

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Inventors: HANSON *et al.*

Confirmation No.: 1042

Applicant: Sarepta Therapeutics, Inc.

Art Unit: 1635

Application No.: 15/993,287

Examiner: Chong, Kimberly

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Title: **EXON SKIPPING OLIGOMER CONJUGATES FOR MUSCULAR DYSTROPHY**

Declaration of Frederick Joseph Schnell, Ph.D., Under 37 C.F.R. § 1.132

Mail Stop Amendment

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Commissioner:

I, Frederick Joseph Schnell, declare and state that:

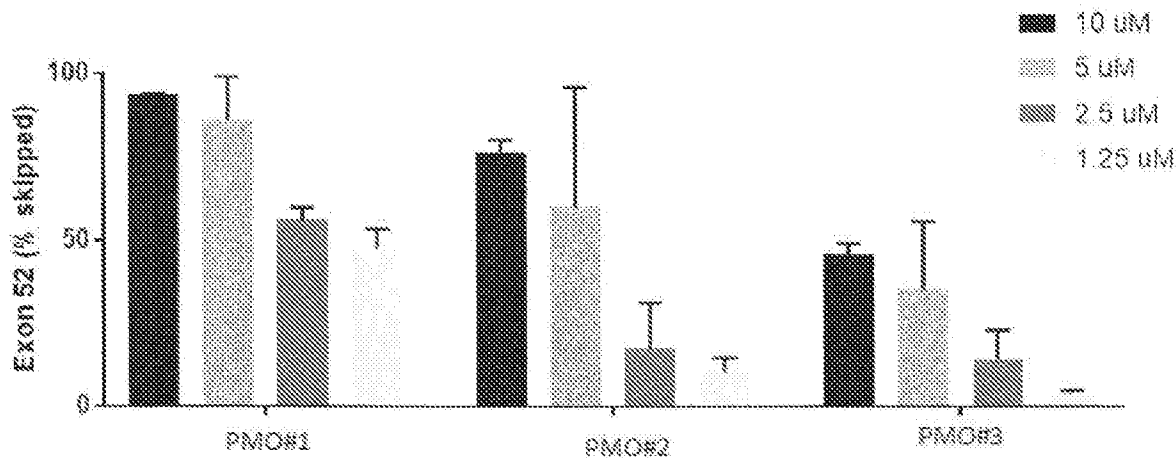
1. I am currently employed by Sarepta Therapeutics, Inc. ("Sarepta"), where I hold the position of Senior Manager, Business Development Search and Evaluation. In a previous role at Sarepta, I was responsible for oversight of antisense oligonucleotide (AON) screening activities for Duchenne muscular dystrophy (DMD) and lead sequence selection. A copy of my CV is attached as Exhibit A.
2. I received my B.S. in Biology from Davidson College in 1998. I earned my Ph.D. in Immunology and Molecular Pathogenesis from Emory University in 2006. I was then a postdoctoral fellow at Emory University from 2006-2008. I joined Sarepta as a Scientist in 2008 and have worked there since. I became a Senior Scientist in 2015 and moved to my current position in Business Development in 2018. During my time as a scientist at Sarepta, I was actively involved in designing and performing biological tests on AONs.

3. I understand that my opinions in this declaration should be based on what an average scientist would have thought as of May 2018 as this was the time at which the instant application and the priority application were filed. I understand that the term used for this average scientist is "a person of ordinary skill in the art."
4. I have been given a copy of each of the following documents: the present application published as US 2019/0365919 A1 on December 5, 2019 ("US '919"), the claim set currently on file at the USPTO, the Office Action issued by the USPTO on August 29, 2019 ("Office Action"), and the prior art documents U.S. Patent No. 10,337,003 to Kaye *et al.* ("Kaye") and U.S. Patent Application Publication No. 2012/0289457 A1 to Hanson ("Hanson"), cited in the Office Action. I have read and understood all of these documents.
5. The Office asserts that Kaye describes PMOs for treating muscular dystrophy including SEQ ID NO: 627 (defined as HU.DMD.exon52.25.006). Office Action, page 3. I understand that the PMOs described in Kaye have been determined by the Office to be the closest prior art to the claimed PPMO antisense oligonucleotide conjugates. The term "PMO" refers to a phosphorodiamidate morpholino oligomer and the term "PPMO" refers to a peptide conjugated PMO.
6. I have been asked to provide my opinion on the results provided herewith comparing the exon 52 skipping effect of three antisense oligonucleotide conjugates: 1) a PMO of SEQ ID NO:1 in the present application targeting the annealing site H52A(-1+24) (hereinafter referred to as "PMO#1"); 2) a PMO of SEQ ID NO:627 in Kaye (referred to as Hu.DMD.exon52.25.006) targeting the annealing site H52A(+1+25) (hereinafter referred to as "PMO#2"); and 3) a PMO of SEQ ID NO:628 in Kaye (referred to as Hu.DMD.exon52.25.006.2) targeting the annealing site H52A(-2+23) (hereinafter referred to as "PMO#3"), in human Rhabdomyosarcoma cells (RD cells).
7. PMO#2 and PMO#3 target a region of exon 52 that is shifted one base 3' and 5', respectively, of the region of exon 52 targeted by PMO#1. Thus, the skilled artisan

would reasonably expect that each of PMOs #1, #2, and #3 would have similar exon skipping activity. However, based on the experimental results presented herein, PMO#1 demonstrated unexpected superior and significant exon 52 skipping as compared to PMO#2 and PMO#3.

8. The exon 52 skipping efficacy of PMO#1, PMO#2, and PMO#3 in RD cells was evaluated using the following procedure. For nucleofection experiments, human RD cells were cultured using standard techniques. Lyophilized PMO were re-suspended in nuclease-free water. To verify molarity, PMO solutions were measured using a NanoDrop 2000 spectrophotometer (Thermo Scientific). PMOs were delivered to RD cells using nucleoporation according to the manufacturer's instructions and the SG kit (Lonza) and allowed to incubate overnight in a 37°C, 5% CO₂ incubator prior to RNA extraction. RNA was extracted from PMO-treated cells using the RNeasy spin 96 well RNA isolation kit from GE Healthcare and subjected to RT-PCR using standard techniques with primers that targeted human DMD exons 51 and 53. Skipping was measured using the Caliper LabChip bioanalyzer and the % exon skipping (i.e., band intensity of the exon-skipped product relative to the full length PCR product) was calculated by the equation: [exon 52 skipped product/(sum of exon 52 skipped and exon 52 unskipped products)*100]. These experiments are standard experiments in the field and provide a reliable and reproducible measure of exon skipping.
9. The results of the comparison of PMO#1 with PMO#2 and PMO#3 are shown in Figure A below:

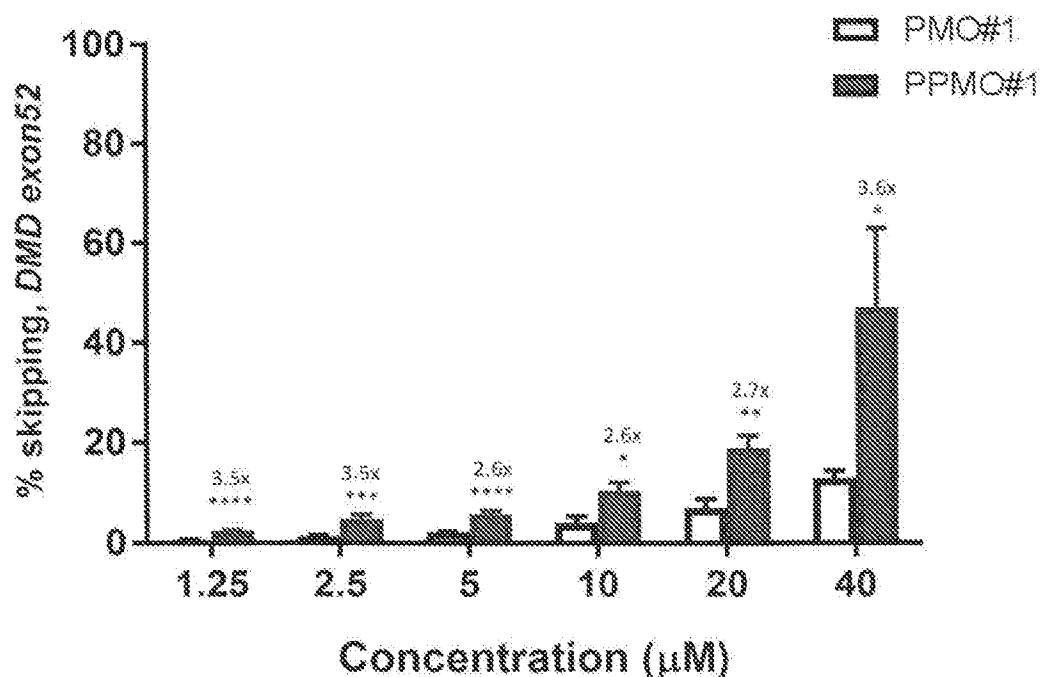
Figure A



10. As can be seen in Figure A, PMO#1 significantly outperformed both PMO#2 and PMO#3 in every tested dose. Specifically, PMO#1 dosed at 1.25 μ M was superior to PMO#2, even when PMO#2 was dosed at 2.5 μ M. More impressively, PMO#1 dosed at 1.25 μ M was superior to PMO#3, even when PMO#3 was dosed at 10 μ M. When dosed at higher levels, PMO#1 demonstrated even larger improvements in exon 52 skipping over both PMO#2 and PMO#3. At 5 μ M, PMO#1 exhibits about 90% exon 52 skipping, whereas PMO#2 and PMO#3 exhibit only about 60% and 30% exon 52 skipping, respectively. At the highest dose of 10 μ M, PMO#1 exhibits about 95% exon 52 skipping compared to only about 75% for PMO#2. In addition, at a dose of 10 μ M, PMO#1 demonstrates about a 2-fold increased exon 52 skipping compared to PMO#3. The magnitude of the increase in exon 52 skipping observed with PMO#1 over PMO#2 and PMO#3 is unexpected.
11. I have also been asked to provide my opinion on how the results of the comparative tests on the PMOs described above in paragraphs 8 and 9 can be applied to compare the effect of exon 52 skipping of PMO#2 and PMO#3 to PPMO#1 of the instant claims.
12. Example 3 of the Specification describes experiments in human myoblasts comparing two compounds that target DMD exon 52, PMO#1 and PPMO#1, both containing the same sequence, i.e., SEQ ID NO:1. Specification, page 123. The experiments provide a

reliable and reproducible comparison of exon skipping by different constructs. As discussed in Example 3, the results of the comparison are shown in Figure 4.

Figure 4



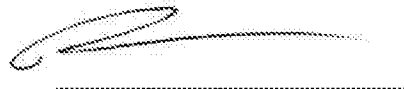
13. As can be seen in the data presented in Figure 4, and as discussed in the Specification in Example 3, significantly higher exon 52 skipping results were obtained in myoblasts when the cells are treated with PPMO#1 as compared to PMO#1 at all concentrations. Specification, page 125. I note that PMO#1 here in Example 3 and shown in Figure 4 is the same compound as PMO#1 of Figure A above. In view of these results, a person of ordinary skill in the art would have concluded that, because PPMO#1 achieved significantly higher exon 52 skipping than PMO#1 in Figure 4 above, and because PMO#1 significantly outperformed both PMO#2 and PMO#3, PPMO#1 would significantly outperform PMO#2 and PMO#3 as well.
14. A person of ordinary skill in the art would have interpreted the results provided herein taken together with the results presented in Figure 4 of the Specification as showing an

unexpected and significant improvement of the claimed PPMOs over the PMOs described in Kaye, including a PMO having SEQ ID NO: 627, e.g. PMO#2 described herein, which the Office has designated as the closest prior art.

15. 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 statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

2/27/2020

Date



Frederick Joseph Schnell, Ph.D.

EXHIBIT A

FRED J. SCHNELL, Ph.D.

1654 NW Crest Place, Corvallis, OR 97330 · (541) 450-2418 · fschnel@gmail.com

SUMMARY

- Seasoned industry professional with drug development experience from lead discovery to negotiating with regulatory agencies for drug approval
- Strong and diverse scientific background in immunology and rare genetic diseases
- Leader in identifying early stage assets to strengthen pipeline

RELATED EXPERIENCE

Sr. Manager, Search and Evaluation, Business Development, Sarepta Therapeutics 2018- present

- Support the Global Business Development team identifying and interrogating strategic opportunities for in-licensing, co-promotion/co-marketing, and acquisition agreements.
- Responsible for leading and coordinating rapid, efficient and credible search processes including strong cross functional collaboration with early R&D, clinical, medical and other franchise stakeholders
- Identify, prioritize and recommend asset opportunities.

Senior Scientist, Translational Research, Sarepta Therapeutics 2015- 2018

- Managed quantitative western blot method optimization and execution for clinical analyses that eventually led to drug approval
- Interacted with senior FDA officials during western blot execution during a regulatory review of the method
- Oversaw development and execution of RNA method for clinical confirmation of drug mechanism of action
- Vetted new technologies for clinical biomarker assays

Scientist II, Discovery Research, Sarepta Therapeutics 2012 - 2015

- Led biological screening efforts for lead candidate selection for clinical development
- Interacted with cross-functional teams from program management to pre-clinical and CMC for lead candidate selection
- Developed high throughput screening assays for identification of lead clinical candidate therapeutics for rare genetic diseases
- Optimized innovative methods for the detection of RNA therapeutics in tissues

Scientist I, Infectious Disease, Sarepta Therapeutics (Formerly AVI BioPharma) 2010 – 2012

- Managed and executed model animal studies testing drug activity and toxicity profiles
- Responsible for development of bioassays for efficacy readouts

Senior Research Associate, Biology, AVI BioPharma 2008 - 2010

- Executed western blot for early-stage animal testing of novel therapeutics

Post-doctoral Fellow, Emory University 2006 - 2008

- Collaborated with immunologists and virologists on three research projects investigating the induction of an unresponsive phenotype in T cells upon recognition of viral escape mutants during chronic infections

Doctoral Candidate, Emory University 2001 - 2006

- Investigated immune cell signaling during development and trafficking

Research Technician, Emory University 1998 - 2001

- Discovered a novel human protein (JAM-A) involved in the maintenance of gut barrier function

EDUCATION

Emory University, Atlanta, GA
Doctor of Philosophy, Immunology/Molecular Pathogenesis

2006

Davidson College, Davidson, NC
Bachelor of Science, Biological Sciences

1998

PEER REVIEWED PUBLICATIONS AND SELECTED PRESENTATIONS

Schnell F. J., Frank D., Fletcher S., Johnsen R., Wilton S. W. 2018 Challenges of interpreting dystrophin content by western blot. *US Neurol.* In press.

Gordish-Dressman H., Willmann R., Dalle Pazzi L., Kreibich A., van Putten M., Heydemann A., Bogdanik L., Lutz C., Davies K., Demonbreun A. R., Duan D., Elsey D., Fukada S. I., Girgenrath M., Patrick Gonzalez J., Grounds M. D., Nichols A., Partridge T., Passini M., Sanarica F., **Schnell F. J.**, Wells D. J., Yokota T., Young C. S., Zhong Z., Spurney C., Spencer M., De Luca A., Nagaraju K., Aartsma-Rus A. 2018. "Of Mice and Measures": A Project to Improve How We Advance Duchenne Muscular Dystrophy Therapies to the Clinic. *J Neuromuscul* 5(4)

Charleston J. S., **Schnell F. J.**, Dworzak J., Donoghue C., Lewis S., Chen L., Young G. D., Milici A. J., Voss J., DeAlwis U., Wentworth B., Rodino-Klapac L. R., Sahenk Z., Frank D., Mendell J. R. 2018. Eteplirsen treatment for Duchenne muscular dystrophy: Exon skipping and dystrophin production. *Neurology* 90(24)

Lu-Nguyen N., Malerba A., Popplewell L., **Schnell F.**, Hanson G., Dickson G. 2016. Systemic Antisense Therapeutics for Dystrophin and Myostatin Exon Splice Modulation Improve Muscle Pathology of Adult mdx Mice. *Mol Ther Nucleic Acids*. 2016;6:15–28

Shorter S. K., **Schnell F. J.**, McMaster S. R., Pinelli D. F., Andargachew R., Evavold B.D. 2016. Viral escape mutant epitope maintains TCR affinity for antigen yet curtails CD8 T cell responses. *PLoS*. 11(2)

Mourich D. V., Oda S. K., **Schnell F. J.**, Crumley S. L., Hauck L. L., Moentenich C. A., Marshall N. B., Hinrichs D. J., Iversen P. L. 2014. Alternative splice forms of CTLA-4 induced by antisense mediated splice-switching influences autoimmune diabetes susceptibility in NOD mice. *Nucleic Acid Ther* 24:114.

Geller B. L., Marshall-Batty K., **Schnell F. J.**, McKnight M. M., Iversen P. L., Greenberg D. E. 2013. Gene-silencing antisense oligomers inhibit acinetobacter growth in vitro and in vivo. *J Infect Dis* 208:1553.

Schnell F. J., Crumley S. L., Mourich D. V., Iversen P. L. 2013. Development of novel bioanalytical methods to determine the effective concentrations of phosphorodiamidate morpholino oligomers in tissues and cells. *Biores Open Access*. 2:61.

Schnell, F. J., Sundholm, S., Crumley, S., Mourich, D. L., Iversen, P. L. 2012. Lymphocytic choriomeningitis virus infection in FVB mouse produces hemorrhagic disease. *PLoS Pathog.*

Schnell, F. J., Alberts-Grill, N., Evavold, B. D. 2009. CD8+ T cell responses to a viral escape mutant epitope: active suppression via altered SHP-1 activity. *J Immunol* 182:1829.

- Zoller A. L., **Schnell F. J.**, Kersh G. J. 2007. Murine pregnancy leads to reduced proliferation of maternal thymocytes and decreased thymic emigration. *Immunology* 121:207.
- Schnell, F. J.**, Zoller A.L., Patel S.R., Williams I.R., and G. J. Kersh. 2005. Early growth response gene 1 provides negative feedback to inhibit entry of progenitor cells into the thymus. *J Immunol* 176:4740.
- Ryan M. R., Shepherd R., Leavey J. K., Gao Y., Grassi F., **Schnell F. J.**, Qian W. P., Kersh G. J., Weitzmann M. N., Pacifici R. 2005. An IL-7-dependent rebound in thymic T cell output contributes to the bone loss induced by estrogen deficiency. *Proc Natl Acad Sci* 102:16735.
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- Shaw S. K., Perkins B. N., Lim Y. C., Liu Y., Nusrat A., **Schnell F. J.**, Parkos C. A., F. W. Luscinskas. 2001. Reduced expression of junctional adhesion molecule and platelet/endothelial cell adhesion molecule-1 (CD31) at human vascular endothelial junctions by cytokines tumor necrosis factor-alpha plus interferon-gamma does not reduce leukocyte transmigration under flow. *Am J Pathol* 159:2281.
- Barton E. S., Forrest J. C., Connolly J. L., Chappell J. D., Liu Y., **Schnell F. J.**, Nusrat A., Parkos C. A., T. S. Dermody. 2001. Junction adhesion molecule is a receptor for reovirus. *Cell* 104:441.
- Liu Y., Nusrat A., **Schnell F. J.**, Reaves T. A., Walsh S., Pochet M., C. A. Parkos. 2000. Human junction adhesion molecule regulates tight junction resealing in epithelia. *J Cell Sci* 2363-74.
- Mazzucchelli L., Burritt J. B., Jesaitis A. J., Nusrat A., Liang T. W., Gewirtz A. T., **Schnell F. J.**, C. A. Parkos. 1999. Cell-specific peptide binding by human neutrophils. *Blood* 93:738.