UNIVERSITY OF TRIESTE

DEPARTMENT OF
LIFE SCIENCES

Ab-based technologies for targeting

Paolo Macor

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SUMMARY

1. Aims of the targeting

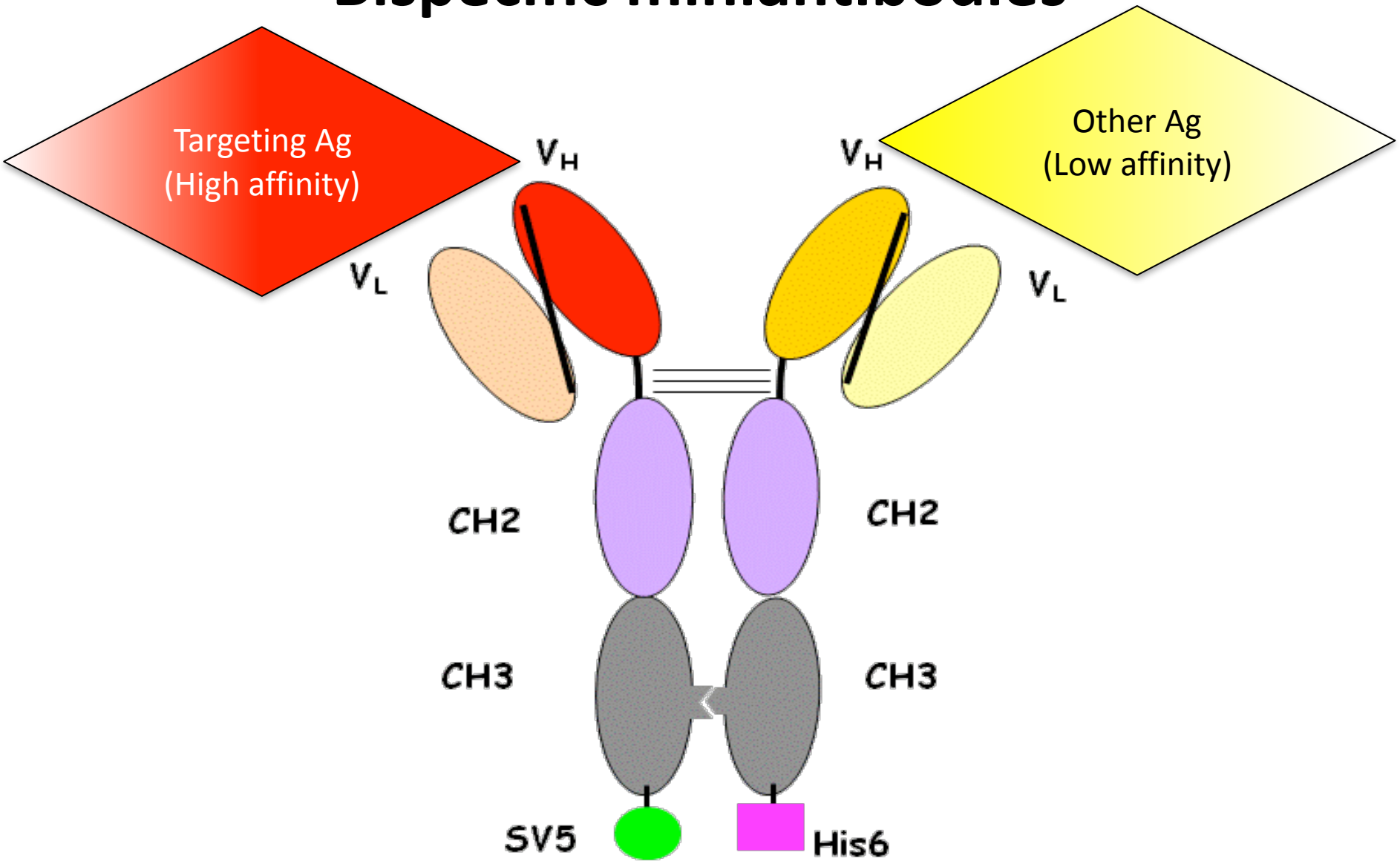
2. Strategy for the targeting

3. Examples of targeting results

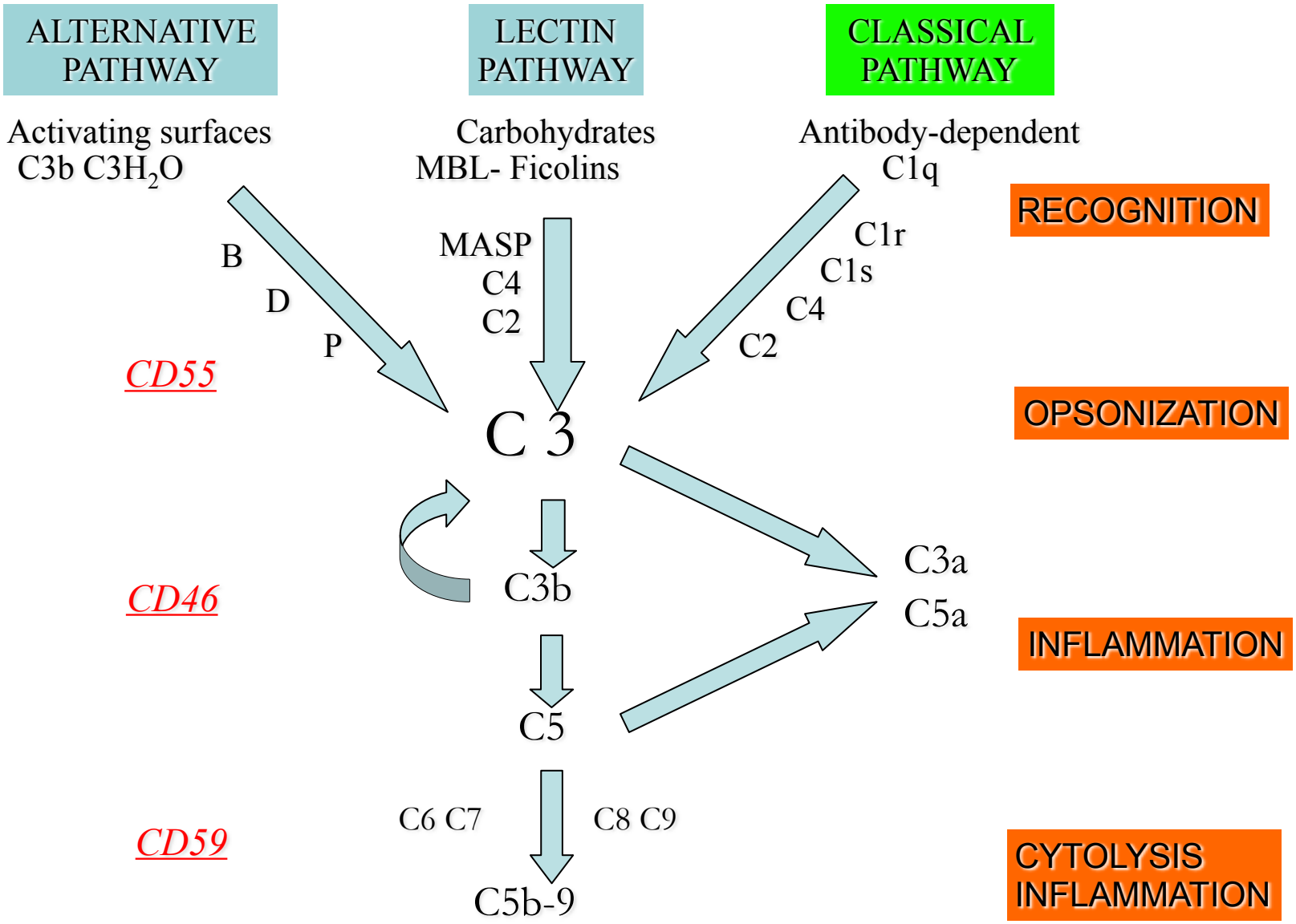
Development of molecules able to target specific cells or tissues in order to:

- ✓ Increase efficacy of the treatments
- ✓ Reduce side effects
- ✓ Reduce doses
- ✓ Reduce costs

Bispecific miniantibodies



The complement system



European Journal of Immunology

Controlling complement resistance in cancer by using human monoclonal antibodies that neutralize complement-regulatory proteins CD55 and CD59

Federica Ziller[1,2], Paolo Macor[2], Roberta Bulla[2], Daniele Sblattero[1], Roberto Marzari[1] and Francesco Tedesco[2]

Research Article

Cancer Res 2007; 67: (21). November 1, 2007

In vivo Targeting of Human Neutralizing Antibodies against CD55 and CD59 to Lymphoma Cells Increases the Antitumor Activity of Rituximab

Paolo Macor,[1] Claudio Tripodo,[4] Sonia Zorzet,[2] Erich Piovan,[5] Fleur Bossi,[1] Roberto Marzari,[3] Alberto Amadori,[5] and Francesco Tedesco[1]

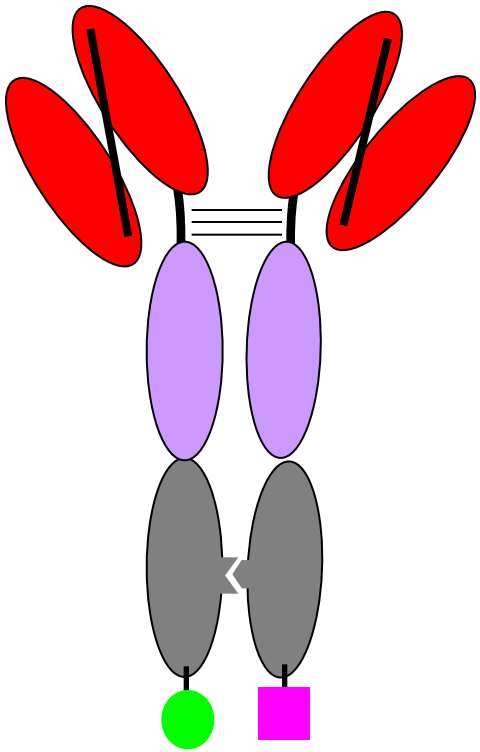
Departments of [1]Physiology and Pathology, [2]Biological Sciences, and [3]Biology, University of Trieste, Trieste, Italy; [4]Department of Human Pathology, University of Palermo, Palermo, Italy; and [5]Department of Oncology and Surgical Sciences, University of Padova, Padova, Italy

Table 1. Complement-mediated killing of lymphoblastoid cell lines

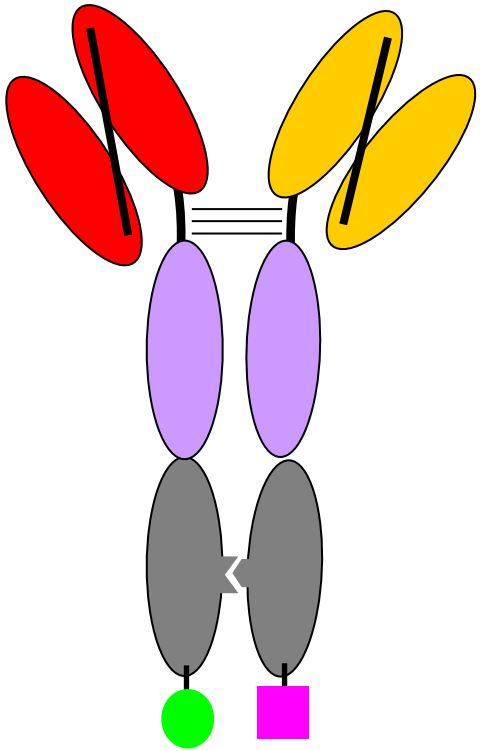
	LCL1	LCL2	LCL3	HuSCID1	HuSCID2	HuSCID3
Rituximab	28 ± 7	13 ± 3	21 ± 4	25 ± 5	31 ± 11	14 ± 5
MB55–MB59	4 ± 2	4 ± 2	5 ± 2	4 ± 3	7 ± 2	7 ± 5
Rituximab + MB55	55 ± 7	49 ± 5	55 ± 7	32 ± 6	41 ± 9	35 ± 9
Rituximab + MB59	64 ± 9	55 ± 6	71 ± 4	53 ± 8	42 ± 12	41 ± 9
Rituximab + MB55–MB59	73 ± 5	84 ± 7	83 ± 11	61 ± 8	63 ± 7	45 ± 7

Bispecific antibodies (BsAbs)

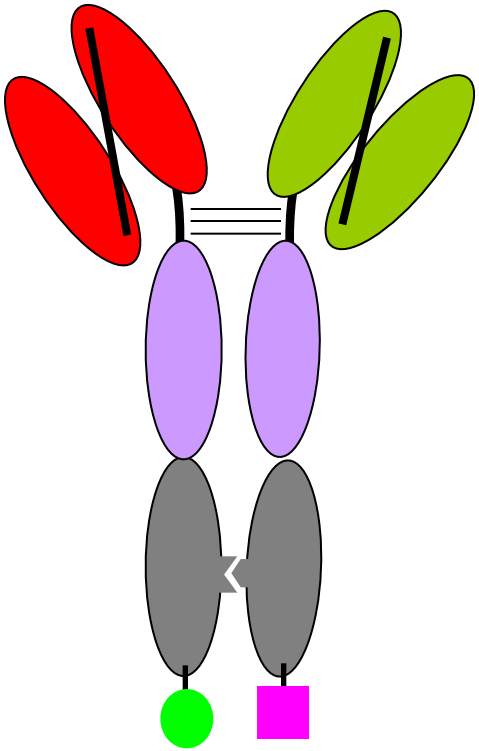
MB20/20



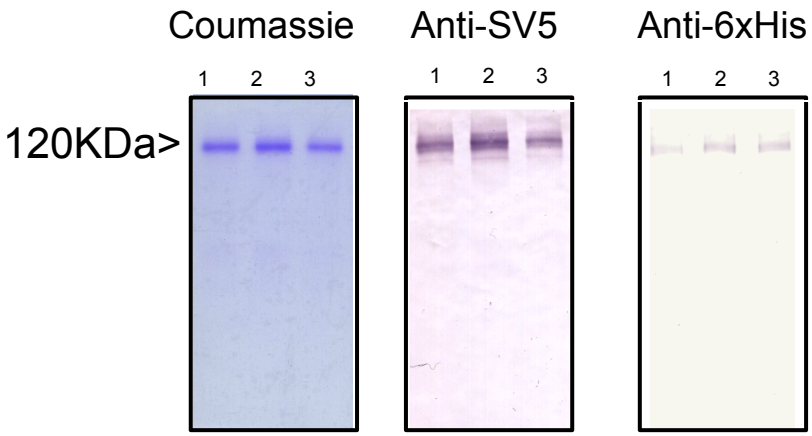
MB20/55



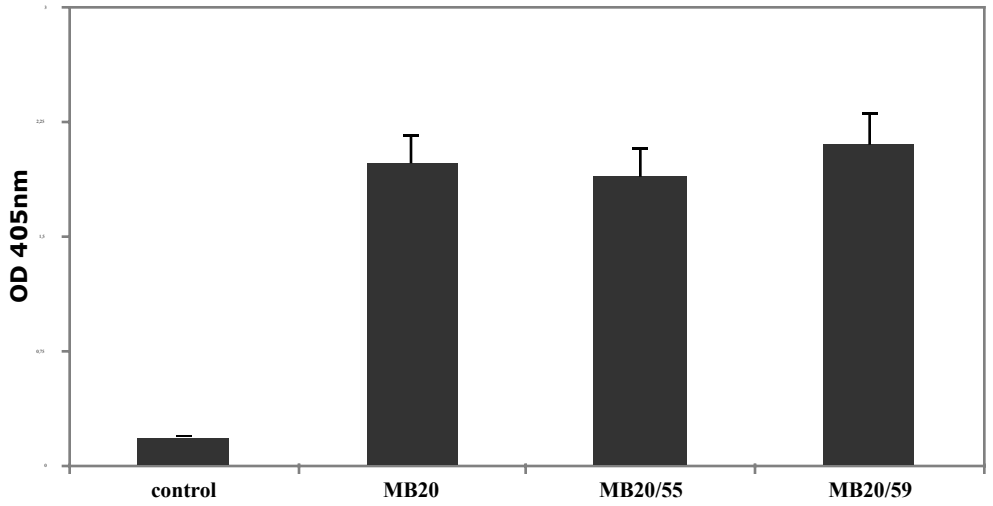
MB20/59



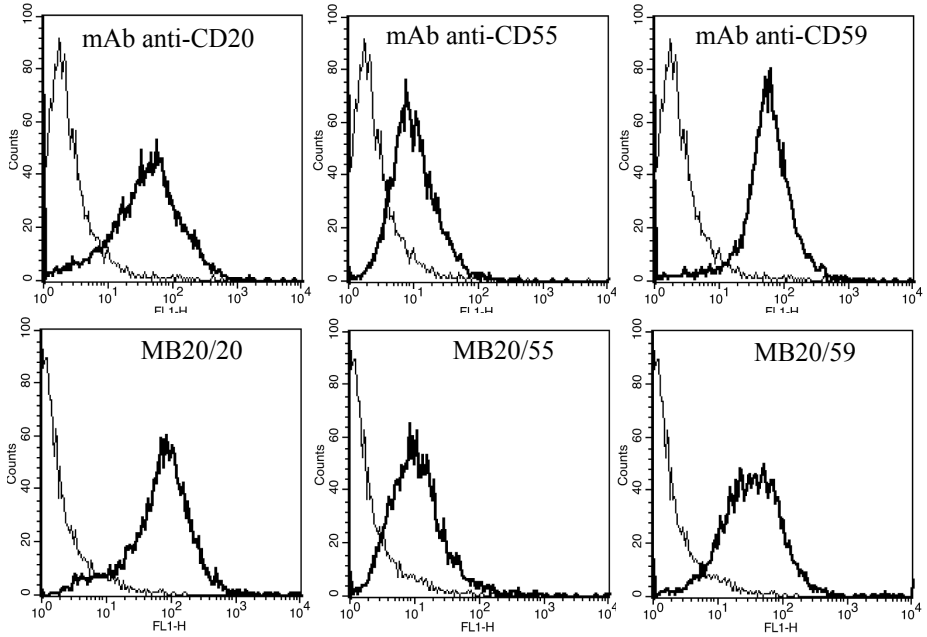
Bispecific Ab production



Heterodimerization

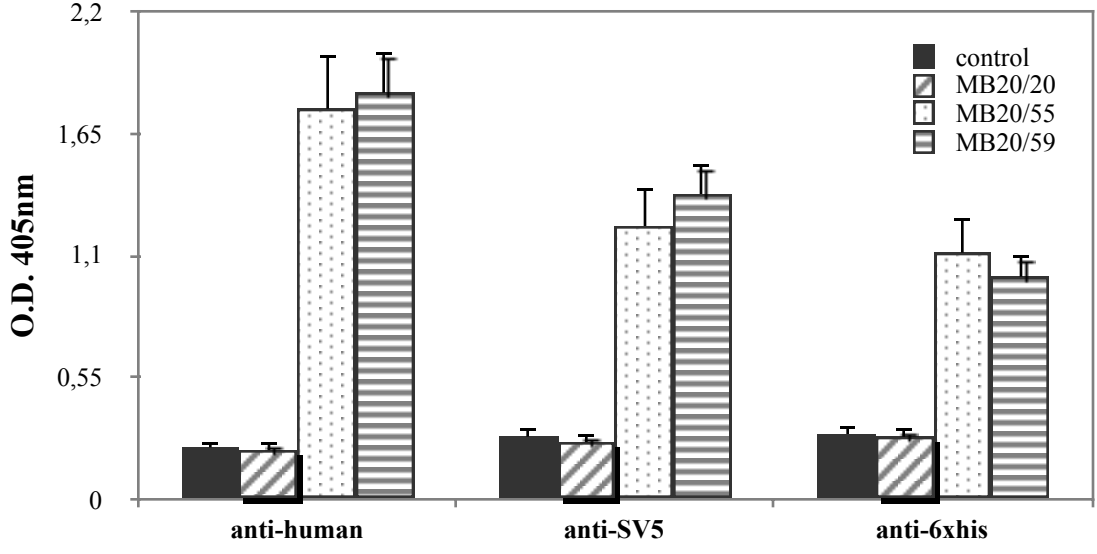


BsAbs binding



BJAB cells

IGROV1 cells

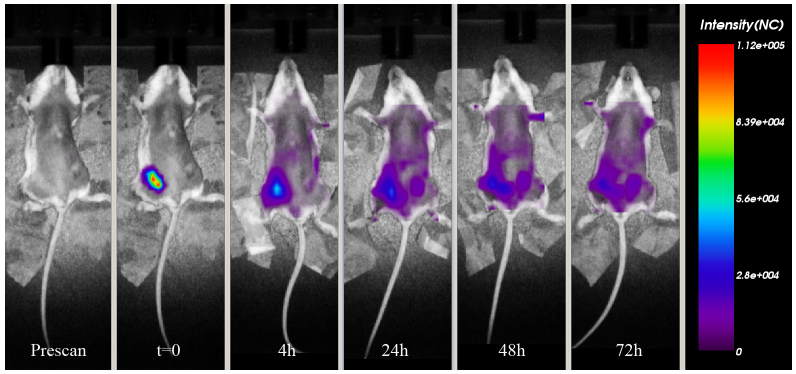


Cytotoxic effect of BsAbs

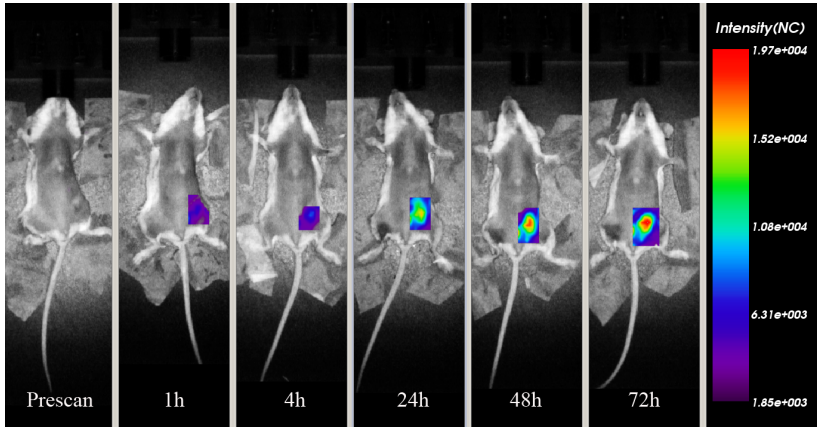
	CD20 (MFI)	Saline	MB20/20	MB20/55	MB20/59	MB20/55 + MB20/59
BJAB	316,8	3,1±1,1	22,0±1,9	31,3±3,9	38,3±4,3	57,8±6,1
MEC1	264,5	0,9±1,3	15±1,9	23,5±3,8	33,4±3,5	44,1±7,0
Pt1	73,6	2,1±1,4	1,3±1,1	33,5±4,5	34,2±5,1	53,9±10,8
Pt2	22,2	1,4±1,4	1,4±1,0	10,7±3,8	9,2±2,8	21,0±5,7
Pt3	65,9	4,3±1,0	9,1±2,1	19,5±3,7	19,7±2,9	43,8±8,4
Pt4	53,6	6,3±1,8	9,9±1,9	18,6±2,1	23,4±4,3	39,7±8,3
Pt5	77,7	1,2±1,0	4,2±2,0	31,4±3,6	38,7±5,0	57,6±10,8

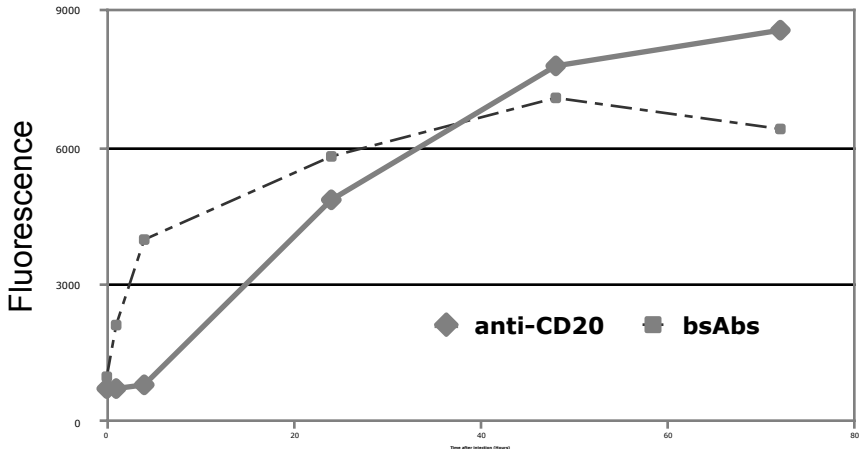
Distribution of BsAbs in tumor bearing mice

Total body



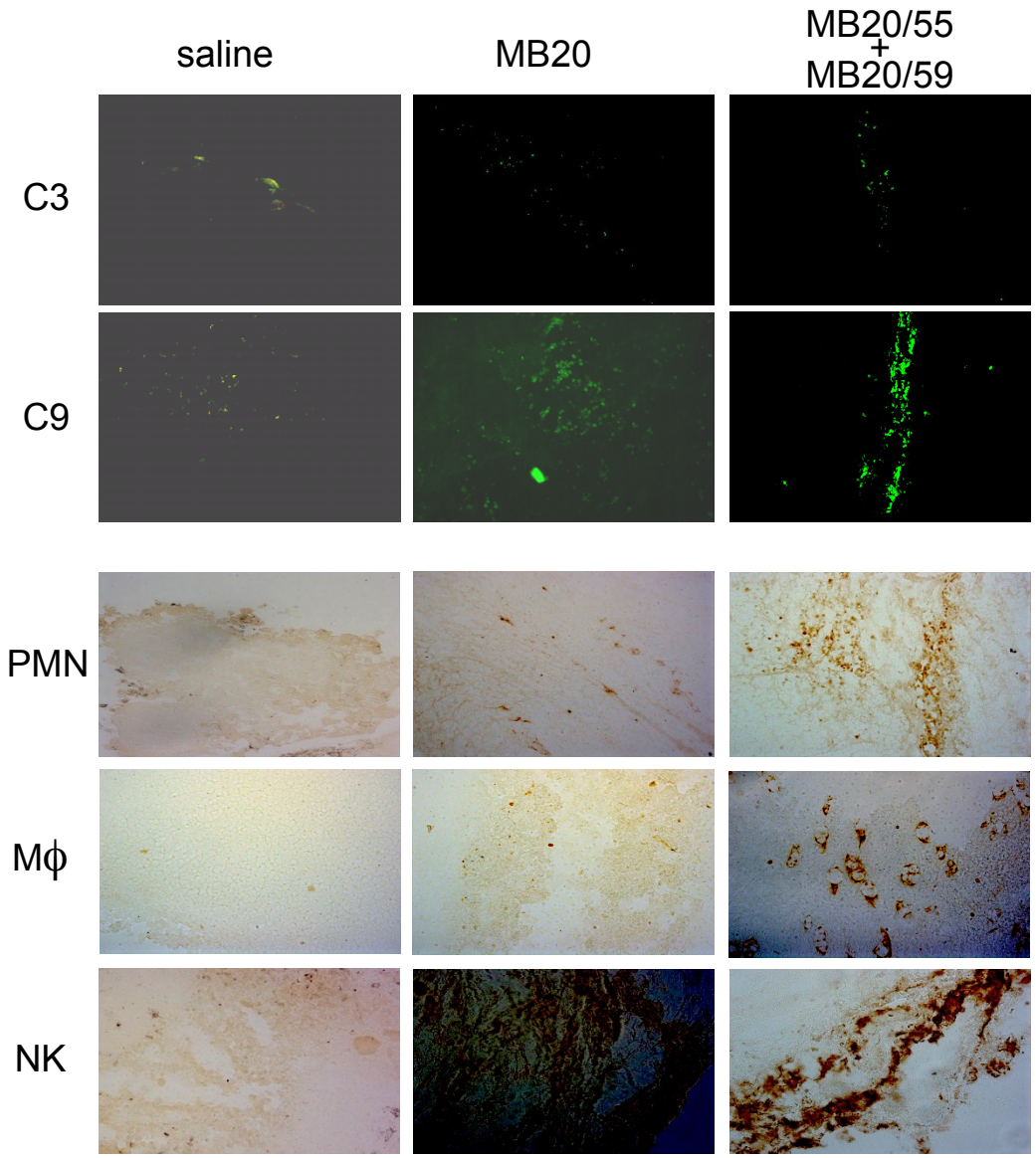
Tumor mass



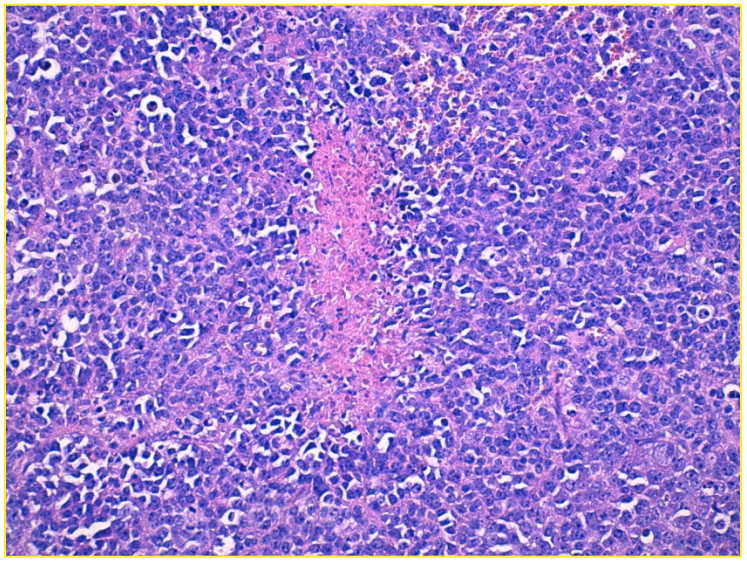


Time after injection (Hours)

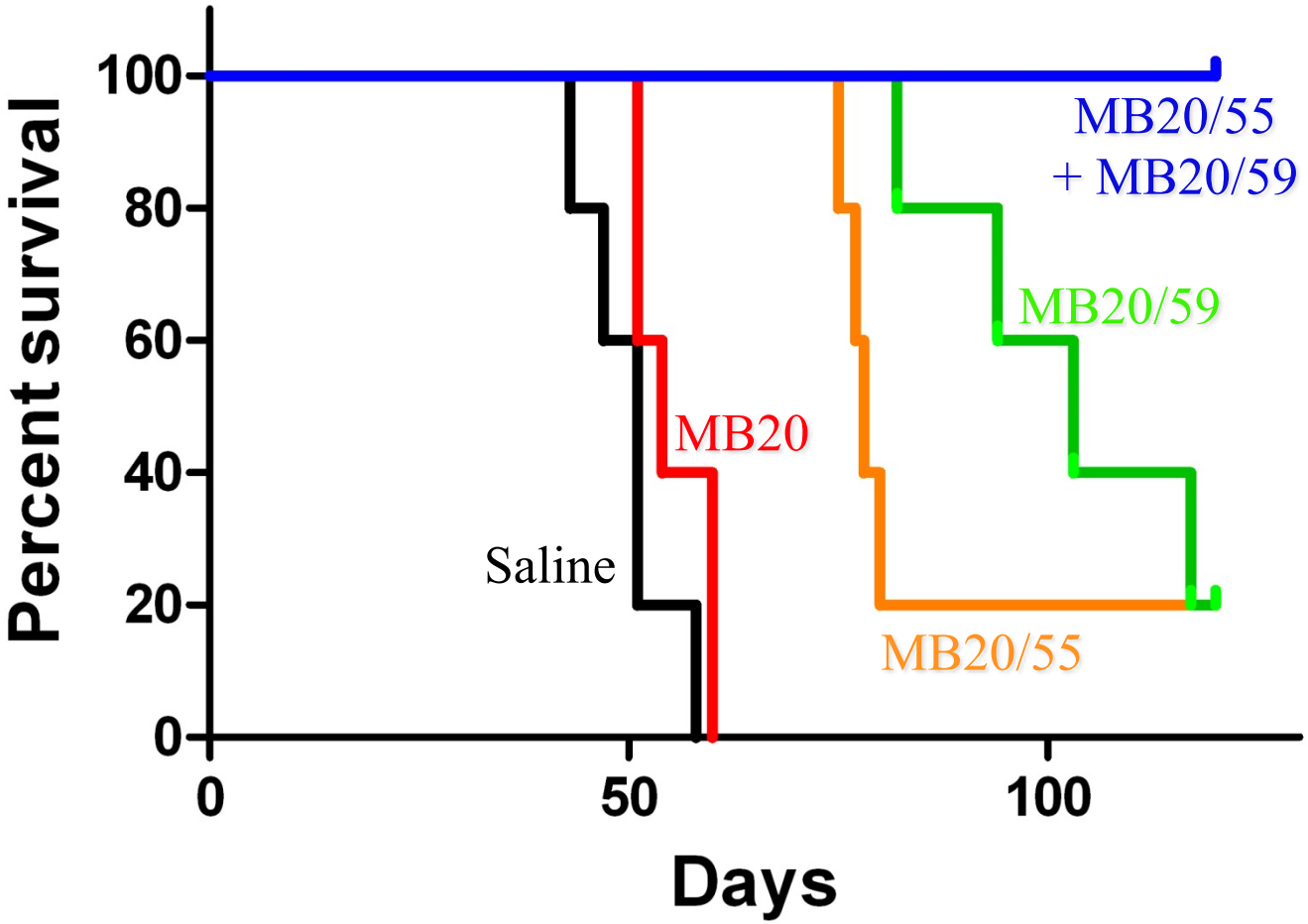
Effect of BsAbs: ex vivo analysis



MB20/55 + MB20/59

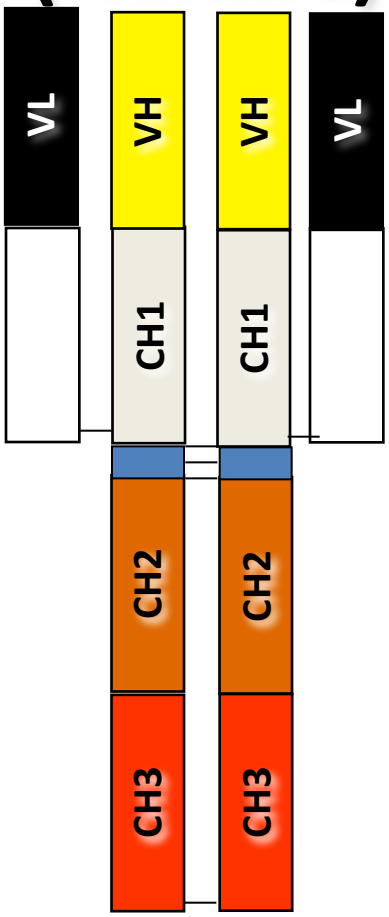


Therapeutic effect of BsAbs

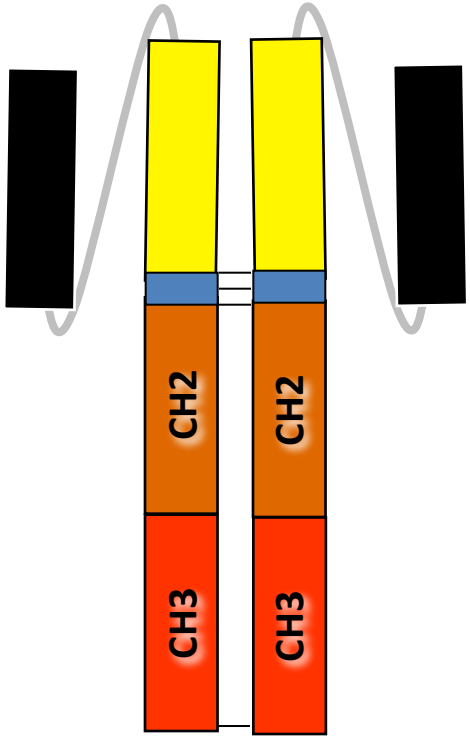


Peptide-guided miniantibodies

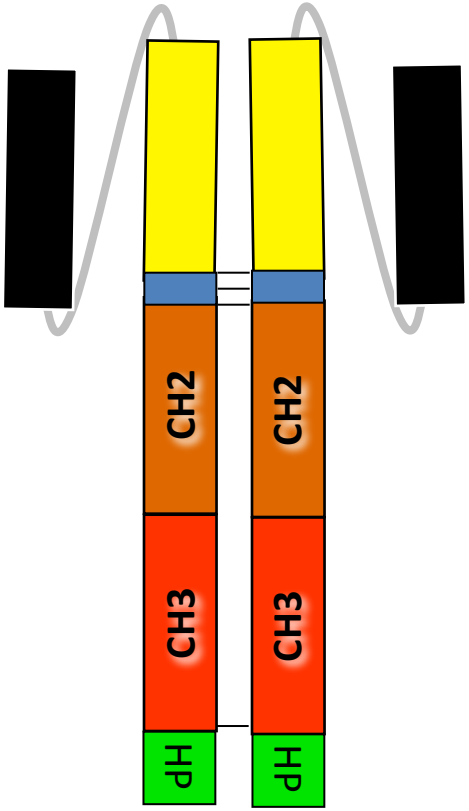
Adalimumab (Humira)



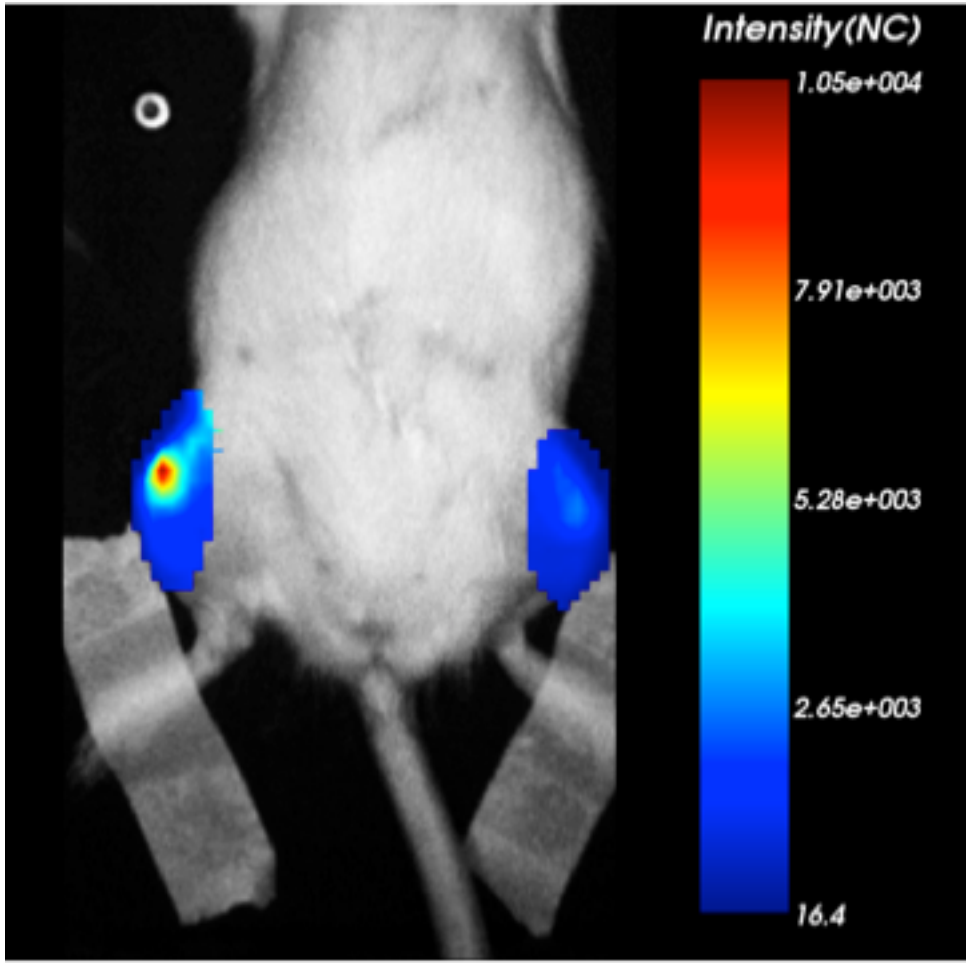
Anti-TNF



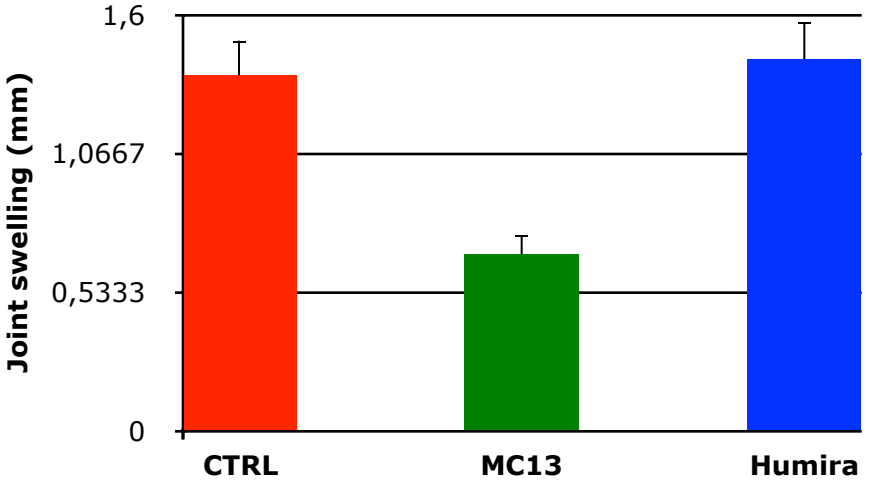
Anti-TNF-HP (MC13)

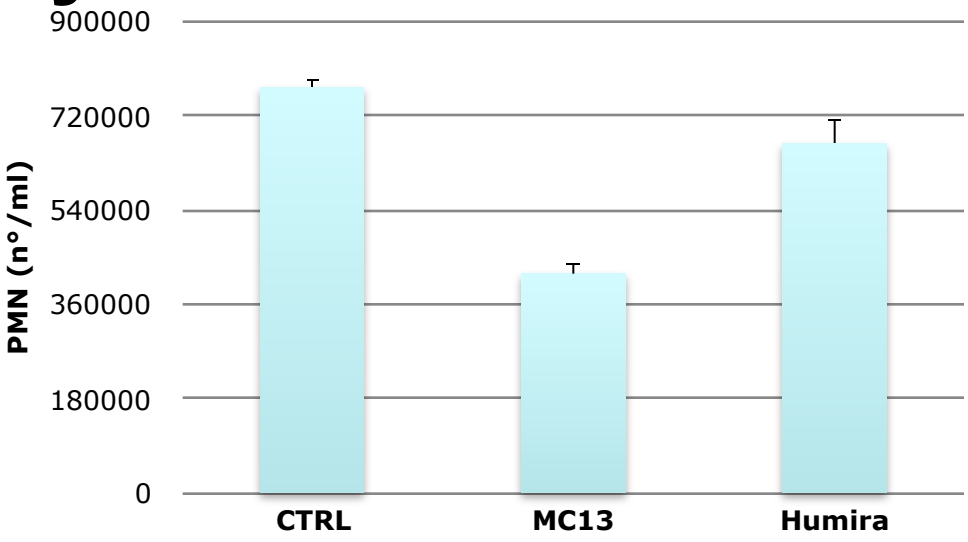


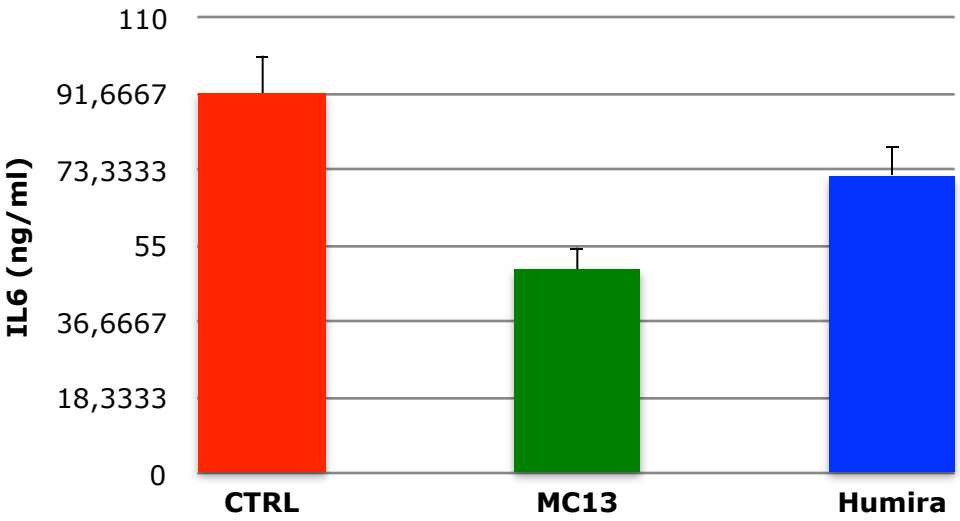
MC13 biodistribution:
optical imaging at 96 hours



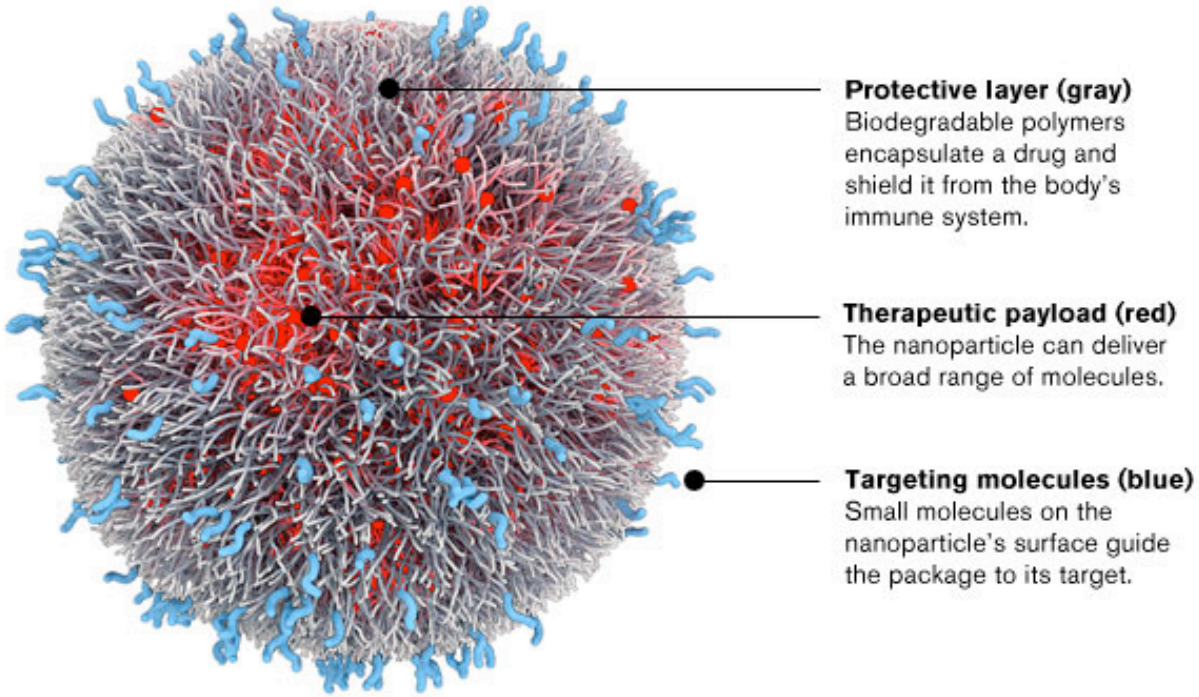
Anti-inflammatory effects of MC13







Targeted polymeric nanoparticles



Targeted nanoparticles

- Ab-targeted *polymeric nanoparticles*
- Peptide-targeted *polymeric nanoparticles*

- Peptide-targeted *liposomes*

- Ab-targeted *silica nanoparticles*

B-CELL MALIGNANCIES

INDOLENT and FOLLICULAR LYMPHOMA

MANTLE CELL LYMPHOMA

WALDENSTROM'S MACROGLOBULINEMIA

HAIRY CELL LEUKEMIA

CHRONIC LYMPHOCYTIC LEUKEMIA (CLL)

BURKITT'S LYMPHOMA

- Chronic lymphoproliferative process
- Accumulation of B-lymphocytes in the blood, bone marrow, lymphonodes and spleen.
- Most frequent in old people
- Late diagnosis
- Asymptomatic disease at the beginning that chance in an aggressive and diffuse process

- High aggressive lymphoma
- Most frequent in young people
- Cells with a very high proliferation rate
- Late diagnosis
- Several organs are involved at the diagnosis (kidneys, liver, spleen, central nervous system, bone marrow, etc.)

TREATMENT OF B-CELL DISORDERS

Poly-Chemotherapy

Alchilant agents: Cyclofosfamide, chlorambucil, ifosfamide
Purine analogues: Fludarabine

✓ Very high anti-tumor effect (first line treatment)

✓ Very high side effects

✓ Chemoresistence

Immunotherapy

Monoclonal antibodies: Rituximab, Ofatumumab, Alemtuzumab, Galiximab, etc.

✓ Specific anti-tumor effect (maintenance treatment)

✓ Low side effects

✓ Down-regulation of tumor-associated antigens

PROPOSAL

Development of a novel therapeutic approach in which the efficacy of high-dose poly-chemotherapy is associated to the specificity and low side effects of antibody-based therapy.

STRUCTURE OF THE IMMUNO-NANOPARTICLES (BNPs)

Production: non-emulsion-polymerization technology in GMP condition (Bio-Target Inc., Lincoln, NE, USA)

Diameter: 200-300nm

Shell: 3 biodegradable biocompatible polimers:

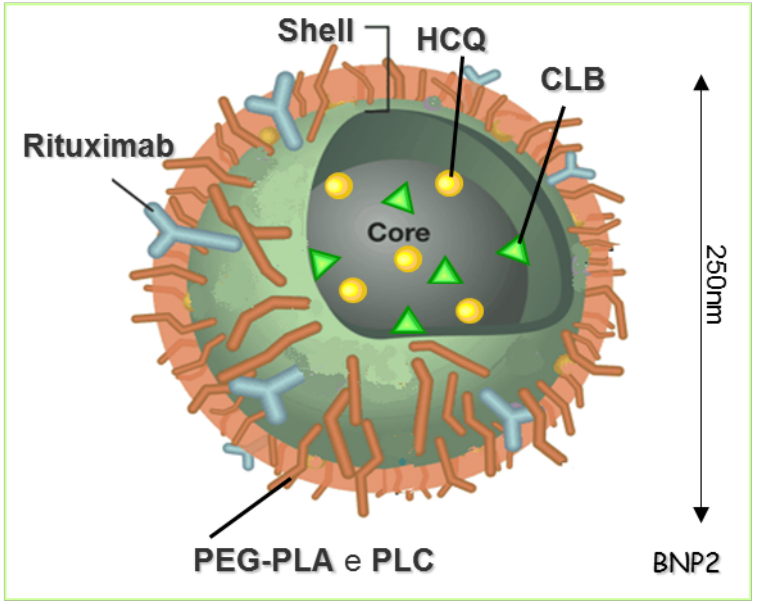
PEG-PLA (polyethylene-glycol / poly(lactic-acid)

PCL (poly-caprolactone)

Anti-CD20 antibody Rituximab

Core: Hydroxichloroquine (HCQ) 5mg/mL

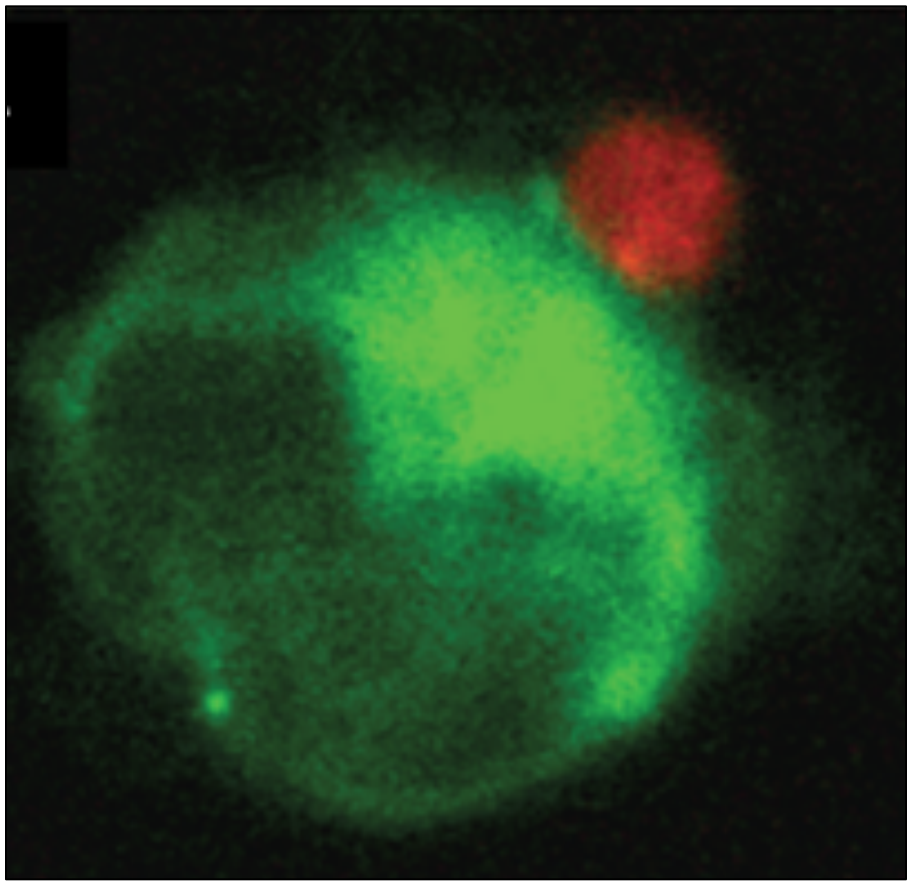
Chlorambucil (CLB) 5mg/mL

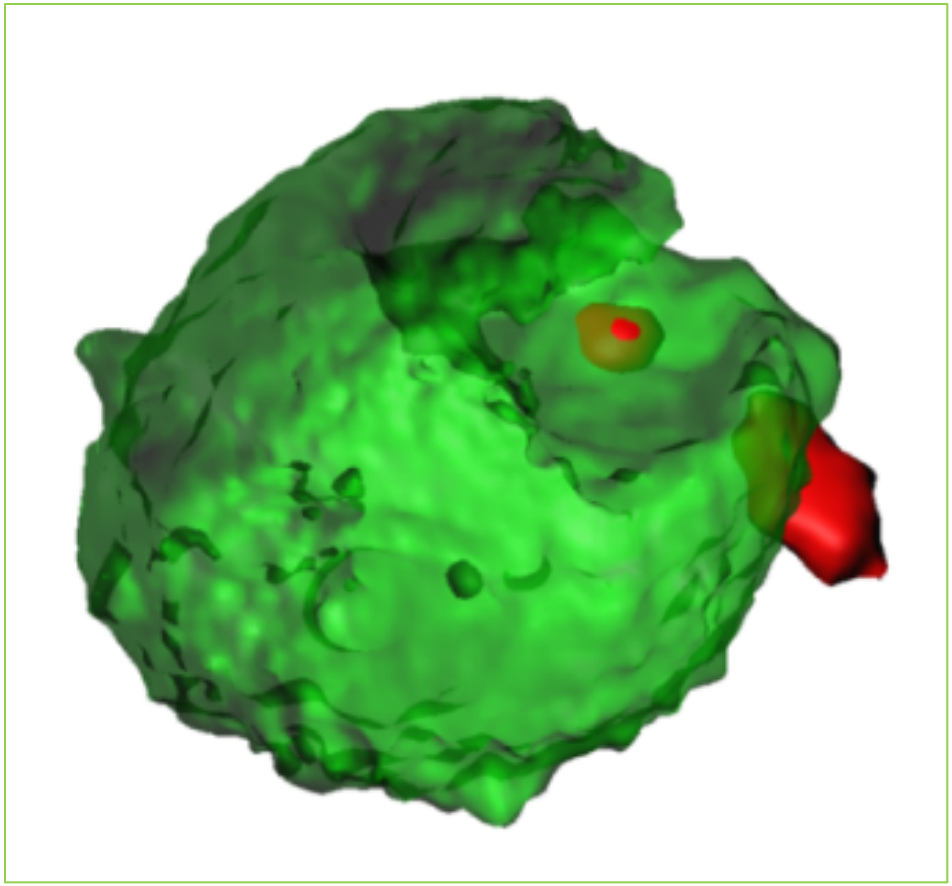


STRUCTURE OF THE IMMUNO-NANOPARTICLES (BNPs)

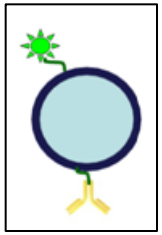
Particle name	Structure	Polymer concentration	Monoclonal antibody	Encapsulated agent
BNP0		1,66mg/ml	none	none
BNP1		1,66mg/ml	Rituximab (9,29µg/ml)	none
BNP2		1,66mg/ml	Rituximab (9,29µg/ml)	HCQ+CLB (5mg/ml each)
BNP3		1,66mg/ml	none	HCQ+CLB (5mg/ml each)

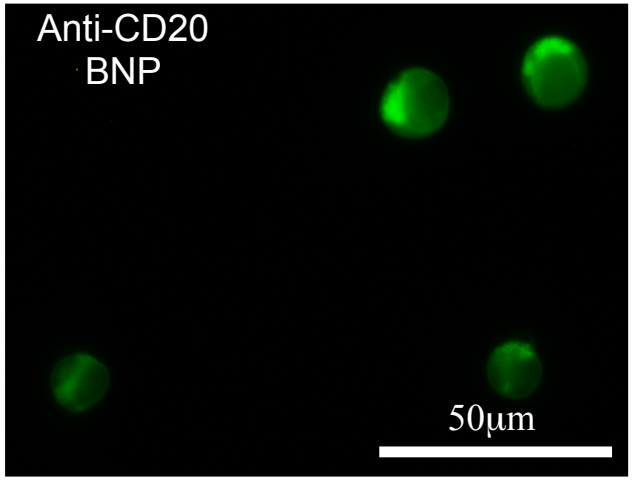
BINDING OF THE ANTI-CD20 BNPs TO BURKITT LYMPHOMA CELLS

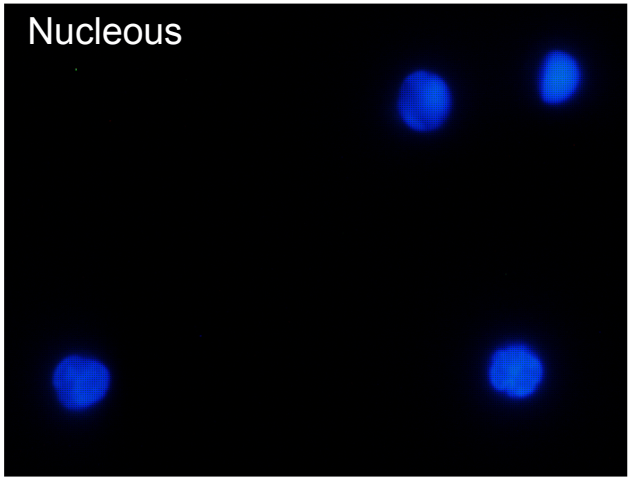


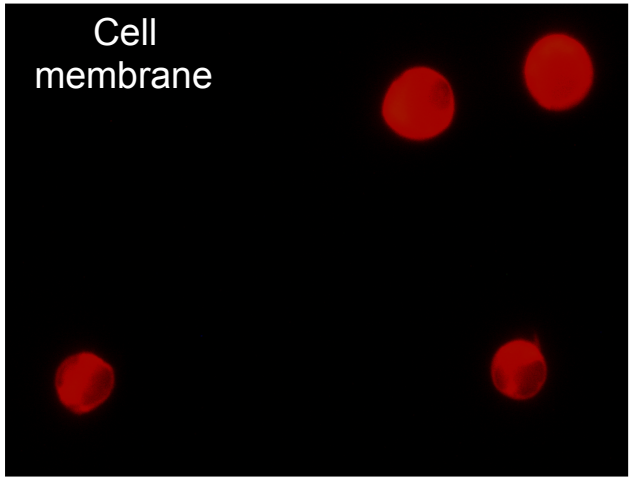


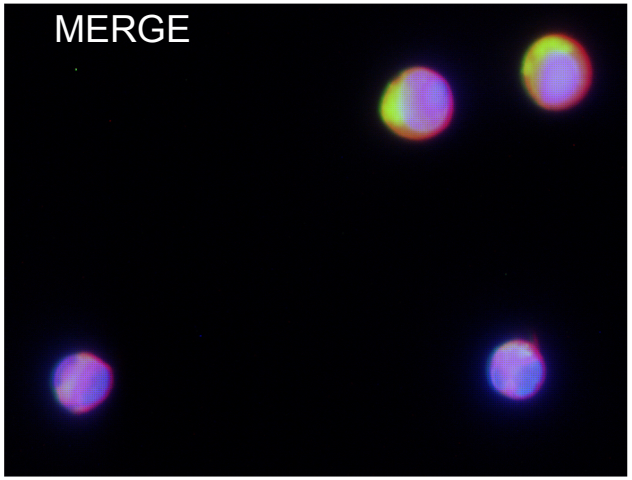
BINDING OF THE ANTI-CD20 BNPs TO CLL CELLS



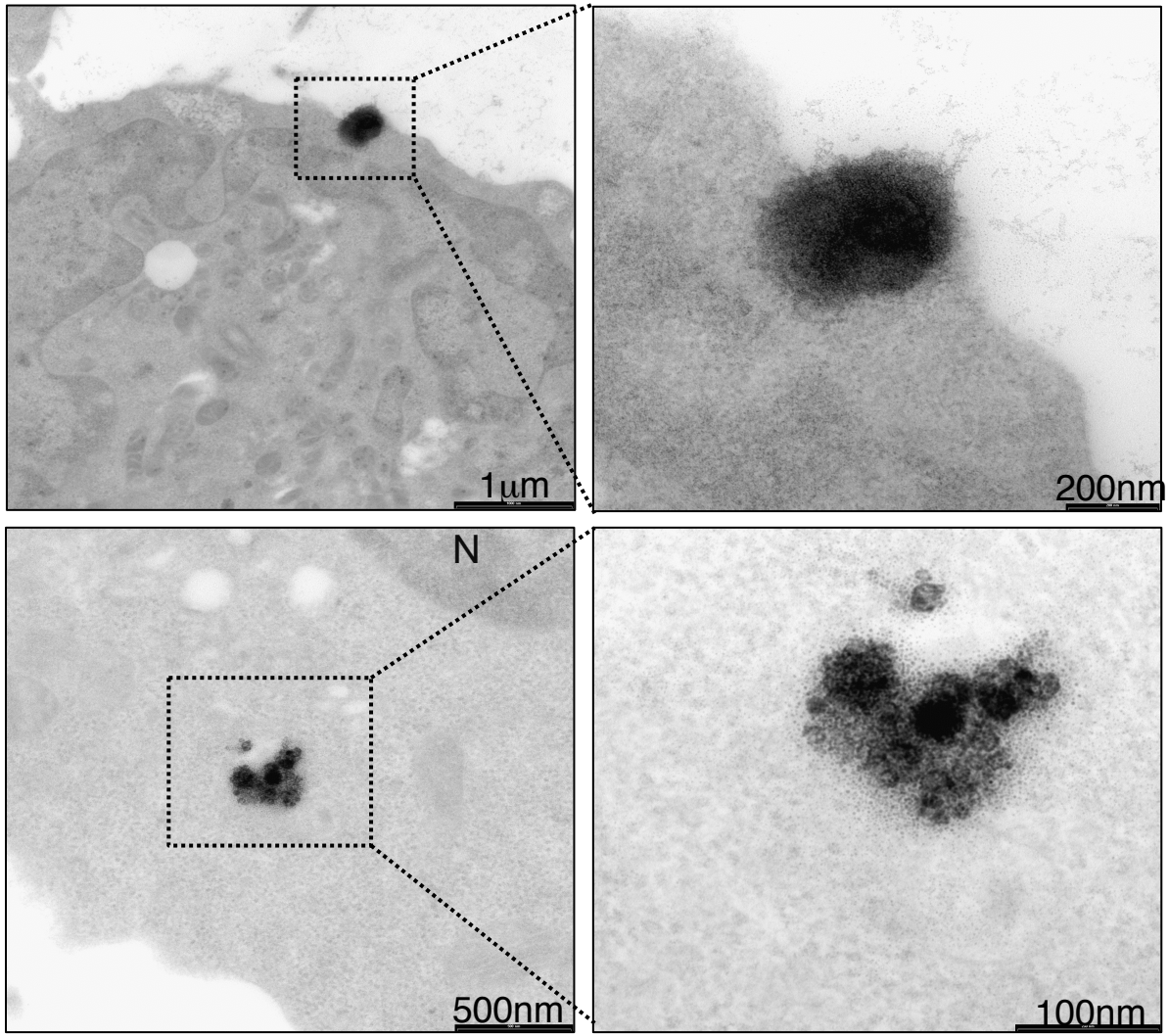








INTERNALIZATION OF THE ANTI-CD20 BNPs INTO LYMPHOMA CELLS



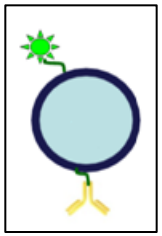
Mezzaroba et al, PlosOne, 2013

ANTI-CD20 BNPs vs RITUXIMAB

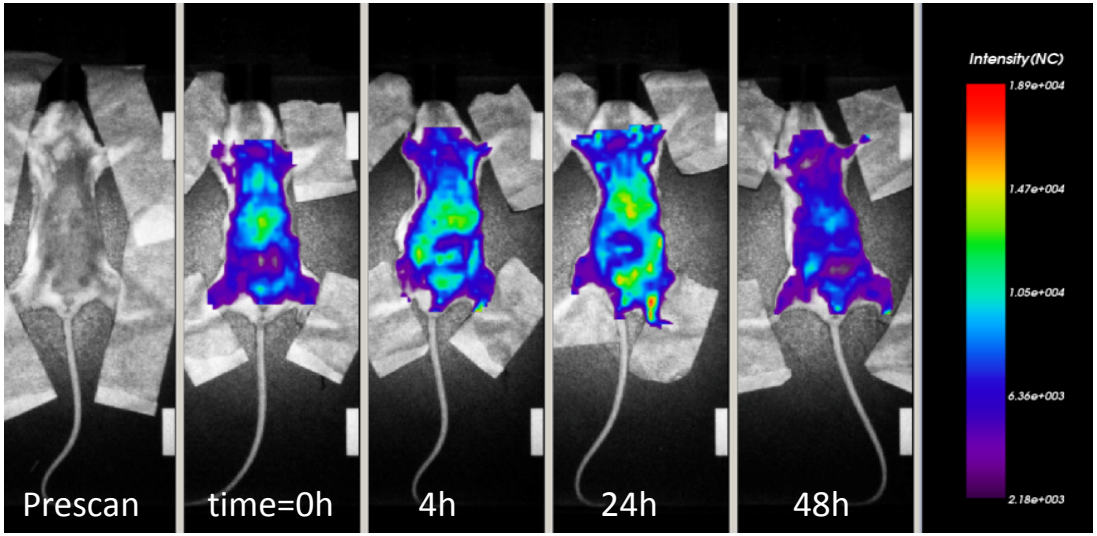
	CD20 (MFI)	BNP2 (% Killing)	RITUXIMAB (% Killing)
BJAB	316	92	18,9
BJAB-High	602	93,2	20,6
BJAB-low	103	83,1	0,6

ANTI-CD20 BNPs DISTRIBUTION IN HEALTHY MICE

In vivo Time-Domain Optical Imaging



- *In vivo* imaging of healthy mice -> lungs, intestine and liver uptake
- 48h after injection -> residual fluorescence in liver and intestine.



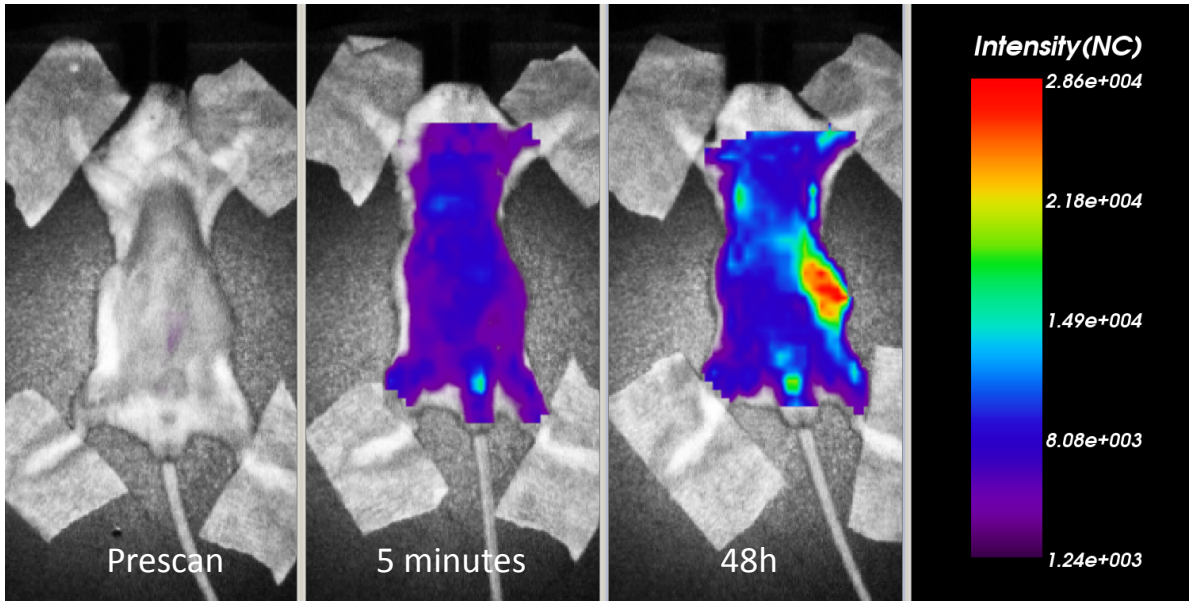
Toxicological study

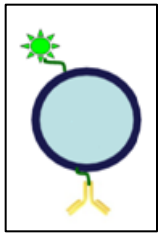
Compound	Dose(µL)	HCQ(µg) + CHL(µg)	Injection	Lethality (n°death/n°total)	Loss of body weight (%)
BNP1	80	/	8	0/5	+ 1
BNP2	10	50+50	4	0/5	+ 2
BNP2	20	100+100	4	0/5	+ 4
BNP2	40	200+200	4	0/5	+ 1
BNP2	80	400+400	4	0/5	+ 1
BNP2	80	400+400	8	0/5	0
BNP3	80	400+400	8	0/5	0
CLB+HCQ	80	400+400	4	0/5	-18
CLB+HCQ	80	400+400	8	5/5	ND

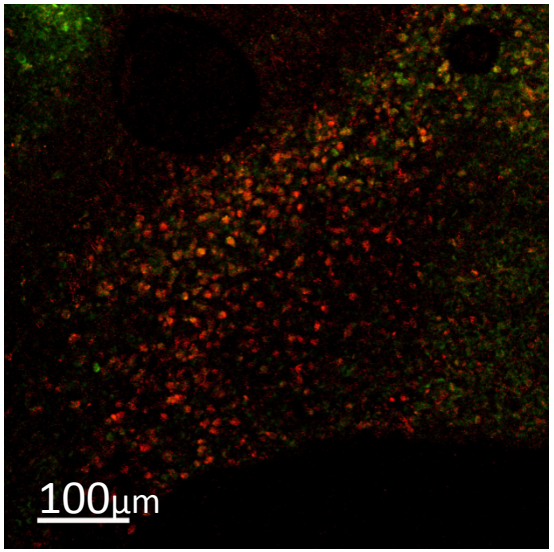
No tissue side effects were documented after the treatments

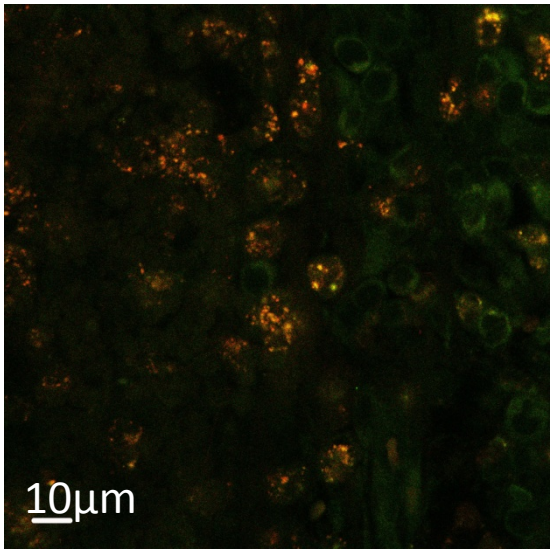
Particle name	Structure
BNP1	
BNP2	
BNP3	

ANTI CD20-BNPs DISTRIBUTION IN A LOCALIZED MODEL OF B-CELL MALIGNANCY

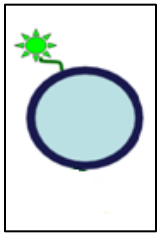


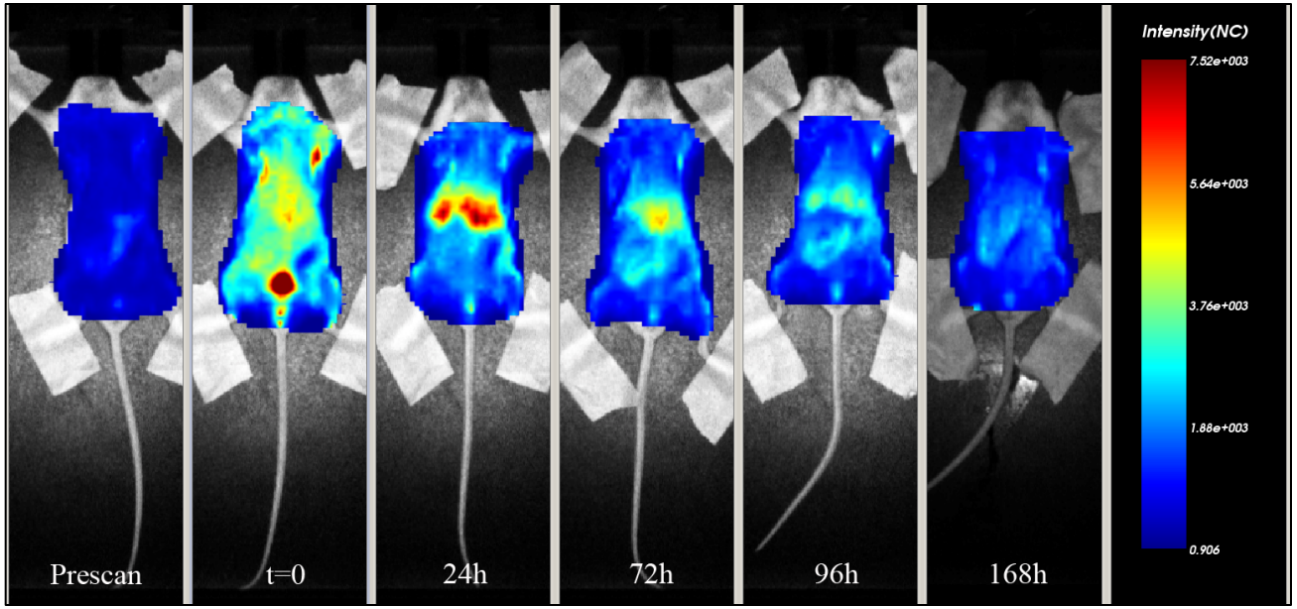


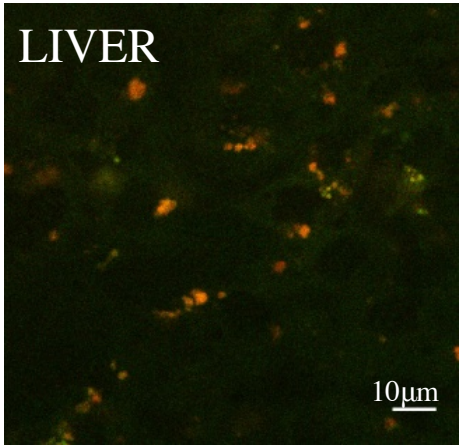


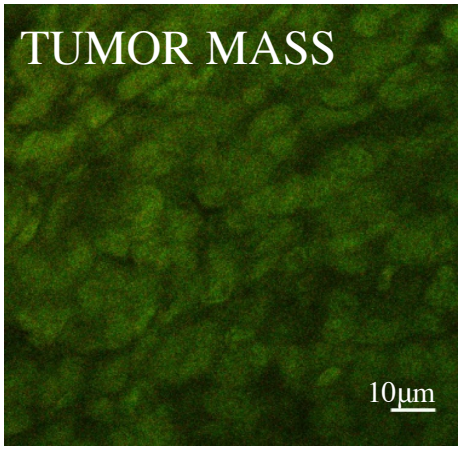


UNTARGETED-BNPs DISTRIBUTION IN A LOCALIZED MODEL OF B-CELL MALIGNANCY

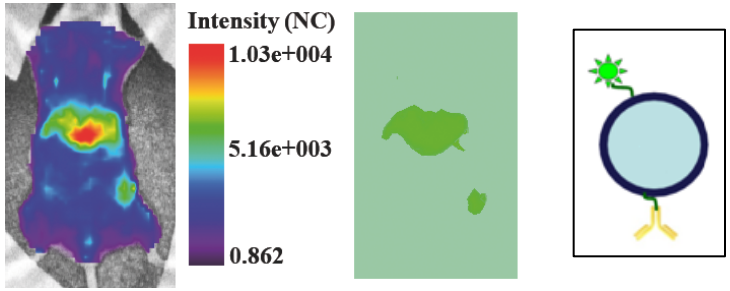




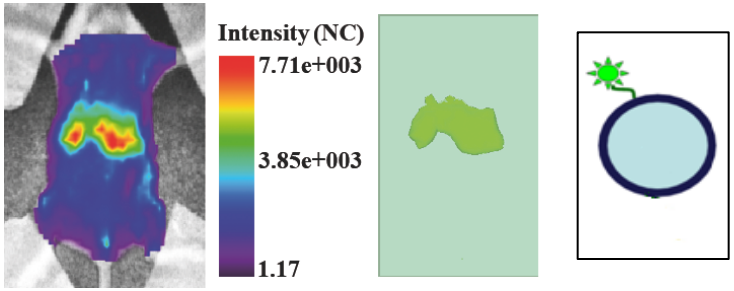




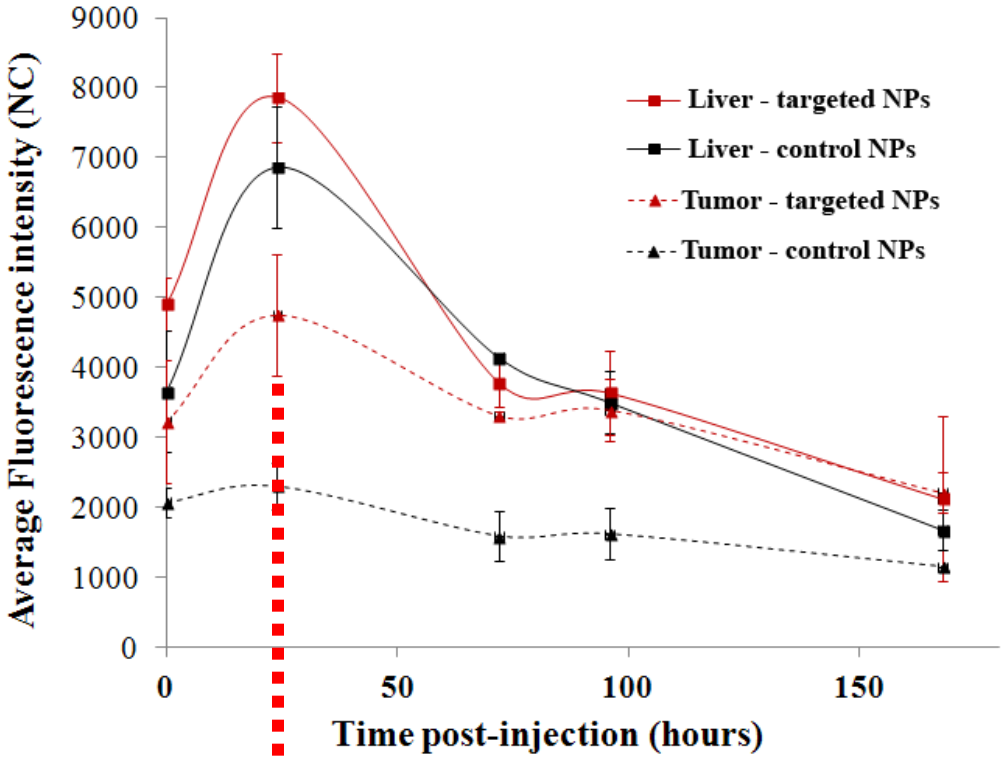
NANOPARTICLES DISTRIBUTION IN A LOCALIZED MODEL OF B-CELL MALIGNANCY

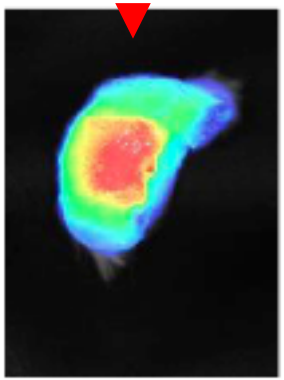


24 hours post injection of targeted NPs



24 hours post injection of control NPs





24 hours post injection of anti-CD20 NPs

DEVELOPMENT OF A BURKITT LYPHOMA MODEL

DAYS

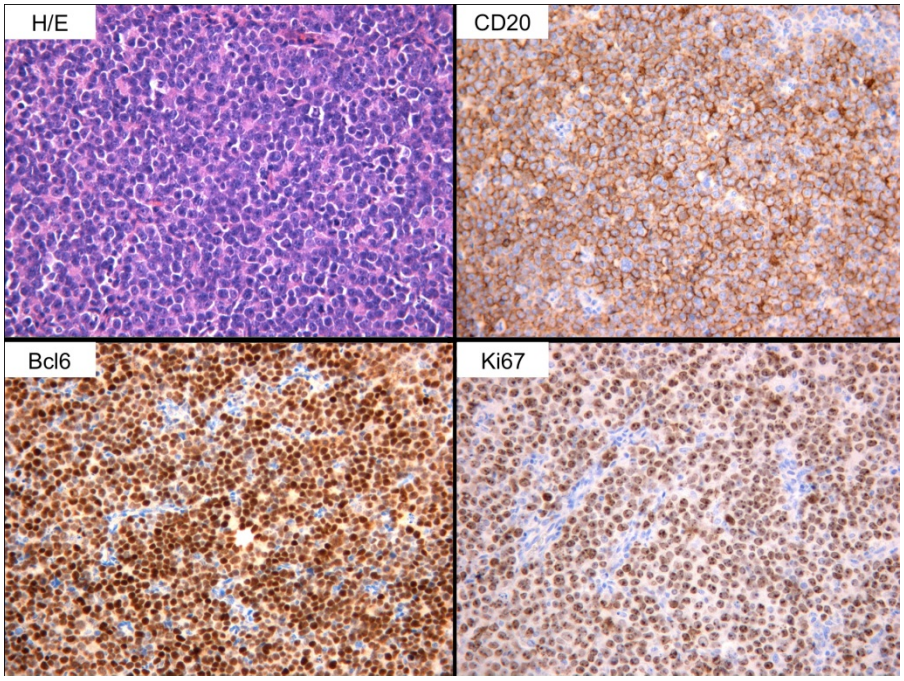
0

BJAB IP

(2x10^6 cells)

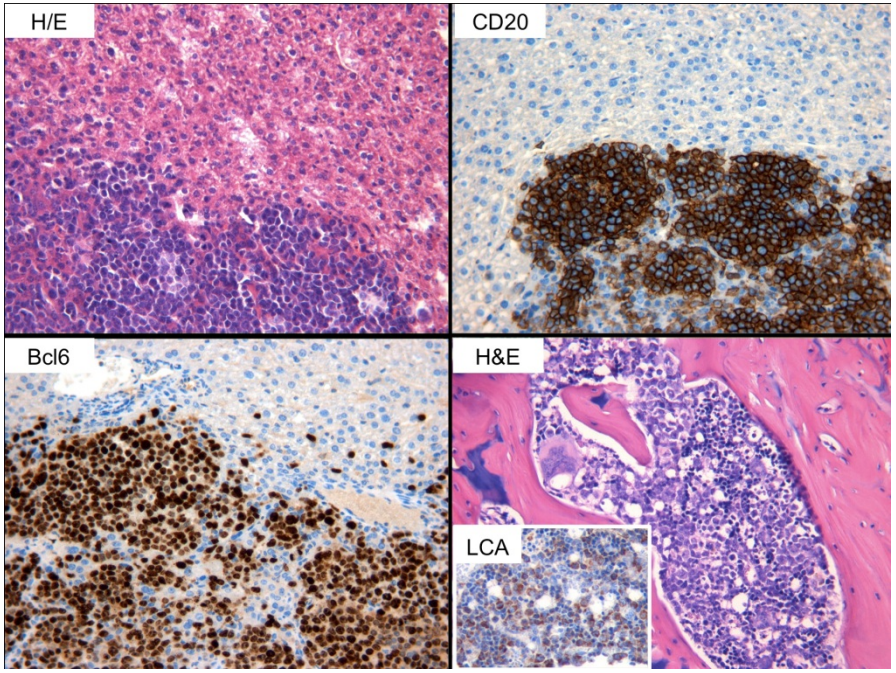
- Survival
- Histological analysis

Peritoneal tumor mass



Peritoneal tumor mass

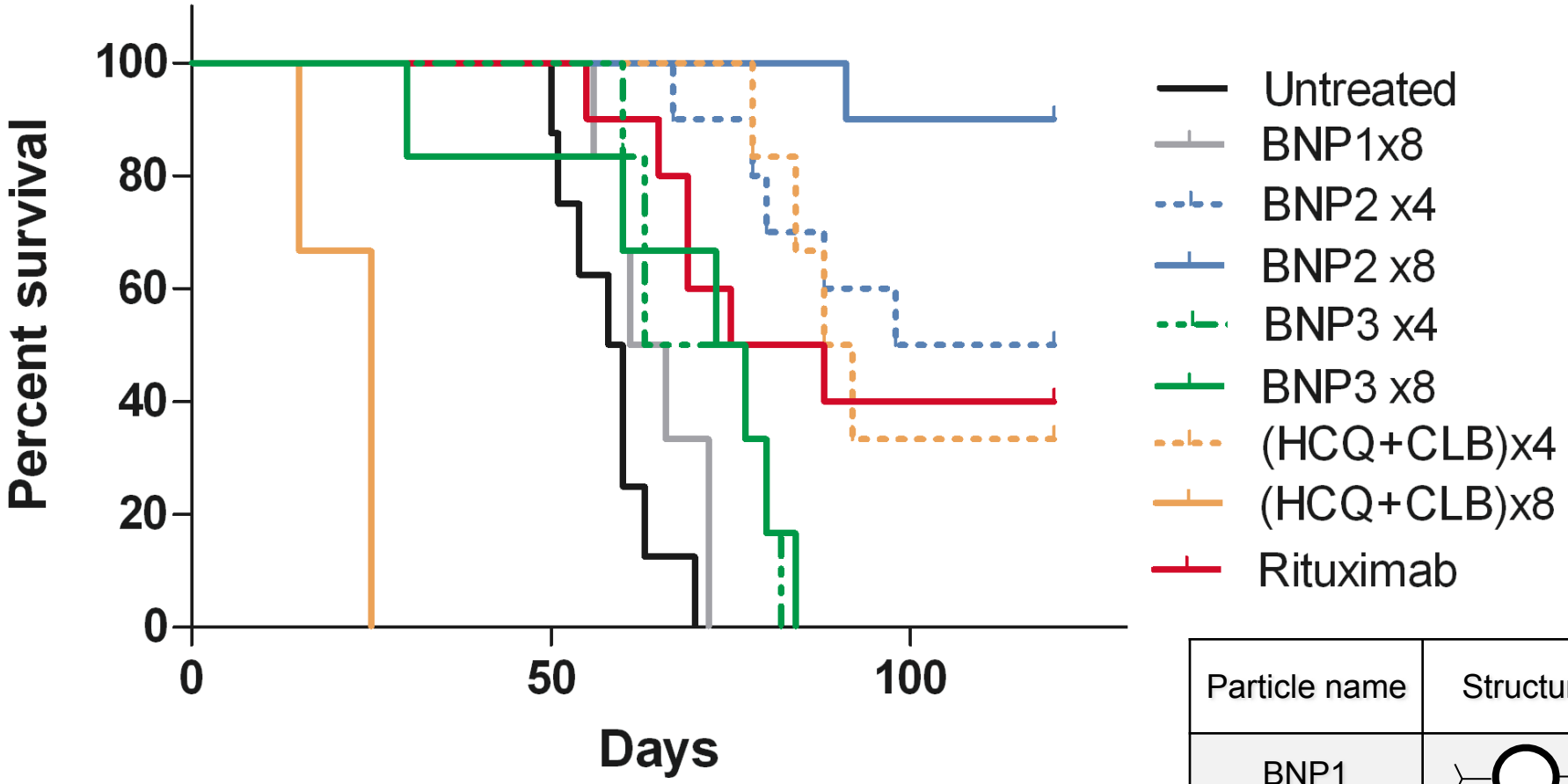
Liver



Liver

Bone marrow

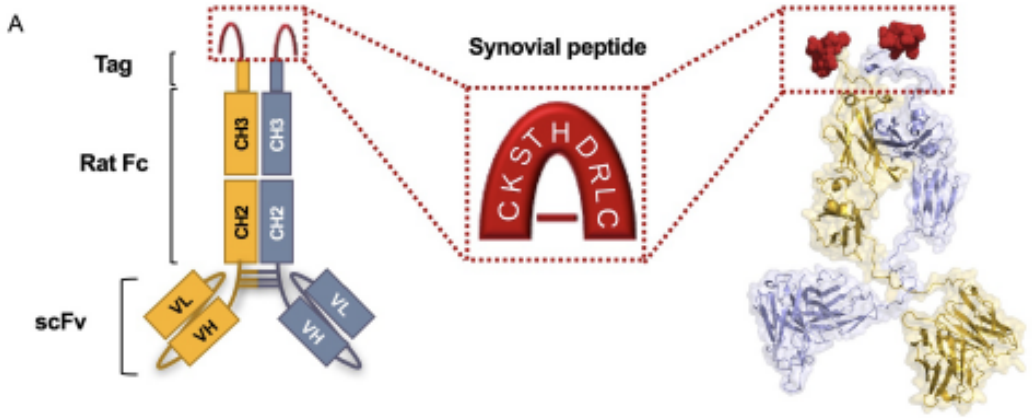
EFFECT OF ANTI CD20-BNPs IN THE TREATMENT OF A BURKITT LYPHOMA MODEL

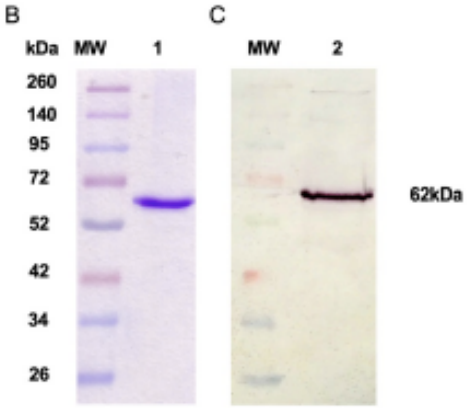


Particle name	Structure
BNP1	
BNP2	
BNP3	

Targeted polymeric nanoparticles for the diagnosis and the treatment of rheumatoid arthritis

Fig. 1


A
Tag
Rat Fc
scFv
CH3
CH3
CH2
CH2
VL
VH
VL
VH
Synovial peptide
C K S T H D R L C


B
C
kDa
MW
1
MW
2
260
140
95
72
52
42
34
26
62kDa

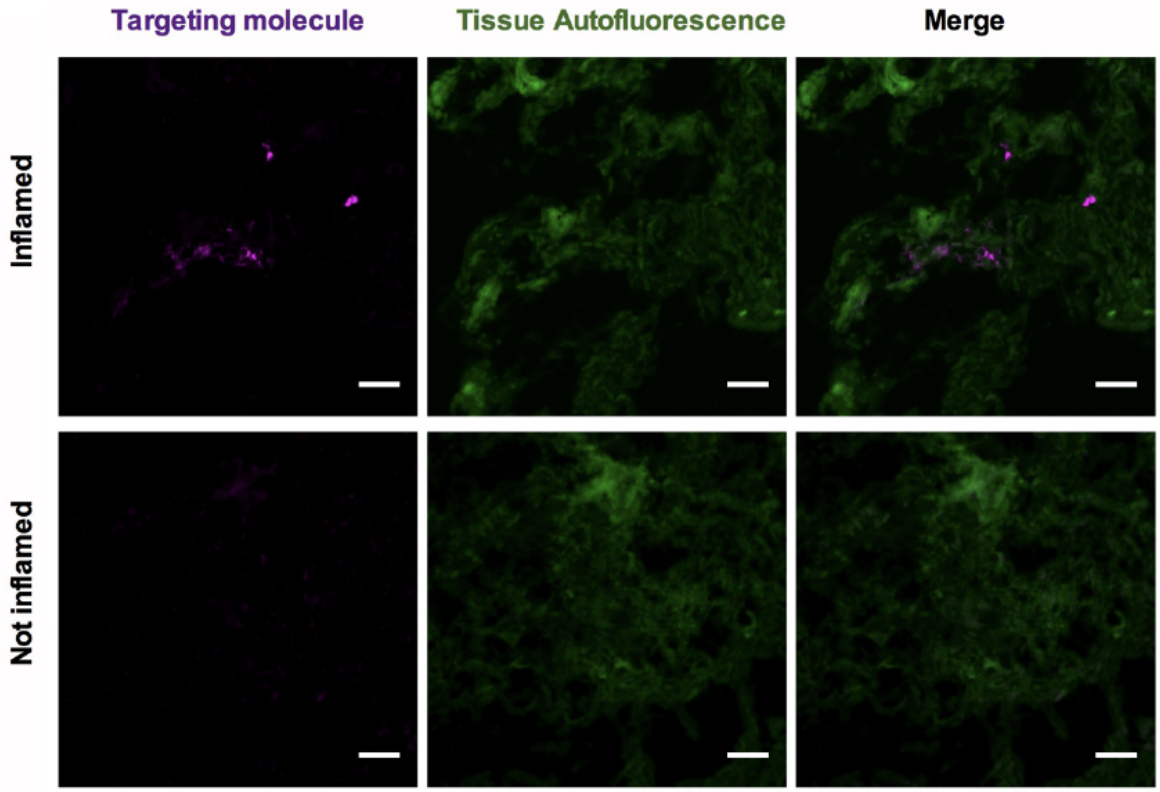
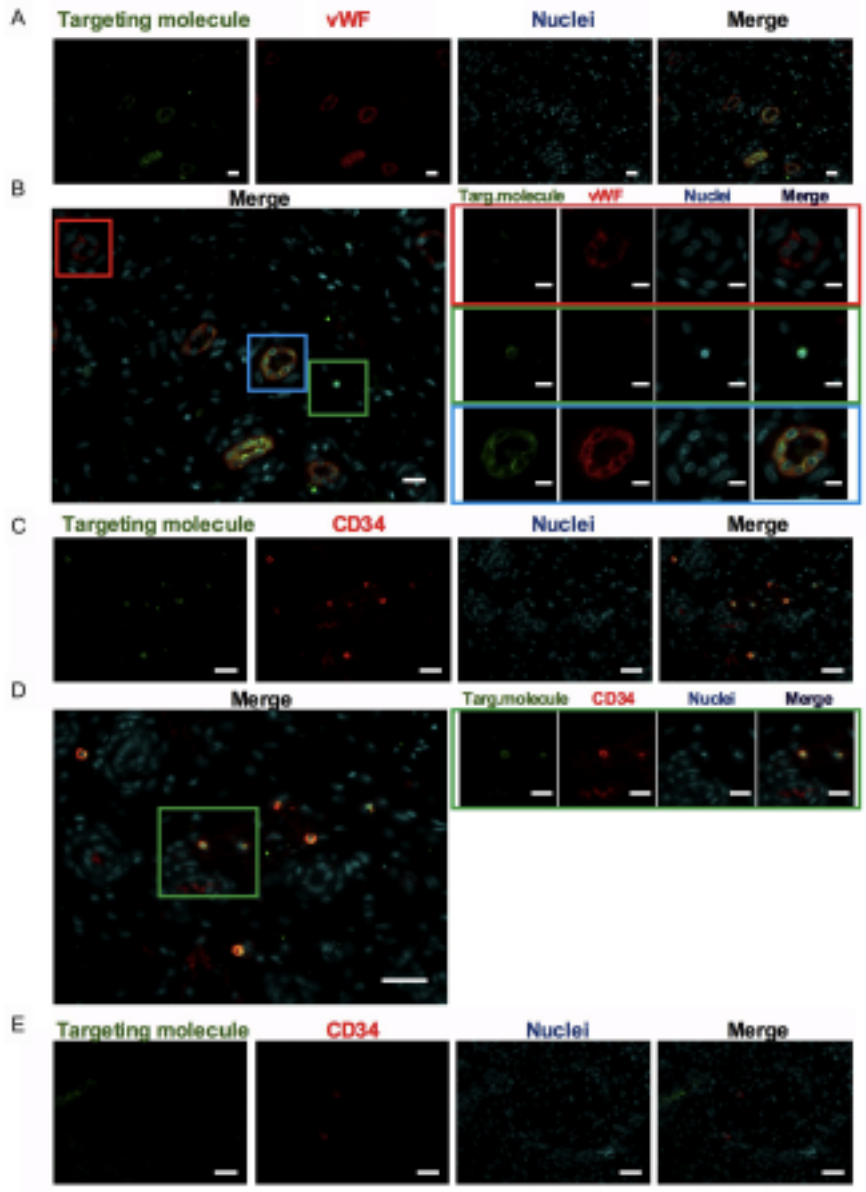
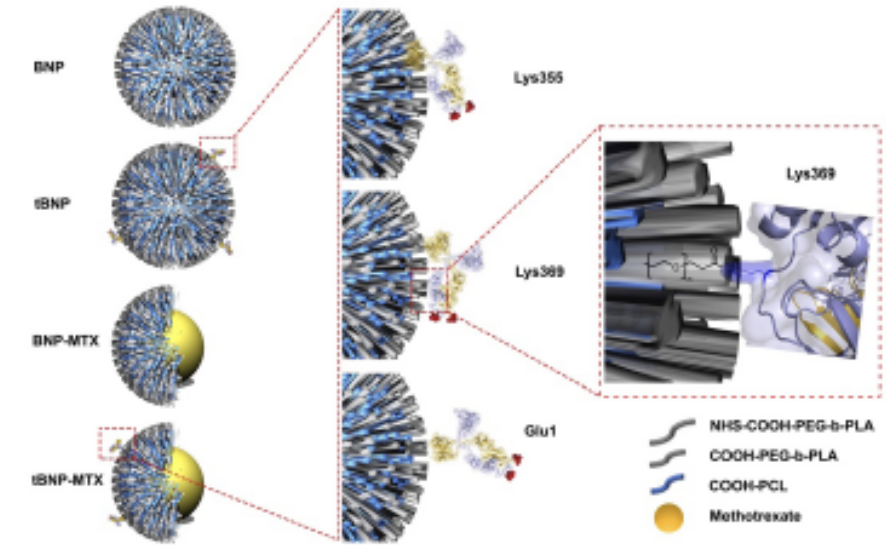

Targeting molecule
Tissue Autofluorescence
Merge
Inflamed
Not inflamed

Fig. 2


A
Targeting molecule
vWF
Nuclei
Merge
B
Merge
Targ.molecule
vWF
Nuclei
Merge
C
Targeting molecule
CD34
Nuclei
Merge
D
Merge
Targ.molecule
CD34
Nuclei
Merge
E
Targeting molecule
CD34
Nuclei
Merge

Targeted polymeric nanoparticles used in the study

A



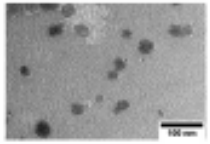
B

Sample	Polymers (mg/mL)	MTX (mg/mL)	Synovial peptide (μg/mL)	Mean diameter (nm)	Particles/mL	PDI
BNPs	1,66	-	-	168	2.5×10^{8}	0.26
tBNPs	1,66	-	12	164	1.7×10^{8}	0.24
BNPs-MTX	1,66	2	-	176	3.6×10^{8}	0.21
tBNPs-MTX	1,66	2	12	160	4.0×10^{8}	0.21

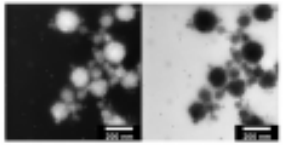
C

Sample	$d_n \pm SD$ (nm)	PDI	$\zeta \pm SD$ (mV)
BNPs	177 ± 2	0.31 ± 0.02	-9.4 ± 2.
tBNPs	176 ± 5	0.33 ± 0.04	-8.0 ± 1.
BNPs-MTX	122 ± 4	0.45 ± 0.07	-9.6 ± 3.
tBNPs-MTX	139 ± 6	0.36 ± 0.06	-7.1 ± 1.

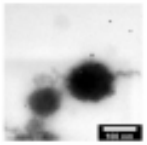
D



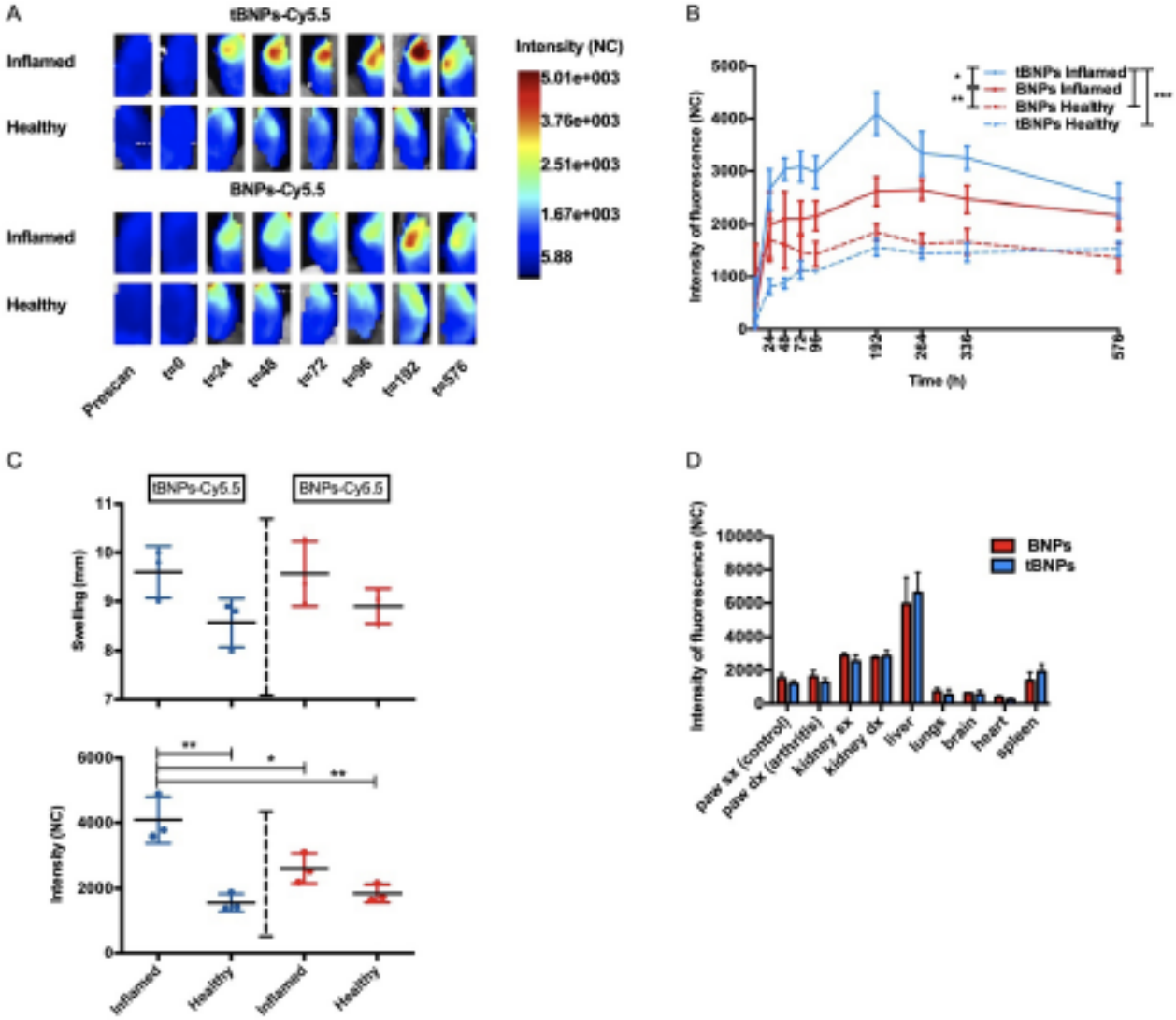
E



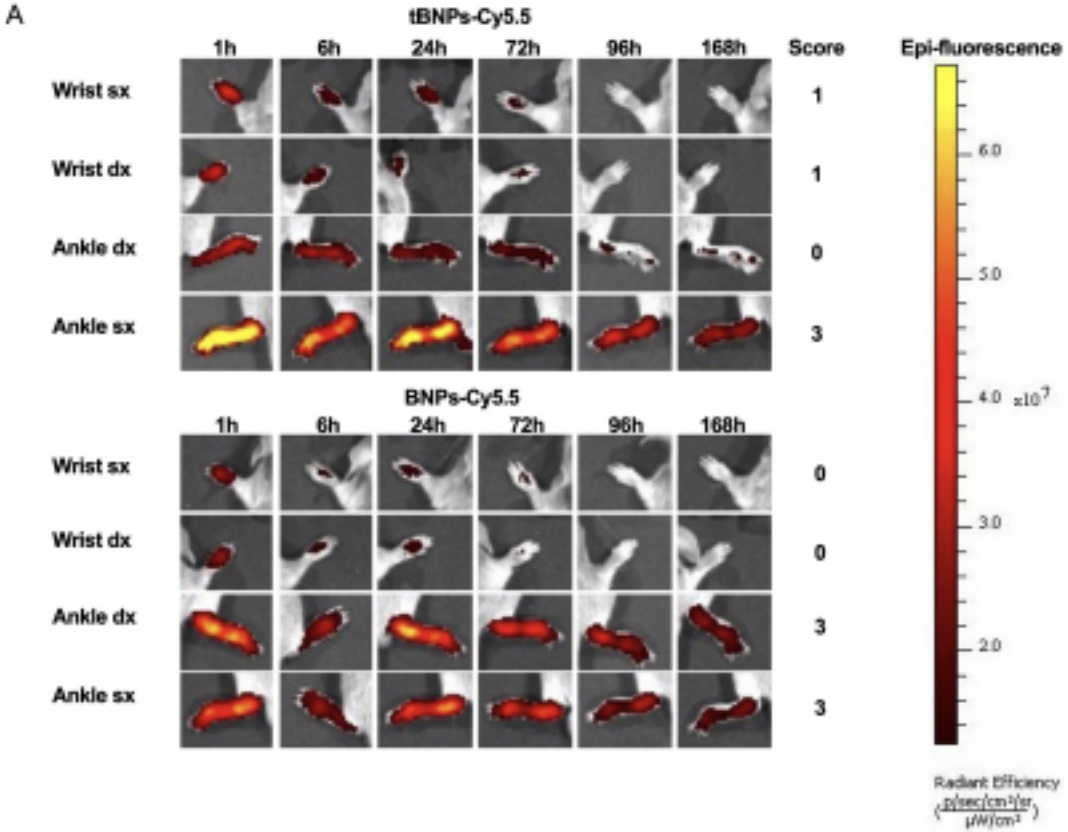
F



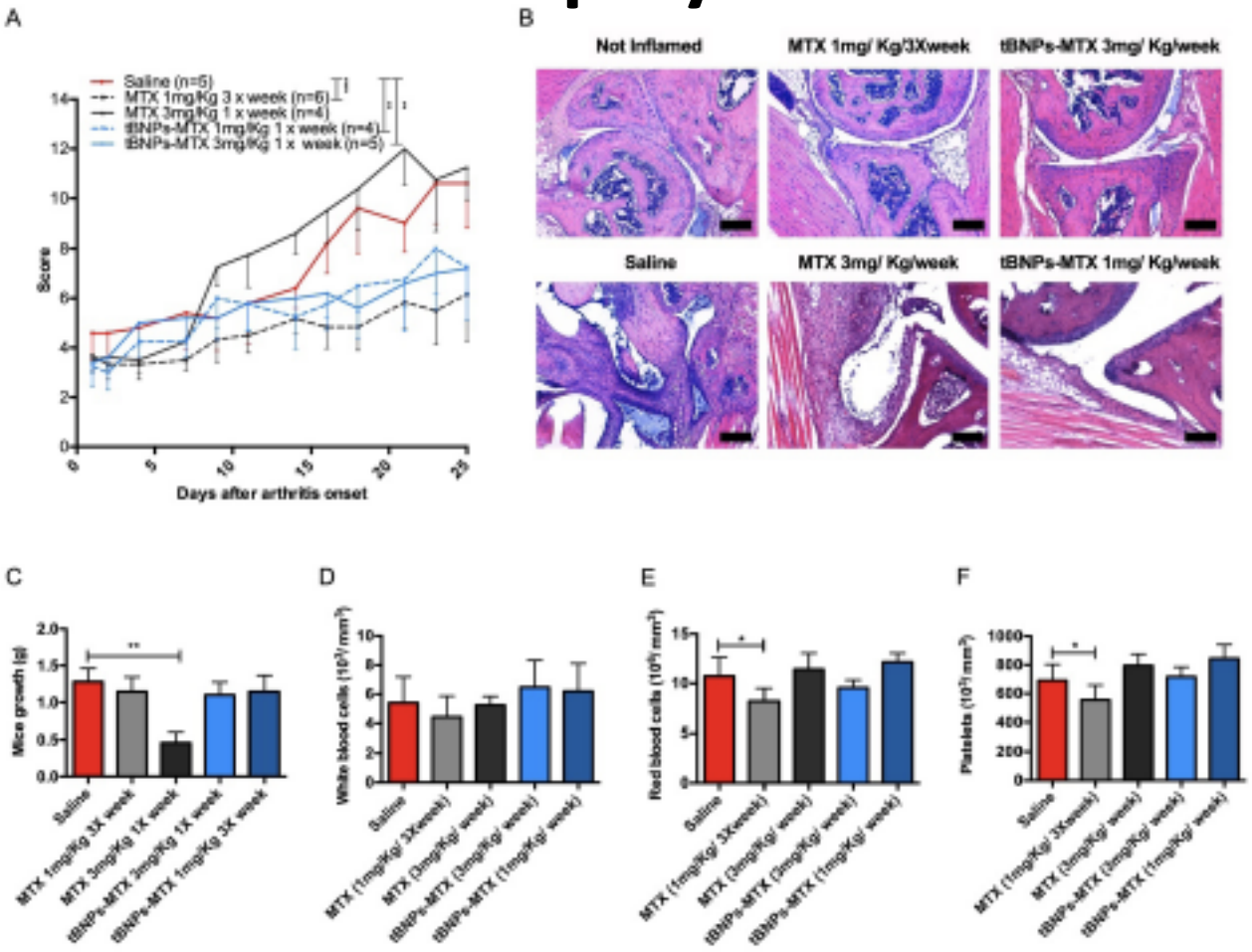
Targeted nanoparticles distribution in a model of mono-arthritis



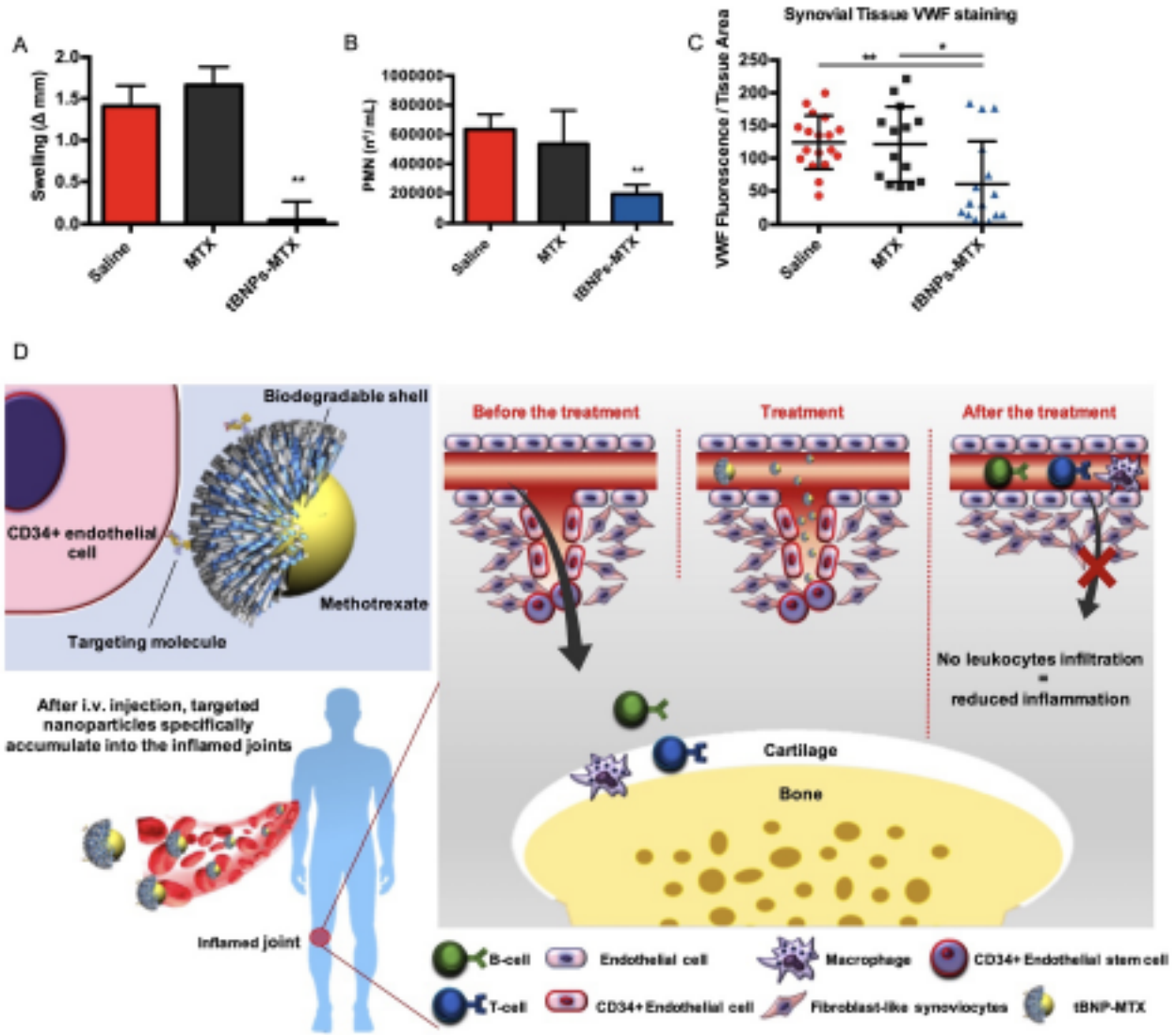
Targeted nanoparticles distribution in a model of poly-arthritis



Therapeutic effect of targeted nanoparticles in a model of poly-arthritis



Proposed mechanism of action



RESULTS