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Before the Patent Trial and Appeal Board

The Regents of the University of California,
University of Vienna, and Emmanuelle Charpentier
Applicants 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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REPLY IN SUPPORT OF SIGMA MOTION 1
(to Substitute Proposed Count 2 for Count 1)

I. INTRODUCTION

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

Contrary to CVC’s assertions, the mere demonstration of non-specific DNA cleavage

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 0.75¹² = 3.2%.
alone—DNA destruction—in a eukaryotic cell (CVC Claim 156) only (partially) addresses the first half of the inquiry, and would not have alleviated a POSITA’s separate concerns directed to the far more complex (and largely uncontrollable) second half of the inquiry, namely, the wholly distinct intracellular process of donor integration via HDR—DNA re-construction. Following cleavage, in 2012 a POSITA could do very little but sit back and hope that HDR might work with this new prokaryotic-derived system. Like many other emergent technical developments in such highly complex and unpredictable biological systems, absent successful testing (or, at a minimum, recognizing these many concerns and explaining how they could be addressed and resolved), mere hope for success is insufficient to demonstrate reasonably expected success.

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

In early December 2012, in the context of the nascent, bacteria-derived CRISPR-Cas9 technology, successfully accomplishing donor integration via HDR in a eukaryotic cell—the pinnacle of genomic engineering DNA re-construction—would not have been obvious in view of simply cleaving a target DNA in a eukaryotic cell—basic DNA destruction. In particular, at that point in time, a POSITA would not have had a reasonable expectation that donor integration via HDR following CRISPR-Cas9 cleavage would be successful. CVC argues that because HDR was a known DNA repair process in eukaryotic cells, and donor integration by HDR had been shown to be possible in eukaryotic cells using DSBs generated by other targeted nucleases, introducing a donor sequence into cells containing DSBs created by CRISPR-Cas9 “would necessarily result in integration by HDR.” CVC Opp’n 1 at 20. But CVC completely ignores that HDR was recognized as the most challenging part of gene editing in 2012, and has remained highly challenging and unpredictable in the decade that followed. See Sigma Mot. 1 at 6-24; Sigma Opp’n Resp. Mot. 1 at 10-24. CVC also ignores the crucial differences between the
nature of the DSBs created by the RNA-guided CRISPR-Cas9 system, and the DSBs created by
the protein-directed TALENs, meganucleases (SceI), and ZFNs (“TMZs”).

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

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

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

CVC argues that the twelve concerns about successful HDR discussed in Sigma Motion 1
were simply resolved by the mere recital in CVC Claim 156 of cleavage in a eukaryotic cell.
CVC Opp’n 1 at 24-27. But Sigma Motion 1 repeatedly acknowledges the hypothetical
framework that CVC Claim 156 is presumed to be prior art. See, e.g., Sigma Mot. 1 at 7, 19, 21.
The twelve concerns identified by Sigma are all premised on that assumption. However, how the
prokaryotic-derived Cas9 could interfere with the DDR processes in a eukaryotic environment to achieve the genomic engineering invention, as claimed by both parties, namely, post-cleavage DNA re-construction by donor integration via HDR, would have been highly unpredictable.

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

CVC argues that “the presence of the template and the existence of DSB was understood to trigger the HDR process.” CVC Opp’n 1 at 14. But CVC’s greatly oversimplified argument overlooks that, in higher eukaryotic cells, NHEJ is the highly preferred repair pathway over HDR at least because: (1) NHEJ is active throughout the cell cycle, while HDR is only available during the late S and G2 phases in dividing cells; (2) NHEJ is faster and more efficient than HDR; (3) components of the DDR process act to promote NHEJ and suppress HDR, such as through the activity of the Ku protein; and (4) HDR is in intense competition with other repair mechanisms. Ex. 2632 (Kass (2010)) at 3703 (“NHEJ is an efficient pathway that functions throughout the cell cycle . . . while HR [occurs] specifically in late S and G2 phases of the cell cycle . . . .”); id. at 3704-05 (“The first protein in [the NHEJ] pathway to bind DNA ends is [Ku]. . . . [S]ince Ku binds DNA ends, it presumably physically blocks access of the end resection machinery.”); Ex. 1032 (Giglia-Mari (2011)) at 5 (NHEJ “very rapidly seals DSBs”); Ex. 1025 (Ceccia (2010)) at 183 (“Alternatively to NHEJ, MRN . . . in competition with Ku, mediates the initial stages of DSB resection . . . to promote homologous recombination in S and G2.”); Ex. 1035 (Grabarz (2012)) at 249 (“[C]ontrolling the initial events of DSB repair is thus an essential step that may be irreversible . . . .”); id. at 255 (“HR is tightly constrained.”); id. at 261 (“[I]n G2, despite the presence of sister chromatids, NHEJ is the first mechanism to act. . . .”); Ex. 1185 (Peng (2015)) at 1227 (NHEJ and HDR “are intensely competitive.”); see Ex. 1184 (Paull (2021)) at 56 (“In . . . budding and fission yeasts . . . Ku is expressed at much lower levels
compared to mammalian cells.”); Ex. 1181 (Hussmann (2021)) at 5653 (“[D]escriptions of DSB repair as decision trees can oversimplify a complex mechanistic landscape and mask gaps and ambiguities that remain in our understanding.”). Indeed, CVC recognized in CVC P3 that “DNA repair by NHEJ is typically induced by a DSB . . . .” Ex. 1013 at [00418] (emphasis added).

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

Citing examples of donor integration by HDR in TMZs, CVC also argues that “[a] complete or comprehensive understanding of the mechanism for HDR was unnecessary to exploit the process for the purpose of integrating a donor sequence at the site of a DSB made by a targeted nuclease.” CVC Opp’n 1 at 14-15. CVC’s argument again greatly oversimplifies the very complicated and not-well-understood DSB repair pathways and ignores factors that would impact repair pathway choice. Ex. 1182 (Millanowska (2011)) at 1-2 (“[I]t is not surprising that DNA repair is a very complicated process, involving many factors. For instance to date, 168 genes encoding proteins involved in DNA repair have been identified in the human genome.”); Ex. 1032 (Giglia-Mari (2011)) at 1 (“A complicated and entangled network of DNA damage
response (DDR) mechanisms, including multiple DNA repair pathways, damage tolerance processes, and cell-cycle checkpoints safeguard genomic integrity.”). In December 2012, it was understood that the eukaryotic DDR processes were highly complex and regulated by numerous cellular enzymes. Ex. 1066 (Thompson (2012)) at 158 (“[T]he elimination of double-strand breaks with minimal nucleotide sequence change involves the spatiotemporal orchestration of a seemingly endless number of proteins . . . .”). And it was also understood that interference with any of those eukaryotic repair enzymes and signal transducers (potentially such as by the introduction of a prokaryote-derived enzyme) could interfere with the cell's DDR:

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

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

CVC further argues that “there were predictable and reliable ways [] to ascertain whether or not HDR-mediated donor integration had occurred . . . .” CVC Opp’n 1 at 24-25. But having predictable ways to ascertain whether HDR had occurred following an experiment is not relevant to predicting beforehand whether HDR would work with CRISPR-Cas9 created DSBs. As Dr. Cannon explained, “[homology-directed repair] really wasn’t . . . a trivial and obvious
technique. It was something that was, certainly in 2012, recognized as . . . the most challenging part of gene editing.” Ex. 2616 (Cannon Tr.) at 104:11 – 105:1. Dr. Cannon further explained that, in 2012, it was known that introducing a donor template into a cell for HDR often led to toxicity and many donor templates did not work. *Id.* at 104:11-17. Improving homology-directed editing was and remains “an area of intense research.” *Id.* at 105:2-11; see Ex. 1181.1 (Hussmann (2021)) at 5653 (“Limitations in our understanding of DSB repair are due in part to technical challenges associated with profiling the determinants of repair outcomes.”).


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

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

Indeed, it was known that Ku promotes NHEJ over HDR because Ku binding to the DNA ends initiates NHEJ pathway and protects cleaved DNA from end resection, which is the first step for HDR. Ex. 1025 (Ciccia (2010)) at 183 (“Double-strand DNA breaks are rapidly bound by [Ku]. . . . Ku localizes within seconds to DSBs, where it . . . initiate[s] NHEJ.”) (emphasis added); Ex. 2632 (Kass (2010)) at 3704 (“The first protein in [NHEJ] pathway to bind DNA ends is [Ku].”); Ex. 1179 (Foster (2011)) at 4387 (“The function of Ku in this regard is not simply to promote NHEJ over HDR . . . DSB end binding by [Ku] influences mitotic DNA repair by limiting access of ExoI for resection.”); Ex. 1180 (Hiom (2010)) at 1258 (“Ku heterodimers . . . [are] particularly important for pairs of broken ends that are less able to associate, for example blunt ends . . . ”). Further, it was also known that Ku prefers blunt ends over overhangs. Ex. 1179 (Foster (2011)) at 4387 (“Ku has a **significantly higher affinity for blunt dsDNA ends** over short ssDNA overhangs.”) (emphasis added). Thus, a POSITA would have understood that
blunt-ended DSBs created by CRISPR-Cas9 would likely be repaired by NHEJ, not HDR.

CVC argues that Jinek (2012) discloses “some blunt and some with overhangs.” CVC Opp’n 1 at 27 (emphasis in original). However, CVC ignores Jinek (2012)’s express teaching that CRISPR-Cas9 produces blunt ends, and that the overhangs observed in Fig. S4B are the result of subsequent trimming. Ex. 2031 (Jinek (2012)) at 816 (“subsequently trimmed”).

Indeed, later work of the CVC inventors further confirms that it was their belief in 2012 that CRISPR-Cas9 creates blunt ends. Ex. 2010 (CVC P2) at Fig. 4 (showing blunt ends after cleavage); Ex. 1178 (Doudna (2014)) at 1077 (“Cas9-sgRNA–mediated DNA cleavage produces a blunt double-stranded break . . . .”); id. at Figs 2C and 4 (showing blunt ends after cleavage).

CVC also argues that “[i]t was also known that resection functions on blunt ends.” CVC Opp’n 1 at 27. However, the passage quoted by CVC is about ends blocked with covalent adducts (a cancer-causing chemical). Ex. 2633 (Nimonkar (2011)) at 359 (“[T]he resection activity of MRN and CtIP is especially critical when the ends are blocked with covalent adducts.”).

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

It was known in 2012 that Cas9-crRNA complex would remain bound to the ends of the cleaved DNA strands after cleavage, which could block the operation of the repair machinery. Ex. 2640 (Gasiunas (2012)) at E2584 (“Interestingly, after DNA cleavage, Cas9-crRNA remains bound to the reaction products (Fig. S11).”); Ex. 1001 ¶ 133; Ex. 2616 (Cannon Tr.) at 92:18-22 (“[G]oing back to 2012 . . . it was known that Cas9 stayed attached to the DNA. And that would bring up to me a lot of questions about whether that in some way inhibited the stages needed for homologous recombination.”); id. at 92:23 – 93:6 (“[R]esection . . . would be blocked by . . . the large Cas9 protein sticking on the DNA ends.”). CVC again attempts to change the express teaching of Gasiunas (2012) by offering recent testimony from Dr. Barrangou. CVC Opp’n 1 at
28. However, it is well established that contemporaneous statements are stronger evidence than statements made in the context of litigation. Ex. 1175 (Decision on Motions (‘115 Int’f)) at 14.

CVC also argues that “the skilled artisan knew that HDR-mediated donor integration occurs even when a nuclease remains covalently bound at the site of the DSB, as is the case with the protein Spo11.” CVC Opp’n 1 at 29. However, Spo11 is involved in meiotic recombination, not HDR integration in mitosis, and after Spo11 cleavage, MRN nicks behind the Spo11 cleavage site to create new sites that will be used for meiotic recombination. Ex. 2643 (Neale (2005)) at Fig. 4 (see Ex. 2619 at 32 for the annotated version); Ex. 1172 (Doyon Tr.) at 399:12 – 400:6, 401:1 – 403:9. Thus, because of Spo11’s attachment, alternate free DNA ends are created by MRN nicking to support the subsequent steps of resection and strand invasion. Id.

CVC also asserts that I-SceI and ZFNs were known to remain bound to the ends of the DSBs. CVC Opp’n 1 at 29. Unlike CRISPR-Cas9, however, I-SceI creates 3’ overhangs, which are preferred for HDR. See Ex. 2619 ¶ 60 (describing the first step of HDR as “[e]xonucleases resect the ends of the DSB, i.e. remove nucleotides, to create an exposed stretch of single-stranded DNA in the form of a 3’ overhang.”). In addition, I-SceI was known to bind only at one end. Ex. 2645 (Perrin (1993)) at 2942 (“This is consistent with the idea that the right hand product of the reaction remains bound to [I-SceI] after cleavage.”). Further, CVC’s reliance on Young (2000) does not support CVC’s assertion that ZFNs were known to remain bound at the ends of DSBs. That study examined derivatives of the natural two zinc finger peptide transcription factor, ADR1, and measured the dissociation rate of ADR1. Ex. 2641 (Young (2000)) at 567. That analysis of the transcription factor ADR1 is not the same as measuring DNA binding or dissociation in the context of an actual ZFN. See Ex. 1080 ¶ 33.

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

CVC argues that because CVC Claim 156 assumes that CRISPR-Cas9 is capable of cleaving eukaryotic DNA, concerns over chromatin are “irrelevant.” CVC Opp’n 1 at 26. But beyond cleavage, chromatin presents separate concerns for DNA repair, including HDR. Ex. 1180 (Hiom (2010)) at 1261 ("Consequently, the detection, processing and mechanics of [DSB]
repair require the manipulation of DNA within a chromatin context. The details of how this occurs are not well understood.

Ex. 1035 (Grabarz (2012)) at 261 (“These data further underline the important role of the chromatin environment during DSB repair and adds another level of regulation of HR events.”); Ex. 1032 (Giglia-Mari (2011) at 6 (“The nucleus is highly structured and functionally compartmentalized in part due to areas of various degrees of chromatin compaction, creating possible obstacles for DDR factor accessibility.”)).

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

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

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

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

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

CVC completely ignores the timeline and history of the development of ZFNs and
TALENs. Both ZFNs and TALENs are engineered nuclease systems that comprise a DNA
binding domain that naturally recognizes eukaryotic DNA, linked to an independent DNA
cleavage domain. Ex. 1080 ¶¶ 29-35. In both of these protein-guided systems, DNA cleavage is
achieved by the *FokI* endonuclease. *Id.* ZFNs were demonstrated to cleave DNA in a sequence-
specific manner *in vitro* by 1996. Ex. 2325 (Kim (1996)) at abstract; Ex. 1172 (Doyon Tr.) at
358:12 – 359:14. In 2001, one group reported cleavage and deletion of intervening sequences
from plasmids containing two copies of the DNA recognition sequence for the QQR ZFN when
injecting into *Xenopus* oocytes. Ex. 2308 (Bibikova (2001)) at 289-290. And it took
investigators two more years to demonstrate cleavage plus donor integration by HDR into
chromosomal DNA in eukaryotic cells with even previously described ZFN reagents. Ex. 1018
(Bibikova (2003) (in Drosophila using a target site in the *yellow* gene)); Ex. 1055 (Porteus
(2003) (in human cells using target sites for QQR)). Similarly for TALENs, roughly a two-year
gap intervened between the initial report that targeted breaks were possible to achieve *in vivo* in
eukaryotic cells (Ex. 1019 (Boch (2009))), and the subsequent demonstration of HDR-mediated
integration (Ex. 1036 (Hockemeyer (2011)); Ex. 1050 (Miller (2011))). Ex. 1001 ¶ 147. Thus, a
POSITA would have known that HDR was far more difficult and challenging than cleavage
alone. These timelines reveal that HDR is very unpredictable, and does not automatically occur
after a DSB is created and a donor template is provided. Ex. 2616 (Cannon Tr.) at 46:9-21.

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

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

Whether HDR Would Have Been Induced By A DSB Created By The
CRISPR-Cas9 System

CVC claims that “contemporaneous evidence [] universally predicted that the CRISPR-
Cas9 system would be a genome editing tool, including for inducing HDR-mediated donor
integration.” CVC Opp’n 1 at 21-22 (citing to recent testimony from Dr. Carroll and Dr.
Barrangou)). This retrospective and compensated testimony is flatly contradicted by the
contemporaneous 2012 evidence. For example, Dr. Carroll, a purported CVC “fact” witness,
was originally skeptical about whether a CRISPR-Cas9-created break would be repaired by
homologous recombination, including donor integration. In his review article published in
September 2012, he placed a conspicuous “?” next to “CRISPR” in Figure 1, and stated that
“[t]he break can be made by any targetable nuclease: zinc-finger nucleases (ZFNs), transcription activator–like effector nucleases (TALENs), homing endonucleases (HEs), or, perhaps, the new CRISPR reagents.” Ex. 2339 at 1659 (emphasis added); Ex. 1171 (Carroll Tr.) at 40:10 – 42:1. Dr. Carroll observed that “[t]he authors [of Jinek (2012)] make the bold prediction that [CRISPR-Cas9] can potentially be used in place of ZFNs or TALENs for targeted genomic cleavage in higher organisms.” Id. (emphasis added). These statements demonstrate that, in September 2012, Dr. Carroll—an expert in the field, with considerably more knowledge and experience than a POSITA—had concerns about whether CRISPR-Cas9 would work in eukaryotic cells, which includes whether CRISPR-Cas9 would support DDR processes. Likewise, Dr. Barrangou expressed similar skepticism in 2012. Ex. 2215 (Barrangou (2012)) at 838 (“[T]here 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 . . . .”) (emphases added). Thus, Dr. Carroll’s and Dr. Barrangou’s 2012 publications do not show that CRISPR-Cas9 was expected to support HDR integration in eukaryotic cells.

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

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

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

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

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

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

In its opposition, CVC complains about Sigma’s analysis showing the correspondence of
CVC’s involved claims to Proposed Count 2. CVC Opp’n 1 at 9-11. But, as Sigma indicated in
its Motion 1, because “CVC has not contested that all of [CVC’s involved] claims are obvious in
view of current Count 1, . . . the Board could reasonably designate all of CVC’s currently
involved claims as corresponding to the proposed Count 2.” Sigma Mot. 1 at 25. In any event,
in Sigma Motion 1, Sigma engaged in a detailed analysis of all 412 of CVC’s involved claims in
all fourteen of CVC’s involved applications, as well as the hundreds of additional claims in
CVC’s extensive family of uninvolved cases. *Id.* at 25-29. At a minimum, that analysis sets forth a *prima facie* showing of claim correspondence. *Id.* And CVC nowhere explains *why* any of that analysis is somehow deficient, nor does CVC offer *any* rebuttal to that analysis (i.e., why any involved CVC claim would be, or would not be, obvious in view of Proposed Count 2).

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

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

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

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

Indeed, when questioned in this regard, Dr. Cannon elaborated further on this issue:

[T]here’s no [] examples, . . . no discussions of what, if anything, might be a consideration for doing this in the situation that’s described in, for example, CVC Claim 164. So I think [] it’s more than just the lack of a working example. It’s the fact that the language is very generic and [] not really teaching me how to [] do that and [] what tricks I might need to use, what to, [] watch out for[].

* * *

Putting myself in the mind of a person of ordinary skill in the art in 2012 would have had, I find that there was inadequate guidance in P3 to help me do that without [] having to make the invention myself and do that.
VII. CVC’S POINTING TO PRIOR ART OF RECORD FAILS TO REBUT SIGMA’S PRIMA FACIE SHOWING THAT PROPOSED COUNT 2 IS PATENTABLE

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

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

CVC’s purported “best proofs” are directed to current Count 1, not Proposed Count 2.
CVC Opp’n 1 at 11-12. While a party’s arguments directed to its purported “best proofs” could be relevant to evaluating the scope of a particular count for a common patentable invention, such arguments completely miss the boat here. Indeed, CVC nowhere even suggests that its best proofs to the parties’ commonly claimed cleavage plus integration invention would counsel for an alternative formulation of Proposed Count 2 (other than simply keeping current Count 1).

IX. CVC’S ARGUMENTS THAT SIGMA IS SEEKING AN INTERFERENCE DIRECTED TO “GENERIC CLEAVAGE” ARE NONSENSICAL

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

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

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

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

In its opposition, CVC correctly states that when there are “two patentably distinct
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inventions . . . the remedy would be a *two-count* interference.” CVC Opp’n 1 at 6-7, 9. But
CVC ignores Sigma’s entirely logical explanation regarding why Sigma Motion 1 seeks only a
single count, namely, because “Sigma would have no interfering subject matter corresponding to
[a cleavage only count]. Thus this interference would only proceed with a single count, namely,
[a cleavage plus integration count] alone.” Sigma List of Proposed Motions (Paper 26) at 4-5.

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

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

XIII. CONCLUSION

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

Dated: April 7, 2022

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Sigma-Aldrich Co. LLC
### LIST OF EXHIBITS CITED

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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 CRISPR-Cas9 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).


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).


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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