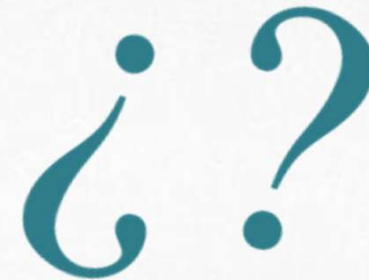


Nanotechnology-based Drug Delivery  
Systems (nanotDDS) against Cancer  
Stem Cells (CSC)

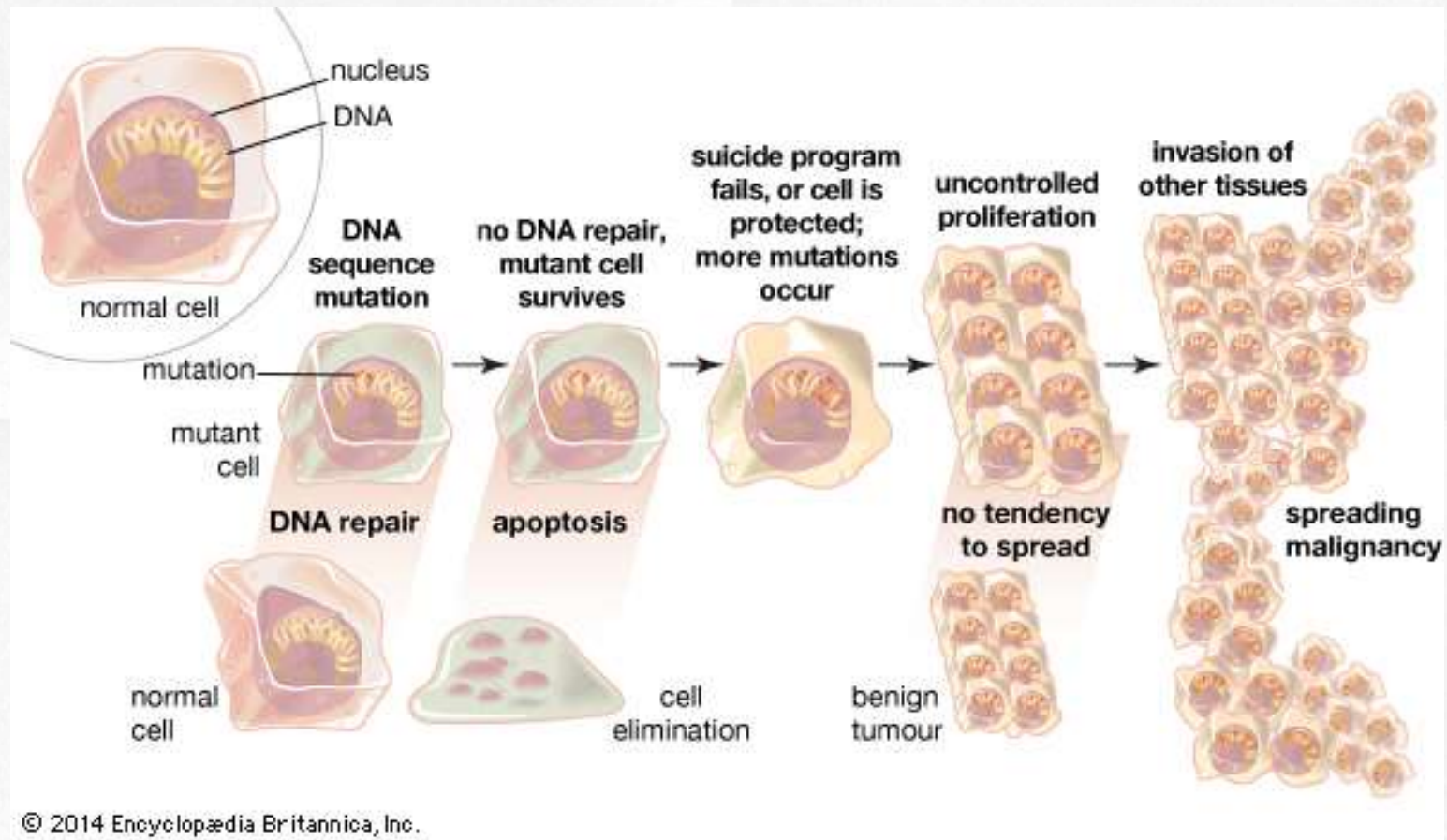
Fernanda da Silva Andrade

## WHY CANCER



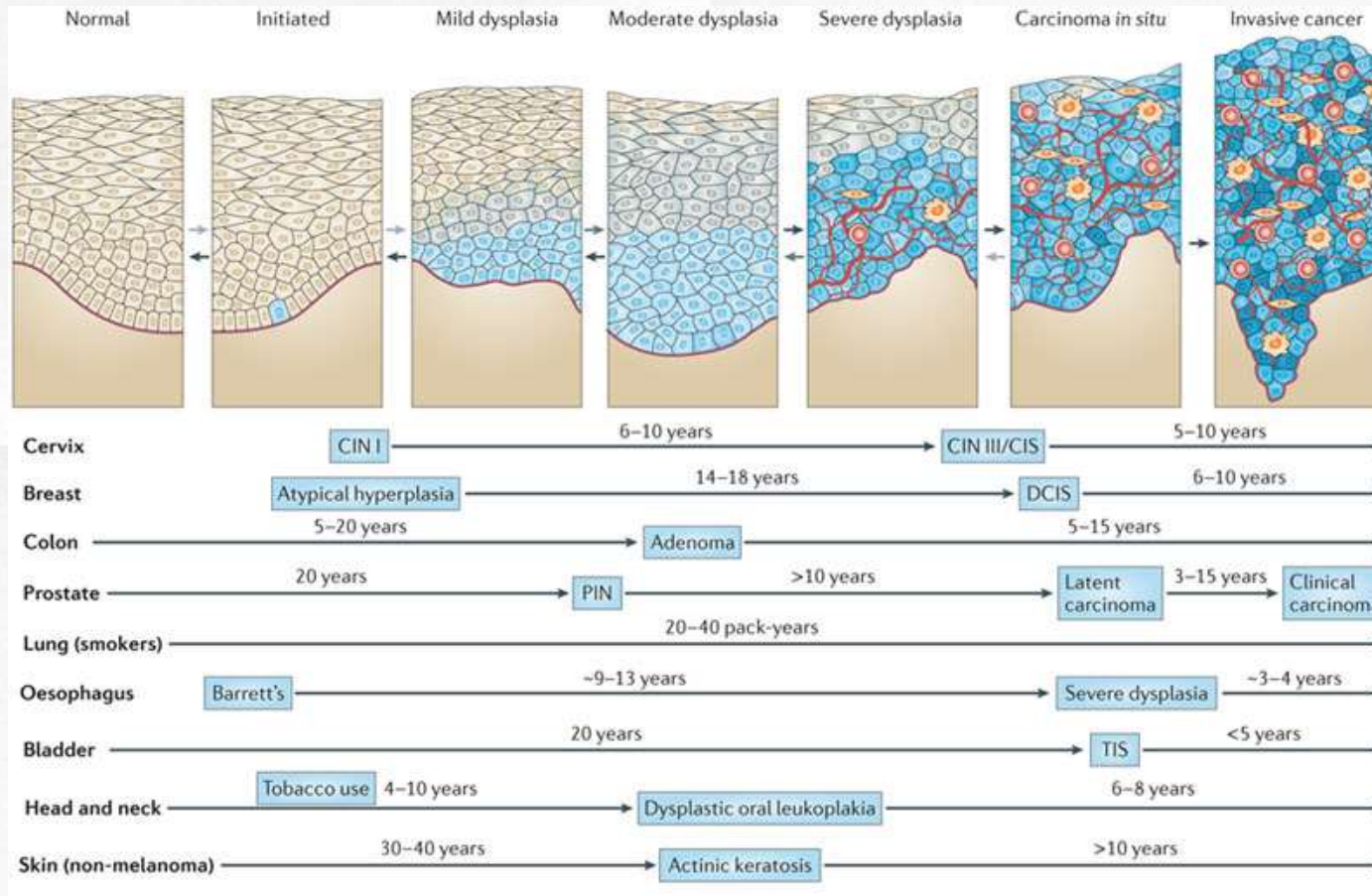
- Cancer is a generic term for a **large group** of diseases that can affect any part of the body.
- The **rapid creation of abnormal cells** that grow beyond their usual boundaries, and which can then invade adjoining parts of the body and spread to other organs.

# Cancer





# Cancer



Asad Umar, et al, *Nature Reviews Cancer* 12, 835-848 (2012)

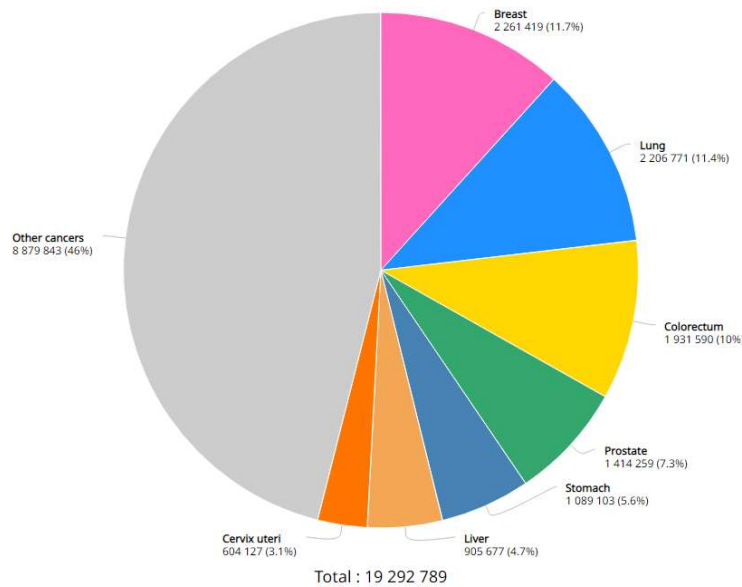


# Cancer Statistics

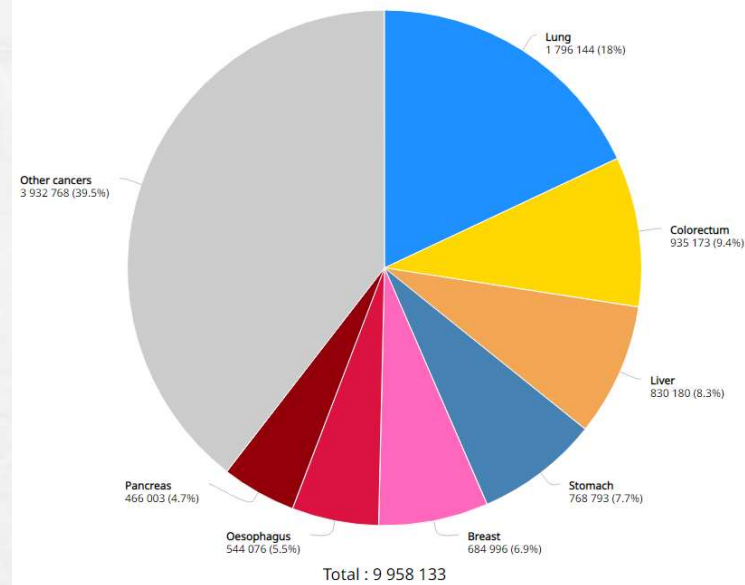
## ▣ In 2020:

- 10 million cancer related deaths
- 19.3 million new cases
- New cases are expected to rise by to 29.5 million up to 2040

Estimated number of new cases in 2020, World, both sexes, all ages



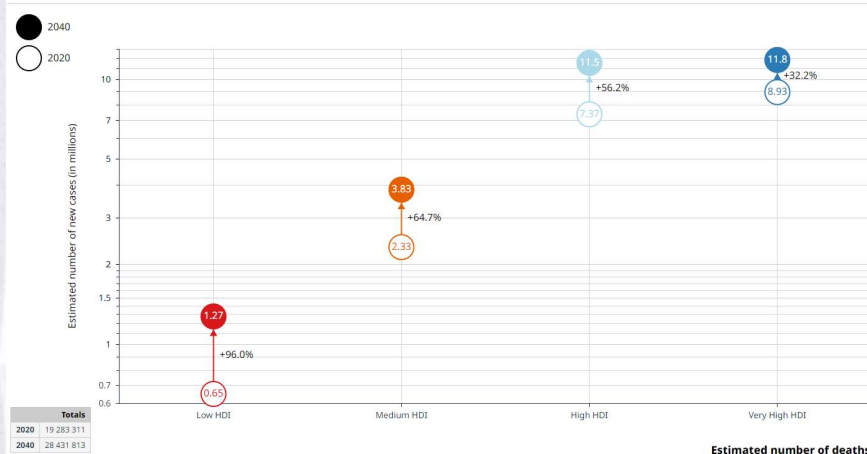
Estimated number of deaths in 2020, World, both sexes, all ages



# Cancer Statistics

## 2040 previsions

Estimated number of new cases from 2020 to 2040, Both sexes, age [0-85+]  
 All cancers



Estimated number of deaths from 2020 to 2040, Both sexes, age [0-85+]  
 All cancers



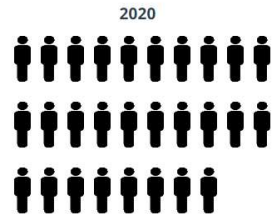
# Cancer Statistics

Data source: GLOBOCAN 2020  
Graph production: Global Cancer Observatory (<http://gco.iarc.fr/>)  
© International Agency for Research on Cancer 2022

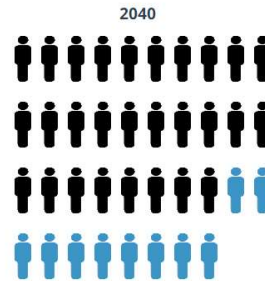
## Spain

Estimated number of new cases from 2020 to 2040, Both sexes, age [0-85+]

All cancers  
Spain



282k

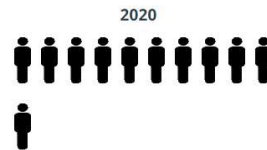


375k

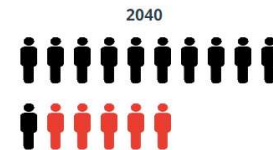


Estimated number of deaths from 2020 to 2040, Both sexes, age [0-85+]

All cancers  
Spain



113k



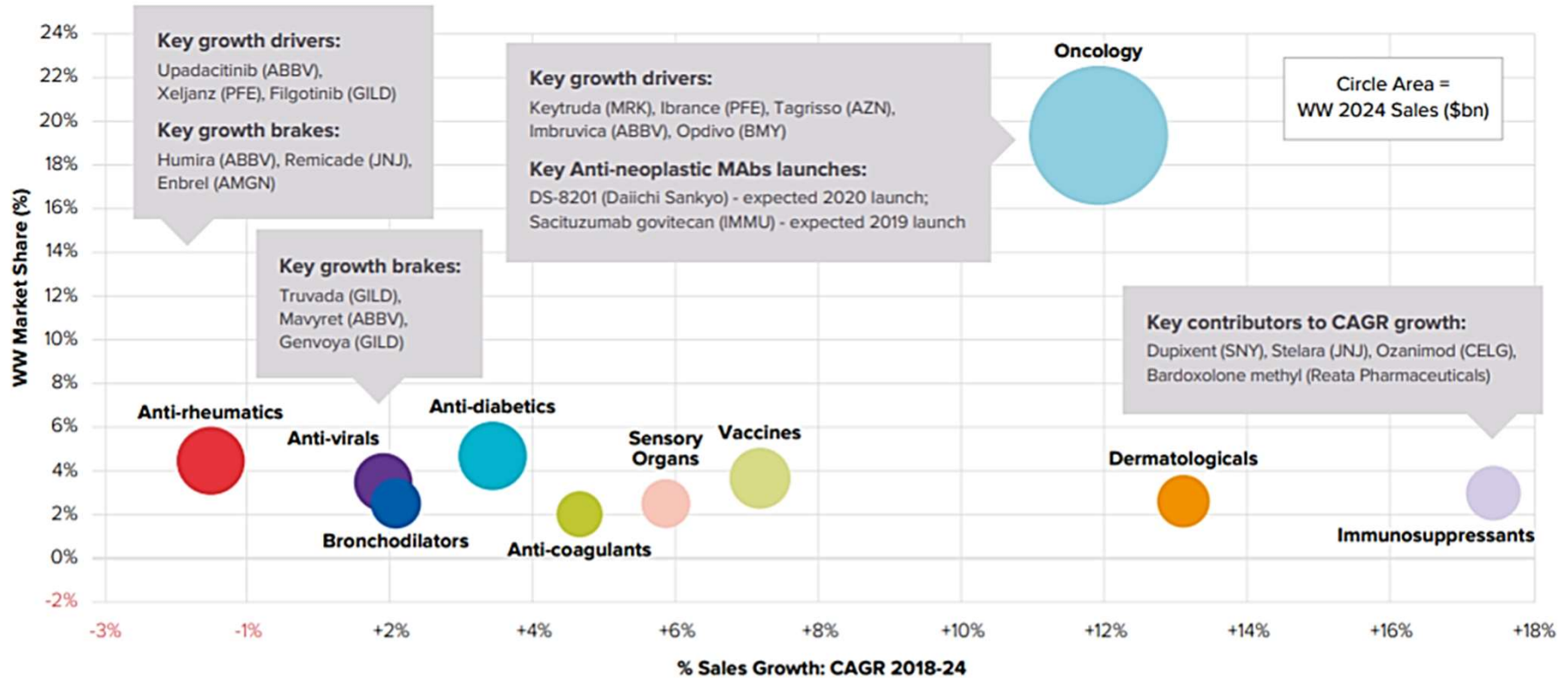
160k





# Cancer Costs

## Top 10 Therapy Areas in 2024, Market Share & Sales Growth



# Cancer Costs



## Worldwide Prescription Drug & OTC Sales by Evaluate Therapy Area (2018 & 2024: Top 10 Categories & Total Market)

Rank	Therapy Area	WW Sales (\$bn)		CAGR % Growth	WW Market Share		Chg. (+/-)	Rank Chg. (+/-)
		2018	2024		2018	2024		
1.	Oncology	123.8	236.6	+11.4%	14.3%	19.4%	+5.0pp	+0
2.	Anti-diabetics	48.5	57.6	+2.9%	5.6%	4.7%	-0.9pp	+1
3.	Anti-rheumatics	58.1	54.6	-1.0%	6.7%	4.5%	-2.3pp	-1
4.	Vaccines	30.5	44.8	+6.6%	3.5%	3.7%	+0.1pp	+1
5.	Anti-virals	38.9	42.2	+1.4%	4.5%	3.5%	-1.0pp	-1
6.	Immunosuppressants	14.2	36.1	+16.9%	1.6%	3.0%	+1.3pp	+6
7.	Dermatologicals	15.8	32.1	+12.6%	1.8%	2.6%	+0.8pp	+4
8.	Bronchodilators	28.0	30.7	+1.6%	3.2%	2.5%	-0.7pp	-2
9.	Sensory Organs	22.3	30.5	+5.3%	2.6%	2.5%	-0.1pp	+0
10.	Anti-coagulants	19.3	24.6	+4.1%	2.2%	2.0%	-0.2pp	+0

# Cancer Costs

## ▨ In 2010:

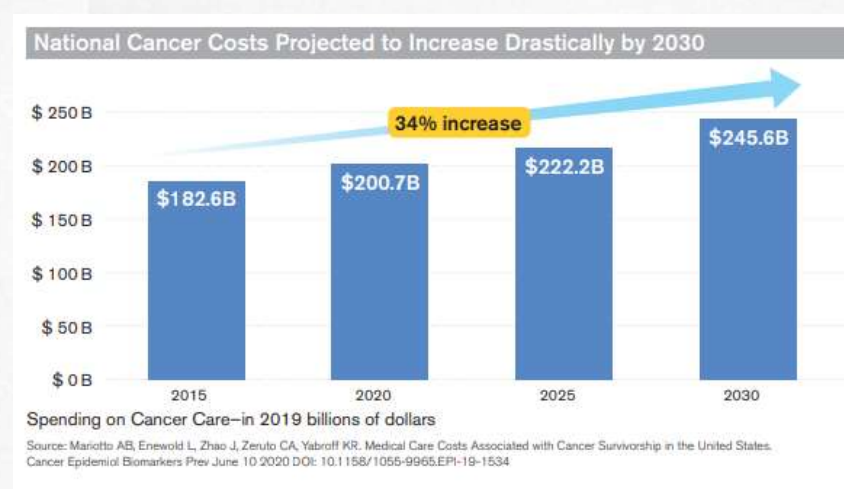
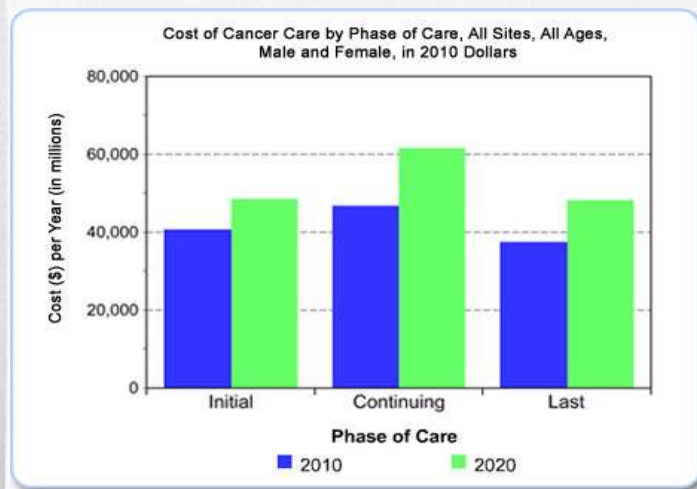
- Costs of cancer care: \$157 billion

## ▨ In 2020:

- Costs of cancer care projected: \$174 billion (calculated \$200 billion)

## ▨ In 2030:

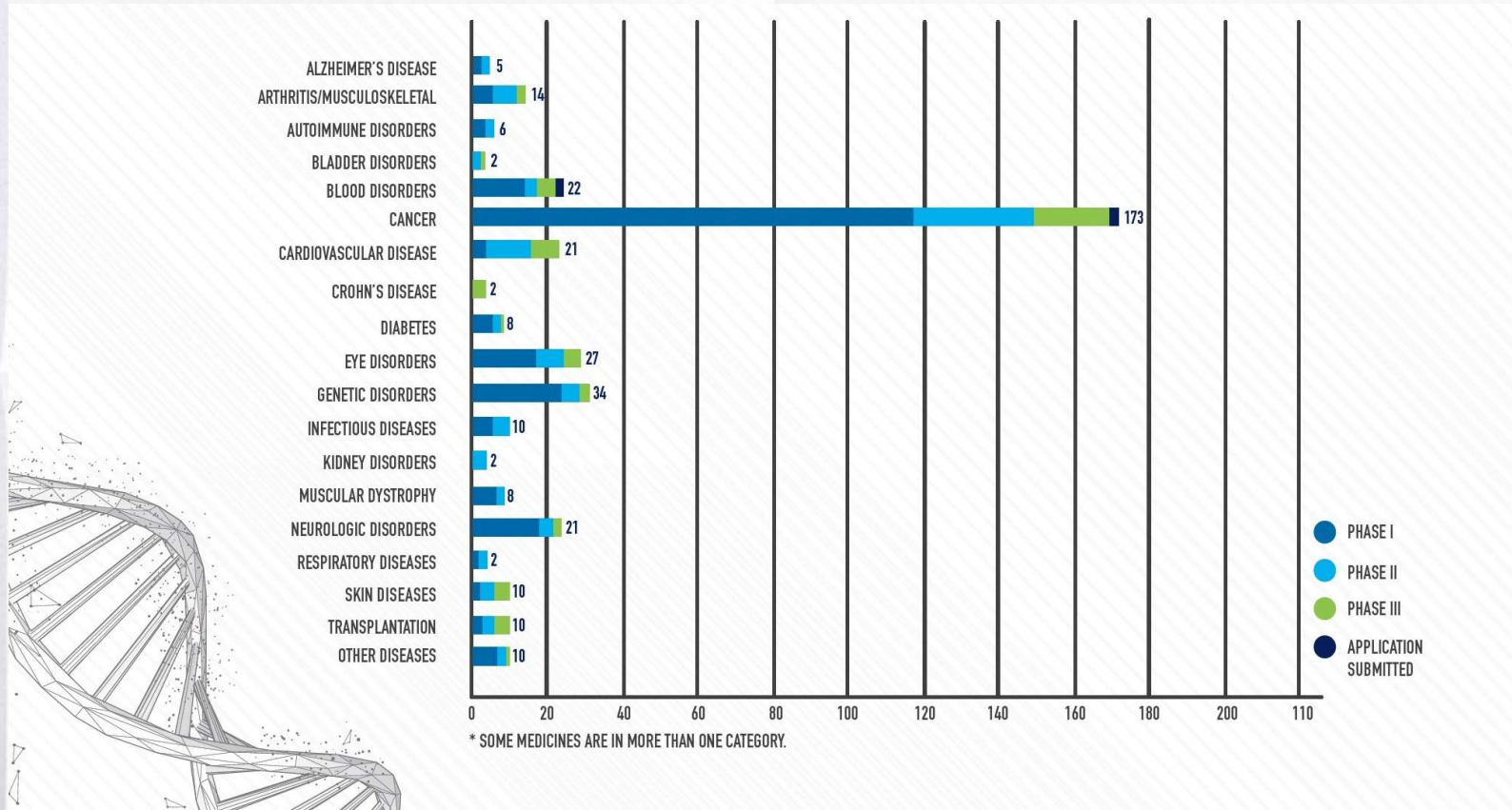
- Costs of cancer care projected: \$246 billion



Data from: USA



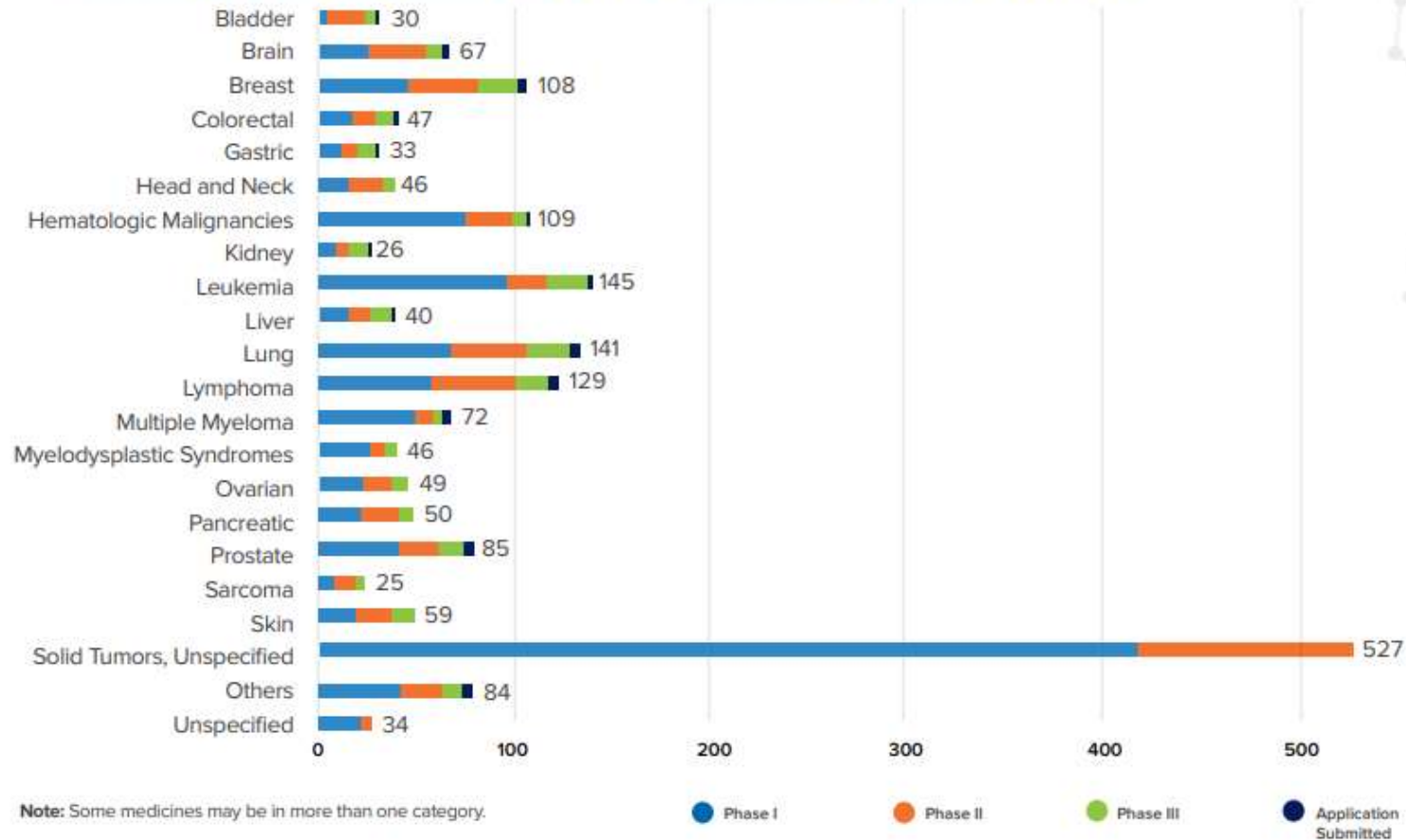
# Medicines Under Development



Data from: 2020

# Medicines Under Development for Cancer

## Medicines and Vaccines in Development for Cancer by Type

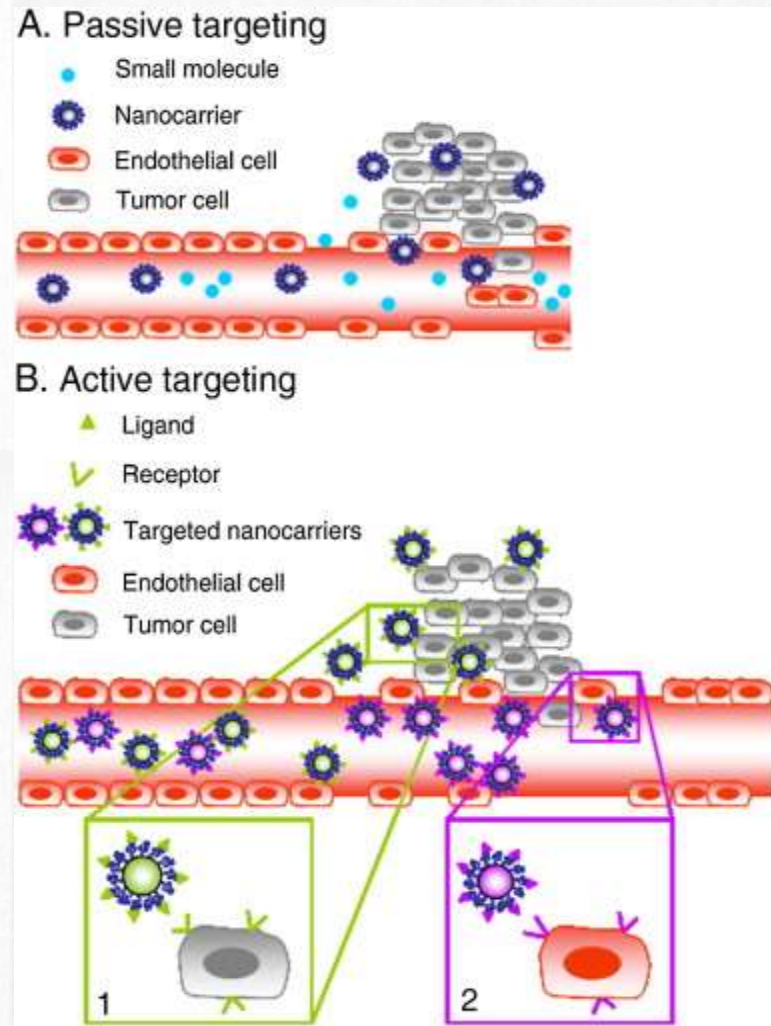


Data from: 2020

# Nanomedicine

▣ Passive – Enhanced Permeability and Retention (EPR) effect

▣ Active Targeting



Danhier, et al, J Control Release. 2010  
1;148(2):135-46



# Nanomedicine

Non Liposomal Drug

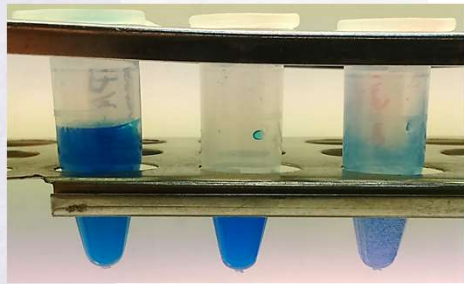


Liposomal Drug



# Nanomedicine

## Biopharmaceutical classification system (BCS)



PM-DID (in PBS) 0.25 mg/ml  
 Free DID in DMSO 0.25 mg/ml  
 Free DID in PBS 0.25 mg/ml

**Class I**  
 High Solubility  
 High Permeability  
 Diazepam, Nifedipine,  
 Diltiazem, Verapamil,  
 Quinidine, Midazolam

**Class II**  
 Low Solubility  
 High Permeability  
 Aciclovir, Captopril,  
 Amoxicillin, Penicillin

**Class III**  
 High Solubility  
 Low Permeability  
 Atorvastatin,  
 Cyclosporin, Tamoxifen,  
 Ketoconazole

**Class IV**  
 Low Solubility  
 Low Permeability  
 Paclitaxel,  
 Amphotericin B

Permeability

Solubility

Increase **solubility and dissolution rate**:  
 increase surface area/size reduction;  
 solid solutions/dispersions;  
 solvents/surfactantes

Increase **solubility and dissolution rate**  
 Increase **trans-epithelial permeability**

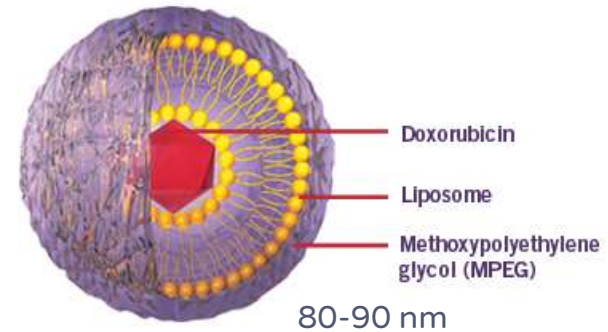
Increase **trans-epithelial permeability**:  
 permeation enhancers;  
 nanotechnology

# Nanomedicine

**Doxil®** - The first FDA-approved nano-drug (1995)

Stealth liposomes of doxorubicin

Ovarian cancer  
AIDS-related Kaposi's Sarcoma  
Multiple Myeloma

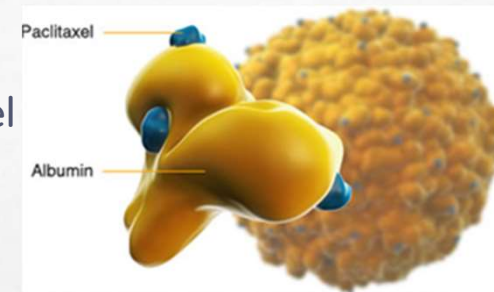


Cardiotoxicity dose:  
Doxorubicin: 570 mg/m<sup>2</sup>  
Doxil: 785 mg/m<sup>2</sup>

**Abraxane®** - FDA-approved nano-drug (2005)

Albumin nanoparticles of paclitaxel

Advanced breast cancer  
Advanced non-small cell lung cancer  
Advanced pancreatic cancer



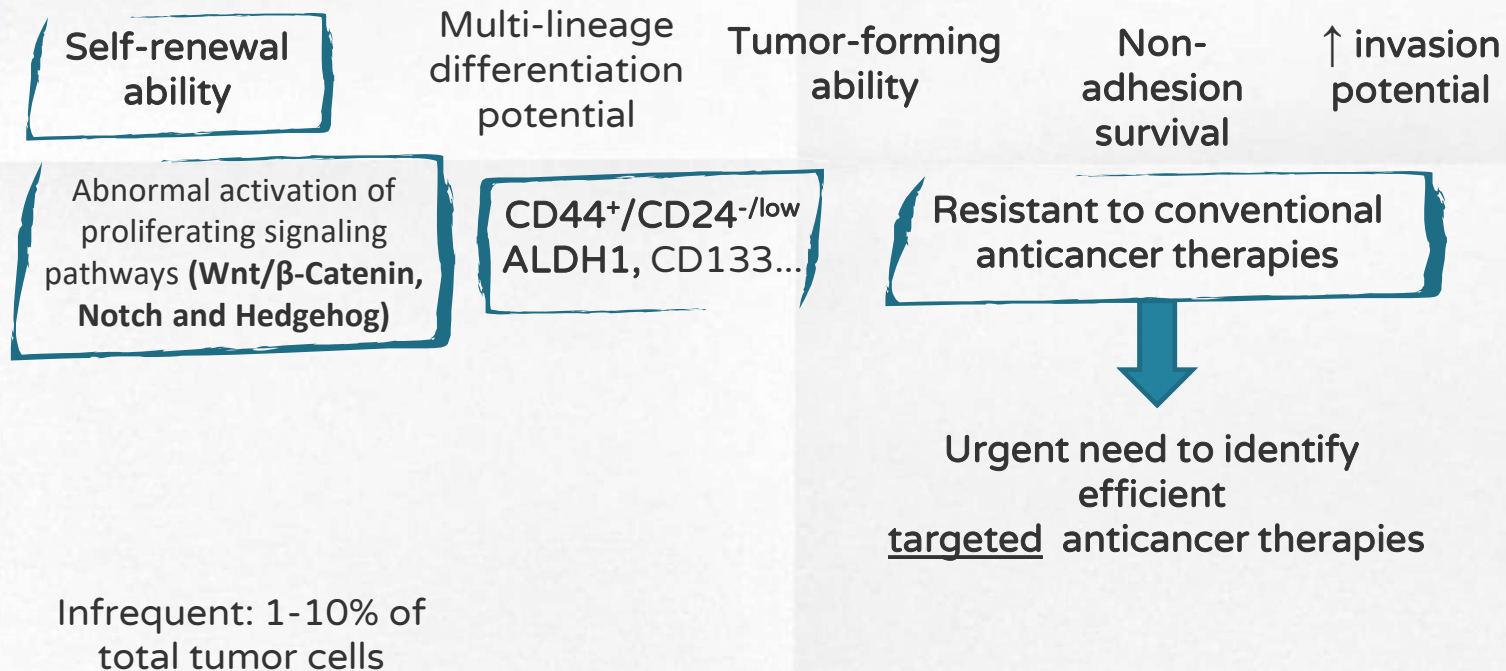
130 nm  
Maximum tolerated dose:  
Taxol: 175 mg/m<sup>2</sup>  
Abraxane: 260 mg/m<sup>2</sup>



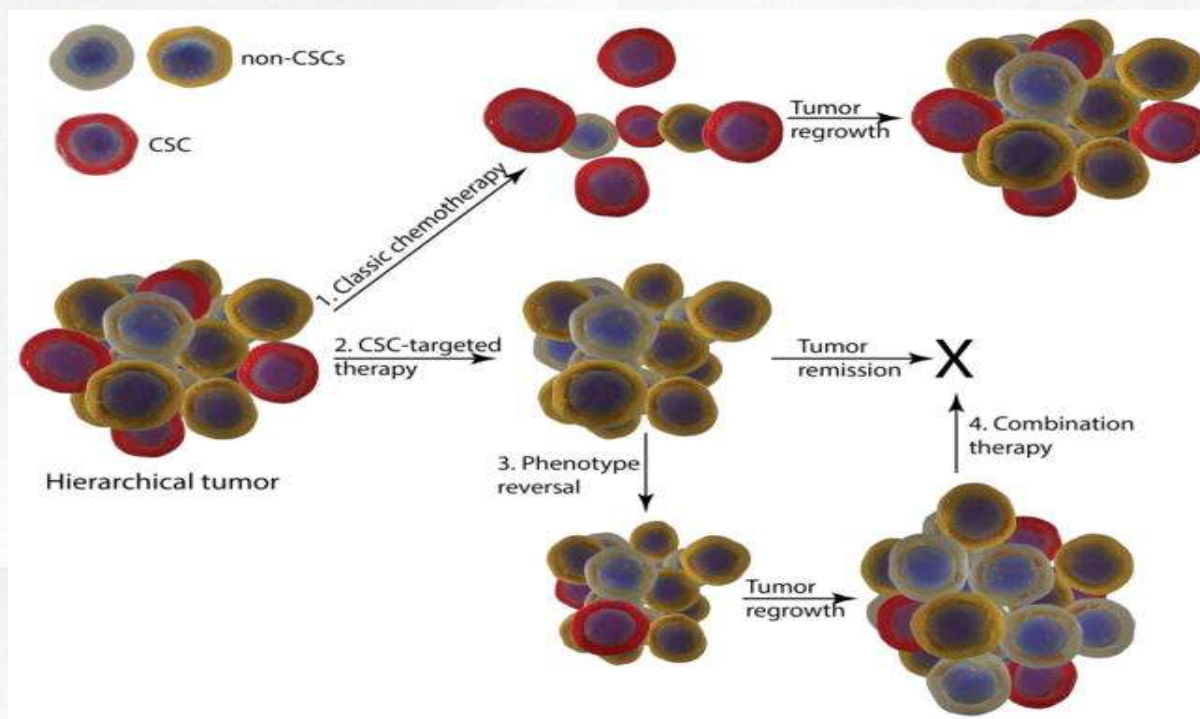
# Cancer Stem Cells (CSC)

## ▣ CSC properties

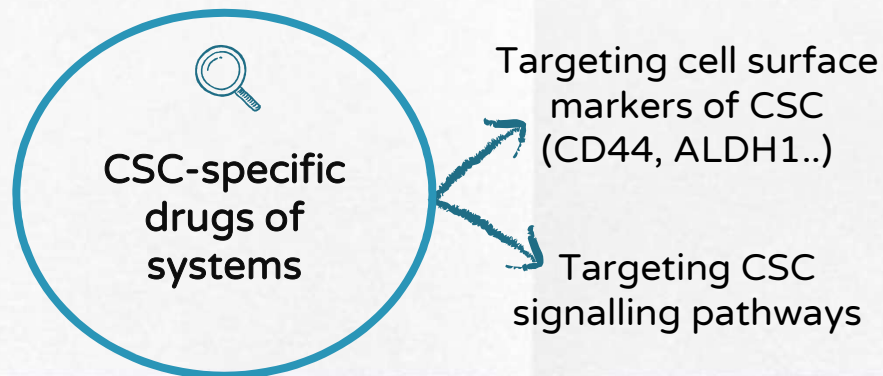
- Support the metastatic spread and tumor resistance reducing overall survival.



# Targeting Cancer Stem Cells (CSC)



*Y. Zhao et al. Adv Drug Deliv Rev, 2013, 65(0): 1763–1783.*

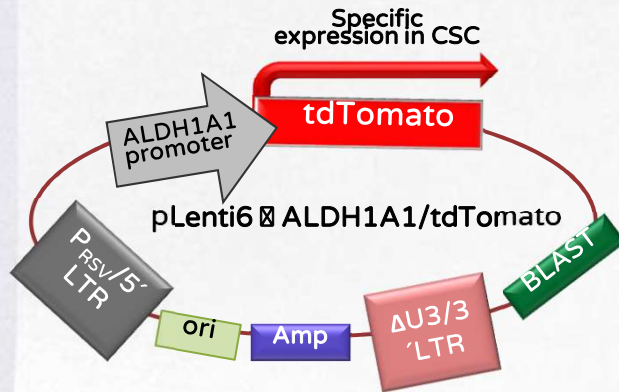


# Advanced Breast Cancer



# Development of CSC fluorescently traceable model

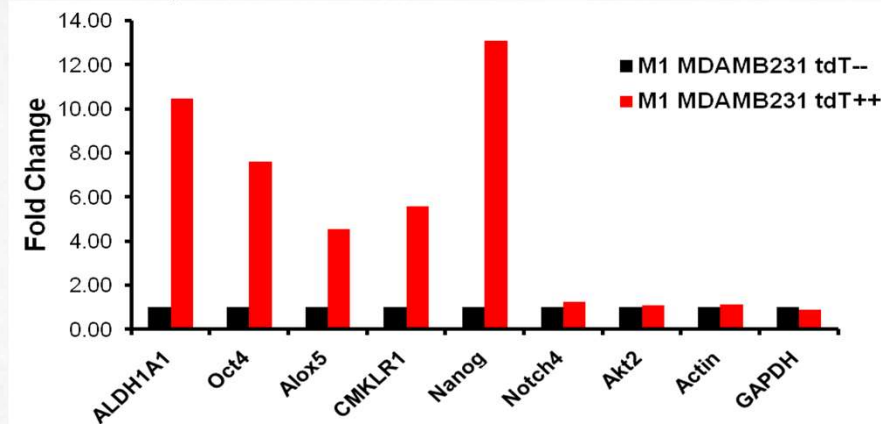
▣ Breast Cancer – most prevalent cancer worldwide (WHO).



## ALDH1A1-tdTomato reporter system

- Permanent expression of reporter in CSC allowing
  - Isolation of CSC from regular cultures
  - Monitoring of CSC within cell culture

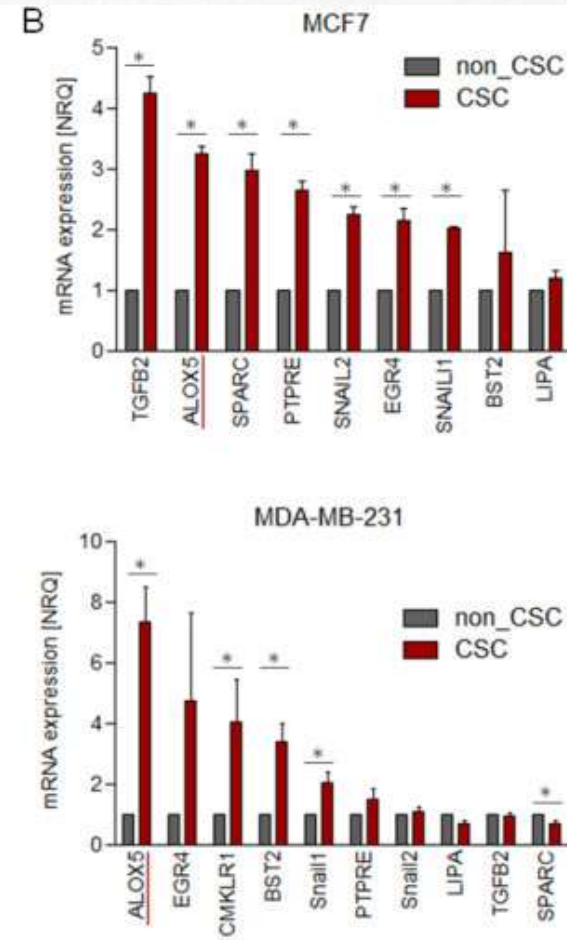
\*ALDH1A1 is overexpressed in bCSCs



# Drug Screening

**A**

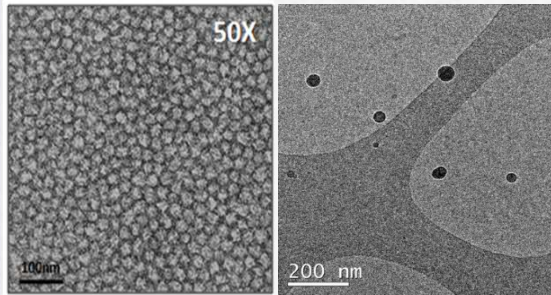
log2ratio_MCF7_CSC vz. MCF7_nonCSC	log2ratio_MDA231_CSC vz. MDA231_nonCSC	log2ratio_CSC vz. nonCSC	GeneName	FC_MCF7_CSC vz. MCF7_nonCSC	FC_MDA231_CSC vz. MDA231_nonCSC	FC_CSC vz. nonCSC	P.Value_CSC vz. nonCSC	Confirmed overexpression in MCF7_CSC	Confirmed overexpression in MDA231_CSC
			PGC	3.76	3.62	3.69	0.01	-	-
			ALOX5	5.15	2.50	3.59	0.00	yes	yes
			EGR4	3.33	2.80	3.05	0.04	no	no
			BST2	2.60	2.62	2.61	0.00	no	yes
			TGFB2	3.38	1.49	2.25	0.02	yes	no
			HLA-DRB5	2.34	2.15	2.24	0.01	-	-
			PTPRE	2.08	2.02	2.05	0.00	yes	no
			SNAI2	1.63	2.57	2.05	0.02	yes	no
			IL10RA	1.23	3.21	1.99	0.10	-	-
			GRM5	1.52	1.96	1.73	0.04	-	-
			LIPA	1.27	1.22	1.24	0.06	no	no
			TLR2	1.55	-1.09	1.19	0.45	-	-
			CMKLR1	-1.02	1.28	1.12	0.36	yes	yes
			GATA4	1.04	1.17	1.11	0.29	no	-
			SNAI1	-1.02	-1.11	-1.06	0.59	yes	yes
			SPARC	-1.03	-1.28	-1.15	0.39	yes	no



▨ Zileuton™ – Anti-asthmatic drug – inhibitor of ALOX5 – overexpressed in CSC

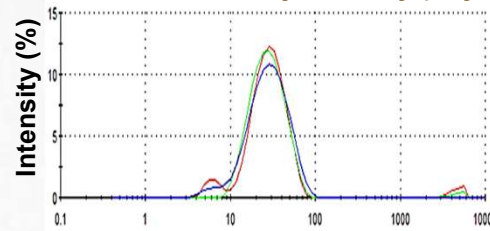
# Polymeric Micelles: Zileuton™ – characterization

## ▣ Morphology (TEM and CryoTEM)



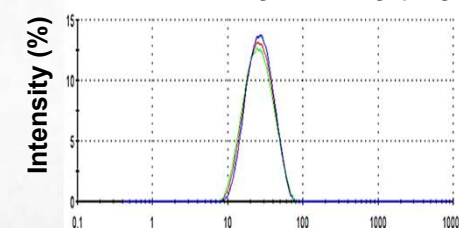
## ▣ Size and Stability over time (day 0 and day 30)

Size Distribution by Intensity (Day 0)



Size (d.nm)

Size Distribution by Intensity (Day 30)

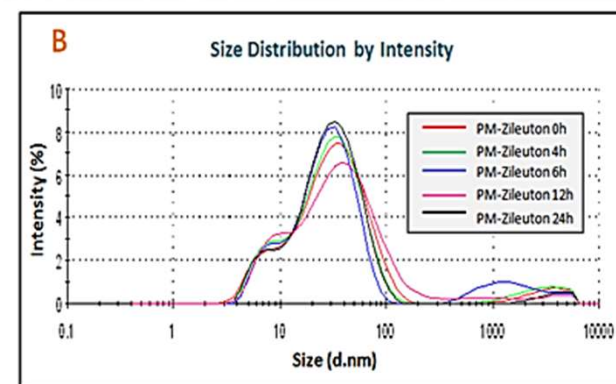
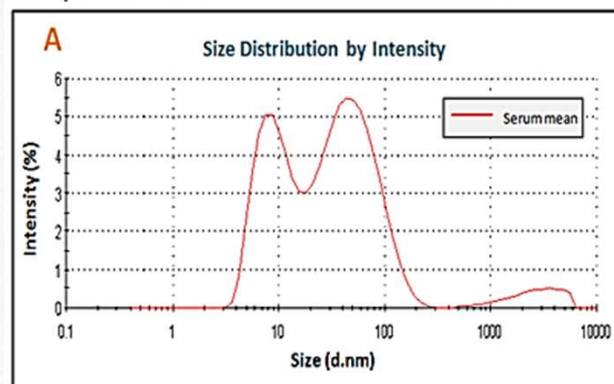


Size (d.nm)

Sample	Size (nm) ± sd	Pdi ± sd
PM-Zil	23.86 ± 0.89	0.226 ± 0.016

Sample	Size (nm) ± sd	Pdi ± sd
PM-Zil	23.93 ± 0.20	0.176 ± 0.004

## ▣ Stability in serum



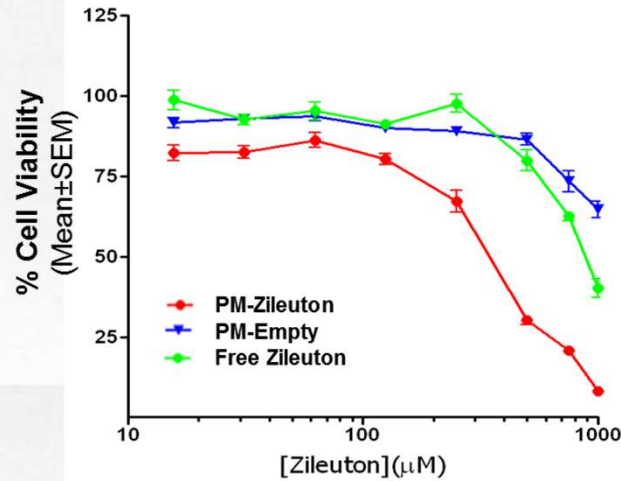


# Polymeric Micelles: Zileuton™ – *in vitro* results

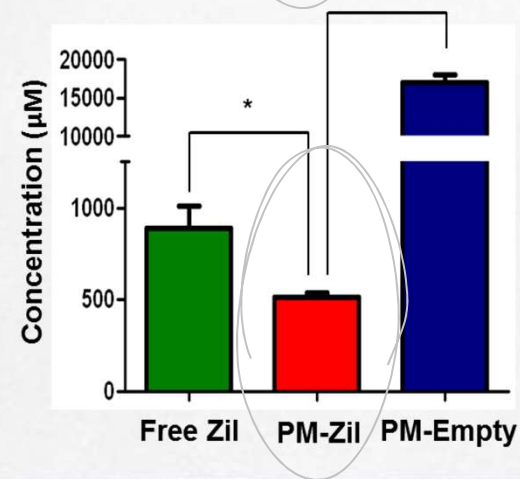
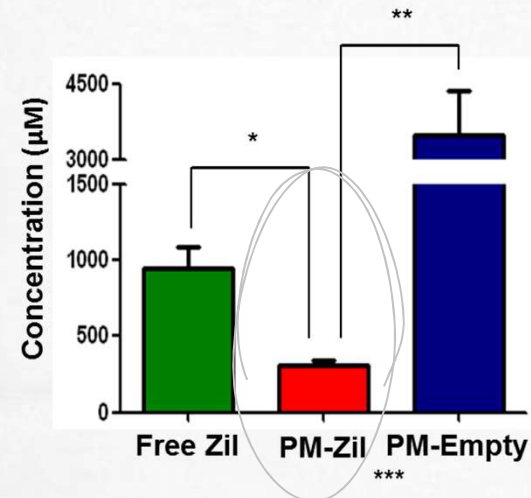
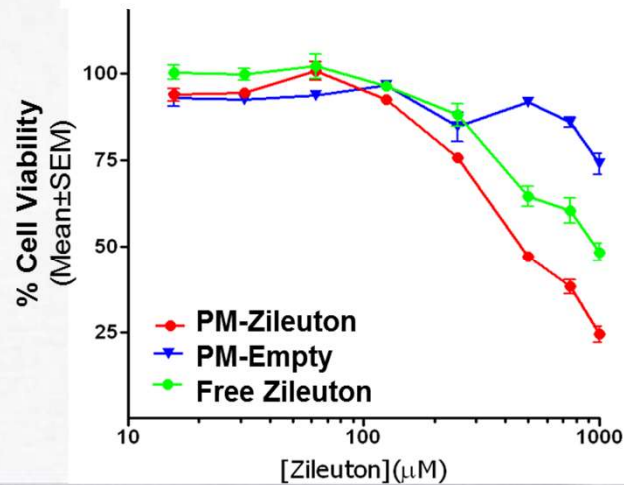


Increased activity of Zileuton™ when encapsulated in PM

MDA-MB-231



MCF-7

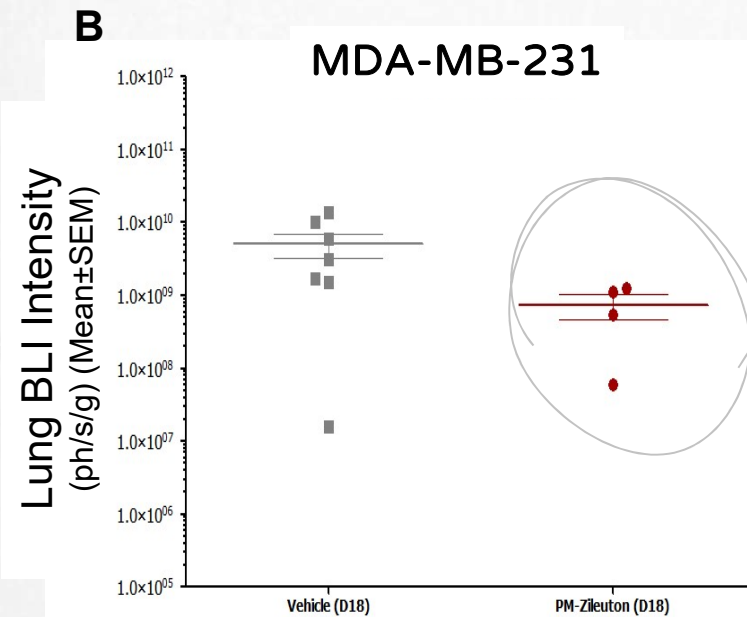
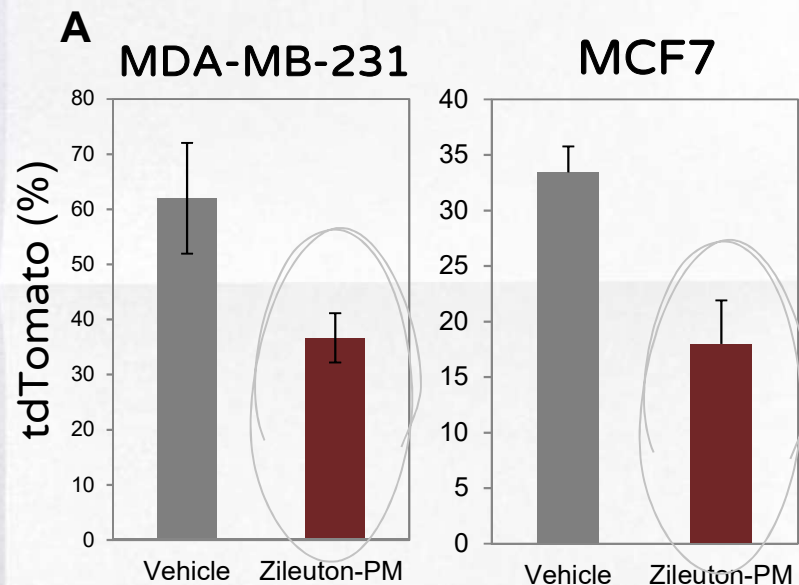


# Polymeric Micelles: Zileuton™ – *in vivo* results

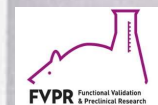


A) Reduction of CSC content in ensuing tumor

B) Abolishment of CTC and reduction of the number of metastasis detected by BLI.



Treatment	CTC incidence	CSC (%)
Vehicle	6/9	35.3
PM-Zileuton	0/7	N/A

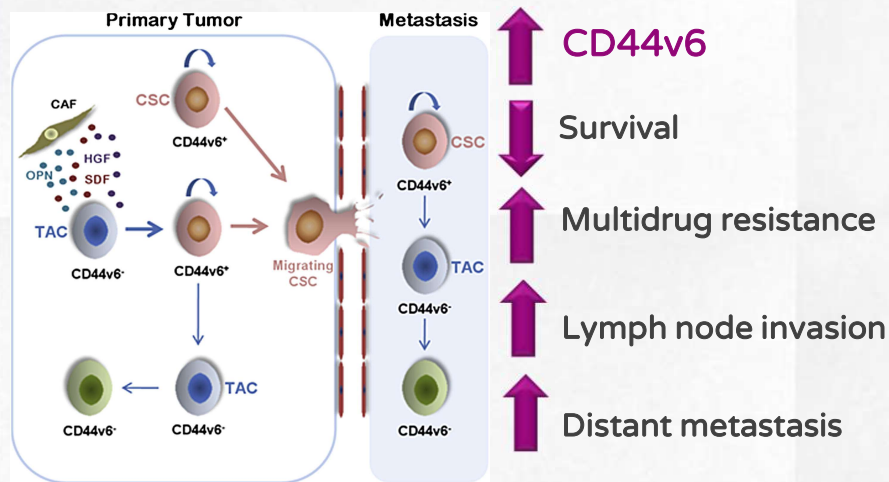


# Advanced Colorectal Cancer



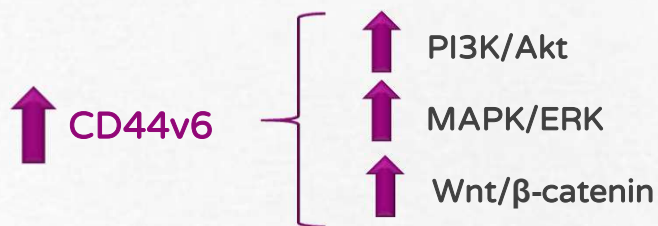
# CD44v6 in Colorectal Cancer

- ▣ Colorectal Cancer (CRC) – 2<sup>nd</sup> leading cause of cancer mortality worldwide (WHO).
- ▣ Metastatic CRC – non-responsive to treatments due to intrinsic and acquired drug resistance.



In gastrointestinal cancers:  
Tumor niche reprograms  
CD44v6<sup>-</sup> CRC progenitors into  
metastatic CD44v6<sup>+</sup> CSC.

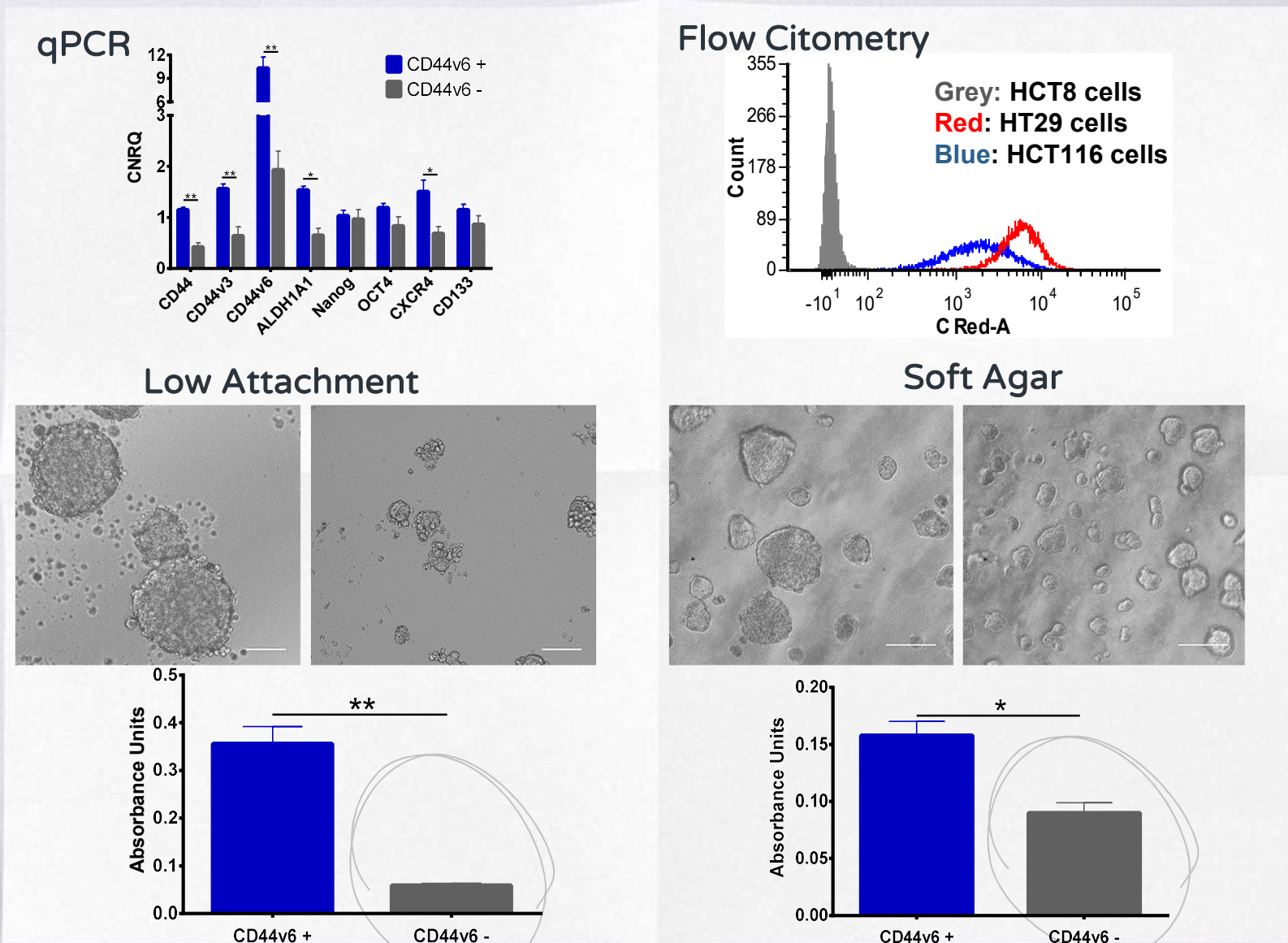
*Todaro, et al, Cell Stem Cell 2014, 14, 342–356*  
*Wang, et al, Oncotarget, 2017, 8(8), 12866-12876*  
*Wang, et al, Mol Med Rep. 2015, 11(5):3505-10*



**CD44v6 – Biomarker of CSC**

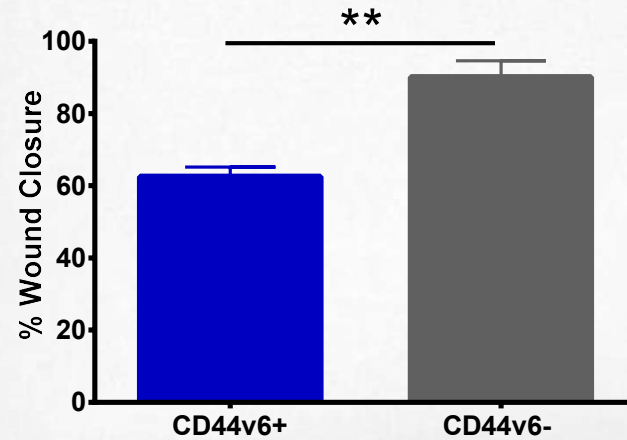
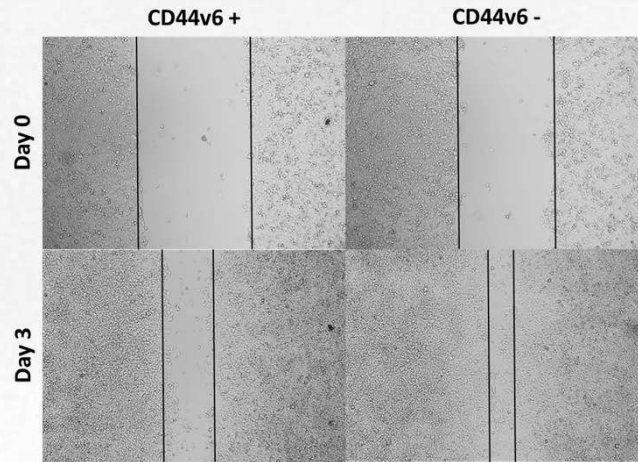
*Ma, et al, Cell Death & Disease 2019, 10:30*

# In vitro validation of CD44v6 as targeting for CSC

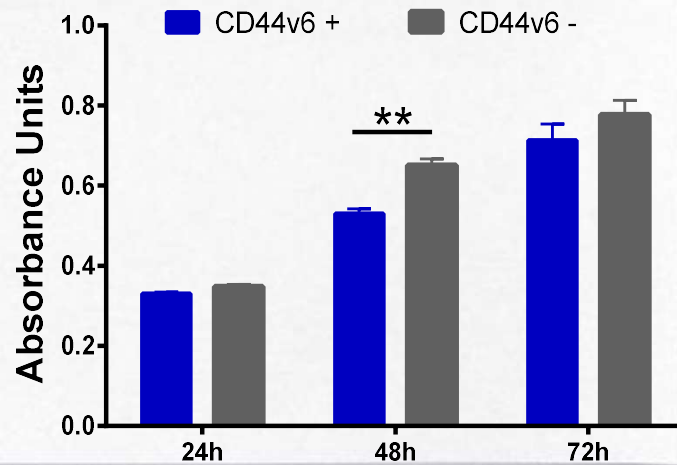


# In vitro validation of CD44v6 as targeting for CSC

## Migration



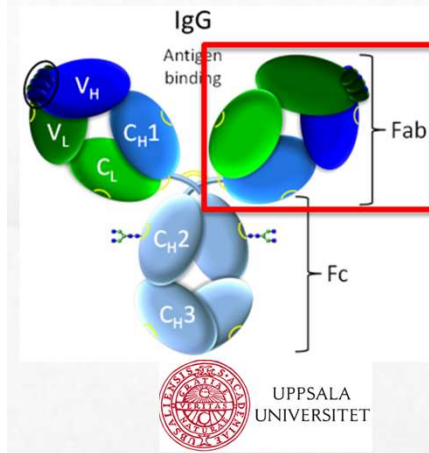
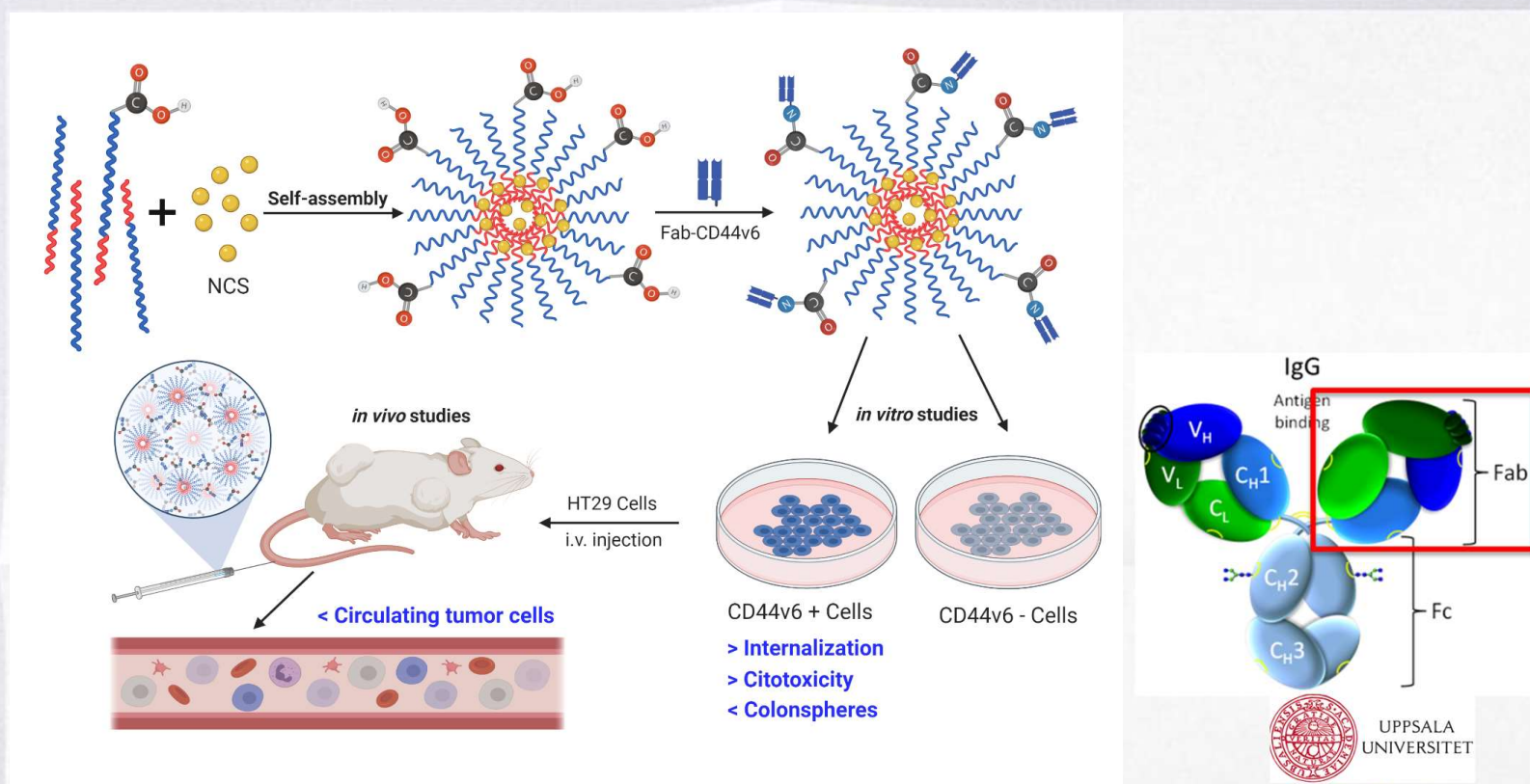
## Proliferation



CD44v6 high expression cells present stemness properties

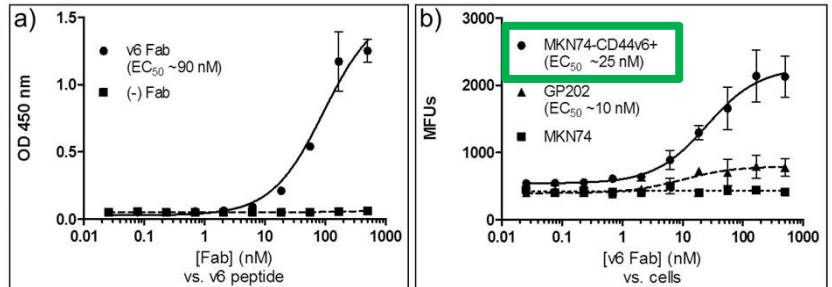


# Objective



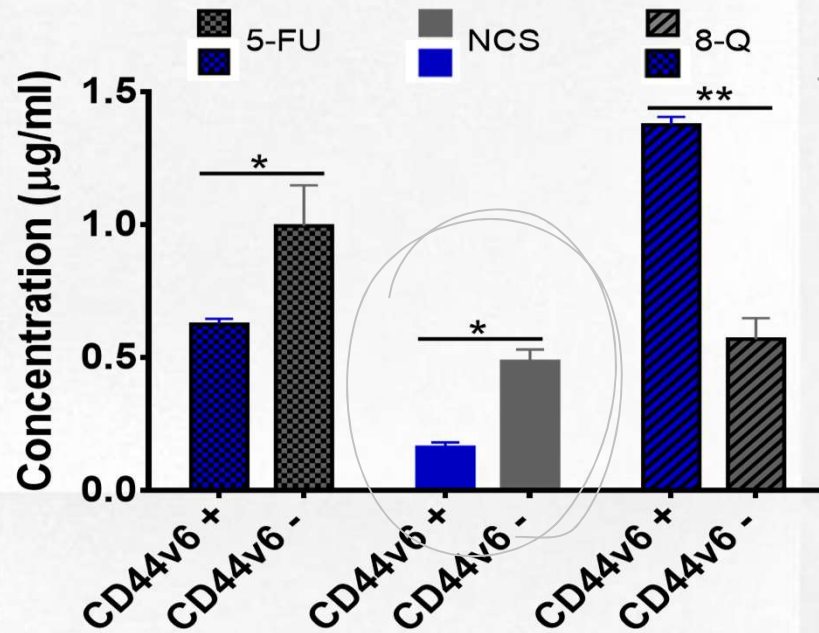
UPPSALA UNIVERSITET  
**Marika Nestor**  
 (HNSCC diagnosis)

## FACS – fixed cells



Antigen Presentation	K <sub>D</sub> (nM)
Recombinant CD44v3-10	6
CD44v6 peptide	60
CD44v6+ cell line	10

# Drug Selection



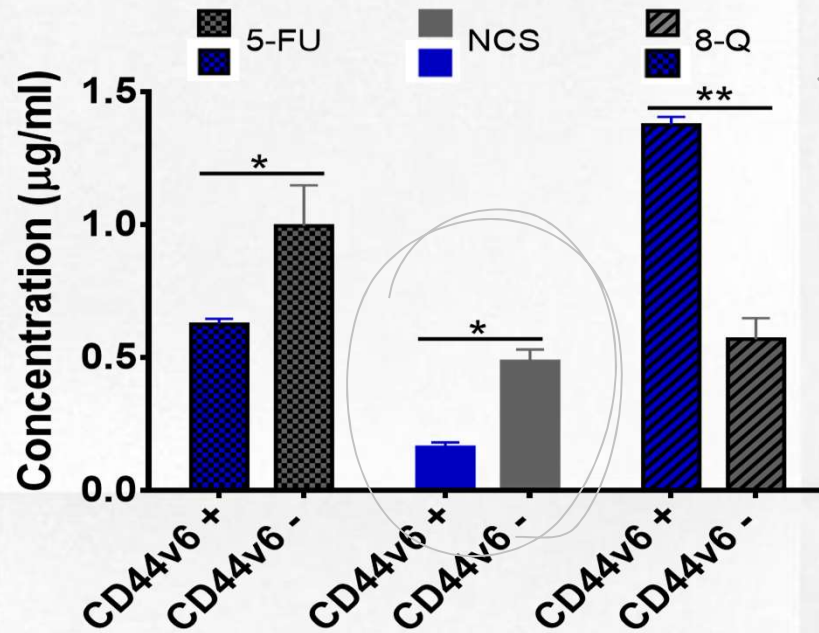
Niclosamide (NCS) present activity against CD44v6+ cells and is more potent than the standard 5-FU

Drug/Cell subpopulation	IC <sub>50</sub> (µg/ml)
5-FU/CD44v6 +	0.62 ± 0.04
5-FU/CD44v6 -	0.99 ± 0.26
NCS/CD44v6 +	0.17 ± 0.03
NCS/CD44v6 -	0.48 ± 0.08
8-Q/CD44v6 +	1.37 ± 0.05
8-Q/CD44v6 -	0.57 ± 0.14



Arend, et al, *Oncotarget*. 2016, 7(52): 86803–86815

# Drug Selection



Niclosamide (NCS) present activity against CD44v6+ cells and is more potent than the standard 5-FU

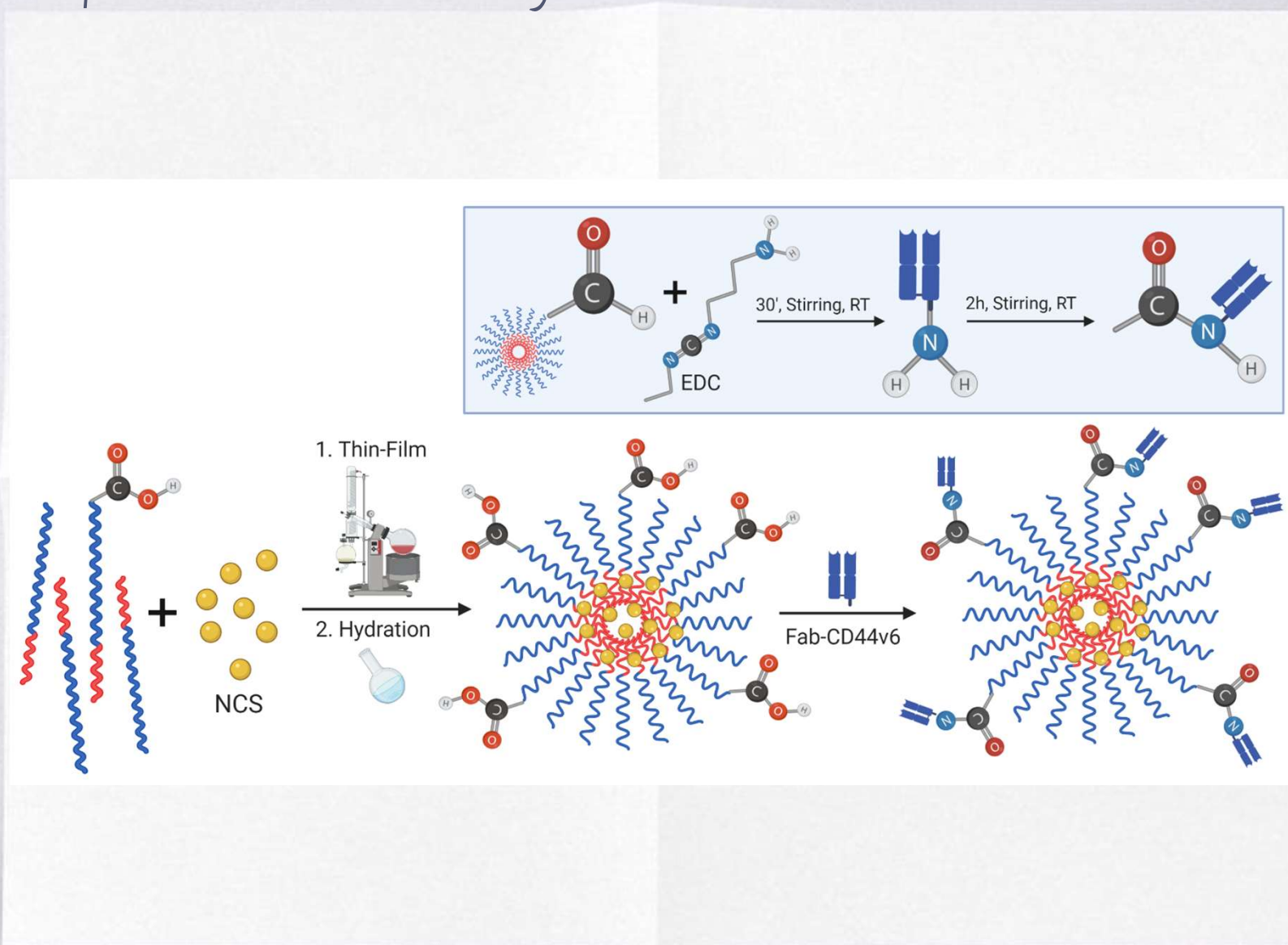
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NCS/CD44v6 -	0.48 ± 0.08
8-Q/CD44v6 +	1.37 ± 0.05
8-Q/CD44v6 -	0.57 ± 0.14



Arend, et al, *Oncotarget*. 2016, 7(52): 86803–86815

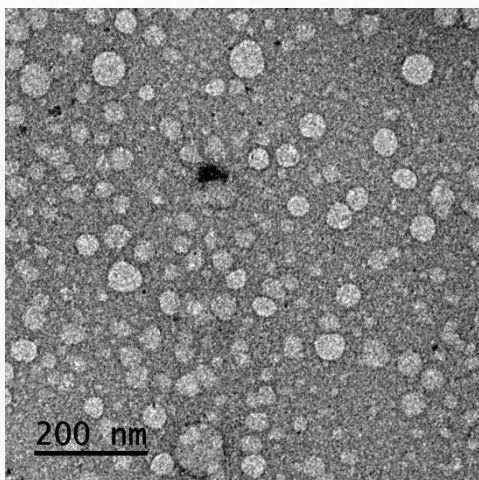


# Polymeric Micelles Design

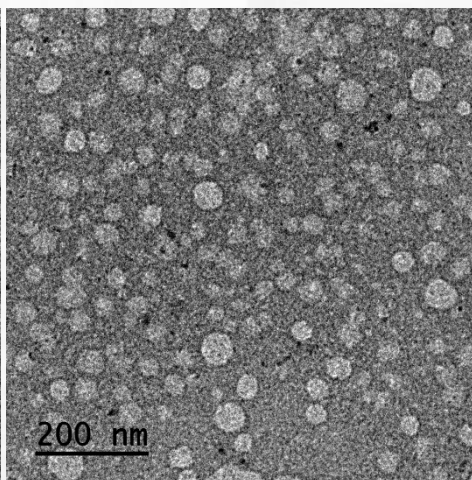


# Polymeric Micelles-Niclosamide:CD44v6 Fab - characterization

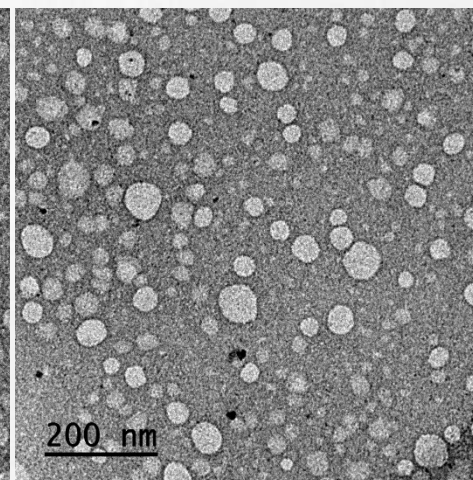
Formulation	Mean Diameter (nm)	Pdl	Zeta Potential (mV)	AE (%)
PM	23.2 ± 1.1	0.332 ± 0.067	-0.7 ± 0.3	N.A.
PM-NCS	24.4 ± 0.7	0.206 ± 0.011	-3.4 ± 2.8	99.8 ± 4x10 <sup>5</sup>
PM-NCS:Fab	29.7 ± 1.2	0.338 ± 0.055	-6.5 ± 0.7	99.7 ± 4x10 <sup>5</sup>



PM



PM-NCS

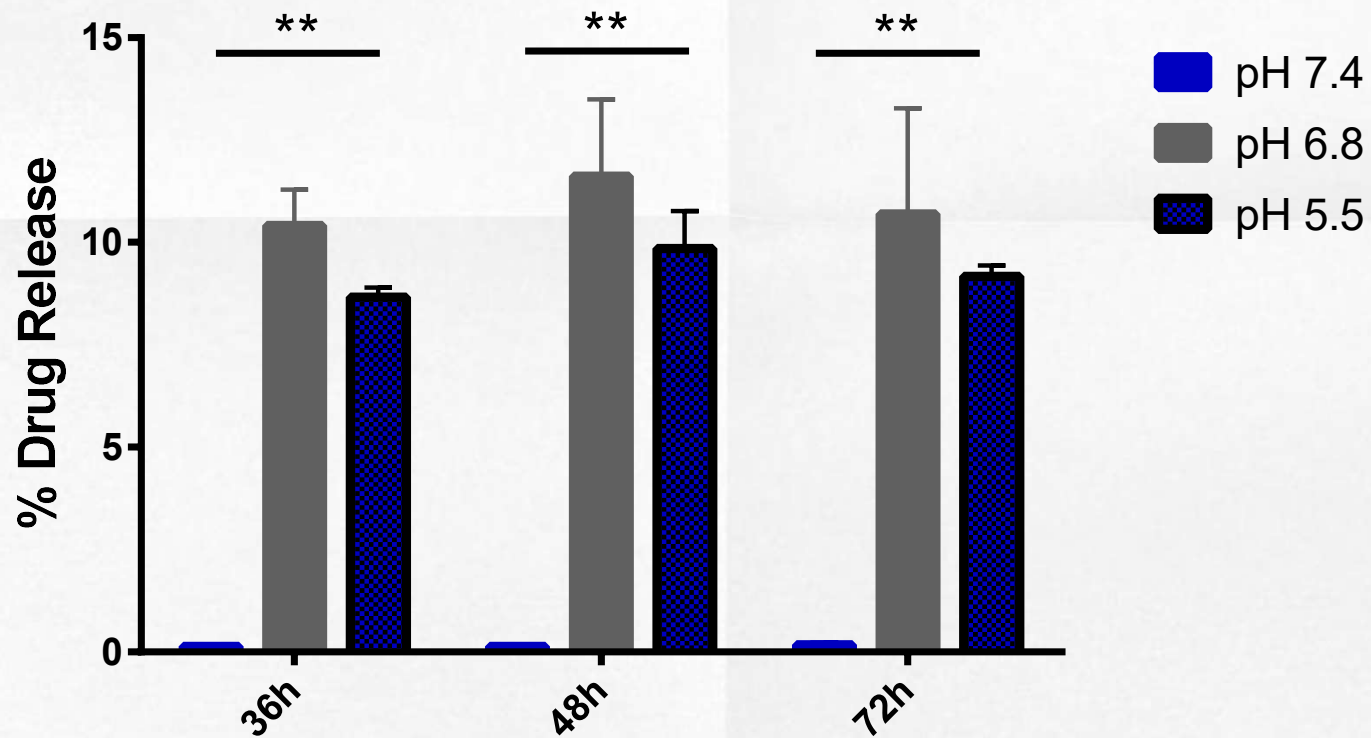


PM-NCS:Fab

# Polymeric Micelles-Niclosamide:CD44v6 Fab - characterization



Drug release dependent on pH  
Preferential release at tumor microenvironment



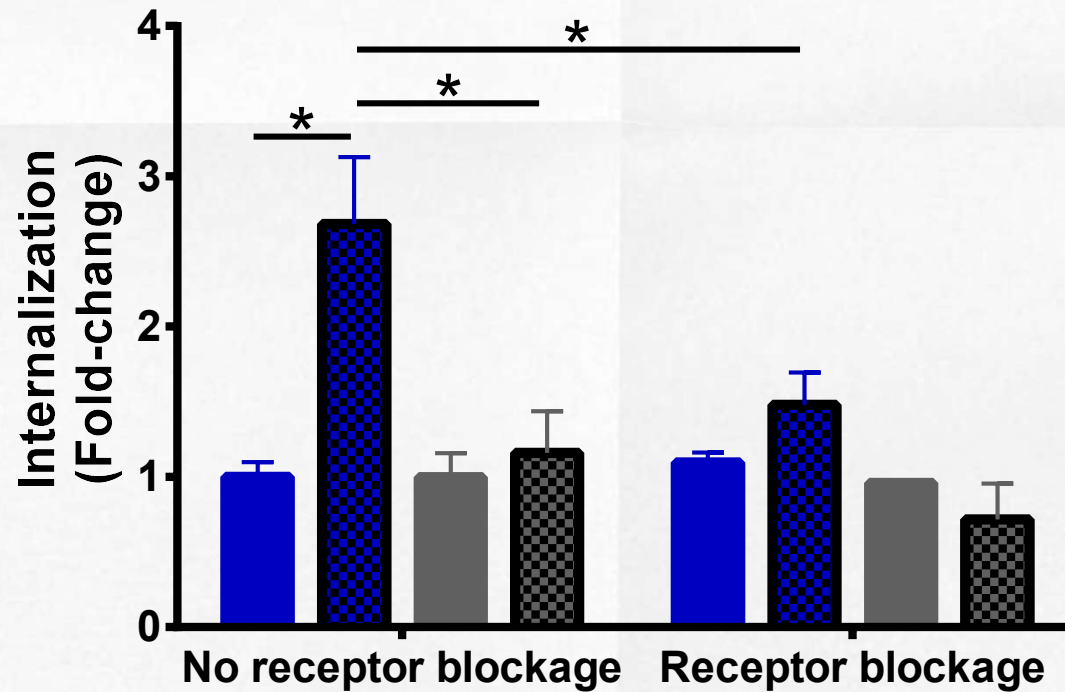


# Polymeric Micelles-Niclosamide:CD44v6 Fab – *in vitro* results



**Internalization:** Fab-CD44v6 PM surface modification increase internalization in CD44v6+ (CSC) population

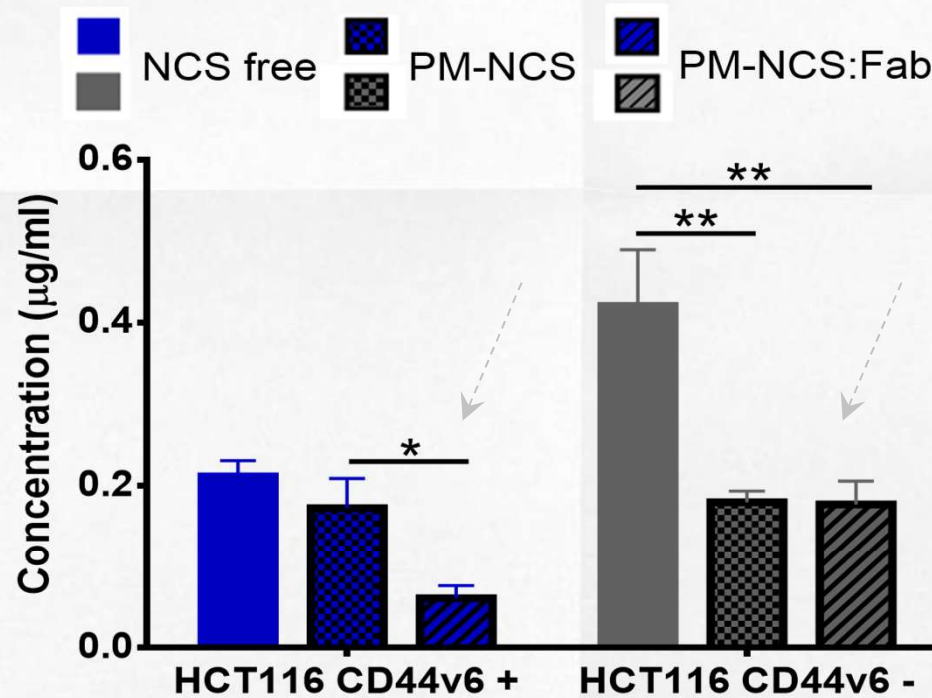
■ CD44v6 + PM      ■ CD44v6 - PM  
■ CD44v6 + PM:Fab      ■ CD44v6 - PM:Fab



# Polymeric Micelles-Niclosamide:CD44v6 Fab – *in vitro* results



**Efficacy:** NCS encapsulation into PM increase its efficacy and Fab presence increase the efficacy in CD44v6+ (CSC) population

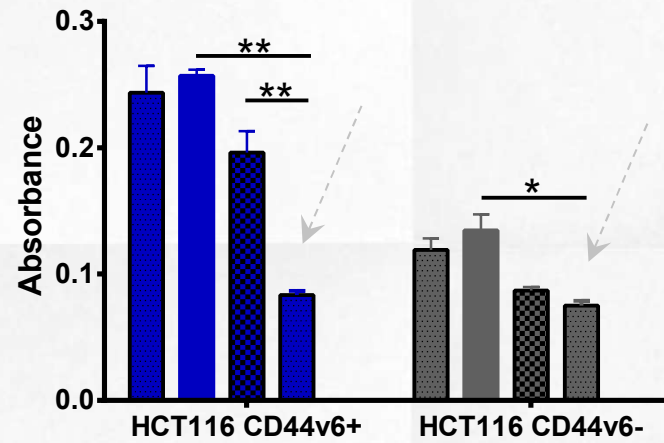


# Polymeric Micelles-Niclosamide:CD44v6 Fab – *in vitro* results



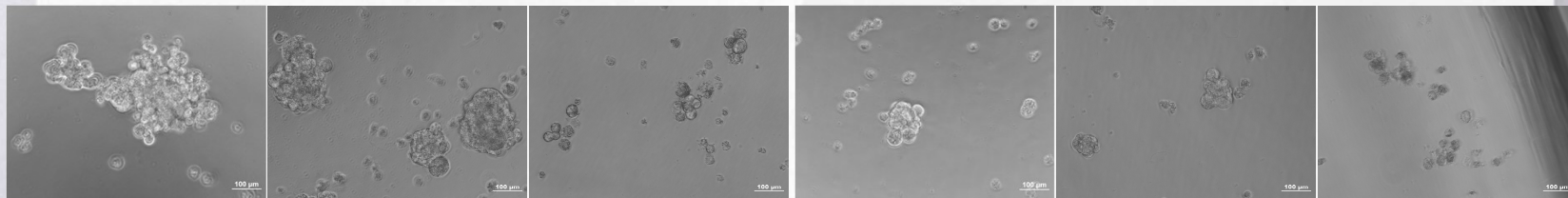
**Efficacy: PM-NCS:Fab impairs colonpheres formation**

■ Control   
 ■ NCS free   
 ■ PM-NCS   
 ■ PM-NCS:Fab



HCT116 CD44v6+

HCT116 CD44v6-



Control

NCS free

PM-NCS-COOH-Fab

Control

NCS free

PM-NCS-COOH-Fab

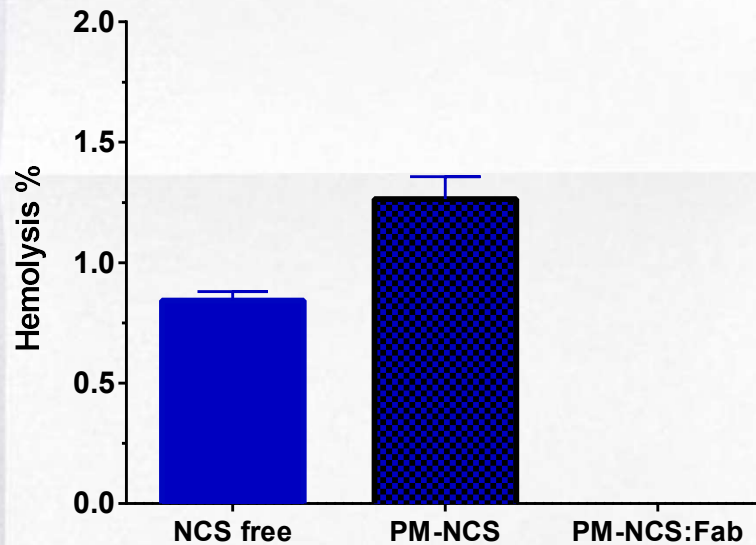


# Polymeric Micelles-Niclosamide:CD44v6 Fab – *in vitro* results



**Hemocompatibility: PM-NCS:Fab are hemocompatible and well tolerated**

## Hemolysis

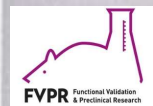


NV: < 5% of hemolysis

## Plasma Coagulation

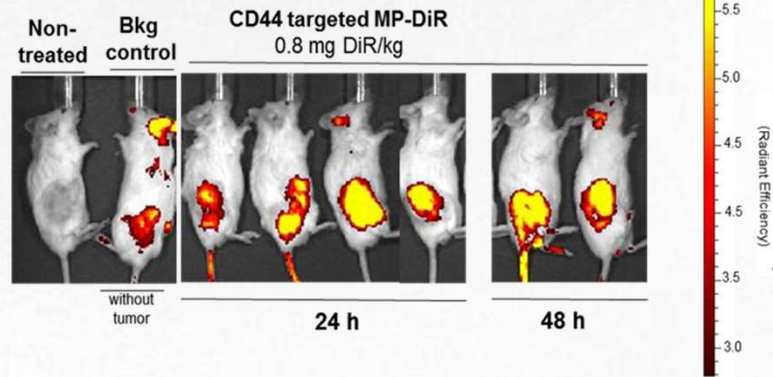
Sample	Time (s)	
	PT	TT
Healthy Patient Control	12.4 ± 0.0	15.9 ± 0.0
Sick Patient Control	19.45 ± 0.1	-
Negative Control	11.65 ± 0.1	17.3 ± 0.3
Control PBS	12.15 ± 0.1	17.45 ± 0.1
Control Methanol	13.1 ± 0.1	15.3 ± 0.1
NCS free	13.8 ± 0.1	19.05 ± 0.2
PM-NCS	12.35 ± 0.2	15.15 ± 0.1
PM-NCS:Fab	12.4 ± 0.1	15.3 ± 0.3

NV: PT ≤ 13.4s and TT ≤ 21s

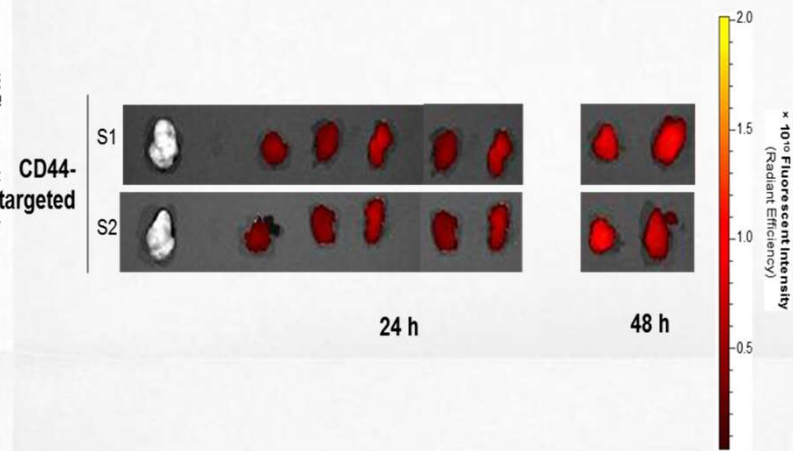


# Polymeric Micelles-Niclosamide:CD44v6 Fab – *in vivo* results

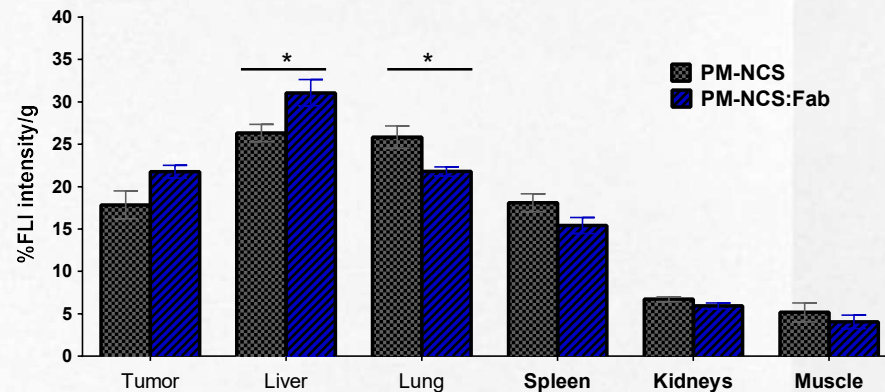
## *In vivo* tumor accumulation



## *Ex vivo* tumor accumulation



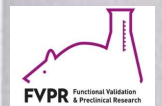
## *Ex vivo* organs accumulation



### Biodistribution:

PM-NCS:Fab reach and accumulates in tumor for at least 48h

NOD-SCID mice bearing subcutaneous HCT116 tumors



# Polymeric Micelles-Niclosamide:CD44v6 Fab – *in vivo* results

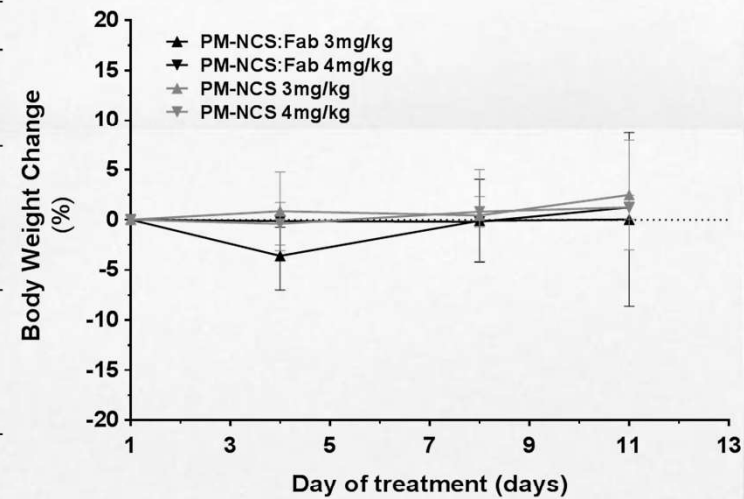


**Safety:** PM reduce the *in vivo* toxicity of NCS

## Survival

Group	Dose (mg/kg)	Number of doses		
		1	2	3
NCS free	0.5	100%	78%	67%
	1	60%	ND	ND
PM-NCS	3	80%	80%	80%
	4	100%	100%	67%
	6	33%	0%	0%
PM-NCS:Fab	3	80%	80%	60%
	4	100%	67%	67%
	6	0%	0%	0%

## Body Weight



NOD-SCID mice bearing subcutaneous HCT116 tumors

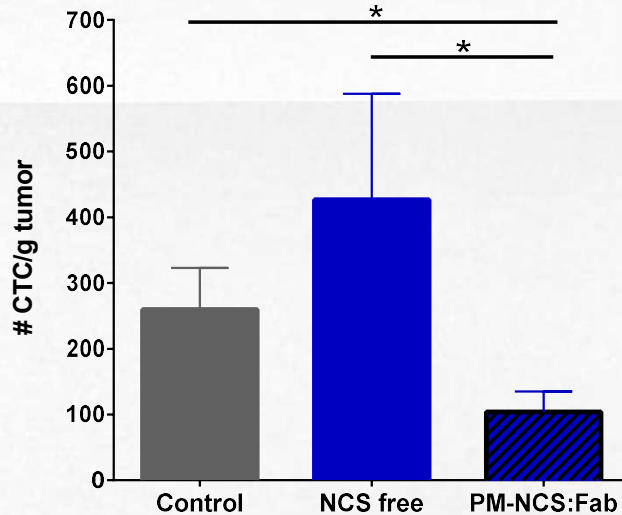


# Polymeric Micelles-Niclosamide:CD44v6 Fab – *in vivo* results



*in vivo* Efficacy .PM-NCS:Fab decrease the tumor circulating cells and are a promising therapeutic adjuvant of CRC treatment to prevent development of mCRC

## Tumor circulating cells



## Plasma Concentration

Group	Dose (mg/Kg)	% of dose injected
NCS free	0.5	45.33 ± 0.54
PM-NCS	4	5.69 ± 0.06
PM-NCS:Fab	4	1.13 ± 2.52

NOD-SCID mice bearing subcutaneous HT29 tumors

## Conclusions



Cancer Stem Cells are responsible for resistance to treatment and tumor relapse.



Targeting CSC through nanomedicine improve treatment outcomes through reduction of circulating tumor cells and metastasis.

# Aknowlegments

## Drug Delivery & Targeting (DDT)

## Area of Functional Validation & Preclinical Research (FVPR)

- Diana Rafael
- Francesc Martinez
- Joaquín Seras-Franzoso
- Zamira V Díaz-Riascos
- Júlia German
- Diego Baranda
- Marc Moltó
- Begoña Fernández
- Belén García
- Sandra Mancilla
- Laura García
- Simó Schwartz Jr.
- Ibane Abasolo



## Collaborations

Bruno Sarmiento



Marika Nestor



**FCT**

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Thank You!



Any questions?

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