IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Application No.	:	16/704,857	Conf. No.: 7285
First Named Inventor	:	Feng Zhang	
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TC/AU	:	1652	
Examiner	:	Raghu, Ganapathiram	
Customer No.	:	198696	
Docket No.	:	BROD-4450US	
Title	:	CAS PROTEINS WITH REDUCED IMMUNOGENICITY AND METHODS OF SCREENING THEREOF	

AMENDMENT AND RESPONSE

FILED VIA EFS-WEB

Commissioner:

In response to the Final Office Action mailed August 29, 2024 ("Office Action"), please enter and consider the following amendments and remarks.

AMENDMENT

In the Claims

Please amend the claims as follows. This listing of claims will replace all prior versions and listings of claims in this application.

1. **(Currently amended)** A method of reducing immunogenicity of a *S. pyogenes* CRISPR-associated (SpCas9) protein as compared to a naturally occurring SpCas9 protein ~~which comprises~~ by mutating one or more immunogenic T cell epitopes in a SpCas9 protein; said method comprising the steps of

determining immunogenicity of the one or more T cell epitopes ~~selected from the group consisting of~~

~~WRQLLNAKL (SEQ ID NO: 223), VITLKSKLV (SEQ ID NO: 224), LFDDKVMKQ (SEQ ID NO: 226), LIHDDSLTF (SEQ ID NO: 227), LVSDFRKDF (SEQ ID NO: 228), FLAAKNLSD (SEQ ID NO: 230), FKTEITLAN (SEQ ID NO: 232), IEKILTFRI (SEQ ID NO: 233), FFYSNIMNF (SEQ ID NO: 234), IYHLRKKLV (SEQ ID NO: 236), LAHMIKFRG (SEQ ID NO: 237), ILSARLSKS (SEQ ID NO: 238), LKALVRQQL (SEQ ID NO: 239), YKFIKPILE (SEQ ID NO: 240), FATVRKVLS (SEQ ID NO: 241), LTLLKALVR (SEQ ID NO: 242), FYKFIKPIL (SEQ ID NO: 243), LFKTNRKVT (SEQ ID NO: 244), MKQLKRRRY (SEQ ID NO: 245), WGRLSRKLI (SEQ ID NO: 246), LKRTARRRY (SEQ ID NO: 247), ARLSKSRRL (SEQ ID NO: 248), IYLALAHMI (SEQ ID NO: 250), MIKFRGHFL (SEQ ID NO: 251), and FLYLASHYE (SEQ ID NO: 252)~~ by measuring affinity of a peptide containing the T cell epitope for one or more MHC molecules,

wherein the amino acid sequence of the peptide is not ILLSDILRV (SEQ ID NO: 225), ILADANLDK (SEQ ID NO: 229), FYSNIMNFF (SEQ ID NO: 231), FKTEITLAN (SEQ ID NO: 232), FFYSNIMNF (SEQ ID NO: 234), YLASHYEKL (SEQ ID NO: 235), FATVRKVLS (SEQ ID NO: 241), LKRTARRRY (SEQ ID NO: 247), or LLFKTNRKV (SEQ ID NO: 249);

ordering the T cell epitopes of the SpCas9 protein based on immunogenicity;

mutating one or more of the most immunogenic T cell epitopes;

determining for the reduced immunogenicity SpCas9 protein, Cas-crRNA complex formation and binding to a PAM-containing target; and

if the reduced immunogenicity SpCas9 protein can bind to a PAM-containing target, performing phage-assisted continuous evolution (PACE) to restore or improve Cas-crRNA complex formation and binding to a PAM-containing target of the reduced immunogenicity SpCas9 protein.

2. (**Canceled**)

3. (**Canceled**)

4. (**Canceled**)

5. (**Previously Presented**) The method of claim 1, which comprises mutating the SpCas9 proteins containing one or more mutations at one or more amino acid positions and screening the mutant proteins for one or more Cas activities.

6. (**Canceled**)

7. (**Canceled**)

8. (**Previously Presented**) The method of claim 1, wherein nuclease activity of the SpCas9 protein is preserved.

9. (**Previously Presented**) The method of claim 1, wherein one or more catalytically active site residues of the SpCas9 protein are unchanged.

10. (**Original**) The method of claim 1, wherein one or more residues that determine complex formation with a guide are unchanged.

11. (**Previously Presented**) The method of claim 1, wherein target specificity of a CRISPR system comprising the SpCas9 protein is maintained or increased.

12. (**Original**) The method of claim 1, which comprises deleting, inserting, or mutating one or more amino acids in the immunogenic T cell epitope.

13. (**Previously Presented**) The method of claim 1, wherein identification of a T cell epitope comprises

determining the sequence of one or more peptides from the SpCas9 protein that bind to an MHC receptor; or

comparison of the CRISPR protein to a database of peptides that bind to an MHC receptor.

14. (**Canceled**)

15. (**Previously Presented**) The method of claim 13, wherein the comparison is *in silico*.

16. (**Previously Presented**) The method of claim 13 wherein the MHC receptor is a class II MHC receptor.

17. (**Canceled**)

18. (**Canceled**)

19. (**Canceled**)

20. (**Canceled**)

21. (**Previously Presented**) The method of claim 1, wherein the SpCas9 protein is associated with a functional domain.

22. (**Previously Presented**) The method of claim 21, wherein the functional domain comprises:

a mutation that reduces immunogenicity;

an activator, a repressor, or a DNA methylase; or

a base editor.

23. (**Canceled**)

24. (**Canceled**)

25. (**Withdrawn**) An engineered Cas protein which comprises at least one mutated T cell epitope, wherein the T cell epitope has reduced immunogenicity as compared to the corresponding T cell epitope of a naturally occurring Cas protein, whereby the engineered Cas protein comprises reduced immunogenicity as compared to the naturally occurring Cas protein.

26. (**Canceled**)

27. (**Withdrawn**) The engineered Cas protein of claim 25, wherein the immunogenicity of the CRISPR protein is measured in a host.

28. (**Canceled**)

29. (**Withdrawn**) The polypeptide of claim 25, wherein the Cas protein comprises at least one T cell epitope mutation.

30. (**Withdrawn**) The polypeptide of claim 29, wherein the mutation comprises an insertion, deletion, or substitution.

31. (**Withdrawn**) The polypeptide of claim 25, wherein the Cas protein is glycosylated.

32. (**Withdrawn**) The polypeptide of claim 25, wherein the polypeptide comprises one or more nuclear localization signals (NLS).

33. (**Withdrawn**) The polypeptide of claim 25, wherein the engineered Cas protein:

comprises a Cas nuclease catalytic site;

is a nickase; or

is catalytically inactive.

34-49. (**Canceled**)

50. (**Withdrawn**) A nucleic acid encoding the engineered Cas protein of claim 28.

51. (**Withdrawn**) A cell comprising the nucleic acid of claim 50.

52. (**Withdrawn**) A composition comprising the engineered Cas protein of claim 25 or a nucleotide sequence encoding the Cas protein, and at least one guide designed to form a complex with the Cas protein or at least one nucleotide sequence encoding the at least one guide, wherein the guide is designed to hybridize with a target sequence of a DNA molecule in a cell.

53. (**Canceled**)

54. (**Canceled**)

55. (**Withdrawn**) The composition of claim 52, wherein the guide comprises

a chimeric RNA; or

a crRNA and a tracrRNA.

56. (**Canceled**)

57. (**Withdrawn**) The composition of claim 52, further comprising a homologous recombination (HR) template.

58-63. (**Canceled**)

64. (**Withdrawn**) A vector system for providing the composition of claim 52, which comprises one or more vectors comprising:

a first regulatory element operably linked to a nucleotide sequence encoding a deimmunized Cas protein, and

i) a) a second regulatory element operably linked to a nucleotide sequence encoding the crRNA, and

b) a third regulatory element operably linked to a nucleotide sequence encoding the tracrRNA,

ii) a second regulatory element operably linked to a nucleotide sequence encoding the crRNA and the tracr RNA, or

iii) a second regulatory element operably linked to a nucleotide sequence encoding a guide sequence.

65. (**Canceled**)

66. (**Canceled**)

67. (**Withdrawn**) The vector system of claim 64, wherein the one or more vectors comprise one or more retroviral, lentiviral, adenoviral, adeno-associated or herpes simplex viral vectors.

68. (**Withdrawn**) A delivery system configured to deliver an engineered Cas protein of claim 25 or a coding sequence thereof,

i) a crRNA comprising

a) a 5' guide sequence designed to hybridize to a target DNA sequence, and

b) a 3' direct repeat sequence, and

ii) a tracr RNA, or a guide,

whereby there is formed a CRISPR complex comprising the Cas protein complexed with the crRNA and the tracr RNA, or the guide.

69-71. (**Canceled**)

72. (**Withdrawn**) The delivery system of claim 68, which comprises a delivery vehicle comprising liposome(s), particle(s), exosome(s), microvesicle(s), a gene-gun or one or more viral vector(s).

73-77. (**Canceled**)

78. (**Withdrawn**) A method of modifying a target nucleic acid, the method comprising contacting the target nucleic acid with one or more engineered compositions comprising:

a) an engineered Cas protein of claim 73, having reduced immunogenicity compared to a naturally occurring Cas protein,

b i) a crRNA comprising a) a guide sequence designed to hybridize to the target DNA sequence, and b) a direct repeat sequence, and ii) optionally a tracr RNA, or a guide,

whereby there is formed a CRISPR complex comprising the Cas protein complexed with the crRNA and, optionally, with the tracr RNA, or the guide,

wherein the guide sequence directs sequence-specific binding to the target nucleic acid sequence in a cell, whereby expression of the target locus of interest is modified.

79-84. (**Canceled**)

85. (**Withdrawn**) A cell comprising a modified target of interest, wherein the target of interest has been modified according to the method of claim 78.

86. (**Withdrawn**) A method of modifying a target DNA in a mammal, which comprises delivering the system of claim 68.

87. (**Withdrawn**) The method of claim 86, wherein the mammal is a human, a non-human primate, a canine, a feline, an bovine, a porcine, an ovine, a rat, a mouse.

88. (**Withdrawn**) The method of claim 86, which further comprises inducing tolerance to the Cas protein.

89. (**Withdrawn**) An engineered composition for site directed base editing comprising a targeting domain and a adenosine or cytidine deaminase, wherein the deaminase has reduced immunogenicity compared to a naturally occurring deaminase.

90-126. (**Canceled**)

127. (**Previously Presented**) The method of claim 1, wherein in the PACE step the selection phage (SP) encodes a catalytically dead mutant of the reduced immunogenicity SpCas9 protein, wherein the SpCas9 protein is fused to the ω subunit of bacterial RNA polymerase, and wherein the accessory plasmid (AP) encodes an sgRNA and a PAM and protospacer upstream of the M13 phage gene III, whereby mutations in the reduced immunogenicity SpCas9 protein restore or improve Cas-crRNA complex formation and binding to the PAM-containing target causing increased gene III expression.

128. (**Previously Presented**) The method of claim 1, wherein ten or more of the most immunogenic T cell epitopes are mutated.

129. **(Currently amended)** The method of claim 1, wherein the ~~immunogenicity of the selected SpCas9 T cell epitope is evaluated in combination with another immunogenic T cell epitope selected from the group consisting of ILLSDILRV (SEQ ID NO: 225), ILADANLDK (SEQ ID NO: 229), FYSNIMNFF (SEQ ID NO: 231), YLASHYEKL (SEQ ID NO: 235) and LLFKTNRKV (SEQ ID NO: 249).~~ T cell epitopes peptides comprise the amino acid sequence of WRQLLNAKL (SEQ ID NO: 223), VITLKSKLV (SEQ ID NO: 224), LFDDKVMKQ (SEQ ID NO: 226), LIHDDSLTF (SEQ ID NO: 227), LVSDFRKDF (SEQ ID NO: 228), FLAAKNLSD (SEQ ID NO: 230), IEKILTFRI (SEQ ID NO: 233), IYHLRKKLV (SEQ ID NO: 236), LAHMIKFRG (SEQ ID NO: 237), ILSARLSKS (SEQ ID NO: 238), LKALVRQQL (SEQ ID NO: 239), YKFIKPILE (SEQ ID NO: 240), LTLLKALVR (SEQ ID NO: 242), FYKFIKPIL (SEQ ID NO: 243), LFKTNRKVT (SEQ ID NO: 244), MKQLKRRRY (SEQ ID NO: 245), WGRLSRKLI (SEQ ID NO: 246), ARLSKSRRL (SEQ ID NO: 248), IYLALAHMI (SEQ ID NO: 250), MIKFRGHFL (SEQ ID NO: 251), or FLYLASHYE (SEQ ID NO: 252).

REMARKS

Status of the Claims

Claims 1, 5, 8–13, 15–16, 21–22, 25, 27, 29–33, 50–52, 55, 57, 64, 67–68, 72, 78, 85–89, and 127–129 are pending, with claims 1, 25, and 89 being independent. Claims 2–4, 6–7, 14, 17–20, 23–24, 26, 28, 34–49, 53–54, 56, 58–63, 65–66, 69–71, 73–77, 79–84, and 90–126 are canceled without prejudice or disclaimer as to the subject matter recited therein. Claims 25, 27, 29–33, 50–52, 55, 57, 64, 67–68, 72, 78, and 85–89 are withdrawn as being directed to the non-elected invention(s) pursuant to the Restriction Requirement of January 6, 2021. Claims 1 and 29 are amended. Claims 1, 5, 8–13, 15–16, 21–22, and 127–129 are under examination on the merits.

Claim 1 is amended to require "wherein the amino acid sequence of the peptide is **not** ILLSDILRV (SEQ ID NO: 225), ILADANLDK (SEQ ID NO: 229), FYSNIMNFF (SEQ ID NO: 231), FKTEITLAN (SEQ ID NO: 232), FFYSNIMNF (SEQ ID NO: 234), YLASHYEKL (SEQ ID NO: 235), FATVRKVLS (SEQ ID NO: 241), LKRTARRRY (SEQ ID NO: 247), or LLFKTNRKV (SEQ ID NO: 249)." (Emphasis added)

Written description for this negative *proviso* can be found, for example, in Example 1, paragraphs [1761] to [1762]. With respect to the written description requirement for negative claim limitations, MPEP 2173.05(i) states:

> Any negative limitation or exclusionary proviso must have basis in the original disclosure. ***If alternative elements are positively recited in the specification, they may be explicitly excluded in the claims.*** See In re Johnson, 558 F.2d 1008, 1019 (CCPA 1977) ("[the] specification, having described the whole, necessarily described the part remaining."). (Emphasis added)

No new matter has been added.

Unless explicitly stated otherwise, none of the amendments to the claims were made for reasons substantially related to the statutory requirements for patentability. Furthermore, unless otherwise indicated, the amendments to the claims were made to make explicit what had been implicit in the claims as originally worded and, therefore, are not narrowing amendments that would create any type of prosecution history estoppel.

Objections to the Claims

In the Office Action, claim 70 is objected to because of alleged informalities. The applicant has amended claim 70 to correct the errors, thereby rendering the objection moot. Withdrawal of the objection is respectfully requested.

Claim Rejections Under 35 U.S.C. § 103 - Obviousness

In the Office Action, the Examiner rejected claims 1, 5, 8-13, 15-16, 21-22 and 127-129 as allegedly being obvious over Payne et al., US 2018/0319850 ("Payne"), now U.S. Patent No. 11,566,052, and further in view of Ferdosi, (Ph.D., Thesis, Arizona State University, December 2017, pages 1-118), ("Ferdosi"), Ewaisha, et al., (US 11,208,640), ("Ewaisha"), Liu et al., US 2020/0172931 ("Liu"), and Hu *et al*. Nature, 2018, Vol. 556:57-63 ("Hu").

Applicant respectfully traverses the rejection.

In response to the Non-Final Office Action, the Examiner alleged the cited primary and secondary references disclose and identify T cell epitopes of claims 1 and 129 as previously presented.

According to the Examiner, Payne discloses T cell epitopes that include the peptide sequences SEQ ID NO: 231, SEQ ID NO: 232, SEQ ID NO: 234, SEQ ID NO: 241; and SEQ ID NO: 247, Ferdosi discloses T cell epitopes that include the peptide sequences ILLSDILRV (SEQ ID NO: 225), YLASHYEKL (SEQ ID NO: 235) & LLFKTNRKV (SEQ ID NO: 249), and Ewaisha discloses T cell epitopes that include the peptide sequences ILADANLDK (SEQ ID NO: 229) and SEQ ID NO: 225; SEQ ID NO: 235 and SEQ ID NO: 249.

The combined teachings of the references cited by the Examiner, therefore allegedly teach the following T cell epitopes having an amino acid sequence of:

ILLSDILRV (SEQ ID NO: 225)
ILADANLDK (SEQ ID NO: 229)
FYSNIMNFF (SEQ ID NO: 231)
FKTEITLAN (SEQ ID NO: 232)
FFYSNIMNF (SEQ ID NO: 234)
YLASHYEKL (SEQ ID NO: 235)
FATVRKVLS (SEQ ID NO: 241)
LKRTARRRY (SEQ ID NO: 247)
LLFKTNRKV (SEQ ID NO: 249)

Claim 1 is amended to require a negative *proviso* that excludes these 9 T cell epitopes.

Claim 129 is amended to require the 21 remaining T cell epitopes that, according to the Examiner, are **not** taught by any cited references, i.e., the peptides having an amino acid sequence of

WRQLLNAKL (SEQ ID NO: 223)
VITLKSKLV (SEQ ID NO: 224)
LFDDKVMKQ (SEQ ID NO: 226)
LIHDDSLTF (SEQ ID NO: 227)
LVSDFRKDF (SEQ ID NO: 228)
FLAAKNLSD (SEQ ID NO: 230)
IEKILTFRI (SEQ ID NO: 233)
IYHLRKKLV (SEQ ID NO: 236)
LAHMIKFRG (SEQ ID NO: 237)
ILSARLSKS (SEQ ID NO: 238)
LKALVRQQL (SEQ ID NO: 239)
YKFIKPILE (SEQ ID NO: 240)
LTLLKALVR (SEQ ID NO: 242)
FYKFIKPIL (SEQ ID NO: 243)
LFKTNRKVT (SEQ ID NO: 244)
MKQLKRRRY (SEQ ID NO: 245)
WGRLSRKLI (SEQ ID NO: 246)
ARLSKSRRL (SEQ ID NO: 248)
IYLALAHMI (SEQ ID NO: 250)
MIKFRGHFL (SEQ ID NO: 251), or
FLYLASHYE (SEQ ID NO: 252).

In summary, claim 1 **excludes** all the T cell epitopes that the Examiner contends are taught by Payne, Ferdosi and Ewaisha. Claim 129, which depends on claim 1, requires those T cell epitopes **not** taught by any cited references.

The combined teachings of Payne, Ferdosi, and Ewaisha therefore disclose T cell epitopes of ILLSDILRV (SEQ ID NO: 225), ILADANLDK (SEQ ID NO: 229), FYSNIMNFF (SEQ ID NO: 231), FKTEITLAN (SEQ ID NO: 232), FFYSNIMNF (SEQ ID NO: 234), YLASHYEKL (SEQ ID NO: 235), FATVRKVLS (SEQ ID NO: 241), LKRTARRRY (SEQ ID NO: 247), or LLFKTNRKV (SEQ ID NO: 249) that are specifically excluded from claim 1. Hence, the combined teachings of Payne, Ferdosi and Ewaisha necessarily **teach away** from the claimed invention.

References that teach away cannot serve to create a *prima facie* case of obviousness. As explained by the Federal Circuit Court of Appeals in *Spectralytics Inc. v. Cordis Corp*., 649 F.3d

1336, 1343 (Fed. Cir. 2011), "'teaching away' does not require that the prior art foresaw the specific invention that was later made and warned against taking that path." The court stated: "A reference may be said to teach away when a person of ordinary skill, upon reading the reference, would be discouraged from following the path set out in the reference or would be led in a direction divergent from the path that was taken by the applicant."

Accordingly, the claimed invention cannot be obvious in view of the references cited by the Examiner. Withdrawal of the rejection is respectfully requested.

No Waiver

All of Applicant's arguments and amendments are without prejudice or disclaimer. Applicant may not have addressed each specific rejection of the independent and dependent claims because Applicant submits that the independent claims are allowable, as discussed above. Applicant has not acquiesced to any such rejection and reserves the right to address the patentability of any additional claim features in the future.

CONCLUSION

Applicant submits the foregoing as a complete response to the Office Action. Applicant submits that this Amendment and Response places the application in condition for allowance and respectfully requests such action. If any issues can be resolved with an Examiner's Amendment or a telephone conference, please contact Applicant's representative at 912-257-4864 (Main) or 478-292-5119 (Direct).

No additional fees are believed due for consideration of this paper. However, the Commissioner is hereby authorized to charge any fee, except excess claim fees, deemed necessary for consideration of this paper, including any extension of time fees, or to credit any overpayment, to Deposit Account Number 50-5193, reference number BROD-4450US.

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