Reimagined SITC 2020 NOVEMBER 9–14

The PD-L1/PD-1 Pathway: Discovery and New Insights

Gordon Freeman, PhD

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DANA-FARBER CANCER INSTITUTE

HARVARD MEDICAL SCHOOL


sitc
Society for Immunotherapy of Cancer

Disclosure Information
Gordon Freeman, PhD

I have the following financial relationships to disclose:

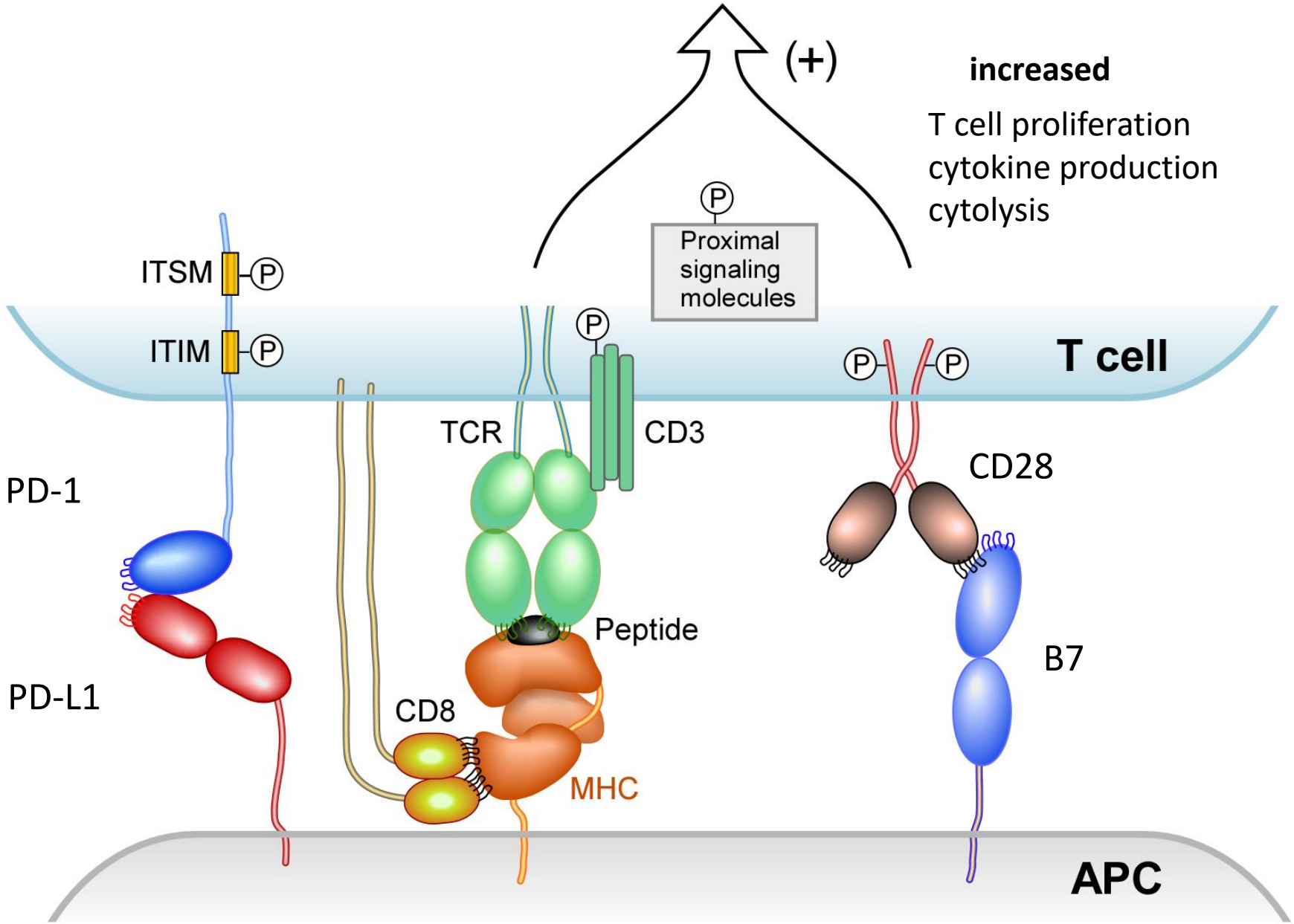
Intellectual Property related to the PD-1 / PD-1 Ligand pathway licensed non-exclusively to: Bristol-Myers-Squibb, Roche, Merck, Merck-Serono, Boehringer-Ingelheim, AstraZeneca, Dako, Leica, Mayo Clinic, Novartis

Intellectual Property related to TIM-3 licensed to Novartis

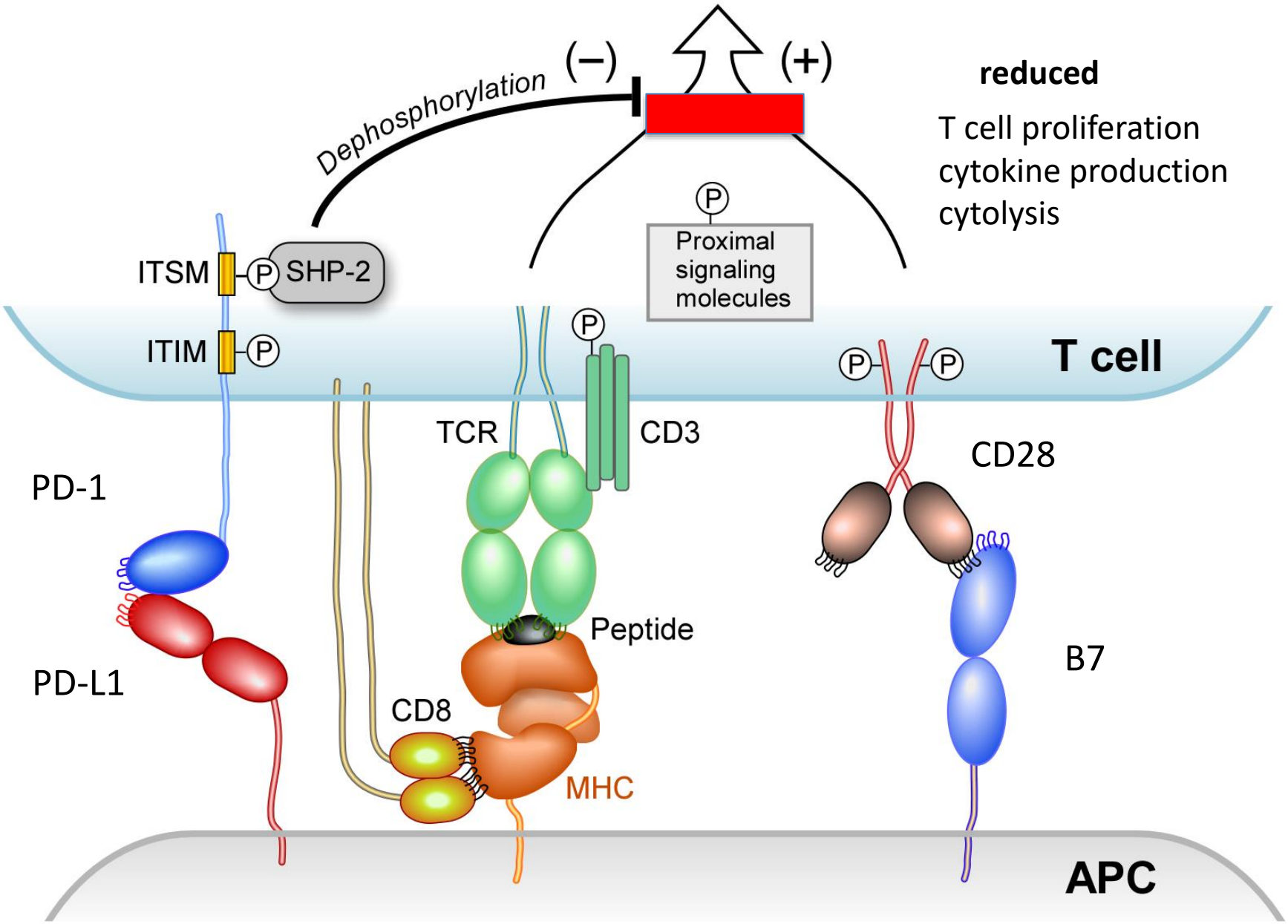
Consultant for: Roche, Bristol-Myers-Squibb, Xios, Origimed, Triursus, iTeos, NextPoint, IgM, Jubilant, Trillium and GV20

Equity in Nextpoint, Triursus, Xios, iTeos, IgM, and GV20

Second signals regulate the outcome of TCR signalling



There are negative second signals



reduced

T cell proliferation
cytokine production
cytolysis

A Tale of Two Paths to Discovery

B7

CD28 mAb costimulates T cells
(Gmunder, Lesslauer, Eur J Biochem 1984)

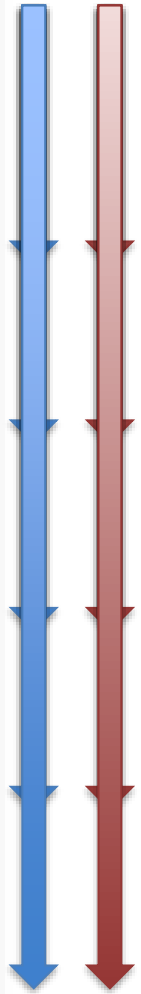
B7-1 cloned
(Freeman...Nadler, J Imm 1989)

B7-1 binds CD28
(Linsley...Ledbetter, PNAS 1990)

B7-1 binds CTLA-4
(Linsley...Ledbetter, J Exp Med 1991)

B7-1 KO shows B7-1 is the minor CD28 ligand
(Freeman...Sharpe, Science 1993)

B7-2 is the major CD28 ligand
(Freeman...Nadler, Science 1993)



PD-1

PD-1 cloned
(Ishida...Honjo, EMBO J 1992)

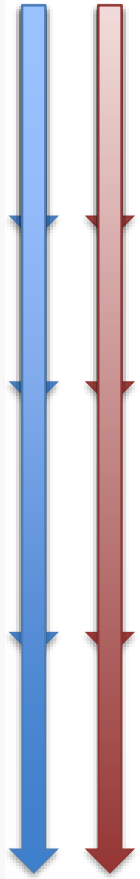
Two Paths to Discovery

B7

CTLA-4 functions as a negative regulator
(Walunas...Bluestone, Immunity 1994)
(Tivol...Sharpe, Immunity 1995)
(Waterhouse...Mak, Science 1995)

CTLA-4 mAb promotes anti-tumor immunity
(Leach...Allison, Science 1996)

Freeman finds a new B7
(1998)



PD-1

PD-1 KO shows PD-1 is a negative regulator
(Nishimura...Honjo Immunity 1999)

What genes look like B7-1?

NCBI **BLAST Search Results** Entrez ?

AA292201 = 292 = B7-4 = PD-L1

gb|AA292201|AA292201 zt50f01.r1 Soares ovary tumor NbHOT Homo sapiens cDNA clone 725785
5'
Length = 497

Score = 40.2 bits (92), Expect = 0.010
Identities = 29/108 (26%), Positives = 51/108 (46%), Gaps = 4/108 (3%)

```
Query: 78  GTYECVVLKYEKDAFKREHLAEVTLSVKADFPTPSISDFEI---PTSNIRRIICSTSGGF 134
           G Y C++  Y    +KR       ++VK + P   I+   +   P ++   + C   G +
Sbjct: 76  GVYRCMI-SYGGADYKR-------ITVKVNAPYNKINQRILVVDPVTSEHELTCQAEG-Y 228

Query: 135 PEPHLSWLENGEE-LNAINTTVSQDPETELYAVSSKLDFNMTTNHSFMCLIK 185
           P+  + W  +  + L+   TT +   E +L+ V+S L  N TTN  F C  +
Sbjct: 229 PKAEVIWTSSDHQVLSGKTTTTNSKREEKLFNVTSTLRINTTTNEIFYCTFR 384
```

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Query: 135 PEPHLSWLENGEE-LNAINTTVSQDPETELYAVSSKLDFNMTTNHSFMCLIK 185
           P+  + W  +  + L+   TT +   E +L+ V+S L  N TTN  F C  +
Sbjct: 229 PKAEVIWTSSDHQVLSGKTTTTNSKREEKLFNVTSTLRINTTTNEIFYCTFR 384
```

Testing the immunological function of 292 (PD-L1)

Prepare DR7 specific alloreactive T cell blasts by activation of allogeneic T cells with $DR7^+$ lymphoblastoid cell line

After one week, harvest T cells and rest overnight in media

COS cells stably transfected with human MHC class II, DR7 = Signal 1

Introduce costimulatory genes by transient transfection = Signal 2

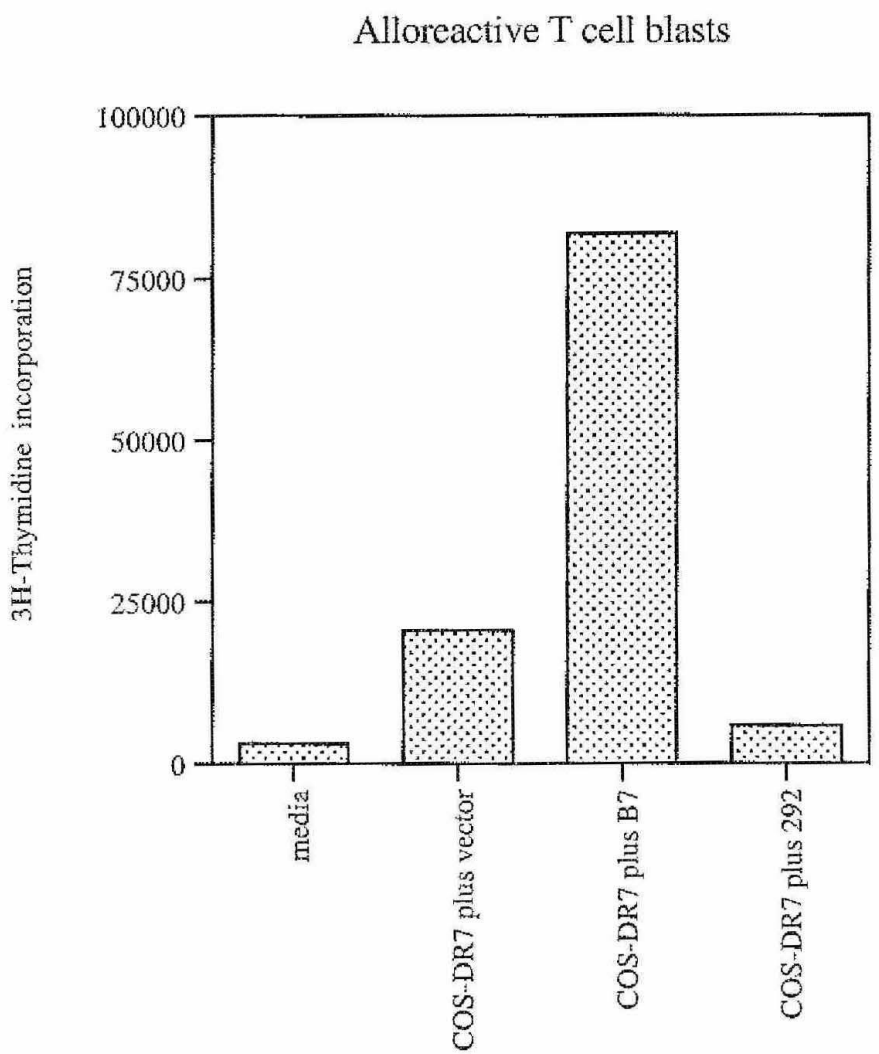
Harvest COS cells after 3 days and treat with Mitomycin C

Incubate alloreactive T cell blasts with transfected COS cells

Assay proliferation and cytokine production

292 inhibits proliferation of previously activated T cells

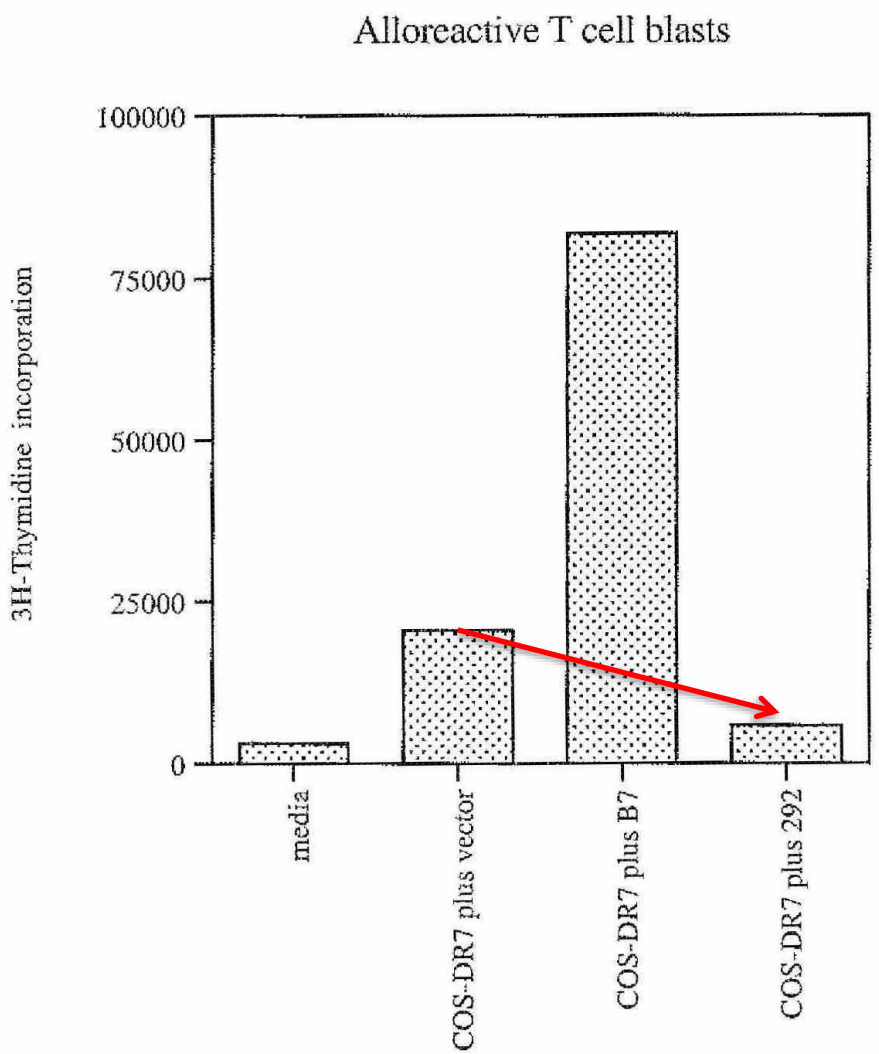
T cell proliferation



Vicki Boussiotis

292 inhibits proliferation of previously activated T cells

T cell proliferation



Vicki Boussiotis

Two Paths to Discovery

B7

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Tivol...Sharpe, Immunity 1995)

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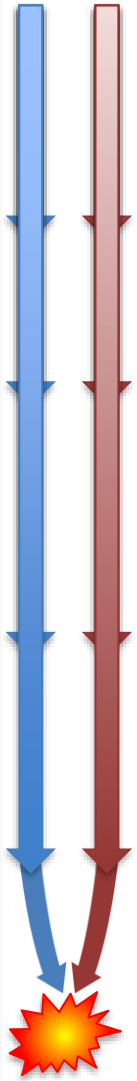
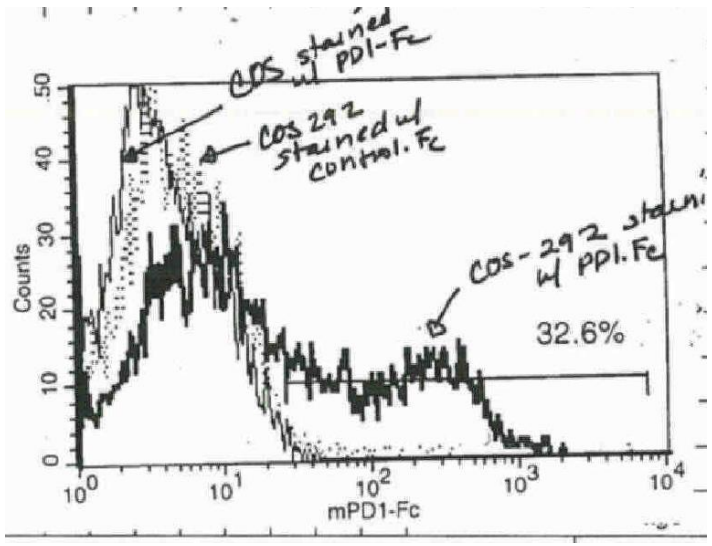
Clive Wood

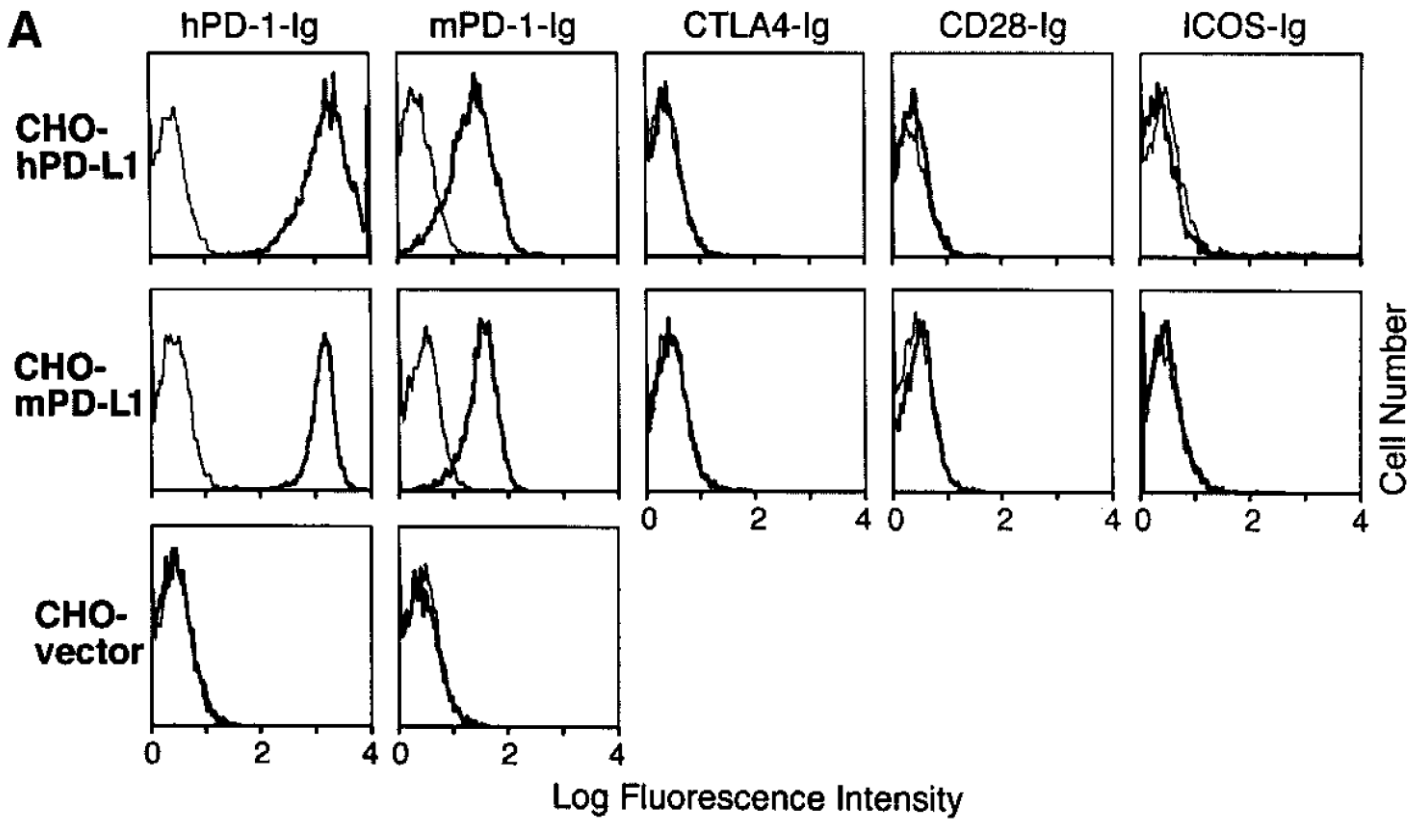


PD-1

PD-1 KO shows it is a negative regulator
(Nishimura...Honjo Immunity 1999)

PD-L1 binds to PD-1





The dawn of a revolution

scientific meeting to discuss PD-L1/PD-1 pathway
October 25, 1999
Genetics Institute, Fresh Pond, Cambridge, MA

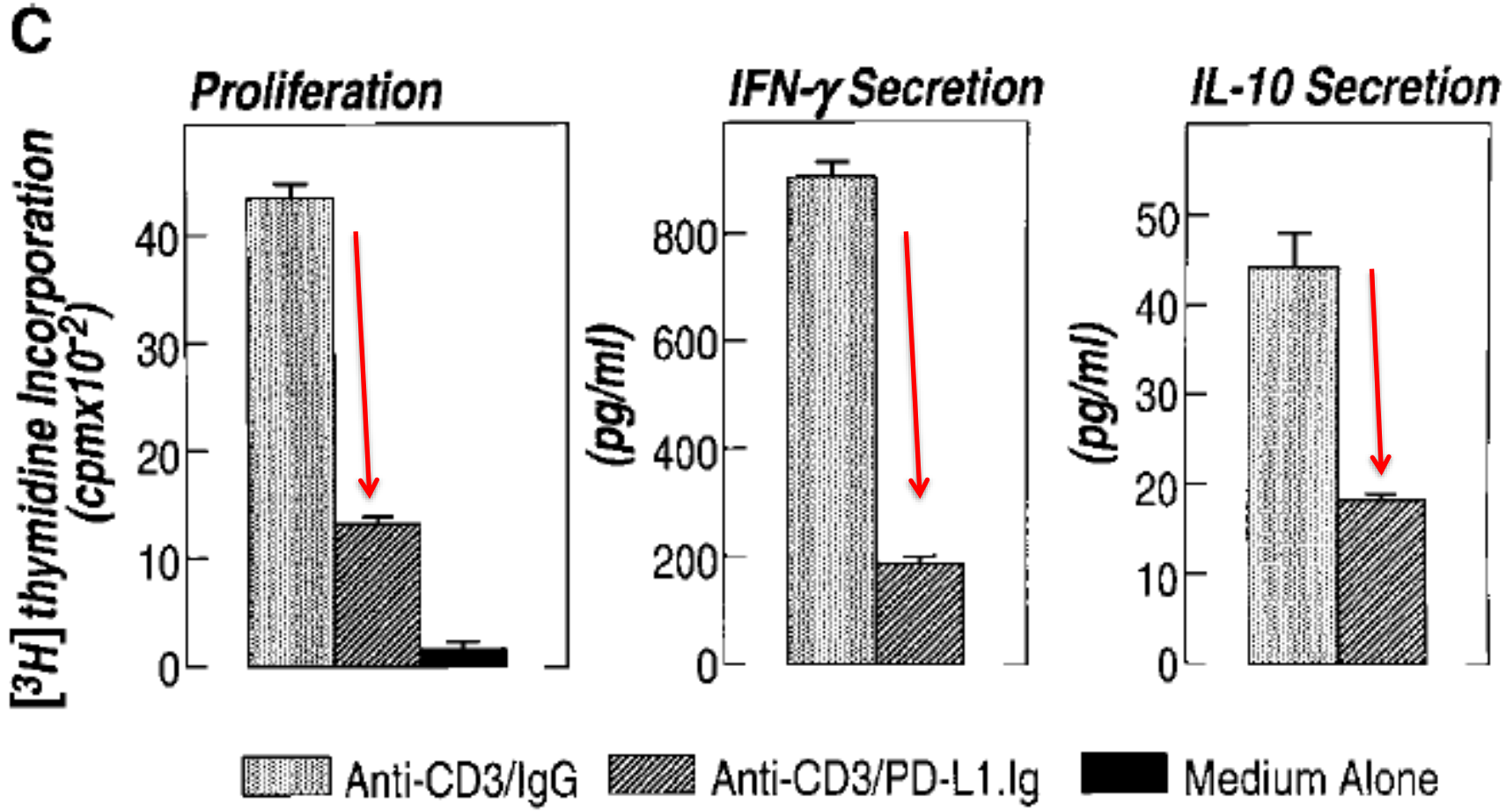
292, A novel B7 related gene

identified by a search of the EST database for genes with protein homology to B7 extracellular domain

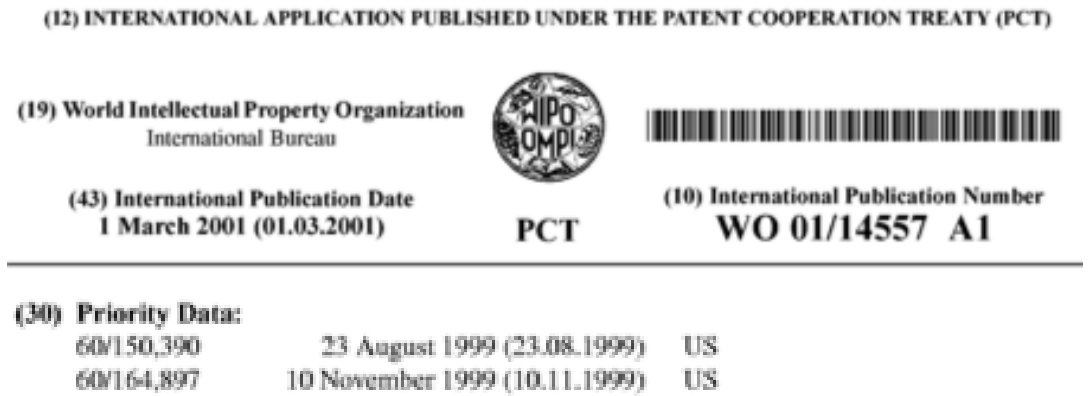
3 ESTs from a human ovary tumor NbHOT

full length cloned by RecA capture and magnetic beads from human placenta/activated keratinocyte cDNA libraries

PD-L1 inhibits T cell activation and cytokine production



The idea for PD-L1/PD-1 cancer immunotherapy



(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
1 March 2001 (01.03.2001)

PCT

(10) International Publication Number
WO 01/14557 A1

(30) Priority Data:

60/150,390	23 August 1999 (23.08.1999)	US
60/164,897	10 November 1999 (10.11.1999)	US

PD-1, a receptor for B7-4, and uses therefor
Clive Wood and Gordon Freeman

1. A method for modulating an immune response comprising contacting an immune cell with an agent that modulates signaling via PD-1...
8. The method of claim 1, wherin the signaling via PD-1 is inhibited using an agent selected from the group consisting of: a blocking antibody that recognizes PD-1....
22. ..., wherein the condition is selected from the group consisting of: a tumor,

PD-L1 = B7-H1

Same molecule, different results

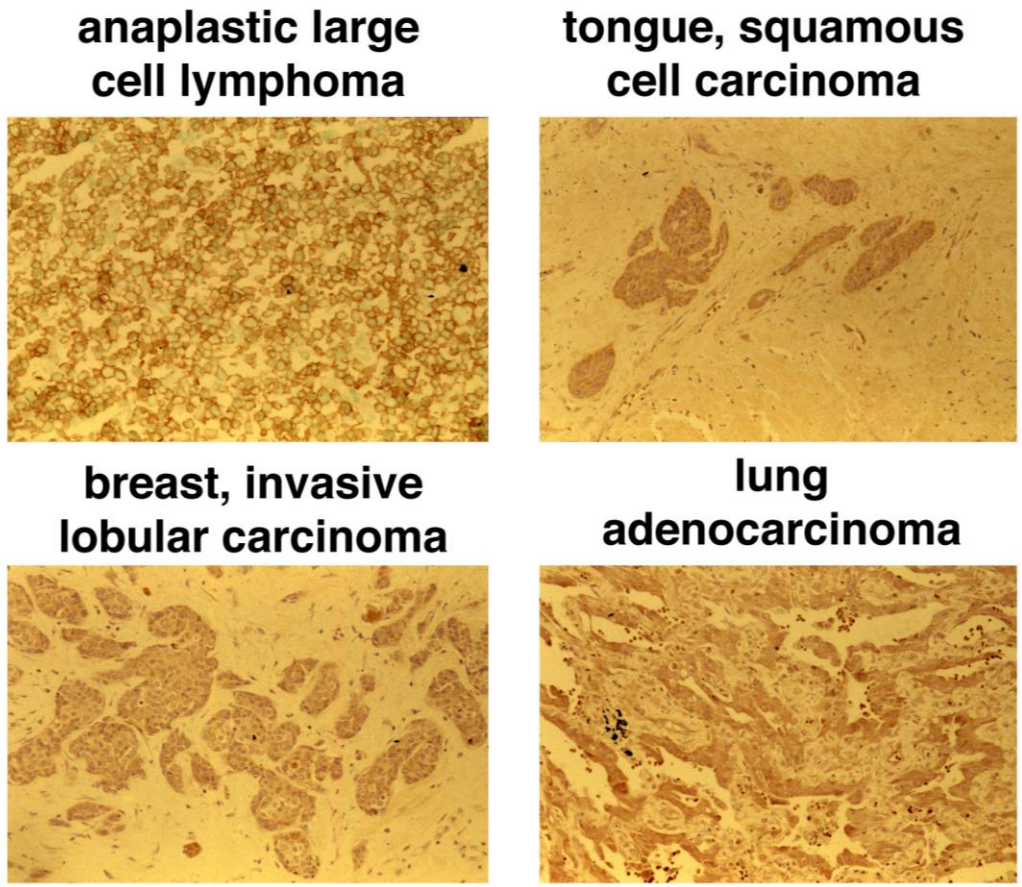
1999-2003

Does it stimulate or inhibit T cells ?

Different results with Fc fusion proteins and transfectants

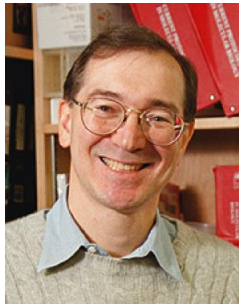
1. B7-H1, a third member of the B7 family, co-stimulates T-cell proliferation and interleukin-10 secretion. Dong H, Zhu G, Tamada K, Chen L. Nat Med. 1999; 5:1365-9

2. Tamura H, Dong H, Zhu G, Sica GL, Flies DB, Tamada K, Chen L. B7-H1 costimulation preferentially enhances CD28-independent T-helper function Blood. 2001; 97:1809-16.



PD-L1 Immunohistochemistry early 2000

David Dorfman



Gordon Freeman

Cancer starts to become a focus

- Meeting at AAI in Seattle, May 13, 2000

Anti-human PD-L1 mAbs

7 mAbs

all block binding of PD-1-Ig to PD-L1 transfected cells

2A3 good for immunohistochemistry of paraffin fixed tissue

5A9 good for immunohistochemistry of frozen sections

Immunohistochemistry of tumors

PD-L1 expressed on

- All thymomas
- Some lung carcinomas
- Some tongue squamous cell carcinomas

PD-L1 is not expressed on B cell neoplasms

Expressed on some T cell neoplasms, primarily anaplastic large cell lymphoma

Freeman et al. J. Exp. Med. 2000

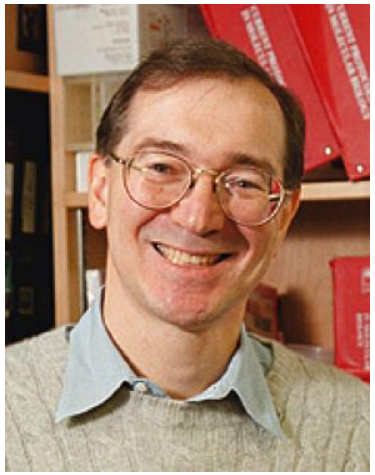
small intestine (16; Fig. 3 D). *PD-L1* is also expressed in some cancers, as three ESTs are from human ovarian tumors. This raises the possibility that some tumors may use *PD-L1* to inhibit an antitumor immune response.

Define the PD-1 pathway
Identified the drug target: block PD-1/PD-L1

Engagement of the PD-1 Immunoinhibitory Receptor by a Novel B7 Family Member Leads to Negative Regulation of Lymphocyte Activation

By Gordon J. Freeman,* Andrew J. Long,‡ Yoshiko Iwai,§ Karen Bourque,‡ Tatyana Chernova,* Hiroyuki Nishimura,§ Lori J. Fitz,‡ Nelly Malenkovich,* Taku Okazaki,§ Michael C. Byrne,‡ Heidi F. Horton,‡ Lynette Fouser,‡ Laura Carter,‡ Vincent Ling,‡ Michael R. Bowman,‡ Beatriz M. Carreno,‡ Mary Collins,‡ Clive R. Wood,‡ and Tasuku Honjo§

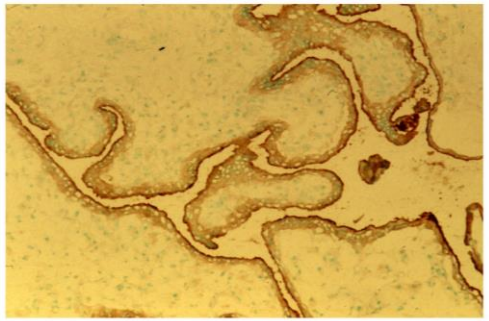
J. Exp. Med. © The Rockefeller University Press • 0022-1007/2000/10/1027/08 $5.00
Volume 192, Number 7, October 2, 2000 1027–1034



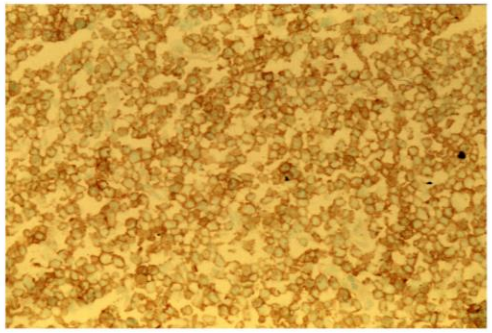
Cancer becomes a focus

- Meeting at Genetics Institute, September 8, 2000

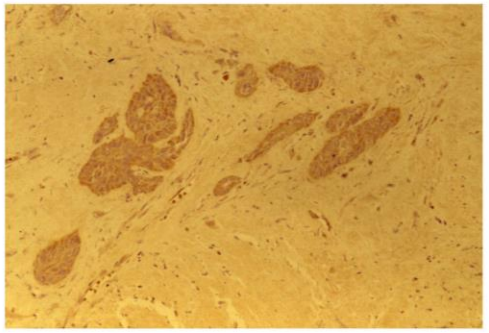
placenta



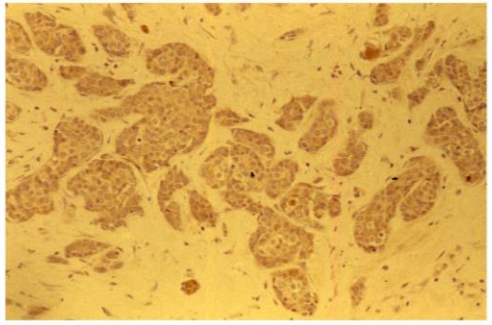
anaplastic large cell lymphoma



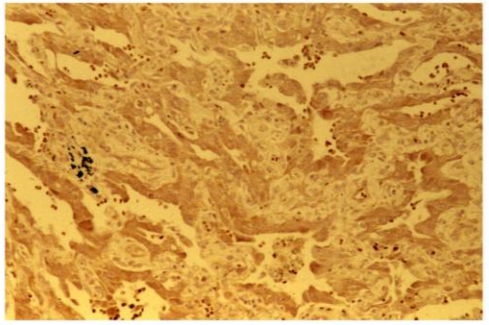
tongue, squamous cell carcinoma



breast, invasive lobular carcinoma



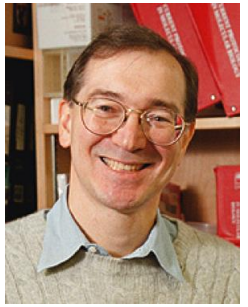
lung adenocarcinoma



PD-L1 tumor Immunohistochemistry presented at September 2000 meeting

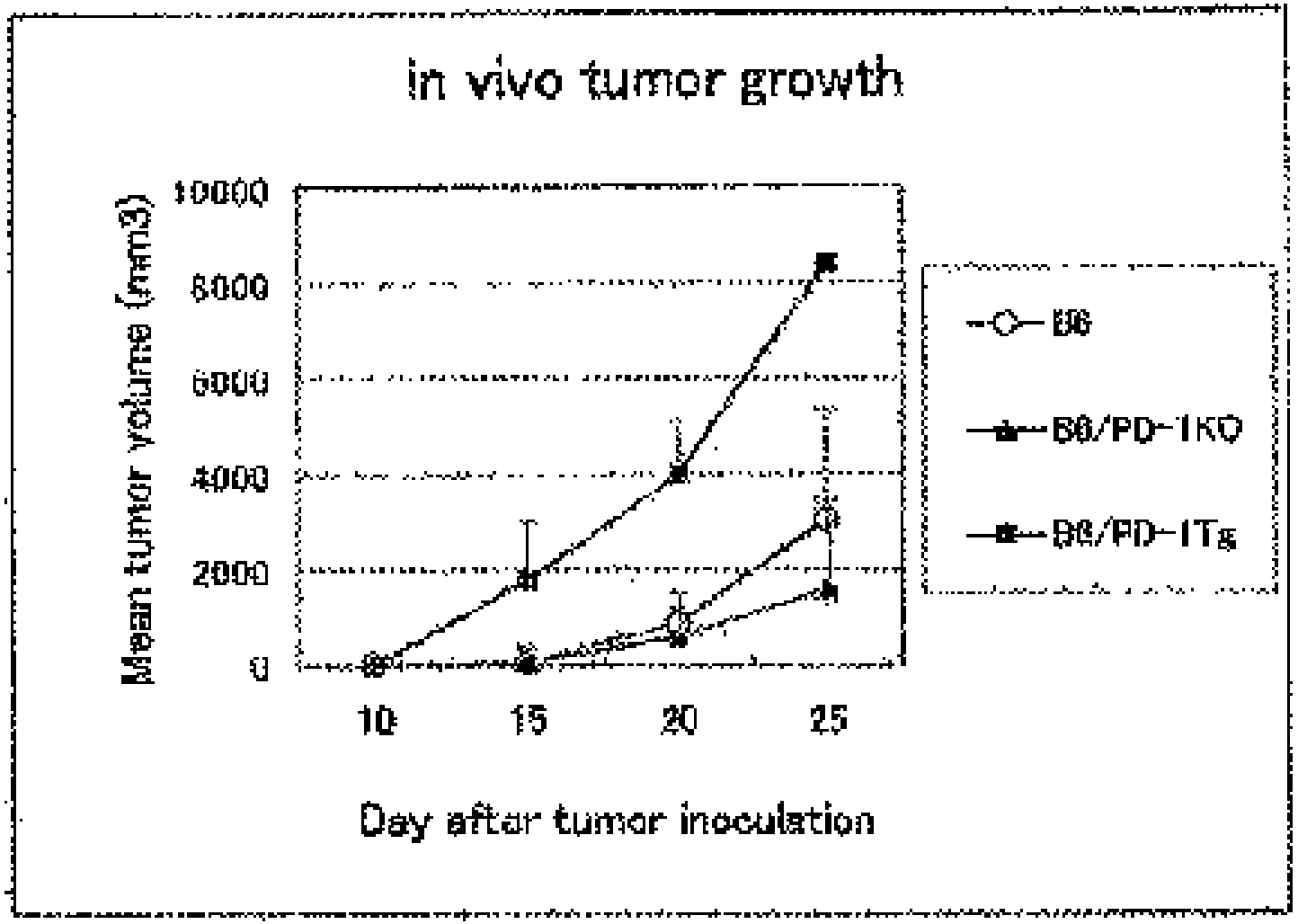
Submitted JEM June 2002, Published in Brown, Dorfman, et al., J Imm 2003; 170:1257-66

David Dorfman



Gordon Freeman

B16F10 transfected with mPD-L1 grows faster in a PD-1 transgenic and slower in a PD-1 KO



Yoshiko Iwai

PD-L2 is a second ligand for PD-1 and inhibits T cell activation

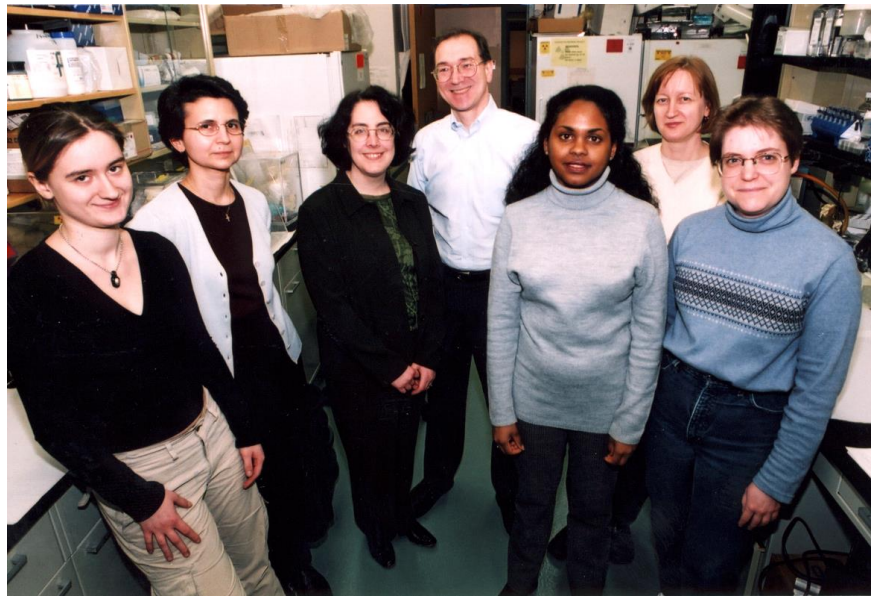
Yvette Latchman[1], Clive R.Wood[2], Tatyana Chernova[3], Divya Chaudhary[2], Madhuri Borde[1], Irene Chernova[3], Yoshiko Iwai[4], Andrew J. Long[2], Julia A. Brown[3], Raquel Nunes[3], Edward A. Greenfield[3], Karen Bourque[2], Vassiliki A. Boussiotis[3], Laura L. Carter[2], Beatriz M. Carreno[2], Nelly Malenkovich[3], Hiroyuki Nishimura[4], Taku Okazaki[4], Tasuku Honjo[4], Arlene H. Sharpe[1,*] and Gordon J. Freeman[3,*]

Discovery may shed light on cancer's shield against the immune system

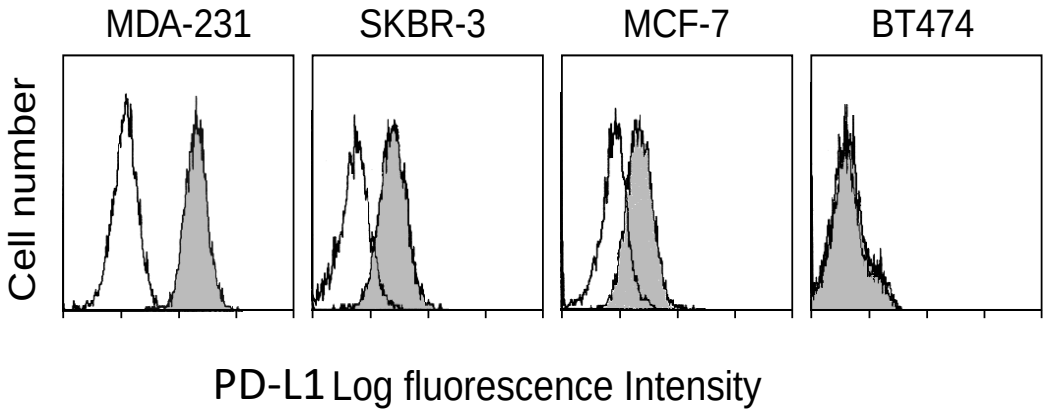
For years, a question has tantalized cancer researchers: why is the immune system, normally so adept at unmasking and eliminating foreign invaders and abnormal cells, not always spry enough to destroy tumor cells?

A new study by Dana-Farber scientists suggests an answer.

In a paper published in the March issue of *Nature Immunology*, investigators led by Gordon Freeman, Ph.D., of Adult Oncology report that a structure

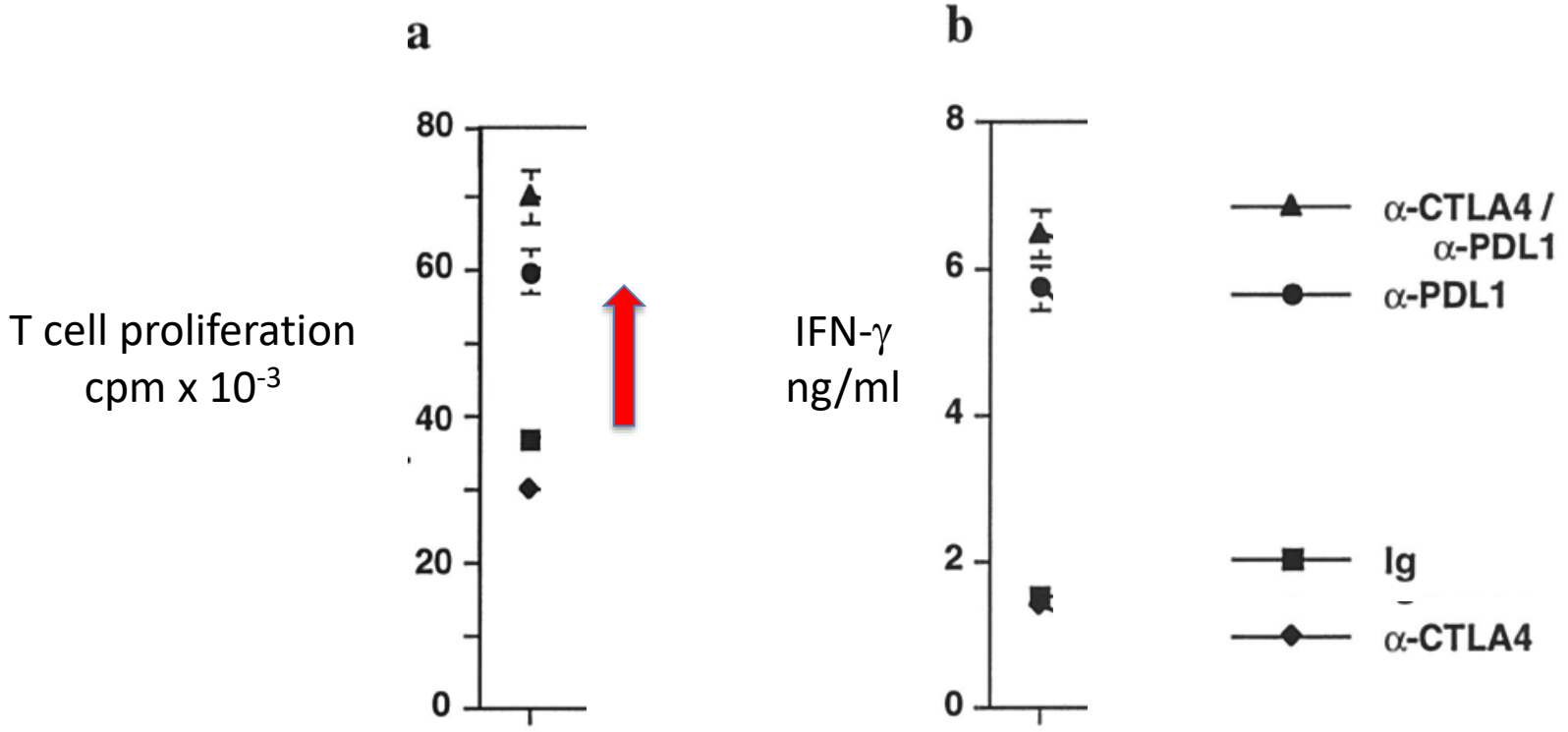


PD-L1 on Breast cancer cell lines



Nat Immunol. 2001 Mar; 2:261

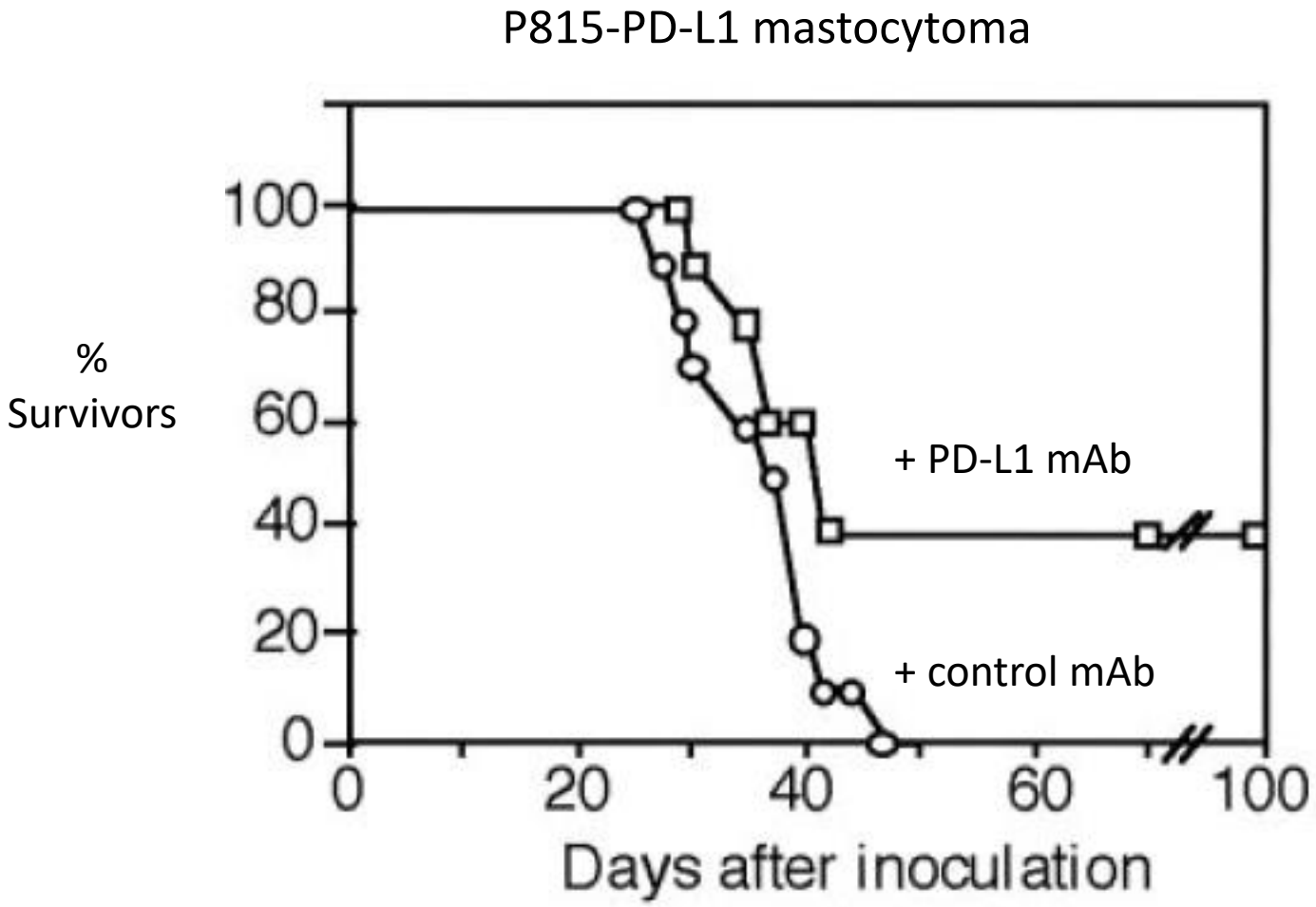
PD-L1 antibody blockade enhances CD4 T cell responses



Human $CD4^{+}CD25^{-}$ T cells
+ anti-CD3 and anti-CD28
+ T depleted accessory cells

Baecher-Allen et al., J Immunol. 2001; 167:1245

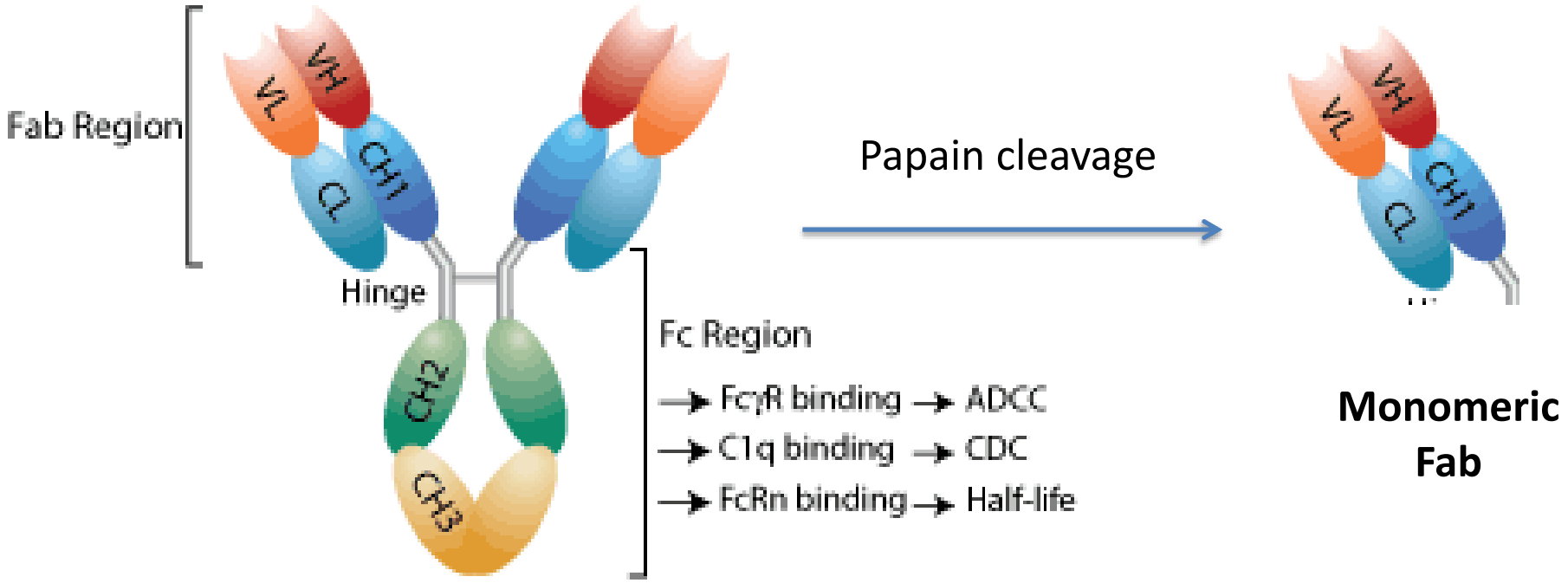
PD-L1 antibody tumor immunotherapy



Yoshiko Iwai

Iwai, et al. PNAS. 2002; 99:12293

What is the right antibody drug strategy: blockade or crosslinking?

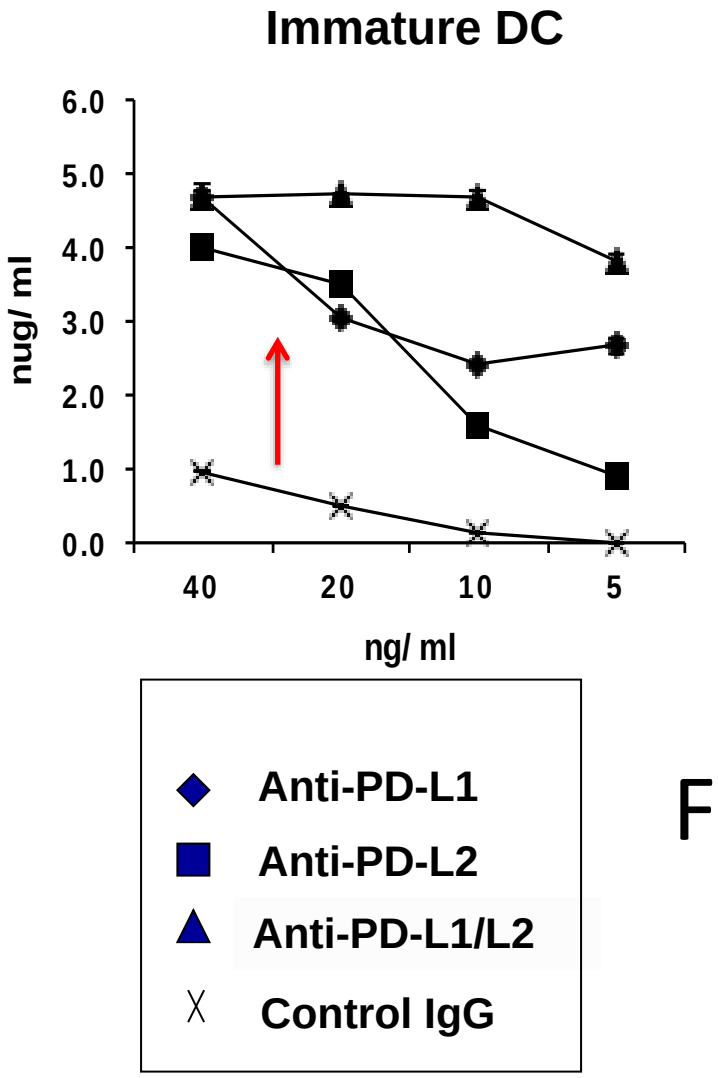


PD-L1 antibody

Monomeric Fab

PD-Ligand blockade with Fab enhances Interferon-γ production in a Mixed Leukocyte Response (MLR)

IFN-γ



Fab

Julia Brown

Brown et al. J. Imm 2003; 170: 1257

Clinical translation:
block the PD-L1/L2 : PD-1 pathway

Drug development of PD-L1 and PD-1 (nivolumab) mAbs begins at Medarex in 2001

Medarex and Ono/Honjo enter into research agreement to develop PD-1 mAb in May 2005

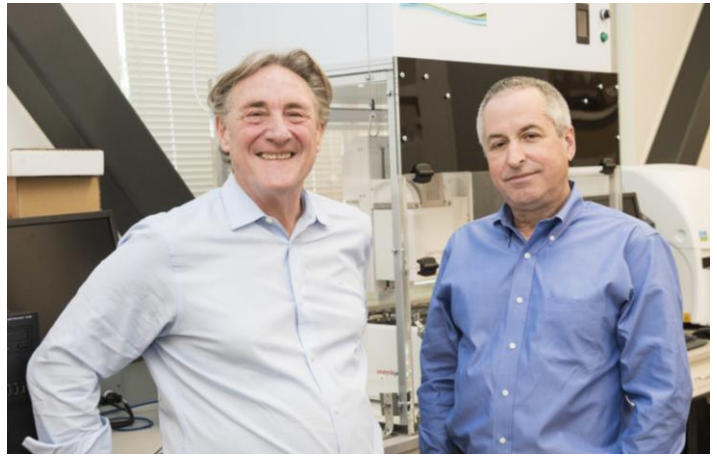
Phase I trial begins 2006

Phase I safety data reported 2008

Medarex acquired by BMS 2009

Pivotal trial reported in 2012

FDA approval in 2014



Alan Korman & Nils Lonberg

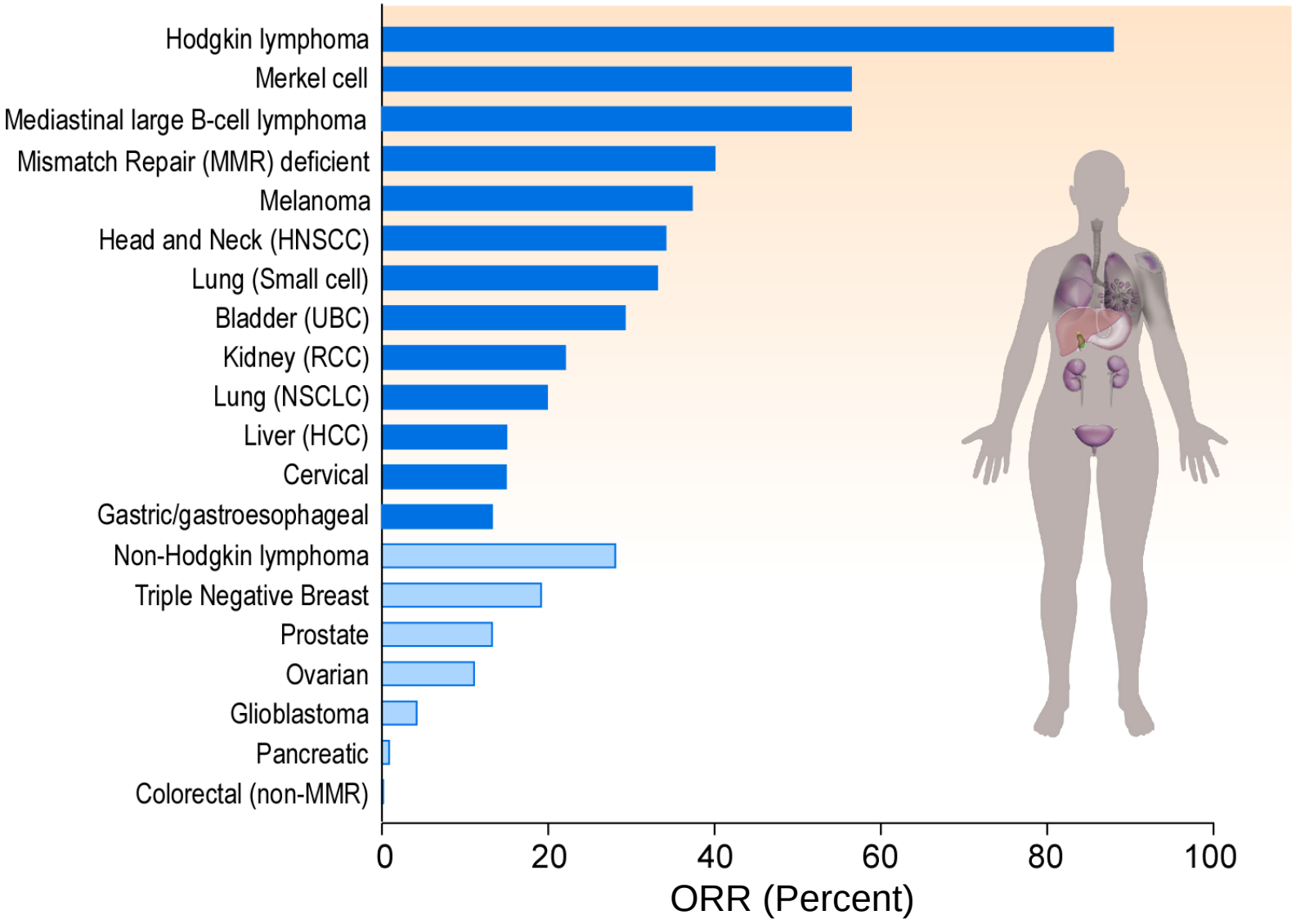
Intellectual property issues of immune checkpoint inhibitors

Ulrich Storz*

mAbs 8:1, 10–26; January 2016;

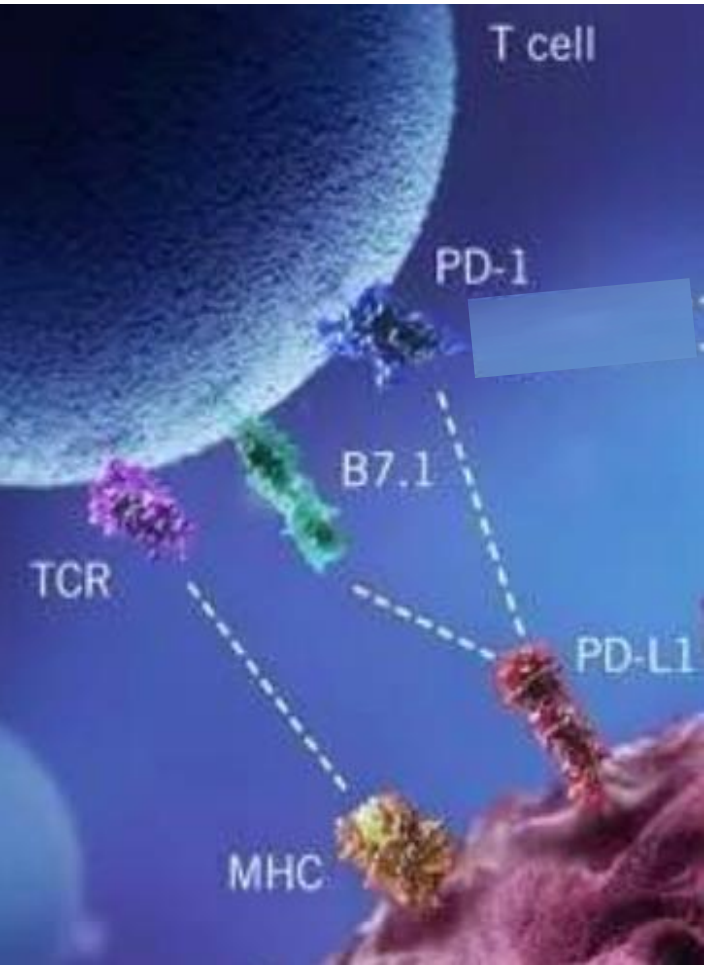
Harvard and Dana-Farber out-licensed 11 patents from this portfolio non-exclusively to BMS, Merck & Co. (Merck), Roche, Novartis, Boehringer Ingelheim, Amplimmune, and MerckSerono (**Table 3**).[16] This open policy has tremendously spurred research on antibodies against PD-1 and its ligands, and today 34 candidates are in clinical studies

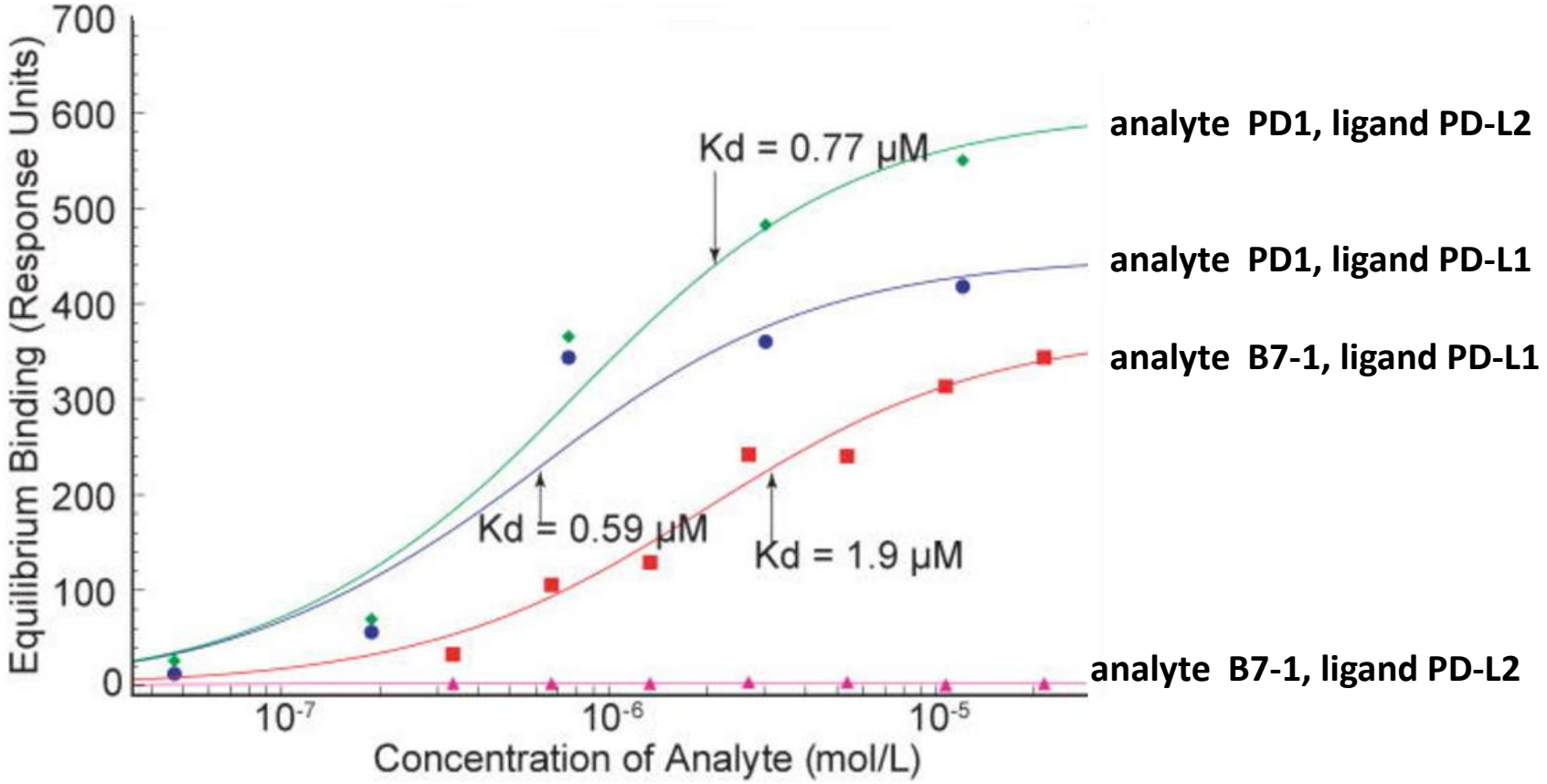
Broad anti-tumor efficacy of anti-PD-1/PD-L1 inhibitors:
Overall Response Rates (ORR)



New Insights

PD-L1 binds to B7-1 (CD80)

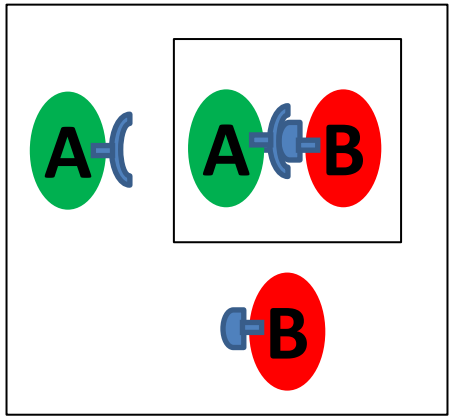




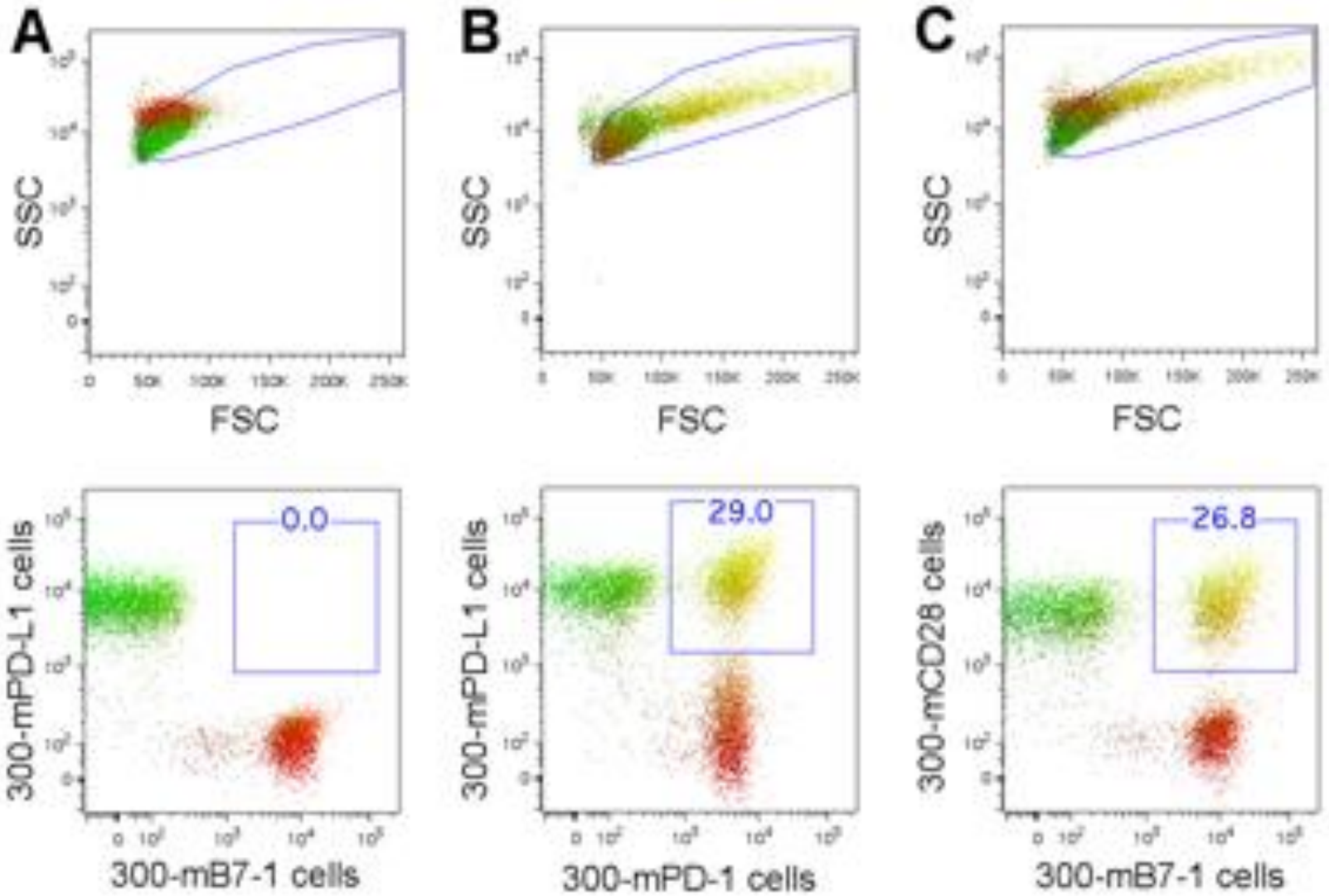
Butte ... Sharpe, Freeman, Immunity. 2007; 27:111

Cell-to cell binding assay:
PD-L1 does not bind to B7-1 in trans

Yanping Xiao

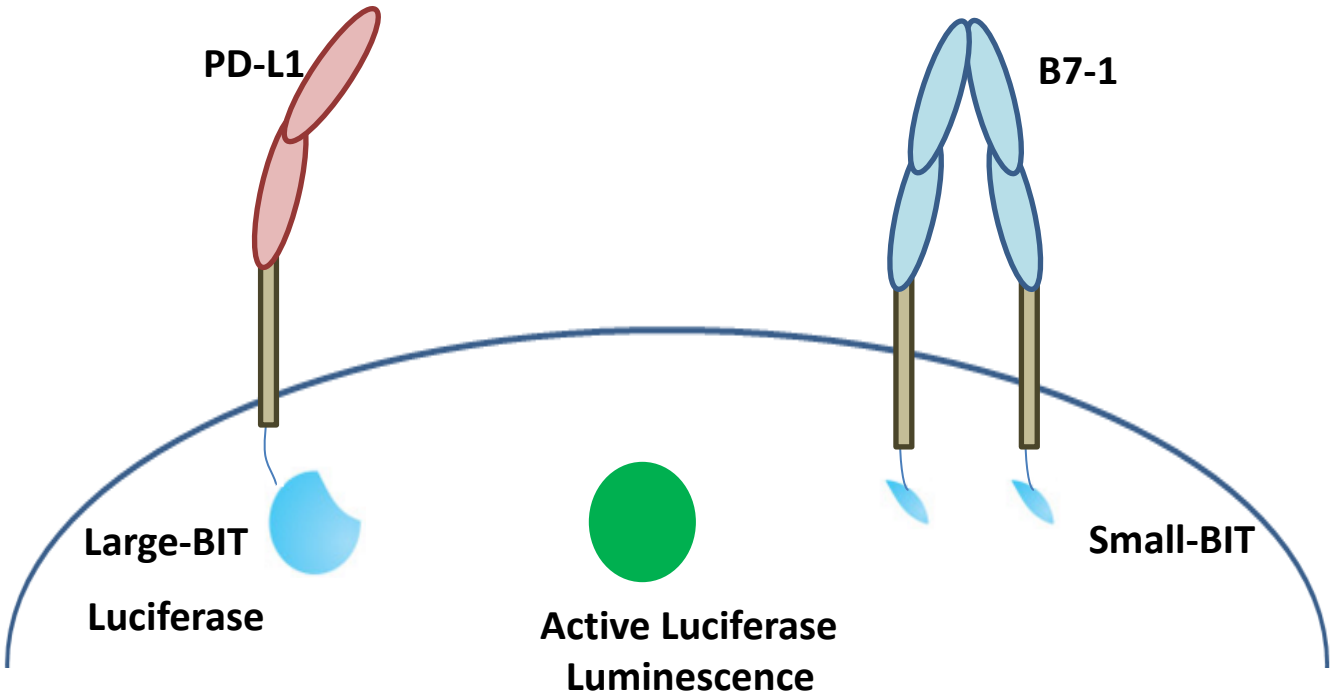


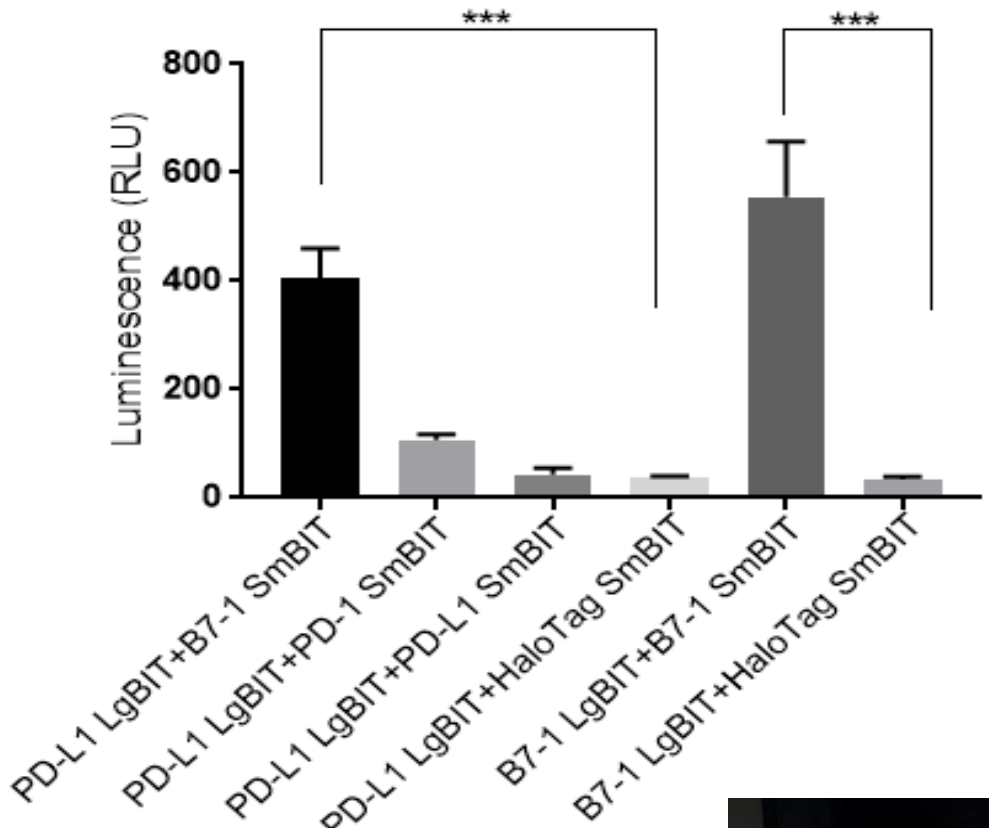
Chaudhri, Xiao... Freeman.
Canc Imm Res. 2018; 6:921



PD-L1 and B7-1 associate in *cis* on the same cell surface

Nanobit proximity assay

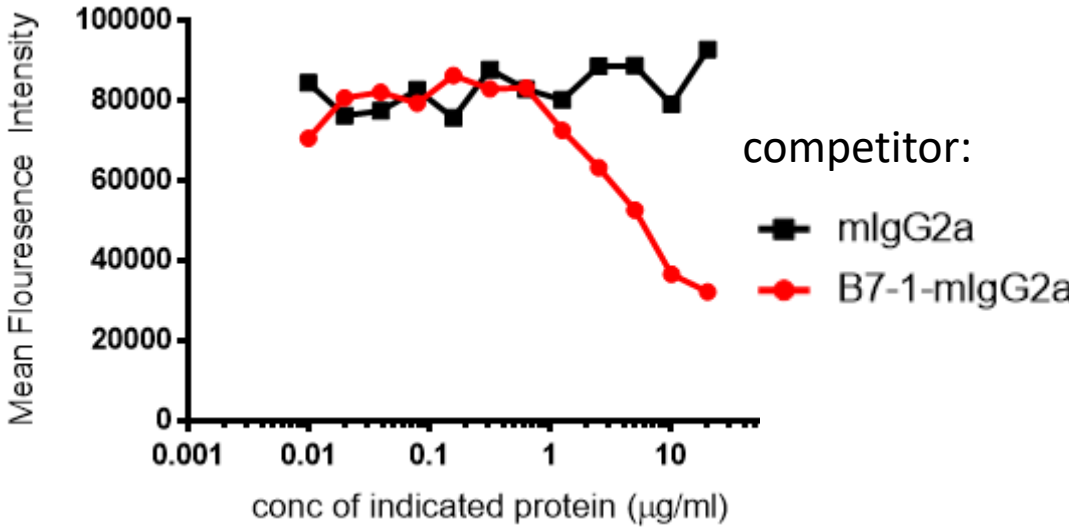




Apoorvi Chaudhri

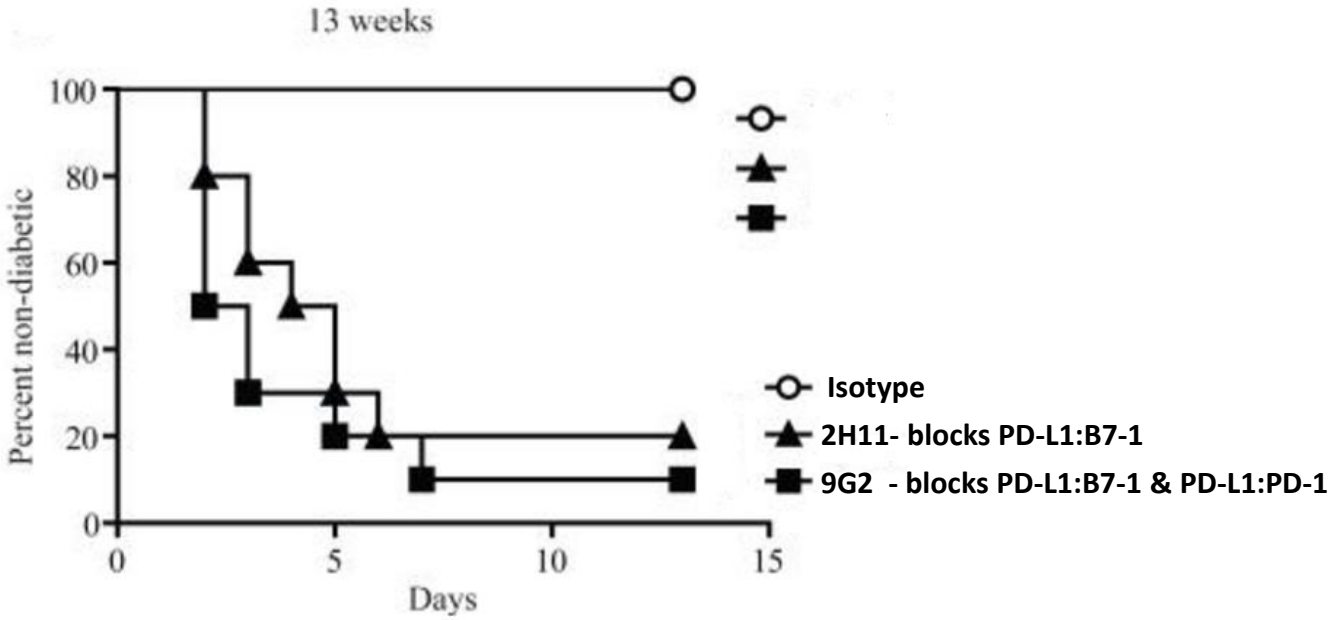
B7-1 competes with PD-1 for binding to PD-L1

Binding of PD-1 to PD-L1



Chaudhri, Xiao... Freeman.
Canc Imm Res. 2018; 6:921

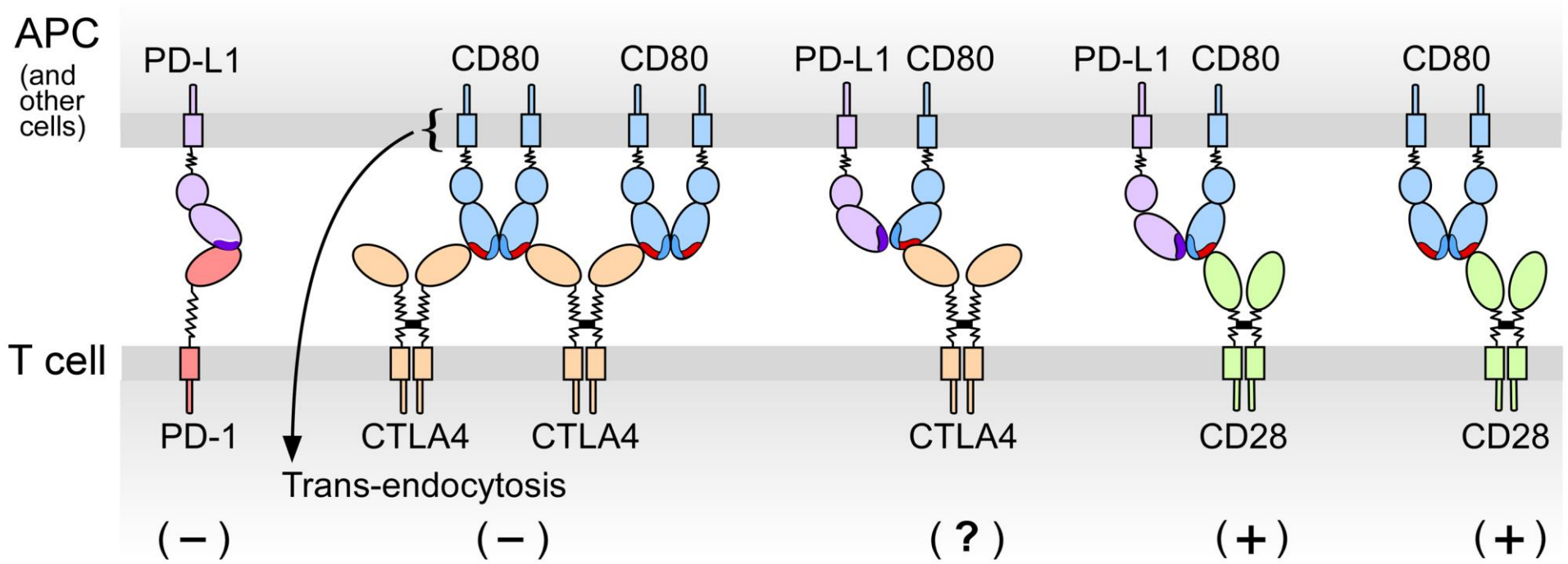
Blockade of PD-L1 : B7-1 accelerates onset of diabetes in NOD



In this case, the net effect of PD-L1 interaction with B7-1 is immunoinhibitory

Paterson...Freeman, Sharpe. J Imm 2011; 187:1097

Dynamic interplay



CD80 **>** PD-L1 favors CD28 signaling, more CTLA-4 trans-endocytosis, less PD-1 signaling

PD-L1 **>** CD80 favors PD-1 signaling, CD28 signaling, less CTLA-4 trans-endocytosis

additional insights from:
Sugiura...Okazaki, Science 2019; 364:558
Zhao...Hui, Immunity 2019; 51:1059

Tsunami of data coming:
1000s of PD-1/PD-L1 and combo clinical trials

- We will learn
  - What works
  - What's safe
  - How it works
  - Who it will work for

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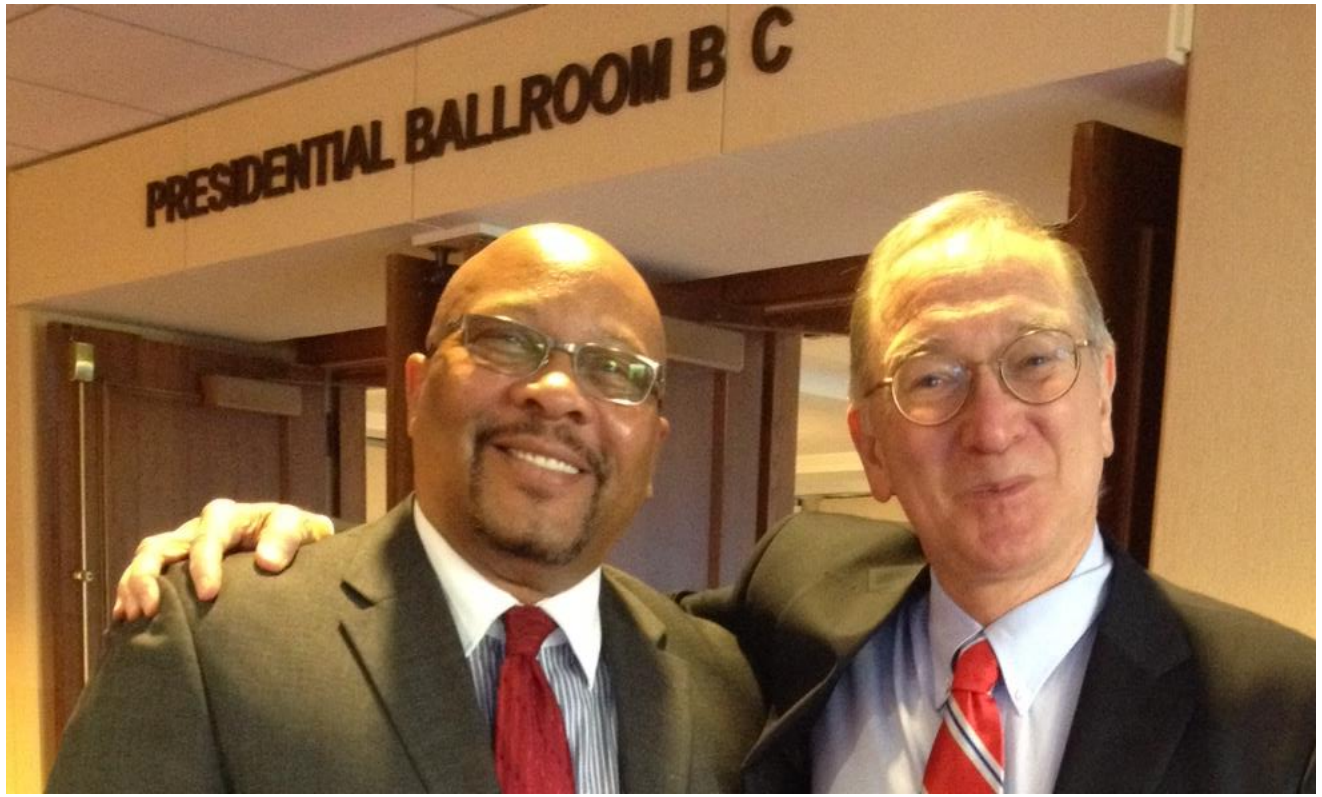
- Tasuku Honjo

The winners: patients and their families !!



Barry Nelson: NSCLC patient 9 years after PD-1 immunotherapy