

UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE PATENT TRIAL AND APPEAL BOARD

BONERGE LIFESCIENCE (HUNAN) CO., LTD.,
Petitioner

v.

NANJING NUTRABUILDING BIO-TECH CO., LTD.,
Patent Owner

U.S. Patent No. 10,278,961
Filing Date: April 19, 2017
Issue Date: May 7, 2019
Title: Administration of berberine metabolites

Inter Partes Review No.: TBD

EXHIBIT 1003

DECLARATION OF DR. RONALD J. SHEBUSKI, SR., PH.D.

Table of Contents

I. SUMMARY OF BACKGROUND AND QUALIFICATIONS.....	5
II. COMPENSATION.....	6
III. MATERIALS REVIEWED.....	7
IV. RELEVANT LEGAL STANDARDS.....	7
V. SUMMARY OF OPINIONS.....	10
VI. OVERVIEW OF THE '961 PATENT.....	10
A. THE '961 PATENT AND SPECIFICATION.....	10
B. THE '961 PATENT FILE HISTORY.....	12
VII. LEVEL OF ORDINARY SKILL IN THE ART FOR THE '961 PATENT....	14
VIII. CLAIM CONSTRUCTION UNDER 37 C.F.R. § 42.104(B)(3).....	15
IX. THE CHALLENGED CLAIMS OF THE '961 PATENT.....	18
A. GROUND 1: CLAIMS 1, 2, 5, 6, 7 ARE OBVIOUS OVER TURNER IN VIEW OF SHAW.....	18
1. Claim 1.....	18
2. Claim 2 - The method of claim 1 wherein the administration of dihydroberberine reduces fasting glucose levels.....	30
3. Claim 5 - The method of claim 1 wherein the dihydroberberine is orally administered as a capsule or tablet.....	31
4. Claim 6 - The method of claim 1 wherein the dihydroberberine is orally administered as at least one of a food product or beverage product.....	33
5. Claim 7 - The method of claim 1 wherein the dihydroberberine is administered at least once daily.....	34
B. GROUND 2: CLAIM 5 IS OBVIOUS OVER TURNER IN VIEW OF SHAW FURTHER IN VIEW OF ZHANG.....	34
1. Claim 5 - The method of claim 1 wherein the dihydroberberine is orally administered as a capsule or tablet.....	35
C. GROUND 3: CLAIMS 1, 2, 5, 7 ARE OBVIOUS OVER ZHANG IN VIEW OF FENG.....	36
1. Claim 1.....	36

2.	Claim 2 - The method of claim 1 wherein the administration of dihydroberberine reduces fasting glucose levels.....	45
3.	Claim 5 - The method of claim 1 wherein the dihydroberberine is orally administered as a capsule or tablet.....	47
4.	Claim 7 - The method of claim 1 wherein the dihydroberberine is administered at least once daily.....	48
D. GROUND 4: CLAIM 6 IS OBVIOUS OVER ZHANG IN VIEW OF FENG FURTHER IN VIEW OF TURNER.....		50
1.	Claim 6 - The method of claim 1 wherein the dihydroberberine is orally administered as at least one of a food product or beverage product.....	50
X. SECONDARY CONSIDERATIONS.....		51
XI. RIGHT TO SUPPLEMENT		51
XII. CONCLUSION.....		52

List of Petitioner's Exhibits

Nos.	Descriptions
1001	U.S. Patent No. 10,278,961
1002	Excerpts of Prosecution File History of U.S. Patent No. 10,278,961
1004	Nigel Turner et al., Berberine and Its More Biologically Available Derivative Dihydroberberine, Inhibit Mitochondrial Respiratory Complex I, Diabetes, Vol. 57, May 2008, 1414-1418 (“Turner”)
1005	Shannon Reagan-Shaw et al., Dose translation from animal to human studies revisited, The FASEB Journal, Vol. 22, March 2007, pp. 659-661 (“Shaw”)
1006	Yifei Zhang et al., Treatment of Type 2 Diabetes and Dyslipidemia with the Natural Plant Alkaloid Berberine, J. Clin Endocrinol Metab, July 2008, 93(7):2559-2565. (“Zhang”)
1007	Ru Feng et al., Transforming berberine into its intestine-absorbable form by the gut microbiota, Nature Scientific Reports, 5:12155, DOI: 10:1038/srep12155, published July 15, 2015 (“Feng”)
1008	Guidance for Industry Estimating the Maximum Safe Starting Dose in Initial Clinical Trials for Therapeutics in Adult Healthy Volunteers, USFDA, Center for Drug Evaluation and Research (CDER), July 2005 (“FDA Guidance”)
1009	Chunqiu Chen et al., A randomized clinical trial of berberine hydrochloride in patients with diarrhea-predominant irritable bowel syndrome, Phytotherapy Research, 29:1822-1827 (2015) (“Chen”)
1010	Li Liu et al., Berberine suppresses intestinal disaccharidases with beneficial metabolic effects in diabetic states, evidences from in vivo and in vitro study, Naunyn-Schmied Arch Pharmacol, March 2013, 381:371–381. (“Liu”)
1011	Aillen JF King, The use of animal models in diabetes research, British Journal of Pharmacology, (2012) 166 877-894. (“King”)
1012	U.S. Application 12/443,401, published on May 6, 2010 as US 2010/0113494. (“Hu”)

I, Ronald J. Shebuski, Sr., declare as follows:

I. Summary of Background and Qualifications

1. This section contains a summary of my educational background, career history, publications, and other relevant qualifications. My full curriculum vitae is attached as Appendix B to this declaration.

2. As a staff Research Scientist at large pharmaceutical companies (Smith Kline & French Laboratories, Inc. and Merck; 1985-1990), and later as Director, Cardiovascular Pharmacology (Pharmacia & Upjohn, Inc.; 1990-1998), my primary responsibilities were to manage and oversee drug discovery and development efforts of cardiovascular projects running simultaneously in my group(s). Those efforts involved high throughput drug screening (“HTS”), in vitro cell- or tissue-based drug candidate assays (as described above), and in vivo drug candidate evaluation of efficacy and preliminary safety. In this capacity, I also worked closely with medicinal chemists who were determining structure activity relationships (“SAR”) for the drug targets we were pursuing. I reviewed results of HTS, identified compounds to move to in vitro cell or tissue-based assays, and eventually selected compounds for in vivo evaluation of pharmacokinetics, pharmacological efficacy (pharmacodynamics), and preliminary safety in animal models. I have published extensively on various stages of drug discovery. My publications include studies related to small molecules, peptide and antibody approaches for pharmaceutical

design, testing, evaluation of pharmacokinetics, pharmacology, preclinical safety and efficacy for various molecules and drug candidates. Additionally, I examined the results of HTS efforts and chose compounds with the greatest potency and selectivity towards the receptor or target being examined for further development in vitro, ex vivo, and in vivo.

3. My expertise in pharmacology also lends itself well to toxicological and clinical studies where I suggest starting doses of the drug candidate based on previous pre-clinical pharmacology dose-response evaluations accomplished in relevant animal models.

4. I am also experienced with regards to the use of animal models to study safety and efficacy of drug candidates. Specifically, I have published frequently on the development and application of animal models for medicinal research. This work includes how non-human primates, pigs, dogs, rabbits, guinea pigs, rats, and mice can be used to study the effect of various proteins, antibodies, and other small molecules in vivo.

II. Compensation

5. For my efforts in connection with the preparation of this declaration, I am being compensated at my standard hourly rate for my time. My compensation is not in any way contingent on my performance, the result of this proceeding, or any of the issues involved therein. I am also being reimbursed for expenses incurred as

a result of activities performed as an expert.

III. Materials Reviewed

6. In preparing this declaration, I have reviewed and/or considered at least the documents cited in the List of Petitioner's Exhibits above, and the documents referenced in this declaration.

IV. Relevant Legal Standards

7. I am not an attorney. For the purposes of this declaration, I have been informed about certain aspects of the law that are relevant to forming my opinions. My understanding of the law is as follows.

8. Petitioner's counsel has informed me that my analysis must be performed from the perspective of a hypothetical "person of ordinary skill in the art" ("POSA") as of the priority date. I understand that POSA is a hypothetical person who is presumed to be aware of all pertinent prior art, has ordinary skill in the field of the invention, and is a person of ordinary creativity. I understand that my analysis should not be performed using a present-day perspective, and that I should not use hindsight or the perspective of the most knowledgeable experts in the field.

9. Petitioner's counsel has informed me that the level of ordinary skill depends on several factors, including: (1) the level of education of the inventor; (2) type of problems encountered in the art; (3) prior art solutions to those problems; (4)

rapidity with which innovations are made; (5) sophistication of the technology; and (6) educational level of active workers in the field.

10. Petitioner's counsel has informed me that a patent claim can be invalidated if it is deemed to be obvious to a POSA at the time of the claimed invention. To be obvious in light of a single prior art reference or multiple prior art references, there must be a reason that would have prompted the POSA to modify the single prior art reference, or combine two or more references, in a manner that provides the elements of the claimed invention. This reason may come from a teaching, suggestion, or motivation to combine, the reference(s) themselves, the knowledge or "common sense" of the POSA, or from the nature of the problem to be solved. The reason that would have prompted the POSA to modify the single prior art reference or combine two or more references may be explicit or implicit from the prior art as a whole.

11. Petitioner's counsel has informed me that a determination of whether a claim would have been obvious should be based upon several factors, including, among others: (i) the level of ordinary skill in the art at the time of the claimed invention; (ii) the scope and content of the prior art; (iii) what differences, if any, existed between the subject matter of the claimed invention and the prior art; and (iv) secondary considerations of non-obviousness.

12. Petitioner's counsel has informed me that secondary or objective

evidence of non-obviousness includes evidence of: (1) a long felt but unmet need in the prior art that was satisfied by the claimed invention; (2) commercial success or the lack of commercial success of the claimed invention; (3) unexpected results achieved by the claimed invention; (4) praise of the claimed invention by others skilled in the art; (5) taking of licenses under the patent by others; (6) deliberate copying of the claimed invention; and (7) contemporaneous and independent invention by others. However, Petitioner's counsel has also informed me that there must be a relationship between any secondary evidence of non-obviousness and the claimed invention. Petitioner's counsel has informed me that evidence on secondary considerations may not be sufficient to overcome a strong *prima facie* case of obviousness.

13. Petitioner's counsel has informed me that in considering obviousness, I should keep in mind that a POSA has ordinary creativity and is not an automaton.

14. Petitioner's counsel has also informed me that in considering obviousness, it is improper to determine obviousness using the benefit of hindsight derived from the patent being considered.

15. Petitioner's counsel has informed me that in some cases, the combination of familiar elements according to known methods is likely to be obvious when it does no more than yield predictable results. Petitioner's counsel has also informed me that in some cases, "obvious to try" is sufficient to establish

obviousness. Petitioner's counsel has also informed me that when there is some recognized reason to solve a problem and there are a finite number of identified, predictable solutions, a POSA has good reason to pursue the known options within his or her technical grasp. If this leads to the anticipated success, it is likely the product of ordinary skill and common sense, not innovation.

V. Summary of Opinions

16. Based on my review of U.S. Patent No. 10,278,961 (Ex. 1001, the "'961 patent") and its prosecution history (Ex. 1002), the other materials I have considered, and my knowledge and experience, my opinions are as follows:

- Claims 1, 2, 5, 6, 7 are obvious over Turner (Ex. 1004) in view of Shaw (Ex. 1005).
- Claim 5 is obvious over Turner (Ex. 1004) in view of Shaw (Ex. 1005), further in view of Zhang (Ex. 1006).
- Claims 1, 2, 5, 7 are obvious over Zhang (Ex. 1006) in view of Feng (Ex. 1007).
- Claim 6 is over Zhang (Ex. 1006) in view of Feng (Ex. 1007), further in view of Turner (Ex. 1004).

VI. Overview of the '961 Patent

A. The '961 Patent and Specification

17. The '961 patent, entitled "Administration of berberine metabolites," is

generally directed to methods of administering dihydroberberine to manage blood glucose levels and/or increase blood ketone levels. Ex. 1001, Abstract. Dihydroberberine is often referred to as dhBBR in the literature. DhBBR is a berberine metabolite. *Id.* at 1:44-48. Berberine is commonly used in China as a nonprescription oral drug to treat gut infections and diarrhea with few side effects. Ex. 1004, Turner at 1414. Berberine is hereafter referred to as BBR.

18. The '961 patent has two independent claims – claims 1 and 9. The '961 Patent has dependent claims 2, 5, 6, and 7 that depend on independent claim 1.

19. The '961 patent acknowledges as prior art background that (i) BBR is a naturally occurring substance that has been administered to humans in the past, (*id.* at 1:21-27), (ii) BBR can lower fasting blood glucose if administered at effective amounts, (*id.* at 1:27-33); (iii) BBR has low bioavailability (*id.* at 1:33-40). At the time of the filing of the '961 patent, dhBBR was commercially available. *Id.* at 4:23-25.

20. The '961 patent claims a novel finding that orally administered dhBBR can lower blood glucose levels but at a lower dosage, and that the improved efficacy of dhBBR compared with BBR is “unexpected.” Specifically, the specification makes the following remarks and claims

The amount of dihydroberberine may be less than the amount of berberine required to achieve the same amount of glucose tolerance (e.g., a predetermined fasting glucose level, a predetermined range of blood glucose, a normal or other blood glucose level as determined by

health guidelines, a normal or other fasting blood glucose as determined by health guidelines, etc.). In some implementations, an amount of dihydroberberine may provide the same level of glucose tolerance as at least double the same amount of berberine (e.g., the dihydroberberine may be at least twice as effective as berberine and thus half or less than half of a first amount of dihydroberberine would be required to achieve the same results as the first amount of berberine). This result is unexpected since one would expect berberine and dihydroberberine to have similar properties. Even with the known increase bioavailability of dihydroberberine over berberine, the ability to administer as little as half as much dihydroberberine as berberine to achieve similar results in glucose tolerance is unexpected.

Id. at 4:64-5:18. This finding, the specification claims, would make dhBBR a better diabetes drug because a lower dosage would mean reduced side effects, eased palatability, eased administration, reduced costs, etc. *Id.* at 5:18-27.

21. The '961 patent disclosed data collected from a few experiments to support its claimed findings. *See id.* at 9:10-67 (Examples 1-4). In these experiments, human subjects received various amounts of either BBR or dhBBR. Blood was withdrawn from the subjects to measure each subject's blood glucose level and changes over time. *Id.* at 9:12-16. The '961 patent provides limited information about these subjects. *See id.* at 9:10-67. There was no disclosure of the subjects' age, gender, health conditions, co-morbidities, or other demographic information.

B. The '961 Patent File History

22. The '961 patent issued from U.S. Patent Application No. 15/491,933 ("the '933 Application"), filed April 19, 2017, claiming the benefit of Provisional Application No. 62/324,794, filed on April 19, 2016. *Id.*, cover page.

23. Applicant filed the '933 Application with 20 claims and made an amendment withdrawing claim 15 and amending claims 16-20 after the Restriction Requirement. Ex. 1002, Amendment and Reply to Restriction Requirement Mailed December 12, 2017, dated February 5, 2018. The Examiner rejected all pending claims as anticipated by Hu et al. (U.S. Pub. 2010/0113494) in an office action dated May 24, 2018. *Id.*, Office Action dated May 24, 2018. Examiner stated that “[Hu] discloses methods for managing glucose tolerance and methods for increasing blood ketone levels in an individual by administering to said individual dihydroberberine.” *Id.* at 3.

24. On November 20, 2018, Applicants had an interview with the Examiner and submitted a response amending independent claims 1 and 10, canceling claim 5, and adding new dependent claims 21-22. *Id.*, Amendment and Reply to Office Action Mailed May 24, 2018, dated November 20, 2018. Specifically, Applicant contended that the added dosage limitation - “wherein the pharmaceutically effective amount of dihydroberberine comprises approximately 25 mg to approximately 800 mg of dihydroberberine” – distinguishes the amended independent claims 1 and 10¹ over the Hu reference. *Id.* at 2-3. Hu, according to the Applicant, taught “the administration of 100 mg/kg/day or 2 g/kg².” *Id.* at 7.

¹ They matured into Claims 1 and 9 of the issued '961 Patent.

² Hu disclosed injecting glucose intraperitoneally to mice at a dose of 2 g/kg of the mice's body weight as part of the set up for the animal study. Ex. 1012 at ¶0219.

25. There is no record in the prosecution history whether the Applicant and the Examiner discussed how to convert the dosage disclosed in Hu's rodent model for application to humans, and whether such a conversion would render the amended claims of the '933 Application obvious. The examiner allowed the amended claims on December 26, 2018. *Id.* Notice of Allowance. The '961 patent issued on May 7, 2019.

VII. Level of Ordinary Skill in the Art for the '961 Patent

26. The relevant time for a POSA with respect to the '961 Patent is April 19, 2016 (earliest purported priority date) or April 19, 2017 (actual filing date of the '961 Patent).

27. Based on my education and experience, as described above and in my CV, as well as consistent with the '961 Patent and the prior art described below, it is my opinion that a POSA during the relevant period would be a researcher engaged in developing pharmaceutical formulation, preclinical and clinical drug development including selecting and evaluating pharmacologically effective amounts of active substances. A POSA would have had the general knowledge in pharmacology, drug metabolism, pharmacokinetics, pharmacodynamics, and toxicology. Furthermore, the POSA would be expected to have knowledge and considerable experience in what is known in the field as "ADME" (Absorption, Distribution, Metabolism, and

Hu did not disclose administering a 2 g/kg dosage of dhBBR to the lab mice.

Excretion The researcher should preferably have advanced pharmaceutical sciences degree such as Ph.D. or Pharm. D. Alternatively, the researcher should have at least a master's degree and preferably a PhD in Pharmacology/Toxicology, Pharmaceutics, or Medicinal Chemistry and several years of experience in pharmaceutical formulation and drug development.

VIII. Claim Construction Under 37 C.F.R. § 42.104(b)(3)

28. For purposes of this proceeding only, Petitioner contends that that, except for the claim term “reduces fasting glucose levels,” no formal claim constructions are necessary because the challenged claims are invalid under their plain and ordinary meanings.

29. In particular, independent claim 1 recites “a method for managing glucose tolerance” in the preamble. Ex. 1001, 11:28. Petitioner contends that this phrase should carry its ordinary meaning. The '961 patent explicitly sets forth “managing glucose tolerance” to include “decrease and/or maintain blood glucose level, decrease and/or maintain fasting blood glucose, increase ability to process glucose, etc.” *Id.* at 2:20-23.

30. Dependent claim 6 recites “dihydroberberine is orally administered as at least one of a food product or beverage product.” Petitioner contends that the term “food product” should carry its ordinary meaning. The '961 patent discloses the following regarding how the dihydroberberine may be orally administered as a food

product. *See* Ex. 1001 at 10:6-18 (“In some implementations, the described composition may be administered via tablet, capsule, powdered supplement; ready-to-drink formulation; topical product including transdermals; cosmeceutical product; foods such bars, cookies, gum, candy, functional foods; toothpaste, sublingual product; injection; intravenous fluids; beverages such as shots or energy shots; inhalers; sublinguals; and/or combinations thereof. The described composition may be provided in a powdered form that allows the described composition to be sprinkled on food, mixed with a liquid to provide a beverage, directly administered.”) *See also id.* at 8:21-24 (“In some implementations, the composition may be provided in a delivery form such powder (e.g., that capable consumed separately, mixed with drinks, and/or food) and/or tablet.”)

31. Dependent claim 2 recites “reduces fasting glucose levels.”

32. The '961 patent specification discloses four experimental studies – Examples 1 through 4. Ex. 1001 at 9:8-67; Figs. 1-4. The effect of dihydroberberine on “fasting glucose levels,” as taught by these experiments, is explained in a single sentence that comments on the results from Example 1 – “an unexpected result of the administration of dihydroberberine is that administration of dihydroberberine may keep blood glucose levels closer *to fasting blood glucose* than berberine.” Ex. 1001 at 9:22-25 (emphasis added).

33. All four experimental studies disclosed in the '961 specification comprise of "glucose challenge tests" studies on human subjects after they were administered varying doses of BBR or dhBBR or compositions containing the same. A "glucose challenge tests" tracks changes in a subject's blood glucose levels within hours of receiving a sugar shot. The '961 specification teaches administering 75 grams of glucose to the testing subjects; it does not disclose how the glucose is taken by the subjects.

34. For the first study (Example 1), the "glucose challenge tests" records five subjects' blood glucose levels at time zero (before 75 grams of glucose were administered) and at 30, 60, 120, and 180 minutes post glucose administration. Three separate "glucose challenge tests" were performed on each subject. The blood glucose levels were measured and averaged for analysis.

35. Because the only disclosure from the '961 experiments supporting the claim 2 limitation is the observation from the first glucose challenge test study that "administration of dihydroberberine may keep blood glucose levels closer to fasting blood glucose than berberine," Petitioner contends that claim 2 would be satisfied if a prior art reference (or a combination of prior art references) demonstrates via a glucose challenge test or similar type of glucose tolerance tests where the administration of dhBBR would keep blood glucose levels closer to fasting blood glucose levels than BBR would.

IX. The Challenged Claims of the '961 Patent

36. For ease of reference, the Challenged Claims are reproduced in Appendix A with numbered claim limitations corresponding to the numbering used throughout my declaration.

A. GROUND 1: Claims 1, 2, 5, 6, 7 Are Obvious over Turner in View of Shaw

1. Claim 1

- a. 1[Pre]: A method of managing glucose tolerance in an individual, the method comprising:

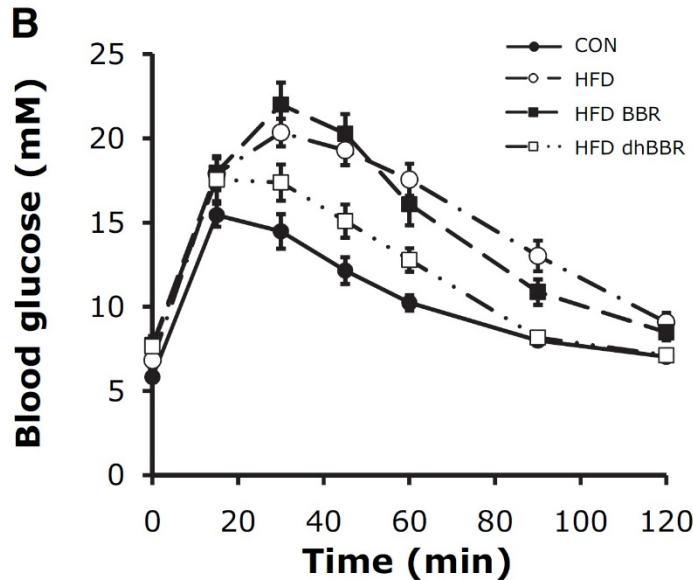
37. To the extent the claim preamble is construed as limiting, Turner teaches a method of managing glucose tolerance in rodent models. *See, e.g.*, Ex. 1004, Turner, 1414 (“The effect of a BBR derivative, dihydroberberine (dhBBR), on ... glucose metabolism was examined in rodents fed a high-fat diet.”), *id.* (“a novel BBR derivative, dhBBR, was identified that displayed improved in vivo efficacy in terms of counteracting ... insulin resistance in high-fat-fed rodents.”), *id.* at 1415 (“In mice fed an HFD, treatment with dhBBR ... markedly ... improved glucose tolerance, compared with HFD controls (Fig. 3).”) Turner further discloses potentially conducting clinical studies of dhBBR for managing glucose tolerance in humans, especially subjects with type 2 diabetes. *Id.* at 1417 (“dhBBR represents an attractive potential therapy for the treatment of type 2 diabetes and other components of the metabolic syndrome.”).

- b. 1[A]: administering, to an individual, a pharmaceutically effective amount of dihydroberberine, wherein the pharmaceutically effective amount of dihydroberberine comprises approximately 25 mg to approximately 800 mg of dihydroberberine

38. Turner discloses potentially conducting clinical studies (including administration) of dhBBR for managing glucose tolerance in humans based on studies of dhBBR's effect on managing glucose tolerance in rodent models. *Id.* at 1414 (“The effect of a BBR derivative, dihydroberberine (dhBBR), on ... glucose metabolism was examined in rodents fed a high-fat diet.”), at 1417 (“dhBBR represents an attractive potential therapy for the treatment of type 2 diabetes and other components of the metabolic syndrome.”).

39. Turner discloses the pharmaceutically effective dosage amount for managing blood glucose levels in rodents. *See id.* at 1414 (“a novel BBR derivative, dhBBR, was identified that displayed improved in vivo efficacy in terms of counteracting ... insulin resistance in high-fat-fed rodents.”), at 1415 (“In mice fed an HFD, treatment with dhBBR (100mg·kg⁻¹·day⁻¹) markedly ... improved glucose tolerance, compared with HFD controls (Fig. 3).”) The improved efficacy of the 100 mg/kg/day dosage is demonstrated via a glucose tolerance test. Specifically, the subject mice were fed with high fat diet. An intraperitoneal glucose tolerance test was performed where 2g/kg of glucose were injected. The mice's blood glucose levels were measured at time zero (before glucose administering) and at 20, 40, 60,

80, 100, and 120 minutes post glucose injection. The blood glucose levels of mice fed with the HFD/dhBBR diet is closer to the fasting blood glucose levels of the



control animals that were not fed a high fat diet. On the other hand, BBR is not as effective at reducing the fasting blood glucose levels at the same dosage. The results were plotted in Fig. 3(B) which is reproduced below.

40. Shaw explained how to use the body surface area (BSA) normalization method to translate drug dosage from animals to humans. Shaw disclosed the BSA conversation formula and included a conversation table based on the BSA method. See Ex. 1005, Shaw at 660. Shaw discloses the conversation formula based on BSA to derive at the human equivalent dosage as follows: $HED (mg/kg) = Animal\ dose (mg/kg) * Animal\ Km / Human\ Km$. *Id.*

Formula for Dose Translation Based on BSA	
HED (mg/kg) = Animal dose (mg/kg) multiplied by	$\frac{Animal\ Km}{Human\ Km}$

41. Shaw discloses that the *Km* factor for adult human is 37, and the *Km* factor for mouse is 3. *Id.* Shaw further notes that “BSA-based dose calculation is the most appropriate method and is far superior to the simple conversion based on body weight.” *Id.* at 661. Shaw advocates the use of BSA method when “converting a dose for translation from animals to humans.” *Id.* at 659, Abstract.

42. Turner discloses that “In mice fed an HFD, treatment with dhBBR (100mg·kg⁻¹·day⁻¹) markedly ... improved glucose tolerance, compared with HFD controls (Fig. 3).” Ex. 1004, Turner at 1415. Based on the rodent dosage amount disclosed in Turner (100 mg/kg/day¹), the human equivalent dosage (“HED”) based on the BSA method is approximately 8.1 mg/kg/day (100 mg/kg/day x 3/37), which is about 486 mg/day dosage for an average human adult weighing about 60 kg.

43. As shown above, the BSA conversion of the Turner rodent dosage amount (486 mg/day dosage for an average human adult weighing about 60 kg) falls within the recited “approximately 25 mg to approximately 800 mg” dosage range.

c. A POSA Would Have Been Motivated to Combine Turner and Shaw with a Reasonable Expectation of Success

44. *First*, Turner discloses that dhBBR is effective for managing glucose tolerance in rodents and opines that dhBBR can be effective for human diabetes treatment. Turner discovered that dhBBR, a BBR derivative, has improved *in vivo* efficacy compared with BBR for managing blood glucose tolerance in animal studies. Specifically, Turner subjected mice on a high-fat-diet (HFD) to glucose

tolerance tests where one group of mice were fed dhBBR for the final two weeks of feeding, and the other group were fed BBR. Ex. 1004, Turner at 1415. The administered dosage for both dhBBR and BBR is 100 mg/kg/day. *Id.* Blood was drawn from the subject mice to measure blood glucose levels and changes over time. The results were plotted and disclosed in Fig. 3(B). This *in vivo* animal study demonstrated that dhBBR has improved efficacy for managing glucose tolerance. *See id.* at 1415-16. (“In mice fed an HFD, treatment with dhBBR ... markedly reduced adiposity and improved glucose tolerance, compared with HFD controls (Fig. 3). At the same dose, BBR had no effect on adiposity or glucose tolerance (Fig. 3), whereas at a dose of 560 mg. kg⁻¹.day⁻¹ (data not shown), we observed the expected effects of BBR.”). Based on these animal studies, Turner opined that dhBBR is “an attractive potential therapy for the treatment of type 2 diabetes and other components of the metabolic syndrome.” *Id.* at 1417. A POSA would find it obvious to study dhBBR’s efficacy for managing human glucose tolerance through clinical studies.

45. *Second*, a POSA would be motivated to apply the teachings of Turner to launch clinical studies of dhBBR for managing glucose tolerance in humans, with reasonable expectation of success. This is because the studies in Turner were conducted on one of the most commonly used animal models for type 2 diabetes research. Moreover, a POSA is expected to use findings from pre-clinical animal

studies as theoretical foundations and experimental evidence to launch clinical studies and applications of a new drug.

46. A POSA understands that high-fat diet mice model is one of the most common animal models to research type 2 diabetes, especially for treatments to improve insulin resistance. *See* Ex. 1011, King at 881-882 (outlining some of “the most commonly used models for type 2 diabetes” in Table 2) & Table 2. King was published in early 2012, well before the earliest priority date (4/19/2016) of the '961 patent. King is entitled “The use of animal models in diabetes research.” Ex. 1011, Title. King described an animal model where the C57BL/6 mice were fed high-fat diet preferable for several weeks to induce more pronounced weight gains. *Id.* at 883. King further commented that the HFD animal model “is thought to model the human [type 2 diabetes] situation more accurately than genetic models of obesity-induced diabetes.” *Id.* at 884. The studies in Turner followed the same HFD animal model that King described for type 2 diabetes. The animal models used in Turner’s diabetes study are obese mice induced by high fat feeding. Ex. 1004, Turner at 1415 & Fig. 3. The mouse strain used in Turner is C57BL/6J. The mice were fed high-fat diet for ten weeks to induce obesity before administration of BBR or dhBBR to study their therapeutic efficacy. *Id.*

47. Researchers in the berberine field already relied on rodent studies to launch clinical studies that yield successes. *See* Ex. 1009, Chen at 1822-23. Chen

was published in late 2015, well before the earliest priority date (4/19/2016) of the '961 patent. Chen is entitled “A randomized clinical trial of berberine hydrochloride in patients with diarrhea-predominant irritable bowel syndrome.” Ex. 1009, Title. Chen cited a number of animal studies of berberine’s effects on gastrointestinal disorders using rodent models. *Id.* at 1822. Using these rodent study findings as “theoretical foundation and experimental evidence,” (*id.* at 1822), Chen performed clinical trials of berberine hydrochloride in patients with irritable bowel syndrome and concluded that berberine hydrochloride is “well tolerated and reduces IBS-D symptoms, which effectively improved patients” quality of life. *Id.* at Abstract.

48. *Third*, a POSA would have experimented with a human equivalent dosage (HED) amount of dhBBR using the BSA method for managing blood glucose levels and would have a reasonable expectation of success. Shaw discloses that the BSA normalization method is the “most appropriate” method to translate drug dosage from animals to humans. Ex. 1005, Shaw at 661. Using BSA method for converting drug doses from animal studies to clinical trials is also recommended by the FDA Guideline document (“normalization to body surface area has remained a widespread practice for estimating an HED based on an animal dose.”). Ex. 1008, FDA Guidance at 6. FDA Guidance was published in July 2005 and well before the earliest filing date of the '961 patent (4/19/2016). FDA Guidance is entitled “Guidance for Industry Estimating the Maximum Safe Starting Dose in Initial

Clinical Trials for Therapeutics in Adult Healthy Volunteers.” *Id.* at Title. The FDA Guideline suggests that a pharmacologically active HED of a new drug can “be derived from a PAD estimate by using a BSA-CF” based on *in vivo* animal studies. *Id.* at 12. PAD is short for “pharmacologically active dose,” which is further defined as “[t]he lowest dose tested in an animal species with the intended pharmacologic activity.” *Id.* at 4, 15. BSA-CF is short for “body surface area conversion factor.” *Id.* at 3.

49. Moreover, the validity of the BSA method for dosage conversion specifically with respect to berberine (and its derivatives) for treating diabetes can be verified by studies done by researchers in this field at the relevant time period. This can be verified by comparing prior art reference Zhang with Liu.

50. Zhang is entitled “Treatment of Type 2 Diabetes and Dyslipidemia with the Natural Plant Alkaloid Berberine.” Ex. 1006, Zhang at Title. Zhang was published in July 2008, well before the earliest priority date of the ’961 patent (4/19/2016). The authors of Zhang conducted clinical studies designed to “evaluate the efficacy and safety of berberine in the treatment of type 2 diabetic patients with dyslipidemia.” *Id.* at 2559. Zhang’s clinical studies demonstrated that a daily dosage of 1.0 gram of berberine is effective in reducing fasting blood glucose levels in type 2 diabetic patients. Ex. 1006, Zhang at 2559. Zhang concluded that berberine is effective and safe in the treatment of type 2 diabetes. *Id.*

51. Liu was published on March 13, 2010, well before the earliest priority date (4/19/2016) of the '961 patent. Liu is entitled “Berberine suppresses intestinal disaccharidases with beneficial metabolic effects in diabetic states, evidences from *in vivo* and *in vitro* study.” Ex. 1010, Liu at Title. The authors of Liu conducted rodent studies and demonstrated that a daily dosage of 200 mg/kg of berberine administered to diabetic rats for five weeks is effective in reducing fasting blood glucose levels. *Id.* at 372, 374 & Fig. 1(b). Specifically, at a daily dosage level of 200 mg/kg of berberine, Liu teaches that berberine “significantly decreased the fasting blood glucose levels” in rats that were induced by STZ to have diabetic symptoms. *See id.* at 374 & Fig. 1(b).

52. Using the BSA conversion formula disclosed in Shaw, the HED of a 200 mg/kg/day dosage administered to rats is approximately 16.2 mg/kg/day, or 972 mg/day (based on an average human weight of 60 kg). Because the HED dosage converted from the Liu rodent studies (972 mg/day) approximates the pharmaceutically effective BBR dosage disclosed in the Zhang clinical studies (1000 mg/day), the BSA normalization method is validated for diabetic research on the therapeutic effects of berberine (or its derivative).

53. *Fourth*, a BSA conversion of the Turner rodent dosage amount falls within the recited human dosage range in the '961 Patent and the converted dosage amount would have a reasonable expectation of success. Applying the BSA

conversion method as taught in Shaw to the dosage amount disclosed in Turner, a POSA can predict, with reasonable expectation of success, that the pharmaceutically effective dosage of dhBBR for application to humans is approximately 8.1 mg/kg/day, or 486 mg/day based on an average human weight of 60 kg. Specifically, Shaw disclosed the BSA conversion formula where HED is calculated from the animal dosage multiplied by animal *Km* and divided by Human *Km*: $HED (mg/kg) = Animal\ dose (mg/kg) \times Animal\ Km / Human\ Km$. Ex. 1005, Shaw at 660. The *Km* value for mouse is 3. *Id.* The *Km* value for an adult human is 37. *Id.* The dosage amount disclosed in Turner that is effective in managing blood glucose is 100 mg/kg/day for mice. Ex. 1004, Turner at 1415-16. The HED value is thus approximately 8.1 mg/kg/day = 100 mg/kg/day x 3/37, *i.e.*, a 486 mg/day dosage for an average human adult weighing about 60 kg. The 486 daily dosage falls within the “approximately 25 mg to approximately 800 mg” dosage range recited in claim 1 of the '961 patent.

54. The reasonable expectation of success of using the BSA conversion can be corroborated by using the BSA conversion method disclosed in Table 1 from the FDA Guidance. Ex. 1008, FDA Guidance, which yields approximately the same HED dosage amount.

55. FDA Guidance recommended a standardized process for selecting the maximum recommended starting dose (MRSD) for first-in-human clinical trials of

new molecular entities in adult healthy volunteers. Ex. 1008, FDA Guidance at 1. According to the guidance, this process “pertains primarily to drug products for which systemic exposure is intended; it does not address dose escalation or maximum allowable doses in clinical trials.” *Id.* at 2. Specifically, part of the process involves extrapolating NOAEL (No Observed Adverse Effect Levels) dosage data derived from animal toxicity studies to a HED (Human Equivalent Dose) dosage. FDA Guidance further teaches that “normalization to body surface area has remained a widespread practice for estimating an HED based on an animal dose.” *Id.* at 6. The process involves further applying a safety factor to the HED to “increase assurance that the first dose in humans will not cause adverse effects.” *Id.* at 4. The default safety factor is 10. *Id.* at 10. FDA Guidance also advises a clinician to consider the pharmacologically active dose as part of MRSD evaluation. *Id.* at 12. FDA Guidance acknowledges that selection of a PAD (pharmacologically active dose) is outside the scope of the guidance but nevertheless discloses that it is appropriate to derive a HED dosage from *in vivo* PAD studies using the BSA-CF (Body Surface Area Conversion Factor) method. *Id.* at 12. BSA-CF is short for Body Surface Area Conversion Factor. *Id.* at 15.

56. FDA Guidance disclosed the BSA conversion formula where HED (measured in mg/kg) is calculated by dividing the animal dosage (also measured in mg/kg) by a predetermined conversion factor. Ex. 1008, FDA Guidance at 7 & Table

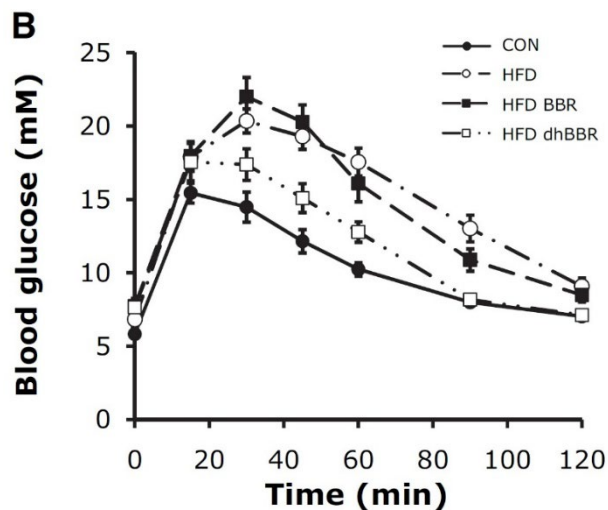
1. The conversion factor for mice is 12.3. *Id* at Table 1. The mice dosage amount disclosed in Turner that is effective in managing blood glucose is 100 mg/kg/day. Ex. 1004, Turner at 1415-16. The HED value is therefore approximately 8.1 mg/kg/day, which equates to 100 mg/kg/day divided by 12.3. For an average human adult weighing about 60 kg, the 8.1 mg/kg/day translates to about 486 mg/day, which falls within the “approximately 25 mg to approximately 800 mg” dosage range recited in claim 1 of the ‘961 patent.

57. The FDA Guidance recommends an additional default safety factor of 10 for selecting the maximum recommended starting dosage for first-in-human trial to “increase assurance that the first dose in humans will not cause adverse effects.” Ex. 1008, FDA Guidance at 4. A POSA would have understood that the pharmaceutically effective dosage of dhBBR for treating humans is likely greater than the recommended maximum recommended *starting* dosage. A POSA may further predict that the HED at 486 mg/day of dhBBR derived from the BSA-normalization method is a pharmaceutically effective dosage. Regardless, even applying the default safety factor of 10 under the FDA Guidance, the maximum recommended starting dosage for an average human adult weighing about 60 kg is approximately 49 mg/day (486 mg/day divided by 10). It still falls within the “approximately 25 mg to approximately 800 mg” dosage range recited in claim 1 of the ‘961 patent.

2. Claim 2 - The method of claim 1 wherein the administration of dihydroberberine reduces fasting glucose levels

58. For reasons I provide above, a POSA would understand that claim 2 would be satisfied if a prior art reference (or a combination of prior art references) demonstrates via a glucose tolerance test that the administration of dihydroberberine would keep blood glucose levels closer to fasting blood glucose levels than berberine would. *See Supra* at §VIII.

59. Turner demonstrates, via a glucose tolerance test, that administration of dihydroberberine would keep mice's blood glucose levels closer to fasting blood glucose levels than berberine. *See Ex. 1004. Turner* at Figs. 3(B) and 3(C). Fig. 3(B) records the mice's blood glucose levels at time zero (before administering 2g/kg of glucose) and at 20, 40, 60, 80, 100, and 120 minutes post glucose administration. The blood glucose levels of mice fed with the HFD/dhBBR diet at a 100 mg/kg/day dosage is closer to the fasting blood glucose levels of the control animals that were not fed a high fat diet. *See Fig. 3(B) reproduced below.*



On the other hand, BBR is not as effective at reducing the blood glucose levels close to the fasting blood glucose level at the same dosage (100 mg/kg/day) in animals fed a high fat diet in this glucose tolerance test study.

60. Similarly, Fig. 3(C) shows that blood glucose area under the curve is significantly reduced by dhBBR, similar to control animals that were not fed a high fat diet. BBR on the other hand is not effective at reducing blood glucose levels at the prescribed dosage (100 mg/kg/day) in animals fed a high fat diet in this experiment.

61. As I explained above in IX.A.1.c, a POSA would have been motivated to combine Turner with Shaw, and would have a reasonable expectation that administering dhBBR to humans would achieve the same success in humans, i.e., the administration of dhBBR would keep blood glucose levels of humans closer to fasting blood glucose levels than berberine, which can be demonstrated via a glucose challenge test. Combining Turner with Shaw therefore would meet the claim 2 limitations thus rendering it obvious.

3. Claim 5 - The method of claim 1 wherein the dihydroberberine is orally administered as a capsule or tablet

62. Turner teaches that BBR is administered orally. *See* Ex. 1004, Turner at 1414 (“BBR is commonly used as a nonprescription oral drug in China to treat

gut infections and diarrhea with few side effects, and its therapeutic potential for the treatment of diabetes ... in humans has been reported.”)

63. Turner teaches that the dihydroberberine is administered orally because dhBBR is administered to the mice the same way BBR is administered in the high-fat-diet (HFD) diet. *Id.* at 1415 (“Mice and rats were fed for 10 weeks and 4 weeks, respectively, and based on pilot testing for dhBBR in mice, **BBR and dhBBR were provided in the HFD** at a dose of $100 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$ for the final 2 weeks of feeding.”) (Emphasis added). Turner teaches that dihydroberberine can be synthesized and kept in a dry and solid form. Ex. 1004, Turner, Supplementary Methods, Preparation of Dihydroberberine (“the filter cake was dried overnight to give dihydroberberine sulfate [5,6-Dihydro-9,10-dimethoxybenzo(g)-1,3-benzodioxolo(5,6-a) quinolizinium sulfate] (2.9 g, yield 75%).”).

64. A POSA would have been motivated to administer dhBBR orally to humans because it is customary in the pharmaceutical field to administer the same medicine (e.g., dhBBR) to humans through the same route (p.o., i.e. per oral) used in preclinical animal studies that demonstrate safety and efficacy. As a matter of fact, the various prior art studies I have reviewed invariably teach administering BBR or its derivatives orally, whether it is an animal study or a clinical trial. *See, e.g.,* Ex. 1004 Turner (orally administration of BBR or dhBBR via mixing with HFD diet to rodents); Ex. 1006 Zhang (oral administration of BBR as tablets to patients);

Ex. 1009 Chen (oral administration of berberine hydrochloride to patients); Ex. 1010 Liu (oral administration of berberine to diabetic rats). A POSA would have been further motivated to administer dhBBR as a capsule or tablet as dhBBR can be synthesized in a stable dry solid form. Administering dhBBR as a tablet would also be obvious to a POSA because it is one of the few well known and common forms for administering solid medications orally.

4. Claim 6 - The method of claim 1 wherein the dihydroberberine is orally administered as at least one of a food product or beverage product

65. Turner teaches that BBR is administered orally to humans. *See* Ex. 1004, Turner at 1414 (“BBR is commonly used as a nonprescription oral drug in China to treat gut infections and diarrhea with few side effects, and its therapeutic potential for the treatment of diabetes ... in humans has been reported.”)

66. Turner teaches that dhBBR can be synthesized and kept in a dry and solid form. Ex. 1004, Turner, Supplementary Methods, Preparation of Dihydroberberine.

67. Turner teaches that the dhBBR is administered orally to the mice because dhBBR is administered the same way BBR is administered in the high-fat-diet (HFD) diet. *Id.* at 1415 (“Mice and rats were fed for 10 weeks and 4 weeks, respectively, and based on pilot testing for dhBBR in mice, **BBR and dhBBR were provided in the HFD** at a dose of 100 mg/kg/day for the final 2 weeks of feeding.”)

(Emphasis added). A POSA would further understand that the dhBBR was mixed with the mice's chow diet. In other words, Turner teaches that dhBBR is orally administered to the mice "as at least one of a food product."

68. A POSA would have been motivated to administer dhBBR orally to humans "as at least one of a food product" because a POSA would have a reasonable expectation of success where the same medicine (e.g., dhBBR) is administered to humans through the same route (p.o.) and form (powder mixed with food) used in the animal studies such as the Turner studies.

5. Claim 7 - The method of claim 1 wherein the dihydroberberine is administered at least once daily

69. Turner teaches that the dihydroberberine is administered daily on rodents. *See* Ex. 1004, Turner at 1415 ("Mice and rats were fed for 10 weeks and 4 weeks, respectively, and based on pilot testing for dhBBR in mice, BBR and **dhBBR** were provided in the HFD at a dose of 100 mg/kg/day for the **final 2 weeks of feeding**." (Emphasis added).

70. As I explained above, a POSA would have a reasonable expectation, based on preclinical animal studies, that administration of dhBBR *daily* to humans would be a successful strategy for managing glucose tolerance.

B. GROUND 2: Claim 5 Is Obvious Over Turner in View of Shaw Further in View of Zhang

1. Claim 5 - The method of claim 1 wherein the dihydroberberine is orally administered as a capsule or tablet

71. Turner teaches that BBR is administered orally. *See* Ex. 1004, Turner at 1414 (“BBR is commonly used as a nonprescription oral drug in China to treat gut infections and diarrhea with few side effects, and its therapeutic potential for the treatment of diabetes ... in humans has been reported.”)

72. Turner teaches that the dihydroberberine is administered orally because dhBBR is administered to the mice the same way BBR is administered in the high-fat-diet (HFD) diet. *Id.* at 1415 (“Mice and rats were fed for 10 weeks and 4 weeks, respectively, and based on pilot testing for dhBBR in mice, **BBR and dhBBR were provided in the HFD animals** at a dose of 100 mg/kg/day for the final 2 weeks of feeding.”) (Emphasis added). Turner teaches that dihydroberberine can be synthesized and kept in a dry and solid form. Ex. 1004, Turner, Supplementary Methods, Preparation of Dihydroberberine (“the filter cake was dried overnight to give dihydroberberine sulfate [5,6-Dihydro-9,10-dimethoxybenzo(g)-1,3-benzodioxolo(5,6-a) quinolizinium sulfate] (2.9 g, yield 75%).”).

73. Zhang teaches that BBR is administered orally in tablet (a dry and solid form) to diabetic patients. EX1006 at 2560. Because Turner teaches that dhBBR can be orally administered in a dry solid form. A POSA therefore would find it obvious to try to administer dhBBR (a derivative of BBR) as an oral drug, also in tablet form.

C. GROUND 3: Claims 1, 2, 5, 7 Are Obvious over Zhang in View of Feng

1. Claim 1

- a. 1[Pre]: A method of managing glucose tolerance in an individual, the method comprising:

74. To the extent the claim preamble is construed as limiting, Zhang teaches a method of managing glucose tolerance in humans because Zhang discloses treatment of diabetic patients. *See* Ex. 1006, Zhang at 2560 (“in the present study, we performed a randomized, double-blind, placebo-controlled trial in four centers to evaluate the efficacy and safety of berberine in the treatment of diabetes and dyslipidemia.”), at 2561 (“As compared with subjects who received placebo, those receiving berberine had a significant improvement in fasting plasma glucose”).

- b. 1[A]: administering, to an individual, a pharmaceutically effective amount of dihydroberberine, wherein the pharmaceutically effective amount of dihydroberberine comprises approximately 25 mg to approximately 800 mg of dihydroberberine

75. As explained above, Zhang discloses a method of managing glucose tolerance in humans. *Id.* at 2560-61.

76. Zhang discloses administration of BBR to patients (an individual). *Id.* at 2560 (“Patients were randomized to receive berberine (0.5 g, twice daily) or placebo prepared in indistinguishable tablets.”). The Zhang clinical trials involved screening and selecting many type 2 diabetes patients across four hospital centers. *Id.* These patients were randomized to receive berberine (0.5 g, twice daily) or

placebo prepared in indistinguishable tablets. *Id.* 0.5 g x 2 (twice daily) equals 1g (1,000 mg)/day. The studies were conducted for up to three months. *Id.* at 2561. The patients' fasting glucose levels were measured and recorded at time zero (before dosing) and at 1, 2 and 3 months post initiation of dosing. *Id.* at 2561, Fig. 3(B).

77. Zhang discloses that the administration of BBR at a dosage of 1g (1,000 mg) / day to type 2 diabetic patients would be pharmaceutically effective for treating diabetes. *Id.* at 2560 (“Eligibility criteria were: 1) age of 25–70 yr; 2) newly diagnosed type 2 diabetes according to the 1999 World Health Organization criteria ...”), 2561 (“As compared with subjects who received placebo, **those receiving berberine had a significant improvement in fasting plasma glucose ...**”) (emphasis added).

78. Prior art reference Feng is entitled “Transforming berberine into its intestine-absorbable form by the gut microbiota.” *Id.* at Title. Feng was published in July 2008, well before the earliest priority date (4/19/2016) of the '961 patent.

79. Feng concludes that “dhBBR is a transient form of BBR in the intestinal lumen, with improved physiochemical characteristics for absorption.” Ex. 1007, Feng at 8. To reach this conclusion, Feng teaches that dhBBR was identified as a metabolite in the intestines of rodents fed BBR in their chow diet. *Id.* at Fig. 1(C). Feng performed additional *in vitro* studies where BBR was incubated with bacteria isolated from rodent intestines as well as *bacteria isolated from human intestines*.

See id. at 2-3, Fig. 1(d). The results confirmed that the bacterial from the guts converted BBR to dhBBR. *Id.* Feng then performed *in vitro* studies using the Caco-2 cell line to examine how dhBBR compares with BBR in terms of the rate of absorption by the intestines. *Id.* at 5, Fig. 3(a). A POSA understands that Caco-2 cell line is a widely used human intestinal epithelial cell model. Feng also administered dhBBR and/or BBR to rats and performed *in vivo* concentration-time curve studies to further examine how dhBBR compares with BBR in terms of the rate of absorption by the intestines. *Id.* at Fig. 3(b)-3(d). Both the *in vivo* and *in vitro* studies confirm that dhBBR has an intestinal absorption rate higher than that of BBR. Finally, Feng performed *in vitro* studies where dhBBR was incubated with homogenates of the small intestines of rats as well as human intestine microsomes. *See id.* at 6, Fig. 4. The results showed dhBBR reverted to BBR via oxidization in intestine tissues. *Id.* Based on their findings, Feng concluded that “the gut microbiota reduces BBR into its absorbable form of dhBBR, which then oxidizes back to BBR after absorption in intestine tissues and enters the blood.” *Id.* at 1.

80. Zhang in view of Feng teaches that administering dhBBR at appropriate dosage would be pharmaceutically effective to manage human subject’s blood glucose levels because dhBBR is a transient form of BBR and that dhBBR gets rapidly converted back to BBR after absorption in intestine tissues and enters the blood. An orally administered dhBBR would remain pharmacologically effective

because BBR would remain as the active drug and dhBBR acts more like a precursor or what is commonly referred to as a pro-drug. *See* Ex. 1007, Feng at 8 (“dhBBR is a transient form of BBR in the intestinal lumen, with improved physiochemical characteristics for absorption.”); *id.* at 1 (“Conclusively, the gut microbiota reduces BBR into its absorbable form of dhBBR, which then oxidizes back to BBR after absorption in intestine tissues and enters the blood.”) A POSA would find that Feng’s findings are further corroborated by Turner. *See* Ex. 1004, Turner at 1417 (“Our pharmacokinetics data were consistent with this prediction, with dhBBR displaying improved absorption compared with BBR. Intriguingly, our data also revealed that once absorbed, dhBBR was rapidly converted back to BBR, highlighting the fact that this is likely the active moiety.”)

81. Feng further discloses that dhBBR “has an intestinal absorption rate 5-fold that of BBR in animals.” *Id.* at 1; *see also* at Abstract (“Here, we show that the gut microbiota converts BBR into its absorbable form of dihydroberberine (dhBBR), which has an intestinal absorption rate 5-fold that of BBR in animals.”). A 5-fold reduction of the dosage of Zhang’s teaching of 1,000 mg/day BBR dosage would bring the dhBBR dosage level to be 200 mg/day. A POSA therefore would have been motivated, with reasonable expectation of success, to administer dhBBR orally to humans at dosage that is approximately one fifth of the BBR dosage clinically proved to be pharmaceutically effective to treat diabetes. The reduced dhBBR

dosage level (200 mg/day dosage for an average human) falls within the recited “approximately 25 mg to approximately 800 mg” dosage range.

c. A POSA Would Have Been Motivated to Modify Zhang based on Feng with a Reasonable Expectation of Success

82. *First*, Zhang discloses clinical studies demonstrating that BBR is effective in managing glucose tolerance of humans. Zhang reported “a randomized, double-blind, placebo-controlled trial in four centers to evaluate the efficacy and safety of berberine in the treatment of diabetes and dyslipidemia.” Ex. 1006, Zhang at 2560. The clinical trials described by Zhang involved screening and selecting many type 2 diabetes patients across four hospital centers. *Id.* These patients were randomized to receive berberine (0.5 g, twice daily) or placebo prepared in indistinguishable tablets. *Id.* The studies were conducted for up to three months. *Id.* at 2561. The patients’ fasting glucose levels were measured and recorded at baseline (prior to BBR dosing) and 1, 2 and 3 months post initiation of BBR dosing. *Id.* at 2561, Fig. 3(B). The results of the study clearly demonstrated that patients “receiving berberine had a significant improvement in fasting plasma glucose” “[a]s compared with subjects who received placebo.” *Id.* at 2561. The studies also found that “[n]o serious adverse events occurred. Mild to moderate constipation occurred in five participants receiving berberine and one participant in the placebo group. However, the frequency of constipation was not significantly different between the berberine and placebo groups (P = 0.207).” *Id.* at 2563. Zhang concluded that

berberine is safe and effective in the treatment of human type 2 diabetes. *Id.* at 2559.

83. *Second*, a POSA is motivated to find a more bioavailable derivative of BBR for diabetes treatment. BBR is widely known to “exhibit[] poor water solubility.” Ex. 1007, Feng at 2. BBR is also “very difficult for intestinal epithelial to absorb.” *Id*; *see also* Ex. 1004, Turner at 1417 (“A potential disadvantage of BBR as an in vivo compound for treatment of diabetes is that we and others have previously reported the need for relatively high doses in rodents to achieve beneficial metabolic effects.”) As such, a POSA would be motivated to find a more bioavailable derivative of BBR.

84. *Third*, a POSA understood that dhBBR is a known BBR derivative with improved absorption characteristics. For instance, dhBBR was predicted to have improved absorption characteristics based on a structural analysis of the molecules where BBR is found to possess “an extremely flat configuration, which is likely to have limited absorption across the intestinal epithelia” but “derivatization to dhBBR was predicted to open up the structure, making it more amenable to uptake.” Ex. 1004, Turner at 1417.

85. The prediction of dhBBR’s improved absorption characteristics was confirmed by preclinical studies, including pharmacokinetic analyses in rats. Turner’s studies showed that following oral administration (by gavage, not in high fat diet) of 20 mg/kg BBR, BBR was not detected in the plasma. In contrast, dhBBR

at the same dose was rapidly detected in the plasma with a C_{\max} of approximately 2.8 ng/ml and half-life of 3.5 hrs. *Id.* at 1416, Supp. Fig. 2. The C_{\max} value for BBR detected in the blood (approximately 12.6 ng/ml) was significantly higher than that detected for the dhBBR in the blood, as was the half-life (9.6 hrs). *Id.* at 1416, Supp. Fig. 2(C). Turner noted that dhBBR gets “rapidly converted back to BBR” soon once it was absorbed based on its pharmacokinetic analyses. *Id.* at 1417.

86. The prediction of dhBBR’s improved absorption characteristics was also confirmed by preclinical studies involving *in vivo* and *in vitro* studies of the metabolism of BBR in gut microbiota. Feng teaches that dhBBR was identified as a metabolite in the intestines of rodents fed on a BBR diet. Ex. 1007, Feng at 2, Fig. 1(C). Feng performed additional *in vitro* studies where BBR was incubated with bacteria isolated from rodent intestines as well as bacteria isolated from human intestines. *See id.* at 2-3, Fig. 1(d). The results confirmed that the bacteria from the guts converted BBR to dhBBR. *Id.* Feng then performed *in vitro* studies using the Caco-2 cell line (a widely used human intestinal epithelial cell model) to examine how dhBBR compares with BBR in terms of the rate of absorption by the intestines. *Id.* at 5 & Fig. 3(a). Feng also administered dhBBR and/or BBR to rats and performed *in vivo* concentration-time curve studies to further examine how dhBBR compares with BBR in terms of the rate of absorption by the intestines. *Id.* at 5-6, Fig. 3(b)-3(d). Both the *in vivo* and *in vitro* studies confirm that dhBBR has an

intestinal absorption rate higher than that of BBR. Feng found that dhBBR gets reverted to BBR via oxidization soon after dhBBR enters the intestine tissues. *Id.* at 6-7. Feng concluded that “the gut microbiota reduces BBR into its absorbable form of dhBBR, which then oxidizes back to BBR after absorption in intestine tissues and enters the blood.” *Id.* at 1.

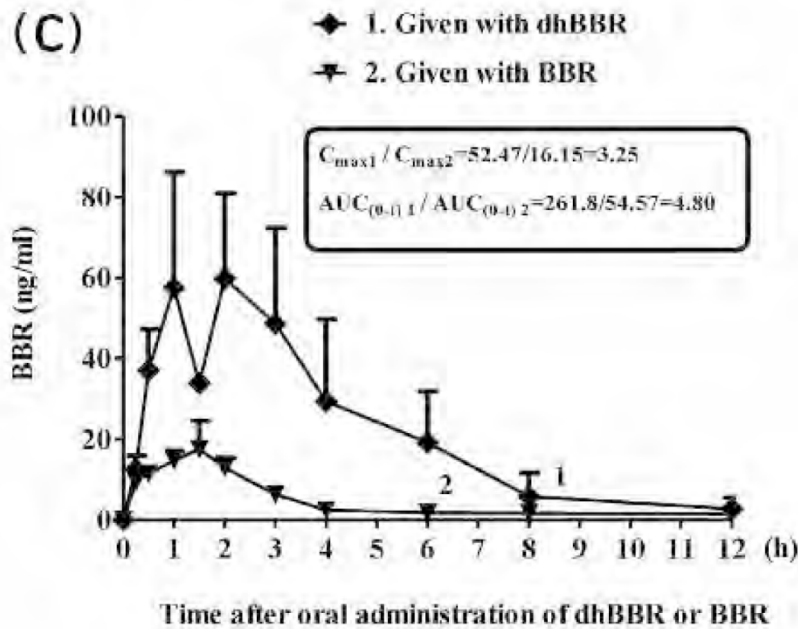
87. *Fourth*, a POSA will have a reasonable expectation of success that dhBBR, as an alternative to BBR for managing blood glucose levels, can be administered at a pharmaceutically effective dosage that is at least five-fold lower than BBR’s dosage. Feng discloses that “dhBBR is a transient form of BBR in the intestinal lumen, with improved physiochemical characteristics for absorption.” Ex. 1007, Feng at 8. As such, a POSA would have been motivated to try to use dhBBR, which readily transforms to berberine after absorption, as a low dose substitute of berberine for oral administration for the treatment of diabetes and managing glucose tolerance in humans. Given that Feng and Turner both teach that dhBBR has improved absorption characteristics, it would have been obvious for a POSA to try dhBBR as an alternative to BBR and expect dhBBR to have improved efficacy at reduced dosage. Also, because Feng and Turner both teach that dhBBR gets rapidly converted back to BBR after absorption in intestine tissues and enters the blood, a POSA would understand that an orally administered dhBBR would remain pharmacologically effective because BBR would remain as the active drug with

dhBBR acting as a pro-drug. Further, since BBR is converted to dhBBR by the gut microbiota after the oral administration of BBR, dhBBR has effectively been administered to human for decades as BBR. Therefore, it is reasonable to assume that dhBBR would be safe to be administered to humans.

88. A POSA would have a reasonable expectation of success that dhBBR can be administered at a dosage that is at least five-fold lower because Feng teaches that dhBBR “has an intestinal absorption rate 5-fold [greater than] that of BBR in animals.” *Id.* at 1. A five-fold reduction of Zhang’s teaching of a daily dosage range for BBR at 1,000 mg dosage would bring the dhBBR dosage level to be 200 mg/daily, which is within the claimed 25 mg to 800 mg dosage range.

89. The reasonable expectation of success is corroborated by the experimental results taught in Feng. Feng demonstrated through *in vivo* animal studies that an orally administered dhBBR would have a higher intestinal absorption and a much higher level of BBR would be detected in the blood over an extended period. Specifically, the blood level of BBR in the dhBBR-treated rats exhibited C_{\max} value that was 3.25-fold higher than those of the BBR-treated rats. *See* Ex. 1007, Feng at 5. The result was documented in Fig. 3(c), which is reproduced below. *Id.* A 3.25-fold reduction of the 1,000 mg/day BBR dosage taught by Zhang would reduce the dhBBR dosage to approximately 300 mg/day, which is within the claimed

25 mg to 800 mg dosage range.



90. Feng also taught the $P_{app(AP-BL)}$ (apparent permeability coefficient of apical-basolateral) coefficient for dhBBR was 11.9-fold higher than that for BBR based on *in vitro* human Caco-2 cell model studies. See Ex. 1007, Feng at 5. Regardless of whether the $P_{app(AP-BL)}$ coefficient accurately reflects how a human's dhBBR intestinal absorption rate compares with the BBR absorption rate, a 12-fold reduction of the BBR dosage amount taught in Zhang would reduce the dhBBR dosage to approximately 83.3 mg/day, which still places the pharmaceutically effective dhBBR dosage within the claimed 25 mg to 800 mg dosage range.

2. Claim 2 - The method of claim 1 wherein the administration of dihydroberberine reduces fasting glucose levels

91. Zhang teaches that administration of BBR reduces fasting glucose levels. See Ex. 1006, Zhang at 2561 ("As compared with subjects who received

placebo, those receiving berberine had a significant improvement in fasting plasma glucose ...”), Fig. 3(B).

92. Feng discloses that “dhBBR is a transient form of BBR in the intestinal lumen, with improved physiochemical characteristics for absorption.” Ex. 1007, Feng at 8. Feng further discloses that dhBBR “has an intestinal absorption rate 5-fold that of BBR in animals.” *Id.* at 1, Abstract.

93. Zhang in view of Feng teaches that administering dhBBR will reduce fasting blood glucose levels because dhBBR gets rapidly converted back to BBR after absorption in intestine tissues and then enters the blood. In other words, an orally administered dhBBR would remain pharmacologically effective because BBR would remain as the active drug and dhBBR acts as a pro-drug. *See* Ex. 1007, Feng at 8 (“dhBBR is a transient form of BBR in the intestinal lumen, with improved physiochemical characteristics for absorption.”), *id.* at 1 (“Conclusively, the gut microbiota reduces BBR into its absorbable form of dhBBR, which then oxidizes back to BBR after absorption in intestine tissues and enters the blood.”) A POSA would find that Feng’s findings are further corroborated by Turner. *See* Ex. 1004, Turner at 1417 (“Our pharmacokinetics data were consistent with this prediction, with dhBBR displaying improved absorption compared with BBR. Intriguingly, our data also revealed that once absorbed, dhBBR was rapidly converted back to BBR, highlighting the fact that this is likely the active moiety.”)

94. As explained above, a POSA would have been motivated to modify Zhang with Feng with a reasonable expectation of success. *See supra* § IX.C.1.c.

3. Claim 5 - The method of claim 1 wherein the dihydroberberine is orally administered as a capsule or tablet

95. Zhang teaches that the BBR is administered as tablets. *See* Ex. 1006, Zhang at 2560 (“Patients were randomized to receive berberine (0.5 g, twice daily) or placebo prepared in indistinguishable tablets.”).

96. Feng teaches that BBR “ha[d] been used orally for decades in China as an over-the-counter (OTC) drug to treat diarrhea with good safety.” Ex. 1007, Feng at 2. Feng discloses that “dhBBR is a transient form of BBR in the intestinal lumen, with improved physiochemical characteristics for absorption.” *Id.* at 8. Feng teaches that dhBBR can be and was indeed administered orally to rodents to study its rate of absorption by the intestines. *Id.* at 5, 12 (“Five SD rats were fasted for 12h and then orally administered 200mg/kg dhBBR. ... BBR served as a control in the study”).

97. Feng further teaches that “the gut microbiota reduces BBR into its absorbable form of dhBBR, which then oxidizes back to BBR after absorption in intestine tissues and enters the blood.” Ex. 1007, Feng at 1. This finding is corroborated by Turner. Ex. 1004, Turner at 1417 (“Our pharmacokinetics data were consistent with this prediction, with dhBBR displaying improved absorption compared with BBR. Intriguingly, our data also revealed that once absorbed, dhBBR

was rapidly converted back to BBR, highlighting the fact that this is likely the active moiety.”) A POSA therefore understands an orally administered dhBBR would remain pharmacologically effective because BBR would enter the blood and remain as the active drug with dhBBR acting as a prodrug in essence.

98. Because BBR has already been administered orally in tablets, and further because orally administered dhBBR would remain pharmacologically effective given BBR would remain as the active drug and dhBBR acts as a pro-drug, a POSA would have been motivated to administrate dhBBR (a transient form of BBR) orally in tablets to improve intestinal absorption rate. As explained above, a POSA would have been motivated to modify Zhang with Feng with a reasonable expectation of success. *See supra* § IX.C.1.c.

4. Claim 7 - The method of claim 1 wherein the dihydroberberine is administered at least once daily

99. Zhang teaches that BBR is administered *at least* once daily. Ex. 1006, Zhang at 2560 (“Patients were randomized to receive berberine (0.5 g, twice daily) or placebo prepared in indistinguishable tablets.”).

100. Feng discloses that “dhBBR is a transient form of BBR in the intestinal lumen, with improved physiochemical characteristics for absorption.” Ex. 1007, Feng at 8. Feng further discloses that dhBBR “has an intestinal absorption rate 5-fold that of BBR in animals.” *Id.* at 1; Abstract.

101. Feng further discloses that “the gut microbiota reduces BBR into its absorbable form of dhBBR, which then oxidizes back to BBR after absorption in intestine tissues and enters the blood.” Ex. 1007, Feng at 1. This finding is corroborated by Turner. Ex. 1004, Turner at 1417 (“Our pharmacokinetics data were consistent with this prediction, with dhBBR displaying improved absorption compared with BBR. Intriguingly, our data also revealed that once absorbed, dhBBR was rapidly converted back to BBR, highlighting the fact that this is likely the active moiety.”) A POSA therefore understands an orally administered dhBBR would remain pharmacologically effective because BBR would enter the blood and remain as the active drug and dhBBR acting as a pro-drug.

102. Because BBR has already been administered orally at least once daily, and further because orally administered dhBBR would remain pharmacologically effective given BBR would remain as the active drug and dhBBR acts as a pro-drug, a POSA would have been motivated to administrate dhBBR similarly - at least once daily. As explained above, a POSA would have been motivated to modify Zhang with Feng with a reasonable expectation of success. *See supra* § IX.C.1.c.

D. GROUND 4: Claim 6 Is Obvious Over Zhang in View of Feng Further in View of Turner

1. Claim 6 - The method of claim 1 wherein the dihydroberberine is orally administered as at least one of a food product or beverage product

103. Turner teaches that dhBBR can be synthesized and kept in a dry and solid form. Ex. 1004, Turner, Supplementary Methods, Preparation of Dihydroberberine.

104. Turner teaches that the dhBBR is administered orally to the mice because dhBBR is administered the same way BBR is administered in the high-fat-diet (HFD) diet. *Id.* at 1415 (“Mice and rats were fed for 10 weeks and 4 weeks, respectively, and based on pilot testing for dhBBR in mice, **BBR and dhBBR were provided in the HFD** at a dose of 100 mg/kg/day for the final 2 weeks of feeding.”) (Emphasis added). A POSA would further understand that the dhBBR was mixed with the mice’s chow diet. In other words, Turner teaches that dhBBR is orally administered to the mice “as at least one of a food product.”

105. A POSA would have been motivated to combine the teachings of Turner with Zhang and Feng because Feng specifically referenced Turner as supporting Feng’s findings. Ex. 1007, Feng at 5 (“DhBBR in its sulfate form (dihydroberberine sulfate) was previously reported to exhibit better absorption in the intestine as compared with BBR.”)

106. A POSA would have been further motivated to administer dhBBR orally to humans “as at least one of a food product” because a POSA would have a reasonable expectation of success where the same medicine (e.g., dhBBR) is administered to humans through the same route (p.o.) and form (powder mixed with food) used in the animal studies such as the Turner studies.

X. Secondary Considerations

107. I am not aware of any relevant information related to secondary considerations of non-obviousness. To the extent Patent Owner comes forward with any such information, I will consider it.

XI. Right to Supplement

108. I reserve the right to supplement my opinions in the future to respond to any arguments that the Patent Owner raises. This declaration represents only those opinions that I have formed to date. I reserve the right to revise, supplement, and/or amend my opinions stated herein based on new information that becomes available to me and on my continuing analysis of the materials already provided.

109. I may utilize the documents cited and/or listed herein, or portions of those documents, as exhibits at any hearing or trial in this proceeding. I may further prepare and use exhibits that summarize portions of my testimony or key terms or concepts presented therein, or other demonstrative exhibits, at any hearing or trial in this proceeding.

110. I reserve the right to supplement my testimony and this report in response to any judicial determinations, in response to the arguments expressed by the Patent Owner or the opinions of the Patent Owner's experts in this proceeding, and/or in light of additional evidence or testimony brought forth at trial or otherwise brought to my attention after the date of my signature below.

XII. Conclusion

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.

Executed on September 26, 2025

By:

Ronald J. Shebuski, Sr.

Ronald J. Shebuski, Sr

APPENDIX A: CHALLENGED CLAIMS

Claim	Limitation
1Pre	A method of managing glucose tolerance in an individual, the method comprising:
1[A]	administering, to an individual, a pharmaceutically effective amount of dihydroberberine, wherein the pharmaceutically effective amount of dihydroberberine comprises approximately 25 mg to approximately 800 mg of dihydroberberine.
2	The method of claim 1 wherein the administration of dihydroberberine reduces fasting glucose levels.
5	The method of claim 1 wherein the dihydroberberine is orally administered as a capsule or tablet.
6	The method of claim 1 wherein the dihydroberberine is orally administered as at least one of a food product or beverage product.
7	The method of claim 1 wherein the dihydroberberine is administered at least once daily.

APPENDIX B: CURRICULUM VITAE

CURRICULUM VITAE

Ronald John Shebuski, PhD

CONTACT INFORMATION

Phone: (906) 458-6212

Email: rshebuski@gmail.com

PERSONAL

Birthplace: Green Bay, WI
Residence: 733 Lake George Drive
Melbourne, FL 32940-2230

EDUCATION

1981-1985 **University of Minnesota-Mpls**, PhD, Pharmacology

1971-1976 **University of Wisconsin-Madison**, BS, Microbiology

PHARMA and BIOTECH EXPERIENCE

2022-present President & CEO
Nytrix, Inc., Athens, GA

1998-present Private Consultant to Pharma and Biotech Industry
Cardiovascular Research Consulting, LLC

2010-2020 President & CEO
Cylerus, Inc., Melbourne, FL

2002-2005 VP, R&D
Afmedica, Inc., Kalamazoo, MI

1998-2002 VP, R&D
CarePoint Medical, Inc., Eden Prairie, MN

1990-1998 Director, Cardiovascular Pharmacology
Pharmacia & Upjohn, Inc., Kalamazoo, MI

1988-1990 Senior Research Fellow, Department of Pharmacology
Merck Sharp & Dohme Research Laboratories, Inc.,
West Point, PA

1985-1988 Senior Investigator, Cardiovascular Pharmacology,
Smith Kline & French Laboratories, Inc., K of P, PA

ACADEMIC APPOINTMENTS

- 2000-2010 Adjunct Associate Professor
Department of Pharmacology
University of Minnesota Medical School
Minneapolis, MN
- 2005-2010 Adjunct Professor
Department of Integrative Biology & Physiology
University of Minnesota Medical School
Minneapolis, MN
- 1992-2000 Adjunct Associate Professor
Department of Pharmacology
University of Michigan Medical School, Ann Arbor, MI

OTHER APPOINTMENTS

- 2009-2017 Special Government Employee (SGE, Grade 15, Step 10)
FDA/CDER-CV/Renal Division, Bethesda, MD
- 2005-2017 General Partner
Apjohn Group, LLC, Kalamazoo, MI
Life Sciences Business Accelerator

CONSULTING CLIENTS (2006-present)

-Abbott Laboratories, Inc., Abbott Park, IL (2009-2010)
(Consultant for Safety Pharmacology group on oncology drug with potential pro-platelet aggregatory activity).

-Accord Biomaterials, Inc., Ann Arbor, MI (2008-2011)
(Retained consultant to provide direction on coating medical devices with anti-microbial and anti-platelet surfaces)

-Ariad Pharmaceuticals, Cambridge, MA (2014-2015)
(Retained hematology/pharmacology consultant to ponatinib project)

-BioMarin, Novato, CA (2010-2012)
(Hourly consultant on external drug development projects)

-InspiRx, Inc., Durham, NC (2015-2021)
(Retained consultant on inhaled interferon gamma for IPF)

-Medivas, Inc., San Diego, CA (2007-2008)
(Retained consultant on drug-eluting polymers for vascular graft failure)

-Medrad/Possis (Division of Bayer, Inc.), Minneapolis, MN and Pittsburgh, PA (2008-2012)
(Retained consultant on drug-eluting angioplasty balloons)

-Mercator Medsystems, Inc., San Leandro, CA (2006-2008)
(Retained consultant on drug delivery to adventitial surface of blood vessels)

-NetScientific, London, UK (2022-present)
(Retained consultant on novel thrombolytic drug for PE and stroke)

-Nostrum Pharmaceuticals/Symmetrix Biotech, Inc., Edison, NJ (2008-2021)
(Retained consultant on novel thrombolytic drug development)

-Proteos, Inc., Kalamazoo, MI (2010-2013)
(Retained consultant on peptide-based PTH analogs)

-SWMF, Kalamazoo, MI (2006-2012)
(Hourly consultant to SWMF Venture Capital group to review CV opportunities when requested)

-Vascular Magnetics, Philadelphia, PA (2012-2014)
(Retained consultant on local treatment of PAD with paclitaxel)

INTELLECTUAL PROPERTY EXPERT WITNESS EXPERIENCE (2010-PRESENT)

-Saffran vs. J&J Cordis (2010-2011)

Retained as testifying expert witness by Dickstein Shapiro, LLP on behalf of Bruce N. Saffran, M.D., Ph.D., in Saffran vs. J&J Corporation et al., 2:07-CV-451 (TJW), US District Court, E.D., Texas, to analyze technical information regarding to the pharmacokinetics of drug release from certain J&J and Cordis Corp. products and technical information, US patent #5,653,760. **Deposed and Jury Trial**

-Sanofi-BMS vs. Apotex (2010-2011)

Retained as testifying expert witness by Gowlings, Lafleur, Henderson, LLP, Toronto, Canada on behalf of Sanofi-BMS in Sanofi-BMS vs. Apotex, Ottawa, Canada to analyze technical information regarding to the anti-platelet drug Plavix® and technical information, Canadian patent #1,336,777. **Deposed and Bench Trial**

-Cook Medical vs. Medrad, PA (2011)

Retained as testifying expert witness by Medrad Inc., in Cook Medical vs. Medrad to analyze technical information regarding to drug-eluting medical devices, US patent # 7,803,149.

-Saffran vs. Abbott (2011)

Retained as testifying expert witness by Dickstein Shapiro, LLP on behalf of Bruce N. Saffran, M.D., Ph.D., in Saffran vs. Abbott Corporation to analyze technical information regarding to the pharmacokinetics of the release of drug from certain Abbott products and technical information, US patent #5,653,760.

-Schering Merck vs. Mylan, New York, NY (2011)

Retained as testifying expert witness by Sidley Austin, LLP, New York, NY on behalf of Schering Merck in Schering Merck vs. Mylan to analyze technical information regarding to drugs for treating and preventing atherosclerosis, US patent # 5,846,966.

-Sanofi vs. Amphastar (2013-2014)

Retained as testifying expert witness by Gibson Dunn & Crutcher on behalf of Sanofi to analyze technical information regarding to Lovenox bioequivalence, US patent # 5,389,618. **Deposed**

-Boehinger Ingleheim vs. Apotex (2016)

Retained as testifying expert witness by Cozen O'Connor on behalf of Apotex to analyze technical information regarding to Pradaxa, US patent # 6,087,380. **Deposed and Bench Trial**

-United Therapeutics Corp. (UTC) vs. Waston/Actavis (2016-2017)

Retained as testifying expert witness by WSGR on behalf of UTC to analyze technical information regarding US patents 8,410,169, 8,747,897, 9,278,901, 9,393,293 and 9,422,223. **Deposed**

-Eli Lilly vs. Apotex (2017)

Retained as testifying expert witness by Belmore Neidrauer (Toronto) on behalf of Eli Lilly to analyze technical information regarding US Patent # 2,432,644.

-Bayer vs. Aurobindo (2017)

Retained as testifying expert witness by Shaw Keller on behalf of Aurobindo to analyze technical information regarding US Patent #7,157,456. **Deposed**

--Grellner vs. Raabe (2016-2018)

Retained as testifying expert witness by Nix, Patterson & Roach on behalf of Grellner to analyze technical information regarding VenaSeal, US Patent # 8,475,492.

-Mass Tort vs. Pfizer (2017-2018)

Retained as testifying expert witness by DLA Piper on behalf of Pfizer to analyze technical information regarding the pharmacokinetics of sildenafil (Viagra).

-BMS Canada Co and BMS Holdings Ireland Unlimited Company vs. Apotex Inc. (2019)

Retained as testifying expert witness by Goodman's LLP on behalf of BMS to analyze technical information regarding to factor Xa. Canadian Patent 2,461,202. **Deposed**

- Sanofi, Bristol-Myers Squibb (2019)

Retained as testifying expert witness by McCarthy Tetrault LLP on behalf of Sanofi/Bristol-Myers Squibb regarding Canadian litigation involving Clopidogrel (Plavix).

-Novartis vs. Alembic (2021)

Retained as testifying expert witness by Venable Law on behalf of Novartis regarding Entresto.

-UTC/Supernus vs. ANI (2022)

Retained as testifying expert witness by McDermott, Will & Emery on behalf of UTC/Supernus regarding Trepstinil. US Patent 9,278,901.

-GW Pharmaceuticals LTD vs. Otsuka LTD (2022)

Retained as testifying expert witness by Williams & Connolly LLP on behalf of GW Pharma regarding Epidiolex.

-Pfizer, Inc. vs. Regor Therapeutics, Inc. (2022-2023)

Retained as testifying expert witness by Quinn Emanuel on behalf of Regor Therapeutics regarding GLP-1 agonists for diabetes.

-United Therapeutics vs. Janssen/Actelion (2025)

Retained as testifying expert witness by McDermott, Will & Emery on behalf of United Therapeutics regarding combination treatments for pulmonary hypertension (IPR case).

-Jazz Pharmaceuticals, Inc. vs. Almaject, Inc. (2025)

Retained as testifying expert witness by Axinn, Veltrop, Harkrider on behalf of Almaject regarding manufacture of a generic version of Defitelio.

-Bonerge vs. Nanjing (2025)

Retained as testifying expert witness by Rimon Law on behalf of Bonerge regarding administration of berberine metabolites (US Patent 10,278,961).

AWARDS

- 1984 Travel Award, Graduate Student, American Society for Pharmacology and Experimental Therapeutics, Annual Meeting, Indianapolis, Indiana
- 1985 Bacaner Basic Science Research Award for Outstanding Graduate Research in Pharmacology - University of Minnesota, Minneapolis, Minnesota
- 1994 1st Annual F.E. Shideman Distinguished Alumnus Award
University of Minnesota, Department of Pharmacology, Minneapolis, Minnesota

THESIS

Influence of Alpha-1 Adrenoceptor Antagonists in the Central Regulation of Blood Pressure and Cardiovascular Reflexes. Submitted to the Faculty of the Graduate School of the University of Minnesota in Partial Fulfillment of the Requirements for the Degree of Doctor of Philosophy, 1985.

AFFILIATIONS

American Association for the Advancement of Science
American Heart Association
American Society for Pharmacology and Experimental Therapeutics
New York Academy of Sciences
Thrombosis Council - American Heart Association

EDITORIAL BOARDS

Expert Opinion on Investigational Drugs (Section Editor, CV and Renal)
Research Communications in Pharmacology and Toxicology, (Section Editor, CV Pharmacology)
Journal of Pharmacology and Experimental Therapeutics
Current Opinion in Pharmacology (CV/Renal Editor)

REVIEWER FOR

Journal of Biochemical Pharmacology
Journal of Cardiovascular Pharmacology
Journal of Pharmacology and Experimental Therapeutics
Circulation
Blood
Cardiovascular Research
American Journal of Physiology
British Journal of Pharmacology

GRANT REVIEW COMMITTEES

Texas Technological Grants - 1989
Special NIH Study Section - Small Business Innovative Research Grants (SBIR) - 1991
Research Grant Committee, American Heart Association of Michigan - 1992-1995
North Carolina Biotechnology Center - 1995
Department of Veterans Affairs - 1995
NIH Study Section/Thrombosis (NHLBI) - 2004
NIH Study Section/Integrative Pharmacology (NIGMS) - 2004
SBIR/STTR Study Section - 2012
SBIR/STTR CV/Hematology Study Section - 2014-2024

OTHER COMMITTEES

ASPET Program Committee - 1992-1995, Reappointed for Term 1995-1997
ASPET Finance Committee- 1998-2002
Founder and Chairman , ASPET Division for Drug Discovery, Drug Development and Regulatory Affairs – 1999-2001

TEACHING ACTIVITIES

University of Minnesota

Medical School Pharmacology, Laboratory Teaching Assistant, 1981-1985
Invited Graduate Lectures, Department of Pharmacology, 2000-2003
Medical School (2nd yr), Cardiovascular Pharmacology Lectures, 2001-2003

Medical College of Wisconsin

Invited Lecturer, Graduate Pharmacology Course, "Mechanisms of Thrombolysis and Restenosis," October, 1991

University of California Santa Barbara

Invited Lecturer, Undergraduate Pharmacology Colloquia Series, "Fibrinogen Receptor Antagonists as Novel Therapeutics," February, 1992

University of Michigan Medical School

Graduate Level Cardiovascular Pharmacology, Medical School Pharmacology, 1993-1995

Western Michigan University

Graduate Thesis Committee, Department of Biological Sciences, 1994-1998

POST-DOCTORAL SCIENTISTS TRAINED

Robert J. Leadley, Ph.D., 1990-1992

Christopher F. Toombs, Ph.D., 1991-1994

Anjali Kumar, Ph.D., 1995 - 1997

EXTRAMURAL MEETINGS ORGANIZED

"Role of Adhesion Molecules in Cardiovascular Pharmacology," ASPET Colloquium, University of Michigan, Ann Arbor, Michigan, June 1994.

"Pharmacological Interventions in Thrombosis and Thrombolysis," ASPET Colloquium, University of Michigan, Ann Arbor, Michigan, June 1995.

"Functional Genomics," ASPET Colloquium, Boston, MA, June 2000.

"Functional Genomics and Proteomics in Drug Discovery and Development, ASPET Symposium, Orlando, FL, April, 2001.

"Protein Therapeutics", ASPET Symposium, New Orleans, LA, April 2002.

EXTRAMURAL INVITED PRESENTATIONS

"Thromboxane Receptor Antagonists and Myocardial Ischemia," organizer and speaker for minisymposium hosted by Philadelphia Physiological Society, Philadelphia, Pennsylvania, September, 1987.

"Thromboxane Receptor Antagonism vs. Thromboxane Synthase Inhibition in Prevention and Interruption of Thrombosis," invited seminar, Department of Pharmacology, Royal College of Surgeons, London, England, November, 1987.

"Role of Thromboxane A₂ in Thrombosis and Thrombolysis," invited seminar, Department of Cardiology, Thomas Jefferson University, Philadelphia, Pennsylvania, February, 1988.

"Thromboxane Receptor Antagonists as Adjunctive Agents to Thrombolytic Therapy," chairman and speaker for ASPET Symposium entitled, "Thromboxane Receptor Antagonists in Acute Myocardial Infarction," Montreal, Canada, October, 1988.

"Thromboxane Receptor Antagonism in Models of Thrombosis and Thrombolysis," invited seminar, Department of Pharmacology, Temple University, Philadelphia, Pennsylvania, January, 1989.

"Animal Models for Thrombosis/Thrombolysis Research," invited seminar, Department of Cardiology, University of Michigan, Ann Arbor, Michigan, September, 1990.

"Fibrinogen Receptor (GPIIb/IIIa) Antagonism," invited lecture, Cardiovascular Discussion Group, Princeton, New Jersey, October, 1990.

"Drug Discovery Targets in Thrombosis," invited lecture, Mid-Atlantic Pharmacology Society, Princeton, New Jersey, October, 1990.

"Fibrinogen Receptor Antagonism as Anti-thrombotic Therapy," symposium speaker, FASEB, Atlanta, Georgia, April, 1991.

"Novel Approaches to Prevention of Restenosis," invited seminar, Department of Pharmacology, Medical College of Wisconsin, Milwaukee, Wisconsin, October, 1991.

"Principles Underlying the Use of Conjunctive Agents with Plasminogen Activators," invited speaker, NYAS, Plasminogen Activation in Fibrinolysis in Tissue Remodeling and Development, Amsterdam, The Netherlands, October, 1991.

"Development of Fibrinogen Receptor Antagonists as Novel Antithrombotics," invited seminar, Department of Pharmacology, University of Michigan Medical School, Ann Arbor, Michigan, February, 1992.

"Development of Fibrinogen Receptor Antagonists as Novel Antithrombotics," invited seminar, Department of Biological Sciences, Western Michigan University, Kalamazoo, Michigan, April, 1992.

"Interruption of Thrombosis and Hemostasis by Anti-Platelet Agents," organizer and speaker in symposium entitled "Perturbation of Hemostasis Following Novel Anti-Platelet, Anticoagulant and Thrombolytic Therapy," Society of Toxicological Pathologists, Phoenix, Arizona, June, 1992.

"Interruption of Smooth Muscle Cell Proliferation Following Balloon Angioplasty in the Rat with the Novel 2 Aminochromone, U-86983," invited seminar, Department of Pharmacology, University of Michigan, Ann Arbor, Michigan, June, 1993.

"Interruption of Smooth Muscle Cell Proliferation Following Balloon Angioplasty in the Rat with the Novel 2 Aminochromone, U-86983," invited seminar, Department of Pharmacology, University of Minnesota, Minneapolis, Minnesota, July, 1993.

"Pharmacological Intervention in Thrombosis," organizer and speaker for ASPET mini-symposium, San Francisco, California, August, 1993.

"Development of Novel Agents for Prevention of Smooth Muscle Cell Proliferation and Restenosis Following Balloon Angioplasty," invited seminar, Physiology Department, Michigan State University, East Lansing, Michigan, September, 1993.

"Development of Novel Agents for Prevention of Smooth Muscle Cell Proliferation and Restenosis Following Balloon Angioplasty," invited seminar, Department of Pharmacology, Uniformed Services University, Bethesda, Maryland, October, 1993.

"Role of Adhesion Molecules in Thrombosis and Restenosis," invited seminar, F.E. Shideman Distinguished Alumnus Awardee Lecture, Department of Pharmacology, University of Minnesota, Minneapolis, Minnesota, April, 1994.

"Role of P-Selectin in Thrombosis and Restenosis," invited seminar, Department of Biological Sciences, Western Michigan University, Kalamazoo, Michigan, September, 1994.

"Role of P-Selectin in Thrombosis and Restenosis," invited seminar, Department of Pharmacology,

University of Michigan, Ann Arbor, Michigan, September, 1994.

"Adhesion Molecules in Cardiovascular Pharmacology," Bronson Methodist Hospital, Kalamazoo, Michigan, March, 1995.

"Adhesion Molecules in Cardiovascular Pharmacology," Borgess Hospital, Kalamazoo, Michigan, March, 1995.

"*In Vivo* Pharmacology of P-Selectin Antagonism," organizer, chair and speaker for ASPET Symposium, San Diego, California, March, 1997.

"Role of P-selectin in Thrombogenesis and Neointimal Proliferation Following Vascular Injury" invited speaker at IBC's 7th annual international conference on cell adhesion molecules and matrix proteins, August, 1997.

"Role of P-selectin in the Pathophysiology and Diagnosis of Acute Coronary Syndromes" invited seminar, Dept. of Pharmacology, University of Minnesota, November, 2000.

"Novel Application of Anti-Platelet GPIIb/IIIa Inhibitors; Prevention of Post-Surgical Adhesions", invited seminar, Dept. of Integrative Biology & Physiology, University of Minnesota, October, 2006.

"A Process for Identifying and Commercializing Novel Academic-Based Technology", invited speaker, Safety Pharmacology Society, Boston, MA, September 2010.

"Competitive Landscape in Anti-Platelets and Anti-Coagulants " invited speaker, SomaLogic, Inc., Boulder, CO, January 2011.

GRANT SUPPORT
Recently Completed

Title: Nitric Oxide releasing ultra-slippy antibacterial surfaces for urological catheter applications
Application Number: 1 R43 DK136411-01A1 (SBIR Phase I)
PI: Shebuski (Nytricx, Inc.) \$337,395
Supporting Agency: NIH/NIDDK
Period of Performance: 7/15/23-7/14/24

Funded (Ongoing)

Title: Achieving homeostasis in traumatic injuries with a nitric oxide-driven antimicrobial dressing combined with a rapid blood clotting agent
Application Number: DM220089 (DoD/USArmy)
PI: Shebuski (Nytricx, Inc.) \$1,114,225
Supporting Agency: DoD/CDMRP/USAMRDC
Period of Performance: 9/01/23-8/30/25

Title: Ultra-low Fouling and Anti-infective and Anti-thrombotic Nitric Oxide Releasing Intravascular Catheters
Application Number: 2 R42 HL149595-02A1 (STTR Phase II)
PI: Shebuski (Nytricx, Inc.) \$2,346,606
Supporting Agency: NIH/NHLBI
Period of Performance: 9/14/23-7/31/25

Title: Enhanced Prosthetic Vascular Graft Function by Local Release of Nitric Oxide (NO)
Application Number: 1R41HL176245-01 (STTR Phase I)
PI: Shebuski (Nytricx, Inc) and Garren (UGA) \$311,824
Supporting Agency: NIH/NHLBI
Period of Performance: 9/1/24-8/31/25

Title: Prevention of ECMO Circuit Thrombosis and Infection by Surface Release of Nitric Oxide (NO)
Application Number: 19-2024-0659-F
PI: Shebuski (Nytricx, Inc.) \$745,973
Supporting Agency: BARDA DRIVe Healing Lungs
Period of Performance: 9/9/24-3/9/26

PUBLICATIONS

Journal and Book Articles

1. Aiken, J.W., Gorman, R.R. and Shebuski, R.J. Prevention of blockage of partially obstructed coronary arteries with prostacyclin correlates with inhibition of platelet aggregation. *Prostaglandins* 17:483-494, 1979.
2. Aiken, J.W., Gorman, R.R. and Shebuski, R.J. Prostacyclin prevents blockage of partially obstructed coronary arteries. In: Prostacyclin (J. R. Vane and S. Bergstrom, eds.), Raven Press, New York, 1979, p. 311.
3. Miller, O.V., Aiken, J.W., Hemker, D.P., Shebuski, R.J. and Gorman, R.R. Prostacyclin stimulation of dog arterial cyclic AMP levels. *Prostaglandins* 18:915-929, 1979.
4. Aiken, J.W. and Shebuski, R.J. Comparison in anesthetized dogs of the anti-aggregatory and hemodynamic effects of prostacyclin and a chemically stable prostacyclin analog, 6a-carba-PGI₂ (carbacyclin). *Prostaglandins* 19:629-643, 1980.
5. Aiken, J.W., Shebuski, R.J. and Gorman, R.R. Blockage of partially obstructed coronary arteries with platelet thrombi: Comparison between cyclooxygenase inhibitors *versus* prostacyclin. In: Advances in Prostaglandin and Thromboxane Research (B. Samuelsson, P. Ramwell and R. Paoletti, eds.), Raven Press, New York, 1980, Vol. 7, p. 635.
6. Miller, O.V., Aiken, J.W., Shebuski, R.J. and Gorman, R.R. 6-Keto-prostaglandin E₁ is not equipotent to prostacyclin (PGI₂) as an antiaggregatory agent. *Prostaglandins* 20:391-400, 1980.
7. Gallagher, K.P., Folts, J.D., Shebuski, R.J., Rankin, J.H.G. and Rowe, G.G. Subepicardial vasodilatory reserve in the presence of critical coronary stenosis in dogs. *Amer. J. Cardiology* 46:67-73, 1980.
8. Gorman, R.R., Miller, O.V., Shebuski, R.J. and Aiken, J.W. Prostacyclin mediated inhibition of canine coronary artery obstruction by platelets: Correlation with platelet and arterial cyclic AMP levels. In: The Regulation of Coagulation (K. G. Mann and F. B. Taylor, eds.). Elsevier Publishing, North-Holland, 1980, Vol. 8, p. 439.
9. Hemker, D.P., Shebuski, R.J. and Aiken, J.W. Release of a prostacyclin-like substance into the circulation of dogs by intravenous adenosine 5'-diphosphate. *J. Pharmacol. Exp. Ther.* 212:246-252, 1980.
10. Shebuski, R.J. and Aiken, J.W. Angiotensin II stimulation of renal prostaglandin synthesis elevates circulating prostacyclin in the dog. *J. Cardiovasc. Pharmacol.* 2:667-677, 1980.
11. Shebuski, R.J. and Aiken, J.W. Angiotensin II induced renal prostacyclin release suppresses platelet aggregation in the anesthetized dog. In: Advances in Prostaglandin and Thromboxane Research (B. Samuelsson, P. Ramwell and R. Paoletti, eds.), Raven Press, New York, 1980, Vol. 7, p. 1149.

12. Aiken, J.W., Shebuski, R.J., Miller, O.V. and Gorman, R.R. Endogenous prostacyclin contributes to the efficacy of a thromboxane synthetase inhibitor for preventing coronary artery thrombosis. *J. Pharmacol. Exp. Ther.* 219:299-308, 1981.
13. Gorman, R.R., Shebuski, R.J., Aiken, J.W. and Bundy, G.L. Analysis of the biological activity of azoprostanoids in human platelets. *Fed. Proc.* 40:19-22, 1981.
14. Taylor, B.M., Shebuski, R.J. and Sun, F.F. Circulating prostacyclin metabolites in the dog. *J. Pharmacol. Exp. Ther.* 224:691-698, 1983.
15. Zollikofer, C.L., Casteneda-Zuniga, W., Einzig, S., Cragg, A., Ryshavy, J., Bruhlmann, W.F., Shebuski, R.J. and Amplatz, K. The role of prostaglandins in percutaneous transluminal angioplasty. *Radiology* 149(3):681-685, 1983.
16. Zeigler, D.W., Shebuski, R.J. and Zimmerman, B.G. Central and peripheral cardiovascular action of urapidil in normotensive and Goldblatt hypertensive animals. *Amer. J. Med.* 77(4A):81-86, 1984.
17. Shebuski, R.J. and Zimmerman, B.G. Suppression of reflex tachycardia following alpha adrenoceptor blockade in conscious dogs: Comparison of urapidil to prazosin. *J. Cardiovasc. Pharmacol.* 6:788-794, 1984.
18. Shebuski, R.J. and Zimmerman, B.G. Comparison of urapidil with the alpha₂-adrenoceptor agonists, clonidine and B-HT 920, on responses of transmurally stimulated guinea pig ileum. *Proc. Soc. Exp. Biol. and Med.* 178:133-138, 1985.
19. Shebuski, R.J. and Zimmerman, B.G. Suppression of reflex tachycardia by central administration of the alpha₁-adrenoceptor antagonists, urapidil and prazosin, in anesthetized dogs. *J. Pharmacol. Exp. Ther.* 234:456-462, 1985.
20. Shebuski, R.J., Fujita, T., Smith, J.M., Jr., Kopaciewicz, L., Blumberg, A.L. and Hieble, J.P. Suppression of sympathetic ganglionic neurotransmission by the selective dopamine-1 agonist, fenoldopam (SK&F 82526), in the anesthetized dog. *J. Pharmacol. Exp. Ther.* 235:735-740, 1985.
21. Rassier, M.E., Shebuski, R.J. and Zimmerman, B.G. Beta₁-adrenoceptor antagonism by urapidil prior to and after treatment with the selective alpha₂-adrenoceptor antagonist, rauwolscine in anesthetized dogs. *Eur. J. Pharmacol.* 122:37-43, 1986.
22. Shebuski, R.J., Fujita, T. and Ruffolo, R.R. Jr. Evaluation of alpha₁- and alpha₂-adrenoceptor-mediated vasoconstriction in the *in situ*, autoperfused pulmonary circulation of the anesthetized dog. *J. Pharmacol. Exp. Ther.* 238:217-222, 1986.
23. Shebuski, R.J., Fujita, T. and Ruffolo, R.R., Jr. Interaction of dopamine (+)-dobutamine and the (-)-enantiomer of dobutamine with alpha- and beta- adrenoceptors in the pulmonary circulation of the dog. *Pharmacology*, 34:201-212, 1987.

24. Shebuski, R.J., Fujita, T., Smith, J.M., Jr. and Ruffolo, R.R., Jr. Comparison of the alpha-adrenoceptor activity of dopamine, ibopamine and epinine in the pulmonary circulation of the dog. *J. Pharmacol. Exp. Ther.*, 241:6-12, 1987.
25. Shebuski, R.J., Ohlstein, E.H., Smith, J.M., Jr. and Ruffolo, R.R., Jr. Enhanced pulmonary alpha₂-adrenoceptor responsiveness under conditions of elevated pulmonary vascular tone. *J. Pharmacol. Exp. Ther.* 242:158-165, 1987.
26. Ohlstein, E.H., Storer, B., Fujita, T. and Shebuski, R.J. Streptokinase and tissue-type plasminogen activator (t-PA) induce platelet hyperaggregability in the rabbit. *Throm. Res.* 46:575-585, 1987.
27. Nichols, A., Smith, J.M., Jr., Shebuski, R.J. and Ruffolo, R.R., Jr. Comparison of the effects of ibopamine, epinine, dopamine and fenoldopam on vascular dopamine receptors in the anesthetized dog. *J. Pharmacol. Exp. Ther.* 242:573-578, 1987.
28. Nichols, A. J., Shebuski, R.J. and Ruffolo, R.R., Jr. Inhibition of plasma cholinesterase prevents the dopamine DA-1 receptor mediated renal vasodilation produced by ibopamine. *Eur. J. Pharmacol.* 141:515-518, 1987.
29. Shebuski, R.J., Smith, J.M., Jr. and Ruffolo, R.R., Jr. Comparison of the renal and pulmonary effects of fenoldopam, dopamine, dobutamine and norepinephrine in the anesthetized dog. *Pharmacology* 36:35-43, 1988.
30. Shebuski, R.J., Smith, J.M., Storer, B.L., Granett, J.R. and Bugelski, P.R. Influence of the selective thromboxane A₂/endoperoxide receptor antagonist sulotroban on lysis time and reocclusion rate following tPA-induced coronary thrombolysis. *J. Pharmacol. Exp. Ther.* 246:790-796, 1988.
31. Shebuski, R.J., Storer, B.L. and Fujita, T. Effect of thromboxane synthase inhibition on the thrombolytic action of tissue-type plasminogen activator (tPA) in a rabbit model of peripheral arterial thrombosis. *Throm. Res.* 52:381-392, 1988.
32. Hieble, J.P., Kopia, G.A., Shebuski, R.J. and Ruffolo, R.R. The role of postjunctional alpha₂-adrenoceptors in the control of vascular resistance, In: Vascular Neuroeffector Mechanisms: Receptors, Ion Channels, Second Messengers and Endogenous Mediators. (Bevan, J.A., Majewski, H., Maxwell, R.A. and Story, D.F., eds), IRL Press, Oxford, 1988, pp. 49-56.
33. Connors, R.W., Sweet, R.W., Noveral, J., Pfavr, D., Trill, T., Shebuski, R.J., Berkowitz, B.A., Williams, D., Franklin, S. and Reff, M. DHFR Coamplification of tPA in DHFR Positive Bovine Endothelial Cells: *In vitro* characterization of the purified serine protease. *DNA*, 7:651-661, 1989.
34. Ohlstein, E.H., Horohonich, S., Shebuski, R.J. and Ruffolo, R.R., Jr. Localization and characterization of the canine pulmonary alpha₂-adrenoceptor. *J. Pharmacol. Exp. Ther.*, 248:233-239, 1989.

35. Shebuski, R.J., Bloom, J.C., Sellers, T.S., Fujita, T., Storer, B., Horohonich, S., Fong, K.L. and Ohlstein, E.H.: Attenuation of the inhibitory effect of prostacyclin on platelet function after tissue plasminogen activator or streptokinase infusion in the rabbit. *Fibrinolysis*, 3:115-123, 1989.
36. Shebuski, R.J., Smith, J.M., Jr. and Ruffolo, R.R., Jr. Effects of dopamine, ibopamine and epinine on alpha- and beta- adrenoceptors in the pulmonary circulation of the dog. *Fund. Clin. Pharmacol.*, 3:211-221, 1989.
37. Shebuski, R.J., Berry, D., Bennett, D., Romoff, T., Storer, B.L., Ali, F. and Samanen, J. Demonstration of Ac-Arg-Gly-Asp-Ser-NH₂ as an antiaggregatory agent in the dog by intracoronary administration. *Throm. Haemo.*, 61:183-188, 1989.
38. Kopia, G.A., Kopaciewicz, L.J., Ohlstein, E.H., Horohonich, S., Storer, B.L. and Shebuski, R.J. Combination of the thromboxane receptor antagonist, sulotroban (BM 13.177, SK&F 95587), with streptokinase: Demonstration of thrombolytic synergy. *J. Pharmacol. Exp. Ther.* 250:877-895, 1989.
39. Fujita, T., Hasan, S., Storer, B.L. and Shebuski, R.J. Effect of selective endoperoxide/thromboxane A₂ receptor antagonism with sulotroban on tPA-induced thrombolysis in a rabbit model of femoral arterial thrombosis. *Fund. Clin. Pharmacol.* 3:643-653, 1989.
40. Shebuski, R.J., Ramjit, D.R., Bencen, G.H. and Polokoff, M.A. Characterization and platelet inhibitory activity of bitistatin, a potent RGD-containing peptide from the venom of the viper, *Bitis arietans*. *J. Biol. Chem.* 264:21550-21556, 1989.
41. Shebuski, R.J., Sitko, G.R., Claremon, D.A., Baldwin, J.J., Remy, D.C. and Stern, A.M. Acceleration of tPA-induced thrombolysis by inhibition of factor XIIIa in a canine model of coronary arterial thrombosis. *Blood*, 75:1455-1459, 1990.
42. Shebuski, R.J., Stabilito, I.J., Sitko, G.R. and Polokoff, M.H. Acceleration of tPA-induced thrombolysis by the RGD-containing peptide bitistatin, and prevention of reocclusion by the combination of heparin and bitistatin in a canine model of coronary thrombosis. *Circulation*, 82:169-177, 1990.
43. Shebuski, R.J., Ramjit, D.R., Lumma, P.K. and Garsky, V.M. Prevention of canine coronary artery thrombosis with echistatin, a potent inhibitor of platelet aggregation from the venom of the viper *Echis carinatus*. *Thromb. Haemo.*, 64:576-581, 1990.
44. Bush, L.R. and Shebuski, R.J. *In vivo* models of arterial thrombosis and thrombolysis: *FASEB Journal*, 4:3087-3098, 1990.
45. Mayer, E.J., Fujita, T., Gardell, S.J., Shebuski, R.J. and Reilly, C.F. The pharmacokinetics of plasminogen activator inhibitor-1 in the rabbit. *Blood*, 76:1514-1520, 1990.

46. Trill, J.J., Fong, K.L., Shebuski, R.J., McDevitt, P., Johanson, K., Williams, D., Boyle, K.E., Sellers, T.S. and Reff, M.E. Expression and characterization of finger protease (FP): a mutant tissue-type plasminogen activator (t-PA) with improved pharmacokinetics. *Fibrinolysis*, 4:131-140, 1990.
47. Reilly, C.F., Mayer, E.J., Hutzelmann, J.E., Sitko, G.R., Gardell, S.J. and Shebuski, R.J. The effect of exogenous plasminogen activator inhibitor-1 in a canine model of occlusive thrombus formation. *Fibrinolysis*, 5:99-104, 1991.
48. Reilly, C.F., Fujita, T, and Shebuski, R.J. Plasminogen activator inhibitor-1 inhibits endogenous fibrinolysis in a canine model of pulmonary embolism. *Circulation*, 84:287-292, 1991.
49. Vlasuk, G.P., Ramjit, D., Dunwiddie, C.T., Nutt, E.M., Fujita, T., Smith, D.E. and Shebuski, R.J. Comparison of the *in vivo* anticoagulant properties of standard heparin and the highly specific factor Xa inhibitors antistasin and tick anticoagulant peptide (TAP) in a rabbit model of venous thrombosis. *Thromb. Haemo.*, 65:257-262, 1991.
50. Gardell, S.J., Ramjit, D.R., Stabilito, I.I., Fujita, T., Lynch, J.J., Cuca, G.C., Deepak, J., Wang, S., Tung, J., Mark, G.E. and Shebuski, R.J. Effective thrombolysis without marked plasminemia following bolus intravenous administration of a vampire bat salivary plasminogen activator in rabbits. *Circulation*, 84:244-253, 1991.
51. Holohan, M.A., Mellott, M.J., Garsky, V.M. and Shebuski, R.J. Prevention of reocclusion following tPA-induced thrombolysis by the RGD-containing peptide, echistatin, in a canine model of coronary thrombosis. *Pharmacology*, 42:340-348, 1991.
52. Shebuski, R.J. Emerging drug discovery targets in thrombosis and coagulation. *Ann. Rep. Med. Chem.*, 26:93-101, 1991.
53. Shebuski, R.J. Principles underlying the use of conjunctive agents with plasminogen activators. *N.Y. Acad. Sci.* 667:382-394, 1992.
54. Toombs, C.F., Norman, N.R., Groppi, V.E., Lee, K.S., Gadwood, R.C., and Shebuski, R.J. Limitation of myocardial injury with the potassium channel opener cromakalim and the non-vasoactive analog U-89,232: Vascular versus cardiac actions in vitro and in vivo. *J. Pharmacol. Exp. Ther.* 263:1261-1268, 1992.
55. Benjamin, C.W., Lin, A.H., Morris, J., Wishka, D.M., Gammill, R.B., Shebuski, R.J. and Gorman, R.R. Inhibition of human platelet aggregation by novel 2-animochromone phospholipase C inhibitors. *Developments in Oncology*, 71: (Eicosanoids Other Bioact. Lipids in Cancer. *Inflammation Radiat. Inj.*) 231-3, ISSN: 0167-4927, 1993.
56. Hartman, J.C., Hullinger, T.G., Wall, T.M., and Shebuski, R.J. Reduction of myocardial infarct size by ramiprilat is independent of angiotensin II synthesis inhibition. *Eur. J. Pharmacol.* 234:229-236, 1993.

57. Hartman, J.C., Wall, T.M., Hullinger, T.G., and Shebuski, R.J. Reduction of myocardial infarct size in rabbits by ramiprilat: Reversal by the bradykinin antagonist, HOE 140. *J. Cardiovas. Pharmacol.* 21:996-1003, 1993.
58. Morris, J., Wishka, D.G., Lin, A.H., Humphrey, W.R., Wiltse, A.L., Gammill, R.B., Judge, T.M., Bisaha, S.N., Bergh, C.L., Cudahy, M.M., Thomas, E.W., Gorman, R.R., Benjamin, C.W., and Shebuski, R.J. Synthesis and biological evaluation of antiplatelet 2-aminochromones. *J. Med. Chem.* 36:2026-2032, 1993.
59. Shebuski, R.J. Pharmacology of anti-platelet agents. Thrombosis and Haemorrhage, Blackwell Scientific Publications, (Ed: Loscalzo and Schafer), pp. 1139-1154, 1993.
60. Leadley Jr., R.J., Lee, P., Erickson, L.A., Shebuski, R.J. The snake venom peptide Sarafotoxin S6b inhibits repetitive platelet thrombus formation in the stenosed canine coronary artery. *J. Cardiovas. Pharmacol. Supplement.* 22(Suppl.8):S199-203, 1993.
61. Toombs, C.F., Wiltse, A.L., Shebuski, R.J. Ischemic preconditioning fails to limit infarct size in reserpinized rabbit myocardium: Implication of norepinephrine release in the preconditioning effort. *Circulation.* 88:2351-2358, 1993.
62. Ramjit, D.R., Lynch Jr., J.J., Sitko, G.R., Mellott, M.J., Holahan, M.A., Stabilito, I.I., Stranieri, M.T., Zhang, G., Lynch, R.J., Manno, P.D., Chang, C.T.-C., Nutt, R.F., Brady, S.F., Veber, D.F., Anderson, P.S., Shebuski, R.J., Friedman, P.A., Gould, R.J. Antithrombotic effects of MK-0852, a novel platelet fibrinogen receptor antagonist, in canine models of thrombosis. *J. Pharmacol. Exp. Ther.* 266:1501-1511, 1993.
63. Shebuski, R.J. Interruption of thrombosis and hemostasis by anti-platelet agents. *Tox. Pathol.* 21:180-189, 1993.
64. Toombs, C.F., Moore, T.L., and Shebuski, R.J. Limitation of infarct size in the rabbit by ischaemic preconditioning is reversible with glibenclamide. *CV Res.* 27:617-622, 1993.
65. Humphrey, W.R., Simmons, C.A., Toombs, C.F., and Shebuski, R.J. Induction of smooth muscle cell proliferation by coronary angioplasty balloon over-inflation: Comparison of feeder pigs to Yucatan mini-pigs. *Am. Heart J.* 127:20-31, 1994.
66. Norman, N.R., Toombs, C.F., Khan, S.A., Buchanan, L.V., Cimini, M.G., Gibson, J.K., Meisheri, K.D., and Shebuski, R.J. Comparative effects of the potassium channels openers cromakalim and pinacidil and the cromakalim analog, U-89232, on isolated vascular and cardiac tissue. *Pharmacology* 49: 86-95, 1994.
67. Toombs, C.F., Moore, T.L., and Shebuski, R.J. Cardioprotection with U-89232 is not reversible with glibenclamide: Pharmacological characterization of a novel anti-ischemic agent derived from cromakalim. *Pharmacology* 49: 96-104, 1994.
68. Hartman, J.C., Kurc, G.M., Hullinger, T.G., Wall, T.M., Sheehy, R.M., and Shebuski, R.J. Inhibition of nitric oxide synthase prevents myocardial protection by ramiprilat. *J. Pharmacol. Exp. Ther.* 270: 1071-1076, 1994.

69. Erickson, L.A., Bonin, P.D., Wishka, D.G., Morris, J., Dalga, R.J., Williams, D.J., Wilson, G.J., Hoover, J.L., Simmons, C.A., Humphrey, S.J., and Shebuski, R.J. In vitro and in vivo inhibition of rat vascular smooth muscle cell migration and proliferation by a 2-aminochromone U-86983. *J. Pharmacol. Exp. Ther.* 271: 415-421, 1994.
70. Morris, J., Wishka, D.G., Humphrey, W.R., Lin, A.H., Wiltse, A.L., Benjamin, C.W., Gorman, R.R. and Shebuski, R.J. Synthesis and biological activity of a potent antiplatelet 7-amino-furochromone. *Bioorganic and Medicinal Chemistry Letters.* 4(21): 2621-2626, 1994.
71. Toombs, C.F. and Shebuski, R.J. U89232 is a novel anti-ischemic agent derived from cromakalim. *Cardiovascular Drug Reviews.* 12(4): 303-316, 1995.
72. Virkhaus, R., Lucchesi, B., Simpson, P.J. and Shebuski, R.J. The role of adhesion molecules in cardiovascular pharmacology; meeting report. *J. Pharmacol. Exp. Ther.* 273: 569-575, 1995.
73. Labhasetwar, V., Song, C., Humphrey, W., Shebuski, R., and Levy, R. Nanoparticles for site specific delivery of U-86983 in restenosis in pig coronary arteries. *Proceed. Intern. Symp. Control. Rel. Bioact. Mater.* 22-25, 1995.
74. Hartman, J.C., Anderson, D.C., Wiltse, A.L., Lane, C.L., Rosenbloom, C.L., Manning, A.M., Humphrey, W.R., Wall, T.M. and Shebuski, R.J. Protection of ischemic/reperfused canine myocardium by CLI8/6, a monoclonal antibody to adhesion molecule ICAM-1. *CV Res.* 30:47-54, 1995.
75. Toombs, C.F., DeGraaf, G.L., Martin, J.P., Geng, J.-G., Anderson, D.C., and Shebuski, R.J. Inhibition of P-selectin with the GA6 monoclonal antibody accelerates pharmacological thrombolysis in a primate model of arterial thrombosis. *J. Pharmacol. Exp. Ther.* 275: 941-949, 1995.
76. Morris, J. and Shebuski, R.J. Small molecule approaches to prevention of restenosis. *Current Pharmaceutical Design.* 1: 469-482, 1995.
77. Manning, A.M., Bell, F.P., Rosenbloom, J.G., Simmons, C.A., Northrup, J.L., Shebuski, R.J., Dunn, C.J., and Anderson, D.C. NF- κ B is activated during acute inflammation in vivo in association with elevated endothelial cell adhesion molecule gene expression and leukocyte recruitment. *J. Inflammation.* 45: 283-296, 1995.
78. Hartman, J.C., Sheehy, R.M., Wiltse, A.L., McGrath J.P., Dunn, C.J., and Shebuski, R.J. Dichloromethylenediphosphonic tetra-acid targets ischemic/reperfused myocardium. *Drug Delivery.* 2: 190-197, 1995.
79. Leadley, R.J., Humphrey, W.R., Erickson, L.A., and Shebuski, R.J. Inhibition of thrombus formation by endothelin-1 in canine models of arterial thrombosis. *Throm. and Haemo.* 74(6): 1583-90, 1995.

80. Mills, C., Northrup, J., Hullinger, T., Simmons C., Shebuski, R., and Jones, D. Temporal expression of *c-fos* mRNA following balloon injury in the rat common carotid artery. *CV Res.*, 32:954-961, 1996.
81. Fedan, J.S. and Shebuski, R.J. Anticoagulant, antiplatelet and fibrinolytic (thrombolytic) drugs. *Modern Pharmacology* Ed: Craig and Stitzel, Little, Brown and Co. 5th Edition, 269-278, 1997.
82. Song, C.X., Labhasetwar, V., Murphy, H., Qu, X., Humphrey, W.R., Shebuski, R.J., and Levy, R.J. Formulation and characterization of biodegradable nanoparticles for intravascular local drug delivery. *Journal of Controlled Release.* 43:197-212, 1997.
83. Humphrey, W.R., Erickson, L.A., Simmons, C.A., Northrup, J.L., Wishka, D.G., Morris, J., Labhasetwar, V., Song, C., Levy, R.J., and Shebuski, R.J. The effect of intramural delivery of polymeric nanoparticles loaded with the antiproliferative 2-aminochromone U-86983 on neointimal hyperplasia development in balloon-injured porcine coronary arteries. *Advanced Drug Delivery Reviews.* 24:87-108, 1997.
84. Kumar, A., Hoover, J.L., Simmons, C.A., Lindner, V., and Shebuski, R.J. Remodeling and neointimal formation in the carotid artery of P-selectin-deficient mice. *Circulation.* 96:4333-4342, 1997.
85. Hullinger, T.G., Jain, K.M., Hartman, J.C., Kumar, A. and Shebuski, R.J. Role of P-selectin in thrombosis and vascular injury. Academic Press. In: *Cell Adhesion Molecules and Matrix Proteins: Role in Health and Diseases*, edited by Shaker A. Mousa, Springer – Verlag and R. G. Landes Company, pgs. 192 –201, 1998.
86. Labhasetwar, V., Song C., Humphrey, W., Shebuski, R., and Levy R.J. Arterial uptake of biodegradable nanoparticles: effect of surface modifications. *J. Pharm. Sci.* 10:1229-34, 1998.
87. Porcari, A.R. and Shebuski, R.J. Functional Genomics: Meeting Report of an ASPET Colloquium. *J. Pharmacol. Exp. Ther.* 295:1291-1295, 2000.
88. Shebuski, R.J. and Ohlstein E.H. Cardiovascular and renal pharmacology: Editorial overview. *Curr. Opin. Pharmacol.* 1(2):107-108, 2001.
89. Shebuski, R.J. and Kilgore, K.S. Role of Inflammatory Mediators in Thrombogenesis. *Perspectives in Pharmacology (PIP), J. Pharmacol. Exp. Ther.*, 300:729-735, 2002.
90. Shebuski, R.J. Utility of point-of-care diagnostic testing in patients with chest pain and suspected acute myocardial infarction. *Curr. Opinion Pharmacol.* 2(2):160-164, 2002.
91. Shebuski, R.J., Bush, L.R., Gagnon, A, Chi, L and Leadley, R.J., Jr. Development and Applications of Animal Models of Thrombosis. Humana Press. In: *Methods in Molecular Medicine: Anticoagulants, Antiplatelets, and Thrombolytics*, edited by Shaker A. Mousa, pgs. 175–219, 2004.

92. Maalej, N., Osman, H.E., Shanmuganayagam, D., Shebuski, R.J. and Folts, J.D.: Antithrombotic properties of the thromboxane A-2/prostaglandin H-2 receptor antagonist, S1886 on prevention of platelet-dependent cyclic flow reductions in dogs. *J. Cardiovasc Pharmacol* 45(5): 389-95, 2005.
93. Shebuski, RJ, Joshi K, Pande A, Sharma R, Prashar Y, Kapeghian J, Sahni G. Preclinical Safety and Efficacy of a Novel Thrombolytic Agent Administered by Rapid Bolus Injection: Clot Specific Streptokinase (CSSK/SMRX-11). *Circulation*. 132 (Suppl 3): A12419, 2015. http://circ.ahajournals.org/content/132/Suppl_3/A12419.

Abstracts

1. Aiken, J.W. and Shebuski, R.J. Analysis of the potency of thromboxane A₂ and prostaglandin H₂ for contracting isolated aortic strips of the rat. Seventh International Congress of Pharmacology, Paris, p. 805, 1978.
2. Folts, J.D., Gallagher, K.P., Shebuski, R.J. and Rowe, G.G. Effects of dipyridamole on total regional blood flow in normal and stenosed coronary arteries in dogs. *Fed. Proc.* 37:318, 1978.
3. Gallagher, K.P., Folts, J.D., Rankin, J.H.G., Shebuski, R.J. and Rowe, G.G. Myocardial blood flow with four degrees of coronary stenosis. *Fed. Proc.* 37:486, 1978.
4. Gallagher, K.P., Folts, J.D., Shebuski, R.J. and Rowe, G.G. Epicardial vasodilatory reserve in the presence of "critical stenosis". *Am. J. Cardiology* 41:363, 1978.
5. Aiken, J.W. and Shebuski, R.J. Relative effectiveness of locally produced versus circulating prostacyclin in preventing blockage of partially obstructed dog coronary arteries with platelet thrombi. *Fed. Proc.*, 38:751, 1979.
6. Hemker, D.P., Shebuski, R.J. and Aiken, J.W. A paradoxical antiaggregatory effect of adenosine 5'-diphosphate in dogs correlates with release of a prostacyclin-like substance. *Fed. Proc.* 38:751, 1979.
7. Aiken, J.W., Shebuski, R.J. and Gorman, R.R. Blockage of partially obstructed coronary arteries with platelet thrombi: Comparison between cyclooxygenase inhibitors versus prostacyclin. Fourth International Prostaglandin Conference Abstracts, p. 1, 1979.
8. Miller, O.V., Aiken, J.W., Shebuski, R.J. and Gorman, R.R. Prostacyclin stimulation of dog arterial cyclic AMP levels. Fourth International Prostaglandin Conference Abstracts, p. 81, 1979.
9. Shebuski, R.J. and Aiken, J.W. Angiotensin II induced renal prostacyclin release suppresses platelet aggregation in the anesthetized dog. Fourth International Prostaglandin Conference Abstracts, p. 106, 1979.
10. Aiken, J.W., Shebuski, R.J. and Gorman, R.R. Local prostacyclin synthesis accounts for the prevention of thrombosis in dog coronary arteries by a thromboxane synthetase inhibitor. Arachidonate Metabolites as Modulators of Cell Function. Abstracts for Satellite Symposium of Eight International Congress of Pharmacology, p. 8, 1981.
11. Aiken, J.W., Shebuski, R.J., Miller, O.V. and Gorman, R.R. Endogenous prostacyclin contributes to the efficacy of a thromboxane synthetase inhibitor for preventing coronary artery thrombosis. Abstracts, Eighth International Congress of Pharmacology, Tokyo, p. 741, 1981.

12. Shebuski, R.J. and Zimmerman, B.G. Comparison of urapidil to alpha₂- agonists on the response of transmurally stimulated guinea pig ileum. *Fed. Proc.* 42:617, 1983.
13. Shebuski, R.J. and Zimmerman, B.G. Suppression of reflex tachycardia by urapidil in conscious dogs. *Fed. Proc.*, 43:734, 1984.
14. Shebuski, R.J. and Zimmerman, B.G. Central administration of alpha₁- adrenoceptor antagonists suppresses reflex tachycardia (RT) in anesthetized dogs. *The Pharmacologist* 26(3):236, 1984.
15. Shebuski, R.J. and Zimmerman, B.G. Unmasking of the alpha- receptor blocking action of urapidil by prior treatment with the selective alpha₂- antagonist, rauwolscine. *Fed. Proc.* 44:879, 1985.
16. Fujita, T. and Shebuski, R.J. Suppression of sympathetic ganglionic neurotransmission by SK&F R-82526 and reversal by the selective dopamine-1 (DA-1) receptor antagonist, SK&F 83566. *The Pharmacologist* 27:215, 1985.
17. Shebuski, R.J., Fujita, T. and Ruffolo, R.R., Jr. Identification of alpha- adrenoceptor subtypes in the pulmonary circulation of the dog. *Fed. Proc.*, 45:1127, 1986.
18. Ruffolo, R.R., Jr., Fujita, T. and Shebuski, R.J. Alpha₂- adrenoceptor activity of dopamine, ibopamine and epinine in the pulmonary circulation of the dog. *Fed. Proc.* 45:555, 1986.
19. Shebuski, R.J. and Ruffolo, R.R., Jr. Effect of ibopamine, a new orally active positive inotropic agent, in the pulmonary circulation of the dog. *X World Congress of Cardiology Abstracts*, p. 186, 1986.
20. Shebuski, R.J., Ohlstein, E.H., Smith, J.M., Jr. and Ruffolo, R.R., Jr. Enhanced responsiveness of pulmonary alpha₂- adrenoceptors under conditions of elevated pulmonary vascular tone. *The Pharmacologist* 28:141, 1986.
21. Ruffolo, R.R., Jr., Smith, J.M., Jr. and Shebuski, R.J. Interaction of ibopamine and epinine with alpha- and beta- adrenoceptors in the pulmonary circulation of the dog. *The Pharmacologist* 28:106, 1986.
22. Smith, J.M., Jr., Ruffolo, R.R., Jr. and Shebuski, R.J. Comparison of the renal and pulmonary effects of fenoldopam, dobutamine, dopamine and norepinephrine in the dog. *The Pharmacologist* 28:104, 1986.
23. Ohlstein, E.H., Shebuski, R.J. and Ruffolo, R.R., Jr. Localization of alpha₂- adrenoceptors in the canine pulmonary vasculature. *The Pharmacologist* 28:141, 1986.
24. Fujita, T., Ruffolo, R.R., Jr. and Shebuski, R.J. Comparisons of the alpha- and beta- adrenergic effects of dopamine (DA) and dobutamine (DB) in the pulmonary circulation of the dog. *The Pharmacologist* 28:151, 1986.

25. Shebuski, R.J., Smith, J.M., Jr. and Ruffolo, R.R., Jr. Enhancement of pulmonary alpha₂-adrenoceptor responsiveness under conditions of elevated pulmonary vascular tone. *Circulation* 74:II-90, 1986.
26. Nichols, A.J., Shebuski, R.J. and Ruffolo, R.R., Jr. Comparison of the DA-1 and DA-2 receptor mediated effects of ibopamine and epinine in the anesthetized dog. *Fed. Proc.* 46:560, 1987.
27. Ohlstein, E.H., Storer, B., Fujita, T. and Shebuski, R.J. Streptokinase (SK) and tissue plasminogen activator (t-PA) induce platelet hyperaggregability. *Fed. Proc.* 46:422, 1987.
28. Smith, J.M., Jr., Ruffolo, R.R., Jr. and Shebuski, R.J. Effects of fenoldopam (F) on enhanced alpha-2 adrenoceptor responsiveness under conditions of elevated pulmonary vascular tone. *Fed. Proc.* 46:1111, 1987.
29. Fujita, T. and Shebuski, R.J. Thrombolytic effect of tissue type plasminogen activator (t-PA) in a rabbit model of peripheral arterial thrombosis. *Fed. Proc.* 46:1145, 1987.
30. Shebuski, R.J., Fujita, T., Storer, B. and Ohlstein, E.H. Loss of the inhibitory effect of prostacyclin (PGI₂) on platelet function after tissue plasminogen activator (t-PA) or streptokinase (SK) infusion in the rabbit. *Fed. Proc.* 46:422, 1987.
31. Ruffolo, R.R., Jr., Shebuski, R.J. and Nichols, A.J. Characterization of the renal vasodilation produced by the inotropic pro-drug, ibopamine, and its active form, epinine. *The Pharmacologist* 29:135, 1987.
32. Shebuski, R.J. Effect of a thromboxane A₂ (TXA) receptor antagonist (TRA) alone and in combination with a thromboxane synthase inhibitor (TSI) on cyclic flow reductions (CFRs) in stenosed coronary arteries. *Circulation* 76:IV-101, 1987.
33. Ohlstein, E.H. and Shebuski, R.J. Tissue-type plasminogen activator (tPA) increases plasma thromboxane levels which is associated with platelet hyperaggregation. *Circulation* 76:IV-100, 1987.
34. Shebuski, R.J. and Ohlstein, E.H. Attenuation of platelet responsiveness to prostacyclin (PGI₂) after tissue-type plasminogen activator (tPA). *Circulation* 76:IV-338, 1987.
35. Hasan, S., Storer, B., Fujita, T., Edmunds, T., Gillies, S. and Shebuski, R.J. Comparison of the fibrinolytic efficacy of Chinese hamster ovary (CHO)-derived tPA to mouse myeloma (MM)-derived tPA *in vitro* and *in vivo*. *FASEB Journal* 2:A391, 1988.
36. Fujita, T. and Shebuski, R.J. Effect of thromboxane synthase inhibition (TSI) on the thrombolytic action of tissue plasminogen activator (tPA) in the rabbit. *FASEB Journal* 2:A391, 1988.
37. Smith, J. and Shebuski, R.J. Lack of an inhibitory effect of tissue plasminogen activator (tPA) on platelet aggregation *in vivo*. *FASEB Journal* 2:A1164, 1988.

38. Ohlstein, E.H., Horohonich, S., Storer, B., Fujita, T. and Shebuski, R.J. Effect of thromboxane synthase inhibition on tissue plasminogen activator (tPA)-induced platelet activation in rabbits. *FASEB Journal* 2:A391, 1988.
39. Shebuski, R.J., Smith, J., Hasan, S. and Fujita, T. Influence of the selective thromboxane receptor antagonist (TRA), BM 13.177, on tPA-induced thrombolysis in animal models of arterial thrombosis. *FASEB Journal* 2:A392, 1988.
40. Connors, R.W., Trill, J., Sweet, R., Shebuski, R.J. and Reff, M. Over-expression of t-PA in bovine endothelial cells and characterization of the protein product. *FASEB Journal* 2:A392, 1988.
41. Fong, K.L.L., Malinoski, L.M., Fujita, A., Chen, T.K., Reff, M., Crysler, C.S., Boyle, K.E., Oka, M., Mai, S., Williams, D., Johansen, H., Mico, B.A. and Shebuski, R.J. Comparative pharmacokinetics of recombinant tissue-type plasminogen activator (tPA) derived from five different cell lines. *Ninth International Congress on Fibrinolysis* 3:29, 1988.
42. Connors, R.W., Sweet, R.W., Noveral, J., Pfarr, S., Trill, J., Shebuski, R., Berkowitz, B.A., Williams, D., Franklin, S. and M. Reff. DHFR Coamplification of t-PA in DHFR positive bovine endothelial cells: *In Vitro* characterization of the purified serine protease. *Ninth International Congress on Fibrinolysis* 2, 11, 1988.
43. Shebuski, R.J., van Erkelens, K., Uebis, R., Etti, H. and Kondor, P. Effect of the selective thromboxane receptor antagonist (TRA) BM 13.177 on coronary thrombolysis in a dog model and in patients with acute myocardial infarction (AMI). *Ninth International Congress on Fibrinolysis*, 2, 5, 1988.
44. Shebuski, R.J. and Smith, J.M. Comparison of aspirin to a thromboxane receptor antagonist on lysis time and reocclusion rate following tPA-induced coronary thrombolysis in the dog. *The Physiologist*, 31:A96, 1988.
45. Reff, M., Trill, J., Fujita, T. Fong, K.L., Cotter, L., Storer, B. and Shebuski, R.J. Pharmacological efficacy and pharmacokinetics of a finger-protease (FP) tPA mutant in a rabbit model of peripheral arterial thrombosis. *J. Cell Biol.* 107:617a, 1988.
46. Granett, J.R., Sulpizio, A., Smith, E.F., Shebuski, R.J., Ohlstein, E.H., Hieble, J.P., Kopia, G.A., Nichols, A.J. and Ruffolo, R.R., Jr. Sulotroban (BM 13.177) is not a partial agonist at vascular thromboxane A₂ receptors. *FASEB Journal* 3:A606, 1989.
47. Shebuski, R.J. and Kopia, G.A. Enhancement of thrombolysis with the thromboxane (Tx) receptor antagonist, sulotroban (BM 13.177, SKF 95587). *J. Mol. Cell. Cardio.* 21:S196, 1989.
48. Shebuski, R.J., Sitko, G.A., Claremon, D.A., Baldwin, J.J., Remy, D.C. and Stern, A.M. Acceleration of tPA-induced thrombolysis by inhibitors of factor XIIIa in a canine model of coronary arterial thrombosis. *Circulation*, 80:II-217, 1989.

49. Shebuski, R.J., Ramjit, D.R., Lumma, P.K. and Garsky, V.M. Prevention of canine coronary artery thrombosis with echistatin, a potent inhibitor of platelet aggregation from the venom of the viper, Echis carinatus. *Circulation*, 80:II-645, 1989.
50. Shebuski, R.J., Ramjit, D.R., Bencen, G., Polokoff, M.A. Effect of bitistatin, a potent RGD-containing peptide from the venom of Bitis Arietens, on platelet aggregation and bleeding time in the dog. *Circulation* 80:II-422, 1989.
51. Niewiarowski, S., Huang, T.-F., Rucinski, B., Cook, J.J., Williams, J.A., Musial, J., Edmunds, L.H., Jr., Gould, R.J., Bush, L., Shebuski, R. and Friedman, P.A. Potential application of RGD-containing peptides from viper venoms (disintegrins) in antiplatelet therapy. *Thromb. Haemo.* 62:1989.
52. Oka, M., Fong, K.L., Carr, S., Chen, T., Fujita, T., Strickler, J., Mai, S. and Shebuski, R. Characterization and biological properties of a recombinant protein produced in *Drosophila* cell culture. *Jap. Assoc. Animal Cell Technology*, 1989.
53. Kopia, G.A., Sulpizio, A., Smith, E.F., Shebuski, R.J., Ohlstein, E.H., Hieble, J.P., Nichols, A.J. and Ruffolo, R.R. The thromboxane receptor antagonist BM 13.177 (sulotroban) is not a partial agonist. *J. Mol. Cell. Pharmacol.*, 21(Supl. II):S195, 1989.
54. Ramjit, D.R., Fujita, T., Smith, D., Dunwiddie, C., Waxman, L., Vlasuk, G. and Shebuski, R.J. Effectiveness of recombinant tick-derived anticoagulant peptide (rTAP) and antistatin (rATS) in the prevention of thromboplastin (TP)-induced clot formation in the jugular vein of the rabbit. *FASEB Journal* 4:A1229, 1990.
55. Holahan, M.A., Mellott, M.J., Garsky, V.M. and Shebuski, R.J. Prevention of reocclusion following t-PA-induced thrombolysis by the RGD-containing peptide, echistatin, in a canine model of coronary thrombosis. *FASEB Journal* 4:A1233, 1990.
56. Mayer, E.J., Fujita, T., Gardell, S.J., Shebuski, R.J. and Reilly, C.F. The catabolism of active and latent plasminogen activator inhibitor-1 *in vivo*. *FASEB Journal* 4:A1233, 1990.
57. Reilly, C.F., Mayer, E.J., Hutzelmann, J.E., Sitko, G.R. and Shebuski, R.J. Characterization of a recombinant plasminogen activator inhibitor-1 *in vitro* and *in vivo*. *FASEB Journal* 4:A1233, 1990.
58. Shebuski, R.J., Sitko, G.R., Stabilito, I.J. and Polokoff, M.A. Acceleration of tPA-induced thrombolysis and prevention of reocclusion by the combination of heparin and bitistatin in a canine model of coronary thrombosis. *FASEB Journal* 4:A1233, 1990.
59. Shebuski, R.J., Fujita, T., Ramjit, D.R., Stabilito, I.J., Cuca, G.C., Gould, S.L., Wang, S., Tung, J.-S., Mark, G.E., Ellis, R.W., Silberklang, M., and Gardell, S.J. Thrombolytic efficacy of i.v. bolus vampire bat salivary plasminogen activator (bPA) in a rabbit model of femoral arterial thrombosis: Comparison to tissue-type plasminogen activator (tPA). *Fibrinolysis*, 4(3):97, 1990.

60. Shebuski, R.J., Siegfried, M.E., and Stern, A.M. Acceleration of streptokinase(SK)-induced thrombolysis and decreased thrombus mass in response to factor XIIIa inhibition in a canine model of femoral venous thrombosis. *Fibrinolysis*, 4(3):23, 1990.
61. Reilly, C.F., Fujita, T., and Shebuski, R.J. The effect of plasminogen activator inhibitor-1 on clot dissolution in the canine lung. *Fibrinolysis*, 4(3):173, 1990.
62. Vlasuk, G.P., Sitko, G.R., Smith, D.E., and Shebuski, R.J. Effect of selective factor Xa inhibition on the rate and incidence of tPA-induced reperfusion in a canine model of coronary arterial thrombosis. *Fibrinolysis*, 4(3):8, 1990.
63. Vlasuk, G.P., Sitko, G.R., and Shebuski, R.J. Specific factor Xa inhibition enhances thrombolytic reperfusion and prevents acute reocclusion in the canine copper coil model of arterial thrombosis. *Circulation*, 82(4):III-603, 1990.
64. Wiltse, A.L., Humphrey, W.R., and Shebuski, R.J. Interaction of the 21-aminosteroid, U-74006F, with tissue-type plasminogen activator (tPA), aspirin and heparin in a canine model of coronary thrombosis. *The Pharmacologist*. 33(3):147, 1991.
65. Hartman, J., Wall, T., Hullinger, T., Williams, L., and Shebuski, R. Ramiprilat reduces myocardial infarct size in a rabbit model of ischemia/reperfusion. *FASEB J*. 6(4):A943, 1992.
66. Toombs, C.F., Norman, N.R., Gadwood, R.C., and Shebuski, R.J. Cromakalim pretreatment reduces infarct size in a rabbit model of regional ischemic-reperfusion injury. *J. Mol. Cell. Cardiol*. 24(Supplement III):S11, 1992.
67. Toombs, C.F., Norman, N.R., and Shebuski, R.J. Reduction of infarct size with U-89232: A non-vasoactive analog of the potassium channel opener cromakalim. *Circulation*. 86(4):I-556, 1992.
68. Wall, T., Linseman, D., Shebuski, R., and Hartman, J. Ramiprilat and DES-ARG bradykinin attenuate hypoxic injury in isolated cardiac myocytes. *FASEB J*. 7(3):A120, 1993.
69. Hullinger, T., Wall, T., Shebuski, R., and Hartman, J. Reduction of myocardial infarct size by ramiprilat is independent of angiotensin II synthesis inhibition. *FASEB J*. 7(3):A120, 1993.
70. Hartman, J., Wall, T., Hullinger, T., and Shebuski, R. Role of bradykinin in myocardial protective action of ramiprilat. *FASEB J*. 7(3):A92, 1993.
71. Humphrey, W.R., Simmons, C.A., Toombs, C.F. and Shebuski, R.J. Induction of neointimal proliferation by rupture of the internal elastic lamina (IEL) following balloon over-inflation in porcine coronary arteries. *FASEB J*. 7(3):A271, 1993.
72. Moore, T.L., Toombs, C.F., and Shebuski, R.J. Myoprotection with the cromakalim analog U-89232 is not reversible with glyburide: Characterization of a novel anti-ischemic agent. *FASEB J*. 7(3):A118, 1993.
73. Toombs, C.F., Moore, T.L. and Shebuski, R.J. Limitation of infarct size in the rabbit by

- ischemic preconditioning is reversible with glyburide. *FASEB J.* 7(3):A418, 1993.
74. Wiltse, A.L., Toombs, C.F., and Shebuski, R.J. Ischemic preconditioning fails to limit infarct size in reserpinized rabbit myocardium. *FASEB J.* 7(3):A418, 1993.
 75. Leadley, R.J., Humphrey, W.R., Wiltse, A.L., Erickson, L.A., and Shebuski, R.J. Endothelin-1 delays thrombus formation induced by electrolytic injury in the stenosed canine coronary artery. *FASEB J.* 7(3):A475, 1993.
 76. Shebuski, R.J., Erickson, L.A., Humphrey, W.R., and Leadley, R.J. Mechanism of endothelin-mediated inhibition of acute platelet thrombus formation in the stenosed canine coronary artery. *FASEB J.* 7(3):A475, 1993.
 77. Morris, J., Wishka, D.G., Gammill, R.B., Bisaha, S.N., Judge, T.M., Erickson, L.A., Bonin, P.D., Boadt, J.A., and Shebuski, R.J. Synthesis and structure activity relationships of antiproliferative 2-aminochromones. *The Pharmacologist*, 35:154, 1993.
 78. Simmons, C.A., Humphrey, S.J., Hoover, J.L., Dalga, R.J., Williams, D.J., Wilson, G.J., Wishka, D.G., Morris, J., and Shebuski, R.J. Antiproliferative effects of U86983 in the rat carotid balloon dilatation model. *The Pharmacologist*, 35:155, 1993.
 79. Hartman, J.C., Kurc, G.M., Hullinger, T.G., and Shebuski, R.J. Role of nitric oxide in the myocardial protective action of ramiprilat. *Circulation*, 88:I-330, 1993.
 80. Wiltse, A., Hartman, J., Hoover, J., McGrath, J., and Shebuski, R. Dichloromethylenediphosphonic acid targets balloon inflation injured carotid artery in the rat. *FASEB Journal* 8(4):A318, 1994.
 81. Sheehy, R., Wiltse, A., McGrath, J., Dunn, C., Shebuski, R., and Hartman, J. Dichloromethylenediphosphonic acid targets ischemic/reperfused myocardium in the rabbit. *FASEB Journal* 8(5):A856, 1994.
 82. Wall, T., Linseman, D., Shebuski, R., and Hartman, J. Preconditioning cardiomyocytes before metabolic inhibition improves viability. *FASEB Journal* 8(5):A855, 1994.
 83. Hartman, J., Wall, T., Sheehy, R., and Shebuski, R. Role of bradykinin in myocardial preconditioning. *FASEB Journal* 8(5):A829, 1994.
 84. Simmons, C., Hoover, J., Humphrey, W., Geng, J., Toombs, C., Hullinger, T., Hartman, J., Anderson, D., and Shebuski, R. Immunohistochemical distribution of P-selectin (CD62p) in rat carotid and porcine coronary arteries after vascular injury. *FASEB Journal* 8(4):A537, 1994.
 85. Toombs, C.F., DeGraaf, G.L., Anderson, D.C., and Shebuski, R.J. In vivo inhibition of P-selectin with the GA6 monoclonal antibody accelerates thrombolysis in a primate model of arterial thrombosis. *FASEB Journal* 8(4):A268, 1994.
 86. Morris, J., Wishka, D.G., Humphrey, W.R., Lin, A.H., Wiltse, A.L., Benjamin, C.W., Gorman, R.R., and Shebuski, R.J. Synthesis and biological activity of a potent antiplatelet 7-

- aminofurochromone. National Meeting of the American Chemical Society, Washington, D.C. MEDI 209 1994.
87. Shebuski, R.J., Humphrey, W.R., Simmons, C.A., Hoover, J.L., DeGraaf, G.L., Geng, J.G., Toombs, C.F., and Anderson, D.C. Role of P-selectin in animal models of thrombosis and restenosis. *Circulation*. 90(4), Part 2: I-142, 1994.
 88. Humphrey, W.R., Simmons, C.A., Northrup, J.L., Wishka, D.G., Morris, J., Labhassetwar, V., Song, C., Levy, R.J., and Shebuski, R.J. Site-specific delivery of polymeric nanoparticles (PNP) loaded with the 2-aminochromone U-86983 can retard intimal hyperplasia in balloon-injured porcine coronary arteries. *FASEB Journal* 9(3): A342, 1995.
 89. Hullinger, T.G., DeGraaf, G.L., Hartman, J.C., and Shebuski, R.J. The effect of P-selectin blockade on neointimal lesion development in a primate carotid injury model. *FASEB Journal* 9(4):A845, 1995.
 90. Northrup, J., Rosenbloom, C., Manning, A., Geng, J.G., Anderson, D., Shebuski, R., and Simmons, C. Immunohistochemical localization of P-selectin and ICAM-1 in a rat model of endotoxic shock. *FASEB Journal* 9(4):A886, 1995.
 91. DeGraaf, G.L., Hartman, J.C., Steele, A.N., Anderson, D.C., Martin, J.P., Herberg, J.T., Kirschner, R.J., Treat, J.C., Beiderman, B., Phillips, M.L., and Shebuski, R.J. Antagonism of P-selectin with CY1748 accelerates thrombolysis in a primate model of arterial thrombosis. *Circulation*. 92:1-487, 1995.
 92. Jain, K., Hullinger, T.G., Humphrey, W.R., and Shebuski, R.J. P-selectin antagonism with the monoclonal antibody, CY1747, prevents venous thrombosis in vivo. *Circulation*. 92:1-487, 1995.
 93. Mills, C., Hullinger, T., Shebuski, R., and Jones, D. Temporal expression of *c-fos* mRNA following balloon injury in the rat common carotid artery. *FASEB. Journal* 10(3):A619, 1996.
 94. Kumar, A. and Shebuski, R.J. Thrombosis in normal and hypercholesterolemic rabbits in response to electrolytic injury. *Ann. Biomed. Eng.* 24(Suppl):S-39, 1996.
 95. Simmons, C.A., Humphrey, W.R., Mills, C.J., Northrup, J.L., and Shebuski, R.J. Inhibition of P-selectin-mediated heterotypic aggregation of platelets and neutrophils: detection by a novel immunohistochemical method. *J. Vasc. Res.*, 33(1):368, 1996.
 96. Kumar, A. and Shebuski, R.J. Assessment of thrombogenicity of carotid arteries in normal and hypercholesterolemic rabbits. *The Pharmacologist*. 39:27, 1997.

97. Kumar, A., Hoover, J.L., Simmons, C.A., Lindner, V., and Shebuski, R.J. Remodeling and neointima formation in the carotid of normal and P-selectin deficient mice. *FASEB Journal*. 11:A638, 1997.
98. Shebuski, R.J., DeGraaf, G.L., Heward, J.E., Stryd, R.P., Peng, G.W., Kirschner, R.J., Martin, J.P., Herberg, J.T., Wolf H.J., Johnson, C.W. Efficacy of CY1748, a monoclonal antibody directed against P-selectin, on deep vein thrombosis in cynomolgus monkeys. *Eur. Heart J*. 18:53, 1997.
99. Wall, T.M., Helmer, K.E., Hartman, J.C., Shebuski, R.J. Preservation of coronary artery endothelial-mediated vasodilation by chronic oral administration of probucol in a unique *in-vivo* rabbit model of cholesterol-induced endothelial dysfunction. *Circulation*. 96(8):I-608, 1997.
100. Kumar, A., Hoover, J.L., Simmons, C.A., Humphrey, W.R., Shebuski, R.J. Role of inflammatory cells and P-selectin following vascular injury. *Circulation*. 96(8):I-493, 1997.
101. Shanmuganayagam, D., Beahm, M.R., Shebuski, R.J. Folts, J.D. Anticoagulation with D-phenylalanine-proline-arginine chloromethyl ketone enables more sensitive and physiological measurement of platelet function when compared to sodium citrate. *FASEB Journal*, 2002.