REPORT

(To be filled by ICMR International Fellow (ICMR-IF)

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

1. Name and designation of ICMR- IF: **Dr. Showket Hussain**

2. Address: **ICMR-National Institute of Cancer Prevention & Research, I-7 Sector 39 Noida India 201301**

3. Frontline area of research in which training/research was carried out: Cervical cancer prevention strategies, anti- HPV therapeutics

4. Name & address of Professor and host institute: **Doris M. Benbrook, PhD**
PHF Presidential Professor
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5. Duration of fellowship with exact date: **February 10th 2023 to July 20th 2023 (Except dates of departure from and arrival to India)**

6. Highlights of work conducted: **Two hypotheses and 4 aims were pursued.**

Hypothesis 1: SHetA2 inhibits complement regulatory proteins in cervical cancer cell lines.

Specific Aim 1: Measure the effects of SHetA2 on CD46, CD59 and CD56 cervical cancer cell lines that are positive for High Risk Human Papillomavirus (HR-HPV) types HPV16 and HPV18, the two most common HPV types in India.

Results: SHetA2 modulated levels of complement regulatory proteins to varying extents depending on the cell line. Palbociclib reduced CD46 complement regulatory protein in 2 of 3 HR HPV+ cervical cancer cell lines in culture. Combination treatment with these two drugs were synergistic in inhibiting the CD55 complement regulatory protein.

Discussion: The results of this study support evaluating SHetA2 and palboclib, administered as single agents, and in combination, for inhibition of cervical cancer development and growth in an immunocompetent experimental model. The down-regulation of complement regulatory proteins in cervical cancer cells by these two drugs is predicted to cause increased immune reaction against the cervical cancer cells.

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Hypothesis 2: SHetA2 and palbociclib complement each other's activity against cervical cancer cell lines by inhibiting HR-HPV E6/E7 proteins and cell survival signaling pathways.
Specific Aim 2: Determine the dose and time dependent kinetics of SHetA2 and/or palbociclib on cervical cancer cell line viability.

Results: Because SHetA2 is known to complement the activity of the cyclin dependent kinase 4/6 (CDK4/6) inhibitor palbociclib, we also studied the combination of these two drugs. Palbociclib is currently being used to treat breast cancer in the US, but has not yet been developed for cervical cancer prevention or treatment. We evaluated effects of multiple dose combinations of SHetA2 or/and plabociclib treatments on metabolic viability of cervical cancer cell lines and evaluated the results using the ZIP Synergy Model. We found that the majority of dose combinations caused additive effects on reducing metabolic viability, while a few were synergistic.

Discussion: SHetA2 and palbociclib are able to inhibit cervical cancer cell line growth, while combination treatment increases the efficacy. To use the combination, a strategic pharmacokinetic approach will be needed to maximize the opportunity for synergistic interaction of the drugs.

Specific Aim 3: Test the effects of SHetA2 and palbociclib in a Drosophila fly model of HPV18 E6-induced dysplasia.

Specific Aim 4: Evaluate effects of SHetA2 and/or palbociclib on levels of HR-HPV E6/E7, AP1, Akt/mTOR and their phosphorylation in cell culture and xenograft cervical cancer models.

Results: We first evaluated SHetA2 effects on High Risk Human Papillomavirus (HR-HPV) Early 6 (E6) and Early 7 (E7) proteins, which are known to cause oncogenesis and support survival of cervical cancer cell lines. We demonstrated for the first time that SHetA2 reduces the levels of E6 and E7 in cervical cancer cells. SHetA2 reduced E6 mRNA and protein in HPV16 positive cervical cancer cell lines in culture and tumor xenografts. This drug also reduced E7 mRNA and protein in HPV16 positive cervical cancer cell lines in culture, but not in tumor xenografts. We found that palbociclib also reduced E6 and E7 protein levels in cervical cancer cell lines, and that the combination of SHetA2 and palboclib had greater effects than either drug when administered as single agents. We then evaluated the effects of SHetA2 on E6 and E7 protein levels in cervical cancer cell line xenograft tumors. SHetA2 reduced growth and E6 expression in the tumors, however in contrast to what was observed with the same cell line in cell culture, SHetA2 increased E7 expression. The drug combination treatment did not interfere with the effects of the individual drugs on E6/E7 expression.

To study the mechanisms of how SHetA2, palbociclib and the combination modulate E6 and E7 levels, we tested the hypothesis that these drugs reduce E6 and E7 gene transcription. Because AP-1 transcription factors subunits, cJun and cFos are known to induce transcription of the E6 and E7 genes from the HR-HPV long control region (LCR), we measured the effects of SHetA2 or/and palbociclib treatment on the two major AP1 proteins (levels of total and phosphorylated cJun and cFos). While the effects of the individual drugs on cJun and cFos protein levels were variable depending on the cell line, the combination consistently increased both cJun and cFos levels. This finding does not support the hypothesis, and therefore future plans are to evaluate the effects of the drugs on phosphorylation and levels of the other AP1 transcription factors, and on transactivation of an HR-HPV LCR reporter plasmid in a cell-based assay.

To further study potential signaling pathway mechanisms of SHetA2, palbociclib and the combination modulate E6/E7 and cervical cancer cell growth, we tested their effects on the Akt/mTOR signaling pathway known to be modulated by E6/E7. We found that the drug combination inhibits Akt and mTOR components, while single drug treatments do not.

Discussion: The synergistic inhibition of Akt/mTOR components offers considerable promise for use of the SHetA2 and palbociclib drug combination in treatment of cervical cancer. Future studies will compare the effects of these drugs on Akt/mTOR at different dosing schedules that we have shown to be additive versus synergistic in order to correlate these kinase signaling regulatory activities with the mechanism of synergy. Also, the ability of constitutively active Akt or mTOR components to prevent the drugs and their combination cervical cancer growth inhibition will be tested to validate their roles in the drugs and combination mechanisms of action.

i) Technique/expertise acquired :

Advanced cell culture techniques relevant to cancer research, Drug synergism, Gene expression studies which include, Western blotting, qRT-PCR, Confocal microscopy, flow cytometry, drug treatment, Immune cell isolation, Drosophila Fly model of HPV-oncogenesis, animal handling and treatment strategies for developing xenograft models for anti-cancer research.

ii) Research results, including any papers, prepared/submitted for publication: **Under preparation.**

iii) Proposed utilization of the experience in India:

The experience gained will certainly help to enhance my ongoing cervical cancer research in India. Since, translational cancer research and/or cancer prevention research is the main mandate of our institute ICMR-NICPR. Hence, this study identifies potential anti-HPV preventative and therapeutic strategies using combination therapy of SHetA2 and palbociclib as future baseline data to start a SHetA2 and/or palbociclib based pre-clinical models and conduct clinical trials of HR-HPV-driven pre-cancerous lesions. Also, the newly acquired skills and training will give further impetuous to the mandate of ICMR-NICPR and to our other cancer research collaborators in the country including my fellow PhD, Postdoc, MD/MS, MCh., trainees and both technical & scientific staff of ICMR-NICPR. This fellowship has opened up doors for collaborating with international collaborators working in the field of cancer prevention and research for joint research collaboration.

Doris M. Benbrook

Signature of ICMR-IF

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