A Rationally-designed Polypeptide-based Combination Conjugate for Treatment of Breast Cancer Brain Metastasis

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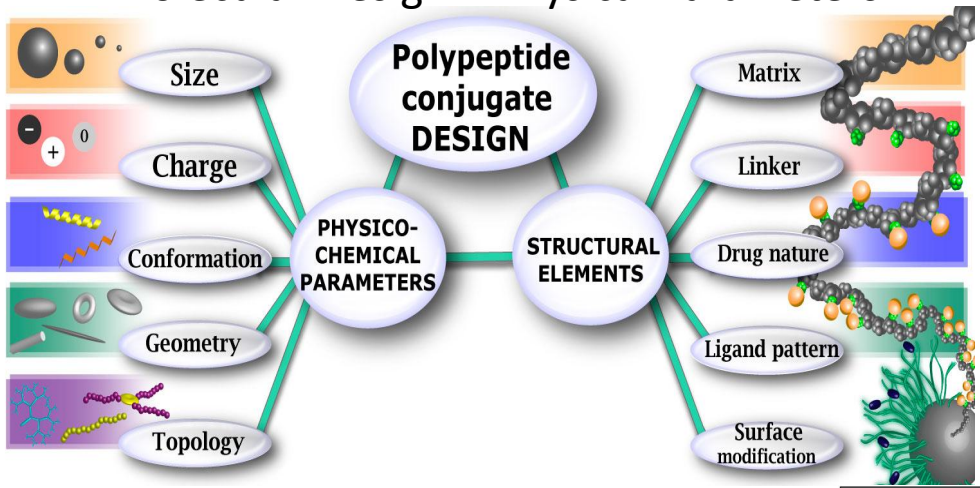
CRS 2022 Annual Meeting & Expo

July 11 – 15, 2022 | Montreal Congress Center, Montreal Canada

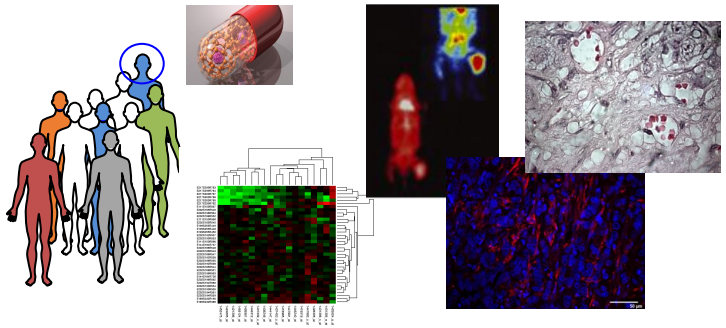
Advanced Delivery Science

Our Motivation:
Polypeptide-based Drug Delivery Systems for Unmet Clinical Needs

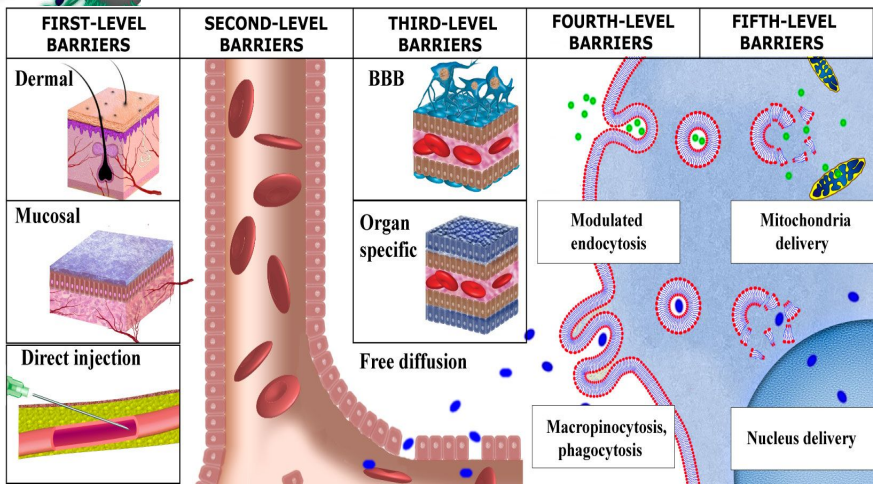
Molecular Design – Physical Parameters



Biological output – Therapeutic performance

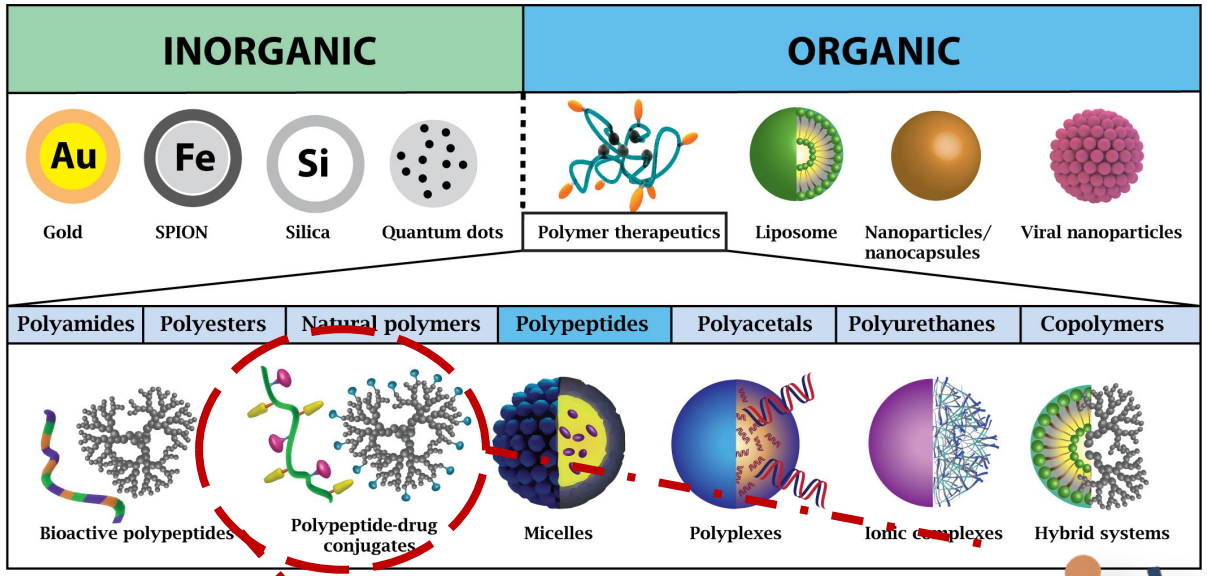


Bio-Nano Interface – Biological Barriers



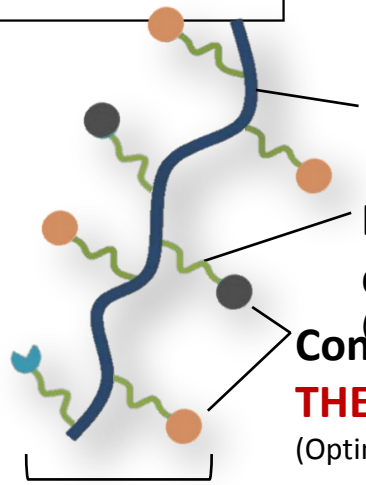
O. Zagorodko, J.J. Arroyo-Crespo, V, J. Nebot, M. J. Vicent, *Macromolecular Bioscience* **2016.**
A. Niño-Pariente, V.J. Nebot, M. J. Vicent, *Current Pharmaceutical Design*, **2016.**

Key Features for a Rational Design of Polypeptide-drug Conjugates



Key Features:

I. Choice of the **polymeric carrier**
II. Choice of the proper **linking chemistry**
III. Characteristics of **drugs** suitable for conjugation
IV. Targeting moieties (CMT through BBB)



Water soluble biodegradable backbone: POLYPEPTIDES
(Increased drug solubility- Topicity-Architecture- control size-shape)

Bioresponsive drug-linkers
(Controlled, site-specific, delivery)

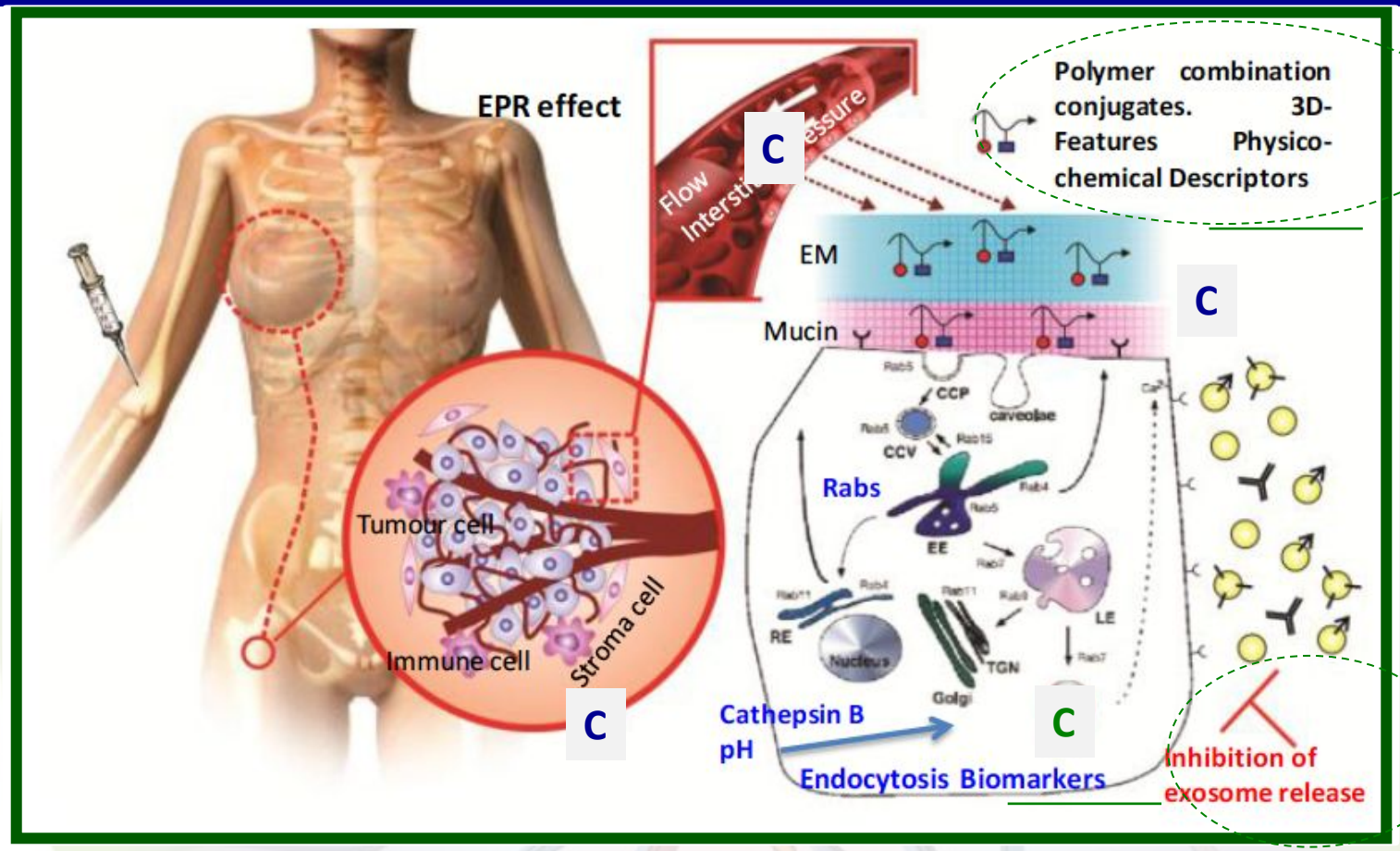
Conjugation of synergistic drugs: COMBINATION THERAPY
(Optimized synergism)

Active Targeting

High molecular weight: Passive Targeting
(Increased accumulation via EPR effect)

T Melnyk, et al Adv Drug Deliv Rev 2020

Design of Personalized Polymer-based combination Therapeutics for Advanced Breast Cancer



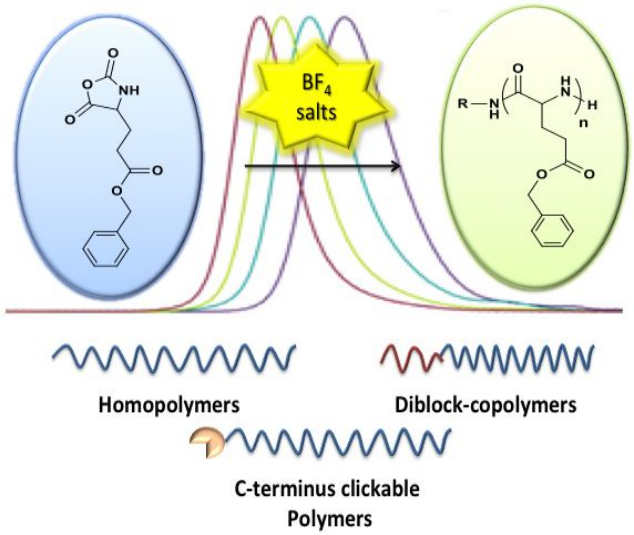
A. Multivalent and Biodegradable Polypeptide-based Carriers with Controlled architecture

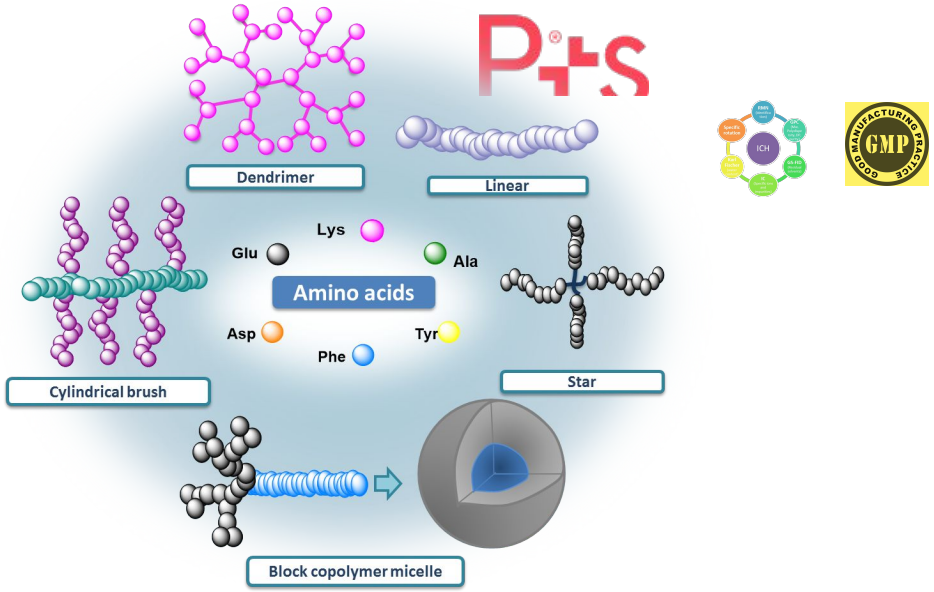
B. Polymer-based Combination Therapy with Novel Molecular Target

C. Physico-chemical Descriptors & Functional Biomarkers. Personalised Therapy

A. Versatile Polypeptides as Carriers

- Biodegradability
- High water solubility
- Multivalency- High Loading capacity
- Examples in the market and in advanced Clinical trials
- Living Polymerization
  Controlled molecular weigth
- Batch-to batch reproducibility
- At the lab: up to 100 g scale.
  At SME at Kg Scale under GMP
- Minimized heterogeneity
- Different architectures
- Undetectable racemization





High quality NCA and precise control on polymerization processes allows accurate PAA engineering

at large scale following ICH guidelines and under GMP

[1]Conejos-Sanchez, I. et a., Polymer Chemistry, **2013**, 4, 3182; M.J. Vicent et al. US PATENT 9623125. Licenced to PTS. [2]Deming T. J., Chem. Rev., **2016**, 116, 786; [3]González-Henríquez C.M. et al., Polymers, **2017**, 9, 551; [4]Byrne M. et al., Macromol. Rapid Commun. **2015**, 36, 1862; [5]Duro-Castaño A., Biomater. Sci., **2015**, 3, 1321; [6]Habraken G. J. M. et al., Macromol. Rapid Commun. **2011**, 33, 272−286; [7] Heise A., Chem. Soc. Rev., **2013**, 42, 7373; O. Zagorodko, *Macromolecular Bioscience,* **2017,** 17 , 1600316*;* A. Duro-Castaño et al Polymer 2014**,**

B. Polymer-based Combination Therapy with Novel Molecular Target

HTS Approach + PK Control + Exosome Release

Current Clinical Treatment AdBC.
Chemotherapy
Endocrine Therapy
Targeted Therapies

Drug Ratio
Drug Kinetics

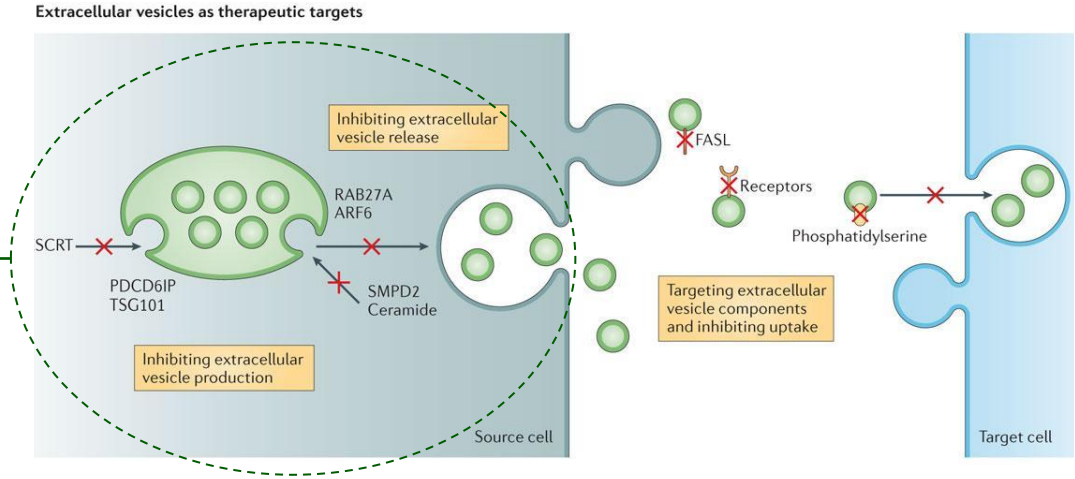
BC Cell models 2D and 3D Organoids:
Clinical Subtypes

1. Luminal A
2. Luminal B
3. HER2
4. **Basal/Triple Negative**

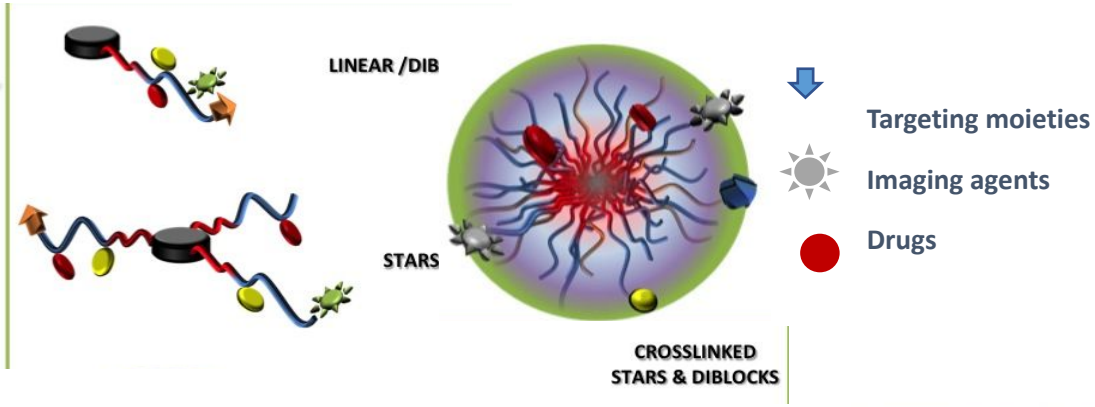
1
2
3
4

Linking Chemistry; PK

Molecular Target: Tumour-derived Exosome Release



S.L. Andaloussi et al. *Nat Rev Drug Discov* 2013



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PRINCIPE FELIPE
CENTRO DE INVESTIGACION

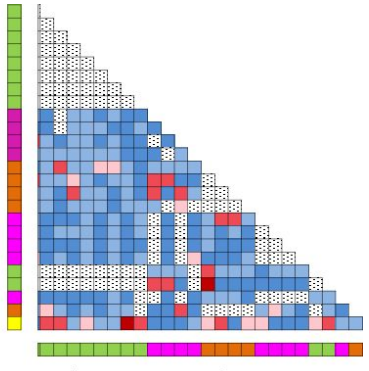
Specialised site Cell-based assays
Nanomedicine

B. Polymer-based combination Therapeutics for Advanced TNBC

Triple negative breast cancer (TNBC):

- Poor prognosis and Highly heterogeneous disease[1]
- High risk of developing metastasis, including brain metastasis[1]
- No effective targeted therapies available[1,2]

Polyglutamate (PGA)-based combination conjugates bearing two synergistic drugs (a chemotherapeutic agent – Dox – and a tyrosine kinase inhibitor – TKi) **as efficient therapies for metastatic TNBC**.



Exosome inhibitors
Chemotherapy
Endocrine therapy
Targeted therapies

Antagonist				*Synergistic*
< -5	-5 to 5	5 to 10	10 to 25	> 25

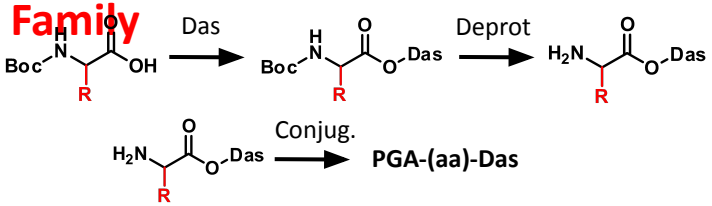
SELECTED DRUG COMBINATIONS
SELECTED DRUG RATIOS
FOR TNBC
Dox: Das

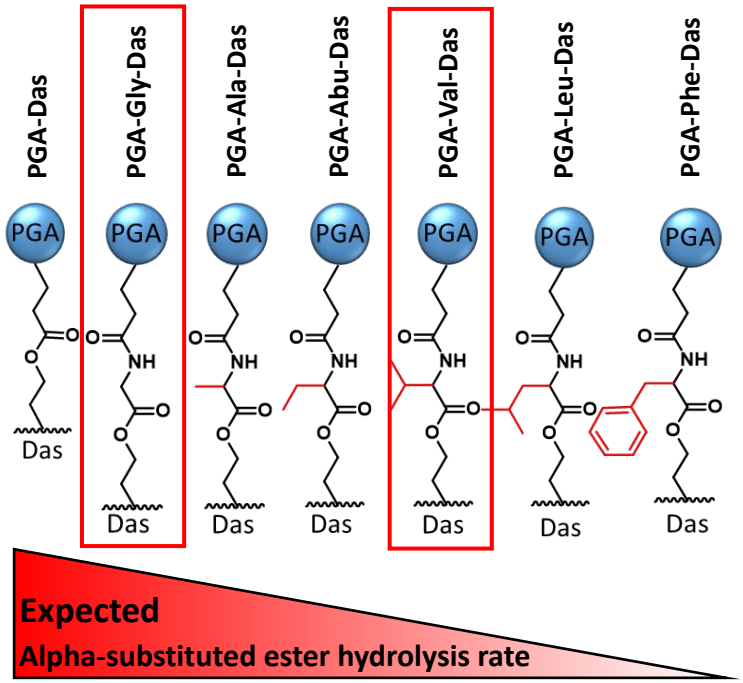
1. http://www.pathophys.org/breast-cancer/#Overview; 2. Palma G, et al. Oncotarget. 2015;6(29):26560-74; 3. Duro-Castano A, et al. Mol Pharm. 2015;12(10):3639-49.

IMPROVEMENT OF DRUG COMBINATION

Importance of the Linking Chemistry

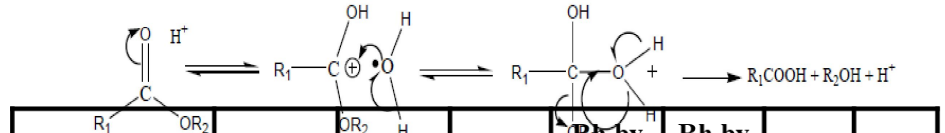
▶ Single Conjugates: PGA-aa-Das Family



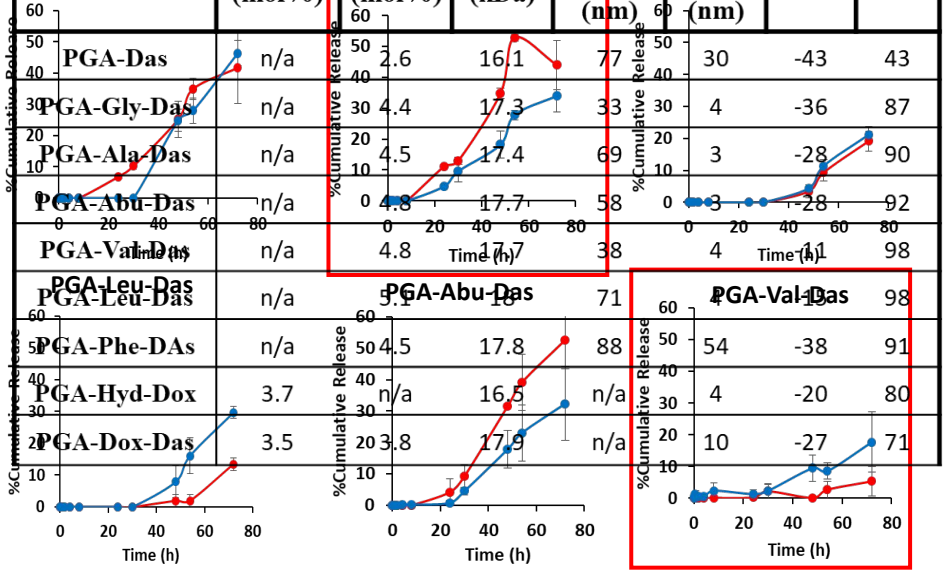


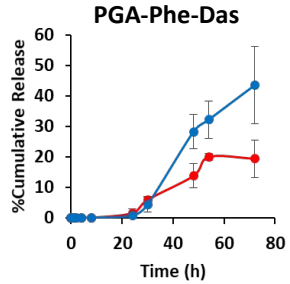
Expected
Alpha-substituted ester hydrolysis rate

▶ pH-mediated Drug Release Kinetics



Polymer-conjugate	Dox loading (mol%)	Das loading (mol%)	Theor. MW (kDa)	Rh by DLS (H_2O) (nm)	Rh by DLS (PBS) (nm)	Z-pot (mV)	C.E. (%)
PGA-Das	n/a	2.6	16.1	77	30	-43	43
PGA-Gly-Das	n/a	4.4	17.3	33	4	-36	87
PGA-Ala-Das	n/a	4.5	17.4	69	3	-28	90
PGA-Abu-Das	n/a	4.8	17.7	58	3	-28	92
PGA-Val-Das	n/a	4.8	17.7	38	4	-11	98
PGA-Leu-Das	n/a	5.1	18	71	4	-13	98
PGA-Phe-DAs	n/a	4.5	17.8	88	54	-38	91
PGA-Hyd-Dox	3.7	n/a	16.5	n/a	4	-20	80
PGA-Dox-Das	3.5	3.8	17.9	n/a	10	-27	71





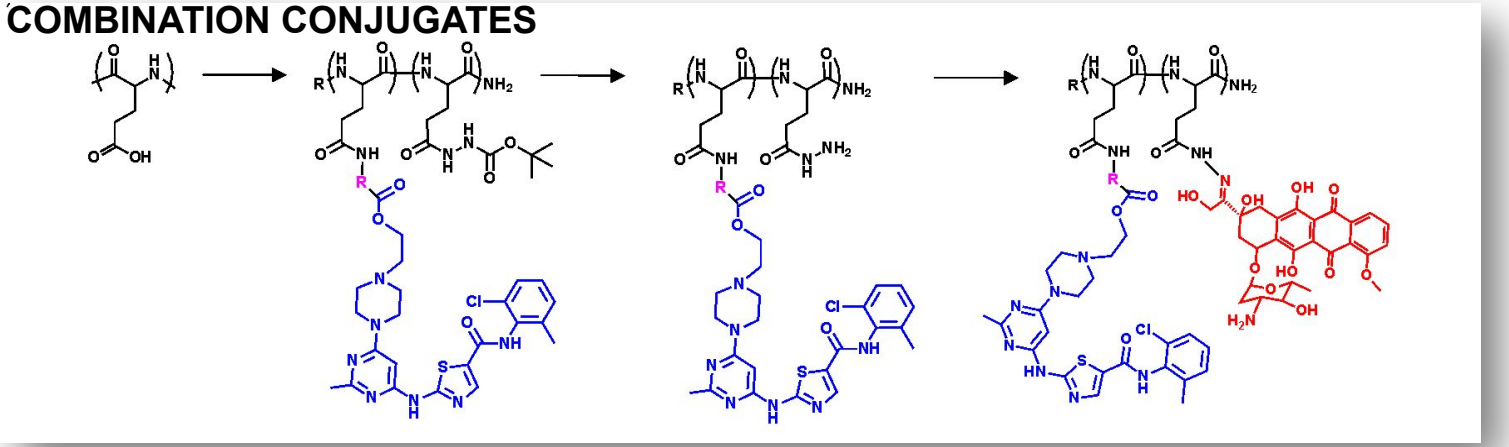
pH = 5
pH = 7.4

Juan J. Arroyo et al. in preparation

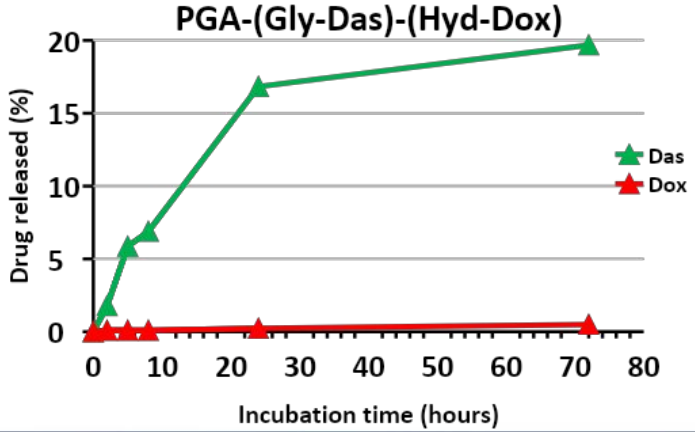
IMPROVEMENT OF DRUG COMBINATION

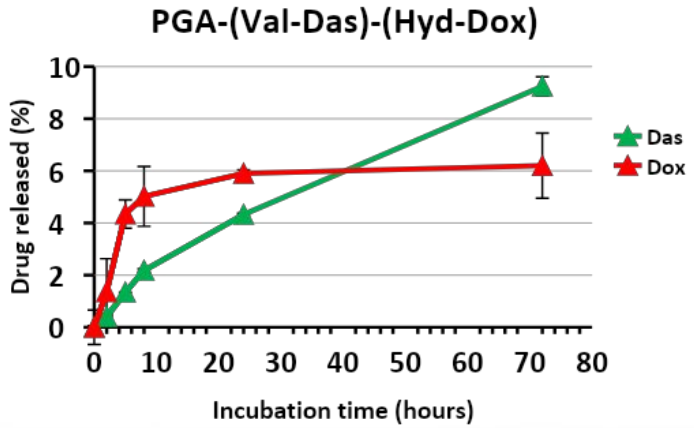
PGA-Das/Dox Combination Conjugates

COMBINATION CONJUGATES



► Cathepsin B-mediated Drug Release Kinetics



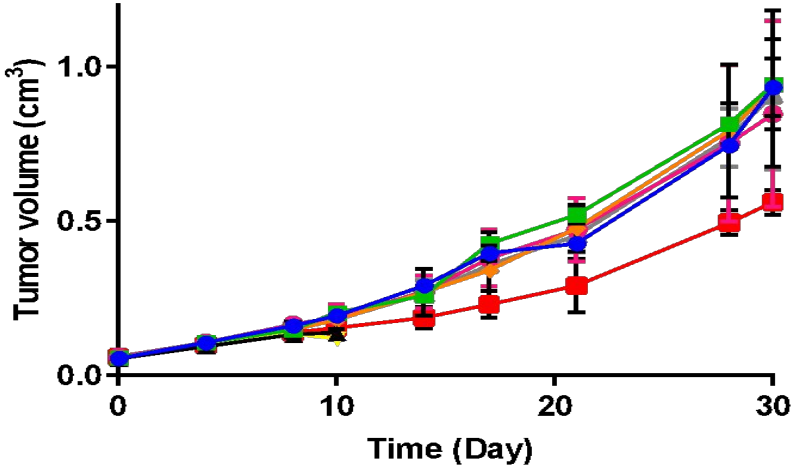


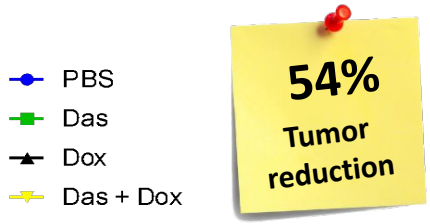
J.J. Arroyo et al. unpublished

IMPROVEMENT OF DRUG COMBINATION

In vivo Evaluation: MDA-MB-231-Luc Human Model

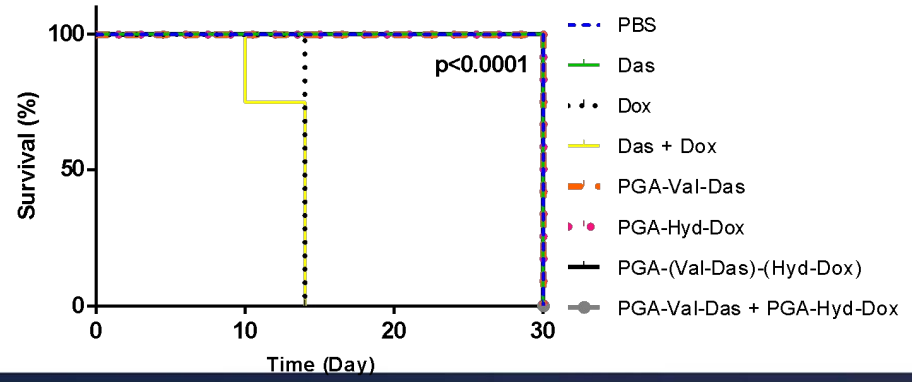
► *In vivo* Evaluation: Orthotopic MDA-MB-231-Luc Model: 1,5mg/Kg Dox eq.

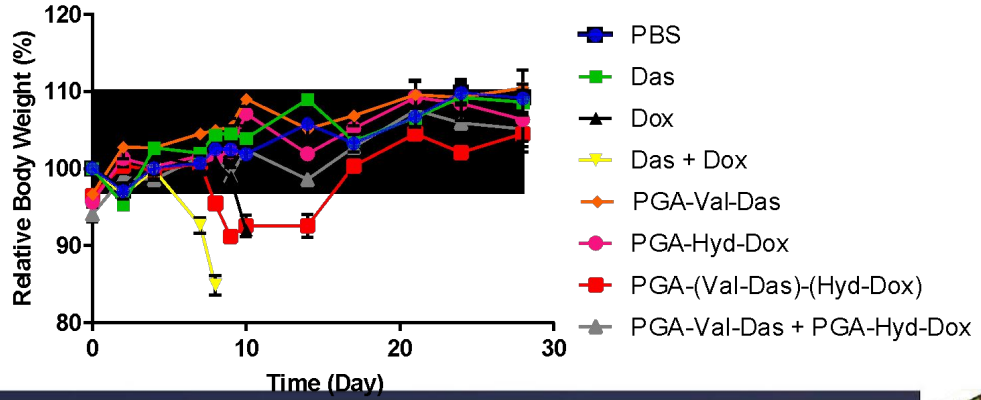




54% Tumor reduction

► *In vivo* Evaluation: Proofs of Safety





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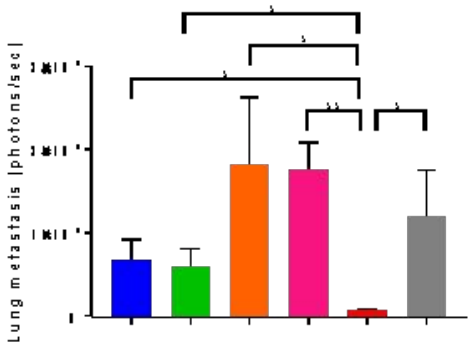
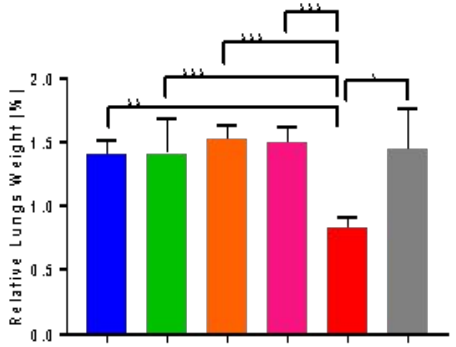
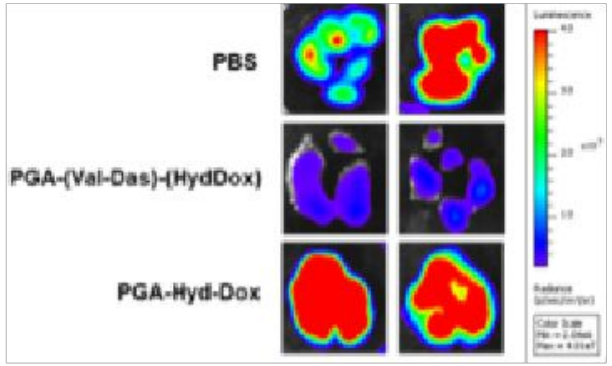
Lung Metastasis

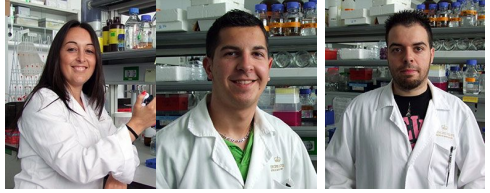
~90% Lung Metastasis reduction





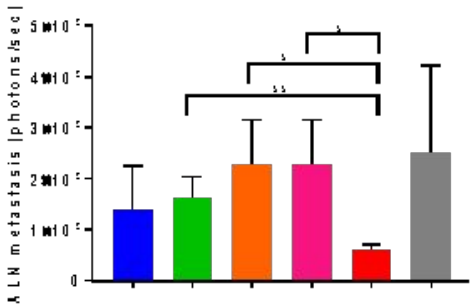
Ex vivo IVIS Quantification

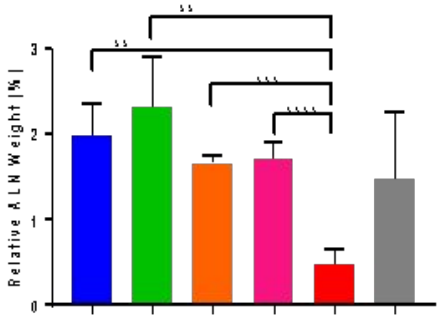




Lymph Node Metastasis

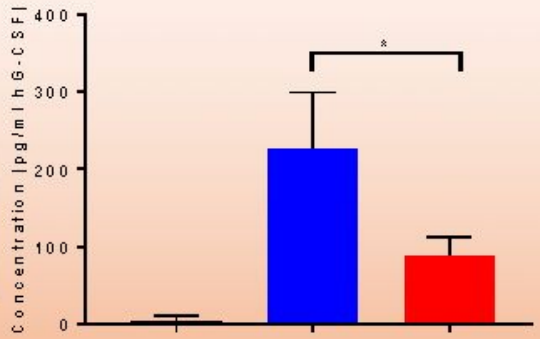
~50% ALN Metastasis reduction





G-CSF Inhibition

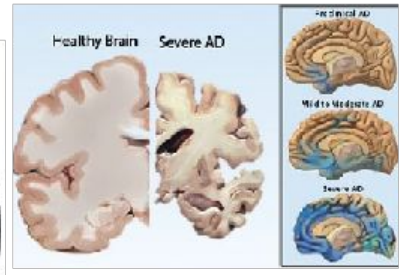
~55% G-CSF inhibition



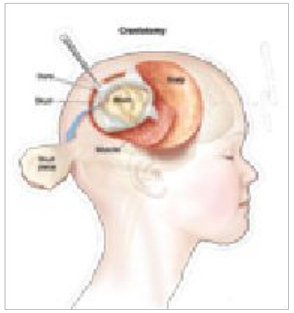
Juan J. Arroyo et al. in preparation

Our CNS targets

Cuesta et al *Nanomedicine:NBM 2021*



Duro-Castano et al *Science Advances 2021*



Melnyk et al *In Preparation*
Coll. A. Montero-Carcaboso

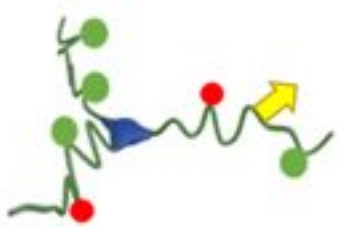
Conejos-Sánchez et al *Nanoscale* 2020

Rodríguez-Otormín et al *In Preparation*
Coll. M. Valiente and G Battaglia

Requejo et al *Biomaterials* 2017
Giraldo et al *Biomaterials* 2021
Bonilla et al Int. J. Mol. Sci 2021
Elkhenany et al. Biomedicines 2021

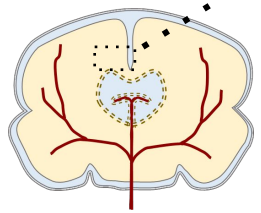
Polymer Conjugates for CNS Delivery. LRP-1 Targeted i.v. administration

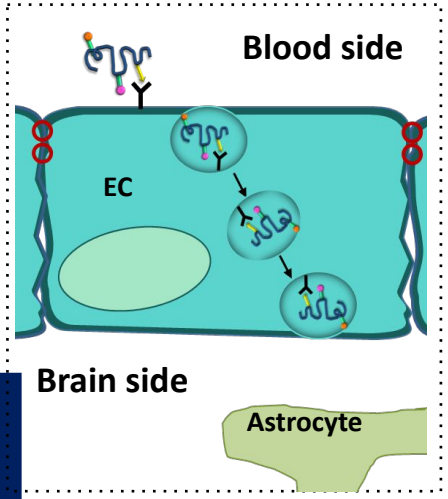
LRP-1 targeted Polymer-based Combination conjugates



- spacer
- targeting residue: Angiopep2
- drugs combination : Dox + TKi

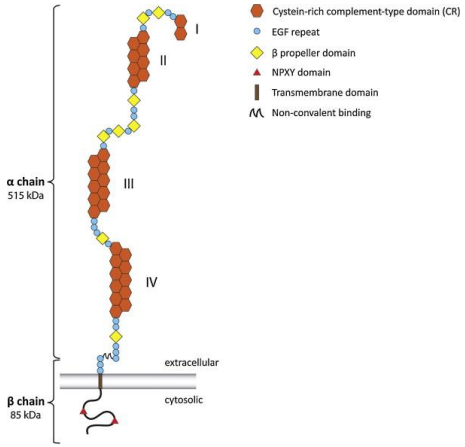
Multi-target therapy





Bypass BBB by active transport

LRP-1 overexpressed in BBB In most cancers, including breast

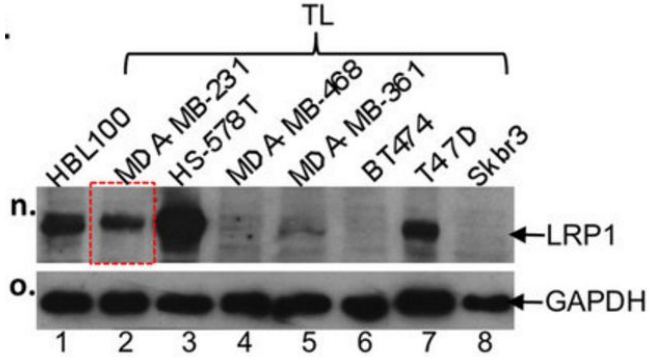


LRP1-Targeted

ANGIOPEP-2 (ANGIOCHEM)

Ac-FFYGGSRGKRNNFKTEEYC (19 aas)

Low-density lipoprotein receptor-Related Proteins (LRP1)

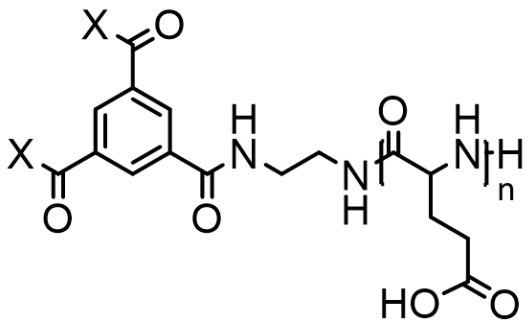


Polymer –based combination nanoconjugates for Brain Metastasis of TNBC

Challenges

- → Identification of effective drug combinations and drug ratios
- → Kinetics of drug release – linking chemistry optimization
- → Loading capacity/optimization
- → Physico-chemical characterization

Star-shaped Poly-L-glutamic Acid (St-PG



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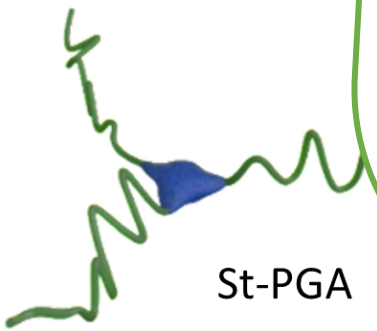
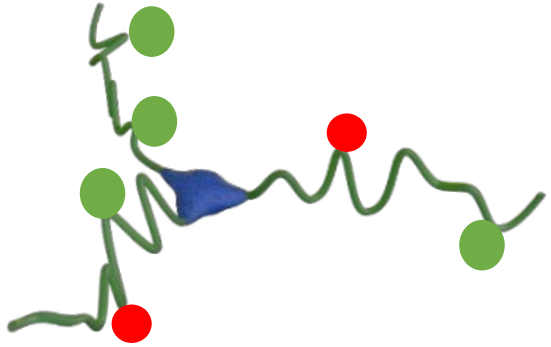
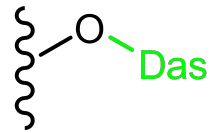
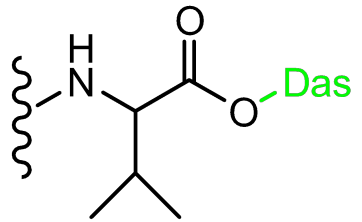




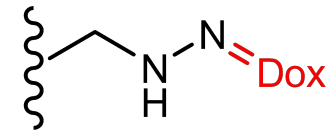
ester-Das



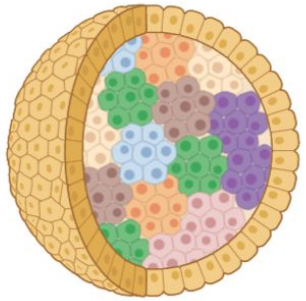
valine-ester-Das



hydrazone-Dox



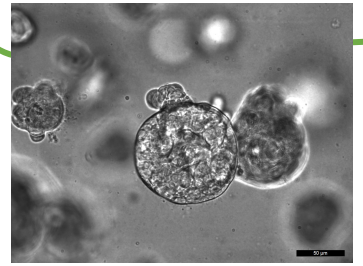
Duro-Castano, A. et al. *Mol. Pharm.* (2015); Duro-Castano, A., Movellan, J. & Vicent, M. J. *Biomater.* (2015); Duro-Castano, A. et al. *Adv. Mater.* (2017).

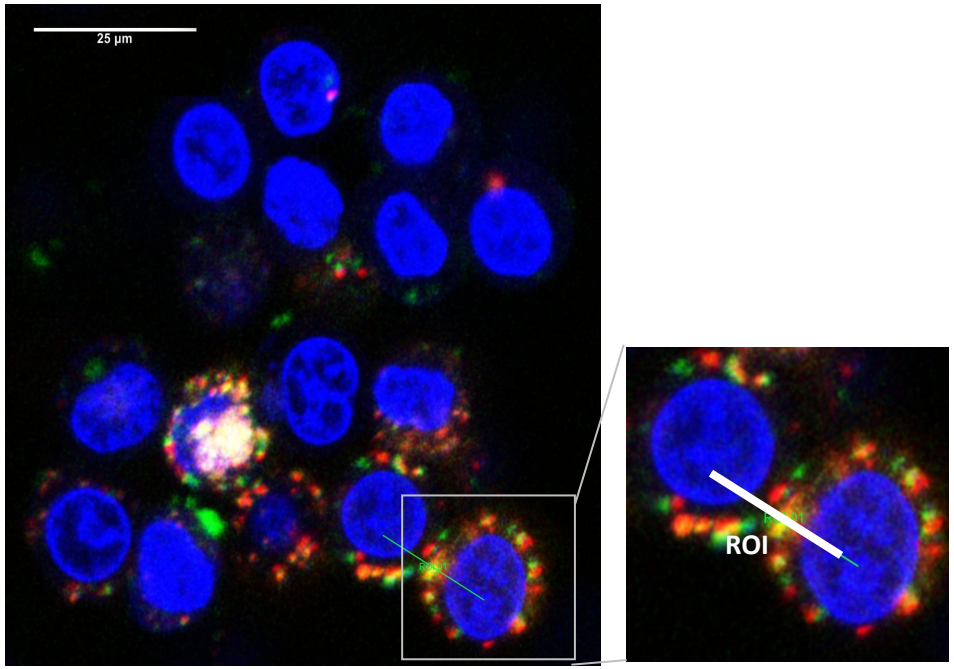


drug release profile

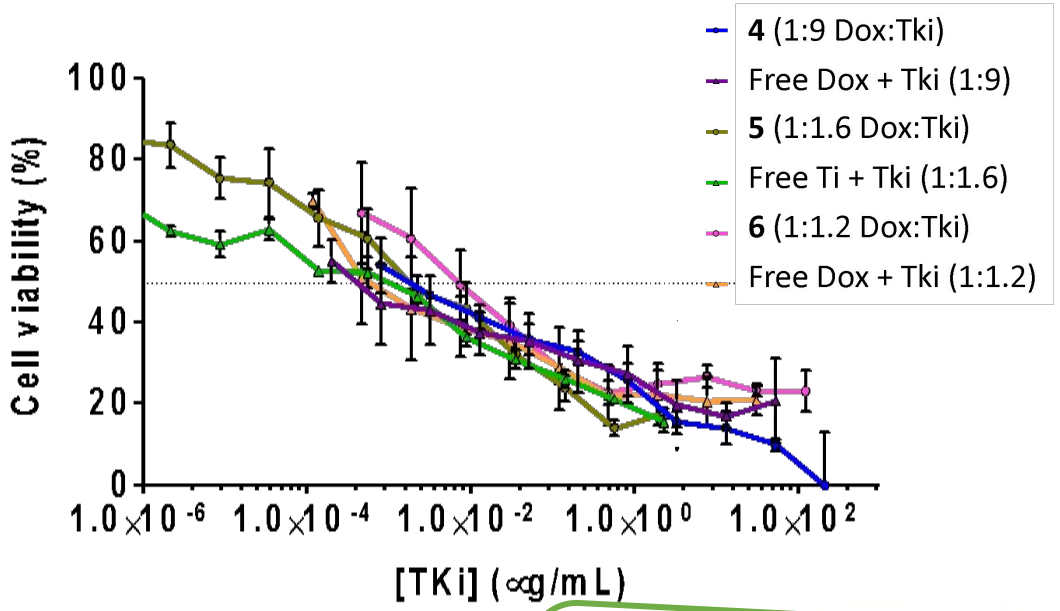
Drug loading does not alter drug release

ER- PR- HER2

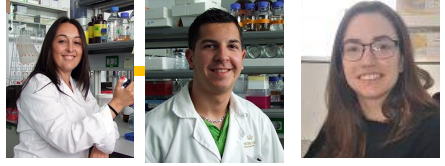




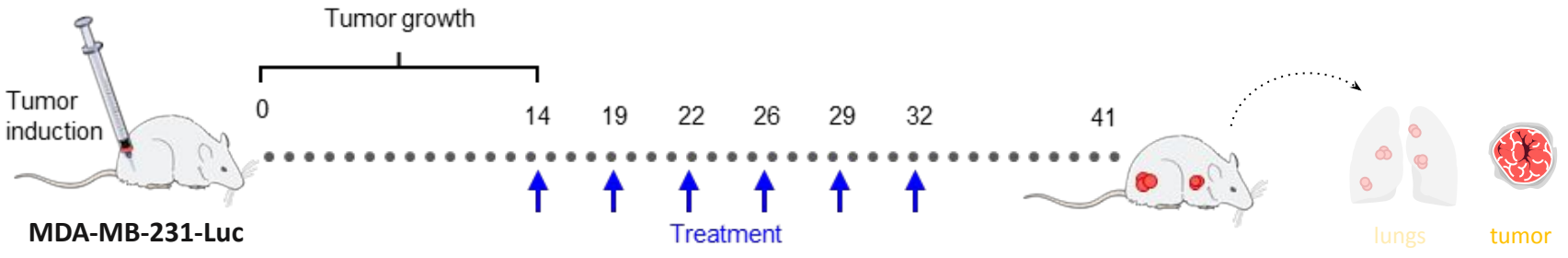
Compound	Ratio Ti:TKi	IC50 (µg Dox/mL)	IC50 (µg Tki (Das)/mL)
4. St-PGA-hyd-Dox$_1$-DAS$_9$	1:9	2.05 x 10^{-4}	1.74 x 10^{-3}
Free Dox + Das	1:9	5.41 x 10^{-5}	4.60 x 10^{-4}
5. St-PGA-hyd-Dox$_1$-DAS$_1$	1:1.6	1.39 x 10^{-3}	2.04 x 10^{-3}
Free Dox + Das	1:1.6	2.86 x 10^{-4}	4.21 x 10^{-4}
6. St-PGA-hyd-Dox$_1$-Val-Das$_1$	1:1.2	4.15 x 10^{-3}	5.16 x 10^{-3}
	1:1.2	8.29 x 10^{-4}	1.03 x 10^{-3}



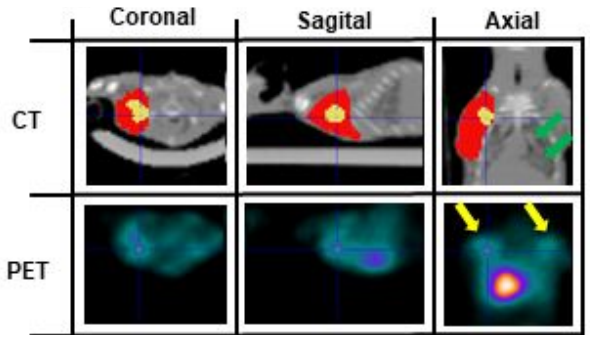
St-PGA-hyd-Dox$_1$-Das$_9$ shows the highest cytotoxic activity



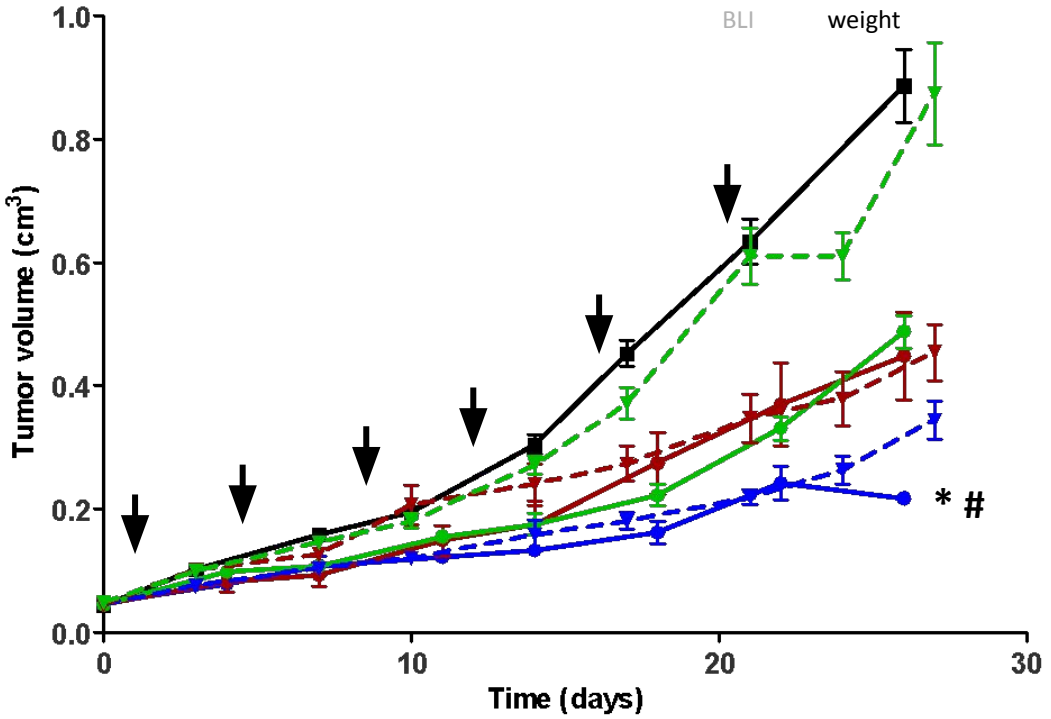
Rational design of an efficient polymer combination conjugate for TNBC: optimization of drug ratio and linking chemistry



St-PGA-hyd-Dox$_1$-Das$_9$ shows the best anti-tumor activity



Juan J. Arroyo et al. *Int. J. Cancer*, 2019



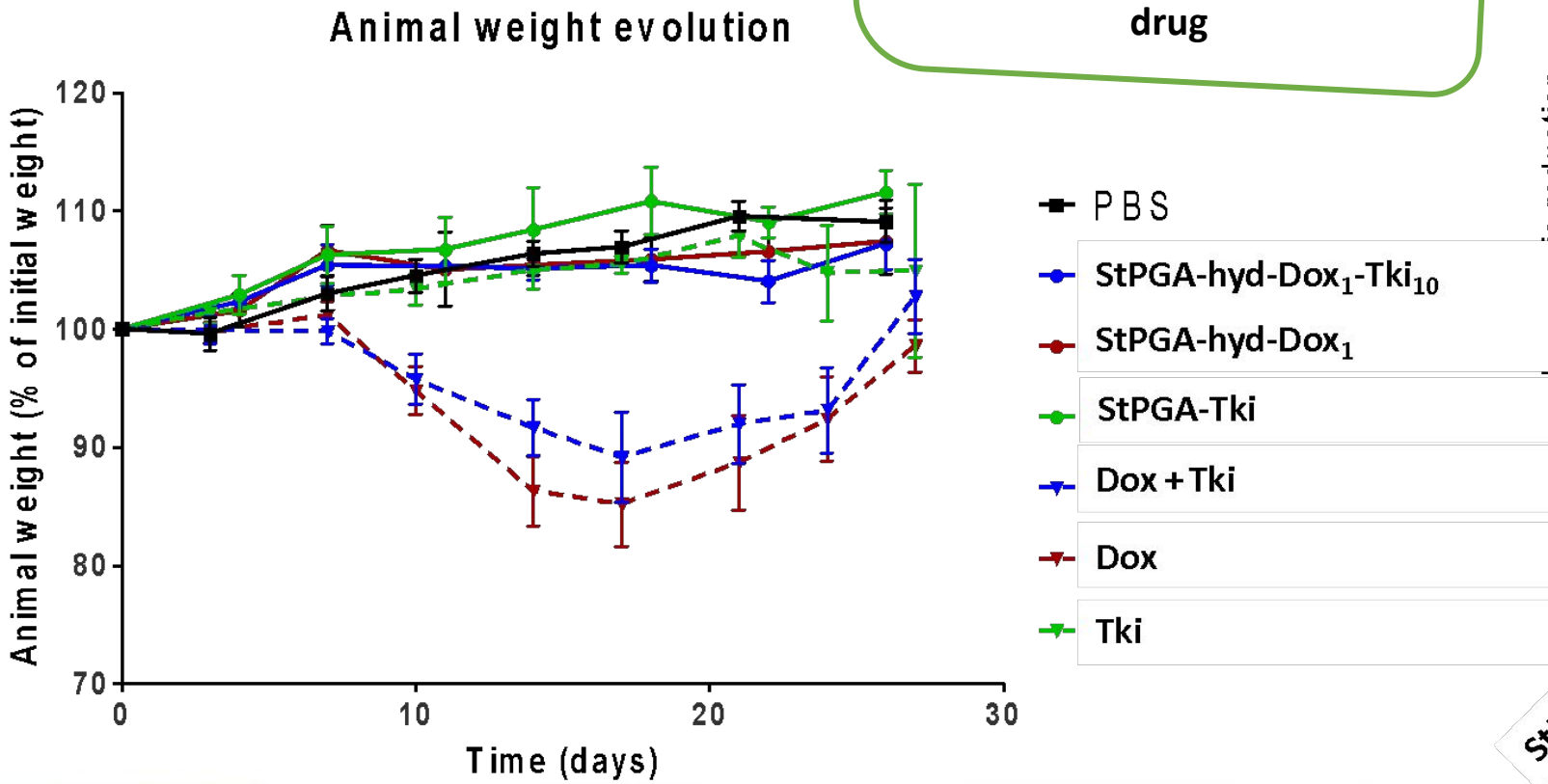
- PBS
- StPGA-hyd-Dox$_1$-Das$_9$
- StPGA-hyd-Dox$_1$
- StPGA-Das
- Dox + Das
- Dox
- Das

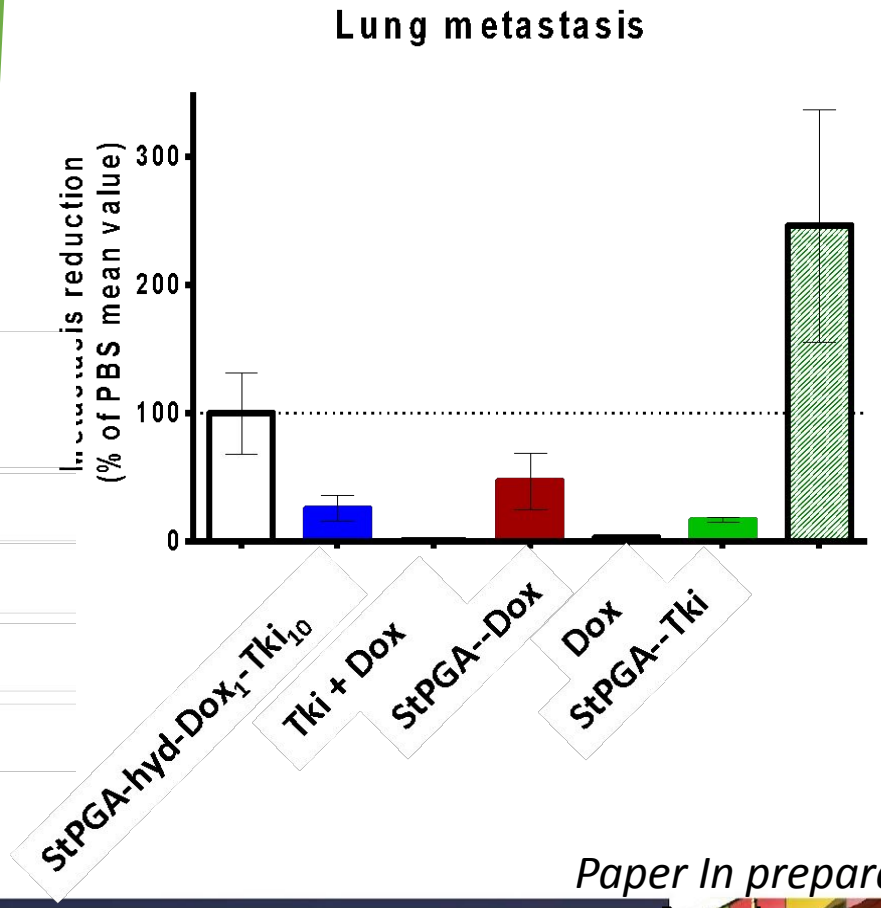
Data represented as average tumor volume ± SEM.
Mice were treated i.v. twice a week with 1.2 mg Dox/Kg and/or 10 mg TKI/Kg.
*p<0.01 versus PBS, #p<0.05 versus Dox 1.2 mg/Kg + TKI 10 mg/Kg

Paper in preparation

Rational design of an efficient polymer combination conjugate for TNBC: optimization of drug ratio and linking chemistry

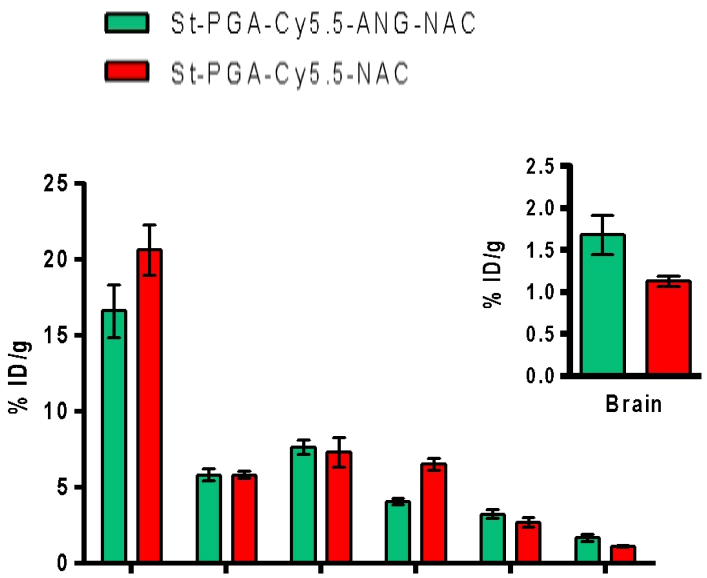
Das conjugation to St-PGA inhibits the pro-metastatic effect of high doses of the free drug



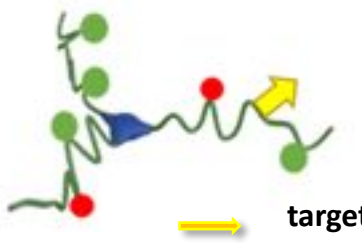


Paper In preparation

LRP1-Targeted STPGA Combination Conjugate reaches the brain in a TNBC metastatic model

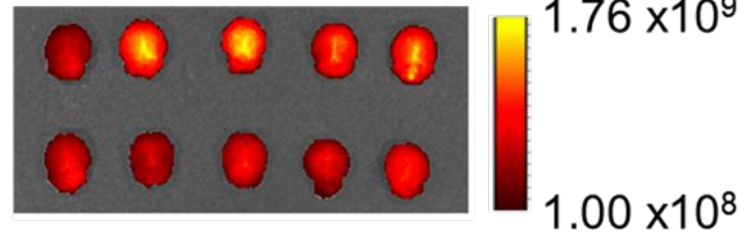


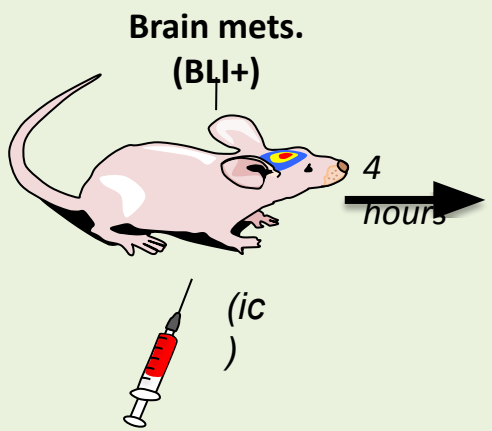
Wild type mice

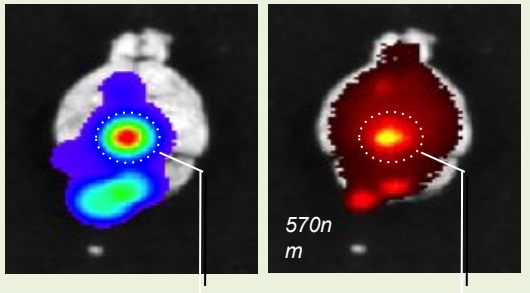


targeting residue: Angiopep2

Through maleimide linker

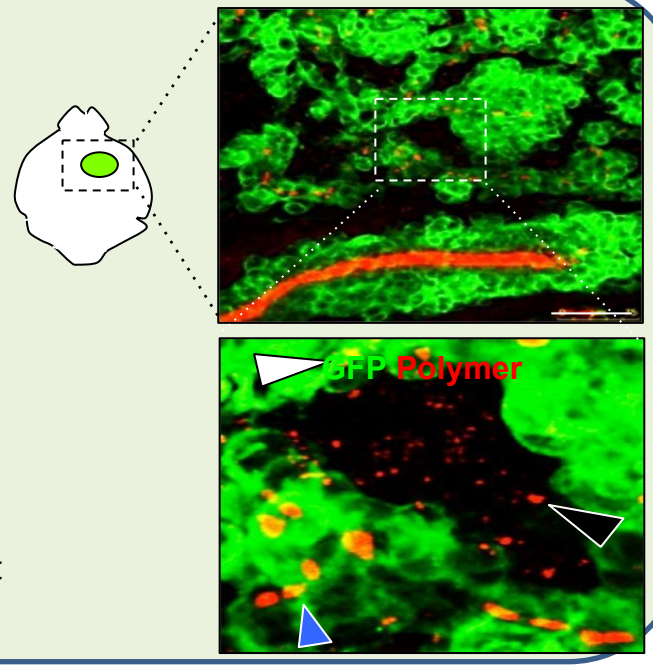






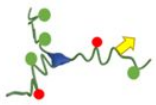
MDA-MB231 Cancer cells

St-CL Conjugate



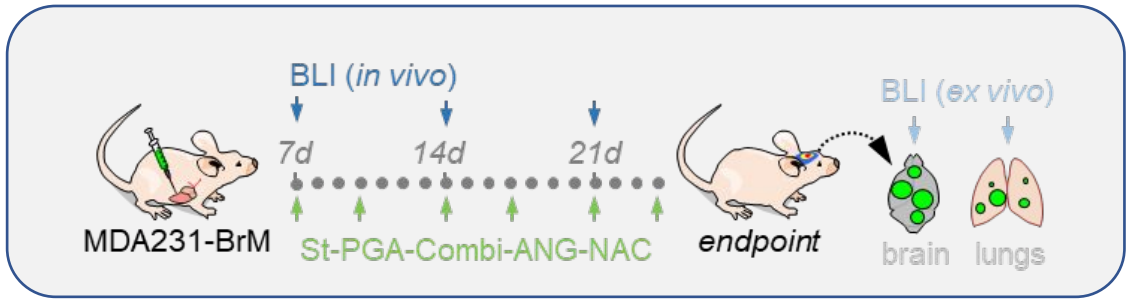
Valiente Lab
BRAIN METASTASIS GROUP

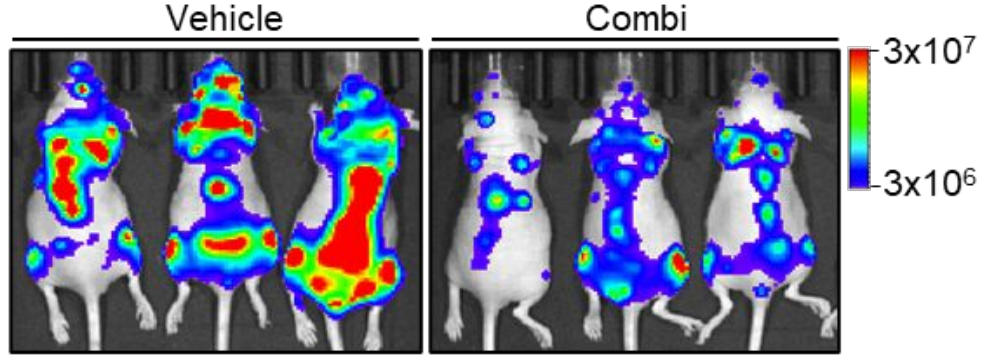
Towards the development of an effective treatment for TNBC brain metastasis: synthesis of LRP-1-targeted combination conjugates

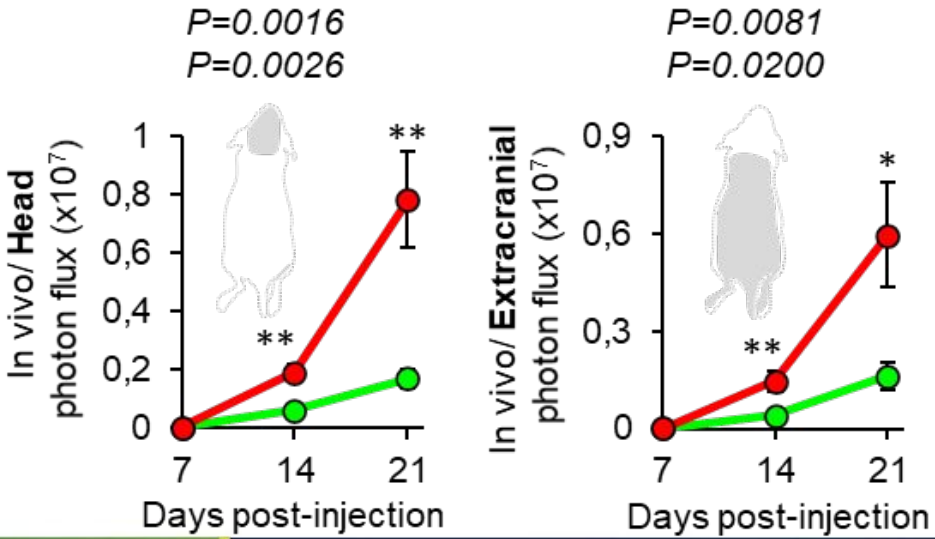


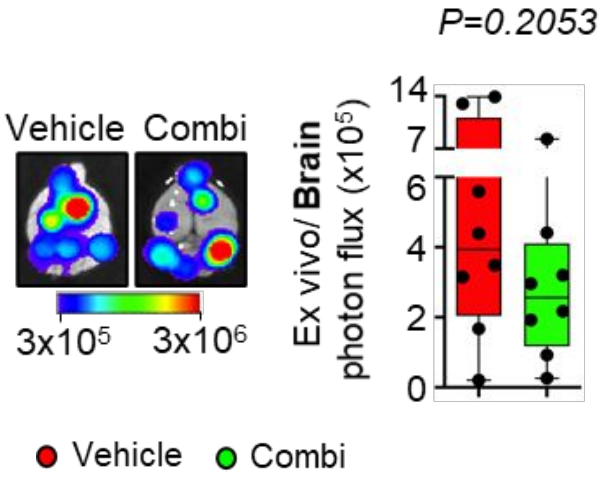
	% mol Dox	% mol Das	% mol ANG	Z-pot (mV)
St-PGA-Combi-ANG	0.76	4.63	1.3	-45.4

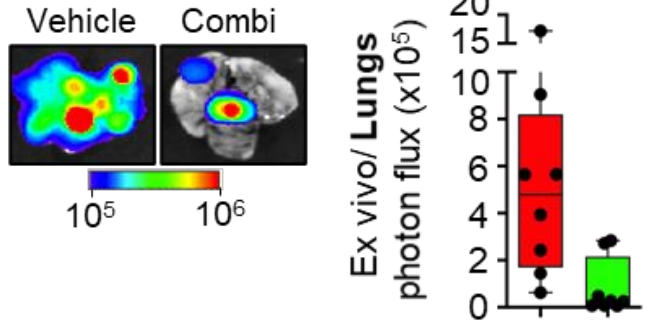
Valiente Lab
BRAIN METASTASIS GROUP











Unpublished data

PRINCIPE FELIPE
CENTRO DE INVESTIGACION

Interdisciplinar Team at the Polymer Therapeutics Lab.

Polymer Therapeutics
Vicent Lab

Collaborators in this project:

molecular bonelabs

Valiente Lab
BRAIN METASTASIS GROUP

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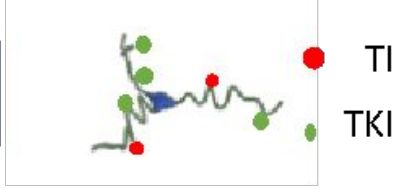
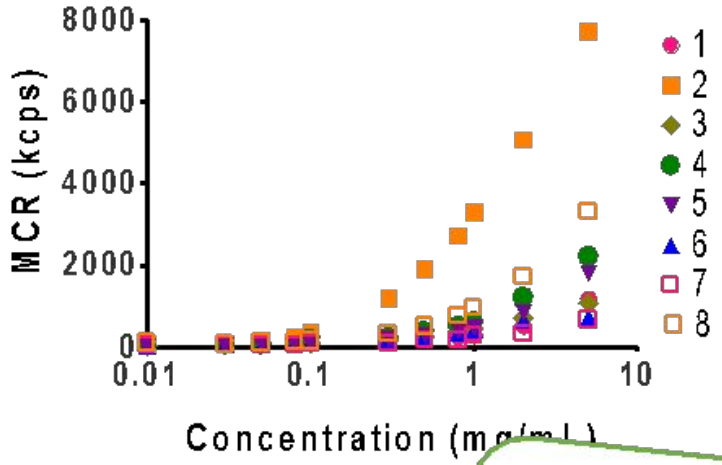
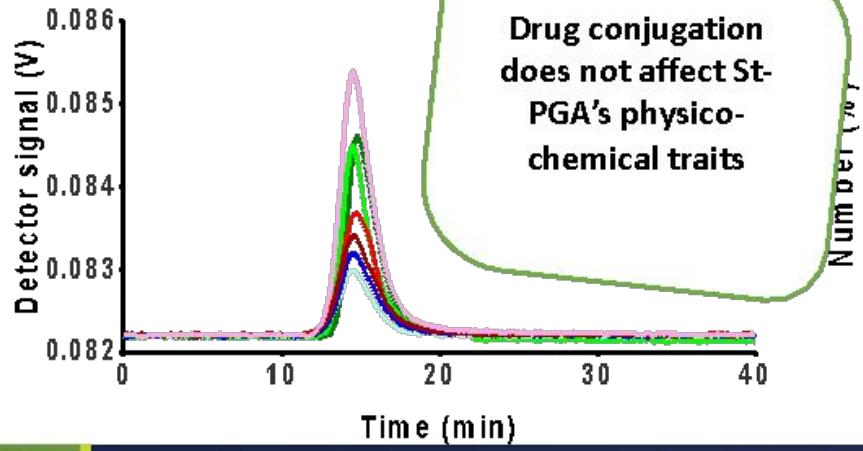
Advanced Delivery Science

Rational design of an efficient polymer combination conjugate for TNBC: optimization of drug ratio and linking chemistry

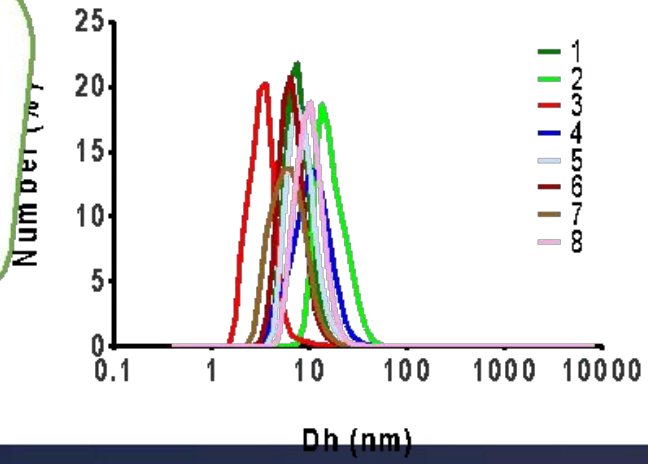


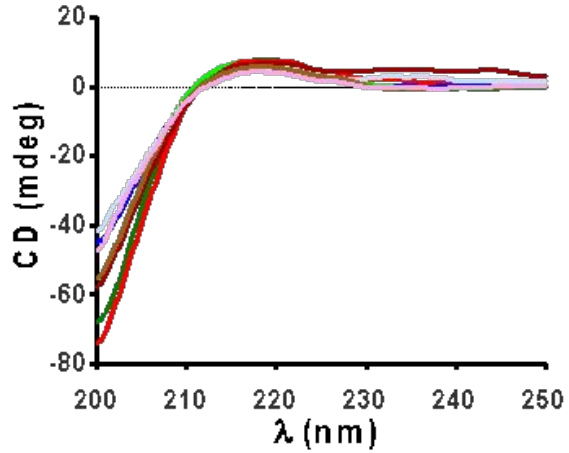


Compound	% mol Ti	% mol TKi	Ratio Ti/TKi	Z-Pot
1. StPGA-TKI	-	3.4	-	-51
2. StPGA-Val-TKI	-	3.1	-	-36
3.StPGA-Hyd-Dox	2.9	-	-	-46
4. St-PGA-hyd-Dox$_1$-TKI$_{10}$	**0.3**	**2.9**	**1:9**	-41
5. St-PGA-hyd-Dox$_1$-TKI$_1$	**1.7**	**2.8**	**1:1.6**	-45
6. St-PGA-hyd-Dox$_1$-Val-TKI$_1$	**2.6**	**3.0**	**1:1.2**	-43
7. St-PGA-HYD	-	-	-	n.d.
8. St-PGA-HYD-TKI	-	4.1	-	-47

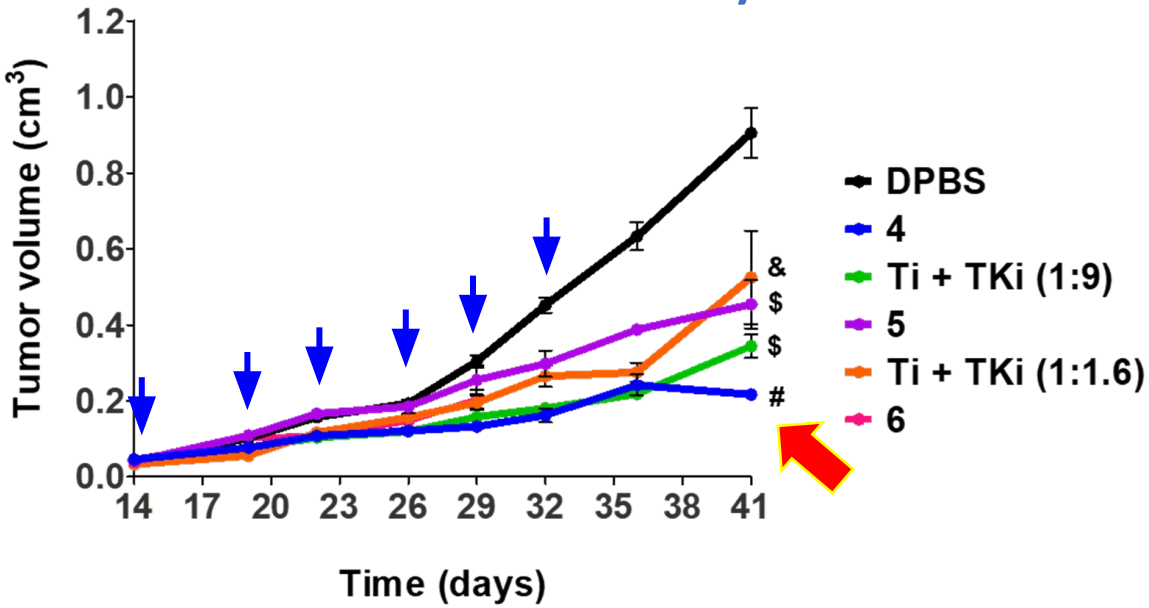


Drug conjugation does not affect St-PGA's physico-chemical traits

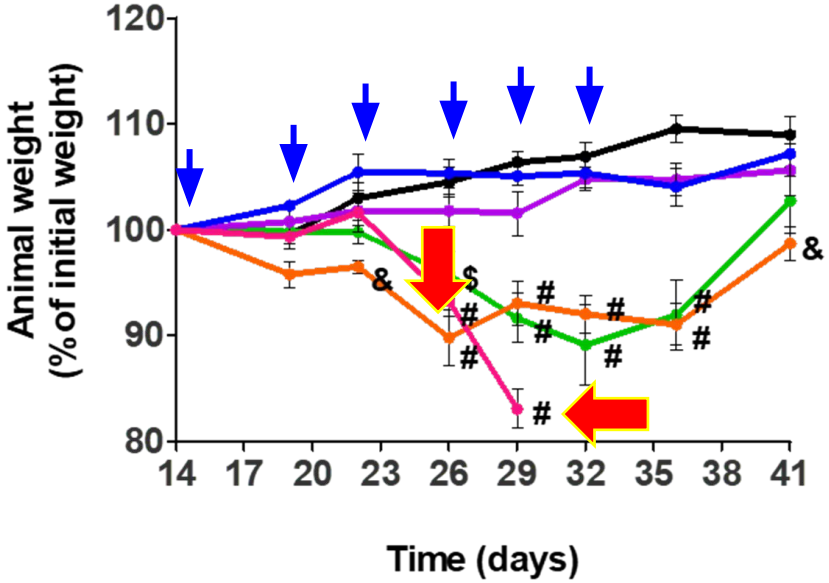




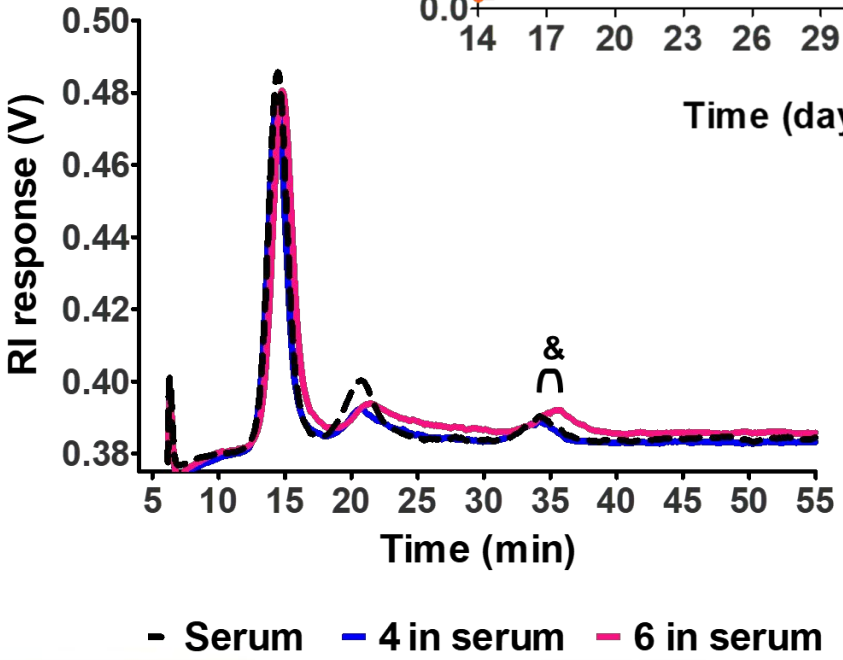
In vivo anti-tumor activity



Animal weight evolution



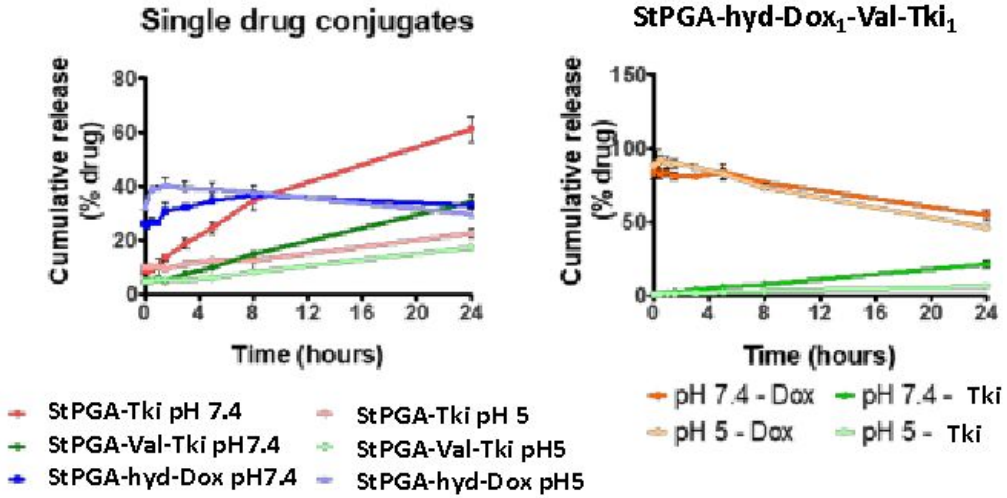
Interaction with serum proteins

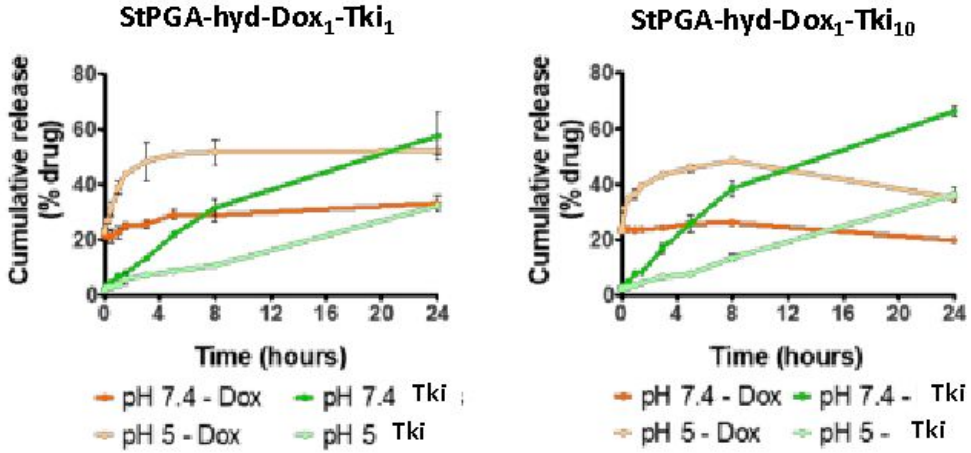


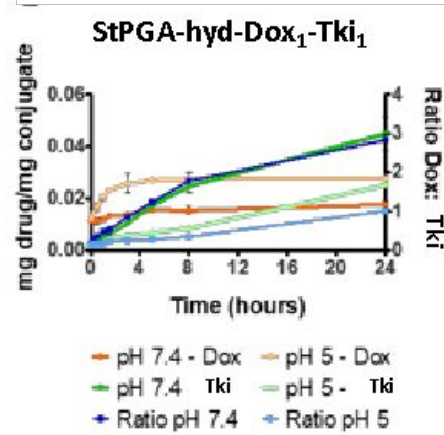
Shift to longer elution times suggests the formation of aggregates with serum proteins

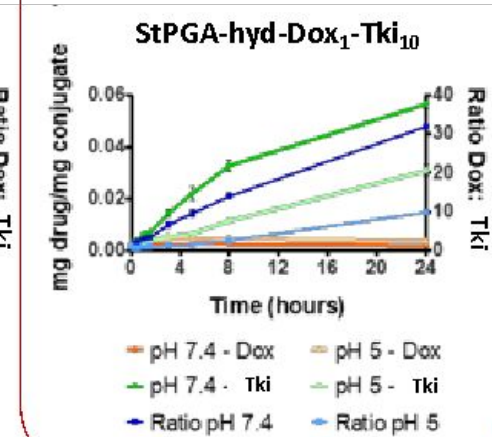
[&]p<0.05, [$]p<0.01, [#]p<0.001 vs. DPBS

pH dependent drug release





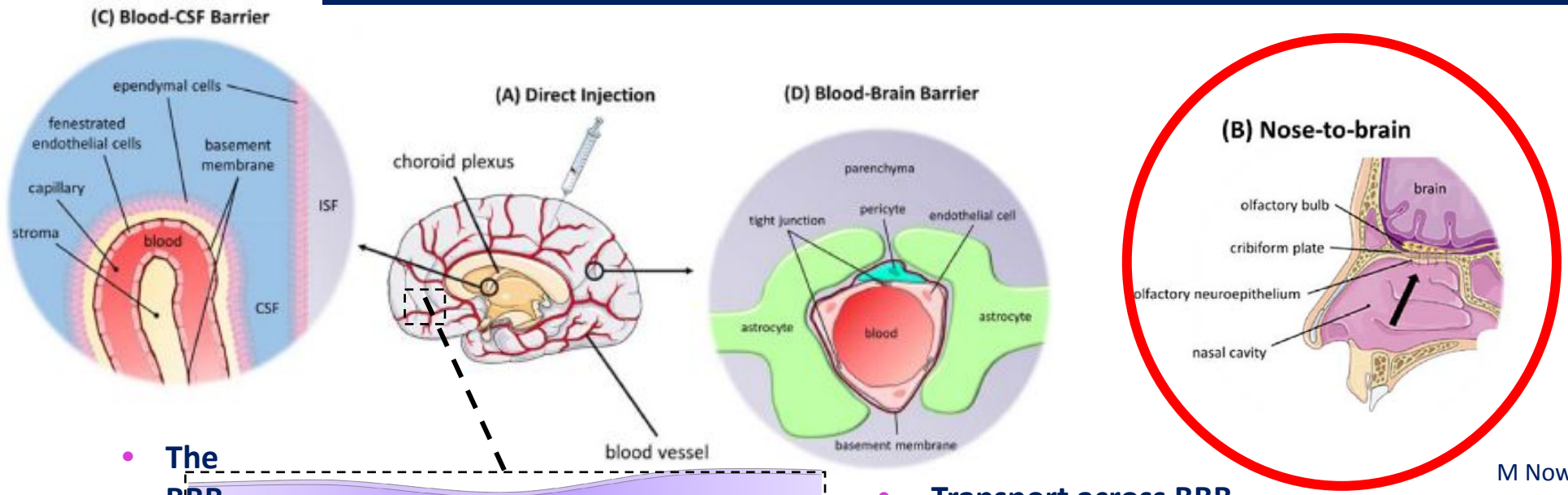




TKi linking chemistry defines drug release profile

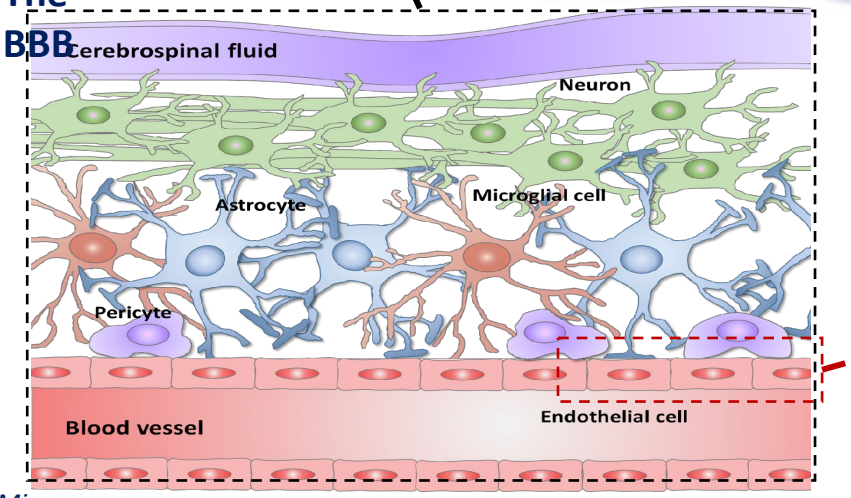
Drug loading does not alter drug release

TNBC Brain Metastasis: The BBB as Major Challenge for CNS Delivery



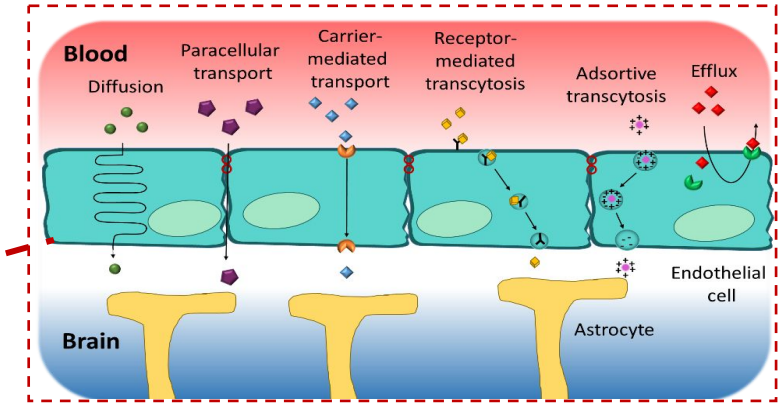
M Nowak et al Adv Ther 2019

- **The BBB**

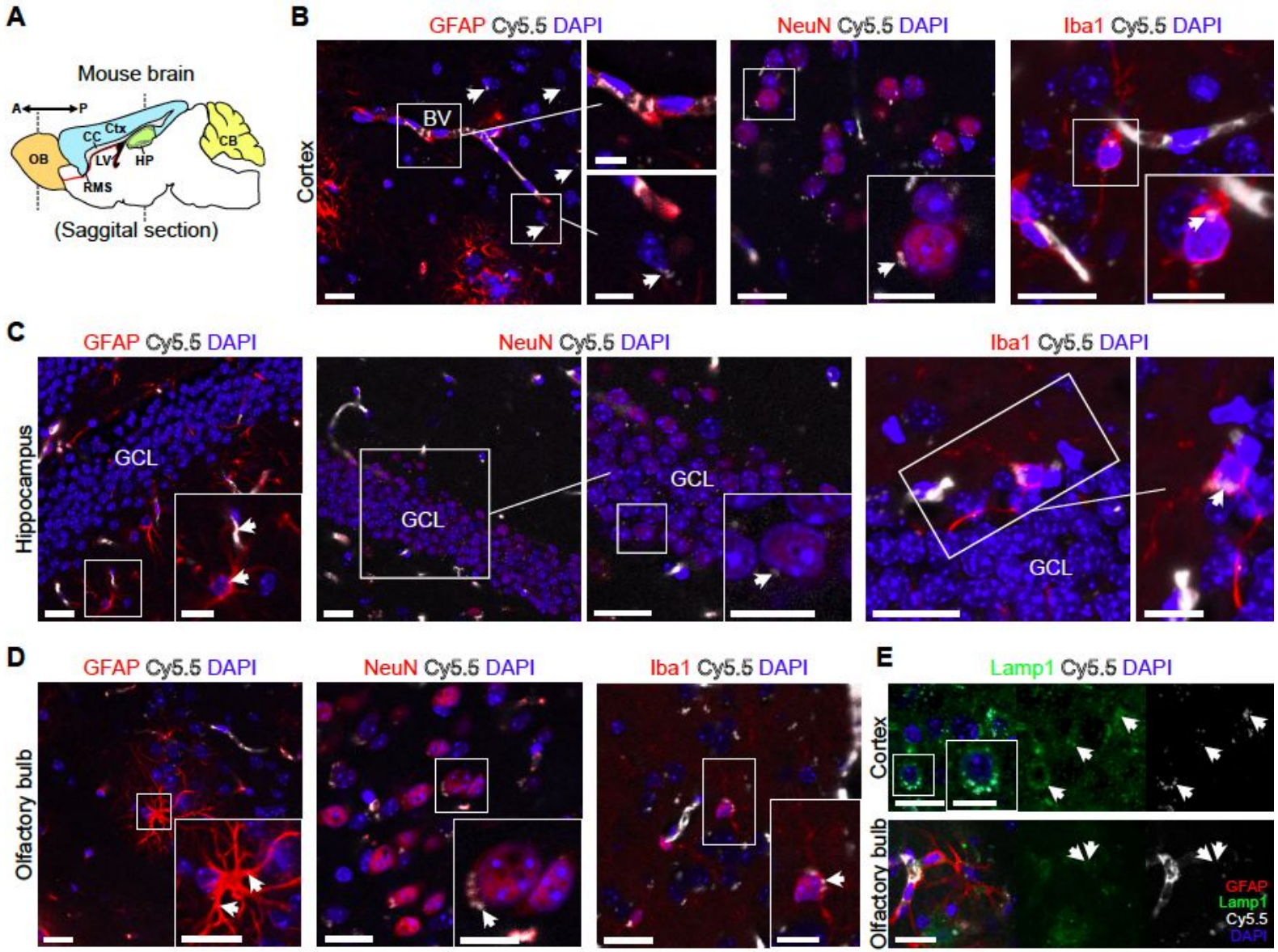


Redrawn from Kim, *Nat Rev Micro*, 2008

- **Transport across BBB.**



Redrawn from www.rsc.org



In Coll. J. Viña and J.M. García-Verdugo; *Duro-CastaNo et al, Science Advances 2021*