Theranostic magnetic siRNA nanocarriers as a novel approach in breast cancer diagnosis and treatment

Appel à projets PAIR Sein 2014 - ARC_INCa_LNCC_7636

Project feedback

Stephanie DAVID

Journée Recherche Cancer du sein

24 Janvier 2020

EA 6295
Nanomédicaments et Nanosondes (NMNS)

INSTITUT NATIONAL DU CANCER

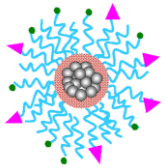
LA LIGUE CONTRE LE CANCER

FONDATION ARC POUR LA RECHERCHE SUR LE CANCER
Reconnue d'utilité publique

université de TOURS
Faculté de pharmacie Philippe-Maupas

Project overview

Coordinator: Dr. Stephanie DAVID
Duration: 36 months (2014 – 2017)
Funding: 382 k€

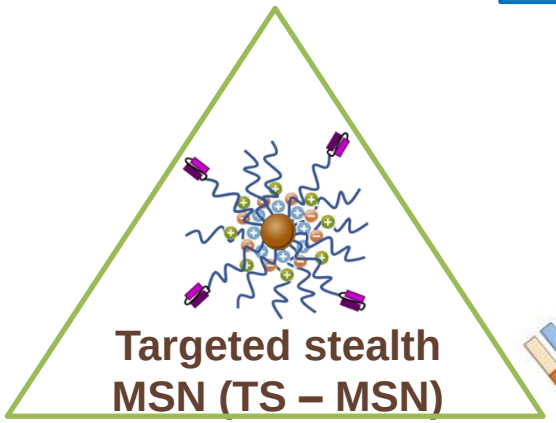


Theranostic hybrid magnetic nanovectors

EA 6295
Nanomédicaments et Nanosondes
Université de Tours (I. Chourpa)

Team 1: NMNS - Tours
Scientific coordinator S. David

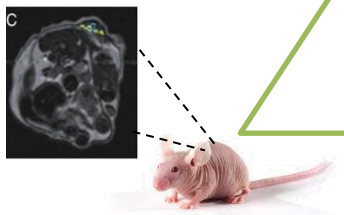
6 PR + 1 PF (2 years)



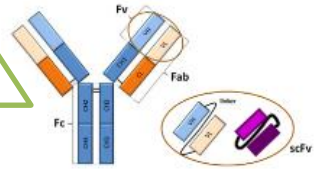
UPR 4301 **IRM, Signaux, images et expression des gènes,**
Centre de Biophysique Moléculaire, CNRS, Orléans (E. Jakob-Toth)

Team 3: CBM - Orléans
Scientific coordinator S. Même

2 PR



MRI on small animals



Antibody fragments

UMR Université INRA ISP 1282
Immunologie Parasitaire, Vaccinologie et Biothérapie Antiinfectieuse,
Université de Tours (I. Dimier-Poisson)

Team 2: IPVBAI - Tours
Scientific coordinator N. Aubrey

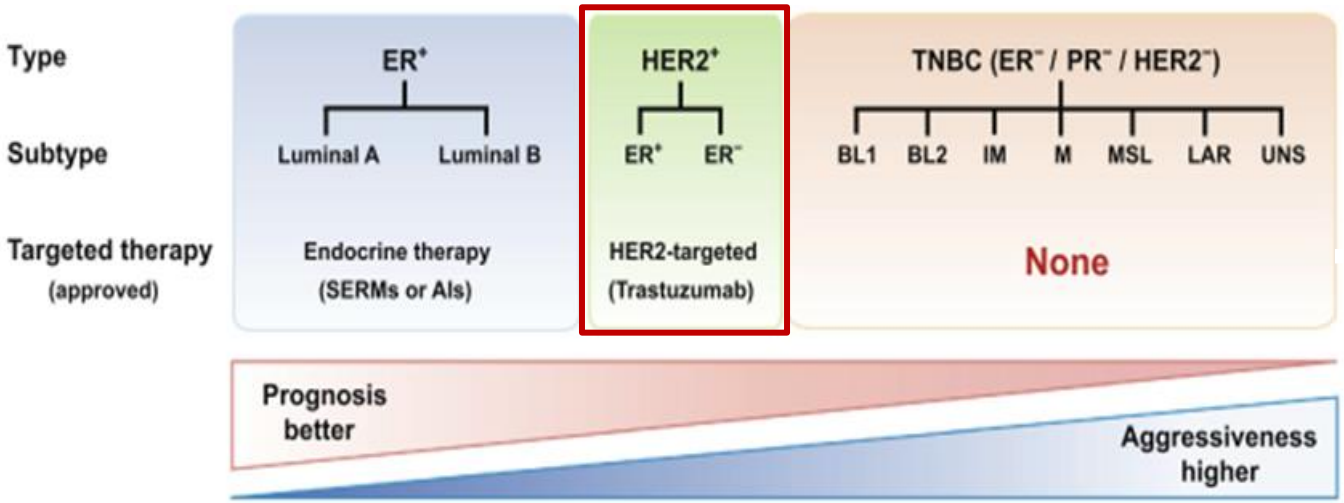
3 PR + 1 PF (1 year)

Theranostic magnetic siRNA nanocarriers (TS-MSN) as a novel approach in breast cancer diagnosis and treatment

PR: permanent researcher, **PF**: post-doctoral fellowship funded through the project

Breast Cancer

- first cause of mortality per cancer in women population



ER: estrogen receptor
PR: progesterone receptor
HER2: human epidermal growth factor receptor 2
TNBC: triple-negative breast cancer
SERM: selective estrogen receptor modulator
AI: aromatase inhibitor
BL: basal-like
IM: immunomodulatory
M: mesenchymal
MSL: mesenchymal stem-like
LAR: luminal androgen receptor
UNS: unstable

HER2+ Breast Cancer

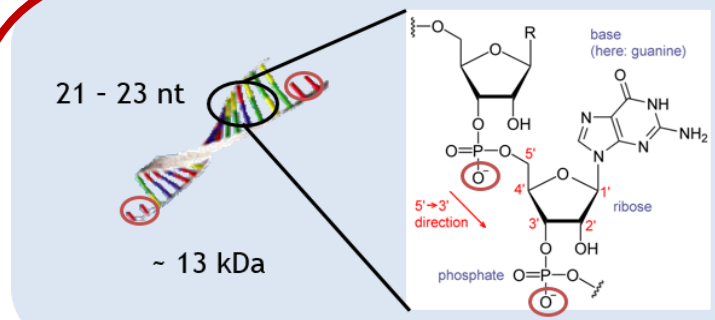
Resistance to classical chemotherapy treatments

Monoclonal antibody Herceptin® (trastuzumab)

- Actually used with adjuvant chemotherapy (after surgery)

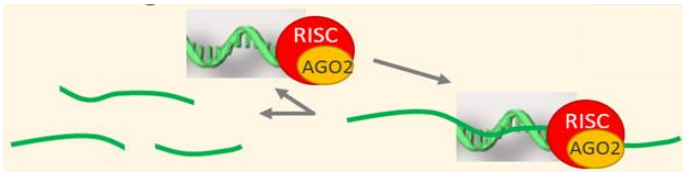
Ma et al., 2018

Gene silencing using small interfering RNA as therapeutic approach for (breast) cancer



Small interfering RNA (siRNA)

RNA interference mechanism



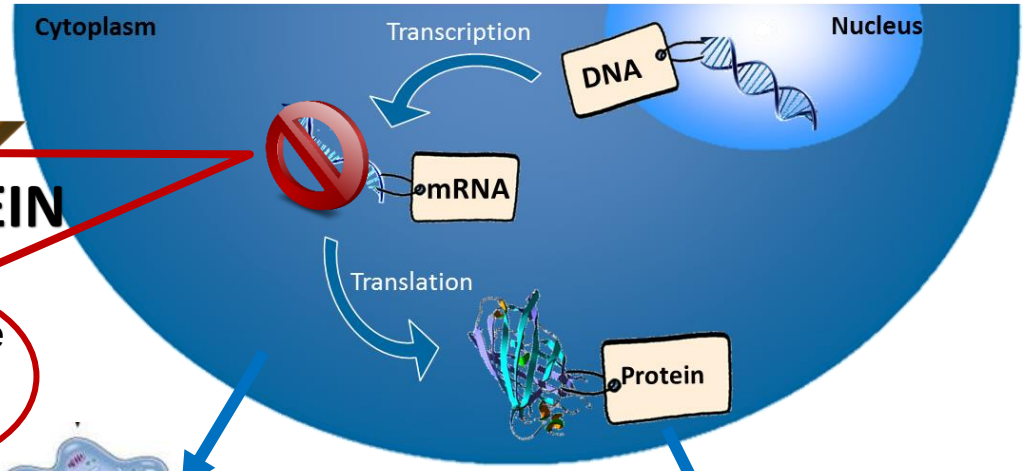
mRNA DEGRADATION

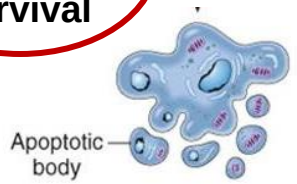
Nobel prize A. Fire and C. Mello, 2006

post-transcriptional gene silencing

PROTEIN

Responsible for the **cell survival**

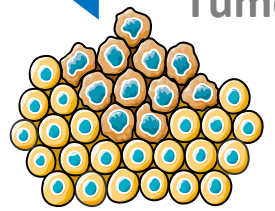




Apoptosis

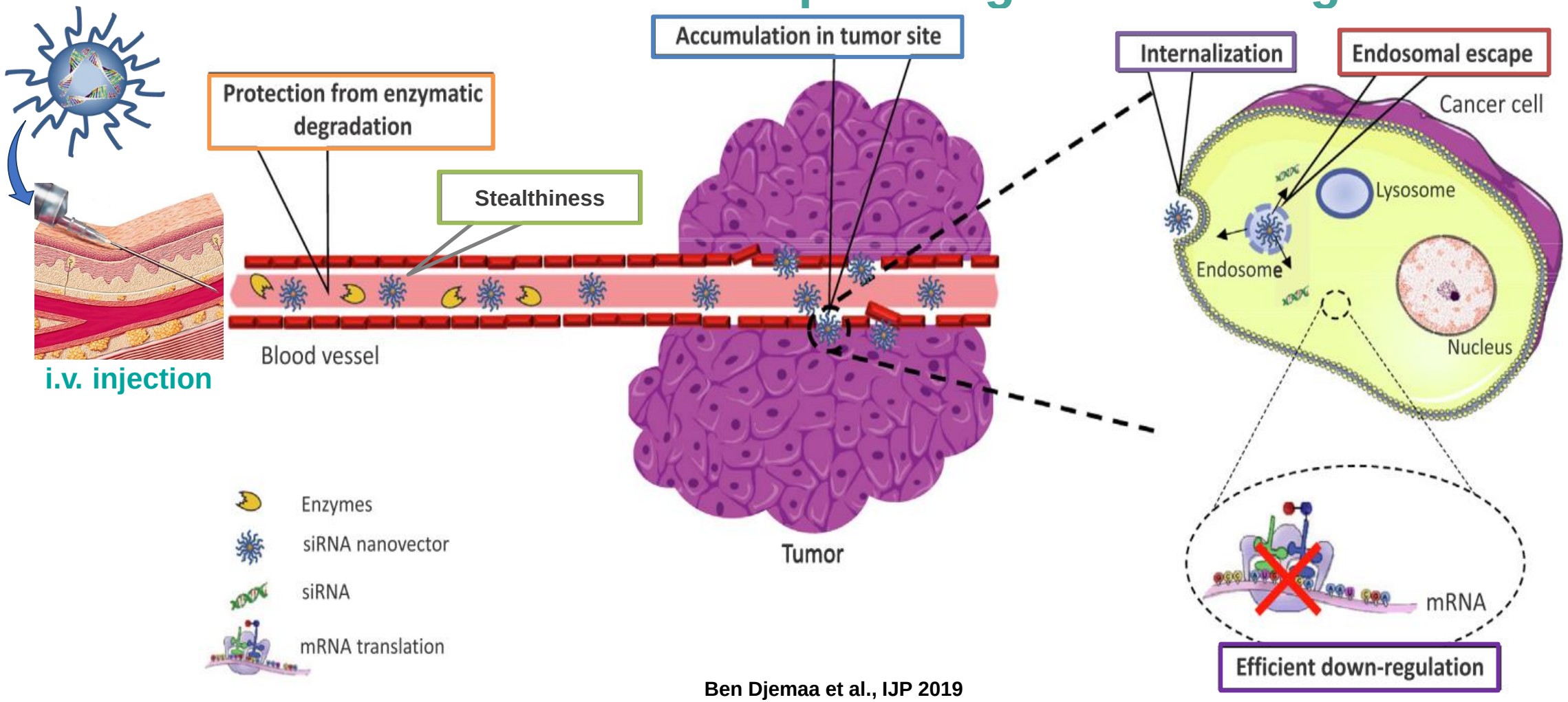
→ **gene silencing**
→ **cell death**

Tumor cells



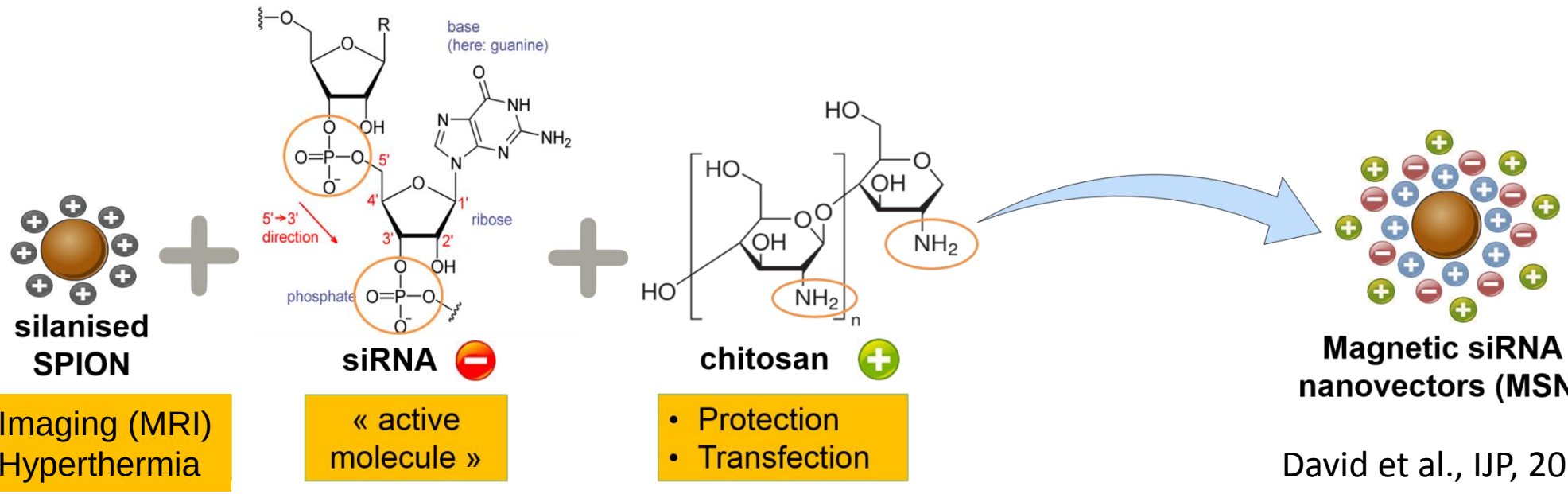
→ **modified** gene expression
→ **uncontrolled** cell proliferation

Nanovectors for improved gene silencing



Ben Djemaa et al., IJP 2019

Magnetic siRNA nanovectors (MSN)



- Imaging (MRI)
- Hyperthermia

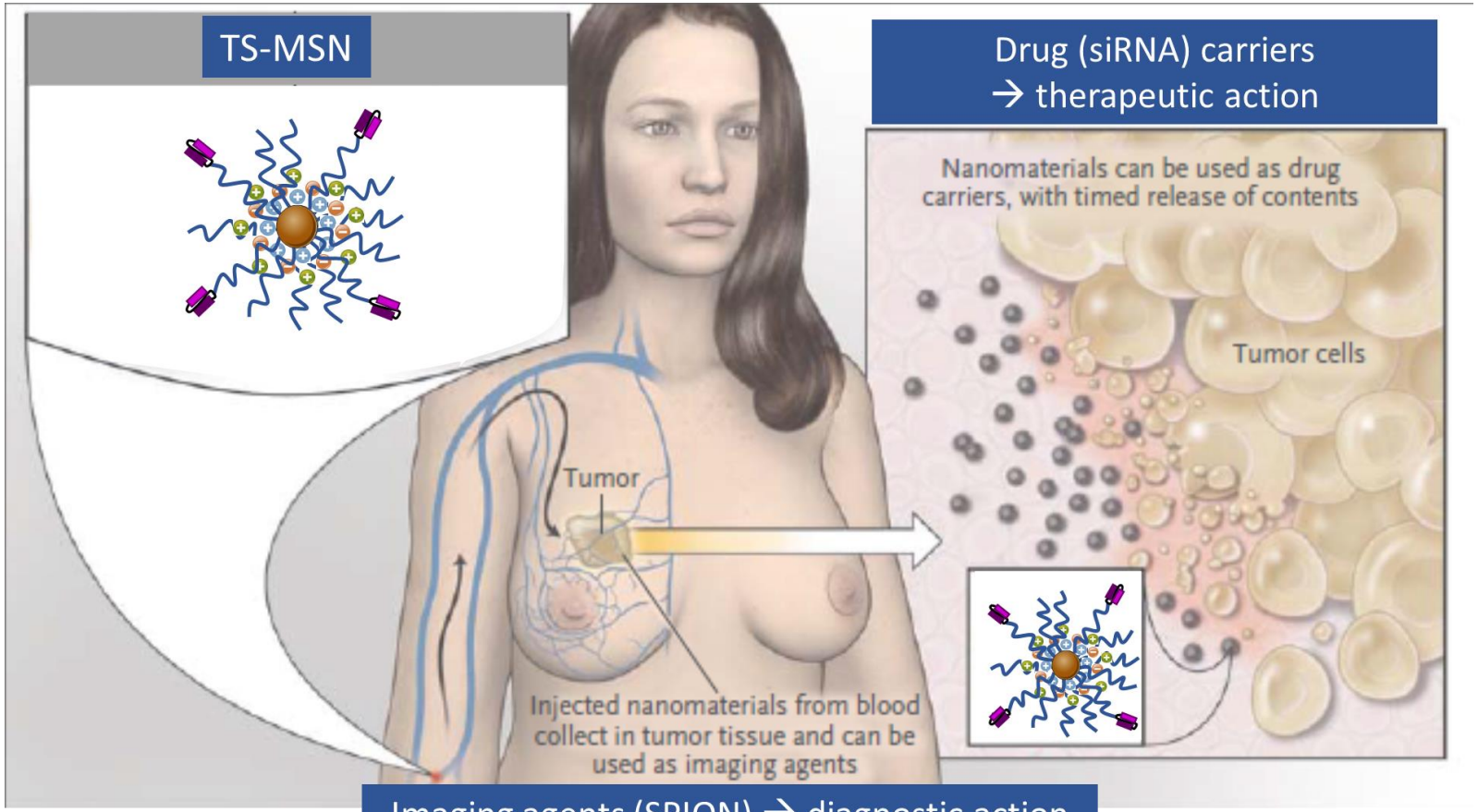
« active molecule »

- Protection
- Transfection

David et al., IJP, 2013

SPION: superparamagnetic iron oxide nanoparticle

Diagnosis and treatment of HER2+ breast cancer



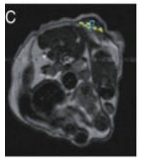
Team 1: NMNS - Tours
Scientific coordinator S. David

Team 2: IPVBAI - Tours
Scientific coordinator N. Aubrey

→ **HER2 targeting**

single chain variable Fragment (**scFv**) of Herceptin® (trastuzumab)

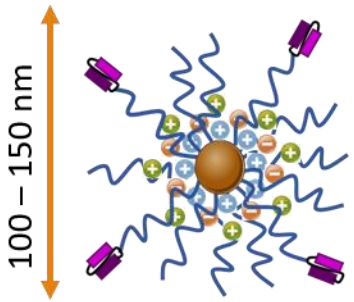
Team 3: CBM - Orléans
Scientific coordinator S. Même



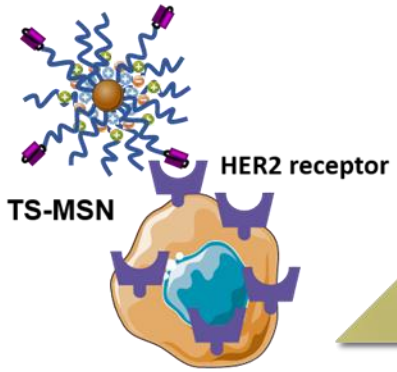
MRI on small animals

TS-MSN = targeted stealth magnetic siRNA nanovector, **SPION** = Superparamagnetic iron oxide nanoparticles, **siRNA** = small interfering RNA

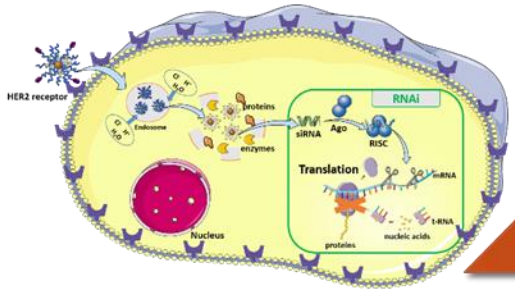
Project objectives



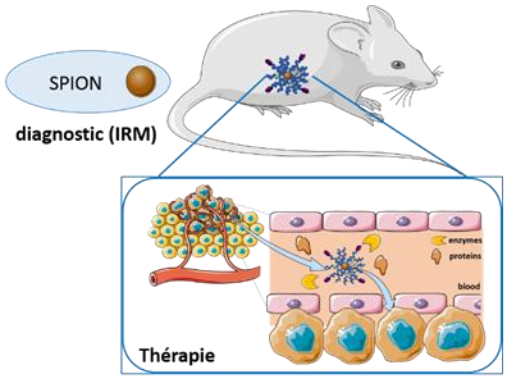
Formulation of TS-MSN and its optimization



Specific targeting of HER2+ breast cancer cells

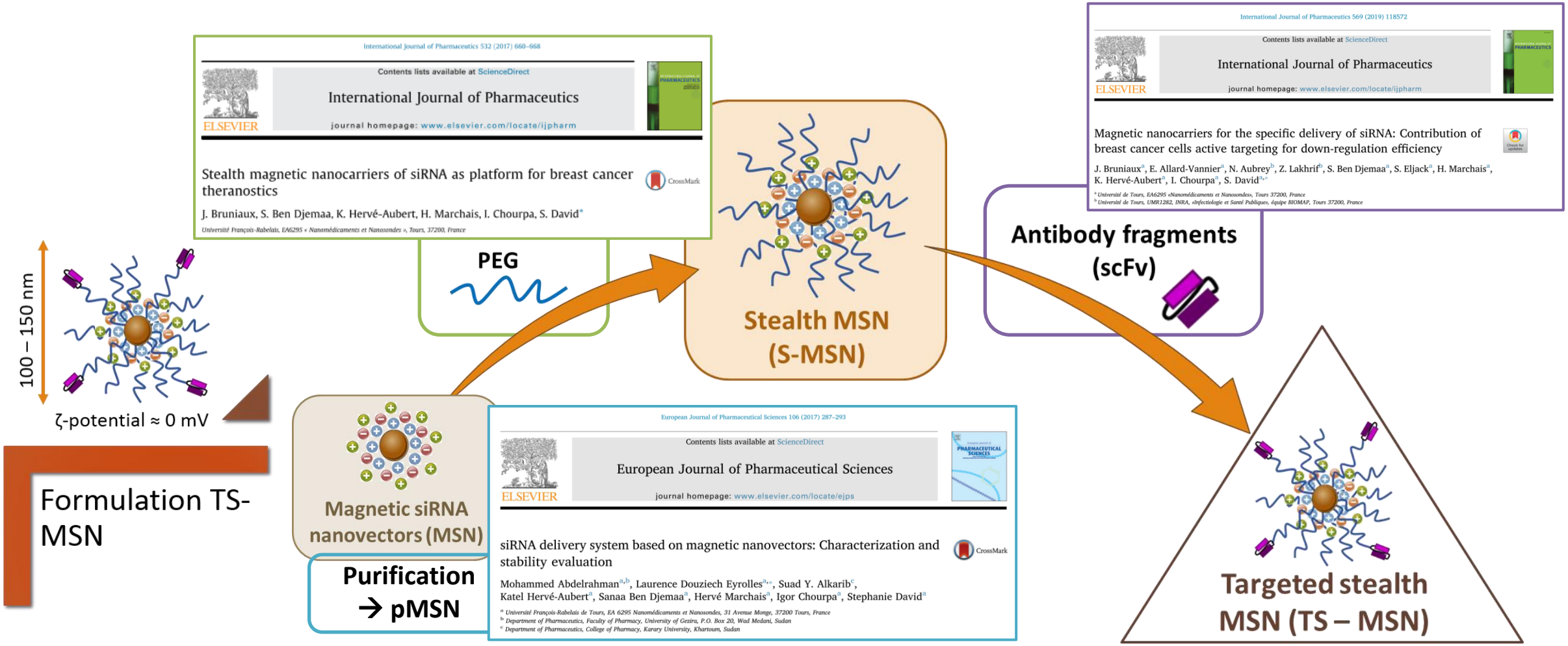


Therapeutic effect due to the action of siRNA on the protein synthesis

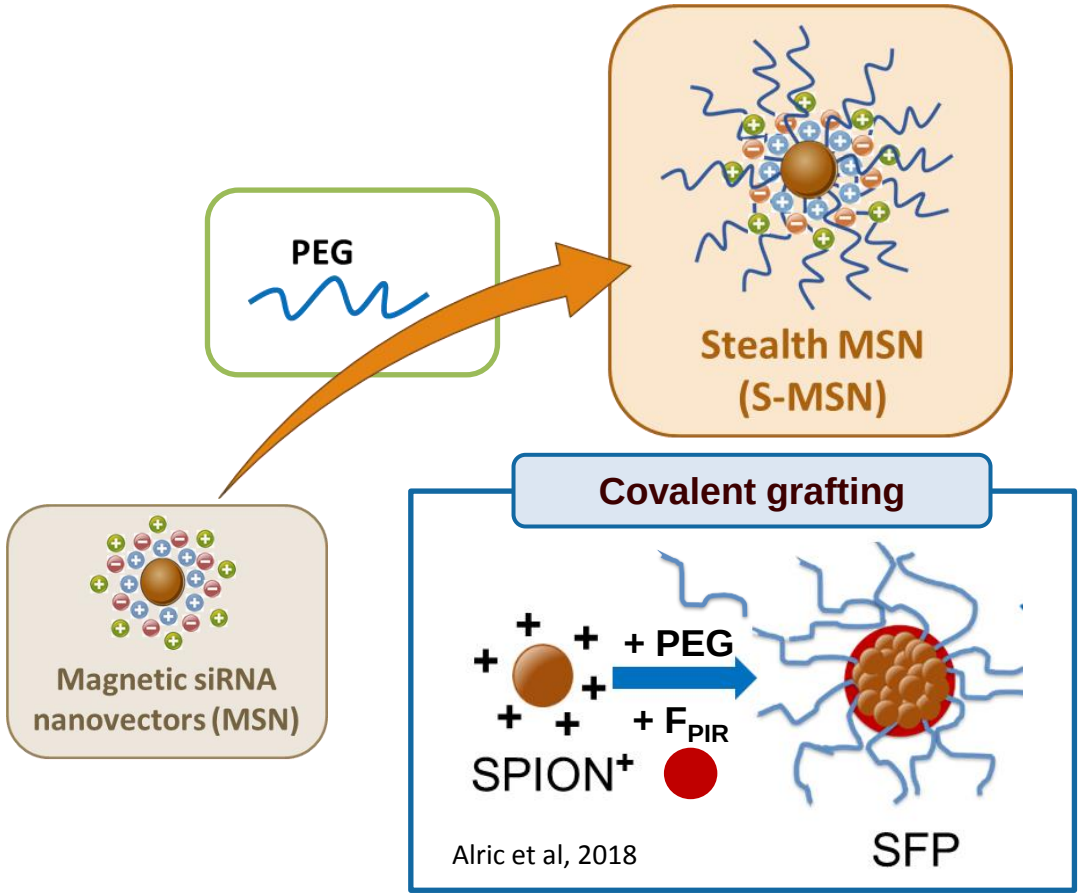


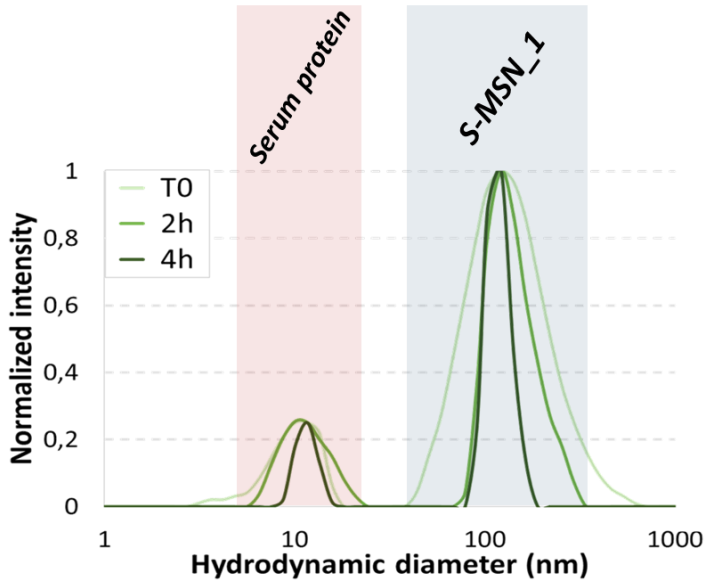
Validation of the "theranostic" concept (therapeutic effect + MRI contrast) *in vivo*

Results - overview

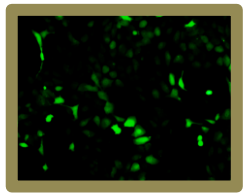


Stealth magnetic siRNA nanovectors



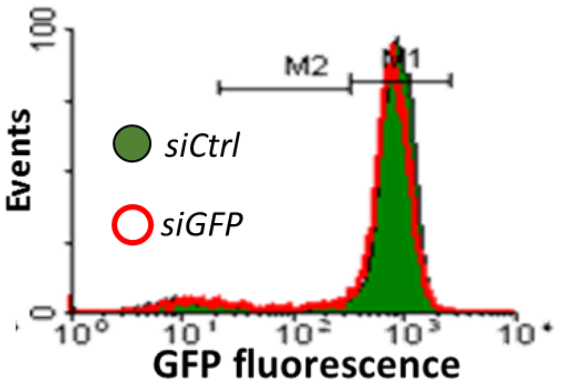


GFP cell culture model



MDA-MB231/GFP

S-MSN_1 (*with chitosan*)



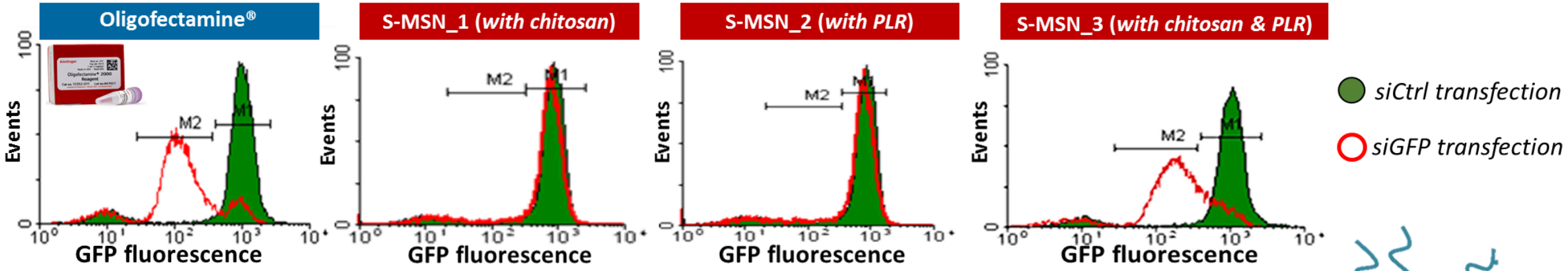
Gene silencing efficiency

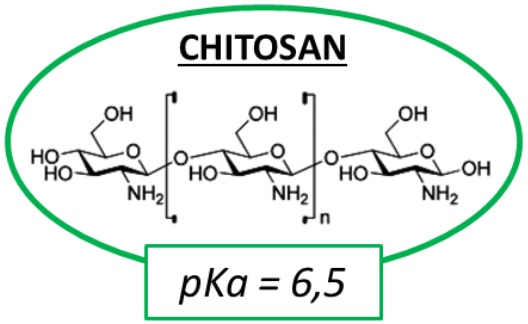
SPION⁺: silanized SPION, **PEG:** polyethylene glycol, **F_{PIR}**: near infrared fluorochrome, **SFP**: stealth fluorescent nanoparticles

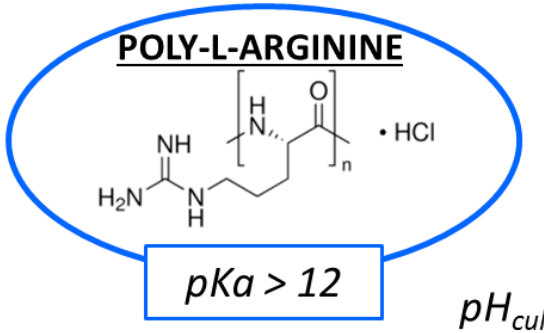
Bruniaux *et al.*, IJP, 2017

Addition of poly-L-arginine in the formulation

[siGFP] = 20 nM
72h transfection on MDA-MB231/GFP

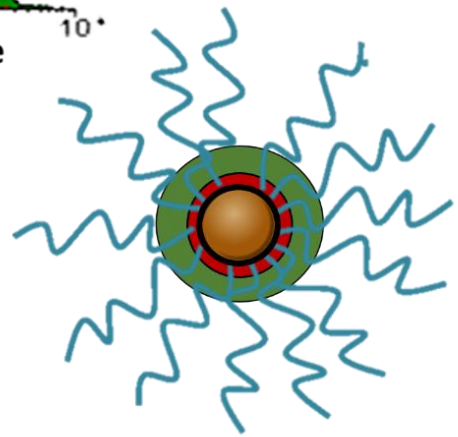






- *Endosomal escape*
- *siRNA complexation*

$pH_{culture\ media} = 7,4$



S-MSN Hydrodynamic diameter: 65-75 nm, Positive charge: 5-10 mV

Bruniaux *et al.*, IJP, 2017

→ Need of both polymers for an efficient gene silencing *in vitro*.

Results - overview


International Journal of Pharmaceutics 569 (2019) 118572

Contents lists available at ScienceDirect

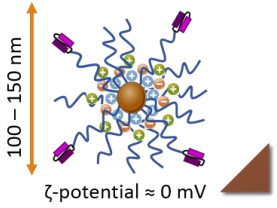
International Journal of Pharmaceutics

journal homepage: www.elsevier.com/locate/ijpharm

Magnetic nanocarriers for the specific delivery of siRNA: Contribution of breast cancer cells active targeting for down-regulation efficiency

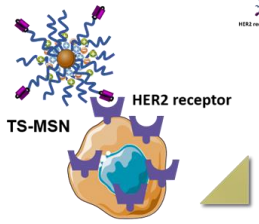
J. Bruniaux[a], E. Allard-Vannier[a], N. Aubrey[b], Z. Lakhrif[b], S. Ben Djemaa[a], S. Eljack[a], H. Marchais[a], K. Hervé-Aubert[a], I. Chourpa[a], S. David[a,*]

[a] Université de Tours, EA6295 «Nanomédicaments et Nanosondes», Tours 37200, France
[b] Université de Tours, UMR1282, INRA, «Infectiologie et Santé Publique», équipe BIOMAP, Tours 37200, France

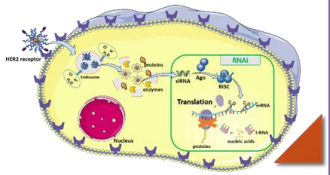


ζ-potential ≈ 0 mV

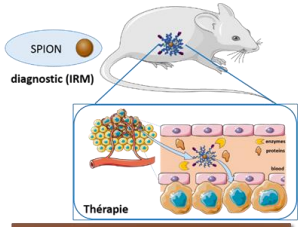
Formulation of TS-MSN and its optimization



Specific targeting of HER2+ breast cancer cells

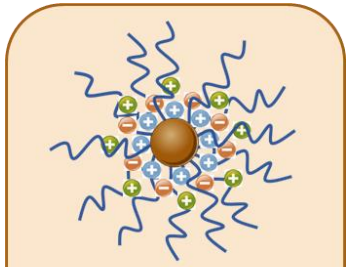


Therapeutic effect due to the action of siRNA on the protein synthesis



Validation of the "theranostic" concept (therapeutic effect + MRI contrast) *in vivo*

→ preliminary results

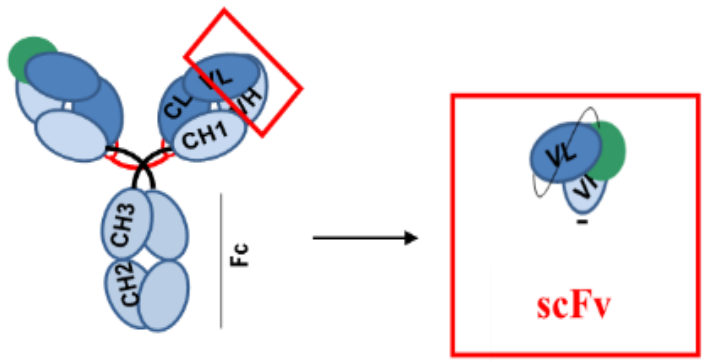


Stealth MSN (S-MSN)

Antibody fragments (scFv)

Targeted stealth MSN (TS – MSN)

Targeted stealth magnetic siRNA nanovectors (TS-MSN)

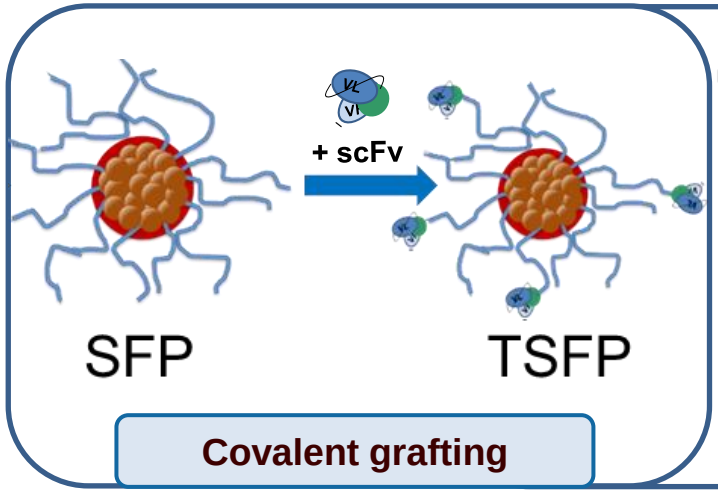


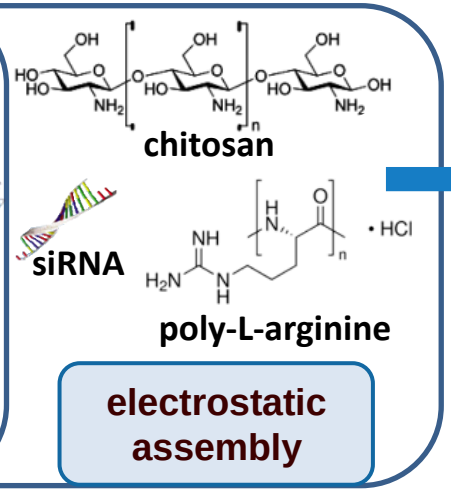
Alric et al, 2016

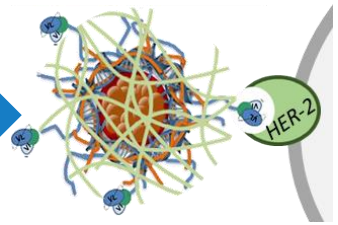
Monoclonal antibody Herceptin® (trastuzumab)

→ HER2 targeting

Team 2: IPVBAI - Tours
Scientific coordinator N. Aubrey



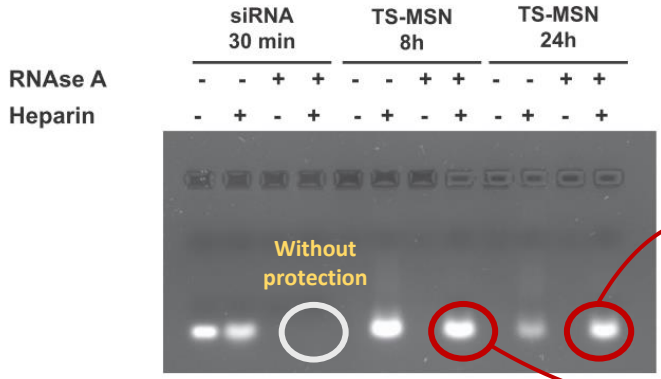




TS-MSN

D_H: 157 +/- 22 nm;
ZP: +17 +/-4 mV;

Protection
1% agarose gel
Heparin 10 mg/mL : used for complexes destabilisation
RNAse A: 2 ng / incubation at 37°C / inactivation during 30' at 70°C

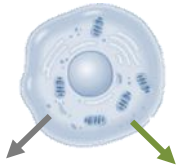


PROTECTION
siRNA integrity
> 95%

Bruniaux *et al.*, IJP, 2019

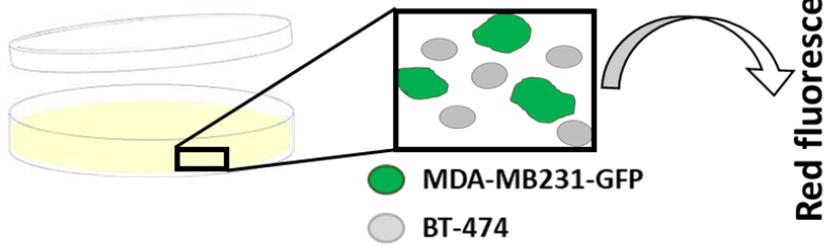
TSFP: targeted stealth fluorescent nanoparticles, **TS-MSN**: targeted stealth magnetic siRNA nanovectors

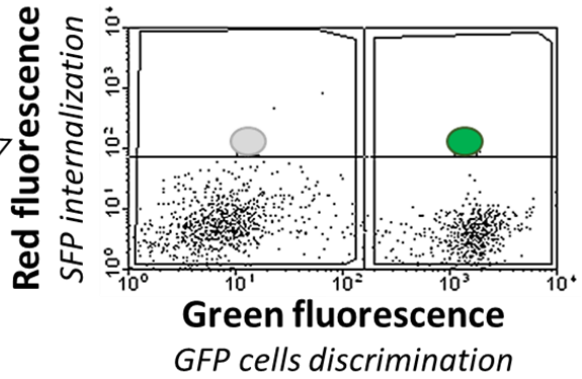
Specific targeting of HER2+ breast cancer cells



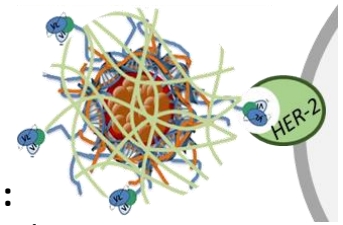
Cells
BT-474
BC-HER2+

Cells
MDA-MB-231-GFP
BC-HER2-

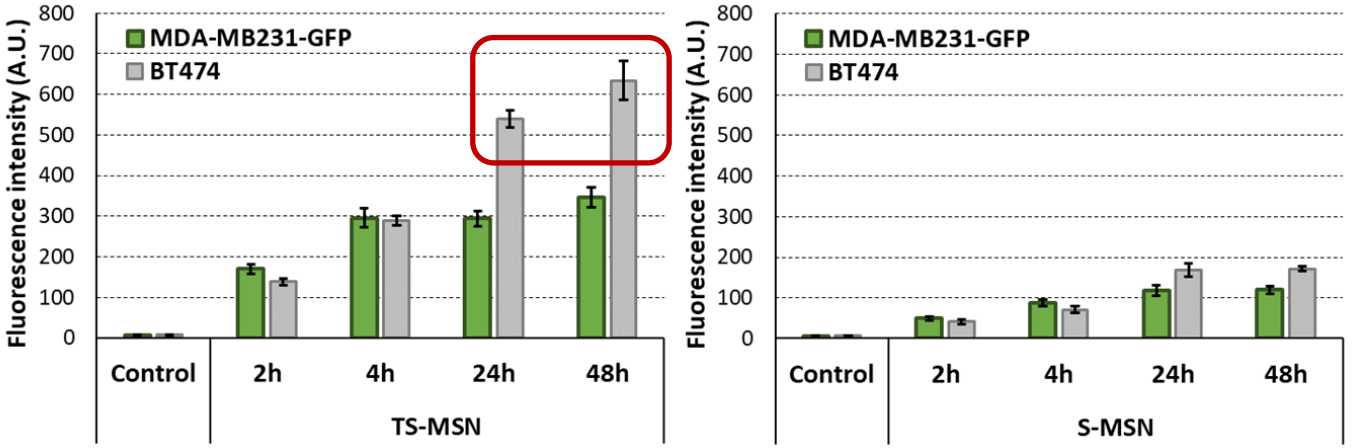




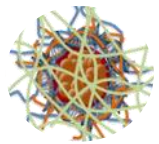
Co-culture experiment to verify the **specific targeting of HER2+ cells**



TS-MSN:
D_H: 157 +/- 22 nm;
ZP: +17 +/-4 mV



→ Enhanced uptake of TS-MSN in HER2+ BC cells for long incubation times (24h and 48h).



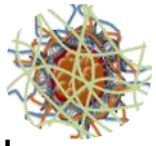
S-MSN
D_H: 73 +/- 6 nm;
ZP: +5 +/-2 mV

Bruniaux *et al.*, IJP, 2019

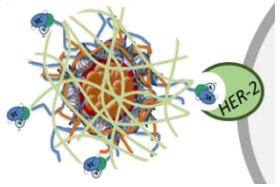
Gene silencing

siRNA anti-survivin

survivin = anti-apoptotic protein implicated in cell survival



S-MSN
D_H: 73 +/- 6 nm;
ZP: +5 +/-2 mV



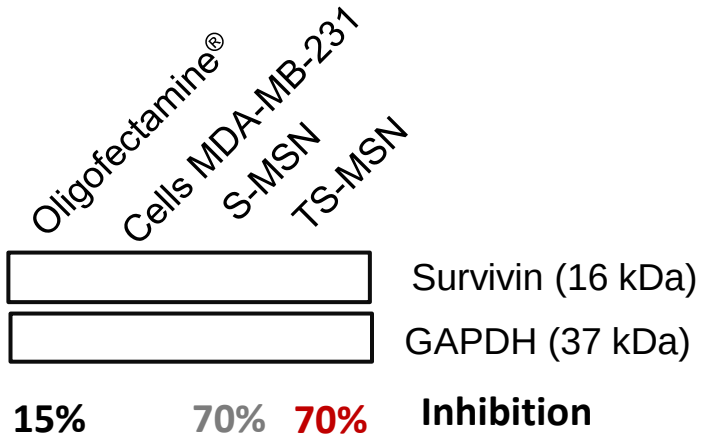
TS-MSN:
D_H: 157 +/- 22 nm;
ZP: +17 +/-4 mV

Cells BT-474 BC-HER2+

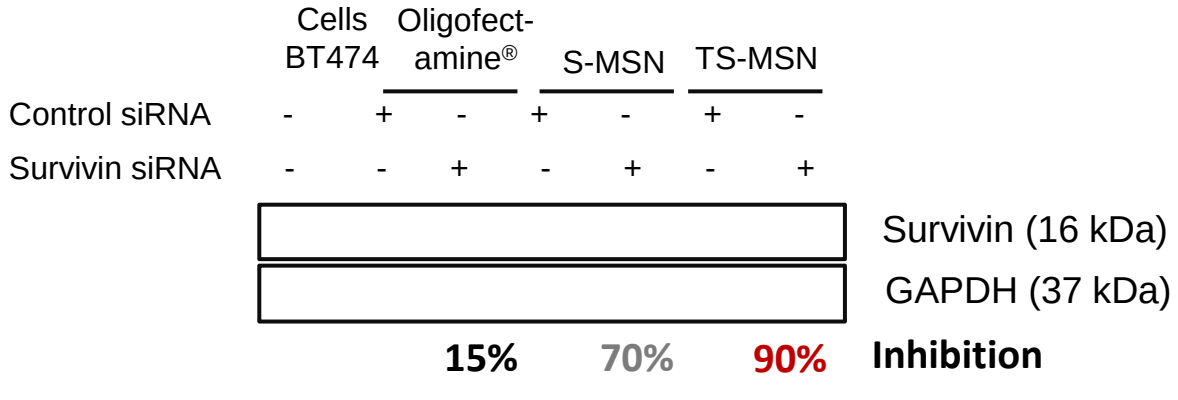
Cells MDA-MB-231 BC-HER2-

Western Blot experiments to verify the **inhibition of the protein synthesis**

Survivin inhibition (HER2- cells)



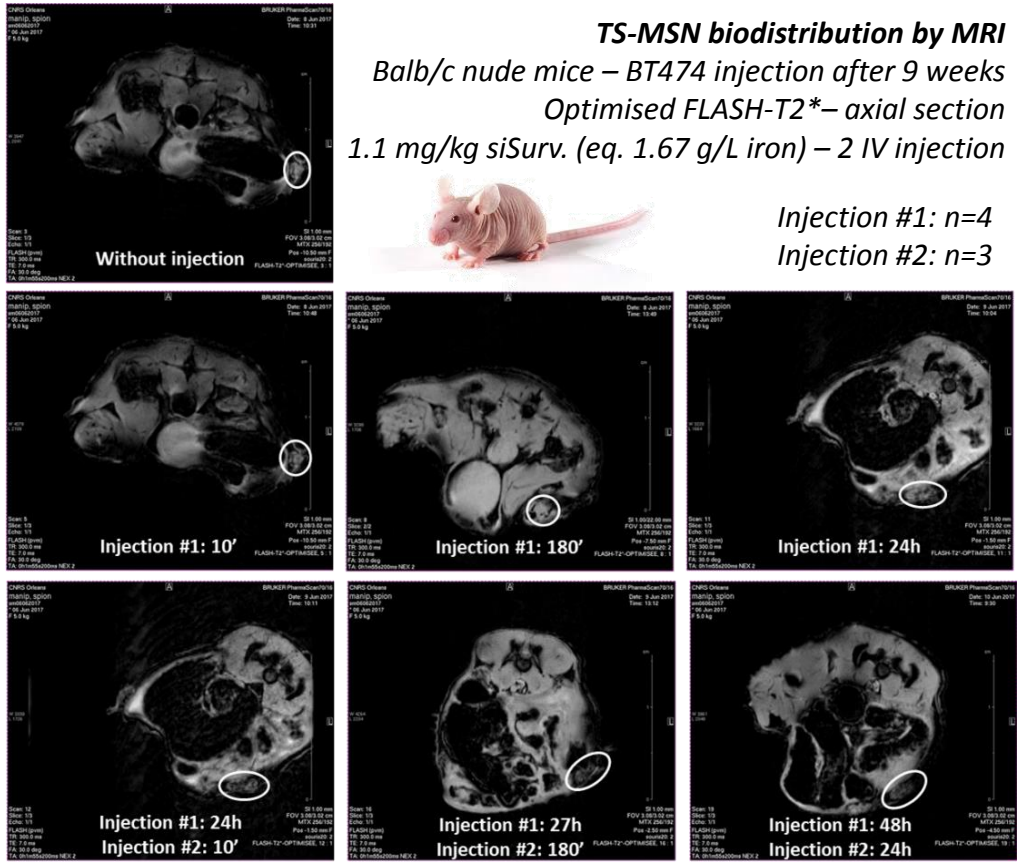
Survivin inhibition (HER2+ cells)



→ Enhanced gene silencing in HER2+ BC cells.

Bruniaux *et al.*, IJP, 2019

TS-MSN administration in mice



TS-MSN biodistribution by MRI

Balb/c nude mice – BT474 injection after 9 weeks

Optimised FLASH-T2– axial section*

1.1 mg/kg siSurv. (eq. 1.67 g/L iron) – 2 IV injection

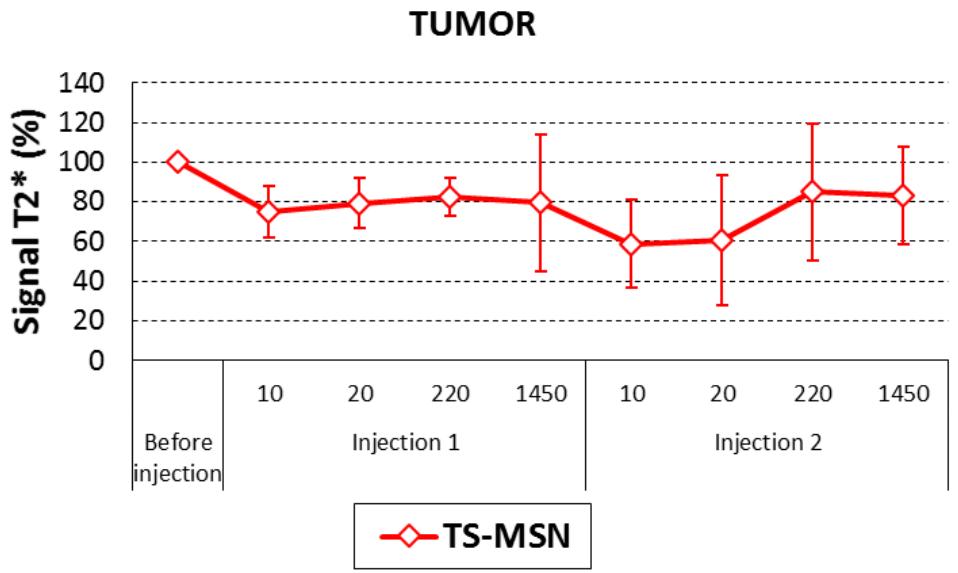
Injection #1: n=4

Injection #2: n=3

Orthotopic HER2 breast cancer mouse model

Team 3: CBM - Orléans

Scientific coordinator S. Même



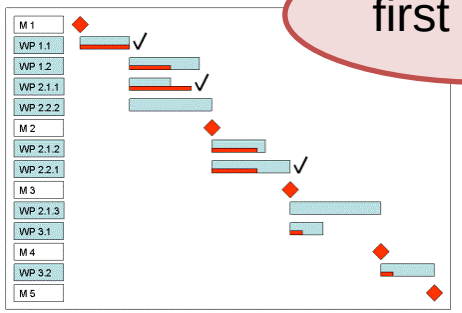
→ TS-MSN accumulation in the tumor can be followed by MRI.

Project feedback

Hurdles

Administrative procedures of the university

People management



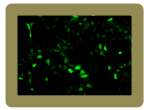
Project time management

first project

Employment

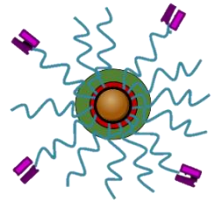
Positive points

Post-doctoral fellowship dedicated 100% to the project



New breast cancer models

Achievement of objectives



New nanovectors

New expertise

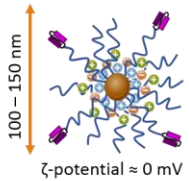
Reports

Funding

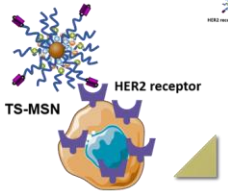
Reinforced collaborations

Conclusion

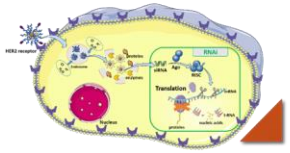
Project objectives



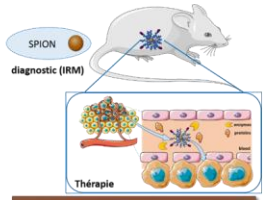
Formulation of TS-MSN and its optimization



Specific targeting of HER2+ breast cancer cells



Therapeutic effect due to the action of siRNA on the protein synthesis



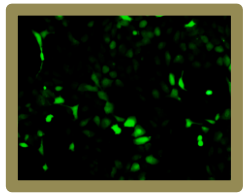
Validation of the "theranostic" concept (therapeutic effect + MRI contrast) *in vivo*

→ Development of targeted stealth magnetic siRNA nanovectors

→ Valorization of the results

→ New biological models, equipment and expertise

MDA-MB231/GFP



BT-474

Western Blot

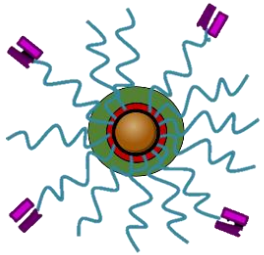
Agarose gel electrophoresis

Gel / Blot imaging system

Spectrofluorimeter

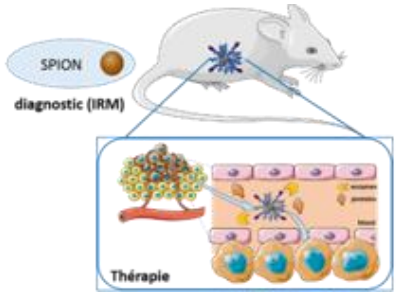
Cell incubator

Perspectives



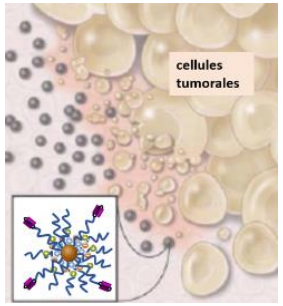
Optimization of the TS-MSN formulation

- PEG corona for better biodistribution *(PhD V. Nguyen)*
- Simplify production for scale-up



Confirmation of the therapeutic effect of TS-MSN

- Gene silencing confirmation at other levels (mRNA, apoptosis) *(PhD S. Ben Djemaa, S. Eljack)*
- Therapeutic effect *in vivo*

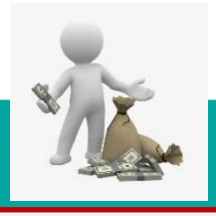


Enhancement of the therapeutic effect of TS-MSN

- Combination of different siRNA sequences and/or siRNA + chemotherapy *(PhD S. Eljack)*
- Application of an external magnetic field (magnetic guidance, magnetic hyperthermia)

… for your attention

Any questions?



… for financial support

- L'institut national du Cancer (INCa)
- La Ligue contre le cancer (LNCC)
- Fondation ARC pour la recherche sur le cancer (ARC)

… for collaborations

EA 6295 Nanomédicaments et Nanosondes (**Tours**)

UMR Université INRA ISP 1282 **IPVBAI** (Tours)

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