

平成 28 年度 委託研究開発成果報告書

I. 基本情報

事業名： (日本語) 免疫アレルギー疾患等実用化研究事業 (移植医療技術開発研究分野)
(英語) Practical Research Project for Allergic Diseases and Immunology
(Research on Technology of Medical Transplantation)

研究開発課題名： (日本語) HLA 不適合血縁者間移植の治療成績を向上し、造血器疾患治療における位置づけを明らかにするための研究
(英語) Hematopoietic stem cell transplantation from an HLA-mismatched related donor: how to improve the outcome and how to select appropriate patients.

研究開発担当者 (日本語) 学校法人 自治医科大学 医学部教授 神田 善伸
所属 役職 氏名： (英語) Division of Hematology, Jichi Medical University, Professor, Yoshinobu Kanda

実施期間： 平成 28 年 4 月 1 日 ～ 平成 29 年 3 月 31 日

分担研究 (日本語) 1. アレムツズマブを用いた HLA 不適合移植の開発
開発課題名： 2. 腫瘍・感染症特異的免疫の研究
3. ATG を用いた HLA 一抗原不適合移植の臨床試験の実施
4. 班研究の統括
(英語) 1. Investigation of haploidentical stem cell transplantation using alemtuzumab
2. Research on tumor-specific and infectious pathogen-specific immunity
3. Clinical study of one antigen mismatched transplantation using ATG
4. Coordination of the whole research project

研究開発分担者 (日本語) 学校法人 自治医科大学 医学部教授 神田 善伸
所属 役職 氏名： (英語) Division of Hematology, Jichi Medical University, Professor, Yoshinobu Kanda

分担研究 (日本語) 1. 低容量 ATG とステロイド剤を用いた HLA 不適合移植の開発
開発課題名 : 2. ホスト由来制御性 T 細胞と樹状細胞の解析
(英語) 1. HLA-haploidentical stem cell transplantation using reduced-intensity conditioning and low dose ATG
2. Analysis of host regulatory T cells and dendritic cells in GVHD

研究開発分担者 (日本語) 学校法人 兵庫医科大学 血液内科教授 小川 啓恭
所属 役職 氏名 : (英語) Division of Hematology, Department of Internal Medicine, Hyogo College of Medicine, Professor and Chairman, Hiroyasu Ogawa

分担研究 (日本語) 造血細胞移植における免疫応答解析
開発課題名 : (英語) Analysis of immunological response during hematopoietic cell transplantation

研究開発分担者 (日本語) 学校法人 東京女子医科大学 血液内科学講座 主任教授 田中 淳司
所属 役職 氏名 : (英語) Department of Hematology, Tokyo Women's Medical University, Professor & Chairman, Junji Tanaka

分担研究 (日本語) ハプロ一致移植における感染症、GVHD 制御の検討
開発課題名 : (英語) Development of cell therapies to improve outcome of HLA haploidentical transplantation

研究開発分担者 (日本語) 国立大学法人 名古屋大学 大学院医学系研究科小児科学 教授 高橋 義行
所属 役職 氏名 : (英語) Department of Pediatrics, Nagoya Graduate School of Medicine, Professor and Chairman, Yoshiyuki Takahashi

分担研究 (日本語) マウスモデルを使った HLA 不適合移植後の免疫寛容の誘導に関する検討
開発課題名 : (英語) Experimental study of the mechanism of tolerance induction in a mouse HLA mismatched allogeneic stem cell transplantation model

研究開発分担者 (日本語) 岡山大学病院 血液・腫瘍内科 講師 前田 嘉信
所属 役職 氏名 : (英語) Department of hematology and oncology, Okayama University hospital, Senior assistant professor, Yoshinobu Maeda

分担研究 (日本語) 臨床研究デザインと臨床データ活用方法に関する調査
開発課題名 : (英語) Investigating trial designs and data usage for clinical researches

研究開発分担者 (日本語) 京都大学大学院医学研究科医学統計生物情報学 教授 森田 智視
所属 役職 氏名 : (英語) Department of Biomedical Statistics and Bioinformatics, Kyoto University Graduate School of Medicine, Professor, Satoshi Morita

分担研究 (日本語) レジストリーデータの統計解析
開発課題名: (英語) Statistical analysis for the registry data

研究開発分担者 (日本語) 一般社団法人 日本造血細胞移植データセンター センター長 熱田 由子
所属 役職 氏名: (英語) The Japanese Data Center for Hematopoietic Cell Transplantation,
Scientific Director, Yoshiko Atsuta

分担研究 (日本語) 造血幹細胞移植後にシクロフォスファミドを用いた HLA 半合致移植に関する研究
開発課題名: (英語) Haploidentical transplantation using post-transplant cyclophosphamide

研究開発分担者 (日本語) 国立大学法人 筑波大学 医学医療系血液内科 講師 栗田 尚樹
所属 役職 氏名: (英語) Department of Hematology, Faculty of Medicine, University of Tsukuba
Assistant Professor, Naoki Kurita

分担研究 (日本語) 低用量アレムツズマブを用いた進行期造血器腫瘍に対する HLA 不適合同種造血
開発課題名: 幹細胞移植の有効性の検討
(英語) HLA-mismatched haploidentical transplantation using low-dose
alemtuzumab for hematological disease

研究開発分担者 (日本語) 学校法人 自治医科大学 准教授 賀古 真一
所属 役職 氏名: (英語) Jichi Medical University, associate professor,
Shinichi Kako

分担研究 (日本語) HLA 不適合移植の移植前処置の至適化
開発課題名: (英語) Optimization of conditioning regimen for HLA mismatched stem cell
transplantation

研究開発分担者 (日本語) 学校法人 自治医科大学 講師 藤原 慎一郎
所属 役職 氏名: (英語) Jichi Medical University, Senior Lecturer,
Shinichiro Fujiwara

II. 成果の概要（総括研究報告）

- ・ 研究開発代表者による報告の場合

【和文】

同種造血幹細胞移植のドナーは患者とヒト白血球型抗原(HLA)が適合していることが望ましいが、HLA 適合ドナーが得られない患者のために HLA 不適合血縁者間移植が開発されてきた。日本では体外での T 細胞除去を行わない HLA 二抗原以上不適合血縁者間移植(以下、ハプロ移植)方法の開発研究が各施設で行われており、世界的にも類を見ない好成績が得られている。本研究は、各施設で異なる方法で行われている HLA 不適合血縁者間移植の臨床研究を一元的に把握し、様々な HLA 不適合移植法の利点、欠点を明確にするとともに、非血縁者間骨髓移植や臍帯血移植との優劣についても評価し、造血器疾患治療における HLA 不適合血縁者間移植の位置づけを明らかにすることを目的として実施された。

自治医大におけるアテムツズマブを用いた HLA 不適合移植の開発研究は、2015 年に低用量アテムツズマブを用いた HLA 不適合移植の臨床試験の登録を完了した。低用量アテムツズマブにおいても GVHD 抑制効果が示され、引き続き背景疾患に応じて標準リスクと高リスクに分けて免疫抑制の強度を変える新規臨床試験を開始した。標準リスク症例の登録はやや滞っているが、高リスク症例については予定を上回る速度で登録が進んでいる。

兵庫医大における低容量 ATG とステロイド剤を用いた HLA 不適合移植の開発研究は、多施設共同第 I/II 相試験を 2014 年に終了し、100 日生存率 88.2%、II 度以上の急性 GVHD 発症率 30.7%、慢性 GVHD(extensive)発症率 20%と優れた成績が得られ、国際専門誌に発表した。2016 年度には非寛解期症例に対する抗腫瘍効果を高めるために前処置の強度を強めた臨床試験を開始した。

名古屋大学小児科におけるドナー由来細胞療法を用い、高用量サイモグロブリンを併用したハプロ移植の開発研究では、ステロイド抵抗性急性 GVHD 症例に対してヒト由来血小板融解産物を用いて培養した間葉系幹細胞を投与する方法を行っている。ハプロ一致移植 27 例の時点で MSC 輸注による急性副反応はなく、急性 GVHD による死亡も完全に抑制されている。

筑波大学における造血幹細胞移植後シクロホスファミドを用いた HLA 半合致移植の開発研究は、骨髓破壊的前処置を用いた臨床試験が目標 20 例中 17 例、骨髓非破壊的前処置を用いた臨床試験が目標 15 例中 8 例の登録を完了し、順調に進捗している。

日本造血幹細胞移植学会主導の多施設共同研究として実施している少量 ATG を用いた HLA 一抗原不適合血縁者間移植は、目標 39 症例中の 31 症例の登録を完了した。

基礎的な研究ではサイトメガロウイルス、EB ウイルスなどに対するテトラマーを用いた抗原特異的細胞傷害性 T 細胞の定量検査や、抗原特異的 T 細胞受容体レパトアの解析を行い、抗原特異的な細胞傷害性 T 細胞の同定に成功した。また、造血幹細胞移植後の NK 細胞回復については各種 NK 細胞マーカーなどの免疫学的手法を用いた解析を進めている。マウス慢性 GVHD モデルの解析では IL-12/IL-23 p40 抗体による臨床的かつ病理組織学的な慢性 GVHD 抑制効果が示され、現在は p40 抗体の肺慢性 GVHD に対する効果を検討している。さらにレジストリーデータから診療に役立つエビデンスを算出するための環境改善、データ活用法の研究も進めている。

これらの研究成果に基づいて、現在、国立研究開発法人日本医療研究開発機構(免疫アレルギー疾患等実用化研究事業(移植医療技術開発研究分野))「移植後シクロホスファミドを用いた血縁者間 HLA 半合致移植法の開発研究」(研究代表者 豊嶋崇徳)と協力しながら、日本造血細胞移植学会の「HLA 不適合

血縁者移植ガイドライン」の改訂作業を行っている。今後の研究としては、患者病態に応じて細やかにATGやアレムツズマブの投与量などを至適化していく臨床試験デザインを検討している。

【英文】

HLA-mismatched hematopoietic stem cell transplantation (HSCT) has been investigated for patients who lack an HLA-matched donor. Especially, T-cell repleted haploidentical SCT has been investigated in Japan using various methods to suppress severe graft-versus-host disease (GVHD). In this study project, we tried to comprehensively organize the studies of haploidentical SCT in Japan and address the characteristics of each method.

In Jichi Medical University, a clinical study of haploidentical SCT using alemtuzumab was completed in 2015 with a very low incidence of GVHD. In a subsequent study, patients were separated into two studies based on their status of background diseases.

In Hyogo College of Medicine, a multicenter phase I/II study of haploidentical SCT using low-dose ATG and steroid was completed in 2014 with excellent results of day 100 survival of 88.2%, grade II-IV acute GVHD incidence of 30.7%, and extensive chronic GVHD incidence of 20%. Currently, a clinical study using an intensified conditioning regimen is ongoing.

In Nagoya University, haploidentical SCT was combined with donor-derived cell therapy including MSC cultured with platelet lysate. Among the 27 patients who underwent haploidentical SCT, none developed acute reactions to MSC infusion, and no patient died of acute GVHD.

In Tsukuba University, two clinical studies of haploidentical SCT are ongoing; one using myeloablative conditioning and the other using reduced-intensity conditioning. Seventeen and eight patients, respectively, have been enrolled thus far.

A multicenter study of HLA one-antigen mismatched related donor SCT is ongoing as an official clinical study of Japan Society for Hematopoietic Cell Transplantation, and 31 patients have been enrolled.

With regard to basic studies, specific cytotoxic T-cells against cytomegalovirus and EB virus were identified using a tetramer assay followed by single cell analysis of the T-cell receptor repertoire. Recovery of NK cells were evaluated using NK-specific markers. In addition, in a mouse model of chronic GVHD, IL-12/IL-23 p40 antibody was shown to effectively suppress chronic GVHD. Currently, the effect of p40 antibody on pulmonary chronic GVHD is being evaluated. From a view of statistical analyses, more efficient methods to analyze registry data have been investigated.

Based on these results, we are currently updating the guideline of haploidentical HSCT of Japan Society of Hematopoietic Cell Transplantation. We are planning a prospective study of personalized GVHD prophylaxis protocol based on the collected clinical data. This study enables ideal GVHD prophylaxis for each patient.

- ・ 研究開発分担者による報告の場合

III. 成果の外部への発表

(1) 学会誌・雑誌等における論文一覧 (国内誌 4 件、国際誌 79 件)

1. Delayed platelet recovery after allogeneic hematopoietic stem cell transplantation: association with chronic graft-versus-host disease and survival outcome. Akahoshi Y, Kimura SI, Gomyo A, Hayakawa J, Tamaki M, Harada N, Kusuda M, Kameda K, Ugai T, Wada H, Ishihara Y, Kawamura K, Sakamoto K, Sato M, Terasako-Saito K, Kikuchi M, Nakasone H, Kako S, Kanda Y. *Hematological Oncology* (in press)
2. Impact of genetic alterations in stem-cell transplantation for myelodysplasia and secondary acute myeloid leukemia. Yoshizato T, Nannya Y, Atsuta Y, Shiozawa Y, Iijima-Yamashita Y, Yoshida K, Shiraishi Y, Suzuki H, Nagata Y, Sato Y, Kakiuchi N, Matsuo K, Onizuka M, Kataoka K, Chiba K, Tanaka H, Ueno H, Nakagawa MM, Przychodzen B, Haferlach C, Kern W, Aoki K, Itonaga H, Kanda Y, Sekeres MA, Maciejewski JP, Haferlach T, Miyazaki Y, Horibe K, Sanada M, Miyano S, Makishima H, Ogawa S. *Blood*. (in press).
3. Increased non-relapse mortality due to high-dose cytarabine plus CY/TBI in BMT/PBSCT for acute lymphoblastic leukemia in adults. Arai Y, Kondo T, Shigematsu A, Tanaka J, Ohashi K, Fukuda T, Kawakita T, Mori T, Hoshino T, Onizuka M, Ozawa Y, Yoshida S, Ueda Y, Mizuno I, Atsuta Y, Mizuta S. *British Journal of Haematology*. (in press)
4. Allogeneic stem cell transplantation for adult patients with acute lymphoblastic leukemia who had central nervous system involvement: a study from the Adult ALL Working Group of the Japan Society for Hematopoietic Cell Transplantation. Shigematsu A, Kako S, Mitsunashi K, Iwato K, Uchida N, Kanda Y, Fukuda T, Sawa M, Senoo Y, Ogawa H, Miyamura K, Takada S, Nagamura-Inoue T, Morishima Y, Ichinohe T, Atsuta Y, Mizuta S, Tanaka J. *International Journal of Hematology* (in press)
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7. Impact of HLA allele mismatch in unrelated bone marrow transplantation with reduced intensity conditioning regimen. Yokoyama H, Kanda J, Kim S, Fukuda T, Ohashi K, Akasaka T, Uchida N, Ueda Y, Eto T, Iwato K, Kobayashi H, Ozawa Y, Kondo T, Ichinohe T, Atsuta Y, Kanda Y. *Biology of Blood and Marrow Transplantation*. 2017;23(2):300-309,2017

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11. Mogamulizumab 投与後に移植後大量 cyclophosphamide を用いて HLA 半合致移植を施行した成人 T 細胞リンパ腫. 日下部学, 栗田尚樹, 錦井秀和, 佐藤利栄, 吉田近思, 横山泰久, 坂田 (柳元) 麻実子, 小原直, 長谷川雄一, 千葉滋. *臨床血液* (採択済み)
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(3) 「国民との科学・技術対話社会」に対する取り組み
なし

(4) 特許出願
なし