Immune escape mechanism of B-cell malignancies on Anti-CD19 Chimeric Antigen Receptor T-cell treatment and solution

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Abstract. Relapse or refractory B-cell malignancies have been reported in multiple clinical trials after treatment of Anti-CD19 Chimeric Antigen Receptor (CAR) T-cells. Many clinical studies have demonstrated the potential immune escape mechanism for B-cell malignancies like genetic mutation, transcriptional deregulation, lineage switch, loss of CAR T-cells, and trogocytosis. The study of these mechanisms can provide us insights in designs of future immunotherapies regarding both B-cell malignancies and even other solid tumors. The potential solution for the immune escape mechanisms regarding CAR T-cell treatment is engineering multispecific CARs. In this article, I review most of the up-to-date immune escape mechanism studies and some multispecific CAR T-cell treatment clinical studies and trials that may prevent the escape route and have to potential to cure B-cell malignancies.

1 Introduction

Despite significant medical treatment advances on B-cell malignancies like B-cell acute lymphoblastic leukemias(B-ALL), relapsed or refractory disease cases in adults is still highly probable and result lower than 50% long-term event-free survival. One of the most innovative treatment against B-ALL is the use of Anti-CD19 CAR T-cells. However, there are still relapsed or refractory disease cases after CAR T cell therapy. In a phase 1 clinical study of CD19-specific CAR T cells in patients with B-ALL, there were four CD19⁺ relapse cases, while CD19⁻ disease relapses were not seen [1]. The relapsed cases were not limited to the treatment of B-ALL. In clinical trial treating patients with B-Cell Lymphomas using CTL019 cells (autologous T cells that express a CD19-directed CAR), both the loss of the CD19 expression and continued expression of CD19 were observed [2]. Recent clinical studies have concluded that relapsed or refractory cases are mostly caused by either the loss of CD19 target protein or the loss of CAR T-cells in blood [3].

2 Immune Escape Mechanism on Anti-CD19 CAR

2.1 Genetic Mutation

For relapses with the loss of CD19 target protein, it may be resulted from genetic mutation or transcriptional deregulation. In genetic mutation cases, a recent study regarding genetic mutational mechanism happened in B-ALL has found that most of CD19⁻ mutations resulted loss of exon 5 in CD19 which is the transmembrane domain of the protein, resulting loss of surface antigen

on tumor cells. Most of the mutations occurred in the form of frameshift insertion or deletion and in some cases single nucleotide variants, but all mutations are unique. The study also found that in 9 available cases, eight patients have acquired loss of heterozygosity, while the remaining cases could be explained by biallelic mutations [4].

Another study about B cell lymphoma immune escape mechanism have reported a CD19⁺ relapse case with a point mutation in exon 3(P. 174 L>V/C.520 C>G) in CD19. The study has shown that though CD19-targeted CAR T-cells possess anti-tumor function against B-cell tumors expressing wildtype CD19, it does not possess anti-tumor function against CD19⁺ B-cells with such point mutation or any CD19⁺ cells with deleted exon 1,2,3 or 4 in CD19 [5]. Such finding alarms us about the incompetence of anti-CD19 CAR T-cells (FMC63) against mutational or transcriptional changes in CD19 target protein and calls for more advanced CAR designs.

2.2 Transcriptional Deregulation

In transcriptional deregulation cases, a study about anti-CD19 CAR T-cells (FMC63) have shown that the SRSF3 (one of the splice factors which accounts for the CD19 mRNA splicing, calculated using AVISPA algorithm) insufficiency could be responsible for the abundance of CD19 Δ ex2 isoform, which caused the loss of the cognate CART-19 epitope. In addition, CD19 Δ ex5-6 isoform, which lacks the transmembrane and cytosolic domains, was found to be another possible splicing-based immune escape mechanism though factors resulting the abundance of Δ ex5-6 isoform were not discussed. Such splicing-based adaptive mechanisms shows the incompetence of the current anti-CD19 CAR

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design and suggested that the future CARs or antibody-based treatments designs should target the essential

exons to prevent such form of splicing-based immune escape [6].

Fig.1. Illustration of Immune Escape mechanisms for Anti-CD19 CAR T-cell treatment. Under ordinary conditions as shown in the top, Anti-CD19 CAR T-cells will be activated through CAR recognition of CD19 target antigen, thus proceed with tumor killing process. However, CAR T-cells will remain inactivated when encounter tumor cells with antigen loss (Genetic mutation or Transcriptional Deregulation), lineage-switched tumor cells expressing different sets of surface antigens (Lineage Switched), self-immunity against murine based CAR (Loss of CAR T-cells), or tumor cells with diminished antigen density (Trogocytosis).

A follow-up study on the immune escape mechanism have shown that CD19 Δ ex2 isoform are commonly expressed in both leukemic blasts at the time of diagnosis and the bone marrow of nonleukemia donors. CD19 Δ ex5-6 isoform was found in only one adult patients, suggesting CD19 Δ ex5-6 isoform immune escape

mechanism are most likely developed only under CAR T-cell therapy pressure. The expression of CD19 Δ ex2 isoform and possible Δ ex5-6 isoform before CAR T-cell therapy further underline the risk of applying anti-CD19 CAR T-cell therapy as a monotherapy due to its high

immune escape risk and calls for novel CAR constructs and combination therapies [7].

However, a recent study on CD19 CARs has reported that the targeting epitope for anti-CD19 CAR T-cells (FMC63) is actually exon 4 rather than exon 2 proposed by the previous study [8]. One recent follow-up study has further investigated the case and proposed a novel immune escape mechanism on CAR T-cell therapy. In some CART19 resistant cells, CD19 variants like CD19 Δ ex2 isoform have disrupted or missing disulfide bonds between Cys38-Cys97 resulting conformational changes in the first Ig-like C2 loops. Such conformational change in the protein shape have caused the protein to remain trapped in the endoplasmic reticulum instead of transporting to Golgi complex for complex glycosylation and later cell surface presentation. Thus, the lack of cell surface expression, these cells with CD19 variants would be immunotherapy-resistant. However, it may still be possible to create CARs targeting neoantigens like CD22 or CD123 to deal with relapsed or refractory B-ALL after CART19 therapy. In addition, it is found that CD19 variants could be recognized and presented by MHC-I and -II, thus it remains possible to target MHC-presented CD19 peptides in those tumors with misfolded CD19 proteins [9].

2.3 Lineage Switch

However, immune escape mechanism regarding the loss of CD19 target antigen are not limited to genetic mutation and transcriptional deregulation on exons of CD19. In a recent clinical study, a novel immune escape mechanism was reported that two patients with mixed lineage leukemia (MLL)-B-ALL transformed to acute myeloid leukemia (AML), resulting the loss of CD19 expression. After examining patients' immunoglobulin heavy chain (IGH) rearrangement patterns in their relapsed myeloid lineage cells, one patient had retention of IGH rearrangement while the other patient does not have IGH rearrangement. Thus, lineage-switch for the first patient is caused by cell reprogramming of a previously committed lymphoid lineage, but the other can only be explained with myeloid differentiation of a noncommitted precursor or selection of a pre-existing myeloid clone [10]. In conjunction with this clinical study, a recent experiment on murine ALL have demonstrated that the immune pressure from CD19 CAR T-cell therapy can induce either a rapid relapsing leukemia resulting previously discussed CD19 Δ ex2 isoforms, or a lineage-switched leukemia which is caused by reprogramming of PAX5 and EBF1 (important B-cell regulatory transcription factors), resulting CD19 CAR resistant tumor [11, 12]. In addition, such linkage-switch immune escape mechanism is not limited to lymphoid to myeloid lineage switch. It had been reported that a patient with chronic lymphocytic leukemia with Richter transformation after CD19 CAR T-cell therapy have relapsed with plasmablastic lymphoma. The case of such lineage switch is a two base-pair insertion/frameshift mutation in TP53 sequence, which resulted transformed plasmablastic lymphoma cell survival without B cell

receptor (like CD19) signaling [13]. The lineage switching mechanism thus have shown the ability of B-cell malignancies to reprogram and escape under pressure from monospecific CAR T-cells. This finding also suggests bispecific immunotherapy since CD22 expression is maintained on the intermediate relapses in some reported cases [10].

2.4 Loss of CAR T-cell

For the loss of CAR T-cells in blood, since all anti-CD19 CAR T-cells used in recent clinical studies incorporate a murine single-chain variable fragment(scFv), it was demonstrated that such construct could result CD8+ T-cell immunity to CAR transgene products. Such immunity against CAR T-cells would result impersistent CAR T-cells in blood and nullify second CAR T-cell infusion. A possible solution to this relapse mechanism is to reduce the immunogenicity of the CAR transgene product by incorporating a human scFv rather than murine scFv into the CAR construct [3]. In a recent study, human CARs with similar epitope and affinity have been derived and tested in vitro as an attempt to reduce the immunogenicity of the synthetic CARs [8]. In addition, during the previous B cell lymphoma clinical trial, human scFv 21D4 had been developed to treat the mutated CD19+ FMC63-resistant B cell lymphoma with some clinical success [5]. Though we have developed various types of humanized CARs and achieved some improvements on the FMC63 CAR, more clinic studies are still required before such fully human CARs can replace the well-tested FMC63 murine CAR.

2.5 Trogocytosis

In addition, it has been reported that a decrease in antigen density could also result in immune escape from the CAR T-cell therapy. A recent experiment has shown its underlying mechanism to be a reversible antigen loss caused by a post-transcriptional process called trogocytosis. It refers to the active process of tumor cells transferring target antigen to T cells, thus losing its antigen density and resulting in fratricide T-cell killing and T-cell exhaustion [14].

3 Solutions on Immune Escape Mechanism

Due to the multitude of the immune escape mechanisms reported, it seems an impossible and fruitless path to develop specific solutions for every single one of the escape routes. Though an improvement on the CAR epitope may result better therapeutic efficacy, since most escape mechanisms include the loss of CD19 surface expression, the relapse rates after CAR T-cell therapy may not be abated dramatically. The current methods in overcoming those relapsed cases and immune escape mechanisms after monospecific CAR T therapy are sequential infusion of different targeting CAR T-cells and dual targeting CAR T-cell therapy which are concluded as multispecific CAR T therapies [14]. In a

recent mini review, multispecific CAR T cells are categorized in to four groups: Bicistronic CAR T cells, Tandem CAR T cells, Coadministration and Cotransduction [15]. Coadministration corresponds to the

generally referred sequential infusion, while bicistronic, tandem CAR T cells and cotransduction can be understood as dual targeting.

Fig. 2. Illustration of multispecific CAR T-cell mechanism. Sequential Infusion used two separate CAR T-cells with different targeting CAR and administered sequentially to patient to reach CAR T-cell treatment's multispecificity. Alternatively, bivalent tandem CARs which recognizes both targeted antigens in one CAR. The simultaneous recognition can also reach enhanced CAR T-cell activity.

3.1 Sequential Infusion

Success in clinical case like lymphoma relapse after anti-CD19 CAR has been reported using sequential anti-CD22 and 20 CAR-T treatments [16]. Pediatric Philadelphia chromosome-like (Ph-like) B-ALL relapse case after anti-CD19 CAR was also successfully treated with sequential infusion of anti-CD22 and anti-CD19 CAR T cells [17]. In addition, clinical studies about anti-CD22 CAR-T therapy on relapsed B-ALL after CART19 treatment and sequential anti-CD19-22 CAR T therapy on B-ALL have shown successes in treating relapsed cases and improvements on CART19 therapy [18, 19]. However, relapse cases after anti-CD22 CAR-T therapy are still appearing with decreased antigen surface expression caused by a post-transcriptional mechanism [18]. The potential explanation could be trogocytosis since the experiment mentioned previously also showed that CARs like anti-CD22 and anti-BCMA for multiple myeloma are also susceptible to this escape mechanism. In addition, the experiment has compared the efficacy between sequential infusion and dual targeting and found the later to be more effective [14].

3.2 Dual Targeting

Many preclinical researches have presented their well-designed CARs against B cell malignancies. Similar CD19/CD20 tandem CAR designs have been presented by Dr. Zah, Dr. Schneider, and Dr. Martyniszyn's teams by linking CD19 scFv and CD20 scFv with a polyglycine-serine chain (GGGGS) [20-22]. The follow up clinical research has shown effectiveness on both CD19+ and CD19- patients using CD19/CD20 tandem CAR treatment, while there are still reported treatment failure cases with CD19 downregulation [23]. Bispecific CARs were not limited to CD19/CD20, successful clinical cases using haploidentical CD19/CD22 tandem CAR-T cell treatment on the patient with relapsed B-ALL were also reported [24]. Promising clinical trials' results have shown that bispecific CD19/CD22 CAR T cells possess the potential to eliminate tumor cell by preempting antigen escape without high grade neurotoxicity. Still, previously discussed antigen escape mechanism like exon 2 deletion and decreased CD22 density were observed in relapsed cases after bispecific CAR T cell treatments [25]. Novel CD19/CD123 CAR was also designed with no CD123- relapse cases observed. CD123 is commonly expressed on hematopoietic progenitor cells and reported to be

expresses in all kinds of B-cell malignancies like B-ALL and AML. Such dual targeting CD19/CD123 CAR, if successfully tested clinically, could prevent the lineage switch escape mechanism as transformed myeloid leukemia would still express CD123 thus susceptible to treatment [26].

4 Conclusion

Clinical researches have reported various kinds of antigen escape mechanism against the commonly used CD19 CAR during the treatment of B-cell malignancies. Though all these tumor escape routes have shown the incompetency of the anti-CD19 CAR T-cell treatment against B-cell malignancies, they also pointed the direction for the development of the future CAR T therapy to the multispecific CAR T-cell treatment. Admittedly, the researches and clinical trials on multispecific CAR T therapy were still limited and the current reported studies still contain treatment failures. However, multispecific CAR T therapy has its clinically meaningful effects against B-cell malignancies and will most likely be the center of future CAR T researches.

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