# Serendipities of acquired immunity

## Nobel Lecture

December 7, 2018


## Tasuku Honjo

Kyoto University Institute for Advanced Study
and Graduate School of Medicine

# My family (1955)

# Cassini: Earth and Saturn
# The Day the Earth Smiled

"The telescopic view of Saturn fascinated me. I dreamed of becoming an astronomer."

**Earth**

Through the brilliance of Saturn's rings, Cassini caught a glimpse of a far-away planet and its moon. At a distance of just under 900 million miles, Earth shines bright among the many stars in the sky, distinguished by its bluish tint.

Saturn.
Actual photo taken on June 5, 2016
https://www.nasa.gov/mission_pages/cassini/multimedia/pia17171.html

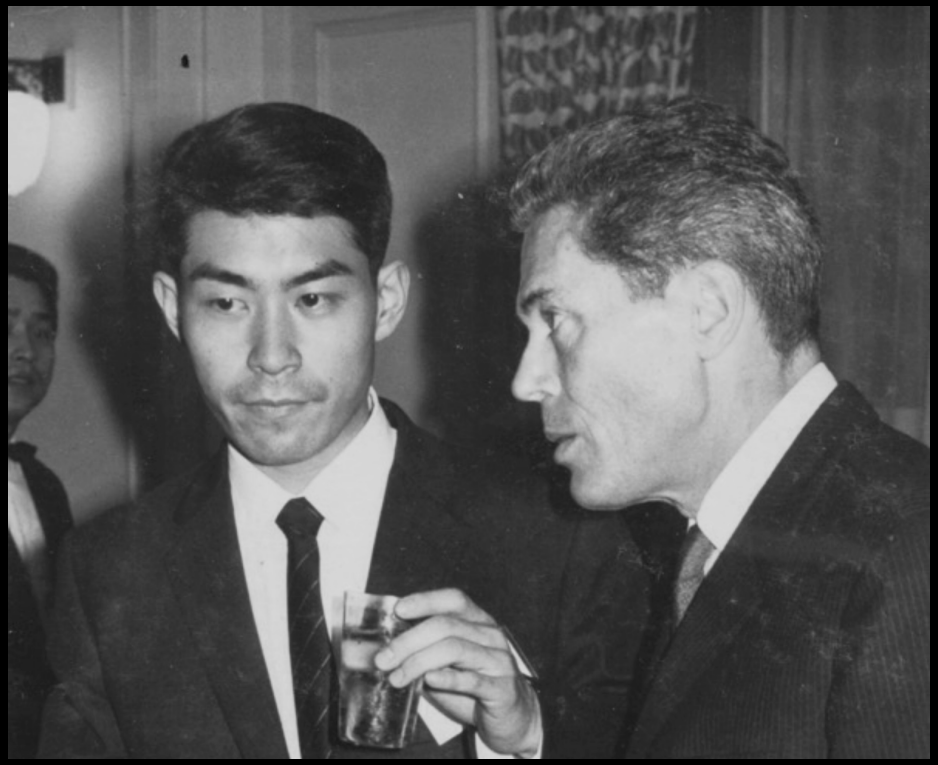
# Inspired by biography of Hideyo Noguchi (1876~1928)

Rockefeller Univ.

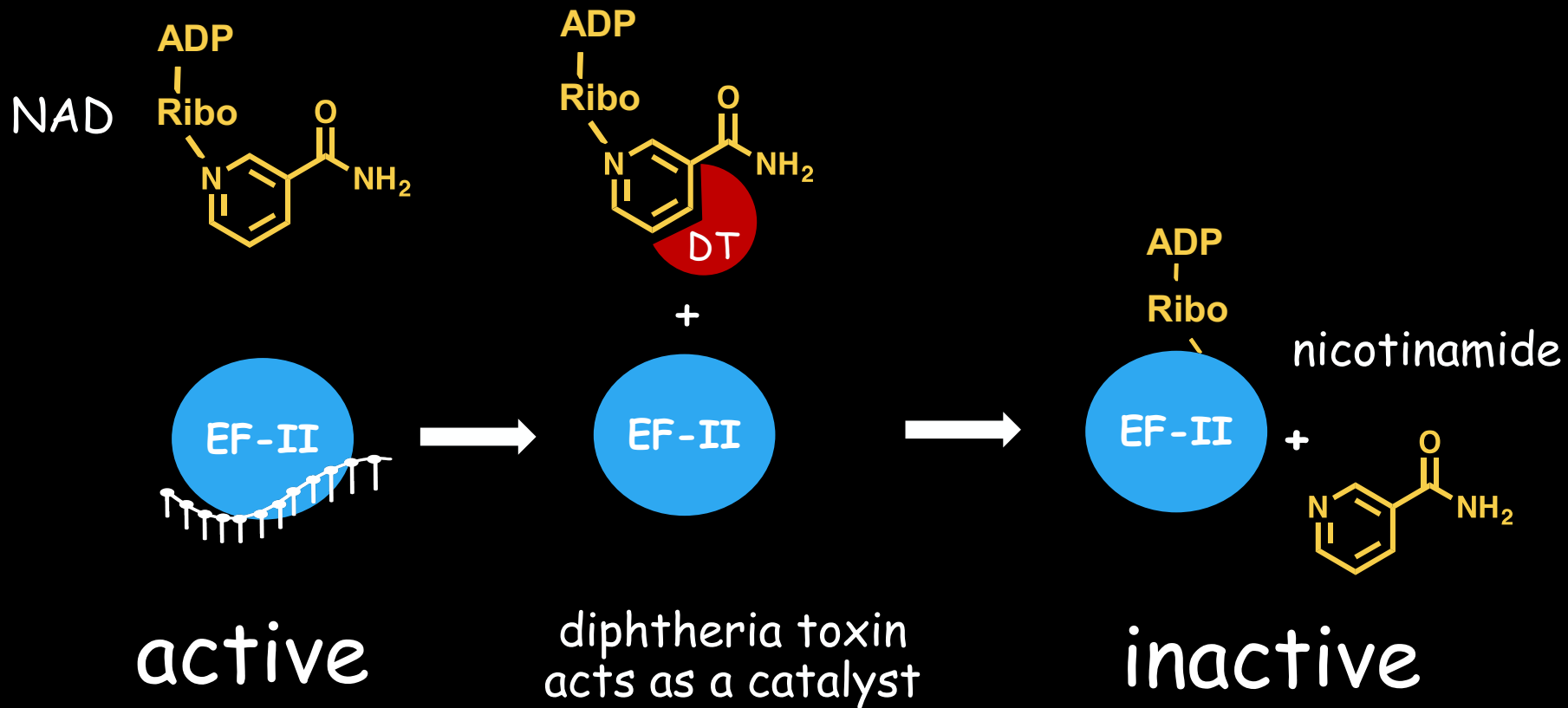

- Identified Syphilis spirochete as the cause of progressive paralysis
- Died in Ghana during pursuit of yellow fever pathogen

# With Osamu Hayaishi

# With Jacques Lucien Monod 1966

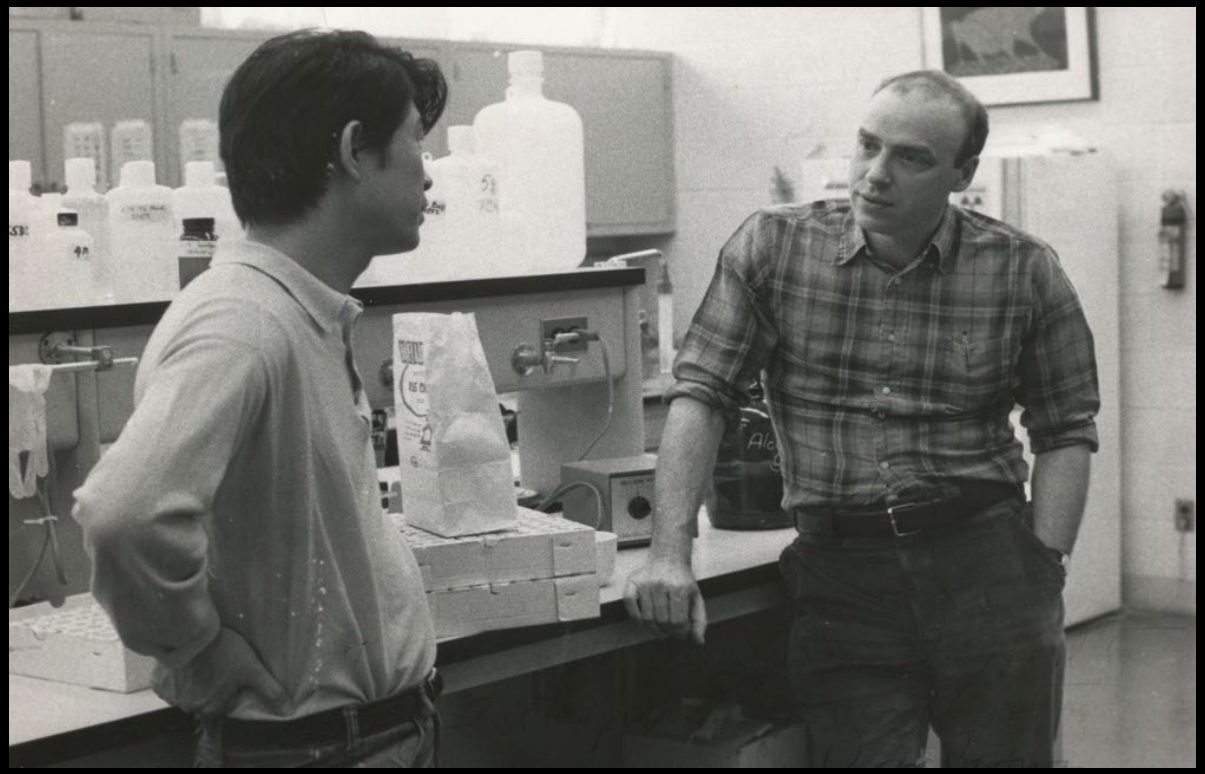


# Diphtheria toxin inactivates protein synthesis factor by ADP-ribosylation




T. Honjo *et al.*, J.Biol.Chem. (1968)

# Donald Brown at Carnegie Institution in Baltimore 1971



# Mystery of immune response in 1950~1970

How can animals generate antibodies specific to an almost infinite number of antigens, including artificial chemicals?

# Why can animals generate specific antibodies to almost all unexperienced compounds?

nitrobenzene -protein → Anti-N-benzene Ab

nitrophenol -protein → Anti-N-phenol Ab

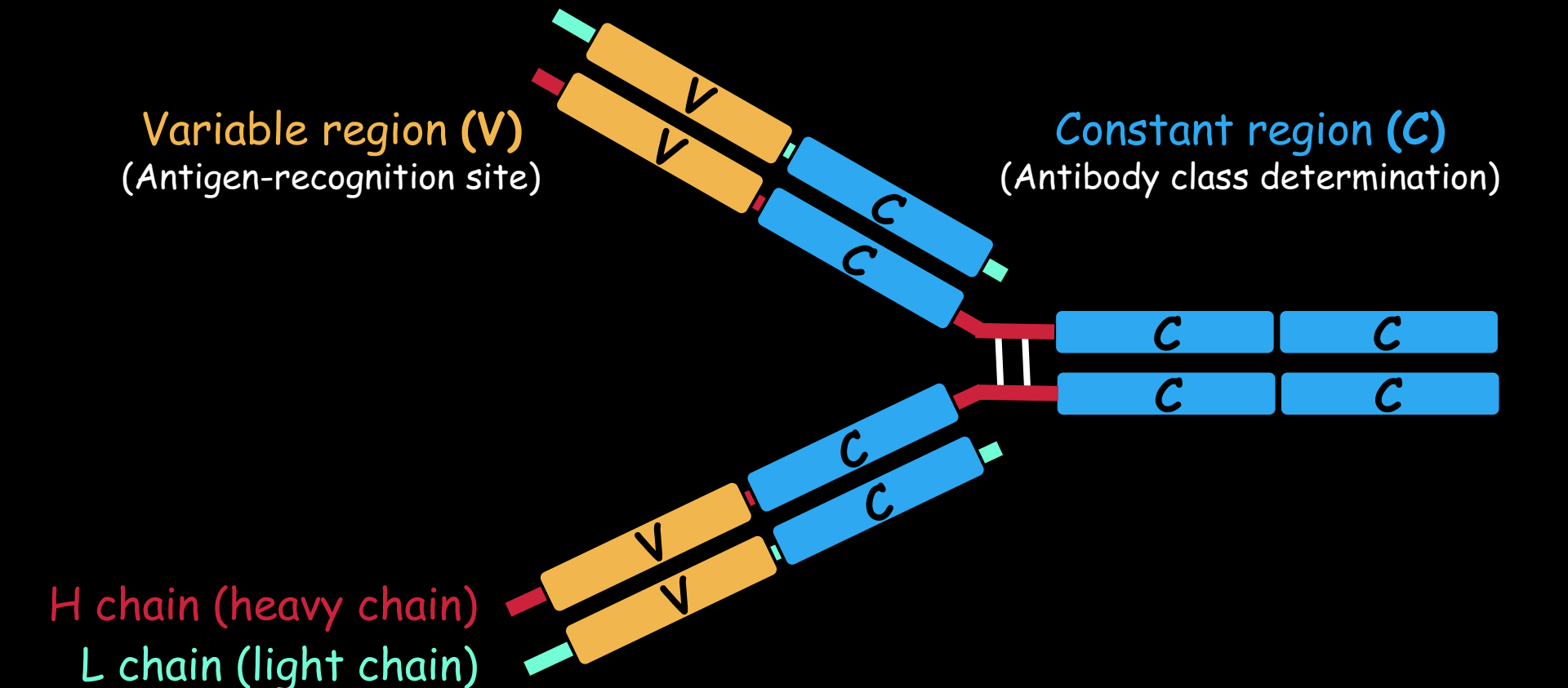
anthracene -protein → Anti-anthracene Ab

toluene diisocyanate -protein → Anti-toluene-DIC Ab

oxazolone -protein → Anti-oxazolone Ab

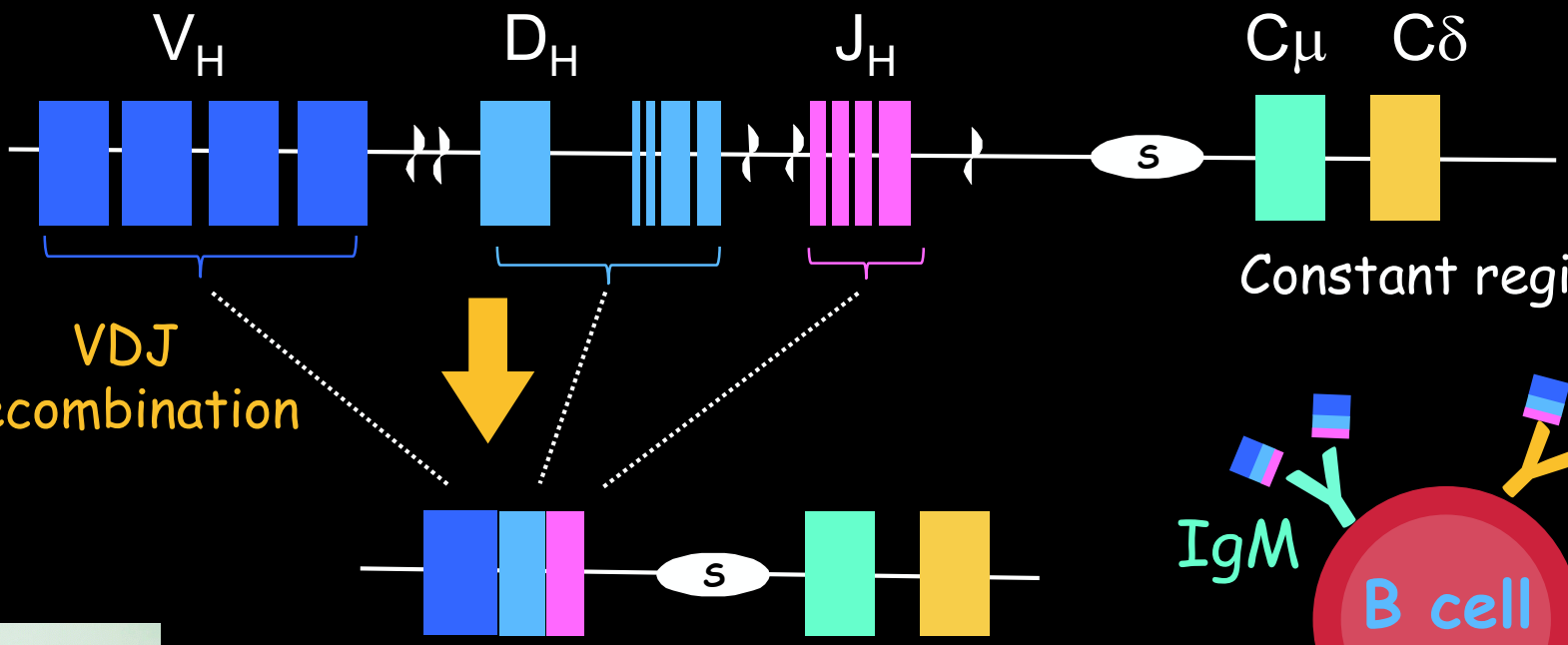
Modified from K. Landsteiner 1919-22

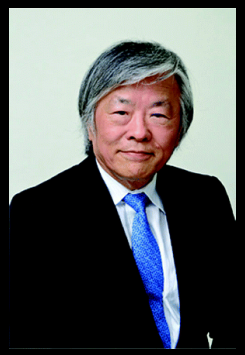
# Structure of antibody identified by 1970



# Philip Leder at NIH 1973

# VDJ recombination generates V region repertoire during differentiation



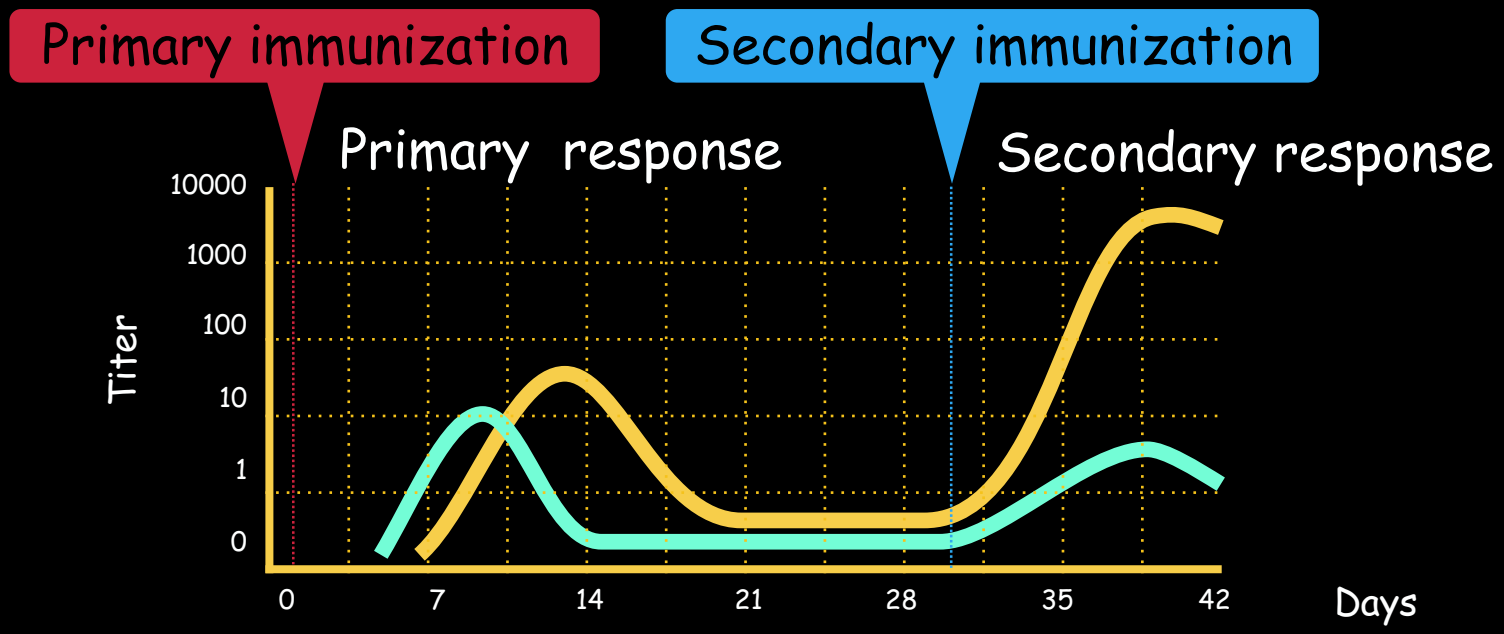



C. Brack *et al.*, Cell (1978)

S. Tonegawa

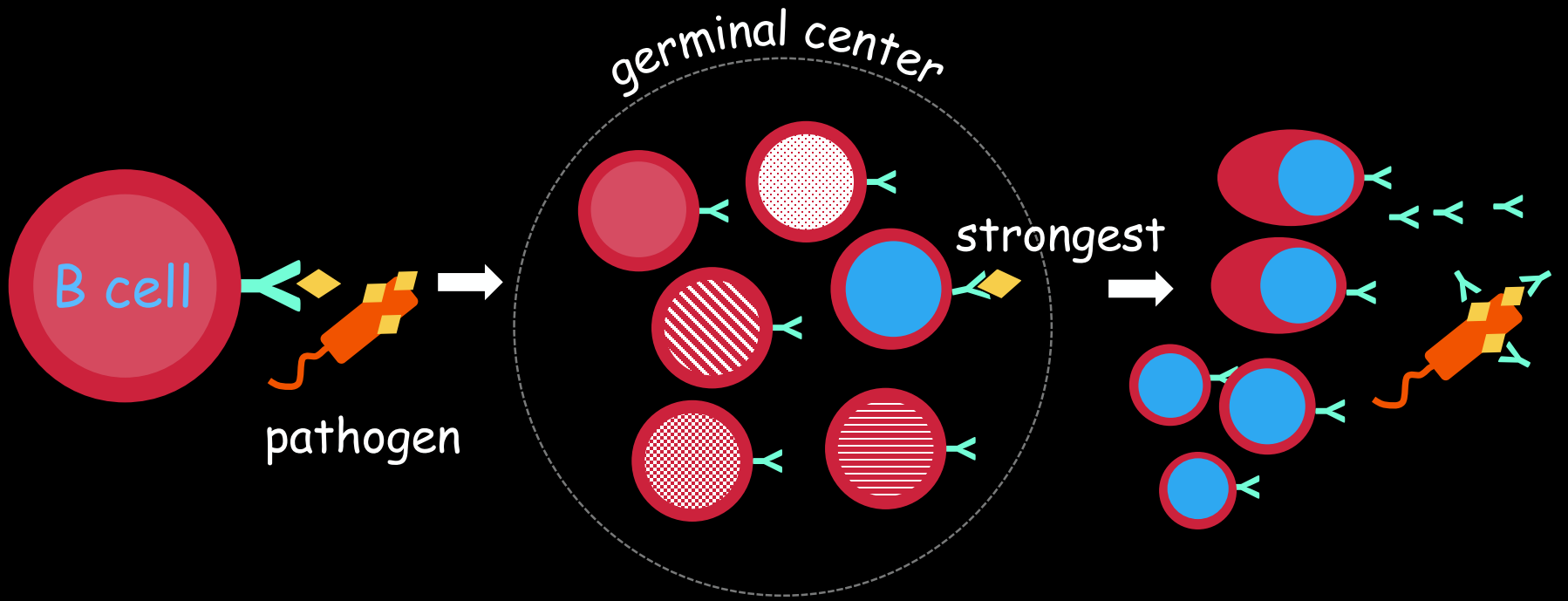
# University of Tokyo, Dept. of Nutrition (Professor Yoshinaga Mano) 1974

# Antibody memory generation by vaccine (antigen) administration



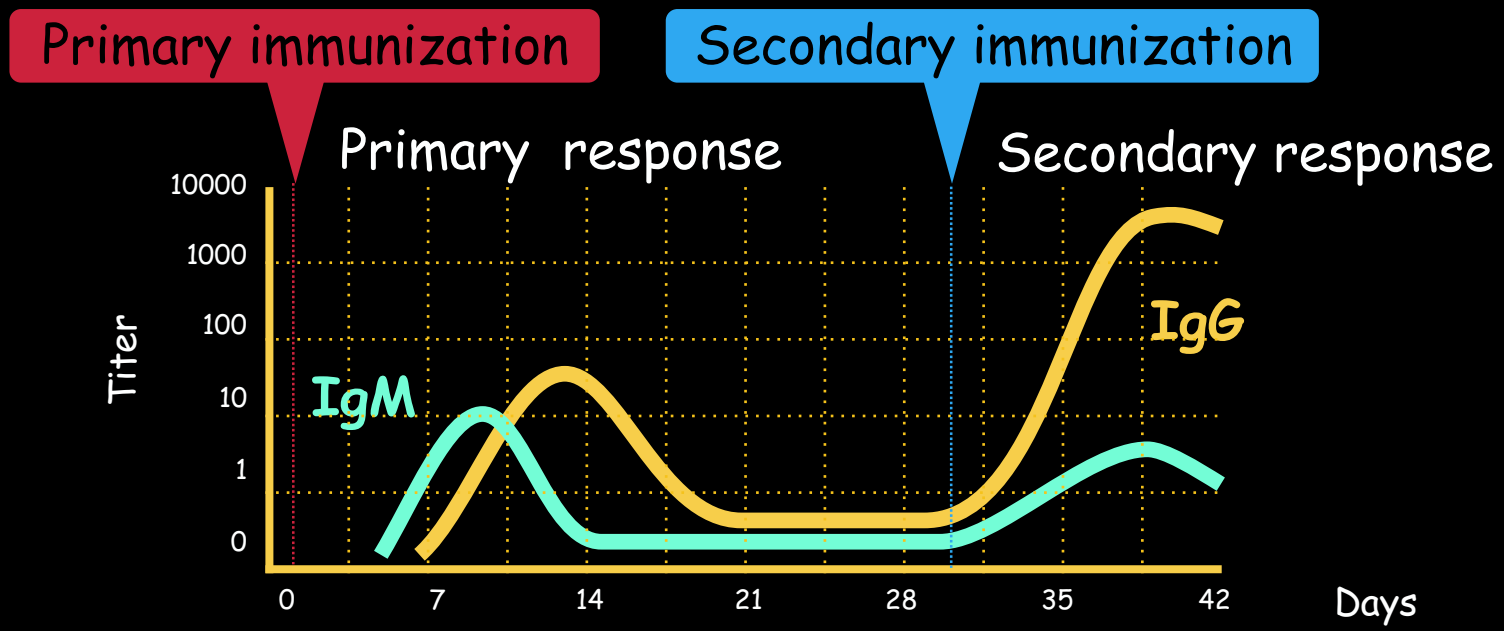

Increase in antigen binding capability
(somatic hypermutation of variable region)

# Somatic hypermutation (SHM) mutates V region and only good antibodies are selected




Darwinian principle

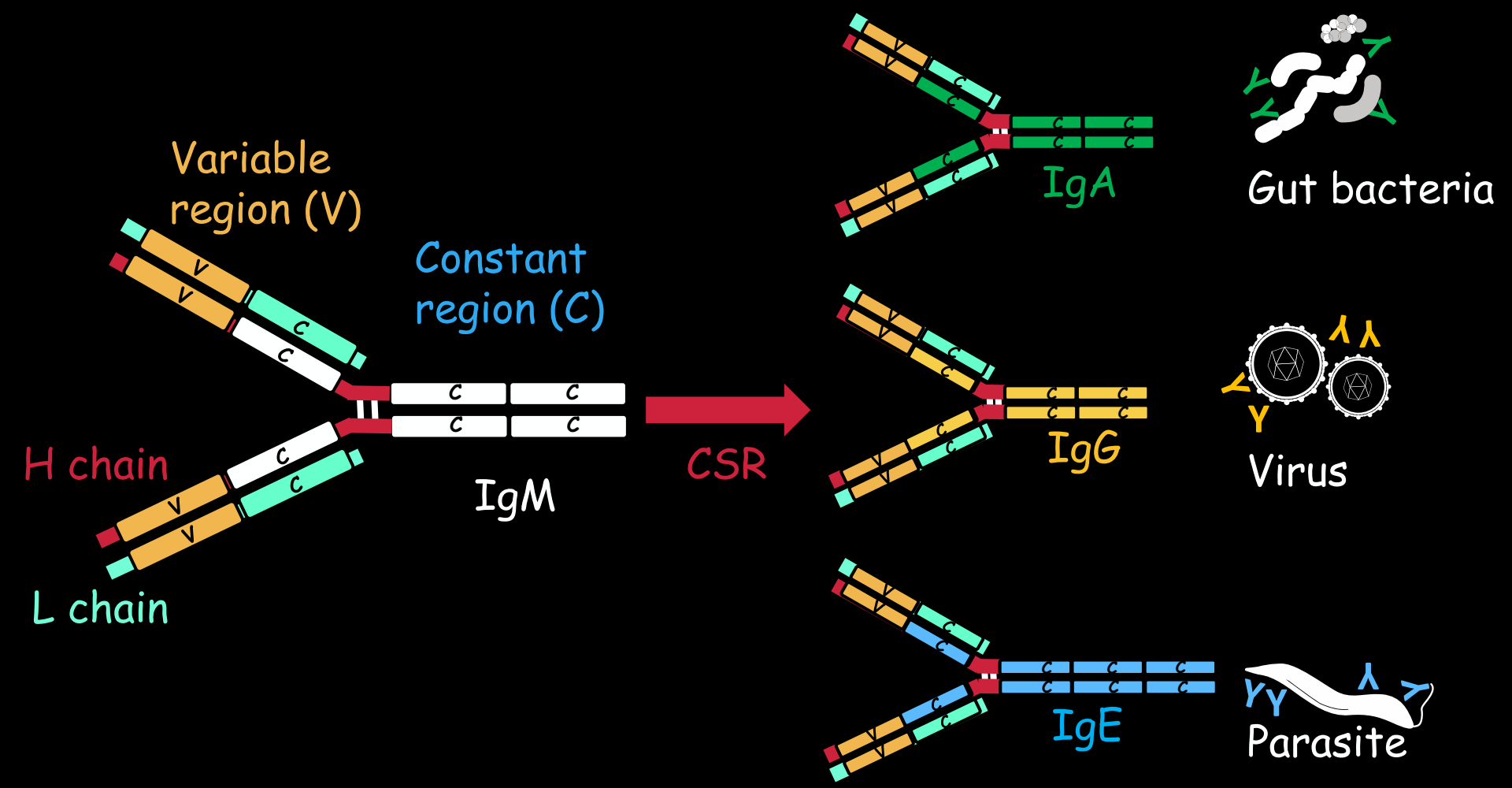
# Antibody memory generation by vaccine (antigen) administration



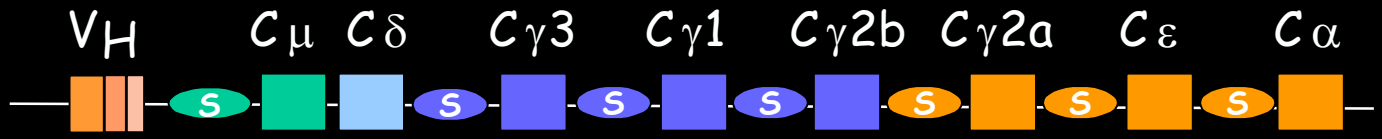

Increase in antigen binding capability
(somatic hypermutation of variable region)

? Increase in antigen processing ability
(class switch of constant region)

# Class switching changes the H chain constant region and antibody function



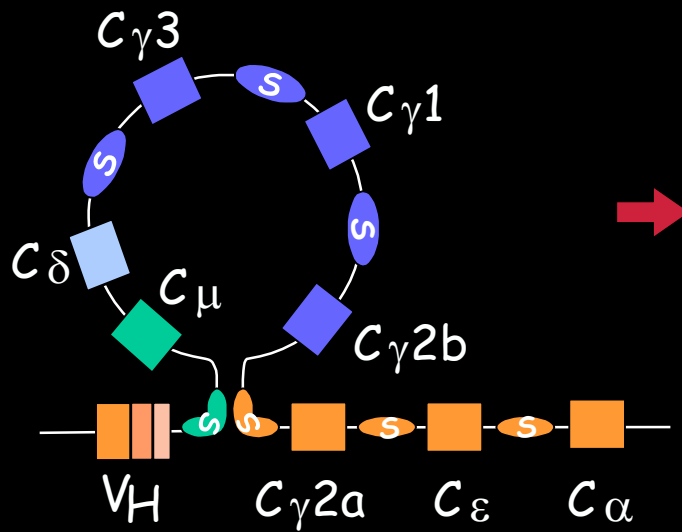
# Class switch recombination takes place by deletion of a large DNA segment




class switch recombination (CSR)

T. Kataoka

A. Shimizu



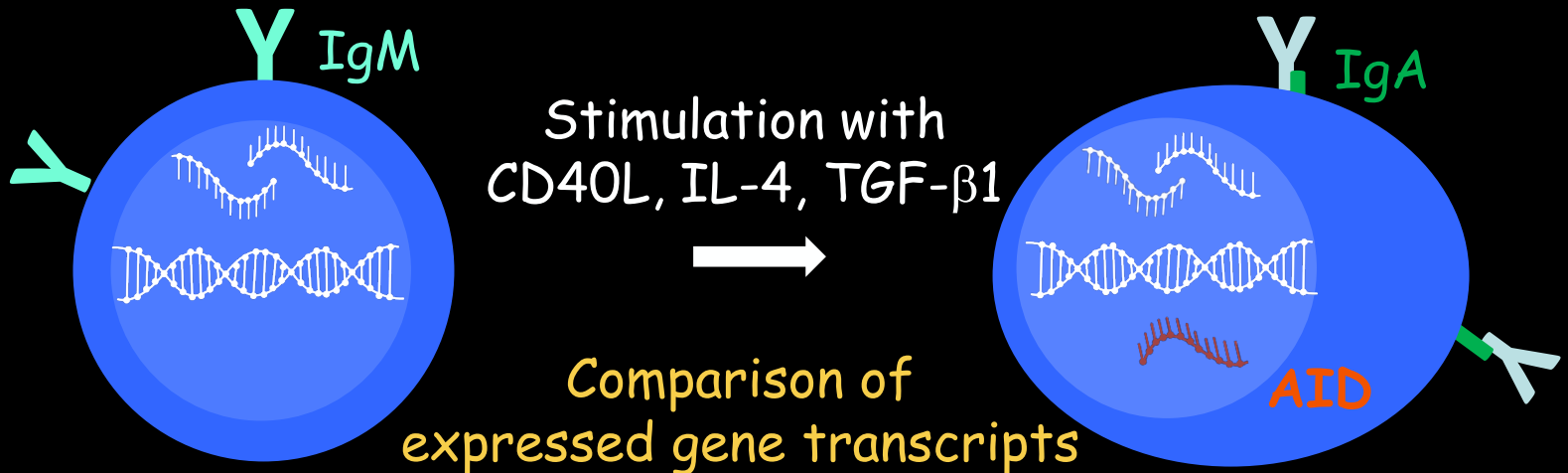

looped-out circular DNA

T. Honjo & T. Kataoka, PNAS (1978)
T. Kataoka *et al.*, PNAS (1980)
A. Shimizu *et al.*, Cell (1982)

# The 55th Nobel Symposium
# "Genetics of the Immune Response"
# Saltsjobaden, Sweden, June 15 - 17, 1982

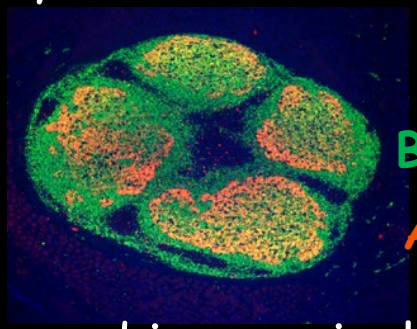

Matthias Wabl, Göran Möller (coorganizer) Leroy Hood

# Discovery of AID by comparison of gene expression before and after CSR



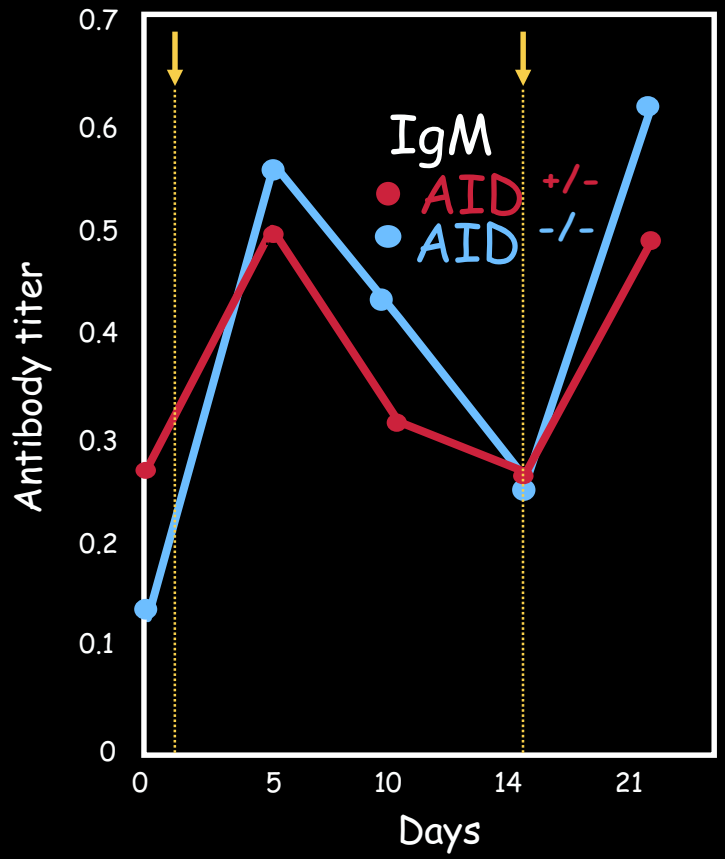

Activation Induced
cytidine Deaminase

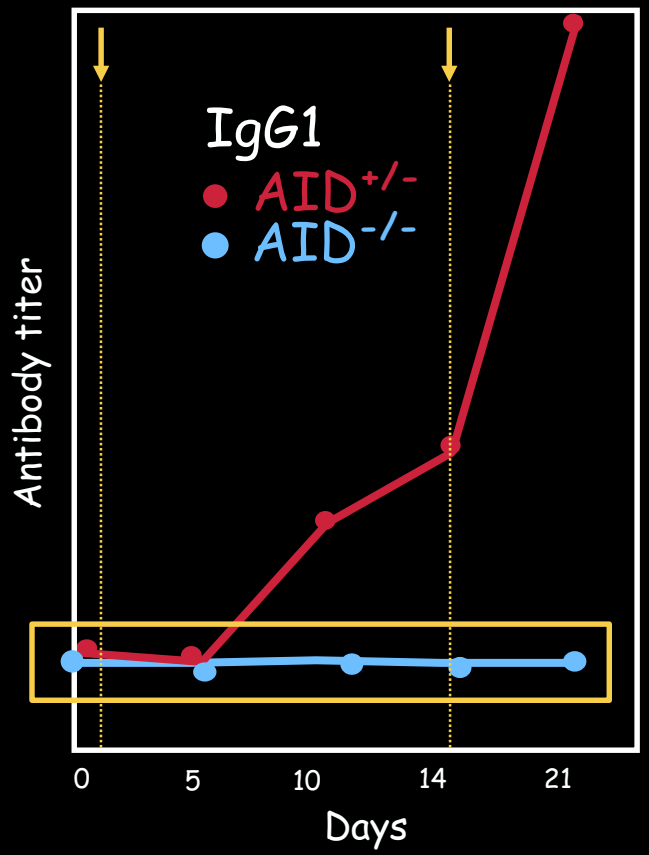
M. Muramatsu *et al.*, J.Biol. Chem. (1999)




Expressed in germinal center

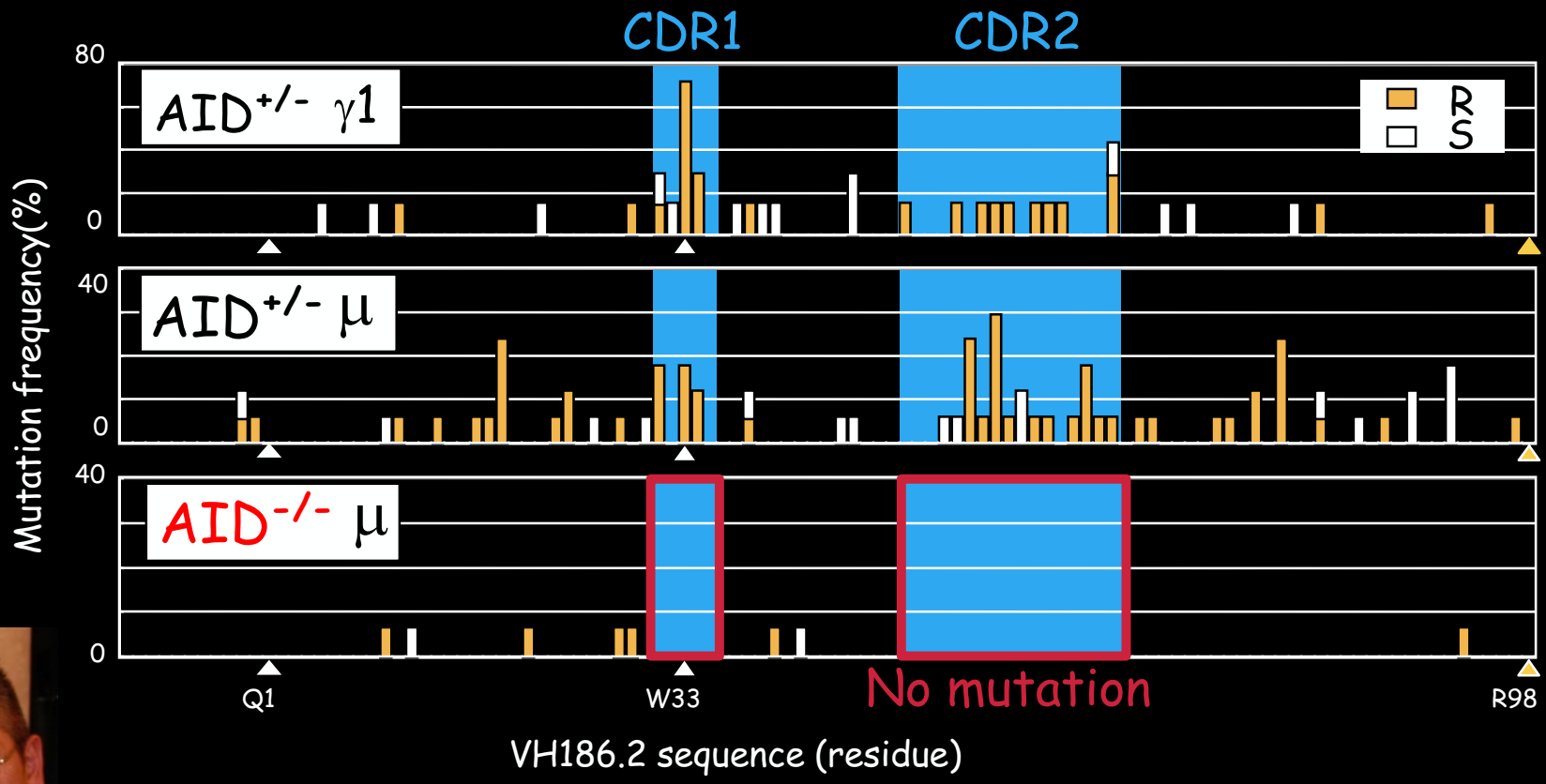
# Defective IgG response to antigens (Sheep Red Blood Cell) in AID deficient mice




M. Muramatsu *et al.*, Cell (2000)

# AID deficient mice fail to accumulate mutations




K. Kinoshita

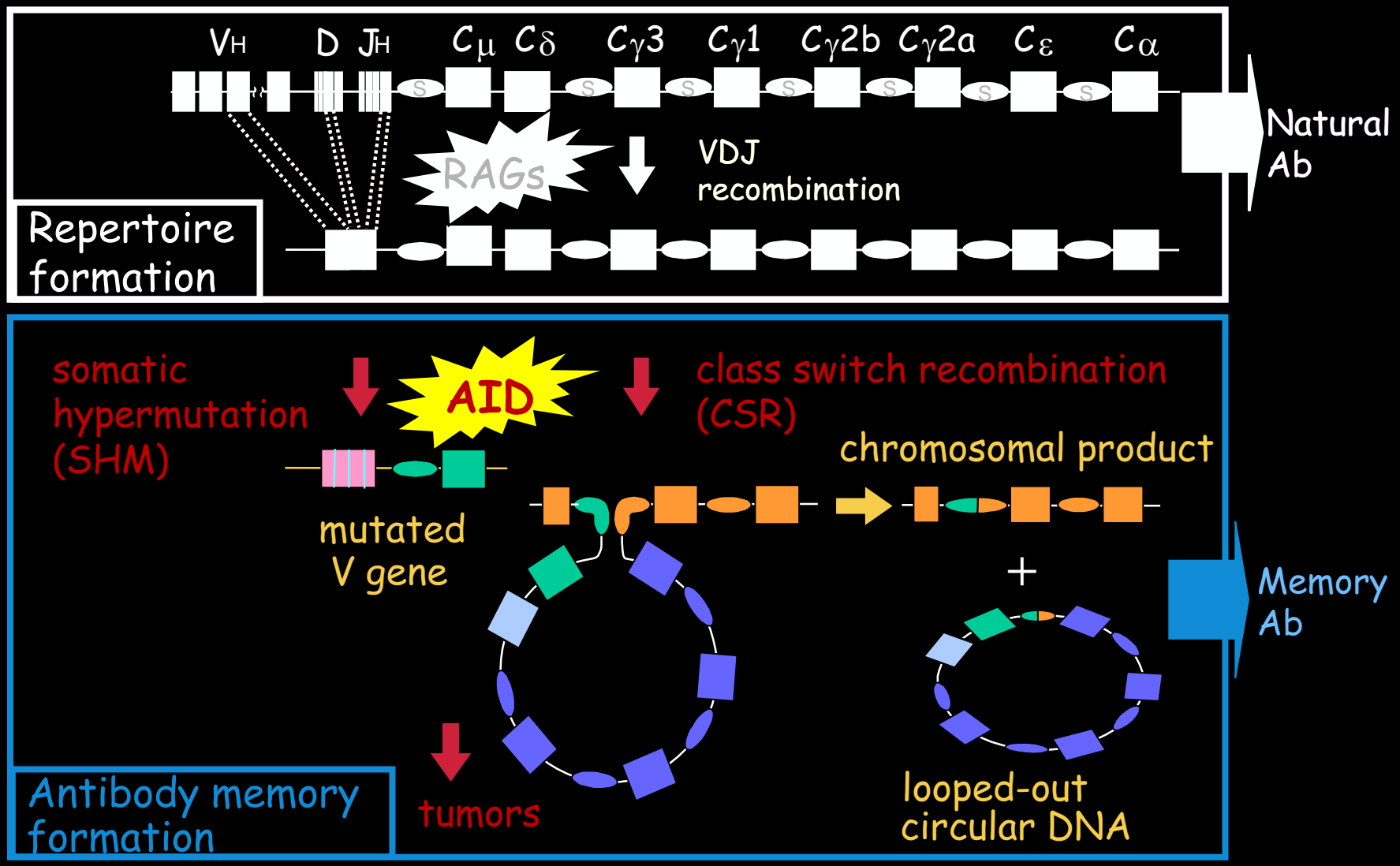
M. Muramatsu *et al.*, Cell (2000)

- AID deficiency in human is the cause of Hyper IgM Syndrome Type II: exactly the same phenotypes as mouse.

P. Revy *et al.*, Cell (2000)

- Thus, AID is the enzyme that engraves antigen memory in the antibody gene, the mechanistic basis of vaccination.

# AID engraves Ab memory in the genome for effective vaccination



# Immune surveillance against cancer

Proposed by
Sir Frank Macfarlane Burnet (1970)

However, numerous attempts to develop immunotherapy were unsuccessful.

# Cancer immunotherapy by boosting accelerators has not given convincing clinical outcomes

1. Cancer vaccine

2. *In vitro* activation of T lymphocytes

3. Cytokine treatment (IFN$\gamma$, IL-2, IL-12 etc)

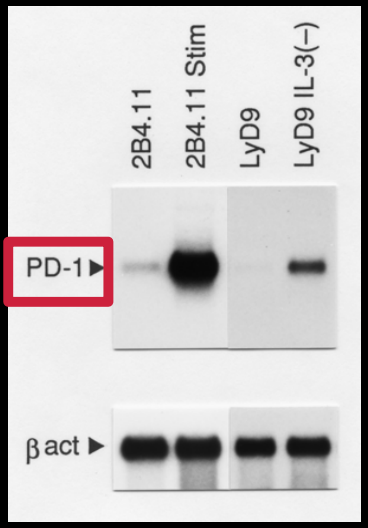
This was because no immune brake molecules were known before 1995

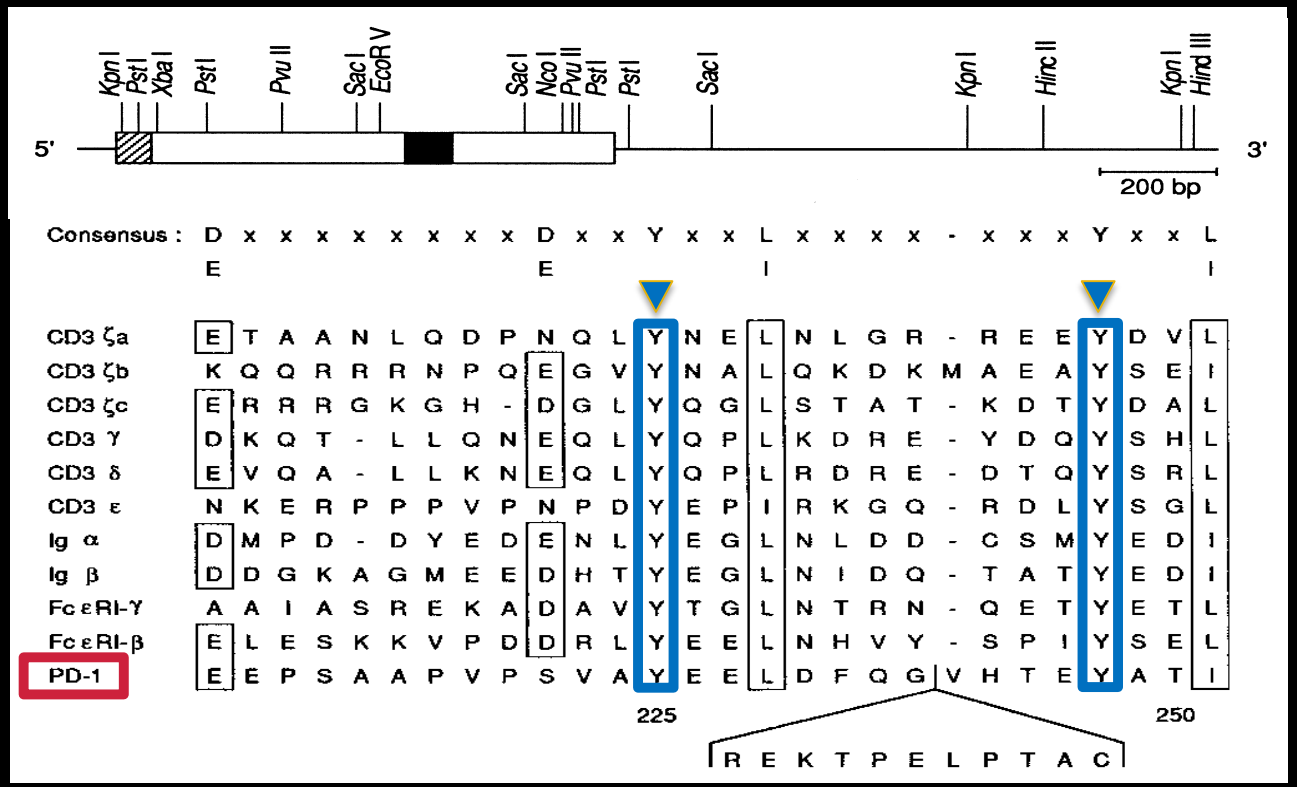
# Brakes and accelerators control immune reactions like those in a car

| action phase | drive | stop | action mode |
|---|---|---|---|
| parking [Activation] | ignition [CD28] | parking brake [CTLA4] | ON/OFF [Drastic] |
| driving [Attack] | accelerator [ICOS] | brake [PD-1] | ~100k/h [Mild] |

# Discovery of PD-1 (programmed death-1) cDNA

Structure of cytoplasmic tail suggests PD-1 is a surface signaling molecule




Y. Ishida Y. Agata

Y. Ishida *et al.*, EMBO J. (1992)

# A long journey to understanding the function of PD-1

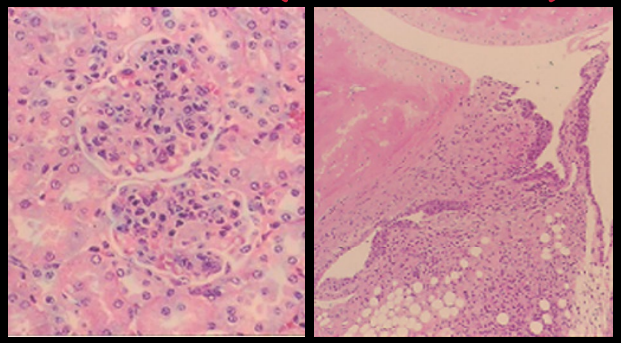
- 1994 PD-1 knock out (KO) on mixed background mice, no phenotype change
- 1996 PD-1 KO on C57BL/6, no phenotype change for 6M

  But over-response to antigen stimulation
- 1997 Nephritis and arthritis after 5M in PD-1 KO x lpr/lpr background
- 1998 Clear autoimmunity in PD-1 KO by 14M

# PD-1 is a negative regulator

Y. Nishimura
*et al.*, Immunity (1999)

C57BL/6
PD-1 KO (Knock Out)



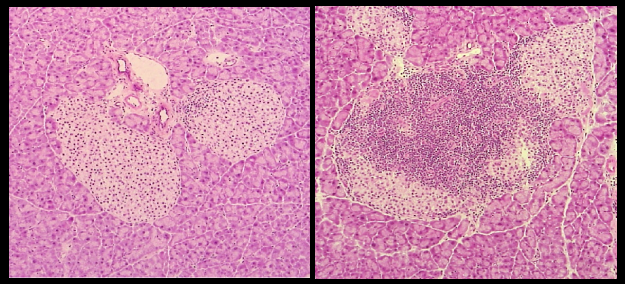
Nephritis Arthritis

BALB/c
WT PD-1 KO

Dilated cardiomyopathy

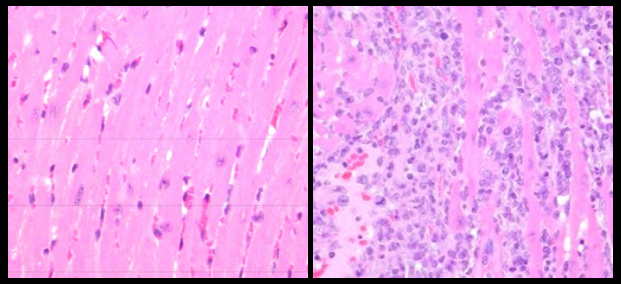
T. Okazaki *et al.*, Nat. Medicine (2003)
J. Wang *et al.*, Int. Immunol. (2010)

NOD NODxPD-1 KO



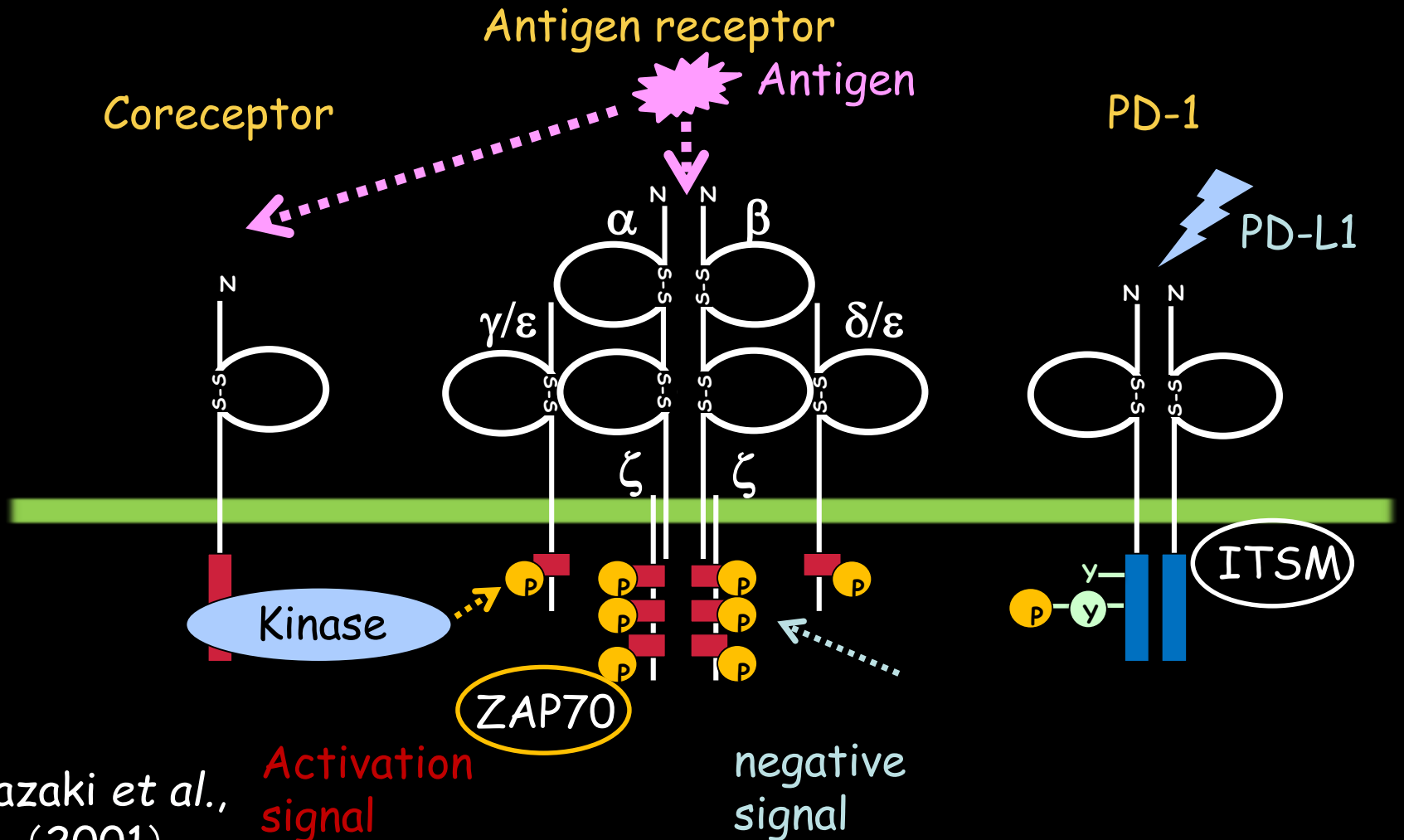
Diabetes

MRL MRLxPD-1 KO



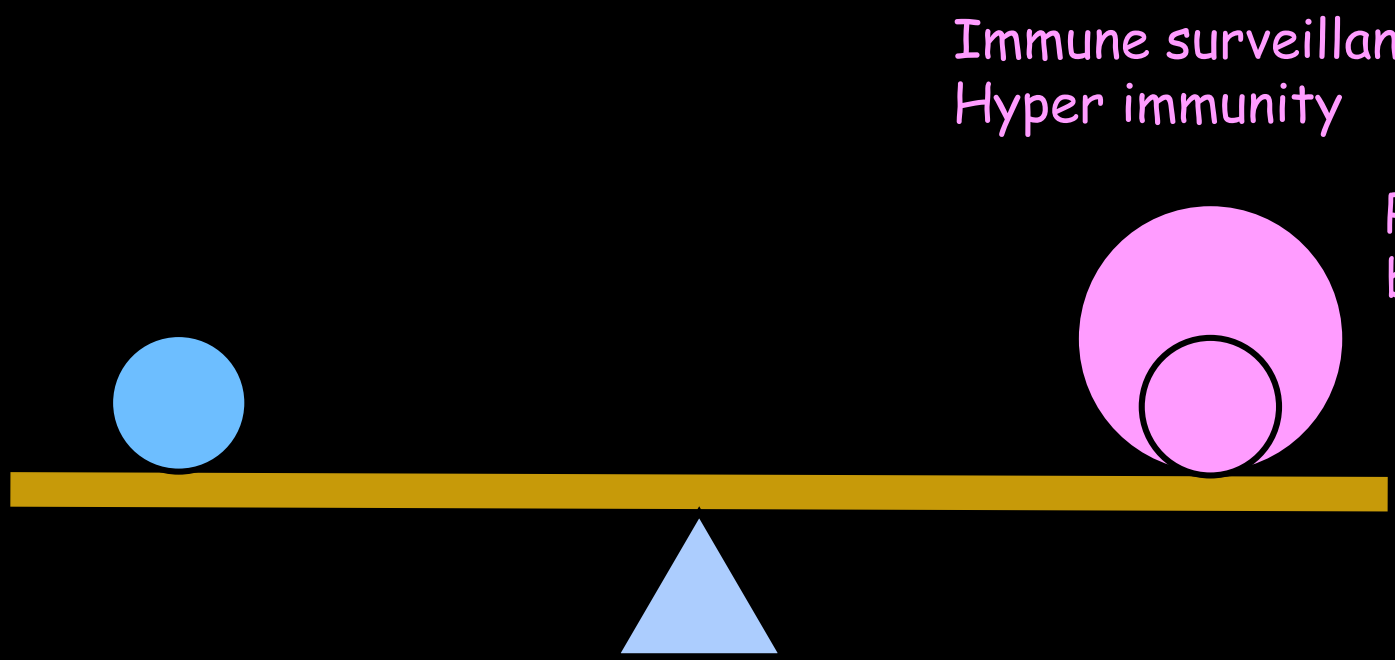
Myocarditis

# Molecular mechanism of immune inhibition by PD-1 signaling



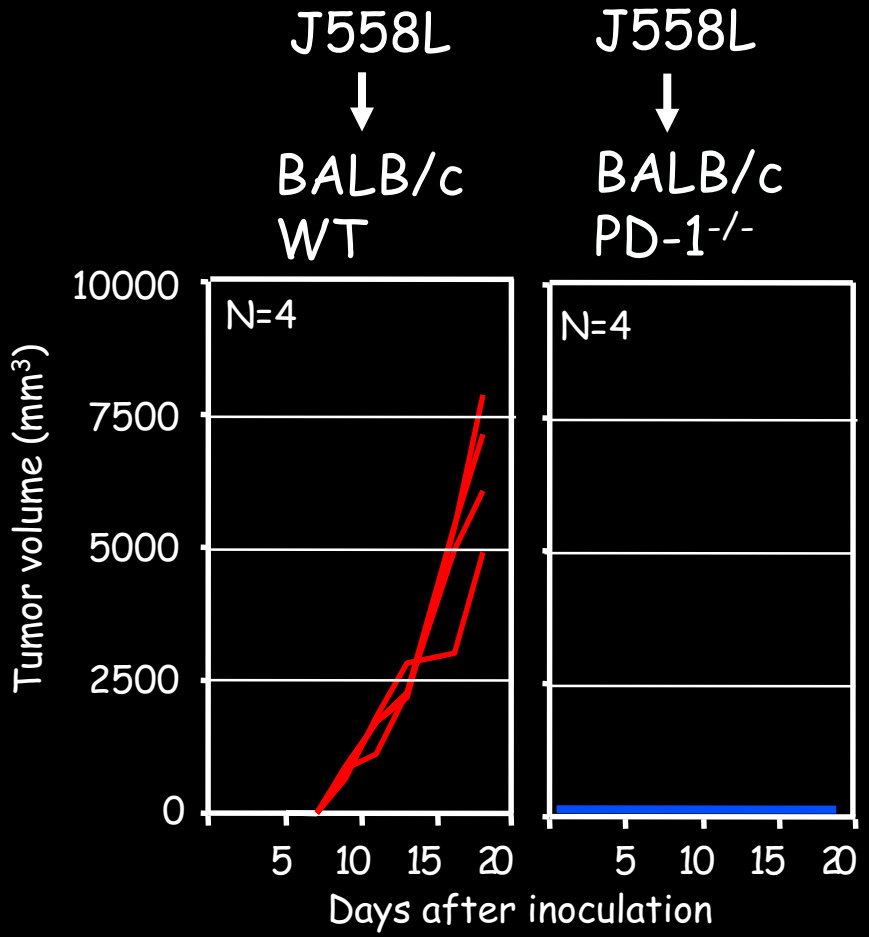

T. Okazaki *et al.*, PNAS (2001)

# Balance between immune surveillance and immune tolerance




Treatment of infectious diseases and cancer
Risk of autoimmunity

# Inhibition of tumorigenesis of myeloma (J558L) in PD-1$^{-/-}$ mice



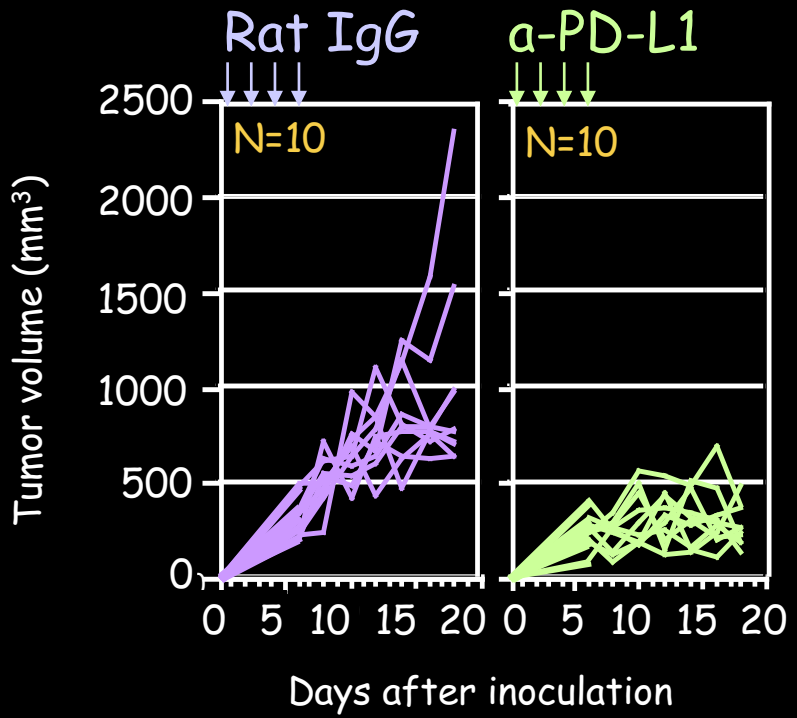

Y. Iwai *et al.*, PNAS (2002)

# Inhibition of tumorigenesis of P815/PD-L1 by anti-PD-L1

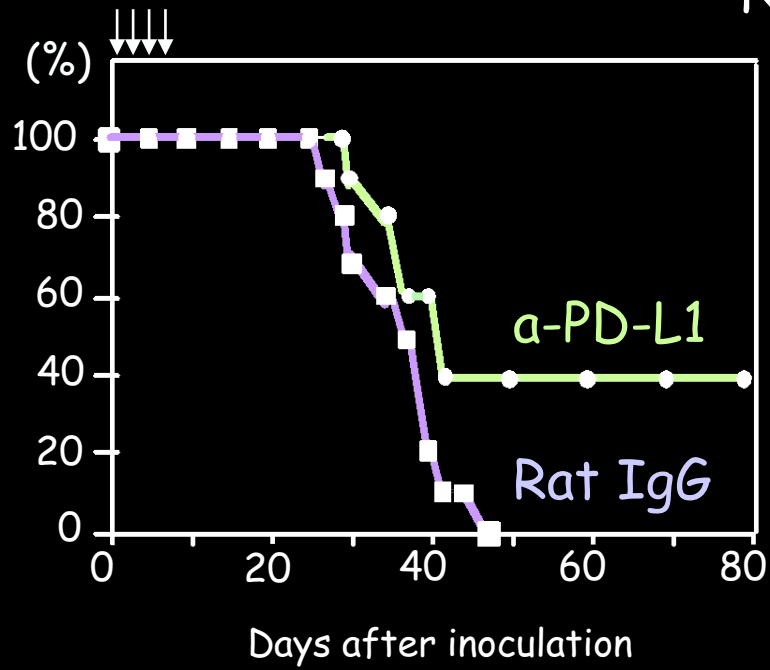
P815/PD-L1 → DBA/2

N. Minato

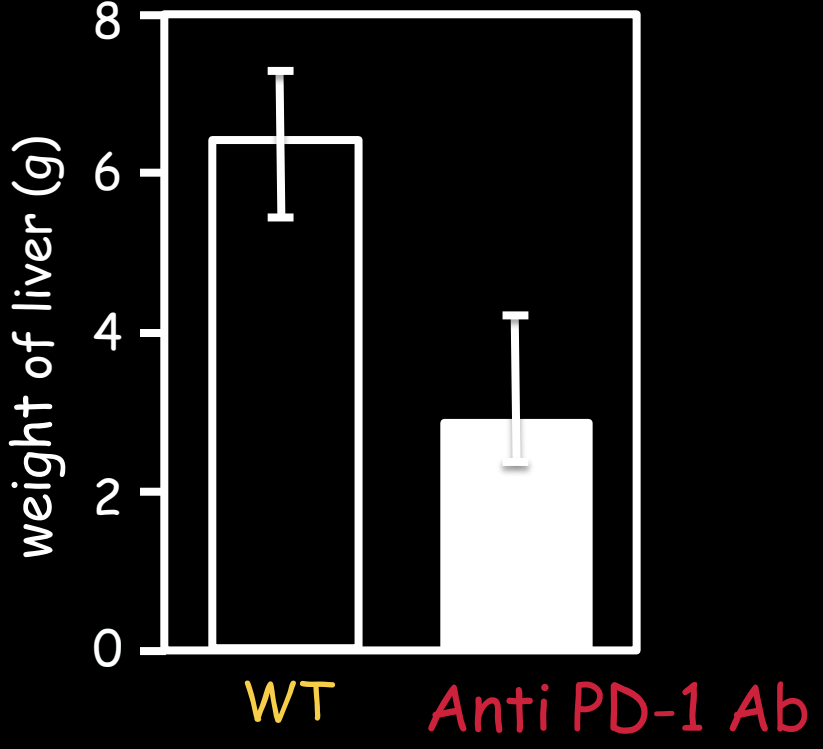
Tumor growth




Survival rate



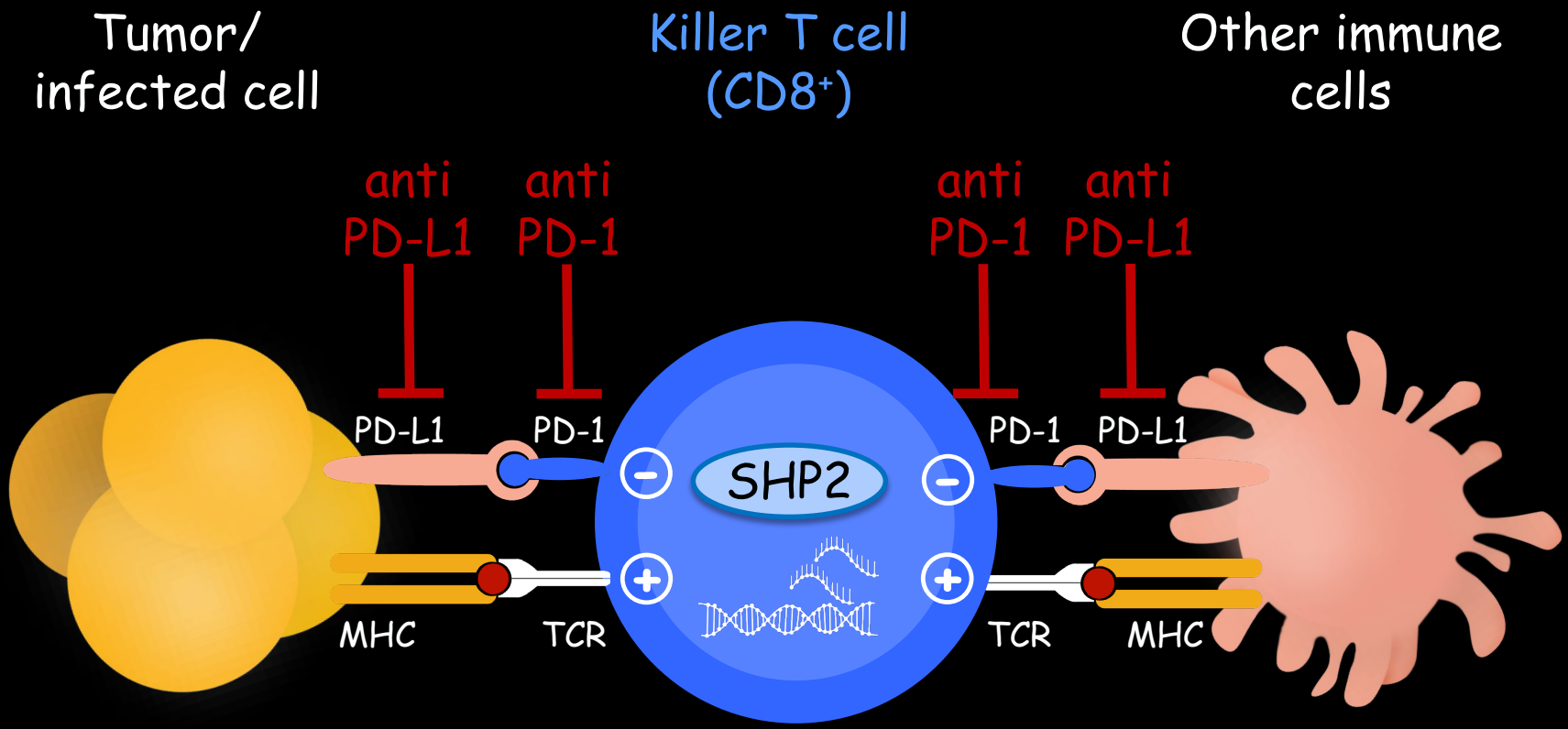
# PD-1 blockade inhibits metastasis of B16 melanoma (mouse model)




Spleen to liver


Y. Iwai *et al.*, Int. Immunol. (2005)

# PD-1 blockade by antibody against either PD-1 or PD-L1 can cure cancer




PD-L1 is expressed on various immune cells

# Human anti-PD-1 antibody

Synthesized in mice containing human immunoglobulin gene by Medarex

Subclass: IgG4S228P

- mutant IgG4 (S228P) stabilizes the protein and reduces ADCC
  (antibody-dependent cell-mediated cytotoxicity)
  KD = 2.6 nmol/L

- Named Nivolumab

**Approved as Investigation New Drug by FDA (USA; Aug 1, 2006)**


La Jolla Institute for Allergy & Immunology
Building Opening Symposium and 5th Ishizaka Lecture
September 14, 2006
Gary Koretzky, Tasuku Honjo, Anjana Rao, Rafi Ahmed, Jim Allison, Ralph Steinman

# Clinical trials began in US (2006) and Japan (2008)

Summary of Phase I clinical trial

296 terminal stage patients recruited
Nivolumab treatment for two years

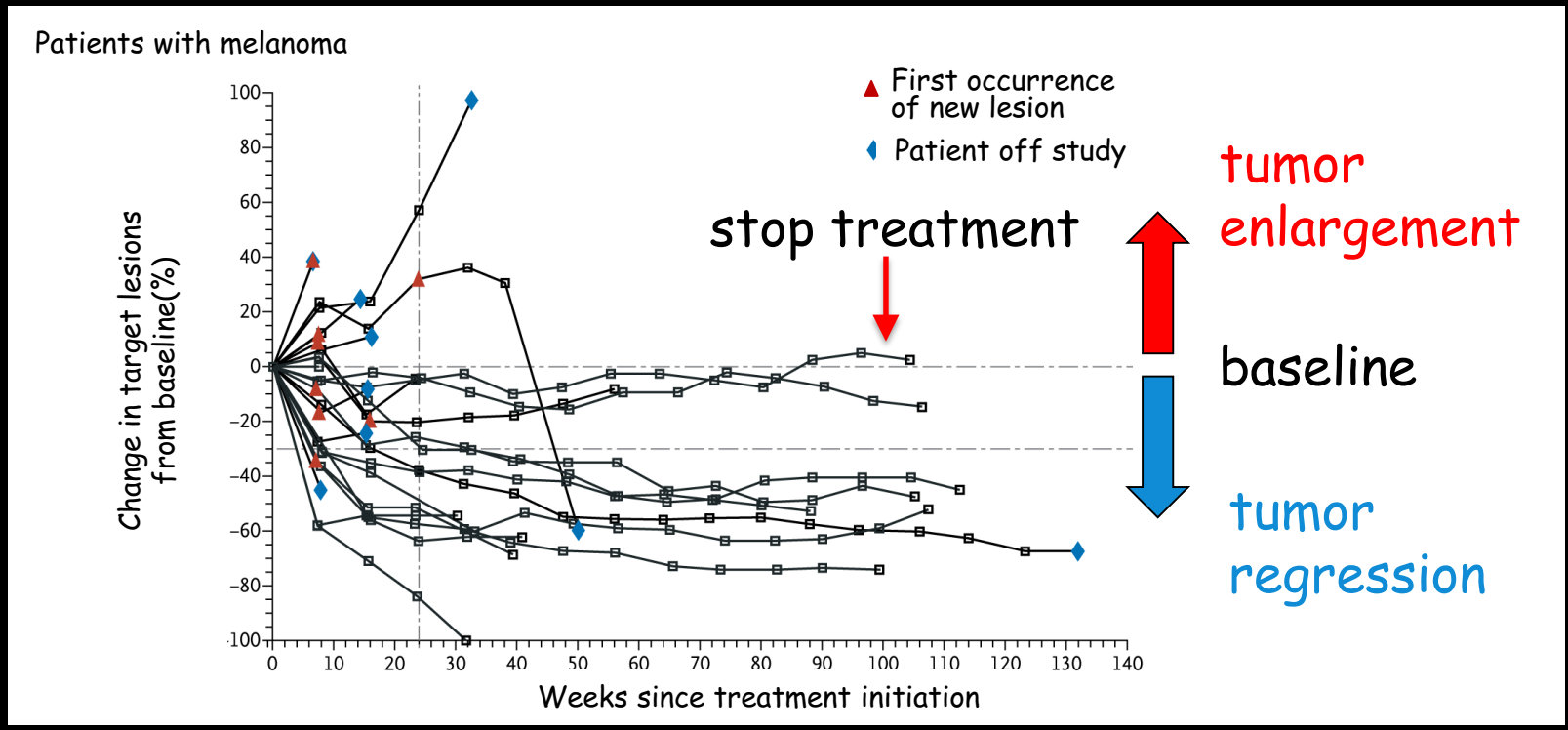
Complete or partial response rates

18% ( 76 patients) of non small cell lung cancer
28% ( 94 patients) of melanoma
27% ( 33 patients) of renal cell carcinoma

S. Topalian *et al.*, NEJM (2012)

# Durable response to PD-1 blockade

"Responses were durable; 20 of 31 responses lasted 1 year or more and some even after stopping therapy"




S. Topalian *et al.*, NEJM (2012)

# Phase II trial of anti-PD-1 antibody in patients with platinum-resistant ovarian cancer

| Dose | total (n) | CR | PR | SD | PD | NE | RR | DCR |
|---|---|---|---|---|---|---|---|---|
| 1 mg/kg | 10 | 0 | 1 | 4 | 4 | 1 | 1/10 (10%) | 5/10 (50%) |
| 3 mg/kg | 10 | 2 | 0 | 2 | 6 | 0 | 2/10 (20%) | 4/10 (40%) |
| Total | 20 | 2 | 1 | 6 | 10 | 1 | 3/20 (15%) | 9/20 (45%) |

Oct 21, 2011-Dec 7, 2014

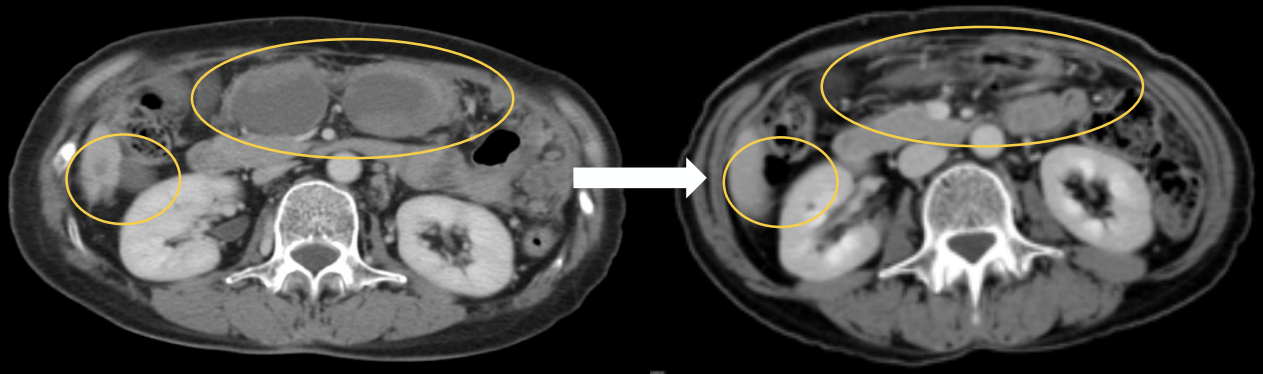
Tumor growth stopped in 40-50% of terminal stage patients

J. Hamanishi *et al.*, J. Clin. Oncol. (2015)

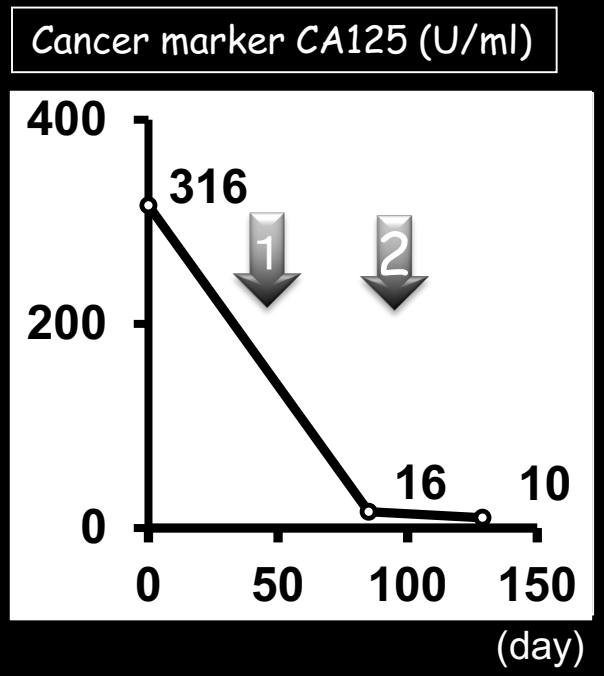
# A responder with ovarian cancer (clear cell): Nivolumab 3mg/kg

History: 60 yr. Stage Ic with progressive disease after
RSO, MMC/CPT11*3, SCH+BSO, CPT/CDDP*5, TC*2

Peritoneal dissemination ⇒ disappeared



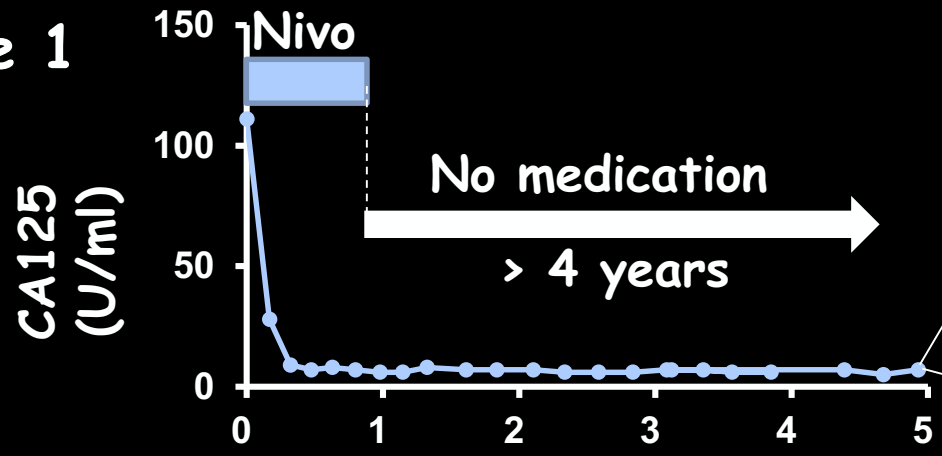
Baseline 4 months



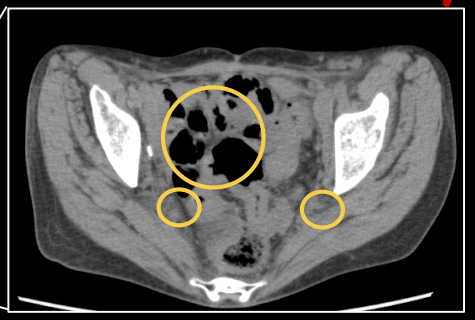

J. Hamanishi *et al.*, J. Clin. Oncol. (2015)

# Durable complete responses of ovarian cancer patients to Nivolumab

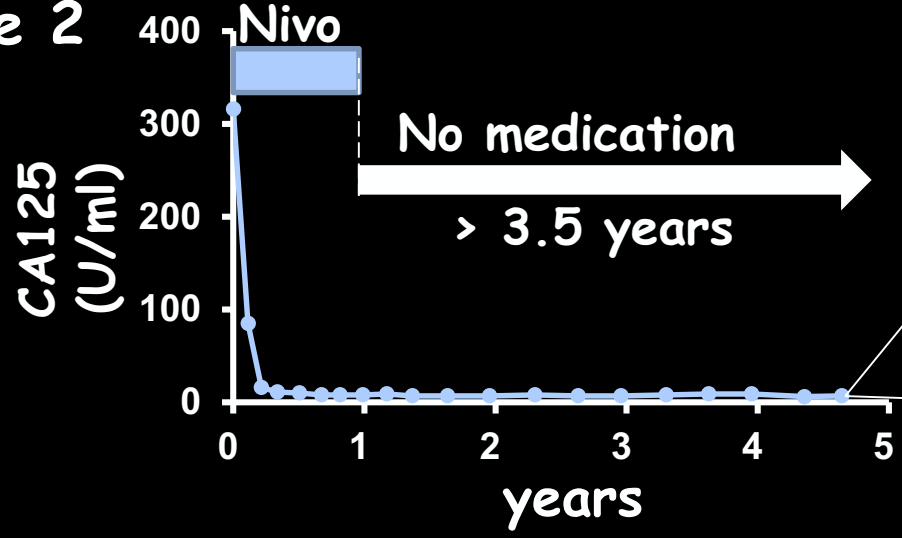
Case 1



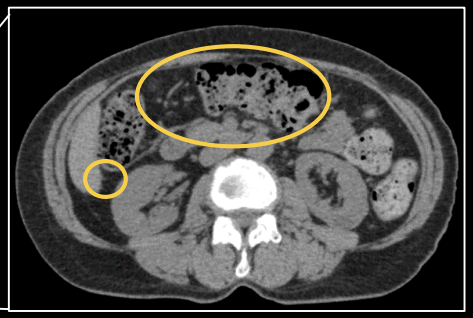

No recurrence > 5 years



Case 2

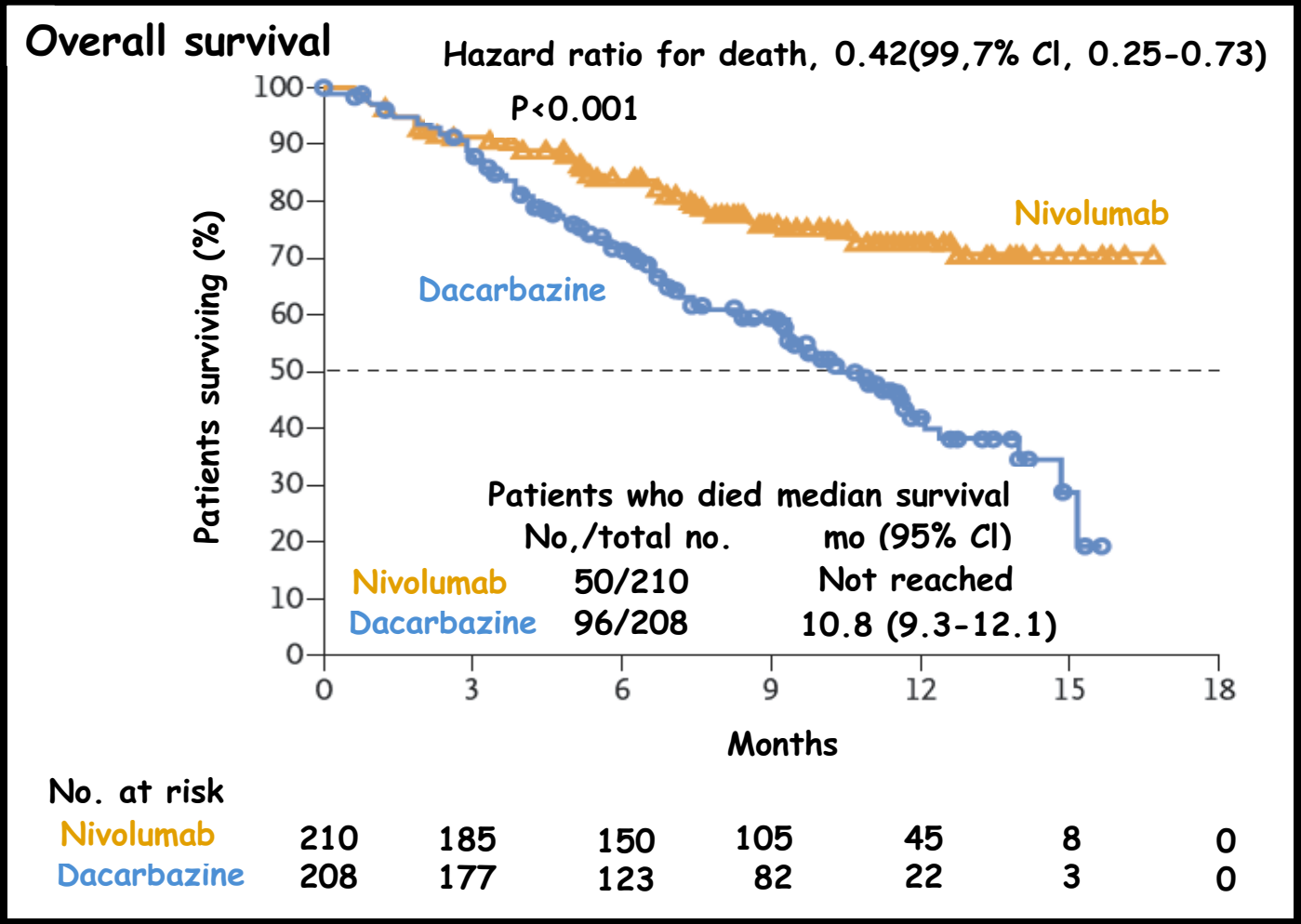



No recurrence > 4.5 years



J. Hamanishi, The International Federation of Gynecology and Obstetrics FIGO (2018)

# Randomized Study on Untreated Melanoma Patients with Nivolumab and Dacarbazine (chemotherapy)




C. Robert *et al.*, NEJM (2015)

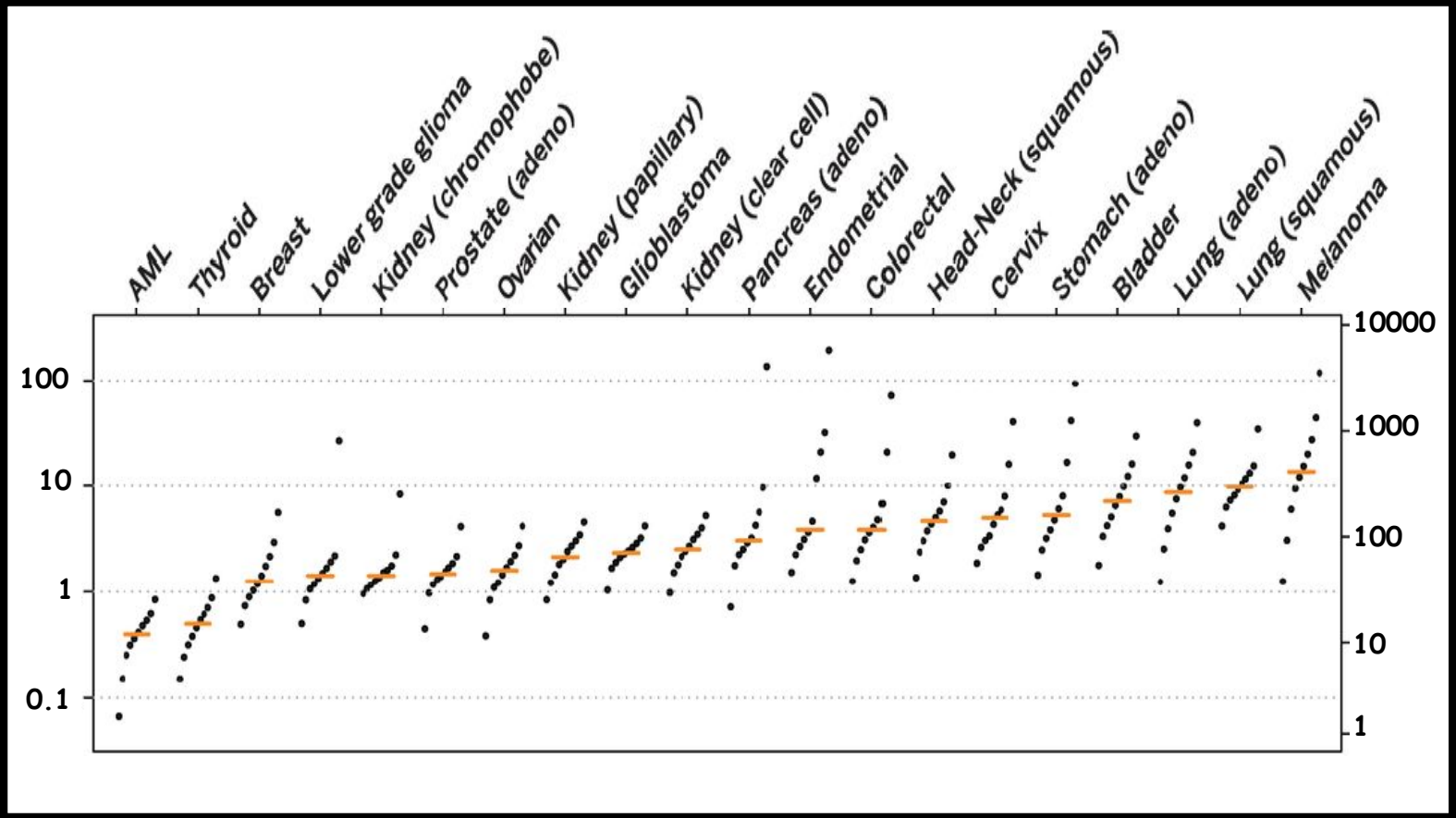
# Cancers approved for PD-1 blockade therapy

- 2014 melanoma
- 2015 lung cancer
- 2016 renal cancer
  Hodgkin's lymphoma
  head and neck cancers
  urothelial cancer
- 2017 colorectal cancer
  gastric cancer
  hepatocellular carcinoma
  Merkel cancer
  all highly mutated cancers
- 2018 cervical cancer
  primary mediastinal large B-Cell lymphoma

# Paradigm shift of cancer therapy by anti-PD-1 treatment

1. Less adverse effects because normal cells are unaffected
2. Effective for a wide range of tumors (more than 1000 clinical trials)
3. Durable effects to responders after stopping treatment

# Cancer cells accumulate mutations




I. Martincorena *et al.*, Science (2015)

# What we learned from huge cancer genome projects

1. Cancer cells accumulate a large number of mutations to express neo-antigens that can be recognized by the immune system as non-self. This is why cancer immunotherapy is effective.

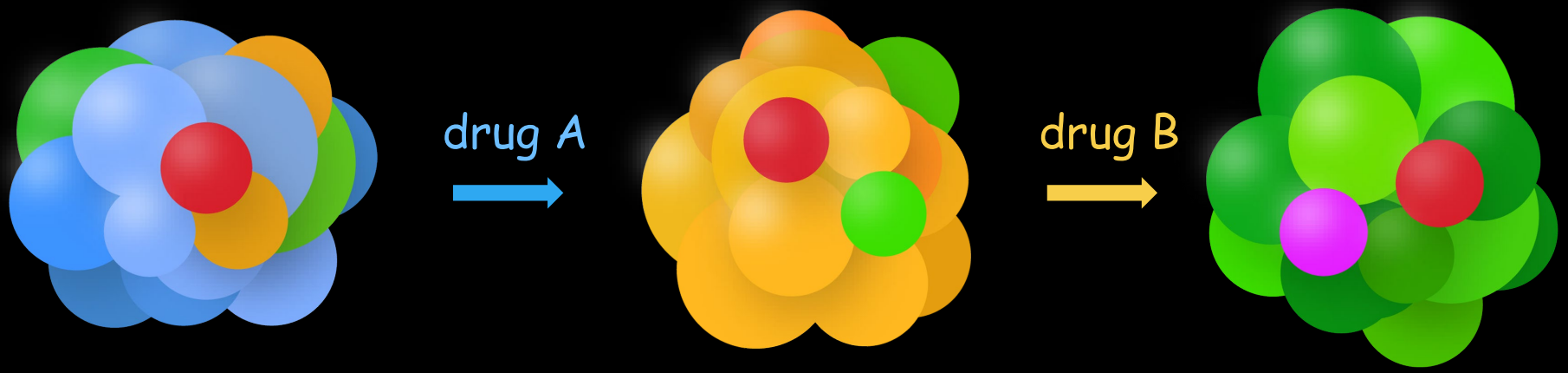
2. Too many mutations to pinpoint the dominant mutations for targeted chemotherapy.

# Continuous mutations generate resistant tumor cells

cancer cells

Selection mutagenesis
Resistant cells grow

Selection mutagenesis
Resistant cells grow




Lymphocytes can recognize many more mutants & attack them

# Current issues in PD-1 blockade therapy

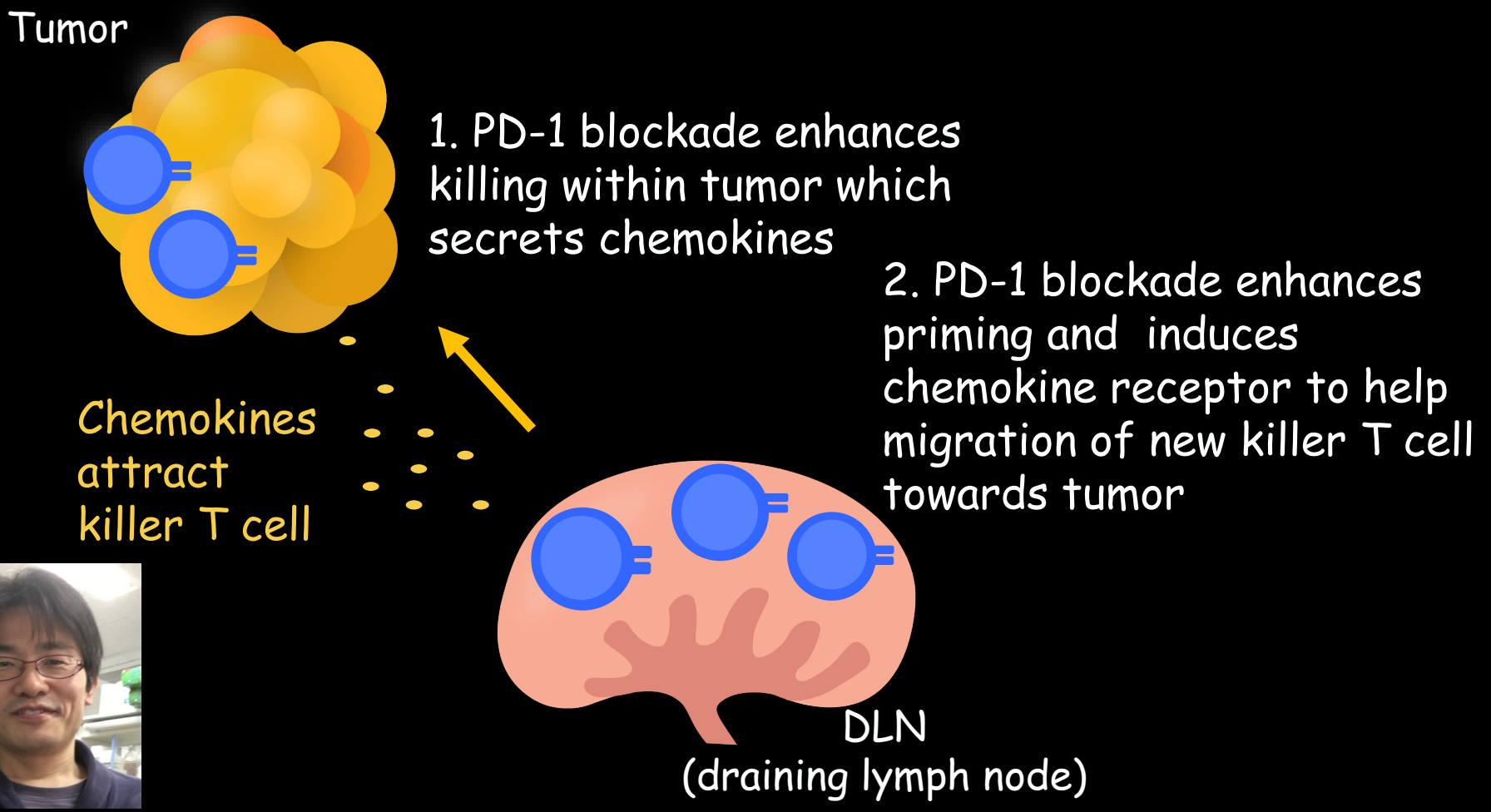
## Biomarkers for responders

- High mutagenesis in tumors
- Potency of individual's immunity

## Improvement of immunotherapy

- Accessibility of killer T cells to tumor sites
- Potentiation of killer T cell function

# PD-1 blockade initiates killer T cell expansion in lymph nodes



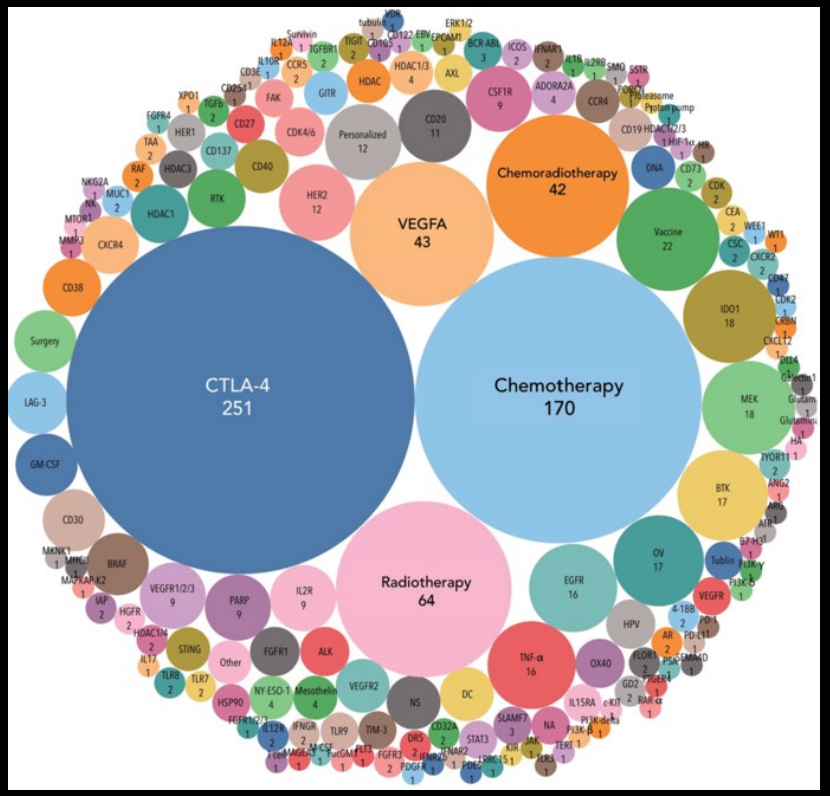



K. Chamoto *et al.*, PNAS (2017)

# Cancer immunotherapy by PD-1-based combination studies underway in 2017

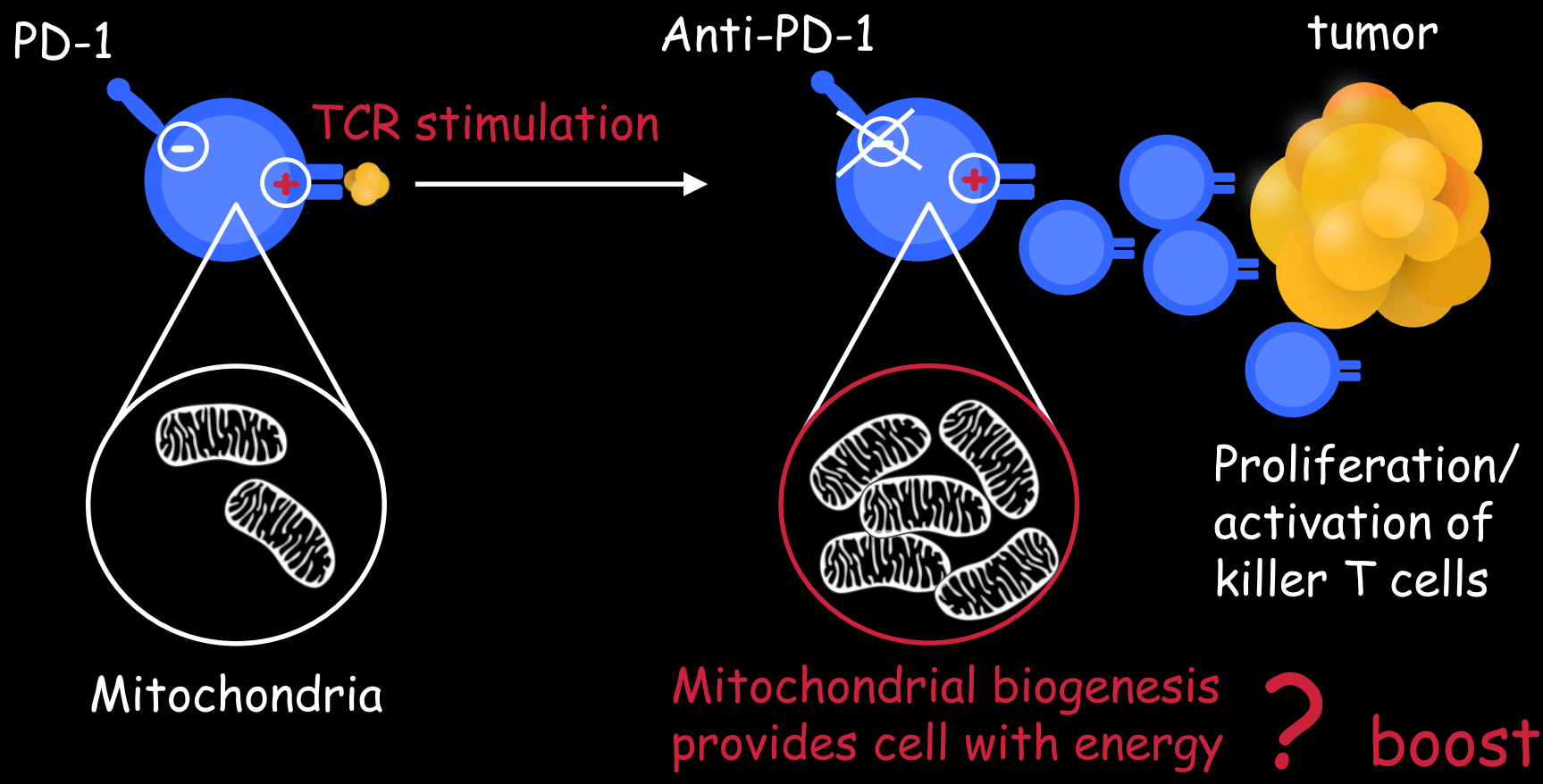
Numbers of PD-1 blockade trials using combinations with :

1. Anti-CTLA-4 agents: 251
2. Chemotherapies: 170
3. Radiotherapies: 64
4. Anti-VEGFA agents: 43
5. Chemoradiotherapy combos: 42

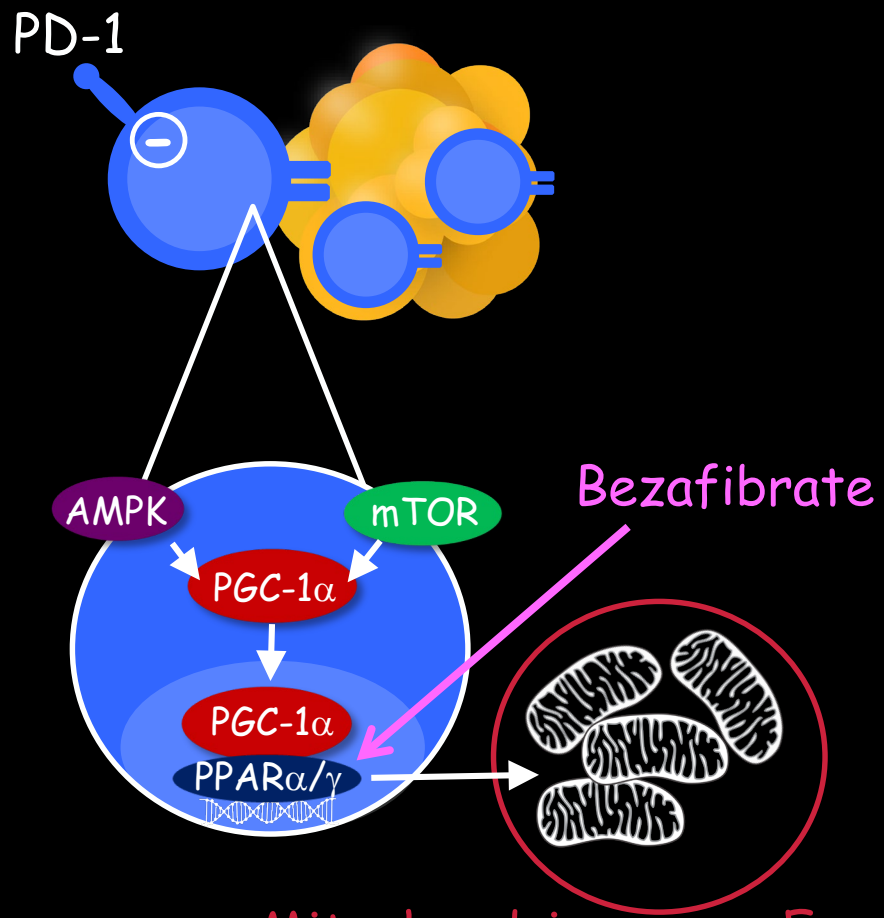


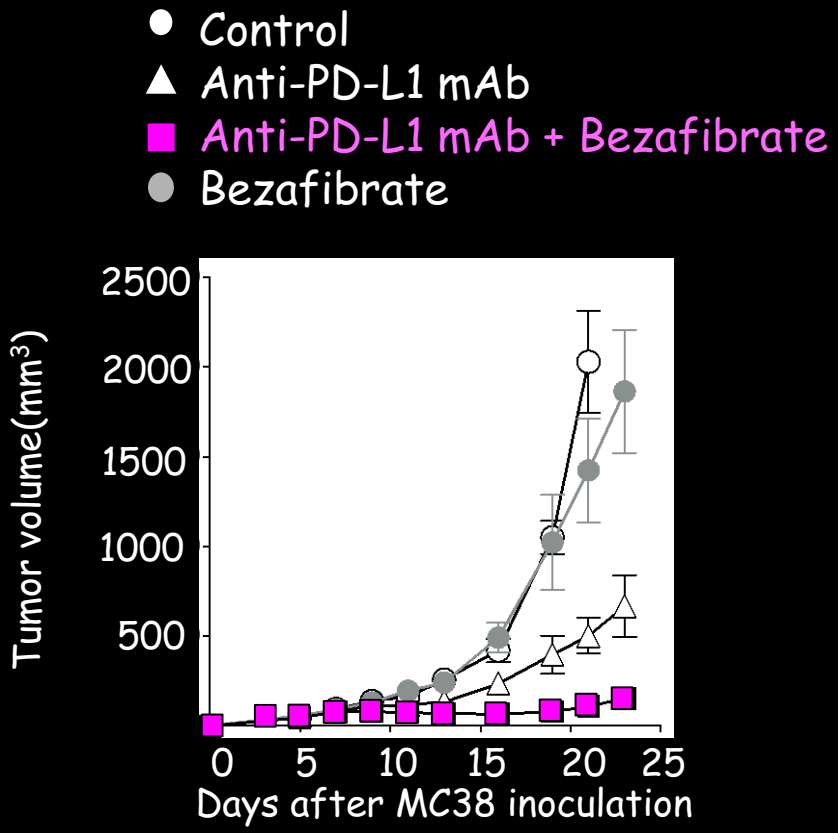

J. Tang *et al.*, Ann. Oncol. (2018)

# Requirement of mitochondrial activation for killer T cell activation and proliferation



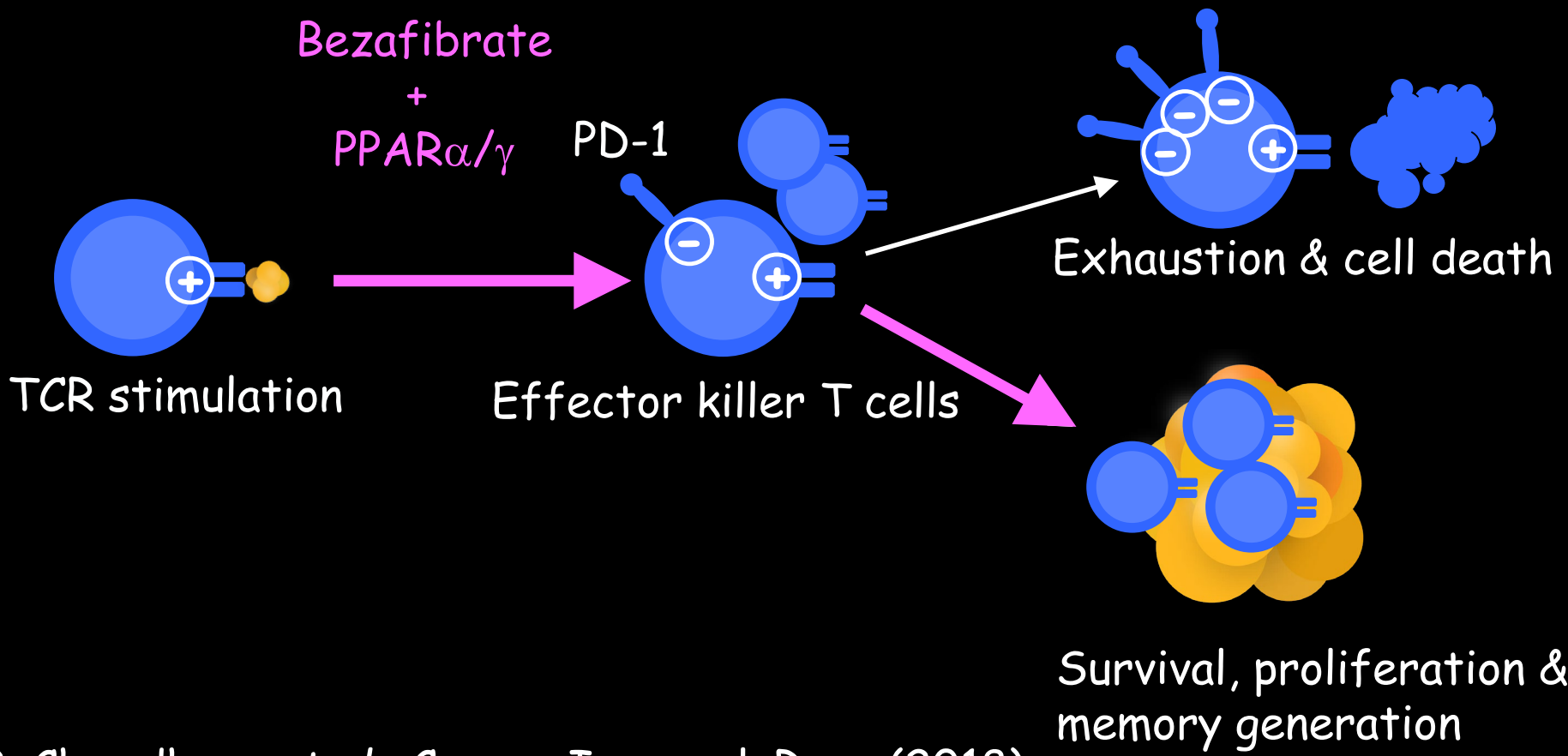
# Activation of PGC-1〈/PPAR complex improves the efficacy of PD-1 blockade



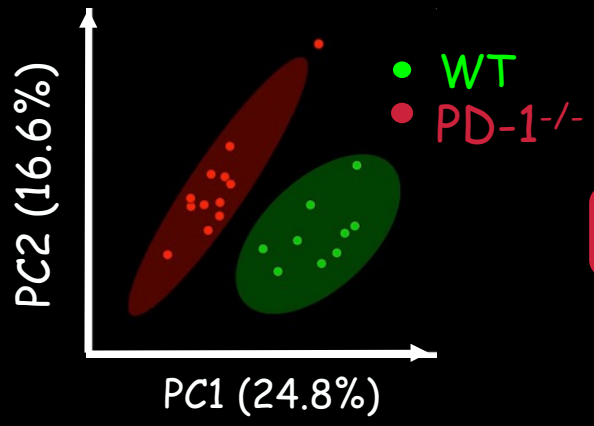

K. Chamoto *et al.*, PNAS (2017)

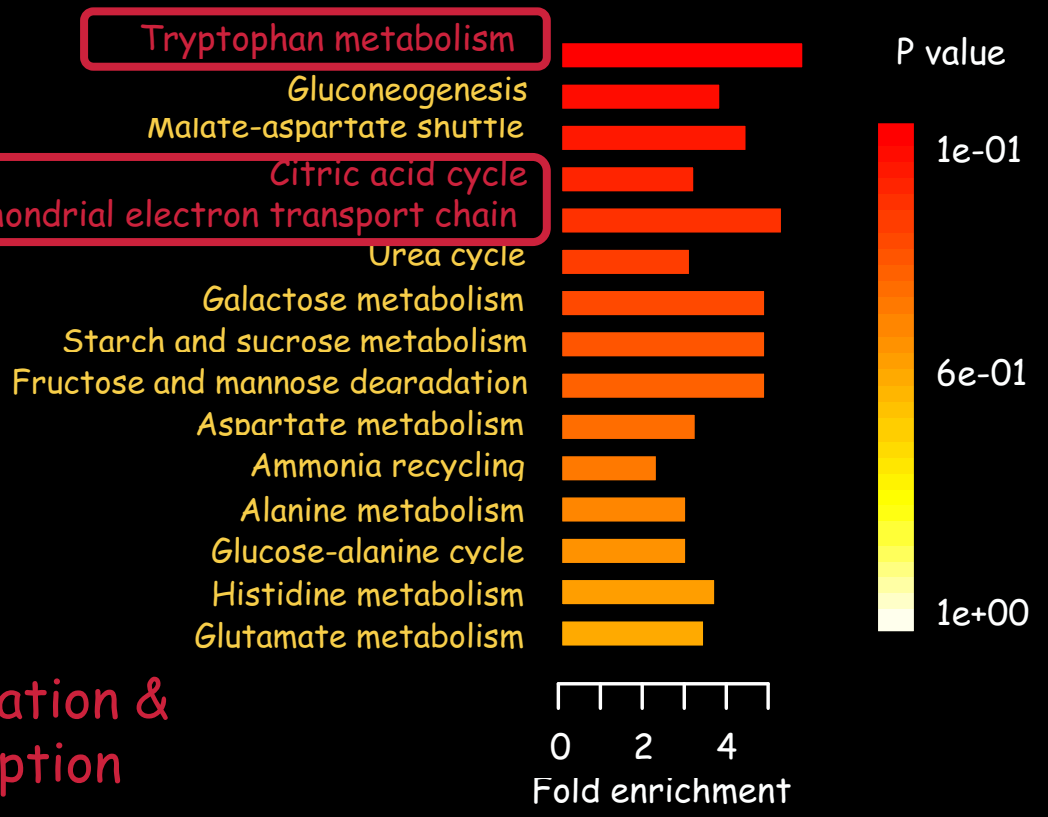
# Bezafibrate increases killer T cell proliferation and blocks cell death




P. Chowdhury *et al.*, Cancer Immunol. Res., (2018)

# Hyperimmune activity can be read in blood biochemistry of PD-1$^{-/-}$ mice



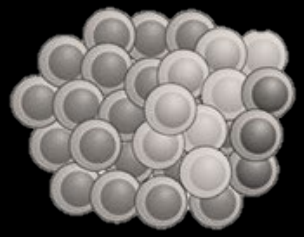




Mitochondrial activation &
Tryptophan consumption

Sidonia Fagarasan

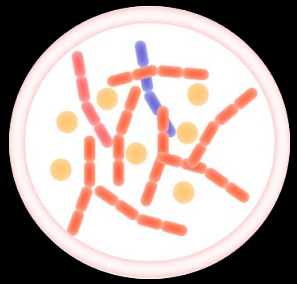
M. Miyajima, B. Zhang *et al.*, Nat. Immunol. (2017)

# PD-1$^{-/-}$ mice biology is very complex



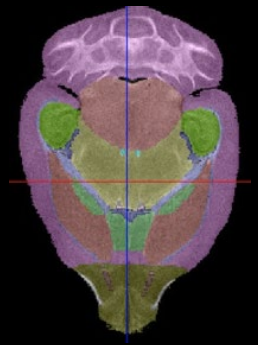
Expansion
T cells

Consumption of
metabolites



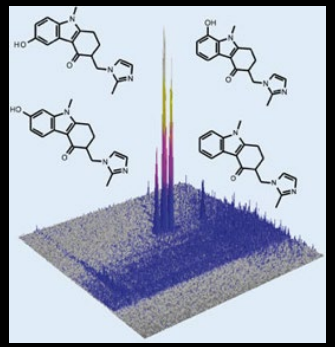
H. Nishimura et al.
Immunity 1999

Gut bacterial
changes



Behavioral
changes

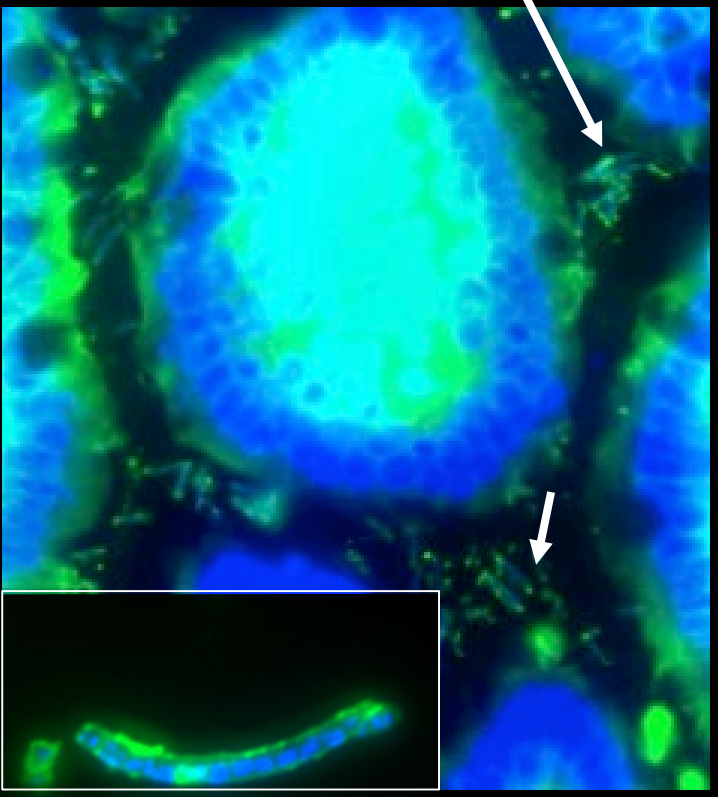
Metabolite
shift



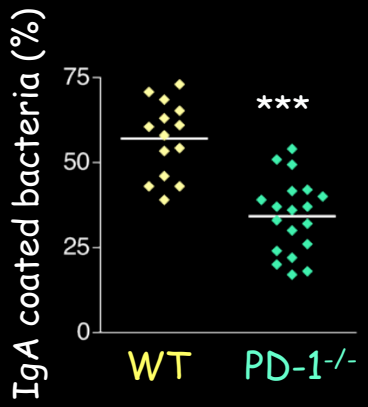
M. Miyajima, B. Zhang *et al.*, Nat. Immunol. (2017)

# PD-1 selects IgA critical to microbiota regulation

IgA-coated bacteria in the gut

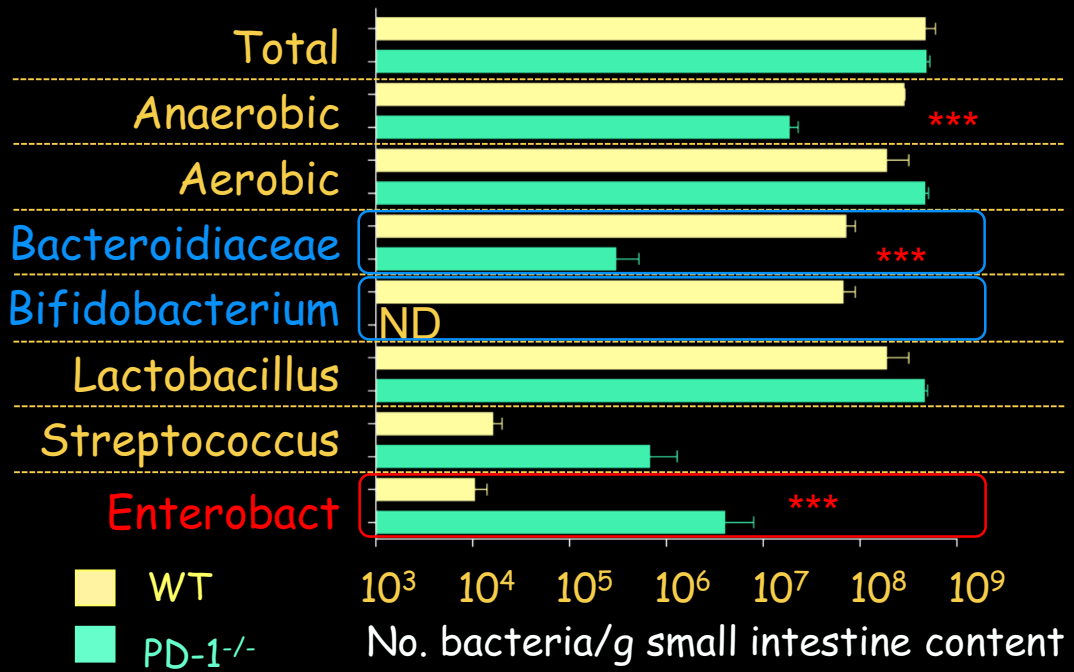


IgA DAPI



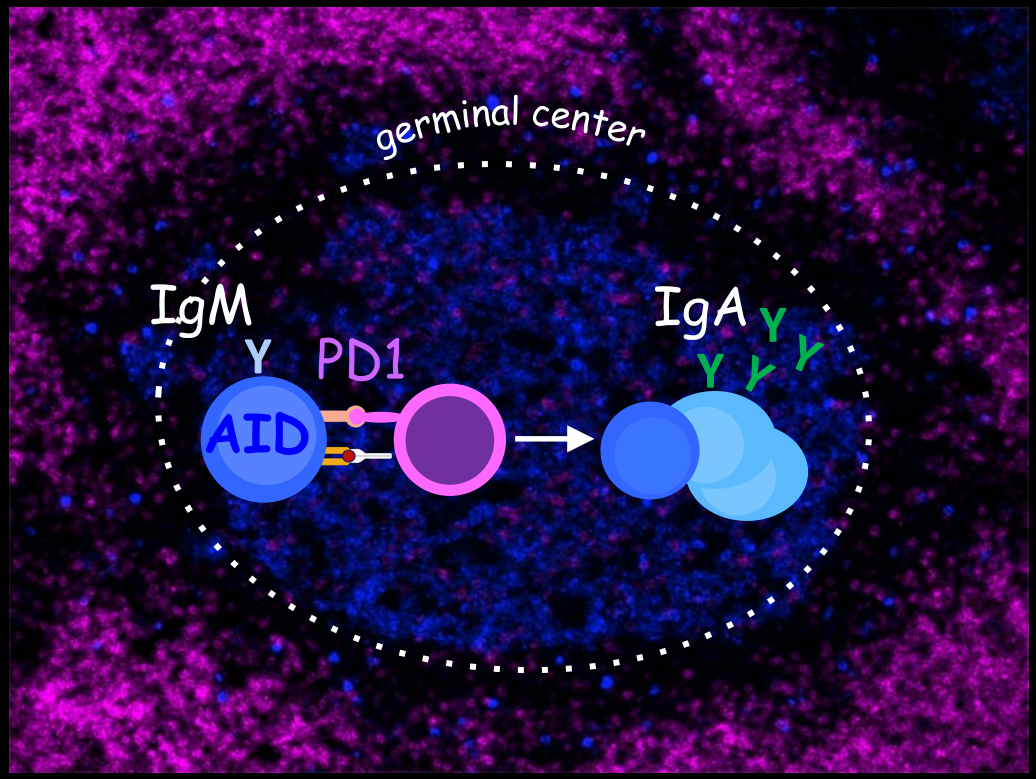

Less IgA-coating of bacteria in PD-1$^{-/-}$ mice

Bacterial dysbiosis




S. Kawamoto *et al.*, Science (2012)

# AID and PD-1 cooperate in germinal centers for high affinity IgA selection to maintain microbiome

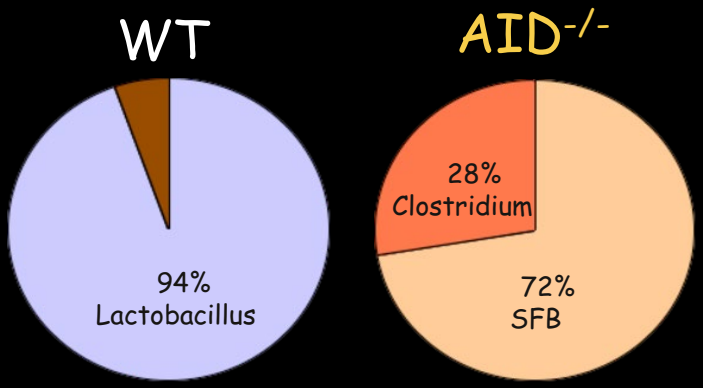



GC B cells
AID$^{hi}$

GC T cells
PD1$^{hi}$

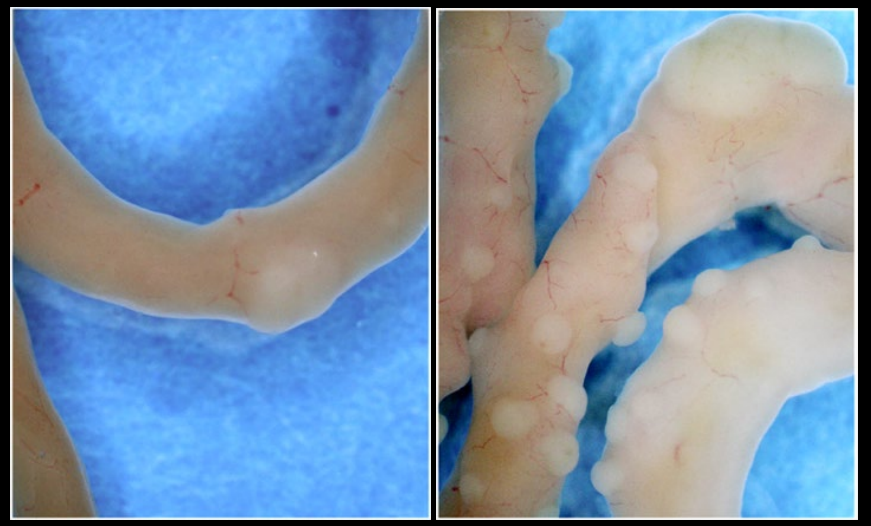
S. Kawamoto *et al.*, Science (2012)

# Critical role of AID for controlling microbiota & whole body immune homeostasis

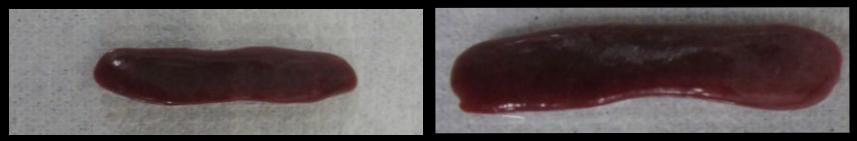




Mucosal immune activation



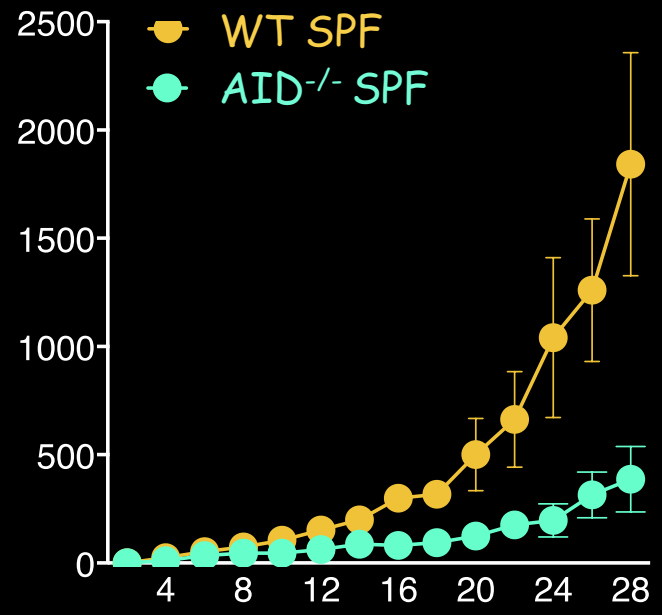

Systemic immune activation, spleen



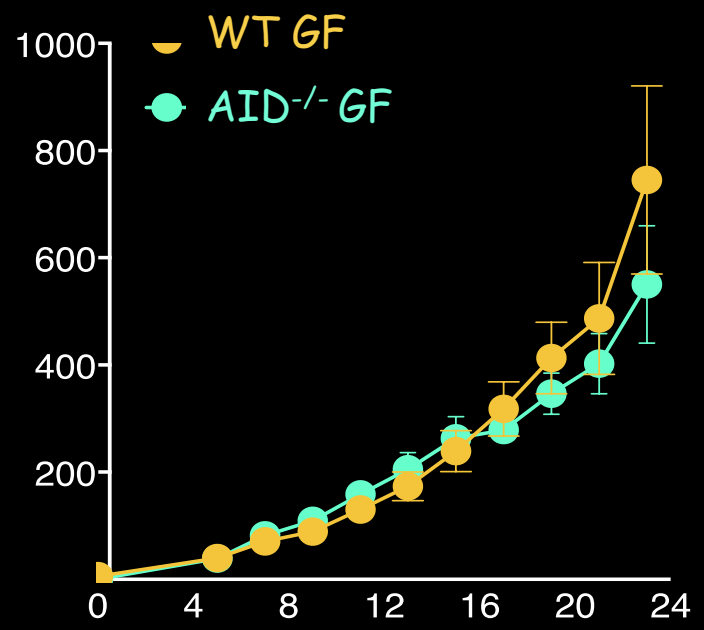

S. Fagarasan *et al.*, Science (2002)
K. Suzuki *et al.*, PNAS (2004)

# Enhanced anti-tumor immunity in AID$^{-/-}$ mice depends on microbiota

Specific-pathogen free

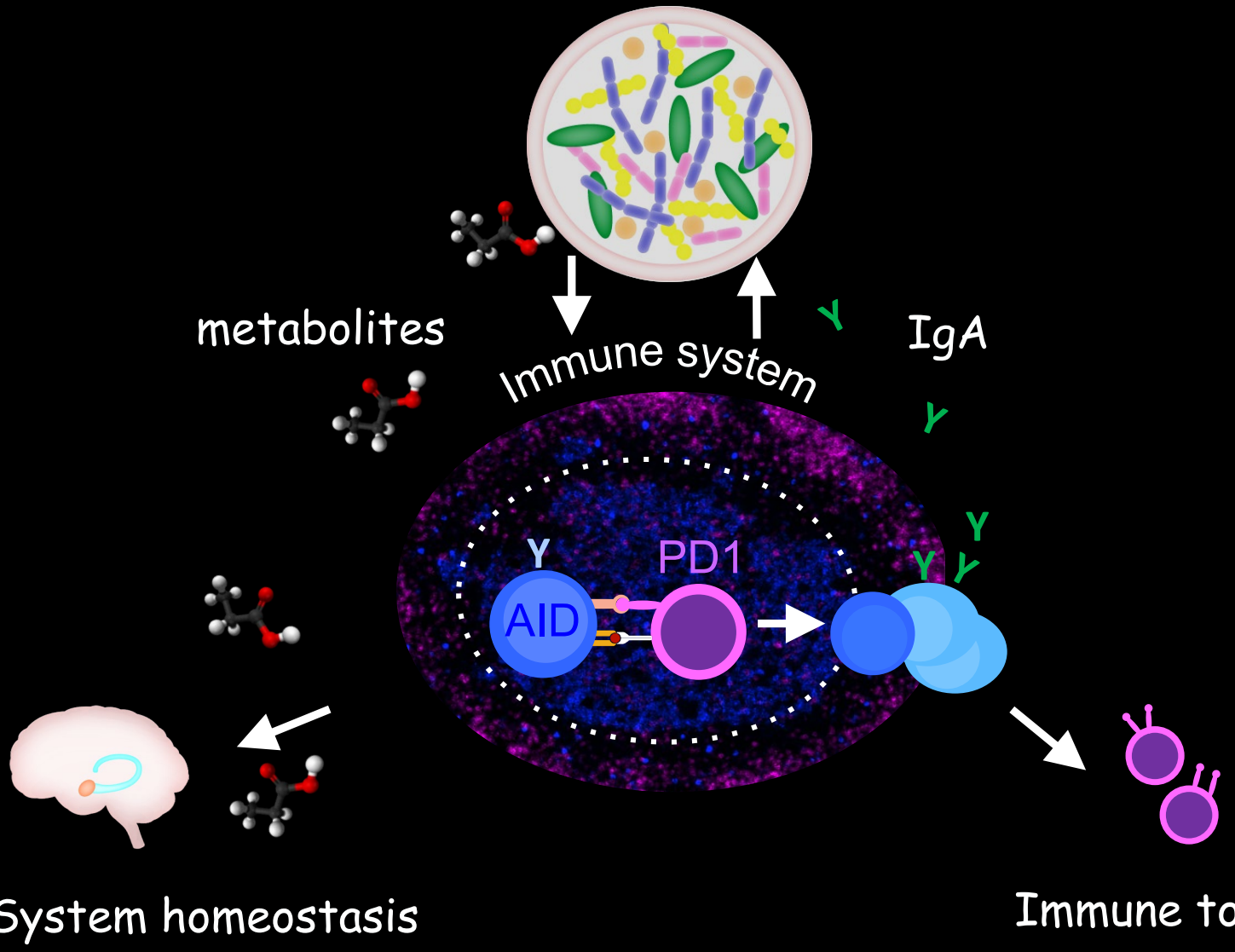



Germ free

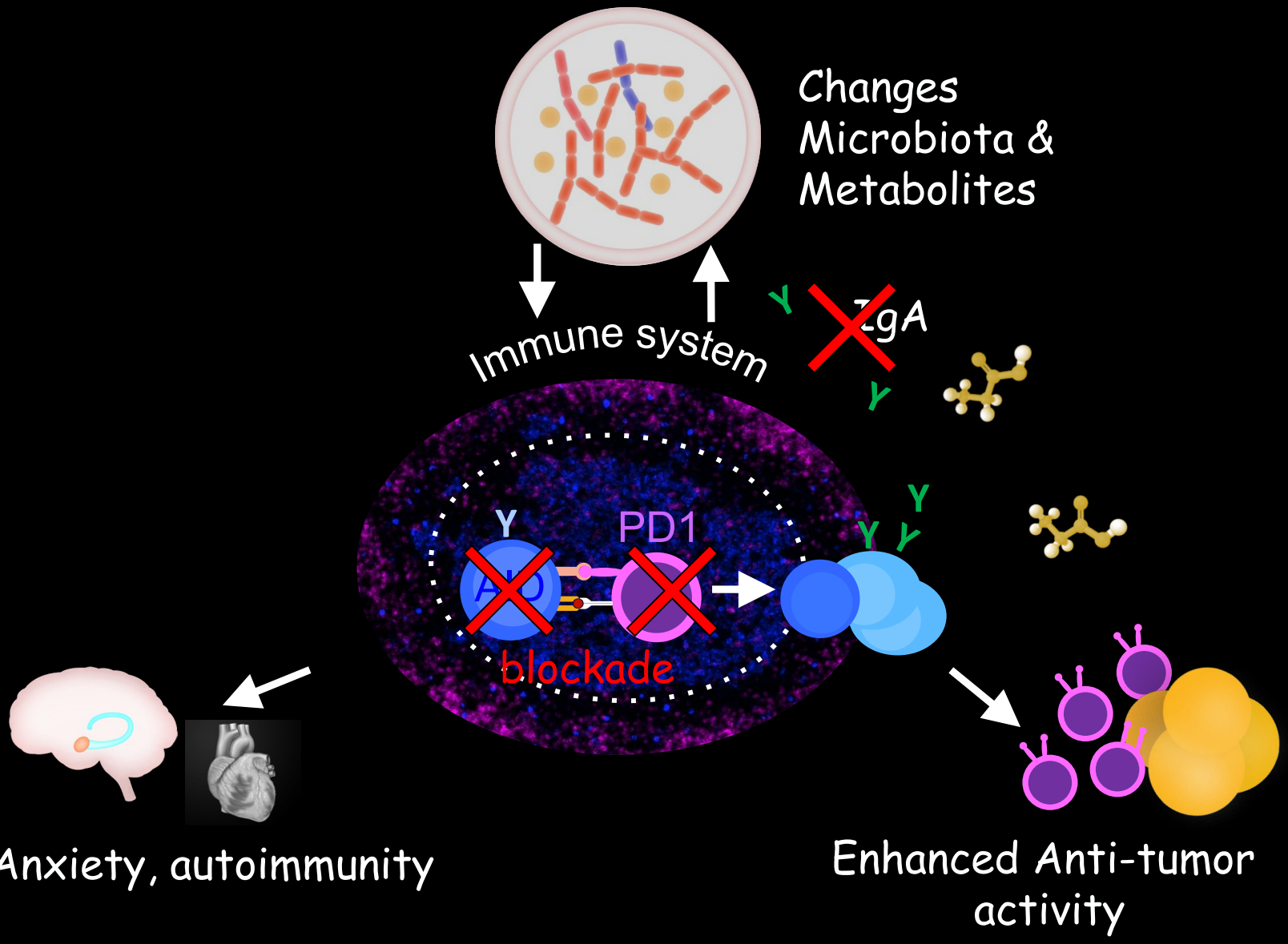


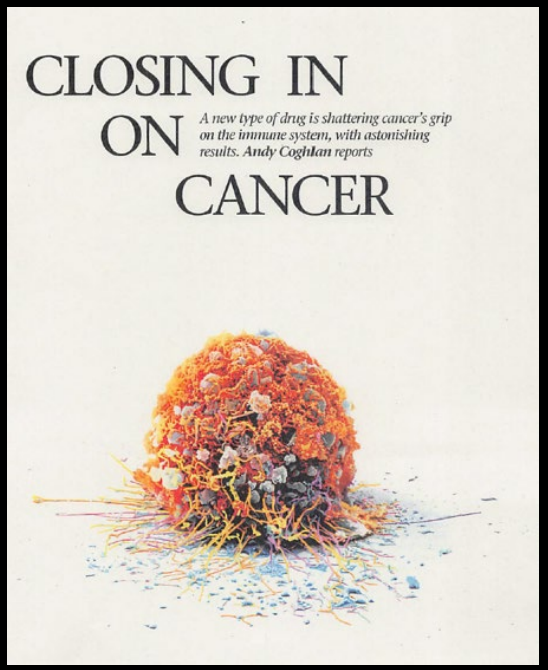

M. Akrami, R. Menzies, M. Miyajima, Y. Nakajima. unpublished data

# Microbiome-immune system regulation



# Microbiome-immune system regulation






# CLOSING IN ON CANCER

Andy Coghlan
New Scientist, 5 March 2016

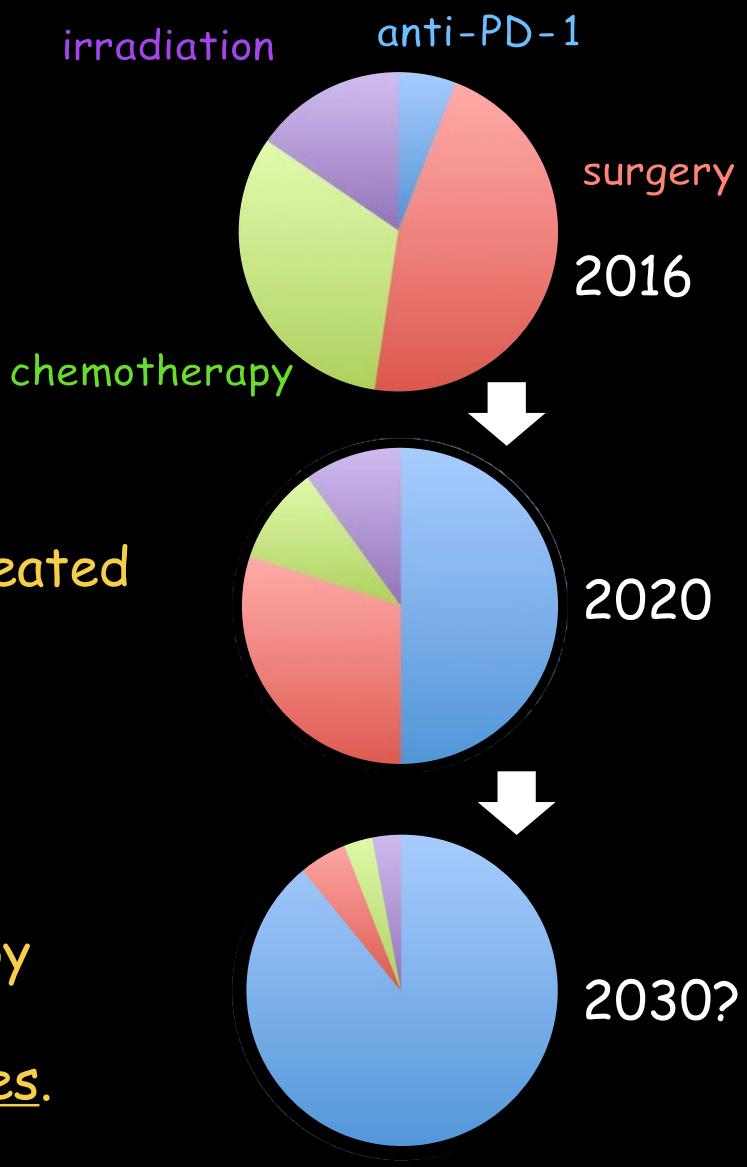
"We're at the point where we've discovered the cancer equivalent of penicillin" says Chen. Although penicillin itself couldn't cure all infections, it gave rise to a whole generation of antibiotics that changed medicine forever, consigning most previously fatal infections to history.

# Future prospects in cancer therapy

1. Efficacy of PD-1 blockade therapy improved.

2. Many more cancers may be treated by immunotherapy.

3. Cancer may not completely disappear, but be controlled by immunotherapy. Cancer may become one of chronic diseases.

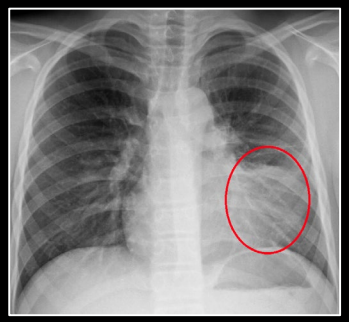


# Enormous benefit by acquired immunity

20th century

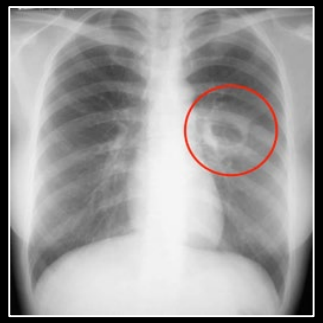
Eradication of infectious diseases
by vaccination and antibiotics

Pneumonia




Penicillin

Tuberculosis

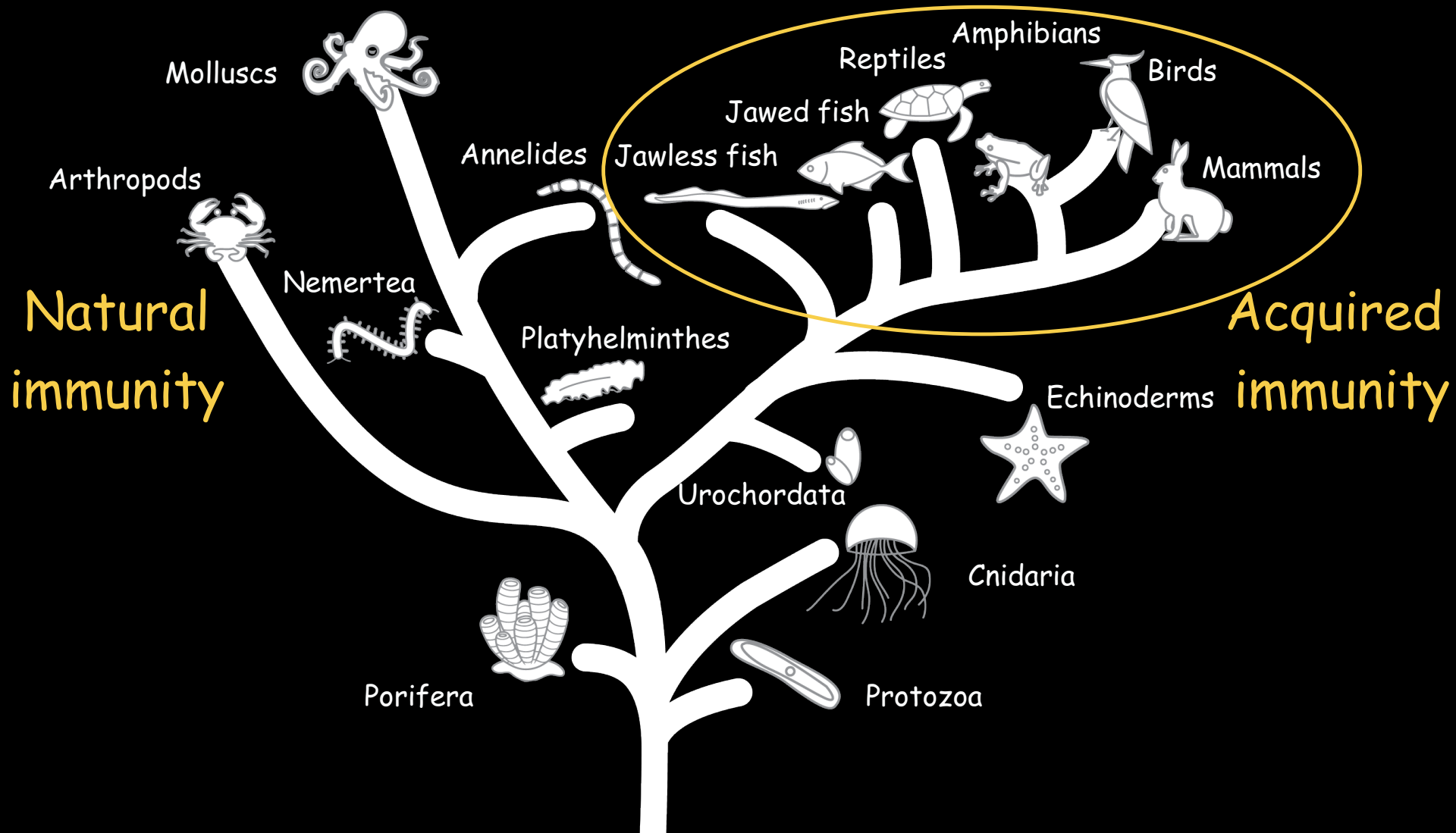



Streptomycin

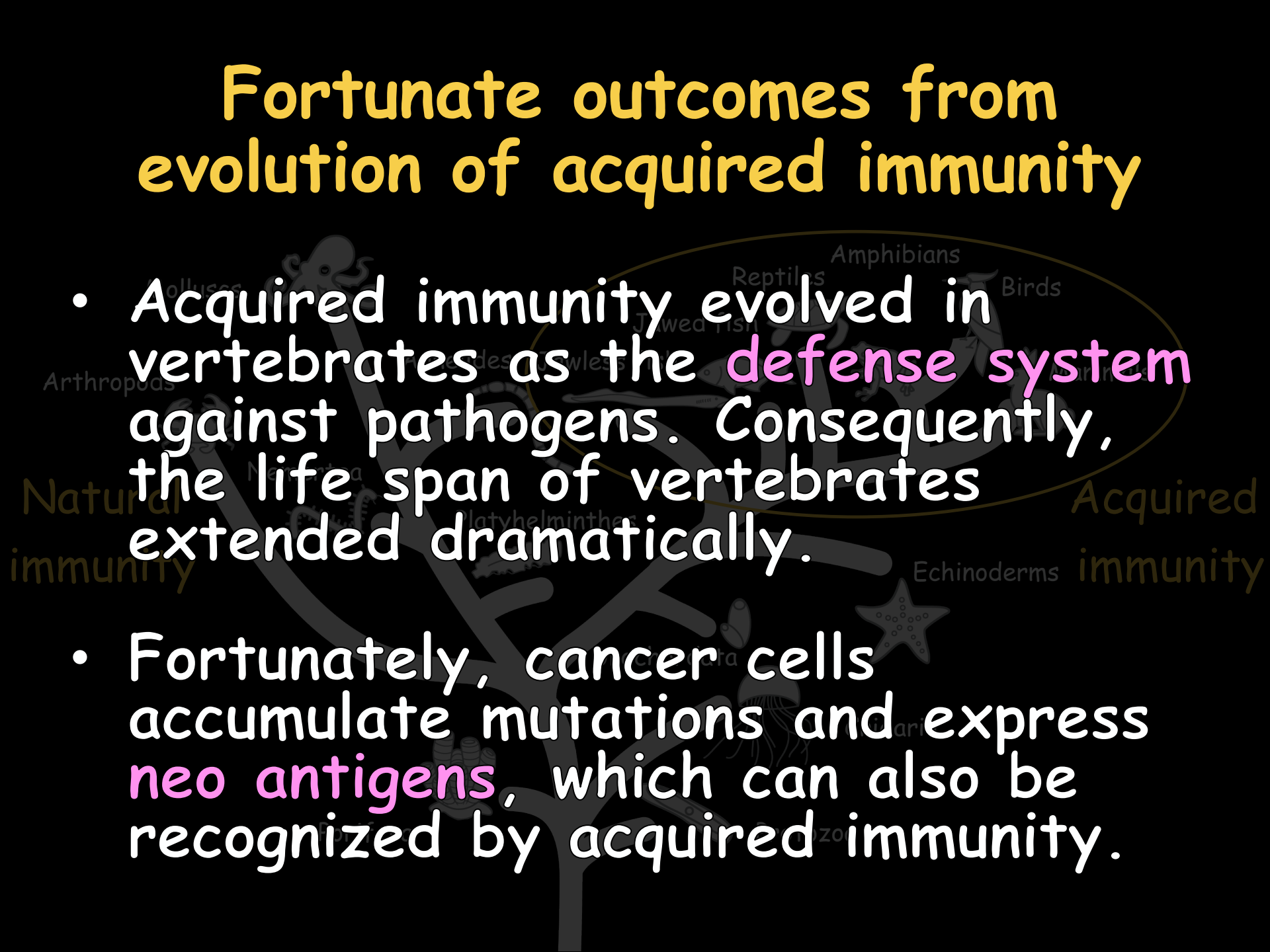
21st century

Cancer may be controlled by
immunotherapy and its improvement
including microbiome manipulation

# Acquired immunity evolved in vertebrates



# Fortunate outcomes from evolution of acquired immunity




- Acquired immunity evolved in vertebrates as the defense system against pathogens. Consequently, the life span of vertebrates extended dramatically.
- Fortunately, cancer cells accumulate mutations and express neo antigens, which can also be recognized by acquired immunity.

# Collaborators

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Graduate School of Medicine, Kyoto University

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IMS, RIKEN

Fumihiko Matsuda
Kyoto University

# Major outside collaborators

## Antibody diversity

E. Severinson (Stockholm Univ.)
F. Alt (Harvard Univ.)
M. Nussenzweig (Rockefeller Univ.)
A. Fischer (Necker Hospital)
A. Durandy (Necker Hospital)
T. Chiba (Kyoto Univ. Hospital)

## Cancer immunotherapy by PD-1 blockade

G. Freeman (Dana Farber Cancer Center)
N. Minato (Kyoto Univ.)
S. Fujii (Kyoto Univ.)
I. Konishi (Kyoto Univ.)

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Thank you for your attention