

UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE PATENT TRIAL AND APPEAL BOARD

MERCK SHARP & DOHME LLC,
Petitioner,

v.

SURROZEN OPERATING, INC.,
Patent Owner.

Case No. PGR2026-00027
Patent No. 12,297,278

DECLARATION OF DR. MICHAEL KAHN

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I. INTRODUCTION

1. I, Michael Kahn, Ph.D., have been retained by Finnegan, Henderson, Farabow, Garrett & Dunner, LLP on behalf of Merck Sharp & Dohme LLC as an independent expert in the field of Wnt signaling and biology to provide my opinions related to the U.S. Patent No. 12,297,278 (“’278 patent”) and explain the relevant state of the art.

II. SUMMARY OF MY OPINIONS

2. As of 2017, Wnt signaling was recognized to be extraordinarily complex yet poorly understood. It was known to involve interactions among numerous ligands, receptors, and co-receptors to mediate a wide variety of downstream signaling pathways, and rather than a single pathway or activation mechanism, there were many. For example, Wnt signaling was understood to encompass both canonical and noncanonical pathways, to vary significantly based on cell type and cellular developmental stages, and to involve multiple ligands, receptors, and co-receptors. As of 2017, no complete Wnt-receptor complex had been crystalized. *See* Section IX.A below.

3. Adding to the complexity of understanding Wnt signaling, as of 2017 there was no one assay recognized to adequately capture or measure all aspects of

Wnt signaling activity.¹ This was due to many factors, including the diversity of Wnt signaling and external factors that influence Wnt signaling such as development stage, cell type, and the presence of modulatory ligands.. *See* Section IX.B below.

4. Based on the state of the art at the time and my review of the '278 patent and file history, it is my opinion that claims 1-5 (all claims) of the '278 patent lack sufficient written description, are not enabled, and are indefinite in scope.

5. To arrive at this opinion, I was first asked to consider what the term “Wnt surrogate molecule” in the preamble of claim 1 means and whether claim 1 requires this molecule to possess any particular function. In my opinion, a POSA would have understood that the term “Wnt surrogate molecule” to mean a molecule that is “biologically active in binding to one or more Fzd receptor and to one or more of LRP5 and LRP6, and in activation of Wnt signaling, i.e., the molecule is a Wnt agonist” as explicitly stated in the specification. A POSA would have also understood that claim 1 requires—therefore is limited to—molecules that are biologically active in activation of Wnt signaling, i.e., Wnt agonists. *See* Section VIII below.

¹ This remains true to date.

6. With that construction in mind, I conclude that all claims of the '278 patent lacks written sufficient description support and enablement. *See* Section X below.

7. Claim 1 of the '278 patent defines tetravalent Wnt surrogate molecules in extremely broad terms. Specifically, claim 1 merely recites that each of these molecules contains two Fzd binding regions and two Lrp5/6 binding regions without restrictions on receptor selection or pairing. The specification permits each Fzd binding region to bind to any one of the ten known Fzd receptors, or to multiple Fzd receptors in numerous combinations, and each Lrp5/6 binding region to bind to Lrp5, Lrp6, or both. As a result, each claimed tetravalent molecule can target at least 35,721 distinct Fzd-Lrp receptor combinations, a conservative estimate considering the stoichiometries and epitopes encompassed by the claim. The specification, however, evaluates a limited number of candidate Wnt surrogate molecules, which are not representative of other, undisclosed molecules that bind to targets within these 35,721 distinct Fzd-Lrp receptor combinations. As one example, it fails to provide *any* species binding to Fzd3, Fzd6, and Fzd10 that agonize Wnt signaling.

8. Additionally, the specification of the '278 patent provides no principled guidance for selecting among the numerous Fzd-Lrp target combinations which influence Wnt signaling activity in different ways, offers limited direction for the stoichiometry and the epitope required for pathway activation, and fails to account

for tissue- and time-dependent variability in receptor expression. Given these unresolved sources of uncertainty, it is my opinion that a POSA would have been required to undertake undue trial-and-error experimentation amounting to many years' worth of assays to determine which of candidate tetravalent, multispecific molecules function as Wnt agonists and fall within the scope of the claims. *See* Section X below.

9. But the amount of work that I estimated assumes that a POSA could determine with reasonable certainty as to whether a candidate molecule works as a Wnt agonist. Here, it is my opinion that a POSA would not be able to do so for numerous reasons. For example, as of 2017, several methods existed to measure Wnt signaling activity at different points along Wnt signaling pathways. However, each of these methods was highly dependent on experimental conditions, such as cell type and the expression levels of Wnt receptors and co-receptors. Different assays and cell types were also known to give conflicting results on Wnt signaling activation for a given Wnt ligand.

10. Despite the lack of a standardized assay applicable across all Wnt signaling pathways, the '278 patent fails to endorse any specific assay or assay conditions for measuring Wnt signaling activity. While the patent primarily reports TOPFlash assay results, the results of this assay are also highly condition-dependent and the specification provides minimal guidance on which conditions to use. For

example, the specification shows that TOPFlash assay outcomes vary unpredictably based on factors such as cell type, the Fzd and Lrp5/6 receptors expressed, the presence of potentiators like R-spondin, and the concentrations of candidate molecules tested, resulting in conflicting data regarding whether a candidate molecule qualifies as a “Wnt surrogate molecule.” Thus, it is my opinion that all claims of the ’278 patent are indefinite. *See* Section X below.

III. QUALIFICATIONS

11. I am currently an Emeritus Professor of Cancer Biology and Molecular Medicine at City of Hope in Duarte, California. I have studied complex biological signaling pathways and biological mimetics for almost 40 years. My research for the past 30 years has focused on the Wnt signaling pathway, particularly in the context of stem cell development and cancer biology. My research not only investigates the basic mechanism of action and regulation of Wnt signaling, but also explores pharmacological intervention of Wnt signaling for disease treatment. My studies on Wnt signaling have led to the development of a novel class of Wnt therapeutic agents, CBP/ β -catenin antagonists. Two of these, PRI-724 and C82, have been clinically evaluated in the context of a number of diseases including cancer, liver fibrosis, and systemic sclerosis. In addition to Wnt signaling in cancers, I have also studied the function and regulation of Wnt signaling in inflammatory

diseases, obesity, neurodegenerative diseases, and metabolic diseases, which cover a wide range of target organs and tissues.

12. I obtained my B.A. in Chemistry from Columbia University in 1978 and my Ph.D. in Chemistry from Yale University in 1983. From 1982 to 1984, I was a National Institute of Health postdoctoral fellow at Columbia University and subsequently a postdoctoral fellow for one year at the Hoffman La Roche Institute for Molecular Biology, where I worked on the design of nonpeptide mimics. From 1985 to 1992, I was a professor at the University of Illinois Chicago where my lab focused on developing peptidic, nonpeptidic, and antibody mimetics. From 1992 to 2006, I was a professor at the University of Washington in Seattle and principal investigator at the Pacific Northwest Research Institute. There, my research group studied both Wnt signaling and pharmaceutical and vaccine applications of peptidomimetics. During that period, I also became the Senior Vice President of Molecumetics, a company that developed small-molecule therapeutics from combinatorial constrained peptido-mimetic libraries. From 2006 to 2017, I worked at the University of Southern California (“USC”), where I was the first appointed provost professor, with joint appointments in the Department of Biochemistry and Molecular Biology at Keck School of Medicine of USC and the Department of Molecular Pharmacology and Toxicology in the USC School of Pharmacy. I was also the co-leader of the GI-Oncology Program at the USC Norris Comprehensive

Cancer Center, as well as the Center for Drug Discovery and Development at USC. My lab at USC focused on studying the role of Wnt signaling in cancer and stem cells. I started my current professorship at City of Hope in 2018, where I continued investigating the role of Wnt signaling in cancer stem cells and developing novel therapeutics targeting both canonical and noncanonical Wnt signaling pathways.

13. I have published around 150 scholarly articles. Most of my publications are articles in peer-reviewed journals, while the others are invited review papers and book chapters. My publications have focused on the modulation of different components of the canonical and noncanonical Wnt signaling pathways and on the development of Wnt signaling pathway modulators for the treatment of cancers and other diseases. I have also published extensively on the design and development of peptidic and non-peptidic mimetics in various biological pathways. From 2014-2025, I was the executive editor for the journal Current Molecular Pharmacology. I have won several awards for my scholarly work and was an American Cancer Society Faculty Fellow (1987-1989), Searle Scholar (1986-1989), NSF Presidential Young Investigator (1987-1992), Camille and Henry Dreyfuss Distinguished Young Faculty Fellow (1986-1991), and an American Heart Association Established Investigator (1990-1995), among other accolades.

14. I have been awarded numerous funding grants from national scientific organizations, including the American Cancer Society, the California Institute of

Regenerative Medicine, the Dhont Family Foundation, the National Cancer Institute, Gateway for Cancer Research, and the National Institutes of Health, for the investigation of, among other topics, the development of novel stem cell lines, pharmacogenetics and cancer biomarkers, and the efficacy of novel Wnt modulators in cancer, fibrosis, and multiple sclerosis.

15. My professional qualifications are summarized in further detail in my curriculum vitae, which is attached as **Appendix A**.

16. I am being compensated for the time I spend on this matter, but my compensation is not contingent in any way upon my opinions, my performance, or the outcome of this or any other proceeding. I have no current or past financial ties with Merck outside of my engagement in this proceeding.

IV. MATERIALS CONSIDERED

17. My opinions in this declaration are based on my education, training, background, and experience, and the documents that I have reviewed and are listed in **Appendix B**. I may consider additional documents and information and form supplemental opinions. I may also modify or expand upon my opinions expressed in this declaration based on any new information provided to me, including any additional declarations and testimony.

V. THE '278 PATENT

A. Disclosures of the '278 Patent

18. The '278 patent relates to “Wnt signaling pathway agonist molecules.” EX1001, 1:34-35. The specification of the '278 patent refers to these Wnt signaling agonist molecules as “Wnt surrogate molecules” and defines the “Wnt surrogate molecules of the present invention” as molecules that are “biologically active in binding to one or more Fzd receptor and to one or more of LRP5 and LRP6, and in activation of Wnt signaling, i.e., the Wnt surrogate molecule is a Wnt agonist.” *Id.*, 16:52-56.

19. According to the '278 patent, “Wnt agonists typically initiate a reaction or activity that is similar to or the same as that initiated by the receptor’s natural ligand.” EX1001, 16:61-63. This reaction or activity may occur in both a “canonical Wnt signaling pathway or a non-canonical Wnt signaling pathway.” *Id.*, 4:49-51. The '278 patent further indicates that “[t]he ability of the Wnt surrogate molecules and other Wnt agonists disclosed herein to mimic the activity of Wnt can be confirmed by a number of assays.” *Id.*, 16:58-61. With respect to measuring canonical Wnt signaling, the specification of the '278 patent also states that

[v]arious methods are known in the art for measuring the level of canonical Wnt/ β -catenin signaling. These include, but are not limited to assays that measure: Wnt/ β -catenin target gene expression; TCF reporter gene expression; β -catenin stabilization; LRP phosphorylation; Axin

translocation from cytoplasm to cell membrane and binding to LRP.

Id., 117:39-44. The '278 patent does not, however, disclose any method or assay to determine the ability of the Wnt surrogate molecules to activate the noncanonical Wnt signaling pathway. *See generally* EX1001.

20. The '278 patent discloses a few working examples. EX1001, 150:20-172:54. Example 1 lists sequence IDs for candidate Wnt surrogate molecules that presumably bind to Fzd and Lrp receptors and induce reporter activity. Examples 2-18 disclose *in vitro* evaluations of candidate Wnt surrogate molecules. The evaluations were primarily performed in an *in vitro* assay called a TOPFlash assay², although in Examples 2 and 3, two of the candidate molecules (R2M3-26 and R2M3-32) were further tested for their abilities to bind to the extracellular domain of Fzd1. *Id.*, cols. 155-56. I include a brief summary of each of these examples below:

21. Examples 2 and 3:³ These examples test candidate Wnt surrogate molecules R2M3-26 and R2M3-32. R2M3-26 and R2M3-32, each made of a Fzd binding sequence (R2M3) and Lrp6 binding sequence (26 or 32). These examples

² See Section IX.B below.

³ Instead of the black-and-white figures of the '278 patent, I have considered corresponding color figures from one of the provisional applications provided by counsel. EX1007.

purport to assess Wnt signaling activation via the TOPFlash assays performed in the 293 reporter cell line, both in the absence and presence of R-spondin. These assays include a negative control, which consisted of a Fzd binding sequence not paired with any Lrp6 binding sequence (“R2M3 IgG”).⁴ These assays do not, however, include a positive control, such as Wnt3A, to provide any standard or threshold for evaluating the TOPFlash reporter activity as indicative of biological Wnt signaling and therefore the specification does not provide support for the statement that these molecules activated Wnt signaling “similar to a natural ligand.” EX1001, 155:34. The specification states that both R2M3-26 and R2M3-32 “induced reporter activity in a dose dependent manner and was enhanced by the presence of R-spondin.” EX1001, 155:32-33; 155:62-63. These examples also report that the fusion of the

⁴ But as discussed below, this is not a meaningful negative control in my view. Standard practice in the field is to include both positive controls (e.g., known pathway activators such as Wnt3a) and negative controls (e.g., basal reporters such as FOPFlash, which do not contain the TCF/LEF binding elements) to distinguish true canonical Wnt/ β -catenin pathway activity from background or non-specific effects. R-spondin alone would also have provided a better understanding as to the baseline signal or noise that exists in the absence of any external factor targeting members of Wnt signaling pathways.

Lrp6 binding arms (26 or 32) “did not affect R2M3 interaction with its target, Fzd.” *Id.*, 155:38-40; 155:12-14, Fig. 2D, Fig. 3D.

22. Example 4: This example tests the ability of soluble Fzd extracellular domain (ECD) or R2M3 IgG to inhibit the binding of candidate molecules R2M3-26 and R2M3-32 to the Fzd1 ECD. The specification reports that both Fzd1 ECD and R2M3 IgG inhibited reporter signaling induced by R2M3-26 and R2M3-32 in TOPFlash assays. EX1001, 156:27-29, Figs. 4A-B.

23. Example 5: This example tests candidate molecules in which the Fzd binding sequence R2M3 is paired with Lrp6 binding sequence 23, 25, 26, 27, 28, 29, 31, 32, 33 or 36. EX1001, col. 156. This example assesses reporter activity with the TOPFlash assay in the 293, Huh7, A375, and BNL.CL2 reporter cell lines, both in the absence and presence of R-spondin. This example uses negative controls, including a R2M3 IgG, as well as R2M3 fused to non-Lrp6 bind sequence 24 (R2M3-24) or 34 (R2M3-34) in the same format as the candidate molecules. The specification states that the tested candidates “activated Wnt signaling to various levels,” whereas the non-binders (R2M3-24 and R2M3-34) “displayed no activity.” *Id.*, 156:45-48, Fig. 5. These assays, however, do not include a positive control, such as Wnt3A, to provide a standard to assess the biological relevance of “various levels” of activation in the TOPFlash assay.

24. Example 6: This example tests candidate molecules with a Fzd binding sequence (18R5) paired with a Lrp6 binding sequence (26, 28, 31, or 32). EX1001, col. 156. This example assesses reporter activity with TOPFlash assays performed in the 293, A375, and BNL.CL2 reporter cell lines, both in the absence and presence of R-spondin. The assays include a negative control, an 18R5 IgG molecule without Lrp6 binders. The specification reports that these candidates “demonstrated ability to activate Wnt signaling.” EX1001, 156:66, Fig. 6. However, this example also lacks a positive control by which a POSA could ascertain whether the candidate molecules truly “demonstrated the ability to activate Wnt signaling.”

25. Example 7: This example tests candidate Wnt surrogate molecules consisting of a Fzd binding sequence (18R5) paired with a Lrp5 binding sequence (5, 7, 8, or 9). EX1001, col. 157. This example assesses reporter signaling with TOPFlash assays performed in the 293 reporter cell line, in the presence of R-spondin. While the specification states that the tested candidate molecules “were able to activate Wnt signaling,” the TOPFlash assays do not include any positive or negative controls, and the assays did not include a condition where R-spondin was absent. *Id.*, 157:6-12, Fig. 7.

26. Example 8: This example evaluates candidate Wnt surrogate molecules with a Lrp6 binding sequence (26) paired with various Fzd binding sequences, including 1R-B05 (binding Fzd1, 2, and 7), 1R-C01 (binding Fzd1, 2, 7, and 9), 1R-

C07 (binding Fzd1, 2, and 7), 1R-E06 (binding Fzd1, 2, and 7), 1R-G05 (binding Fzd1, 2, and 7), and 1R-G06 (binding Fzd1, 2, 7, and 9). EX1001, 157:20-22, Fig. 8. This example assesses Wnt signaling activity based on TOPFlash assays in the 293 reporter cell line. The assays are performed in the presence of R-spondin only and do not include any positive or negative controls. The specification states that these candidates “were able to activate Wnt signaling,” but, without a positive control to set a reporter activity threshold for biological Wnt signaling, there is no basis for this conclusion. *Id.*, 157:28-29. While Example 8 also purports to include two additional Fzd binders (1R-H04-26 and 1R-E01-26), the ’278 patent neither includes them in the table of Wnt surrogate sequences (Table 3) nor reports the results for these binders in TOPFlash assays. *See id.*, Fig. 8.

27. Example 9: This example tests candidate Wnt surrogate molecules (R2M3-26, R2M3-32, and 18R5-26) made of a Fzd binding sequence (R2M3 or 18R5) and a Lrp6 binding sequence (26 or 32). EX1001, col. 157. The example evaluates four different arrangements of these sequences, and names those arrangements based on where in an IgG molecule the Lrp6 binding sequence is attached to: N-terminus of the IgG heavy chain (“NH”); N-terminus of the IgG light chain (“NL”); C-terminus of the IgG heavy chain (“CH”); C-terminus of the IgG light chain (“CL”). *Id.*, 157:35-44. This example assesses reporter activity with TOPFlash assays in the 293 reporter cell line. The assays are performed in the

presence of R-spondin only and do not include any positive or negative controls. While the specification claims that these candidates “activated Wnt signaling to various levels,” Figure 9 of the ’278 patent demonstrates that the candidate molecules R2M3-26 CL and 18R5-26 CL only produce minimal to no reporter signal even at the highest concentrations tested. *Id.*, 157:46-47, Fig. 9. Without a positive control to set a reporter activity threshold for biological Wnt signaling, it is impossible to know whether the reporter activity at these “various levels” has biological relevance. The specification recognizes that “the attachment location of the fusion and the geometry between the Fzd and LRP binding domains play roles in the ability of the Wnt surrogates to activate Wnt signaling.” *Id.*, 157:48-50.

28. Examples 10 and 11: These examples test candidate Wnt surrogate molecules consisting of a Fzd binding sequence (R2M3) in the Fab format and a Lrp6 binding sequence (26 or 32). EX1001, cols. 157-58. The Fzd and Lrp6 binding sequences purport to be in 1:1 stoichiometry in these molecules. *Id.*, Figs. 10A, 11A, Table 2A. In these examples, the Wnt surrogate molecules are purified by Ni-NTA affinity column followed by a size exclusion chromatography, and the fractions are tested for their reporter activity in TOPFlash assays performed in the 293 reporter cell line. Because “the peak of the reporter activity from the R2M3 in the Fab format did not correlate with the peak of the proteins,” the ’278 patent reports that the

R2M3-26 and R2M3-32 “fusion in the Fab format is ineffective in inducing canonical Wnt signaling.” *Id.*, 158:1-6; 158:25-30, Figs. 10-11.

29. Example 12: This example evaluates the candidate molecule R2M3-26 in a “HeteroIg” format. EX1001, col. 158. Like the molecules tested in Examples 10 and 11, the Fzd and Lrp6 binding sequences also purport to be in 1:1 stoichiometry in this molecule. *Id.*, 150:47-56, Fig. 12A. This example assesses reporter activity with TOPFlash assays in the 293 reporter cell line. As shown in Figure 12B, the assays are performed in the presence of R-spondin and the activities of the Hetero-Ig format are compared to those of a normal R2M3-26 molecule. The specification states that “R2M3-26 HeteroIg was ineffective in inducing canonical Wnt signaling.” *Id.*, 158:45-46, Fig. 12.

30. Example 13: This example tests candidate molecules consisting of a Fzd binding sequence, 17SB9, and a Lrp6 binding sequence, 26, arranged in different formats and with three different linker lengths. EX1001, 158:54-159:3. The candidate molecules are tested in TOPFlash assays in the 293 reporter cell line and in the absence or presence of R-spondin. The specification reports that the tested molecules “all activated Wnt signaling to various levels in the presence of 20 nM R-

spondin.” EX1001, 159:1-3, Fig. 13.⁵ However, without a positive control to set a reporter activity threshold for biological Wnt signaling, there is no basis for this conclusion.

31. Example 14: This example evaluates candidate molecules consisting of the Fzd binder 18R5 and the Lrp6 binder 1115.3 (binding to the E1E2 domain of Lrp6) or YW211.31.57 (binding to the E3E4 domain of Lrp6) in various “tandem scFv formats,” with linkers of 5, 10, or 15 amino acids. EX1001, 159:10-20. This example assesses reporter activity with the TOPFlash assay in the 293 reporter cell line. In relevant part, the specification states that while the formats in Figures 14A-G “activated Wnt signaling, potency and overall maximal efficacy may differ depend[ing] on the binder combination, linker length, and relative orientation.” *Id.*, 159:36-39. However, because the assays did not include any positive control, such as a natural Wnt ligand, it is difficult to interpret whether a candidate is “biologically active” when it only induced miniscule reporter assay activity (e.g., 18R5:Fc:1115.3 in Fig. 14E). When the Fzd and Lrp6 binders are connected such that “bispecific but monovalent binding to each of Fzd or LRP” molecules are made, the specification

⁵ In the '278 patent, Figure 13B appears to be identical to Figure 13E. Figure 13B of the underlying provisional (EX1007) aligns with the description of the figure in the specification.

reports that those molecules “were ineffective in activating Wnt signaling in the presence of 20 nM R-spondin.” *Id.*, 159:40-46, Fig. 14.

32. Example 15: This example tests candidate molecules in a “Fab-IgG format” with TOPFlash assays in the 293 reporter cell line. EX1001, cols. 159-160. The specification reports that candidate molecule 421.1-R2M3 (which has a Fzd binder that binds to Fzd1, 2, 5, 7, and 8 and a Lrp6 binder that binds to the E1E2 domain of Lrp6) “dose-dependently activated Wnt signaling.” *Id.*, 160:28-29, Fig. 15B. However, without a positive control to set a threshold of biological activity it is impossible to discern if the reporter activity has biological relevance. The candidate molecule 1RC07-10SA7 (which has a Fzd binder that binds to Fzd1, 2, and 7 and a Lrp6 binder with undisclosed epitope) “activated Wnt signaling” at three different linker lengths. *Id.*, 160:30-32, Fig. 15C. These assays also lack a positive control. Candidates 1RC07-10SG7 and 10SG7-1RC07 (which have a Fzd binder that binds to Fzd1, 2, and 7 and a Lrp binder with undisclosed target Lrp) with three different linker lengths “activated Wnt signaling while some preference of orientation and linker length was observed.” *Id.*, 160:38-40, Figs. 15D-E. However, without a positive control it is impossible to discern whether these differences in reporter activity have biological relevance. The candidates 1115.3-R2M3 (which has a Fzd binder that binds to Fzd1, 2, 5, 7, and 8 and a Lrp6 binder that binds to the E1E2 domain of Lrp6) and 1RC07-10SA7 in a crossover format “also activated Wnt

signaling.” *Id.*, 160:41-49, Figs. 15F-G. All of these candidates are tested with the TOPFlash assay in the 293 reporter cell line. However, because the assays do not include positive controls, such as a natural Wnt ligand, it is difficult to determine whether the low-level activations seen in the reporter assays demonstrated that the tested molecules were “biologically active.”

33. Example 16: This example tested the candidate molecule R2M3-26 in the F(ab')₂ format. EX1001, 160:53-57. This example measured reporter activity with the TOPFlash assay in the 293 reporter cell line and reported that the candidate “was able to activate Wnt signaling.” *Id.*, 160:66-67, Fig. 16. However, without a positive control to set a reporter activity threshold for biological Wnt signaling there is no basis for this conclusion.

34. Example 17: This example evaluates “additional” candidate molecules with different combinations of Fzd and Lrp specificities:

- Figure 17B: 1RC07-3 (Fzd1, 2, 7; Lrp5) and 1RC07-36 (Fzd1, 2, 7; Lrp6 E3E4);
- Figure 17C: R2M13-3 (Fzd5, 8; Lrp5); R2M13-26 (Fzd5, 8; Lrp6 E3E4); and R2M13-36 (Fzd5, 8; Lrp6 E3E4);
- Figure 17D: R2M3-3 (Fzd1, 2, 5, 7, 8; Lrp5) and R2M3-36 (Fzd1, 2, 5, 7, 8; Lrp6 E3E4);

- Figure 17E: 3SD10-3 (Fzd4; Lrp5); 3SD10-26 (Fzd4; Lrp6 E3E4); and 3SD10-36 (Fzd4; Lrp6 E3E4);
- Figure 17F: 4SD1-3 (Fzd4; Lrp5); 4SD1-26 (Fzd4; Lrp6 E3E4); and 4SD1-36 (Fzd4; Lrp6 E3E4);
- Figure 17G: 14SB6-3 (Fzd9; Lrp5); 14SB6-26 (Fzd9; Lrp6 E3E4); and 14SB6-36 (Fzd9; Lrp6 E3E4);
- Figure 17H: R2M9-3 (Fzd2; Lrp5); R2M9-26 (Fzd2; Lrp6 E3E4); and R2M9-36 (Fzd2; Lrp6 E3E4);

EX1001, col. 161. In this example, the reporter activity is measured with TOPFlash assays in the 293 reporter cell line (Figures 17B, C, D, H) or in these cells transfected with either Fzd4 (Figures 17E and F) or Fzd9 (Figure 17G). The specification states that the tested candidate molecules “activated Wnt signaling with varying levels of potency and efficacy.” *Id.*, 161:15-17, Fig. 17. However, all the TOPFlash assays are performed in the presence of 20 nM R-spondin, and none of the assays include either a positive or a negative control to set thresholds for biological activity and background luminescence. Furthermore, in Fig 17C, the concentration required (x-axis) and the TOPFlash reporter activity achieved (RLU; y-axis) is dramatically different between R2M13-3 and R2M13-26, further muddling understanding of whether R2M13-26’s reporter activity translates to a biologically significant response. *See id.*, Fig. 17C.

35. Example 18: This example evaluates candidate molecule 10SG11-1RC07 in a “2Fv-Ig format.” EX1001, 161:21-33. This molecule binds to Fzd1, 2, 7 but its Lrp specificity is not disclosed. Reporter activity is tested in TOPFlash assays in 293 reporter cell lines in the presence or absence of R-spondin. The specification states that the candidate molecule “demonstrated the ability to activate Wnt signaling.” *Id.*, 161:31-32, Fig. 18. However, without a positive control to set a reporter activity threshold for biological Wnt signaling, there is no basis for this conclusion.

36. In sum, the specification discloses candidate Wnt surrogate molecules that show varying levels of response in TOPFlash assays. However, *none* of the *in vitro* assays reported in the '278 patent include a positive control, such as a natural Wnt ligand, to establish a standard for biological activation. In addition, most examples do not include a true negative control to distinguish the signal induced by the candidate molecules from unspecific activation or background noise. Of all the examples that evaluate TOPFlash reporter activity, only two (Examples 7 and 17) contain candidate molecules with Lrp binding regions that bind to Lrp5. Moreover, *none* of the examples tested for Wnt agonist activity include molecules with Fzd binding regions that bind to Fzd3, 6, or 10. In addition, several examples (Examples 7-9, 17) assessed Wnt signaling activity only in the presence of R-spondin, which is not required for the activation of Wnt signaling by natural ligands and reflects at

most the responses amplified by R-spondin. *See, e.g.*, EX1063, 5; EX1060, 9-11; EX1053, 4-5; EX1093, 1-3. Also notably, none of the examples include an R-spondin only control, which would reflect background responses or background noise induced by R-spondin in the absence of candidate molecules. *See generally* EX1001.

37. Examples 19-26 of the '278 patent disclose *in vivo* experiments for *three* of the tested candidate Wnt surrogate molecules (R2M3-26, 1R-C07-3, and 1R-C07-26) and a molecule 18R5-DKK1c previously disclosed by others. EX1001, cols. 161-72 (“18R5-DKK1c . . . in PCT Publication WO2016/040895”). Collectively, these examples test R2M3-26, 1R-C07-3, or 1R-C07-26 or the prior art molecule 18R5-DKK1c *only* in animal models of liver or bone disease. The candidate Wnt surrogate molecules *only* collectively encompass binding to Fzd1, Fzd2, Fzd5, Fzd7, and Fzd8 and either Lrp5 or Lrp6 (E3E4 domain). Due to at least differences in receptor composition and tissue-specific response to Wnt, as discussed in Section IX.A below, I would not expect these molecules to demonstrate Wnt activation in every tissue or model system. These experiments include:

38. Example 19: *in vivo* PK/PD characterization of one tested molecule (R2M3-26) in liver samples where Wnt activation is assessed via Axin2 mRNA expression;

39. Example 20: *in vivo* characterization of a prior art Wnt surrogate molecule (18R5-DKK1c) delivered by AAV in a bone model;
40. Example 21: *in vivo* characterization of three tested molecules (R2M3-26, 1R-C07-3, 1R-C07-26) delivered by intraperitoneal injection in an ovariectomy induced osteoporosis model where Wnt activation is assessed via RNA transcript levels of genes such as Axin2, Runx2, Alp, and Dkk1;
41. Example 22: *in vivo* characterization of a prior art Wnt surrogate molecule (18R5-DKK1c) delivered by AAV in a liver regeneration model;
42. Example 23: *in vivo* characterization of two tested molecules (R2M3-26 and 1R-C07-26) delivered by intraperitoneal injection in a liver regeneration model where mRNA expression of genes such as Ki67 and cyclinD1 was assessed⁶;
43. Example 24: *in vivo* characterization of a prior art Wnt surrogate molecule (18R5-DKK1c) delivered by AAV in a chronic liver injury model;
44. Example 25: *in vivo* characterization of one tested Wnt surrogate molecule (R2M3-26) delivered by AAV in a chronic liver injury model where Wnt activation is assessed via Axin2 expression levels;

⁶ Ki67 is a proliferation marker and not necessarily Wnt dependent, whereas CyclinD1 is a defined Wnt activation gene in some tissues but not all.

45. Example 26: *in vivo* characterization of one tested Wnt surrogate molecule (R2M3-26) delivered by AAV in an acute liver injury model where Wnt activation is assessed via mRNA expression of genes such as Ki67 and cyclinD1.

B. Claims

46. The '278 patent contains one independent claim and four dependent claims. Claim 1 recites:

1. A soluble, tetravalent, multispecific Wnt surrogate molecule, wherein the Wnt surrogate molecule comprises:

(i) two Fzd binding regions that specifically bind to two Frizzled (Fzd) receptors, wherein each of the two Fzd binding regions comprises an antibody or antigen-binding fragment of the antibody; and

(ii) two LRP5/6 binding regions that specifically bind to a Low-density lipoprotein (LDL) receptor-related protein 5 (LRP5) and/or a Low-density lipoprotein (LDL) receptor-related protein 6 (LRP6), wherein each of the two LRP5/6 binding regions comprises an antibody or antigen-binding fragment of the antibody, and

wherein each of the two LRP5/6 binding regions is fused to one of the two Fzd binding regions via a linker moiety.

EX1001, cl. 1.

47. The dependent claims (claims 2-5), which are also reproduced below, include additional limitations. Claim 2 recites that the Fzd binding regions comprise a full IgG and the Lrp5/6 binding regions comprise a VHH. Claims 3 and 5 specify fusion locations, and claim 4 recites a pharmaceutical composition.

2. The Wnt surrogate molecule of claim 1, wherein the Fzd binding regions comprise a full immunoglobulin G (IgG), comprising two light chains and two heavy chains, and the LRP5/6 binding regions comprise a VHH.

3. The Wnt surrogate molecule of claim 2, wherein the LRP5/6 binding region is fused to the N-terminus or the C-terminus of the heavy chain or is fused to the N-terminus or the C-terminus of the light chain.

4. A pharmaceutical composition comprising a physiologically acceptable excipient, diluent, or carrier, and the Wnt surrogate molecule according to claim 1.

5. The Wnt surrogate molecule of claim 2, wherein each of the two LRP5/6 binding regions is fused to one of the light chains via a linker moiety.

48. While each of the five claims requires a “Wnt surrogate molecule,” the claims include no limitation as to how to determine whether a candidate molecule is

a “Wnt surrogate.” Specifically, the claims do not provide a specific assay or specific assay conditions for measuring the activity of the Wnt signaling pathway. The claims also do not provide any indication on the threshold value for determining whether a candidate molecule is a “Wnt surrogate” in any specific assay or under any specific assay conditions.

49. Each of claims 1-5 recites “two Fzd binding regions that specifically bind to two Frizzled (Fzd) receptors.” Because the claims impose no restrictions on the specific Fzd receptors, it is my opinion that the claims cover molecules having binding regions to one or more of Fzd1-10.

50. The claims also recite “two LRP5/6 binding regions that specifically bind to a Low-density lipoprotein (LDL) receptor-related protein 5 (LRP5) and/or a Low-density lipoprotein (LDL) receptor-related protein 6 (LRP6).” Because the claims only require that the Lrp5/6 regions bind to Lrp5 and/or Lrp6, it is my opinion that the claims cover molecules that have binding regions to Lrp5 alone, Lrp6 alone, or both Lrp5 and Lrp6.

C. Prosecution History

51. In rendering my opinion, I have also reviewed the prosecution history of the patent application that led to the '278 patent.

52. During prosecution of the '278 patent, the Examiner rejected the then-pending claims as obvious over Garcia US (EX1011) in view of Wu (EX1012).

EX1008, 2973, 3982-83. The Examiner stated that Garcia US taught molecules that bind to both Fzd and Lrp5/6 proteins “and directly activate canonical wnt pathway signaling.” *Id.* According to the Examiner, while Garcia US did not teach a specific “dual variable domain (DVD) format,” this particular format was obvious based on Wu’s disclosure that the “DVD-Ig format bispecific antibody is highly stable and exhibits excellent, IgG-like physicochemical and pharmacokinetic properties.” *Id.*, 2973-74, 3983-84.

53. In response, the Applicant relied on the language in the preamble of claim 1 to distinguish Wu. EX1008, 3956, 4005-06. The Applicant argued that Wu taught Wnt *antagonists* “that allow the simultaneous blockade of multiple targets . . . which is very different than the claimed molecules, which simultaneously engage two cell surface coeffectors molecules (Fzds and Lrp5/6) required to agonize the canonical Wnt signaling cascade This represents an important biological distinction between the molecules of Wu and those presently claimed.” *Id.*, 4006 (emphasis in original).

54. The Applicant also submitted a declaration from one of the named inventors, Yang Li. EX1008, 4167-72. In the declaration, Li stated that “a Wnt surrogate molecule . . . must bind both Fzd receptor(s) and Lrp5/6 receptors simultaneously, in order to agonize the Wnt signaling pathway.” *Id.*, 4168. Li also stated that “Wu et al. relates to bivalent molecules that allow the simultaneous

blockade of multiple targets . . . which is very different than the claimed molecules, which simultaneously engage two cell surface coeffectors (Fzd and Lrp5/6) required to agonize the canonical Wnt signaling cascade.” *Id.*, 4168 (emphasis in original).

55. The Examiner then allowed the claims but did not provide any reasons for the allowance. EX1008, 4207-14.

VI. PATENT LEGAL REQUIREMENTS

56. I am not an attorney but have been advised by counsel on the following legal principles. I have applied these principles to the '278 patent based on the perspective of a Person of Ordinary Skill in the Art (“POSA”), as discussed further in Section VII below.

57. Claim Construction: As discussed in further detail in Section VIII below, I understand that the meaning of claims and their specific “limitations” are interpreted or “construed” from the perspective of a POSA as of the patent’s effective filing date. From this perspective, they are interpreted first based on patent’s “intrinsic evidence,” which I understand to be the language and context of the claims, the inventors’ statements in the specification, and the prosecution history record from the Patent Office’s examination. I also understand that so-called “extrinsic evidence”—such as the use of terms in the dictionaries outside the context of the patent—may be considered to understand the technology and ordinary

meaning of a term. I understand that extrinsic evidence cannot be used to contradict the meaning of a term according to the intrinsic evidence.

58. I have also been informed by counsel that a claim term in the preamble, which I understand is the part of claim before the first “comprise” or “comprising,” can limit the scope of the claim if it is necessary to give life, meaning, and vitality to the claim, and the intrinsic evidence shows that the inventors regarded the preamble as an important feature of their invention.

59. Written Description Requirement: I understand that the specification must provide sufficient information for a POSA to recognize that the inventors possessed the full scope of the claimed subject matter. I have been informed that the specification must disclose either a representative number of species (here, “tetravalent, multispecific Wnt surrogate molecules”) falling within the scope of the claimed genus (i.e., the entire group of Wnt surrogate molecules encompassed by the claims of the '278 patent) or structural features common to the members of the genus such that a POSA can “visualize or recognize” the members of the claimed genus.

60. Enablement Requirement: I understand that the specification must enable a POSA to make and use the full scope of the claimed subject matter without undue experimentation as of the effective filing date. I further understand that the specification may call for a reasonable amount of experimentation to make and use

a patented invention, and that what is reasonable in any case depends on the nature of the invention and the underlying art.

61. To determine whether the amount of experimentation required would be undue or reasonable, I understand that the following factors can be considered: (1) the breadth of the claims, (2) the nature of the claimed subject matter, (3) the state of the prior art, (4) the level of predictability in the art, (5) the level of one of ordinary skill, (6) the amount of direction or guidance provided by the named inventors, (7) the presence or absence of working examples, and (8) the quantity of experimentation needed to make or use the claimed subject matter based on the content of the disclosure.

62. Definiteness Requirement: I understand that under the “definiteness requirement,” patent claims must particularly point out and distinctly claim the subject matter that the named inventors regard as their invention. I understand that a claim fails to meet this standard—that it is *indefinite*—when it fails to inform a POSA of the claim’s scope with reasonable certainty when read in view of the specification and the prosecution history. In this regard, I understand that a claim may be indefinite if there are multiple ways to measure a claimed parameter that would yield different results but the patent and its prosecution history fail to specify a measurement method to use.

VII. LEVEL OF ORDINARY SKILL IN THE ART

63. I understand that my analysis of the '278 patent claims should be based on what a POSA would have known or understood at the effective filing date of the application that became the '278 patent. The '278 patent claims priority to three provisional applications, filed December 19, 2017, March 9, 2018, and June 4, 2018, respectively. EX1001, 1; EX1005-1007. I was instructed to assume December 19, 2017 as the effective filing date of the '278 patent claims. But my opinion regarding the '278 patent would not change if the effective filing date is December 19, 2018, which I understand is the filing date of the first non-provisional application. EX1001, 1.

64. I have considered the following factors in determining the level of ordinary skill in the art: (1) the type of problems encountered in the art; (2) the prior-art solutions to those problems; (3) the rapidity with which innovations are made; (4) the sophistication of the technology; and (5) the educational level of active workers in the field.

65. Given the nature of the subject matter claimed in the '278 patent, in my opinion and based on my experience in the field at the relevant time, a POSA for the subject matter of the '278 patent in December 2017 would have had a Ph.D. degree in immunology, molecular biology, chemistry, or biochemistry with at least five years of experience in antibody engineering or Wnt signaling. A POSA with respect

to the '278 patent may also have had access to other POSAs with complementary experience in antibody engineering or Wnt signaling.

VIII. CLAIM CONSTRUCTION OF “WNT SURROGATE MOLECULES”

66. I was asked by counsel to consider the meaning of the term “Wnt surrogate molecule,” which appears in the preamble of claim 1. I was also asked by counsel to consider whether the term “Wnt surrogate molecule” is limiting. In my opinion, the term “Wnt surrogate molecule” means a “molecule that is biologically active in binding to one or more Fzd receptor and to one or more of LRP5 and LRP6, and in activation of Wnt signaling, i.e., the molecule is a Wnt agonist.” As explained below, I also conclude that the term “Wnt surrogate molecule” is part of the definition of and is limiting on the scope of the claimed subject matter.

67. The specification of the '278 patent makes clear that the inventors expressly defined the term “Wnt surrogate molecule” as “molecule that is biologically active in binding to one or more Fzd receptor and to one or more of LRP5 and LRP6, and in activation of Wnt signaling.”

68. The '278 patent specification states:

*Wnt surrogate molecules of the present invention are biologically active in binding to one or more Fzd receptor and to one or more of LRP5 and LRP6, and in activation of Wnt signaling, i.e., the Wnt surrogate molecule is a **Wnt agonist**.*

EX1001, 16:52-56 (emphasis added). This statement demonstrates that the inventors have clearly defined that the claimed “Wnt surrogate molecules” of the “present invention” must possess these functional features: (1) they are biologically active in binding to one or more Fzd receptor and to one or more of LRP5 and LRP6; and (2) they are also biologically active “in activation of Wnt signaling.” In my opinion, by providing such an explicit definition, a POSA would understand that the inventors assigned this specific meaning to the term “Wnt surrogate molecule.”

69. It is also my opinion that the term “Wnt surrogate molecule” would also be understood by a POSA to limit the scope of the claims of the ’278 patent. That is, in my opinion, this term limits the claims of the ’278 patent to only include those molecules that are “biologically active in binding to one or more Fzd receptor and to one or more of LRP5 and LRP6, and in activation of Wnt signaling” and excludes those molecules that are not “biologically active” in performing these functions.

70. In my opinion, both the specification and the prosecution history indicate to a POSA that this term is necessary to give life, meaning, and vitality to the claims and, therefore, is limiting.

71. As I explained above, the specification of the ’278 patent expressly defines the “Wnt surrogate molecule” of the “present invention” to be a molecule that is “biologically active in binding to one or more Fzd receptor and to one or more of LRP5 and LRP6, and in activation of Wnt signaling.” EX1001, 16:52-55. That

definition further includes the statement that “the Wnt surrogate molecule is a *Wnt agonist*.” *Id.*, 16:55-56 (emphasis added). The specification of the ’278 patent also repeatedly emphasizes that the claimed subject matter is an agonist of the Wnt signaling pathway. *E.g., id.*, Abstract, 1:34-35 (the “present invention relates generally to Wnt signaling pathway *agonist* molecules”) (emphasis added).

72. Furthermore, as I explained above (*see supra* Section V.A), in every example of the ’278 patent that evaluated the biological activity of candidate molecules in an *in vitro* assay, the assay of choice was invariably a TOPFlash assay. Notably, the named inventors appear to treat positive responses in this assay as a proxy for Wnt signaling activation. *See, e.g.*, EX1001, 155:25 (Example 2: “*activate* canonical Wnt signaling”) (emphasis added); 156:66 (Example 6: “demonstrated ability to *activate* Wnt signaling”) (emphasis added); 157:11-12 (Example 7: “were able to *activate* Wnt signaling”) (emphasis added). Indeed, the ’278 patent does not exemplify any “Wnt surrogate molecule” that modulates the function of the Wnt signaling pathway by a purported mechanism other than activation. Therefore, in my opinion, the ’278 patent specification clearly shows that the inventors considered the “biologically active” “agonist” functional property as an essential requirement for the claimed molecules.

73. The prosecution history also supports my opinion that the preamble is limiting. During prosecution of the ’278 patent, the Applicant relied on the preamble

of claim 1 to overcome a prior art rejection partly based on Wu. EX1008, 4005-06.

According to the Applicant, Wu taught antagonists:

that allow the simultaneous blockade of multiple targets . . . which is very different than the claimed molecules, which simultaneously engage two cell surface coeffectors molecules (Fzds and Lrp5/6) required to agonize the canonical Wnt signaling cascade . . . This represents an important biological distinction between the molecules of Wu and those presently claimed.

Id., 4006 (emphasis in original).

74. The declaration from the named inventor Dr. Li also reflects that Wnt surrogate molecules of the invention must activate Wnt signaling pathways. Dr. Li contended that “a Wnt surrogate molecule . . . must bind both Fzd receptor(s) and Lrp5/6 receptors simultaneously, in order to agonize the Wnt signaling pathway.”

EX1008, 4168. Dr. Li repeated the Applicant’s assertion that:

Wu et al relates to bivalent molecules that allow the simultaneous blockade of multiple targets . . . which is very different than the claimed molecules, which simultaneously engage two cell surface coeffectors molecules (Fzd and Lrp5/6) required to agonize the canonical Wnt signaling cascade.

Id. (emphasis in original).

75. For the reasons discussed above, it is my opinion that the preamble term “Wnt surrogate molecule” is limiting and means a “molecule that is biologically active in binding to one or more Fzd receptor and to one or more of LRP5 and LRP6, and in activation of Wnt signaling, i.e., the molecule is a Wnt agonist.”

IX. BACKGROUND AND STATE OF THE ART

A. Wnt Signaling Is Complex and Was Poorly Understood in 2017

76. The Wnt signaling pathway is one of the most, if not the most complicated cellular pathways known. Since the discovery of the first Wnt gene, Wnt1, in mouse tumor cells in 1982, researchers have been exploring this intricate network of proteins and signaling cascades that regulate a wide range of cellular processes. EX1060, 1. By 2017, it was understood that Wnt signaling operates through both canonical and noncanonical pathways. *Id.*, 2. Canonical Wnt signaling is mediated by Wnt ligands that stabilize β -catenin, allowing it to accumulate in the nucleus and activate TCF/LEF-dependent transcription of target genes. Noncanonical Wnt signaling, by contrast, is β -catenin-independent and includes pathways such as the planar cell pathway (“PCP”) and the Wnt/Ca²⁺ pathway. EX1043, 2-4. As discussed in detail below, all of these signaling mechanisms begin with the binding of extracellular Wnt ligands to cell-surface receptors, which initiate intracellular cascades that ultimately regulate gene transcription. *Id.*

77. Wnt signaling is involved in a multitude of biological processes, from regulating tissue stem cells during embryonic development to maintaining adult tissue homeostasis by modulating cell proliferation, differentiation, migration, and apoptosis. EX1063, 1; EX1043, 1. For example, Wnt signaling regulates the self-renewal as well as the differentiation of stem cells across different tissues and

systems in the body, such as hair follicles, intestines, mammary glands, kidneys, and the hematopoietic system. EX1063, 7.

78. As Wnt signaling is involved in many biological functions, aberrant regulation of the Wnt signaling pathway has been associated with the pathology of a variety of diseases, including many cancers, metabolic diseases, neurodegenerative diseases, and growth-related pathologies. EX1063, 1; EX1043, 1. For instance, in some cancers, Wnt ligands are overexpressed leading to aberrant cell growth. EX1043, 4. Mutations in Wnt signaling proteins and receptors is known to cause neural tube defects during development. *Id.*, 5.

79. While the field in 2017 recognized the therapeutic potential of targeting the Wnt signaling pathway, “a number of factors . . . thwarted progress in this field,” including the fact that “the WNT signaling cascade is bewilderingly complex.” EX1043, 1. As discussed in detail below, Wnt signaling involves multiple ligands, receptors, and co-receptors, triggers diverse downstream responses through both the canonical β -catenin pathway and noncanonical pathways, and exhibits complex tissue and cell-type specificity.

80. Complex Wnt ligands, receptors, and co-receptors. By 2017, the Wnt signaling network was known to involve interactions between multiple ligands, receptors, and co-receptors. EX1043, 1. However, the mechanisms by which individual Wnt and non-Wnt ligands engage specific receptors and co-receptors to

activate Wnt signaling were poorly understood, and were recognized to vary from ligand to ligand.

81. As of 2017, “19 WNT ligands and more than 15 receptors and co-receptors distributed over seven protein families in mammals” had been identified. EX1043, 1. While these Wnt ligands were known to activate either the canonical Wnt signaling pathway (through β -catenin) or noncanonical Wnt signaling pathways, “the activity of WNTs depends on the cellular context and the receptor, and thus WNTs cannot be rigorously subdivided according to the pathway they induce.” EX1060, 2. In other words, whether and how Wnt ligands trigger downstream signaling processes depended on not only the binding partners but many other factors external to the ligand-receptor interaction. Thus, it was known that Wnt ligands cannot be neatly sorted into “canonical” or “non-canonical” groups because their effects change depending on the situation.

82. As of 2017, multiple receptors and co-receptors were known to interact with Wnt ligands. Among these receptors and co-receptors are ten Frizzled (Fzd) receptors, Fzd1-10, and two low-density lipoprotein receptor-related (Lrp), Lrp5 and Lrp6. EX1063, 1, 3. By 2017, it was well recognized that extensive cross-reactivity existed between different Wnt ligands and Fzd, Lrp5, and Lrp6 receptors. *Id.*, 1. Multiple studies have demonstrated that certain Wnt ligands preferentially interact with specific Fzd and/or Lrp receptors. The same Wnt ligand could interact with

multiple Fzd receptors and bind to these receptors with different affinities. For example, in one study, Wnt3A was shown to bind strongly across many Fzd receptors, including Fzd4, Fzd5, Fzd7, and Fzd8. EX1025, 3-5. In contrast, other Wnt ligands, such as Wnt4, Wnt5A, Wnt5B, were more selective, only strongly binding to Fzd8 over other Fzd receptors. *Id.* This study also demonstrated that different Wnt ligands can attach to several types of Fzd receptors, but they do not all attach with the same strength. *Id.*

83. The specificity between Wnt ligands and Lrp receptors is also diverse. For example, Holmen and colleagues evaluated the diverse pattern of Wnt ligand interaction with Fzd and Lrp receptors using 293T cells. EX1033, Abstract. The study showed that, when Wnt11 and Wnt5A were fused to Fzd5, the fusion proteins were able to induce β -catenin signaling in cells when the cells express Lrp6, but not Lrp5. *Id.*, 6, Fig. 5. The study also showed that, when Wnt8 was fused to different Fzd receptors, Fzd3, Fzd4, Fzd5, Fzd6, and Fzd7, the fusion proteins induced β -catenin signaling in cells differently. *Id.*, 5-6, Figs. 5-6. For example, Wnt8-Fzd4 fusion induced the signaling in the presence of Lrp5 or Lrp6, but Wnt8-Fzd5 fusion induced the signaling only in the presence of Lrp6. *Id.*, 6, Fig. 6. A similar, distinct pattern across different Wnt ligands was reported in another study from Goel and colleagues. This study reported that the presence of both Lrp5 *and* Lrp6 is required for signaling transduction by Wnt1, Wnt9B and Wnt10B in mammary basal cells,

while Wnt3A can induce signaling with only either Lrp5 or Lrp6 and does not require the presence of both receptors. EX1082, 1. These findings demonstrate that different Wnt ligands require different Fzd and Lrp receptors to induce Wnt signaling, and the biological activity of one Wnt-Fzd-Lrp complex in one cell type does not necessarily translate to another complex in another cell type.

84. Even for the same ligand-receptor pair, Wnt signaling activity can be dependent on which epitopes on the Lrp receptor the ligand binds. Lrp6, for example, was known to contain multiple epitopes where Wnt ligands could bind, including in E1, E2, E3, and E4 domain(s). EX1016, 1, 3. In a binding assay reporting β -catenin fluorescence, Wnt3A stabilized β -catenin when bound to domains E1E2 or E3E4 but the same Wnt3A ligand inhibited β -catenin stabilization when bound to domain E1E4. *Id.*, 3, Fig. 1B. As another example, Wnt1, Wnt2, Wnt2B, Wnt6, Wnt8A, Wnt9A, Wnt9B, and Wnt10B all preferentially bind to Lrp6 domain E1E2, whereas Wnt3 and Wnt3A preferentially binds to Lrp6 domain E3E4. EX1054, 6-7, Fig. 2.

85. Due to the complexity and diversity of Wnt-Fzd-Lrp interactions, the field in 2017 struggled to come up with a coherent interaction model that applies to Wnt ligands generally. For example, one model proposed a 1:1:1 (Fzd:Wnt:Lrp) stoichiometry for certain Wnt ligands based on the observation that Wnt8, Wnt5A or Wnt11 fused to Fzd5, Fzd4, and Fzd7 were able to interact with Lrp5/6 and induce Wnt signaling. EX1033, Fig. 8. Another model proposed a 2:2:1 (Fzd:Wnt:Lrp)

stoichiometry where Wnt3A and Wnt9B interact with two separate Fzd receptors but share one Lrp6 receptor and induce Wnt signaling. EX1016, Fig. 5C. Yet another model proposed the ability of Fzd receptors to aggregate and form an oligomer of four interconnected Fzd receptors upon binding of Wnt. EX1022, 6-7, Fig. 7. These competing models illustrate that Wnt signaling was thought to occur through different receptor–ligand arrangements and stoichiometry, highlighting how incomplete the field’s understanding of Wnt receptor architecture and signaling dynamics still was.

86. The field in 2017 relied on these simulated models in part because no complete Wnt-Fzd-Lrp complex had been crystalized. *See, e.g.*, EX1046, 2. Available crystal structures included only subparts of the complex and often involved non-human species or the combination of human and non-human species. For example, one study analyzed Wnt-Fzd interactions through the pairing of xenopus Wnt8 and mouse Fzd8. EX1038, 6, 10, Fig. 2. Another group studied the structure of the cysteine-rich domain of Fzd4 crystalized with palmitoleic acid. EX1022, 2-3, Fig. 1. Having a complete crystal structure of the full Wnt-Fzd-Lrp complex would have been helpful in designing therapeutic moieties, which could mimic Wnt ligands and their interaction with Fzd/Lrp receptors. But having the crystal structure is only the first step in that process. Ultimately, one must understand whether this crystalized complex is relevant in the physiological system,

i.e., how the Wnt-Fzd-Lrp complex exists and functions in the human body. Unfortunately, as of 2017, the “unambiguous demonstration of this receptor complex *in vivo* [was] lacking.” EX1053, 3.

87. To further complicate the ligand-receptor interactions in the Wnt signaling pathway, it was also known that ligands structurally unrelated to Wnt ligands can interact with Fzd and Lrp receptors. EX1063, 3-4. Just like Wnt ligands, these non-Wnt ligands can activate Wnt signaling and compete for binding to Fzd and Lrp receptors. One example of non-Wnt ligands is Norrin, which was reported to bind to Fzd4 and Lrp5/6 and mediate the development of the ear, brain, and female reproductive system, and maintain the integrity of the blood-brain and blood-retina barriers. EX1044, 1-2.

88. As of 2017, it was understood that endogenous Wnt ligands could activate downstream signaling without the aid of a biological potentiator in certain biological processes. See, e.g., EX1063, 5; EX1060, 9-11; EX1053, 4-5; EX1093, 1-3. But it was also known that the body expresses several external potentiators, which can synergize with Wnt ligands and boost downstream signaling. One example of such potentiators is R-spondin. EX1063, 5. It was shown that R-spondin inhibits factors that destabilize and degrade Fzd receptors, resulting in the stabilization of Fzd receptors and thereby longer, stronger Wnt signaling. *Id.*

89. As illustrated above, by 2017, much of the knowledge about Wnt signaling was derived from studies in non-human cells and organisms or *in vitro* assays using isolated human cells. This situation hampered the identification of optimal therapeutic strategies due to an incomplete understanding of signaling circuitry in the human body. Tellingly, as of 2017, there was no FDA-approved drug that was developed to specifically target Wnt signaling. See EX1043, 11 (FDA-approved drugs that may affect Wnt signaling did so only “nonspecifically”). That remains true to date.

90. Diverse downstream signaling responses. As discussed above, as of 2017, it was understood that Wnt signaling operates through both canonical and noncanonical pathways. EX1060, 2. Wnt signaling operated as a highly interconnected network in which downstream responses were shaped not by any single ligand or receptor, but by the specific combination of Wnt proteins, Fzd receptors, co-receptors, and intracellular factors involved, functioning through both canonical and noncanonical pathways.

91. In 2017, it was thought that canonical Wnt signaling is initiated when a Wnt ligand binds to a Fzd receptor and its co-receptor Lrp5/6 on the cell surface, leading to activation of the intracellular protein Dishevelled. Dishevelled promotes phosphorylation of the cytoplasmic tail of Lrp5/6, which in turn promotes Axin binding and causes Axin to be recruited from the cytoplasmic β -catenin destruction

complex to the cell membrane, leading to the inhibition of β -catenin phosphorylation and proteasomal degradation. As a result, stabilized β -catenin accumulates in the cytoplasm and translocates into the nucleus, where it associates with TCF/LEF transcription factors and activates expression of Wnt target genes such as *c-myc* and *cyclin-D1*. EX1043, 1-3.

92. Compared to canonical Wnt signaling pathways, noncanonical Wnt signaling pathways were much less understood, in part due to overlap with other signaling pathways. EX1045, 2. Noncanonical Wnt signaling pathways activate gene transcription through signaling proteins such as PLC, calcineurin, and NFAT for the Wnt/ Ca^{2+} pathway and proteins such as RAC1, JNK and JUN in the planar cell polarity (“PCP”) pathway. EX1060, 2.

93. The distinction between canonical and noncanonical Wnt signaling is not always clear-cut. For example, calcium acts downstream of noncanonical Wnt signaling receptors in both the PCP pathway and Wnt/ Ca^{2+} pathway and is known to mediate the antagonism of the canonical β -catenin pathway. EX1045, 2, 5. As another example, heterotrimeric G-proteins, once thought to only be involved in noncanonical Wnt signaling, were also known to induce TCF/ β -catenin mediated reporter gene activity. EX1060, 4-5.

94. Wnt ligands were initially classified as canonical or noncanonical based on their primary pathway activation in biological assays used to assess activity at the

time. EX1065, 3-4. For example, Wnt1, Wnt3A, and Wnt8 were classified as canonical pathway activators while Wnt5A and Wnt11 were classified as noncanonical pathway ligands. *Id.* This classification, however, was not straightforward. For example, while noncanonical signaling typically occurs through Wnt interactions with Fzd and co-receptors such as Ryk and Ror, Lrp6 was known at the time to also mediate noncanonical signaling. EX1029, 1. As another example, Wnt11, classified as a noncanonical ligand, was known to activate canonical β -catenin signaling during axis specification. EX1065, 3-4.

95. Wnt pathway receptors, particularly Fzd and Lrp, also participate in both the canonical and noncanonical signaling pathways. Different Fzd receptors, such as Fzd4, were known to activate canonical Wnt signaling while others, for example, such as Fzd8 and Fzd9, were known to activate noncanonical signaling, and yet some Fzd receptors, for example, Fzd1, Fzd2 and Fzd7, were capable of activating both. EX1077, 3-8. While Fzd-Lrp interactions primarily mediate the activation of canonical Wnt signaling, Fzd8-Lrp6 interactions were shown to inhibit Fzd8-mediated noncanonical pathway activation. EX1066, 1, 5-6. As of 2017, the “determinants of Wnt-Frizzled specificity [were] still unknown, and there [was] a strong suspicion that additional modulatory/specificity proteins remain[ed] to be discovered.” EX1077, 11. This complexity indicated that “differences in affinities

of different Wnts and Fzds and their local concentrations . . . would determine to which signaling branch a particular Wnt couples.” EX1065, 4.

96. Even within the canonical Wnt signaling pathway, downstream signaling responses were known to be complex. Signaling through the β -catenin pathway depends on the activity of four protein complexes, including 1) the Wnt-Fzd-Lrp complex, 2) the β -catenin destruction complex involving multiple proteins such as Axin and APC, 3) the nuclear TCF/LEF transcriptional complex, and 4) the nuclear transcriptional repressor complex. EX1034, 1; EX1070, 1. Wnt-dependent activity at each of these levels can be modulated based on the interactions with multiple proteins and downstream signaling molecules. *See, e.g.*, EX1070, 3-5, 7; EX1034, 1, 6-7.

97. At the level of the β -catenin destruction complex, proteins Axin, APC, GSK-3 and CK1 work in concert to phosphorylate β -catenin, targeting it for destruction in the proteasome. EX1070, 2. The “relative levels of all components appear to be critical for both homeostasis and responsiveness to Wnt” as mutations or imbalances in the levels of proteins in this complex, such as APC, alter canonical Wnt signaling. *Id.*, 5. In the nucleus, β -catenin binds to TCF/LEF and cofactors such as CBP or p300, BCL9/LGS, and Pygo to initiate transcription of canonical Wnt signaling genes. EX1034, 1. In the absence of β -catenin, TCF/LEF bind to co-repressors such as CtBP and groucho to inhibit the transcription of canonical Wnt

pathway genes. *Id.* TCF and LEF activity is modulated by the binding of SUMO, small ubiquitin-related modifiers; when bound to LEF, SUMO inhibits transcriptional activation, however when bound to TCF, SUMO enhances transcriptional activity. *Id.*, 7. The determination of Wnt-dependent activity in the canonical pathway therefore requires assessment of key regulators at each of these levels. EX1065, 4 (“[T]he analysis of a particular Wnt ligand or Frizzled receptor in a certain biological context always requires an investigation of how the Wnt signal is transduced.”).

98. *Highly tissue- and cell-dependent.* By 2017, it was known that Wnt and non-Wnt ligands and their receptors function in distinct tissue and cellular contexts. For example, Norrin was known to play important roles in the development of the eye, ear, brain, and female reproductive system. EX1044, 1. Wnt4 was known to specifically express in the gonads during development of the female reproductive system and inactivation of Wnt4 in mice led to reproductive deficiencies. EX1043, 9. The loss of Wnt gene activity was known to result in numerous unique phenotypes, corresponding to the tissue and cellular expression profiles of those Wnt and non-Wnt ligands. EX1063, 1. For example, Wnt1 deficiency led to midbrain defects in mice, while Wnt4 deficiency caused kidney development abnormalities. *Id.*

99. Even within a given tissue or system, different Wnts regulate different cell types. For example, in the intestines, Wnt3, Wnt6, and Wnt9B, along with Fzd4,

Fzd6, and Fzd7, were reported to be expressed in crypt epithelial cells and induce proliferation and cell positioning. EX1030, 1-2. In subepithelial mesenchymal cells in the intestine and colon, Wnt2B, Wnt4, Wnt5A, and Wnt5B, along with Fzd4 and Fzd6, were expressed and influenced cell differentiation. *Id.*, 1. In the immune system, receptor expression varies by cell type: microglia expressed Fzd3, Fzd5, Fzd7 and Fzd8, whereas myeloid progenitor cells exhibited little to no Fzd receptor expression. EX1025, 5, Fig. 2.

100. In sum, Wnt signaling is an extraordinarily complex system. It encompasses numerous ligands, receptors, and co-receptors and mediates a wide spectrum of downstream responses through canonical β -catenin signaling as well as noncanonical pathways, with signaling outputs that are highly dependent on cellular and tissue context. As a result of this complexity, determining the effects of Wnts or Wnt mimetics on downstream signaling is an extremely time-intensive process that requires testing Wnts in multiple contexts and cell types in combination with testing the binding capabilities to Fzds1-10 and Lrp5/6 epitopes.

B. Methods for Measuring Wnt Signaling Activity in 2017 Were Highly Condition-Dependent

101. As of 2017, several different methods were used in the field to assess Wnt signaling activity. However, no one assay could adequately capture all Wnt signaling activity as 1) each assay had significant limitations and 2) Wnt signaling

could be influenced by other pathways and modulators at each stage, i.e., receptor-ligand interactions, signal transduction, and gene transcription.

102. This lack of a single standardized assay stemmed in part from the complex mechanisms underlying canonical and noncanonical Wnt signaling, which generally rendered assays developed for canonical Wnt signaling inapplicable to noncanonical signaling, and vice versa. However, even assays that could measure common upstream modulators, such as Dishevelled, were problematic because both canonical and noncanonical Wnt ligands could induce phosphorylation of a common region on the Dishevelled protein, leading to an unclear interpretation, as a standalone assay, of which pathway was activated with no indication of whether this phosphorylation resulting in downstream gene transcription. EX1028, 4.

103. Assays frequently used for canonical Wnt signaling by 2017 included LRP phosphorylation assay, Axin translocation assay, and several β -catenin-based assays such as β -catenin stabilization assay, Wnt/ β -catenin target gene expression assay, and TCF reporter gene expression assay. As discussed further below, results for a given molecule could and did vary from assay to assay. Moreover, the results of each type of assay could and did vary depending on the cell type and other conditions used.

104. The LRP phosphorylation assay measures the phosphorylation of the cytoplasmic tail of Lrp5/6 induced by Wnt ligands. As discussed above (*see supra*

Section IX.B), in the absence of Wnt signaling stimulation, Lrp5/6 is largely unphosphorylated. Binding of canonical Wnt ligands to Fzd and Lrp5/6 triggers recruitment of a complex containing CK1 and GSK3, which phosphorylate the cytoplasmic tail of Lrp5/6. EX1063, 3. LRP phosphorylation is typically detected using phospho-specific antibodies by Western blotting, immunoprecipitation, or immunofluorescence microscopy. Because the noncanonical Wnt pathways do not signal through Lrp5/6 phosphorylation, this assay can only report the activation of canonical Wnt signaling. EX1086, 1.

105. The Axin translocation assay measures the relocalization of Axin. As discussed above (*see supra* Section IX.B), in the absence of Wnt signaling, Axin resides predominantly in the cytoplasm, where it facilitates phosphorylation and degradation of β -catenin. Upon stimulation of canonical Wnt signaling, the cytoplasmic tail of Lrp5/6 is phosphorylated, which promotes Axin binding and its recruitment from the cytoplasm to the plasma membrane. EX1063, 3. This Wnt-induced translocation of Axin can be detected using immunofluorescence microscopy, live-cell imaging, or biochemical fractionation approaches. The Axin translocation assay, like the LRP phosphorylation assay, does not assess the activation of noncanonical Wnt signaling.

106. The β -catenin stabilization assay measures cytoplasmic accumulation of β -catenin. As discussed above (*see supra* Section IX.B), in the absence of Wnt

ligands, cytoplasmic β -catenin is constitutively phosphorylated by the destruction complex, leading to its ubiquitination and proteasomal degradation. Upon stimulation with canonical Wnt ligands (such as Wnt3A), the destruction complex is inhibited, resulting in the accumulation and stabilization of cytoplasmic β -catenin. EX1043, 2. The stabilization of β -catenin can be measured biochemically by immunoblotting or immunofluorescence, typically using antibodies specific for total or non-phosphorylated (active) β -catenin.

107. The Wnt/ β -catenin target gene expression assay measures downstream transcriptional activity of canonical Wnt signaling. Upon stabilization and nuclear translocation, β -catenin associates with TCF/LEF family transcription factors to activate transcription of a number of endogenous Wnt-responsive target genes, including AXIN2, MYC (*c-Myc*), and CCND1 (*cyclin D1*). EX1043, 1-3. The activity of canonical Wnt signaling can therefore be assessed by measuring changes in expression of these endogenous target genes using methods such as quantitative PCR and RNA sequencing.

108. The TCF reporter assays are an artificial cell-based assay, which could approximate the transcriptional activity of the canonical Wnt signaling pathway using a reporter system. These assays typically use cells transfected with synthetic reporter constructs containing multimerized TCF/LEF binding sites positioned upstream of a minimal promoter that drives expression of a reporter gene, such as

luciferase (also commonly referred to as “TOPFlash” assays). Upon activation of canonical Wnt signaling, stabilized β -catenin enters the nucleus and forms transcriptional complexes with TCF/LEF transcription factors, resulting in increased reporter gene expression. Reporter activity is then quantified using luminescence or fluorescence measurements. TCF reporter assays include the steps of cell culture, reporter construct transfection, ligand treatment, and reporter readout. The entire process typically takes about a week from start to finish. As the TCF reporter assays respond specifically to β -catenin-mediated transcriptional activation, they do not detect noncanonical Wnt signaling pathway activity, which proceeds independently of β -catenin.

109. Unlike the β -catenin stabilization assay and the Wnt/ β -catenin target gene expression assay, TCF reporter assays rely on synthetic transcription elements, whose responses do not always reflect endogenous transcriptional regulation. Reporter activity can vary depending on the design, preparation, and transfection of the construct, making interpretation of small activation signals challenging without appropriate controls. Thus, standard practice in the field is to include *both* positive controls (e.g., known pathway activators such as Wnt3a) and negative controls (e.g., basal reporters such as FOPFlash, which do not contain the TCF/LEF binding elements) to distinguish true canonical Wnt/ β -catenin pathway activity from background or non-specific effects.

110. Despite their widespread use, TCF reporter assays, including TOPFlash, have significant limitations and cannot “capture all the important transcriptional changes caused by activated WNT signaling.” EX1043, 3. *First*, multiple pathways can activate β -catenin signaling independently of Wnts, which can confound the results of β -catenin reporter assays and obscure whether the measured signal reflects Wnt-dependent or Wnt-independent processes. EX1043, 11. *Second*, because Wnt-dependent transcription depends on the assembly of activating or repressing transcriptional complexes, β -catenin nuclear translocation “does not imply functional Wnt signaling.” EX1055, 1 (“[I]n some cellular contexts, LEF/TCF members recruit transcriptional inhibitors and maintain the promoter in an OFF state even in the presence of activated β -catenin.”). *Third*, β -catenin was known to be “highly underestimated by some of these readouts (for example, TOPFlash)” because β -catenin binds to a spectrum of transcription factors beyond TCF/LEF, allowing it to “modulate a plethora of downstream biological processes.” EX1043, 11. *Finally*, β -catenin signaling readouts are “highly cell-type dependent.” *Id.* Taken together, these limitations show that TOPFlash and related TCF reporter assays provide, at best, an incomplete and often misleading picture of true Wnt/ β -catenin pathway activity. Notably, many of these limitations with TCF receptor assays, e.g., cell type dependence, are also applicable to other assays discussed above (e.g., gene expression assays or *in vivo* assays) because this

variability is inherent to Wnt signaling pathways, which vary from one cell type to another. Moreover, the limitations of these assays mean that one could obtain conflicting results from one assay to another. As noted above, by 2017, it was well known that TCF reporter assays such as a TOPFlash assay could (and did) underestimate the canonical signaling activation because β -catenin can activate other transcription factors. As such, a negative result on a TOPFlash assay did not necessarily indicate the lack of canonical Wnt signaling activation, and other assays such as gene expression assays, which may capture other transcription factors, could still show responses. This phenomenon, known as “ghost response,” illustrates that determining whether Wnt signaling activation occurred was far from straightforward in 2017, and in fact, often ambiguous and conflicting depending on the assay of choice, and the assay condition of choice.

111. Studies with Wnt5A illustrate this complexity and uncertainty in measuring Wnt signaling activity. In a TOPFlash assay using 293 cells, Wnt5A inhibited the activation of canonical Wnt signaling. EX1056, 2. However, when those cells were engineered to express Fzd4 and Lrp5, Wnt5A induced the activation of canonical Wnt signaling. *Id.*, 5. The ability of Wnt5A “to toggle between two considerably diverse forms of signaling” demonstrates that cell receptor expression significantly influences Wnt signaling outcomes. *Id.*, 11. As another example, in ST2 cells, Wnt3A was shown to activate both canonical signaling through β -catenin

and noncanonical signaling through G-protein activation of PKC δ , while Wnt3A activity in other cell types has been classically defined as canonical. EX1087, 9. This cell-type dependency reflects the understanding that Wnt activity cannot be determined by the Wnt molecule itself, but rather can only be evaluated based on specific biological contexts which influences receptor density and availability.

112. In sum, each of the methods discussed above measures only specific components of the Wnt signaling pathway, and their applicability is highly dependent on experimental conditions, including cell type and the expression levels of Fzd and Lrp receptors. Thus, determining whether and to what extent Wnt signaling was activated by experimental approaches was challenging in 2017.

X. ANALYSIS OF THE '278 PATENT

A. The '278 Patent Claims Cover Wnt Surrogate Molecules with an Exceedingly Broad Scope of Fzd-Lrp Target Combinations

113. Claim 1 of the '278 patent recites that the claimed “Wnt surrogate molecule” comprises “two Fzd binding regions that specifically bind to two Frizzled (Fzd) receptors” and “two LRP5/6 binding regions that specifically bind to a Low-density lipoprotein (LDL) receptor-related protein 5 (LRP5) and/or a Low-density lipoprotein (LDL) receptor-related protein 6 (LRP6).” EX1001, col. 1.

114. With respect to the claimed “Fzd binding regions,” claim 1 does not impose any restriction on the specific Fzd receptors that the “Fzd binding regions” can bind to. The specification states that any of the “Fzd binding regions” can bind

to “one or more” of Fzd1, Fzd2, Fzd3, Fzd4, Fzd5, Fzd6, Fzd7, Fzd8, Fzd9 and Fzd10, or “two or more” of Fzd1, Fzd2, Fzd3, Fzd4, Fzd5, Fzd6, Fzd7, Fzd8, Fzd9 and Fzd10. EX1001, 2:31-40. The specification further lists eight specific combinations where any of the “Fzd binding regions” can bind to **three or more** Fzd receptors: (a) Fzd1, Fzd2, Fzd7, Fzd9; (b) Fzd1, Fzd2, Fzd7; (c) Fzd5, Fzd7, Fzd8; (d) Fzd1, Fzd4, Fzd5, Fzd8; (e) Fzd1, Fzd2, Fzd5, Fzd7, Fzd8; (f) Fzd5, Fzd8, Fzd10; (g) Fzd4, Fzd5, Fzd8; and (h) Fzd1, Fzd5, Fzd7, Fzd8. *Id.*, 2:41-46.

115. For each of the two claimed “Fzd binding regions,” if it binds to one of the 10 Fzd receptors, there are 10 possibilities. If it cross-reacts with two of the 10 Fzd receptors, there are 45 possibilities (the combination of 10 items taken 2 at a time). Plus the eight specifically disclosed combinations of three or more of Fzd receptors, there are a total of 63 possibilities (10 + 45 + 8) of Fzd or Fzd combinations for each of the two claimed “Fzd binding regions.” For example, a tetravalent molecule comprising two Fzd binding regions, each of which cross-react with Fzd1, Fzd2, and Fzd7, would bind to two Fzd molecules, each selected from Fzd1, Fzd2 and Fzd7. In contrast, if the Fzd binding region reacts with Fzd1 only, the tetravalent molecule would still bind to two Fzd molecules, but both would be Fzd1. This calculation underestimates the possible Fzd or Fzd combinations, because “one or more” and “two or more” of Fzd1-10 covers many more combinations of three or more of Fzd receptors than the eight combinations

specifically called out in the specification. For my calculation, however, I use the conservative number of 63 possibilities.

116. With respect to the claimed “LRP5/6 binding regions,” because each of these regions must bind to Lrp5 “and/or” Lrp6, there are at least three possibilities for each of the two “LRP5/6 binding regions”: (1) Lrp5 only; (2) Lrp6 only; and (3) both Lrp5 and Lrp6.

117. Claim 1 does not have any limitation regarding the choices of Fzd receptors between the two “Fzd binding regions.” That is, the choice of a specific Fzd or Fzd combination for the first of the two “Fzd binding regions” does not affect the choice of a specific Fzd or Fzd combination for the second of the two “Fzd binding regions.” Similarly, claim 1 does not have any limitation regarding the choices of Lrp5/6 receptors between the two “LRP5/6 binding regions.” That is, the choice from the three possibilities of Lrp5 and/or Lrp6 for the first of the two “LRP5/6 binding regions” does not affect the choice from the three possibilities of Lrp5 and/or Lrp6 for the second of the two “LRP5/6 binding regions.” Moreover, claim 1 also does not have any limitation regarding the combination of Fzd-Lrp receptors. That is, the choice of a specific Fzd or Fzd combination for each of the “Fzd binding regions” does not affect the choice of Lrp5 and/or Lrp6 for each of the “LRP5/6 binding regions.”

118. Applying the numbers and principles explained above, *each* tetravalent molecule covered by claim 1 can bind to one of at least **35,721** Fzd-Lrp target combinations. The calculation is as follows: 63 possibilities for the first Fzd binding region $\times$ 63 possibilities for the second Fzd binding region $\times$ 3 possibilities for the first LRP5/6 binding region $\times$ 3 possibilities for the second LRP5/6 binding region = 35,721 combinations. As I explained above, this number *significantly underestimates* the combinations covered by claim 1 because my calculation only accounts for the eight combinations of three or more Fzd receptors specifically called out in the specification and ignores the many other Fzd combinations covered by claim 1. In my opinion, the actual possible Fzd-Lrp target combinations for each of the tetravalent molecules covered by claim 1 would far exceed 35,721.

119. While the examples of the '278 patent evaluated a limited number of target combinations, the claims are not limited to the tested combinations. Moreover, these tested combinations, in my opinion, do not demonstrate that the inventors were in possession of the full scope of the claimed molecules that could bind other, untested target combinations. As summarized in Table 3 of the '278 patent and discussed above, only 14 Fzd binding sequences were tested in the examples, and those sequences are reported to bind just eight Fzd combinations: (1) Fzd2; (2) Fzd4; (3) Fzd8; (4) Fzd9; (5) Fzd5 and Fzd8; (6) Fzd1, Fzd2, and Fzd7; (7) Fzd1, Fzd2, Fzd7, and Fzd9; and (8) Fzd1, Fzd2, Fzd5, Fzd7, and Fzd8. EX1001, Tables 1B &

3. The specification fails to provide *any* species binding to Fzd3, Fzd6, and Fzd10 that agonize Wnt signaling. *Id.* In other words, the specification evaluated only 14 of 63 possible Fzd target combinations, and even what's tested is limited in quality.

120. Moreover, these 14 Fzd binding sequences were paired with either Lrp5 or Lrp6 binding sequences only, i.e., there is no example in which these Fzd binding sequences were paired with one Lrp5 binding sequence and one Lrp6 binding sequence. EX1001, Tables 2B & 3. Overall, the tested target combinations are a significantly small fraction of possible 35,721 combinations.

121. The manner in which these target combinations were tested is also limited. As discussed above, the named inventors used a TOPFlash assay as a proxy of Wnt signaling activation. *See supra* Section IX.B. As also discussed above, this TOPFlash assay measures canonical Wnt signaling only. *See supra* Section IX.B. As such, the specification fails to disclose even a single molecule that could activate noncanonical Wnt signaling. And in view of the complicated web of Wnt and non-Wnt ligands and receptors involved in both canonical and noncanonical Wnt signaling, one cannot extrapolate positive responses in a TOPFlash assay exhibited by one candidate Wnt surrogate molecule to other candidate molecules that binds to completely a different Fzd/Lrp target combination, which may not even be involved in activating canonical Wnt signaling. *See supra* Section IX. In other words, a POSA reading the specification would have concluded that the tested molecules are

not representative of other untested molecules that could bind untested target combinations.

122. For the same reason, a POSA reading the specification would not have been able to identify any structural feature that correlates with the activation of Wnt signaling. As discussed above, it was well appreciated that the activation of Wnt signaling involves not just the Wnt-Fzd-Lrp complex, but also many external factors. *See supra* Section IX.A. The manner in which the ligand binds to Fzd and Lrp5/6 (e.g., epitope and strength) was known to be important. *See supra* Section IX.A. Yet, the specification fails to explain why some tested molecules (e.g., “1R-G05-26” in Figure 8) did not produce positive responses in a TOPFlash assay, even though these molecules purportedly bind (or at least have binding regions) to Fzd and Lrp5/6. *See supra* Section V.A. The specification also fails to teach specific epitopes and binding strengths of each tested tetravalent, multispecific molecule. *See supra* Section V.A. As such, a POSA would not have been able to identify any common structure that differentiate molecules capable of activating Wnt signaling from those that are not capable of doing so.

123. For at least these reasons, my opinion is that claim 1 lacks written description. I understand that claims 2-5 directly or indirectly depend from claim 1 and thus incorporate claim 1’s “Fzd binding regions” and “LRP5/6 binding regions” limitations. In my opinion, none of these dependent claims have any limitation that

would restrict the possibility of Fzd-Lrp target combinations. Therefore, my opinion with respect to claim 1 applies in equal force to claims 2-5.

B. The '278 Patent Does Not Resolve the Unpredictability of Wnt Signaling Recognized in the Art as of 2017

124. As explained above, the Wnt signaling pathway is particularly complex on multiple levels and, as of 2017, the mechanism of action for Wnt signaling was poorly understood. *See supra* Section IX.A. Therefore, identifying a molecule that was effective in activating the Wnt signaling pathway was highly unpredictable in 2017. Having reviewed the '278 patent, it is my opinion that the disclosures of the '278 patent do not provide sufficient guidance to address, resolve, or even reduce the unpredictability. Thus, as of 2017, a POSA would have been required to perform undue trial-and-error experimentation to determine which of candidate tetravalent, multispecific molecules could activate the Wnt signaling pathway. Here, I briefly summarized the state of the art regarding Wnt signaling and notable deficiencies of the specification of the '278 patent.

125. *First*, as of 2017, the mechanism by which each Wnt and non-Wnt ligand engaged specific Fzd and Lrp5/6 molecules to trigger Wnt signaling was poorly understood, and the mechanisms varied from ligand to ligand. *See supra* Section IX.A. However, the '278 patent does not provide meaningful guidance on the target specificity for the claimed genus of “Wnt surrogate molecule.” As discussed above, for the claimed “LRP5/6 binding regions,” the specification merely

discloses that each of the “LRP5/6 binding regions” binds to Lrp5 “and/or” Lrp6. EX1001, 2:5-9. For the claimed “Fzd binding regions,” the specification expressly permits these “Fzd binding regions” to bind to “one or more” and “two or more” of Fzd1-10. *Id.*, 2:2-5, 2:31-40. While the specification lists eight specific combinations of three or more Fzd receptors, these combinations are disclosed as “particular embodiments,” and do not in any way limit the scope of the claimed “Fzd binding regions.” *Id.*, 2:40-46. Thus, as discussed above, even only taking into account the specifically listed combinations of three or more Fzd receptors, the specification expressly contemplated over 35,000 different Fzd-Lrp target combinations (*see supra* Section X.A).

126. The specification provides no principled guidance as to which of over 35,000 different Fzd-Lrp target combinations would confer the ability to activate Wnt signaling. Often referring to Fzd and Lrp5/6 generically, the specification fails to appreciate different functions and pathways mediated by different Fzds, Lrp5, and Lrp6. Nor does the specification identify and distinguish molecules that bind to combinations of Fzds, Lrp5, and Lrp6 and activate Wnt signaling from those that bind to other combinations and fail to activate Wnt signaling.

127. While the examples of the ’278 patent evaluated a limited number of target combinations, the claims are not limited to these tested combinations. Moreover, given the variability of reporter assay responses in the examples, they do

not provide sufficient guidance for a POSA to determine which combinations will exhibit Wnt surrogate activity. *See supra* Section V.A, V.C. The specification refers to several specific Fzd combinations as “particular embodiments,” but does not teach whether candidate molecules with these Fzd combinations would be expected to activate Wnt signaling. EX1001, 2:40-46. Indeed, the specification does not even characterize these specific Fzd combinations in the context of Lrp5/6 combinations. The specification also does not provide guidance as to which Lrp-Fzd combinations would likely lead to Wnt signaling activation. Therefore, in my opinion, due to the insufficient guidance in the specification of the ’278 patent, a POSA would have needed to perform undue trial-and-error experimentation to distinguish candidate tetravalent, multispecific Wnt surrogate molecules that lead to Wnt signaling activation from those that do not.

128. *Second*, as I have explained above, there was no consensus in 2017 regarding the stoichiometry of the Wnt-Fzd-Lrp complex. EX1054, 8. *See supra* Section IX.A. But the ’278 patent provides no guidance on the stoichiometric relationships between the Fzd and Lrp receptors that are necessary to activate Wnt signaling. As a result, the POSA is left without instruction on how to configure the two Fzd binding regions and two Lrp binding regions for optimal activation of Wnt signaling—for example, should the molecule be configured to bind to two separate

Fzd receptors and one Lrp receptor or two separate Fzd receptors and two separate Lrp receptors?

129. As discussed above (*see supra* Section IX.A), Lrp receptors have multiple epitopes for Wnt binding, and depending on which of these different epitopes Wnt ligands bind to, the effects on Wnt signaling can be either activating or inhibiting. EX1016, 3. Thus, for a molecule with multiple Lrp binding regions, these binding regions can bind to epitopes on multiple different Lrp receptors, but it is also possible that these binding regions may bind to multiple different epitopes on the same Lrp receptor.

130. Claim 1 of the '278 patent requires, among other things, that the claimed tetravalent, multispecific Wnt surrogate molecules have “two LRP5/6 binding regions that specifically bind to a Low-density lipoprotein (LDL) receptor-related protein 5 (LRP5) and/or a Low-density lipoprotein (LDL) receptor-related protein 6 (LRP6).” EX1001, col. 1. Neither claim 1 nor the specification of the '278 patent, however, indicate whether these two LRP5/6 binding regions must bind to two different Lrp receptors or can bind to different epitopes on the same Lrp receptor. Thus, each tetravalent molecule that falls within the scope of claim 1 could engage Fzd and Lrp5/6 molecules in five different stoichiometries: (1) two Fzds and one Lrp5; (2) two Fzds and two Lrp5; (3) two Fzds and one Lrp6; (4) two Fzds and two Lrp6; or (5) two Fzds, one Lrp5, and one Lrp6. The specification is completely

silent on how these different stoichiometries impact the ability of a tetravalent molecule to activate the Wnt signaling pathway. In my opinion, the lack of guidance from both the prior art and the specification regarding the stoichiometric relationships between the Fzd and Lrp receptors further made it necessary to perform undue empirical trial-and-error experimentation in order to identify functional tetravalent, multispecific Wnt surrogate molecules.

131. *Third*, as discussed above (*see supra* Section IX.A), by 2017, it was well recognized that the expression profiles of Fzd and Lrp5/6 vary across tissues, cell types, and developmental stages. The differential expression profiles of these receptors means that there is also a corresponding difference in the spatial distance between these receptors on the cell surface across tissues and over time. This also means that Wnt signaling strength varies depending on the receptor density and availability on a given cell.

132. When these receptors are expressed at low levels, their distributions on the cell surface are sparse and the distances between these receptors are relatively large. Under this circumstance, a Wnt surrogate molecule may need to have greater flexibility and extended reach such that its Fzd and Lrp5/6 binding regions can effectively and simultaneously engage both Fzd and Lrp5/6. Conversely, when these receptors are expressed at high levels, their distributions on the cell surface are dense and the distances between these receptors are relatively small. In this case, a Wnt

surrogate molecule may not need to stretch its Fzd and Lrp5/6 binding regions in order to simultaneously interact with both Fzd and Lrp5/6. A more compact format of Wnt surrogate molecule may be used or even preferred under this circumstance. For these reasons, the flexibility and reach required for a tetravalent, multispecific molecule must be determined case by case, based on the expression levels of Fzd and Lrp5/6 receptors which can vary dependent on cell type and disease or developmental state.

133. In my opinion, the specification of the '278 patent provides no meaningful guidance to address the variability and unpredictability of Wnt agonist activity resulted from tissue- and time-dependent expression profiles of Fzd and Lrp5/6 receptors. The specification acknowledges that the distance between binding domains must be enforced to modulate signal strength “depending on the desired use of the Wnt surrogate molecule.” EX1001, 116:48-64. However, I do not see any disclosure in the specification that teaches what the enforced distance between binding domains should be to induce Wnt signaling, let alone how to enforce that distance when the expression levels of Fzd and Lrp5/6 receptors are constantly changing throughout the developmental stage and during the adulthood. The specification includes some broad, generic disclosure about the use of linkers between the Fzd and Lrp5/6 binding regions, but the disclosure only suggests that a wide range of linker compositions and lengths might be effective, including peptidic

or non-peptidic linkers of “less than about 100 angstroms,” whether “rigid” or “flexible.” *Id.*, 17:52-54, 116:52-58. The disclosure provides no guidance on how these parameters should be modulated to accommodate the differing expression levels of Fzd and Lrp5/6 receptors.

134. *Fourth*, as explained above (*see supra* Section IX.A), as of 2017, different Wnt and non-Wnt ligands were known to interact with their receptors at different epitopes and with different affinities. EX1025, 3-5; EX1054, 6-7. It was recognized that “differences in affinities of different Wnt and [Fzds] and their local concentrations . . . would determine to which signaling branch a particular Wnt couples,” and that “the analysis of a particular Wnt ligand or Frizzled receptor in a certain biological context always requires an investigation of how the Wnt signal is transduced.” EX1065, 4. In sum, in order to mimic the biological activity of Wnt and non-Wnt ligands, one must target comparable epitopes in Fzd/Lrp receptors bound by those ligands with comparable strengths.

135. In my opinion, the specification of the '278 patent provides no meaningful guidance to account for the different epitopes and binding affinities required for activating the Wnt signaling pathway. The specification provides no specific guidance as to which attachment locations within Fzd or Lrp5/6 would correspond to the activation of Wnt signaling. The specification also lacks meaningful guidance on binding affinity. While the specification defines “specific

binding” as “more frequently, more rapidly, with greater duration and/or with greater affinity,” it does not teach which frequency, rapidity, duration, or affinity correlates with Wnt signaling activation. *Id.*, 120:66-121:3. Thus, in my opinion, the ’278 patent lacks any meaningful guidance for a POSA to avoid undue trial-and-error experimentation.

136. *Fifth*, as discussed above (*see supra* Section IX.B), as of 2017, many different methods were used for measuring Wnt signaling activity, but there was no one standard assay universally applicable to all Wnt systems. Indeed, the available methods at that time only measured certain aspects of the Wnt signaling pathway, and the applicability of these methods were highly dependent on different assay conditions, including cell type and the expression level of Fzd and Lrp receptors. As an example, the measurement of Wnt signaling activity induced by Wnt5a illustrates that, even for the same molecule, diametrically different results can be generated in different assays and under different assay conditions (*see supra* Section IX.B). EX1056, 2, 5, 11. Notably, these conflicting results from Wnt5a were reported even though researchers had been studying Wnt5a for several years by then. When a candidate molecule of interest is being tested without any background knowledge regarding that molecule, the risk of obtaining conflicting results—appearing to be an agonist, ineffective, or an antagonist depending on the assay and conditions used—and the inability of interpreting such results would be much greater than the

Wnt5a example. Thus, whether the same molecule is an agonist, ineffective, or an antagonist can be entirely dependent on the assays and assay conditions used.

137. The specification of the '278 patent does not resolve the unpredictability arising from the lack of a reliable method for measuring the activity of all Wnt systems under all conditions. The '278 specification does not list any assays for measuring noncanonical signaling. For canonical signaling, the specification lists the following five assays: “Wnt/ β -catenin target gene expression; TCF reporter gene expression; β -catenin stabilization; LRP phosphorylation; Axin translocation from cytoplasm to cell membrane and binding to LRP.” EX1001, 117:39-44. However, notwithstanding the known assay dependence in 2017, the specification fails to endorse any particular assay or assay condition for measuring canonical or noncanonical Wnt signaling.

138. The specification of the '278 patent primarily uses the TOPFlash assay as a proxy for Wnt signaling activation. *See supra* Section V.A. However, as discussed above, the TOPFlash assay is also condition dependent, and the specification does not endorse a specific condition for the TOPFlash assay. *See supra* Section IX.B. As I will discuss in detail below (*see* Section X.C), the '278 patent presents conflicting data from the TOPFlash assay as to whether a candidate molecule qualifies as a “Wnt surrogate molecule” depending on assay conditions, including which cells were used, which Fzd and Lrp5/6 were expressed in those cells,

whether a potentiator like R-spondin was used, and what concentrations were tested. Thus, this variability would have required a POSA to test each candidate molecule in multiple assays targeting different points along the Wnt signaling pathways and under multiple assay conditions.

139. Given these multiple levels of unpredictability recognized in the art as of 2017, which were not resolved by the teachings of the '278 patent, a POSA would have been required to perform undue empirical trial-and-error experimentation in order to identify and distinguish tetravalent, multispecific Wnt surrogate molecules that fall under the scope of the '278 patent claims from those that do not. This would have required a tremendous amount of work.

140. As explained above, a single run of TOPFlash assay typically takes about a week from cell seeding to signal detection. *See supra* Section IX.B. In 2017, one could run this assay in a relatively high throughput format, i.e., testing several candidate molecules in a single run. Typically, the assay was scaled by the number of plate wells available, e.g., 6-well, 12-well, 48-well, 96-well, and 384-well plates.⁷ But scaling also came with various drawbacks. As the number of wells increases, the amount of cells seeded per well decreases, meaning that the intensity of signal

⁷ Plates with 1536 wells were in existence at the time, but were very seldomly used at labs and institutions.

generated per well decreases and the background noise can overpower the signal. Also, only a large research institute had the capability of reading 384-well plates, and many small laboratories in 2017 still relied on a conventional plate reader, which could read at most 96-well plates at a time.

141. But even assuming that a POSA had the capability of reading 384-well plates with reasonable accuracy, the POSA would have taken a significant amount of time in making and testing the full scope of the claimed subject matter. Consider the possible 35,721 combinations discussed above. Even evaluating one candidate molecule per target combination means that a POSA would have to evaluate at least 35,721 candidate molecules. Here, due to the inherent limitations of the TOPFlash assay, one would have to test the same candidate molecules in multiple cell types at multiple concentrations. For my conservative estimate, I assumed that each candidate is evaluated in two different cell types with two different concentrations in the presence of R-spondin. This conservative estimate translates to 142,884 reactions ($35,721 \text{ candidate molecules} \times 2 \times 2$), requiring at least 372 independent TOPFlash assays using 384-well plates ($142,884 \text{ reactions} / 384 \text{ wells}$). Running these assays consecutively would take 372 weeks, i.e., about 7 years of work. This is a highly conservative estimate because it does not account for the time required to make candidate molecules. It also does not account for the assay variability that a POSA would typically encounter in this situation.

142. Similarly, I was asked to estimate the amount of time required to evaluate candidate molecules with 130 million different tetravalent, multispecific formats. Assuming that one candidate molecule per tetravalent format is tested in two cell types at two concentrations with R-spondin, there would be 4 reactions per candidate molecule. Thus, 520 million reactions would be needed for candidate molecules with 130 million different tetravalent, multispecific formats. As the TOPFlash assay could be performed in a high throughput manner (e.g., 384 reactions per run), 520 million reactions would require about 1.4 million TOPFlash assay runs. As explained above (*see supra* Section IX.B), a TOPFlash assay in 2017 typically took about a week, from growing cells to reading reporter signals. Completing 1.4 million TOPFlash assay runs would thus need 1.4 million weeks, which amounts to more than 26,000 years (1.4 million weeks / 52 weeks per year). This estimate is again highly conservative as it does not account for the uncertainty of negative assay results given the variability and unpredictability associated with different assay conditions.

143. For at least these reasons, my opinion is that claim 1 is not enabled. Claims 2-5 do not solve the unpredictability problems identified above: none of these claims limits the target combinations, stoichiometry, epitope, binding affinity, and other elements that result in the need of undue trial-and-error experimentation. Therefore, my opinion with respect to claim 1 applies in equal force to claims 2-5.

C. The '278 Patent Presents Conflicting Data as to Whether a Candidate Molecule Qualifies as a “Wnt Surrogate Molecule”

144. As explained above, notwithstanding the known assay dependence as of 2017, the specification of the '278 patent does not specify any particular assay or assay conditions for measuring canonical or noncanonical Wnt signaling. Although the '278 patent reports results generated using a TOPFlash assay, that assay itself is condition dependent, and the specification does not endorse any specific TOPFlash assay conditions. *See supra* Sections V.A, IX.B, X.B. Rather, the '278 patent presents conflicting TOPFlash assay data regarding whether a candidate molecule qualifies as a “Wnt surrogate molecule,” with the outcome varying based on assay conditions, including the cell type used, the particular Fzd and Lrp5/6 receptors expressed, the presence or absence of potentiators such as R-spondin, and the concentrations tested.

145. *First*, while the specification includes negative controls for some examples, there is a *complete lack* of positive controls in the TOPFlash assay used in the '278 patent. The specification expressly defines that the “Wnt surrogate molecules of the present invention” must be “**biologically active** in . . . activation of Wnt signaling.” EX1001, 16:52-55 (emphasis added). In my opinion, “biologically active” requires activity that is biologically meaningful. There is, however, no basis to conclude that a small response in the TOPFlash assay, while active in the sense

that it is non-zero under those assay conditions in a technical sense, will be “biologically active.”

146. The omission of positive controls in *all* TOPFlash assays is quite puzzling to me, when including a positive control is routinely done in the field, and in fact, deemed necessary for evaluating potential Wnt activity. Indeed, in my opinion, to determine whether assay activity is reflective of being biologically active, there must at least be a biologically relevant positive control, which in this case could be a Wnt protein. Comparing the reporter activity induced by a Wnt protein to reporter activity of a candidate Wnt surrogate would provide the information necessary to determine if a candidate is a Wnt surrogate, i.e., if the candidate exhibits similar or greater reporter activity as produced by a natural Wnt ligand at similar concentrations to a natural Wnt ligand, then that candidate would be considered to have Wnt surrogate activity. However, none of the TOPFlash assays reported in the specification include a positive control. Without a positive control, a POSA would not be able to determine whether or not a low level TOPFlash response corresponds to being “biologically active.”

147. The candidate molecule “18R5:Fc:1115.3” illustrates the problem with not including a positive control in a TOPFlash assay. EX1001, Fig. 14E. In the figure reproduced below, “18R5:Fc:1115.3” exhibited a much lower response in the TOPFlash assay compared to that of another candidate molecule “1115.3:Fc:18R5.”

While the specification characterizes “18R5:Fc:1115.3” as being able to “activate[] Wnt signaling,” *id.*, 159:33-36, that conclusion is necessarily limited to it having a response above zero under the specific assay conditions and there is no disclosure—or basis to conclude—as to whether “18R5:Fc:1115.3” is “biologically active.” Without a positive control for comparison, a POSA is left without a reasonable certainty for how to determine whether “18R5:Fc:1115.3” constitutes a “biologically active” Wnt surrogate molecule. This example also illustrates the problem of not including R-spondin as a standalone control. By comparing to responses induced by R-spondin alone, a POSA may be able to discern whether responses induced by “18R5:Fc:1115.3” were mediated by the molecule or were background noise present in this system. Without a R-spondin control, a POSA would not be able to unequivocally make that determination, further complicating the interpretation of these results.

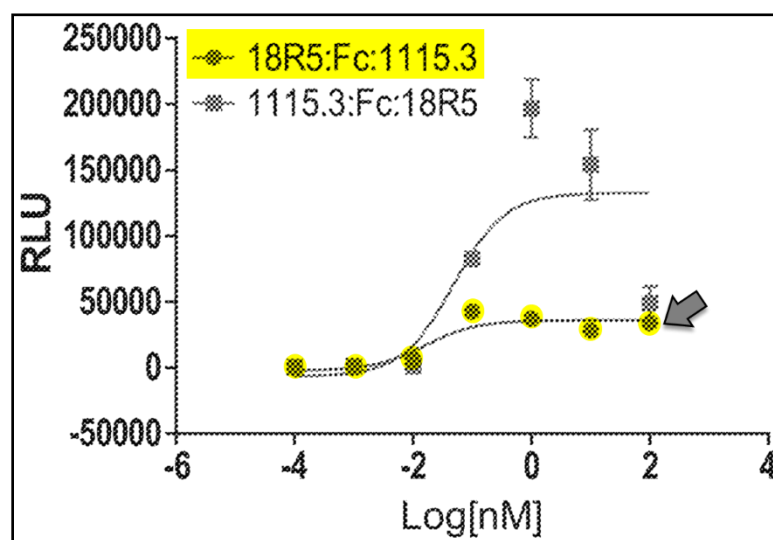


Figure 1. Annotated Figure 14E of the '278 Patent

148. *Second*, the '278 patent reports conflicting data depending on the presence or absence of R-spondin. The specification states that “Wnt agonists typically initiate a reaction or activity that is ***similar to or the same as*** that initiated by the receptor’s natural ligand.” EX1001, 16:61-63 (emphasis added). As explained above (*see supra* Section IX.A), naturally occurring Wnt and non-Wnt ligands are capable of activating Wnt signaling in the absence of additional potentiating factors such as R-spondin, i.e., they can activate Wnt signaling independent of potentiating factors such as R-spondin. The specification, however, adds uncertainty as to the scope of “Wnt surrogate” by claiming that candidate molecules tested *only* in the presence of R-spondin exhibit “Wnt signaling activity.”

149. As shown in the figure reproduced below, several candidate molecules (R2M3-26, R2M3-32, and 26-17SB9) failed to activate Wnt signaling in TOPFlash assays conducted in the absence of R-spondin. EX1001, Figs. 2D, 3D, 13B. There is no appreciable difference between the gray response curves, which reflect responses in the absence of R-spondin, and the blue curves, which the specification states “did not induce reporter activity.” EX1001, 155:35-36, 155:65. In my opinion, because these molecules are unable to activate Wnt signaling without R-spondin, the data in the patent suggests that they are not biologically active in activating Wnt signaling. Nevertheless, when focusing solely on responses observed in the presence of R-spondin (red curves), the specification characterizes these same molecules as

“induc[ing] canonical Wnt signaling.” *Id.*, 158:61-63. This characterization, in my opinion, would cause confusion to a POSA as to whether these molecules qualify as “Wnt surrogate molecules” in view of their apparent inability to produce a response in the absence of R-spondin. Further, without a positive control in these assays to set the baseline for reporter activity levels that are biologically relevant, it is impossible to determine whether the reporter activity in the presence or absence of R-spondin of the examples confers “Wnt surrogate activity.” The conflicting condition-dependent results and the specification’s inconsistent characterization of what it means to be “biologically active” and “initiate a reaction or activity that is similar to or the same as that initiated by the receptor’s natural ligand” would leave a POSA unable to determine with reasonable certainty whether a given candidate molecule constitutes a “Wnt surrogate molecule.”

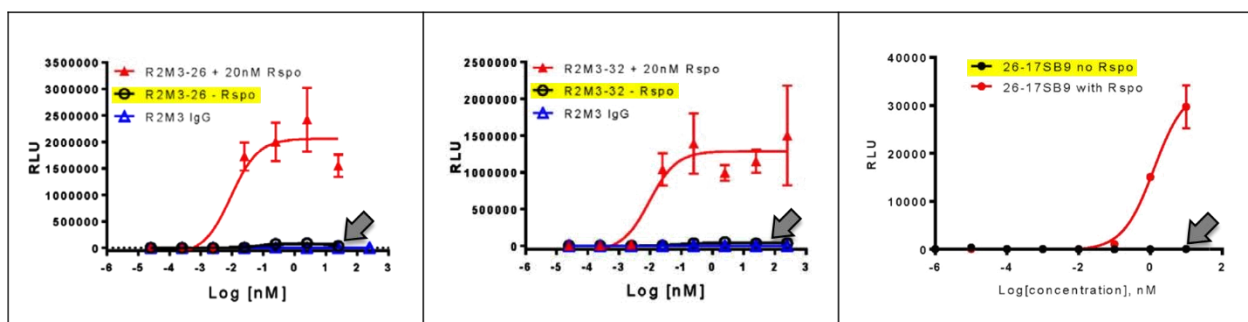


Figure 2. Annotated Figures 2D, 3D, 13B⁸ of the '278 Patent

⁸ As discussed above, Figure 13B of the '278 patent appears to be identical to Figure 13E, and is inconsistent with the description of Figure 13B in the specification. Thus,

150. *Third*, the '278 patent reports conflicting data depending on different cell types. As I have explained (*see supra* Section IX.A.), as of 2017, it was well-recognized that Wnt and non-Wnt ligands play tissue- and cell-specific roles, and that the ability to activate or inhibit Wnt signaling can depend on the ligand's biological targets as well as the particular Fzd/Lrp receptors expressed in the cell or tissue of interest. EX1056, 2-5. Reflecting this known uncertainty, the '278 patent reports inconsistent TOPFlash assay responses across different cell types. The cell-type dependence of reporter assays—and the possibility for differing and inconsistent results—adds to the lack of reasonable certainty to determine if a candidate molecule is a claimed “Wnt Surrogate.”

151. As shown in the figure reproduced below, the candidate molecule “18R5-28” failed to activate Wnt signaling in 293 cells in a TOPFlash assay conducted without R-spondin, yet the same molecule “18R5-28” was reported to activate Wnt signaling in A375 cells. The '278 patent offers no explanation for these contradictory cell-type-dependent results. *See* EX1001, 156:54-67, Fig. 6. A lack of response in one cell type does not provide the POSA with reasonable certainty that the candidate Wnt surrogate molecule would or wouldn't work in another cell

I reviewed Figure 13B of the underlying provisional application (EX1007), which appears to match the description of Figure 13B in the specification.

type—if a given candidate did not work in one cell type, the POSA would have to test in other cell types, ad nauseum, to determine if the candidate was a Wnt surrogate molecule. In my opinion, because the TOPFlash assay outcomes vary depending on the cell type used, a POSA would be unable to determine with reasonable certainty whether a candidate molecule such as “18R5-28” qualifies as a “Wnt surrogate molecule.” This figure also exemplifies differences in activity metrics reported across TOPFlash graphs. For example, the Y-axis in the two graphs in the figure below are different i.e. RLU versus fold induction, further complicating comparative activity in the 2 cell lines.

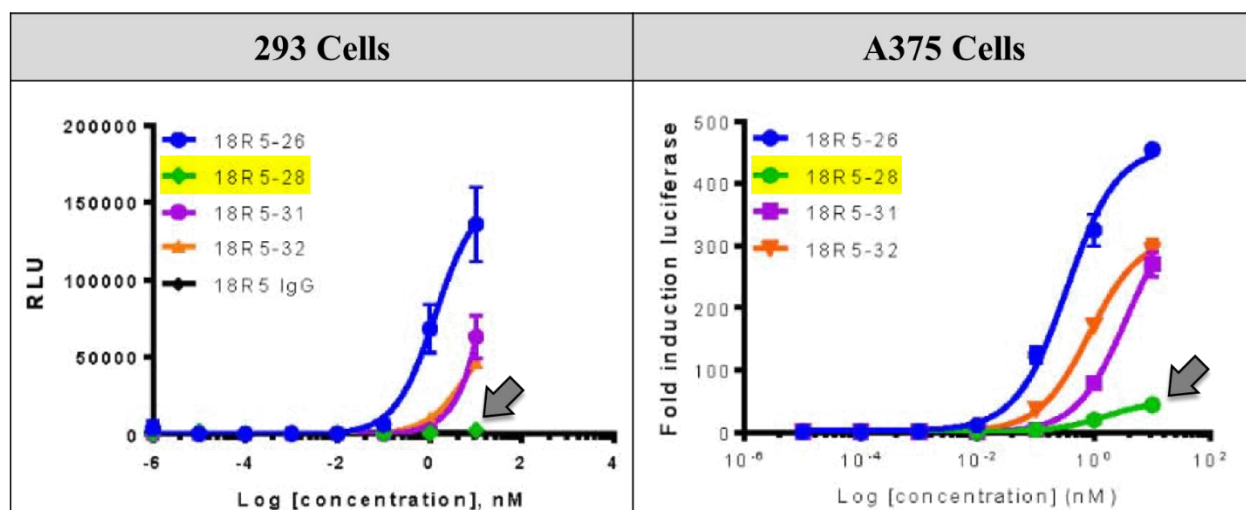


Figure 3. Annotated Figures 6A and 6B of the '278 Patent

152. *Fourth*, the '278 patent reports TOPFlash assay data exhibiting inconsistent dose-response behavior. Although some candidates in TOPFlash assays produced dose responsive signals, i.e., the assay readout increases with increasing

concentrations of a candidate Wnt surrogate molecule, the responses of certain other candidates unexpectedly decreased at higher concentrations of the candidate molecules, as shown in the figure reproduced below. EX1001, Figs. 14A, 15G.

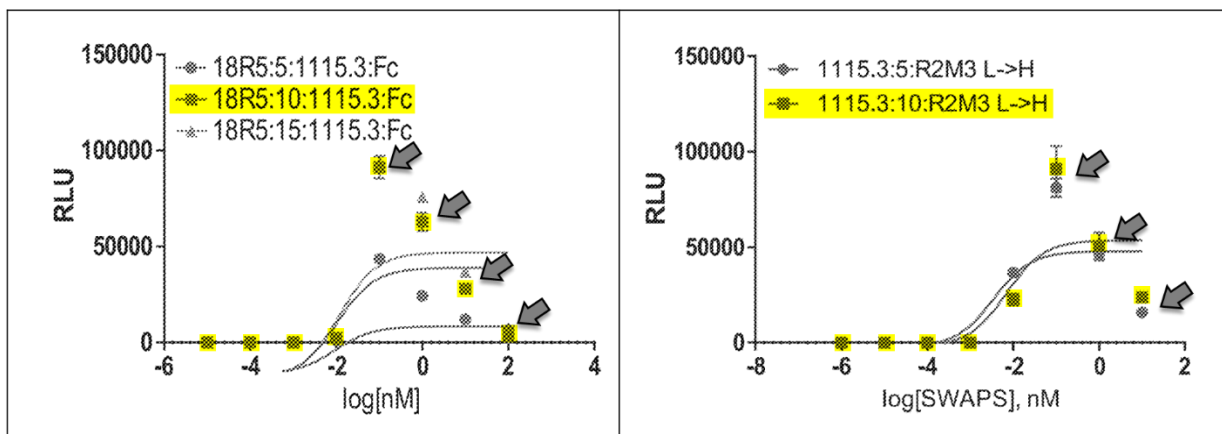


Figure 4. Annotated Figures 14A (Left) and 15G (Right) of the '278 Patent

153. Notably, not all tested molecules follow this pattern. In other words, it is unpredictable whether a given molecule would follow a dose-response curve or not, and moreover, a POSA may conclude that a candidate molecule could produce positive responses in a TOPFlash assay at one concentration (e.g., 0.1 nM) while the POSA may arrive at an opposite conclusion at another concentration (e.g., 100 nM). But the specification provides no guidance on how to account for this unpredictable effect and offers no explanation as to why some, but not all, candidate molecules deviate from a dose-response curve.⁹

⁹ Importantly, other assays mentioned or used in the '278 patent (e.g., *in vivo* assays) suffer from the same problems as TOPFlash assays. The specification did not

154. In sum, the '278 patent identifies numerous variables that affect the outcome of the TOPFlash assay, each of which independently influences assay results in an unpredictable manner. The specification, however, does not define or endorse the conditions under which these variables should be applied. Because the determination of whether a candidate molecule is “biologically active” in activating Wnt signaling turns on the choice of assay conditions, the absence of defined assay

endorse any of these assays as “the” assay for evaluating candidate molecules as the specification repeatedly characterizes candidate molecules as allegedly “Wnt surrogate molecules” based on *in vitro* TOPFlash assays. *See, e.g.*, EX1001, cols. 156-161. Although *in vivo* assays were used in certain examples, the specification does not endorse any specific *in vivo* assay condition. This is problematic because, just like TOPFlash assays, *in vivo* assays would show condition-dependent results (e.g., tissue-type or dose of molecules). Moreover, the specification evaluated only three tetravalent candidate molecules *in vivo*, which is more complicated than TOPFlash assays and would take weeks to months to conduct. Requiring an *in vivo* assay to determine whether a candidate molecule is a “Wnt surrogate molecule” further supports my opinion that the '278 patent lacks written description support and is not enabled.

conditions would prevent a POSA from determining, with reasonable certainty, whether a candidate molecule qualifies as a “Wnt surrogate molecule.”

155. For at least these reasons, my opinion is that claim 1 is indefinite. Claims 2-5 do not solve the unpredictability problems identified above: none of these claims identifies a specific assay or assay condition for determining whether a given molecule is a “Wnt surrogate molecule.” Therefore, my opinion with respect to claim 1 applies in equal force to claims 2-5.

XI. CONCLUSION

156. I hereby declare that all statements made herein of my own knowledge are true and that statements made on information and belief are, to the best of my knowledge, believed to be true; and further that these statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code.

Dated: 02/10/26

By: 

Dr. Michael Kahn

APPENDIX A

Curriculum Vitae

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Education

1978 B.A., Chemistry Columbia University, New York, NY
1983 Ph.D., Chemistry Yale University, New Haven, CT

Professional Experience

NIH Postdoctoral Fellow (Mentor: Professor Gilbert Stork)	1982 – 1984
Research Associate, Hoffman-LaRoche, Nutley, NJ	1984 – 1985
Assistant Professor, University of Illinois – Chicago	1985 – 1988
Associate Professor, University of Illinois – Chicago	1988 – 1992
Associate Professor, University of Washington	1992 – 1995
Research Associate Professor, University of Washington	1995 – 2006
Scientific Founder and Consultant, Molecumetics, Bellevue, WA	1992 – 1994
Chief Scientific Officer, Molecumetics, Bellevue, WA	1994 – 1997
Consultant, Asahi Chemical Corporation, Japan	1995 – 2007
Senior Vice-President, Molecumetics, Bellevue, WA	1998 – 2000
Consultant, Choong Wae Pharmaceutical Corporation, Korea	2000 – 2005
Principal Investigator, Pacific Northwest Research Institute, Seattle, WA	2000 – 2004
Scientific Advisory Board, Acucela Corporation, Seattle, WA	2001 – 2006
Consultant & Scientific Advisory Board, International Therapeutics, Seattle, WA	2001 – 2004
Scientific Director, Institute for Chemical Genomics, Seattle, WA	2004 – 2017
Provost Professor of Medicine & Pharmacy, University of Southern California	2006 – present
Visiting Professor (Sabbatical Research), Osaka University, Japan	July 2015 – June 2016
Consultant and Head of Scientific Advisory Board, Prism Biolab, Japan	2006 – 2015
Scientific Advisory Board, Presidio Pharmaceuticals, San Francisco, CA	2007 – 2010
Consultant, Fuji Film – Pharmaceutical Division, Japan	2010 – 2020
Scientific Founder, Makucell Inc, Los Angeles, CA	2011
Editor in Chief, Current Molecular Pharmacology	2014 – 2025
Professor & Chair, Dept of Molecular Medicine, Beckman Research Institute – City of Hope	2017 – 2022
Professor, Beckman Research Institute – City of Hope	2017 – 2025

Awards

Camille and Henry Dreyfuss Distinguished Young Faculty Fellow	1986 – 1991
Searle Scholar	1986 – 1989
NSF Presidential Young Investigator	1987 – 1992
American Cancer Society Faculty Fellow	1987 – 1989
American Cyanamid Faculty Award	1989
University Scholar – University of Illinois	1989
American Heart Association Established Investigator	1990 – 1995
Camille and Henry Dreyfuss Teacher Scholar	1991 – 1993

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APPENDIX B

LIST OF MATERIALS CONSIDERED

Exhibit	Description
1001	U.S. Patent No. 12,297,278
1005	U.S. Provisional Application No. 62/607,875
1006	U.S. Provisional Application No. 62/641,217
1007	U.S. Provisional Application No. 62/680,522
1008	File History of U.S. Patent No. 12,297,278
1011	U.S. Publication No. 2017/0306029 A1 (“Garcia US”)
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