Analysis on How CRISPR Technology Facilitates Anticancer Therapeutics

Sitian Wang
Faculty of Medicine, Imperial College London, SW7 2AZ, London, U.K.

Keywords: Component, CRISPR, Immunotherapy, Cancer, TCR Therapy, CAR-T Therapy.

Abstract: As a novel treatment modality, immunotherapy, based on the principle of boosting the antitumour response, help patients to fight against cancer. Out of many different types of immunotherapies, adoptive T cell therapy, characterized by enhancing immunity and specificity by ex vivo manipulation on patient-derived T cells, has aroused great attention of scholars, who expect to apply it to future cancer treatment. Several CAR-T therapies have been officially approved in clinical use. Nevertheless, post-transfer T cell exhaustion and immunosuppression within the tumour region still constitute the major technical limitations. As an indispensable gene-editing tool with strong capacity in both biomedical research and clinical fields, CRISPR technology has strong potentials in facilitating adoptive T cell therapies to overcome the current barriers through full gene knockout on the engineered T cells. In this paper, the clinical feasibility and future prospect of the combined use of CRISPR-Cas9 and adoptive T cell therapies are analyzed using two latest studies for discussion and comparison. The studies indicate that CRISPR-Cas9 has facilitated to increase T cell persistence and potency in TCR therapy and CAR-T therapy respectively with acceptable safety profile, and it has shed the light for clinical use of CRISPR-Cas9 to increase the therapeutic effectiveness of adoptive T cell therapy. Future investigations are still needed to further assess its clinical safety and to understand the underlying mechanism of how CRISPR-Cas9 helps to extend the survival and increase anti-tumour response of the T cells within the tumour region.

1 INTRODUCTION

CRISPR, which stands for Clustered Regularly Interspaced Short Palindromic Repeats, is an indispensable tool in biological research. It was firstly found in archaea by Mojica et al. in 1995 (Mojica, Ferrer, Juez and Rodríguez-Valera 1995), and then later experimentally verified by Barrangou et al. in 2007 as the adaptive immune system of bacteria to fight against the invading viruses (Barrangou, Fremaux, Deveau, Richards, Boyaval and Moineau et al 2007). After years of development, CRISPR technology can now be modified to target specific sequence of the genetic code and perform gene-editing at a relatively precis location, and it has been widely applied in various field including biomedicine and agriculture. The function of CRISPR technology was mainly dependent on the CRISPR-associated (Cas) genes flanked by the sequence of CRISPR. Out of the various types of CRISPR-technology, the most widely applied one is CRISPR-Cas9, where Cas9 is an endonuclease guided by sgRNA (single guide RNA).

When Cas9 enters the nucleus, sgRNA facilitates the recognition of the PAM sequence (protospacer adjacent motif) and the target sequence, which consequently leads to the activation of PAM-dependent Cas9 nuclease. The consequent induced DNA cleavage will generate double strand breaks (DSB) and activate homologous recombination (HR) or non-homologous end joining (NHEJ) to achieve gene-editing (Sternberg, Redding, Jinek, Greene and Doudna 2014). Compared with previous gene-editing tools like Zinc Finger Nucleases (ZFNs) and Transcription activator-like effector nucleases (TALENs), CRISPR technologies allow rapid retargeting of DNA sequence without the requirement for manufacturing novel proteins for each target site. Such advantage and the high genome-editing efficiency of CRISPR allows it to facilitate the progress in oncology research and anticancer therapies through different modalities. In addition to genetic screening to discover potential

Wang, S.
Analysis on How CRISPR Technology Facilitates Anticancer Therapeutics.
DOI: 10.5220/0011197300003444
In *Proceedings of the 2nd Conference on Artificial Intelligence and Healthcare (CAIH 2021)*, pages 128-133
ISBN: 978-989-758-594-4

therapeutic targets, CRISPR technologies can be used to generate cell lines with specific gene deletions or to manipulate multiple genes to explore human malignancies. Compared with the traditional method of manipulations of germline cells to introduce driver mutation, CRISPR-Cas 9 can establish cancers more directly in animal models, which is less time consuming. More importantly, the combination of CRISPR-Cas9 and cancer immunotherapy can be a powerful therapeutic strategy in increasing clinical safety and efficacy (Yin, Xue and Anderson 2019). The aim of this review is to analyse the clinical feasibility of using CRISPR-Cas9 in cancer immunotherapy, especially in adoptive T cell therapy, and discuss the current limitation and the prospect of this direction in the future. Two latest studies are analyzed in this review to discuss how CRISPR-Cas9 has facilitated adoptive T cell therapy, where they used CRISPR-Cas9 to ex vivo knock out the genes of T cells to increase its therapeutical effectiveness. With the progress in gene-editing technologies and advances in immunotherapy, the investigations so far show that the combined use of CRISPR-Cas9 and immunotherapy has high translational potential for clinical use, and some of them have even been proved safe and effective in incipient clinical pilot study. Future studies still need to be carried out to expand our understanding in the underlying mechanism of the engineered T cells in tumour microenvironment and further prove its feasibility for wide clinical use.

2 CRISPR-CAS9 IN TCR THERAPY AND CAR-T THERAPY

2.1 CRISPR-Cas9 Knockout of the Gene Encoding PD-1 And Endogenous TCR Increases the Persistence of the Engineered T Cells in TCR Therapy

Adoptive T cell therapy is a type of immunotherapy that involves the direct extraction of T cells from the patient and conduct certain manipulation to increase its anti-tumour effectiveness. One of the adoptive T cell therapy called engineered T cell receptor (TCR) therapy, where T cells from the patients are isolated and genetically manipulated in vitro to express the synthetic T cell receptor that specifically target the cancer cells. However, previous studies have illustrated that the expression of α and β chains in endogenous TCR is related with the reduced the expression of therapeutic TCR due to the competitive expression, and programmed cell death protein 1 (PD-1) is negatively associated with the antigen response and persistence of the engineered T cells in the tumour region (Hamilton, Doudna 2020). The consequently reduced therapeutic efficacy constituted the major limitation of TCR therapy, and CRISPR-Cas9 may be a promising approach to overcome it through disrupting the genes associated with T cell exhaustion and the reduced antigen response.

In an article published in Science in 2020, Stadtmauer et al. conducted the first-in-human phase 1 clinical trial, where they aimed to investigate the use of CRISPR-Cas9 in improving the effectiveness and safety of TCR therapy on patients with advanced and refractory cancer (Stadtmauer, Fraietta, Davis, Cohen, Weber and Lancaster et al 2020). They hypothesised that the deletion of the genes encoding PD-1 and the α and β chain in endogenous TCR would improve the persistence of the engineered T cells and increase the feasibility of the initial TCR therapy. Referring to the graphical abstract (see Fig.1), CRISPR-Cas9 was used to knock out TRAC, TRBC, and PDCD1 over isolated T cells from the patients, and then they introduced the synthetic TCR transgene NY-ESO-1, which can specifically target at myeloma, melanoma, and sarcoma, through lentiviral transduction into the cells. The CRISPR-Cas9-engineered T cells were later infused back into the 3 patients with advanced and refractory cancer. The T cells were then tracked and monitored in vivo to determine if they could persist longer with better safety profile after the CRISPR-Cas9 modification. The results of the In vitro assessment indicated that cells modified by CRISPR-Cas9 has higher cytotoxicity than those retained the endogenous TCR, suggesting a higher potency. After the cell infusion, there is no evidence of T cell genotoxicity or overt side effect observed in the patients, and high level and sustained persistence of the engineered cells were found with antigen-specific cytotoxicity. Overall, the results of this trial overall indicated the acceptable safety profile of the CRISPR-modified transgenic T cells with higher sustained level and higher specificity targeting the tumour. However, the potential mechanism of the extended survival of the T cells was not investigated in this study, and whether the longer half-life of the engrafted transgenic T cells is attributed to PD-1 deficiency was not explained. Hence the transcriptional state of the modified T cells

within the tumour micro-environment can be investigated next step to see how the cytotoxicity and persistence are increased. In addition, only one patient out of the total three had the highest level of engraftment, which restricted the in vivo single-cell analysis, herein more patients for infusion of the cells with higher editing efficiencies and engraftment level are needed to fully assess the safety and feasibility in the use of CRISPR-Cas9 in TCR therapy in the future.

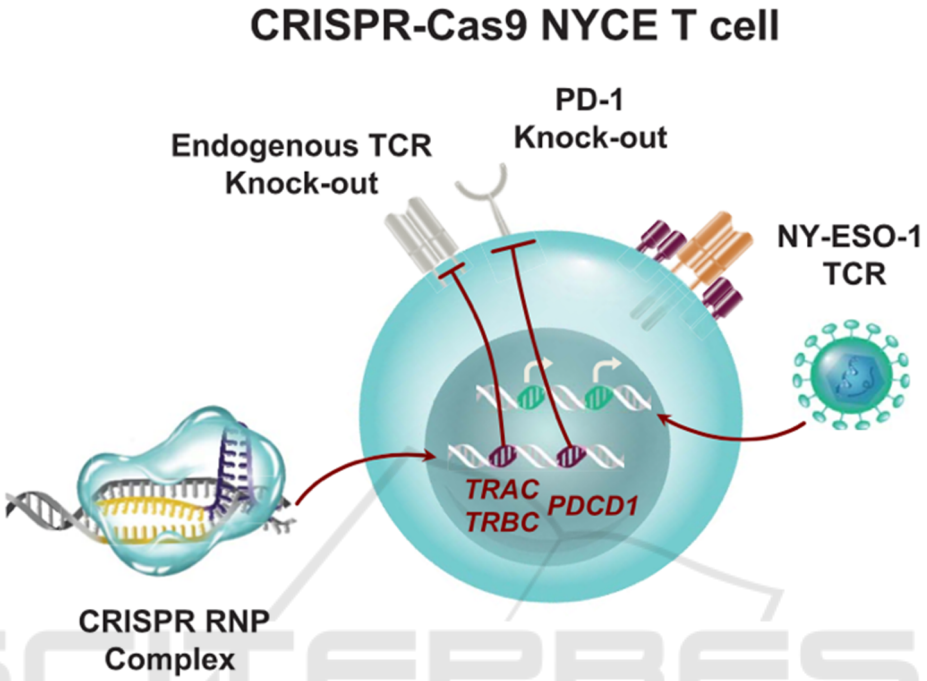

Figure 1: Graphical Abstract of using CRISPR-Cas9 to knock out the gene encoding PD-1 and endogenous TCR (Stadtmauer, Fraietta, Davis, Cohen, Weber and Lancaster et al 2020).

2.2 CRISPR-Cas9 Improves the Efficacy of CAR-T Therapy via Knockout of Adenosine Receptor

Similarly, CRISPR-Cas9 also shows great potential in improving the effectiveness of CAR-T therapy, which is another type of adoptive T cell therapy. Similar to TCR therapy, CAR-T therapy involves the process of T cell extraction with ex vivo transduction of the T cell with chimeric antigen receptor (CAR), which can specifically recognize a defined tumour antigen. Compared to TCR therapy, the introduction of CAR transgene in TRAC gene can knock out the gene encoding TCR simultaneously (Roth, Puig-Saus, Yu, Shifrut, Carnevale and Li et al 2018). However, one of the major barriers of CAR-T therapy is the effect of immunosuppression. Out of the multiple immunosuppressive pathways, the hypoxia-adenosine link is relatively prominent in tumour region, where adenosine binding to the receptor A2AR reduces the accumulation of intracellular cAMP and increases the production of anti-inflammatory factors in immune cells to suppress the immune response (Raker, Becker and Steinbrink 2016). Previous studies indicated that pharmacological blockade of A2AR is able to enhance the T-cell-mediated antitumour effect (Halpin-Veszeleiova, Hatfield 2020). Hence, these findings suggest the potential of targeting the immunosuppressive pathway via CIRSPR-Cas9 to improve the efficacy of adoptive T-cell therapy.

In May 2021, an article published in Nature by Giuffrida et al. It indicated that A2AR deletion can enhance the efficacy of CAR-T therapy via CRISPR-Cas9, which further proves the strong potential of the use of CRISPR technology in improving anticancer therapies compared with other methodologies (Giuffrida, Sek, Henderson, Lai, Chen and Meyran et al 2021). They delivered the recombinant Cas9 and sgRNA targeting at the gene encoding A2AR into naïve splenocytes via electroporation, followed by retroviral transduction of CAR targeting human Her2 cells. With the aim to investigate whether CRISPR-Cas9-mediated A2AR deletion could enhance CAR-T cell function, the level of cAMP signalling, in vivo

antitumour efficacy in mice models, and transcriptional profile of the engineered T cells were all assessed and analysed. It was then found the editing efficiency of CRISPR-Cas9 on the gene encoding A2AR could achieve more than 75% in human CAR-T cells and result in potent attenuation in the level of intracellular cAMP. Because of the high sensitivity of A2AR towards adenosine, A2AR knockdown via short hairpin RNA (shRNA) or pharmacological blockade is not effective enough in attenuating the conversion of ATP to cAMP. Instead, the full knockout of A2AR is sufficient to significantly suppress the immunosuppressive pathway mediated by adenosine in the function of CAR-T cells. The survival of the tumour bearing mice was significantly prolonged due to the enhanced inhibition of tumour growth by CAR-T cells after A2AR deletion, and the memory recall responses was able to get evoked. Through the analysis of the transcriptional profile, the suppressive effect on the production of pro-inflammatory cytokines like IFNγ and TNF by CD4+ and CD8+ mediated by hypoxia-adenosine pathway was also significantly reduced, indicating the enhanced therapeutic efficacy. It has been found that the increased CAR-T cell activation mediated by the knockdown or knockout of A2AR could enhance the expression of several effector-related genes including PD-1, granzyme B and Ki-67, which may compromise the persistence the T cells. Surprisingly, the deletion of A2AR by CRISPR-Cas9 had minimal effect in the persistence of the CAR-T cells compared with the control group, unlike knockdown or pharmacological blockade. However, the mechanisms that full knockout of A2AR by CRISPR-Cas9 uses to circumvent the reduction in persistence is unknown yet, and it may be related with the production of pro-survival factors in memory T cells. For next step, it would be interesting to analyse the difference in the expression of the memory associated genes between knockdown and knockout CAR-T cells to investigate the underlying mechanisms of the uncompromised persistence in CRISPR-modified CAR-T cells.

3 DISCUSSIONS

As discussed above, T-cell exhaustion and immunosuppression are major technical barriers that result in reduced efficacy and potency of the adoptive T cell therapy when the cells are infused back into the patients. Gene-editing technology like CRISPR-Cas9 can be an influential tool to overcome these limitations with increasing editing efficiencies and precisions over these years. Herein, two latest studies from 2020 and 2021 were discussed in this review to demonstrate how CRISPR-Cas9 helps with improving the effectiveness of CAR-T therapy and TCR therapy.

In these 2 studies, different pathways were targeted with similar aims, and both show promising future for clinical application. The first study used CRISPR-Cas9 to prevent the engineered T cell exhaustion through suppressing the apoptosis pathway via PD-1 knockout and to improve the expression of the synthetic TCR through disrupting the expression of endogenous TCR, and it has been proved safe and effective to improve the cell persistence in the first-in-human pilot study. Whereas the second study distinctively targeted the immunosuppressive pathway via A2AR knockout to increase the antitumour response, and it has been proved effective in improving the potency of the CAR-T cells in animal models. This has shed the light of utilizing CRISPR-Cas9 to target multiple immunosuppressive genes to improve the therapeutic efficacy of CAR-T therapy in the future. In contrast to the first study, the second study showed that A2AR deletion via CRISPR-Cas9 in T cells has increased the expression of PD-1. However, the persistence of the CAR-T cells is not significantly affected but with increased cytokine production, and this achieved a well-balanced trade-off between cell persistence and therapeutic efficacy. Compared with other immunosuppressive pathways, the adenosine-activated pathway is more prominent in hypoxia tumour microenvironment, which also equips it with advantageous efficacy profile. In terms of frequency of editing, the editing efficiencies of TRAC, TRBC, and PDCD in the first study are 45%, 15%, and 20% respectively, and it might be due to the limited progress in the CRISPR-based technology back in 2016 when their clinical trial application was approved, leading to higher off-target effect. Whereas the editing efficiency of A2AR reached over 75% in the latest paper here, suggesting the strong potential of CRISPR technology in facilitating anticancer therapies with increasing on-target editing efficiency over the years.

However, safety considerations are still important considerations regarding the permanent deletion of certain genes using CRISPR-Cas9. There are still limited studies using gene-editing technologies to target immunosuppressive pathways in CAR-T cells. Despite the in vivo assessment in mice models proved that A2AR-edied CAR-T cells are well tolerated with good safety profile through liver and

kidney toxicity analysis. Whether permanent A2AR deletion will induce excessive immune response against the host still needs further assessment and observation. In addition, PD-1 is not the only indicator of T-cell exhaustion which is also related with other immunoregulatory pathways like soluble factors IL-10 and regulatory T cells (Wherry 2011). Moreover, the underlying mechanism of increased persistence and improved potency of the modified T cells after PD-1 and A2AR knockout are not clear yet. Hence, despite the positive conclusion in early clinical trials and incipient in vivo assessment, the wide feasibility and long-term safety of CRISPR-modified engineered T cells still needs future investigations.

Nowadays, CRISPR-based technology has shown promising future in improving the effectiveness of immunotherapy to enhance its efficacy and reduce the toxicity. Immunotherapy is mainly based on the innate and adaptive immune system of the host to activate the specific immune response or reduce the immunosuppressive effect, including monoclonal antibodies, vaccine therapies, checkpoint inhibitors, and adoptive T cell therapies. The general principle of adoptive T cell therapy is to boost the antitumour response through ex vivo manipulation of the T cells to increase their ability and specificity targeting the tumour when they are infused back into the patient. The modalities include ex vivo expansion of tumour-infiltrating lymphocytes (TILs), the introduction of transgenic TCR that targets the major histocompatibility complex (MHC) to eradicate tumour cells, and gene transfer of chimeric antigen receptors that target specific antigen presented on the surface of the tumour cells . With the high response rate compared with conventional chemotherapy, adoptive T cell therapy has made huge progress in recent years. Several therapies have been approved for clinical use with large number of them in the stage of clinical trials. Notwithstanding such success in its early clinical application, adoptive T cell therapy are still facing various challenges. For example, CAR-T therapy is mainly used in haematological cancers, because of the high heterogeneity of the solid tumours leading to challenges for transgenic TCR targeting the tumours. Moreover, the nature of adoptive T cell therapy being highly personalized also make the manufacturing cost unexpectedly high and difficult to get industrialized. Hence allogeneic CAR T cells from have become a promising direction to reduce the manufacturing cost and simplify the procedure. However, the main barrier of using allogeneic T cells from healthy donors are the rejection by the immune system of the recipient and the toxicity resulted from the non-self antigen grafted from the recipient to the donor cells (Graham, Jozwik, Perpper and Benjamin 2018). Thus, in addition to improving the efficacy of the current adoptive T cell therapies, CRISPR-Cas9 can also be a useful tool to knock out the related genes expressed on the surface of the allogeneic T cells to reduce the rejection and toxicity (see Fig.2) and make the therapies “off -the-shelf” for most of the patients.

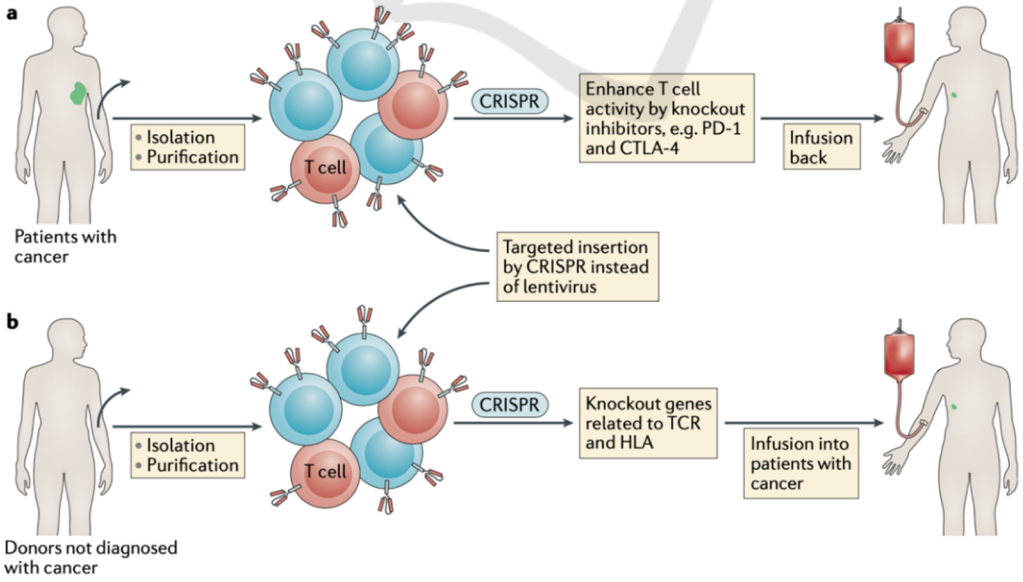

Figure 2: Graphical Abstract of using CRISPR-Cas9 on allogeneic T cells for CAR-T cells (Yin, Xue and Anderson 2019).

4 CONCLUSIONS

To sum up, this paper discusses the clinical feasibility of CRISPR-Cas9 being applied into adoptive T cell therapy and addresses the limitations of the current investigations. Gene-editing technology has overall showed high clinical translational potential in overcoming T cell exhaustion and immunosuppression to improve the therapeutical effectiveness of adoptive T cell therapy. Moreover, it is also promising to contribute to the development of allogeneic CAR-T therapy through reducing the toxicity and rejection effect via gene knockout and to further simplify the manufacturing process. However, safety considerations are still major concerns regarding the application of gene editing technologies. The underlying mechanisms of CRISPR-Cas9 in improving adoptive T cell therapies are still not fully understood yet, and the clinical feasibility and safety of CRISPR-Cas9 application in anticancer therapies still need further investigations, despite the incipient success.

REFERENCES

C.Graham, A. Jozwik, A. Pepper, R. Benjamin. Allogeneic CAR-T Cells: More than Ease of Access?. Cells. 2018;7(10):155.

E. Stadtmauer, J. Fraietta, M. Davis, A. Cohen, Weber, Lancaster E et al. CRISPR-engineered T cells in patients with refractory cancer. Science. 2020; 367(6481): eaba7365.

E.Wherry. T cell exhaustion. Nature Immunology. 2011; 12(6):492-499.

F. Mojica, C. Ferrer, G. Juez, F. Rodríguez-Valera. Long stretches of short tandem repeats are present in the largest replicons of the Archaea Haloferax mediterranei and Haloferax volcanii and could be involved in replicon partitioning. Molecular Microbiology. 1995;17(1):85-93.

H. Yin, W. Xue, D. Anderson. CRISPR–Cas: a tool for cancer research and therapeutics. Nature Reviews Clinical Oncology. 2019;16(5):281-295.

J. Hamilton, J. Doudna. Knocking out barriers to engineered cell activity. Science. 2020;367(6481):976-977.

K.Halpin-Veszeleiova, S. Hatfield. Oxygenation and A2AR blockade to eliminate hypoxia/HIF-1α-adenosinergic immunosuppressive axis and improve cancer immunotherapy. Current Opinion in Pharmacology. 2020; 53:84-90.

L.Giuffrida, K. Sek, M. Henderson, J. Lai, A. Chen, D. Meyran et al. CRISPR/Cas9 mediated deletion of the adenosine A2A receptor enhances CAR T cell efficacy. Nature Communications. 2021;12(1).

R. Barrangou, C. Fremaux, H. Deveau, M. Richards, P. Boyaval, S. Moineau et al. CRISPR Provides Acquired Resistance Against Viruses in Prokaryotes. Science. 2007;315(5819):1709-1712.

S. Sternberg, S. Redding, M. Jinek, E. Greene, J. Doudna. DNA interrogation by the CRISPR RNA-guided endonuclease Cas9. Nature. 2014;507(7490):62-67.

T. Roth, C. Puig-Saus, R.Yu , E.Shifrut , J. Carnevale, Li P et al. Reprogramming human T cell function and specificity with non-viral genome targeting. Nature. 2018;559(7714):405-409.

V. Raker, C. Becker, K.Steinbrink. The cAMP Pathway as Therapeutic Target in Autoimmune and Inflammatory Diseases. Frontiers in Immunology. 2016;7.