Ueno T, Toi M, Ishiguro H, Mikami Y, Tanaka S,Togashi K. MRI evaluation of residual tumor size after neoadjuvant endocrine therapy vs. neoadjuvant chemotherapy. Eur J Radiol. 2011 Jun 9.
12. Iima M, Le Bihan D, Okumura R, Okada T, Fujimoto K, Kanao S, Tanaka S, Fujimoto M, Sakashita H, Togashi K. Apparent diffusion coefficient as an MR imaging biomarker of low-risk ductal carcinoma in situ: a pilot study. Radiology. 2011 Aug;260(2):364-72.
13. Takuwa H, Ueno T, Ishiguro H, Mikami Y, Kanao S, Takada M, Sugie T, Toi M. A case of metaplastic breast cancer that showed a good response to platinum-based preoperative chemotherapy. Breast Cancer. 2011 28. [Epub ahead of print]
14. Fukutani K, Someda Y, Taku M, Asao Y, Kobayashi S, Yagi T, Yamakawa M, Shiina T, Sugie T, and Toi M. Characterization of photoacoustic tomography system with dual illumination, Proc. of SPIE, vol. 7899, 78992J-1 (2011).
15. Bu S, Kondo K, Yamakawa M, Shiina T, Fukutani K, Someda Y, and Asao Y. Adaptive and quantitative reconstruction algorithm for photoacoustic tomography, Proc. of SPIE, vol. 7899, 78992G-1 (2011).
16. Tanji K, Watanabe K, Fukutani K, Asao Y, Yagi T, Yamakawa M, and Shiina T. Advanced model-based reconstruction algorithm for practical three-dimensional photoacoustic imaging, Proc. of SPIE, vol. 7899, 78992K-1 (2011).
17. Kumagai Y, Toi M, Kawada K, Kawano T. Angiogenesis in superficial esophageal squamous cell carcinoma: magnifying endoscopic observation and molecular analysis.Dig Endosc. 2010 Oct;22(4):259-67.
18. Inamoto T, Takada M, Toi M. Results of questionnaire survey of breast cancer

specialists about optimal surgery for patients with breast cancer. Local and systemic management of primary breast cancers. Edited by Toi M and Winer EP. Kyoto University press. 2010

19. Bu S, Yamakawa M, Shiina T. Adaptive Depth Compensation Algorithm for Photoacoustic Tomography. Proc. of 2010 IEEE Ultrasonics Symposium, pp.2139−2142, 2010.
20. Fukutani K, Miyasato T, Nakajima T, Someda Y, Asao Y, Yagi T, Yamakawa M, Shiina T. Dual illumination mode photoacoustic tomography for quantitative imaging. Proc. of 2010 IEEE Ultrasonics Symposium, pp.2143-2146, 2010.
21. Taki H, Sakamoto T, Yamakawa M, Shiina T, Sato T. Calculus Detection for Ultrasonography Using Decorrelation of Forward Scattered Wave, J. Med. Ultasonics , Vol.37, No.3, pp.129-135, 2010.
22. Kido A, Kataoka M, Yamamoto A, Nakamoto Y, Umeoka S, Koyama T, Maetani Y,Isoda H, Tamai K, Morisawa N, Saga T, Mori S, Togashi K. Diffusion tensor MRI of the kidney at 3.0 and 1.5 Tesla. Acta Radiol. 2010 Nov;51(9):1059-63.
23. Fujimoto K, Nakai A, Okada T, Ikeuchi T, Satogami N, Daido S, Yakami M,Togashi K. Effect of hyoscine butylbromide (HBB) on the uterine corpus:quantitative assessment with T2-weighted (T2W) MRI in healthy volunteers. J Magn Reson Imaging. 2010 Aug;32(2):441-5.
24. Taki H, Sakamoto T, Yamakawa M, Shiina T, and Sato T. Small Calculus detection for medical acoustic imaging using cross-correlation between echo signals. Proc. IEEE Ultrason. Symp., pp. 2398-2401, 2009.
25. Kataoka, M. Kido, A. Yamamoto, A. Nakamoto, Y. Koyama, T. Isoda, H. Maetani, Y. Umeoka, S. Tamai, K. Saga, T. Morisawa, N. Mori, S, Togashi, K. Diffusion tensor imaging of kidneys with respiratory triggering: optimization of parameters to demonstrate anisotropic structures on fraction anisotropy maps. J Magn Reson Imaging 29(3): 736-44.2009.
26. Okada, T. Kanao, S. Ninomiya, A. Sato, S. Kuhara, S. Kamae, T. Gotoh, K, Togashi, K. Whole-heart coronary magnetic resonance angiography with parallel imaging: comparison of acceleration in one-dimension vs. two-dimensions. Eur J Radiol 71(3): 486-91.2009.
27. Okada, T. Kuhara, S. Kanao, S. Ninomiya, A. Sato, S. Kamae, T. Gotoh, K, Togashi, K. Facilitated acquisition of whole-heart coronary magnetic resonance angiography with visual feedback of respiration status. Int J Cardiovasc Imaging 25(4): 397-403.2009.
28. Kido A, Togashi K, Hatayama H, Nakayama T, Yamamoto A, Kataoka M, Tulandi T.Uterine peristalsis in women with repeated IVF failures: possible therapeutic effect of hyoscine bromide. J Obstet Gynaecol Can. 2009 Aug;31(8):732-5.

2. 学会発表

1. M. Torii, T. Kitai, M. Kataoka, S. Kanao, T. Sugie, T. Shiina and M. Toi. Characterization of brast cancer by Photoacoustic mammography, European Congress of

Radiology 2012, March 1 - 5, 2012, Vienna, Austria

2. Kanao S, Kataoka M, Iima M, Sakaguchi R, Onishi N, Okada T, Porter DA, Feiweier T, and Togashi K. Feasibility of High Resolution Diffusion Weighted MRI of the Breast using Readout Segmented EPI or Single Shot EPI, The 20th Annual Scientific Meeting and Exhibition of the International Society of Magnetic Resonance in Medicine, May 5-12, 2012 Melbourne, Australia
3. Taki H, Sakamoto T, Yamakawa M, Shiina T, and Sato T. Evaluation of Small Calcification Indicator in Ultrasonography Using Decorrelation between Adjacent Scan Lines. Proc. 17th International Meeting of the European Society of Gynaecological Oncology, Sep. 11-14, 2011, Milan.
4. Taki T, Sakamoto T, Yamakawa M, Shiina T and Sato T. 医用超音波イメージングにおける微細ワイヤーを用いた微小石灰化モデル, Annual Scientific Meeting of the Japan Society of Ultrasonics in Medicine, May 27-29, 2011, Tokyo.
5. Kanao S, Okada T, Takeda K, Kubo T, Umeoka S, Sugie T, Matsumura Y, Toi M, Mikami Y, Togashi K. Differentiating Breast Mass with Hyper-intensity Area on T2 Weighted Image: Value of Diffusion Weighted Imaging. European Congress of Radiology 2011, Vienna, Austria, March 3-7, 2011
6. Torii M, Kanao S, Ueno T, Sugie T, Takeuchi M, Tsuji W, Toi M. Response Evaluation of Breast Cancer after Neoadjuvant Chemotherapy: Dynamic MRI vs. FDG-PET. European congress of Radiology 2011, March 2011, Vienna
7. Iima M, Le Bihan D, Okumura R, Okada T, Fujimoto K, Kanao S, Tanaka S, Togashi K. Potential of diffusion MRI as a biomarker of low-risk DCIS. European Congress of Radiology 2011, March 3-7, 2011, Vienna, Austria
8. Takeda K, Kanao S, Okada T, Kubo T, Umeoka S, Togashi K. MRI evaluation of residual tumor size of the breast cancer after neoadjuvant endocrine therapy vs neoadjuvant chemotherapy: is there any difference? European Congress of Radiology 2011, March 3-7, 2011, Vienna, Austria
9. Kanao S, Evaluation of Presurgical Systemic Therapy by Breast MRI. Kyoto Breast Cancer Consensus Conference International Convention 2011, April 14-16, 2011, Kyoto, Japan
10. Kanao S, Okada T, Iima M, Takeda K, Umeoka S, Kubo T, Porter D, Feiweier T, Togashi K, 3.0 T Breast Diffusion Weighted MRI Using Readout Segmented EPI: Comparison With Single Shot EPI, The 19th Annual Scientific Meeting and Exhibition of the International Society of Magnetic Resonance in Medicine, May 6-13, 2011 Montreal, Canada
11. Iima M, Le Bihan D, Okumura R, Okada T, Fujimoto K, Kanao S, Tanaka S, Togashi K. Potential of diffusion MRI as a biomarker of low-risk DCIS. The 19th Annual Scientific Meeting and Exhibition of The International Society for Magnetic Resonance in Medicine. May 6-13, 2011, Montreal, Canada
12. Iima M, Le Bihan D, Okumura R, Okada T, Fujimoto K, Kanao S, Togashi K. Potential of

breast diffusion MRI for the management of low grade DCIS patients. Journées Françaises de Radiologie, October 21-25, 2011, Paris, France

13. 鍛利幸　浅尾恭史　杉江知治　金尾昌太郎　三上芳喜　椎名毅　戸井雅和、光超音波マンモグラフィを用いた乳癌診断法の開発、 第５０回　日本生体医工学会大会　JSMBE、2011 年 4 月 29 日　東京
14. 鍛利幸　杉江知治　戸井雅和　金尾昌太郎　三上芳喜　椎名毅　浅尾恭史、光超音波マンモグラフィを用いた乳癌診断法の開発、第１９回　日本乳癌学会学術総会、 2011 年 9 月 2－4 日　仙台
15. 戸井雅和　杉江　知治、乳癌におけるセンチネルリンパ節生検と腋窩手術、第５１回日本核医学会学術総会、2011 年 10 月 28 日、筑波
16. 布樹輝, 近藤健悟, 山川誠, 椎名毅, 福谷和彦, 染田恭宏, 浅尾恭史, “3-D 平面走査光超音波トモグラフィーのための定量的な再構成方法,” 日本超音波医学会第８４回学術集会, 2011 年 5 月 29 日、 東京
17. 富樫　かおり、Functional MR imaging of the uterus、日本ーフィンランド「メディカルサイエンス」シンポジウム、2011 年 12 月 13 日、フィンランド
18. 富樫　かおり、MRI による腫瘍モニタリングと予後予測：形態から数値へ、第 49 回日本癌治療学会学術集会、2011 年 10 月 27 日、愛知
19. 金尾昌太郎　中本裕士　三宅可奈江　富樫かおり、FDG-PET でリング状集積を示す乳癌の画像・病理所見－全身用 PET と乳房専用 PET の比較、第 70 回日本医学放射線学会総会、web 開催、2011 年 5 月 9-20 日
20. 金尾昌太郎　岡田知久　上野貴之　杉江知治　竹内恵　辻和香子　戸井雅和　三上芳喜　富樫かおり、MRI による乳癌術前化学療法の効果判定－長径縮小率と体積縮小率の比較、第 19 回日本乳癌学会学術総会、2011 年 9 月 2-4 日、仙台
21. 金尾昌太郎　片岡正子　飯間麻美　阪口怜奈　武田和奈　大西奈都子　富樫かおり、RESOLVE を用いた高分解能拡散強調画像による DCIS の描出、第 39 回日本磁気共鳴医学会、2011 年 9 月 29 日～10 月 1 日、小倉
22. 大西 奈都子　金尾 昌太郎　片岡 正子　富樫かおり　戸井雅和　三上芳喜、DCIS を合併した mucocele-like lesion の 2 例、第 47 回日本医学放射線学会秋季臨床大会　2011 年 10 月 21-23 日、下関
23. 大西奈都子　金尾昌太郎　片岡正子　飯間麻美　阪口怜奈　富樫かおり拡散強調 MRI による乳房粘液癌の評価、、第 71 回日本医学放射線学会総会、2011 年 4 月 12-15 日、横浜
24. Toi M. Latest research outcome regarding Angiogenesis and Breast Cancer. The 3rd CCH International Breast Cancer Conference, August 2010, Taiwan
25. Togashi K. Cine, diffusion and 3T magnetic resonance Imaging of the female Pelvis、The 13th Asian-Oceanian Congress of Radiology （AOCR2010） March 20, 2010, Taiwan
26. Togashi K. MRI diagnosis of endometriosis and uterine myomas、The 13th Asian-Oceanian Congress of Radiology （AOCR2010） March 20, 2010, Taiwan
27. Togashi K. Recent Progress in MR imaging diagnosis in the female pelvis、The 26th International Congress of Radiology(ICR2010) April 11, 2010, China
28. Togashi K. Malignant Diseases of the Uterus、The 18th International Society for Magnetic Resonance in Medicine(ISMRM2010) May 2, 2010, Sweden
29. Togashi K. Functional uterine MR imaging、

The 15th workshop of the German-Japanese Radiological Affiliation. May 21-23, 2010, Tokyo

30. Kanao S, Okada T, Takeda K, Kubo T, Umeoka S, Togashi K. Differentiating Granulomatous Lobular Mastitis from Breast Cancer at MRI: Value of Diffusion Weighted Imaging AOCR (Asian Oceanian Congress of Radiology) 2010 March 2010, Taipei
31. Bu S, Yamakawa M, and Shiina T. Interpolation Method for Model-based 3-D Planar Photoacoustic Tomography Reconstruction. The 3rd Biomedical Engineering International Conference, Auust 28, 2010, Kyoto.
32. 瀧 宏文, 阪本 卓也, 山川 誠, 椎名 毅, 佐藤 亨, 前方散乱波の相関低下を用いた医用超音波微小石灰化検出法における送信ビーム形成, Spring Meeting of Acoustic Society of Japan, Mar. 8-10, 2010, Tokyo.
33. 金尾昌太郎　岡田知久　武田和奈　久保武　梅岡成章　富樫かおり、 3.0T MRI を用いた乳癌初期全身療法の効果判定、 第 69 回日本医学放射線学会総会　2010 年 4 月　横浜
34. Taki G, Sakamoto T, Yamakawa M, Shiina T, and Sato T Small Calculus detection for medical acoustic imaging using cross-correlation between echo signals. Proc. IEEE Ultrason. Symp., Sep. 20-23, 2009, Roma.
35. Taki H, Sakamoto T, Yamakawa M, Shiina T, and Sato T. Experimental study of small calculus detction for medical acoustic imaging using correlation between echo signals, Proceedings of Symposium on Ultrasonnic Electronics, Nov. 18-20, 2009, Kyoto.
36. Togashi K. Recent Progress in Imaging Diagnosis of Female Pelvis、The 2nd Asian Congress of Abdominal Radiology (ACAR2009) June 6, 2009 Korea
37. Togashi K. Uterine contractility evaluated with Cine MR Imaging、The 36th Congress of the International Union of Physiological Sciences(IUPS2009) July 29, 2009 Kyoto

H. 知的財産権の出願・登録状況

（予定を含む。）

1. 特許取得

（出願）

1) 浅尾恭史、中嶌隆夫、音響信号受信装置、特願2011-117942

2) 浅尾恭史、中嶌隆夫、音響信号受信装置、特願2012-048397

（登録）

なし

2. 実用新案登録

なし。

3. その他

なし。

研究成果の刊行に関する一覧表

雑誌

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
Toi M, Saeki T, Iwata H,et al.	A multicenter phase II study of TSU-68, an oral multiple tyrosine kinase inhibitor, in combination with docetaxel in metastatic breast cancer patients with anthracycline resistance	Breast Cancer	[Epub ahead of print]		2012
Gasparini G,Torino F, Ueno T et al.	A phase II study of neoadjuvant bevacizumab plus capecitabine and concomitant radiotherapy in patients with locally advanced rectal cancer	Angiogenesis	5(1)	141-50	2012
Kitai T, Torii M, Sugie T et al.	Photoacoustic mammography: initial clinical results	Breast Cancer	[Epub ahead of print]		2012
Takeda K, Kanao S, Okada G et al.	Assessment of CAD-generated tumor volumes measured using MRI in breast cancers before and after neoadjuvant chemotherapy	Okada T et al. Eur J Radiol.	[Epub ahead of print]		2012
Bu S, Liu Z, Shiina, Kondo K, et al.	Model-Based Reconstruction IntegratedWith Fluence Compensation for Photoacoustic Tomography	IEEE Trans. on Biomedical Engineering	Vol. 59, No. 5	1354-1363	2012
Taki H, Taki K, Sakamoto T et al.	High Range Resolution Ultrasonographic Vascular Ima ging Using Frequency Domain Interferometry with the Capon	IEEE Trans. on Medical Imaging	Vol. 31, No. 2	417-429	2012
Tang Y, Xu F, Tao K, et al.	Clinical applications of sentinel lymph node biopsy in ductal carcinoma in situ of the breast: a dilemma	Tohoku J Exp Med.	224(1)	1-5	2011
Taki H, Sakamoto T, Yamakawa M et al.	Small calcification depiction in ultrasound B-mode images using decorrelation of echoes caused by forward scattered wa	J. Med. Ultasonics	Vol.38, No.2	73-80	2011
Kido A, Ascher S M, Kishimoto K, et al. :	Comparison of uterine peristalsis before and after uterine artery embolization at 3-T MRI	AJR Am J Roentgenol Erratum in: AJR Am J Roentgenol.	196(6) 8(2)	1431-5 488.	2011 2012

Okada T, Kanao S, Kuhara S, et al.	Whole-heart coronary MR angiography under a single breath-hold: a comparative study with respirat ory-gated acquisition using a multi-element phased- array coil	Clin Radiol	66(11)	1060-3.	2011
Takeda K, Kanao S, Okada T, et a	MRI evaluation of residual tumor size after neoadjuvant endocrine therapy vs. neoadjuvant chemotherapy	Eur J Radiol	[Epub ahead of print]		2011
Iima M, Le Bihan D, Okumura R et al.	Apparent diffusion coefficient as an MR imaging biomarker of low-risk ductal carcinoma in situ: a pilot study	Radiology	260(2)	364-72.	2011
Takuwa H, Ueno T, Ishiguro H, et al.	A case of metaplastic breast cancer that showed a good response to platinum-based preoperative chemotherapy	Breast Cancer	[Epub ahead of print]		2011
Fukutani K, Someda Y, Taku M, et al.	Characterization of photoacoustic tomography system with dual illumination	Proc. of SPIE	7899	78992J-1	2011
Bu S, Kondo K, Yamakawa M, et al.	Adaptive and quantitative reconstruction algorithm for photoacoustic tomography	Proc. of SPIE	7899	78992G-1	2011
Tanji K, Watanabe K, Fukutani K, et al.	Advanced model-based reconstruction algorithmfor practical three-dimensional photoacoustic imaging	Proc. of SPIE	7899	78992K-1	2011
Kumagai Y, Toi M, Kawada K, et al.	Angiogenesis in superficial esophageal squamous cell carcinoma: magnifying endoscopic observation and molecular analysis	Dig Endosc	22(4)	259-67	2010
Bu S, Yamakawa M, Shiina T	Adaptive Depth Compensation Algorithm for Photoacoustic Tomography	Proc. of 2010 IEEE Ultrasonics Symposium		2139−2142	2010

Fukutani K, Miyasato T, Nakajima T, et al.	Dual illumination mode photoacoustic tomo graphy for quantitative imaging	Proc. of 2010 IEEE Ultrasonics Symposium		2143-2146	2010
Taki H, Sakamoto T, Yamakawa M et al.	Calculus Detection for Ultrasonography Using Decorrelation of Forward Scattered Wave	J. Med. Ultasonics	Vol.37, No.3	129-135	2010
Kido A, Kataoka M, Yamamoto A, et al.	Diffusion tensor MRI of the kidney at 3.0 and 1.5 Tesla	Acta Radiol	51(9)	1059-63.	2010
Fujimoto K, Nakai A, Okada T, et al.	Effect of hyoscine butyl bromide (HBB) on the uterin e corpus:quantitative assessment with T2-weighted(T2W) MRI in healthy volunteers	J Magn Reson Imaging	32(2	441-5.	2010
Taki H, Sakamoto T, et al.	Small Calculus detection for medical acoustic imaging using cross-correlation between echo signals	Proc. IEEE Ultrason. Symp		2398-2401	2009
Kataoka, M. Kido, A. Yamamoto, A. et al..	Diffusion tensor imaging of kidneys with respiratory triggering: optimization of parameters to demonstrate anisotropic structures on fracti on an isotropy maps	J Magn Reson Imaging	29(3)	736-44	2009
Okada, T. Kanao, S. Ninomiya, A et al.	Whole-heart coronary magnetic resonance angiography with parallel imaging: comparison of acceler ation in one-dimension vs. two-dimensions	Eur J Radiol	71(3)	486-91	2009
Okada, T. Kuhara, S. Kanao, S et al.	Facilitated acquisition of whole-heart coronary magnetic resonanc e angiography with visual feedback of respiration status	Int J Cardiovasc Imaging	25(4)	397-403	2009
Kido A, Togashi K, Hatayama H, et al.	Uterine peristalsis in women with repeated IVF failures: possible therapeutic effect of hyoscine bromide	J Obstet Gynaecol Can	31(8)	32-5	2009

Breast Cancer
DOI 10.1007/s12282-012-0344-3

ORIGINAL ARTICLE

A multicenter phase II study of TSU-68, an oral multiple tyrosine kinase inhibitor, in combination with docetaxel in metastatic breast cancer patients with anthracycline resistance

Masakazu Toi · Toshiaki Saeki · Hiroji Iwata · Kenichi Inoue · Yutaka Tokuda · Yasuyuki Sato · Yoshinori Ito · Kenjiro Aogi · Yuichi Takatsuka · Hitoshi Arioka

Received: 11 November 2011 / Accepted: 30 January 2012

Abstract
Background TSU-68 is a novel multiple tyrosine kinase inhibitor that inhibits vascular endothelial growth factor receptor-2, platelet-derived growth factor receptor, and fibroblast growth factor receptor. This open-label, non-comparative, multicenter phase II study evaluated TSU-68 in combination with docetaxel in patients with metastatic breast cancer that had relapsed within 1 year despite prior treatment with an anthracycline-containing regimen.
Methods TSU-68 was orally administered on days 1–21, and docetaxel was intravenously delivered on day 1. The regimen was repeated every 21 days. Primary endpoint was objective response rate according to the RECIST guidelines version 1.0.
Results TSU-68 in combination with docetaxel produced objective responses in 21.1% and clinical benefits in 42.1% of the patients, respectively (1 complete response, 3 partial response, and 4 stable disease for at least 24 weeks, $n = 19$). Median time to progression was 148 days, and median overall survival was 579 days. The common adverse drug reactions were leukopenia, neutropenia, nail disorder, malaise, dysgeusia, alopecia, and edema.
Conclusions TSU-68 in combination with docetaxel showed a promising antitumor response with manageable

M. Toi (✉)
Department of Breast Surgery, Graduate School of Medicine, Kyoto University, 54 Kawaracho, Shogoin, Sakyo-ku, Kyoto 606-8507, Japan
e-mail: toi@kuhp.kyoto-u.ac.jp

M. Toi
Department of Surgery, Tokyo Metropolitan Komagome Hospital, 3-18-22 Honkomagome, Bunkyo-ku, Tokyo 113-8677, Japan

T. Saeki
Department of Breast Oncology, Saitama International Medical Center, Saitama Medical University, 1397-1 Yamane, Hidaka, Saitama, Japan

T. Saeki · K. Aogi
Department of Breast Oncology, National Hospital Organization Shikoku Cancer Centre, 160 Minamiumemoto-machi-ko, Matsuyama, Ehime 791-0280, Japan

H. Iwata
Department of Breast Oncology, Aichi Cancer Center Hospital, 1-1 Kanokoden, Chikusa-ku, Nagoya, Aichi 464-8681, Japan

K. Inoue
Division of Breast Oncology, Saitama Cancer Centre, 818 Komuro, Ina, Kitaadachi, Saitama 362-0806, Japan

Y. Tokuda
Department of Surgery, Tokai University School of Medicine, 143 Shimokasuya, Isehara, Kanagawa 259-1193, Japan

Y. Sato
Department of Surgery, National Hospital Organization Nagoya Medical Center Hospital, 4-1-1 Sannomaru, Naka-ku, Nagoya, Aichi 460-0001, Japan

Y. Ito
Department of Medical Oncology, The Cancer Institute of Japanese Foundation for Cancer Research, 3-10-6 Ariake, Koto-ku, Tokyo 135-8550, Japan

Y. Takatsuka
Department of Surgery, Kansai Rosai Hospital, 3-1-69 Inabaso, Amagasaki, Hyogo 660-8511, Japan

H. Arioka
Division of Medical Oncology, Yokohama Rosai Hospital, 3211 Kozukue-cho, Kohoku-ku, Yokohama 222-0036, Japan

Published online: 02 March 2012

toxicity in patients with anthracycline-resistant metastatic breast cancer. Further studies are warranted in a different population of breast cancer or other solid cancers.

Keywords Receptor tyrosine kinase inhibitor · Phase II study · Anthracycline-resistant breast cancer

Introduction

Angiogenesis plays a vital role in the growth and metastasis of solid tumors and vascular endothelial growth factor (VEGF) has been implicated in pathological angiogenesis associated with tumors [1–3]. For instance, bevacizumab, a humanized monoclonal antibody against the VEGF, plus chemotherapy showed a significant increase in clinical response and prolongation of time to disease progression as compared with chemotherapy alone in a wide variety of cancers such as colorectal cancer, non-small-cell lung cancer (NSCLC), and breast cancer [4–8]. However, it has been revealed that not only VEGF but also other endothelial growth factors are critical in the induction and maintenance of tumor angiogenesis. Through the platelet-derived growth factor-BB (PDGF-BB)/PDGF receptor signaling pathway, the contact between endothelial cells and pericytes and smooth muscle cells stabilizes new blood vessels, promotes endothelial survival, and inhibits endothelial cell proliferation [9, 10]. PDGF-BB and fibroblast growth factor (FGF) are two frequently expressed non-VEGF pro-angiogenic factors in tumors, and the expression levels of these factors are correlated with cancer progression and metastasis [11]. Activated endothelial cells in growing blood vessels are also important sources of PDGF-BB production. Malignant tumor transition could activate FGF secretion from tumor cells. Since FGF modulates the PDGF signaling system in endothelial cells, PDGF-BB and FGF together promote primary tumor growth in mice more than either of them could do so alone [12].

TSU-68 is a novel multiple tyrosine kinase inhibitor that competitively inhibits the adenosine triphosphate-binding domain of Flk-1/KDR tyrosine kinase, PDGF receptor tyrosine kinase, and FGF receptor tyrosine kinase. Animal studies showed that TSU-68 has a strong antitumor effect against several established human cancer xenografts as well as a broad antitumor spectrum without apparent adverse drug reactions [13–15]. In addition, TSU-68 in combination with taxane has exhibited synergic efficacy against established human breast cancer xenografts compared to TSU-68 or taxane alone [16].

On the basis of the results of the animal studies and three phase I studies with a dose-escalation design to determine recommended dose and dosing schedule of TSU-68 alone in patients with advanced solid tumor in Japan [17], we conducted a single-arm, open-label, multicenter phase II study to evaluate the efficacy and safety of TSU-68 in combination with docetaxel in patients with anthracycline-resistant metastatic breast cancer.

Patients and methods

Eligibility criteria

Female patients aged 20–75 years with histologically or cytologically confirmed metastatic breast cancer that had relapsed within 1 year (52 weeks) despite prior treatment with an anthracycline-containing regimen for metastatic disease or in the neo/adjuvant setting were eligible. Patients who had metastatic breast cancer relapsed after no less than 6 months (24 weeks) after completion of a treatment with taxane in the neo/adjuvant setting were allowed to be enrolled into the study but not mandated. Patients were required to have at least one measurable lesion according to the Response Evaluation Criteria for Solid Tumors (RECIST) version 1.0 guidelines [18]; World Health Organization performance status (WHO PS) of 0–2; adequate bone marrow, hepatic and renal function [white blood cell (WBC) count at least 4,000/m^3 to no greater than 12,000/m^3 or absolute neutrophil count at least 2,000/m^3; platelet count at least 100,000/m^3; hemoglobin level at least 9.0 g/dL; aspartate aminotransferase (AST) and alanine aminotransferase (ALT) no greater than 100 U/L; total bilirubin no greater than 1.5 mg/dL; serum creatinine no greater than 1.5 mg/dL]; no clinically significant electrocardiogram (ECG) abnormalities; and life expectancy of at least 3 months (90 days).

Patients were excluded if they had uncontrolled ascites, pleural effusion, or pericardial fluid requiring drainage, symptomatic brain metastasis, and active infection. Also patients who had received chemotherapy except for a 5-FU derivative agent and radiotherapy within 4 weeks of the initiation of study treatment were excluded, as were patients who had received immunotherapy, biological response modifiers (BRMs), hormonal therapy, or a 5-FU derivative agent within 2 weeks of the initiation of study treatment.

Written informed consent had been obtained from all patients before they were registered in this study. The study protocol was approved by the institutional review board or the ethical review board of respective study centers. This study was conducted by following the guidelines of Good Clinical Practice and in compliance with the Declaration of Helsinki.

Study design and treatment

This was open-label, non-comparative, multicenter phase II study. Nineteen qualified patients were to be enrolled in the study. This study did not have a multi-stage design based on statistical methods; however, the tolerability of TSU-68 in combination with docetaxel was non-statistically evaluated in the initial 6 patients by an independent data monitoring committee (IDMC) because this was the first study to assess TSU-68 in combination with docetaxel in patients with metastatic breast cancer. In addition, pharmacokinetic analysis was also performed in the initial 6 patients. Enrollment of additional patients was placed on hold until completion of tolerability confirmation of TSU-68 in combination with docetaxel by the IDMC.

TSU-68 was orally administered at a dose of 800 mg twice a day on days 1–21 and docetaxel was intravenously delivered at a dose of 60 mg/m^2, which is a common treatment dose for patients with metastatic breast cancer in Japan, over 1 h on day 1. This regimen was repeated every 21 days. After completion of cycle 6, patients were allowed to be treated with TSU-68 alone at the investigator's discretion. The study treatment was continued until the occurrence of unacceptable adverse drug reaction, withdrawal of informed consent, disease progression, or death.

Assessments

Tumor assessments by the investigator were performed at baseline (within 4 weeks before the initiation of study treatment) and every even cycle until disease progression according to the RECIST guidelines version 1.0. Objective response evaluated by the investigators was reviewed by an independent review committee. Complete response (CR) and partial response (PR) were confirmed at least 4 weeks later. Stable disease (SD) was required to last at least 6 weeks. Time to progression (TTP) was defined as days from the date of initiation of study treatment to disease progression or death.

Baseline evaluations for safety assessment included physical examination, vital signs, laboratory tests (hematology, blood chemistry, and urinalysis), and ECG (12-lead). Hematology and blood chemistry were performed weekly in cycle 1, on day 8, and day 15 (if clinically required) in the subsequent cycle, and at the end of the cycle. Urinalysis was measured before each cycle, if clinically required. Adverse drug reactions (ADRs) were graded according to the National Cancer Institute Common Toxicity Criteria (NCI-CTC) version 2.0 [19]. The criteria for cessation of TSU-68 or dose modification of docetaxel were determined as follows. The dosage of TSU-68 should not be decreased on shifting to the next cycle. If an adverse reaction is observed during a cycle, TSU-68 should be stopped. If any adverse reactions, such as febrile neutropenia of grade 3, thrombocytopenia of grade 3, and other grade 2 toxicity, making it difficult to continue the administration are observed during the previous cycle, the dosage of docetaxel may be decreased to 50 mg/m^2 for the next cycle.

Angiogenesis-related factors including plasma VEGF, tissue plasminogen activator (t-PA), vascular cell adhesion molecule-1 (VCAM-1), and plasma plasminogen activator inhibitor-1 (PAI-1) were measured at baseline and the end of cycle 1. Blood specimens were centrifuged at 5°C for 15 min at 3,000 rpm to separate plasma, which was collected in plastic tubes and stored at −20°C until analysis. Plasma VEGF, t-PA, and VCAM-1 were measured by enzyme-linked immunosorbent assay (ELISA). Line probe assay (LPIA) was used to measure PAI-1.

Blood samples were collected on days 1 and 2 of cycle 1 for PK analysis of TSU-68 and docetaxel plasma concentrations using an ultraviolet high-performance liquid chromatography (UV-HPLC) assay and a gas chromatography–mass spectrometry assay, respectively, in initial 6 patients. Samples were collected at baseline, 1, 3, 4, 6, and 9 h on day 1 and 1, 2, and 4 h on day 2 after morning dosing of TSU-68. Docetaxel was intravenously delivered after 1 h of morning dosing of TSU-68 on day 1 only in cycle 1. Parameters included area under the curve (AUC), maximum concentration (C_{max}), time to maximum concentration (T_{max}), and elimination half-life ($T_{1/2}$). Noncompartmental PK parameters (AUC_{0-t}, $AUC_{0-infinity}$, and $T_{1/2}$) for TSU-68 and docetaxel were calculated using WinNonlin software version 3.1 (Pharsight Corporation, California, USA) and PhAST software version 2.3 (MDS Pharma Services, Pennsylvania, USA), respectively.

Statistical analysis

Assuming a historical objective response rate of approximately 35% in Japanese patients with anthracycline-resistant metastatic breast cancer [20], a sample size of 19 patients would provide 80% power for testing that the objective response rate for TSU-68 in combination with docetaxel was 60% or more (overall 1-sided significance level of 0.05, exact binomial test). If 10 or more responses were observed, then the lower bound of the 90% confidence intervals (CI) would exclude 30%. CIs for parameters to be estimated were calculated with a significance level of 0.05. The exact 95% CI was calculated for the efficacy variables. Primary endpoint was objective response rate defined in the RECIST guidelines version 1.0. Secondary endpoints included clinical benefit rate, defined as an objective response (CR or PR) and SD for at least 24 weeks, TTP, overall survival (OS), exploratory

assessment of angiogenesis-related factors in plasma, and safety of TSU-68 in combination with docetaxel. TTP was analyzed by using the Kaplan–Meier curves. Patients who received at least one dose of study treatment were included in a population for efficacy analysis.

Results

Patient characteristics

Nineteen patients were enrolled from February 2003 through January 2005. All patients who received the study treatment at least once were included in full analysis set in the efficacy and safety analyses. The baseline patient characteristics are summarized in Table 1.

Hormone receptor status, estrogen receptor (ER) and progesterone receptor (PgR), and HER2 status were assessed according to the standard method used at respective study centers. All 19 patients had been treated with an anthracycline-containing regimen for metastatic disease and/or in the neo/adjuvant setting. Two patients had been treated with a taxane in the neo/adjuvant setting.

Efficacy

The efficacy of TSU-68 was evaluated in 19 patients. TSU-68 in combination with docetaxel produced objective responses in 21.1% and clinical benefits (CR, PR, and SD for at least 24 weeks) in 42.1% of all patients, respectively (Table 2). Median TTP was 148 days (95% CI 85–246 days; Fig. 1) and median OS was 579 days (95% CI 449–899 days; Fig. 1). Median number of cycles was 6.6 (range 1–44+) and median duration of treatment was 20.1 weeks at this analysis. One patient was treated with this combination chemotherapy for over 1,683 days and is still continuing. Stratification according to hormone receptor status and HER2 status showed the response results as follows: ER+ 4/15 (26%), ER− 0/4 (0%), PgR+ 3/10 (30%), PgR− 1/9 (11%), HER2+ 0/1 (0%), and HER2− 4/18 (22%).

Pharmacokinetics

Pharmacokinetics (PK) and drug–drug interaction at administration of TSU-68 in combination with docetaxel were assessed in initial 6 patients in this study. PK parameters of TSU-68 on day 1 were similar to those at administration of TSU-68 alone in a phase I study in Japan [17], suggesting no affect of docetaxel on the PK of TSU-68 (Fig. 2; Table 3). PK parameters of docetaxel on day 1 were also similar to those in a Japanese phase I study of docetaxel [21].

Table 1 Patient characteristics

Characteristics	Number of patients ($n = 19$)	%
Age (years)		
Median (range)	54 (29–72)	
<65	15	78.9
≥65	4	21.1
WHO performance status		
0	16	84.2
1	3	15.8
Menopausal status		
Premenopausal	1	5.3
Postmenopausal	18	94.7
Dominant disease site		
Soft tissue	4	21.1
Bone	4	21.1
Viscera	11	57.9
ER status		
+	15	78.9
−	4	21.1
PgR status		
+	10	52.6
−	9	47.4
HER2 status		
1+	12	63.2
2+	0	0.0
3+	1	5.3
−	6	31.6
Prior chemotherapy		
Anthracycline-containing regimen only	17	89.5
Anthracycline-containing regimen and taxane	2	10.5
Treatment setting of anthracycline-containing regimen		
Neo/adjuvant setting only	4	21.1
For metastatic disease only	11	57.9
Both	4	21.1

ER estrogen receptor, *PgR* progesterone receptor, *HER2* human epidermal growth factor receptor-2

Angiogenesis-related factors in plasma

Plasma specimens were available in 17 of 19 patients and 4 factors (plasma VEGF, plasma t-PA, VCAM-1, and PAI-1; Table 4) were assessed. No significant change in plasma VEGF, plasma t-PA, VCAM-1, and PAI-1 was observed at the end of cycle 1 ($p = 0.531$, 0.991, 0.782, and 0.530, respectively).

Safety

The safety of TSU-68 in combination with docetaxel was assessed in 19 patients. The compliance of TSU-68 administration was 89.5% (17/19 patients) in cycle 1 (21 days). Hematologic and non-hematologic ADRs that commonly occurred in the patients are summarized by grade in Table 5. The most common hematologic ADRs were neutropenia (94.7%), leukopenia (94.7%), and hemoglobin decreased. Of 19 patients, 7 patients experienced thrombocytopenia; however, no patient experienced grade 3 or 4. Severe non-hematologic ADRs rarely occurred, and they were mild or moderate in severity. Three patients experienced rash (two patients with grade 2 and one patient with grade 1) and only one patient experienced grade 1 skin reaction. One patient experienced grade 2 hypertension. No episode of death related to study treatment occurred in 19 patients.

Table 2 Objective response

Overall responses	*n*	%
Objective response rate	4	21.1
Exact 95% confidence limits		6.1–45.6
Clinical benefit rate	8	42.1
Exact 95% confidence limits		20.3–66.5
Complete response (CR)	1	5.3
Partial response (PR)	3	15.8
Stable disease (SD) ≥ 24 weeks	4	21.1
Stable disease	5	26.3
Progression of disease (PD)	3	15.8
Not evaluable (NE)	3	15.8

Discussion

TSU-68 is a multiple tyrosine kinase inhibitor for VEGF receptor-2, PDGF receptor, and FGF receptor. This open-label, non-comparative, multicenter phase II study was conducted in patients with metastatic breast cancer that had relapsed within 1 year despite prior treatment with an anthracycline-containing regimen, mainly as the second-line treatment. In this situation, TSU-68 in combination with docetaxel achieved clinical benefit in 42% of patients and an objective response rate of 21%. It seems possible to say that some combination effect is occurring, although a comparative study is required to prove this. So far many chemotherapy agents have been applied for the treatment of breast cancer and it has been elucidated that the impact on prognosis is limited in the situation of second- or third-line therapy. In the first-line therapy, several studies have shown a significant benefit of anti-angiogenesis therapy plus chemotherapy in improving antitumor effect and progression free survival when compared with chemotherapy alone. A representative result was reported from a phase III study, which enrolled 722 patients and compared the efficacy and safety of paclitaxel in combination with bevacizumab with paclitaxel alone as initial treatment for metastatic breast cancer, although no significant difference in OS was observed [5]. Another phase III study comparing docetaxel plus bevacizumab with docetaxel alone also showed a similar result [6]. These results suggest that it is possible to increase response and relapse-free survival with a therapy targeting VEGF combined with cytotoxic chemotherapy such as taxane, although it seems difficult to produce an impact on OS.

As to safety, it is known well that bevacizumab causes hypertension, bleeding, and thrombosis [4–8]. Sunitinib and sorafenib, which are VEGF tyrosine kinase inhibitors, induce hand foot skin reaction [22, 23]. This

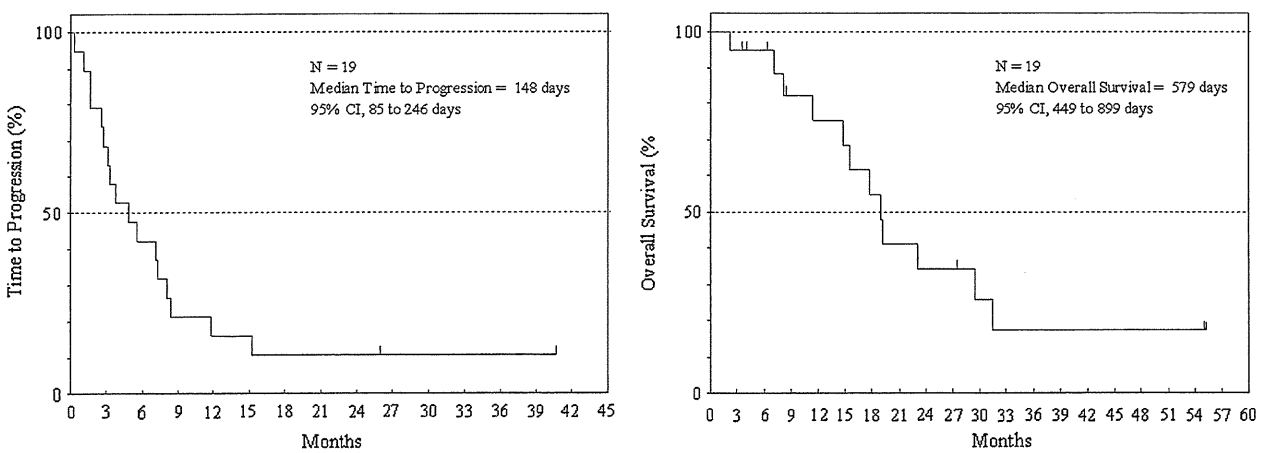


Fig. 1 Kaplan–Meier curve for TTP and OS

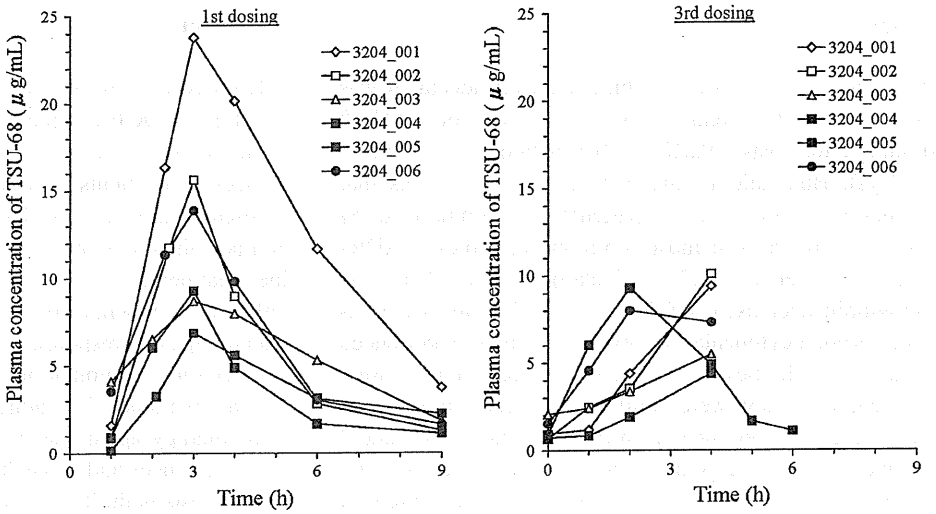


Fig. 2 Plasma concentrations of TSU-68 at morning dosing on day 1–2 and PK parameters of TSU-68/docetaxel on day 1

Table 3 PK parameters of TSU-68 at morning dosing and docetaxel on day 1

PK parameter	TSU-68 ($n = 6$)	Docetaxel ($n = 6$)
T_{max} (h)	3.00 ± 0.0	–
C_{max} (μg/mL)	13.02 ± 6.22	1.62 ± 0.74
$AUC_{0-infinity}$ (μg h/mL)	58.30 ± 28.60	1.91 ± 0.69
$T_{1/2}$ (h)	2.40 ± 0.75	9.31 ± 6.95

Mean ± SD

ADR and others commonly associated with other VEGF inhibitors, e.g., hypertension, were observed in a few patients and were mild or moderate in severity in the present study. In addition, only one patient experienced grade 2 hypertension. This unique safety profile of TSU-68 may suggest insufficient inhibition of VEGF; however, it should be considered that major tyrosine kinase inhibitors have often given rise to safety concerns, which require frequent and rapid dose reductions, dose interruptions, and dose delays in the treatment when the inhibitors are administered with other chemotherapy agents or other molecular target agents [24–27]. Furthermore, if there is an increase in the frequency of unacceptable ADRs that prevent long-term treatment, it would mean that the regimen does not sufficiently confer its efficacy benefits. Although only 19 patients were treated with TSU-68 in combination with docetaxel in this study, no unexpected or unacceptable ADRs leading to patients suffering or requiring difficult management were observed. In this point, TSU-68 has an advantage compared with other VEGF inhibitors. On the other hand, a higher frequency of edema was observed in this study, although this is a common ADR of docetaxel itself. In phase I studies in Japanese patients with solid tumor, edema was one of the common ADRs of TSU-68; this ADR is also associated with imatinib, which inhibits PDGF tyrosine kinase as well as Bcr-Abl [28]. The biological mechanism of TSU-68-induced edema is still unclear. However, as TSU-68 strongly inhibits PDGF tyrosine kinase as well as VEGF [13], edema might be associated with TSU-68.

PK study on drug–drug interactions confirmed that docetaxel exhibited no effect of on the PK of TSU-68. The result on day 1 was similar to that shown in a Japanese phase I study of docetaxel [21].

It is known that anti-VEGF therapy could elicit an increase in VEGF expression [29]. Nevertheless, we detected no increase or decrease in the levels of plasma VEGF. In addition, no significant change was observed for plasma t-PA, VCAM-1, and PAI-1. We need to encourage further careful analysis on the up- or downregulation of angiogenesis-associated molecules; it is important to know about the changes in VEGF and VCAM-1 induced by TSU-68.

Thus, TSU-68 in combination with docetaxel was tolerable in patients with anthracycline-resistant metastatic breast cancer. To evaluate if TSU-68 in combination with docetaxel uniquely shows synergistic benefit compared with docetaxel alone, further appropriate randomized phase II study would be required in the future.