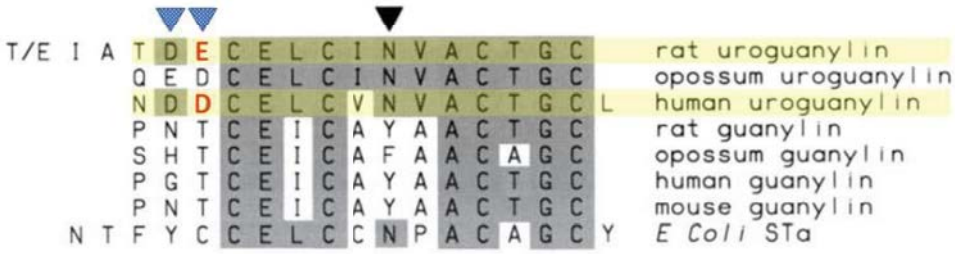
and rat uroguanylin peptide sequences. Second, a skilled artisan would have known that replacing third-position aspartic acid with glutamic acid would at least maintain, if not broaden, the intestinal surface susceptible to acid-enhanced uroguanylin signaling—thereby increasing potency. Third, a skilled artisan would have recognized that a glutamic acid substitution would reduce undesirable aspartimide formation during peptide synthesis.

124. My analysis below identifies exemplary disclosures of the cited references relative to the corresponding claim elements and is not meant to be exhaustive. As I set forth in my analysis below, a skilled artisan would have understood claim 1 to have been obvious in view of the combined teachings of Currie and Li.

'786 Patent Claim	**Currie and Li**
1. A peptide consisting of the amino acid sequence of SEQ ID NO:20.	**Currie teaches:** A "novel ***peptide***…which has the following amino acid sequence. (NDDCELCVNVACTGCL) Asn-Asp-Asp-Cys-Glu-Leu-Cys-Val-Asn-Val-Ala-Cys-Thr-Gly-Cys-Leu This peptide, also referred to herein as ***human uroguanylin***, has been isolated from human urine and has been chemically

	synthesized by solid phase peptide synthesis. In its oxidized active biologic form, the novel peptide has two disulfide bridges, one between cysteine residues at positions 4 and 12 and the other between cysteine residues at positions 7 and 15." EX1005, 1:45-63 (emphasis added). **Li teaches:** "Fig. 6. ***Amino acid … sequences of rat*** [and human] ***uroguanylin***. (a) ***Alignment of the amino acid sequence*** of the purified duodenal peptide (top) with published sequences of guanylin, uroguanylin, and STa. Amino acid identities are indicated by shading. ***The arrowheads denote structural features described in the text***." EX1006, 52 (emphasis added).  EX1006, 52, FIG. 6(a) (annotated). "Alignment of the sequence[s]…with the appropriate regions of rat guanylin and [human] uroguanylin (Fig. 6a) reveals that" rat uroguanylin "is more closely related to [human] uroguanylin (80% identity when compared across species, with ***nearly all differences representing conservative amino acid substitutions***)." EX1006, 53 (emphasis added).

	"Thus, features that are found in uroguanylin, but not in guanylin, offer information about structural elements that specify the strength of the ligand/receptor interaction. ***Of particular interest are two residues*** that are basic or uncharged in guanylin but ***acidic in uroguanylin (stippled arrowheads)***." EX1006, 54 (emphasis added).

125. Claim 1 recites only [Glu^3]-human uroguanylin, nothing more. Currie identified human uroguanylin. EX1005, 1:45-63. Li further identified the glutamic acid substitution in aligning rat and human uroguanylin. EX1006, 52-54, FIG. 6(a). Thus, Currie and Li together disclose each element of [Glu^3]-human uroguanylin.

A. Currie Suggests Uroguanylins For Treating Constipation

126. Well before 2002, Currie expressly suggested that human uroguanylin treated clinical constipation. EX1005, 2:20-24. Currie empirically validates its suggestion in three ways. First, it confirms that human uroguanylin binds to the intestinal guanylate cyclase. EX1005, 2:53-65, FIG. 3(b). Second, Currie confirms that human uroguanylin has activity upon intestinal guanylate cyclase in that it "stimulate[s] increases in cyclic GMP levels in a manner similar to guanylin and the STs." *Id.*, 2:8-9. Third, Currie confirms that "[h]uman uroguanylin added to the mucosal reservoir of rat colon mounted in an Ussing chamber also caused a sustained rise in Isc," or Short-Circuit Current. *Id.*, 6:29-31.

127. Currie noted that human uroguanylin is an "endogenous stimulator of intestinal guanylate cyclase." EX1005, 2:6-8. Intestinal guanylate cyclase is a transmembrane enzyme expressed on the surface of cells lining the intestinal lumen. *Id.*, 1:23-30 ("This form of the enzyme is predominantly found in the intestinal epithelial cells with the largest number of receptors oriented towards the lumen. Recently, the intestinal form of guanylate cyclase has been cloned and expressed from rat small intestinal mucosa. This enzyme is characterized by an extracellular receptor binding region, a transmembrane region, an intracellular protein kinase-like region and a cyclase catalytic domain.").

128. Human uroguanylin binds to intestinal guanylate cyclase, thereby increasing cGMP. Following further downstream events, cGMP causes the physiological effects associated with treating constipation or, when present in too great an amount, the pathology of diarrhea. EX1005, 1:34-39. As Currie describes, this effect is mediated by, among other things, the downstream effect of chloride secretion from intestinal endothelial cells. *Id.*, 2:18-20. Chloride secretion decreases (and reverses) water absorption. *Id*. Human uroguanylin was thus known by 2002 to provide therapeutic benefits for clinical constipation.

129. Given the above, a skilled artisan would have had good reason to modify human uroguanylin for developing a treatment for clinical constipation.

130. Moreover, though human uroguanylin was known to be naturally

occurring, in view of Currie, a skilled artisan would have had good reason to turn to chemical synthesis over isolation from a natural source. For example, Currie teaches that human uroguanylin was "both isolated and chemically synthesized in a homogeneously purified form which did not exist in human urine from which it was initially obtained. That is, it has been prepared in a form which is essentially free of other low molecular weight peptides, and free from higher molecular weight material and other cellular components and tissue matter." EX1005, 1:64-2:3.

131. Currie also teaches more specifically that human uroguanylin "can be prepared by known solution and solid phase peptide synthesis methods." EX1005, 3:7-9; *see also id.*, 5:17-20 (teaching how "[u]roguanylin was synthesized by the solid-phase method on an Applied Biosystems Model 430A peptide synthesizer and purified by reverse-phase C_{18} chromatography"). This synthetic approach works by adding one amino acid to another. *See id.*, 3:25-28 ("This procedure, though using many of the same chemical reactions and blocking groups of classical peptide synthesis, provides a growing peptide chain anchored by its carboxy terminus to a solid support."). As noted in Section VII, skilled artisans enjoyed a reasonable expectation of success in preparing synthetic analogs of high activity using these routine and conventional solid-state peptide synthesis techniques.

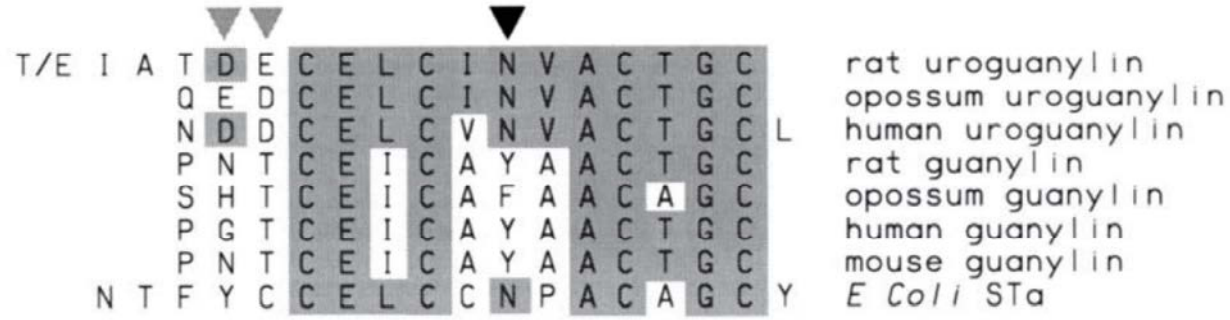
B. Reason to Look to Synthetic Analogs of Human Uroguanylin

132. By 2002, a skilled artisan would have had good reason to make a synthetic analog of the human uroguanylin taught by Currie for developing a therapeutic for treating constipation. As described above in Section VII, this approach was routine well before 2002. For example, as illustrated in my discussion of GnRH, skilled artisans routinely looked to synthetic analogs to improve the activity of peptide hormones and to convert them into drugs. *See* Section VII.E, *above*.

133. Currie demonstrates the conventional nature of evaluating the potency of peptide analogs when discussing the differences between human uroguanylin and guanylin that made uroguanylin more potent than guanylin. EX1005, 6:13-16 (confirming "[h]uman uroguanylin appeared to be more potent than human guanylin … for activation of GC-C in T84 cells"). From Currie, a skilled artisan would have known that uroguanylin and guanylin comprise conserved and divergent portions. *See id.*, 5:66-6:2 (noting that "human uroguanylin shares homology with guanylin"). From here, a skilled artisan would have had good reason to compare and contrast uroguanylin and its paralog guanylin to identify mechanistic insights for achieving enhanced activity in a synthetic analog.

134. This analysis was performed by Li, which not only compared human uroguanylin with guanylin, but also compared other related sequences in animals.

For example, Li aligns and compares the peptide sequence of human uroguanylin with related sequences including rat uroguanylin and human guanylin in Figure 6(a), reproduced below.



EX1006, 52, FIG. 6(a).

135. In so doing, Li teaches that "[t]he affinity of GCC for uroguanylin (opossum or human) is about 10-fold higher than its affinity for guanylin (rat or human). Thus, features that are found in uroguanylin, but not in guanylin, offer information about structural elements that specify the strength of the ligand/receptor interaction." *Id.*, 54. In particular, Li notes that "[o]f particular interest are two residues that are basic or uncharged in guanylin but acidic in uroguanylin (stippled [*i.e.*, grey] arrowheads)" in Figure 6(a), above. *Id.* Thus, a skilled artisan would have had good reason to investigate synthetic analogs of human uroguanylin with a particular focus on optimizing the residues identified by Li as being of particular interest given their apparent involvement in ligand/receptor interactions.

136. Skilled artisans routinely made and screened large numbers of

synthetic analogs of naturally-occurring peptides, in part because of the wide availability and ease of peptide synthesis methods. As discussed above in Sections VII.D-E, multiple synthetic strategies were known and routinely used by skilled artisans for the development of synthetic analogs. For example, skilled artisans routinely sought to stabilize electrostatic pairing between hormone and receptor to strengthen or increase binding potential. *See* Section VII.E.3, above. Skilled artisans also recognized naturally occurring homologs as promising avenues for activity- or stability-enhancing substitutions. *See* Section VII.E.2, above.

137. When preparing synthetic analogs to human uroguanylin, a skilled artisan would have had good reason to look first to making conservative changes to the peptide. The target receptor, as Currie discloses, is part of a "group of proteins that share structural characteristics relative to the enzymatic function of producing cyclic GMP, but differ quite remarkably in their selective activation by ligands." EX1005, 1:7-11. Thus, as Currie notes, the guanylate cyclases are only selectively activated by their ligands. Moreover, as described above in Section VII, skilled artisans routinely began analog synthesis with conservative substitutions to avoid causing immunogenicity or ablating activity altogether.

138. By 2002, a skilled artisan would have had other good reasons to look first to making conservative changes to the human uroguanylin peptide because the potential therapeutic acts as an agonist, not an antagonist. *See* EX1050, 68 ("if you

wish to design a drug to effect a certain response, an agonist would be desired; if you wish to design a drug to prevent a particular response …, an antagonist would be required").[44] The structural requirements for agonists are more stringent than those for antagonists because agonists need to "interact[] with [the receptor] in the specific way required to elicit a response," while antagonists only need to "block[] a receptor site." *Id.*, 69-70. "In general, there are great structural similarities among a series of agonists, but little structural similarity exists in a series of competitive antagonists." *Id.*, 69. Making conservative substitutions, especially conservative substitutions consistent with homologous sequences already existing in nature, thus would have been particularly attractive to a skilled artisan as the relevant time.

C. Currie and Li Suggest a Glu^3 Substitution as in Rat Uroguanylin

139. As noted above, in view of Currie and Li, a skilled artisan would have had good reason to seek out information about the structure-function relationship of human and rat uroguanylin with respect to their activity on the human receptor, intestinal guanylate cyclase.

140. Currie also gave good reason to align rat uroguanylin to human uroguanylin. For example, Currie teaches that human uroguanylin acts on the rat

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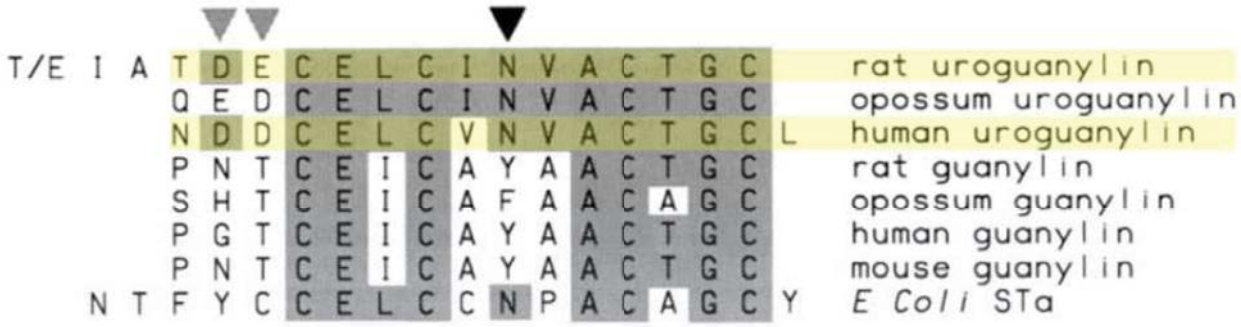
[44] Silverman, R. B., *Chapter 3: Receptors*, THE ORGANIC CHEMISTRY OF DRUG DESIGN AND DRUG ACTION, (Academic Press, Inc.) **1992**, 52-97 ("Silverman," EX1050).

receptor, which is displayed on the endothelial cells of rat tissue. EX1005, 2:16-21 ("Human uroguanylin has been further demonstrated to act in an isolated intestinal rat preparation to stimulate an increase in short circuit current.").

141. Li also provided good reason to look to the alignment of human and rat uroguanylin for promising substitutions for human uroguanylin. Li discloses the amino acid sequences of various mammalian uroguanylins and aligns their sequences to illustrate their homology. EX1006, FIG. 6(a). As can be seen below, Li teaches that rat and human uroguanylin have substantial overlap:



EX1006, FIG. 6(a) (annotated with yellow highlight for ease of comparing the rat and human uroguanylin sequences).

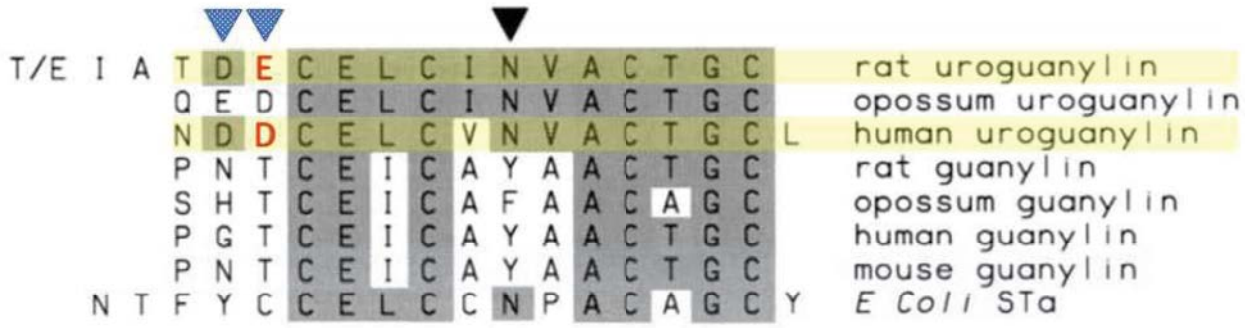
142. In view of teachings of Currie and Li, a skilled artisan would have had good reason to look to the amino acids that differ between rat and human uroguanylin sequences in identifying promising, conservative amino acid substitution in designing a synthetic human uroguanylin analog. Indeed, a skilled artisan would have known that rat uroguanylin was highly likely to stimulate the human receptor. As discussed above in Section VII, well before 2002, skilled

artisans understood that conformation fit determined receptor-ligand binding, and that such fit may depend on pairing of hydrophobic groups between the ligand and receptor and/or pairing of charged groups with opposite charge. Because human uroguanylin binds to the rat receptor, as Currie teaches, a skilled artisan would have reasonably expected that human uroguanylin and the rat receptor have a good conformational fit. *See* EX1005, 2:17-19. So too for the binding of the human ligand-receptor and the rat ligand-receptor.

143. Moreover, Li demonstrates that rat uroguanylin activates cyclic GMP synthesis in ***human*** T84 cells, providing further evidence for the activity of the rat peptide on the human receptor. EX1006, 46-47, 54. Because human uroguanylin binds to both the human and rat receptor, and the rat receptor binds to both human and rat uroguanylin, a skilled artisan would have had good reason to reasonably expect conformational fit between rat uroguanylin and the human receptor.

144. Applying the principle of conservative substitutions first, a skilled artisan would have read Li's homology as a map. This homology shows that uroguanylin orthologs generally do not vary much among mammals, with the third position being a notable and rare exception that a skilled artisan would have readily focused on. *See* EX1006, FIG. 6(a). Indeed, Li expressly notes that "[o]f particular interest are two residues that are basic or uncharged in guanylin but acidic in uroguanylin (stippled arrowheads)" in Figure 6(a), which I have annotated again

below, highlighting in blue the stippled arrowheads indicating the residues of interest identified by Li. *Id.*



EX1006, FIG. 6(a) (annotated).

145. As can be seen above, the residues "of particular interest" are those that are aligned with the second and third positions of the human uroguanylin sequence. While the second position (left blue arrow) is an aspartic acid that is conserved between the rat and human uroguanylin sequence, the third position (right blue arrow) is not. The two sequences instead demonstrate a Glu^3 substitution, in which the aspartic acid appearing at the third position in human guanylin, highlighted in red above, is instead a glutamic acid (E/Glu) in the aligned rat uroguanylin sequence, also highlighted in red. Thus, the Glu^3 substitution of human uroguanylin was a natural, expected, and conventional substitution that a skilled artisan would have readily identified in view of the prior art.

146. Because a skilled artisan would have had good reason to believe that [Glu^3]-human uroguanylin would be an effective analog of human uroguanylin and

that there was sufficient tolerance for this substitution at the third amino acid position, they would have had good reason to replace the aspartic acid at the third amino acid position of human uroguanylin with glutamic acid, as disclosed in Li. EX1006, 54, FIG. 6(a).

147. A skilled artisan would have had a reasonable expectation of success in making the [Glu^3]-human uroguanylin analog. And as I noted previously in this declaration, as a general principle, conservative substitutions, such as changing aspartic acid to glutamic acid, are typically favored to maintain or even increase activity without undue toxicity.

148. Thus, for the reasons discussed above, a skilled artisan's attention immediately would have been drawn to the only three amino acid positions on uroguanylin that were available for substitution while preserving the mammalian consensus sequence for uroguanylin. The three conservative mammalian substitutions disclosed by Li were Asp^2-to-Glu^2 (human → opossum), Asp^3-to-Glu^3 (human → rat), and Val^8-to-Ile^8 (human → opossum or rat). *See* EX1006, FIG. 6(a). Prominent among these three, Li specifically discloses the Glu^3 substitution that is the only difference between the human uroguanylin disclosed in Currie and the claimed peptide in Shailubhai. Li suggests that this substitution would result in a functional human uroguanylin analog, noting that rat uroguanylin "follows the consensus sequence of uroguanylin rather than that of guanylin, and thus we would

expect its affinity to be comparable to that of opossum or human uroguanylin." *Id.*, 54.

149. A skilled artisan also would have reasonably expected that applying this substitution would maintain or improve the efficacy of human uroguanylin. As described above, skilled artisans knew that human and rat uroguanylin had activity against the human receptor. As Li demonstrates, rat and human uroguanylin are homologs. *See* EX1006, FIG. 6(a). And a skilled artisan would reasonably expect at least some recombinants to exceed the activity of human uroguanylin. The Glu^3 substitution is one such recombinant, but because of the high homology between human and rat uroguanylin, possible recombinants are finite. A skilled artisan would have applied the well-known assays Currie recites to confirm the therapeutic activity of [Glu^3]-human uroguanylin with a reasonable expectation of success. These long-known assays are summarized above in Section IX.A, and discussed in even more detail in Section VII.

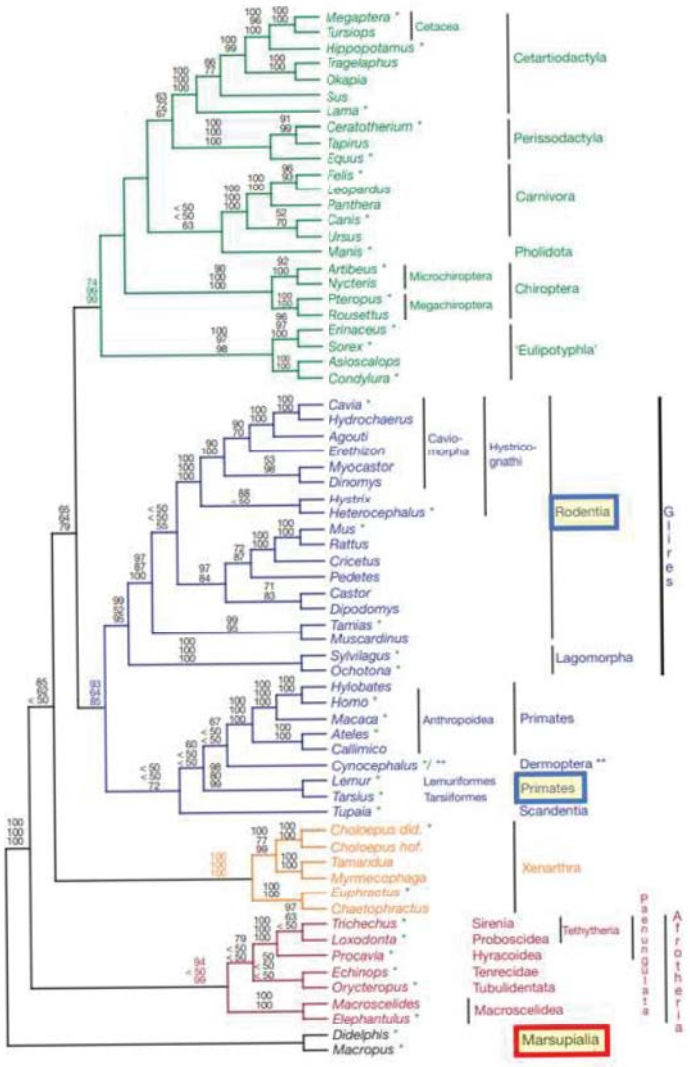
150. I also note that by 2002, skilled artisans also had identified that mouse uroguanylin sequences also have the same Glu^3 substitution found in rat uroguanylin. EX1051, FIG. 1B, 1001-02.[45] A skilled artisan would have understood

[45] Whitaker, T. L., *et al.*, *Uroguanylin and Guanylin: Distinct but Overlapping Patterns of Messenger RNA Expression in Mouse Intestine,* GASTROENTEROLOGY, 113, **1997**, 1000–1006 ("Whitaker," EX1051).

this ubiquity as showing broad functionality for that particular modification with respect to the intended target receptor.

151. I also note that while Li also aligns a sequence of opossum uroguanylin in Figure 6(a), there are several reasons why a skilled artisan would have focused first on comparing human uroguanylin with rat (and mouse) uroguanylin in designing analogs. Among them, humans were known to be more closely related to mice and rats than to opossums, which are marsupials. *See*, *e.g.*, EX1052, 616, FIG. 1.[46] Figure 1 of Murphy, reproduced below, shows a phylogenetic mapping of mammals. Both human ("Primates") and rats ("Rodentia") are in the same clade (blue text, highlighted with blue outline). Possum ("Marsupialia") are as far as possible from humans while still being mammals, in a separate clade (black text, highlighted with red outline).

[46] Murphy, W. J., *et al.*, *Molecular Phylogenetics and the Origins of Placental Mammals*, NATURE, 409, **2001**, 614-618 ("Murphy," EX1052).



EX1052, 616, FIG. 1 (annotated).

152. Moreover, uroguanylin signaling was known to be especially similar in humans and rats. EX1053, 1331-32.[47] For example, Forte 1999 confirms that the "GC-C receptors of the rat are more closely related to human and pig GC-C in the ligand-binding domains with these proteins sharing ~70% identity in this region."

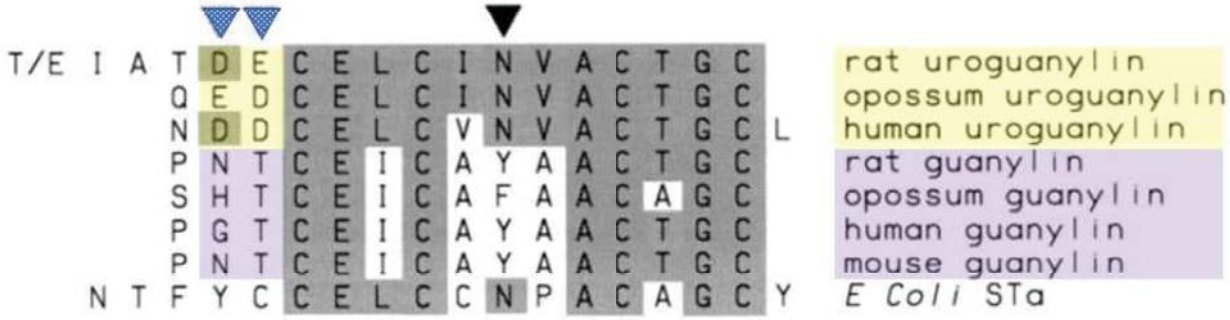
[47] Forte, L. R., *et al.*, *Guanylin Peptides: Cyclic GMP Signaling Mechanisms*, BRAZ. J. MED. BIOL. RES., 32, **1999**, 1329-1336 ("Forte 1999," EX1053).

Id., 1332.

D. The Known Chemical Properties of Uroguanylins and Amino Acids Would Have Further Provided a Skilled Artisan with a Reasonable Expectation of Improving Constipation-Related Activity through Glu^3 Substitution

153. Currie teaches that natural human uroguanylin outclasses natural human guanylin for constipation-related biochemical activity. EX1005, 6:12-22 ("Human uroguanylin appeared to be more potent than human guanylin."); *see also id.*, 2:6-24. One year later, Li provided mechanistic and biochemical insight as to why.

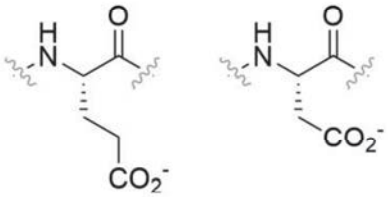
154. Li's mechanistic insight has to do with the different kinds of ionizable groups present on the amino acids of the guanylin and uroguanylin orthologs that align with the second and third positions of human uroguanylin. This position of interest was expressly identified by Li and is indicated by the two stippled arrows present in Figure 6(a). *Id.*, 54. I have reproduced this figure below for convenience, highlighting the stippled arrows in blue. I have highlighted the uroguanylin orthologs in yellow and the guanylin orthologs in purple.



EX1006, 52, FIG. 6(a) (annotated).

155. In the uroguanylin orthologs (yellow highlighting), these positions are filled only by either aspartic acid (D) or glutamic acid (E). *Id.*, 54. For example, human uroguanylin has aspartic acid (D) at both the second and third positions. *Id.*, FIG. 6(a). Rat uroguanylin has an aspartic acid (D) followed by a glutamic acid (E). *Id.* Opossum uroguanylin also displays only aspartic or glutamic acid residues at these positions. *Id.*

156. Aspartic and glutamic acid are the only two amino acids that possess an acidic side chain. That is, both of these amino acids possess carboxylic acid groups that become deprotonated under certain physiological conditions. For convenience, and to assist in visualizing these amino acids, I again provide the chemical structures of these residues below, shown in their deprotonated state.



Glu **Asp**

157. Fundamental organic chemistry teaches that carboxylic acid groups (-CO_2H) are neutral when they are protonated (*i.e.*, have an H as in -CO_2**H**). This happens under acidic conditions, predominantly where the pH of the environment is lower than the acid strength (pKa) of the -CO_2**H** in a given carboxylic acid.

When a carboxylic acid's pKa equals the pH, it will be 50% protonated, *i.e.*, half of the molecules will have that carboxylic acid protonated (-CO_2**H**) and half will be deprotonated (-CO_2^-), just as the glutamate and aspartate residues are in the structures I provided above. When the pH becomes higher (*i.e.*, more basic) than the carboxylic acid's pKa, the deprotonated, negatively charged state (-CO_2^-) will dominate. EX1012, 97-99; *see also* Section VII.

158. On the other hand, the guanylin orthologs, which are highlighted in the annotated Figure 6(a) above in purple, bear amino acids at these positions that have either neutral (uncharged) or positively charged (cationic) chemical groups under physiological conditions. EX1006, 52, FIG. 6(a); *id.*, 54.

159. Li expressly identifies this difference, noting, "[o]f particular interest are two residues that are basic or uncharged in guanylin but acidic in uroguanylin (stippled arrowheads)." *Id.*, 54. Li further expressly notes that "affinity of GCC for uroguanylin (opossum or human)," which have only aspartic or glutamic acid at these important positions, "is about 10-fold higher than its affinity for guanylin (rat or human)," which do not. *Id.* (noting these "features that are found in uroguanylin, but not in guanylin, offer information about structural elements that specify the strength of the ligand/receptor interaction"). Thus, in view of Li, a skilled artisan would have understood that the acidity and ionizability of the amino acids at these positions of interest were important to uroguanylin's increased activity as

compared to guanylin.

160. As discussed above, in view of the teachings of Currie and Li, a skilled artisan would have had good reason to make [Glu^3]-human uroguanylin, and would have done so with a reasonable expectation of success in maintaining or improving the efficacy of human uroguanylin. This substitution would have been further supported by these known chemical properties of aspartic acid (Asp/D) and glutamic acid (Glu/E), as well as what was known about the structure/activity relationship in uroguanylins.

161. More specifically, in view of the teachings of Li, a skilled artisan would avoid substituting substitute an amino acid that did not bear an acidic side chain for the aspartic acid at the 3-position. However, a skilled artisan would have reasonably expected that a Glu^3 substitution, which preserves the acidic amino acid that Li indicated was responsible for the increased activity of uroguanylin over guanylin, would maintain or even enhance activity.

162. Hamra 1997 confirms that this is especially so in relatively acidic environments (pH 6-7) in the human large intestine as well as more acidic regions of the microenvironment of intestinal mucosa (pH 5), both target tissues for treating clinical constipation using the receptor at issue. For example, Hamra 1997 teaches that uroguanylin shows 10-fold greater affinity for T84 cells at a pH of 5.0 compared to a higher pH of 8.0. EX1021, 2707, FIG. 1; *id.*, 2709. In contrast,

guanylin was known to be 10-fold more potent at pH 8.0 than pH 5.0. *Id.* Thus, "[a]t an acidic mucosal pH of 5.0, uroguanylin is 100-fold more potent than guanylin." *Id.*, 2705.

163. Like Li, Hamra 1997 related this activity difference to the "striking difference" of "the appearance of two acidic amino acids at the N terminus of uroguanylin," (*i.e.*, positions 2 and 3 of human uroguanylin) compared to the lack of acidic residues at this position in guanylin. EX1021, 2709. Hamra 1997 also notes that "[d]eletion of the N-terminal acidic amino acids in uroguanylin," *i.e.*, positions 2 and 3, "demonstrated that these residues are responsible for the increase in binding affinities that are observed for uroguanylin at an acidic pH." *Id.*, 2705; *see also id.*, 2709 (similar). Hamra 1997 thus confirms the skilled artisan's understanding that the acidic amino acids at positions 2 and 3 in human uroguanylin were directly involved in the increased binding affinity of this peptide to receptors in the tissues relevant to treating conditions such as constipation.

164. Hamra 1997 explains that "[i]t is likely that acidic conditions influence the ionization and/or conformational state of the uroguanylin molecule as a molecular mechanism for the increased biological activity of uroguanylin in this circumstance." EX1021, 2709. A skilled artisan would have understood this comment on ionization to refer to the degree of protonation of the acidic amino acids of interest. A skilled artisan also easily would have recognized that, at the

acidic mucosal pH of 5.0, the acidic side-chain of Asp, which has a pKa of 3.65 is mostly deprotonated, while the acidic side-chain of Glu, which has a pKa of 4.25, would be comparably more protonated. *See* EX1012, 118, Table 5-1 (providing pKas of amino acids). Given the increased activity of uroguanylins at acidic pHs, in which a higher percentage of these acidic residues would be protonated, a skilled artisan would have reasonably suspected that making a substitution that allowed for a higher degree of protonation at these acidic residues would yield a further improvement in activity.

165. More specifically, a skilled artisan would have recognized that the [Glu^3]-human uroguanylin analog, made obvious by Currie and Li, would be comparably more protonated than native human uroguanylin in acidic environments in the large intestine and intestinal mucosa compared to native human uroguanylin. Thus, a skilled artisan would have reasonably expected [Glu^3]-human uroguanylin to have improved activity compared to native human uroguanylin in these environments.

166. Moreover, a skilled artisan also would have reasonably expected that the comparably more acidic native human uroguanylin leaves many receptors under-stimulated in less acidic sections of the intestine. For example, Fan teaches that uroguanylin is "a more effective agonist for regulating receptor-GC activity" when the "lumen of the intestine and the mucosal (microclimate) surface is

acidified when chyme containing HCl enters the duodenum." EX1016, E963; *see also* EX1019, G714. Increasing the degree to which a uroguanylin analog may be protonated at comparably less acidic pHs merely involves swapping the more acidic Asp residue for the more easily protonated Glu residue. The Glu^3 substitution thus broadens the intestinal zone of high, or protonated, uroguanylin activity. This more permissive protonation allows for protonated [Glu^3]-human uroguanylin to maximally activate receptors further away from human uroguanylin's native maximal zone where the intestinal mucosal is acidified by chyme. EX1016, E963.

167. A skilled artisan would have recognized the desirability of enhanced affinity of [Glu^3]-human uroguanylin in these areas of the large intestine and intestinal mucosa because it was known that the large intestine is the primary target for relieving clinical constipation using human uroguanylin analogs that trigger cGMP signaling. *See*, *e.g.*, EX1005, 2:20-25; EX1016, E957, E962; EX1020, FIG. 2; *see also* Section VII.C, *above*. For example, Hamra 1996 notes that uroguanylin's target receptor, intestinal guanylin cyclase, is expressed "throughout the entire length of the small and large intestine." EX1019, G714. Because of the relative increase in protonation of [Glu^3]-human uroguanylin compared to native human uroguanylin, a skilled artisan reasonably would have expected this analog to have greater effect on the intestine by increasing its area of action along the

intestinal mucosa.

168. Simply put, a skilled artisan would have expected [Glu^3]-human uroguanylin's more permissive protonation to cause the stimulation of more receptor-bearing cells, not just those in the most acidified microenvironments where human uroguanylin works best. With more cells so stimulated, more cGMP would be produced. With more cGMP, more chloride ion would be secreted. With more secretion of chloride ions, more water would be drawn from the body into the intraluminal space. This pathway was known, and as discussed above in Section VII.H, was already linked to treating constipation.

169. Thus, because of Li and Hamra 1997's mechanistic insights, a skilled artisan would have reasonably expected [Glu^3]-human uroguanylin to bind the receptor tighter, elicit more cGMP, and cause more chloride flux associated with constipation-related activity than native human uroguanylin, in the environment of the large intestine.

170. A skilled artisan thus would have reasonably expected that applying the conservative Glu^3 substitution observed in mammals in nature to human uroguanylin, as made obvious by Currie and Li, would maintain or improve the efficacy of human uroguanylin. Moreover, in view of the known mechanistic insights of Li and Hamra 1997, as well as standard biochemistry, a skilled artisan would have had a reasonable expectation of success in increasing constipation-

related activity of human uroguanylin by making such a substitution.

E. Routine and Conventional Testing of Synthetic Analogs

171. Having made a peptide analog, it would have been routine and conventional to confirm the analog's potential efficacy for treating clinical constipation. A skilled artisan would have simply applied the same well-known assays used in Currie for evaluating human uroguanylin. As discussed above, the assays employed by Currie were conventional and described in extensive detail in the art. *See* Section VII.F, *above*.

172. For example, Currie tested uroguanylin and its naturally occurring analogs guanylin and STa using three different assays, including the Radioligand Binding Assay to confirm that human uroguanylin had binding affinity for intestinal guanylate cyclase. EX1005, 6:7-10. The Radioligand Binding Assay is described in depth above in Section VII.F.1. *See also* EX1005, 2:58-65; 4:62-5:7, 6:15-17; FIG. 3(b).

173. Currie also tested uroguanylin for activity on intestinal guanylate cyclase via downstream biochemical signaling in formation of cGMP. EX1005, 2:53-57; 3:55-4:9, 6:11-15, FIG. 3(a). The cGMP assay is described in depth at above in Section VII.F.2.

174. Currie also tested uroguanylin for Short-Circuit Current (ISc) in rat colon. EX1005, 2:66-3:3, 5:8-16, 6:23-32, FIG. 4. The Short-Circuit Current assay

is described in depth above in Section VII.F.3. Just as Currie did for human uroguanylin, a skilled artisan would have used these tests to confirm [Glu^3]-human uroguanylin as a useful human therapeutic for treating constipation.

F. The Known Issue of Aspartimide Formation in Solid-Phase Peptide Synthesis Would Have Further Supported a Glu^3 Analog of Human Uroguanylin

175. A skilled artisan would have noted additional benefits of the [Glu^3] substitution suggested by Currie and Li, given the known issue of aspartimide formation from aspartic acid in solid-phase peptide synthesis. As I discussed above in Section VII.D, aspartic acid was known to participate in side-reactions during solid-phase peptide synthesis that led to the formation of an impurity known as aspartimide. *See* EX1022, 63 ("Aspartimide formation is one of the best-documented side reactions in peptide synthesis"); EX1023, 107.

176. In particular, skilled artisans observed aspartimide formation in peptide residues containing a protected Asp-Asp sequence as well as a protected Asp-Cys sequence. *See*, *e.g.*, EX1024, 197 ("Aspartimide formation occurred for [Asp-X] for X = … Asp(O*t*Bu), Cys(Acm)."), 201, Table 1. Notably, no such impurity-producing side reactions were reported when a protected glutamic acid residue was placed next to aspartic acid. *Id.*

177. As set forth by Currie, human uroguanylin has two aspartic acid residues: one at position 2 and one at position 3, shown in bold below:

Asn[1] **Asp[2] Asp[3]** Cys[4] Glu[5] Leu[6] Cys[7] Val[8] Asn[9] Val[10] Ala[11] Cys[12] Thr[13] Gly[14] Cys[15] Leu[16]

EX1005, [57], 1:45-55. In view of the known issue of aspartimide formation when aspartic acid residues reside next to each other, or next to a cysteine residue, a skilled artisan would have readily noticed the synthetically problematic [Asp^2]-[Asp^3] and [Asp^3]-[Cys^4] in the human uroguanylin sequence.

178. Substituting [Asp^3] with [Glu^3] would eliminate both of these potential sources of aspartimide. *See* EX1024, 197, 199, 201 (noting that no aspartimide formation was observed when a protected glutamic acid was placed next to aspartic acid). A skilled artisan would reasonably expect the conservative [Glu^3] substitution suggested by Currie and Li, which would eliminate both problematic [Asp^2]-[Asp^3] and [Asp^3]-[Cys^4] sequences, would provide an additional benefit during solid-phase peptide synthesis of uroguanylin analogs. For this additional reason, making [Glu^3]-human uroguanylin would have been even more attractive to a skilled artisan than making the Glu^2 analog.

179. In view of the above, a skilled artisan would have found [Glu^3]-human uroguanylin to have been obvious given the teachings of Currie and Li. Claim 1 as a whole was thus obvious in view of Currie and Li.

G. Minimal Difference Between Currie's Human Uroguanylin and the Claimed [Glu^3]-Human Uroguanylin.

180. The only difference between human uroguanylin disclosed in Currie

and Claim 1's peptide is the Glu^3 substitution, *i.e.*, a replacement of aspartic acid with glutamic acid at the third position. As I have noted previously, glutamic acid and aspartic acid are identical except that glutamic acid's side chain contains one extra methylene unit ($-CH_2-$). *See* EX1012, 119, FIG. 5-5; *see also* Sections IV & IX.D, above. Thus, the only structural difference between the claimed peptide and Currie's human uroguanylin is, in fact, just one $-CH_2-$ unit. As I set forth above, however, this difference between the claim and the prior art disappears in view of the combined teachings of Currie and Li, which render [Glu^3]-human uroguanylin obvious.

X. GROUND 2. CLAIMS 2, 4, AND 5 WERE OBVIOUS OVER CURRIE, LI, AND NARAYANI

181. Independent claim 2 and dependent claims 4 and 5 recite conventional and routine unit dose formulations, such as a tablet or capsule, comprising [Glu^3]-human uroguanylin and one or more excipients.

182. As set forth above in Ground 1, Currie and Li render obvious [Glu^3]-human uroguanylin. Currie and Li further render obvious employing [Glu^3]-human uroguanylin as a therapeutic for treating constipation. For example, Currie teaches that human uroguanylin is useful for a variety of therapeutic applications, including regulating intestinal fluid and electrolyte transport by targeting receptors on the intestinal endothelium. EX1005, 1:20-25, 1:34-44, 2:6-24; *see also* EX1006, 45, 53-54; EX1018, G641-42 (noting oral administration of uroguanylin

successfully targets these receptors to stimulate intestinal fluid secretion). In particular, human uroguanylin can "act as a laxative and be useful in patients suffering from constipation, e.g. cystic fibrosis patients who suffer with severe intestinal complications from constipation." EX1005, 2:16-24.

183. In view of these teachings, a skilled artisan would have had good reason to formulate [Glu^3]-human uroguanylin into a unit dose form that targets the intestinal tract for, *e.g.*, administering to patients who suffer from constipation. Because the prior art renders such an application obvious, a skilled artisan would have good reason to turn to a publication such as Narayani, which teaches methods of formulating peptides with intestinal targets for oral administration.

184. I note that Shailubhai provides no explanation for how to formulate [Glu^3]-human uroguanylin as claimed. The only relevant disclosure appears as follows:

> The guanylate cyclase receptor agonists of the present invention (Table 2; SEQ ID NOs:2-5 and Table 3; SEQ ID NOs:6-21), as well as uroguanylin, guanylin and/or bacterial enterotoxin ST, may be combined or formulated with various excipients, vehicles or adjuvants for oral, local or systemic administration. Peptide compositions may be administered in solutions, powders, suspensions, emulsions, tablets, capsules, transdermal patches, ointments, or other formulations. Formulations and dosage forms may be made using methods well known in the art (see, e.g., *Remington's Pharmaceutical*

Sciences, 16th ed., A. Oslo ed., Easton, Pa. (1980)).

EX1001, 13:21-30.

185. Shailubhai thus presumes that a skilled artisan would have had the requisite skill to formulate [Glu^3]-human uroguanylin into a known unit dose form without further instruction or undue experimentation. *See also* Section V (defining the level of ordinary skill in the art as an individual with a Ph.D. in chemistry or protein engineering or related field, or an individual with a master's degree in one of these fields and two-to-five years of experience in drug development, who would have worked in consultation with a pharmaceutical chemist and/or pharmacist).

186. I also note that Shailubhai does not suggest that a particular type of unit dose form, be it a solution, inhalation formulation, capsule, or tablet, is critical for successful delivery of [Glu^3]-human uroguanylin to a particular target tissue of interest.

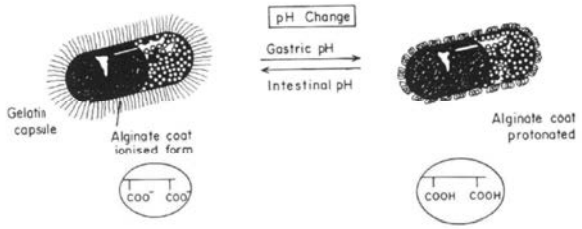
187. The analysis provided here thus exceeds what Shailubhai discloses. For example, as I set forth below, the prior art renders obvious not only the general concept of formulating peptide compositions in generic unit dose forms like tablets and capsules. The prior art also provided the skilled artisan with good reason to formulate [Glu^3]-human uroguanylin with excipients to ensure the composition survives the highly acidic environment of the stomach and release the peptide at

the targeted intestinal tissues of interest, to disguise the ordinary unpleasant taste of drugs, to eliminate gastrointestinal irritation, and to sustain drug release.

188. Thus, in view of the teachings of Currie and Li, in view of Narayani, a "unit dose," (claim 2) such as a tablet, capsule, solution, or inhaled formulation, (claim 4) comprising [Glu^3]-human uroguanylin along with one or more excipients (claim 5) also would have been obvious.

189. The chart below, as well as the analysis that follows, identifies exemplary disclosures of the cited references relative to the corresponding claim elements and is not meant to be exhaustive.

'786 Patent Claim	**Currie, Li, and Narayani**
2. A composition in unit dose comprising . . .	***Narayani teaches:*** "[E]nteric ***capsules*** for dumping microspheres ***containing therapeutically active proteins and peptides***…or other drugs, that are well absorbed in the intestine but need protection against degradation, selectively in the intestine." EX1007, 40 (emphasis added). "[S]uitable devices" for the "oral delivery of protein and peptide drugs," including "therapeutic agent-incorporated microspheres" within "***[g]elatin capsules*** coated with 20% w/v" of

	"sodium alginate [] cross-linked with appropriate concentrations of calcium chloride." EX1007, 39 (emphasis added), *see also id.*, 47, FIG. 10 (reproduced below, depicting the described capsule-based unit dose form).  **Figure 10.** Schematic representation of gelatin capsules in different pH conditions of the gastro intestinal tract.
. . . a guanylate cyclase receptor agonist peptide consisting of the amino acid sequence of SEQ ID NO:20.	*See* Section IX (Ground 1), *above*, demonstrating that a guanylate cyclase receptor agonist peptide consisting of the amino acid sequence of SEQ ID NO:20 was obvious in view of Currie and Li.
4. The composition of either claim **2** or **3**, wherein the unit dose form is selected from the group consisting of a tablet, a capsule, a solution and an inhalation formulation.	*See* claim 2, *above*, highlighting Narayani's disclosure of a capsule formulation.
5. The composition of either claim **2** or **3**, further comprising one or more excipients.	"The ***gelatin*** capsules were packed…and then ***coated with sodium alginate*** for *in vivo* tests." EX1007, 41 (emphasis added to identify excipients).

A. Formulating [Glu^3]-Human Uroguanylin in a Unit Dose Form as Recited in Claim 2 Was Obvious

190. Independent claim 2 recites:

2. A composition in unit dose comprising a guanylate cyclase receptor agonist peptide consisting of the amino acid sequence of SEQ ID NO:20.

191. As set forth above in Ground 1, Currie and Li render obvious [Glu^3]-human uroguanylin. As I discussed above, this peptide would have been understood to be a guanylate cyclase receptor agonist. Thus, for the same reasons as those discussed above in Ground 1, Currie and Li render obvious "a guanylate cyclase receptor agonist peptide consisting of the amino acid sequence of SEQ ID NO: 20," as recited in claim 2. A composition in unit dose form comprising [Glu^3]-human uroguanylin was also obvious in view of the further teachings of Narayani.

192. For example, Narayani discloses the formulation of peptides into capsules with excipients for oral administration for delivery to the intestines. EX1007, 39. Narayani notes that formulating protein and peptide drugs for oral administration as "single unit systems such as tablets" or pellets was known in the art. *Id.* Narayani further teaches that a particular method of formulating unit dose forms of protein and peptide drugs as capsules allows for enhanced delivery of said drugs to intestinal targets of interest. *Id.*, 39-40.

193. More specifically, Narayani teaches that "enteric coated multiple unit

delivery system[s] such as microspheres may be administered enclosed in a gelatin capsule" that is coated "with a natural polymer such as alginate and cross-linking with calcium chloride" to enhance delivery to the intestine. *Id.*, 40, 47; *see also id.*, 39 (noting such capsule-based dosage forms "are becoming an increasingly popular method for providing controlled drug release in the gastrointestinal (GI) tract").

194. I note that though Narayani describes this formulation as a "multiple unit delivery system" because it contains microspheres, the final composition itself is a single unit dose form. That is, the end product of Narayani's formulation is a single capsule, as depicted below in Figure 10.

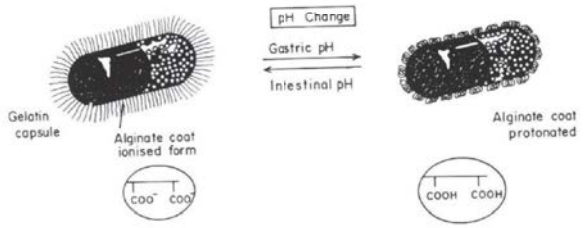


Figure 10. Schematic representation of gelatin capsules in different pH conditions of the gastro intestinal tract.

EX1007, 47, FIG. 10.

195. A skilled artisan would have had good reason to formulate [Glu^3]-human uroguanylin in a unit dose form such as a capsule, taught by Narayani, because Narayani teaches that these "polymer-coated gelatin capsules will facilitate the routine use of the oral route of drug delivery for protein and peptide drugs." EX1007, 47. Narayani further teaches that this formulation is also useful in

"disguis[ing] the unpleasant taste of drugs, eliminat[ing] gastrointestinal irritation, and sustain[ing] drug release." *Id.*, 39. Indeed, as discussed in more detail in Section VII.G, these types of unit dose forms were routinely employed for peptide therapeutics with intestinal targets. *See*, *e.g.*, EX1046, 28; EX1047, 3708-09.

196. A skilled artisan would have had a reasonable expectation of success in formulating [Glu^3]-human uroguanylin in unit dose form because preparing a unit dose form such as a capsule was routine and required only conventional techniques. *See*, *e.g.*, EX1010, 1553-1576 (describing routine preparation of tablets),1576-82 (describing routine preparation of capsules), 1582-83 (describing routine preparation of pills), 1583 (describing other solid dosage forms); *see also id.*, 1553 (noting "[d]rug substances are most frequently administered orally by means of solid dosage forms such as tablets and capsules"). I note again that Shailubhai presumes a skilled artisan possessed the skills necessary to formulate such a peptide without explicit instruction. *See* EX1001, 13:21-30.

197. Narayani's capsule formulation also involves coating with commercially available ingredients, using the conventional techniques of 3-minute drop coating and quick drying. EX1007, 40 (noting that, *e.g.*, "sodium alginate (Riedel, Germany), calcium chloride (BDH, England), and gelatin capsules ('0' size, hard) (Shibi Capsules Lts., India) were used as received"), 42. Narayani also teaches that its capsule formulations may be readily optimized using conventional

techniques such as "*in vitro* disintegration tests." EX1007, 40, FIGS. 2-10. Thus, in view of the teachings of Narayani, a skilled artisan would have had a reasonable expectation of success in formulating the capsule with an optimized amount of alginate cross-linking to ensure capsule disintegration at the desired region of the gastrointestinal tract.

198. Moreover, a skilled artisan would have had good reason to make a composition in unit dose form, as taught by Narayani, comprising [Glu^3]-human uroguanylin, as suggested by Currie and Li, in view of [Glu^3]-human uroguanylin's intestinal target—the receptor intestinal guanylate cyclase, which is expressed on the intestinal endothelium. EX1005, 1:23-44, 2:8-9, 2:16-24, 2:54-65, 6:11-22, FIGS. 3(a) & (b).

199. For example, Narayani teaches that gelatin capsules with a double coating of crosslinked 20% w/v sodium alginate and 24% w/v calcium chloride quickly disintegrate in simulated intestinal fluid, but only slowly disintegrate in simulated stomach fluid. EX1007, 41-42, FIGS. 2-10. Narayani confirms this successful intestinal delivery by radiographically tracking the coated capsules by packing them with barium sulphate. *Id.*

200. This resistance to degradation in the harsh environment of the stomach would have been especially notable to a skilled artisan because otherwise "exposure of…the bioactive substance, especially acid and protease sensitive

protein-based drugs to the gastric environment, will result in the inactivation and proteolytic degradation of the therapeutic agent" before the drug can "reach the intestine for therapeutic action or absorption." EX1007, 40; *see also* Section VII.G (citing, *e.g.*, EX1046, 28, demonstrating that delivering peptides to the intestinal endothelium with therapeutic efficacy was routine well before 2002; EX1047, 3708, demonstrating delivery of a protein enzyme to alleviate pathological supra-activity from human uroguanylin's receptor). Thus, a skilled artisan would have reasonably expected that a composition in a coated-capsule unit dose form, such as taught by Narayani, would similarly "remain[] intact" while "retained in the stomach (up to 3 h) and then migrate[] to the ileocecal region of the intestine [for] disintegrat[ion]." *Id.*, 39.

201. Thus, for the reasons set forth above, a skilled artisan would have found a composition in unit dose form comprising a guanylate cyclase receptor agonist peptide consisting of the amino acid sequence of SEQ ID NO:20 to be obvious in view of the teachings of Currie, Li, and Narayani.

B. Formulating [Glu^3]-Human Uroguanylin in a Capsule as Recited in Claim 4 Was Obvious

202. Dependent claim 4 recites:

4. The composition of either claim 2 or 3, wherein the unit dose form is selected from the group consisting of a tablet, a capsule, a solution and an inhalation formulation.

203. As set forth above in Section X.A, a composition in unit dose form comprising [Glu^3]-human uroguanylin would have been obvious in view of the teachings of Currie, Li, and Narayani. More specifically, Narayani renders obvious formulating [Glu^3]-human uroguanylin in a capsule. *See* Section X.A (discussing EX1007). Thus, for the same reasons as those set forth for claim 2, claim 4 would have been obvious.

C. Formulating [Glu^3]-Human Uroguanylin in a Unit Dose Form along with One or More Excipients as Recited in Claim 5 Was Obvious

204. Dependent claim 5 recites:

5. The composition of either claim 2 or 3, further comprising one or more excipients.

205. As set forth above in Section X.A, a composition in unit dose form comprising [Glu^3]-human uroguanylin would have been obvious in view of the teachings of Currie, Li, and Narayani. More specifically, Narayani renders obvious formulating [Glu^3]-human uroguanylin in a gelatin capsule coated "with a natural polymer such as alginate" that is "cross-link[ed] with calcium chloride." EX1007, 40, 47; *see also* my discussion of EX1007 in Section X.A.

206. A skilled artisan would have understood the gelatin and alginate present in Narayani's unit dose formulation to be excipients as recited in claim 5. *See*, *e.g.*, EX1044, 237 (stabilizing a vaccine with "an ***excipient*** blend of cellulose,

starch, sucrose and ***gelatin***" (emphasis added)); EX1045, 1063 (noting that the "***excipients used were … sodium alginate***" (emphasis added)).

207. As explained in Section VII.G, an excipient is a substance that is included in a pharmaceutical dosage form not for any direct therapeutic effect but to aid in manufacturing or delivery of the dosage form, such as by providing stability or protection for the active, to improve palatability of the dosage form for the patient, or to increase bioavailability of the active. EX1043, 210. Excipients may serve as the vehicle or medium for the active substance, and may include fillers, preservatives, stabilizers, coloring agents, and protective coatings. *Id.* As noted by Remington's, "[l]arge-scale production methods" used for the production of tablets, capsules, and pills "require the presence of other materials in addition to the active ingredients. Additives may also be included in the formulation to enhance the physical appearance, improve stability, and aid in disintegration after administration." EX1010, 1553. Thus, the inclusion of excipients in these types of formulation was routine and conventional.

208. As discussed above in Section X.A, a skilled artisan would have had good reason to formulate [Glu^3]-human uroguanylin into a capsule further comprising excipients such as gelatin and alginate because Narayani teaches such a formulation as "facilitat[ing] the routine use of the oral route of drug delivery for protein and peptide drugs" that have intestinal targets. EX1007, 47. More

specifically, Narayani teaches that this excipient-containing dose form allows the peptide drug to "spread out more uniformly in the GI tract and have relatively reproducible upper GI transit times, minimize the risk of local irritation, and dose dumping when compared to tablets and pellets." *Id.*, 39. It also prevents "inactivation and proteolytic degradation of the therapeutic agent" in the stomach because "[c]oating the gelatin capsules with a natural polymer such as alginate and cross-linking with calcium chloride had made them resistant to the gastric environment." *Id.*, 40, 47.

209. Thus, in view of the reasons stated above, as well as those discussed in Section X.A, above, a skilled artisan would have found a composition in unit dose form comprising a guanylate cyclase receptor agonist peptide consisting of the amino acid sequence of SEQ ID NO:20, and further comprising one or more excipients, to have been obvious in view of the teachings of Currie, Li, and Narayani.

XI. GROUND 3. CLAIMS 3-5 WERE OBVIOUS OVER CURRIE, LI, NARAYANI, AND CAMPIERI

210. Independent claim 3 and dependent claims 4 and 5 recite conventional and routine unit dose formulations, such as a tablet or capsule, comprising [Glu^3]-human uroguanylin and one or more excipients, along with "at least one compound selected from the group consisting of: a cGMP-dependent phosphodiesterase inhibitor, an anti-inflammatory agent, an antiviral agent and an anticancer agent."

211. As set forth above in Ground 1, Currie and Li render obvious [Glu^3]-human uroguanylin. Currie and Li further render obvious employing [Glu^3]-human uroguanylin as a therapeutic for treating constipation. For example, Currie teaches that human uroguanylin is useful for a variety of therapeutic applications, including regulating intestinal fluid and electrolyte transport. EX1005, 1:34-44, 2:6-24; *see also* EX1006, 45, 53-54. In particular, human uroguanylin can "act as a laxative and be useful in patients suffering from constipation, e.g. cystic fibrosis patients who suffer with severe intestinal complications from constipation." EX1005, 2:16-24.

212. In view of these teachings, a skilled artisan would have had good reason to look to Narayani in formulating [Glu^3]-human uroguanylin into a unit dose form for, *e.g.*, administering to patients who suffer from constipation. Moreover, a skilled artisan would have good reason to turn to publications such as Campieri, which further contemplates the administration of anti-inflammatory agents such as budesonide or prednisolone to patients suffering from constipation.

213. As I set forth below, in view of the teachings of Currie, Li, and Narayani, in view of Campieri, a "composition in unit dose form," (claim 3) such as a tablet, capsule, solution, or inhaled formulation, (claim 4) comprising such a peptide along with one or more excipients (claim 5) as well as, *e.g.*, an anti-inflammatory agent (claim 3), also would have been obvious.

214. The chart below, as well as the analysis that follows, identifies exemplary disclosures of the cited references relative to the corresponding claim elements and is not meant to be exhaustive.

'786 Patent Claim	Currie, Li, Narayani, and Campieri
3. A composition in unit dose form comprising: a) a guanylate cyclase receptor agonist peptide consisting of the amino acid sequence of SEQ ID NO: 20; and . . .	*See* Sections IX (Ground 1) and X (Ground 2) above, demonstrating that unit dose form comprising a guanylate cyclase receptor agonist peptide consisting of the amino acid sequence of SEQ ID NO:20 was obvious in view of Currie, Li, and Narayani.
. . . b) at least one compound selected from the group consisting of: a cGMP-dependent phosphodiesterase inhibitor, an anti-inflammatory agent, an antiviral agent and an anticancer agent.	**Campieri discloses:** "As one of the first aims in ***treating patients with inflammatory bowel*** disease is the prompt disappearance of symptoms, this goal was most clearly achieved with ***budesonide*** once daily and ***prednisolone*** within the first two weeks." EX1008, 213 (emphasis added). "To date, glucocorticoids (GCS)—***prednisone or prednisolone***—have been the most effective drugs in

	inducing clinical remission in these patients with ***Crohn's disease***." EX1008, 209 (emphasis added).
4. The composition of either claim **2** or **3**, wherein the unit dose form is selected from the group consisting of a tablet, a capsule, a solution and an inhalation formulation. **5.** The composition of either claim **2** or **3**, further comprising one or more excipients.	*See* Ground 2, *above*, highlighting Narayani's disclosure of a capsule formulation comprising peptides that target intestinal tissues as well as excipients such as gelatin and alginate.

A. Formulating [Glu^3]-Human Uroguanylin in a Unit Dose Form as Recited in Claim 3 Was Obvious

215. Independent claim 3 begins:

3. A composition in unit dose form comprising:

a) a guanylate cyclase receptor agonist peptide consisting of the amino acid sequence of SEQ ID NO: 20; and . . .

216. As set forth above in Ground 1, Currie and Li render obvious [Glu^3]-human uroguanylin. As I discussed above, this peptide would have been understood to be a guanylate cyclase receptor agonist. Thus, for the same reasons as those discussed above in Ground 1, Currie and Li render obvious "a guanylate cyclase receptor agonist peptide consisting of the amino acid sequence of SEQ ID NO: 20," as recited in claim 3.

217. As set forth in Ground 2, in view of Narayani, a composition in unit dose form, as recited in the preamble of claim 3, would also have been obvious.

218. Thus, for the same reasons as those set forth for claim 1 in Ground 1 and claim 2 in Ground 2, a composition in unit dose form comprising a guanylate cyclase receptor agonist peptide consisting of the amino acid sequence of SEQ ID NO: 20 would have been obvious.

219. Independent claim 3 continues, reciting that the composition further comprises:

> ***. . . b) at least one compound selected from the group consisting of: a cGMP-dependent phosphodiesterase inhibitor, an anti-inflammatory agent, an antiviral agent and an anticancer agent.***

220. The inclusion of at least one compound selected from the group consisting of a cGMP-dependent phosphodiesterase inhibitor, an anti-inflammatory agent, an antiviral agent, and an anticancer agent in the composition discussed above would have been obvious in view of the teachings of Campieri.

221. For example, Campieri discloses remission of inflammatory symptoms upon administration of anti-inflammatory agents to patients suffering from constipation associated with Crohn's disease—a chronic inflammatory disorder. EX1008, Abstract, 209. More specifically, Campieri teaches the administration of the anti-inflammatory agents budesonide and prednisolone to "patients with active Crohn's disease affecting the ileum and/or the ascending

colon." *Id.*, Abstract. Thus, Campieri teaches the administration of an anti-inflammatory agent to patients suffering from constipation resulting from an inflammatory bowel disease.

222. According to Campieri: "As one of the first aims in ***treating patients with inflammatory bowel*** disease is the prompt disappearance of symptoms, this goal was most clearly achieved with ***budesonide*** once daily and ***prednisolone*** within the first two weeks." EX1008, 213. Thus, a skilled artisan would have had good reason to combine [Glu^3]-human uroguanylin, as suggested by Currie and Li, with an anti-inflammatory agent, as taught by Campieri, in a unit dose form as taught by Narayani.

223. I note that a skilled artisan would have readily recognized the ability to treat both constipation and inflammation simultaneously, as these disorders coincide with the same pathophysiology. Because it was known that both constipation and inflammation required treatment in the same patients at the same time, combination therapy would have been obvious. Moreover, the administration of an anti-inflammatory agent to patients suffering from constipation resulting from, *e.g.*, inflammatory bowel disease, was routine. *See*, *e.g.*, EX1049, 693 (finding anti-inflammatory agents to be helpful in the treatment of constipation).

224. A skilled artisan would have had a reasonable expectation of success in preparing such a combination unit dose form as Narayani teaches that its enteric

capsules, described in more detail above in Ground 2, were appropriate unit dose forms not just for "therapeutically active proteins and peptides," but for "other drugs" as well. EX1007, 40.

225. I also note that, again, Shailubhai provides no explanation for how to formulate [Glu^3]-human uroguanylin in a combination dosage form as claimed. The only relevant disclosures appear as follows:

> The invention also encompasses combination therapy utilizing a guanylate cyclase receptor agonist administered either alone or together with an inhibitor of cGMP-dependent phosphodiesterase, an anti-inflammatory agent or an anticancer agent. These agents should be present in amounts known in the art to be therapeutically effective when administered to a patient.

EX1001, 3:45-60.

> Agonists may be administered as either the sole active agent or in combination with other drugs, e.g., an inhibitor of cGMP-dependent phosphodiesterase. In all cases, drugs should be administered at a dosage that is therapeutically effective using the existing art as a guide.

EX1001, 5:37-44.

226. Thus, Shailubhai presumes that a skilled artisan would have had the requisite skill to formulate [Glu^3]-human uroguanylin into a combination dosage form without further instruction or undue experimentation. *See also* Section V

(defining the level of ordinary skill in the art as an individual with a Ph.D. in chemistry or protein engineering or related field, or an individual with a master's degree in one of these fields and two-to-five years of experience in drug development, who would have worked in consultation with a pharmaceutical chemist and/or pharmacist); *see also* EX1008, 209 (confirming therapeutically effective dosages of the anti-inflammatory agents budesonide and prednisolone were known).

227. Thus, for the reasons set forth above, a skilled artisan would have found a composition in unit dose form comprising a guanylate cyclase receptor agonist peptide consisting of the amino acid sequence of SEQ ID NO:20 as well as an anti-inflammatory agent to be obvious in view of the teachings of Currie, Li, Narayani, and Campieri.

B. Formulating [Glu^3]-Human Uroguanylin with an Anti-Inflammatory Agent in a Capsule with One or More Excipients as Recited in Claims 4 and 5 Would Have Been Obvious

228. Dependent claims 4 and 5 recite:

4. The composition of either claim 2 or 3, wherein the unit dose form is selected from the group consisting of a tablet, a capsule, a solution and an inhalation formulation.

5. The composition of either claim 2 or 3, further comprising one or more excipients.

229. As set forth above in Section XI.A, a composition in unit dose form

comprising [Glu^3]-human uroguanylin and an anti-inflammatory agent would have been obvious in view of the teachings of Currie, Li, Narayani, and Campieri. More specifically, Narayani renders obvious formulating [Glu^3]-human uroguanylin, as suggested by Currie and Li, along with an anti-inflammatory agent, as taught by Campieri, in a capsule, as recited in claim 4. *See* Sections X.A & XI.A (discussing EX1007). As set forth earlier in this declaration, the capsule-based unit dose forms taught by Narayani comprise one or more excipients (*e.g.*, alginate and gelatin), as recited in claim 5.

230. Thus, for the same reasons as those set forth for claim 3 in Section XI.A, in view of the relevant teachings of Narayani discussed in Section X, claims 4 and 5 would have been obvious.

231. Thus, in view of the reasons stated above, as well as those discussed in Section XI.A for claim 3, and Section X regarding Narayani's disclosures, a skilled artisan would have found a composition in unit dose form, such as a capsule, as recited in claim 4, comprising a guanylate cyclase receptor agonist peptide consisting of the amino acid sequence of SEQ ID NO:20 and an anti-inflammatory agent, as recited in claim 3, further comprising one or more excipients, as recited in claim 5, to have been obvious in view of the teachings of Currie, Li, Narayani, and Campieri.

XII. GROUND 4. CLAIM 6 WAS OBVIOUS OVER CURRIE, LI, AND EKWURIBE

232. Independent claim 6 recites:

6. A peptide conjugate comprising polyethylene glycol (PEG) attached to a peptide consisting of the amino acid sequence SEQ ID NO:20.

233. As set forth above in Ground 1, Currie and Li render obvious [Glu^3]-human uroguanylin. Thus, for the same reasons as those set forth in Ground 1, "a peptide consisting of the amino acid sequence SE ID NO:20" would have been obvious. Currie and Li further render obvious employing [Glu^3]-human uroguanylin as a therapeutic for treating constipation. For example, Currie teaches that human uroguanylin is useful for a variety of therapeutic applications, including regulating intestinal fluid and electrolyte transport. EX1005, 1:34-44, 2:6-24; *see also* EX1006, 45, 53-54. In particular, human uroguanylin can "act as a laxative and be useful in patients suffering from constipation, e.g. cystic fibrosis patients who suffer with severe intestinal complications from constipation." *Id.*, 2:16-24. In view of these teachings, a skilled artisan would have had good reason to formulate [Glu^3]-human uroguanylin to administer to patients with constipation in a way that allows for enhanced absorption.

234. Because Currie and Li render such an application obvious, a skilled artisan had good reason to turn to a publication such as Ekwuribe, which teaches oral administration of peptide conjugates comprising polyethylene glycol attached to a given peptide, wherein said conjugation improves peptide absorption and

stability.

235. Thus, from the teachings of Currie and Li, in view of Ekwuribe, a peptide conjugate comprising polyethylene glycol (PEG) attached to a peptide consisting of the amino acid sequence SEQ ID NO:20 would have been obvious.

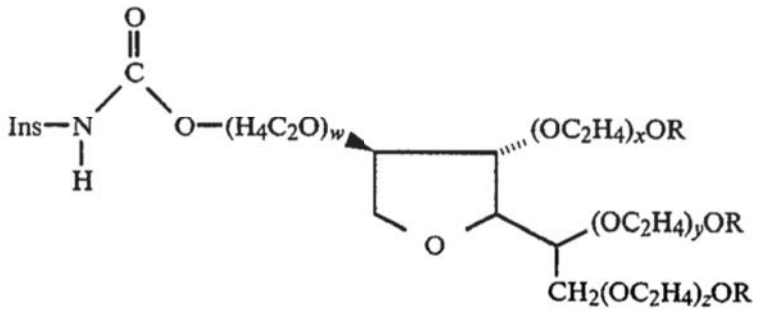
236. The chart below, as well as the analysis that follows, identifies exemplary disclosures of the cited references relative to the corresponding claim elements and is not meant to be exhaustive.

'786 Patent Claim	**Currie, Li, and Ekwuribe**
6. A peptide conjugate comprising polyethylene glycol (PEG) attached to a peptide consisting of . . .	**Ekwuribe discloses:** A "conjugated peptide complex comprising ***a peptide stabilizingly and conjugatively coupled to a polyethylene glycol***." EX1009, claim 15 (emphasis added). "The resulting ***polymer-peptide conjugates*** thus will be: stabilized (to chemical and enzymatic hydrolysis); water-soluble, ***due to the PEG residue***; and, by virtue of the fatty acid-hydrophobic domain interactions, not prone to aggregation." EX1009, 12:57-61 (emphasis added).
. . . [a peptide consisting of] the amino acid	*See* Section IX (Ground 1) above, demonstrating that a peptide consisting of the amino acid sequence

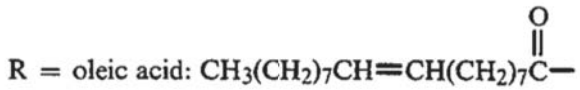
sequence SEQ ID NO:20.	of SEQ ID NO:20 was obvious in view of Currie and Li.

237. Ekwuribe teaches a "conjugated peptide complex comprising a peptide conjugatively coupled to a polymer." EX1009, [57]. Ekwuribe claims, among other conjugations, "conjugated peptide complex comprising a peptide stabilizingly and conjugatively coupled to a polyethylene glycol." EX1009, claim 15. More specifically, Ekwuribe discloses a "conjugated peptide complex comprising a physiologically active peptide covalently coupled to a physiologically compatible polyethylene glycol." EX1009, 7:66-8:2. For example, "Conjugate 1 features commercially available polysorbate monooleate at the center of the polymeric system, a sugar derivative used in many pharmaceutical applications." *Id.*, 13:44-52. The selected peptide "is attached through a carbamate linkage adjacent to the PEG region of the polymer." *Id.*, 13:50-52. Conjugate 1 of Ekwuribe is reproduced below, in which the "Ins" on the left side of the structure represents the peptide (in this case, insulin) attached to the PEG polymer.

Conjugate 1:



wherein:
w + x + y + z = 20: and



EX1009, 13:17-33.

238. Methodologically, Ekwuribe discloses that, between peptide and polyethylene glycol, "covalent bonding may be either direct, e.g., to a hydroxy terminal functionality of the polyethylene glycol group, or alternatively, the covalent bonding may be indirect, e.g., by reactively capping the hydroxy terminus of the polyethylene glycol group with a terminal carboxy functionality spacer group, so that the resulting capped polyethylene glycol group has a terminal carboxy functionality to which the physiologically active moiety may be covalently bonded." EX1009, 7:57-65. Ekwuribe teaches that this conjugation yields "a labile covalent bond at a free amino acid group of the polypeptide, wherein the liable covalent bond is scissionable in vivo by biochemical hydrolysis and/or proteolysis." *Id.*, 8:2-9.

239. Ekwuribe discloses a working example of how insulin, as an

exemplary protein hormone, is conjugated to polyethylene glycol. EX1009, 27:19-34. A skilled artisan would have had a reasonable expectation of success in carrying out this procedure on [Glu^3]-human uroguanylin without undue experimentation because Ekwuribe expressly suggests that its peptide-polymer conjugates can be modified to include a different peptide, including "peptide hormones." EX1009, 21:19-36; *see also id.*, claims 31-33. To accommodate these other peptides, Ekwuribe contemplates varying "alignments of hydrophilic and lipophilic regions relative to the point of attachment of the polymer to the peptide" to "result in polymers which protect lipophilic and hydrophilic domains." *Id.*, 13:66-14:3.

240. A skilled artisan would have had good reason to PEGylate [Glu^3]-human uroguanylin. For example, Ekwuribe teaches that "[l]ipophilic and absorption enhancing properties are imparted by the oleic acid chain, while the polyethylene glycol (PEG) residues provide a hydrophilic (hydrogen bond accepting) environment." *Id.*, 13:47-50. PEGylation was also known to "endow the polymer-peptide with high aqueous solubility." EX1009, 14:51-55; *see also id.*, 2:35-41 (teaching PEGylation of proteins rendered the proteins "soluble and non-immunogenic"). In addition, Ekwuribe teaches that linking polyethylene glycol to proteins can result in "increased stability against denaturation and enzymatic digestion." *Id.*, 2:60-65; *see also id.*, 31:38-40 (noting "polymer-enzyme

conjugates had enhanced thermal stability over the native enzyme"). Depending on the application, Ekwuribe further teaches that PEGylation may also reduce the frequency of dosing, "resulting in reduced costs and increased patient compliance." *Id.*, 14:26-30.

241. Ekwuribe further notes that such peptide conjugates may be employed in oral unit dose forms. For example, Ekwuribe suggests an "oral administration dosage form for the mediation of insulin deficiency, comprising a pharmaceutically acceptable carrier and a stable, aqueously soluble, conjugated insulin complex comprising insulin or proinsulin covalently coupled to a physiologically compatible polyethylene glycol." *Id.*, 8:34-40; *see also id.*, 24:35-42 (describing "orally administered dosages"), 29:55-58 (describing administering an oral dosage form comprising 1.56 mg/kg of an insulin-based peptide conjugate or a conjugate of alkaline phosphatase).

242. While PEGylation is not without challenges, Shailubhai presumes that the skilled artisan would have had sufficient skill to PEGylate [Glu^3]-human uroguanylin without additional instruction or undue experimentation. Indeed, Shailubhai provides little, if any, explanation for how to form the peptide conjugate as claimed. In fact, Shailubhai does not describe or disclose covalent attachment or the conjugation of anything, much less polyethylene glycol, to a uroguanylin analog. Instead, the only relevant disclosures provided by Shailubhai

are:

> Dosage forms include preparations for inhalation or injection, solutions, suspensions, emulsions, tablets, capsules, topical salves and lotions, transdermal compositions, other known peptide formulations and pegylated peptide analogs.

EX1001, 5:24-35.

> Other formulations, such as microspheres, nanoparticles, liposomes, pegylated protein or peptide, and immunologically-based systems may also be used. Examples include formulations employing polymers (e.g., 20% w/v polyethylene glycol) or cellulose, or enteric formulations and pegylated peptide analogs for increasing system half-life and stability.

EX1001, 13:40-52.

243. Thus, Shailubhai presumes that a skilled artisan would have had the requisite skill to form a peptide conjugate comprising polyethylene glycol attached to [Glu^3]-human uroguanylin without further instruction or undue experimentation. *See also* Section V (defining the level of ordinary skill in the art).

244. The instruction provided by Ewkuribe exceeds what Shailubhai discloses. Ewkuribe renders obvious not only the general concept of conjugating a peptide by attaching it to polyethylene glycol, it provided the skilled artisan with good reason to form such a conjugate and instructions for how to do so with a reasonable expectation of success.

245. A skilled artisan would have been able to perform this conjugation without undue experimentation. As Ewkuribe notes, "[i]n some instances, it may be difficult to avoid coupling and therefore masking the activity of these important residues, but some activity may be traded for increased stability while maintaining the endowed beneficial properties." EX1009, 14:22-27. Moreover, Ekwuribe teaches that even with some activity masking, "full activity of the component peptide is realized when the polymer is completely cleaved from the peptide" in the body. *Id.*, 14:48-51.

246. Thus, in view of the reasons stated above, a skilled artisan would have found a peptide conjugate comprising polyethylene glycol (PEG) attached to a peptide consisting of the amino acid sequence SEQ ID NO:20, as recited in claim 6, to have been obvious in view of the teachings of Currie, Li, and Ekwuribe.

247. Accordingly, for the reasons I have discussed above, in Grounds 1-4, it is my opinion that each of claims 1-6 would have been obvious to a person having ordinary skill in the art to which Shailubhai pertains before the earliest claimed priority date of that patent.

XIII. SECONDARY CONSIDERATIONS

248. As noted above in Section III, I understand that a determination of obviousness also involves an inquiry into secondary considerations of non-obviousness, should secondary considerations exist. I have been instructed that

these considerations may include, *e.g.*, commercial success, long-felt need, failure of others, copying, unexpected results, praise, lack of simultaneous invention, and teaching away, should there be a nexus between evidence of these considerations and the claimed invention. I understand that this nexus should involve an aspect of the claim not already in the prior art.

249. To my knowledge, Bausch has not identified any secondary considerations of non-obviousness relating to the claims of Shailubhai, and I am not aware of any other evidence supporting the existence of these secondary considerations.

250. I note, however, that, as I set forth above, the only difference between human uroguanylin disclosed in, *e.g.*, Currie and the [Glu^3]-human uroguanylin recited in Shailubhai claims is that an aspartic acid residue at the third position in the peptide has been exchanged with a glutamic acid residue. *See* Section X. As evidenced by Li, nature already explored a glutamic acid residue at the third position in, *e.g.*, the rat uroguanylin sequence. *Id.*

251. I also note that Shailubhai admits that such a substitution "do[es] not differ substantially" from the human uroguanylin sequence. Indeed, Shailubhai specification expressly states that "[f]or the purpose of the present application, a peptide differs substantially if its structure varies by more than three amino acids from a peptide of SEQ ID NOs:2-21." EX1001, 3:23-31. As I note above, the

claimed peptide differs from the known human uroguanylin peptide sequence by only one amino acid residue. *See* Sections IV & X. Thus, by the Shailubhai's own admission, the peptide recited in Shailubhai claims does not differ substantially from the human uroguanylin sequence described in the prior art. This is consistent with how these substitutions were characterized in the prior art as well. For example, Li describes the few amino acids that differ between rat and human uroguanylin sequences by noting "nearly all differences represent[] ***conservative amino acid substitutions***." EX1006, 53 (emphasis added).

252. As I have also noted previously, glutamic acid and aspartic acid are identical except that glutamic acid's side chain contains one extra methylene unit (-CH_2-). *See* EX1012, 119, FIG. 5-5; *see also* Sections IV & IX.D, above. Thus, the only structural difference between the claimed peptide and Currie's human uroguanylin is, in fact, just one -CH_2- unit. This difference between the claim and the prior art disappears in view of the combined teachings of Currie and Li, which render [Glu^3]-human uroguanylin obvious. *See* Section IX.

253. I note that Table 4 of Shailubhai presents the "biological activity" of various peptides, including human uroguanylin and [Glu^3]-human uroguanylin using "the T84 cell bioassay." EX1001, 16:1-20, Table 4. [Glu^3]-human uroguanylin is noted as having a cGMP level of 315 pmol/well, compared to 205 pmol/well for human uroguanylin. *Id.* This means that human uroguanylin

provided 65.1% of the T84 cell bioassay activity provided by the [Glu^3]-human uroguanylin. These results are not unexpected given what was known about human uroguanylin and rat uroguanylin, discussed above in Section IX. These results indicate that [Glu^3]-human uroguanylin provided the same kind of effect as human uroguanylin, though perhaps to a somewhat greater degree (consistent with what would have been reasonably expected in view of the art). As discussed in detail above, in view of the known chemical properties of uroguanylins and amino acids, a skilled artisan would have reasonably expected the efficacy of [Glu^3]-human uroguanylin to match if not exceed that of human uroguanylin. *See* Section X.D. Thus, the observed activity of [Glu^3]-human uroguanylin compared to human uroguanylin would not have been surprising nor unexpected.

XIV. CONCLUDING STATEMENTS

254. In signing this declaration, I understand that the declaration will be filed as evidence in a contested case before the Patent Trial and Appeal Board of the United States Patent and Trademark Office. I acknowledge that I may be subject to cross-examination in this case and that cross-examination will take place within the United States. If cross-examination is required of me, I will appear for cross-examination within the United States during the time allotted for cross-examination.

255. I declare that all statements made herein of my knowledge are true,

and that all statements made on information and belief are believed to be true, and that these statements were 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: **3/21/22**

By: **/Blake R. Peterson/**

Blake R. Peterson, Ph.D.

XV. APPENDIX – LIST OF EXHIBITS

Exhibit No	Description
1001	U.S. Patent No. 7,041,786, *Guanylate Cyclase Receptor Agonists for the Treatment of Tissue Inflammation and Carcinogenesis*, issued **May 9, 2006** to Shailubhai, K., *et al.*
1003	*Curriculum Vitae* of Dr. Blake R. Peterson, Ph.D.
1004	Prosecution History of U.S. Patent No. 7,041,786
1005	U.S. Patent No. 5,489,670, *Human Uroguanylin*, issued **Feb. 6, 1996** to Currie, M. G., *et al.*
1006	Li, Z., *et al.*, *Purification, cDNA Sequence, and Tissue Distribution of Rat Uroguanylin*, REGUL. PEPT., 68, **1997**, 45-56
1007	Narayani, R., *et al.*, *Polymer-Coated Gelatin Capsules as Oral Delivery Devices and their Gastrointestinal Tract Behaviour in Humans*, J. BIOMATER. SCI. POLYM. ED., 7(1), **1995**, 39-48
1008	Campieri, M., *et al.*, *Oral Budesonide Is as Effective as Oral Prednisolone in Active Crohn's Disease*, GUT, 41, **1997**, 209-214
1009	U.S. Patent No. 5,359,030, *Conjugation-Stabilized Polypeptide Compositions, Therapeutic Delivery and Diagnostic Formulations Comprising Same, and Method of Making and Using the Same*, issued **Oct. 25, 1994** to Ekwuribe, N. N.
1010	King, R. E., *Chapter 89: Tablets, Capsules, and Pills*, REMINGTON'S PHARMACEUTICAL SCIENCES, 16th ed., (ed. A. Oslo, ed., Mack Publishing Co.) **1980**
1011	Rehfeld, J. F., *The New Biology of Gastrointestinal Hormones*, PHYSIOL. REV., 78(4), **1998**, 1087-1108
1012	Nelson, D. L., *et al.*, *Chapters 4-5, 7*, LEHNINGER PRINCIPLES OF BIOCHEMISTRY, 3rd ed. (eds. Ryan, M., *et al.*, Worth Publishers) **2000**
1013	Segaloff, D. L., *et al.*, *Chapter 9: Internalization of Peptide Hormones and Hormone Receptors*, HORMONES AND THEIR

Exhibit No	Description
	ACTIONS, PART I, (eds. Cooke, B. A., *et al.*, Elsevier) **1988**, 133-149
1014	Chipens, G., *et al.*, *Recognition of Peptide Hormones and Kinins: Molecular Aspects of the Problem*, FRONTIERS OF BIOORGANIC CHEMISTRY AND MOLECULAR BIOLOGY, (ed. Ananchenko, S. N., Pergamon Press) **1980**, 99-103
1015	Unson, C. G., *et al.*, *Positively Charged Residues at Positions 12, 17, and 18 of Glucagon Ensure Maximum Biological Potency*, J. BIOL. CHEM., 273(17), **1998**, 10308-10312
1016	Fan, X., *et al.*, *Structure and Activity of Uroguanylin and Guanylin from the Intestine and Urine of Rats*, AM. J. PHYSIOL. ENDOCRINOL. METAB., 273(5), **1997**, E957-E964
1017	Thomson, A. B. R., *et al.*, *Small Bowel Review: Part I*, CAN. J. GASTROENTEROL., 14(9), **2000**, 791-816
1018	Joo, N. S., *et al.*, *Regulation of Intestinal Cl^- and HCO_3^- Secretion by Uroguanylin*, AM. J. PHYSIOL., 274(4), **1998**, G633-G644
1019	Hamra, F. K., *et al.*, *Opossum Colonic Mucosa Contains Uroguanylin and Guanylin Peptides*, AM. J. PHYSIOL. GASTROINTEST. LIVER PHYSIOL., 270, **1996**, G708-G716
1020	Nakazato, M., *Guanylin Family: New Intestinal Peptides Regulating Electrolyte and Water Homeostasis*, J. GASTROENTEROL., 36, **2001**, 219-225
1021	Hamra, F. K., *et al.*, *Regulation of Intestinal Uroguanylin/Guanylin Receptor-Mediated Responses by Mucosal Acidity*, PROC. NATL. ACAD. SCI. USA, 94, **1997**, 2705-2710
1022	Mergler, M., *et al.*, *Systematic Investigation of the Aspartimide Problem*, PEPTIDES: THE WAVE OF THE FUTURE, (ed. Lebl, M., *et al.*, American Peptide Society) **2001**, 63-64
1023	Wade, J. D., *et al.*, *Base-Induced Side Reactions in Fmoc-Solid Phase Peptide Synthesis: Minimization by Use of Piperazine as N^{α}-Deprotection Reagent*, LETT. PEPT. SCI., 7, **2000**, 107-112
1024	Lauer, J. L., *et al.*, *Sequence Dependence of Aspartimide Formation*

Exhibit No	Description
	during 9-Fluorenylmethoxycarbonyl Solid-Phase Peptide Synthesis, LETT. PEPT. SCI., 1, **1994**, 197-205
1025	Karten, M. J., *et al.*, *Gonadotropin-Releasing Hormone Analog Design. Structure-Function Studies Toward the Development of Agonists and Antagonists: Rationale and Perspective*, ENDOCR. REV., 7(1), **1986**, 44-66
1026	French, S., *et al.*, *What is a Conservative Substitution?*, J. MOL. EVOL., 19, **1983**, 171-175
1027	Tager, H. S., *et al.*, *Peptide Hormones*, ANN. REV. BIOCHEM., 43, **1974**, 509-538
1028	Noble, S. L., *et al.*, *Insulin Lispro: A Fast-Acting Insulin Analog*, AM. FAM. PHYSICIAN, 57(2), **1998**, 279-286
1029	Galloway, J. A., *New Directions in Drug Development: Mixtures, Analogs, and Modeling*, DIABETES CARE, 16(Supp 3), **1993**, 16-23
1030	Mishra, V. K., *et al.*, *Interactions of Synthetic Peptide Analogs of the Class A Amphipathic Helix with Lipids: Evidence for the Snorkel Hypothesis*, J. BIOL. CHEM., 269(10), **1994**, 7185-7191
1031	Currie, M. G., *et al.*, *Guanylin: An Endogenous Activator of Intestinal Guanylate Cyclase*, PROC. NATL. ACAD. SCI. USA, 89, **1992**, 947-951
1032	Visweswariah, S. S., *et al.*, *Characterization and Partial Purification of the Human Receptor for the Heat-Stable Enterotoxin*, EUR. J. BIOCHEM., 219, **1994**, 727-736
1033	Krause, W. J., *et al.*, *Distribution of* Escherichia coli *Heat-Stable Enterotoxin/Guanylin/ Uroguanylin Receptors in the Avian Intestinal Tract*, ACTA ANAT., 153, **1995**, 210-219
1034	Forte, L. R., *et al.*, Escherichia coli *Enterotoxin Receptors: Localization in Opossum Kidney, Intestine, and Testis*, AM. J. PHYSIOL., 257(2), **1989**, F874-F881
1035	Hyun, C. S., *et al.*, *Interaction of Cholera Toxin and* Escherichia coli *Enterotoxin with Isolated Intestinal Epithelial Cells*, AM. J. PHYSIOL., 247(6:1), **1984**, G623-G631

Exhibit No	Description
1036	Nguyen, T. D., *et al.*, *Stimulation of Secretion by the T_{84} Colonic Epithelial Cell Line with Dietary Flavonols*, BIOCHEM. PHARMACOL., 41(12), **1991**, 1879-1886
1037	Guarino, A., *et al.*, *T_{84} Cell Receptor Binding and Guanyl Cyclase Activation by* Escherichia coli *Heat-Stable Toxin*, AM. J. PHYSIOL., 253, **1987**, G775-G780
1038	Bakre, M. M., *et al.*, *Dual Regulation of Heat-Stable Enterotoxin-Mediated cGMP Accumulation in T84 Cells by Receptor Desensitization and Increased Phosphodiesterase Activity*, FEBS LETT., 408, **1997**, 345-349
1039	Lin, M., *et al.*, *Heat-Stable Toxin from* Escherichia coli *Activates Chloride Current via cGMP-Dependent Protein Kinase*, CELL PHYSIOL. BIOCHEM., 5, **1995**, 23-32
1040	Tien, X.-Y., *et al.*, *Neurokinin A Increases Short-Circuit Current Across Rat Colonic Mucosa: A Role for Vasoactive Intestinal Polypeptide*, J. PHYSIOL., 437, **1991**, 341-350
1041	Muflih, I. W., *et al.*, *Sugars and Sugar Derivatives which Inhibit the Short-Circuit Current of the Everted Small Intestine of the Rat*, J. PHYSIOL., 263, **1976**, 101-114
1042	Helbock, H. J., *et al.*, *The Mechanism of Calcium Transport by Rat Intestine*, BIOCHIM. BIOPHYS. ACTA, 126, **1966**, 81-93
1043	Baldrick, P., *Pharmaceutical Excipient Development: The Need for Preclinical Guidance*, REGUL. TOXICOL. PHARMACOL., 32, **2000**, 210-218
1044	Duncan, J. D., *et al.*, *Comparative Analysis of Oral Delivery Systems for Live Rotavirus Vaccines*, J. CONTROL. RELEASE, 41, **1996**, 237-247
1045	Gerogiannis, V. S., *et al.*, *Floating and Swelling Characteristics of Various Excipients Used in Controlled Release Technology*, DRUG DEV. IND. PHARM., 19(9), **1993**, 1061-1081
1046	Mynott, T. L., *et al.*, *Oral Administration of Protease Inhibits Enterotoxigenic* Escherichia coli *Receptor Activity in Piglet Small Intestine*, GUT, 38, **1996**, 28-32

Exhibit No	Description
1047	Mynott, T. L., *et al.*, *Efficacy of Enteric-Coated Protease in Preventing Attachment of Enterotoxigenic* Escherichia coli *and Diarrheal Disease in the RITARD Model*, INFECT. IMMUN., 59(10), **1991**, 3708-3714
1048	Rao, S. S. C., *et al.*, *Symptoms and Stool Patterns in Patients with Ulcerative Colitis*, GUT, 29, **1988**, 342-345
1049	Jalan, K. N., *et al.*, *Faecal Stasis and Diverticular Disease in Ulcerative Colitis*, GUT, 11, **1970**, 688-696
1050	Silverman, R. B., *Chapter 3: Receptors*, THE ORGANIC CHEMISTRY OF DRUG DESIGN AND DRUG ACTION, (Academic Press, Inc.) **1992**, 52-97
1051	Whitaker, T. L., *et al.*, *Uroguanylin and Guanylin: Distinct but Overlapping Patterns of Messenger RNA Expression in Mouse Intestine,* GASTROENTEROLOGY, 113, **1997**, 1000–1006
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