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UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE PATENT TRIAL AND APPEAL BOARD

THE REGENTS OF THE UNIVERSITY OF CALIFORNIA, UNIVERSITY OF VIENNA, AND EMMANUELLE CHARPENTIER

Application No. 15/947,680; 15/947,700; 15/947,718; 15/981,807; 15/981,808; 15/981,809; 16/136,159; 16/136,165; 16/136,168; 16/136,175; 16/276,361; 16/276,365; 16/276,368; and 16/276,374

Junior Party,

v.

TOOLGEN, INC.
Senior Party

Application 14/685,510

Patent Interference No. 106,127 (DK)

TOOLGEN OPPOSITION 3
(opposing adding the claims of ToolGen patent 10,851,380)

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Senior Party, ToolGen, Inc. ("ToolGen"), requests that the Board deny CVC's Motion 3 (Paper 363), because: (1) the claims of ToolGen's U.S. Patent No. 10,851,380 ("the '380 patent") do not correspond to Count 1 of the interference; and (2) CVC's Motion 3 fails to comply with the requirements of 37 C.F.R. §§ 41.202 and 41.203.

I. DESCRIPTION OF APPENDICES

Appendix A is a list of Exhibits cited in this motion. Appendix B is the Statement of Material Facts ("F_").

II. REASONS WHY CVC'S MOTION 3 SHOULD BE DENIED

A. LEGAL STANDARDS

"A suggestion to add . . . [a] patent to an interference . . . must . . . show how the claims [of the patent] correspond to the count[.]" Standing Order ("S.O.") (Paper 2), ¶ 203.2. "A claim corresponds to a count if the subject matter of the count, treated as prior art to the claim, would have anticipated or rendered obvious the subject matter of the claim." 37 C.F.R. § 41.207(b)(2); *see* S.O. ¶ 208.3.1 ("A motion to have a claim designated as corresponding to a count must show why the subject matter of the count, if treated as prior art, would have anticipated or rendered obvious the subject matter of the claim.").

"[A] claim is anticipated if each and every limitation is found either expressly or inherently in a single prior art reference." *Celeritas Techs. Ltd. v. Rockwell Intl. Corp.*, 150 F.3d 1354, 1360 (Fed. Cir. 1998); *see* 35 U.S.C. § 102 (pre-AIA). A claim is obvious "if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art" ("POSA"). 35 U.S.C. § 103(a) (pre-AIA).

Obviousness is a question of law based on underlying factual determinations including:

(1) the "level of ordinary skill in the pertinent art"; (2) the "scope and content of the prior art"; (3) the "differences between the prior art and the claims at issue"; and (4) "secondary considerations" of non-obviousness. *KSR Int'l Co. v. Teleflex Inc.*, 550 U.S. 398, 406 (2007) (quoting *Graham v. John Deere Co.*, 383 U.S. 1, 17–18 (1966)). "An obviousness determination requires finding both that a skilled artisan would have been motivated to combine the teachings of the prior art and that the skilled artisan would have had a reasonable expectation of success in doing so." *In re Stepan Co.*, 868 F.3d 1342, 1345–46 (Fed. Cir. 2017).

CVC, as the moving party, bears the burden of proving "that it is entitled to the requested relief." 37 C.F.R. § 41.121(b).

B. THE CLAIMS OF THE '380 PATENT DO NOT CORRESPOND TO COUNT 1

On page 5, line 18 to page 12, line 15 of CVC's Motion 3 ("Mot."), CVC argues that "the claims of the '380 patent correspond to Count 1[.]" Mot. 5:18 (capitalization normalized); *see id.*, 5:18–12:15. The response is that the claims of the '380 patent do *not* correspond to Count 1, because the claims are neither anticipated nor rendered obvious by Count 1 alone or in combination with Martin Jinek *et al.*, A Programmable Dual-RNA-Guided DNA Endonuclease in Adaptive Bacterial Immunity, 337 *Science* 816 (Aug. 17, 2012) (Ex. 2031) ("Jinek").

1. THE CLAIMS OF THE '380 PATENT ARE NEITHER ANTICIPATED NOR RENDERED OBVIOUS BY COUNT 1

CVC does not argue—nor could it—that the claims of the '380 patent are anticipated by Count 1. In fact, CVC and its expert, Dr. Scott Bailey, admit that Count 1 fails to disclose "each and every limitation" recited in the claims, namely, the limitation "wherein the guide RNA comprises two guanines at its 5' end, and there are no additional nucleic acid residues between the two guanines at the 5' end and the crRNA portion of the guide RNA" ("the 5'-GG limitation"). Ex. 2011, '380 patent, 179:32–36; *see, e.g.*, Mot. 6:14–19 ("The only element of [independent]

claim 1 that Count 1 does not recite is [the 5'-GG limitation]."); Ex. 2015, (Bailey Decl.) ¶ 129 (similar).

Likewise, CVC does not argue that Count 1 alone renders the claims of the '380 patent obvious. Rather, by its reliance—indeed, substantial reliance—on Jinek, CVC acknowledges the need to go beyond the subject matter of Count 1 and hence, that no *prima facie* case of obviousness exists in view of Count 1 alone. Accordingly, the claims of the '380 patent are neither anticipated nor rendered obvious by Count 1.

2. INDEPENDENT CLAIM 1 OF THE '380 PATENT IS NOT RENDERED OBVIOUS BY COUNT 1 IN VIEW OF JINEK

On page 5, line 19 to page 9, line 9 of CVC's Motion 3, CVC argues that independent claim 1 of the '380 patent ("independent claim 1") "would have been obvious in view of Count 1 and Jinek[.]" Mot. 5:19–20; *see id.*, 5:19–9:9. The response is that independent claim 1 would not have been obvious in view of Count 1 and Jinek, at least because as of October 23, 2012, the priority date of the '380 patent ("the priority date"): (1) a POSA would not have been motivated to combine Count 1 and Jinek; (2) a POSA would not have had an expectation of success in implementing the CRISPR/Cas9 system of independent claim 1 in eukaryotic cells; and (3) the claims of the '380 patent exhibit superior properties and advantages that a POSA would have found surprising or unexpected.

Above all, CVC's reliance on Jinek is misplaced for a number of reasons. First, as the Board previously recognized, Jinek describes only the use of a prokaryotic CRISPR/Cas9 system *in vitro*, "that is, in a non-cellular experimental environment." *Broad Inst., Inc. v. Regents of the Univ. of California*, Interference No. 106,048, 2017 WL 657415, at *7 (P.T.A.B. Feb. 15, 2017), *aff'd*, 903 F.3d 1286 (Fed. Cir. 2018); F20. Moreover, Jinek does not report the results of *any* experiments using CRISPR/Cas9 in eukaryotic cells, *Broad Inst., Inc.*, 2017 WL 657415, at *7;

F21, as required by independent claim 1. Likewise, Jinek says *nothing* of cell introduction, F22, which too is required by independent claim 1. Finally, Jinek was addressed at length during prosecution of the '380 patent, and the examiner ultimately determined that independent claim 1—and in particular, the 5'-GG limitation—was nonobvious in view of Jinek, a fact that CVC's expert did not consider. Ex. 2120, 8386; Ex. 1550, June 25, 2021 Dep. Transcript of Dr. Bailey, 132:11–134:11; F23; *see Harris Corp. v. Fed. Exp. Corp.*, 502 F. App'x 957, 968 (Fed. Cir. 2013) (holding that the fact that a party "directly addressed and overcame" a prior art reference during prosecution was a "reasonable consideration[]" in subsequently determining obviousness based on the same reference); *Glaxo Grp. Ltd. v. Apotex, Inc.*, 376 F.3d 1339, 1348 (Fed. Cir. 2004) (holding that the burden of proving anticipation or obviousness is "'especially difficult' when, as is the present case, the [challenger] attempts to rely on prior art that was before the patent examiner during prosecution").

(a) A POSA WOULD NOT HAVE BEEN MOTIVATED TO COMBINE COUNT 1 AND JINEK

On page 7, line 13 to page 8, line 15 of CVC's Motion 3, CVC argues that "[a] POSA would have been motivated to modify Count 1 by using Jinek['s] . . . method of preparing RNA with a 5'-GG to arrive at [independent] claim 1." Mot. 7:16–17; *see id.*, 7:13–8:15. The response is that a POSA would not have been motivated to combine Count 1 and Jinek, at least because *nothing* in the prior art—cited by CVC or otherwise—"suggest[s] the desirability" of RNA transfection—a method necessitated by Jinek—as a method of introducing sgRNA (or "guide RNA") into eukaryotic cells as of the priority date. *In re Fulton*, 391 F.3d 1195, 1200 (Fed. Cir. 2004) (emphasis omitted); *see Forest Labs., LLC v. Sigmapharm Labs., LLC*, 918 F.3d 928, 934 (Fed. Cir. 2019) ("An invention is not obvious simply because all of the claimed limitations were known in the prior art at the time of the invention. Instead, we ask whether there is a reason,

suggestion, or motivation in the prior art that would lead one of ordinary skill in the art to combine the references, and that would also suggest a reasonable likelihood of success."). Rather, the prior art suggests the desirability of pursuing other methods of RNA introduction, namely, plasmid transfection.

Independent claim 1 of the '380 patent recites a method of "*introducing* into [a] eukaryotic cell" a CRISPR/Cas9 system comprising an sgRNA having, among other things, "two guanines at [the] 5' end[.]" Ex. 2011, 179:32–36 (emphasis added); F24. As of the priority date, a POSA would have understood that introducing RNA into eukaryotic cells might be accomplished using various methods of transfection, among them plasmid transfection and RNA transfection. Ex. 2065, 1228–30 (paragraphs describing "Methods of Inducing RNAi" and "Delivery Strategies for siRNA"); Ex. 1412 (Turchi. Decl.), ¶39; F25. Plasmid transfection is a mechanism of introducing plasmid DNA into a cell, where DNA segments are then transcribed, within the cell, into RNA. Ex. 1247, Mellon *et al.*, Identification of DNA Sequences Required for Transcription of the Human αl-Globin Gene in a New SV40 Host-Vector System, 27 *Cell* 279, 286 (paragraph describing "COS Cell Transfection") (1981); Ex. 1412, ¶39; F26. RNA transfection, on the other hand, is a method of introducing RNA that is *in vitro* transcribed and purified, directly into a cell. Ex. 2417, Malone *et al*., Cationic Liposome-Mediated RNA Transfection, 86 *Proceedings of the Nat'l Acad. of Sci.* 6077, 6077–78 (paragraph describing "RNA Transfection of Cells Mediated by N-[1-(2,3-Dioleyloxy)propyl]-N,N,N-trimethylammonium Chloride (DOTMA)") (1989); Ex. 1412, ¶39; F27.

Jinek describes a method of *preparing* RNA, specifically, the method of "[*i*]*n vitro* transcription [(or "IVT")] and purification of RNA . . . using [a] T7 Flash *in vitro* Transcription Kit . . . and [polymerase chain reaction]-generated DNA templates carrying a T7 promoter

sequence" ("the Jinek method"). Ex. 2031, "Supplementary Materials and Methods"; F28. A POSA would have understood that *in vitro* transcribed RNA, such as that prepared using the Jinek method, could only be effectively introduced into eukaryotic cells using RNA transfection. Ex. 1412, ¶40; F29. As of the priority date, however, RNA transfection was not routinely employed in the art for introducing RNA into eukaryotic cells. Ex. 1412, ¶41; F30. In fact, as Dr. Turchi explains, literature searches show fewer than forty papers published on lipid-based RNA transfection up to 2012 and less than fifty papers using other chemical means. Ex. 1412, ¶45; F31. This is not surprising because POSAs understood RNA transfection to be especially problematic, particularly in eukaryotic cells. Ex. 1412, ¶41; F32. Ichim—on which CVC and Dr. Bailey rely—acknowledges this fact, Ex. 2065, Thomas E. Ichim *et al*., RNA Interference: A Potent Tool for Gene-Specific Therapeutics, 4 *American J. of Transplantation* 1227, 1232 (paragraph describing "Clinical applicability of siRNA") (2004) ("The promise of siRNA-therapeutics is held back by the question of delivery."); F33; a fact that remained as of the priority date, Ex. 1412, ¶41; *see* Ex. 1559, Yan Gao *et al.*, Research Progress on siRNA Delivery with Nonviral Carriers, 6 Int'l J. of Nanomedicine 1017, 1017 (Abstract) (2011) ("Gao") ("Unfortunately, siRNA duplexes are not ideal drug-like molecules. Problems hindering their effective application fundamentally lie in their delivery[.]"); F34. Jinek fails to acknowledge, let alone provide *any* guidance as to how a POSA might address and overcome the numerous problems associated with direct RNA transfection in eukaryotic cells of bacterial RNA. F35. In fact, Jinek is entirely silent concerning methods of RNA introduction even in prokaryotic cells, to say nothing of introduction in eukaryotic cells. F36. Notably, in its Motion 3, CVC addresses neither the problematic nature of RNA transfection nor the lack of guidance in Jinek, relying instead on "nothing more than impermissible hindsight" to suggest a motivation to combine.

InTouch Techs., Inc. v. VGO Commc'ns, Inc., 751 F.3d 1327, 1348 (Fed. Cir. 2014).

Unlike RNA transfection, plasmid transfection was widely employed in the art for introducing DNA sequences encoding RNA molecules into eukaryotic cells. Ex. 1248, Paul *et al.*, Effective Expression of Small Interfering RNA in Human Cells, 20 *Nature Biotech.* 505, 505 (Abstract) (2002); Ex. 1412, ¶42; F37. In fact, as of the priority date, POSAs understood plasmid transfection to be the most desirable and most common method of RNA introduction given its ease of use and numerous benefits over other methods, particularly RNA transfection. Ex. 1243, Molecular Cloning: A Laboratory Manual (3rd ed. Cold Spring Harbor Laboratory Press 2001), Chapter 16 (describing numerous protocols for "introducing cloned genes into cultured mammalian cells" (capitalization normalized)); Ex. 1412, ¶42; F38. Notably, this fact was confirmed by Dr. Bailey during cross-examination numerous times, for example:

> Q. I mean, over the last ten years it's been well-known that you could use expression vectors like plasmids with a gene on it to express RNA in a cell?
>
> A. Yes.

Ex. 1550, 61:3–7; *see id.*, 77:14–18 ("Q. . . . And you can transfect HEK 293T cells with plasmids, right? A. You can, yes."); F39.

Among other material benefits, a POSA would have understood plasmid transfection to provide continuous and sustained RNA expression and chemical stability within eukaryotic cells. Ex. 1412, ¶42; *id.*, ¶44 ("Cellular transcription of a transfected plasmid also has the advantage of allowing for post-transcriptional capping of small RNAs to increase cellular stability."); F64. Ichim recognizes "several [other] advantages[.]" Ex. 2065, 1229 (right column, first full paragraph); *see id.* (explaining that "express[ing siRNA] from a plasmid . . . possesses several advantages . . . : (1) The siRNA could be constitutively expressed, allowing for a higher level of

silencing; (2) regulatory elements could be added to the promoter region of the plasmid such that tissue-specific silencing occurs with a systemically administered plasmid; and (3) permanent gene 'knock-down' cell lines can be established for *in vitro* work, or for generation of 'knock-down' animals through cloning."); F39. Paddison—on which CVC and Dr. Bailey also rely—likewise recognizes numerous other benefits of plasmid transfection. Ex. 2027, Patrick J. Paddison *et al*., Short Hairpin RNAs (shRNAs) Induce Sequence-Specific Silencing in Mammalian Cells, 16 *Genes & Development* 948, 956 (right column, first paragraph) (Mar. 8, 2002) (explaining that "shRNAs . . . synthesized in vivo from RNA polymerase III promoters . . . enable[s] the creation of continuous cell lines in which suppression of a target gene is stably maintained by RNAi"); F40. The benefits of plasmid transfection are rendered all the more important here by the presence of sgRNA, which, because of its single-stranded structure, is significantly more susceptible to degradation compared to double-stranded RNA structures, e.g. siRNA and shRNA, Ex. 1412, ¶44; F42—a distinction that neither CVC nor Dr. Bailey substantively address. Additionally, as Dr. Turchi explains, "given that CRISPR/Cas9 is a multi-component complex, wherein the Cas9 protein portion is most commonly expressed in a cell following plasmid transfection, it would be most convenient to use plasmid transfection for the RNA portion as well." Ex. 1412, ¶43; F63.

Moreover, while *in vitro*-transcribed sgRNA prepared by the Jinek method *may* result in RNA having two guanines at the 5' end, this is due to the fact that using T7 RNA polymerase in conjunction with the T7 promoter *requires* one or more guanines at the 5' end of the RNA transcript. Ex. 1245, Rosenberg *et al.*, Vectors for Selective Expression of Cloned DNAs by T7 RNA Polymerase, 56 *Gene* 125, 132 (paragraph bridging the right and left columns) (1987); Ex. 1412, ¶ 46; F43. Neither Jinek nor any other prior-art reference, however, specifies two guanines at the 5' end nor attributes the use of one or more guanines at the 5' end as having *any* import with respect

to CRISPR/Cas9 function. Rather, it was ToolGen that first recognized its significance. Ex. 2011, 36:28–49; F44. In fact, CVC's expert, Dr. Dana Carroll, admitted exactly this in a 2017 presentation, explaining: "Another thing that Jin-Soo Kim"—a named inventor of ToolGen's '380 patent—"found was that if you . . . put a couple of extra Gs on the 5' end of the guide RNA . . . it actually improves the specificity[.]" Ex. 1283, Dana Carroll, *Issues in CRISPR-Cas Editing*, YOUTUBE, 32:42–32:57 (Nov. 4, 2017), https://www.youtube.com/watch?v=5bHKz142FHs; F45. Notably, during this presentation, Dr. Carroll referenced only "data from [Dr. Kim's] paper"; he never referenced Jinek or any other CVC disclosure as teaching or suggesting the use or significance of two guanines at the 5' end despite the obvious opportunity to do so. Ex. 1283, 32:58–33:04. Thus, as of the priority date, a POSA would have understood the Jinek method as having inherent limitations, i.e., the requirement of one or more guanines at the 5' end, without any known benefit. Ex. 1412, ¶46.

On the other hand, an advantage of eukaryotic promoters, e.g., the U6 promoter, which drive expression of RNA via RNA polymerase III used in plasmid transfection of eukaryotic cells, is that they do not have the limiting feature of requiring the one or more guanines at the 5' end. Ex. 1412, ¶46; F47. Thus, as of the priority date, a POSA would have also found the ease and flexibility of plasmid transfection desirable given its lack of inherent limitations as compared to the Jinek method. Ex. 1412, ¶46. Moreover, a POSA would have understood the T7 promoter as being used exclusively in *in vitro*, non-cellular experiments. Ex. 1412, ¶47; F48. The U6 promoter, on the other hand, was being used in zinc finger nucleases ("ZFN") and transcription activator-like effector nucleases ("TALEN") systems as of the priority date, and thus, would have been the natural choice of POSAs pursuing cellular experiments. Ex. 1412, ¶47; F49. In fact, in its Motion 1 (Paper 368), CVC admits that the U6 promoter "was commonly used" for this exact

purpose. CVC Motion 1, 13:15; F50.

For all these reasons, a POSA, desiring to introduce sgRNA into eukaryotic cells as of the priority date, would have selected plasmid transfection, not RNA transfection, using standard, well-established methods. Ex. 1243 (describing numerous protocols for "introducing cloned genes into cultured mammalian cells" (capitalization normalized)); Ex. 1248, 505 (Abstract); Ex. 1412, ¶45. Indeed, CVC, in its own experiments described in U.S. Provisional Application No. 61/757,640—filed three months *after* the priority date—utilized DNA plasmids to introduce CRISPR RNA into eukaryotic cells. Ex. 2003, U.S. Provisional Application No. 61/757,640, ¶¶ 408–23; F51. This is not surprising, as *nothing* in the prior art—cited by CVC or otherwise—"suggested the desirability" of using RNA transfection over plasmid transfection for the introduction of sgRNA in eukaryotic cells. *In re Fulton*, 391 F.3d 1195, 1200 (Fed. Cir. 2004) (emphasis omitted); *see id.* ("The question is whether there is something in the prior art as a whole to suggest the *desirability*, and thus the obviousness, of making the combination[.]"); F52. In fact, CVC goes no farther than to say that RNA transfection was *known* as of the priority date, *see, e.g.*, Mot. 3:18–19, 8:3–5; but, it offers no evidence of the desirability of using RNA transfection over plasmid transfection, F53; *see In re Gordon*, 733 F.2d 900, 902 (Fed. Cir. 1984) ("The mere fact that the prior art could be so modified would not have made the modification obvious unless the prior art suggested the desirability of the modification."); *see also Forest Labs.*, 918 F.3d at 934. CVC's cited references instead evidence the contrary proposition—they describe the desirability of *plasmid* transfection for introducing sgRNA into eukaryotic cells. Ex. 2027, 56 (right column, first paragraph); Ex. 2065, 1229 (right column, first full paragraph). While CVC now claims that the Jinek method of RNA *preparation* would have been preferable, *see* Mot. 2:2–5 ("[A] POSA aware of Count 1 as prior art would have been motivated to modify Count 1 to use Jinek['s] . . .

IVT method to *make* a guide RNA because of the method's low cost, efficiency, and accuracy[.]"), this says nothing whatsoever about the desirability of RNA transfection as a method of *introduction* of sgRNA in eukaryotic cells.

Dr. Bailey's declaration fails to make up for these deficiencies in CVC's arguments. For example, Paddison—on which Dr. Bailey relies to support the contention that "*in vitro* transcription with T7 RNA polymerase was a well-known and commonly used method for making RNA to introduce into eukaryotic cells," Ex. 2015, ¶77—provides that "[d]elivery of siRNAs can be accomplished by any of a number of transient transfection methodologies," Ex. 2027, 952–53 (paragraph bridging pages 952 and 953), but offers no evidence whatsoever that RNA transfection was a desirable method of introducing sgRNA into eukaryotic cells, F54; *see Forest Labs.*, 918 F.3d at 934. Rather, as noted above, Paddison—like Ichim—recognizes the benefits of plasmid transfection. Ex. 2027, 956 (right column, first paragraph); Ex. 2065, 1229 (right column, first full paragraph); F40, 41. The remaining mRNA references on which Dr. Bailey relies, namely, Qu (Ex. 2067) and Liao (Ex. 2068), are likewise unsupportive of CVC's contentions. For example, a POSA would have appreciated that the mRNA molecules prepared by Qu and Liao, include additional structural elements rendering the mRNA molecules more stable, and therefore not comparable to sgRNA. Ex. 1412, ¶48.

Accordingly, a POSA would not have been motivated to modify Count 1 in view of Jinek, because Jinek requires RNA transfection, which a POSA would have found undesirable, particularly given the availability of other more advantageous methods of RNA introduction.

(b) A POSA WOULD NOT HAVE HAD AN EXPECTATION OF SUCCESS IN IMPLEMENTING CRISPR/CAS9 IN EUKARYOTES

On page 8, line 16 to page 9, line 9 of CVC's Motion 3, CVC argues that "[a] POSA would have had a reasonable expectation of success in performing [independent] claim 1's method by

using Jinek['s] guide RNA made with IVT in a CRISPR-Cas9 system of Count 1[.]" Mot. 8:16–18; *see id.*, 8:16–9:9. The response is that a POSA would not have had an expectation of success in implementing CRISPR/Cas9 in eukaryotic cells as recited in independent claim 1 as of the priority date.

In fact, the Board previously held, and the Federal Circuit affirmed, that as of December 2012—two months *after* the priority date—a POSA "would not have reasonably expected success" in implementing CRISPR/Cas9 in eukaryotic cells. *Broad Inst.*, 2017 WL 657415, at *12; *see id.*, *9 (concluding that "at the time of Jinek" a POSA would not have "reasonably expected the CRISPR-Cas9 system to work in eukaryotic cells"); F55. This conclusion is bolstered here by: (1) the earlier priority date; (2) the recitation of additional novel and nonobvious limitations, e.g., the 5'-GG limitation, not previously considered by the Board; and (3) the limitations of RNA transfection as of the priority date. Ex. 1412, ¶¶42, 49. Indeed, for the reasons noted above, there would have been no expectation of success in using RNA transfection to introduce sgRNA into eukaryotic cells. *Id.*, ¶49. Thus, it necessarily follows that a POSA would not have had an expectation of success in implementing the CRISPR/Cas9 systems of independent claim 1 in eukaryotic cells. *Id.*; *see Institut Pasteur & Universite Pierre Et Marie Curie v. Focarino,* 738 F.3d 1337, 1345-46 (Fed. Cir. 2013) (finding no reasonable expectation of success for the claimed methods of introducing site-directed, double-stranded breaks in chromosomal DNA, in part, because the prior art failed to show success in eukaryotic cells).

A similar argument was made in Interference No. 106,115 ("the '115 interference"). While the count in the '115 interference was based on a later priority date and did not recite the additional limitations recited in independent claim 1, like Count 1, the count in the '115 interference recited the use of CRISPR/Cas9 in eukaryotic cells. The senior party "argu[ed] that none of the

publications" cited by the junior party—including Jinek—"show[ed] success in eukaryotic cells," which the Board held in Interference No. 106,048 ("the '048 interference") to be "necessary for a reasonable expectation of success." *Regents of the Univ. of California v. Broad Institute, Inc.*, Interference No. 106,115, slip op. at 66 (P.T.A.B. Sept. 10, 2020) (Doc. No. 877); F56. The Board was "not persuaded" by this argument. *Regents of the Univ. of California*, Interference No. 106,115, slip op. at 66. As the Board explained, "the issue in the ['048] interference was whether a CRISPR-Cas9 system would have been expected to work in a eukaryotic cell," but in the '115 interference, "[t]hat issue [was] assumed under the framework of 37 C.F.R. § 41.207(b)(2)[.]" *Id.*; F57

CVC understands the Board's assumption in the '115 interference to mean that "[e]xpectation of success in eukaryotes would not be in doubt" here, as of the priority date. Mot. 9:3–4. This is incorrect as a matter of fact and law. Concerning the former, as noted above, both the Federal Circuit and the Board have held otherwise. Moreover, CVC fails to acknowledge, let alone address, the earlier priority date and recitation of additional limitations in independent claim 1, as well as the problematic nature of RNA transfection as of the priority date. Concerning the latter, § 41.207(b) provides *only* that "the subject matter of the count"—*and nothing else*—is to be "treated as prior art[.]" 37 C.F.R. § 41.207(b)(2). To be sure, while "other evidence may be relied upon to establish the obviousness of the differences between the count and the claims," the prior-art assumption of § 41.207(b) applies only to "the subject matter of the count," but not to other evidence, such as the disclosure of "a party's specification," the knowledge of a POSA, or teachings in the art. Ex. 1108, Decision on Motions, *Desjardins v. Wax*, Interference No. 105,915, at 19 n.3 (P.T.A.B. Jan. 21. 2014) (Doc. No. 125); *see id.*, 19. Thus, to interpret § 41.207(b) as providing for an assumption of a reasonable expectation of success—as CVC suggests—would be

inconsistent with the plain language of the rule and Board precedent. Moreover, such an interpretation would effectively nullify the requisite obviousness analysis of § 41.207(b). *Broad Inst.*, 903 F.3d at 1291 ("An obviousness determination requires finding that a person of ordinary skill in the art . . . would have had a reasonable expectation of success in [combining or modifying the teachings in the prior art]."); *Medichem, S.A. v. Rolabo, S.L.*, 437 F.3d 1157, 1162 (Fed. Cir. 2006) ("Determining obviousness requires consideration of . . . whether the prior art would have also revealed . . . a reasonable expectation of success."). This would be particularly egregious here, where neither Count 1 nor Jinek offer *any* reason why a POSA would have expected success in implementing CRISPR/Cas9 in eukaryotic cells, let alone using RNA transfection to do so. Rather, Count 1 simply recites "eukaryotic cell" and "CRISPR/Cas" in the same claim, but this is not enough. *See Institut Pasteur*, 738 F.3d at 1346 ("'[K]nowledge of the goal does not render its achievement obvious,' and obviousness generally requires that a skilled artisan have reasonably expected success in achieving that goal." (quoting *Abbott Labs. v. Sandoz, Inc.*, 544 F.3d 1341, 1352 (Fed. Cir. 2008)).

CVC's remaining argument is unpersuasive. CVC contends that a POSA "would have reasonably expected success in performing claim 1's method by modifying Count 1 to use Jinek['s] . . . guide RNA comprising a 5'-GG directly adjacent to the crRNA portion of the guide RNA because doing so would require only application of a proven and reliable method." Mot. 8:19–22. Specifically, CVC asserts that "RNA molecules made with IVT using T7 RNA polymerase were *regularly and successfully* used in eukaryotic cells, and Jinek . . . and Deltcheva had already tested and proven the method in the context of CRISPR systems." Mot. 8:22–24 (emphasis added). This assertion is—at best—false. In fact, neither Jinek nor Deltcheva (Ex. 2029) disclose use of CRISPR/Cas9 in eukaryotic cells, much less that such use was made "regularly and

successfully" as of the priority date. Ex. 2029; Ex. 2031; F58.

Therefore, a POSA would not have had an expectation of success in implementing CRISPR/Cas9 in eukaryotic cells as recited in independent claim 1.

(c) INDEPENDENT CLAIM 1 OF THE '380 PATENT EXHIBITS SUPERIOR PROPERTIES AND ADVANTAGES THAT A POSA WOULD HAVE FOUND SURPRISING AND UNEXPECTED AS OF THE PRIORITY DATE

On page 10, line 15 to page 12, line 15 of CVC's Motion 3, CVC argues that "no objective indicia support the patentability of the '380 patent's claims[.]" Mot. 10:15; *see id.*, 10:15–12:15. The response is that independent claim 1 of the '380 patent—particularly, the 5'-GG limitation—exhibits superior properties and advantages that a POSA would have found surprising and unexpected as of the priority date. *See In re Soni*, 54 F.3d 746, 750 (Fed. Cir. 1995) ("One way for a patent applicant to rebut a *prima facie* case of obviousness is to make a showing of 'unexpected results,' i.e., to show that the claimed invention exhibits some superior property or advantage that a person of ordinary skill in the relevant art would have found surprising or unexpected.").

The '380 patent discloses that ToolGen "tested whether the addition of two guanine nucleotides at the 5' end of sgRNA could make [RNA-guided endonucleases] more specific by comparing 5'-GGX_{20}; (or 5'-$GGGX_{19}$) s[g]RNA with 5'-GX_{19} sgRNA." Ex. 2011, 36:28–31; F59. To ToolGen's great surprise, "GGX_{20} sgRNAs discriminated off-target sites effectively. In fact, the T7E1E assay barely detected [RNA-guided endonuclease]-induced indels at six out of the seven validated off-target sites when [ToolGen] used the four GGX_{20} sgRNAs[.]" Ex. 2011, 36:34–37; F60. As applicants explained,

> [t]hese results show that the extra nucleotides at the 5' end can affect mutation frequencies at on-target and off-target sites, perhaps by altering guide RNA stability, concentration, or secondary structure. These results suggest that three

> factors-the use of synthetic guide RNA rather than guide RNA-encoding plasmids, dualRNA rather than sgRNA, and GGX_{20} sgRNA rather than GX_{19} sgRNA-have cumulative effects on the discrimination of off-target sites.

Ex. 2011, 36:40–49; F61. A POSA would have found these results surprising and unexpected, as the addition of nucleotides not part of the targeting sequence would have been expected to have minimal effect on specificity and to effect each on-target and off-target genes similarly. Ex. 1412, ¶50. This is critical, particularly in light of the fact that RNA was understood as having problematic "off-target effects." Ex. 1559, 1017 (Abstract). In fact, Dr. Carroll, during the same 2017 presentation referenced above, while addressing "off-target effects and delivery hurdles when using CRISPR-Cas technology for genome engineering," referred to Dr. Kim's use of two guanines at the 5' end not only as a "method[] to improve specificity," but as a "high-fidelity modification[]" compared to "basic [CRISPR/Cas] systems." Ex. 1283, 39:10–40:44; F62. Such praise by CVC's own expert should not be overlooked.

CVC's arguments to the contrary are unpersuasive. First, CVC argues that "ToolGen cannot attribute the . . . unexpected increase in specificity to the 5'-GG, because ToolGen argued that the same allegedly unexpected increase in specificity is obtained with systems that did not include the 5' GG." Mot. 11:7–10. This argument relies on the mistaken proposition that no more than one inventive feature can yield the same (or similar) surprising or unexpected results. This proposition lacks any legal support, and CVC cites none. CVC's argument also defies common sense, particularly here, where the unexpected results were accomplished in a new and unpredictable field of endeavor. *See In re Mayne*, 104 F.3d 1339, 1343 (Fed. Cir. 1997) ("The principle [of unexpected results] applies most often to the less predictable fields, such as chemistry, where minor changes in a product or process may yield substantially different results."). Finally, CVC's argument is incorrect as a matter of fact. As the '380 patent makes clear, CRISPR/Cas9

can achieve increased specificity compared to prior technologies, e.g., ZFN and TALEN, with *or* without two guanines at the 5' end, while at the same time, achieving *even greater* specificity when using sgRNA having two guanines at the 5' end. Ex. 2011, 35:53–36:49, Figs. 14A–15B.

Next, CVC argues that "the *prima facie* case here is extraordinarily strong and would not be outweighed by data showing increased specificity of the '380 patent's claimed method." Mot. 11:17–18. This is incorrect. CVC's argument presumes that its showing of obviousness is "extraordinarily strong," which—as already noted above—it is not. Rather, CVC's purported *prima facie* showing of obviousness is stitched together by overstating both the content of the prior art and the contributions of a POSA's knowledge. *See, e.g.*, *id.*, 8:22–23 (arguing that, as of the priority date, "RNA molecules made with IVT using T7 RNA polymerase were *regularly and successfully* used in eukaryotic cells" (emphasis added)). Indeed, were the Board to accept CVC's characterization of the state of the art as of the priority date, it is difficult to imagine how anyone could obtain a patent in such a "well-known" and "predictable" field as CRISPR technology, *id.*, 12:1–4, let alone, receive the Nobel Prize in Chemistry eight years *after* the priority date.

Finally, CVC argues that "discovering the effect of including a 5'-GG does not render the *prima facie* obvious combination of Count 1 and Jinek . . . patentable." *Id.*, 12:5–6. This argument, however, fails for the same reasons. Specifically, CVC premises its argument on the assertion that the combination of Count 1 and Jinek would have involved nothing more than "simply measuring the results of an *obvious method*" or "merely discovering . . . a new benefit of an *old process*." *Id.*, 12:6–12 (emphases added). Again, CVC's characterization of the state of the art is patently false, lacking any support in the record.

Accordingly, independent claim 1 exhibits superior properties and advantages that a POSA would have found surprising and unexpected as of the priority date.

3. CLAIMS 2–10 OF THE '380 PATENT ARE NEITHER ANTICIPATED NOR RENDERED OBVIOUS BY COUNT 1 ALONE OR IN COMBINATION WITH JINEK

On page 9, line 10 to page 10, line 14 of CVC's Motion 3, CVC argues that "claims 2–10 of the '380 patent would have been obvious in view of Count 1[.]" Mot. 9:10–11 (capitalization normalized); *see id.*, 9:10–10:14. The response is that claims 2–10 of the '380 patent, which depend from independent claim 1, are neither anticipated nor rendered obvious by Count 1, alone or in combination with Jinek, for at least the reasons discussed above with reference to independent claim 1.

C. THE CLAIMS OF THE '380 PATENT DO NOT INTERFERE IN FACT WITH THE CLAIMS INVOLVED IN THE INTERFERENCE

On page 5, line 18 to page 12, line 15 of CVC's Motion 3, CVC argues that "the claims of the '380 patent interfere in fact with [the] CVC claims involved in the interference[.]" Mot. 12:16–17 (capitalization normalized); *see id.*, 12:16–13:19; *see also* 37 C.F.R. § 41.203(a) ("An interference exists if the subject matter of a claim of one party would, if prior art, have anticipated or rendered obvious the subject matter of a claim of the opposing party and vice versa."). The response is that the claims of the '380 patent do not interfere in fact with the claims involved in the interference for at least the reasons discussed above with reference to independent claim 1, much less claims with the additional limitations of claims 2–10.

D. CVC'S MOTION 3 SHOULD BE DENIED AS IT FAILS TO COMPLY WITH THE REQUIREMENTS OF 37 C.F.R. §§ 41.202 AND 41.203

CVC's Motion 3 fails to comply with the Board's rules governing interferences, namely, 37 C.F.R. §§ 41.202 and 41.203. Section 41.203 provides that "[a] party may suggest the addition of a patent . . . to the interference[.]" 37 C.F.R. § 41.203(d). The suggestion, however, "should make the showings required under § 41.202(a)[.]" *Id.* Section 41.202(a), in turn, provides that "[t]he suggestion must[,]" among other things, "[e]xplain in detail why the applicant will prevail

on priority[.]" 37 C.F.R. § 41.202(a)(4). CVC made no such showing in its Motion 3. The Board has denied similar motions for the same failure. *See, e.g.*, *Australia v. Leiden*, Interference No. 106,007 (RES), 2016 WL 1752729, at *24 (P.T.A.B. Apr. 29, 2016) (denying a junior party's motion "requesting an additional interference" because it "ha[d] not provided argument nor directed [the Board] to evidence sufficient to establish a reasonable basis upon which it w[ould] prevail on priority").

Accordingly, CVC's Motion 3 should be denied because it fails to comply with the requirements of 37 C.F.R. §§ 41.202 and 41.203.

III. CONCLUSION

For the foregoing reasons, ToolGen, Inc. respectfully requests that CVC's Motion 3 be denied.

Respectfully submitted,

Dated: July 15, 2021

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APPENDIX A: LIST OF EXHIBITS CITED

Ex.	Description
1108	Decision on Motions, *Desjardins v. Wax*, Interference No. 105,915 (P.T.A.B. Jan. 21. 2014) (Doc. No. 125).
1243	Molecular Cloning: A Laboratory Manual (3rd ed. Cold Spring Harbor Laboratory Press 2001), Chapter 16 [Substitute]
1245	Rosenberg *et al.*, Vectors for Selective Expression of Cloned DNAs by T7 RNA Polymerase, 56 *Gene* 125 (1987).
1247	Mellon *et al*., Identification of DNA Sequences Required for Transcription of the Human αl-Globin Gene in a New SV40 Host-Vector System, 27 *Cell* 279 (1981).
1248	Paul *et al.*, Effective Expression of Small Interfering RNA in Human Cells, 20 NATURE BIOTECH. 505 (2002).
1283	Dana Carroll, *Issues in CRISPR-Cas Editing*, YOUTUBE, 32:42–32:57 (Nov. 4, 2017), https://www.youtube.com/watch?v=5bHKz142FHs.
1412	July 15, 2021 Declaration of Dr. John J. Turchi
1550	June 25, 2021 Deposition Transcript of Dr. Scott Bailey, *Regents of the University of California v. ToolGen, Inc.*, Interference No. 106,127.
1559	Yan Gao *et al.*, Research Progress on siRNA Delivery with Nonviral Carriers, 6 *Int'l J. of Nanomedicine* 1017 (2011).
2011	U.S. Patent No. 10,851,380
2015	May 19, 2021 Declaration of Dr. Scott Bailey
2027	Patrick J. Paddison *et al.*, Short Hairpin RNAs (shRNAs) Induce Sequence-Specific Silencing in Mammalian Cells, 16 *Genes & Development* 948 (Mar. 8, 2002).
2029	Elitza Deltcheva *et al*., CRISPR RNA Maturation by Trans-Encoded Small RNA and Host Factor RNase III, 471 *Nature* 602 (2011).
2031	Martin Jinek *et al.*, A Programmable Dual-RNA-Guided DNA Endonuclease in Adaptive Bacterial Immunity, 337 *Science* 816 (Aug. 17, 2012).

Ex.	Description
2065	Thomas E. Ichim et al., RNA Interference: A Potent Tool for Gene-Specific Therapeutics, 4 *American J. of Transplantation* 1227 (2004).
2067	Xianghu Qu et al., ndrg4 is Required for Normal Myocyte Proliferation During Early Cardiac Development in Zebrafish, 317 *Development Biology* 486 (March 6, 2008).
2068	Xinsheng Liao et al., Transfection of RNA Encoding Tumor Antigens Following Maturation of Dendritic Cells Leads to Prolonged Presentation of Antigen and the Generation of High-Affinity Tumor-Reactive, 9 *Molecular Therapy* 757 (Apr. 8, 2004)
2120	File History for U.S. Patent No. 10,851,380
2417	Malone *et al.*, Cationic liposome-mediated RNA transfection, *Proc. Natl Acad. Sci.,* 86, 6077–6081 (1989).

APPENDIX B: STATEMENT OF MATERIAL FACTS

Junior Party's Alleged Facts 1-20 and Senior Party's Answers

1. By Oct. 23, 2012, methods of preparing RNA using *in vitro* transcription ("IVT") with T7 RNA polymerase were known in the art. Ex. 2064, 59; Ex. 2065, 1229.

Response: Admitted that, by October 23, 2012, IVT with T7 RNA polymerase was a known method of preparing some RNA. Otherwise, denied.

2. By Oct. 23, 2012, a POSA would have known that the consensus promoter for T7 RNA polymerase ("T7 RNAP") is TAATACGACTCACTATAGG. Ex. 2069, 180; Ex. 2064, 59-60.

Response: Denied.

3. Using T7 RNAP and the T7 RNAP promoter TAATACGACTCACTATAGG to transcribe RNA results in a 5'-GG on the transcribed RNA. Ex. 2069, 180; Ex. 2064, 59-60.

Response: Admitted that using T7 RNAP and the T7 RNAP promoter TAATACGACTCACTATAGG to transcribe RNA may result in a GG at the 5' end. Otherwise, denied.

4. By Oct. 23, 2012, a POSA would have known that including the terminal GG sequence in the T7 RNA polymerase promoter is critical for transcriptional yield with T7 RNAP. Ex. 2070, 30 Ex. 2064, 60; Ex. 2027, 952.

Response: Denied.

5. Jinek 2012 discloses making sgRNA and crRNA with IVT using T7 RNA polymerase. Ex. 2031, Fig. 5 and Suppl. Methods.

Response: Admitted.

6. Jinek 2012 discloses using TAATACGACTCACTATAGG as the promoter for making RNA with IVT using T7 RNAP. Ex. 2031, Table S3.

Response: Admitted that Jinek discloses using TAATACGACTCACTATAGG as a

promoter for making RNA in some instances. Otherwise, denied.

7. Jinek 2012 discloses making crRNA with primers that, when used for IVT with T7 RNAP, would have resulted in a 5'-GG directly adjacent to the targeting region of the crRNA. Ex. 2031, Table S3; Ex. 2069, 180; Ex. 2064, 59-60.

Response: Admitted that Jinek discloses instances of making crRNA with primers that, when used for IVT with T7RNAP may have resulted in a GG at the 5' end. Otherwise, denied.

8. Jinek 2012 demonstrated that RNA made with IVT using T7 RNAP and the consensus T7 RNAP promoter was functional in a CRISPR-Cas9 system, including in cleaving eukaryotic DNA sequences. Ex. 2031, Figs. 1-5.

Response: Denied.

9. Deltcheva (Ex. 2029) discloses making crRNA and tracrRNA with IVT using T7 RNA polymerase. Ex. 2029, Suppl. Methods.

Response: Admitted.

10. Deltcheva discloses making crRNA and tracrRNA with promoters that, when used for IVT with T7 1 RNAP, would have resulted in a 5'-GG on the transcribed RNAs. Ex. 2029, Suppl. Table 10; Ex. 2069, 180; Ex. 2064, 59-60.

Response: Admitted that Deltcheva discloses making RNA with promoters that, when used for IVT with T7 RNAP, may have resulted in a GG at the 5' end. Otherwise, denied.

11. By Oct. 23, 2012, a POSA would have known that IVT of RNA with T7 RNAP had been used to make RNA that was subsequently introduced into eukaryotic cells. Ex. 2027, 952; Ex. 2065, 1228-1229; Ex. 2067, 487; Ex. 2068, 763.

Response: Admitted only as to mRNA, shRNA, and siRNA. Otherwise, denied.

12. By Oct. 23, 2012, NLS tags were commonly placed at the C-terminus of proteins to obtain nuclear localization. Ex. 2117, 42189; Ex. 2118, 24391.

Response: Admitted that Ex. 2117 and Ex. 2118 disclose the use of multiple NLSs. Otherwise, denied.

13. By Oct. 23, 2012, human cells were used for genome editing experiments with ZFN and TALEN systems. Ex. 2111, 9284-9285, 9291; Ex. 2110, 3-4.

Response: Admitted that, by October 23, 2012, some genome editing experiments in human cells used ZFN and TALEN systems. Otherwise, denied.

14. By Oct. 23, 2012, using vectors was a known method for expressing exogenous proteins within eukaryotic cells. Ex. 2111, 9284-9285, 9291; Ex. 2110, 3-4.

Response: Admitted that, by October 23, 2012, using vectors was a known method for expressing some exogenous proteins within eukaryotic cells. Otherwise, denied.

15. The only difference between the CRISPR-Cas9 system in '380 patent's claims and Count 1 is the presence of a 5'-GG on the guide RNA. Ex. 2011, 179:18-38.

Response: Denied.

16. During prosecution of the '510 patent, ToolGen argued that the claims that would become Count 1 were commensurate in scope with an unexpected increase in specificity. Ex. 2012, 6774-6775, 6910-6914, 6939-6940.

Response: Denied.

17. By Oct. 23, 2012, injection of *in vitro* transcribed RNA was known as an essential quick and robust tool for exploring gene function in the zebrafish embryos. Ex. 2071, 1.

Response: Denied.

18. The '380 patent's method claims recite no active steps beyond a general instruction to

"introduce" a CRIPSR-Cas9 system into cells. Ex. 2011, 179:18-180:38.

Response: Denied.

19. By Oct. 23, 2012, a POSA would understand a chimeric guide RNA to comprise a crRNA and a tracrRNA linked together with intervening nucleotides. Ex. 2031, Fig. 5.

Response: Denied.

ToolGen, Inc.'s Additional Material Facts

20. Jinek describes only the use of a prokaryotic CRISPR/Cas9 system *in vitro*, that is, in a non-cellular experimental environment. Ex. 2031.

21. Jinek does not report the results of any experiments using CRISPR/Cas9 in eukaryotic cells. Ex. 2031.

22. Jinek says nothing of cell introduction. Ex. 2031.

23. Jinek was addressed during prosecution of the '380 patent, and the examiner determined that independent claim 1—and in particular, the 5'-GG limitation—was nonobvious in view of Jinek. Ex. 2120, 8386.

24. Independent claim 1 of the '380 patent recites a method of "introducing into [a] eukaryotic cell" a CRISPR/Cas9 system comprising an sgRNA having, among other things, "two guanines at [the] 5' end[.]" Ex. 2011, 179:32–36.

25. As of the priority date, a POSA would have understood that introducing RNA into eukaryotic cells might be accomplished using various methods of transfection, among them plasmid transfection and RNA transfection. Ex. 2065, 1228–30; Ex. 1412, ¶ 39.

26. Plasmid transfection is a mechanism of introducing plasmid DNA into a cell, where DNA segments are then transcribed, within the cell, into RNA. Ex. 1247, 286; Ex. 1412, ¶ 39.

27. RNA transfection is a method of introducing RNA that is *in vitro* transcribed and purified, directly into a cell. Ex. 1245, 132; Ex. 1246, 6077–78; Ex. 1412, ¶ 39.

28. Jinek describes a method of preparing RNA, specifically, the method of "[*i*]*n vitro* transcription [(or "IVT")] and purification of RNA . . . using [a] T7 Flash *in vitro* Transcription Kit . . . and [polymerase chain reaction]-generated DNA templates carrying a T7 promoter sequence." Ex. 2031, "Supplementary Materials and Methods."

29. As of the priority date, a POSA would have understood that *in vitro* transcribed RNA, such as that prepared using the Jinek method, could only be effectively introduced into eukaryotic cells using RNA transfection. Ex. 1412, ¶ 40.

30. As of the priority date, RNA transfection was not routinely employed in the art for introducing RNA into eukaryotic cells. Ex. 1412, ¶ 41.

31. Fewer than forty papers were published on lipid-based RNA transfection up to 2012 and less than fifty papers using other chemical means. Ex. 1412, ¶ 45.

32. As of the priority date, a POSA would have understood RNA transfection to be problematic, particularly in eukaryotic cells. Ex. 1412, ¶ 41.

33. Ichim discloses that RNA transfection was problematic, particularly in eukaryotic cells. Ex. 2065, 1232.

34. Gao discloses that RNA transfection was problematic, particularly in eukaryotic cells. Ex. 1559, 1017.

35. Jinek fails to acknowledge, let alone provide guidance as to how a POSA might address and overcome problems associated with direct RNA transfection in eukaryotic cells of bacterial RNA. Ex. 2031.

36. Jinek is entirely silent concerning methods of RNA introduction in both prokaryotic cells and eukaryotic cells. Ex. 2031.

37. As of the priority date, plasmid transfection was widely employed in the art for introducing DNA sequences encoding RNA molecules into eukaryotic cells. Ex. 1248, 505 (Abstract); Ex. 1412, ¶ 42.

38. As of the priority date, a POSA would have understood plasmid transfection to be the most desirable and most common method of RNA introduction given its ease of use and numerous

benefits over other methods, particularly RNA transfection. Ex. 1248, 505 (Abstract); Ex. 1550, 61:3–7, 77:14; 1243; Ex. 1412, ¶ 42.

39. Dr. Bailey indicated on cross-examination that plasmid transfection was a well-known technique to introduce RNA into eukaryotic cells. Ex. 1550, 61:3-7; *see also id.*, 77:14-18.

40. Ichim discloses "several advantages" of using plasmid transfection in eukaryotic cells. Ex. 2065, 1229.

41. Paddison recognizes several benefits of plasmid transfection. Ex. 2027, 952–53, 956.

42. sgRNA is more susceptible to degradation compared to double-stranded RNA structures, e.g. siRNA and shRNA. Ex. 1412, ¶ 48.

43. While *in vitro*-transcribed sgRNA prepared by the Jinek method may result in RNA having two guanines at the 5' end, this is due to the fact that using T7 RNA polymerase in conjunction with the T7 promoter requires one or more guanines at the 5' end of the RNA transcript. Ex. 1245, 132.

44. It was ToolGen that first recognized the significance of using two guanines at the 5' end. Ex. 2011, 36:28–49.

45. During a 2017 presentation, Dr. Carroll, stated: "Another thing that Jin-Soo Kim found was that if you . . . put a couple of extra Gs on the 5' end of the guide RNA . . . it actually improves the specificity[.]" Ex. 1283, 32:42–32:57.

46. During a 2017 presentation, Dr. Carroll referenced only "data from [Dr. Kim's] paper" in discussing the use and significance of two guanines at the 5' end; he never referenced Jinek or any other CVC disclosure as teaching or suggesting the use or significance of two guanines at the 5' end. Ex. 1283, 32:58–33:04.

47. Eukaryotic promoters do not require one or more guanines at the 5' end. Ex. 1412, ¶ 47.

48. As of the priority date, a POSA would have understood the T7 promoter as being used exclusively in *in vitro*, non-cellular experiments. Ex. 1412, ¶ 47.

49. As of the priority date, the U6 promoter was being used in ZFN and TALEN systems. Ex. 1412, ¶ 47.

50. In its Motion 1 (Paper 368), CVC admits that the U6 promoter "was commonly used" in ZFN and TALEN systems. CVC Motion 1, 13:15.

51. CVC, in its own experiments described in U.S. Provisional Application No. 61/757,640, utilized DNA plasmids to introduce CRISPR RNA into eukaryotic cells. Ex. 2003, ¶¶ 408–23.

52. As of the priority date, nothing in the prior art suggested the desirability of using RNA transfection over plasmid transfection for the introduction of sgRNA in eukaryotic cells.

53. In its Motion 3, CVC offers no evidence of the desirability of using RNA transfection over plasmid transfection.

54. Paddison provides that "[d]elivery of siRNAs can be accomplished by any of a number of transient transfection methodologies," Ex. 2027, 952–53 (paragraph bridging pages 952 and 953), but offers no evidence that RNA transfection was a desirable method of introducing sgRNA into eukaryotic cells.

55. The Board previously held, and the Federal Circuit affirmed, that as of December 2012, a POSA "would not have reasonably expected success" in implementing CRISPR/Cas9 in eukaryotic cells. *Broad Inst.*, 2017 WL 657415, at *12.

56. The senior party in the '115 interference "argu[ed] that none of the publications" cited by the junior party—including Jinek—"show[ed] success in eukaryotic cells," which the Board held in Interference No. 106,048 to be "necessary for a reasonable expectation of success." *Regents of the Univ. of California*, Interference No. 106,115, slip op. at 66.

57. In the ’115 Interference, the Board explained that “the issue in the [’048] interference was whether a CRISPR-Cas9 system would have been expected to work in a eukaryotic cell,” but in the ’115 interference, “[t]hat issue [was] assumed under the framework of 37 C.F.R. § 41.207(b)(2)[.]” *Regents of the Univ. of California*, Interference No. 106,115, slip op. at 66.

58. Neither Jinek nor Deltcheva disclose use of CRISPR/Cas9 in eukaryotic cells, much less that such use was made “regularly and successfully” as of the priority date. Ex. 2029; Ex. 2031.

59. The ’380 patent discloses that ToolGen “tested whether the addition of two guanine nucleotides at the 5' end of sgRNA could make [RNA-guided endonucleases] more specific by comparing 5'-GGX_{20}; (or 5'-$GGGX_{19}$) s[g]RNA with 5'-GX_{19} sgRNA.” Ex. 2011, 36:28–31.

60. The ’380 patent discloses that “GGX_{20} sgRNAs discriminated off-target sites effectively. In fact, the T7E1E assay barely detected [RNA-guided endonuclease]-induced indels at six out of the seven validated off-target sites when [ToolGen] used the four GGX_{20} sgRNAs[.]” Ex. 2011, 36:34–37.

61. The ’380 patent discloses that “[t]hese results show that the extra nucleotides at the 5' end can affect mutation frequencies at on-target and off-target sites, perhaps by altering guide RNA stability, concentration, or secondary structure. These results suggest that three factors-the use of synthetic guide RNA rather than guide RNA-encoding plasmids, dualRNA rather than sgRNA, and GGX_{20} sgRNA rather than GX_{19} sgRNA-have cumulative effects on the discrimination of off-target sites.” Ex. 2011, 36:40–49.

62. Dr. Carroll, during a 2017 presentation, while addressing “off-target effects and delivery hurdles when using CRISPR-Cas technology for genome engineering,” referred to Dr. Kim’s use of two guanines at the 5' end not only as a “method[] to improve specificity,” but as a “high-fidelity modification[]” compared to “basic [CRISPR/Cas] systems.” Ex. 1283, 39:10–40:44.

63. Because CRISPR/Cas9 is a multi-component complex, wherein the Cas9 protein portion is most commonly expressed in a cell following plasmid transfection, it would be most convenient to use plasmid transfection for the RNA portion. Ex. 1412, ¶ 43.

64. As of the priority date, a POSA would have understood plasmid transfection to provide continuous and sustained RNA expression and chemical stability within eukaryotic cells. Ex. 1412, ¶¶ 42, 44.

CERTIFICATE OF SERVICE

I hereby certify that the foregoing **TOOLGEN OPPOSITION 3** was filed via the Interference Web Portal on July 15, 2021 by 5:00 PM ET, and thereby served on the attorneys of record for the Junior Party pursuant to ¶ 105.3 of the Standing Order. Pursuant to agreement of the parties, service copies are being sent by email by 11:00 pm ET to counsel for Junior Party as follows:

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