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Applicant: Neurimmune Holding AG
Title: HUMAN-DERIVED ANTI-DIPEPTIDE REPEATS (DPRS) ANTIBODY
Our ref.: BIO20104PCTEP
Date: December 13, 2019

Declaration

Mia Marie Rushe

Principal Scientist, Biologics Drug Discovery, Biogen, Inc

I. Summary

1. I am **Mia Marie Rushe**. I have worked in the field of protein biochemistry.
2. In preparing this declaration, I have reviewed European patent application EP3201228 entitled "HUMAN-DERIVED ANTI-DIPEPTIDE REPEATS (DPRS) ANTIBODY", filed on September 30, 2015 ("the present application") and the currently pending claims. I have also reviewed the Communication pursuant to Rule 164(2)(b) and Article 94(3) EPC dated July 10, 2019 and the prior art cited in the communication, namely: D1 (WO 2014/114303 A1), D2 (K. Mori et al.), D3 (K. Mori et al.), D4 (Ian R. Mackenzie et al.), D5 (WO 2014/114660 A1), and D6 (May S. et al.).
3. Furthermore, I have been made familiar with the objection of the Examining Division concerning the alleged lack of inventive step and lack of sufficiency of disclosure.
4. In particular, I understand that the Examining Division has rejected the subject matter of the claims of the present application based on antibodies described in prior art references D4 or any one of D5, D1 and D6. I also understand that the Examining Division is of the opinion that a therapeutic effect of the claimed antibodies in the treatment/prevention of a disease associated with a DPR protein or aggregated forms thereof is merely speculative.
5. The purpose of this Declaration is to show that antibodies comprising the specific CDR sequences comprised in SEQ ID NOs: 8 and 10 of the present application have advantageous properties making them particularly useful for diagnostic and also therapeutic purposes. The results of the experiments described below demonstrate the therapeutic efficacy of the claimed antibodies.
6. Precisely, Antibody A which comprises the specific VH CDRs 1, 2 and 3 comprised in SEQ ID NO: 8 (i.e., positions 32-36, 51-67 and 100-113, respectively) and VL CDRs 1, 2 and 3 comprised in SEQ ID NO: 10 (i.e., positions 25-35, 51-57 and 90-98, respectively) of the present application unexpectedly reduced poly(GA) in cell line experiments in vitro. Treatment with Antibody A decreased poly(GA) levels in a dose dependent manner in C9orf72 iPSC derived motor neurons and also the amount of poly(GA) aggregates in T98G human glioblastoma cell lines. Further, data

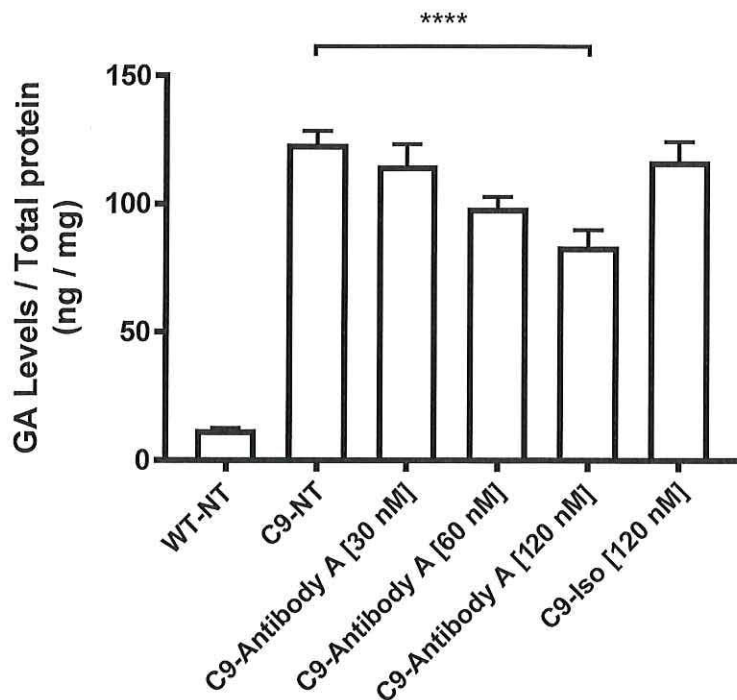
is provided illustrating that treatment with Antibody B, i.e. a mouse chimeric analog of Antibody A, provided a statistically significant survival benefit in C9orf72-500R human BAC Tg mice.

II. Antibody A Reduced Poly(GA) Levels in a Dose Dependent Manner in C9orf72 iPSC derived motor neurons

7. The effects of Antibody A on poly(GA) protein clearance in wild-type (WT) and C9ORF72 patient derived iPSC motor neurons was examined. iPSCs were differentiated into spinal motor neurons using a protocol adapted from Maury Y. et al. (Nat Biotechnol. 2015 Jan;33(1):89-96), which generates motor neuron precursors by 14 days in vitro (DIV) and ~75% beta-tubulin positive (TUJ1+) neurons by 28 DIV. 30 nM, 60 nM and 120 nM control hlgG1 and Antibody A antibodies were administered to culture media starting at 5 DIV and replenished with media and antibody changes every three days until 15 DIV. Cells were lysed in RIPA buffer at 15 DIV, supernatant was subsequently boiled in 1% SDS/1mM TCEP and analyzed for poly(GA) levels using a Meso scale discovery (MSD) immunoassay as described in Nguyen et al. (Neuron (2019), <https://doi.org/10.1016/j.neuron.2019.11.007>).
8. In WT cells, no poly(GA) was detected and all WT cells treated with this antibody behaved similarly in the poly(GA) MSD assay. In C9ORF72 patient derived cultures, treatment with Antibody A resulted in a dose dependent decrease in poly(GA) levels compared to untreated cells and cells treated with an isotype control antibody (see Figure 1, below).

Figure 1: Poly(GA) Levels in Antibody A Treated iPSC Neurons

GA Levels in iPSC Cultures Treated with Antibody A



Antibody A = hIgG1 antibody comprising VH CDRs 1 (NHAMH), 2 (VISYDGENTYYADSIEG) and 3 (GGRRGHFTSYLDY), and VL CDRs 1 (RASQNIDKYLN), 2 (AASSLHS) and 3 (QQSYSSFRT).

GA = glycine-alanine; iPSC = induced pluripotent stem cell; NT = not treated; WT = Wild-type cells, C9 = C9ORF72 cells, C9-Iso = C9ORF72 cells treated with hIgG1 isotype antibody; C9-Antibody A = C9ORF72 cells treated with Antibody A.

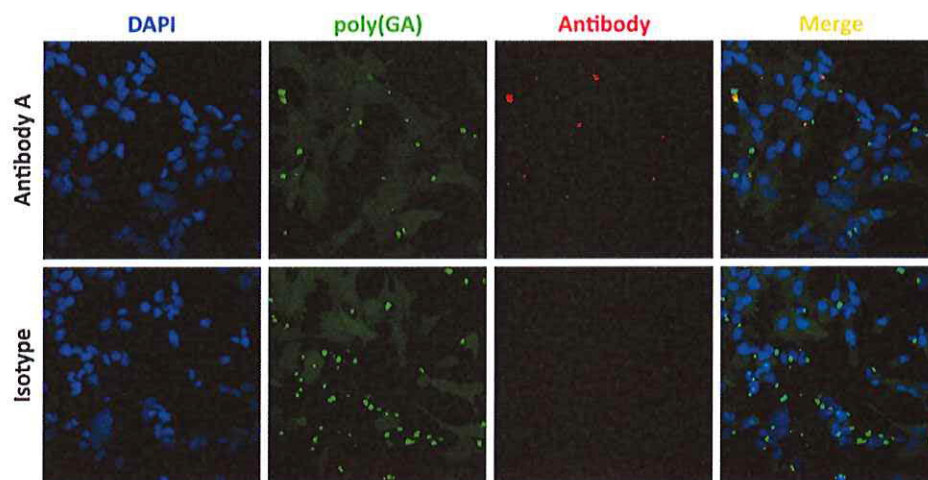
Quantitative measurement of poly(GA) in iPSC neurons using MSD (n = 2/treatment group, where poly(GA) levels were measured twice per sample), one-way ANOVA with Tukey multiple comparison analyses. Compared to untreated or IgG controls, iPSC patient derived motor neurons treated with Antibody A for 8 days exhibit reduced GA levels, respectively, as determined by MSD.

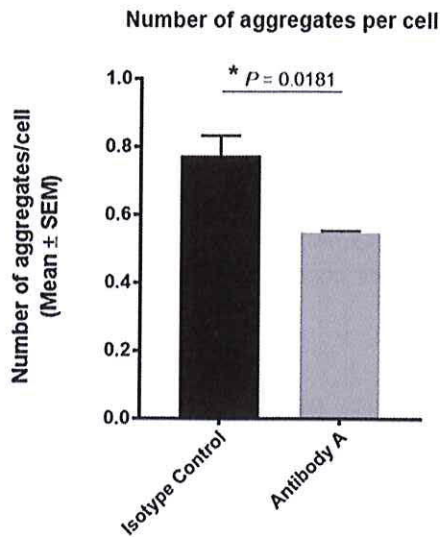
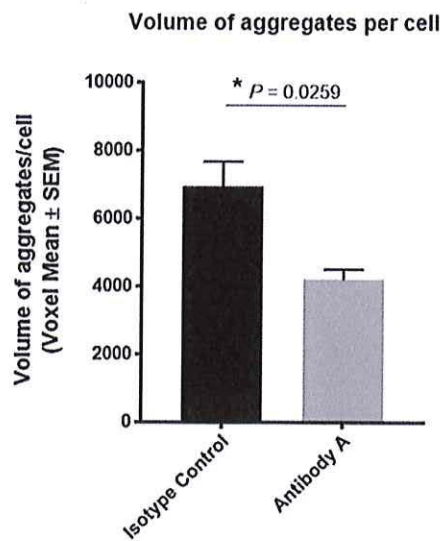
III. Antibody A reduced poly(GA) aggregates in T98G human glioblastoma cell lines

9. In order to examine whether treatment of poly(GA) expressing cells with Antibody A reduces the number and size of poly(GA) aggregates, stable, inducible T98G glioblastoma cells overexpressing ATG initiated non-hairpin-forming (GA)₁₆₁-GFP were treated with 100 nM Antibody A or human IgG1 control antibody for 72 hours. Following treatment, cells were fixed, stained with AlexaFluor 647 conjugated anti-human IgG, and imaged.
10. Image analysis demonstrated that Antibody A colocalized with poly(GA) aggregates while isotype control was not observed in cells (see Figure 2A, below). GFP fluorescence was used to quantitate the number and volume (voxels) of poly(GA) aggregates per cell.
11. Treatment with Antibody A reduced the number of aggregates per cell by 29% (see Figure 2B, below) and reduced the volume (voxel) of poly(GA) aggregates per cell by 39% (see Figure 2C, below) relative to treatment with isotype control.

Figure 2: Poly(GA) Aggregate Quantitation in Antibody A Treated T98G Cells

A



B**C**

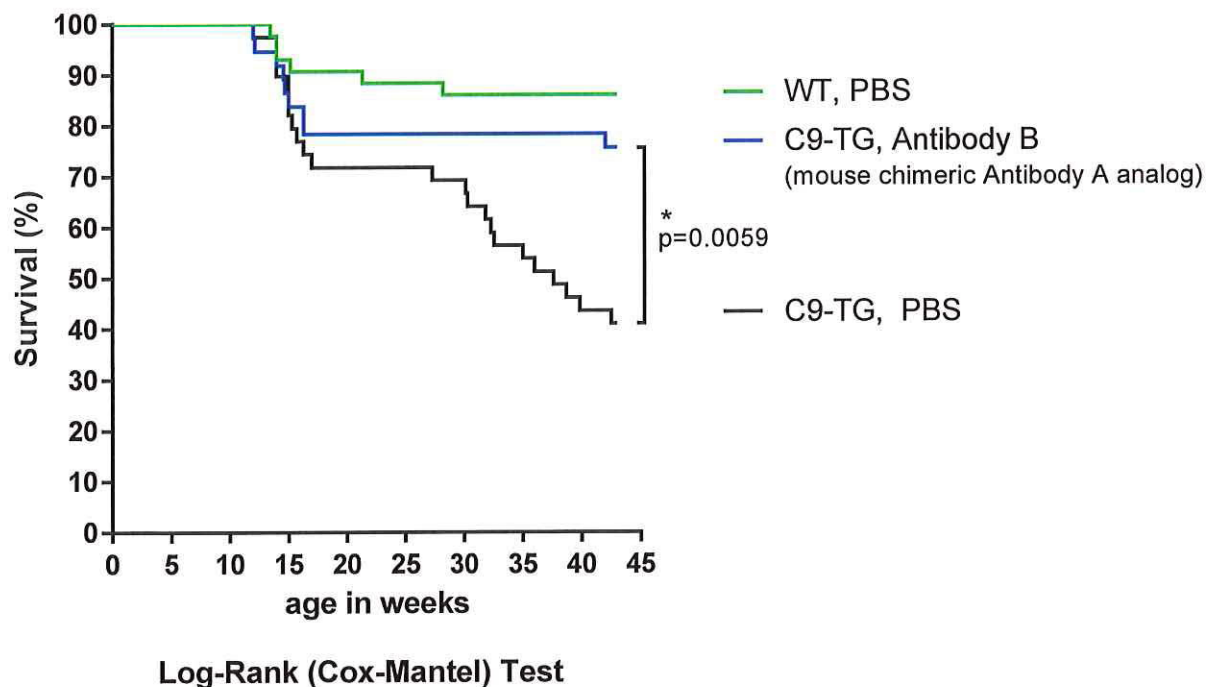
SEM = standard error of the mean

(A) Double immunofluorescence of stable inducible T98G cells expressing (GA)₁₆₁-GA (green) and treated with 100 nM antibody (red) for 72 h. Colocalization observed in cells treated with Antibody A but not in cells treated with human IgG1 isotype control antibody. (B, C) Compared with IgG isotype control treated cells, Antibody A treated cells exhibited a significant reduction in the number of poly(GA) aggregates per cell (B) and significant reduction in volume of aggregates per cell (C). Antibody treatment was performed in biological triplicate and 10 or 11 distinct fields were captured for each biological replicate. Bars represent mean $\pm$ SEM; statistical analyses were performed using unpaired two-tailed t-test; ns $P > 0.05$, * $P \leq 0.05$, ** $P \leq 0.01$.

IV. Antibody B Treatment Improves Survival in C9ORF72-500R Human BAC Tg Mice

12. To assess the ability of the poly(GA) antibodies of the present application to improve survival in vivo, a transgenic mouse model was used. In detail, C9ORF72-500R BAC Tg mice (see Liu et al., 2016 May 4, Neuron 90,521-534) were treated with either Antibody B (a mouse chimeric analog of Antibody A containing the specific CDRs of Antibody A and mouse IgG2a constant regions) or PBS.
13. As shown in Figure 3, Antibody B treatment significantly prolonged survival time in treated mice. Mice treated with Antibody B displayed a 76% survival versus a 41% survival of the C9ORF72-500R Tg BAC mice treated with placebo. The difference in survival was found to be statistically significant ($p = 0.0169$).

Figure 3: Increased Survival in *C9ORF72*-500R Human BAC Tg Mice Administered Antibody B



BAC = bacterial artificial chromosome; GA = glycine-alanine; GP = glycine-proline; IP = intraperitoneal; PBS = phosphate-buffered saline; WT = wild-type; WT, PBS = wild-type mice with PBS; C9-TG, Antibody B = *C9ORF72*-500R transgenic mice treated with the mouse chimeric analog of Antibody A (containing Antibody A variable regions and mouse IgG2a constant regions); C9-TG, PBS = *C9ORF72*-500R transgenic mice treated with PBS

Kaplan-Meier survival curve of animals treated with Antibody B directed against poly(GA). Weekly IP injections of Antibody B at 30 mg/kg significantly improved *C9ORF72*-500R survival ($p = 0.0059$). A log-rank (Mantel-Cox) statistical test was performed to determine significance of survival benefit.

14. Taken together, these data show that antibodies comprising the specific VH and VL CDR sequences of claim 1 reduced poly(GA) levels and poly(GA) aggregation in cell cultures in vitro and significantly prolonged survival of treated mice in vivo. The provided data thus confirms that the specific combination of CDRs leads to properties making the antibodies particularly useful for therapeutic purposes.

Executed on the 13 of Dec, 2019 in Cambridge, MA.

Mia Marie Rushe
Mia Marie Rushe

Enclosures
Curriculum Vitae

Mia Rushe
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Everett, MA 02149
617-669-5365
Mia.rushe@gmail.com

Summary

- Protein biochemist with 10+ years experience in drug development, including leadership of programs at all phases of development through IND
- Experienced leader of cross-functional research and development project teams
- Experienced manager of associate scientists and PhD scientists
- Diverse background includes support of structural biology and small molecule targets
- Enjoys working in collaborative environment furthering discovery and development of novel human therapies

Professional Experience

Biogen, Cambridge, MA

Principal Scientist, Biologics Drug Discovery **Senior Scientist, Biologics Drug Discovery**

May 2019-present
Feb 2015-April 2019

- Manage group of 4 scientists (PhD level and associates). Group responsible for production, purification and characterization of candidate protein therapeutics to support early and late stage discovery projects. CHO expression (transient and stable) in shake flasks up to 20+ liter controlled stirred tank bioreactors. Parallel purification of 8+ proteins at 1-100 mg scale and multigram antibody preparations for *in vivo* studies. Manage all aspects of protein production from transfection through purification and characterization.
- R&D project lead for therapeutic targeting neurodegenerative disease from early discovery through IND filing.

Scientist II, Chem and Molecular Therapeutics **Scientist I, Exploratory Protein Sciences**

March 2013-Jan 2015
July 2012-March 2013

- Lead group of four associate scientists responsible for production, purification and characterization of protein reagents. Reagents include antibodies and engineered Fc fusion proteins to support biologic projects and also protein reagents to support small molecule projects.
- Lead protein chemist on several early research projects (small molecule and biologic). Responsibilities include devising strategy and overseeing all aspects of protein production from construct design to purified protein.
- Responsible for allocating resources of the group and coordinating activities with other groups across research.
- Responsible for maintaining and improving insect cell/baculovirus capabilities.
- Manage outsourced production of protein reagents at CROs.
- Provide biochemistry and strategic support for several exploratory projects; propose and lead exploratory projects.
- Responsible for incorporating new technologies to improve capability and capacity into existing workflows.

Scientist I, Protein Drug Discovery

Feb. 2009-July 2012

- Proposed a new exploratory project to explore a potential immunomodulator of macrophage activity; designed, cloned, and purified multiple reagents for proof of concept studies; coordinated activities of team members.
- Lead protein biochemist for anti-viral antibody project: played central role in the rational re-engineering of candidates resulting in lead with improved affinity. Medium throughput (20-30) parallel purification and evaluation of antibody candidates; development of a robust automated ELISA assay.
- Developed an improved method for the production of virus-like particles (VLP) used in a serological assay to identify JCV-positive Tysabri patients; transferred method to outsourcing partner; responsible for overseeing biochemical characterization of VLP preparations.
- Lead protein biochemist for an oncology antibody project: purified and biophysically characterized multiple engineered antibodies for *in vivo* studies, played a major role in lead candidate selection, established robust purification protocol for lead; coordinated activities of other groups to maintain project timelines. Involved in strategic planning of project and team goals.

Senior Associate Scientist, Protein Drug Discovery**June 2006-Feb. 2009**

- Provided protein chemistry support for an early stage oncology antibody project: purified and characterized proteins for immunization, assisted in screening of antibody panels, developed ligand blocking ELISA for antibodies, purified monoclonal antibodies.
- Provided protein chemistry support for a toxin-conjugated antibody anti-cancer therapeutic in development/pre-clinical stage.
- Archived results in research reports, presented work regularly in project and group meetings, trained colleagues on AKTA, HPLC, and other purification/characterization equipment.

Novartis Institutes for Biomedical Research, Cambridge, MA**Jan. 2006-June 2006****Scientist II, Protein Structure**

- Produced multiple constructs of an infectious disease target for structural analysis by x-ray crystallography and NMR; Included construct design and molecular biology, production of isotopically labeled proteins for NMR analysis, biophysical analysis of oligomeric state, and initial crystallization.

Biogen Idec, Cambridge, MA**April 2002-Sept. 2005****Senior Associate Scientist, Physical Biochemistry**

- Provided specialized protein chemistry support for crystallography and NMR. Included isotopic (NMR) and metabolic labeling (seleno-methionine for crystallography) of proteins and protein production for structural work (molecular biology, protein expression, and purification).
- Identified a crystallizable NEMO/IKK complex. Involved cloning, production, determination of novel folding conditions and purification of multiple rationally designed constructs; resulted in publication of a first-author paper.

Massachusetts Institute of Technology, Cambridge, MA**Sept. 1994-April 2002****Research Technician, Chemistry Department (Laboratory of Dr. Lawrence Stern)**

- Developed an *in vitro* folding procedure for soluble MHC class II protein HLA-DR1 from inclusion bodies expressed in *E. coli*.
- Produced multiple proteins in *E. coli* and baculovirus expression system (insect cells); optimized purification and refolding methods, evaluated the activity of proteins using ELISA's, FRET kinetic binding assay's, and surface plasmon resonance (BIAcore); used CD/thermal denaturation to study stability of protein-ligand complexes.
- Additional responsibilities: assisted in setting up research laboratory by ordering startup equipment and supplies, instructed graduate students in laboratory techniques, presented results in lab group meetings.

Harvard University, Cambridge, MA**Nov. 1989-Sept. 1994****Research Technician, Chemistry Department (Laboratory of Professor Don Wiley)**

- Produced Influenza C virus in MDCK cells and in chicken embryos, isolated virus from cell culture medium, and isolated and crystallized hemagglutinin protein from the virus.
- Established protocols for expression of MHC's in baculovirus expression system; expressed, purified and crystallized HLA-DR1.

Technical Expertise

Molecular Biology/Protein Expression: cDNA cloning, PCR, site-directed mutagenesis, recombinant protein design and expression in bacterial, insect, and mammalian cell lines, high density *E. coli* fermentation, production of isotopically labeled proteins in *E. coli* for NMR and X-ray crystallography

Protein Purification/Characterization: Ion exchange, gel filtration, immunoaffinity, hydrophobic interaction and reverse phase chromatography using, AKTA and HPLC proficiency, Native and SDS-PAGE, UV/Vis spectroscopy, protein assays, ELISA, western blot

Biophysical analysis/protein-protein interactions: SEC/static light scattering, protein crystallization, velocity and equilibrium analytical ultracentrifugation, circular dichroism/thermal denaturation, kinetic analysis using fluorescence-based assays, isothermal titration calorimetry, surface plasmon resonance (BIAcore)

Protein Biochemistry: protein modification (biotinylation, fluorescent labeling, lipidation of peptides and proteins), proteolytic fragmentation (Fab production), limited proteolysis

Cell assays: FACS assays, proliferation assays, pulse-chase metabolic labeling, immunoprecipitation.

Education

Northeastern University, Boston, MA

Mississippi University for Women, Columbus, MS

Mississippi State University, Starkville, MS

B.S., Biological Science, May 2000

1988-1989

1985-1988

Publications

Almeida, S., Krishnan, G., **Rushe, M.**, Gu, Y., Kankel, MW, Gao FB. (2019) Production of poly(GA) in C9ORF72 patient motor neurons derived from induced pluripotent stem cells. *Acta Neuropathol.* 2019 Dec;138(6):1099-1101.

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Carven, G.J., Chitta, S., Hilgert, I., **Rushe, M.M.**, Baggio, R.F., Palmer, M., Arenas, J.E., Strominger, J.L., Horejsi, V., Santambrogio, L., and Stern, L.J. (2004). Monoclonal antibodies specific for the empty conformation of HLA-DR1 reveal aspects of the conformational change associated with peptide binding. *J Biol Chem* 279, 16561-16570.

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Sato, A.K., Zarutskie, J.A., **Rushe, M.M.**, Lomakin, A., Natarajan, S.K., Sadegh-Nasseri, S., Benedek, G.B., and Stern, L.J. (2000). Determinants of the peptide-induced conformational change in the human class II major histocompatibility complex protein HLA-DR1. *J Biol Chem* 275, 2165-2173.

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Patents

Assay for JC Virus Antibodies. US Patent Application number US 13/521,311, Publication No. US20130022961 A1 Issued Jan 24, 2014. Inventors Leonid Gorelik, Kenneth J. Simon, Meena Subramanyam, **Mia Marie Rushe**

JCV Neutralizing Antibodies. US WO2013142299A1. Issued Sept 26, 2013. Inventors Kenneth Simon, Thomas Cameron, Deping Wang, Joseph Arndt, **Mia Rushe**, Justin Caravella, Eric Day.