Peer Review File

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Reviewer A

Comment 1: The English language is very poor, and the manuscript lacks a proper flow of information, and reading the manuscript is very difficult and boring. It looks as if the authors have opened many related articles and just taken the information, without any editing to make a continues flow of information.

Reply 1: Thanks for your very useful comments. We tried our best to improve the manuscript and made some changes to the manuscript. These changes will not influence the content and framework of the paper. And here we did not list the changes but corrections using the "Track Changes" function of word processing program in revised paper. We appreciate for Editors/Reviewers' warm work earnestly and hope that the correction will meet with approval. In addition, we invite William C. Cho for his help to polish our paper. The revisions may be incomplete but I'm willing to continue to make additions.

Comment 2: The review is full of abbreviations, most of the abbreviations have not been provided with full form, when mentioned at their first place. For example, Cas9, crRNA, PAM, HLA, PD1, CTLA-4, LAG-3, TIM-3, OCA-B, etc. Please check all, and better to provide a list of abbreviations and their full form at the end of manuscript.

Reply 2: Thanks for your very useful comments. We have checked all abbreviations but failed to list the abbreviations in the text due to problems with the word limit of the paper.

Changes in the text:

Page 2, line 17-18: Clustered regularly interspaced short palindromic repeats-CRISPR-associated 9 (CRISPR-Cas9);
Page 4, line 80: CRISPR RNA (crRNA)-trans-activated crRNA (tracRNA);
Page 5, line 85-86: protospacer adjacent motif (PAM);
Page 7, line 127-128: leukocyte antigen (HLA);
Page 7, line 128-129: programmed cell death protein 1 (PD1);
Page 7, line 130-131: cytotoxic T lymphocyte associate protein-4 (CTLA-4);
Page 7, line 131-132: lymphocyte activation gene-3 (LAG-3);
Page 7, line 132: T cell immunoglobulin domain and mucin domain-3 (TIM-3)
Page 8, line 159: Oct co-activator from B cells (OCA-B).
HNH and RuvC are two nuclease domains of Cas9.

Comment 3: Follow a pattern in writing abbreviation or words, e.g., BCL6 or Bcl6? [Line 180, 184], CRISPR-Cas9 or CRISPR Cas 9 [Line 1, 61] etc. Check it throughout the manuscript for all such cases.

Reply 3: Thanks for your very useful comments. We have checked all abbreviation or words to follow a pattern.
Changes in the text: BCL6 (see Page 8, line 169; Page 9, line 171); CRISPR-Cas9 (see Page 1, line 1; Page 4, line 60).

Comment 4: Some sentences are vague, without any proper flow of information [Line 47-49].
Reply 4: Thanks for your very useful comments. We have presented this sentence in detail.
Changes in the text: According to 2016 World Health Organization (WHO) reclassification of hematopoietic and lymphoid tissue tumors (1), 11 types of lymphoma are B-cell in origin, accounting for the most (see Page 3, line 44-47).

Comment 5: References have been mentioned as superscript or normal font. Just keep one format.
Reply 5: Thanks for your very useful comments. We have checked all references mentioned in the paper to keep one format.

Comment 6: What is the meaning of Cellphone anemia [Line 60], thru [Line 173].
Reply 6: Thanks for your very useful comments. We are really sorry for our careless mistakes. We have corrected the typo and checked in the entire paper.
Changes in the text: sickle cell anemia (SCD) (see Page 3, line 57); Through (see Page 8, line 160).

Comment 7: Some sentences are very small e.g., Line 71, Line 472 etc.
Reply 7: Thanks for your very useful comments. We have presented this sentence (Line 472) in detail. “The search strategy is summarized in Table 1.” This sentence is the journal usual practice.
Changes in the text: The treatment of B-cell lymphoma is mostly chemotherapy combined with biological therapy but patients are susceptible to drug resistance (see Page 18, line 384-385).

Comment 8: Some sentences are very big, e.g., [Line 124-130], Line 163-167, Line 209-213,
Reply 8: Thanks for your very useful comments. We have rewritten this sentence and broken these sentences down into several small sentence.
Changes in the text:
Delivery of bound CAR and crRNA led to the injury of TCR and β-2-microglobulin (B2M), and the loss of human leukocyte antigen (HLA) type I molecules and programmed cell death protein 1 (PD1), to avoid graft-versus-host responses (GVHD) and other immune responses (31). T cell suppressors [including cytotoxic T lymphocyte associate protein-4 (CTLA-4), PD1, lymphocyte activation gene-3

(LAG-3)] and T cell immunoglobulin domain and mucin domain-3 (TIM-3), and Fas receptor/Fas ligand (FasL) induced T cell apoptosis (32-35) (see Page 7, line 126-133).
CREBBP deficiency was found to decrease histone H3 acetylation and MHCII expression, causing B-cell hyperproliferation and MYC-driven lymphomas, ultimately leading to immune escape (46) (see Page 9, line 179-182).
Mo et al found that CC-122 redirected cereblon, the substrate receptor of CUL4/DDB1/RBX1/CRBN E3 ubiquitin ligase complex (CRL4CRBN), to induce IKAROS zinc finger protein (IKZF1/3) ubiquitination and degradation and mediate antiproliferative activity in DLBCL (see Page 10, line 200-203).

Comment 9: Conclusion should not be supported by any reference. So, remove reference 70 from conclusion.
Reply 9: Thanks for your very useful comments. According to your suggestion, we have removed reference from conclusion.

Comment 10: There are so many minor mistakes, please check it carefully.
Reply 10: Thanks for your very useful comments. We are very sorry for our careless mistakes and we have thoroughly checked grammatical errors, typos, and punctuation in the entire manuscript.

Comment 11: To increase the standard of this manuscript, some important diagrams are missing as:
a. a. Provide a simple diagram showing some genes as an example and the role of CRISPR/Cas9 in modulating these genes.
b. Similarly, provide a diagram with some signaling pathways mentioned in manuscript and the role of CRISPR/Cas9 in these pathways.
Reply 11: Thanks for your very useful comments. According to your suggestion, we have chosen the BCL6 that it was associated with many genes, pathways to drawn the figure.
Changes in the text:

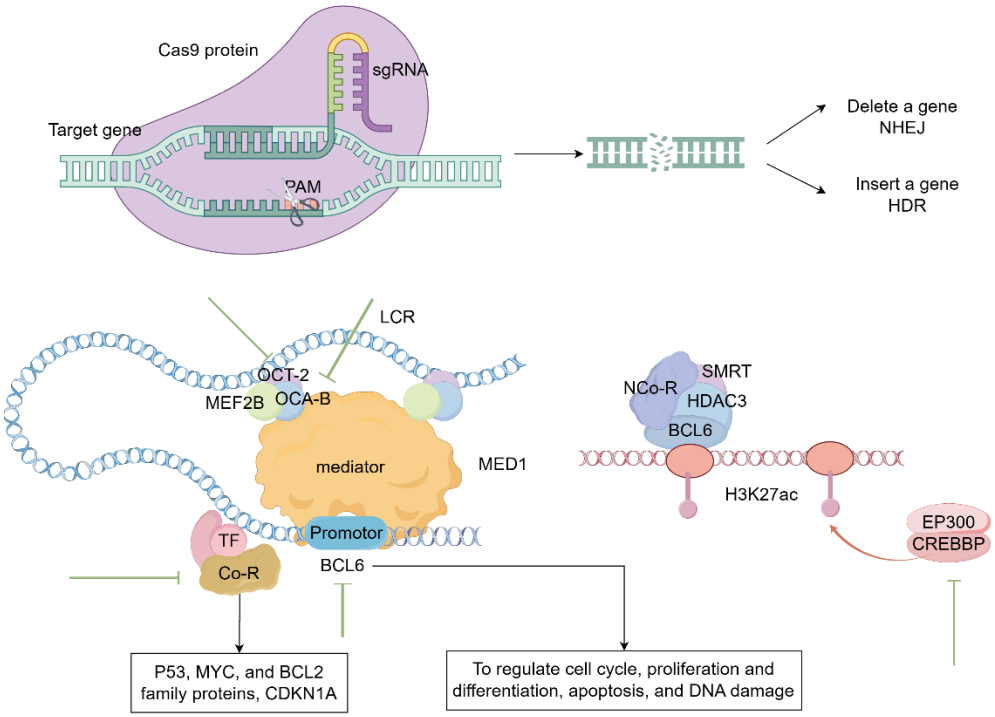


Figure 2. Mechanism of CRISPR-Cas9 in modulating BCL6 and many genes, pathways associated with BCL6.
Mediator complex submit 1 (MED1) consist of mediator, organic cation transporter 2 (OCT-2), Oct co-activator from B cells (OCA-B) and myocyte enhancer factor 2B (MEF2B) (OCT2→OCA-B→MEF2B) activate LCR to impact BCL6 promoter. In addition, histone acetyltransferase (HAT), consist of CREB binding protein (CREBBP) and E1A-binding protein p300 (EP300), inhibited the enhancer/super-enhancer network via the BCL6/ silencing mediator of retinoic acid and thyroid hormone (SMRT)/ histone deacetylase 3 (HDAC3) complex to regulate the development of GC B cells. Design of gRNA with 20 nucleotide base complementary pairing with the target DNA strand. Then Cas9 protein recognize and cleave to generate site-specific double-strand breaks (DSB), inducing nonhomologous end joining (NHEJ) or homology directed repair (HDR) repair (see Page 28-29, line 637-651).

Comment 12: The targeting approaches of CRISPR/Cas9 in B-cell should be added as a section.

Reply 12: Thanks for your very useful comments. According to your suggestion, we have added the targeting approaches of CRISPR-Cas9.

Changes in the text:

1.2 Mechanism of CRISPR Cas9 gene editing

Design of gRNA with 20 nucleotide (NT) base complementary pairing with the target DNA strand. The proximal seed region sequence of gRNA preformed in the A-type conformation, and Cas9 protospacer adjacent motif (PAM) interaction structural

domain binds to the PAM sequence and initiates local DNA strand separation (15-17). The Cas9 phosphate lock loop interacts with +1 phosphate on the DNA backbone of the adjacent PAM target strand, stabilizing the unwound target DNA strand and causing the first base to flip toward the gRNA (18). PAM proximal end forms the R-loop, and gRNA is significantly base-paired with the target DNA, resulting in a conformational change in HNH nuclease domain. Cas9 nuclease is activated and DNA double-strand is dissociated to form a heterologous RNA-DNA duplex. And the non-target strand is directed to the RuvC nuclease domain for cleavage (19). Then approximately -3 nucleotides prior to the original PAM sequence generate site-specific double-strand breaks (DSB), inducing error-prone nonhomologous end joining (NHEJ) or high-fidelity homology directed repair (HDR) repair (20) (see Page 5, line 82-99).

Comment 13: Mention some clinical trials based on CRISPR/Cas9 in modulating B-cells

Reply 13: Thanks for your very useful comments. CAR-T therapy is currently mainly applied in clinical trials based on CRISPR-Cas9 technology. We have introduced in the paragraph about the origin of CAR-T therapy, the current problems and possible solutions.

Changes in the text:

2 Clinical Trial of CRISPR-Cas9 in B-Cell Lymphoma-CART therapy

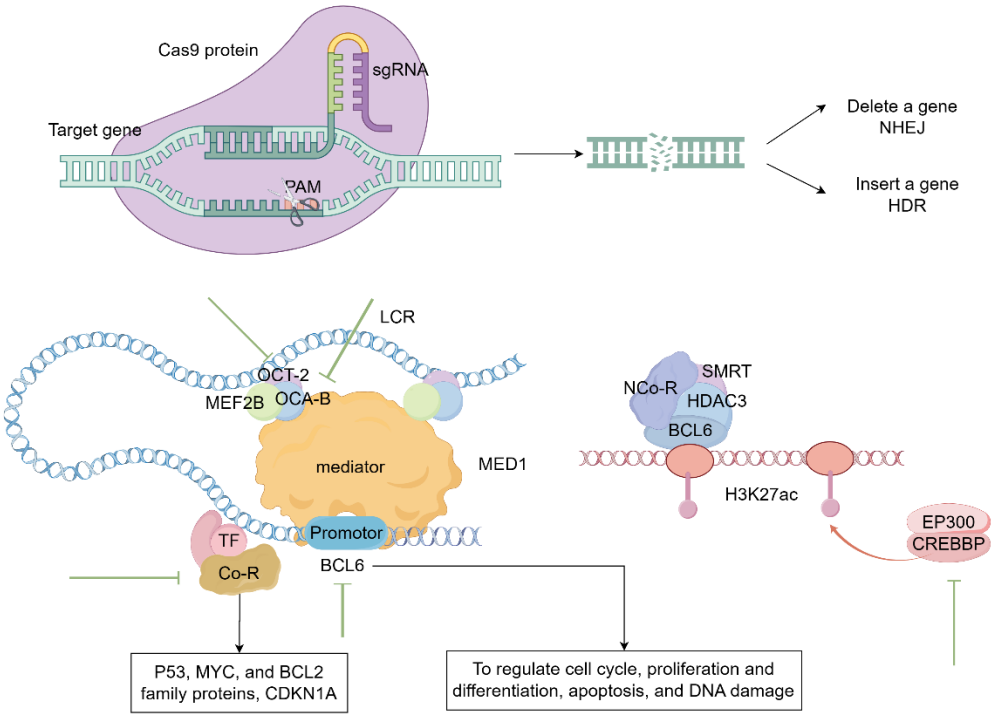
One of the most successful innovations in the area of hematology was the CRISPR-Cas9-edited chimeric antigen receptor (CAR)-T cell, which has been approved by FDA for the treatment of leukemia and lymphoma (27, 28). Gross et al first suggested structural and functional similarities between B-cell antibodies and endogenous αβT cell receptors (TCRs). CAR-T combined the immunoglobulin V and TCR C regions, which could recognize target cells carrying 2,4,6-trinitrophenyl (TNP) semi-antigenic motif. B cells specifically express CD19, and CAR-T cells, modified by CRISPR-Cas9, can recognize CD19 particularly to kill tumor cells, apart from T-lymphocyte cytotoxicity (29, 30).

However, a variety of factors influenced the efficacy of CAR-T therapy, including the type of CAR structure, the quality of T cells, and host-specific factors such as tumor heterogeneity and tumor microenvironment. Delivery of bound CAR and crRNA led to the injury of TCR and β-2-microglobulin (B2M), and the loss of human leukocyte antigen (HLA) type I molecules and programmed cell death protein 1 (PD1), to avoid graft-versus-host responses (GVHD) and other immune responses (31). T cell suppressors [including cytotoxic T lymphocyte associate protein-4 (CTLA-4), PD1, lymphocyte activation gene-3 (LAG-3)] and T cell immunoglobulin domain and mucin domain-3 (TIM-3), and Fas receptor/Fas ligand (FasL) induced T cell apoptosis (32-35). To improve anti-tumor efficacy, knockout of these elements via CRISPR-Cas9 could decrease T cell apoptosis, amplify and decorate CAR-T cells. In addition, bispecific CAR-T cells, such as anti-CD19/CD22 and anti-CD19/CD20 were

promising options currently in clinical trials (36) (see Page 6-7, line 111-137).

Comment 14: Write some challenges, limitation and future prospects of CRISPR/ Cas9 technology in modulating B cells.
Reply 14: Thanks for your very useful comments. Due to the problem of word limit in the article, it has not been described in detail, that in the conclusions, we have written some challenges, limitations and future prospect of CRISPR-Cas9 technology in modulating B cells.
Changes in the text: However, many challenges of CRISPR-Cas9 remain to be addressed, such as the survival efficacy of edited cells, low transfection efficiency, low-efficient delivery methods, oncogenicity and off-target effect. In addition, it is difficult to carry out in clinic because of ethical issues and the inevitable effects of gene knockout on the function of other systems in the body. However, the success of SCD and DMD has also made researchers see the tremendous application foreground of the technology. Even though there are still some problems to be resolved, with the exploration of scientists and the advancement of the technology, screening for knockout of suitable genes without significantly impairing the function of organism will eventually make it possible to treat and alleviate clinically intractable diseases. (see Page 19, line 394-405).

Reviewer B
Comment 1: The authors should add summarized figures for each section, 2.1, 2.2, 2.3, 2.4. It is beneficial for readers to understand the concept.
Reply 1: Thanks for your very useful comments. According to your suggestion, we have chosen the BCL6 that it was associated with many genes, pathways to drawn the figure.
Changes in the text:



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