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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 REPLY IN SUPPORT OF SIGMA MOTION 1
(to Substitute Proposed Count 2 for Count 1)

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1 **REPLY IN SUPPORT OF SIGMA MOTION 1**
2 **(to Substitute Proposed Count 2 for Count 1)**

3 **I. INTRODUCTION**

4 Sigma moves to substitute Proposed Count 2 for Count 1 because the current count
5 encompasses two patentably distinct inventions: (1) CRISPR-Cas9-mediated ***cleavage only*** in a
6 eukaryotic cell; and (2) CRISPR-Cas9-mediated ***cleavage plus integration of a donor***
7 ***polynucleotide via HDR*** ("cleavage plus integration") in a eukaryotic cell. In Motion 1, Sigma
8 sets forth a dozen reasons why in early December 2012 a POSITA would have had considerable
9 uncertainty whether a double-stranded break ("DSB") cleaved by CRISPR-Cas9 would be
10 successfully repaired by donor integration via HDR in a eukaryotic cell. Sigma Mot. 1 at 6-24.
11 This uncertainty would have resulted from the combination of both general concerns about the
12 complex and unknown operation of the largely uncontrollable DNA damage repair ("DDR")
13 processes in eukaryotic cells, as well as very specific concerns about how the bacterial Cas9
14 defense system that evolved to destroy invading viral DNA could impair the highly intricate and
15 precisely regulated HDR process in eukaryotic cells. *Id.* Indeed, many of these concerns
16 persisted for years after 2012. *See* Sigma Opp'n Resp. Mot. 1 at 10-24. Importantly in this
17 context, even if the probability of any particular concern could arguably be categorized as
18 "favorable" (which is not the case here), the ***compound probability*** of these concerns, when
19 considered collectively, leads to the inescapable conclusion that in early December 2012
20 successful donor integration by HDR would not have been reasonably predicted.[1]
21 Contrary to CVC's assertions, the mere demonstration of non-specific DNA cleavage

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[1] Solely for purposes of illustrating the statistical concept of "compound probability", the probability of 12 events successfully occurring, when each event has a 75% chance of successful occurrence, is $0.75^{12} = 3.2\%$.

1 alone—DNA ***destruction***—in a eukaryotic cell (CVC Claim 156) only (partially) addresses the
2 first half of the inquiry, and would not have alleviated a POSITA's separate concerns directed to
3 the far more complex (and largely uncontrollable) second half of the inquiry, namely, the wholly
4 distinct intracellular process of donor integration via HDR—DNA ***re-construction***. Following
5 cleavage, in 2012 a POSITA could do very little but sit back and ***hope*** that HDR ***might*** work
6 with this new prokaryotic-derived system. Like many other emergent technical developments in
7 such highly complex and unpredictable biological systems, absent successful testing (or, at a
8 minimum, recognizing these many concerns and explaining how they could be addressed and
9 resolved), mere hope for success is insufficient to demonstrate reasonably expected success.

10 II. COUNT 1 ENCOMPASSES TWO PATENTABLY DISTINCT INVENTIONS:
11 (1) CLEAVAGE ONLY; AND (2) CLEAVAGE PLUS INTEGRATION VIA HDR

12 In early December 2012, in the context of the nascent, bacteria-derived CRISPR-Cas9
13 technology, successfully accomplishing donor integration via HDR in a eukaryotic cell—the
14 pinnacle of genomic engineering DNA re-construction—would not have been obvious in view of
15 simply cleaving a target DNA in a eukaryotic cell—basic DNA destruction. In particular, at that
16 point in time, a POSITA would not have had a reasonable expectation that donor integration via
17 HDR following CRISPR-Cas9 cleavage would be successful. CVC argues that because HDR
18 was a known DNA repair process in eukaryotic cells, and donor integration by HDR had been
19 shown to be possible in eukaryotic cells using DSBs generated by other targeted nucleases,
20 introducing a donor sequence into cells containing DSBs created by CRISPR-Cas9 "would
21 necessarily result in integration by HDR." CVC Opp'n 1 at 20. But CVC completely ignores
22 that HDR was recognized as the most challenging part of gene editing in 2012, and has remained
23 highly challenging and unpredictable in the decade that followed. *See* Sigma Mot. 1 at 6-24;
24 Sigma Opp'n Resp. Mot. 1 at 10-24. CVC also ignores the crucial differences between the

1 nature of the DSBs created by the RNA-guided CRISPR-Cas9 system, and the DSBs created by
2 the protein-directed TALENs, meganucleases (SceI), and ZFNs ("TMZs").

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A. Abundant Evidence Demonstrates That A POSITA Would Have Had Many Concerns About CRISPR-Cas9's Ability To Support Donor Integration Via HDR

6 As explained in Sigma Motion 1 and in this Reply, a POSITA would have had many
7 concerns about whether the DSBs created by CRISPR-Cas9 would support donor integration via
8 HDR, and a POSITA would not have had a reasonable expectation that HDR would be
9 successful with this new and largely unknown system, particularly in a highly unpredictable and
10 complex eukaryotic cellular environment. And while the hypothetical legal construct of this
11 motion presumes cleavage in a eukaryotic cell of a target DNA molecule ***somewhere*** in its
12 genome, CVC Claim 156 recites nothing about, *inter alia*, the site specificity of the cleavage
13 (*compare* Sigma Claim 31 ("a double-stranded break *at the target site*")); whether the cleavage
14 results in a SSB or a DSB (*compare* CVC dependent claim 180 ("cleaving only one strand")); the
15 extent of likely off-target effects ("*comprising* . . . cleaving the target DNA molecule"); or repair
16 of the DNA by any DDR process at all, even NHEJ (which had never been demonstrated). Thus,
17 assuming CVC Claim 156 is prior art, a POSITA would still not have known whether CRISPR-
18 Cas9 would create DSBs that would be capable of being repaired by donor integration via HDR.

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21

1. Concerns 1-12 Have Assumed That DNA Cleavage By CRISPR-Cas9 In A Eukaryotic Cell (CVC Claim 156) Was Known As Of December 6, 2012

22 CVC argues that the twelve concerns about successful HDR discussed in Sigma Motion 1
23 were simply resolved by the mere recital in CVC Claim 156 of cleavage in a eukaryotic cell.
24 CVC Opp'n 1 at 24-27. But Sigma Motion 1 repeatedly acknowledges the hypothetical
25 framework that CVC Claim 156 is presumed to be prior art. *See, e.g.,* Sigma Mot. 1 at 7, 19, 21.
26 The twelve concerns identified by Sigma are all premised on that assumption. However, how the

1 prokaryotic-derived Cas9 could interfere with the DDR processes in a eukaryotic environment to
2 achieve the genomic engineering invention, ***as claimed by both parties***, namely, post-cleavage
3 DNA ***re-construction*** by donor integration via HDR, would have been highly unpredictable.

4 **2. In 2012, HDR Was Understood To Be The Disfavored Repair**
5 **Pathway, And HDR-Mediated Donor Integration Was Recognized As**
6 **The Most Challenging Part Of Genome Engineering**

7 CVC argues that "the presence of the template and the existence of DSB was understood
8 to trigger the HDR process." CVC Opp'n 1 at 14. But CVC's greatly oversimplified argument
9 overlooks that, in higher eukaryotic cells, NHEJ is the highly preferred repair pathway over HDR
10 at least because: (1) NHEJ is active throughout the cell cycle, while HDR is only available
11 during the late S and G2 phases in dividing cells; (2) NHEJ is faster and more efficient than
12 HDR; (3) components of the DDR process act to promote NHEJ and suppress HDR, such as
13 through the activity of the Ku protein; and (4) HDR is in intense competition with other repair
14 mechanisms. Ex. 2632 (Kass (2010)) at 3703 ("NHEJ is an efficient pathway that functions
15 throughout the cell cycle . . . while HR [occurs] specifically in late S and G2 phases of the cell
16 cycle . . . ."); *id.* at 3704-05 ("The first protein in [the NHEJ] pathway to bind DNA ends is [Ku].
17 . . . [S]ince Ku binds DNA ends, it presumably physically blocks access of the end resection
18 machinery."); Ex. 1032 (Giglia-Mari (2011)) at 5 (NHEJ "very rapidly seals DSBs"); Ex. 1025
19 (Ceccia (2010)) at 183 ("Alternatively to NHEJ, MRN . . . in competition with Ku, mediates the
20 initial stages of DSB resection . . . to promote homologous recombination in S and G2."); Ex.
21 1035 (Grabarz (2012)) at 249 ("[C]ontrolling the initial events of DSB repair is thus an essential
22 step that may be irreversible . . . ."); *id.* at 255 ("HR is tightly constrained."); *id.* at 261 ("[I]n G2,
23 despite the presence of sister chromatids, NHEJ is the first mechanism to act. . . ."); Ex. 1185
24 (Peng (2015)) at 1227 (NHEJ and HDR "are intensely competitive."); *see* Ex. 1184 (Paull
25 (2021)) at 56 ("In . . . budding and fission yeasts . . . Ku is expressed at much lower levels

1 compared to mammalian cells."); Ex. 1181 (Hussmann (2021)) at 5653 ("[D]escriptions of DSB
2 repair as decision trees can oversimplify a complex mechanistic landscape and mask gaps and
3 ambiguities that remain in our understanding."). Indeed, CVC recognized in CVC P3 that "DNA
4 repair ***by NHEJ*** is typically induced by a DSB . . . ." Ex. 1013 at [00418] (emphasis added).

5 In the natural cellular environment, the sister chromatid, which is only available during
6 S/G2 phases, is the primary repair template for HDR. Ex. 2632 (Kass (2010)) at 3704 ("[T]he
7 primary repair template in mammalian cells is the sister chromatid, which is not present in G1
8 cells."). Thus, a POSITA would have known that a donor template with homologous sequences
9 would have to compete with the sister chromatid as the repair template, and the sister chromatid
10 would often have an advantage because it is held in close proximity. *See* Ex. 2620 (Moynahan
11 (2010)) at 199 ("It is perhaps not surprising that inter-homologue HR is so much less efficient, as
12 sister chromatids are held in proximity by cohesion, whereas homologues are more distant from
13 each other in the nuclear volume."). Accordingly, artificial donor integration via HDR is even
14 less likely to occur than natural HDR, which was known to occur at extremely low frequency.
15 *See* Ex. 1188 (Yang (2020)) at 3 ("[T]he frequency of HDR in nature is extremely low[.]").

16 Citing examples of donor integration by HDR in TMZs, CVC also argues that "[a]
17 complete or comprehensive understanding of the mechanism for HDR was unnecessary to
18 exploit the process for the purpose of integrating a donor sequence at the site of a DSB made by
19 a targeted nuclease." CVC Opp'n 1 at 14-15. CVC's argument again greatly oversimplifies the
20 very complicated and not-well-understood DSB repair pathways and ignores factors that would
21 impact repair pathway choice. Ex. 1182 (Millanowska (2011)) at 1-2 ("[I]t is not surprising that
22 DNA repair is a very complicated process, involving many factors. For instance to date, 168
23 genes encoding proteins involved in DNA repair have been identified in the human genome.");
24 Ex. 1032 (Giglia-Mari (2011)) at 1 ("A complicated and entangled network of DNA damage

1 response (DDR) mechanisms, including multiple DNA repair path ways, damage tolerance
2 processes, and cell-cycle checkpoints safeguard genomic integrity."). In December 2012, it was
3 understood that the eukaryotic DDR processes were highly complex and regulated by numerous
4 cellular enzymes. Ex. 1066 (Thompson (2012)) at 158 ("[T]he elimination of double-strand
5 breaks with minimal nucleotide sequence change involves the spatiotemporal orchestration of a
6 seemingly endless number of proteins . . . ."). And it was also understood that interference with
7 ***any*** of those eukaryotic repair enzymes and signal transducers (potentially such as by the
8 introduction of a prokaryote-derived enzyme) could interfere with the cells DDR:

> 9 DNA repair is carried out by a plethora of enzymatic activities that chemically
> 10 modify DNA to repair DNA damage, including nucleases, helicases, polymerases,
> 11 topoisomerases, recombinases, ligases, glycosylases, demethylases, kinases, and
> 12 phosphatases. These repair tools must be precisely regulated, because ***each in its***
> 13 ***own right can wreak havoc on the integrity of DNA if misused or allowed to***
> 14 ***access DNA at the inappropriate time or place***.

15 Ex. 1025 (Ciccia (2010)) at 179-180 (emphasis added).

16 CVC also argues that techniques for donor integration via HDR were "routine" in 2012.
17 CVC Opp'n 1 at 15-16. But CVC's argument is directed to enablement, which is not at issue in
18 this motion. In that context, as Dr. Cannon explained, "pretty much everything in all gene
19 editing patents is using techniques that are in routine use." Ex. 2616 (Cannon Tr.) at 103:9-12.
20 The use of routine techniques for donor integration has little relevance to determining whether, *a*
21 *priori*, donor integration via HDR would have been reasonably expected to be successful.

22 CVC further argues that "there were predictable and reliable ways [] to ascertain whether
23 or not HDR-mediated donor integration had occurred . . . ." CVC Opp'n 1 at 24-25. But having
24 predictable ways to ascertain whether HDR ***had occurred*** following an experiment is not
25 relevant to predicting ***beforehand*** whether HDR would work with CRISPR-Cas9 created DSBs.
26 As Dr. Cannon explained, "[homology-directed repair] really wasn't . . . a trivial and obvious

1 technique. It was something that was, certainly in 2012, recognized as . . . the most challenging
2 part of gene editing." Ex. 2616 (Cannon Tr.) at 104:11 – 105:1. Dr. Cannon further explained
3 that, in 2012, it was known that introducing a donor template into a cell for HDR often led to
4 toxicity and many donor templates did not work. *Id*. at 104:11-17. Improving homology-
5 directed editing was and remains "an area of intense research." *Id*. at 105:2-11; *see* Ex. 1181.1
6 (Hussmann (2021)) at 5653 ("Limitations in our understanding of DSB repair are due in part to
7 technical challenges associated with profiling the determinants of repair outcomes.").

8 9 **3. Single-Cell Yeast Fungus Relies On Different Repair Factors And Is Not Representative Of Eukaryotes**

10 CVC argues that "ZFNs and TALENs stimulat[e] HDR-mediated donor integration in
11 yeast." CVC Opp'n 1 at 25. However, it was known that single-cell yeast fungi process DSBs
12 differently than higher eukaryotes. Ex. 1187 (Stark (2004)) at 9306 ("[W]e provide evidence
13 that mammalian RAD52 promotes SSA but not HDR, highlighting a striking difference between
14 yeast and mammalian cells in the reliance on particular repair factors."); Ex. 1080 (Hiom (2010))
15 at 1260 ("Why vertebrates [compared to yeast] have an extra level of regulation is a question of
16 considerable interest."); Ex. 1183 (Moertle (2008)) at 1894 ("Yeast cells preferentially employ
17 HDR, whereas in mammalian cells NHEJ is considered to be the major pathway of DSB
18 repair."). Indeed, Dr. Cannon doubted that yeast would be a good system to study HDR of
19 CRISPR-Cas9 induced DSBs because in yeast "[HDR] is efficient in the absence of a DNA
20 break." Ex. 2616 at 126:8-14. Thus, HDR-mediated donor integration in yeast was not
21 predictive of whether HDR-mediated donor integration would work in eukaryotes generally. *See*
22 *In re Vaeck*, 947 F.2d 488, 496 (Fed. Cir. 1991) ("[I]n cases involving unpredictable factors,
23 such as most chemical reactions and physiological activity . . . it is not obvious from the
24 disclosure of one species, what other species will work.") (citing MPEP § 2164.03).

1 2 **4. Blunt Ends Are Preferred By NHEJ Over HDR And Can Inhibit The HDR Pathway**

3 Unlike the overhang DSBs produced by TMZs, it was understood that the DSBs
4 produced by CRISPR-Cas9 have blunt ends. Ex. 2031 (Jinek (2012) at 816 ("Plasmid DNA
5 cleavage produced blunt ends at a position three base pairs upstream of the PAM sequence."));
6 Ex. 2616 (Cannon Tr.) at 118:16-18 ("I think contemporaneous opinions and discussion of
7 CRISPR-Cas9 in 2012 was always [] that this was an enzyme that produced blunt ends."); Ex.
8 2619 ¶ 63 ("I-SceI meganuclease was known to create a DSB with a short 3′ overhang of
9 nucleotides, whereas the *Fok*I nucleases (ZFNs and TALENs) were known to create a DSB with
10 a 5′ overhang of nucleotides."). Thus, it was uncertain whether the DSBs created by CRISPR-
11 Cas9 would be effectively resected by the complex DDR processes to support HDR. *See id.* ¶ 60
12 (describing end resection as the first step of HDR-mediated donor integration); Ex. 1001 ¶ 133.

13 Indeed, it was known that Ku promotes NHEJ over HDR because Ku binding to the DNA
14 ends initiates NHEJ pathway and protects cleaved DNA from end resection, which is the first
15 step for HDR. Ex. 1025 (Ciccia (2010)) at 183 ("Double-strand DNA breaks are rapidly bound
16 by [Ku]. . . . Ku localizes ***within seconds*** to DSBs, where it . . . initiate[s] NHEJ.") (emphasis
17 added); Ex. 2632 (Kass (2010)) at 3704 ("The first protein in [NHEJ] pathway to bind DNA ends
18 is [Ku]."); Ex. 1179 (Foster (2011)) at 4387 ("The function of Ku in this regard is not simply to
19 promote NHEJ over HDR . . . DSB end binding by [Ku] influences mitotic DNA repair by
20 limiting access of ExoI for resection."); Ex. 1180 (Hiom (2010)) at 1258 ("Ku heterodimers . . .
21 [are] particularly important for pairs of broken ends that are less able to associate, for example
22 blunt ends . . . ."). Further, it was also known that Ku prefers blunt ends over overhangs. Ex.
23 1179 (Foster (2011)) at 4387 ("Ku has a ***significantly higher affinity for blunt dsDNA ends*** over
24 short ssDNA overhangs.") (emphasis added). Thus, a POSITA would have understood that

1 blunt-ended DSBs created by CRISPR-Cas9 would likely be repaired by NHEJ, not HDR.

2 CVC argues that Jinek (2012) discloses "some blunt *and some with overhangs*." CVC
3 Opp'n 1 at 27 (emphasis in original). However, CVC ignores Jinek (2012)'s express teaching
4 that CRISPR-Cas9 produces blunt ends, and that the overhangs observed in Fig. S4B are the
5 result of subsequent trimming. Ex. 2031 (Jinek (2012)) at 816 ("subsequently trimmed").
6 Indeed, later work of the CVC inventors further confirms that it was their belief in 2012 that
7 CRISPR-Cas9 creates blunt ends. Ex. 2010 (CVC P2) at Fig. 4 (showing blunt ends after
8 cleavage); Ex. 1178 (Doudna (2014)) at 1077 ("Cas9-sgRNA–mediated DNA cleavage produces
9 a blunt double-stranded break . . . ."); *id.* at Figs 2C and 4 (showing blunt ends after cleavage).
10 CVC also argues that "[i]t was also known that resection functions on blunt ends." CVC Opp'n
11 1 at 27. However, the passage quoted by CVC is about ends blocked with covalent adducts (a
12 cancer-causing chemical). Ex. 2633 (Nimonkar (2011)) at 359 ("[T]he resection activity of
13 MRN and CtIP is especially critical when the ends are blocked with covalent adducts.").

14 **5. CVC's Reliance On Spo11, I-SceI, And ZFNs Supports The Concern**
15 **That By Remaining Bound To The Cleaved DNA Ends, Cas9 Could**
16 **Preclude HDR**

17 It was known in 2012 that Cas9-crRNA complex would remain bound to the ends of the
18 cleaved DNA strands after cleavage, which could block the operation of the repair machinery.
19 Ex. 2640 (Gasiunas (2012)) at E2584 ("Interestingly, after DNA cleavage, Cas9-crRNA remains
20 bound to the reaction products (Fig. S11)."); Ex. 1001 ¶ 133; Ex. 2616 (Cannon Tr.) at 92:18-22
21 ("[G]oing back to 2012 . . . it was known that Cas9 stayed attached to the DNA. And that would
22 bring up to me a lot of questions about whether that in some way inhibited the stages needed for
23 homologous recombination."); *id.* at 92:23 – 93:6 ("[R]esection . . . would be blocked by . . . the
24 large Cas9 protein sticking on the DNA ends."). CVC again attempts to change the express
25 teaching of Gasiunas (2012) by offering recent testimony from Dr. Barrangou. CVC Opp'n 1 at

1 28. However, it is well established that contemporaneous statements are stronger evidence than
2 statements made in the context of litigation. Ex. 1175 (Decision on Motions ('115 Int'f)) at 14.
3 CVC also argues that "the skilled artisan knew that HDR-mediated donor integration
4 occurs even when a nuclease *remains covalently bound* at the site of the DSB, as is the case with
5 the protein Spo11." CVC Opp'n 1 at 29. However, Spo11 is involved in meiotic recombination,
6 ***not*** HDR integration in mitosis, and after Spo11 cleavage, MRN nicks behind the Spo11
7 cleavage site to create new sites that will be used for meiotic recombination. Ex. 2643 (Neale
8 (2005)) at Fig. 4 (*see* Ex. 2619 at 32 for the annotated version); Ex. 1172 (Doyon Tr.) at 399:12
9 – 400:6, 401:1 – 403:9. Thus, because of Spo11's attachment, alternate free DNA ends are
10 created by MRN nicking to support the subsequent steps of resection and strand invasion. *Id.*
11 CVC also asserts that I-SceI and ZFNs were known to remain bound to the ends of the
12 DSBs. CVC Opp'n 1 at 29. Unlike CRISPR-Cas9, however, I-SceI creates 3′ overhangs, which
13 are preferred for HDR. *See* Ex. 2619 ¶ 60 (describing the first step of HDR as "[e]xonucleases
14 resect the ends of the DSB, *i.e.* remove nucleotides, to create an exposed stretch of single-
15 stranded DNA in the form of a 3′ overhang."). In addition, I-SceI was known to bind only at one
16 end. Ex. 2645 (Perrin (1993)) at 2942 ("This is consistent with the idea that the right hand
17 product of the reaction remains bound to [I-SceI] after cleavage."). Further, CVC's reliance on
18 Young (2000) does not support CVC's assertion that ZFNs were known to remain bound at the
19 ends of DSBs. That study examined derivatives of the natural two zinc finger peptide
20 transcription factor, ADR1, and measured the dissociation rate of ADR1. Ex. 2641 (Young
21 (2000)) at 567. That analysis of the transcription factor ADR1 is not the same as measuring
22 DNA binding or dissociation in the context of an actual ZFN. *See* Ex. 1080 ¶ 33.

23 24 **6. Predicted CRISPR-Cas9 Off-Target Cleavages Could Trigger Cell Cycle Arrest And Inhibit HDR**

1 In 2012, CRISPR-Cas9 was known to tolerate sequence mismatches between gRNA and
2 target DNA. Ex. 1038 (Jinek (2012)) at 4 ("[U]p to six contiguous mismatches in the 5′-terminal
3 region of the protospacer are tolerated (Fig. 3E)."); *id.* at Fig. 3E (showing mismatches were
4 tolerated). These mismatches would lead to off-target effects and be of particular concern in
5 eukaryotes because of their large genome size. Ex. 1080 ¶ 48. Further, a POSITA would not
6 have known whether a long guide sequence would mitigate the off-target problems because, in
7 2012, the extent of tolerable mismatches was unknown. *Id.* The PAM sequence NGG occurs
8 frequently and therefore may not be sufficient to lessen the off-targeting problems. Ex. 1084
9 (Doyon Tr.) at 215:13-18 ("[I]n general, . . . [NGG] would be present approximately every eight
10 base pair."). Indeed, Dr. Carroll was concerned with this issue and agreed that "initial studies
11 indicate that this is a more significant problem [for CRISPR-Cas9] than it is for TALENs as
12 mismatches near the 5′ end of the guide RNA are rather well tolerated." Ex. 1171 (Carroll Tr.) at
13 75:3-14. Thus, a POSITA would have been concerned that CRISPR-Cas9 cleavage in eukaryotic
14 cells might not be specific enough to support HDR. Ex. 1080 ¶ 48. And off-target effects could
15 also lead to cell arrest that prevents cell cycle progression. *Id.* It was known that "very few or
16 even a single unrepaired DSB can be sufficient to trigger p53-dependent G1 arrest in human
17 cells." Ex. 1054 (Polo (2011)) at 422. Because HDR is only available during late S/G2 phases,
18 even a low level of off-target activity could inhibit donor integration via HDR.

19 **7. Chromatin Poses A Separate Concern For Impairing Eukaryotic**
20 **DNA Damage Repair Processes, Particularly HDR**

21 CVC argues that because CVC Claim 156 assumes that CRISPR-Cas9 is capable of
22 cleaving eukaryotic DNA, concerns over chromatin are "irrelevant." CVC Opp'n 1 at 26. But
23 beyond cleavage, chromatin presents separate concerns for DNA repair, including HDR. Ex.
24 1180 (Hiom (2010)) at 1261 ("Consequently, the detection, processing and mechanics of [DSB]

1 repair require the manipulation of DNA within a chromatin context. The details of how this
2 occurs are not well understood[.]"); Ex. 1035 (Grabarz (2012)) at 261 ("These data further
3 underline the important role of the chromatin environment during DSB repair and adds another
4 level of regulation of HR events."); Ex. 1032 (Giglia-Mari (2011) at 6 ("The nucleus is highly
5 structured and functionally compartmentalized in part due to areas of various degrees of
6 chromatin compaction, creating possible obstacles for DDR factor accessibility.").

7 **8. Post-2012 Publications Continue To Demonstrate That Donor**
8 **Integration Via HDR Following CRISPR-Cas9 Cleavage Remain**
9 **Challenging And Unpredictable**

10 CVC argues that "[t]he dearth of evidence supporting these concerns confirms that they
11 are post-hoc litigation positions and not a reflection of what a skilled artisan would have thought
12 as of December 6, 2012." CVC Opp'n 1 at 22. But CVC ignores that the first time CRISPR-
13 Cas9 was even shown to cleave DNA in an extra-cellular test tube environment was June 28,
14 2012. Ex. 2031 (Jinek (2012)) at 816. Indeed, by December 6, 2012, not a ***single*** publication
15 had shown whether any CRISPR-Cas9-mediated DSB could be repaired by ***any*** DDR
16 mechanism. Ex. 1172 (Doyon Tr.) at 456:17 – 457:1. Thus, it is not surprising that less than six
17 months after Jinek (2012) there was little evidence regarding the fledgling CRISPR-Cas9 system
18 at all, let alone evidence reporting concerns a POSITA would have had about using that system
19 for donor integration via HDR. Indeed, a large body of literature has since confirmed many of
20 the concerns identified in Sigma Motion 1. *See* Sigma Opp'n Resp. Mot. 1 at 10-24; Ex. 1080
21 ¶¶ 58-75; *see also, e.g.*, Ex. 1177 (Devkota (2016)) at 439 ("[T]aming endogenous cellular HDR
22 machinery to insert a desired DNA sequence has proven to be a tricky business . . . .").

23 **B. A POSITA Could Not Have Predicted Whether Cleavage By CRISPR-Cas9**
24 **Would Support HDR Based On Prior Experience With Other Targeted**
25 **Nucleases Because Of The Fundamental Differences In Those Systems**

26 CVC argues that because HDR-mediated donor integration after DSBs created by TMZs

1 were achieved before 2012, a POSITA would have reasonably expected that donor integration
2 via HDR would be successful in CRISPR-Cas9 cleaved DSBs. CVC Opp'n 1 at 16-21. CVC
3 also argues that because concerns 1-5 apply to "all targeted nucleases" and HDR-mediated donor
4 integration was routinely achieved in DSBs created by TMZs, a POSITA "would not reasonably
5 have expected the so-called concerns to be impediments to using CRISPR-Cas9 to induce HDR-
6 mediated donor integration in a eukaryotic cell." *Id.* at 24. But as explained in more detail
7 below, CVC's greatly oversimplified argument ignores the crucial differences between DSBs
8 created by CRISPR-Cas9 and DSBs created by TMZs, and relies on impermissible hindsight.

9 **1. After Demonstrating Cleavage, It Took Several Years To**
10 **Demonstrate HDR-Mediated Integration Using ZFNs And TALENs**

11 CVC completely ignores the timeline and history of the development of ZFNs and
12 TALENs. Both ZFNs and TALENs are engineered nuclease systems that comprise a DNA
13 binding domain that naturally recognizes eukaryotic DNA, linked to an independent DNA
14 cleavage domain. Ex. 1080 ¶¶ 29-35. In both of these protein-guided systems, DNA cleavage is
15 achieved by the *Fok*I endonuclease. *Id.* ZFNs were demonstrated to cleave DNA in a sequence-
16 specific manner *in vitro* by 1996. Ex. 2325 (Kim (1996)) at abstract; Ex. 1172 (Doyon Tr.) at
17 358:12 – 359:14. In 2001, one group reported cleavage and deletion of intervening sequences
18 from plasmids containing two copies of the DNA recognition sequence for the QQR ZFN when
19 injected into *Xenopus* oocytes. Ex. 2308 (Bibikova (2001)) at 289-290. And it took
20 investigators two more years to demonstrate cleavage plus donor integration by HDR into
21 chromosomal DNA in eukaryotic cells with even previously described ZFN reagents. Ex. 1018
22 (Bibikova (2003) (in Drosophila using a target site in the *yellow* gene)); Ex. 1055 (Porteus
23 (2003) (in human cells using target sites for QQR)). Similarly for TALENs, roughly a two-year
24 gap intervened between the initial report that targeted breaks were possible to achieve *in vivo* in

1 eukaryotic cells (Ex. 1019 (Boch (2009))), and the subsequent demonstration of HDR-mediated
2 integration (Ex. 1036 (Hockemeyer (2011)); Ex. 1050 (Miller (2011))). Ex. 1001 ¶ 147. Thus, a
3 POSITA would have known that HDR was far more difficult and challenging than cleavage
4 alone. These timelines reveal that HDR is very unpredictable, and does not automatically occur
5 after a DSB is created and a donor template is provided. Ex. 2616 (Cannon Tr.) at 46:9-21.

6 7 8 **2. A POSITA Would Have Been Concerned That CRISPR-Cas9 Would Interfere With The Eukaryotic DNA Damage Repair Processes, Especially HDR**

9 CVC also fails to consider the whether the differences between CRISPR-Cas9 and TMZs
10 would affect the very complex and highly regulated DNA repair pathways in eukaryotic cells.
11 For example, the donor template must be delivered along with targeted nucleases, and be present
12 at the cleavage sites when the repair is occurring. TMZs are proteins while CRISPR-Cas9 is an
13 RNA-guided protein complex. Ex. 1080 ¶ 35; Ex. 1062 (Silva (2011)) at 14. Thus, merely
14 based on the experience with TMZs, a POSITA would not have known whether a donor template
15 would be properly delivered and located with a CRISPR-Cas9 complex. Ex. 1001 ¶ 117.

16 17 18 **3. Carroll (2012) and Barrangou (2012) Show Skepticism Regarding Whether HDR Would Have Been Induced By A DSB Created By The CRISPR-Cas9 System**

19 CVC claims that "contemporaneous evidence [] universally predicted that the CRISPR-
20 Cas9 system would be a genome editing tool, including for inducing HDR-mediated donor
21 integration." CVC Opp'n 1 at 21-22 (citing to recent testimony from Dr. Carroll and Dr.
22 Barrangou)). This retrospective and compensated testimony is flatly contradicted by the
23 contemporaneous 2012 evidence. For example, Dr. Carroll, a purported CVC "fact" witness,
24 was originally skeptical about whether a CRISPR-Cas9-created break would be repaired by
25 homologous recombination, including donor integration. In his review article published in
26 September 2012, he placed a conspicuous "**?**" next to "CRISPR" in Figure 1, and stated that

1 "[t]he break can be made by any targetable nuclease: zinc-finger nucleases (ZFNs), transcription
2 activator–like effector nucleases (TALENs), homing endonucleases (HEs), or, ***perhaps***, the new
3 CRISPR reagents." Ex. 2339 at 1659 (emphasis added); Ex. 1171 (Carroll. Tr.) at 40:10 – 42:1.
4 Dr. Carroll observed that "[t]he authors [of Jinek (2012)] make the ***bold prediction*** that [CRISPR
5 -Cas9] can potentially be used in place of ZFNs or TALENs for targeted genomic cleavage in
6 higher organisms." *Id*. (emphasis added). These statements demonstrate that, in September
7 2012, Dr. Carroll—an expert in the field, with considerably ***more*** knowledge and experience
8 than a POSITA—had concerns about whether CRISPR-Cas9 would work in eukaryotic cells,
9 which includes whether CRISPR-Cas9 would support DDR processes. Likewise, Dr. Barrangou
10 expressed similar skepticism in 2012. Ex. 2215 (Barrangou (2012)) at 838 ("[T]here are
11 ***intriguing possibilities*** for genome editing and genome engineering of eukaryotes. This will
12 ***require testing*** whether crRNA-Cas systems can efficiently cleave chromatin DNA in vivo . . .
13 .") (emphases added). Thus, Dr. Carroll's and Dr. Barrangou's 2012 publications do ***not*** show
14 that CRISPR-Cas9 was expected to support HDR integration in eukaryotic cells.

15 **C. Spacer Integration, Which Is Part Of CRISPR-Cas9 Mediated Bacterial**
16 **Immunity, Was Known To Require Additional Components And Is Not**
17 **Involved In Mechanisms Similar To HDR**

18 CVC argues that because "it was [] known in the context of bacterial immunity that Cas9
19 was required both for cleavage and the recombinational integration of spacer sequences sampled
20 from invading DNA into the bacterial chromosome . . . CRISPR-Cas9 was assumed to be
21 compatible with mechanisms similar to HDR." CVC Opp'n 1 at 23. CVC is incorrect for at
22 least three reasons. First, the mechanism of spacer integration was unknown in 2012. Ex. 2134
23 (Wiedenheft (2012)) at 332 ("[T]he mechanism of spacer integration and replication of the repeat
24 sequence is still unknown . . . ."); Ex. 1173 (Barrangou Tr.) at 154:7-13 (in agreement). Second,
25 Csn2 (Cas7), Cas1 and Cas2 were known to be involved in spacer acquisition. Ex. 2134

1 (Wiedenheft (2012)) at 332 ("Mutational analysis of the cas genes in *S. thermophilus*
2 demonstrated that csn2 (previously known as cas7) is required for new spacer sequence
3 acquisition. . . . [Other] genetic studies suggest a role for Cas1 and Cas2 in the integration of
4 foreign DNA into the CRISPR."); Ex. 1173 (Barrangou Tr.) at 154:7-13 (in agreement). Thus, a
5 POSITA would have known that Cas9 might have played a role in, but likely would be
6 insufficient alone for, spacer integration. *See* Ex. 2128 (Horvath (2010)) at Fig. 2(A) & Ex. 2134
7 (Wiedenheft (2012)) at Fig. 2 (CRISPR-Cas mechanism of action). Third, a POSITA would
8 have understood that spacer insertion would not involve any mechanisms similar to HDR, at
9 least because there is no homology between the invading nucleic acid and the CRISPR array.
10 *See* Ex. 2128 (Horvath (2010)) at Fig. 2(A); Ex. 2134 (Wiedenheft (2012)) at Fig. 2. Thus, a
11 POSITA would have understood that spacer integration was not analogous to HDR integration,
12 and would not have assumed CRISPR-Cas9 to be compatible with mechanisms similar to HDR.

13 **III. CVC DOES NOT DISPUTE THAT ALL OF SIGMA'S INVOLVED CLAIMS**
14 **CORRESPOND TO PROPOSED COUNT 2**

15 In its Opposition 1, CVC nowhere disputes Sigma's showing that all of Sigma's involved
16 claims correspond to Proposed Count 2. Sigma Mot. 1 at 24-25.

17 **IV. CVC PROVIDES NO SUBSTANTIVE ANALYSIS DISPUTING SIGMA'S**
18 **ASSESSMENT OF THE CORRESPONDENCE OF CVC'S INVOLVED CLAIMS**

19 In its opposition, CVC complains about Sigma's analysis showing the correspondence of
20 CVC's involved claims to Proposed Count 2. CVC Opp'n 1 at 9-11. But, as Sigma indicated in
21 its Motion 1, because "CVC has not contested that all of [CVC's involved] claims are obvious in
22 view of current Count 1, . . . the Board could reasonably designate all of CVC's currently
23 involved claims as corresponding to the proposed Count 2." Sigma Mot. 1 at 25. In any event,
24 in Sigma Motion 1, Sigma engaged in a detailed analysis of all 412 of CVC's involved claims in
25 all fourteen of CVC's involved applications, as well as the hundreds of additional claims in

1 CVC's extensive family of uninvolved cases. *Id.* at 25-29. At a minimum, that analysis sets
2 forth a *prima facie* showing of claim correspondence. *Id.* And CVC nowhere explains ***why*** any
3 of that analysis is somehow deficient, nor does CVC offer ***any*** rebuttal to that analysis (*i.e.*, why
4 any involved CVC claim would be, or would not be, obvious in view of Proposed Count 2).

5 **V. CVC SETS FORTH NO SUBSTANTIVE CHALLENGE TO SIGMA'S**
6 **ENTITLEMENT TO THE BENEFIT OF SIGMA P1 FOR PROPOSED COUNT 2**

7 In its opposition, CVC complains about Sigma's demonstration of its entitlement to
8 Sigma P1 for Proposed Count 2. CVC Opp'n 1 at 30-31. But Sigma Motion 1 sets forth a *prima*
9 *facie* showing of entitlement to that benefit. Ex. 1001 ¶¶ 63-99. And CVC nowhere explains
10 ***any*** purported deficiency, or offers any rebuttals to any claim limitations purportedly not shown.

11 **VI. CVC FAILS TO SUBSTANTIVELY REBUT THAT CVC IS NOT ENTITLED TO**
12 **THE BENEFIT OF CVC P3 OR CVC P4 FOR PROPOSED COUNT 2**

13 In Opposition 1, CVC argues that it is entitled to the benefit of CVC P3 for Proposed
14 Count 2. CVC Opp'n 1 at 31-32. But CVC's arguments fail to provide any substantive ***evidence***
15 that the CVC inventors actually possessed the invention of Proposed Count 2. CVC relies on the
16 basic textbook and boilerplate disclosures in CVC P3 regarding HDR, but fails to address the
17 glaring omission of any discussion of a donor or integration of any kind in CVC's experimental
18 endeavors. Dr. Cannon's thoughtful analysis explains why CVC P3 (and CVC P4) do not show
19 that the CVC inventors demonstrated possession of donor integration. Ex. 1001 ¶¶ 50-58.
20 Indeed, when questioned in this regard, Dr. Cannon elaborated further on this issue:

21 [T]here's no [] examples, . . . no discussions of what, if anything, might be a
22 consideration for doing this in the situation that's described in, for example, CVC
23 Claim 164. So I think [] it's more than just the lack of a working example. It's
24 the fact that the language is very generic and [] not really teaching me how to []
25 do that and [] what tricks I might need to use, what to, [] watch out for[.]
26 * * *
27 [P]utting myself in the mind of a person of ordinary skill in the art in 2012 would
28 have had, I find that there was inadequate guidance in P3 to help me do that
29 without [] having to make the invention myself and do that.

1 Ex. 2616 (Cannon Tr.) at 85:16-25, 86:13-17.

2 VII. CVC'S POINTING TO PRIOR ART OF RECORD FAILS TO REBUT SIGMA'S 3 *PRIMA FACIE* SHOWING THAT PROPOSED COUNT 2 IS PATENTABLE

4 CVC questions whether Proposed Count 2 is patentable, premising its arguments solely
5 on the prior art of record in the prosecution of Sigma's involved '204 application, namely the
6 Kim references and Sun (2012). Opp'n 1 at 12-13. But those references were cited by the
7 Examiner during prosecution, and were successfully distinguished by Sigma in extensive
8 dialogues with the USPTO. Ex. 1174. *See* Ex. 1174, *viz.*, Ex. 1174.19 at 5245, Ex. 1174.13 at
9 3715 (Examiner's citations of Sun (2012)); Ex. 1174.9 at 2839-2844, Ex. 1174.7 at 2293-2294
10 ¶¶ 163-167, Ex. 1174.5 at 1707-1712; Ex. 1174.2 at 439-440 ¶¶ 163-167 (Sigma's analyses of
11 Sun (2012)); Ex. 1174.1 at 34 (Examiner's indication of allowability); *see also* Ex. 1174.19 at
12 5225-5234 (Examiner's citation of the Kim references); Ex. 1174.13 at 4169 ¶ 9, Ex. 1174.14 at
13 4226 ¶ 9 (Sigma 131 Declarations, which are wholly consistent with Sigma's Priority Statement.
14 Priority Statement ¶ 3 (Paper 479) (filed under seal Nov. 19, 2021)); Ex. 1174.13 at 3789 (the
15 USPTO's determination that the Examiner was required to consider the declarations); *id.* at 3782
16 (the Examiner's acknowledgement that 131 Declarations "will be considered on the merits to
17 determine whether they are sufficient to establish invention of the subject matter of the rejected
18 claims prior to the effective date of [the Kim references]"); Ex. 1174.13 at 3680 ("The
19 Declarations filed on 10/17/2017 under 37 CFR 1.131(a) are sufficient to overcome the Kim
20 references . . . ."). Thus, contrary to CVC's arguments, the prosecution history of Sigma's '204
21 application leaves no "lingering question or doubt" of the patentability of Claim 31 (Sigma's half
22 of Proposed Count 2) over the cited references, including Sun (2012) and the Kim references.

23 VIII. CVC'S PURPORTED BEST PROOFS FOR A CLEAVAGE ONLY INVENTION 24 ARE WHOLLY IRRELEVANT TO THIS MOTION

25 CVC's purported "best proofs" are directed to current Count 1, not Proposed Count 2.

1 CVC Opp'n 1 at 11-12. While a party's arguments directed to its purported "best proofs" could
2 be relevant to evaluating the scope of a particular count for a ***common patentable invention***,
3 such arguments completely miss the boat here. Indeed, CVC nowhere even suggests that its best
4 proofs to the parties' commonly claimed ***cleavage plus integration invention*** would counsel for
5 an alternative formulation of Proposed Count 2 (other than simply keeping current Count 1).

6 IX. CVC'S ARGUMENTS THAT SIGMA IS SEEKING AN INTERFERENCE 7 DIRECTED TO "GENERIC CLEAVAGE" ARE NONSENSICAL

8 In its opposition, CVC argues that "Sigma is not entitled to an interference that involves
9 claims to generic cleavage." CVC Opp'n 1 at 6. But Proposed Count 2 does not involve claims
10 to so-called "generic cleavage." Indeed, the entire objective of this motion is to change CVC's
11 half of the count from "cleavage only" (Claim 156) to "cleavage plus integration" (Claim 164),
12 thereby mirroring Sigma's "cleavage plus integration" half of the count in this respect. Thus,
13 substituting Proposed Count 2 for current Count 1 would ***not*** result in Sigma obtaining any
14 cleavage only claims, nor Sigma obtaining priority of invention to any cleavage only invention.

15 X. CVC'S INOPPORTUNE PROPOSAL FOR THE BOARD TO RENDER 16 JUDGMENT ON COUNT 1 SHOULD BE DISREGARDED

17 CVC argues in its Opposition 1 that at this juncture in the interference, the Board should
18 render judgment on current Count 1. CVC Opp'n 1 at 30-32. Such an inopportune proposal
19 ignores both the fundamental nature and basic procedures of interference practice, in which the
20 contested count is determined in the motions phase, and thereafter priority of invention—based
21 on that count—is determined in the priority phase. This motion addresses the former, and the
22 parties will have an opportunity to address the latter in the next phase of the interference.

23 XI. CVC'S PROPOSAL OF A TWO-COUNT INTERFERENCE IGNORES SIGMA'S 24 RATIONAL EXPLANATION THAT A FIRST CLEAVAGE-ONLY COUNT 25 WOULD HAVE NO CORRESPONDING SIGMA CLAIMS

26 In its opposition, CVC correctly states that when there are "two patentably distinct

1 inventions . . . the remedy would be a *two-count* interference." CVC Opp'n 1 at 6-7, 9. But
2 CVC ignores Sigma's entirely logical explanation regarding why Sigma Motion 1 seeks only a
3 single count, namely, because "Sigma would have no interfering subject matter corresponding to
4 [a cleavage only count]. Thus this interference would only proceed with a single count, namely,
5 [a cleavage plus integration count] alone." Sigma List of Proposed Motions (Paper 26) at 4-5.

6 XII. CVC'S EXTENSIVE ARGUMENTS REGARDING 37 C.F.R. § 41.202(c) ARE 7 BOTH INCORRECT AND IRRELEVANT HERE

8 CVC's opposition argues about the purported operation of 37 C.F.R. § 41.202(c) in this
9 interference. Opp'n at 7-9. But CVC misleadingly fails to quote the entire rule, which states that
10 "An examiner may ***require*** an applicant to add a claim to provoke an interference. Failure to
11 satisfy the ***requirement*** . . . ." 37 C.F.R. § 41.202(c) (emphasis added). And even CVC
12 acknowledges here that the Examiner only "recommended" a cleavage only claim. CVC Opp'n
13 1 at 8; Ex. 1174.2 at 493, 559. Moreover, CVC ignores Sigma's express explanation at that time
14 that "a restriction requirement between potential CRISPR cleavage-only claims (not presented
15 herein) and its CRISPR cleavage + donor sequence integration claims (as presented herein)
16 would potentially be necessary if both types of claims were presented in this single application."
17 *Id.* at 494. In any event, this issue is entirely irrelevant to this interference because Sigma is not
18 pursuing any cleavage only claims in Sigma's involved '204 application (nor is Sigma currently
19 pursuing any such claims in any other Sigma patent prosecution). And CVC's apparent belief
20 that Sigma could, in this interference, somehow obtain any of ***CVC's*** cleavage only claims
21 represents a fundamental misunderstanding of the relief afforded by the Board in an interference.

22 XIII. CONCLUSION

23 For the reasons set forth above and in Sigma's Motion 1, Sigma requests that the Board
24 substitute Proposed Count 2 for Count 1, and re-declare this interference accordingly.

1 Respectfully submitted,

2

3 Dated: April 7, 2022 By: /*Brenton R. Babcock*/

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APPENDIX 1

LIST OF EXHIBITS CITED

Exhibit No.	Description
1001	Cannon Decl.
1013	CVC P3 - Prov. Appl. No. 61/757,640, filed Jan. 28, 2013
1018	Bibikova (2003)
1019	Boch (2009)
1025	Ceccia (2010)
1032	Giglia-Mari (2011)
1035	Grabarz (2012)
1036	Hockemeyer (2011)
1038	Jinek (2012)
1050	Miller (2011)
1054	Polo (2011)
1055	Porteus (2003)
1062	Silva (2011)
1066	Thompson (2012)
1080	Supp. Cannon Decl.
1084	Doyon Depo. Tr. 02/03/2022
1171	Carroll Depo. Tr. 03/16/2022
1172	Doyon Depo. Tr. 03/22/2022
1173	Barrangou Depo. Tr. 03/24/2022
1174	U.S. Pat. Appl. No. 15/456,204 File History
1175	Decision on Motions ('115 Int'f)
1176	Decision on Priority ('115 Int'f)
1177	Devkota (2016)
1178	Doudna (2014)
1179	Foster (2011)
1180	Hiom (2010)
1181	Hussmann (2021)
1182	Millanowska (2011)
1183	Moertle (2008)
1184	Paull (2021)
1185	Peng (2015)
1187	Stark (2004)
1188	Yang (2020)
2010	CVC P2 - Prov. Appl. No. 61/716,256, filed October 19, 2012
2031	Jinek (2012)
2128	Horvath (2010)
2134	Wiedenheft (2012)
2215	Barrangou (2012)
2308	Bibikova (2001)
2325	Kim (1996)
2339	Carroll (2012)
2616	Cannon Tr. 01/27/2022

Exhibit No.	Description
2619	Third Doyon Decl.
2620	Moynahan (2010)
2632	Kass (2010)
2633	Nimonkar (2011)
2640	Gasiunas (2012)
2641	Young (2000)
2643	Neale (2005)
2645	Perrin (1993)

APPENDIX 2

APPENDIX 2 – STATEMENT OF MATERIAL FACTS

Sigma's Statement of Material Facts

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

Response: Denied.

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

Response: Denied.

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

Response: Denied.

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

Response: Admitted.

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

Response: Denied.

6. Sigma P1 Examples 1-5 (esp. Figures 1-5) adequately describe and enable the successful integration of a donor polynucleotide via HDR into a target DNA site cleaved by a CRISPR- Cas9 system in a eukaryotic cell. *Id.* ¶¶ 41-49, 63-99, & Appx. F-G.

Response: Denied.

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

Response: Denied.

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

Response: Denied.

CVC's Statement of Material Facts

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

Response: Denied.

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

Response: Admitted.

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

Response: Denied.

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

Response: Denied (particularly with respect to "nucleases").

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

Response: Denied.

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

Response: Admitted.

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

Response: Admitted.

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

Response: Denied (particularly with respect to "first step").

17. Before December 6, 2012, it was known in the art that DSBs with a 3' overhang made by I-SceI meganuclease and DSBs with a 5' overhang made by *FokI* (ZFNs and TALENs) induced HDR-mediated donor integration in eukaryotic cells. Ex. 2619, ¶¶ 85, 138; Ex. 1001, ¶ 131; Ex. 2622, 148; Ex. 2646, 1083, Figs. 1A, 2, Tbl. 1; Ex. 2617, ¶¶ 5−12; Ex. 2618, ¶¶ 18−27.

Response: Denied (particularly with respect to "induced").

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

Response: Denied (to the extent "improve" implies predictable success).

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

Response: Denied.

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

Response: Admitted.

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

Response: Admitted.

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

Response: Denied.

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

Response: Denied.

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

Response: Denied.

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

Response: Denied (with respect to the knowledge of a POSITA by Dec. 6, 2012).

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

Response: Admitted.

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

Response: Denied.

28. CVC has submitted to the Board proofs of conception coupled with diligence to actual reductions to practice of cleavage or editing by sgRNA CRISPR-Cas9, by Aug. 9, 2012, in fish, and by Oct. 31, 2012, in human cells; a donor sequence was not added. Ex. 2628; Ex. 2232; Ex. 2233; Ex. 2033; Ex. 2642; Ex. 2399; Ex. 2399; Ex. 2630; Ex. 2652; Ex. 2653; Ex. 2654.

Response: Denied (*see* Ex. 1176).

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

Response: Admitted.

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

Response: Denied.

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

Response: Denied.

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

Response: Admitted.

CERTIFICATE OF FILING AND SERVICE

I hereby certify that:

I. The following paper was filed April 7, 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 REPLY IN SUPPORT OF SIGMA MOTION 1
(to Substitute Proposed Count 2 for Count 1)

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 April 7, 2022, via e-mail:

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