ULTRASOUND TARGETED PACLITAXEL MICROBUBBLES TO IMPROVE ONCOLYTIC VIROTHERAPY

Kishan S. Italiya[1], Bourgeois-Daigneault M.C.[2,3,4], François T.H. Yu[1,3,5]

[1]*Microbubble Theranostics Laboratory, CRCHUM: "Centre Hospitalier de l'Université de Montréal" Research Centre, Montreal, Canada*

[2]*Cancer axis, CRCHUM: "Centre Hospitalier de l'Université de Montréal" Research Centre, Montreal, Canada*

[3]*Institut du cancer de Montréal, Montreal, Canada*

[4]*Département de Microbiologie, Infectiologie et Immunologie, Faculté de Médecine, Université de Montréal, Montreal, Canada*

[5]*Department of radiology, radio-oncology and nuclear medicine, Université de Montréal, Montreal, Canada*

3 February 2022
Virtual Seminar 2022

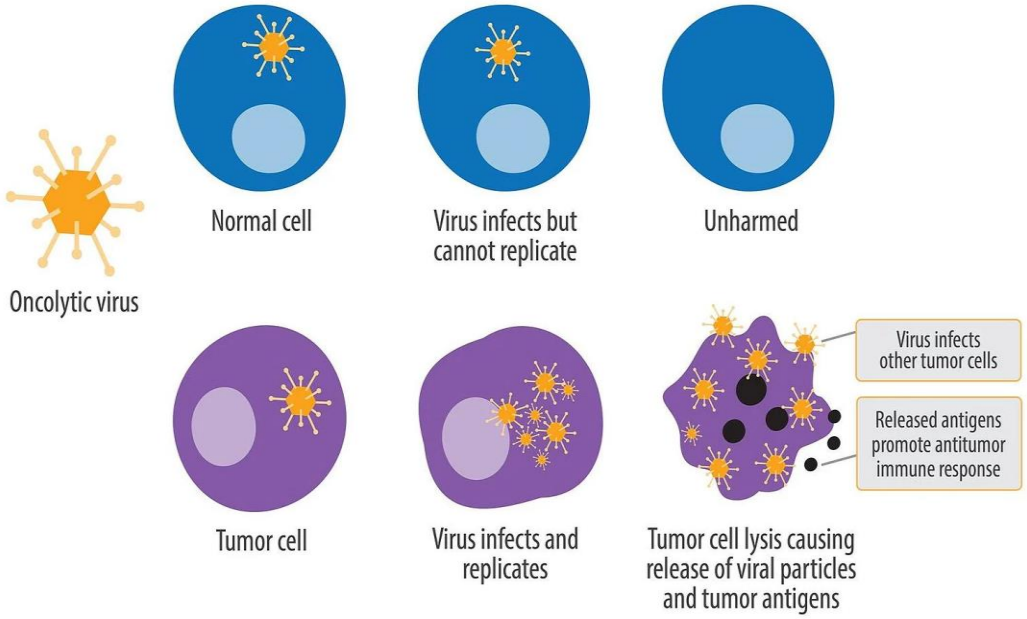
Département de radiologie, radio-oncologie et médecine nucléaire
Faculté de médecine
Université de Montréal

Disclosures

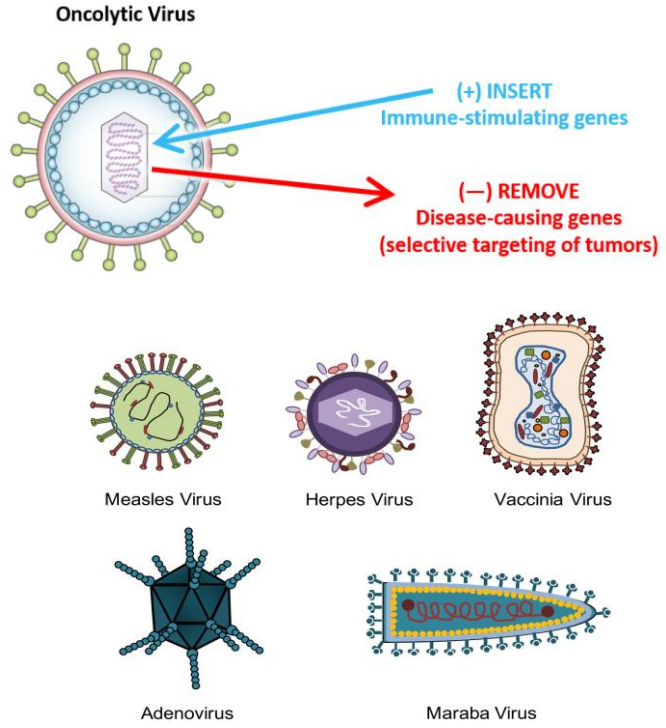
N/A

Introduction: Oncolytic virotherapy

- Oncolytic viruses (OVs) are an emerging class of **bio-therapeutics** with the capacity to selectively kill **cancer**
- **OV T-VEC (Imlygic®)** was approved as the first oncolytic virus therapy by USFDA for advanced melanoma treatment in the year 2015



How oncolytic virus therapy is changing cancer treatment



Examples of OVs

http://www.epicentrx.com
https://blog.crownbio.com/oncolytic-viruses-immuno-therapy

Département de radiologie, radio-oncologie et médecine nucléaire
Faculté de médecine
Université de Montréal

Introduction: Oncolytic virotherapy

Challenges for delivery of OVs in cancer:

- **Cancer response** to intratumorally injected OVs **is heterogeneous** (types, locations, intra and inter lesion), means it is extremely difficult to predict which cancers will be responsive
- While intravenous (IV) delivery is highly desirable (simple and targets metastases), the systemic delivery of OVs is not very efficient as most of the **viruses are neutralized by IV delivery**
- Very **limited viral replication** within the tumor tissue, which **hampers the therapeutic response**

Oncolytic virus sensitizers can improve virotherapy

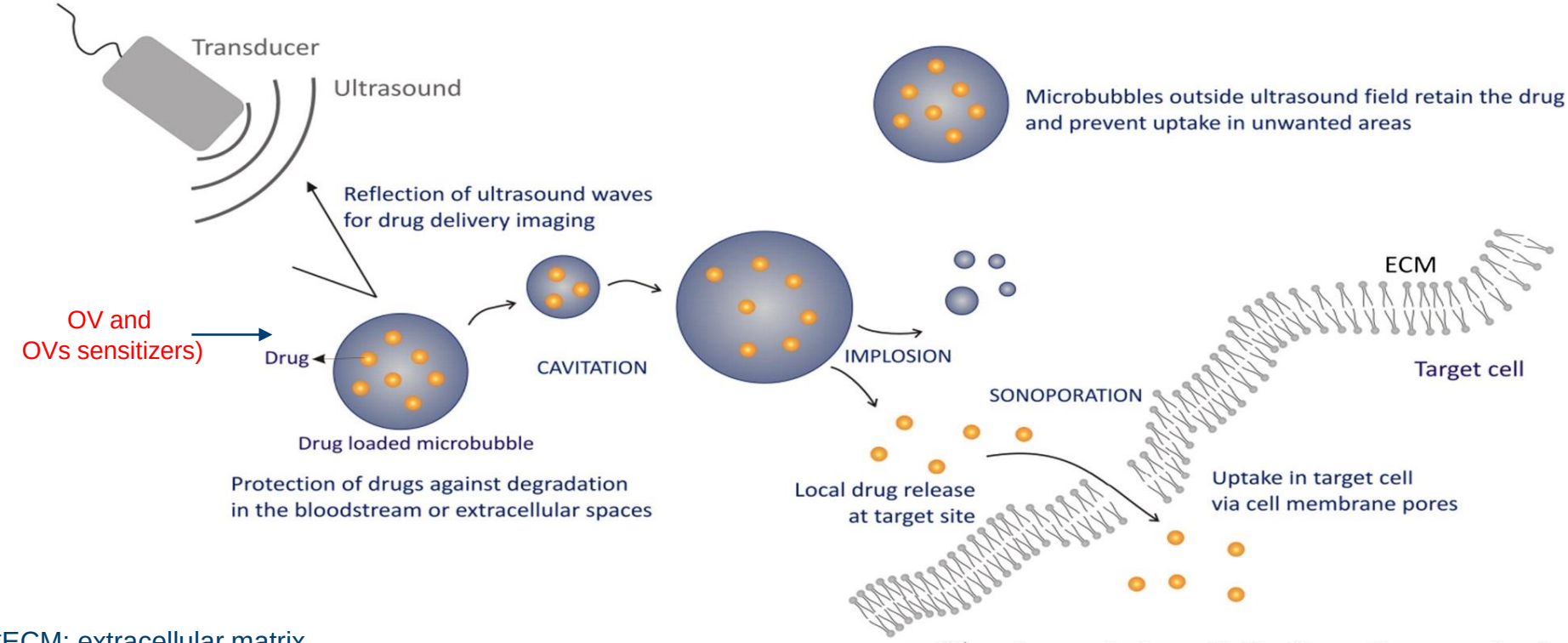
- **Paclitaxel**, mTOR and novel kinase inhibitors (e.g. BI-D1870, KA-019 and others) can **increase OV replication** 1000-folds
- **Systemic delivery of these drugs** is often associated with **severe side effects**, and when combined with systemic OV-therapy, can also **compromise the tumor specificity** of the virus

Bourgeois-Daigneault, M.-C. *et al.* Breast Cancer Res. 18, 83 (2016)
Zakaria, C. *et al*. PLOS Pathog. 14, e1007264 (2018)

Hypothesis: Ultrasound targeted OVs and OVs sensitizer (e.g. Paclitaxel) loaded microbubbles

- Ultrasound targeted microbubbles can increase viral load as well as replication of **virus** and also increase the local concentration of **OV sensitizer (Paclitaxel)** within tumor tissue

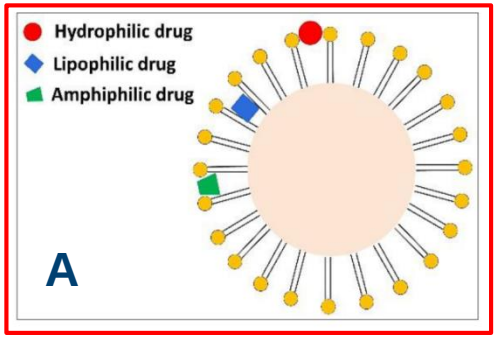
- Local disruption of MB has been shown to increase vascular permeability and improve reperfusion, which could ameliorate subsequent drug/virus delivery

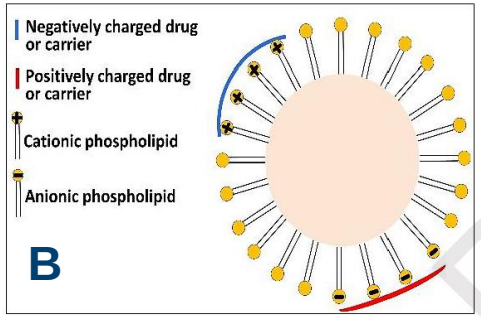


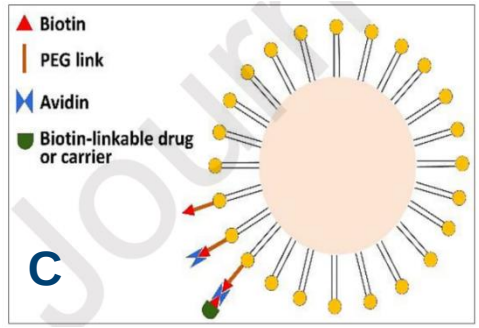
*ECM: extracellular matrix

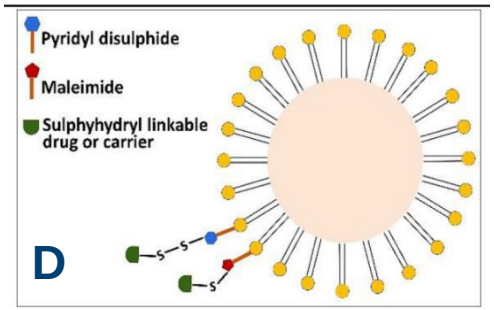
Geers, Bart, *et al*. *Journal of controlled release* 164.3 (2012): 248-255.

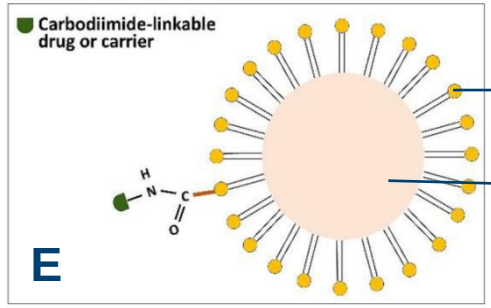
Drug loading techniques & microbubble











Outer lipid layer of MB

Inner core of MBs (Perfluorocarbon gas)

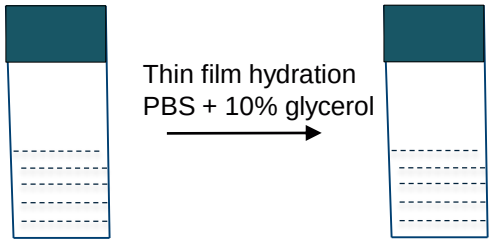
Technique A is used for Microbubble preparation

Here, drug is Paclitaxel (PTX) which is cytotoxic and hydrophobic in nature

Département de radiologie, radio-oncologie et médecine nucléaire
Faculté de médecine
Université de Montréal

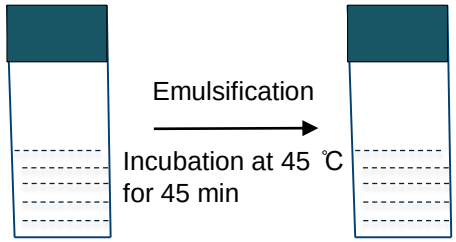
Al-Jawadi, S. *et al*.,(2020). *International journal of pharmaceutics*, 119559.

Paclitaxel (PTX) loaded Lipid Microbubbles (PTXLPMB)



Lipids and drug in Chloroform

Thin film hydration
PBS + 10% glycerol



Homogenous lipid suspension (DPPC,DSPE) with drug

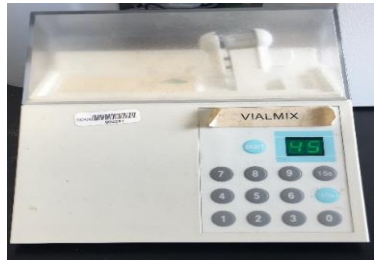
Emulsification

Incubation at 45 ℃ for 45 min

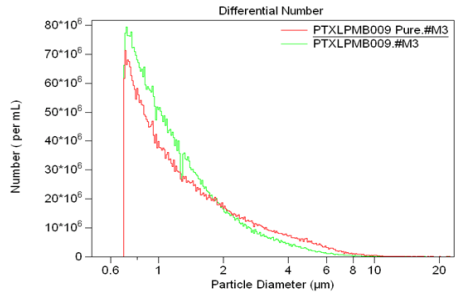
Degassing

C_4F_{10} gas

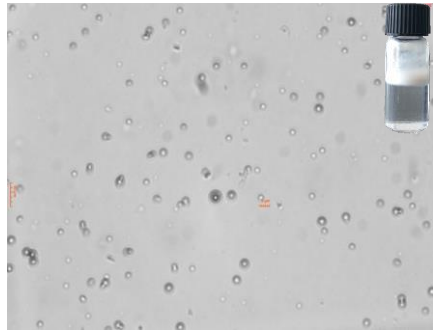
Shaking for 60 s



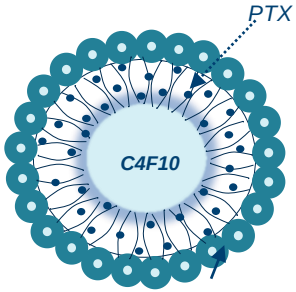
MBs Purified by centrifugation 500 rpm for 5 min (× 3 cycles)



PTX loaded Lipid MBs (Size: ~1.6 µm; MB Count: 3.8 × 10^9/ mL)



Microscopy



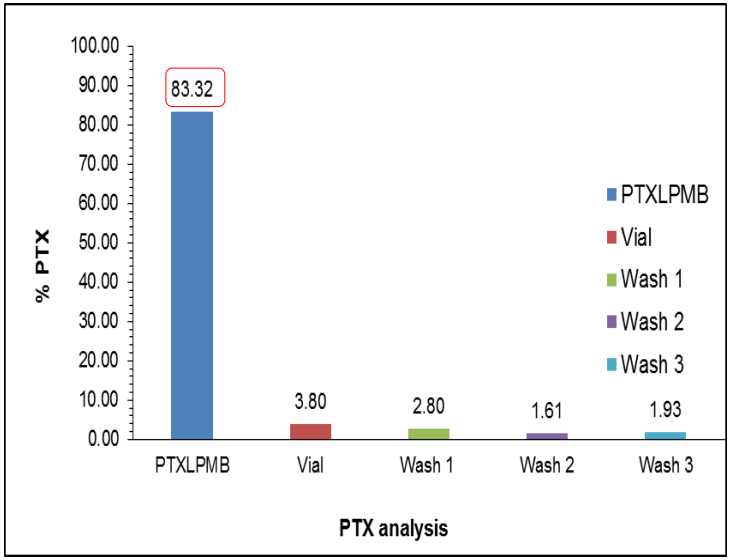
Lipid shell (DPPC, DSPE)

PTX loaded lipid MBs

Département de radiologie, radio-oncologie et médecine nucléaire
Faculté de médecine
Université de Montréal

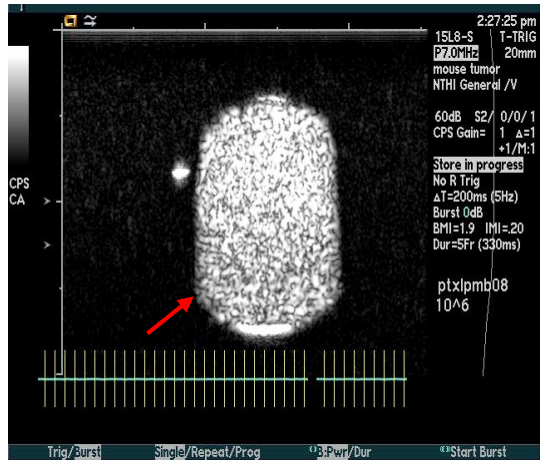
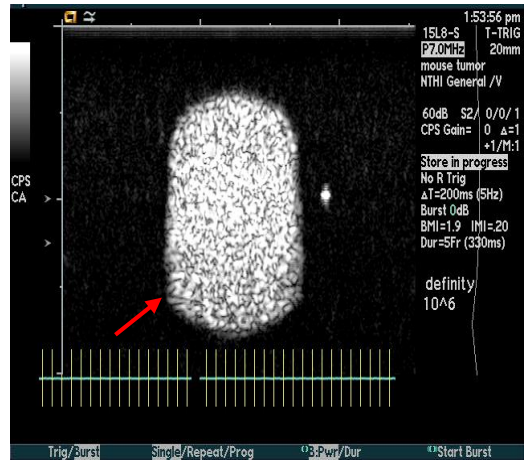
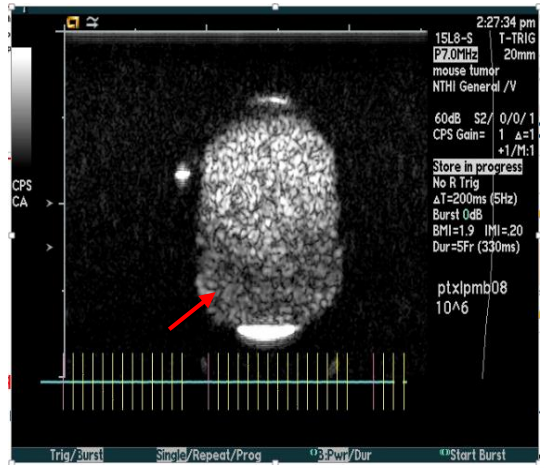
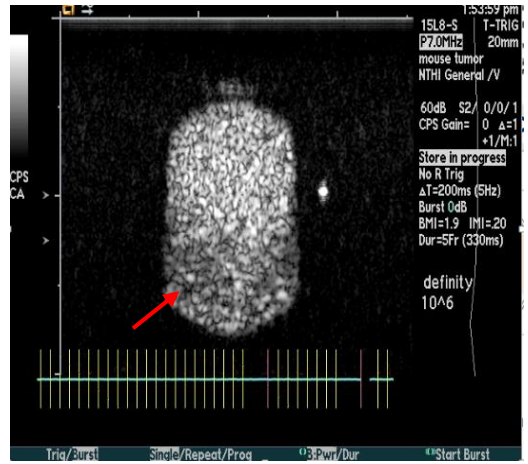
Paclitaxel (PTX) loaded Lipid Microbubbles (PTXLPMB)

PTX quantification by HPLC



PTX amount: 30,48 µg/10^8 MBs

Ultrasound Imaging

	PTXLPMB	DEFINITY® (Marketed MB)
Before US		
After US		

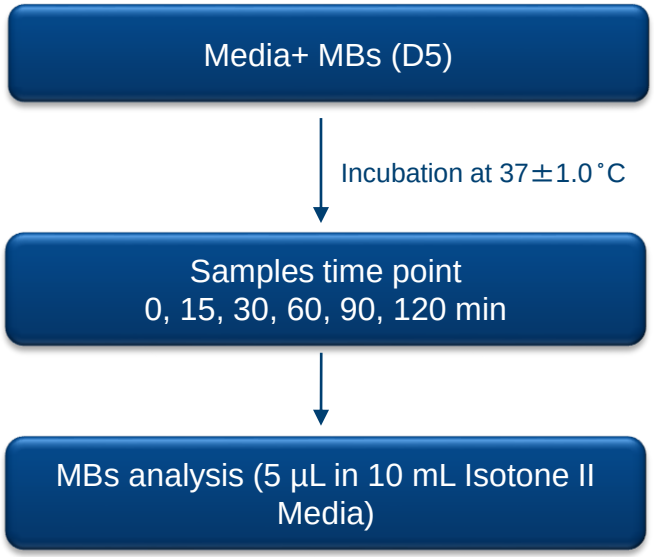
Département de radiologie, radio-oncologie
et médecine nucléaire
Faculté de médecine
Université de Montréal

PTXLPMB stability studies:

Stability study medium:

1). Cell culture media (DMEM + 10 % FBS, Sterile)
2). Pig Plasma (from Pig (Yorkshire/Landrace hybrid)

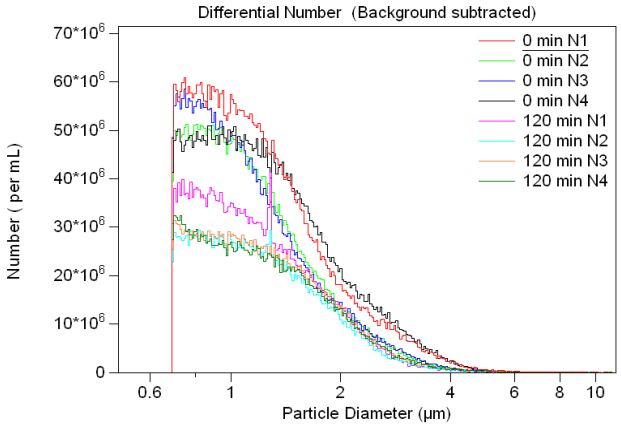
Protocol:

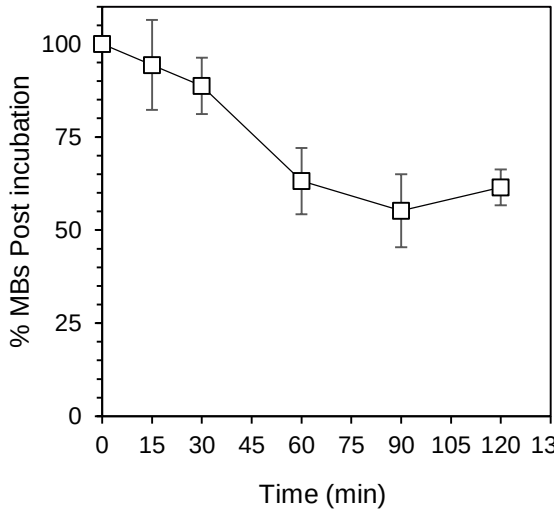


*D5:dilution 5 times with media

MB count and size analysis was performed using Beckman coulter counter

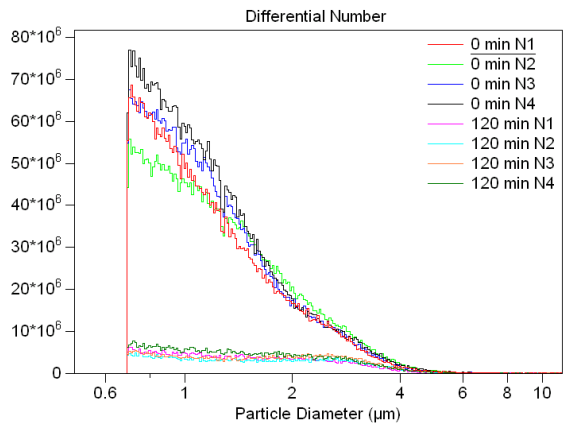
1. Cell culture media

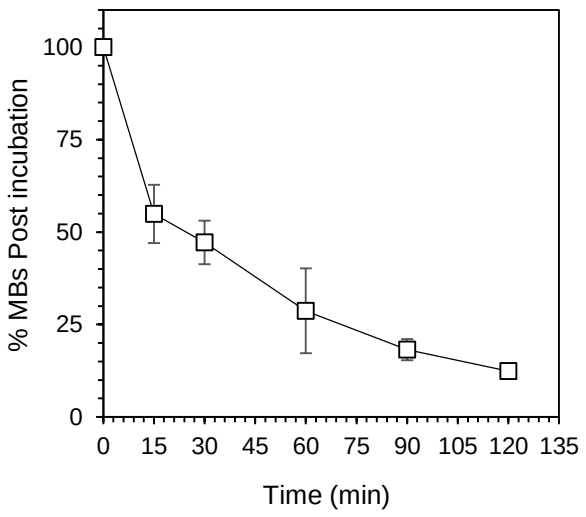




$t_{50\%}$ > 60 min

2. Pig plasma





$t_{50\%}$ > 15 min

Département de radiologie, radio-oncologie et médecine nucléaire
Faculté de médecine
Université de Montréal

Conclusion

- **MB and US targeted delivery** has the unique opportunity to **increase viral load** within the tumour tissues, which we believe will **strongly improve efficacy for cancer** therapy.

- We are expecting to have developed efficient techniques to incorporate viral sensitizing drugs, as well as OVs, into MBs and provided a proof of concepts showing that **OVs**, viral sensitizing drugs, **MB and US** are **complementary approaches to treat cancers.**

Acknowledgments

CRCHUM CENTRE DE RECHERCHE

MTL

Université de Montréal

New Frontiers in Research Fund
Fonds Nouvelles frontières en recherche

Département de radiologie, radio-oncologie et médecine nucléaire
Faculté de médecine
Université de Montréal