DISEÑO DE NANOVEHÍCULOS MULTIFUNCIONALES: DESAFIANDO LAS BARRERAS BIOLÓGICAS

3-05-2018

Lucia Policastro

CONICET

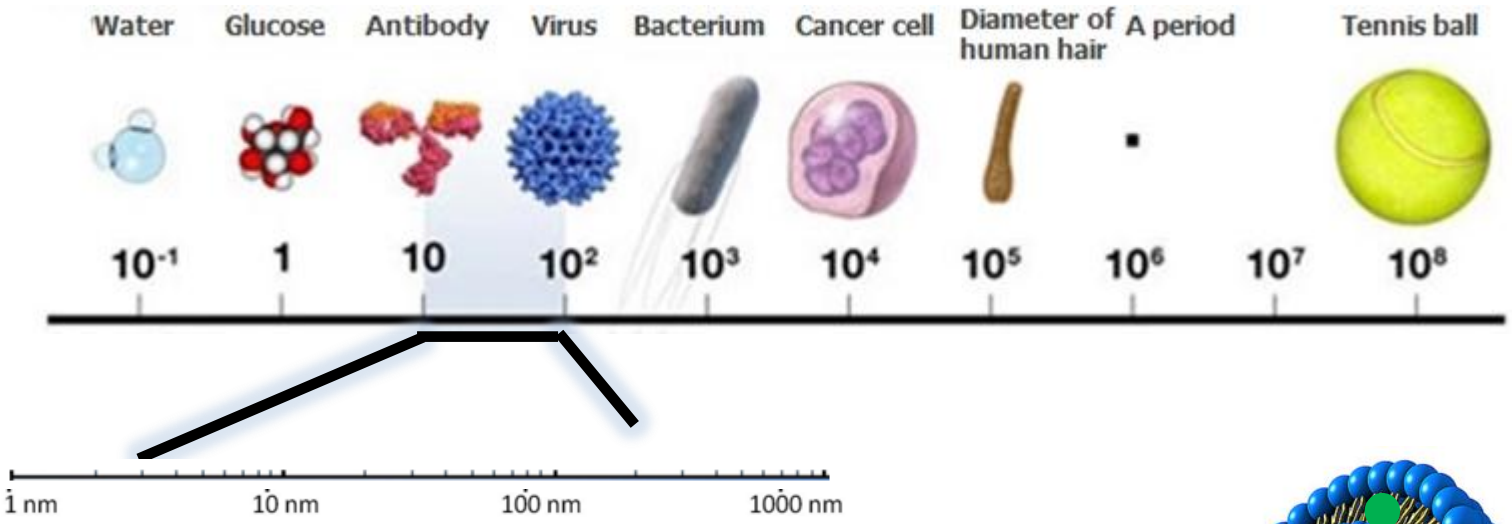
INN CNEA-CONICET
Instituto de Nanociencia y Nanotecnología

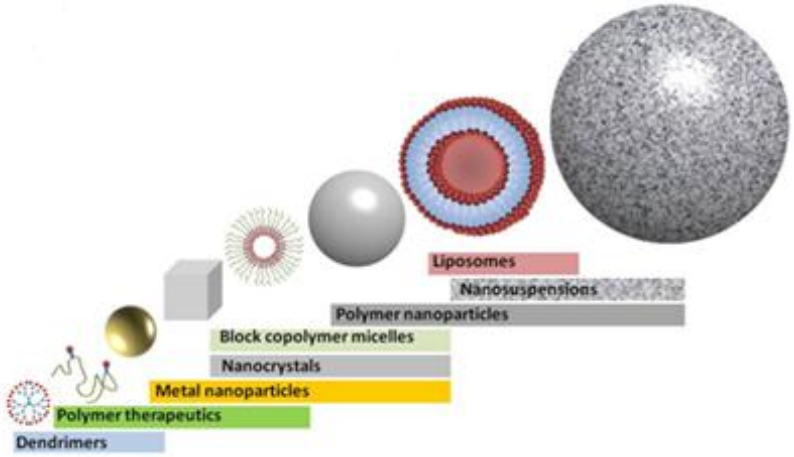
CNEA

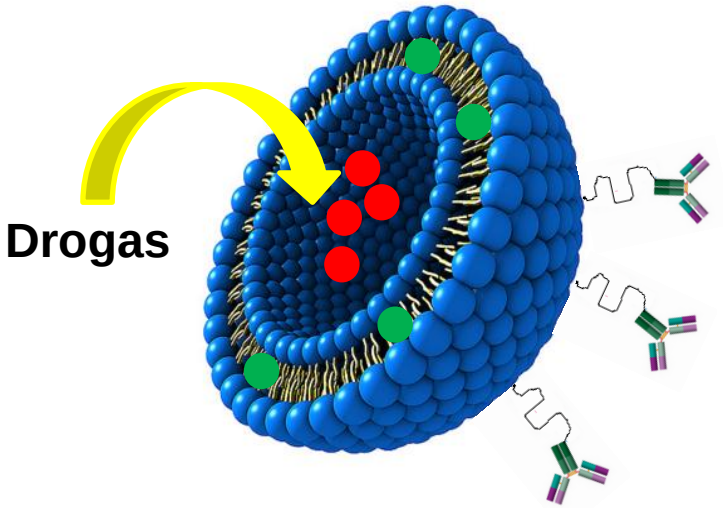
AGENCIA

UNSAM

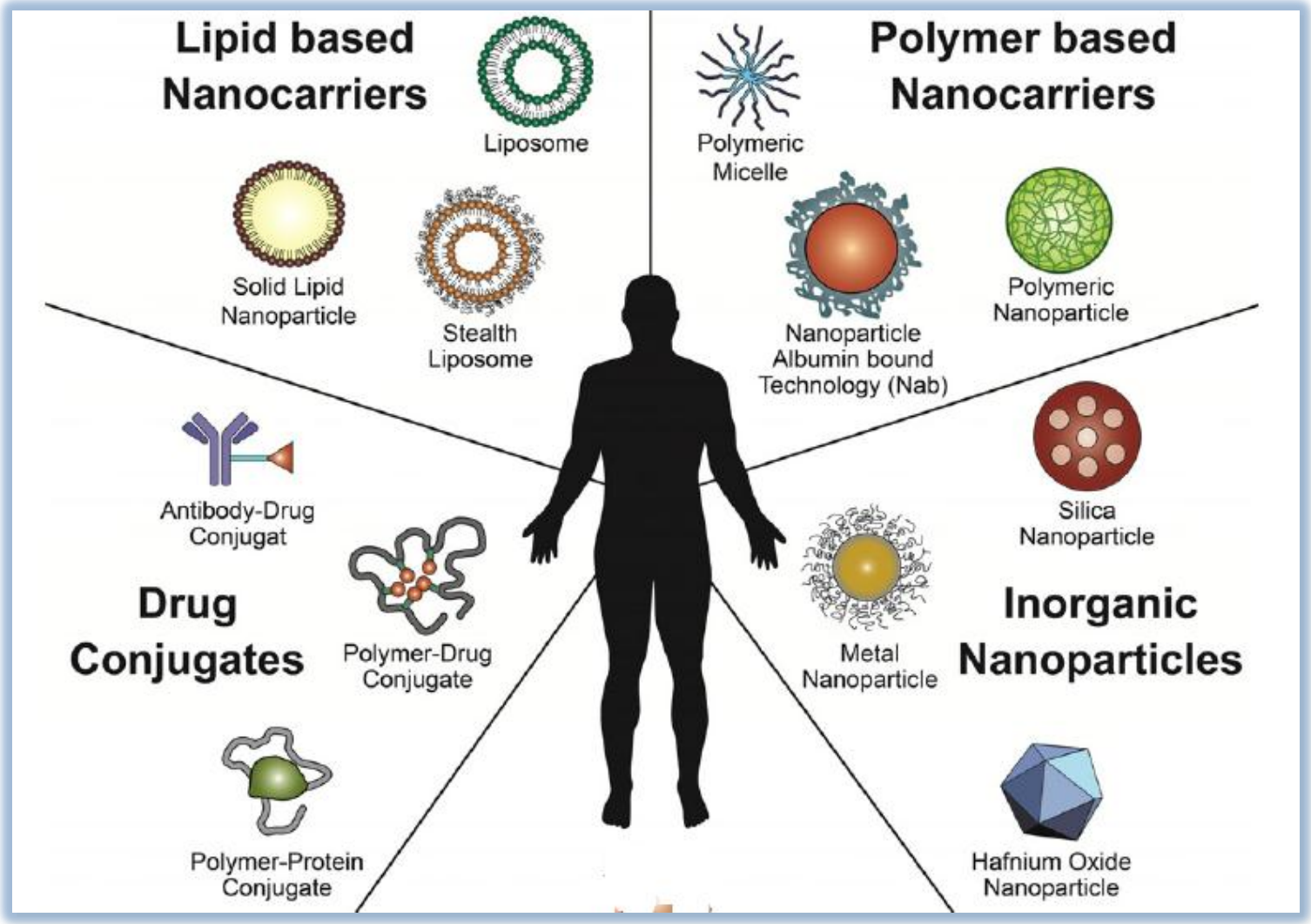
Que es una Nanomedicina?



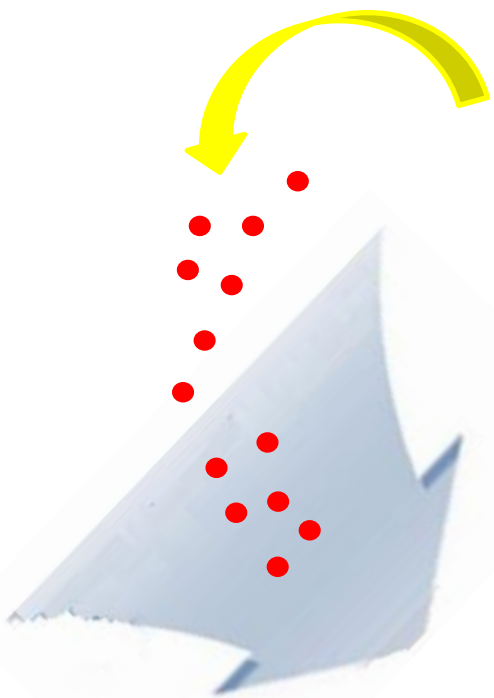




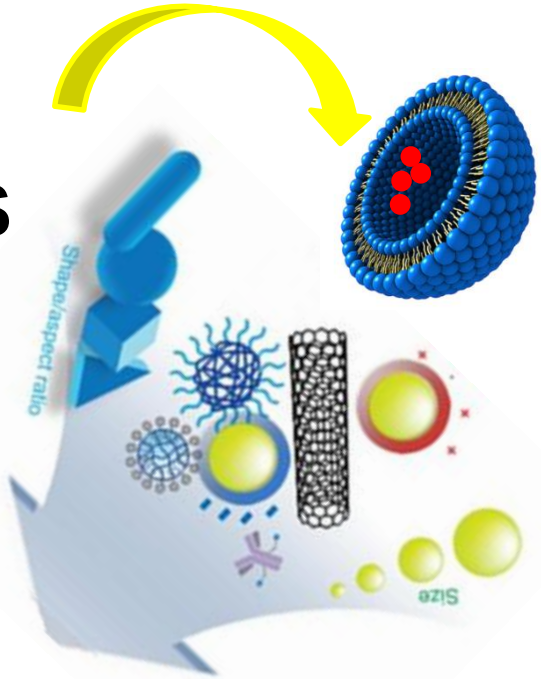
Plataformas más utilizadas para la generación de Nanomedicinas

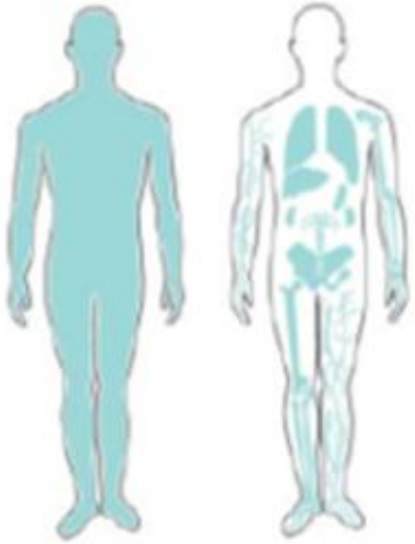


Nanomedicinas



DROGAS

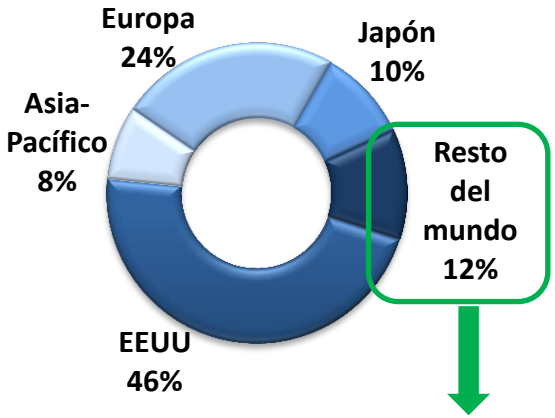




- **Mayor tiempo de circulación**
- **Menor toxicidad**
- **Mayor acumulación en los Tejidos blancos**

Nanomedicinas actuales y proyecciones

Number of approved and investigational nanomedicinal products.	
Products	**Number**
Approved products:	
EMA	43
EU	47
FDA	71
EMA and FDA	40
Investigational products:	
Phase I	29
Phase II	47
Phase III	25
Total of investigational products	101



América del Sur <2%

x2 en 5 años!

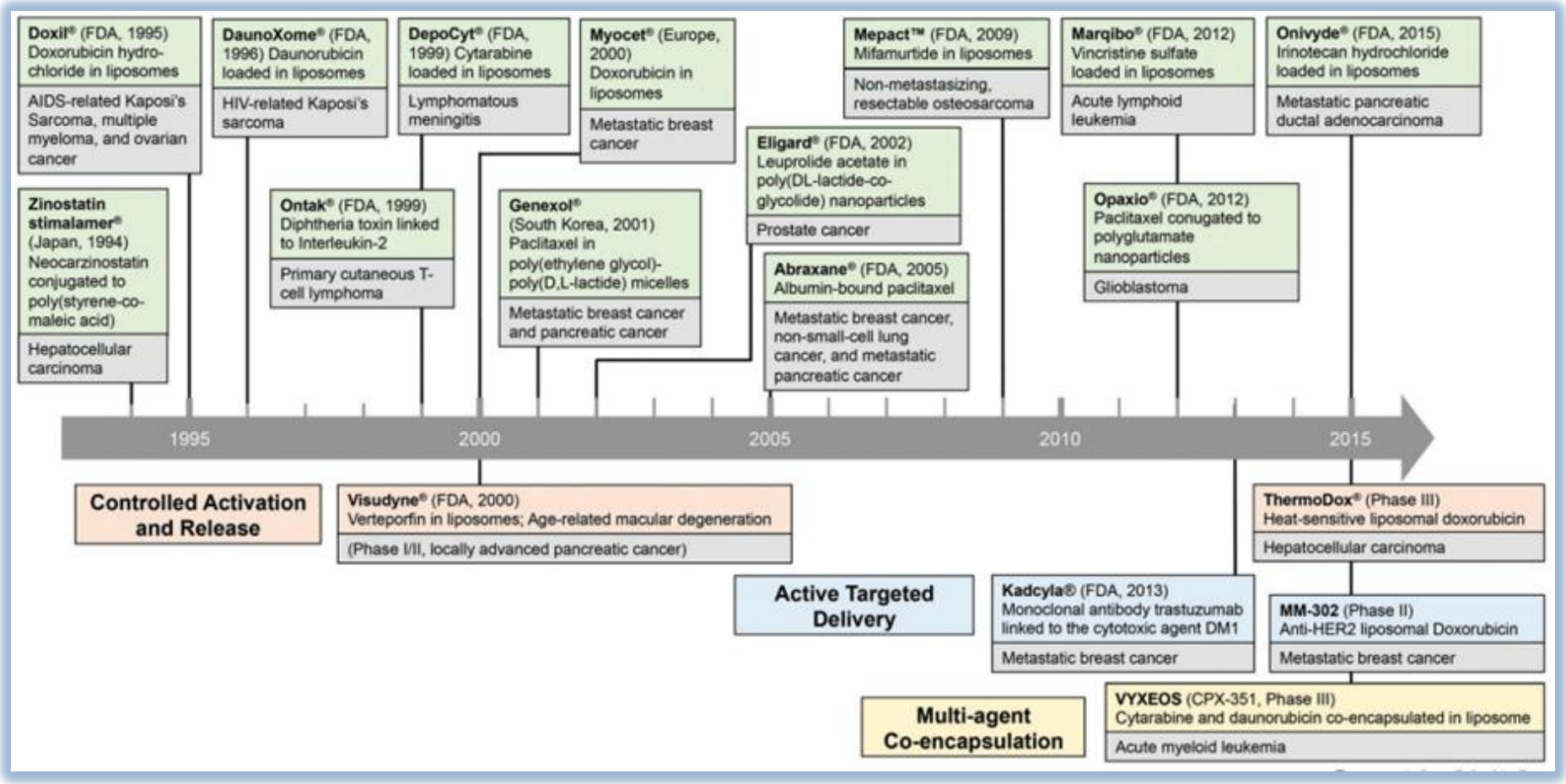
2020

USD 180 mil millones

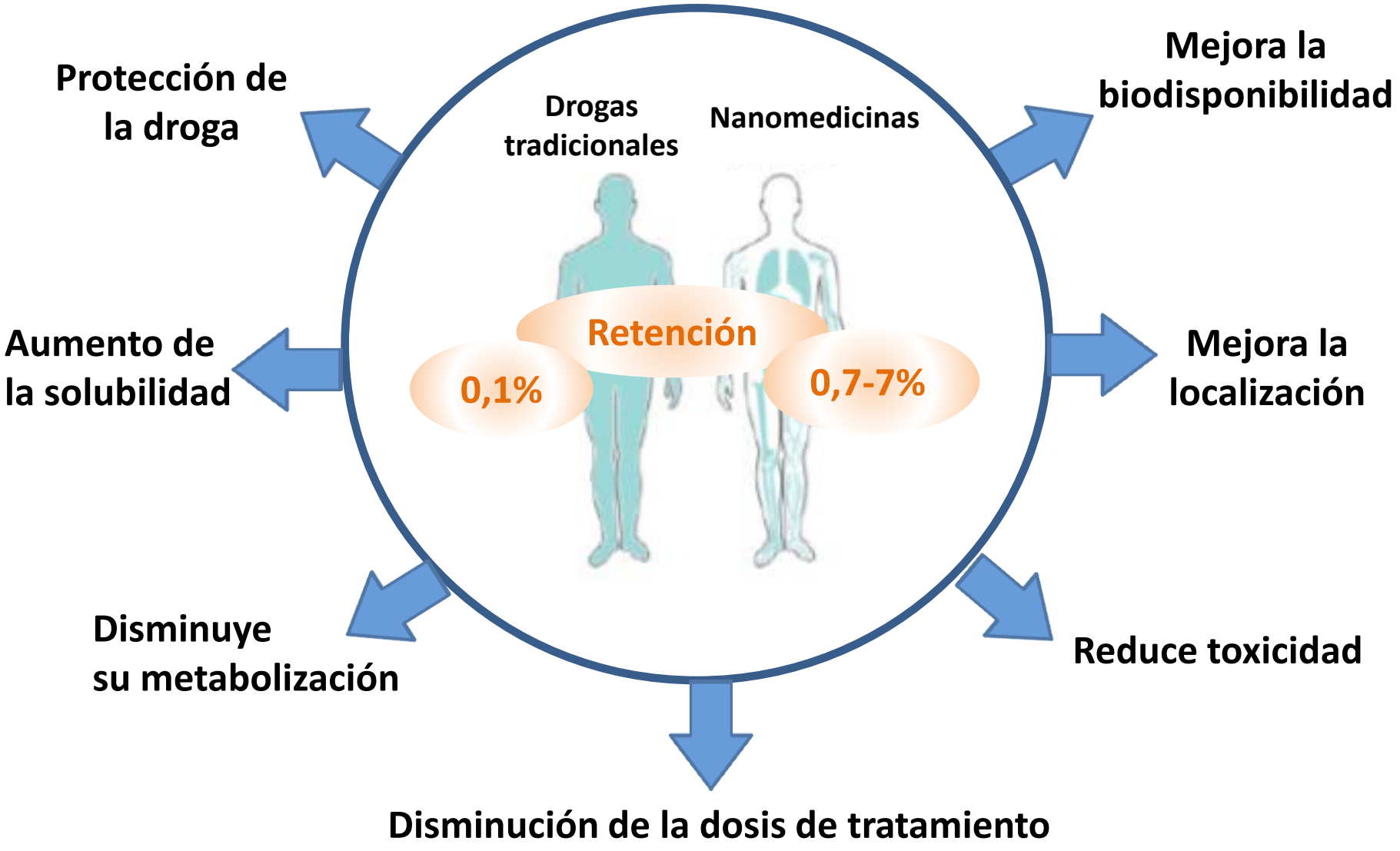
40% del mercado de la Ind. Farm.

Nanomedicine.10(10):1599-608, 2015

Nanomedicinas:aprobadas y en investigación clínica

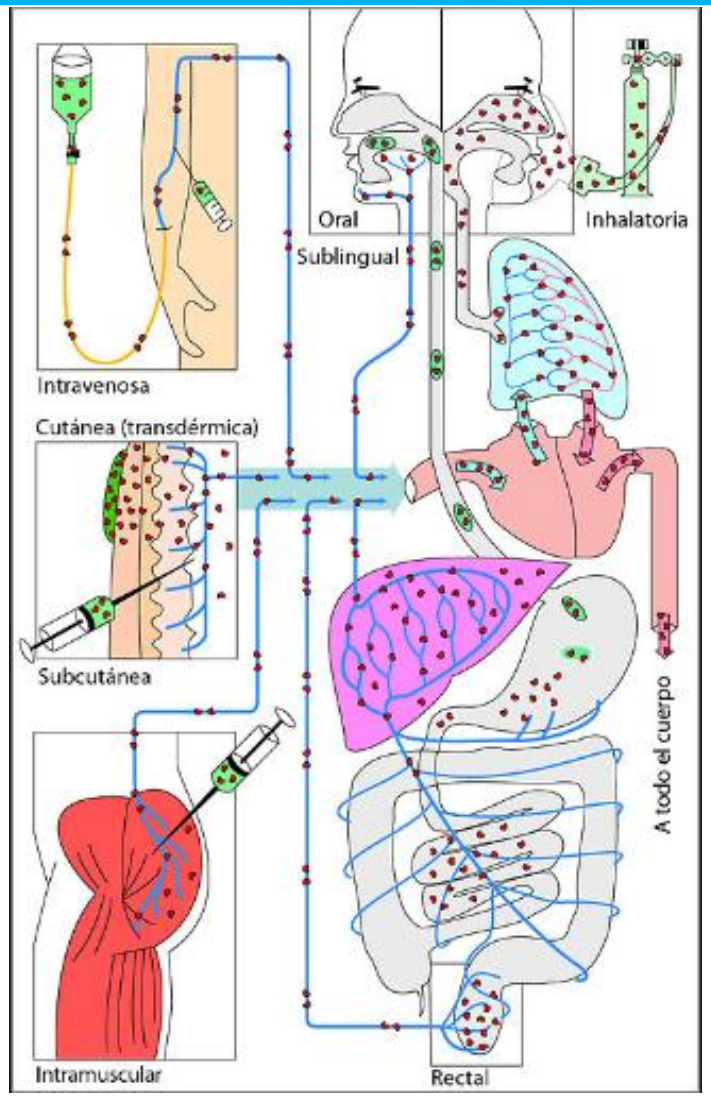


Nanomedicinas



Vías de administración de un Nanovehículo

- **Vía Enteral**



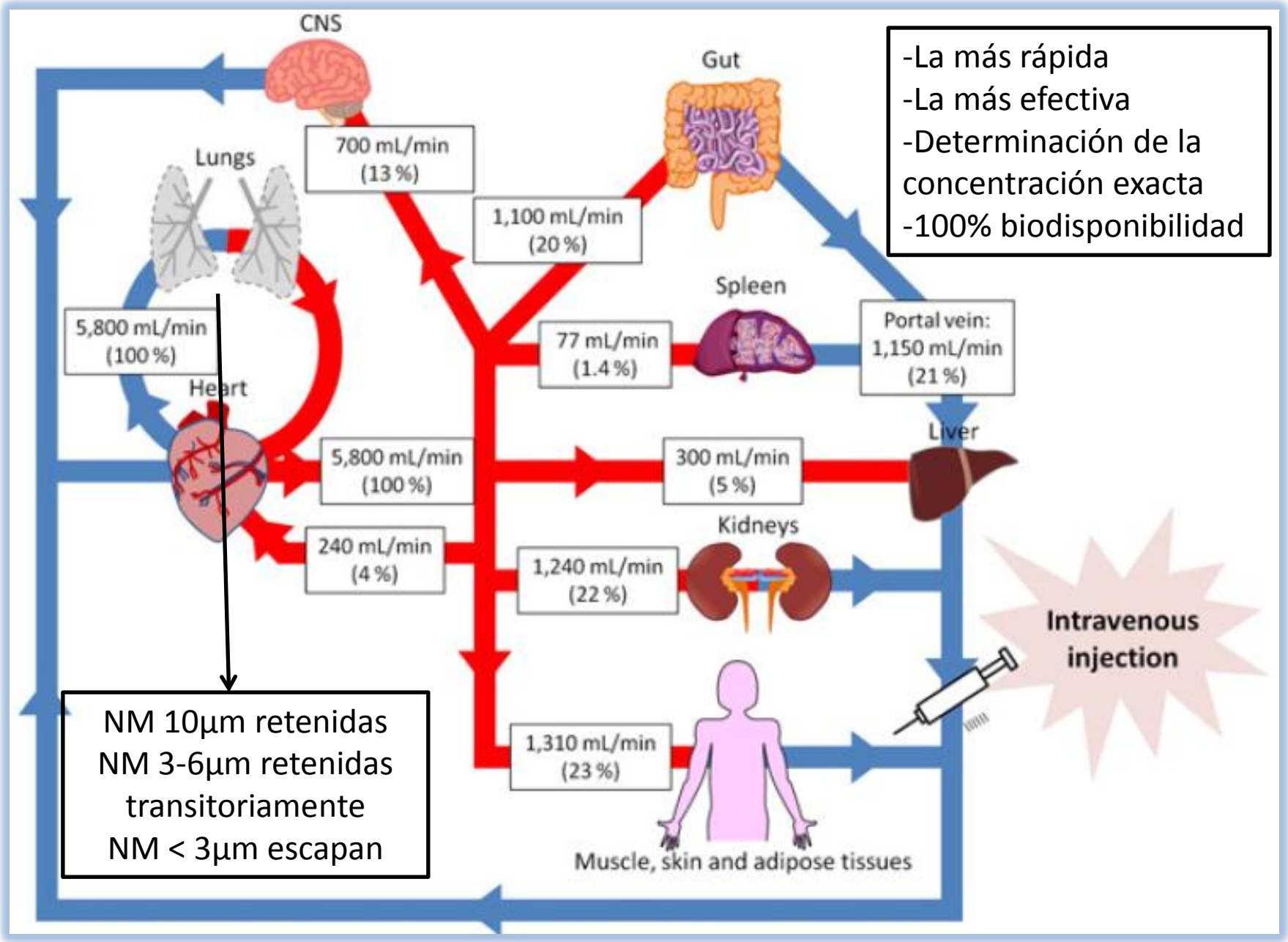
Vía Parenteral

Subcutanea
Intradérmica
Intramuscular
Intraperitoneal
Intracardíaca
Intratecal
Intraósea

Intravenosa

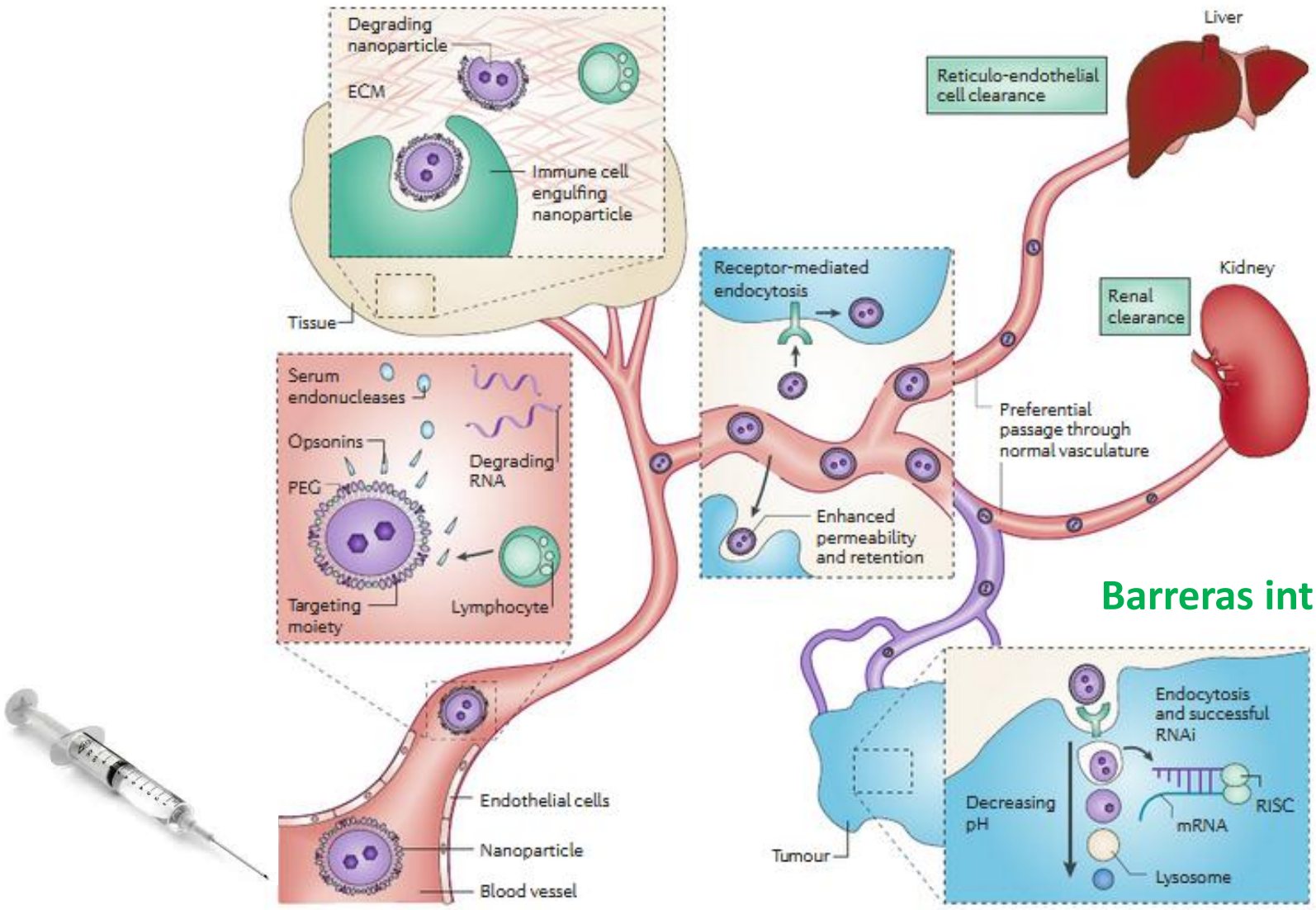
-La más rápida
-La más efectiva
-Determinación de la concentración exacta
-100% biodisponibilidad

- **Vía Tópica**
- **Vía Aerea**


CNS
Gut
Lungs
700 mL/min
(13 %)
1,100 mL/min
(20 %)
Spleen
5,800 mL/min
(100 %)
77 mL/min
(1.4 %)
Portal vein:
1,150 mL/min
(21 %)
Heart
5,800 mL/min
(100 %)
300 mL/min
(5 %)
Liver
Kidneys
240 mL/min
(4 %)
1,240 mL/min
(22 %)
Intravenous
injection
1,310 mL/min
(23 %)
Muscle, skin and adipose tissues
-La más rápida
-La más efectiva
-Determinación de la concentración exacta
-100% biodisponibilidad
NM 10µm retenidas
NM 3-6µm retenidas transitoriamente
NM < 3µm escapan

BARRERAS BIOLÓGICAS

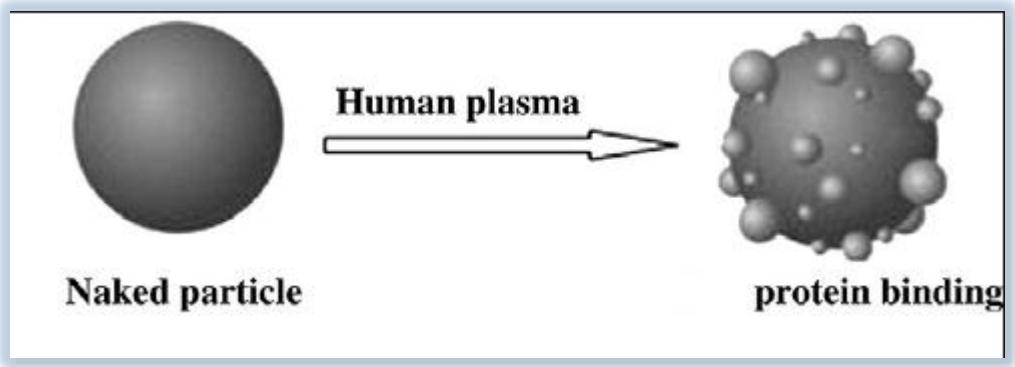
Barreras extracelulares



Barreras intracelulares

Opsonización

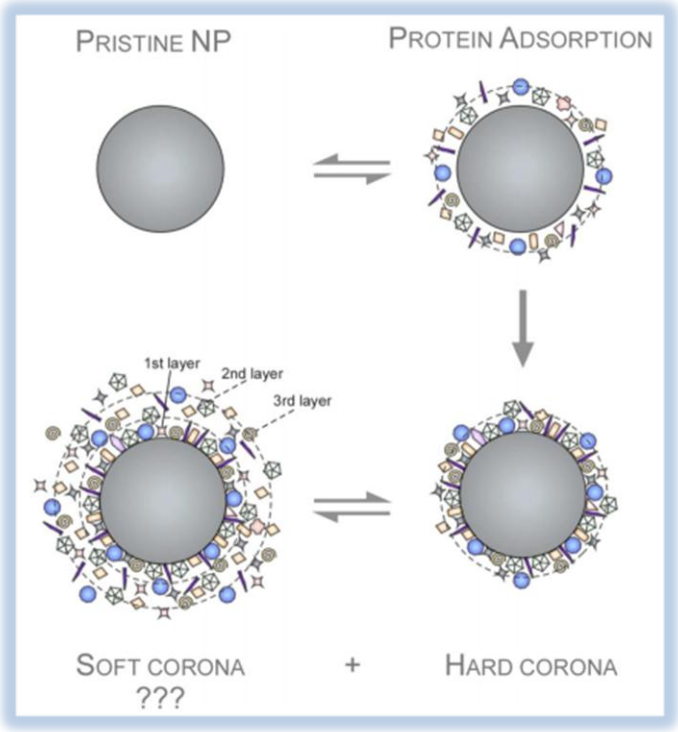
Adsorción de proteínas (opsoninas) a la superficie del NP



Sup. hidrofóbicas > hidrofílica
Sup. cargadas > neutras

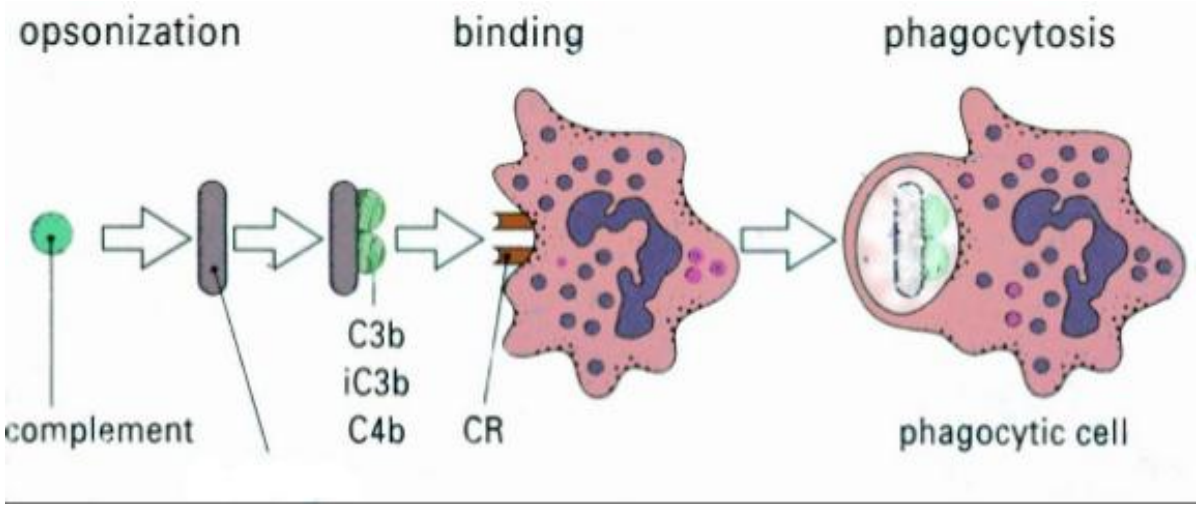
La absorción tiene que ver con la abundancia y afinidad de las proteínas

Albúmina : 55% proteina plasma. Uniones débiles
Efecto VROMAN
Inmunoglobulinas (IgG e IgM)
Lipoproteínas y Apoliproteinas
Fibronectina
Proteínas del complemento (C3,C4,C5)



Opsonización: respuesta inmunológica

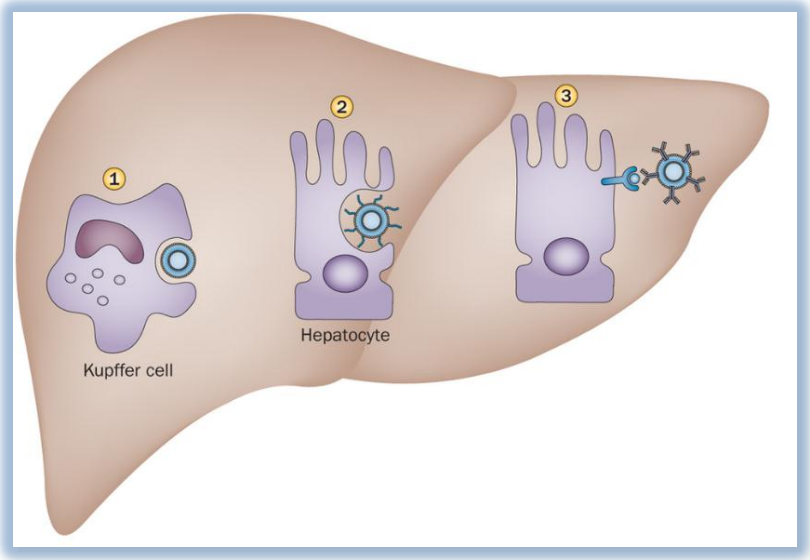
Los NV opsonizados son reconocidos por el **Sistema Fagocítico Mononuclear (MPS)**: macrófagos circulantes, en hígado (células de Kupffer), bazo y médula ósea. Reconocimientos principalmente

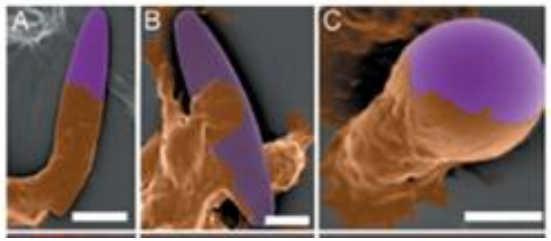


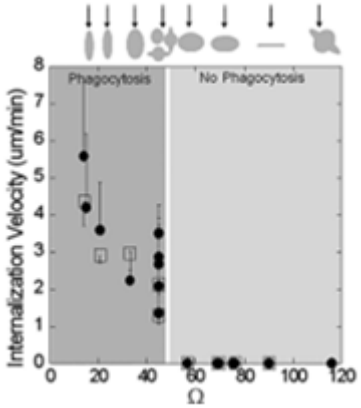
Paso hepático: eliminación del 30-99% NM

80-90% de los macrófagos del cuerpo están en el hígado (células Kupffer)

30-99% de las Nanopartículas son eliminadas por las células de Kupffer



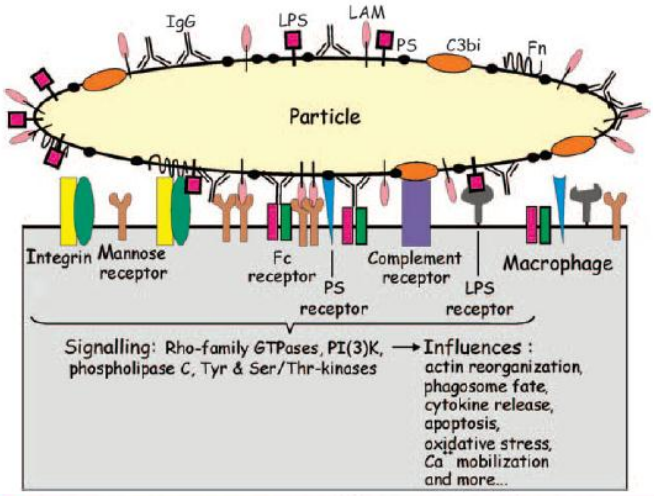


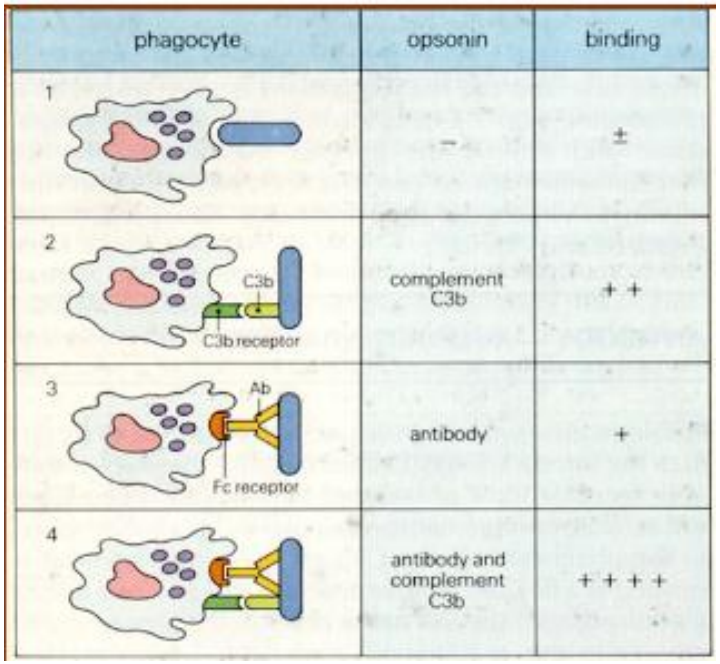


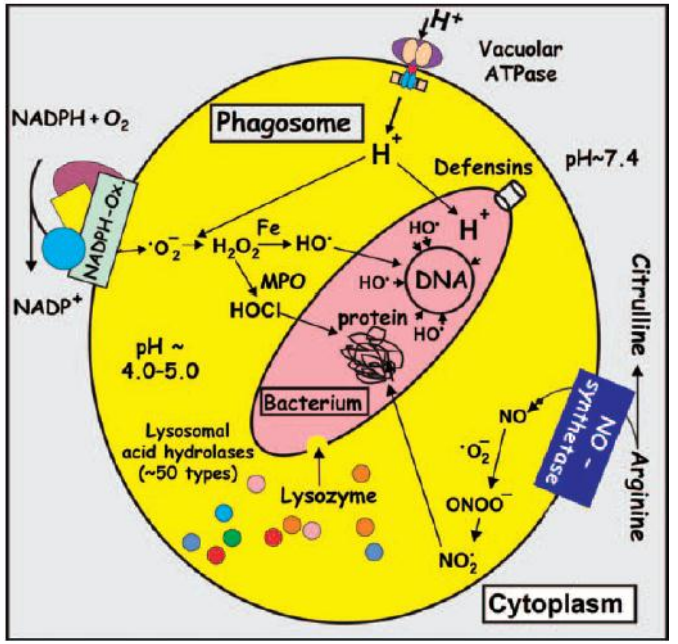
La eliminación está relacionada con el tamaño de la partícula, carga superficial y con ligandos adheridos a la superficie

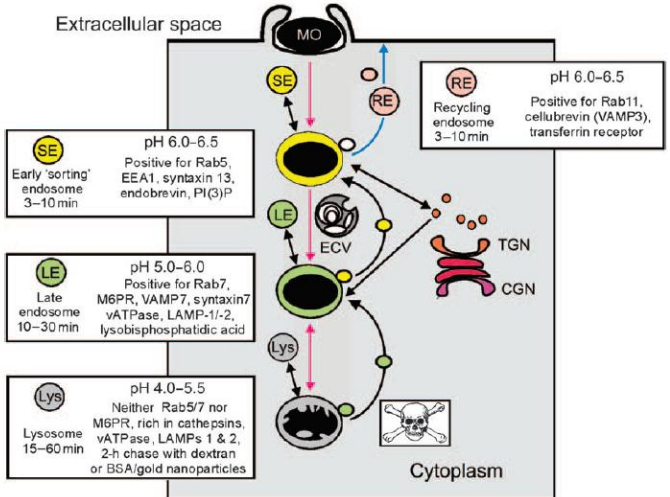
NP no biodegradable: acumulación y toxicidad en MPS

Fagocitosis en macrófagos



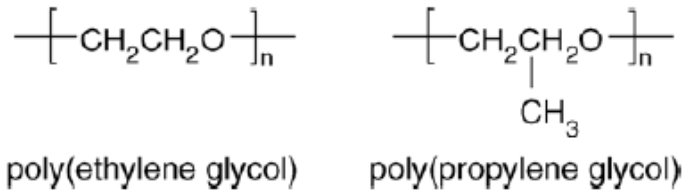




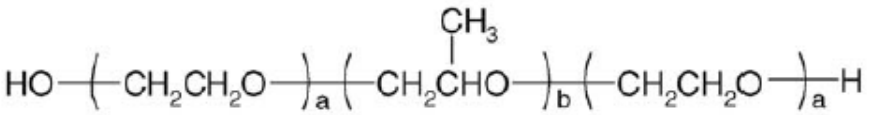


Estabilización estérica e invisibilidad inmunológica

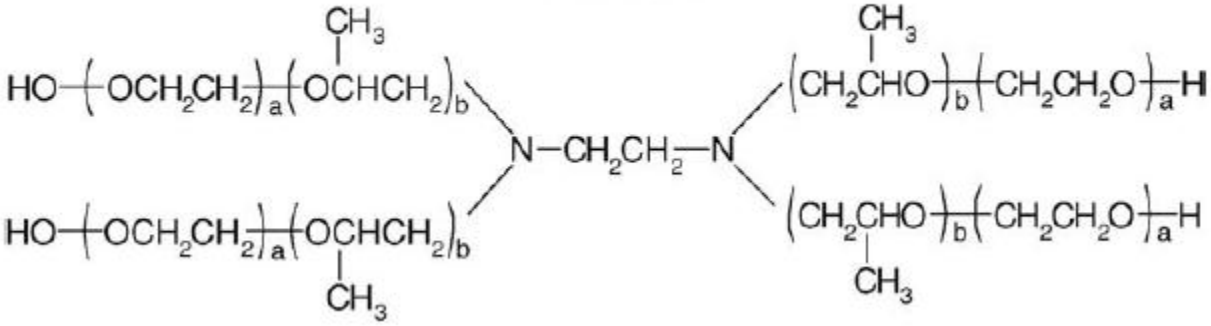
- **Polímeros para aumentar la hidrofilicidad de la superficie del NV**
- **Polietylenglicol o polipropylen glycol**
- **Decrecen la interacción con proteínas, repulsión estérica**
- **Optimo largo, configuración y densidad**



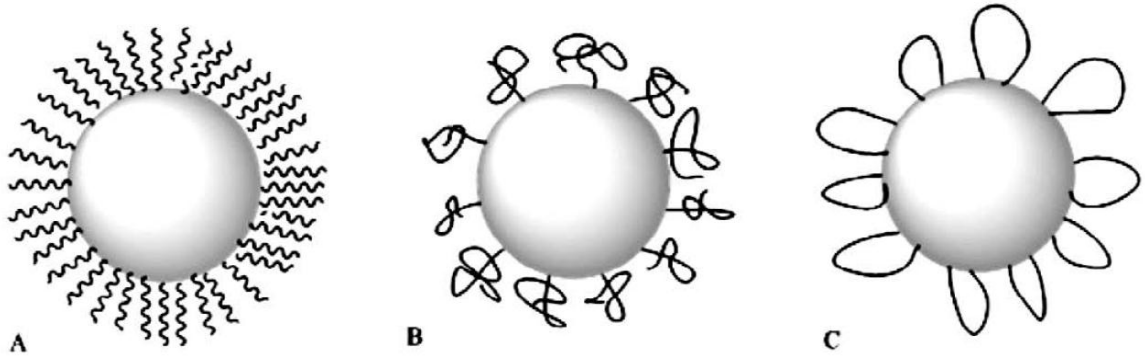
Poloxamers

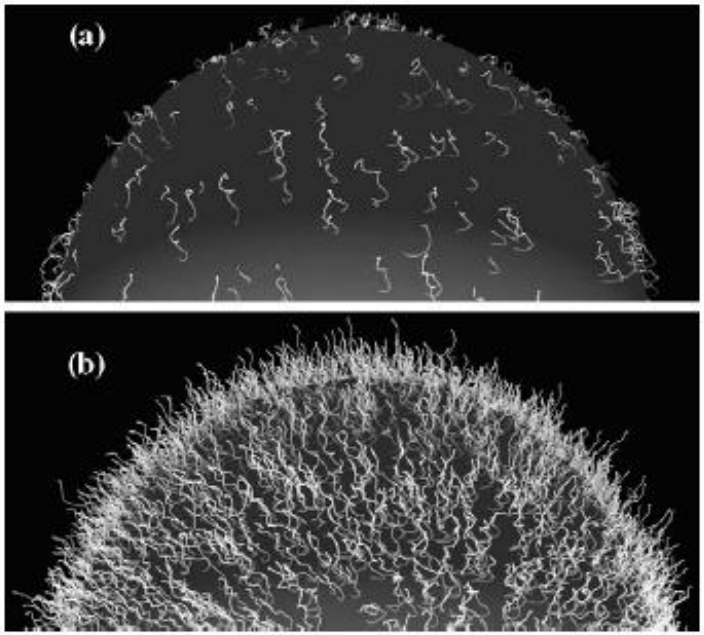


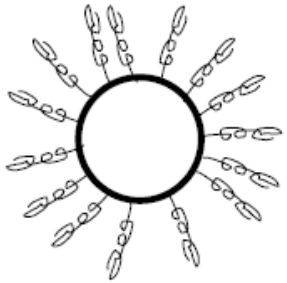
Poloxamines

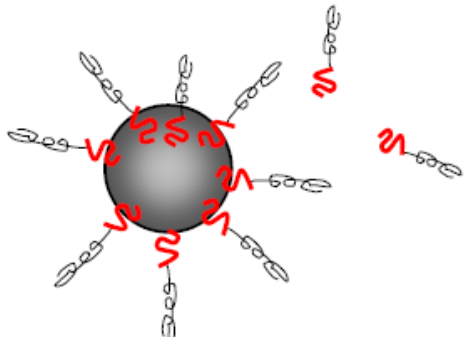


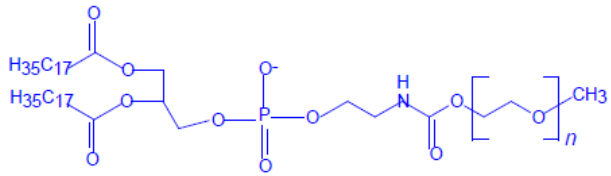
Estabilizadores estéricos e invisibilidad inmunonológica











DSPE-PEG 2000

(A) “Brush” regimen (high density). (B) “Mushroom” configuration (low density). (C) “PEG loops

Liposomas pegilados vs liposomas lisos

Table 1
In vivo tissue distribution of PEG-SL

Molecular weight of PEG-DSPE	% injected dose in tissues	
	Blood	MPS
0	2.0 ± 0.8	30.7 ± 1.3
120	1.0 ± 1.3	12.9 ± 1.4
750	3.2 ± 1.3	9.7 ± 9.1
2000	21.2 ± 6.8	7.3 ± 0.9
5000	21.0 ± 1.5	9.6 ± 2.0

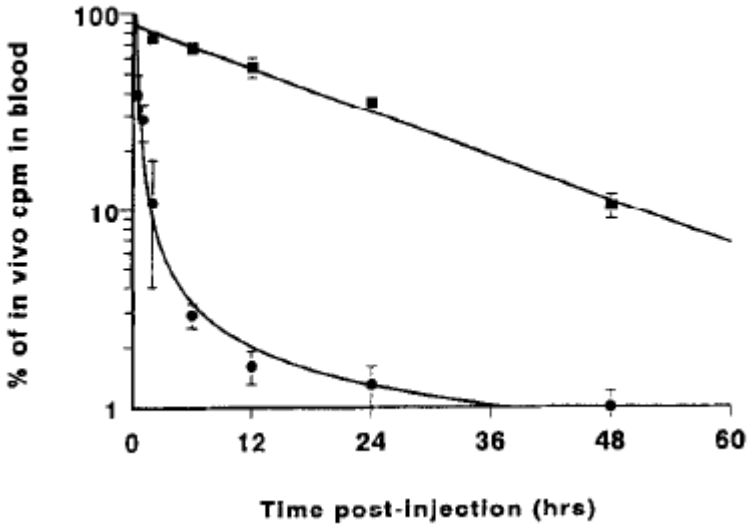
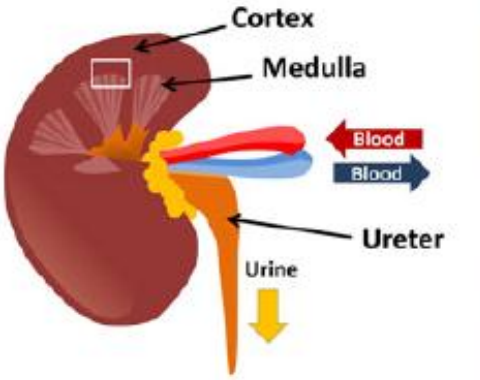


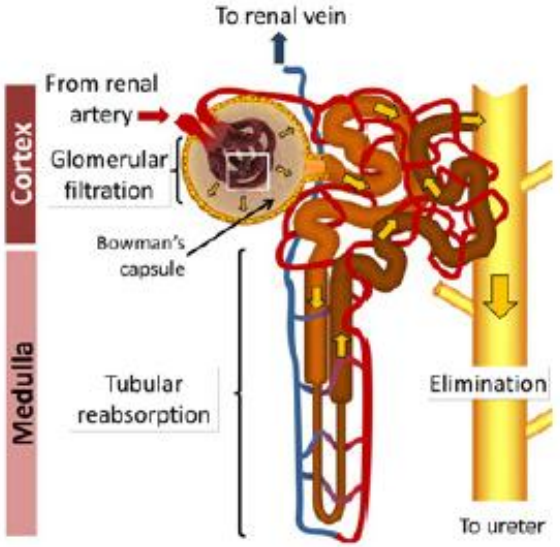
Table 3
A comparison of pharmacokinetic parameters of free doxorubicin (DXR) and liposome-encapsulated DXR formulati and humans

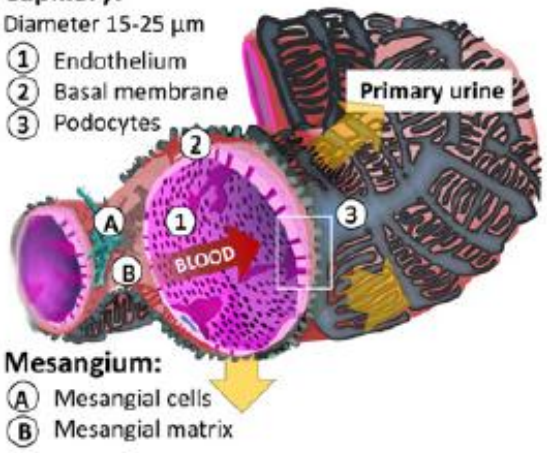
Treatment	Species	Dose (mg/kg)	$t_{1/2}\alpha$ (h)	$t_{1/2}\beta$ (h)	Clearance (l/h)	AUC (mg · h/l)	V_d (l)
FreeDXR	mouse	10	0.08	8.6	0.051	3.9	0.64
HSPC:CHOL:HPI (DXR-HPI-SL)	mouse	10	2.0	15.5	0.0002	1099	0.004
Free DXR	man	50	0.013	20.0	25.3	3.5	≈1000
HSPC:CHOL:PEG (DXR-SL)	man	50	1.4	45.9	0.09	1096	4.8
EggPC:CHOL (DXR-CL)	man	25	0.29	6.7	23	58.5	18.8

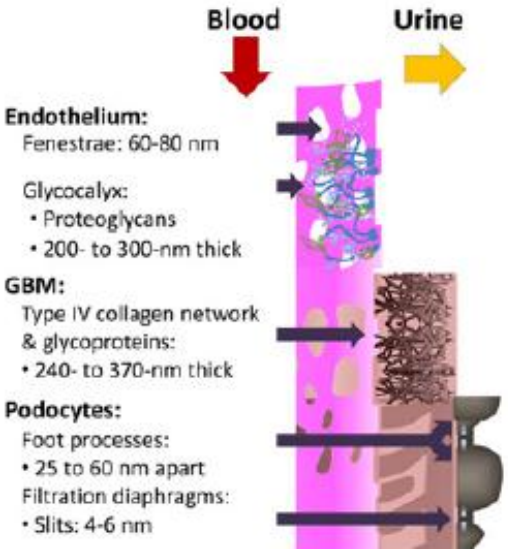
Filtración renal



Blood flow: 1 240 mL/min (both kidneys)



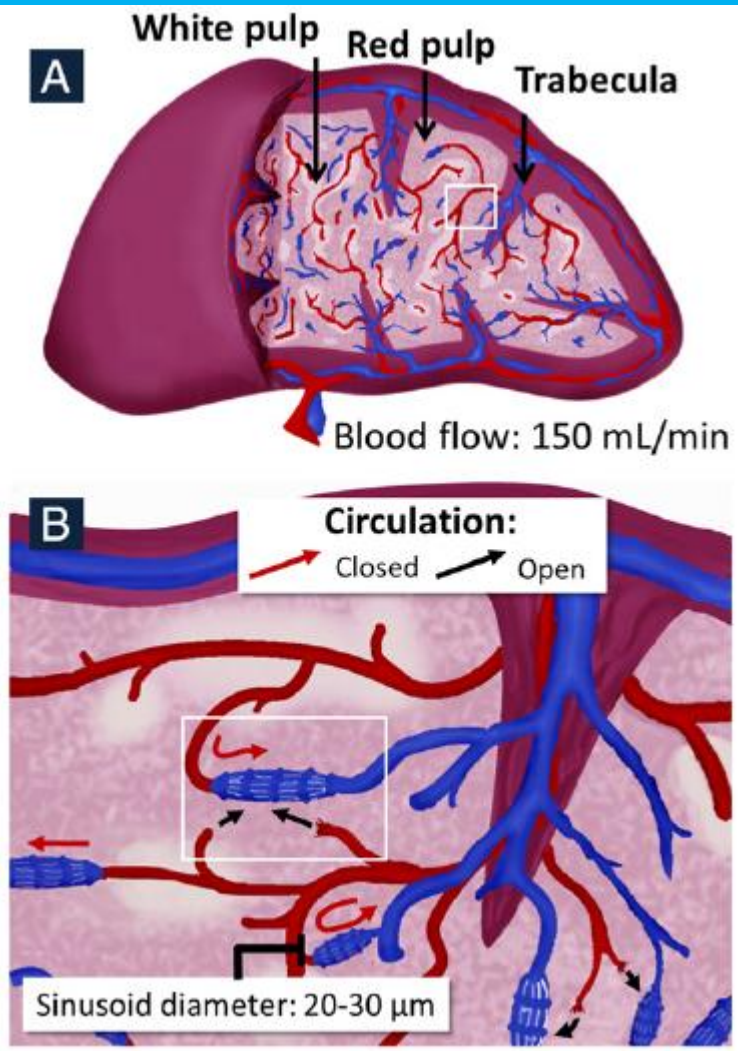


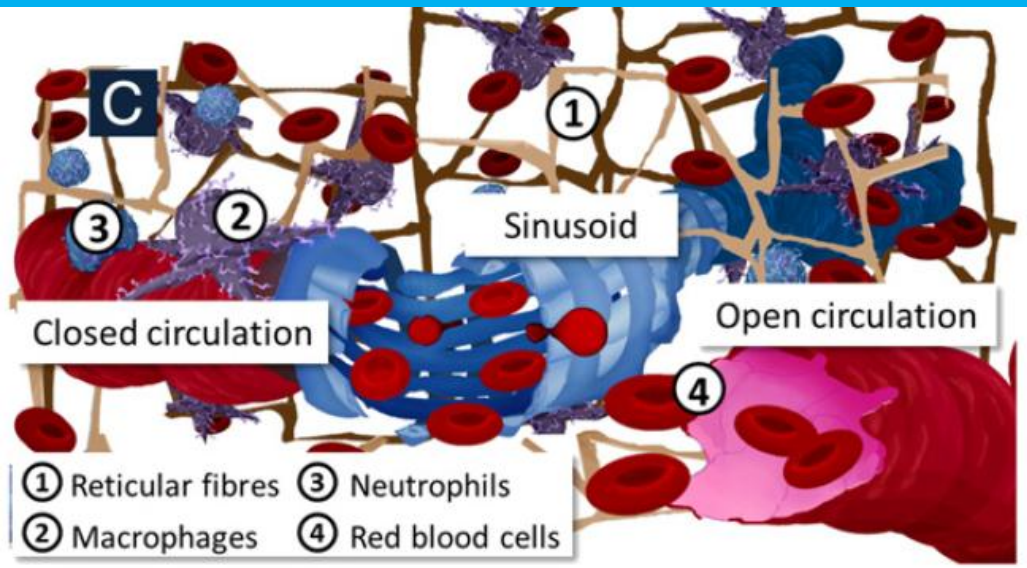


Filtración de proteínas globulares de 5-6nm diam (49-60kD)

QD, NTC, Dentrimeros son facilmente filtrados

Filtración esplénica

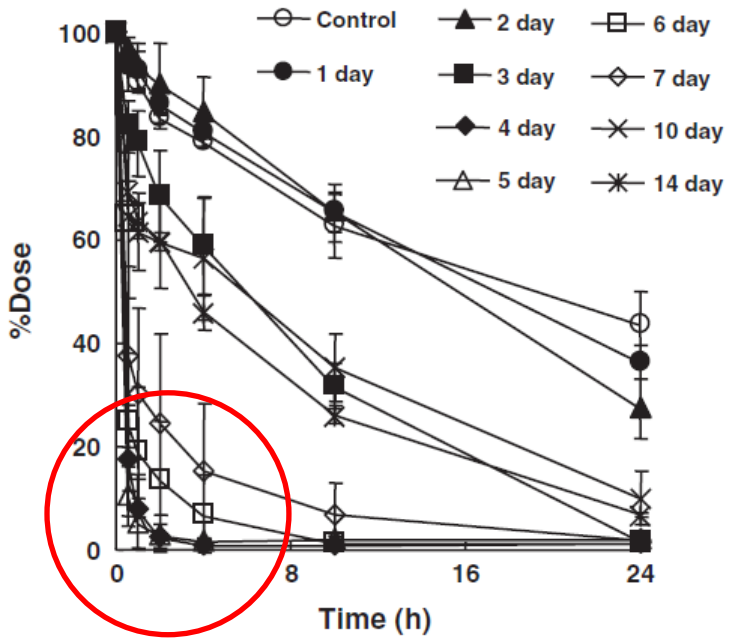


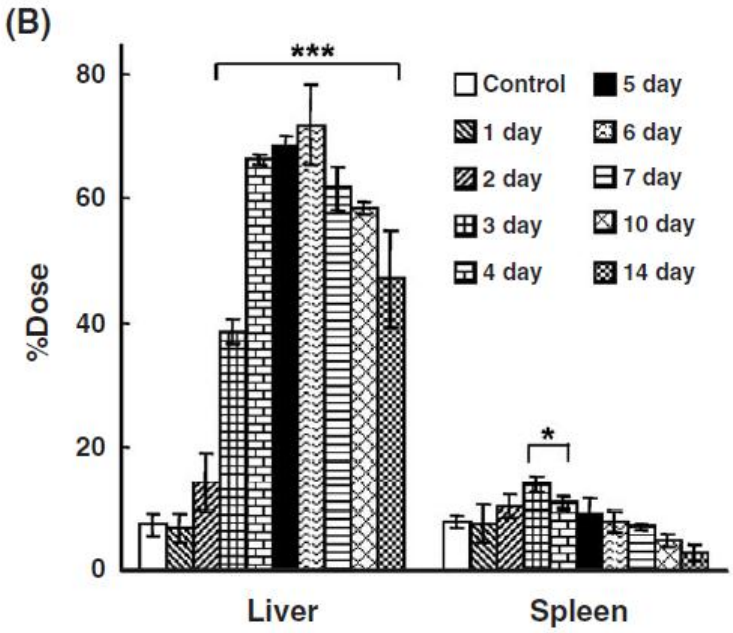


Se favorece la retención de NV rígidos
Humanos y ratas tienen sinusoides fenestrados y ratones sinusoides lisos
Relacionado con el efecto ABC

Journal of Controlled Release 161 (2012) 152–163

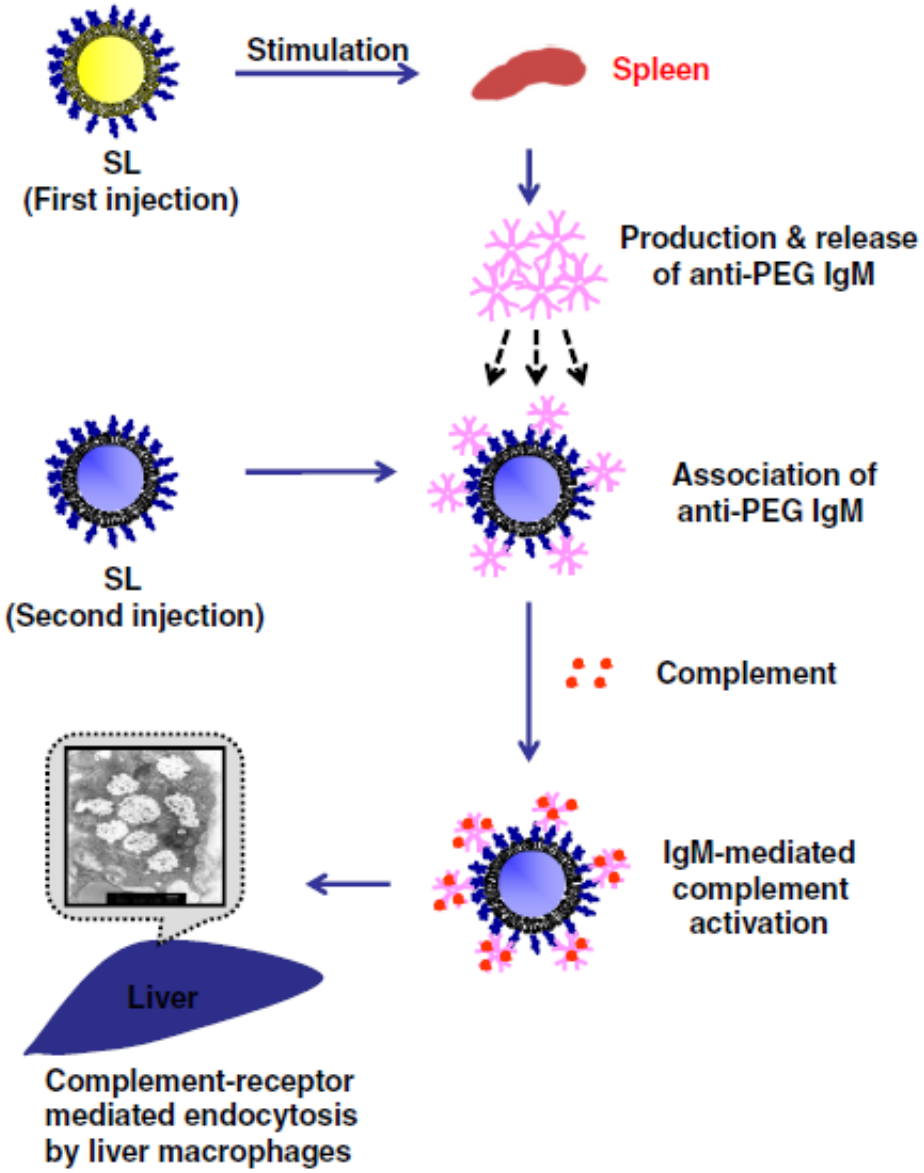
Accelerated Blood Clearance: Efecto ABC



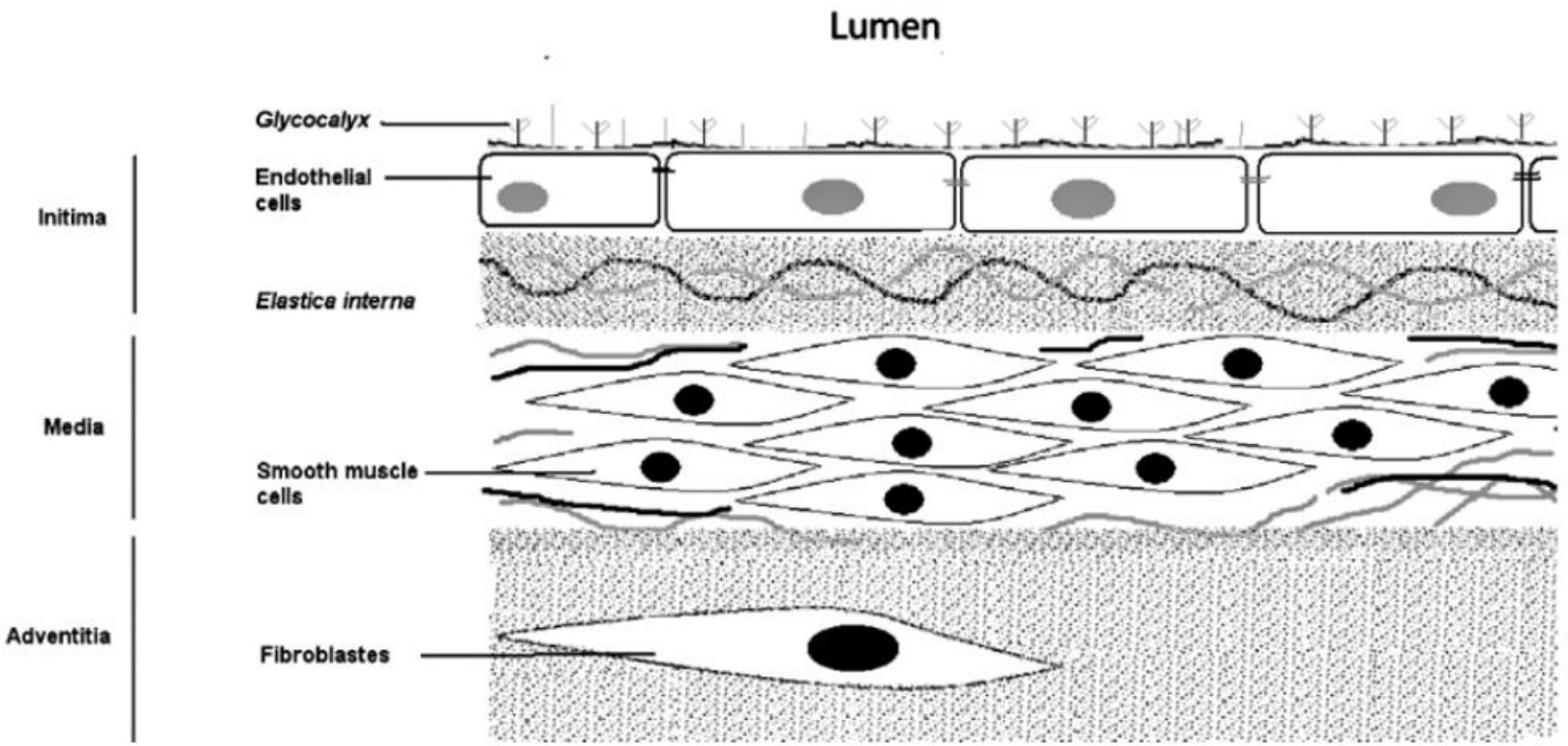


Time after first injection (day)	Elimination half-life (h) (mean of three values±S.D.)
Control	14.76±0.95
1	16.99±1.42
2	13.32±1.57
3	5.89±0.39
4	0.50±0.18***
5	0.31±0.08***
6	1.83±0.64***
7	4.16±2.82***
10	9.00±2.20*
14	7.02±0.24***

Accelerated Blood Clearance: Efecto ABC

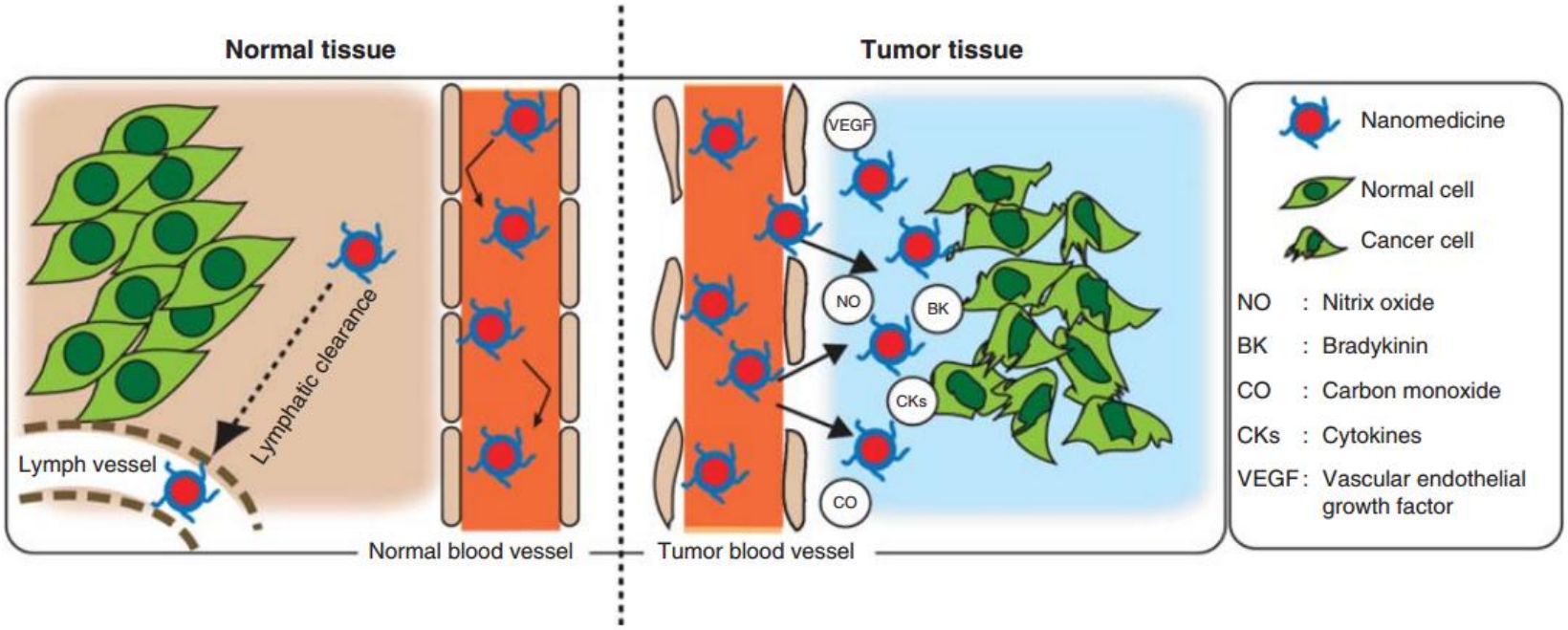


EXTRAVASACIÓN DEL NV: ARQUITECTURA DE VASOS SANGUINEOS



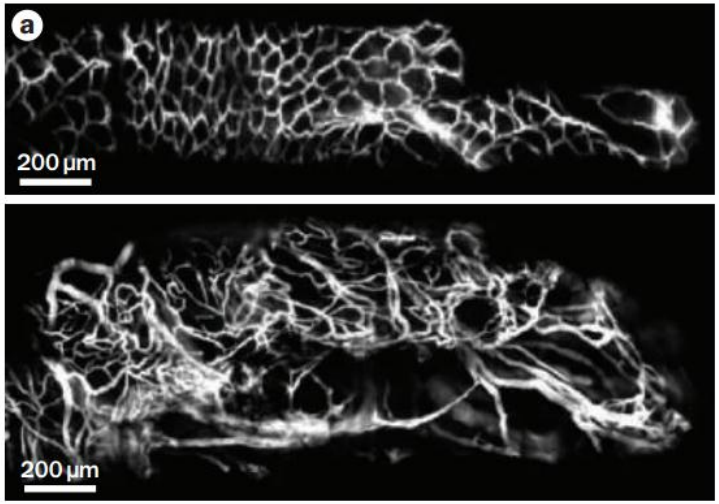
Extravación: Efecto de aumento de la permeabilidad y retención (efecto EPR)

- Matsumura and Maeda 1986



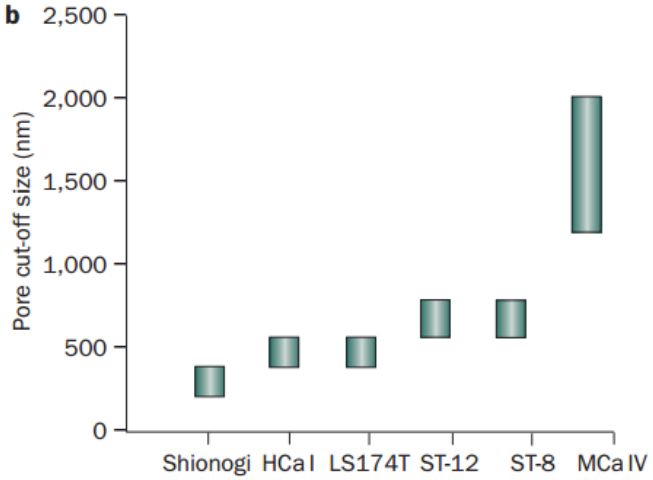
1-Poros 100-780 nm entre las células endoteliales
2-Alta producción de mediadores vasculares como bradikinina y oxido nítrico
3-Drenaje linfático deteriorado.
4-Vasos muy irregulares: aumento del tiempo de transito

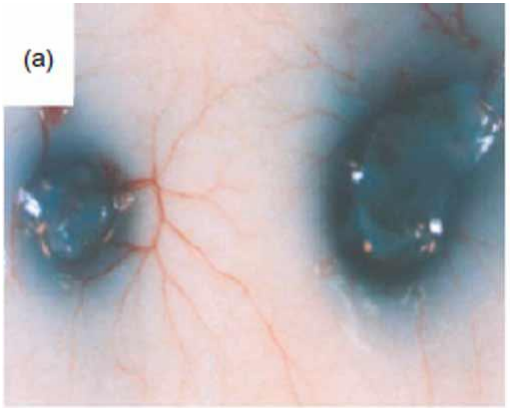
Efecto EPR

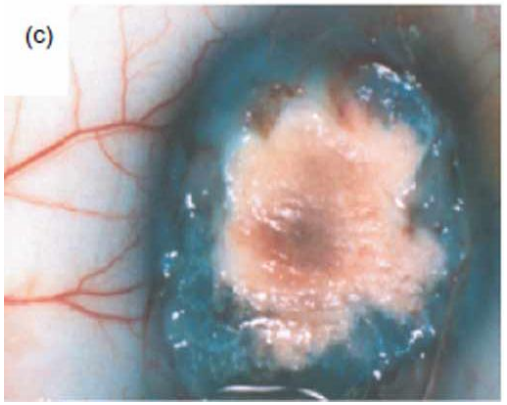


Vasculatura normal

Vasculatura tumoral







Poros 200-780 nm

Kim, P. et al. Nat. Methods 7, 303–305 (2010

EPR:
Dependiente del tamaño NV

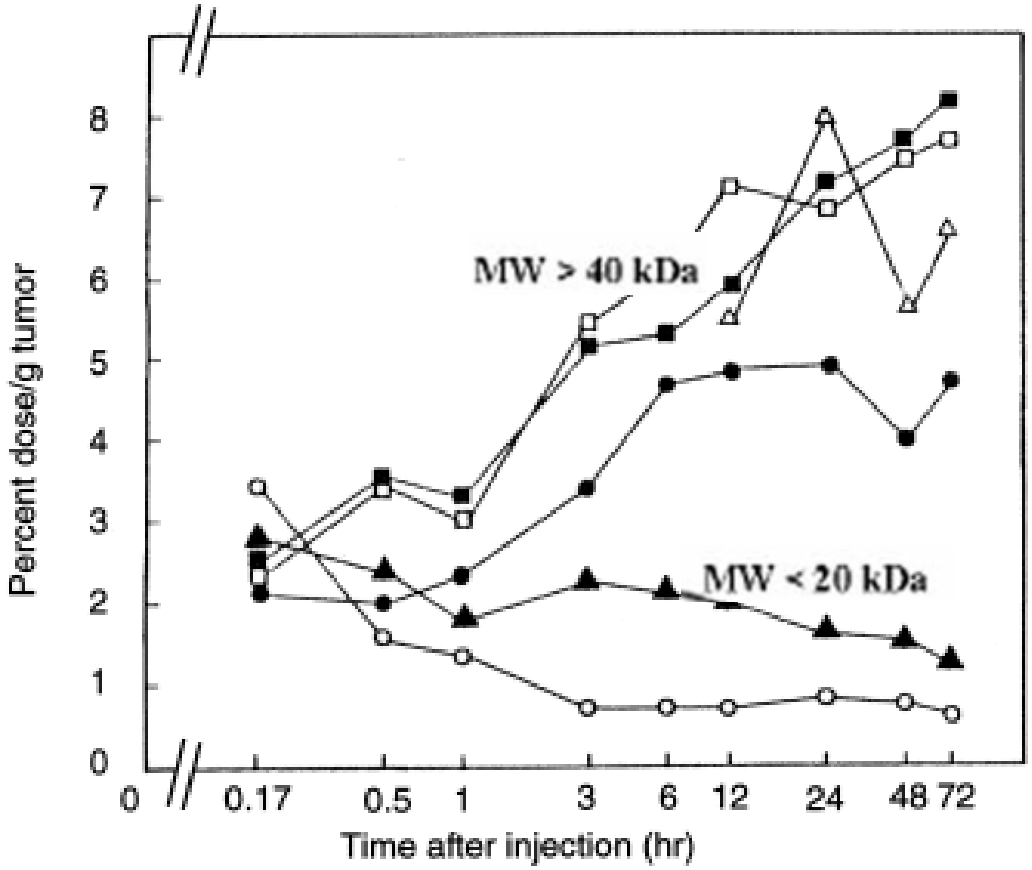
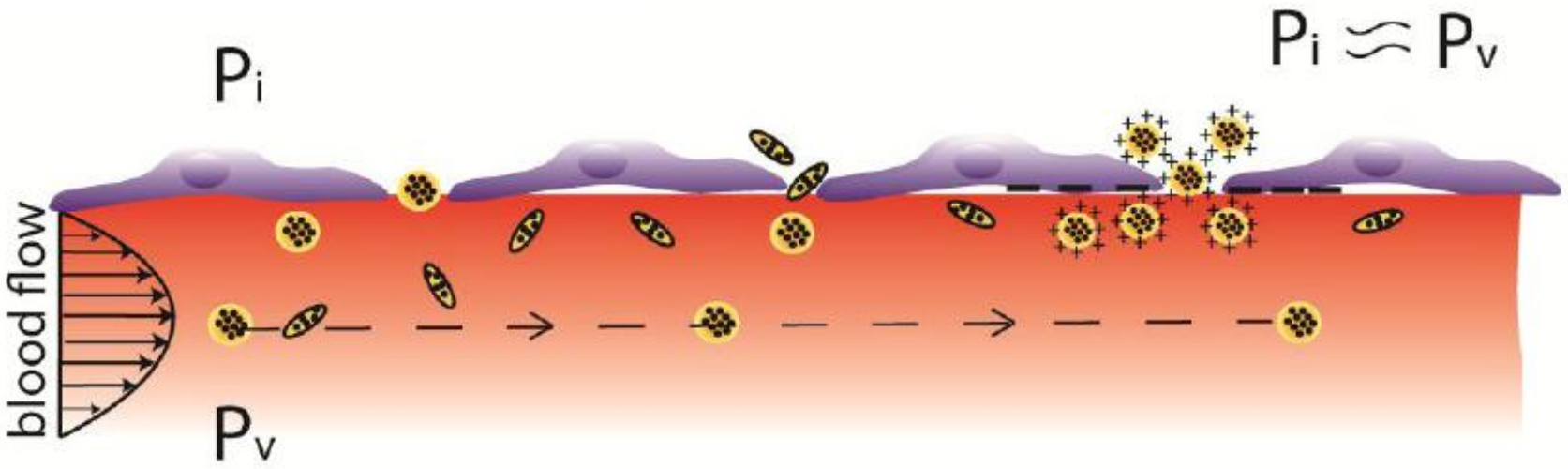


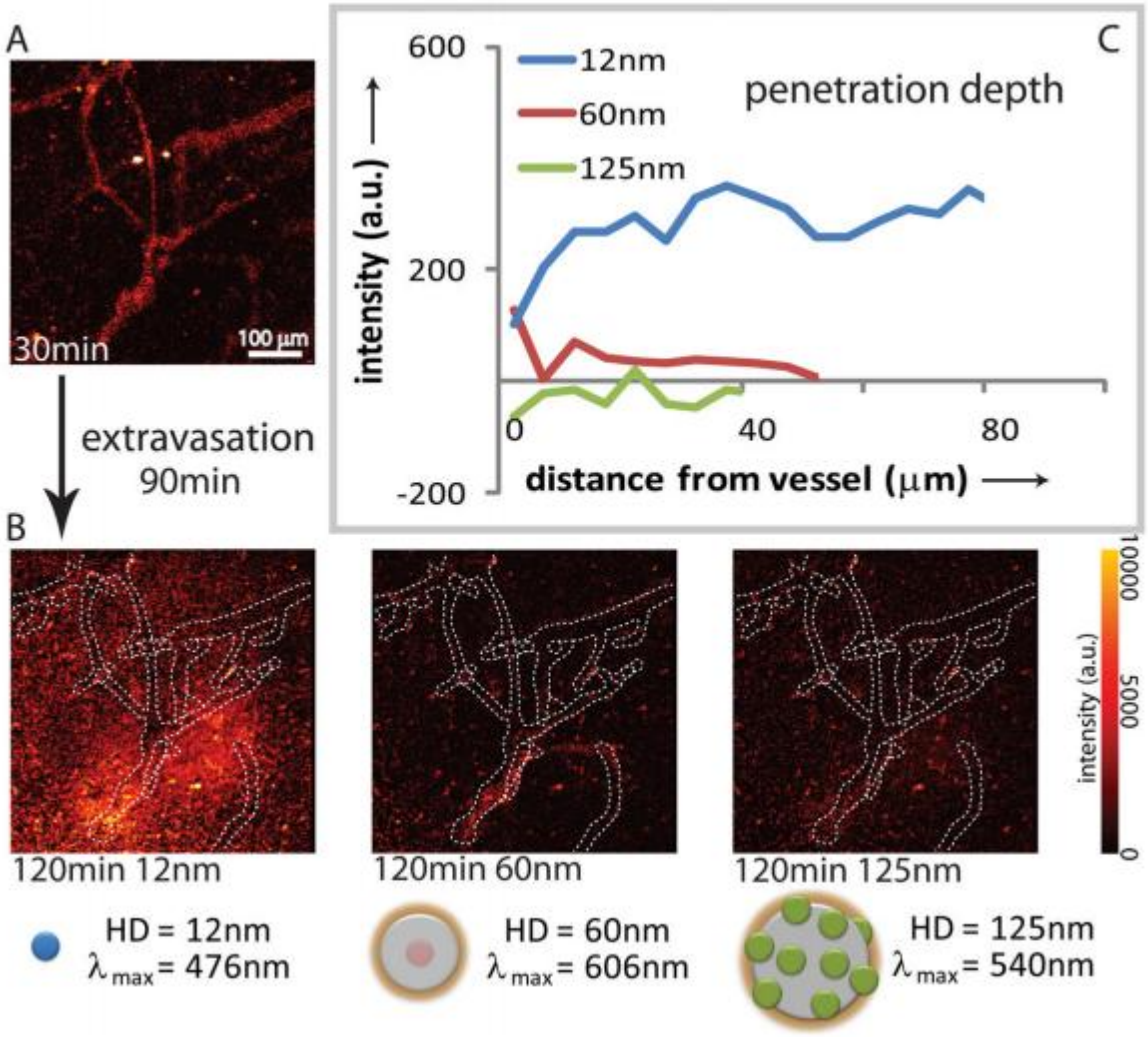
Fig. 1. Intratumor accumulation of various ^{51}Cr-tagged proteins in S-180 solid tumor-bearing mice: ○, neocarzinostatin (NCS) (12 kDa); ●, SMANCS (16 kDa); ▲, ovomucoid (29 kDa); □, BSA (69 kDa); ■, mouse serum albumin (68 kDa); △, mouse IgG (160 kDa). Radioactive proteins were injected i.v. at time zero. Values are based on radioactivity (from Ref. [1], with permission).

Transporte transvascular: efecto del tamaño, carga y forma de los NVs

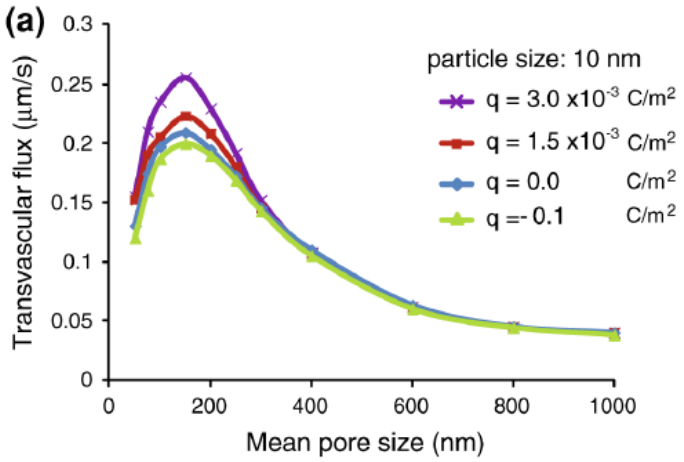


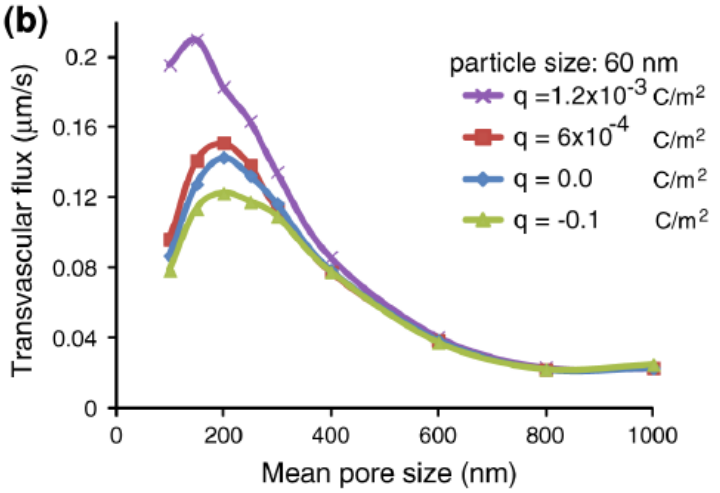
Interacciones: estéricas, hidrodinámicas y eléctricas

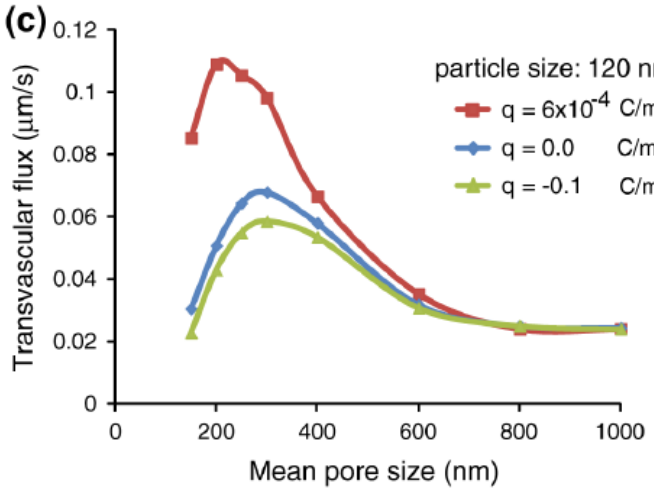
Transporte vascular: efecto del tamaño



Transporte vascular: Efecto de la carga



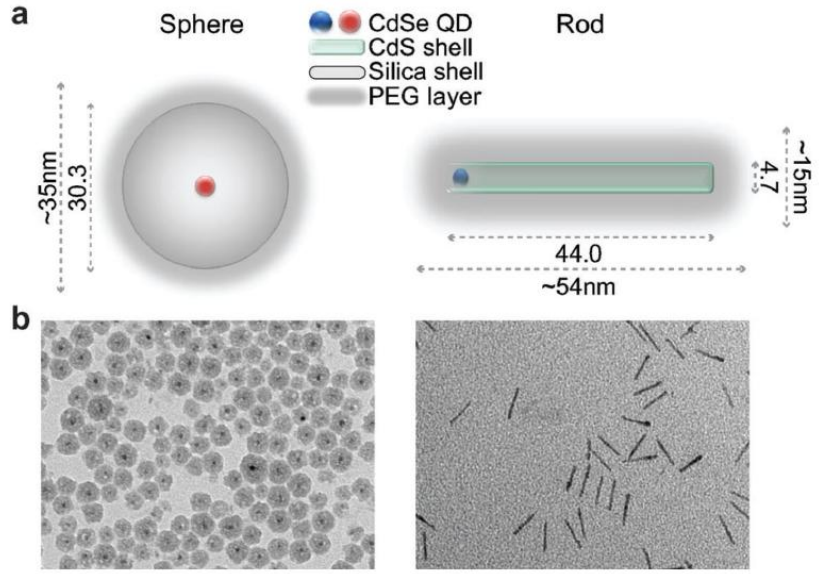


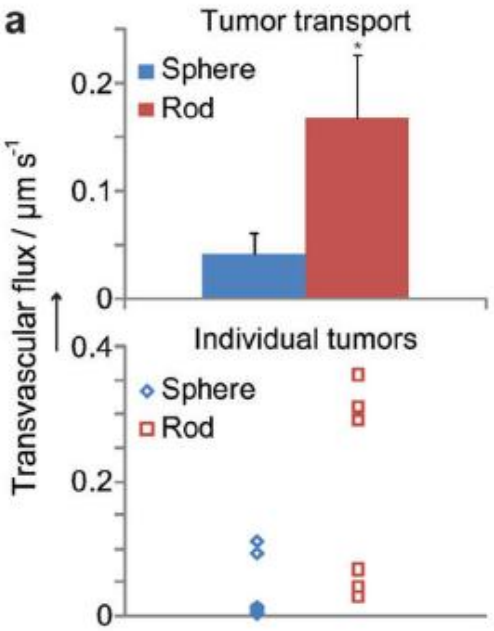


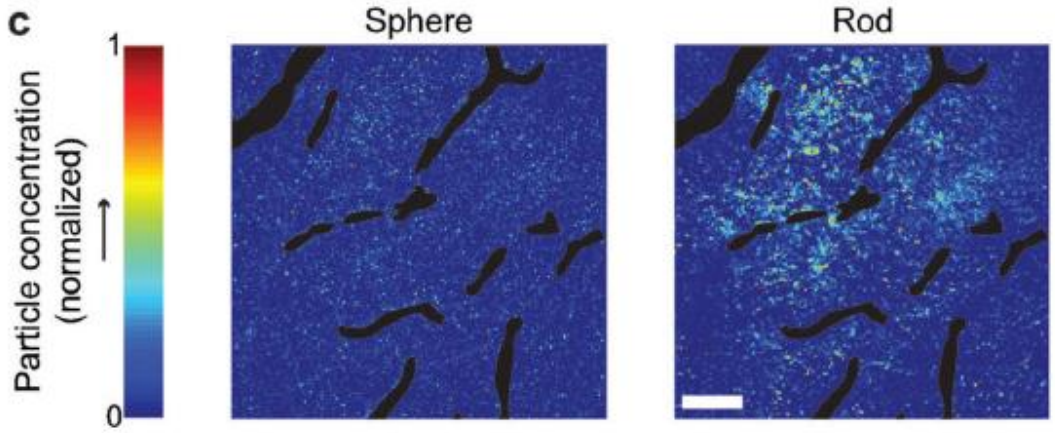
Physiological parameter values.

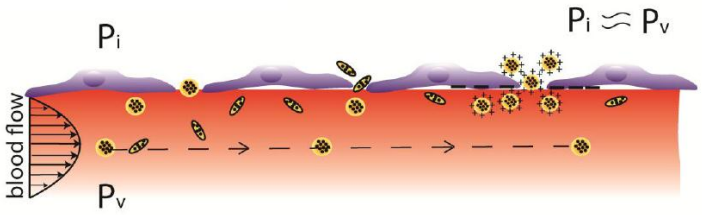
Model parameters	Value	References
Vessel wall pore size	400 ± 60 nm	8,16
Vessel wall charge density	−0.05 C/m^2	34
Blood viscosity	3×10^{-5} mmHg s	19
Vascular pressure at the inlets	25 mmHg	3
Vascular pressure at the outlets	5 mmHg	3
Vascular density	100 cm^{-1}	44
Vessel wall thickness	5 μm	18
Vessel diameter	15 μm	45
Interstitial space conductivity	8×10^{-7} cm^2/mmHg s	25

Transporte vascular: Efecto de la forma

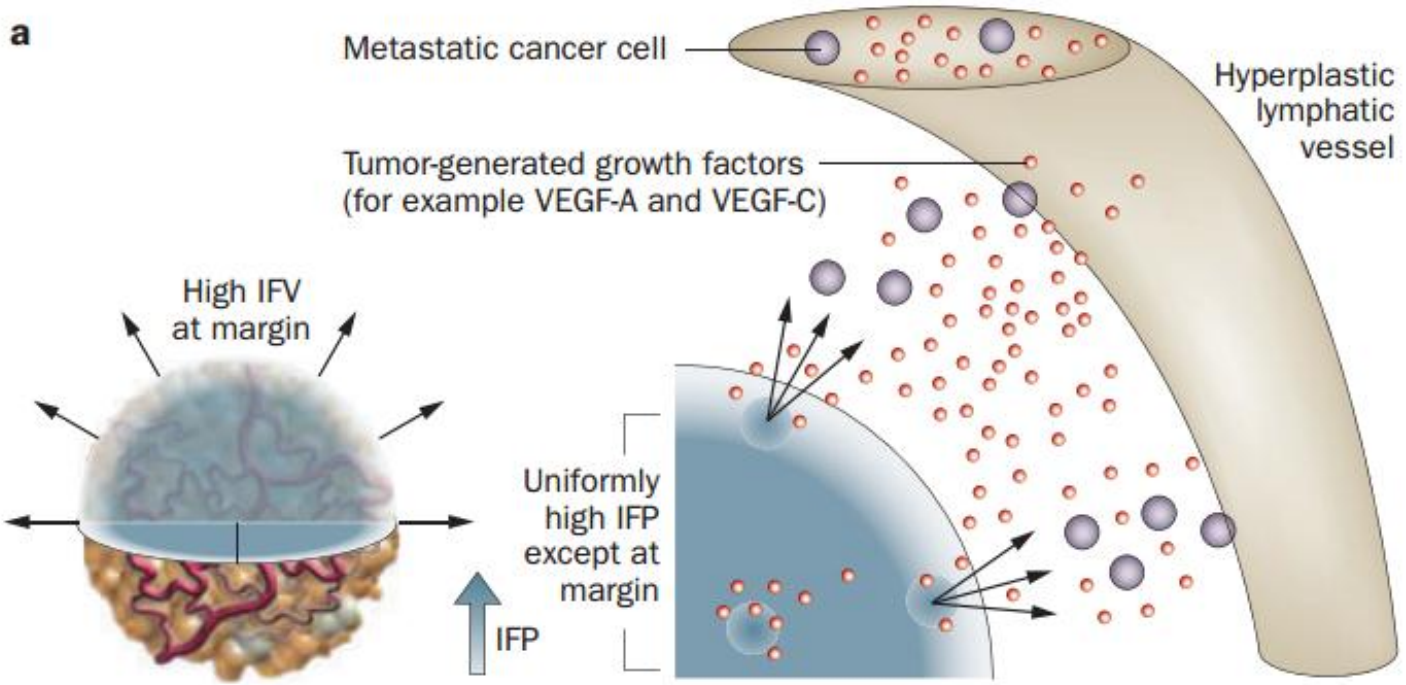




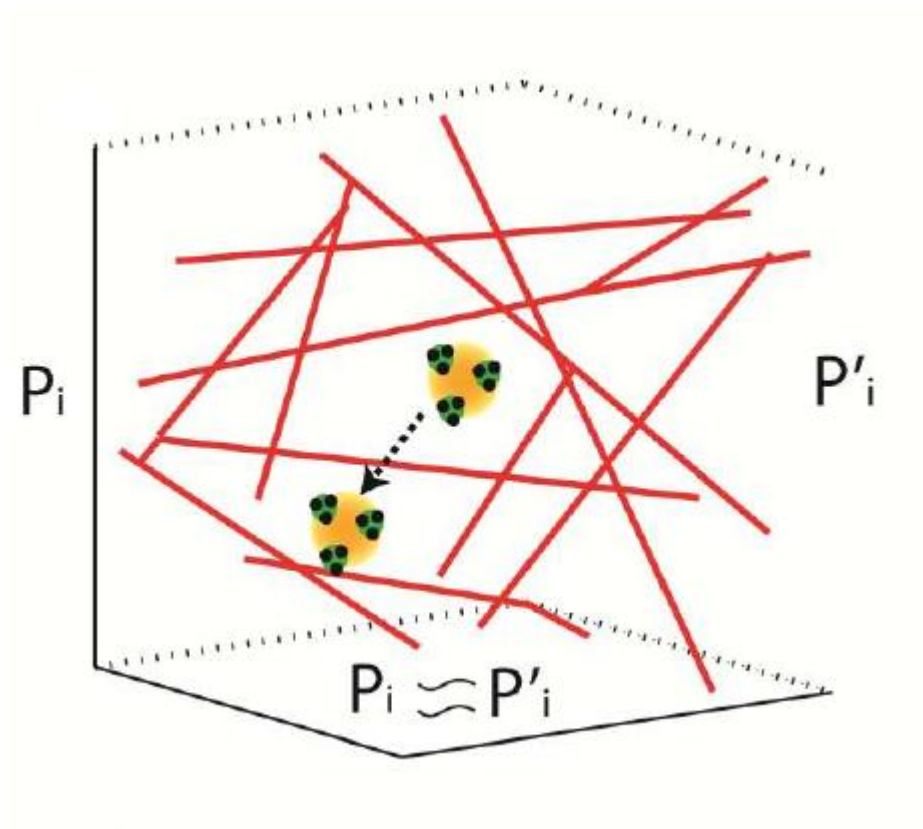




Transporte intersticial: presión intersticial



Transporte intersticial:
Difusión NV depende de su tamaño y de las interacciones con las fibras extracelulares



<u>Difusión</u> NV es inversamente proporcional a su tamaño

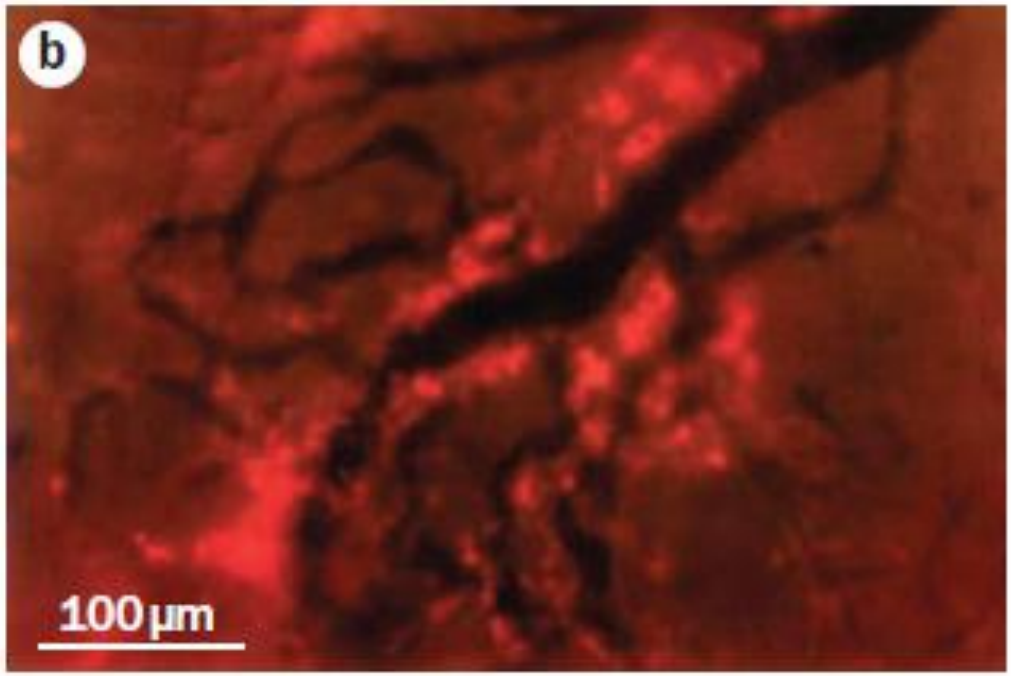
<u>Interacciones entre NV y los poros intersticial:</u>

Estéricas,
Electrostáticas
Hidrodinámicas

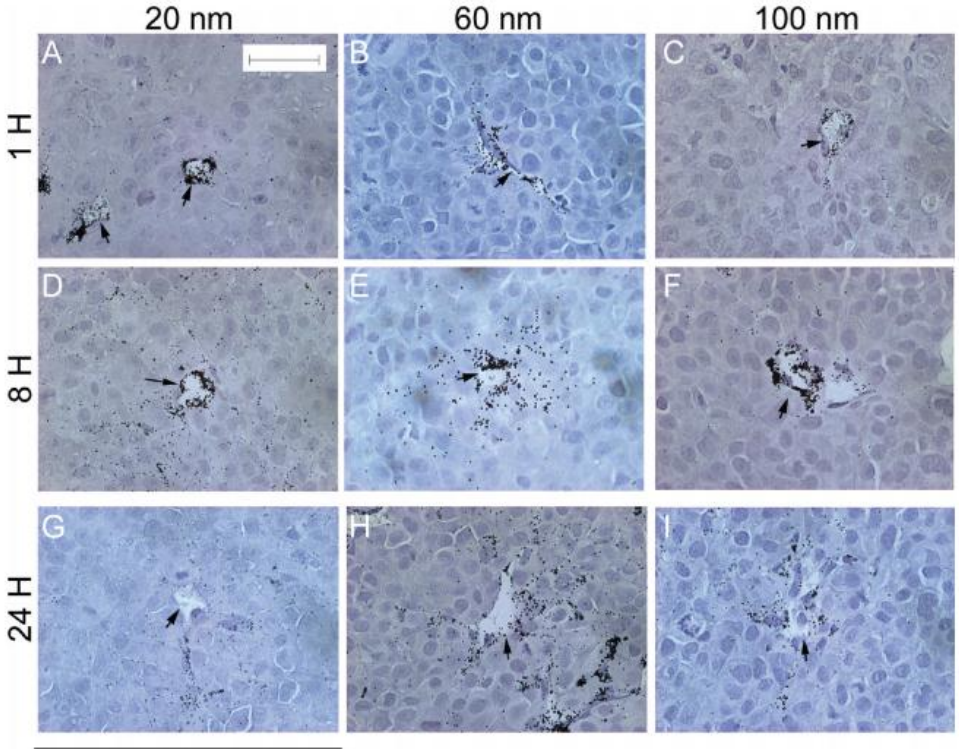
Transporte intersticial

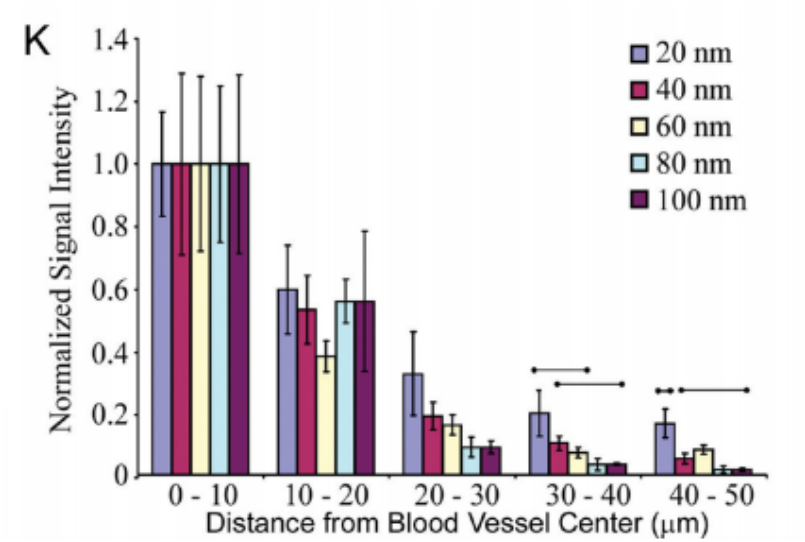
NV se acumulan alrededor de los vasos

Difusión de liposomas de 90nm



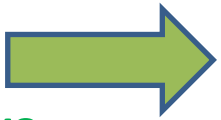
Transporte Intersticial: efecto del tamaño NV





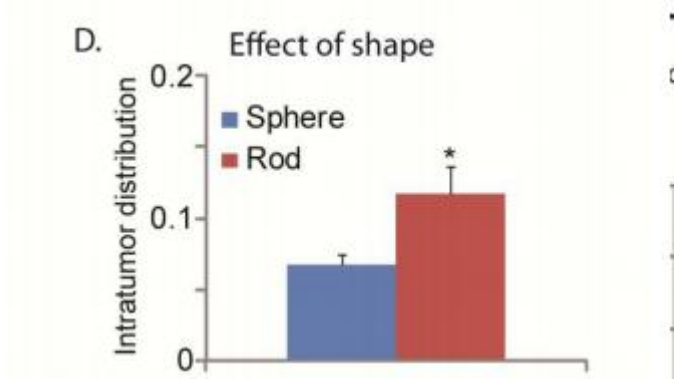
Transporte intersticial: efecto de la carga y de la forma de los NV

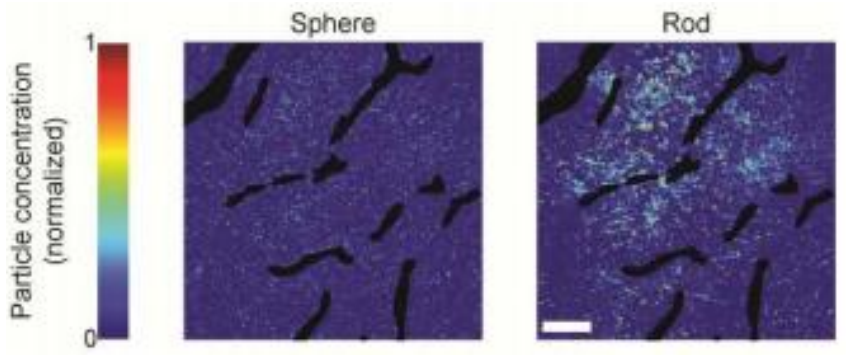
Colágeno es levemente positivo
Ácido Hialurónico es altamente negativo



NV cargados eléctricamente forman agregados

NV neutros tienen mejor transito intersticial





Biophys J 2010, 99 1342-9

Targeting Activo

- 1 Utilización de anticuerpos o ligandos específicos sobre la superficie de los NV

- 2-Aumentos de tiempos de permanencia en el sitio tumoral en algunos casos

- 3-Aumenta la internalización, que es necesaria para determinado tipo de agentes terapéuticos (DNA, siARN, emisores de electrones) .

Criterios de elección del anticuerpo

Altos niveles de expresión (104-105 copias/célula)

Accesibilidad

Celular uptake

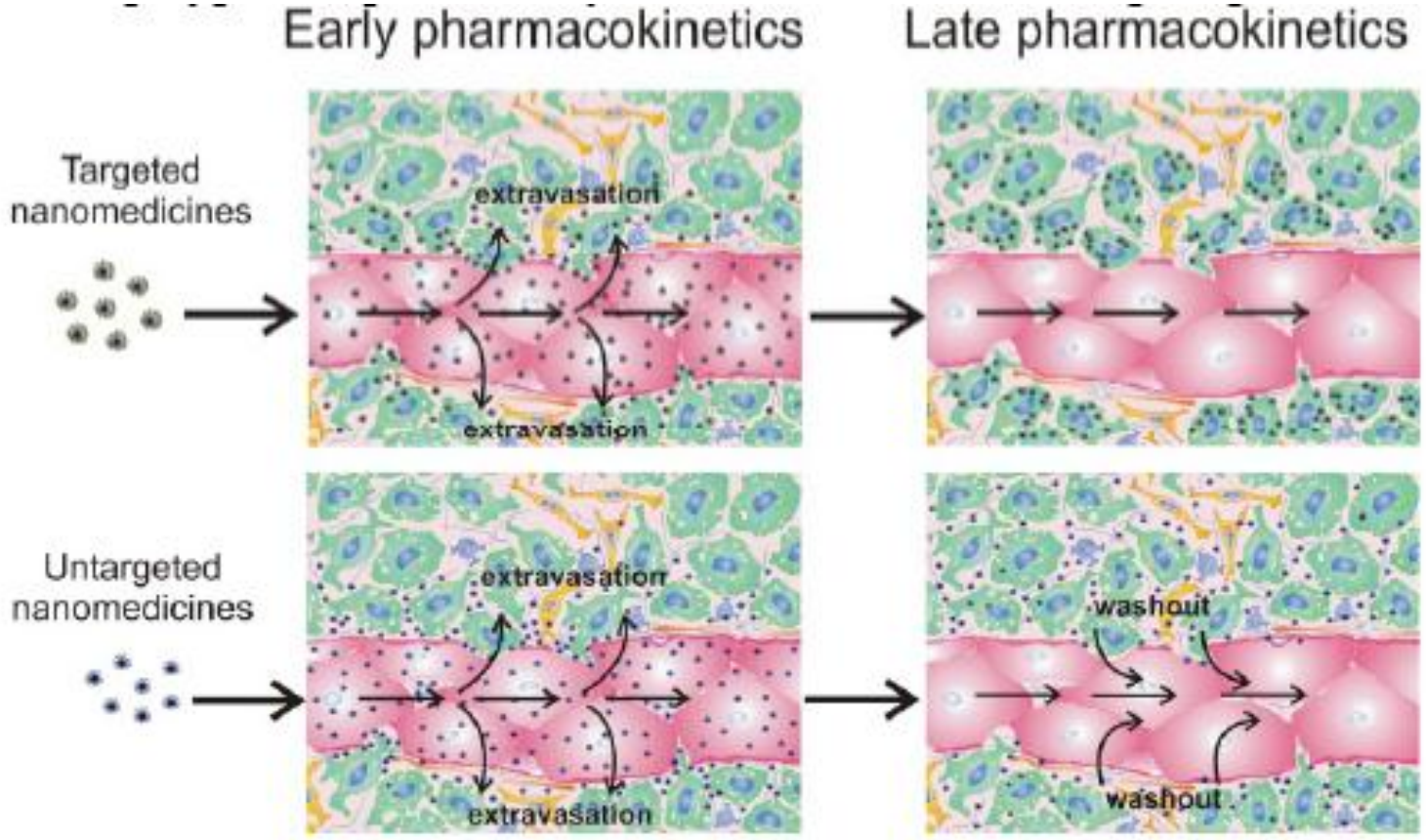
Especificidad y afinidad

Baja inmunogenicidad: humanización

Capacidad de conjugación con el NV o con la droga

Targeting Activo

Aumento de retención por internalización: importante en moléculas pequeñas y en terapias



Aumento de endocitosis pero no del reclutamiento de los NV en el tumor

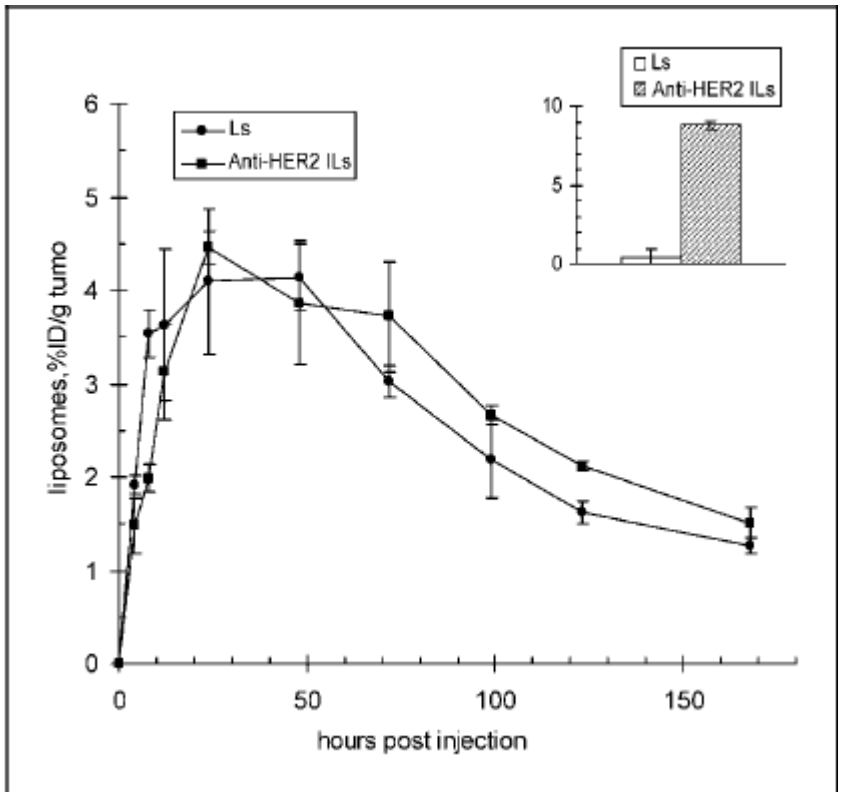


Figure 1. Tumor pharmacokinetics of ^{67}Ga-labeled anti-HER2 immunoliposomes (Fab′ targeted) versus control pegylated liposomes (*Ls*) in s.c. BT-474 breast cancer xenografts in nude mice. Liposomes/immunoliposomes were administered i.v. at 40 μmol phospholipid/kg. *Points*, mean; *bars*, ±SD; three animals per group. *Inset*, uptake of anti-HER2 immunoliposomes (*cross-hatched column*) versus control liposomes (*solid column*) in HER2-overexpressing breast cancer cells (SK-Br-3) *in vitro*. Uptake units, nmol liposome phospholipid/10^6 cells (mean ± SD; $n = 4$ experiments).

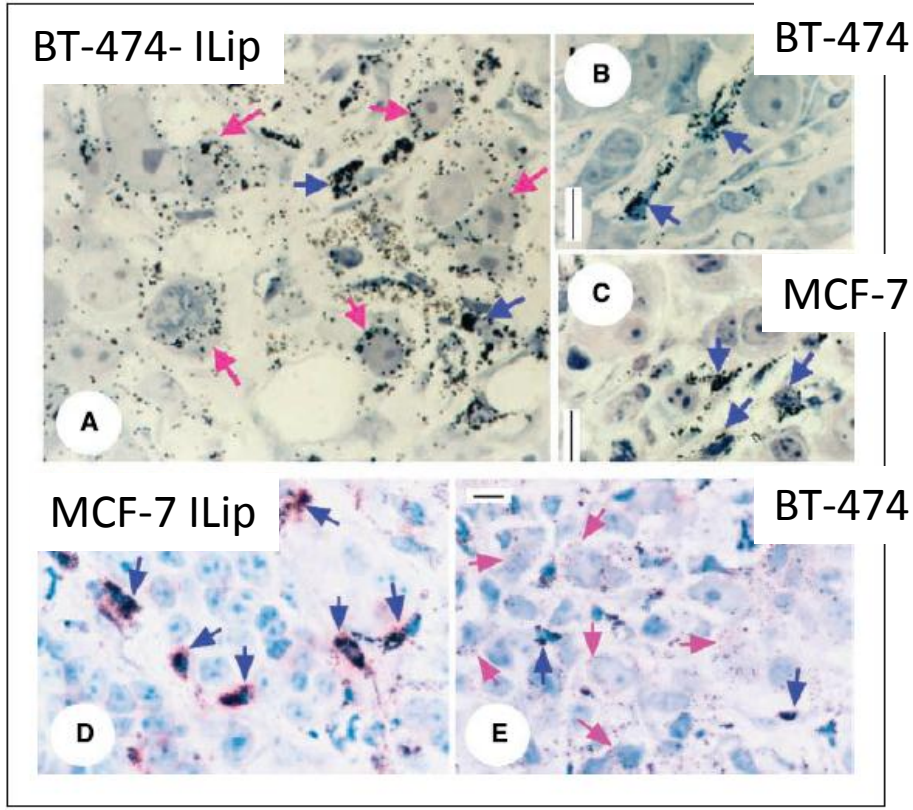


Figure 3. Detailed histologic analysis of the cellular internalization of anti-HER immunoliposomes (Fab′ targeted) versus control pegylated liposomes in breast cancer xenografts in nude mice. Colloidal gold-encapsulating immunoliposomes/liposomes were administered i.v. in nude mice bearing either HER2-overexpressing BT-474 tumors (*A*, *B*, and *E*) or nonoverexpressing MCF-7 tumors (*C* and *D*). Forty-eight hours postinjection, tumors were excised and colloidal gold was visualized by silver enhancement. At high-power magnification (×100 objective; *bar*, 10 μm/L), cancer cells could be identified as large cells, often in clusters, with large nuclei and prominent nucleoli (*magenta arrows*). Macrophages could be identified as smaller, elongated, or pyramidal cells, typically within stromal gaps, with small densely stained nuclei (*blue arrows*). *A-C*, high-power magnification of immunoliposome and liposome uptake in cells within BT-474 or MCF-7 tumors. *A*, anti-HER2 immunoliposomes showed marked internalization in BT-474 tumor cells and in macrophages. *B*, nontargeted liposomes showed internalization only in macrophages within BT-474 tumor tissue, with minimal tumor cell uptake. *C*, anti-HER2 immunoliposomes showed internalization only in macrophages within MCF-7 tumor tissue, with minimal tumor cell uptake in this HER2-low tumor. *D-E*, high-power magnification with cytochemical staining of macrophages in MCF-7 or BT-474 tumor tissue from animals injected with anti-HER2 immunoliposomes. Macrophages were stained for nonspecific esterase activity (*red*) and tissue was counterstained with Gill's hematoxylin. Immunoliposomes were confirmed to be restricted to macrophage uptake in HER2-low MCF-7 tumors (*D*), but in contrast, had predominantly internalized within tumor cells in HER2-overexpressing BT-474 tumors (*E*).

"Binding site barrier"

- **Los anticuerpos de mayor afinidad se unen a sus blancos en la proximidad de los vasos sanguíneos**

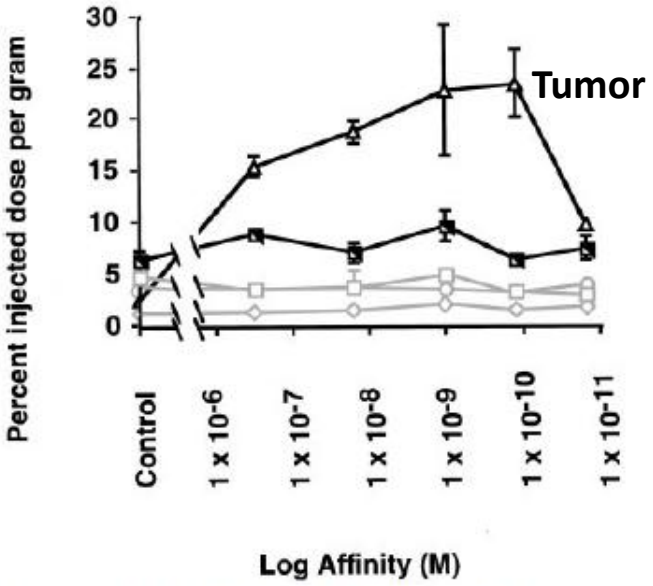


Fig. 2. Twenty-four h biodistribution of ^{125}I-labeled anti-HER-2/*neu* scFv affinity mutants in anephric SK-0V-3 tumor-bearing *scid* mice. Cohorts of three mice were given 1 μg of one of the affinity mutants and were then euthanized 24 h later. The percentage of the injected dose of each affinity mutant localized per gram of tumor (—△—), blood (—◪—), liver (—□—), muscle (—◇—), and spleen (—○—) is presented. *Bars*, SE.

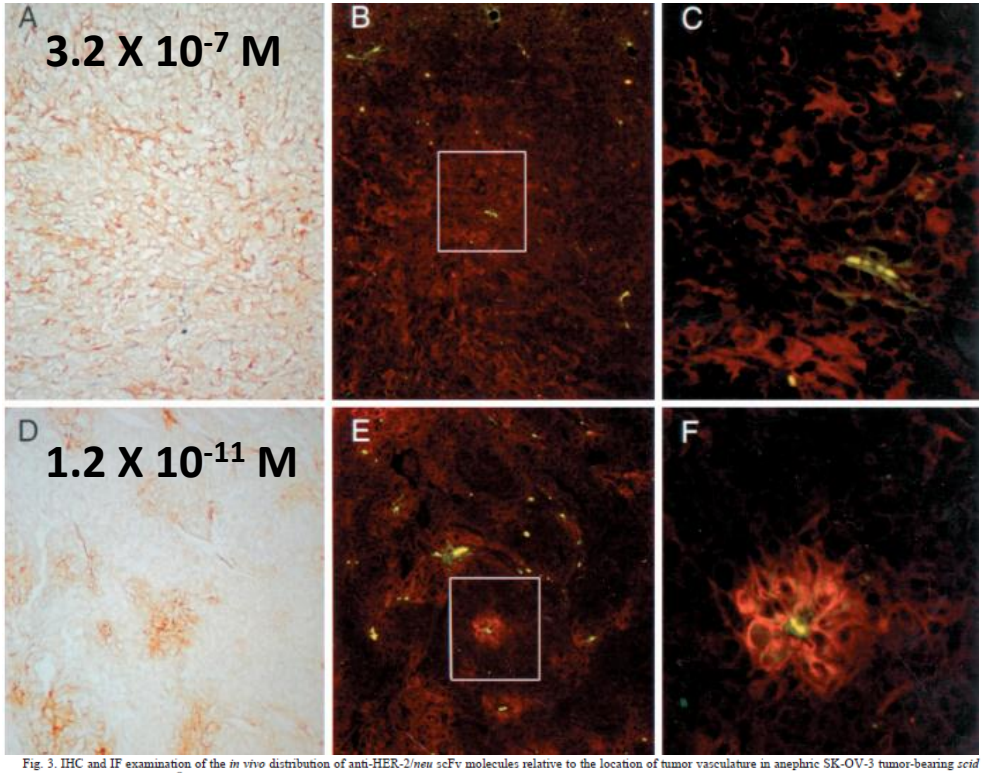
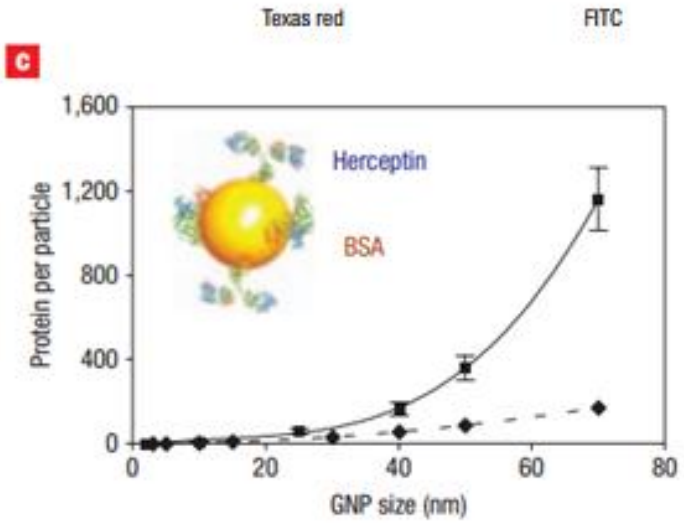
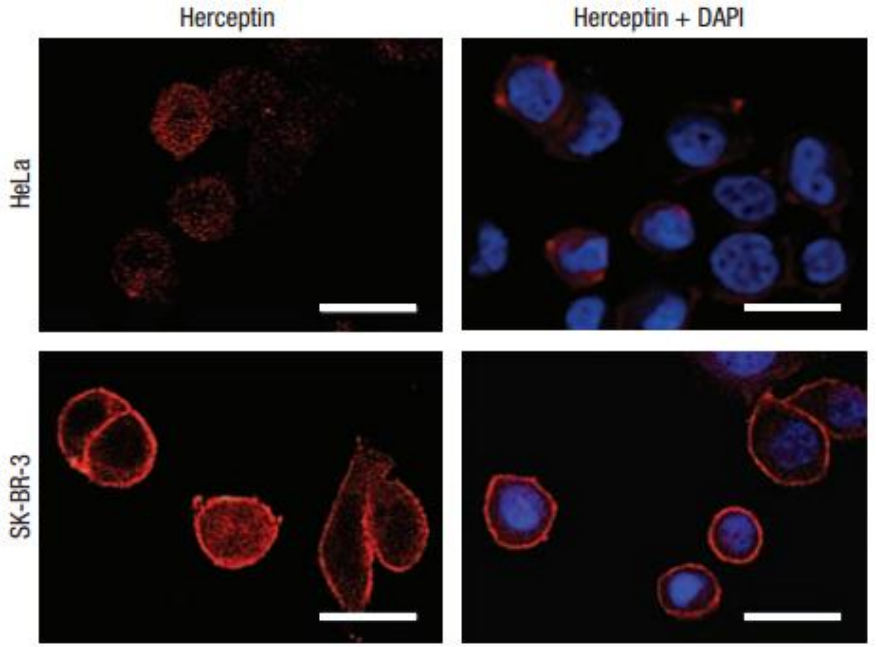


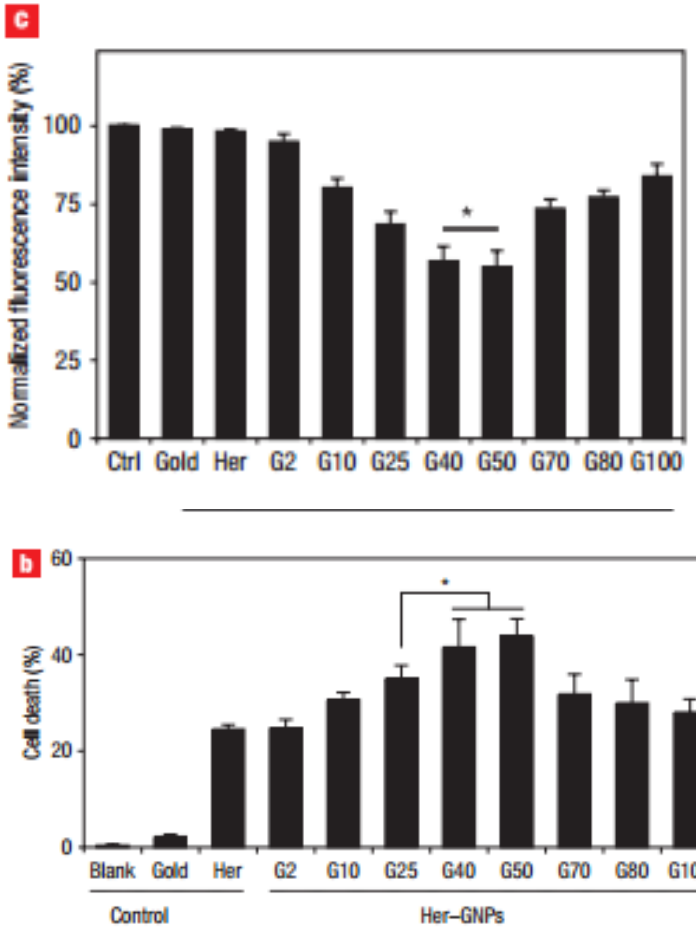
Fig. 3. IHC and IF examination of the *in vivo* distribution of anti-HER-2/*neu* scFv molecules relative to the location of tumor vasculature in anephric SK-OV-3 tumor-bearing *scid* mice. *A*, low affinity (3.2×10^{-7} M) anti-HER-2/*neu* scFv (*brown*) displays a diffuse staining pattern relative to tumor blood vessels (*black/blue*) by IHC. In *B*, IF examination of nearby section of same tumor (3.2×10^{-7} M anti-HER-2/*neu* scFv = *red* and tumor blood vessels = *green*) again indicates diffusion from the blood vessels. *C*, higher magnification of boxed area in *B*. In *D*, IHC examination of the localization of the high affinity (1.5×10^{-11} M) anti-HER-2/*neu* scFv (*brown*) relative to tumor blood vessels (*black/blue*) reveals a focal staining pattern with short diffusion from the vascular bed. In *E*, IF examination of nearby section of same tumor (1.5×10^{-11} M anti-HER-2/*neu* scFv = *red* and tumor blood vessels = *green*) again indicates a lack of diffusion from the tumor blood vessels. *F*, higher magnification of boxed area in *E*. Original magnifications: ×10 (*A*, *B*, *D*, and *E*) and ×40 (*C* and *F*).

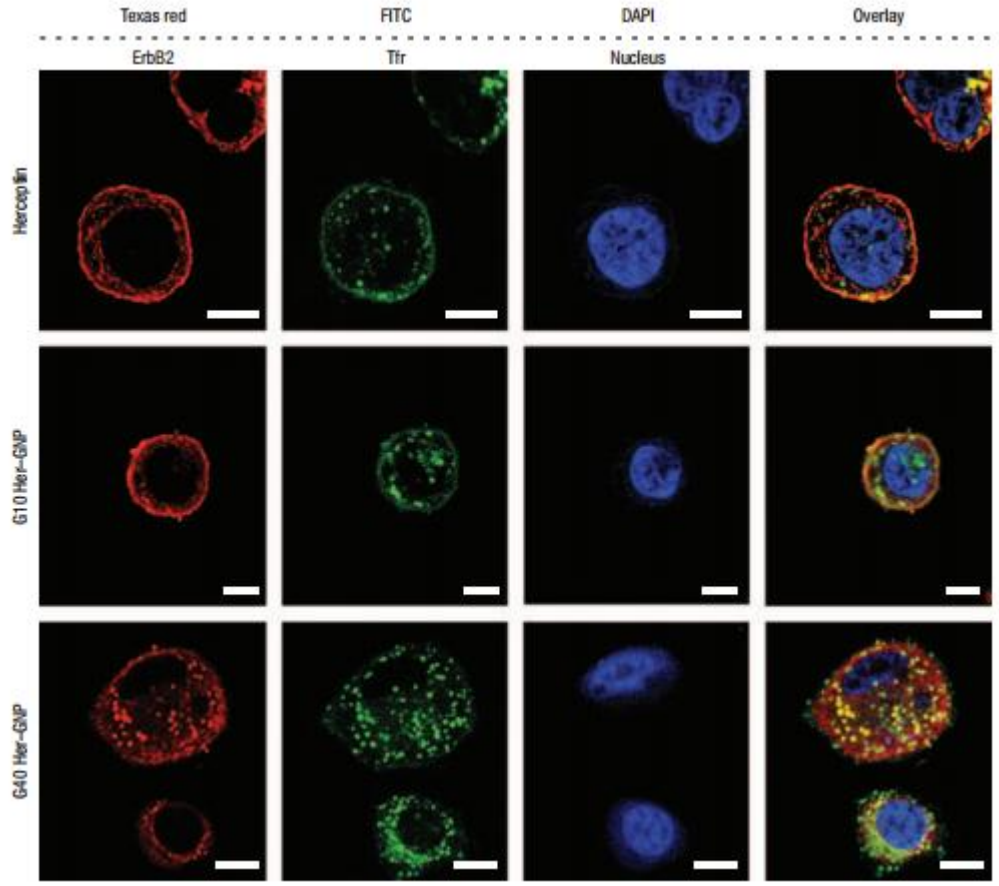
Influencia del tamaño del NV y la endocitosis mediada por receptor



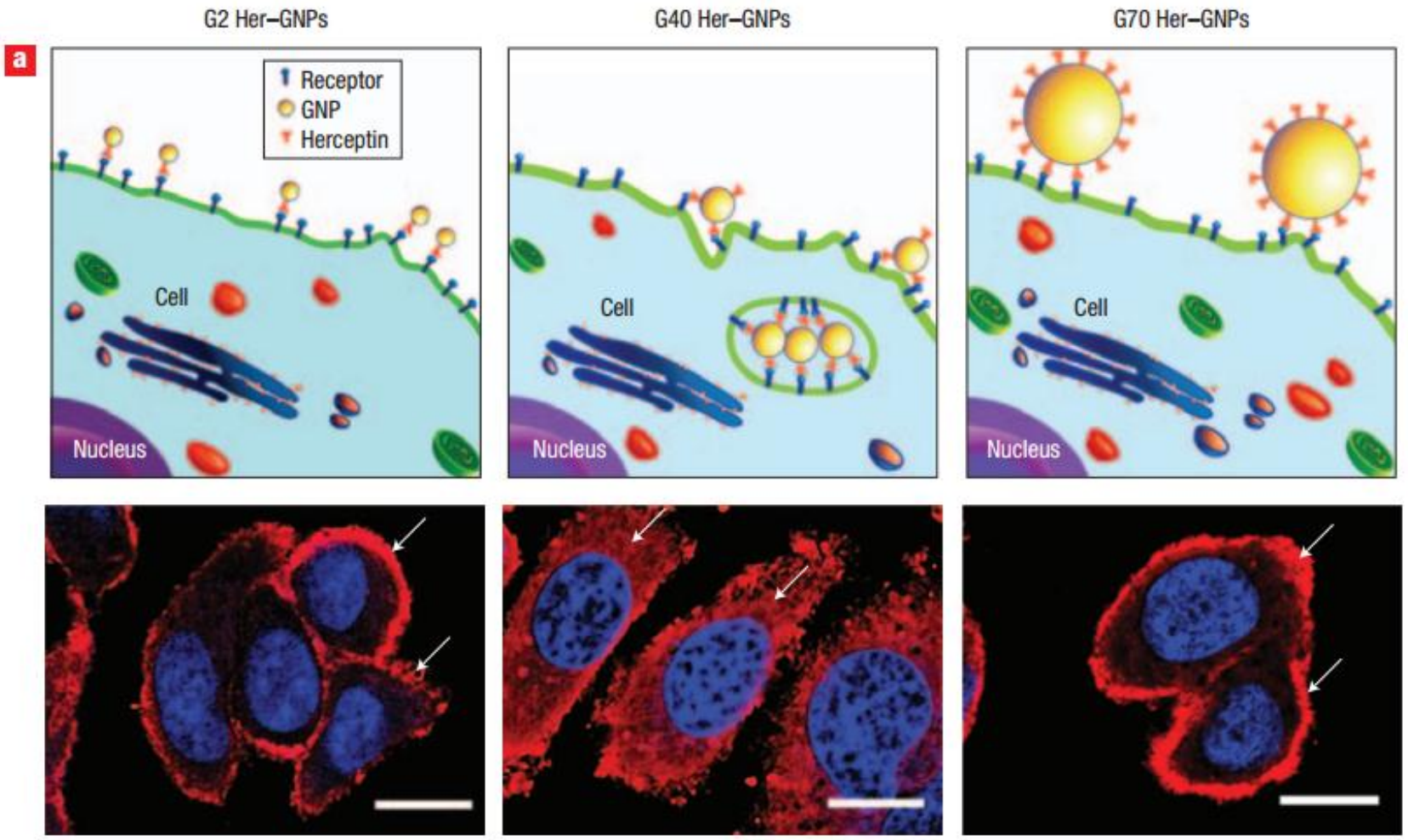


Influencia del tamaño del NV y la endocitosis mediada por receptor



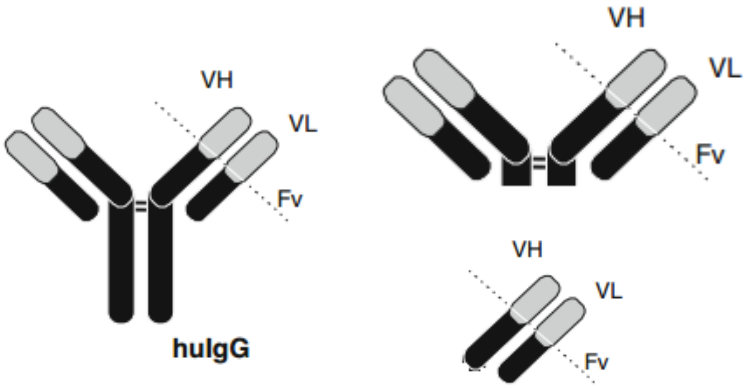


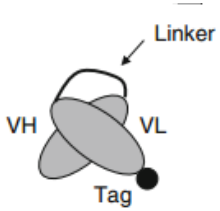
Influencia del tamaño del NV y la endocitosis mediada por receptor

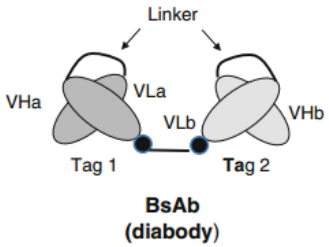


Targeting Activo: Variabilidad de anticuerpos

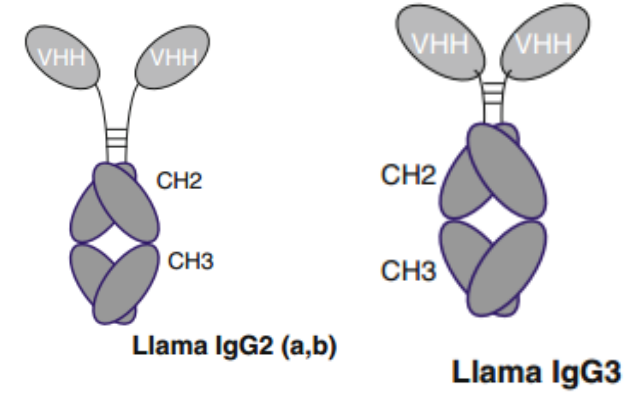
Anticuerpos convencionales



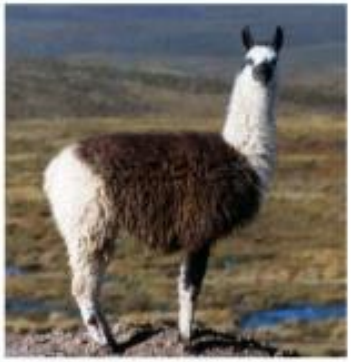


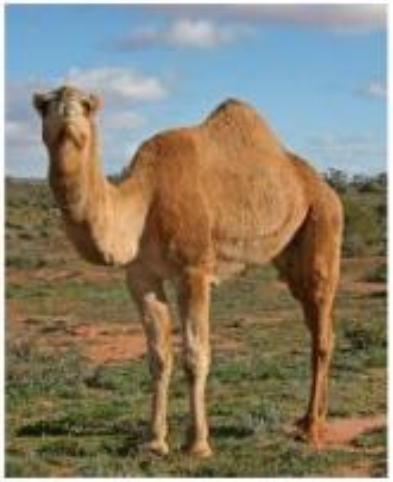


Anticuerpos de camélidos: de dominio único

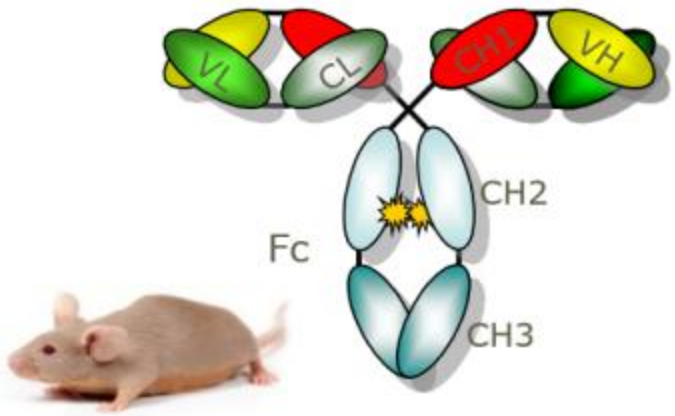


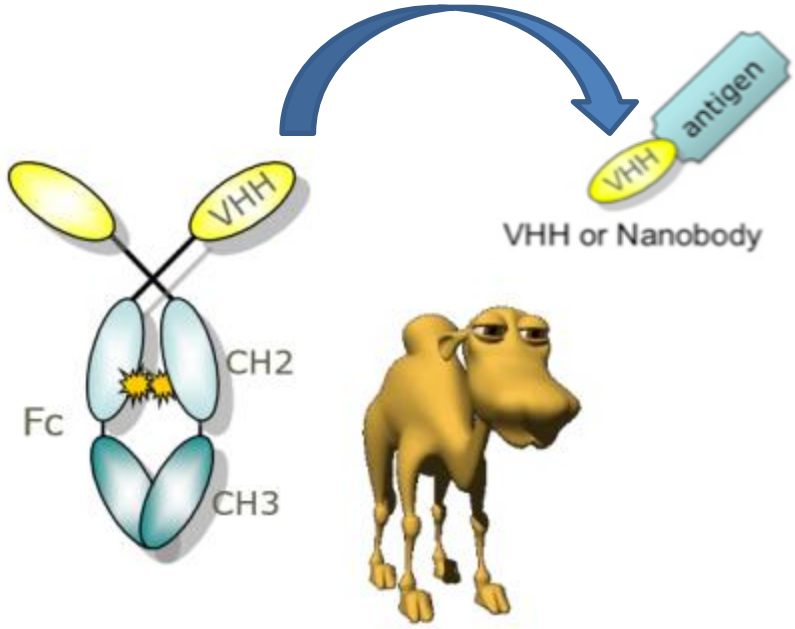
Nanoanticuerpos





Nanoanticuerpos

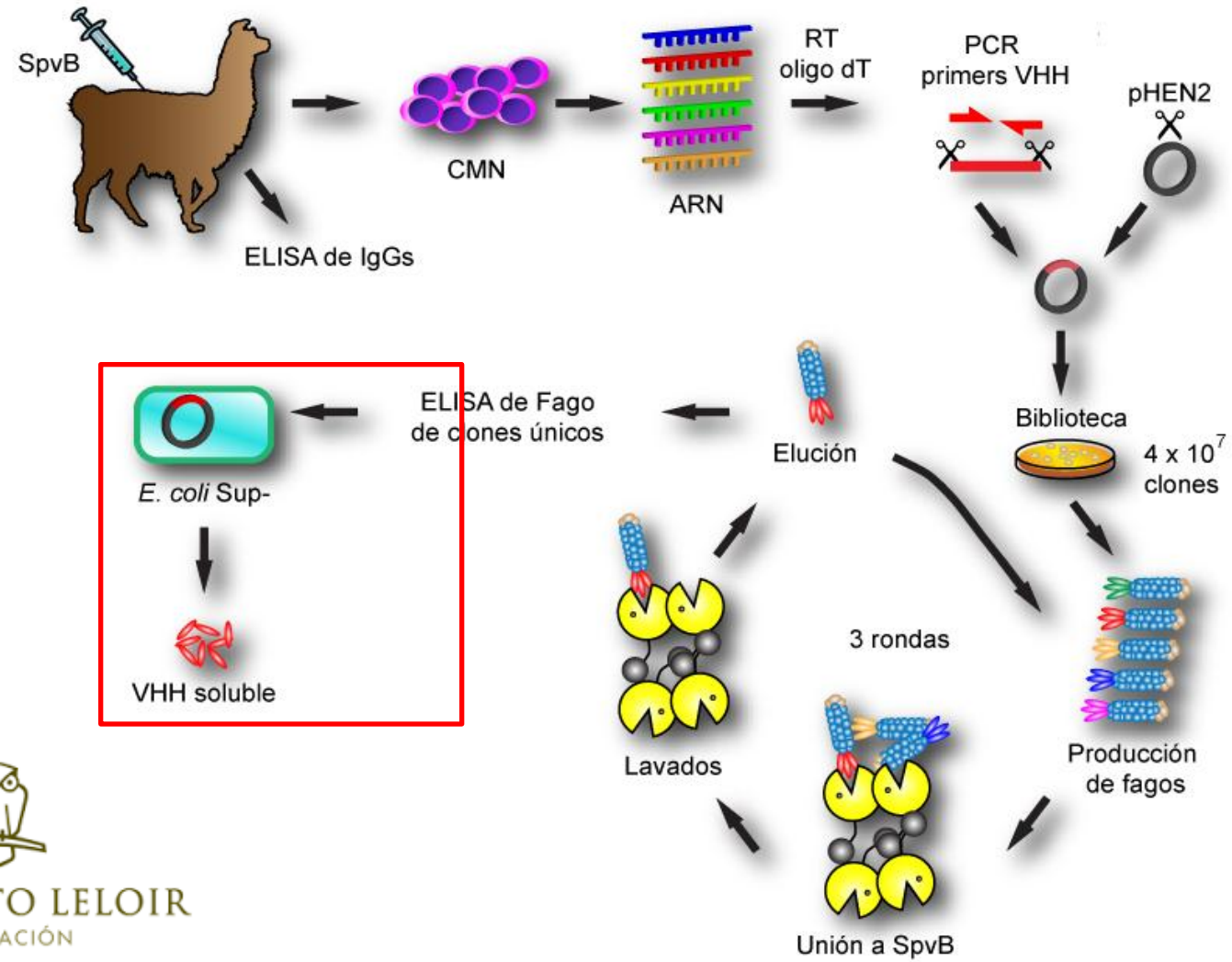




VHH:
Variable chain of
Heavy chain of
Heavy antibodies

- Tamaño reducido (1/10 respecto Ab)
- Alta estabilidad (temperatura, acidez)
- Poco inmunogénicos
- Bajos costos de producción
- Fácil manipulación genética

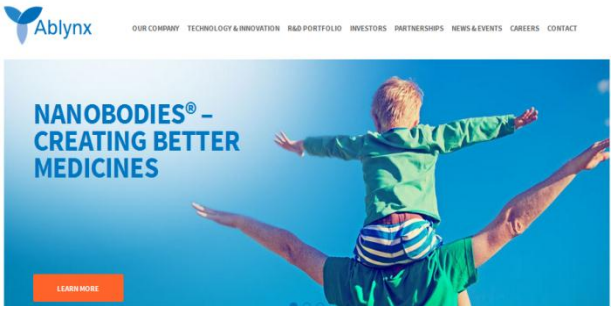
Obtención de Nanoanticuerpos



INSTITUTO LELOIR
FUNDACIÓN

PICT- Start up 2010-1263
Empretecno 2010-50

Nanoanticuerpos de Ablynx



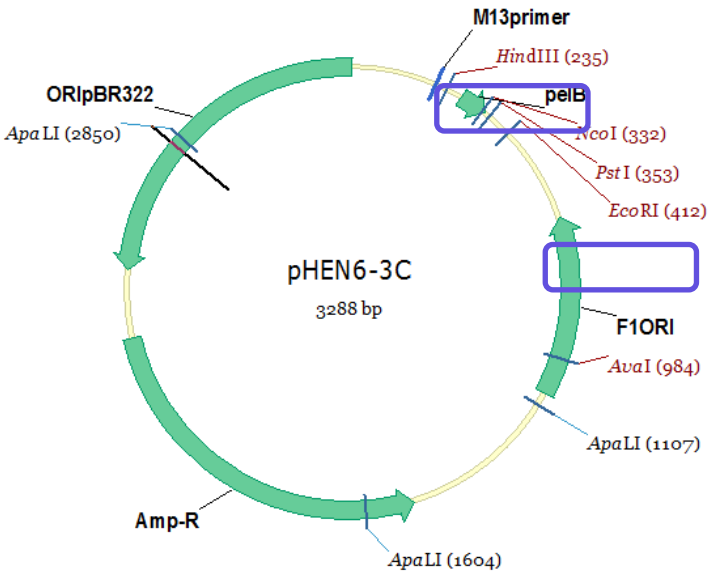
Clinically validated targets
First-in-class

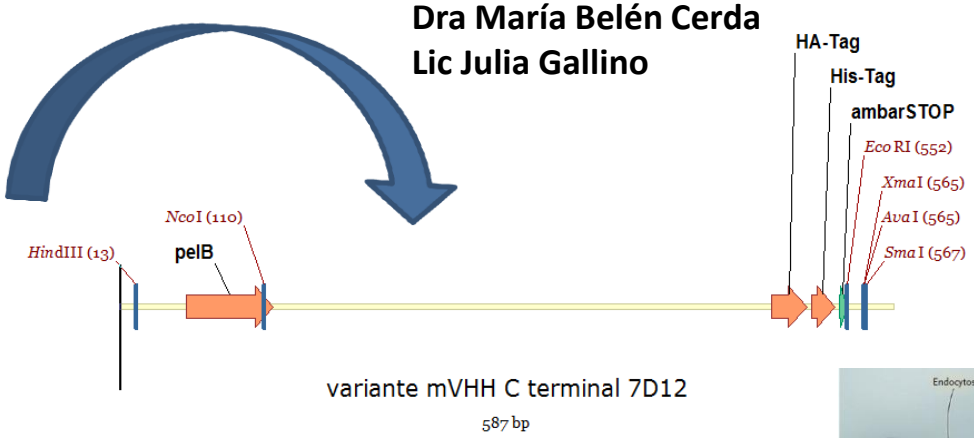
	Therapeutic area	Product name	Target	Discovery	Pre-clinical	Phase I	Phase II	Phase III	Filing
FULLY OWNED	Haematology	caplacizumab	vWF						
	Respiratory	ALX-0171	RSV						
	Oncology/ Immuno-oncology	Various							
	Inflammation/ Immunology	Various							
	Ocular	Various							
	Other	Various							
PARTNERED	Inflammation/ Immunology	ALX-0061	IL-6R						
		ALX-0761	IL-17F/IL-17A						
		ozoralizumab	TNFα				Japan		
							Greater China		
		Various							
	Oncology/ Immuno-oncology	Various	VEGF/Ang2						
	Bone disorders	ALX-0141	RANKL				Greater China		
	Neurology								
	Various		CXCR2						
	Other	Various							

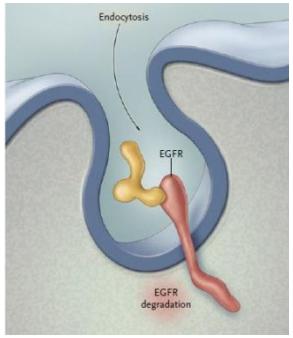
abbvie
Merck
TAISHO
EDDINGE PHARM
Merck
Boehringer Ingelheim
Boehringer Ingelheim
MERCK
Merck
EDDINGE PHARM
MERCK
genzyme A SANOFI COMPANY
NOVARTIS
Boehringer Ingelheim
novo nordisk

Obtención de Nanoanticuerpos contra EGFR

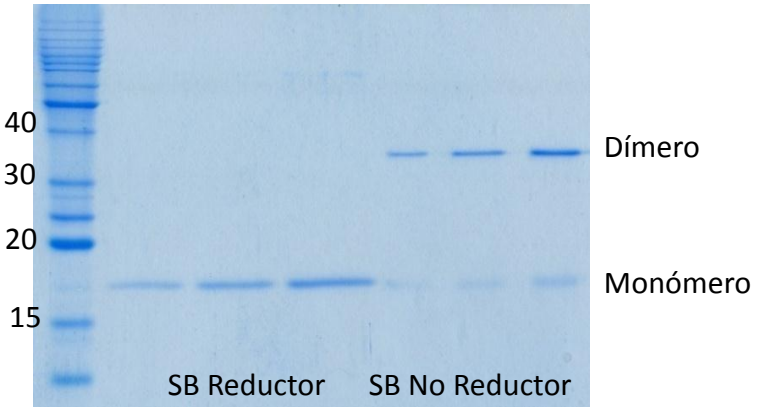
Dra María Belén Cerda
Lic Julia Gallino

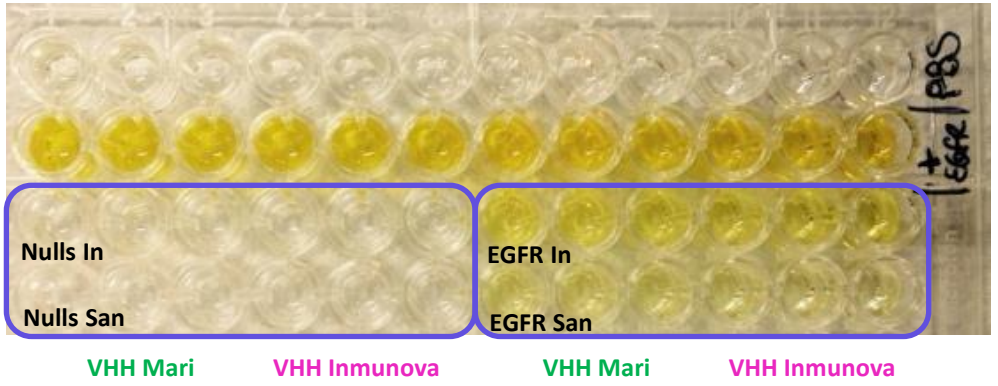




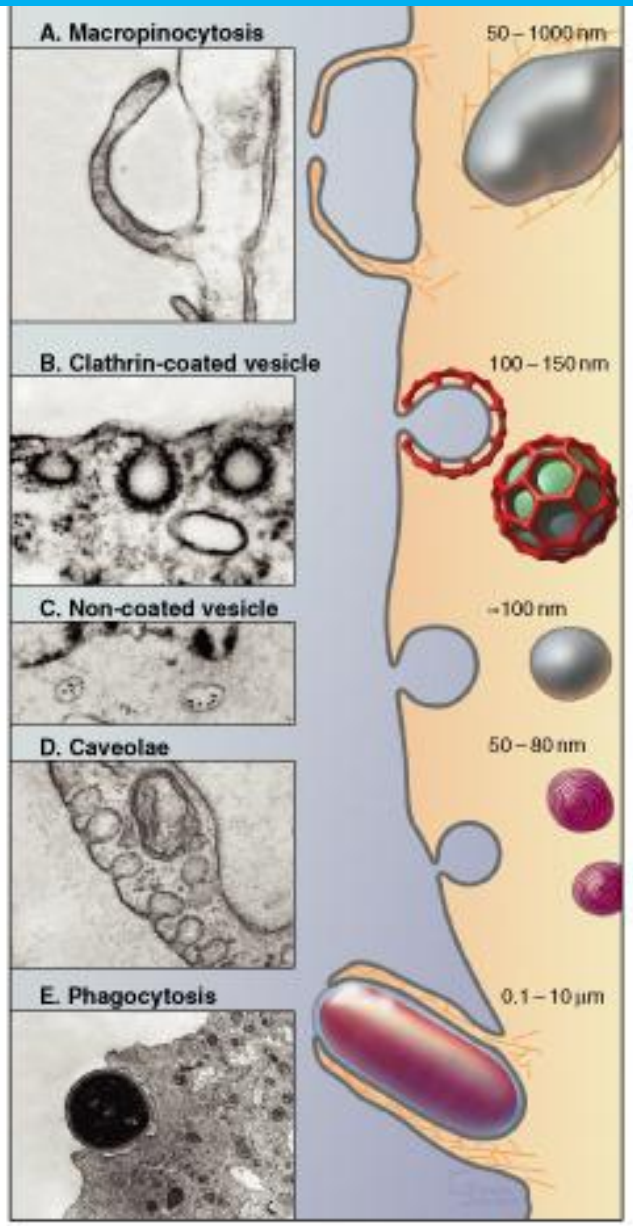


Periplasma de E. coli WK6 → Purificación HisTrap HP → Diálisis y concentración

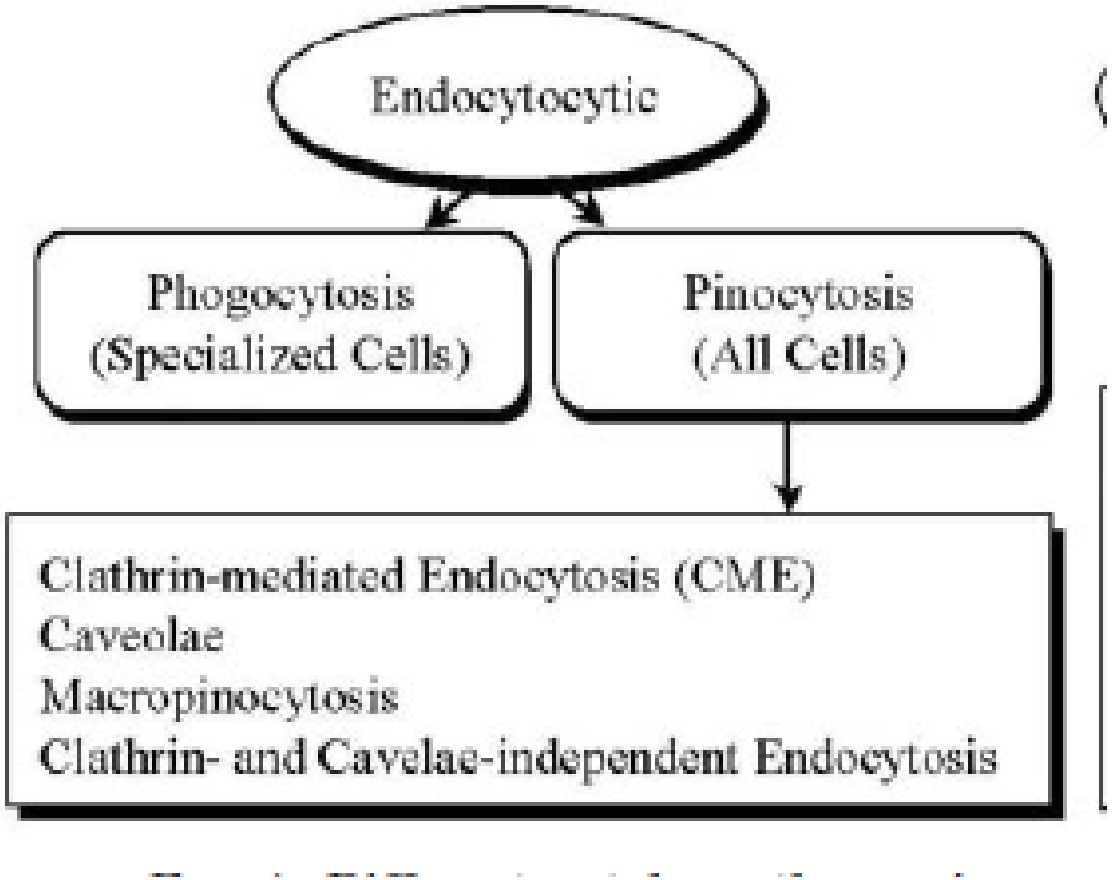




Trafico intracelular



Vías endocíticas

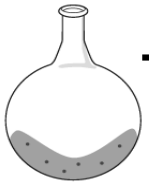


Consideraciones para el diseño de NV

Particle property	Vascular transport	Transvascular transport	Interstitial transport	Cellular uptake	Optimal overall condition
Size	> 12 nm and < 200 nm	> 12 nm < 60 nm	> 5 nm < 50 nm	~ 50 nm	12-50 nm
Surface charge	Neutral	Cationic	Neutral	Cationic	Neutral/slightly positive
Shape	Any shape	Elongated (high aspect ratio)	Elongated (high aspect ratio)	Not conclusive	Elongated

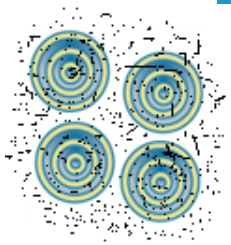
Tecnologías de síntesis: Desarrollo de un inmunoliposoma

Lípidos en solventes



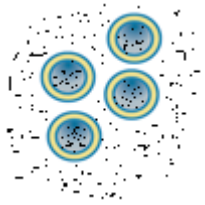
Secado film lipídicos

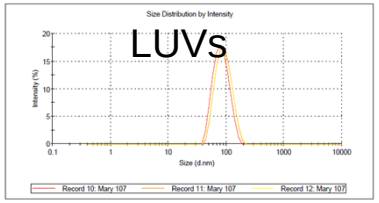
Encapsulados
Moléculas hidrófílicas e hidrofóbicas

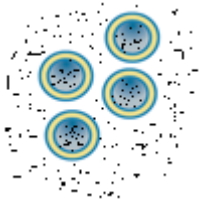


MLVs

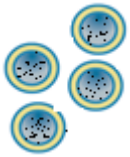
Extrusion

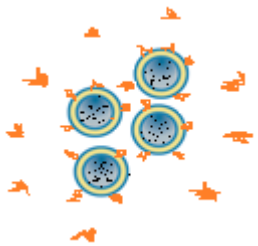




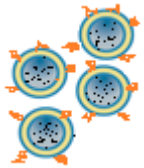


Diálisis o cromatografía





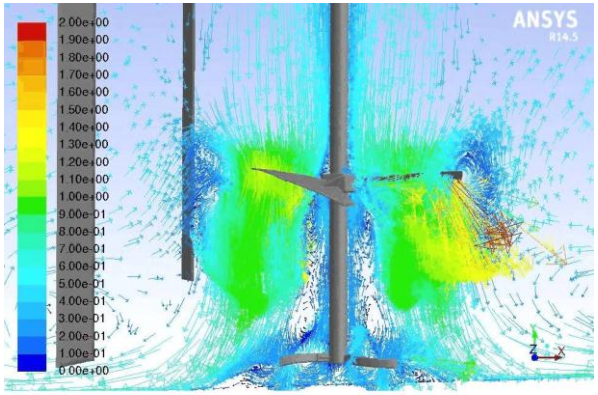
Diálisis o cromatografía

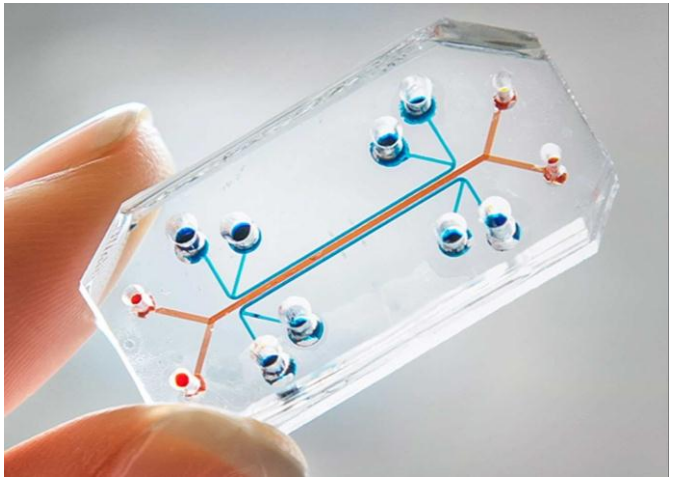


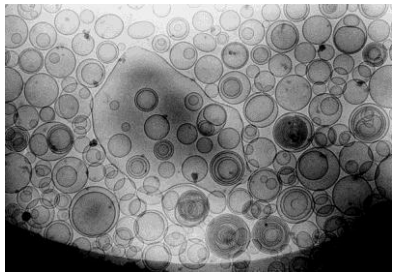
➢Caracterización del producto final: cuantificación de lípidos, cuantificación de molécula encapsulada, medida del tamaño, carga superficial, etc

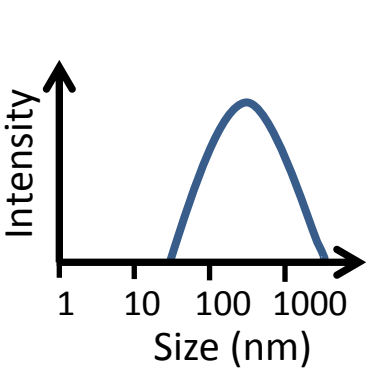
Tecnologías tradicionales

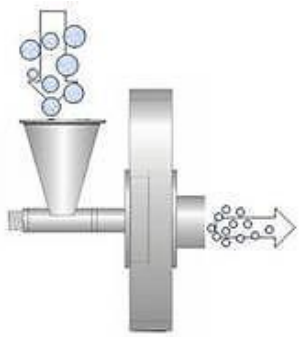
Tecnología Microfluidica

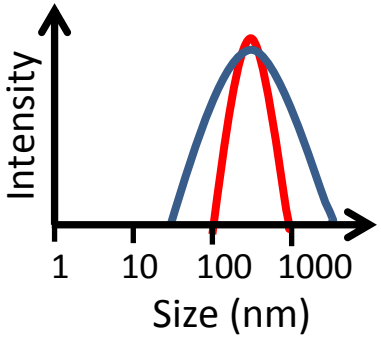












Variaciones lote a lote ✗

Baja eficiencia de encapsulation ✗

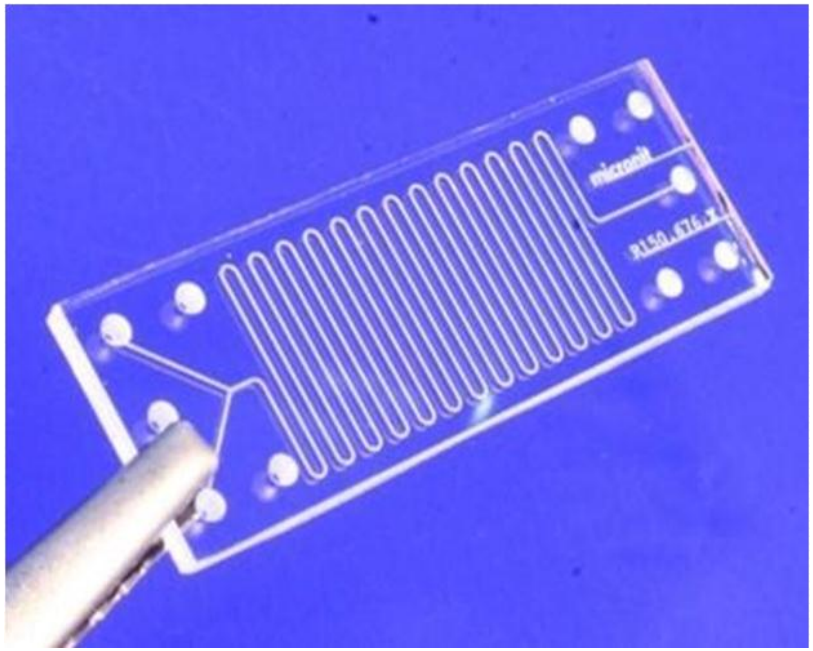
Alto consumo de tiempo ✗

Alto costo ✗

¿Qué es la microfluídica?

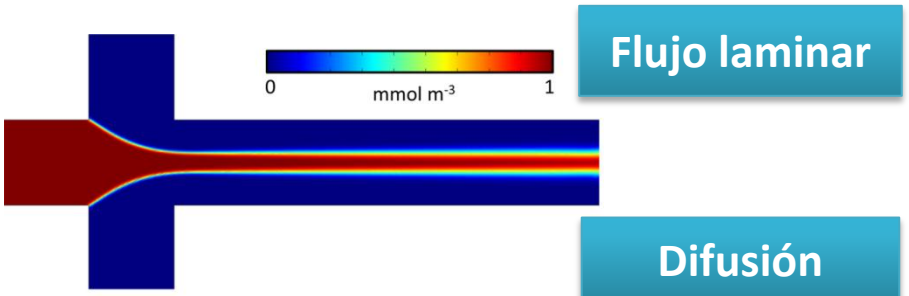
"Es el manejo de los fluidos a escala microscópica"

- **Difusión**
- **Flujo laminar**
- **Elevada relación superficie/volumen**
- **Predecible matemáticamente**



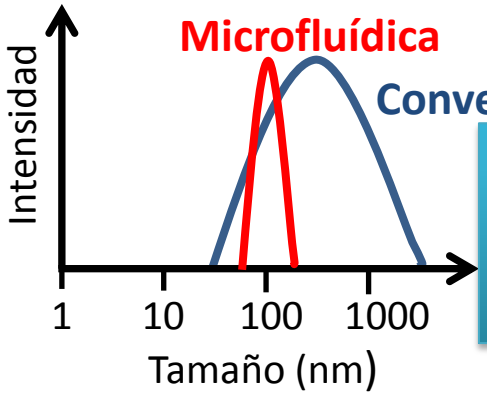
- Reducción del tamaño de los dispositivos
- Reducción de los volúmenes de reactivos y muestras
- Reducción de costos

Síntesis de nanomedicinas por microfluídica



Flujo laminar

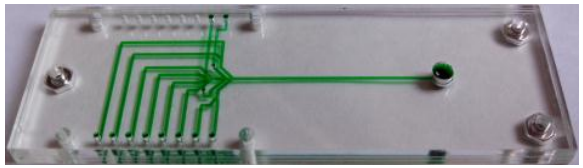
Difusión

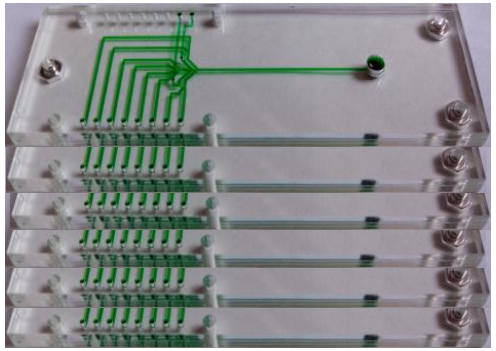


Poblaciones homogéneas sin post-procesado

Reproducibilidad ✓

Rendimiento ✓



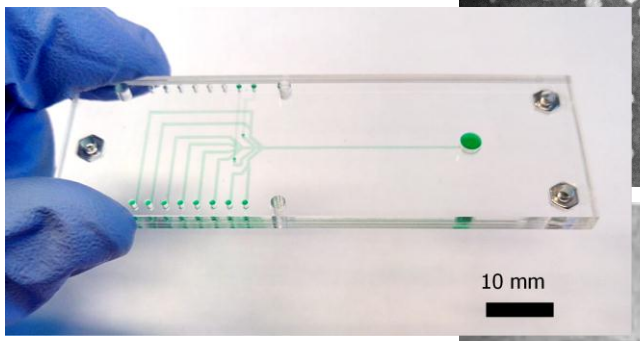


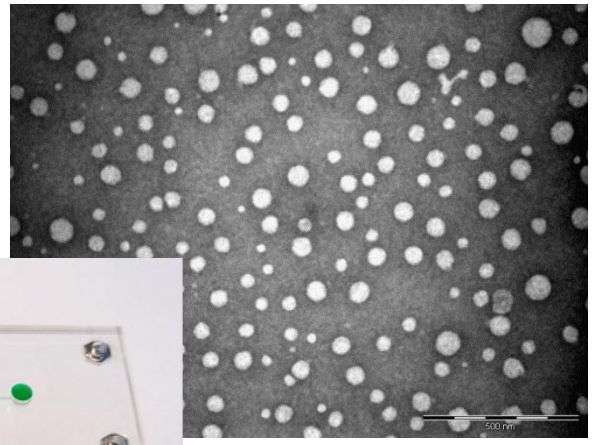
Escalado y Costos ✓

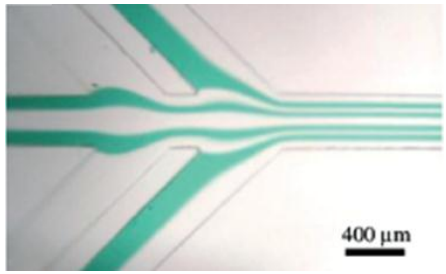

Plamic
Desarrollamos Nanoproductos a Medida
con Tecnología Microfluídica
IB50K 2015 QUINTA EDICIÓN
CONCURSO
DE PLANES DE NEGOCIO
PARA JÓVENES EMPRENDEDORES
IB50K
AGENCIA
NACIONAL DE PROMOCION
CIENTIFICA Y TECNOLOGICA
PICT-START-UP 2016-0013

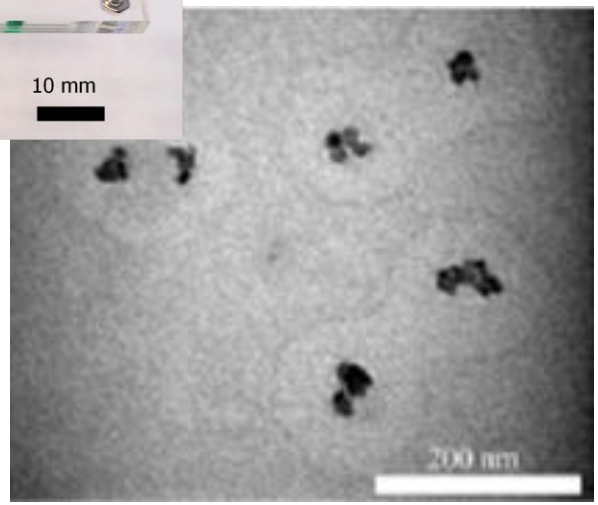
PLAMIC

Dr. Alvaro Conde

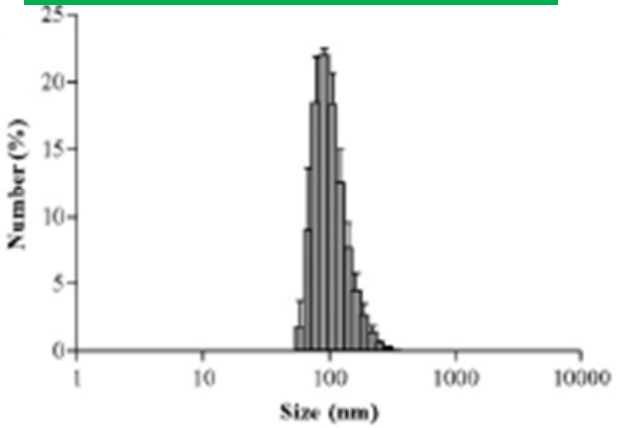






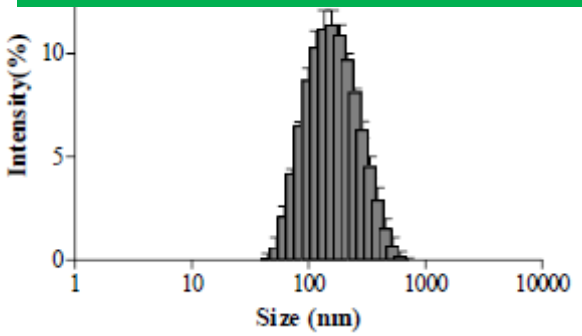


Liposomas



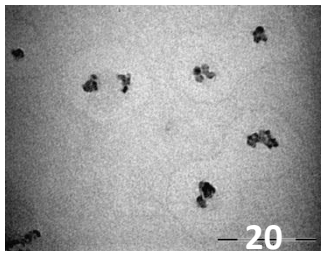
DLS: 90 ± 28 nm (PI 0.165)

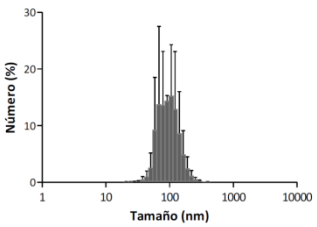
Magnetoliposomas

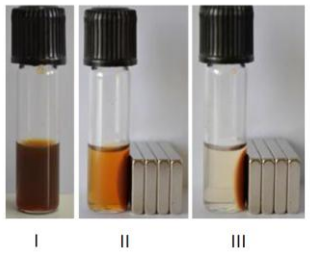


Desarrollos en proceso

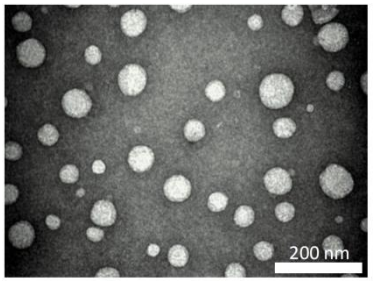
Nanopartículas Magnéticas

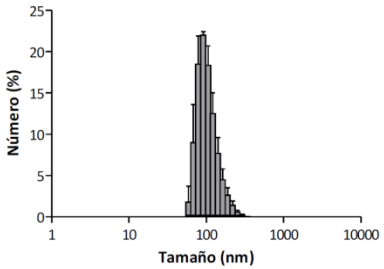






Conde et al. **Lab Chip**, 2014, 14, pp. 4506-4512





Oxaliplatino (Droga hidrofílica)

Method	Size (nM)	PdI	Drug/lipid ratio
Conventional	167,0	0,12	2,3
Microfluidic	116,4	0,17	6,4

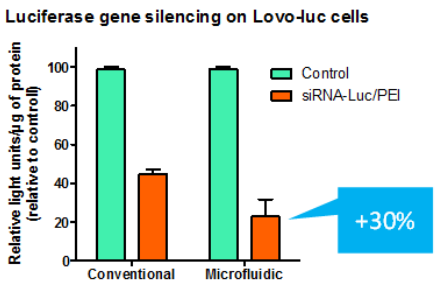
+278%

Lactsil-carborano (Droga hidrofóbica)

Method	Size (nM)	PdI	B/P ratio
Conventional	144,4	0,24	2,4
Microfluidic	89,6	0,18	3,5

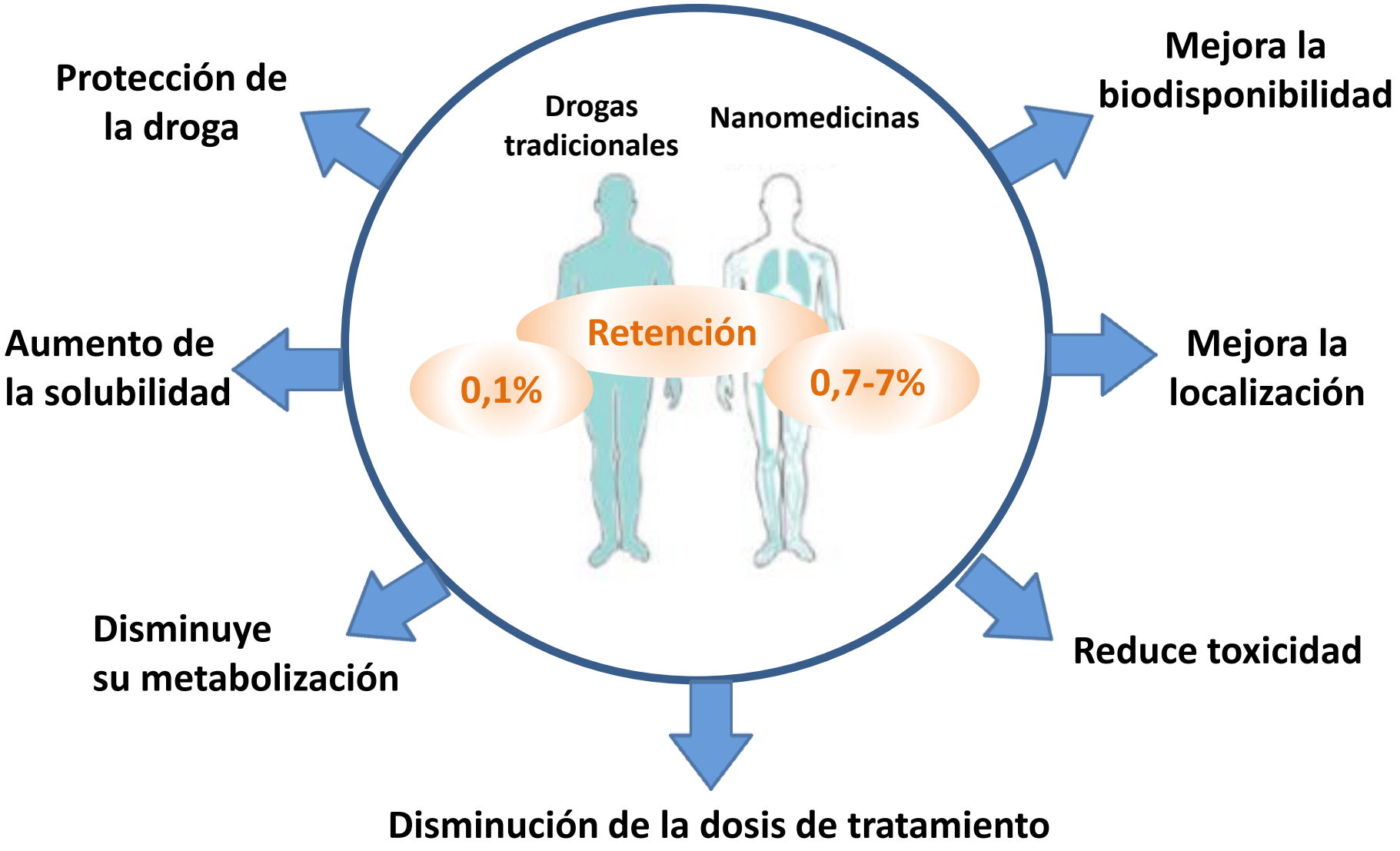
+46%

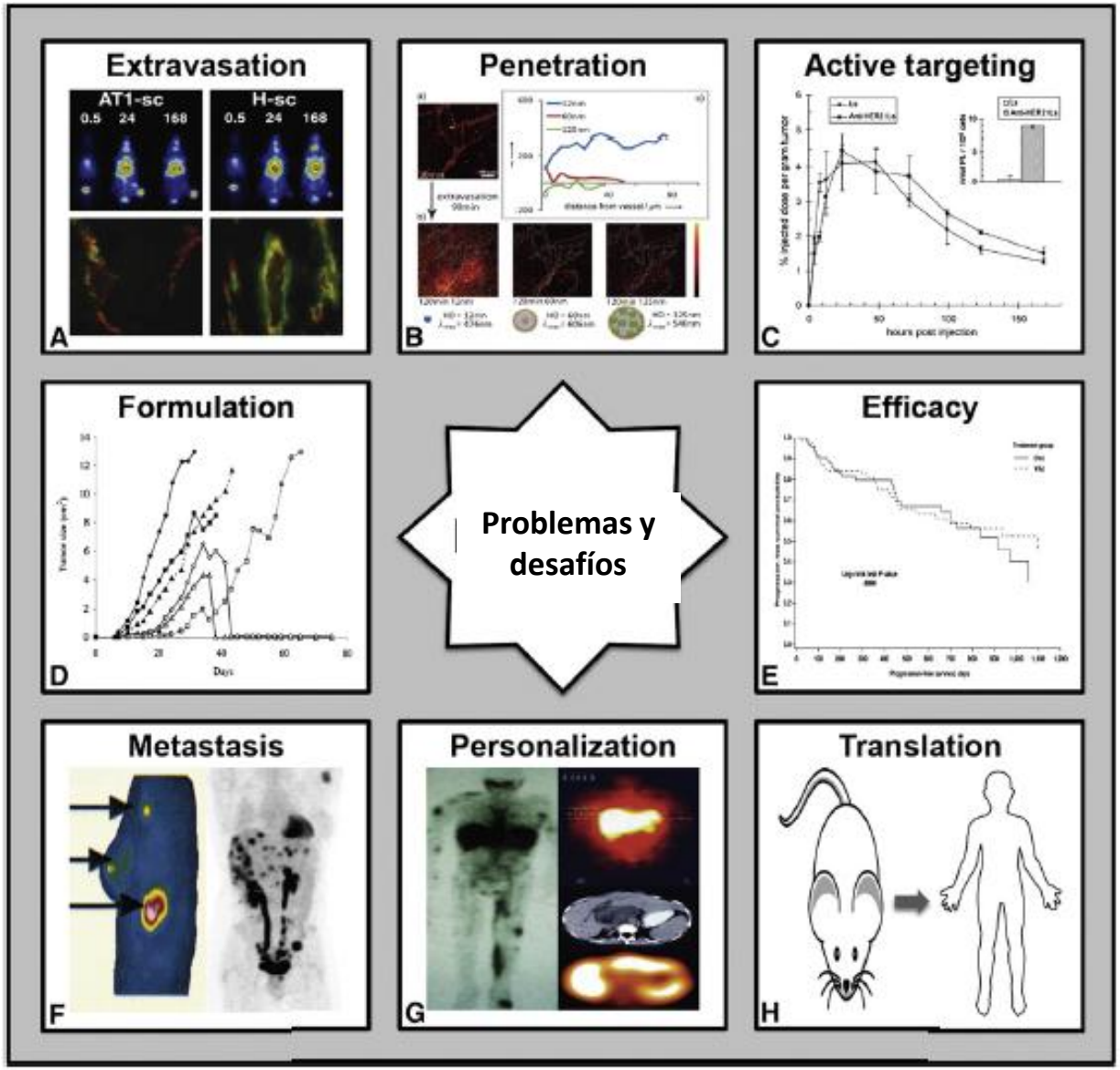
Complejos siARN/PEI

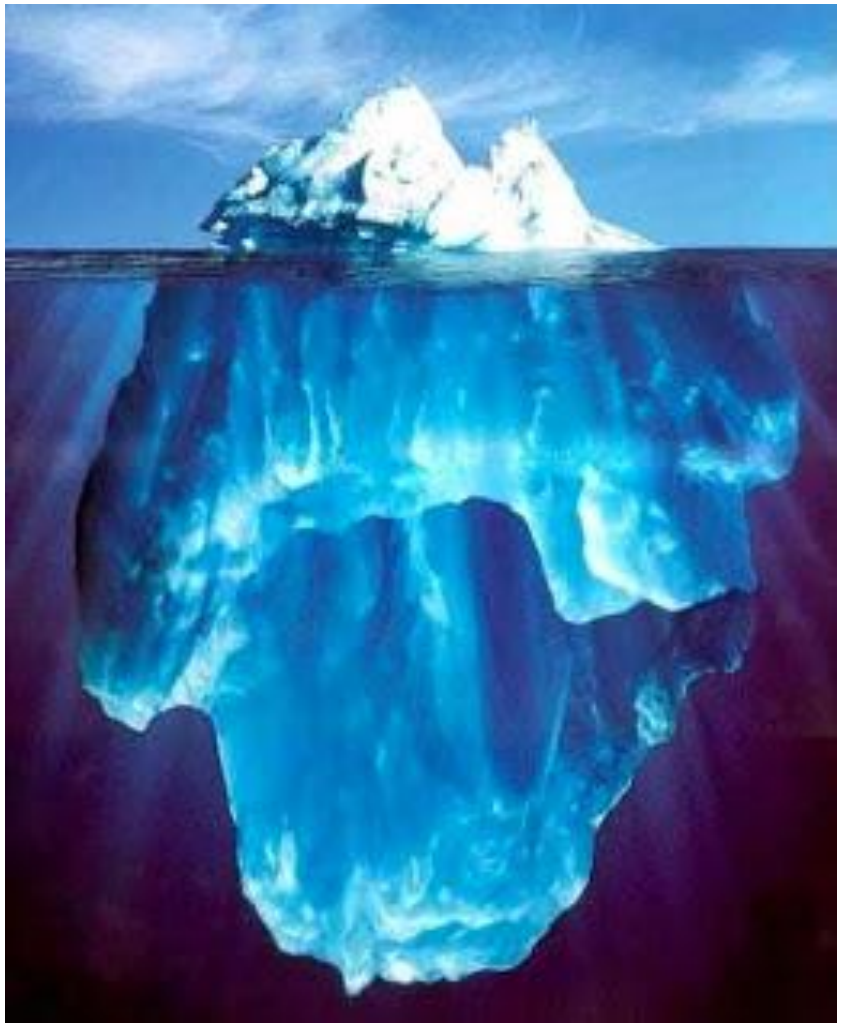


	Conventional	Microfluidic
Total time of process	8 hours	1,5 hours
Synthesis time (per sample)	2,5 hours	5 min

Nanomedicinas




Extravasation
AT1-sc
H-sc
0.5 24 168
0.5 24 168
A
Penetration
B
Active targeting
C
Formulation
D
Problemas y desafíos
Efficacy
E
Metastasis
F
Personalization
G
Translation
H



En el 1985 muchas cosas nos parecían imposibles de pensar…que ocurrieran en el 2015

Video Conferencias

Gafas Inteligentes

Televisores Smart TV

1. Animaciones 3D

Cobro Electrónico

Drones

Hologramas

3. Patineta Voladora

Laboratorio Nanomedicina

- Dra. Lucia Policastro
- Dra. María Belén Cerda
- Dr. Alvaro Conde
- Lic. Rodrigo Lloyd
- Lic. Florencia Giannoni
- Lic. Julia Gallino.

- Ing. Mario Gadan
- María Silvia Olivera

Colaboraciones

- José Luis Crudo (CAE)
- Noemí Nevares (CAE)
- Manuel Sztenjberg(CAE)
- Sara Gonzalez (CAC)
- Agustina Portu (CAC)
- Osvaldo Podhajcer (FIL)
- María Bastianello (CEMIC)
- Patricia Gargallo (CEMIC) Cecilia Albornoz (CAC)
- Gabriela Leyva (CAC)
- Lic. María Elena Iezzi (FIL)
- Luigi Pansa (Universidad Piamonte, Italia)
- Chirstian Plank (Universidad Técnica Munich, Alemania)
- Pablo Brumovsky (Universidad Austral)


Muchas gracias!
policast@cnea.gov.ar
luciapolicastro@yahoo.com