REPORT

Report on participation of the ICMR International Fellow (ICMR-IF) in Training/Research abroad.

1. Name and designation of ICMR- IF : Dr Sanjay Yadav; Additional Professor

2. Address : Room 313, Department of Biochemistry, Medical College, AIIMS Raebareli

3. Frontline area of research in which training/research was carried out : Neurobiology

4. Name & address of Professor and host institute : Dr. Avindra Nath, M.D.
Senior Investigator and Clinical Director
Section of Infections of the Nervous System
Building 10-CRC, Room 7C103
10 Center Drive
Bethesda, MD 20814
NINDS: National Institute of Health
Bethesda, USA.

5. Duration of fellowship with exact date : Six Months (27/03/2023 to 26/09/2023)

6. Highlights of work conducted :

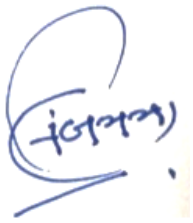
i) Technique/expertise acquired : Generation of iPSCs and development of different types of brain cells like dopaminergic neurons or astrocytes from iPSCs. Exosome Biology

ii) Research results, including any papers: (Figures in Annexure I)

Research Results: At NIH, I have isolated and characterized exosomes from 60 human serum samples (20 each i.e., control, PD and ALS). Exosomes were characterized using Spectradyne system, which shows around 10 fold variation in number of exosomes between different samples. However, most of the exosomes are below 100 nM in size. Using TaqMan system, we have profiled expression of 13 miRNAs as proposed in the project, however only 7 miRNAs are substantially present (miR-29b-3p, miR-34-5p, miR-139, miR-186, miR-150, miR-139, miR-145v and miR-191) in the exosome fractions isolated from serum samples. We have developed a copy number assay of these miRNAs using RNA oligo and measured number of copies of these 7 miRNAs. Our studies have identified miR-29b-3p is most significantly and specifically up-regulated miRNA in exosome of PD patients, which was unaltered in ALS patients. In contrast, miR-34a-5p was present in substantially high amount in exosomes of ALS patients and unaltered in PD patients. Interestingly, copies of miR-186 and miR-150 were present in higher number in both PD and ALS exosomes. For studying the mechanism of these miRNAs, we have generated iPSCs (4 lines) from PBMC of PD patients and controls and converted them into dopaminergic neurons. These iPSC lines

and some more lines (10 control and 10 PD lines) generated by core facility of NIH will be shipped to my lab for our future studies.
The studies conducted will be published with joint authorship of NIH and AIIMS Raebareli in reputed peer review journal.

iii) Proposed utilization of the experience in India : At AIIMS Raebareli, we are working on development of exosome-based biomarker for Parkinson disease and need expertise in stem cell biology to study the mechanism of neurodegeneration, the expertise developed at NIH will be used in our current ICMR project (2021-8340/F1) and future projects.



Signature of ICMR-IF
ICMR Sanction No. INDO/FRC/452/Y-02/2022-23-IH & HRD
Date: 19/10/2022

Annexure I

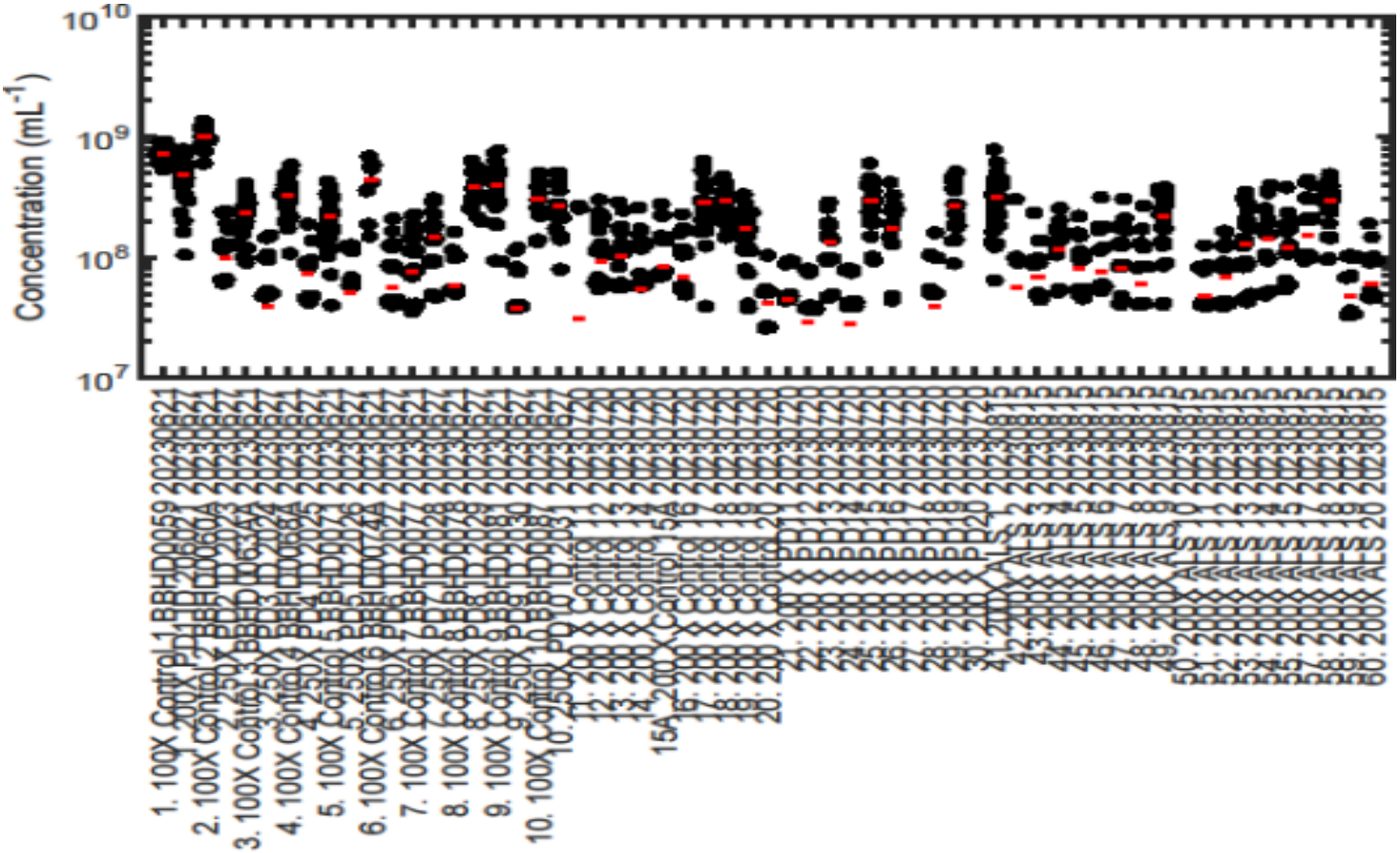


Figure 1: Comparison of amount and size of Exosomes isolated from 20 control/ 20PD and 20 ALS samples using the Spectradyne nCS1 particle analyser. Samples are diluted either 100X or 200X and run with the bead of 220 nM beads as size standard.

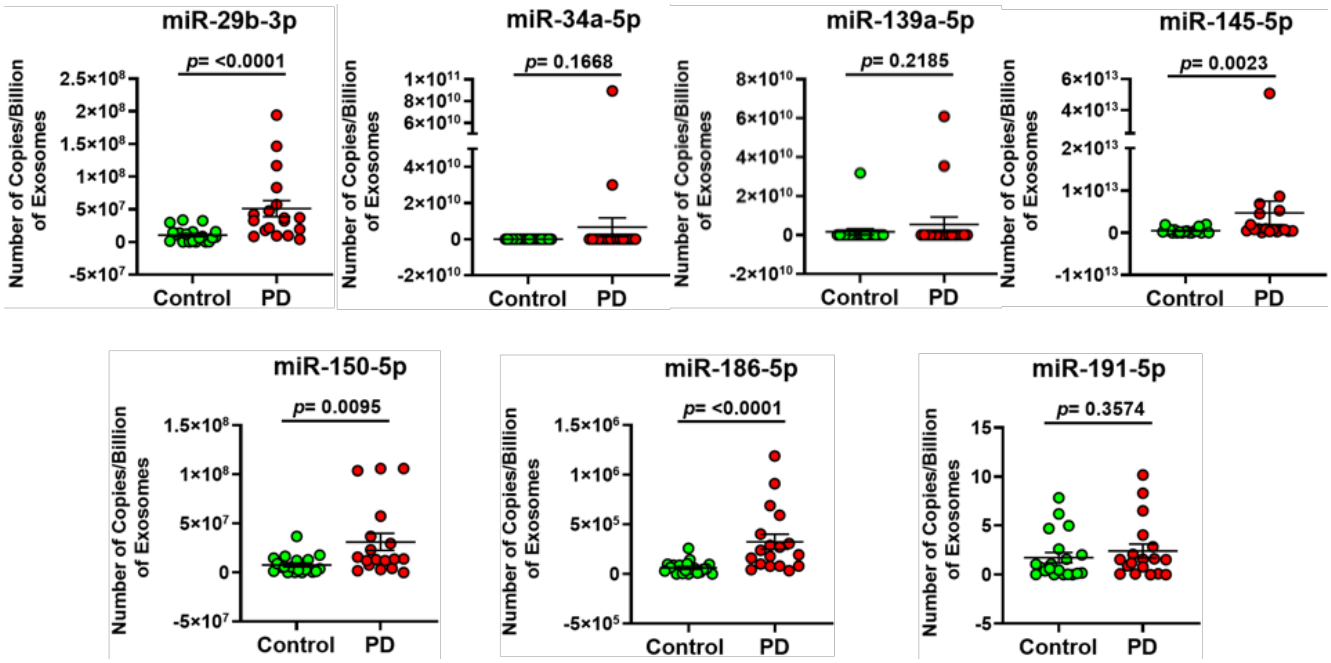


Figure. 2: Number of copies of selected miRNAs in exosome isolated from serum of control and PD patients (n=20 in each group). Number of copies are calculated from standard curve generated with known amount of synthetic RNA oligos of sequence matching to mature miR-29b-3p.

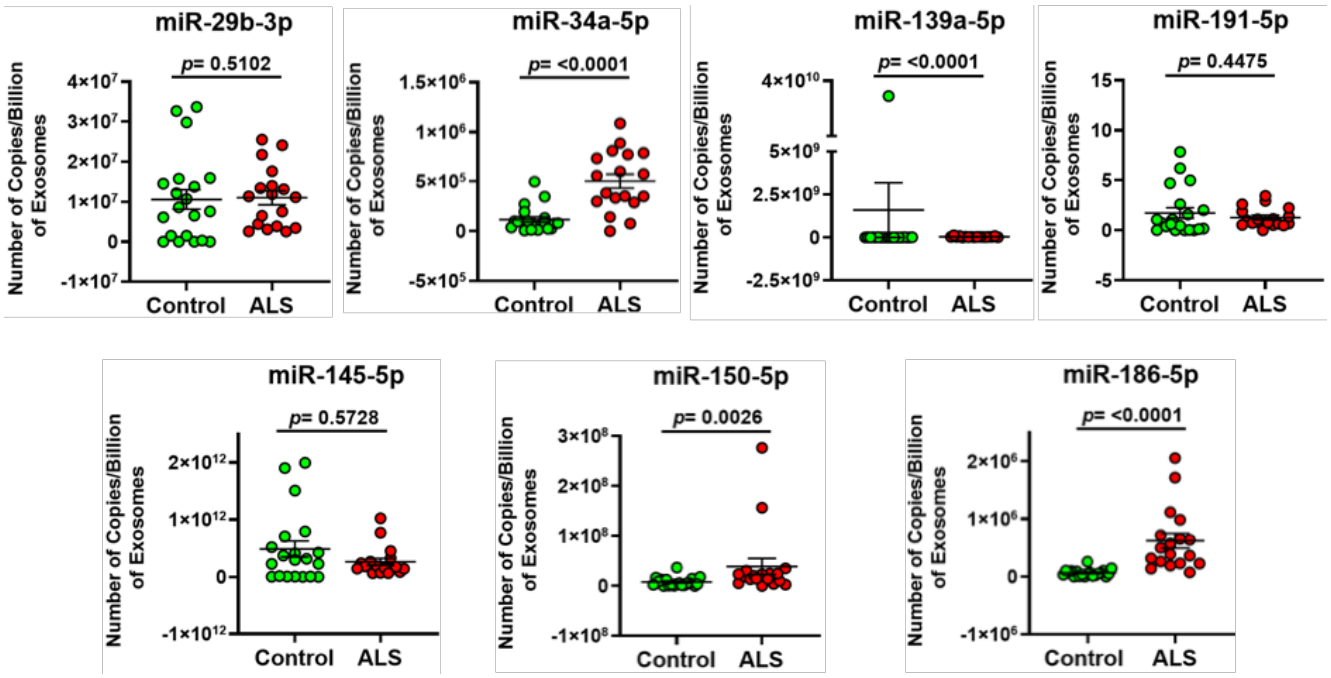


Figure. 3: Number of copies of selected miRNAs in exosome isolated from serum of control and ALS patients (n=20 in each group). Number of copies are calculated from standard curve generated with known amount of synthetic RNA oligos of sequence matching to mature miR-29b-3p.

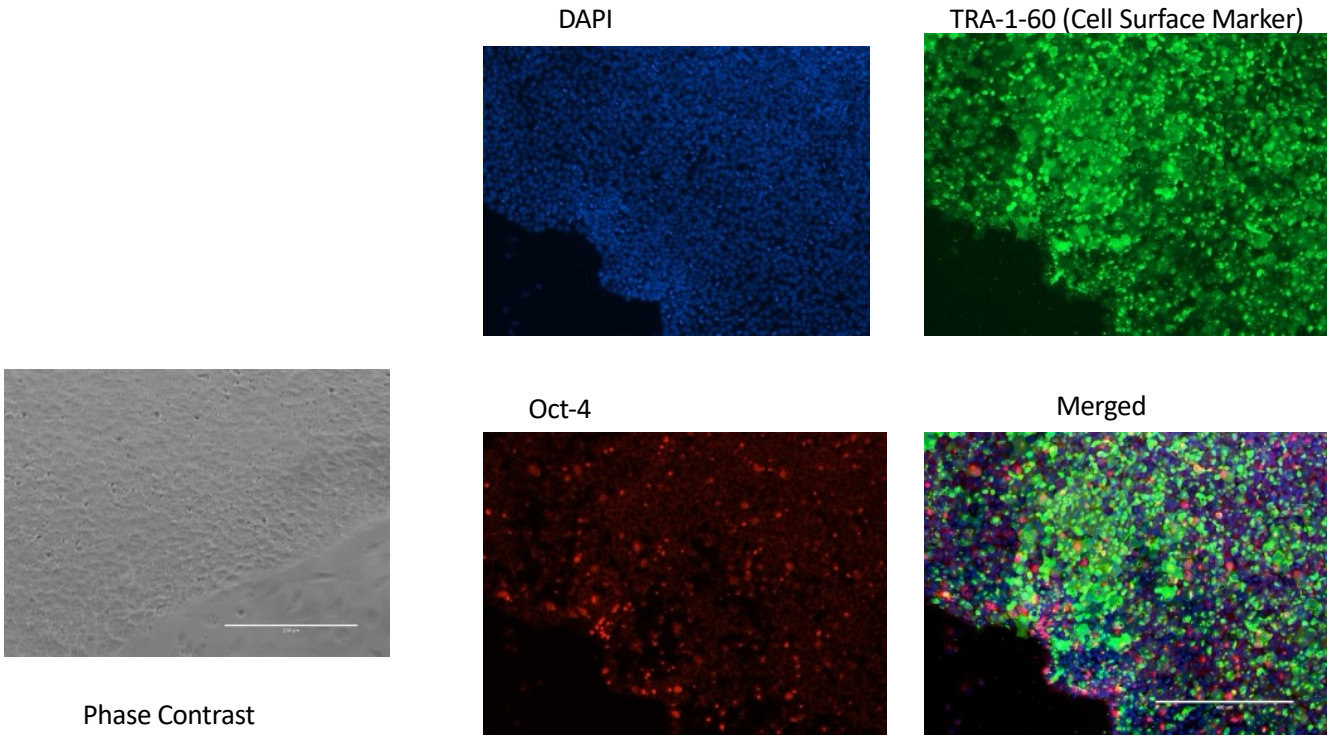


Figure. 4: Generation of CD34+ iPSC lines from PBMC of PD patients.

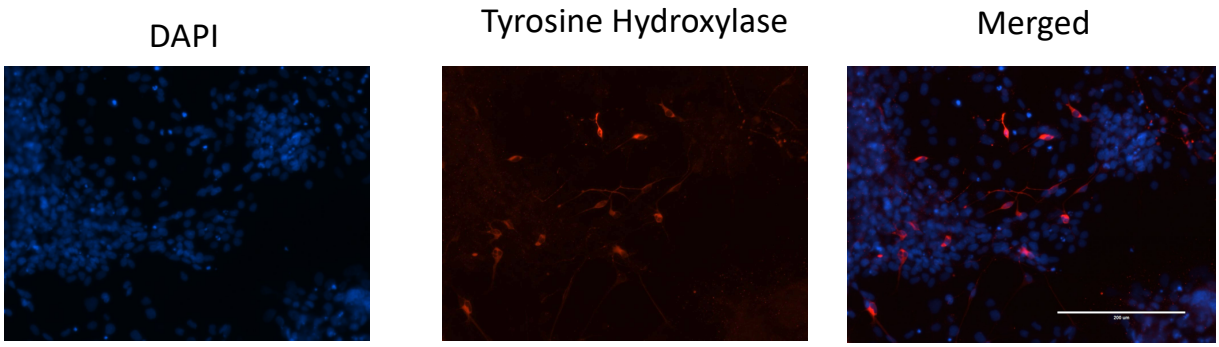


Figure. 5: Development of dopaminergic neurons (Tyrosine Hydroxylase positive) from CD34+ iPSC lines developed from PBMC of PD patients.