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Best CAR design based on the analyses of ligand-independent activation of CD19-CAR T cells

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Background

Adoptive immunotherapy using the Chimeric Antigen Receptor (CAR) gene-modified T cells is a promising strategy to treat patients with malignancy and autoimmune diseases. The latest results of CD19 CAR-T immunotherapy clinical trials have demonstrated impressive potential in a range of B-lymphoid malignancies. In spite of the recent great success, serious adverse events occurred after infusion of CAR T cells including "on-target off-organ" activation, and cytokine release syndrome has been observed in a number of CAR T-cell therapies as a result of excessive T-cell activation. To improve the efficacy and safety of CAR- T cells, selection of the target tumor-associated antigen, that are expressed only on tumor cells, and the suitable CAR constructs showing the optimal T cell activities are essential, thereby minimizing the risk of side effects.

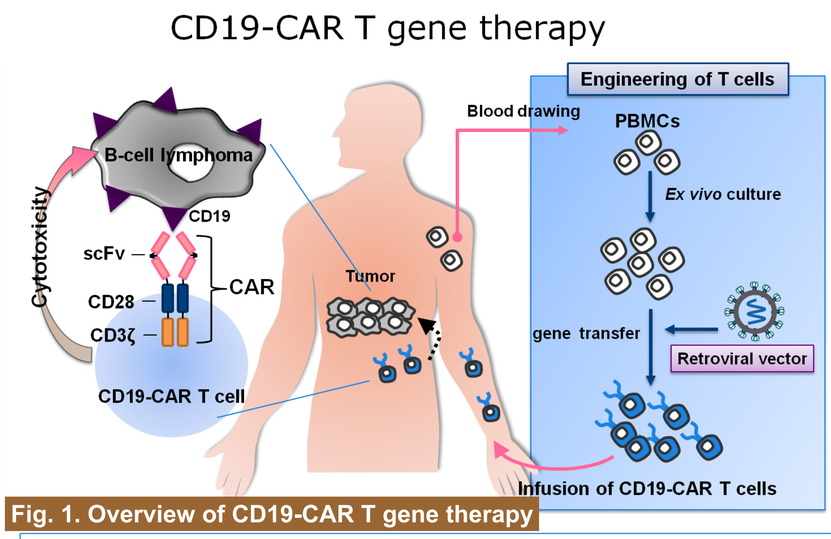


Fig. 1. Overview of CD19-CAR T gene therapy

CD19-CAR T cells, which are engineered to express extracellular single-chain immunoglobulin variable fragments to CD19, linked to cytoplasmic T cell activation domains including CD3-ζ, showed remarkable therapeutic benefits toward CD19+ B cell malignancies.

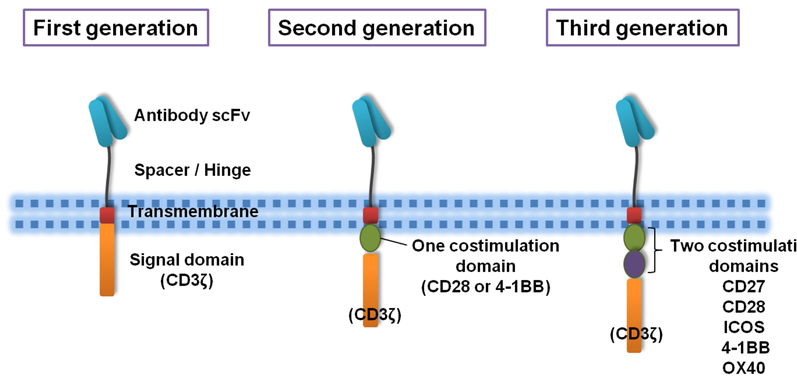


Fig. 2. Chimeric antigen receptors

CARs target surface antigens in an MHC-independent fashion and consist of an ectodomain, hinge domain, transmembrane domain, and endodomain. The first-generation CARs have a single cytoplasmic domain. Current trials are testing second- and third-generation CARs that have combinations of signaling domains.

Methods

Aiming to select the optimal design of CD19 CAR for effective and safe immunotherapy, using anti-CD19 antibody, clone FMC63, we performed detail analysis of non-specific activation of CD19 CAR-T cells caused by the design of scFv (e.g.the leader sequences, the order of VH and VL, the spacer sequences between VH and VL, and the extracellular-spacer domains).
PBMCs from healthy donors were stimulated with anti-CD3 monoclonal antibody (OKT3) together with recombinant fibronectin fragment (RetroNectin®) and transduced with CAR-retroviral vectors and further expanded. The resultant CAR-T cells were assessed for their non-specific activation via expression analyses of activation markers CD25 and CD69. CD19 ligand specific activation was analyzed by intracellular cytokine secretion such as IFN-γ and TNF-α in the presense of CD19 positive Raji cells.

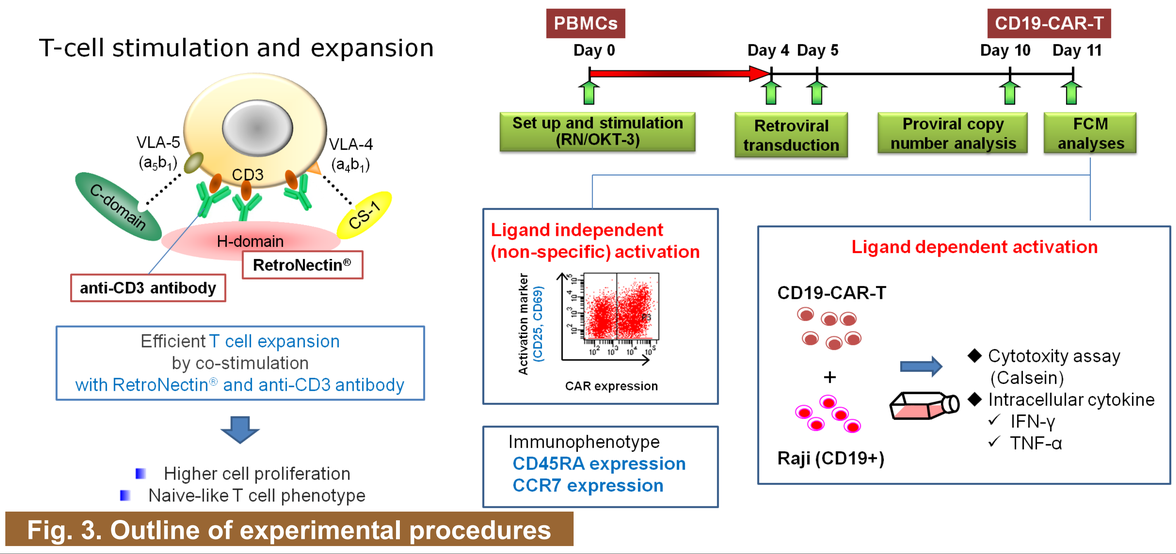


Fig. 3. Outline of experimental procedures

Evaluation of hinge

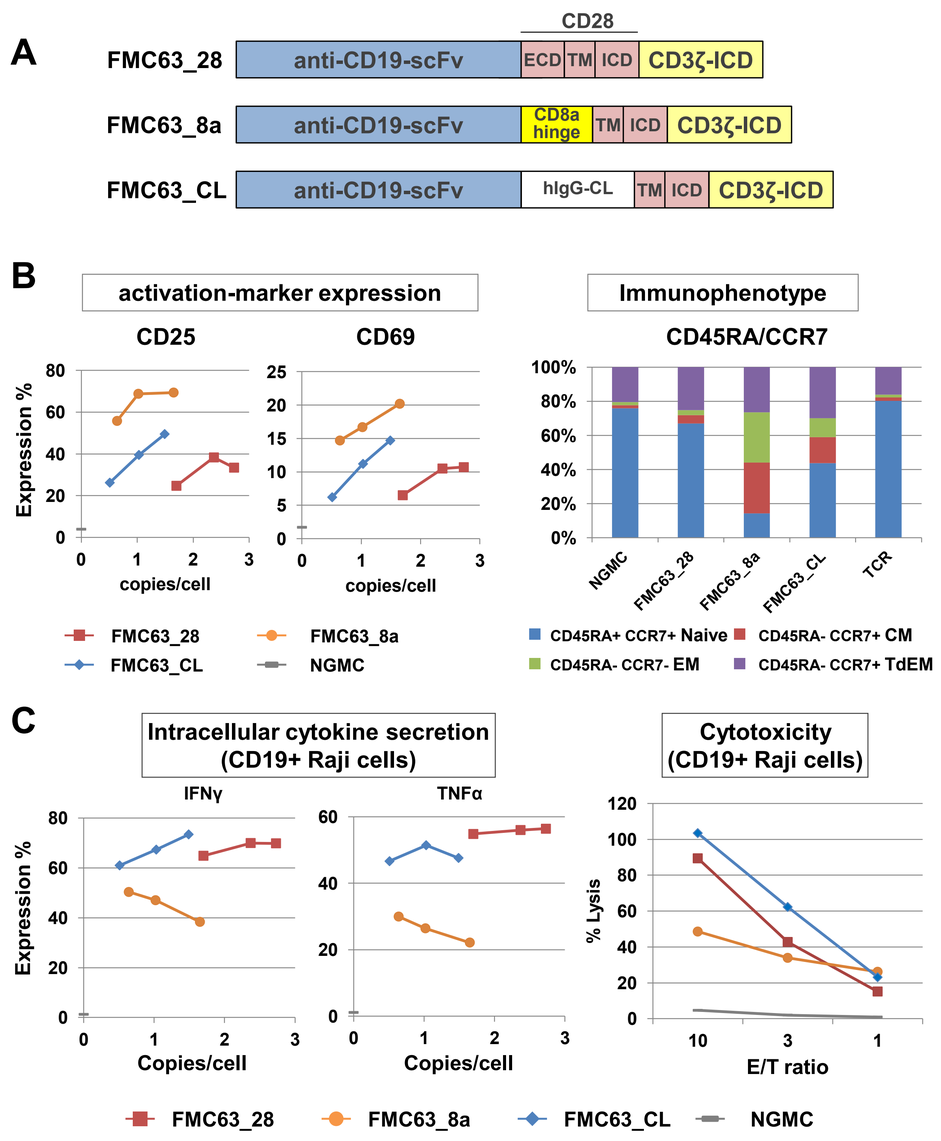


Fig. 4. Evaluation of hinge sequences flanked by scFv of clone FMC63.

Three kinds of hinge sequence were compared with the same backbone of scFv and intracellular signal domain.

A; Vector constructs used in this study. Hinge sequence were chosen from CD28, CD8α or human immunoglobulin G constant region of light chain (hIgG-CL).

B; Antigen-non-specific activation by measuring the CD25 and CD69 expression level of CAR-transduced T cells compared to the non gene modified T cells (left panel) and Immunophenotype analysis (right panel).

C; CD19-antigen specific activation of CAR-transduced T cells. Intracellular cytokine secretion analysis (left panel) and cytotoxity assay of calsein labeled tumor killing assay (right panel).

CAR construct having CD8α hinge represented higher CD25 and CD69 activation without ligand mediated stimulation. Consequently, highly activated CAR-T cells with CD8α hinge showed lower antigen specific reactivity of intracellular cytokine secretion activity and cytotoxity activity. CAR construct having CD28 extracellular domain showed the lowest non-specific activation of CAR-T cells.

Evaluation of scFv; FMC63 VH/VL and linker

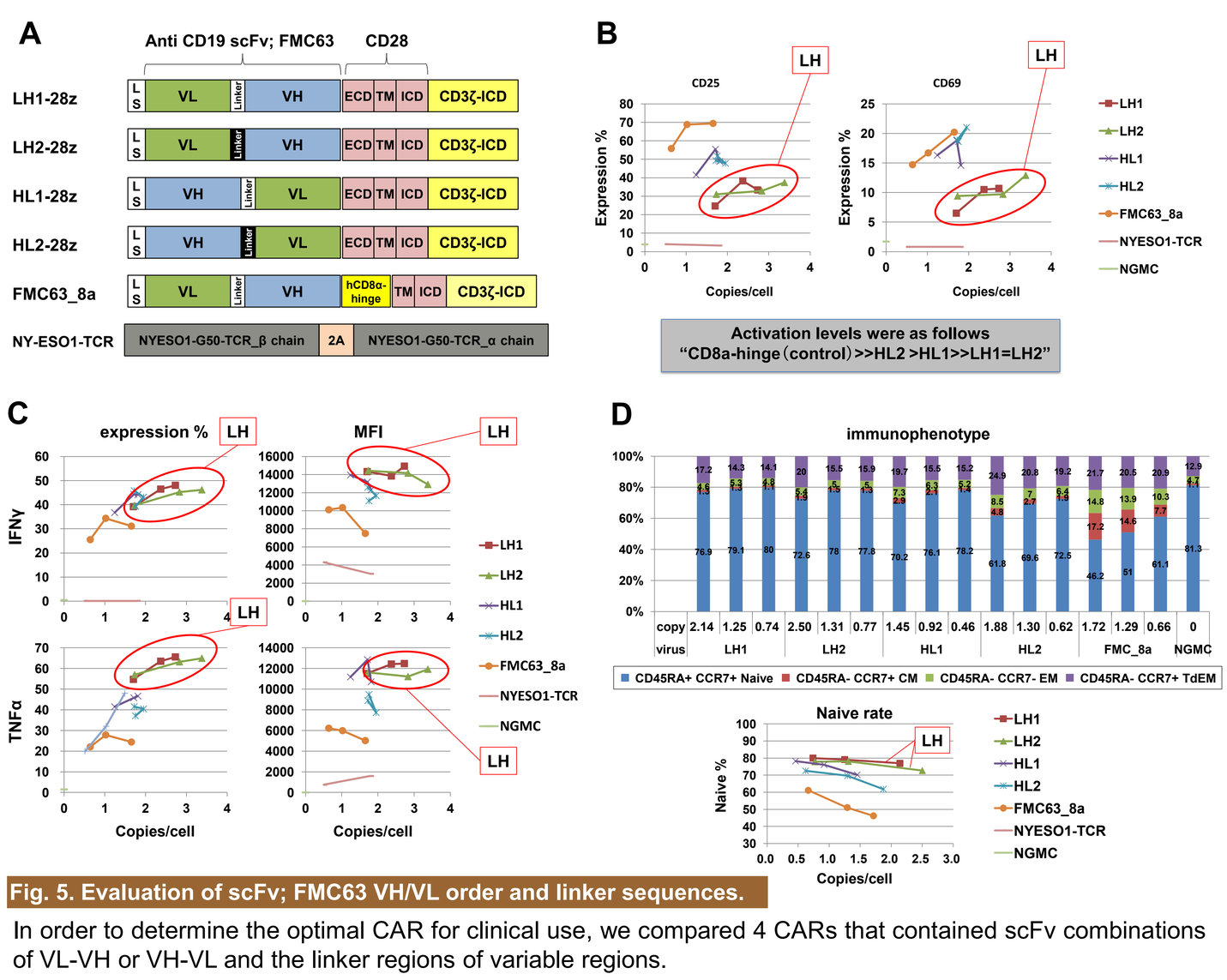
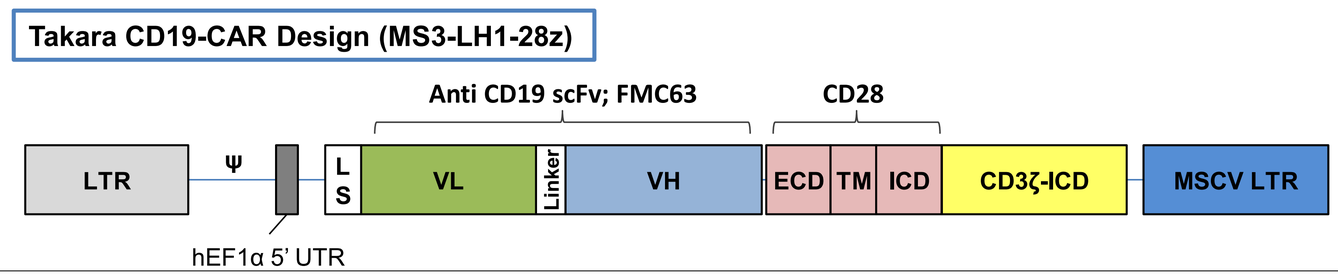


Fig. 5. Evaluation of scFv; FMC63 VH/VL order and linker sequences.

In order to determine the optimal CAR for clinical use, we compared 4 CARs that contained scFv combinations of VL-VH or VH-VL and the linker regions of variable regions.

A; Vector constructs used in this study.
B; Antigen-non-specific activation by measuring the CD25 and CD69 expression level of CAR-transduced T cells.
C; CD19-antigen specific activation of CAR-transduced T cells by Intracellular cytokine secretion analysis.
D; Immuno phenotype analysis of CAR-transduced T cells.
NGMC, non gene modified cells; CM, central memory; EM, effector memory, TdEM, terminally differentiated effector memory.

Highly activated CAR-T cells showed lower antigen specific reactivity of intracellular cytokine secretion activity. Finally we chose the order of VL-VH (LH1) flanked by CD28 extracellular domain and CD28 co-stimulatory molecules as our CAR design.



Conclusions/Discussions

There was a trend that higher expression of CAR resulted in higher ligand-independent activation and lower CD19-specific activity. Especially, the CD19-CAR T cells with CD8 hinge domain were highly activated with CD19-independent manner. The excessive non-specific activation lowered the cytokine secretion and the cytotoxic activities against CD19-expressing tumor cells.

Because of the nature of CAR construct by artificial synthesis, the exact mechanism of ligand-independent activation by CD8 hinge domain is still unclear. However, it is desired to select the optimal design of CAR constructs which shows high antigen-specific activities with low non-specific activities for the safe and effective CAR-gene modified T cell therapy. A number of ongoing and planned clinical trials in the world will navigate the future direction toward the best CD19-CAR design.

[Disclosure]

Hideto Chono: Takara Bio Inc., Employment,
Yasunori Amaishi: Takara Bio Inc., Employment,
Zheng Pei: Takara Bio Inc., Employment,
Sachiko Okamoto: Takara Bio Inc., Employment,
Junichi Mineno: Takara Bio Inc., Membership on Board of Directors.

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