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

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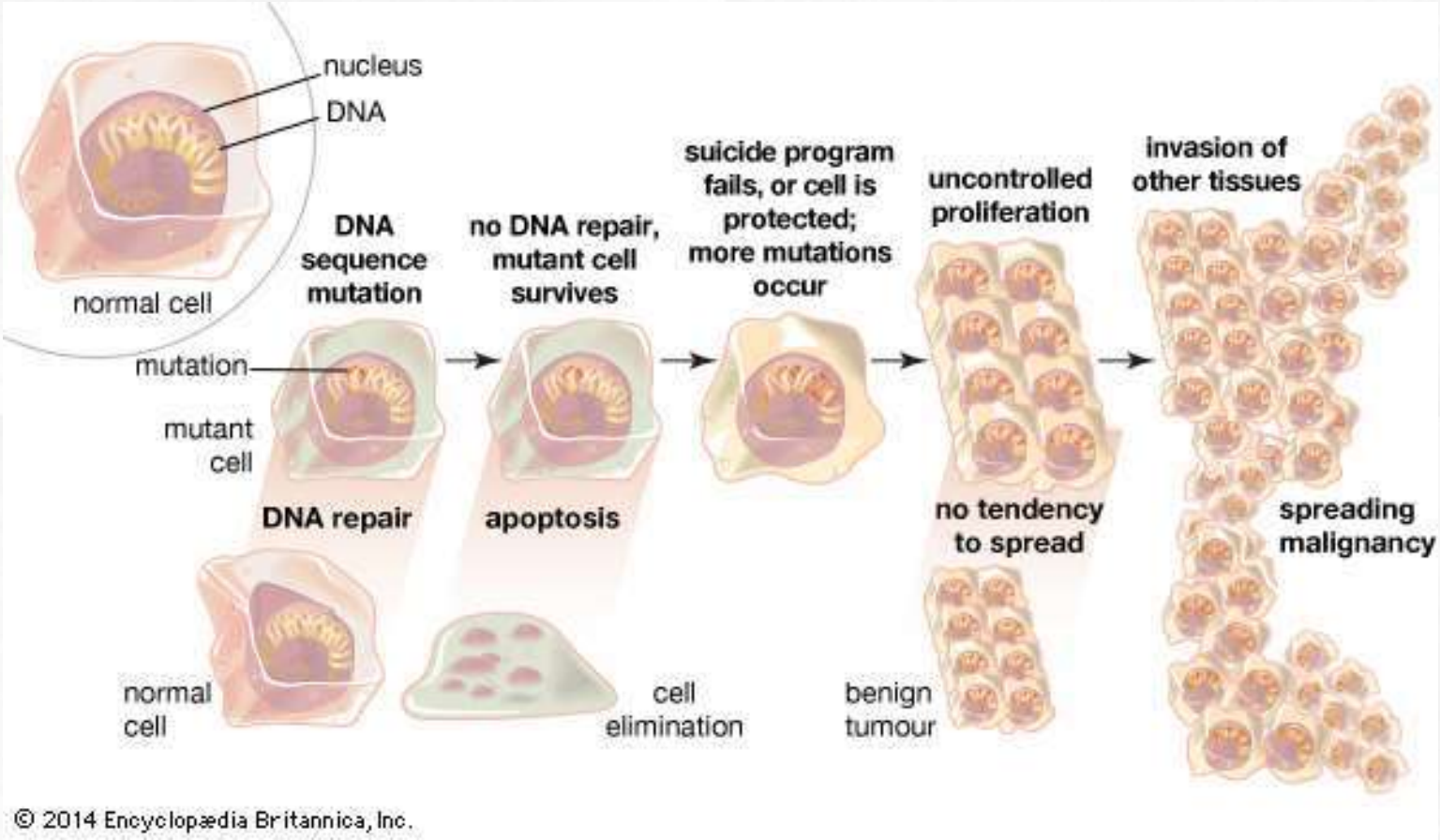
17/11/2022

International Agency for Research on Cancer
World Health Organization

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


nucleus
DNA
normal cell
DNA sequence mutation
no DNA repair, mutant cell survives
suicide program fails, or cell is protected; more mutations occur
uncontrolled proliferation
invasion of other tissues
mutation
mutant cell
DNA repair
apoptosis
no tendency to spread
spreading malignancy
normal cell
cell elimination
benign tumour
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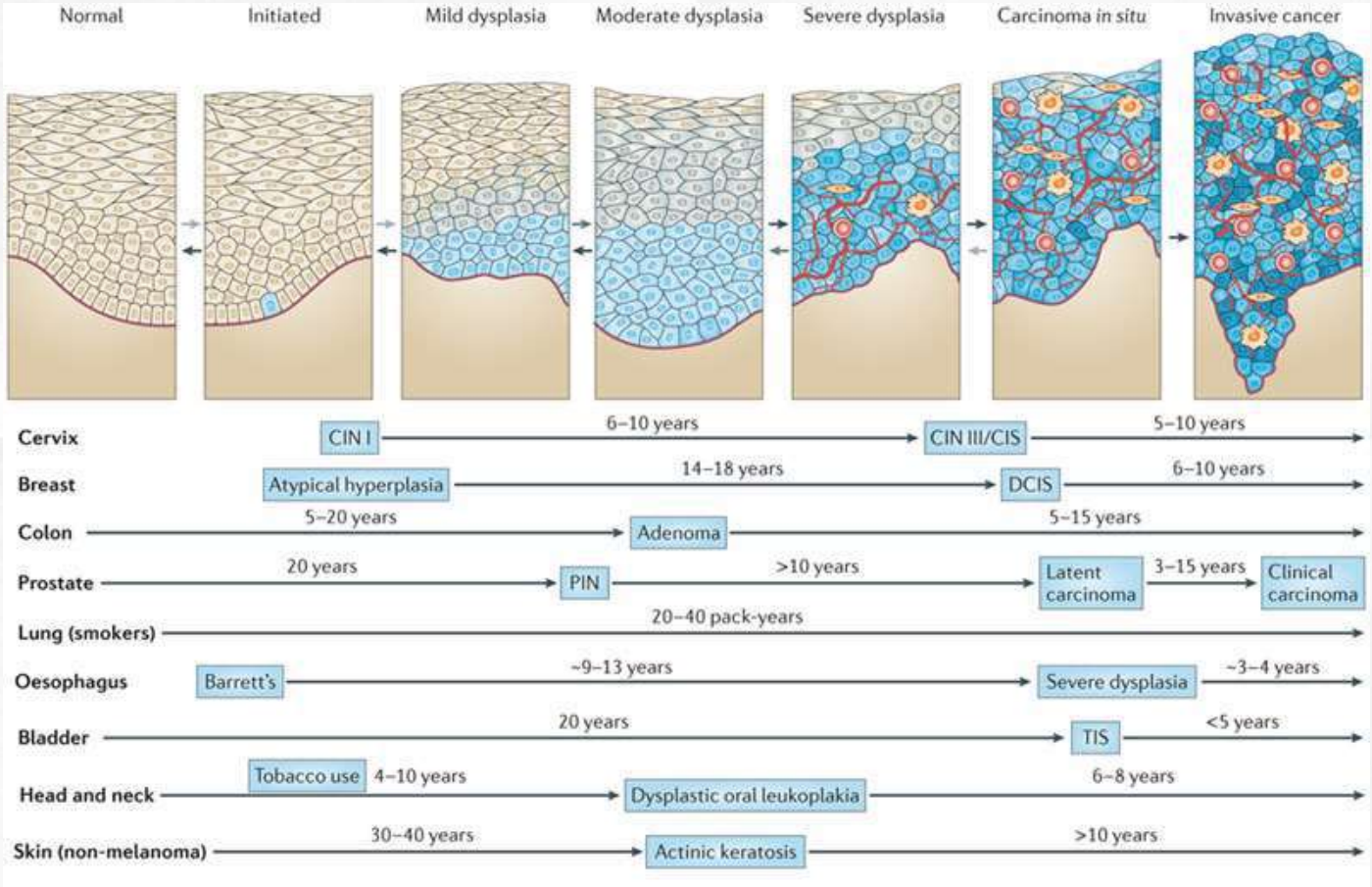
Cancer



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

Cancer Statistics

International Agency for Research on Cancer
World Health Organization

Data source: GLOBOCAN 2020
Graph production: Global Cancer Observatory (http://gco.iarc.fr/)
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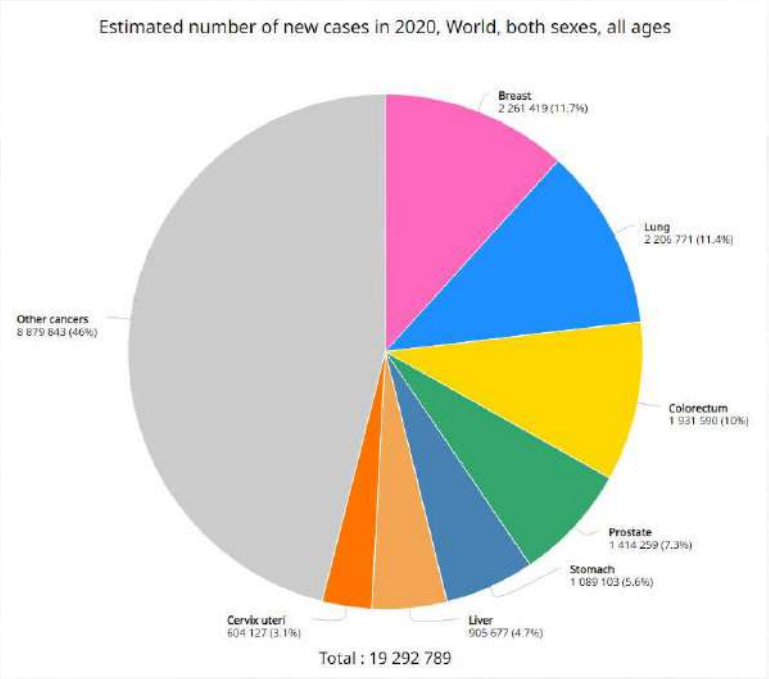
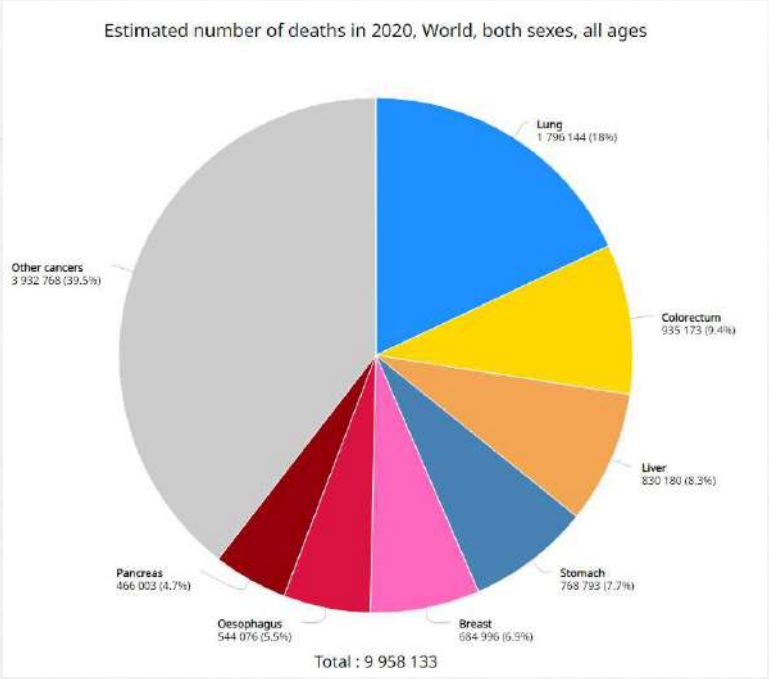
- In 2020:
  - 10 million cancer related deaths
  - 19.3 million new cases
  - New cases are expected to rise by to 29.5 million up to 2040



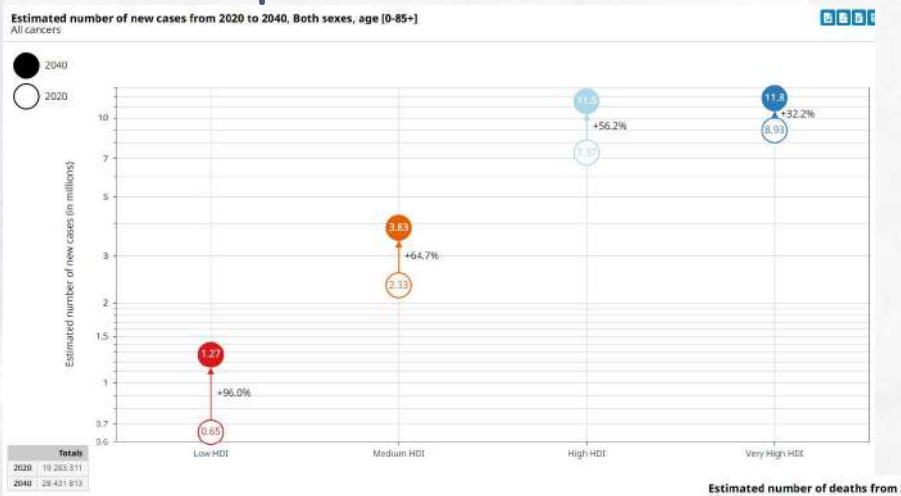


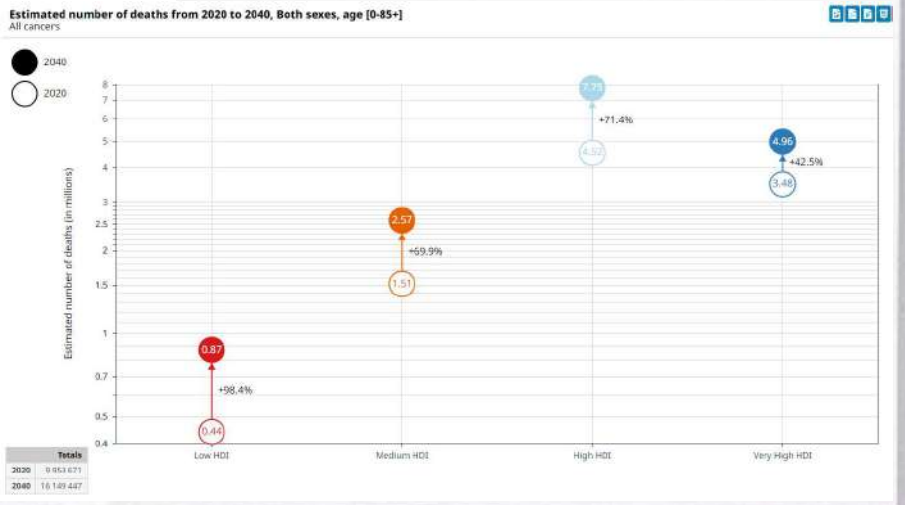
Cancer Statistics

International Agency for Research on Cancer

World Health Organization

2040 previsions





Cancer Statistics

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World Health Organization

Data source: GLOBOCAN 2020
Graph production: Global Cancer Observatory (http://gco.iarc.fr/)
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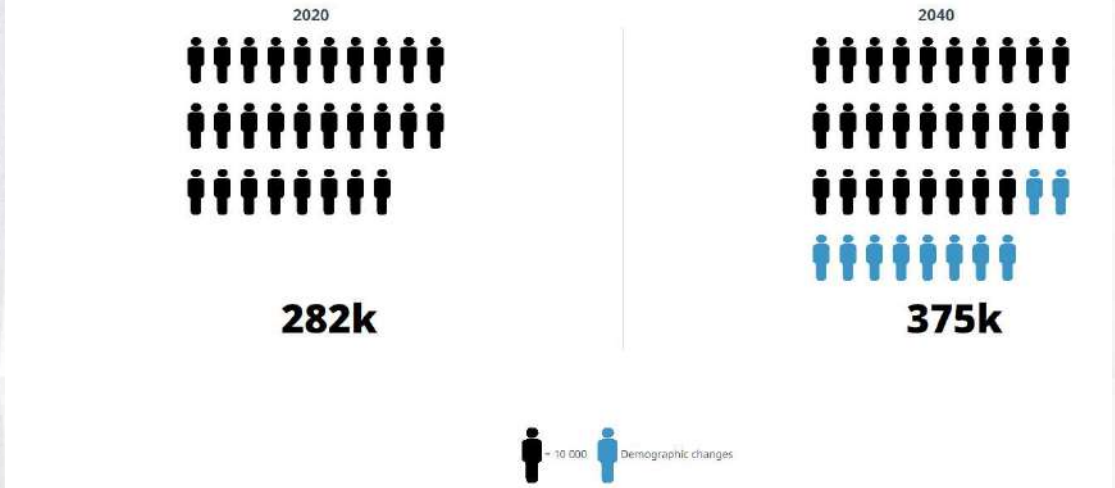
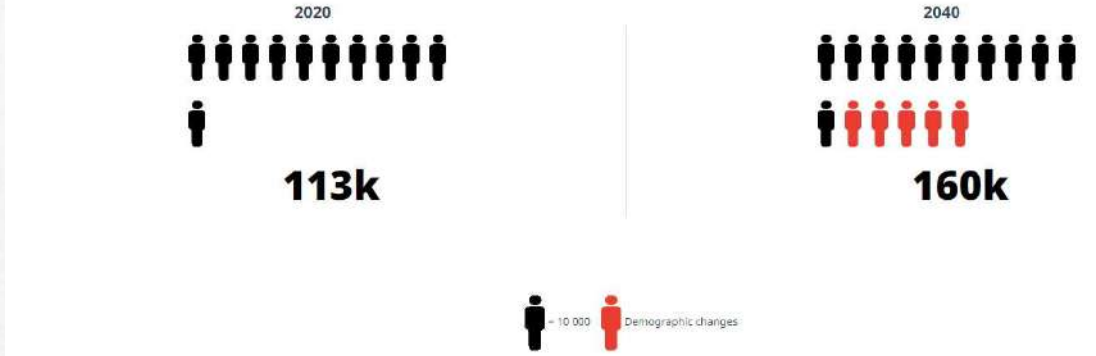
Spain

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



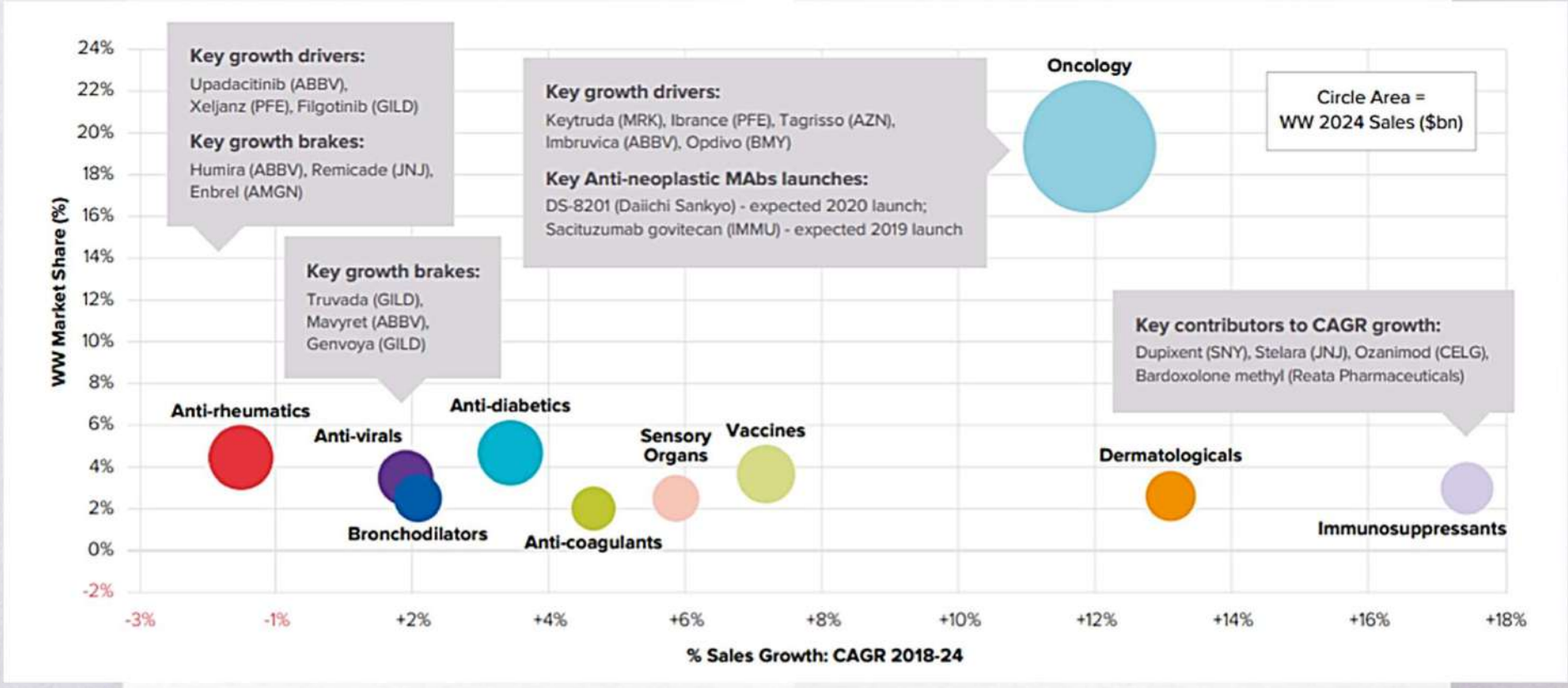
Estimated number of deaths from 2020 to 2040, Both sexes, age [0-85+]
All cancers
Spain



Cancer Costs

EvaluatePharma

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



Cancer Costs

EvaluatePharma

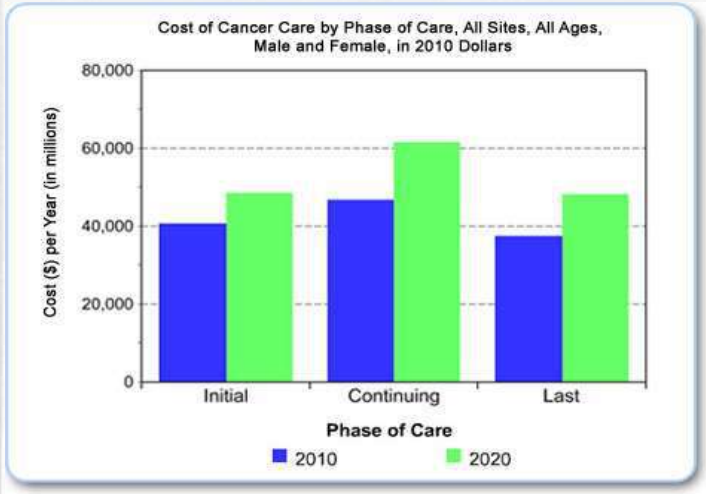
▧ Worldwide Prescription Drug & OTC Sales by Evaluate Therapy Area (2018 & 2024: Top 10 Categories & Total Market

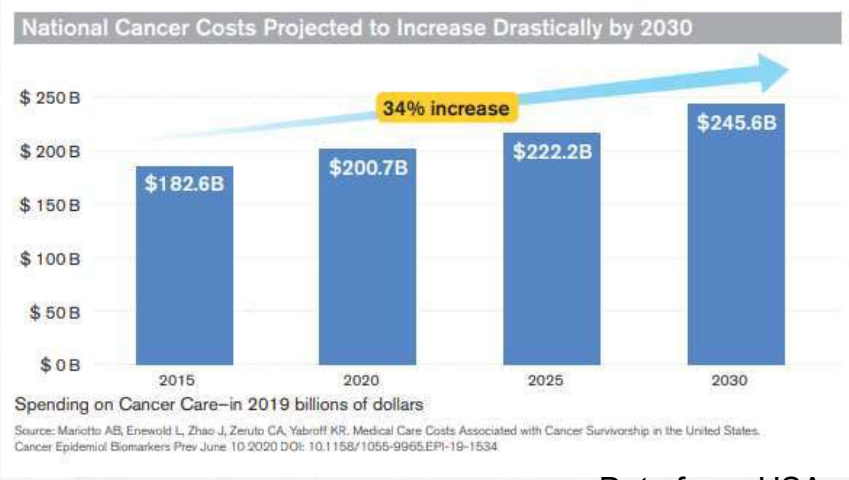
Rank	Therapy Area	WW Sales ($bn) 2018	2024	CAGR % Growth	WW Market Share 2018	2024	Chg. (+/-)	Rank Chg. (+/-)
1.	Oncology	123.8	236.6	+11.4%	14.3%	19.4%	+5.0pp	+0
2.	Anti-diabetics	48.5	57.6	+2.9%	5.6%	4.7%	-0.9pp	+1
3.	Anti-rheumatics	58.1	54.6	-1.0%	6.7%	4.5%	-2.3pp	-1
4.	Vaccines	30.5	44.8	+6.6%	3.5%	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

NIH NATIONAL CANCER INSTITUTE
Cancer Prevalence and Cost of Care Projections

- 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

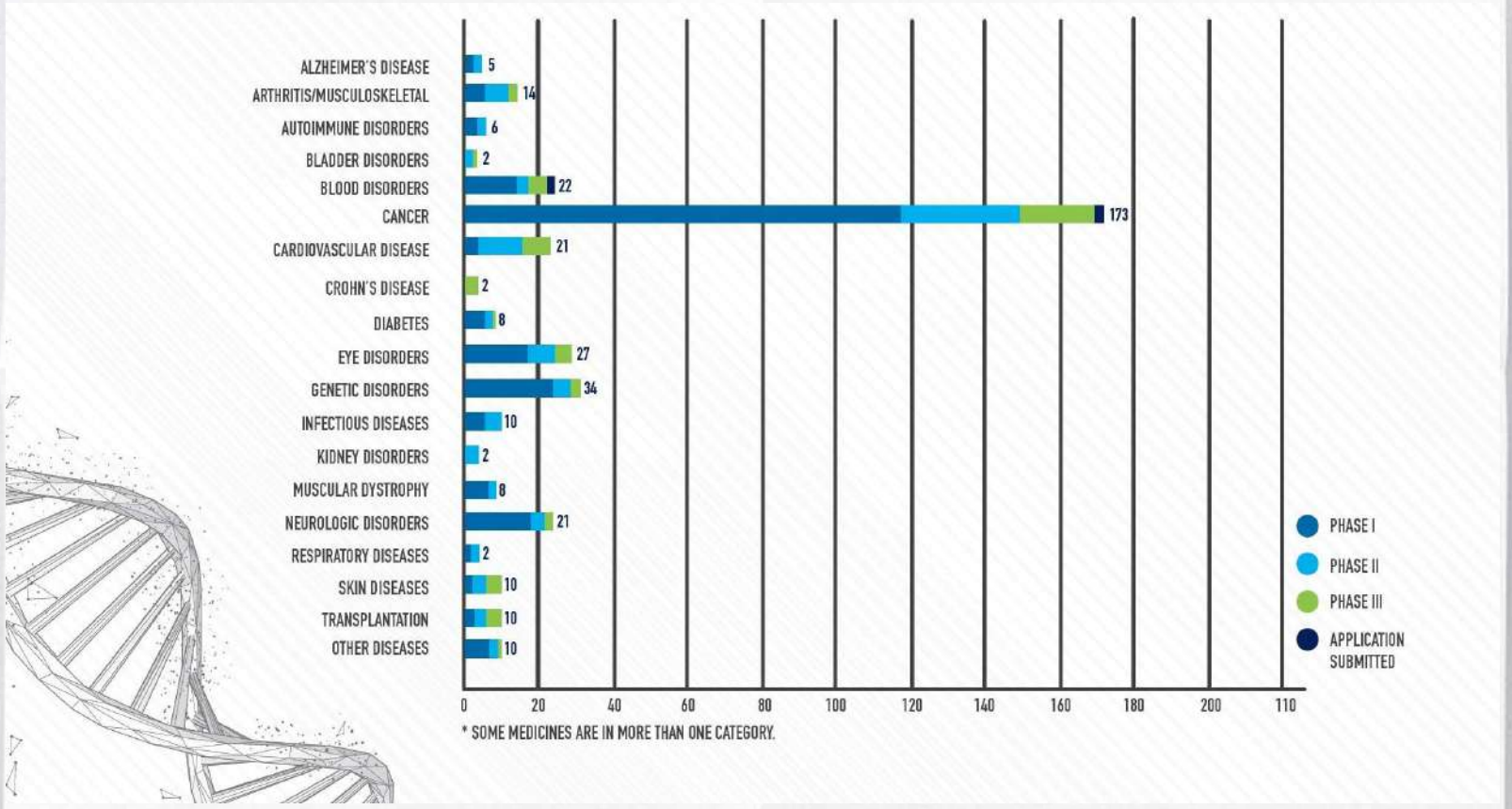




Data from: USA

Medicines under Development

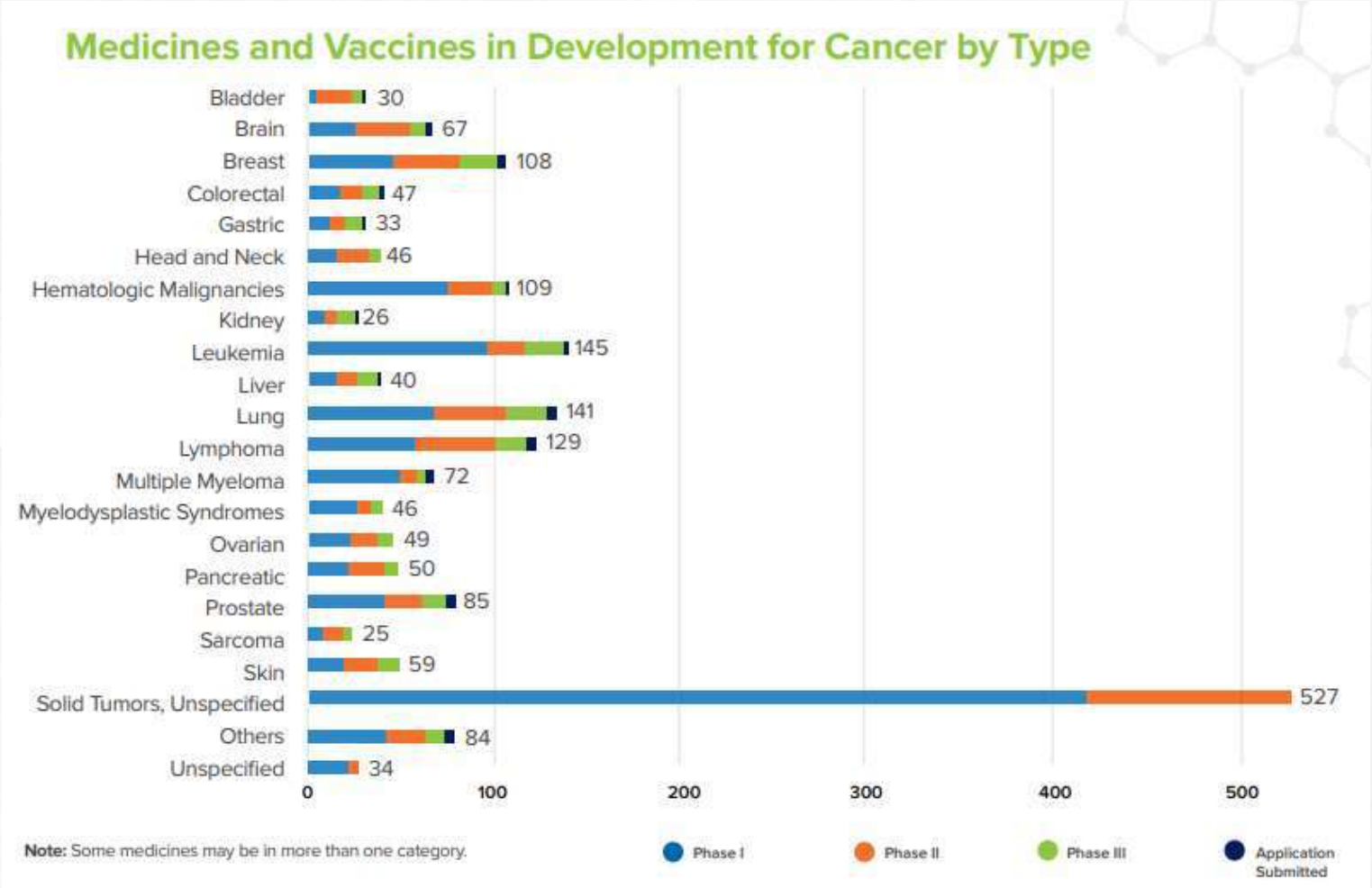
PhRMA
RESEARCH • PROGRESS • HOPE



Data from: 2020

Medicines under Development for Cancer

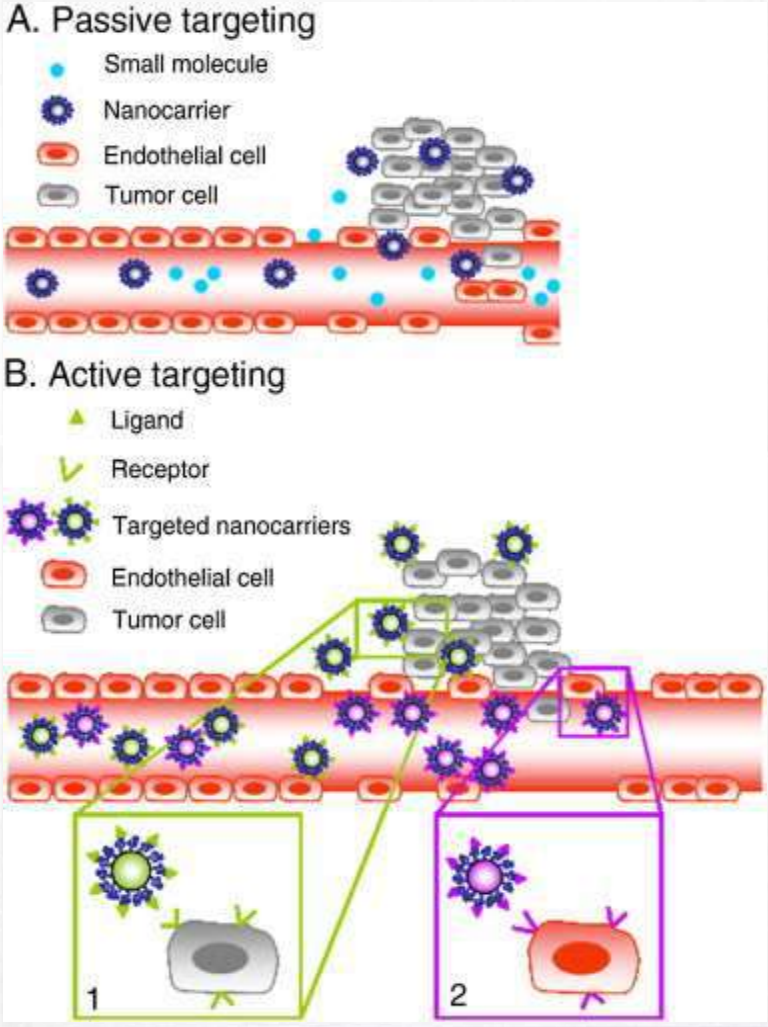
PhRMA
RESEARCH • PROGRESS • HOPE



Data from: 2020

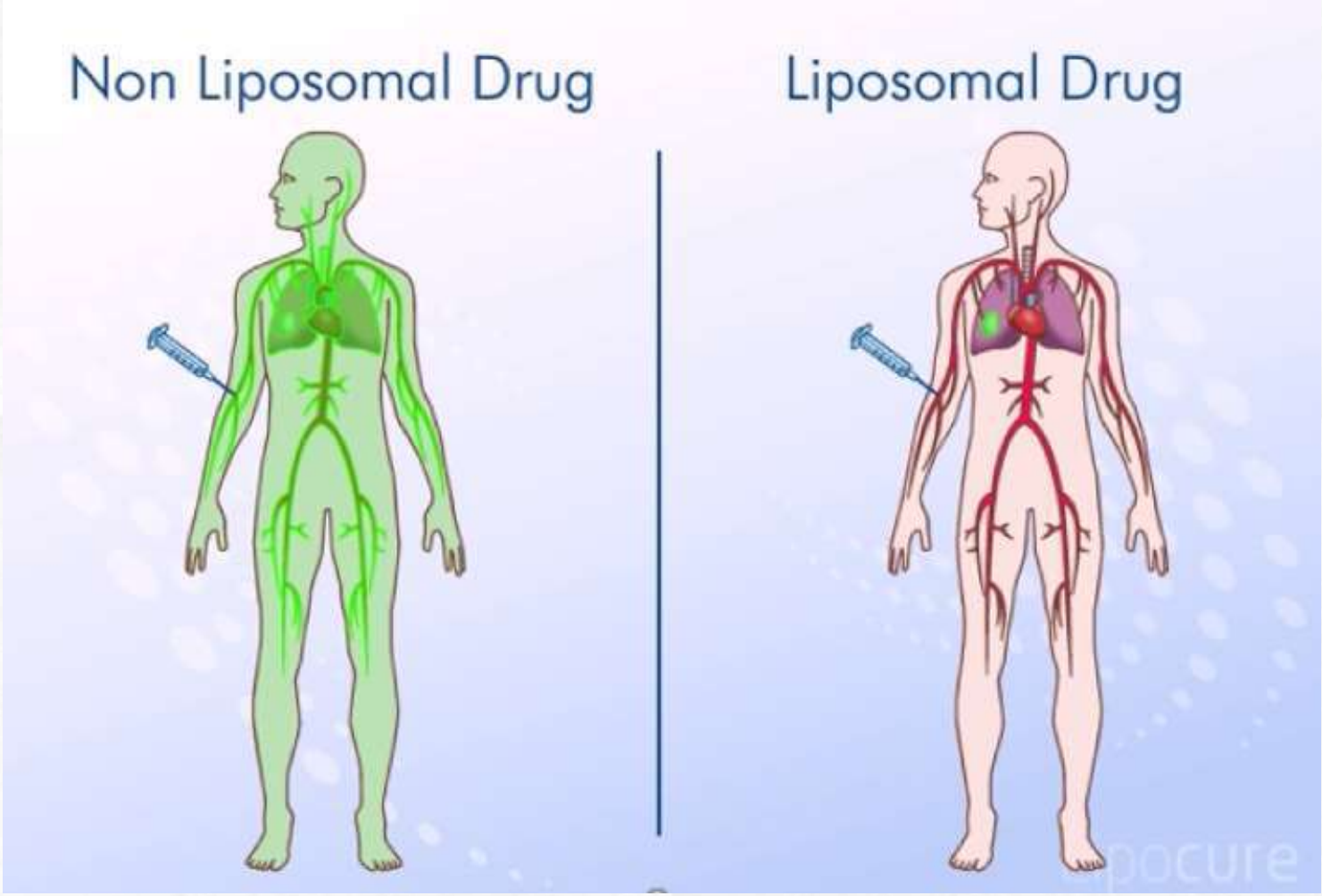
Nanomedicine

- Passive – Enhanced Permeability and Retention (EPR) effect
- Active Targeting



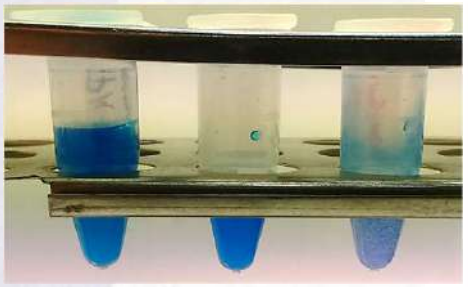
Danhier, et el, J Control Release. 2010 1;148(2):135-46

Nanomedicine



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**

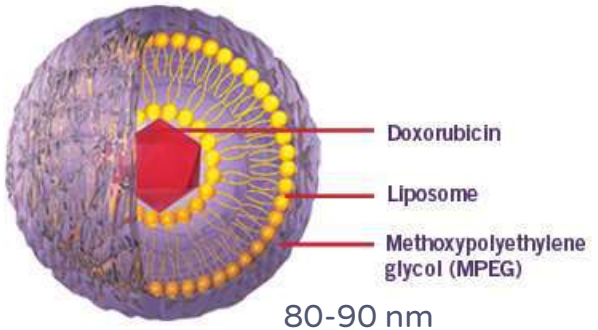
Increase **trans-epithelial permeability**: permeation enhancers; nanotechnology

Nanomedicine

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

Stealth liposomes of doxorubicin

Ovarian cancer
AIDS-related Kaposi's Sarcoma
Multiple Myeloma



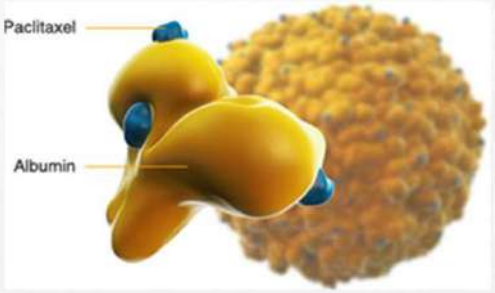
80-90 nm

Cardiotoxicity dose:
Doxorubicin: 570 mg/m^2
Doxil: 785 mg/m^2

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^2
Abraxane: 260 mg/m^2

Cancer Stem Cells (CSC)

CSC properties

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

Self-renewal ability

Abnormal activation of proliferating signaling pathways **(Wnt/β-Catenin, Notch and Hedgehog)**

Multi-lineage differentiation potential

$CD44^{+}/CD24^{-/low}$ ALDH1, CD133…

Tumor-forming ability

Non-adhesion survival

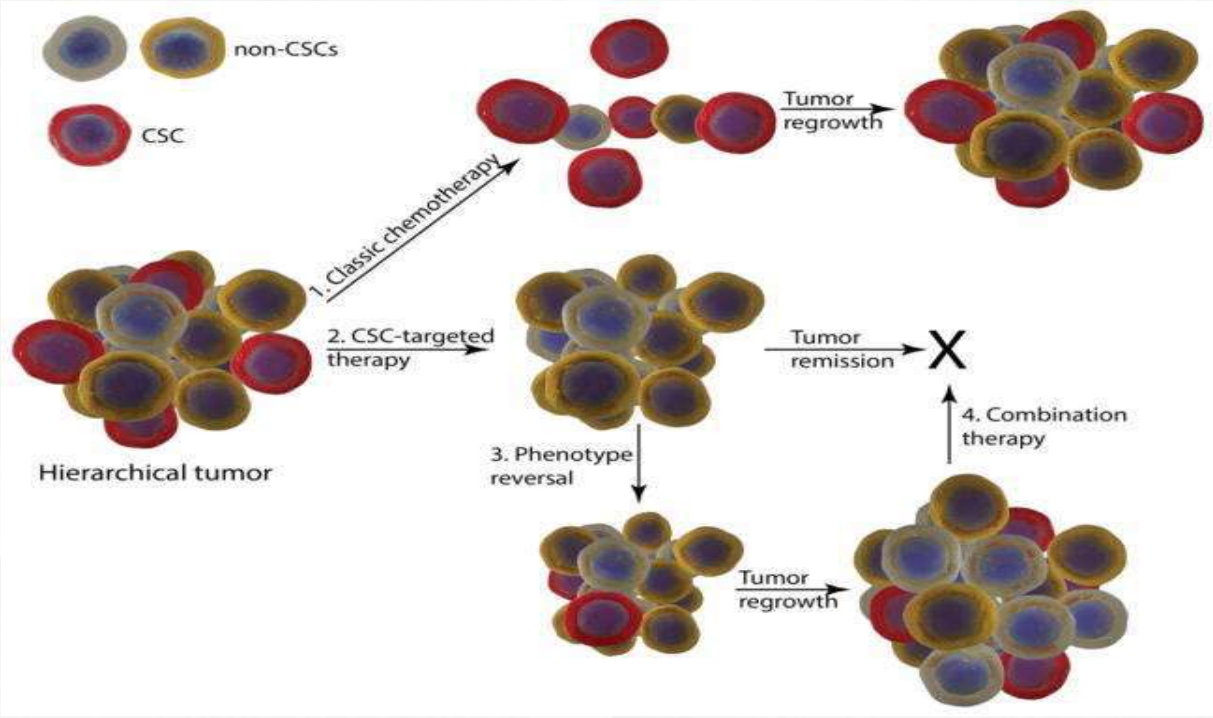
↑ invasion potential

Resistant to conventional anticancer therapies

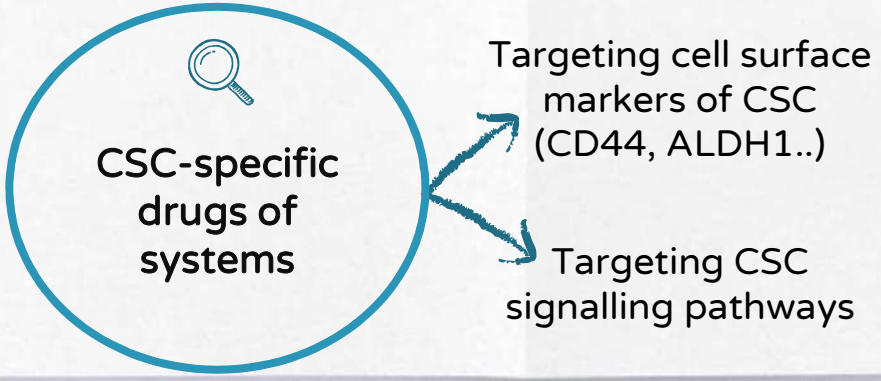
Urgent need to identify efficient targeted anticancer therapies

Infrequent: 1-10% of total tumor cells

Targeting Cancer Stem Cells (CSC)



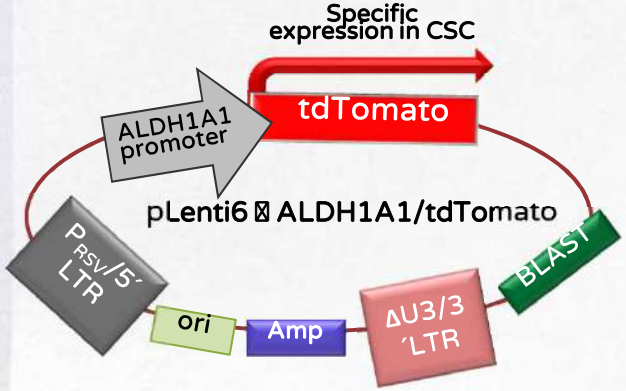
Y. Zhao et al. Adv Drug Deliv Rev, 2013, 65(0): 1763–1783.



Advanced Breast Cancer

Development of CSC fluorescently traceable model

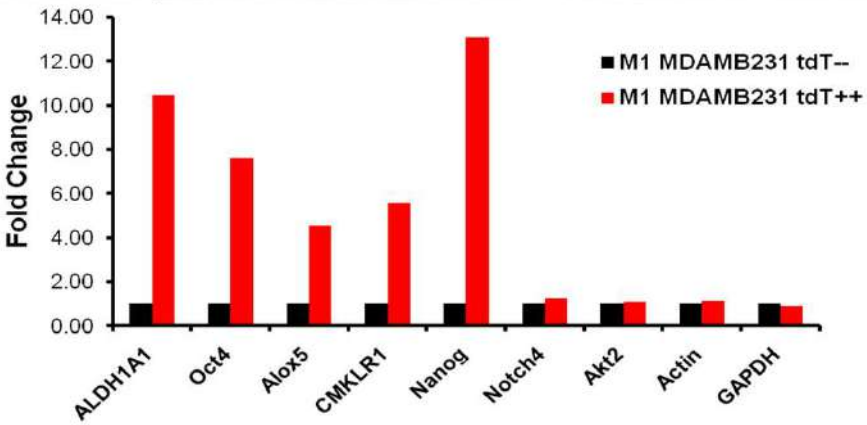
- Breast Cancer – most prevalent cancer worldwide (WHO).

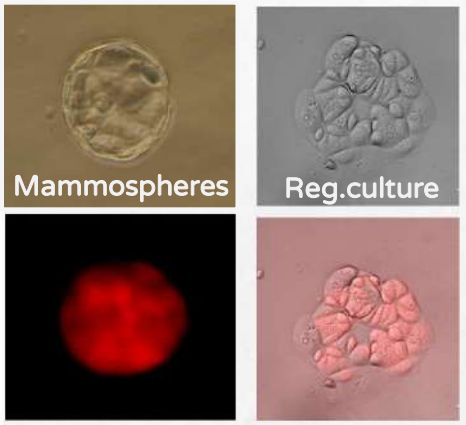


ALDH1A1-tdTomato reporter system

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

*ALDH1A1 is overexpressed in bCSCs





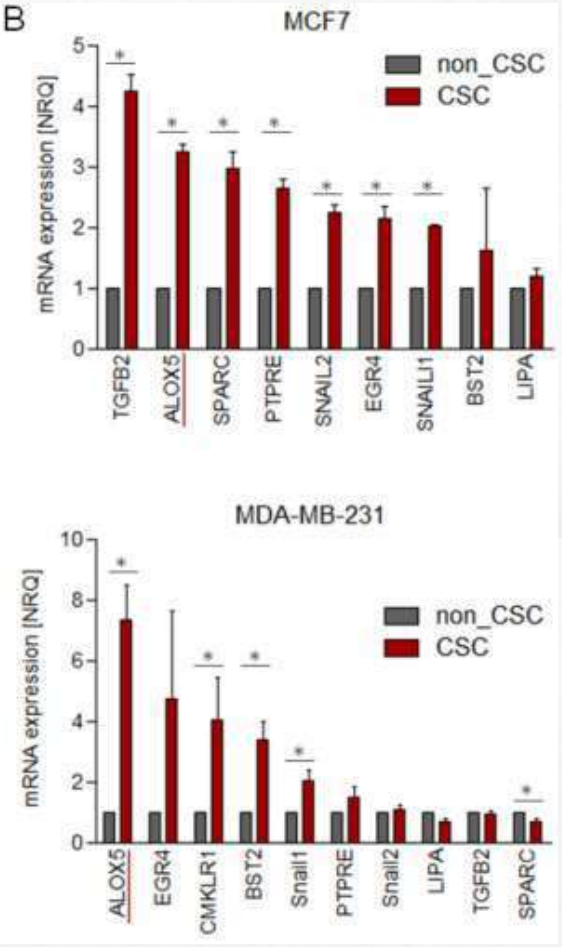
Gener, P. et al, Nanomedicine. 2015 11(8):1883-92

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_nnCSC	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	3.36	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

B

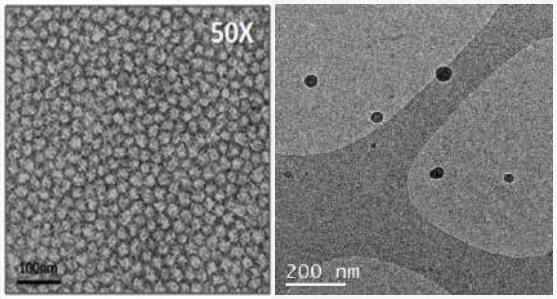


- Zileuton™ – Anti-asthmatic drug – inhibitor of ALOX5 – overexpressed in CSC

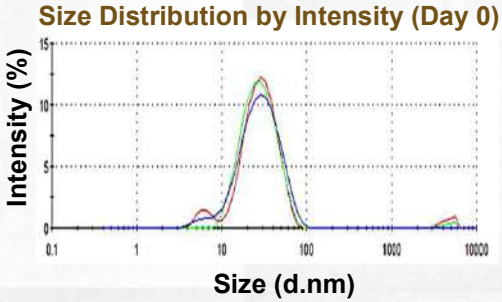
Gener, P. et al, Nanomedicine. 2020 Feb;24:102106

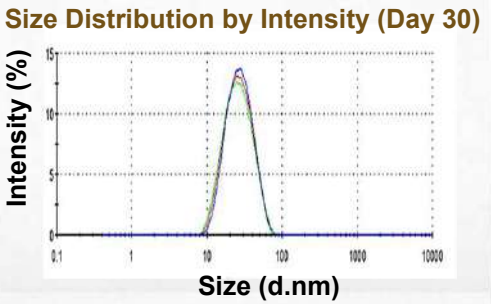
Polymeric Micelles: Zileuton™ – characterization

- Morphology (TEM and CryoTEM)



- Size and Stability over time (day 0 and day 30)

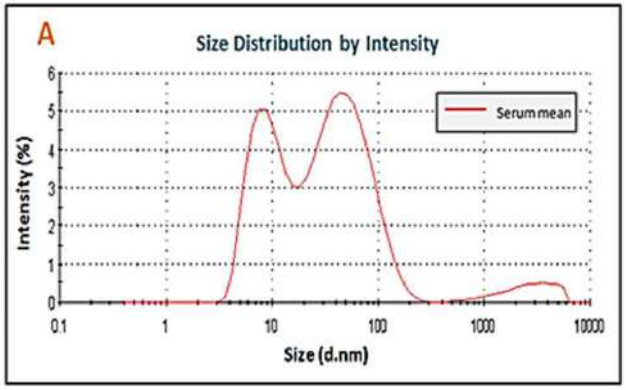


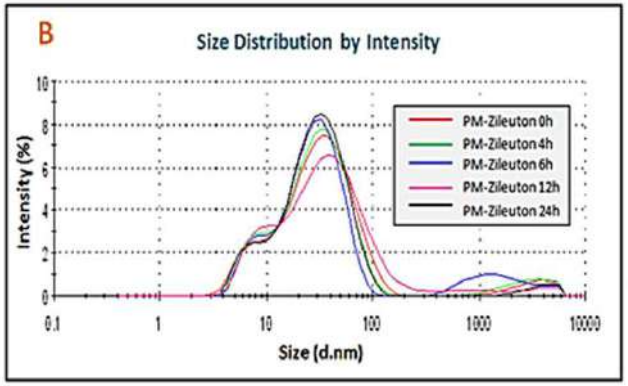


Sample	Size (nm) ± sd	Pdi ± sd
PM-Zil	23.86 ± 0.89	0.226 ± 0.016

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

- Stability in serum

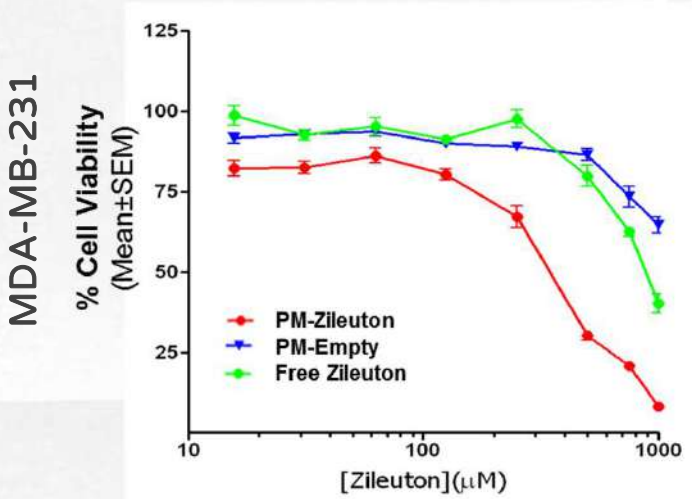


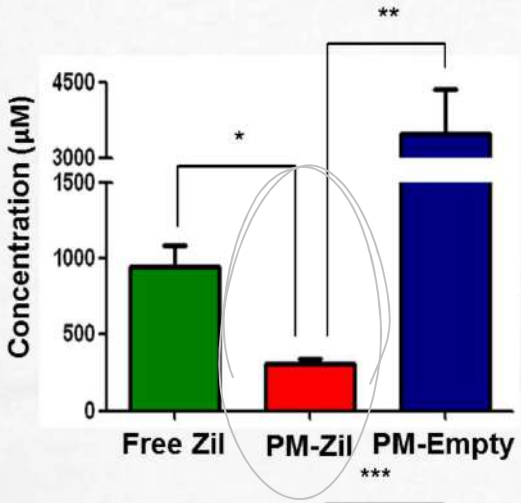


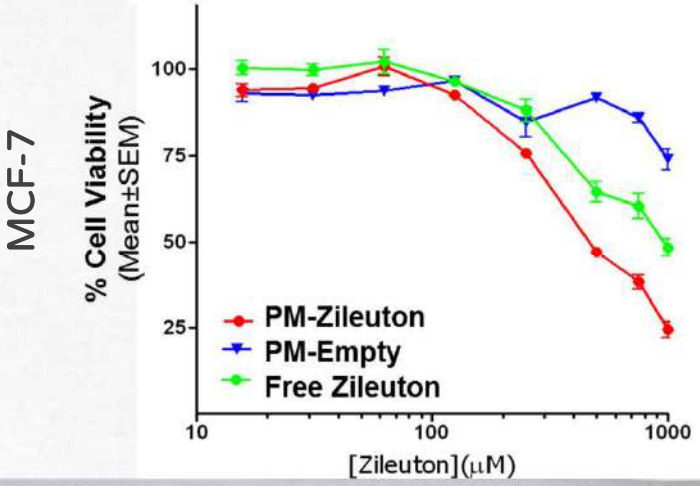
Gener, P. et al, Nanomedicine. 2020 Feb;24:102106

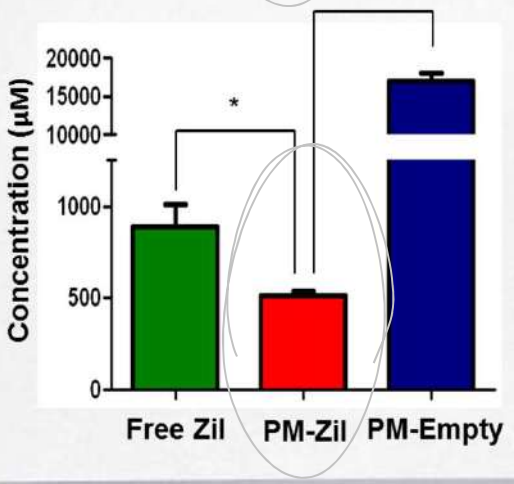
Polymeric Micelles: Zileuton™ – *in vitro* results

Increased activity of Zileuton™ when encapsulated in PM







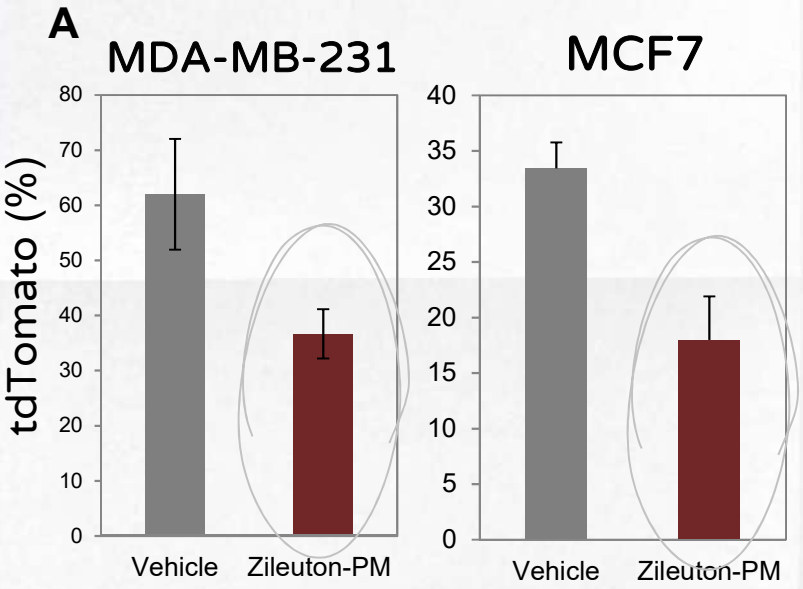


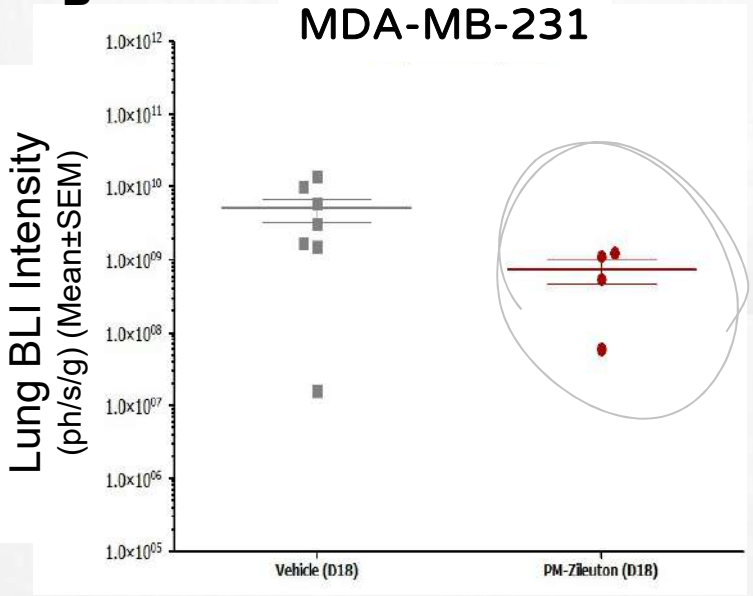
Gener, P. et al, Nanomedicine. 2020 Feb;24:102106

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

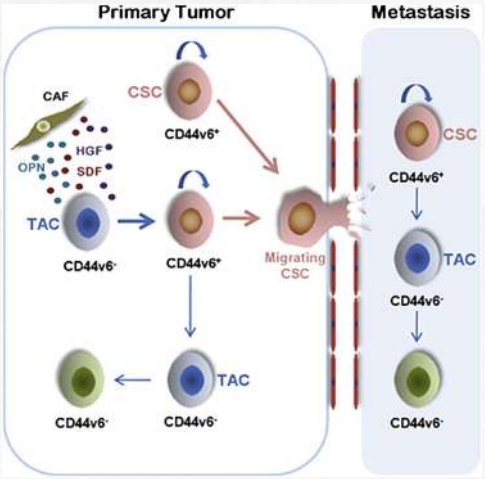
Gener, P. et al, Nanomedicine. 2020 Feb;24:102106

Experiment conducted with the collaboration of FVPR/U20 ICTS Nanbiosis

Advanced Colorectal Cancer

CD44v6 in Colorectal Cancer

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



CD44v6

Survival

Multidrug resistance

Lymph node invasion

Distant metastasis

In gastrointestinal cancers:
Tumor niche **reprograms** CD44v6– CRC progenitors into metastatic CD44v6+ CSC.

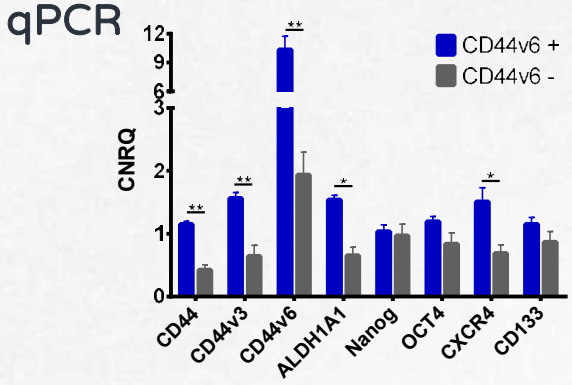
Todaro, et al, Cell Stem Cell 2014, 14, 342–356
Wang, et al, Oncotarget, 2017, 8(8), 12866-12876
Wang, et al, Mol Med Rep. 2015, 11(5):3505-10

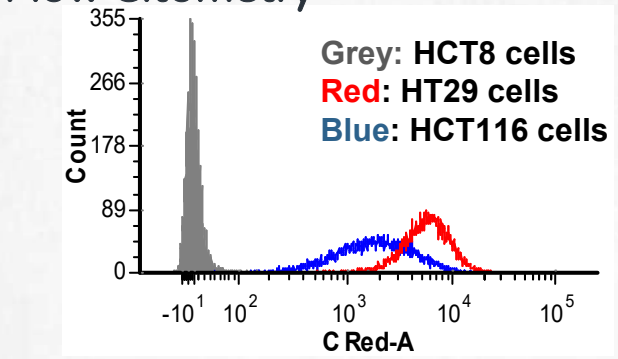
CD44v6 → PI3K/Akt, MAPK/ERK, Wnt/β-catenin

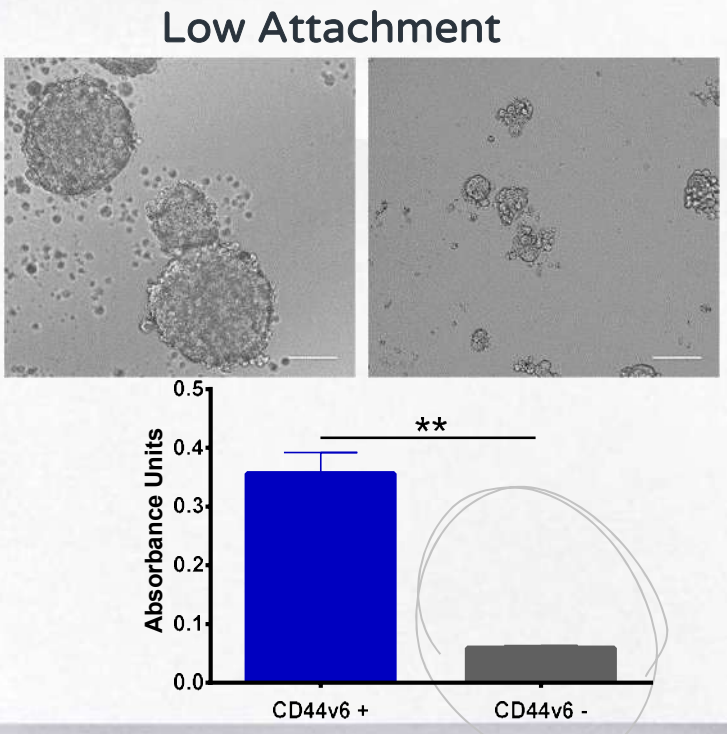
Ma, et al, Cell Death & Disease 2019, 10:30

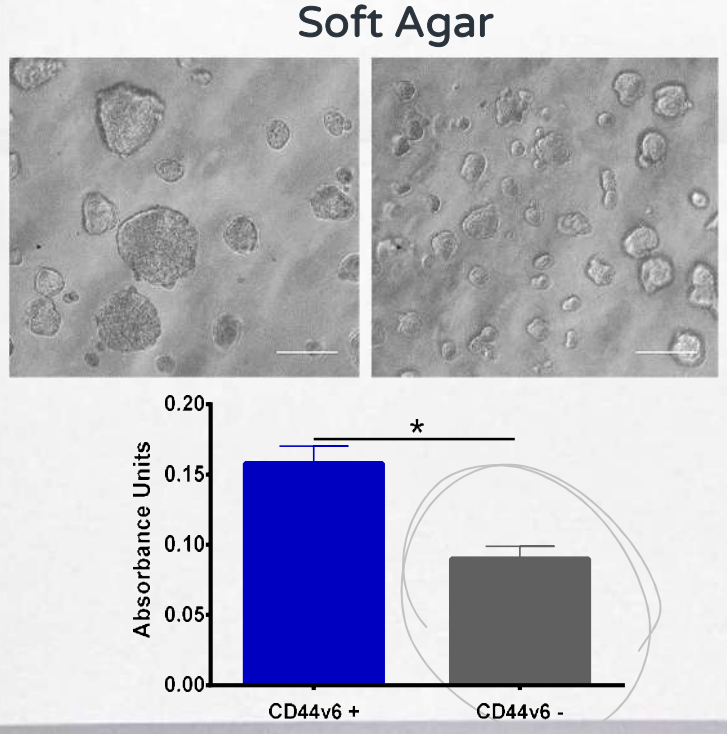
CD44v6 – Biomarker of CSC

In vitro validation of CD44v6 as targeting for CSC





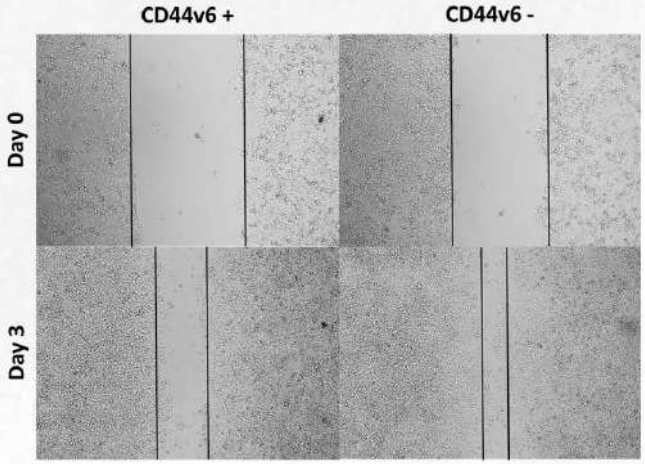


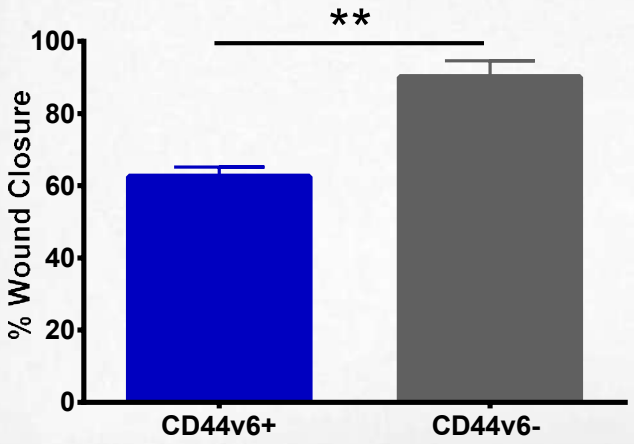


Andrade, F. et al, J Contr. 2021;331(2021):198-121

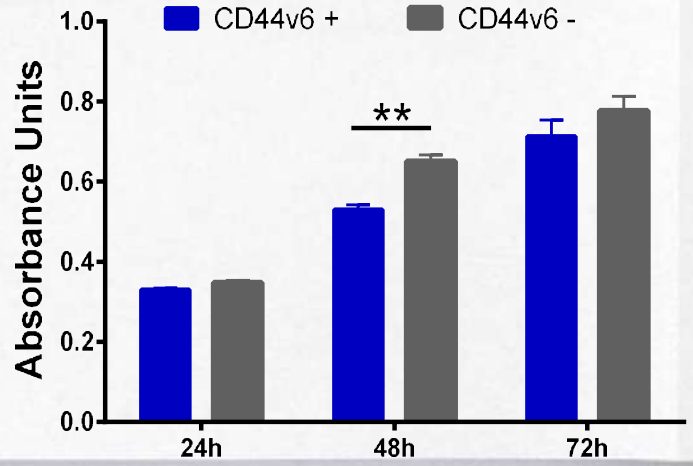
In vitro validation of CD44v6 as targeting for CSC

Migration





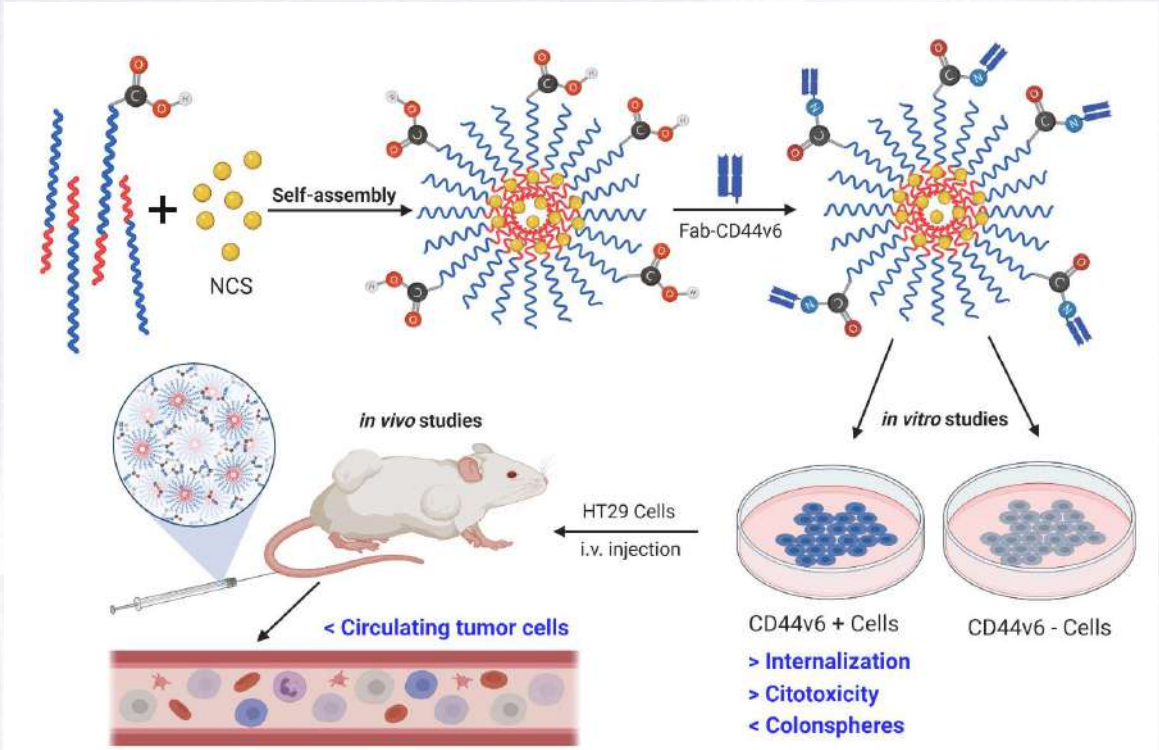
Proliferation

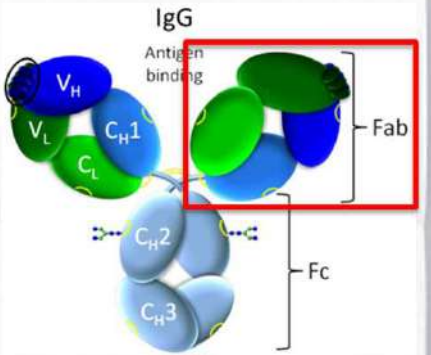


CD44v6 high expression cells present stemness properties

Andrade, F. et al, J Contr. 2021;331(2021):198-121

Objective

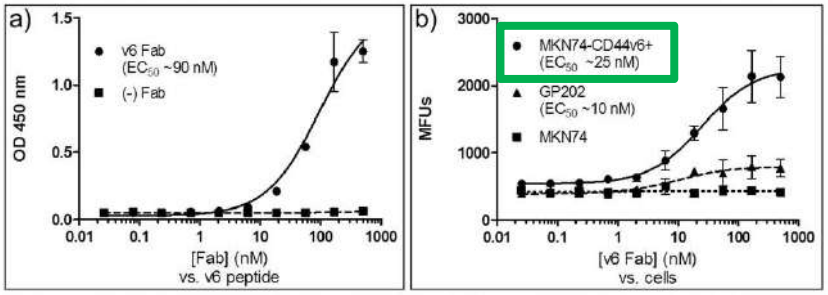




UPPSALA UNIVERSITET

Marika Nestor
(HNSCC diagnosis)

FACS – fixed cells

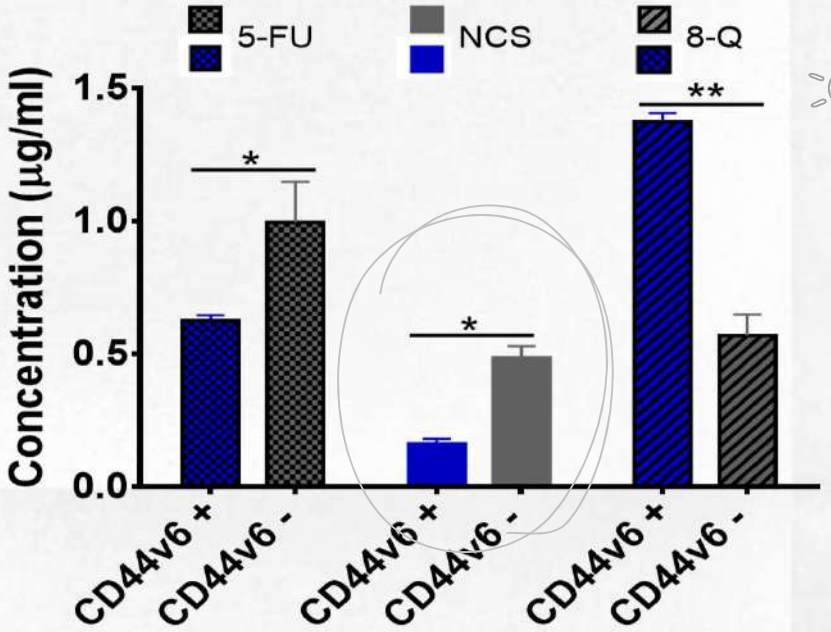


Antigen Presentation	K_D (nM)
Recombinant CD44v3-10	6
CD44v6 peptide	60
CD44v6+ cell line	10

Andrade, F. et al, J Contr. 2021;331(2021):198-121

Nilvebrant et al. Biotechnol Appl Biochem 2012
Kennedy et al, Acta Biomaterialia, 2018, 81, 208-218

Drug Selection



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

Drug/Cell subpopulation	IC_{50} (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

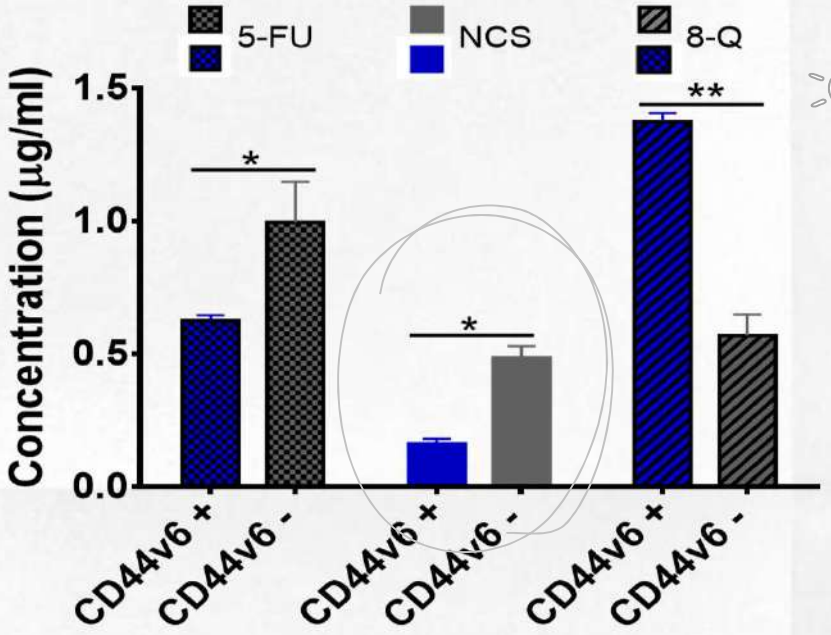
Niclosamide ↓ Wnt/β-catenin

Niclosamide ↓ mTOR/STAT3

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

Andrade, F. et al, J Contr. 2021;331(2021):198-121

Drug Selection



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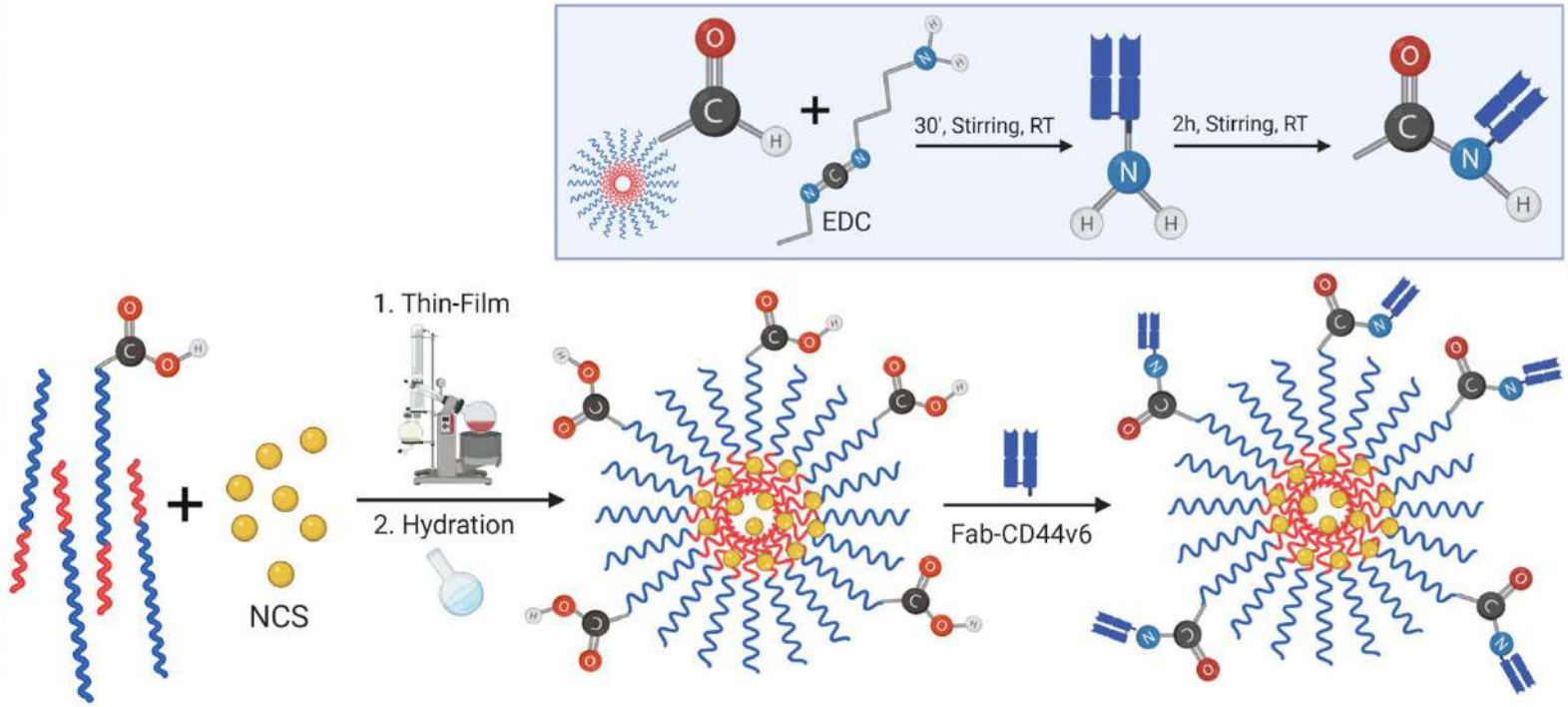
Niclosamide ↓ Wnt/β-catenin

mTOR/STAT3

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

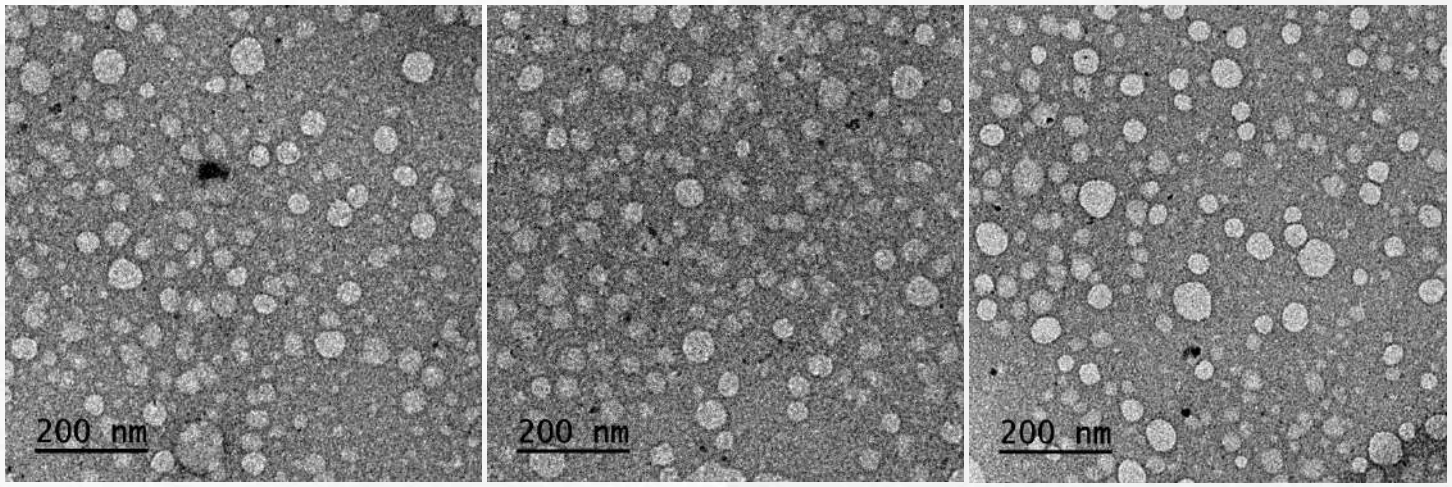
Andrade, F. et al, J Contr. 2021;331(2021):198-121

Polymeric Micelles Design



Polymeric Micelles-Niclosamide:CD44v6 Fab - characterization

Formulation	Mean Diameter (nm)	PdI	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 ± 4x10^5
PM-NCS:Fab	29.7 ± 1.2	0.338 ± 0.055	-6.5 ± 0.7	99.7 ± 4x10^5

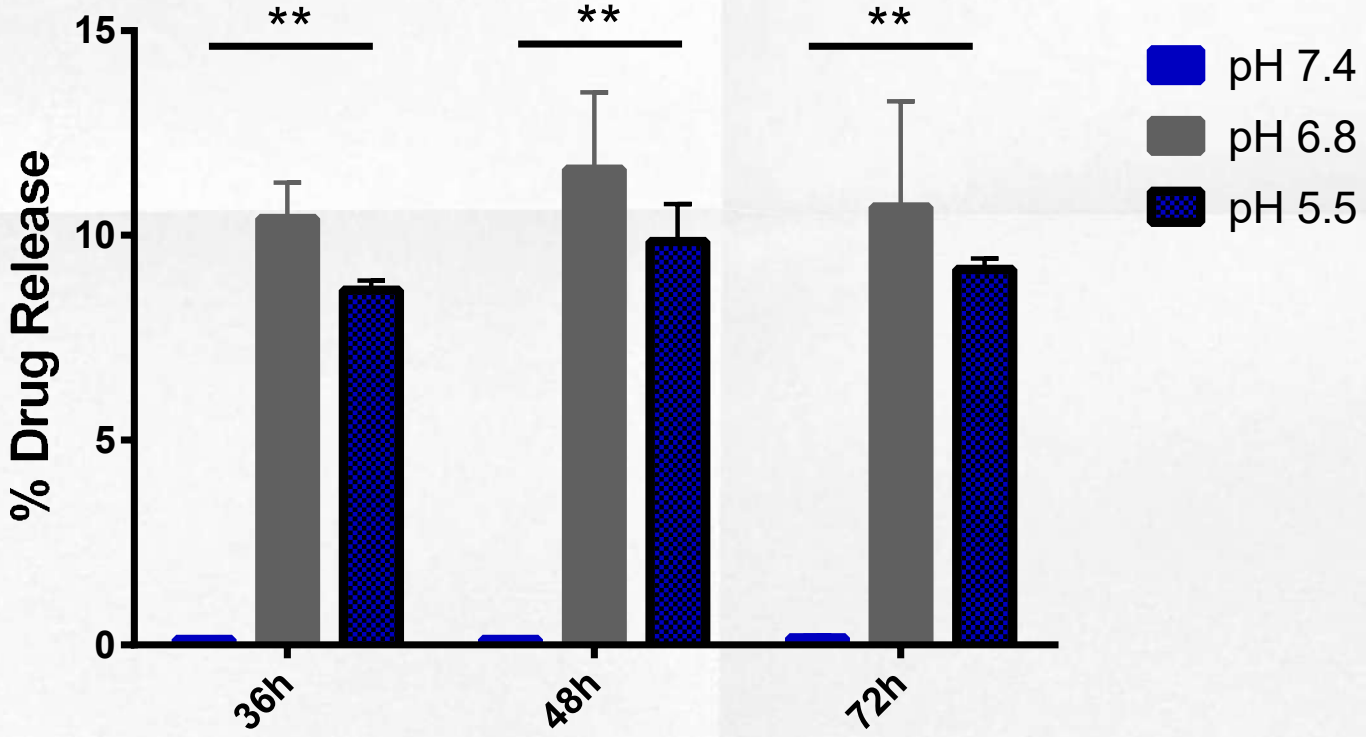


PM PM-NCS PM-NCS:Fab

Andrade, F. et al, J Contr. 2021;331(2021):198-121

Polymeric Micelles-Niclosamide:CD44v6 Fab - characterization

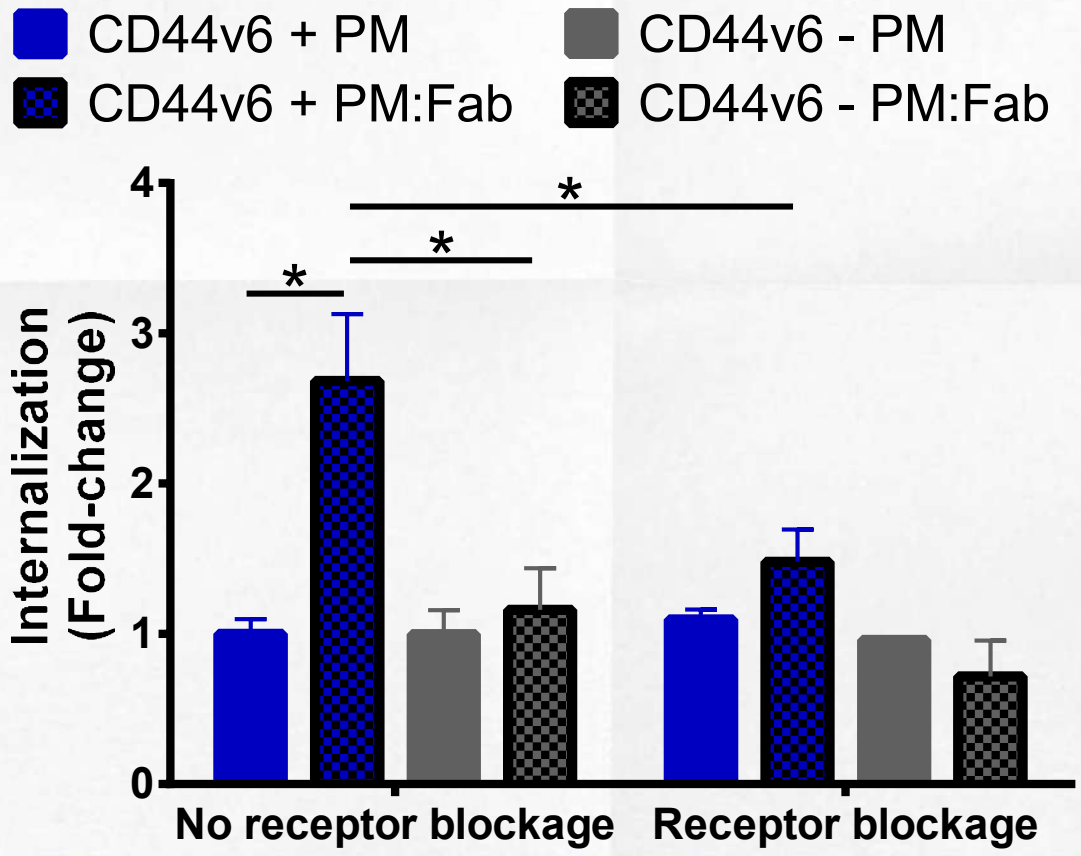
Drug release dependent on pH
Preferential release at tumor microenvironment



Andrade, F. et al, J Contr. 2021;331(2021):198-121

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

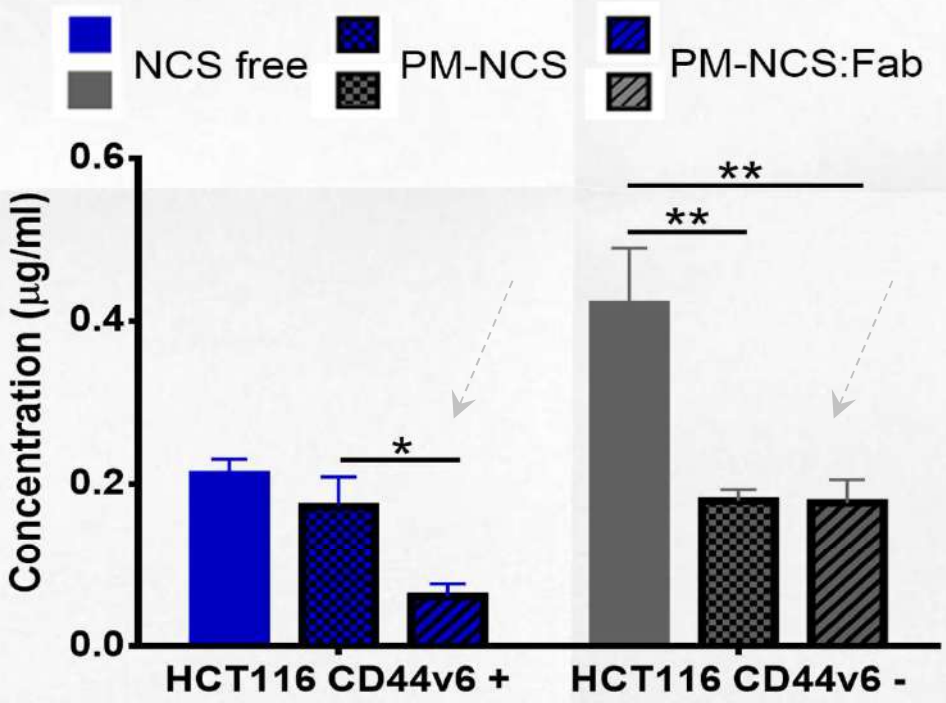
Internalization: Fab-CD44v6 PM surface modification increase internalization in CD44v6+ (CSC) population



Andrade, F. et al, J Contr. 2021;331(2021):198-121

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

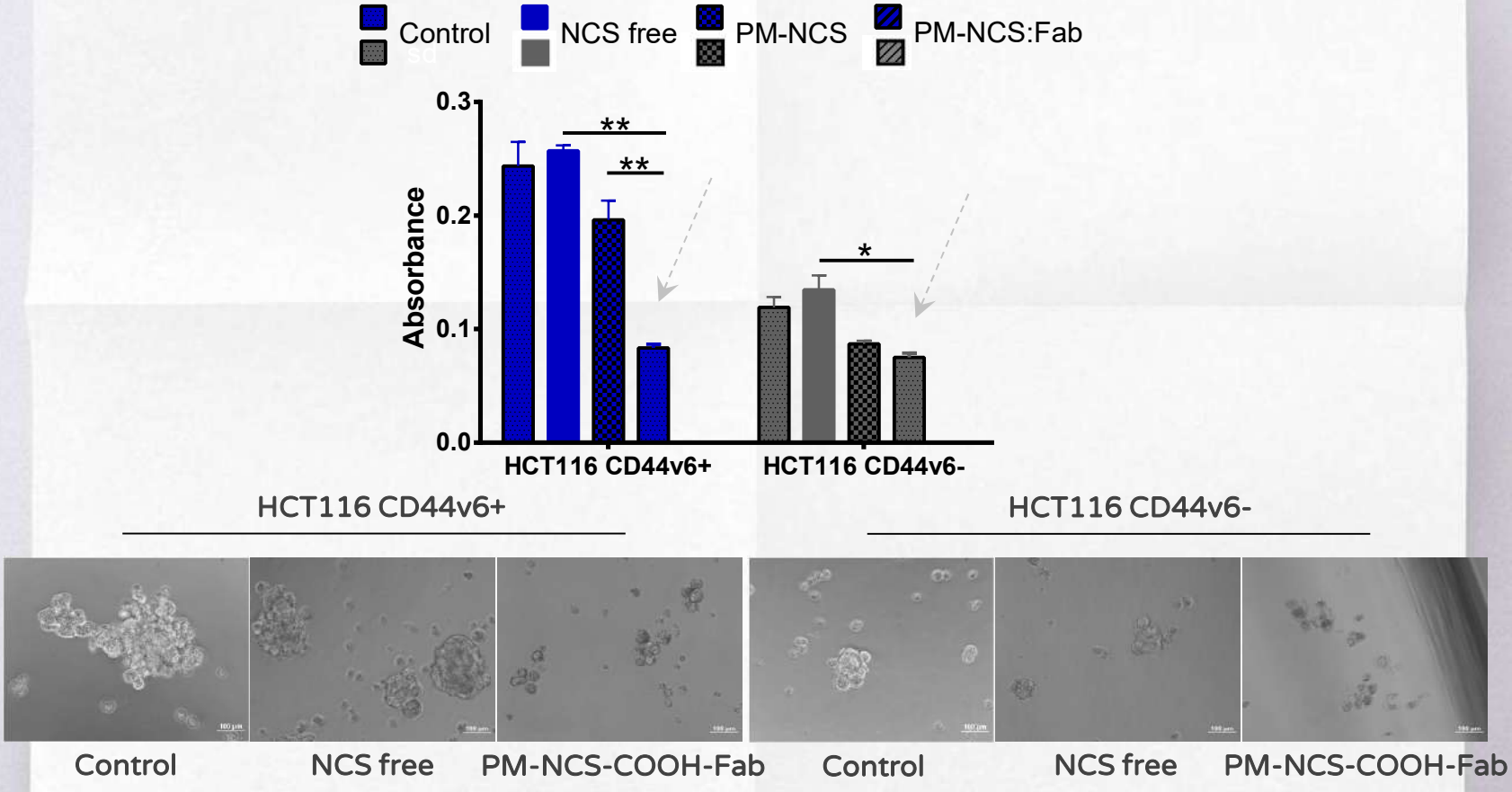
Efficacy: NCS encapsulation into PM increase its efficacy and Fab presence increase the efficacy in CD44v6+ (CSC) population



Andrade, F. et al, J Contr. 2021;331(2021):198-121

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

Efficacy: PM-NCS:Fab impairs colonpheres formation

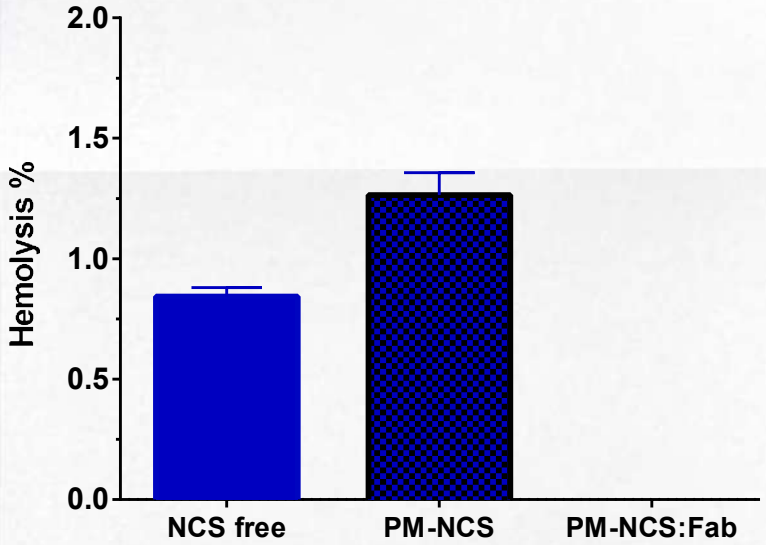


Andrade, F. et al, J Contr. 2021;331(2021):198-121

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

Hemocompatibilty: 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

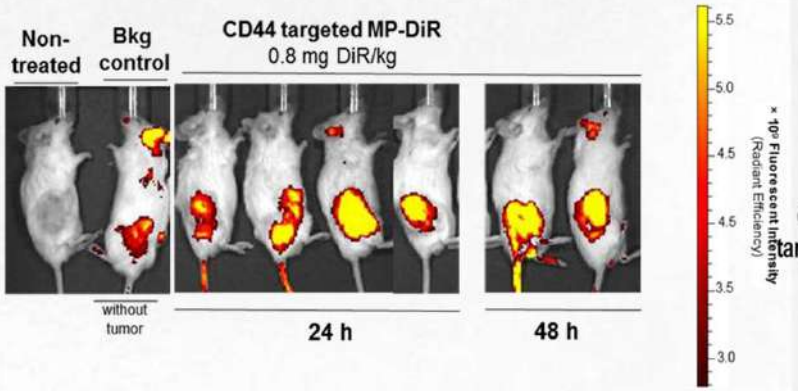
NV: PT ≤ 13.4s and TT ≤ 21s

Andrade, F. et al, J Contr. 2021;331(2021):198-121

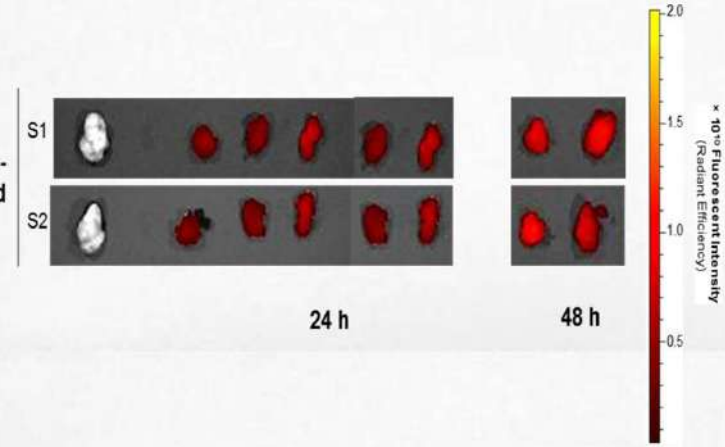
Experiment conducted with the collaboration of FVPR/U20 ICTS Nanbiosis

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

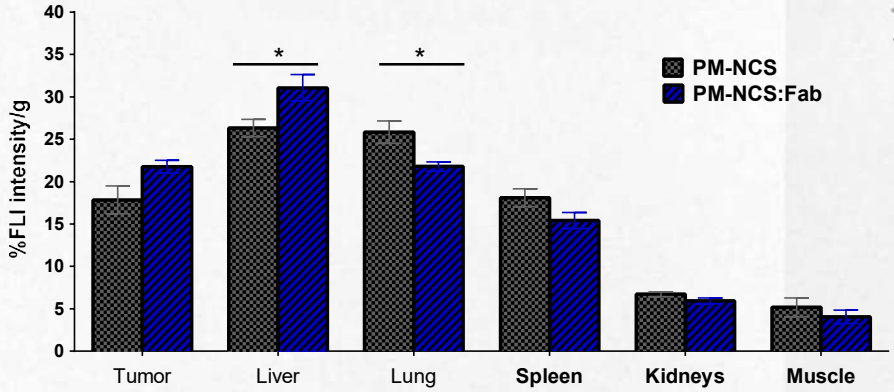
In vivo tumor accumulation



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

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Experiment conducted with the collaboration of FVPR/U20 ICTS Nanbiosis

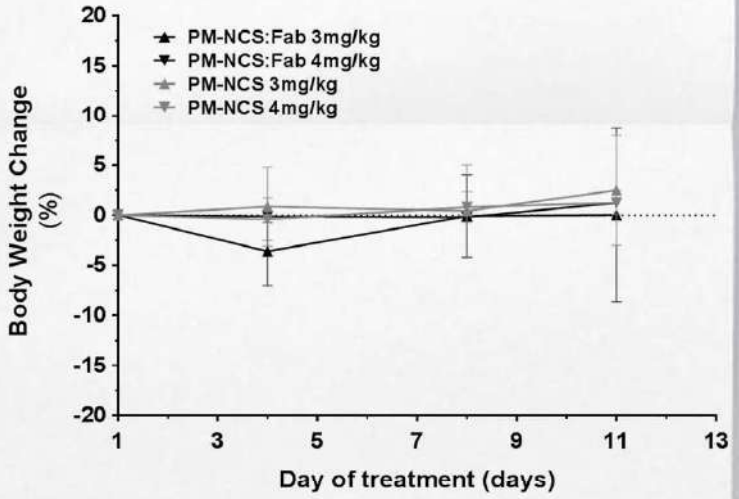
Polymeric Micelles-Niclosamide:CD44v6 Fab – *in vivo* results

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

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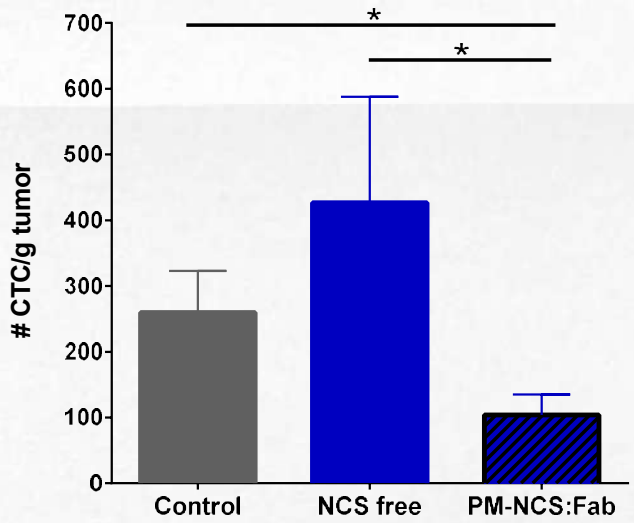
Experiment conducted with the collaboration of FVPR/U20 ICTS Nanbiosis

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

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Experiment conducted with the collaboration of FVPR/U20 ICTS Nanbiosis

Conclusions

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

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

Aknowlegments

Drug Delivery & Targeting (DDT)
Area of Functional Validation & Preclinical Research (FVPR)

- Diana Rafael
- Francesc Martinez
- Joquín 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 Sarmento

Marika Nestor

Thank You!

Any questions?

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