厚生労働科学研究費補助金
がん対策推進総合研究事業
（革新的がん医療実用化研究事業）

進行非小細胞肺癌を対象としたエルロチニブとYM155の分子標的治療薬併用第I相試験

平成２４年度～２６年度　総合研究報告書

研究代表者　中川　和彦

平成２７（２０１５）年　３月

別添１

厚生労働科学研究費補助金総合研究報告書表紙

別添２

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研究成果の刊行に関する一覧表

別紙１

厚生労働科学研究費補助金

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別紙２

目　　次

別紙３

厚生労働科学研究費補助金（がん対策推進総合研究事業（革新的がん医療実用化研究事業））
（総合）研究報告書

進行非小細胞肺癌を対象としたエルロチニブとYM155の分子標的治療薬併用第I相試験

研究代表者　中川　和彦
近畿大学医学部内科学腫瘍内科部門　教授

研究要旨　進行非小細胞肺癌患者を対象に、EGFRチロシンキナーゼ阻害剤(EGFR-TKI)エルロチニブに併用するサバイビン阻害薬YM155の推奨投与量の設定、及び用量制限毒性（DLT）を明らかにし、推奨投与量における安全性と抗腫瘍効果および効果に関わるバイオマーカーを探索する

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西尾　和人　（近畿大学医学部ゲノム生物学教室　教授）
坂井　和子　（近畿大学医学部ゲノム生物学教室　助教）
千葉　康敬　（近畿大学医学部附属病院臨床研究センター　講師）

A．研究目的

EGFR陽性進行非小細胞肺癌患者を対象に、EGFRチロシンキナーゼ阻害剤(EGFR-TKI)エルロチニブに併用するサバイビン阻害薬YM155の推奨投与量の設定、及び用量制限毒性（DLT）を明らかにし、推奨投与量における安全性と抗腫瘍効果および効果に関わるバイオマーカーを探索する。

B．研究方法

[研究計画・方法]

分子標的治療薬併用第Ｉ相臨床試験（医師主導治験）として、進行非小細胞肺癌に対する化学療法を受ける患者を対象にエルロチニブとYM155併用投与の両薬剤推奨投与量の設定、用量制限毒性（DLT）および最大耐用量（MTD）を明らかにし、両分子標的治療薬の推奨投与量における安全性と抗腫瘍効果について検討する。

[対象症例]

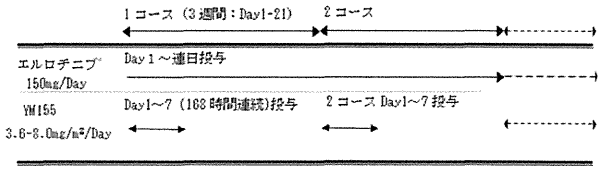
進行非小細胞肺癌に対する化学療法を受ける患者、20歳以上、ECOG Performance Status (PS) 0-2、主要臓器機能が保持された症例。患者本人の自由意思による文書同意を必須とする。

【Primary endpoint】

エルロチニブとYM155併用投与の安全性プロファイル（有害事象）、用量制限毒性（DLT : dose limiting toxicity）、最大耐用量（MTD : maximum tolerated dose）および推奨投与量の決定。

【Secondary endpoint】

推奨投与量における安全性と抗腫瘍効果、及び抗腫瘍効果に関わるバイオマーカーの探索。



エルロチニブは１日１錠(150mg)の連日投与とし、YM155（アステラス製薬より供給予定）は（シリンジポンプを用いた）168時間（7日間）の持続点滴静脈内投与とする。併用治療開始時点を1コースday１とする。エルロチニブは連日投与、YM155は1週間(168時間)投与2週間休薬をもって1コース（21日間隔）とする。以後、腫瘍の増悪、新病変の出現または投与継続が困難な有害事象の発現を認めるまで、1コースを21日間隔として治療を継続する。パート1(dose escalation cohort)の症例では、プロトコール本文に記載のスケジュールにてエルロチニブ及びYM155の薬物動態測定（血漿及び尿検体）を行う。また同意が得られた患者に対し、抗腫瘍効果に関わるバイオマーカーの探索として1)YM155投与前後における腫瘍組織中のサバイビン蛋白質量の測定とアポトーシス誘導の有無を確認、2)肺癌組織の体細胞変異解析にあたり、LungCarta、Bio-plex (Ligand panel)等のマススクリーニングパネルを用いた半網羅的体細胞変異解析を行う。

[予定症例数及び研究期間]

医師主導治験による第Ｉ相臨床試験として、12-24例。試験期間は2012年12月1日より2015年11月31日（準備期間：1年、登録期間: １年、追跡期間：1年）とする。

[研究体制]

研究代表者（医師主導治験実施責任者）は研究の統括・計画を実施する。研究分担者は近畿大学医学部腫瘍内科において研究の計画・測定・解析を実施、症例登録を行う。バイオマーカーの測定は近畿大学医学部ゲノム生物学教室で測定する。近畿大学医学部・医学部附属病院および外部CROであるクインタ

イルズ・ジャパン・データマネジメント部および日本臨床研究オペレーションズ（Japan Clinical Research Operations:JCRO)は近畿大学医学部腫瘍内科と共同して本医師主導治験運用に必須であるセンターデータマネージメント、モニタリング業務、治験薬管理（治験薬剤提供元企業との連携）、CRC業務およびローカルデータマネージメント業務を遂行する。統計解析は近畿大学医学部臨床研究センター腫瘍統計学部門および外部CROであるクインタイルズ・ジャパン・データマネジメント部が行う。研究実施環境については研究施設・研究資料・研究フィールド・現在の研究環境の状況等インフラ整備されており問題はない。

（倫理面への配慮）
試験に関係するすべての研究者は、ヘルシンキ宣言および臨床研究に関する倫理指針にしたがって本試験を実施し，以下の事項を厳守する.
1. 登録に先立って、すべてに患者に施設の倫理審査委員会（IRB）承認が得られた説明文書を用いて十分な説明を行い，考慮の時間を設けた後に患者自身の自由意志による同意を文書にて取得るする。
2. 個人情報および診療情報などのプライバシーに関する情報は個人の人格尊重の理念の下、厳重に保護され慎重に取り扱われるべきものと認識し、万全な管理対策を講じ、プライバシー保護に努める。データの取り扱いに関しては直接個人を識別できる情報を用いず，データベースのセキュリティーを確保し，個人情報の保護を厳守する。
本研究に組み込まれるバイオマーカー研究は蛋白発現、体細胞DNAを対象に解析するものであり、「ヒトゲノム・遺伝子解析研究に関する倫理指針」の対象ではないが、その趣旨を踏まえた対応を行う。

C．研究結果
EGFR陽性進行非小細胞肺癌患者を対象に、分子標的治療薬併用第Ⅰ相臨床試験としてEGFR阻害薬エルロチニブ併用時における新規サバイビン阻害薬YM155の推奨投与量の設定、用量制限毒性（DLT）および最大 耐用量（MTD）を明らかにし、両分子標的治療薬の推奨投与量における安全性と抗腫瘍効果の検討及び抗腫瘍効果に関わるバイオマーカー探索を実施した。現況として当初の治験実施計画規定に基づく第１コホートレベル～第4コホートレベル迄の合計4段階用量漸増計画のうち、同事業完了時点において現在引き続き当施設に於いて残る最終の第4コホートレベル（合計3～6名予定）の被験者に対して治験薬を投与中であり、同医師主導治験完遂まで実施予定である。併用第Ⅰ相臨床試験の第3コホートレベル終了時までにおける研究結果として、
①安全性に関する評価に関しては第1コホートレベルから第3コホートレベル（合計12例）においては用量制限毒性（DLT）発現は全12症例中1症例のみに認められ（血清クレアチニン値上昇 2.4mg/dl NCI-CTC グレード2）、治験薬休薬中止にて可逆的に完全回復した。治療との因果関係が否定出来ない毒性に関してNCI CTC-AEグレード3以上の毒性に関してはYM155 第2コホートレベルにおいて1例のみ（下痢：グレード3）を認めたのみであり、最も高頻度の毒性に関しては皮疹（グレード2：45.5%、グレード1：45.5%）、疲労（グレード1：23.9%）、下痢（グレード3：9.1%、グレード2：9.1%、グレード1：18.2%）、尿中β2-ミクログロブリン上昇（グレード1：23.9%）、尿中NAG上昇（グレード1：23.9%）、血清クレアチニン上昇（グレード2：9.1%、グレード1：9.1%）、ヘモグロビン低下（グレード2：9.1%、グレード1：9.1%）、蛋白尿（グレード1：18.2%）、発熱（グレード1：9.1%）、低Na血症（グレード1：9.1%）、味覚異常（グレード1：9.1%）であり、全般的に忍容性は良好であった。
②有効性に関する評価に関しては全12症例中2症例において（それぞれYM155 第1コホートレベルおよび第3コホートレベル）6か月間以上の画像上の病勢安定（RECIST判定基準においてSD：Stable disease）および腫瘍縮小効果が認められた。
③抗腫瘍効果に関わるバイオマーカー探索研究として、治験薬投与前後（YM155投与前および2サイクル目投与期間中）の腫瘍組織採取（気管支鏡下肺生検もしくは転移病巣からの経皮的腫瘍針生検等）が採取施行可能例には被験者の同意取得のもとに実施されており、抗腫瘍効果に関わるバイオマーカーの探索として1)YM155投与前後における腫瘍組織中のサバイビン蛋白質量の測定（Survivin IHC、Survivin RT-PCR）とアポトーシス誘導の有無を確認、2)肺癌組織の体細胞変異解析にあたり、LungCarta Panel、Ion Ampliseq Panel （NGS：次世代シーケンサー）、Luminex Panel（血漿タンパク質解析）等のマススクリーニングパネルを用いた半網羅的体細胞変異解析を施行した。腫瘍組織中のサバイビン蛋白発現に関しては免疫組織染色（Survivin IHC）においてYM155投与前後において有意にサバイビン蛋白発現が低下する傾向を認めた。血液検体を用いたLuminex Panel（血漿タンパク質解析）においては抗腫瘍効果判定においてNon-PD（Progressive disease）群はPD群と比較してDay7以降のIL-1Ra, IL-2, IL-7,IL-12, IL-13, G-CSF, TNF-αが高値を示す傾向が認められた。効果予測因子となりうる血清バイオマーカーに関してEGFR阻害薬エルロチニブに関しては治療前のHGFおよびVEGF-A高値が、併用薬YM155に関しては治療前のIL-10, IL-12およびVEGF-A高値が予後不良と相関性傾向を示した。

同医師主導治験実施期間中に治験薬供給元であるアステラス製薬株式会社において他の開発品との優先度等を総合的に勘案し製薬企業側の戦略的観点から、治験薬YM155の今後の開発中止が決定された旨をアステラス製薬株式会社より報告を受けた。既に現在までに合計3回の外部委員による効果安全性委員会開催が施行されており、また治験薬に関する安全性・有効性以外の製薬企業理由による薬剤開発中止を受けた後における医師主導治験実施継続に関する妥当性に関してPMDA審査マネジメント部・アステラス製薬株式会社より問題はないものとの回答を得ており、平成27年4月20日当施設倫理委員会にての審議承認を経て、治験実施計画書に準じて予定通り最終コホートレベルである第4コホートレベルにおいて現在も医師主導試験実施中である。治験薬の安定性試験（延長申請済み：アステラス製薬品質保証部より再試験期限変更済み）結果に基づいた予定治験薬使用期限である平成27年9月末までに最終コホートレベルである第4コホートレベルを終了完結すべく現在も同内容に関して被験者へ十分なインフォームドコンセントを行ったうえで（同意説明文書改訂済・施設倫理委員会承認済）医師主導治験を実施中である。

D．考察

抗腫瘍効果に関わるバイオマーカー探索研究として、治験薬投与前後（YM155投与前および2サイクル目投与期間中）の腫瘍組織採取（気管支鏡下肺生検もしくは転移病巣からの経皮的腫瘍針生検等）が採取施行可能例には被験者の同意取得のもとに実施されており、抗腫瘍効果に関わるバイオマーカーの探索として1)YM155投与前後における腫瘍組織中のサバイビン蛋白質量の測定（Survivin IHC、Survivin RT-PCR）とアポトーシス誘導の有無を確認、2)肺癌組織の体細胞変異解析にあたり、LungCarta Panel、Ion Ampliseq Panel （NGS:次世代シーケンサー）、Luminex Panel（血漿タンパク質解析）等のマススクリーニングパネルを用いた半網羅的体細胞変異解析を施行した。腫瘍組織中のサバイビン蛋白発現に関しては免疫組織染色（Survivin IHC）においてYM155投与前後において有意にサバイビン蛋白発現が低下する傾向を認めた。血液検体を用いたLuminex Panel（血漿タンパク質解析）においては抗腫瘍効果判定においてNon-PD（Progressive disease）群はPD群と比較してDay7以降のIL-1Ra, IL-2, IL-7,IL-12, IL-13, G-CSF, TNF-αが高値を示す傾向が認められた。効果予測因子となりうる血清バイオマーカーに関してEGFR阻害薬エルロチニブに関しては治療前のHGFおよびVEGF-A高値が、併用薬YM155に関しては治療前のIL-10, IL-12およびVEGF-A高値が予後不良と相関性傾向を示した。臨床的に長いPFSを示した群においては抗アポトーシスケモカイン及びVEGFが低値を示し、抗アポトーシスが弱い腫瘍細胞においては、YM155によるアポトーシス易誘導可能性が示唆された。今後も適切な症例選択に基づくサバイビン阻害薬によるEGFR阻害薬耐性克服メカニズム可能性に関してバイオマーカーを含めた更なる探索が求められる。

論文発表

1. Azuma K, Okamoto I, Kawahara A, Taira T, Nakashima K, Hattori S, Kinoshita T, Takeda M, Nakagawa K, Takamori S, Kuwano M, Ono M, Kage M. Association of the Expression of Mutant Epidermal Growth Factor Receptor Protein as Determined with Mutation-Specific Antibodies in Non-small Cell Lung Cancer with Progression-Free Survival after Gefitinib Treatment. J Thorac Oncol. 7(1):122-7, 2012.
2. ○Okamoto K, Okamoto I, Hatashita E, Kuwata K, Yamaguchi H, Kita A, Yamanaka K, Ono M, Nakagawa K. Overcoming erlotinib resistance in EGFR mutation-positive non-small cell lung cancer cells by targeting survivin. Mol Cancer Ther. 11(1):204-13, 2012.
3. Takeda M, Okamoto I, Tsurutani J, Oiso N, Kawada A, Nakagawa K. Clinical Impact of Switching to a Second EGFR-TKI After a Severe AE Related to a First EGFR-TKI in EGFR-mutated NSCLC. Jpn J Clin Oncol. 42(6):528-33, 2012.
4. Nakagawa K, Kudoh S, Ohe Y, Johkoh T, Ando M, Yamazaki N, Seki A, Takemoto S, Fukuoka M. Postmarketing surveillance study of erlotinib in Japanese patients with non-small-cell lung cancer (NSCLC): an interim analysis of 3488 patients (POLARSTAR). J Thorac Oncol. 7(8):1296-303, 2012.
5. ○Aoyama Y, Nishimura T, Sawamoto T, Satoh T, Katashima M, Nakagawa K. Pharmacokinetics of sepantronium bromide (YM155), a small-molecule suppressor of survivin, in Japanese patients with advanced solid tumors: dose proportionality and influence of renal impairment. Cancer Chemother Pharmacol. 70(3):373-80, 2012.
6. Hayashi H, Kurata T, Fujisaka Y, Kawakami H, Tanaka K, Okabe T, Takeda M, Satoh T, Yoshida K, Tsunoda T, Arao T, Nishio K, Nakagawa K. Phase I trial of OTS11101, an anti-angiogenic vaccine targeting Vascular Endothelial Growth Factor Receptor 1 in solid tumor. Cancer Science 104(1):98-104, 2012.
7. Kiyota H, Okamoto I, Takeda M, Daga H, Naito T, Miyazaki M, Okada H, Hayashi H, Tana

ka K, Terashima M, Azuma K, Murakami H, Takeda K, Yamamoto N, Nakagawa K. Phase I and pharmacokinetic study of gefitinib and S-1 combination therapy for advanced adenocarcinoma of the lung. Cancer Chemother Pharmacol. 71(4):859-65, 2013

8. Kogure Y, Ando M, Saka H, Chiba Y, Yamamoto N, Asami K, Hirashima T, Seto T, Nagase S, Otsuka K, Yanagihara K, Takeda K, Okamoto I, Aoki T, Takayama K, Yamasaki M, Kudoh S, Katakami N, Miyazaki M, Nakagawa K. Histology and Smoking Status Predict Survival of Patients with Advanced Non-Small-Cell Lung Cancer: Results of West Japan Oncology Group (WJOG) Study 3906L. J Thorac Oncol. 8(6):753-758, 2013
9. Akamatsu H, Inoue A, Mitsudomi T, Kobayashi K, Nakagawa K, Mori K, Nukiwa T, Nakanishi Y, Yamamoto N. Interstitial Lung Disease Associated with Gefitinib in Japanese Patients with EGFR-mutated Non-small-cell Lung Cancer: Combined Analysis of Two Phase III Trials (NEJ 002 and WJTOG 3405). Jpn J Clin Oncol. 43(6):664-8, 2013
10. Seto T, Kiura K, Nishio M, Nakagawa K, Maemondo M, Inoue A, Hida T, Yamamoto N, Yoshioka H, Harada M, Ohe Y, Nogami N, Takeuchi K, Shimada T, Tanaka T, Tamura T. CH5424802 (RO5424802) for patients with ALK-rearranged advanced non-small-cell lung cancer (AF-001JP study): a single-arm, open-label, phase 1-2 study. Lancet Oncol. 14(7):590-8, 2013
11. Shaw AT, Kim DW, Nakagawa K, Seto T, Crinó L, Ahn MJ, De Pas T, Besse B, Solomon BJ, Blackhall F, Wu YL, Thomas M, O'Byrne KJ, Moro-Sibilot D, Camidge DR, Mok T, Hirsh V, Riely GJ, Iyer S, Tassell V, Polli A, Wilner KD, Jänne PA. Crizotinib versus chemotherapy in advanced ALK-positive lung cancer. N Engl J Med. 368(25)2385-94., 2013
12. Yang JC, Wu YL, Chan V, Kurnianda J, Nakagawa K, Saijo N, Fukuoka M, McWalter G, McCormack R, Mok TS. Epidermal growth factor receptor mutation analysis in previously unanalyzed histology samples and cytology samples from the phase III Iressa Pan-ASia Study (IPASS). Lung Cancer. S0169-5002(13):00535-7, 2013
13. Takeda M, Okamoto I, Nakagawa K. Survival outcome assessed according to tumor response and shrinkage pattern in patients with EGFR mutation-positive non-small-cell lung cancer treated with gefitinib or erlotinib. J Thorac Oncol. 9(2):200-4, 2014.
14. Hayashi H, Arao T, Matsumoto K, Kimura H, Togashi Y, Hirashima Y, Horita Y, Iwasa S, Okita NT, Honma Y, Takashima A, Kato K, Hamaguchi T, Shimada Y, Nakagawa K, Nishio K, Yamada Y. Biomarkers of reactive resistance and early disease progression during chemotherapy plus bevacizumab treatment for colorectal carcinoma. Oncotarget. 5(9)2588-95, 2014
15. ○Yoshida T, Zhang G, Smith MA, Lopez AS, Bai Y, Li J, Fang B, Koomen J, Rawal B, Fisher KJ, Chen AY, Kitano M, Morita Y, Yamaguchi H, Shibata K, Okabe T, Okamoto I, Nakagawa K, Haura EB. Tyrosine phosphoproteomics identifies both codrivers and cotargeting strategies for T790M-related EGFR-TKI resistance in non-small cell lung cancer. Clin Cancer Res. 20(15)4059-74. 2014
16. Kawakami H, Okamoto I, Okamoto W, Tanizaki J, Nakagawa K, Nishio K. Targeting MET Amplification as a New Oncogenic Driver. Cancers (Basel). 6(3)1540-52, 2014
17. Hayashi H, Okamoto I, Tanizaki J, Tanaka K, Okuda T, Kato A, Nishimura Y, Nakagawa K. Cystic Brain Metastasis in Non-Small-Cell Lung Cancer With ALK Rearrangement. J Clin Oncol. 32(36)e122-4, 2014
18. Seto T, Kato T, Nishio M, Goto K, Atagi S, Hosomi Y, Yamamoto N, Hida T, Maemondo M, Nakagawa K, Nagase S, Okamoto I, Yamanaka T, Tajima K, Harada R, Fukuoka M, Yamamoto N. Erlotinib alone or with bevacizumab as first-line therapy in patients with advanced non-squamous non-small-cell lung cancer harbouring EGFR mutations (JO25567): an open-label, randomised, multicentre, phase 2 study. Lancet Oncol. 15(11)1236-44, 2014
19. Gemma A, Kudoh S, Ando M, Ohe Y, Nakagawa K, Johkoh T, Yamazaki N, Arakawa H, Inoue Y, Ebina M, Kusumoto M, Kuwano K, Sakai F, Taniguchi H, Fukuda Y, Seki A, Ishii T, Fukuoka M. Final safety and efficacy of erlotinib in the phase 4 POLARSTAR surveillance study of 10 708 Japanese patients with non-small-cell lung cancer. Cancer Sci. 105(12)1584-90, 2014
20. Solomon BJ, Mok T, Kim DW, Wu YL, Nakagawa K, Mekhail T, Felip E, Cappuzzo F, Paolini J, Usari T, Iyer S, Reisman A, Wilner KD, Tursi J, Blackhall F; PROFILE 1014 Investigators. First-line crizotinib versus chemo

therapy in ALK-positive lung cancer. N Engl J Med. 371(23)2167-77, 2014
21. Kawakami H, Okamoto I, Yonesaka K, Okamoto K, Shibata K, Shinkai Y, Sakamoto H, Kitano M, Tamura T, Nishio K, Nakagawa K. The anti-HER3 antibody patritumab abrogates cetuximab resistance mediated by heregulin in colorectal cancer cells. Oncotarget. 5(23)11847-56, 2014
22. Okamoto I, Miyazaki M, Takeda M, Terashima M, Azuma K, Hayashi H, Kaneda H, Kurata T, Tsurutani J, Seto T, Hirai F, Konishi K, Sarashina A, Yagi N, Kaiser R, Nakagawa K. Tolerability of Nintedanib (BIBF 1120) in Combination with Docetaxel: A Phase 1 Study in Japanese Patients with Previously Treated Non-Small-Cell Lung Cancer. J Thorac Oncol. 10(2)346-52, 2015
23. Sakai K, Takeda M, Okamoto I, Nakagawa K, Nishio K. Multiple regulatory mechanisms of hepatocyte growth factor expression in malignant cells with a short poly(dA) sequence in the HGF gene promoter. Oncol Lett. 9(1)405-410, 2015
24. Nishio M, Horiike A, Murakami H, Yamamoto N, Kaneda H, Nakagawa K, Horinouchi H, Nagashima M, Sekiguchi M, Tamura T. Phase I study of the HER3-targeted antibody patritumab (U3-1287) combined with erlotinib in Japanese patients with non-small cell lung cancer. Lung Cancer. S0169-5002(15)00162-2, 2015
25. Tsurutani J, Kuroi K, Iwasa T, Miyazaki M, Nishina S, Makimura C, Tanizaki J, Okamoto K, Yamashita T, Aruga T, Shigekawa T, Komoike Y, Saeki T, Nakagawa K. Phase I study of weekly nab-paclitaxel combined with S-1 in patients with human epidermal growth factor receptor type 2-negative metastatic breast cancer. Cancer Sci. 2015 inpress

G．知的財産権の出願・登録状況

1. 特許取得　なし
2. 実用新案登録　なし
3. その他　　なし

研究名:

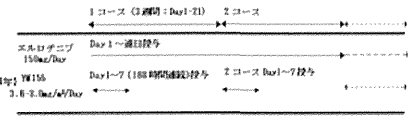
EGFR陽性進行非小細胞肺癌を対象としたYM155/エルロチニブ併用臨床第Ⅰ相試験

試験の概要

分子標的治療薬併用第Ⅰ相臨床試験(医師主導治験)

・ 試験の目的

EGFR陽性進行非小細胞肺癌患者を対象に、分子標的治療薬併用第Ⅰ相臨床試験としてEGFR阻害薬エルロチニブ併用時における病変サバイビン阻害薬YM155の推奨投与量の設定、用量制限毒性(DLT)および最大耐用量(MTD)を明らかにし、両分子標的治療薬の推奨投与量における安全性と抗腫瘍効果の検討及び抗腫瘍効果に関わるバイオマーカー探索を行う。



計画症例数及び登録期間

第Ⅰ相試験として、12-36例

試験期間:2013年1月1日～2015年12月31日

(準備期間:1年、登録期間:1年、追跡期間:1年)

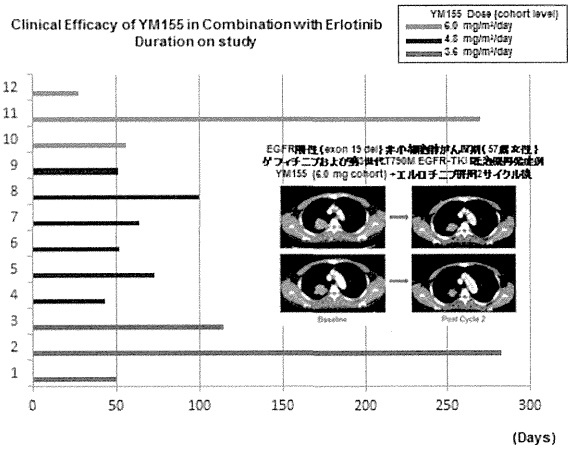
Part 1 (用量漸増パート)

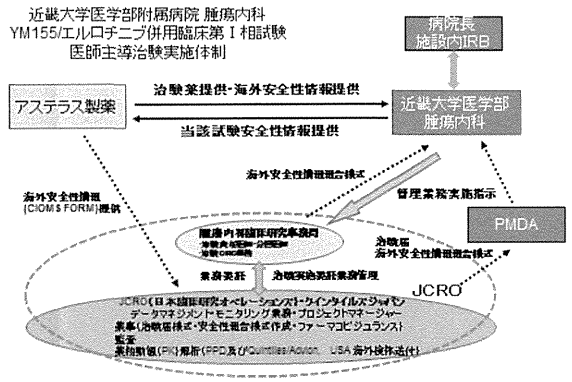
Level	Erlotinib	YM155	No. of patients
(0)	150 mg/day	3.6 mg/m²/day	(3-6)
1	150 mg/day	4.8 mg/m²/day	3-6
2	150 mg/day	6.0 mg/m²/day	3-6
3	150 mg/day	8.0 mg/m²/day	3-6

Part 2 (拡大コホート:MTD)

エルロチニブ	YM155	症例数
150mg/Day	推奨投与量	～12

	YM155単剤Phase 1 MTD	DLT	Publication
米国	4.8 mg/m²/day	血清クレアチニン上昇	JCO 2008
日本	8.0 mg/m²/day	血清クレアチニン上昇	CCR 2009



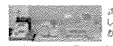


平成24年6月27日 本研究計画に関する探索通知(厚生労働省)
平成24年7月 医師主導治験実施体制準備開始
平成24年10月23日 治験届 治験審査委員会(IRB)承認
平成24年11月22日 PMDA当局へ治験届提出
平成24年12月10日 PMDA当局より照会事項を確認
平成25年1月25日 コホートH 第1症例 治験登録 First Patient in
平成25年3月25日 コホートH 独立安全性評価委員会
平成25年4月 3日 コホートE開始
平成25年5月 2日 コホートE追加3症例 登録開始
平成26年4月 9日 コホートB開始
平成27年4月 21日 コホートH(最終コホート)開始予定中

バイオマーカー解析実施内容

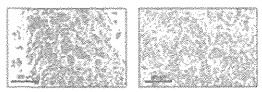
" 腫瘍組織採取可能症例検体において実施
" 血球検体は全例において実施

- Survivin 解析 (IHC・RT-PCR)
- LungCarta Panel
- Ion Ampliseq Panel(次世代シーケンサー)

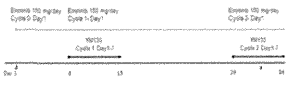


次世代シーケンサー(Ion PGM)の解析技術を用い、Cancer Panel(体細胞変異測定)の測定が可能となった。

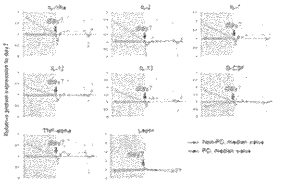
- Luminex Panel(血漿タンパク質解析)



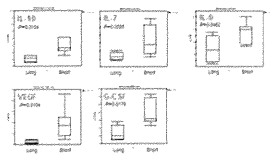
Changes of expression of survivin (IHC) at liver metastatic lesion biopsies on YM155 4.8mg/m²/day cohort



Serum biomarker (induction of serum proteins) for YM155



■YM155投与前 (day 0)時の血中サイトカイン/ケモカイン濃度と効果持続期間との関係
■PFS≧100日(中央値:193日、4例)とPFS<100日(中央値:62日、7例)を比較検討
■PFS≧100日群では、抗アポトーシスタンパクが高値であった(Mann-Whitney U test)
■抗VEGF抗体薬を含む
■抗アポトーシスタンパク高値では、YM155によるアポトーシス誘導増可能性が示唆された



医師主導治験症例登録状況 (平成27年2月1日現在)
および安全性評価(全12症例)要約

用量制限毒性(DLT)発現
1/12例 (可逆的血清クレアチニン値上昇 grade 2: 2.4mg/dl)

治療との因果関係が否定出来ない≧grade 3毒性に関しては1例のみ(下痢: grade 3)であり、殆どがgrade 1-2以下であり全般的にYM155+Erlotinib併用療法忍容性は良好であった。

Level	YM155	Erlotinib	DLT	症例数
1	3.6 mg/m²/day	150 mg/day	なし	3
2	4.8 mg/m²/day	150 mg/day	血清クレアチニン上昇:1例	6
3	8.0 mg/m²/day	150 mg/day	なし	3
4	8.0 mg/m²/day	150 mg/day	※平成27年4月 同時登録中	3-6

Treatment-Related Adverse Events*	CTCAE Grade					
N=12	Grade 1 n (%)	Grade 2 n (%)	Grade 3 n (%)	Grade 4 n (%)	Grade 5 n (%)	Any Grade n (%)
Rash	6 (50.0)	5 (41.7)	0 (0.0)	0 (0.0)	0 (0.0)	11 (91.7)
Fatigue	4 (33.3)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	4 (33.3)
Diarrhea	2 (18.2)	1 (9.1)	1 (9.1)	0 (0.0)	0 (0.0)	4 (33.3)
Urinary β2-microglobulin elevation	4 (33.3)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	4 (33.3)
Urinary N-acetyl-β-D-glucosaminidase elevation	4 (33.3)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	4 (33.3)
Creatinine elevation	1 (8.3)	1 (8.3)	0 (0.0)	0 (0.0)	0 (0.0)	2 (16.7)
Anemia	1 (8.3)	1 (8.3)	0 (0.0)	0 (0.0)	0 (0.0)	2 (16.7)
Proteinuria	2 (16.7)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	2 (16.7)
Fever	2 (16.7)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	2 (16.7)
Hyponatremia	1 (8.3)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (8.3)
Dysgeusia	1 (8.3)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (8.3)

Included data up to 28 days after last dose of study drug. Highest grade reported for each subject.
* Reported by ≥8% of subjects in any dose group (n=12)

別紙４

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	出版年	巻号	ページ
Azuma K, Okamoto I, Kawahara A, Taira T, Nakashima K, Hattori S, Kinoshita T, Takeda M, Nakagawa K, Takamori S, Kuwano M, Ono M, Kage M.	Association of the Expression of Mutant Epidermal Growth Factor Receptor Protein as Determined with Mutation-Specific Antibodies in Non-small Cell Lung Cancer with Progression-Free Survival after Gefitinib Treatment.	J Thorac Oncol	2012	7(1)	122-127
○Okamoto K, Okamoto I, Hatashita E, Kuwata K, Yamaguchi H, Kita A, Yamanaka K, Ono M, Nakagawa K.	Overcoming erlotinib resistance in EGFR mutation-positive non-small cell lung cancer cells by targeting survivin	Mol Cancer Ther	2012	11(1)	204-213
Takeda M, Okamoto I, Tsurutani J, Oiso N, Kawada A, Nakagawa K.	Clinical Impact of Switching to a Second EGFR-TKI After a Severe AE Related to a First EGFR-TKI in EGFR-mutated NSCLC.	Jpn J Clin Oncol	2012	42(6)	528-533
Nakagawa K, Kudoh S, Ohe Y, Johkoh T, Ando M, Yamazaki N, Seki A, Takemoto S, Fukuoka M.	Postmarketing surveillance study of erlotinib in Japanese patients with non-small-cell lung cancer (NSCLC): an interim analysis of 3488 patients (POLARSTAR).	J Thorac Oncol	2012	7(8)	1296-1303
○Aoyama Y, Nishimura T, Sawamoto T, Satoh T, Katashima M, Nakagawa K.	Pharmacokinetics of sepantronium bromide (YM155), a small-molecule suppressor of survivin, in Japanese patients with advanced solid tumors: dose proportionality and influence of renal impairment.	Cancer Chemother Pharmacol	2012	70(3)	373-380
Hayashi H, Kurata T, Fujisaka Y, Kawakami H, Tanaka K, Okabe T, Takeda M, Satoh T, Yoshida K, Tsunoda T, Arao T, Nishio K, Nakagawa K.	Phase I trial of OTS11101, an anti-angiogenic vaccine targeting Vascular Endothelial Growth Factor Receptor 1 in solid tumor.	Cancer Science	2013	104(1)	98-104
Kiyota H, Okamoto I, Takeda M, Daga H, Naito T, Miyazaki M, Okada H, Hayashi H, Tanaka K, Terashima M, Azuma K, Murakami H, Takeda K, Yamamoto N, Nakagawa K.	Phase I and pharmacokinetic study of gefitinib and S-1 combination therapy for advanced adenocarcinoma of the lung.	Cancer Chemother Pharmacol	2013	71(4)	859-865
Kogure Y, Ando M, Saka H, Chiba Y, Yamamoto N, Asami K, Hirashima T, Seto T, Nagase S, Otsuka K, Yanagihara K, Takeda K, Okamoto I, Aoki T, Takayama K, Yamasaki M, Kudoh S, Katakami N, Miyazaki M, Nakagawa K.	Histology and Smoking Status Predict Survival of Patients with Advanced Non-Small-Cell Lung Cancer: Results of West Japan Oncology Group (WJOG) Study 3906L.	J Thorac Oncol	2013	8(6)	753-758
Akamatsu H, Inoue A, Mitsudomi T, Kobayashi K, Nakagawa K, Mori K, Nukiwa T, Nakanishi Y, Yamamoto N.	Interstitial Lung Disease Associated with Gefitinib in Japanese Patients with EGFR-mutated Non-small-cell Lung Cancer: Combined Analysis of Two Phase III Trials (NEJ 002 and WJTOG 3405).	Jpn J Clin Oncol	2013	43(6)	664-668
Seto T, Kiura K, Nishio M, Nakagawa K, Maemondo M, Inoue A, Hida T, Yamamoto N, Yoshioka H, Harada M, Ohe Y, Nogami N, Takeuchi K, Shimada T, Tanaka T, Tamura T.	CH5424802 (RO5424802) for patients with ALK-rearranged advanced non-small-cell lung cancer (AF-001JP study): a single-arm, open-label, phase 1-2 study.	Lancet Oncol	2013	14(7)	590-598

Shaw AT, Kim DW, Nakagawa K, Seto T, Crinó L, Ahn MJ, De Pas T, Besse B, Solomon BJ, Blackhall F, Wu YL, Thomas M, O'Byrne KJ, Moro-Sibilot D, Camidge DR, Mok T, Hirsh V, Riely GJ, Iyer S, Tassell V, Polli A, Wilner KD, Jänne PA.	Crizotinib versus chemotherapy in advanced ALK-positive lung cancer.	N Engl J Med	2013	368(25)	2385-2394
Yang JC, Wu YL, Chan V, Kurnianda J, Nakagawa K, Saijo N, Fukuoka M, McWalter G, McCormack R, Mok TS.	Epidermal growth factor receptor mutation analysis in previously unanalyzed histology samples and cytology samples from the phase III Iressa Pan-ASia Study (IPASS).	Lung Cancer	2013	83(2)	174-181
Takeda M, Okamoto I, Nakagawa K.	Survival outcome assessed according to tumor response and shrinkage pattern in patients with EGFR mutation-positive non-small-cell lung cancer treated with gefitinib or erlotinib.	J Thorac Oncol	2014	9(2)	200-204
Hayashi H, Arao T, Matsumoto K, Kimura H, Togashi Y, Hirashima Y, Horita Y, Iwasa S, Okita NT, Honma Y, Takashima A, Kato K, Hamaguchi T, Shimada Y, Nakagawa K, Nishio K, Yamada Y.	Biomarkers of reactive resistance and early disease progression during chemotherapy plus bevacizumab treatment for colorectal carcinoma.	Oncotarget	2014	5(9)	2588-2599
○Yoshida T, Zhang G, Smith MA, Lopez AS, Bai Y, Li J, Fang B, Koomen J, Rawal B, Fisher KJ, Chen AY, Kitano M, Morita Y, Yamaguchi H, Shibata K, Okabe T, Okamoto I, Nakagawa K, Haura EB.	Tyrosine phosphoproteomics identifies both codrivers and cotargeting strategies for T790M-related EGFR-TKI resistance in non-small cell lung cancer.	Clin Cancer Res	2014	20(15)	4059-4074
Kawakami H, Okamoto I, Okamoto W, Tanizaki J, Nakagawa K, Nishio K.	Targeting MET Amplification as a New Oncogenic Driver.	Cancers (Basel)	2014	6(3)	1540-1552
Hayashi H, Okamoto I, Tanizaki J, Tanaka K, Okuda T, Kato A, Nishimura Y, Nakagawa K.	Cystic Brain Metastasis in Non-Small-Cell Lung Cancer With ALK Rearrangement.	J Clin Oncol	2014	32(36)	e122-124
Seto T, Kato T, Nishio M, Goto K, Atagi S, Hosomi Y, Yamamoto N, Hida T, Maemondo M, Nakagawa K, Nagase S, Okamoto I, Yamanaka T, Tajima K, Harada R, Fukuoka M, Yamamoto N.	Erlotinib alone or with bevacizumab as first-line therapy in patients with advanced non-squamous non-small-cell lung cancer harbouring EGFR mutations (JO25567): an open-label, randomised, multicentre, phase 2 study.	Lancet Oncol	2014	15(11)	1236-1244
Gemma A, Kudoh S, Ando M, Ohe Y, Nakagawa K, Johkoh T, Yamazaki N, Arakawa H, Inoue Y, Ebina M, Kusumoto M, Kuwano K, Sakai F, Taniguchi H, Fukuda Y, Seki A, Ishii T, Fukuoka M.	Final safety and efficacy of erlotinib in the phase 4 POLARSTAR surveillance study of 10 708 Japanese patients with non-small-cell lung cancer.	Cancer Sci	2014	105(12)	1584-1590
Solomon BJ, Mok T, Kim DW, Wu YL, Nakagawa K, Mekhail T, Felip E, Cappuzzo F, Paolini J, Usari T, Iyer S, Reisman A, Wilner KD, Tursi J, Blackhall F; PROFILE 1014Investigators.	First-line crizotinib versus chemotherapy in ALK-positive lung cancer.	N Engl J Med	2014	371(23)	2167-2177
Kawakami H, Okamoto I, Yonesaka K, Okamoto K, Shibata K, Shinkai Y, Sakamoto H, Kitano M, Tamura T, Nishio K, Nakagawa K.	The anti-HER3 antibody patritumab abrogates cetuximab resistance mediated by heregulin in colorectal cancer cells.	Oncotarget	2014	5(23)	11847-11856

Okamoto I, Miyazaki M, Takeda M, Terashima M, Azuma K, Hayashi H, Kaneda H, Kurata T, Tsurutani J, Seto T, Hirai F, Konishi K, Sarashina A, Yagi N, Kaiser R, Nakagawa K.	Tolerability of Nintedanib (BIBF 1120) in Combination with Docetaxel: A Phase 1 Study in Japanese Patients with Previously Treated Non-Small-Cell Lung Cancer.	J Thorac Oncol	2015	10(2)	346-352
Sakai K, Takeda M, Okamoto I, Nakagawa K, Nishio K.	Multiple regulatory mechanisms of hepatocyte growth factor expression in malignant cells with a short poly(dA) sequence in the HGF gene promoter.	Oncol Lett	2015	9(1)	405-410
Nishio M, Horiike A, Murakami H, Yamamoto N, Kaneda H, Nakagawa K, Horinouchi H, Nagashima M, Sekiguchi M, Tamura T.	Phase I study of the HER3-targeted antibody patritumab (U3-1287) combined with erlotinib in Japanese patients with non-small cell lung cancer.	Lung Cancer	2015	88(3)	275-281
Tsurutani J, Kuroi K, Iwasa T, Miyazaki M, Nishina S, Makimura C, Tanizaki J, Okamoto K, Yamashita T, Aruga T, Shigekawa T, Komoike Y, Saeki T, Nakagawa K.	Phase I study of weekly nab-paclitaxel combined with S-1 in patients with human epidermal growth factor receptor type 2-negative metastatic breast cancer.	Cancer Sci	2015		inpress

Association of the Expression of Mutant Epidermal Growth Factor Receptor Protein as Determined with Mutation-Specific Antibodies in Non-small Cell Lung Cancer with Progression-Free Survival after Gefitinib Treatment

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Introduction: Somatic mutations in the epidermal growth factor receptor (*EGFR*) gene are associated with an increased response to EGFR tyrosine kinase inhibitors (TKIs) such as gefitinib in patients with non-small cell lung cancer (NSCLC). Although most NSCLC patients with EGFR mutations benefit from EGFR-TKI treatment, the efficacy of such treatment varies among individuals. Molecular markers for prediction of EGFR-TKI treatment efficacy in EGFR mutation-positive NSCLC have not been well defined.

Methods: The expression of mutant EGFR proteins was quantitated by immunohistochemical analysis with mutation-specific antibodies in tumor specimens from 47 NSCLC patients with postoperative recurrent disease who harbored activating EGFR mutations. The expression score was determined from both the staining intensity and the proportion of tumor tissue expressing the mutant EGFR.

Results: The median progression-free survival after the start of gefitinib treatment was significantly longer in patients with a high score for mutant EGFR expression than in those with a low score (12.2 versus 3.4 months, $p < 0.001$), whereas no significant difference in median overall survival was apparent between the two groups (24.9 versus 17.7 months, respectively, $p = 0.144$). This association between the expression score for mutant EGFR and progression-free survival was apparent both in patients with deletions in exon 19 of EGFR and in those with the L858R mutation in exon 21.

Conclusions: Quantitative analysis of mutant EGFR expression by immunohistochemical analysis with mutation-specific antibodies may predict the efficacy of gefitinib treatment for EGFR mutation-positive NSCLC.

Key Words: Activating *EGFR* mutation, Mutation-specific antibody, Immunohistochemistry, Non-small cell lung cancer, Gefitinib.

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Lung cancer is the leading cause of cancer death worldwide.[1] Somatic mutations in the epidermal growth factor receptor (*EGFR*) gene have been identified as a major determinant of the clinical response to treatment with EGFR tyrosine kinase inhibitors (TKIs) such as gefitinib and erlotinib in individuals with non-small cell lung cancer (NSCLC). Most of these mutations occur in exons 19 to 21, which encode the tyrosine kinase domain of the receptor, with the most common being deletions in exon 19 (such as delE746-A750) and the L858R point mutation in exon 21. These mutations are found more frequently in female patients, in individuals who have never smoked, and in patients of East Asian ethnicity.[2–5] Prospective clinical trials of EGFR-TKI treatment in NSCLC patients with *EGFR* mutations have revealed radiographic response rates of 55 to 91%.[6–17] Most NSCLC patients with *EGFR* mutations thus benefit from treatment with EGFR-TKIs. Nevertheless, the clinical efficacy of EGFR-TKIs differs among such patients, and almost all individuals eventually develop resistance to these drugs. Recently, Yu et al[18] prepared antibodies that specifically recognize EGFRs that harbor the delE746-A750 or L858R

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mutations, allowing the development of a simple immunohistochemical method for identification of such mutations in human tissue. We and others subsequently demonstrated the potential value of these *EGFR* mutation-specific antibodies for analysis of clinical tumor specimens.[19–23] We have now investigated whether the expression score for EGFR mutant proteins is related to the efficacy of gefitinib treatment in *EGFR* mutation-positive NSCLC.

PATIENTS AND METHODS

Patients and EGFR Mutation Analysis

In this retrospective study, we screened 110 consecutive NSCLC patients with postoperative recurrent disease who underwent surgery between 1995 and 2009. All the patients received gefitinib (250 mg) orally once a day for recurrent disease. *EGFR* mutations were identified either by the PCR-Invader method (BML, Tokyo, Japan)[24] in 40 patients or by the peptide nucleic acid-locked nucleic acid PCR clamp method[25] in 70 patients. Forty-seven patients were found to harbor activating *EGFR* mutations (either exon 19 deletions or L858R in exon 21). Complete clinical information and tissue blocks suitable for additional analysis were available for all 47 individuals. A computed tomography scan was performed for tumor assessment within 28 days of initiation of treatment and was repeated after 2 to 3 months. All responses were defined according to RECIST. Response was confirmed at least 4 weeks (for a complete or partial response) or 6 weeks (for stable disease) after it was first documented. Progression-free survival (PFS) was calculated from the date of initiation of gefitinib treatment either to the date of disease progression or to the date of last contact. This study conforms to the provisions of the Declaration of Helsinki and was approved by the Institutional Review Board of participating institutions.

Immunohistochemical Analysis of EGFR Mutant Proteins in Clinical Samples from NSCLC Patients

Paraffin-embedded tumor tissue was sectioned at a thickness of 4 μm, and the sections were mounted on glass slides and then incubated with mutation-specific antibodies to EGFR that specifically recognize the delE746-A750 mutation in exon 19 (clone 6B6; Cell Signaling Technology) or the L858R mutation in exon 21 (clone 43B2; Cell Signaling Technology) for immunohistochemical analysis with the use of a Dako autostainer (Dako Cytomation).[18] The proportion of tumor cells found to express an EGFR mutant (proportion score) was assessed according to the following scale: 0, none (0%); 1, 1 to 10%; 2, 11 to 30%; 3, 31 to 50%; 4, 51 to 70%; and 5, 71 to 100% of tumor cells. The intensity of staining (intensity score) was evaluated according to the following scale: 0, no staining; 1, weak staining; 2, moderate staining; and 3, strong staining in >10% of cancer cells. As previously described,[26,27] the proportion score and intensity score were added to yield a total expression score ranging from 0 to 8. We classified expression scores of 0 to 3 as low expression and those of 4 to 8 as high expression for exon 19 deletions and expression scores of 0 to 6 as low expression and those of 7 or 8 as high expression for the L858R mutation, given that the corresponding median scores for the two types of mutation were 4 and 7, respectively. All immunohistochemical analysis was evaluated by two experienced observers (A.K. and M.K.) who were unaware of the conditions of the patients.

Statistical Analysis

We used Fisher's exact test to evaluate the significance of relations between the expression score for EGFR mutants and other patient characteristics. Survival curves for both PFS and OS were estimated by the Kaplan-Meier method, and the difference between the curves for patients with a high or low expression score for EGFR mutants was evaluated by the log-rank test. The Cox proportional hazards model was applied to examine whether the expression score for mutant EGFR proteins was associated with PFS or OS even after adjustment for other prognostic factors. All tests were two sided, and a p value of <0.05 was considered statistically significant. Statistical analysis was performed with R version 2.90 and SAS version 9.2 software (SAS Institute, Cary, NC).

RESULTS

Patient Characteristics

The clinical characteristics of the 47 patients are shown in Table 1 (also see Supplemental Table, Supplemental Digital Content 1, http://links.lww.com/JTO/A151). Thirty-five (74%) patients were female and 36 (77%) were never-smokers, with the median age of all patients being 65 years (range, 46–82 years). Forty-five (96%) patients had adenocarcinoma, 31 (66%) had a good performance status (Eastern Cooperative Oncology Group 0), and 15 patients (32%) received EGFR-TKI treatment as first-line chemotherapy. With regard to the type of *EGFR* mutation, 27 patients had deletions in exon 19, and 20 patients had the L858R missense mutation in exon 21.

Immunohistochemical Analysis of Activating EGFR Mutations in NSCLC Patients

Representative images for immunohistochemical staining of tumor specimens with antibodies specific for the two different types of *EGFR* mutation are shown in Figure 1. According to the protocol definition, the expression of the mutant EGFR proteins was evaluated on the basis of both the proportion of stained cells and staining intensity. There was a strong correlation between the proportion score and the intensity score (Spearman $p = 0.760$). We determined the expression score for the mutant proteins as the sum of the proportion score and the intensity score and divided the patients in the study into two groups according to the expression score. Twenty-eight and 19 patients were thus found to have high and low expression scores, respectively. We examined the possible relation between the expression score of the EGFR mutants and various clinical characteristics, but no significant association was found with age, sex, tumor histology, smoking status, or performance status (Table 2).

TABLE 1. Patient Characteristics

Characteristic	
Age (yr)	
Median	65
Range	46–82
Sex	
Male	12
Female	35
Histology	
Adenocarcinoma	45
Squamous cell carcinoma	1
Adenosquamous cell carcinoma	1
Smoking status	
Never-smoker	36
Smoker	11
Performance status	
0	31
1	9
2	7
Gefitinib	
First line	15
Second line	20
Third line	11
Fourth line	1
EGFR mutation status	
L858R	20
Exon19 deletions	27
Metastases	
Lung	30
Brain	20
Bone	16
Liver	5
Lymph node	2
Adrenal	1
Skin	1

Relation of Expression Score for EGFR Mutants to Survival

At the time of analysis, the median follow-up time was 15.0 months (range, 1.5–57.9 months). The median PFS was 6.7 months (range, 0.7–36.0 months), and the median OS was 15.0 months (range, 1.5–57.9 months). At this time, three patients were still receiving gefitinib treatment. The median duration of gefitinib treatment in patients with a high or low expression score was 12.2 (range, 0.3–36.0) and 3.4 (range, 0.7–17.2) months, respectively. Kaplan-Meier analysis of PFS and OS after the start of gefitinib treatment is shown in Figure 2. The log-rank test revealed that gefitinib treatment resulted in a significantly longer PFS in patients with a high expression score for EGFR mutants than in those with a low expression score (median of 12.2 versus 3.4 months, $p < 0.001$; Figure 2*A*), whereas there was no significant difference in OS between the two groups of patients (median, 24.9 versus 17.7 months, respectively, $p = 0.144$; Figure 2*B*). This difference in PFS between patients with high and low expression scores was apparent for both types of *EGFR* mutation (Figure 2*C*, *D*). Univariate analysis revealed that a high expression score for EGFR mutants ($p < 0.001$) was significantly associated with PFS and that performance status ($p = 0.034$) was significantly associated with OS (Table 3). None of the other factors examined was significantly associated with either PFS or OS. Finally, Cox regression analysis revealed that expression score for the EGFR mutants was significantly associated with PFS (hazard ratio, 0.265; 95% confidence interval, 0.132–0.531; $p < 0.001$) independently of performance status (Table 4). The relation between expression score for EGFR mutants and OS was close to achieving statistical significance after adjustment for performance status (hazard ratio, 0.503; 95% confidence interval, 0.231–1.093; $p = 0.083$).

Poststudy Treatment

Nine (32%) of the 28 patients who had a high expression score for EGFR mutants received subsequent treatment, whereas 8 (42%) of the 19 patients with a low expression score received such treatment. There was thus no significant difference in poststudy treatment between the two groups (χ^2, $p = 0.4866$).

DISCUSSION

With the use of mutation-specific antibodies, we have performed immunohistochemical analysis of the expression of mutant EGFR proteins in tumor specimens obtained from relapsed NSCLC patients with *EGFR* mutations. We found that the expression score for mutant EGFR proteins, as determined by quantitation of both staining intensity and the proportion of tumor cells expressing the mutant proteins, was significantly associated with PFS after the onset of gefitinib treatment. We previously showed that *EGFR* mutation was significantly associated with *EGFR* amplification in NSCLC cell lines and that the mutant EGFR proteins in such cells with both of these types of *EGFR* alteration were activated constitutively, resulting in an increased sensitivity to EGFR-TKIs.[28] These findings suggested that *EGFR* mutant alleles are amplified selectively and that increased expression of the mutant EGFR proteins confers susceptibility to EGFR-TKIs.[28,29] In this study, we found that a high staining intensity for mutant EGFR proteins was associated with a longer PFS in *EGFR* mutation-positive NSCLC patients treated with gefitinib (see Supplemental Figure, Supplemental Digital Content 2, http://links.lww.com/JTO/A151). In addition to staining intensity, we measured the proportion of tumor tissue in which the mutant EGFR protein was expressed. Many types of cancer have been found to manifest tissue heterogeneity with regard to the detection of tumor suppressor genes or oncogenes.[30,31] Previous studies have also suggested that such heterogeneity is also the case for *EGFR* mutations in NSCLC cell lines and tumor tissue.[24,32] Consistent with these findings, we have now shown that mutant EGFR protein detected with mutation-specific antibodies was expressed heterogeneously in individual tumors. Although intratumoral heterogeneity for *EGFR* mutations may explain the variable clinical efficacy of EGFR-TKIs in *EGFR* mutation-positive NSCLC patients, this issue has not previously been clinically addressed. We have now found that a high proportion score

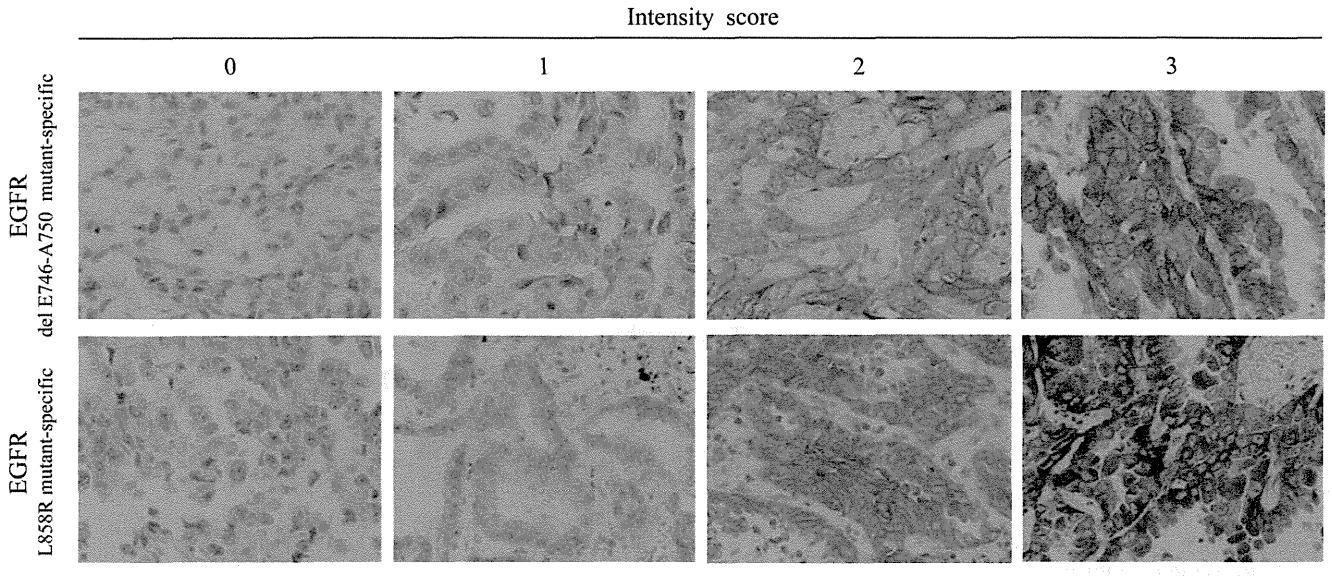

FIGURE 1. Immunohistochemical staining of NSCLC adenocarcinoma specimens with antibodies specific for delE746-A750 or L858R mutant forms of EGFR. Representative staining patterns for each of the four intensity levels are shown (original magnification, ×400).

TABLE 2. Relation Between Expression Score for EGFR Mutants and Various Patient Characteristics

Characteristic	*n*	Expression Score		*p*[a]
		Low	High	
Age (yr)				
High (≥65)	26	12	14	0.550
Low (<65)	21	7	14	
Sex				
Male	12	6	6	0.505
Female	35	13	22	
Histology				
Adenocarcinoma	45	18	27	1.000
Squamous + adenosquamous	2	1	1	
Smoking status				
Never-smoker	36	14	22	0.736
Smoker	11	5	6	
Performance status				
0	31	14	17	0.531
1 or 2	16	5	11	
Response rate				
PR or SD	38	12	26	0.021
PD	9	7	2	

[a] Determined by Fisher exact test.
PR, partial response; SD, stable disease; PD, progressive disease.

for mutant EGFR proteins was associated with a longer PFS in *EGFR* mutation-positive NSCLC patients treated with gefitinib (see Supplemental Figure, Supplemental Digital Content 2, http://links.lww.com/JTO/A151). Together, these findings suggest that the combination of the proportion score and intensity score for EGFR mutants might prove useful for predicting the efficacy of EGFR-TKIs in NSCLC patients harboring *EGFR* mutations.

The efficacy of EGFR-TKIs varies among *EGFR* mutation-positive NSCLC patients, but no clear candidate for a molecular marker able to predict treatment response in such patients has been identified. The T790M mutation of *EGFR* has been associated with acquired resistance to EGFR-TKIs in *EGFR* mutation-positive NSCLC patients, and this mutation was recently shown to be present in 35% of such patients before treatment with gefitinib and to be associated with de novo resistance to this drug.[33,34] A low expression level of the endogenous NF-κB inhibitor IκB was recently shown to be predictive of a poor clinical outcome in a cohort of erlotinib-treated NSCLC patients harboring an activating *EGFR* mutation but lacking evidence of the T790M mutation.[35] In this study, we have demonstrated that quantitative analysis of EGFR mutant expression in tumor tissue predicts the efficacy of EGFR-TKIs in NSCLC patients harboring *EGFR* mutations. Several highly sensitive methods for the detection of *EGFR* mutations have been described which can detect such mutations in specimens containing only a low percentage of mutation-positive cancer cells. Although these methods are useful for diagnosis of *EGFR* mutation-positive cancer, they are qualitative rather than quantitative.[24,25] One advantage of immunohistochemical diagnosis is that it provides a quantitative measurement of the expression level of the mutant protein in the cancer cells from individual patients. A potential drawback of this technique is that the sensitivity of the antibodies that detect exon 19 deletions is slightly inferior to that of the antibodies specific for the L858R mutant.[18–23] Consistent with this difference, we found that the sensitivity for the immunohistochemical detection of exon 19 deletions or the L858R mutation was 78 and 100%, respectively. These results thus indicate that not all EGFR

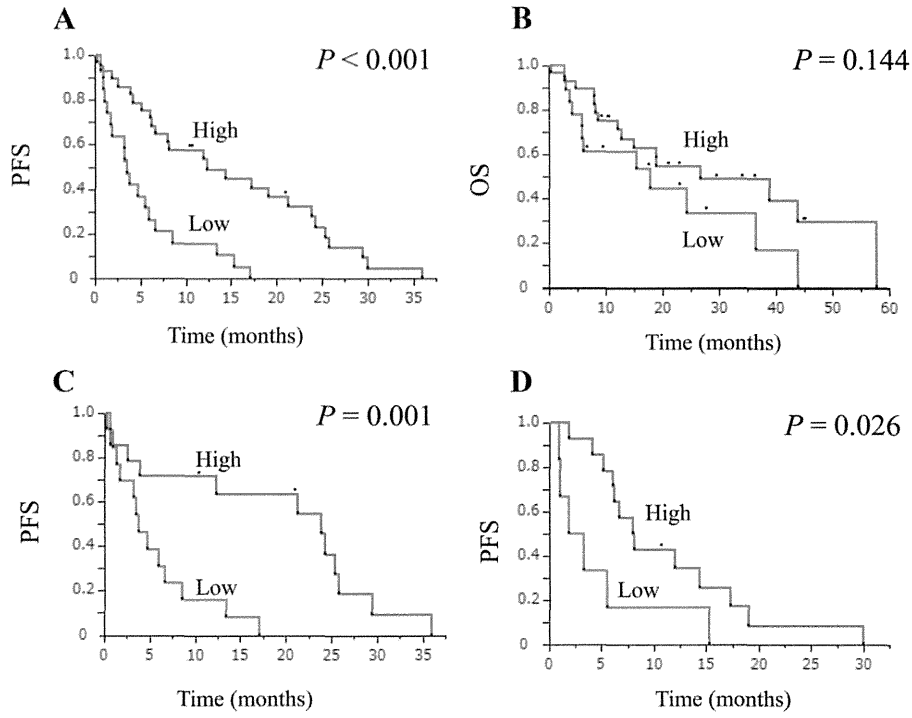

FIGURE 2. Kaplan-Meier survival curves according to expression score for EGFR mutants. PFS (*A*) and OS (*B*) for patients with high or low expression scores for either type of EGFR mutant. PFS for patients with high or low expression scores for exon 19 deletion (*C*) or L858R (*D*) mutant forms of EGFR.

TABLE 3. Factors Associated with PFS and OS

Factor	*n*	Median PFS (mo)	*p*[a]	Median OS (mo)	*p*[a]
Age (yr)					
High (≥65)	26	7.4	0.872	21.6	0.711
Low (<65)	21	5.4		18.2	
Sex					
Male	12	3.4	0.857	10.3	0.537
Female	35	8.1		25.5	
Histology					
Adenocarcinoma	45	6.5	0.170	23.7	0.941
Squamous + adenosquamous	2	4.2		13.5	
Smoking					
Never-smoker	36	7.4	0.640	25.5	0.339
Smoker	11	3.1		11.2	
Performance status					
0	31	8.3	0.143	35.3	0.034
1 or 2	16	5.6		12.5	
EGFR-mutant expression score					
Low	19	3.4	<0.001	17.7	0.144
High	28	12.2		24.9	

[a] Univariate analysis by log-rank test.
PFS, progression-free survival.

TABLE 4. Multivariate Analysis of PFS and OS

	Parameter	HR (95% CI)	*p*[a]
PFS	EGFR-mutant expression score (high vs. low)	0.265 (0.132–0.531)	<0.001
	Performance status (0 vs. 1 or 2)	1.720 (0.901–3.283)	0.100
OS	EGFR-mutant expression score (high vs. low)	0.503 (0.231–1.093)	0.083
	Performance status (0 vs. 1 or 2)	2.546 (1.137–5.702)	0.028

[a] Multivariate analysis by Cox proportional hazards model.
PFS, progression-free survival; HR, hazard ratio; CI, confidence interval.

mutant protein in cancer cells could be detected by immunohistochemical analysis.

The PFS benefit of gefitinib treatment in patients with a high expression score for mutant EGFR relative to those with a low expression score did not translate into an OS benefit. One explanation for this finding is that the sample size was too small to detect a clinically significant difference in OS. In addition, the data for OS were premature, with 40% of patients still being alive when censored. Although the frequency of *EGFR* mutations is only ~20 to 30% in East Asians and ~10% in Caucasians, efforts are ongoing to confirm our findings in larger cohorts.

In conclusion, we found that a high expression score for mutant EGFR protein is associated with a longer PFS in *EGFR* mutation-positive NSCLC patients treated with gefitinib. Our clinical findings demonstrate that quantitative analysis of EGFR mutant expression may predict the efficacy of EGFR-TKIs for treatment of *EGFR* mutation-positive NSCLC. Further study is warranted to clarify the clinical utility of immunohistochemical analysis for EGFR mutant proteins in determination of the optimal treatment for *EGFR* mutation-positive NSCLC.

REFERENCES

1. Parkin DM, Bray FI, Devasa SS. Cancer burden in the year 2000: the global picture. *Eur J Cancer* 2001;37:4–66.
2. Lynch TJ, Bell DW, Sordella R, et al. Activating mutations in the epidermal growth factor receptor underlying responsiveness of non-small-cell lung cancer to gefitinib. *N Engl J Med* 2004;350:2129–2139.
3. Paez JG, Janne PA, Lee JC, et al. EGFR mutations in lung cancer: correlation with clinical response to gefitinib therapy. *Science* 2004;304: 1497–1500.
4. Pao W, Miller V, Zakowski M, et al. EGF receptor gene mutations are common in lung cancers from "neversmokers" and are associated with sensitivity of tumors to gefitinib and erlotinib. *Proc Natl Acad Sci U S A* 2004;101:13306–13311.
5. Ono M, Kuwano M. Molecular mechanisms of epidermal growth factor receptor activation and response to gefitinib and other EGFR-targeting drugs. *Clin Cancer Res* 2006;12:7242–7251.
6. Maemondo M, Inoue A, Kobayashi K, et al. Gefitinib or chemotherapy for non-small-cell lung cancer with mutated EGFR. *N Engl J Med* 2010;362:2380–2388.
7. Mitsudomi T, Morita S, Yatabe Y, et al. Gefitinib versus cisplatin plus docetaxel in patients with non-small-cell lung cancer harbouring mutations of the epidermal growth factor receptor (WJTOG3405): an open label, randomized phase 3 trial. *Lancet Oncol* 2010;11:121–128.
8. Mok TS, Wu YL, Thongprasert S, et al. Gefitinib or carboplatin-paclitaxel in pulmonary adenocarcinoma. *N Engl J Med* 2009;361:947–957.
9. Inoue A, Suzuki T, Fukuhara T, et al. Prospective phase II study of gefitinib for chemotherapy-naive patients with advanced non-small-cell lung cancer with epidermal growth factor receptor gene mutations. *J Clin Oncol* 2006;24:3340–3346.
10. Asahina H, Yamazaki K, Kinoshita I, et al. A phase II trial of gefitinib as first-line therapy for advanced non-small cell lung cancer with epidermal growth factor receptor mutations. *Br J Cancer* 2006;95:998–1004.
11. Sutani A, Nagai Y, Udagawa K, et al. Gefitinib for non-small-cell lung cancer patients with epidermal growth factor receptor gene mutations screened by peptide nucleic acid-locked nucleic acid PCR clamp. *Br J Cancer* 2006;95:1483–1489.
12. Yoshida K, Yatabe Y, Park JY, et al. Prospective validation for prediction of gefitinib sensitivity by epidermal growth factor receptor gene mutation in patients with non-small cell lung cancer. *J Thorac Oncol* 2007;2:22–28.
13. Sunaga N, Tomizawa Y, Yanagitani N, et al. Phase II prospective study of the efficacy of gefitinib for the treatment of stage III/IV non-small cell lung cancer with EGFR mutations, irrespective of previous chemotherapy. *Lung Cancer* 2007;56:383–389.
14. Tamura K, Okamoto I, Kashii T, et al. Multicentre prospective phase II trial of gefitinib for advanced non-small cell lung cancer with epidermal growth factor receptor mutations: results of the West Japan Thoracic Oncology Group trial (WJTOG0403). *Br J Cancer* 2008;98:907–914.
15. Sugio K, Uramoto H, Onitsuka T, et al. Prospective phase II study of gefitinib in non-small cell lung cancer with epidermal growth factor receptor gene mutations. *Lung Cancer* 2009;64:314–318.
16. Sequist LV, Martins RG, Spigel D, et al. First-line gefitinib in patients with advanced non-small-cell lung cancer harboring somatic EGFR mutations. *J Clin Oncol* 2008;26:2442–2449.
17. Okamoto I, Mitsudomi T, Nakagawa K, et al. The emerging role of epidermal growth factor receptor (EGFR) inhibitors in first-line treatment for patients with advanced non-small cell lung cancer positive for EGFR mutations. *Ther Adv Med Oncol* 2010;2:301–307.
18. Yu J, Kane S, Wu J, et al. Mutation-specific antibodies for the detection of EGFR mutations in non-small-cell lung cancer. *Clin Cancer Res* 2009;15:3023–3028.
19. Kawahara A, Yamamoto C, Nakashima K, et al. Molecular diagnosis of activating EGFR mutations in non-small-cell lung cancer using mutation-specific antibodies for immunohistochemical analysis. *Clin Cancer Res* 2010;15:3163–3170.
20. Kitamura A, Hosoda W, Sasaki E, et al. Immunohistochemical detection of EGFR mutation using mutation-specific antibodies in lung cancer. *Clin Cancer Res* 2010;16:3349–3355.
21. Kato Y, Peled N, Wynes MW, et al. Novel epidermal growth factor receptor mutation-specific antibodies for non-small cell lung cancer. *J Thorac Oncol* 2010;5:1551–1558.
22. Kozu Y, Tsuta K, Kohno T, et al. The usefulness of mutation-specific antibodies in detecting epidermal growth factor receptor mutations and in predicting response to tyrosine kinase inhibitor therapy in lung adenocarcinoma. *Lung Cancer* 2011;73:45–50.
23. Brevet M, Arcila M, Ladanyi M. Assessment of EGFR mutation status in lung adenocarcinoma by immunohistochemistry using antibodies specific to the two major forms of mutant EGFR. *J Mol Diagn* 2010; 12:169–176.
24. Tadokoro K, Kobayashi M, Yamaguchi T, et al. Classification of hepatitis B virus genotypes by the PCR-Invader method with genotype-specific probes. *J Virol Methods* 2006;138:30–39.
25. Nagai Y, Miyazawa H, Huqun, et al. Genetic heterogeneity of the epidermal growth factor receptor in non-small cell lung cancer cell lines revealed by a rapid and sensitive detection system, the peptide nucleic acid-locked nucleic acid PCR clamp. *Cancer Res* 2005;65:7276–7282.
26. Allred DC, Clark GM, Elledge R, et al. Association of p53 protein expression with tumor cell proliferation rate and clinical outcome in node-negative breast cancer. *J Natl Cancer Inst* 1993;85:200–206.
27. Hammond ME, Hayes DF, Dowsett M, et al. American Society of Clinical Oncology/College of American Pathologists guideline recommendations of immunohistochemical testing of estrogen and progesterone receptors in breast cancer. *J Clin Oncol* 2010;28:2784–2795.
28. Okabe T, Okamoto I, Tamura K, et al. Differential constitutive activation of the epidermal growth factor receptor in non-small cell lung cancer cells bearing EGFR gene mutation and amplification. *Cancer Res* 2007;67:2046–2053.
29. Morinaga R, Okamoto I, Fujita Y, et al. Association of epidermal growth factor receptor (EGFR) gene mutations with EGFR amplification in advanced non-small cell lung cancer. *Cancer Sci* 2008;99:2455–2460.
30. Klein CA, Blankenstein TJ, Schmidt-Kittler O, et al. Genetic heterogeneity of single disseminated tumour cells in minimal residual cancer. *Lancet* 2002;360:683–689.
31. Kitago M, Ueda M, Aiura K, et al. Comparison of K-ras point mutation distributions in intraductal papillary-mucinous tumors and ductal adenocarcinoma of the pancreas. *Int J Cancer* 2004;110:177–182.
32. Sakurada A, Lara-Guerra H, Liu N, et al. Tissue heterogeneity of EGFR mutation in lung adenocarcinoma. *J Thorac Oncol* 2008;3:527–529.
33. Kobayashi S, Boqqon TJ, Dayaram T, et al. EGFR mutation and resistance of non-small-cell lung cancer to gefitinib. *N Engl J Med* 2005;352:786–792.
34. Rosell R, Molina MA, Costa C, et al. Pretreatment EGFR T790M mutation and BRCA1 mRNA expression in erlotinib-treated advanced non-small-cell lung cancer patients with EGFR mutations. *Clin Cancer Res* 2011;17:1160–1168.
35. Bivona TG, Hieronymus H, Parker J, et al. FAS and NF-κB signaling modulate dependence of lung cancers on mutant EGFR. *Nature* 2011; 471:523–526.

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Molecular Cancer Therapeutics

Preclinical Development

Overcoming Erlotinib Resistance in *EGFR* Mutation–Positive Non–Small Cell Lung Cancer Cells by Targeting Survivin

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Abstract

Loss of PTEN was recently shown to contribute to resistance to epidermal growth factor receptor (EGFR) tyrosine kinase inhibitors (TKI) in *EGFR* mutation–positive non–small cell lung cancer (NSCLC) through activation of the protein kinase AKT. We previously showed that downregulation of the expression of the antiapoptotic protein survivin by EGFR–TKIs contributes to EGFR–TKI-induced apoptosis in *EGFR* mutation–positive NSCLC cells. We have now investigated the role of survivin expression in EGFR–TKI resistance induced by PTEN loss. The EGFR–TKI erlotinib did not affect survivin expression or induce apoptosis in *EGFR* mutation–positive NSCLC cells with PTEN loss. Downregulation of survivin either by transfection with a specific short interfering RNA or by exposure to the small-molecule survivin suppressor YM155 reversed erlotinib resistance in such cells *in vitro*. Furthermore, combination therapy with YM155 and erlotinib inhibited the growth of tumors formed by *EGFR* mutation–positive, PTEN-deficient NSCLC cells in nude mice to a greater extent than did treatment with either drug alone. These results thus indicate that persistent activation of signaling by the AKT–survivin pathway induced by PTEN loss underlies a mechanism of resistance to erlotinib-induced apoptosis in *EGFR* mutation–positive NSCLC. They further suggest that the targeting of survivin has the potential to overcome EGFR–TKI resistance in *EGFR* mutation–positive NSCLC. *Mol Cancer Ther; 11(1); 204–13. ©2011 AACR.*

Introduction

Approximately 70% of individuals with non–small cell lung cancer (NSCLC) who harbor somatic mutations in exons of the epidermal growth factor receptor (*EGFR*) gene that encode the tyrosine kinase domain of the receptor experience substantial tumor regression when treated with the EGFR tyrosine kinase inhibitors (TKI) gefitinib or erlotinib (1). However, most patients, even those who show a marked response to initial treatment, develop acquired resistance to EGFR–TKIs after varying periods of time (2). To date, several major mechanisms of such acquired resistance, including secondary mutation of *EGFR*, amplification of *MET*, and overexpression of hepatocyte growth factor, have been identified, and the development of pharmaceutical agents that target these mechanisms is underway (3–7). In addition, some patients are intrinsically resistant to EGFR–TKIs, even though their tumors harbor activating mutations of *EGFR* (8). Further characterization of the mechanisms of EGFR–TKI resistance is thus important to provide a basis for the development of effective therapies for patients who develop such resistance.

The deletion or inactivation of the *PTEN* gene occurs in a variety of tumor types, including melanoma as well as lung, bladder, renal, breast, endometrial, and thyroid cancer, and there are no related proteins that can compensate for the loss of PTEN function (9). The loss of PTEN results in misregulation of AKT-dependent signaling, which plays a key role in the progression of malignant cancer (10). Recent studies have shown that PTEN loss contributes to EGFR–TKI resistance in *EGFR* mutation–positive lung cancer through activation of the protein kinase AKT (11, 12). We recently found that EGFR–TKIs downregulate survivin expression through inhibition of the phosphoinositide 3-kinase (PI3K)–AKT signaling pathway and that such downregulation of survivin contributed to EGFR–TKI-induced apoptosis in *EGFR* mutation–positive NSCLC cells (13). Survivin is a member of the inhibitor of apoptosis (IAP) family of proteins and has been shown to inhibit caspases and to prevent caspase-mediated cell death (14). Persistent survivin expression might therefore be expected to result in resistance to EGFR–TKIs in *EGFR* mutation–positive NSCLC cells with PTEN loss.

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Note: Supplementary material for this article is available at Molecular Cancer Therapeutics Online (http://mct.aacrjournals.org/).

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