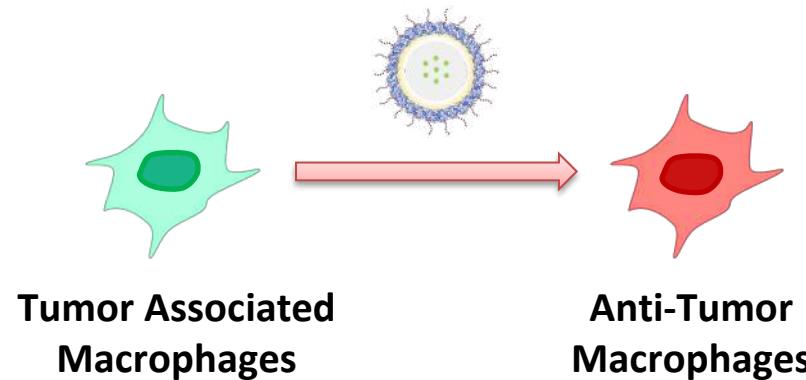


# Nanomedicines to Target and Reprogram

## Tumor Associated Macrophages



Fernando Torres Andón



María Jose Alonso

Paola Allavena



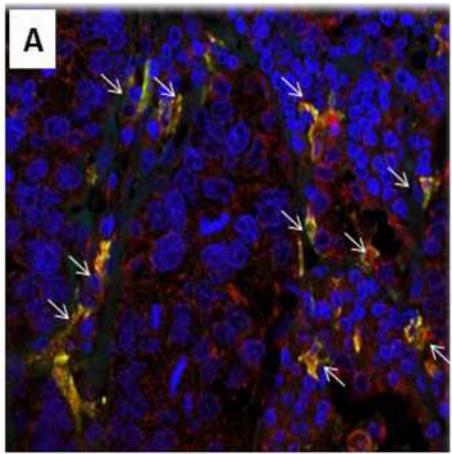
María José Oliveira



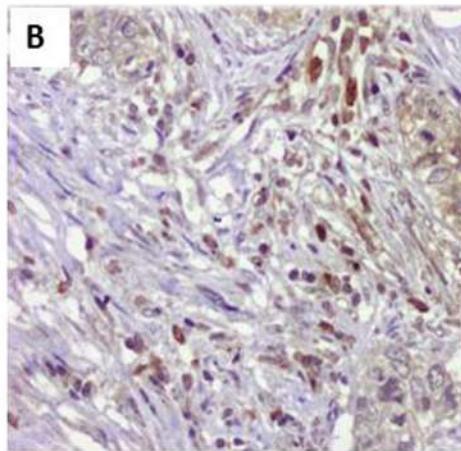
# Tumor Associated Macrophages

## TAM in Human Lung Cancer

### Non-Small-Cell Lung Carcinoma



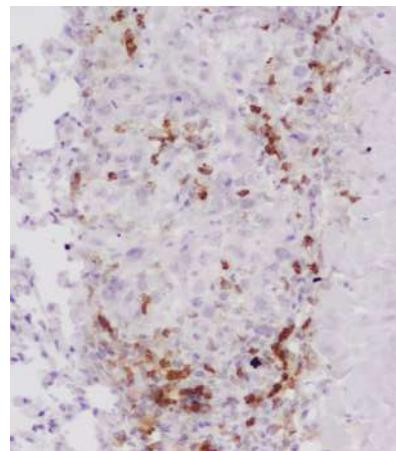
Immunofluorescence



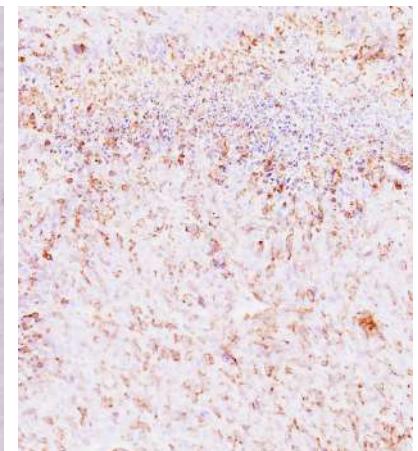
Immunohistochemistry

## TAM in Immunocompetent Murine Models

### CMT167 Lung Tumor

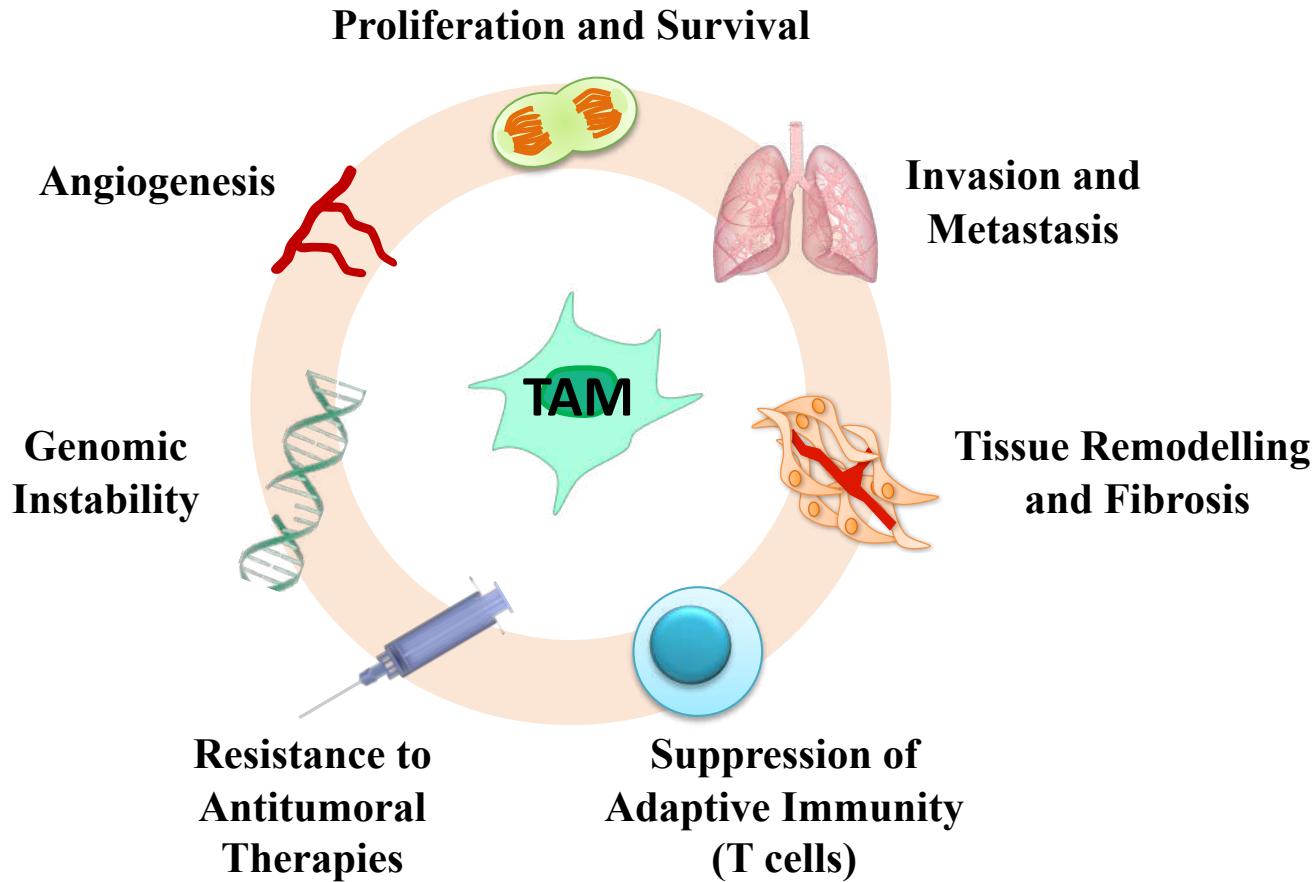


### MN/MCA Fibrosarcoma



Immunohistochemistry

# Pro-tumor Functions of Tumor Associated Macrophages

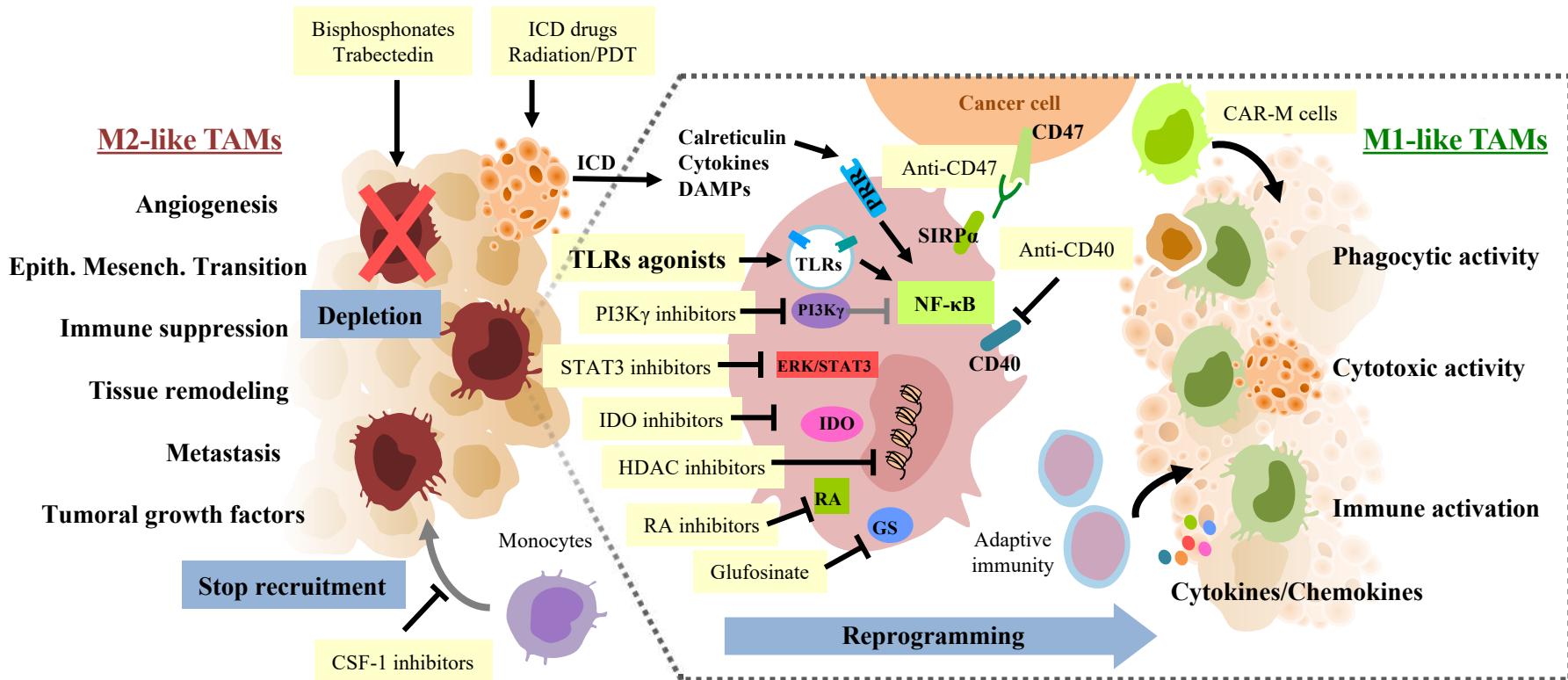


# Prognostic value of TAMs in Solid Tumors

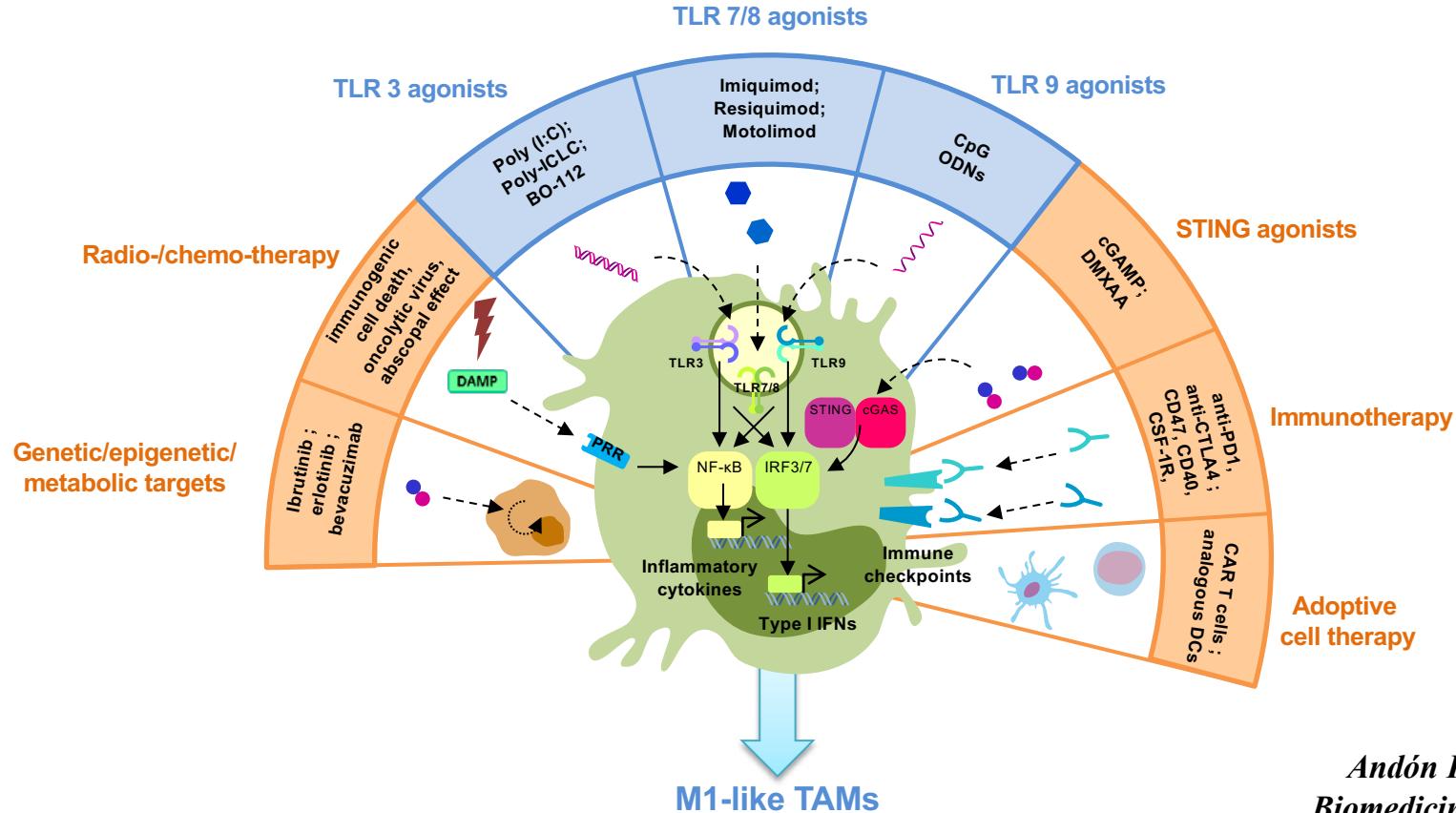
Tumor Type	Markers of TAMs	Prognostic Impact
Glioma	CD68, CD163, CD204	Bad
	IBA, CD204	Bad
	CD163/CCL3 ratio	Bad (if high ratio)
	CD68, CD163/AIF ratio	Bad (if high ratio)
Mesothelioma	CD68	Bad
	CD163	Bad
	CD68	Good (in tumor islets/bad in tumor stroma)
Lung cancer (NSCLC)	CD68/iNOS (for M1); zqx CD68/CD163 (for M2)	Bad (if M2 > M1)
Pancreatic cancer	CD68, CD204	Bad
	CD163	Bad
Breast cancer	CD68, CD163	Bad
Colorectal cancer	iNOS	Good (if together with high CD8+ cells)
	CD68	Good (at the invasive front)
	Wnt5a, CD68	Bad
Melanoma	CD68, CD163	Bad (CD163 at tumor stroma and CD68 at invasive front)
Bladder cancer	CD68	Bad

TAM: tumor-associated macrophages; NSCLC: non-small-cell lung cancer.

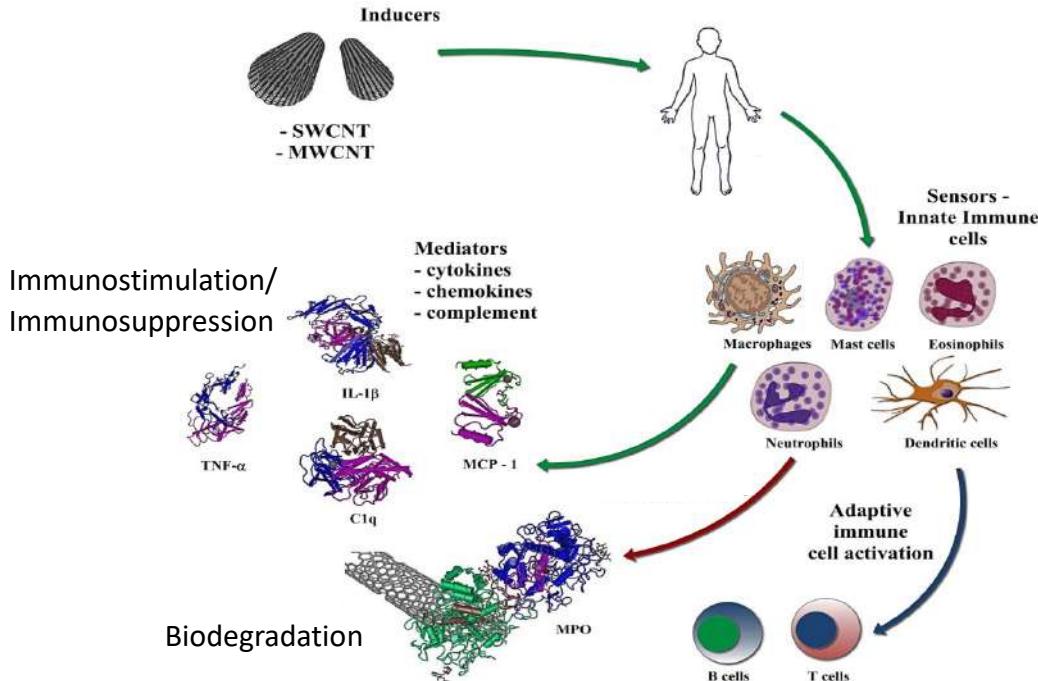
# Therapeutic Manipulation of Tumor Associated Macrophages



# TLR agonists and Combination with other Anti-tumoral Treatments



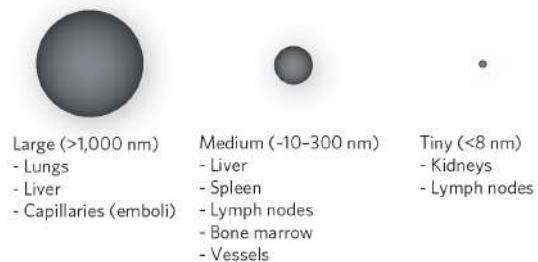
# Uptake of Nanoparticles by Macrophages



Nanoparticle uptake

Macrophages  
Dendritic cells  
Ly-6Ch<sup>hi</sup> monocytes  
Ly-6Cl<sup>lo</sup> monocytes  
Neutrophils  
NK cells  
Cancer cells  
Tubular cells  
Endothelial cells  
T cells  
B cells  
Other cells

Nanoparticle distribution



Bhattacharya K et al.  
Adv Drug Deliv Rev. 2013

Weissleder et al.  
Nat Materials 2014

# Delivery of Nanoparticles to Macrophages in Tumors

Analysis of nanoparticle delivery  
to tumours

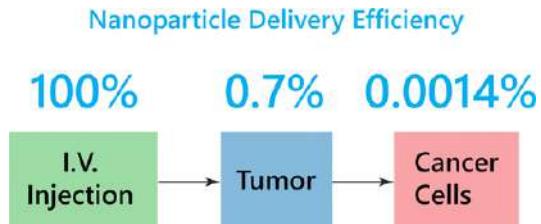
Stefan Wilhelm, Anthony J. Tavares, Qin Dai, Seiichi Ohta, Julie Audet,  
Harold F. Dvorak and Warren C. W. Chan

**0.7%**  
Median in 2006

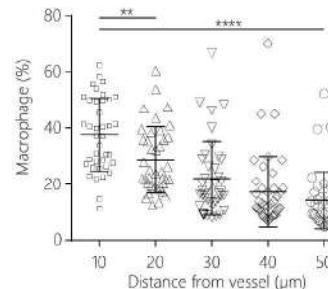
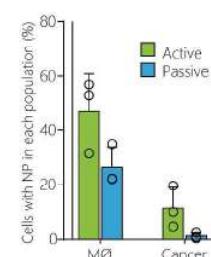
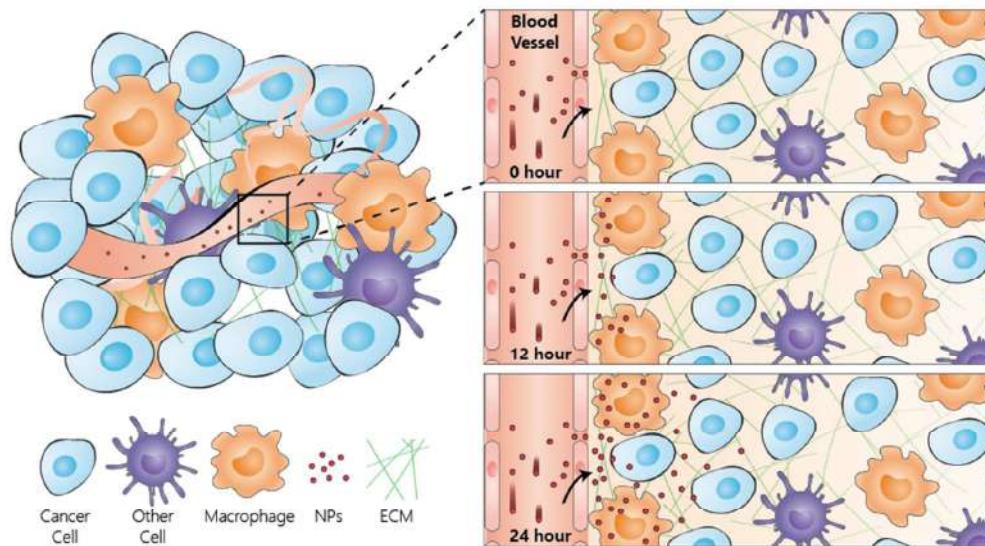
Wilhelm S et al *Nature Reviews Materials* 2016

## Quantifying the Ligand-Coated Nanoparticle Delivery to Cancer Cells in Solid Tumours

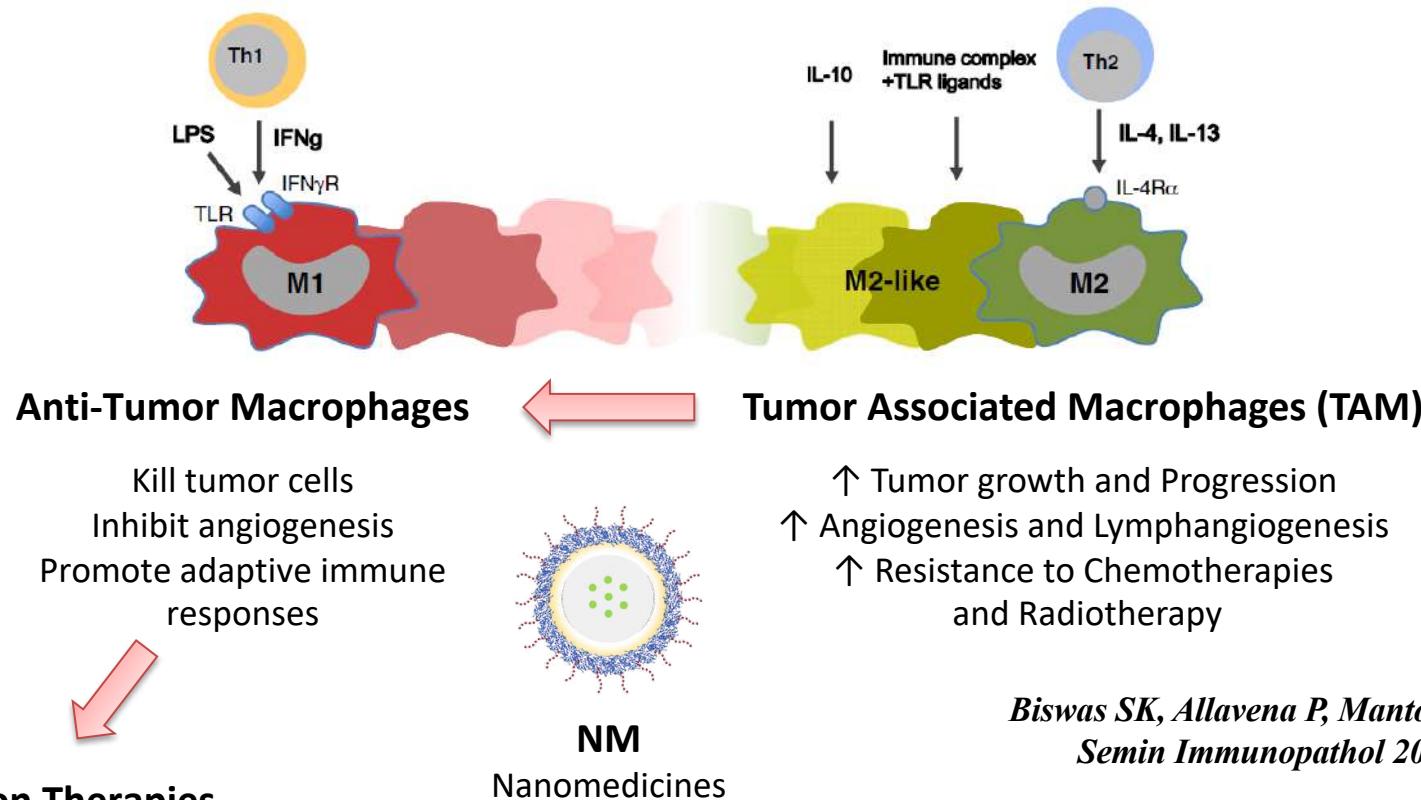
Qin Dai, Stefan Wilhelm, Ding Ding, Abdullah Muhammad Syed, Shrey Sindhwani,  
Yuwei Zhang, Yih Yang Chen, Presley MacMillan, and Warren C.W. Chan



Dai Q et al *ACS Nano* 2018



# Re-education of Tumor Associated Macrophages



## Combination Therapies

- Radiotherapy
- Immunecheckpoint Inhibitors or CAR-T cells
- Immunogenic Cell Death Activators
- Targeted Chemotherapy

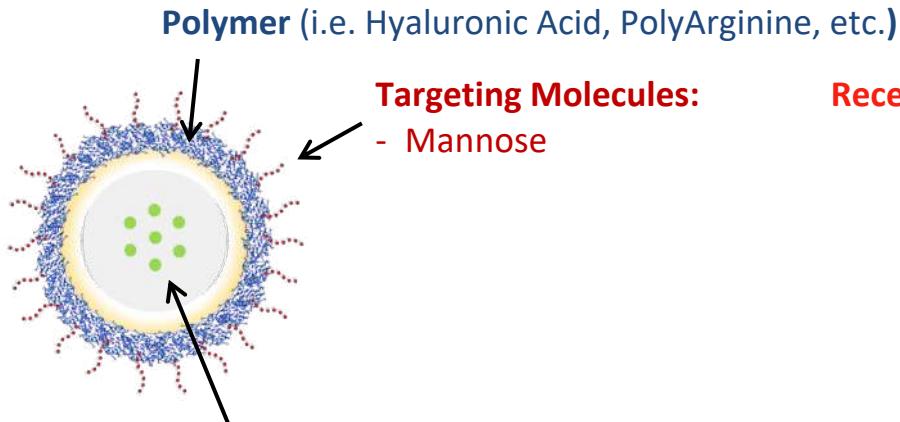


Prof. Alberto Mantovani  
Prof. Paola Allavena

**HUMANITAS**  
RESEARCH HOSPITAL

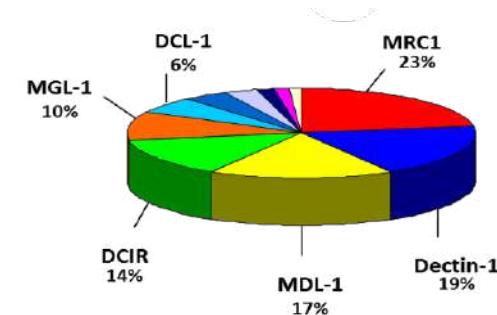
*Biswas SK, Allavena P, Mantovani A.  
Semin Immunopathol 2013*

# Molecular Rational for Targeting and Re-education of TAM



Receptors on TAM surface:

- CD206



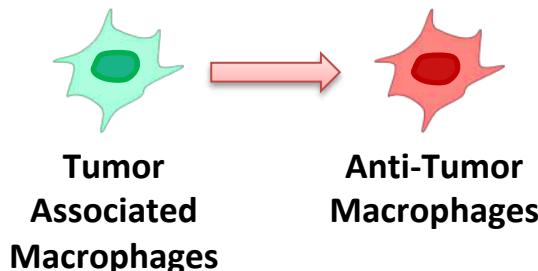
Analysis of C-type lectin  
receptors expressed in TAM  
from Human Cancer

Molecules to stimulate M1-polarization:

- Imiquimod / Gardiquimod
- Resiquimod
- Poly I:C

Receptor Inside TAM:

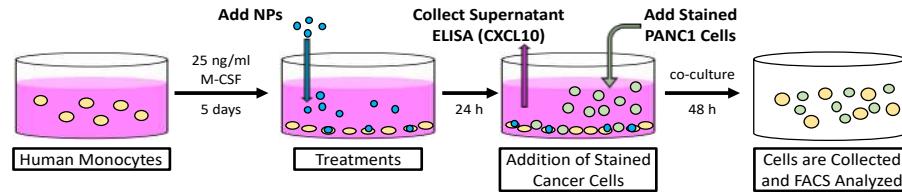
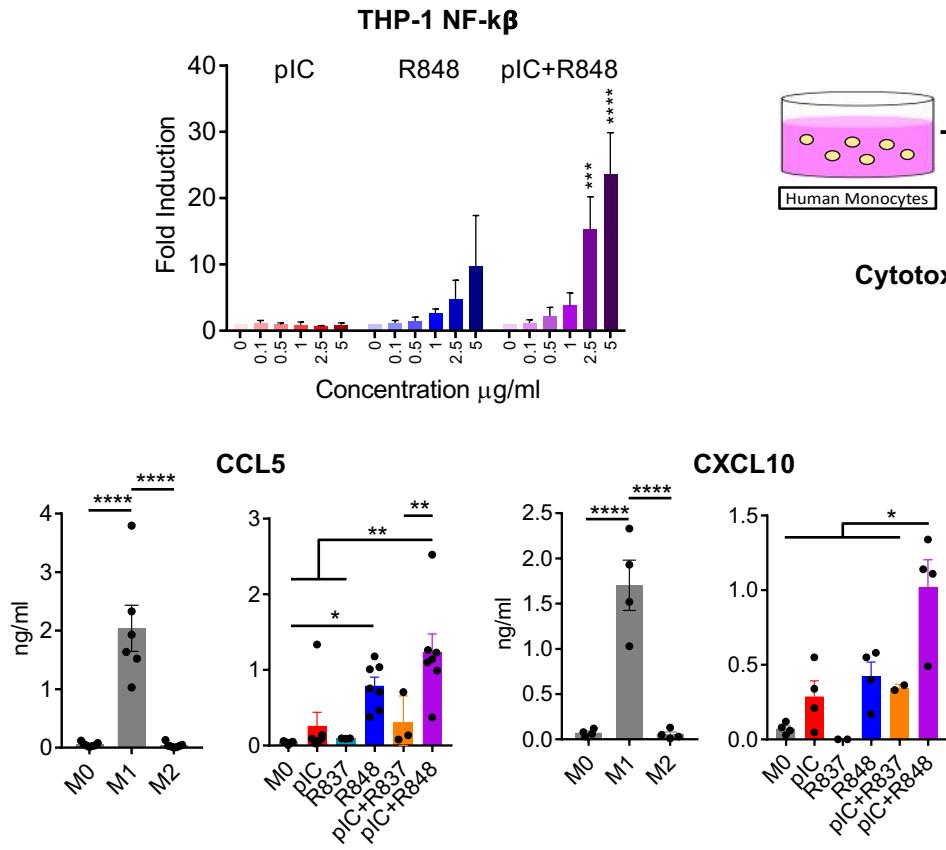
- TLR7
- TLR7/8
- TLR3



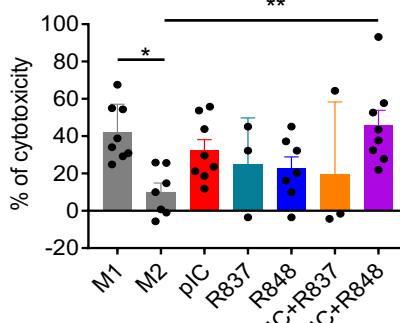
Prof. María Jose Alonso



# Poly(I:C) + Resiquimod polarize macrophages towards M1-like antitumor effector cells *in vitro*

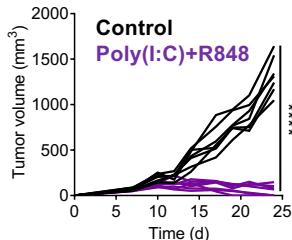
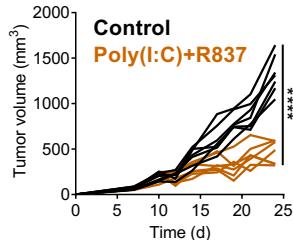
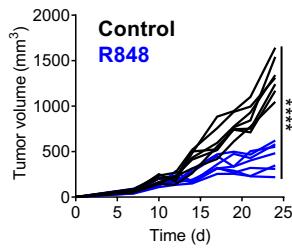
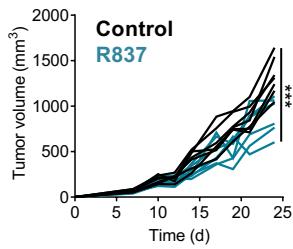
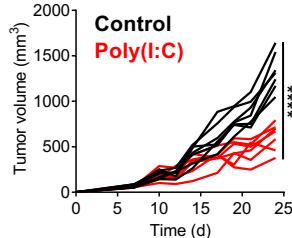
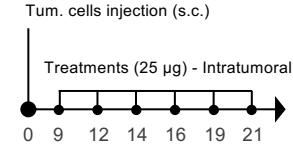


Cytotoxicity of Human Macrophages towards Cancer Cells

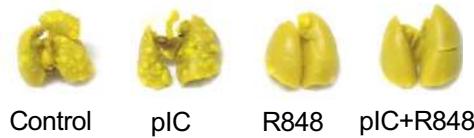
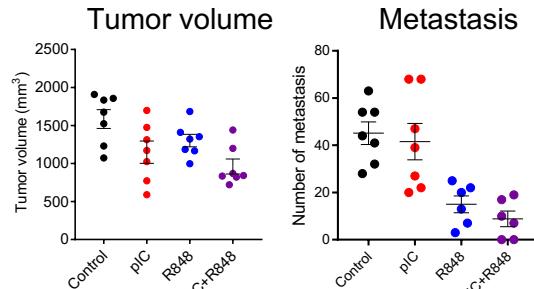
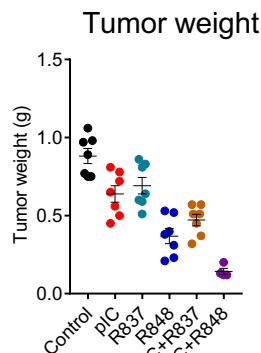
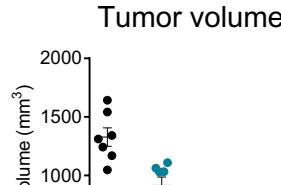


Anfray C, ... Andon FT. JITC 2021

# Poly(I:C) + Resiquimod inhibit primary tumor growth and metastasis in immunocompetent tumor models



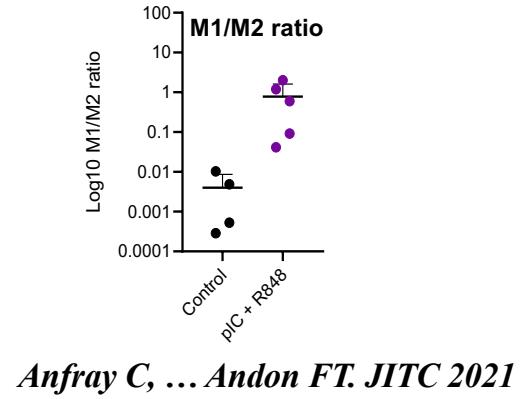
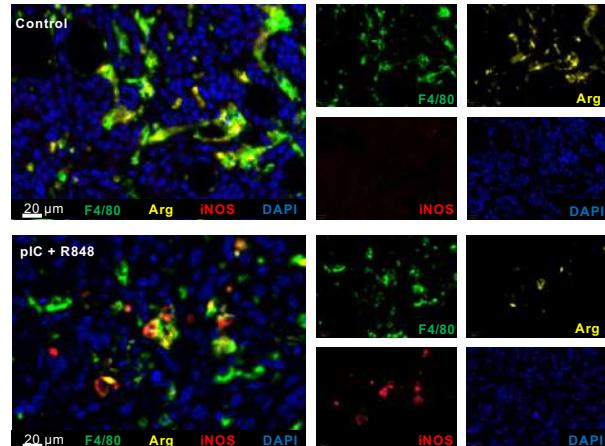
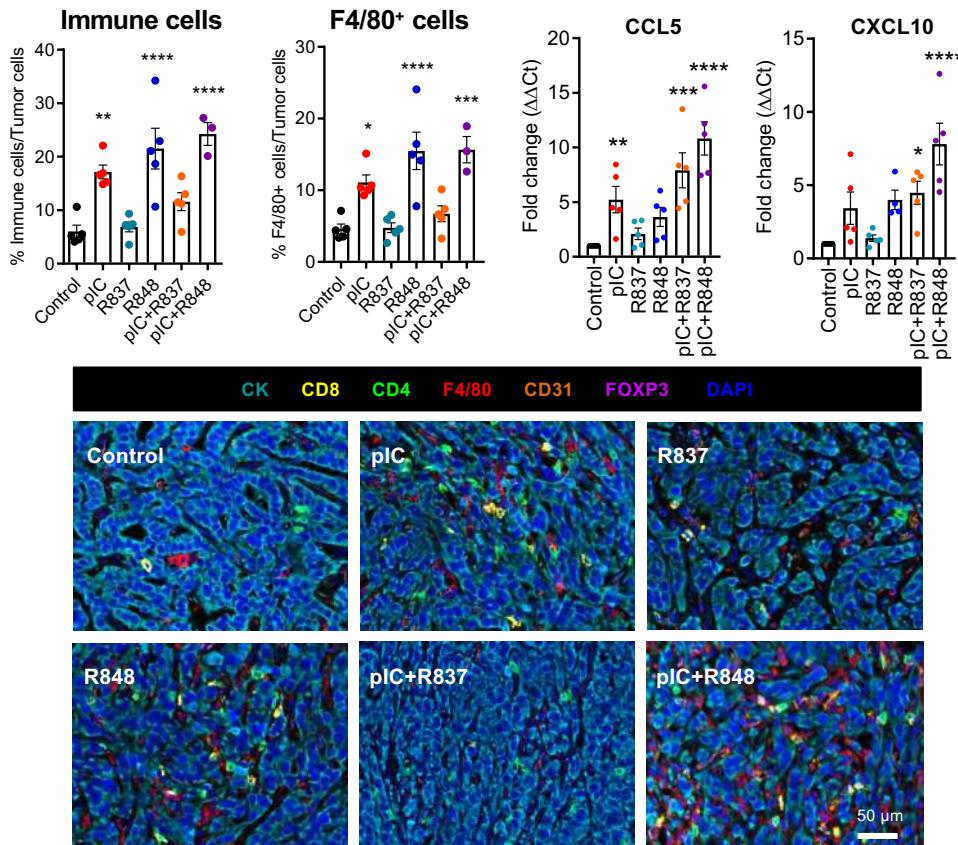
CMT167 Lung Cancer Model



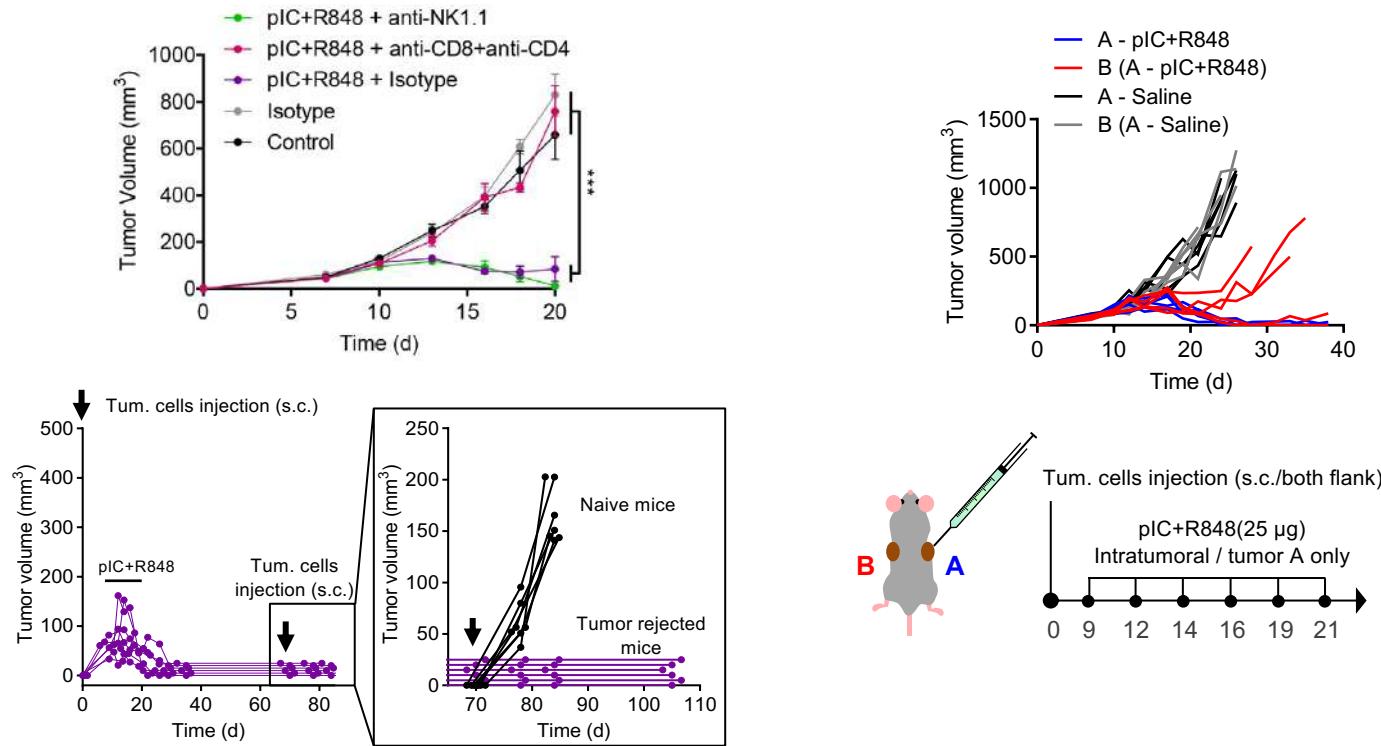
Fibrosarcoma Model with Lung Metastasis

Anfray C, ... Andon FT. JITC 2021

# Poly(I:C) + Resiquimod reprogram TAMs *in vivo*



# Poly(I:C) + Resiquimod combination induces systemic response, adaptive immunity and antitumoral memory



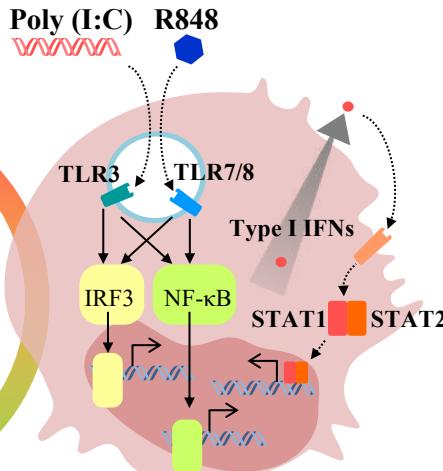
Anfray C, ... Andon FT. JITC 2021

# Combination of Poly(I:C) + Resiquimod reprograms Tumor Associated Macrophages

## Tumor Associated Macrophages



Cancer cell  
Immune suppression, angiogenesis, epithelial to mesenchymal transition, tissue remodeling, metastasis, tumoral growth factors...



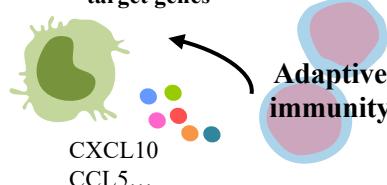
## Anti-tumoral TAMs



Phagocytic activity



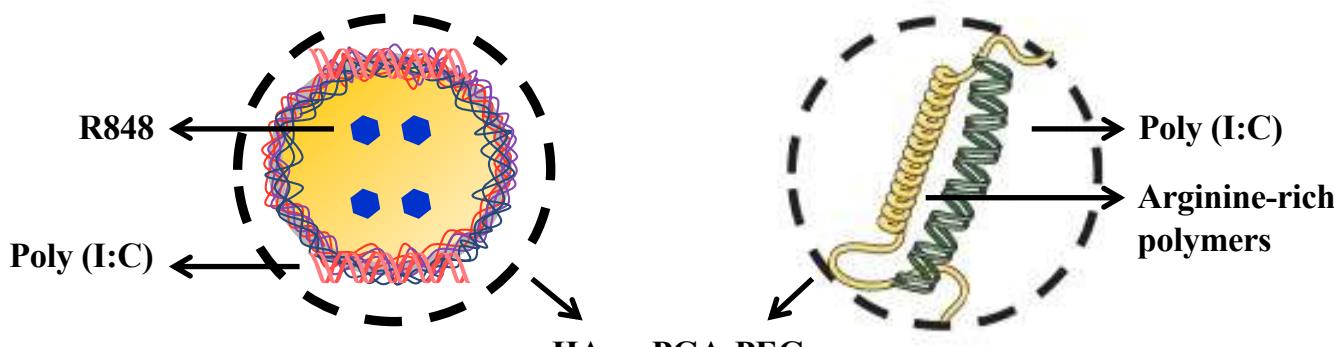
Cytotoxic activity



Immune activation

- Inhibition of tumor growth
- Prevention of metastasis
- Antitumor immune memory

# Nanomedicines to Target and Reprogram TAM



**Drugs to stimulate M1-polarization:**

- Resiquimod
- Poly (I:C)

**Receptors inside TAM:**

- TLR7 / TLR8
- TLR3

## Characterization:

Size: 70 - 150 nm  
Free Endotoxin Contamination

**Nanocapsules**

**Polymers:**

- Inulin / Dextran sulfate
- Polysialic Acid (30 KDa)
- Hyaluronic Acid (50 KDa)
- Polyarginine
- Protamine / Chitosan

HA or PGA-PEG functionalized

**Nanocomplexes**

**Polymers:**

- Polyarginine
- C12-Octarginine

**Targeting ligands:**

- Mannose

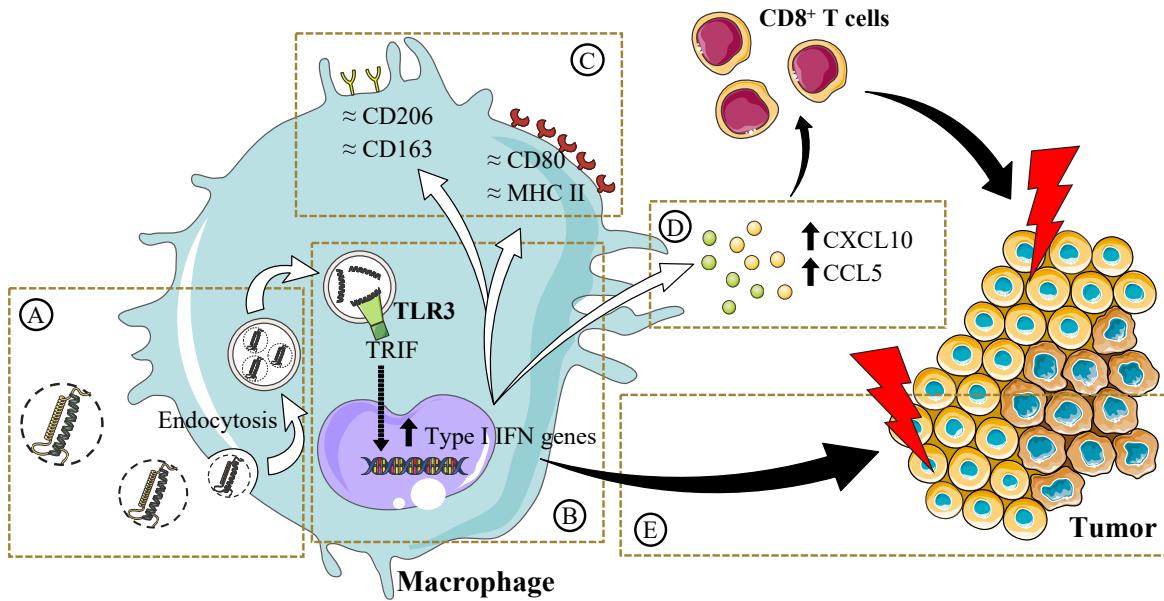
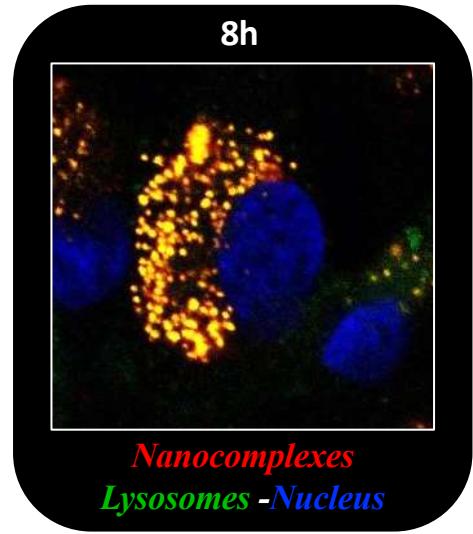
**Receptors on TAM surface:**

- CD206

Surface Charge: +50 mV / -30 mV

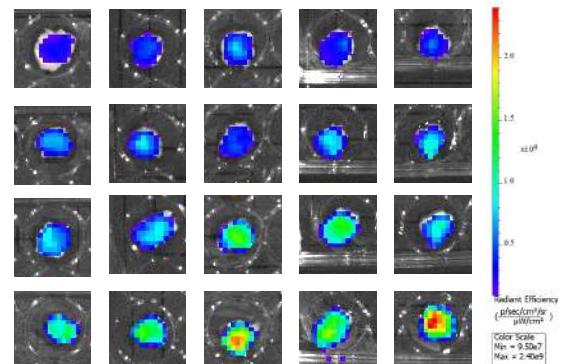
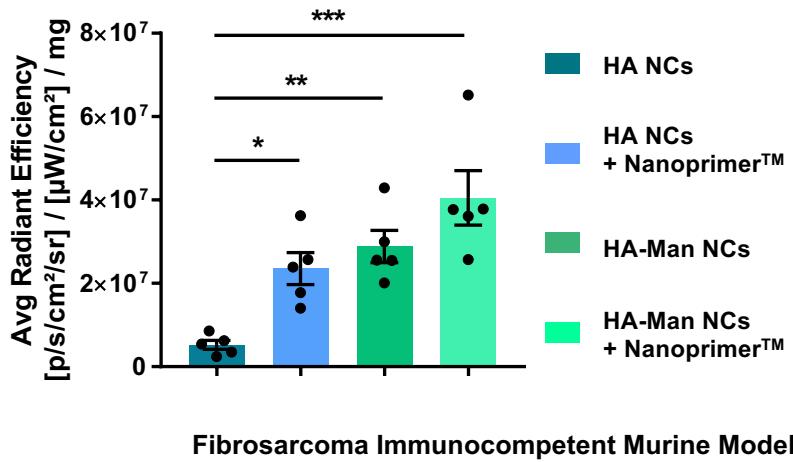
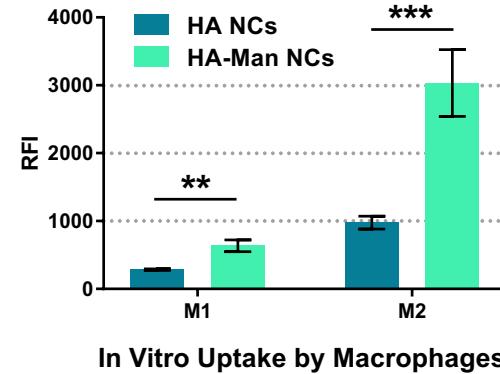
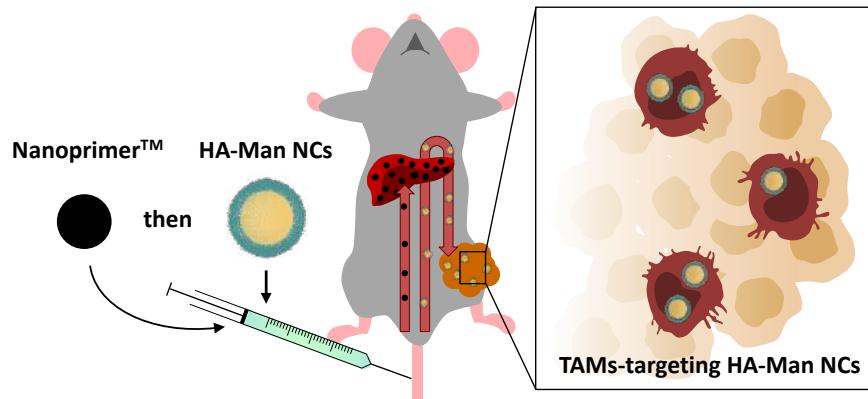
Jose Crecente Campo  
Tamara Gomez Dacoba  
Carmen Fernandez Varela  
Iago Fernandez Mariño

# Arginine-based Poly(I:C)-nanocomplexes polarize macrophages towards M1-like antitumoral effectors

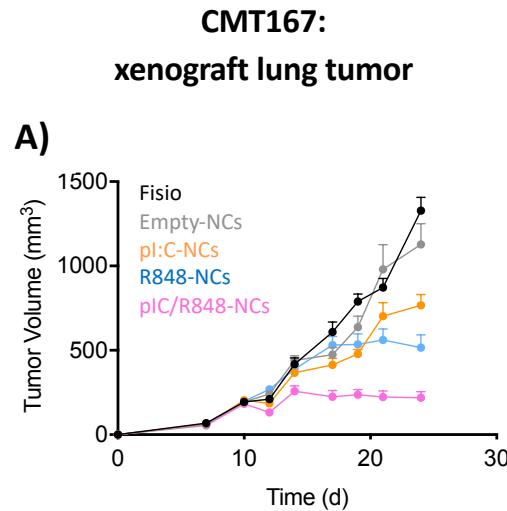


Dacoba TG, ... Andon FT, Crecente-Campo J. *Front Immunol* 2020

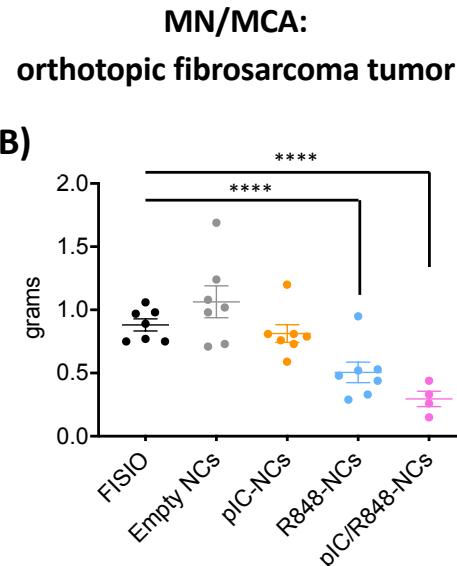
# Tumor Accumulation of Mannose-HA-functionalized-NCs



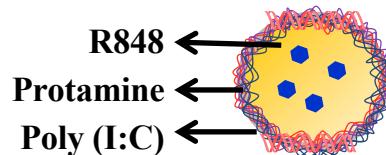
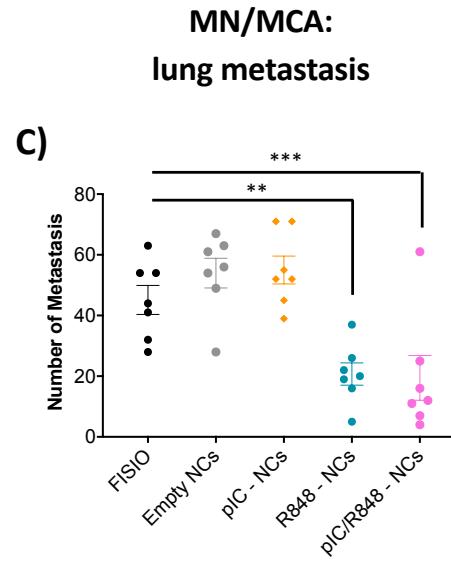
# Antitumoral Efficacy of Poly(I:C) + R848-protamine-NCs Intratumorally Injected in Immunocompetent Models



3 x 25 µg of poly(I:C)+R848 HA-Mannose-NCs



Mean ± s.e.m.; \*(p<0.05) vs control; N = 6

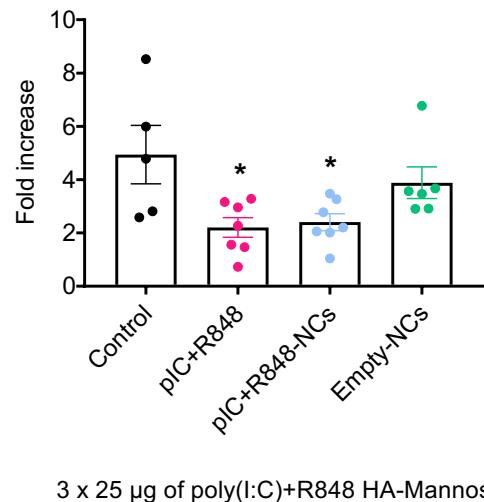


Intratumoral injection of poly(I:C) + R848-protamine-NCs:

- A) prevents tumor growth in lung and B) fibrosarcoma tumor models
- C) and metastasis to the lungs

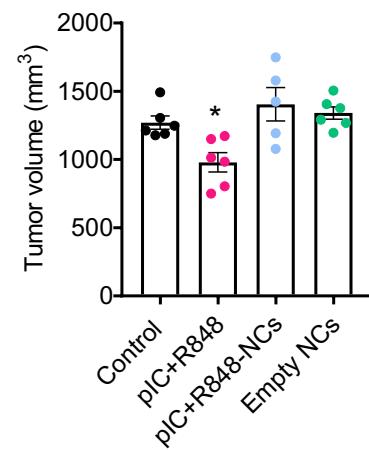
# Antitumoral Efficacy of Poly(I:C) + R848-HA-Mannose-NCs Intravenously Injected in Orthotopic Models

CMT167 - Lung cancer model

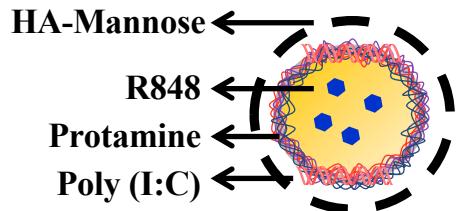


3 x 25 µg of poly(I:C)+R848 HA-Mannose-NCs

MN/MCA - Fibrosarcoma model with lung metastasis



Mean ± s.e.m.; \*(p<0.05) vs control; N = 6

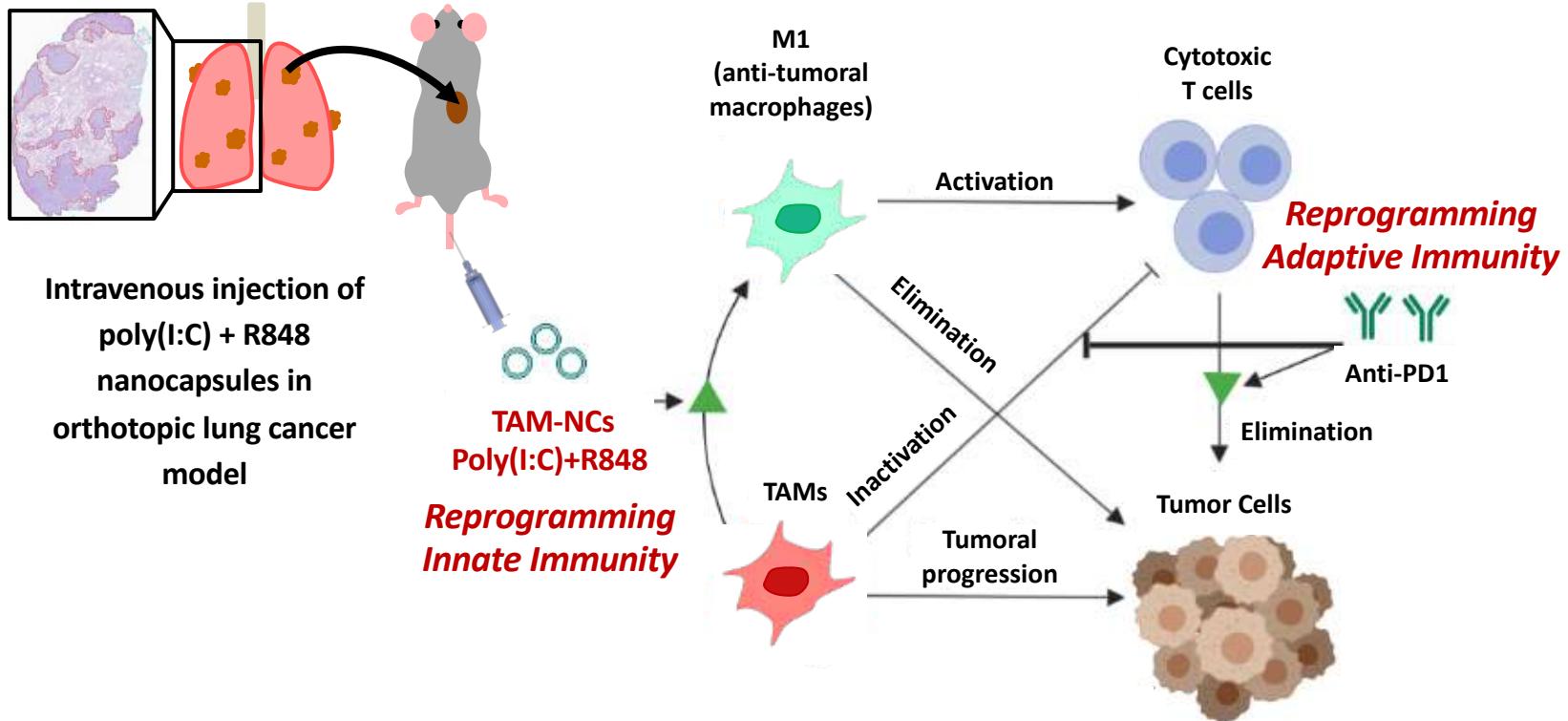


## Intravenous injection of poly(I:C)+R848-NCs:

- prevents tumor growth in a lung cancer model
- metastasis to lungs in an orthotopic model of fibrosarcoma
- best in terms of immunotoxicity??

*under preparation for publication*

# Combination Antitumoral Immunotherapy



## Other possible Combination Therapies:

- Radiotherapy
- Immunogenic Cell Death Activators
- Targeted therapies
- Immunecheckpoint Inhibitors or CAR-T cells

## Other Tumor Models:

- Pancreatic
- Glioma
- Colon Cancer
- Breast Cancer

# Antitumoral Efficacy of Poly(I:C) + R848 in 3D spheroids

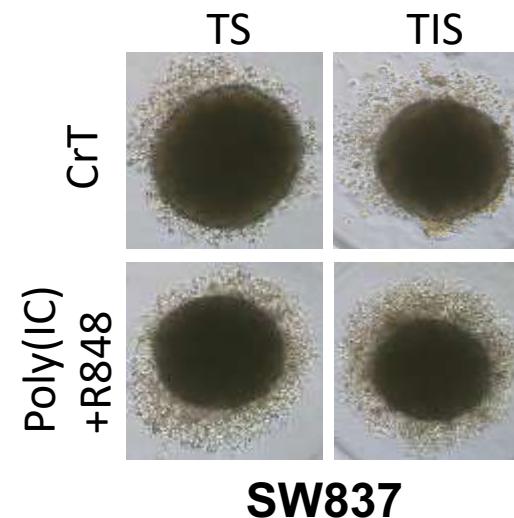
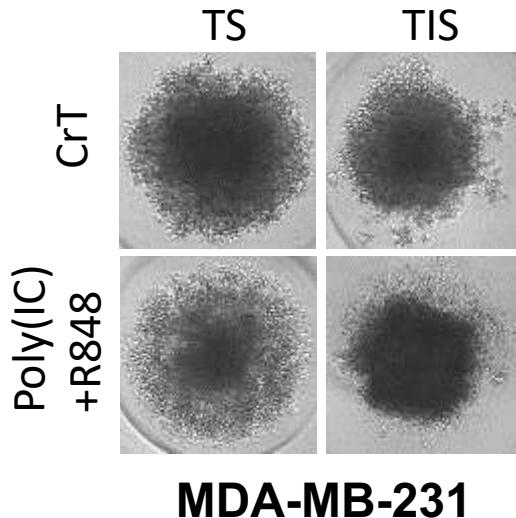
## Tumor Spheroids (TS):

- MDA-MB-231 breast cancer cells
- SW837 rectal cancer cells spheroids

## Tumor-immune Spheroids (TIS):

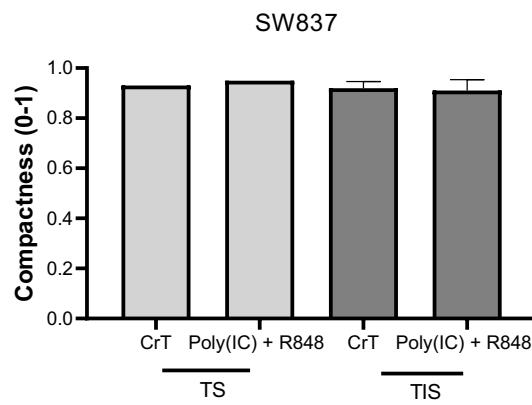
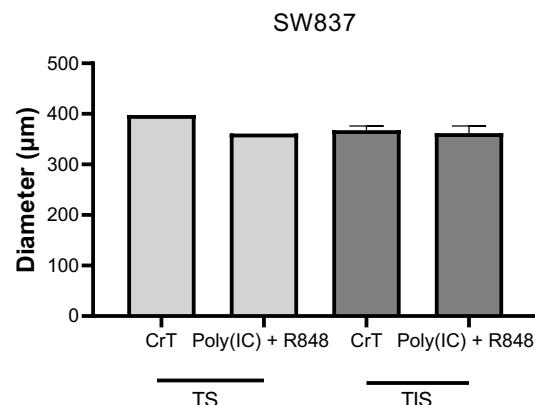
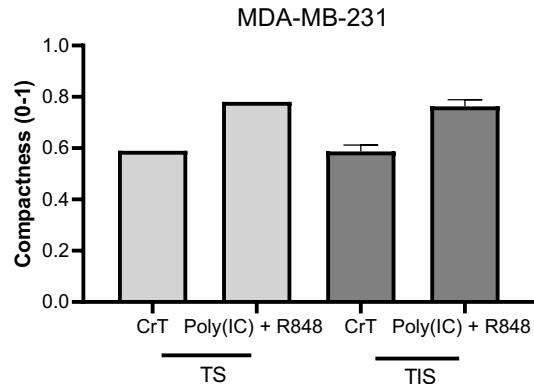
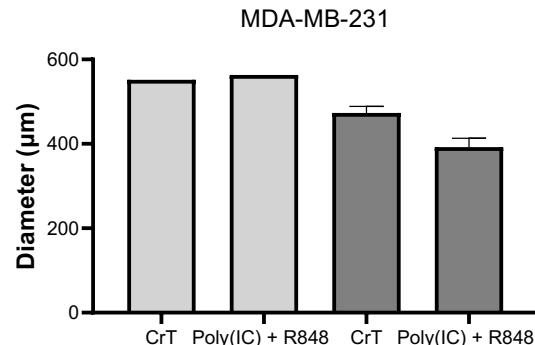
- MDA-MB-231 breast cancer cells + monocytes (35%:65%)
- SW837 rectal cancer cells + monocyte spheroids (80%:20%)

(We have used 3 donors n=3)



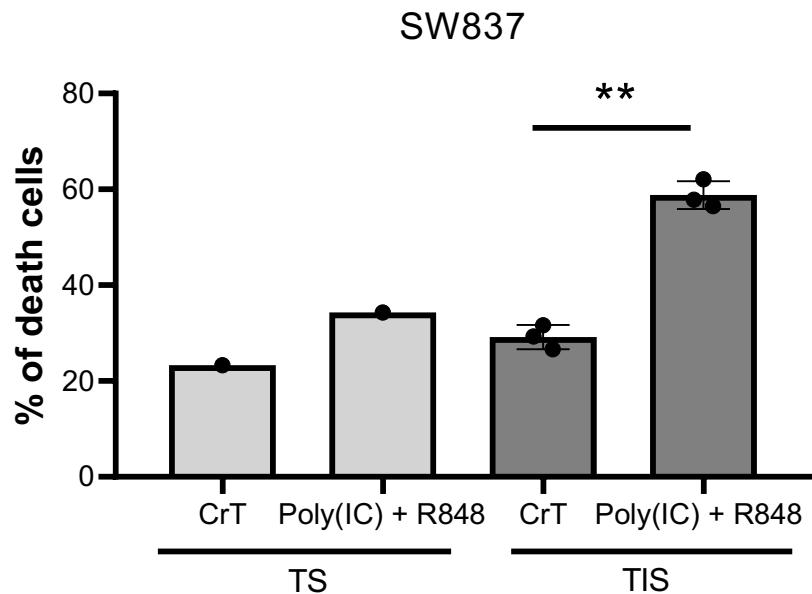
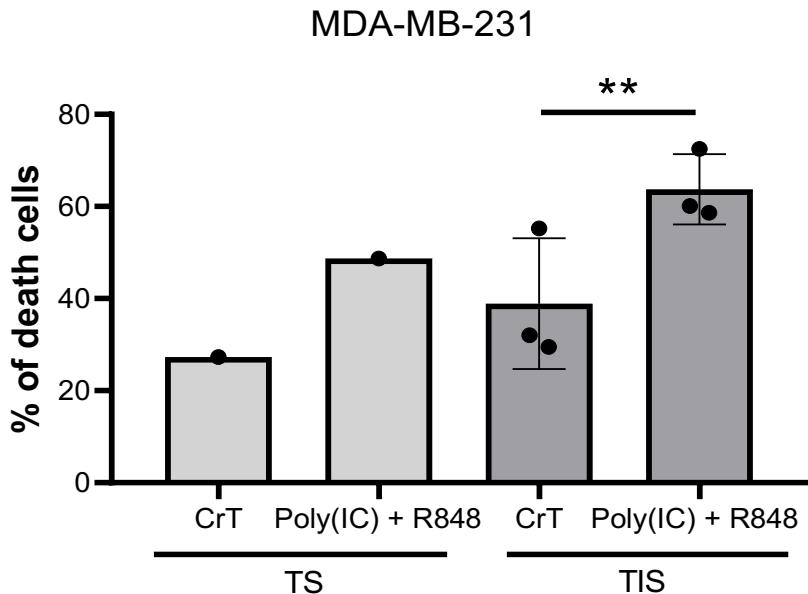
*not published*

# Morphology of 3D spheroids treated with Poly(I:C) + R848



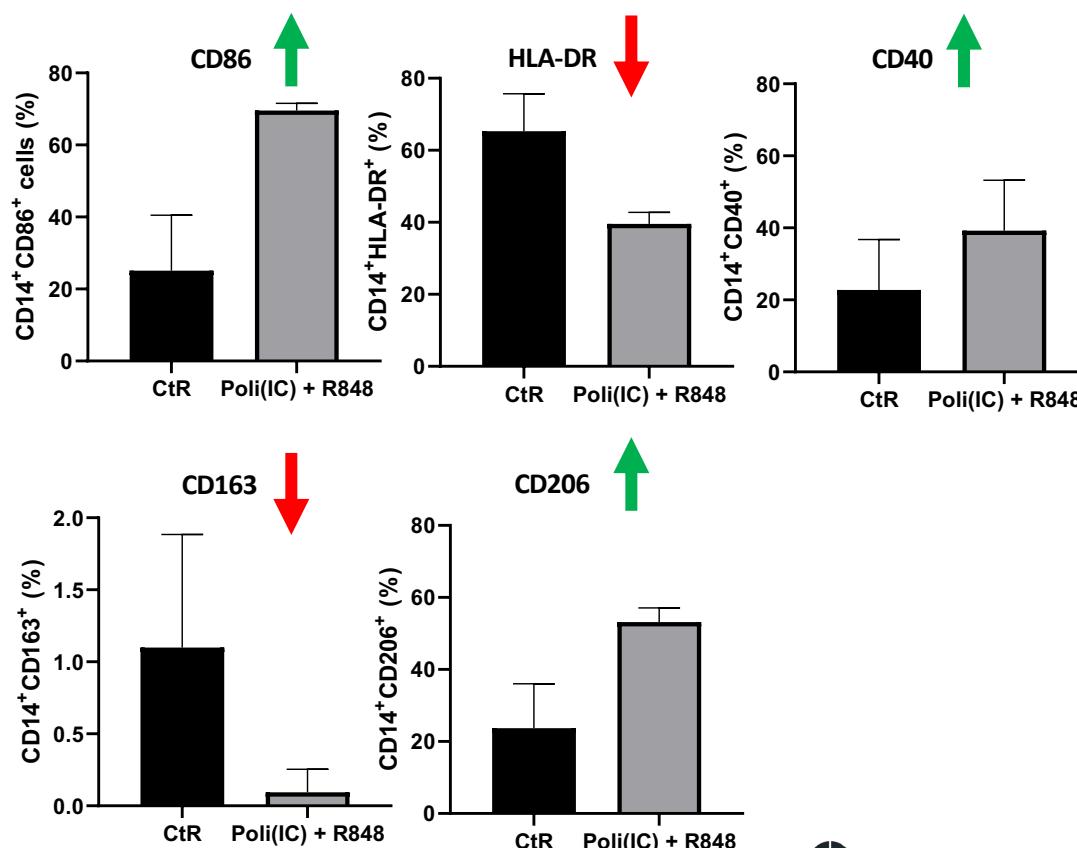
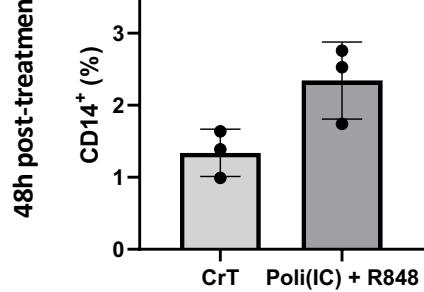
*not published*

# Cell death in 3D spheroids treated with Poly(I:C) + R848



not published

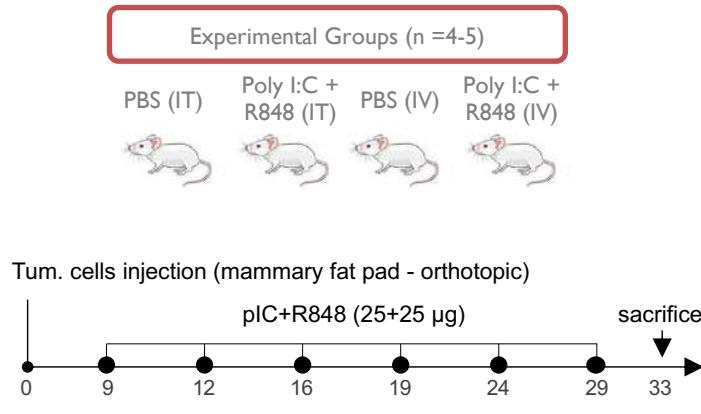
# Cell death in 3D spheroids treated with Poly(I:C) + R848



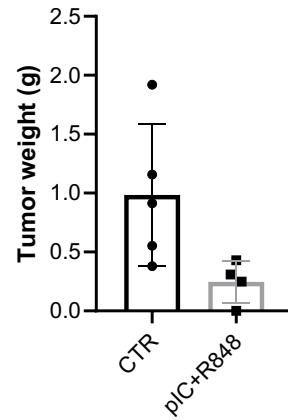
The % of macrophages decreased from 20% to ~1-3% from 0 to 72h, having the spheroids treated with Poly(IC) + R848 a > % of macrophages

not published

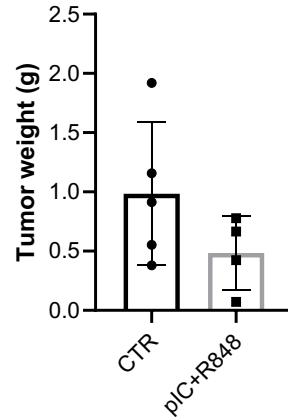
# Antitumoral Efficacy of Poly(I:C) + R848 in 4T1 Breast Cancer Model *In Vivo*



## INTRAVENOUS ADMINISTRATION



## INTRATUMORAL ADMINISTRATION



### On-going:

- A) No toxicity as evaluated by weight of mice along the experiment.
- B) Analysis of tumor microenvironment by Immunohistochemistry, FACS and RNA.
- C) Metastasis quantification in lungs.
- D) Next experiment: evaluation of Poly(I:C) + R848 loaded NPs.

*not published*



María José Alonso

Jose Crecente Campo

Tamara Gómez Dacoba

Carmen Fernández Varela

Iago Fernández Mariño



Universidad de Navarra

Alfonso Calvo

# Acknowledgements

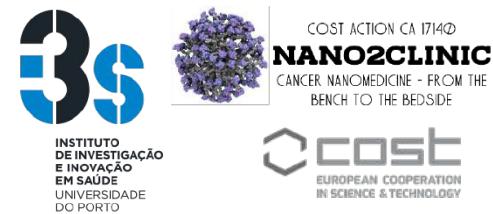


Paola Allavena

Alberto Mantovani

Clement Anfray

Aldo Ummarino



María José Oliveira

Flavia Castro

Tania Cruz