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Local delivery of nanomedicines-loaded hydrogel for the treatment of glioblastoma

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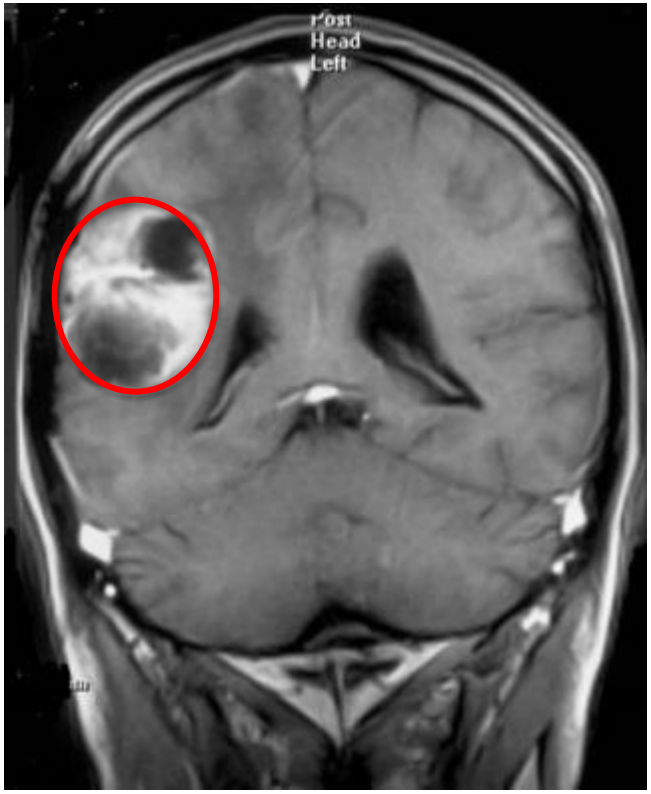
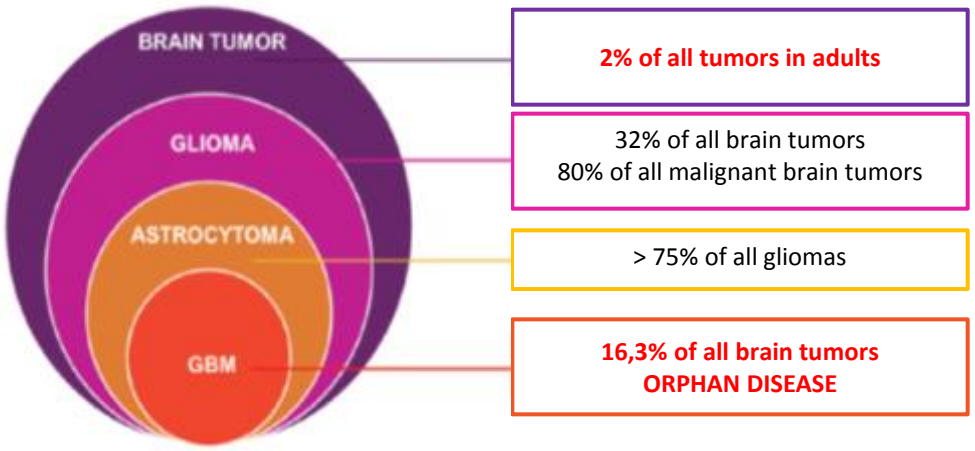
European Doctorate in nanomedicine and pharmaceutical innovation

Glioblastoma (GBM)

Most common and aggressive malignant brain tumor in adults



http://www.info-radiologie.ch/glioblastome.php



Grade IV Central Nervous System (CNS) tumor: Cytological malignant, mitotically active neoplasm associated widespread **invasion**, **rapid proliferation**, **recurrence** after all forms of therapy and **fatal outcome**.

Aparicio-Blanco *et al. J Biomed Nanotech* (2015)

Glioblastoma (GBM)

- Rapid proliferation and propensity to infiltrate healthy brain tissue
- Standard-of-care therapy:

SURGICAL RESECTION + RADIOTHERAPY + CHEMOTHERAPY with Temozolomide

→ **Incurable**: median survival 12-15 months with 5 years survival rate < 10%

→ **Chemoresistance**

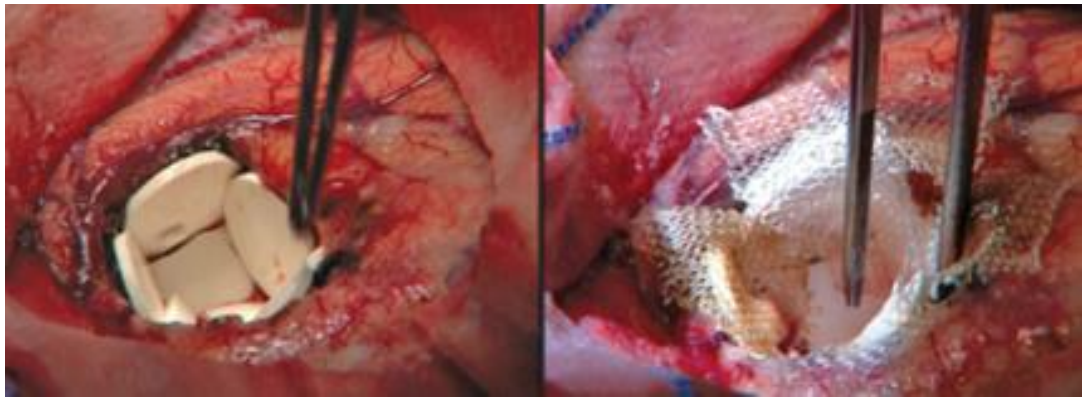
→ High tendency of **recurrences** after surgical resection due to micrometastatis undetectable by MRI

UNMET MEDICAL NEEDS:
URGENT NECESSITY TO FIND NEW TREATMENT STRATEGIES

Batich *et al. Exp Opin Pharmacother* (2014)

Alternative

- Gliadel® wafer: first intracereblal implant for the treatment of GBM approved by the FDA in 1996
- Local delivery of carmustine



RELEASE OF ACTIVE COMPOUND DIRECTLY INTO THE CNS BY DIFFUSION AND DEGRADATION:
PROMISING STRATEGY FOR THE TREATMENT OF GLIOBLASTOMA

BUT :
- migration of implants
- release of 80% carmustine in 1 week
- many side effects : intracranial abscess, meningitis, impaired wound healing, cerebrospinal fluid leak, seizures and tumor cyst formation

Juratli *et al. Pharmacol Ther* (2013)

Local delivery

Advantages:

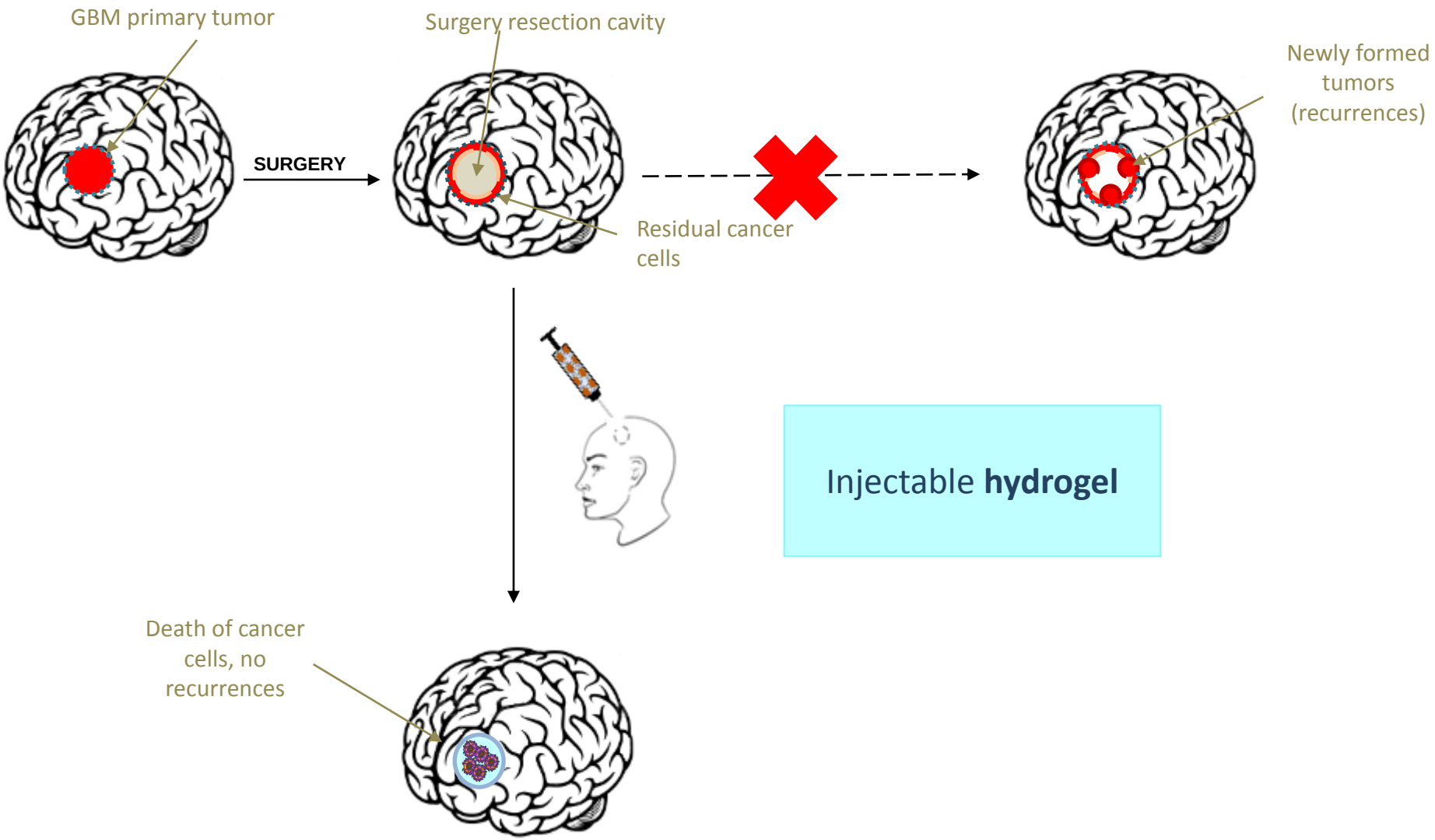
- Reduce systemic side effects
- Avoid the BBB
- Concentrate the drug to the target tissue

Challenges:

- Controlled and sustained release of the drug
- Fitting with the resection cavity
- Injectability
- Biocompatibility and biodegradability

Keep in mind the clinical relevance !!!!

Aim / hypothesis

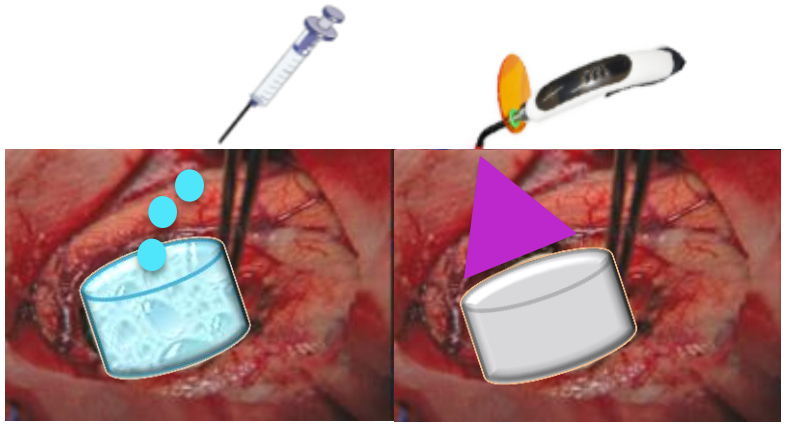


Hydrogels

PEG-DMA **hydrogel**

+ Temozolomide (TMZ)

- Polyethylene glycol dimethacrylate (PEG-DMA)®
  + Lucin TPO® (photoinitiator)
- Photopolymerization (UV light)
- Prevent cell infiltration (PEG)
- Commercially available (GMPc)

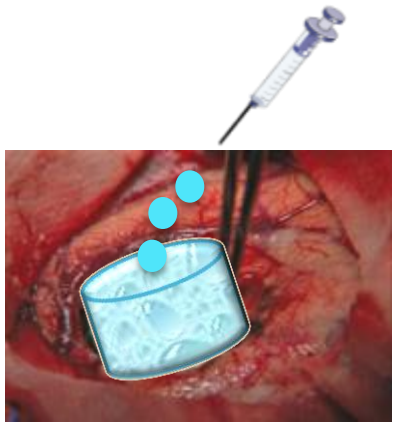


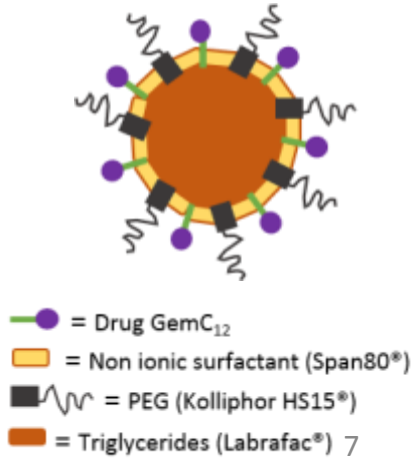
Fourniols *et al. J. Control. Rel* (2015)

Lipid nanocapules (LNC) **hydrogel**

+ Gemcitabine derivative ($GemC_{12}$)

- Labrafac®, Span 80®, Kolliphor®
- Gelation in the syringe
- No polymers, no gelling agents nor application of external stimuli
- Gem has the potent to overcome the resistance of GBM to conventional chemotherapy

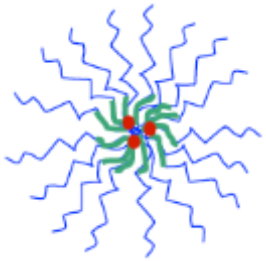




Bastiancich *et al. J. Control. Rel* (2016)

Nanomedicines : physico-chemical characterization

TMZ-loaded PEG-DMA **hydrogel**



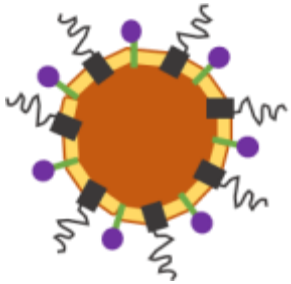
Solubilization of TMZ in **polymeric micelles**
PEG-p(CL-co-TMC) 50:50

Size (nm): 35 ± 1.5
PDI: 0.058
ζ potential: -5.2 ± 12.4
TMZ conc (mg/ml): 2 ± 0.1

Irradiation 15s, 750 mW/cm2, 400 nm

Δt^{max}= 5.6 ° C

$GemC_{12}$-LNC **hydrogel**



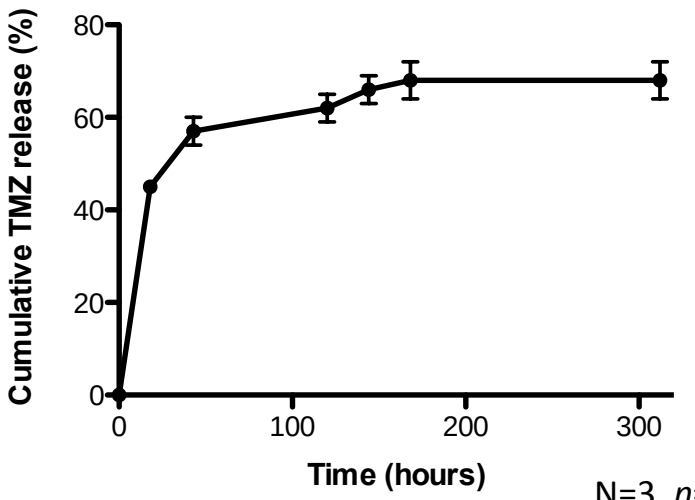
Hydrogel: when the drug is a key player of the **nanoparticle**

Size (nm): 69 ± 4
PDI: 0.27
ζ potential: -2.5 ± 0.2
$GemC_{12}$ conc (mg/ml): 16.6

Adapted rheological properties:
Near to the brain tissue moduli (1kPa)

In vitro drug release in artificial cerebrospinal fluid

TMZ-loaded PEG-DMA **hydrogel**

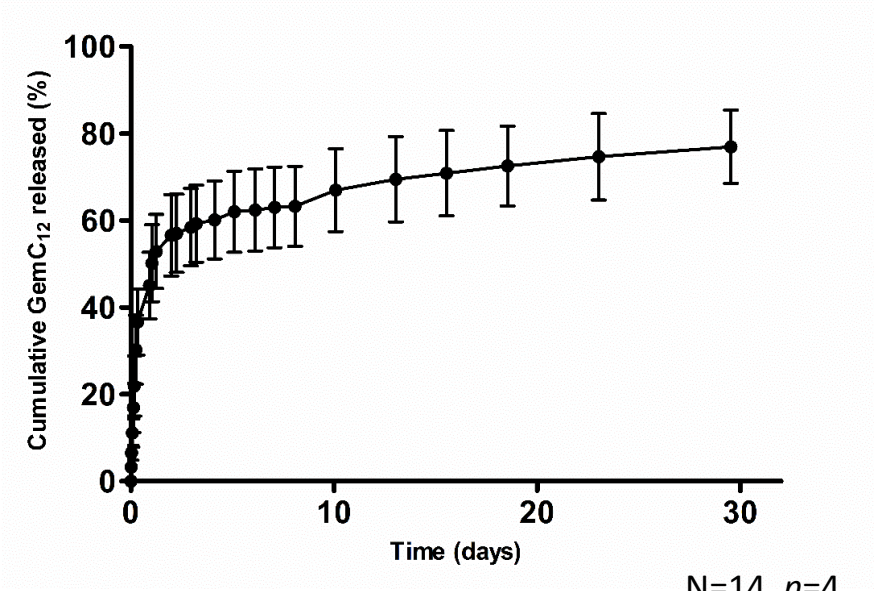


N=3, *n*=3

Burst release (45% in 1 day)
20% more **over 1 week**
Release plateaued to reach 70%

Similar profile to Gliadel®
Should be improved

$GemC_{12}$-LNC **hydrogel**



N=14, *n*=4

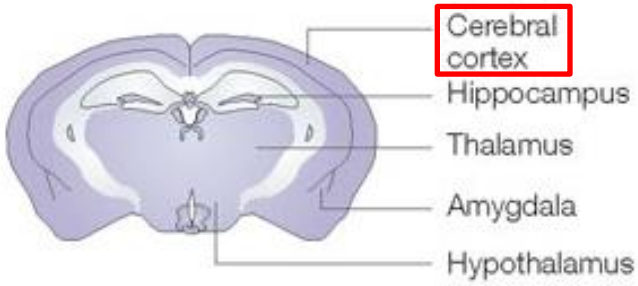
Burst release (56% in 2 days)
20% more **over 1 month**
Release plateaued to reach 77%

Degradation of the gel = release of the drug

In vivo tolerability (short term)

DAY 1

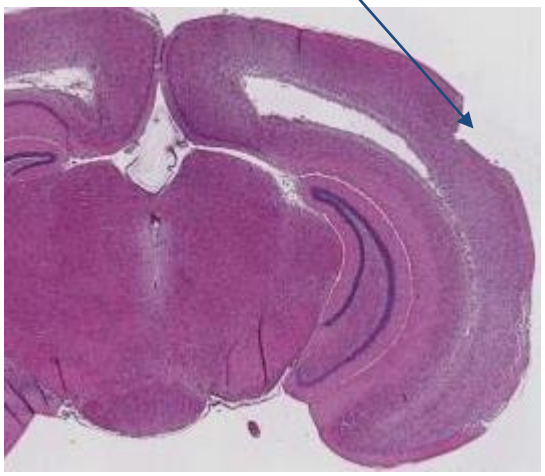
- Creation of a cavity in the brain of the left frontal lobe of 8-weeks old NMRI mice
- Injection of 10 µl PBS, unloaded LNC, $GemC_{12}$, $GemC_{12}$-LNC in the cortex



DAY 8

- Sacrifice of the mice and extraction of the brain
- Embed the brains in paraffin and cut in 10 µm sections

CAVITY



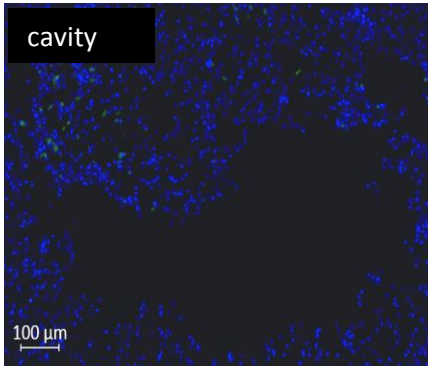
- EVALUATION OF THE CELLULAR AND INFLAMMATORY RESPONSE IN THE CAVITY BY

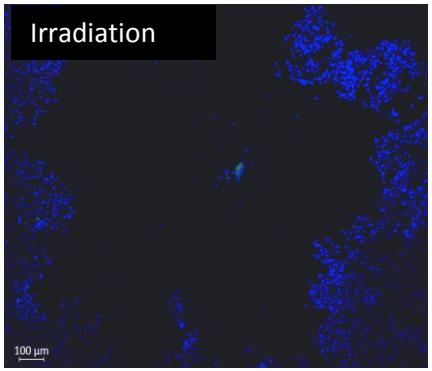
A) Terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) assay

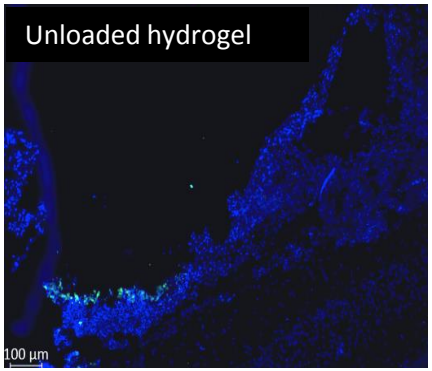
B) Microglia activation by Iba-1 immunostaining

In vivo tolerability: TUNEL

TMZ-loaded PEG-DMA **hydrogel**





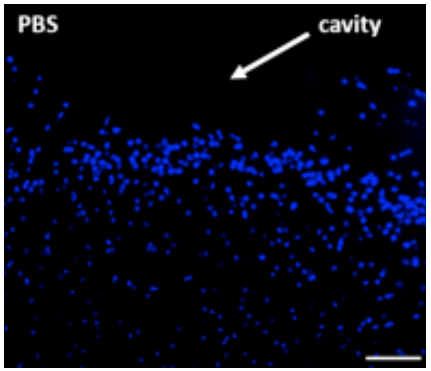


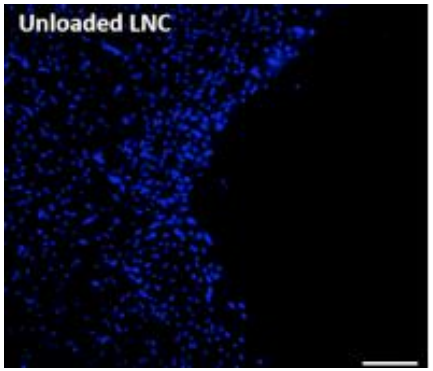
Blue: living cells nuclei (DAPI); Green: apoptotic cells (TUNEL)

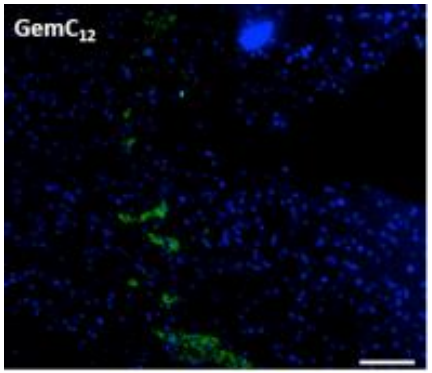
N=3, n=3

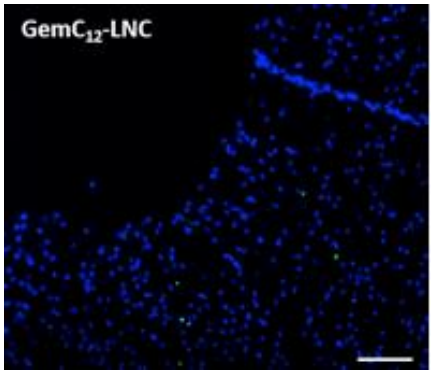
——— : 100 µm

$GemC_{12}$-LNC **hydrogel**









Blue: living cells nuclei (DAPI); Green: apoptotic cells (TUNEL)

N=3, n=3

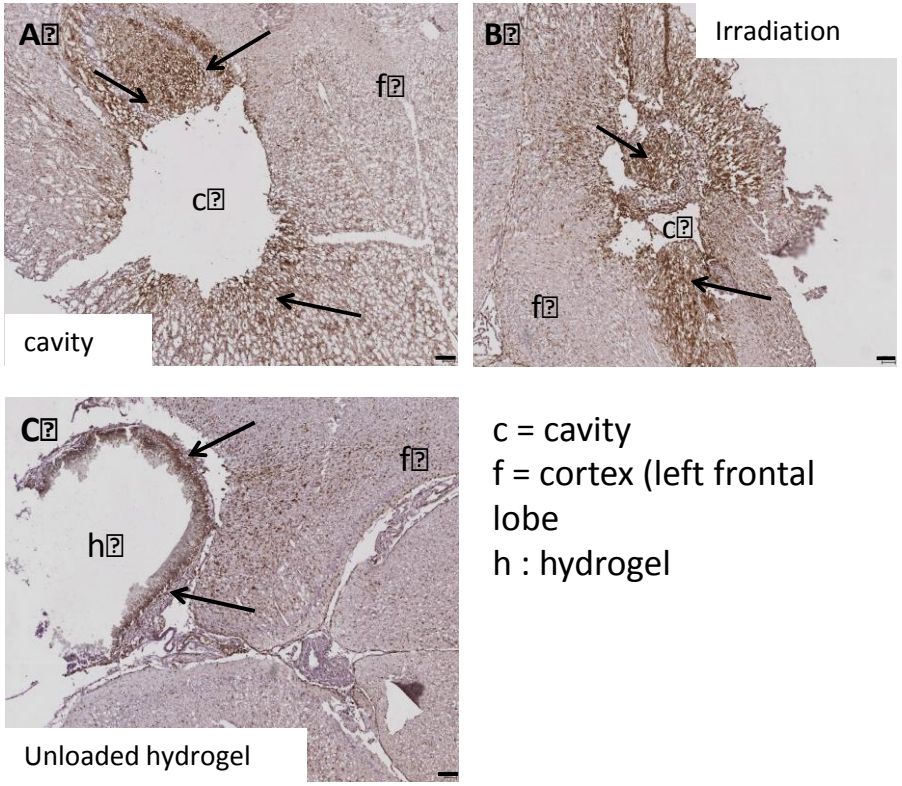
——— : 100 µm

Low toxicity of the unloaded hydrogel

No cell infiltration in the cavity

In vivo tolerability: Iba-1 staining (microglia activation)

TMZ-loaded PEG-DMA **hydrogel**

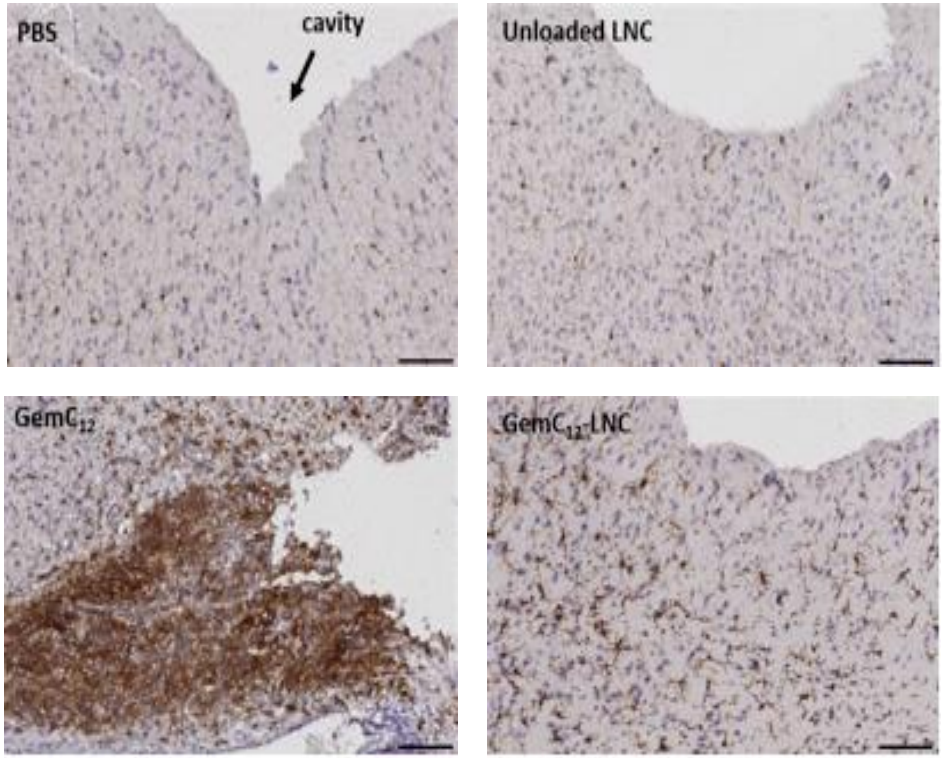


c = cavity
f = cortex (left frontal lobe
h : hydrogel

N=3, n=3

—— : 100 µm

$GemC_{12}$-LNC **hydrogel**



N=3, n=3

—— : 100 µm

The microglial activation is due to the surgery

In vivo anti-tumor efficacy

SUBCUTANEOUS GLIOBLASTOMA MODEL

DAY 1

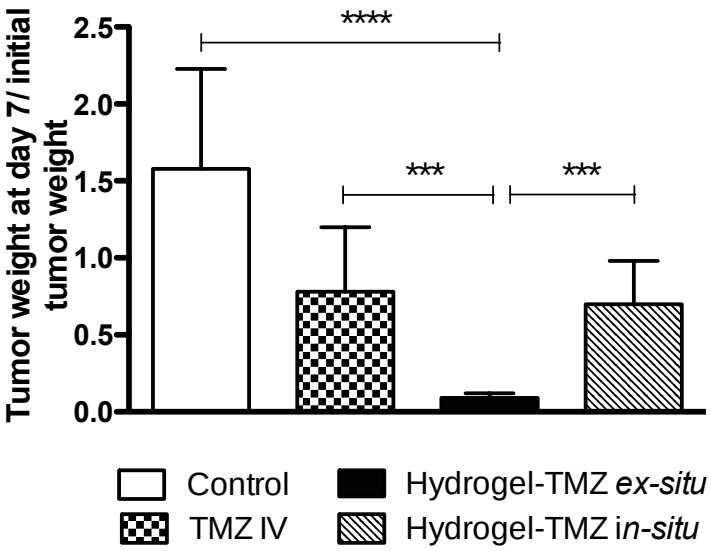
- Sc injection of U87MG cells in NMRI nude mice in the right flank
  → $2x10^6$ cells/mouse

DAY 10-18

- Let the tumor grow and reach a volume of 35 mm^3
- Inject intratumorally the treatment
- At day 8 after injection of the treatment sacrifice the animal, extract and weight the tumor

In vivo anti-tumor efficacy

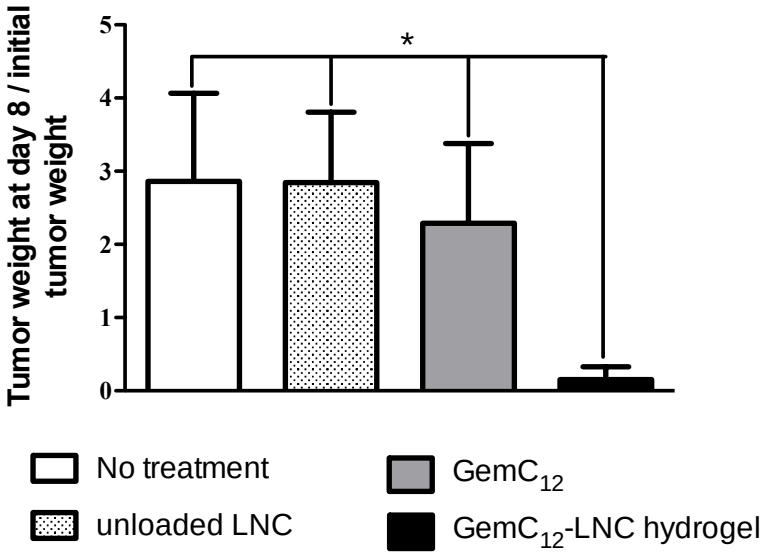
TMZ-loaded PEG-DMA **hydrogel**



TMZ dose: 4.75 mg/kg
Ex situ = polymerization before implantation
In situ = polymerization through the skin

N=5 to 7

$GemC_{12}$-LNC **hydrogel**



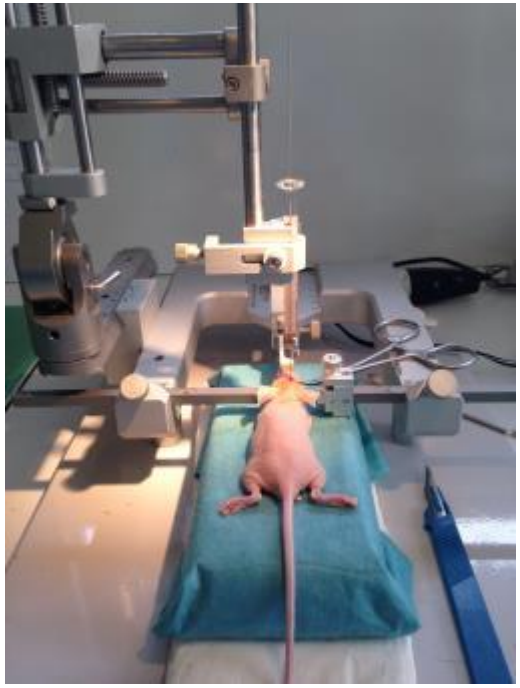
$GemC_{12}$ dose: 19 mg/kg
Ex situ = polymerization before implantation
In situ = polymerization through the skin

N=5 to 7

Proof of concept:
Significant reduction in tumor weight one week after injection of drug-loaded hydrogel compared to other groups.

In vivo orthotopic glioblastoma model (on going)

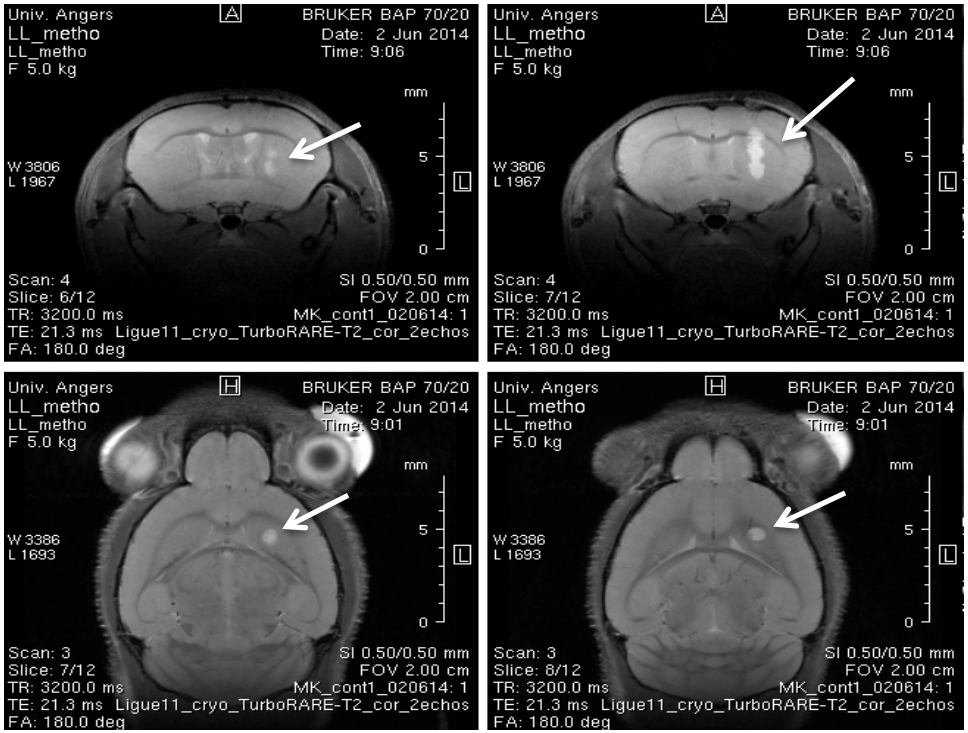
Stereotactic injection of $3x10^4$ cells (5µL)



Right frontal lobe (striatum)

Coordinates:
2.1 mm lateral from bregma
0.5 mm anterior
3 mm deep from the border of the cranium

In vivo orthotopic glioblastoma model (on going)



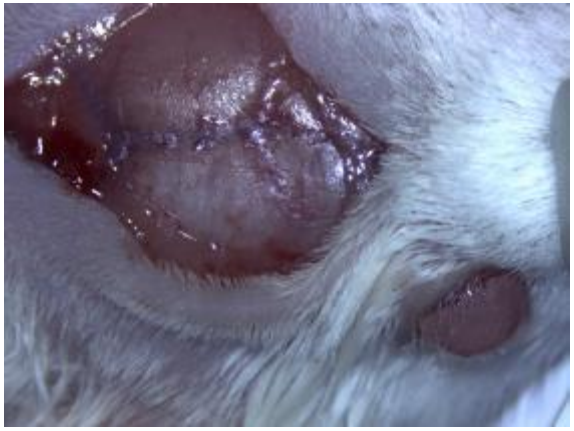
Positive contrast: hyper-intense zone

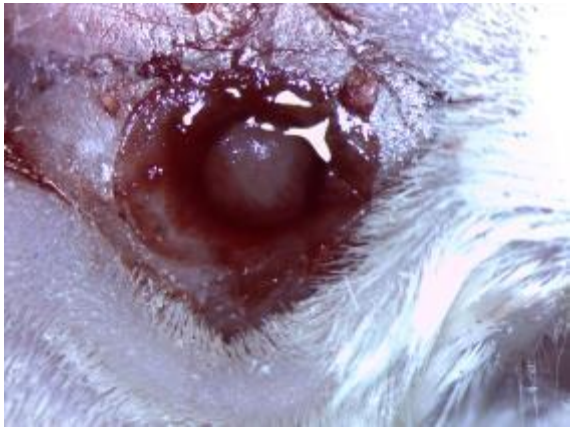
7 T scanner Biospec 70/20 Avance III, Bruker

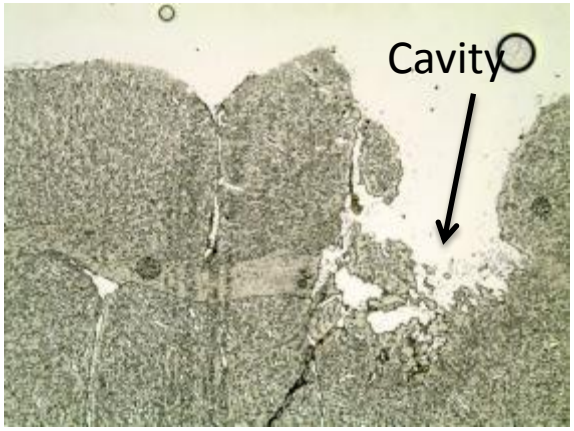
RARE sequence: TR=3200ms; effective echo time = 21,3 ms;
acceleration factor = 4; FOV= 2x2 cm

Danhier *et al.*, Int. J. Pharm (2015)

In vivo resection glioblastoma model (on going)







Conclusions

Injectable hydrogels with slow and controlled release

Adapted rheological properties

Good short-term tolerability in the brain

Decreased tumor growth

Proof-of-concept of the two projects has been established

Perspectives:

In vivo anti-tumor efficacy in a **resection model**

Clinical relevance

Prof. Veronique Préat
Prof. Anne des Rieux
Chiara Bastiancich
Dr. John Bianco
Dr. Julian Leprince
Thibaut Fourniols
Luc Randolph
Marline Ndiaye
Mengnan Zhao
Bernard Ucakar
Kevin Vanvarenberg

The Advanced Drug Delivery & Biomaterials team!

Prof. Frédéric Lagarce
Prof. Guillaume Bastiat
Marion Pitorre

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European Doctorate in nanomedicine and pharmaceutical innovation

Thank you for your attention