厚生労働科学研究費補助金
医療技術実用化総合研究事業

RET融合遺伝子陽性の進行非小細胞肺癌に対する新規治療法の確立に関する研究

平成26年度　総括研究報告書

研究代表者　後藤　功一

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Ⅰ.総括研究報告書

厚生労働科学研究費補助金（医療技術実用化総合研究事業）
（総括）研究報告書

RET 融合遺伝子陽性の進行非小細胞肺癌に対する新規治療法の確立に関する研究

研究代表者　後藤　功一
独立行政法人国立がん研究センター東病院
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研究要旨

2012年に発見された肺癌の新規ドライバー遺伝子であるRET融合遺伝子の臨床応用を目指した研究を実施した。RET 融合遺伝子陽性肺癌（RET 肺癌）は全肺癌の 1-2%と頻度が低いため、全国規模の遺伝子診断ネットワーク（LC-SCRUM-Japan）を立ち上げ、平成 25 年 2 月より遺伝子スクリーニングを開始した。同時に、RET 肺癌に対する Vandetanib の医師主導治験（LURET study）を世界で初めて開始した。平成 27 年 1 月 31 日現在、LC-SCRUM-Japan には 190 施設が参加し、1438 例が登録された結果、RET 肺癌が 32 例スクリーニングされ、このうち 18 例が LURET study に登録されている。

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A.　研究目的

希少疾患である RET 融合遺伝子陽性の進行非小細胞肺癌（RET 肺癌）を対象に、国内未承認の医薬品である RET チロシンキナーゼ阻害薬 Vandetanib（治験成分記号：ZD6474）の薬事承認申請を目指した多施設共同非無作為化非盲検第Ⅱ相試験（医師主導治験）を実施する。

B.　研究方法

RET 肺癌の頻度は、肺癌全体の 1-2%と非常に低

いため、患者のスクリーニングが重要となる。このため、日本全体から多施設が参加する遺伝子診断ネットワークを構築し、この中で「RET 融合遺伝子等の低頻度の遺伝子変化陽性肺癌の臨床病理学的、分子生物学的特徴を明らかにするための前向き観察研究」に基づいて RET 肺癌のスクリーニングを行う。施設倫理委員会で本研究が承認された施設のみ、遺伝子スクリーニングへの参加が可能とした。RET 融合遺伝子の診断は、国立がん研究センターで開発した RT-PCR 法、FISH 法を用いて行うこととし、この診断技術を株式会社エスアールエル（SRL）へ技術移管し、実際の臨床検体の遺伝子解析は SRL が行った。同時に Vandetanib の医師主導治験を 7 施設（国立がん研究センター東・中央病院、がん研有明病院、静岡がんセンター、兵庫県立がんセンター、四国がんセンター、九州がんセンター）で開始し、スクリーニングされた RET 肺癌は、医師主導治験へ登録して、Vandetanib の有効性を検討する方針とした。

医師主導治験である「RET 融合遺伝子陽性の局所進行/転移性非扁平上皮非小細胞肺癌患者を対象とした Vandetanib（ZD6474）の多施設共同非無作為化非盲検第Ⅱ相試験」（LURET study）のプライマリーエンドポイントは、奏効割合。セカンダリーエンドポイントは、無増悪生存期間、病勢制御割合、奏効期間、全生存期間、有害事象、前・後化学療法の有効性とした。予定登録数 17 例、登録期間 2 年、追跡期間 1 年であり、主な適格規準は、1)年齢 20 歳以上、2)扁平上皮癌以外の非小細胞肺癌、3)局所療法不能の III 期又は IV 期、4)RET 融合遺伝子陽性（RT-PCR 法及び FISH 法でいずれも陽性）、5)EGFR 遺伝子変異陰性、ALK 融合遺伝子陰性、6)1 レジメン以上の化学療法を実施後、7)PS=0-2、8)測定可能病変あり、9)主要臓器機能が保持、10)患者本人から文書による同意が必要とした。治療方法は、21 日を 1 コースとして、Vandetanib 300mg を 1 日 1 回朝食後に経口投与として、疾患の増悪、または許容できない毒性が認められるまで投与を継続することにした。Vandetanib（治験成分記号：ZD6474）は、治験薬提供者であるアストラゼネカ株式会社から無償提供され、治験薬の製造の記録、品質保証の記録も併せて提供される。医師主導治験への登録、モニタリング、安全性情報の管理、データセンター、統計解析については、国立がん研究センター 早期・探索臨床研究センター 臨床試験支援室において行うこととした。

倫理面への配慮としては、患者の人権保護のため、医師主導治験に関係するすべての研究者は、ヘルシンキ宣言、ICH Harmonized Tripartite Guidelines for Good Clinical Practice、「医薬品の臨床試験の実施の基準に関する省令」（平成 9 年厚生省令第 28 号）およびその改正、関連通知を遵守して本治験を実施する。医師主導治験を実施するにあたり、治験実施計画書、説明同意文書等の関連文書は事前に、「医薬品の臨床試験の実施の基準に関する省令」（平成 9 年厚生省令第 28 号）に規定する治験審査委員会の承認を取得した。患者への説明は、治験審査委員会で承認が得られた研究の内容、費用及び補償の有無、利益相反の有無等について記載された説明文書を用いて行い、登録前に充分な説明と理解に基づく自発的同意を本人より文書で得ることを規定した。また、医師主導治験が適正に行われていることを確保するため、中央モニタリングに加えて原資料との照合を行う施設訪問モニタリングをサンプリングにて実施することを規定した。監査は、国立がん研究センター 研究支援センター 研究管理部が行う。データマネージメントはデータセンターで行い、データの取り扱い上、患者氏名等直接個人が識別できる情報を用いず、かつデータベースのセキュリティを確保し、個人情報保護を厳守した。

更に、薬事承認後の実地診療における確実な患者選択のために、RET 融合遺伝子のコンパニオン診断薬の開発も同時に行うこととした。本研究では、RT-PCR 法、FISH 法を用いて遺伝子診断を行い、患者のスクリーニングを行うが、新鮮凍結検体から RNA の抽出が必要となる RT-PCR 法は、臨床現場では実施困難な場合が多いと予想されるため、ターゲットキャプチャー法を応用したゲノム DNA からの RET 融合遺伝子診断法の開発も同時に行った。このため、スクリーニングのために全国から収集した検体は保存し、今後のコンパニオン診断薬の開発のために二次利用することにした。

C. 研究結果

RET 肺癌のスクリーニングのため、全国規模の遺伝子診断ネットワークとして、Lung Cancer Genomic Screening Project for Individualized Medicine in Japan（LC-SCRUM-Japan）を組織し、平成 25 年 2 月 7 日より遺伝子スクリーニングを開始した。平成 27 年 1 月 31 日現在、LC-SCRUM-Japan には 190 施設が参加し、施設倫理審査委員会で研究が承認された 169 施設で順調に遺伝子スクリーニングが進行中である。平成 27 年 1 月 31 日までに LC-SCRUM-Japan には 1438 例の登録があり、既に 32 例（3%）の RET 肺癌がスクリーニングされている。更に、希少な ROS1 融合遺伝子陽性肺癌 57 例(4%)、ALK 融合遺伝子陽性肺癌 22 例(2%)も同時にスクリーニングされている。

LURET study は、平成 24 年 11 月 19 日に PMDA の薬事戦略相談を受け、平成 25 年 1 月 29 日に治験計画届けを厚生労働省へ提出し、2 月 21 日より患者登録を開始した。平成 27 年 1 月 31 日までに LC-SCRUM-Japan でスクリーニングされた RET 肺癌 32 例のうち 18 例（内 2 例は不適格例）が LURET

study に登録され、Vandetanib による治療が進行中であり、予想通りの治療効果が認められている。あと 1 例で目標症例数に到達するため、平成 26 年度で予定通りの登録完了を目指している。

これらと並行して、RET 融合遺伝子を含む複数の肺癌ドライバー変異を一度にかつ迅速に検出できるmultiplex 遺伝子診断法の開発も行っており、微量のゲノム DNA (50ng～) から、ターゲットキャプチャーを用いて目的ゲノム領域を濃縮し、次世代シーケンサーで変異を検出するキットの開発が進行中である。

D. 考察

肺癌はがん死亡原因第一位の難治性がんであり、2010 年の年間死亡者数は約 7 万人で、がん死亡の約 2 割を占めている。非小細胞肺癌（主に腺癌、扁平上皮癌、大細胞癌）は、肺癌全体の約 85%を占めるが、約 2/3 は発見時にすでに切除不能の進行癌であり、これらの患者に対しては化学療法が行われる。しかし、非小細胞肺癌は一般に化学療法の感受性が低く、現在の化学療法による治療成績は 1 年生存率が約 40%と不良であり、非小細胞肺癌の治療成績の向上のためには優れた分子標的薬による個別化治療の推進が不可欠である。

近年、非小細胞肺癌における個別化治療の標的となる遺伝子異常（EGFR 遺伝子変異、ALK 融合遺伝子)が同定され、EGFR 遺伝子変異例に対する EGFR チロシンキナーゼ阻害薬（ゲフィチニブ、エルロチニブ）や、ALK 融合遺伝子陽性例に対する ALK チロシンキナーゼ阻害薬（クリゾチニブ）の臨床応用によって、従来の化学療法と比較して、著しい治療成績の改善が認められている。

RET 融合遺伝子は、2012 年に報告された非小細胞肺癌の新しいドライバー遺伝子であり、新規の治療標的となることが期待される。RET 肺癌は、肺癌全体の 1-2%と頻度は低いが、基礎研究において RET チロシンキナーゼ阻害薬である Vandetanib の有効性が確認されており、臨床試験に基づいた Vandetanib の有効性の確認、早期臨床応用が期待されている。

Vandetanib はアストラゼネカ株式会社が開発中の国内未承認薬である。既に米国 FDA では、2011 年 4 月に切除不能または進行性の甲状腺髄様癌に対する治療薬として承認されている。国内では、肺癌を含む固形がんを対象に第 I 相試験が行われ、推奨用量は海外と同じ 300 mg/day と設定され、更に、進行非小細胞肺癌に対する第 II 相試験において、主な有害事象は下痢、皮疹、高血圧、頭痛などで、これまでの報告とほぼ同じであり、安全性の確認は完了している。

RET 肺癌は、頻度が 1-2%という稀少疾患であるため、治療開発は医師主導治験以外では困難であり、Vandetanib の有効性を評価して薬事承認申請を目指す医師主導治験の実施が必須となる。この Vandetanib の有効性を評価する本試験は、世界初の試みであると同時に、今後も明らかになる新たな遺伝子異常を伴う希少がんに対する分子標的治療薬の開発方法を考える上で、非常に重要な意味を持つと考えられる。

更に、本研究において、我が国初の全国規模の遺伝子診断ネットワーク LC-SCRUM-Japan が構築され、希少肺癌のスクリーニングが成功したことは非常に重要である。少数施設で 1-2%の頻度の希少な肺癌をスクリーニングして、新規治療法の開発を実施するのは不可能なため、希少肺癌では、従来とは異なる治療開発の方法が模索されてきた。本研究の中で LC-SCRUM-Japan という希少肺癌の遺伝子スクリーニング基盤が実際に構築されたことは大きな意義を持つと考えられる。また、希少肺癌の治療開発においては、正確な診断及び、スクリーニングが可能となるコンパニオン診断薬の同時開発が必須とされている。今後、LC-SCRUM-Japan は、遺伝子変化を伴うその他の希少肺癌のスクリーニングにも応用可能であり、また、全国から集めた多くの検体を利用し、multiplex 診断薬を含めたコンパニオン診断薬の開発を担える組織として、更に存在意義が高まっていくと予想される。

E. 結論

本研究では、2012 年に我が国で発見された肺癌の新規ドライバー遺伝子である RET 融合遺伝子の臨床応用を目指した研究を行っている。RET 肺癌は希少頻度の肺癌であるが、全国規模の遺伝子診断ネットワークである LC-SCRUM-Japan において順調にスクリーニングが進行している。更に、スクリーニングされた RET 肺癌は、Vandetanib の医師主導治験に登録され、治療開発も同時に進行している。このような民間企業の研究と公的資金に基づく医師主導治験との連携による治療開発システムの構築は、今後も明らかになる遺伝子異常を伴う希少がんに対する分子標的治療薬の効率的な治療開発のモデルケースとして注目されており、個別化治療の発展への大きな貢献が期待される。

F. 健康危険情報

なし

G. 研究発表

1. 論文発表

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3. Yoshida A, Tsuta K, Wakai S, Arai Y, Asamura H, Shibata T, Furuta K, Kohno T, Kushima R Immunohistochemical detection of ROS1 is useful for identifying ROS1 rearrangements in lung cancers. Modern Pathol.2014, 27(5):711-720.
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H. 知的財産権の出願・登録状況

1. 特許取得
知財(特許出願)
なし

2. 実用新案登録
なし

3. その他
なし

Ⅱ.研究成果の刊行に関する一覧表

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
Kohno T, Tsuchihara K, Ogiwara H, Ichikawa H.	RET and other genes: therapeutic targets in lung adenocarcinoma.	Lung Cancer Manag	3	219-226	2014
Tsuta K, Kohno T, Yoshida A, Shimada Y, Asamura H, Furuta K, Kushima R.	RET-rearranged non-small-cell lung carcinoma: a clinicopathological and molecular analysis.	Br J Cancer	110(6)	1571-1578	2014
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Mizukami T, Shiraishi K, Shimada Y, Ogiwara H, Tsuta K, Ichikawa H, Sakamoto H, Kato M, Shibata T, Nakano T, Kohno T.	Molecular mechanisms underlying oncogenic RET fusion in lung adenocarcinoma.	J Thoracic Oncol	9(5)	622-630	2014

Ⅲ.研究成果の刊行物・別刷

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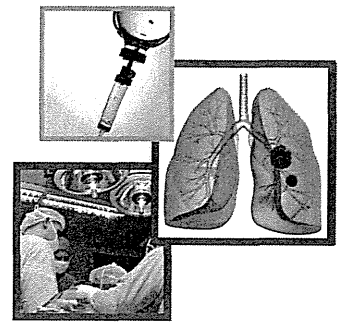
Lung Cancer Management



RET and other genes: therapeutic targets in lung adenocarcinoma

Takashi Kohno*[1,2], Katsuya Tsuchihara[2], Hideaki Ogiwara[1] & Hitoshi Ichikawa[2]

Practice Points

- *RET* fusion was identified as a new gene aberration driver in 1–2% of lung adenocarcinomas (LADCs) that results in constitutive activation of RET kinase.
- Two RET tyrosine kinase inhibitors approved by the US FDA for the therapy of medullary thyroid cancer showed a positive therapeutic response in a small number of patients with *RET* fusion-positive LADC.
- Phase II clinical trials have been initiated to investigate the therapeutic effects of RET tyrosine kinase inhibitors on patients with *RET* fusion-positive non-small-cell lung cancer.
- Personalized LADC therapy targeting seven druggable oncogene aberrations, including *RET* fusion, will cover >60 and >20% of east Asia and US/European LADC patients, respectively.
- Multiplex diagnosis of aberrations in driver oncogenes in clinical samples will facilitate the design of personalized therapy for LADC.

SUMMARY The *RET* fusion gene was recently identified as a new druggable driver gene present in 1–2% of lung adenocarcinomas (LADCs). Vandetanib (ZD6474) and cabozantininb (XL184), two RET tyrosine kinase inhibitors approved by US FDA for the therapy of medullary thyroid cancer, have demonstrated therapeutic effectiveness in a few *RET* fusion-positive LADC patients. Several clinical trials are under way to address the therapeutic effects of RET tyrosine kinase inhibitors, including these two drugs. Multiplex diagnosis of aberrations in druggable driver oncogenes, such as *EGFR, ALK, RET, ROS1, HER2/ERBB2, BRAF* and others, in clinical samples will facilitate the design of personalized therapies for LADC based on protein kinase inhibitors. The development of therapeutic methods targeting aberrations of other genes, such as chromatin remodeling genes, is necessary to further improve the treatment of LADC.

KEYWORDS

• chromatin remodeling gene • driver gene • multiplex diagnosis • *RET* fusion gene • synthetic lethality

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Current personalized therapies for lung adenocarcinoma targeting driver oncogenes

A driver gene is defined as one whose aberrations are causally implicated in oncogenesis or tumor survival [1]. To date, seven oncogenes have been identified as representative driver genes in lung adenocarcinomas (LADCs) (**Table 1**): *EGFR*, *KRAS*, *ALK*, *RET*, *ROS1*, *HER2/ERBB2* and *BRAF*. Aberrations of these genes occur in a mutually exclusively manner in LADCs. Molecular-targeted therapy combined with the identification of driver oncogene aberrations is a powerful and promising personalized therapy for the treatment of LADC [2].

The *EGFR* gene is activated by missense or in-frame deletion mutations in approximately 5–15% of LADC cases in the USA and in approximately 50% of cases in east Asia (**Table 1 & Figure 1**) [2]. Tumors with *EGFR* mutations respond to EGFR tyrosine kinase inhibitors (TKIs), such as erlotinib and gefitinib, leading to the improvement of progression-free survival and quality of life [3,4]. In addition, 3–6% of LADCs harbor fusions that result in the activation of ALK tyrosine kinase, mainly associated with an inversion in chromosome 2. ALK TKIs, such as crizotinib and CH5424802, show marked therapeutic effects against *ALK* fusion-positive LADCs [5–7]. Treatment of LADC patients carrying either *EGFR* or *ALK* aberrations with EGFR or ALK TKIs has begun to replace conventional chemotherapy using cytotoxic drugs, even for first-line use [2].

RET-fusion gene as a new driver gene & therapeutic target

The *RET*-fusion gene was recently identified as a new driver oncogene aberration in LADC, occurring in a mutually exclusively manner with other known driver gene aberrations (**Figure 1 & Table 1**) [8–10,24,25]. The screening of approximately 5000 LADC cases in several studies revealed that *RET* fusion occurs in 1–2% of LADC patients of both east Asian and European descent [10,11,26–30]. To date, four fusion partner genes, *KIF5B*, *CCDC6/PTC/H4*, *NCO4/PTC3/ELE1* and *TRIM33/PTC7*, have been identified [28]. *KIF5B–RET* and *CCDC6–RET* are the most frequent types of *RET* fusion, and they are generated by inversions in chromosome 10.

Proteins encoded by all types of *RET* fusion genes show common characteristics including RET kinase activation; the coiled-coil domain(s) in the N-terminal fusion partners causes the RET domains to dimerize, resulting in constitutive activation of the RET tyrosine kinase in the absence of ligands [8,9,24,28]. The *RET* fusion associated with LADC is a druggable aberration since TKIs against RET kinase suppress the activation of the RET fusion protein. A LADC cell line, LC-2/ad, which has a *CCDC6-RET* fusion, was shown to be sensitive to RET TKI *in vitro* and *in vivo*, indicating that LADC cells with *RET* fusion are in a state of 'oncogenic addiction' to constitutive RET kinase activation [31,32].

The *RET* fusion is most likely to occur in young and/or never/light-smoker patients [8,9,11,33].

Table 1. Representative driver oncogenes in lung adenocarcinoma.

Gene	Discovery of aberration (year)	Aberration causing activation	Frequency of aberration (%)		Molecular targeting drug approved for lung cancer therapy
			East Asia	*USA/Europe*	
EGFR	2004	In-frame deletion and missense mutation	40–55	5–15	Gefitinib, erlotinib
KRAS	1984	Missense mutation	8–10	20–30	–
ALK	2007	Fusion with *EML4*, *KIF5B* and others	3–5	3–6	Crizotinib
ROS1	2007	Fusion with *CD74*, *ROS1*, *SLC34A2* and others	2–3	1–2	Crizotinib[†]
RET	2012	Fusion with *KIF5B*, *CCDC6* and others	1–2	1–2	–
HER2	2004	In-frame insertion	2–3	2–3	–
BRAF	2002	Missense mutation	0.5–1	2–3	–

[†]Approved for *ALK* fusion-positive cancer.

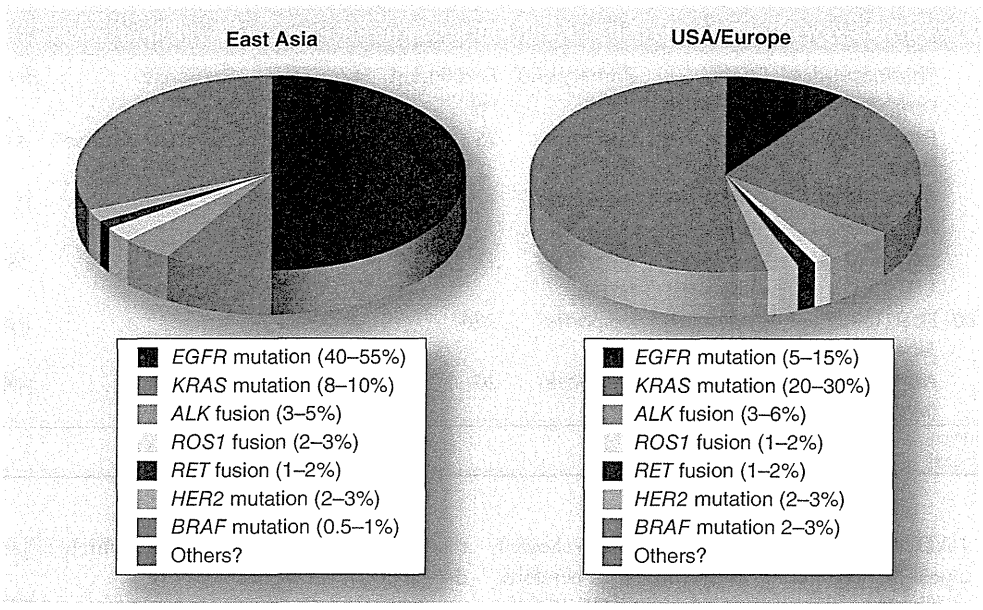

Figure 1. The fraction of lung adenocarcinomas that harbor aberrations of seven representative driver oncogenes. Data on patients in east Asia (Japan, Korea and China) and of European descent were generated by summarizing the results of previous reports [8–23]. Activation of other oncogenes, such as *MET, NRAS, AKT1, MEK1, JAK2* and *PIK3CA*, are also observed in a proportion of lung adenocarcinomas, and they are also thought to be drivers for lung carcinogenesis [21]. Since mutually exclusiveness of these alterations with the seven oncogenes shown here has not been well characterized, so, such alterations are not included in the pie chart.

LADCs harboring *RET* fusion genes show well or moderately differentiated histological features, similar to LADCs harboring *EGFR* mutations, whereas a subset of LADCs that harbor *RET* fusion genes show a mucinous cribriform feature, similar to *EML4–ALK* fusion-positive LADCs [8,32].

Clinical trials of RET TKIs

RET is a driver oncogene both for hereditary and sporadic thyroid cancer [34]. The US FDA has approved two multi-kinase inhibitors with RET TKI activity, vandetanib (ZD6474) and cabozantinib (XL184), for the treatment of advanced medullary thyroid cancer, in which activating *RET* mutations are observed in >50% of cases. There are several other commercially available multikinase inhibitors with RET TKI activity, such as sorafenib, sunitinib, lenvatinib (E7080) and ponatinib (AP24534).

Several Phase II clinical trials have been initiated to investigate the therapeutic effects of the multikinase inhibitors described above on patients with *RET* fusion-positive non-small-cell lung cancer (**Table 2**) [12]. All of these trials have open-label and single-arm designs, with response rate as the primary end point. One study conducted at Memorial Sloan–Kettering Cancer Center [35] reported that the responses of the first three patients treated with cabozantinib were promising (**Table 2**) [28]. Another Phase II clinical trial is currently being conducted in Japan by our group and colleagues (UMIN00001009) [12]. This trial, designated LURET, is investigating the therapeutic effects of vandetanib (**Table 2**). To select patients with *RET* fusion-positive tumors, screening is being conducted in >120 hospitals throughout Japan by a consortium designated LC-SCRUM. In this trial, RNAs from frozen biopsy tissue or pleural effusion from patients with nonsquamous non-small-cell lung carcinomas without *EGFR* mutations are subjected to reverse transcription PCR that enables us to detect all *KIF5B–RET* and *CCDC6–RET* variants identified to date [28]. The positive cases are subsequently subjected to break-apart and fusion FISHs to validate the reverse transcription PCR results [12]. The therapeutic results will be obtained within 2 years; therefore, the effectiveness of vandetanib for the treatment of *RET* fusion-positive LADC is still unknown. In a recent study, however, one patient with

Table 2. Phase II clinical trials of RET-tyrosine kinase inhibitors in patients with RET fusion-positive non-small-cell lung cancer.

Trial number†	Drug	Pharmaceutical company	Study design	Primary end point	Enrollment (n)	Study start	Response	Ref.
NCT01639508	Cabozantinib/ XL184	Exelixis (CA, USA)	Open-label, single arm	Response rate	25	July 2012	2: partial response‡ 1: stable disease	[35]
UMIN000010095	Vandetanib/ ZD6474	AstraZeneca (DE, USA)	Open-label, single arm	Response rate	17	February 2013	–	[37]
NCT01823068	Vandetanib/ ZD6474	AstraZeneca	Open-label, single arm	Response rate	17	April 2013	–	[38]
NCT01877083	Lenvatinib/E7080	Eisai (Tokyo, Japan)	Open-label, single arm	Response rate	≤20	April 2013	–	[39]
NCT01813734	Ponatinib/ AP24534	ARIAD (MA, USA)	Open-label, single arm	Response rate	20	June 2013	–	[40]

†Detailed information is available at [41] and [37].
‡Results for three patients were published in [28].

LADC harboring a *KIF5B–RET* fusion showed a positive response to vandetanib [36]. Therefore, molecular targeted therapy using RET TKIs is highly promising.

Three other driver oncogenes encoding protein kinases (*ROS1, HER2*, and *BRAF*) have been identified as druggable targets (**Table 1**). *ROS1* is activated by gene fusion with several partner genes, with *CD74*, *EZR* and *SLC34A2* as the main ones [8,13,14,42]. A significant number of LADC patients with *ROS1* fusions, among those enrolled in a clinical trial, have responded to crizotinib, a FDA-approved drug for the treatment of *ALK* fusion-positive LADC [43]. A LADC patient harboring *ROS1*-fusion was also reported to have responded to therapy with crizotinib [44]. This was attributed to the high structural similarity between the ALK and ROS1 tyrosine kinase domains [45]. *HER2* and *BRAF* are activated by in-frame insertion and missense mutations, respectively [46–52]. Patients with LADCs harboring *HER2* mutations are likely to respond to therapy with anti-HER2 antibodies and HER2 TKIs, as suggested by a recent retrospective analysis [15]. The authenticity of the therapeutic efficacy will be clarified by ongoing clinical trials examining HER2-directed therapies. LADC cases harboring *BRAF* V600E mutation responded to therapy with vemurafenib, an FDA-approved drug for the treatment of melanoma with the same mutation [53,54]. Preliminary data from the phase II trial of dabrafenib in NSCLC with a *BRAF* V600E mutation also supports the fact [55]. On the other hand, although *KRAS* was the first discovered driver gene in LADC (**Table 1**) [56–59], efficient therapeutic strategies against *KRAS* mutation positive LADC have not been established because the KRAS protein is a GTPase, which is less druggable than a kinase [60].

Activation of other oncogenes such as *MET, NRAS, AKT1, MEK1, JAK2* and *PIK3CA* are also observed in a proportion of LADCs, and are also promising therapeutic targets [16], although mutual exclusiveness of these aberrations with aberrations of the seven oncogenes in LADC (in **Table 1**) is not fully examined. Thus, LADC is a disease with multiple promising therapeutic targets that lead to substantial therapeutic improvements.

Conclusion

RET-fusion is the latest driver mutation found in lung cancer, therefore, more in-depth research on pathogenesis and mechanisms of action of RET TKIs is essential to realize the therapy targeting *RET*-fusion. Personalized therapy for LADC will be further expanded in the near future by establishing therapies targeting *RET* fusion, *ROS1* fusion, and *HER2* and *BRAF* mutations in addition to the existing therapies targeting *EGFR* mutations and *ALK* fusion. Although the percentage of LADC patients with these aberrations is small, future therapies including all these druggable targets will cover >50% and >20% of east Asia and US/European LADC patients, respectively.

Future perspective

• Other genes as therapeutic targets

As indicated by the pie chart in **Figure 1**, >30% and >50% of LADCs in east Asia and US/Europe, respectively, are negative for seven driver oncogene aberrations, and they are referred to as 'pan-negative' cases. Therefore,

the development of efficient therapeutic methods against such tumors is necessary to further improve the treatment of LADC. Recent genome-wide sequencing studies have identified a set of genes that are mutated, amplified or fused in LADC and various other human cancers [61,62]. Representative genes are summarized in **Supplementary Table 1** (see online at www.futuremedicine.com/doi/suppl/10.2217/lmt.13.77) as an aberration landscape. Notably, LADCs carry inactivating aberrations of several (candidate) tumor suppressor genes, such as *CDKN2A/p16*, *PTEN*, *RB1*, *BRG1/SMARCA4*, *ARID1A*, *STK11* and *TP53*, as other types of cancers (**Supplementary Table 1**). Synthetic lethality-based therapy targeting gene inactivation in lung cancer cells, such as a that of a chromatin remodeling factor *BRG1/SMARCA4* and an antioxidant factor *KEAP1*, might be effective as suggested by us and others (**Figure 2**) [63–67].

• Clinical sequencing

LADC represents a type of cancer in which 'precision cancer medicine' [69] based on gene aberrations will be achieved, as described above. For this purpose, multiplex and rapid diagnosis of aberrations of druggable driver genes, such as *EGFR*, *ALK*, *RET*, *ROS1*, *HER2* and *BRAF*, is essential in clinical practice. The sequential examination of each of these genes individually by different systems is time consuming and requires large amounts of sample material, which may interfere with the timely diagnosis within a period allowing the selection of therapy. Notably, cases with aberrations in multiple driver genes have been observed. Furthermore, the acquisition of drug resistance by second-site mutations, not only in driver oncogenes, but also in other oncogenes, is a serious problem for therapies based on TKIs [5,43,70]. Therefore, 'clinical sequencing' – that is, the bed-side identification of gene aberrations in tumor DNA by sequencing of biopsy sample DNA/RNA, is critical both for diagnosis and following up during therapy. Systems of clinical sequencing that are properly designed according to purpose, sample type and time schedule are being developed by us and others [69].

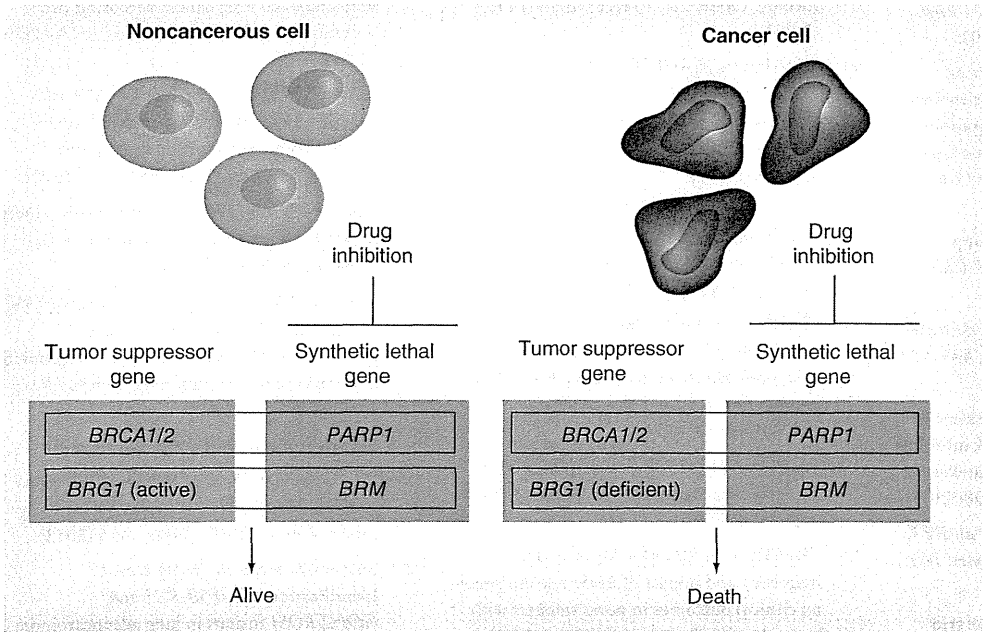

Figure 2. Molecular targeting therapy based on synthetic lethality. Synthetic lethality arises when a combination of mutations in two genes leads to cell death, whereas a mutation in only one of these genes does not. *BRCA1/2* and *PARP1* are shown as a representative gene set for synthetic lethality therapy in human cancer; poly (ADP-ribose) polymerase inhibitors specifically kills hereditary breast and ovarian cancer cells with *BRCA1* or *BRCA2* deficiency (which is caused by a combination of germline and somatic alterations) [68]. In the same way, inhibitors of BRM/SMARCA2 ATPase might be effective for the treatment of lung cancer cells with *BRG1/SMRCA4* deficiency (which is caused by somatic alterations).

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RET-rearranged non-small-cell lung carcinoma: a clinicopathological and molecular analysis

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Background: To elucidate clinicopathological characteristics of non-small-cell lung carcinoma (NSCLC) cases carrying *RET* rearrangements causing oncogenic fusions to identify responders to therapy with RET tyrosine kinase inhibitors.

Methods: We investigated 1874 patients with carcinomas, including 1620 adenocarcinomas (ADCs), 203 squamous cell carcinomas (SCCs), 8 large cell carcinomas, and 43 sarcomatoid carcinomas (SACs). Fluorescence *in situ* hybridisation (FISH) and/or reverse transcription–PCR (RT–PCR) were performed to detect *RET* gene rearrangement.

Results: In all, 22 cases (1.2%) showed *RET* rearrangements; all cases were of ADC histology. Of the 22 patients, 19 possessed *KIF5B–RET* fusion genes, whereas 3 possessed *CCDC6–RET* fusion genes. The *RET*-rearranged tumours were significantly more common in younger patients ($P=0.038$) and tended to occur in patients with no history of smoking ($P=0.051$). In addition, *RET* rearrangements were not associated with gender, occupational history (particularly radioactive exposure), tumour size, lymph node status, tumour stage, or patient survival. The predominant growth pattern in *RET*-rearranged ADCs was lepidic in 6 cases, papillary in 9 cases, acinar in 2 cases, micropapillary in 1 case, and solid in 4 cases. Cells with cytoplasmic mucin production were at least focally present in 12 of the 22 (54.5%) *RET*-rearranged ADC cases. Among the 21 analysed *RET*-rearranged tumours, RET immunopositivity was observed in 15 cases (71.4%), and was significantly associated with *RET* rearrangement ($P<0.001$).

Conclusions: The *RET* rearrangements were observed in 1.2% of NSCLCs. All cases of *RET* rearrangement were ADCs. The *RET* rearrangements were more likely to be observed in younger patients. Although cytoplasmic mucin production was at least focally present in 54.5% of *RET*-rearranged ADCs, specific histological features were not detected.

After the discovery of crucial 'driver' oncogenic mutations in the *epidermal growth factor receptor* (*EGFR*) gene, the EGFR tyrosine kinase inhibitor (TKI) was found to improve survival in non-small-cell lung carcinoma (NSCLC) patients possessing an *EGFR* mutation (Lynch *et al*, 2004; Paez *et al*, 2004; Pao *et al*, 2004). A genomic alteration involving the transforming fusion gene joining the *echinoderm microtubule-associated protein-like 4* gene (*EML4*) and the *anaplastic lymphoma kinase* gene (*ALK*) was identified in 3–13% of patients with NSCLC (Soda *et al*, 2007; Koivunen *et al*, 2008; Yoshida *et al*, 2011). A dramatic response has been observed in patients with *ALK* rearrangements under treatment with an ALK TKI crizotinib (PF-02341066) during a recent clinical trial (Kwak *et al*, 2010).

The *rearranged during transfection* (*RET*) proto-oncogene encodes a receptor tyrosine kinase for members of the glial cell line-derived neurotrophic factor family of extracellular signalling molecules (Knowles *et al*, 2006). This proto-oncogene is involved in the growth and differentiation of neural crest-derived tissues

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