厚生労働科学研究費補助金

第３次対がん総合戦略研究事業

ヒト化抗 CD20 抗体を細胞外ドメインとした新規キメラ抗原レセプター（CAR）遺伝子導入 T 細胞の作成と評価

平成 25 年度　総括・分担研究報告書

研究代表者　寺　倉　精　太　郎

（名古屋大学医学部附属病院）

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(第３次対がん総合戦略事業)

平成25年度　総括・分担研究報告書

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Ⅰ．研　究　組　織

【ヒト化抗 CD20 抗体を細胞外ドメインとした新規キメラ抗原レセプター（CAR）遺伝子導入 T 細胞の作成と評価】　平成 25 年度名簿

	氏名	所属・職名
主任研究者	寺倉　精太郎	名古屋大学医学部附属病院 血液内科医員
分担研究者	村田　誠	名古屋大学医学部附属病院 血液内科講師

Ⅱ. 総　括　研　究　報　告

厚生労働省科学研究費補助金（第３次対がん総合戦略事業）

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ヒト化抗 CD20 抗体を細胞外ドメインとした新規キメラ抗原レセプター（CAR）遺伝子導入 T 細胞の作成と評価

研究代表者：寺倉　精太郎　名古屋大学医学部附属病院　血液内科医員

研究要旨：

ヒト化抗 CD20 抗体を細胞外ドメインとした新規キメラ抗原レセプター(CAR) を開発し、これを遺伝子導入した T 細胞を用いてその評価を行った。CD20 を特異的に認識する CAR 遺伝子を開発し得た。現在臨床で用いられる抗 CD20 抗体は、連用することで腫瘍細胞表面上の CD20 発現が低下し、抗 CD20 抗体療法が不応になることが知られているが、CD20-CAR+ T 細胞はこうした CD20 低発現細胞株も有効に認識・傷害した。また、腫瘍細胞表面上の CD20 が低発現となった患者からの臨床分離株においても有効な認識・傷害を示した。

これまで用いてきた CD28 細胞内ドメインに加え、4-1BB および CD27 細胞内ドメインをもつ CAR を作成した。細胞内シグナルを詳細に検討するため、T 細胞が活性化すると蛍光を発するよう vector を遺伝子導入したが、刺激後にうまく発現しなかった。さらに抗体部分の affinity の異なる CAR を複数種類作成し、affinity と細胞内ドメインの最適な組み合わせについて検討した。

研究分担者氏名・所属研究機関名及び所属研究機関における所属

村田誠・名古屋大学医学部附属病院　講師

A.研究目的

本研究ではヒト化 CD20 抗体を細胞外ドメインとして用いた CAR を作成･評価し前臨床試験までを行うことを目的とした。既に抗体の Affinity が報告されているヒト化 CD20 抗体の遺伝子情報を用いて開発に着手できる。ヒト化抗体を用いた CAR の開発はまだ報告が少ないが、導入した遺伝子産物に対する免疫反応が起こりにくいと考えられるヒト化抗体を用いて免疫反応を避ける必要性は高い。本研究で用いる CD20 抗体は Affinity が既知であるために、標的細胞側の抗原発現量がリガンド結合後の T 細胞機能に及ぼす影響を、異なる Affinity の CAR を用いて検討可能である。CD20 低発現細胞株や臨床分離細胞を用いて CD20 低発現の標的に対する CD20-CAR の作用について検討する。さらに Affinity/avidity を変化させたときに CD28/CD27/4-1BB の細胞内ドメインの違いが及ぼす影響について、とくに抗原刺激後の T 細胞の増殖・メモリー化に及ぼす影響について in vivo で検討する。

CD20 刺激後のサイトカイン分泌や細胞分裂能から最適な CAR の構造を決定し、これを用いた臨床試験の準備を行う。分担研究者において既に稼働している Cell processing center を用いて実際の患者から分離した T 細胞を用いた細胞調整の試験を数例程度行い、GMP 基

準に則った細胞調整が可能であることを確認する。

開発したCD20-CARを用いて臨床試験を行い、実際に臨床的有用性が示されれば、現在臨床で使用されている維持抗体療法・化学療法の代替としてより副作用が少なく、維持療法よりもむしろ安価な治療として認知されることが期待される。

B. 研究方法

これまで用いてきたCD28細胞内ドメインに替えて4-1BB/CD27の細胞内ドメインを組み込んだプラスミド・ベクターを作成した。上記同様にレトロウイルス・ベクターを作成した。CARがCD20に結合した後、伝達されるシグナルを比較検討するため、Jurkat細胞株にreporter vectorを組み込んだものを作成した。これにより、より定量的にシグナル伝達を評価できるものと考えた。

CARの細胞外ドメインとなる抗体のaffinityとCARの有効性との関連は詳細には検討されていない。5種類のaffinityの異なるCD20抗体を用いてCARを作成した。これをT細胞に遺伝子導入し、CAR-Tの細胞傷害活性などのT細胞活性に及ぼす影響を検討する。また、細胞内ドメインのaffinityの組み合わせの違いによってT細胞機能にどのような影響が出るのか検討する。

（倫理面への配慮）

患者あるいはドナーから細胞その他の材料を採取する場合には、当院 IRB で審査を受け、適切なインフォームド・コンセントのもと行う。研究遂行にあたって必要な倫理指針などを遵守して行う。

C. 研究結果

新規にヒト化抗 CD20 抗体を細胞外ドメインとして用いた CAR を作成し、細胞表面上に発現する CD20 の抗原量と CD20-CAR+ T 細胞の反応しうる限界について検討した。新たに作成した CD20-CAR を遺伝子導入した CD20-CAR+ T 細胞は CD20 特異的に標的細胞を認識・傷害した。この細胞を用いて様々な程度の CD20 を発現する CEM 細胞株群に対する細胞傷害活性を検討した。今回作成した CD20CAR-T 細胞は標的細胞あたり約 200 分子の CD20 を認識して傷害することが分かった。同様にして CD20CAR-T 細胞を活性化するために必要な CD20 分子密度を検討した。CD20CAR-T を活性化するために必要な CD20 抗原は標的細胞あたり約 2000 分子程度であることが分かった。さらに、CD20 低発現となり臨床的に抗 CD20 抗体療法に対して不応となった慢性リンパ性白血病患者から樹立された細胞株・臨床分離検体に対しても十分高い認識・細胞傷害活性を示した。

CD27 細胞内ドメインを用いた CAR を CD28 あるいは 4-1BB の細胞内ドメインを用いた CAR と比較して有用性を検討することを目的として、これまで用いてきた CD28 細胞内ドメインに替えて、4-1BB/CD27 細胞内ドメインに入れ替えたものを作成した。これらのプラスミド・ベクターを用いてレトロウイルス・ベクターを作成した。細胞内シグナルを詳細に検討するため、Reporter vector を遺伝子導入した Jurkat 細胞にこれらの CD20-CAR を遺伝子導入し、CD20 刺激後に比較検討する系を樹立した。しかしながら、刺激後に蛍光は定量的に検討できなかった。これは Jurkat および SUPT1 などの今回用いた T 細胞腫瘍細胞株では、文献的には刺激後の活性化は見られることになっているが、実際に手持ちの細胞株では刺激後の再活性化が見られなかったためと考えている。ATCC から購入した SUPT1 細胞を用いてみたが同様の結果であった。

最適と考えられる CD20-CAR の構造が決定した後、これを用いた臨床試験の準備として GMP 基準に則った細胞調製が可能かどうかについて検討することにしていたが、cell processing center (CPC) での細胞調製は臨床試験開始後にしか認められておらず、断念し

た。そのため、Large scale 培養の検討は実験室にて行った。

D. 考察

CAR の標的抗原が腫瘍組織以外に発現していると、CAR がその抗原を標的として正常組織をも攻撃することが懸念される。そのため CAR の標的抗原は腫瘍組織以外に発現がないことが極めて厳密に求められてきた。そのためになかなか新しい CAR の標的抗原の同定はこれまで困難であった。一方で、これまで抗体療法の標的としての腫瘍特異抗原の探索は広く行われてきたが、その場合には腫瘍特異性と同時にその抗原が腫瘍において高発現していることが求められてきた。こちらも同様になかなか新規に良い標的抗原は出てこなかった。

本研究の結果から、CAR は標的抗原が細胞あたり 200 分子程度発現していれば、傷害活性を示すことが出来、また 2000 分子程度発現していれば抗原刺激によって分裂・増殖などの活性化を示すことが分かった。すなわち、抗体の認識しうる範囲よりも低発現の標的でも十分認識しうることが示され、腫瘍抗原の探索範囲をこれまでよりも低発現の範囲に広げることによって新たな腫瘍抗原が得られる可能性が考えられた。そのような新しい戦略によって比較的発現の低い腫瘍抗原を CAR の標的抗原として同定出来れば、CAR の臨床応用の可能性も高まることが期待される。

現在、CAR の affinity と細胞内ドメインの最適な組み合わせについて検討を行っており、今後 CD20-CAR の最適構造が決定されれば CPC における細胞調製試験を経て臨床試験の開始を目指していく。

E. 結論

新規 CD20-CAR を作成し、T 細胞に遺伝子導入を行った。これらの CD20-CAR+ T 細胞は CD20 を特異的に認識・傷害した。これらの細胞を用いて CD20 低発現細胞株・臨床分離検体に対する反応を検討した。極めて低発現の細胞株や臨床分離検体でも認識・傷害しうることがわかった。CAR のこういった特性を生かして、低発現であるが腫瘍特異性の極めて高い標的抗原の探索という新しい戦略が考えられた。

F. 健康危険情報

特になし

G. 研究発表

1. 論文発表

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3) Keisuke Watanabe, Seitaro Terakura, Tatsunori Goto, Ryo Hanajiri, Nobuhiko Imahashi, Kazuyuki Shimada, Tetsuya Nishida, Akihiro Tomita, Makoto Murata, Tomoki Naoe. Anti-CD20 chimeric antigen receptor transduced T cells can recognize very low antigen expression: Determination of the lower threshold required to activate the CAR-Tcells. 第 55 回米国血液学会総会、New Orleans, USA, 2013

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H. 知的財産権の出願・登録状況

(予定を含む)

1. 特許取得出願

ヒト化抗 CD20 キメラ抗原レセプター 発明者：寺倉精太郎、渡邊慶介 権利者：名古屋大学 産業財産権の種類、番号：特願 2013-234784、出願年月日：2013 年 11 月 13 日

2. 実用新案登録

なし

3. その他

なし

Ⅲ. 分 担 研 究 報 告

厚生労働科学研究費補助金（第３次対がん総合戦略研究事業）
分担研究報告書

Cell processing centerにおける実際の遺伝子導入および細胞調製試験

研究分担者　村田　誠　　名古屋大学医学部附属病院　血液内科　講師

研究要旨
細胞免疫療法を臨床応用するためには、信頼性の高い細胞調製法の確立が必須である。そのため、３−６例程度の患者から分離されたT細胞に対して、実際に作成したCD20-CARを遺伝子導入し、SOPを作成するとともに、遺伝子導入効率などを検討する。実際に細胞を調製することで問題点の洗い出しを目的とした。
患者に投与する細胞しかCPCでは調製が許されておらず、患者細胞を用いた細胞調製は行わなかった。ドナー細胞を用いた細胞調製を実験室で行った。これまでの方法を踏襲し、かなり再現性よく細胞調製が行われた。今後患者細胞を用いた細胞調製を行い、さらに問題点の洗い出しを行っていくことにしている。

A. 研究目的

信頼性の高い細胞調製法の確立を目指して、CD20-CAR遺伝子導入T細胞作成のtest-runを実際のcell processing center (CPC)で行い、問題点を洗い出すことを目的とした。

B. 研究方法

治療抵抗性の悪性リンパ腫患者からインフォームドコンセントの上で末梢血を採取し、CPCにおいてCD20-CAR遺伝子導入細胞を作成する。手順の詳細を記録し、Standard Operation Procedure (SOP)として作成する。問題点があればこれらは別途記録し、対応策を講じる。

（倫理面への配慮）

厚生労働省研究の遂行にあたっては、厚生労働省臨床研究の倫理指針に従い、患者の利益を最優先し、研究実施計画書・同意説明書・同意書等を策定し、倫理審査委員会の承認を得る。

C. 研究結果

院内CPCの内規から、CPCでは実際に患者に投与する予定の細胞しか調製できないことが分かったため、通常用いている実験室でLarge scaleの細胞調製を行った。CD20-CAR遺伝子およびウイルスベクターの作成・遺伝子導入は、24穴プレートを用いるこれまで通りの方法を用いた。その後、バッグに移して培養を行った。今回は患者細胞でなく、ドナー細胞を用いたこともあり、概ね予定通りの遺伝子導入効率および培養増幅効率が得られた。今後、患者由来細胞を用いた細胞調製の実施に向けて、仮のSOPの作成を作成し、さらに研究計画作成・施設IRBへの申請を準備している。

D. 考察

今回ドナー由来の細胞を用いた細胞調製を行い、多くの問題点を知ることが出来た。細胞療法の実施に向けたCPCにおける細胞調製のtest-runは、臨床試験の実施に向けて重要な段階である。今後患者由来細胞を用いたTest-runによってさらに多くの問題点が明らかになるものと期待される。

E. 結論

CD20-CAR遺伝子導入T細胞のCPCにおける作成を目指して、CPCにおけるSOP/protocolを準備中である。

F. 研究発表

1. 論文発表

1) 本研究に関する今年度の論文は未発表

2. 学会発表

未発表

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

Ⅳ．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

書籍

著者氏名	論文タイトル名	書籍全体の編集者名	書　籍　名	出版社名	出版地	出版年	ページ
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v．研究成果の刊行物・別刷

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Randomized controlled trial comparing ciprofloxacin and cefepime in febrile neutropenic patients with hematological malignancies

Takahiko Yasuda[a], Ritsuro Suzuki[b,*], Yuichi Ishikawa[a], Seitaro Terakura[a], Yoshihiro Inamoto[a,c], Masamitsu Yanada[a,d], Hirokazu Nagai[e], Yukiyasu Ozawa[f], Kazutaka Ozeki[g], Yoshiko Atsuta[b], Nobuhiko Emi[a,d], Tomoki Naoe[a]

[a] *Department of Hematology and Oncology, Graduate School of Medicine, Nagoya University, Nagoya, Japan*
[b] *Department of HSCT Data Management & Biostatistics, Graduate School of Medicine, Nagoya University, 1-1-20 Daiko-Minami, Higashi-ku, Nagoya 461-0047, Japan*
[c] *Department of Hematology, Chukyo Hospital, Nagoya, Japan*
[d] *Department of Hematology, Fujita Health University School of Medicine, Toyoake, Japan*
[e] *Department of Hematology, National Hospital Organization, Nagoya Medical Center, Nagoya, Japan*
[f] *Department of Hematology, Japanese Red Cross, Nagoya First Hospital, Nagoya, Japan*
[g] *Department of Hematology, Konan Kosei Hospital, Konan, Japan*

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SUMMARY

Background: Ciprofloxacin (CPFX) is a potential alternative in patients with febrile neutropenia (FN) because of its activity against Gram-negative organisms. We conducted a non-inferiority, open-label, randomized controlled trial comparing intravenous CPFX and cefepime (CFPM) for FN patients with hematological malignancies.

Methods: Patients aged from 15 to 79 years with an absolute neutrophil count of $<0.500 \times 10^{9}$/l were eligible, and were randomized to receive 300 mg of CPFX or 2 g of CFPM every 12 h. Initial treatment efficacy, overall response, and early toxicity were evaluated.

Results: Fifty-one episodes were included in this trial, and 49 episodes (CPFX vs. CFPM: 24 vs. 25) were evaluated. Treatment efficacy at day 7 was significantly higher in the CFPM group (successful clinical response: nine with CPFX and 19 with CFPM; $p = 0.007$). The response was better in high-risk patients with neutrophil counts of $\leq 0.100 \times 10^{9}$/l ($p = 0.003$). The overall response during the study period was similar between the CPFX and CFPM groups ($p = 0.64$). Adverse events were minimal, and all patients could continue the treatment.

Conclusions: We could not prove the non-inferiority of CPFX in comparison with CFPM for the initial treatment of FN. CFPM remains the standard treatment of choice for FN.

1. Introduction

The goal of initial empiric antibiotic therapy for febrile neutropenia (FN) with hematologic malignancies is to prevent serious morbidity and mortality due to bacterial pathogens, until the results of blood cultures are available to guide more precise antibiotic choices. Although Gram-positive bacteria have increased as pathogens in FN during the past 20 years, Gram-negative bacteria are associated with a greater mortality.[1] In particular, *Pseudomonas aeruginosa* infection is associated with a higher mortality,[2] and coverage of this organism remains an essential component of the initial empiric antibiotic regimen. A commonly used therapy for FN is a combination of β-lactam antibiotic and aminoglycoside, which offers a broad spectrum of initial coverage, including *P. aeruginosa*.[3,4]

Although combination therapy with a β-lactam antibiotic and an aminoglycoside has been reported to be highly effective for neutropenic patients,[3,4] aminoglycosides have some serious adverse effects such as renal dysfunction and ototoxicity. Antibiotics as monotherapy are generally less toxic, less costly, and more convenient to administer to patients than combination therapy,[5] so monotherapy with a fourth-generation cephem or carbapenem has been applied and compared to combination therapy in randomized controlled trials; these did not show diminished effectiveness of monotherapy.[6–9] Monotherapy is now also recommended as standard therapy in the Infectious Diseases Society of America (IDSA) guidelines 2010.[10]

However, the incidence of drug-resistant bacterial species in the institute should be taken into consideration when using monotherapy, because resistant bacteria would tend to result in

* Corresponding author. Tel.: +81 52 719 1974; fax: +81 52 719 1973.
E-mail address: r-suzuki@med.nagoya-u.ac.jp (R. Suzuki).

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treatment failure in the case of monotherapy compared with combination therapy.[5] In fact, extended-spectrum β-lactamase (ESBL)- and metallo-β-lactamase-producing Gram-negative bacteria are emerging at an increasing rate, and these cause significant mortality.[11–13] In this context, alternative effective regimens other than β-lactams are warranted for neutropenic patients to overcome the resistant bacteria.

Ciprofloxacin (CPFX) is an attractive drug that has wide coverage against Gram-negative organisms including *P. aeruginosa*, good pharmacokinetic characteristics, and an absence of the need for drug level monitoring.[14,15] A number of studies have demonstrated that CPFX combined with a β-lactam is effective for neutropenic patients.[16–18] Furthermore, CPFX inhibits DNA gyrase of prokaryotic organisms,[14] and the drug mechanism is completely different from that of β-lactams. Therefore, CPFX may be active for some organisms resistant to β-lactams and it would be acceptable for those who are allergic to β-lactams.[19] In this context, CPFX is a potential alternative for the empiric treatment of patients with FN. However, monotherapy with CPFX has been less well reported and is not well established in the treatment of FN patients.

To assess the possibility of increasing the choice of initial treatment for FN, we designed a randomized controlled trial of intravenous CPFX vs. cefepime (CFPM) in FN patients. This trial aimed to prove its non-inferiority compared to CFPM, a standard therapy for FN.

2. Materials and methods

From January 2005 to December 2009, a non-inferiority, open-label, randomized, multicenter trial was conducted to evaluate the efficacy of intravenous CPFX for FN. The study was approved by both the protocol committee and the institutional review board of each institution. Informed consent was obtained from all patients before registration in this study. The study was registered in the University Hospital Medical Information Network Clinical Trials Registry (UMIN-CTR number: UMIN C000000083) and at ClinicalTrials.gov (identifier: NCT00137787). Randomization was performed automatically, stratified by primary disease and balanced in each institute, at the time of enrollment, on a website operated by the Center for Supporting Hematology-Oncology Trials (C-SHOT) data center.

3. Definitions

Fever was defined as an axillary temperature of not less than 38 °C, or of 37.5–38 °C sustained for more than 1 h. Resolution of fever was defined as a maximum temperature of less than 37.5 °C sustained for three successive days, and the first day was defined as the date the fever disappeared. Fever was considered to be worse when at least one of the following criteria was met: more than 1 °C elevation in maximum body temperature, change from remittent fever to continued fever, emergence of new infectious foci, blood culture positivity after administration of antibiotics, more than 10% fall in arterial O_2 pressure or oxygen saturation, and a decline of performance status.

Episodes of fever were classified as microbiologically documented infection, clinically documented infection, or fever of unknown origin (FUO). Microbiologically documented infection was defined as the isolation of microorganisms. Clinically documented infection was considered when there were foci of infection on physical examination or clinical data, without microbiological documentation. FUO was considered when there was no clinical or microbiological evidence of infection in a febrile episode.

Neutropenia was defined as an absolute neutrophil count (ANC) of $<0.500 \times 10^9$/l or that from 0.500×10^9/l to 0.100×10^9/l showing a decline compared with the level at the last examination. Recovery of neutropenia was defined as an ANC of $\geq 0.500 \times 10^9$/l sustained for 24 h after ANC had dropped to $<0.500 \times 10^9$/l. The first day was considered to be the recovery date.

3.1. Patients

Patients had to meet all of the following criteria for inclusion in the study: age 15–79 years, at least one episode of fever, neutropenia within 72 h, total bilirubin of 2.0 times the upper limit of normal (ULN) or less, creatinine of 1.5 times ULN or less, and giving informed consent. Patients were excluded if they had a history of allergic reaction to antibiotics, HIV infection, were pregnant or lactating, had a family history of deafness, had received antibiotics in the last 14 days, had received an antifungal or antiviral agent, ketoprofen, or sodium valproate, were infected with bacteria resistant to agents used in this study, were in septic shock, or other inappropriate cases as judged by a physician. If the ANC did not recover to $\geq 1.000 \times 10^9$/l after the last episode of fever, the patient was also ineligible for this study.

3.2. Treatment

Patients received 300 mg of CPFX or 2 g of CFPM intravenously every 12 h immediately upon the development of FN. Treatment was continued until patients met the criteria for treatment discontinuation as follows: fever absent for more than 48 h (ANC of $\geq 0.500 \times 10^9$/l) or for more than 5 days (ANC from 0.100×10^9/l to 0.500×10^9/l) without any symptoms. If the associated symptoms worsened or were sustained during the study period, the treatment was modified according to the study protocol (Figure 1). From 72 h to 120 h after the study started, an aminoglycoside was added to the treatment if fever symptoms worsened. From 120 h to 168 h, the initial antibiotic was discontinued and the combination therapy of carbapenem (meropenem or imipenem), aminoglycoside, and antifungal agents was started. After 168 h, patients were allowed to receive any treatment as required if fever persisted. Patients could receive granulocyte colony-stimulating factor, if required, at any time.

3.3. Clinical and laboratory evaluations

Clinical symptoms were monitored daily. Blood cell counts were obtained at least twice a week, and biochemical parameters were measured at least once a week. Blood culture, serum endotoxin, β-D-glucan, and chest radiographs were obtained before starting antibacterial therapy and in the case of a sustained or worsened pattern of fever.

3.4. Response criteria

The primary endpoint of this study was the rate of the initial treatment success at day 7. Response to treatment at day 7 was divided into four groups as follows: very effective: fever disappeared with a temperature below 37.5 °C within 4 days and an afebrile state remained for more than 3 days; effective: maximum temperature decreased 1 °C or more within 4 days and an afebrile (below 37.5 °C) state persisted for 7 days; partial response: maximum temperature decreased 1 °C or more within 7 days accompanied by the improvement of clinical symptoms; not effective: maximum temperature did not decrease by 1 °C or more within 7 days and/or no improvement of febrile symptoms. The response to treatment was categorized as a success if patients were

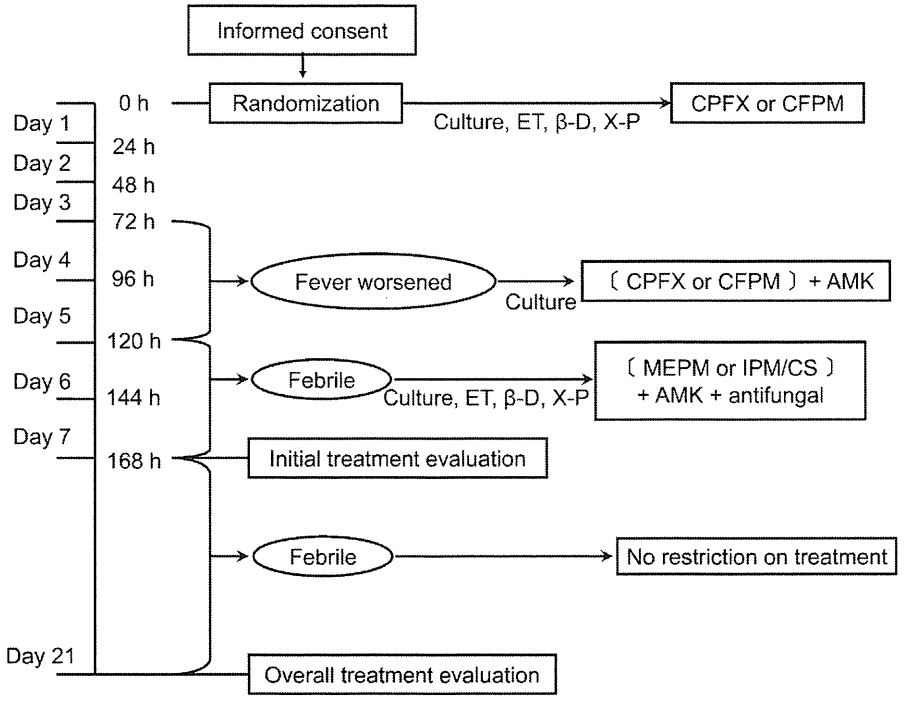

Figure 1. Treatment algorithm for febrile neutropenia. For febrile neutropenia, we treated patients according to the treatment algorithm. Treatment evaluation and treatment modification were performed as shown (CPFX, ciprofloxacin; CFPM, cefepime; ET, endotoxin; β-D, β-D-glucan; X-P, X-ray picture; AMK, aminoglycoside; MEPM, meropenem; IPM/CS, imipenem/cilastatin).

included in either the very effective group or the effective group at day 7.

We also evaluated the overall response rate at day 21 as a secondary endpoint. If patients were able to discontinue the treatment according to the criteria described above, it was considered to be successful.

3.5. Adverse events

Adverse events, regardless of whether they appeared to be related to the use of the study medication, were carefully recorded throughout the study. Causal relationships between the study drugs and adverse events were analyzed using six stages: definitive, probable, possible, unlikely, not related, and not assessable. Adverse events were considered related to the study drug if the stage was definitive, probable, or possible. The severity of the adverse events was classified according to the National Cancer Institute Common Toxicity Criteria version 2.0 (http://ctep.cancer.gov/).

3.6. Statistical analysis

Percentages of comparability of the treatment arms, treatment response, and treatment modification were compared by Chi-square test or Fisher's exact test. Quantitative variables were analyzed by Mann–Whitney test.

The success rates of the CFPM and CPFX arms were estimated to be 50% and 60%, respectively.[7] The δ value of non-inferiority was set to be −15% in accordance with previous reports. The CFPM arm was the reference. To prove the non-inferiority of the CPFX arm, the lower limit of the 95% confidence interval (CI) of the difference of efficacy should exceed the δ value. With a statistical power of 90% and a one-sided type I error of 2.5%, the number of patients required for this study was calculated to be 82 in each arm using a binominal analysis method. Therefore, the total number of accrual was planned to be 100 patients in each arm.

4. Results

4.1. Characteristics of the study population

From January 2005 to December 2009, 51 patients were registered from seven participating institutes in Japan. Forty-nine patients (24 in the CPFX arm and 25 in the CFPM arm) were eligible for assessment, but two patients were excluded because they did not meet the inclusion criteria. Ten patients were enrolled in the study more than once via different episodes of FN. Although we planned to include 200 patients, this study was closed in December 2009 due to slow accrual.

The clinical characteristics of the patients in both treatment arms are listed in Table 1. The distribution of patient sex, diagnosis, treatment for primary disease, neutrophil count at randomization, and duration of neutropenia did not differ between the arms. Acute leukemia was the most common disease in this study (55.1%). Patient age was younger in the CPFX arm than in the CFPM arm (median age 53 vs. 61 years; $p = 0.02$). Four patients were excluded from further analysis of the duration of neutropenia because their neutrophil counts did not exceed $0.500 \times 10^{9}/l$ ($n = 3$), or their neutrophil counts did not drop below $0.500 \times 10^{9}/l$ ($n = 1$).

4.2. Type of infection and microbiological outcomes

Of 49 episodes, the responsible bacterium was identified in 11 (22.4%). A Gram-positive coccus was cultured in eight episodes, consisting of one each of methicillin-sensitive *Staphylococcus aureus*, *Staphylococcus haemolyticus*, and *Staphylococcus epidermidis*, and five *Streptococcus* species (Table 2). A Gram-negative bacillus was isolated in three episodes: one each for *P. aeruginosa*, *Klebsiella pneumoniae*, and Pasteurella (Table 2). Ten of the 11 episodes were diagnosed with sepsis and one with meningitis. The other two clinically documented episodes were diagnosed with pneumonia and peritonitis, but no responsible organisms were identified.

Table 1
Characteristics of patients enrolled in the study

Characteristic	CPFX (n=24)	CFPM (n=25)	p-Value
Patient sex			
Male	16 (67%)	14 (56%)	0.44
Female	8 (33%)	11 (44%)	
Patient age			
Median	53	61	0.02
Range	21–65	21–79	
Diagnosis			
AML	9 (38%)	11 (44%)	0.72
ALL	4 (17%)	3 (12%)	
CML	3 (13%)	2 (8%)	
MDS	1 (4%)	0	
ML	5 (21%)	5 (20%)	
MM	1 (4%)	3 (12%)	
ATLL	0	1 (4%)	
Myeloid sarcoma	1 (4%)	0	
Treatment for primary disease			
HSCT	0	1 (4%)	0.32
Chemotherapy	24 (100%)	24 (96%)	
Neutrophil count at start of study			
$<0.100 \times 10^9$/l	15 (63%)	18 (72%)	0.19
$0.100–0.500 \times 10^9$/l	6 (25%)	7 (28%)	
$0.501–1.000 \times 10^9$/l	3 (13%)	0	
Duration of neutropenia			
≤7 days	5 (24%)	7 (29%)	0.75
>7days	16 (76%)	17 (71%)	

CPFX, ciprofloxacin; CFPM, cefepime; AML, acute myeloid leukemia; ALL, acute lymphoblastic leukemia; CML, chronic myelogenous leukemia; MDS, myelodysplastic syndrome; ML, malignant lymphoma; MM, multiple myeloma; ATLL, adult T-cell leukemia/lymphoma; HSCT, hematopoietic stem cell transplantation.

4.3. Treatment modification

Ten patients (41.7%) treated with CPFX and 15 patients (60.0%) treated with CFPM received the same treatment without modification (Table 3). For the patients who were judged as febrile by physicians, treatment modifications were performed according to the algorithm described in Figure 1. In the CPFX arm, an aminoglycoside was added to the treatment regimen for 13 patients (54.2%) and CPFX was replaced by other antibiotics for 10 patients (41.7%). In the CFPM arm, an aminoglycoside was added for eight patients (32.0%) and CFPM was replaced for five patients (20.0%). Vancomycin was added for four patients (16.7%) in the CPFX arm, but not in the CFPM arm.

4.4. Efficacy of CPFX and CFPM

The treatment was effective in nine patients (37.5%) in the CPFX arm and 19 (76.0%) in the CFPM arm at day 7 (Figure 2). The difference of the effective proportion was −38.5% (95% CI −64% to −13%), and the lower limit (−64%) did not exceed the δ value of −15%. Furthermore, the efficacy was significantly lower in the CPFX arm (p = 0.007). However, the overall efficacy at day 21 was similar between the two arms (CPFX 83.3% vs. CFPM 88.0%, p = 0.64). Patients for whom treatment failed were rescued by treatment modification.

Table 2
Microbiological blood culture results on day 0

Infecting microorganisms	CPFX (n=24)	CFPM (n=25)
Gram-positive organisms	5 (21%)	3 (12%)
Coagulase-positive Staphylococcus	1 (4%)	-
Coagulase-negative Staphylococcus	2 (8%)	-
Streptococcus	2 (8%)	3 (12%)
Gram-negative organisms	-	3 (12%)
Pseudomonas aeruginosa	-	1 (4%)
Klebsiella pneumoniae	-	1 (4%)
Pasteurella species	-	1 (4%)

CPFX, ciprofloxacin; CFPM, cefepime.

Table 3
Treatment modification

Treatment modification	CPFX (n=24)	CFPM (n=25)	p-Value
Initial treatment continued	10 (42%)	15 (60%)	0.20
Modification			
Initial treatment replaced	10 (42%)	5 (20%)	0.10
Add aminoglycoside	13 (54%)	8 (32%)	0.12
Add vancomycin	4 (17%)	0	0.05
Add antifungal agents	8 (33%)	7 (28%)	0.69
Add antiviral agents	2 (8%)	1 (4%)	0.61

CPFX, ciprofloxacin; CFPM, cefepime.

For patients from whom the responsible bacteria were isolated, a treatment response at day 7 was achieved in 20.0% in the CPFX arm and 66.7% in the CFPM arm (p = 0.12, Table 4). Gram-positive coccus infection (16.3%) was more common than Gram-negative bacillus infection (6.1%). The efficacy was better in the CFPM arm (66.7%) than in the CPFX arm (20.0%), but the difference was not statistically significant (p = 0.12). For patients retaining FUO, a treatment response was achieved in 47.1% of patients in the CPFX arm and 78.9% of patients in the CFPM arm (p = 0.05, Table 4).

Since patients with prolonged neutropenia of more than 7 days or profound neutropenia (ANC of $\leq 0.100 \times 10^9$/l) are regarded as at high risk in the IDSA guidelines 2010,[10] a subgroup analysis of this population was also conducted. Fewer patients in the CPFX arm than in the CFPM arm had a good clinical response (Table 4).

4.5. Adverse events

Table 5 shows all adverse events within 21 days in both arms. Six events in the CPFX arm compared to two in the CFPM arm were associated with the drug. The most common toxicity was liver dysfunction (16.7% in the CPFX arm and 8.0% in the CFPM arm). Two severe adverse events of grade 3 were observed in the CPFX arm (liver dysfunction and skin rash), and one event in the CFPM arm (liver dysfunction). All patients could continue the study medication without cessation of the therapy due to adverse events.

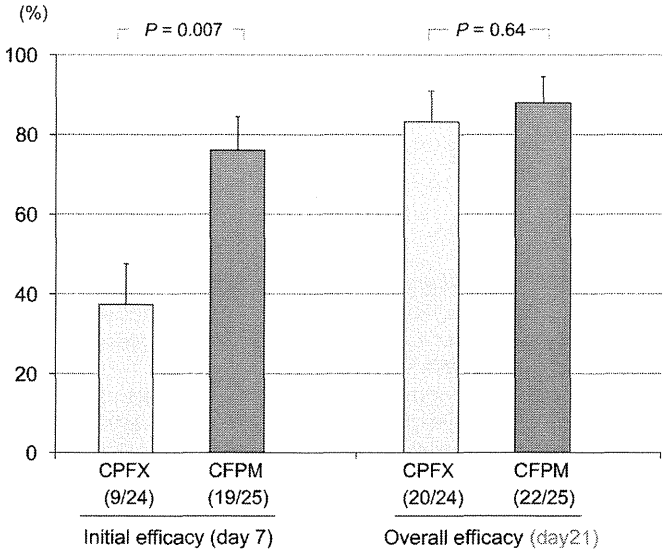

Figure 2. Clinical efficacy of ciprofloxacin and cefepime. Initial treatment evaluation showed a significantly better response in the CFPM arm than in the CPFX arm (76.0% vs. 37.5%, p = 0.007). Overall, treatment evaluation showed almost the same efficacy between the two arms (CPFX, ciprofloxacin; CFPM, cefepime).