

MPOC Tocotrienol Webinar

2<sup>nd</sup> June 2021

# Palm Tocotrienols: **Immune-enhancing Potential**

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# Presentation outline

- Overview of the immune system
- Factors that affect immune system
- Vitamin E: **Tocotrienols**
- Evidence from research
  - ❖ Vaccination (clinical trial and mouse model)
  - ❖ Breast cancer (mouse model)





**TRF**

**Immunity: vaccine or invading pathogens**

**Pathogens/Vaccines**

**1. Prevent infections**

**2. Fight pathogens**

**3. Acquired immunity against pathogens/vaccine**

**Identify & destroy abnormal cells**

**Cancer cells, damaged cells, mutated cells**

**Immune System**

**Repair & Healing**

**Remove old cells (e.g. old RBC)**

**Remove dead cells (e.g. tissue debris)**

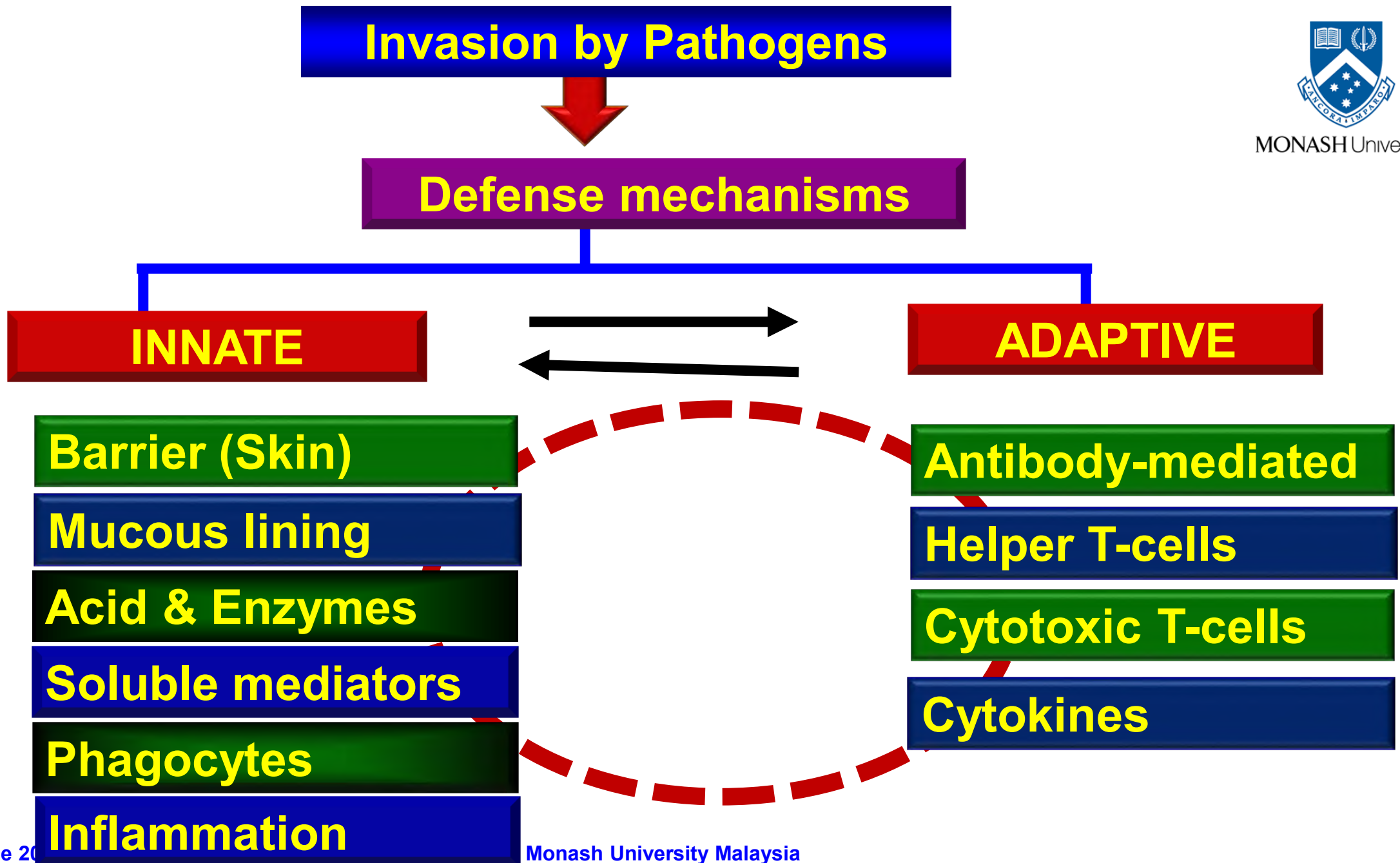
**Undesirable effects**

**Immunodeficiency**

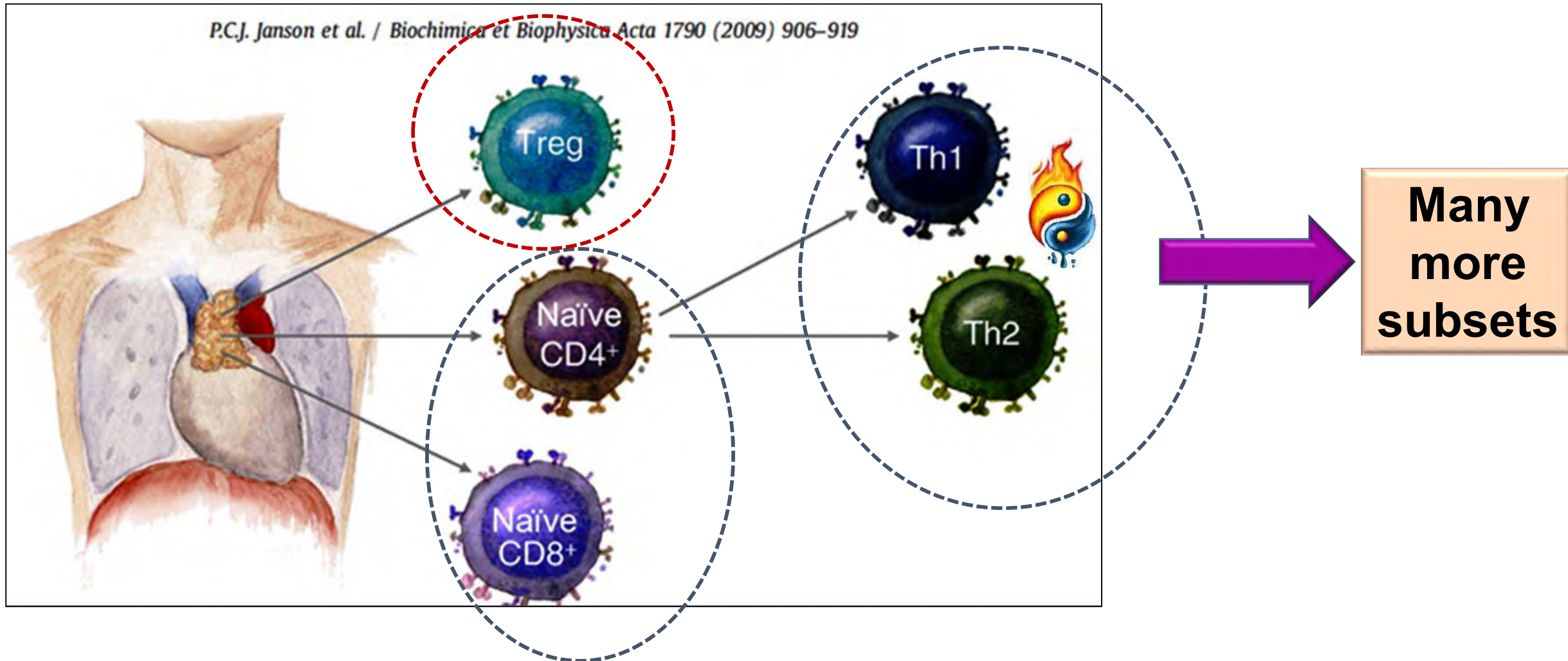
**Allergy**

**Autoimmune diseases**

**Rejection of transplants**



# Major T-cell Subsets

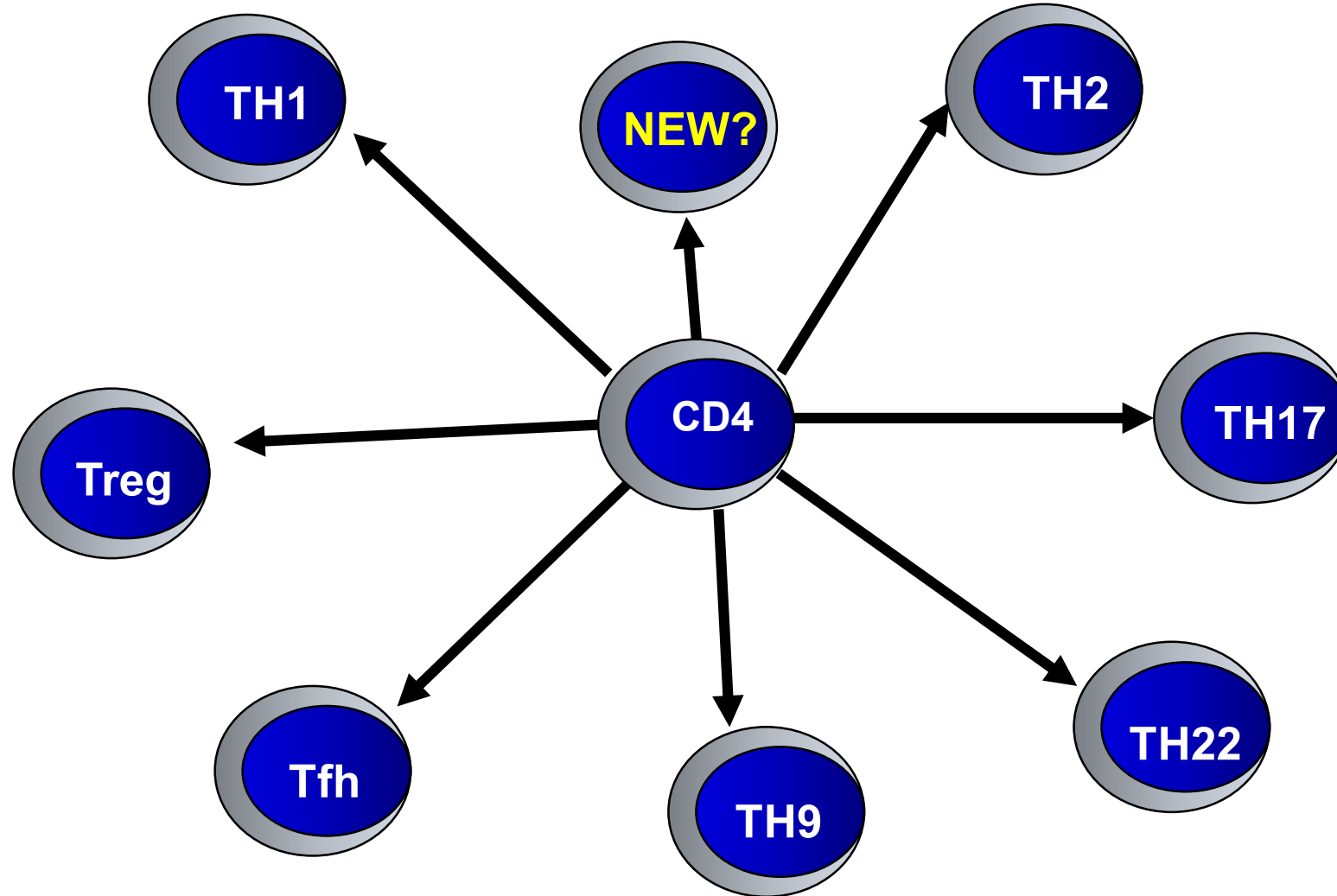


# Subsets of CD4<sup>+</sup> T-Cell:

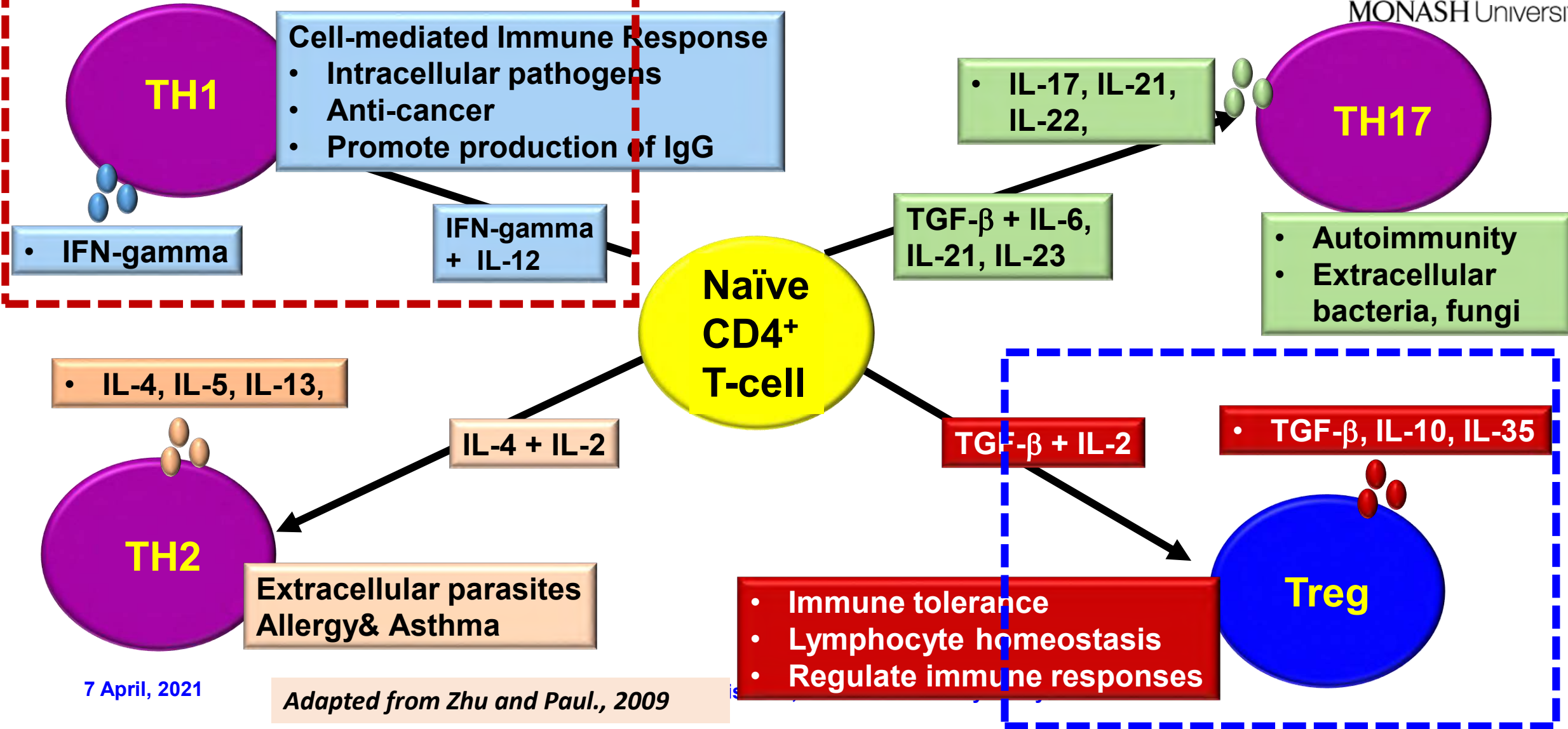
## Current



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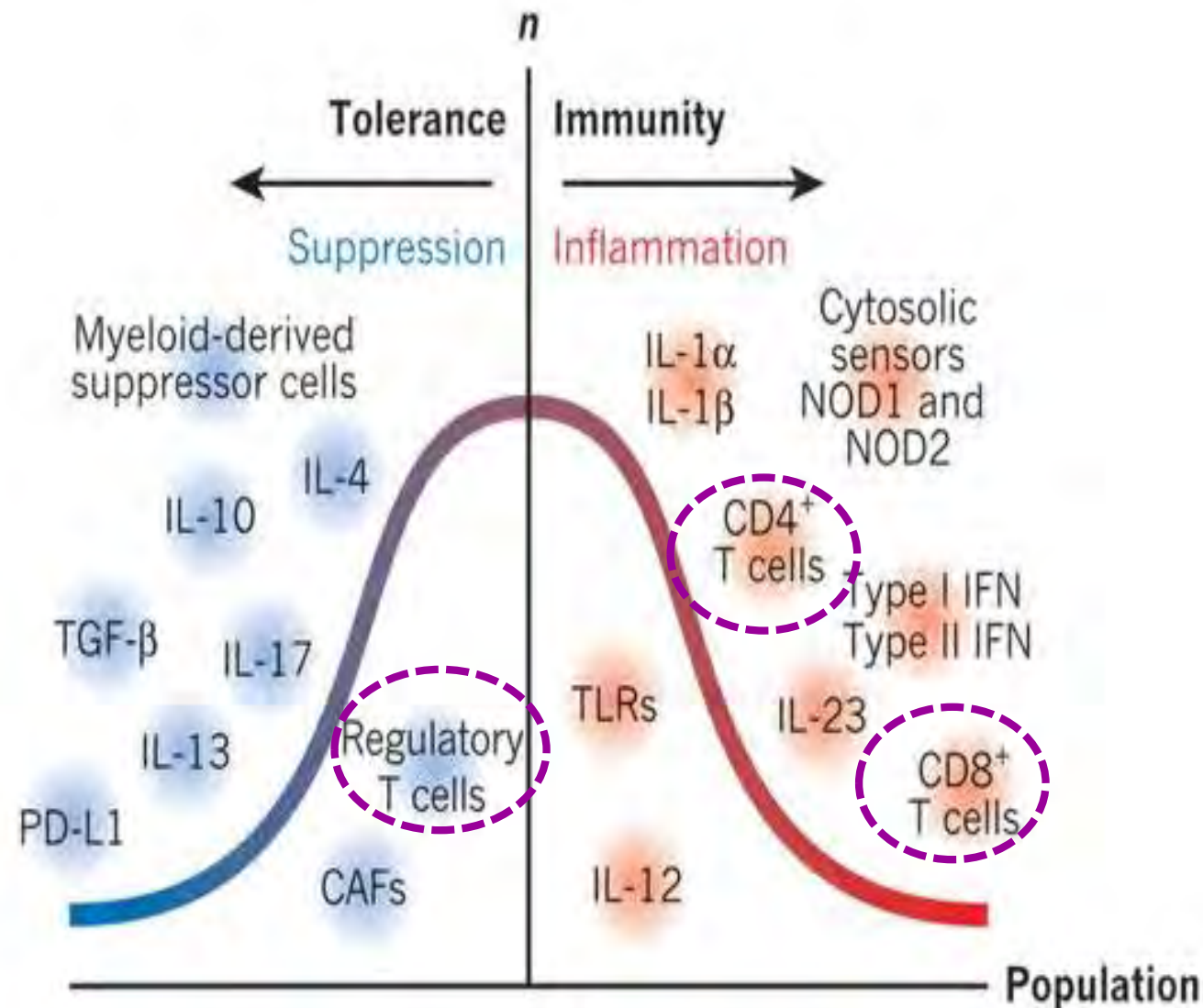
# Functions CD4<sup>+</sup> T-cell



# Tolerance or Immune Response?



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**Our ability to interact with  
the world and remain healthy  
largely depends on a healthy  
immune system**

**NUTRITION**

**MODERATE  
EXERCISE**

**STRESS**

**FACTORS THAT  
AFFECT HOST  
IMMUNE SYSTEM**

**OTHERS**  
(Drug-abuse; substance-  
abuse; Smoking)

**SLEEP**

# Nutrition and the Immune System



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- **Medical science has established**
  - ❖ Good **NUTRITION** → one of the **most important** factors that support a healthy & balanced immune system
- **Immune function decreases as we age**
  - ❖ Literature suggests that maintaining healthy nutrition may retard or stop this decline

# Nutrition and the Immune System



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- **Poor nutrition: → Dysfunctional immune system**
- **↑ Susceptibility to**
  - ❖ **↑ Infections**
  - ❖ **↑ Cancer**
  - ❖ **Slow healing from injury and infections**

# Nutrition and the Immune System

## MACRONUTRIENTS

- ❖ CARBOHYDRATES
- ❖ PROTEINS
- ❖ LIPIDS
- ❖ FIBRES\*\*\*

## MICRONUTRIENTS

- ❖ VITAMIN A
- ❖ VITAMIN C
- ❖ VITAMIN D
- ❖ VITAMIN E\*\*\*
- ❖ VITAMIN K

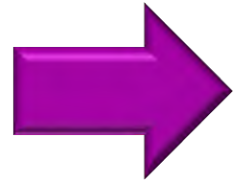
## SUPPLEMENTS

- COLOSTRUM
- PROBIOTICS
- ANTIOXIDANTS

## BIOACTIVE AGENTS

- CURCUMIN
- GENISTEIN
- APIGENIN
- RESVERATROL
- EPIGALLOCATECHIN-3-GALLATE
- SULPHORAPHANE

**NATURAL  
VITAMIN E**



**TOCOTRIENOL-RICH FRACTION (TRF)**

**Tocopherols**

**Tocotrienols**



- **80% Tocotrienols**
  - **22% alpha-tocotrienol**
  - **12% delta-tocotrienol**
  - **46% gamma-tocotrienol**
- **20% alpha-tocopherol**

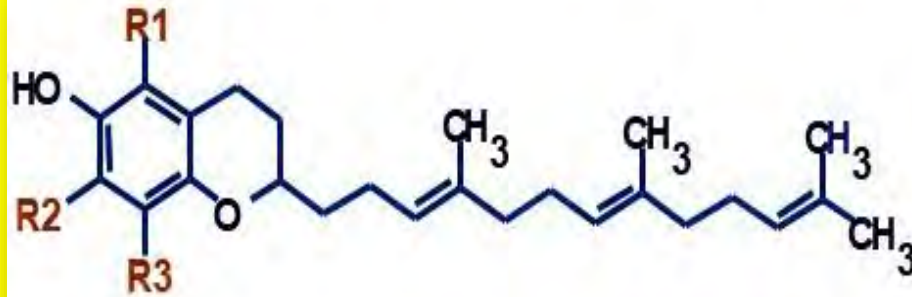
## Tocotrienols

Alpha ( $\alpha$ T3)

Beta ( $\beta$ T3)

Delta ( $\delta$ T3)

Gamma ( $\gamma$ T3)



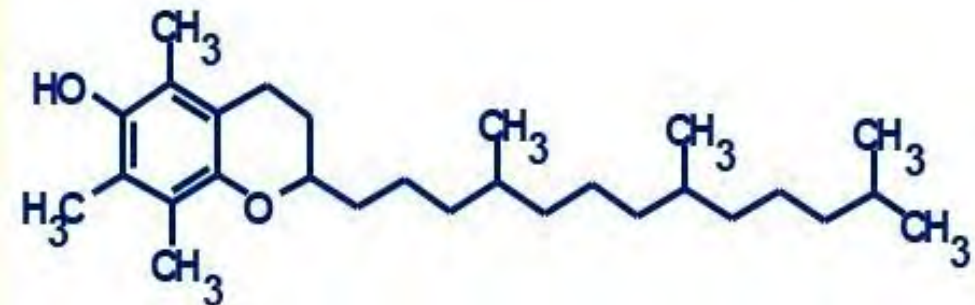
## Tocopherols

Alpha ( $\alpha$ T)

Beta ( $\beta$ T)

Delta ( $\delta$ T)

Gamma ( $\gamma$ T)



# Emergence of research in Tocotrienols



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**1964-1966**

**The discovery of tocotrienol**

**1980s**

**Ability to lower lipid peroxidation**

**1989**

**Anti-cancer effects in rodent**

**1991-1995**

**Anti cancer studies (Breast)**

**1997-1999**

**Further evidence appeared**

**2000-2008**

**Other beneficial health effects**

**2008-2010**

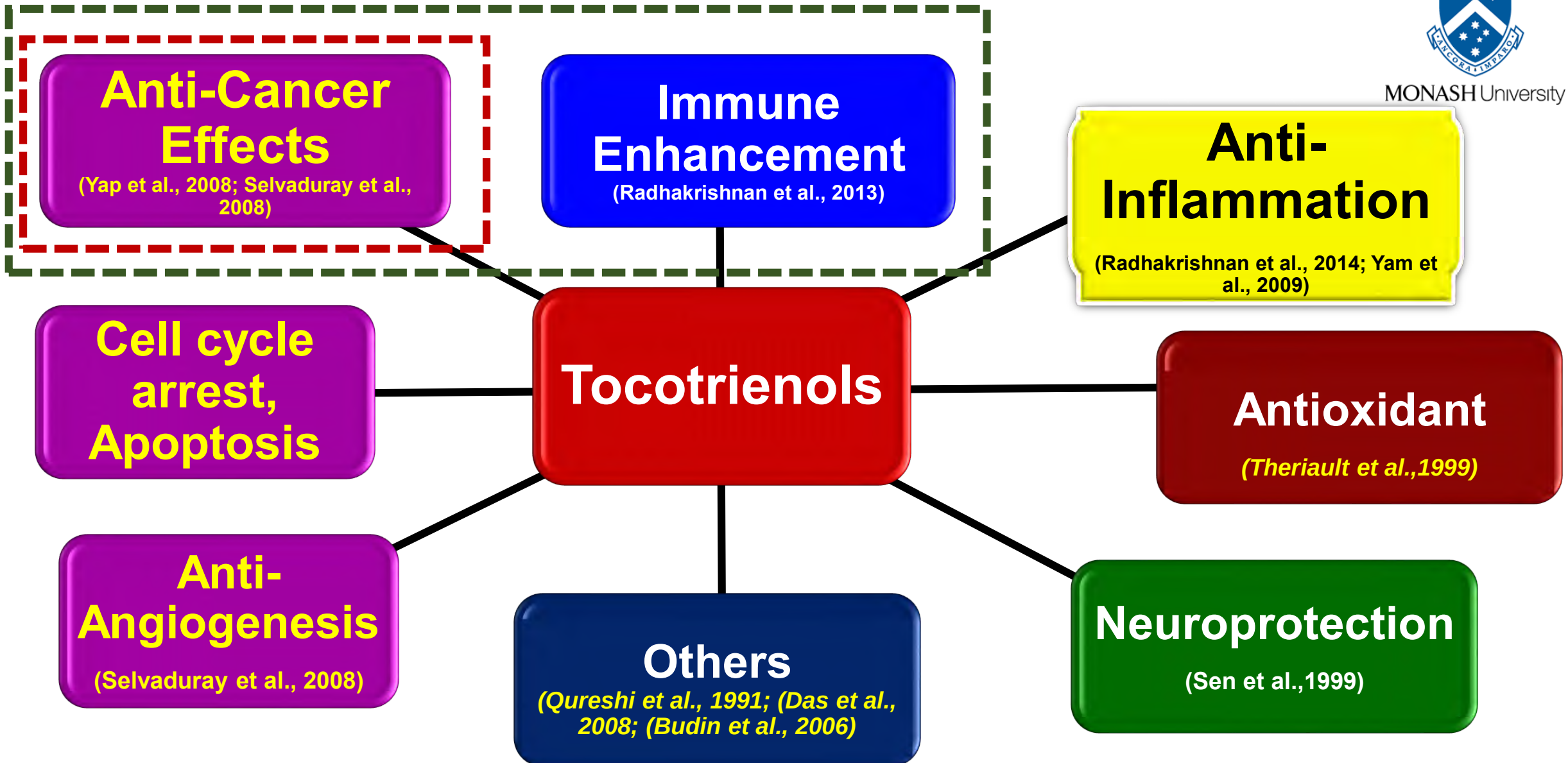
**Clinical trials initiated**

**2011-2015**

**Chip-based Genomics**

**2016-2020**

**Quantitative Proteomics**





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# Enhancing Immune Response to Vaccine (Tetanus Toxoid Vaccine)



# Study 1: Clinical Trial

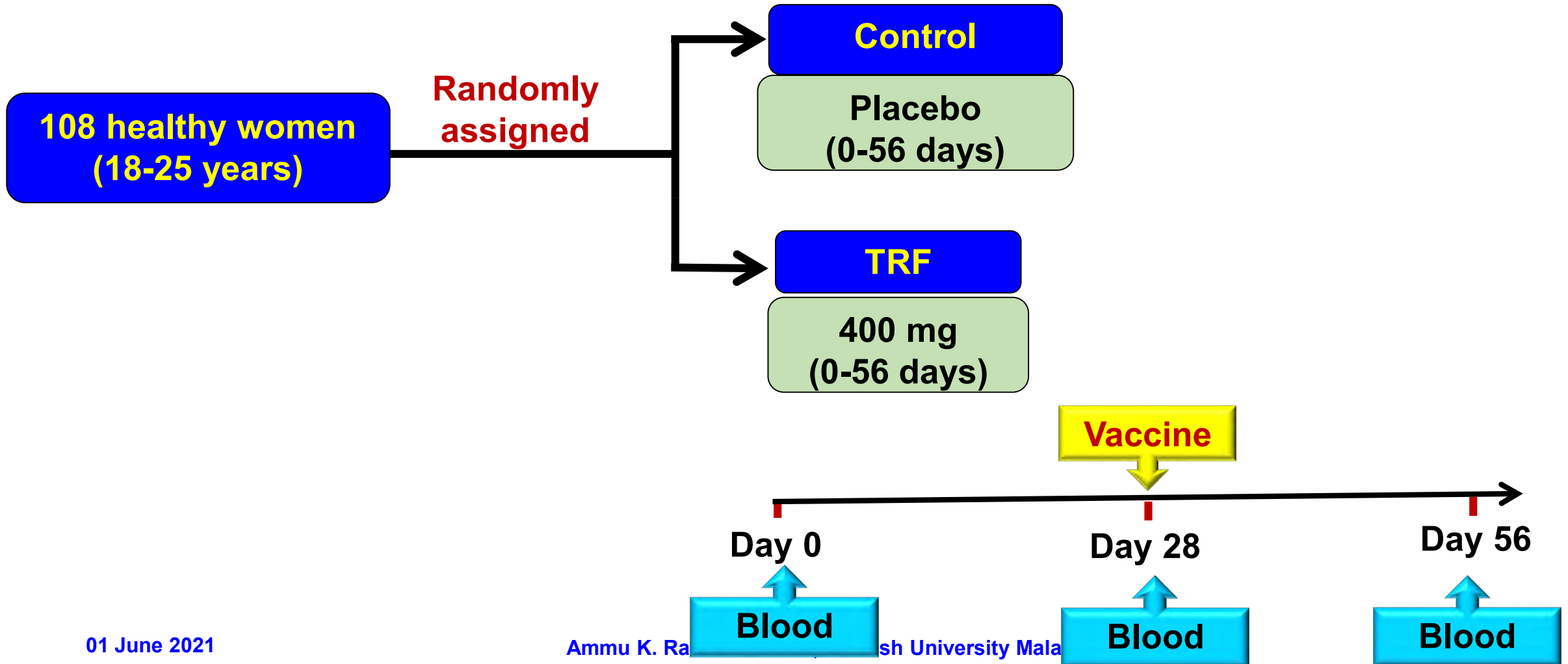
- To assess the effects of palm TRF supplementation on immune response following **tetanus toxoid vaccine (TT)** challenge in healthy female volunteers.
  - ❖ **TRF**: tocotrienol-rich fraction

## Experimental Approach

Randomised, double-blinded, placebo-controlled clinical trial



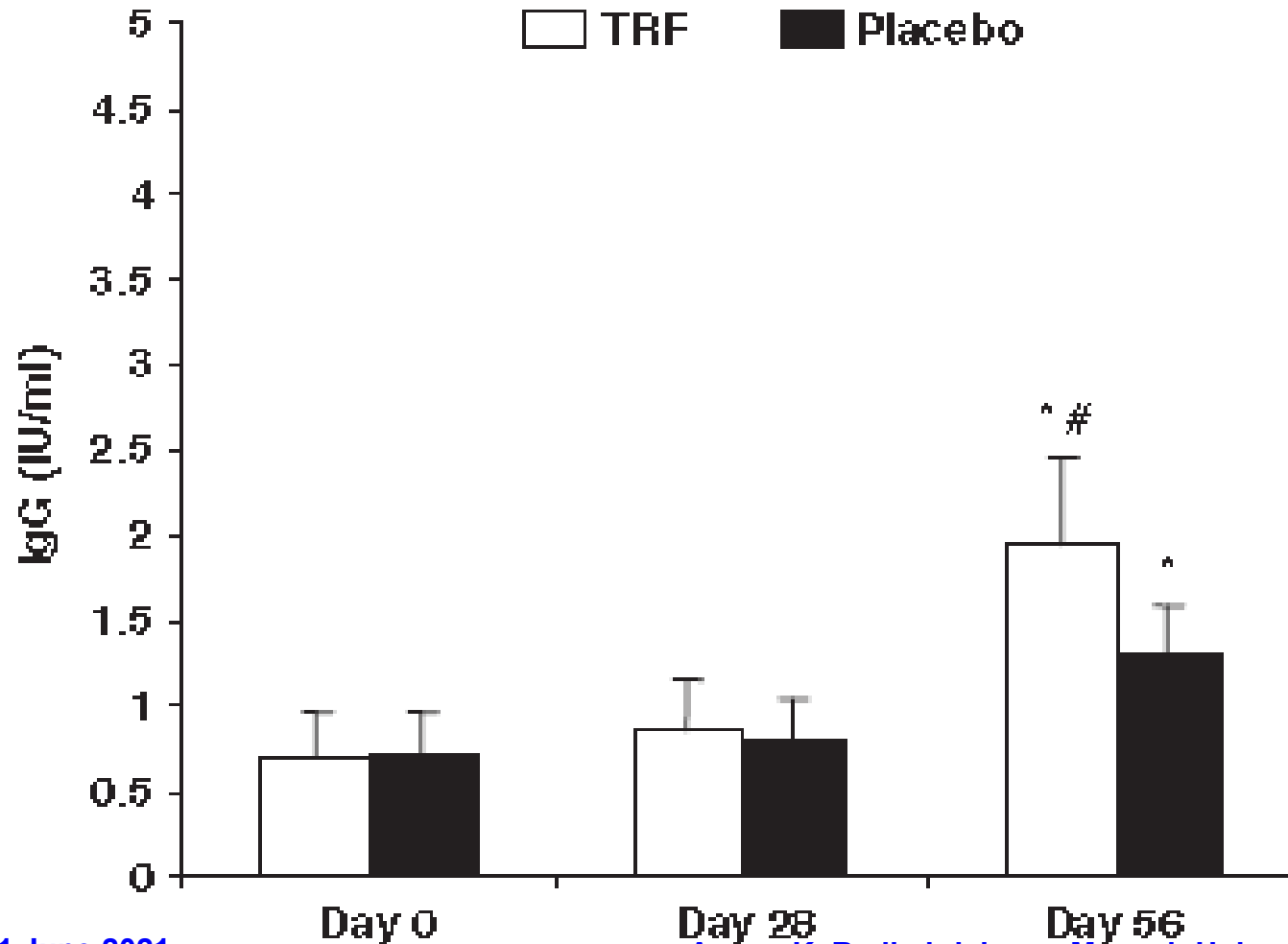
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# Anti TT antibody: IgG



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01 June 2021

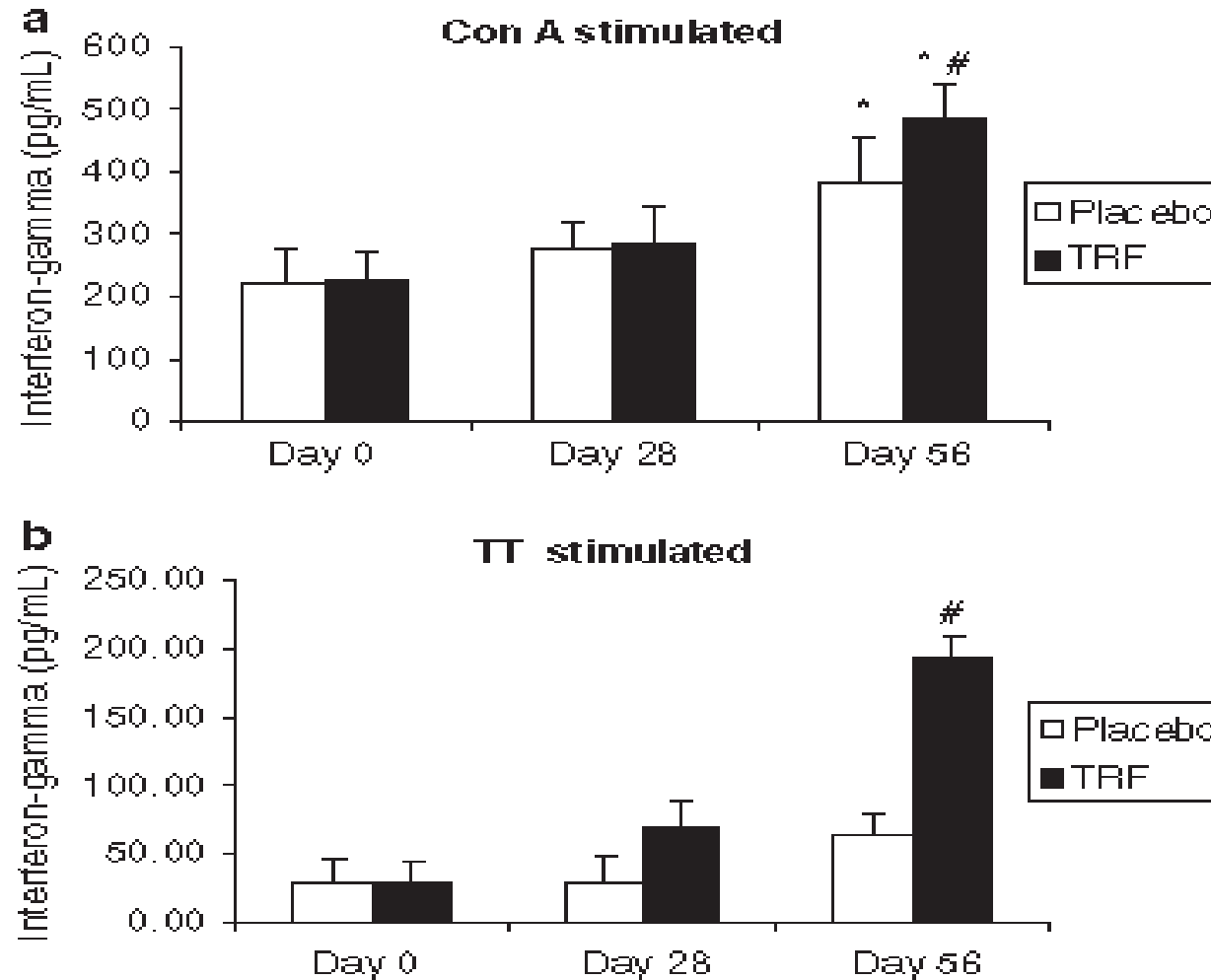
Ammu K. Radhakrishnan, Monash University Malaysia

- **↑ concentrations in the plasma of TRF- and placebo-supplemented volunteers ( $p < 0.05$ )**
- **Marked difference in production of anti-TT between day 0 and day 56 \* ( $P < 0.001$ )**

# IFN-gamma



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- **↑ production of IFN- $\gamma$  peripheral blood leucocytes (PBL) from TRF- and supplemented volunteers ( $p < 0.05$ )**
- **Marked difference in the production of IFN- $\gamma$  between day 0 and day 56**  
**\*( $P < 0.001$ )**

## ORIGINAL ARTICLE

# Effects of supplementation with tocotrienol-rich fraction on immune response to tetanus toxoid immunization in normal healthy volunteers

D Mahalingam<sup>1,2</sup>, AK Radhakrishnan<sup>2</sup>, Z Amom<sup>3</sup>, N Ibrahim<sup>2</sup> and K Nesaretnam<sup>1</sup>

<sup>1</sup>Malaysian Palm Oil Board, Selangor, Malaysia; <sup>2</sup>International Medical University, Kuala Lumpur, Malaysia and <sup>3</sup>University Putra Malaysia, Selangor, Malaysia

**Background/Objectives:** Vitamin E is an essential fat-soluble vitamin that has been shown to induce favorable effects on animal and human immune systems. The objective of this study was to assess the effects of tocotrienol-rich fraction (TRF) supplementation on immune response following tetanus toxoid (TT) vaccine challenge in healthy female volunteers.

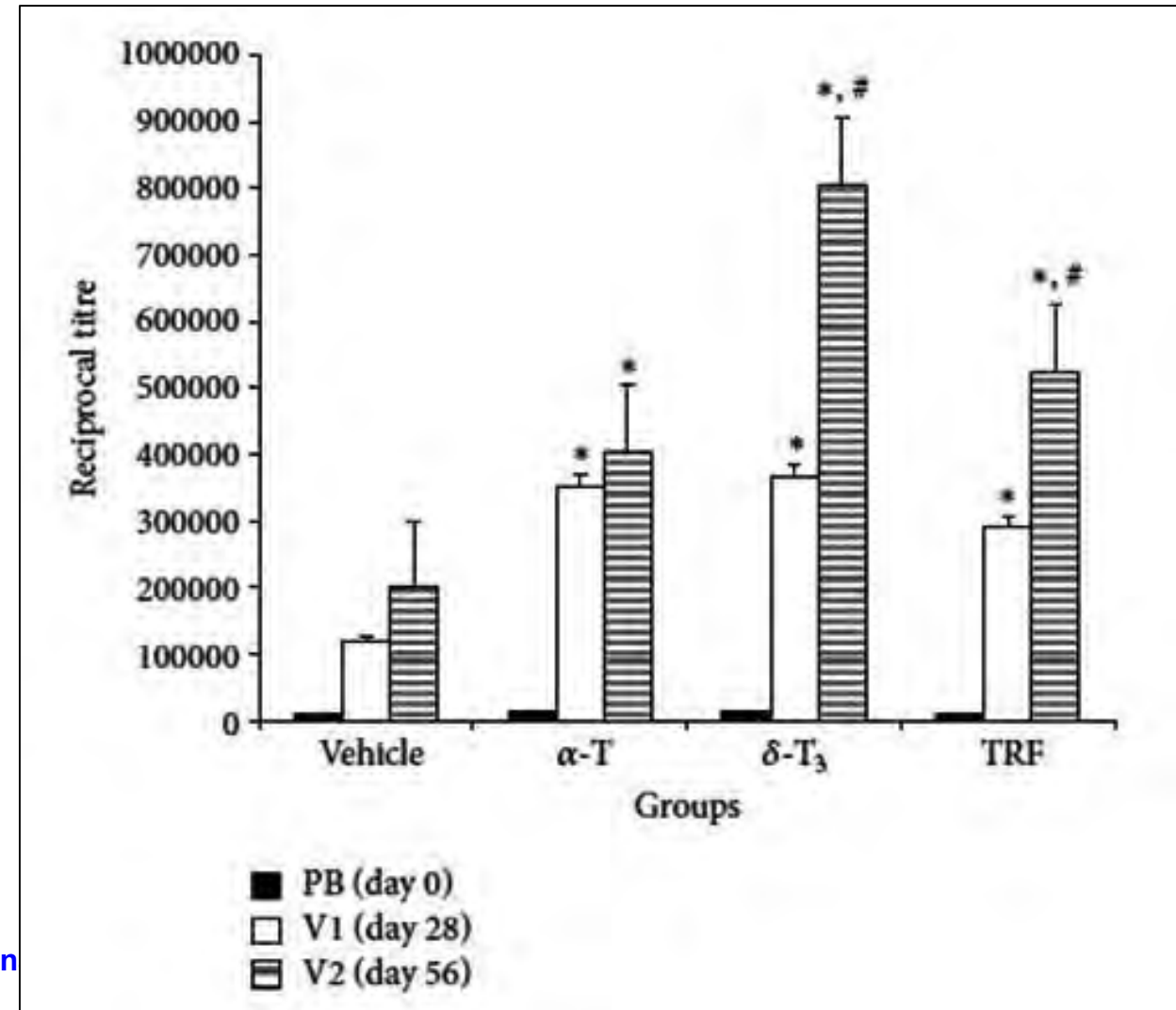
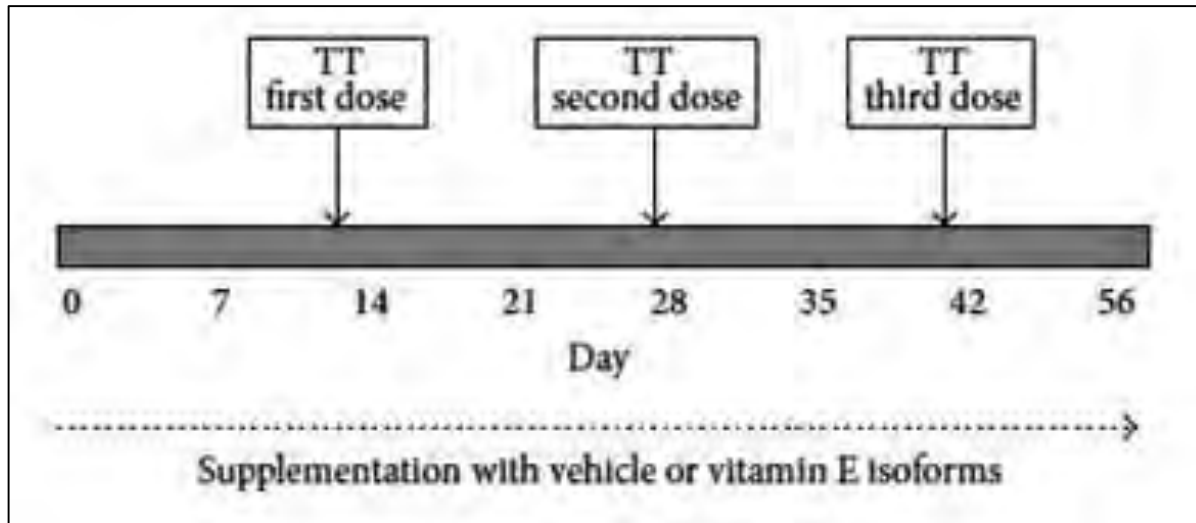
**Subjects/Methods:** In this double-blinded, placebo-controlled clinical trial, participants were randomly assigned to receive either placebo (control group) or 400 mg of TRF (study group) supplementation daily. Over the 2-month period of the study, volunteers were asked to attend three clinical sessions (that is, on days 0, 28 and 56) and blood samples were obtained from the volunteers during the follow-up. On day 28, all volunteers were also vaccinated with the TT vaccine (20 Lf) intramuscularly.

**Results:** The results from the clinical trial showed that TRF supplementation significantly increased the total vitamin E level in the plasma of the TRF-supplemented volunteers compared with the placebo group, indicating overall compliance. Volunteers supplemented with TRF showed a significantly ( $P < 0.05$ ) enhanced production of interferon- $\gamma$  and interleukin (IL)-4 by the mitogen or TT-stimulated leukocytes. The TRF-supplemented group produced significantly ( $P < 0.05$ ) lower amounts of IL-6 compared with the placebo group. Interleukin-10 production was also significantly ( $P < 0.05$ )

## Study 2: Mouse model of TT

- To assess the effects of vitamin E supplementation on immune response following **tetanus toxoid (TT)** vaccine challenge in a mouse model
- Vitamin E tested
  - ❖ Alpha-tocopherol ( **$\alpha$ Toc**)
  - ❖ Tocotrienol-rich fraction (**TRF**)
  - ❖ Delta-tocotrienol ( **$\delta$ T3**)
- Compared against vehicle

# Key Findings



## Research Article

# Supplementation with Natural Forms of Vitamin E Augments Antigen-Specific TH1-Type Immune Response to Tetanus Toxoid

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Kanga Rani Selvaduray,<sup>2</sup> and Kalanithi Nesaretnam<sup>2</sup>

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This study compared the ability of three forms of vitamin E [tocotrienol-rich fraction (TRF), alpha-tocopherol ( $\alpha$ -T), and delta-tocotrienol ( $\delta$ -T3)] to enhance immune response to tetanus toxoid (TT) immunisation in a mouse model. Twenty BALB/c mice were divided into four groups of five mice each. The mice were fed with the different forms of vitamin E (1 mg) or vehicle daily for two weeks before they were given the TT vaccine [4 Lf] intramuscularly (i.m.). Booster vaccinations were given on days 28 and 42. Serum was collected (days 0, 28, and 56) to quantify anti-TT levels. At autopsy, splenocytes harvested were cultured with TT or mitogens. The production of anti-TT antibodies was augmented ( $P < 0.05$ ) in mice that were fed with  $\delta$ -T3 or TRF compared to controls. The production of IFN- $\gamma$  and IL-4 by splenocytes from the vitamin E treated mice was significantly ( $P < 0.05$ ) higher than that from controls. The IFN- $\gamma$  production was the highest in animals supplemented with  $\delta$ -T3 followed by TRF and finally  $\alpha$ -T. Production of TNF- $\alpha$  was suppressed in the vitamin E treated group compared to vehicle-supplemented controls. Supplementation with  $\delta$ -T3 or TRF can enhance immune response to TT immunisation and production of cytokines that promote cell-mediated (TH1) immune response.

## 1. Introduction

Tocopherol and tocotrienol are bioactive plant derivatives

[8–10]. Both tocopherol and tocotrienol supplementation induced immunomodulatory effects resulting in Brown Norway rats [11]. When T-helper cells are appropriately activated,



# **Enhancing Immune Response to fight CANCER**

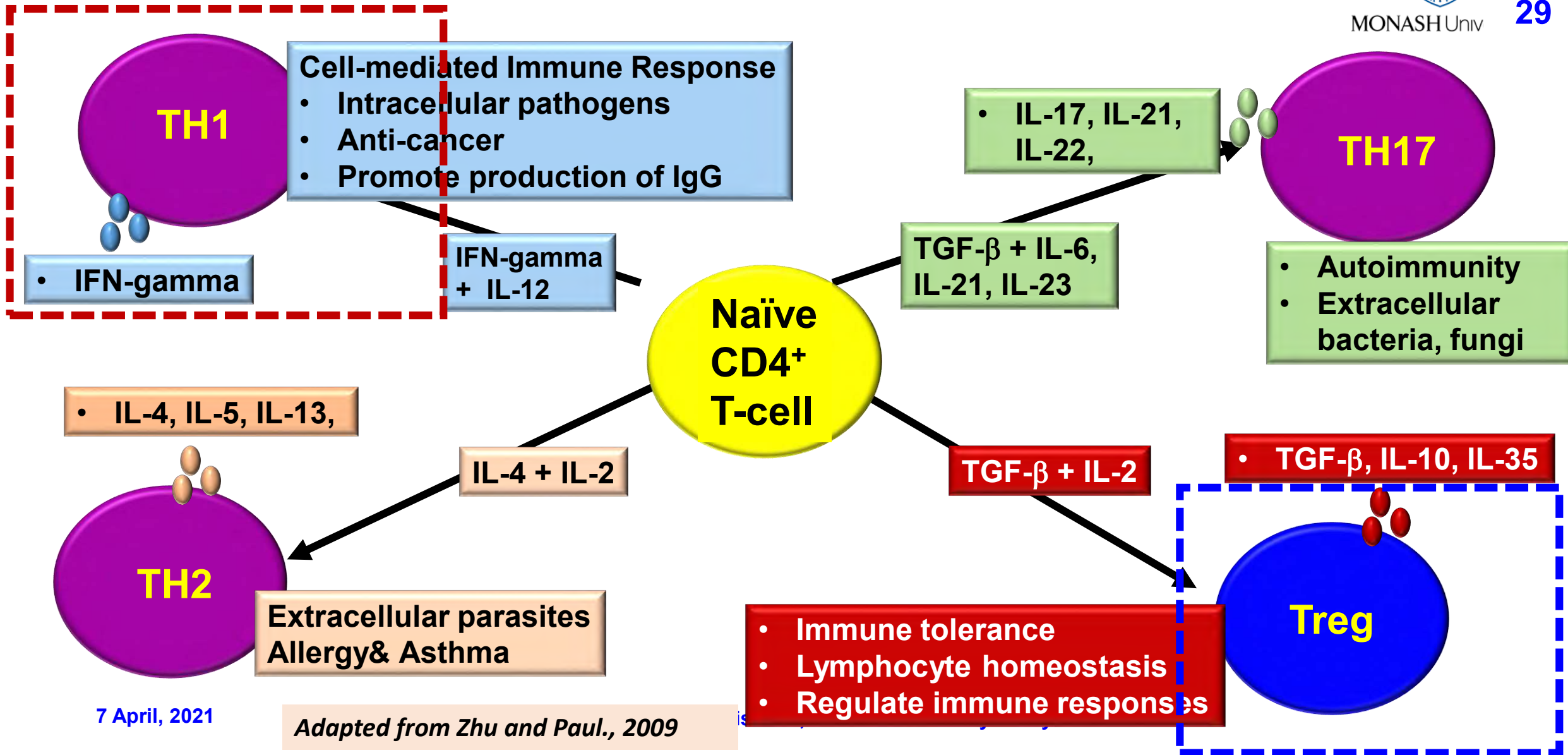
## **(Syngeneic mouse model of breast cancer)**



# Study 3: Mouse Model of Breast Cancer

- To assess if the anti-cancer effects of tocotrienol supplementation work through regulation of T-helper-1 (Th1) and T-regulatory (Treg) cells

# Functions CD4<sup>+</sup> T-cell





Mice fed with  $\gamma$ T3 or vehicle (0.5 mg twice daily)

Mice inoculated with mouse breast cancer

Mice treated with vehicle or  $\gamma$ T3 and sacrificed every 7 days until termination of study

**Blood:**

Flow  
cytometry  
analysis

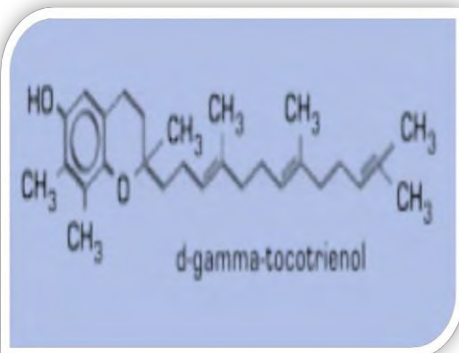
Histopathology

H & E: lung,  
liver, kidney

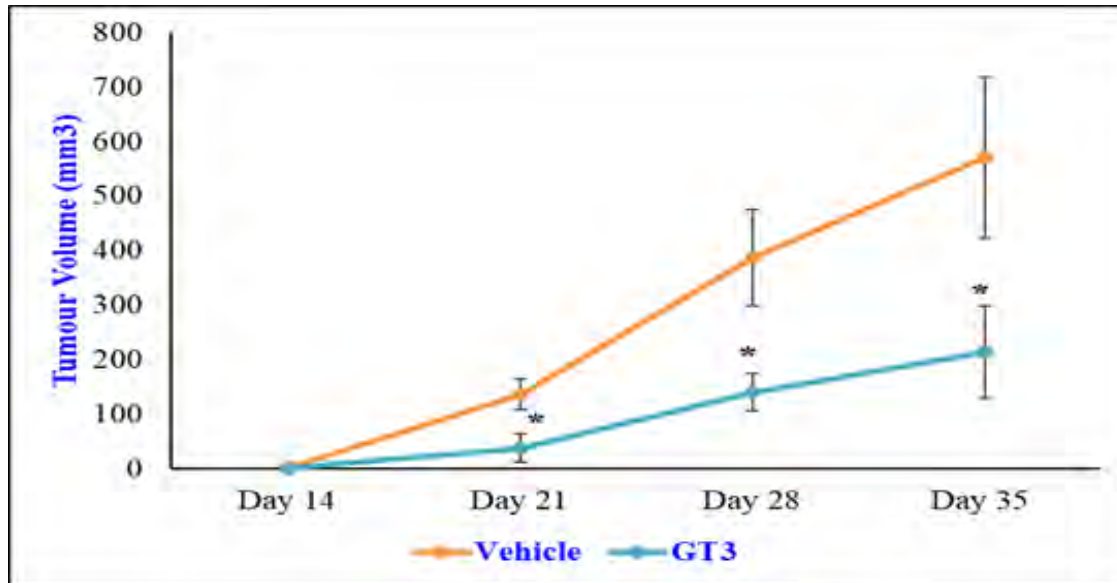
IHC: tumour

**Spleen:**

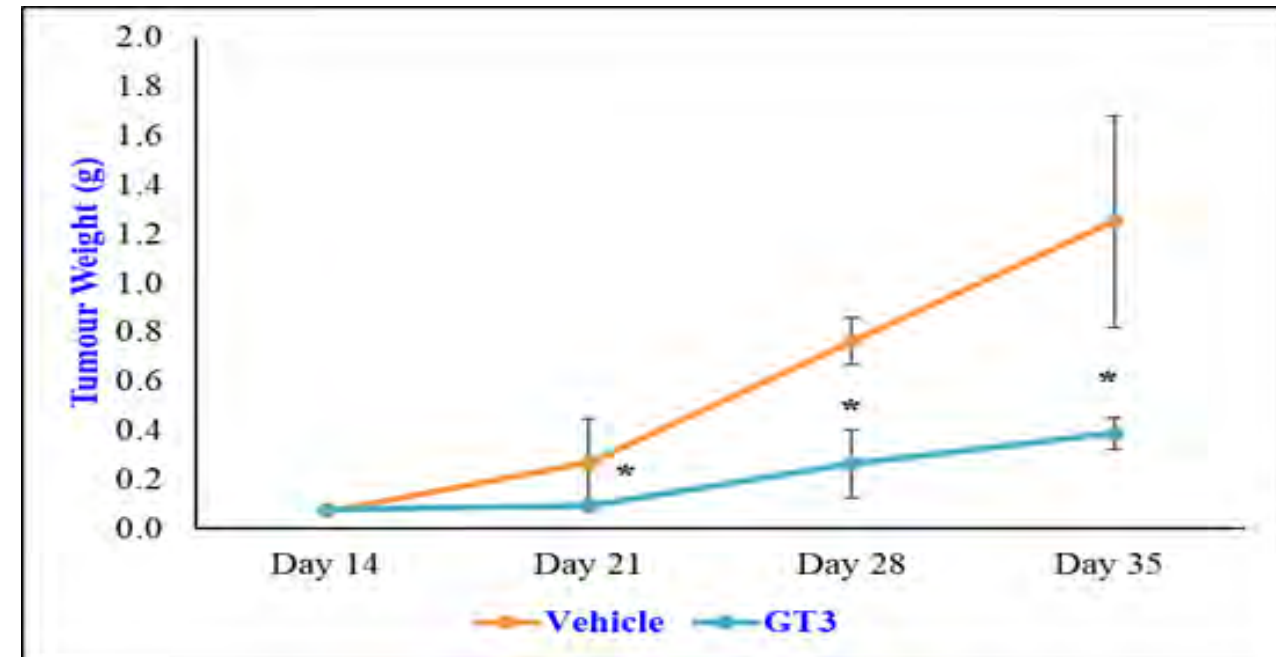
MTT assay, ELISA,  
CD4<sup>+</sup> isolation



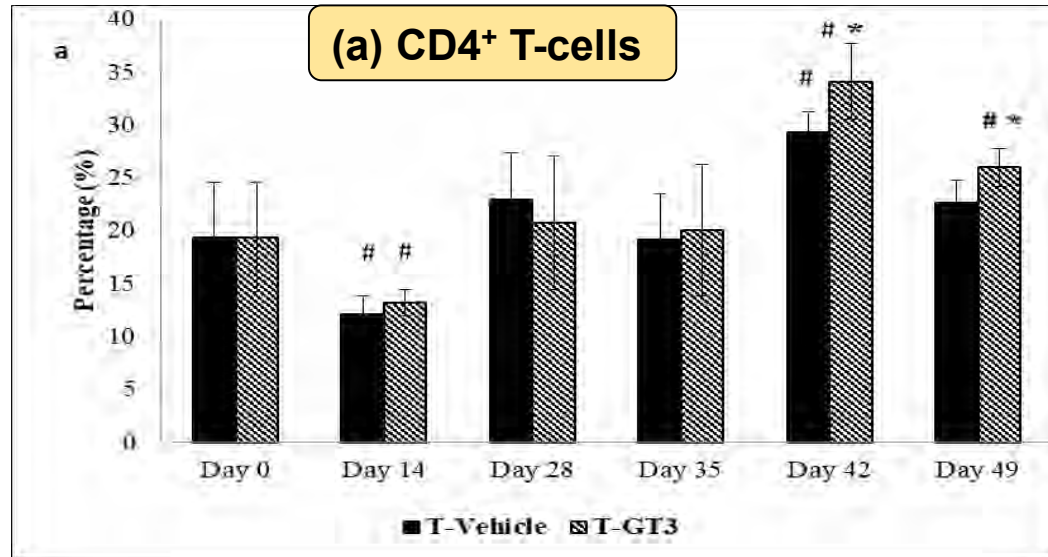
# Tumour Growth



**\*P < 0.05**



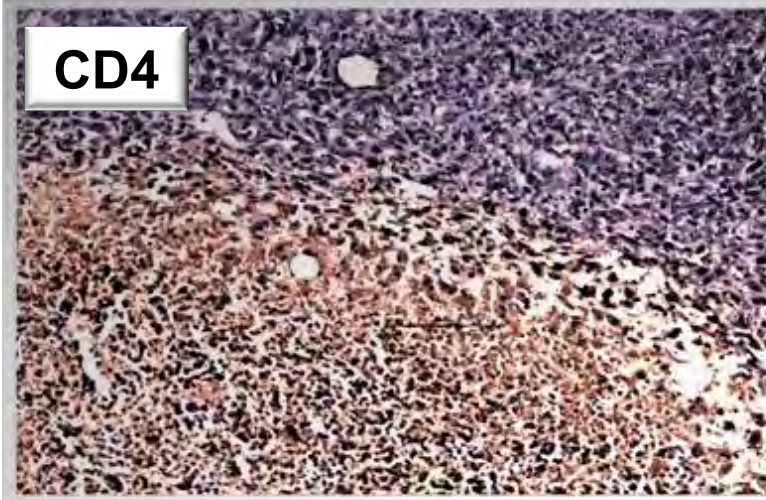
# Blood Immune Markers



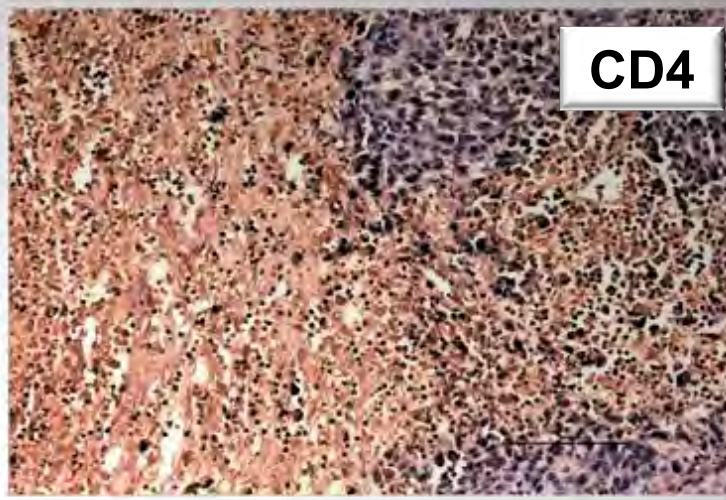
**Vehicle**

**Tocotrienol**

**CD4**



**CD4**



**CD4+ T-cells**

**T-regulatory cells**

# Conclusion

➤ **TRF supplementation enhanced immune response to booster TT vaccine**

❖ **↑ Anti-TT antibody**

❖ **↑ IFN-gamma levels**

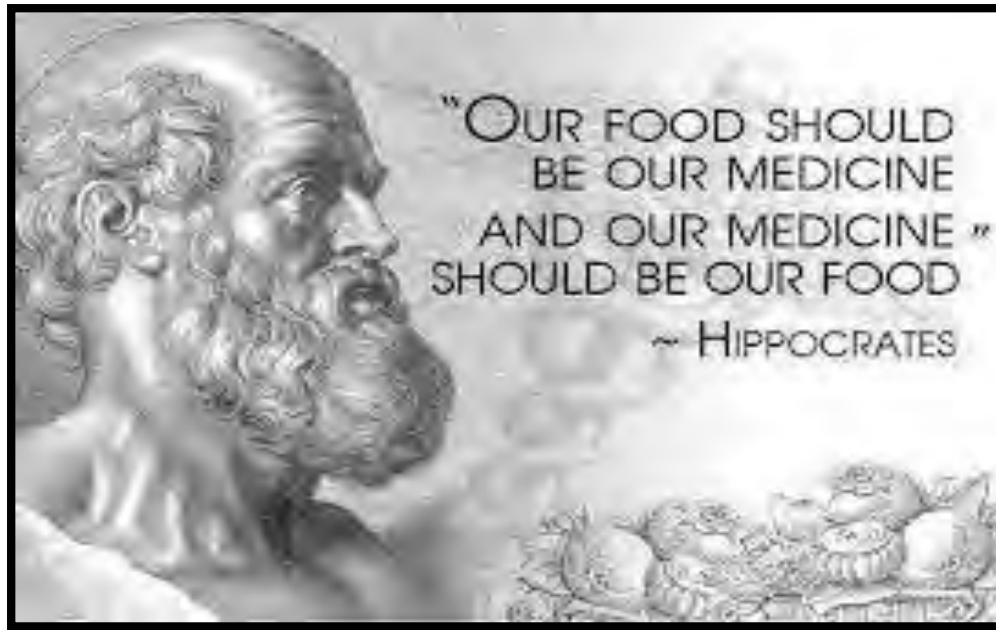
# Conclusion

- **Tocotrienol supplementation activated host anti-tumour immune response**
  - ❖ **↑ CD4 and CD8 count in PBL**
  - ❖ **↓ Treg cells in PBL**
  - ❖ **↑ CD4<sup>+</sup> cells count in tumour microenvironment**
  - ❖ **↓ Treg cells in tumour microenvironment**

**Tocotrienols supplementation can enhance host immune system**



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# THANK YOU

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