



Facultad de Medicina
Clínica Alemana - Universidad del Desarrollo



Fundamentos Biológicos Quimioterapia y Terapias Biológicas



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Oncología Molecular
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Objetivos

- Comprender los mecanismos básicos involucrados en la acción de drogas antineoplásicas: tradicionales, biológicas
- Conocer ejemplos específicos de algunas drogas y sus aplicaciones
- Conocer limitaciones de las terapias actuales

Terapias Tradicionales

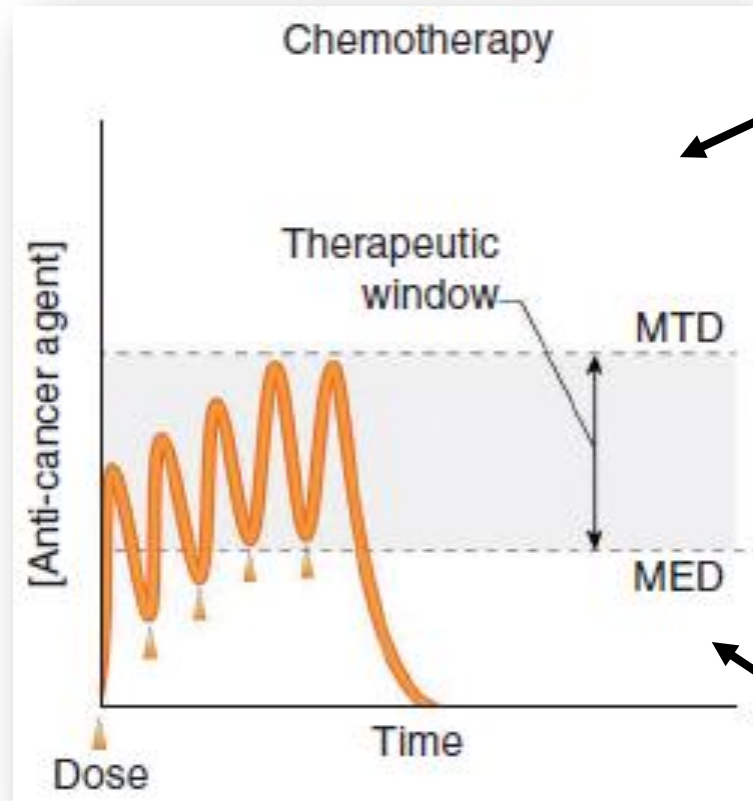
Quimioterapia: Principios

- Situación ideal: terapia curativa, «Total Kill»
- Agentes citotóxicos: mayor toxicidad en células tumorales → definición de ventana terapéutica
- Varias drogas dependientes de ciclo celular (células en estadio altamente proliferativo)
- Blancos: ADN (síntesis, modificación directa), componentes citoesqueleto (microtúbulos), síntesis de proteínas; modulación hormonal

Ventana Terapéutica

- Limitada por mínimo efectivo y máximo tolerado
- Para quimioterapia, relativamente similar entre pacientes

Ventana
Terapéutica



Daño a tejidos sanos: efectos colaterales

Dosis Máxima Tolerada

Dosis Mínima Efectiva

No efectivo

Mecanismos de Acción ("MOA")

M Phase Specific

Antimicrotubule Agents

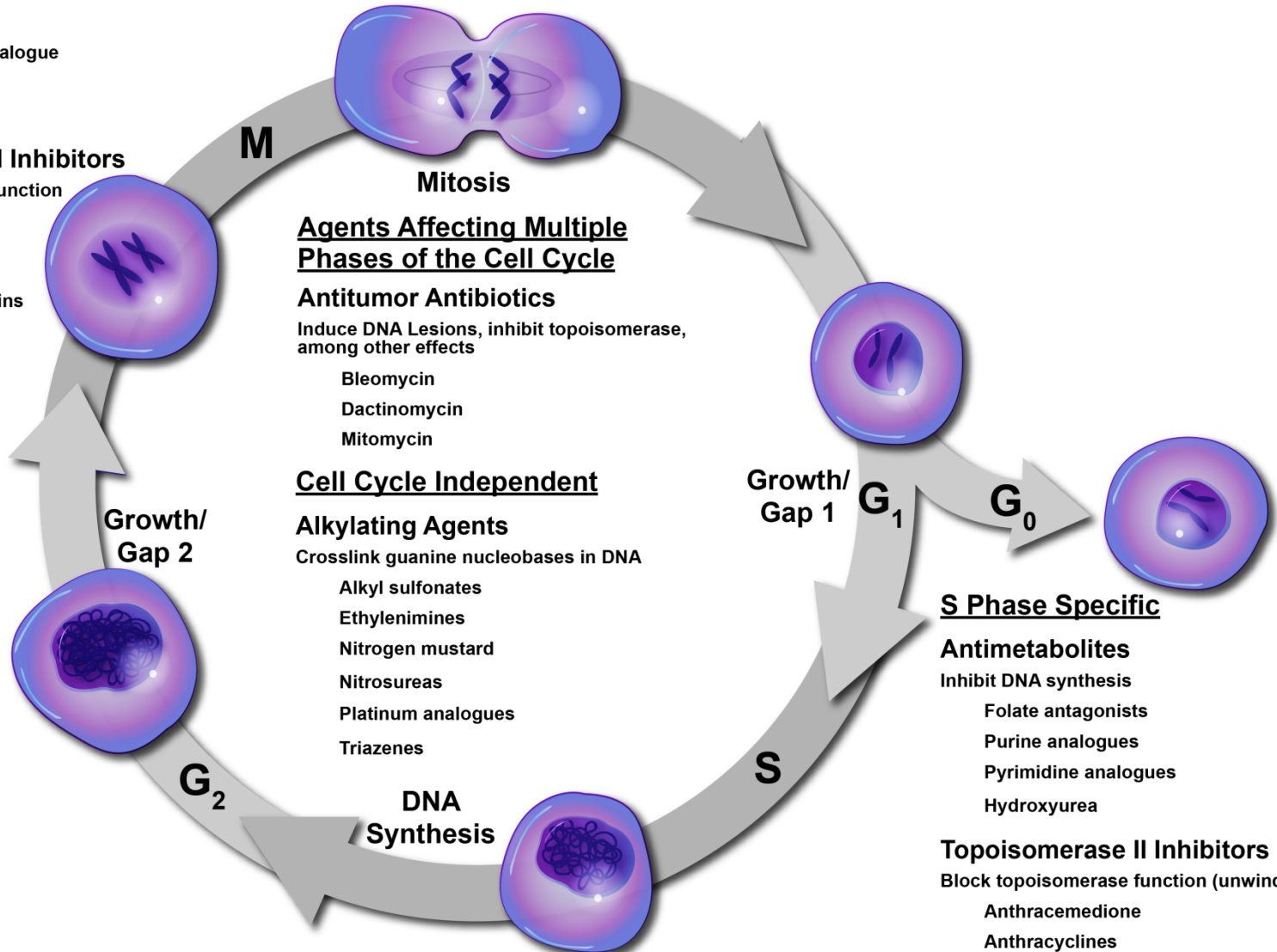
Inhibit function of microtubules

- Epothilones
- Halichondrin B analogue
- Taxanes
- Vinca alkaloids

Topoisomerase II Inhibitors

Block topoisomerase function (unwinding DNA)

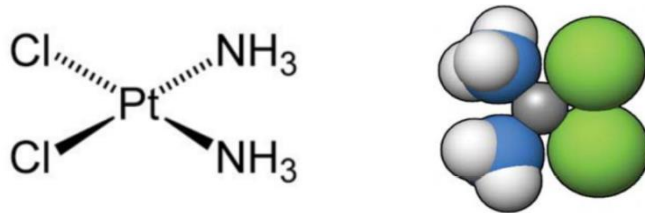
- Anthracedione
- Anthracyclines
- Epipodophyllotoxins



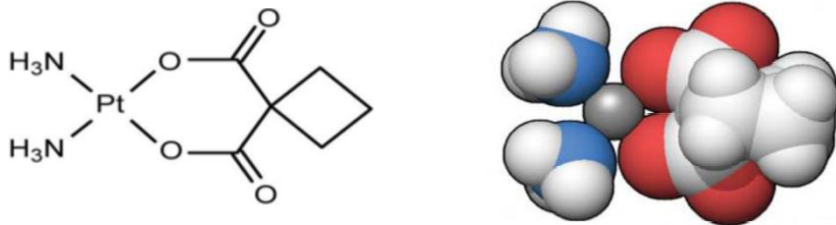
Agentes Alquilantes

- Independientes de ciclo celular
- Modificación directa del ADN

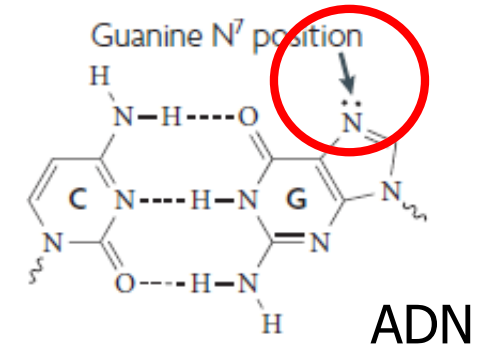
Ej: Compuestos platinados



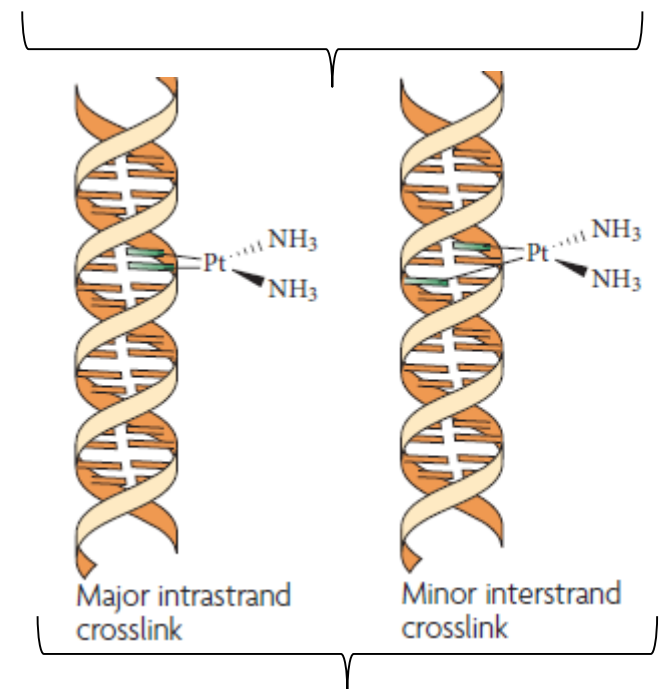
Cisplatino



Carboplatino

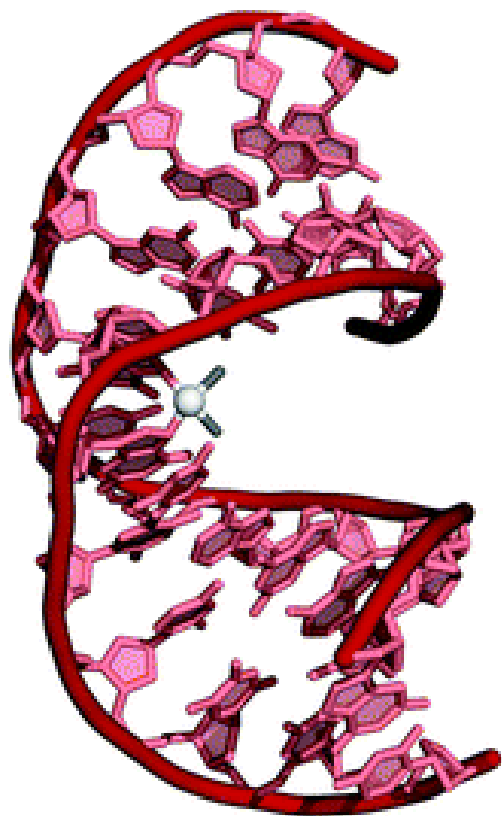


ADN

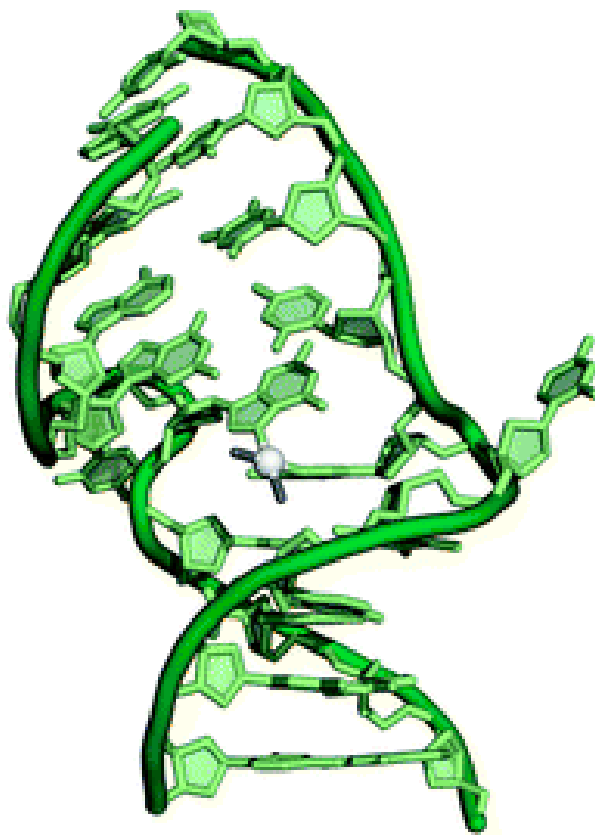


Daño al ADN, reparación
Detención transcripción
Muerte celular

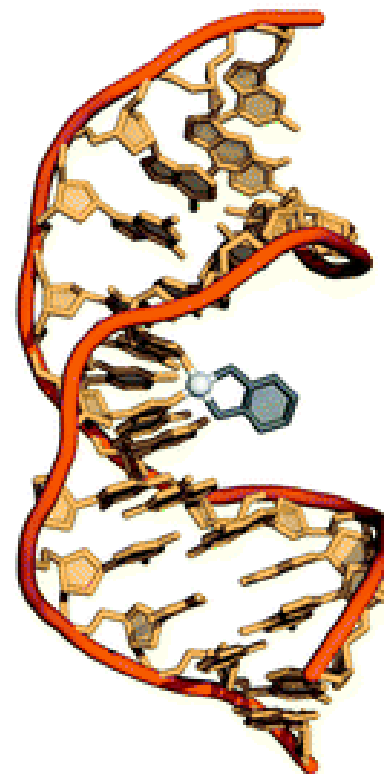
Agentes Alquilantes



Cisplatin 1,2-d(GpG)
intrastrand cross-
link



Cisplatin interstrand
cross-link



Oxaliplatin 1,2-
d(GpG) intrastrand
cross-link

Agentes Alquilantes

TYPE OF AGENT	NONPROPRIETARY NAMES	DISEASE
Nitrogen mustards	Mechlorethamine	Hodgkin's disease
	Cyclophosphamide Ifosfamide	Acute and chronic lymphocytic leukemia; Hodgkin's disease; non-Hodgkin's lymphoma; multiple myeloma; neuroblastoma; breast, ovary, lung cancer; Wilms' tumor; cervix, testis cancer; soft-tissue sarcoma
Methylhydrazine derivative	Melphalan	Multiple myeloma
	Chlorambucil	Chronic lymphocytic leukemia; macroglobulinemia
	Procarbazine (<i>N</i> -methylhydrazine, MIH)	Hodgkin's disease
Alkyl sulfonate	Busulfan	Chronic myelogenous leukemia, bone marrow transplantation
Nitrosoureas	Carmustine (BCNU)	Hodgkin's disease; non-Hodgkin's lymphoma; glioblastoma
	Streptozocin (streptozotocin)	Malignant pancreatic insulinoma; malignant carcinoid
Triazenes	Bendamustine	Non-Hodgkin's lymphoma
	Dacarbazine (DTIC; dimethyltriazenoimidazole carboxamide),	Malignant melanoma; Hodgkin's disease; soft-tissue sarcomas; melanoma
Platinum coordination complexes	Temozolomide	Malignant gliomas
	Cisplatin, carboplatin, oxaliplatin	Testicular, ovarian, bladder, esophageal, lung, head and neck, colon, breast cancer

Agentes Antimitóticos

- Actúan durante fase M del ciclo celular
- Alteración estructural de microtúbulos por interacción directa
- Vinca alcaloides : Vincristine, vinblastine, vindesine, vinorelbine
- Taxanos: Paclitaxel, Docetaxel



Vinca rosea

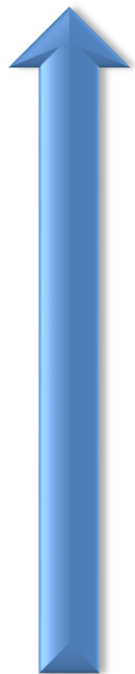


Taxus brevifolia,
Taxus buccata

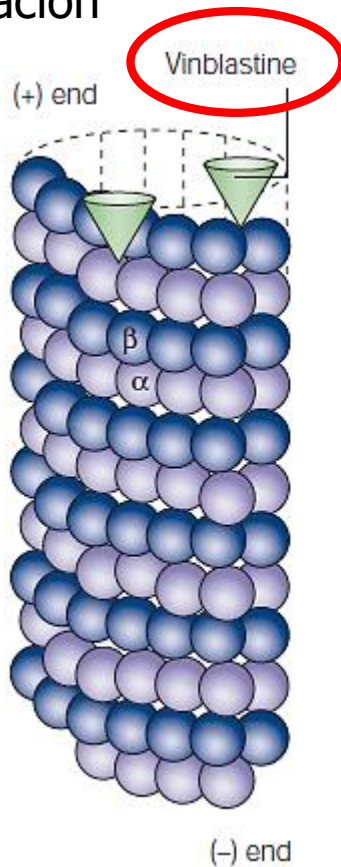
TYPE OF AGENT	NONPROPRIETARY NAMES	DISEASE
Vinca alkaloids	Vinblastine	Hodgkin's disease; non-Hodgkin's lymphoma; testis cancer
	Vinorelbine	Breast and lung cancer
	Vincristine	Acute lymphocytic leukemia; neuroblastoma; Wilms' tumor; rhabdomyosarcoma; Hodgkin's disease; non-Hodgkin's lymphoma
Taxanes	Paclitaxel, docetaxel	Ovarian, breast, lung, prostate, bladder, head, and neck cancer

Agentes Antimitóticos: Mecanismo

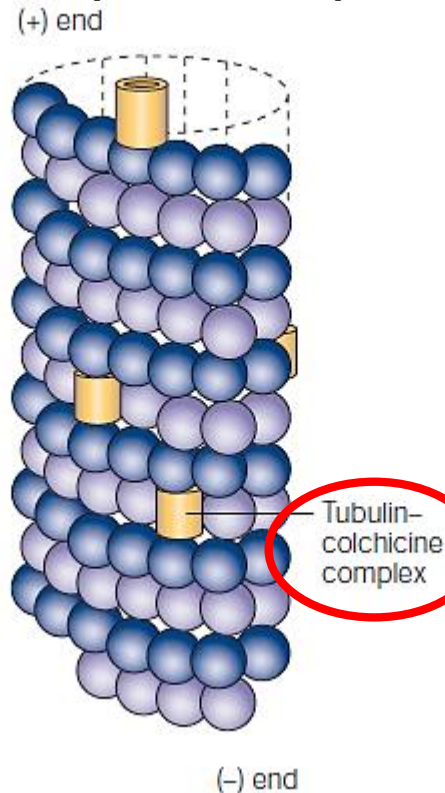
Vinca
alcaloides:
Polimerización
inhibida
(+ end)



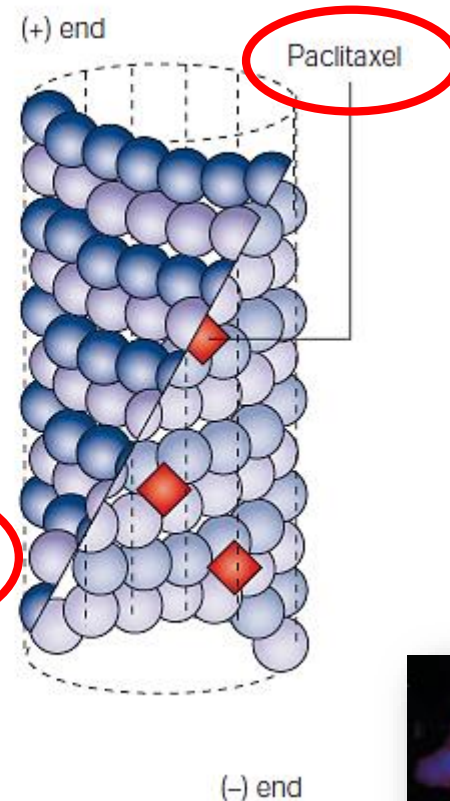
POLIMERIZACION



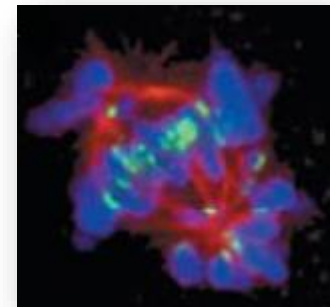
Dinámica
inhibida
(intercalado)



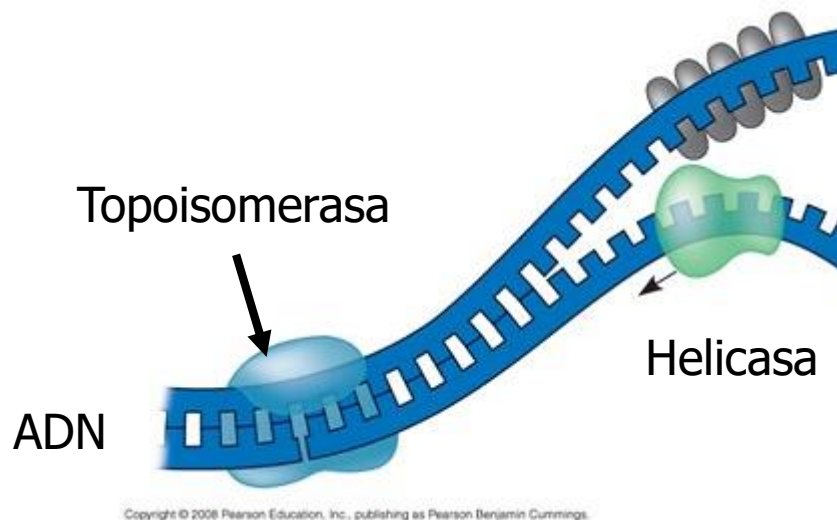
Taxanos:
Dinámica inhibida,
rigidez (unión interna)



Muerte celular por fallas huso mitótico



Inhibidores de Topoisomerasa II

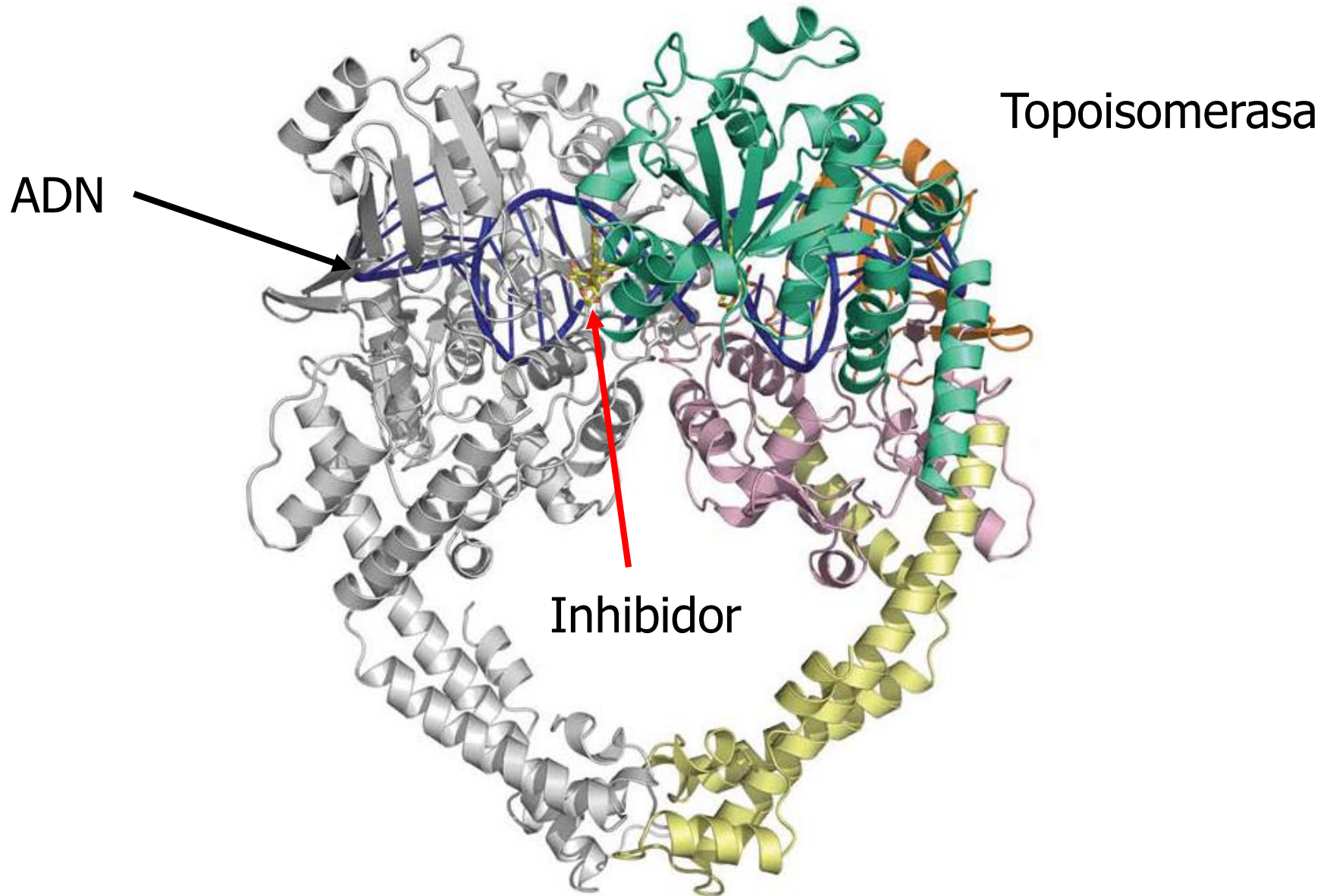


Replicación del
ADN

- Derivados semisintéticos de podophyllo-toxinas (glicósidos de *Podophyllum peltatum*):
Etoposide, tenoposide
- Activos en fase M y S ciclo celular



Inhibidores de Topoisomerasa II



Inhibidores de Topoisomerasa II

- Topoisomerasa: permite “desenrollar” hebra de ADN para su replicación (giro, ruptura y reunión, elimina tensión torsional)
- Inhibidores actúan en fase M o S de ciclo celular
- Lesiones en ADN por unión covalente de Topo II, o inhibición de su actividad catalítica
- Induce quiebres en el ADN, muerte celular

Antibióticos Antitumorales

- Actúan en varias fases del ciclo celular
- Varios mecanismos:
 - Intercalantes en ADN (bloquean síntesis)
 - Generación de radicales libres oxígeno: quiebres del ADN
 - Interferencia con Topo II
- Aislados de *Streptomyces*, *Streptococcus*

Antibiotics	Dactinomycin (actinomycin D) Daunorubicin (daunomycin, rubidomycin) Doxorubicin	Choriocarcinoma; Wilms' tumor; rhabdomyosarcoma; testis; Kaposi's sarcoma Acute myelogenous and acute lymphocytic leukemia Soft-tissue, osteogenic, and other sarcoma; Hodgkin's disease; non-Hodgkin's lymphoma; acute leukemia; breast, genitourinary, thyroid, lung, and stomach cancer; neuroblastoma and other childhood and adult sarcomas
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Antibióticos Antitumorales

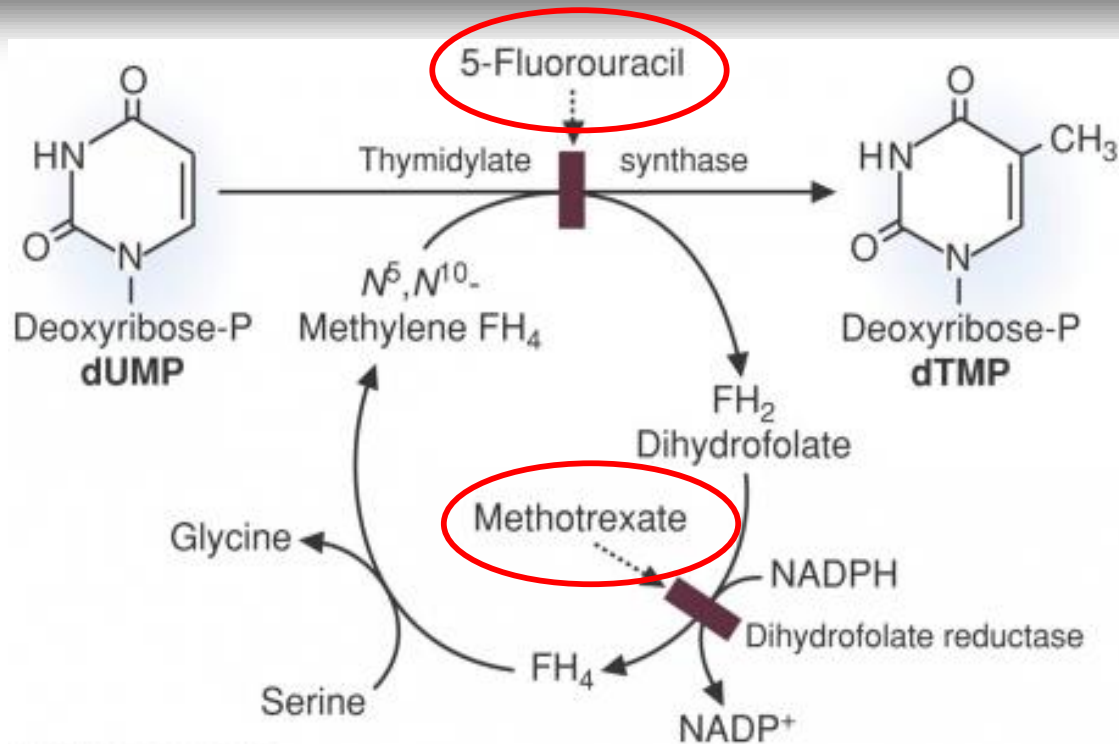
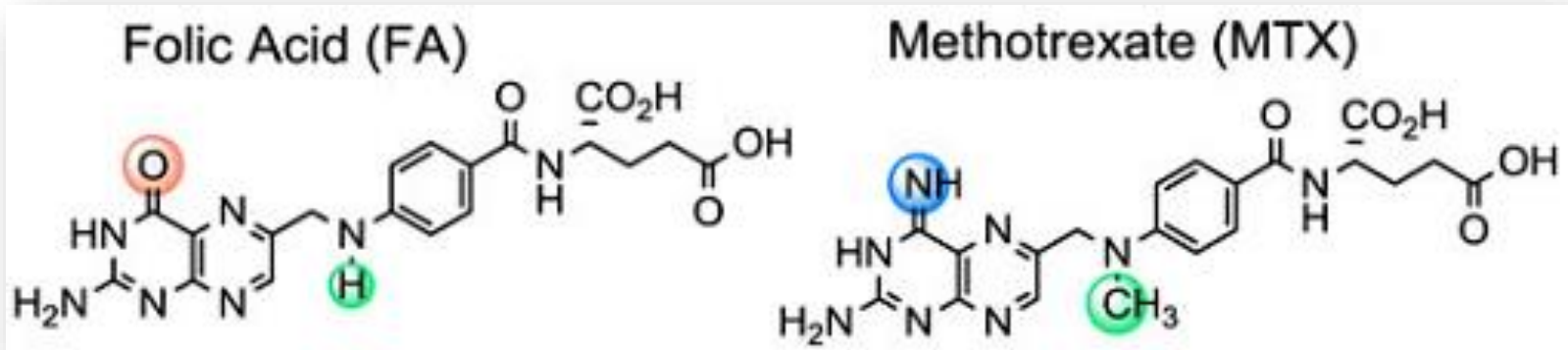
<https://www.youtube.com/watch?v=AX2mRRxVbNo>

Antimetabolitos

- Estructuralmente relacionados a moléculas celulares
- Interfieren con la disponibilidad de precursores (purinas y pirimidinas) para la síntesis de nucleótidos
- Máxima efectividad en fase S del ciclo celular
- Antagonistas de folatos, purinas, pirimidinas
- Ej: Metotrexato, 5-FU, gemcitabina

Antimetabolitos

- Metotrexato: análogo de folato



Hormonoterapias

- Agonistas o antagonistas
- Tratamiento (p.ej. Prevenir recurrencias) y manejos de síntomas (p.ej. Cáncer de próstata)
- Pueden bloquear producción hormonal, o interferir con su función
- Cáncer de mama: Tamoxifeno (anti-estrógeno no esteroidal)
- Cáncer de próstata: antagonistas de receptor de andrógenos; inhibidores síntesis de testosterona

Hormonoterapias

TYPE OF AGENT	NONPROPRIETARY NAMES	DISEASE
Adrenocortical suppressants	Mitotane (<i>o,p'</i> -DDD)	Adrenal cortex cancer
Adrenocortico-steroids	Prednisone (other equivalent preparations available)	Acute and chronic lymphocytic leukemia; non-Hodgkin's lymphoma; Hodgkin's disease; breast cancer, multiple myeloma
Progestins	Hydroxyprogesterone caproate, medroxyprogesterone acetate, megestrol acetate	Endometrial, breast cancer
Estrogens	Diethylstilbestrol, ethinyl estradiol (other preparations available)	Breast, prostate cancer
Anti-estrogens	Tamoxifen, toremifene	Breast cancer
Aromatase inhibitors	Anastrozole, letrozole, exemestane	Breast cancer
Androgens	Testosterone propionate, fluoxymesterone (other preparations available)	Breast cancer
Anti-androgen	Flutamide, casodex	Prostate cancer
GnRH analog	Leuprolide	Prostate cancer

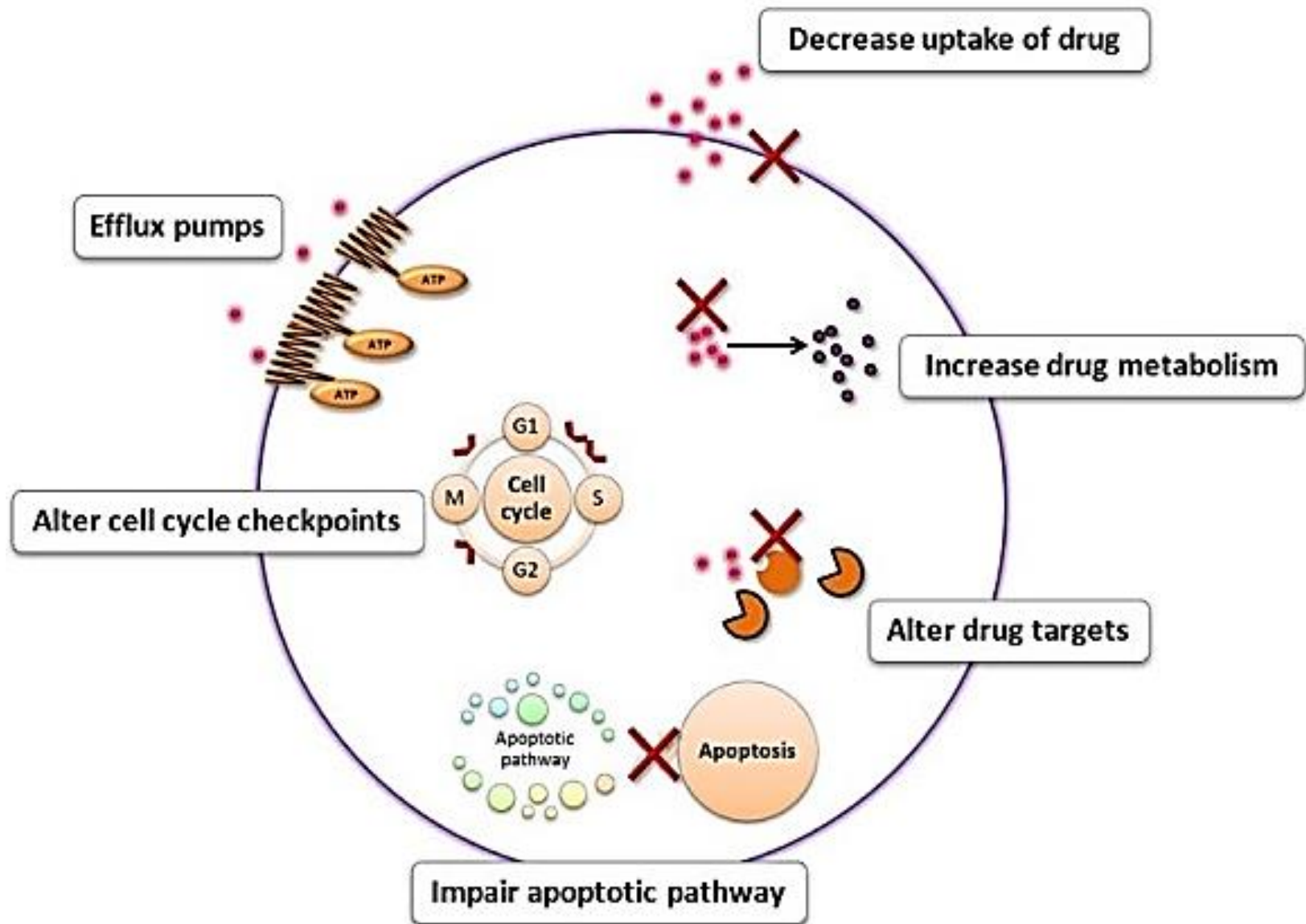
Quimioterapia: Limitaciones

- Efectos adversos (Anemia, infecciones, toxicidad hepática, hemorragia, etc.)



- Resistencia a drogas
Selección de clones resistentes, generación de nuevas mutaciones, terapias no logran curación

Resistencia a Drogas (MDR)



Terapias Biológicas

Definición

- Terapias Biológicas:

«Involucran organismos vivos, sustancias derivadas de organismos vivos, o variantes de éstas producidas en el laboratorio, y que se aplican para el tratamiento de enfermedades»

- Ejemplos:

Vacunas	}	Modulación de respuesta inmune
Bacterias		

Anticuerpos	}	Interacción directa con célula tumoral
Ácidos nucleicos (ADN, ARN)		

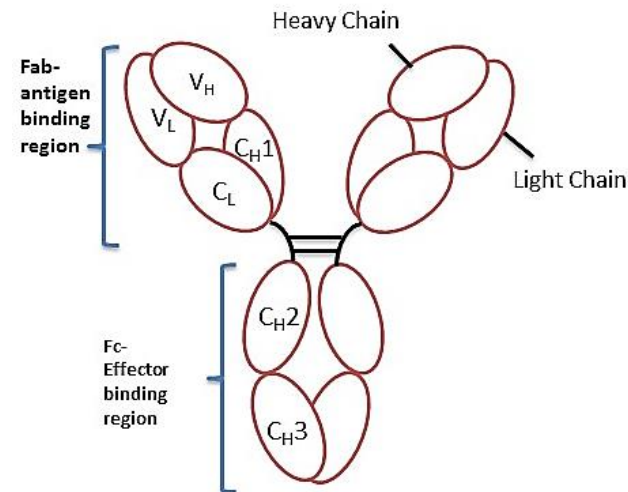
Terapias Dirigidas

Anticuerpos Monoclonales

- Estimulación de producción de células B por inyección de antígenos (ratón)
- Las células B producen anticuerpos contra el antígeno
- Las células B se aíslan y fusionan con células inmortales (mieloma): cultivo por tiempo indefinido

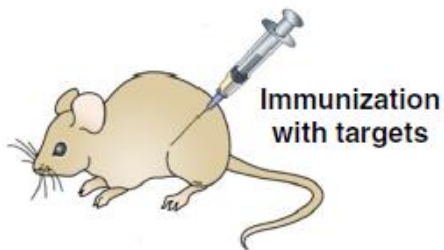
Anticuerpos monoclonales:

- Idénticos entre sí (homogéneos)
- Altamente específicos
- Obtención de grandes cantidades



Generación de Anticuerpos

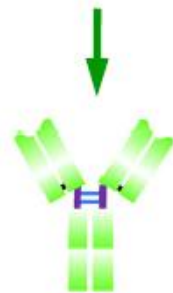
(A) Mouse hybridoma



Harvest splenocytes, generate hybridomas



Screening



Mouse mAb

Chimerization

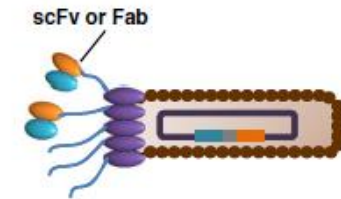
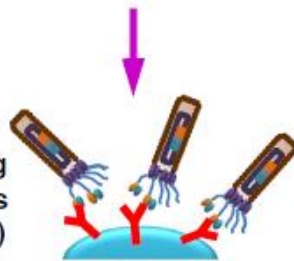
CDR graft

Chimeric mAb

Humanized mAb



Biopanning with targets (3-5 cycles)

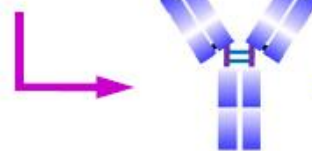


Phage-displayed Ab libraries

Screening

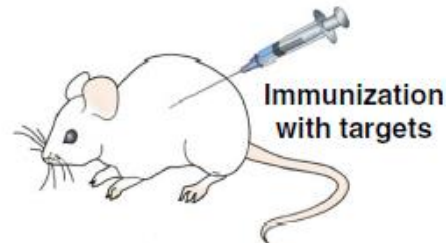


Construction of Human IgG

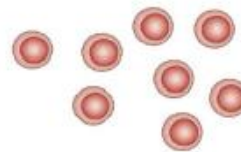


Human mAb

(C) Transgenic mouse



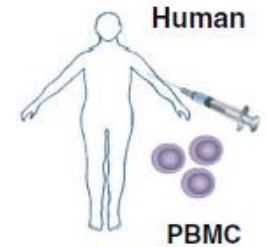
Harvest splenocytes, generate hybridomas



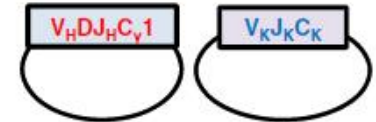
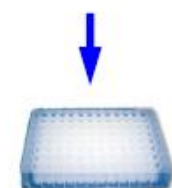
Screening



(D) Single B cell



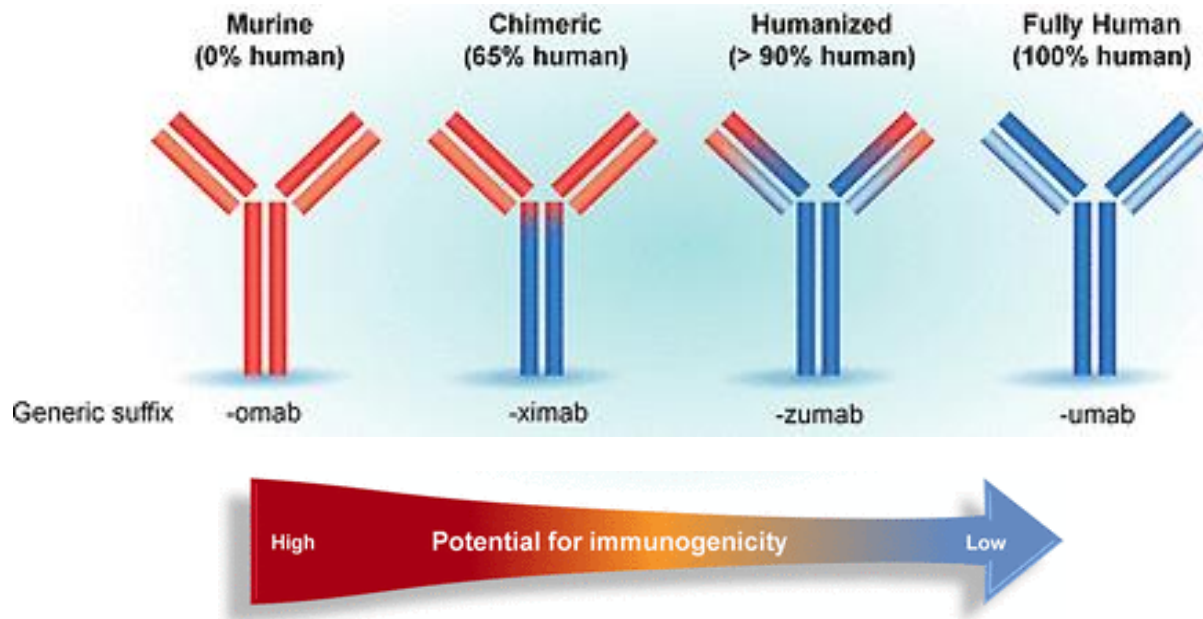
Sort B cells with labeled antigens



PCR, construct V_H and V_L

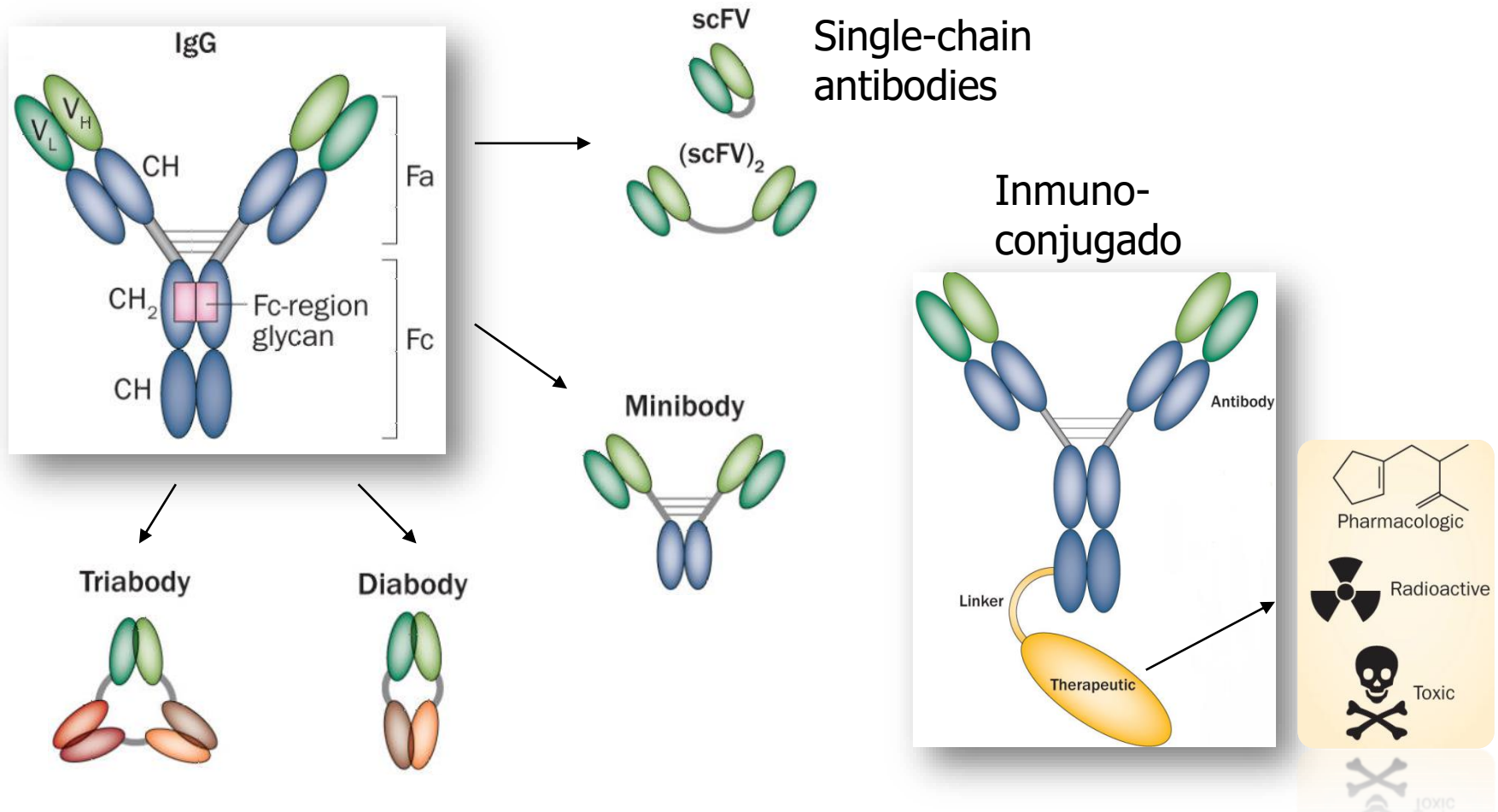
Anticuerpos Modificados

- Manipulación tecnológica para reemplazar las secuencias de ratón que pueden ser inmunogénicas → interferencia con respuesta inmune antitumoral
- Anticuerpos "humanizados"



Y Otras Formas de Potenciarlos...

- Manipulación tecnológica para crear nuevas estructuras → inmunoconjugados



Posibles Aplicaciones

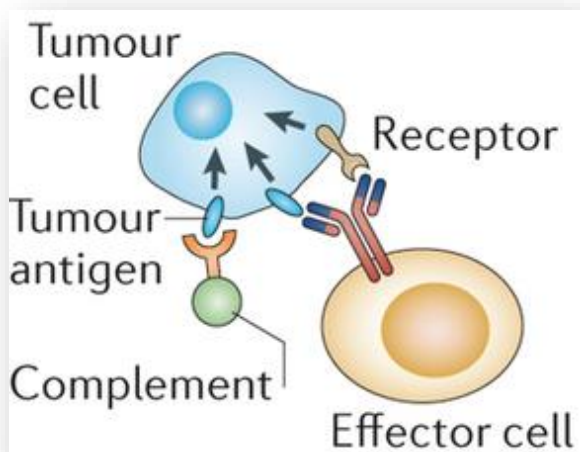
- Tratamiento, imágenes, dirigir drogas al tumor

Antibody constructs	Examples of targets	Potential clinical use
scFv	CC49, ERBB2 and Le ^y	Imaging and cell targeting
Diabody	Le ^y and TAG-72	Imaging and drug delivery
Affibody	ERBB2	Imaging and drug delivery
Minibody	CEA and ERBB2	Imaging and drug delivery
Protein-Fc	Angiopoietin 1, angiopoietin 2, VEGFR1 and VEGFR2	Imaging and therapy
Intact IgG	CD20, CD33, EGFR, ERBB2 and VEGF	Imaging therapy and drug delivery
IgE and IgM	GM2	Therapy
Drug conjugates	CD30, CD33 and ERBB2	Therapy
Loaded nanoparticles	A33, EGFR and transferrin	Drug delivery
Bispecifics	CD19-CD3, EPCAM-CD3 and gp100-CD3	Therapy

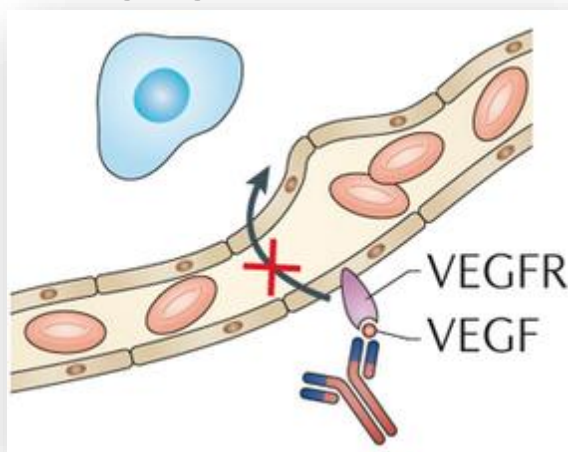
Mecanismos de Acción



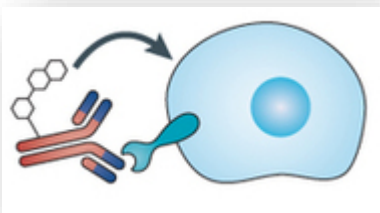
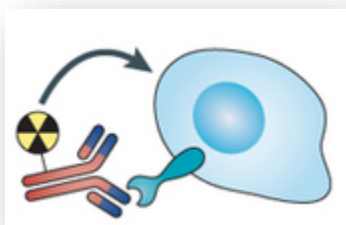
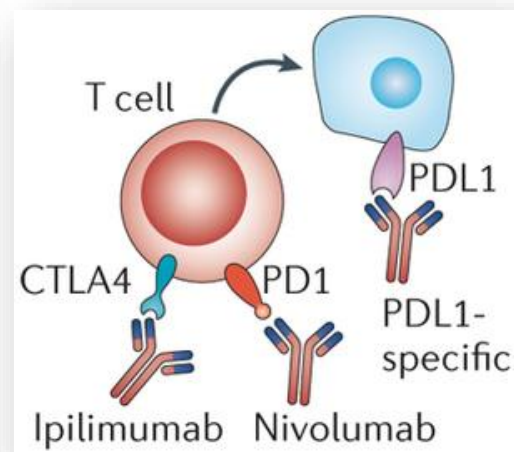
Anticuerpos tumor-específicos



Inhibición de angiogénesis



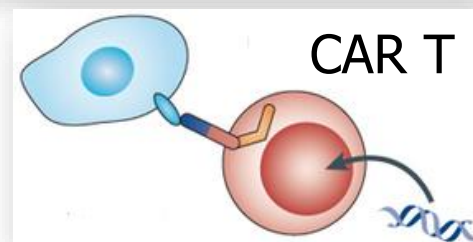
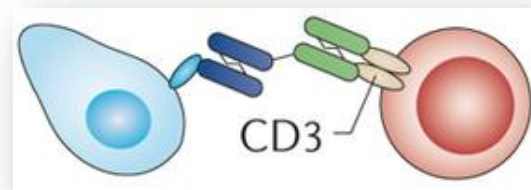
Desbloqueo de check-points



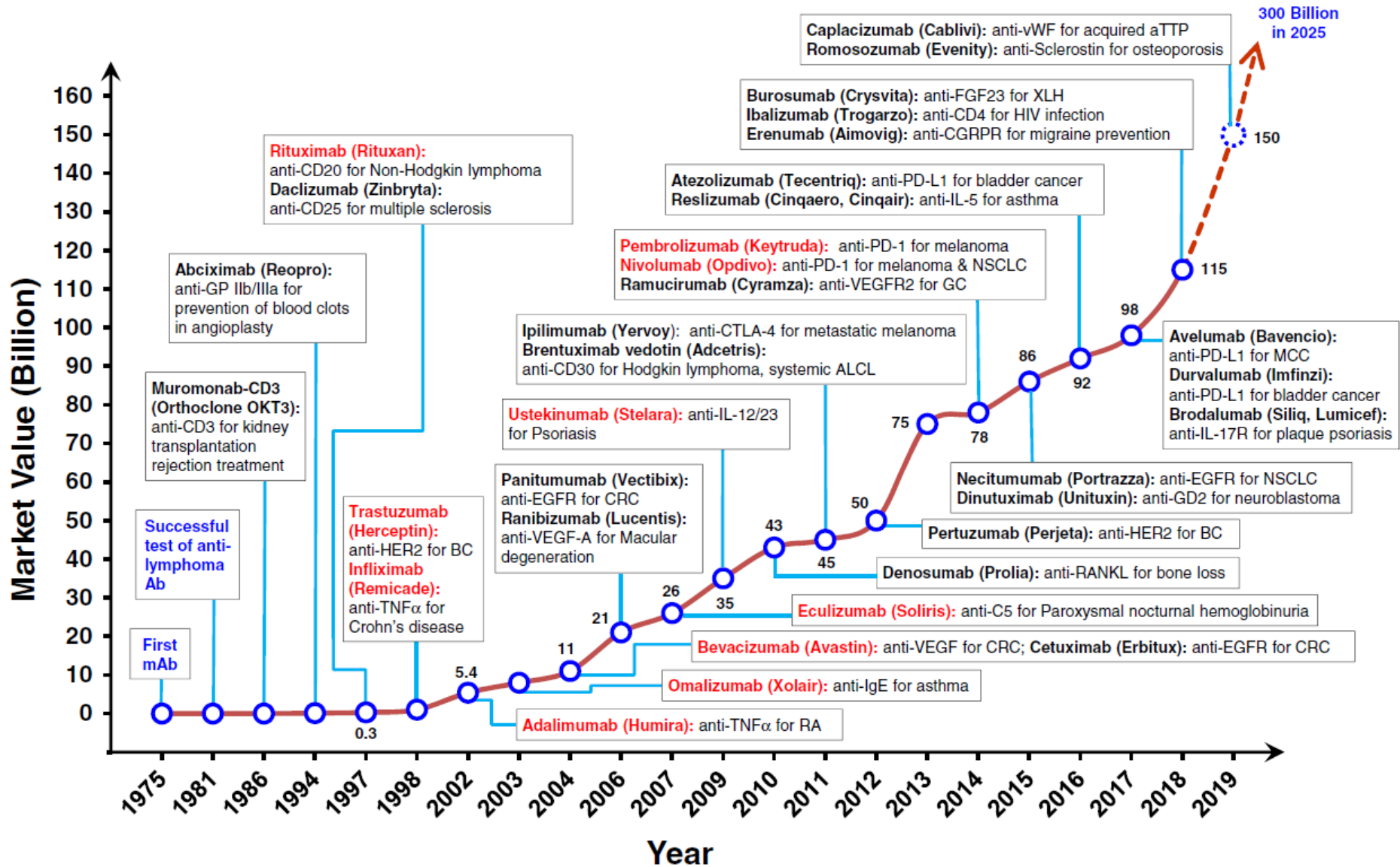
Inmuno-conjugados

Redireccionamiento de inmunidad celular

Anticuerpo biespecífico



Desarrollo Anticuerpos Terapéuticos



Limitaciones, Desafíos

Antibody function	Lack of response
Targeting	Antigen or receptor heterogeneity or mutation, downregulation of expression
Pharmacokinetics	Antibody stability, half-life, immunogenicity
Tumor penetration	Vascular permeability, interstitial pressure, antibody size and affinity
Receptor binding	Low affinity, ineffective blockade of receptor dimerization
Pharmacodynamics	Signaling pathway irrelevant for tumor growth and progression incl. resistance, redundant survival signaling
Effector function (FcR)	Immune escape (FcR polymorphisms, low T cell and NK potency), immune suppression (regulatory T cells)

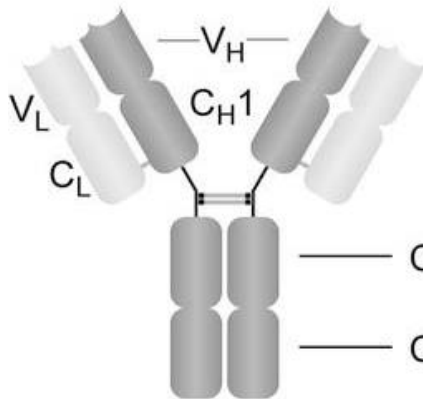
Y en el Futuro...

- Nanobodies: anticuerpos de camélidos
- Bajo: tamaño, costo de producción, inmunogenicidad
- Alta: estabilidad, solubilidad

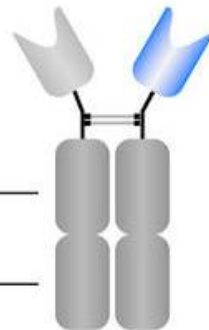


Llama (*Lama glama*)

Conventional
Antibody - 150 kDa



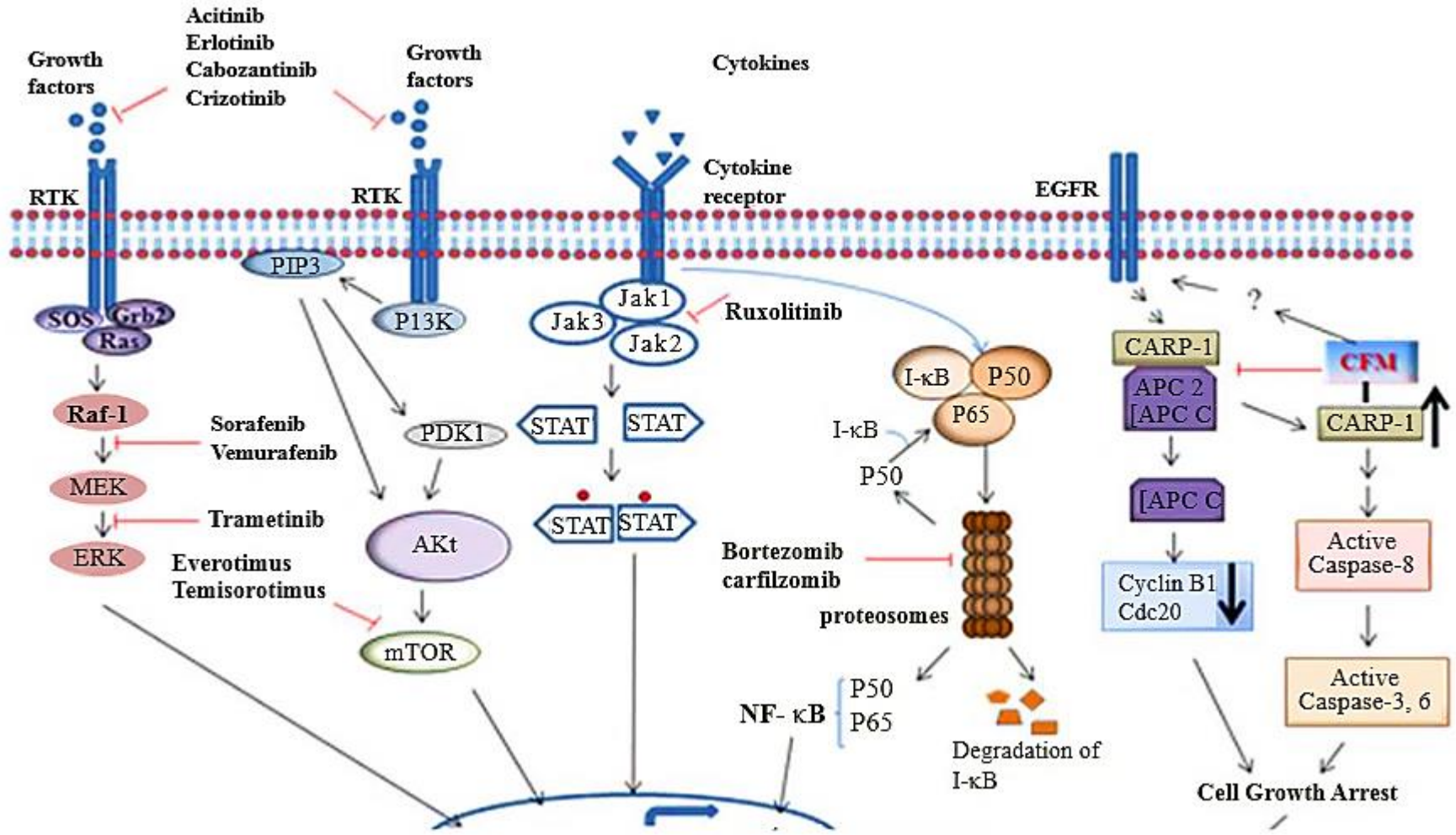
Camelidae
Antibody



V_H fragment



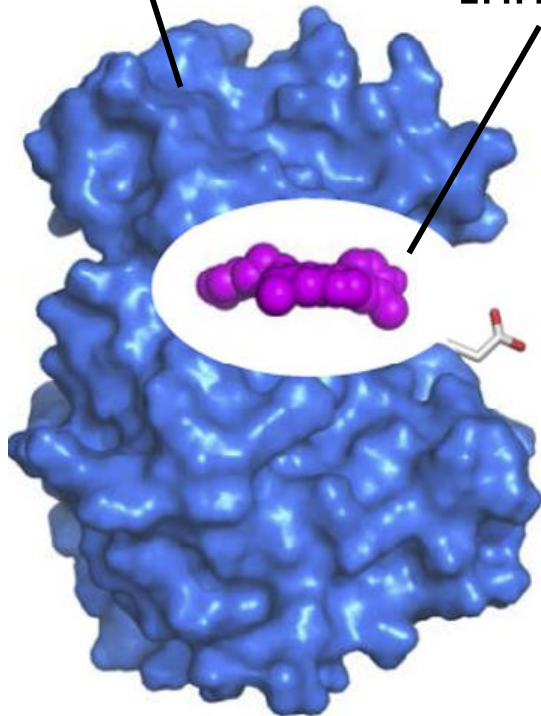
Moléculas Pequeñas (Inhibidores)



Ejemplo: Inhibidores de Quinasas

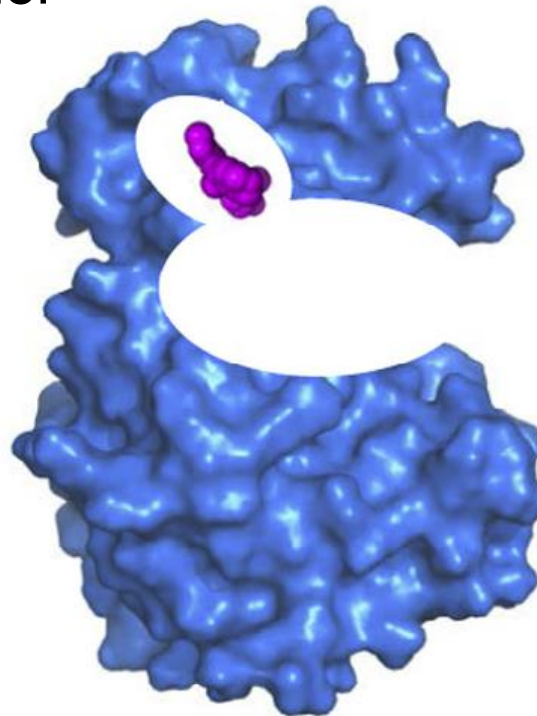
Quinasa

Inhibidor



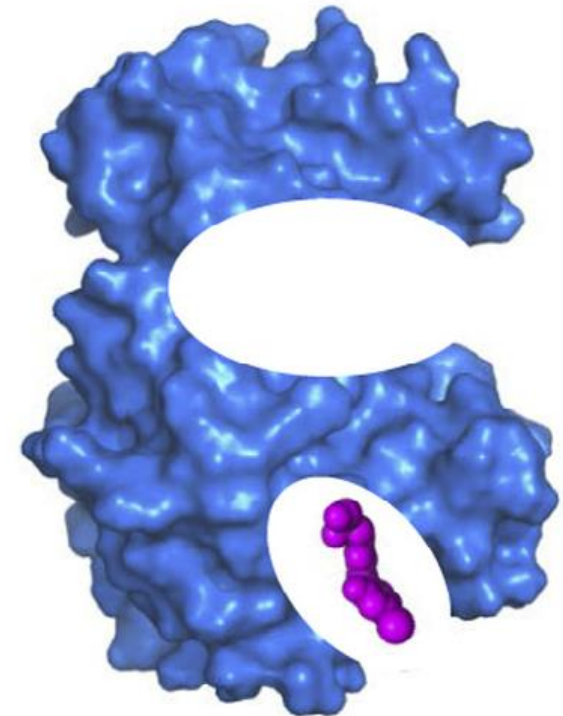
Interfiere con
unión de ATP

(Tipo II)



Unión cercana
a dominio ATP

(Tipo III)

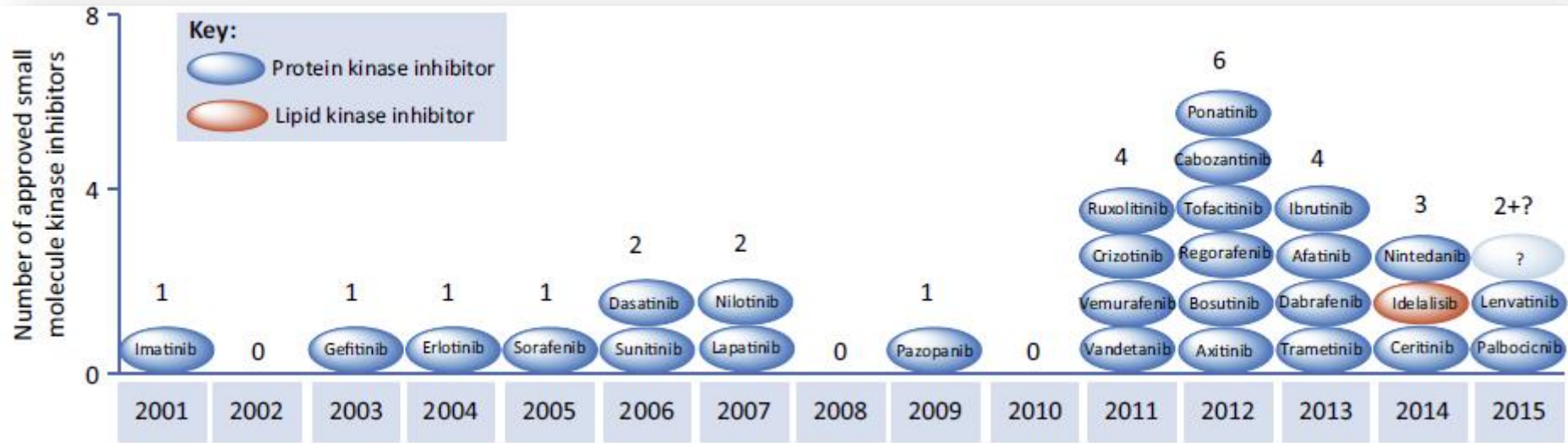


Unión en otros
dominios

(Tipo IV)

Inhibidores de Quinasas (FDA)

> 28 aprobados por FDA desde 2001-2015



TIPS 2015. <http://dx.doi.org/10.1016/j.tips.2015.04.005>

Otros blancos terapéuticos:

- Apoptosis (Bcl2, Bcl-xL)
- Proteosoma (20S, 26S)
- Proteínas «heat-shock» (Hsp90)
- Metaloproteinasas

Molecular Target	Small Molecule Drug	Specific Target	Cancer Targeted	Clinical Status
1. Tyrosine & serine/threonine Kinases	Imatinib (Gleevec)	Bcr-Abl	Philadelphia chromosome-positive chronic myelogenous leukemia	FDA approved in 2001
	Gefitinib (Iressa)	EGFR	Non small cell lung cancer	FDA approved in 2003
	Erlotinib (Tarceva)	EGFR	Non small cell lung cancer, pancreatic cancer	FDA approved in 2005
	Sunitinib (sutent)	VEGFR2,RET PDGFR, FLT-3, KIT, CSF-1	Renal cell carcinoma	FDA approved in 2006
	Lapatinib (Tykerb)	EGFR, HER2/neu	Breast cancer	FDA approved in 2007
	Nilotinib (Tasigna)	Bcr-Abl, KIT,LCK	Chronic myelogenous leukemia	FDA approved in 2007
	Sorafenib (Nexavar)	B-Raf, VEGFR2 EGFR,PDGFR	hepatocellular carcinoma	FDA approved in 2007
	Temsirolimus (CCI-779)	mTOR	Renal cell carcinoma	FDA approved in 2007
	Everolimus (afinitor)	mTOR	Renal cell carcinoma	FDA approved in 2009
	Pazopanib (Votrient)	c-KIT, FGFR, PDGFR and VEGFR	Renal cell carcinoma, soft tissue sarcoma	FDA approved in 2009
	Crizotinib (Xalkori)	HGFR	Non small cell Lung Cancer	FDA approved in 2011
	Ruxolitinib (jafaki)	Jak1,Jak2	Primary Myelofibrosis	FDA approved in 2011
	Vandetenib (Caprelsa)	VEGFR, EGFR,RET	Medullary thyroid cancer	FDA approved in 2011
	Axitinib (Inlyta)	VEGFR1-3, cKIT, PDGFR	Renal cell carcinoma	FDA approved in 2012
	Bosutinib (Bosulif)	Src, Bcr-Abl	Philadelphia chromosome-positive chronic myelogenous leukemia	FDA approved in 2012
Cabozantinib (Cometriq)	c.Met, VEGFR2	Medullary thyroid cancer	FDA approved in 2012	
Ponatinib (Iclusig)	Bcr-Abl	Chronic myeloid leukemia, acute lymphoblastic leukemia	FDA approved in 2012	

Terapia Génica

Cancers 2011. doi:[10.3390/cancers3010368](https://doi.org/10.3390/cancers3010368)

DELIVERY VECTOR

VIRAL

- Ad
- Rv/Lv
- AAV
- HSV
- VACV
- SV40

NON VIRAL

- Naked DNA
- Liposomes
- Polymers

CELLULAR

DELIVERY ROUTE

INTRAVENOUS

INTRATUMORAL

INTRAPERITONEAL

KEY ELEMENTS IN CANCER GENE THERAPY

TUMOR TARGETING

VIRAL GENE MUTATION

TRANSCRIPTIONAL

TRANSDUCTIONAL

- Genetic modifications
- Pseudotyping / Chimerism
- Molecular conjugates

THERAPEUTIC SYSTEMS

VIROTHERAPY

SUICIDE GENE THERAPY

CORRECTION

- Mutated genes
- Altered miRNAs

GENES

- Apoptotic
- Immunomodulatory

Terapias Inmunomoduladoras

Inmunosupresión y Cáncer

- Evidencia la importancia del sistema inmune en la prevención del cáncer

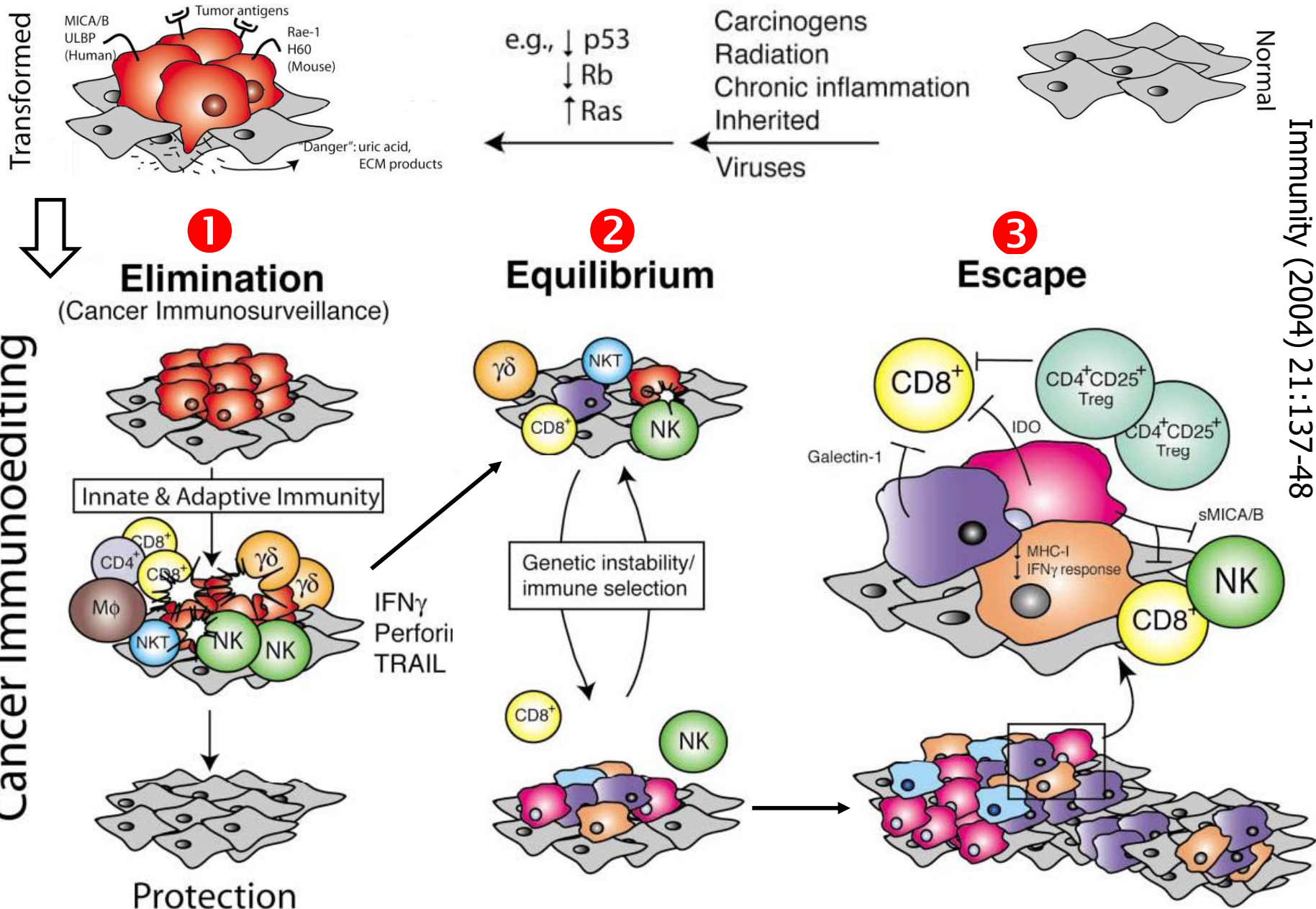


Sarcoma de Kaposi's en pacientes con SIDA



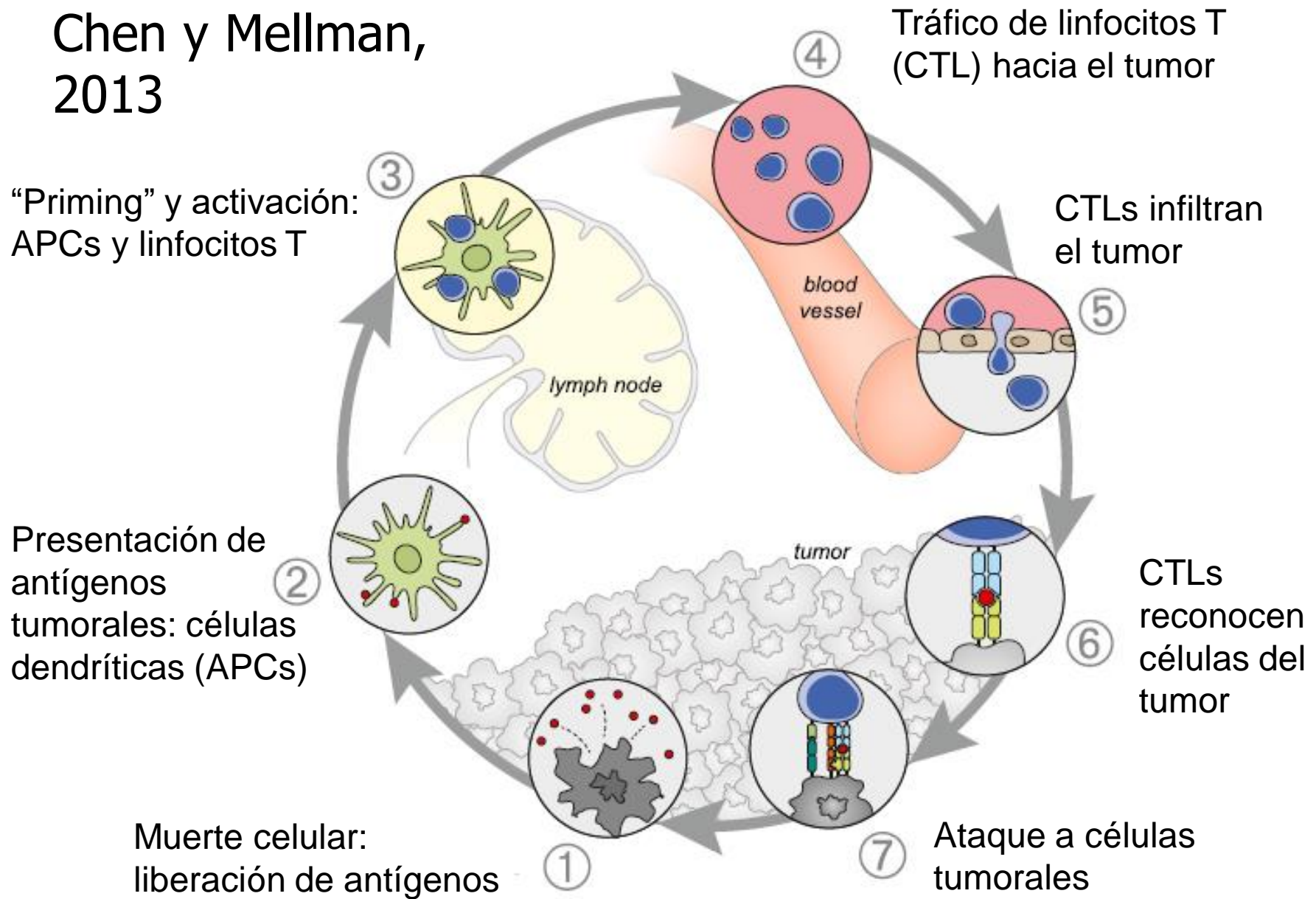
Cáncer de piel en pacientes trasplantados

Inmuno-Edición Tumoral



"Cancer Immunity Cycle"

Chen y Mellman,
2013



Ciclo Regulado

Check-Point
immune

CD28/B7.1
CD137/CD137L
OX40/OX40L
CD27/CD70
HVEM
GITR
IL-2
IL-12

CTLA4/B7.1
PD-L1/PD-1
PD-L1/B7.1
prostaglandins

TNF- α
IL-1
IFN- α
CD40L/CD40
CDN
ATP
HMGB1
TLR

IL-10
IL-4
IL-13

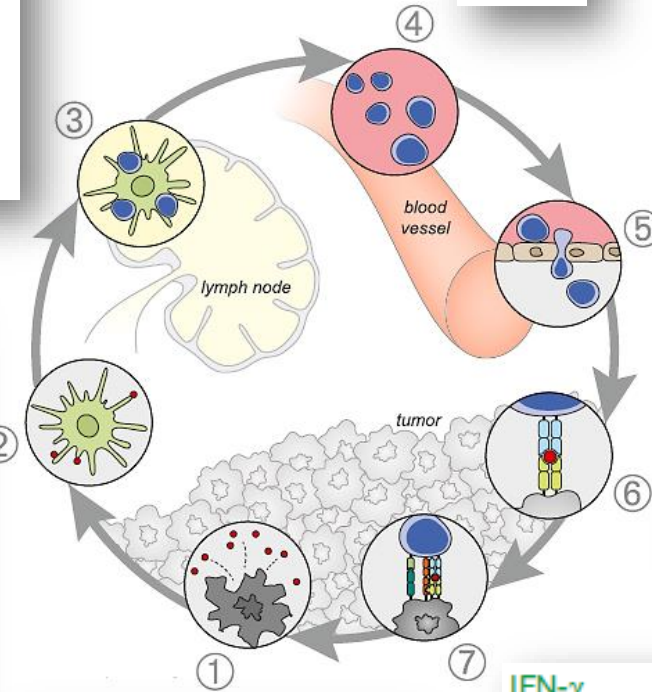
CX3CL1
CXCL9
CXCL10
CCL5

LFA1/ICAM1
Selectins
VEGF
Endothelin B receptor

T cell receptor
Reduced pMHC on cancer cells

IFN- γ
T cell granule content

PD-L1/PD-1
PD-L1/B7.1
IDO
TGF- β
BTLA
VISTA
LAG-3
Arginase
MICA/MICB
B7-H4
TIM-3/phospholipids

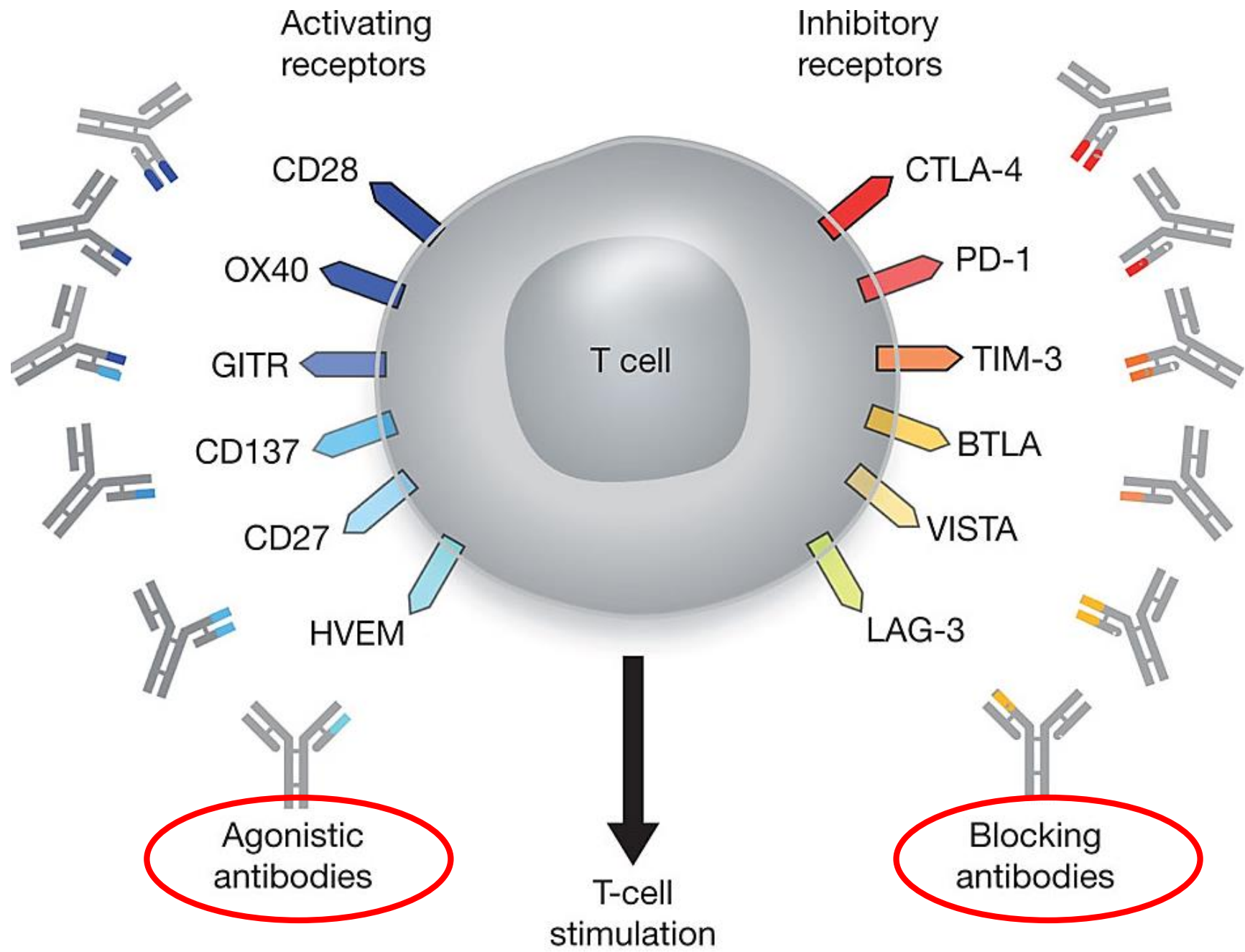


Immunogenic cell death
Tolerogenic cell death

Reostato
immune

■ Stimulatory factors

■ Inhibitors



Inhibidores de Check-Points Inmunes

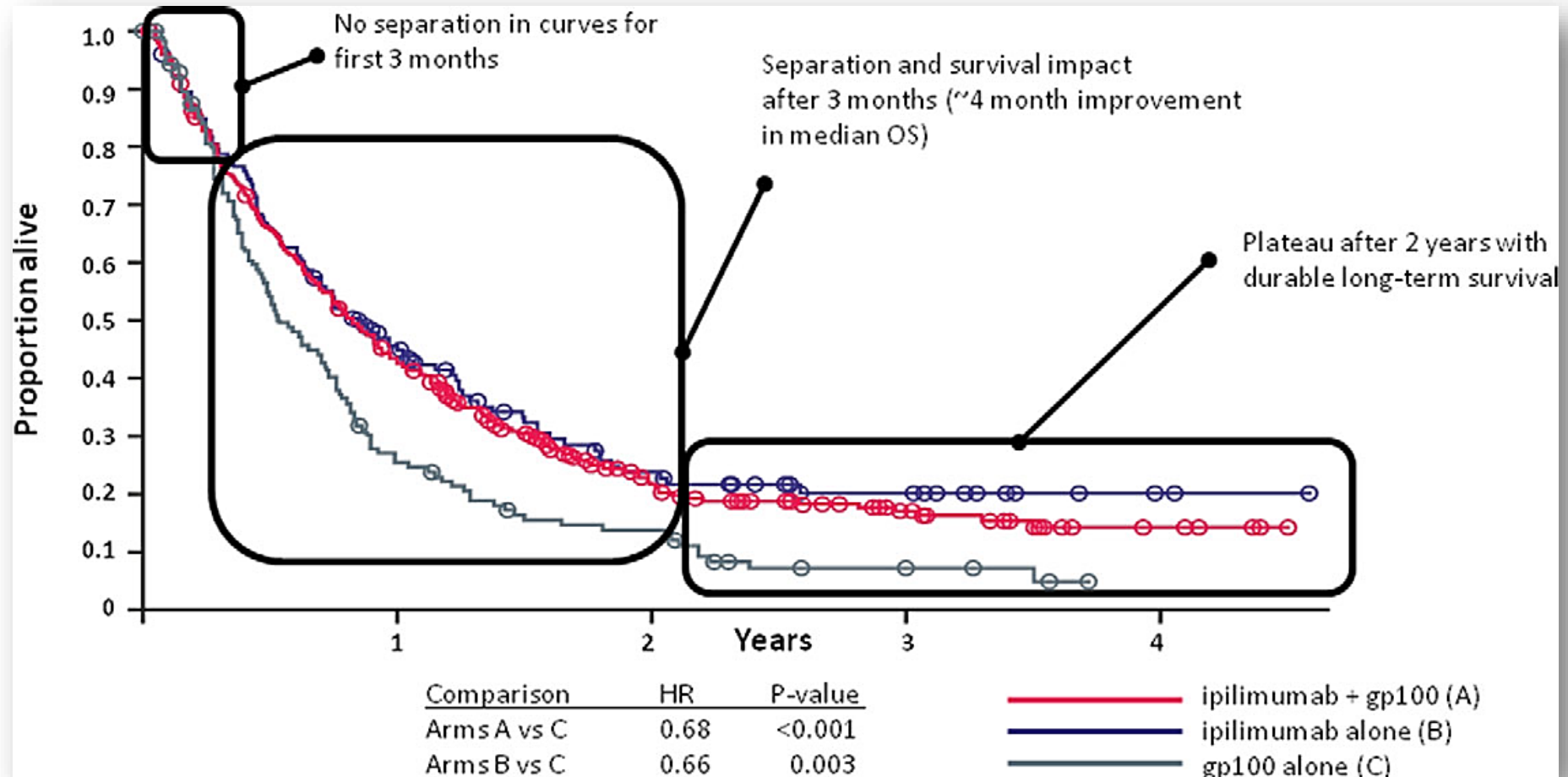
Aprobados por FDA:

Targets	Checkpoint Inhibitors	Approved Indications
CTLA-4	Ipilimumab	Metastatic melanoma
PD-1	Nivolumab	Metastatic melanoma Recurrent NSCLC Recurrent Hodgkin lymphoma Recurrent renal cell carcinoma
	Pembrolizumab	Metastatic melanoma PD-L1–positive NSCLC Head & neck squamous cell cancer
CTLA-4 + PD-1	Ipilimumab + nivolumab	Metastatic melanoma
PD-L1	Atezolizumab	Recurrent urothelial cancer

CTLA-4 = cytotoxic T lymphocyte–associated antigen 4; NSCLC = non–small-cell lung cancer;
PD-1 = programmed death 1; PD-L1 = programmed death ligand 1.

Ipilimumab: Melanoma

- Estudio Fase III MDX010-20, melanoma avanzado



Citoquinas

- Proteínas producidas por linfocitos
- Regulación de hematopoiesis, respuesta inmune, inflamación

Interferones (INF- α) } Tratamiento
Interleucinas (IL2) }

Factores crecimiento hematopoiéticos:
contrarrestar algunos efectos adversos de
quimioterapia

→ Eritropoietina, GM-CSF, G-CSF, IL-11

Citoquinas

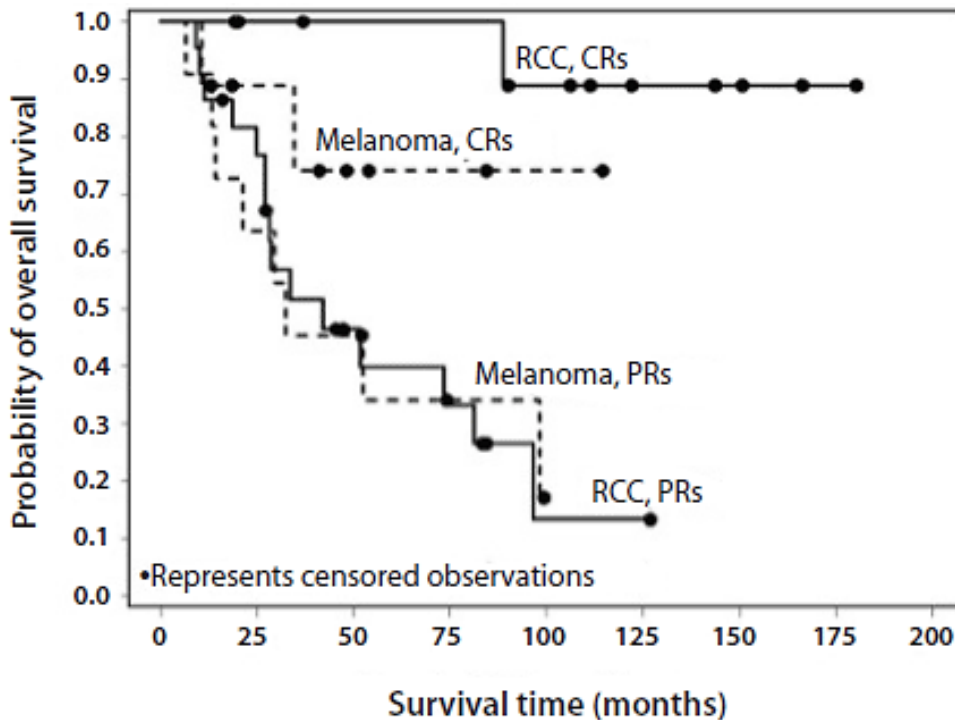
Cytokine	Primary Cell Source	Primary Target Cell	Biological Activity
GM-CSF	T cells Macrophages Endothelial cells Fibroblasts Mast cells	Bone marrow progenitor cells DC Macrophages NKT cells	Promotes antigen presentation T cell homeostasis Hematopoietic cell growth factor
IL-2	T cells NK cells	T cells NK cells B cells Monocytes	Cell growth/ activation
IFN- α	Plasmacytoid DC NK cells T cells B cells Macrophages Fibroblasts Endothelial cells Osteoblasts	Macrophages NK cells	Anti-viral Enhances MHC expression

Aprobadas FDA

Cytokines

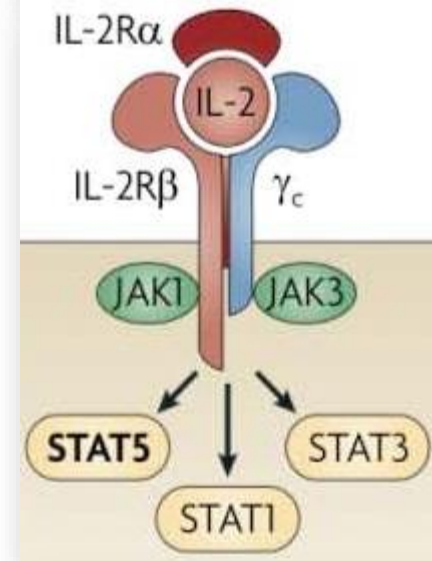
MOA	Agent	Year	Indication ^c
Cytokine	IL-2	1992	RCC
		1998	Melanoma
Cytokine	IFN- α	1986	HCL
		1988	AIDS-related Kaposi's Sarcoma Melanoma
		1995	Melanoma
		1997	NHL

Clin Transl Sci 2016. doi:10.1111/cts.12391



Cytokine produced by:

IL-2: T cells
and DCs



Receptor expressed by:

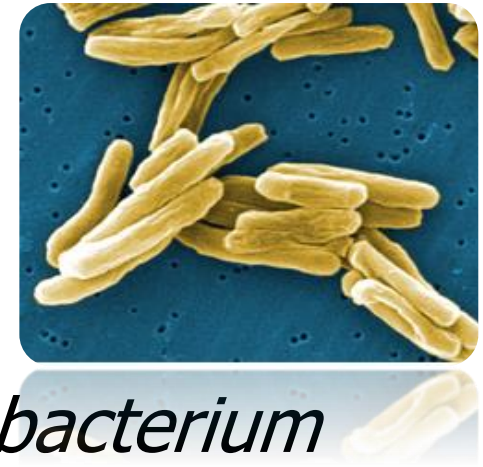
T cells, B cells
and NK cells

IL2 en alta dosis:

Melanoma avanzado (n=117)

Carcinoma renal (n=104)

Otros Tratamientos



Bacilo Calmette-Guérin (BCG)

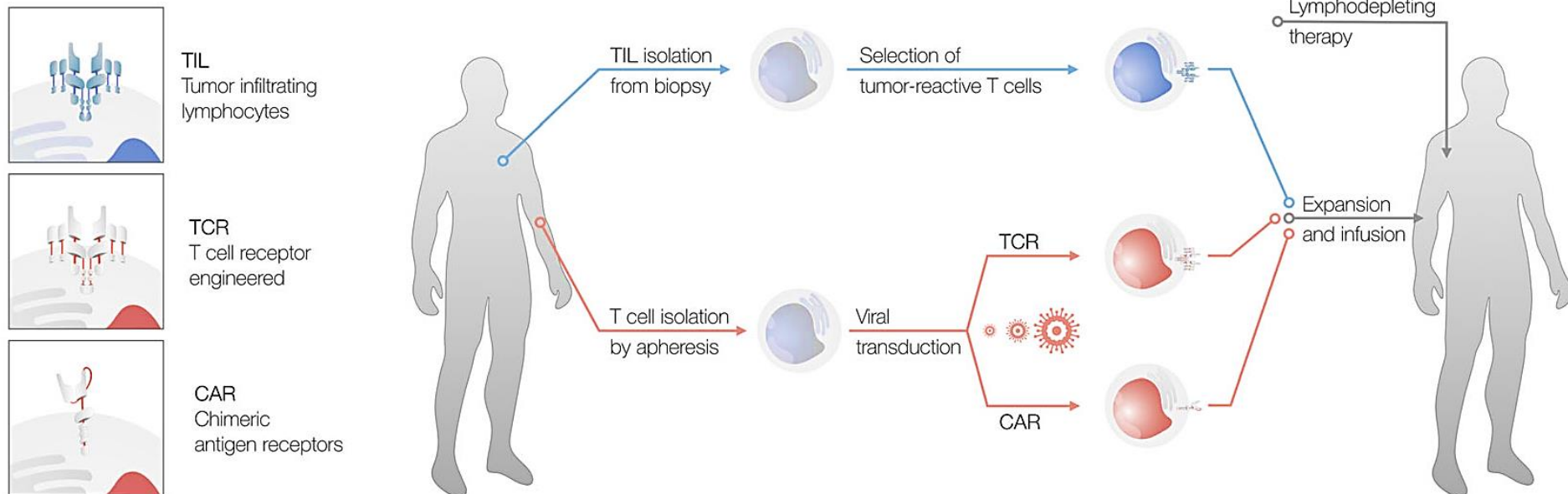
- Vacuna contra tuberculosis (*Mycobacterium bovis*)
- En cáncer de vejiga: infección con lisis celular, liberación de neoantígenos, respuesta inmune antitumoral

Vistos en clases anteriores:

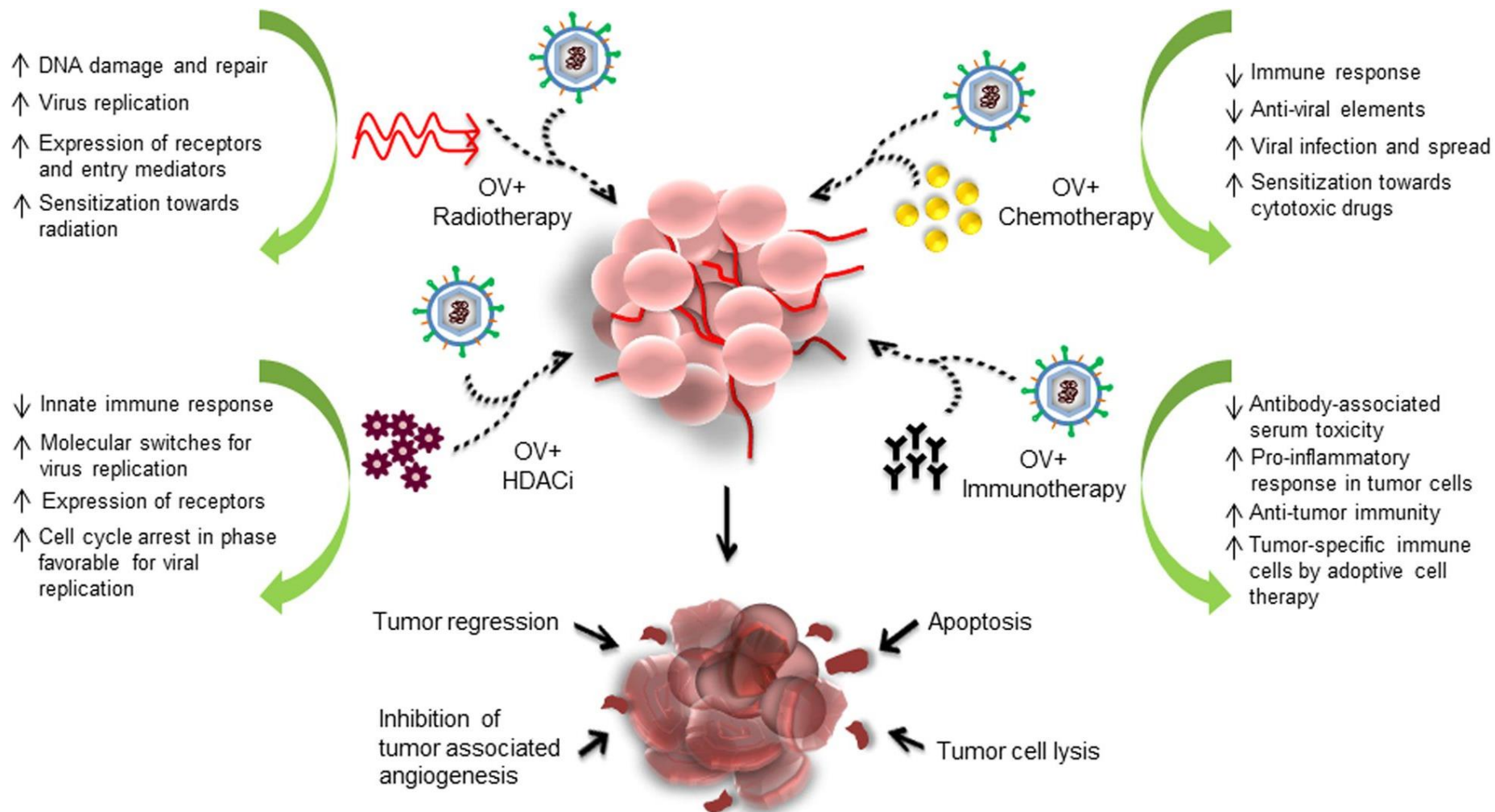
- Vacunas (inmunomodulación, respuesta inmune)

Terapia Adoptiva Células T

Adoptive T cell therapy modalities



Virus Oncolíticos



Aprobado FDA: talimogen laherparepvec (Imlygic®), o T-VEC, para melanoma.

Referencias de Interés

- Immunotherapy and Novel Combinations in Oncology: Current Landscape, Challenges, and Opportunities. Morrissey 2106. Clin Transl Sci (2016) 9, 89–104; doi:10.1111/cts.12391
- Immunoterapias (NIH):
<https://www.cancer.gov/about-cancer/treatment/types/immunotherapy/biotherapies-fact-sheet>
- Virus oncolíticos (NIH):
<https://www.cancer.gov/news-events/cancer-currents-blog/2018/oncolytic-viruses-to-treat-cancer>