ELSEVIER

Blood 142 (2023) 2057–2058

The 65th ASH Annual Meeting Abstracts

POSTER ABSTRACTS

703.CELLULAR IMMUNOTHERAPIES: BASIC AND TRANSLATIONAL

Analysis of Metabolic Mechanism of CD19CAR-T with CD79A/CD40 Co-Stimulatory Domain Based on Comparison with Established CAR-Ts

Yuki Takeuchi, MD[1], Seitaro Terakura, MDPhD[2], Shiho Hirano, MD[3], Hirofumi Yokota, MD[4], Shihomi Kuwano, MD[3], Masahide Osaki, MD[4], Yoshitaka Adachi, MD PhD[4], Kanae Imai, MD[3], Jakrawadee Julamanee, MDPhD[5], Ryo Hanajiri, MD PhD[3], Makoto Murata, MD, PhD[6], Hitoshi Kiyoi, MD PhD[7]

[1] Nagoya University Graduate School of Medicine, Anjo, Japan
[2] Department of Hematology and Oncology, Nagoya University Graduate School of Medicine, Nagoya, JPN
[3] Nagoya University Graduate School of Medicine, Nagoya, Japan
[4] Nagoya University Graduate School of Medicine, Nagoya, JPN
[5] Prince of Songkla University, Songkhla, Thailand
[6] Nagoya University Hospital, Nagoya, JPN
[7] Department of Hematology and Oncology, Nagoya University Graduate School of Medicine, Nagoya, Japan

Introduction: Anti-CD19 chimeric antigen receptor T cell (CD19CAR-T) therapy has achieved significant clinical outcomes and changed treatment strategy in B-cell malignancies. However, recurrence after CAR-T therapy remains a major obstacle due to poor peak expansion and short persistence of CAR-T cells. We have reported that CD19CAR-T incorporating a co-stimulatory domain consisting of CD79A and CD40 (79A40CAR) provided superior anti-tumor efficacy and survival compared to CD19CAR-T with CD28 or 4-1BB (CD28CAR/4-1BBCAR) in mice xenograft models. 79A40CAR exhibited sustained NF-κB phosphorylation and superior in vivo efficacy compared to the CD28CAR or 4-1BBCAR. (Molecular Therapy 2021, Julamanee J et al.) In this study, we aimed to clarify the molecular mechanism of 79A40CAR by comparing with CD28/4-1BBCAR in terms of T-cell metabolism.

Methods: CD8+T cells were isolated from PBMCs of healthy donors and retrovirally transduced with either CD28IC-, 4-1BBIC-, or 79A40IC-CD19CARs. CD28CAR, 4-1BBCAR, or 79A40CAR were cocultured with 100 Gy irradiated NALM6 at E:T ratio = 1:5, respectively. Repeated antigen stimulations (Ag-stim) were performed, and day 7 after each stimulation was subjected to analysis. RNA-seq, expansion capacity, T-cell phenotype, and metabolic evaluation using a seahorse flux analyzer (Agilent Technologies) were performed. After Ag-stim, CD45RA negative CD62L positive and CD45RA negative CD62L positive CAR-T cell fractions were sorted as central memory (T_{CM}) and effector memory (T_{EM}), respectively. T_{CM} and T_{EM} received Ag-stim. After Ag-stim, we performed evaluation of mitochondrial functionality with MitoTracker based on mitochondrial mass and membrane potential.

Results: All CD19CAR genes were successfully transduced into primary CD8+ T-cells. To understand the differences between co-stimulatory domains, we first confirmed bulk RNA-seq, expansion capacity, and T-cell phenotype. After Ag-stim, 79A40CAR showed enhancement of the NF-κB pathway. We detected few DEGs when 79A40CAR compared to 4-1BBCAR, however, we detected 162 upregulated DEGs when 79A40CAR compared to CD28CAR. Principal component analysis of gene expression demonstrated similarity between 4-1BBCAR and 79A40CAR. We confirmed that 79A40CAR had superior expansion capacity than CD28, or 4-1BBCAR after repeated Ag-stim. PD-1 and Tim-3 expression in 79A40CAR was lowest after repeated Ag-stim. In the evaluation of T-cell differentiation, 4-1BB and 79A40CAR showed an earlier transition to the effector phenotype compared to CD28CAR after Ag-stim.

To evaluate differences in terms of T-cell metabolism, we performed metabolic analysis using a seahorse flux analyzer. After repeated Ag-stim, 4-1BB and 79A40CAR showed a predominant oxidative phosphorylation metabolic pathway (Fig1A) and higher spare respiratory capacity (SRC), which has been correlated with mitochondrial functionality (Fig1B), than CD28CAR. After repeated Ag-stim, 4-1BB and 79A40CAR demonstrated higher ECAR than CD28CAR.

Because we observed differences in the expression of T-cell differentiation phenotype, we performed cell sorting to evaluate T-cell metabolism of each differentiation phenotype. After applying repeated Ag-stim to T_{CM} and T_{EM}-derived CD19CAR-T, mitochondrial functionality was evaluated using Mitotracker. T_{CM}- (Fig1C) and T_{EM}- (Fig1D) derived 4-1BB and 79A40CAR had more functional mitochondria and fewer dysfunctional mitochondria compared to CD28CAR.

Conclusion: 79A40CAR seems to preserve the mitochondrial oxidative phosphorylation-dominant metabolic pathway and supply energy demands for a longer period, especially when compared to CD28CAR. 79A40CAR showed functionally very similar to 4-1BBCAR. We showed that CD19CAR-Ts had different metabolic pathway according to the different co-stimulatory domains. To reveal additional metabolic pathway differences between 4-1BBCAR and 79A40CAR, we are now conducting proteomics analysis.

Disclosures Kiyoi: *Asahi Kasei:* Research Funding; *SymBio:* Honoraria; *AstraZeneca:* Honoraria; *Ono Pharmaceuticals:* Honoraria; *Eisai:* Honoraria, Research Funding; *Sumitomo Dainippon Pharma:* Research Funding; *Amgen:* Honoraria; *Nippon Kayaku:* Honoraria; *Zenyaku Kogyo:* Research Funding; *Bristol-Myers Squibb:* Honoraria; *Novartis:* Honoraria; *Chugai:* Honoraria, Research Funding; *Astellas Pharma:* Honoraria, Research Funding; *CURED:* Research Funding; *AbbVie:* Honoraria, Research Funding; *Daiichi Sankyo:* Honoraria, Research Funding; *Perseus Proteomics:* Research Funding; *Meiji Seika:* Honoraria; *Pfizer:* Honoraria; *Otsuka Pharmaceutical:* Research Funding; *Kyowa Hakko Kirin:* Research Funding; *Towa:* Honoraria; *MSD:* Honoraria; *Pharma Essentia Japan:* Honoraria.

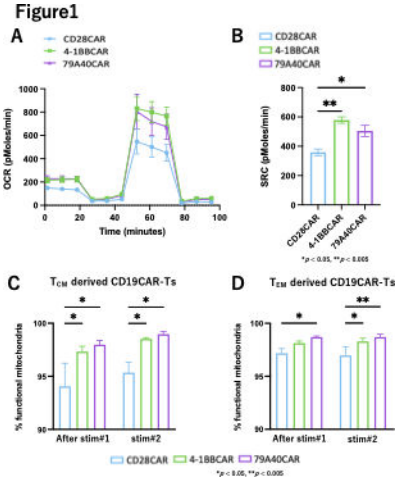

Figure 1

https://doi.org/10.1182/blood-2023-179842