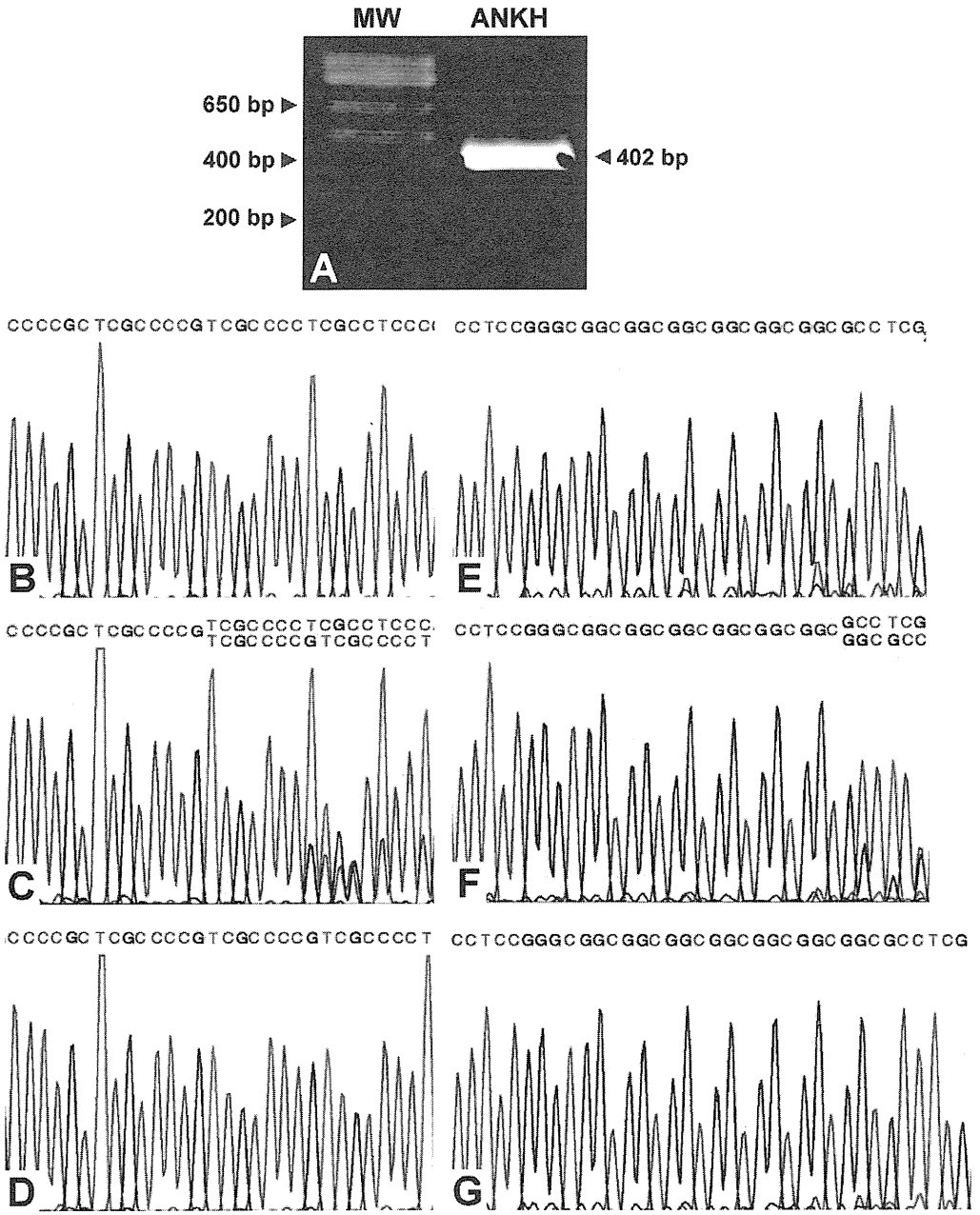

Figure 2. Results of ANKH mRNA expression in the TMJ and sequence traces of ANKH polymorphic sites. (A) Assessment of ANKH mRNA expression in RT-PCR. The sizes of the molecular weight markers (MW) are displayed on the left. Sizes are given in base pairs (bp). (B to G) Sequence traces of identified polymorphic sites on the ANKH gene in the human sample of the study. (B) Genotype 1/1 of ANKH-OR. (C) Genotype 1/2 of ANKH-OR. (D) Genotype 2/2 of ANKH-OR. (E) Genotype 7/7 of ANKH-TR. (F) Genotype 7/8 of ANKH-TR. (G) Genotype 8/8 of ANKH-TR.
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Figure 3 shows the right TMJ of an ANKH-OR homozygous (Genotype 2/2) participant with closed lock. Type I TMJ ankylosis (fibrous adhesion) was identified in the pre-operative arthroscopic view. Quntitative analysis of the arthroscopic data was unattainable as only 2 patients with closed lock in this sample consented to have their arthroscopic images used for research and demonstration. None of the TMJ clicking subjects were managed with this invasive procedure.

Discussion

Fibrous ankylosis was notably found in the TMJ of *ank/ank* mutant mice. Joint space narrowing found in this group represents a relevant pathological trait of fibrous ankylosis [29] and/or osteoarthritis [30]. This finding was consistent with a previous study reporting narrowed joint spaces in the hind limb interphalangeal joints of *ank/ank* mutant mice [16]. Past studies have identified the ANKH protein as serving a critical role in the transport of pyrophosphate ions (PPi) [31] which inhibit ectopic mineralisation in bones [32] as well as joints [33]. Although it was not found in this study, an impaired *ank* function has been associated with calcified debris, excessive calcification and cartilage erosion in the joints of *ank/ank* mutant mice [16]. The discrepancy in the occurrence of calcified changes could be due to a concurrence of crystal deposition and osteoarthritis in most

Table 1. Frequency distribution of TMJ internal derangement by gender, age and genotypes of ANKH polymorphisms in the sample of the study (n = 55).

	Closed lock [n (%)]	Clicking [n (%)]	All [n (%)]	Unadjusted OR[a] (95% CI)	p-values	Adjusted OR[b] (95% CI)	p-values	Adjusted OR[c] (95% CI)	p-values
Genotypes of ANKH-OR polymorphisms									
Heterozygotes	13 (52.0%)	12 (48.0%)	25 (45.5%)	1				1	
Homozygotes	26 (86.7%)	4 (13.3%)	30 (54.5%)	6 (1.6–22.3)	0.005			7.7 (1.6–36.5)	0.011
Genotypes of ANKH-TR polymorphisms									
Heterozygotes	17 (60.7%)	11 (39.3%)	28 (50.9%)	1		1			
Homozygotes	22 (81.5%)	5 (18.5%)	27 (49.1%)	2.9 (0.8–9.8)	0.090	1.9 (0.5–7.9)	0.363		
Gender									
Female	34 (77.3%)	10 (22.7%)	44 (80.0%)	1		1		1	
Male	5 (45.5%)	6 (54.5%)	11 (20.0%)	0.3 (0.1–0.96)	0.038	0.3 (0.1–1.4)	0.130	0.3 (0.1–1.7)	0.174
Age									
	43.0±17.2[d]	25.6±10.6[d]	38.0±17.4[d]	2.2 (1.3–3.7)[e]	0.003	2.1 (1.2–3.6)[e]	0.009	2.4 (1.3–4.3)[e]	0.005

[a]results based on univariate logistic regression statistics.
[b]results based on multivariate logistic regression statistics when excluding genotypes of ANKH-OR polymorphisms.
[c]results based on multivariate logistic regression statistics when excluding genotypes of ANKH-TR polymorphisms.
[d]mean ± standard deviation (years of age).
[e]each additional 10 years of age.
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synovial joints except the TMJ [34]. Utilisation of 3-to-5-month-old mice in this study might be responsible for the absence of erosive changes in the TMJ. According to a biglycan and fibromodulin double-deficient mouse model, osteoarthritis-related cartilage and bony defects in the TMJ were not identified until the age of 9 months although some histological changes in the condylar cartilage were found amongst 3-month-olds [35]. With the exception of joint space narrowing, histological signs of osteoarthritis [36] and synovitis [37], such as joint calcification, joint erosion, proliferative fibrocartilage and lymphocyte infiltration, were rarely seen in this study. Thus, this study could not direct its findings to an association with osteoarthritis and/or synovitis. Consequently, a connection can only be inferred between mutations of the murine *ank* gene and fibrous ankylosis of the TMJ.

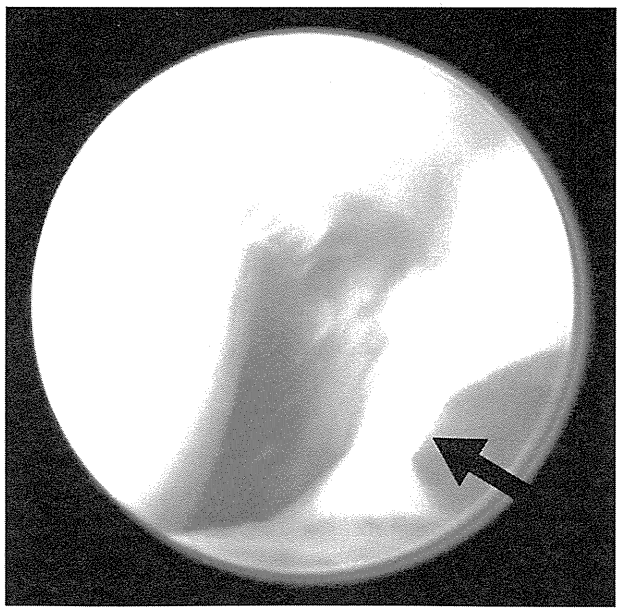

Figure 3. Pre-operative arthroscopic view of the right TMJ from an ANKH-OR homozygote (Genotype 2/2) with closed lock. The white strip at the right portion of the view indicated fibrous tissues adhering the joint (arrow).
doi:10.1371/journal.pone.0025503.g003

This study has demonstrated for the first time an enhancing effect of homozygous ANKH-OR polymorphisms on closed lock of the human TMJ. ANKH-OR homozygotes were approximately 8 times more likely to develop closed lock than their control group counterparts. A past study has reported a more serious phenotype of craniometaphyseal dysplasia amongst homozygous *ank* mutant mice than their heterozygous littermates, based on an autosomal dominant trait of the disorder [38]. Since both types of TMJ internal derangement were observed in all ANKH-OR genotypes, a higher morbidity of closed lock in the homozygous genotypes could be attributable to more complicated molecular mechanisms and/or genetic interactions. A previous study has suggested ANKH-OR and ANKH-TR polymorphisms to be in complete linkage disequilibrium [17]. The disproportionate numbers of ANKH-OR and ANKH-TR homozygotes in the sample of this current study did not corroborate complete linkage disequilibrium of these loci, although it should be noted that genetic interactions were not examined. The discrepancy encourages further investigation into expressions and interactions of ANKH polymorphisms.

Age was related to the type of TMJ internal derangement in this sample and this agreed with a previous study [39]. It was observed that closed lock occurred several months, or at times more than 10 years after milder TMJ symptoms [4]. This long incubation time could explain why closed lock was seen more frequently in older patients. Hence, the risk of a transit from TMJ clicking to closed lock could increase with age amongst those younger ANKH-OR homozygotes who did not exhibit the severer condition. However, a lower response rate could have resulted in a potential of sampling bias in this study. Application of a cohort study method would be able to explore the influence of age on TMD. Compared to closed lock, TMJ clicking was not gravely regarded. A high percentage of patients having joint sounds hesitated to participate

in this study as they were unwilling to take a blood test to assess such an unproblematic condition. Buccal smear for DNA collection may be a more encouraging alternative. Since there are no previous studies using a buccal smear technique to examine ANKH polymorphisms, its feasibility requires further investigation.

This study identified ANKH expression in TMJ synovial cells. It was found that mutations and polymorphisms of the ANKH gene predisposed closed lock in humans and fibrous ankylosis in mice. Although this study did not indicate an equivalence between fibrous ankylosis in mice and closed lock in humans, past studies have reported relationships and similarities between the two disorders.. Fibrous adhesion, known as Type I TMJ ankylosis [10], has been noticed in a number of closed lock cases [4]. This fibrous change has shown a positive correlation with closed lock and a negative correlation with clicking [9]. The pathological duration of closed lock was related to the formation of fibrous adhesions, however TMJ clicking did not exhibit this relationship [40]. Of further note, inflammatory cells that were absent in most ankylosed murine TMJs in this study were also rarely seen in the human TMJs with internal derangement [41]. The ANKH gene, a suggested genetic marker for closed lock in this study, is a human homolog of the murine *ank* gene [17] which contributed to fibrous ankylosis in mice. Thus, it could be deduced that a mouse model is adequate for the simulation of human ANKH-related TMJ internal derangement. In humans, the ANKH gene has been associated with a variety of skeletal and joint defects including ankylosing spondylitis [17], cuff tear arthropathy [42], craniometaphyseal dysplasia [43] and calcium pyrophosphate dihydrate disease [44]. The ANKH protein has been found to be pertinent to the transport of PPi across the plasma membrane and it has been observed as a rescue to certain phenotypes of the above diseases [31]. Hence, future biochemical approaches to the role of PPi on TMD development and rescue are indicated.

Conclusions

This study has manifested that the ANKH-OR polymorphism is a genetic marker associated with TMJ closed lock. ANKH expression in the TMJ has been confirmed. In addition, fibrous ankylosis in the TMJ of *ank* mutant mice has been identified by this study.

TMD has become an important issue in public health and dentistry. A genetic approach to the pathogenesis adds to current understanding of aetiology of TMD. Succeeding investigations into the influence of the ANKH gene on mechanisms of TMD development are indicated.

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Author Contributions

Conceived and designed the experiments: KT KF AS SK T. Sato KB. Performed the experiments: BH KT T. Sakata HK MS. Analyzed the data: BH T. Sato. Contributed reagents/materials/analysis tools: KT KF AS T. Sato KB. Wrote the paper: BH.

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0. 二重盲検とPROBE法を知る：各々の長所と短所を学ぶ

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Introduction

最近，循環器領域の臨床試験で，PROBE法という研究デザインが多用されるようになった。これはProspective Randomized Open Blinded Endpointの略であり，20年ほど前に北欧で開発されたものである。PROBE法とは，前向きの並行群間比較試験で，患者にも医師にも割り付けを知らせるが，エンドポイントの評価者には盲検化をする，という手法である。
本稿では，これまでスタンダードとされてきた二重盲検法と，PROBE法を比較して，バイアスの制御など両者の比較をしたい。

臨床試験で起こるバイアスとは

臨床試験で得られる結果の，真値からの系統的な誤差（偶然誤差とは異なり，繰り返し実験を行ってもその大きさが推定不可能なもの）をバイアスという（p48「臨床試験のバイアスを見抜く：正しい解釈のための第一歩」参照）。臨床試験で起こる主なバイアスとして，
①対象選択のバイアス
②試験の実施に伴う，情報収集や評価のバイアス
③交絡
の3つがあげられる[1]。

(1)対象選択バイアス

このなかで，対象選択バイアスを制御するためには，全数調査や後述のランダム抽出が有効であるが，臨床試験ではこれらを実施することはほぼ不可能である。したがって，特定の臨床試験を実施している医療機関に受診した患者のなかで，臨床試験への参加に同意した協力的な被験者（多くは新薬への期待が大きく研究に理解のある人々）というような，選択バイアスのかかった集団を対象にせざるをえない。

(2)情報バイアス

一方，情報バイアスとは，被験薬群とか対照薬群に被験者が割り付けられたという情報を医師/評価者が知るこ

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とによって，例えば，医師が被験薬群の効果を無意識に期待するあまり，被験薬群は効果を大きめに評価するとか，被験薬を飲んでいないと疾患発症のリスクが高いかもしれないので，イベント予防のために早めに入院させるとか，などのバイアスが発生しうる可能性がある。また対照薬群に割り付けられたことを知っている患者が，軽い症状であっても，「(被験薬を飲んでいないので不安なので)念のため入院させてほしい」と申し出て，その結果入院に至る可能性もある。したがって，臨床試験の評価項目として「入院」などのソフトエンドポイントを設定した場合には，医師や患者の希望・意思・判断によりイベントを増減することが可能であり，割り付け群という情報を医師や患者が知っていると，その情報がバイアスの原因となる可能性がある。情報バイアスの制御には盲検化が有効である。

(3)交絡

交絡とは，治療などの介入とエンドポイントの両者に関連する別の要因であるが，これを制御するためにランダム化が用いられる。交絡についてはp48「臨床試験のバイアスを見抜く：正しい解釈のための第一歩」を参照されたい。

ランダム化とランダム抽出

臨床試験で，標準的治療を対照として，ある治療の効果を正しく評価するために，医師や被験者の意思や判断によらずに，被験者を確率的なメカニズムで割り付けることをランダム化という。これにより，評価したい治療群と対照群の背景因子の分布は，未知の要因についても平均的には等しくなり，背景因子の不均等により結果が偏る交絡因子を，平均的には避けることが可能となる。したがって確率的な評価が可能となる。ランダム化とは，このように交絡因子などを制御して研究対象集団の中の，内的妥当性(両群の比較可能性)を確保する手段である。なお，ランダム抽出とは，結論を適用する母集団からの無作為抽出であり，選挙の出口調査や世論調査などに代表されるが，外的妥当性(標本集団から得られた結果を母集団に外挿する)を確保する手段である(p65「EBMにおけるエビデンスの妥当性とは：内的妥当性と外的妥当性」参照)。

ランダム化によって割り付けられた介入の種類を，被験者や医療従事者，評価者が知り得ないようにすることで，それぞれの思い込みが引き起こすバイアス(情報バイアス)を減らす技術を盲検化とよぶ。被験者のみを盲検化するのが単盲検(一重盲検)，被験者と医師を盲検化するのが二重盲検である。被験薬と対照薬を用いた比較試験の場合は，両者の色・形・においなどを識別不可能な状態(カプセル薬など)にしてそれぞれの割り付け群に投薬されることが多いが，両者の薬効が明らかに異なる場合(実薬とプラセボなど)には，二重盲検をしても，自覚症状や他覚所見から容易に被験者や医師に割り付け群がわかってしまうこともある。なお，エンドポイント評価者や統計解析者の盲検まで含めて三重盲検，四重盲検とよぶ場合もある(表1)[2]。

表1　それぞれのレベルの盲検化と研究デザイン

	一重盲検	二重盲検	三重盲検	四重盲検	PROBE
患者	○	○	○	○	×
医師	×	○	○	○	×
アウトカム評価者	×	×	○	○	○
解析者	×	×	×	○	×

市販されている薬剤を用いて，実際の治療環境のなかで併用法も含め治療ガイドライン全体を介入するようなreal worldの試験の場合には，PROBE法が用いられることがある。

PROBE法と二重盲検法

PROBE法とは，Prospective Randomized Open Blinded Endpointの略であり，1992年にスウェーデンイェテボリ大学のDahlöfらにより，発表された[3]。前向きの臨床試験で，ランダム化で交絡を制御し，エンドポイント

表2 わが国でPROBE法を用いて実施された，循環器領域の主な臨床試験

	MEGA study	JELIS study	JIKEI HEART Study	CASE-J
割り付け群	食事療法群(3,966人) 食事療法+プラバスタチン群(3,866人)	EPA+スタチン群(9,326人) スタチン群(9,319人)	従来降圧治療強化群(1,540人) 従来降圧療法+バルサルタン群(1,541人)	カンデサルタン群(2,354人) アムロジピン群(2,349人)
エンドポイント	複合エンドポイント 心筋梗塞，狭心症，心臓死・突然死，冠動脈血管再建術	複合エンドポイント 冠動脈イベント(心臓突然死，心筋梗塞，不安定狭心症，冠動脈血管再建術)	複合エンドポイント 脳卒中やTIAによる入院，心筋梗塞，心不全による入院，狭心症による入院，解離性動脈瘤，血清クレアチニンの2倍以上の上昇，透析導入	複合エンドポイント 突然死，脳卒中，TIA，心不全，狭心症，心筋梗塞，血清クレアチニン4mg/dL以上または2倍以上への上昇，末期腎不全，解離性動脈瘤，ASO

コラム
propensity analysisとpropensity score

コホート研究やランダム化されていない介入研究では，ランダム化比較試験と異なり，比較する各群の背景因子が異なるので，介入の影響を評価することが困難である。また，臨床現場では，医師がある治療法を知らなかったり，この患者には従来の治療法で十分と考えたり，といった理由で，ある治療法の適応を待つ患者群のなかで，実際にその治療を受けたものと受けなかったものが出てくる。患者背景によって治療選択を変えるのは臨床医として当然だが，患者の状態が対等であったらどうなるだろうか，という問いに，介入群と非介入群の背景因子のバランスを調整して答える解析方法がpropensity analysisである。

Propensity analysisは，ある介入を行う傾向(propensity)を，介入に関連する他の因子で表現する解析であり，propensity scoreとは，各対象者がある介入群に属する確率を，その群に属することに影響を与えると考えられる一群の独立変数を用いて計算したものである。

具体的には，まず，ある群に属するかどうか(介入を受けるかどうかなど)に影響を与える可能性のある変数ー年齢，性別，疾患の重篤度，人種などーの背景因子を決める。次にこれらの独立変数を用いて，多重ロジスティック回帰分析などを行い，ある群に属する確率を推定するモデルを作成する。そして，これを用いて，それぞれの患者についてpropensity scoreを算定する。propensity scoreは，多変量回帰モデルの独立変数とすることができるし，このscoreが近い患者同士をマッチングしてペアを抽出し，その2群で比較をすることもできる。またscoreにより対象集団を層別化して，各層ごとにランダム化比較試験をしたと推定して，仮想のメタ解析をすることも可能である。

Propensity analysisを用いて解析したコホート研究の例として，ConnorsらのSUPPORT研究を紹介する[4]。この研究では，ICU入室患者にSwan-Ganz(S-G)カテーテルを行ったほうが生命予後がよいかどうかを調べるため，入室後24時間以内にS-Gカテーテル術を受ける確率を推定し，各患者についてpropensity scoreが算出された。このscoreを他の変数とともに比例ハザードモデルに投入すると，S-G患者はS-G術を受けなかった患者より30日目の死亡リスクが1.21倍高いという結果が出た。

Propensity analysisは，非ランダム化デザインで治療効果を評価する研究で，ベースラインの違いを調整するには良い方法であり，特に，エンドポイントがまれな事象で，かつ各研究群の対象者の人数が比較的均等な場合には，有効である。ただし，propensity scoreが調整できるのは，既知の交絡因子のみであること，重要な背景因子が測定されていないと研究の精度が低下すること，また，2群でスコアの重なりが少なければ背景因子を調整することは困難であるし，case-control研究では使用できないことなどに留意する必要がある。

評価者の盲検化により情報バイアスを制御する試みであるが，医師や患者への盲検化は行わないので，彼らが割り付けを知ることによる情報バイアスは制御できない。したがってPROBE法では，エンドポイントの選定には十分に留意する必要がある。狭心症や一過性脳虚血発作（transient ischemic attack：TIA）のように，主として患者の訴えや医師からの臨床情報で診断されるような疾患は，医師・患者からの情報バイアスが避けられない。また「…の疾患による入院」，「…の治療のための手術」などをエンドポイントに選んでも，入院や手術の決定には医師や患者の意思・判断が影響するので同様のことが起こりうる。ハードエンドポイントと考えられている，心筋梗塞や脳卒中などをエンドポイントとして選択する場合にも，エンドポイント評価委員会には，（割り付け群を知っている）医師から種々の臨床症状を伝えられていることに留意すべきである。PROBE法には「死亡」のようなハードエンドポイントを選択するのが最も好ましいということになるが，日本人は欧米人に比べて心血管疾患の発症やそれによる死亡が少ないので，ハードなエンドポイントを選択すると，目標症例数または観察期間が非常に増え，実施困難になる可能性がある。下記にわが国でPROBE法を用いて実施された循環器領域の大規模臨床試験の例として，MEGA study[5]，JELIS[6]，JIKEI Heart Study[7]，CASE-J[8]をあげるが，いずれも狭心症，入院，治療，TIAなど，ソフトなエンドポイントを含む複合エンドポイントを採用している（表2）。

PROBE法と二重盲検法は，対象の無作為化（ランダム割り付け）という点では共通しており，どちらも交絡については制御できる。PROBE法は，患者も医師も治療内容を理解しているという実地臨床と類似した環境での臨床試験であり，健康保険制度の下にフリーアクセスが可能な日本の患者にも受け入れられやすく，コストも低いが，前述の情報バイアスは回避できない。したがって，エンドポイントの選択に注意が必要である。一方，二重盲検法は，情報バイアスもかなり制御できるが，被験薬と識別不可能な対照薬をつくるコストや，薬剤管理の手間などが非常に大きいし，被験者の服薬アドヘランスも低下しやすい。どちらも利点と欠点があるので，状況に応じてすぐれたデザインを選択する必要がある。なお，治験以外の臨床試験で，識別不可能なカプセル薬などを作成して多施設に配布するのは薬事法に抵触するのではないか，という懸念がかつてあったが，最近は厚労省医薬食品局監視指導・麻薬対策課に相談をしてから，プラセボやカプセル薬を作成することが可能になり，実際にこれらを用いた医師主導臨床試験も実施されている。

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第10回CRCと臨床試験のあり方を考える会議2010 in 別府／2010年10月3日・ビーコンプラザ

●シンポジウム5／臨床研究に携わるCRCのアドバンススキル／講演1

臨床研究に携わるCRCのアドバンススキル
—プロトコルコーディネートとデータマネジメント—

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はじめに

世の中で実際に行われている研究者主導臨床試験（以下，臨床研究）は治験より多いといわれているが，その実体はわからない。今までわが国の大多数の臨床研究は，一部の多施設共同試験を除き，医師のみあるいは秘書等が手伝うだけで行われることが多かった。しかし，「臨床研究に関する倫理指針」の改訂で今まで以上に質の向上が求められるようになり，医師だけでは手に負えなくなったため，データセンターを持たない大学・病院では，治験管理室等に対して臨床研究の支援要請が増大している。

アドバンススキルを得たいと考えているCRCに，本稿が臨床研究支援のヒントとなれば幸いである。

データマネジメント

臨床研究におけるデータマネジメント（DM）とは，データエラーを低くコントロールし，研究を科学的，倫理的，効率的に行って正しい結論を導くための技術体系である[1,2]。DMは，医学や生物統計学，情報学といった既存の学問に基礎をおき，コンピュータ技術の進歩とともに発展してきたが，いまだ未成熟な発展途上の実学である[2]。このため，DMは臨床研究のデータの品質を保証する重要な手段であるものの，日々その手法が変化しているため，教科書だけではその実際はなかなか理解できない。

このため，医師研究者が，より良い治療・診断法の開発や，エビデンス創成を目的として臨床研究を企画・運営しようとする場合，統計解析とともにDMは作業がわからないという点から，臨床研究支援部門にもっとも支援を求める事項である。この2つは，臨床研究の歴史のなかでも早期に医師から独立したため，通常は臨床現場にそのノウハウは蓄積されていない。また，医学とは異なる統計学，情報学といった専門的知識と技術を要することも，医師が支援を求める要因である。

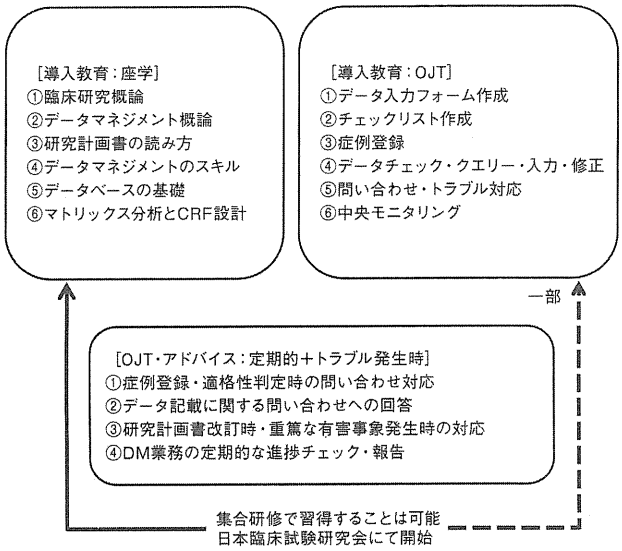

図1　CRCに対するDM教育の試み（導入研修と継続的アドバイス）

他にならおうとしても，企業治験のDMは効率性の面からそのまま導入できず，多施設共同臨床試験グループのDMは実現可能性の面から小規模臨床研究にそのまま導入できない。非専門家が見よう見まねで行うDMにはピットホールがあり，座学で学んでも実務がわからないといった問題が出てきている。このため，当院では，臨床研究の専門的なデータセンターである探索医療センター検証部が，各医局で小規模臨床研究を担当しているCRCを支援する試みを行った。具体的には，**図1**のように，

座学と on the jog training（OJT）を組み合わせた教育訓練と，実際に臨床研究のDM業務を行うCRCに対して，継続的にアドバイスを行うという方法である。

実際に複数の研究事務局を対象に，この教育とアドバイスを行ったところ，導入教育の座学とOJTは，集合研究で習得が可能であることがわかった[3]。これにより，2010年より日本臨床試験研究会において，「研究者主導臨床試験(臨床研究）支援シリーズセミナー」として，データマネジメント・モニタリング基礎知識・技術の習得を目的としたセミナーを開始した。

プロトコルコーディネート

プロトコルは，本来は企画者である医師自身が作成すべきものだが，臨床研究の要件が厳密となり，診療を主業務とする医師だけでプロトコルを作成することは非常に困難となってきている。また，臨床研究のしくみが複雑化し，分業，専門分化が進んだことにより，医師がしくみ全体を把握し，詳細な業務分担を記載することは不可能に近い。このため，多施設，大規模，早期の相，未承認薬・機器を用いる，試験治療が複雑，特殊な試験デザインや統計解析手法，といった臨床研究においては，プロトコル作成は多種専門家による共同作業とならざるを得ない。そこで，この業務を適切にスムーズに遂行させるために生まれたのがプロトコルコーディネーターという役割である[4]。

プロトコル作成は，ほとんどを1人で書く場合(プロトコルライター制）と，分担して書く場合(プロトコルコーディネーター制）がある。1人で書く場合，統一性や整合性は高いが，書ける人が少なく，1人の作業量も限定されるため時間がかかる。一方，分担して書く場合は，専門家が得意なところだけを書くため早いが，統一性や整合性が落ちるため，コーディネートやマネージメントが必須となる。

当院の探索医療センターは，トランスレーショナルリサーチの支援を専門とする部門であるが，メディカルライティング専門のスタッフはいない。このため，プロトコルコーディネート制を導入した。

プロトコルコーディネーターの業務は，基本はチームで協働するための調整をすることであるが，成果物がプロトコルという明確なものであり，期限も評価もはっきりしている。このため，短期業務として兼任が可能である。作業としては，分担して記述した計画書の各パーツを1つの電子ファイルに組み込み，用語や表現を統一し，内容の整合性をとり，長期および多人数による利用に耐えられるよう，ファイル形式やバージョン管理，ログ管理を適切に保持することである。

プロトコルには本来，①臨床的に意味があること(科学性），倫理的であること(倫理性），③法規・ガイドラインに従っていること(順守性・遵守性），④実行でき，守れること(実施可能性），⑤早くタイムリーに研究が始められること(時機性），⑥人・費用・時間がかからないこと(効率性）が求められるが，プロトコルコーディネーター制の導入は，特に⑤時機性と⑥効率性の点で効果があると考えられる。

ま と め

DMとプロトコルコーディネートは，CRCにとって新たな知識と技術を要求するが，医療機関で臨床研究を支援するには，避けて通れない役割である。CRCが臨床研究の支援という新たなステージに入るには，自分たちも企画者および運営者の一部となることが求められる。しかし，これはCRCにとっては，キャリアアップ，キャリアチェンジの機会を与えるものとなるであろう。

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ORIGINAL ARTICLE

Safety of erlotinib treatment in outpatients with previously treated non-small-cell lung cancer in Japan

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Abstract
Purpose Erlotinib is the first epidermal growth factor receptor–tyrosine kinase inhibitor shown to provide a survival benefit for advanced non-small-cell lung cancer (NSCLC) patients. Adverse drug reactions of erlotinib in Japanese, which may be very different from those in Caucasians because of differences in genetic background, have not been fully reported. Therefore, we aimed to clarify the safety profile of erlotinib.

Methods Forty-eight patients with pretreated NSCLC were treated with erlotinib between March 2008 and January 2009 in this historical cohort study at Kyoto University Hospital Outpatients Oncology Unit. Erlotinib 150 mg/day was administered until progressive disease or discontinuation due to adverse events. The primary endpoint was frequency and degree of adverse events, and secondary endpoints were clinical efficacy including response rate, disease control rate, progression-free survival and overall survival.

Results Of 48 patients, 3 patients experienced erlotinib-induced interstitial pneumonitis, which appeared on day 15 and 70 in 2 patients who recovered and on day 8 in 1 patient who died. The incidences of pruritus, dry skin, diarrhea and stomatitis rapidly increased within 14 days after the start of medication with erlotinib. However, these adverse events were well controllable in outpatients treated with erlotinib. Overall response rate was 10% and disease control rate was 68%. The median progression-free survival was 58 days (95% confidence interval 30–118) and the median overall survival was 229 days (95% confidence interval 135–not available).

Conclusions Outpatients with NSCLC can be treated with initial administration of erlotinib by careful management.

Keywords Erlotinib · Epidermal growth factor receptor · Epidermal growth factor receptor–tyrosine kinase inhibitor · Adverse events · Non-small-cell lung cancer · Japanese outpatients

Abbreviations

ADR	Adverse drug reaction
AE	Adverse event
CBC	Complete blood count
CT	Computed tomography

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CTCAE	Common terminology criteria for adverse events
CR	Complete response
DCR	Disease control rate
EGFR	Epidermal growth factor receptor
IP	Interstitial pneumonitis
NSCLC	Non-small-cell lung cancer
PD	Progressive disease
PFS	Progression-free survival
PR	Partial response
PS	Performance status
RECIST	Response evaluation criteria in solid tumors
SD	Stable disease
TKI	Tyrosine kinase inhibitor

Introduction

Erlotinib is an epidermal growth factor receptor (EGFR)–tyrosine kinase inhibitor (TKI), as is gefitinib [1], which showed significant prolongation of overall survival especially in never-smoker or Asian-origin patients but not in the overall population in scheduled subgroup analysis in a randomized phase III trial [2]. A report of a randomized, placebo-controlled trial (BR.21 study) indicated that erlotinib prolonged the median overall survival (OS) (6.7 vs. 4.7 months, $p = 0.001$) and progression-free survival (PFS) in non-small-cell lung cancer (NSCLC) patients after one or two regimens of chemotherapy compared with placebo [3, 4], resulting in approval in clinical indication for previously treated advanced/metastatic NSCLC.

In Japan, phase I (JO16564) [5] and phase II (JO16565) [6] studies regarding erlotinib treatment for pretreated NSCLC experienced progression after prior chemotherapy demonstrated its efficacy and safety, resulting in an approval for its clinical use in Japan since October 2007. However, in the BR.21 study, adverse drug reactions (ADRs) to erlotinib, including interstitial pneumonitis (IP), dermal disorders and diarrhea, similar to gefitinib, were observed within 4 weeks after its administration [3]. Several studies indicated that the incidence of gefitinib-induced IP in Japanese was higher than that in Caucasians [6–9]. Furthermore, another study reported that the ADRs of gefitinib depended on race due to genetic backgrounds [2]. On the other hand, a phase III study concerning erlotinib for NSCLC has not been performed in Japan. These observations suggest that ADRs to erlotinib observed in Caucasians may not apply to Japanese.

In Japan, initial treatment with erlotinib for NSCLC for 3–4 weeks requires admission to hospital for dealing with unpredicted severe ADRs, as with gefitinib, according to the package insert. Several reports describe common toxicities caused by erlotinib such as skin rash (98%), dry skin (81%), and diarrhea (74%) in inpatients with pretreated NSCLC [6]. However the details of timing and degree of adverse events (AEs) are unclear. Furthermore, in Japan, the performance of cancer treatment has been gradually changed year by year from inpatient to outpatient for the convenience of patients and the reduction of national medical expenditure. Therefore, we thought that we should collect data on incidence, timing, and degree of AEs in Japanese outpatients treated with erlotinib in order to manage the initial treatment prophylactically, in an observational study.

Patients and methods

Study design

We conducted a historical cohort study in the Kyoto University Hospital Outpatients Oncology Unit. A total of 48 patients with NSCLC who met the following two criteria were included in this analysis: (1) patients with advanced disease such as stage IIIB or IV, or post-operative relapse, who underwent at least one prior regimen of chemotherapy; and (2) patients giving written informed consent for this study. This study was approved by the ethical committee of Kyoto University Graduate School of Medicine on June 13, 2008 (E-467).

Treatment

Erlotinib 150 mg/day was administered once a day at least 2 h after a meal according to the package insert and critical paths until progressive disease (PD) or occurrence of AEs which might limit continuation of medication with erlotinib.

When AEs equal to grade 1 or higher interstitial lung disorder, or other grade 4 AEs except for hematologic toxicity were observed, administration of erlotinib was discontinued. If grade 2 AEs did not subside regardless of supportive care, or grade 3 AEs were observed, erlotinib administration was suspended. Erlotinib administration was then resumed with reduction of erlotinib by 25 mg/day after recovery from the AEs.

If AEs which limited continuation of treatment occurred even after supportive care for the AEs, erlotinib was reduced by a further 25 mg/day. For the prevention of competitive inhibition, any agents which may modulate cytochrome P450 activity in liver were changed for another agent which did not influence cytochrome P450 activity [10].

Endpoints

The primary endpoint was to estimate incidence and degree of AEs during and after erlotinib treatment in outpatients with pretreated NSCLC. Secondary endpoints were response rate (RR), disease control rate (DCR), PFS after the start of erlotinib treatment and OS. Tumor response to erlotinib was assessed by response evaluation criteria in solid tumors (RECIST) [11] using computed tomography (CT) scans.

Evaluation of adverse events

AEs were graded according to the National Cancer Institute common terminology criteria for adverse events (CTCAE), version 3.0 [12]. Observed AEs were recorded in the electronic medical record and open-database, CyberOncology®, developed in the Kyoto University Hospital Outpatients Oncology Unit [13].

At the start of erlotinib treatment, patients were evaluated by physical examinations such as percutaneous arterial oxygen saturation, complete blood count (CBC) and biochemical tests. To detect and record subjective symptoms, the patients were obliged to keep a diary every day. Patients were evaluated by physical examination, CBC and biochemical tests every week during treatment with erlotinib for the first month. To detect lung injury including IP due to erlotinib treatment, we took chest X-rays every week during the first month after the start of treatment with erlotinib and chest CT scan every 4 weeks for the first 2 months. After a month, patients were evaluated by physical examination, CBC, biochemical tests and chest X-ray every 2 weeks.

Supportive care for adverse events

Positive therapeutic intervention was performed to treat exanthema and diarrhea. At the start of erlotinib treatment, heparin analogue ointment, difluprednate and hydrocortisone butyrate ester ointment were prescribed and treated to prevent exanthema. Lactomin-amylolytic bacillus mixture was prescribed to prevent diarrhea.

Evaluation of response to treatment and follow-up assessments

To assess response to erlotinib treatment, we compared identifiable tumor sizes using CT scan every 4 weeks until 2 months after the start of erlotinib administration. RR was evaluated according to RECIST criteria [11]. A CT scan was performed every 2 months or every 3 months if patients achieved stable disease (SD) or partial response (PR), respectively. If necessary, CT scans of the brain, chest or abdomen were performed appropriately to assess PD.

Statistical methods

The safety of erlotinib was assessed by examining the incidences of AEs. The relationship between the achievement of disease control or response to erlotinib by RECIST [11] and the following clinical factors were assessed by using the Mantel–Haenszel chi-squared test in univariate analysis and logistic regression in multivariate analysis: age, gender, performance status (PS), histological classification of tumor tissue, smoking history, EGFR mutation, previous gefitinib treatment, previous thoracic radiotherapy, number of regimens before erlotinib treatment, skin AEs and skin AEs within 7 days after the start of erlotinib. We planned that all the factors significant in the Mantel–Haenszel chi-squared test were to be further assessed by logistic regression analysis.

Results

Patients' characteristics

Forty-eight patients with pretreated NSCLC were treated by erlotinib between March 2008 and January 2009 in this cohort and analyzed in the current analysis.

Among them, 40 patients were evaluable for response to erlotinib with CT scan. Eight of the 48 patients could not continue erlotinib treatment until the first estimation of IP with chest CT scan after the start of the therapy and were not evaluable because of reasons as follows: five patients who experienced AEs (IP in 2 patients, pruritus in 2, and nausea in 1) stopped erlotinib medication, 2 patients refused to continue the therapy before an evaluation CT scan after the start of therapy, and one had worsening of PS level (PS 4) and did not continue the therapy.

The patients' backgrounds are shown in Table 1. There were 25 males (52%). Thirty-one patients (65%) had a history of smoking. The histological type was diagnosed as adenocarcinoma in 37 patients (77%) and squamous cell carcinoma in 6 (13%). The stage was evaluated as IIIB in 13 patients (27%) and IV in 19 (40%). Postoperative relapse was detected in 16 patients (33%). Of 21 patients (44%) in whom EGFR mutation could be evaluated, it was detected in 13 (27%), but not in 8 (17%). The median number of previous chemotherapeutic regimens was 3. Twenty-three patients (48%) had received gefitinib therapy as a previous therapy. Duration of erlotinib therapy ranged between 1 and 333 days with a median of 35.5 days.

Table 1 Characteristics of 48 patients with advanced non-small-cell lung cancer

	Number of persons	Percentage
Median age (range)	62 (30–82)	
Age		
<65 years	26	54
≥65 years	22	46
Gender		
Male	25	52
Female	23	48
Performance status		
0	37	77
1	10	21
2	0	0
3 or higher	1	2
Histological classification		
Adenocarcinoma	37	77
Squamous cell carcinoma	6	13
Others	5	10
Smoking		
Smoker	31	65
Non-smoker	17	35
Staging		
IIIB	13	27
IV	19	40
Post-operative relapse	16	33
Epidermal growth factor receptor mutation		
Mutant type	13	27
Wild type	8	17
Previous thoracic radiotherapy		
Present	10	21
Absent	38	79
Number of previous chemotherapeutic regimens		
1	6	12
2	7	15
3	13	27
4 or more	22	46
Previous gefitinib therapy		
Present	23	48
Absent	25	52

Adverse events

AEs in 48 patients who were treated with erlotinib are shown in Table 2. Diarrhea, anorexia, pruritus, dry skin, desquamation and stomatitis were observed in more than 50% of the patients. Diarrhea was the most frequent toxicity. There was no hematologic toxicity. Most of the AEs were evaluated as grade 1 or 2 and erlotinib treatment was tolerable. Grade 3 or 4 AEs were noted in 9 patients with 11 incidences: stomatitis in 1 patient, anorexia in 1, acne in 2, fatigue in 1, dyspnea in 2, an increase in the total bilirubin level in 1, muscular weakness of the lower limbs in 1 and IP in 2.Only IP was observed in a patient as a grade 4 or higher AE. The incidence of grade 3 or 4 AEs was 31.8% in the elderly group (≥65 years old), and 15.3% in the younger group (<65 years old). In 3 of 7 patients, excluding 2 with IP, erlotinib therapy could be resumed. Overall, 10 patients (20.8%) required suspension. In 11 (22.9%), the dose was decreased. In 39 (81.2%), analysis was performed during the discontinuation period. Of these, administration was terminated due to disease progression, AEs and patients' desire in 25 (52.0%), 11 (22.9%) and 3 (6.3%) patients, respectively.

Concerning frequent symptoms, we examined the association between time after the start of erlotinib and the incidences of pruritus (Fig. 1a), diarrhea (Fig. 1b), dry skin (Fig. 1c) and stomatitis (Fig. 1d). The incidence of grade 1 or higher pruritus rapidly increased to approximately 50% within 10 days after the start of administration, and then gradually rose (Fig. 1a). Furthermore, the incidence of grade 2 or higher pruritus increased to 10% within 14 days after the start of administration, and then reached a plateau and stayed at a constant rate on the basis of reduction in incidence of AEs, or termination of medication with erlotinib either by unbearable AEs or by experience of PD (Fig. 1a). The incidence of grade 1 or higher diarrhea increased to approximately 40% within 7 days after the start of administration, and stayed at a constant rate until day 21 (Fig. 1b). However, it gradually decreased to approximately 25% thereafter (Fig. 1b); that of grade 2 or higher diarrhea increased to approximately 5% within 14 days after the start of administration, and then persisted until day 30 (Fig. 1b). However, there was no grade 2 or higher diarrhea after day 30 (Fig. 1b). The incidence of grade 1 or higher dry skin increased to approximately 30% within 10 days after the start of administration, and rose continuously to 76% thereafter (Fig. 1c); that of grade 2 or higher dry skin after 1 month of administration was approximately 4%, and remained at 4–5% thereafter (Fig. 1c). The incidence of grade 1 or higher stomatitis increased to approximately 30% within 7 days after the start of administration, and then gradually rose to 40% (Fig. 1d); that of grade 2 or higher stomatitis was approximately 4% a few days after the start of administration, and persisted until day 40 (Fig. 1d). However, there was little stomatitis of grade 2 or higher after 40 days (Fig. 1d). Furthermore, each figure shows the relationship between the number of patients treated with erlotinib and the time after the start of administration as bar graphs (Fig. 1a–d).

IP was observed in 3 patients, occurring on days 8, 15, and 70, respectively. The main symptoms were fever,

Table 2 Adverse events observed in the 48 patients during erlotinib treatment

Adverse events	Number of patients						
	Grade 1	Grade 2	Grade 3	Grade 4	Grade 3/4	Total (*n*)	%
Diarrhea	23	8	0	0	0	31	64.6
Anorexia	23	5	1	0	1	29	60.4
Pruritus	21	8	0	–	0	29	60.4
Dry skin	24	1	0	–	0	25	52.1
Desquamation	14	11	0	0	0	25	52.1
Stomatitis	21	2	1	0	1	24	50.0
Acne	15	5	2	–	2	22	45.8
Hand–foot skin reaction	16	1	0	–	0	17	35.4
Fatigue	7	6	1	0	1	14	29.2
Dyspnea	10	1	2	0	2	13	27.1
Nausea	11	1	0	0	0	12	25.0
Taste alteration	10	1	–	–	–	11	22.9
Vomiting	10	0	0	0	0	10	20.8
Pneumonitis	0	1	1	1	2	3[a]	6.3
Gait/walking	1	0	1	0	1	2	4.2
Bilirubin	0	0	1	0	1	1	2.1

[a] One patient died after incidence of pneumonitis

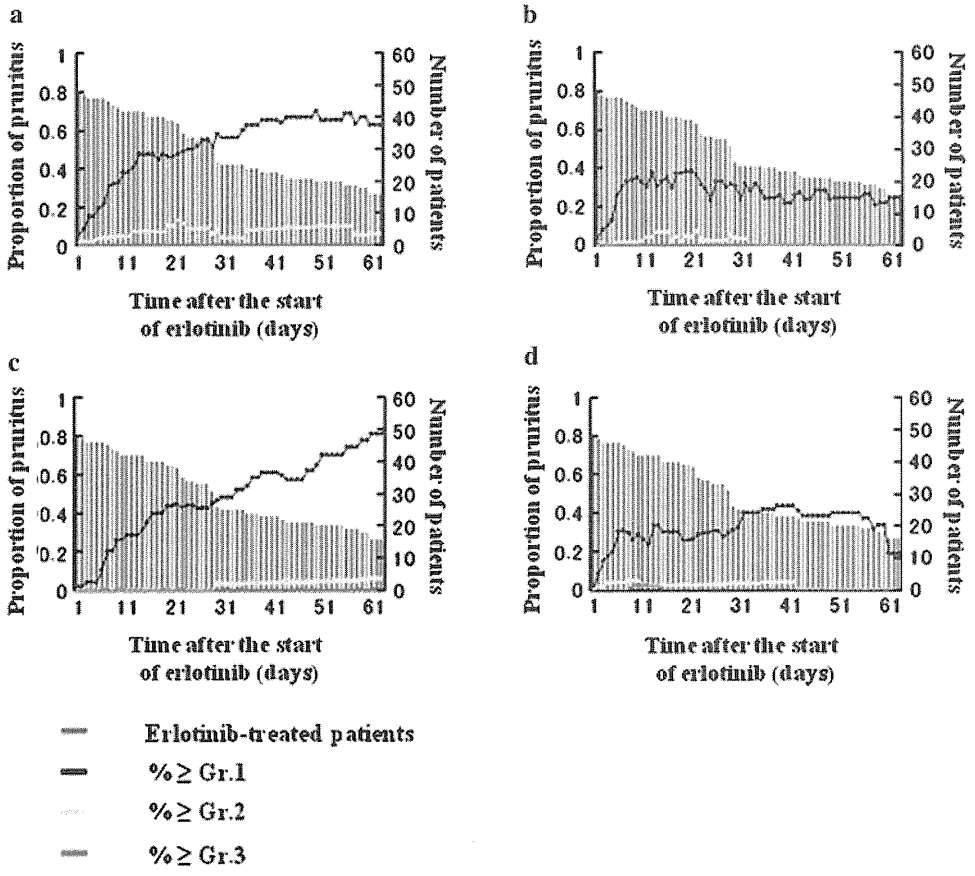

Fig. 1 Incidence of adverse events including pruritus, diarrhea, dry skin and stomatitis. **a** *Left vertical scale* indicates proportion of pruritus (%). *Right vertical scale* indicates number of patients. *Horizontal scale* indicates days after start of erlotinib. **b** *Left vertical scale* indicates proportion of diarrhea (%). *Right vertical scale* indicates number of patients. *Horizontal scale* indicates days after start of erlotinib. **c** *Left vertical scale* indicates proportion of dry skin (%). *Right vertical scale* indicates number of patients. *Horizontal scale* indicates days after start of erlotinib. **d** *Left vertical scale* indicates proportion of stomatitis (%). *Right vertical scale* indicates number of patients. *Horizontal scale* indicates days after start of erlotinib

dyspnea and cough. Chest CT scan showed ground-glass opacity in 2 of 3 patients and consolidation like organizing pneumonia in the lung field in one. Of 3 patients with IP, 2 patients recovered and one died (Table 3).

Efficacy

No patient showed complete response. PR was achieved in 4 patients (8.3%), SD was noted in 23 patients (48.0%) and

Table 3 Patients with interstitial pneumonia

	Age	Histological type	Smoking status	Status of EGFR gene mutation	Physical findings	Day of onset confirmation	Imaging findings	Therapeutic intervention	Clinical course	Pretreatment regimens
Case 1	80	Adenocarcinoma	Smoker	Wild type	Dyspnea (grade 3) Hypoxia (grade 2)	Day 15	NSIP pattern	Only termination	Survival	CBDCA + PTX VNR GEM
Case 2	52	Non-squamous cell carcinoma	Smoker	Not evaluated	Fever (grade 1) Cough (grade 1)	Day 70	OP pattern	mPSL pulse →PSL 1 mg/kg/day	Survival	CBDCA + PTX GEM + VNR DTX + S-1 CDDP + CPT-11
Case 3	67	Adenocarcinoma	Smoker	Not evaluated	Fever (grade 1) Dyspnea (grade 3) Hypoxia (grade 2)	Day 8	Extensive bilateral GGO pattern	mPSL pulse →PSL 1 mg/kg/day	Death	CDDP + DTX

CBDCA carboplatin, *CDDP* cisplatin, *CPT-11* irinotecan, *DTX* docetaxel, *GEM* gemcitabine, *GGO* ground-glass opacity, *mPSL* methylprednisolone, *NSIP* non-specific interstitial pneumonia, *OP* organizing pneumonia, *PSL* prednisolone, *PTX* paclitaxel, *S-1* oral fluoropyrimidine formulation that combines tegafur, 5-chloro-2,4-dihydroxypyridine, and potassium oxonate, *VNR* vinorelbine

PD in 13 (27.1%), and eight patients were not evaluable. The RR was 10% (4 of 40 patients), and the DCR was 67.5% (27 of 40). The median follow-up time was 117 days (1–358 days). The median PFS was 58 days (95% confidence interval 30–118), and the median OS was 229 days (95% confidence interval 135–not available).

In 21 of 48 patients, EGFR mutation could be evaluated: thirteen of 21 patients had EGFR mutation. In 10 of 13 mutant-type patients, the response to erlotinib treatment could be evaluated: PR was achieved in 3 patients, and SD and PD were noted in 4 and 3 patients, respectively. In 7 of 8 wild-type patients, the erlotinib treatment response could be evaluated: no patient showed PR in wild-type, while SD and PD were noted in 5 and 2 patients, respectively. The RR in the mutant- and wild-type patients was 30.0 and 0%, respectively. However, the DCR was 70.0 and 71.4%, respectively, showing no difference between them.

In the elderly group, the RR and DCR were 18.7 and 68.7%, and in the younger group, 4.2 and 66.7%, respectively.

We also explored association between RR or DCR and clinical factors including age, gender, PS, histological classification of tumor tissue, smoking history, EGFR mutation, previous gefitinib treatment, previous thoracic radiotherapy, regimen number of erlotinib treatment, skin AEs and skin AEs within 7 days after the start of erlotinib. However, no significant association was observed with the Mantel–Haenszel test.

Discussion

We demonstrated here that erlotinib showed high DCR, as previously described [6, 14]. Furthermore, its AEs were tolerable and seemed to be equivalent to those in the Asian population as reported previously, but the incidence of IP in this study is much higher than that observed in Caucasians, as shown in the BR.21 study [3]. On the other hand, never-smoking, EGFR mutation and adenocarcinoma did not associate with response to erlotinib in logistic regression analysis. Interestingly, the incidence of common toxicities in outpatients in this study has no significant differences compared with that in inpatients as reported previously [6].

We conducted initial treatment by erlotinib in outpatients with pretreated NSCLC. However, neither its safety nor efficacy in Japanese patients in clinical practice has been fully demonstrated. In this study, we examined the safety and efficacy of erlotinib in patients with previously treated NSCLC; 65% diarrhea, 60% pruritus and 52% dry skin were observed, which appears to be similar to the previous report [6], in which initial treatment with erlotinib before approval of clinical use in Japan was managed in

inpatients with pretreated NSCLC and 98% rash, 81% dry skin and 74% diarrhea were observed. We think that careful management of initial treatment with erlotinib enables outpatients to receive a level of therapy equivalent to admission to a hospital.

IP has been reported to be a lethal toxicity related to erlotinib treatment, and is the most critical matter in clinical practice [3, 6]. In our study, IP was observed in 3 patients whose onset was days 8, 15, and 70, respectively, and the patient who developed IP on day 8 did not show any response to steroid therapy, resulting in death. Incidence of IP by erlotinib and its mortality were reported to be 6 and 1.6% in a phase II trial [6] and 4.5 and 1.6% in post-marketing surveillance (3000 patients). These data were closely equivalent to our data (6% IP occurrence and 2% death due to IP). However, the size of our study was small, and we should carry out further large-scale outcome research to demonstrate the possibility of safely managing initial treatment with erlotinib in outpatients with pretreated NSCLC.

In a post-marketing observational study in Japan, safety is not guaranteed in elderly patients 65 or more years old. Furthermore, in the BR.21 study, the elderly group ($\geq$70 years old), showed a higher rate of grade 3 or 4 AEs (35%) than that observed in the younger group (<70 years old; 18%) [15]. These data are also very similar to our data. DCR in the BR.21 study was 78% in the elderly group and 68% in the younger group, which is also similar to our data (69% in the elderly group and 67% in the younger group). Therefore, to treat elderly patients with pretreated NSCLC with erlotinib, more attention should paid to them than to younger patients, although the clinical benefit may be almost the same among the two groups.

We examined the incidences of frequent AEs, such as diarrhea, pruritus, dry skin and stomatitis, as well as the timing of their appearance. The incidence of grade 1 AEs rapidly increased 7–10 days after the start of administration, reaching a plateau thereafter or showing a gradual increase; that of grade 2 AEs reached a plateau 10–14 days after the start of administration. However, dry skin was observed after 1 month of administration. These results suggest the importance of close follow-up of AEs to erlotinib, including IP, especially for the initial 2 weeks after the start of administration.

In this study, we assessed the independent factors associated with disease control with erlotinib in univariate analysis. We found that there were no associations between achievement of disease control or response to erlotinib and independent factors including smoking history, adenocarcinoma in tumor histology, presence of EGFR mutation in cancer cells and skin adverse effects. These are not consistent with the results previously described [3, 6, 14]. The reasons are unclear. However, the small number of data concerning EGFR mutation in cancer cells, and differences in prior treatment with gefitinib, may contribute to the results of the present research. We should study the risk factors for disease control or response to erlotinib in a larger-scale outcome research in order to efficiently manage pretreated NSCLC in Japanese.

We reported that erlotinib showed high DCR in outpatients with pretreated NSCLC and its AEs were well controllable. From the standpoint of both this study and a post-marketing surveillance, outpatients with non-small-cell lung cancer can be treated with initial administration of erlotinib by careful management.

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TRIALS

STUDY PROTOCOL Open Access

Design paper: A phase II study of Bevacizumab and Erlotinib in patients with non-Squamous non-small cell lung cancer that is refractory or relapsed after 1-2 previous Treatment (BEST)

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Abstract

Background: Combination of erlotinib and bevacizumab is a promising regimen in advanced non-squamous non-small-cell lung cancer (NSCLC). We are conducting a single arm phase II trial which aims to evaluate the efficacy and safety of this regime as a second- or third-line chemotherapy.

Methods: Key eligibility criteria were histologically or cytologically confirmed non-squamous NSCLC, stage III/IV or recurrent NSCLC not indicated radical chemoradiation, prior one or two regimen of chemotherapy, age 20 years or more, and performance status of two or less. The primary endpoint is objective response rate. The secondary endpoints include overall survival, progression-free survival, disease control rate and incidence of adverse events. This trial plans to accrue 80 patients based on a two-stage design employing a binomial distribution with an alternative hypothesis response rate of 35% and a null hypothesis threshold response rate of 20%. A subset analysis according to EGFR mutation status is planned.

Discussion: We have presented the design of a single arm phase II trial to evaluate the efficacy and safety of combination of bevacizumab and erlotinib in advanced non-squamous NSCLC patients. In particular we are interested in determining the merit of further development of this regimen and whether prospective patient selection using EGFR gene is necessary in future trials.

Trial registration: This trial was registered at the UMIN Clinical Trials Registry as UMIN000004255 (http://www.umin.ac.jp/ctr/index.htm).

Background

Chemotherapy for advanced non-small-cell lung cancer (NSCLC) patients with good performance status improves survival time and quality of life [1]. Platinum doublet therapies with third-generation agents are thought as the standard in first-line for NSCLC patients, of which response rate is 30-40%, one year survival rate is 26-36% and median survival time is 8-13 months [2-4]. For patients who had relapsed or did not respond to first-line chemotherapy, docetaxel [5-7] and pemetrexed [8] are effective. Erlotinib, an oral epidermal growth factor receptor tyrosine kinase inhibitor (EGFR-TKI), was also shown to improve progression-free survival (PFS) and overall survival (OS) modestly with acceptable toxicity in second- or third-line setting for advanced NSCLC [9,10]. On third-line treatment only erlotinib is recommended by the National Comprehensive Cancer Network guideline [11] and no established treatment options exist for patients who have experienced erlotinib failure.

Several lines of evidence lent support to the notion that combining bevacizumab, a monoclonal antibody targeting the vascular endothelial growth factor (VEGF), with erlotinib for advanced NSCLC might confer additional clinical benefit. Two large phase III trials confirmed that bevacizumab improves survival of advanced non-squamous NSCLC patients when combined with carboplatin plus

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paclitaxel or cisplatin plus gemcitabine as first-line chemotherapy [12,13]. A significant improvement in PFS and objective response rate (ORR) by the addition of bevacizumab with carboplatin plus paclitaxel was also shown in a randomize phase II trial of Japanese patients [14]. Finally, a recent randomized phase II trial of combination of bevacizumab with erlotinib, combination with cytotoxic drug, and cytotoxic drugalone showed results for PFS and OS favour the combination regimens over cytotoxic drug alone in the second-line setting, although not statistically significant [15].

Objective

The primary objective of the trial is to evaluate the efficacy and safety of combination of bevacizumab and erlotinib as a second- or third-line chemotherapy for advanced non-squamous NSCLC. Specific hypotheses to be tested are (1) one-sided hypothesis that the ORR of combination of bevacizumab and erlotinib is higher than a pre-specified threshold of 20%, (2) whether this regimen are safe and feasible, and (3) whether the ORR is higher in patients with EGFR mutation than in patients with EGFR wild type.

Methods

Design and setting

This study is an open-label, multi-institute, single arm phase II clinical trial. The coordinating office is at Kyoto University Hospital. Registration and data collection are conducted with the use of the web system and the electronic case report form (e-CRF).

Ethical consideration and registration

The study protocol is according to the Helsinki declaration [16] and the Ethics Guidelines for Clinical Research by the Ministry of Health, Labor, and Welfare [17]. We obtained approval by the ethical committee at Kyoto University on October 27, 2010 (C-453). This trial was registered at the UMIN Clinical Trials Registry as UMIN000004255 (http://www.umin.ac.jp/ctr/index.htm).

Eligibility criteria

Staging was according to the 7th Edition of the TNM Classification for Lung Cancer [18]. Inclusion criteria are as follows:

1) Histologically or cytologically confirmed non-squamous NSCLC.

2) Stage III/IV or recurrent NSCLC not indicated radical chemoradiation, and prior one or two regimen of chemotherapy.

3) Age 20 years or more at the date of informed consent.

4) The Eastern Cooperative Oncology Group Performance Status of two or less.

5) Presence of measurable lesion.

6) Sufficient hematologic, hepatic, and renal and lung function in laboratory tests 14 days before registration.

7) Expected survival time more than three months.

8) Expected interval more than 28 days after surgery if the patient received a major surgery.

9) Written informed consent by the patient.

Exclusion criteria are as follows:

1) Prior EGFR-TKI.

2) Serious complications.

3) Hemoptysis or bloody sputum of 2.5 mL or more, or history of clinically significant hematemesis, coagulation disorder or thrombosis.

4) A cavitating lesion, a central lesion or a lesion abutting major blood vessels.

5) History of myocardial or cerebral infarction within six months before registration.

6) Refusal of contraception or woman with on-going or contemplating pregnancy or breast-feeding.

7) Brain metastasis with a bleeding risk.

8) Interstitial pneumonia confirmed by computer tomography.

9) Difficulty in ingestion.

10) Pleural effusion which is uncontrolled by local therapy and requires other treatments

11) Patients judged inappropriate for the trial by investigators.

Patient registration

After confirming eligibility criteria and obtaining informed consent. Eligible patients are registered and then investigators initiate the planned treatment. The accrual started in November 2010 and is to continue for two years.

Treatment

Patients enrolled in this trial receive the protocol treatment with bevacizumab and erlotinib within 15 days. Dose of the protocol treatment is based on the prior trials [15,19]. Bevacizumab is administered at a dose of 15 mg/kg on the first day of each 3-week cycle. No dose reductions are allowed for bevacizumab. Bevacizumab is terminated if either of the following adverse events occurs.

1) Grade 2 to 4 hemorrhage

2) Grade 3 to 4 thrombosis

3) Delay of administration of each cycle over 23 days

Erlotinib is administered initially at 150 mg/day orally. Tablets are taken at least one hour before or two hours after a meal, preferably in the morning. Dose of erlotinib are reduced by one or two levels of five doses, 150, 125, 100, 75 and 50 mg, if either of the following adverse events occurs.

1) Unacceptable skin toxicity

2) An increase in AST or ALT up to Grade 3 to 4