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

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

**Project overview**

**Targeted stealth MSN (TS – MSN)**

**Theranostic hybrid magnetic nanovectors**

**Antibody fragments**

**MRI on small animals**

**Team 1: NMNS - Tours**
Scientific coordinator S. David
6 PR + 1 PF (2 years)

**Team 2: IPVBAI - Tours**
Scientific coordinator N. Aubrey
3 PR + 1 PF (1 year)

**Team 3: CBM - Orléans**
Scientific coordinator S. Même
2 PR

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

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

**Coordinator:** Dr. Stephanie DAVID
**Project feedback, Paris 24/01/20 – S. DAVID**

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Nanomédicaments et Nanosondes (NMNS)
Breast Cancer

- first cause of mortality per cancer in women population

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

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Nanovectors for improved gene silencing

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Protection from enzymatic degradation

Stealthiness

Accumulation in tumor site

Internalization

Endosomal escape

Blood vessel

Enzymes

siRNA nanovector

siRNA

mRNA translation

Ben Djemaa et al., IJP 2019

Efficient down-regulation

Cancer cell

Lysosome

Nucleus

i.v. injection

Tumor

Nanovectors for improved gene silencing

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Magnetic siRNA nanovectors (MSN)

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Magnetic siRNA nanovectors (MSN)

David et al., IJP, 2013

SPION: superparamagnetic iron oxide nanoparticle
Diagnosis and treatment of HER2+ breast cancer

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

**Team 1**: NMNS - Tours  
Scientific coordinator S. David

**Team 2**: IPVBAI - Tours  
Scientific coordinator N. Aubrey

**Team 3**: CBM - Orléans  
Scientific coordinator S. Même

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

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**Diagnosis and treatment of HER2+ breast cancer**

- TS-MSN
- Drug (siRNA) carriers → therapeutic action
- Imaging agents (SPION) → diagnostic action

**Team 1: NMNS - Tours**

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**Team 2: IPVBAI - Tours**

Scientific coordinator: N. Aubrey

**Team 3: CBM - Orléans**
Scientific coordinator: S. Même

HER2 targeting

MRI on small animals

**TS-MSN**

- Targeted stealth magnetic siRNA nanovector

**SPION**

- Superparamagnetic iron oxide nanoparticles

**siRNA**

- Small interfering RNA

**MRI**

- Magnetic resonance imaging on small animals

**Single chain variable Fragment (scFv)**

- HER2-targeting

**Herceptin® (trastuzumab)**
Theranostic magnetic siRNA nanocarriers as a novel approach in breast cancer diagnosis and treatment

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Formulation of TS-MSN and its optimization

Specific targeting of HER2+ breast cancer cells

Validation of the “theranostic” concept (therapeutic effect + MRI contrast) in vivo

Therapeutic effect due to the action of siRNA on the protein synthesis
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Results - overview

Stealth magnetic nanocarriers of siRNA as platform for breast cancer therapeutics

J. Brunioux, S. Ben Djerzaa, K. Hervé-Aubert, H. Marchais, I. Chourpa, S. David

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

J. Brunioux, E. Allard-Vannier, N. Aubry, Z. Lakhdar, S. Ben Djerzaa, S. Elpak, H. Marchais, K. Hervé-Aubert, I. Chourpa, S. David

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Stealth magnetic siRNA nanovectors

Covalent grafting

Stealth MSN (S-MSN) + PEG + SPION+ + FPIR

Gene silencing efficiency

GFP cell culture model

MDA-MB231/GFP

S-MSN_1 (with chitosan)

Bruniaux et al., IJP, 2017

SPION+: silanized SPION, PEG: polyethylene glycol, FPIR: near infrared fluorochrome, SFP: stealth fluorescent nanoparticles

Gene silencing efficiency

Colloidal stability in complete culture medium

DMEM 10% FBS

Incubation at 37°C

Stability > 4h

Alric et al, 2018

SFP

Bruniaux et al., IJP, 2017
Addition of poly-L-arginine in the formulation

\[[\text{siGFP}] = 20 \text{ nM}\]
72h transfection on MDA-MB231/GFP

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Oligofectamine®
S-MSN_1 (with chitosan)
S-MSN_2 (with PLR)
S-MSN_3 (with chitosan & PLR)

\[\text{CHITOSAN}\]
\[\text{POLY-L-ARGININE}\]

\[\text{pK}_a = 6.5\]
\[\text{pK}_a > 12\]

\[\text{pH}_{\text{culture media}} = 7.4\]

\[\rightarrow\] Need of both polymers for an efficient gene silencing \textit{in vitro}.

\[\text{siCtrl transfection}\]
\[\text{siGFP transfection}\]

Bruniaux \textit{et al.}, \textit{IJP}, 2017

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Targeted stealth magnetic siRNA nanovectors (TS-MSN)

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Monoclonal antibody
Herceptin®
(trastuzumab)

\[ \text{TSFP: targeted stealth fluorescent nanoparticles, TS-MSN: targeted stealth magnetic siRNA nanovectors} \]

**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

** TEAM 2: IPVBAl - Tours**

Scientific coordinator N. Aubrey

**Protections**

- siRNA integrity > 95%

Bruniaux et al., IJP, 2019

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Specific targeting of HER2+ breast cancer cells

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

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

Bruniaux et al., IJP, 2019

Scientific context

Specific targeting of HER2+ breast cancer cells

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Scientific context
Gene silencing

Survivin inhibition (HER2- cells)

Survivin (16 kDa)
Survivin siRNA
GAPDH (37 kDa)
- - - - - - -
15% 70% 70%
Inhibition

Survivin inhibition (HER2+ cells)

Survivin (16 kDa)
Survivin siRNA
GAPDH (37 kDa)
- - + - + - +
15% 70% 90%
Inhibition

Western Blot experiments to verify the inhibition of the protein synthesis

Enhanced gene silencing in HER2+ BC cells.

Bruniaux et al., IJP, 2019

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**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

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

- Administrative procedures of the university
- People management
- Project time management
- Employment

Positive points

- Post-doctoral fellowship dedicated 100% to the project
- New breast cancer models
- Achievement of objectives
- Reports
- Funding
- Reinforced collaborations
- New nanovectors
- New expertise

Achievement of objectives

- Positive points

People management

Right person for the right job!

First project

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Valorization of the results

New biological models, equipment and expertise

→ Development of targeted stealth magnetic siRNA nanovectors

→ 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

→ Valorization of the results

MITO-MSN
HER2 receptor

MDA-MB231/GFP
BT-474

Agarose gel electrophoresis
Western Blot

Gel / Blot imaging system
Spectrofluorimeter

Cell incubator

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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 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)
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UPR 4301 IRM, CBM, CNRS (Orléans)

Any questions?