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UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE PATENT TRIAL AND APPEAL BOARD

Ex parte JIN-SOO KIM, SEUNG WOO CHO, and SOJUNG KIM

Appeal 2019-001990
Application 14/685,510
Technology Center 1600

Before JOHN E. SCHNEIDER, RYAN H. FLAX, and CYNTHIA M. HARDMAN, *Administrative Patent Judges*.

FLAX, *Administrative Patent Judge*.

DECISION ON APPEAL

This is a decision on appeal under 35 U.S.C. § 134(a) involving claims to a method of introducing a site-specific, double-stranded break at a target nucleic acid sequence in a mammalian cell or an isolated mammalian cell using a Type II CRISPR/Cas system. Appellant appeals the Examiner's rejections of claims 66–68, 70–74, 83, 85–88, and 90–94 under 35 U.S.C. § 103.[1,2] We have jurisdiction under 35 U.S.C. § 6(b).

We reverse.

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[1] "Appellant" herein refers to the "applicant" as defined by 37 C.F.R. § 1.42. Appellant identifies "TOOLGEN INCORPORATED" as the real party-in-interest. Appeal Br. 1.

[2] Oral argument was heard on March 2, 2020; a transcript of the hearing ("Hr'g Tr.") is a part of the record.

RELATED MATTERS

Appellant states "[t]here are no other appeals, interferences, trials before the Board or judicial proceedings that involve an application or patent owned by the appellant or assignee that is related to, may directly affect, or be affected by the Board's decision in the pending appeal." Appeal Br. 1.

STATEMENT OF THE CASE

The application on appeal has a filing date of April 13, 2015, but claims priority to international application PCT/KR2013/009488, which was filed October 23, 2013, and further claims the benefit of three provisional applications: 61/837,481, filed June 20, 2013; 61/803,599, filed March 20, 2013; and 61/717,324 ("the '324 provisional"), filed October 23, 2012. *See* Bib Data Sheet. The Examiner does not challenge the application's claim to the earliest, October 23, 2012, effective date for priority. *See generally* Final Action; Answer; *see also* Appeal Br. 4 ("Appellant was the first to engineer a Type II CRISPR/Cas system to successfully introduce site-specific, double-stranded breaks in target sequences of *mammalian* cells. *See* U.S. Provisional Application No. 61/717,324 filed October 23, 2012,[ ] to which the instant application claims priority.").

Independent claims 66 and 85 are reproduced below and are illustrative of the claims on appeal:

> 66. A method of introducing a site-specific, double-stranded break at a target nucleic acid sequence in a mammalian cell, the method comprising introducing into the mammalian cell a Type II Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)/Cas system, wherein the CRISPR/Cas system comprises:
>
> a) a nucleic acid encoding a Cas9 polypeptide, wherein the Cas9 polypeptide comprises a nuclear localization signal and

wherein said nucleic acid is codon-optimized for expression in mammalian cells, and

b) a chimeric guide RNA comprising a CRISPR RNA (crRNA) portion fused to a trans activating crRNA (tracrRNA) portion,

wherein the target nucleic acid sequence comprises a first strand having a region complementary to the crRNA portion of the chimeric guide RNA and a second strand having a trinucleotide protospacer adjacent motif (PAM), and

whereby the site-specific, double-stranded break at the target nucleic acid sequence is introduced in the mammalian cell.

85. An isolated mammalian cell comprising a Type II Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)/Cas system for site-specific, cleavage of a double-stranded target nucleic acid sequence in the isolated mammalian cell, wherein the CRISPR/Cas system comprises:

a) a nucleic acid encoding a Cas9 polypeptide, wherein the Cas9 polypeptide comprises a nuclear localization signal and wherein said nucleic acid is codon-optimized for expression in mammalian cells, and

b) a chimeric guide RNA comprising a CRISPR RNA (crRNA) portion and a trans-activating crRNA (tracrRNA) portion,

wherein the target nucleic acid sequence comprises a first strand having a region complementary to the crRNA portion of the chimeric guide RNA and a second strand having a trinucleotide protospacer adjacent motif (PAM), and

wherein the Cas9 polypeptide and the chimeric guide RNA form a Cas9/RNA complex in the isolated mammalian cell and mediate double stranded cleavage at the target sequence.

Appeal Br. 74, 75–76 (Claims Appendix).

The Specification states that the "invention relates to targeted genome editing in eukaryotic cells or organisms," and more particularly, "to a composition for cleaving a target DNA in eukaryotic cells or organisms

comprising a guide RNA specific for the target DNA and Cas protein-encoding nucleic acid or Cas protein, and use thereof." Spec. ¶ 3.

As background, the Specification states that "CRISPRs (Clustered Regularly Interspaced Short Palindromic Repeats) are loci containing multiple short direct repeats that are found in [prokaryotic] genomes." Spec. ¶ 4. The Specification further explains that "CRISPR functions as a prokaryotic immune system, in that it confers resistance to exogenous genetic elements such as plasmids and phages." *Id.* Further, "Cas9, an essential protein component in the Type II CRISPR/Cas system, forms an active endonuclease when complexed with two RNAs termed CRISPR RNA (crRNA) and trans-activating crRNA (tracrRNA)," which enables the system to "slic[e] foreign genetic elements in invading phages or plasmids to protect the host cells." *Id.* ¶ 5. The Specification explains that it was discovered by a research team including Martin Jinek and Jennifer Doudna,[3] among others, that this two-RNA system could be replaced by a single-chain chimeric fused crRNA-tracrRNA (a guide RNA) to form a functional endonuclease with Cas9. *Id.*; *see also id.* ¶¶ 87, 93, 94.

The Specification further states that "the present inventors have made many efforts to develop a genome editing method based on CRISPR/Cas system and finally established a programmable RNA-guided endonuclease that cleave DNA in a targeted manner in eukaryotic cells and organisms." Spec. ¶ 11. The Specification further states that "[i]t is an object of the present invention to provide a composition for cleaving target DNA in eukaryotic cells or organisms comprising a guide RNA specific for target

[3] *See infra* notes 5 and 8 (the references cited by the Examiner as prior art are publications by these researchers).

DNA or DNA that encodes the guide RNA, and Cas protein-encoding nucleic acid or Cas protein." *Id.* ¶ 13.

The following rejections are on appeal[4]:

Claims 66–68, 70–74, 83, 85–88, and 90–94 stand rejected under 35 U.S.C. § 103 over Doudna,[5] Gustafsson,[6] and Chiu.[7] Answer 4; *see also* Final Action 3 (reflecting the same rejection, but including subsequently cancelled claims).

Claims 66–68, 70–74, 83, 85–88, and 90–94 stand rejected under 35 U.S.C. § 103 over Jinek,[8] Chen,[9] Close,[10] Gustafsson, and Chiu. Answer

[4] Rejections of cancelled claims 58–60, 62–65, 75–77, 79–82, and 84 and a double patenting rejection were withdrawn. Answer 3.
[5] US 2014/0068797 A1 (published Mar. 6, 2014) ("Doudna"). Doudna was filed as U.S. Application 13/842,859 on March 15, 2013; it indicates priority to four provisional applications: 61/652,086, filed May 25, 2012 ("Doudna '086 provisional"); 61/761,256, filed October 19, 2012 ("Doudna '256 provisional"); 61,757,640, filed January 28, 2013 ("Doudna '640 provisional"); and 61/765,576, filed February 15, 2013 ("Doudna '576 provisional").
[6] Claes Gustafsson et al., *Codon bias and heterologous protein expression*, 22(7) TRENDS IN BIOTECH. 346–53 (2004) ("Gustafsson").
[7] Wan-ling Chiu et al., *Engineered GFP as a vital reporter in plants*, 6(3) CURRENT BIO. 325–30 (1996) ("Chiu").
[8] Martin Jinek et al., *A Programmable Dual-RNA-Guided DNA Endonuclease in Adaptive Bacterial Immunity*, 337 SCIENCE 816–21 (2012) ("Jinek").
[9] Chung-Pin Chen et al., *Transfection and expression of plasmid DNA in plant cells by an arginine-rich intracellular delivery peptide without protoplast preparation*, 581 FEBS Letts. 1891–97 (2007)) ("Chen").
[10] Dan Close et al., *Expression of Non-Native Genes in a Surrogate Host Organism*, in GENETIC ENGINEERING – BASICS, NEW APPLICATIONS AND RESPONSIBILITIES 3–34 (Hugo A. Barrera-Saldaña ed., 2012) ("Close").

13; *see also* Final Action 6–7 (reflecting the same rejection, but including subsequently cancelled claims).

DISCUSSION

I. LEGAL STANDARDS

"[T]he examiner bears the initial burden, on review of the prior art or on any other ground, of presenting a *prima facie* case of unpatentability. [Once] . . . that burden is met, the burden of coming forward with evidence or argument shifts to the applicant." *In re Oetiker*, 977 F.2d 1443, 1445 (Fed. Cir. 1992).

The Supreme Court in *KSR International Co. v. Teleflex Inc.*, 550 U.S. 398 (2007), reaffirmed the framework for determining obviousness as set forth in *Graham v. John Deere Co.*, 383 U.S. 1 (1966). The *KSR* Court summarized the four factual inquiries set forth in *Graham* (383 U.S. at 17–18) that are applied in determining whether a claim is unpatentable as obvious under 35 U.S.C. § 103(a) as follows: (1) determining the scope and content of the prior art; (2) ascertaining the differences between the prior art and the claims at issue; (3) resolving the level of ordinary skill in the pertinent art; and (4) considering objective evidence indicating obviousness or non-obviousness. *KSR*, 550 U.S. at 406.

"The combination of familiar elements according to known methods is likely to be obvious when it does no more than yield predictable results." *KSR*, 550 U.S. at 416. The "case law is clear that obviousness cannot be avoided simply by a showing of some degree of unpredictability in the art so long as there was a reasonable probability of success. . . . [T]he proper standard [is that] the expectation of success need only be reasonable, not absolute." *Pfizer, Inc. v. Apotex, Inc.*, 480 F.3d 1348, 1364 (Fed. Cir. 2007);

see also In re O'Farrell, 853 F.2d 894, 903–04 (Fed. Cir. 1988) (reasonable expectation of success, not absolute predictability, is required). However, evidence showing there was no reasonable expectation of success may support a conclusion of nonobviousness. *In re Rinehart*, 531 F.2d 1048, 1054 (CCPA 1976); *see also Amgen, Inc. v. Chugai Pharm. Co.*, 927 F.2d 1200, 1207–08 (Fed. Cir. 1991) (regarding a biotechnology invention, testimony supported the conclusion that the references did not show that there was a reasonable expectation of success).

Expectation of success is assessed from the perspective of a person of ordinary skill in the art, at the time the invention was made. *See Life Techs., Inc. v. Clontech Labs., Inc.*, 224 F.3d 1320, 1326 (Fed. Cir. 2000). ("Reasonable expectation of success is assessed from the perspective of the person of ordinary skill in the art. That the inventors were ultimately successful is irrelevant to whether one of ordinary skill in the art, at the time the invention was made, would have reasonably expected success." (citation omitted)). Even where there is motivation to try a claimed course of action, some degree of predictability is required, as is the anticipation of success, for such a path to be considered obvious under the law. *KSR*, 550 U.S. at 421.

"[R]ejections on obviousness grounds cannot be sustained by mere conclusory statements; instead, there must be some articulated reasoning with some rational underpinning to support the legal conclusion of obviousness." *Id.* at 418 (quoting *In re Kahn*, 441 F.3d 977, 988 (Fed. Cir. 2006)). When a prima facie case of obviousness has not been established, the rejection must be reversed. *In re Fine*, 837 F.2d 1071, 1075 (Fed. Cir. 1988). "[D]ependent claims are nonobvious if the independent claims from

which they depend are nonobvious." *In re Fritch*, 972 F.2d 1260, 1266 (Fed. Cir. 1992).

With these standards in mind, we address the Examiner's rejections and Appellant's arguments there-over.

II. ANALYSIS

In support of its arguments, Appellant has submitted the Declaration of Bryan R. Cullen, Ph.D. under 37 C.F.R. § 1.132, dated June 24, 2016 ("Cullen Declaration"). As a preliminary matter, the only direct evidence of the definition of the person of ordinary skill in the art (the "skilled artisan") is found in Appellant's expert's declaration. Dr. Cullen states:

> [A]s of October 23, 2012, one of ordinary skill in the art relevant to the field of the invention claimed in the '510 Application would have had a life sciences Ph.D. (such as a Ph.D. in microbiology, genetics, virology, molecular biology, cell biology, *etc.*) and been actively involved for at least 1-2 years post-Ph.D. in research related to manipulation of gene expression in eukaryotes. I understand that one of ordinary skill in the art at that time would have been aware of all the relevant prior art, including both scientific literature and patent documents.

Cullen Declaration ¶ 13. This definition is uncontested by the Examiner and we conclude it is consistent with the other evidence of record illustrating the level of skill in the art, including the prior art and the Specification. When necessary herein, it is from the perspective of such a skilled artisan that we consider the appealed claims and the prior art.

OBVIOUSNESS OVER DOUDNA, GUSTAFSSON, AND CHIU

The Examiner determined, as to the independent claims, that Doudna (specifically the Doudna '086 provisional) teaches using a CRISPR/Cas system for introducing a site-specific, double-stranded break at a target nucleic acid sequence in a mammalian cell by introducing the CRISPR/Cas

system into the cell. Answer 4, 7–11. The Examiner further determined that the Doudna '086 provisional teaches a nucleic acid encoding a Cas9 polypeptide and that this polypeptide comprises a nuclear localization signal (NLS) (at "page 31, lines 13–17"), a chimeric guide RNA with a crRNA portion fused to a tracrRNA portion, where the crRNA is complementary to the target nucleic acid sequence, where the target also has a trinucleotide protospacer adjacent motif ("PAM") (trinucleotide 5'-NGG-3') at a second strand. *Id.* at 4 (citing Doudna '086 provisional Fig. 3B), 8. The Examiner determined that the Doudna '256 provisional teaches that a protein transduction domain ("PTD") (nona-arginine (R9)) can be at the C-terminus of Cas9, which teaches the NLS of the independent claims. *Id.* at 10 (citing Doudna '256 provisional at 37).

The Examiner stated that "Doudna et al First ['086] and Second ['256] Provisionals differ from the instant base claims 66 and 85 in not disclosing that the nucleic acid encoding the Cas9 protein is codon-optimized for expression in mammalian cells." Answer 11 (emphasis omitted). The Examiner determined that modification of the sequence encoding a prokaryotic protein via codon optimization was well known if expression in eukaryotic cells was intended. *Id.* at 11–12 (citing Gustafsson as disclosing the usefulness of codon optimization and Chiu as disclosing modifying jellyfish proteins with favored human codons to enhance expression in eukaryotic plant cells). The Examiner determined that the skilled artisan would have been motivated to optimize codons to optimize the expression of eukaryotic Cas9 protein in a eukaryotic cell, and that the techniques were performed routinely for almost twenty years as evidenced by Chiu. *Id.* at 12.

The Examiner took the position that "the only modification to the prokaryotic Type II CRISPR/Cas 'system' recited in the claims at issue compared to Doudna et al was codon-optimization of a nucleic acid encoding a Cas9 protein." Answer 18–19 (emphasis omitted). The Examiner reasoned that the above rationale supports a reasonable expectation of success, once Doudna is paired with the other cited prior art. *Id.* at 19.

Appellant argues Doudna has not been established to be prior art and there would not have been a reasonable expectation of success in using a CRISPR/Cas system in mammalian cells. Appeal Br. 6. In support of its arguments, Appellant relies on the Cullen Declaration.

Regarding whether Doudna is prior art, Appellant asserts "Doudna '797 cannot be accorded priority to either [of its] provisional's filing date[s] because the Examiner did not (and cannot) meet her burden of proving entitlement to that date under Sections 102(d)(2) and 112." Appeal Br. 21. Appellant cites, *inter alia*, *Dynamic Drinkware, LLC v. Nat'l Graphics, Inc.*, 800 F.3d 1375 (Fed. Cir. 2015). *See* Reply Br. 29. We find *Dynamic Drinkware*, and the subsequent *Amgen Inc. v. Sanofi*, 872 F.3d 1367 (Fed. Cir. 2017), to be controlling on this issue.

In *Dynamic Drinkware*, a review of the Board's decision in an *inter partes* review, the Federal Circuit held that the party having the burden of proof, there the petitioner, but here the Examiner, must prove that a prior art patent is entitled to priority to its provisional application under § 119(e), if such provisional date is necessary to establish that the patent is indeed prior art. *Dynamic Drinkware*, 800 F.3d at 1380. To establish such priority, the Federal Circuit held that the claims of the asserted prior art patent must be

compared to the disclosure of the provisional because "[a] reference patent is only entitled to claim the benefit of the filing date of its provisional application if the disclosure of the provisional application provides support *for the claims* in the reference patent in compliance with § 112, ¶ 1." *Id.* at 1381 (emphasis added). Here, Doudna is not a patent, but is a published application. However, in *Amgen* the Federal Circuit confirmed that the *Dynamic Drinkware* enablement and written description analysis must also be applied to published applications, such that a comparison must be made between the disclosure of the related provisional(s) and the claims of the published application to establish that the published application is prior art as of its provisional's filing date. *Amgen*, 872 F.3d at 1380 (citing *Dynamic Drinkware*, 800 F.3d at 1378). Although the Office has indicated that it follows these requirements of *Dynamic Drinkware* when determining whether a reference qualifies as prior art under pre-AIA 35 U.S.C. § 102(e), the MPEP is silent with respect to whether these requirements are applicable to § 102 as amended by the AIA.

For purposes of this decision, we understand that the requirements of *Dynamic Drinkware* and *Amgen* apply here. Although the Examiner extensively discussed the disclosures of the Doudna '086 provisional and the Doudna '256 provisional and explained why she determined they disclosed the claimed subject matter, the Examiner did not establish that Doudna, which incorporates these provisionals by reference (*see* Doudna ¶ 1), is prior art. The Examiner never compared any claim of Doudna to the disclosures of the Doudna '086 provisional or the Doudna '256 provisional. *See generally* Final Action; Answer. It was the Examiner's initial burden to do

so, but even were it not, it became the Examiner's obligation to do so once Appellant argued that Doudna was not prior art under *Dynamic Drinkware*.

The Examiner has not satisfied her burden of proving the claims would have been obvious over the cited prior art. Thus, Appellant's argument that the claims are non-obvious because Doudna is not prior art is persuasive.

However, even were Doudna established to be prior art, Appellant contends the unpredictability of the technology foreclosed a person of ordinary skill in the art from reasonably expecting success in arriving at the claimed invention. The Examiner has accounted for each element of the independent claims in the asserted prior art combination, but we are persuaded by Appellant's arguments and evidence that there would not have been a reasonable expectation of success.

It is worth noting what the claimed invention requires, because it is not merely using a functioning CRISPR/Cas endonuclease to cleave mammalian DNA. *See* Appeal Br. 74–77 (Claims Appendix). The claimed invention requires introducing into a mammalian cell a codon-optimized (for mammalian cell expression) genetic sequence that *encodes* a Cas9 polypeptide having an NLS, and introducing into the mammalian cell a fused crRNA-tracrRNA chimeric guide RNA, where the crRNA portion has a complementary region to the target nucleic acid sequence (DNA), which has a PAM. Furthermore, the system of these components causes a site-specific, double-stranded break in target nucleic acid sequence (DNA). To achieve this claimed result, it is undisputed that the Cas9 protein has to be expressed by the mammalian cell and then couple with the fused chimeric guide RNA so as to provide a functioning CRISPR/Cas endonuclease. *See,*

e.g., Spec. ¶ 81; Answer 7–8 (discussing what Doudna must teach to cover the claims); Appeal Br. 4–6. It is further undisputed that this functioning endonuclease system then must enter the mammalian cell's nucleus to access the DNA and then perform the claimed function of the site-specific, double strand break therein.

Regarding whether the skilled artisan would have had a reasonable expectation of success (as of October 23, 2012) in achieving the claimed invention based on the cited prior art combination, the evidence of record supports Appellant's assertion that the technology was unpredictable.

The Cullen Declaration evidences the art was unpredictable. *See* Appeal Br. 29–37. Dr. Cullen opines that one of ordinary skill in the art would have had no basis to reasonably believe the claimed invention would work in mammalian cells. Cullen Declaration ¶ 14. Dr. Cullen states that he, himself, "was aware of and read the Jinek paper [cited by the Examiner] at the time of its publication" and that his "first thought upon reading Jinek 2012 at the time of its publication was that the Type II CRISPR/Cas system described by Jinek 2012, while interesting, would not be useful in eukaryotic cells." *Id.* ¶ 15. Dr. Cullen's reasons for this opinion were

> (i) the drastic size differences between bacterial and mammalian genomes, (ii) the limited accessibility of genomic DNA within eukaryotic cells, (iii) known attempts to introduce bacterial systems into mammalian cells that had failed, (iv) uncertainties arising from placing a bacterial system into a eukaryotic cell environment, and (v) uncertainties known to have arisen from modification of proteins like Cas9,

which Dr. Cullen stated

> point out the unpredictability that existed at the time regarding the applicability of Type II CRISPR/Cas9 for gene editing in eukaryotic cells, and any of these would have left one of ordinary

> skill in the art with no reasonable expectation that a Type II CRISPR/Cas9 system could successfully be used to introduce double-stranded breaks into a target nucleic acid sequence in a eukaryotic cell.

Id. Dr. Cullen further stated

> the intracellular environment of a eukaryotic cell is wholly incomparable to the controlled, artificial environment afforded by a test tube, and as such, the *in vitro* data in Jinek 2012 and [Doudna] would have provided nothing to one of ordinary skill in the art as of October 23, 2012 that would have contributed to a reasonable expectation of success in eukaryotic cells.

Id. ¶ 17 (boldface omitted). Dr. Cullen opined that, even though the techniques of codon optimization (as evidenced by Gustafsson and Chiu) and the use of nuclear localization signals (as evidenced by Chen) were known in the prior art, the skilled artisan would not have reasonably expected the Type II CRISPR/Cas system to function in eukaryotic cells. *Id.* Dr. Cullen explained that the concern over the size of the mammalian genome relates to the claimed system's ability to sort through the condensed chromatin's highly compact structure of eukaryotic-specific components to target a specific sequence for cleaving. *Id.* ¶¶ 21–23. Regarding access to eukaryotic DNA, Dr. Cullen explained that in eukaryotic cells, the genome is packed inside the nucleus, separated from the cytoplasm, where, per the claim requirements, the Cas9 polypeptide would be made. *Id.* ¶ 24. Dr. Cullen explained that this was different from prokaryotic cells, such as the bacterial origins of the CRISPR/Cas9 system, which do not have nuclei. *Id.* Dr. Cullen discussed several failures in the field to get bacterial enzymes to function in eukaryotic cells due to ionic requirements and the presence of chromatin in eukaryotic cells. *Id.* ¶¶ 26–28. Dr. Cullen also addressed

several uncertainties regarding applying a prokaryotic enzyme to a eukaryotic environment and in modifying proteins like Cas9. *Id.* ¶¶ 30–39.

Among other things, Appellant also argues that

> the field of Appellant's invention was highly unpredictable at the time of its priority application, and the mere mention in [Doudna] '797 P1/P2 of the potential possibility of the use of CRISPR/Cas9 in eukaryotic cells is not supported by evidence showing possession of this invention but rather represents a research plan at best. In fact, the [Doudna] '797 P1/P2 ['086 and '256 provisionals] inventors demonstrated skepticism that the CRISPR/Cas9 would work in mammalian cells. *See, e.g.*, Jinek *et al*, 2013, eLIFE e00471 at pp. 1-2 ("it was not known whether such a bacterial system would function in eukaryotic cells"). Moreover, other scientists expressed similar doubt based upon the lack of examples or data in the specification. *See, e.g.*, Carroll, 2012, Molecular Therapy 20(9):1658-1660, at p. 1660 ("[t]here is no guarantee that Cas9 will work effectively on a chromatin target or that the required DNA-RNA hybrid can be stabilized in that context . . . .)

Appeal Br. 23; *see also id.* at 37–41. Appellant points to this as evidence of unpredictability and skepticism directed to the results required by the appealed claims.

The evidence of record also includes a January 7, 2014 article about Dr. Jennifer Doudna, the first named inventor of the Doudna reference and one of the foundational researchers who discovered CRISPR/Cas9 and first fused the system into a single chimeric system. Melissa Pandika, *Jennifer Doudna, CRISPR Code Killer*, OZY, Rising Stars, www.ozy.com/rising-stars-and-provocateurs/jennifer-doudna-crispr-code-killer/4690, Apr. 13, 2015 ("Pandika"). In the Pandika article, Dr. Doudna is quoted as stating that she experienced "many frustrations" getting CRISPR to work in human cells, but she knew if she succeeded, CRISPR would be "a profound

discovery" — and maybe even a powerful gene therapy technique. *Id.* at 5. The article states that it was Harvard geneticist, George Church, who first experienced some success in getting CRISPR to work in human cells, information that was relayed to Dr. Doudna thereafter. *Id.* Drs. Doudna's and Church's results showing that CRISPR could work to cut genes in human cells were both published in January 2013. *Id.*

The evidence of record on this issue also includes a January 2013 publication by Martin Jinek and Jennifer Doudna; Dr. Jinek, like Dr. Doudna, was one of the foundational researchers who discovered CRISPR/Cas9 and first fused the system into a single chimeric system. Martin Jinek et al., *RNA-programmed genome editing in human cells*, eLIFE 1–9 (2013) ("Jinek 2013"). Jinek 2013 states that it "show[s] here that Cas9 assembles with hybrid guide RNAs in human cells and can induce the formation of double-stranded DNA breaks (DBSs) at a site complementary to the guide RNA sequence in genomic DNA." *Id.* at 1 (Abstract). Jinek 2013 discusses the background of CRISPR technology, specifically discussing the very Jinek publication cited by the Examiner in the appealed second rejection (*see supra* note 8). *Id.* at 1–2, 9 (identifying the reference). As a summary to this background, Jinek 2013 concludes: "[h]owever, it was not known whether such a bacterial system would function in eukaryotic cells." *Id.* at 2. Ultimately, Jinek 2013 reported that, among other things, codon-optimization of Cas9 and the provision of an NLS at the C-terminus of Cas9 were modifications to the CRISPR system enabling its functioning in human cells, but this was not discussed in the Jinek (2012) prior art reference cited in the Examiner's rejection. *Id.* at 2, 5.

Also of record on this issue is a July 9, 2014 article in Catalyst Magazine focused on Dr. Doudna and her CRISPR research. College of Chemistry, University of California, Berkeley, Catalyst Magazine, *The CRISPR revolution*, http://catalyst.berkeley.edu/slideshow/the-crispr-revolution/, Apr. 13, 2015 ("Catalyst"). In the Catalyst article, Dr. Doudna is quoted as stating "[o]ur 2012 paper [(understood as referring to Jinek)] was a big success, but there was a problem. We weren't sure if CRISPR/Cas9 would work in eukaryotes—plant and animal cells." *Id.* at 3. Catalyst then explains that, "[u]nlike bacteria, plant and animal cells have a cell nucleus, and inside, DNA is stored in tightly wound form, bound in a structure called chromatin." The article further states that the research team had determined that CRISPR would work in eukaryotes "[b]y the end of 2012" and published their results in January 2013. *Id.* at 4. Such a discovery, however, would have only occurred around the priority date of the appealed claims.

The evidence of record on this issue also includes a September 2012 publication by Dana Carroll, of the Department of Biology at the University of Utah's School of Medicine, discussing the work of Drs. Doudna and Jinek and the Jinek publication. Dana Carroll, *A CRISPR Approach to Gene Targeting*, 20(9) MOLECULAR THERAPY 1658–60 (2012) ("Carroll"). After reviewing the background on CRISPR technology and these foundational researchers' work and publications, Carroll calls Jinek's "prediction that this system can potentially be used in place of ZFNs or TALENs for targeted genomic cleavage in higher organisms" "bold."[11] *Id.* at 1659. Carroll, in

[11] ZFN refers to zinc-finger nucleases, which are enzymes engineered to target specific desired DNA sequences, which enables zinc-finger nucleases

comparing CRISPR to ZFN and TALEN, states "[t]here is no guarantee that Cas9 will work effectively on a chromatin target or that the required DNA-RNA hybrid can be stabilized in that context," and "[o]nly attempts to apply the system in eukaryotes will address these concerns." *Id.* at 1660. Carroll concludes, "[w]hether the CRISPR system will provide the next-next generation of targetable cleavage regents remains to be seen, but it is clearly well worth a try. Stay tuned." *Id.*

Thus, the record bolsters Dr. Cullen's view that the technology was unpredictable enough that success in its use in mammalian cells (eukaryotes), as claimed, was quite uncertain, certainly not reasonably predictable. We conclude this supports Appellant's argument that the skilled artisan would not have had a reasonable expectation of success in achieving the claimed invention.

To conclude, on the present record, the Examiner has not demonstrated that all references in the cited combination qualify as prior art. However, even had the Examiner done so, we determine that the Examiner has not established a prima facie case of obviousness. Although the Examiner accounted for each claim element in the cited prior art combination, "[o]bviousness requires more than a mere showing that the prior art includes separate references covering each separate limitation in a claim under examination." *Unigene Labs., Inc. v. Apotex, Inc.*, 655 F.3d 1352, 1360 (Fed. Cir. 2011). Proof of obviousness requires a showing of at least a reasonable expectation of success from the perspective of a person of

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to target unique sequences within complex genomes. TALEN refers to transcription activator-like effector nucleases, which are also engineered enzymes to cut specific sequences of DNA.

ordinary skill in the art. *In re Vaeck*, 947 F.2d 488, 493 (Fed. Cir. 1991); *Life Techs.*, 224 F.3d at 1326. Here the balance of evidence of record supports that the relevant field was highly unpredictable, which diminishes any reasonable expectation of success in achieving the claimed invention. Considering the evidence of record, on balance it supports Appellant's positions. We therefore reverse the Examiner's rejection over Doudna, Gustafsson, and Chen.

OBVIOUSNESS OVER JINEK, CHEN, CLOSE, GUSTAFSSON, AND CHIU

This rejection is similar to the first obviousness rejection, discussed above, but replaces Doudna with Jinek and Chen, and supplements Gustafsson and Chiu with Close. Jinek, like Doudna, represents the work of the foundational research team concerning CRISPR/Cas9 systems, as discussed above.

The Examiner determined that Jinek teaches a chimeric guide RNA of crRNA and tracrRNA, fused in a Cas9 complex to form a functional endonuclease, for targeting a nucleic acid sequence with a PAM (5'-NGG-3'), and suggests using it for genome editing in cells and organisms and contemplating use in human cells (because ZFNs and TALENs were used in mammalian cells). Answer 13–14 (citing Jinek 817, 820, Figs. 1, 5). The Examiner stated:

> **Jinek et al** teach a CRISPR/Cas system comprising a Cas9 polypeptide, but does not disclose that the Cas9 polypeptide comprises a nuclear localization signal specifically located at the C terminus of the Cas9 polypeptide. However, although Jinek et al do not disclose that the Cas9 polypeptide has a nuclear localization signal or specifically located at the C terminus of the Cas9 polypeptide (per instant claim 74), before the time of the presently claimed invention it was well-known in the art to modify the sequence encoding a prokaryotic (i.e., bacterial)

> protein if the prokaryotic protein was intended to be expressed in a eukaryotic (i.e., plant, mammal, human) cell by a process known as codon optimization and addition of an NLS.

Id. at 15–16.

The Examiner cited Chen as teaching introducing bacterial genes into eukaryotic cells using nona-arginine (R9) as an efficient PTD (protein transduction domain) and NLS (at the C-terminus). Answer 16 (citing Chen 1891–97). The Examiner cited Close as suggesting that codon-optimization is routine and effective for expressing bacterial proteins in eukaryotic cells. *Id.* (citing Close's Section 3). As in the first rejection discussed above, the Examiner cited Gustafsson as disclosing the usefulness of codon optimization when expressing functional bacterial proteins in human or mammalian cells. *Id.* at 16–17 (citing Gustafsson 346–53). Also, as in the first rejection, the Examiner cited Chiu as evidencing that a jellyfish protein was engineered to have favored codons of highly expressed human proteins to enhance expression in eukaryotic cells. *Id.* at 17 (citing Chiu 325–30).

The Examiner took the position that Jinek demonstrates a reasonable expectation of success because it specifically suggests the expectation of cleavage in eukaryotic cells and improvement over ZFN and TALEN systems, which were the two closest known, prior art DNA-targeting systems using bacterial nucleases to cleave eukaryotic/mammalian target DNA. *Id.* at 26.

Appellant's arguments over this rejection are largely the same as those discussed above, except there is no argument that Jinek is not prior art. Appellant argues:

> This rejection is even weaker than the rejection over '797 P1/P2 and the secondary references discussed above. Jinek 2012 is a scientific publication corresponding to the '797 P1/P2 patent

> application filings. If anything, Jinek 2012 contains less information than '797 Pl/P2. Jinek 2012 ***does not mention*** eukaryotic cells (and certainly does not mention mammalian cells).

Appeal Br. 59. Appellant cites Dr. Cullen's opinion that Jinek's data, which are directed to experiments in bacteria, cannot be extrapolated to and provide a reasonable expectation of success in mammalian cells. *Id.* at 60 (citing Cullen Declaration ¶¶ 19–36). Appellant argues that, like the evidence discussed above, Chen, Close, Gustafsson, and Chiu may verify that NLSs and codon-optimization were known techniques, but they do not allay the concerns of those of skill in the art concerning transitioning the use of CRISPR/Cas9 from prokaryotic cells to eukaryotic cells. *Id.*

We are again persuaded by Appellant's arguments and evidence. As discussed above, the evidence of record supports that there was a high level of uncertainty and unpredictability in the art and that the skilled artisan would not have had a reasonable expectation of successfully transitioning the CRISPR/Cas9 technology to eukaryotic cells, e.g., mammalian cells as claimed. This evidence, on the whole, supports Appellant's contentions that the claims would not have been obvious. Therefore, for the same reasons as discussed above, we reverse this rejection as well.

CONCLUSION

In summary, the obviousness rejections are reversed.

Claims Rejected	35 U.S.C. §	References/Basis	Affirmed	Reversed
66–68, 70–74, 83, 85–88, 90–94	103	Doudna, Gustafsson, Chiu		66–68, 70–74, 83, 85–88, 90–94
66–68, 70–74, 83, 85–88, 90–94	103	Jinek, Chen, Close, Gustafsson, Chiu		66–68, 70–74, 83, 85–88, 90–94
Overall Outcome				66–68, 70–74, 83, 85–88, 90–94

REVERSED