Filed on behalf of: **Junior Party, Broad** Paper No. ____

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UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE PATENT TRIAL AND APPEAL BOARD

THE BROAD INSTITUTE, INC., MASSACHUSETTS INSTITUTE OF TECHNOLOGY, and PRESIDENT AND FELLOWS OF HARVARD COLLEGE,

Patents 8,697,359; 8,771,945; 8,795,965; 8,865,406; 8,871,445; 8,889,356; 8,889,418; 8,895,308; 8,906,616; 8,932,814; 8,945,839; 8,993,233; 8,999,641; and 9,840,713; and Applications 14/704,551 and 15/330,876,

Junior Party,

v.

TOOLGEN, INC.,
Application 14/685,510,

Senior Party.

Patent Interference No. 106,126 (DK)
(Technology Center 1600)

BROAD REPLY 3

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I. PRECISE RELIEF REQUESTED

The claims currently designated as corresponding to Count 1 include claims that are patentably distinct from the subject matter of Count 1, and Proposed Count 2. These patentably distinct inventions fall into five categories: (a) vector delivery; (b) use of SaCas9; (c) chimeric Cas9 formed of two fragments, each from a different Cas9 ortholog; (d) Cas9 having two or more NLSs; and (e) Cas9 with fused and heterologous protein domains. These claims should be designated as not corresponding to Count 1 and, if Broad's Motion 1 is granted, should not be designated as corresponding to Proposed Count 2.

Additionally, if the Interference proceeds with Count 1 (it should not as shown in Motion 1 and Reply 1), then Broad's generic RNA claims should be designated as not corresponding to Count 1. Broad's involved claims include claims that cover dual-molecule or single-molecule RNA, and indeed claims that do not recite any RNA at all. If the Interference proceeds with Count 1, limited as it is to just the single-molecule RNA species and therefore, prohibiting reliance on dual-molecule RNA experiments to establish priority, it would be unjust to put at risk Broad's generic RNA claims. Broad would be subject to the risk of losing its generic RNA claims, while at the same time precluded from introducing proofs showing it invented the generic RNA subject matter before ToolGen. This is manifestly unfair. ToolGen's blithe response is that there is no support in the interference rules for considering "fairness and equity." Opp. 3 at 33. Broad respectfully submits that, contrary to ToolGen's contention, the PTAB has the power, authority, and responsibility to conduct interferences in a manner consistent with "fairness and equity."

II. DESCRIPTION OF APPENDICES

Appendix A is a List of Exhibits Cited, Appendix B is the Statements of Material Fact and Responses.

III. ARGUMENT

A. Broad's Claims Limited To Vector Delivery Should Be Designated As Not Corresponding To Count 1, And Do Not Correspond To Proposed Count 2

Broad's Motion 3 set forth the dispositive facts establishing that claims requiring the use of vectors for delivery of CRISPR-Cas9 components are novel and non-obvious over Count 1 and Proposed Count 2. ToolGen does not dispute that these claims are not anticipated by either count. Instead, at 2:13-14, ToolGen argues that a POSA would have had a motivation to use and a reasonable expectation of success in using vector delivery to eukaryotic cells. Opp. 3.

The first response is that ToolGen's litigation positions were contradicted by its own expert, who admitted at his deposition that by 2012 "plasmids [a vector] being an undesirable or suboptimal delivery vehicle was definitely being recognized by investigators and POSAs right around then, including my own lab." Ex. 2459, Stoddard Dep. at 186:16-19; *see also id*. 187:10-11 (agreeing that "you would generally call a plasmid a vector."). Indeed, the most Dr. Stoddard could say for delivery of SpCas9 via AAV vectors in 2012 was that it "might be possible under certain conditions" but it would be "problematic." *Id.* at 139:5-10. That is, even though as Dr. Stoddard stated in his declaration that AAV vectors were "available in the art and were commonly used" by 2012 (Ex. 1420, ¶ 87), and SpCas9 was one of the best-characterized CRISPR systems by 2012 (Ex. 2454, Seeger Decl. ¶ 197), the 2012 POSA would have concluded that successful use of such a system would be "problematic" but "might be possible under certain conditions." This is a far cry from the required "reasonable expectation of success."

The further response is that ToolGen concedes that "Broad is correct that '[d]elivery of RNA components of a CRISPR-Cas9 system can be accomplished in multiple ways.'" Opp. at 3:5-6. ToolGen also does not dispute that there is no teaching or suggestion in Count 1 relevant to vector delivery to eukaryotic cells and does not point to any prior art disclosing vector delivery of

CRISPR-Cas9 systems to eukaryotic cells. Instead, ToolGen points to the generic delivery of (non-CRISPR-Cas9) RNA molecules to eukaryotic cells via vector. But merely because "the use of vectors was well-known" for delivery of other RNA molecules does not provide any reason why a POSA would have selected vector delivery specifically for a CRISPR-Cas9 system that includes both protein and RNA components nor does it provide reasonably expected success. Indeed, ToolGen offers no reason why a POSA would select an "undesirable or suboptimal delivery vehicle" from a wide range of choices, particularly for use in the challenging and unpredictable eukaryotic cell environment. ToolGen offers only hindsight analysis that such systems are now successful and common. If anything, such success is an objective indicia of *non-obviousness*.

At 3:3-13, ToolGen contends it is "improper" to consider other methods of delivery in evaluating the non-obviousness of vector delivery and that Broad's arguments on this point "should be ignored." Opp. 3. The response is that the opposite is true under the law: where, as here, the prior art provides a number of choices, without any reason to make a particular selection, this militates for a finding of non-obviousness. *See Ortho-McNeil Pharm., Inc. v. Mylan Labs.*, Inc., 520 F. 3d 1358, 1364 (Fed. Cir. 2008). ToolGen attempts to distinguish *Ortho-McNeil* as only directed to a selection from an infinite number of choices, not a "finite, and in the context of the art, small or easily traversed, number of options." But the choices in *Ortho-McNeil* were in fact finite, and the Federal Circuit warned that hindsight analysis such as ToolGen's seeks to "retrace[] the path of the inventor" by "discount[ing] the number and complexity of the alternatives." *Id*.

ToolGen also cites to *Bestway (USA), Inc. v. Intex Marketing Ltd.*, 2017 WL 1969760, at *6 n.2 (P.T.A.B 2017), as allegedly distinguishing *Ortho-McNeil* as limited to only inventions with "unpredictable alternatives." But as set forth in Motion 1, delivery of CRISPR-Cas9 to eukaryotic cells was developed in 2012 and remains an unpredictable and difficult task—a fact

that is not disputed by ToolGen, and indeed a fact with which the Federal Circuit agreed. *See* Ex. 2648, Lino 2018, at Abstract; *Regents of Uni. of Cal. v. Broad Institute, Inc.*, 903 F.3d 1286, 1294 (Fed. Cir. 2018)("[S]ubstantial evidence supports the Board's finding that the success in ***applying similar prokaryotic systems in eukaryotes was unpredictable*** …."). In fact, ToolGen's own expert Dr. Cullen has opined that "the order of providing elements may be an important feature to getting the CRISPR/Cas9 system to work in eukaryotic cells" specifically, given their unpredictability. Ex. 2069, 28 October 2016 Interview Summary in 14/685,568. And, as noted above, given the wide number of alternatives for delivery of CRISPR-Cas9 systems to eukaryotic cells, that vectors were recognized as an undesirable delivery vehicle further eliminates any reason for a POSA to select them among the various options in such an unpredictable circumstance.

At 5:7-10, ToolGen argues that Broad and its expert expressed a "factual inaccuracy" in claiming that "'[t]here is no prior art that delivered CRISPR-Cas9 systems to eukaryotic cells at all and so, of course, there is none directed to vector delivery of the RNA.'" Opp. 3 (citing Mot. 6:15–17). But ToolGen does not identify any prior art showing vector delivery of CRISPR-Cas9 to a eukaryotic cell, whether single-molecule or dual-molecule RNA. ToolGen merely claims, without any support or citation that "[a]ll of the claims require RNA for the CRISPR-Cas9 system, [and] expression of RNA using a vector was and is the most common way to do it." Opp. 3 at 5:16-17. ToolGen's inability to cite a single example as of December 2012 is telling.

At 6:5-8, ToolGen argues there was only a small set of possibilities for delivery because vectors "were the first among 'three . . . commonly reported' 'CRISPR/Cas9 cargoes." Opp. 3 (citing Exhibit 2648, 1241). This is clearly impermissible hindsight analysis of how "common" vectors are based on a reference published in 2018. Vectors were not "common" for delivery of CRISPR-Cas9 to eukaryotic cells in December 2012—indeed, there had been no published reports

of the use of CRISPR-Cas9 in eukaryotic cells at all. ToolGen's citation to a 2018 reference falling well after Broad's own publication of highly successful eukaryotic experiments using vectors, after which it made available its own plasmids for delivery, is without merit. What ToolGen's citation proves is that Broad's teachings on vector delivery—teachings that came after the relevant date and are not in the prior art—have shown that vector delivery produces unexpectedly positive and commercially successful results. Ex. 2454, Seeger Decl. ¶¶ 225.

As to reasonable expectation of success, Broad showed why a POSA would have no such expectation with regards to vector use as of 2012. Ex. 2454, Seeger Decl. ¶¶ 212-27; Ex. 2110, Paper 893, 048 Decision on Motions; Ex. 2121, Paper 877, 115 Decision on Motions. For that additional reason, the use of vector-delivery would not have been obvious. *See Novartis Pharm. Corp., v. West-Ward Pharm. Int'l Ltd*., 923 F.3d 1051 (Fed. Cir. 2019) (affirming that claims were not obvious even where the POSA would be motivated to pursue the claimed species if there was not a reasonable expectation of success given the uncertainty in the art). Tellingly, ToolGen nowhere addresses in its Opposition whether a POSA would have a reasonable expectation, other than to suggest vectors were widely employed in non-CRISPR-Cas9 contexts. This is unsurprising given vector delivery, even with a system that could otherwise work successfully in a eukaryotic cell (as is presumed by the Counts), would be unpredictable, as the state of the art was still unpredictable as to the impact of delivery. MF 7-8; Ex. 2454, Seeger Decl. ¶¶ 225.

At 6:13-7:17, ToolGen argues that Broad has not established commercial success or unexpectedly positive results, focusing on the nexus between Broad's evidence and the vector aspect of the claims. Opp. 3. Broad has cited in particular to the wide use of the vectors it makes available from AddGene, which are specifically for plasmids—vector delivery—of CRISPR-Cas9 systems. The nexus between this success and the use of vectors is clear. ToolGen speculates that

the popularity of Broad's vectors could be attributed to "any number of features" including "marketing and advertising." But ToolGen offers no evidence to support its conjecture and Broad was not required to negate all possible alternatives as part of its Motion.

B. Broad's Claims Limited To SaCas9 Should Be Designated As Not Corresponding To Count 1, And Do Not Correspond To Proposed Count 2

Broad's Motion set forth the dispositive facts establishing that claims limited to SaCas9 are novel and non-obvious over Count 1 and Proposed Count 2. With respect to anticipation, neither count recites that the Cas9 is SaCas9. MF 9; Paper 1 at 12-13; Broad Motion 1 at 4. Thus, neither count anticipates claims that require SaCas9 (Ex. 2454, Seeger Decl. ¶¶ 228-48), a fact ToolGen does not contest.

ToolGen contends that the POSA would have had a motivation to select SaCas9 out of all the other known orthologs, despite the facts that in 2012 the best characterized and most widely studied systems were the SpCas9 and StCas9, that only SpCas9 had been validated *in vitro*, and that only StCas9 had been shown to function heterologously (*i.e.*, in a bacterial species other than *S. thermophilus*). As to the number of available Cas9 orthologs by 2012, ToolGen's expert admitted that he did not know how many were actually available by 2012, but at least 65 were known by 2011 and his expectation would be that even more would be known in 2012. Ex. 2459, Stoddard Dep. at 126:9-127:15.

At 8:25-9:1, ToolGen contends there was motivation to select SaCas9 from this long list because SaCas9 was allegedly an "established" model for studying CRISPR, citing Ex. 1311 (Sapranauskas 2011). Opp. 3. But a POSA would find no motivation to use SaCas9 in eukaryotic cells from Sapranauskas. Sapranauskas makes a passing reference to the existence of the *S. aureus* CRISPR system, but then only reports results showing heterologous expression using *S. Thermophilus* Cas9. If anything, the Sapranauskas reference would have motivated the POSA to

attempt the use of StCas9, not to blaze a new trail down the unmarked *S. Aureus* path. Further discouraging the POSA from SaCas9, the only work on SaCas9 cited by the Sapranauskas reference was done by Drs. Marraffini and Sontheimer—who speculated on eukaryotic CRISPR systems starting in 2008 yet, as a 2012 POSA would have known, four years later they had never reported any success at all in eukaryotic cells, let alone with SaCas9. Ex. 2054, ¶¶ 47-53.

ToolGen's reliance on its expert's testimony is similarly misplaced. While Dr. Stoddard opines in his declaration that the POSA would be motivated to select SaCas9 over StCas9 or SpCas9 because it would fit on an AAV vector, he admitted that both SpCas9 and StCas9 likewise fit on AAV vectors. Ex. 1420, Stoddard Decl., ¶¶ 87-91. The only motivation for selecting a smaller size Cas9 that Dr. Stoddard identifies is the Wu 2010 publication, reporting that expression is less efficient from AAV vectors when the payload is more than 5 KB. Ex. 1314. But, SpCas9 and StCas9 are both less than 5 kb, as Dr. Stoddard concedes. Ex. 1420, Stoddard Decl., ¶¶ 87-91. There is simply no motivation to select SaCas9 identified by Dr. Stoddard that would not also apply to selecting StCas9 or SpCas9—both better characterized systems.

Moreover, Dr. Stoddard admitted at his deposition that he does not know nor could even guess how many Cas9s were known to POSAs by 2012. So not only has Dr. Stoddard failed to identify any reason to select specifically the SaCas9 needle out of the haystack of other Cas9s, but he does not even know how big the haystack is.

And as to SaCas9, Dr. Stoddard himself—despite all the alleged motivation to use SaCas9—has *never* in his own research used SaCas9. Tellingly, in his work using CRISPR-Cas9 starting in 2013, he opted to use SpCas9 because there "the most information was available at the time" on that particular ortholog. Ex. 2459, Stoddard Dep. at 82:6-19. If Dr. Stoddard, who ToolGen asserts to be an expert on genome engineering, opted to use the best characterized Cas9

for his work, there should be no question the POSA would have done likewise—they would not have taken up a new and unexplored system.

ToolGen argues Broad did not show how many small orthologs were known before December 12, 2012. But it is beyond reasonable dispute that a POSA would have known of hundreds of Cas9 orthologs other than SaCas9 that are smaller than SpCas9 by December 12, 2012. And CVC's expert in the 115 Interference, Dr. Carroll, testified there were hundreds known by 2012. Ex. 2408, Carroll Dep. at 171:15-21 ("[T]here were several hundred Cas9 orthologs that were known as of December 12, 2012, that were smaller than SpCas9; right? A. Yes."). This accords with Dr. Stoddard's admission when confronted with the Makarova reference (Ex. 1207) that there were at least 65 by 2011, with his expectation being that more would be known by 2012. Ex. 2459, Stoddard Dep. at 128:22-131:5.

At 9:16-11:2, ToolGen concocts a hindsight-influenced scenario in which it contends that a POSA would have been motivated to use SaCas9 for human therapeutics, specifically in combination with AAV vectors for "tissue-specific" applications. Opp. 3. But the Count is not limited to human therapeutics, let alone tissue specific applications, and in any event both SpCas9 and StCas9 can be delivered with AAV vectors. *Id.* at 8:22-9:15. That SaCas9 can successfully be used with AAV vectors in human therapeutics now, in 2021, using Broad's invention, cannot be used to retroactively manufacture a 2012 motivation for a POSA to choose to attempt to use SaCas9 over SpCas9, StCas9, or any of the other known Cas9 orthologs.

As to objective indicia of non-obviousness, at 18:10-12, ToolGen argues "unexpected results 'must be shown to be unexpected compared with the closest prior art.'" Opp. 3. As addressed in Broad's Motion 3, here, the closest prior art would be the smaller StCas9, which was found to be less efficient that SpCas9. ToolGen does not dispute that SaCas9 was unexpectedly

better than StCas9, only that because the evidence on which Broad relies was published after the priority date, it is not relevant. This is legally incorrect. A party can rely on evidence, such as test results, created after the patent issued to show that at the time of the invention one of skill in the art would have found the results achieved by the claimed invention unexpected. *Knoll Pharm. Co., Inc. v. Teva Pharm. USA, Inc.*, 367 F.3d 1381, 1384-85 (Fed. Cir. 2004) ("Evidence developed after the patent grant is not excluded from consideration, for understanding of the full range of an invention is not always achieved at the time of filing the patent application."); *Sanofi-Aventis Deutschland GmbH v. Glenmark Pharm. Inc., USA*, 748 F.3d 1354, 1360 (Fed. Cir. 2014) ("Glenmark also argues that later-discovered benefits cannot be considered in an obviousness analysis…. That is incorrect; patentability may consider all of the characteristics possessed by the claimed invention, whenever those characteristics become manifest.").

C. Broad's Claims Limited To Chimeric Cas9 Should Be Designated As Not Corresponding To Count 1, And Do Not Correspond To Proposed Count 2

Broad's motion set forth the dispositive facts establishing that claims reciting a chimeric Cas9 are novel and non-obvious over Count 1 and Proposed Count 2. ToolGen does not dispute that Count 1 or Proposed Count 2 fail to anticipate Broad's claims directed to use of a chimeric Cas9. With respect to obviousness, nothing in either count or in the prior art teaches, suggests, or provides motivation to a POSA to design a chimeric Cas9 that is comprised of two fragments from different organisms. MF 19; Ex. 2454, Seeger Decl., ¶¶ 249-57.

At 16:12-17, ToolGen argues that Broad has waived its argument regarding chimeric Cas9. ToolGen characterizes Broad's argument as "three conclusory sentences" that do not address the merits. Opp. 3. First, Broad's argument spans from 13:20-14:5, and addresses both the lack of motivation to design a Cas9 comprised of two fragments from different organisms and establishes the unexpected benefits of chimeric Cas9. It also cites and discusses the eight paragraphs of Dr.

Seeger's declaration addressing this point. Moreover, from 16:21-18:17, ToolGen spends two pages addressing the merits of Broad's position. Opp. 3. ToolGen was able to understand the merits of Broad's position. Broad has not waived its argument.

At 16:21-17:15, ToolGen argues that because chimeric proteins were known to be used in non-CRISPR-Cas9 systems, "it was well-known in the art to design chimeric proteins in both prokaryotic and eukaryotic cells." Opp. 3. ToolGen does not point to any reference relating to Cas9 itself, nor any reason why a POSA would believe the use of chimeric proteins in other applications would motivate a POSA to modify wild-type Cas9 proteins—particularly prior to any public demonstration of a successful CRISPR-Cas9 system. A POSA would not be motivated to make such a modification, which would further increase the unpredictability of the system.

ToolGen claims that chimeric proteins had the "known ability to alter the functions of the wild-type Cas9 protein," but it does not cite to any evidence showing successful alteration of the complex Cas9 protein as of December 2012, let alone alterations to modify the functions of wild-type Cas9. Opp. 3 at 17:5-7. Moreover, ToolGen does not identify any known limitations or concerns with wild-type Cas9s ***as of 2012*** that would be solved by the proposed alteration.

At 17:18-18:17, ToolGen argues the result of altering wild-type Cas9s would be "expected." Opp. 3. But what Dr. Stoddard testified is that "a POSA would have *expected* a chimeric Cas9 protein to have altered results…." Ex. 1420, Stoddard Decl., ¶149. ToolGen has argued successfully to the Patent Office that the smaller modification of adding an NLS to the Cas9 protein could have potentially "rendered it inactive upon expression in a eukaryotic cell," citing problems caused by prior modifications of proteins in the art. Ex. 2460, Cullen Decl., ¶ 38. Even more so, the POSA would have understood that the "altered results" expected from a

chimeric Cas9 might be inactivity, *i.e.*, failure, and so there was nothing motivating a POSA to proceed in that way.

ToolGen also argues that Broad failed to "compare the results obtained from using chimeric Cas9 with the closest prior art, *i.e.*, known chimeric variants of the Cas protein…." Opp. 3 at 18:12-14. But ToolGen does not identify any known chimeric variants of the Cas protein at all—there was no such "closest prior art"—highlighting the fact that a POSA as of 2012 would not have reasonably expected successful results from use of a chimeric Cas9 protein.

D. Broad's Claims Limited To Use Of Two Or More NLSs Should Be Designated As Not Corresponding To Count 1, And Do Not Correspond To Proposed Count 2

Broad's motion set forth the dispositive facts establishing that claims reciting two or more NLSs are novel and non-obvious over Count 1 and Proposed Count 2. ToolGen does not dispute that neither Count 1 nor Proposed Count 2 anticipate Broad's claims directed to the use of two or more NLSs. With respect to obviousness, the prior art did not provide any teaching or suggestion to use two or more NLSs in a CRISPR-Cas9 system in a eukaryotic cell nor was there a reasonable expectation of success in using two or more NLSs. Ex. 2454, Seeger Decl., ¶¶ 258-78.

As to motivation, at 19:12-2:9, ToolGen contends POSAs knew that attaching additional NLSs to non-Cas9 proteins had further increased nuclear localization in prior art systems. The response is that this is an inaccurate characterization of the art. As Dr. Seeger explained, adding NLSs to a protein such as Cas9 can alter its folding, resulting in the functional domains being shielded or buried or otherwise inactivating the protein or inhibiting its function. Ex. 2454, Seeger Decl. ¶ 271. Adding two or more NLSs would further increase this risk, as was the case in other systems identified by Dr. Seeger such as with the mTOR protein. As Dr. Seeger explained, adding NLSs had the deleterious impact of preventing the needed trafficking of the protein into and out

of the nucleus. There is no dispute about this as Dr. Stoddard conceded adding additional NLSs to mTOR caused this problem:

> Q. Was it a problem with adding additional NLSs to the mTOR protein, that the mTOR protein was being prevented from exiting the nucleus?
>
> A. In this particular experimental paper and the experiments described in this study, adding four copies of a nuclear localization signal affected cellular function of the protein, because nuclear export was affected in a significant manner. Yes.

Ex. 2459, Stoddard Dep. at 178:14-179:1 (objections omitted).

ToolGen also claims that this is just Broad and Dr. Seeger's "speculation that protein folding may impair the function of Cas9." Opp. 3 at 20:18-21:9. But ToolGen here takes a position directly contrary to its prosecution statements supported by its expert Dr. Cullen who opined that a POSA "would have also recognized that modification of Cas9, a large modular protein, for example by tagging it with NLS and/or optimizing its codon sequence, could have rendered it inactive upon expression in a eukaryotic cell," citing problems with prior modifications of proteins in the art when using an NLS. Ex. 2460, Cullen Decl. ¶ 38. Now, having secured allowance and in this Interference with Broad, ToolGen has hired a new expert who takes the opposite opinion—that the POSA would not have recognized that tagging Cas9 with an NLS and/or codon optimizing could have rendered it inactive upon expression. The contradiction between ToolGen's prosecution expert's position and its current expert's opinions could not be more striking:

ToolGen's Prosecution Expert Dr. Cullen	**ToolGen's Interference Expert Dr. Stoddard**
38. Further, in my opinion, one of ordinary skill in the art as of October 23, 2012 would have also recognized that modification of Cas9, a large modular protein, for example by tagging it with a NLS and/or optimizing its codon sequence, could have rendered it inactive upon expression in a eukaryotic cell.	Dr. Stoddard, would you agree that a POSA, as of 2012, would have recognized that modification of Cas9 by tagging it with an NLS could have rendered it inactive upon expression in a eukaryotic cell? A. No, I don't particularly agree with that statement.

Ex. 2460, Cullen Decl. ¶ 38	Q. Why do you disagree with that statement? A. I would expect an NLS to affect the folding of the protein, if at all, only if the protein were an extremely small, single-domain protein. Cas9, I would expect a small NLS to almost certainly not affect its folding or function. Ex. 2459, Stoddard Dep. at 158:6-21 (objections omitted).

Dr. Stoddard's opinions on the impact of adding NLSs to Cas9 should be disregarded because they directly contradict Dr. Cullen's opinions that led to allowance of the ToolGen claims at issue. ToolGen cannot have it both ways.

As to expectation of success, at 20:10-21:18, ToolGen argues that use of two or more NLSs with prokaryotic proteins in non-CRISPR Cas9 systems would have led to an expectation of success that use of two or more NLSs would increase nuclear localization with CRISPR-Cas9 specifically. Opp. 3. The response is that none of the examples of alleged use of multiple NLSs cited by ToolGen support its over-simplistic characterization. As the PTAB has already noted, prior art eukaryotic systems such as TALENS and ZFNs on which ToolGen relies are hybrids of both eukaryotic and prokaryotic elements and would not provide a reasonable expectation of success for CRISPR-Cas9. Ex. 2110, 048 Interference, Paper 893, at 40-41. They are not informative here. ToolGen further discusses Lac proteins and Fieck, but Fieck found that adding an NLS to the Lac repressor protein in many cases made the protein completely ineffective. Ex. 2258 at 1788-89. Thus, whether a particular prokaryotic protein will work after the addition of two or more NLSs is dependent on many factors that are specific to the protein and the location where the NLSs are placed. Ex. 2454, Seeger Decl., ¶¶ 258-78.

At 20:15-17, ToolGen cites to Jinek's attachment of a four amino-acid tag to a Cas9 in an *in vitro* context as allegedly supporting a POSAs expectation of success. Opp. 3. But during prosecution, ToolGen expressly argued that expression of the Cas9 ***in a eukaryotic cell*** with NLS was problematic—Jinek's *in vitro* work is irrelevant to that problem. Ex. 2460, Cullen Decl. ¶ 38.

At 21:21-22:22, ToolGen argues that Broad has not established objective indicia of non-obviousness. Opp. 3. Citing *Galderma Labs., L.P. v. Tolmar, Inc*., 737 F.3d 731, 739 (Fed. Cir. 2013), ToolGen argues that Broad's evidence shows a difference in "degree, but not kind" and that this is insufficient as a matter of law to show unexpected success. Opp. 3. This misstates the law. *Galderma* holds that the modification achieved must be "within the capabilities of one skilled in the art at the time." *Goldman,* 737 F.3d. at 739. ToolGen nowhere argues that a POSA could have achieved the same results Dr. Zhang did with multiple NLSs as of 2012. As Broad showed in Motion 3, there is nothing in the art suggesting that POSAs (as opposed to those of extraordinary skill) understood how to modify Cas9 proteins such that their function in eukaryotic cells would not be eliminated by multiple NLSs.

Finally, at 22:6-22:22, ToolGen does not dispute the unexpected improvement caused by Broad's use of two NLSs, but quizzically claims that the data cited by Broad "only shows the expected nuclear localization of the Cas9 protein, which is not the same as its function." Opp. 3. But localizing the NLS is intended to improve access of the CRISPR-Cas9 system to target DNA in the nucleus—thus improving the Cas9's function.

E. Broad's Claims Limited To Cas9 Fused To Specified Protein Domains Or Including Heterologous Domains Should Be Designated As Not Corresponding To Count 1, And Do Not Correspond To Proposed Count 2

Broad's motion set forth the dispositive facts establishing that claims that are limited to a Cas9 fused to specified protein domains or including heterologous domains are novel and non-obvious over Count 1 and Proposed Count 2.

At 23:16-19, ToolGen argues that Broad has waived its argument regarding these claims. As with its prior waiver argument, ToolGen characterizes Broad's argument as "six conclusory sentences" that do not address the merits. Opp. 3. First, Broad's argument on the merits spans from 18:23-19:17 and addresses both the lack of motivation and unexpected benefits of a Cas9 fused to specified protein domains or including heterologous domains. *Id.* It also cites and discusses the five paragraphs of Dr. Seeger's declaration addressing this point. ToolGen clearly understood the merits of Broad's position, as it addresses them from 23:23-24:21 of its opposition.

Substantively, ToolGen does not contend that either Count would anticipate Broad's claims. Instead, ToolGen contends that a POSA would have been motivated to fuse Cas9 to specified protein domains with a reasonable expectation of success. As with its prior arguments regarding chimeric Cas9 and more than 2 NLSs, ToolGen points to use of fused proteins in non-CRISPR Cas9 contexts as the only motivations for use, arguing such modifications were routine. These arguments fail for the same reasons set out in Section III.C-D. ToolGen does not point to any reference relating to Cas9 itself supporting motivation, nor any reason why a POSA would believe the use of fused proteins in other applications would motivate a POSA—prior to any public demonstration of a successful CRISPR-Cas9 system—to attempt to modify a wild-type Cas9 protein through fusion, adding to the unpredictability of the system. ToolGen also claims that fused proteins could be used to "purify" proteins or detect localization, but it does not cite to any evidence of modified Cas9 protein being functional as of December 2012, let alone use of a fused domain with such a complex protein prior to any successful public demonstration in the art. ToolGen has not identified any teaching or suggestion in Count 1, Proposed Count 2, or the prior art directing a POSA to modify the naturally occurring Cas9 protein sequences as set forth in the claims, because there was no such teaching or suggestion.

F. If The Interference Proceeds With A Single-Molecule RNA Count (It Should Not), Then Broad's Generic RNA Claims Should Be De-Designated

If the Interference proceeds with Count 1 (it should not), then Broad's involved claims that are not limited to single-molecule RNA should also be designated as not corresponding to Count 1, which is so limited. Broad's generic RNA claims include claims that do not limit the RNA configuration at all, including claims that do not recite any RNA component. They also include claims that do not use the term "guide RNA." Thus, even if it is determined that "guide RNA" is limited to single-molecule RNA—contrary to how the term was used in the field, in Broad's specifications, and in ToolGen's specification—it remains the case that Broad has involved claims that are *not* limited to single-molecule RNA. In any event, the term "guide RNA," should not be construed as limited only to single-molecule RNA.

At 30:14-31:9, ToolGen argues that there was no plain meaning of "guide RNA" in 2012, despite the generic use of that term in Jinek 2012 and other prior art. Opp. 3. At deposition, however, ToolGen's expert admitted that the "reasonable simplest meaning" of "guide RNA" to a 2012 POSA was "an RNA molecule that guides another molecule to a target of some sort":

> Q. And what was that "at least one understood meaning of guide RNA"?
>
> A. The simplest meaning would be an RNA molecule that guides another molecule to a target of some sort.
>
> Q. And that's the simplest meaning in 2012?
>
> A. To the best of my memory, yes, I think that's a reasonable simplest explanation.

Ex. 2459, Stoddard Dep. at 91:18-92:5 (objections omitted). Dr. Stoddard's "reasonable simplest" usage accords with how "guide RNA" is used in Broad's specification, namely as a generic term describing the function of the RNA component, not limited to either the single-molecule RNA or dual-molecule RNA species. Moreover, as Dr. Stoddard further admitted, a definition of "guide

RNA" as encompassing both single- and dual-molecule RNA would be a "reasonable" and "acceptable" definition to a POSA in 2012. Ex. 2459, Stoddard Dep. at 88:15-89:9.

ToolGen attempts to avoid certain evidence of plain meaning cited by Broad as either from after the December 12, 2012 benefit date or as coming from the Doudna and Charpentier inventors. Neither of these critiques carry water. Evidence of plain meaning need not be contemporaneous, and ToolGen identifies no reason why there would be a change in the usage before and after December 12, 2012. *See Application of Glass*, 492 F.2d 1228, 1232 n.6 (C.C.P.A. 1974) (instructing that later-issuing publications may be used to construe claim language). Moreover, a POSA would certainly consider how "guide RNA" was used in Jinek 2012, given the prominence of this work. And as Dr. Stoddard admitted, Jinek 2012 "use[s] [] the term 'guide RNA' to refer to dual RNA formats" in its supplementary material. Ex. 2459, Stoddard Dep. at 153:1-7.

At 26:13-30:11, ToolGen contends that Broad's involved claims reciting the term "guide RNA" should be construed to cover only single-guide RNA because the Broad inventors acted as lexicographers based on one sentence in the specification stating that "***in aspects of the invention***" the term "guide RNA" is "interchangeable" with various species of RNA, such as "chimeric RNA" and "synthetic guide RNA." Opp. 3; Ex. 1021, U.S. Pat. No. 8,697,359 at 12:6-16.

As set out in Broad's Motion 3 at 27:9-28:21, this sentence does not "clearly express an intent" to depart from the plain meaning of "guide RNA." *See Ex Parte Charles John Berg, Jr. & John David Norcom*, Appeal No. 2010-004063, 2012 WL 1744485, at *2 (B.P.A.I. Apr. 25, 2012). It instead refers to the use of guide RNA only "in aspects of the invention," not in *all* aspects of the invention or the invention as a whole. ToolGen's expert confirmed that his interpretation of this sentence as a definition is dependent on interpreting "in aspects of the invention" to mean "in ***all*** aspects of the invention," improperly re-writing the express specification language. Ex. 2459,

Stoddard Dep. at 147:21-148:3 ("Q. So you interpreted this term, 'in aspects of the invention,' to mean in all aspects of the invention; right? A. Yes, that's what I believe I am saying.") (objection omitted). This is improper.

Moreover, the word "interchangeable" confirms there is a ***difference*** among the types of RNAs recited in the sentence relied on by ToolGen, but that they can still each be substituted for the other to achieve the same function in aspects of the invention. For example, it makes no sense to say incandescent bulbs can be "used interchangeably" with incandescent bulbs, but it does make sense to say LED bulbs can be "used interchangeably" with incandescent bulbs for "aspects" of lighting a house. Interchangeable things are not the ***same*** thing, but when it comes to their primary function, interchangeable things can be changed out for each other ***despite their differences***.

At 27:19-30:11, ToolGen seeks to dismiss the fact that its interpretation of "guide RNA" violates the claim construction canons of claim differentiation, and against rendering claim language superfluous. Opp. 3. ToolGen does not substantively dispute that these issues are present—just that they can be ignored because, ToolGen claims, the alleged specification definition controls. But ToolGen misapprehends the required inquiry for claim construction. The specification and the claims must be considered as a whole in considering whether overall there is an explicit, clear, and unambiguous definition. As set out in Broad's opening motion, the claim differentiation issues and broader use of "guide RNA" in the specification make it clear that no such definition was intended. The rest of the specification that injects ambiguity into, and demonstrates, ToolGen's misreading of a single sentence therein cannot be ignored.

The limited substantive challenges raised by ToolGen as to the preferred embodiments at 29:7-21-30:8 are premised on ignoring the specification's plain language. Opp. 3. For instance, Example 6 describes an "optimized guide RNA," and in the example, dual-molecule RNA and

single-molecule RNA systems are compared, with an improved guide RNA shown in certain embodiments. Thus, the improvements, including to both dual- and single-molecule RNA are described as an "optimization" of the broader genus of guide RNA that includes "dual molecule RNA." If the two species were not both encompassed in the term "guide RNA," then guide RNA would be described as "superior" or "inferior" to dual-molecule guide RNA, not an "optimization" of the broader genus.

At 32:12-33:3, ToolGen addresses claims 15, 17–26, and 28–41 of the 713 Patent and claims 1–24 of the 418 Patent, which do not use the term "guide RNA." Opp. 3. While the header of this section claims these are single-guide claims, ToolGen does not appear to so contend. Instead, ToolGen properly refers to them as "generic" claims and appears to argue that because they are generic claims, they should be designated to the species Count. ToolGen argues that Broad made no argument as to why these claims should be de-designated and that such argument is waived. But these claims were addressed as part of ***all*** of the generic claims in Broad's Motion—there is no waiver, and they should be de-designated as set forth there and further discussed below.

ToolGen does not dispute that under Count 1, priority will be determined based on only single-molecule RNA proofs. ToolGen also does not dispute that Broad could lose its generic RNA claims even if Broad was the first to conceive and reduce to practice the generic RNA invention. Instead, at 33:7-34:6, ToolGen argues that 37 C.F.R. § 41.207(b)(2) is an inflexible rule. But the very title of this rule ("Presumptions") cannot be ignored under proper interpretative practice. *See Tesoro Hawaii Corp. v. United States*, 405 F.3d 1339, 1346 (Fed. Cir. 2005) ("We construe a regulation in the same manner as we construe a statute, by ascertaining its plain meaning."); *Gorman v. Nat'l Transp. Safety Bd.*, 558 F.3d 580, 588 (D.C. Cir. 2009); *Phelps Dodge Corp. v. Fed. Mine Safety & Health Review Comm'n*, 681 F.2d 1189, 1192 (9th Cir. 1982). The PTAB's

own interpretation of Rule 207(b)(2) faithfully treats the two-way test just as the Rule's title states—as a *presumption*. Rules of Practice Before the Board of Patent Appeals and Interferences, 69 Fed. Reg. 49960 (Aug. 12, 2004) ("It simply creates a presumption that must be addressed.").

Finally, at 33:25-34:6, ToolGen states that Broad's "argument presumes, without proving, that Broad was first to invent the subject matter of its generic claims." Opp. 3. The response is that, while Broad proffered proof that it was the first to invent the generic RNA invention in connection with Motion 1, it was not necessary to prove that its dual-molecule RNA proofs came before its experiments with single-molecule RNA as in Count 1 at this time. Unless Motion 1 is granted, if ToolGen successfully attacks Broad's single-molecule RNA proofs, Broad would lose its generic RNA claims even if Broad's dual-molecule RNA experiments were successful and came before ToolGen's single-molecule RNA work.

IV. CONCLUSION

For the foregoing reasons, Broad respectfully requests that the PTAB grant its Motion 3.

Dated: September 24, 2021

Respectfully submitted,

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APPENDIX A: LIST OF EXHIBITS CITED

EX.	DESCRIPTION
1021	U.S. Patent No. 8,967,359, issued April 15, 2014, to Feng Zhang.
1207	Makarova et al., Evolution and Classification of the CRISPR-Cas Systems, Nature Review Microbiology, 9, 467–77 (2011).
1311	Sapranaukas et al., The Streptococcus thermophiles CRISPR/Cas system provides immunity in Escherichia coli, Nucleic Acids Research, 39(21), 9275–9282 (2011).
1314	Wu et al., Effect of Genome Size on AAV Vector Packaging, The American Society of Gene & Cell Therapy, 18(1), 80–86 (2010).
1420	August 6, 2021 Declaration of Dr. Barry Stoddard, Ph.D.
2054	U.S. Patent Publication No. 2010/0076057, published on March 25, 2010 to Sontheimer et al.
2069	Interview Summary in Application No. 14/685568, October 28, 2016
2110	Paper 893, Decision on Motions 37 C.F.R. § 41.125(a), Interference 106,048, February 15, 2017.
2121	Paper 877, Declaration of Interference, Interference 106,015, September 10, 2020.
2258	Fieck, A., et al., Modifications of the E. coli Lac repressor for expression in eukaryotic cells: effects of nuclear signal sequences on protein activity and nuclear accumulation, Nucleic Acids Res. 20(7) 1785-1791 (1992) (Ex. 1235)
2408	Second Deposition Transcript of Dr. Dana Carroll, September 13, 2016.
2454	Declaration of Christoph Seeger, executed May 28, 2021.
2459	Deposition Transcript of Barry Stoddard, Ph.D., September 16, 2021.
2460	Declaration of Bryan R. Cullen, PH.D. Application No. 14/685,510 Prosecution History, dated June 24, 2016.
2648	Lino, et al. Delivering CRISPR: a review of the challenges and approaches, Drug Delivery, VOL. 25, NO. 1, 1234–1257 (2018). https://doi.org/10.1080/10717544.2018.1474964

APPENDIX B: STATEMENT OF MATERIAL FACTS

Broad's Material Facts And ToolGen's Responses

1. Count 1 is an "or" count drawn (first half) to a eukaryotic CRISPR-Cas9 system comprising Cas9 and RNA that comprises a guide sequence fused to a tracr sequence that targets and hybridizes to a DNA target sequence (Ex. 2011, '359 patent, claim 18), or (second half) a mammalian cell comprising a CRISPR-Cas9 system comprising Cas9 or a nucleic acid encoding Cas9 and a chimeric guide RNA (Ex. 2062, ToolGen, claim 85). Paper 1 at 12-13.

RESPONSE: Admitted.

2. Proposed Count 2 substitutes the Broad half of the current "or" count with allowed claim 1 of the 710 application that encompasses both dual-molecule and single-molecule RNA. Broad Motion 1 at 4.

RESPONSE: Admitted.

3. Under Count 1 or Proposed Count 2, one may deliver the system via non-vector means such as microinjection of the system as a mature RNP or a RNA and mRNA. Paper 1 at 12-13; Broad Motion 1 at 4.

RESPONSE: Admitted.

4. There is no teaching or suggestion in either Count 1 or Proposed Count 2, or in the prior art, directing a POSA to vector-delivered RNA for use in eukaryotic cells, nor would a 2012 POSA have had a reasonable expectation of success in so doing. *See generally* Ex. 2454, Seeger Decl. ¶¶ 212-27; Ex. 2110, Paper 893, 048 Decision on Motions; Ex. 2121, Paper 877, 115 Decision on Motions; Paper 1 at 12-13; Broad Motion 1 at 4.

RESPONSE: Denied.

5. There are many ways that the RNA components of the system can be delivered to a eukaryotic cell, many of which performed poorly or failed outright. *See* Ex. 2454, Seeger Decl.

¶¶ 212-27.

RESPONSE: The statement is too vague to be admitted or denied, therefore, ToolGen is unable to admit or deny this fact.

6. ToolGen's priority application (Kim P1) discloses for delivery to the eukaryotic cell only a sequential process that includes adding extraordinarily high amounts of already-translated RNA to cells previously transfected with Cas9. *Id*. ¶¶ 219-22; Ex. 2062 at 9.

RESPONSE: Denied.

7. Dr. Zhang's vector systems are among the most requested CRISPR vectors by researchers in the field. Exs. 2502-2505; Ex. 2454, Seeger Decl. ¶¶ 225-27.

RESPONSE: Denied.

8. Broad licensee Editas Medicine is conducting a clinical trial using CRISPR-Cas9 systems that include vector delivery to human patients. *Id*.; Ex. 2687, MacLeod 2021; Exs. 2517, 2686-87.

RESPONSE: Admitted, but only to the extent the clinical trial involves two patients; otherwise, denied.

9. Neither Count 1 nor Proposed Count 2 recites any particular ortholog of Cas9 protein, including SaCas9. Paper 1 at 12-13; Broad Motion 1 at 4.

RESPONSE: Admitted.

10. There is no teaching or suggestion in Count 1, Proposed Count 2, or the prior art to use SaCas9 in CRISPR-Cas9 systems in eukaryotic cells.

RESPONSE: Denied.

11. CRISPR-Cas9 systems using SaCas9 possess a combination of small size and high efficacy in eukaryotes. Ex. 2017, 406 Patent, 83:1-25-84:1-23; Ex. 2454, Seeger Decl. ¶¶ 228-48.

RESPONSE: This statement is too vague to be admitted or denied, therefore, ToolGen is unable to admit or deny this fact.

12. Small size and high efficacy in eukaryotes render CRISPR-SaCas9 systems advantageous for use in eukaryotic cell-based applications where vector delivery using the highly versatile adeno-associated virus (AAV) is favored because AAV vectors are space-constrained. Ex. 2454, Seeger Decl. ¶¶ 237, 245; Ex. 2017, 406 Patent, 83:1-25-84:1-23.

RESPONSE: This statement is too vague to be admitted or denied, therefore, ToolGen is unable to admit or deny this fact.

13. As of 2012, the most commonly studied Cas9 ortholog in prokaryotes was from *Streptococcus pyogenes* ("SpCas9"), and more than 600 bacterial Cas9 orthologs that had been identified. Ex. 2226; Ex. 2454, Seeger Decl. ¶ 234.

RESPONSE: Denied.

14. SpCas9 is larger than SaCas9. Ex. 2454, Seeger Decl. ¶¶ 235, 242-43, 245.

RESPONSE: Admitted.

15. As of 2012, there was nothing in the art pointing the POSA to use SaCas9 in eukaryotic cells including nothing point to SaCAs9 out of the many known "small" Cas9 orthologs that are similar in size to, or smaller than, SaCas9. Ex. 2454, Seeger Decl. ¶¶ 230-44.

RESPONSE: Denied.

16. Broad determined that using Cas9 from *Streptococcus aureus* in a CRISPR-Cas9 system for DNA cleavage or editing or for modulating transcription in a eukaryotic cell provides a surprising combination of benefits not taught or suggested by the prior art, namely high efficiency and small size. *See* Ex. 2454, Seeger Decl. ¶¶ 228-48.

RESPONSE: Denied.

17. SaCas9 is used for more therapeutic applications than any other Cas9 ortholog.

Ex. 2454, Seeger Decl. ¶ 246; *see* Ex. 2017, 406 Patent at 83:25-84:23; Exs. 2687, 2517, 2686.

RESPONSE: The statement is too vague to be admitted or denied, therefore, ToolGen is unable to admit or deny this fact.

18. All claims of Broad's 418 Patent (Ex. 2060) are directed to the improvement in which the Cas9 is not taken from a single organism but rather is a chimeric Cas9 that includes two fragments from different Cas9, which has unexpected benefits. *Id*. at 83:45-52; Ex. 2454, Seeger Decl. ¶¶ 249-57.

RESPONSE: Admitted to the extent the Broad Ex. 2060 Claim 1 is directed to a chimeric Cas9 that includes two fragments from different Cas9; otherwise, denied.

19. Count 1, Proposed Count 2, and the prior art do not teach, suggest, or provide motivation to a POSA to design a chimeric Cas9 that is comprised of two fragments from different organisms. Ex. 2454, Seeger Decl. ¶¶ 249-57; Paper 1 at 12-13; Broad Motion 1 at 4.

RESPONSE: Denied.

20. Ex. 2060 418 Patent, 4:21-25 recites "[t]hese chimeric Cas9 proteins may have a higher specificity or a higher efficiency than the original specificity or efficiency of either of the individual Cas9 enzymes from which the chimeric protein was generated."

RESPONSE: Admitted.

21. The 418 Patent (Ex. 2060 at 83:45-52) also recites benefits of a chimeric Cas9.

RESPONSE: Admitted to the extent Ex. 2060 recites benefits of a chimeric Cas9 without providing any supporting data; otherwise, denied.

22. Neither Count 1 nor Proposed Count 2 recite that the Cas9 has any NLSs at all nor do they recite use of two or more NLSs. Paper 1 at 12-13; Broad Motion 1 at 4.

RESPONSE: Denied.

23. The prior art did not provide any teaching or suggestion to use two or more NLSs

in a CRISPR-Cas system in a eukaryotic cell nor was there a reasonable expectation of success in using two or more NLSs. Ex. 2454, Seeger Decl. ¶¶ 258-78.

RESPONSE: Denied.

24. Broad determined that, unexpectedly, the use of two or more NLSs resulted in CRISPR-Cas9 systems with significantly improved localization to the nucleus, as compared to systems lacking two or more NLSs. *See generally* Ex. 2454, Seeger Decl. ¶¶ 258-78; Ex. 2001, Zhang B1, Figure 1B; Ex. 2201, Cong 2013, Figure 1A; Ex. 2793.

RESPONSE: Denied.

25. A POSA would have understood that adding amino acids to a protein such as Cas9 could alter its folding affecting its structure and function in ways that were not predictable. Ex. 2454, Seeger Decl. ¶¶ 271-76; Ex. 2258 at 1785-1790; Exs. 2263-2265.

RESPONSE: Admitted.

26. Neither Count 1 nor Proposed Count 2 recites that the Cas9 is fused to specified protein domains. Paper 1 at 12-13; Broad Motion 1 at 4.

RESPONSE: Denied.

27. There is no teaching or suggestion in Count 1, Proposed Count 2, or the prior art directing a POSA to modify the naturally occurring Cas9 to include protein domains. Ex. 2454, Seeger Decl. ¶¶ 279-84.

RESPONSE: Denied.

28. The 233 Patent notes that there is a benefit to fusing functional domains to Cas9, as doing so can "to turn the Cas9/gRNA CRISPR system into a generalized DNA binding system [which] can execute functions beyond DNA cleavage." Ex. 2024 at 73:22-37.

RESPONSE: Admitted to the extent the quoted language appears in Ex 2024; otherwise, denied.

29. Broad's 713 Patent (Ex. 2043), includes claims, including claims 15-26 and 28-41, that are not limited to single-molecule RNA and do not contain the term "guide RNA." *See* Ex. 2454, Seeger Decl. ¶¶ 150-51.

RESPONSE: Denied.

30. Independent claim 1 and dependent claims 2-24 of Broad's 418 Patent (Ex. 2060) do not recite any RNA component. *See* Ex. 2454, Seeger Decl. ¶¶ 152-54.

RESPONSE: Denied.

31. In U.S. Patent No. 8,895,308, claim 1 describes a method using CRISPR-Cas system having "guide RNA" that hybridizes to the target sequence and claim 6 covers the "method of claim 1, wherein the guide RNA comprises a guide sequence and a tracr sequence." Ex. 2013.

RESPONSE: Admitted to the extent the quoted language appears in Ex 2013; otherwise, denied.

32. In U.S. Patent No. 8,906,616, claim 1 describes a CRISPR-Cas system having a "guide RNA" polynucleotide sequence comprising a guide sequence, a tracr mate sequence, and a tracr sequence, claim 2 covers the "composition of claim 1, wherein the modified guide RNA comprises a chimeric guide sequence and a tracr sequence" and claim 5 covers the "composition of claim 1, wherein the modification comprises fusing the tracr mate sequence and the tracr sequence through an artificial loop." Ex. 2014.

RESPONSE: Admitted to the extent the quoted language appears in Ex 2013; otherwise, denied.

33. In the 965 Patent (Ex. 2012), none of the dependent claims include limitations that the components of the guide RNA be fused or bound to one another in a chimeric manner.

RESPONSE: Denied.

34. The Broad patents disclose preferred embodiments that are dual-molecule RNA systems. Ex. 2011, 359 Patent at 43:49-53, 44:5-8; Ex. 2454, Seeger Decl. ¶¶ 179-81.

RESPONSE: Denied.

35. On April 13, 2015, ToolGen filed the 510 Application as a continuation of PCT application PCT/KR2013/009488 (the "ToolGen PCT application") (Ex. 2067), which had been filed on October 23, 2013, and claiming priority to provisional patent applications dating back to October 23, 2012. Ex. 2062, 510 Application; Ex. 2067, ToolGen PCT.

RESPONSE: Admitted.

36. ToolGen's patent applications explicitly define "guide RNA" to encompass both dual- and single-molecule RNA configurations:

> In the present invention, ***the guide RNA may consist of two RNA***, i.e., CRISPR RNA (crRNA) and transactivating crRNA (tracrRNA) ***or be a single-chain RNA*** (sgRNA) produced by fusion of an essential portion of crRNA and tracrRNA.

Ex. 2068, ¶¶ [168]-[0170]; Ex. 2067, ToolGen PCT, ¶¶ [0168]-[0169]; Ex. 2062, 510 Application, ¶¶ [0094]-[0095] (emphasis added).

RESPONSE: Admitted to the extent the quoted language appears in Ex 2068, Ex 2067, and Ex 2062; otherwise, denied.

37. In the original claims of the ToolGen PCT application and the 510 Application, the inventors included claims reciting "guide RNA," without any restriction as to RNA configuration. Ex. 2067, ToolGen PCT; Ex. 2062, 510 Application original claims.

RESPONSE: Denied.

38. In the original claims of the ToolGen PCT application and the 510 Application, the inventors included claims 3 and 4, which respectively limited that "guide RNA" to a dualRNA (a dual molecule RNA) and a "single-chain" guide RNA (a single molecule RNA). *Id*.

RESPONSE: Denied.

39. In the disclosures from Ex. 2067 and 2063 referenced in MFs 4 and 5, ToolGen used the term "guide RNA" consistent Jinek 2012's use of the term to include both sgRNA and dualRNA.

RESPONSE: Denied.

40. Jinek 2012 states: "In this ternary complex, the dual tracrRNA:crRNA structure acts as guide RNA that directs the endonuclease Cas9 to the cognate target DNA." Ex. 2202, Jinek 2012, at Figure S1 description.

RESPONSE: Admitted.

Broad's Responses to ToolGen's Additional Material Facts

41. As of the priority date, the use of vectors, *e.g.*, plasmid vectors, was well known and widely employed for introducing DNA sequences encoding RNA molecules into eukaryotic cells. Ex. 1420, ¶¶43–45.

RESPONSE: Denied.

42. Vectors are molecules that are delivered into a cell for RNA expression. Ex. 1420, ¶43.

RESPONSE: Denied.

43. In 2012, the prior art was replete with descriptions of the use and benefits of vectors in eukaryotic cells. *See, e.g.*, Ex. 1309, 1229; Ex. 1310, 956; Ex. 1420, ¶143.

RESPONSE: Denied.

44. In 2012, a POSA would have understood that vectors provided the advantage of continuous and sustained RNA expression and chemical stability within eukaryotic cells. Ex. 1420, ¶144.

RESPONSE: Denied.

45. sgRNA, with its single-stranded structure, is significantly more susceptible to degradation compared to double-stranded RNA structures. Ex. 1420, ¶144.

RESPONSE: Denied.

46. In 2012, a POSA would have known about vectors, as well as their numerous benefits. Ex. 1248, 505 (Abstract); Ex. 1243, 28, 30, 64; Ex. 1309, 1229; Ex. 1310, 956; Ex. 1420, ¶145.

RESPONSE: Denied.

47. Dr. Seeger in paragraph 239 of his declaration articulates the number of "Cas9 orthologues smaller than SpCas9," not SaCas9; and the cited Figs. 4A–D do not identify the proteins that were as small or smaller than SaCas9 or SpCas9. Ex. 2017, Figs. 4A–D; Ex. 1420, ¶97.

RESPONSE: Denied.

48. As of the priority date, it was well known in the art to design chimeric proteins in both prokaryotic and eukaryotic cells. Ex. 1317, Abstract; Ex. 1318, Abstract.

RESPONSE: Denied.

49. As of the priority date, several naturally occurring chimeric variants of Cas proteins were well known in the art. Ex. 1207, Abstract; Ex. 1420, ¶148.

RESPONSE: Denied.

50. As of the priority date, a chimeric Cas9 protein was known for the ability to alter the functions of the wild-type Cas9 protein. Ex. 1420, ¶148.

RESPONSE: Denied.

51. As of the priority date, PAM sequences were known to differ between Cas proteins. Ex. 1207, 467; Ex. 1420, ¶148.

RESPONSE: Admitted.

52. Designing a chimeric Cas protein to modify target PAM sequences would have been routine for a POSA as of the priority date. Ex. 1420, ¶148.

RESPONSE: Denied.

53. U.S. Patent No. 8,889,418 ("the '418 patent") provides no data or testing results to support Broad's argument that chimeric Cas9 has unexpected benefits. Ex. 2060, 83:45–52.

RESPONSE: Denied.

54. Before the priority date, a POSA knew to attach, and had attached, NLSs to Cas proteins of Type I and III CRISPR systems, and to proteins of Zinc Finger Nucleases, TALENs, Rec A, Lac, and HaloTag™ proteins. Ex. 1022, ¶¶0054, 0058; Ex. 1319, Abstract; Ex. 1320 , Fig. 1(b); Ex. 1321, 3095; Ex. 2258, 1785 (last paragraph); Ex. 1322, 11; Ex. 1420, ¶115.

RESPONSE: Denied.

55. Park (Ex. 2262) notes that the degree of nuclear localization corresponded to the number of NLSs present. Ex. 2262, 31427; Ex. 1420, ¶122.

RESPONSE: Denied.

56. Before the priority date, several well-known and readily available commercial eukaryotic expression vectors attached two or more NLSs to proteins expressed from the vector. Ex. 1323, 24391; Ex. 1325, 42189 ; Ex. 1420, ¶127.

RESPONSE: Denied.

57. Before the priority date, POSAs routinely used NLSs to localize prokaryotic and eukaryotic proteins, with different sequence and three-dimensional structure, to the nucleus. Ex. 1420, ¶130.

RESPONSE: Denied.

58. Before the priority date, POSAs routinely attached two or more NLSs to proteins to increase nuclear localization while maintaining protein function. Ex. 1420, ¶¶130–31.

RESPONSE: Denied.

59. Jinek 2012 attached a four-amino-acid tag to a Cas9 while retaining function. Ex. 2202; Ex. 1420, ¶¶130–31.

RESPONSE: Denied.

60. In the 115 interference, the Board found that "Fieck teaches that one NLS position produced 'efficient nuclear accumulation, strong repressor activity and greater sensitivity to IPTG induction.'" Ex. 1101, 64:2–4 (quoting Ex. 2258, Abstract).

RESPONSE: Denied.

61. Broad's exhibits 2258, 2263, 2264, and 2265 each shows successful attachment of peptide tags to proteins after routine experimentation. Ex. 2258, 1785; Ex. 2263, Abstract, 6111; Ex. 2264, 7738, 7744; Ex. 2265, 265, 268; Ex. 1420, ¶¶137–39.

RESPONSE: Denied.

62. Before the priority date, given Count 1 or Proposed Count 2 as prior art, a POSA would have expected that attaching multiple NLSs to a protein, including two or more NLSs, would improve nuclear localization. Ex. 1420, ¶141.

RESPONSE: Denied.

63. Zhang 2012 and Cong 2013 show that a POSA would have expected increased nuclear localization of the Cas9 protein with two NLSs compared to when only one or no NLS is used. Ex. 2001, Fig. 1B; Ex. 2201, Fig. 2A; Ex. 1420, ¶141.

RESPONSE: Denied.

64. Zhang 2012 and Cong 2013 fail to show that a Cas9 protein with two NLSs was "unexpectedly more efficient." Ex. 2001, Fig. 1B; Ex. 2201, Fig. 2A; Ex. 1420, ¶141.

RESPONSE: Denied.

65. Neither Zhang 2012 nor Cong 2013 provide data on efficiency, *i.e.*, function of the Cas9 protein. Ex. 2001, Fig. 1B; Ex. 2201, Fig. 2A; Ex. 1420, ¶141.

RESPONSE: Denied.

66. Zhang 2012 and Cong 2013's data only shows the expected nuclear localization of the Cas9 protein. Ex. 2001, Fig. 1B; Ex. 2201, Fig. 2A; Ex. 1420, ¶141.

RESPONSE: Denied.

67. Table M of Example 14 of the '445 patent does not show an unexpected or significant increase in efficiency with two or more NLSs. Ex. 2029, 124:27–125:21; Ex. 1420, ¶141.

RESPONSE: Denied.

68. Table M of Example 14 of the '445 patent shows that the Cas9 protein with one NLS attached to its N-terminus, one NLS attached to its C-terminus, and an NLS attached to both its N-and C-termini, had the following activity: 6.45% (± 0.63), 5.10% (± 0.33), and 8.9% (± 0.60), respectively. Ex. 2029, 124:27–125:21; Ex. 1420, ¶141.

RESPONSE: Admitted.

69. As of the priority date, a POSA would have understood that an NLS attached to a Cas9 protein as a fusion. Ex. 1420, ¶150.

RESPONSE: Denied.

70. As of the priority date, it was well known in the art to fuse proteins to specified protein domains. Ex. 1327, Abstract; Ex. 1328, Abstract; Ex. 1329, Abstract.

RESPONSE: Denied.

71. Before the priority date, generating protein fusions had been used as a method of purifying proteins, and fusing green fluorescent protein (GFP) to proteins was also used as

method of detecting protein localization in prokaryotic and eukaryotic cells. Ex. 1329; Ex. 1420, ¶151.

RESPONSE: Denied.

72. Generating protein fusions would have been routine for a POSA as of the priority date. Ex. 1420, ¶151.

RESPONSE: Denied.

73. U.S. Patent No. 8,993,233 does not provide any data or testing results supporting unexpected benefits from fusing functional domains to Cas9. Ex. 2024, 73:22–29.

RESPONSE: Denied.

74. Broad's patent specifications define "guide RNA" as a single-molecule guide. Ex. 2011, 12:6–10; Ex. 1420, ¶53–54.

RESPONSE: Denied.

75. U.S. Patent No. 8.697,359 ("the '359 patent") states: "In aspects of the invention the terms "*chimeric RNA*", "*chimeric guide RNA*", "*guide RNA*", "*single guide RNA*" and "synthetic guide RNA" are *used interchangeably* and *refer to the polynucleotide sequence* comprising the guide sequence, the tracr sequence and the tracr mate sequence." Ex. 2011, 12:6–10

RESPONSE: Denied.

76. All of Broad's specifications include this limiting definition: "In aspects of the invention the terms "*chimeric RNA*", "*chimeric guide RNA*", "*guide RNA*", "*single guide RNA*" and "synthetic guide RNA" are *used interchangeably* and *refer to the polynucleotide sequence* comprising the guide sequence, the tracr sequence and the tracr mate sequence." Ex. 2016; Ex. 2013; Ex. 2011.

RESPONSE: Denied.

77. The Board previously held in the 115 interference that Broad defines a "guide RNA" as a singular "polynucleotide sequence" comprising the "guide sequence, the tracr sequence[,] and the tracr mate sequence." Ex. 1101, 29:22–30:5; Ex. 1420, ¶54.

RESPONSE: Denied.

78. A POSA as of the priority date would have understood that "guide sequence" and "tracr mate sequence" refer to the crRNA component of the CRISPR-Cas system and "tracr sequence" refers to the tracrRNA component of the CRISPR-Cas system. *See, e.g.*, Ex. 2011, 12:10–15, 21:18–25, 21:41–45; Ex. 2202, Fig. 5(A); Ex. 2214, Fig. 4; Ex. 1420, ¶55.

RESPONSE: Denied.

79. A "guide RNA," as defined by Broad, comprises both RNA components of the CRISPR-Cas system—crRNA and tracrRNA—in a singular polynucleotide sequence, *i.e.*, a single-molecule guide. Ex. 1420, ¶55.

RESPONSE: Denied.

80. Broad's specifications use "guide RNA" interchangeably with "chimeric RNA" and "single guide RNA," which are single-molecule guides. Ex. 1420, ¶56.

RESPONSE: Denied.

81. Broad's specifications use "guide RNA" interchangeably with "chimeric RNA" and "single guide RNA" with no qualifying language. Ex. 2011, 12:6–10; Ex. 1420, ¶57–58.

RESPONSE: Denied.

82. Broad's specifications do not disclose a single embodiment that is a dual-molecule RNA system. Ex. 2016; Ex. 2013; Ex. 2011.

RESPONSE: Denied.

83. The term "guide RNA" is used throughout Broad's specifications to refer to single-molecule guide RNA; different terminology is used to refer to dual-molecule guide RNA. *See, e.g.*, Ex. 2016, 105:1–106:16; Ex. 2013, 38:33–43; Ex. 2011, 21:41–45, 43:49–53, 43:56–50:5.

RESPONSE: Denied.

84. Example 6 of U.S. Patent No. 8,889,356 uses the term "guide RNA" to refer to only to single-molecule guides. Ex. 2016, 105:1–106:16; Ex. 1420, ¶64.

RESPONSE: Denied.

85. Example 6 of U.S. Patent No. 8,889,356 uses "tracrRNA" and "direct repeat sequence" to refer to a dual-molecule guide, but uses "chimeric guide RNA" or "guide RNA" to refer to a single-molecule guide. Ex. 2016, 105:1–106:16; Ex. 1420, ¶64.

RESPONSE: Denied.

86. Example 6 of U.S. Patent No. 8,889,356 only reports the results of the single-molecule guides, concluding that the "optimized chimeric guide RNA works better as indicated in Fig. 3." Ex. 2016, 106:9–10; Ex. 1420, ¶64.

RESPONSE: Denied.

87. Example 6 of U.S. Patent No. 8,889,356 referenced Fig. 3 also only shows the results of guide sequences 1, 2, and 3, which the specification describes as single-molecule guides. Ex. 2016, 105:1–106:16, Fig. 3; Ex. 1420, ¶67.

RESPONSE: Denied.

88. Example 6 of U.S. Patent No. 8,889,356 is titled "[o]ptimization of the Guide RNA," referring to only the single-molecule guides that were tested. Ex. 2016, 105:4–5; Ex. 1420, ¶64.

RESPONSE: Denied.

89. When the disclosures in Broad's specifications are read in proper context, none of them uses the term "guide RNA" to refer to a dual-molecule guide. Ex. 1420, ¶¶65–70.

RESPONSE: Denied.

90. Column 38, lines 33 through 43 of U.S. Patent No. ("the '308 patent") section relates to single-guide molecules and uses the phrase "or combination of tracrRNA and crRNA" to describe the term "chimeric guide RNA." Ex. 2013, 38:33–43; Ex. 1420, ¶65.

RESPONSE: Denied.

91. The portions of the '359 patent to which Dr. Seeger cites, Ex. 2454, ¶181 (citing Ex. 2011, 21:41–45, 43:57–50:5 (Example 1)), fail to recite the term "guide RNA," and do not use it in reference to a dual-molecule guide, Ex. 1420, ¶67.

RESPONSE: Denied.

92. The term "guide RNA" did not have a plain and ordinary meaning in the art as of the priority date. Ex. 1420, ¶76.

RESPONSE: Denied.

93. Jinek 2012 (Ex. 2202), and U.S. Patent Application 61/652,086 ("the '086 application") (Ex. 2002) demonstrate that there was no plain and ordinary meaning as of the priority date for "guide RNA." Mot. 27:4–6; Ex. 2454, ¶¶164–65, 167; Ex. 1420, ¶77.

RESPONSE: Denied.

94. Jinek 2012 recites "guide RNA"; the '086 application does not. Ex. 2202, 830; Ex. 2002.

RESPONSE: Denied.

CERTIFICATE OF FILING AND SERVICE

I hereby certify that on September 24, 2021, a true and complete copy of the foregoing **BROAD REPLY 3** is being filed and served by 5:00 pm PT /8:00 pm ET via the Interference Web Portal and by agreement served by email on the Senior Party by 8:00 pm PT / 11:00 pm ET to:

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