Università degli Studi di Padova

Corso di Laurea in

BIOTECNOLOGIE

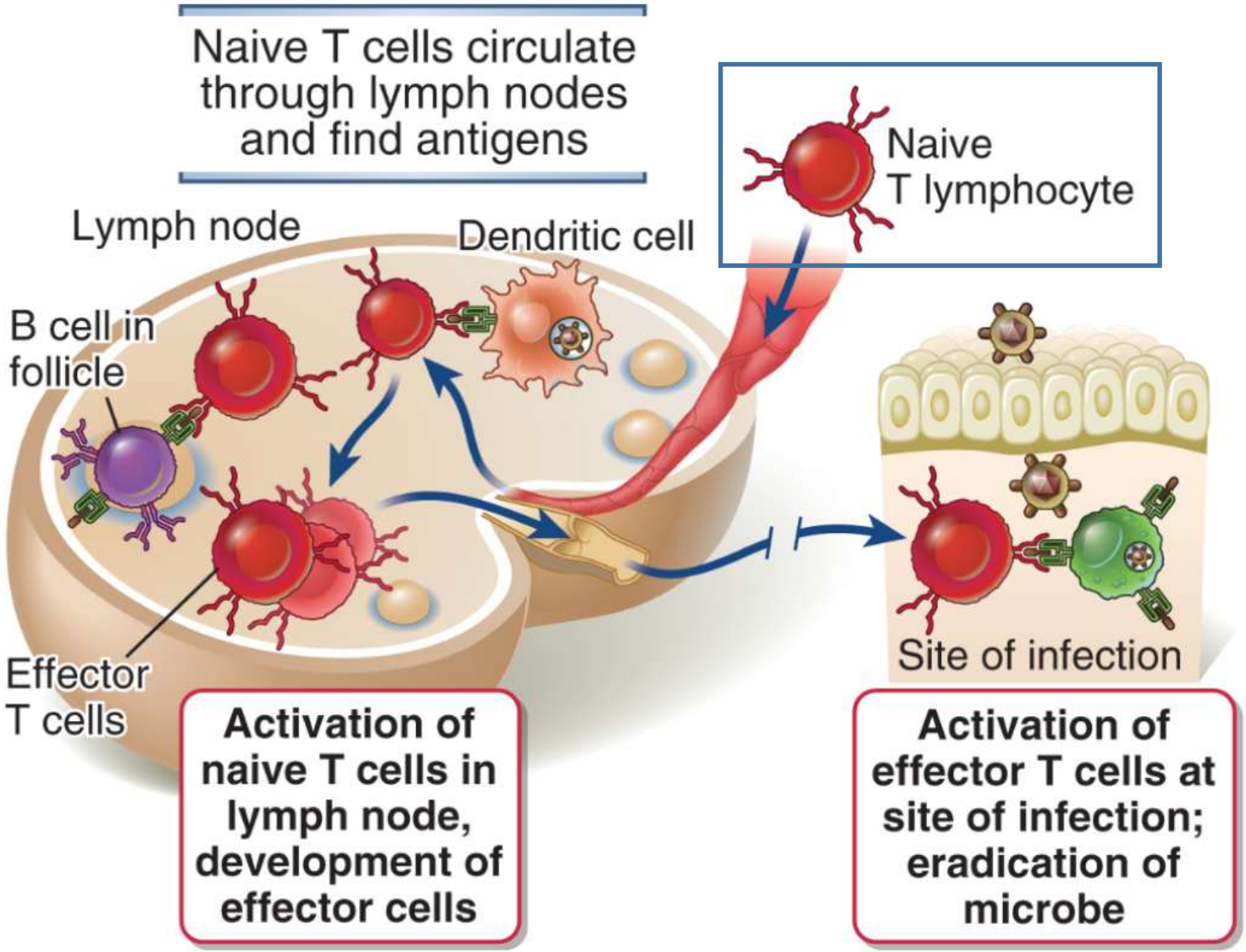
Piano di studi Farmaceutico

Anno Accademico 2024-2025

Immunologia Farmaceutica

Attivazione dei linfociti T

Activation of naive and effector T cells by antigen



Risultato degli eventi di attivazione nei linfociti T e B naive

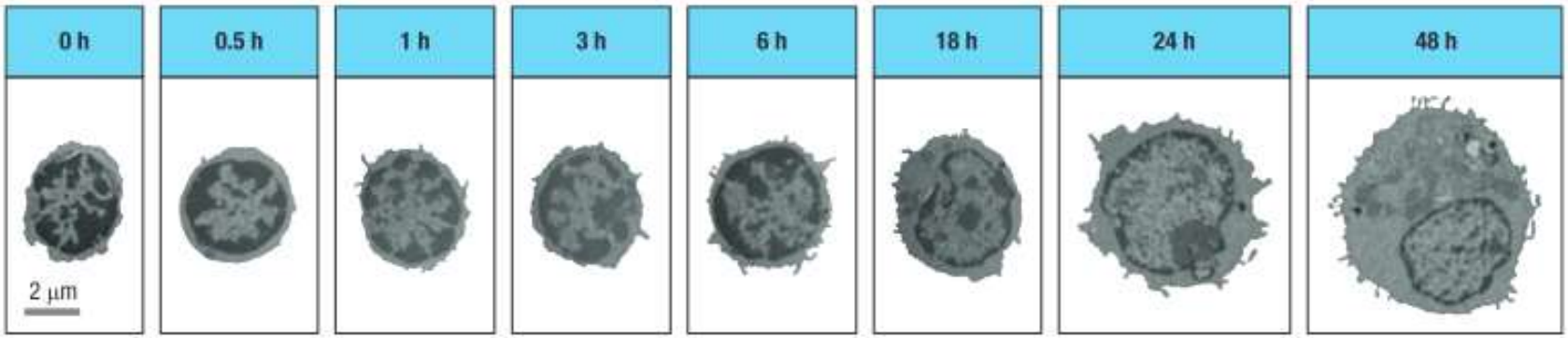
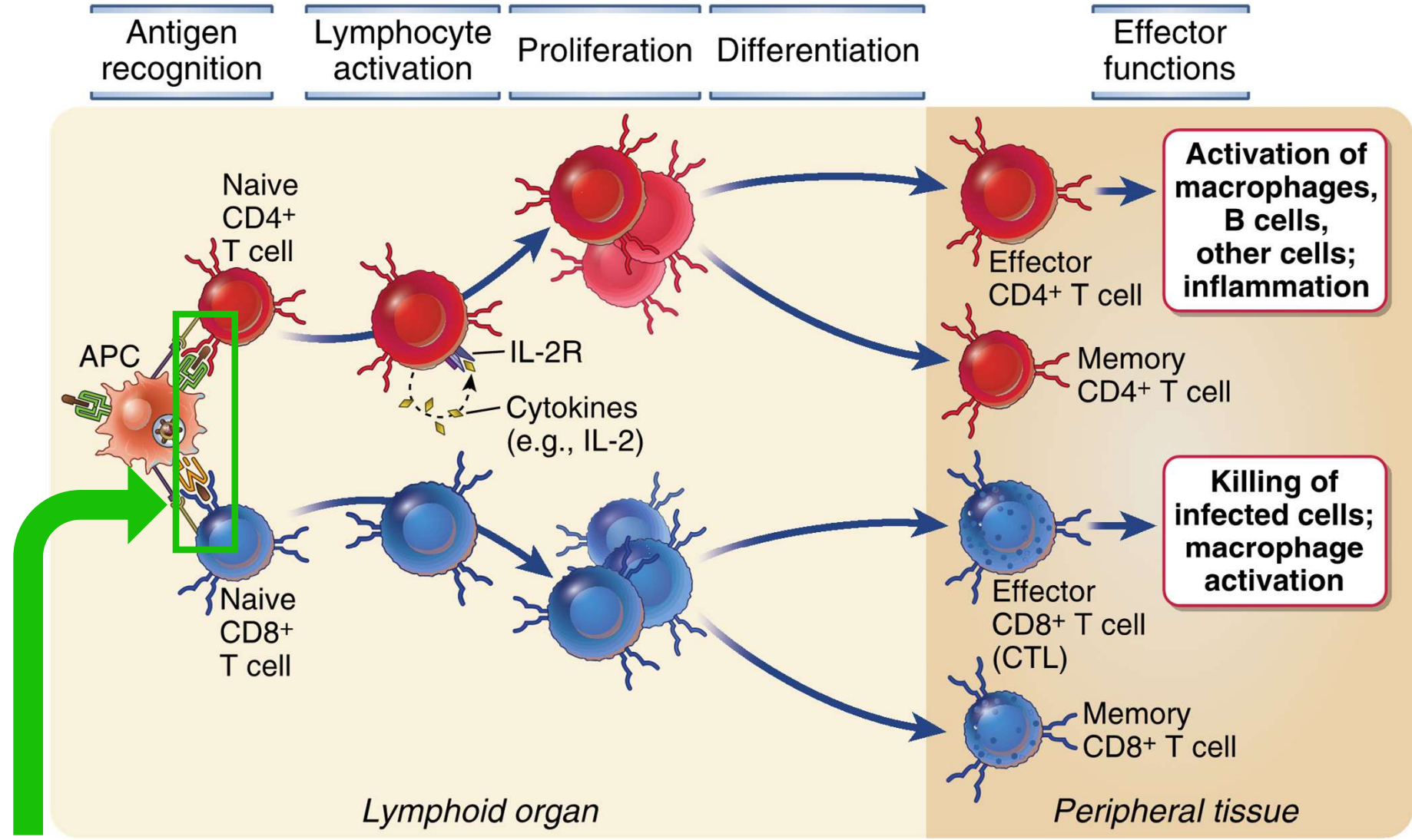


Fig. 7.1 Lymphocyte activation converts small nondividing cells into effector cells. Transmission electron microscopy was used to view resting naive T cells before and after stimulation with anti-CD3 antibodies. Among the changes that can be seen are the increased size of the cell, the expanded volume of the cell cytoplasm, and the decondensation of the chromatin in the cell nucleus.

Sequence of events in T cell responses



Signal transduction and lymphocyte activation

Riassunto delle vie di segnale del recettore per l'antigene

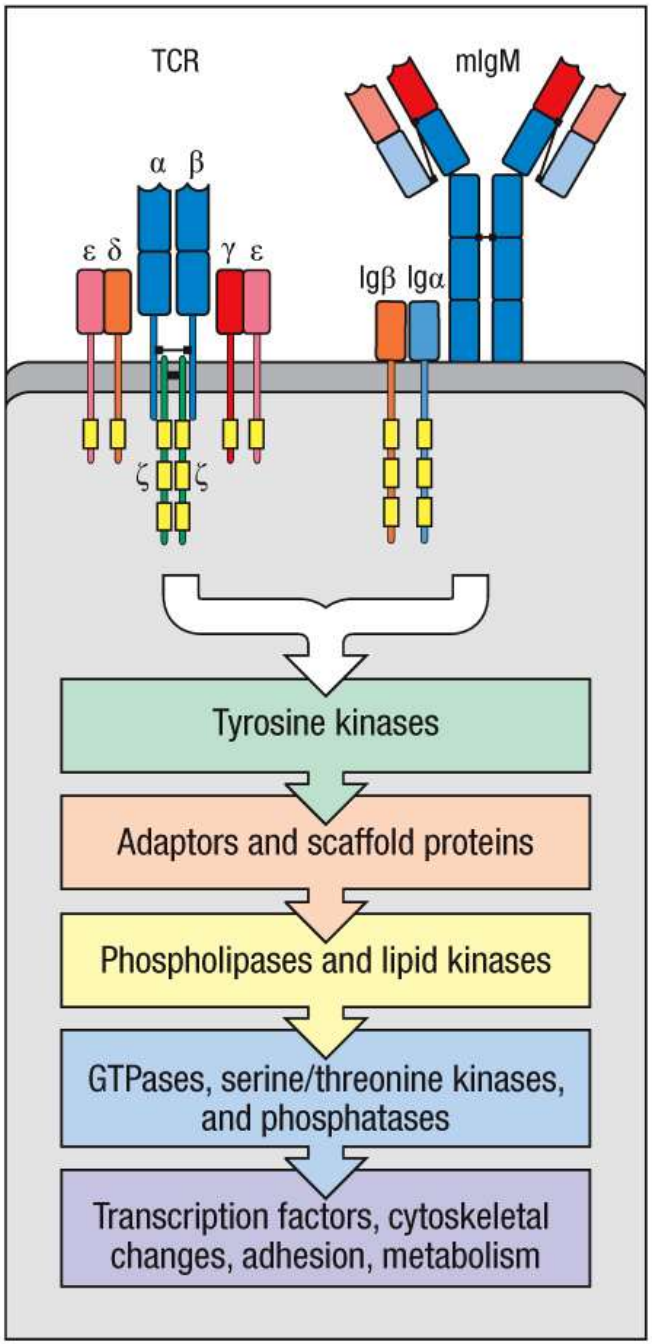
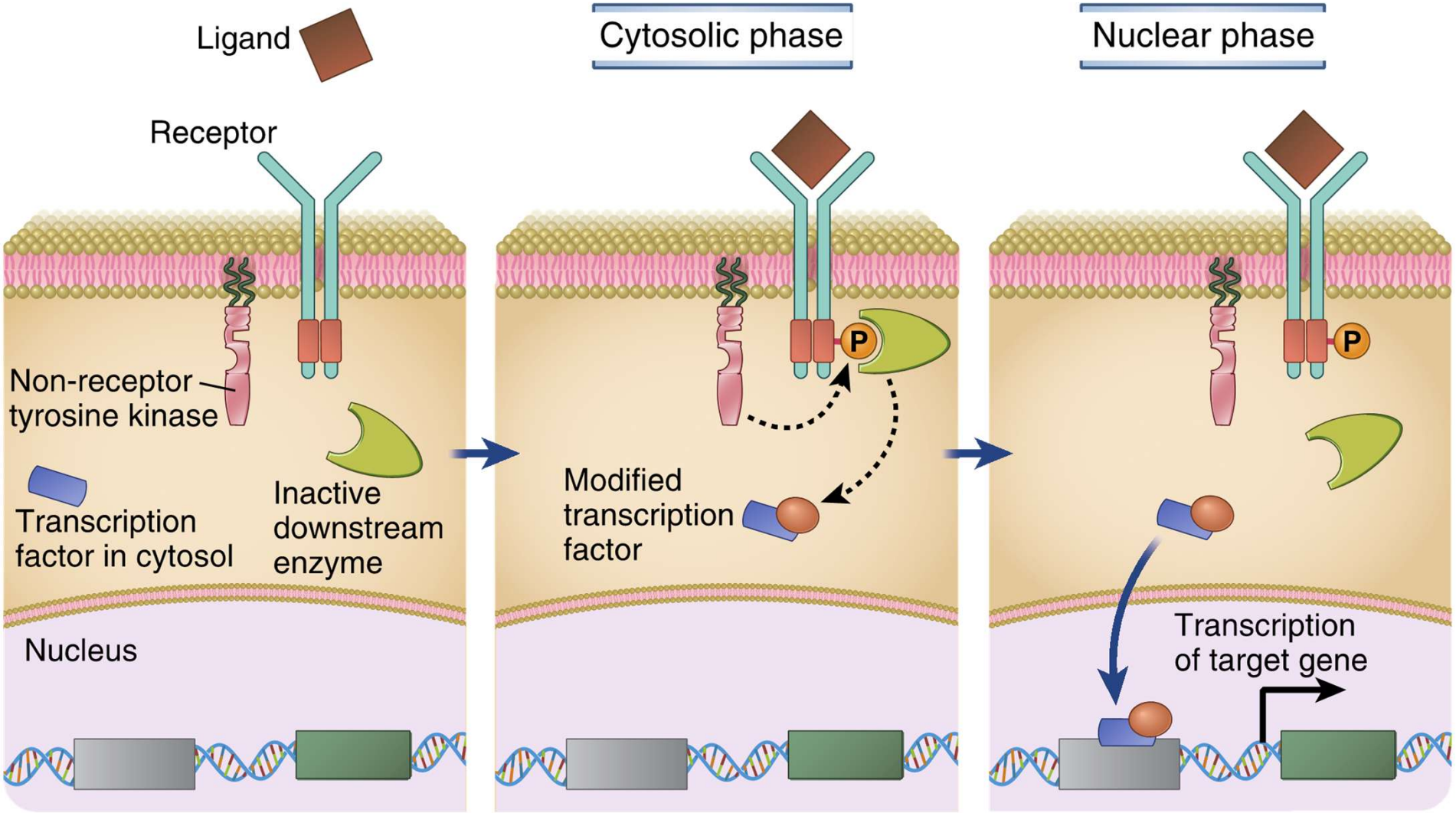


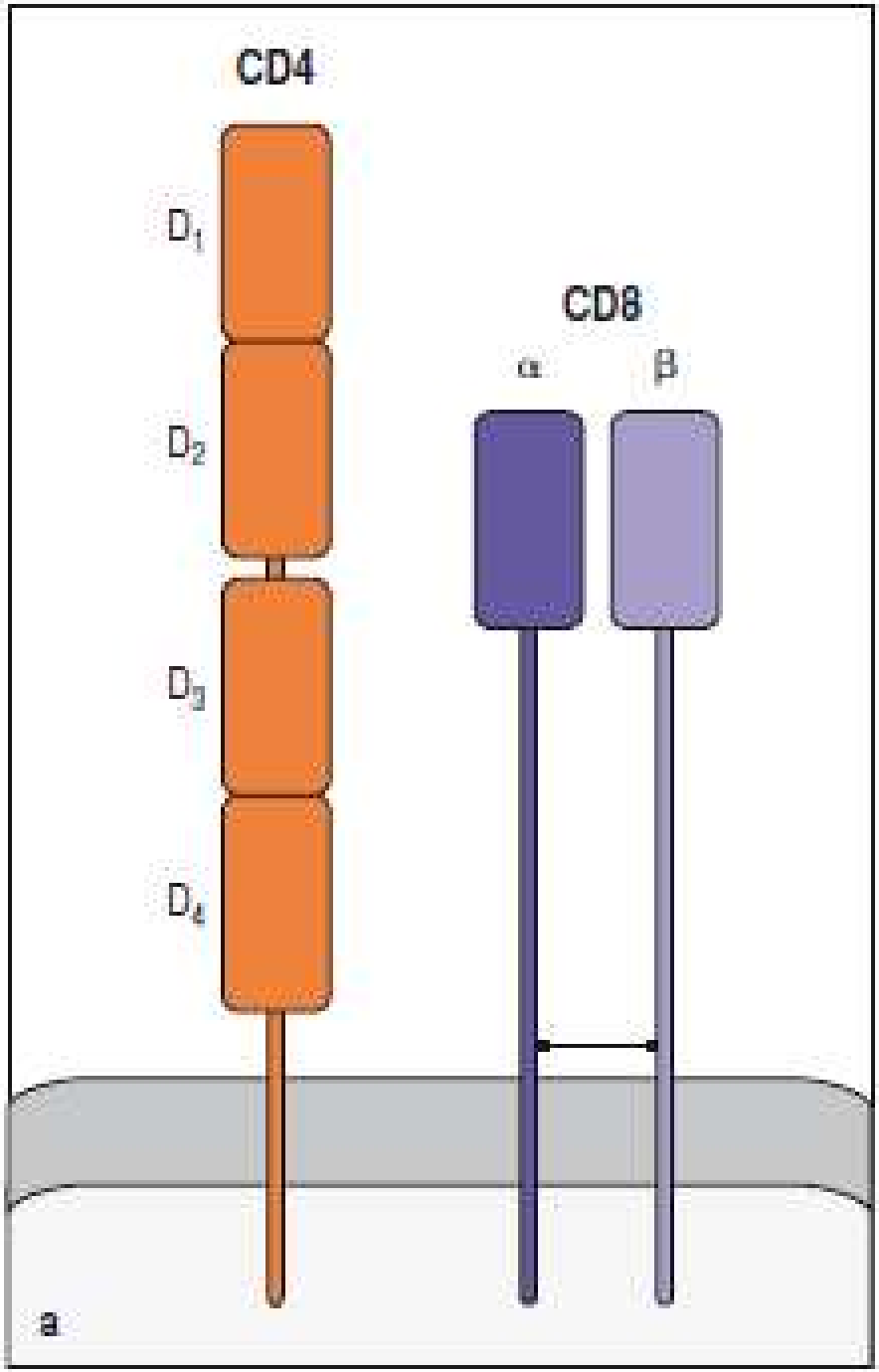
Figure 7.28 Janeway's Immunobiology, 9th ed. (© Garland Science 2017)

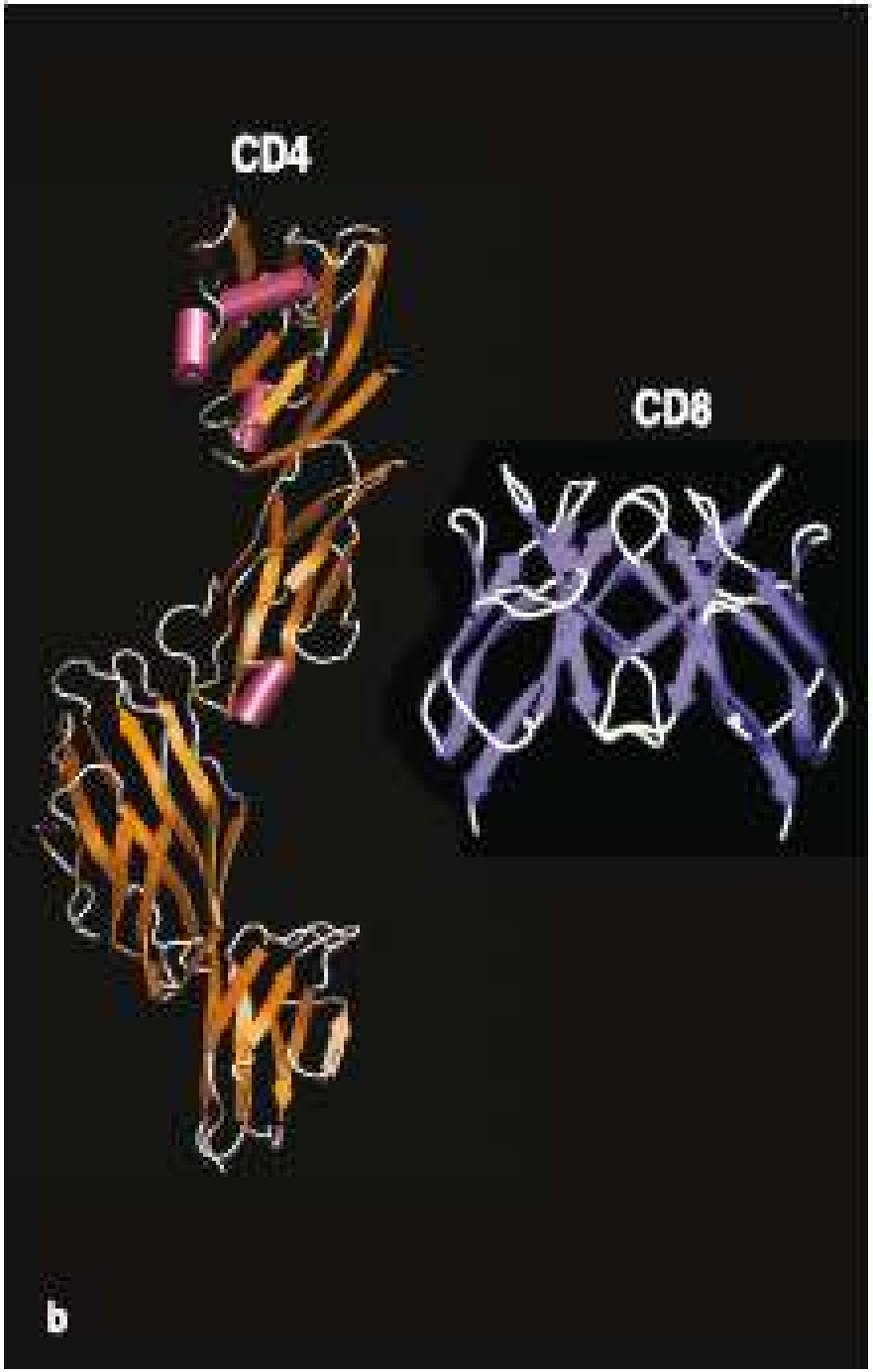
Serie orchestrata di stadi che coinvolgono molte categorie di proteine che producono cambiamenti diffusi nelle cellule

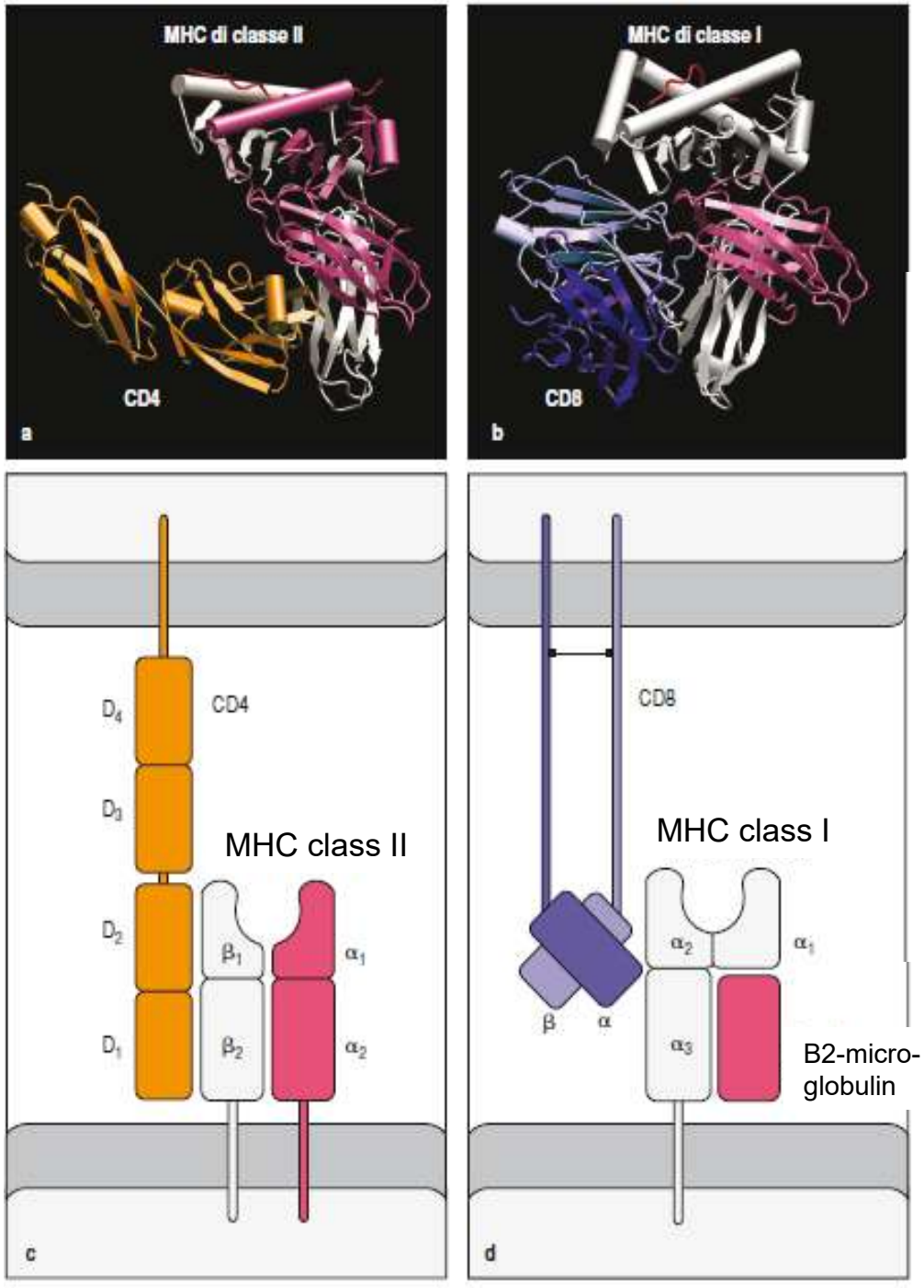
Signaling from the cell surface involves cytosolic and nuclear phases

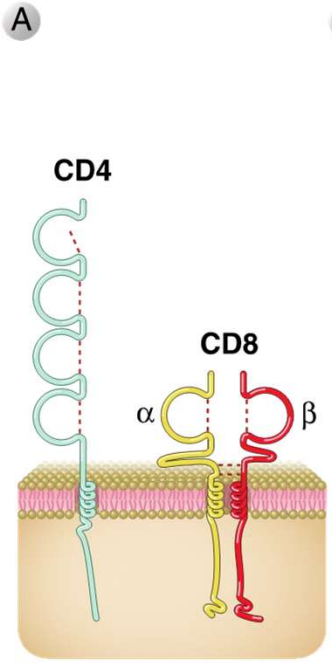


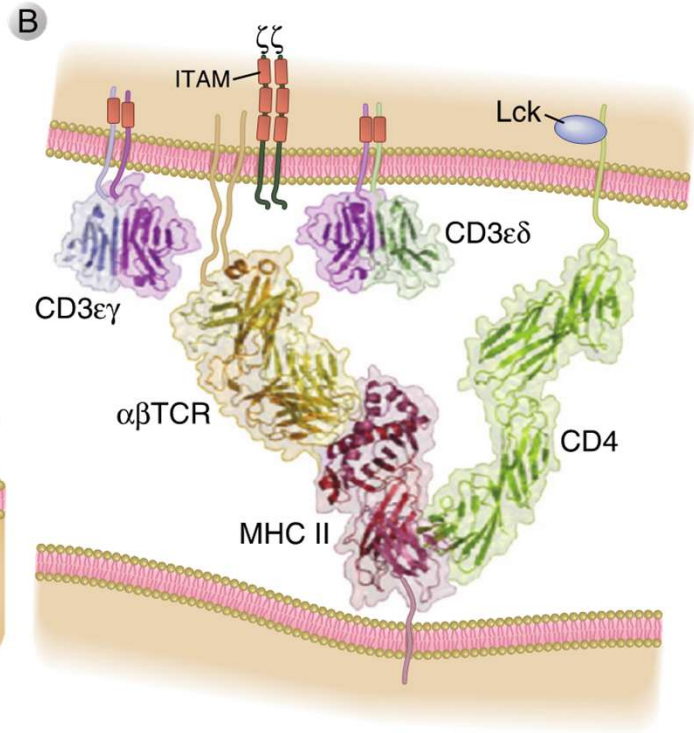
The T lymphocyte co-receptors





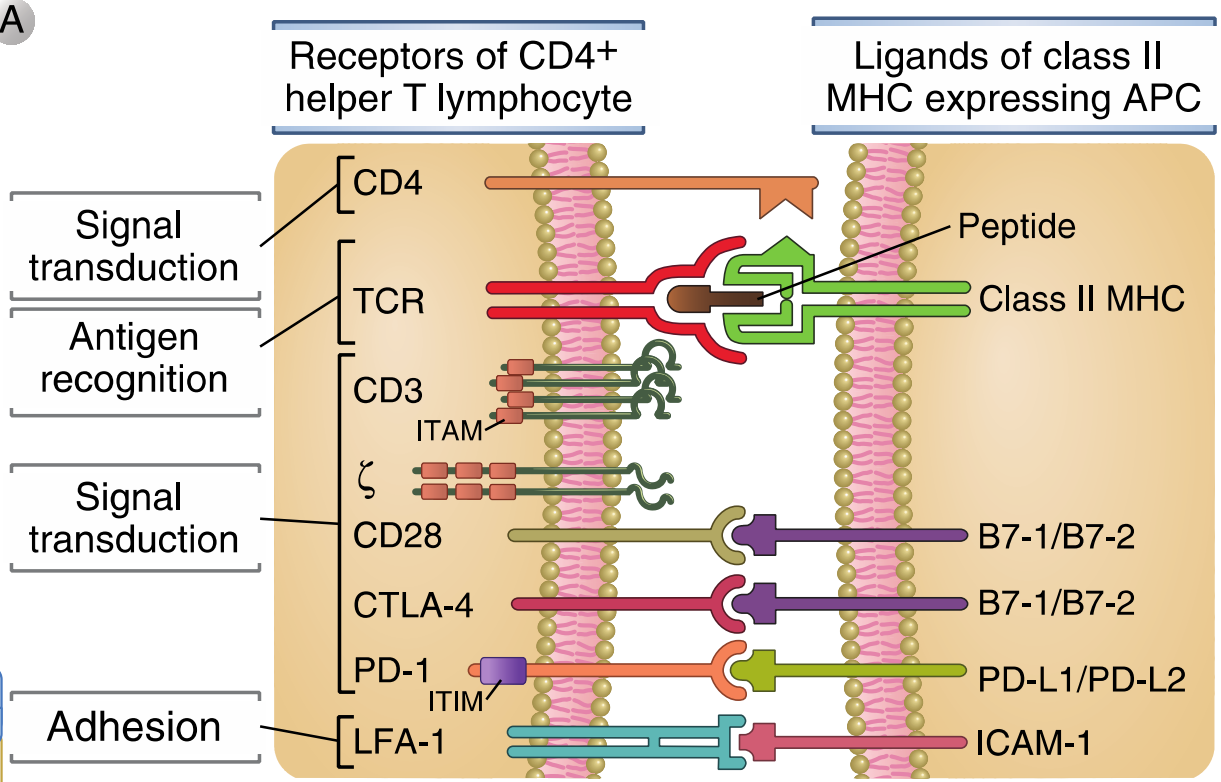

MHC di classe II
CD4
a
MHC di classe I
CD8
b
D4
CD4
D3
MHC class II
D2
β1
α1
D1
β2
α2
c
CD8
MHC class I
α2
α1
β
α
α3
B2-micro-
globulin
d


A
CD4
CD8
α
β


B
ζζ
ITAM
Lck
CD3εδ
CD3εγ
αβTCR
CD4
MHC II

Ligand-receptor pairs involved in T cell activation

A



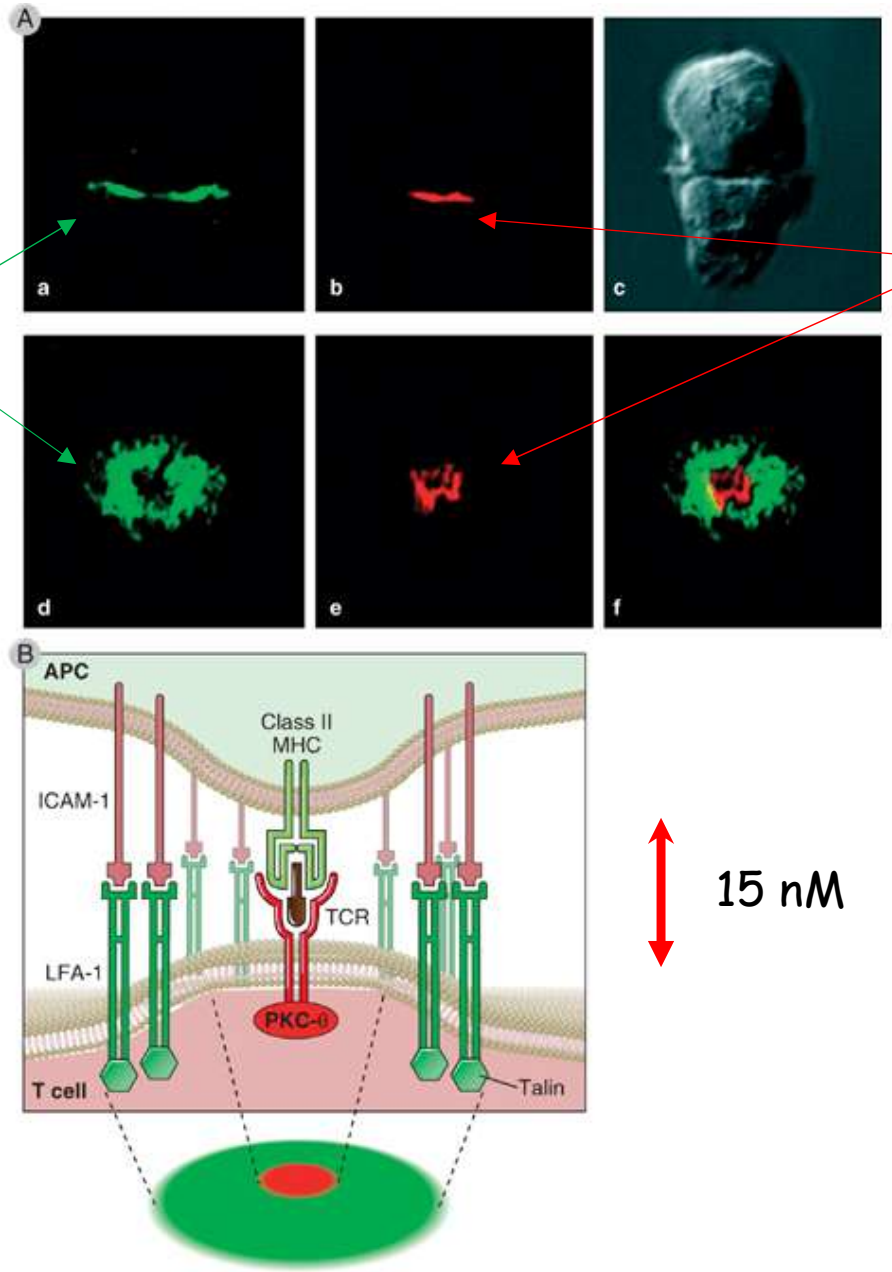
B

T cell molecule	Function	Ligand	
		Name	Expressed on
CD3	Signal transduction by TCR complex	None	
ζ	Signal transduction by TCR complex	None	
CD4	Signal transduction	Class II MHC	Antigen presenting cells
CD8	Signal transduction	Class I MHC	All nucleated cells
CD28	Signal transduction (costimulation)	B7-1/B7-2	Antigen presenting cells
CTLA-4	Inhibition	B7-1/B7-2	Antigen presenting cells
PD-1	Inhibition	PD-L1/PD-L2	Antigen presenting cells, tissue cells, tumor cells
LFA-1	Adhesion	ICAM-1	Antigen presenting cells, endothelium

Formation of a specialized structure: the immunological synapse (SMAC)

Talina associata a LFA-1

PKCΘ associata al TCR



40 nM

15 nM

Lipid rafts
TCR Microclusters

Not only signalling but also orientation of effector function

Supramolecular activation cluster (SMAC): center (c) and periphery (p)

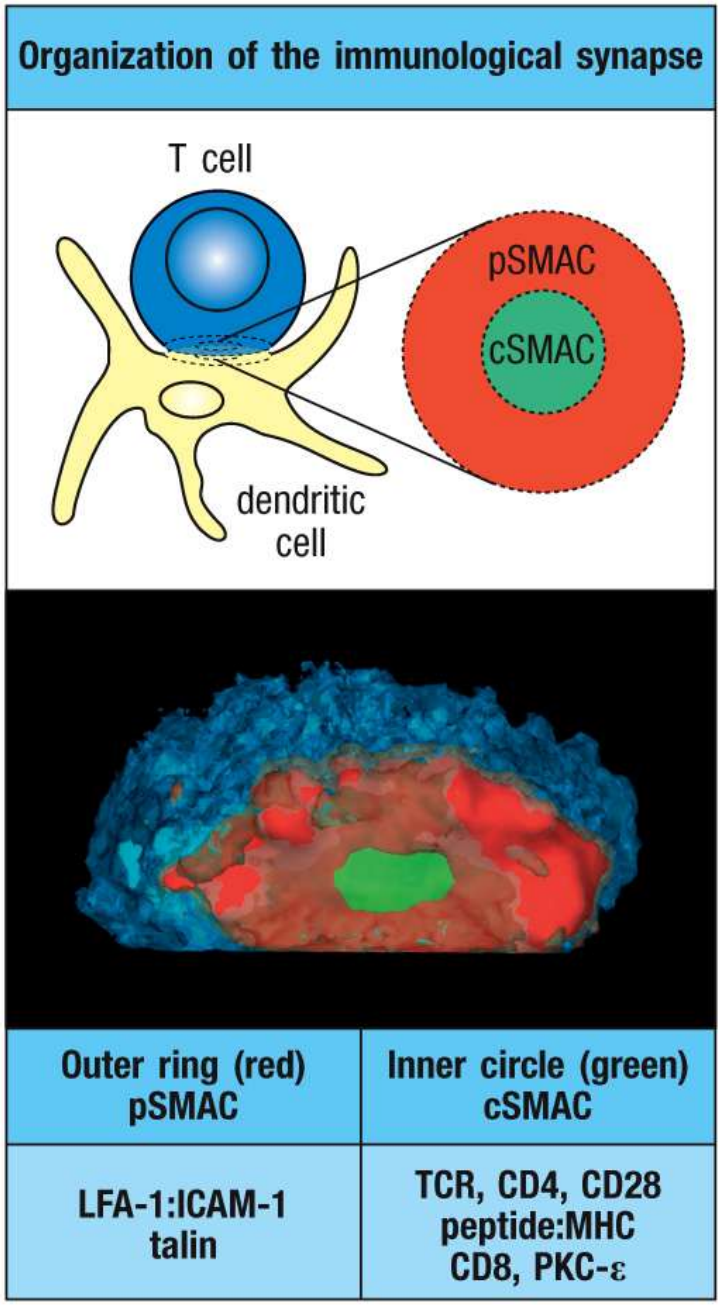
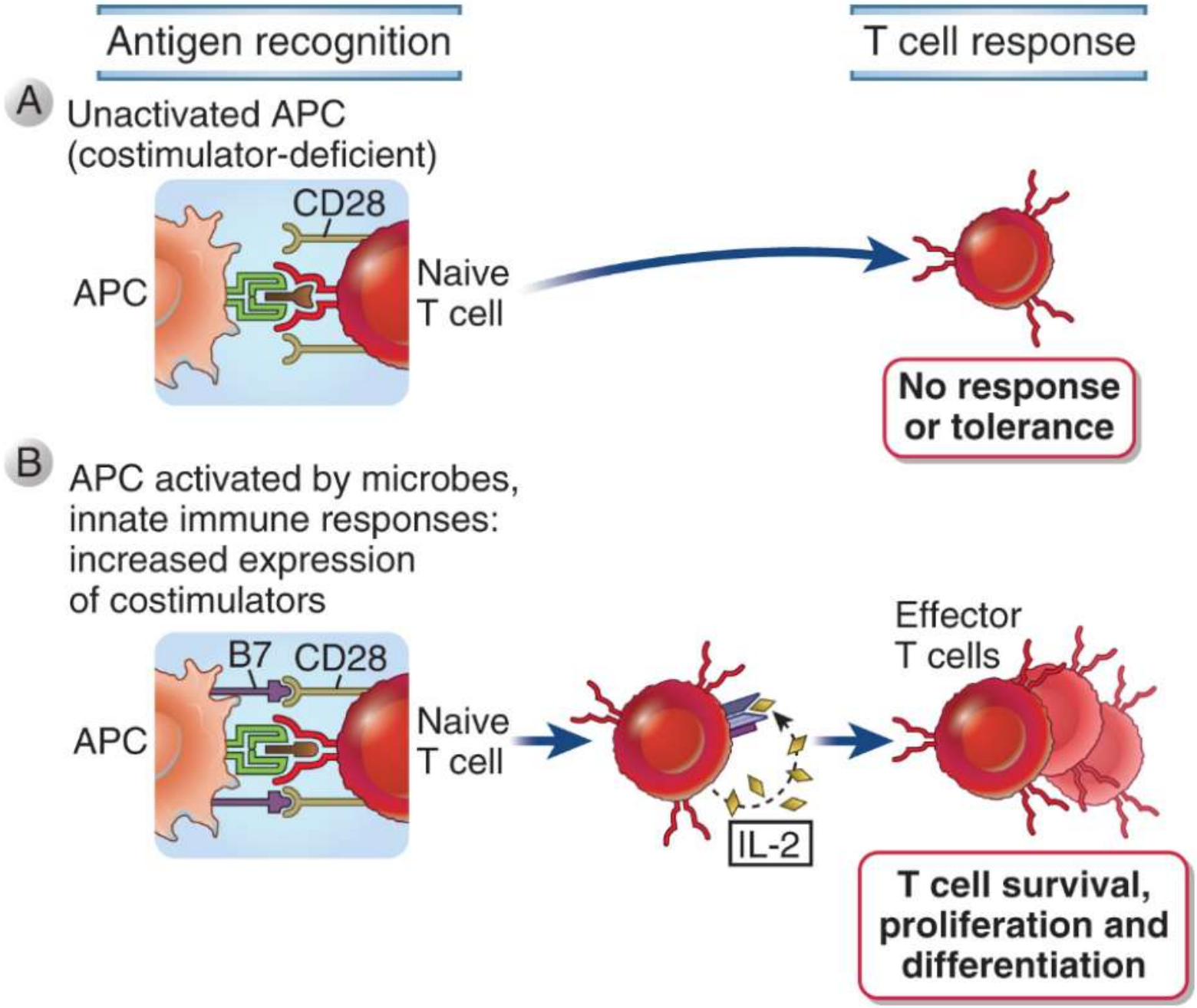
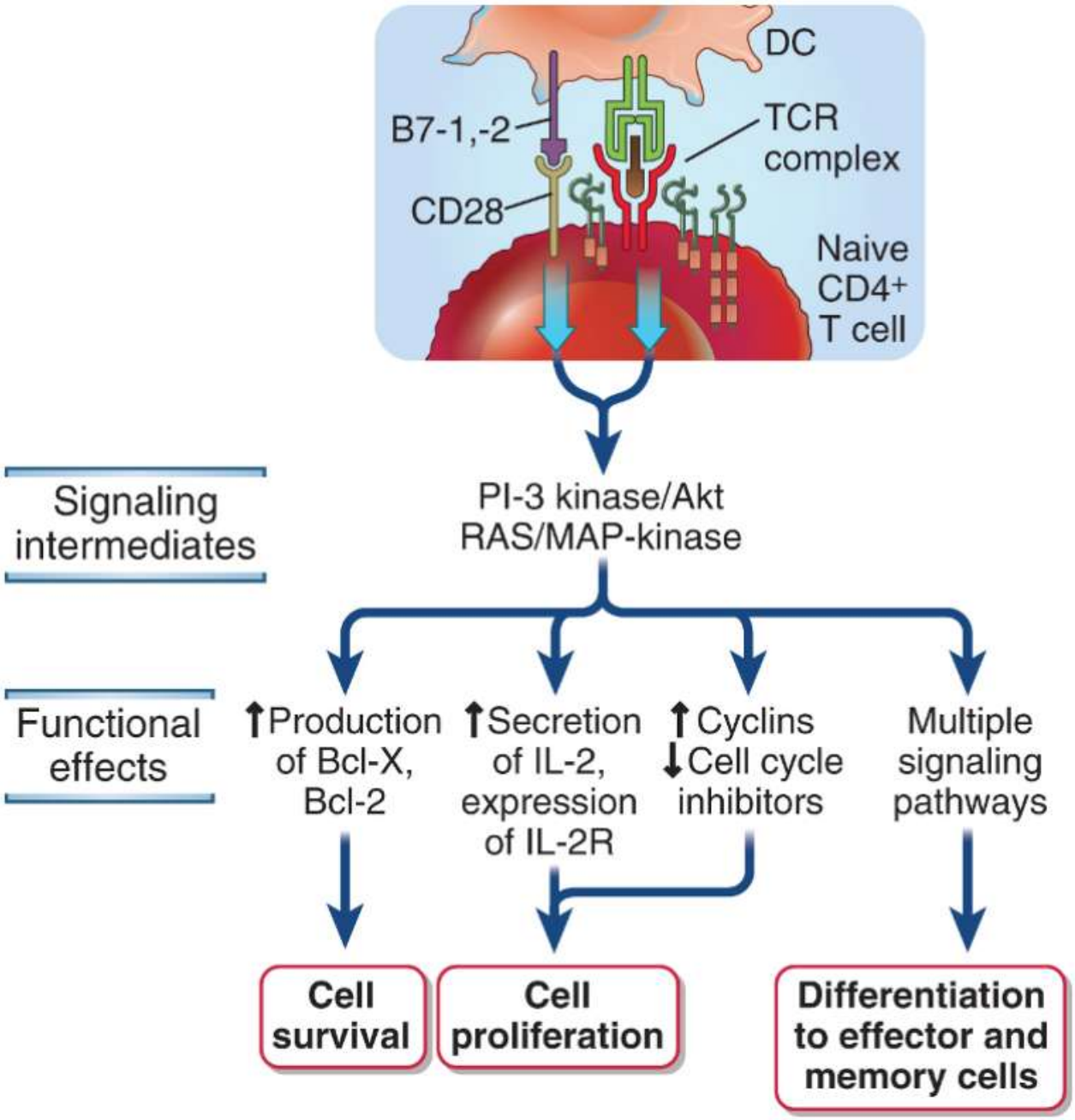


Figure 9.37 Janeway's Immunobiology, 9th ed. (© Garland Science 2017)

Functions of costimulators in T cell activation



Mechanisms of T cell costimulation by CD28



Multiple signalling pathways converge on the IL2 promoter

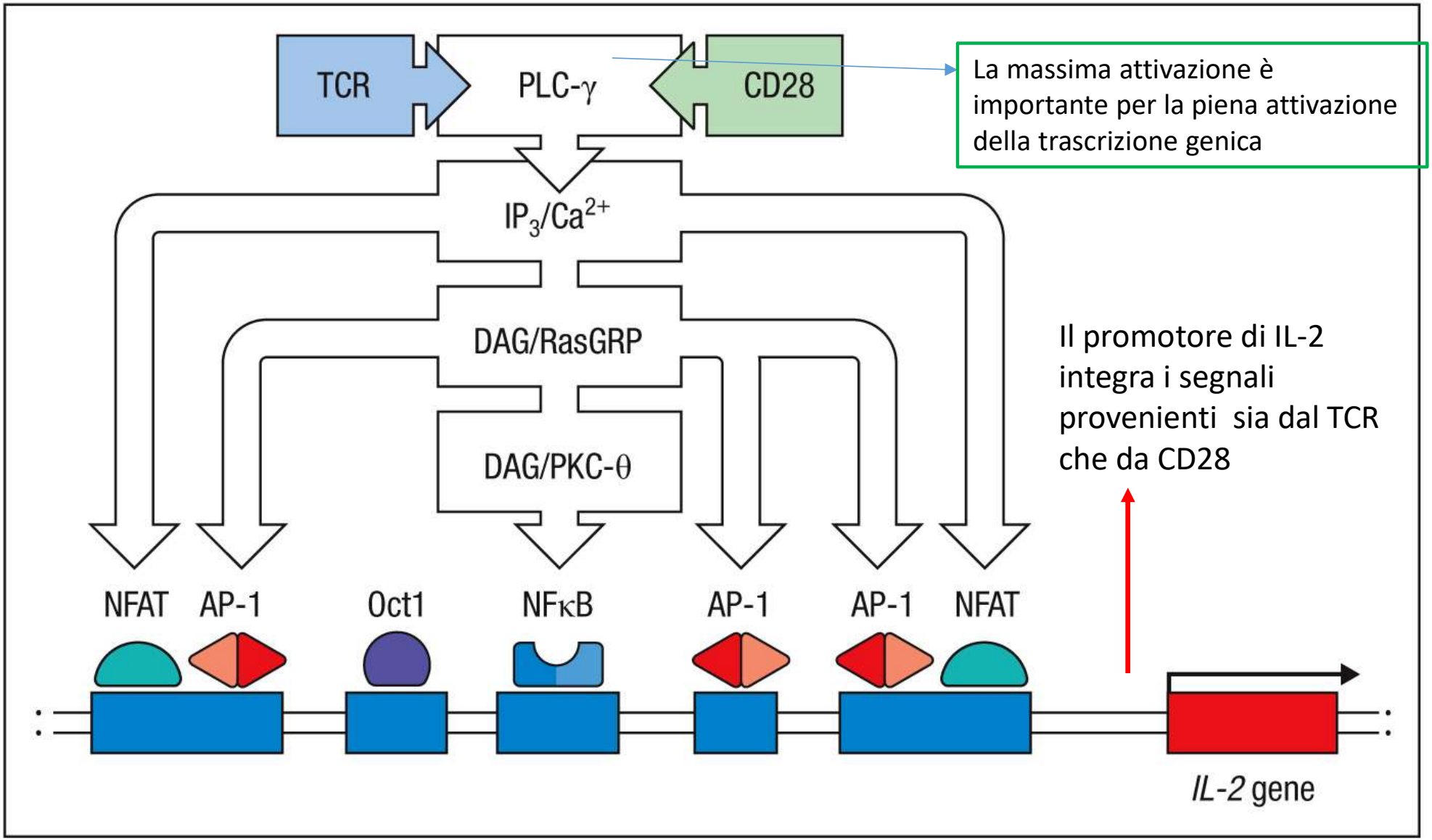
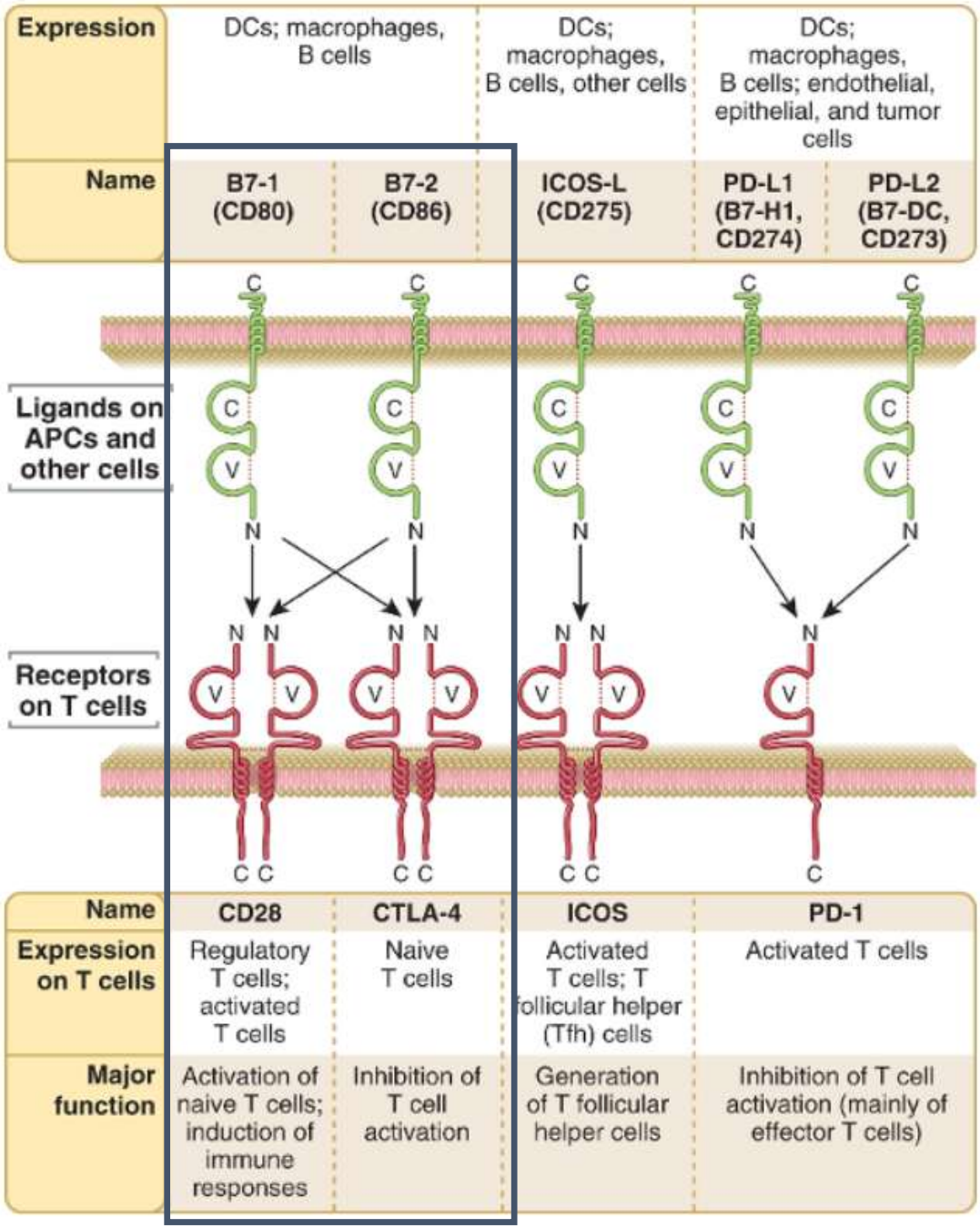


Figure 7.30 Janeway's Immunobiology, 9th ed. (© Garland Science 2017)

The major members of the B7 and CD28 families



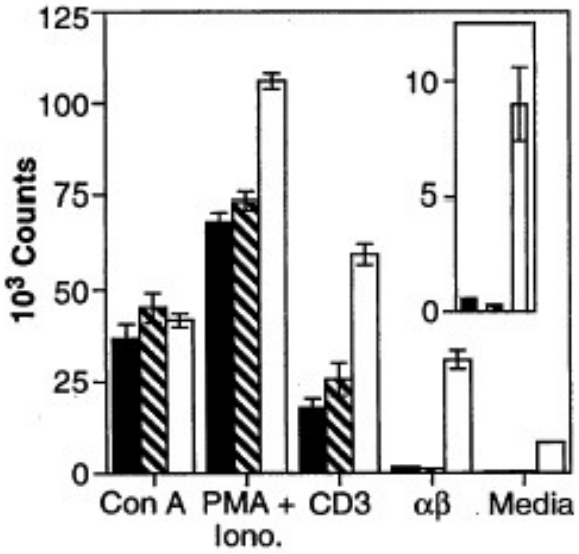
CTLA-4 down-regulates co-stimulation.

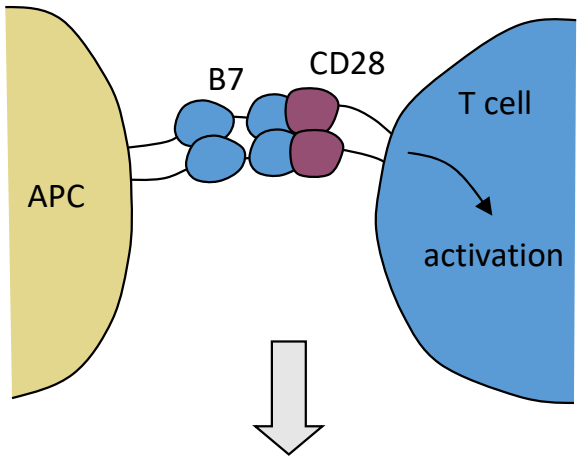
CTLA-4 deficiency leads to Lymphoproliferation disorder

Geno-type	Wet weight (mg)		Lymphocytes (10^7)	
	Lymph nodes	Spleen	Lymph nodes	Spleen
Ctla-4$^{+/+}$	71	69	1.3	3.1
Ctla-4$^{+/-}$	97	77	1.7	3.1
Ctla-4$^{-/-}$	540	145	28.0	7.7
Ctla-4$^{-/-}$	380	501	12.0	16.5

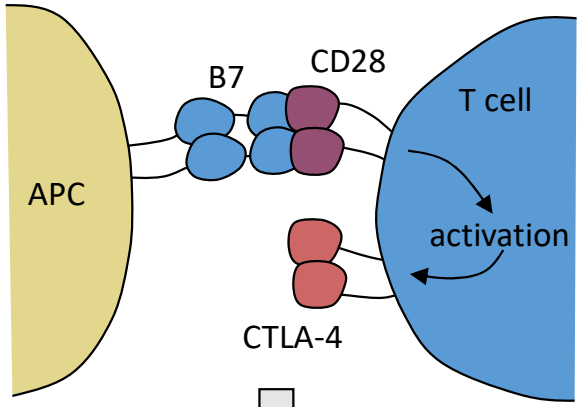
The mice die 3-4 weeks after birth.
The mice suffer massive tissue destruction.

Hyper-proliferation of T cells



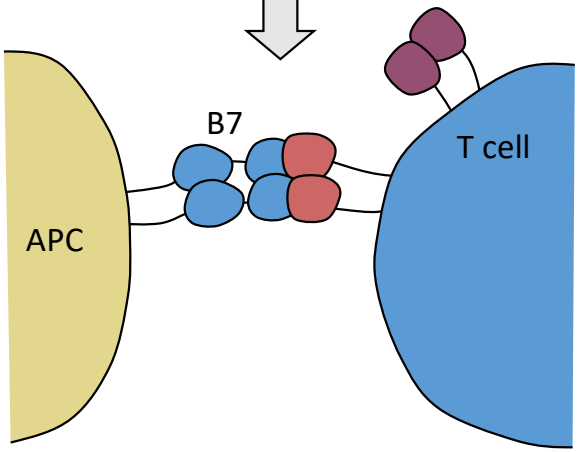


CD28 is expressed on resting T cells.



CTLA-4 is induced after T cell activation.

CTLA-4 is homologous to CD28.



CTLA-4 binds B7 tighter than CD28.

Inhibitory receptors on lymphocytes downregulate immune responses by interfering with co-stimulatory signaling pathways

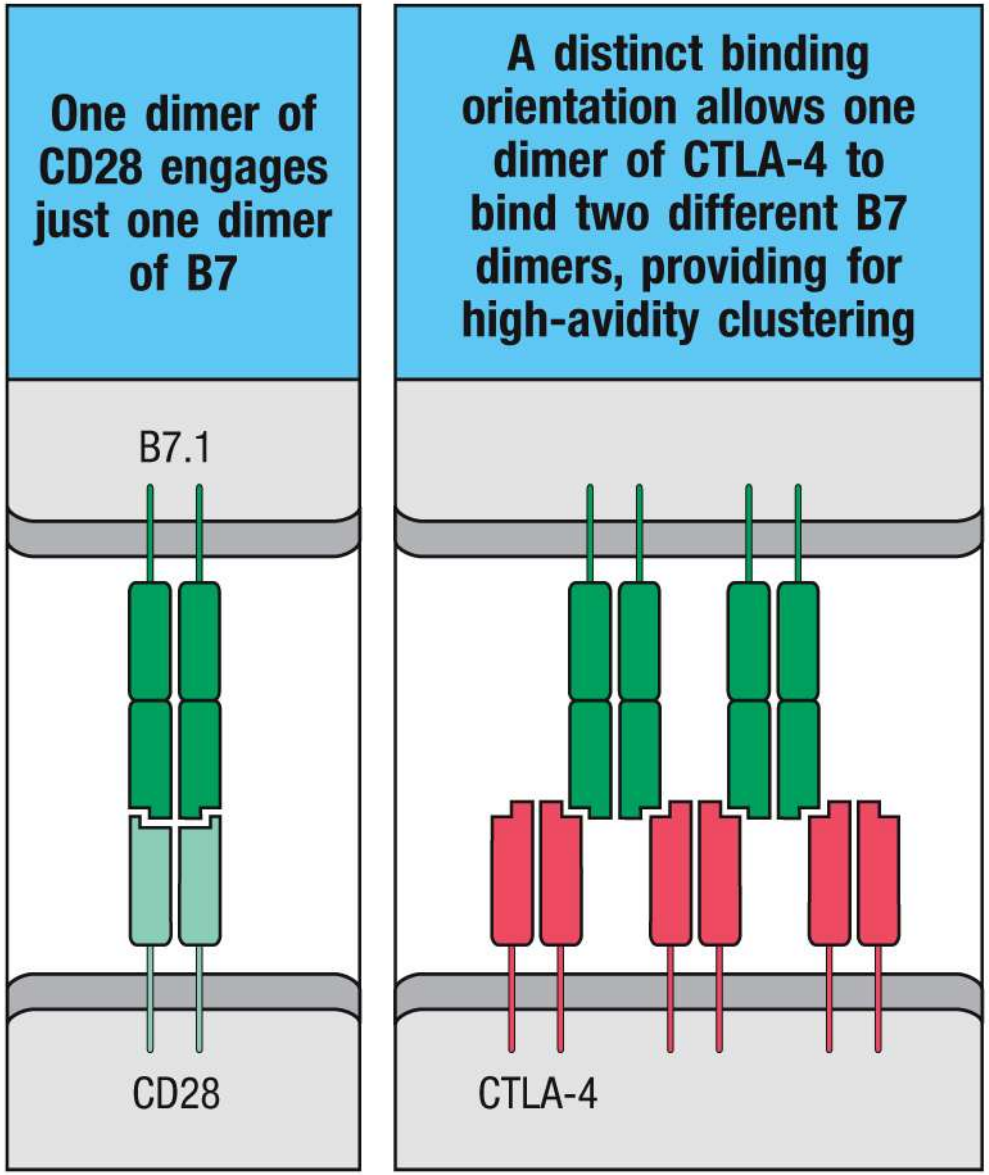
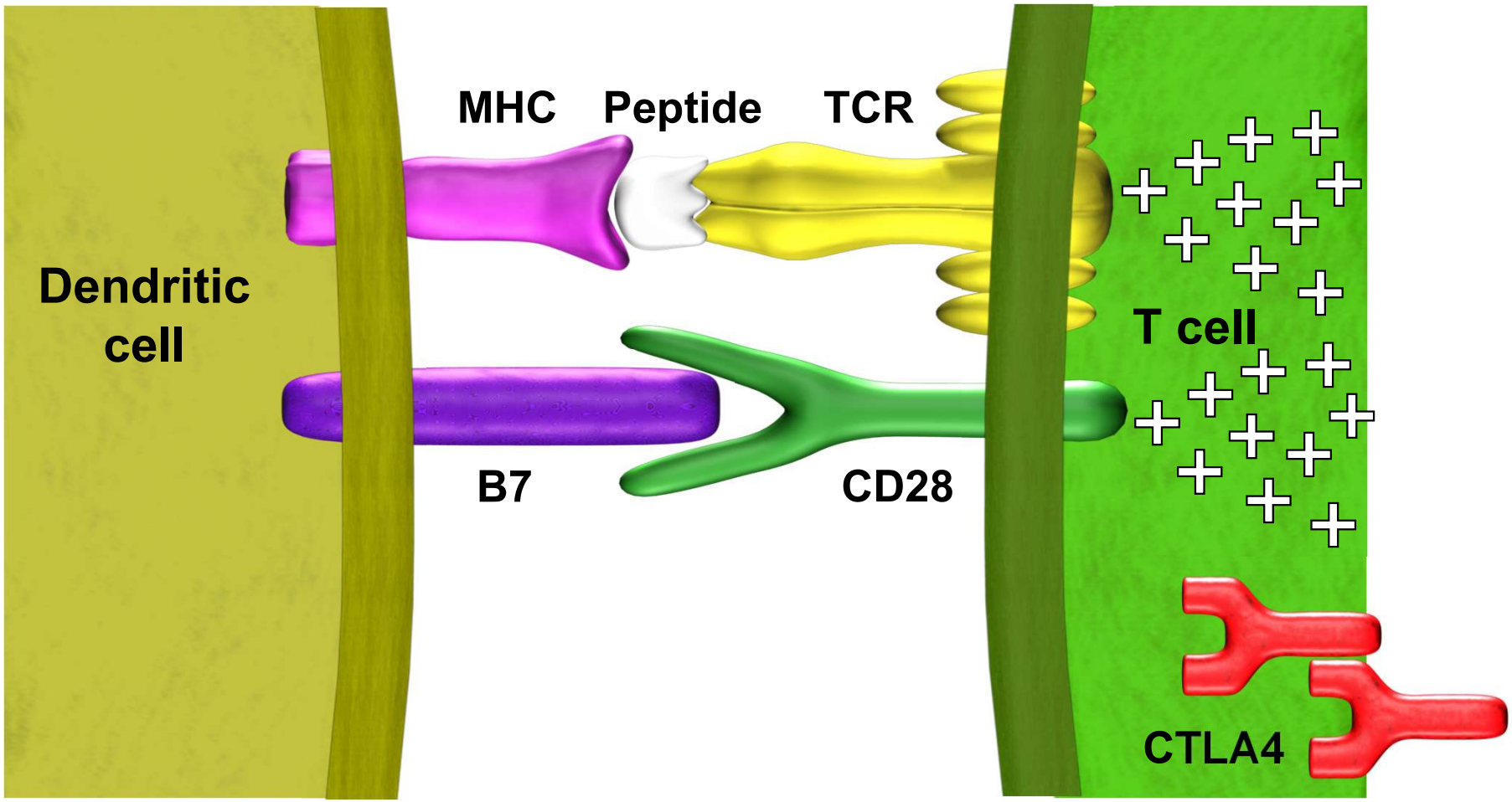


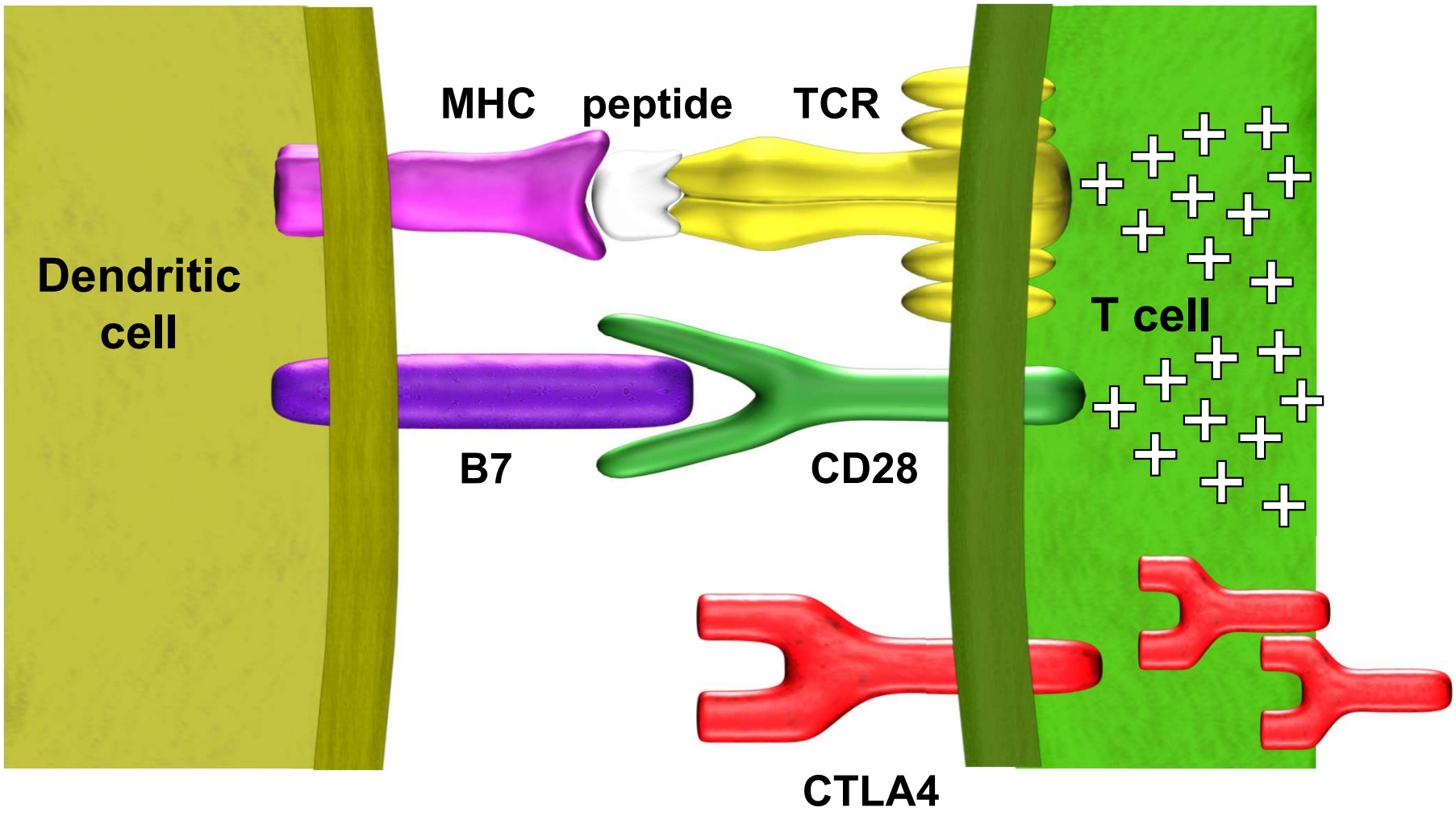
Figure 7.32 Janeway's Immunobiology, 9th ed. (© Garland Science 2017)

CTLA-4 has a higher affinity than CD28 for B7 and engages it in a multivalent orientation

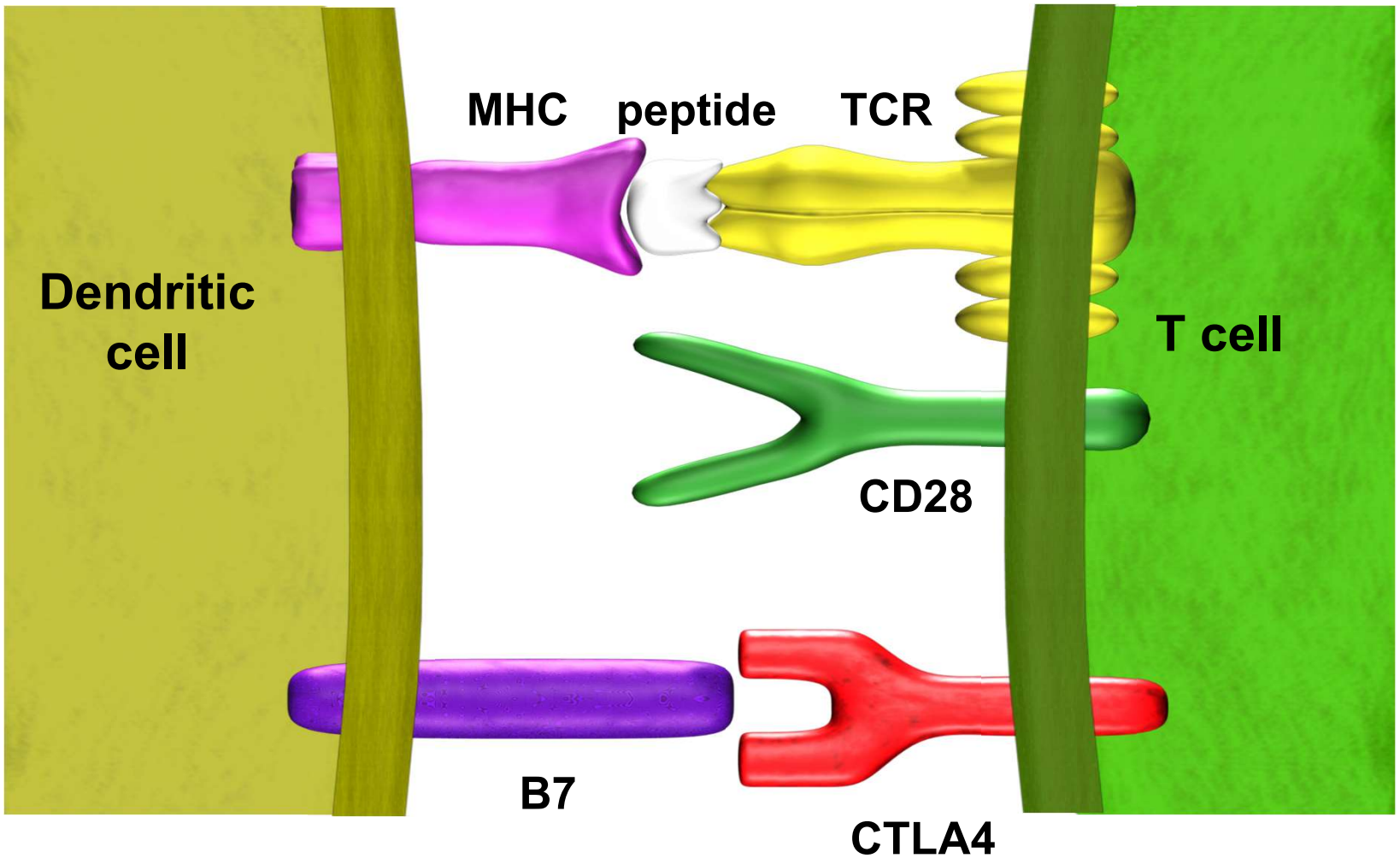
T Cell Activation by TCR and Co-stimulation Through CD28



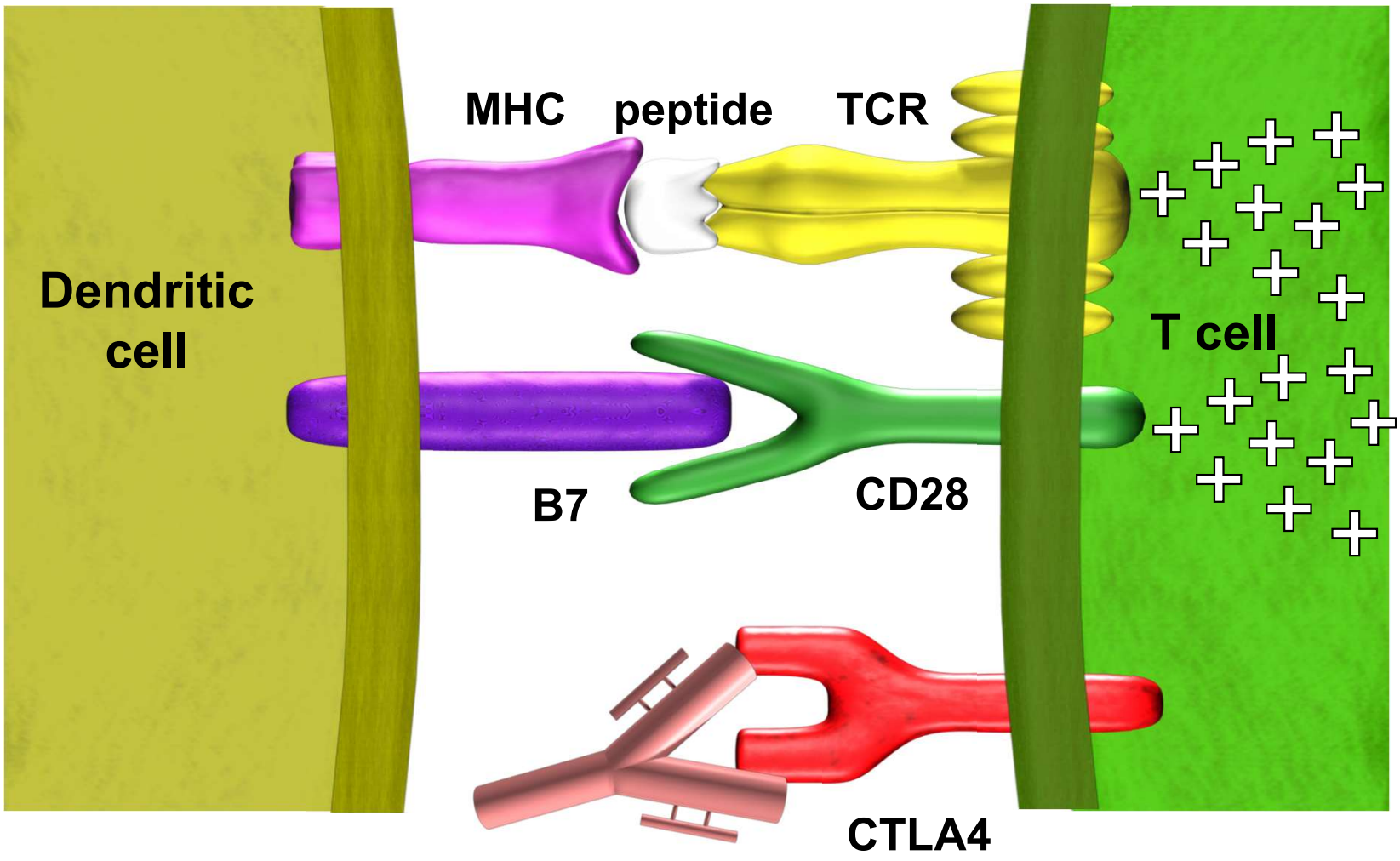
CTLA4 Receptors Are Up-Regulated Following T-Cell Activation



CTLA4 Negatively Modulates T-Cell Activation



Blocking Antibodies to CTLA4 Allow Positive Signaling from Costimulatory Molecules to T Cells



Ipilimumab(Yervoy) in Treatment of Cancer

- CTLA-4:
  - Down-regulates T-cell activation
- Ipilimumab(Yervoy):
  - Fully human monoclonal antibody
  - Blocks CTLA-4 receptor
  - Potentiates T cell activation

Immune checkpoints:

an alternative term for coinhibitory molecules, generally referring to inhibitory signals that immune cells must overcome to perform full effector functions

Immune checkpoint blockade:

Therapeutic strategy based on monoclonal antibodies whose goal is to enhance T cell responses by inhibiting inhibitory receptors

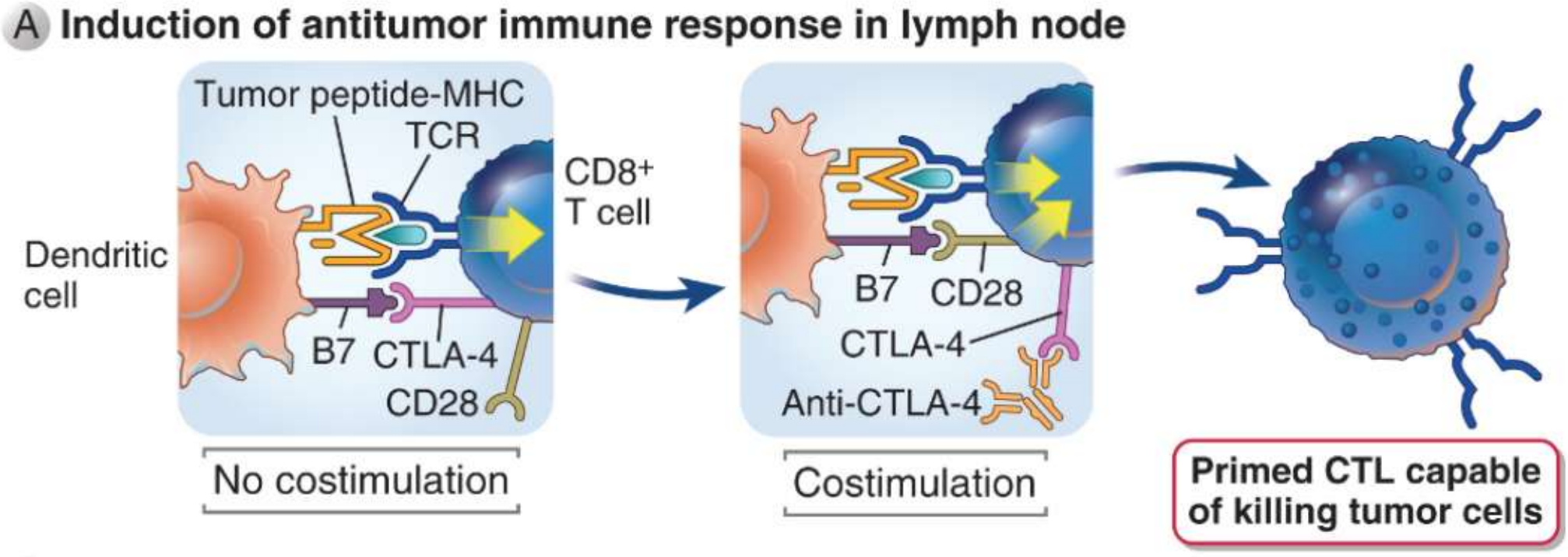
The NEW ENGLAND JOURNAL of MEDICINE

ESTABLISHED IN 1812 AUGUST 19, 2010 VOL. 363 NO. 8

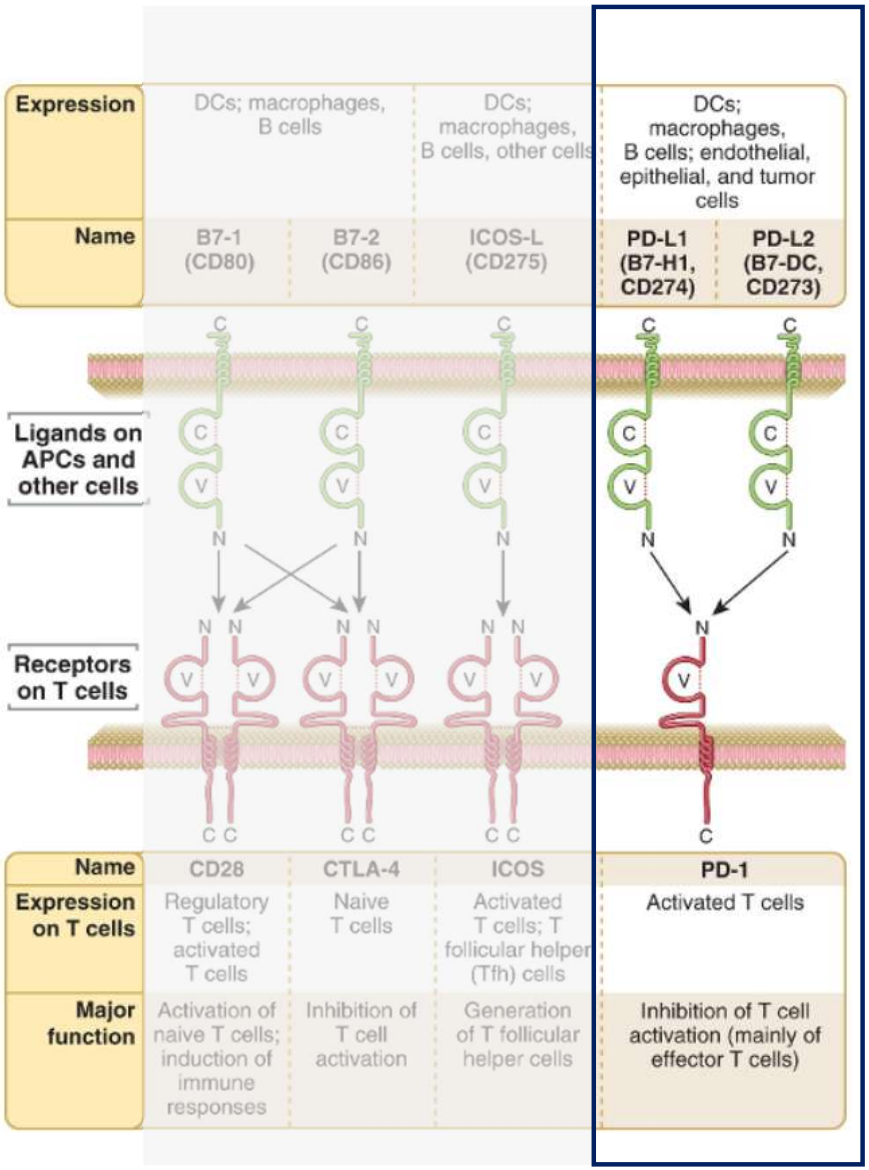
Improved Survival with Ipilimumab in Patients with Metastatic Melanoma

F. Stephen Hodi, M.D., Steven J. O'Day, M.D., David F. McDermott, M.D., Robert W. Weber, M.D., Jeffrey A. Sosman, M.D., John B. Haanen, M.D., Rene Gonzalez, M.D., Caroline Robert, M.D., Ph.D., Dirk Schadendorf, M.D., Jessica C. Hassel, M.D., Wallace Akerley, M.D., Alfons J.M. van den Eertwegh, M.D., Ph.D., Jose Lutzky, M.D., Paul Lorigan, M.D., Julia M. Vaubel, M.D., Gerald P. Linette, M.D., Ph.D., David Hogg, M.D., Christian H. Ottensmeier, M.D., Ph.D., Celeste Lebbé, M.D., Christian Peschel, M.D., Ian Quirt, M.D., Joseph I. Clark, M.D., Jedd D. Wolchok, M.D., Ph.D., Jeffrey S. Weber, M.D., Ph.D., Jason Tian, Ph.D., Michael J. Yellin, M.D., Geoffrey M. Nichol, M.B., Ch.B., Axel Hoos, M.D., Ph.D., and Walter J. Urba, M.D., Ph.D.

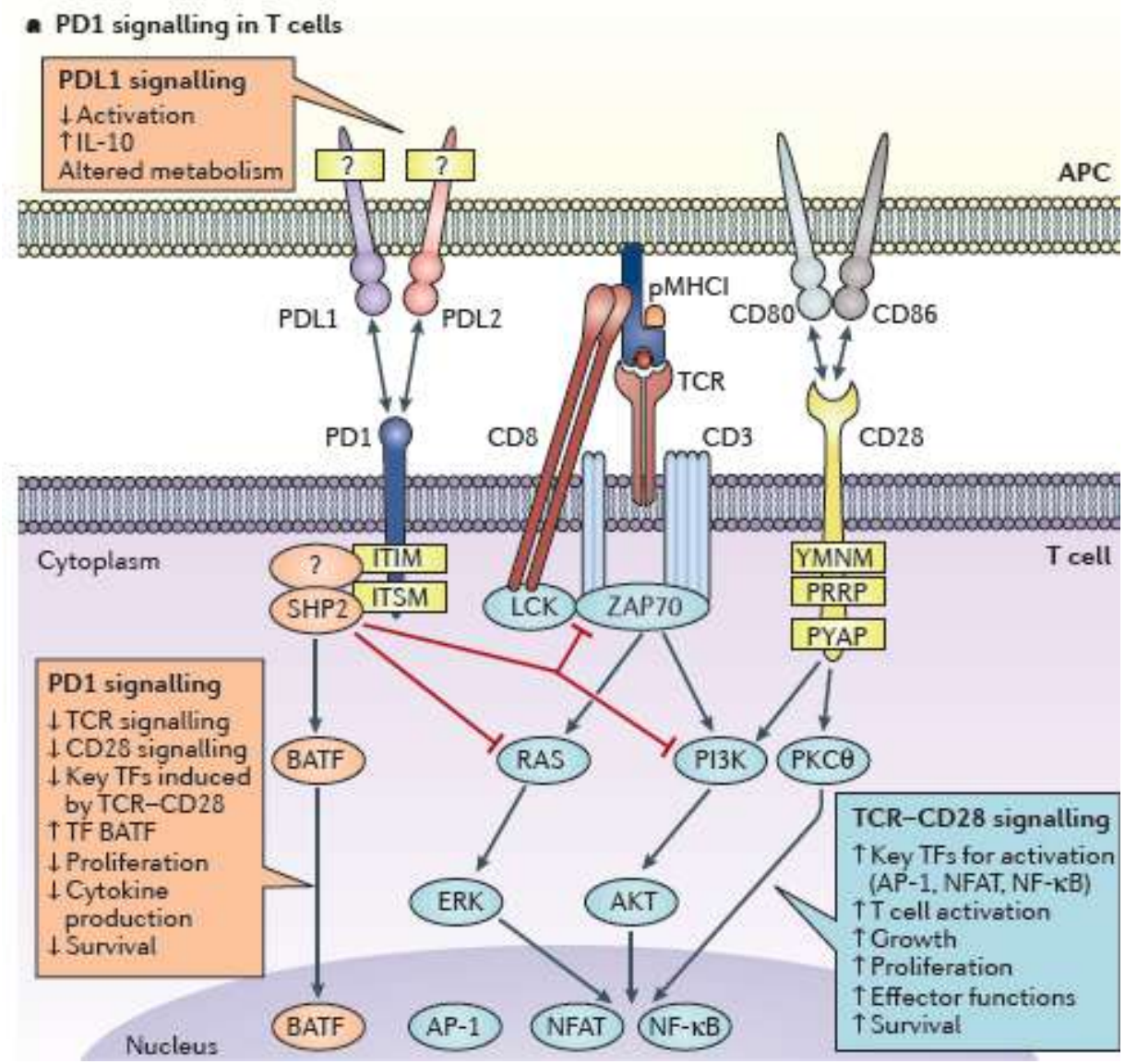
Checkpoint blockade: blocking T cell inhibitory pathway



Regulation of the T cell response by PD1 inhibitory receptor

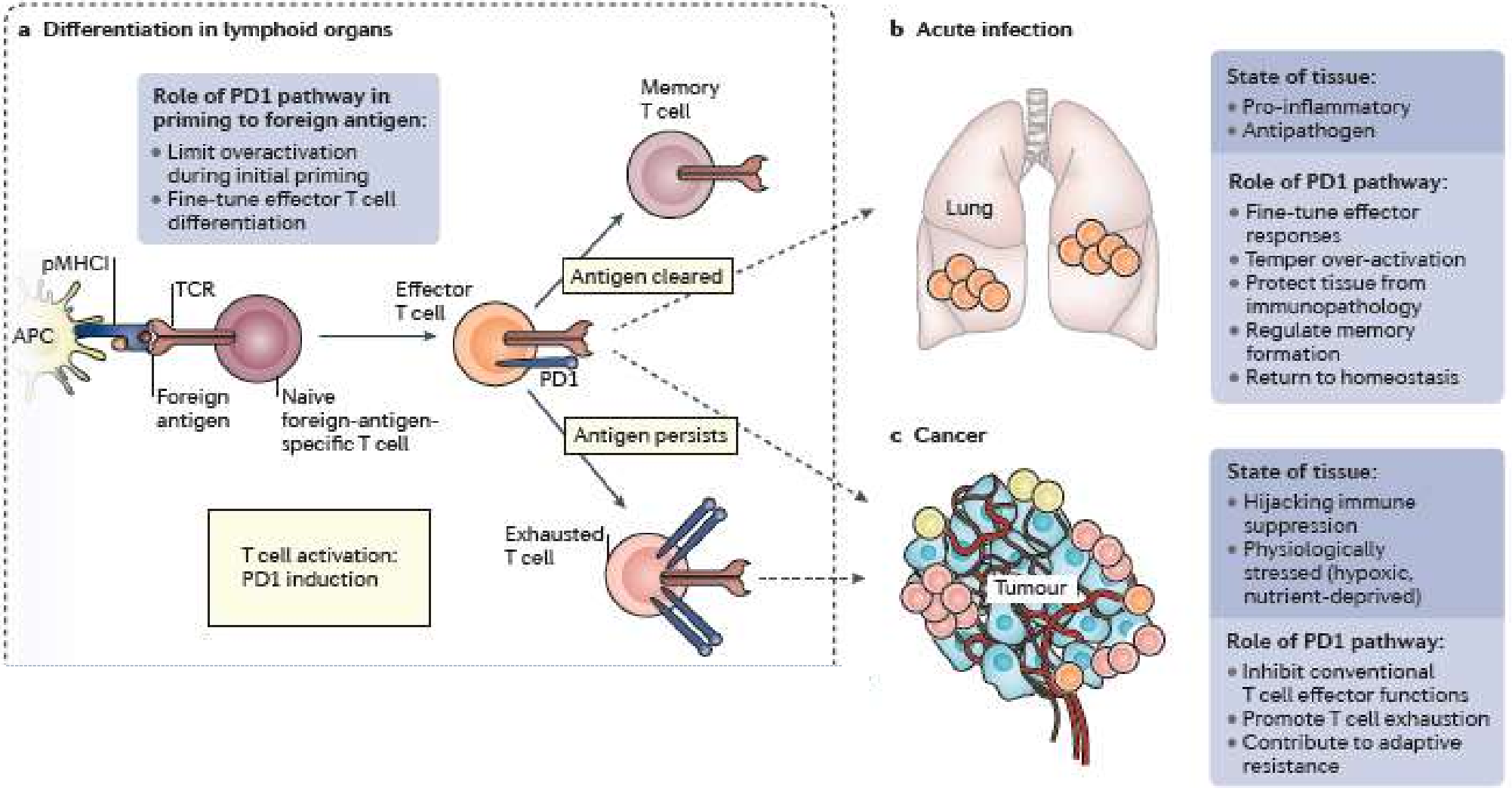


Functions of the PD-1 inhibitory pathway

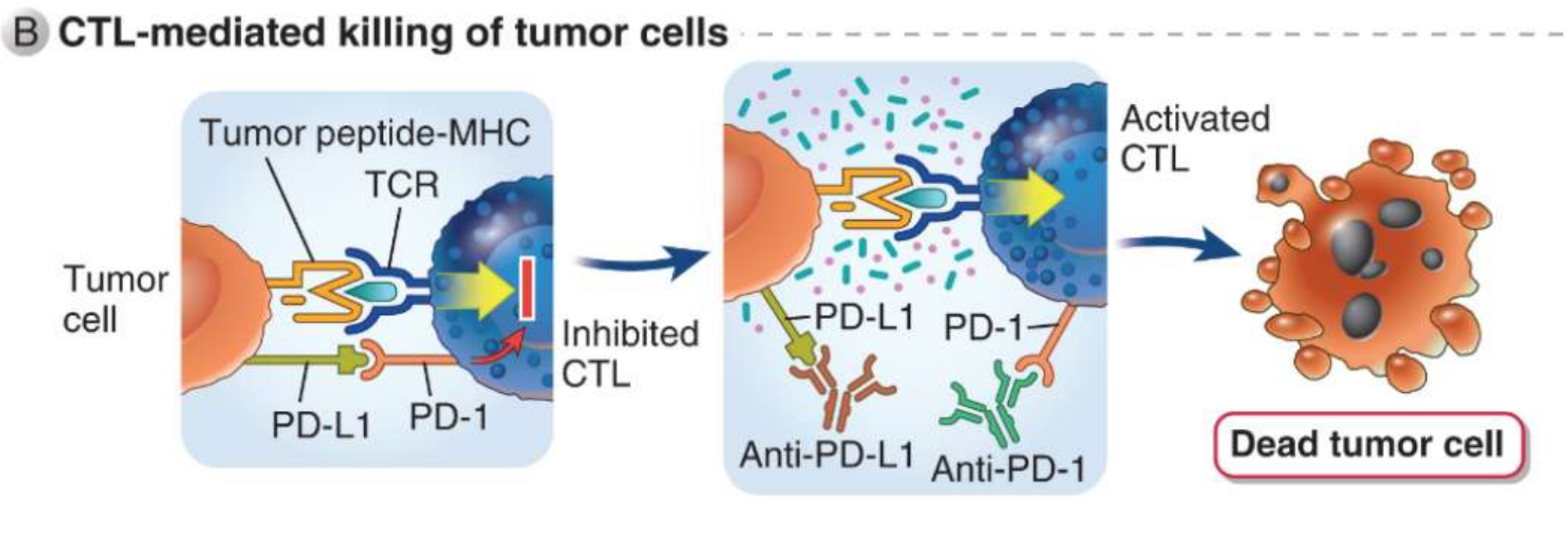


From: Sharpe and Pauken, Nature Reviews in Immunology, 2017

Roles of PD1 in acute infection and cancer



Checkpoint blockade : targeting the PD-1 pathway



FDA Approval

BMS

Yervoy®

Ipilimumab

CTLA-4 blocking antibody

2011

2014

Keytruda®

Pembrolizumab

PD-1 blocking antibody

Merck

BMS

Opdivo®

Nivolumab

PD-1 blocking antibody

2014

The FDA approved Opdivo® (nivolumab) for the treatment of patients with metastatic squamous non-small cell lung cancer (NSCLC) after prior therapy. Opdivo is the first immunotherapy agent approved for the treatment of lung cancer.

The FDA approved Opdivo® (nivolumab) for melanoma patients. Opdivo, made by Bristol-Myers Squibb (BMS), belongs to a class of immunotherapies called checkpoint inhibitors. BMS's Opdivo is the second FDA-approved checkpoint inhibitor targeted at an immune protein called PD-1. Opdivo is FDA-approved for treating metastatic melanoma in patients who have failed prior treatment. Ongoing studies suggest it may benefit patients with many different cancers, including lung, brain, head and neck, stomach, and kidney cancers.

Major FDA Approvals of PD-1 / PD-L1 Inhibitors

Drug	Commercial name	Owner	Target	First approval date
Pembrolizumab	Keytruda	MSD	PD-1	September 2014
Nivolumab	Opdivo	BMS	PD-1	December 2014
Atezolizumab	Tecentriq	Roche	PD-L1	May 2016
Avelumab	Bevancio	EMD and Pfizer	PD-L1	March 2017
Durmalumab	Imfinzi	AstraZeneca	PD-L1	May 2017

U.S. FDA Approved Immune-Checkpoint Inhibitors

Name	Company	Target	Indications	Details
Nivolumab (Opdivo®)[1]	**Bristol-Myers Squibb**	**PD-1**	· 1L Inoperable or Metastatic Melanoma	· Single Agent for BRAF-WT and BRAF-MU *or* in Combination with Yervoy®
			· 2L Metastatic Non-Small Cell Lung Cancer	· Failure on Platinum-Doublet Chemotherapy · Failure on Targeted Agent (if Applicable)
			· 2L Advanced Renal Cell Carcinoma	· After Prior Treatment with Anti-Angiogenic Treatment
			· 4L Classical Hodgkin Lymphoma	· After Prior Auto-HSCT and Brentuximab Vendotin Treatment
			· 2L Recurrent or Metastatic Head and Neck Squamous Cell Carcinoma	· Disease progression on *or* after Platinum-Based Chemotherapy
			· 1L/2L Locally Advanced or Metastatic Urothelial Carcinoma	· Failure on Prior Platinum-Based Chemotherapy · PD<12 Months after (Neo)Adjuvant Platinum-Based Chemotherapy
			· Microsatellite Instability-High (MSI-H) or Mismatch-Repair Deficient (dMMR) Metastatic Colorectal Cancer	· Adult and Paediatric Patients (≥12 years) · PD following FOLFOXIRI
Pembrolizumab (Keytruda®)[2]	**Merck (MSD)**	**PD-1**	· 1L Inoperable or Metastatic Melanoma	· Single Agent
			· 2L Metastatic Non-Small Cell Lung Cancer with PD-L1 Expression	· Failure on Platinum-Doublet Chemotherapy · Failure on Targeted Agent (if Applicable) · Tumour Proportion Score (TPS) ≥1%
			· 1L Metastatic *Non-Squamous* Non-Small Cell Lung Cancer	· In combination with Carboplatin and Pemetrexed · Regardless of Tumour Proportion Score (TPS)
			· 1L Metastatic Non-Small Cell Lung Cancer with high PD-L1 Expression	· No Prior Systemic Treatments · No Known Tumour-Driver Mutations · Tumour Proportion Score (TPS) ≥50%
			· 2L Recurrent or Metastatic Head and Neck Squamous Cell Carcinoma	· Disease Progression on *or* after Platinum-Based Chemotherapy
			· 4L Refractory Classical Hodgkin Lymphoma	· Adult and Paediatric Patients · Disease Relapse after 3 Prior Treatments
			· 1L/2L Locally Advanced or Metastatic Urothelial Carcinoma	· Ineligible for Cisplatin-based Chemotherapy · Failure on Prior Platinum-Based Chemotherapy · PD<12 Months after (Neo)Adjuvant Platinum-Based Chemotherapy
			· Microsatellite Instability-High (MSI-H) or Mismatch-Repair Deficient (dMMR) Cancers	· Adult and Paediatric Patients · Solid Tumours Progressed Following Prior Treatment and Without Satisfactory Alternative Treatment Options · Colorectal Cancer PD following FOLFOXIRI · **Limitation of Use**: Safety and Effectiveness of Pembrolizumab not Established in Paediatric Patients with MSI-H Central Nervous System (CNS) Cancers
Ipilimumab (Yervoy®)[3]	**Bristol-Myers Squibb**	**CTLA-4**	· 1L Inoperable or Metastatic Melanoma	· Adult and Paediatric Patients (≥12 years) · Single Agent *or* in Combination with Opdivo® (see Opdivo® USPI)
			· Adjuvant Treatment of Stage IIIa Melanoma	· At Least One Metastasis >1 mm, no In-Transit Metastasis · Undergone Complete Resection, Including Total Lymphadenectomy
Atezolizumab (Tecentriq®)[4]	**Roche Genentech**	**PD-L1**	· 1L/2L Locally Advanced or Metastatic Urothelial Carcinoma	· Ineligible for Cisplatin-based Chemotherapy · Failure on Prior Platinum-Based Chemotherapy · PD<12 Months after (Neo)Adjuvant Platinum-based Chemotherapy
			· 2/L Metastatic Non-Small Cell Lung Cancer	· Failure on Platinum-Doublet Chemotherapy · Failure on Targeted Agent (if Applicable)
Avelumab (Bavencio®)[5]	**Merck Serono Pfizer**	**PD-L1**	· 1L Metastatic Merkel Cell Carcinoma (MCC)	· Adult and Pediatric Patients (≥12 years)
			· 1L/2L Locally Advanced or Metastatic Urothelial Carcinoma	· Failure on Prior Platinum-Based Chemotherapy · PD<12 Months after (Neo)Adjuvant Platinum-based Chemotherapy
Durvalumab (Imfinzi®)[6]	**AstraZeneca**	**PD-L1**	· 1L/2L Locally Advanced or Metastatic Urothelial Carcinoma	· Failure on Prior Platinum-Based Chemotherapy · PD<12 Months after (Neo)Adjuvant Platinum-based Chemotherapy

1 Prescribing information nivolumab (Opdivo®), revised: 04/2017
2 Prescribing information pembrolizumab (Keytruda®), revised: 07/2017
3 Prescribing information ipilimumab (Yervoy®), revised: 03/2017
4 Prescribing information atezolizumab (Tecentriq®), Revised: 04/2017
5 Prescribing information avelumab (Bavencio®), Revised: 5/2017
6 Prescribing information durvalumab (Imfinzi®), Revised: 4/2017

Updated on 03-Aug-2017 - medi-paper.com

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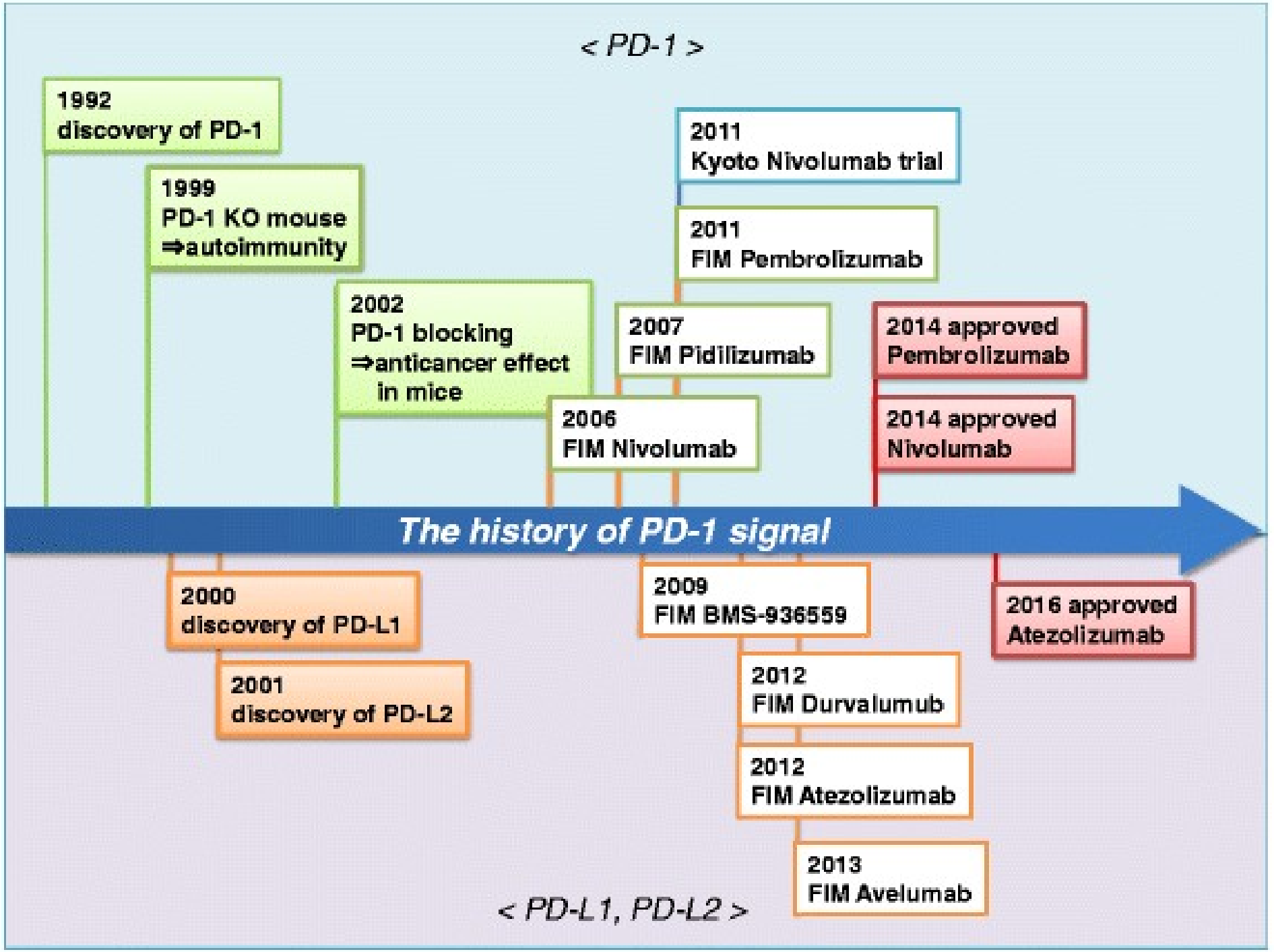
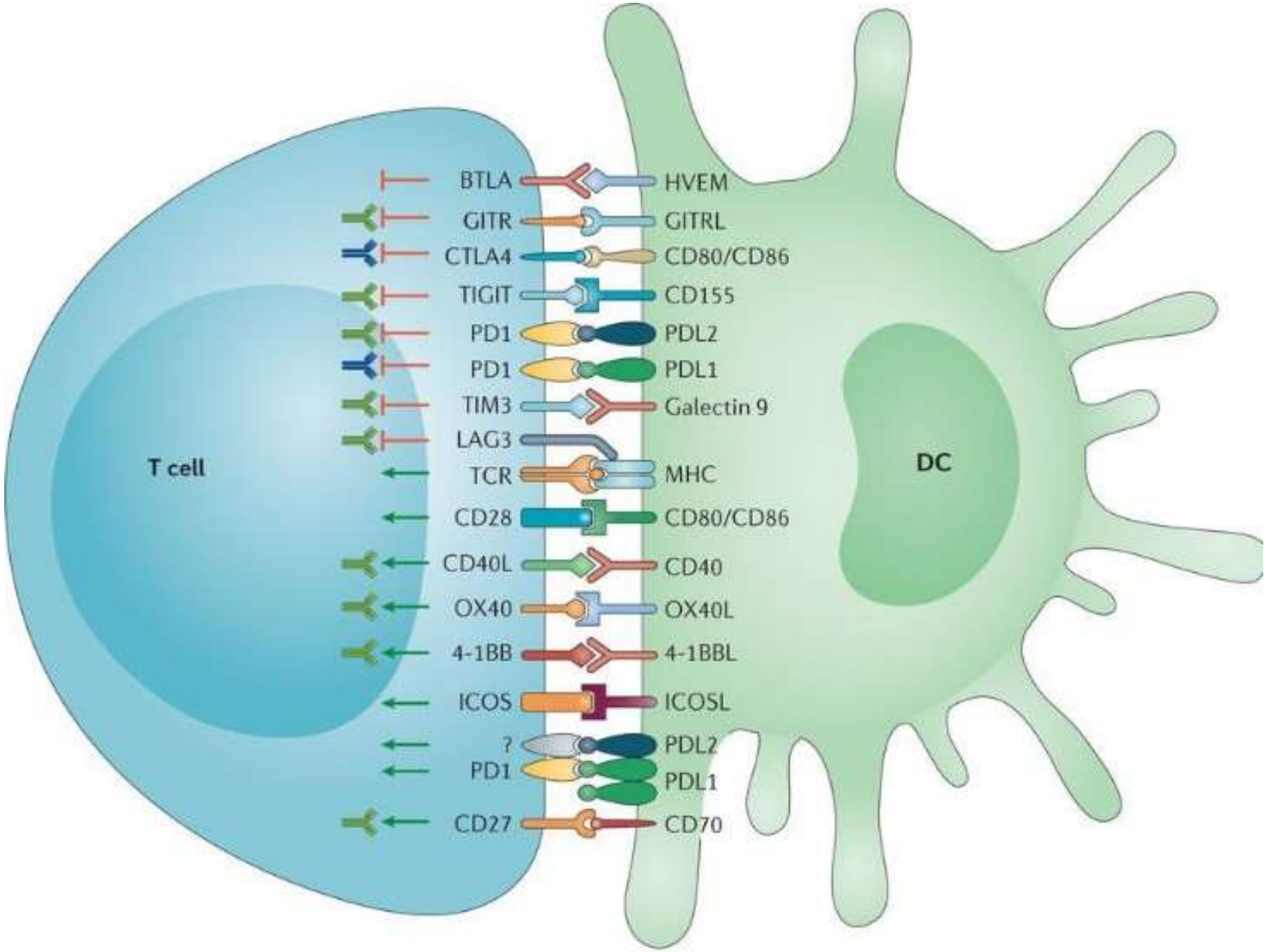
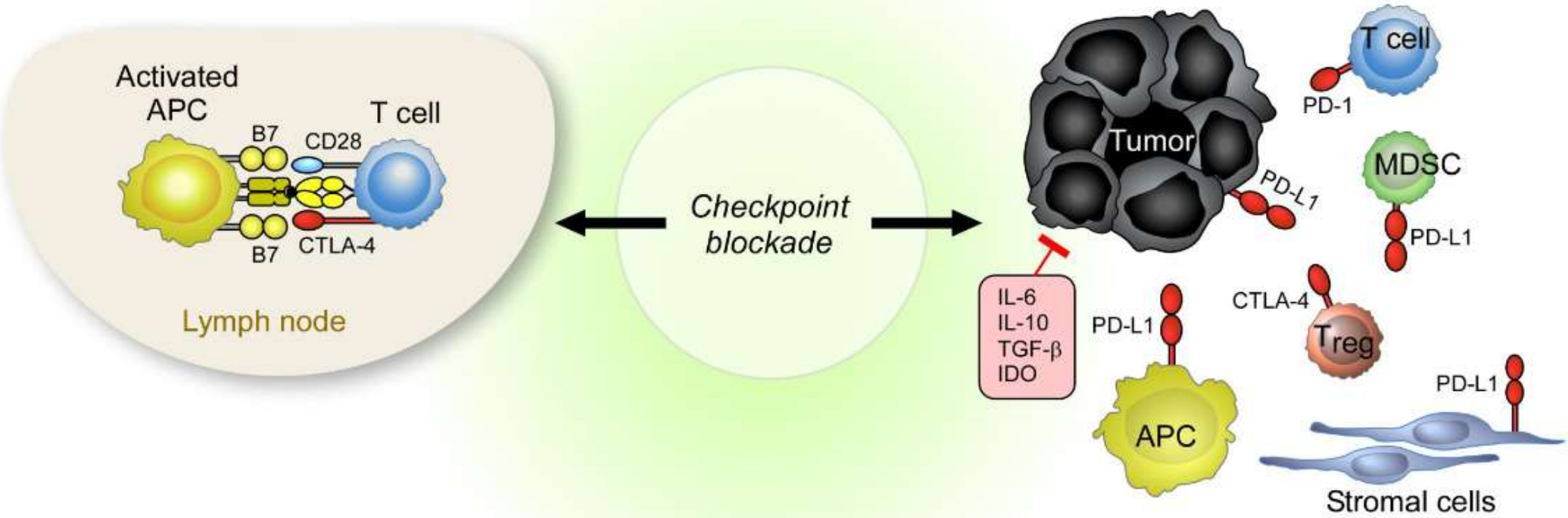
The race for PD-1/PD-L1 therapy




T cell
DC
BTLA
HVEM
GITR
GITRL
CTLA4
CD80/CD86
TIGIT
CD155
PD1
PDL2
PD1
PDL1
TIM3
Galectin 9
LAG3
TCR
MHC
CD28
CD80/CD86
CD40L
CD40
OX40
OX40L
4-1BB
4-1BBL
ICOS
ICOSL
?
PDL2
PD1
PDL1
CD27
CD70

Where does checkpoint blockade function?



CTLA-4 in the lymph node

PD-1 in the tumor

Checkpoint Inhibitors | A Famous Patient

- Late stage melanoma with brain and liver metastases.
- Given Merck's KEYTRUDA in September 2015
- Cancer was no longer showing up on scans in December 2015.

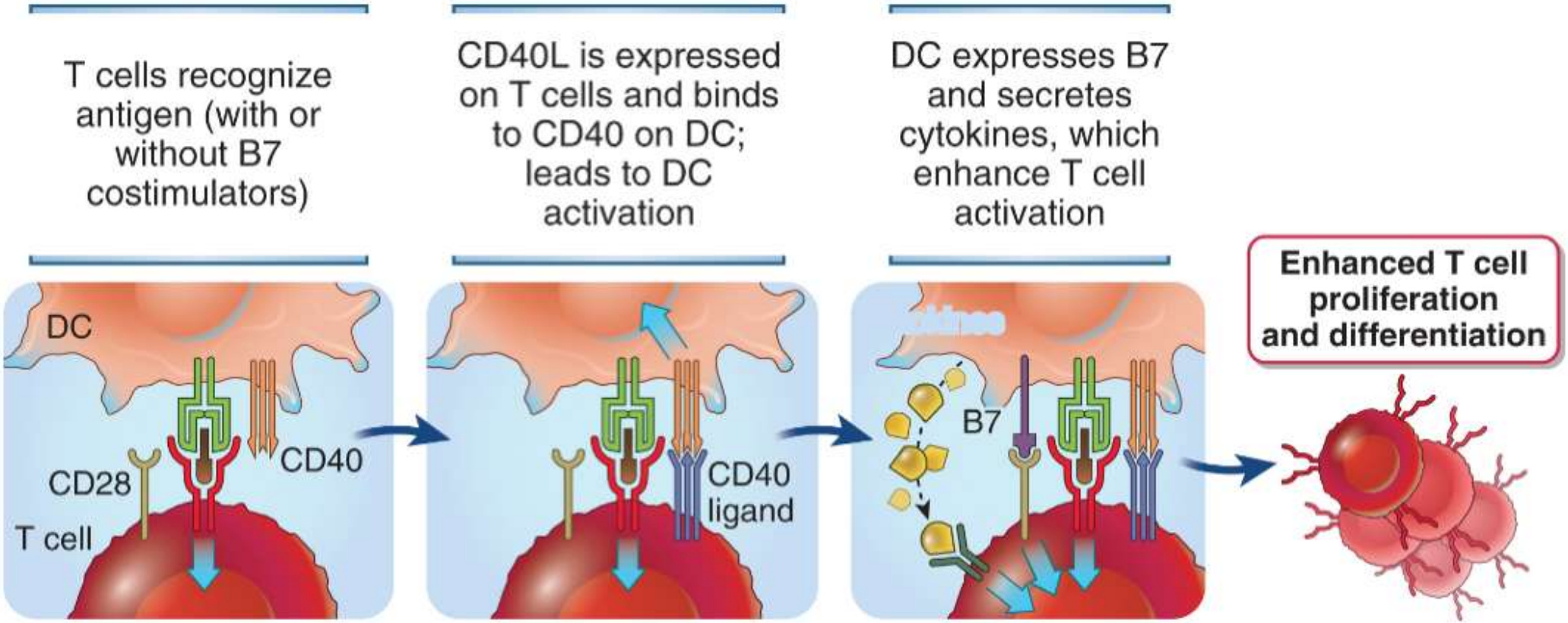
Tolerability and quality of life can be just as important as efficacy.

- President Carter was 91 years old.
- You might not even offer chemotherapy to someone that age because it can be so debilitating.
- He was able to continue daily life while on the immunotherapy.

Altre vie di costimolazione….

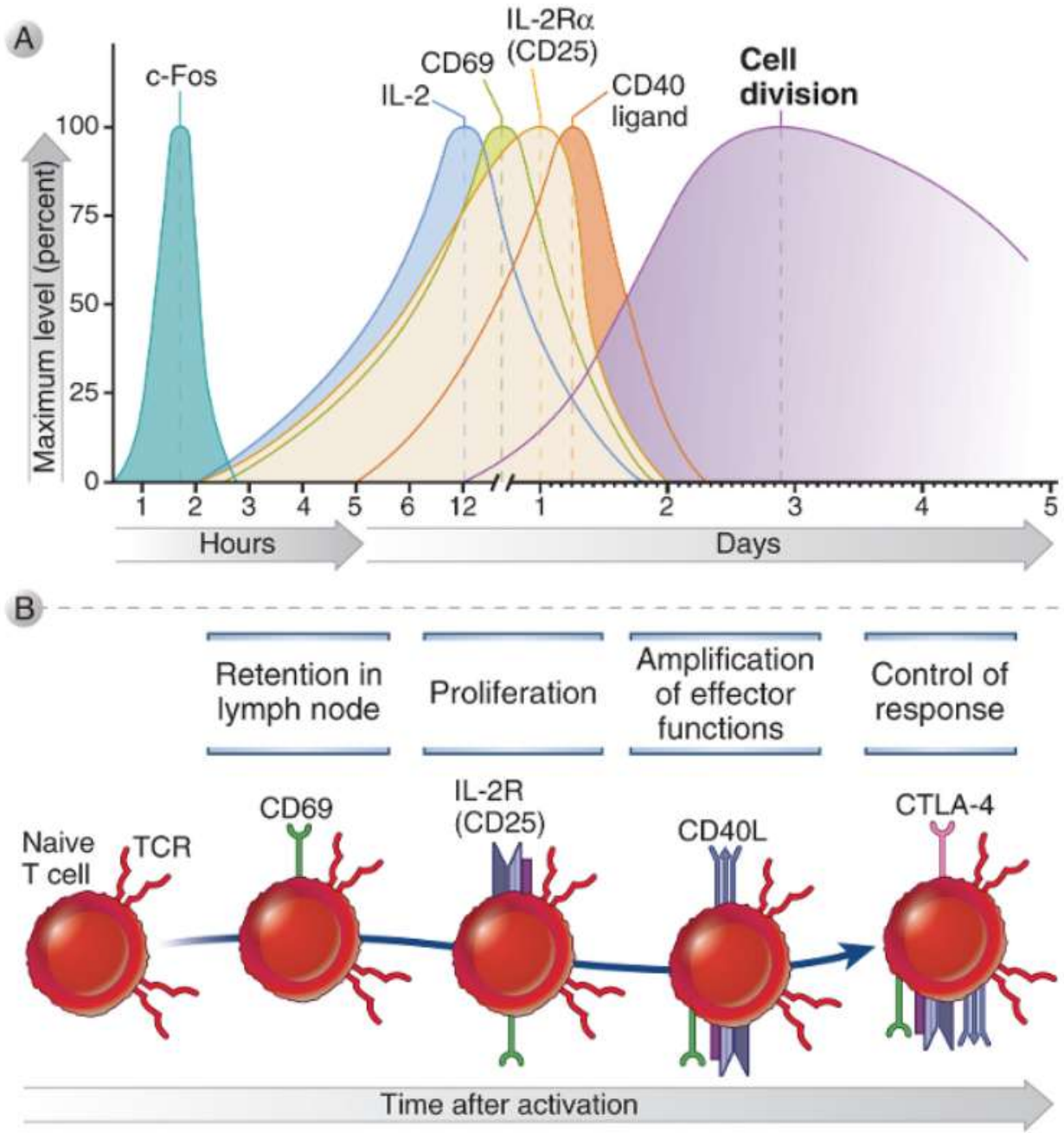
Ruolo di CD40 nell'attivazione delle cellule T: potenziamento indiretto delle risposte T grazie all'attivazione delle APC

(e nei linfociti B…)



- CD40 è un membro della superfamiglia del recettore del TNF
- I membri di questa famiglia attivano principalmente la via di NFkB a cui consegue un aumento della sopravvivenza
- CD40 è espresso costitutivamente sui linfociti B
- Membri della superfamiglia del recettore del TNF sono espressi sulle cellule T (es. OX40, 4-1BB…)

Cambi nelle molecole di superficie dopo attivazione delle cellule T



Le cellule di Langherans catturano l'antigene nella cute, migrano negli organi linfoidi periferici e presentano gli antigeni alle cellule T

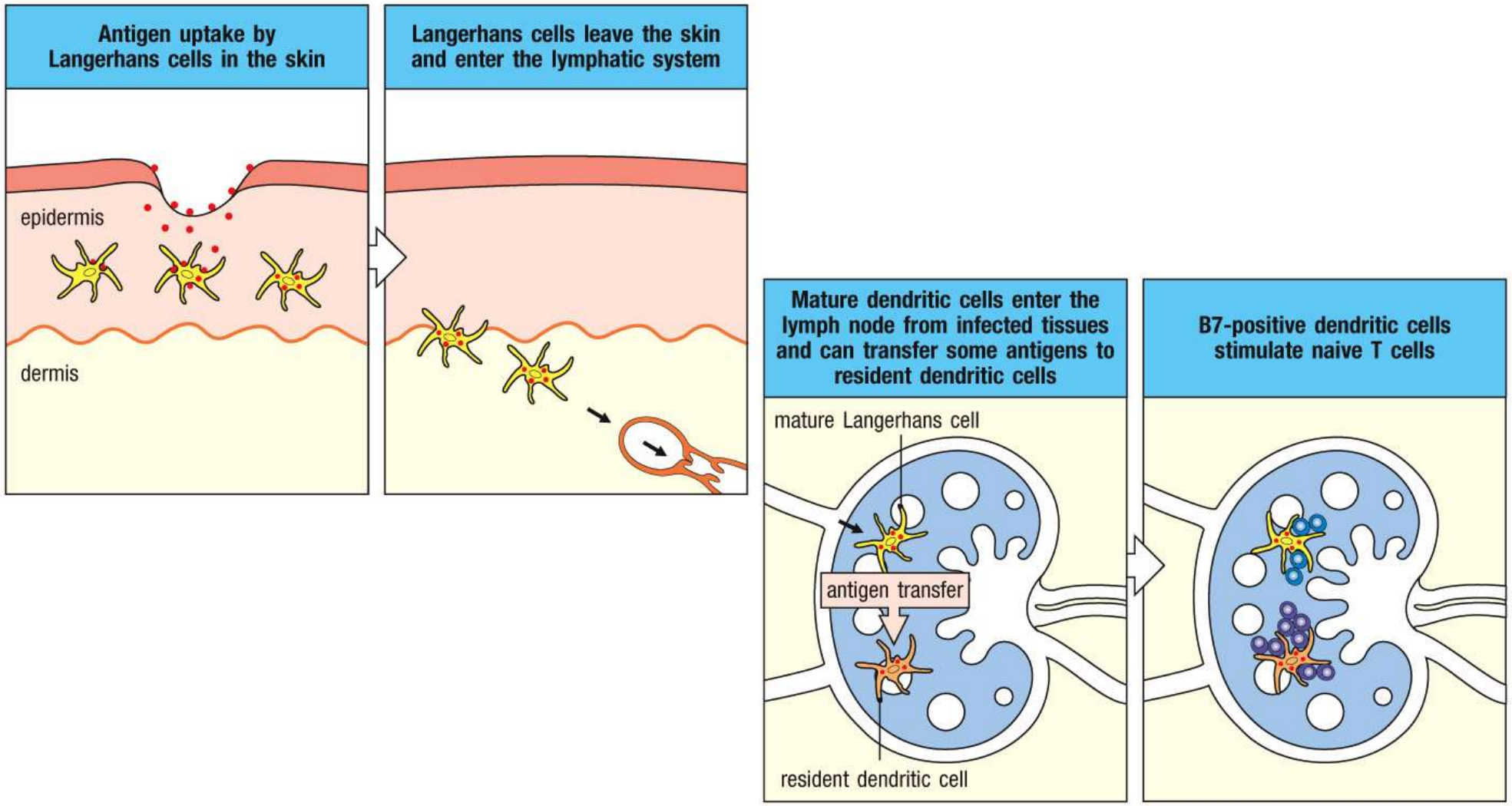


Figure 9.16 Janeway's Immunobiology, 9th ed. (© Garland Science 2017)

Naive T cells migrate through secondary lymphoid tissues, sampling peptide:MHC complexes on dendritic cells

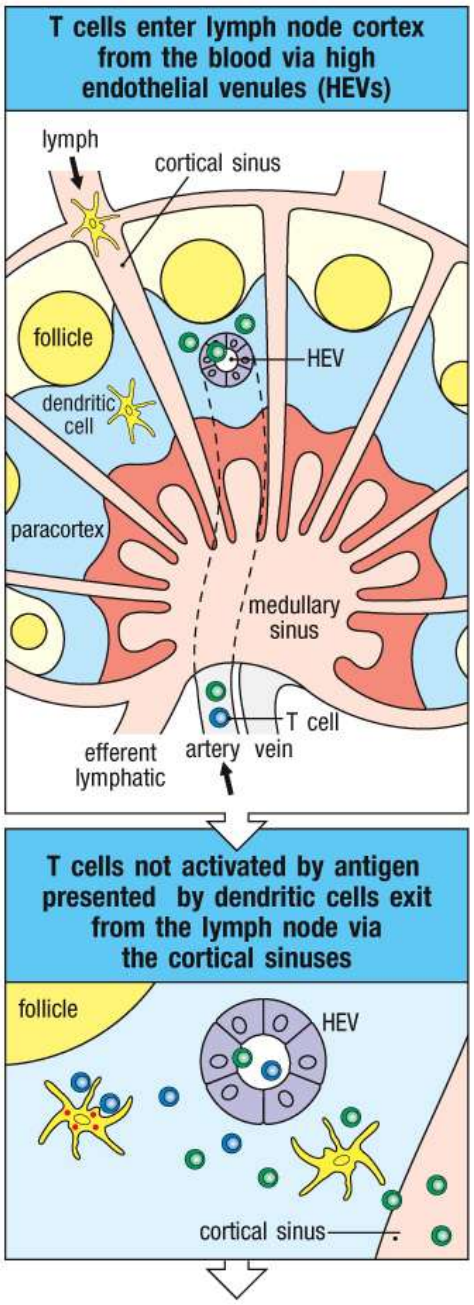
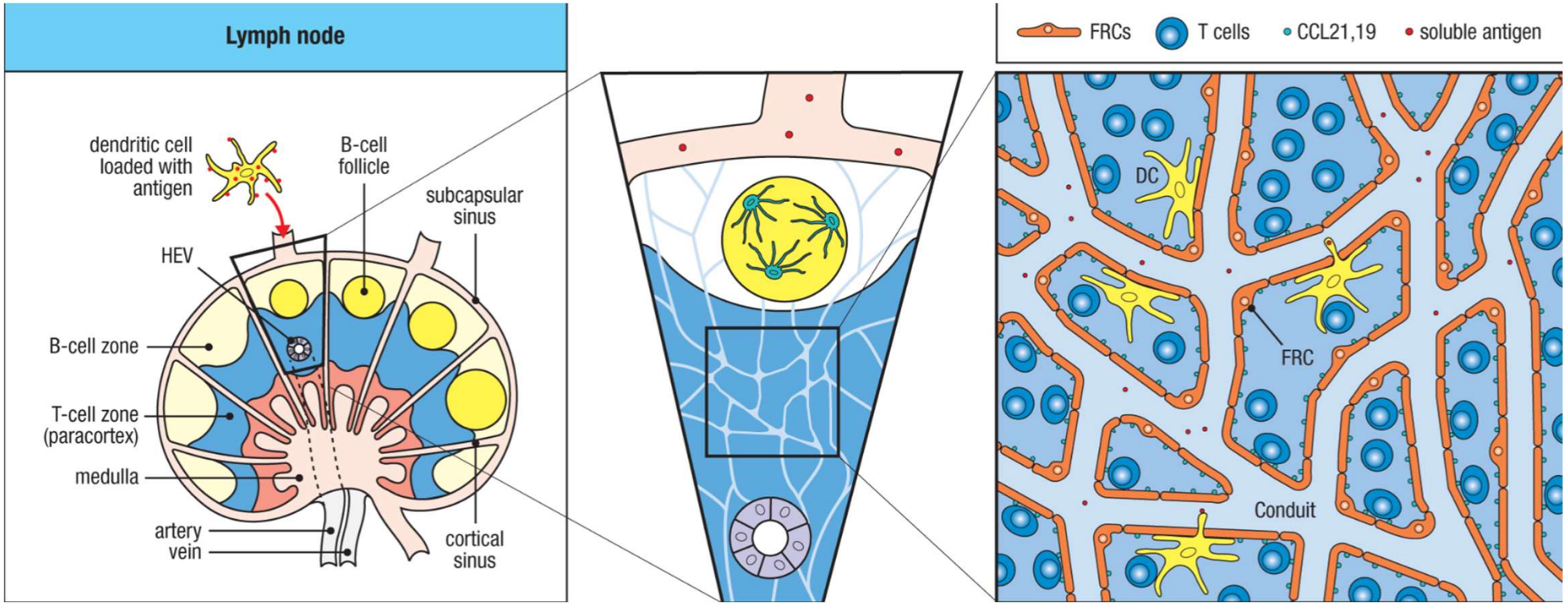


Figure 9.4 (part 1 of 3) Janeway's Immunobiology, 9th ed. (© Garland Science 2017)

Le cellule reticolari fibroblastiche formano una rete nelle zone delle cellule T dei tessuti linfoidi, facilitando le interazioni tra le cellule T naive e le DC



Le FRC all'interno delle zone delle cellule T negli organi linfoidi secondari producono una matrice extracellulare contenente reticolina e fibrille di collagene, che formano una struttura reticolare chiamata condotti linfoidi, rivestita da uno strato continuo di FRC.

Naive T cells migrate through secondary lymphoid tissues, sampling peptide:MHC complexes on dendritic cells

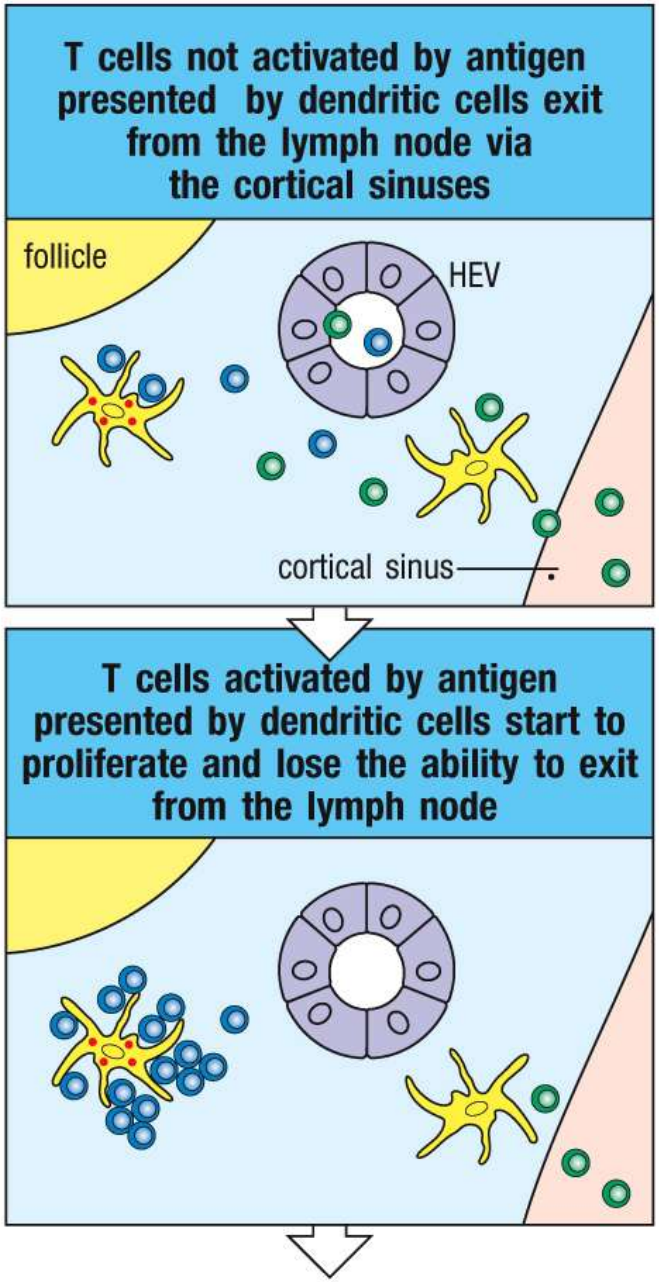


Figure 9.4 (part 2 of 3) Janeway's Immunobiology, 9th ed. (© Garland Science 2017)

Naive T cells migrate through secondary lymphoid tissues, sampling peptide:MHC complexes on dendritic cells

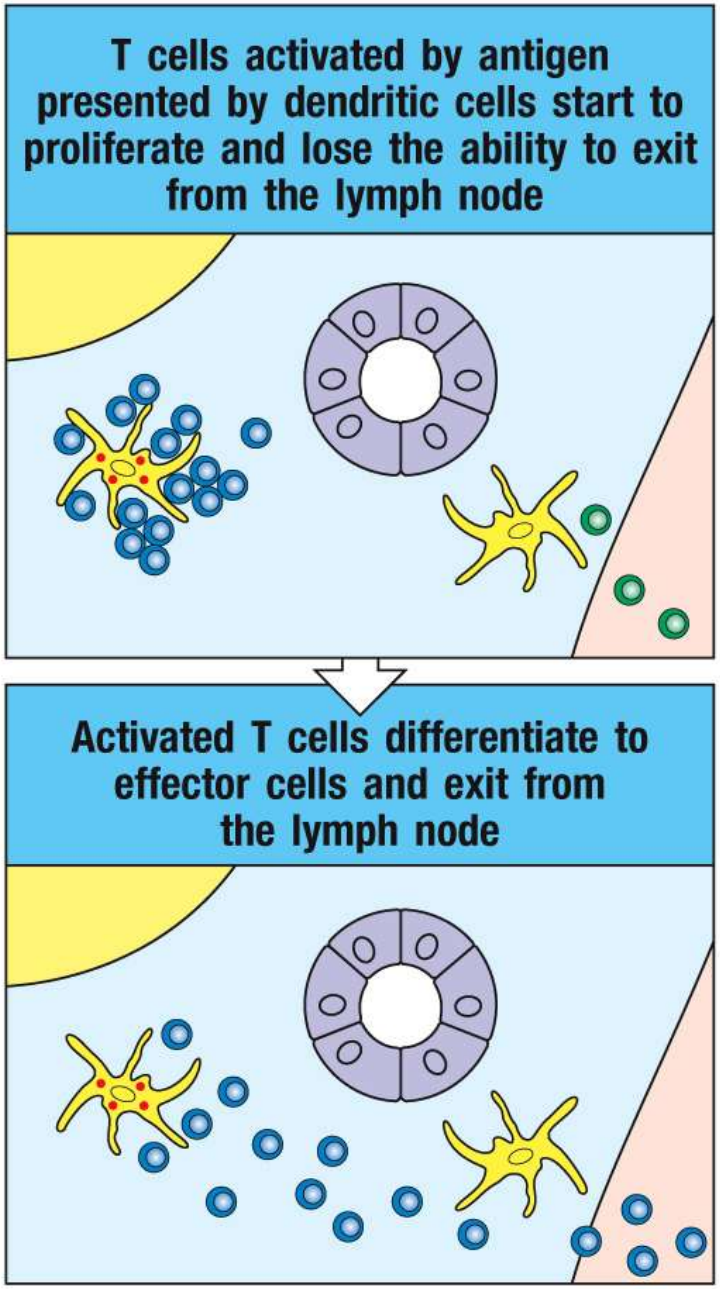


Figure 9.4 (part 3 of 3) Janeway's Immunobiology, 9th ed. (© Garland Science 2017)

Naive T cells migrate through secondary lymphoid tissues, sampling peptide:MHC complexes on dendritic cells

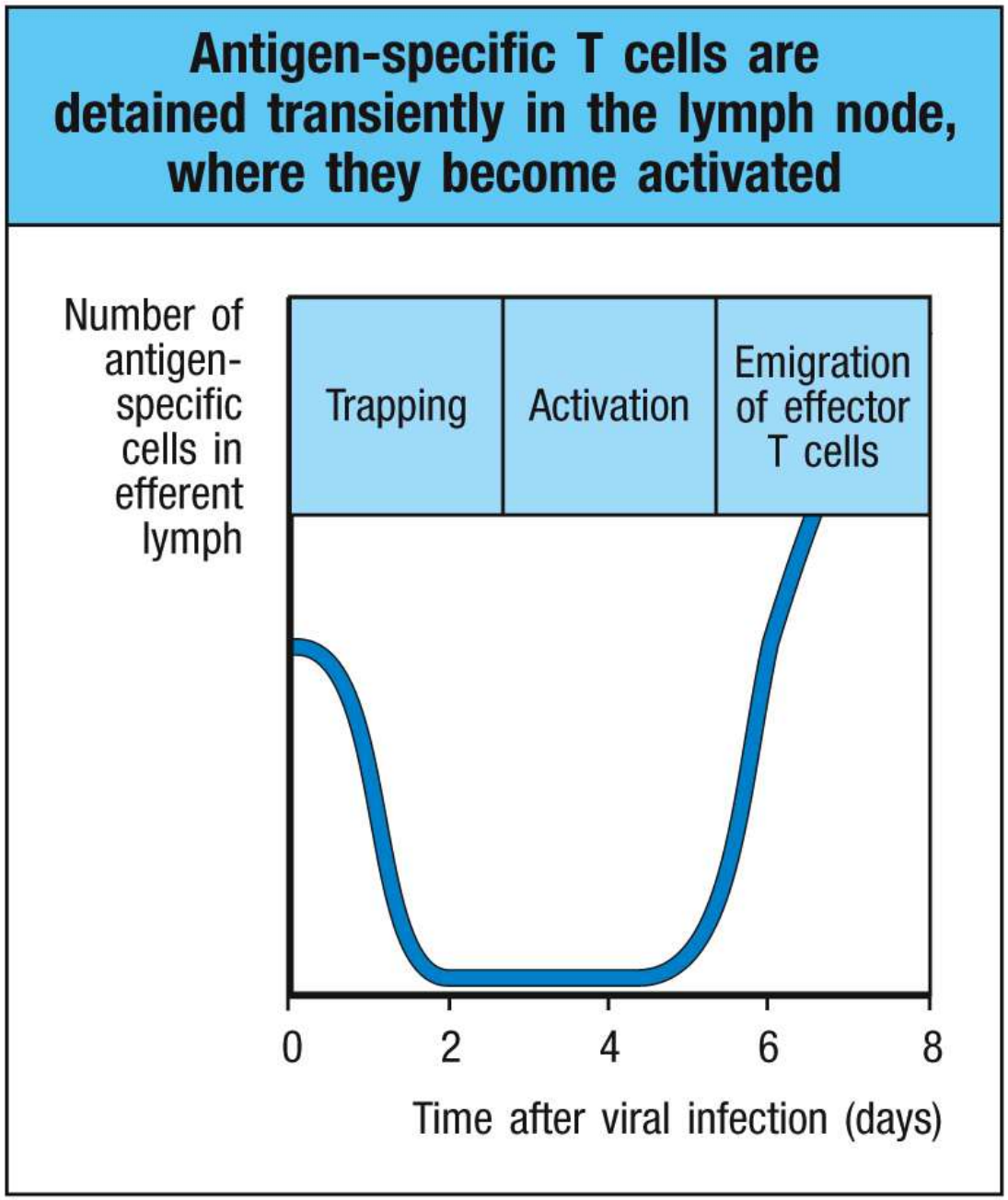


Figure 9.5 Janeway's Immunobiology, 9th ed. (© Garland Science 2017)

La fuoriuscita delle cellule T dal linfonodo è controllata da un fattore chemiotattico di origine lipidica

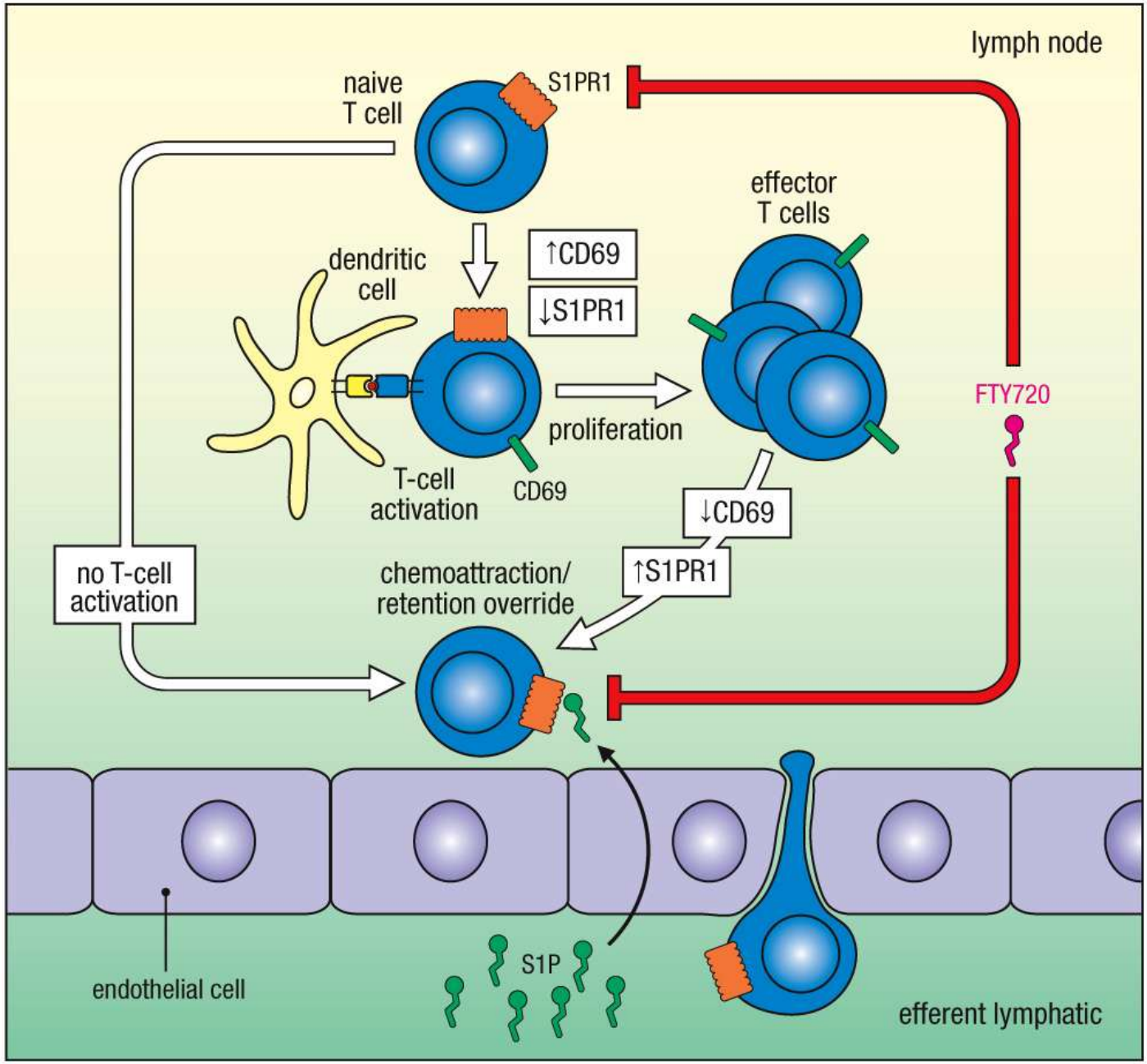


Figure 9.11 Janeway's Immunobiology, 9th ed. (© Garland Science 2017)

FTY720 inhibits S1P-S1P1 axis-mediated lymphocytes egress from the SLO

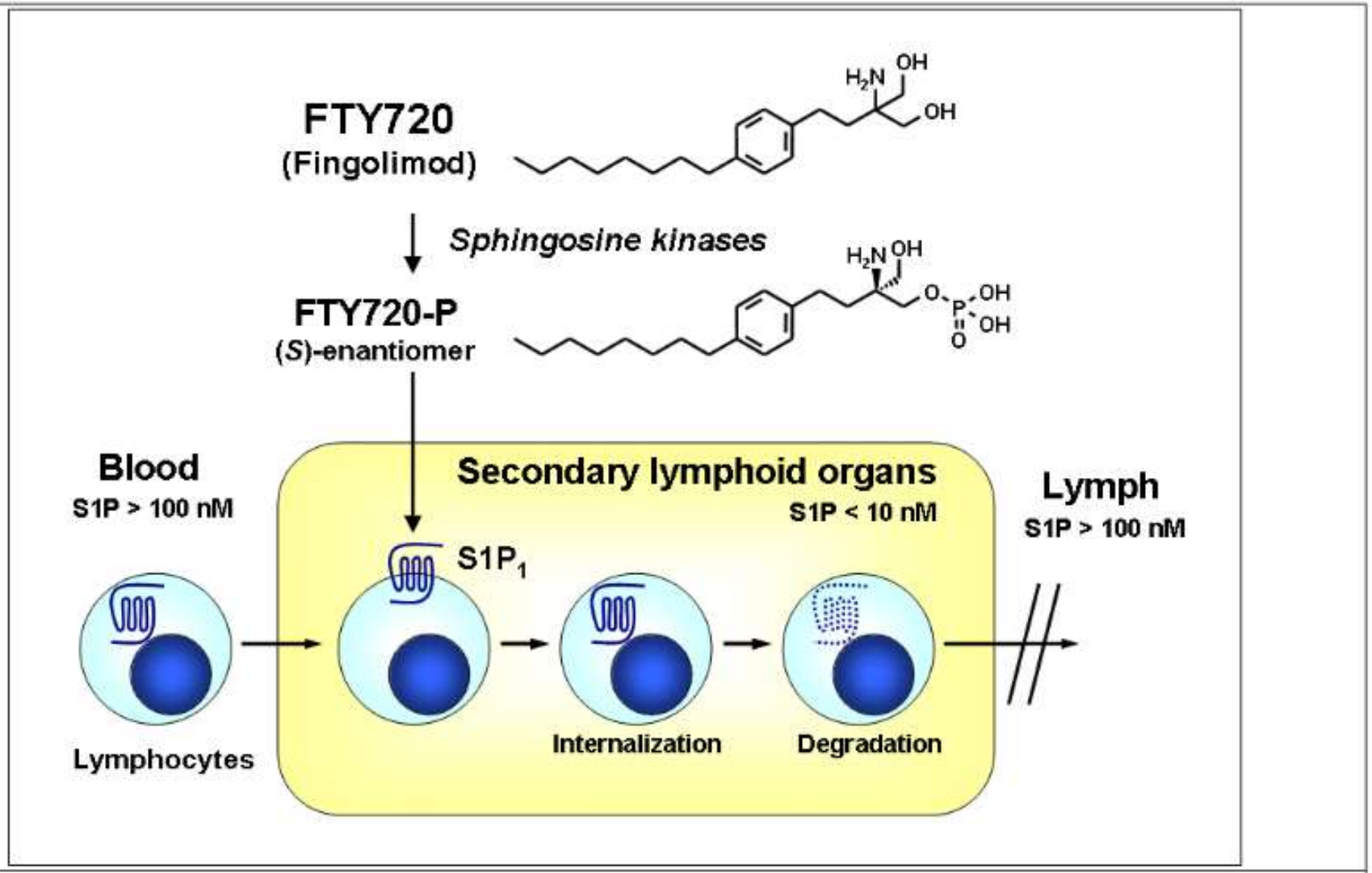


Fig. 1

FTY720-P converted from FTY720 acts as a functional antagonist at lymphocytic $S1P_1$ by internalization and degradation of the receptor, and inhibits S1P-$S1P_1$ axis-mediated lymphocyte egress from the SLO.

Fingolimod

Gylenia

Azienda: Novartis Europharm

Principio attivo: fingolimod

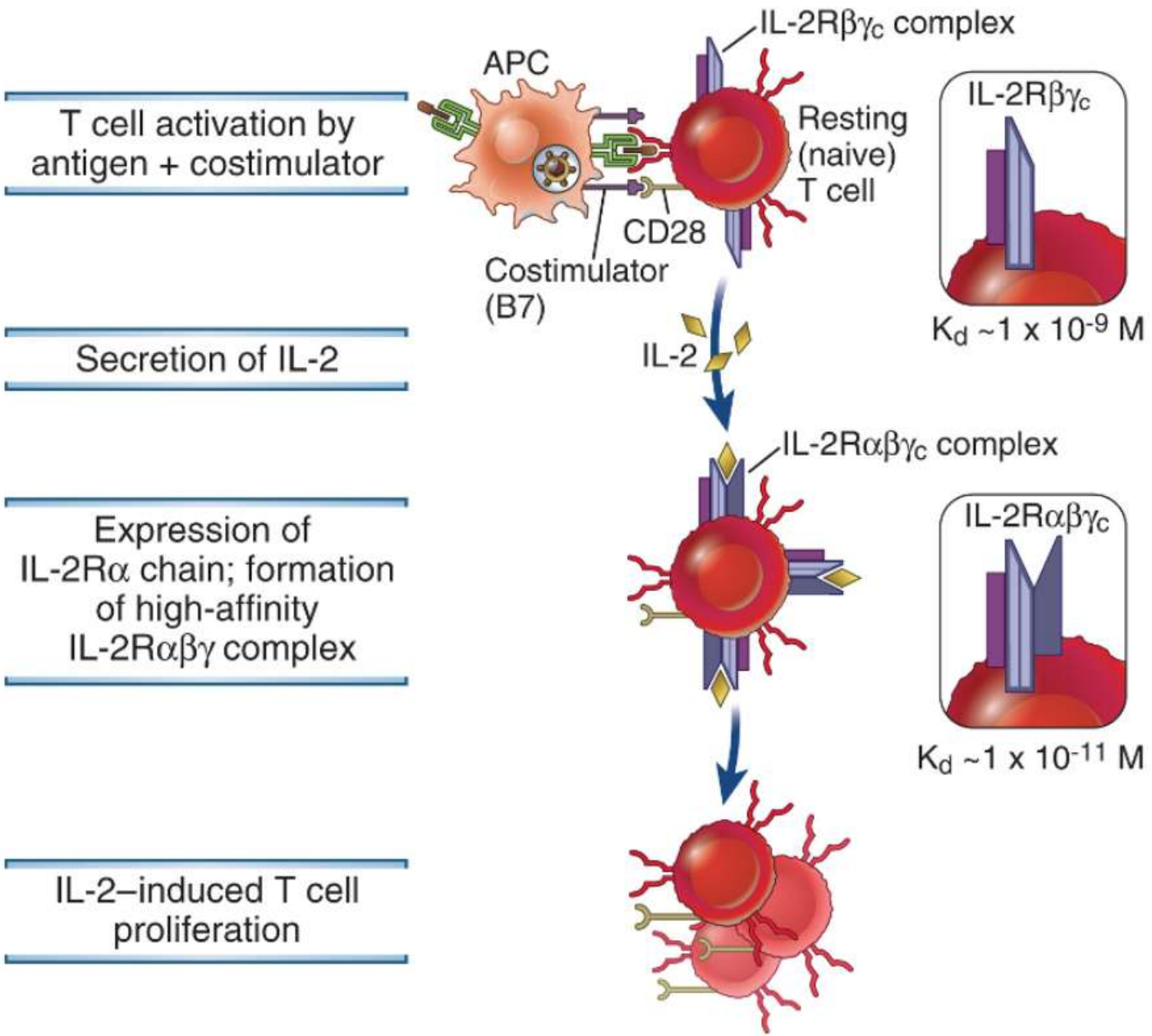
Modalità di somministrazione: orale

Posologia: tutti i giorni

Indicazioni: Gilenya è indicato in monoterapia, come farmaco modificante la malattia, nella sclerosi multipla recidivante-remittente ad elevata attività nei seguenti gruppi di pazienti adulti:
- persone con un‟elevata attività di malattia nonostante la terapia con interferone-beta. Questi pazienti possono essere definiti come coloro che non hanno risposto ad un ciclo terapeutico completo ed adeguato (normalmente almeno un anno di trattamento) con interferone beta. I pazienti devono avere avuto almeno 1 recidiva nell‟anno precedente mentre erano in terapia, e presentare almeno 9 lesioni iperintense in T2 alla RM cerebrale o almeno 1 lesione captante gadolinio. Un paziente non responder può anche essere definito come un paziente che presenta, rispetto all‟anno precedente, un tasso di recidive invariato o aumentato o che presenta recidive gravi.
- persone con sclerosi multipla recidivante-remittente grave ad evoluzione rapida, definita da due o più recidive disabilitanti in un anno, e con 1 o più lesioni captanti gadolinio alla RM cerebrale o con un aumento significativo del carico lesionale in T2 rispetto ad una precedente RM recentemente effettuata.

Effetti collaterali comuni: aumento rischio di infezioni, tosse, cefalea, dolore alla schiena, diarrea

Regolazione dell'espressione del recettore di IL-2



IL-2 is key to antigen-induced T cell proliferation and differentiation

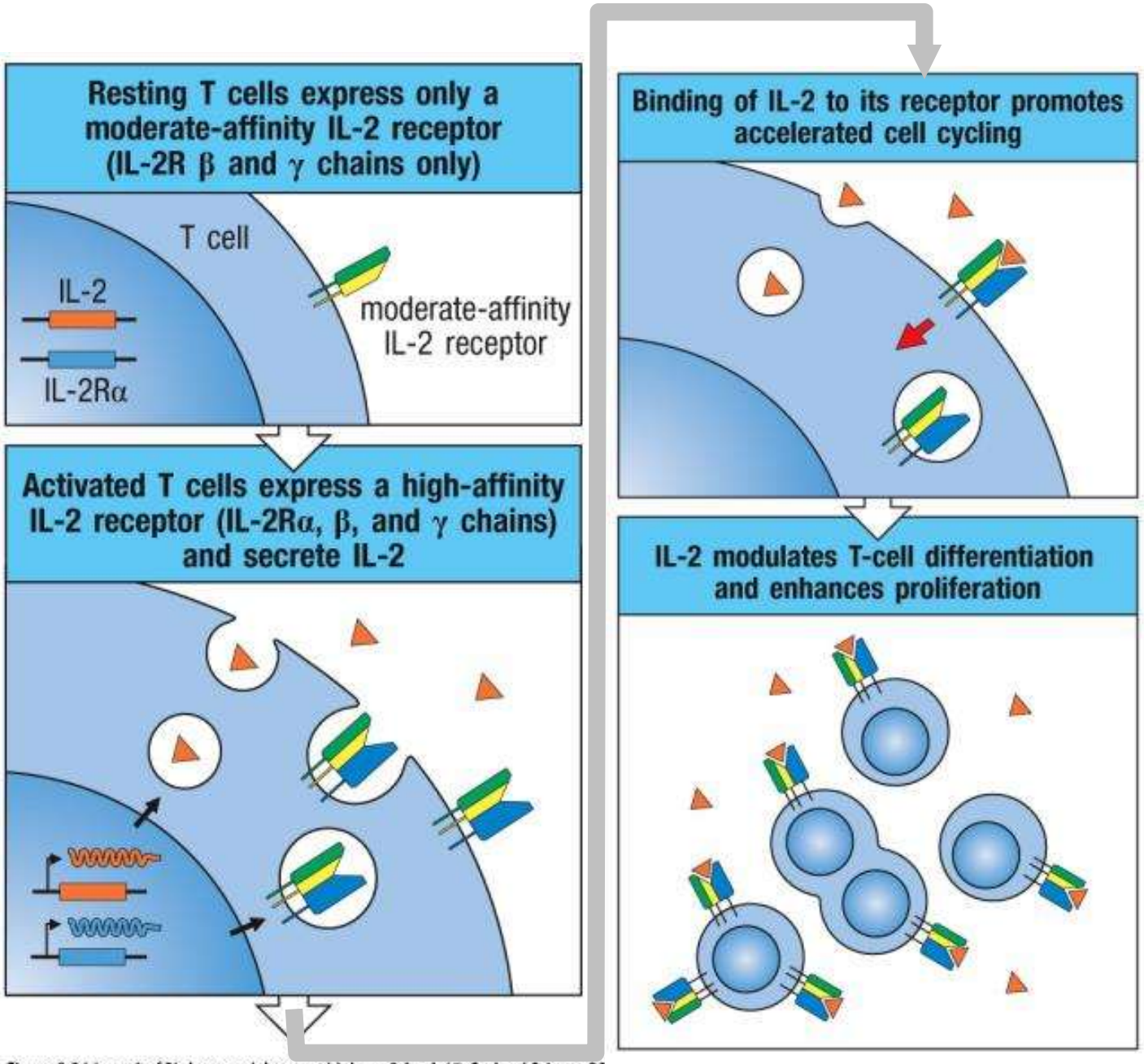
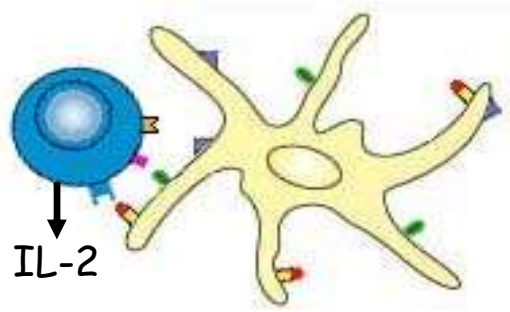


Figure 9.24 (part 1 of 3) Janeway's Immunobiology, 9th ed. (© Garland Science 20

Figure 9.24 (part 3 of 3) Janeway's Immunobiology, 9th ed. (© Garland Science 2017)



Autocrine stimulation

(a cell makes a hormone that is secreted and stimulate the cell that made it)

Cells divide 2-3 times per day for 4 or 5 day ($2^{12} = 4096$)

IL-2 used to be called "T cell growth factor"

Once T cells are activated and have acquired their effector function, they do not need co-stimulation (signal 2) to carry out the effector function.

Co-stimulation required for activation

Proliferate and acquire effector function (signals 1 and 2 and cytokines) (differentiate)

No co-stimulation is required for activated cell to carry out the effector function

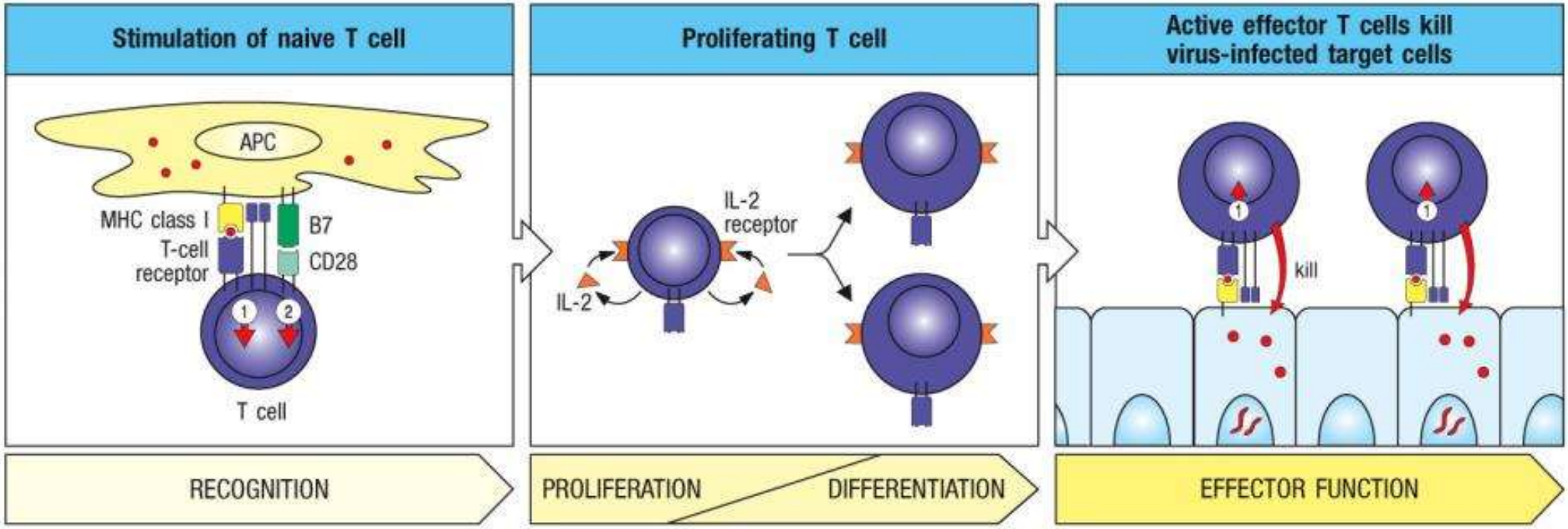
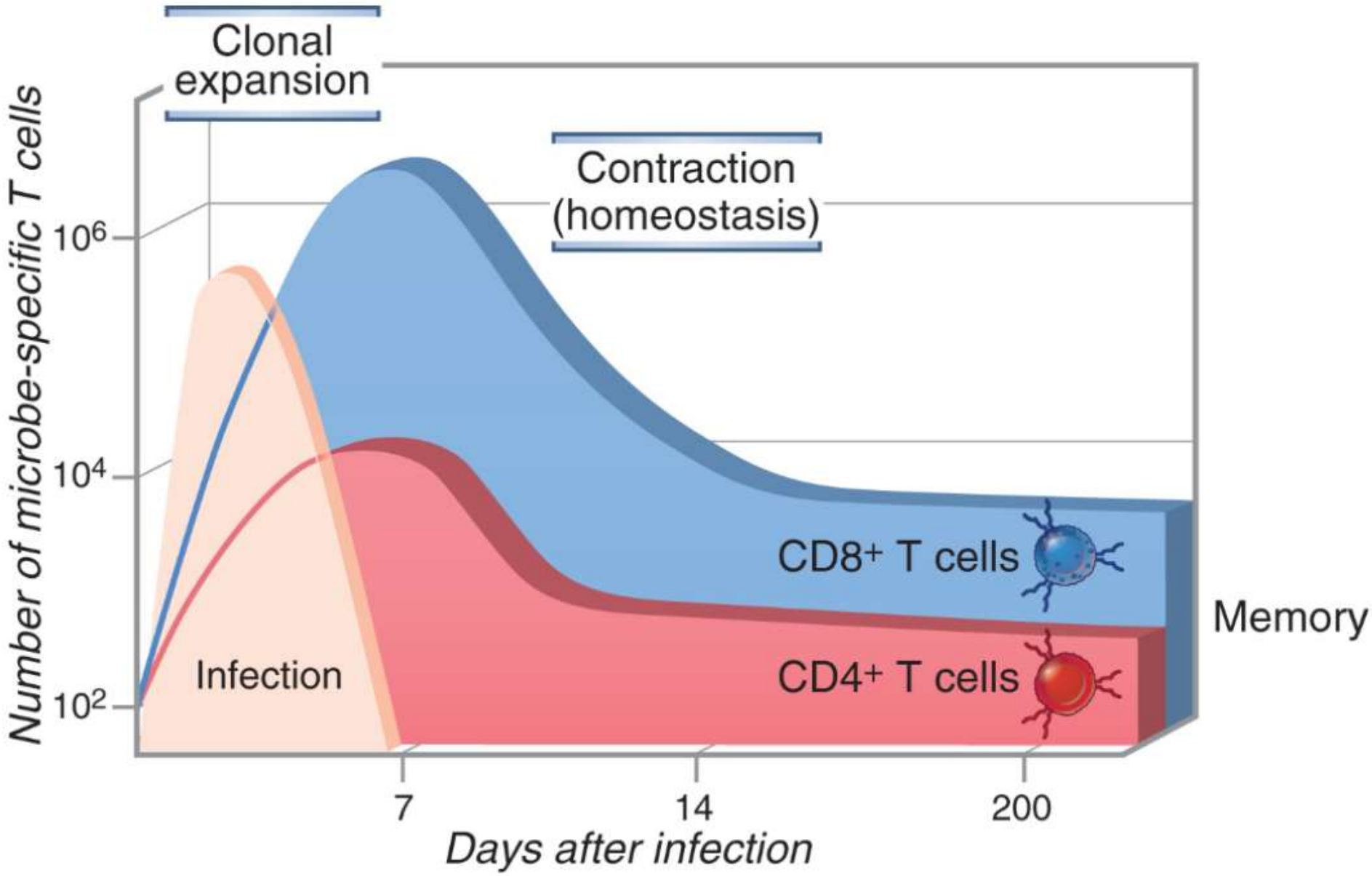


Figure 9.26 Janeway's Immunobiology, 9th ed. (© Garland Science 2017)

Espansione clonale delle cellule T

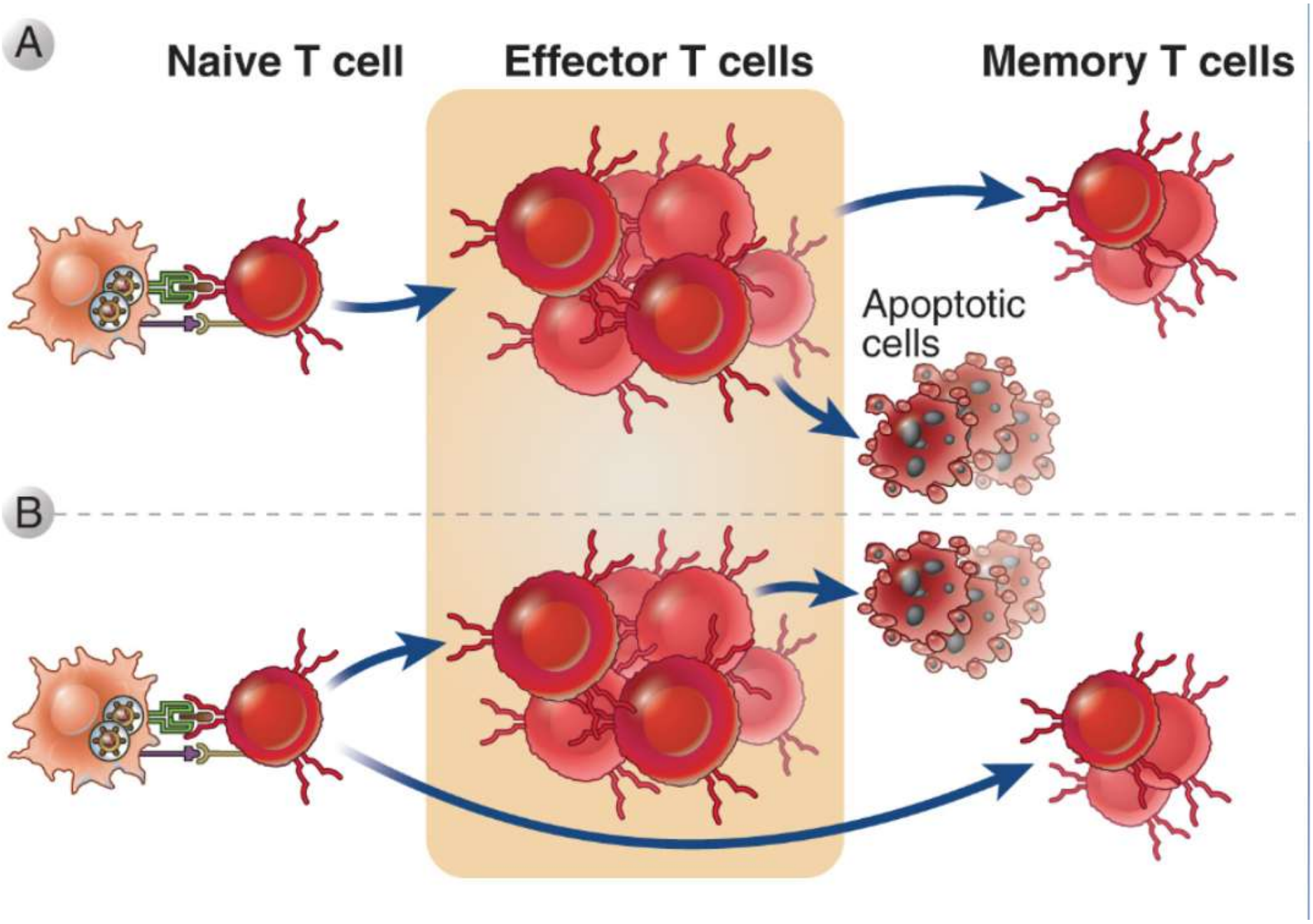


General principles of controlling immune responses

- Responses against pathogens decline as the infection is eliminated
  - Apoptosis of lymphocytes that lose their survival signals (antigen, etc)
  - Memory cells are the survivors

- Active control mechanisms may function to limit responses to persistent antigens (self antigens, possibly tumors and some chronic infections)
  - Often grouped under "tolerance"

Sviluppo delle cellule T di memoria



Change in proportions of naive and memory T cells with age

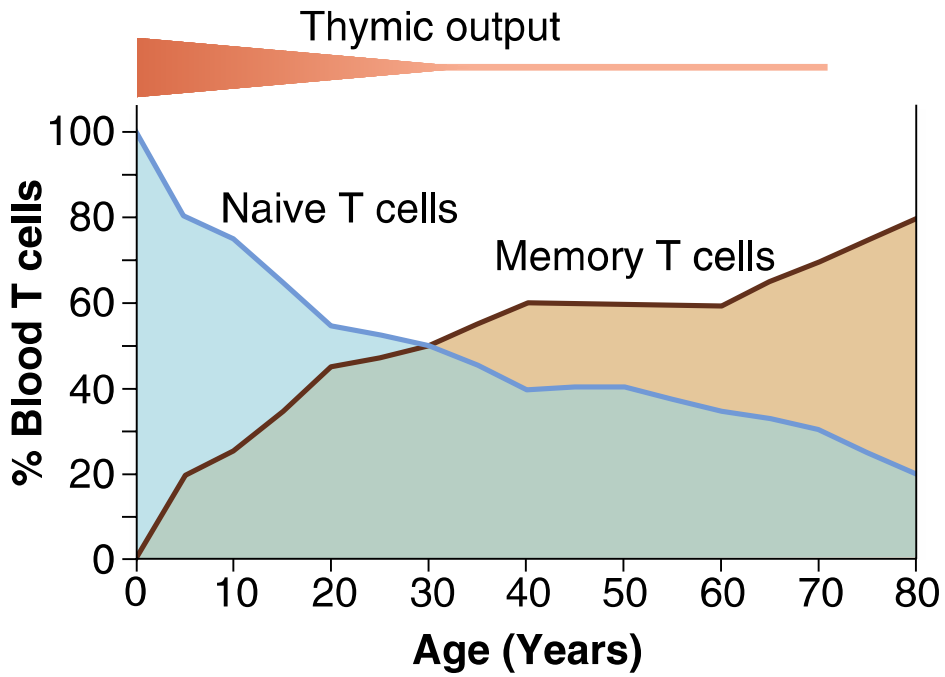


FIGURE 2.10 Change in proportions of naive and memory T cells with age. The proportions of naïve and memory T cells are based on data from multiple healthy individuals. The estimate of thymic output is an approximation. *(Courtesy of Dr. Donna L. Farber, Columbia University College of Physicians and Surgeons, New York.)*