IMMUNOLOGY SECTION

Objectives

Tumor immunology

To understand tumor-immune interactions in the microenvironment and apply this understanding to:

1. Identify prognostic markers in cancer (cancer progression/chemo-radio-resistance/immunosuppression)

2. Develop immunotherapies that strengthen the immune system and can be used as adjuvants in cancer treatment along with chemo/radio therapies

Low dose radio biology

To understand the

1. Effects of low dose radiation on the immune system

2. Effects of diagnostic/medical exposures on DNA damage response, immune response and antioxidant status in cancer

Immunosuppressive Tumor microenvironment

- **Tumor secretes mediators like PGE2**
- **Immune cells are dysfunctional**
- **Immune cells secrete T cell suppressive cytokines like TGF-β and IL-10**
- **Radiotherapy increases secretion of TGF-β**
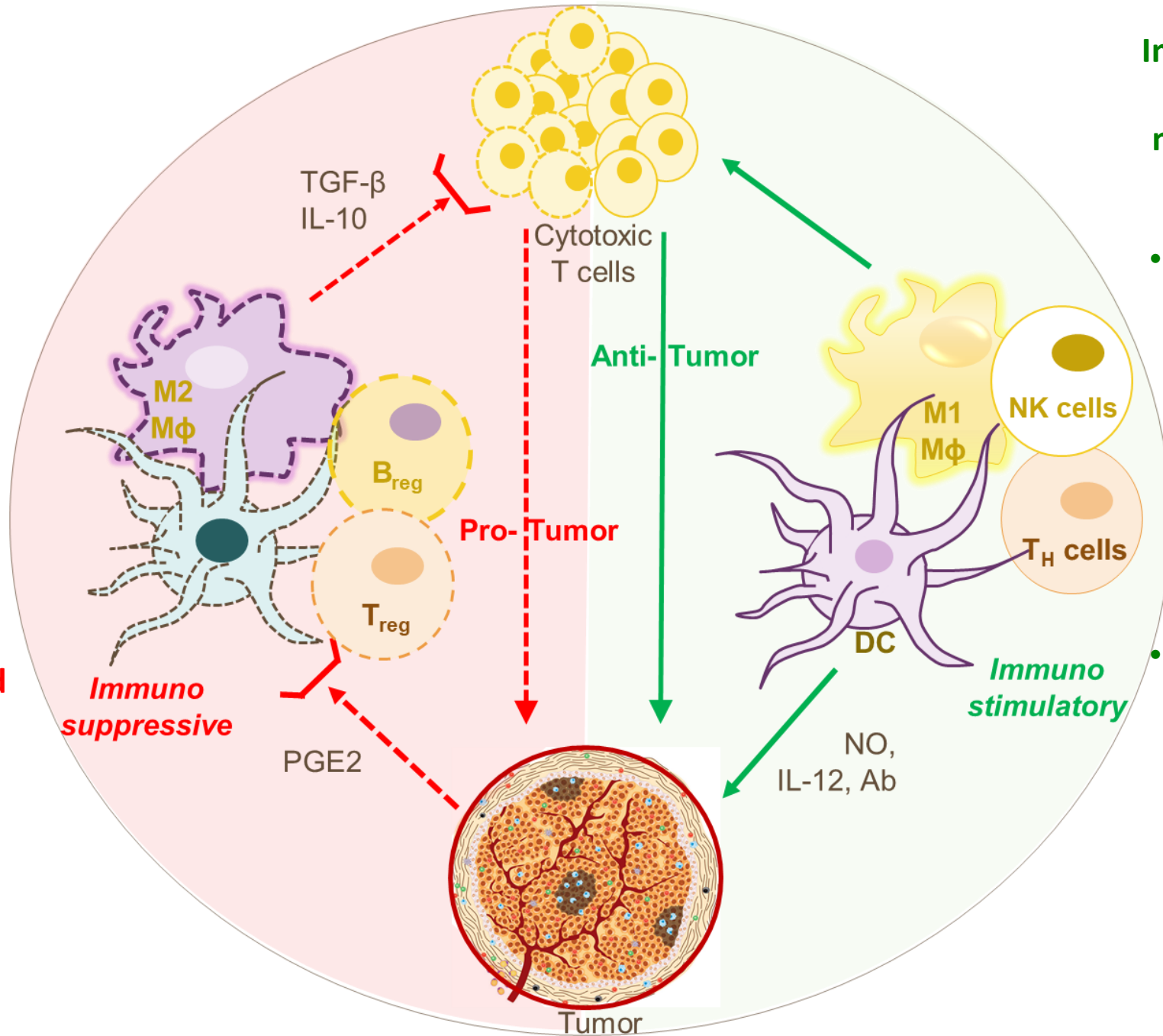
Immunostimulatory Tumor microenvironment

- **Immune cells are functional and can directly kill the tumor cells through cell-cell contact**
- **They can also kill the tumor cells through mediators like nitric oxide.**

TGF-β
IL-10

Cytotoxic T cells

Anti- Tumor

Pro- Tumor

M2 Mϕ

B_reg

T_reg

Immuno suppressive

PGE2

M1 Mϕ

NK cells

T_H cells

DC

Immuno stimulatory

NO, IL-12, Ab

Tumor

1. Identify prognostic markers in cancer

Cancer progression

(a) A novel 15 gene signature was identified from macrophage-tumor interactions in breast cancer and has prognostic significance

8 gene set identified by macrophage-tumor interactions
(TNF-α, IL-6, IL-1β, MMP1, MMP9, TGF-β1, TGFβRII, EGFR)

Enrichment genes with significant co-occurrence
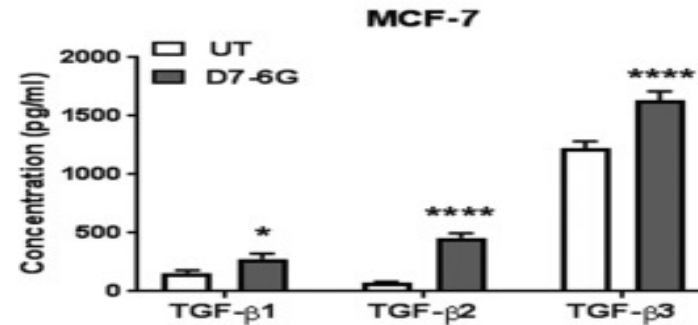(TP53, WTAPP1, SLC12A5, PSAT1, ESR1, TPD52,PRKCD)

15 Gene signature

Altered in 63.6% TCGA samples

Singh et al., BBA, 2018

Cancer radioresistance

(b) TGF-β signaling was increased in radio resistant breast cancer cells resulting in hybrid epithelial-mesenchymal phenotype and enrichment of cancer stem cells

Increased TGF-β in radioresistant cells

Increased tumor formation of radioresistant cells in SCID mice

MCF-7 D7-6G TUMOR

MCF-7 UT TUMOR

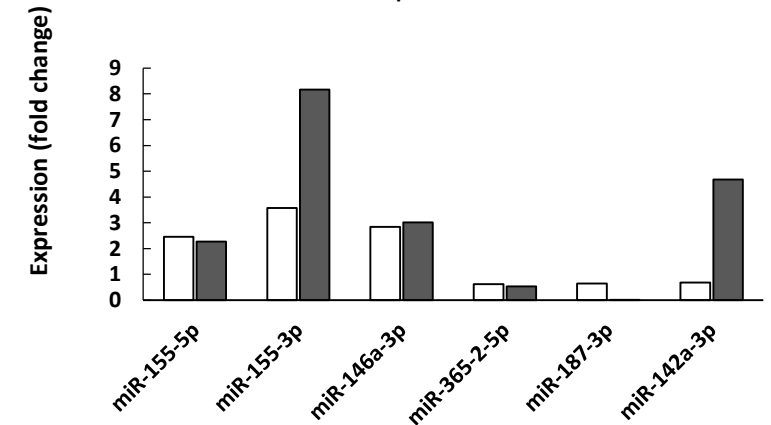Yadav and Shankar, Biomedicine & Pharmacotherapy, 2019

Immunosuppression

(c) Tumor induced alterations in miRNAs can serve as markers of dendritic cells with lowered immunogenicity
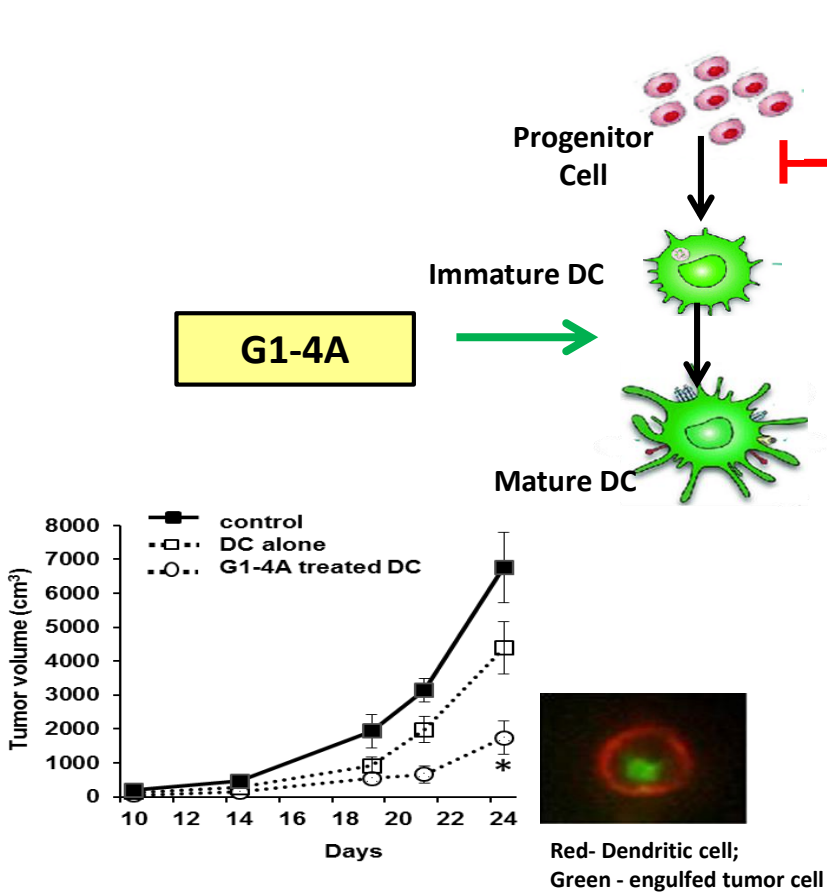
miRNA profile (iDC TCM vs iDC)
□ RNA-seq ■ RT-PCR

miR-155-5p, miR-155-3pand miR-146a-3p Upregulated in dysfunctional DC

miR-362-2-5p, miR-187-3pand miR-142a-3p Downregulated in dysfunctional DC

(2.a) Molecules tested as immunotherapeutics in pre-clinical models:

G1-4A, polysaccharide from *T.cordifolia* induced killer Dendritic cell phenotype and DC mediated reduction of tumor burden in Lymphoma model

Pandey et al, Int. Immunophar, 2012,2014
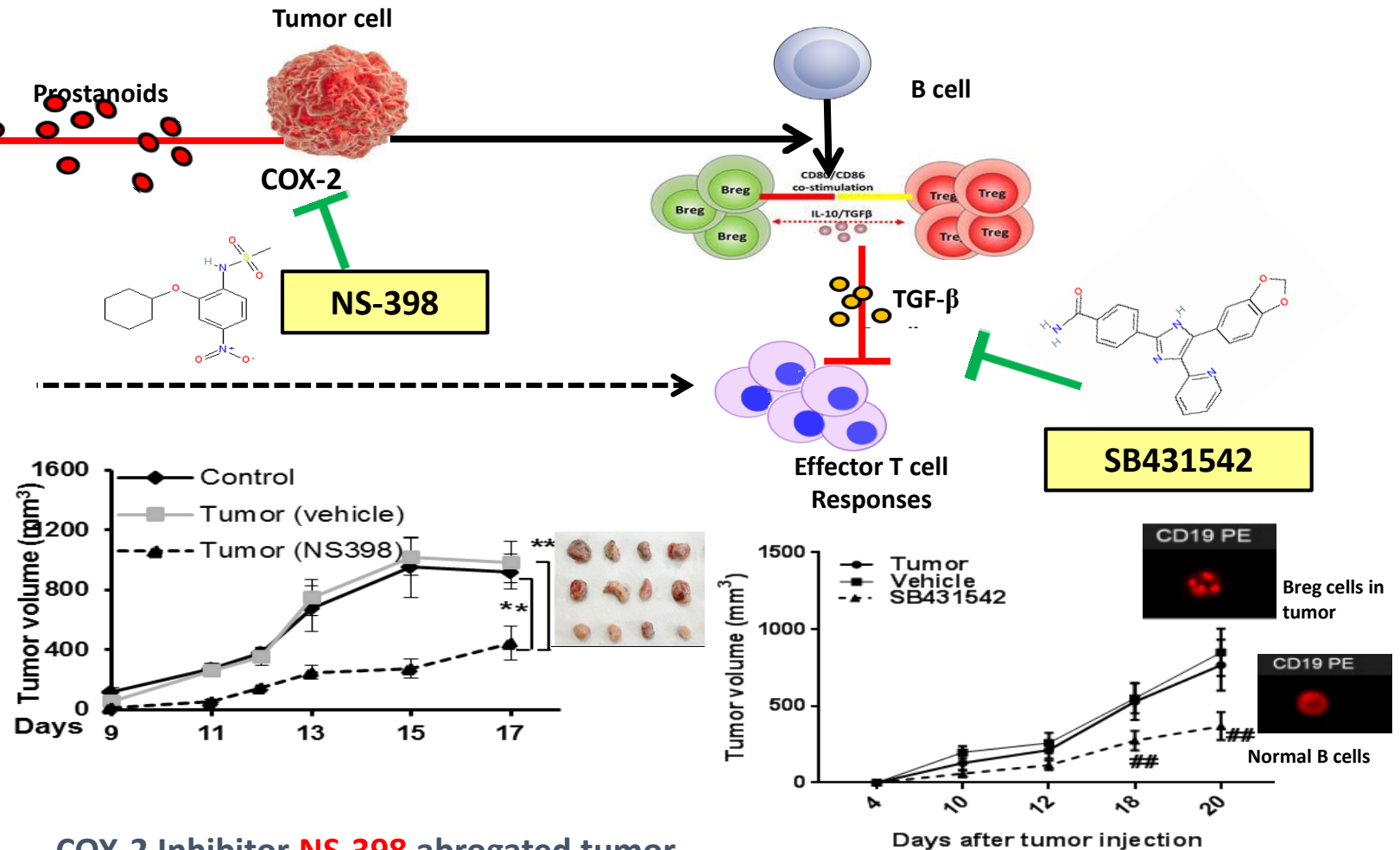
COX-2 Inhibitor **NS-398** abrogated tumor induced DC dysfunction and decreased tumor burden in Lymphoma model
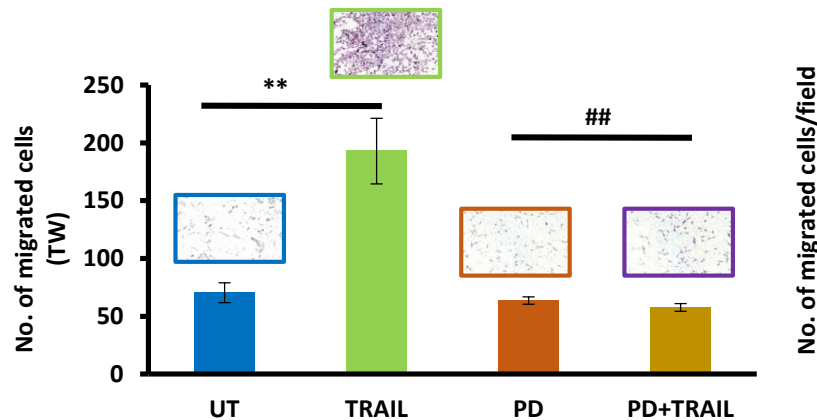
Pandey et al, Immunology letters, 2017

TGF-β Receptor I inhibitor **SB431542** inhibited Breg-Treg axis and reduced tumor burden in Fibrosarcoma model

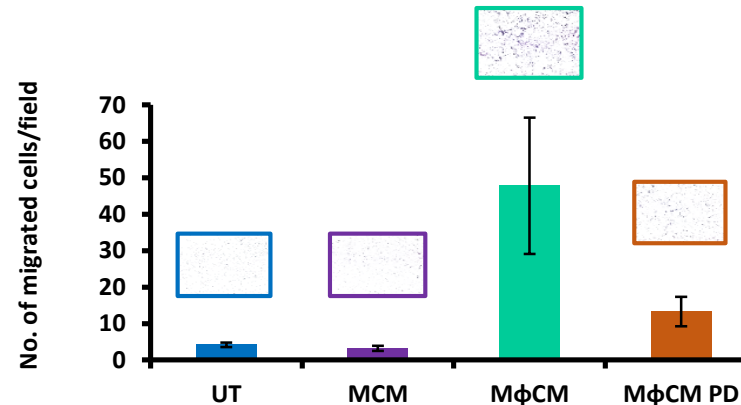# (2.b) Molecules tested as immunotherapeutics in cell culture systems

**PD98059
(MEK inhibitor)**

**Sulphoraphane
(derived from broccoli)**

(1) ERK inhibitor abrogated TRAIL induced increase in epithelial-mesenchymal transition in lung cancer cell lines with mutant KRAS

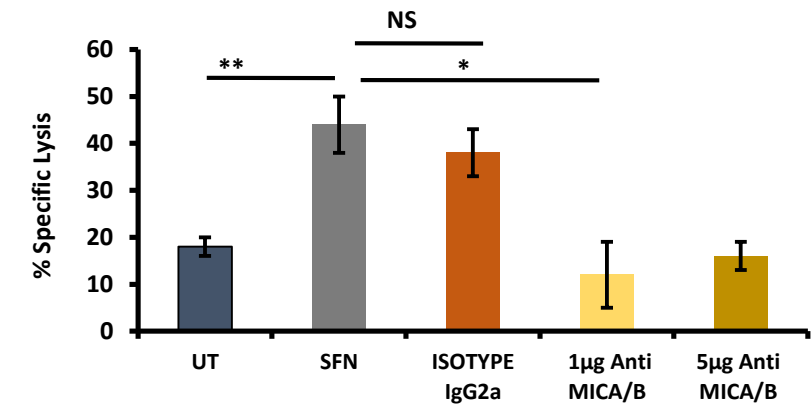**(2) ERK inhibitor abrogated macrophage induced increase in epithelial-mesenchymal transition; cancer stem cells; migration and invasion of breast cancer cells.**

(3) Sulforaphane up-regulated NKG2D ligands in lung cancer cell lines thereby activating NK cell-mediated killing.

Pal et al, Cancer microenvironment, 2016

Amin and Shankar, LifeSciences, 2015

c) Molecules being screened as immunotherapeutics

(1)Epigenetic drug library for identification of inhibitors of T regulatory cell differentiation

(2)FDA approved drug library for identification of COX-2 inhibitors

A. Effects of low dose radiation on the immune system (murine model)

BALB/c Radio-Sensitive Strain

MAPK ↑
Ca²⁺ ↑
RNS ↑

Priming Dose PD (0.1 Gy)

Challenge Dose (2 Gy)

+ConA, 24 h

C57BL/6 Radio-Resistant strain

MAPK ↑
Ca²⁺ ↓
RNS ↓

Radio-adaptive response observed in BALB/c mice and not in C57BL/6 mice

Radio-adaptive Response

IL-2 (pg/mL): Control, 0.1 Gy, 2 Gy, 0.1+2 Gy **

IL-2 (pg/mL): Control, 0.1 Gy, 2 Gy, 0.1+2 Gy

No adaptive Response

PD (0.1 Gy) increased Calcium and RNS in BALB/c and not in C57BL/6 mice

FLUO-3AM (AU): Ca²⁺ Control, 0.1 Gy **
DAF-FM (AU): RNS Control, 0.1 Gy **
FLUO-3AM (AU): Ca²⁺ Control, 0.1 Gy
DAF-FM (AU): RNS Control, 0.1 Gy

Combination of NO donor and Ca²⁺ ionophore mimic PD and induced RAR in both strains

Radio-adaptive Response

% CD69+ Cells: UT, NO/Ca, 2 Gy, NO/Ca+ 2 Gy **

% CD69+ Cells: UT, NO/Ca, 2 Gy, NO/Ca+ 2 Gy **

Radio-adaptive Response

Premkumar and Shankar, IJRB, 2016,
Premkumar et al, IJRB, 2019

B. Biological effect of low and high dose radiation exposure on human peripheral blood mononuclear cells and tissues of cancer patients: a prospective *in-vivo* study

**Principal Investigator: Dr. R. Badwe, Director, TMC**

Lead Investigators from BARC: Dr. Birajalaxmi Das, Head, LLRRS; Dr. Bhavani Shankar , Head, Immunology Section

Lead Investigators ACTREC : Dr. Jayant S. Goda, Dr. Supriya J. Sastri, Dr. Sarbani Ghosh Laskar, Dr. Sudeep Gupta, Dr. S. Chiplunkar

Objective: **To determine the effects of medical exposures (diagnostic/therapeutic) in blood cells and tissues of normal & cancer patients using multiple endpoints.**

PBMC

- DNA damage and repair studies : Analysis of γ-H2AX (positive cells/foci) by flow cytometry and fluorescence microscopy
- Immune response : Cytokine expression by ELISPOT and ELISA
- Gene expression profile : DNA Damage Response and DNA repair genes by RT-qPCR
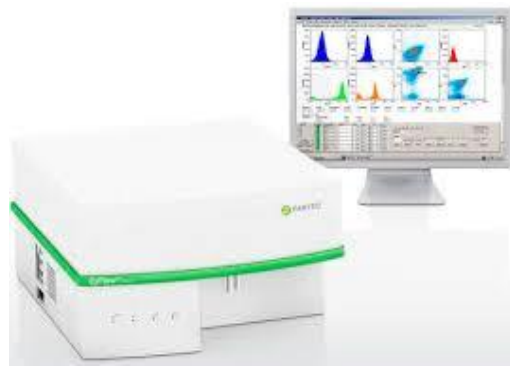- Antioxidant status: Lipid peroxidation, lactate dehydrogenase, and levels of Antioxidant enzyme status

γ-H2AX foci

γ-H2AX foci

Gene expression

ELISPOT

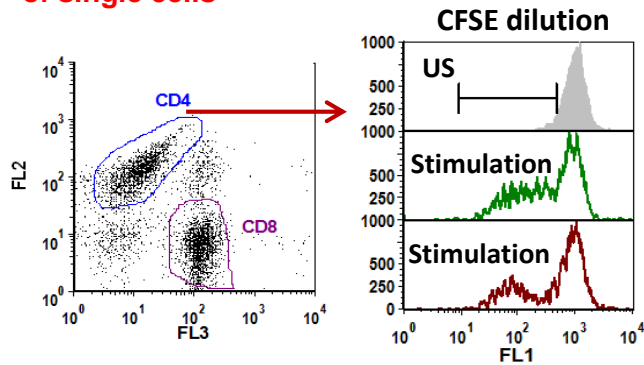FLUOROSPOT

FLUOROSPOT
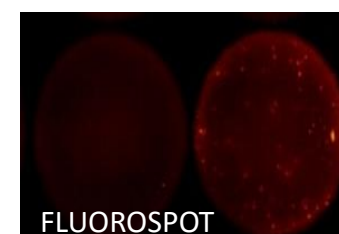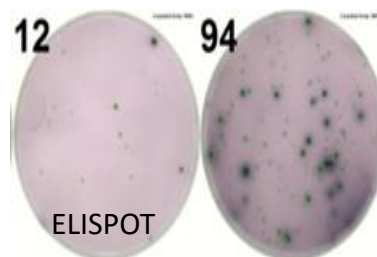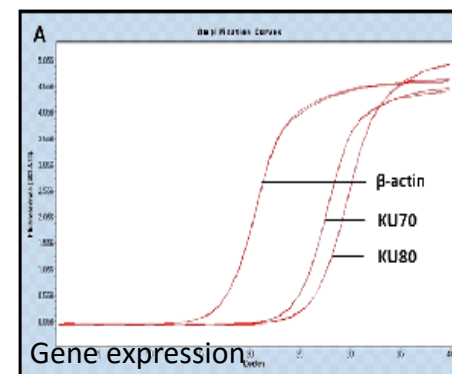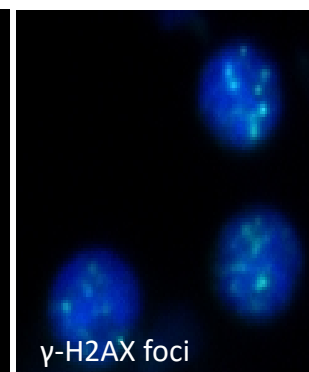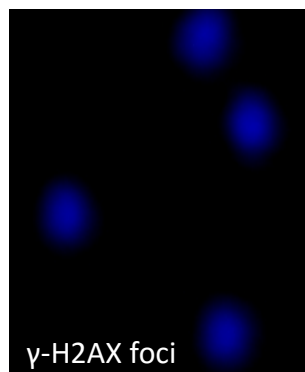
Tumor tissues

- Transcriptome sequencing
- Exome sequencing
- miRNA sequencing

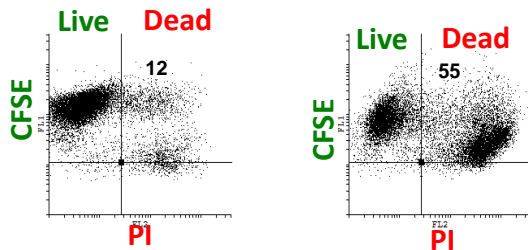**Collaborative project with TMH-ACTREC**

FLOW CYTOMETER

• Provides simultaneous multi-parameter analysis of single cells

Proliferation by CFSE dilution in CD4$^+$ T cells

• Cell-cell interaction in co-culture

**Immune cell mediated tumor killing in co-culture**

IMAGING CYTOMETER

• Combines advantages of microscopy and cytometry for high-throughput cellular analysis

• Multichannel digital images of hundreds of thousands of individual cells can be captured within minutes

• Can obtain single-cell morphological and intracellular localization measurements of different cell markers

Phagocytosis of E.coli Bioparticle by macrophages
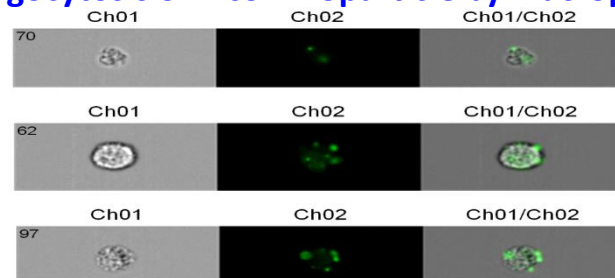
Proliferation of B cells in tumor
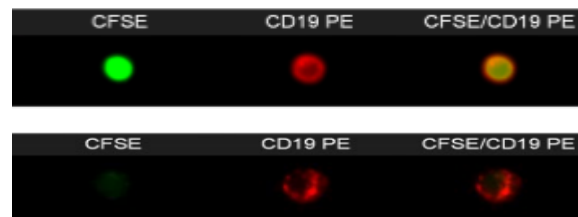
ELISPOT READER

• Measures the frequency of cytokine-secreting cells at the single-cell level.

• Each spot corresponds to an individual cytokine-secreting cell.

• Very sensitive and can detect frequencies in $<10^4$ cells

ELISpot read-out of cytokine secreting cells