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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
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

Junior Party

v.

SIGMA-ALDRICH CO. LLC
Application 15/456,204

Senior Party

Patent Interference No. 106,132 (DK)
(Technology Center 1600)

SIGMA OPPOSITION TO CVC MOTION 1 (for Priority Benefit of CVC P1 and CVC P2)

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OPPOSITION TO CVC MOTION 1
(for Priority Benefit of CVC P1 and CVC P2)

I. INTRODUCTION

CVC did not invent a CRISPR-Cas9 system that is capable of cleaving or editing a target DNA ***in a eukaryotic cell*** or modulating transcription of at least one gene encoded by the target DNA, as recited in Count 1. By the filing date of CVC P2 (October 19, 2012), despite their wishes and hopes, the CVC inventors were only able to disclose a CRISPR-Cas9 that could cleave short DNA sequences in test tubes. Around that time, the CVC inventors themselves, as well as many others in the field, doubted whether the prokaryotic CRISPR-Cas9 could even work in eukaryotic cells. This is because of the significant differences between prokaryotic and eukaryotic environments, the past failures and challenges in adapting a prokaryotic system to eukaryotic cells, and the unique nature of CRISPR-Cas9. In the '115 Interference, the Board conducted a thorough analysis of this issue and determined that CVC P1 and P2 do not satisfy the written description requirement of Section 112 because the disclosures of P1 and P2 fail to demonstrate that the CVC inventors had possession of a CRISPR-Cas9 system that cleaves or edits a target DNA in a eukaryotic cell. No material facts have changed since that decision.

In its Motion 1, CVC claims that it has uncovered "new evidence" and presented "new" arguments. However, all of CVC's purported "new evidence" was available to it during the '115 Interference, and all of CVC's so-called "new" arguments have already been expressly or implicitly rejected by the Board. CVC has not shown—and cannot show—that CVC P1 or P2 address the many concerns a person of ordinary skill in the art ("POSITA") would have had in adapting CRISPR-Cas9 to eukaryotes. Yet, CVC now argues that ***later*** results from ***other*** groups that demonstrated working examples of eukaryotic CRISPR-Cas9 support CVC's possession in P1 and P2. CVC also argues that P1 and P2 have disclosed eukaryotic embodiments because P1

and P2 recite ***all cell types from all organisms during their entire cellular life***. These arguments are completely untenable, both legally and factually. Thus, CVC's Motion 1 should be denied for the same reasons that the Board found in the '115 Interference.

II. PRECISE RELIEF REQUESTED

Sigma requests that the PTAB deny CVC's Motion 1, which seeks the benefit of each of the filing dates of CVC P1 and P2.

III. LEGAL STANDARDS

To be accorded benefit of an earlier-filed application, a party must show a constructive reduction to practice of an embodiment within the scope of the count. *Hunt v. Treppschuh*, 523 F.2d 1386, 1389 (C.C.P.A. 1975). To show a constructive reduction to practice, the embodiment must satisfy the written description and enablement requirements of 35 U.S.C. § 112. *Id.*; *accord* 37 C.F.R. § 41.201. To satisfy the written description requirement, the application must "reasonably convey[] to those skilled in the art that the inventor had possession of the claimed subject matter as of the filing date." *Ariad Pharm., Inc. v. Eli Lilly & Co.*, 598 F.3d 1336, 1351 (Fed. Cir. 2010). "When determining whether a specification contains adequate written description, one must make an 'objective inquiry into the four corners of the specification from the perspective of a person of ordinary skill in the art.'" *Boston Sci. Corp. v. Johnson & Johnson*, 647 F.3d 1353, 1366 (Fed. Cir. 2011). A prior application "must describe an invention, and do so in sufficient detail that one skilled in the art can clearly conclude that the inventor invented the claimed invention as of the filing date sought." *Lockwood v. Am. Airlines, Inc.*, 107 F.3d 1565, 1572 (Fed. Cir. 1997).

To satisfy the enablement requirement, the specification must teach those in the art to make and use the invention without undue experimentation, taking into consideration several factors in evaluating "undue experimentation":

> (1) the quantity of experimentation necessary, (2) the amount of direction or guidance presented, (3) the presence or absence of working examples, (4) the nature of the invention, (5) the state of the prior art, (6) the relative skill of those in the art, (7) the predictability or unpredictability of the art, and (8) the breadth of the claims.

In re Wands, 858 F.2d 731, at 736-737 (Fed. Cir. 1988).

IV. LEVEL OF ORDINARY SKILL IN THE ART

CVC P1 and P2 should be analyzed from the perspective of a POSITA at the time of filing, which is May 25, 2012, and October 19, 2012, respectively. Sigma's expert, Dr. Paula Cannon, explains that, at the time CVC P1 and P2 were filed, a POSITA would have had an academic and research background in molecular biology and/or biochemistry, with an understanding of the techniques needed to clone, express, isolate, purify, and manipulate proteins and nucleic acids in the context of both *in vitro* and *in vivo* experiments, including in extra-cellular, prokaryotic, and eukaryotic environments. Ex. 1080 (Cannon Supp'l Decl.) ¶ 17. More specifically, a POSITA would have had a Ph.D. degree in a life sciences discipline (*e.g.*, chemistry, biochemistry, genetics, cell biology, molecular biology, or neurobiology) and at least two years of relevant post-doctoral experience. *Id.*

V. NO PURPORTED "NEW" CVC EVIDENCE WARRANTS ANY CHANGE TO THE BOARD'S PREVIOUS DETERMINATION IN THE '115 INTERFERENCE THAT CVC P1 AND P2 DO NOT DEMONSTRATE POSSESSION OF A CRISPR-CAS9 SYSTEM IN A EUKARYOTIC CELL

In the '115 Interference, the Board concluded that CVC P1 and P2 do not satisfy the written description requirement of Section 112 because they do not contain sufficient disclosures that would have demonstrated to a POSITA that the CVC inventors had possession of a CRISPR-Cas9 system in eukaryotic cells. *See, e.g.,* '115 Int'f Decision on Motions, paper 877 ("'115 Decision") at 104:12-17, 105:9-22. It is undisputed that CVC P1 and P2 only show CRISPR-Cas9 activity in a cell-free environment, not in any eukaryotic cells. *See e.g.* Ex. 2009

¶¶ 248-251; Ex. 2010 ¶¶ 311-357; '115 Decision at 81:15-16 ("CVC does not direct us to a disclosure in P1 of results from a CRISPR-Cas system in any of [fish, human, or fruit fly] cells."); Ex. 1084 (Depo. Tr. of CVC expert Dr. Doyon) at 179:17 – 180:13; Ex. 2543 ¶ 152. The Board concluded that a POSITA would have considered specific instructions or conditions for a CRISPR-Cas9 activity in a eukaryotic cell to be necessary to show that the CVC inventors had possession of the claimed invention, given the experiences in the art with similar systems. '115 Decision at 90:6 – 91:6. The Board found that CVC P1 and P2 do not address possible obstacles a POSITA would have considered, let alone disclose any instructions on how to overcome those obstacles or instructions that specific conditions were not necessary to adapt the prokaryotic CRISPR-Cas9 system to eukaryotic cells. *See e.g. id*. at 87:18 – 88:2 (RNA degradation), 94:18 – 95:15 (PAM sequences), 100:1 – 101:7 (chromatin access). Thus, the Board concluded that a POSITA would not have considered that the CVC P1 and P2 inventors had possession of an embodiment of Count 1 at the time of filing P1 or P2. *Id*. at 102:13 – 103:2, 105:20-22.

In its latest attempt to recraft the inadequate disclosures of P1 and P2, CVC now argues that it deserves another bite at the same apple because CVC has uncovered "new evidence" and presents four "new" arguments. CVC Mot. 1 at 5:11 – 6:1. As discussed below, all of this purportedly "new" evidence was previously available to CVC—and most was even cited—in the '115 Interference. In addition, all CVC's so-called "new" arguments were either expressly or implicitly rejected by the Board's decision in the '115 Interference. Thus, CVC's instant Motion 1 for benefit has presented no cognizable new evidence or new argument, and should be denied for the same reasons that the Board denied the same request in the '115 Interference.

A. All Of CVC's Purported "New" Evidence Was Previously Available To CVC And Most Was Cited In The '115 Interference

Procedurally, CVC had ample opportunities to raise these arguments during the motions

phase of the '115 Interference, and CVC does not cite here any reference or fact that was not available to CVC at the time of its motions in the '115 Interference. CVC's first two arguments claim that the Board did not address certain purported disclosures of P1. CVC Mot. 1 at 5. But those purported disclosures are not new, as they have remained the same since the filing of P1.

CVC's third argument claims that the Board's decision in the '115 Interference "did not consider evidence that has since come to light" and refers to Section V.B. of its motion. CVC Mot. 1 at 5:19-21. However, Section V. B. only cites references reporting the results of several groups using CRISPR-Cas9 for gene editing in eukaryotic cells and their previous work using ZFNs or TALENs for gene editing. All the evidence cited was publicly available by 2013 and most were cited as exhibits in the '115 Interference.[1] Thus, CVC is incorrect in arguing that the evidence has "since come to light." CVC's argument claiming that the Board "did not consider [such] evidence" is also baseless because most of the exhibits were before the Board in the '115 Interference and the Board is not required to discuss each exhibit in its decision.

CVC's last argument essentially accuses the Board of misinterpreting statements made by Drs. Doudna and Carroll. CVC Mot. 1 at 5:22 – 6:1. Again, CVC was fully aware of those statements and cited and/or responded to those statements in its motions in the '115 Interference, particularly in the context of CVC's Motion 1 (for accorded benefit).

[1] *See e.g.* Ex. 2306 (Zhang 2011) cited as Ex. 4620 in the '115 Int'f; Ex. 2329 (Sanjana 2012) cited as Ex. 5130 in the '115 Int'f; Ex. 2344 (Cong 2012 manuscript) cited as Ex. 3564 in the '115 Int'f; Ex. 2333 (Briggs 2012) cited as Ex. 5281 in the '115 Int'f; Ex. 2345 (Mali 2013) cited as Ex. 3623 in the '115 Int'f; Ex. 2154 (Cho 2013) cited as Ex. 4076 in the '115 Int'f; Ex. 2331 (Sander 2011) cited as Ex. 5236 in the '115 Int'f; Ex. 2028 (Hwang 2013) cited as Ex. 4084 in the '115 Int'f; Ex. 2327 (Chen 2011) cited as Ex. 5022 in the '115 Int'f.

Indeed, as the movant, CVC bore the burden to establish that it is entitled to the priority benefit of P1 and P2 in the '115 Interference. S.O. ¶ 121.3. Thus, CVC had all the incentives to present these arguments in the '115 Interference, but either failed to do so or decided not to proceed with these arguments in the '115 Interference.

B. The Board Has Already Rejected CVC's Purportedly "New" Arguments In The Decision In The '115 Interference, And The Board Should Do So Again Here For The Same Reasons

CVC's first argument is that the Board's decision in the '115 Interference "did not address whether direct injection of a pre-assembled RNP complex into an embryo . . . would trigger the same alleged concerns as embodiments relying on vector expression. *Infra* § VI.A." CVC Mot. 1 at 5. As explained in further detail below, the concerns identified in the '115 Decision are not unique to embodiments relying on vector expression, and injection of a pre-assembled ribonucleoprotein ("RNP") complex into an embryo would not obviate most of the concerns a POSITA would have had in using CRISPR-Cas9 in eukaryotic cells. *See infra* Part VI.A.4; Ex. 1080 ¶¶ 49-51. For example, a POSITA would have considered whether a prokaryotic RNP complex would remain stable and functional in a eukaryotic cell given the differences between prokaryotic and eukaryotic environments and between test tubes and eukaryotic cells. *Id.* at ¶ 49. A POSITA would also have considered whether the RNP complex would recognize the target DNA embedded in the complex environment of eukaryotic chromatin. *Id*. A POSITA would have been concerned about the stability of RNA/DNA hybrids that form when a guide RNA binds to its target DNA, because there are enzymes in eukaryotic cells that specifically target and remove RNA/DNA hybrids. *Id*. at ¶ 50. CVC P1 and P2 do not address any of those concerns. *See infra* Part VI.B.

Second, CVC argues that the Board's decision in the '115 Interference "did not address descriptions in P1 regarding: the analogous nature of ZFNs and TALENs; routine uses of nuclear

localization signals and codon selection; and the inventors' appreciation of the dynamic nature of chromatin." CVC Mot. 1 at 5. But the Federal Circuit has affirmed the Board's finding that ZFNs and TALENs "are not analogous to CRISPR-Cas9 because they have their origins in eukaryotic domains. . . ." *Regents of the Univ. of Cal. v. Broad Inst., Inc.*, 903 F.3d 1286, 1293 (Fed. Cir. 2018). In addition, the Board considered CVC's arguments concerning NLSs, codon selection, and chromatin in the '115 Interference. *See, e.g.,* '115 Decision at 95:16 – 102:12. Thus, this second argument has been raised and thoroughly addressed in the '115 Interference. As explained in more detail below, a POSITA would not have considered TALENs or ZFNs to be analogous to CRISPR-Cas9, and the purported descriptions in CVC P1 and P2 would not have indicated to a POSITA that the CVC inventors of CVC P1 or P2 had possession of an embodiment within the scope of Count 1. *See infra* Part VI.A.3, Part VI.C.

CVC's third argument claims that "evidence that has since come to light [] undermines the initial allegations by Broad that CVC P1 and P2 fail to disclose necessary 'special' instructions and adaptations for applying CRISPR-Cas9 in eukaryotic cells. *Infra* § V.B." CVC Mot. 1 at 5:19-21. In Section V.B. of CVC's Motion, CVC claims that several other groups were able to apply CRISPR-Cas9 in eukaryotic cells within a year after the publication of Jinek (2012) "without any special adaptions or conditions." CVC Mot. 1 at 13. CVC misses the point; the issue is not ***enablement*** under Section 112, but rather ***written description***. The Board repeatedly (and correctly) explained, "we are not persuaded that questions about the ultimate requirements for CRISPR-Cas function in eukaryotes are relevant to the issue of written description because whether a disclosure indicates possession is viewed from what one of ordinary skill in the art would have considered at the time of filing." '115 Decision at 87:14-18; *see also id.* at 96:5-8 ("[t]he ultimate determination that a feature is not required for a CRISPR-Cas9 system as recited in Count 1 is not relevant to whether those of ordinary skill in the art would have considered a

disclosure to show possession at the time of filing."); 104:9-11 ("we consider the evidence the parties present of what one of ordinary skill in the art would have considered at the time P1 was filed, not what was later determined to be needed for CRISPR-Cas9 function."). CVC P1 and P2's failure to address any special instructions or conditions necessary for applying CRISPR-Cas9 in eukaryotic cells, ***or to indicate that no specific instructions or conditions were necessary***, demonstrates that the CVC inventors did not have possession of a CRISPR-Cas9 system in eukaryotic cells at the time of filing CVC P1 and P2. *See id.* at 91:1-6 ("If the P1 applicants did not disclose specific instructions or conditions necessary for CRISPR-Cas9 activity in a eukaryotic cell, ***or indicate that no specific instructions or conditions were necessary***, we are not persuaded that one of ordinary skill would have considered there to be possession, given the experiences in the art with the similarly complex Group II intron RNA/protein system.") (emphases added); *see also infra* Part VI.B.

Finally, CVC argues that "Broad [Institute et al.]'s arguments in the '115 interference relied on a misinterpretation of quotes by Doudna and Carroll." CVC Mot. 1 at 5:22-23. CVC further argues that testimony recently crafted for purposes of this interference proceeding "inform[s] what people in the field thought and the expectation that CRISPR-Cas9 would work in eukaryotes." *Id*. at 5:23 – 6:1. CVC's argument completely contradicts the Board's final judgment—as affirmed on appeal—that a POSITA would ***not*** have had a reasonable expectation of success in applying the CRISPR-Cas9 system in a eukaryotic cell. 106,048 Int'f Decision on Motions, paper 893 ("'048 Decision"), Section III C. Indeed, in its affirmance, the Federal Circuit determined that the Board "performed a thorough analysis of the factual evidence and considered a variety of statements by experts for both parties and the inventors" and concluded that "substantial evidence supports the Board's finding that there was not a reasonable expectation of success . . . ." *Broad*, 903 F.3d at 1296.

Courts typically afford contemporaneous statements made at the time of the event considerably more weight than statements prepared long thereafter in the context of litigation. *United States v. U.S. Gypsum Co.*, 333 U.S. 364, 395-96 (1948); '048 Decision, at 14:3-15 ("giv[ing] significant weight to the statements by those in the art at the time of Jinek (2012) regarding expectation of success in using a CRISPR-Cas9 system in eukaryotic cells."). Thus, the Board should give little if any weight to CVC's manufactured testimony, submitted in 2019-2021 for purposes of the interference proceedings to undermine—and even directly contradict—the contemporaneous statements made by Drs. Carroll and Doudna shortly after the publication of Jinek (2012). As further explained below, the testimony relied on by CVC either is irrelevant to the issues of the instant motion or does not support CVC's arguments. *See infra* Part VIII.

In sum, none of CVC's purported "new evidence" or so-called "new arguments" should alter the finding that neither CVC P1 nor P2 describes an embodiment of the invention within the scope of Count 1. Thus, CVC's Motion 1 for benefit should be denied.

VI. CVC P1 AND P2 DO NOT DESCRIBE AN EMBODIMENT WITHIN THE SCOPE OF COUNT 1

CVC's part of the Count 1 recites "a ***eukaryotic cell*** comprising a target DNA molecule" and "a CRISPR-Cas system … whereby said system is capable of cleaving or editing the target DNA molecule or modulating transcription of at least one gene encoded by the target DNA molecule." Declaration (Paper 1) at 5-6 (emphases added). Thus, to meet the written description requirement, CVC must show that the disclosures of CVC P1 or P2 would have demonstrated to a POSITA that, at the time of filing, the CVC inventors possessed a CRISPR-Cas9 system that "is capable of cleaving or editing a DNA molecule or modulating transcription of at least one gene encoded by the target DNA molecule" in a ***eukaryotic*** cell. Despite CVC's attempts to fabricate some illusory embodiments purportedly disclosed in CVC P1 and P2, a POSITA would

not have concluded that the CVC inventors possessed such a CRISPR-Cas9 system. This is because a POSITA would have understood that adapting the prokaryotic CRISPR-Cas9 system to function in eukaryotic cells would face significant challenges and uncertainties that were nowhere addressed in CVC P1 or P2.

A. A POSITA Would Have Been Concerned About Trying To Adapt CRISPR-Cas9 To A Eukaryotic Cell

Unlike other known DNA nucleases that use a protein domain to recognize a target DNA, CRISPR-Cas9 recognizes and binds the target DNA through an RNA component. Ex. 1080 ¶¶ 19, 35. This unique feature, as well as the differences between prokaryotic and eukaryotic environments, would have raised many concerns to a POSITA when adapting the prokaryotic CRISPR-Cas9 system to a eukaryotic cell. *Id.* at ¶¶ 19-28, 36-48.

1. The Unique Nature Of CRISPR-Cas9 Systems Would Have Led A POSITA To Doubt That CRISPR-Cas9 Would Function In A Eukaryotic Cell Without Special Conditions Or Specific Instructions

"[A] CRISPR-Cas9 system is a combination of protein and ribonucleic acid ("RNA")[.]" '048 Decision, at 2:17-20; *see* Ex. 1080 ¶ 19. In their native environment, CRISPR-Cas9 systems protect bacteria against viral infections by cleaving and destroying the invading viral DNA. *Id.* Specifically, Cas9 protein is guided by the RNA component to introduce double-stranded breaks in the target viral DNA. *Id.* CRISPR-Cas9 is a prokaryotic system that evolved to function in prokaryotic cells, and is not known to occur naturally in eukaryotic cells. *Id.*; '048 Decision at 6:13 – 7:2; Ex. 1083 (Depo. Tr. of CVC Expert Dr. Doyon) at 47:9-10, 48:4-6.

Past experience of adapting prokaryotic systems to eukaryotic cells has shown that this process can be challenging and unpredictable. Ex. 1080 ¶¶ 22-28. Because CRISPR-Cas9 is an RNA-guided nuclease, a POSITA would have considered experiences with other RNA-based systems for guidance. *Id.* ¶ 22.

Group II introns would be the most analogous system because they share several structural and functional similarities with CRISPR-Cas9. Ex. 1080 ¶¶ 23-25; Ex. 1092 (Lambowitz 2017) ¶ 22. Both Group II introns and CRISPR-Cas9 originate in bacteria and function as RNP complexes; both systems use an RNA to interact with a DNA target and to direct the action of the RNP complex; and the consequences of both CRISPR-Cas9 and Group II introns are sequence-specific changes to prokaryotic DNA that involve endonuclease activities. Ex. 1080 ¶¶ 23-25. Group II introns comprise an RNA component and an intron encoded protein ("IEP"). *Id.* LtrA protein encoded by L1.LtrB intron is the best characterized IEP. *Id.* Both LtrA and Cas9 have an HNH endonuclease domain that cleaves a DNA target. *Id.*; Ex. 2367 at 4; Ex. 2009 ¶ 5. Group II introns are capable of site-specifically cleaving DNA, or additionally inserting sequences into DNA. Ex. 1080 ¶ 25; Ex. 1092 ¶ 24. Because of this, Group II introns have been utilized in prokaryotes for gene targeting applications. Ex. 1092 ¶ 25. This function is mediated by the RNP particle containing the IEP and an excised intron RNA, with DNA target specificity determined by base pairing of the intron RNA to the DNA target sequence, with additional support from the IEP. *Id.* at ¶¶ 22-24; Ex. 1080 ¶¶ 24-25. Therefore, the intron RNA acts as a guide to the DNA target, while the events required for targeted insertion are carried out by the entire RNP complex. Ex. 1080 ¶¶ 24-25.

Despite successful use in prokaryotes for gene targeting, only moderate activity of Group II introns has been obtained in eukaryotic cells. Ex. 1080 ¶ 26; Ex. 1092 ¶¶ 25-26. Mastroianni (2008) reported no integration of Group II introns in eukaryotic cells under natural conditions, without the addition of high levels of Mg^{2+}. Ex. 2150 at 3 and Table 1. And only moderate integration of Group II introns was achieved by adding exogenous Mg^{2+}, resulting in an increase of "intracellular Mg^{2+} concentration by ~9mM[.]" *Id.* at 3-4; Ex. 1092 ¶¶ 25-26. The authors of Mastroianni (2008) concluded that "we show that group II intron-based gene targeting reactions

can occur efficiently in eukaryotes, but are dependent upon the injection of additional Mg^{2+}." Ex. 2150 at 9. Despite this early optimism, the senior author of Mastroianni (2008) later acknowledged the impracticality of this requirement, commenting that "[s]uch high Mg^{2+} concentrations are deleterious to eukaryotic cells, a limitation that could not be overcome despite considerable effort on our part." Ex. 1092 ¶ 26. Thus, a POSITA would have understood that Group II introns cannot function in eukaryotic cells without addition of high level of Mg^{2+} and had not been successfully adapted for use in eukaryotic cells. Ex. 1080 ¶ 26. CVC agrees. CVC Mot. 1 at 11-12 ("Group II introns . . . require specific concentrations of magnesium, higher than those usually found in eukaryotic cells."); Ex. 1081 (Depo. Tr. of CVC expert Dr. Zamore) at 109:18-22 (the Mg^{2+} concentration in eukaryotic cells is 1-2 mM). This issue is different from the situation in bacteria, the natural hosts of Group II introns, where Group II introns function without any requirement of addition of Mg^{2+}. Ex. 1080 ¶ 26. The finding that Group II intron activity in eukaryotic cells depends on supraphysiological levels of Mg^{2+} further highlights the differences between prokaryotic and eukaryotic systems. *Id*. In addition, a POSITA would have understood that the need to artificially raise the Mg^{2+} concentration likely reflects the fact that the Group II intron RNP was not able to form in the natural eukaryotic environment. *Id.* As discussed further in Part VI.A.2.f, the intracellular Mg^{2+} concentration, which varies between prokaryotic and eukaryotic cells, is a critical parameter for RNA folding and function. *Id.* at 46. Thus, a POSITA would have been very concerned about adapting CRISPR-Cas9 to eukaryotic cells based on the experience with Group II introns. *Id*.

Riboswitches are another example of RNA-based systems that require special instructions or conditions to function in eukaryotic environments. Ex. 1080 ¶¶ 27-28. Riboswitches "regulate expression of enzymes that catalyze key chemical transformations" in bacteria. Ex. 2574 (Link (2009)) at 1190. Despite great interest in adapting riboswitches to

control gene expression in non-bacteria context, only a few riboswitches have been validated in eukaryotes. *Id.* at 1192. A number of factors that prevent riboswitches from becoming useful genetic switches have been identified, including that "the functions of most aptamers have not been validated in cells, the folding of RNA constructs might differ between test tube and cell, or the ribozyme chosen for RNA switch construction might not be appropriate for controlling gene expression." *Id.* at 1190. Thus, a POSITA would have understood that RNA folding may prevent riboswitches from proper functioning in eukaryotic cells. Ex. 1080 ¶ 28. This is particularly relevant because CRISPR-Cas9 systems also rely on proper folding of the guide RNA molecule. *Id*. Thus, a POSITA would have expected that special conditions or instructions are needed to use CRISPR-Cas9 systems in eukaryotic cells. *Id*.

CVC argues that because Cas9 has a RuvC domain, which is found in both prokaryotes and eukaryotes, "[a] POSITA would [] have expected a nuclease having the RuvC nuclease domain to function under the same conditions." CVC Mot. 1 at 12. CVC also argues that a POSITA would have known that a nuclease with an HNH domain would function in eukaryotic cells because an HNH domain was known to be present in KpnI, an enzyme that cleaves DNA under eukaryotic cell levels of Mg^{2+}. *Id*. However, CVC's arguments fail for at least two reasons. First, the fact that Cas9 has enzymatic domains that also exist in some other enzymes that could function in eukaryotic cells does not address all of the issues a POSITA would have considered for applying CRISPR-Cas9 to eukaryotic cells. For example, CRISPR-Cas9 is an RNP complex and requires both the RNA component and protein component to be properly folded and form the correct complex. Ex. 1080 ¶¶ 19-20. Thus, even assuming its RuvC domain and HNH domain could work in eukaryotes, a POSITA would still have doubts whether the entire CRISPR-Cas9 complex would function in eukaryotic cells. *Id.* at ¶ 20. Second, having an HNH domain does not guarantee that a prokaryotic nuclease would work in eukaryotic cells

under normal conditions. As explained above, the IEP of the Group II introns has an HNH domain but does not function under the normal conditions found in eukaryotic cells. *Id.* at ¶¶ 20, 26; Ex. 1092 ¶ 26. Thus, a POSITA would not have known that Cas9 would work in eukaryotic cells simply because Cas9 has a RuvC domain and an HNH domain. Ex. 1080 ¶ 20.

Contrary to CVC's assertion that "a POSA would not have felt skepticism, uncertainty, or doubt about [Group II introns'] ability to function," (CVC Mot. 1 at 12) the Board found that a POSITA would have known that transferring other prokaryotic, RNA-based systems, such as Group II introns and riboswitches, into eukaryotic environments "required ***specific tailoring*** of conditions" and thus a POSITA "would have expected that the CRISPR-Cas9 system would have also required its own set of unique conditions." '048 Decision, at 36-39 (emphases added). Indeed, there is not a set of common factors or conditions that work for all these RNA-based systems: Group II introns require high concentrations of Mg^{2+} and introduced DNA targets are highly sensitive to chromatinization of the target DNA, while riboswitches are sensitive to influences on the proper RNA folding. Ex. 1080 ¶¶ 24-28. Thus, based on prior experiences, a POSITA would have considered specific instructions a necessity for transferring CRISPR-Cas9, a prokaryotic, RNA-based system, into a eukaryotic environment. *Id.* ¶ 22.

2. A POSITA Would Have Recognized Several Potential Challenges To Applying CRISPR-Cas9 In A Eukaryoic Cell Because Of The Many Differences Between Prokaryotic And Eukaryotic Environments And The Past Failures Of Adapting Other Prokaryotic Systems To Eukaryotic Cells

In addition to prior experiences with similar RNA-based systems, a POSITA would have expected several potential challenges in applying a prokaryotic system, such as CRISPR-Cas9, in eukaryotic cells due to the inherent differences between prokaryotes and eukaryotes and between *in vitro* and cellular environments. The following section illustrates some potential challenges a POSITA would have considered in adapting the prokaryotic CRISPR-Cas9 to eukaryotic cells.

a. Stability of RNA / RNA Degradation

A POSITA would have known that eukaryotic cells contain ribonucleases that can rapidly degrade RNA. Ex. 1080 ¶ 37. While 5′ capping and 3′ poly-adenylation protect eukaryotic mRNAs from rapid degradation, the prokaryotic guide RNA of the CRISPR-Cas9 has no such protection. *Id.* The RNA component of CRISPR-Cas9 is necessary for the Cas9 protein to achieve DNA binding and DNA cleavage function. *Id.* Therefore, a POSITA would not have known whether the prokaryotic guide RNA would be degraded by exonucleases in eukaryotic cells, which would lead to loss of function of CRISPR-Cas9. *Id.*.

In addition, since CRISPR guide RNA binds to its target DNA and creates an RNA:DNA hybrid, and RNase H is a eukaryotic nuclease that effectively recognizes and cleaves the RNA strand of RNA:DNA hybrids in eukaryotic cells, a POSITA would also be concerned about the ability of CRISPR-Cas9 to create these necessary hybrid molecular complexes. Ex. 1080 ¶ 38; Ex. 1096 (Cerritelli (2009)) at Abstract; Ex. 1124 (Wahba (2011)) at 978. Indeed, Dr. Carroll expressed this concern in his commentary: "[The CRISPR-Cas9:target DNA hybrid] may be a substrate for RNA hydrolysis by ribonuclease H and/or FEN1, both of which function in the removal of RNA primers during DNA replication." Ex. 1023 (Carroll (2012)) at 1660.

Furthermore, a POSITA would have understood that some eukaryotic proteins cause RNA degradation as part of anti-viral responses to double-stranded RNA ("dsRNA"). Ex. 1100 (Karpala (2005)) at Table 1. This would present additional challenges to the stability of the guide RNA because part of the guide RNA is double-stranded. Ex. 1080 ¶ 39.

Another concern a POSITA would have had was whether RNA would have folded correctly and be associated with Cas9 in eukaryotic cells so that the CRISPR-Cas9 complex would remain functional. Ex. 1080 ¶ 20. The experience with riboswitches shows that RNA folding is always a concern in adapting an RNA-based system to eukaryotes. *Id.* ¶ 28. The *in*

vitro experiments disclosed in P1 and P2 were conducted in test tubes with purified components that do not represent the much more complex conditions in eukaryotes. *Id.* ¶¶ 43, 53. Thus, a POSITA would also have been concerned whether the guide RNA and Cas9 protein would be properly folded, correctly associated and remain functional in eukaryotic cells. *Id.* ¶ 28.

b. Macromolecular Crowding

Molecular crowding refers to the phenomenon that macromolecules such as proteins and nucleotides occupy a large proportion of the intracellular volume of a cell, which can make molecules in cells behave in radically different ways than in test-tube assays. Ex. 1080 ¶ 41. Molecular crowding influences protein folding, macromolecular interactions, and protein function. *Id.* For example, it was reported that "[p]roteins that refold spontaneously in dilute solution aggregate in crowded solution and require chaperones to refold." Ex. 1121 (Minton (2006)) at 2866 Table 1; *see also* Ex. 1098 (Ellis (2001)) at 597-600; Ex. 1111 (Zimmerman (1993)) at 175, 181-183; Ex. 1107 (Richter (2008)) at 2104-05. Thus, a POSITA would have understood that proteins and RNAs can fold and function differently in a crowded cellular environment versus an *in vitro* (i.e., in an extra-cellular environment) system. Ex. 1080 ¶ 41.

Indeed, CVC P1 and P2 report experiments conducted in test tubes with purified proteins, in buffered solutions, and using short, non-eukaryotic DNA targets. Ex. 2009 ¶¶ 248-251; Ex. 2010 ¶¶ 311-357. Thus, a POSITA would not have extrapolated results from those *in vitro* experiments to an *in vivo* (i.e., in a living cell) situation (such as inside a eukaryotic cell). Ex. 1080 ¶ 41. Nor would a POSITA have predicted CRISPR-Cas9 activity in a eukaryotic cell based on the *in vitro* data reported in Jinek (2012). *Id.*

c. Eukaryotic Genomic DNA

As of 2012, a POSITA would have known that eukaryotic genomes are significantly larger than bacterial genomes. For example, the *S. pyogenes* genome is about 1.8 Mbp, while the

human genome is about 3,100 Mbp per haploid genome, which is over 1,700 times larger than the *S. pyogenes* genome. Ex. 1080 ¶ 42; Ex. 1101 (Lander (2011)) at 875; Ex. 1104 (McShan (2008)) at 7774. Moreover, CRISPR-Cas9 has evolved to survey and find its target in invading viruses entering bacterial cells, which have even smaller genomes. Ex. 1080 ¶¶ 19, 42. A POSITA would have been concerned whether CRISPR-Cas9 could scan and identify target DNA molecules within eukaryotic genomes that are over a thousand times bigger than even the *S. pyogenes* genome. *Id.* ¶ 42.

CVC P1 reported experiments conducted in test tubes with purified proteins and RNAs at concentrations of 50-fold molar excess as compared to the target DNA, and using an extremely simplified system where the target DNA was provided as a synthesized oligonucleotide and not in the context of vastly outnumbered competing sequences. Ex. 1080 ¶ 43; Ex. 2009 ¶ 249. Those conditions disclosed in CVC P1 would greatly simplify the process whereby a guide RNA-Cas9 complex needed to scan, identify and act on the target DNA. Ex. 1080 ¶ 43. Thus, this *in vitro* result says nothing about whether CRISPR-Cas9 would be able to scan and recognize target DNA in eukaryotic genomes. *Id*.

d. Chromatin

A POSITA would have also considered access to DNA targets within the tightly bound chromatin in the nucleus of eukaryotic cells as a potential challenge. Ex. 1080 ¶ 44. Unlike bacterial DNA, eukaryotic DNA is embedded in the complex environment of eukaryotic chromatin, which is enclosed in a membrane-bound nucleus. *Id.* Thus, even if CRISPR-Cas9 could navigate the much larger eukaryotic genomes, it must also find and correctly interact with its correct target while enclosed in chromatin. *Id.*

CVC's reliance on prior experience of ZFNs and TALENs completely ignores the key differences between ZFNs/TALENs and CRISPR-Cas9. As explained below, the DNA binding

domains of ZFNs and TALENs have evolved to function in eukaryotic environments and in particular to associate with DNA contained in eukaryotic chromatin, while Cas9 originates from bacteria and does not exist in eukaryotic cells. Ex. 1080 ¶¶ 19, 35. In addition, the DNA binding of CRISPR-Cas9 is through the RNA component while ZFNs/TALENs are through protein components. *Id.* Thus, whether those protein-only systems that have evolved to function in eukaryotic genomes can successfully survey eukaryotic genomic DNA is not relevant to whether the completely different prokaryotic CRISPR-Cas9 machinery would be able to function on eukaryotic genomic DNA. *Id*. ¶ 35.

CVC cites Mastroianni (2008) (Ex. 2150) for stating that "DNA replication can mitigate effects of chromatinization." CVC Mot. 1 at 27. This quote is completely taken out of context. Mastroianni (2008) reported that no integration was observed in native embryos by microinjection of Group II introns, and only some integration was obtained when additional Mg^{2+} was injected to cells. Ex. 2150 at 9; Ex. 1092 ¶¶ 25, 26. Mastroianni (2008) recognizes that chromatinization inhibits Group II introns' function, presumably by impeding access to target DNA, and successful integration requires high concentration of Mg^{2+}:

> ***Chromatinization of the target DNA inhibits both types of targeting reactions***, presumably by impeding RNP access. However, by using similar RNP microinjection methods, we show efficient Mg^{2+}-dependent group II intron integration into plasmid target sites in zebrafish (Danio rerio) embryos and into plasmid and chromosomal target sites in Drosophila melanogster embryos, indicating that DNA replication can mitigate effects of chromatinization.

Ex. 2150, Abstract ("These findings indicate that ***chromatin poses a significant barrier to group II intron gene targeting reactions***, presumably because it impedes access of group II intron RNPs to their DNA target sites.") (emphases added). Even through chromatinization might be mitigated by DNA replication, any sufficient integration requires extra Mg^{2+}. *Id*. at Table 5 (data indicating dependence upon the injection of additional Mg^{2+}); Ex. 1092 ¶¶ 25, 26. Thus, a

POSITA reading Mastroianni (2008) would have understood that chromatin access would be a challenge even during DNA replication, such as in developing embryos. Ex. 1080 ¶¶ 44, 51.

CVC argues that chromatin is dynamic and, during DNA replication, chromatin is in an open stage so access to chromatin is not an issue. CVC Mot. 1 at 5, 27, 35. This ignores the fact that even during replication, the eukaryotic DNA is still associated with histones. Ex. 1080 ¶ 44. In addition, only some DNA may be exposed at any given moment while other DNA encoding "inactive genes" are not exposed. *See* Ex. 1081 at 102:20 – 121:1 (agreeing that for any given cell types, some genes are active and some genes are inactive); 121:12-16 (agreeing that the chromatin for the inactive genes are likely to be in the closed stage); 122:4-13 (admitting that "the genes that are in a closed chromatin state [] are less accessible to CRISPR-cas9").

Indeed, several experts in the field also expressed the same concerns in 2012. Dr. Carroll, CVC's expert in related proceedings, stated in his 2012 commentary: "There is no guarantee that Cas9 will work effectively on ***a chromatin target*** . . . ." Ex. 1023 at 1660 (emphases added). Similarly, Dr. Barrangou (a CVC witness) commented: "[Applying CRISPR-Cas9 in eukaryotes] will require testing whether crRNA-Cas systems can efficiently **cleave chromatin DNA** *in vivo* . . . ." Ex. 1015 at 838 (emphases added).

e. PAM Sequences

As the Board found in the '115 Interference, a POSITA would have known that a PAM sequence played a role in CRISPR DNA editing in ***bacterial*** cells. '115 Decision, at 93-95. However, a POSITA would not have known whether PAM sequences would play any role in ***eukaryotic*** environment. *Id*. Indeed, the requirement for a PAM is a significant aspect of the use of CRISPR-Cas9 for gene editing because it represents an unusual and unexpected aspect of DNA targeting mechanism. Ex. 1080 ¶ 45; *see* Ex. 1084 at 214:3-17 ("Zinc fingers [and TALENs] do not require a sequence adjacent, such as [PAM sequence] NGG, next to its binding

site."). Thus, a POSITA would have expected at least discussion of the role of a PAM sequence in ***eukaryotic cells*** is required to show possession of a CRISPR-Cas9 system in eukaryotes. *Id*.

CVC argues that "P1 discloses PAM sequences in Figure 3C" because "a POSITA would have immediately recognized the PAM sequences GGG and TGG adjacent to the target sequences …." CVC Mot. 1 at 38. However, CVC does not and cannot identify any disclosure in P1 that discuss ***the role*** of PAM sequences in ***eukaryotic*** cells.

CVC next argues that:

> P2 provides expanded discussion about PAMs, consistent with what a POSITA would have known and understood: "[I]n the S. pyogenes type II system, the PAM conforms to an NGG consensus sequence, containing two G:C base pairs that occur one base pair downstream of the crRNA binding sequence, within the target DNA." Ex. 2010, [00350]; Ex. 2543, ¶¶ 54-64, 249- 259; Ex. 2548, ¶¶ 13-19; MF 22. Further, P2 cites Sapranauskas (Ex. 2132), Deveau (Ex. 2125), Mojica (Ex. 2127), Makarova (Ex. 2130), and Wiedenheft (Ex. 2134) which discuss PAMs.

CVC Mot. 1 at 38-39. However, CVC's expert admitted that all the discussions are limited to PAMs in prokaryotic context, which may or may not be the same for eukaryotes. Ex. 1080 ¶ 45; Ex. 1083 at 108:18 – 109:7 ("[Exs. 2125-2134, 2200, 2404] describe several aspect [sic] of the CRISPR-Cas system in bacteria and archaea."). Thus, all the disclosures relied on by CVC would not have indicated to a POSITA that the CVC inventors had possession of a CRISPR-Cas9 system in eukaryotic cells at the time of filing.

f. Ion Concentrations and Other Intracellular Conditions

Intracellular ion concentrations can significantly impact protein activities. For example, Mastroianni (2008) teaches that Group II introns require extra Mg^{2+} to function in eukaryotic cells. Ex. 2150, at 9. A POSITA would have known that Mg^{2+} concentrations can differ greatly between bacterial and eukaryotic cells. Ex. 1110 (Walker (1994)) at Table 2. Mg^{2+} concentrations can impact RNA and protein functions. Ex. 1120 (Leipply (2010)) at 1843 ("For

many years, researchers have studied the striking dependence of RNA tertiary structure stability on Mg^{2+} ions . . . ."); Ex. 1122 (Romani (2011)) at 13 ("Mammalian cells contain high concentrations of total and free magnesium ion (Mg^{2+}). These concentrations are essential to regulate numerous cellular functions and enzymes, including ion channels, metabolic cycles, and signalling pathways."). Thus, a POSITA would have understood that ion concentrations would be another challenge in adapting CRISPR-Cas9 to eukaryotes. Ex. 1080 ¶ 46.

Citing paragraph 119 of Dr. Doyon's Declaration, CVC argues that "[a] POSITA would have understood that the temperature, pH, and ion concentrations in a fish embryo, e.g., a zebrafish embryo, are not materially different from the conditions in *S. pyogenes*, which is the Cas9 of CVC P1 and P2. Ex. 2543, ¶ 119." CVC Mot. 1 at 26. Paragraph 119 of Ex. 2543 only cites Ex. 2106, page 707 for support. However, nowhere on that page discusses the differences or similarities of temperature, pH, and ion concentrations between *S. pyogenes* and zebrafish or between bacterial and eukaryotic cells. *See* Ex. 1084 at 242:5-11 ("[The pH or ion concentrations in fish embryos] are not disclosed in [Ex. 2106.]"). Thus, CVC's conclusory assertion is not supported.

g. Toxicity

A POSITA would have understood that the presence of the guide RNA can trigger an interferon response, leading to toxicity. Ex. 1080 ¶ 47. For example, RIG-I is a receptor that can sense viral RNAs bearing 5′-triphosphate, which will activate host defense mechanisms via interferon. *Id.*; Ex. 1106 (Pichlmair (2006)) at Abstract; Ex. 1108 (Schmidt (2012)) at Abstract. In addition, dsRNA can be recognized as 'foreign' in a eukaryotic cell and stimulate protective responses such as the activation of type 1 interferon, which will lead to cellular toxicity. Ex. 1100 at Abstract. Because guide RNA of CRISPR-Cas9 comprises 5′-triphosphate and contains stretches of dsRNAs, thus a POSITA would have been concerned that CRISPR-Cas9 could

trigger interferon response and toxicity. Ex. 1080 ¶ 47.

A POSITA would have also been concerned about the potential toxicity due to non-specific targeting by prokaryotic gene editing systems when used in eukaryotic cells. Ex. 1080 ¶ 48. For example, Cre recombinase only requires 8-10 matches in its 13-bp binding sites – loxP – to induce recombination in its native prokaryotic environment. Ex. 1102 (Loonstra (2001)) at 9209. Such binding specificity is adequate in prokaryotic cells because of their smaller genomes, but was completely inadequate in eukaryotic cells, where the protein could also target peudo-loxP sites in large genomes such as human. Ex. 1109 (Thyagarajan (2000)) at 47, 54. Thus, careful titration of Cre was required for its use in mammalian cells because high levels of Cre activity can lead to chromosomal aberrations in mammalian cells as a result of non-specific targeting. Ex. 1102 (Loonstra (2001)) at Abstract. Similarly, CRISPR-Cas9 also tolerates sequence mismatches between guide RNA and target DNA, which increases the potential for off-target recognition. Ex. 1038 (Jinek (2012)) at Fig. 3E (mismatches distal to PAM region tolerated). Thus, even if CRISPR-Cas9 could target DNA in eukaryotic cells, a POSITA would have been concerned that it could also cause cleavage of non-specific targets that leads to toxicity to eukaryotic cells. Ex. 1080 ¶ 48.

CVC argues the use of a relatively long recognition sequence and the requirement of a PAM sequence would minimize off-target effects. CVC Mot. 1 at 28, 36. This argument has several problems. First, CVC's argument incorrectly assumes that a POSITA would have known the role of PAM in eukaryotic cells. Second, even assuming the role of PAM were known, off-target effects would still be problematic because PAM appears quite frequently in 1/8 of the genome, as CVC's expert Dr. Doyon concedes. Ex. 1084 at 215:13-18. Third, although the full extent of tolerable mismatches was not known, Jinek 2012 showed that "up to six contiguous mismatches in the 5-terminal region of the protospacer are tolerated (Fig. 3E)" (Ex. 1038 at 4-5)

so a POSITA would have had significant concerns about whether the recognition sequence was long enough to minimize off-target effects. Ex. 1080 ¶ 48.

h. The Experience of CVC Inventors Also Confirmed Many Concerns A POSITA Would Have Had In Attempting To Apply CRISPR-Cas9 To A Eukaryotic Cell

CVC's inventors' own experience also confirmed that a POSITA would have had many of the concerns discussed above. For example, Dr. Jinek considered chromatin might be a barrier to DNA target. Ex. 1091 at 1 ("we are not targeting the right piece of DNA (due to chromatin structure etc)"). He also speculated that guide RNA was not stable due to exonucleases ("RNA . . . turns over too fast to associate with Cas9 posibly [sic] due to degradation by exonucleases") or "RNA is stable but does not associate with Cas9 at the right place and at the right time." *Id*. at 1-2. Thus, the CVC inventors' own experience further highlights the challenges and uncertainties that a POSITA would have had in applying CRISPR-Cas9 to eukaryotic cells.

3. A POSITA Would Have Not Considered ZFNs And TALENs To Be Analogous Systems Because They Use Completely Different Mechanisms, And Therefore Prior Experience With ZFNs And TALENs Would Not Have Alleviated A POSITA's Concerns About Applying CRISPR-Cas9 To A Eukaryotic Cell

CVC argues that "the relevant comparison [of CRISPR-Cas9 system] is to ZFNs and TALENs" because they are "the most analogous systems[.]" CVC Mot. 1 at 8-11. As an initial matter, this argument has already been rejected by the Board and the Federal Circuit. '048 Decision, at 39-41; *Broad*, 903 F.3d at 1293 (affirming the Board's finding that "the prior art TALEN and [ZFN] systems were not analogous to CRISPR-Cas9 because they have their origins in eukaryotic domains[.]"). Furthermore, as explained below, a POSITA would not have considered ZFNs/TALENs to be analogous to CRISPR-Cas9 due to fundamental differences between ZFNs/TALENs and CRISPR-Cas9.

a. Neither CVC P1 Nor P2 Teaches That ZFNs Or TALENs Are Analogous Systems With Respect To CRISPR-Cas9

CVC argues that ZFNs and TALENs are the most analogous system because CVC P1 "compares CRISPR-Cas9 directly to . . . ZFNs and TALENs[.]" CVC Mot. 1 at 9. However, P1 does not describe any similarities between ZFNs/TALENs and CRISPR-Cas9 or explain how these systems are analogous. For example, paragraph 1 of P1 describes ZFNs and TALENs as "[t]wo major technologies for engineering site-specific DNA nucleases have emerged, both of which are based on the construction of chimeric endonuclease enzymes in which a sequence non-specific DNA endonuclease domain is fused to an engineered DNA binding domain." Ex. 2009 ¶ 1. This description merely teaches that ZFNs/TALENs are engineered chimeric DNA nucleases for gene editing. *Id*. Paragraphs 158 and 169 of P1 describe expression vectors and do not specifically cite to ZFNs or TALENs. *Id*. ¶¶ 158, 169. Paragraph 174 describes techniques of transferring exogenous DNA, RNA or protein to cells using transfection or microinjection, including injection of a ZFN. *Id.* ¶ 174. Thus, none of these paragraphs relied upon by CVC compare ZFNs/TALENs and CRISPR-Cas9 or teach that ZFNs/TALENs and CRISPR-Cas9 are analogous systems. At most, a POSITA reading CVC P1 would have understood that ZFNs/TALENs and CRISPR-Cas9 can be used for DNA cleavage. *Id*. A POSITA would not have recognized that ZFNs/TALENs are analogous to CRISPR-Cas9 based on the disclosure of CVC P1. *Id*.; Ex. 1080 ¶¶ 29-35.

b. Scientific Literature Demonstrates That ZFNs And TALENs Are Not Analogous To CRISPR-Cas9 Due To Fundamental Differences In Their Respective Origins, Components, And Functions

Indeed, CVC does not, and cannot, cite any literature to support its bald assertion that ZFNs and TALENs are the most analogous systems to CRISPR-Cas9. It was well-established that, at the time of filing CVC P1 and P2, both ZFNs and TALENs were artificial restriction

enzyme systems that could be engineered to cut DNA in a sequence specific manner. Ex. 1080 ¶¶ 30-35; Ex. 1081 at 110:14-111:5; Ex. 1083 at 59:10-22. Both systems comprise a DNA binding domain and a separate DNA cleavage domain. Ex. 1118 (Carlson (2011)) at Fig. 1 ("Each ZFN polypeptide consists of two functional domains, a DNA-binding domain comprising a chain of finger modules (ZFs) that each typically recognize a unique 3-base pair sequence of DNA and a DNA-cleaving domain composed of the nuclease domain of the *FokI* nuclease."); Ex. 1119 (Huang (2014)) at Abstract ("[TALENs] are engineered endonucleases composed of a customized transcription activator-like effector (TALE) DNA-binding domain and a FokI DNA cleavage domain."). The DNA binding domains of ZFNs/TALENs can be engineered to bind any desired DNA sequence, without the requirement for a PAM sequence, and once bound, the separate DNA cleavage domain can cut the target DNA. Ex. 1080 ¶¶ 30-35; Ex. 1084, 214:3-17. Thus, a POSITA would have understood that ZFNs and TALENs are not analogous systems at all because of several fundamental differences. Ex. 1080 ¶¶ 29-35.

First, a POSITA would have understood that ZFNs and TALENs would not have had problems in accessing a DNA target in a eukaryotic cell, which is embedded in the complex environment of eukaryotic chromatin, because the DNA binding domains of ZFNs and TALENs were derived from components known to function in eukaryotes. Ex. 1080 ¶¶ 30-35. The DNA binding of ZFNs is achieved via arrays of Zinc Finger peptides ("ZFPs"), which is a eukaryotic DNA binding motif. Ex. 1099 (Gonzalez (2012)) at 791-793; Ex. 1083 at 60:13-18. TALENs utilize a protein TALE for DNA binding, which is secreted by the plant pathogen *Xanthomonas*. Ex. 1097 (Christian (2010)) at 757. In the most natural setting, TALE binds promoter sequences in the host plant and activates the expression of plant genes that aid bacterial infections. *Id.*; Ex. 1083 at 62:12-18. Thus, TALE has evolved to function within a eukaryotic cell and to recognize a eukaryotic genomic sequence. Ex. 1080 ¶ 31. In contrast, the entirely prokaryotic CRISPR-

Cas9 system was known to function only in prokaryotic cells as of the filing date of CVC P1 and P2. *Id.* ¶ 19.

CVC argues that "CRISPR-Cas9 is akin to ZFNs and TALENs because it is a *nuclease* guided by a DNA-targeting and binding domain." CVC Mot. 1 at 11 (emphasis original). This argument completely ignores a fundamental difference between CRISPR-Cas9 and ZFNs/TALENs: the former achieves DNA targeting and binding through an RNA while the later through a protein. Ex. 1080 ¶¶ 19, 35; Ex. 1083 at 63:22 – 64:12 (ZFNs and TALENs "bind to DNA through protein-DNA context."). CVC also argues that "ZFNs and TALENS are [] analogous because these particular systems, like CRISPR-Cas9, have cleavage domains that are bacteria-derived nucleases (FokI and Cas9 respectively.)". CVC Mot. 1 at 11. However, the *FokI* DNA cleavage domain cannot recognize any specific target DNA sequence. Ex. 1080 ¶¶ 30-35. It is the DNA binding domain of ZFNs and TALENs, not the cleavage domain, that accesses, scans, and recognizes target DNA sequences in eukaryotic chromatin. *Id.*

Second, a POSITA would have understood that concerns related to the RNA-DNA binding of CRISPR-Cas9 do not exist in ZFNs/TALENs because ZFNs/TALENs use protein-DNA binding and do not have an RNA component. Ex. 1080 ¶¶ 30-35; Ex. 1083 at 63:22 – 64:12. This difference in binding mechanisms has several implications. For example, ZFNs/TALENs do not require the formation of a RNP complex, or DNA unwinding and melting activity to produce strand separation to allow RNA hybridization to target DNA. Ex. 1080 ¶ 35. In addition, ZFNs/TALENs do not have any RNA components, thus the concerns directed to the stability and function of RNA and the RNP complex of the CRISPR-Cas9 do not apply to ZFNs/TALENs. *Id.*

Third, ZFNs and TALENs use a bi-module system, where the DNA binding conferred by the ZFPs or TALEs is completely distinct from the subsequent enzymatic step of cleavage that is

provided by an associated *FokI* enzyme. Ex. 1080 ¶¶ 30-35. The essential component of a ZFN or TALEN is conferred by the DNA-binding capability of the ZFN and TALE as other enzymes can freely replace the *FokI* component. *Id.* ¶ 35. Thus, ZFNs and TALENs comprise linked but functionally separate DNA binding and DNA cleavage domains. *Id.* In contrast, CRISPR-Cas9 consists of an RNP complex that has to accommodate three different types of macromolecular interactions: Cas9/RNA, RNA/target DNA, and Cas9/target DNA. *Id.* ¶ 20. These interactions are inextricably linked because neither DNA binding nor DNA cleavage is possible if any one of the components is missing. *Id.*

Indeed, Dr. Carroll had highlighted some of the differences between ZFNs/TALENs and CRISPR-Cas9 and doubted whether CRISPR-Cas9 would be used in eukaryotes despite success of using ZFNs/TALENs in eukaryotes:

> What about activity of the system in eukaryotic cells? ***Both zinc fingers and TALE modules come from natural transcription factors that bind their targets in a chromatin context. This is not true of the CRISPR components. There is no guarantee that Cas9 will work effectively on a chromatin target or that the required DNA–RNA hybrid can be stabilized in that context.*** This structure may be a substrate for RNA hydrolysis by ribonuclease H and/or FEN1, both of which function in the removal of RNA primers during DNA replication. ***Only*** attempts to apply the system in eukaryotes will address these concerns.

Ex. 2339 at 1660 (emphases added); *see also* Ex. 2566 at 87:20 – 88:8. He also admitted during deposition that he would not consider that ZFNs and TALENs were analogous to CRISPR-Cas9 in 2012. Ex. 2566 at 87:14-19.

As explained above, the components, mechanisms of action, and origins of ZFNs and TALENs are all fundamentally different from CRISPR-Cas9. Thus, the success of using ZFNs/TALENs in eukaryotic cells would not have alleviated concerns a POSITA would have had in adapting CRISPR-Cas9 to eukaryotic cells.

c. CVC's Evidence, Including The CVC Inventors' Emails And References Published After The Filing Dates Of CVC P1 And P2, Is Either Irrelevant Or Does Not Support CVC's Position

CVC also claims that inventors viewed ZFNs and TALENs as the most analogous systems. CVC Mot. 1 at 9-10. However, the email correspondence between inventors merely shows that they were aware of ZFNs/TALENs and interested in comparing their gene editing efficiency with CRISPR-Cas9. In one email, Dr. Doudna discussed with Dr. Jinek a recent article using ZFNs for gene editing by Dr. Drubin's group, and she wanted to "compare ***efficiencies*** to what [Drubin] observed." Ex. 2250 at 1 (emphases added). In another email, Dr. Charpentier suggested testing CRISPR-Cas9 in fish by microinjection, which worked for TALENs. Ex. 2399 at 1. Nothing in these emails even remotely suggests that ZFNs and TALENs are analogous to CRISPR-Cas9, let alone the ***most*** analogous. The fact that the inventors later used some techniques referenced in those emails had no bearing on whether a POSITA would have considered that ZFNs and TALENs were analogous to CRISPR-Cas9 at the time of filing CVC P1 or P2. Even if the ***CVC inventors*** had viewed ZFNs and TALENs as the most analogous systems, which is not supported by any evidence, their subjective belief is irrelevant to the written description analysis because the focus is "the four corners of the specification from the perspective of ***a person of ordinary skill in the art***." *Boston Sci.*, 647 F.3d at 1366 (emphases added).

CVC also argues that CRISPR-Cas9 was being compared with ZFNs and TALENs because Sigma's '204 application applied "techniques previously used for ZFNs" for introducing CRISPR-Cas9 into eukaryotic cells. CVC Mot. 1 at 10. As a threshold matter, the '204 application was published in 2017, several years after the filing dates of CVC P1 and P2. Thus, a POSITA would not have known the teachings of the '204 application at the time of filing P1 or P2. Further, the fact the '204 application compares ZFNs and TALENs with CRISPR-Cas9 does

not support that the applicants of the '204 application considered these systems to be analogous. Indeed, the reference to ZFNs and TALENs in the '204 applications is simply directed to the fact that both ZFNs/TALENs and CRISPR-Cas9 are gene editing tools.

CVC also relies on references published between September 2012 and February 2013 that "expressly compare[] CRISPR/Cas9 to ZFNs and TALENs." Ex. 2543 ¶ 308. However, all but two references were published after the filing date of CVC P2, thus they are not prior art and are irrelevant. Two remaining references are commentaries by Drs. Carroll (Ex. 2339) and Barrangou (Ex. 2215). As discussed above, Dr. Carroll explained that, because ZFNs/TALENs were quite different systems, the success of ZFNs/TALENs in eukaryotic gene editing cannot address many concerns a POSITA would have in applying CRISPR-Cas9 to eukaryotic cells. Ex. 2339 (Carroll (2012)) at 1660. Dr. Barrangou expressed similar sentiments:

> Although immediate applications of this new tool include customized DNA nicking and/or cleavage in bacteria, there are intriguing ***possibilities*** for genome editing and genome engineering of eukaryotes. ***This will require testing whether crRNA-Cas systems can efficiently cleave chromatin DNA in vivo and be readily transferred into organisms of interest***, notably yeast and fungi, but also plants, for crop and agricultural applications, and human cells, for medical purposes. ***Only the future will tell whether this programmable molecular scalpel can outcompete ZFN and TALEN DNA scissors for precise genomic surgery***.

Ex. 2215 (Barrangou (2012)) at 838 (emphases added).

Indeed, discussion of ZFNs/TALENs and CRISPR-Cas9 by these references is simply because ZFNs/TALENs were leading gene editing tools in 2012, and if CRISPR-Cas9 could be adapted in eukaryotic cells, ZFNs/TALENs would be the standard for comparing gene editing efficiencies. Ex. 1080 ¶ 29. Nothing in these references describes or even suggests that ZFNs/TALENs and CRISPR-Cas9 are analogous systems. *Id*. Thus, a POSITA, at the time of filing of CVC P1 and P2, would not have considered the experience with ZFNs and TALENs instructive for overcoming obstacles a POSITA would have considered in adapting CRISPR-

Cas9 to eukaryotic cells. *Id*. ¶¶ 29-35.

4. Microinjection Of An RNP Complex Would Not Obviate Most Technical Challenges

Although microinjection could obviate some potential challenges a POSITA would have anticipated when translating the prokaryotic CRISPR-Cas9 systems to eukaryotes, such as suboptimal expression of a prokaryotic protein, it cannot get around most challenges discussed above in Part VI.A.2. Ex. 1080 ¶ 49. For example, a POSITA would have been concerned whether such a prokaryotic RNP complex could have retained function in a eukaryotic cell, including whether the complex and/or the CRISPR RNA was stable, whether the complex could screen and recognize a DNA target sequence in the context of large eukaryotic genome size and the presence of eukaryotic chromatin, and whether the RNA/DNA hybrid would remain stable. *Id.* Mastroianni (2008), which CVC relies upon, reported results of microinjection of a RNP (Group II introns) into embryos. Ex. 2150 at 2. Their study shows that limited success was observed only with addition of extra Mg^{2+}, which would result in Mg^{2+} concentrations that "are deleterious to eukaryotic cells." Ex. 1092 (Lambowitz (2017)) ¶ 26. Thus, a POSITA would have understood that microinjection of a RNP into embryos, without addition of Mg^{2+}, would not make the RNP work in eukaryotic environment. Ex. 1080 ¶ 26.

Citing Deltcheva (2011), CVC argues that "[a] POSITA would have understood that RNA degradation is not a concern when microinjecting a pre-assembled RNP complex of sgRNA/Cas9, because the Cas9 protects the RNA guide from RNases and any other factors in the environment, particularly when the RNP is injected directly into the nucleus." CVC Mot. 1 at 26. There are several problems with CVC's arguments. First, Deltcheva (2011) does not support CVC's position because it merely states that "Csn1 ***might help*** protect tracrRNA and pre-crRNA against other host RNases, as suggested by the strongly reduced accumulation of

tracrRNA in the absence of csn1." Ex. 2029 at 604 (emphasis added). Second, it was unclear whether a RNP would shield all of the RNA from cleavage. Ex. 1080 ¶ 50. Indeed, several review articles depict CRISPR RNAs sticking out beyond the limits of the Cas9 protein. Ex. 2130 at Fig. 1; Ex. 2131 at Fig. 4. Thus, a POSITA would not have known whether Cas9 would cover and protect all of the tracrRNA and crRNA from degradation even in bacterial cells. Ex. 1080 ¶ 50. Third, even if Cas9 could protect the CRISPR RNAs, a POSITA would have considered that the protection afforded in bacterial cells might be missing in eukaryotic cells. *Id.* ¶¶ 37, 39, 50. Eukaryotic cells have a different set of RNases than prokaryotic cells. *Id.* Thus, protection from bacterial RNases would not necessarily guarantee protection in a eukaryotic cell. *Id*. And finally, the RNA:DNA hybrids formed by CRISPR-Cas9 binding to its target DNA would be susceptible to cleavage by the eukaryotic RNase H, which does not exist in prokaryotic cells. *Id.* ¶¶ 38, 50. In sum, without more definitive support, a POSITA would not have known that a guide RNA would be shielded from RNA degradation in a eukaryotic environment. *Id.* ¶ 50. Accordingly, a POSITA would have remained concerned about the stability of guide RNA and a CRISPR-Cas9 complex. *Id*. In addition, a POSITA would have understood that microinjection of RNP does not reduce the size of eukaryotic genomes or make DNA more accessible by changing chromatin structures. *Id.* ¶¶ 49-50. Thus, a POSITA would not have thought that microinjection of RNP would improve access to eukaryotic genomic DNA or facilitate scanning and recognizing target DNA sequences within a eukaryotic genome. *Id.*

Furthermore, a POSITA would have understood that microinjection of RNPs would cause some unique challenges. Ex. 1080 ¶ 52. To account for natural degradation as well as dilution by cell division, RNPs would need to be introduced at high concentrations. *Id.* High concentrations of RNPs would be expected to exacerbate off-target effects, creating more toxicity and acting as decoys to the intended target sites. *Id.* High concentrations of RNP would

also cause more cellular toxicity. *Id.*

CVC only cites three examples of microinjection of RNP into eukaryotic cells, but none supports its argument that "[m]icroinjecting a pre-assembled RNP into a fish embryo obviates the concerns alleged in the '115 interference. . . ." CVC Mot. 1 at 26; Ex. 1084 at 216:1 – 217:4 ("I don't have other example [of using microinjection to deliver an RNP to a eukaryotic cell] in mind."). Ex. 2148 describes experiments of microinjecting a RNP complex from a eukaryotic source (silk moth) to Drosophila embryo. Ex. 1080 ¶ 51. Thus, it does not address the concerns a POSITA would have had about whether the prokaryotic CRISPR-Cas9 would also work in a eukaryotic cell. *Id.* Ex. 2149 reports microinjection of an RNP complex, which consists of components of a virus that naturally infects eukaryotic cells, resulted in production and release of the virus. *Id.* The function of the viral RNP reported in Ex. 2149 has nothing to do with DNA or gene editing and the RNP does not even need to be in the nucleus. *Id.* Ex. 2150 describes microinjection of Group II introns into embryos but the gene editing function of Group II introns requires addition of high levels of Mg^{2+}. *Supra* Part VI.A.1. The authors of Ex. 2150 expressly acknowledge that chromatinization inhibits Group II introns' function. Ex. 2150 at Abstract, 11; Ex. 1080 ¶¶ 44, 51. Thus, a POSITA would not have considered that microinjection of an RNP into a fish embryo would have obviated concerns such as chromatin impeding access, RNA and RNP stability, and inadequate ion concentration. Ex. 1080 ¶ 51. Accordingly, the examples cited by CVC either contradict CVC's argument or are inapposite.

B. The Lack Of Any Discussion In CVC P1 And P2 Of Potential Challenges And Specific Instructions On How To Adapt CRISPR-Cas9 To Eukaryotes Would Have Demonstrated To A POSITA That The CVC P1 And P2 Inventors Did Not Possess An Embodiment Within The Scope Of Count 1

In 2012, CRISPR-Cas9 was a nascent technology. Ex. 1001 ¶ 100. The Board has found, and the Federal Circuit affirmed, that a POSITA would not have expected that CRISPR-

Cas9 would work in eukaryotic cells. '048 Decision, at 45-46; *Broad*, 903 F.3d at 1296. The key prior art reference in that case was Jinek (2012) (Ex. 1038). Like CVC P1 and P2, Jinek (2012) only discloses using CRISPR-Cas9 in test tubes to cleave DNA. Ex. 1080 ¶ 21. Thus, a POSITA reading CVC P1 and P2 would have not expected that CRISPR-Cas9 would have worked in eukaryotic cells. *Id.*

As explained above, a POSITA would have considered several obstacles and uncertainties in applying a prokaryotic system to a eukaryotic system. *See supra* Part VI.A; Ex. 1080 ¶¶ 36-48. This is particularly true for CRISPR-Cas9 due to its prokaryotic origin, the requirements of both an RNA and a protein component to function together, the lack of a perfectly analogous system in eukaryotes, the past challenges and failures for applying prokaryotic RNA and RNP systems to eukaryotes, and the many differences between prokaryotic and eukaryotic cellular and genomic environments. *Id*. Thus, a POSITA "would have expected that the CRISPR-Cas9 system would have also required its own set of unique conditions." '048 Decision, at 6-10. Based on prior experiences, a POSITA would have expected to see that the CVC inventors considered or at least recognized the potential challenges and uncertainties in adapting CRISPR-Cas9, a prokaryotic system, to a eukaryotic environment. *Id.* ¶ 55. Yet, CVC P1 and P2 fail to address any of the potential challenges or obstacles discussed above, or provide any instructions or special conditions on how to use CRISPR-Cas9 in eukaryotic cells. *Id.*

CVC claims that the potential obstacles and challenges "are addressed in P1 . . . and could be easily addressed using techniques well within the skill and knowledge of a POSITA." CVC Mot. 1 at 32-36. CVC argues that "P1 [] contemplates ways to minimize the potential for RNA degradation" because paragraph 107 of P1 discloses "using modified nucleic acids to provide 'stability towards 3'-exonucleolytic degradation.'" *Id*. at 32. This disclosure, at best, would have indicated to a POSITA that the CVC inventors were aware of one way to potentially

make the guide RNA more stable. Ex. 1080 ¶ 53. CVC P1 does not teach that the disclosure was intended for applying CRISPR-Cas9 to eukaryotic cells. *Id.* In addition, the modified RNA was never used in any experiment disclosed in CVC P1 or P2, or discussed in P1 or P2 as a way to overcome the challenges of RNA degradation in eukaryotic cells. *Id*. Indeed, CVC P1 and P2 do not address other potential challenges of RNA degradation, such as eukaryotic proteins disclosed in Karpala (2005) (Ex. 1100) that degrade RNA. *Id.* Nor do CVC P1 and P2 address other issues concerning RNA stability, including whether RNA would have been properly folded and remained in complex with Cas9 to create an RNP complex, or one that was capable of performing DNA targeting and cleavage in eukaryotic cells, and whether an RNA:DNA hybrid would have been formed properly and remained functional, *i.e.*, whether RNase H would have cleaved the RNA strand of the RNA:DNA hybrid. *Id.* ¶¶ 38-40, 53.

With respect to "Cellular Conditions," CVC does not identify any disclosures in P1 other than paragraphs 248-252. CVC Mot. 1 at 33-34. However, those paragraphs of P1 only disclose an *in vitro* experiment showing targeted DNA cleavage in test tubes and do not discuss the differences in cellular conditions between prokaryotes and eukaryotes or between *in vivo* and *in vitro* applications. Ex. 1080 ¶ 46. Nor do those paragraphs discuss whether any special conditions would be required for using CRISPR-Cas9 in eukaryotic cells. *Id.* For example, a POSITA would have been concerned about the sufficiency of the concentration of Mg^{2+}, as illustrated in the Group II intron case. Ex. 2150 at 9. And, a POSITA would further understand that Mg^{2+} has pleiotropic effects, influencing protein and RNA structures and function. *Id*. Furthermore, a POSITA would not have extrapolated the results from the oversimplified conditions in test tubes disclosed in paragraphs 248-252 to the much more complicated cellular environments of eukaryotes. Ex. 1080 ¶ 41. For example, a POSITA would have been concerned about molecular crowding, which is not addressed at all by CVC P1 or P2. *Id.* Thus,

CVC fails to identify any disclosures in CVC P1 or P2 that address the potential challenges a POSITA would have considered due to the differences in cellular conditions. *Id*. ¶ 46.

CVC argues that "[a] POSA would also have considered ion concentrations, such as magnesium ion concentrations, to be a parameter that could be optimized and there were straightforward techniques for adjusting ion concentrations, including magnesium." CVC Mot. 1 at 33-34. But this argument has already been rejected by the Board in the '115 Interference:

> CVC's arguments fail to persuade us that those of ordinary skill in the art would not have considered specific instructions or conditions for a CRISPR-Cas9 activity in a eukaryotic cell to be necessary. ***Possession of an innovation is not indicated by the need for optimization to obtain it*** because "[t]he question is not whether a claimed invention is an obvious variant of that which is disclosed in the specification. Rather, a prior application itself must describe an invention, and do so in sufficient detail that one skilled in the art can clearly conclude that the inventor invented the claimed invention as of the filing date sought." *Lockwood v. Am. Airlines, Inc., 107 F.3d 1565, 1572* (Fed. Cir. 1997).

'115 Decision at 90 (emphases added).

CVC next asserts that "P1 contemplates and discusses nucleosomes, which are a structural unit of a eukaryotic DNA, consisting of a length of DNA coiled around a core of histone." CVC Mot. 1 at 35. CVC is only able to cite paragraphs 164 and 174 to support this argument. *Id*. However, paragraph 164 of P1 does not contemplate the impact of histones on CRISPR-Cas9 access to target DNA, but instead describes a potential application of CRISPR-Cas9 that does not rely on target DNA cleavage, by modifying histone proteins:

> Histone proteins are known in the art to bind DNA and form complexes known as nucleosomes. Histones can be modified (e.g., by methylation, acetylation, ubiquitination, phosphorylation) to elicit structural changes in the surrounding DNA, thus controlling the accessibility of potentially large portions of DNA to interacting factors such as transcription factors, polymerases and the like. . . . Thus, a site-directed modifying polypeptide with histone-modifying activity finds use in the site specific control of DNA structure and can be used to alter the histone modification pattern in a selected region of target DNA. Such methods find use in both research and clinical applications.

Ex. 2009 ¶ 164; *see* Ex. 1080 ¶ 54. Paragraph 164 does not explain whether chromatin would be

a barrier to adapting CRISPR-Cas9 in eukaryotic cells, nor does it provide any instructions on how to overcome the barrier. Ex. 1080 ¶ 54. For example, paragraph 164 does not discuss whether modification of histones is required for CRISPR-Cas9 activity. *Id.* Thus, the disclosure of paragraph 164 would not have indicated to a POSITA that the CVC inventors considered that access to target DNA within eukaryotic chromatin would be a challenge or provided any instructions on how to overcome the challenge. *Id.*

Citing paragraph 174 of P1, CVC also argues that "[a] POSITA would have understood that the structure of DNA changes throughout the cell cycle, and is often in an open conformation that exposes DNA and makes it accessible, such as during cell division." CVC Mot. 1 at 35. However, paragraph 174 teaches techniques of transferring polynucleotides and proteins to cells without even mentioning chromatin or any conformation changes that allow better access to DNA. Ex. 1080 ¶ 54. Therefore, contrary to CVC's assertion, P1 and P2 do not discuss chromatin structure as a potential challenge to applying CRISPR-Cas9 in eukaryotes.

CVC further argues that "P1 describes and contemplates the use of a relatively long recognition sequence (e.g., 20 nucleotides) in combination with a PAM adjacent to the target, providing a high degree of specificity," and cites paragraph 48 of P1 for support. CVC Mot. 1 at 36; *see also id*. at 28. There are several problems with this argument. First, paragraph 48 does not even reference PAM. Second, although paragraph 48 discloses that "[t]he DNA-targeting RNA provides target specificity to the complex by comprising a nucleotide sequence that is complementary to a sequence of a target DNA[,]" it does not discuss anything about the degree of specificity conferred, or whether off-target recognition would be a concern, or whether off-target cleavage would lead to toxicity. Finally, this argument does not address the possibility of an RNA-triggered interferon response, which would also result in toxicity, as discussed above. *See supra* Part VI.A.2.g.

CVC also argues, without explanation, that the potential challenges or obstacles "are not recited elements of the count and [] are not required for practicing an embodiment within the scope of the count." CVC Mot. 1 at 32-36. Again, this argument has been expressly rejected by the Board in the '115 Interference: "The ultimate determination that a feature is not required for a CRISPR-Cas9 system as recited in Count 1 is not relevant to whether those of ordinary skill in the art would have considered a disclosure to show possession at the time of filing." '115 Decision at 96; *see also id*. at 87 ("[W]e are not persuaded that questions about the ultimate requirements for CRISPR-Cas function in eukaryotes are relevant to the issue of written description because whether a disclosure indicates possession is viewed from what one of ordinary skill in the art would have considered at the time of filing.").

Indeed, the lack of discussion of any potential concerns would have indicated to a POSITA that the CVC inventors had not even begun to consider the type of information required for possession of a CRISPR-Cas9 system in eukaryotic cells. Ex. 1080 ¶ 55. Thus, without at least a discussion of these potential challenges or obstacles, a POSITA would not have considered that the CVC inventors had possession of the alleged embodiments at the time of filing CVC P1 and P2. *Id.*

C. A POSITA Would Have Understood That The *In Vitro* Studies Reported In CVC P1 And P2 Were Insufficient To Demonstrate Possession Of An Embodiment Within The Scope Of Count 1 Because *In Vitro* Experiments Do Not Predict *In Vivo* Activities

The disclosures of CVC P1 and P2, which only show CRISPR-Cas9 activities in test tubes, would not have indicated to a POSITA that the CVC inventors had possession of a eukaryotic CRISPR-Cas9 system for additional reasons. Ex. 1080 ¶¶ 56-57. The Board in the '115 Interference found that *in vitro* experiments do not predict the activities of *in vivo* activity. '115 Decision, at 91:19 – 93:3. For example, Koseki (1999) reported that "the efficacy of

ribozymes in vitro is not necessarily correlated with functional activity in vivo." Ex. 2575 at 1868. That study identifies several factors for effective ribozyme activity *in vivo*, including "high level of ribozyme expression, the intracellular stability of the ribozyme, colocalization of the ribozyme and its target RNA in the same cellular compartment, and the cleavage activity of the transcribed ribozyme." *Id*. (internal citations omitted). Similarly, Link (2009) identifies "the folding of RNA constructs might differ between test tube and cell" as a reason that prevents RNA switches from becoming useful genetic tools. Ex. 2574 at 1190.

Like ribozymes and riboswitches, which are RNA molecules that have the ability to catalyze biochemical reactions, CRISPR-Cas9 also has an RNA component and a protein component that are both required to target and cleave DNA. Ex. 1080 ¶¶ 19-20. Given the differences between test tubes and *in vivo*, a POSITA would not have been convinced that *in vitro* studies, such as those disclosed in CVC P1 and P2, would have predicted CRISPR-Cas9 activities *in vivo*. *Id.* ¶¶ 56-57.

These differences are particularly relevant here because as of May 25, 2012, it was not fully known how Cas9 worked and whether there were possibly additional unknown components. CVC's expert Dr. Doyon agrees that by May 25, 2012 "the necessary and sufficient components of the Type II CRISPR-Cas9 DNA-cleavage complex remained unknown in the art." Ex. 2543 ¶ 53 (citing two articles that describe "nucleases that have not yet been identified" and "activity that has not yet been identified" of the CRISPR-Cas9 complex.); *see also id.* ¶¶ 61, 62. Although Jinek (2012), CVC P1, and/or CVC P2 describe the necessary components for CRISPR-Cas9 to work in test tubes, a POSITA would have considered additional unknown component(s) beyond Cas9 and guide RNA that would be needed for CRISPR-Cas9 to work even in bacterial cells, let alone eukaryotic cells. Ex. 1080 ¶¶ 56-57. For example, a POSITA would have considered additional components necessary to protect the

stability of the CRISPR-Cas9 complex in the much more complicated eukaryotic environment. *Id.* A POSITA would also have considered additional component(s) to assist with surveying the DNA of a eukaryotic genome for a target DNA when it was not simply presented as a short oligonucleotide or a plasmid in higher than natural concentration in test tubes. *Id.* Thus, a POSITA would have understood that the CVC inventors had not demonstrated the ***sufficient*** components of CRISPR-Cas9 system for DNA cleavage *in vivo* in eukaryotes. *Id.*

Given the significant differences between prokaryotic and eukaryotic environments (*see supra* Part VI.A.2.f), a POSITA would have considered that additional component(s) could be required for CRISPR-Cas9 to function in eukaryotic cells. Ex. 1080 ¶¶ 56-57. Thus, CVC P1 and P2 would not have indicated to a POSITA that the CVC inventors had possession of a CRISPR-Cas9 embodiment in a eukaryotic cell. *Id.*

D. The CVC Inventors' Expression of Doubt And Subsequent Failures Further Support A Lack Of Possession Of An Embodiment Within The Scope Of Count 1

The named inventors of CVC P1 and P2 repeatedly admitted that they were not sure whether CRISPR-Cas9 would work in eukaryotic cells in 2012, and they had encountered "many frustrations" getting CRISPR-Cas9 to work in eukaryotic cells. For example, Drs. Jinek and Doudna, the inventors named in CVC P1 and P2, stated in an article reporting their experiments using CRISPR-Cas9 in human cells:

> These findings [reported in Jinek 2012] suggested the exciting possibility that Cas9:sgRNA complexes might constitute a simple and versatile RNA-directed system for generating DSBs that could facilitate site-specific genome editing. However, ***it was not known whether such a bacterial system would function in eukaryotic cells***.

Ex. 1039 (Jinek (2013)) at 1-2 (emphases added). This concession reveals that the inventors did not know in 2012, following the publication of Jinek (2012), whether CRISPR-Cas9 would function in eukaryotic cells. Therefore, a POSITA would have understood that the inventors did

not have possession of CRISPR-Cas9 at the time of filing CVC P1 and P2. Consistent with this, Dr. Doudna stated in an interview by the Catalyst Magazine as follows:

> Our 2012 [Jinek] paper was a big success, but there was a problem. ***We weren't sure if CRISPR/Cas9 would work in eukaryotes—plant and animal cells***. Unlike bacteria, plant and animal cells have a cell ***nucleus***, and inside, DNA is stored in a tightly wound form, bound in a structure called ***chromatin***.

Ex. 1089 (Catalyst (2014)) at 3 (emphases added).

In another interview, Dr. Doudna was quoted for "experience[ing] '***many frustrations***' getting CRISPR to work in human cells" and considering getting CRISPR to work in human cells "a profound discovery[.]" Ex. 1105 (Pandika (2014) at 3; Ex. 1090 (Cheng-Doudna emails (2012)) at 1 (Dr. Doudna stating that "none of Aaron's data ended up in paper since the initial result back in August turned out to be irreproducible, and in light of subsequent experiments was probably some kind of contamination.").

Even in October 2012, the CVC inventors had encountered significant obstacles and did not know how to get CRISPR-Cas9 to work in eukaryotic cells. Ex. 1091 (Jinek-Doudna emails (2012)) at 1-2. They considered "RNA expression/stability/Cas9 assembly/localization" as potential problems that need to be resolved. *Id.* at 1. They contemplated several approaches to increase RNA stability/block RNA degradation, including switching to a different expression vector and extending the length of guide RNA at 5′ and 3′ termini. *Id.* at 1-2. The CVC inventors' failures further demonstrate a lack of possession of the invention of Count 1.

E. CVC P1 And P2 Do Not Disclose Required Elements Of Count 1

Count 1 requires a CRISPR-Cas9 system with a guide RNA and a Cas9 protein that can form a complex and cleave a target DNA in a eukaryotic cell. Declaration (Paper 1) at 5-6. CVC claims that CVC P1 and P2 disclose three embodiments of Count 1: a fish cell, a human cell, and a fruit fly cell. However, neither P1 nor P2 disclose any cleavage of a target DNA in a

eukaryotic cell. '115 Decision at 81 ("CVC does not direct us to a disclosure in P1 of results from a CRISPR-Cas system in any of these eukaryotic cells."). As discussed above, all that CVC P1 and P2 show are *in vitro* experiments conducted in test tubes, which is described in Fig. 3A of P1 and P2. CVC Mot. 1, Appendix 3, at XII-6, XIII-8. In addition, the disclosed 20 nucleotide target DNA molecules in Fig. 3C are identical to known natural targets of the prokaryotic CRISPR-Cas9 system, as indicated by their presence as protospacers in the *S. pyogenes* genome, but which are not present in eukaryotic DNA. Ex. 2009, Fig. 3C; Ex. 2029 (Deltcheva (2011)). Thus, CVC P1 and P2 fail to disclose elements 1, 4, 7, and 8 of CVC's half of Count 1, which require a target DNA in a eukaryotic cell. Thus, CVC P1 and P2 do not disclose the required elements of Count 1.

In addition, Dr. Peterson, CVC's expert in the '115 Interference, admitted during cross-examination that he used ***the Count*** as his guide to "select" elements and "imagine" the three embodiments. Ex. 1123 at 76:8-11, 122:13-18. But he failed to identify any guidance in ***CVC P1*** that would allow a POSITA to arrive at the purported embodiments. Here, the relevant portions of CVC's expert Dr. Doyon's declaration are nearly identical to Dr. Peterson's declaration submitted in the '115 Interference. *See* Ex. 2543 (¶¶ 44-238, App. 2, App. 3) and Ex. 2457 (¶¶ 37-186, App. 2, App. 3); *see e.g.* Ex. 1084 at 291:5 – 292:14 (admitting no difference between ¶ 44 of Ex. 2543 and ¶ 37 of Ex. 2457). Thus, the purported embodiments are merely *post hoc* creations that CVC's experts have fabricated using Count 1 as the roadmap and with the benefit of hindsight.

F. Accepting CVC's Illusory Embodiments Would Amount To Granting CVC A Hunting License

The only reference of eukaryotic cells in CVC P1 is paragraph 165, which is boilerplate patent application language listing virtually all cells of "[a]ny type" from "any organism" during

their entire cellular life:

> In some of the above applications, the subject methods may be employed to induce DNA cleavage and DNA modification in mitotic or post-mitotic cells ***in vitro and/or ex vivo and/or in vitro*** (e.g., to produce genetically modified cells that can be reintroduced into an individual). Because the DNA-targeting RNA provide specificity by hybridizing to target DNA, a mitotic and/or post-mitotic cell of interest in the disclosed methods may include ***a cell from any organism (e.g. a bacterial cell, an archaeal cell, a cell of a single-cell eukaryotic organism, a plant cell, an animal cell, a cell from an invertebrate*** animal (e.g. fruit fly, cnidarian, echinoderm, nematode, etc.), ***a cell from a vertebrate animal*** (e.g., fish, amphibian, reptile, bird, mammal), a cell from a mammal, a cell from a rodent, a cell from a human, etc.). ***Any type of cell may be of interest*** (e.g. a stem cell, e.g. an embryonic stem (ES) cell, an induced pluripotent stem (iPS) cell, a germ cell; a somatic cell, e.g. a fibroblast, a hematopoietic cell, a neuron, a muscle cell, a bone cell, a hepatocyte, a pancreatic cell etc.). . . .

CVC P1, ¶ 165 (emphases added). This paragraph merely reflects the CVC inventors' wish that CRISPR-Cas9 might be used in non-prokaryotic environments, such as in eukaryotic cells. "A 'mere wish or plan' for obtaining the claimed invention is not adequate written description." *Centocor Ortho Biotech, Inc. v. Abbott Labs*., 636 F.3d 1341, 1348 (Fed. Cir. 2011). This exceedingly broad disclosure does not demonstrate that the CVC inventors knew how to make CRISPR-Cas9 work in all eukaryotic cells. Indeed, the inventors still did not know how to get CRISPR-Cas9 to work in eukaryotic cells around the time of filing CVC P2. *See supra* Part VI.D.; Ex. 1091 at 1-2.

If one were to accept (incorrectly) that CVC P1 discloses embodiments of CRISPR-Cas9 in eukaryotic cells, even though CVC P1 and P2 only disclose experiments in test tubes and show no possession of any eukaryotic embodiment, that determination would amount to granting CVC a hunting license, which has long been prohibited by fundamental patent law:

> Patents are not awarded for academic theories, no matter how groundbreaking or necessary to the later patentable inventions of others. "[A] patent is not a hunting license. It is not a reward for the search, but compensation for its successful conclusion." . . . . Requiring a written description of the invention limits patent protection to those who actually perform the difficult work of "invention"—that is, conceive of the complete and final invention with all its claimed limitations—

and disclose the fruits of that effort to the public.

Ariad, 598 F.3d at 1353 (internal citations and quotations omitted). A finding that CVC P1 or P2 discloses a CRISPR-Cas9 embodiment in eukaryotes would also mean that a patent applicant could meet the written description requirement by simply including anything and everything in its disclosure, not by showing that the applicants actually describe their requisite possession of the invention. Such a determination would completely defeat the purpose of the written description requirement. Thus, CVC's Motion should be denied for failure to describe any embodiment of subject matter within the scope of Count 1.

VII. CVC P1 AND P2 DO NOT ENABLE AN EMBODIMENT WITHIN THE SCOPE OF COUNT 1

To carry its burden on this motion, CVC must show that CVC P1 and/or CVC P2 provide a described ***and enabled*** embodiment within the scope of Count 1. The foregoing discussion demonstrates that both CVC P1 and P2 lack written description under 35 U.S.C. § 112, which alone is sufficient to defeat CVC's motion. *See supra* Part VI. While CVC P1 and P2 also fail to enable the subject matter of Count 1, Sigma will conserve its and the Board's resources by not addressing that additional deficiency here:

> We need not determine whether the P1 disclosure would have enabled an embodiment of Count 1 because we have determined that the P1 disclosure does not sufficiently describe an embodiment of Count 1.

CVC v. Broad, Int'f No. 106, 115, Decision on Motions, at 104-105 (citing *Ariad Pharm., Inc. v. Eli Lilly & Co.*, 598 F.3d 1336, 1344 (Fed. Cir. 2010) ("We agree with Lilly and read the statute to give effect to its language that the specification 'shall contain a written description of the invention' and hold that § 112, first paragraph, contains two separate description requirements: a 'written description [i] of the invention, *and* [ii] of the manner and process of making and using [the invention']. 35 U.S.C. § 112, ¶ 1 (emphasis added).").

VIII. FACT WITNESSES' TESTIMONY IS CONTRARY TO FINAL JUDGMENT, IRRELEVANT, AND/OR DOES NOT SUPPORT CVC'S POSITIONS.

CVC purports to show expectation of success of using CRISPR-Cas9 in eukaryotic cells by testimony from some researchers. *See* CVC Mot. 1 Section VII. However, as discussed above, this completely contradicts the Board's final judgment—as affirmed on appeal—that a POSITA would not have had a reasonable expectation of success in applying the CRISPR-Cas9 system in a eukaryotic cell. '048 Decision, Section III C. CVC's argument also suffers from other serious flaws. For example, the proper inquiry should focus on "the four corners of the specification from the perspective of a person of ordinary skill in the art." *Boston Sci.*, 647 F.3d at 1366. Yet none of the witness offers testimony from the perspective of a POSITA or considers the disclosures of CVC P1 or P2. Thus, all the fact witnesses' testimony is irrelevant.

In addition, CVC offers testimony from Drs. Barrangou, Carroll, and Doudna in an effort to re-cast their contemporaneous statements from 2012 in a light more favorable to CVC today. Courts typically afford contemporaneous statements made at the time of the events considerably more weight than statements prepared long thereafter in the context of litigation. Accordingly, the newly crafted testimony from Drs. Barrangou, Carroll, and Doudna prepared solely for interference proceedings should not be afforded any weight.

IX. CONCLUSION

For the foregoing reasons, CVC is not entitled to the accorded benefit of CVC P1 or P2 because that those applications do not demonstrate possession of the invention of Count 1.

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Respectfully submitted,

Dated: February 18, 2022

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APPENDIX 1

LIST OF EXHIBITS CITED

Exhibit No.	Description
1001	Cannon Decl.
1015	Barrangou (2012)
1023	Carroll (2012)
1038	Jinek (2012)
1039	Jinek (2013)
1073	Zamore Tr. 06/21/2021
1074	Zamore Tr. 04/26/2021
1075	Cannon Decl.
1076	Doyon Tr. 01/08/2021
1077	Doyon Tr. 04/22/2021
1078	Doyon Tr. 07/02/2021
1079	Doyon Tr. 08/12/2021
1080	Supp. Cannon Decl.
1081	Zamore Tr. 01/19/2022
1082	Bailey Tr. 01/24/2022
1083	Doyon Depo. Tr. 02/01/2022
1084	Doyon Depo. Tr. 02/03/2022
1085	EPO Opposition of EP 3138911 B1 (Schlich)
1086	In re Schlich (SDNY 2017)
1087	Broad P1
1088	ToolGen P1
1089	Catalyst (2014)
1090	Cheng-Doudna Emails (2012)
1091	Jinek-Doudna Emails (2012)
1092	Lambowitz Decl. (2017)
1093	Mohr (2000)
1094	Mastroianni (2008)
1095	Mirkin Decl.
1096	Cerritelli (2009)
1097	Christian (2010)
1098	Ellis (2001)
1099	Gonzalez (2010)
1100	Karpala (2005)
1101	Lander (2001)
1102	Loonstra (2001)
1103	Marraffini Tr. 03/11/21
1104	McShan (2008)
1105	Pandika (2014)
1106	Pichlmair (2006)
1107	Richter (2008)
1108	Schmidt (2012)
1109	Thyagarajan (2000)

Exhibit No.	Description
1110	Walker (1994)
1111	Zimmerman (1993)
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CERTIFICATE OF FILING AND SERVICE

I hereby certify that:

I. The following paper was filed February 18, 2022, with the Patent Trial and Appeal Board via:

✓ Interference Web Portal at https://acts.uspto.gov/filing/. Under SO ¶ 105.3, a paper filed through the Interference Web Portal is considered served. The web portal e-filing system is to send email notification of the filing to counsel for Junior Party THE REGENTS OF THE UNIVERSITY OF CALIFORNIA, UNIVERSITY OF VIENNA, AND EMMANUELLE CHARPENTIER.

SIGMA OPPOSITION TO CVC MOTION 1
(for Priority Benefit of CVC P1 and CVC P2)

II. A courtesy copy of the above paper is being sent to counsel for Junior Party THE REGENTS OF THE UNIVERSITY OF CALIFORNIA, UNIVERSITY OF VIENNA, AND EMMANUELLE CHARPENTIER at the address(es) below on February 18, 2022, via e-mail:

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