A Novel Kefir Product, PFT, Exerts Anticancer Effects

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WJ

CANCER:

THE ENEMY FROM WITHIN

We are in great need for new, effective, non-toxic agents for the treatment of cancer

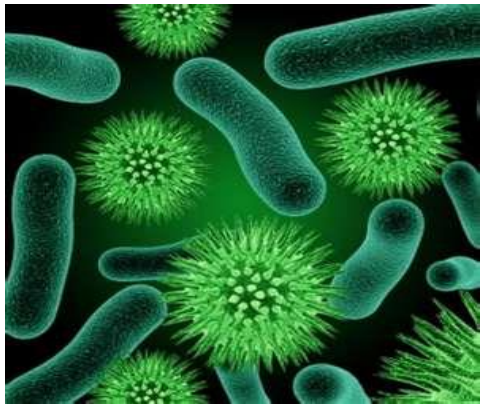
Kefir Product: PFT

(Probiotics Fermentation Technology)

Paitos Co., Ltd. Yokohama, Kanagawa, Japan.

What are probiotics?

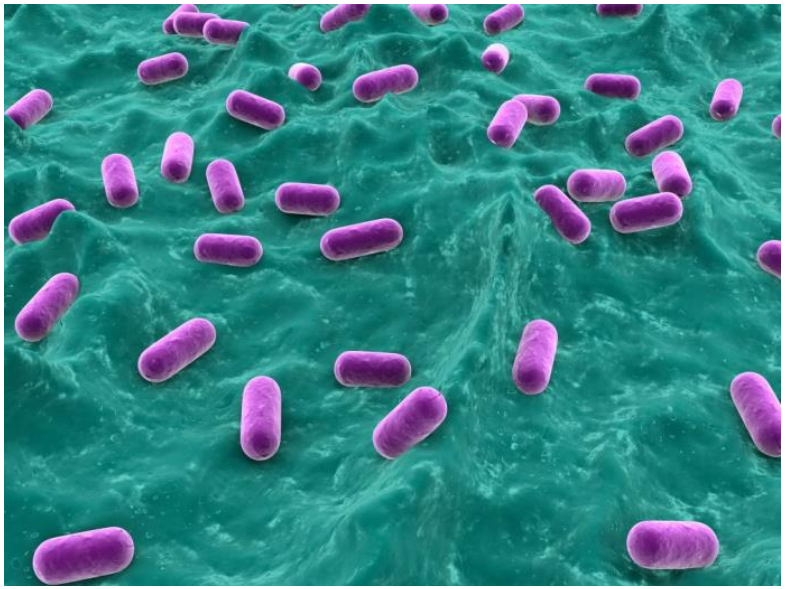
- Any microbe (ei. Bacteria or fungus) that provides a benefit to its host is considered probiotic



Probiotic Examples

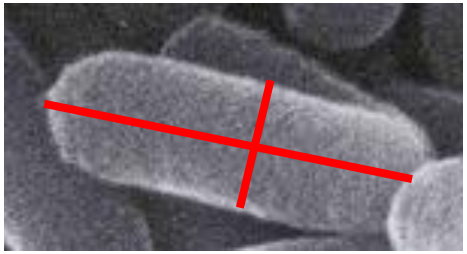
There are <u>many</u> types of probiotic examples which are <u>beneficial for human health!</u>

In our study we focus on **Lactic Acid Bacteria (LAB)**



Lactobacillus

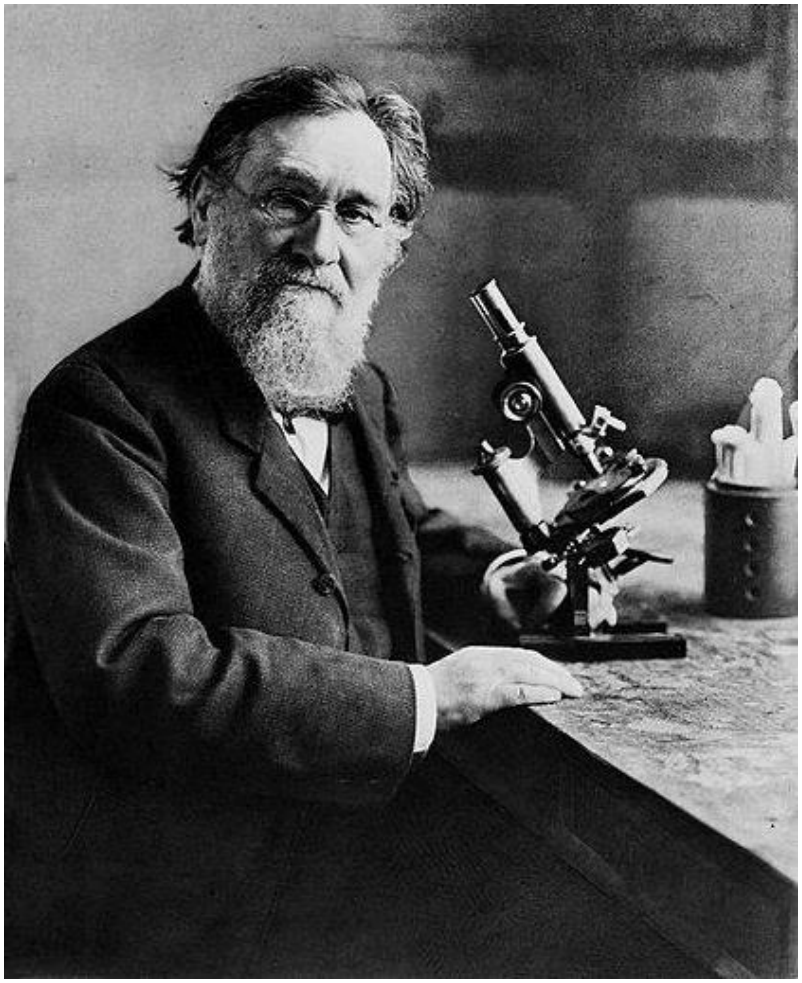
Cell Size: 0.9 x 3.0 micrometer (µm)



History of Probiotics

Eli Metchinkoff

(1845 – 1916)



•Russian scientist who received the Nobel Prize in medicine (immunity)in 1908

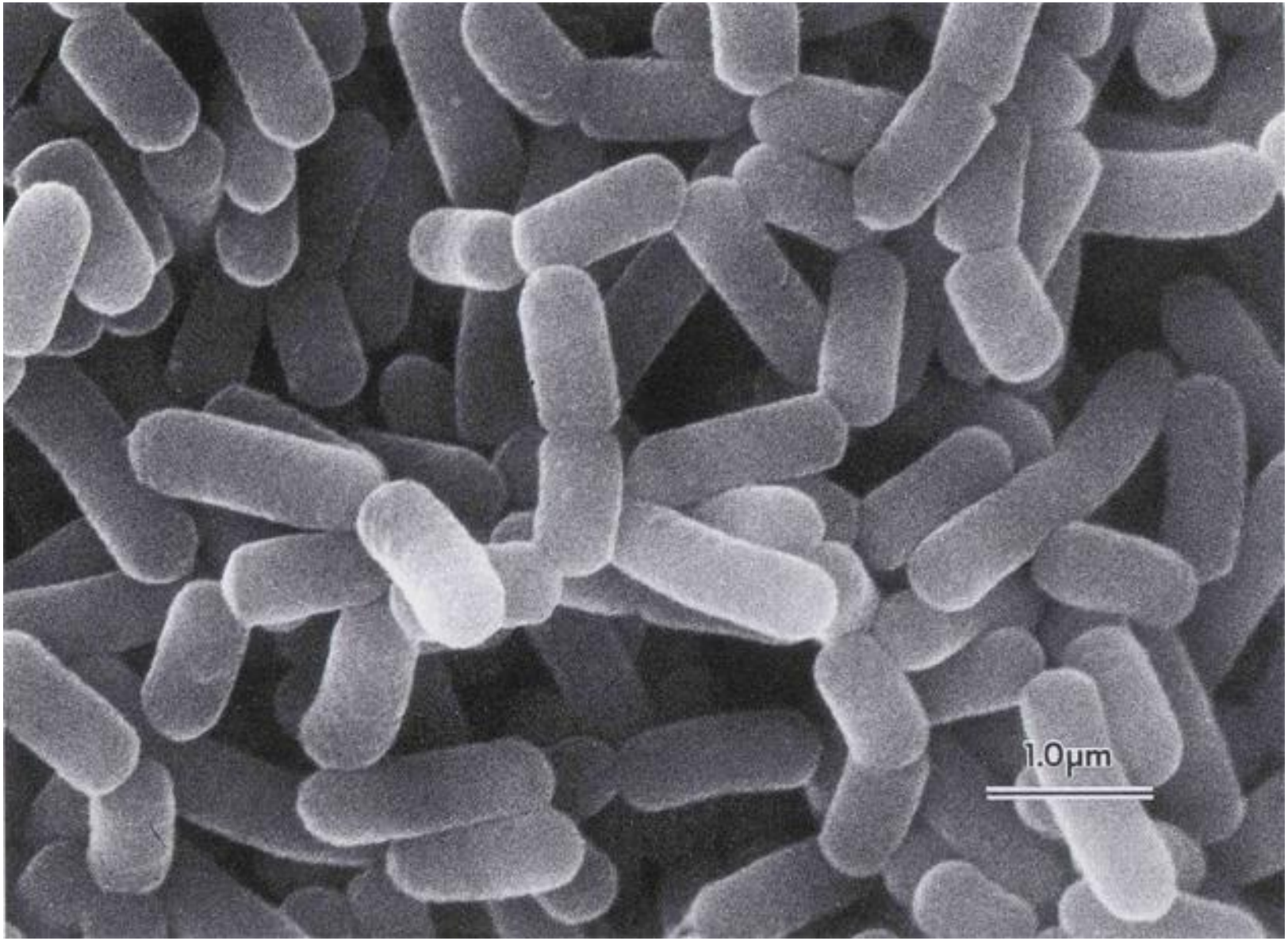
•He suggested that LAB might be an effective tool in prolonging life over one century ago

What is PFT?

Origin of PFT

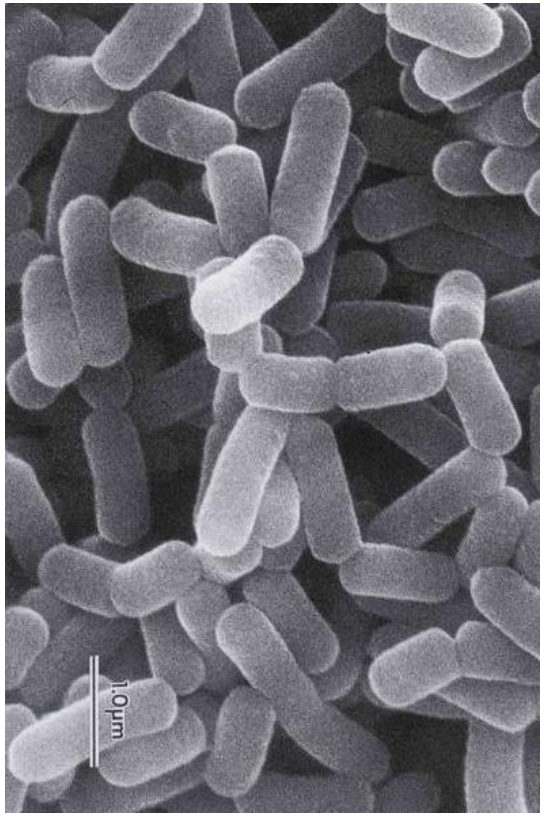
Kefir is thought to originate in the Caucasus mountains in Russia and Turkey.

Lactobacillus kefiri P-IF



L. kefiri P-IF

•***Lactobacillus kefiri P-IF (90 %)***



PFT

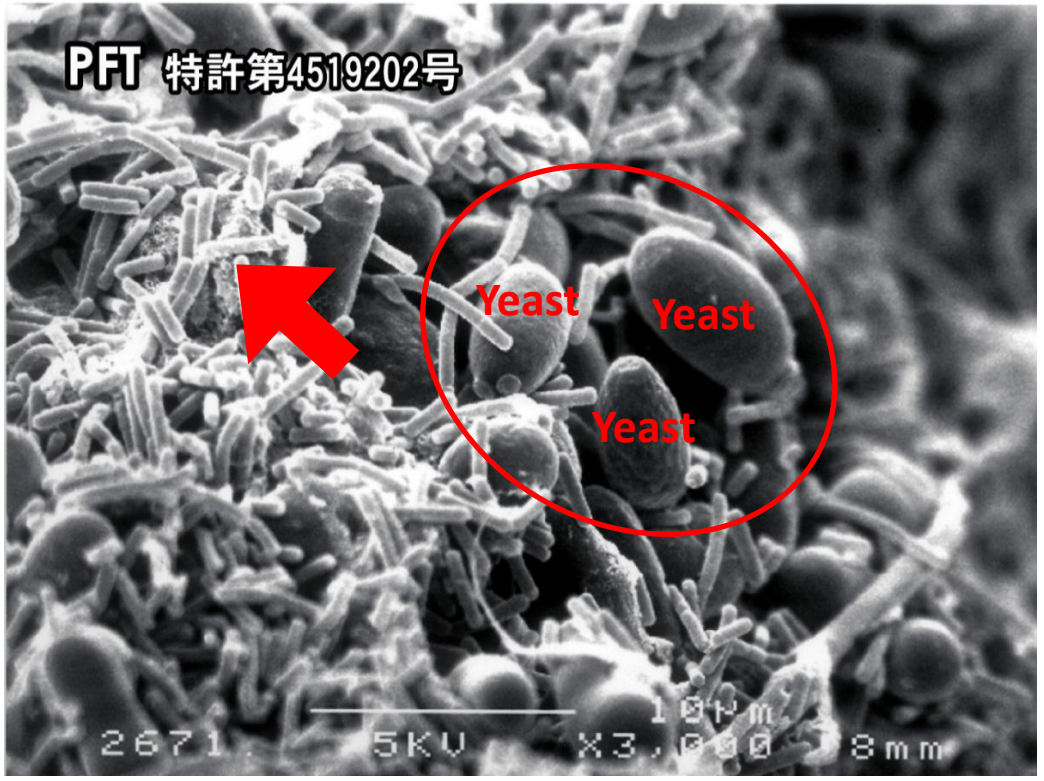
PFT* is a probiotic composed of:

2 bacteria: Red arrows

- ***Lactobacillus kefiri P-IF (90 %) (large red arrows)***
- ***Lactobacilus kefiri P-B1 (2-3%)***

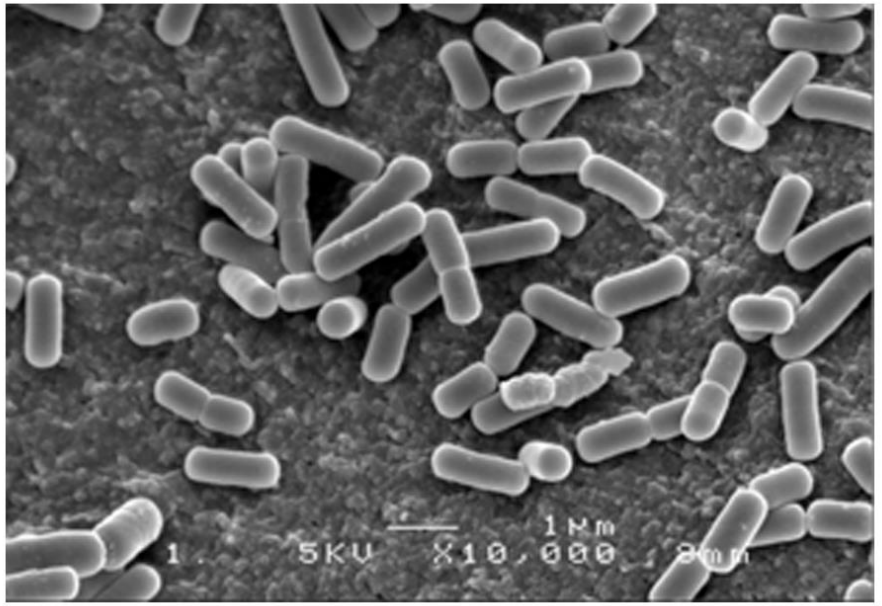
3 yeast:

- ***Kazachstania turicensis (2-3%)***
- ***Kazachstania unispora (2-3%)***
- ***Kluyveromyces marxianus (2-3%)***



Unique P-IF Characteristics

- **Grows three dimensionally (3D) due to unique cell wall composition**
  - The special cell wall could contribute to its effectiveness as a probiotic
- **Grows in low pH and produces acid**
  - Acid production helps to kill off pathogenic bacteria
- **Uses galactose as an energy source**
  - P-IF can help to regulate toxic levels of galactose



Electron microscope image of *L. kefiri* P-IF strain (Ghoneum and Gimziewski, 2014)

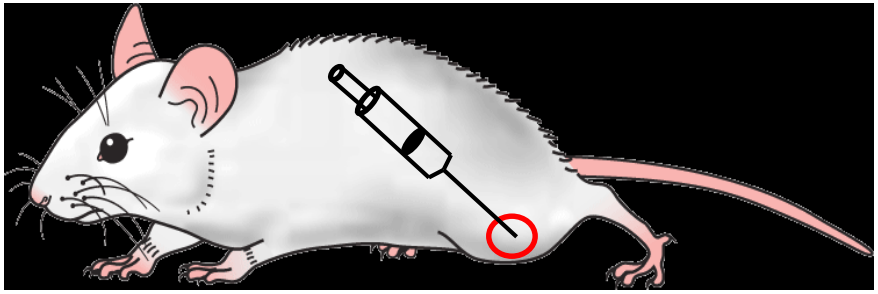
Does PFT Exert Anti-Cancer Activity?

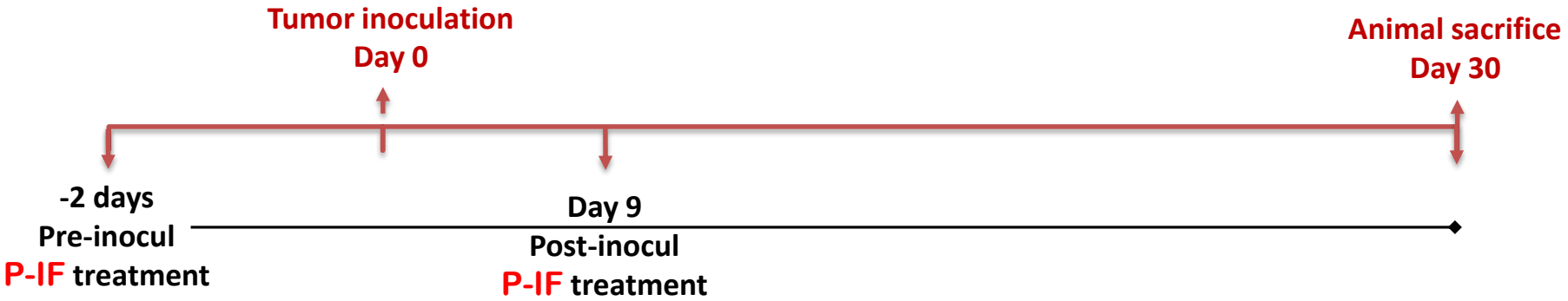
Experimental Design

Tumor Inoculation

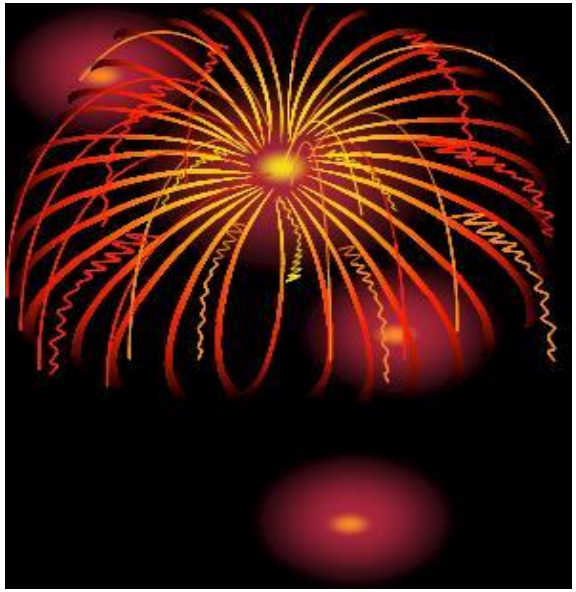
Ehrlich Ascites Carcinoma is poorly differentiated malignant tumor derived from breast carcinoma.

Female Swiss albino mice



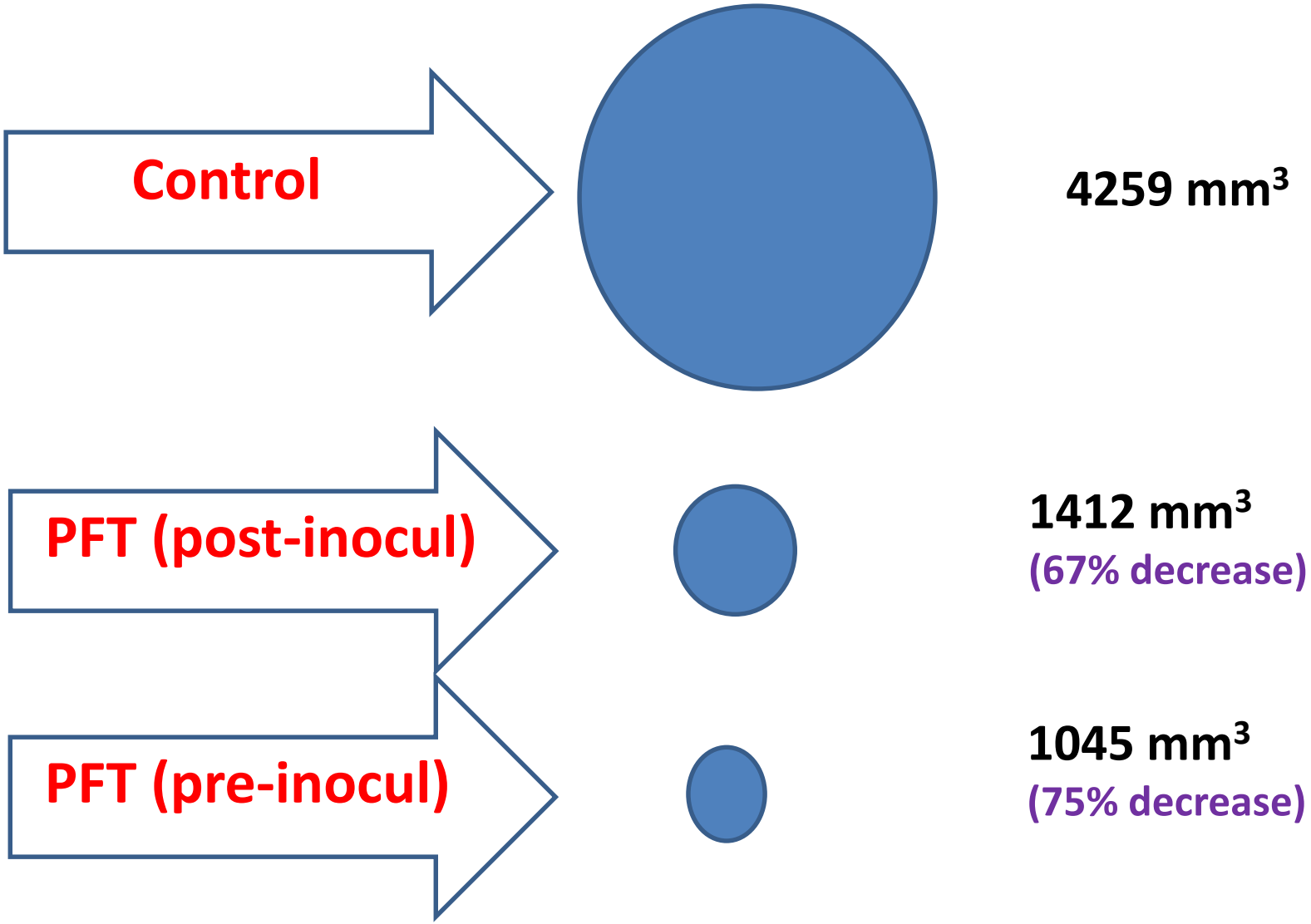


1. On day 0, **mice were inoculated intramuscularly with Ehrlich ascites carcinoma cells (2.5 x 10^6 cells) in the right thigh of the lower limb to develop solid tumor.**
2. ***L. kefiri* P-IF was administered orally (2g/kg/day)** to mice 6 days/week, either two days before tumor cell inoculation or nine days after inoculation to mice bearing tumor mass (~300 mm^3) that developed within 9 days.

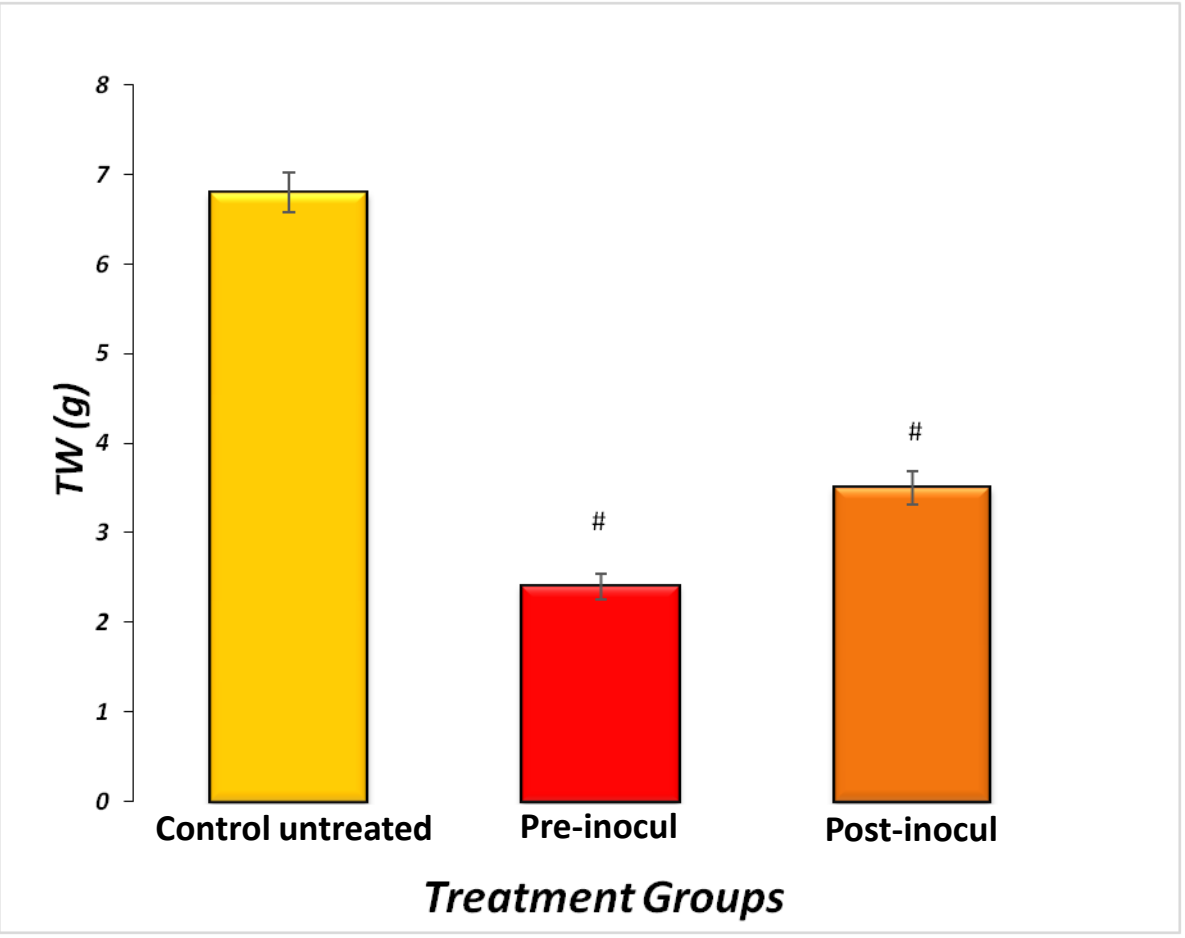


RESULTS

Tumor Volume



2-Tumor weight/g.



-64%

- P-IF (pre-inocul)

-48%

- P-IF (post-inocul)

Each value represents the mean±SE.
Number of mice/group: **Control untreated** (11), **Pre-inocul** (10), **post-inocul** (16).
significantly different from the TW of Control untreated group at *p<0.01* level.

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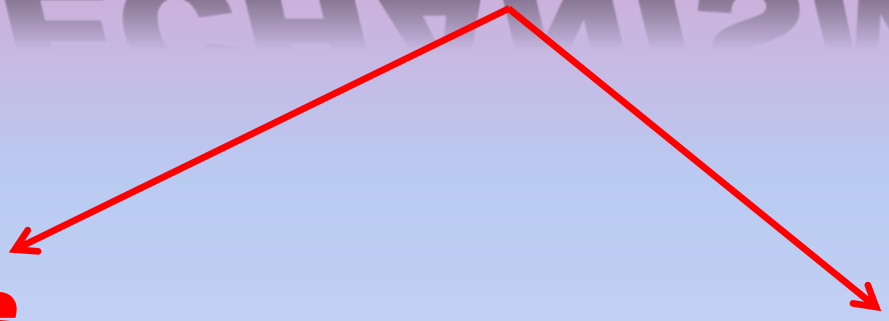
MECHANISMS



I. Immune modulation

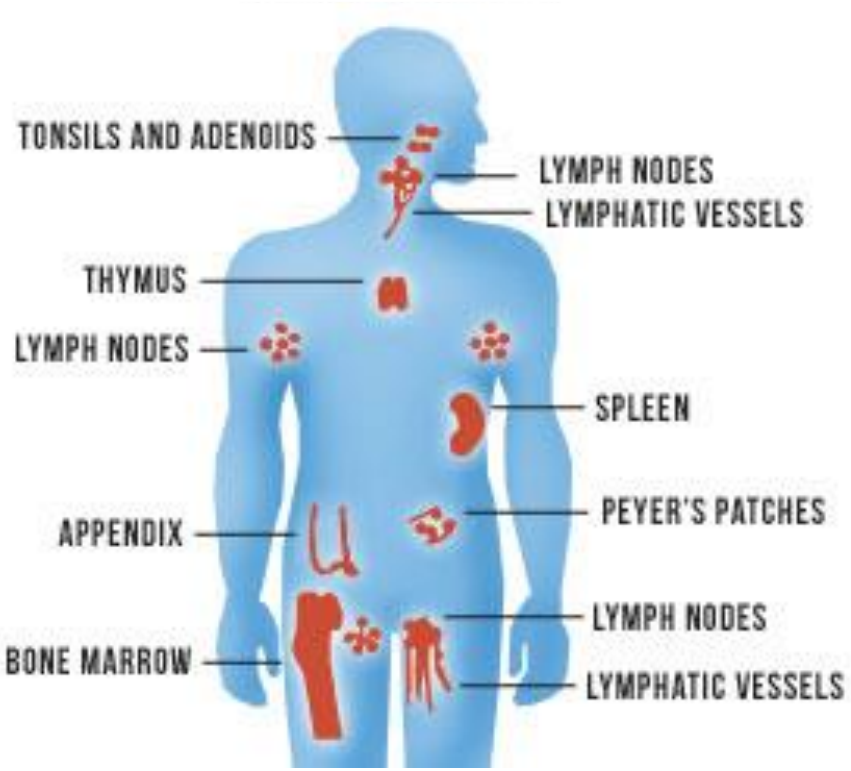
II. Induction of Apoptosis

1.

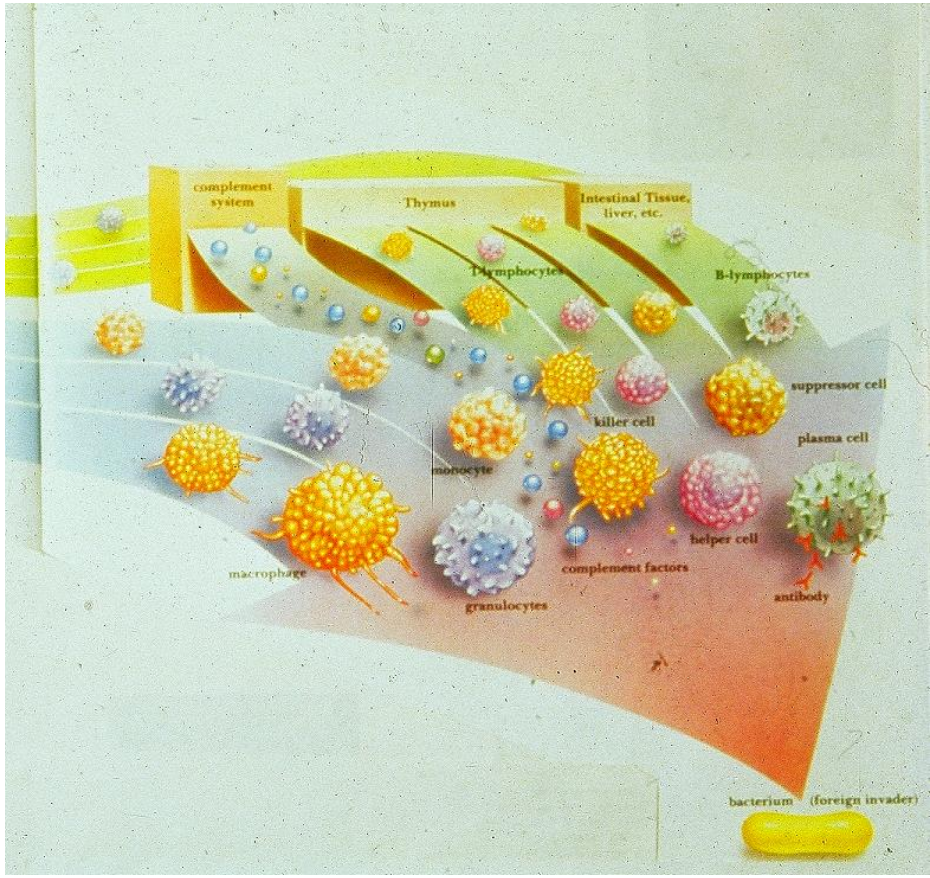
PFT AS AN IMMUNE MODULATOR

(ACTIVATES THE IMMUNE SYSTEM)

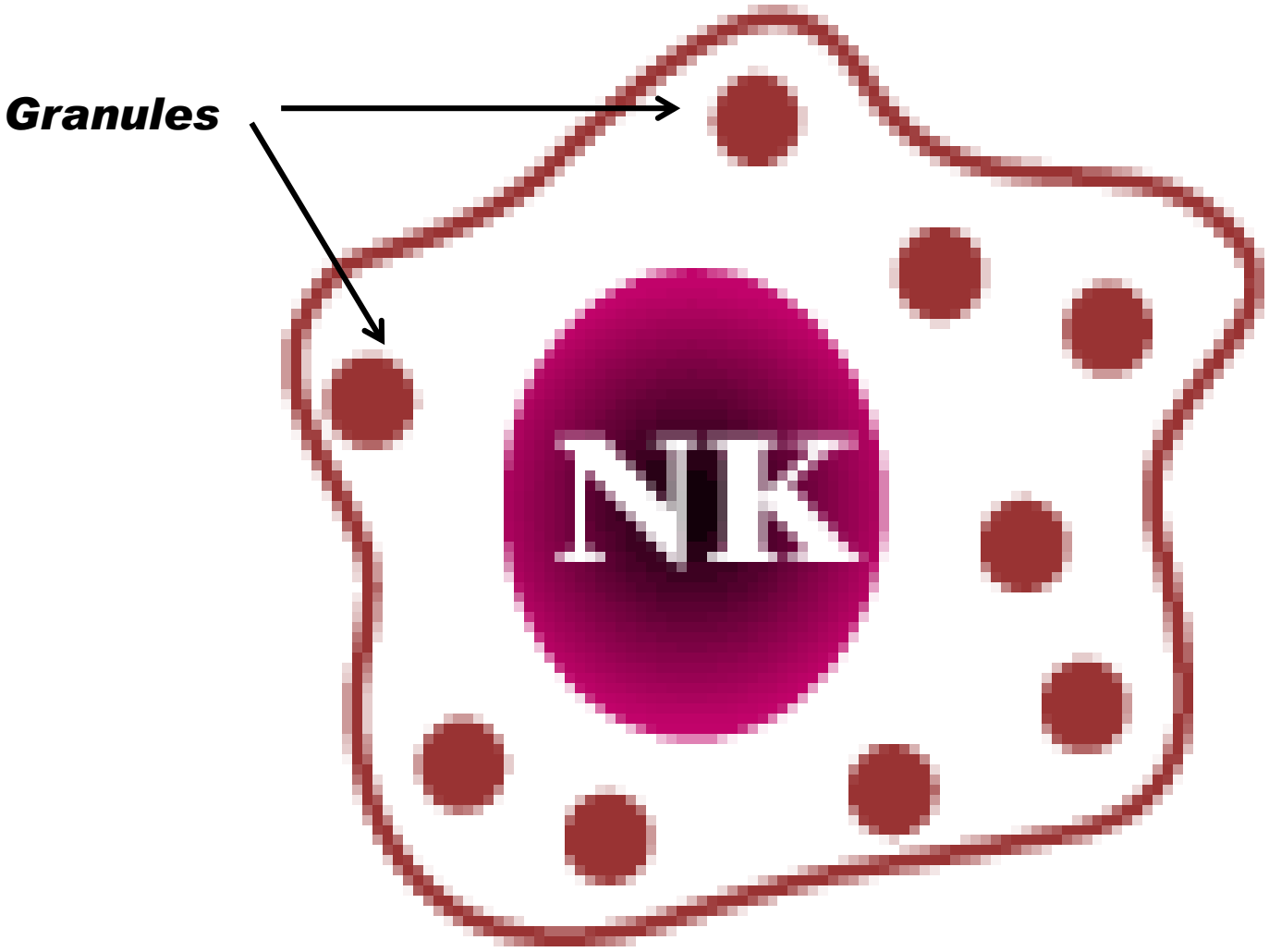
1. Immune tissues



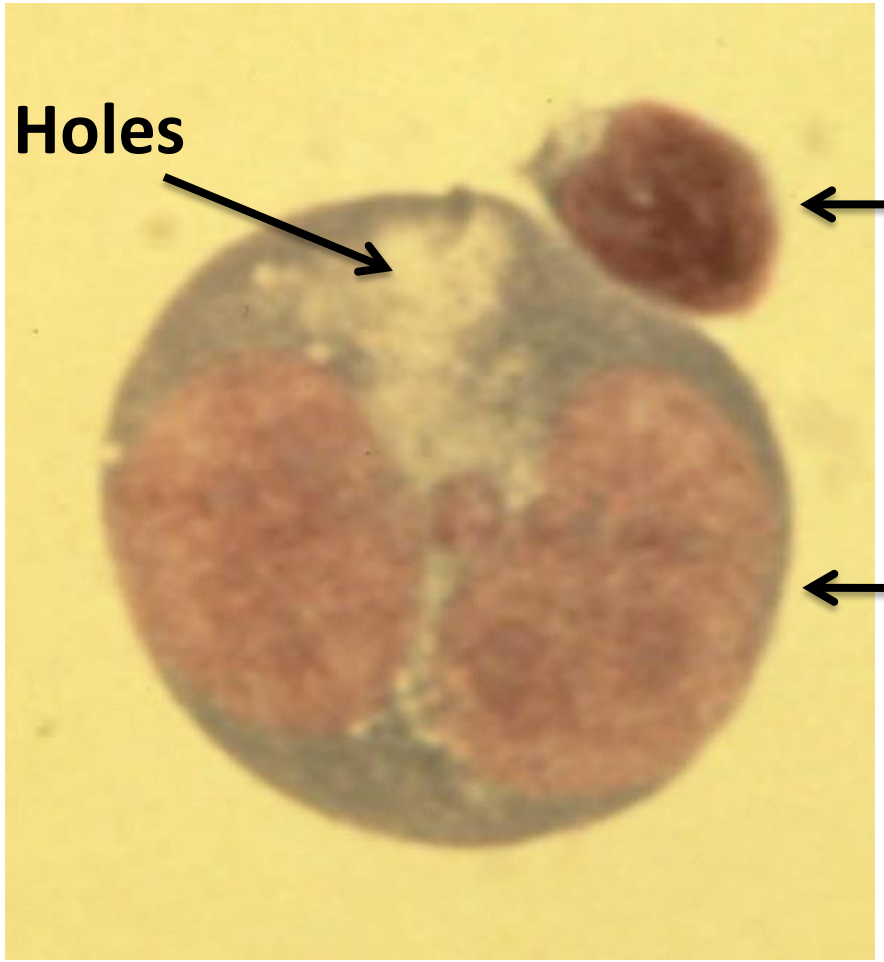
2. Immune cells



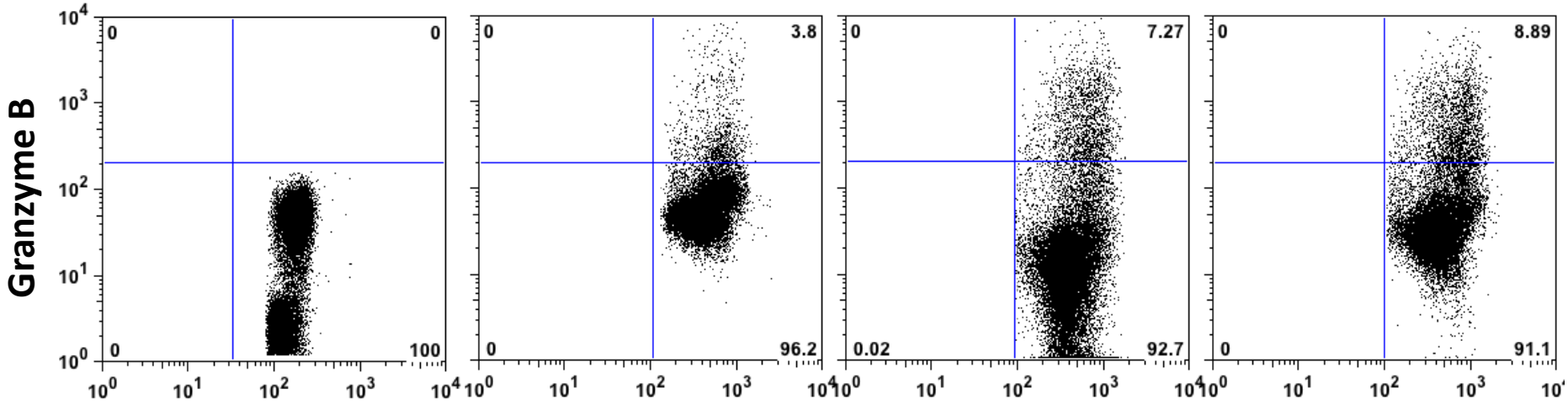
Natural Killer (NK) Cells



NK Cell binds to cancer cell, injects granules that induce holes and ultimately kill cancer cell



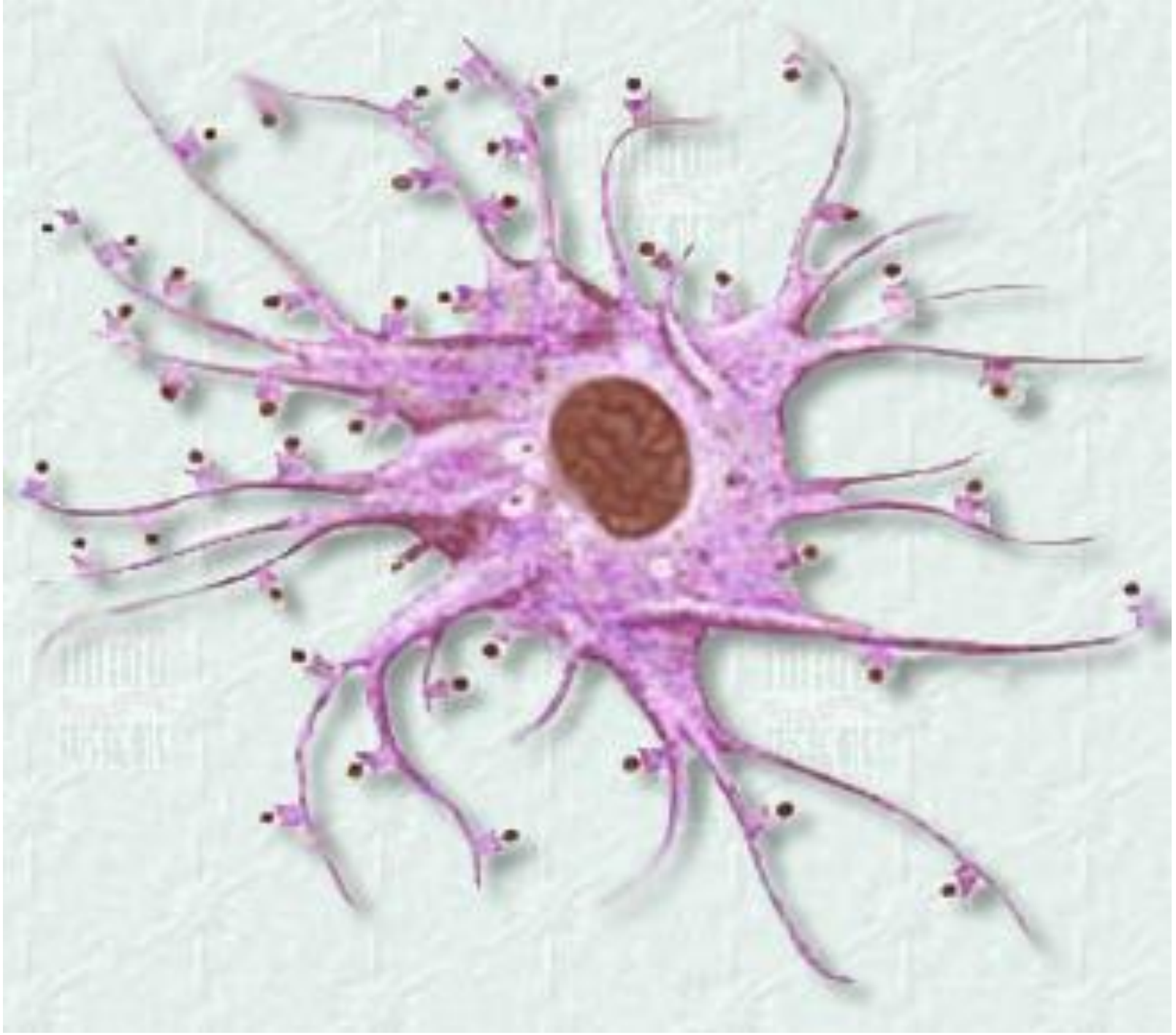
PFT Induces Granzyme-B in CD8^{+}T cells



PFT stimulated Pan-DCs prime activate CD8^{+}T cells. DCs were stimulated PFT (50 and 100 μg/ml) for 24 h and then cultured with CD8^{+}T cells for 7 days. CD8^{+}T cells were stained with Granzyme-B. One representative experiment is shown from 3 individual experiments.

Dendritic Cell (DC)

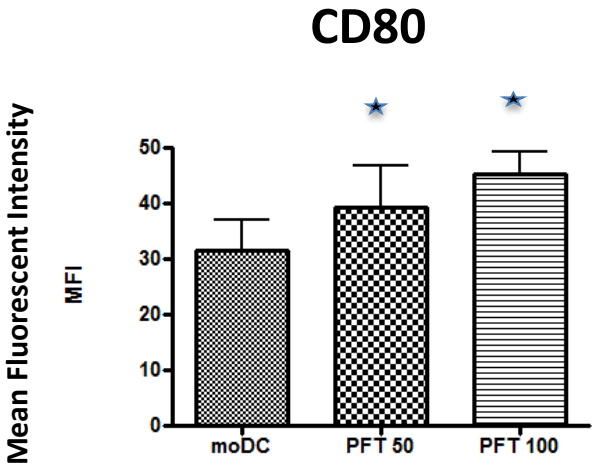
(The MOST Efficient Antigen Presenting Cells)

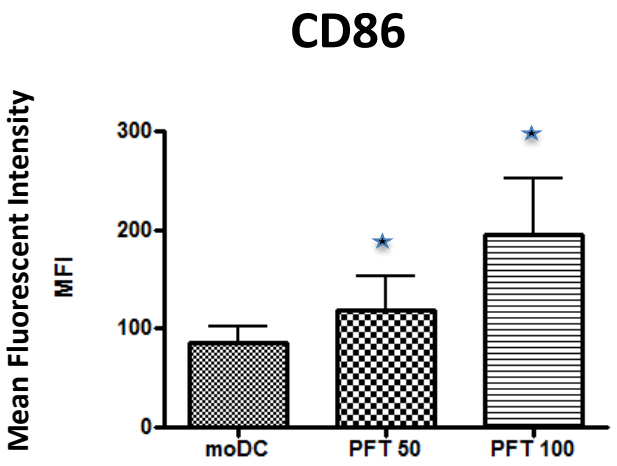


PFT Effect on DC:

A. **Induces maturation of DCs**
B. **Production of Cytokines**

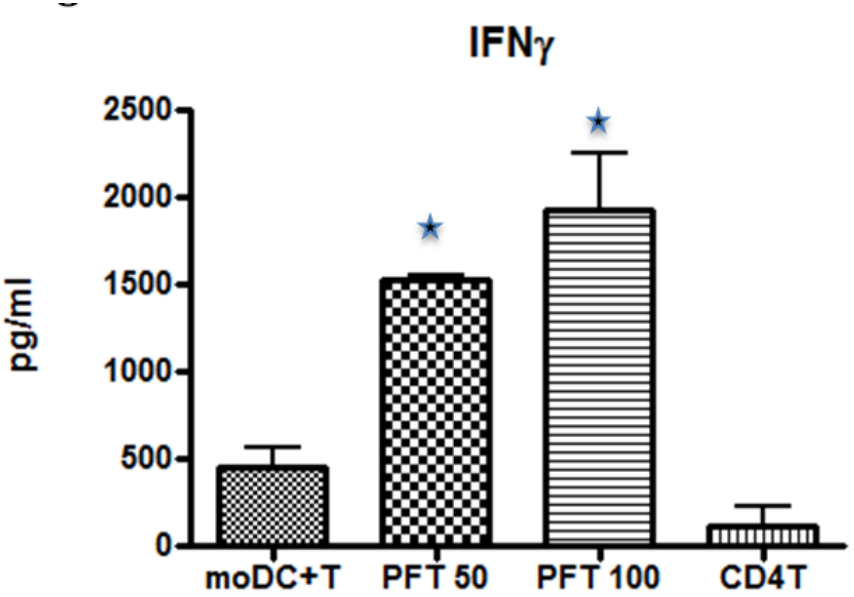
PFT Increases Expression of Co-Stimulatory and Maturation Markers CD80, CD86, and HLADR





Monocyte-derived DCs were treated for 24 h with PFT (50 and 100 μg/ml). Isotype antibody was used as a negative control. Expression of cell surface markers was determined by flow cytometry. A) One representative cytofluorograph is shown from 4 individual experiments. B) The density of mean florescent intensity (MFI) of CD80, CD86 and HLA DR in DCs in the absence or presence of PFT. Data represents the mean +/- SE of 4 experiments (★$p<0.01$ as compared to DCs alone).

PFT Stimulated CD4+T Cells to Secrete Interferon-gamma



PFT stimulated DCs prime regulatory type $CD4^+$T cells and secrete IFN-γ, IL-10, and TNF-α. The data are the mean ± SD from 5 individual experiments; $p<0.05$ as compared to DCs-$CD4^+$T cells alone.

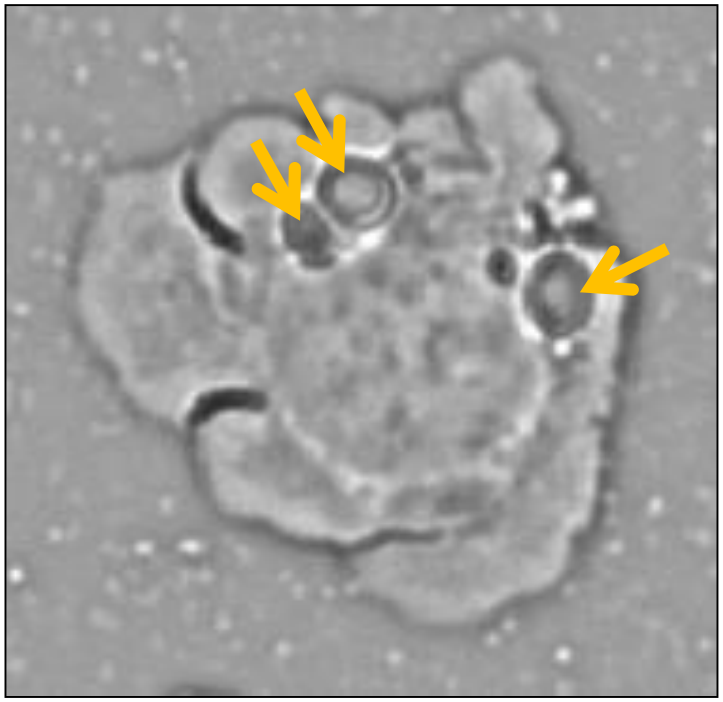
International Journal of Immunopathology and Pharmacology

Ghoneum M, Felo N, Agrawal S, Agrawal A.

A novel kefir product (PFT) activates dendritic cells to induce CD4+T and CD8+T cell responses in vitro.

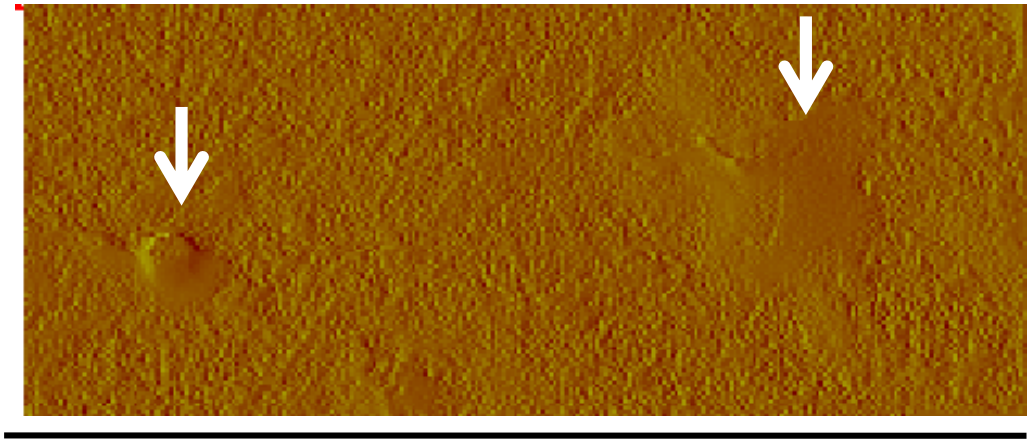
Int J Immunopathol Pharmacol. 28:488-96 (2015)

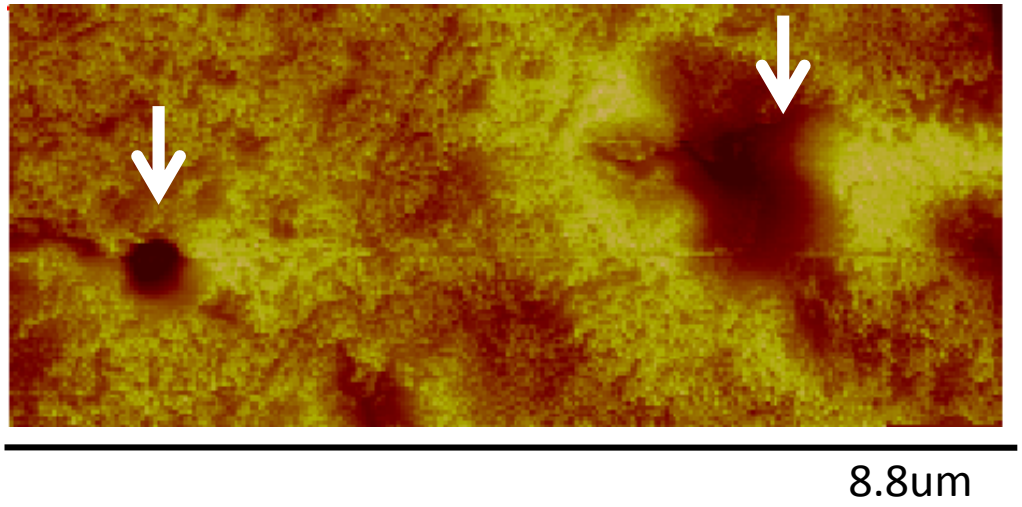
PFT Induces Holes in HL60/AR (MDR) Cancer Cells



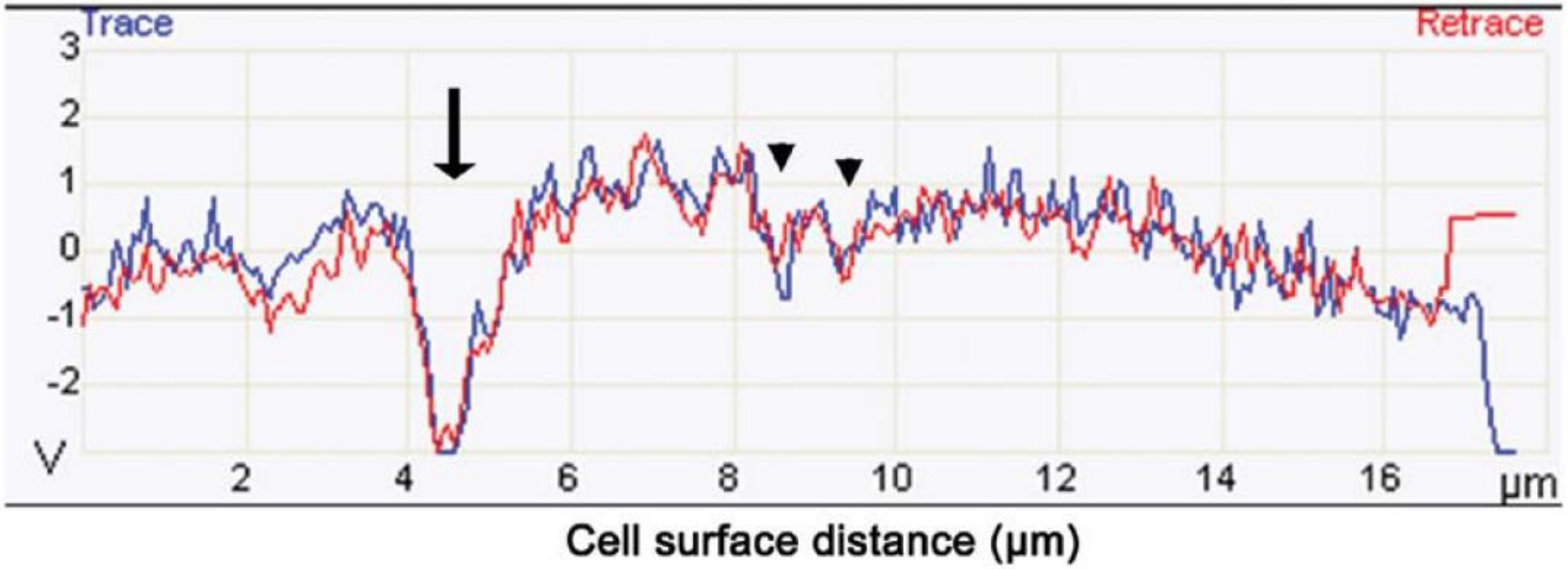
PFT Induces Holes in MDR Cancer Cells (Peak Force Imaging)

Imaging was done with atomic force microscope (AFM)
CNSI Facility at UCLA





Hole Characteristics: Depth (in μm) and Number



Determining the depth of PFT-induced hole formation. The red and blue lines indicate the surface contour of an HL60/AR cell treated with PFT. The arrow indicates a large hole detected by the SNL tip and arrowheads indicate smaller holes. This image is representative of many HL60/AR cells during PFT treatment.

INTERNATIONAL JOURNAL OF ONCOLOGY
SPANDIDOS PUBLICATIONS

MAMDOOH GHONEUM and JAMES GIMZEWSKI

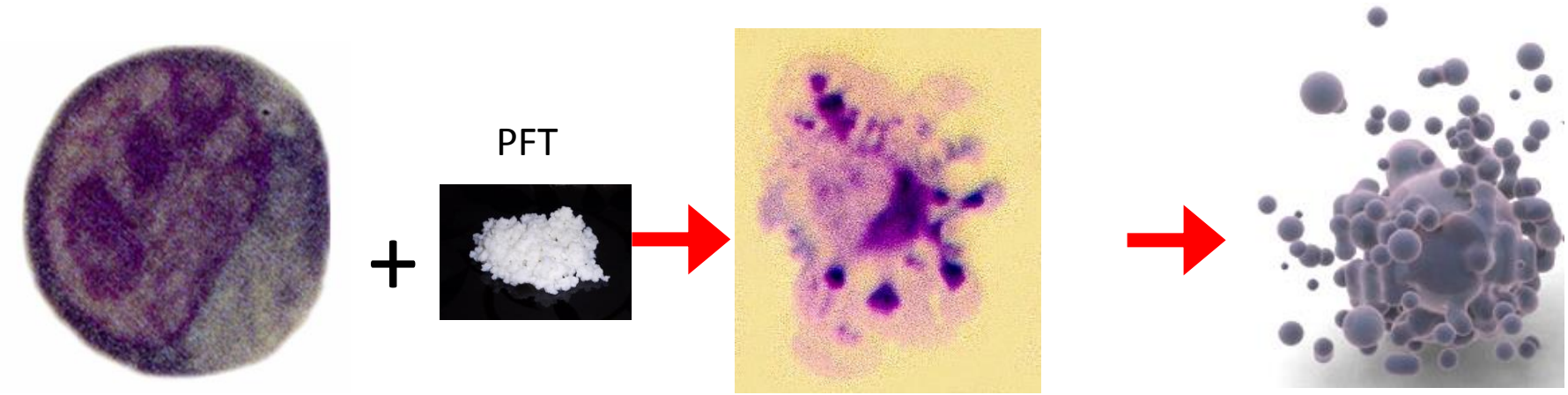
Apoptotic effect of a novel kefir product, PFT, on multidrug-resistant myeloid leukemia cells via a hole-piercing mechanism.

Int J Oncol. 2014 Mar; 44(3): 830–837

2.

PFT AS AN APOPTOTIC AGENT (PROGRAM CELL DEATH)

Apoptosis



Viable cancer cell

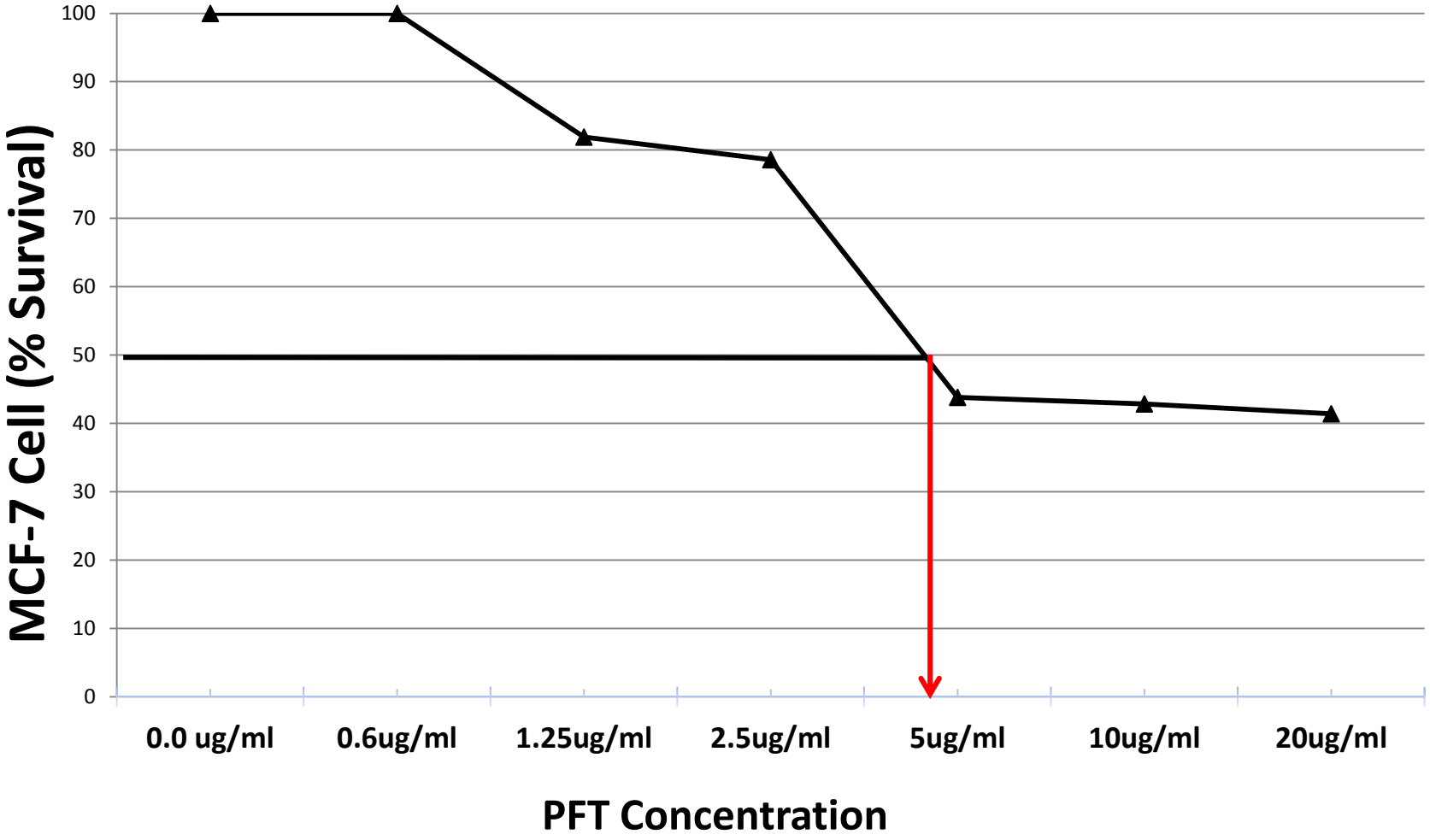
Apoptosis

PFT can kill different cancer cell lines

Cancer cell lines include:

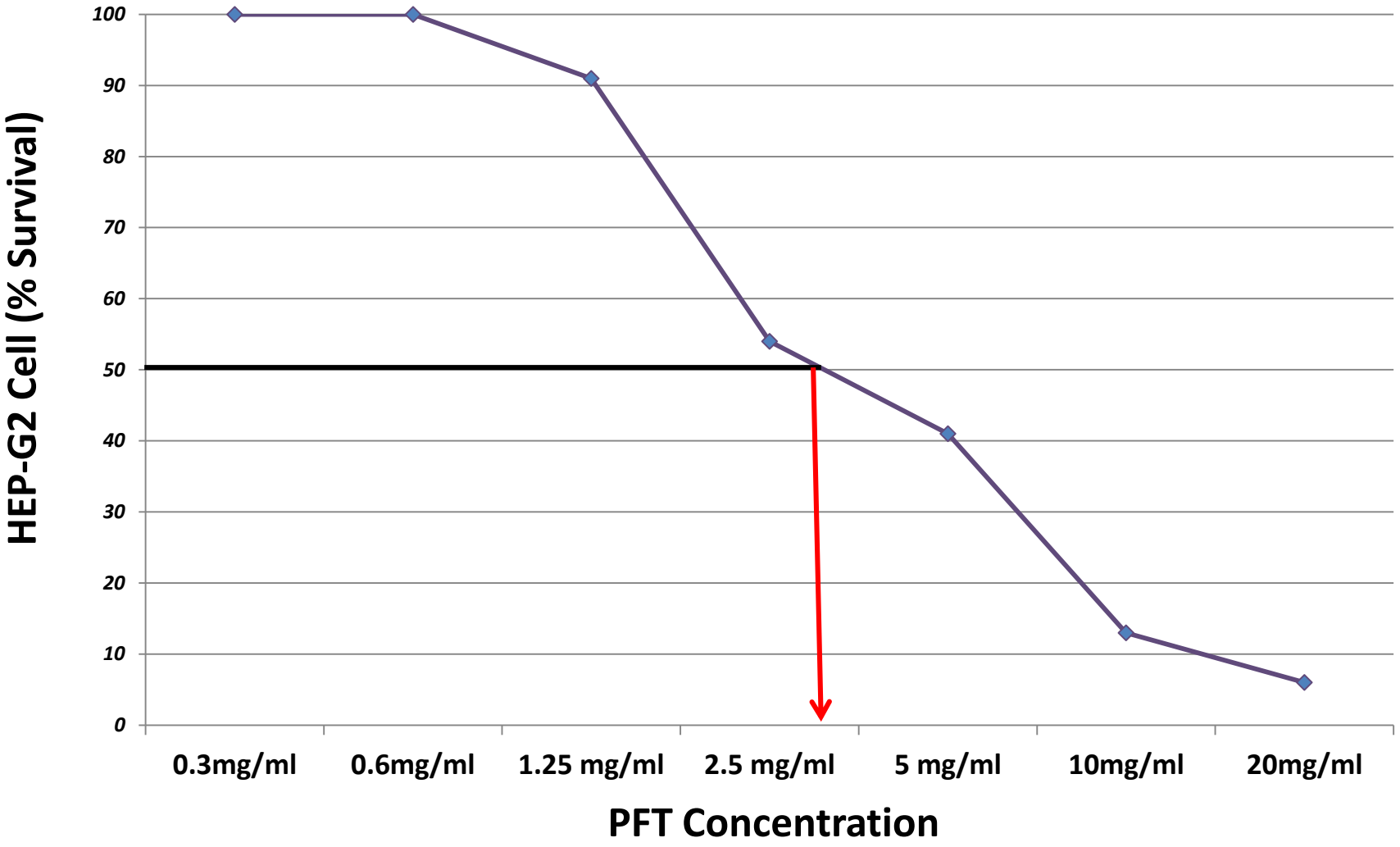
- Human breast cancer,
- Human prostate cancer,
- Human stomach cancer,
- Human liver cancer,
- multi-drug resistance (NDR) cancer cells
- Mice EAC cancer cells

PFT Suppresses the Growth of Human Breast Cancer MCF-7



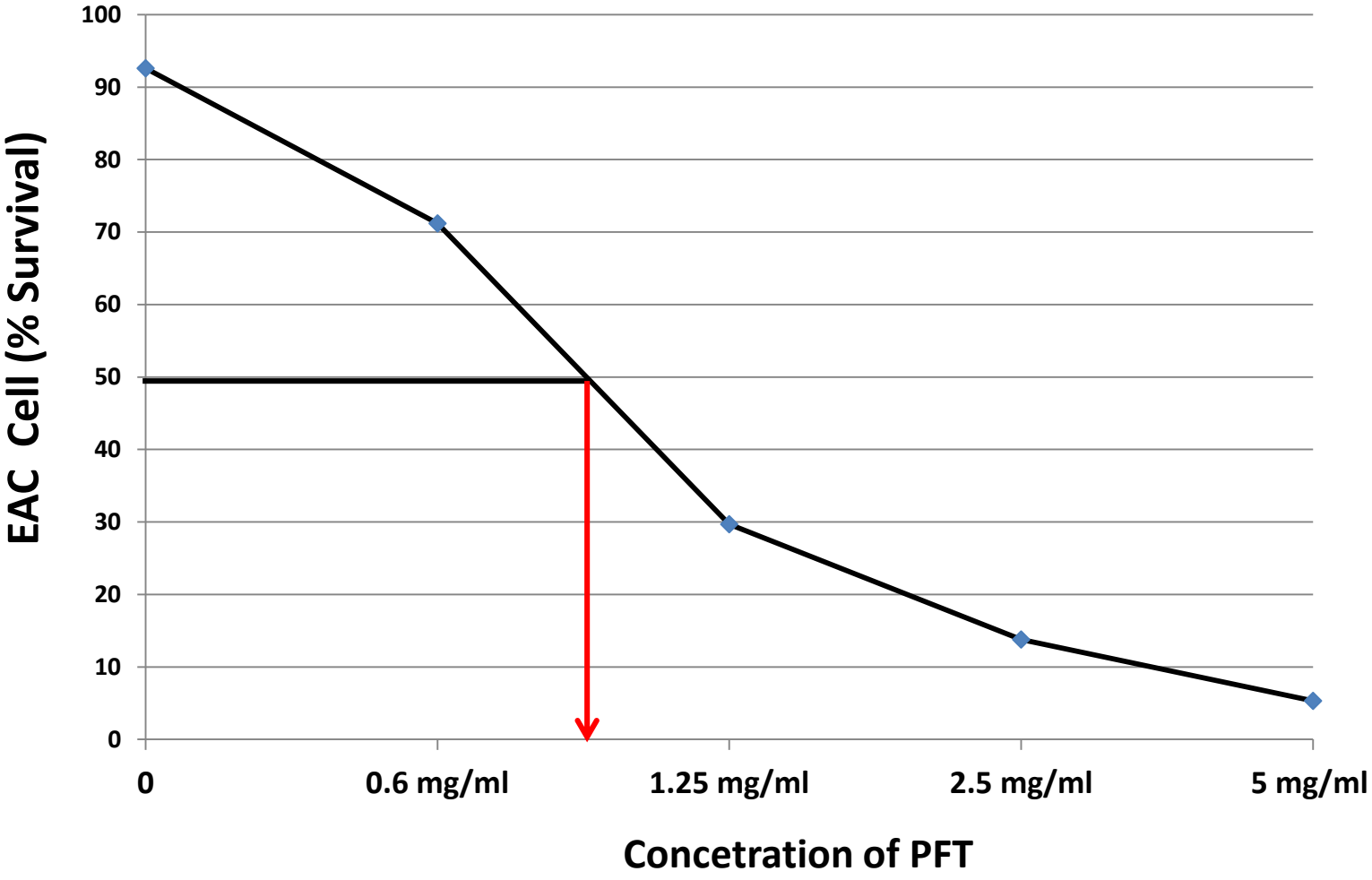
24hrs, MTT Assay

PFT Suppresses the Growth of Human Hepatocellular Carcinoma (HEP-G2)



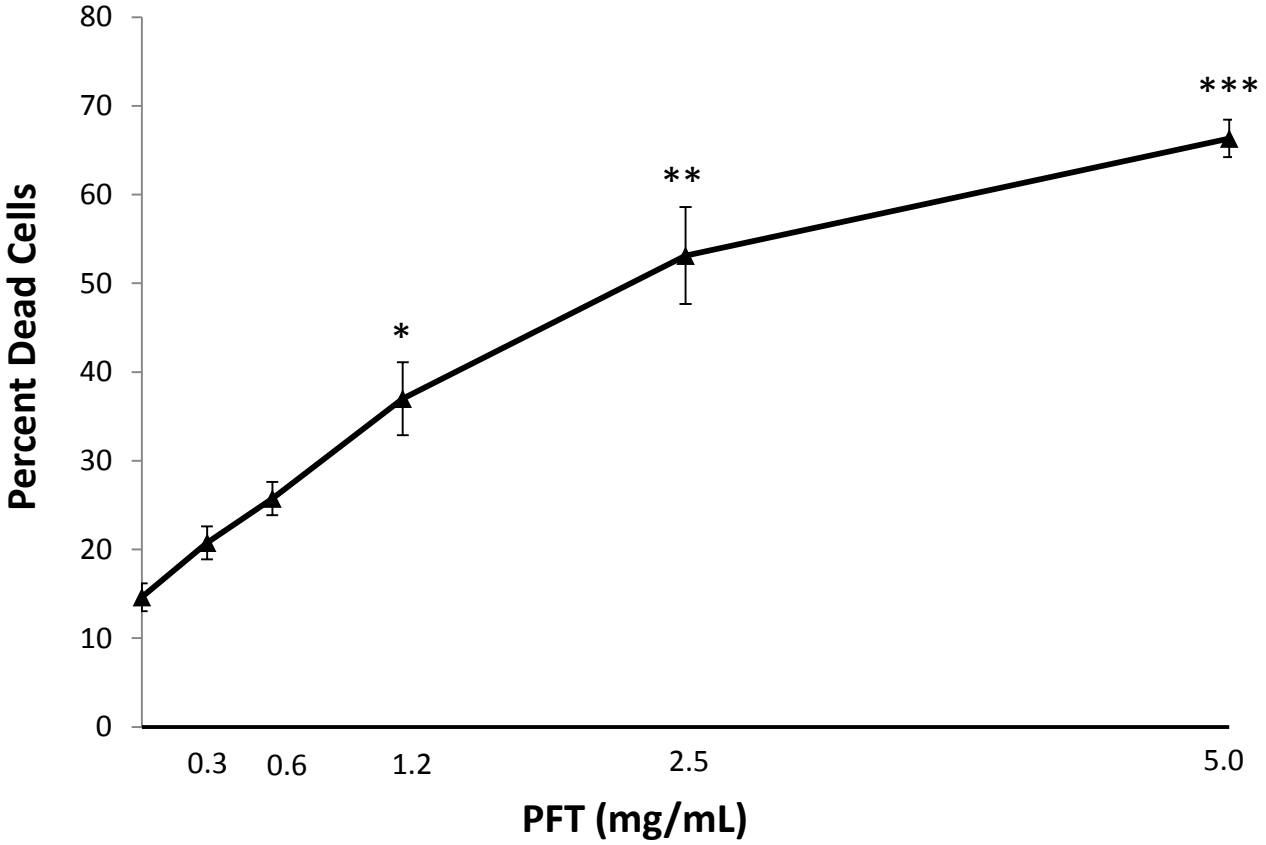
24hrs, MTT Assay

PFT Suppresses the Growth of Erlich's Ascites Carcinoma (EAC)



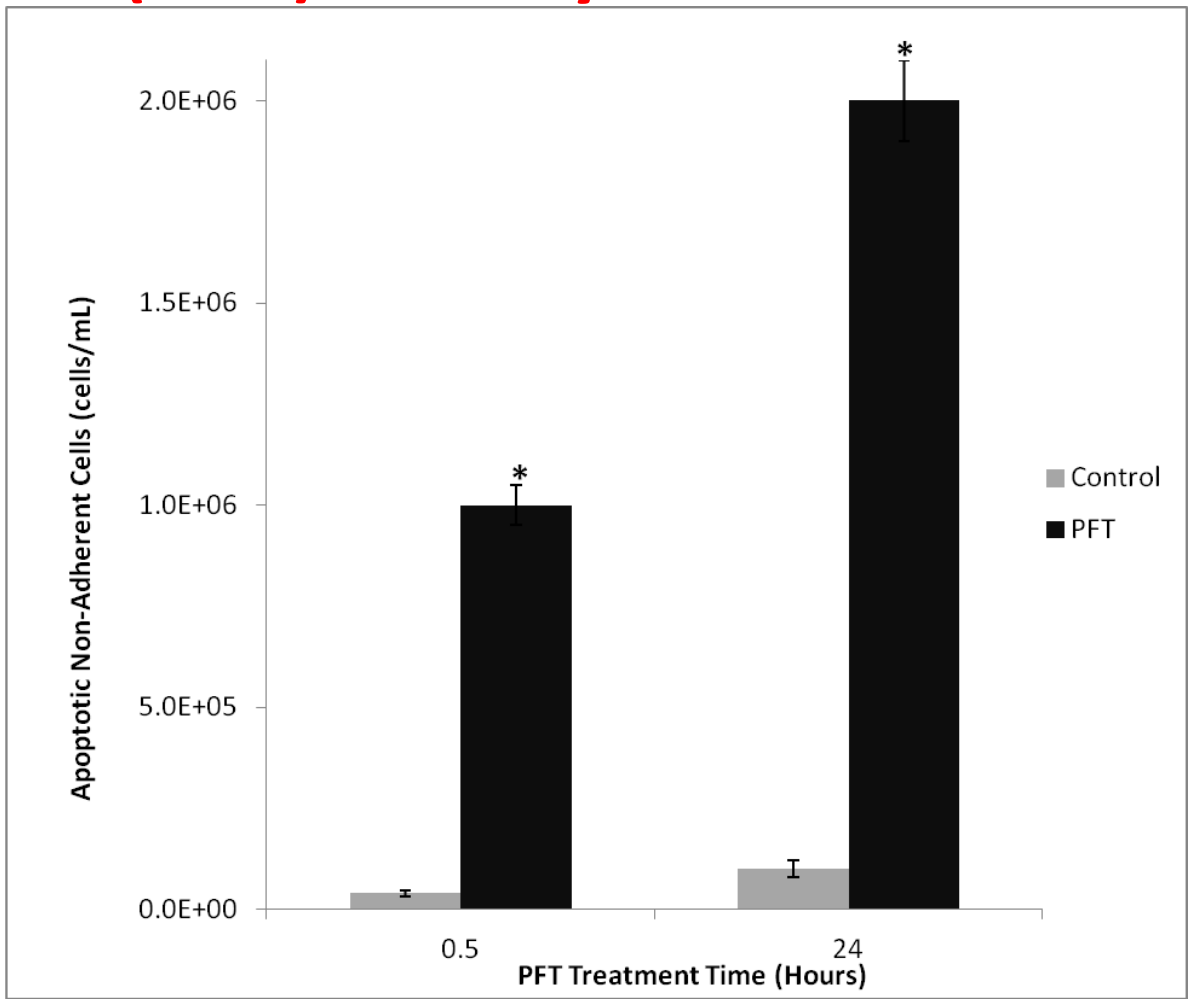
24hrs, MTT Assay

PFT Suppresses the Growth of Human Gastric Cancer (AGS)



Effect of PFT on the percentage of apoptotic cancer cells by flow cytometry. AGS ($1x10^5$) were cultured with PFT at concentrations of 0-5 mg/ml for 3 days. Cell death was determined by flow cytometry using 7AAD dye. Data represents the mean +/- SE of 4 experiments at each concentration. *p<0.05, **p<0.001, ***p<0.0001.

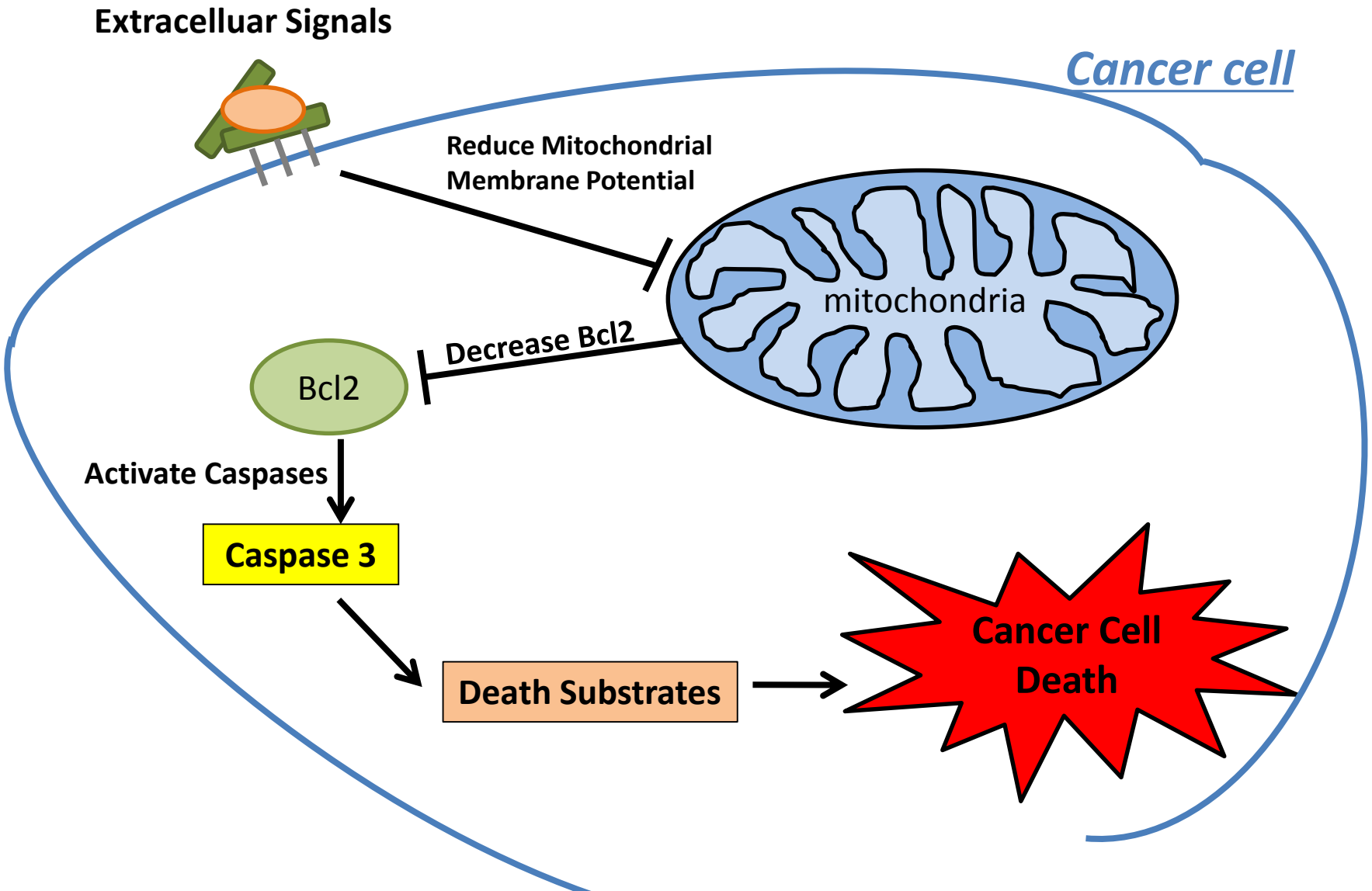
PFT Effect Against Human Gastric Cancer (AGS) as Early as 30 Minutes



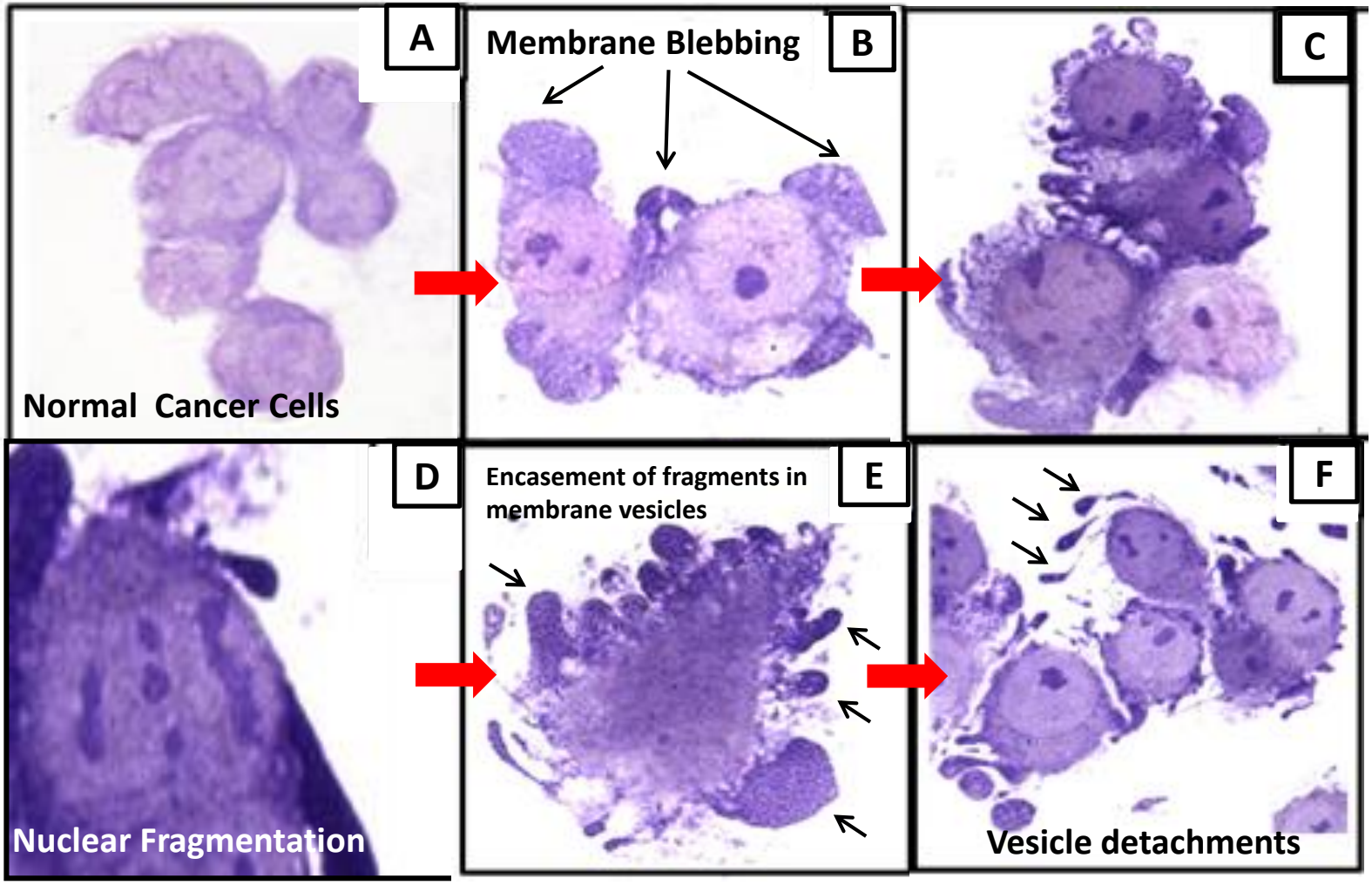
Number of apoptotic non-adherent AGS cells post-culture with PFT (5.0 mg/ml). Tumor cells were cultured in the absence (grey) or presence of PFT (black). The number of non-adherent apoptotic tumor cells was determined at 0.5 and 24 hours using a hemocytometer. Data represents the mean +/- SD of 3 experiments. *$p < 0.001$ as compared to control untreated cells.

II- *Induction of Apoptosis*

Mechanism of Programmed Cell Death



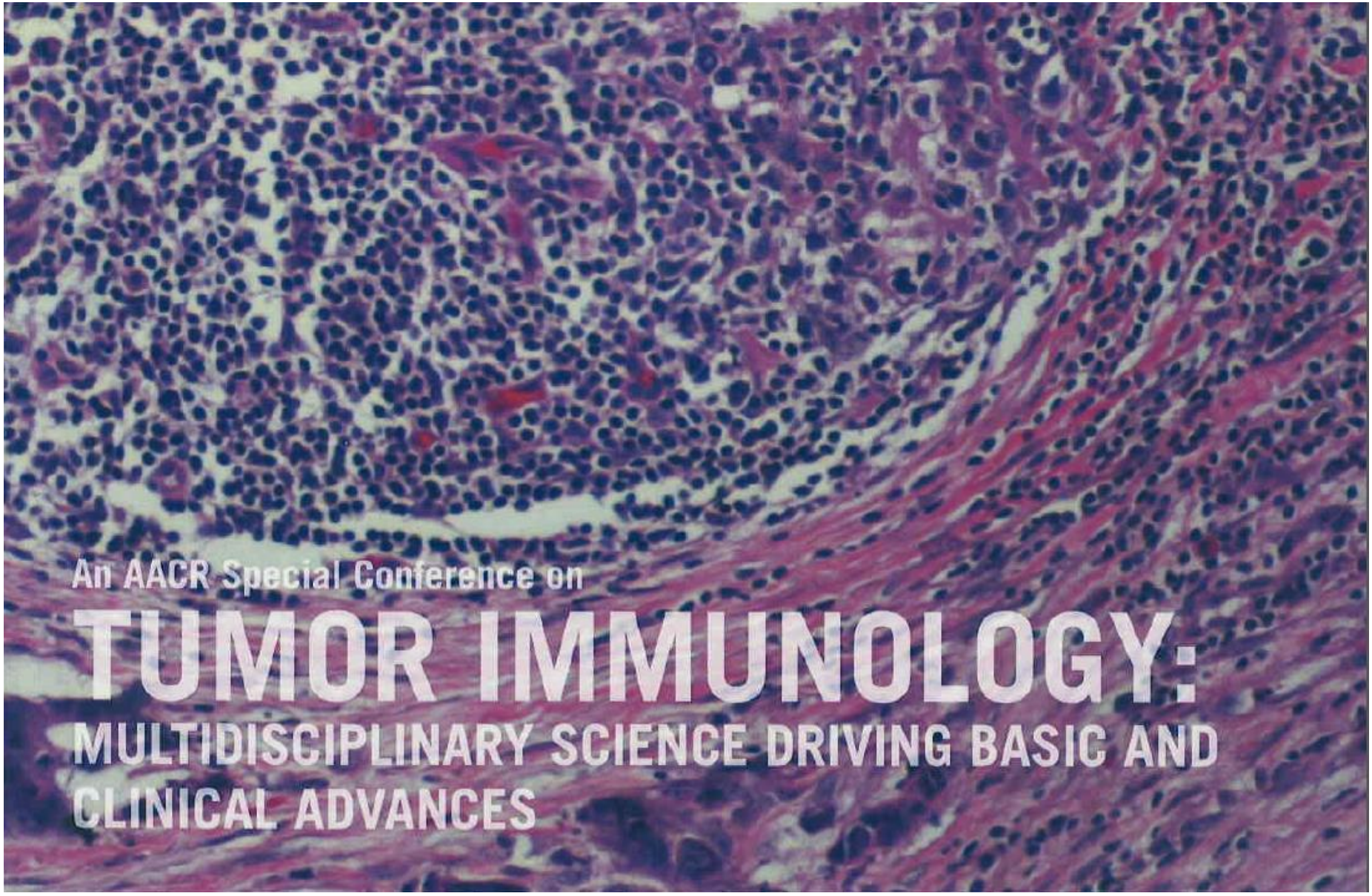
Gastric Cancer Cells (AGS) undergoes Apoptosis After Exposure with PFT



Cytospin preparation of adherent AGS cancer cells showing signs of apoptosis post treatment with PFT. Monolayer AGS cells grown on cover glass were cultured with PFT (5.0 mg/ml) for 24 hours and stained with Giemsa

American Association for Cancer Research-AACR

Miami-FL- December 2-5, 2012



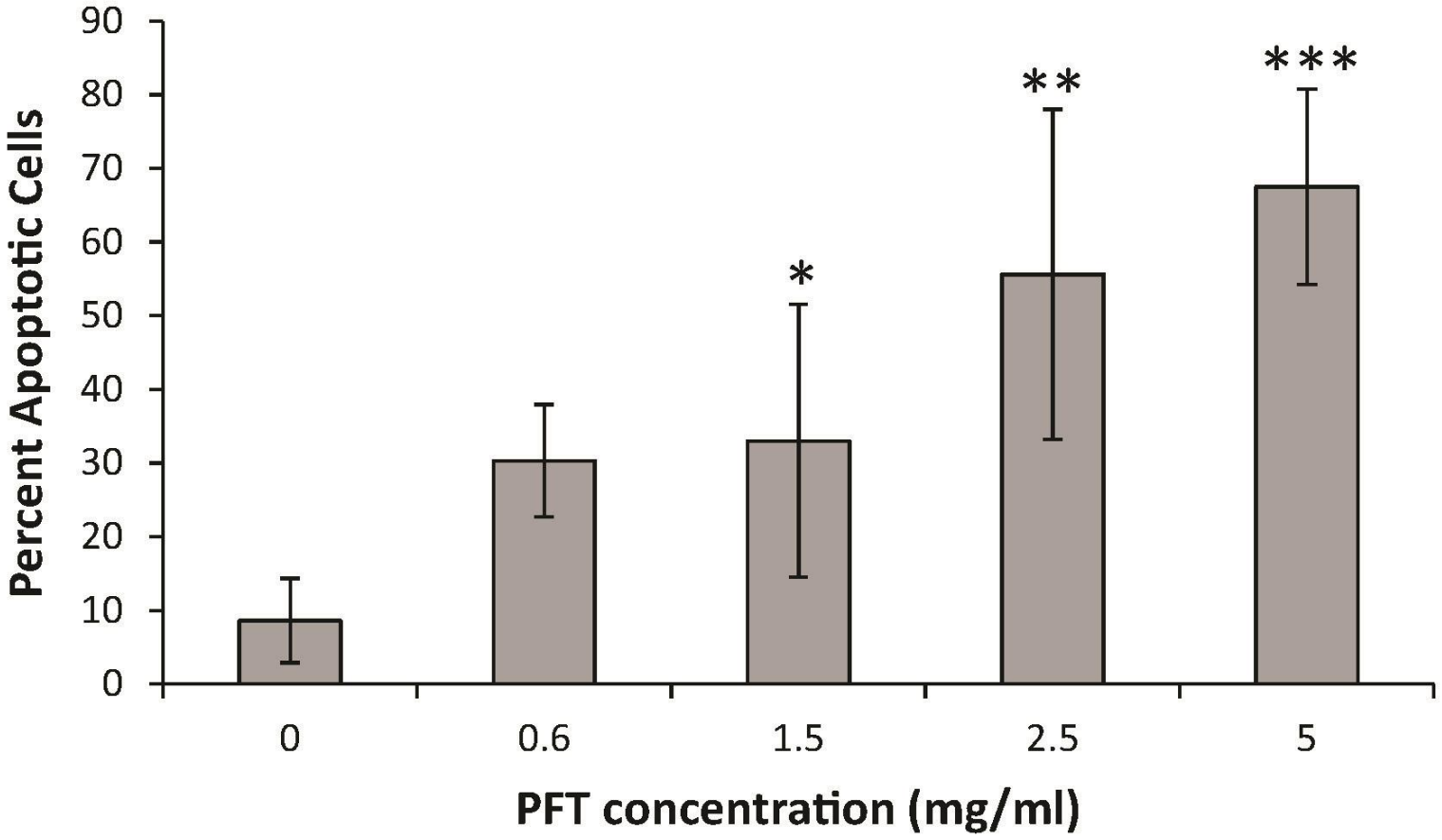
ONCOLOGY REPORTS
SPANDIDOS PUBLICATIONS

Authors: Mamdooh Ghoneum and Nouran Felo

Selective induction of apoptosis in human gastric cancer cells by *Lactobacillus kefiri* (PFT), a novel kefir product

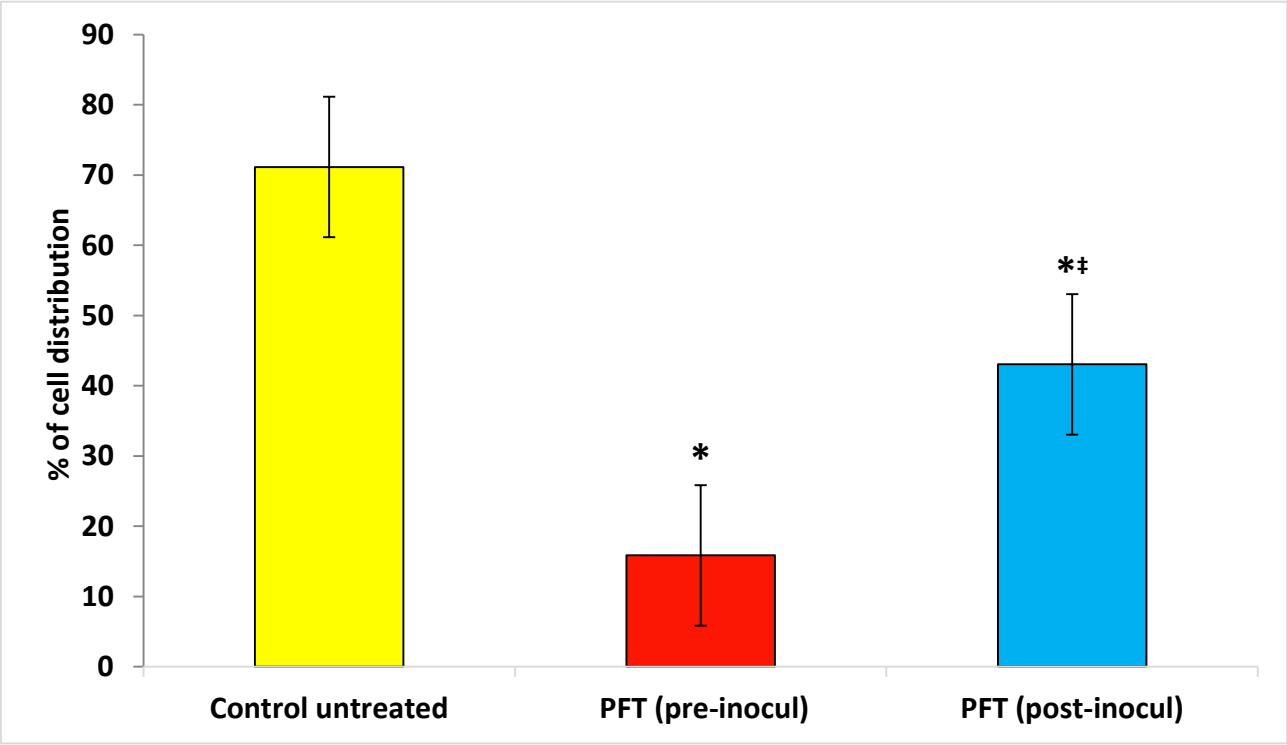
Oncol Rep. 2015 Oct;34(4):1659-66

PFT Induces Apoptosis Against Human Myeloid Leukemia HL60/AR Cancer Cells (MDR)



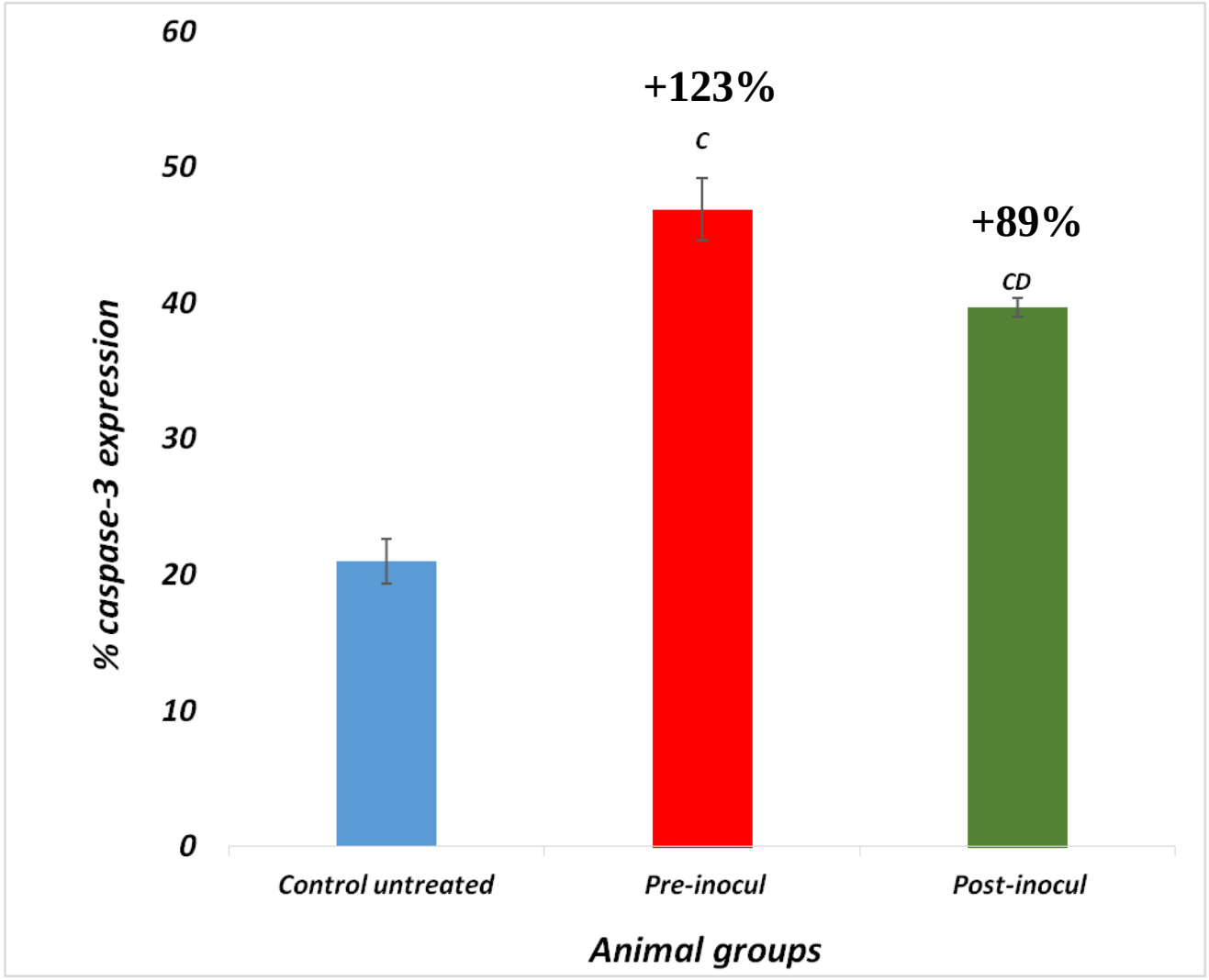
*p< 0.05, **p<0.0005, ***p<0.0001

1- Mitochondrial Membrane Potential (MMP)



Effect of PFT on MMP in tumor tissues. Each value represents the mean ± SE of 6 mice/group.
*Significantly different from inocul control group at p ≤0.01 level.
‡Significantly different from PFT pre-inoculated group at p ≤0.01 level.

2- Caspase-3 protein expression



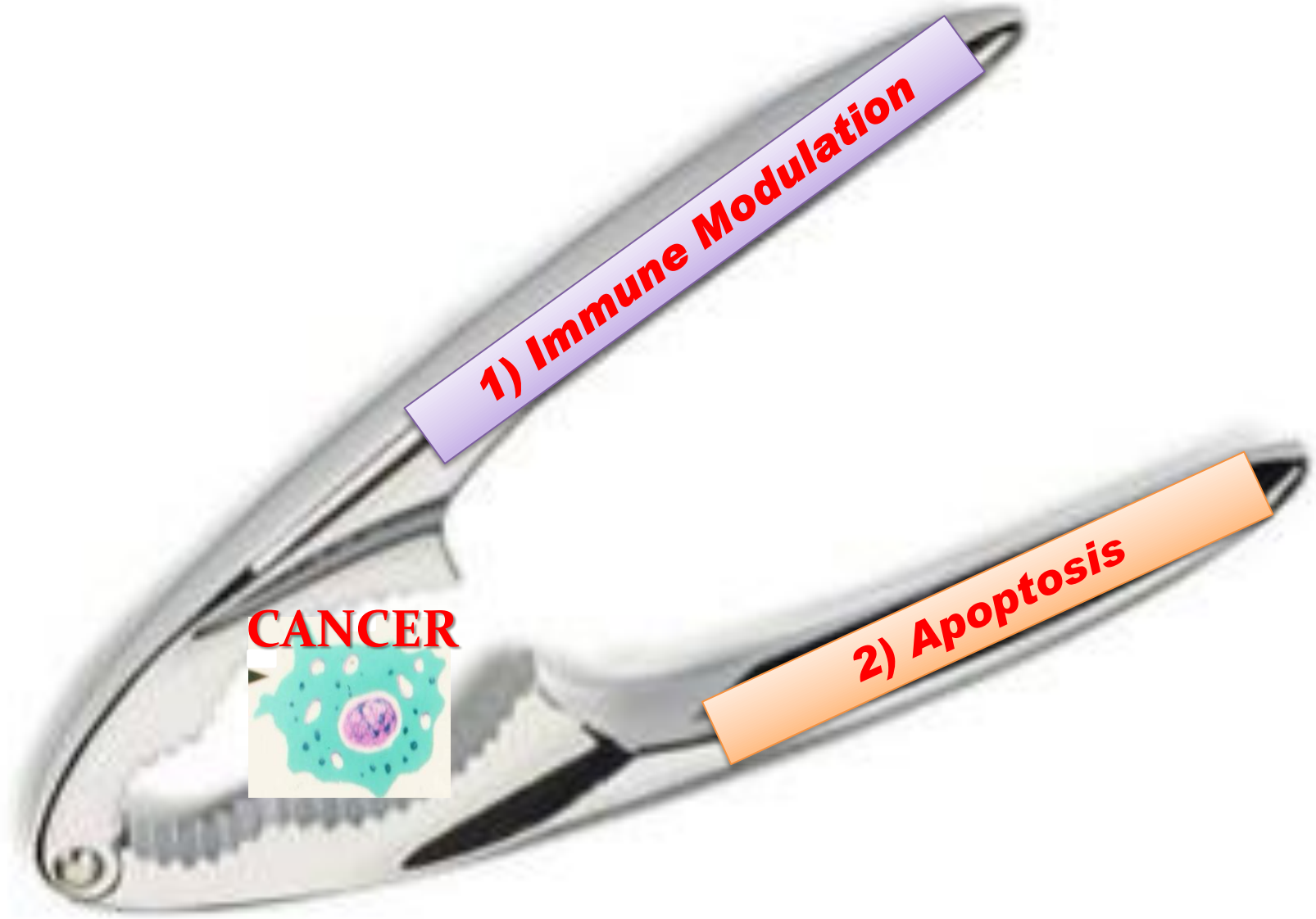
Each value represents the mean±SE of 6 mice/group.
C significantly different from the Control untreated group at p <0.01 level.
D Significantly different from Pre-inocul group at p<0.05.

Chemopreventive potential of *Lactobacillus kefiri* P-IF, a novel kefir product, on Ehrlich ascites carcinoma cells

AACR American Association for Cancer Research ANNUAL MEETING 2017 WASHINGTON, DC

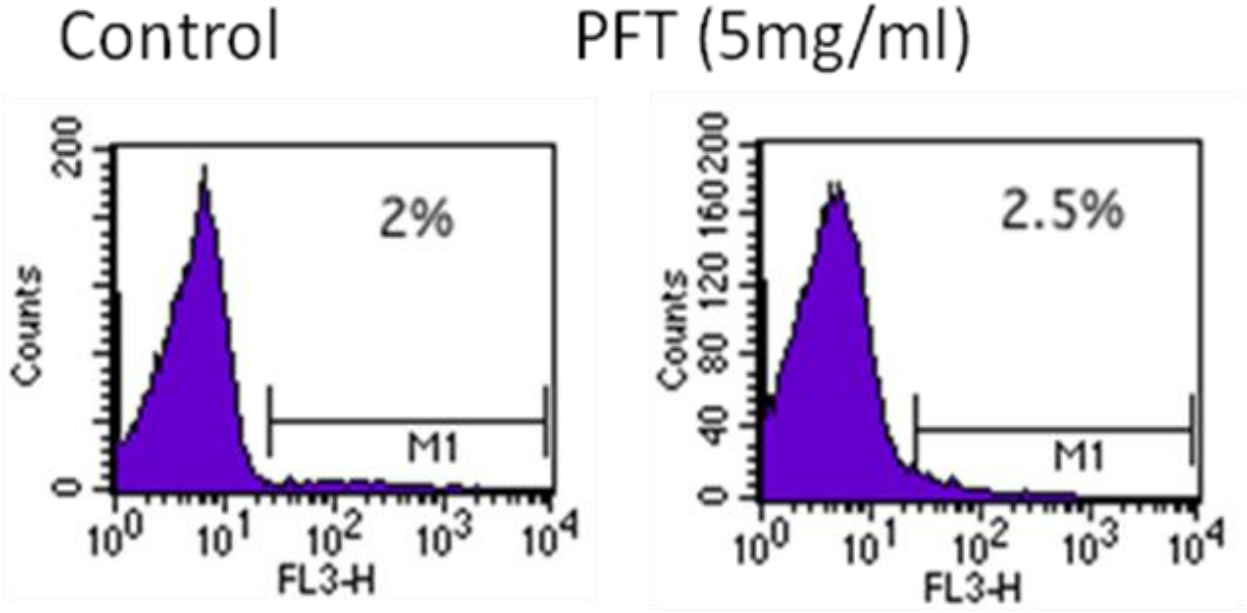
RESEARCH PROPELLING CANCER PREVENTION AND CURES

Mechanisms by which PFT kills cancer cell



PART III:
Is PFT safe?

PFT Does Not Induce Apoptosis on Human Peripheral Blood Mononuclear Cells (PBMC)



Effect of PFT on the apoptosis of PBMC (Peripheral Blood Mononuclear Cells). PBMC ($1x10^6$ cell/ml) were cultured in the absence or presence of PFT (5.0 mg/ml) for 3 days. Apoptotic cells were determined by PI technique using FACS Calibur flow cytometer.

Toxicity in Mice Studies

PFT has been shown to be a non-toxic agent.

Results of mice treated with PFT showed no macroscopic or histopathological abnormalities were detected in different organs post treatment.

AH21研究結果

マウスによる乳酸菌並行複合発酵産生物質の生体影響および安全性に関する検討

Toshiyuki	Original Jikei University School of Medicine	Asari, Masao	Azabu University Veterinary Medicine
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Koyubi	Seitoku	Hisashi	Azabu University Veterinary Medicine
Takatoshi	Mitsubishi SMBC El	Murayama	
Suzuki		Satomi	
Shingon			

Conclusion

L. kefiri P-IF, a novel symbiotic microbe, may have Chemopreventive potential to reduce tumor incidence and tumor growth in mice.

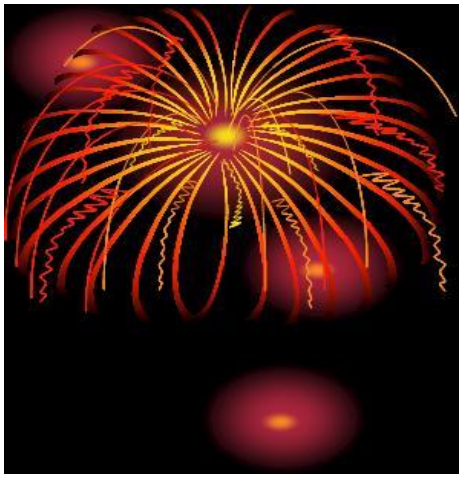
Mechanisms underlying its effect may include:

- Activating immune system
- inducing apoptosis in cancer cells

- Safe, nontoxic agent

L. kefiri P-IF was provided by Paitos Co., Ltd. Yokohama, Kanagawa, Japan.

Published Article in Peer Review Journal



Apoptotic effect of a novel kefir product, PFT, on multidrug-resistant myeloid leukemia cells via a hole-piercing mechanism.

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Publications Related to the Project

1. Ghoneum M, Gill G, Stein E, Salem F and Cooper E. Phagocytosis of large granular lymphocytes and other leukocytes by tumor cells. Abstracts in: Nat. Immunity and Cell Growth Regul. 4(5):249-250 (1985) presented at Int. Symp. Nat Immunity and biol response modification for th therapy of cancer and other diseases. Honolulu, Hawaii, Nov. 10-12, (directed by R. Herberman).*
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11. Ghoneum M, Golapudi S, Hamilton J, Brown J and Ninomiya K. Human Squamous Cell Carcinoma of the Tongue Exhibits Phagocytosis against Baker's Yeast. Annual Meeting of the American Academy of Otolaryngology-Head and Neck Surgery Foundation in New York, New York, September 19-22, 2004.*
12. Ghoneum M, Gollapudi S. Phagocytosis of candida albicans by metastatic and non-metastatic human breast cancer cell lines in vitro. Cancer Detec.& Preven. 28:17-26 (2004).
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14. Ghoneum M, Gollapudi S. Induction of Apoptosis in Breast Cancer Cells by Saccharomyces Cerevisiae, the Baker's Yeast, In Vitro. Anticancer Res. 24:1455-1464 (2004).
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Publications Related to the Project Continued

16. Ghoneum M, Gollapudi S. Modified arabinoxylan rice bran (MGN-3/Biobran) enhances yeast-induced apoptosis in human breast cancer cells in vitro. Anticancer Res. 25:859-870 (2005).
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