# Petitioner's Demonstratives

***Mylan Pharmaceuticals Inc., MSN Laboratories Private Ltd., and MSN Pharmaceuticals Inc.,***

***v.***

***Bausch Health Ireland Limited***

IPR2022-00722
U.S. Patent No. 7,041,786

WILSON SONSINI

DEMONSTRATIVE EXHIBIT – NOT EVIDENCE

June 14, 2023

# *The Challenged Claims Are Obvious*

- Good reason existed to look to the body's natural laxative peptide, uroguanylin, that was designed to add fluid to the intestines naturally.

- Good reason existed to make one, conservative substitution specifically suggested by the prior art.

- Reasonable expectation of success existed for making the modified peptide.

Pet., 1-2, 32, 34-34; Reply, 1-2, 4-6, 11-12.

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# *Introduction*

GC-C and Human Uroguanylin

# *Uroguanylin Treats Constipation*

Human uroguanylin acts as a natural laxative by activating GC-C receptors on the intestinal endothelium, drawing water into the intestinal lumen.

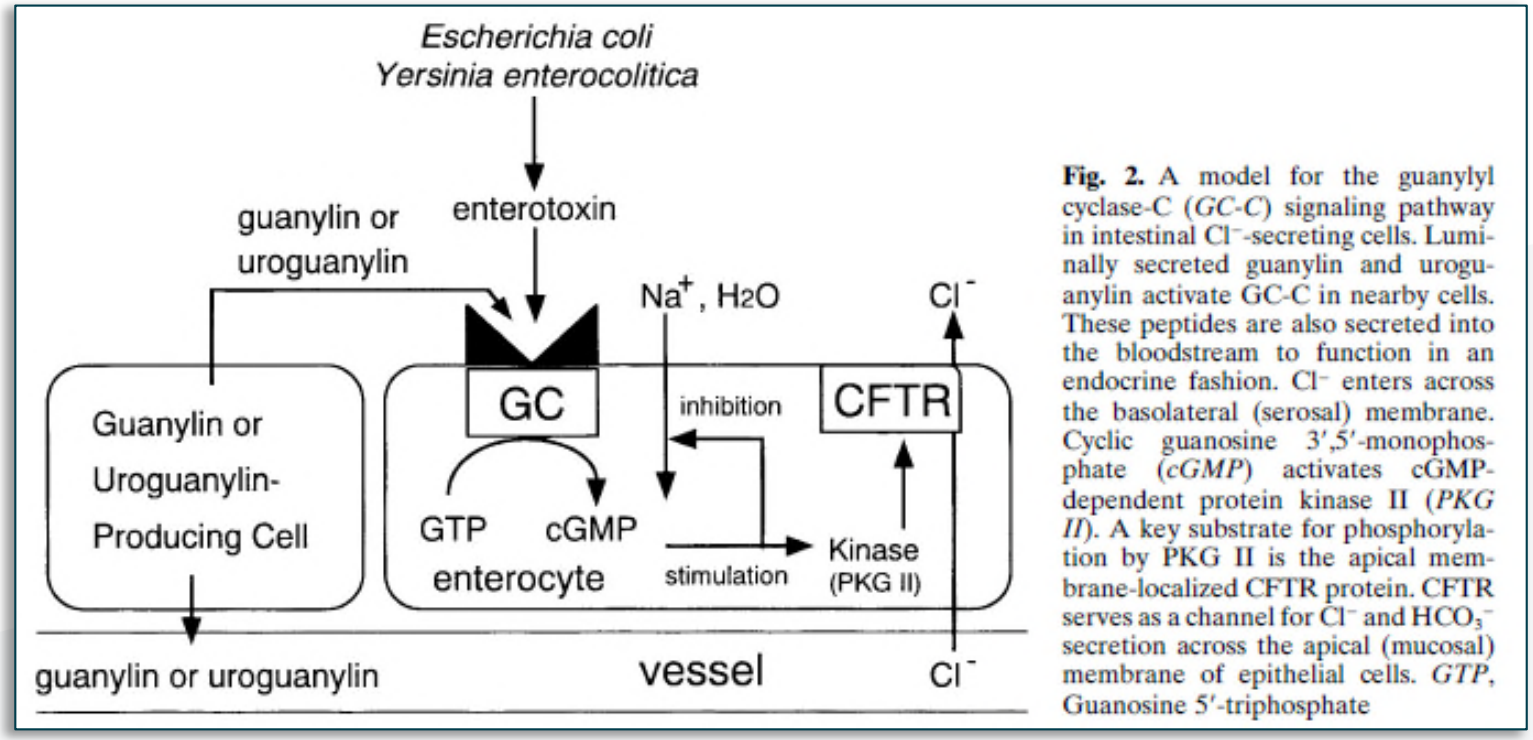



**Fig. 2.** A model for the guanylyl cyclase-C (*GC-C*) signaling pathway in intestinal $Cl^-$-secreting cells. Luminally secreted guanylin and uroguanylin activate GC-C in nearby cells. These peptides are also secreted into the bloodstream to function in an endocrine fashion. $Cl^-$ enters across the basolateral (serosal) membrane. Cyclic guanosine 3′,5′-monophosphate (*cGMP*) activates cGMP-dependent protein kinase II (*PKG II*). A key substrate for phosphorylation by PKG II is the apical membrane-localized CFTR protein. CFTR serves as a channel for $Cl^-$ and $HCO_3^-$ secretion across the apical (mucosal) membrane of epithelial cells. *GTP*, Guanosine 5′-triphosphate

EX1020 (Nakazato), Figure 2.

Pet., 1, 6, 17, 24; EX1002 (Peterson), ¶¶58-59; EX1063 (Peterson), ¶9; Reply, 4; EX1062 (Waldman), 62:7-67:7; *see also* EX1016 (Fan), E957; EX1064 (Epstein), ¶¶23-24.

# *Locus of GC-C Receptors/Ligands*

GC-C is disposed throughout small and large intestines, with higher density in the small intestine.

58. Uroguanylin is a small peptide that stimulates intestinal guanylate cyclase (GC-C), a receptor displayed in the mucosa of the intestinal endothelium. *See, e.g.*, EX1016, E957.[7] Thomson reports that skilled artisans knew by 2000 that "signal density for uroguanylin is greatest in the small intestine." EX1017, 807[8]; *see also* EX1018, G635-36, G639-41 (noting high activity in the proximal duodenal epithelium due to "a higher receptor density compared with other segments of the intestinal tract").[9] And as others noted by 1996, "All species of mammals and birds examined express GC-C-like receptor activity on the apical surface of enterocytes throughout the intestine." EX1019, G708.[10] Furthermore, according to Hamra 1996, intestinal guanylate cyclase is expressed on "apical membranes of cells throughout the entire length of the small and large intestine." *Id.*, G714. Accordingly, uroguanylin was known before 2002 to target signaling pathways in the intestines.

EX1002 (Peterson), ¶¶58-59.

Pet., 17, 36-37; Reply, 4; EX1063 (Peterson), ¶9; EX1064 (Epstein), ¶24; *see also* EX2021 (WIPO), 3:1-2 (both natural ligands produced throughout the intestinal mucosa).

# *Small Intestines Designed to Add Water to Lumen*

Uroguanylin is the body's endogenous ligand for adding water to the lumen:

So direction -- the channels conduct ions into the lumen up in the small intestine. The water follows the ions. And in the colon, the ions are sucked back into the cells and the water follows. And that's the characteristics of those two very divergent areas of the intestine. The small intestine pumps out water; the colon is the vacuum cleaner for water, sucks up the water.

A. It will act in the proximal small intestine. It will act -- so it will act in the duodenum, all four parts of it. It will act in the jejunum. It will act in the ilium, at least the proximal ilium. It will act in all of those places.

EX1062 (Waldman), 57:3-11, 65:9-19.

and the small intestines, it was well understood that the small intestines were the primary GI tissue for fluid secretion into the intestinal lumen (as Dr. Waldman admitted) and that this action was useful for treating those with CIC and IBS-C. This was because any water content added to the small intestines would have continued on to the colon, and thus would have promoted better formation and consistency of stool that would help address symptoms associated with constipation (*e.g.*, hard and lumpy stool). The body's natural processes were

EX1064 (Epstein), ¶¶34-35.

EX1063 (Peterson), ¶9; EX1002 (Peterson), ¶¶59, 63, 90, 127-28; Reply, 4, 6, 20-21; EX2025 (Waldman), ¶39; *see also* EX1062, 62:7-63:14, 65:9-19 (endogenous ligand for water secretion).

# ***Undisputed:*** *Known to Draw Water Into Lumen of Small and Large Intestines to Treat Constipation*

Bausch's expert Dr. Waldman admits it was known to treat constipation by using laxatives to induce water flow into the intestinal lumen:

**(3) Hyperosmolar agents**

33. Hyperosmolar agents include mixed electrolyte solutions containing polyethylene glycol and nonabsorbable sugars such as lactulose and sorbitol. Ex. 2050 at 921. Sorbitol and lactulose are degraded by colonic bacteria to low molecular weight acids that increase stool acidity and osmolarity. *Id.* These agents create an osmotic gradient that promotes water and electrolyte secretion into the intestinal lumen, thereby increasing stool volume and peristalsis. *Id.*

**(4) Saline Laxatives**

34. Saline laxatives contain relatively nonabsorbable cations and anions, such as magnesium hydroxide, that exert on osmotic effect to increase intralumenal water content. Ex. 2050 at 921. Because an appreciable amount of magnesium may be absorbed, there is a risk from magnesium toxicity. *Id.*

EX2025 (Waldman), ¶¶27, 29-30, 33-34, 37.

the colon. So it's fair to say that when you state intestinal lumen, you are including the entire luminal tract, the entire tract from the small intestine into the colon?

A. Yes.

Q. Everywhere from the small intestine to the colon?

A. All the way to the end.

EX1062 (Waldman), 53:5-14, 51:18-22.

POR, 7; Reply, 4-6, 20-21; EX1002 (Peterson), ¶¶59, 63, 90, 127-28; *see also* EX1064 (Epstein), ¶¶33-36; EX1063 (Peterson), ¶¶10-11, 106-07.

# Claims 2-6 (Grounds 2-4) Fall With Claim 1

Petitioner provided fulsome obviousness arguments against claims 2-6 in Grounds 2-4.

Pet., 40-53.

POR failed to provide independent arguments against these additional grounds; instead, they stand or fall with claim 1.

**V. Grounds 2-4: Claims 2-6 Would Not Have Been Obvious Over Combinations Based on Currie and Li**

Grounds 2-4 do not raise any additional arguments regarding the alleged obviousness of plecanatide as recited in claim 1. Rather, Grounds 2-4 address only the additional elements recited by claims 2-6. Because plecanatide would not have been obvious for the reasons discussed above, the compositions and peptide conjugates comprising plecanatide as recited in claims 2-6 also would not have been obvious. Ex. 2024 ¶¶ 244-253. Accordingly, Petitioner fails to establish unpatentability of claims 2-6.

**VI. Conclusion**

POR, 67.

Pet., 40-53; Reply, 1; EX1002 (Peterson), ¶¶181-247.

# *The Claims Are Defined by Linear Sequence*

1. A peptide consisting of the amino acid sequence of SEQ ID NO:20.

Q. When we look at Sequence ID No. 20, which you've reproduced in Paragraph 117 of your declaration, are there any aspects of tertiary structure that are specified in that sequence ID number?

A. That just gives you the linear sequence.

Q. When you say that Sequence ID No. 20 just gives you the linear sequence --

A. The order -- sorry. The order in which the amino acids are joined together.

EX1060 (Davies), 111:17-112:5.

Q. Does Claim 1, as you understand it, require a method of treatment of inflamed precancerous or cancerous tissue or polyps in a mammalian subject?

A. I think Claim 1 is for a peptide of the given sequence, and that's all.

EX1060 (Davies), 20:3-8.

Pet., 11-12; Reply, 1-2; EX1001, 37:1-38:10; EX1002 (Peterson), ¶¶32-33, 44-45, 124.

# *The Patent Claims [Glu³]-Human Uroguanylin*

## *"A peptide consisting of the amino acid sequence of SEQ ID NO: 20"*

### *Independent claim 1:*

**1**. A peptide consisting of the amino acid sequence of SEQ ID NO:20.

### *Independent claims 2, 3, 6, and dependent claims 4 and 5 rise or fall with Claim 1.*

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

**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 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.

**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.

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

EX1001, 37:1-38:10; Pet., 11-12; Reply, 1; EX1002 (Peterson), ¶¶31-37.

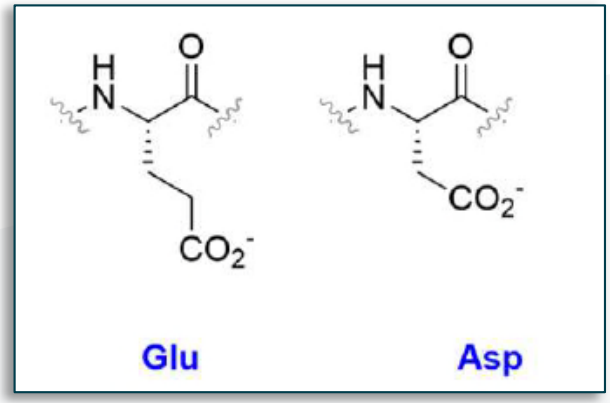
# *Graham Factor: Comparing the Claims to the Prior Art*

A single conservative substitution is all that differentiates Seq ID No. 20 (top sequence) from human uroguanylin (bottom sequence):

Asn[1] Asp[2] **Glu**[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]

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]

EX1002 (Peterson), ¶23.




EX1002 (Peterson), ¶25.

EX1002, ¶¶23-25, 73-75; Pet., 7, 17, 20-21; Reply, 14-16.

# *Making the [Glu³] Analogue Was Obvious*

"[T]he cases establish that if [a challenger] has found prior art close enough to the claimed invention to give one skilled in the relevant chemical art the motivation to make close relatives (homologs, analogs, isomers, etc.) of the prior art compound(s), then there arises what has been called a presumption of obviousness or a *prima facie* case of obviousness."

*In re Dillon*, 919 F.2d 688, 696 (Fed. Cir. 1990) (en banc).

The motivation to modify that lead compound can come from any number of sources and need not necessarily be explicit in the art. "[I]t is sufficient to show that the claimed and prior art compounds possess a 'sufficiently close relationship . . . to create an expectation,' in light of the totality of the prior art, that the new compound will have 'similar properties' to the old." *Otsuka Pharm. Co., Ltd. v. Sandoz, Inc.*, 678 F.3d 1280, 1293 (Fed.Cir. 2012) (quoting *In re Dillon*, 919 F.2d 688, 692 (Fed.Cir.1990) (en banc)). Whether a

*Bristol-Myers Squibb v. Teva Pharms.*, 752 F.3d 967, 973 (Fed. Cir. 2014).

Pet., 31; Reply 3; EX1002 (Peterson), ¶¶15-22, 117-25;
*see also KSR Int'l Co. v. Teleflex Inc.*, 550 U.S. 398, 406 (2007).

# *Four Reasons to Make Synthetic Uroguanylin Analogue*

- Uroguanylin is naturally produced by the body to draw water into the intestinal lumen, is "useful for the control of intestinal absorption," able to displace ST binding, and may "act as a laxative and be useful in patients suffering from constipation[.]" EX1005 (Currie), 2:6-24; EX1016 (Fan), E957, E962; EX1020 (Nakazato), 222 & Fig. 2; EX1018 (Joo), G635-36, G639-41; EX1019 (Hamra 96), G708; EX1017 (Thomson), 807; EX1002 (Peterson), ¶¶59, 63, 71, 90, 126-31; EX1064 (Epstein), ¶¶23-24.

- Oral administration of human uroguanylin stimulated intestinal fluid secretion for treatment of constipation. EX1018, G641-G642; EX1002, ¶¶99, 59-60, 85-86, 107; EX1005, 1:34-44, 1:50-55, 2:6-24, 2:53-65, 6:11-22; EX1063 (Peterson), ¶10; EX2021 (WIPO), 2:28-3:1.

- At relevant intestinal pH, human uroguanylin has enhanced potency over guanylin attributed to acidic residues at positions 2 and 3. EX1002, ¶¶61-65, 91; EX1021 (Hamra 97), 2705, 2709; EX1063, ¶13.

- Currie acknowledged placement and import of uroguanylin's disulfide bridges and that it lacked ST's toxic potency, but taught its "physiological characteristics" made it "important to medical science in the study of regulators of guanylate cyclase." EX1005, 1:47-63, 2:3-7; EX1002, ¶¶60, 86, 88, 105.

Pet., 1, 5, 17-22; Reply, 2, 4-6; EX1002 (Peterson), ¶¶132-38.

# *Six Reasons to Make [Glu³]-Substitution*

- Li narrows substitution choices at position 3 to Glu.

  EX1002 (Peterson), ¶¶137-52.

- Conservative substitution likely to retain excellent GC-C activity.

  EX1002, ¶¶73-75.

- Conserved in homologous species.

  EX1002, ¶¶132-36.

- Fine tune pH response.

  EX1002, ¶¶165-70.

- Eliminate pairing causing aspartimide formation.

  EX1002, ¶¶175-79.

- Routine synthesis and characterization.

  EX1002, ¶¶66-67.

Pet., 21-22, 34-39; Reply, 11-12; EX1063 (Peterson), ¶50.

# Reasonable Expectation of Successfully Making [Glu³]-Human Uroguanylin

[11] [12] The reasonable expectation of success requirement refers to the likelihood of success in combining references to meet the limitations of the claimed invention. "[F]ailure to consider the appropriate scope of the ... patent's *claimed invention* in evaluating the reasonable expectation of success ... constitutes a legal error that [is] review[ed] without deference." *Allergan*, 754 F.3d at 966 (emphasis added). Under the Board's uncontested construction, "claim 1 does not require removal of the protecting group to allow subsequent nucleotide incorporation," let alone quantitative removal. *Intelligent Bio–Sys., Inc.*, 2015 WL 996355, at *4. Accordingly, it is of no moment that Zavgorodny's protecting group would not be removed quantitatively in Tsien or Ju's sequencing method—removal is simply not required by the claim of the #537 patent. The Board seemed to believe that the "reasonable expectation of success" inquiry looked to whether one would reasonably expect the prior art references to operate as those references intended once combined. That is not the correct inquiry—one must have a motivation to combine accompanied by a reasonable expectation of achieving what is claimed in the patent-at-issue. The Board's reliance

*Intelligent Bio-Systems v. Illumina Cambridge*, 821 F.3d 1359, 1367 (Fed. Cir. 2016).

Making a desired peptide using the proteogenic amino acids was a matter of routine skill.

> The novel peptide of this invention can be prepared by known solution and solid phase peptide synthesis methods.

EX1005 (Currie), 3:8-45.

Skilled artisans routinely used solid-phase peptide synthesis to synthesize large numbers of peptides with ease.

> In solid-phase synthesis, an amino acid molecule is covalently bound to a solid support material and amino acids are added to the chain residue by residue. Solid-phase synthesis methods improved the efficiency, throughput, simplicity, and speed of the synthesis of earlier solution phase methods where the synthesized amino acid chains were not bound to a solid support. The chemistry and processes involved in solid-phase peptide synthesis were developed decades ago and were quite routine before 2002.

EX1002 (Peterson), ¶¶66-67; *id.*, ¶¶130-31.

Pet., 21-22, 24, 35-36; Reply, 2; EX1002, ¶¶142-49.

# *Unrebutted* Reasonable Expectation of Success

THE WITNESS: I think in terms of the chemistry, they would have been able to do it. But that's not what the goal would be.

EX1060 (Davies), 127:5-128:4.

Q. Okay. Let's assume for the purposes of this question that the person asked to make the Glu 3 human uroguanylin is a peptide chemist with experience using solid-phase peptide synthesis.

Would that individual have a reasonable expectation of being able to successfully make the peptide?

* * *

THE WITNESS: Well, I think they would have been able to make the peptide.

EX1060 (Davies), 130:9-20.

formation during synthesis (*id.* ¶¶ 175–179). *See* Pet. 34–39. On the current record, this evidence, which is largely unrebutted, is sufficient to show that a POSA would have been motivated to substitute glutamic acid for the $Asp^3$ in human uroguanylin and would have had a reasonable expectation of success in doing so.

Paper 16 (Institution Decision), 20.

Pet., 21-22, 31, 35-36; Reply, 2-3; EX1002 (Peterson), ¶66-67, 130-31, 147; EX1063 (Peterson), ¶¶114-17.

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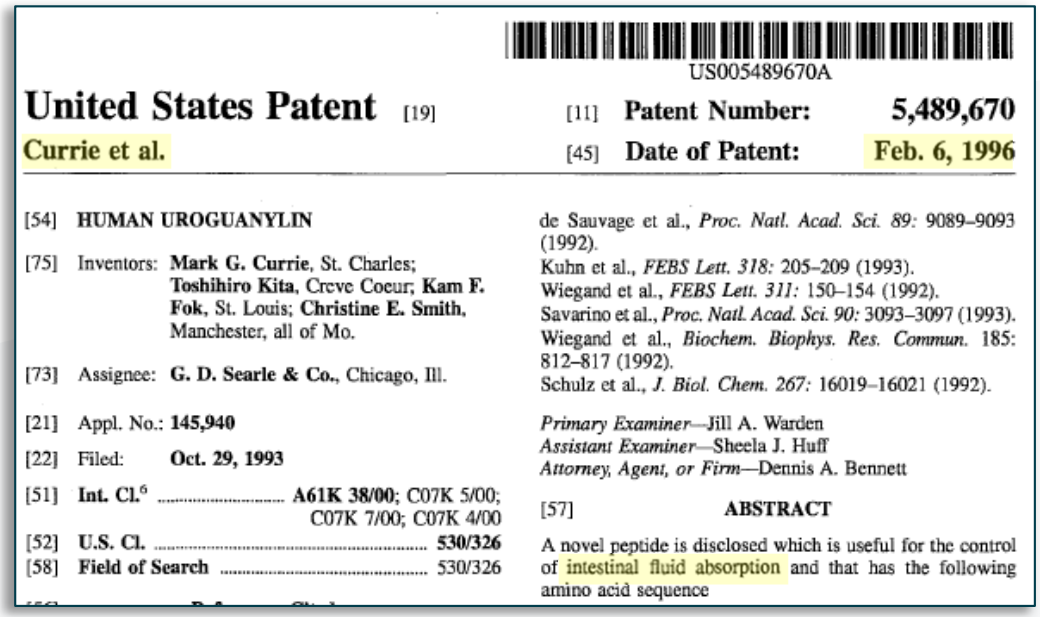
# Ground 1: Currie & Li

EX1005 and EX1006



US005489670A

**United States Patent** [19]

**Currie et al.**

[11] **Patent Number: 5,489,670**

[45] **Date of Patent: Feb. 6, 1996**

[54] **HUMAN UROGUANYLIN**

[75] Inventors: **Mark G. Currie**, St. Charles; **Toshihiro Kita**, Creve Coeur; **Kam F. Fok**, St. Louis; **Christine E. Smith**, Manchester, all of Mo.

[73] Assignee: **G. D. Searle & Co.**, Chicago, Ill.

[21] Appl. No.: **145,940**

[22] Filed: **Oct. 29, 1993**

[51] **Int. Cl.**[6] ..... **A61K 38/00**; C07K 5/00; C07K 7/00; C07K 4/00

[52] **U.S. Cl.** ..... **530/326**

[58] **Field of Search** ..... 530/326

de Sauvage et al., *Proc. Natl. Acad. Sci. 89:* 9089–9093 (1992).
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Savarino et al., *Proc. Natl. Acad. Sci. 90:* 3093–3097 (1993).
Wiegand et al., *Biochem. Biophys. Res. Commun.* 185: 812–817 (1992).
Schulz et al., *J. Biol. Chem. 267:* 16019–16021 (1992).

*Primary Examiner*—Jill A. Warden
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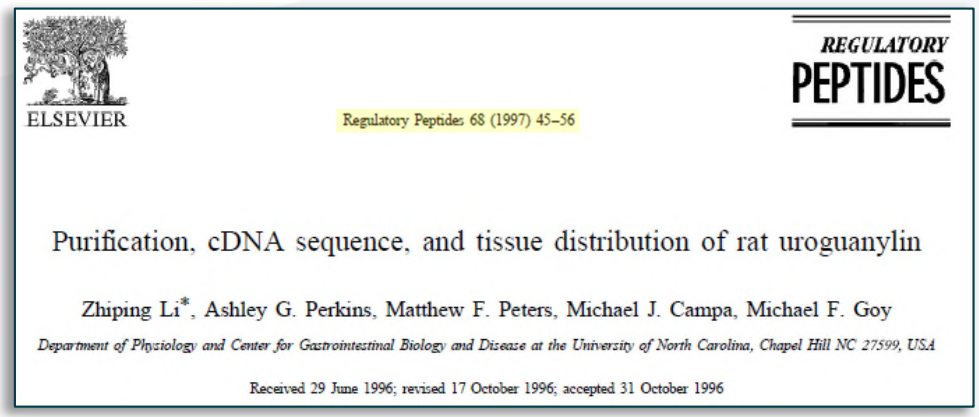
*Attorney, Agent, or Firm*—Dennis A. Bennett

[57] **ABSTRACT**

A novel peptide is disclosed which is useful for the control of intestinal fluid absorption and that has the following amino acid sequence



ELSEVIER

Regulatory Peptides 68 (1997) 45–56

REGULATORY PEPTIDES

Purification, cDNA sequence, and tissue distribution of rat uroguanylin

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Received 29 June 1996; revised 17 October 1996; accepted 31 October 1996

# *Claim 1 Was Obvious Over Currie & Li*

**Currie** (EX1005) taught natural GC-C ligand human uroguanylin:

- Laxative effect for treating constipation. EX1005, 2:21-25.
- Enhanced activity over guanylin; not toxic like ST. EX1005, 1:31-44, 3:65-4:9, Fig. 3B.
- Works on rat intestines. EX1005, 5:5-20, 6:19-32.
- Synthetic analogues easily and routinely made. EX1005, 3:8-45.

Pet., 22-24, 32-34.

**Li** (EX1006) taught $Glu^3$-uroguanylin:

- $Glu^3$ was a conservative and homologous substitution for human uroguanylin
- Likely to retain enhanced receptor affinity and potency at acidic pH. EX1006, 53-54.
- Rat uroguanylin (with $Glu^3$ substitution) works on a cell line from human intestines. EX1006, 47, 54.

Pet., 24-26, 32-34.

**The Combination** provides:

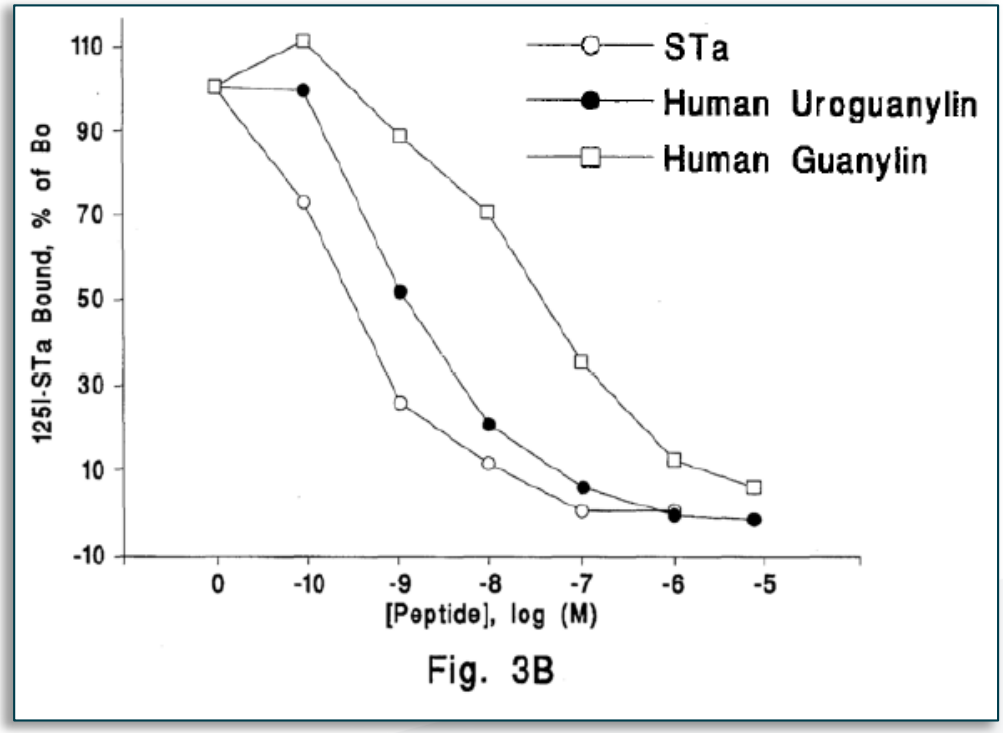
- Good reason to make the [$Glu^3$]-analogue of human uroguanylin with a reasonable expectation of success.

Pet., 32-34.

*See also* EX1002 (Peterson), ¶¶117-25.

# *Currie (EX1005)*

Human uroguanylin stimulated laxative activity via GC-C activation:




EX1005, Figure 3B (with key from Figure 3A).

Human uroguanylin appeared to be more potent than human guanylin, but less potent than ST for activation of GC-C in T84 cells. A different profile of relative affinity was obtained using the competitive binding assay with $^{125}$I-ST$_{5-18}$ as the radioligand. ST and human uroguanylin had similar affinities for the receptors on T84 cells and human guanylin had a much lower affinity (FIG. 4*b*). The data indicate that these peptides all possess the ability to stimulate GC-C and share similar binding sites with varying degrees of relative affinities for the receptors in T84 cells.

EX1005, 6:11-21.

Human uroguanylin has been further demonstrated to act in an isolated intestinal rat preparation to stimulate an increase in short circuit current. This action is believed to be the physiologic driving force for eliciting chloride secretion and ultimately decreased water absorption. The human uroguanylin may thus 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, 2:21-25.

Pet., 22-24; EX1002 (Peterson), ¶¶65, 84-87; Reply, 5-6; *see also* EX1005, 3:7-8 ("The novel peptide of this invention can be prepared by known solution and solid phase peptide synthesis methods.").

# *Currie (EX1005)*

The prior art provided good reason to make a synthetic analogue of the natural human uroguanylin GC-C ligand used by the body to draw water into the intestinal lumen.

fluid at the large intestines). The fact that uroguanylin was known to be the body's natural ligand for increasing water content in the intestinal lumen with the highest receptor density provided a strong indication that it was both safe and effective, and also was well-calibrated to work for this purpose without causing adverse effects. EX1002, ¶¶59-60 (discussing uroguanylin's "more controlled" version of activating the cGMP pathway).

EX1063 (Peterson), ¶9.

Q. -- but does your answer apply -- is it fair to say that how you -- what you explained applies to uroguanylin?

A. Yeah. So to put a fine point on it, one of the things that uroguanylin does is it stimulates fluid and electrolyte secretion. That's one of the things that it does.

EX1062 (Waldman), 62:7-67:6.

Pet., 22-24; EX1002 (Peterson), ¶¶104-06, 124-38; Reply, 4; EX1063, ¶9.

# *Currie (EX1005)*

Heat stable enterotoxins (STs) were known to have toxic properties;

Human uroguanylin was a desirable alternative.

Pathogenic strains of E. coli and other bacteria produce a family of heat stable entertoxins (STs) that activate intestinal guanylate cyclase. STs are acidic peptides 18–19 amino acids in length with six cysteines and three disulfide bridges that are required for full expression of bioactivity (7). The increase of intestinal epithelial cyclic GMP elicited by STs is thought to cause a decrease in water and sodium absorbtion and an increase in chloride secretion (8,9). These changes in intestinal fluid and electrolyte transport then act to cause secretory diarrhea. In developing countries, the diarrhea due to STs is the cause of many deaths, particularly in the infant population (10). STs are also considered to be a major cause of traveler's diarrhea in developed countries (11). STs have also been reported to be a leading cause of morbidity in domestic animals (12).

EX1005, 1:31-44.

colon would increase the likelihood of diarrhea). A person of ordinary skill would have understood that replicating the potency, pH-independence, and "stability" of enterotoxins could increase the risk of causing severe diarrhea, which can result in death, especially in children, domestic animals, or adults of small stature. Given the excellent known potency of uroguanylin, and its natural ability to increase water accumulation in the intestinal lumen, attaining pH-independent and toxic levels of potency associated with enterotoxin would not be a clear favorite of person of ordinary skill in the art, and certainly would not dissuade them from modifying uroguanylin.

EX1063 (Peterson), ¶¶16-19.

Pet., 22-24; EX1002 (Peterson), ¶¶59-65; Reply, 5-6.

# *STs Were Molecular Mimics Designed to Cause Massive Diarrhea*

"They create a massive diarrhea, a massive fluid and electrolyte secretion event, massive diarrhea, and the bugs are expelled into the environment so that they can find a new host. That's the purpose of the molecular mimicry."

Dr. Waldman

What purpose does it serve for the bacteria to produce enterotoxins?

* * *

A. Okay. The reason they do that -- so -- so the bugs get in there, and they set up shop, and they colonize the intestine, which is where they live. And they're enjoying their environment, and they're sucking up all the glucose.

And they get -- there is so much of them. At some point they suck up all the glucose, and there are no resources anymore.

* * *

The bugs massively secrete ST. They create a massive diarrhea, a massive fluid and electrolyte secretion event, massive diarrhea, and the bugs are expelled into the environment so that they can find a new host. That's the purpose of the molecular mimicry.

EX1062 (Waldman), 77:15-79:20.

Pet., 34-39; Reply, 6-7; EX1002 (Peterson), ¶¶59-65.

# Li (EX1006)


EX1006, 52, Fig. 6A (annotated).

The affinity of GCC for uroguanylin (opossum or human) is about 10-fold higher than its affinity for guanylin (rat or human) [28,29]. 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), and one residue that contains an aromatic ring in guanylin but an acid amide in uroguanylin (solid arrowhead). At all three positions, our duodenal peptide 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. Dose/response curves with synthetic rat peptide will be required to test this idea directly. It will be particularly of interest to determine

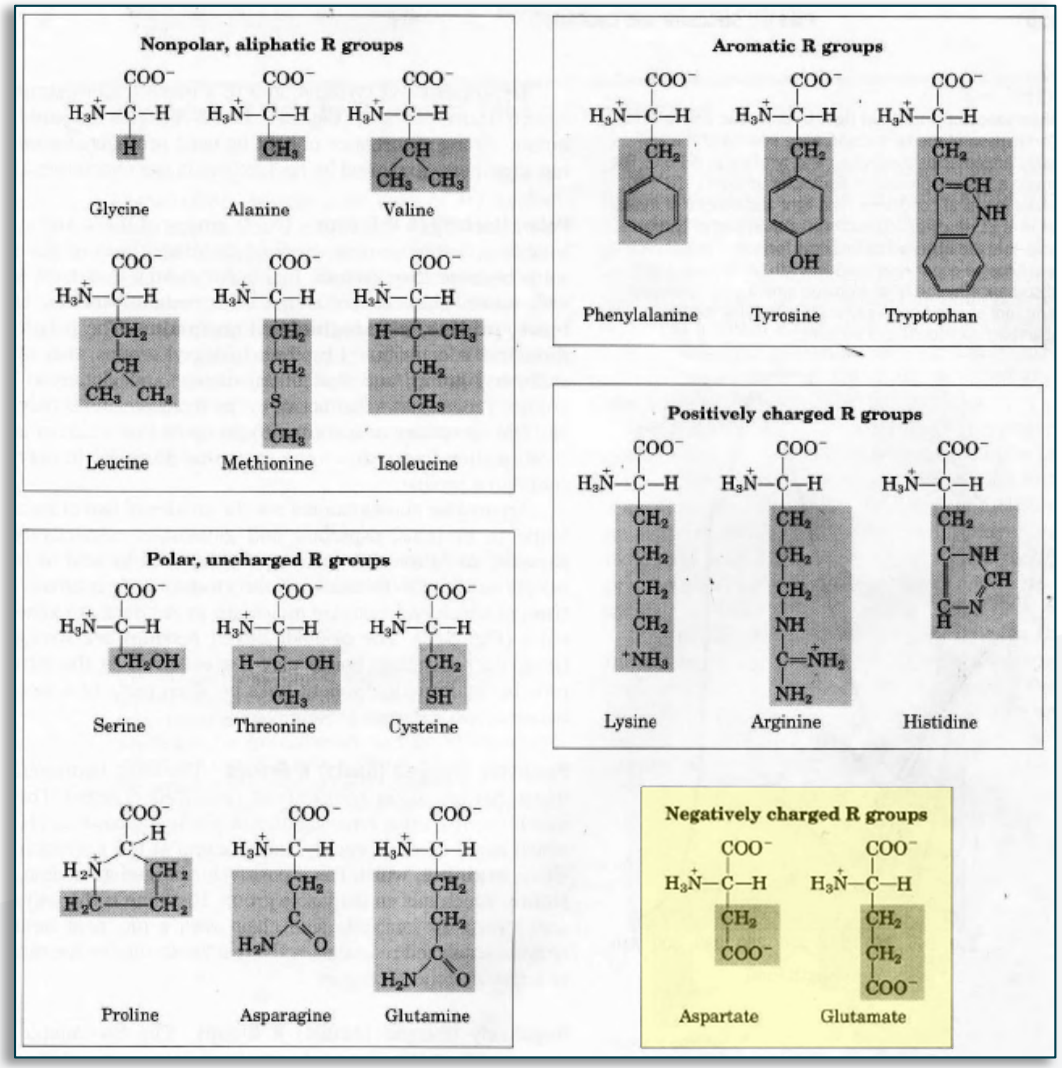
EX1006, 54.

Li indicates the conservative replacement of $Asp^3$ of uroguanylin with $Glu^3$, as found naturally in a mammal closely related to humans, was expected to retain relevant enhanced receptor affinity of uroguanylin.

EX1006, 47, 54.

Pet., 24-26; EX1002 (Peterson), ¶¶108-10; EX1006, 45, 53.

# One Conservative Substitution




EX1012 (Nelson), Fig. 5-5.

Aspartate (Asp) and Glutamate (Glu) "are the only amino acids having a second carboxyl group within the side chain and therefore were known for their characteristic, negatively charged functional groups." "[T]he only structural difference between them is…one additional methylene…."

EX1002 (Peterson), ¶54.

Pet., 16-17; Reply, 14-16; EX1002, ¶¶53-54.

# *Hamra Confirms Glu-Asp Substitution at Position 3*

Hamra 1997: "All uroguanylin peptides have aspartate or glutamate residues at these positions" and the "acidic residues" should not be deleted. EX1021, 2709.

A striking difference in the primary structure of uroguanylin compared with guanylin is the appearance of two acidic amino acids at the N terminus of uroguanylin (Fig. 6). All uroguanylin peptides have aspartate or glutamate residues at these positions (8, 9). Deletion of the N-terminal residues ($Gln^{95}$–$Glu^{96}$–$Asp^{97}$) of opossum $uroguanylin^{95–109}$ converted the truncated $uroguanylin^{98–109}$ into a uroguanylin analogue that possessed the pharmacological property that is characteristically observed in the guanylin subfamily of peptide agonists. The truncated $uroguanylin^{98–109}$ was actually somewhat more potent at pH 8.0 than at pH 5.0. We conclude that the N-terminal acidic residues of uroguanylin are required for the increased binding affinities, and therefore, the enhanced potency of uroguanylin for activation of receptors under acidic conditions.

EX1021, 2709.

Pet., 34-39; Reply, 16-19; EX1002 (Peterson), ¶¶64-65, 163-65; *see also* POR, 22, 45-51.

# *One Conservative Substitution*

This conservative substitution was expected to retain or improve activity.

> 74. Because the chemical and physical properties of the amino acids involved in a conservative substitution are similar, their impact on the structural and chemical properties of the protein may be similar enough to preserve functionality (*e.g.*, ligand binding activity) of the protein. Where conservative substitutions are found at a given position in natural homologs, especially across closely-related species, this provided a strong indication to skilled artisans that the particular substitution was biologically acceptable to retain or even improve functionality.

EX1002 (Peterson), ¶74.

Pet., 21; Reply, 11; *see also* EX1063 (Peterson), ¶62.

# *Prior Art Supports the Conservative Substitution*

Bausch contends conservative substitutions simultaneously have minimal effect, and yet are wildly unpredictable.

POR, 22, 41-42.

Yet Bausch's exhibits confirm an obvious, conservative modification was expected to retain activity.

Reply, 14-16.

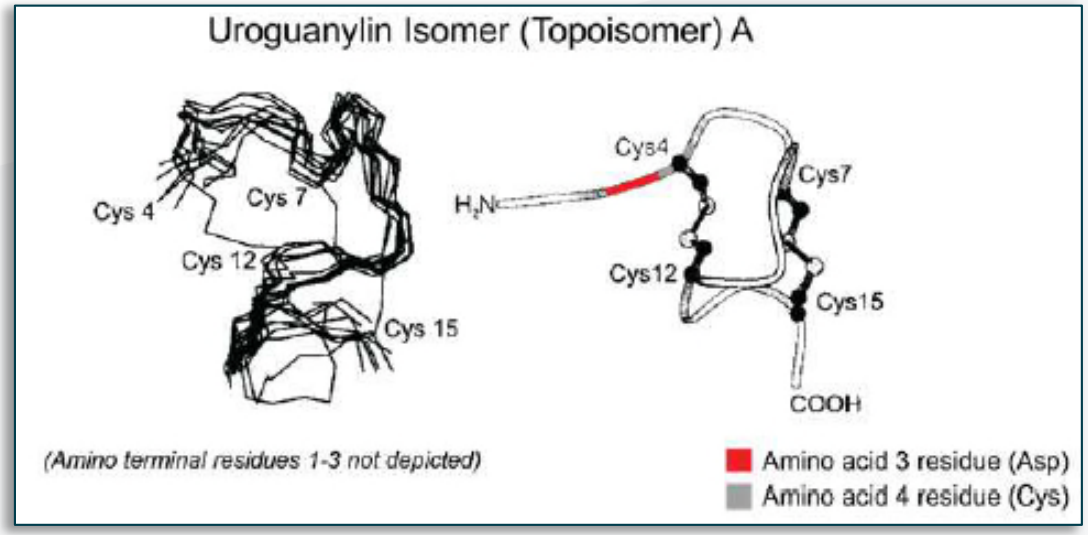
"In protein engineering the concept of conservative mutations is frequently used."

EX2035 (Jonson), 397.

"In the case of aspartic acid, the obvious replacement is glutamic acid…these are certainly the only really conservative substitutions that would be possible."

EX2041 (Grossman), 37-38.

Substitution would not negatively affect peptide structure.




EX2010 (Marx), Fig. 4 (annotated with red coloring).

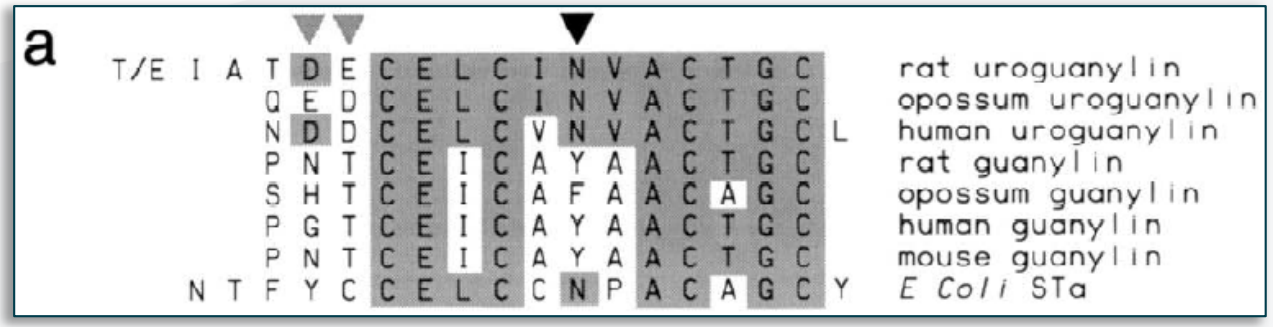
Pet., 34-39; Reply, 14-16; EX1002 (Peterson), ¶¶73-76, 137-38; *see also* POR, 40-45.

# *Orthologous Substitution Was Routine*

POSAs routinely looked to orthologs (i.e., a peptide performing the same function in a different species, such as rat uroguanylin).

> "[S]killed artisans routinely investigated orthologous peptides, the same peptide hormone but in a different animal, for potential amino acid substitutions.... [E]valuating uniformity verses variance in the sequence of orthologs across various species was a routine practice[.]"
>
> EX1002 (Peterson), ¶78; *see also* EX1025 (Karten).

> "[H]omology shows that uroguanylin orthologs generally do not vary much among mammals."
>
> EX1002, ¶144 (citing EX1006 (Li), Figure 6A (reproduced below)).




EX1006 (Li), Figure 6A.

Pet., 21; EX1002, ¶¶73-81, 137-38.

# *Functional Overlap Between Human and Rat GC-C*

Human uroguanylin has been further demonstrated to act in an isolated intestinal rat preparation to stimulate an increase in short circuit current. This action is believed to be the physiologic driving force for eliciting chloride secretion and ultimately decreased water absorption. The human uroguanylin may thus 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 (Currie), 2:16-24.

"[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."

EX1002 (Peterson), ¶142.

POSAs understood rat uroguanylin was informative to human uroguanylin:

- **Currie:** human uroguanylin acted on isolated intestinal rat preparation. EX1005, 2:16-24.
- **Li:** rat uroguanylin activated GC-C in human-derived T84 cells. EX1006, 47, 54.

Pet., 1-2; EX1002, ¶¶139-52.

# *Enhanced Activity at Acidic pH*

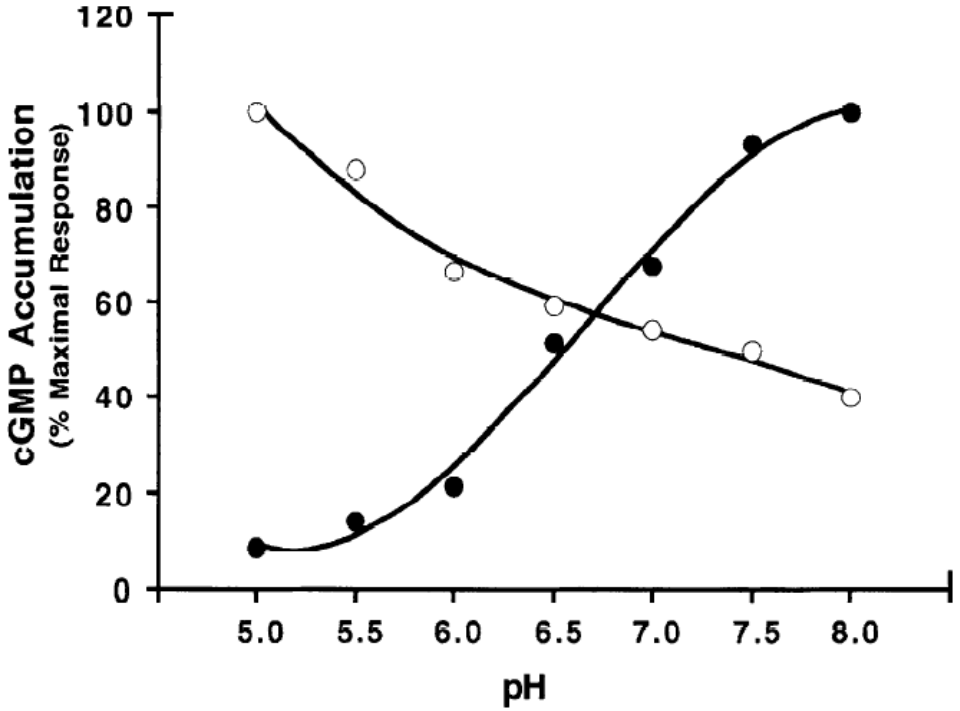



Fig. 1. Effects of medium pH on uroguanylin (○) and guanylin (●)-stimulated guanosine 3′,5′-cyclic monophosphate (cGMP) accumulation in T84 cells. Vehicle, 30 nM synthetic opossum uroguanylin, and 30 nM synthetic opossum guanylin were suspended in buffered assay medium previously adjusted to pH values indicated, as described in MATERIALS AND METHODS. Levels of T84 cell cGMP accumulation (pmol/well, average of 3 wells) elicited by vehicle and peptides in this experiment when tested at pH 5.0 and pH 8.0, respectively, were as follows: basal (vehicle control) = 0.45 and 0.78, uroguanylin = 43.9 and 17.5, and guanylin = 0.85 and 10.0. Data are representative of 4 experiments with similar results.

EX1019 (Hamra 96), Fig. 1.

Uroguanylin evolved to have enhanced potency in acidic mucosa in the intestines.

EX1019, G710; EX1002 (Peterson), ¶62-63; Pet., 18-19; Reply, 20-21; EX1063 (Peterson), ¶¶105-06.

# *Undisputed* Enhanced Activity at Acidic pH

Human uroguanylin "works better in the acidic environment of the small intestine[.]"

POR, 54.

tion via intracellular cGMP. Because the potency of uroguanylin is markedly enhanced when the intraluminal pH is acidic and an acidic pH markedly decreases the potency of guanylin, it may be postulated that the secretion of uroguanylin is increased when acidic chyme is delivered from the stomach to the duodenum (12, 13, 15). The relative potencies of guanylin and uroguanylin for activation of intestinal receptor GCs and the stimulation of transepithelial $Cl^-$ secretion are markedly influenced by mucosal acidity (12). In the present study, uroguanylin isolated from rat duodenum (or urine) stimulates cGMP accumulation in T84 cells to a greater magnitude at a medium pH of 5.5 than at pH 7.5. Guanylin isolated from the intestine increased cGMP to a greater level in T84 cells at pH 7.5 than at pH 5.5. Thus uroguanylin and guanylin isolated from rat intestine exhibit properties similar to those previously defined for the homologous peptides derived from human subjects and opossums (12, 13, 15). Evolution of the

EX1016 (Fan), E962.

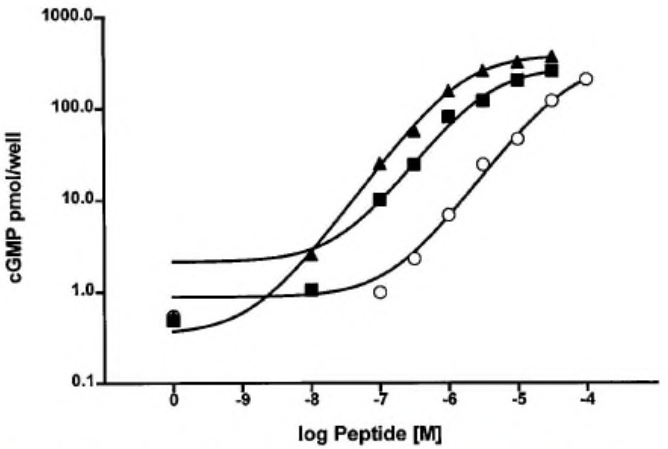



Fig. 9. Bioactivity of synthetic uroguanylin and guanylin in T84 cells. Values are representative of 3 experiments conducted with cultured T84 cells and are means of duplicate assays at each peptide concentration. ■, Rat guanylin (PNTCEICAYAACTGC); ▲, rat uroguanylin (TDECELCINVACTGC); ○, 12-residue portion of uroguanylin (CELCINVACTGC). Disulfide bonds in these synthetic peptides occur between 1st to 3rd and 2nd to 4th cysteine residues. Medium is DMEM at pH 7.4 for this assay.

determined. The amino acid sequence of rat uroguanylin has been recently elucidated by the isolation of cDNA clones encoding prepro-uroguanylin and by purification of uroguanylin from duodenum and $NH_2$-terminal sequence analysis (1, 26, 29). These studies revealed that the sequence of the 15 COOH-terminal residues for rat uroguanylin is TDECELCINVACTGC, which agrees with the partial sequence that we obtained in the present study. A synthetic peptide prepared according to this sequence activated the T84 cell receptor GC with potency and efficacy similar to the activation elicited by synthetic rat guanylin.

Pet., 17, 36-37; Reply, 4;
EX1002 (Peterson), ¶¶65, 166-67.

# *Bausch Misinterpreted Li*

Bausch wrongly asserts Li Fig. 3 teaches Glu$^3$ reduced activity.
EX1063 (Peterson), ¶¶82-87; 1006 (Li), 49-51.

- Dr. Davies read but did not analyze Li:

> Is that something that you analyzed in coming to your opinions in this case?
>
> A. Well, I would have read it whether I analyzed it or not. If I don't talk about it, I -- I don't recall.

EX1060 (Davies), 139:19-142:5.

- Dr. Davies mistakenly thought the uroguanylin bar graph in Fig. 3 was extracted from rat intestines to compare its activity to other peptides because a POSA would not assay activity of the synthetic standards:

> You wouldn't be using them in an assay.

EX1060 (Davies), 144:8-20.

> Sitting here right now, you don't know whether the uroguanylin bar graph in Figure 3 is a synthetic standard that was analyzed or whether it is uroguanylin that was extracted from Li's rats; is that correct?
>
> * * *
>
> THE WITNESS: I think he says that it's rat uroguanylin that he has extracted.

EX1060 (Davies), 172:19-173:4.

> Q. Okay. So in Figure 3, you believe that the uroguanylin bar graph with the N equals 2 is what Li is referring to when it says it extracted rat uroguanylin from its tissues?
>
> * * *
>
> THE WITNESS: I'm not sure I can identify where the uroguanylin comes from.

EX1060 (Davies), 172:12-173:19.

Pet., 34-39; Reply, 16-19; EX1060, 139:19-142:5, 160:15-166:12, 168:2-171:22, 172:1-173:19.

# *Bausch Misinterpreted Li*

Dr. Davies eventually confirmed Li Fig. 3 uses rat guanylin and opossum uroguanylin synthetic standards merely to show whether preincubation makes a difference in activity levels. EX1063 (Peterson), ¶¶82-87; 1006, 49-51.

Q. So you agree that Li Figure 3, when it says: "Guanylin N equals 3," that that refers to synthetic guanylin, correct?

A. That's the implication, yes.

Q. You agree that when Li Figure 3 has a bar graph for uroguanylin N equals 2 that that refers to synthetic uroguanylin, correct?

A. That's the implication.

EX1060 (Davies), 184:4-185:1.

Q. Okay. So now I want to go back up. They are comparing -- they are looking to see whether incubation makes a difference in the activity of the peptides in Figure 3.

Do you agree?

***

THE WITNESS: That's the implication.

EX1060 (Davies), 182:14-21.

Q. And Li's conclusion with respect to Li Figure 3 is that none of the synthetic guanylin, synthetic uroguanylin, or commercially-purified STA in Figure 3 showed a difference when they were incubated as opposed to preincubation?

***

THE WITNESS: That's his conclusion.

EX1060 (Davies), 185:7-186:2.

Pet., 34-39; Reply, 16-19; *see also* POR, 22, 45-51.

# Bausch Misinterpreted Li

Li does not indicate the same amount of each peptide was used:

> 87. Li (Fig. 3) does not provide data permitting a comparison of activity between peptides. For example, Li does not indicate it used identical amounts of the different peptides. To the contrary, Li extracted the peptides (example, guanylin in fraction 21, uroguanylin in fractions 23/24) having varying concentrations from the duodenum of rat intestines (EX1006, 46) and then measured the activity of the fraction. In contrast, Li obtained synthetic rat guanylin and synthetic opossum uroguanylin from other labs, rather than from a fraction,

EX1063 (Peterson), ¶87.

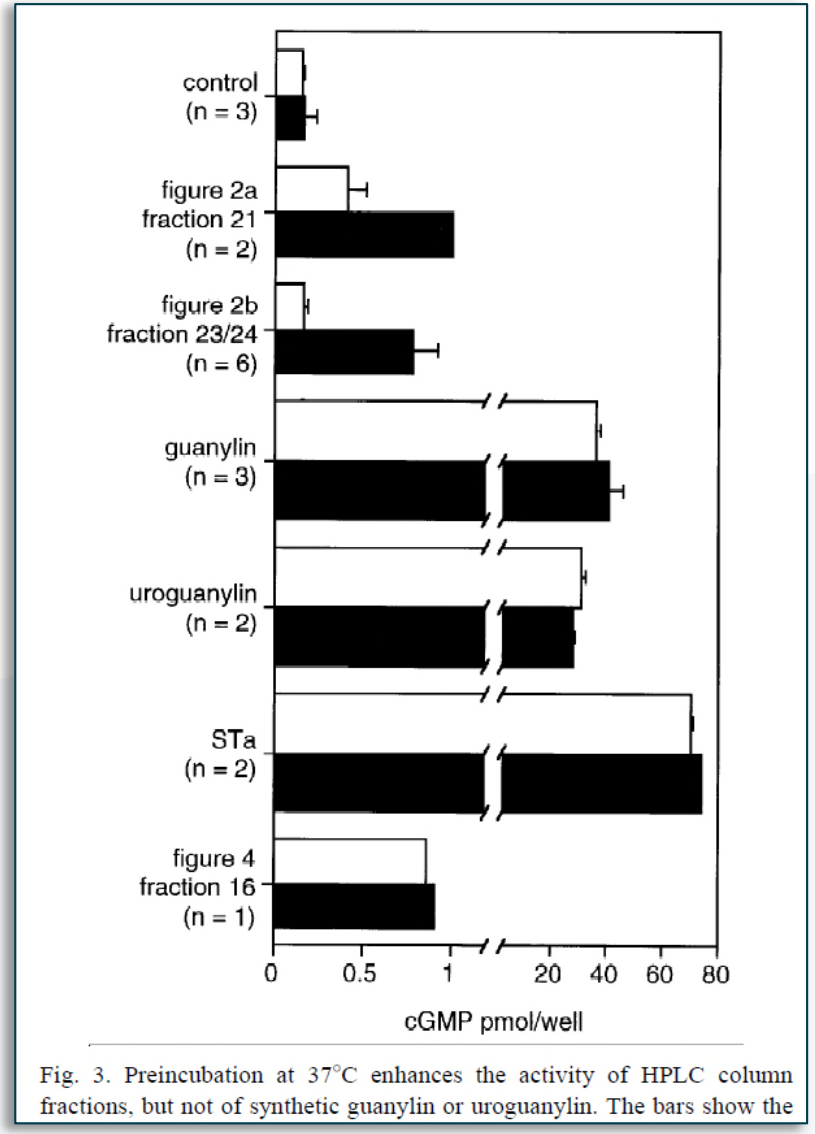



Fig. 3. Preincubation at 37°C enhances the activity of HPLC column fractions, but not of synthetic guanylin or uroguanylin. The bars show the

EX1006 (Li), 49 (Fig. 3).

This misunderstanding infects testimony of both Drs. Davies and Waldman. EX2024 (Davies), ¶¶75, 79, 161-65; EX2025 (Waldman), ¶57.

Pet., 34-39; Reply, 16-19; *see also* POR, 22, 45-51.

# *Asp At Positions 2 and 3 Not Required for Activity*

FIG. 7

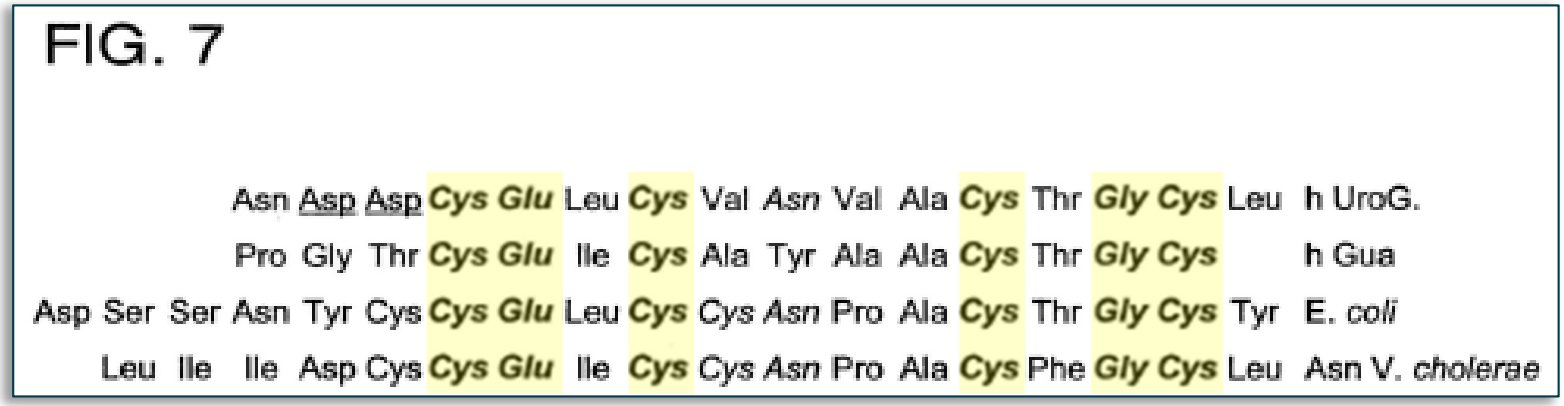



Figure 7 depicts the primary structure of human uroguanylin (*h UroG*) [identified as SEQ. ID. 2], human guanylin (*h Gua*) [identified as SEQ. ID. 3], and bacterial enterotoxins (*E.coli* [identified as SEQ. ID. 4] & *V.cholerae* [identified as SEQ. ID. 5]). Bold and italic letters represent the similar residues in these peptides. These residues are believed to be required for the functional activity of these peptides. *E. coli ST* has three additional intestinal pH. Two underlined (Asp-Asp) residues are believed to be important for regulating the functional activity of uroguanylin only at the acidic environment of the intestinal mucosa.

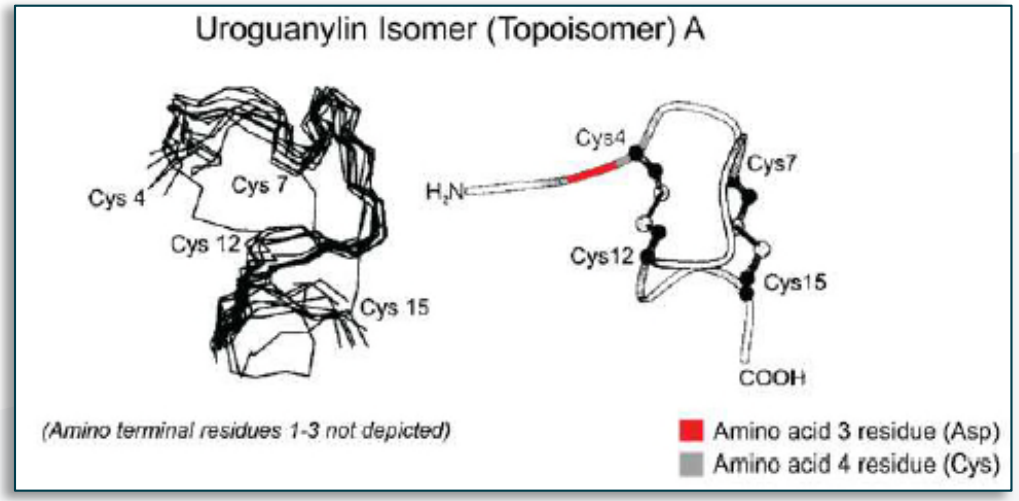
EX2021 (WIPO), 7:26-8:3 & Fig. 7.

Pet., 34-39; Reply, 16-19; EX1002 (Peterson), ¶¶177-79.

# *Glu³-Substitution Fine Tunes pH Response*

POSA expected Glu[3] substitution to result in protonated glutamate at higher pH for better activity in the less acidic environment further from stomach.
EX1002 (Peterson), ¶¶157-61; EX1012 (Nelson), 118, Table 5-1.

Bausch's contention that buried-residue pKa values showed unpredictability is erroneous; position 3 is not buried. EX1063 (Peterson), ¶¶102-03; EX2010 (Marx), 235, Fig. 4A-C.




EX2010 (Marx), Fig. 4. (select portions, labeled, annotated with red coloring)

The relative *difference* between pKas would benefit intestinal absorption.
EX1063, ¶¶98-100, 104; EX2026 (Peterson), 100:3-13.

Pet., 16-17, 36-37; Reply, 20-21; EX1002, ¶¶55-57, 157, 164-68.

# *Avoid Unneeded Aspartimide Pairings*

110. I explained in my first declaration that an additional motivation for the [Glu$^3$]-substitution was that it would eliminate two amino acid pairings (Asp$^2$Asp$^3$ and Asp$^3$Cys$^4$) that were known to be synthetically problematic because of their propensity to form aspartimide despite the use of conventional protecting groups. EX1002, ¶¶68-70, 175-78; EX1022, 63; EX1023, 107; EX1024, 197, 199, 201, Table 1. Lauer, for example, reported significant aspartimide formation when the carboxyl end of any one of Asn, Asp, Cys, Thr, Gly, Ser, or Thr is bound to the amino end of an Asp residue, even though these residues were protected. *See, e.g.*, EX1024, 197 Abstract (Val-Lys-Asp-X-Tyr-Ile, where X = Asp (OtBu), Cys(Acm), etc.).

EX1063 (Peterson), ¶110.

EX1002 (Peterson), ¶¶175-79; Pet., 21-22, 34-39; Reply, 11-12, 21-22.

# *Routine Synthesis and Characterization*

> Skilled artisans thus did not wait for proof of efficacy to make a new synthetic peptide but instead were quite willing to synthesize even very large numbers of analogs simply to learn more about the functionality of the peptide and its interactions with the receptor.

EX1002 (Peterson), ¶¶66-67.

EX1002, ¶¶120-25, 179; Pet., 21-22; Reply, 2.

# *POSA Would Not Be Dissuaded By Topoisomers*

- Oral uroguanylin already shown to naturally add fluid to lumen.
  EX1063 (Peterson), ¶27; EX1018 (Joo), G641-G642; EX2021 (WIPO), 2:28-3:1; EX1002 (Peterson), ¶¶99, 59-60, 85-86, 107; EX1005 (Currie), 1:34-44, 1:50-55, 2:6-24, 2:53-65, 6:11-22.
- No requirement to expose uroguanylin to pH 4.5 for 24 hours at 37°C.
- No more than 1% interconversion *in vivo*. EX1063, ¶¶25-27, 29, 31-32, 58; EX1064 (Epstein), ¶¶25-31, 39.
- Topoisomerism was easily managed. EX1063, ¶¶23-30, 34-40, 52; EX1064, ¶32; EX1002, ¶¶97-98; EX2010 (Marx), 236-39; EX2020 (Klodt), 227-28.
- Purification was straightforward. EX1063, ¶¶34, 40.
- Marx taught the ionizable side chains at positions 2 and 3 "may be involved in the control of stabilization of the two isomers." EX2010, 236, 238.
- No reference says not to make uroguanylins because of topoisomerism.

Pet., 34-39; Reply, 7-11.

# *Oral Uroguanylin Known to be GI Stable & Active*

per gastrointestinal tract (25). In vivo studies have found that oral guanylin is ineffective as an intestinal secretagogue unless the peptide is coadministered with the protease inhibitor chymostatin (22). In contrast, oral administration of uroguanylin markedly stimulates intestinal fluid secretion. Third, uroguanylin ap-

Finally, recent in vivo studies in mice have demonstrated that orally administered uroguanylin resists luminal proteolysis and stimulates net intestinal fluid accumulation (22, 25). On the basis of its action to

EX1018 (Joo), G641-G642.

304. Uroguanylin is an acid-stable and proteolysis-resistant peptide, which will remain in tact to act on the intestinal lumen directly rather than being absorbed systemically. Uroguanylin and guanylin are produced throughout the intestinal mucosa and in the myocardium.

EX2021 (WIPO), 2:28-3:1.

the agonistic *Escherichia coli* heat-stable enterotoxin. Compared with guanylin isomers, the conformational interconversion of uroguanylin isomers is retarded significantly. As judged from chromatography and NMR spectroscopy, both uroguanylin isoforms are stable at low temperatures, but are subject to a slow pH-dependent mutual isomerization at 37°C with an equilibrium isomer ratio of approximately 1:1. The conformational exchange is most likely under the sterical control of the carboxy-terminal leucine. These results imply that GC-C is activated by ligands

EX2010 (Marx), 229.

bridged peptides and proteins (38). A fundamental difference between uroguanylin and guanylin is the velocity of interconversion between the respective isomers. Both guanylin isomers are present simultaneously in an equimolar ratio under any conditions. In contrast, the conversion between uroguanylin isomers follows significantly slower kinetics and is hindered substantially by the carboxy terminal leucine. Therefore, the two isomers of human uroguanylin may exist physiologically in a nonequimolar ratio. From

EX2010 (Marx), 239.

Pet., 48; Reply, 4, 7-8, 19; EX1063 (Peterson), ¶27; EX1002 (Peterson), ¶¶95-99, 59-60, 85-86, 107.

# *pH 4.5 at 37°C for 24 Hours Not Required*

Bausch overstates the likelihood of interconversion.

EX1063 (Peterson), ¶¶24-27, 29, 31-32, 58.

25. But the 25% interconversion Drs. Davies and Waldman rely upon was observed after storage in solution for 24 hours at a ***pH of 4.5***. EX2010, 236 (left column). Drs. Davies and Waldman concede that this degree of acidic pH is ***not*** generally observed in the small or large intestines (pH 5.5-8.0), most commonly being relegated to the stomach during a fasting state (pH 1.4-2.1) or perhaps in the stomach with food (pH 4.3-5.4). EX2024, ¶¶43-44; *see also* EX2025, ¶¶20-22. As Dr. Epstein explains, a clinician would have understood that uroguanylin would clear the stomach within about 30 minutes in a fasting state and within about 60 minutes when taken with food. EX1064, ¶¶25-31. Any topoisomerism interconversion experiment performed at a pH of 4.5 or lower for longer than one hour is ***not*** representative of biological conditions for oral administration of uroguanylin. The topoisomeric interconversion rate Drs. Davies and Waldman cite

EX1063 (Peterson), ¶25.

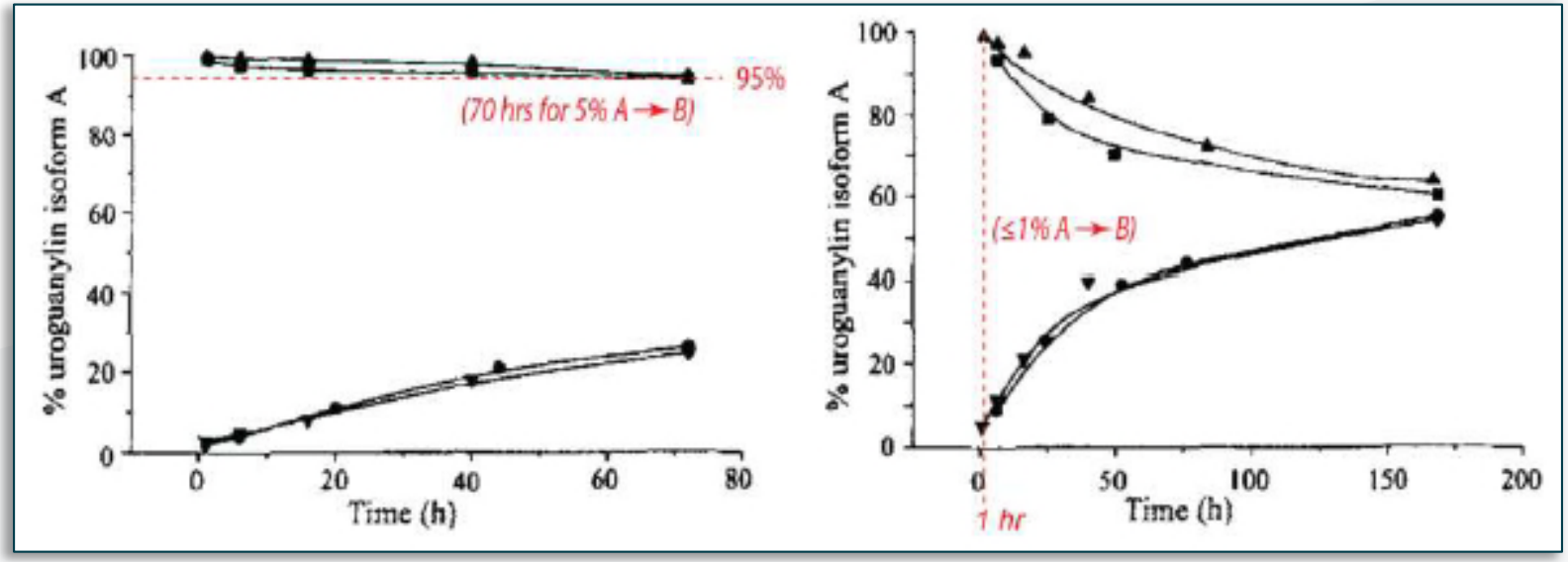
Reply, 11-14; EX1064 (Epstein), ¶¶37-41; *see* POR, 39-40.

# *No More Than 1%* In Vivo

POSA would anticipate at most 1% conversion following oral administration.
EX1063 (Peterson), ¶¶24-27, 29, 31-32, 58.

> 26. Indeed, Figure 6C of Marx 1998, the same data (below, right) that indicate a 25% interconversion after 24 hours at pH 4.5, indicates at most 1% interconversion of topoisomer A to topoisomer B after one hour. I added a vertical

EX1063 (Peterson), ¶26.




EX2010 (Marx), Figure 6C (annotated by Dr. Peterson, EX1063 ¶26).

Pet., 34-39; Reply, 11-14; *see* POR, 39-40.

# *Topoisomerism Easily Managed*

Topoisomerism was addressable via formulation. EX1063 (Peterson), ¶¶27-28, 34-40, 52.

> 28. A person of ordinary skill in the art also would not have been dissuaded from human uroguanylin based on potential *in vivo* topoisomeric conversion in the stomach because this would have been easily avoided by administering it in a dosage form designed to time its release specifically for the intestines rather than the stomach. EX1002, ¶¶97-98, 112, 189, 193; EX1007, 39-40, 47; EX1046, 28-29; EX1047, 3708.

EX1063 (Peterson), ¶28.

> stored at reduced temperature. As discussed above, Marx 1998 and Klodt 1997 teach that uroguanylin was relatively stable with regard to topoisomeric conversion. While both references consider topoisomerism, neither reference teaches that topoisomerism presented any barrier to synthesis or formulation.

EX1063 (Peterson), ¶40.

Pet., 34-39; Reply, 11-14; *see* POR, 39-40.

# *Topoisomerism Easily Managed*

Topoisomeric purification was straightforward. EX1063 (Peterson), ¶¶27-28, 34-40, 52.

purification would be by HPLC. You would expect the topoisomers to run at different retention time by HPLC, so you would be purifying one out of -- one of them out of the rest.

EX1060 (Davies), 114:19-115:10.

15. After formation of the second disulfide, two stereoisomers were obtained and separated by reversed-phase HPLC at a temperature of 15 °C. Uroguanylin-24 was prepared cor

The isomers of uroguanylin-16 and the amino-terminally extended uroguanylin-24 were synthesized and separated using standard chromatography techniques as described under "Experimental Procedures". The GC-C-activating

EX2010 (Marx), 230.

*3.2. Stability of compounds I and II of human uroguanylin*

Compounds I and II have distinctly different retention times on RP HPLC at 40°C, and thus could be separately isolated at purities greater than 99%. The purities of the iso

EX2011 (Chino), 29.

Pet., 34-39; Reply, 11-14; *see* POR, 39-40.

# *Acidic Residues at Positions 2-3 Stabilize Isomers*

POSA knew the ionizable terminal carboxylic acid side chains at positions 2 and 3 were points of interest for stabilizing uroguanylin. EX2010 (Marx), 236, 238.

The slow development of the equilibrium between uroguanylin isomers at alkaline pH indicates that the ion ization state of the isomeric molecules strongly influences the kinetics of transition between the isomers of uroguanylin 16 and uroguanylin 24. Thus, the terminal carboxyl, ionizable side-chains of Asp2, Asp3 and Glu5, or those groups able to form intrachain hydrogen bonds, may be involved in the control of stabilization of the two isomers. After 3 days at alkaline pH, both isoforms decom-

EX2010 (Marx), 236.

Pet., 34-39; Reply, 11-14; *see* POR, 39-40.

# *No Reference Says Not To Make Uroguanylins*

Bausch's references instead called for "systematic substitution of amino acids contained in uroguanylin and guanylin." EX2010 (Marx), 236.

minimum concentration of $10^{-6}$ M. To understand the individual contribution of single amino acids for receptor binding and activation, further experiments using systematic substitution of amino acids contained in uroguanylin and guanylin are necessary.

EX2010 (Marx), 236.

33. Contrary to the testimony of Dr. Davies that topoisomeric interconversion would have dissuaded a person of ordinary skill in the art from human uroguanylin, none of the literature he cites says that a person of ordinary skill in the art should avoid uroguanylin (as opposed to providing a good reason to isolate and evaluate any topoisomers). Indeed, Klodt 1997 and Marx 1998 show

EX1063 (Peterson), ¶33.

Pet., 34-39; Reply, 11-14; *see* POR, 39-40.

# *Bausch Errs in Arguing an Alternate Lead Compound*

Bausch argues a POSA necessarily would have modified enterotoxin. POR, i-ii, 2-3, 26, 41.

The Board already cautioned Bausch against this.

> further development at trial. We also note that our reviewing court has cautioned that it would be overly restrictive to view the "lead compound test" to require "that the prior art must point to only a single lead compound for further development." *Altana Pharma AG. v. Teva Pharms. USA, Inc.*, 566 F.3d 999, 1008 (Fed. Cir. 2009). This suggests that the parties should

Paper 16 (Institution Decision), 20-23.

Petitioner need only identify "some reason" to modify a known compound.

*Bristol-Myers Squibb v. Teva Pharms.*, 752 F.3d 967, 973 (Fed. Cir. 2014).

*Altana Pharma AG. v. Teva Pharms.*, 566 F.3d 999, 1007-08 (Fed. Cir. 2009) (impermissible to require prior art "point to only a single lead compound").

*In re Fulton*, 391 F.3d 1195, 1200 (Fed. Cir. 2004) (no requirement "that the combination is the most desirable combination available").

Pet., 31; Reply, 2-4; EX1002 (Peterson), ¶¶29, 122-23, 126-79.

WILSON SONSINI

# *Bausch's "Unexpected Results" Are Unsupported*

# *No Significant Improvement in Activity*

Bausch contends plecanatide is unexpectedly more active in producing cGMP than uroguanylin.

POR, 60-64.

Yet the difference is within the level of experimental error. No real, material difference was shown.

EX1063, ¶¶118-35.

Dr. Shailubhai's testimony confirmed the cGMP values of Table 4 can't be read to conclude a statistical difference existed between tested peptides.

Q. Okay. And do you recall earlier we talked about the P value stated under -- underneath Table 4?

A. Yes, we did.

Q. What was the comparison being made for that P value?

A. Please clarify your question.

Q. Did you calculate the P value stated there?

A. One of my team members did that.

Q. In calculating that P value, what were they comparing SP304 against?

A. I don't recall that.

EX1061 (Shailubhai), 33:19-34:15.

Pet., 31-32, 50, 54; Reply, 24-26; EX1002 (Peterson), ¶253; *see also* POR, 40-45.

# *No Material Improvement in Activity*

Any nominal difference reflects experimental variability, not unexpected results.
EX2023, ¶¶9, 16; EX1063, ¶¶126-29; EX2027, 20.

**Table 1: Effects of SP-304, Uroguanylin, and Other Test Peptides Stimulation Bioassay**

| Test Material | Concentration | | cGMP Levels (pmol/well) * |
|---|---|---|---|
| | Molar | ng/mL | |
| SP-301 (uroguanylin) | 0 | 0 | 0 |
| | $10^{-9}$ M | 1.668 | 0 |
| | $10^{-8}$ M | 16.668 | 12 |
| | $10^{-7}$ M | 166.8 | 82 |
| | $10^{-6}$ M | 1668 | 205 |
| | $10^{-5}$ M | 16680 | 254 |
| SP-302 | 0 | 0 | 0 |
| | $10^{-9}$ M | 1.696 | 0 |
| | $10^{-8}$ M | 16.96 | 8 |
| | $10^{-7}$ M | 169.6 | 62 |
| | $10^{-6}$ M | 1696 | 185 |
| | $10^{-5}$ M | 16960 | 248 |
| SP-303 | 0 | 0 | 0 |
| | $10^{-9}$ M | 1.682 | 0 |
| | $10^{-8}$ M | 16.82 | 12 |
| | $10^{-7}$ M | 168.2 | 82 |
| | $10^{-6}$ M | 1682 | 195 |
| | $10^{-5}$ M | 16820 | 254 |
| SP-304 | 0 | 0 | 0 |
| | $10^{-9}$ M | 1.682 | 0 |
| | $10^{-8}$ M | 16.82 | 17 |
| | $10^{-7}$ M | 168.2 | 149 |
| | $10^{-6}$ M | 1682 | 320 |
| | $10^{-5}$ M | 16820 | 315 |

SP-PH-001 Fig. 1 (EX2027): cGMP Stimulation Assay

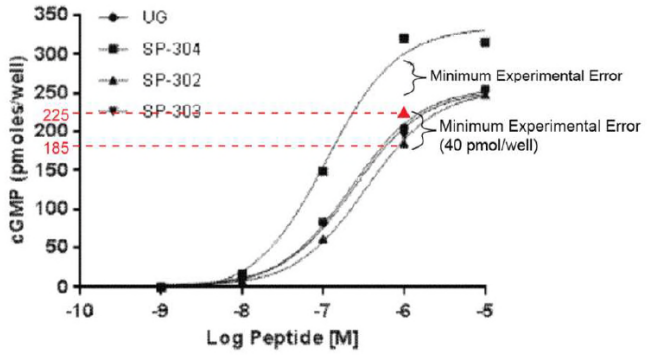



TABLE 4

Peptide agonists evaluated for biological activity in the T84 cell bioassay.

| SEQ ID NO.* | Compound Code | cGMP Level** (pmol/well) |
|---|---|---|
| 1 | SP301 | 205 |
| 6 | SP302 | 225 |
| 7 | SP303 | 195 |
| 20 | SP304 | 315 |
| 14 | SP306 | 0 |
| 4 | SP310 | 0 |
| 21 | SP316 | 275 |

EX1063 (Peterson), ¶¶126-29 (annotating and comparing Table 1, EX2027, 20 (left) with Table 4, EX1001 (right)).

Pet., 31-32, 50, 54; Reply, 24-26; *see also* POR, 40-45.

# *No Unexpected Improvement in Activity*

Dr. Davies glosses over the error-riddled data, arguing observed differences were not real differences; merely "a different way to interpret the data."

EX1060, 57:8-10, 54:2-4, 68:8-18; EX1063, ¶¶124-29.

> Q. So in some cases depending on how you process the data, you might get one value, and after processing the data, you might get a value that is 40 percent different. Is that your opinion?
>
> * * *
>
> THE WITNESS: The 40 percent, it doesn't mean anything. It's just a different way of processing the numbers.

EX1060 (Davies), 74:8-17.

Pet., 31-32, 50, 54; Reply, 24-26; *see also* POR, 40-45.

# *No Unexpected Improvement in Activity*

Bausch's Table 2 reports ***human uroguanylin*** was ~20% more active.

**Table 2: Raw Data (pH sensitivity assays)**

| Test Peptide | Concentration | cGMP Levels (pmol/well) * | | | |
|---|---|---|---|---|---|
| | | pH 5.5 | pH 6.0 | pH 6.5 | pH. 7.0 |
| Uroguanylin | 0.1 µM (1.67 µg/mL) | 24.6 | 21.36 | 24.6 | 21.72 |
| SP-304 | 0.1 µM (1.68 µg/mL) | 24.9 | 43.8 | 36.68 | 18.18 |
| SP-302 | 0.1 µM (1.67 µg/mL) | 14.80 | 18.6 | 22.98 | 17.94 |
| SP-303 | 0.1 µM (1.68 µg/mL) | 17.46 | 22.26 | 28.14 | 13.62 |

* cGMP levels in T84 cells after a 30-minute incubation at 37°C.

EX2023, ¶24 (Table 2).

Bausch's Table 3 reports ***plecanatide*** was the least active of the tested peptides.

**Table 3: Raw Data (thermostability assays)**

| Test Peptide | Concentration | cGMP Levels (pmol/well) * | | | | | |
|---|---|---|---|---|---|---|---|
| | | 0 | 15 min | 30 min | 45 min | 60 min | 90 min |
| Uroguanylin | 0.1 µM (1.67 µg/mL) | 21.72 | 20.92 | 21.68 | 20.88 | 18.76 | 18.96 |
| SP-304 | 0.1 µM (16.8 µg/mL) | 20.44 | 21.12 | 20.64 | 21.4 | 20.8 | 21.88 |
| SP-302 | 0.1 µM (1.67 µg/mL) | 23.16 | 20.44 | 21.12 | 20.32 | 20.36 | 20.34 |
| SP-303 | 0.1 µM (1.68 µg/mL) | 21.64 | 20.0 | 20.56 | 20.68 | 20.36 | 19.8 |

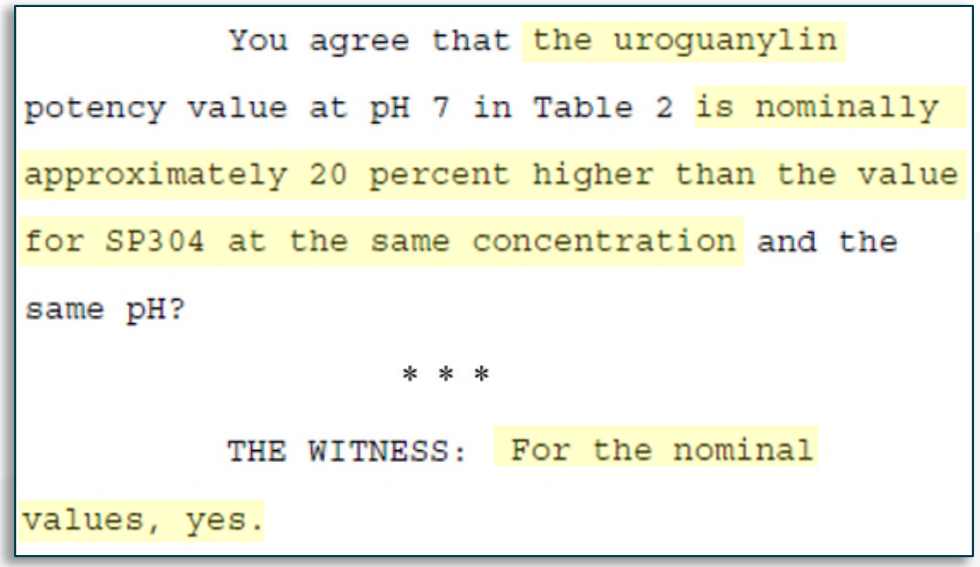
* cGMP levels in T84 cells after heat treatment at 95°C for the indicated times. Samples at different times of treatment were withdrawn and assayed for their ability to stimulate cGMP synthesis.

EX2023, ¶29 (Table 3).

EX1063 (Peterson), ¶¶134-41; EX1060 (Davies), 79:9-82:19; Pet., 31-32, 50, 54; Reply, 24-26; *see also* POR, 40-45.

# *No Unexpected Improvement in Activity*

Variation in nominal values for the same peptides at the same concentrations was as high as 29-59%; yet even a two-fold variation is common and does not show unexpected results. EX1063 (Peterson), ¶¶135-36; EX2028, 14, Table 3.



You agree that the uroguanylin potency value at pH 7 in Table 2 is nominally approximately 20 percent higher than the value for SP304 at the same concentration and the same pH?

* * *

THE WITNESS: For the nominal values, yes.

EX1060 (Davies), 79:9-82:19.

Pet., 31-32, 50, 54; Reply, 24-26; *see also* POR, 40-45.

# No Unexpected Improvement in Activity

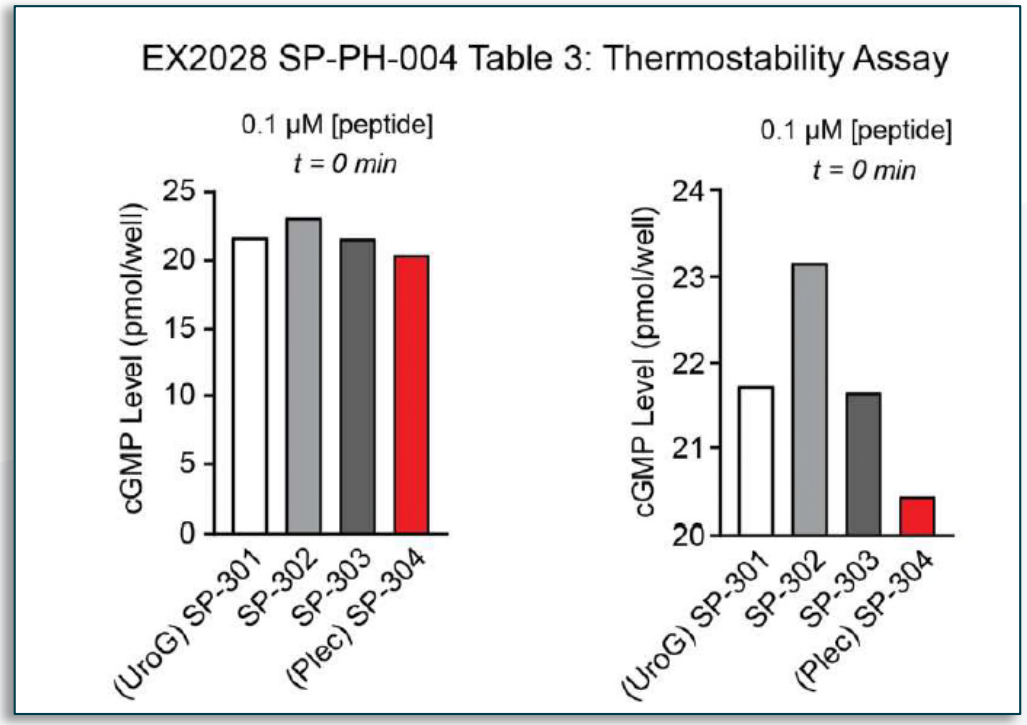
Bausch's data shows experimental error is greater than the asserted 56% improvement.

| 0.1 μM cGMP Levels (pmol/well) | | | |
|---|---|---|---|
| | Table 1 | Table 2 | Table 3 (T=0) |
| SP-301 | 82 | 21.72 | 21.72 |
| SP-302 | 62 | 17.94 | 23.16 |
| SP-303 | 82 | 13.62 | 21.64 |
| SP-304 | 149 | 18.18 | 20.44 |

EX1063 (Peterson), ¶131.

| 0.1 μM cGMP Levels (pmol/well) | | | |
|---|---|---|---|
| | Table 2 | Table 3 (T=0) | % Difference |
| SP-301 | 21.72 | 21.72 | 0% |
| SP-302 | 17.94 | 23.16 | 29% |
| SP-303 | 13.62 | 21.64 | 59% |
| SP-304 | 18.18 | 20.44 | 12% |

EX1063 (Peterson), ¶135.




EX1063 (Peterson), ¶136 (Data of EX2023 Table 3 at T=0; full scale (left) and zoomed-in on nominal differences (right)).

Pet., 31-32, 50, 54; Reply, 24-26; *see also* POR, 40-45.

# No Difference in Kind

Table 1 was amended, eliminating the purported 10-fold potency difference.

EX1063 (Peterson), ¶124; EX2027, 20.

Q. You agree that Table 1 in Paragraph 219 of your declaration does not disclose that there is a tenfold difference between the EC50 values for SP301 and SP304, correct?

* * *

THE WITNESS: Well, there isn't a tenfold difference.

Q. Is there a onefold difference?

A. Well, the difference is between 2.3 times ten to the minus 7 and 1.1 times ten to the minus 7 done over twice.

EX1060 (Davies), 66:1-70:5.

**Results and Conclusions**: Plecanatide, UG, and SP-338 potently stimulated cGMP production in T84 cells. Plecanatide was the most potent with an $EC_{50}$ value of 480 nM, and UG was nearly equipotent with an $EC_{50}$ of 560 nM. SP-338 was less potent with an $EC_{50}$ value of 2000 nM.

In the receptor binding assay, all 3 peptides displaced binding of the $^{125}$I-labelled STY72F peptide. The $IC_{50}$ for plecanatide was 1.9 nM compared with 2.8 nM for UG. SP-338 was only slightly less potent, with an $IC_{50}$ of 5 nM.

EX1069, 30.

**Table 1. Effects of SP-304, Uroguanylin, and Other Test Peptides in the T84 cGMP Stimulation Bioassay**

| Test Material | Concentration | | cGMP Levels (pmol/well) * | $EC_{50}$ ** | |
|---|---|---|---|---|---|
| | Molar | ng/mL | | Molar | ng/mL |
| SP-301 (uroguanylin) | 0 | 0 | 0 | ~~$10^{-6}$ M~~ **$2.3\times10^{-7}$M** | ~~1668~~ **383.6** |
| | $10^{-9}$ M | 1.668 | 0 | | |
| | $10^{-8}$ M | 16.668 | 12 | | |
| | $10^{-7}$ M | 166.8 | 82 | | |
| | $10^{-6}$ M | 1668 | 205 | | |
| | $10^{-5}$ M | 16680 | 254 | | |
| SP-302 | 0 | 0 | 0 | ~~$10^{-6}$ M~~ **$3.5\times10^{-7}$M** | ~~1696~~ **593.6** |
| | $10^{-9}$ M | 1.696 | 0 | | |
| | $10^{-8}$ M | 16.96 | 8 | | |
| | $10^{-7}$ M | 169.6 | 62 | | |
| | $10^{-6}$ M | 1696 | 185 | | |
| | $10^{-5}$ M | 16960 | 248 | | |
| SP-303 | 0 | 0 | 0 | ~~$10^{-6}$ M~~ **$2.4\times10^{-7}$M** | ~~1682~~ **403.7** |
| | $10^{-9}$ M | 1.682 | 0 | | |
| | $10^{-8}$ M | 16.82 | 12 | | |
| | $10^{-7}$ M | 168.2 | 82 | | |
| | $10^{-6}$ M | 1682 | 195 | | |
| | $10^{-5}$ M | 16820 | 254 | | |
| SP-304 | 0 | 0 | 0 | ~~$10^{-7}$ M~~ **$1.1\times10^{-7}$M** | ~~168.2~~ **185.0** |
| | $10^{-9}$ M | 1.682 | 0 | | |
| | $10^{-8}$ M | 16.82 | 17 | | |
| | $10^{-7}$ M | 168.2 | 149 | | |
| | $10^{-6}$ M | 1682 | 320 | | |
| | $10^{-5}$ M | 16820 | 315 | | |

* cGMP levels in T84 cells after a 30-minute incubation.
** $EC_{50}$: median effective concentration (required to induce a 50% effect)

EX2027, 20.

Pet., 31-32, 50, 54; Reply, 24-26; *see also* POR, 40-45.

# No Material Improvement in Heat Stability

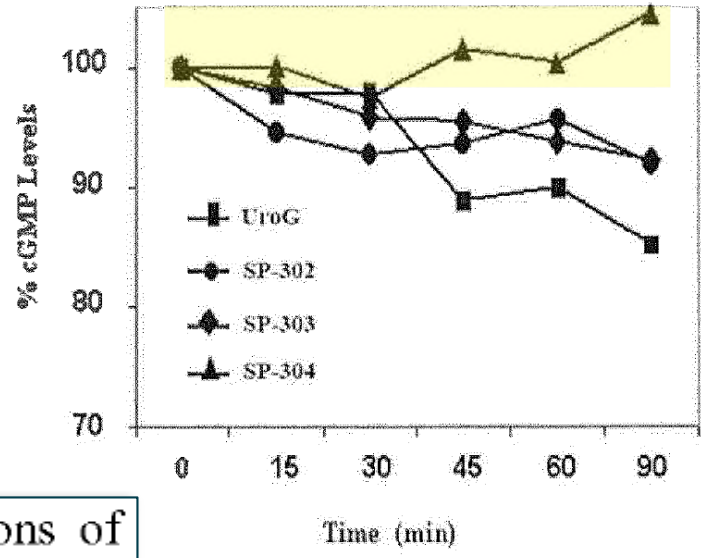
No material difference in kind after boiling peptides (95°C) for 90 minutes).

Reply, 26-27; EX1063 (Peterson), ¶140.

Glu[3]'s cGMP values drifted ~7% higher after heat treatment.

EX1063 (Peterson), ¶¶137-140.

**Figure 2: Thermostability Results with Uroguanylin, SP-304 and other Test Peptides on cGMP Production Following Heat Treatment at 95°C in T84 Cells**




EX1067, 129.

To examine heat stability, 10 micromolar solutions of peptide analogs were heated at 95° C. for up to 90 minutes. At specific times during the treatment, samples were tested for their biological activity in the T84 cell-based assay. Biological activity of SP301, SP302, SP303 and SP304 did not change significantly after 60 minutes of heating. After 90 minutes, the activities of SP301, SP302 and SP303 were reduced to about 80% of their original values, whereas the biological activity of SP304 remained unaltered. This indi-

EX1001, 16:19-28; EX1060 (Davies), 47:3-22.

Reply, 26-27.

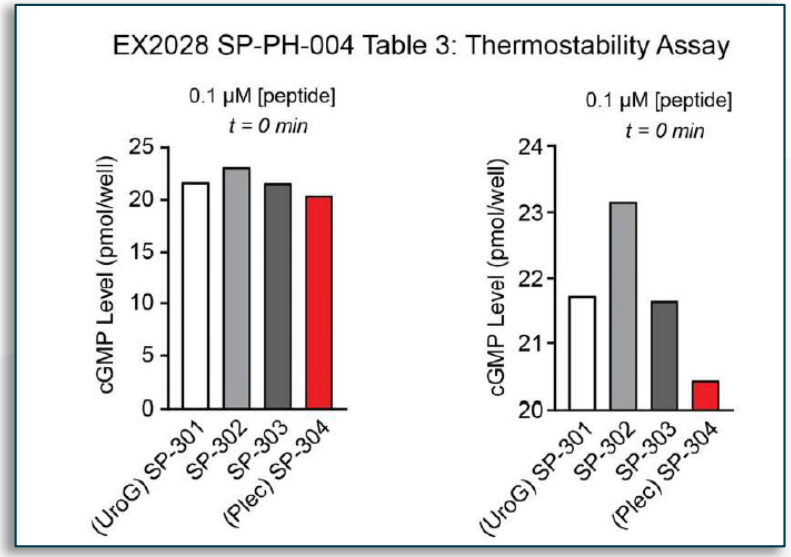
# *No Significant Improvement in Heat Stability*

Bausch's presentation of data as % of starting activity gives false impression of significant or material differences.

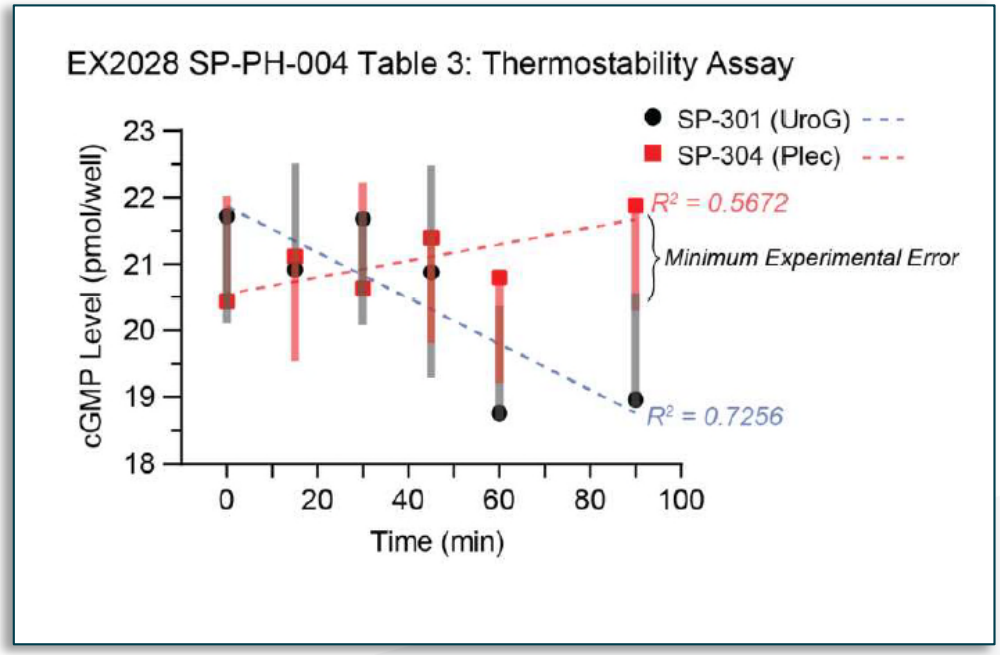
Reply, 26-27; EX1063 (Peterson), ¶¶136-43.

Uroguanylin and Glu[2] have initial cGMP values 6-13% greater than Glu[3].

Reply, 26-27; EX1063 (Peterson), ¶¶136-43 & n.8.




EX1063 (Peterson), ¶136.




EX1063 (Peterson), ¶141.

**Table 3: Raw Data (thermostability assays)**

| Test Peptide | Concentration | cGMP Levels (pmol/well) * | | | | | |
|---|---|---|---|---|---|---|---|
| | | 0 | 15 min | 30 min | 45 min | 60 min | 90 min |
| Uroguanylin | 0.1 µM (1.67 µg/mL) | 21.72 | 20.92 | 21.68 | 20.88 | 18.76 | 18.96 |
| SP-304 | 0.1 µM (16.8 µg/mL) | 20.44 | 21.12 | 20.64 | 21.4 | 20.8 | 21.88 |
| SP-302 | 0.1 µM (1.67 µg/mL) | 23.16 | 20.44 | 21.12 | 20.32 | 20.36 | 20.34 |
| SP-303 | 0.1 µM (1.68 µg/mL) | 21.64 | 20.0 | 20.56 | 20.68 | 20.36 | 19.8 |

* cGMP levels in T84 cells after heat treatment at 95°C for the indicated times. Samples at different times of treatment were withdrawn and assayed for their ability to stimulate cGMP synthesis.

EX2028, 14, Table 3.

Pet., 31-32, 50, 54; Reply, 26-27; EX1063, ¶¶136-43 & n.8; POR, 64-65.

# *No Material Improvement in Topoisomeric Conversion*

Bausch has not shown relevance of topoisomeric interconversion at pH 3 at 37°C for 16 hours:

> EX2024, ¶¶210-15 (discussing EX2011, 27, 30; EX2020, 223, 229, 236). As discussed above, however, Dr. Davies does not establish that incubation for 16 hours at 37°C at pH 3.0 is representative of any conditions expected in the body, or during manufacturing, purification, or storage. The alleged improvement simply has no relevance to the factors to which Dr. Davies attributes them.

EX1063 (Peterson), ¶177.

> stomach with food (pH 4.3-5.4). EX2024, ¶¶43-44; *see also* EX2025, ¶¶20-22. As Dr. Epstein explains, a clinician would have understood that uroguanylin would clear the stomach within about 30 minutes in a fasting state and within about 60 minutes when taken with food. EX1064, ¶¶25-31. Any topoisomerism interconversion experiment performed at a pH of 4.5 or lower for longer than one hour is *not* representative of biological conditions for oral administration of uroguanylin. The topoisomeric interconversion rate Drs. Davies and Waldman cite

EX1063 (Peterson), ¶25.

EX1067, 130; Pet., 31-32, 50, 54; Reply, 27-28; EX1063, ¶¶24-29, 31-32, 162, 167, 177.

# *No Unexpected Reduction in Topoisomers*

- Acidic residues at positions 2 and 3 contributed to stabilization.

> 178. Moreover, it was not unexpected that the [Glu$^3$]-substitution would reduce topoisomeric conversion. *See* Section IV.A above. Marx 1998 merely teaches that *extending* the N-terminus was not likely to decrease interconversion, and specifically suggests that the acidic residues 2 and 3 may contribute to the stabilization. EX2010, 236 (right column: "the ionization state of the isomeric

EX1063 (Peterson), ¶178.

- Sterical bulk reduces topoisomeric conversion.

> of NMR resonances (data not shown). Chino *et al.* (21) suspected that the carboxy-terminal leucine residue present in uroguanylin-(97–112) (**14**) may stabilize the isomers because the absence of this leucine residue resulted in a peptide showing a HPLC characteristic similar to guanylin. On the other hand, uroguanylin **19** with a carboxy-terminal cysteine was homogeneous during reverse-phase HPLC. Summarizing the present data, it is possible that a C-terminally attached elongation is crucial for the stability of topoisomers, but other residues, in particular between the inner cysteine residues, also influence this phenomenon by their sterical bulk.

EX2020 (Klodt), 228.

- Substituting Glu$^3$ thus provided no unexpected difference in kind.

Pet., 31-32, 50, 54; Reply, 27-28; EX1002 (Peterson), ¶253; EX1063, ¶178.

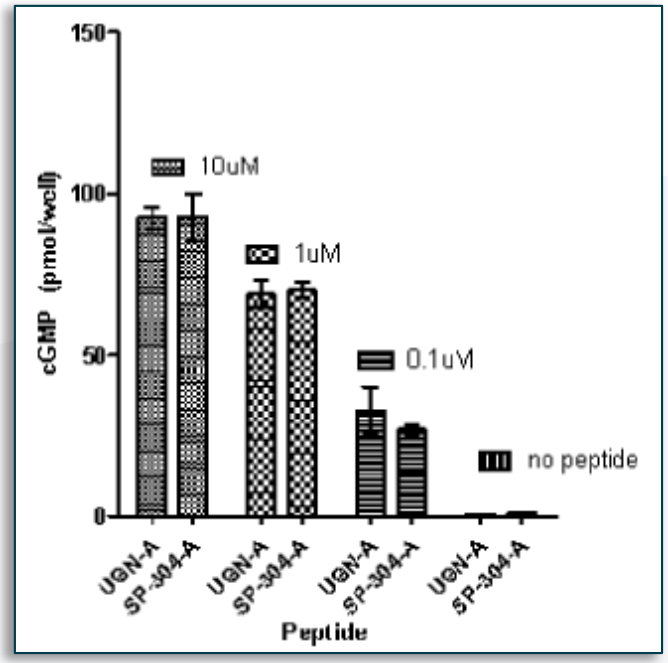
# Bausch's Deficiencies: cGMP Studies

Indicia of reliability, including error bars and evaluation of statistical significance between peptides, absent from Bausch reports .

EX1063 (Peterson), ¶¶166-67; EX1067, 93-94, 120-22, 140.

The results of the T84 cell-based assay are shown below in Figure 1 and Table 1. The results demonstrate that the purified A isomers of SP-304 (SP-304-A) and human uroguanylin (UGN-A) are indistinguishable in their biological activity. As shown in Table 1, there were no statistically significant differences in biological activity for these two peptides (p-values for all concentrations were non-significant (ns) using a two-tailed paired t-test). These results support the conclusion that SP-304 does not exhibit improved biological activity compared with human uroguanylin.

EX1067, 121 (Annex A).




EX1067, 121, Figure 1.

| Concentration | p-value | Significance |
|---|---|---|
| 10 µM | 0.9889 | ns |
| 1 µM | 0.8190 | ns |
| 0.1 µM | 0.5402 | ns |

EX1067, 121, Table 1.

Pet., 31-32, 50, 54; Reply, 24-26; *see also* POR, 40-45.

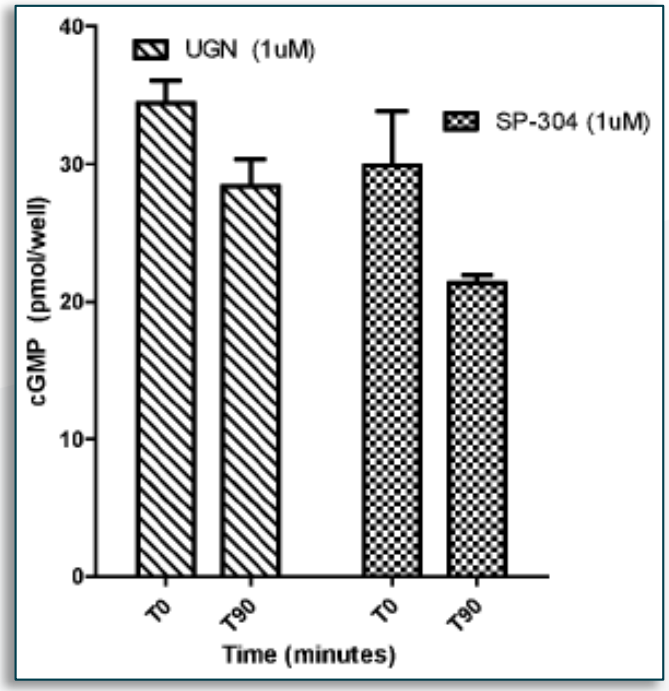
# *Bausch's Deficiencies: Heat Stability*

Indicia of reliability, including error bars and evaluation of statistical significance between peptides, absent from Bausch reports.

EX1063 (Peterson), ¶¶166-67; EX1067, 93-94, 120-22, 140.

The results of the heat stability study are shown below in Figure 2 and Table 2. The results demonstrate that the purified A isomers of SP-304 and human uroguanylin (UGN) exhibit comparable reductions in biological activity after heating for 90 minutes at 95°C (p-values were non-significant). In fact, uroguanylin showed less degradation (83.4% remaining at T90 compared to T0) than SP-304 did (73.7% remaining at T90 compared to T0). These results support the conclusion that SP-304 does not exhibit improved heat stability compared with human uroguanylin.

EX1067, 122 (Annex A).




EX1067, 122, Figure 2.

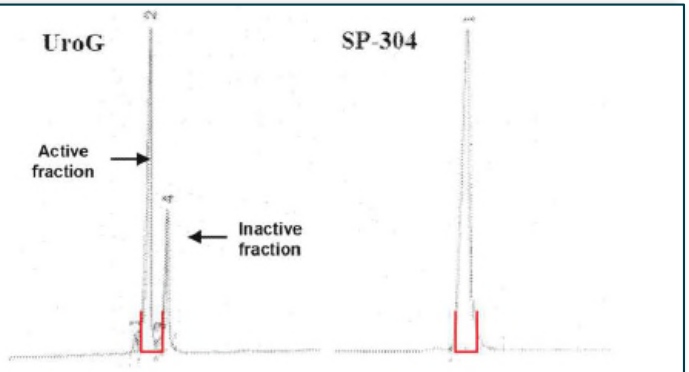
| Time point | p-value | Significance |
|---|---|---|
| T0 | 0.2522 | ns |
| T90 | 0.1008 | ns |

EX1067, 122, Table 2.

Pet., 31-32, 50, 54; Reply, 24-26; *see also* POR, 40-45.

# *Bausch's Deficiencies: Topoisomers*

Bausch has not shown the same HPLC protocol was capable of resolving both peaks for both peptides.




147. The peak Dr. Shailubhai obtained for SP-304 is relatively broad with some degree of tailing, raising the question of whether the HPLC protocol employed was simply lacking in optimization to resolve the topoisomers of this peptide from one another. If SP-304 was homogeneous, the peak could have been taller instead of wider than the uroguanylin peak in the chromatogram. I have

EX1063 (Peterson), ¶¶146-47
(annotating EX2028, 10, Fig. 1).

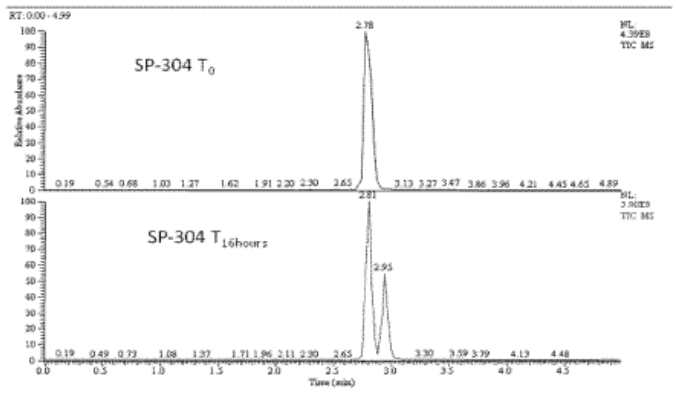



Figure 1

It can be seen from Figure 1 that, after incubating the purified active (A) isomer of the SP-304 peptide for 16 hours at pH 3 and at a temperature of 37 °C (as in Patentee's experiments), a significant and similar portion of the peptide had converted to the inactive (B) isomer.

EX1067, 91.

EX1063, ¶¶146-47; EX1067, 90-91 ;Pet., 31-32, 50, 54; Reply, 27-28.

# Bausch's Deficiencies: Claims Not Limited to Purified Topoisomer A

Claim 1 recites no topoisomer limitation:

> **1**. A peptide consisting of the amino acid sequence of SEQ ID NO:20.

> Q. Does Claim 1, as you understand it, require a method of treatment of inflamed precancerous or cancerous tissue or polyps in a mammalian subject?
>
> A. I think Claim 1 is for a peptide of the given sequence, and that's all.

EX1060 (Davies), 20:3-8;
*see also id.*, 111:17-112:13, 108:22-110:15.

"[N]o principle of law…would authorize us to read into a claim an element which is not present."

*McCarty v. Lehigh Valley R.R. Co.*,
160 U.S. 110, 116 (1895).

Unexpected results must be "commensurate in scope with the claims."

*DuPont v. Synvina*,
904 F.3d 996, 1012 (Fed. Cir. 2018).

Bausch's later patents state the '786 patent synthetic methods produce mixed topoisomers. EX1068 ('346 patent), 3:30-41.

Pet., 11, 14, 32-34; Reply, 22-24; EX1002 (Peterson), ¶¶44-45, 117-19.

# *Bausch's Deficiencies: Apples-to-Oranges Comparison*

Bausch previously argued plecanatide in "the form in which it is therapeutically administered" was compared to uroguanylin as "a mixture of isoforms."

EX1063 (Peterson), ¶¶148-49, 152; EX1067, 102-03, 139-40.

> For completeness, we note that, because of the rapid isomeric interconversion of uroguanylin (and the much slower interconversion of $E^3$-uroguanylin (SP-304)), it is impossible to compare the affinity of the active conformation (only) of uroguanylin to the affinity of the active conformation of $E^3$-uroguanylin (SP-304). Testing of the activity or affinity of the active form (only) of uroguanylin is not possible, as it rapids converts to, and eventually reaches equilibrium with, the inactive conformation.

EX1067, 139-40.

But Bausch could have—and should have—compared the active form of uroguanylin against the active form of plecanatide.

EX1063 (Peterson), ¶168; EX1060 (Davies), 114:19-116:10, 41:6-17, 43:12-19.

> Opponent 1 arrives at this conclusion because, when the purified biologically active (A) isomers of the peptides are compared, it is observed that SP-304 is no more active than human uroguanylin. In this regard, Opponent 1 files herewith the results of experiments in which the biological activity of the biologically active (A) isomers of SP-304 and human uroguanylin were compared (see the enclosed Annex A, Experiment 1). It can be seen from the results filed herewith that, when the purified A isomers are compared, SP-304 is no more potent than human uroguanylin. Furthermore, experiments were conducted comparing the heat stability of the A isomer of SP-304 to the A isomer of human uroguanylin. These results demonstrate that SP-304 is no more stable to heat denaturation than human uroguanylin (see Annex A, Experiment 2). Thus, contrary to Patentee's assertions, the claimed peptides do not exhibit improved biological activity or increased heat stability compared with human uroguanylin.

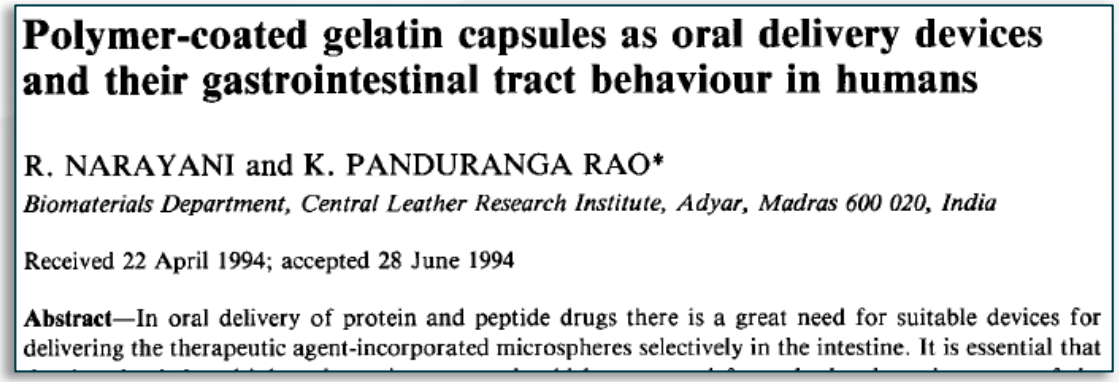
EX1067, 125-26.

Pet., 11, 14, 32-34; Reply, 22-24.

WILSON SONSINI

# Ground 2: Currie, Li, & Narayani

EX1005, EX1006, and EX1007



**Polymer-coated gelatin capsules as oral delivery devices and their gastrointestinal tract behaviour in humans**

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**Abstract**—In oral delivery of protein and peptide drugs there is a great need for suitable devices for delivering the therapeutic agent-incorporated microspheres selectively in the intestine. It is essential that

# *Claim 2 Was Obvious Over Currie, Li, & Narayani*

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

Currie & Li obviated Glu$^3$-human uroguanylin (Plecanatide; SEQ ID NO: 20) as previously discussed.

Currie also taught:

- Human uroguanylin could be used for its natural purpose as a laxative to treat constipation in human patients. EX1005, 2:20-24.
- Uroguanylin and its analogs act in the intestinal endothelium. EX1005, 1:20-25.

Narayani taught:

- Unit doses of peptides in gelatin capsules facilitates passage through the stomach and drug delivery to the intestine. EX1007, 47.

Combination taught:

- Good reason to use a capsule as a unit dose of Glu$^3$ analog of human uroguanylin to deliver the peptide to the intestine.

Pet., 40-42; EX1002 (Peterson), ¶¶181-89, 190-201.

# *Narayani (EX1007)*

It was known to deliver peptide drugs to the intestine while avoiding degradation in the stomach using natural polymer coating (e.g., alginate).

> The present study was directed towards the development of enteric capsules for dumping microspheres containing therapeutically active proteins and peptides (for example, insulin) or other drugs, that are well absorbed in the intestine but need protection against degradation, selectively in the intestine. Sodium alginate which is a natural, biodegradable polysaccharide was chosen for coating the gelatin capsules. The gelatin capsules coated with this pH-sensitive biopolymer will pass through the stomach unaffected by the acidity of the gastric juice and disintegrate in the intestinal fluid where it can dump the microspheres. The microspheres will then provide controlled release of the drug in the intestine. The viability of the polymer-coated gelatin capsules for the oral delivery has been demonstrated using human volunteers.

EX1007, 40.

> The results of this study clearly suggested that alginate-coated gelatin capsules are safe candidates as oral delivery devices to carry microspheres containing bioactive peptides and proteins and dump them selectively in the large intestine where therapeutic action or drug absorption is desired. The polymer-coated gelatin capsules will facilitate the routine use of the oral route of drug delivery for protein and peptide drugs.

EX1007, 47

Pet., 26-27; EX1002 (Peterson), ¶¶111-12, 181-89, 190-201.

# *Claims 4-5 Were Obvious Over Currie, Li, & Narayani*

> **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.

Narayani taught:

- Unit doses of peptides in *polymer-coated gelatin capsules* facilitates passage through the stomach and drug delivery to the intestine. EX1007, 47.

Plain meaning of "excipient" includes coating agents:

> Excipients are materials used in the formulation of pharmacologically active drugs; currently over 1000 such materials are used in marketed pharmaceuticals. They have a variety of roles including diluents/fillers/bulking agents, binders/adhesives, propellants, disintegrants, lubricants/glidants, colors, flavors, coating agents, polishing agents, fragrances, sweetening agents, polymers, and waxes; vaccine adjuvants also represent an excipient form. Excipients can be broadly

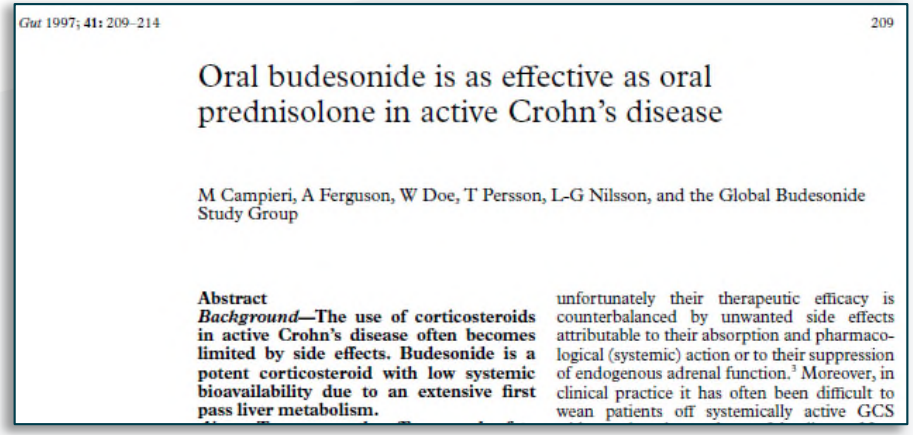
EX1043 (Baldrick), 210.

Pet., 14, 42-45; EX1002 (Peterson), ¶¶202-09.

WILSON SONSINI

# Ground 3: Currie, Li, Narayani, & Campieri

EX1005, EX1006, EX1007, and EX1008



*Gut* 1997; **41:** 209–214 209

## Oral budesonide is as effective as oral prednisolone in active Crohn's disease

M Campieri, A Ferguson, W Doe, T Persson, L-G Nilsson, and the Global Budesonide Study Group

**Abstract**

***Background*—The use of corticosteroids in active Crohn's disease often becomes limited by side effects. Budesonide is a potent corticosteroid with low systemic bioavailability due to an extensive first pass liver metabolism.**

unfortunately their therapeutic efficacy is counterbalanced by unwanted side effects attributable to their absorption and pharmacological (systemic) action or to their suppression of endogenous adrenal function.[3] Moreover, in clinical practice it has often been difficult to wean patients off systemically active GCS

# *Claim 3 Obvious Over Currie, Li, Narayani & 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 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.

Currie, Li, and Narayani obviated unit dose forms of Glu$^3$-human uroguanylin (Plecanatide; SEQ ID NO: 20), including as capsules, to treat constipation as previously discussed.

Campieri taught:

- Using steroidal anti-inflammatory agents (prednisolone, budesonide) to treat intestinal inflammation. EX1008, 213.
- Application in treatment of Chrohn's disease, a chronic inflammatory disorder. EX1008, 209.

Combination taught:

- Good reason to include an anti-inflammatory agent in a capsule dose of Glu$^3$ analog of human uroguanylin.

Pet., 45-50; EX1002 (Peterson), ¶¶113-14, 210-14, 215-27.

# Campieri (EX1008)

POSA knew administration of budesonide and prednisolone would promptly treat symptoms of inflammatory bowel disease.

The CDAI scores for patients on prednisolone or budesonide once daily decreased in a similar fashion, with a less rapid decline in the budesonide twice daily group. 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. These

EX1008, 213.

Campieri orally administered the steroids as tablets, consistent with a capsule.

***Patients and methods*—One hundred and seventy eight patients were randomised to receive budesonide controlled ileal release (CIR) capsules 9 mg once daily or 4.5 mg twice daily, or prednisolone tablets 40 mg once daily. The treatment period was 12 weeks. The primary efficacy variable was clinical remission, defined as a Crohn's Disease Activity Index (CDAI) of 150 or less.**

* * *

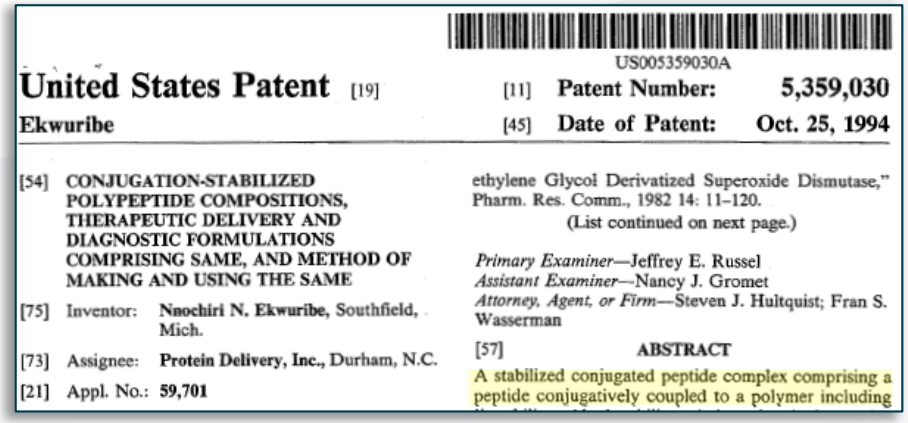
***Conclusions*—Budesonide CIR, administered at 9 mg once daily or 4.5 mg twice daily, is comparable to prednisolone in inducing remission in active Crohn's disease. The single dose administration is as promptly effective as prednisolone and represents a simpler and safer therapeutic approach, with a considerable reduction in side effects.**

EX1008, 209.

Pet., 27-28; EX1002, ¶¶113-14.

# Ground 4: Currie, Li, & Ekwuribe

EX1005, EX1006, and EX1009



US005359030A

**United States Patent** [19]

**Ekwuribe**

[11] **Patent Number: 5,359,030**

[45] **Date of Patent: Oct. 25, 1994**

[54] **CONJUGATION-STABILIZED POLYPEPTIDE COMPOSITIONS, THERAPEUTIC DELIVERY AND DIAGNOSTIC FORMULATIONS COMPRISING SAME, AND METHOD OF MAKING AND USING THE SAME**

[75] Inventor: **Nnochiri N. Ekwuribe**, Southfield, Mich.

[73] Assignee: **Protein Delivery, Inc.**, Durham, N.C.

[21] Appl. No.: **59,701**

ethylene Glycol Derivatized Superoxide Dismutase," Pharm. Res. Comm., 1982 14: 11–120.

(List continued on next page.)

*Primary Examiner*—Jeffrey E. Russel
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[57] **ABSTRACT**

A stabilized conjugated peptide complex comprising a peptide conjugatively coupled to a polymer including

# *Claim 6 Obvious Over Currie, Li, & Ekwuribe*

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

Currie & Li obviated Glu$^3$-human uroguanylin (Plecanatide; SEQ ID NO: 20) as previously discussed.

Ekwuribe taught:

- Benefits of peptide conjugates comprising PEG attached to the peptide.
  EX1009, [57], 33:50-55 (claim 15); EX1002 (Peterson), ¶¶234, 237-39.

Combination taught:

- Good reason to conjugate polyethylene glycol (PEG) to plecanatide.

Pet., 50-53; EX1002, ¶¶232-36, 237-47.

# *Ekwuribe (EX1009)*

Ekwuribe teaches advantages to modifying peptides so physiological activities are maintained.

indicate that properly formulated (poly)peptides and proteins may be administered by the oral route, with retention of sufficient biological activity for their intended use. If, however, it were possible to modify these peptides so that their physiological activities were maintained totally, or at least to a significant degree, and at the same time stabilize them against proteolytic enzymes and enhance their penetration capability through the intestinal mucosa, then it would be possible to utilize them properly for their intended purpose. The product so obtained would offer advantages in that more efficient absorption would result, with the concomitant ability to use lower doses to elicit the optimum therapeutic effect.

EX1009, 1:46-59.

Ekwuribe conjugated the peptide insulin to PEG, then to a linker with more PEG, providing a hydrophilic environment.

EX1009, 13:44-53.

Ekwuribe further teaches PEGylation "endow[s] the polymer-peptide with high aqueous solubility" and provided "increased stability against denaturation and enzymatic digestion."

EX1009, 14:50-55, 2:60-3:1.

Pet., 27-28; EX1002 (Peterson), ¶¶113-14, 232-36, 237-47.