PATENT EVALUATION

miRNA let-7b inhibitors by treatment of diabetic retinopathy: evaluation patent US2019093106

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Abstract

Introduction: diabetic retinopathy is a clinical complication that affects to a 93 million people suffering from diabetes and is responsible for more hospitalizations than any other complication of diabetes. Authors of US2019093106 patent propose a method for treatment of diabetic retinopathy. Areas covered: US2019093106 describes a method that consists of the administration of inhibitor of miRNA let-7b, in patients with diabetic retinopathy; additionally describe a method to inhibiting the effects of increased levels or activity of miRNA let-7b. Expert opinion: The results pre-clinical trials support the therapy's efficacy; however, the invention is not new considering art state, whereby, new studies will be necessary to determine other administration routes, e.g. topical, dose and time of administration.

Keywords: miRNA let-7b, diabetic retinopathy, patent, diabetes, inhibitor

Introduction

Globally, diabetes mellitus has been recognized as the third cause of premature mortality due to hyperglycemia. The data suggests that about 415 million people live with diabetes, of which 75% live in low- and middle-income countries [1]. Similarly, it is estimated that by 2040 there will be 642 million people with diabetes [2]. Various clinical complications are observed in patients with type 2 diabetes mellitus, including cardiovascular disease, diabetic foot ulcer, retinopathy, neuropathy and nephropathy [3-7].

According to Yau et al., diabetic retinopathy affects to 93 million people suffering from diabetes [8]. Similarly, Leasher et al. has shown that diabetic retinopathy was responsible for the visual impairment of 3.7 million people and the blindness of 0.8 million people [9]. Diabetic retinopathy is a progressive disease that, according to its severity, is divided into non-

proliferative and proliferative. The first is characterized by micro-aneurysms and intraretinal microvascular anomalies, while the second is characterized by neovascularization of the optic disc and preretinal and vitreous hemorrhage [10]. There are several treatment modalities for diabetic retinopathy, which include the topical application of growth factors, synthetic drugs and natural products. For example, calcium dobesilate inhibits apoptosis of vascular endothelial cells in blood vessels and the expression of ICAM-1 [11-13]. Similarly, Aflibercept is a recombinant fusion protein from promotions of extracellular domains of VEGF receptors 1 and 2 fused to the Fc portion of IgG [14-15]. In turn, Ranibizumab is a fragment of a monoclonal antibody that does not contain the Fc region and with affinity for VEGF-A [16-17]. On the other hand, several synthetic drugs such as triamcinolone acetonide, dexamethasone or fluocinolone acetonide have been employed in the treatment of diabetic retinopathy [18-21]

Continuous development of new alternatives for the treatment of diabetic retinopathy is necessary, and consequently this article evaluates the US2019093106 patent application [22], which describes the application of miRNA let-7b inhibitors as an alternative for healing diabetic retinopathy. miRNA let-7b, a microRNA that regulates cell proliferation and mediates immune responses [23], play a role in the pathogenesis of type 2 diabetes mellitus [24], and represents a potential target for the treatment of renal fibrosis in diabetic nephropathy [25]. These inhibitors are owned by Novo Nordisk and patent applications were filed at the Patent Cooperation Treaty (WO2017062659), Europe (EP3359553) and the United States (US2019093106).

Chemistry and Biology

US2019093106 patent was published on March 28, 2019. Patent application claims and describes two methods. First method includes the administration of an inhibitor of miRNA let-7b to a patient with retinopathy (claims 1-23, 27). Second method includes the administration of a miRNA let-7b to a patient for inhibits the effects of increased levels or activity of miRNA let-7b associated with any injury, disease, or disorder (23-26). Further claims cover type inhibitors, route of administration, and their applications in diabetic retinopathy (Table I).

Key inhibitors of the invention are those having the nucleotide sequences SEQ ID 1 (AACCACACAACCUACUACCUCA) and SEQ ID 2 (AACCACACAACCUA). Those specific claims for said inhibitors (circles and filled cells) are observed in Table 1. In particular, the SEQ ID 1 inhibitor can be modified, in one or more nucleotides, through 2′-O-methylating, 2′-O-methoxyethylating, phosphorothioate linkages,

cholesterol hydroxyprolinol linkage, or locked nucleic acid.

From the results of biological experiments, in a murine model of diabetic retinopathy, it can be concluded that the injection of a let-7b microRNA inhibitor reduces retinal capillary dropout, decreases microvascular leakage and prevents hyperproliferation of microvascular cells. Was observed that miRNA let-7b expression is upregulated in the retina during diabetic retinopathy of Akimba mouse model. This miRNA let-7b over-expression induced autophagy and accelerate the diabetic retinal degeneration, by directly targeting and downregulating the genes upstream of mTORC1 and FoxO3A pathway.

In another series of experiments, the authors determined the potential of miRNA let-7b inhibitors to protect or restore large-scale loss of retinal vessels and aberrant neovascularization. The sequence of the inhibitors used are described in Table II. When intravitreally injected in male mice, the antagomiR let-7b inhibitor conjugated with Dy-547 showed that it was located uniformly, from 8 weeks to 6 months, throughout the entire retinal vasculature. Additionally, treatment with the inhibitor reduced the ratio between the fall and the total retinal area by 24%; also, a protection of the thickness of the retina and the cellularity was observed.

Table I. Claims of US2019093106

Claim	Problem to solve (treatment to retinopathy)	Technical feature
1	**Method** of treatment to retinopathy	Administer inhibitor of miRNA let-7b in a **therapeutically effective** amount to a subject in need
2	**Method** of treatment to retinopathy	Inhibitor is a nucleic acid
3	**Method** of treatment to retinopathy	Nucleic acid is an **antagomir** of miRNA let-7b
4-5	**Method** of treatment to retinopathy	Nucleic acid comprises **SEQ ID 1** or a biologically active homolog of fragment thereof modified or not
6, 8-13,15-16	**Method** of treatment to retinopathy	Modification can be a detectable label, phosphorothioate, 2'-O-methyl modification, 2'-O-methoxiethyl modification, 3'-cholesterol modification, and locked nucleic acid modification at each nucleotide residue
7	**Method** of treatment to retinopathy	Nucleic acid comprises two modifications
14	**Method** of treatment to retinopathy	Nucleic acid comprises five modifications
17	**Method** of treatment to retinopathy	Biologically active homolog of fragment has **SEQ 2** or a modification thereof
18	**Method** of treatment to retinopathy	Inhibitor can be Antagomir-let7b (ant-let7b), LNA-Chl antisense of let-7b, LNA antisense let-7b, MOE antisense let-7b, truncated ant-let7b, LNA-Chl antisense let-7b mini, LNA antisense let-7b mini, or MOE antisense let-7b mini.
19	**Method** of treatment to retinopathy	Inhibitor is administered intravitreally
20	**Method** of treatment to retinopathy	Stimulates vascular stabilization and an increase in retinal thickness
21	**Method** of treatment to retinopathy	Reduces hyperproliferation of microvascular cells, retinal capillary dropout, and microvascular leakage
22	**Method** of treatment to retinopathy	Retinopathy is diabetic retinopathy
23	**Method** to inhibiting the effects of increased levels or activity of miRNA let-7b associated with an injury, disease, or disorder	Administer inhibitor of miRNA let-7b in a **therapeutically effective** amount to a subject in need
24	**Method** to inhibiting the effects of increased levels or activity of miRNA let-7b associated with an injury, disease, or disorder	Inhibitor is a nucleic acid comprising the sequence SEQ ID NO: 1 or a biologically active homolog or fragment thereof and said sequence is modified.
25	**Method** to inhibiting the effects of increased levels or activity of miRNA let-7b associated with an injury, disease, or disorder	Wherein increased levels or activity of miRNA let-7b are associated with diabetic retinopathy.
26	**Method** to inhibiting the effects of increased levels or activity of miRNA let-7b associated with an injury, disease, or disorder	Wherein said injury, disease, or disorder is a microvasculature injury, disease or disorder.
27	**Method** of treatment to retinopathy	A kit for treating diabetic retinopathy, said kit comprising at least one inhibitor of claim 1, an applicator, and an instructional material for the use thereof.

Table II. MiRNA let-7b inhibitors

Inhibitor	Sequence
AntagomiR let-7b	Dy547mA(*)mA(*)mCmCmAmCmAmCmAmAmCmCmUm AmCmUmAmCmCmU(*)m C(*)mA(*)(3′-Chl)
AntagomiR Let-7b Mini	mA(*)mA(*)mCmCmAmCmAmCmAmAmCmC(*)mU(*)mA (3′-Chl)
LNA-Chl Anti-Sense let-7b	lAlAlClClAlClAlClAlAlClClUlAlClUlAlClClUlClA(3′-Chl)
LNA-Chl Anti-Sense let-7b Mini	lAlAlClClAlClAlClAlAlClClUlAlClClUlA(3′-Chl)
LNA Anti-Sense let-7b	lAlAlClClAlClAlClAlAlClClUlAlClUlAlClClUlAlClUlAlClC lUlClA
LNA Anti-Sense let-7b Mini	lAlAlClClAlClAlClAlAlClClUlAlClClUlA
MOE Anti-Sense let-7b	meAmeAmeCmeCmeAmeCmeAmeCmeAmeAmeCmeCmeU meAmeCmeUmeAme CmeCmeUmeCmeA
MOE Anti-Sense let-7b Mini	meAmeAmeCmeCmeAmeCmeAmeCmeAmeAmeCmeCmeU meA

"m" indicates 2-O-Methyl moiety; * indicates Phosphorothioate and a cholesterol molecule at the 3′; Dy-547 is a fluorescent molecule for detection; "l" indicates LNA modification; (3′-Chl) is a cholesterol molecule at the 3′; "l" indicates LNA modification; "me" indicates 2′-O-methoxyethyl modification.

Expert opinion

US2019093106 patent describes the potential application of inhibitors of miRNA let-7b for the treatment of diabetic retinopathy. As mentioned in the Biology section, the inventors do show data regarding the efficacy of miRNA let7-b's treatment. Therefore, the patent is shown scientific data concerning effect on the protection of the thickness of the retina. Authors have not yet reported their results in scientific publications, nor have clinical trials started on such inhibitors. However, despite these positive premises, data supporting the invention are still limited; further studies aimed at investigating dose–response relationship, long-term effects, are indeed necessary to assess the clinical applicability of the invention.

Various claims of the patent appear not to be involved inventive step. For example, Shen et al. [26] describe the use of nucleic acids modified through 2′-O-methylating, 2′-O-methoxyethylating, phosphorothioate linkages, cholesterol hydroxyprolinol linkage, or locked nucleic acid, in such a way that the claims 1-7, 8, 9, 11, 12 and 15 are preceded by Shen's work. However, Shen does not describe a sequence comprising SEQ ID 1 or SEQ ID 2 or a biologically active homolog or fragment of US2019093106. Similarly, Olson et al, [27] shown nucleic acids inhibitors of miRNA let-7b to treat or prevent cardiovascular diseases, anticipates claims 12, 14 and 15. These nucleic acids can be modified through 2′-O-methylating, 2′-O-methoxyethylating, phosphorothioate linkages, cholesterol hydroxyprolinol linkage, or locked nucleic acid. Particularly, said nucleic acid comprise SEQ ID 1 or a biologically active homolog or fragment of the US2019093106 invention. Likewise, Sallustio et al. [28] describes a new method for the diagnosis of glomerulonephritis which includes the use of antisense miRNAs. These nucleic acids show 100% sequence identity with SEQ ID 1 of US2019093106 patent, whereby claims 4-9 and 12-16 lack an inventive step. Although the patents described do not describe the application in the treatment of diabetic retinopathy with the miRNA let-7b inhibitors, SEQ ID and SEQ 2, the US2019093106's applicant claims the invention very broadly. This invention only demonstrates its novelty in the case of sequences SEQ 1 and SEQ 2.

In conclusion, the application of inhibitors of miRNA let-7b for the treatment of diabetic retinopathy is not new or has inventive activity. Also, additional studies are necessary that include other types of additives that allow a greater permanence of the drug in the damaged tissue. Similarly, new studies will be necessary to

determine, at the topical level, the dose and time of administration of inhibitors of miRNA let-7b.

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Declaration of interest

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

References

[1]. International Diabetes Federation (IDF) Diabetes Atlas. 3rd edition. Brussels, Belgium: International Diabetes Federation; 2017.

[2]. International Diabetes Federation (IDF) Diabetes Atlas. 3rd edition. Brussels, Belgium: International Diabetes Federation; 2015.

[3]. Pozo L, Bello F, Suarez A, et al. Novel pharmacological therapy in type 2 diabetes mellitus with established cardiovascular disease: Current evidence. World journal of diabetes, 2019, 10(5), 291-303.

[4]. Zhang P, Lu J, Jing Y, et al. Global epidemiology of diabetic foot ulceration: a systematic review and meta-analysis. Annals of medicine, 2017,*49*(2), 106-16.

[5]. Whitehead M, Wickremasinghe S, Osborne A, et al. Diabetic retinopathy: a complex pathophysiology requiring novel therapeutic strategies. Expert opinion on biological therapy, 2018,18(12), 1257-70.

[6]. Sloan G, Shillo P, Selvarajah D, et al. A new look at painful diabetic neuropathy. Diabetes research and clinical practice. 2018, 144:177-191.

[7]. Kopel J, Pena-Hernandez C, Nugent K. Evolving spectrum of diabetic nephropathy. World journal of diabetes, 2019,10(5), 269.

[8]. Yau JW, Rogers SL, Kawasaki R, et al. Global prevalence and major risk factors of diabetic retinopathy. Diabetes Care. 2012;35:556–64.

[9]. Leasher JL, Bourne RR, Flaxman SR, et al. Global estimates on the number of people blind or visually impaired by diabetic retinopathy: a meta-analysis from 1990 to 2010. Diabetes Care. 2016;39:643-9.

[10]. Wu L, Fernandez-Loaiza P, Sauma J, et al. Classification of diabetic retinopathy and diabetic macular edema. World J Diabetes. 2013;4:290-4.

[11]. Zhou Y, Qi C, Li S, et al. Diabetic Nephropathy Can Be Treated with Calcium Dobesilate by Alleviating the Chronic Inflammatory State and Improving Endothelial Cell Function. Cellular Physiology and Biochemistry, 2018;51(3):1119-1133.

[12]. Graber R, Farine JC, Losa GA. Calcium Dobesilate protects human peripheral blood mononuclear cells from oxidation and apoptosis. Apoptosis, 1998;3:41-9.

[13]. Opreanu M, Lydic TA, Reid GE, et al. Inhibition of cytokine signaling in human retinal endothelial cells through downregulation of sphingomyelinases by docosahexaenoic acid. *Investigative ophthalmology & visual science*, 2010;51(6):3253-63.

[14]. Bressler SB, Liu D, Glassman AR, et al. Change in diabetic retinopathy through 2 years: secondary analysis of a randomized clinical trial comparing aflibercept, bevacizumab, and ranibizumab. JAMA ophthalmology, 2017;135(6):558-68.

[15]. Sivaprasad S, Prevost AT, Vasconcelos JC, et al. Clinical efficacy of intravitreal aflibercept versus panretinal photocoagulation for best corrected visual acuity in patients with proliferative diabetic retinopathy at 52 weeks (CLARITY): a multicentre, single-blinded, randomised, controlled, phase 2b, non-inferiority trial. The Lancet, 2017;389(10085):2193-203.

[16]. Stewart MW. A review of ranibizumab for the treatment of diabetic retinopathy. Ophthalmology and Therapy, 2017;6(1):33-47.

[17]. Hutton DW, Stein JD, Bressler NM, et al. Cost-effectiveness of intravitreous ranibizumab compared with panretinal photocoagulation for proliferative diabetic retinopathy: secondary analysis from a diabetic retinopathy clinical research network randomized clinical trial. JAMA ophthalmology, 2017;135(6), 576-84.

[18]. Watanabe A, Tsuzuki A, Arai K, et al. Efficacy of intravitreal triamcinolone acetonide for diabetic macular edema after vitrectomy. Journal of Ocular Pharmacology and Therapeutics, 2016;32(1):38-43.

[19]. Yilmaz T, Cordero-Coma M, Lavaque A, et al. Triamcinolone and intraocular sustained-release delivery systems in diabetic retinopathy. Current Pharmaceutical Biotechnology, 2011;12(3):337-46.

[20]. Querques L, Parravano M, Sacconi R, et al. Ischemic index changes in diabetic retinopathy after intravitreal dexamethasone implant using ultra-widefield fluorescein angiography: a pilot study. Acta Diabetologica, 2017;54(8): 769-773.

[21]. Wykoff CC, Chakravarthy U, Campochiaro PA, et al. Long-term effects of intravitreal 0.19 mg fluocinolone acetonide implant on progression and regression of diabetic retinopathy. Ophthalmology, 2017;124(4):440-9.

[22]. Dey BK, Yates P, University of Virginia. Compositions and methods for treating diabetic retinopathy. United States patent US2019093106, 2019 March 28.

[23]. Bao MH, Feng X, Zhang YW, et al. Let-7 in cardiovascular diseases, heart development and cardiovascular differentiation from stem cells. International Journal of Molecular Sciences, 2013;14(11):23086-102.

[24]. Liang YZ, Dong J, Zhang J, et al. Identification of neuroendocrine stress response-related circulating microRNAs as biomarkers for type 2 diabetes mellitus and insulin resistance. Frontiers in Endocrinology, 2018;9:132.

[25]. Wang B, Jha JC, Hagiwara S, et al. Transforming growth factor-β1-mediated renal fibrosis is dependent on the regulation of transforming growth factor receptor 1 expression by let-7b. Kidney International, 2014;85(2):352-361.

[26]. Shen J, Kelnar K, Shelton J, et al. Compositions and method related to miRNA modulation of neovascularization or angiogenesis, United States patent US2013065951, 2013 Mar 14.

[27]. Olson E, Rooij EV, Frost R, University of Texas. Targeting of the miR-30 family and Let-7 family as a treatment for heart disease. Worldwide patent WO2010120969, 2010 Oct 21.

[28]. Sallustio F, Serino G, Schena FP, Universita degli studi di Bari. Method and kit for the diagnosis of IgA nephropathy. Worldwide patent WO2012056282, 2012 May 3.