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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24年度 総括・分担研究報告書

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## 平成24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 24 年度名簿

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## II. 総括研究報告

## 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 発現が落ち、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。今後 *in vivo* でも CD20 低発現株を有効に認識しうるかどうか検討する予定である。

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。今後細胞内に伝わるシグナルの異同について定性的・定量的評価を加える予定である。評価系として腫瘍細胞株に蛍光レポーター・ベクターを発現させて用いるため、遺伝子導入を行った。

#### 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

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#### 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

新規ヒト化抗CD20抗体の可変領域を細胞外ドメインとするCARを分子生物学的手法を用いて作成した。作成したCD20-CARをレトロウイルス作成用のプラスミド・ベクターに組み込み、レトロウイルス・ベクターを作成した。作成したレトロウイルス・ベクターを用いてドナー末梢血T細胞にCD20-CARを遺伝子導入し、CD20-CAR+ T細胞を作成した。さらにCD8陽性細胞あるいはCD4陽性細胞に純化して実験に用いた。種々のレベルのCD20を発現するCD20-CEM細胞を標的としてクロム放出試験を行った。また、本学にて富田らが樹立・報告しているCD20低発現細胞株およびCD20低発現臨床分離細胞を標的としてクロム放出試験や細胞内インターフェロン $\gamma$ 染色にて、CD20-CAR+ T細胞が標的をCD20特異的に認識・傷害するかどうかについて検討した。細胞上清に含まれるインターフェロン $\gamma$ やIL-2はELISAを用いて測定した。

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ている。これにより、より定量的にシグナル伝達を評価できる。

(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。CD20-CAR+ T 細胞は極めて低い CD20 発現を示す CD20+ CEM 細胞に対しても高い細胞傷害活性を示した。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患者から樹立された細胞株・臨床分離検体に対しても比較的高い認識・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。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ている。

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、CAR 遺伝子の最適化の途中であり、本学 CPC を用いた Large scale 培養の検討には至っていない。

## 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を示され、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。

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・傷害しうるということがわかった。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

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of CD19-chimeric antigen receptor modified CD8+ T cells derived from virus-specific central memory T cells. Blood. 119(1): 72-82, 2012.

<和文>

なし

## 2. 学会発表

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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

なし

### 2. 実用新案登録

なし

### 3. その他

なし

### Ⅲ. 分 担 研 究 報 告

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

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### 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

CD20-CAR 遺伝子およびウイルスベクターの作成は概ね予定通り進捗しているが、もともと CPC における細胞調製は2年目に予定していたこともあり、これまでのところ CPC における細胞調製は行っていない。H25 年度中の CPC における test-run の実施に

向けて、仮の SOP の作成、および研究計画、施設 IRB への申請を準備している。

### D. 考察

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。Test-run によって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 test-run 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
なし							

雑誌

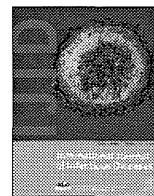
発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
Yasuda T, Suzuki R, Ishikawa Y, <u>Terakura S</u> , <u>Naoe T</u> , et al.	Randomized controlled trial comparing ciprofloxacin and cefepime in febrile neutropenic patients with hematological malignancies.	Int J Infect Dis.	17(6)	e385-390	2013
Kanda J, Ichinohe T, Kato S, Uchida N, <u>Terakura S</u> , et al.	Unrelated cord blood transplantation vs related transplantation with HLA 1-antigen mismatch in the graft-versus-host direction.	Leukemia	27(2)	286-294	2013
Kuwatsuka Y, Kohno A, <u>Terakura S</u> , <u>Naoe T</u> , et al.	Phase II study of dose-modified busulfan by real-time targeting in allogeneic hematopoietic stem cell transplantation for myeloid malignancy.	Cancer Sci.	103(9)	1688-1694	2012
Kato T, <u>Terakura S</u> , <u>Naoe T</u> , et al.	Escape of leukemia blasts from HLA-specific CTL pressure in a recipient of HLA one locus-mismatched bone marrow transplantation.	Cell Immunol.	276(1-2)	75-82	2012
<u>Terakura S</u> , et al.	Generation of CD19-chimeric antigen receptor modified CD8+ T cells derived from virus-specific central memory T cells.	Blood	119(1)	72-82	2012

## 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

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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.

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### 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.<sup>1</sup> In particular, *Pseudomonas aeruginosa* infection is associated with a higher mortality,<sup>2</sup> 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  $\beta$ -lactam antibiotic and

aminoglycoside, which offers a broad spectrum of initial coverage, including *P. aeruginosa*.<sup>3,4</sup>

Although combination therapy with a  $\beta$ -lactam antibiotic and an aminoglycoside has been reported to be highly effective for neutropenic patients,<sup>3,4</sup> 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,<sup>5</sup> 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.<sup>6–9</sup> Monotherapy is now also recommended as standard therapy in the Infectious Diseases Society of America (IDSA) guidelines 2010.<sup>10</sup>

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

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treatment failure in the case of monotherapy compared with combination therapy.<sup>5</sup> In fact, extended-spectrum  $\beta$ -lactamase (ESBL)- and metallo- $\beta$ -lactamase-producing Gram-negative bacteria are emerging at an increasing rate, and these cause significant mortality.<sup>11–13</sup> In this context, alternative effective regimens other than  $\beta$ -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.<sup>14,15</sup> A number of studies have demonstrated that CPFX combined with a  $\beta$ -lactam is effective for neutropenic patients.<sup>16–18</sup> Furthermore, CPFX inhibits DNA gyrase of prokaryotic organisms,<sup>14</sup> and the drug mechanism is completely different from that of  $\beta$ -lactams. Therefore, CPFX may be active for some organisms resistant to  $\beta$ -lactams and it would be acceptable for those who are allergic to  $\beta$ -lactams.<sup>19</sup> 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<sub>2</sub> 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  $0.500 \times 10^9/l$  to

$0.100 \times 10^9/l$  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

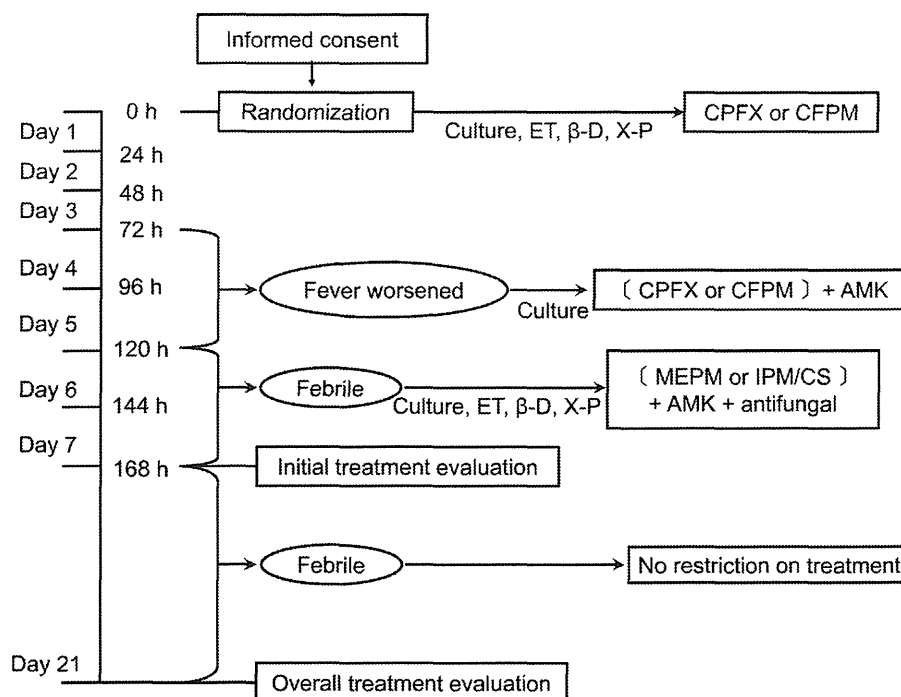
Patients received 300 mg of CPFX or 2 g of CFPM intravenously every 12 h immediately upon the development of FN. Treatment was continued until patients met the criteria for treatment discontinuation as follows: fever absent for more than 48 h (ANC of  $\geq 0.500 \times 10^9/l$ ) or for more than 5 days (ANC from  $0.100 \times 10^9/l$  to  $0.500 \times 10^9/l$ ) 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,  $\beta$ -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.<sup>7</sup> The  $\delta$  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  $\delta$  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 binomial 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 × 10 <sup>9</sup> /l	15 (63%)	18 (72%)	0.19
0.100–0.500 × 10 <sup>9</sup> /l	6 (25%)	7 (28%)	
0.501–1.000 × 10 <sup>9</sup> /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  $\delta$  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%,

**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%)
<i>Pseudomonas aeruginosa</i>	-	1 (4%)
<i>Klebsiella pneumoniae</i>	-	1 (4%)
<i>Pasteurella</i> 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.

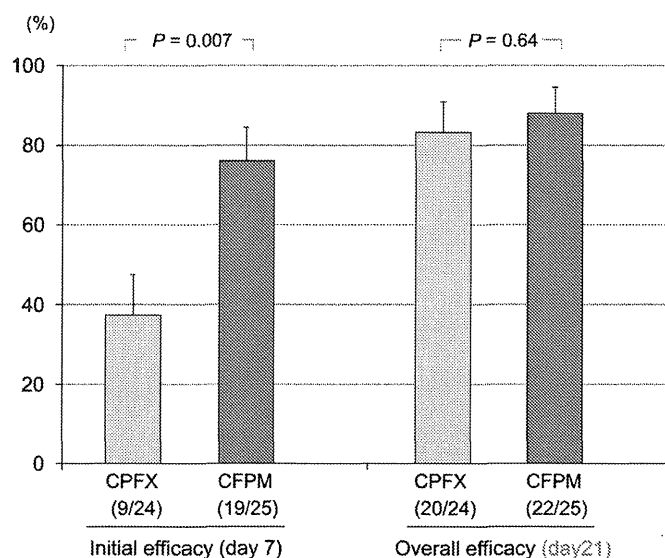
$p = 0.64$ ). Patients for whom treatment failed were rescued by treatment modification.

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,<sup>10</sup> 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).

**Table 4**  
Response at day 7 by the cause of fever and severity of neutropenia

	CPFX	CFPM	p-Value
Cause of fever			
Microbiologically documented infection	1/5 (20%)	4/6 (67%)	0.12
Sepsis	1/4 (25%)	4/6 (67%)	
Meningitis	0/1	-	
Clinically documented infection (pneumonia and peritonitis)	0/2	-	-
Unknown origin	8/17 (47%)	15/19 (79%)	0.05
Duration of neutropenia <sup>a</sup>			
>7 days	5/16 (31%)	13/17 (77%)	0.02
≤7 days	3/5 (60%)	5/7 (71%)	0.68
Baseline neutrophil count			
≤0.100 × 10 <sup>9</sup> /l	5/15 (33%)	15/18 (83%)	0.003
>0.100 × 10 <sup>9</sup> /l	4/9 (44%)	4/7 (57%)	0.61

CPFX, ciprofloxacin; CFPM, cefepime.

<sup>a</sup> Four patients were excluded from the analysis because the neutrophil count did not recover to 0.500 × 10<sup>9</sup>/l (n=3), or the neutrophil count did not drop to <0.500 × 10<sup>9</sup>/l (n=1).

## 5. Discussion

The efficacy and safety of CPFX monotherapy for neutropenic patients has not been well investigated. One study showed that patients treated with CPFX had a significantly lower overall success rate than those treated with piperacillin plus amikacin.<sup>20</sup> In contrast, another study comparing CPFX monotherapy with β-lactam plus aminoglycoside showed that the response rate was similar.<sup>19</sup> Furthermore, a prospective randomized study comparing ceftazidime and CPFX as initial therapy also demonstrated that the levels of efficacy were equal.<sup>21</sup> These results imply that the role of CPFX monotherapy for FN has been controversial and needs further assessment because β-lactam-resistant organisms are on the increase.

In this randomized, controlled, open-label trial, we could not prove the non-inferiority of CPFX monotherapy compared with

**Table 5**  
Adverse events within 21 days

Adverse events <sup>b</sup>	Grade	Number of patients in CPFX group (n=24)	Number of patients in CFPM group (n=26)
Cardiovascular	1	0	2
Gastrointestinal	1	2	3
	2	1	1
	3	2	1
	4	1	0
Liver	1	5 (2) <sup>a</sup>	10 (1) <sup>a</sup>
	2	4 (2) <sup>a</sup>	0
	3	1 (1) <sup>a</sup>	1 (1) <sup>a</sup>
Renal	1	1	0
Blood sugar	1	2	3
	2	2	0
Electrolytes	1	3	4
	2	1	1
	3	2	0
Neurological	1	1	1
	2	0	1
	3	0	0
	3	1	0
Cutaneous	1	0	1
	2	2	1
	3	1 (1) <sup>a</sup>	0

CPFX, ciprofloxacin; CFPM, cefepime.

<sup>a</sup> Numbers in the parenthesis indicate the drug-associated events (judged as 'possible' or more).<sup>b</sup> Adverse events grade is based on the National Cancer Institute (NCI) Common Toxicity Criteria version 2.0.

CFPM. One of the possible reasons for this is that, although CPFX has strong activity against Gram-negative rods, the coverage and activity for Gram-positive cocci including viridans were insufficient.<sup>22,23</sup> In fact, our microbiological data show that the treatment success rate for Gram-positive organisms tended to be inferior in the CPFX arm, and the use of vancomycin was applied only in the CPFX arm.

Another possible reason is that the blood concentration of CPFX might not be adequate because CPFX was administered at a dose of 600 mg/day in this study, a dose that is allowed under the health insurance system in Japan. A recent study demonstrated that only high-dose CPFX (regimens of 400 mg every 8 h or 400 mg every 12 h) can provide good coverage for pathogens with a minimum inhibitory concentration (MIC) of 0.5 μg/ml.<sup>24</sup> This was also confirmed by previous clinical studies, in which monotherapy with CPFX at a low dose (400 mg/day) was not comparable to the standard therapies, but CPFX at a relatively high dose (600 mg/day) was equally effective.<sup>19,20,25</sup> A precise pharmacokinetic study and the provision of an appropriate concentration of CPFX might have led to a better response for FN.<sup>26</sup>

Previous studies have demonstrated that various therapies of CPFX combined with β-lactams such as benzylpenicillin,<sup>27</sup> teicoplanin,<sup>28</sup> and azlocillin,<sup>29</sup> are comparable with the standard therapy for neutropenic patients. In a meta-analysis comparing CPFX plus β-lactam and aminoglycoside plus β-lactam, the former showed better outcomes.<sup>17</sup> Furthermore, CPFX plus β-lactam is reported to be less toxic in terms of nephro- and oto-toxicities.<sup>30</sup> These results suggest that the combination of CPFX with a β-lactam may be a valuable alternative to the more commonly used aminoglycoside plus β-lactam combination in the management of FN.

Assessment of the risk of complications in severe infection is important to determine the type of empiric antibiotic therapy (oral vs. intravenous), the venue for treatment (inpatient vs. outpatient), and the duration of antibiotic therapy.<sup>10</sup> The IDSA guidelines have demonstrated that monotherapy with oral CPFX is acceptable for low-risk patients.<sup>10,31</sup> On the other hand, the guidelines do not recommend monotherapy with CPFX as standard therapy for high-risk patients.<sup>10</sup> We further tried to assess the link between initial treatment response and risk status using both the duration of neutropenia and the neutrophil count as simple biomarkers.<sup>7</sup> Our subgroup analysis showed that among patients at a 'high risk' of neutropenia, those who received CPFX had significantly lower response rates at day 7. In contrast, no significant difference was found for low-risk patients. These results suggest that CPFX monotherapy might be applicable for low-risk FN.

In terms of safety, the two agents appear to have similar safety profiles. The most common adverse event possibly related to the therapies was liver dysfunction, and all the patients could continue therapy.

This trial was prematurely terminated due to slow patient accrual, but not by the predefined early stopping-rule of superiority of CFPM. Prophylactic oral CPFX was not allowed in this study, which might have hindered the accrual. The significance of prophylactic CPFX has been legitimized in recent years.<sup>10</sup> Another possible reason is that this was an open-label randomized controlled trial. Since physicians were able to observe the efficacy of the allocated agents, their impressions might have influenced the slow accrual. Furthermore, the randomization procedure was cumbersome for physicians because fever could occur at any time.

In conclusion, we could not verify the non-inferiority of monotherapy with CPFX to that with CFPM at day 7, although the overall response was similar in both arms. When selecting monotherapy for the treatment of neutropenic patients, CFPM remains the standard initial treatment of choice. CPFX is better for prophylactic than empiric use.