



# PANDEMIC COVID-19



## Co-expression of PML-II and Spike proteins Augments type I interferon response (Tackling viruses by antiviral cellular proteins)



The 3<sup>rd</sup> International conference of ArPHA  
The Health sector Post Covid-19, Challenges and  
Opportunities

Cairo, 12-13 March 2023

By

Prof. Dr. Zeena Atwan

# Introduction

COVID-19

## Anti-Infectious Cellular Proteins

Many cellular genes exhibit specific virus gene inhibitory activity either by preventing the binding of them to cells, or by inducing or repressing cellular factors that in turn influence viral gene expression.

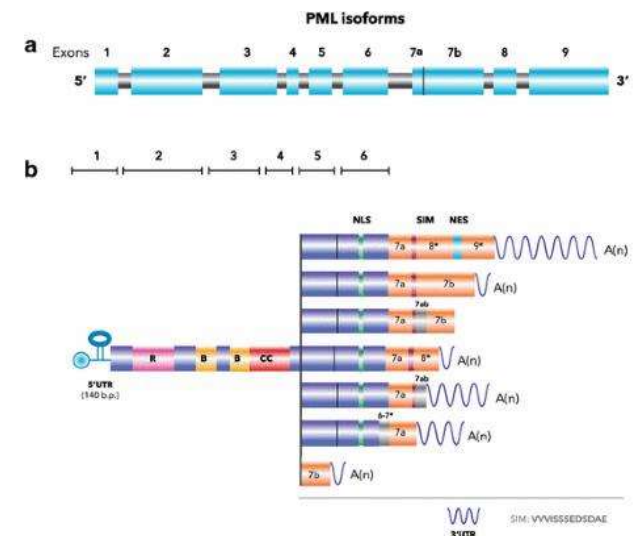


# Introduction

COVID-19

## Promyelocytic Leukemia proteins (PML)

PMLs are crucial for apoptosis, transcriptional regulation, tumor suppression, and innate immune responses. PML affects the innate immune signaling as a suppressor of viral gene expression and regulator of interferon (IFN) responses. PML-II isoform inhibits the replication of many viruses such as adenovirus and rotavirus through regulating the interferon response



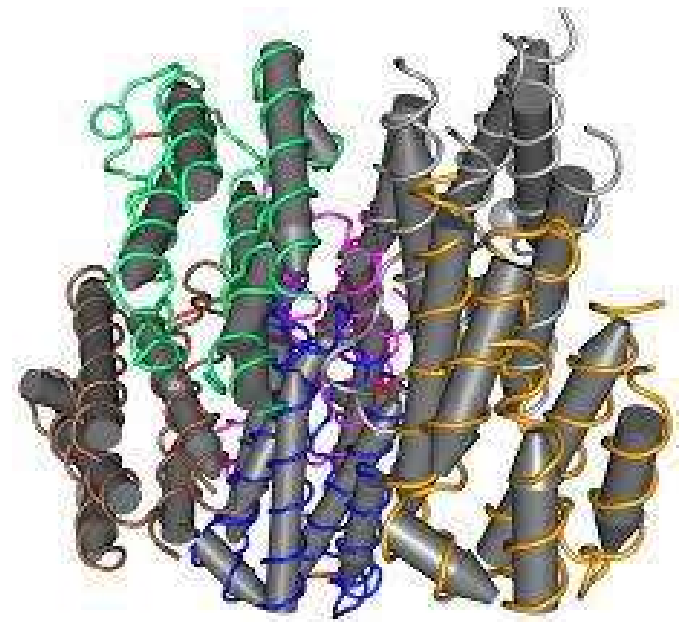
# An Introduction

COVID-19

## Use of interferon in treating virus infections

IFN $\beta$  is an antiviral cytokine secreted by host cells in response to virus infection (PML-II affected and affecting gene)

Recombinant IFN- $\alpha$  is used to treat chronic hepatitis C virus infection certain forms of chronic hepatitis B virus infections. IFNs have also been used in other viral infections



# An Introduction

COVID-19

## Use of interferon in treating virus infections

IFN $\beta$  is an antiviral cytokine secreted by host cells in response to virus infection (PML-II affected and affecting gene)

Recombinant IFN- $\alpha$  is used to treat chronic hepatitis C virus infection certain forms of chronic hepatitis B virus infections. IFNs have also been used in other viral infections



# Aim of study

COVID-19

## The aim of the study

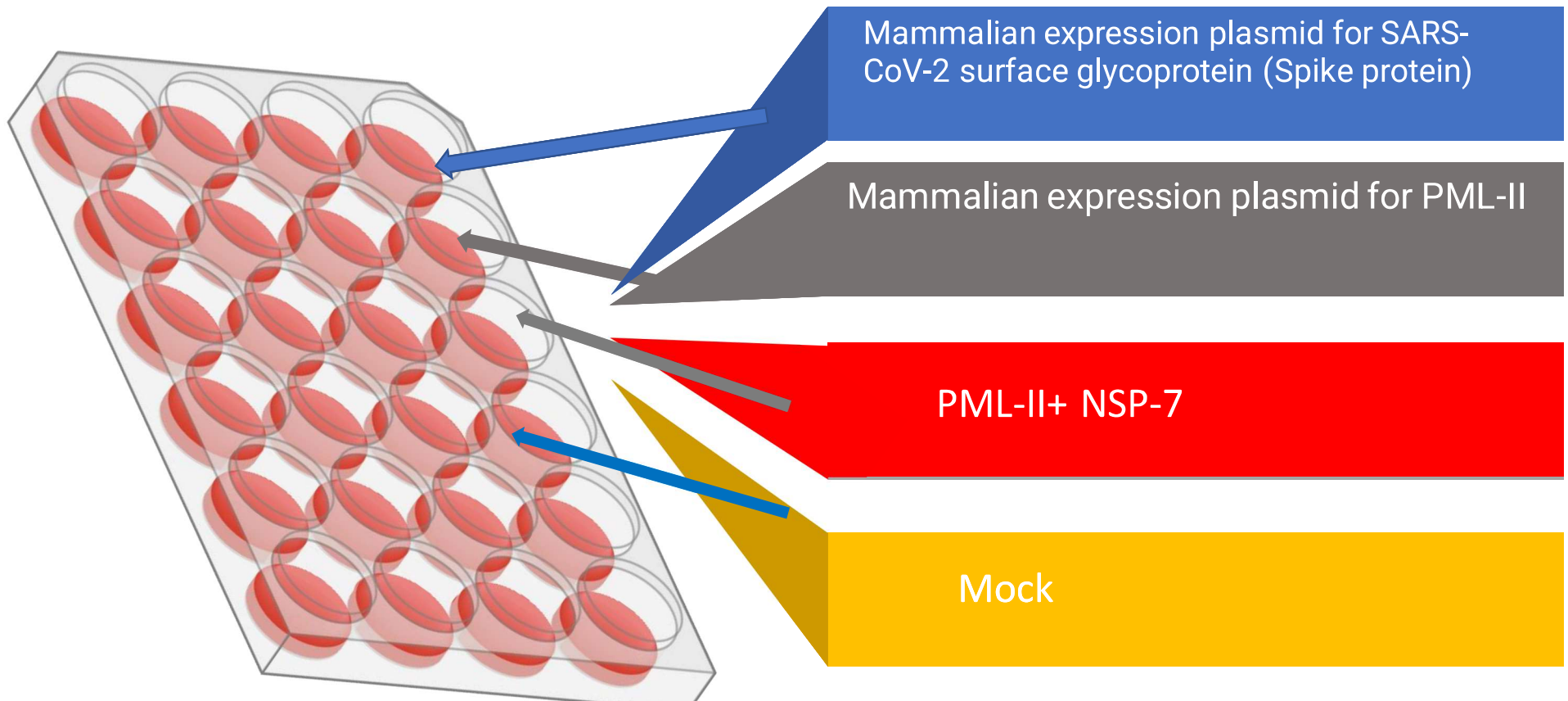
Is to investigate the effect of overexpression of PML-II coincides with the expression of the SARS-CoV-2 Spike on innate immune response including IFN, ISG20, IFITM2, IFITM3 in Colon cancer SW 1417 and 293HEK cells.

# Material and Method

# The Experimental Design

COVID-19

SW1417 were grown in 24-well plate





# DESIGN OF STUDY

COVID-19

## In VITRO ANALYSIS

**Subculturing  
cells**

**Transfection**

**Isolation of  
Total RNA from  
Cell Line**

**Gene Expression  
by Real-Time  
PCR**

# The Results

# The Results

COVID-19

## Calculation of expression Results

- First step

$\Delta\text{Ct}$ : Normalisation to Endogenous Control

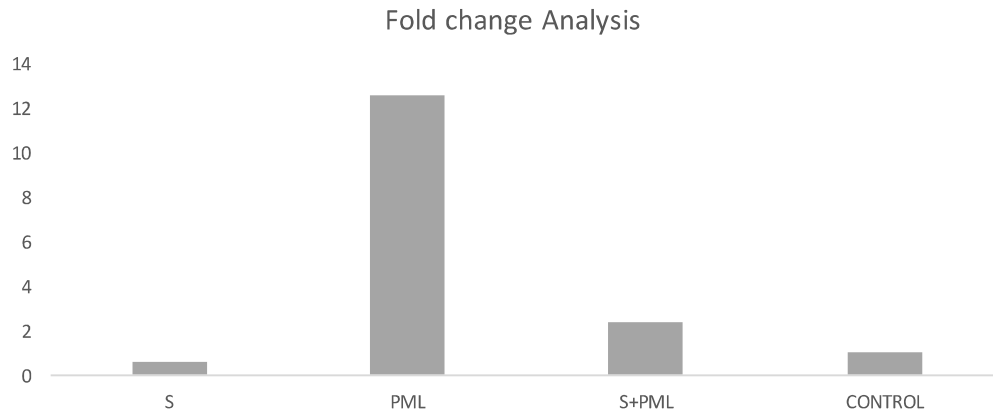
- Second step

$\Delta\Delta\text{Ct}$ : Normalisation to Calibrator



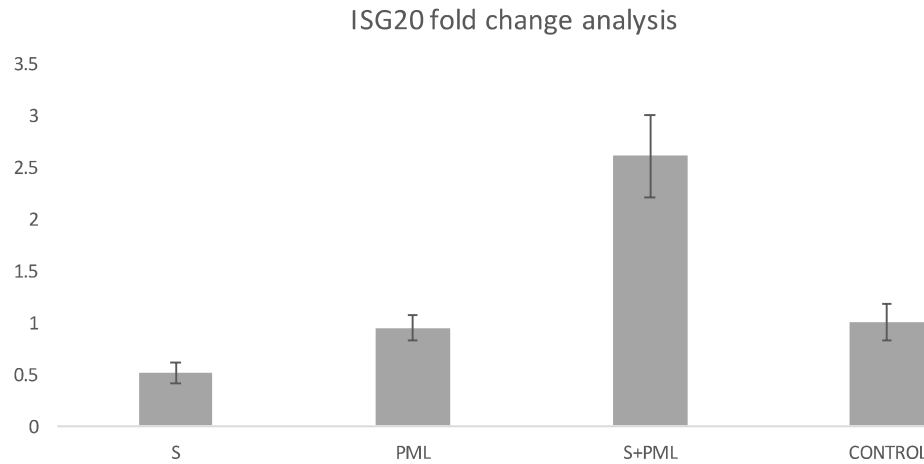
# *IFIT-2*

COVID-19



**Figure (2): *IFIT-2* fold change analysis with or without the overexpression of S or PML-II or S+PML-II plasmids in 293 cells.**

Equivalent cultures of 293 were transfected with S, PML-II, S+PML-II, or left as control without any treatment. Total RNA was extracted at 24 hours post transfection, reverse transcribed and the synthesized DNA were used as a template for qPCR relative expression assay using SYBR green master mix. Data were analyzed by  $\Delta\Delta$ .CTs and normalized to (GAPDH) house-keeping gene.



**Figure (4): *IFIT-2* fold change analysis with or without the overexpression of S or PML-II or S+PML-II plasmids in 293 cells.**

Equivalent cultures of 293 were transfected with S, PML-II, S+PML-II, or left as control without any treatment. Total RNA was extracted at 24 hours post transfection, reverse transcribed and the synthesized DNA were used as a template for qPCR relative expression assay using SYBR green master mix. Data were analyzed by  $\Delta\Delta$ . CTs and normalized to (GAPDH) house-keeping gene.

# Conclusions and Recommendations



## Conclusions & Recommendations

Elevated levels of type I IFN and IFN stimulated genes due to the overexpression of PML-II is critical to control the virus at very early stages.

IFN alpha & Beta could be considered as a bridge between the innate and adaptive immune response and hence this could be considered in treating Covid-19 cases.

We recommend

Verifying the idea using more techniques

Use of normal cell lines



**THANK YOU**

