



**IJPPR**

INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH  
An official Publication of Human Journals

ISSN 2349-7203



Human Journals

**Research Article**

August 2018 Vol.:13, Issue:1

© All rights are reserved by Haiqing Yu et al.

# Applications of the CRISPR–Cas9 System in Cancer Therapy, an Emphasis on Three Clinical Trials



**Fanding Gao<sup>1,2</sup>, Qing Hong<sup>3</sup>, Hong Yu<sup>4</sup>, Haiqing Yu<sup>5\*</sup>**

*1. University of Missouri of Missouri, Columbia, MO  
65212, USA*

*2. Northwestern University Feinberg School of Medicine,  
Chicago, IL 60611, USA*

*3. Mechanical & Aerospace Engineering, University of  
Missouri, Columbia, MO 65211, USA*

*4. Department of Medical Pharmacology and Physiology,  
University of Missouri, Columbia, MO 65212, USA*

*5. Department of Internal Medicine, University of  
Missouri Hospital and Clinics, Columbia, MO 65212,  
USA*

**Submission:** 24 July 2018

**Accepted:** 31 July 2018

**Published:** 30 August 2018

**Keywords:** CRISPR-Cas9; genome editing; Cancer; tumor; Immunotherapy

## ABSTRACT

The clustered regularly interspaced short palindromic repeats (CRISPR) and CRISPR-associated protein 9 (Cas9) system are much more precise and efficient than other methods in the field of genome editing. It also brings new insights into cancer therapy. Currently, no recent review on its application in the field of clinical cancer therapy is presented. This review summarizes the applications of the CRISPR-Cas9 system in the field of cancer therapy, in which three widely reported clinical trials were emphasized. The CRISPR-Cas9-based clinical application is focusing on cancer immunotherapy by modifying primary human T cells, especially for the disruption of the PD-1 gene.



HUMAN JOURNALS

[www.ijppr.humanjournals.com](http://www.ijppr.humanjournals.com)

## INTRODUCTION

The clustered regularly interspaced short palindromic repeats (CRISPR) and CRISPR-associated protein 9 (Cas9) systems, CRISPR-Cas9 system, is a genome-editing technique patented in 2012 in the United States. It is a natural occurred prokaryotic immune system in most bacteria and archaea that have the machinery to defense invading viruses and phages, and to change genes within organisms [1, 2]. After using in the field of genome editing of eukaryotic cells and animal models of human disease, the technique becomes very popular due to its simplicity, ease of use, adaptability and flexibility to different targets [3, 4].

Cancer is the second most common cause of death in the United States, running only behind heart disease. About 1,735,350 new cancer cases are expected to be diagnosed in 2018. It is also estimated that cancer was responsible for 609,640 deaths in the United States in 2018 [5]. One reason for a high death rate in cancer patients is that cancer is a genetically complex disease. It is characterized by the accumulation of multiple genetic and epigenetic alterations in oncogenes and tumor-suppressor genes [6]. Another reason is that patients are often countered by the cancer therapeutic resistance. Despite the use of surgery, chemotherapy, radiation therapy and immunotherapy, the overall outcome for cancer cure continues to be disappointing in clinics [7]. Therefore, new strategies to combat cancer are still needed in clinics.

Among the newly developed strategies, the CRISPR-Cas9 system brings new insights into cancer therapy. Although this cutting-edge technology is widely used in genome editing, no recent review on its application in the field of cancer therapy is presented so far. In this review, we try to give a summary regarding CRISPR-Cas9 and cancer therapy for the most recent five years.

## MATERIAL AND METHODS

Based on the published paper in PubMed or report online (up to June 30, 2018), Information items were searched using the keywords 'CRISPR-Cas9 and cancer' or 'CRISPR-Cas9 and tumor', and the application of CRISPR-Cas9 in cancer therapy is summarized in the current

review with an emphasis on three widely reported clinical trials.

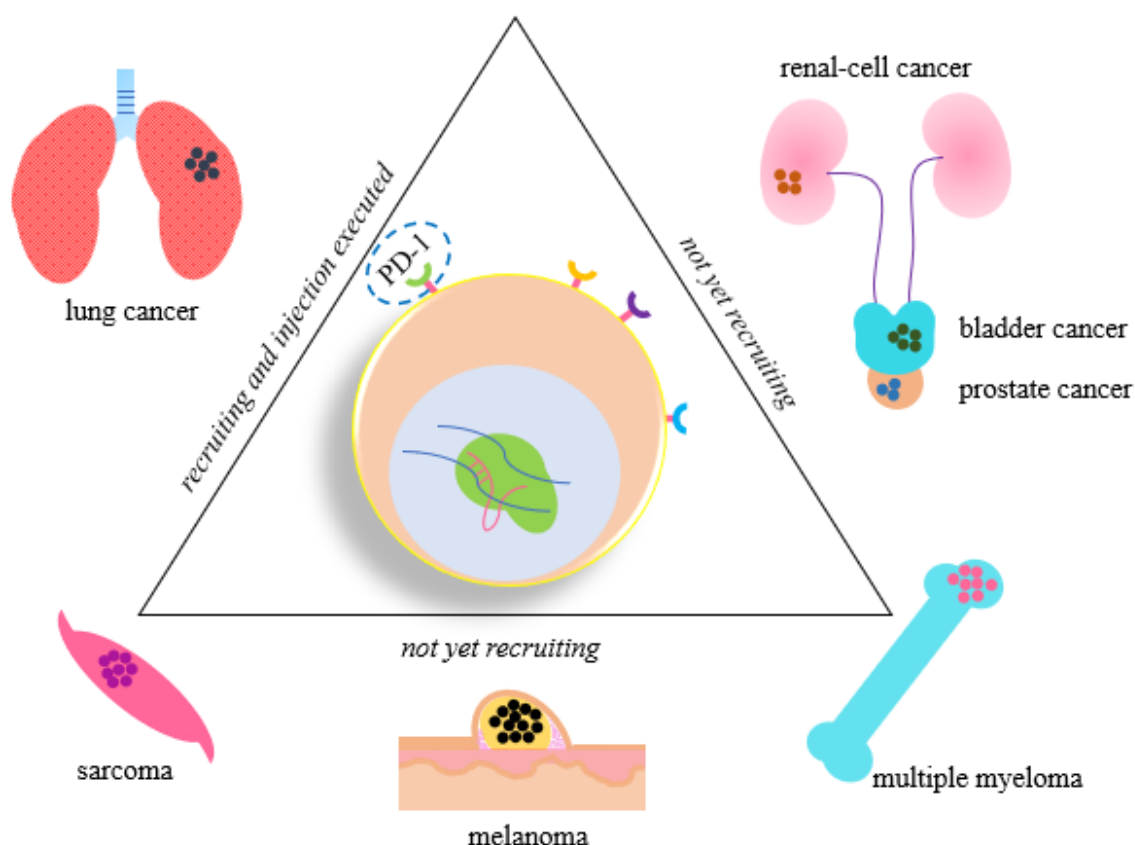
## RESULTS AND DISCUSSION

Totally, 18 hits were found involving the CRISPR-Cas9 and cancer therapy in the current search (**Table 1**), in which three clinical trials were widely reported (**Figure 1**). This information is listed and discussed as below.



**Table 1: CRISPR-Cas9 system and its application in the field of cancer therapy.**

No.	Delivery methods of CRISPR-Cas9	CRISPR gene editing	Acceptors	Potential types of cancer treatment	Year	References
1	Electroporation	Knockout of programmed death-1 (PD-1) gene in primary human T cells	M14 cells, AGS-EBV cells	Melanoma Stomach adenocarcinoma	2016	9
2	Electroporation	Disruption of programmed cell death protein 1 (PD-1) gene in primary human chimeric antigen receptor (CAR) T cells	K562 cells, a xenograft tumor mouse model	Myelogenous leukemia	2017	10
3	N/A	Knockout of programmed cell death protein 1 (PDCD1) gene in patient T cells	Patients at phase 1	Metastatic non-small cell lung cancer	2016	11
4	N/A	Knockout of programmed cell death protein 1 (PDCD1) gene in patient T Cells	Patients at phase 1	Renal-cell cancer Castration-resistant prostate cancer Muscle-invasive bladder cancer	2016	11, 12, 13
5	N/A	Knockout of programmed cell death protein 1 (PDCD1) gene and editing other two genes (insert one and remove one) in patient T cells	Patients at phase 1	Multiple myelomas Sarcoma Melanoma	2016	14
6	Polyethyl enimine	Knockdown of urothelial carcinoma-associated 1 (UCA1) gene in bladder cancer cells	5637 and T24 cells, nude mice	Bladder cancer	2017	15
7	Lipofectamine 2000	Disruption of the LacI gene of an engineered vector in bladder cancer cells	T24, 5637, SW-780 and J82 cells	Bladder cancer	2014	16
8	Electroporation	Correction of additional sex combs-like 1 (ASXL1 mutation in myeloid leukemia cell	KBM5 cells	Chronic myeloid leukemia	2015	17
9	Lentiviral transduction	Knockout p53 gene by introducing small indel mutations and long deletions	H460 and HCT116 cells	Large cell lung cancer Colorectal carcinoma	2016	18
10	Electroporation	Mutagenesis scanning of genes sensitive to three cancer drugs	HAP1 and K-562 cells	Chronic myeloid leukemia Myelogenous leukemia cell line	2018	19



**Figure 1: Three widely reported clinical trials that are approved for cancer therapy (outside the triangle, references [11-14]) by using CRISPR-Cas9-mediated gene editing in primary human T-cells (inside the triangle). The blue dash oval indicates the PD-1 disruption. The small dots represent the cancer cells growing in different tissues.**

Cancer immunotherapy by using CRISPR-Cas9 genome editing technique has become one of the most attractive applications in cancer treatment, particularly for chimeric antigen receptor (CAR) T cells that recognize specific antigens on cancer cells [8]. Recently, the CRISPR-Cas9-based gene editing raises the prospect of enhanced CAR T cell therapy via gene modification and/or disruption by establishing an sgRNA-Cas9-based effective gene disruption method for the highly efficient disruption of PD-1 on primary human T cells, which mediated cellular immune response and activate cytotoxic T cells on cancer cell lines [9]. Levis et al. demonstrated the improvement of anti-tumor efficacy by combining CRISPR-mediated gene editing with lentiviral transduction of CAR T cells [10].

In 2015, Sichuan University's West China Hospital in Chengdu received ethical approval to

test the cells in people with lung cancer, which is the first trial of CRISPR-Cas9 application in people [11]. Other clinical trials of testing CRISPR-Cas9 modified human T cells to treat castration-resistant prostate cancer and muscle-invasive bladder cancer are underway [12, 13]. Meanwhile, US National Institutes of Health (NIH) approved the clinical trial for the use of the CRISPR-Cas9 technique to engineer human T cells to attack melanoma, multiple myeloma and sarcoma [14].

The CRISPR-Cas9 system has also been applied in correcting gene mutations as an anticancer therapy, combating oncogenic virus-associated cancers, and screening anticancer drug target and resistant gene. The CRISPR-Cas9 system is used to inhibit the up-regulator of cancers, which successfully activated tumor suppressors, effectively inhibited cancer cell proliferation, migration, and invasion, induced cancer cell apoptosis, and decreased cell motility [15, 16]. By mediating certain tumor suppressors, CRISPR-Cas9 gene editing therapy shows a great promising to address cancer-caused mutations [17]. The CRISPR-Cas9 system is also used in specific drug target validation of resistance mutation and screening for drug resistance genes [18, 19].

Overall, the CRISPR-Cas9 genome editing technique has been a popular star for cancer modeling and treatment explorations to precisely target genes in the cancer cellular genome [20]. The technique is revolutionizing gene therapy for cancer genome editing, including engineering of immune cells and oncolytic viruses for cancer immunotherapeutic applications [21, 22].

## CONCLUSION

Although there are still technical limitations by using CRISPR-Cas9 to edit cancer genes in patients as a therapeutic strategy, the potential of this technology for developing anticancer approaches is making a big process. Its clinical application is focusing on cancer immunotherapy by modifying primary human T cells, especially for the disruption of the PD-1 gene. In future, successful immunotherapy clinical trials will be most likely reported, and the CRISPR-Cas9 technique may also show a great success in curing patients with

genetic mutations linked to certain cancers.

## ACKNOWLEDGMENTS

The authors contribute equally to the present study.

## CONFLICT OF INTEREST

There is no conflict of interest.

## REFERENCES

1. Isakov N. Future Perspectives for Cancer Therapy Using the CRISPR Genome Editing Technology. *J Clin Cell Immunol.* 2017; 8: e120.
2. Bhaya D, Davison M, Barrangou R, CRISPR-Cas systems in bacteria and archaea: versatile small RNAs for adaptive defense and regulation. *Ann Rev of Gen.* 2011; 45: 273-297.
3. White MK, Khalili K. CRISPR/Cas9, and cancer targets: future possibilities and present challenges. *Oncotarget.* 2016; 7(11): 12305.
4. Ran FA, Hsu PD, Wright J, Agarwala V, Scott DA and Zhang F. Genome engineering using the CRISPR-Cas9 system. *Nat Protoc.* 2013; 8: 2281-2308.
5. Rebecca L. Siegel MPH, Kimberly D. Miller MPH, and Ahmedin Jemal DVM. Cancer statistics, 2018. *CA Cancer J CLIN.* 2018; 68(1):7-30.
6. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. *Cell.* 2011; 144(5): 646-674.
7. Baskar R, Dai J, Wenlong N, Yeo R and Yeoh KW. Biological response of cancer cells to radiation treatment. *Frontiers in molecular biosciences.* 2014; 1: 24.
8. Ren J, Zhao Y. Advancing chimeric antigen receptor T cell therapy with CRISPR/Cas9. *Protein & cell.* 2017; 8(9): 634-643.
9. Su S, Hu B, Shao J, Shen B, Du J, Du Y, Zhou J, Yu L, Zhang L, Chen F, Sha H, Cheng L, Meng F, Zou Z, Huang X and Liu B. CRISPR-Cas9 mediated efficient PD-1 disruption on human primary T cells from cancer patients. *Scientific reports.* 2016; 6: 20070.
10. Rupp LJ, Schumann K, Roybal KT, Gate RE, Ye CJ, Lim WA and Marson A. CRISPR/Cas9-mediated PD-1 disruption enhances anti-tumor efficacy of human chimeric antigen receptor T cells. *Scientific reports.* 2017; 7(1): 737.
11. Cyranoski D. CRISPR gene-editing tested in a person for the first time. *Nature.* 2016; 539(7630): 479.
12. PD-1 knockout engineered t cells for castration-resistant prostate cancer. *ClinicalTrials.gov*, <https://clinicaltrials.gov/ct2/show/NCT02867345?term=CRISPR&rank=2> 2016.
13. PD-1 knockout engineered T cells for muscle-invasive bladder cancer. *ClinicalTrials.gov*, <https://clinicaltrials.gov/ct2/show/NCT02863913?term=CRISPR&rank=3> 2016.
14. Reardon S. First CRISPR clinical trial gets the green light from the US panel. *Nature News.* 2016.

15. Zhen S, Hua L, Liu YH, Sun XM, Jiang MM, Chen W, Zhao L and Li X. Inhibition of long non-coding RNA UCA1 by CRISPR/Cas9 attenuated malignant phenotypes of bladder cancer. *Oncotarget*. 2017; 8(6): 9634.
16. Liu Y, Zeng Y, Liu L, Zhuang C, Fu X, Huang W, Cai Z. Synthesizing AND gate genetic circuits based on CRISPR-Cas9 for identification of bladder cancer cells. *Nature communications*. 2014; 5: 5393.
17. Valletta S, Dolatshad H, Bartenstein M, Yip BH, Bello E, Gordon S, Yu Y, Shaw J, Roy S, Scifo L, Schuh A, Pellagatti A, Fulga TA, Verma A and Boulton J. ASXL1 mutation correction by CRISPR/Cas9 restores gene function in leukemia cells and increases survival in mouse xenografts. *Oncotarget*. 2015; 6(42): 44061.
18. Wanzel M, Vischedyk JB, Gittler MP, Gremke N, Seiz JR, Hefter M, Noack M, Savai R, Mernberger M, Charles JP, Schneikert J, Bretz AC, Nist A and Stiewe T. CRISPR-Cas9-based target validation for p53-reactivating model compounds. *Nature chemical biology*. 2016; 12(1): 22.
19. Neggers JE, Kwanten B, Dierckx T, Noguchi H, Voet A, Ball L, Minner K, Massant B, Kint N, Delforge M, Vercruyse T, Baloglu E, Senapedis W, Jacquemyn M and Daelemans D. Target identification of small molecules using large-scale CRISPR-Cas mutagenesis scanning of essential genes. *Nature communications*. 2018; 9(1): 502.
20. Sánchez-Rivera FJ, Jacks T. Applications of the CRISPR– a Cas9 system in cancer biology. *Nature reviews cancer*. 2015; 15(7): 387.
21. Sayin VI, Papagiannakopoulos T. Application of CRISPR-mediated genome engineering in cancer research. *Cancer letters*. 2017; 387: 10-17.
22. Plummer M, de Martel C, Vignat J, Ferlay J, Bray F and Franceschi S. Global burden of cancers attributable to infections in 2012: a synthetic analysis. *The Lancet Global Health*. 2016; 4(9): e609-e616.

