

**UNITED STATES PATENT AND TRADEMARK OFFICE**

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**BEFORE THE PATENT TRIAL AND APPEAL BOARD**

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GlaxoSmithKline LLC,  
Petitioner

v.

FibroGen, Inc.,  
Patent Owner

U.S. Patent No. 8,466,172

Appl. No. 12/928,119 filed December 2, 2010

Issued: June 18, 2013

Title: Stabilization of Hypoxia Inducible Factor (HIF) Alpha

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*Inter Partes* Review No. IPR2016-01315

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**DECLARATION OF PAUL SCHUMACKER, PH.D. IN SUPPORT OF  
GLAXOSMITHKLINE PETITION FOR *INTER PARTES* REVIEW  
OF U.S. PAT. NO. 8,466,172**

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Declaration of Paul T. Schumacker in Support of  
GlaxoSmithKline Petition for *Inter Partes* Review of U.S. Pat. No. 8,466,172

1. I, Paul T. Schumacker, have been retained by Mayer Brown LLP, counsel for GlaxoSmithKline LLC ("GSK"). I understand that GSK has petitioned for *inter partes* review of U.S. Patent No. 8,466,172 ("the '172 patent") and requests that the United States Patent and Trademark Office cancel Claims 1-8 of the '172 patent as unpatentable. The following discussion and analyses address the bases for GSK's petition.

**I. BACKGROUND AND QUALIFICATIONS, PREVIOUS TESTIMONY, AND COMPENSATION**

**A. Background and Qualifications**

2. I have been an academic faculty investigator-scientist studying oxygen metabolism, hypoxia, and ischemia for more than 31 years. During that time, my research has been supported by competitive grants from the National Institutes of Health, as well as by grants from private foundations, such as the American Heart Association. A major component of my work since the mid-1990s has focused on the molecular mechanisms of oxygen sensing by cells, and the mechanisms regulating the family of transcription factors known as hypoxia-inducible factors.

3. I have authored or co-authored the following papers that are directly relevant to the '172 patent:

- Waypa, G.B. *et al.* Superoxide generated at mitochondrial complex III triggers acute responses to hypoxia in the pulmonary

circulation. *Am. J. Respir. Crit Care Med.* 187, 424-432 (2013).

- Schriewer, J.M., Peek, C.B., Bass, J., & Schumacker, P.T. ROS-Mediated PARP Activity Undermines Mitochondrial Function After Permeability Transition Pore Opening During Myocardial Ischemia-Reperfusion. *J. Am. Heart Assoc.* 2, e000159 (2013).
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- Schumacker, P.T. Nitric oxide quenches the fire in heart mitochondria. *Nat. Med.* 19, 666-667 (2013).
- Mungai, P.T. *et al.* Hypoxia triggers AMPK activation through reactive oxygen species-mediated activation of calcium release-activated calcium channels. *Mol. Cell Biol.* 31, 3531-3545 (2011).
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- Schumacker, P.T. SIRT3 controls cancer metabolic reprogramming by regulating ROS and HIF. *Cancer Cell* 19, 299-300 (2011).
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- Waypa, G.B. & Schumacker, P.T. Hypoxia-induced changes in pulmonary and systemic vascular resistance: where is the O<sub>2</sub> sensor? *Respir. Physiol Neurobiol.* 174, 201-211 (2010).
- Evens, A.M. *et al.* Hypoxia-inducible factor-1 {alpha} expression predicts superior survival in patients with diffuse large B-cell lymphoma treated with R-CHOP. *J. Clin. Oncol.* 28, 1017-1024 (2010).
- Waypa, G.B. *et al.* Hypoxia triggers subcellular compartmental redox signaling in vascular smooth muscle cells. *Circ. Res.* 106, 526-535 (2010).
- Desiredi, J.R., Farrow, K.N., Marks, J.D., Waypa, G.B., & Schumacker, P.T. Hypoxia increases ROS signaling and cytosolic Ca(2+) in pulmonary artery smooth muscle cells of mouse lungs slices. *Antioxid. Redox. Signal.* 12, 595-602 (2010).
- Chi, A.Y., Waypa, G.B., Mungai, P.T., & Schumacker, P.T. Prolonged hypoxia increases ROS signaling and RhoA activation in pulmonary artery smooth muscle and endothelial cells. *Antioxid. Redox. Signal.* 12, 603-610 (2010).
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- Robin, E. *et al.* Oxidant stress during simulated ischemia primes cardiomyocytes for cell death during reperfusion. *J. Biol. Chem.* 282, 19133-19143 (2007).
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- Iwase, H. *et al.* Nitric oxide during ischemia attenuates oxidant stress and cell death during ischemia and reperfusion in cardiomyocytes. *Free Radic. Biol. Med.* 43, 590-599 (2007).
- Guzy, R.D., Mack, M.M., & Schumacker, P.T. Mitochondrial complex III is required for hypoxia-induced ROS production and gene transcription in yeast. *Antioxid. Redox. Signal.* 9, 1317-1328 (2007).
- Subramanian, R.M., Chandel, N., Budinger, G.R., & Schumacker, P.T. Hypoxic conformance of metabolism in primary rat hepatocytes: a model of hepatic hibernation. *Hepatology* 45, 455-464 (2007).
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- Schumacker, P.T. Hypoxia-inducible factor-1 (HIF-1). *Crit Care Med.* 33, S423-S425 (2005).
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- Waypa, G.B. *et al.* Mitochondrial reactive oxygen species trigger calcium increases during hypoxia in pulmonary arterial myocytes. *Circ. Res.* 91, 719-726 (2002).
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- Chandel, N.S. *et al.* Reactive oxygen species generated at mitochondrial Complex III stabilize HIF-1-alpha during hypoxia: A mechanism of O<sub>2</sub> sensing. *J. Biol. Chem.* 275, 25130-25138 (2000).
- Chandel, N.S. & Schumacker, P.T. Cellular oxygen sensing by mitochondria: old questions, new insight. *J. Appl. Physiol* 88, 1880-1889 (2000).
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- Chandel, N.S., Vander Heiden, M.G., Thompson, C.B., & Schumacker, P.T. Redox regulation of p53 during hypoxia.

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- Vanden Hoek, T.L., Becker, L.B., Shao, Z., Li, C., & Schumacker, P.T. Reactive oxygen species released from mitochondria during brief hypoxia induce preconditioning in cardiomyocytes. *J. Biol. Chem.* 273, 18092-18098 (1998).
- Chandel, N.S. *et al.* Mitochondrial reactive oxygen species trigger hypoxia-induced transcription. *Proc. Natl. Acad. Sci. USA* 95, 11715-11720 (1998).

4. I hold no patents.

5. I am currently the Patrick M. Magoon Distinguished Professor of Neonatology Research in the Department of Pediatrics at Northwestern University Feinberg School of Medicine ("FSM"). I hold joint secondary appointments in the Department of Medicine (Pulmonary) and the Department of Cell & Molecular Biology at the same Institution. I have been a tenured Professor at Northwestern University FSM since October 1, 2004.

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6. Prior to my appointment at Northwestern University FSM, I was a tenured Professor in the Department of Medicine at the University of Chicago (Section of Pulmonary and Critical Care). My appointment at the University of Chicago as an Assistant Professor (Research Associate) began in March of 1983.

7. I have received the following awards: American Thoracic Society Research Achievement Award; NIH Research Career Development Award (NIH), and multiple grants from the National Institutes of Health.

8. I have been an expert in the field of tissue and cellular hypoxia and ischemia since 1985, and in the field of HIF signaling since 1997. I am qualified to provide an opinion as to what a person of ordinary skill in the art would have understood, known, or concluded as of 2001.

9. Exhibit 1047 is my *curriculum vitae* which sets forth my education, experience, employment history, professional affiliations, and publications.

**B. Previous Testimony**

10. I have never served as an expert in any court proceedings.

**C. Compensation**

11. I am currently being compensated for my time at a rate of \$550 per hour. I have no personal financial interest in any of the entities involved in this litigation and my compensation does not depend in any way on my testimony, my conclusions, or the outcome of my analysis.

## **II. MATERIALS CONSIDERED**

12. Exhibit 1030 is a listing of the documents that I have reviewed and relied upon in connection with providing this report.

## **III. THE '172 PATENT AND FILE HISTORY**

13. I have reviewed the '172 patent and its file history. The '172 patent (Ex. 1001) describes a method for treating a hypoxic or ischemic disorder or condition in a subject by administering to the subject an effective amount of a heterocyclic carboxamide compound that stabilizes the alpha subunit of hypoxia inducible factor ("HIF $\alpha$ "). The '172 patent describes hypoxia as a state of reduced oxygen that can occur when the lungs are compromised or blood flow is reduced, and ischemia as a reduction in blood flow that can be caused by the obstruction of an artery or vein by a blood clot (thrombus), by any foreign circulating matter (embolus), or by a vascular disorder such as atherosclerosis. Ex. 1001 ('172 patent), at col. 2, lns. 34-39.

14. The '172 patent includes one independent claim, claim 1, and several dependent claims (i.e., claim 2 to 8). Independent claim 1 recites:

A method for treating a hypoxic or ischemic disorder or condition in a subject, the method comprising administering to the subject an effective amount of a heterocyclic carboxamide compound that stabilizes the alpha subunit of hypoxia inducible factor (HIF $\alpha$ ). *Id.*, at col. 63, lns. 33-37

15. The '172 Patent describes diseases or disorders associated with these

conditions, and discloses several non-limiting examples of "ischemic conditions," "ischemic disorders," "hypoxic conditions," and "hypoxic disorders." *Id.*, at col. 21, lns. 29-43; lns. 50-62. Examples of ischemic disorders or conditions include, but are not limited to, acute ischemic conditions, such as "myocardial infarction, ischemic stroke, pulmonary embolism, perinatal hypoxia, circulatory shock including, e.g., hemorrhagic, septic, cardiogenic, etc., mountain sickness, acute respiratory failure, etc.," and chronic ischemic conditions, such as "atherosclerosis, chronic venous insufficiency, chronic heart failure, cardiac cirrhosis, diabetes, macular degeneration, sleep apnea, Raynaud's disease, systemic sclerosis, nonbacterial thrombotic endocarditis, occlusive artery disease, angina pectoris, TIAs, chronic alcoholic liver disease, etc." *Id.*, at col. 21, lns. 29-40.

16. Examples of hypoxic disorder or conditions include, but are not limited to, "ischemic disorders (ischemic hypoxia) such as those listed above, wherein hypoxia results from reduced circulation; pulmonary disorders (hypoxic hypoxia) such as COPD, severe pneumonia, pulmonary edema, pulmonary hypertension, hyaline membrane disease, and the like, wherein hypoxia results from reduced oxygenation of the blood in the lungs; anemic disorders (anemic hypoxia) such as gastric or duodenal ulcers, liver or renal disease, thrombocytopenia or blood coagulation disorders, cancer or other chronic illness,

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cancer chemotherapy and other therapeutic interventions that produce anemia, and the like, wherein hypoxia results from a decreased concentration of hemoglobin or red blood cells; and altitude sickness, etc." *Id.*, at col. 21, lns. 50-63.

17. Based on the broad description and disclosure of several non-limiting examples of hypoxic and ischemic disorders and conditions, the '172 patent encompasses as a hypoxic or ischemic disorder or condition any disorder linked to low oxygen levels or low blood flow levels. The '172 patent does not appear to place any limitation on the hypoxic or ischemic disorder or condition.

18. A broad class of heterocyclic carboxamide compounds are disclosed in the '172 patent, including compounds according to formulas I (*id.*, at col. 26, lns. 26-40), II (*id.*, at col. 36, ln. 33 to col. 37, ln. 38) and III (*id.*, at col. 37, lns. 53-64), as well as heterocyclic carboxamide compounds incorporated-by-reference from other patents and publications. Examples of heterocyclic carboxamide compounds include a number of compounds previously disclosed in prior publications. For example, the '172 Patent cites Bickel (as discussed below) as an example of the use of a heterocyclic carboxamide of the invention for the treatment following induction of toxic-ischemic hepatic injury (*id.*, at col. 59, lns. 15-37). The '172 patent also states that U.S. Patent No. 6,098,730 ("Weidmann") discloses exemplary compounds that can be used in the methods of the invention (*id.*, at col.

36, lns 1-2), and lists and tests many of the compounds disclosed in the examples of Weidmann. In particular, the '172 patent discloses several tests of Compound 9 of Weidmann, which is referenced as Compound B of the '172 patent.

#### **IV. SUMMARY OF OPINIONS**

19. It is my opinion that United States Patent No. 6,093,730 (Ex. 1019), Weidmann, contains each of the claim elements, either expressly or inherently, of claims 1, 2, 3, 5, 6, and 8 of the '172 patent and therefore, these claims of the '172 patent are anticipated by this prior art reference.

20. My opinion that claims 1, 2, 3, 5, 6 and 8 are anticipated by the Weidmann patent is based on the following disclosure in the prior art as of the effective filing date of the '172 patent: The administration of heterocyclic carboxamide compounds, as inhibitors of prolyl hydroxylase, to a subject in need of treatment of ischemic conditions would result in the treatment of ischemia (Weidmann). Although not appreciated by the inventors of Weidmann, the administration of such inhibitors of prolyl hydroxylase resulted in the stabilization of HIF.

21. It is my opinion that:

- Claims 1, 2, 3, 5, and 6 of the '172 patent are rendered obvious by the combination of Ivan 2001 in view of Bickel (Ex. 1027);

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- Claims 4 and 7 of the '172 patent are rendered obvious by the combination of Ivan 2001 in view of Bickel and Semenza 2000 (Ex. 1009); and
- Claim 8 of the '172 patent is rendered obvious by the combination of Ivan 2001 in view of Bickel and Weidmann.

22. My opinion that claims 1-8 are obvious, as provided in ¶ 21, is based on the following understanding, known within the prior art as of the effective filing date of the '172 patent:

- Hypoxia inducible factor (HIF) acted as a central mediator for a broad range of protective responses triggered by hypoxia (low oxygen), including to provide oxygen to tissues (Ivan 2001, Semenza 2000).
- Compounds that stabilized HIF and increased its activity are useful in the treatment of conditions such as ischemia (Ivan 2001).
- Such compounds include heterocyclic carboxamides, which were shown to provide treatment to fibrotic and ischemic conditions (Bickel 1998).



## **V. LEGAL STANDARDS**

### **A. Legal Principles**

23. I have been advised by counsel for Petitioner on certain relevant legal principles that I accept for the purposes of my analysis. For instance, I am informed that 35 U.S.C. § 102 governs the determination of anticipation and that 35 U.S.C. § 103 governs the determination of obviousness. I have also been advised regarding certain patent law concepts such as "effective filing date" of a patent and "person of ordinary skill in the art." These legal principles and concepts are discussed herein.

### **B. Anticipation**

24. It is my understanding that for a patent claim to be invalid as anticipated, all elements of the claim must be disclosed in a single piece of prior art, either expressly or inherently. I understand that a claim element is inherent in the prior art if it is necessarily present in the prior art reference, even though a person of ordinary skill in the art (defined below) would not necessarily recognize or appreciate the presence of the inherent disclosure in the prior art at the time of the filing of the patent. Further, I understand that a prior art reference does not need to anticipate every possible embodiment within the scope of the claim: it anticipates if it discloses an embodiment that is within the scope of the claim. Moreover, it is my understanding that anticipation does not require actual

performance and/or examples in a disclosure; it only requires that a reference teach a person skilled in the art how to implement the disclosure of a reference without undue experimentation.

**C. Obviousness**

25. It is my understanding that a patent claim is invalid as obvious if the claim would have been obvious to a person of ordinary skill in the relevant art at the time the invention was made. In determining whether a patent claim is invalid because of obviousness, it is my understanding that one must consider the scope and content of the prior art, the differences between the prior art and the claimed invention, and the level of ordinary skill in the art.

26. It is my understanding that a prior art reference is pertinent to the obviousness analysis if it discloses information designed to solve the same problems faced by the patent's inventor(s) or if the reference discloses information that has obvious uses beyond its main purpose that a person of ordinary skill in the art would reasonably expect to solve the same problem(s) faced by the inventor(s).

27. In undertaking an obviousness analysis, I also understand that I may take into account the inferences and creative steps that a person of ordinary skill in the art would have employed in reviewing the prior art at the time of the invention. If the claimed invention combines elements known in the prior art and the

combination yields results that would have been predictable to a person of ordinary skill in the art at the time of the invention, then this evidence would make it more likely that the claim was obvious.

28. I understand that I should also consider whether a reason existed at the time of the invention that would have prompted a person of ordinary skill in the art in the relevant field to combine the known elements in the way the patent claim does. The reason could come from the prior art, the background knowledge of one of ordinary skill in the art, the nature of the problem to be solved, market demand, or common sense. If a reason existed at the time of the invention to combine the elements of the prior art to arrive at the claimed invention, this evidence would make it more likely that the claimed invention was obvious.

## **VI. A PERSON OF ORDINARY SKILL IN THE ART**

29. I am informed that anticipation and obviousness are analyzed from the perspective of a hypothetical person of ordinary skill in the art at the time of the invention.

30. In my opinion, a person of ordinary skill in the art related to the '172 patent would have a Ph.D. in physiology or biochemistry (or a related discipline) or a M.D. degree. In addition to one of these degrees, this person would have 1 or 2 years of research experience in cardiology, nephrology, or pulmonology. The

research experience may overlap with the period of education.

## **VII. CLAIM CONSTRUCTION AND EFFECTIVE DATE OF THE PATENT**

### **A. "Heterocyclic Carboxamide"**

31. I understand that I should apply the broadest reasonable interpretation of the claim consistent with the disclosure in the patent specification. The term "heterocyclic carboxamide" appears in each of the claims. It is my opinion that a person of ordinary skill in the art would understand the term "heterocyclic carboxamide" as used in the patent claims and specification, to simply require the existence of a heterocyclic ring and a carboxamide moiety anywhere in the compound. A carboxamide moiety has the general formula  $R-CO-NR'R''$  wherein R, R' and R'' are organic substituents or hydrogen.

### **B. The Provisional Applications' Disclosures Compared to My Understanding of the Claim Scope**

32. I understand that applicants can rely upon an earlier application(s) with the United States Patent and Trademark Office ("PTO") as the "effective filing date" of a patent application. I understand that the term "effective filing date" allows a person of ordinary skill in the art to determine whether a publication will be considered prior art to the patent application.

33. The '172 patent lists four provisional applications on its face. Ex. 1001 ('172 patent), at cover. I understand that the filing dates for these provisional

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applications may serve as the effective filing date for the claims of the '172 patent provided the provisional applications meet the written description and enablement requirements of the patent statute. I understand that an earlier filed application will meet the written description requirement provided a person of ordinary skill in the art would understand that the applicants were in possession of the full scope of the issued claims. I also understand that an earlier filed application meets the enablement requirement if a person of ordinary skill in the art can practice the full scope of the issued claims without undue experimentation.

34. As each of the issued claims of the '172 patent include the term "heterocyclic carboxamide," I understand that the provisional applications will have to meet the written description and enablement requirements for the scope of that term as described above in Section VIII.A.<sup>1</sup> I have reviewed the provisional applications and did not see the term "heterocyclic carboxamide". The provisional applications mention heterocyclic carbonyl glycine—a subset of heterocyclic carboxamides. The provisional applications provide no explanation as to what chemical element(s) of a compound are necessary for that compound to inhibit HIF prolyl hydroxylase. The provisional applications do not disclose any of the

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<sup>1</sup> When I use the term heterocyclic carboxamide in this section, I am using that term to encompass the scope I describe in section VIII.A, *supra*.

formulas that the applicants included in application no. 10/313,551, filed on December 6, 2002.

35. Without these formulas, a person of ordinary skill in the art would not know where to start looking for compounds other than those included in the provisional applications. In my opinion, without these formulas and the absence of "heterocyclic carboxamide" from the provisional applications, a person of ordinary skill in the art would not understand that the applicants were in possession of using heterocyclic carboxamide compounds as a class to stabilize HIF $\alpha$  to treat ischemia or hypoxia.

## **VIII. TECHNICAL BACKGROUND AND STATE OF THE ART**

### **A. Hypoxia and Ischemia**

36. Cells require oxygen for energy production and for the production of important cellular metabolites (i.e., building blocks) used for cellular maintenance and function. Hypoxia is a condition in which oxygen levels in cells or tissues is decreased from normal physiological levels (i.e., normoxic); when sufficiently severe this condition becomes inadequate to support normal function. Tissue hypoxia may develop because of low oxygen content of blood or because of inadequate blood flow to tissue (i.e., ischemia). Ex. 1043 (Reimer 1992), at 1875-6.

37. Low oxygen content of blood can be due to low concentration of red

blood cell hemoglobin (i.e., anemia) or due to inadequate oxygenation of hemoglobin. Inadequate oxygenation of hemoglobin may be a result of low concentration of oxygen in inhaled air (e.g. high altitude), impaired lung gas exchange in which deoxygenated venous blood is admixed with oxygenated blood, inadequate pulmonary ventilatory function, or due to cardiac or vascular disease. Ex. 1046 (Leff 1993), at 69-81.

38. Ischemia is a restriction in the blood flow to tissues that results in, among other things, the reduced delivery of oxygen and nutrients needed for cellular metabolism. This can cause the cells to transition from a normoxic condition to a hypoxic condition. Ischemia is generally caused by an obstruction or vascular disorder. Ex. 1043 (Reimer 1992), at 1878-81.

39. Tissue hypoxia or ischemia elicits a number of compensatory and potentially corrective local and systemic vascular and biochemical responses that act to restore the delivery of oxygen to tissue (e.g., by decreasing constriction in nearby blood vessels to allow increased blood flow in the region, or by stimulating the production of new microvasculature through the development of new blood vessels from preexisting blood vessels, a response known as angiogenesis), increase the efficiency of available oxygen (e.g., by decreasing tissue metabolic activity to reduce the need for oxygen), engage in anaerobic metabolism, or

increase the extraction of oxygen from red blood cells. Ex. 1009 (Semenza 2000).

Other systemic adaptations to hypoxia include an increase in the production of erythropoietin by the kidneys and liver, which in turn stimulates increased production of red blood cells in the bone marrow, and increases iron metabolism.

40. One cause of ischemia arises when blood clots dislodge from the sites where they have formed and are carried by the blood until they reach vessels that are too small to accommodate them. Consequently, they lodge at those sites and partially or completely obstruct blood flow to the tissue distal to the obstruction, causing hypoxia and ischemia. Ischemia can also arise if the smooth muscle cells in the wall of an artery or vein undergo uncontrolled constriction, a state known as vasospasm. A third cause of ischemia involves a pathological remodeling of arteries supplying blood to organs or tissues. This remodeling can decrease the caliber of the vessels and thereby limit the passage of blood flow. In the above cases where the limitation of blood flow is incomplete, reductions in blood flow may still provide sufficient oxygen and nutrients to prevent ischemic cell death. However, if the oxygen and nutrient demands of the compromised tissue increases, for example during exercise, then pain and tissue injury may ensue as the imbalance between oxygen delivery and oxygen demand becomes more severe. Ex. 1043 (Reimer 1992), at 1891-1919.



41. Certain ischemic disorders involve a temporary or transient interruption of blood flow and oxygen/nutrient supply. Such a condition can arise when therapeutic interventions restore blood flow by removing the obstruction or otherwise correcting the blood flow compromise. Indeed, the most effective treatment of ischemic disorders involves minimizing the duration or severity of the blood flow limitation. However, during ischemia certain molecules involved in the production of cellular energy accumulate because the lack of oxygen prevents their normal metabolism. When the tissue oxygen level is restored, these metabolites are rapidly processed in biochemical reactions that result in the generation of excessive levels of reactive oxygen species ("ROS"). These toxic molecules then attack the tissue and can induce cellular injury or death. The cellular injury that occurs when oxygen supply is restored to an ischemic tissue is termed *ischemia-reperfusion injury*. Ex. 1043 (Reimer 1992), at 1891-1919). Such injury is paradoxical in the sense that the standard of treatment for ischemia involves minimizing its duration, yet the restoration of blood flow can itself cause cellular injury. *Id.*, at 1931-32. Antioxidant compounds, or the expression of genes that protect cells from this oxidant stress, are highly protective against ischemia-reperfusion injury. Ex. 1042, (Ambrosio 1986)

42. Nitric oxide is a reactive molecule generated in cells by a class of

enzymes referred to as nitric oxide synthase ("NOS"). Nitric oxide is highly protective against ischemia-reperfusion injury when it is synthesized by a cell or when it is supplied to cells before or during ischemia. Ex. 1031(Bolli), at 975 (column 1). Nitric oxide protects against ischemia-reperfusion injury by suppressing the generation of ROS during reperfusion. It therefore follows that the increased expression of NOS confers protection against ischemia-reperfusion injury. Similarly, heme oxygenase 1 ("HO-1") is also known to limit oxidant stress or mitigate the damage caused by such ROS. Ex. 1026 (Lee 1997). Hypoxia can induce expression of both NOS and HO-1. Ex. 1032 (Melillo), at 1689; Ex. 1026 (Lee 1997).

43. These mechanisms can be exploited to provide cellular protection against hypoxia and ischemia by a technique known as ischemic preconditioning. Ischemic preconditioning is a phenomenon whereby brief periods of non-lethal ischemic or hypoxic stress are applied, followed by a lethal period of ischemia. These conditions simulate ischemia-reperfusion injury wherein the non-lethal ischemic or hypoxic stress induces the protective effects of, for example, NOS and HO-1, which protects against the subsequent lethal period of ischemia. Indeed, nitric oxide and HO-1 has been shown to contribute importantly to the protection against ischemia-reperfusion injury conferred by ischemic preconditioning. Ex.

1031 (Bolli); Ex. 1026 (Lee 1997)

44. If the oxygen supply to the body is decreased because of lung gas exchange dysfunction, hypo-ventilation, or decreases in the concentration of red blood cells (anemia), survival may be threatened. Those conditions elicit autonomic responses that attempt to restore oxygen supply, and molecular responses that help to protect the cells of the body from the consequences of tissue hypoxia. For example, decreases in the concentration of oxygen in arterial blood caused by these conditions evokes an increase in the rate and depth of breathing, while autonomic constriction of blood vessels in many organs acts to preserve blood flow in areas of greatest need. The activation of hypoxia-inducible factor ("HIF") leads to the increased expression of genes that contribute to the cellular, tissue, and organismal responses to these forms of hypoxia.

**B. Biological Response to Hypoxia and Ischemia**

45. HIF is a key regulator of gene expression in response to hypoxia, affecting diverse physiological responses including angiogenesis, erythropoiesis, ventilation, and cellular metabolism targeted at increasing oxygen delivery to tissues and decreasing oxygen consumption Ex. 1008 (Semenza 1998); Ex. 1009 (Semenza 2000); Ex. 1033 (Semenza 2001). HIF acts by migrating into the nucleus of the cell, where it associates with other proteins at specific sites on DNA,

known as promoters or enhancers, and causes the expression of genes in adjacent regions of DNA. Ex. 1008 (Semenza 1998); Ex. 1009 (Semenza 2000); Ex. 1033 (Semenza 2001). In the early 2000s, scientists had identified many proteins related to glucose/energy metabolism and cell proliferation/viability, erythropoiesis, iron metabolism, vascular development/remodeling and vascular tone for which HIF impacted the expression of those proteins. These proteins included ceruloplasmin, erythropoietin ("EPO"), transferrin, transferrin receptor, vascular endothelial growth factor ("VEGF"), VEGF receptor, HO-1 and nitric oxide synthase 2 ("NOS2"). Ex. 1009 (Semenza 2000), at 1984, Table 1.

46. HIF<sup>2</sup> is a heterodimer composed of  $\alpha$  and  $\beta$  subunits. As the HIF $\beta$  subunit exists in excess in the body, the activation of HIF is primarily determined by the stabilization of the  $\alpha$  subunit. Ex. 1024 (Huang 1998); Ex. 1034 (Pugh 1997); Ex. 1035 (Jiang 1996). Under normoxic conditions, HIF $\alpha$  is continuously transcribed and translated to HIF $\alpha$  protein, which is subsequently hydroxylated and rapidly degraded by an ubiquitin-proteosome pathway. Ex. 1009 (Semenza 2000). The cellular concentration of HIF is increased during hypoxia because the

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<sup>2</sup> Many publications use the term HIF and HIF-1. HIF is a broader category of proteins than HIF-1, but it would be understood that any disclosures discussing HIF are equally applicable to HIF-1 and *vice versa*.

destruction of HIF $\alpha$  is suppressed under hypoxic or ischemic conditions. Ex. 1008 (Semenza 1998); Ex. 1009 (Semenza 2000); Ex. 1033 (Semenza 2001). During hypoxia, the decrease in degradation of HIF $\alpha$  allows HIF to accumulate in the cell, migrate into the nucleus, and activate gene expression. By this mechanism, hypoxia or ischemia increases the activation of many genes through an increase in the abundance of HIF $\alpha$ . Ex. 1009 (Semenza 2000)

47. While it was known in the late 1990s that hypoxic stabilization of HIF $\alpha$  led to its accumulation in cells, the oxygen sensor responsible for the regulation of this process was not understood. A major breakthrough came in 1999 when scientists determined that the tumor suppressor gene, von Hippel-Lindau (vHL), binds to the HIF $\alpha$  subunit and targets it for ubiquitin-mediated degradation by acting as the recognition component of a multiprotein ubiquitin ligase complex. Ex. 1029, Maxwell 1999. In 2001, the fundamental oxygen-sensitive step underlying the degradation of HIF $\alpha$  was defined as oxygen-dependent hydroxylation of specific proline residues on HIF $\alpha$ , a modification that directs the binding of vHL. The enzyme responsible for the proline hydroxylation was identified as prolyl hydroxylase, and specifically HIF prolyl hydroxylase. Ex. 1025 (Ivan 2001); Ex. 1007 (Jaakkola 2001).

48. Prolyl hydroxylases are key regulators of HIF $\alpha$  degradation. Ex. 1025

(Ivan 2001); Ex. 1007 (Jaakkola 2001). These are iron- and 2-oxoglurate-dependent dioxygenases. As such, prolyl hydroxylases have an absolute requirement for dioxygen (i.e., O<sub>2</sub>) as a substrate, along with iron and 2-oxoglurate.<sup>3</sup> (Epstein 2001, *Id.*) Prolyl hydroxylases catalyze the hydroxylation of proline amino acids on HIF $\alpha$ .

49. HIF $\alpha$  is marked for destruction by a subset of prolyl hydroxylases known as HIF prolyl hydroxylases. Ex. 1007 (Jaakkola 2001); Ex. 1025 (Ivan 2001). HIF prolyl hydroxylases are active under normoxic conditions, while under hypoxia their activity is suppressed. This is because the hypoxic condition deprive prolyl hydroxylases of dioxygen (i.e., O<sub>2</sub>); a necessary component for hydroxylation of HIF $\alpha$  by prolyl hydroxylases. Hydroxylation of HIF $\alpha$  by HIF prolyl hydroxylase constitutes the initial step in the degradation pathway for the protein.

50. As a result of these biochemical pathways, the cellular concentration of HIF $\alpha$  is directly regulated in accordance with the level of cellular oxygen such

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<sup>3</sup> Additional co-factors may be necessary for specific prolyl hydroxylase. For example, collagen prolyl hydroxylase requires ascorbate as a co-factor.

Nonetheless, all prolyl hydroxylase are at least O<sub>2</sub> -, iron-, and 2-oxoglutarate-dependent.

that it remains low during normoxia and increases during hypoxia or ischemia. If normoxia is restored to an ischemic or hypoxic cell, this pathway results in degradation of HIF $\alpha$  leading to a rapid return of HIF $\alpha$  (and in turn HIF) to normoxic levels within 1 to 5 minutes Ex. 1024 (Huang 1998).

**C. Use of Prolyl Hydroxylase Inhibitors to Stabilize HIF**

51. Prolyl hydroxylase inhibitors act to suppress the ability of HIF prolyl hydroxylases to modify the HIF $\alpha$  subunits. Ex. 1025 (Ivan 2001); Ex. 1007 (Jaakkola 2001). The hydroxylation of HIF $\alpha$  normally facilitates the interaction of the  $\alpha$  subunit with von Hippel Lindau protein ("vHL"), an ubiquitin ligase that chemically attaches ubiquitin to HIF $\alpha$ . Ubiquitin is a protein tag that destines the labeled protein (i.e., HIF $\alpha$ ) for degradation by the cellular proteasomes. By blocking the initial hydroxylation step, prolyl hydroxylase inhibitors prevent the recognition of HIF $\alpha$  by vHL, allowing HIF $\alpha$  to accumulate in the cell, migrate into the nucleus, bind to its HIF $\beta$  partner (forming HIF), and activate the expression of a wide range of different genes. As with other prolyl hydroxylases, HIF prolyl hydroxylase activity is dependent on three co-substrates: ferrous iron (i.e., Fe<sup>2+</sup>), 2-oxoglurate, and dioxygen. Ex. 1025 (Ivan 2001); Ex. 1007 (Jaakkola 2001).

52. In the absence of any of its three co-substrates, prolyl hydroxylases

become inactive. Inactivation of the enzyme can therefore be effected by sequestering iron in the cell (using chelating agents such as desferrioxamine ("DFO") or by altering the iron state through cobaltous ions), by depriving the cell of oxygen (by hypoxia, anoxia or ischemia) or by interfering with the availability or binding of 2-oxoglutarate. Ex. 1025 (Ivan 2001); Ex. 1007 (Jaakkola 2001); Ex. 1006, (Epstein 2001).

53. Prior to the discovery that proline hydroxylation is involved in regulating its stability (Ex. 1025 (Ivan 2001)), methods for inducing the activation of HIF to regulate downstream genes existed throughout the 1990s (Ex. 1008 (Semenza 1998)). Previous studies had shown that hypoxia, cobalt chloride, or DFO had the ability to induce expression of the downstream genes controlled by HIF. Ex. 1033 (Semenza 2001), at 347. Hypoxia or anoxia (i.e., lack of dioxygen) regulates HIF $\alpha$  stability by suppressing the activity of HIF prolyl hydroxylase. Ex. 1006 (Epstein 2001). Cobalt chloride suppresses the activity of prolyl hydroxylase by catalyzing the generation of reactive oxygen species in cells (Ex. 1022 (Chandel 1998)), which could inhibit prolyl hydroxylases by altering the oxidation state of the iron atom (from ferrous, Fe<sup>2+</sup>, to ferric, Fe<sup>3+</sup>), or by other means. The ability of DFO to elicit expression of erythropoietin in normoxic cells led early investigators to postulate the involvement of an iron-containing heme protein as an O<sub>2</sub> sensor.



Ex. 1023 (Goldberg 1988). However, the actual mechanism involving the removal of the iron atom became clear upon the discovery that prolyl hydroxylases regulate HIF $\alpha$  stability. Ex. 1025 (Ivan 2001); Ex. 1007 (Jaakkola 2001). That discovery was made independently and contemporaneously by two research laboratories and the resulting papers were published side-by-side as Ivan 2001 (Ex. 1025) and Jaakkola 2001 (Ex. 1007) in the journal *Science*. Those reports clarified the understanding of the mechanism by which hypoxia mimetics (e.g., cobalt chloride, DFO) lead to HIF $\alpha$  stabilization. They also teach that small molecules that interfere with the ability of 2-oxoglutarate to interact with prolyl hydroxylase can suppress the activity of the enzyme and thereby mimic hypoxia. Ex. 1025 (Ivan 2001); Ex. 1007 (Jaakkola 2001).

54. One report demonstrated that HIF prolyl hydroxylase can be inhibited by depleting the cell of 2-oxoglutarate, or by inhibiting its interaction with 2-oxoglutarate using the structural analog, N-oxalylglycine. Ex. 1007 (Jaakkola 2001). The second report recognized that HIF prolyl hydroxylase is distinct from the collagen prolyl hydroxylases, and suggested that inhibitors of the latter, described as antifibrotics, would in principle represent potential therapeutics in the treatment of chronic hypoxia or ischemia. Ex. 1025 (Ivan 2001). Ivan 2001 expressly states that "[s]everal small-molecule proline hydroxylase inhibitors [that]

have been developed as antifibrotic agents [] can now be tested in ischemia models", including the compound disclosed in Bickel. Ex. 1025 (Ivan 2001), at 467 (references 22 – Kivirikko (1998), 32 – Bickel (1998), 33 – Franklin (2001), and 34 – Friedman (2001)). These reports teach that regulators of prolyl hydroxylase, including small molecules that prevent interaction of 2-oxoglutarate with the enzyme, represent important means of controlling the tissue response to hypoxia or ischemia, through their ability to prevent the degradation of HIF $\alpha$ .

## **IX. SCOPE AND CONTENT OF THE PRIOR ART**

### **A. Central Role HIF in Hypoxic Regulation**

55. HIF activation leads to the increased expression of genes that protect individual cells from the potentially lethal effects of hypoxia or ischemia. These genes encode enzymes involved in glucose metabolism, membrane transporters involved in the cellular uptake of glucose, regulators of cell survival and proliferation, protein regulators of cell death pathways, and those involved in the regulation of cellular pH. Ex. 1008 (Semenza 1998). HIF-dependent activation of genes that regulate glucose uptake, glucose metabolism, and pH can protect cells from hypoxic or ischemic damage by enhancing their ability to sustain energy production in the absence of molecular oxygen. Such enhancement can allow a cell to survive longer during ischemia or hypoxia, thereby lessening the number of cells in the tissue that succumb to that stress. HIF also regulates genes that

enhance the ability of cells to withstand oxidant stress caused by the generation of free radicals (or ROS). As oxidant stress is centrally involved in the cellular damage associated with ischemia-reperfusion injury, HIF-dependent expression of proteins such as NOS and HO-1, which limit oxidant stress or that mitigate the damage caused by oxidants, can confer protection against that condition. Ex. 1026 (Lee 1997).

56. HIF-dependent genes can also act to enhance or restore oxygen delivery either locally within tissues or systemically (i.e., to the entire organism). These include proteins that regulate vascular tone and local blood flow, that regulate the systemic lung ventilatory response to low arterial blood oxygenation, and that regulate the generation of new blood vessels through angiogenesis. Ex. 1008 (Semenza 1998); Ex. 1009 (Semenza 2000). Some genes induced by HIF can protect against hypoxia and ischemia by multiple mechanisms. For example, erythropoietin stimulates the production of new red blood cells by interacting with bone marrow progenitor cells that give rise to new red blood cells, and also by binding to receptors on other cells including neurons (Ex. 1028 (Marti 1996)), where it confers protection against ischemic stress (Ex. 1036 (Kilic 2005)).

## **B. HIF Regulation**

57. The HIF $\alpha$  gene is constitutively expressed, and HIF $\alpha$  protein is

continuously generated in cells, regardless of the O<sub>2</sub> concentration. Some cells tend to express HIF-1 $\alpha$  preferentially, while other cells tend to express HIF-2 $\alpha$ , but the regulation of these two forms by HIF prolyl hydroxylase is similar. HIF prolyl hydroxylase is constitutively present in the cell, along with iron and 2-oxoglutarate. Under normoxic conditions, newly synthesized HIF $\alpha$  protein interacts with HIF prolyl hydroxylase at a region of the protein referred to as the Oxygen Degradation Dependent Domain (ODDD). Ex. 1024 (Huang 1998). Recognition of HIF $\alpha$  by the prolyl hydroxylase results in the hydroxylation of two proline residues through a reaction that converts 2-oxoglutarate to succinate and consumes one molecule of molecular oxygen. Ex. 1006 (Epstein 2001). Hydroxylation of HIF $\alpha$  then facilitates its interaction with vHL protein, which acts to ligate ubiquitin to a lysine residue on HIF $\alpha$ . Ex. 1025 (Ivan 2001); Ex. 1007 (Jaakkola 2001). Ubiquitin ligation effectively identifies the protein for degradation by the proteasome system. Hence, by controlling the proximal step in that process, HIF prolyl hydroxylases are responsible for the low abundance of HIF $\alpha$  in normoxic cells.

58. Loss of HIF prolyl hydroxylase activity occurs when the enzyme is deprived of oxygen (e.g., under hypoxic conditions), iron (e.g. in the presence of iron chelators), or 2-oxoglutarate, or when the oxidation state of the iron increases

from the  $\text{Fe}^{2+}$  to the  $\text{Fe}^{3+}$  state (e.g. presence of cobaltous ions)<sup>4</sup>. Ex. 1006 (Epstein 2001); Ex. 1025 (Ivan 2001); Ex. 1009 (Semenza 2000). In each case, the resulting suppression of HIF $\alpha$  degradation leads to an increase in its abundance in the cell (and consequently an increase in HIF) and an increase in expression of its target genes.

**C. Prolyl Hydroxylase Inhibitors as Anti-Fibrotic Agents**

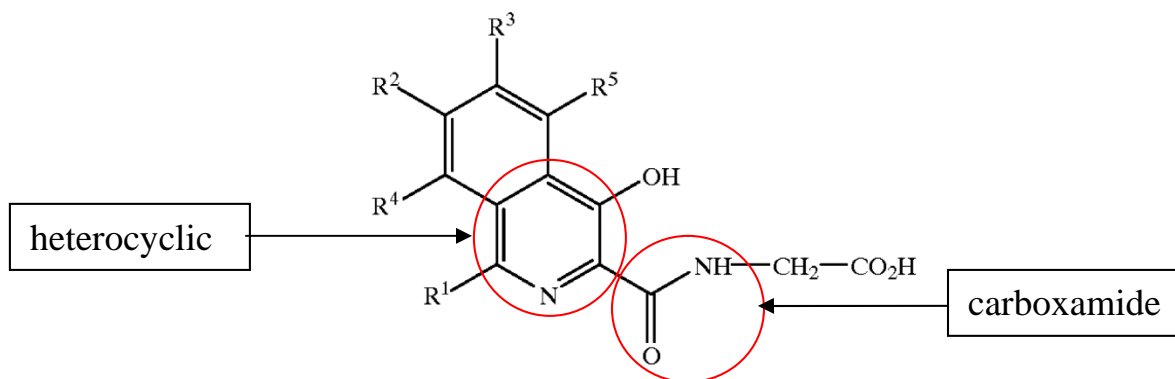
59. Weidmann (Ex. 1019) teaches that isoquinoline-3-carboxamides, i.e., heterocyclic carboxamides, of Formula I have a strong inhibitory action on collagen biosynthesis *in vivo*, through their ability to inhibit prolyl 4-hydroxylase enzymes. Ex. 1019 (Weidmann), at col. 1, lns. 64-67) The inhibition of prolyl 4-hydroxylase impacts the hydroxylation of a proline residues on collagen—resulting in a "nonfunctional, underhydroxylated collagen molecule." (*Id.*, at col. 1, lns. 17-24) Through that effect, these compounds are proposed as potential therapeutics for the treatment of fibrotic diseases of the lung, liver, and skin, and also in atherosclerosis. (*Id.*, at col. 1, lns. 25-30),

60. Formula I of Weidmann is represented below. Ex. 1019 (Weidmann),

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<sup>4</sup>The administration of iron chelators, 2-oxoglutarate mimetics, and cobaltous ions shows an ability to mimic hypoxia by allowing an increased level of HIF by reducing the level of the co-factors iron and 2-oxoglutarate.

at col. 2, lns. 1-15. A compound falling within the scope of Formula I of Weidmann will be a heterocyclic carboxamide.



61. In addition to Formula I, Weidmann provides several examples of heterocyclic carboxamide compounds. *See, e.g.*, Ex. 1019 (Weidmann), at col. 13, lns. 13-24 (Example 1, N-((1-Chloro-4-hydroxyl-7-(2-propyloxy) isoquinolin-3-yl)carbonyl)glycine); col. 16, lns. 43-47 (Example 7, N-((6-Benzyloxy-1-chloro-4-hydroxyisoquinolin-3-yl)carbonyl)glycine); col. 16, lns. 62-65 (Example 8, N-((7-Benzyloxy-1-chloro-4-hydroxyisoquinolin-3-yl)carbonyl)glycine); col. 17, lns. 12-14 (Example 9, N-((1-Chloro-4-hydroxyisoquinolin-3-yl)carbonyl)glycine); col. 18, lns. 25-28 (Example 13, N-((7-(1-Butyloxy)-4-hydroxyisoquinolin-3-yl)carbonyl)glycine)

62. Interestingly, the '172 patent discloses many of the compounds disclosed in the examples of Weidmann. Table 1 below provides a list of such compounds. Despite the difference in name, the structure of the compounds are the same.

**TABLE 1. Compounds Disclosed in Both the '730 and '172 Patents**

'730 PATENT		'172 PATENT	
Compound 7	N-((6-Benzoyloxy-1-chloro-4-hydroxyisoquinolin-3-yl)carbonyl)glycine	Compound J	N-((6-benzoyloxy-1-chloro-4-hydroxy-isoquinoline-3-carbonyl)-amino)-acetic acid
Compound 8	N-((7-Benzoyloxy-1-chloro-4-hydroxyisoquinolin-3-yl)carbonyl)glycine	Compound L	N-((7-benzoyloxy-1-chloro-4-hydroxy-isoquinoline-3-carbonyl)-amino)-acetic acid
Compound 9	N-((1-Chloro-4-hydroxyisoquinolin-3-yl)carbonyl)glycine	Compound B	N-((1-chloro-4-hydroxy-isoquinoline-3-carbonyl)-amino)-acetic acid
Compound 13	N-((7-(1-Butyloxy)-4-hydroxyisoquinolin-3-yl)carbonyl)glycine	Compound M	N-((7-butoxy-4-hydroxy-isoquinoline-3-carbonyl)-amino)-acetic acid

63. Of particular interest, Compound 9 (N-((1-Chloro-4-hydroxyisoquinolin-3-yl)carbonyl)glycine) of Weidmann, which is the same compound disclosed in the '172 patent as Compound B (N-((1-chloro-4-hydroxy-isoquinoline-3-carbonyl)-amino)-acetic acid, was shown to stabilize HIF $\alpha$  (Example 1 and 2), induce expression of HIF-target genes (Example 3), and treat myocardial infarction (*id.*, Example 6, experiment II) and renal ischemia-reperfusion injury (*id.*, Example 8). Compound 9 (i.e., Compound B) is a heterocyclic carboxamide.

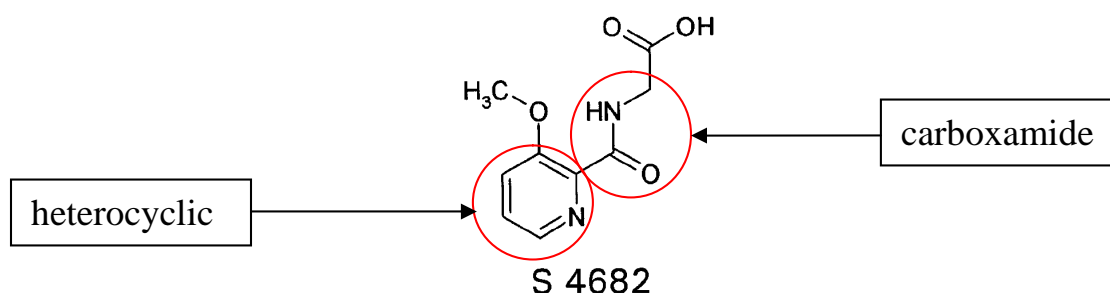
64. Weidmann reports the *in vitro* concentration required to inhibit 50% (IC<sub>50</sub>) of the antifibrotic activity for the example compounds of Formula I. Ex. 1019 (Weidmann), at col. 9, lns. 40-60. Weidmann also describes testing of rats that had been administered carbon tetrachloride ("CCl<sub>4</sub>") for 4 weeks to induce chronic liver fibrosis.<sup>5</sup> *Id.*, at col. 9, ln. 62 to col. 10, ln. 51. Administration of isoquinoline-3-carboxamides to such rats decreased the extent of liver fibrosis through their ability to inhibit prolyl 4-hydroxylases as assessed by the ratio of hydroxyproline to total proline content. *Id.*, at Table II (compounds 1, 9, and 19 of Weidmann inhibited hydroxyproline formation by 39% to 71%).

65. Bickel (Ex. 1027) teaches the use of a prolyl 4-hydroxylase inhibitor for treatment of liver fibrosis. Ex. 1027 (Bickel), at Abstract ("S4682 reduced hepatic collagen accumulation, decreased prevalence of ascites, and lowered serum procollagen type III N-peptide (PIIINP) levels."). In particular, S4682, a heterocyclic carboxamide in the form of a heterocyclic carbonyl glycine, was "designed as an analog of the enzyme's cosubstrate, 2-oxoglutarate." *Id.*, at 404. The structure of S4682 is shown below.

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<sup>5</sup> This is the same ischemia model used in Bickel. Ex. 1027 (Bickel), at 405. The '172 patent admits that this model shows induction of toxic-ischemic injury to the liver. Ex. 1001 ('172 patent), at col. 59, lns. 16-18.





*Id.*, at pg. 405. Bickel proposes that S4682, a prolyl 4-hydroxylase inhibitor, would inhibit the hydroxylation of collagen resulting in underhydroxylated collagen, which "is not stable at body temperature [and] . . . is retained and rapidly degraded inside the collagen-producing cells" resulting in immature collagen formation and decreased fibrosis. *Id.*, at 404.

66. S4682 was shown to inhibit prolyl 4-hydroxylase *in vitro* and in cell culture (*id.*, at 406, Table I), and decrease the formation of hepatic collagen in healthy rats (*id.*, at 407). In a CCl<sub>4</sub> model of chronic hepatic injury in rats, S4682 was shown to "reduced collagen accumulation in the liver and PIINP in serum significantly, and attenuated the morbidity of the animals as documented by the striking reduction of the occurrence of ascites (Table 3)." *Id.*, at 409. This is consistent with its ability to inhibit the interaction of 2-oxoglutarate with collagen prolyl 4-hydroxylase. S4682 "is the first compound whose antifibrotic properties in experimental liver fibrosis induced by long-term treatment with CCl<sub>4</sub> can be

attributed exclusively to inhibition of P4H activity." *Id.*, at 410. Further, Bickel suggests "the concept that direct inhibition of P4H is a promising approach toward reducing." *Id.*

67. The excessive deposition of collagen in the liver caused by chronic injury, such as CCl<sub>4</sub>, results in fibrosis and toxic-ischemic injury. CCl<sub>4</sub> is metabolized by the cytochrome P450 system, which results in the generation of ROS and damages the liver. These reactive intermediates then cause oxidative stress in the hepatic cells (i.e., hepatocytes) and in adjacent hepatic stellate cells ("HSC"), resulting in the oxidation of lipids, proteins, and DNA. This oxidative stress stimulates the HSC to proliferate, to increase the synthesis of collagen, and to decrease the production of enzymes that degrade extracellular proteins, such as collagen. Similarly, hypoxia and ischemia-reperfusion injury each generates oxidant stress that promotes liver fibrosis by stimulating HSC cells. HIF activation triggers the expression of genes that attenuate oxidant stress such as HO-1 (Ex. 1026 (Lee 1997); Ex. 1009 (Semenza 2000), at 1984, Table 1), and thereby confers protection by this secondary effect.

68. First, the antioxidant enzymes protect the cells from oxidant-mediated damage to lipids, proteins, and DNA. Second, antioxidant enzymes attenuate the oxidant-mediated stimulation of HSC cells, which lessens the production of

collagen and resulting fibrosis. Indeed, Bickel shows that hepatocellular injury is lessened by S4682, based on decreased release of alanine aminotransferase ("ALT") and alkaline phosphatase in CCl<sub>4</sub>-treated rats. Ex, 1027, Bickel, Table 2. Such protection is likely the result of the induction of protective antioxidant genes caused by HIF activation resulting from HIF prolyl hydroxylase inhibition. Bickel also shows that S4682 lessens serum levels of procollagen type III peptide, a marker of collagen synthesis. While the inhibitory effect of S4682 on collagen proline hydroxylation could explain that effect, HIF-mediated up-regulation of antioxidant genes would also attenuate oxidant stress, a principal driver of HSC cell collagen synthesis. Thus, the protective effects of S4682 in the CCl<sub>4</sub> model of hepatic fibrosis likely represent the combined effects of collagen prolyl 4-hydroxylase and HIF prolyl hydroxylase inhibition.

69. Kivirikko (Ex. 1021) discloses that prolyl 4-hydroxylase may be "a potential target for therapeutic modulation of excessive collagen accumulation" associated with various fibrotic disease. (Ex. 1021, Kivirikko, at 357) Kivirikko describes a number of peptide and non-peptide inhibitors of prolyl 4-hydroxylase. In particular, Kivirikko states that "interest has been focused especially on competitive inhibitors with respect to 2-oxoglutarate" (id., at 362), and discloses a number of 2-oxoglutarate competitive inhibitors as potential anti-fibrotic drugs

(*id.*, at 363). Although additional research is necessary, Kivirikko concludes that it "seems possible to develop prolyl 4-hydroxylase inhibitors into antifibrotic drugs." *Id.*, at 363.

70. Franklin 2001 (Ex. 1020) discloses novel phenanthrolinones that were shown to be potent competitive inhibitors of prolyl 4-hydroxylase, and of collagen hydroxylation.. Ex. 1020 (Franklin 2001) at 337 (column 1). Compound 5, a heterocyclic carboxamide, is a "competitive antagonist of the co-substrate, 2-oxoglutarate, [and was] well tolerated in the rat at doses producing sustained inhibition of collagen hydroxylation in several tissues *in vivo*." *Id.* Franklin 2001 concludes that "[t]he *in vivo* data presented here, together with those from earlier reports of *in vitro* systems, may have implications for the potential use of inhibitors of prolyl 4-hydroxylase in the treatment of life-threatening progressive fibrotic disorders of the liver, lungs, kidneys and other tissues." *Id.*, at 337 (column 2).

71. Earlier, Franklin 1991 (Ex. 1045) discloses inhibitors of prolyl 4-hydroxylase as anti-fibrotic agents for the treatment of progressive fibro-proliferative diseases. Ex. 1045 (Franklin 1991), at 812. Similar to Bickel, Franklin targeted prolyl 4-hydroxylase because of its role in collagen biosynthesis. Specifically, Franklin pursued structural analogs (or mimetics) of 2-oxoglutarate as "possible antagonist of the enzyme" prolyl 4-hydroxylase. *Id.*, at 813. More

specifically, Franklin proposed heterocyclic carbonyl glycines (i.e., heterocyclic carboxamides) as potential inhibitors of prolyl 4-hydroxylase:

We think it possible that the heterocyclic derivatives of N-oxalylglycine (heterocyclic carbonylglycines) may co-ordinate the postulated  $\text{Fe}^{2+}$  at the active centre of the enzyme through the nitrogen atom of the heterocyclic ring *ortho* to the side-chain and through the amide carbonyl group (Fig. 1). A free C-group is also necessary for inhibition (data not shown) and this may interact with a complementary positively charged amino-acid side chain in the enzyme.

*Id.*, (Franklin), at 814. Compounds C and D, both heterocyclic carboxamides, "showed more promising activity against intracellular hydroxylation although both compounds were less active against the isolated enzyme (Table 1)" and "both C and D gave significant inhibition" of prolyl 4-hydroxylase *in vivo* in rats. *Id.*

72. Friedman (Ex. 1017) describes a model system for the discovery of small-molecule inhibitors of prolyl 4-hydroxylase. Ex. 1017 (Friedman), at 4736. Friedman discloses examples of small-molecule inhibitors of prolyl 4-hydroxylase, and states "[i]nhibitors of prolyl 4-hydroxylase have received much interest as potential antifibrotic agents" (*Id.*, at 4740)

73. Nwogu *et al.* (Ex. 1018) described the beneficial effects of FG-041 administration on the cardiac function and survival response after myocardial infarction in rats. That study demonstrated that 7/29 control rats succumbed between days 7 and 28, whereas only 3/29 rats treated with FG-041 beginning 48

hr after the infarction died. Ex. 1018 (Nwogu), at 2218. The authors concluded that "prevention of interstitial fibrosis in the noninfarcted myocardium alters the pattern of LV [left ventricular] enlargement and produces partial recovery of LV function after MI [myocardial infarction]" *Id.*, at 2219. Yet, there was no statistical difference in the weight of scar tissue, which is principally collagen, in the infarcted area of the control compared to treated animals. *Id.*, at 2218 ("The weights of the scar tissue in the infarcted area of both MI-control and MI-FG041 animals were similar (93 versus 91 mg, respectively.") Echocardiographic evidence suggested that the treated animals exhibited enhanced contractility (*id.*, at 2220), and surprisingly, the principal effect was restricted to the non-infarcted myocardium where collagen deposition does not occur. Their data therefore suggested that the inhibitor of prolyl 4-hydroxylase was protecting the heart in areas that were not involved in collagen synthesis.

74. A more likely explanation for that finding is that the protection conferred against the injury was mediated by HIF $\alpha$  stabilization induced by the prolyl 4-hydroxylase inhibition in the normal, non-infarcted regions of the heart. Chronic ischemia in the heart elicits HIF activation in hypoxic regions. Ex. 1037 (Lee 2000). HIF activation in those regions, in response to the prolyl 4-hydroxylase inhibitor, likely improved cardiac repair by enhancing angiogenesis,

improving glycolytic metabolism, and altering the metabolism in a manner that promoted maintenance and recovery of contractility.

75. Although the aforementioned references discuss prolyl 4-hydroxylase inhibitors in terms of inhibition of collagen biosynthesis, it was well known from Ivan 2001 (Ex. 1025) and Jaakkola 2001 (Ex. 1007) that HIF prolyl hydroxylase plays a central role in a number of hypoxia regulated processes, including, among other things, angiogenesis, erythropoiesis, and glucose metabolism. As discussed above, HIF prolyl hydroxylase is a 2-oxoglutarate-dependent enzyme. These same 2-oxoglutarate mimetics could also inhibit HIF prolyl hydroxylase and its attendant biological processes. Ivan 2001 suggests as much.

**D. Prolyl Hydroxylase Inhibitors Stabilize HIF $\alpha$**

76. Ivan 2001 (Ex. 1025) is a seminal paper elucidating the mechanism for the degradation of HIF $\alpha$  by the vHL protein. Ivan 2001 deduces that the prolyl hydroxylase responsible for HIF $\alpha$  regulation is distinct from the prolyl 4-hydroxylases involved in collagen maturation. Ex. 1025, (Ivan 2001), at 467 ("It is therefore unlikely that HIF is modified by the type I and type II prolyl 4-hydroxylases.") Ivan 2001 also notes that "[t]issue ischemia is a major cause of morbidity and mortality. In principle, drugs that stabilize HIF may augment angiogenesis and the adaptation to chronic hypoxia." *Id.* Finally, Ivan 2001 states

"[s]everal small-molecule proline hydroxylase inhibitors have been developed as anti-fibrotic agents and can now be tested in ischemia models." *Id.*, at 467 (references 22 – Kivirikko (1998), 32 – Bickel (1998), 33 – Franklin (2001), and 34 – Friedman (2001)). Notably, the '172 patent "contemplates the use of these compounds [which Ivan 2001 explicitly teaches] in the methods provided herein" and as claimed in the '172 patent. Ex. 1001 ('172 patent), at col. 26, lns. 10-18. Ivan 2001 identifies the heterocyclic carboxamides used by Bickel as compounds that should be tested in ischemia models (Ivan 2001). Ex. 1025 (Ivan 2001), at 467, 468, end note 32. Several months after the publication of Ivan 2001, the applicants of the '172 patent made a similar observation in the provisional patent application filed on December 6, 2001. Ex. 1001 ('172 patent), at 16 ("Several small molecule inhibitors of prolyl 4-hydroxylase have been identified. (See, e.g., . . . Bickel et al. (1998) *Hepatology* 28:404-411 . . .) The present invention contemplates the use of these compounds in ischemic disorders.").

77. Jaakkola 2001 (Ex. 1007) demonstrates that compounds capable of disrupting the interaction of 2-oxoglutarate with HIF prolyl hydroxylase can prevent HIF $\alpha$  hydroxylation and abolish its interaction with vHL protein. Ex. 1007 (Jaakkola 2001), at 471. Similarly, Jaakkola 2001 demonstrates that depletion of 2-oxoglutarate from the cell produces an effect comparable to hypoxia



and DFO. *Id.* Like Ivan 2001, Jaakkola 2001 deduces that a prolyl hydroxylase is responsible for HIF $\alpha$  regulation, but this is distinct from the prolyl hydroxylases involved in collagen processing. Ex. 1007 at 470 ("These results revealed that the enzymatic activity promoting interaction of HIF-1 $\alpha$  with pVHL is a prolyl-hydroxylase.") Jaakkola applies the term 'HIF prolyl hydroxylase' to describe the responsible enzyme. *Id.*

78. In 2001, Epstein 2001 (Ex. 1006) identified a family of prolyl hydroxylases (i.e., HIF-prolyl hydroxylase), highly conserved in species ranging from *C. elegans* to mammals, that was responsible for regulating hydroxylation of HIF $\alpha$  subunits. Ex. 1006 (Epstein 2001), at Abstract. Epstein 2001 concludes that "the identification of the HIF-PHs also raises the therapeutic possibilities. Inhibitors of HIF-PHs might be used to activate HIF and enhance angiogenesis in ischemic/hypoxic disease." *Id.*, at 52. Inhibitors of HIF-prolyl hydroxylase or HIF-PH include 2-oxoglutarate analogs. *Id.*, at 48.

**E. Heterocyclic Carboxamides as HIF Prolyl Hydroxylase Inhibitors**

79. The '172 patent teaches that the compounds of Bickel and Weidmann can be used in the methods described and claimed in the '172 patent. Specifically, both Bickel and Weidmann disclose, among other things, heterocyclic carboxamide compounds that inhibit prolyl 4-hydroxylase and their use as

antifibrotic agents. The '172 patent admits that these compounds also inhibit HIF prolyl hydroxylase and stabilizes HIF $\alpha$ . *See, e.g.*, Ex. 1001 ('172 patent), at col. 26, lns 10-17 and Example 7 (Bickel) and col. 36, lns. 1-22 (Weidmann).

80. Similarities in enzyme structure are shared widely among members of the family of prolyl hydroxylase enzymes, including collagen prolyl hydroxylase and HIF prolyl hydroxylase. Myllyharju (Ex. 1038) teaches that coordination of iron at the catalytic site of the  $\alpha$  subunit of human prolyl 4-hydroxylase is facilitated by its interaction with histidine412, asparagine414 and histidine483. Ex. 1038 (Myllyharju), at 1177. Epstein 2001 deduces that HIF prolyl hydroxylase also coordinates an iron via histidine residues, and that mutations to HIF prolyl hydroxylase involving replacement of a critical histidine with another amino acid can abrogate enzymatic activity. Ex. 1006 (Epstein 2001), at 51. 2-oxoglutarate and its structural analogs, including pyridine 2,4-dicarboxylates, also bind, in part, to this site. Ex. 1038 (Myllyharju), at 1178. Thus, the binding of iron, 2-oxoglutarate, and proline by collagen prolyl hydroxylase and HIF prolyl hydroxylase is similarly regulated by the molecular structure of the enzyme. Hence, structural analogs of 2-oxoglutarate that inhibit collagen prolyl 4-hydroxylase are likely to inhibit HIF prolyl hydroxylases as well.

**F. Rationale to Treat Hypoxia/Ischemia with Prolyl Hydroxylase Inhibitors, and in Particular, a Heterocyclic Carboxamide.**

81. A number of investigators employed prolyl 4-hydroxylase inhibitors as antifibrotic agents to treat ischemic disorders or conditions, including hepatic and cardiovascular ischemia. For example, Bickel teaches that S4682, an analog of 2-oxoglutarate, decreased hepatic collagen hydroxylation in a CCl<sub>4</sub>-model of liver ischemia, and significantly decreased the ALT and alkaline phosphatase levels in serum consistent with a protective effect of S4682 (*see* ¶¶ 57-60, *supra*). Similarly, Nwogu teaches that an inhibitor of prolyl 4-hydroxylase confers significant beneficial effects in recovery from ischemic injury in the heart (*see* ¶ 64, *supra*).

82. Lee 2000 (Ex. 1037) teaches that HIF-1 $\alpha$  is increased in the heart as an early response to myocardial ischemia and infarction. Ex. 1037 (Lee 2000), at Abstract. Bergeron 2000 (Ex. 1039) reports that in young rats, obstruction of blood flow to one side of the brain followed by exposure to environmental hypoxia (8% O<sub>2</sub>) causes cerebral infarction on the side with compromised blood flow. However, rats exposed to 8% O<sub>2</sub> for 3 hours and then allowed to recover for 24 hr before hypoxia-ischemia are protected from infarction. Ex. 1039 (Bergeron 2000), at 291. Similar protection is conferred by cobalt chloride and desferrioxamine (DFX), which induce HIF-1 $\alpha$  stabilization and HIF-dependent gene expression.

*Id.*, at 292. These results teach that HIF-1 activation is induced by hypoxia and ischemia, and that HIF activation regulates a large number of genes that can confer protection against ischemia, including VEGF as well as genes involved in energy production and antioxidant function. Ex. 1009 (Semenza 2000).

83. Semenza 2000 (Ex. 1009) provides a review of the role of HIF-1 on various physiological and pathophysiological processes. Semenza 2000 explains that "under hypoxic conditions, HIF-1 $\alpha$  protein levels increase dramatically and the fraction that is ubiquitinated decreases." Ex. 1009 (Semenza 2000), at 1983. The increase of HIF resulting from increased availability of HIF-1 $\alpha$  leads "to the transcriptional activation of VEGF and several dozen other known target genes." *Id.* The genes that HIF regulates affect several physiological processes including glucose/energy metabolism and cell proliferation/viability, erythropoiesis, iron metabolism, vascular development/remodeling, and vasomotor tone. *Id.* (Semenza 2000), at 1984, Table 1, 1987, Figure 1. These physiological processes affect several hypoxic and ischemic conditions, such as ischemic cardiovascular disorders, cerebral ischemia, retinal ischemia, pulmonary hypertensions, pregnancy disorders and cancer. *Id.*, (Semenza 2000), at 1984-1985. Further, Semenza 2000 discusses the potential role of HIF in ischemic preconditioning through induction of HIF-mediated genes, such as *Nos2*, which is protective against a subsequent

lethal ischemic challenge. *Id.*, (Semenza 2000), at 1984. Semenza 2000 proposes that therapeutic strategies that increase HIF-1 $\alpha$  expression can promote biological responses that will assist in the treatment of these conditions. *Id.*, (Semenza 2000), at 1984. Target conditions that could be treated in this manner include atherosclerosis, myocardial infarction, and reperfusion injury.

84. Ivan 2001 (Ex. 1025) and Jaakkola 2001 (Ex. 1007) found that HIF prolyl hydroxylase regulates the activity of HIF by regulating the degradation of HIF $\alpha$ . (*see* ¶¶ 66 and 67, respectively, *supra*). Specifically, under normoxic conditions, HIF $\alpha$  is hydroxylated by HIF prolyl hydroxylase and rapidly degraded by the ubiquitin-proteasome pathway. On the other hand, under hypoxic conditions, HIF prolyl hydroxylase activity is suppressed, resulting in the stability of HIF $\alpha$  and accumulation of HIF. It was known in the art that prolyl hydroxylases were O<sub>2</sub><sup>-</sup>, iron-, and 2-oxoglutarate-dependent enzymes. It was also known that iron chelators mimicked hypoxia. Ivan 2001 (Ex. 1025) and Jaakkola 2001 (Ex. 1007) teach that small molecules that interfere with the ability of 2-oxoglutarate to interact with HIF prolyl hydroxylase can suppress the activity of the enzyme and thereby also mimic hypoxia. Ex. 1025 (Ivan 2001); Ex. 1007 (Jaakkola 2001).

85. Based on these findings, Ivan 2001 concludes that "drugs that stabilize HIF may augment angiogenesis and the adaptation to chronic hypoxia" and

suggests that "[s]everal small-molecule proline hydroxylase inhibitors have been developed as anti-fibrotic agents and can now be tested in ischemia models." Ex. 1025 (Ivan 2001), at 467. Specifically, one of the heterocyclic carboxamide compounds that Ivan 2001 (Ex. 1025) suggests should be tested in ischemia models is Bickel (Ex. 1027), and, likewise, it is my opinion that the heterocyclic carboxamide compounds of Weidmann (Ex. 1019) would also be included within this suggestion. Thus, in my opinion, a heterocyclic carboxamide would represent an obvious candidate in selecting such a compound.

86. Based on the structural similarity of prolyl hydroxylases, there is a reasonable expectation that these small molecule inhibitors of prolyl 4-hydroxylase would also inhibit HIF prolyl hydroxylase. Indeed, the '172 patent (Ex. 1001) confirms that the heterocyclic carboxamide compounds of Bickel and Weidmann (Ex. 1019) inhibit HIF prolyl hydroxylase and stabilize HIF $\alpha$ , resulting in the accumulation of HIF and expression of HIF-targeted genes, and treatment of ischemic or hypoxic disorders or conditions.

**X. CLAIMS 1, 2, 3, 5, 6, AND 8 OF THE '172 PATENT ARE ANTICIPATED**

87. I have reviewed the claims of the '172 patent. (Ex. 1001) and it is my opinion that all of the limitations of the claims are found (either expressly or

inherently) within Weidmann<sup>6</sup> (Ex. 1019). Below, I demonstrate that each and every limitation of the '172 patent are met by Weidmann.

**A. Claims 1, 2, 3, 5, 6, and 8 Are Anticipated By Weidmann**

**1. Independent Claim 1 is Anticipated By Weidmann**

88. Claim 1 is an independent claim, and it reads:

A method for treating a hypoxic or ischemic disorder or condition in a subject, the method comprising administering to the subject an effective amount of a heterocyclic carboxamide compound that stabilizes the alpha subunit of hypoxia inducible factor (HIF $\alpha$ ).

89. Weidmann (Ex. 1019) discloses substituted isoquinoline-3-carboxamides, a heterocyclic carboxamide, for use as inhibitors of prolyl-4-hydroxylase. Ex. 1019 (Weidmann), at col. 1, lns. 8-10. Such inhibitors are suitable for use in the treatment of, among other things, liver ischemia and atherosclerosis. Atherosclerosis is a hardening and narrowing of the arteries due to the formation of atheromatous plaques. It is a chronic and cumulative disease which, if left untreated, results in reduction or cessation of blood flow (i.e., ischemia), leading to infarction. Weidmann proposes that substituted isoquinoline-

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<sup>6</sup> I understand that U.S. Patent No. 5,658,933 ("the '933 patent") was raised by the Examiner during prosecution of the '172 patent. The '933 patent is a related patent to Weidmann (both Weidman, *et al.*), and will be discussed further below.

3-carboxamides would be "suitable substances in the therapy of disorders in which the deposition of collagen decisively contributes to the clinical picture," such as atherosclerosis. *Id.*, at col. 1, lns 25-30.

90. Weidmann provides examples that show the antifibrotic activity of the heterocyclic carboxamide compounds *in vitro*<sup>7</sup> and *in vivo* in a CCl<sub>4</sub>-model of liver ischemia. Weidmann reports the *in vitro* IC<sub>50</sub> concentration for the antifibrotic activity for each of example compounds for Formula I. *Id.*, at col. 9, lns. 40-60, Example Nos. 9, 7, 8 and 13, respectively. This table includes data for Compounds B, J, L & M of the '172 patent (*see* ¶ 61, Table 1, *supra*).

91. In the *in vivo* testing, Weidmann discloses the use of heterocyclic carboxamides for the treatment of liver ischemia in a CCl<sub>4</sub> model of hepatic injury.<sup>8</sup> CCl<sub>4</sub> is a hepatotoxin that induces fibrosis and liver ischemia. Rats were administered twice weekly with CCl<sub>4</sub> for 4 weeks and treated with control or heterocyclic carboxamide compounds. *Id.*, at col. 19, ln. 62 to col. 10, ln. 51. Weidmann shows that the heterocyclic carboxamide compounds inhibited prolyl 4-

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<sup>7</sup>The *in vitro* inhibition of prolyl 4-hydroxylase was determined as described in Kaule and GiinZler, *Anal. Biochem.* 184:291-297 (1990).

<sup>8</sup> The '172 patent admits that CCl<sub>4</sub> is a model of toxic-ischemic liver injury. Indeed, the '172 patent references Bickel as such as model in Example 7.



hydroxylase as demonstrated by the inhibition of hydroxyproline formation of collagen (i.e., procollagen III and collagen IV).

92. Notwithstanding the role of collagen biosynthesis, substituted isoquinoline-3-carboxamides would have inherently inhibited HIF prolyl hydroxylase and naturally resulted in the stabilization HIF $\alpha$ . HIF would then activate the adaptive responses to low oxygen tension in the arteries resulting in cellular protective mechanisms, such as the expression of glucose transporters, glycolytic enzymes, antioxidant enzymes including HO-1, and other proteins that limit oxidant stress, enhance glycolytic metabolism, enhance angiogenesis, enhance local blood flow, and regulate cell survival.

93. CCl<sub>4</sub>-induced liver injury and ischemia-reperfusion injury are mediated by an increase in the generation of ROS. Ex. 1040 (Stoyanovsky 1996); Ex. 1041 (Wasser 2001). These ROS can induce cellular injury by reacting with proteins, lipids, and DNA. Accordingly, antioxidants administered in CCl<sub>4</sub> models of hepatotoxicity confer protection against cellular injury (Ex. 1042 (Yi 2000)), and antioxidant enzymes confer protection in models of ischemia-reperfusion injury (Ex. 1042 (Ambrosio 1986)). HIF regulates the expression of proteins that enhance cell survival under conditions where the supply of oxygen is compromised, such as the expression of antioxidant enzymes, including HO-1,

enzymes known to provide protection against oxidant stress, and NOS2. Ex. 1044 (Choi 1996); Ex. 1009 (Semenza 2000), at 1984. HO-1 and NOS2 expression are increased by HIF activation (Ex. 1026 (Lee 1997); Ex. 1008 (Semenza at 1988), Table 1), and HIF activation is stimulated by inhibition of HIF prolyl 4-hydroxylase. Hence, HIF activation would be expected to protect cells and tissues from conditions where oxidant stress is increased, including CCl<sub>4</sub>-induced liver injury and ischemia-reperfusion injury. In those models, administration of a heterocyclic carboxamide, or other molecular analogs of 2-oxoglutarate that inhibit prolyl 4-hydroxylase, would therefore be expected to confer protection against CCl<sub>4</sub>- or ischemia-reperfusion-induced injury because they enhance the expression of protective genes under the control of HIF.

94. In allowing the patent application that issued as the '172 patent, the Examiner failed to appreciate that the compounds of the '172 patent stabilizes HIF $\alpha$  by inhibiting prolyl hydroxylase just the same as the compounds in prior art, e.g., U.S. Patent No. 5,658,933 and Weidmann. Likewise, the compounds of Weidmann inhibit prolyl hydroxylase thereby stabilize HIF $\alpha$ . The Examiner mistakenly believed that compounds that inhibit of prolyl hydroxylase do not stabilize HIF $\alpha$ , which is scientifically incorrect.

95. As explained above, prolyl hydroxylase marks HIF $\alpha$  for proteosome

degradation by hydroxylation of proline residues. Inhibition of prolyl hydroxylase blocks the hydroxylation and subsequent degradation, and results in the stabilization of HIF $\alpha$  by the same mechanism they inhibit fibrogenesis. Thus, the heterocyclic compounds of U.S. Patent No. 5,658,933, in fact, stabilize HIF $\alpha$ . Weidmann has a similar disclosure as U.S. Patent No. 5,658,933, and teaches heterocyclic compounds that stabilize HIF $\alpha$  by inhibiting prolyl hydroxylase. In fact, Weidmann discloses many of the same heterocyclic carboxamide compounds as the '172 patent (*supra*, Table I at ¶ 65), which inhibit HIF prolyl hydroxylase and stabilize HIF $\alpha$ .

96. Accordingly, Weidmann teaches a method of treating a hypoxic or ischemic condition by administering a heterocyclic carboxamide, i.e., substituted isoquinoline-3-carboxamides, which stabilizes HIF $\alpha$ . As such, Weidmann anticipates claim 1 of the '172 patent.

**2. Dependent Claims 2, 3, 5, 6, and 8 are Anticipated By Weidmann**

97. Weidmann also anticipates dependent claims 2, 3, 5, 6, and 8 of the '172 patents. Claim 2 depends from claims 1 and recites, in relevant part, "wherein the heterocyclic carboxamide compound inhibits HIF prolyl hydroxylase enzyme activity." Ex. 1001 ('172 patent), at col. 63, lns. 37-39. Weidmann teaches that isoquinoline-3-carboxamides inhibit prolyl-4-hydroxylase. Ex. 1019 ('730 patent),

at col. 1, lns. 8-10. 2-oxoglutarate is a co-substrate required for the enzymatic activity of both collagen prolyl hydroxylase and HIF prolyl hydroxylase.

Isoquinoline-3-carboxamides are 2-oxoglutarate mimetics, and would inherently and naturally result in the inhibition of HIF prolyl hydroxylase. The '172 patent admits as much. The '172 patent states that Weidmann teaches compounds that inhibit HIF prolyl hydroxylase—"Exemplary compounds according to Formula (Ib) are described in U.S. Pat. No. 6,093,730. All compounds listed in U.S. Pat. No. 6,093,730, in particular, those listed in the compound claims and the final products of the working examples, are hereby incorporated into the present application by reference herein." Ex. 1001 ('172 patent), at col. 36, lns. 1-6. Thus, Weidmann anticipates claim 2 of the '172 patent.

98. Claim 3 depends from claims 1 and recites, in relevant part, "wherein the hypoxic or ischemic disorder or condition is associated with an ischemic event." *Id.*, at col. 63, lns. 40-41. The '172 patent defines "ischemic event" as "acute or transient, or chronic" and includes, among other things, "hypertension, diabetes, occlusive arterial disease, chronic venous insufficiency, Raynaud's disease cirrhosis, congestive heart failure, systemic sclerosis, etc." *Id.*, at col. 5, lns. 20-30. Weidmann teaches that the isoquinoline-3-carboxamides can be used to treat, among other things, atherosclerosis, an occlusion of the arteries and

reduction in blood flow, which is an ischemic event. Ex. 1019 ('730 patent), at col. 1, lns. 28-30. Further, Weidmann teaches the use of the isoquinoline-3-carboxamide compounds to treat a CCl<sub>4</sub>-model of liver fibrosis in rats, which is also an ischemic event (as admitted by Ex. 1001 ('172 patent), at col. 59, lns. 17-19). Accordingly, claim 3 of the '172 patent is anticipated by Weidmann.

99. Claim 5 depends from claim 3, and recites, in relevant part, "wherein the ischemic event is associated with surgery, organ transplantation, infarction, trauma, or injury." Ex. 1001 ('172 patent), at col. 64, lns. 32-34. Weidmann teaches the use of isoquinoline-3-carboxamide compounds to treat atherosclerosis, which can be characterized as an injury to the arterial wall leading to occlusion of the arteries and infarction. Further, as discussed above, prolonged treatment with CCl<sub>4</sub>, a known hepatotoxin, induces chronic liver injury and fibrosis. Ex. 1019 ('730 patent), at col. 9, lns. 62-63. In Weidmann, isoquinoline-3-carboxamide compounds were shown to improve liver function in chronic liver fibrosis induced by CCl<sub>4</sub>. Accordingly, Weidmann anticipates claim 5 of the '172 patent.

100. Claim 6 depends from claim 1, and recites, in relevant part, "wherein the ischemic condition is chronic." Ex. 1001 ('172 patent), at col. 64, lns. 35-36. Weidmann teaches the treatment of a chronic ischemic disorder in the CCl<sub>4</sub>-induced model of liver fibrosis. Ex. 1019 ('730 patent), at col. 9, ln. 62 – col. 10,

ln. 51 (discussing administering CCl<sub>4</sub> twice weekly for 4 weeks)). The administration of CCl<sub>4</sub> to rats twice weekly for 4 weeks results in chronic liver fibrosis. Thus, Weidmann anticipates claim 6 of the '172 patent.

101. Claim 8 depends from claim 1, and recites, in relevant part, "wherein the administering is oral administration." Ex. 1001 ('172 patent), at col. 64, lns. 41-42. Weidmann teaches the oral administration of the substituted isoquinoline-3-carboxamides. Ex. 1019 ('730 patent), at col. 9, ln. 65-66. Thus, Weidmann anticipates claim 8 of the '172 patent.

**XI. CLAIMS 1-8 OF THE '172 PATENT WOULD HAVE BEEN OBVIOUS TO A PERSON OF ORDINARY SKILL IN THE ART**

102. I have reviewed the claims of the '172 patent (Ex. 1001) and it is my opinion that all of the claim limitations of the '172 patent are found within the teaching of Ivan 2001 in view of the Bickel (Ex. 1027) or Weidmann (Ex. 1019) and further in view of Semenza 2000 (Ex. 1009).

**A. Claims 1, 2, 3, 5 and 6 are Obvious in view of the Combination of Ivan 2001 and Bickel**

**1. Claim 1 is Obvious over Ivan 2001 in view of Bickel**

103. Claim 1 describes a method for treating an hypoxic or ischemic disorder or condition by administering a heterocyclic compound that stabilizes HIF $\alpha$ . This method would have been obvious to a person of ordinary skill in the art in light of: (1) the disclosure by Ivan 2001 that inhibitors of prolyl hydroxylase

inhibitors could stabilize the HIF $\alpha$  coupled with, (2) Bickel 1998 that teaches the use of a heterocyclic carboxamide compound that inhibits prolyl hydroxylase in the treatment of liver ischemia.

104. Ivan 2001 describes the importance of prolyl hydroxylases in the regulation of HIF $\alpha$ —that is, HIF hydroxylation by prolyl hydroxylase (i.e., HIF prolyl hydroxylase (Jaakkola (Ex. 1007))) mediates the proteosomal degradation of HIF $\alpha$ . Accordingly, Ivan 2001 suggests that inhibitors of prolyl hydroxylase can prevent the hydroxylation of HIF $\alpha$  (and thus stabilize HIF $\alpha$ ), resulting in the accumulation of HIF and induction of gene expression of HIF-targeted genes. Ivan 2001 states that "[i]n principle, drugs that stabilize HIF may augment angiogenesis and the adaption to chronic hypoxia" Ex. 1025 (Ivan 2001), at 467. Although Ivan 2001 does not specifically discuss heterocyclic carboxamides as inhibitors of HIF prolyl hydroxylase, Ivan 2001 recognizes that "[s]everal small-molecule proline hydroxylase inhibitors have been developed as antifibrotic agents and can now be tested in ischemia models." *Id.* Ivan 2001 expressly instructs that the compounds disclosed in four earlier publications, including Bickel, should be tested in ischemia models. *Id.*, Footnote 33 (Bickel).

105. As discussed above, Bickel teaches the use of a heterocyclic carboxamide, S4682, for the treatment of liver ischemia in a CCl<sub>4</sub> model of toxic-

ischemic hepatic injury. (*supra*, ¶ 66)

106. Both Ivan 2001 and Bickel are in the field of HIF and, among other things, the treatment of ischemic disorders or conditions. A person of ordinary skill in the art would follow the instructions of Ivan 2001 to test the heterocyclic compound of Bickel (S4682) to treat ischemic disorders and conditions. The administration of S4685 would have inherently stabilized HIF $\alpha$ , resulting in the accumulation of HIF and expression of HIF-targeted genes. Indeed, a person of ordinary skill in the art would have expected such results based on the disclosure of Ivan 2001, which suggests that small-molecule proline hydroxylase inhibitors may stabilize HIF and augment adaptive physiological processes to hypoxia and ischemia. Ex. 1025 (Ivan 2001), at 467.

107. The '172 patent admits that in Bickel "[t]he improvement in liver function is attributed to stabilization of HIF $\alpha$  by the methods of the invention." Ex. 1001 ('172 patent), at col. 59, lns. 65-67. This method would have been obvious to a person of ordinary skill in the art in light of the disclosure by Ivan 2001 that inhibitors of prolyl hydroxylase, such as Bickel, could stabilize the HIF $\alpha$  coupled with Bickel that teaches a heterocyclic carboxamide, S 4682, inhibits prolyl hydroxylase for the treatment of liver ischemia. This is exactly what the inventor of '172 patent did—they simply followed the express instruction of Ivan 2001 to



test such compounds as Bickel for the treatment of ischemic disorders or conditions.

108. Based on the foregoing, claim 1 of the '172 patent is obvious over Ivan 2001 in view of Bickel.

**2. Dependent Claims 2, 3, 5, and 6 Are Obvious over Ivan 2001 in view of Bickel**

109. Claim 2 depends from claims 1 and recites, in relevant part, "wherein the heterocyclic carboxamide compound inhibits HIF prolyl hydroxylase enzyme activity." Ex. 1001 ('172 patent), col. 63, lns. 37-39. Ivan 2001 (Ex. 1025) teaches that the degradation of HIF occurs through an enzyme and notes that Jaakkola 2001 (Ex. 1007) came to the same conclusion.<sup>9</sup> Jaakkola 2001 named the enzyme HIF prolyl hydroxylase. Further, the Bickel compound, S4682, is a heterocyclic carboxamide and, as admitted in the '172 patents, inhibits HIF prolyl hydroxylase enzyme activity. Specifically, the '172 patents states, "[t]he present invention contemplates the use of these compounds [Bickel] in the methods provided herein . . . Such compounds may inhibit the target 2-oxoglutarate dioxygenase enzyme family" (*id.*, at col. 26, lns. 10-25), that is, HIF prolyl hydroxylase. Moreover,

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<sup>9</sup> Both Ivan 2001 and Jaakkola 2001 published in Issue 292 of Science. Ex. 1025 (Ivan 2001); Ex. 1007 (Jaakkola 2001).

Declaration of Paul T. Schumacker in Support of  
GlaxoSmithKline Petition for *Inter Partes* Review of U.S. Pat. No. 8,466,172

Ivan 2001 identifies "[s]everal small-molecule proline hydroxylase inhibitors" (i.e., HIF prolyl hydroxylase), including the heterocyclic carboxamide (S 4682) compound of Bickel. Accordingly, it would have been obvious to one of ordinary skill in the art that the heterocyclic carboxamide compound of Bickel would inhibit HIF prolyl hydroxylase enzyme activity and stabilize HIF $\alpha$ .

110. Claim 3 depends from claims 1 and recites, in relevant part, "wherein the hypoxic or ischemic disorder or condition is associated with an ischemic event." *Id.*, at col. 63, lns. 40-41. Bickel teaches the use of a heterocyclic carboxamide compound for the treatment of liver ischemia in a CCl<sub>4</sub> model of liver injury. The '172 patent admits that CCl<sub>4</sub> is a toxic-ischemic liver injury, i.e., an ischemic event. *Id.*, at col. 59, lns. 17-19. As such, it would have been obvious to a person of ordinary skill in the art to treat an ischemic event, such as a toxic-ischemic liver injury, with a heterocyclic carboxamide compound that stabilizes HIF $\alpha$ .

111. Claim 5 depends from claim 3, and recites, in relevant part, "wherein the ischemic event is associated with surgery, organ transplantation, infarction, trauma, or injury." *Id.*, at col. 64, lns. 32-34. Bickel teaches a CCl<sub>4</sub> model of liver injury. CCl<sub>4</sub> is a known hepatotoxin and causes ischemic injury of the liver. Moreover, the '172 patent admits that CCl<sub>4</sub> is a toxic-ischemic liver injury, i.e., an

ischemic event. *Id.*, at col. 59, lns. 17-19. It would have been obvious to a person of ordinary skill in the art to treat an ischemic event associated with a toxic-ischemic liver injury with a heterocyclic carboxamide compound that stabilizes HIF $\alpha$ .

112. Claim 6 depends from claim 1, and recites, in relevant part, "wherein the ischemic condition is chronic." *Id.*, at col. 64, lns. 35-36. Bickel teaches a CCl<sub>4</sub> model of liver injury. Treatment with CCl<sub>4</sub> induces chronic liver injury that leads to liver fibrosis, ischemia, and cirrhosis. In Bickel, CCl<sub>4</sub> was administered to rats over a long-term period (9 weeks) to induce chronic liver injury. S4682 was shown to improve liver function in chronic hepatic ischemia. As such, it would have been obvious to a person of ordinary skill in the art to treat a chronic ischemic condition with a heterocyclic carboxamide compound that stabilizes HIF $\alpha$ .

**B. Claim 8 is Obvious in view of the Combination of Ivan 2001, Bickel, and Weidmann**

113. Claim 8 depends from claim 1, and recites, in relevant part, "wherein the administering is oral administration." *Id.*, at col. 64, lns. 41-42. As discussed above, the limitation of claim 1 are obvious in view of Ivan 2001 (Ex. 1025) and Bickel (Ex. 1027). In general, a person of ordinary skill in the art would have understood that oral administration of a compound is the preferred mode of administration unless indicated otherwise. No reference is needed for such

teaching. Other modes of administration, e.g., parenteral, are more cumbersome, painful, and/or, generally, lack patient compliance. Thus, it would have been obvious to one of ordinary skill in the art to pursue an orally administered heterocyclic carboxamide compound. Nevertheless, Weidmann teaches the oral administration of the substituted isoquinoline-3-carboxamides. Ex. 1019 ('730 patent), at col. 9, ln. 65-66. Thus, it would have been obvious to combine Ivan 2001 and Weidmann to treat a hypoxic or ischemic disorder or condition by orally administering a heterocyclic carboxamide compound that stabilizes HIF $\alpha$ . Thus, it would have been obvious to one of ordinary skill in the art to pursue an orally administered heterocyclic carboxamide compound that stabilizes HIF $\alpha$  to treat a hypoxic or ischemic disorder or condition.

**C. Claims 4 and 7 are Obvious in view of the Combination of Ivan 2001, Bickel, and Semenza 2000**

**1. Dependent Claim 4 is Obvious over Ivan 2001 in view of Bickel and Semenza 2000**

114. Claim 4 depends from claim 3, and recites, in relevant part, "wherein the ischemic event is acute." Ex. 1001 ('172 patent), at col. 63, lns. 41-42. As discussed above, Ivan 2001 combined with Bickel renders claim 3 obvious. Semenza 2000 teaches that HIF is involved in a number of human pathologies, including ischemic cardiovascular disease, hypertension, and cancer. Specifically, Semenza 2000 teaches that an increase in HIF-1 $\alpha$  mRNA and protein expression

are induced during an acute ischemic event such as a myocardial infarction,<sup>10</sup> which induces vascular endothelial growth factor (VEGF) expression and, in turn, angiogenesis Ex. 1009 (Semenza 2001) at 1984. Semenza 2000 then states that "therapeutic strategies designed to increase HIF-1 $\alpha$  expression may promote angiogenesis within ischemic myocardium." *Id.*

115. A person of ordinary skill in the art would have understood that a compound that stabilizes HIF $\alpha$  and induces gene expression of HIF-targeted genes, such as glucose transporters or glycolytic enzymes, could be used to treat an acute ischemic event. Further, HIF-mediated expression of VEGF, along with other angiogenic or survival factors, such as angiopoietin-2 or possibly Insulin-like Growth Factor-2 ("IGF-2"), can stimulate the growth of new blood vessels into the ischemic region, thereby improving blood flow and oxygen delivery. Ex. 1009 (Semenza 2000). As described above, Ivan 2001 teaches that inhibitors of prolyl hydroxylase can stabilize HIF $\alpha$  and Bickel discloses heterocyclic carboxamide compounds as prolyl hydroxylase inhibitors. Indeed, Ivan 2001 suggests using prolyl hydroxylase inhibitors, as described by Bickel, to inhibit HIF prolyl hydroxylase to stabilize HIF $\alpha$ . Semenza teaches HIF $\alpha$  is induced during acute

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<sup>10</sup> The '172 patent defines "acute ischemic conditions" as including myocardial infarction. Ex. 1001 ('172 patent), at col. 21, lns. 29-31.

ischemic event. Thus, a person of ordinary skill in the art would have combined the teachings of Ivan 2001, Bickel, and Semenza 2000 to treat an acute ischemic event with a heterocyclic compound that stabilize HIF $\alpha$ . As such, claim 4 is obvious.

**2. Dependent Claim 7 is Obvious over Ivan 2001 in view of Bickel and Semenza 2000**

116. Claim 7 depends from claim 1, and recites, in relevant part, "wherein the hypoxic or ischemic disorder or condition is associated with ischemic reperfusion injury in a subject." A person of ordinary skill in the art would have understood that a compound that stabilizes HIF $\alpha$  and induces gene expression of HIF-targeted genes, such as HO-1 or NOS, could be used to treat a condition involving ischemia-reperfusion injury. Ischemia-reperfusion injury is a type of cellular injury that occurs when oxygen supply is restored to an ischemic tissue. HIF-mediated expression of HO-1 can protect cells during ischemia-reperfusion injury by enhancing the scavenging of reactive oxygen species, thus lessening the oxidant-induced cellular injury associated with that condition. Similarly, expression of NOS can lead to the generation of nitric oxide, which can act as a trigger to further activate HIF and also as a molecular mediator of cellular protection. Ex. 1009 (Semenza 2000), at 1984 ("Furthermore, NO has shown to induce HIF-1 $\alpha$  expression under non-hypoxic conditions.").

117. The cellular protection afforded by the HIF-mediated response would

also derive from the increased expression of glucose transporters, glycolytic enzymes, and other genes that provide an alternative source of energy in cells deprived of oxygen. Scientists already established that HIF regulated the expression of several proteins, including glucose transporters, that impact glucose/energy metabolism and cell proliferation/viability. Ex. 1009 (Semenza 2000), at 1984, Table 1. These mechanisms would enhance the survival of cells during a lethal ischemic event. Because inhibitors of prolyl hydroxylase, such as Bickel, can stabilize HIF $\alpha$  and induce HIF-mediated gene expression of enzymes, such as HO-1 and NOS, and glucose metabolic proteins, a person of ordinary skill in the art would have combined the teachings of Ivan 2001, Bickel, and Semenza 2000 to treat a condition of ischemic reperfusion injury with a heterocyclic compound that stabilizes HIF $\alpha$ . As such, claim 7 is obvious.

Declaration of Paul T. Schumacker in Support of  
GlaxoSmithKline Petition for *Inter Partes* Review of U.S. Pat. No. 8,466,172

**XII. CONCLUSION**

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

Dated: June 29, 2016

By:   
Paul Schumacker, Ph.D.