EUROPEAN PATENT 4,357,457
TOOLGEN INCORPORATED
INITIAL ARGUMENTS TO SUPPORT OPPOSITION February 2025

1. INTRODUCTION and REQUESTS

1.1. European patent 4357457 is opposed by Vertex Pharmaceuticals Incorporated under Article 99 EPC and Rule 76 EPC, as set out in the accompanying Form 2300. The opponent requests revocation of the Patent under Article 101(2) EPC. If the opposition division cannot revoke the Patent on the basis of the written submissions then the opponent requests oral proceedings under Article 116 EPC.

1.2. The documents relied upon in the opposition are listed in section 5 on page 10. The opponent reserves the right to rely additionally upon other documents cited in the patent itself, and documents filed in the opposition proceedings by any other opponent or by the patentee.

1.3. At this stage the opponent provides brief arguments under Articles 100(a) and 100(c) EPC, but may supplement these before the 9 month opposition deadline of 16th July 2025. It is enough for now to show that the claims go beyond the content of the application as filed and that, anyway, they lack priority basis and so lack novelty and/or inventive step.

2. THE CLAIMS GO BEYOND THE CONTENT OF THE PARENT APPLICATION AS FILED

2.1. The patent is a second-generation divisional application filed from EP-3372679, which was a first-generation divisional of the original PCT application. The granted claims were all included in the second-generation divisional at the time of filing, but none of them finds basis in either the original PCT application (grandparent) or in the first-generation divisional application (parent), so the claims do not meet the requirements of Article 76(1) EPC.

2.2. Granted claim 1 reads:

> 1. A composition comprising a single-chain guide RNA (sgRNA) and a *Streptococcus pyogenes* Cas9 protein, wherein the sgRNA and the Cas9 protein are present in a molar ratio ranging from 29 : 14.0 to 29 : 1.4.

Thus the claim requires a mixture of sgRNA and *Streptococcus pyogenes* Cas9 (SpCas9), with sgRNA present at a molar excess in the approximate range of 2.07:1 and 20.7:1.

2.3. As basis for this claim the applicant pointed to [329] on page 29 of the PCT application, and this paragraph indeed contains the application's only reference to the molarities which were used to derive the claimed range of molar ratios, as it refers to an experiment where 29 µM sgRNA was used with 1.4-14 µM Cas9. However, there is no basis for extracting these values from the specific context of Example 2 and using them to derive a general molar ratio, so claim 1 extends beyond the content of the application as filed, and it is a typical example of an unallowable **intermediate generalisation**.

2.4. Although the claimed molar ratio range was used in Example 2, it was disclosed only in the context of a very specific experiment, using a specific sgRNA targeting a specific gene, using a specific form of Cas9, performed on specific cells under specific conditions, and at a specific scale which leads to specific amounts and concentrations of both Cas9 and sgRNA. In the

claim, however, the molar ratio range has been stripped away from this context and generalised to any sgRNA and any form of SpCas9, used at any concentration. The application as filed does not provide any basis for making this generalisation. For instance, the disclosure to use 29 µM sgRNA with 1.4-14 µM Cas9 is not a disclosure of using any concentration of these components at the same molar ratio. For instance, there is no disclosure in Example 2 that the concentrations could be scaled up 10x (*i.e.* to use 290 µM sgRNA with 14-140 µM Cas9), or scaled down by half (*i.e.* to use 14.5 µM sgRNA with 0.7-7µM Cas9), *etc*. Rather, Example 2 discloses the use of particular molarities but does not disclose that the ratio between these molarities is a parameter of interest.

2.5. The basic requirement for disclosure under Article 76(1) EPC was set out by the Enlarged Board in G2/10: *"the skilled person may not be presented with new technical information"* (¶4.5.1). The prohibition on providing new information is also stated in the *Guidelines for Examination* in the passage which deals with intermediate generalisations (H-V,3.2.1):

> These conditions are to be understood as an aid to assessing, in the particular case of an intermediate generalisation, if the amendment fulfils the requirements of Art. 123(2). In any case it has to be ensured that the skilled person is not presented with information which is not directly and unambiguously derivable from the originally filed application, even when account is taken of matter which is implicit to a person skilled in the art using the common general knowledge.

2.6. Contrary to this requirement, the molar ratio range in claim 1 certainly provides the skilled person with new technical information. In short, a skilled person would not know from the original patent application that the ratio of SpCas9 and sgRNA (still less their molar ratio) is a relevant parameter. They can see that this range was used in Example 2, but there is no suggestion here, or anywhere else in the application, that this range would be generally applicable to SpCas9 or to sgRNA in general.

2.7. Immediately prior to the passage quoted above, section H-V,3.2.1 of the *Guidelines* also states that a feature cannot be used as the basis of an amendment unless:

> the overall disclosure justifies the generalising isolation of the feature and its introduction into the claim.

but the "overall disclosure" of the present application cannot justify a generalisation from Example 2. Perhaps if the description had disclosed in general terms that the ratio of Cas9 and sgRNA could be varied or controlled then Example 2 might provide a pointer to selecting specific ratios, but there is no such general disclosure. On the contrary, the Cas9/sgRNA molar ratio was varied only within the specific context of Example 2. By introducing this feature into claim 1, the applicant provided to the skilled person for the first time the new technical information that the Cas9/sgRNA molar ratio is a parameter of interest. The claim violates Article 76(1) EPC because it unallowably generalises from a single specific experiment to claim all forms of SpCas9 and all sgRNAs having that ratio range, but there was nothing in the original disclosure to justify this generalisation. The values specified in claim 1 are merely the specific molar amounts which were used for one experiment, and they were not disclosed as values to be used for defining a general range of ratios.

2.8. Looking again at [329], the applicant might as well have generalised to claim the use of any Cas9 which carries a hexa-histidine tag (see [329], line 3), or any experiment where SpCas9 & sgRNA are *"incubated for 48hr"* (line 12) — but none of these specific features is fit for generalisation within the context of the application as a whole. The applicant might be able to justify claiming a specific Cas9 protein having a hexa-histidine tag, mixed with the specific sgRNA shown in Table 2, in the amounts disclosed in [329], but there is no justification for claiming a mixture which does not have these limitations.

2.9. A further problem with the ratios in claim 1 is the precision which is specified in the smaller ratio. Whereas [329] refers to a sgRNA concentration of "29 µM", and a Cas9 range from "1.4-14 µM", the claim refers to a molar ratio of 29:14.0. The claim therefore specifies three significant figures of precision, whereas the original disclosure used only two. Whereas the original disclosure of "14 µM" might be seen as covering 13.5-14.4 µM, a claim to "14.0 µM" would instead cover from 13.95 to 14.04 µM (*"rounding-off convention"*; G/L G-VI,7.1), and these differences change the understanding of the end-point of the ratio range:

Cas9 value	Rounding-off	Upper rounding	Lower rounding	End-point
14	13.5 - 14.4	29:13.5 = 2.148	29:14.4 = 2.014	2.014 - 2.148
14.0	13.95 - 14.04	29:13.95 = 2.079	29:14.04 = 2.066	2.066 - 2.079

2.10. The change in precision therefore means that the claim defines a different end-point from the disclosure in [329]. There is no technical justification for defining the claims using the number "14.0" rather than the original disclosure "14", so the claim goes beyond the content of the original application for this reason as well.

2.11. This issue was already considered by a board of appeal in T2203/14, which is referred to in the CLBA 'white book' at II.E.1.5.3. In that case the term "5 microns" was amended to "5.0 microns" and the board held that this change was not allowable because the two values did not have the same meaning. In rejecting the claim, the board noted that the extra digit of precision changed the boundary of the claim: *"the appearance of the alloy may therefore be different if the thickness is 5.4 microns – a thickness covered by claim 1 as filed – compared to 5.04 microns – the upper limit of claim 1 as granted"* (section 1.4).

2.12. This point can also be seen by asking why the Cas9 molarity was changed from 14 to 14.0 at the lower end of the range, but at the upper end the value of 1.4 was not changed to 1.40. Similarly, the value for the sgRNA is given as 29 rather than being amended to 29.0. The claim provides new technical information by changing the precision at one end of the claimed range, and there is no technical justification for adding a third digit of precision to the highest molar amount of Cas9 but taking a different approach for the lowest molar amount of Cas9 or for the molar amount of sgRNA.

2.13. Furthermore, although the molarity of the sgRNA is given as 29 µM in [329], it is clear that this value is only approximate. Table 2 on page 30 gives the sequence for the sgRNA, and this can be used to calculate a molecular weight of 33954 Da. In the specified volume of 100 µL ([329], line 9) this gives a concentration of 29.45 µM. The unambiguous disclosure within Example 2 is therefore that the disclosed concentration of 29 µM is an approximation. Turning to the Cas9, it is impossible to determine if the specified concentrations of 1.4 µM and 14 µM are approximations because the sequence of the Cas9 protein is not disclosed.

The presence of a hexa-histidine tag is mentioned in [329], but the remainder of the sequence is unclear *e.g.* it could also include an epitope tag and a nuclear localisation sequence (as used in Example 1; see [292]). Overall, it is impossible to know the protein's molecular weight and so it is impossible to know if the 1.4 µM and 14 µM values are precise or approximations. Either way, there is no technical justification for defining the ratios in claim 1 by referring to (i) a value for the sgRNA which is necessarily less precise than can be inferred from Example 2, and (ii) a value for the SpCas9 which has been made more precise than can be inferred.

2.14. Overall, therefore, claim 1 extends beyond the content of the application as filed because: (i) the skilled reader would not directly and unambiguously derive that the range of ratios used specifically in Example 2 could be applied more generally, not least because the application as a whole does not mention this parameter as having any relevance; and (ii) the reference to a ratio of 29:14.0 in claim 1 provides different technical information from the ratio of 29:14 which was used in Example 2. Claim 1 therefore adds matter contrary to Article 76(1) EPC.

2.15. The same issue also affects the dependent claims, but many of these have further problems of their own:

- Claims 2 and 3 similarly add matter because they are also unallowable generalisations from Example 2. Although [329] mentions *"recombinant Cas9 protein"* which was *"purified from E. coli"*, there is no basis for extracting these two features on their own *e.g.* without also including the information that this recombinant protein expressed by *E. coli* expressly includes a hexa-histidine tag.
- Claim 4 specifies that the sgRNA comprises a fusion of a crRNA to a tracrRNA, but this merely re-states the structure of a sgRNA with additional words. Claim 4 thus implies that claim 1 extends to embodiments in which the sgRNA does not comprise a crRNA fused to a tracrRNA, which would provide an additional point against claim 1 under Article 76(1) EPC, as there is no basis for defining claim 1 in terms of such embodiments. In short, the only sgRNA which is contemplated in Example 2 is a sgRNA as defined in claim 4.
- When the applicant filed claims 5-7 it again pointed to Example 2 as basis. As already explained, Example 2 cannot be generalised and so these three claims add matter.
- Claim 9 specifies that a human cell is transfected by electroporation. The applicant pointed to [217] on page 19 as basis, or original claim 39, but in both these passages electroporation is part of a long list of transfection techniques. There is no basis for singling out electroporation from this list, or for specifying that the techniques are used with human cells.
- Claim 10 specifies the use of nucleofection. When filing this claim the applicant pointed to Example 2 as basis but, as already explained, Example 2 cannot be generalised.

2.16. Thus these dependent claims add matter not only because they include the unallowable features of claim 1, but also because their additional features cannot be derived from the original disclosure.

3. THE CLAIMS DO NOT HAVE BASIS IN THE PRIORITY APPLICATIONS

3.1. Even if the opposition division would disagree with the arguments in section 2 above, and find that the molar ratio range in claim 1 has basis in Example 2 of the PCT application, the relevant disclosure is not found in any of the priority applications. The claim would thus at

best be entitled to the filing date of 23rd October 2013, by which time document D1 had been published (its publication date in August 2013 is confirmed by its PubMed entry, cited as D1a). As explained in more detail below, the claims are invalid in view of this prior art.

3.2. Example 2 of the PCT application is not present in the earliest two priority applications ('P1' in October 2012, or 'P2' in March 2013), and nor does P1 or P2 contain any other disclosure relevant to the claimed molar ratio range. Example 2 was added for the first time in the third priority application ('P3' in June 2013), but in P3 it does not include the molarities of the sgRNA and Cas9, and instead refers only to mass amounts (*i.e.* in µg units). Nor is there any other disclosure in P3 of molar amounts or ratios of sgRNA and SpCas9, so the ratio range in granted claim 1 cannot be derived from P3.

3.3. The differences between the relevant text in P3 and in the PCT application are shown below:

P3	mM KCl, 1 mM DTT, and 10% glycerol). Cas9 protein/sgRNA complex was introduced directly into K562 cells by nucleofection: $1x10^6$ K562 cells were transfected with 22.5-225 µg of Cas9 protein mixed with 100ug of *in vitro* transcribed sgRNA (or crRNA 40ug and tracrRNA 80ug) using the 4D-Nucleofector, SF Cell Line 4D-Nucleofector X Kit, Program FF-120 (Lonza). according to the manufacturer's protocol. After 48hr,
PCT	150 mM KCl, 1 mM DTT, and 10% glycerol). Cas9 protein/sgRNA complex was introduced directly into K562 cells by nucleofection: $1x10^6$ K562 cells were transfected with 22.5-225 (1.4-14 µM) of Cas9 protein mixed with 100ug (29 µM) of *in vitro* transcribed sgRNA (or crRNA 40ug and tracrRNA 80ug) in 100µl solution using the 4D-Nucleofector, SF Cell Line 4D-Nucleofector X Kit, Program FF-120 (Lonza) according to the manufacturer's protocol. After nucleofection, cells were placed in

3.4. Although the mass amounts of Cas9 and sgRNA are the same in P3 and in the PCT application, in P3 these figures do not enable the skilled person to derive molar amounts (and thus molar ratios) because this calculation would also require disclosure of the molecular weights of the relevant components, but this information is neither disclosed nor derivable from P3.

3.5. The molecular weight of the Cas9 protein depends on various factors, including any additional modifications which are made to the native form of the protein (*e.g.* addition of epitope tags, nuclear localisation sequence, *etc.*), but this information cannot be derived from P3 for the reasons already discussed in section 2.13 above *i.e.* critical technical details are absent from Example 2. In contrast, the molecular weight of the sgRNA can be derived because its sequence is shown in Table 2, but this sequence differs between P3 and the PCT application. Therefore, although the molar amount of sgRNA can be derived from Example 2 of P3, this is different from the molar amount which can be derived from the PCT application, and so the molar ratio used in the two examples must necessarily be different.

3.6. Going into more detail, the sgRNA sequences shown in Table 2 of P3 and of the PCT application are shown below. The difference between them is already clear from the lengths shown in the right-hand column — P3 refers to '103bp' (NB: 'bp' is an error here as the molecules are single-stranded), whereas the PCT application refers to '104bp' but then shows a 105nt sequence which has an additional GG dinucleotide at the 5' end:

P3

RNA type	RNA sequence (5' to 3')	Length
sgRNA	UGACAUCAAUUAUUAUACAUGUUUUAGAGCUAGAAAUAGC AAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGU GGCACCGAGUCGGUGCUUUUUUU	103bp

PCT

RNA type	RNA sequence (5' to 3')	Length
sgRNA	GGUGACAUCAAUUAUUAUACAUGUUU UAGAGCUAGAAAUAGCAAGUUAAAAU AAGGCUAGUCCGUUAUCAACUUGAAAA AGUGGCACCGAGUCGGUGCUUUUUUU	104bp

3.7. These sequences allow calculation of molecular weights for the sgRNA molecules: 33954 Da in the PCT application, but only 33264 Da in P3 (*i.e.* a difference of 690 Da). Because they have different molecular weights then the molar amount of 100µg sgRNA is necessarily different in the two examples — 30.06 µM in P3, but only 29.45 µM in the PCT application.

3.8. For completeness, although Figure 10 of the PCT application shows both molarity and mass amounts for sgRNA and Cas9, Figure 10 in P3 shows only mass amounts and so it does not provide any information beyond the text of Example 2.

3.9. The molar ratios of 29:14.0 and 29:1.4 therefore were not explicitly or implicitly disclosed in P3 because the molar amount of the Cas9 enzyme cannot be derived and the molar amount of the sgRNA differs from the disclosure in the PCT application. P1 and P2 also provide no useful disclosure and so, as noted above, the prior art also includes documents published prior to October 2013.

4. NOVELTY and INVENTIVE STEP: D1 (Cho *et al.*)

Claim 1

4.1. D1 describes generating Cas9-sgRNA RNP complexes which were then injected into *C. elegans*, leading to successful *in vivo* mutagenesis of the *dpy-3* and *unc-1* genes to give their characteristic 'dumpy' and 'uncoordinated' mutant phenotypes (see Figure 1). As explained below, the compositions which were injected have a molar excess of sgRNA, with a sgRNA:SpCas9 molar ratio of about 3:1, which is squarely within the range of claim 1. Claim 1 therefore lacks novelty.

4.2. The molar ratios can be derived based on the information in D1's Supplementary Materials and Methods. This section explains that Cas9-sgRNA complexes were formed by mixing

equal volumes of (i) an 8 µg/µL Cas9 protein solution and (ii) a pair of sgRNA molecules, each at 2.5 µg/µL, targeting one site upstream of the target gene and one downstream. These quantities permit the molar concentrations of SpCas9 protein and sgRNAs in the transfection mixture to be calculated, as shown in more detail below.

4.3. The "*Cas9 protein and sgRNA preparation*" section of D1's Supplementary Materials and Methods explains that *"templates for sgRNA in vitro transcription were generated by oligo-extension using Phusion polymerase"*. The templates themselves are shown in Table S1 on the final page of D1:

Table S1 Oligonucleotide sequences used in this study

Templates for *in vitro* transcription

Target gene	Forward or Reverse	Sequence
Dpy-L	F	GAAATTAATACGACTCACTATAGGGGCTAATGTTGCCGACGCAAGTTTTAGAGCTAGAAATA GCAAGTTAAAATAAGGCTAGTCCG
Dpy-R	F	GAAATTAATACGACTCACTATAGGTGTACGTGATTGTAGACAAGGTTTTAGAGCTAGAAATA GCAAGTTAAAATAAGGCTAGTCCG
Unc-L	F	GAAATTAATACGACTCACTATAGGCCAATCGTCTCGTAATCAGGGTTTTAGAGCTAGAAATAG CAAGTTAAAATAAGGCTAGTCCG
Unc-R	F	GAAATTAATACGACTCACTATAGGATGCGTTACAAGCTCTTTCAGTTTTAGAGCTAGAAATAG CAAGTTAAAATAAGGCTAGTCCG
sgRNA	R	AAAAAAGCACCGACTCGGTGCCACTTTTTCAAGTTGATAACGGACTAGCCTTATTTTAACTTGC

4.4. Thus there are four different forward templates ('F') and a single common reverse template ('R'). The reverse template is complementary to the 3' portion of the forward templates, thus leading to a 24bp duplex with a long single-stranded overhang at both ends. For instance, with the Dpy-L forward template the duplex is:

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Dpy-L: GAAATT...CGCAAGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCG
                                        ||||||||||||||||||||||||
sgRNA:                                  CGTTCAATTTTATTCCGATCAGGCAATAGTTGAACTTTTT...GAAAAAA
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and *"oligo-extension with Phusion polymerase"* of this duplex fills in the single-stranded overhangs and gives a fully double-stranded template ready for *in vitro* transcription (IVT).[1]

4.5. D1 states that the sgRNA templates were *"transcribed as previously reported (CHO et al. 2013)"*, and this earlier Cho *et al.* paper is enclosed as D2. The 'Supplementary Methods' section in D2 explains on page 2 that *"RNA was in vitro transcribed through run-off reactions by T7 RNA polymerase"*. The IVT templates are shown in the table on the final page, which confirms the presence of a T7 promoter sequence (24bp, underlined). In D2, comparison of the CCR5 templates with the sgRNA shown in Figure 1 confirms that transcription begins with the final two nucleotides of the underlined sequence (GG), which is the recognised start site for the T7 promoter, and continues all the way to the 3' end. The patent also confirms that

[1] The same approach of hybridising two partially overlapping single-stranded templates, followed by the use of Phusion polymerase to provide a full duplex, is described in [0138] of the opposed patent. The forward & reverse sequences then shown in Table 9 are identical to Table S1 of D1, except for the 20nt target sequence in the forward template.

this is the start site for transcription from this promoter sequence (*e.g.* compare the 'Foxn #3' template in Table 3 (page 15) with the sgRNA in Figure 5a).

4.6. The templates in Table S1 of D1 include the same promoter sequence as used in D2 and the patent, so IVT will begin at the same GG dinucleotide same position, leading to 104nt sgRNAs with the following sequence, where N_{20} is the portion which matches the target sequence:

$^{5'}GGN_{20}GUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCUUUUUU^{3'}$

4.7. For completeness, this 104nt sequence is derived as follows:

Position	Origin
1-2	Transcription start site for T7 polymerase; nucleotides 23-24 of 'F' template
3-22	Nucleotides 25-44 of 'F' template, which match the target sequence
23-40	Nucleotides 45-62 of 'F' template
41-64	Common to 'F' & 'R' templates *i.e.* nucleotides 63-86 of 'F', and the reverse complement of nucleotides 64-41 of 'R'
65-104	Reverse complement of nucleotides 40-1 of 'R'

4.8. The resulting sgRNA molecules disclosed in D1 are therefore as follows, and their sequences can be used to calculate precise molecular weights, and thus to derive molarities of each sgRNA, and of their combination, at the disclosed concentration of 2.5 µg/mL:

Target	N_{20}	MW (Da)	Molarity (µM)	sgRNA mixture
Dpy-L	GGCUAAUGUUGCCGACGCAA	33772	74.02	148.0 µM
Dpy-R	UGUACGUGAUUGUAGACAAG	33798	73.97	
Unc-L	CCAAUCGUCUCGUAAUCAGG	33693	74.20	148.5 µM
Unc-R	AUGCGUUACAAGCUCUUUCA	33655	74.28	

4.9. The Cas9 used in D1 is implicitly SpCas9. The sequence downstream of N_{20} in a sgRNA is the portion which corresponds to the natural tracrRNA and interacts with the Cas9 enzyme, rather than hybridising to the target sequence. The skilled person would recognise that the 3' sequence of the sgRNA molecules in D1 matches the known tracrRNA and sgRNA sequences of the SpCas9 system, as disclosed in *e.g.* D3 (see Figures 1E & 5B) and D2 (see Figure 1). This downstream sequence thus tells the skilled person that the authors of D1 had used SpCas9.

4.10. Wild-type SpCas9 has a molecular weight of 160 kDa (*e.g.* see D4, page 2 'Results') which, at 8 µg/µL, corresponds to 50 µM. In combination with the molar concentrations of the sgRNA mixtures calculated in section 4.7 above (*i.e.* just under 150 µM), this means that both of the compositions in the pre-injection mixtures of D1 had a molar excess of sgRNA with a molar ratio of sgRNA to SpCas9 of ~3:1 *i.e.* within the range in claim 1.

4.11. The 'Supplementary Materials and Methods' in D1 explain that it used SpCas9 modified to include *"an HA-epitope and an N-terminal nuclear localization signal"*. Although the precise sequences for these features is not disclosed, this point is not relevant because they are much shorter than the whole SpCas9, so they do not have a meaningful impact on the molar concentration (*e.g.* see the sequences used in Supplementary Figure 1 of D2, where the NLS and a HA epitope together have only 21aa *i.e.* <2% of the overall sequence). Furthermore, adding these tags increases the molecular weight of the protein and so decreases its molar concentration for a given mass. Thus the added epitope and NLS would increase the molar

excess of sgRNA relative to Cas9, to be slightly more than 3:1, but it would still fall within the broad range specified in claim 1.

4.12. If the opposition division would decide that the lack of specific details in D1 about the Cas9 protein means that the skilled person would have residual doubts about the molar ratio which was used, routine work would lead the skilled person to an embodiment falling within the scope of the claim in an obvious way. A skilled person reading D1 when it was published in August 2013 must of course have the right to repeat its experiments without being inhibited by a patent having an effective date which is 2 months later. To repeat those experiments they would need to choose an appropriate Cas9 protein[2]. The tracr portion of the sgRNA would already reveal to the skilled person that they should choose a SpCas9, which naturally has a molecular weight of 160 kDa (see sections 4.9-4.10 above). Their only remaining task would be to insert *"an HA-epitope and an N-terminal nuclear localization signal"* into SpCas9, but this would have involved only routine work:

- The HA epitope was a well-known molecular biology tool, being a short sequence derived from the HA (hemagglutinin) protein of influenza virus which is easily detected by anti-HA antibodies. It was routinely used at the priority date *e.g.* see D5 from Millipore in 2008, confirming the commercial availability of antibodies for detecting the HA tag (which *"has been extensively used as a general epitope tag"*) having the 9aa sequence YPYDVPDYA (*i.e.* as also seen at the C-terminus of the sequence used in D2).
- The nuclear localization signal ('NLS') is explained in undergraduate textbook *Molecular Biology of the Cell* (D6) as a short amino acid sequence which targets a protein to the nucleus of a cell. D7 describes them in more detail as *"short peptide motifs that mediate the nuclear import of proteins by binding to their receptors"*. D7 further explains that there are two types of classical NLS: *"monopartite NLSs having a single cluster of basic amino acid residues and bipartite NLSs having two clusters of basic amino acids separated by a 10–12-amino acid linker"*. Many variants were known, and their amino acid sequences are given in Table 1 on page 482, but they do not exceed 20aa in length. In principle the skilled person could choose any of these options, although a typical choice would be the SV40 'large T antigen NLS' which has the sequence PKKKRKV (D7, page 1, right-hand column; see also Figure 12-13 of D6).

4.13. Thus a skilled person wanting to repeat D1 before October 2013 would easily be able to put its technical teaching into effect, and in doing so would arrive at an embodiment falling within the scope of claim 1. Indeed, to fall outside the molar ratio in claim 1 the skilled person would have to modify the SpCas9 protein so that its molecular weight would either be less than 112 kDa or more than 1119 kDa, neither of which is reasonable or feasible when the wild-type SpCas9 protein is 160 kDa. In more detail, the upper limit in the claim (29:1.4 *i.e.* a molar ratio of 20.7:1) would not be reached until the protein's concentration was less than 7.15 µM, which would require its molecular weight to be 1119 kDa or more. Similarly, the lower limit in the claim (29:14 *i.e.* a molar ratio of 2.07:1) would not be reached until the protein's concentration was more than 71.5 µM, which would require its molecular weight to be 112 kDa or less. Thus, regardless of the precise modifications made when incorporating *"an HA-epitope and an N-terminal nuclear localization signal"* into SpCas9, the skilled person

[2] On the assumption that this information is not already unambiguously derivable from D1, which the opponent denies, but which is being accepted here solely for the sake of argument.

would arrive at a molar ratio which falls within the range specified in claim 1 and would do so without an inventive step.

Dependent claims

4.14. The SpCas9 used in D1 is a recombinant Cas9 because it is modified to include a HA-epitope and a NLS (*cf.* the definition of 'recombinant' in [0030]). Thus claim 2 adds nothing beyond claim 1.

4.15. *E.coli* is a standard host for expression of recombinant proteins and so claim 3 would have been an obvious choice for expressing the SpCas9 used in D1. The patentee has not proposed that this choice of expression host provides any technical effect and so it is merely an arbitrary choice.

4.16. The sgRNAs used in D1 comprises a crRNA portion fused to a tracrRNA portion and so claim 4 adds nothing beyond claim 1.

4.17. D1 used its system to edit the genome of the model animal *C. elegans*, but SpCas9/sgRNA was already known for use in editing the genome of human cells as well *e.g.* in D2. Therefore, with the success seen in D1 with *C. elegans* the skilled person would be confident that the same approach, using a composition with the same molar ratio of sgRNA:SpCas9, would also be effective when used in human cells. Claim 5 would therefore have been obvious.

4.18. Compared to claim 5, claim 6 merely requires *"inducing a targeted disruption of an endogenous gene",* but this is the specific purpose of using a Cas9 system where the sgRNA sequence targets a specific gene (*e.g.* specific portions of the *dpy-3 or unc-1* genes in D1, or of the *CCR5* or *C4BPB* genes in D2). Claim 6 therefore adds nothing beyond claim 5.

4.19. Claims 7 and 8 are merely re-wordings of claims 5 and 6, so these two claims do not add anything patentable.

4.20. Claims 9 and 10 specify that the composition is transfected using electroporation, or more specifically nucleofection. D2 already reported the use of the *"4D-Nucleofector"* apparatus for achieving transfection in human cells (Supplementary Information, page 3) and as its name suggests, the *"4D-Nucleofector"* was used for nucleofection. A skilled person would therefore have known to use this specific electroporation technique for transfection of human cells, and claims 9 and 10 would also have been obvious.

5. CITED DOCUMENTS

D1: Cho *et al.* (2013) *Genetics* 195:1177-1180 + supporting information.
D1a: Confirmation of publication date of D1.
D2: Cho *et al.* (2013) *Nature Biotechnology* 31:230-232 + supplementary information.
D3: Jinek *et al.* (2012) *Science* 337:816-821.
D4: Jinek *et al.* (2013) *eLife* 2:e00471.
D5: Millipore certificate of analysis for 'Anti-HA Tag', 2008.
D6: *Molecular Biology of the Cell* (ed. Alberts *et al.*), 3rd edition 1994; pages 563-564.
D7: Kosugi *et al.* (2009) *J Biol Chem* 284:478-485.