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BEFORE THE PATENT TRIAL AND APPEAL BOARD

Ex parte ANDREW MARK CIGAN, PHILLIP P. PATTEN, and JOSHUA K. YOUNG

Appeal 2020-001237
Application 14/913,177
Technology Center 1600

Before ERIC B. GRIMES, TIMOTHY G. MAJORS, and MICHAEL A. VALEK, *Administrative Patent Judges*.

VALEK, *Administrative Patent Judge.*

DECISION ON APPEAL

Appellant[1] submits this appeal[2] under 35 U.S.C. § 134(a) involving claims to a method for modifying a target site in the genome of a cell that

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[1] We use the word "Appellant" to refer to "applicant" as defined in 37 C.F.R. § 1.42(a). Appellant identifies Pioneer Hi-Bred International, Inc. and E.I. DuPont de Nemours & Company as the real parties in interest. Appeal Br. 3. Herein, we refer to the Final Action mailed October 18, 2018 ("Final Act."); Appellant's Appeal Brief filed May 10, 2019 ("Appeal Br."); Examiner's Answer mailed October 4, 2019 ("Ans."); and Appellant's Reply Brief filed December 3, 2019 ("Reply Br.").

[2] This Appeal is related to Appeal 2019-003067 (Application 14/463,687) and Appeal 2020-001230 (14/913,614), Decisions affirming the rejections of record entered June 24, 2020 and September 10, 2020 respectively.

have been rejected for anticipation under 35 U.S.C. § 102 and obviousness under 35 U.S.C. § 103. We have jurisdiction under 35 U.S.C. § 6(b).

We AFFIRM.

STATEMENT OF THE CASE

"Recombinant DNA technology has made it possible to insert foreign DNA sequences into the genome of an organism, thus, altering the organism's phenotype." Spec. 1. According to the Specification,

> [a]lthough several approaches have been developed to target a specific site for modification in the genome of a cell, there still remains a need for more efficient and effective methods for producing an organism, such as but not limited to yeast and fertile plants, having an altered genome comprising specific modifications in a defined region of the genome of the cell.

Id. at 2.

Appellant's Specification describes "compositions and methods for genome modification of a target sequence in the genome of a cell" that "employ a guide polynucleotide/Cas endonuclease system to provide an effective system for modifying target sites within the genome of a cell." Spec. 19. A Cas endonuclease is a protein encoded by a "CRISPR-associated (Cas)" gene such as Cas9. *Id.* at 20–21. "Once a genomic target site is identified, a variety of methods can be employed to further modify the target sites such that they contain a variety of polynucleotides." *Id.* at 2, 19–20.

Claims 16–18, 25, 26, and 28 are on appeal and can be found in the Claims Appendix of the Appeal Brief. Claim 16 is illustrative of the claims on appeal. It reads as follows:

> 16. A method for modifying a target site in the genome of a cell, the method comprising providing a guide polynucleotide

> and a Cas endonuclease to a cell, wherein said guide polynucleotide comprises at least one deoxyribonucleotide, wherein said guide polynucleotide and Cas endonuclease form a guide polynucleotide Cas endonuclease complex, wherein the Cas endonuclease introduces a double strand break at said target site.

Appeal Br. 17.

Appellant seeks review of the following rejections:

I. Claims 16, 18, 25, and 26 under 35 U.S.C. § 102(a)(2) as anticipated by Joung;[3] and

II. Claims 17 and 28 under 35 U.S.C. § 103 as unpatentable over Joung in view of Zhang.[4]

Appeal Br. 6.

I. ANTICIPATION REJECTION OVER JOUNG

Issue

Appellant does not argue the rejection of dependent claims 18, 25, and 26 separately from the rejection of independent claim 16. Accordingly, we focus our analysis on claim 16, and the other claims stand or fall with claim 16. *See* 37 C.F.R. § 41.37(c)(iv).

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[3] US 2016/0024524 A1, published Jan. 28, 2016 ("Joung"). Joung claims priority to a number of applications, including provisional application no. 61/838,178, filed on June 21, 2013 ("'178 Provisional").

[4] US 2014/0186919 A1, published July 3, 2014 ("Zhang"). Zhang claims priority to a number of provisional applications filed prior to the earliest possible effective filing date of Appellant's present application. Appellant does not address the disclosure in any of Zhang's provisional applications in its briefing, nor does Appellant otherwise dispute that Zhang is prior art to the present application.

The issue for this rejection is whether a preponderance of the evidence supports Examiner's conclusion that Joung anticipates the method recited in claim 16.

Findings of Fact

FF1. Joung discloses "methods [for increasing specificity] of genome editing using the CRISPR/Cas system, e.g., using Cas9 or Cas9-based fusion proteins." Joung ¶¶ 3, 5; '178 Provisional 1.[5]

FF2. One such method disclosed in Joung involves the use of "a synthetic guide ribonucleic acid . . . wherein one or more of the nucleotides is a deoxyribonucleic acid." Joung ¶ 6; '178 Provisional 3. In particular, Joung discloses "methods for inducing a single or double-stranded break in a target region of a double-stranded DNA molecule, e.g., in a genomic sequence in a cell" comprising the step of:

> expressing in or introducing into the cell: a Cas9 nuclease or nickase; and (a) a guide RNA that includes one or more deoxyribonuclotides (e.g., where the sequence may also be partially or wholly DNA but with thymine in place or [sic] uracil), e.g., a guide RNA that includes a sequence of 17-20 nucleotides that are complementary to the complementary strand of a target sequence, preferably a target sequence immediately 5' of a protospacer adjacent motif (PAM), e.g., NGG, NAG, or NNGG, wherein the guide RNA includes one or more deoxyribonuclotides (e.g., where the defined sequence may also be partially or wholly DNA but with thymine in place or [sic] uracil), e.g., a hybrid nucleic acid as described herein.

[5] We include parallel citations to supporting disclosure in the '178 Provisional. The '178 Provisional was filed prior to the earliest possible effective filing date of Appellant's present application. Appellant does not dispute that the '178 Provisional provides written description support for one or more claims in Joung.

Joung ¶¶ 22, 36–37, 72; '178 Provisional 3–4.

FF3. Joung discloses that the synthetic guide RNA in its method forms "complexes" with Cas9 "to improve the genome-wide specificity of the CRISPR/Cas9 nuclease system." Joung ¶ 77; '178 Provisional 18; *see also* Joung Fig. 1; '178 Provisional Fig. 1 (depicting a guide polynucleotide/Cas9 complex bound to a target DNA site).

FF4. Joung explains the rationale for its method of using "DNA-Based Guide Molecules" as follows:

> Existing Cas9-based RGNs [RNA-guided nucleases] use gRNA-DNA heteroduplex formation to guide targeting to genomic sites of interest. However, RNA-DNA heteroduplexes can form a more promiscuous range of structures than their DNA-DNA counterparts. In effect, DNA-DNA duplexes are more sensitive to mismatches, suggesting that a DNA-guided nuclease may not bind as readily to off-target sequences, making them comparatively more specific than RNA-guided nucleases. To this end, we propose an engineered Cas9-based RGN wherein a short DNA oligonucleotide replaces all or part of the complementarity region of a gRNA (for example, see FIG. **4**). This DNA-based molecule could replace either all or part of the gRNA in a single gRNA system or alternatively might replace all of part of the crRNA in a dual crRNA/tracrRNA system. Such a system that incorporates DNA into the complementarity region should more reliably target the intended genomic DNA sequences due to the general intolerance of DNA-DNA duplexes to mismatching compared to RNA-DNA duplexes.

Joung ¶¶ 85–86; '178 Provisional 19–20. Joung further discloses that "[m]ethods for making such duplexes are known in the art." *Id.* (citing references).

FF5. At least claim 7 of Joung is supported by the written description in the '178 Provisional. *See* '178 Provisional 3–4, 8, 10, 19–20 (referring to "Strategy 2B: *DNA-based guide molecules*").

Analysis

Examiner finds that Joung discloses "a method comprising providing a guide polynucleotide and a Cas endonuclease to a cell" wherein the guide polynucleotide comprises "a crNucleotide and a tracrRNA" that is "a deoxyribonucleotide sequence or a combination of a deoxyribonucleotide and ribonucleotide sequence" and "wherein the guide polynucleotide and Cas endonuclease are capable of forming a complex that enables the Cas endonuclease to introduce a double strand break at a target site." Final Act. 4–5. Examiner further determines these disclosures are supported by the '178 Provisional and, therefore, Joung is entitled to the date of the '178 Provisional for purposes of assessing Joung as prior art. *Id.* at 6; *see also* Ans. 9 (explaining that Joung is "now issued as a patent, U.S. Patent No. 9,885,033" and finding that claim 1 of that '033 patent is supported by the disclosure in the '178 Provisional).

We agree with, and adopt, Examiner's findings of fact and reasoning supporting the determination that Appellant's claims are anticipated by Joung. *See* Final Act. 3–7; Ans. 3–5, 8–21; FF1–FF5. We are not persuaded by Appellant's arguments, as explained below.

We are not persuaded by Appellant's argument that "Joung was at best 'ambiguous' in merely mentioning DNA" in the guide RNA of the method it discloses. Appeal Br. 7–8 (citing *Wasica Finance GmbH v. Continental Automotive Systems Inc.*, 853 F.3d 1272 (Fed. Cir. 2017)). Unlike the prior art reference considered in *Wasica*, Joung specifically and

sufficiently describes the method of claim 16, including the limitation requiring "at least one deoxyribonucleotide" in the guide polynucleotide. FF2–FF3. Moreover, Joung explains that the rationale for including one or more deoxyribonucleotides in its guide RNA is that "DNA-DNA duplexes are more sensitive to mismatches" and, therefore, a guide RNA comprising at least some DNA will be "comparatively more specific" and "more reliably target the intended genomic DNA sequences." FF4. Given these disclosures, there is no ambiguity in Joung's disclosure of a method comprising providing a Cas endonuclease and a DNA-containing guide polynucleotide to a cell, as recited in claim 16.

Appellant further asserts that Joung "does not qualify as an enabling prior art" because "every example related to methods and results for guide **RNA polynucleotides only**." Appeal Br. 7. In other words, Appellant contends that the method disclosed in Joung is not enabled because Joung does not provide a working example demonstrating that method with a guide RNA that "included ***any*** DNA." *Id.* We are not persuaded.

As Examiner correctly points out, Joung need not provide a working example demonstrating the claimed method to anticipate. Ans. 10; *see Bristol-Myers Squibb Co. v. Ben Venue Labs., Inc.*, 246 F.3d 1368, 1379 (Fed. Cir. 2001) (holding that "anticipation does not require actual performance of suggestions in a disclosure"). What is required is that the prior art reference disclose a method comprising all of the steps "arranged or combined in the same way as in the claim." *See In re Gleave*, 560 F.3d 1331, 1334 (Fed. Cir. 2009) (quotations omitted). We agree with Examiner that Joung discloses such a method. *See* FF1–FF4. That disclosure is "presumptively enabling." *See In re Antor Media Corp.*, 689 F.3d 1282,

1287 (Fed. Cir. 2012). It is the patent applicant who bears the burden to "submit rebuttal evidence" to show these teachings are not enabled. *Id.* at 1289.

Appellant, however, has not persuasively explained why, much less provided the rebuttal evidence required to show, the anticipating disclosure in Joung (*see* FF1–FF5) is not enabled. Appellant's argument that Joung does "**not** demonstrate a guide polynucleotide comprising anything other than RNA" (Appeal Br. 8) is not persuasive because Appellant has not offered evidence-backed argument explaining why Joung's detailed description of the claimed method is insufficient to enable a skilled artisan to practice that method. The only specific argument Appellant advances is that "Joung only contemplates methods for the introduction of only DNA molecules into a cell that must necessarily be capable of expressing a protein," i.e., "DNA-only expression plasmids," in which all of the components "including the guide polynucleotide – are transcribed into all RNA within the target cell after transformation." Appeal Br. 10–11. According to Appellant, the "DNA component of a guide polynucleotide cannot be provided to the recipient cell via a DNA expression plasmid" and, therefore, Joung is not enabled. *Id.* at 11. We are not persuaded because, as Examiner points out, "Joung repeatedly refers to providing the required components indirectly (expressing) or directly (introducing into)" the cell. Ans. 14 (citing Joung ¶¶ 22, 36, 37). Thus, Appellant's argument that Joung *only* discloses transformation with DNA-only expression plasmids is rebutted by Joung's express teaching that the DNA-containing guide RNA it discloses may also be directly "introduced" into the cell. FF2.

The record also supports Examiner's finding that "a person of ordinary skill in the biotechnological arts would recognize that a guide RNA that includes one or more deoxyribonucleotides (DNA) could be provided to the recipient cell directly, since the direct introduction of nucleic acids into cells was known and practiced in the [prior] art." Ans. 14. In particular, Examiner also cites Beetham,[6] which describes techniques for the direct introduction of chimeric RNA/DNA oligonucleotides into plant cells. *Id.*; *see* Beetham 8776 (describing the introduction of such molecules by "microparticle bombardment"). Appellant responds,[7] urging that Beetham "does not mention any method of delivering ***both*** an endonuclease ***and*** a guide polynucleotide to a cell." Reply Br. 7. But that argument misses the point. It is Joung that discloses the introduction of *both* a Cas endonuclease and a guide RNA to form a Cas endonuclease complex that induces targeted double-strand breaks in the genome of a cell. FF1–FF5. Examiner points to Beetham merely as further evidence that techniques for directly introducing polynucleotides into cells were known in the art. Appellant has not presented persuasive argument, much less offered rebuttal evidence, on this

[6] Peter R. Beetham et al., *A Tool for Functional Plant Genomics: Chimeric RNA/DNA Oligonucleotides Cause In Vivo Gene-Specific Mutations*, 96 Plant Biology 8774–78 (1999) ("Beetham").

[7] Inasmuch as Appellant contends it was prejudiced by not having an "opportunity to address allegations related to [Beetham] in any previous response," (Reply Br. 7) it could have petitioned to re-open prosecution (*i.e.*, to have the alleged new rationale from Examiner be designated a new ground). *See* 37 C.F.R. § 41.40. Appellant did not, but instead elected to respond in its Reply Brief, thus maintaining the appeal. 37 C.F.R. § 41.39(b)(2).

point. Accordingly, we agree with Examiner that Beetham further demonstrates that the anticipatory disclosure in Joung is enabled.

For these reasons, Examiner's rejection of claim 16 as anticipated by Joung is supported by the preponderance of the evidence. We, therefore, affirm the rejection. We affirm the anticipation rejection of claims 18, 25, and 26 for the same reasons.

II. OBVIOUSNESS REJECTION OVER JOUNG AND ZHANG

Issue

The issue for this rejection is whether a preponderance of the evidence supports Examiner's conclusion that Joung in combination with Zhang renders the method of claims 17 and 28 obvious.

Additional Findings of Fact

FF6. Zhang teaches that in the context of a CRISPR system "formation of a CRISPR complex (comprising a guide sequence hybridized to a target sequence and complexed with one or more Cas proteins) results in cleavage of one or both strands in or near . . . the target sequence." Zhang ¶ 158; *see also id.* ¶ 152 (teaching that a CRISPR system comprising "Cas9 generates double stranded breaks at target site sequences"). Zhang explains that the various "elements of a CRISPR system" may be introduced into a host cell on one or more vectors. *Id.* ¶ 158. Zhang further teaches that "[a] variety of delivery systems can be introduced [sic] to introduce Cas9 (DNA or RNA) and guide RNA (DNA or RNA) into the host cell." *Id.* ¶ 171; *see also id.* ¶ 239 (listing techniques).

FF7. Zhang teaches that, along with the other elements of a CRISPR system, a "recombination template" may be provided "to serve as a template in homologous recombination, such as within or near a target sequence

nicked or cleaved by a CRISPR enzyme as part of a CRISPR complex." Zhang ¶ 174; *see also id.* ¶ 210 (teaching that "[t]he break created by the CRISPR complex can be repaired by . . . high fidelity homology-directed repair" in which "an exogenous polynucleotide template can be introduced" and "used to modify a genome sequence"). Zhang ¶ 210; *see also* ¶ 174 (teaching that "a recombination template is designed to serve as a template in homologous recombination, such as within or near a target sequence nicked or cleaved by a CRISPR enzyme as part of a CRISPR complex"). Zhang explains that this "exogenous polynucleotide template comprises a sequence to be integrated (e.g., a mutated gene)" such as "polynucleotides encoding a protein." *Id.* ¶ 212. Zhang further teaches that the "recombination template may be . . . contained in a separate vector, or provided as a separate polynucleotide" from the other elements of the CRISPR system introduced into the cell. *Id.* ¶ 174.

Analysis

Examiner relies upon the same findings regarding the teachings in Joung discussed for the anticipation rejection above. Final Act. 8. Examiner acknowledges that Joung does "not teach a method further comprising providing a donor DNA to the cell or a method further comprising introducing a polynucleotide modification template into the cell," as recited in claims 17 and 28. *Id.* Examiner finds that the additional limitations of claims 17 and 28 are taught by Zhang. *Id.* Moreover, Examiner determines it would have been obvious to combine these teachings with Joung's method:

> [g]iven the teachings of Joung . . . that the target site in the genome of a cell may be modified by providing a guide polynucleotide and a Cas endonuclease to the cell, given the

> teachings of Zhang . . . that the target site in the genome of a cell may also be modified by providing a guide polynucleotide, a Cas endonuclease and a donor DNA or a modification template to the cell, and given the further teachings of Joung . . . that the guide polynucleotide may comprise at least one deoxyribonucleotide which may reduce off-target effects, it would have been *prima facie* obvious . . . to modify a target site in the genome of a cell by providing a guide polynucleotide comprising at least one deoxyribonucleotide, a Cas endonuclease and a donor DNA or a modification template to the cell. One skilled in the art would have been motivated to do so in order to modify a target site in the genome of a cell and potentially reduce off-target effects as well. One skilled in the art would have had a reasonable expectation of success, given the success of both Joung . . . and Zhang . . . in modifying a target site in the genome of a cell.

Id. at 9.

We agree with, and adopt, Examiner's findings and reasoning as well as Examiner's conclusion of obviousness. *See* Final Act. 7–9; Ans. 6–8, 22–32; FF1–FF7. We are not persuaded by Appellant's arguments, as explained below.

Appellant's argument that the prior art provides "no guidance for the usage of a guide polynucleotide comprising DNA" is not persuasive. Appeal Br. 11–13 (emphasis omitted). As explained above, Joung specifically discloses the use of a guide polynucleotide comprising at least one deoxyribonucleotide in a CRISPR-Cas system for the purpose of inducing targeted double-strand breaks in the genome of a cell. FF1–FF3. Moreover, Joung evidences that one of ordinary skill in the art would be motivated to do so because the inclusion of DNA in the guide RNA promotes specificity and potentially reduces off-target effects. FF4.

Notwithstanding these teachings, Appellant argues "[t]here can be no expectation of success in the absence of demonstrable evidence in Joung and/or Zhang." Appeal Br. 13–15. Specifically, Appellant contends Examiner's finding that there would have been a reasonable expectation of success is too "conclusory" and "speculative" to support the rejection "because neither [reference] demonstrated success of modifying a target site in the genome of a cell with a Cas endonuclease and a guide polynucleotide comprising DNA" and Examiner has "erroneously equat[ed] the alleged success of Zhang in modifying a target site with a guide polynucleotide made of **only RNA** to one of skill in the art achieving reasonable success with a guide polynucleotide made of **both RNA and DNA**." *Id.* at 13–14.

Appellant's argument is unpersuasive for several reasons. First, it is well-settled that "[t]he reasonable expectation of success requirement for obviousness does not necessitate an absolute certainty for success." *Par Pharm., Inc. v. TWI Pharms., Inc*., 773 F.3d 1186, 1198 (Fed. Cir. 2014). Thus, obviousness is not avoided merely because the cited references do not provide a working example or other data to demonstrate Joung's teachings and rationale for using a guide polynucleotide comprising at least one deoxyribonucleotide.

Second, Examiner found, and Appellant does not dispute, that Joung provides examples successfully demonstrating the use of a Cas endonuclease and guide RNA to induce targeted double-strand breaks in the genome of a cell. *See* Ans. 28–29. Appellant has failed to persuasively explain why a skilled artisan would not reasonably expect that results successfully demonstrated using a purely RNA guide polynucleotide could also be obtained when one or more of the nucleotides in that guide RNA was

replaced with a deoxyribonucleotide, as taught in Joung. *See, e.g.*, Joung ¶ 36 (teaching that the same polynucleotide guide sequences can be used "wherein the guide RNA includes one or more deoxyribonucleotides (e.g., where the defined sequence may also be partially or wholly DNA but with thymine in place or [sic] uracil)").[8]

For these reasons, we determine that Examiner's rejection is supported by the preponderance of the evidence and, therefore, affirm the rejection of claims 17 and 28 as obvious over the combination of Joung and Zhang, as articulated by Examiner.

CONCLUSION

In summary:

Claims Rejected	35 U.S.C. §	Reference(s)/Basis	Affirmed	Reversed
16, 18, 25, 26	102	Joung	16, 18, 25, 26	
17, 28	103	Joung, Zhang	17, 28	
Overall Outcome			16–18, 25, 26, 28	

No time period for taking any subsequent action in connection with this appeal may be extended under 37 C.F.R. § 1.136(a)(1)(iv).

[8] Appellant asserts that "[t]he demonstration in the instant application, **that a functional Cas9 complex can be formed with a guide comprising both RNA and DNA**, was surprising and unexpected." Appeal Br. 9. This argument is unpersuasive because Appellant has not offered evidence to show that the results in the Specification would have been surprising or unexpected to a skilled artisan in light of the teachings in Joung. *See* FF2–FF4; *see also In re De Blauwe*, 736 F.2d 699, 705 (Fed. Cir. 1984) (explaining that arguments and conclusions unsupported by factual evidence carry no evidentiary weight).

AFFIRMED