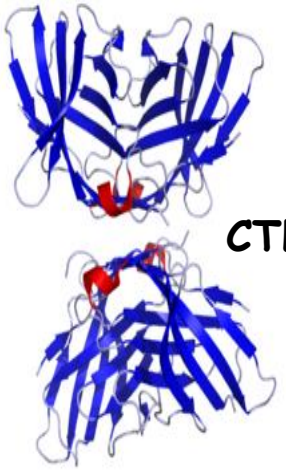
Cancer Therapy by Inhibition of Immune Checkpoints

Allison and Honjo

PD-1



CTLA-4

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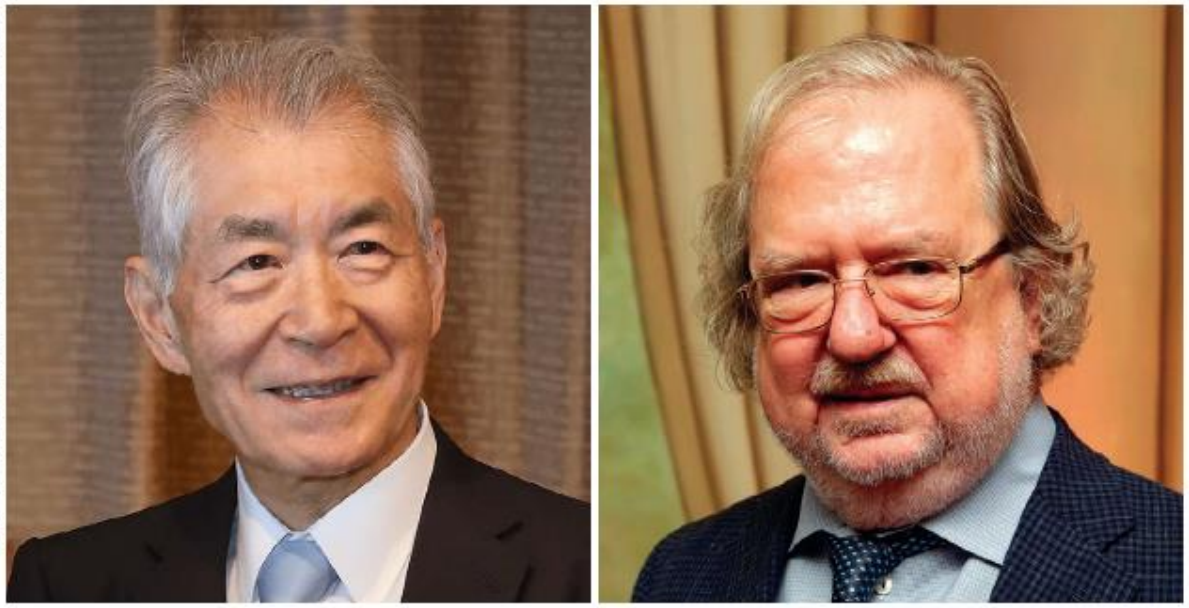
Alagappa University, April 5, 2019

Discovery of checkpoint inhibitors

- **James Allison** at the University of Texas MD Anderson Cancer Center in Houston and **Tasuku Honjo** at Kyoto University in Japan have won the 2018 Nobel Prize in Physiology or Medicine.
- They showed how proteins on immune cells can be used to manipulate the immune system so that it attacks cancer cells.



LEFT: MEXT/CC BY 4.0; RIGHT: RICHARD DREW/AP/SHUTTERSTOCK

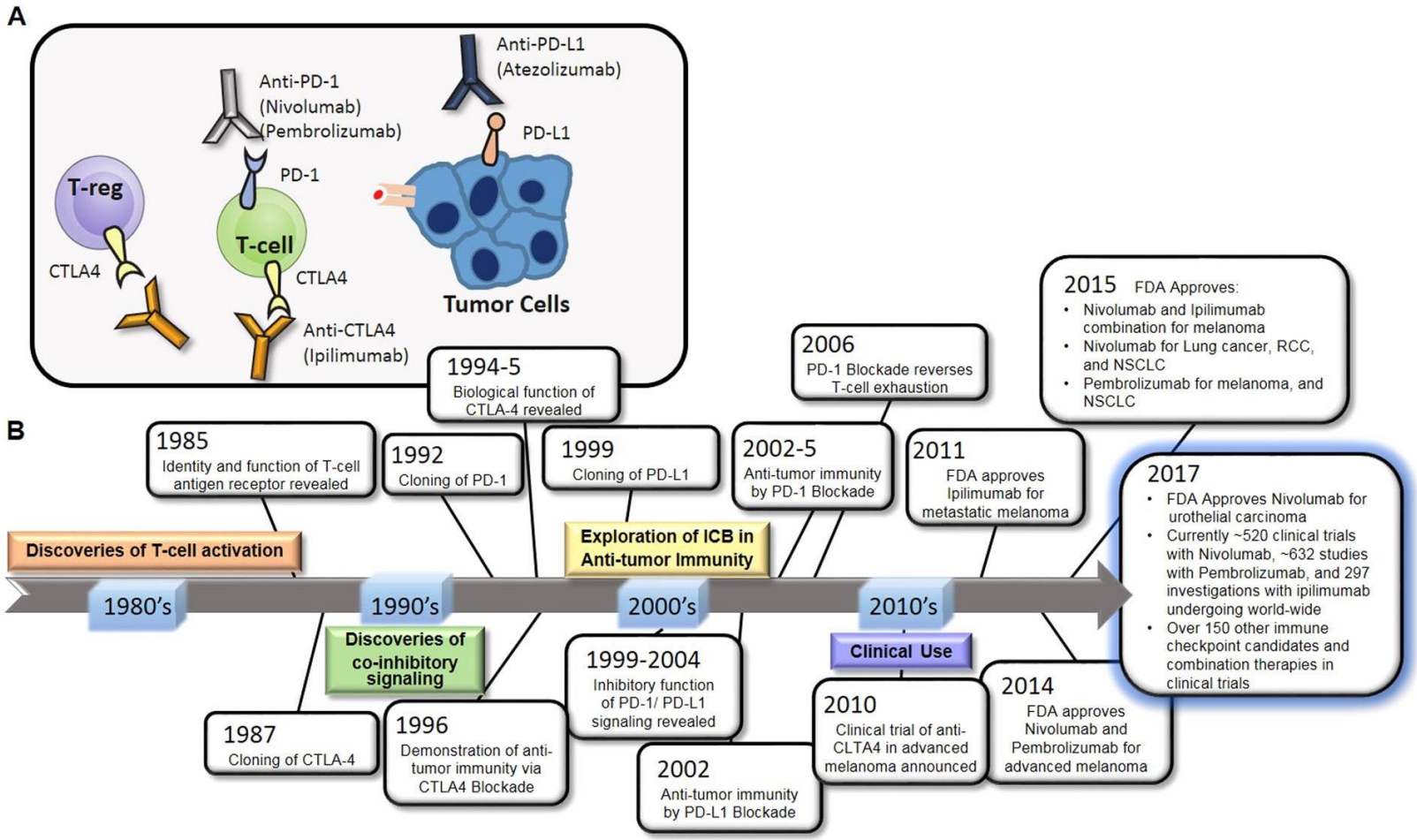
Tasuku Honjo (left) and James Allison share the 2018 Nobel Prize in Physiology or Medicine.

Why Immunotherapy?

- **Cancer:**
  - Myriad gene mutations
  - High genome instability
  - Many different diseases, each with distinct genetic alterations
  - Targeting single mutations with targeted inhibitors always almost leads to disease relapse.

- **Immunotherapy:**
  - Specificity
  - Memory
  - Adaptability

Timeline of ICB therapy development within the last three decades



Tasuku Honjo

Tasuku Honjo
Born: 27 January 1942, Kyoto, Japan

- Honjo discovered the T-cell protein PD-1, which acts as a brake on the immune system by a different mechanism.
- Treatments that block PD-1 have proved to be effective in lung and renal cancers, lymphomas and melanoma.

Checkpoint inhibitors: Important early discoveries

- Gordon Freeman, an immunologist at the Dana-Farber Cancer Institute in Boston, Massachusetts

- along with immunologists Arlene Sharpe at Harvard Medical School in Boston and

- Lieping Chen at Yale University in New Haven also studied checkpoint proteins, as well as a molecule that binds to PD-1 called PD-L1.

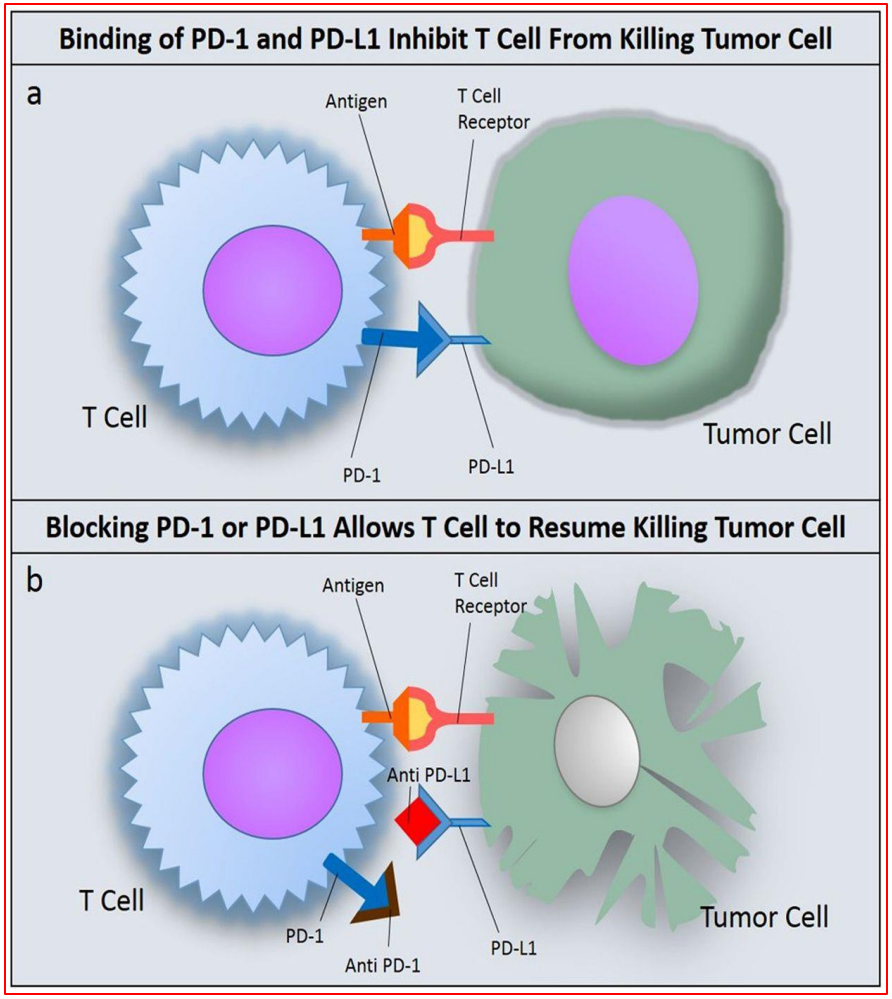
Drawbacks of Conventional Immunotherapies

- ✓ Extracting antigens from cancer cells and injecting larger doses of those antigens.
- ✓ Cell activation therapy – collecting lymphocytes and re-injecting them to the same patient after activation.
- ✓ Interferon therapy and other cytokine therapies.

  (All these attempts remain unsuccessful)

Finally, Honjo and his team discovered that releasing PD-1 "brake" can activate the immune system for treating cancer.

Tumors escape from host immune response



- Honjo demonstrated a novel mechanism
- Engagement of PD-1 by PD-L1 leads to the inhibition of Tcell proliferation and cytokine production.
- Tumors escape from the host immune response by expressing PD-L1 on their surface.
- This effect negatively regulates T-cell immune response through the interaction with PD-1, an immunoinhibitory receptor from CD28 family.

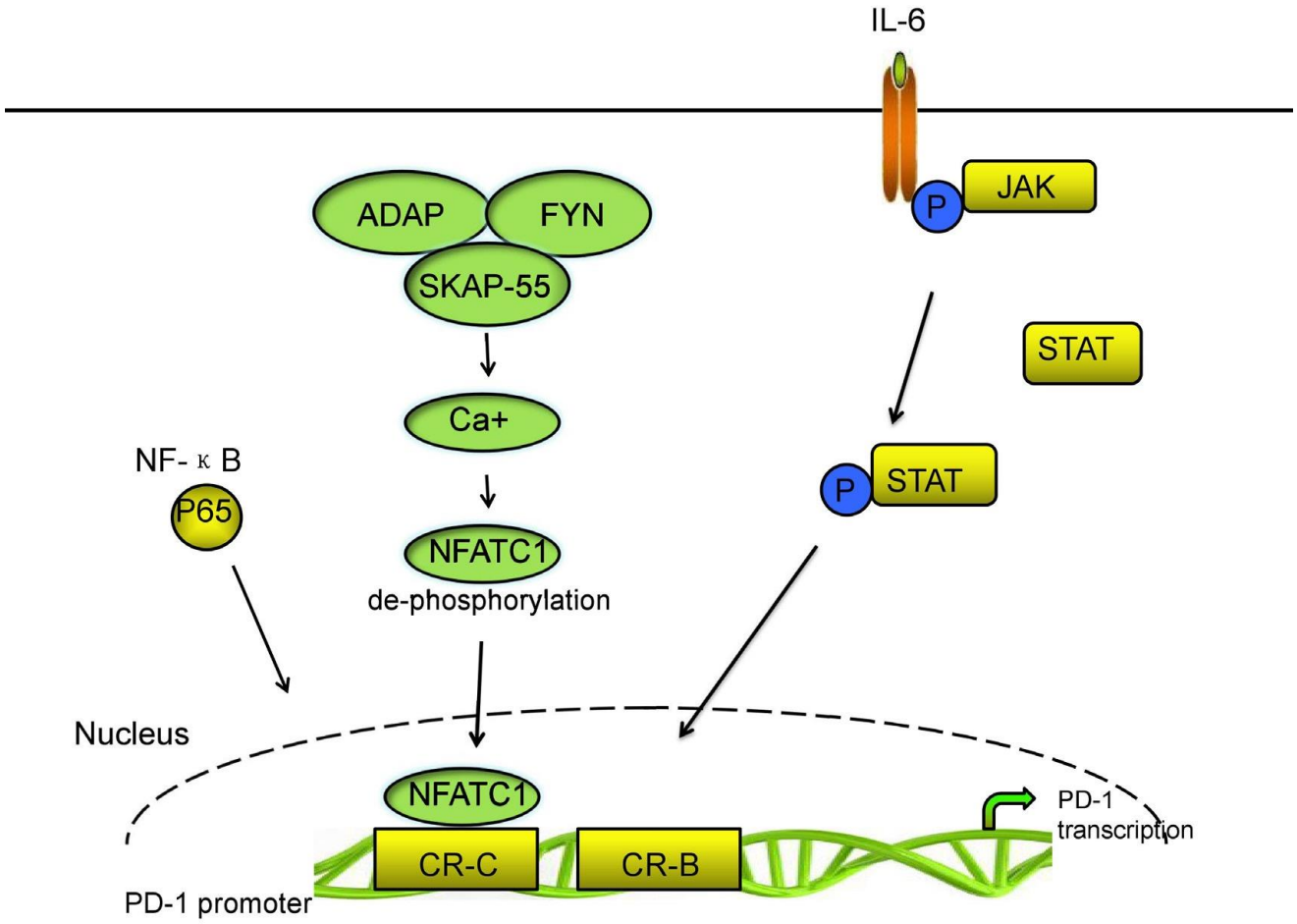
PD-1 (programmed death-1)

- PD-1 is also a 288 amino acid (55 kDa) type I transmembrane protein of the immune globulin superfamily, comprising an extracellular N-terminal IgV-like domain, a transmembrane domain, and a cytoplasmic tail
- There are two tyrosine residues in the cytoplasmic tail of PD-1;
- the N-terminal of which is involved in a sequence defined as the immunoreceptor tyrosine-based inhibitory motif (ITIM, I/L/VXYXXL/V);
- the C-terminal tyrosine is engaged in a sequence defined as immunoreceptor tyrosine-based switch motif (ITSM, TxYxxL).
- The amino acid sequence around the C-terminal tyrosine (TEYATIVF) of PD-1 is well conserved between mouse and human and is related to SHP-1 and SHP-2.

PD-1 (programmed death-1)

- PD-1 (also called CD279) was first isolated from 2B4.11 (a murine T-cell hybridoma) and interleukin-3 (IL-3)-deprived LyD9 (a murine hematopoietic progenitor cell line) by using subtractive hybridization technique
- PD-1 is encoded by the Pdcd1, which is located on chromosome 2 (*2q37*).
- PD-1 is one of the member of B7/CD28 family

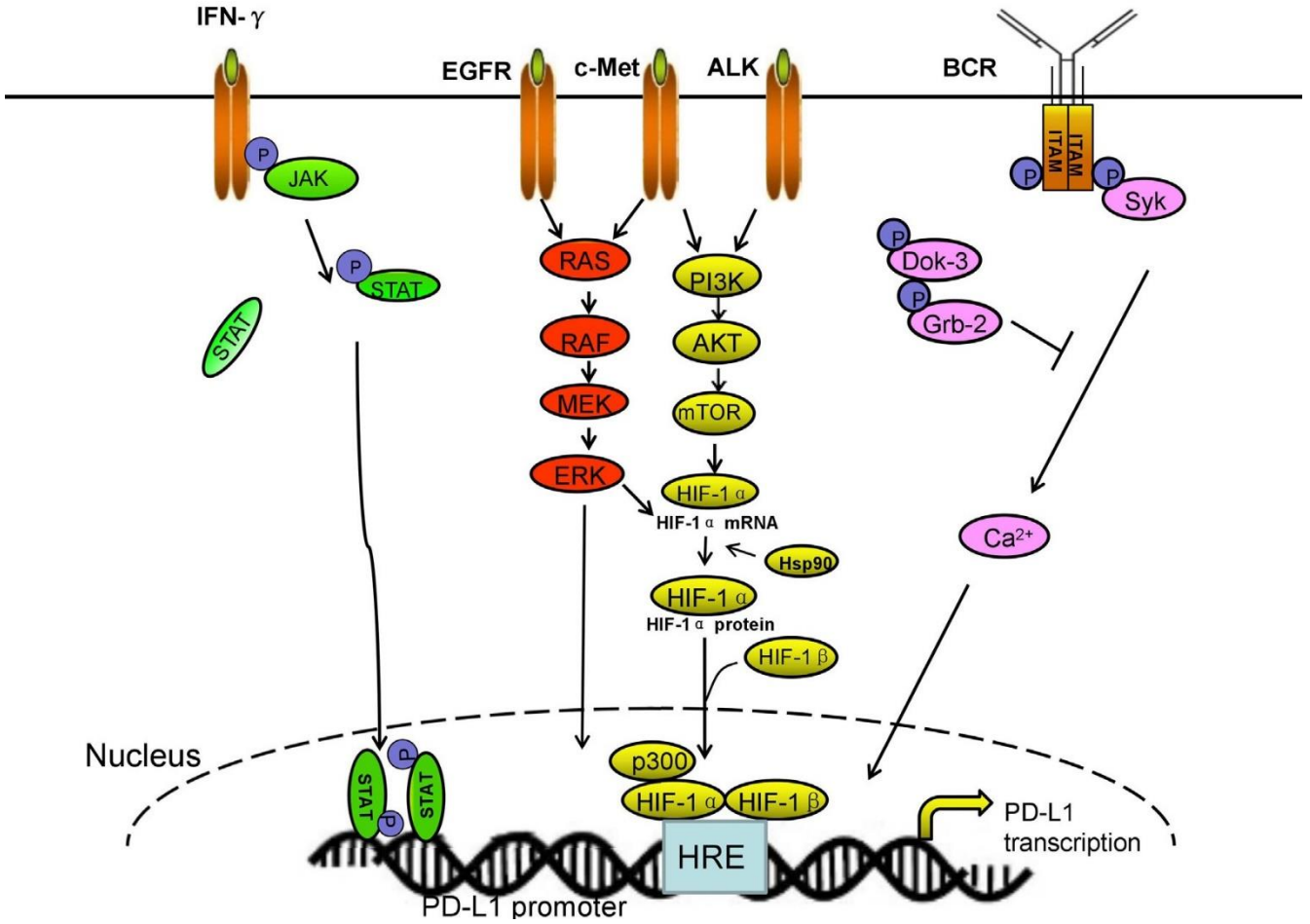
The main signal pathways of PD-1 transcriptional regulation



PDL (programmed death ligand)

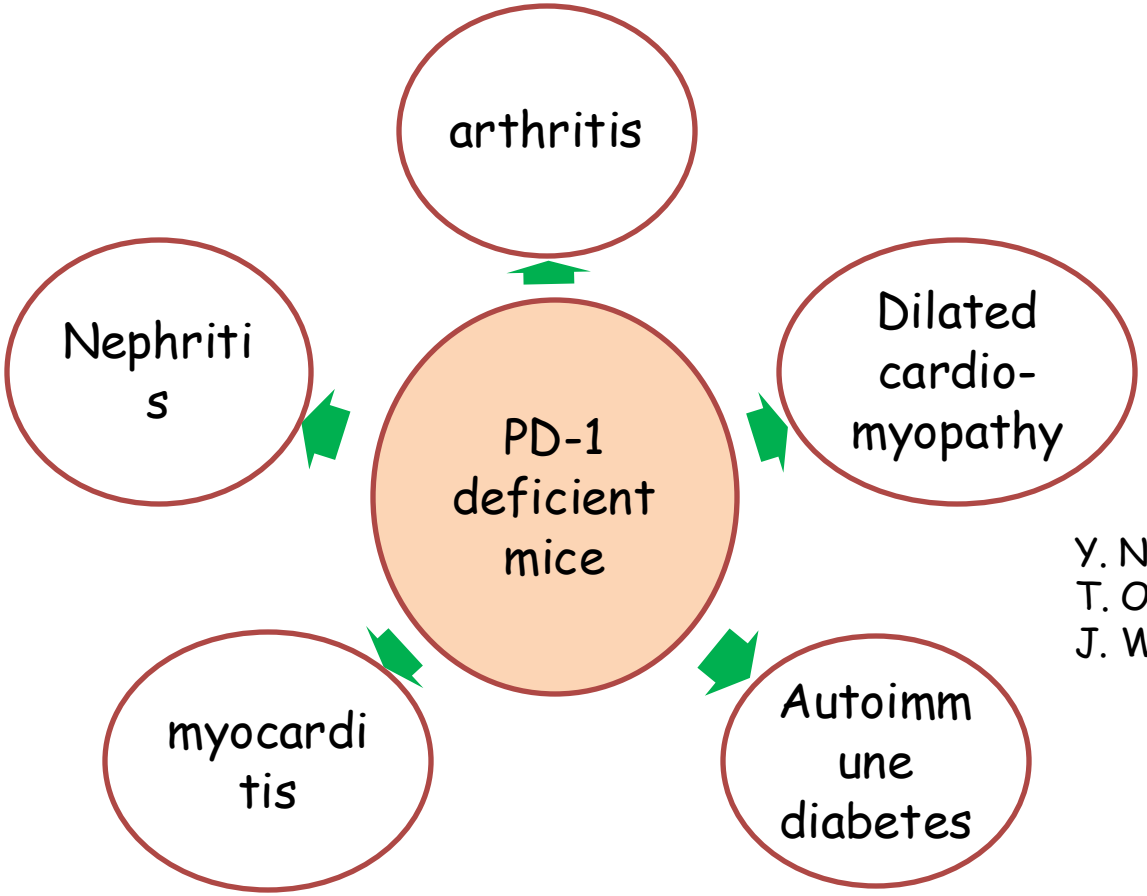
- PD-1 is involved in immune tolerance by suppressing activated immune cells via interaction with its ligands. Two known ligands of PD-1 are PD-L1 and PD-L2
- B7-H1 was originally named as the first gene homolog of B7 molecules, and B7-H1 was renamed as PD-L1 after it has been identified as the first ligand of the receptor PD-1 (CD279) in the murine system
- PD- L1 is a 290-amino-acid transmembrane glycoprotein.
- The second known counter-receptor of PD-1, called B7-DC or PD-L2, is also a member of the B7 family
- While PD-L2 is exclusively induced on APCs, PD-L1 is expressed on tumor cells, epithelial cells, and immune cells. These molecules inhibit TCR downstream signaling when ligated with PD-1

The main signal pathways of PD-L1 transcriptional regulation



PD-1 is a negative regulator

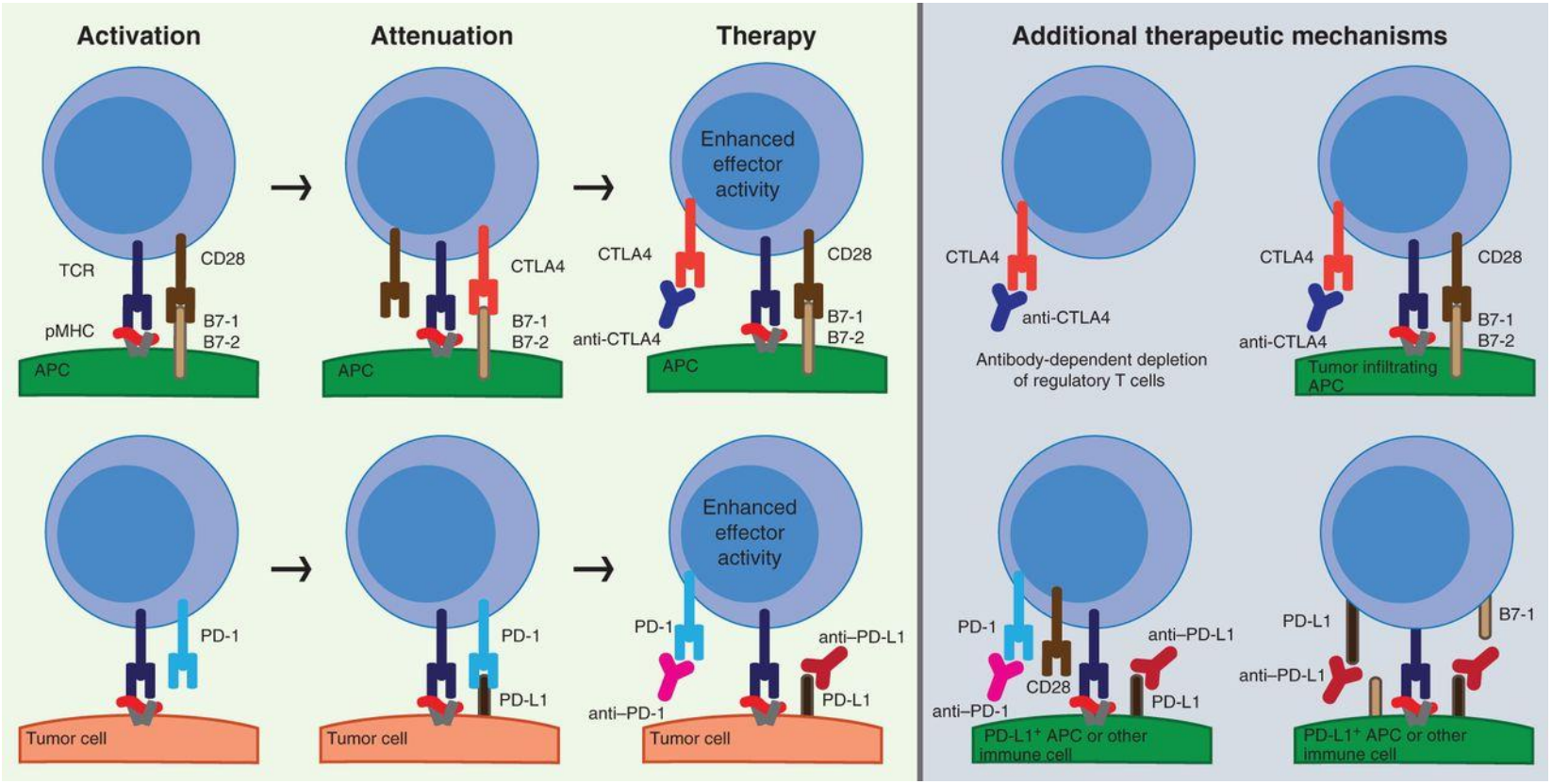
- PD-1 knockout mice (black and white), developed different autoimmune diseases in tumour free mice (5 yrs).



Y. Nishimura *et.al.*, Immunity (1999)
T. Okazaki *et al.*, Nat. Medicine (2003)
J. Wang *et.al.*, Int. Immunol. (2010)

This finding suggest that mice without PD-1 exhibit overactive immune system which acts as brake on immune response.

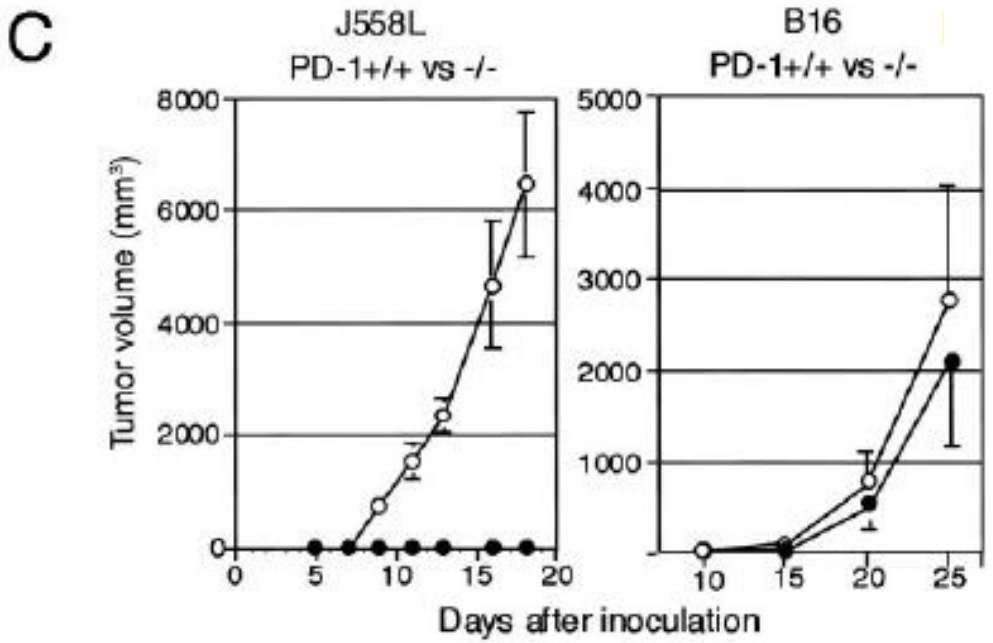
Schematic of the molecular mechanisms of action of CTLA4 and PD-1 blockade.



Spencer C. Wei et al. Cancer Discov 2018;8:1069-1086

AACR American Association for Cancer Research
CANCER DISCOVERY

Tumors in PD-1 knockout mice versus normal mice

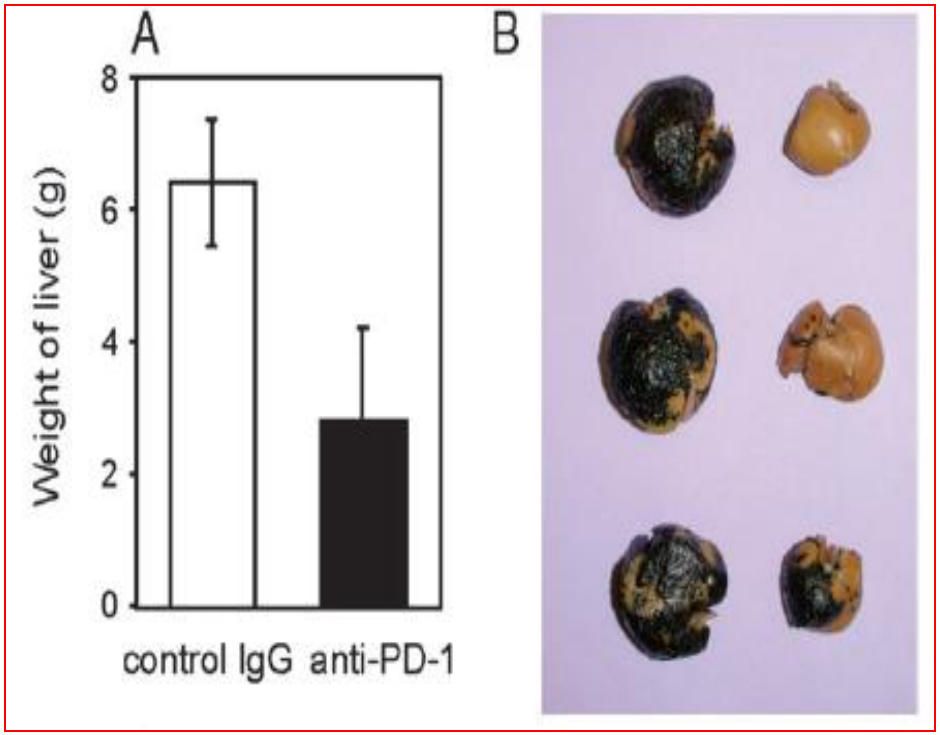


These results suggest lack of inhibitory signals in cross-priming reactions.

- The tumors on the wild-type mice, proliferated in a linear fashion.
- The tumors of PD-1 knockout mice injected with J558L cells (expressing PD-L1), reduced the tumor growth.
- The tumors of PD-1 knockout mice injected with B6 cells (not expressing PD-L1) did not reduce the tumor growth.

Iwai *et al.*, PNAS (2002)

PD-1 blockade inhibits metastasis of B16 melanoma in mouse model



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

- Injection with anti-PD-1 mAb inhibited tumor growth in the liver, while injection of control IgG had no therapeutic effect.
- The weight of liver from mice treated with anti-PD-1 mAb was almost half that of control mice.

Human anti-PD-1 antibody

Named Nivolumab

Synthesized in mice containing human Immunoglobin gene by Medarex

Subclass: IgG4S228P

Mutant IgG4(S228P) stabilizes the protein and reduces ADCC

(antibody-dependent cell-mediated cytotoxicity)

Nivolumab demonstrated durable clinical activity with less severe side effects than ipilimumab (CTLA-4)

Approved as Investigation New Drug by FDA

(USA: Aug 1,2006)

Clinical trails began in US (2006) and Japan (2008)

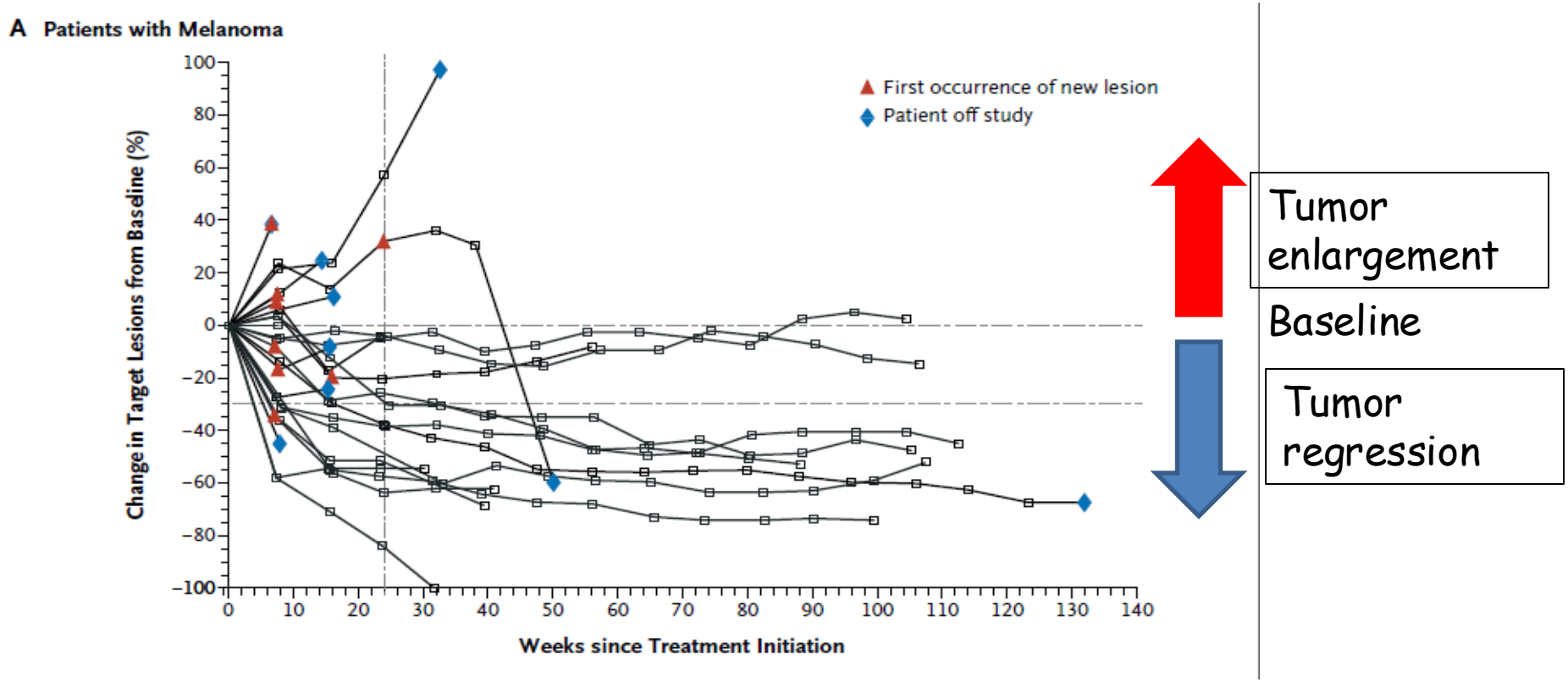
Summary of Phase I clinical trial

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

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

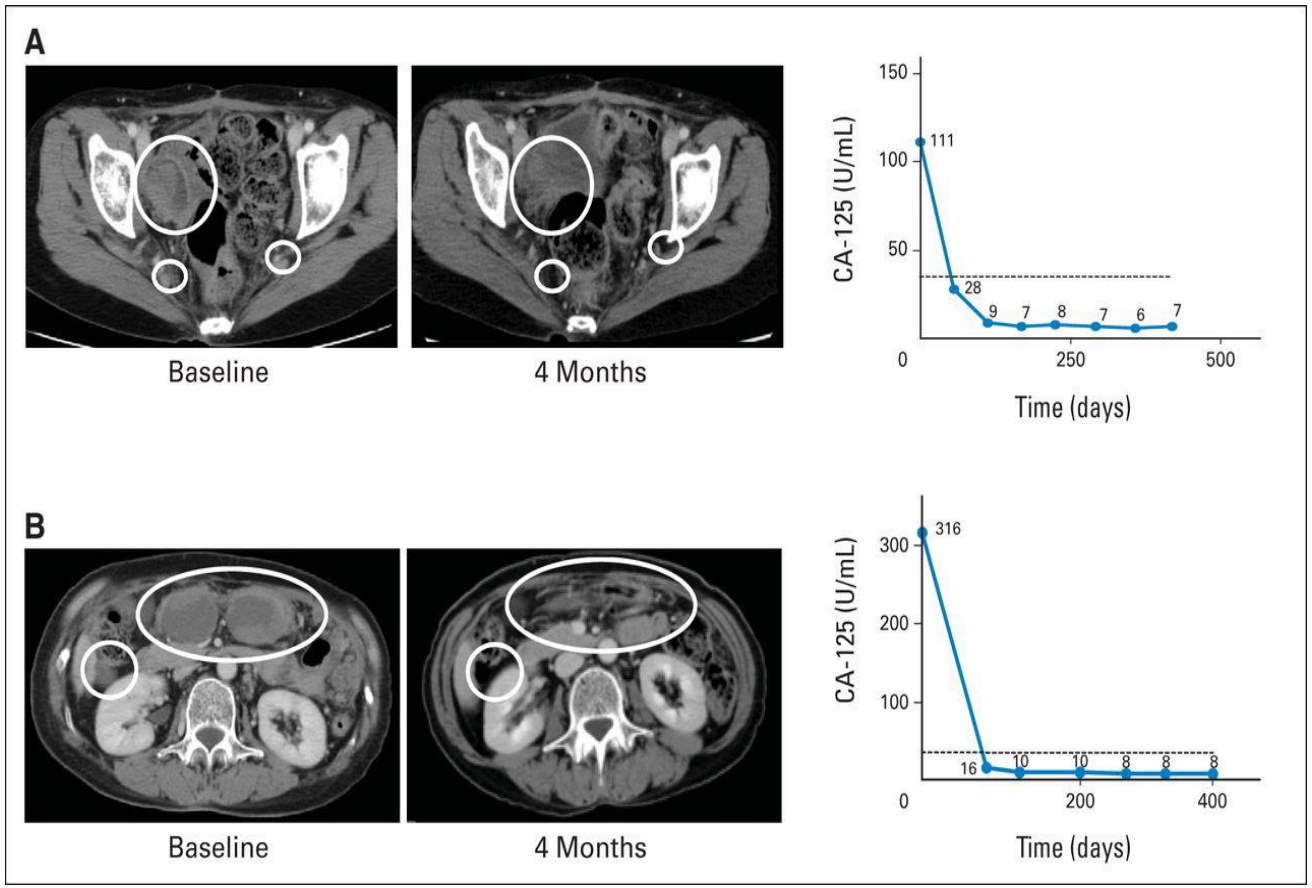
Dose	Total (n)	CR	PR	SD	PD	NE	RR	DCR
1 mg/kg	10	0	1	4	4	1	1/10 (10%)	5/10 (50%)
3 mg/kg	10	2	0	2	6	0	2/10 20%	4/10 (40%)
Total	20	2	1	6	10	1	3/20 15%	9/20 (45%)

CR, complete response; DCR, disease control rate (ie, CR, PR and SD)
PR-partial response, SD-stable disease,

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

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

A responder with ovarian cancer: Nivolumab 3mg/kg



Disappearance of lymph node metastases

Disappearance of peritoneal dissemination

Tumor marker CA-125 decreased to normal range after one course of nivolumab

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

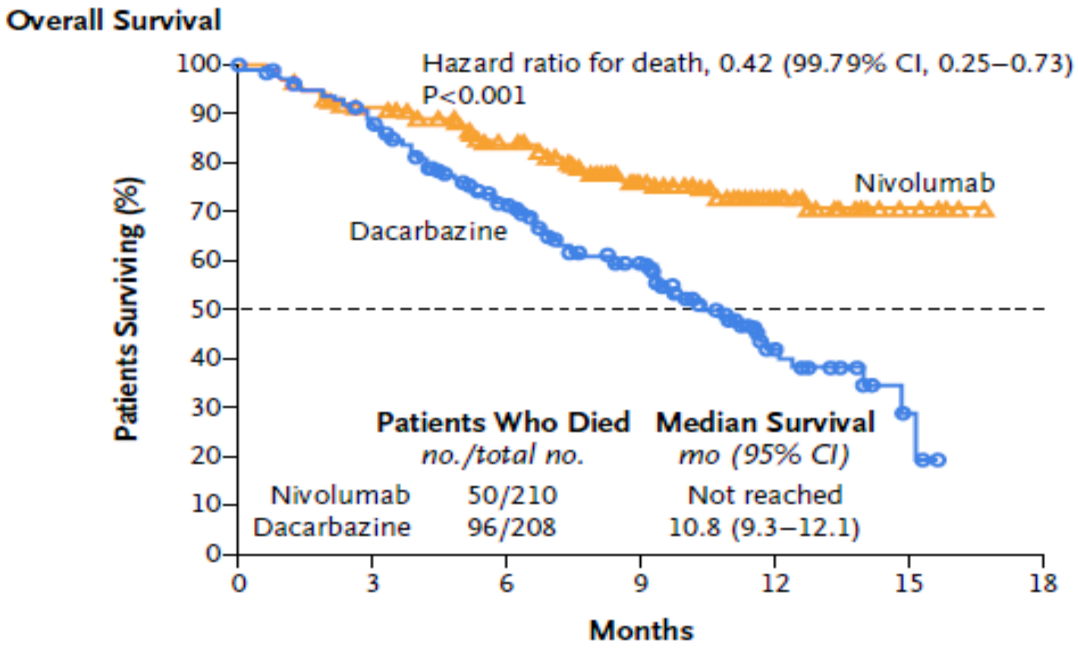
Cancers approved for PD-1 blockade therapy

- 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

Effectiveness 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

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

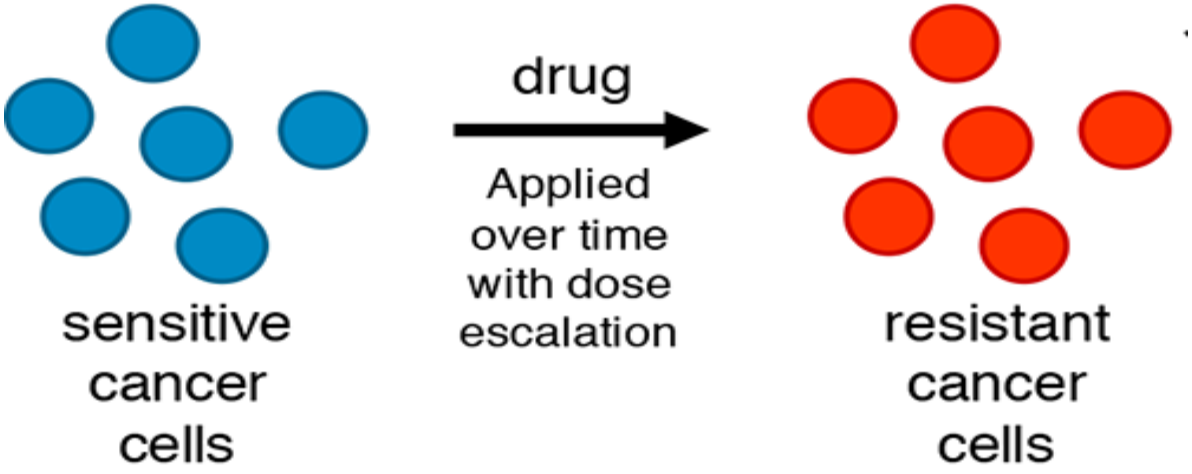


C. Robert *et al.*, NEJM (2015)

What we learned from huge cancer genome projects

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

Continuous mutations generate resistant tumor cells



Recurrent cancer cells gain additional mutation and provide new resistant Cancer phenotype.

Lymphocytes can recognize many more mutants and attack them with long lasting effect.

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.

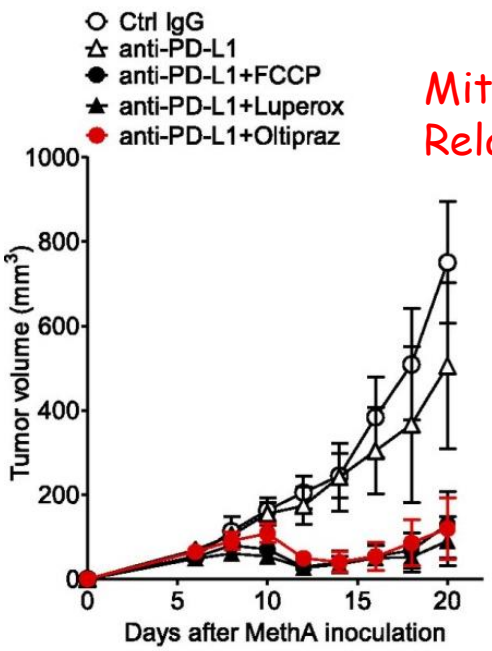
Strategies to enhance PD-1 blockade therapy

- Around 30 to 50% of patients remain unresponsive, or less responsive, to PD-1 blockade therapy.
- PD-1 blockade therapy combined with various treatments such as inhibition of negative co-receptors (Lag3 and Tim3), cancer vaccines, mild irradiation, and low doses of chemotherapy.
- No striking synergistic effects have so far been reported.

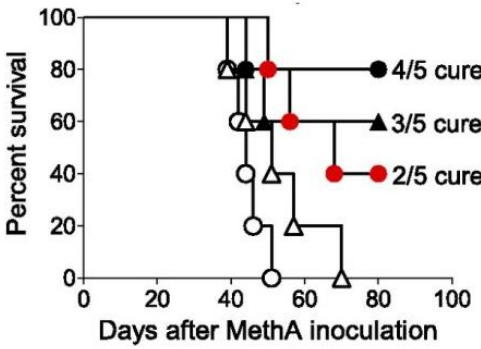
Requirement of mitochondrial activation for killer T cell activation and proliferation

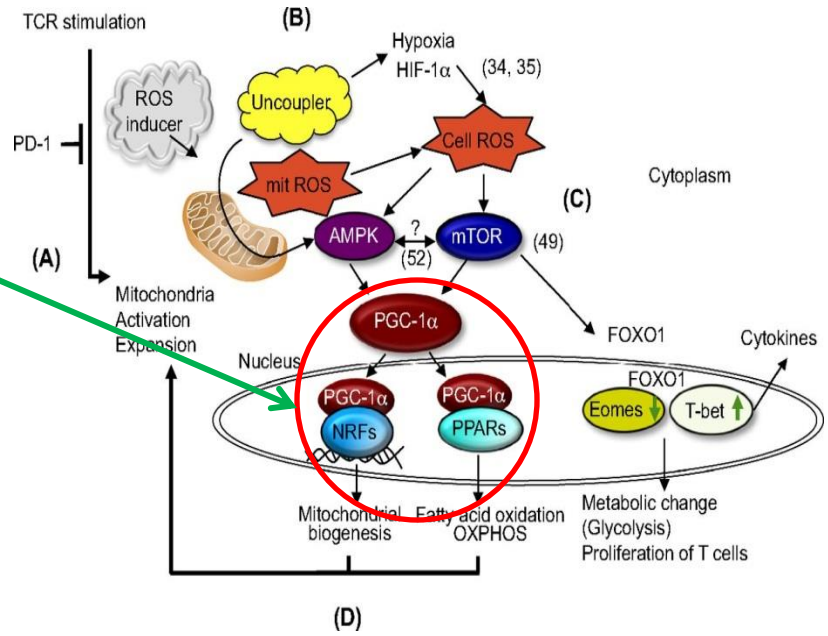
- PGC-1α, a transcriptional cofactor regulated by either AMPK or mTOR, is known to enhance mitochondrial biogenesis and oxidative phosphorylation
- Chemical activators of PGC-1α, known to increase mitochondrial activity, synergize with PD-1 blockade for tumor-growth suppression

PGC-1a Activators Enhance PD-1 Blockade Therapy: A synergistic effect



Mitochondrial activation Related chemicals

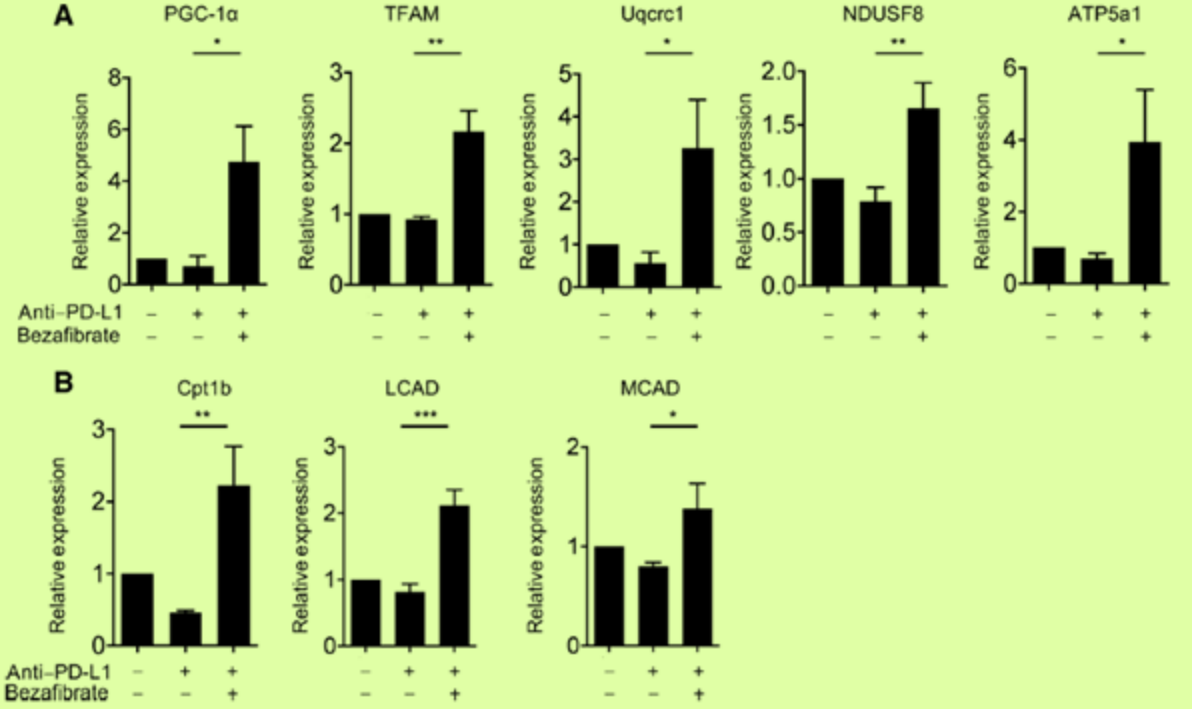




- All of the chemicals tested had strong synergistic effects with anti-PD-L1 on murine skin carcinoma growth suppression
- These chemicals alone did not show any effect on tumor growth.

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

Treatment with Bezafibrate and PD-1 blockade enhances expression of genes associated with mitochondrial biogenesis in T cells.



Less mitochondrial activation in effector T cells results in less tumor clearance.

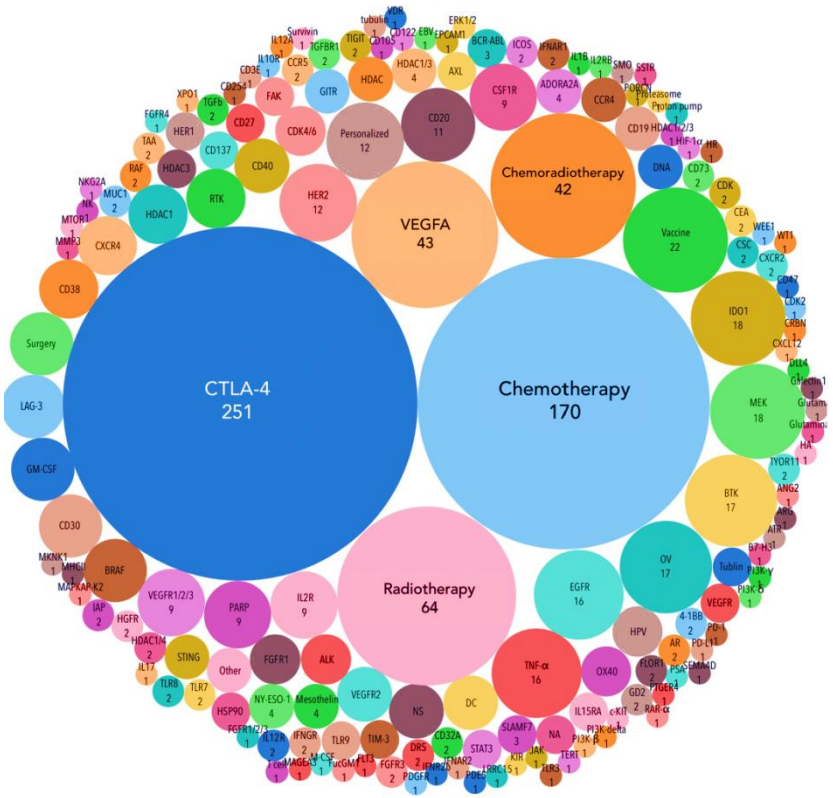
- Bezafibrate facilitates more proliferation of naive T cells leading to enhanced antitumor immunity.
- Also, increased the fatty acid oxidation (FAO) and mitochondrial respiratory capacity, by providing extra energy demands.

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

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

Numbers of PD-1 blockade trials using combination with:

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



The size of the bubble correlates to the target enrollment of patients. Multiple bubbles of the same color represent multiple cancer types that are being tested in these trials.

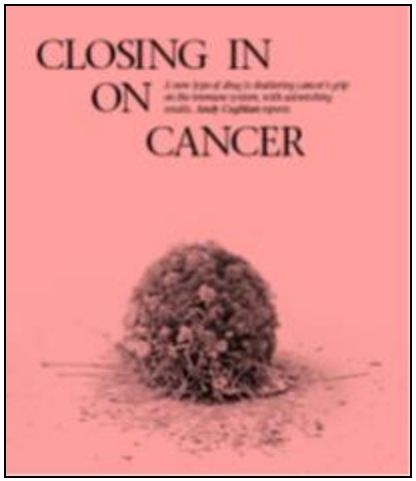
J. Jang *et al.*, Ann. Oncol. (2018)

Future prospects in cancer therapy

1. Efficacy of PD-1 blockade therapy improved. (2016)

2. Many more cancers may be treated by immunotherapy. (2020)

3. Cancer may not completely disappear, but be controlled by immunotherapy. Cancer may become one of chronic disease. (2030 ??)



CLOSING IN ON CANCER

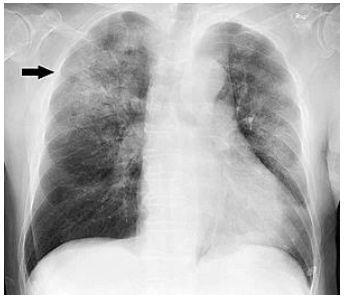
Andy Coghhlon
New Scientist, 5 March 2016

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

Enormous benefit by acquired immunity

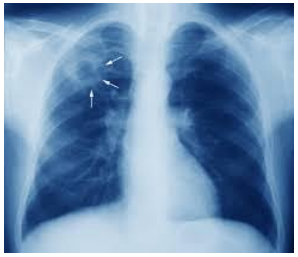
20th century — Eradication of infectious disease by vaccination and antibiotics.

Pneumonia



Penicillin

Tuberculosis



Streptomycin

21st century — Cancer may be controlled by immunotherapy and its improvement including microbiome manipulation

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.


THANK YOU!