EXCECUTIVE SUMMARY UGC (major) project F.No 41-1294/2012(SR)

UGC reference no F.No 41-1294/2012(SR) date 30.7.2012

PROJECT TITLE: Generation of monoclonal antibodies against a nuclear receptor 'Pregnane & Xenobiotic Receptor' for utility as immunological and diagnostic tool.

Final Report Submitted by

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Submitted to

MAJOR RESEARCH PROJECT SECTION
UNIVERSITY GRANTS COMMISSION
BAHADUR SHAH ZAFAR MARG
NEW DELHI – 110 002

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PROFORMA FOR SUBMISSION OF INFORMATION AT THE TIME OF SENDING THE FINAL REPORT OF THE WORK DONE ON THE PROJECT

1. NAME AND ADDRESS OF THE PRINCIPAL INVESTIGATOR

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2. NAME AND ADDRESS OF THE INSTITUTION

Special Centre for Molecular Medicine,

Jawaharlal Nehru University,

New Delhi. Pin code: 110067

3. UGC APPROVAL NO. AND DATE

F.No 41=1294/2012 (SR) Date 30.7.2012

4. DATE OF IMPLEMENTATION

01.07.2012

5. TENURE OF THE PROJECT .....

01.07.2012 to 30.06.2015 (a further extension was given without additional funds)

6. TOTAL GRANT ALLOCATED

Rs. 8,88,000

7. TOTAL GRANT RECEIVED .....

Rs. 8,08,000

8. FINAL EXPENDITURE

Rs. 7,90,218

9. TITLE OF THE PROJECT

Generation of monoclonal antibodies against a nuclear receptor 'Pregnane & Xenobiotic Receptor' for utility as immunological and diagnostic tool

10. OBJECTIVES OF THE PROJECT .....

This work has been divided into three major objectives:

1. Generation of monoclonal antibody against full-length human nuclear receptor PXR (Pregnane & Xenobiotic Receptor).
2. Characterization of PXR monoclonal antibody.
3. Analysis of expression of PXR protein in normal and pathological states.

11. WHETHER OBJECTIVES WERE ACHIEVED

Yes. A detailed report submitted to UGC.

12. ACHIEVEMENTS FROM THE PROJECT

Monoclonal antibodies have been successfully raised. These antibodies are working efficiently with most immunological tools. Other than publications (either published/communicated) we are also processing an application to file an Indian patent with the support from IPM Cell of Jawaharlal Nehru University.

13. SUMMARY OF THE FINDINGS (in 500 WORDS)

Pregnane & Xenobiotic Receptor (PXR) is also referred to as Steroid and Xenobiotic Receptor (SXR) or Pregnane X Receptor (PXR, NR1I2) and is one of the 48 members of the Nuclear Receptor Superfamily of ligand-activated transcription factors. PXR is a 434 amino acid long sequence and has a NCBI sequence accession number O75469. In the present project murine monoclonal antibodies have been successfully raised against human PXR utilizing the purified full-length protein as an antigen.

Unlike the polyclonal antibodies, the monoclonal antibodies are produced from a replenishing cellular source which extends to the advantage of being economical, reliable and useful immunological tool in biological research with potential use in immunodiagnostics. Also, it is apparent from the literature that expression of nuclear receptor PXR in different cancerous states like endometrial cancer, breast cancer, prostate cancer and epithelial ovarian carcinoma etc. is relatively higher. But due to apparent lack of specific antibodies as well as domain specific monoclonal antibodies against PXR, the expression of PXR and its potential isoforms in different pathological states could not been concretely explored. So, this part of the study was based on

generation of specific monoclonal antibodies which could specifically recognize PXR and/or its isoforms. To address this issue we generated polyclonal and monoclonal antibodies which were raised against full-length PXR protein with ability to detect various potential isoforms of PXR. Western blot analysis with a polyclonal antibody generated against full-length PXR, reveals reactivity against all the major domains of PXR. Though the monoclonal antibodies against DBD and LBD of PXR have been successfully raised we were not successful in retrieving the monoclonal antibody against amino-terminal part (NTD) of PXR. Specificity and efficacy of these monoclonal antibodies were determined by utilizing different immunological tools like isotype mapping, western blot analysis, domain mapping, flow-cytometry and immunocytochemistry / immunohistochemistry, immunoprecipitation, analyses. However, our preliminary analysis with EMSA using one of the monoclonal antibodies has not been successful so far. Overall, at least two of the monoclonal antibodies have exhibited their efficacy with most of the immunological tools and studies. These monoclonal antibodies are expected to be useful immunological tool for detection of nuclear receptor PXR under different experimental requirements.

14. CONTRIBUTION TO THE SOCIETY

Monoclonal antibodies serve as important research and diagnostic tools in multiple studies and analyses. Monoclonal antibodies can replenish, therefore, the results once optimized can be uniformly reproduced everytime. Since nuclear receptor PXR is projected to be a potential prognostic marker in several metabolic disorders these antibodies are expected to serve not only as a research tools but also in immuno-clinical tests.

15. WHETHER ANY PH.D. ENROLLED/PRODUCED OUT OF THE PROJECT

Yes. One PhD and support to other researchers and studies.

16. NO. OF PUBLICATIONS OUT OF THE PROJECT

Five papers published

1. Kotiya D, Jaiswal B, Ghose S, Kaul R, Datta K and **Tyagi RK** (2016) Role of PXR in hepatic cancer: its influences on liver detoxification capacity and cancer progression. *PLoS One*.**(in press) (IF 2014 = 3.5)**
2. Rana M, Devi S, Gourinath S, Goswami R, **Tyagi RK** (2016) A comprehensive analysis and functional characterization of naturally occurring non-synonymous variants of nuclear receptor PXR. *Biochimica et Biophysica Acta – Gene Regulatory Mechanisms* 1859: 1183–1197. **(IF 2014 = 6.3)**
3. Priyanka, Kotiya D, Rana M, Subbarao N, Puri N and **Tyagi RK** (2016) Transcription regulation of nuclear receptor PXR: role of SUMO-1 modification and NDSM in receptor function. *Molecular and Cellular Endocrinology* 420:194-207. **(IF 2014 = 4.5)**

4. Saradhi M, Kumari S, Rana M, Mukhopadhyay G and **Tyagi RK** (2015) Identification and interplay of sequence specific DNA binding proteins involved in regulation of human Pregnane & Xenobiotic Receptor Gene *Experimental Cell Research* 339:187-196. **(IF 2014 = 3.25)**
5. Dash AK, Yende AS, Kumar S, Singh SK, Kotiya D, Rana M and **Tyagi RK** (2014) The Constitutive Androstane Receptor (CAR): a Nuclear Receptor in Health and Disease. *Journal of Endocrinology and Reproduction* 18: 59-74. (Indian Journal)

Paper in communication:

Priyanka, Puri N and **Tyagi RK** (2016) Novel alternative translational isoforms of nuclear receptor PXR: potential relevance in transcription modulation and metabolic disorders (*in communication process*)

Rakesh K Tyagi

(PRINCIPAL INVESTIGATOR)

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Registrar 19.10.2016

(REGISTRAR/PRINCIPAL)

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(CO-INVESTIGATOR)