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

By: Eldora L. Ellison, Ph.D., Esq.
Eric K. Steffe, Esq.
David H. Holman, Ph.D., Esq.
Byron Pickard, Esq.
Paul A. Ainsworth, Esq.
John Christopher Rozendaal, Esq.
Michael E. Joffre, Ph.D., Esq.
Pauline M. Pelletier, Esq.
STERNE, KESSLER, GOLDSTEIN & FOX, P.L.L.C.
1100 New York Avenue, NW
Washington, DC 20005
Telephone: (202) 371-2600
Facsimile: (202) 371-2540
eellison-PTAB@sternekessler.com
esteffe-PTAB@sternekessler.com
dholman-PTAB@sternekessler.com
bpickard-PTAB@sternekessler.com
painsworth-PTAB@sternekessler.com
jcrozendaal-PTAB@sternekessler.com
mjoffre-PTAB@sternekessler.com
ppelletier-PTAB@sternekessler.com

By: Li-Hsien Rin-Laures, M.D., Esq.
RINLAURES LLC
321 N. Clark Street, 5th Floor
Chicago, IL 60654
Telephone: (773) 387-3200
Facsimile: (773) 929-2391
lily@rinlauresip.com

Sandip H. Patel, Esq.
MARSHALL GERSTEIN & BORUN LLP
233 South Wacker Drive
6300 Willis Tower,
Chicago, IL 60606
Telephone: (312) 474-6300
Facsimile: (312) 474-0448
spatel@marshallip.com

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
Junior Party

(Applications 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),

v.

SIGMA-ALDRICH CO., LLC.
Senior Party

(Application 15/456,204).

Patent Interference No. 106,132 (DK)

CVC'S OPPOSITION TO SIGMA'S MOTION 1 (to Substitute Proposed Count 2 for Count 1)

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I. INTRODUCTION

Sigma's motion to substitute Count 1 should be denied as contrary to law and equity. Sigma has not met its burden to overcome the presumption that Count 1 recites a single invention. But even if Sigma had rebutted that presumption, its motion requests improper and inequitable relief.

To appreciate the inequity of Sigma's request, one must go back to Sigma's maneuvering during prosecution to avoid an interference with CVC over the subject matter of CRISPR-Cas9 for cleaving or editing target DNA in a eukaryotic cell—not limited to donor integration by HDR.[1] During prosecution of its involved application, the examiner required that Sigma add a claim to the subject matter of CVC's half of Count 1 ("generic cleavage") for purposes of an interference, including with CVC. Remarkably, Sigma *refused to add such a claim*, instead seeking allowance limited to the subject matter of CRISPR-Cas9 cleavage or editing in a eukaryotic cell followed by donor integration by homology-directed repair ("donor integration by HDR"). Yet in refusing to add a claim to generic cleavage during prosecution, Sigma conceded priority to that subject matter pursuant to 37 C.F.R. § 41.202(c). Rule 202(c) reflects the longstanding disclaimer doctrine that when the examiner suggests a claim to an applicant for purposes of a future interference, and the applicant refuses, such refusal operates as a "concession" that the subject matter of the suggested claim was the prior invention of another. *In re Ogiue*, 517 F.2d 1382, 1391 (C.C.P.A. 1975).

Sigma continued its efforts to provoke an interference with CVC based on a count limited to donor integration by HDR. This interference ensued and was declared, not according to Sigma's suggested count but with Count 1: a *McKelvey* count that encompasses both generic cleavage and

[1] Sigma's motions refers to this invention as "cleavage only," however, that term is a misnomer because CVC's half of Count 1 in fact recites "cleaving *or editing or modulating transcription*," and it is inclusive of subsequent donor integration by HDR. Paper 1, 6 (emphasis added).

donor integration by HDR, as represented by CVC claim 156 and Sigma claim 31 respectively.

Sigma tried—but failed—to provoke an interference with CVC limited to donor integration by HDR. Sigma's motion tries, yet again, to engineer a priority contest limited to donor integration by HDR. The Board should not grant that relief, as it finds no support in the law and would result in the gross inequity of excluding CVC's best proofs. The only remedy supported by the rules and precedent is a *two-count* interference—which Sigma made impossible when it deliberately refused to add a claim to generic cleavage. Having conceded the generic cleavage priority contest before it started, Sigma is not entitled to an interference that involves claims to generic cleavage.

Making matters worse, Sigma asserts that 186 of CVC's claims correspond to this narrow embodiment, and must rise or fall under Proposed Count 2. Yet Sigma admits that *only 10* of those claims are drawn to donor integration by HDR. Sigma further admits that CVC's generic cleavage claims do not interfere-in-fact with the subject matter of Proposed Count 2. Thus, after conceding priority during prosecution for the invention represented by 176 of CVC's claims, Sigma now has the temerity to request a single narrow count and claim correspondences, hoping to obtain priority for the very invention it conceded. The Board should not condone such flagrant manipulation.

Sigma's motion is also defective for a slew of other reasons. Sigma's claim correspondence explanation is conclusory and unsupported. As is Sigma's showing under 37 C.F.R. § 41.208(c)(2), which fails to address the clear question of patentability over the prior art raised during prosecution of Sigma's involved application. Each of these defects justifies denial of Sigma's motion.

There is a "presumption that the initial count is limited to a single patentable invention." *Lee v. Mcintyre*, 55 U.S.P.Q.2d 1406 (B.P.A.I. 2000). Sigma has not rebutted that presumption. In light of Sigma's concession during prosecution, CVC is entitled to a judgment of priority for Count 1 because donor integration by HDR is patentably indistinct from the invention Sigma conceded. M.P.E.P. § 2304.04 ("Refusal to add a required claim will operate as a concession of priority for

the subject matter of the required claim. The applicant would then be barred from claiming, *not only* the subject matter of the required claim, but any subject matter that would have been anticipated *or rendered obvious* if the required claim were treated as prior art.") (emphasis added).

If the Board grants Sigma's motion, then CVC is entitled to a judgment of priority in its favor as to CVC's half of Count 1 and CVC's 176 claims that are drawn to the subject matter that Sigma conceded under Rule 202(c). Such a judgment is the inevitable and logical consequence of (a) Sigma's concession that it did not invent the subject matter represented by CVC's half of Count 1 and 176 generic cleavage claims, coupled with (b) Sigma's concession (Mot. 27:2−4) that the subject matter of CVC's 176 generic cleavage claims does not interfere with Proposed Count 2.

II. STATEMENT OF MATERIAL FACTS AND EVIDENCE

Appendix 1 is a list of exhibits cited. Appendix 2 is a Statement of Material Facts.

III. BACKGROUND

For purposes of whether Proposed Count 2 is patentably distinct, CVC's half of Count 1 is "treated as prior art." Fed. Reg. Vol. 69, No. 155, 49990−91 (Aug. 12, 2004). CVC's half of Count 1 recites a "eukaryotic cell comprising" a sgRNA CRISPR-Cas9 system "capable of hybridizing with a target sequence" and "cleaving or editing … target DNA." Paper 1, 5−6; MF 9. The prior art thus includes a CRISPR-Cas9 system that successfully cleaves target DNA in a eukaryotic cell.

The parties agree that the level of ordinary skill is high and and the person of ordinary skill ("skilled artisan") would have a Ph.D. in a life sciences discipline and multiple years of experience manipulating DNA, including in eukaryotic environments. *See* Ex. 1001, ¶ 26; Ex. 2619, ¶ 30.

Before 2012, the skilled artisan knew that prokaryotic and eukaryotic cells alike possess natural mechanisms to repair double stranded breaks ("DSBs") in DNA created by various sources of DNA damage (e.g., radiation, chemicals, nucleases, etc.). Ex. 2619, ¶¶ 41, 129; MF 12. HDR, the most common form of which is homologous recombination ("HR"), and non-homologous end

joining ("NHEJ") are two naturally-occurring and highly-conserved DNA repair processes which, before 2012, were routinely exploited for genome-editing applications in which a meganuclease, zinc finger nuclease ("ZFN"), or transcription activator-like effector nuclease ("TALEN") created targeted DSB in the DNA. Ex. 2619, ¶¶ 45−59; MF 10, 14. The skilled artisan knew that a DSB is the trigger for both of these DNA repair processes. Ex. 2619, ¶¶ 45, 96; MF 10, 12. NHEJ repairs DSBs by ligation of the broken ends and HDR uses a homologous sequence to direct editing of the target DNA. Ex. 2619, ¶¶ 42−44. Before 2012, HDR had been regularly exploited to integrate donor sequences efficiently into the DNA of various eukaryotic cells and organisms, such as fruit fly, by creating targeted DSBs using a meganunucleases, ZFN, or TALEN. Ex. 2619, ¶¶ 45, 74−75.

The skilled artisan knew that HDR-mediated donor integration is triggered by the existence of a DSB and that it occurs *independent* of how the break is created. Ex. 2619, ¶¶ 63, 85, 104; Ex. 2618, ¶ 19; MF 10, 12, 14, 17, 26. The skilled artisan knew that different types of DSBs supported HDR-mediated donor integration. For example, the skilled artisan knew that I-SceI meganuclease created DSBs having a 3' overhang of nucleotides, whereas the *FokI* nuclease used in ZFNs and TALENs created DSBs having a 5' overhang. Ex. 2619, ¶ 63. Yet both types of DSBs were known to induce HDR-mediated donor integration for genome-editing in various eukaryotic cell types. Ex. 2619, ¶ 63. The skilled artisan also knew that DSBs created by nucleases that remained bound to the cleavage site after cleavage induced HDR. Ex. 2619, ¶¶ 62, 104, 138, 142; MF 10, 22.

While Sigma's expert, Dr. Cannon, characterizes HDR as a limited pathway active during certain phases of the cell cycle, she conceded that it "is a naturally occurring repair process that takes place even without special measures to enhance its happening." Ex. 2616, 46:23−47:2; MF 13. She also conceded that the steps to achieve HDR-mediated donor integration were "routine." Ex. 2616, 103:2−16, 105:12−106:20; MF 19. On this, the experts agree. Ex. 2619, ¶¶ 83−87.

IV. ARGUMENT

A. Sigma has not met its burden to show that Count 1 should be substituted.

1. The Office has already *twice* rejected Sigma's argument that donor integration by HDR is patentably distinct from generic cleavage.

Sigma "has the burden of proof to establish that it is entitled to the requested relief." 37 C.F.R. 12 § 41.121(b). Sigma must also overcome the "presumption that the initial count is limited to a single patentable invention." *Lee*, 55 U.S.P.Q.2d 1406. On page 1 lines 3−6, Sigma argues that Count 1 "encompasses two patentably distinct inventions: (1) CRISPR-Cas9 in a eukaryotic cell to cleave a target DNA; and (2) CRISPR-Cas9 in a eukaryotic cell to cleave a target DNA *and subsequently to integrate a donor DNA sequence into the target DNA via homology-directed repair ('HDR')*." The response is that Sigma has already failed, twice, to demonstrate that these are two separately patentable inventions. Sigma's motion fails yet again to justify a different result here.

First, during prosecution of its involved '204 application, Sigma argued that cleavage by the CRISPR-Cas9 system in a eukaryote followed by donor integration by HDR was nonobvious before December 6, 2012. Ex. 2622, 25. The examiner disagreed, explaining that this would have been obvious and citing Sun 2012 (Ex. 2646), which the examiner stated "teaches that homologous recombination with a donor polynucleotide is a well-established technology to edit a mammalian genome, where editing occurs after a double-strand break by a meganuclease, a zinc finger nuclease, [or] a transcription-activator-like nuclease, because the double-strand break increases the efficiency of homologous recombination" and, in view of this, "one would expect to be able to integrate a donor polynucleotide as taught by Doudna et al." Ex. 2622, 148 (citing 2646, 1083).

Second, when Sigma suggested this interference, it asked for a count matching Proposed Count 2. Ex. 2622, 180−186; MF 32. The Board declared this interference, but not with that count. Paper 1. Instead, the Board adopted Count 1, which is not limited to donor integration by HDR.

Under *Lee*, there is a presumption that donor integration by HDR is not separately patentable from generic cleavage. As explained below, Sigma has not carried its burden to rebut that presumption.

2. Sigma is not entitled to the relief it requests because no authority supports substitution and Rule 202(c) bars a two-count interference.

Even if Proposed Count 2 were regarded as patentably distinct, Sigma's motion should be denied because Sigma is not entitled to the relief it requests. On page 1 lines 14−25 of the motion (emphasis added), Sigma argues: "When an interference is declared with a single count directed to two patentably distinct inventions, a motion to *substitute* the count is appropriate." The response is that no authority supports Sigma's argument that Count 1 should be *substituted*. Rather, if there were two patentably distinct inventions here, the remedy would be a *two-count* interference. Yet Sigma has made such an interference impossible by refusing to add a claim to generic cleavage.

First, Sigma cites 37 C.F.R. § 41.201 for the proposition that Count 1 should be substituted because Proposed Count 2 is patentably distinct from Count 1. Mot. 1:12−14. However, Rule 201 states that: "Where there is *more than one count*, *each count* must describe a patentably distinct invention." 37 C.F.R. § 41.201 (emphasis added). In other words, Rule 201 concerns interferences having more than one count and does not support, or provide for, the *substitution* of any count.

Second, Sigma cites to Examples 18 and 20 in M.P.E.P. § 2309.01, Formulation of Counts (7th ed. July 1998). Mot. 1:16−23. Yet both examples provide that the proper relief in this type of situation is to *add* a count to the separately patentable invention: "If a party believes benzene and chloroform define separate patentable inventions, the party could move … to substitute a count (benzene) for (Markush group of benzene or chloroform) *and to add a count (chloroform).*" M.P.E.P. § 2309.01 (7th ed. July 1998), Example 18 (emphasis added); Example 20 (same).

Third, Sigma's quotation from *Godtfredsen v. Banner*, 598 F.2d 589, 592 (C.C.P.A. 1979) (Mot. 2:1−11) expressly observes that "the primary purpose of an interference" in cases involving

patentably distinct inventions is for the Board to determine priority "as to *each* of the common inventions claimed by the parties." 598 F.2d 589, 592 (C.C.P.A. 1979) (emphasis in original).

Sigma's reliance on *Ashurst v. Stampf*, 2008 WL 2781979 (B.P.A.I. 2008) and *Edelman v. Stomp*, 83 U.S.P.Q.2d 1200 (B.P.A.I. 2006) is misplaced. Neither case is precedential and both are distinguishable. Both *Ashurst* and *Stampf* involved situations where the movant sought to remove from the interference subject matter that *it alone claimed*. Here, by contrast, Sigma seeks to remove from the count subject matter that *CVC alone claims*, while nevertheless keeping CVC's claims to that subject matter ensnared in an interference and also excluding CVC's best proofs. MF 28.

None of the cited authority supports substituting the count here. Rather, the cited authority provides that the remedy where there are two separately patentable inventions is to conduct a two-count interference, consistent with the Board's "primary purpose" of entering a "determination of priority as to [e]ach of the common inventions claimed by the parties." *Godtfredsen*, 598 F.2d at 592. Yet Sigma is not entitled to a two-count interference because Sigma does not have any claims that are drawn to generic cleavage. Why? Because Sigma refused to add such a claim, either during prosecution or by seeking leave to add a claim during this interference. Ex. 2622, 166; MF xx.

Sigma has thus *created* a situation in which it is ineligible for the only relief supported by the rules—i.e., a two-count interference. Sigma cannot request such relief for the first time in its reply. 37 C.F.R. § 41.208(b) ("To be sufficient, *a motion* must provide a showing, supported with appropriate evidence, such that, if unrebutted, it would justify the relief sought") (emphasis added); *see also Nau v. Ohuchida*, Interference No. 104,258, Paper 57 at 10 (B.P.A.I. 1999) (precedential) ("The Trial Section does not deem it fair to an opponent when a party 'gets its licks in' for the first time at the reply stage after the opponent can no longer submit evidence and/or argument.").

Sigma's refusal to add a generic cleavage claim during prosecution, despite the examiner's requirement that it do so in contemplation of an interference, bars Sigma from obtaining the relief

it has requested. Specifically, during prosecution, Sigma faced prior art rejections based on CVC's U.S. 2014/0068797 ("Doudna et al.") and ToolGen's U.S. 2015/0322457 and U.S. 2015/0344912 ("the Kim references"). Ex. 2622, 2−3. Following the Board's September 10, 2020 Decision on Motions in Interference No. 106, 115, which denied CVC the priority benefit of its May 25, 2012 provisional application, and a decision by the Office of Petitions allowing Sigma to swear behind the Kim references, the examiner withdrew the remaining prior art rejections and requested that Sigma "file a second set of claims in the instant application based upon the language used in claim 1 *but without the requirement for a donor polynucleotide in the independent and dependent claims*." Ex2622, 161 (emphasis added); MF 31. In its response, Sigma acknowledged that this discussion was in connection with potential interferences, including with CVC. Ex. 2622, 164; MF 31. Then, in remarks dated October 13, 2020, Sigma stated: "[T]he Examiner recommended the inclusion of additional claims directed to a method for modifying a chromosomal sequence in a eukaryotic cell, in which a donor polynucleotide is not present and only a double-stranded break is introduced ('CRISPR cleavage-only'). Respectfully, the Applicant has **not** included CRISPR cleavage-only claims in this Amendment and Response." Ex. 2622, 166 (emphasis in original); MF 31. Thus, the prosecution history clearly shows that the examiner requested that Sigma add a claim to generic cleavage for purposes of an interference and Sigma deliberately refused to do so.

Sigma's refusal to add a generic cleavage claim "operate[s] as a concession of priority for the subject matter of the claim" under 37 C.F.R. § 41.202(c). Rule 202(c) mirrors the longstanding disclaimer doctrine that when the examiner suggests a claim to the applicant for purposes of an interference, and the applicant refuses, such refusal operates as a concession that the subject matter of the claim was the prior invention of another. *In re Ogiue*, 517 F.2d at 1391 (holding that the applicant's failure to copy claims resulted in "concession" that the subject matter of the claims was the prior invention of another); *In re Williams*, 62 F.2d 86, 88 (C.C.P.A. 1932) ("[A]ppellants

having admitted that they are not entitled to the interference counts, they also are not entitled to any subject-matter failing to define invention thereover."); *In re McKellin*, 529 F.2d 1324, 1328 (C.C.P.A. 1976) ("Such consequences are imposed when an applicant, by refusing to copy claims, prevents an award of priority based on evidence of record."); *Ethyl Gasoline Corp. v. Coe*, 139 F.2d 372, 373 (D.C. Cir. 1943) ("Assuming the validity of [the predecessor rule], its effect is to establish, under certain circumstances, an estoppel against the applicant who declines to present a suggested claim."); *see also* M.P.E.P. § 2304.04 ("Refusal to add a required claim will operate as a concession of priority for the subject matter of the required claim."); Fed. Reg. Vol. 69, No. 155, 49992 (Aug. 12, 2004) ("The requirement that an applicant either comply by adding the proposed claim or concede priority of the proposed subject matter is not new ….") (citing *In re Ogiue*).

Sigma's deliberate refusal to add a claim to generic cleavage during prosecution operates as a concession that Sigma did not invent that subject matter and precludes Sigma from contesting priority over that subject matter. It also means that Sigma is not entitled to a two-count interference, which is the only relief supported by the rules. Accordingly, Sigma's motion should be denied.

3. Sigma's claim correspondence proposal is unreasonable and seeks to ensnare 176 CVC claims drawn to the invention Sigma conceded.

As the movant, Sigma must satisfy its "burden of proof to establish that it is entitled to the requested relief"—including that various CVC claims be designated as corresponding to Proposed Count 2. 37 C.F.R. § 41.121(b); Mot. 25:7−29:5. Sigma concedes that CVC's claims "directed to cleavage alone" without a "subsequent step of incorporating or inserting a donor sequence" do not interfere with Sigma's claims. Mot. 26:12−27:4. And Sigma admits that *only 10* of CVC's involved claims—one in each of 10 different CVC applications—recite donor integration by HDR. Mot. 27:4−5. Despite this, Sigma proposes that 186 of CVC's claims should be designated as corresponding to Proposed Count 2. Mot. 28:4−5. In other words, Sigma overreaches by proposing

to have 176 of CVC's claims that are *admittedly not drawn to interfering subject matter* rise or fall with Proposed Count 2. Mot. 29:4−5. Sigma's contention, that 186 of CVC's claims correspond to Proposed Count 2 when only 10 claims (or a mere 5%) are "drawn to" that subject matter—is a complete perversion of the reasoned claim correspondence analysis that Sigma was obligated to perform. *Grose v. Plank*, 15 U.S.P.Q.2d 1338 (B.P.A.I. 1990) ("[T]he motion must show that the claims … are drawn to the same patentable invention as the counts to which they correspond").

The Board should reject Sigma's claim correspondence proposal because Sigma forfeited the right to contest priority over the subject matter of CVC's 176 claims, which are drawn to the invention of generic cleavage and not limited to donor integration by HDR. *See supra* § IV.A.2−3.

Additionally, Sigma has failed to make out a *prima facie* case as to why any of CVC's 176 non-interfering claims correspond to Proposed Count 2. Neither Sigma nor its expert provides an evidence-based analysis explaining how CVC's 176 claims are anticipated or rendered obvious by Proposed Count 2. Sigma bears the burden of proof to establish that CVC's claims correspond to Proposed Count 2. *See* 37 C.F.R. § 41.208(b) ("To be sufficient, a motion must provide *a showing, supported with appropriate evidence*, such that, if unrebutted, it would justify the relief sought.") (emphasis added). With respect to obviousness, which is all that Sigma alleges, that burden is no less than "an examiner rejecting claims or … a requestor for reexamination." *Cf. Horton v. Stevens*, 7 U.S.P.Q.2d 1245 (B.P.A.I. 1988). Yet Sigma's motion and accompanying expert declaration—which parrots the motion—both lack such a *prima facie* showing. Mot. 28:6−10, Ex. 1001, ¶¶ 36, 37. Dr. Cannon's declaration provides no explanation of her understanding of the legal standards she applied to arrive at the conclusory and unsubstantiated assertion that 176 CVC claims "would have been obvious in view of proposed Count 2." Ex. 1001, ¶¶ 36, 37. On cross-examination, Dr. Cannon admitted that she merely compared CVC's claims to Sigma's claim 31 to see if they were "analogous, similar, identical." Ex. 2616, 60:13−61:8; *see also id.*, 62:2−63:14. Beyond that, she

relied on Sigma's lawyers. *Id.*, 62:10−16; 73:15−21. The Board should disregard her conclusory and unsupported testimony. 37 C.F.R. § 41.158(a); *In re Kahn*, 441 F.3d 977, 988 (Fed. Cir. 2006) ("[O]bviousness grounds cannot be sustained by mere conclusory statements; instead, there must be some articulated reasoning with some rational underpinning."). Sigma's motion fares no better, as it mirrors the deficient testimony. Thus, Sigma has not shown that CVC's claims correspond.

4. No authority permits the Board to narrow the count to exclude CVC's best proofs; doing so would be highly prejudicial and unfair to CVC.

Proposed Count 2 should be denied because it would exclude CVC's best priority proofs. It is "an accepted reason in interference practice" to redefine a count when a party's "best proofs" lie outside the scope of the count. *Grose v. Plank*, 15 U.S.P.Q.2d 1338 (B.P.A.I. 1990); *accord Univ. of S. California v. DePuy Spine, Inc.*, 473 F. App'x 893, 895 (Fed. Cir. 2012) ("This failure is particularly troublesome because a party typically should be allowed to rely on its best priority proofs."). The same rationale supports rejecting Sigma's request to narrow Count 1, because doing so would exclude CVC's best proofs. Accordingly, the Board should deny Sigma's motion.

As shown by CVC's priority proofs in the '115 interference, CVC conceived and diligently reduced to practice a sgRNA CRISPR-Cas9 system that cleaved target DNA in zebrafish embryos and human cells. A proffer of these proofs is provided here. Ex. 2628; Ex. 2232; Ex. 2233; Ex. 2033; Ex. 2642; Ex. 2399; Ex. 2399; Ex. 2630; Ex. 2652; Ex. 2653; Ex. 2654; MF 28. These actual reductions to practice involved experiments where no donor sequence was added to induce HDR. *See, e.g.*, Ex. 2033, Fig. 1E (description of "[m]utated alleles of the CLTA gene in HEK293T cells as a result of Cas9-induced NHEJ"); Ex. 2399 (noting that experiments would involve NHEJ).

Building on that work, CVC applied the sgRNA CRISPR-Cas9 system in a eukaryotic cell with a donor sequence and demonstrated efficient HDR-mediated donor integration, and published those results in 2014. *See, e.g.*, Ex. 1046, 1 (reporting 38% efficient HDR, high fidelity genome-

editing, and minimal cell mortality); MF 29. Proposed Count 2 would exclude CVC's proofs for its actual reductions to practice of cleavage or editing by sgRNA CRISPR-Cas9 in a eukaryotic cell, actually by August 9, 2012, in fish and by October 31, 2012, in human cells. MF 28, 29.

Exacerbating this unfair prejudice, Sigma would also have CVC's 176 generic cleavage claims that are directed to the invention *represented* by those proofs rise or fall with a count that *excludes* those proofs. This is unjust and underscores that Sigma is not entitled to its requested relief, particularly because Sigma has not alleged that Count 1 would exclude Sigma's best proofs.

5. Sigma's motion and its expert fail to address the clear question of patentability over the prior art evident from the prosecution history.

Under 37 C.F.R. § 41.208(c)(2): "A party moving to add or amend a count must show how the count is patentable over prior art." As stated in *Louis v. Okada*: "[The movant] has the burden of accounting for and explaining away any potential question of patentability over prior art, if any such question is self-apparent or manifestly evident from the prosecution history of its involved patent or application. An interference cannot proceed with a count over which a lingering question or doubt as to patentability arises from the face of the moving party's prosecution history." 59 U.S.P.Q.2d 1073 (B.P.A.I. 2001) (precedential). The prosecution history of Sigma's involved '204 application leaves self-apparent questions about the patentability of Proposed Count 2 over the prior art. Claim 31 of Sigma's '204 application (which is also Sigma's half Count 1) was allowed due to a hypertechnicality: a decision by the Office of Petitions dated August 24, 2018, permitting Sigma to swear-behind ToolGen's Kim references—one application of which is now involved in interferences—because ToolGen's claims were not then in condition for allowance. Ex. 2622, 65.

The Kim references and Sun 2012 (Ex. 2646) are prior art against Sigma's '204 application, and Sigma's motion had to explain how Proposed Count 2 is patentable in light of those references, at least as articulated by the examiner before Sigma was able to swear-behind. *Id.*, 147−148. But

neither Dr. Cannon nor Sigma's motion addresses the Kim references or Sun 2012 as prior art. Ex. 1001, Appx' A; MF 30. In fact, Dr. Cannon did not consider the prosecution history of Sigma's '204 application *at all*, despite offering the opinion in her declaration that "[m]ultiple references have been cited during the prosecution of Sigma's and CVC's applications" and "[n]one of the art prior to that date, alone or in proper combination, anticipates or renders obvious the subject matter of Proposed Count 2." Ex. 1001, ¶ 59; *see* Ex. 2616, 120:8−20; Mot. 33:15−16. Such conclusory opinions are insufficient. *In re Dembiczak*, 175 F.3d 994, 999 (Fed. Cir. 1999) ("Broad conclusory statements regarding the teaching of multiple references, standing alone, are not evidence.").

Rather than address the prosecution history, Sigma's motion argues that donor integration by HDR should be automatically patentable based on the decisions in Interference No. 106,048. Mot. 33:17−34:21. Yet this argument also ignores the Kim references (which are prior art against Sigma) and Sun 2012 which the examiner cited as "evidence of predictability." Ex. 2622, 148.

Having failed to address the pertinent rejections during prosecution, and relying on expert testimony that did not consider the prosecution history, Sun et al., or the Kim references, Sigma's showing under Rule 208(c)(2) is deficient under *Louis*. Sigma cannot address these issues for the first time in its reply. 37 C.F.R. § 41.208(b); *Nau*, Interference No. 104,258, Paper 57 at 10−12.

B. Sigma has not rebutted the presumption that Count 1 recites one invention.

Sigma asserts that Count 1 recites two patentably distinct invnetions. However, there is a "presumption that the initial count is limited to a single patentable invention." *Lee*, 55 U.S.P.Q.2d 1406. To rebut this presumption, Sigma must demonstrate that the subject matter of its Proposed Count 2 is patentably distinct from the subject matter of CVC's half of Count 1. *Hester v. Allgeier*, 687 F.2d 464, 466 (C.C.P.A. 1982); *see also* M.P.E.P. § 2309.01(A). In this exercise, the subject matter of Count 1 is "treated as prior art." Fed. Reg. Vol. 69, No. 155, 49990−91 (Aug. 12, 2004). For the reasons explained in detail below, Sigma has not rebutted the presumption under *Lee*.

1. Before 2012, donor integration by HDR was regularly achieved by creating DSBs in eukaryotic cells using targeted nucleases.

Before December 6, 2012, the skilled artisan would have known that HDR is a natural and highly-conserved DNA repair process, present in prokaryotes and eukaryotes alike, that is "very active" in the late S and G2 phases of the cell cycle. Ex. 2632, 3703; Ex. 2619, ¶¶ 41−44; MF 10. The skilled artisan would have known that HDR is triggered by a DSB in DNA, independent of the source of the DSB—which could be induced by a variety of different types of DNA damage (e.g., ionizing radiation, genotoxic agents, nuclease cleavage). Ex. 2619, ¶¶ 41, 129; MF 10, 12.

The skilled artisan would have known the general steps of the HDR pathway, including when it was available in the cell cycle and what was required to initiate the pathway: exonuclease resection of the ends of the DSB to create a long 3' overhang. Ex. 2619, ¶¶ 60−65; MF 10, 16.

The skilled artisan would have understood that HDR results in integration of a homologous sequence from a donor repair template at the site of the DSB and could therefore be exploited as a precise method for repairing DSBs in the DNA. Ex. 2619, ¶¶ 45−59; MF 10−16. It is possible for the sister chromatid to serve as the repair template or for an exogenous donor sequence to be added for integration. Ex. 2619, ¶¶ 41−44; MF 10−16. In both cases, the presence of the template and the existence of a DSB was understood to trigger the HDR process. Ex. 2619, ¶¶ 45, 46; MF 10−16.

A complete or comprehensive understanding of the mechanism for HDR was unnecessary to exploit the process for the purpose of integrating a donor sequence at the site of a DSB made by a targeted nuclease. Ex. 2619, ¶ 65; MF 20. Before 2012, skilled artisans were regularly exploiting HDR to perform targeted genome editing in a variety of eukaryotic cells and organisms, including nematodes, *Xenopus laevis* oocytes, fruit flies (*Drosophila melanogaster*), plants, stem cells, and mammalian cell types in culture (e.g., HEK293, HEK293T, mouse). Ex. 2619, ¶¶ 45, 55, 116, 171; MF 10. Indeed, these applications were well-developed by 2012. Several groups had shown before

2000 that DSBs generated by I-SceI meganuclease in "mammalian chromosomes can be repaired by either HR or NHEJ and that DSBs stimulate HR." Ex. 2624, 8353; Ex. 2619, ¶¶ 44−45; MF 10−16. The laboratory techniques developed for achieving targeted donor integration by HDR in the case of DSBs created by meganucleases were applied to ZFNs in the 2000s, and then likewise to TALENs soon after they were developed as a targeted nuclease. Ex. 2619, ¶¶ 53−55; MF 18−21.

Indeed, by 2012, there were "three different platforms for creating designer nucleases: re-engineered homing endonucleases, zinc-finger nucleases (ZFNs) and transcription activator–like effector nucleases (TALENs)." Ex. 2597, 28; Ex. 2619, ¶¶ 53−59; MF 10−16. The skilled artisan knew that DSBs created by any of these targeted nucleases would significantly increase "the yield of specific homologous recombination events"—allowing for the predictable exploitation of HDR for genome-editing applications in various eukaryotes. Ex. 2308, 289; Ex. 2619, ¶ 53; MF 10−16.

By 2012, laboratory techniques for engineering donor templates having suitable homology were routine and the task of synthesizing donors was "trivial." Ex. 2327, 755; Ex. 2617, ¶ 19; Ex. 2619, ¶ 50−55, 66; Ex. 2616, 103:2−16; MF 18−21. Techniques for introducing donor templates and targeted nucleases into cells were also routine. Ex. 2619, ¶¶ 74−79, 115−118; Ex. 2617, ¶¶ 8, 12−19; Ex. 2616, 103:2−16; MF 18−21. The skilled artisan would also have known of techniques for increasing HDR frequency in eukaryotic cells, including by arresting cells in the S or G2 phase, optimizing the donor template, simply increasing the amount of donor template, or inhibiting the NHEJ pathway—for example, through the use of drugs or genetically manipulating the target cell or organism. Ex. 2619, ¶¶ 53, 54, 66, 86; Ex. 2617, ¶¶ 13−19; Ex. 2616, 37:24−38:4; MF 18−21.

Before 2012, it was also a well-understood principle in the context of genome-editing that, within any population of growing cells treated for genome editing with a targeted nuclease, some cells would have gone through the S and G2 phases of cell cycle and would have integrated a donor sequence by HDR. Ex. 2619, ¶¶ 50−52, 94, 102−103; Ex. 2617, ¶ 17; MF 21. The skilled artisan

would not have needed every cell in the population to undergo HDR-mediated donor integration, but would have screened the population for cells having HDR-mediated donor integration, which was routinely done using FACS or PCR. Ex. 2619, ¶ 103; Ex. 2616, 105:18−106; MF 18−21. In doing so, the skilled artisan would have expected to find some cells showing HDR-mediated donor integration and would select those for expansion. Ex. 2619, ¶ 103. Screening and selection methods were used routinely in genome-editing applications with meganucleases, ZFNs, and TALENs. *Id.*

The skilled artisan further understood that the same donor-integration techniques used for genome-editing applications with meganucleases, ZFNs, and TALENs would apply when using sgRNA CRISRPR-Cas9 to create the targeted DSB. Ex. 2619, ¶¶ 62, 63, 85. This is because the downstream process of donor integration by HDR was regarded as independent from the source and nature of the targeted DSB. Ex. 2618, ¶¶ 19, 22; Ex. 2619, ¶¶ 62, 63, 85, 138; MF 10, 13, 14.

The skilled artisan would therefore have applied that understanding of the HDR process to CRISPR-Cas9 when it burst onto the scene in June of 2012 as the next major category of targeted nuclease for genome editing. Ex. 2619, ¶¶ 71−74; Ex 2617, ¶¶ 5−12; Ex. 2618, ¶¶ 18-26; MF 10.

2. Proposed Count 2 is not patentably distinct from CVC's half of Count 1, which recites CRISPR-Cas9 cleavage or editing of target DNA.

Sigma cannot rebut the presumption that Count 1 is limited to a single patentable invention because: (1) Count 1, which is treated as prior art, teaches "a eukaryotic cell comprising" a sgRNA CRISPR-Cas9 system that is "capable of cleaving or editing the target DNA molecule" (Paper 1, 5−6); (2) as shown below, a skilled artisan would have been highly motivated to follow the explicit suggestions in Carroll 2012 to introduce CRISPR-Cas9 and a donor sequence for integration by HDR and thereby perform precise genome editing in a eukaryotic cells, for example, in fruit fly embryos as described in Beumer 2008, using direct injection to introduce the system and a donor sequence (*see infra* § IV.B.b.); (3) in doing so the skilled artisan would have reasonably expected

to be successful, given the prior successes that were regularly achieved before 2012 using ZFNs, TALENs, and meganucleases to create targeted DSBs in DNA followed by HDR-mediated donor integration across a range of eukaryotic cells types, including fruit flies (*see infra* § IV.B.c.); and (4) Sigma's motion does not allege nor are there any objective indicia of nonobviousness (*see infra* § IV.B.e.). Accordingly, Proposed Count 2 would have been obvious before December 6, 2012.

a. Count 1 is prior art and teaches cleavage or editing of target DNA by a CRISPR-Cas9 system in a eukaryotic cell.

CVC's half of Count 1 recites a sgRNA CRISPR-Cas9 system "capable of hybridizing with a target sequence" and "cleaving or editing the target DNA" in "a eukaryotic cell." Paper 1, 5−6; MF 1. The prior art therefore includes a sgRNA CRISPR-Cas9 system in a eukaryotic cell that has reached the nucleus, hybridized with the target DNA, and cleaved the target DNA to create a DSB.

The only difference between CVC's half of Count 1 and CVC's half of Proposed Count 2 is that the DSB resulting from the cleavage is "repaired by a homology-directed repair mechanism which incorporates a sequence of a donor polynucleotide into the target DNA molecule, thereby editing the target DNA molecule." Mot. 6:14−18. Donor integration by HDR occurs naturally in the cell in response to a DSB when there is a donor sequence present. Ex. 2619, ¶¶ 82−84; MF 13.

Sigma and its expert Dr. Cannon fixate on alleged uncertainty regarding whether CRISPR-Cas9 was expected to function in a eukaryotic cell. *See, e.g.*, Mot. 6:33−7:15, 22:7−23:2; Ex. 1001, ¶¶ 142, 60, 61, 122−130. But this is legally irrelevant and misleading because Count 1 assumes the successful application of sgRNA CRISPR-Cas9 in a eukaryotic cell. Paper 1, 5−6; MF 1.

Sigma and Dr. Cannon mischaracterize the articles by Dr. Dana Carroll (Ex. 2339) and Dr. Rodolphe Barrangou (Ex. 2215) published in September of 2012. Mot. 22:16−23:2; Ex. 1001, ¶¶ 143−145, 153−157. Both authors provide testimony rebutting Sigma's suggestion that their articles convey anything but clear *confidence* that CRISPR-Cas9 would function as a genome-editing tool,

including in eukaryotes and including for applications involving HDR-mediated donor integration. Ex. 2617, ¶¶ 5−12; Ex. 2618, ¶ 18−27. Both described, and illustrated, the CRISPR-Cas9 system as being in the same category as meganucleases, ZFNs, and TALENs. Ex. 2617, ¶ 12; Ex. 2618, ¶ 20. Both noted that the same techniques applied with those targeted nucleases would apply equally to genome-editing applications involving CRISPR-Cas9. Ex. 2617, ¶¶ 8, 9; Ex. 2618, ¶¶ 19−24.

b. Sigma does not deny that a skilled artisan would have been motivated to add a donor sequence for integration by HDR.

Dr. Cannon agrees that Carroll 2012 (Ex. 2339) "provide[s] an accurate representation of the knowledge of the skilled person at the start of December 2012 …." Ex. 1001, ¶ 25; MF 10. Carroll 2012 was published in the journal *Molecular Therapy* in September of 2012. Carroll 2012 provides a discussion of the system described in Jinek 2012 (an embodiment of sgRNA CRISPR-Cas9 of CVC's half of Count 1), explaining in detail how the CRISPR-Cas9 system would function as a tool for genome-editing and comparing it to existing targeted nucleases. Ex. 2339, 1658−1660; Ex. 2619, ¶¶ 71−74; Ex. 2617, ¶¶ 7−9. In this context, Carroll 2012 explains that "[w]hen modified donor DNA is also provided, repair by homologous recombination will lead to introduction of donor sequences at the target" and that "[t]hese break-induced modifications can be very efficient, in the range of 10% or more of all targets in a single treatment." *Id.*, 1658. Figure 1 of Carroll 2012 illustrates that—regardless of which "targetable nuclease" creates the DSB (whether a ZFN, TALEN, meganuclease, or the "new CRISPR reagents")—the DSB would be repaired by NHEJ or HDR. *Id.*, 1659; MF 14, 17. Carroll 2012 Figure 1 illustrates that if "Donor DNA" is added ("+") this "result[s] in replacement of genomic sequences," utilizing a "manipulated donor DNA as a template." *Id*. Thus, Carroll 2012 describes that HDR would be induced by a DSB created by the sgRNA CRISPR-Cas9 system, and that this results in HDR-mediated donor integration. *Id.*, 1659, Fig. 1. Carroll 2012 also describes *how* CRISPR-Cas9 would be introduced into a eukaryotic

cell, for example: "In many animals, direct injection of nuclease-encoding messenger RNAs into early embryos has proved quite effective in generating germline modifications." *Id.*, 1660.

Carroll 2012 cites to Carroll 2011 (Ex. 2401) and Urnov 2010 (Ex. 2135), which catalogue successful applications of ZFNs for HDR-mediated donor integration in various eukaryotic cell types. *Id.*, 1660. Carroll 2011 and Urnov 2010 both cite Beumer 2008 (Ex. 2123). Ex. 2619, ¶¶ 74, 78; MF 10, 11. Dr. Carroll is an author for Carroll 2012, Carroll 2011, and Beumer 2008. Ex. 2619, ¶ 74; MF 10, 11. Jinek 2012 cites to Urnov 2010 (Ex. 2031, 821). Thus, these references are heavily interconnected, underscoring the relationship between their teachings. Ex. 2619, ¶88; MF 10, 11.

Beumer 2008 discloses detailed information, including parameters for optimizing the donor DNA structure as well as a specific protocol for coinjecting donor DNA along with mRNAs encoding the targeted nuclease to achieve reliable and efficient HDR-mediated donor integration Ex. 2123, 19821–19826; Ex. 2617, ¶¶ 13−15; Ex. 2619, ¶¶ 75−79. Consistent with the suggestion in Carroll 2012 to directly inject messenger RNAs, Beumer 2008 "recommend[s] injecting ZFN mRNAs and the desired donor DNA into *lig4* mutant embryos because the frequency of HR is very high and very few NHEJ products are produced," noting "the results were quite dramatic," in that "essentially all of the mutants were the result of HR, with very rare NHEJ products." Ex. 2123, 19824−19825; MF 11. Beumer 2008 reports that by using "embryos mutant for DNA ligase IV, a component of the canonical NHEJ pathway (28), the bias [between these two repair pathways] was shifted strongly toward products of HR with a coinjected donor DNA." *Id.*, 19821; MF 11, 15.

Given CVC's half of Count 1, the skilled artisan would be motivated to apply the recited sgRNA CRISPR-Cas9 system for genome-editing in eukaryotes, as expressly suggested in Carroll 2012. Ex. 2619, ¶¶ 77−79. Further, Carroll 2012 expressly suggests that targeted DNA cleavage, as predicted in Jinek 2012, would have applications for genome editing by HDR-mediated donor integration, as had been done successfully with meganucleases, ZFNs, and TALENs in eukaryotes.

Id. Carroll 2012 explains that this could be done using the same techniques used with the existing targeted nucleases. Ex. 2339, 1660 (recommending direct injection of mRNAs into embryos).

Carroll 2011 and Urnov 2010 are cited in Carroll 2012, and each cites Beumer 2008 for its protocol for performing efficient HDR-mediated donor integration in a fruit fly model. Ex. 2619, ¶ 78. A skilled artisan would have been motivated to combine CVC's half of Count 1 with Carroll 2012 and further with Beumer 2008, because Beumer 2008 offers a "straightforward" protocol for performing gene replacement with a targeted nuclease (i.e., a ZFN), one that "greatly simplifies both targeted mutagenesis and targeted gene replacement" and thus makes "ZFN-directed genome modification much more accessible." Ex. 2123, 19823−19824; Ex. 2617, ¶ 13−15; MF 10, 11. As a publication highlighted in both Carroll 2011 and Urnov 2010 as providing a successful model, the skilled artisan would be highly motivated to apply Beumer 2008. Ex. 2619, ¶ 79; MF 10, 11.

Beumer 2008 and Carroll 2012 both comment on the limitations of ZFNs in terms of the labor associated with programming them for different targets. Ex. 2123, 19824 ("Each time a new gene is selected for targeting, new ZFNs must be designed and constructed."); Ex. 2339, 1658 (noting that CRISPR-Cas9 provides a solution to the "design challenge" of developing new DNA binding domains, "which can be dauntingly laborious"); MF 10. A skilled artisan would thus have been strongly motivated to substitute the ZFN with sgRNA CRISPR-Cas9. Ex. 2619, ¶ 79.

Tellingly, Sigma does not dispute that the prior art provided the skilled artisan with such a motivation. Mot. 7:9−12, 8:11−12. Rather, Sigma focuses exclusively on whether a skilled artisan would have expected success in achieving donor integration by HDR in a eukaryotic cell.

c. The skilled artisan would have expected that adding a donor sequence would necessarily result in integration by HDR.

By applying the teachings of Carroll 2012 in combination with the detailed protocol of Beumer 2008, a skilled artisan would have reasonably expected to observe HDR-mediated donor

integration resulting from cleavage by the sgRNA CRISPR-Cas9 system of Count 1. Ex. 2619, ¶¶ 82−94. Applying sgRNA CRISPR-Cas9 in fruit flies would have been a matter of combining the prior art sgRNA CRISPR-Cas9 system "in a eukaryotic cell" with well-established techniques for adding a donor sequence for integration by HDR, as described in both Carroll 2012 and Beumer 2008. Ex. 2619, ¶¶ 67, 79. The skilled artisan would have simply substituted one known targeted nuclease (ZFN) with another (sgRNA CRISPR-Cas9) and followed Beumer 2008's protocol. *Id.*

During prosecution of Sigma's involved application, the examiner made a similar finding, citing as "evidence of predictability" Sun 2012 for its teaching of successful integration of donor sequences by HDR in eukaryotic cells following the creation of DSBs by each of ZFNs, TALENs, and meganucleases. Ex. 2622, 147−148; MF 10, 14. As shown below, the examiner's reasoning is consistent with the contemporaneous evidence, which universally predicted that the CRISPR-Cas9 system would be a genome editing tool, including for inducing HDR-mediated donor integration.

d. Sigma does not allege, nor are there, any objective indicia supporting the nonobviousness of Proposed Count 2.

In its motion Sigma does not assert nor are there any objective indicia of nonobviousness. Sigma cannot assert any for the first time in its reply. 37 C.F.R. § 41.208(b); *Nau*, Interference No. 104,258, Paper 57 at 10−12. Accordingly, no objective indicia support nonobviousness in this case.

3. Sigma's hypothetical concerns about the expectation of achieving HDR-mediated donor integration are fundamentally flawed.

At page 6 line 3 to page 22 line 3 of the motion, Sigma argues that a skilled artisan would not expect success based on 12 hypothetical concerns. The response is that Sigma's concerns are unfounded because the skilled artisan knew that DSBs created by a variety of sources—including three different prior art targeted nucleases—all induced HDR-mediated donor integration, which had been reliably exploited for over a decade to perform successful genome editing in eukaryotes. Ex. 2619, ¶¶ 45−59. Those in the field had high confidence that this would be true for CRISPR-

Cas9 as well, as documented in their 2012 publications. Ex. 2617, ¶¶ 5−12; Ex. 2618, ¶¶ 18−26.

Moreover, Sigma's alleged concerns, while itemized, amount to a generalized requirement that a skilled artisan have certainty. But the law does not require "absolute certainty." *Par Pharm., Inc. v. TWi Pharms., Inc.*, 773 F.3d 1186, 1198 (Fed. Cir. 2014). "[O]bviousness 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." *Pfizer, Inc. v. Apotex, Inc.*, 480 F.3d 1348, 1364 (Fed. Cir. 2007).

Tellingly, Sigma's concerns do not call for any specific adaptations or novel techniques to facilitate donor integration by HDR. Rather, as Dr. Cannon conceded, the steps required to achieve donor integration by HDR were "routine." Ex. 2616, 103:2−16, 105:12−106:20; MF 19. Indeed, Sigma's provisional does not describe any novel techniques or methods. *See* Ex. 2616, 103:2−16.

Moreover, while resisting the notion that prior success with ZFNs and TALENs predicted success with CRISPR-Cas9, Sigma itself relies on the ZFN precedents to fill gaps in its provisional disclosure. For example, Dr. Cannon points to Moehle 2007 (Ex. 1052), a publication describing successful HDR-mediated green fluorescent protein ("GFP") integration in human cells after ZFN cleavage, to explain how Sigma's use and analysis of that insert was consistent with prior practice. Ex. 1001, ¶ 70 ("ZFNs, also a type of targeted nuclease, could direct the site-specific insertion of a DNA donor sequence containing the GFP gene at a targeted genomic site."). Indeed, Dr. Cannon analogizes Moehle 2007's approach using a ZFN to achieve targeted insertion of a GFP sequence to Sigma's experiments in its P1 using sgRNA CRISPR-Cas9 to do the same thing. *Id.*, ¶¶ 70−75.

The dearth of evidence supporting these concerns confirms that they are post-hoc litigation positions and not a reflection of what a skilled artisan would have thought as of December 6, 2012.

a. HDR-mediated donor integration following DSBs made by other targeted nucleases sufficiently predicted success.

The success that had been achieved consistently before 2012 using meganucleases, ZFNs,

and TALENs predicted that DSBs created by the sgRNA CRISPR-Cas9 system would trigger the cell's natural HDR process, which could be exploited to integrate a donor sequence. Ex. 2619, ¶¶ 82−94. A skilled artisan knew that different types of DSBs generated by the targeted nucleases induced HDR-mediated donor integration, including DNA cleavage producing as 3' overhang (I-SceI) and DNA cleavage producing a 5' overhang (*FokI*). Ex. 2619, ¶ 63; Ex. 1001, ¶ 131; MF 17.

A skilled artisan knew that different types of cleavage mechanisms would induce HDR-mediated donor integration. For example, nucleases that remained bound to the ends of the DSB still induced HDR (e.g., Spo11, ZFNs, I-SceI). Ex. 2619, ¶¶ 62, 104, 138, 142; MF 10, 22, 26.

This tolerance for different types of cuts and different mechanisms of cutting, is consistent with the fact that HDR is a highly conserved process, and its role in protecting genomic integrity against diverse sources of damage—from ionizing radiation to genotoxic chemicals. Ex. 2619, ¶¶ 41, 46, 72; Ex. 2616, 19:4−24, 19:24-20:4 (Q: "As you sit here today, can you think of a type of double-stranded DNA break that homology-directed repair can't repair?" A: "No, I can't.").

Conversely, it was also known in the context of bacterial immunity that Cas9 was required both for cleavage *and* the recominational integration of spacer sequences sampled from invading DNA into the bacterial chromosome. Ex. 2618, ¶¶ 9−17, 22, 24, 30−32 (citing Ex. 2129, 67); Ex. 2619, ¶ 129. Thus, CRISPR-Cas9 was assumed to be compatible with mechanisms similar to HDR.

b. Sigma's fabricated post-hoc concerns either have nothing to do with CRISPR-Cas9 or are premised on faulty assumptions.

Sigma's motion presents a laundry list of alleged concerns that a skilled artisan would have had. Each concern is fundamentally flawed and contradicted by the contemporaneous evidence.

First, Sigma's alleged concerns about the complexity and frequency of HDR versus NHEJ (concerns [1−5]) would apply equally to meganucleases, ZFNs, and TALENs—all of which were successful genome-editing tools before 2012—and thus would not be concerns to a skilled artisan

in 2012. Second, alleged concerns about CRISPR-Cas9's ability to cleave DNA in a eukaryotic cell (concerns [6−7] [10−12]) are obviated by the fact that CVC's half of Count 1 is prior art.

Sigma's remaining concerns regarding "blunt ends" (concern [8]) and Cas9 allegedly being "sticky" (concern [9]) rest on inaccurate premises and are contradicted by evidence that the skilled artisan knew that HDR was not prevented by blunt ends or whether the nuclease remained bound.

i. Concerns 1−5 apply to all targeted nucleases, and therefore have nothing to do with CRISPR-Cas9.

Accepting for the sake of argument that there were concerns that the mechanism of HDR was not fully characterized [1], that in higher eukaryotes HDR occurs at a lower frequency than NHEJ [2], that HDR is available only during specific phases of the cell cycle [3], that NHEJ makes errors preventing target recognition and cleavage [4], and that NHEJ was generally more likely to occur than HDR [5], all of these considerations *also* apply to meganucleases, ZFNs, and TALENs, which were all successful by 2012 nonetheless. Ex. 2619, ¶¶ 97−99, 108−125. The frequency of HDR and NHEJ, as competing repair pathways in any given cell, was known to apply to the prior art targeted nucleases, yet by 2012 HDR-mediated donor integration was being routinely achieved. *Id.*, ¶¶ 97−99. Indeed, by December 2012, ZFNs were even being tested in clinical trials. Ex. 2617, ¶¶ 15−18. The skilled artisan would not reasonably have expected the so-called concerns to be impediments to using CRISPR-Cas9 to induce HDR-mediated donor integration in a eukaryotic cell. Ex. 2619, ¶¶ 97−99. This is particulary true in light of the teachings of Beumer 2008, which reported the achievement of high efficiency HDR in fruit flies with a simplified protocol. *Id.*, ¶ 75.

Regardless of whether every aspect of the mechanism of HDR was characterzed in 2012, Dr. Cannon concedes that there were predictable and reliable ways (including FACS and PCR) to ascertain whether or not HDR-mediated donor integration had occurred. Ex. 2616, 36:23−37:18, 105:18-106:1. Thus, not knowing the exact mechanism of HDR did not prevent those in the field

from predictably and reliably exploiting HDR in genome editing applications. Ex. 2619, ¶¶ 60−65.

Separately, many alleged concerns relate to whether HDR would occur *efficiently* [2, 3, 5]. Ex. 2619, ¶¶ 100−104. But, whether HDR occurs efficiently or is the "preferred" pathway for a given cell type is irrelevant because Proposed Count 2 requires no such thing. Mot. 11:19−12:14. It suffices that HDR would occur, which Dr. Cannon admits *is the case* across the spectrum of cell types in which this highly-conserved repair system exists and functions. Ex. 2616, 46:23−47:2.

Dr. Cannon also concedes that, before December 2012, the skilled artisan knew that HDR could be promoted, including by "arresting cells at the specific stage of the cell cycle where homology-directed repair is able to occur." Ex. 2616, 37:24−38:4; Ex. 2619, ¶¶ 66, 86; MF 18.

Nor does Proposed Count 2 require that the eukaryotic cell be that of a "higher eukaryote" [2], [3]. Mot. 11:19−14:17. Setting aside the unfounded premise that HDR is somehow not viable in higher eukaryotes, Proposed Count 2 is broad enough to include single-celled eukaryotes such yeast (*Saccharomyces cerevisiae*). Ex. 2619, ¶ 107. Dr. Cannon admits that it was known in the art before 2012 that yeast preferentially repairs DSBs by HDR, not NHEJ. Ex. 2616, 126:4-8; MF 10. Consistent with this knowledge, the prior art provides many examples of ZFNs and TALENs stimulating HDR-mediated donor integration in yeast. Ex. 2619, ¶¶ 45, 58, 102, 107; MF 10, 12.

Moreover, it was standard practice to perform genome-editing experiments on a population of cells, with the understanding that only a subset would undergo HDR-mediated donor integration and that those cells would be detected and selected out using well-known techniques such as FACS analysis, which Sigma admits was routinely used in genome-editing applications involving ZFNs and TALENs before December 2012. Ex. 2616, 36:23−37:18, 105:18−106:1; Ex. 2619, ¶¶ 102, 103; MF 20, 21. The population selected cells could then be expanded. Ex. 2619, ¶¶ 102, 103.

ii. Concerns 6−7 and 10−12 about CRISPR-Cas9 ignore that Count 1 *assumes* targeted cleavage in a eukaryote.

Sigma's alleged concerns [6−7] and [10−12] revolve around whether CRISPR-Cas9 would be capable of creating a DSB at the target DNA in a eukaryotic cell. For example, Sigma alleges that the skilled artisan would have been concerned about "accessibility of a target sequence in a eukaryotic genome to the prokaryotic CRISPR-Cas9 complex" because of chromatin [7] and "access the eukaryotic nucleus" because CRISPR-Cas9 originates in bacteria [12]. These alleged concerns are irrelevant because Count 1 *assumes* that the sgRNA CRISPR-Cas9 system is capable of both "hybridizing with a target sequence in the target DNA molecule" and "cleaving or editing the target DNA" in "a eukaryotic cell." Paper 1, 5−6; Ex. 2619, ¶¶ 105, 106, 126−135, 147−163. The only question is whether *donor integration by HDR* would have been obvious. Indeed, it was.

Sigma's generalized concern that CRISPR-Cas9 was a "*prokaryotic* CRISPR-Cas9 DNA degradation system" [6], [7], [12] is contradicted by contemporaneous evidence and Dr. Carroll's and Dr. Barrangou's testimony. Ex. 2617, ¶¶ 5−19; Ex. 2618, ¶¶ 9−11, 18−27, 30−32; Ex. 2545, ¶¶ 3−15; Ex. 2547, ¶¶ 14-21. Indeed, their September 2012 publications expressly identified HDR-mediated donor integration as an application of CRISPR-Cas9. Ex. 2617, ¶ 12; Ex. 2618, ¶¶ 19−24.

Sigma's alleged concerns regarding specificity [10] and off-target activity [11] apply also to ZFNs and TALENs, which had limited specificity but were successful. Ex. 2619, ¶¶ 152−154. Moreover, as described in Jinek 2012, CRISPR-Cas9 was known to be programmable with a 20-nucleotide crRNA, which accounts for the system's specificity for a given DNA target. Ex. 2617, ¶ 7; Ex. 2618, ¶¶ 25−26; Ex. 2031, 818. As Dr. Carroll stated: "Watson–Crick pairing can be very specific, and a match of 16–20 base pairs is sufficient to ensure recognition of a unique sequence in a complex genome." Ex. 2339, 1660; Ex. 2617, ¶ 7. Dr. Carroll called Watson–Crick pairing the "gold standard" for achieving specificity. Ex. 2339, 1658; Ex. 2617, ¶ 7. Barrangou 2012 and Brouns 2012 echo this. Ex. 2215, 837 ("This synthetic *tour de force* introduces a new endonuclease family to the genome engineering arsenal that enables synthetic RNA-mediated reprogramming

cleavage specificity"); Ex. 1021, 809 ("Jinek *et al.* realized that a highly specific customizable RNA-directed DNA nuclease could be sueful to edit whole genomes."). Indeed, both Carroll 2012 and Barrangou 2012 highlight the added target specificity provided by the PAM requirement—as yet another reason to expect that CRISPR-Cas9 would achieve greater specificity for genome-editing applications than ZFNs and TALENs. Ex. 2339, 1659; Ex. 2618, ¶¶ 25−26. These three articles, which Dr. Cannon concedes "provide an accurate representation of the knowledge of the skilled artisan at the start of December 2012," disprove alleged concerns [11−12]. Ex. 1001, ¶ 25.

Even the specification of Sigma's involved '204 application admits: "Since the specificity [of CRISPR-Cas9] is provided by the guide RNA, the RNA-based endonuclease is universal and can be used with different guide RNAs to target different genomic sequences … Furthermore, the targeting is specific *with limited off target effects*." Ex. 2585, ¶ [0015] (emphasis added).

iii. Concern 8 is unfounded because CRISPR-Cas9 can leave staggered ends, and blunt ends are not a problem.

The premise of Sigma's "blunt ends" argument is that the CRISPR-Cas9 system cuts DNA and leaves ends having no overhangs. Mot. 17:22−18:17. That is incorrect. In Jinek 2012, CVC's inventors reported that CRISPR-Cas9 generates DSBs, some blunt *and some with overhangs*. Ex. 2619, ¶¶ 136−140; Ex. 2618, ¶ 32; Ex. 2031, 816, Fig. 1E, Fig. S4B; MF 25. This description is consistent with CVC's provisional applications, which disclose that "DNA cleavage can result in the production of either blunt ends *or staggered ends*." Ex. 2009, ¶ [0042] (emphasis added); *id.*, Fig. 4 (depicting a "DSB" having an overhang or "staggered" ends); Ex. 2619, ¶ 137; MF 25. Thus, the skilled artisan would have understood from Jinek 2012 that CRISPR-Cas9 cleavage produces ends with overhangs; Sigma and its expert failed to consider this. Ex. 2616, 116:23−118:18.

Regardless, any allegation of concern is contradicted by evidence that blunt ends would, in fact, induce HDR-mediated donor integration. Ex. 2619, ¶ 138 (citing various references). It was

known that HDR occurs regardless of the nature of the ends, and that cut ends with a 5' overhang achieve HDR similarly to cut ends with a 3' overhang. Ex. 2619, ¶ 63; MF 17, 26, 27. The skilled artisan understood that HDR is a versatile process, adapted to repair diverse DNA damage, and was not sensitive to nature of the cut or the mechanism by which it was made. Ex. 2619, ¶¶ 63, 85.

Moreover, the first step of HDR involves resection of the ends of the DNA to create a long stretch of single stranded DNA in the form of a 3' overhang. *Id.*, ¶¶ 61, 66, 138; MF 10, 16. It was also known that resection functions on blunt ends. *Id.*, ¶¶ 62, 138, 139. Given these precedents, the skilled artisan expected HDR to occur at cleavage sites having blunt ends. Westmoreland 2012 does not prove otherwise. The authors were not investigating whether or not HDR-mediated donor integration can be induced based on blunt-ended cuts, as they did not add any donor polynucleotide to promote HDR. Ex. *Id.*, ¶ 139. In any event, Westmoreland 2012 did report some "survivors" which, although rare, were thought to be repaired by HDR despite the challenging conditions. *Id.*

iv. Concern 9 is unfounded because Cas9 was not known to be "sticky" and that would not be a concern anyway.

Sigma's alleged concern that Cas9 would somehow interfere with HDR by sticking to the cleavage site [9] is premised on a flawed interpretation of Gasiunas 2012 (Ex. 1031), co-authored by Dr. Barrangou. Mot. 18:18−19:6. As Dr. Barrangou explains, Dr. Cannon mischaracterizes this article by interpreting it as reporting that Cas9 is "sticky" and remains bound to the target, thereby interfering with HDR. Ex. 2618, ¶¶ 28−31; Ex. 2619, ¶ 144. Rather, Gasiunas 2012 reported, in passing, that *some* Cas9-crRNA remained bound to the reaction products in a specific experiment. Ex. 2618, ¶ 29 (discussing Fig. S11, which Dr. Cannon did not consider); MF 23. Dr. Barrangou explains that his article does not state or imply that Cas9 is "sticky" or that it interferes with HDR.

Regardless, the skilled artisan would have known that the CRISPR-Cas9 complex, like any other DNA-binding domain, would be displaced during DNA replication and transcription, e.g., in

growing cells such as those that would commonly be the subject of genome-editing experiments. Ex. 2619, ¶¶ 141−143. Further, as Dr. Barrangou explains, it was known in the art that Cas9 cutting was fully compatible with the integration of new spacer sequences into the bacterial chromosome. Ex. 2618, ¶¶ 10, 13-16, 22, 30−31 (citing Ex. 2129, 67). Indeed, the genetic signatures associated with this recombination functionality had led Makarova et al. to suggest that CRISPR-Cas systems *were* DNA repair systems. *Id.*, ¶ 16; Ex. 2621, 482. Because Cas9 was known to be required for integrating DNA in to the bacterial chromosome, Cas9 would not have been expected to interfere with such a process. *Id.*, ¶¶ 22, 28, 30. Rather, the skilled artisan would have expected that CRISPR-Cas9 activity would be fully compatible with the HDR process. *Id.*, ¶¶ 10, 11, 14, 21, 30.

The skilled artisan also would have expected, based on Jinek 2012, that Cas9 was a multiple turnover enzyme, meaning that it dissociates from the cut DNA rapidly. Ex. 2619, ¶ 144; MF 24.

Even assuming CRISPR-Cas9 remained bound after cleavage, this would not have been regarded as problematic because the skilled artisan knew that HDR-mediated donor integration occurs even when a nuclease *remains covalently bound* at the site of the DSB, as is the case with the protein Spo11. Ex. 2619, ¶ 138; MF 22. Similarly, targeted nucleases I-SceI meganuclease and ZFNs were known to remain bound to the ends of the DSBs, and yet they routinely induced HDR-mediated donor integration. Ex. 2619, ¶ 104; MF 22. Given this, the skilled artisan would not have expected Cas9 to interfere with HDR or otherwise preclude HDR-mediated donor integration.

C. If the Board does not adopt Proposed Count 2, then Rule 41.202(c) compels a judgment of priority for CVC on Count 1 based on Sigma's concession.

As shown above (*see supra* § IV.A.2−3), Sigma conceded priority under Rule 202(c) of the generic cleavage invention represented by CVC's half of Count 1 by deliberately refusing to add a claim to that subject matter during prosecution for purposes of an interference. Proposed Count 2 is not patentably distinct from the subject matter represented by CVC's half of Count 1.

In view of these two circumstances, the Board should deny Sigma's motion and also enter a judgment of priority in favor of CVC for Count 1 by operation of Rule 202(c), terminating the interference. *In re Ogiue*, 517 F.2d at 1390; *In re Williams*, 62 F.2d at 88; *In re McKellin*, 529 F.2d at 1328. This is the inevitable and only logical consequence of Sigma having conceded that it did not invent the generic cleavage subject matter in combination with the presumption, which Sigma has failed to rebut, that Sigma's half of Count 1 is the same invention that Sigma conceded during prosecution. *Lee*, 55 U.S.P.Q.2d 1406; M.P.E.P. § 2304.04 ("The applicant would then be barred from claiming, not only the subject matter of the required claim, but any subject matter that would have been anticipated *or* rendered obvious if the required claim were treated as prior art."). Equity favors this result, as "consequences are imposed when an applicant, by refusing to copy claims, prevents an award of priority based on evidence of record." *In re McKellin*, 529 F.2d at 1328.

D. If the Board adopts Proposed Count 2, then it should enter a judgment of priority for CVC on CVC's half of Count 1 and 176 generic cleavage claims.

If, despite the numerous deficiencies identified here, the Board grants Sigma's motion, then the Board should still enter a judgment of priority, by operation of 37 C.F.R. § 41.202(c), in CVC's favor for the subject matter represented by CVC's half of Count 1, including with respect to CVC's 176 generic cleavage claims. This is the only logical consequence of Sigma's concession during prosecution that it was not first to invent the subject matter represented by CVC's half of Count 1 in combination with Sigma's concession in its motion that the subject matter of CVC's 176 claims does not interfere with Proposed Count 2. Mot. 26:12−27:4. Niether the law nor equity justifies subjecting CVC's 176 generic cleavage claims to an interference with Sigma's Proposed Count 2. And in any event, CVC's 176 noninterfering claims to generic cleavage should not be designated as corresponding to Proposed Count 2, for the reasons detailed above. *See supra* § IV.A.3−4.

E. If the Board adopts Proposed Count 2, Sigma should not get benefit to its P1

at least because it failed to include the affidavit required by Rule 158(b).

At page 29, line 9 of the motion, Sigma argues that it is entitled to the benefit of Sigma P1 for Proposed Count 2. The response is that Sigma's assertion of priority benefit relies on "tests and data" but lacks the required support in the form of an affidavit under 37 C.F.R. § 41.158(b). Sigma relies on data from technical tests, namely the results of FACS analysis and PCR, the results of which Sigma states are shown in "Examples 4 and 5 (referencing Figures 4 and 5, respectively)." Mot. 29:15−16. Sigma's arguments rely on Dr. Cannon, who opines in a conclusory manner that the "experimental details provided in Sigma P1 would have enabled a POSITA to practice the invention of Sigma Claim 31." Ex. 1001, ¶¶ 43−46. Dr. Cannon's declaration testimony does not establish that she has knowledge of the facts surrounding why FACS or PCR data were selected, or how Sigma performed the FACS and PCR tests to generate the data relied upon, or how the data was analyzed to determine the values relied upon, e.g. adjustment for controls or background. *Id*.

For these reasons, Dr. Cannon's declaration does not satisfy Rule 158(b)'s requirement for an affidavit and Sigma's attempt to demonstrate entitlement to priority benefit is deficient.

F. If the Board adopts Proposed Count 2, CVC is entitled to the priority benefit of its third provisional application ("P3") filed on January 3, 2012.

At page 32, line 12, to page 33, line 11 of the motion, Sigma argues that CVC is not entitled to benefit of its P3 for the subject matter of Proposed Count 2. The response is that Sigma's motion in no way undermines CVC's entitlement to the benefit of P3. Dr. Doyon shows that P3 provides a constructive reduction to practice for CVC's half of Proposed Count 2. Ex. 2612, ¶¶ 256−263; Ex. 2619, ¶¶ 176−190 Sigma's arguments amount to a demand for a working example, which the law does not require. *Ariad Pharms., Inc. v. Eli Lilly and Co.*, 598 F.3d 1336, 1352 (Fed. Cir. 2010) (en banc); *see also Dana-Farber Cancer Inst., Inc. v. Ono Pharm. Co.*, Ltd., 964 F.3d 1365, 1372 (Fed. Cir. 2020). Dr. Cannon admitted that, when considering CVC's entitlement to priority

benefit to P3, she required a working example because, "a person of skill in the art in 2012 would have had a lot of questions and concerns about … the CRISPR-Cas9 system and its ability to cleave DNA" and "whether that could be harnessed to also support homology-directed integration of a donor polynucleotide." Ex. 2616, 86:1−11, 96:9−97:3. This is an incredible premise, given that before the filing date of P3, by January 3, 2012, two other groups had already published articles in *Science* reporting genome editing by the sgRNA CRISPR-Cas9 system in mammalian cells, including *with donor integration by HDR*. Ex. 2619, ¶¶ 185, 190; Ex. 2030, 822; Ex. 2345, 824.

It is clear that Dr. Cannon applied the wrong legal standard and ignored relevant evidence.

V. CONCLUSION

For the foregoing reasons, Sigma's Motion 1 should be denied and the Board should issue a judgment of priority in favor of CVC by operation of Rule 202(c). If Sigma's motion is granted, the Board should still issue a judgment of priority in favor of CVC by operation of Rule 202(c) for the subject matter represented by CVC's half of Count 1 and CVC's 176 generic cleavage claims, and dismiss those claims from any interference that may continue on Sigma's Proposed Count 2.

Respectfully submitted,

By /Eldora L. Ellison/
Eldora L. Ellison, Ph.D., Esq.
Lead Attorney for UC and UV
Registration No. 39,967
STERNE, KESSLER, GOLDSTEIN & FOX P.L.L.C.
1100 New York Avenue, NW
Washington, D.C. 20005

Date: February 18, 2022

By /Li-Hsien Rin-Laures/
Li-Hsien Rin-Laures, M.D., Esq.
Lead Attorney for EC
Registration No. 33,547
RINLAURES LLC
321 N. Clark Street, 5th floor
Chicago, IL 60654

Date: February 18, 2022

VI. APPENDIX 1 – LIST OF EXHIBITS

Exhibit No.	Description
1001	Declaration of Dr. Paula Cannon
1021	Brouns, S.J.J., "A Swiss Army Knife of Immunity," *Science* 337:808-809 (2012)
1031	Gasinuas, G., *et al*., "Cas9–crRNA ribonucleoprotein complex mediates specific DNA cleavage for adaptive immunity in bacteria," *PNAS* E2579–E2586 (2012)
1046	Lin, S., *et al*., "Enhanced homology-directed human genome engineering by controlled timing of CRISPR/Cas9 delivery," *eLife* 3: e04766 (2014)
1052	Moehle, E.A., *et al*., "Targeted gene addition into a specified location in the human genome using designed zinc finger nucleases," *PNAS* 104(9): 3055-3060 (2007)
2009	Prov. Appl. No. 61/652,086, filed May 25, 2012
2030	Cong, L., *et al*., "Multiplex Genome Engineering Using CRISPR/Cas Systems," *Science* 339(6121):819-823, Supplemental Material (2013)
2031	Jinek, M., *et al*., "A Programmable Dual-RNA–Guided DNA Endonuclease in Adaptive Bacterial Immunity," *Science* 337(6096):816-821, with Supplementary Information (2012)
2033	Jinek, M., *et al*., "RNA-programmed genome editing in human cells," *eLife* 2:e00471, 1-9 (2013)
2123	Beumer, K.J., *et al*., "Efficient gene targeting in *Drosophila* by direct embryo injection with zinc-finger nucleases," *Proc. Natl. Acad. Sci*, 105(50):19821-19826 (2008)
2129	Garneau, J.E., *et al*., "The CRISPR/Cas bacterial immune system cleaves bacteriophage and plasmid DNA," *Nature* 468:67-72 (2010)
2135	Urnov, F.D., *et al*., "Genome editing with engineered zinc finger nucleases," *Nat Rev Genet* 11:636-646 (2010)
2308	Bibikova, M., *et al*., "Stimulation of Homologous Recombination through Targeted Cleavage by Chimeric Nucleases," *Molecular and Cellular Biology* 21(1):289-297 (2001)
2327	Chen, F., *et al*., "High-frequency genome editing using ssDNA oligonucleotides with zinc-finger nucleases," *Nature Methods* 8(9):753-755, Supplementary Information (2011)
2339	Carroll, D., "A CRISPR Approach to Gene Targeting", *Molec. Therapy* 20:1658 (2012)
2345	Mali, P. *et al*., "RNA-Guided Human Genome Engineering via Cas9," *Science* 339(6121): 823-826 (2013)
2401	Carroll, D., "Genome Engineering with Zinc-Finger Nucleases," *Genetics* 188:773-782 (2011)
2545	Declaration of Dana Carroll, Ph.D.
2547	Declaration of Rodolphe Barrangou, Ph.D.
2585	U.S. Appl. No. 15/456,204 with current claims appended

2597	McMahon, M.A., *et al*., "Gene editing: not just for translation anymore," *Nature Methods* 9(1): 28-31 (2012)
2612	Second Declaration of Yannick Doyon, Ph.D.
2616	Deposition Transcript of Paula Cannon, Ph.D., (January 27, 2022)
2617	Second Declaration of Dana Carroll, Ph.D.
2618	Second Declaration of Rodolphe Barrangou, Ph.D.
2619	Third Declaration of Yannick Doyon, Ph.D.
2622	U.S. Patent Appl. No. 15/456,204 file history excerpt, 224 pages
2624	Lin, Y., *et al*., "Multiple Pathways for Repair of DNA Double-Strand Breaks in Mammalian Chromosomes," *Molecular and Cellular Biology* 19(12): 8353–8360 (1999)
2632	Kass, E.M. and Jasin, M., "Collaboration and competition between DNA double-strand break repair pathways," *FEBS Letters* 584:3703–3708 (2010)
2646	Sun, N., *et al*., "Recent advances in targeted genome engineering in mammalian systems," *Biotechnol. J.* 7:1074-1087 (2012)
2628	CVC Substantive Motion 2 (for judgment based on priority), *The Regents of the University of California v. The Broad Institute, Inc.*, Patent Interference No. 106,115 (October 30, 2020)
2232	Martin Jinek, Ph.D., laboratory notebook excerpt
2233	Martin Jinek, Ph.D., second laboratory notebook excerpt
2033	Jinek, M., *et al*., "RNA-programmed genome editing in human cells," *eLife* 2:e00471, 1-9 (2013)
2642	Email from Alexandra East to Aaron Cheng, dated October 31, 2012, with attachment, 3 pages
2399	Email from Jennifer Doudna to Emmanuelle Charpentier, dated June 28, 2012, 4 pages
2630	Florian Raible Laboratory Notebook No. 835, November 21, 2008, 12 pages
2652	Image - SNAP-185336-0001, 1 page
2653	Image - SNAP-185435-0003, 1 page
2654	Image - SNAP-185406-0002, 1 page

VII. APPENDIX 2 – STATEMENT OF MATERIAL FACTS

Senior Party Alleged Material Facts

1. To a POSITA in early December 2012: 1. A number of complex and unknown general issues regarding repair of a DSB in eukaryotic cells existed such that a POSITA would not have had a reasonable expectation of succesfully achieving donor integration via HDR of a CRISPR-Cas9 induced DNA break in a eukaryotic cell. Ex. 1001 ¶¶ 104 & 106-125.

Response: Denied.

2. A POSITA would have had specific concerns about using the recent CRISPRCas9 system in a eukaryotic cell to achieve HDR-mediated integration. Id. ¶¶ 105 & 126-140.

Response: Denied.

3. Previous success in ZFN and TALEN systems would not have predicted a likelihood of success in the very different CRISPR-Cas9 system in a eukaryotic cell to achieve HDR-mediated integration. Id. ¶¶ 141-147.

Response: Denied.

4. All of Sigma's involved claims (Claims 31-63) in the Sigma '204 application would have been obvious in view of proposed Count 2. Id. ¶¶ 27-28 & Appx. B.

Response: Admitted.

5. All of CVC's involved claims would have been obvious in view of proposed Count 2, except those directed to: (a) > 1 targeting RNA (aka "multiplexing"); (b) a Cas9 protein that includes a Protein Transduction Domain (PTD); (c) one or more mutation(s) in the Cas9 RuvC/HNH domain(s); (d) a nickase for a creating a "nick" or a single stranded break in the target DNA; and (e) a chimeric Cas9 protein. Id. ¶¶ 29-40 & Appx. C-E.

Response: Denied.

6. Sigma P1 Examples 1-5 (esp. Figures 1-5) adequately describe and enable the successful

integration of a donor polynucleotide via HDR into a target DNA site cleaved by a CRISPR-Cas9 system in a eukaryotic cell. Id. ¶¶ 41-49, 63-99, & Appx. F-G.

Response: Denied.

7. CVC P3 and CVC P4 provide no adequate description, experimental evidence, or analysis to demonstrate the inventors' possession of successful integration of a donor polynucleotide via HDR into a target DNA cleaved by a CRISPR-Cas9 system. Id. ¶¶ 50-58.

Response: Denied.

8. Sigma Claim 31 is patentable over the prior art. Id. ¶¶ 59-62.

Response: Denied.

Junior Party's Statement of Material Facts

9. CVC's half of Count 1 recites a sgRNA CRISPR-Cas9 system "capable of hybridizing with a target" and "cleaving or editing the target DNA" in "a eukaryotic cell." Paper 1, 5−6.

10. Carroll 2012 (Ex. 1023 or 2339), Barrangou 2012 (Ex. 1015 or 2215), Brouns 2012 (Ex. 1021), and Urnov 2010 (Ex. 1067 or 2135) provide an accurate representation of the knowledge of the skilled person at the start of December 2012. Ex. 1001, ¶ 25; Ex. 2616, 168:9−14; Ex. 2619, ¶¶ 67−81; Ex. 2617, ¶¶ 1−19; Ex. 2618, ¶¶ 1−32; Ex. 2545, ¶¶ 1−16; Ex. 2547, ¶¶ 1−22.

11. Urnov 2010 cites Beumer 2008 (Ex. 2123) for "ZFNs targeting exonic sequences can be delivered via mRNA injection into the early fly embryo; up to 10% of the progeny produced … is mutated for the gene of interest." Ex. 2135, 638; Ex. 2619, ¶¶ 67−80; Ex. 2617, ¶¶ 13−15.

12. Before December 6, 2012, it was known that prokaryotic and eukaryotic cells possess natural mechanisms to repair DSBs in DNA that could be created by different sources of DNA damage (e.g., radiation, chemicals, nucleases). Ex. 2619, ¶¶ 41−44, 129; Ex. 2616, 19:4−20:4.

13. When asked if HDR "is a naturally occurring repair process that takes place even without special measures to enhance its happening" Dr. Cannon stated: "Yes." Ex. 2616, 46:23−47:2.

14. Before December 6, 2012, it was known in the art that HDR-mediated donor integration had been induced by DSBs created by meganucleases, ZFNs, or TALENs. Ex. 2619, ¶¶ 45−59.

15. Before December 6, 2012, it was known in the art that HDR-mediated donor integration had been induced in fruit fly embryos by ZFNs. Ex. 2619, ¶ 75, 78, 78; Ex. 2123, 19821–19826.

16. Before December 6, 2012, it was known in the art that the first step of HDR involves resection of the ends of the cleaved DNA to create a 3' overhang. Ex. 2619, ¶¶ 60−65.

17. Before December 6, 2012, it was known in the art that DSBs with a 3' overhang made by I-SceI meganuclease and DSBs with a 5' overhang made by *FokI* (ZFNs and TALENs) induced HDR-mediated donor integration in eukaryotic cells. Ex. 2619, ¶¶ 85, 138; Ex. 1001, ¶ 131; Ex.

2622, 148; Ex. 2646, 1083, Figs. 1A, 2, Tbl. 1; Ex. 2617, ¶¶ 5−12; Ex. 2618, ¶¶ 18−27.

18. Before December 6, 2012, techniques were known in the art to improve HDR frequency in eukaryotic cells, including arresting the cells in the S or G2 phase or by inhibiting the NHEJ pathway. Ex. 2619, ¶ 66; Ex. 2616, 37:24−38:4; Ex. 2617, ¶¶ 14, 17; Ex. 2123, 19821–19826.

19. Before December 6, 2012, introducing a donor template into a eukaryotic cell for HDR-mediated integration was a routine technique. Ex. 2616, 105:12−106:20; Ex. 2617, ¶ 19.

20. Before December 6, 2012, techniques were known in the art for determining that HDR-mediated donor integration had occurred. Ex. 2619, ¶ 52; Ex. 2616, 36:23−37:18.

21. Before December 6, 2012, techniques were known in the art for detecting and selecting cells in a population subject to a genome-editing agent that had undergone HDR-mediated donor integration. Ex. 2616, 36:23−37:18, 105:18−106:1; Ex. 2619, ¶¶ 102, 103; Ex. 2617, ¶ 17.

22. Before December 6, 2012, it was known in the art that DSBs created by nucleases that remained bound after cleavage (e.g., Spo11) could induce HDR. Ex. 2619, ¶¶ 62, 104, 138, 142.

23. Supplemental Fig. S11 of Gasiunas 2012 (Ex. 1031, Ex. 2640) shows free DNA products migrating at the bottom of the gel after DNA cleavage. Ex. 2618, ¶¶ 28−31; Ex. 2619, ¶ 144.

24. Jinek 2012 states: Cas9 is a "multiple-turnover enzyme." Ex. 2031, 816; Ex. 2619, ¶ 143.

25. The ends of DNA cleaved by sgRNA CRISPR-Cas9 can have an overhang. Ex. 2619, ¶ 137; Ex. 2618, ¶ 32; Ex. 2031, 816, Fig. 1E, Fig. S4B; Ex. 2009, [0042], Fig. 4.

26. Nimonkar 2011 (Ex. 2633) states: "Regardless of the nature of the ends, MRN functions as an important recruiting factor to target DNA helicases and resection nucleases to the ends, a function that is conserved in *S. cerevisiae* as well." Ex. 2633, 359; Ex. 2919, ¶¶ 136−140.

27. Before December 6, 2012, it was not documented in the prior art that DSBs with blunt ends are incapable of inducing HDR. Ex. 2619, ¶¶ 62, 99, 136−140; Ex. 2618, ¶ 32.

28. CVC has submitted to the Board proofs of conception coupled with diligence to actual

reductions to practice of cleavage or editing by sgRNA CRISPR-Cas9, by Aug. 9, 2012, in fish, and by Oct. 31, 2012, in human cells; a donor sequence was not added. Ex. 2628; Ex. 2232; Ex. 2233; Ex. 2033; Ex. 2642; Ex. 2399; Ex. 2399; Ex. 2630; Ex. 2652; Ex. 2653; Ex. 2654.

29. CVC applied the sgRNA CRISPR-Cas9 system in human cells with a donor sequence and demonstrated HDR-mediated donor integration in results published in 2014. Ex. 1046, 1.

30. Dr. Cannon's Appendix A does not list U.S. 2015/0322457, U.S. 2015/0344912, Sun 2012 (Ex. 2646), or the prosecution history of Sigma's '204 application. Ex. 1001, Appendix A.

31. During prosecution of its involved '204 application, Sigma refused to add any "CRISPR cleavage-only claims." Ex. 2622, 160−179.

32. During prosecution of its involved '204 application, Sigma suggested an interference with CVC and proposed a count that is identical to Proposed Count 2. Ex. 2622, 180−223.

CERTIFICATE OF SERVICE

I hereby certify that the foregoing **CVC OPPOSITION TO SIGMA MOTION 1** is being filed via the Interference Web Portal by 8:00 PM Eastern Time on February 18, 2022, pursuant to the Order Authorizing Motions and Setting Times ("Order"; Paper 30), and thereby served on the attorney of record for the Senior Party pursuant to ¶ 105.3 of the Standing Order. Pursuant to the Order, the foregoing was also served via email by 11:00 PM Eastern Time on counsel for the Senior Party at:

Brenton R. Babcock
Dan Liu, Ph.D.
LOEB & LOEB LLP
10100 Santa Monica Blvd., Ste. 2200
Los Angeles, CA 90067
bbabcock@loeb.com
dliu@loeb.com
BoxSigma132@loeb.com

Benjamin J. Sodey
SIGMA-ALDRICH CORP.
3050 Spruce St.
Saint Louis, MO 63103
benjamin.sodey@milliporesigma.com

Benjamin I. Dach, Ph.D.
LOEB & LOEB LLP
345 Park Ave.
New York, NY 10154
bdach@loeb.com

STERNE, KESSLER, GOLDSTEIN & FOX P.L.L.C

/Eldora L. Ellison/
Eldora L. Ellison, Ph.D., Esq.
Lead Attorney for UC and UV
Registration No. 39,967

Date: February 18, 2022

STERNE, KESSLER, GOLDSTEIN & FOX P.L.L.C.
1100 New York Avenue, NW
Washington, DC 20005-3934