

# Active targeting and Small Molecule Delivery to Individual Leukemia Cells Utilizing Mesoporous Silica Nanoparticle-Supported Bilayers (Protocells)

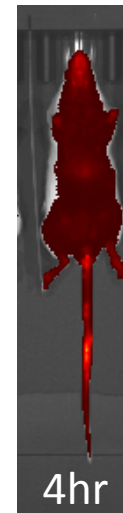
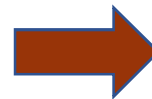
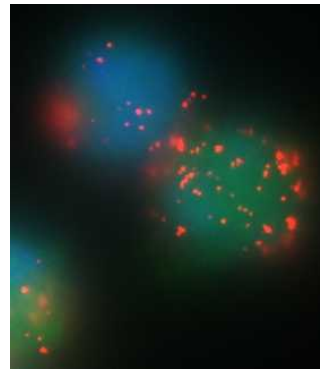
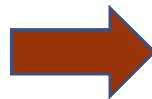
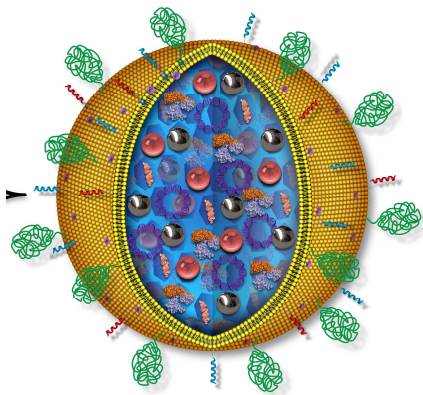
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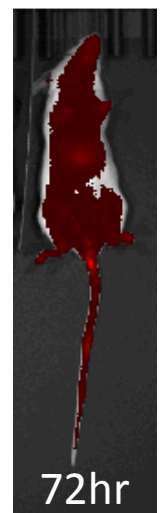
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4hr



72hr

# Challenges in Leukemia for Nanoparticle Therapeutics

The enhanced permeability and retention (EPR) effect where particles accumulate due to leaky vasculature has limited utility in this disease

Leukemia is a disseminated disease which makes **active** targeting advantageous to treat circulating cells

Active targeting is advantageous, but demands *in vivo* nanoparticle stability for prolonged circulation and binding to individual cells

Effective targeted nanocarrier for leukemia

- Uniform and controllable particle size and shape

- High colloidal stability under physiological conditions

- Minimal non-specific binding interactions

- High specificity for disease cells

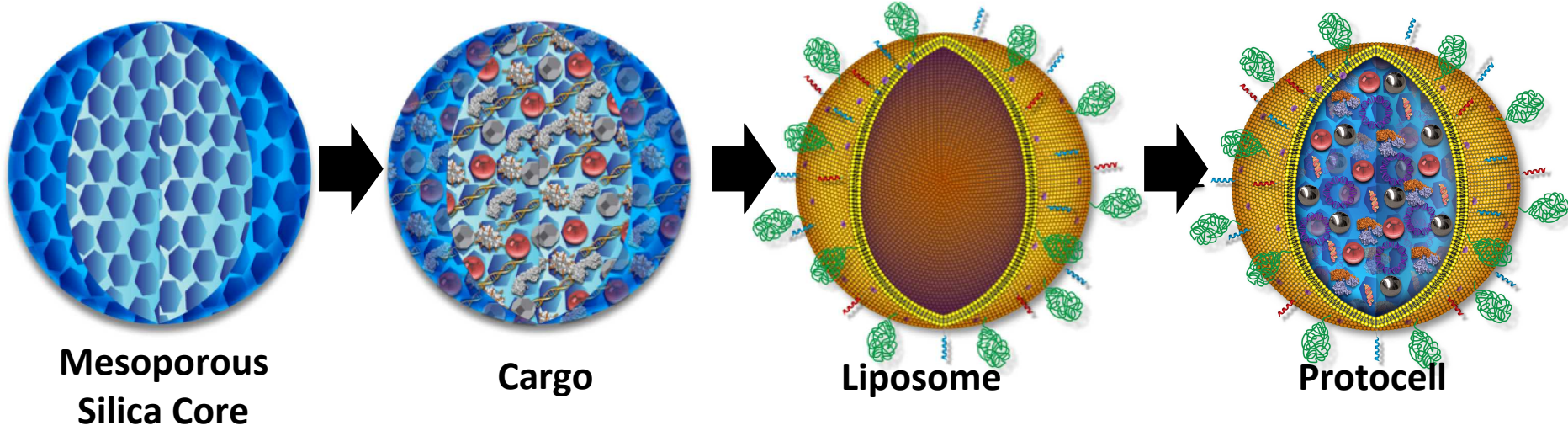
- High capacity for and precise release of diverse therapeutic cargos

- Low cytotoxicity

# 'Protocell' Nanoparticle Delivery Platform

Mesoporous silica NP encapsulated within lipid bilayer

Modular design enables engineering and optimization



Core:

Controlled: particle size, shape, pore size, pore surface chemistry

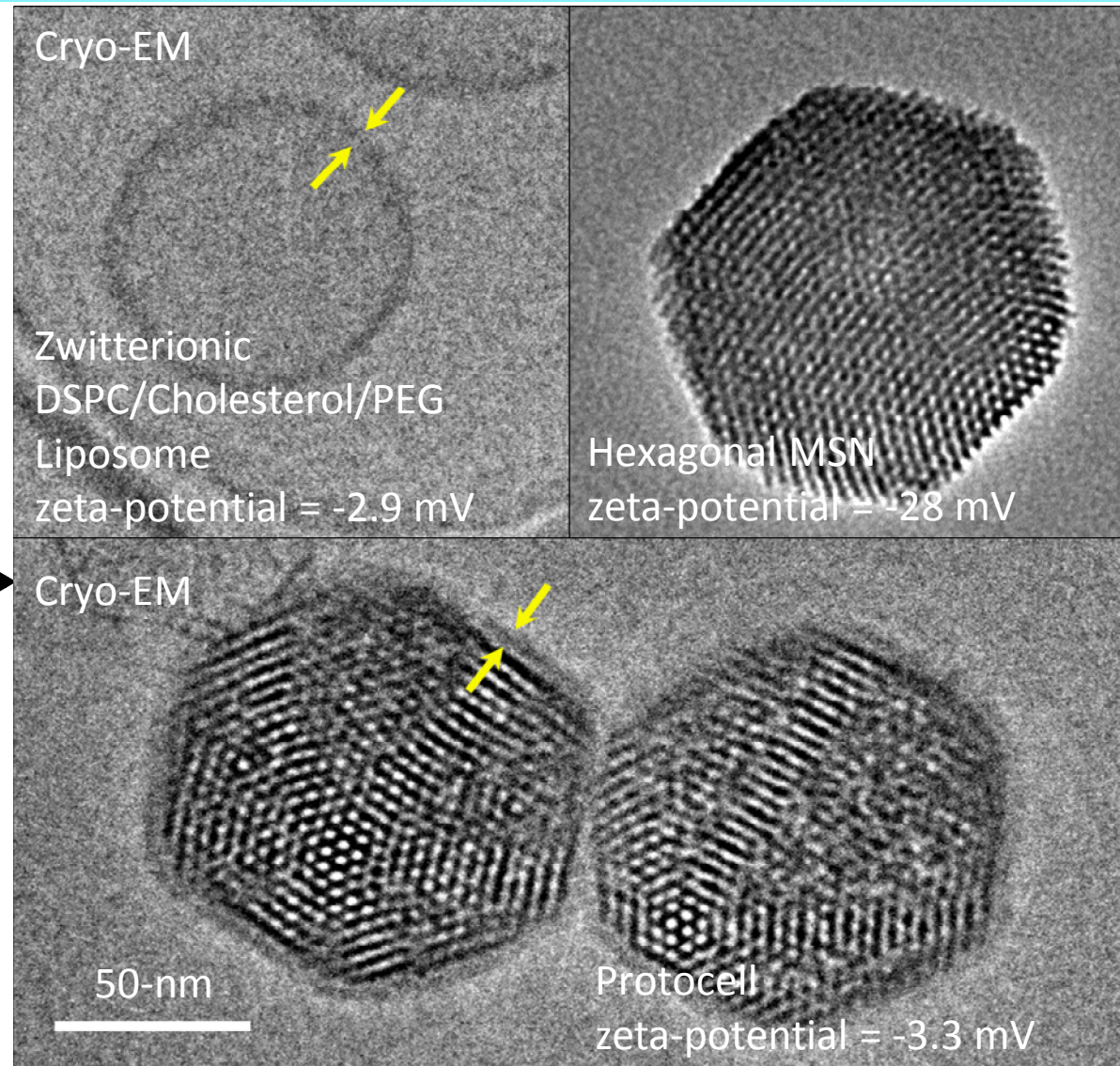
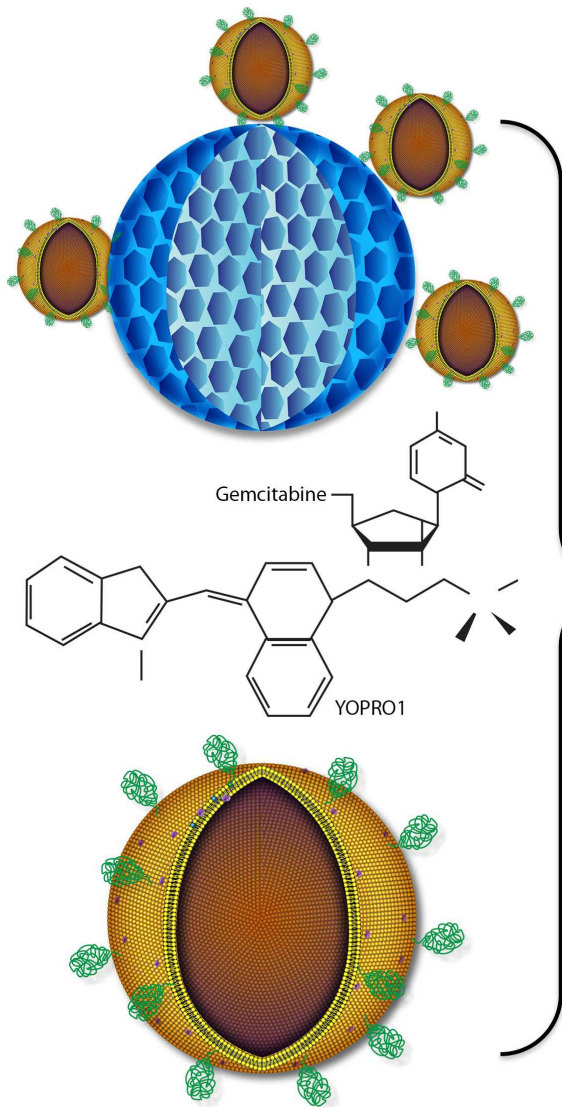
Cargo:

Variable cargo type, combinations, cargo loading and release

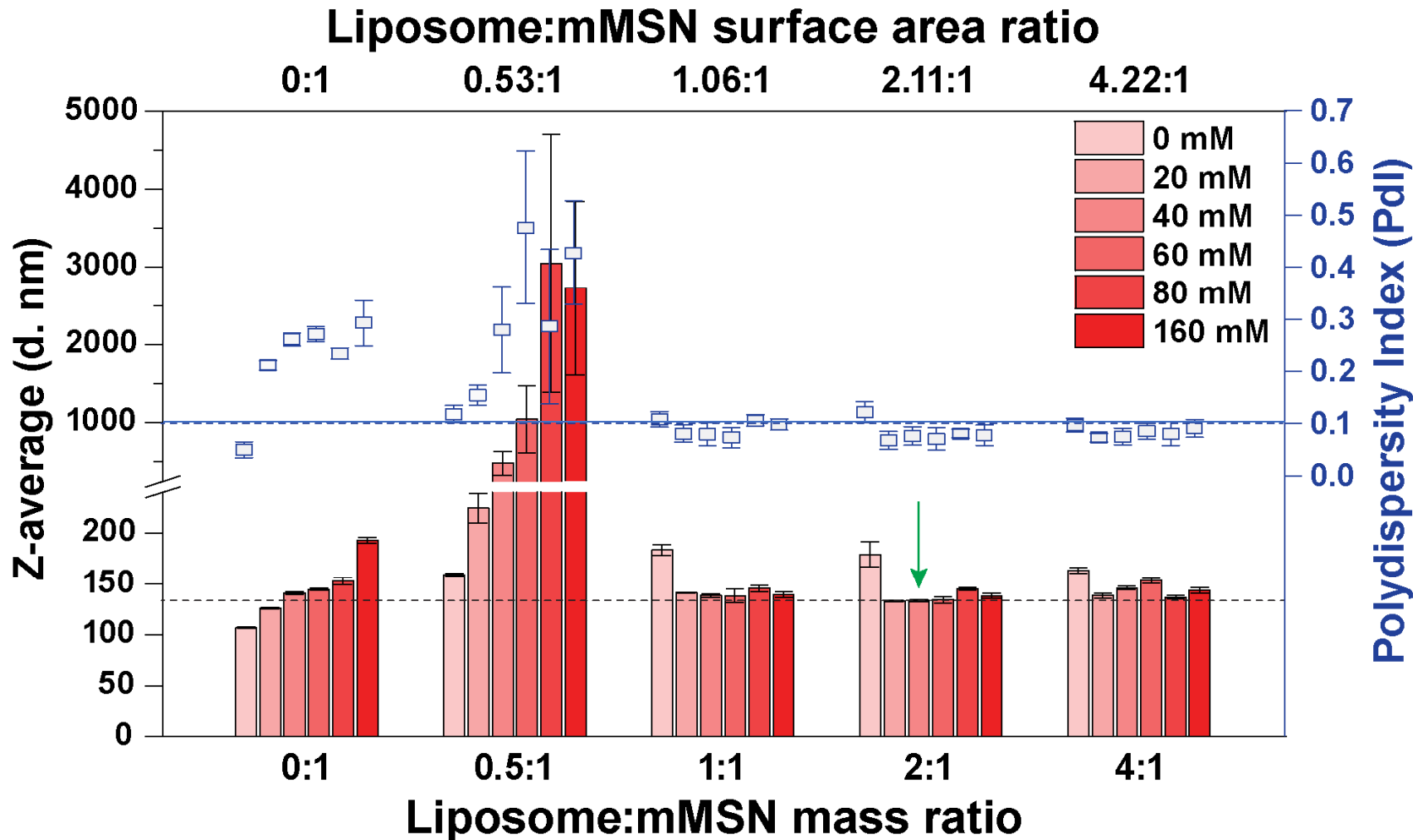
Liposome:

Lipid composition, PEG (or alternative), conjugation chemistry of targeting ligands, targeting ligand density

# We established robust conditions to create monosized and colloiddally stable protocells



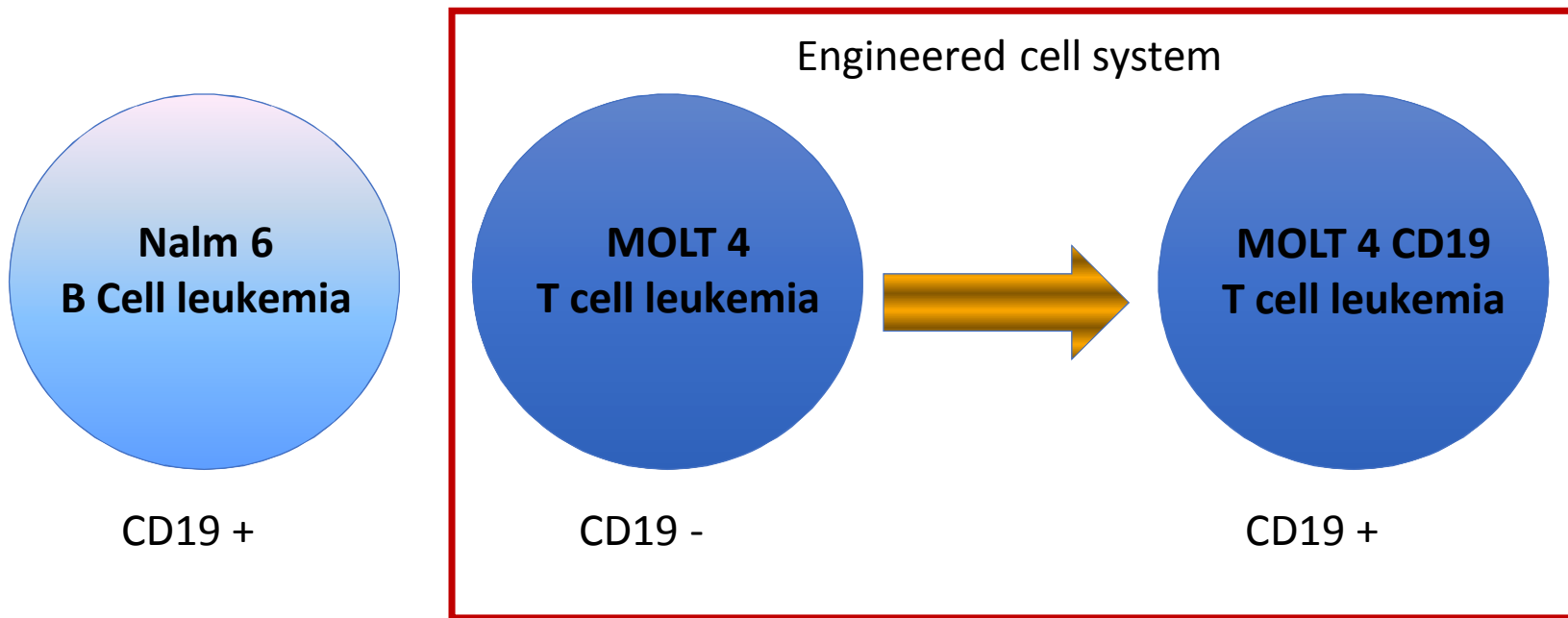
# Criteria for Formation of Monosized, Non-Aggregated Protocells



Criteria: small hydrodynamic diameter and Pdl below 0.1 (monosized)  
 Lipid:MSN Surface Area Ratios > 1:1 and Ionic Strength  $\geq$  20 mM\*

# Development of specific leukemia cell targeting: target selection and cell model system

**CD19** is a B-cell surface protein expressed throughout B-cell development and expressed on nearly all B-cell malignancies

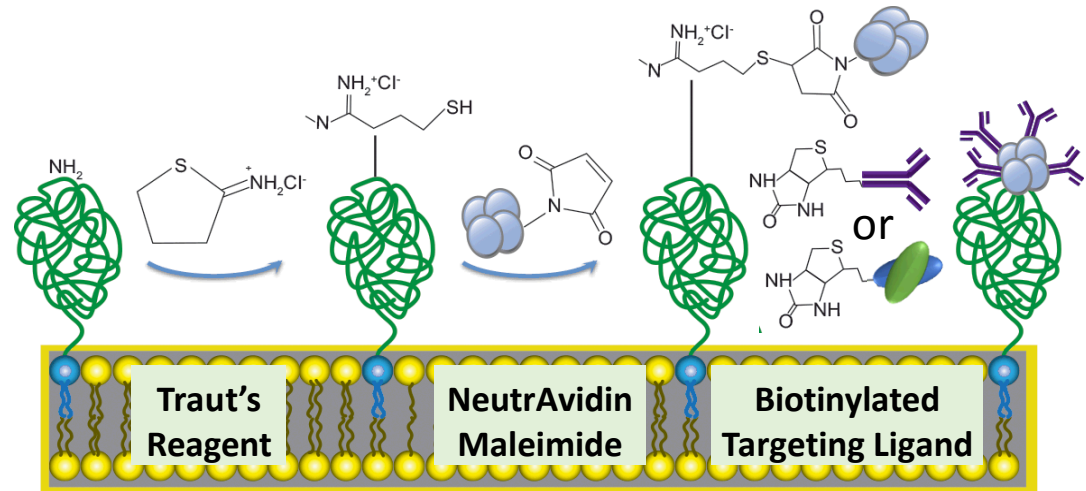


## Areas of Characterization

- Specific binding: Molt4 Parental versus the engineered Molt4 CD19
- Internalization of protocells
- Cargo delivery
- Selective drug delivery

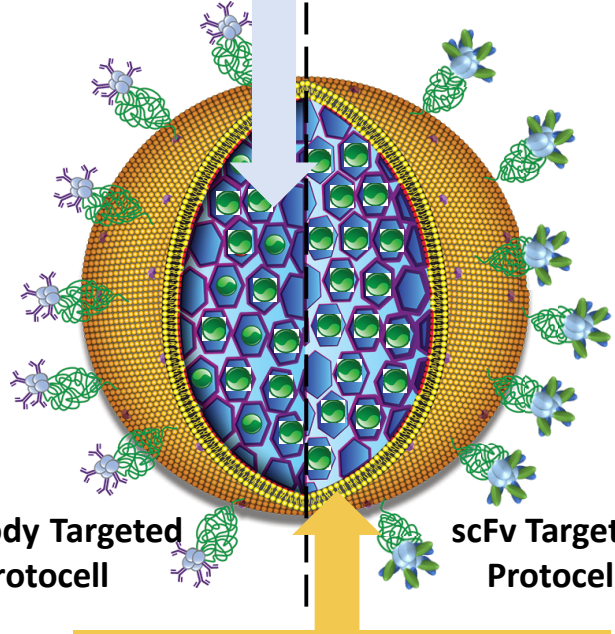
# Conjugation of targeting moieties to the protocell surface

After protocell formation, CD19 antibodies or scFvs were added to the surface to provide targeting



## Mesoporous Silica Core

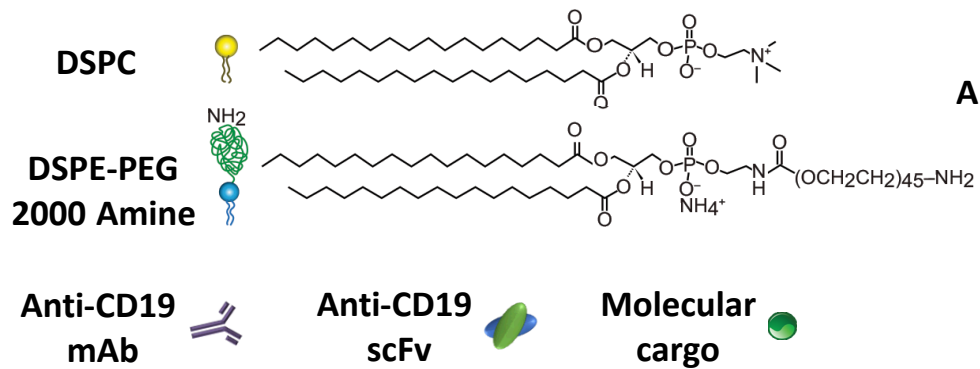
- Variable size
- Variable surface chemistry
- Variable shape
- Variable pore size
- Variable pore morphology



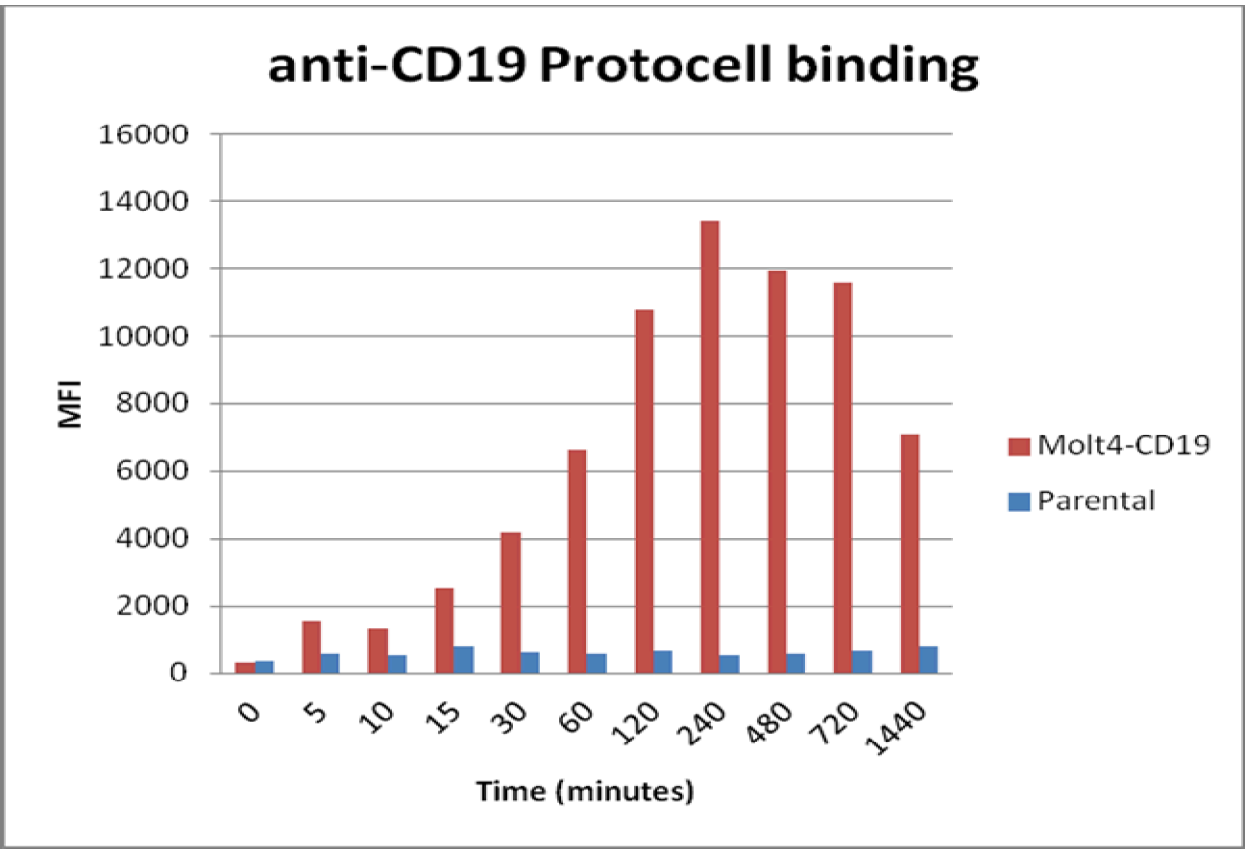
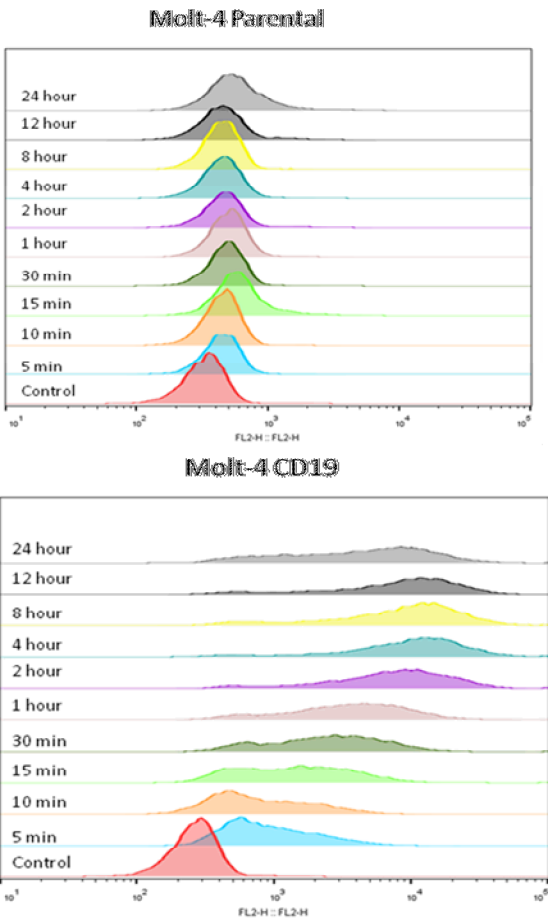
Antibody Targeted Protocell      scFv Targeted Protocell

## Supported Lipid Bilayer

- Variable fluidity
- Variable cholesterol content and extent of PEGylation



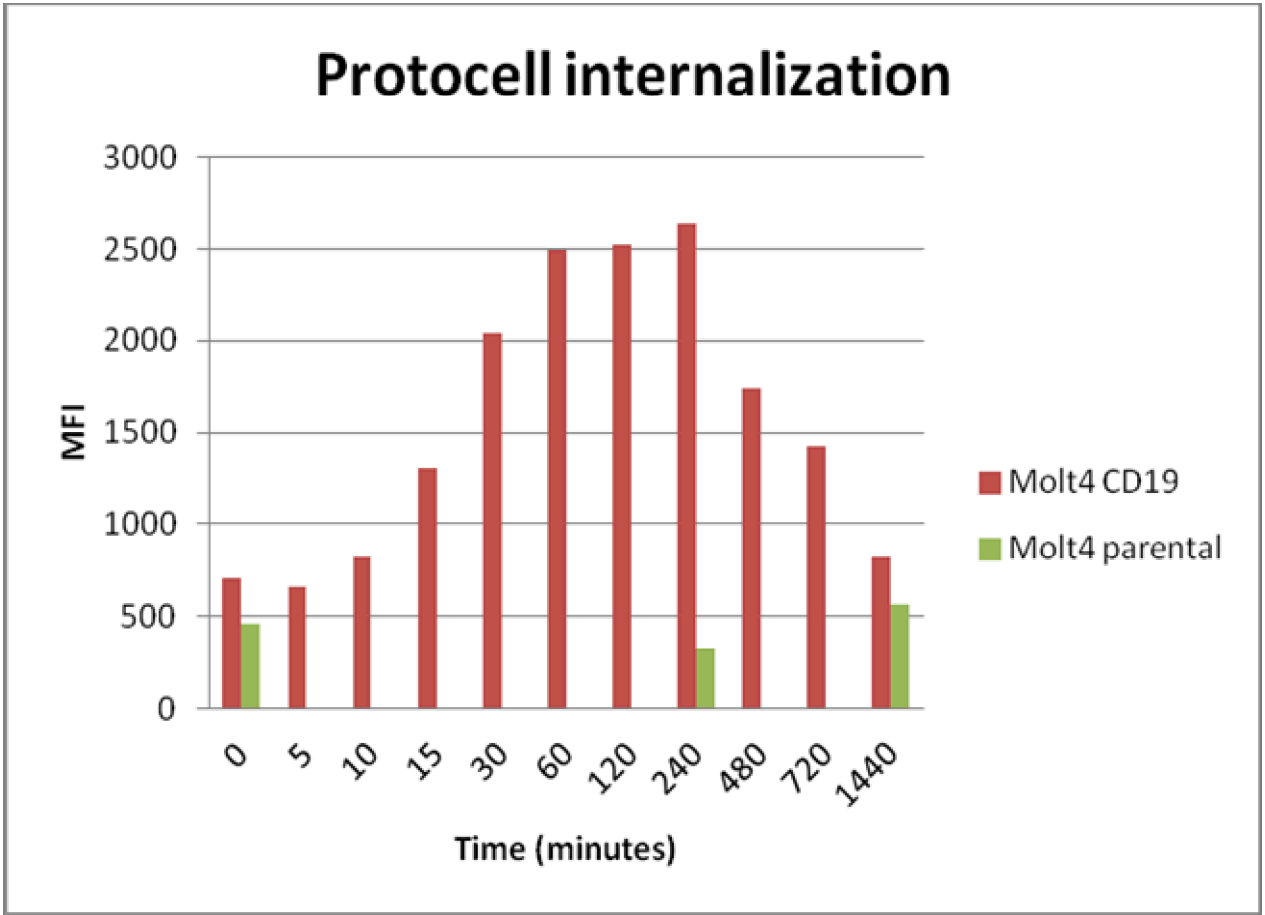
# Characterization of anti-CD19 mAb protocells binding to MOLT-4 cells



CD19 targeted protocells bind specifically to CD19 expressing cells and do not bind to cells which do not express CD19



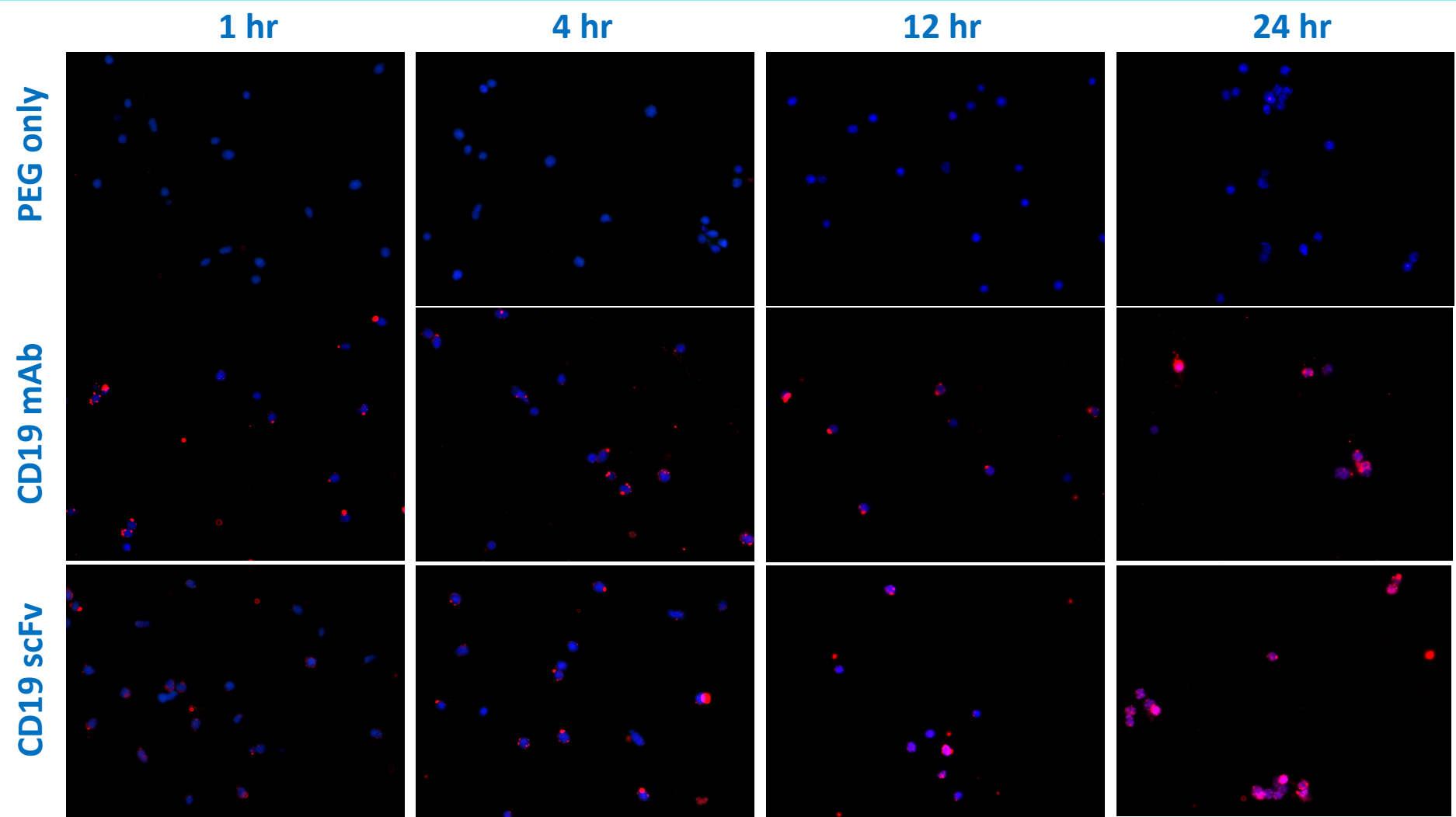
# Internalization of anti-CD19 mAb protocells binding to MOLT-4 cells



- Molt4 cells were exposed to anti-CD19 protocells for 1 hour on ice.
- Samples were washed to remove unbound protocells and transferred to 37C
- Samples were collected over time and a mild acid was used to remove surface associated protocells

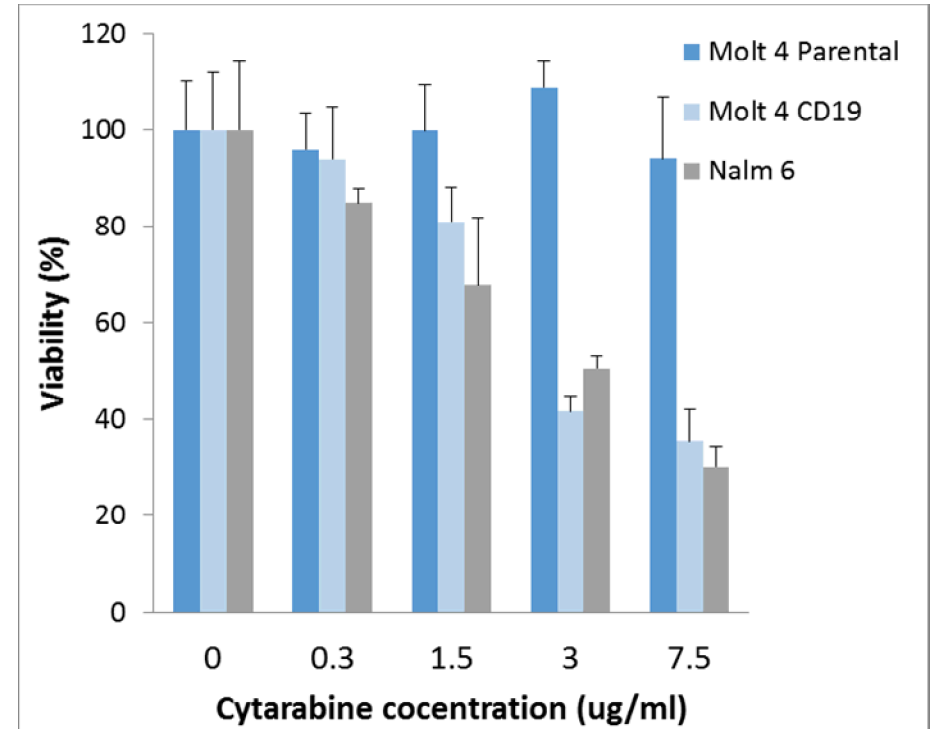
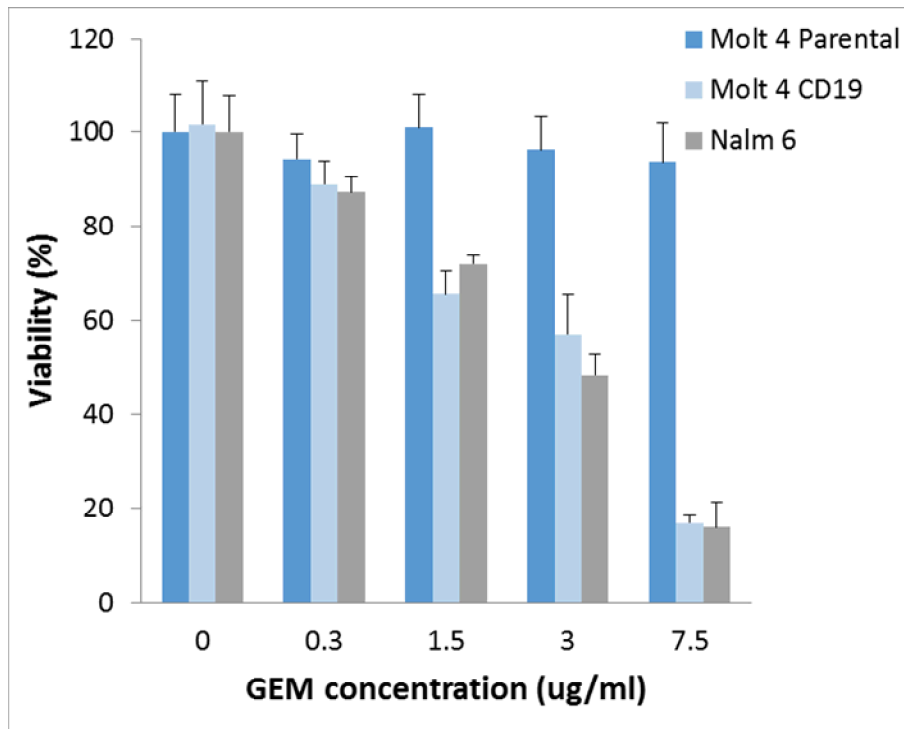
CD19 targeted protocells internalize overtime specifically into cells expressing CD19. Maximal internalization was at ~60 minutes.

# Binding of anti-CD19 protocells targeted by scFv or antibody to MOLT-4 CD19 cells



CD19 antibody and scFv targeted protocells (red) show strong binding by 60 minutes to CD19 expressing cells (blue). Non-targeted protocells do not bind CD19 expressing cells.

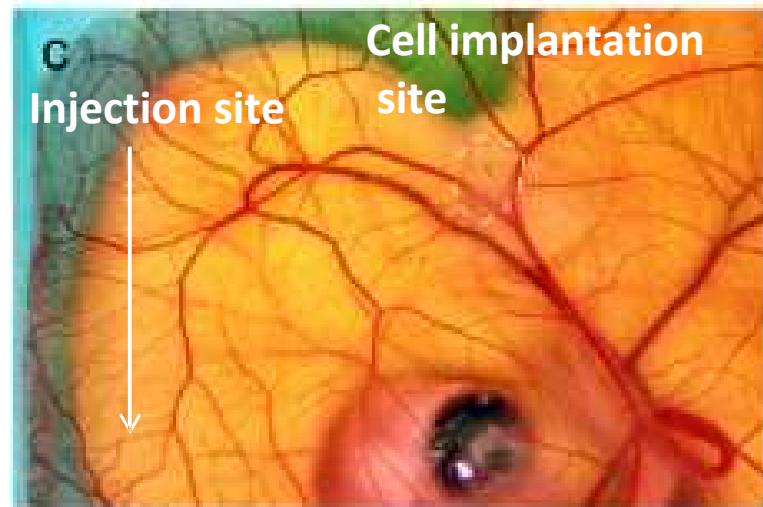
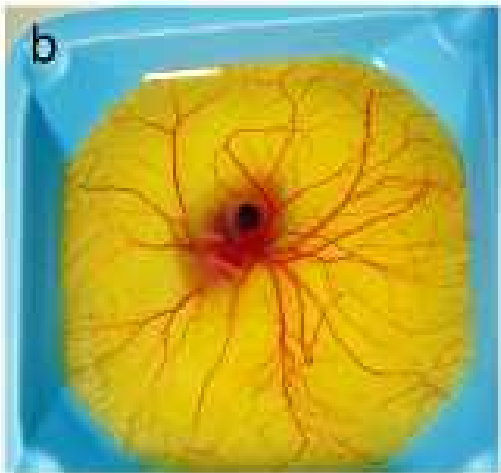
# Selective delivery of therapeutic agents by CD-19 antibody targeted protocells



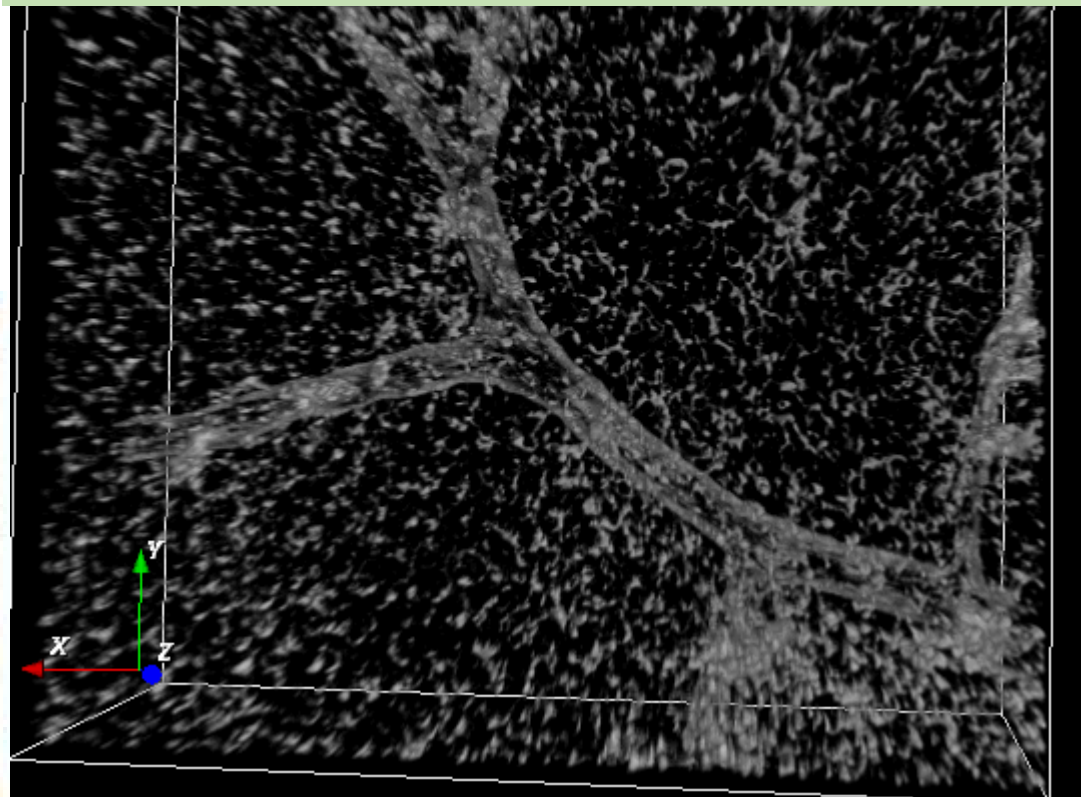
CD19 targeted protocells can specifically deliver loaded therapeutic agents, Gemcitabine and cytarabine, to cells expressing CD19 on the cell surface (Molt4 CD19 and Nalm6) while sparing cells which do not express CD19 (Molt 4 parental)

# Chicken Chorioallantoic membrane (CAM) model for understanding vascular behavior

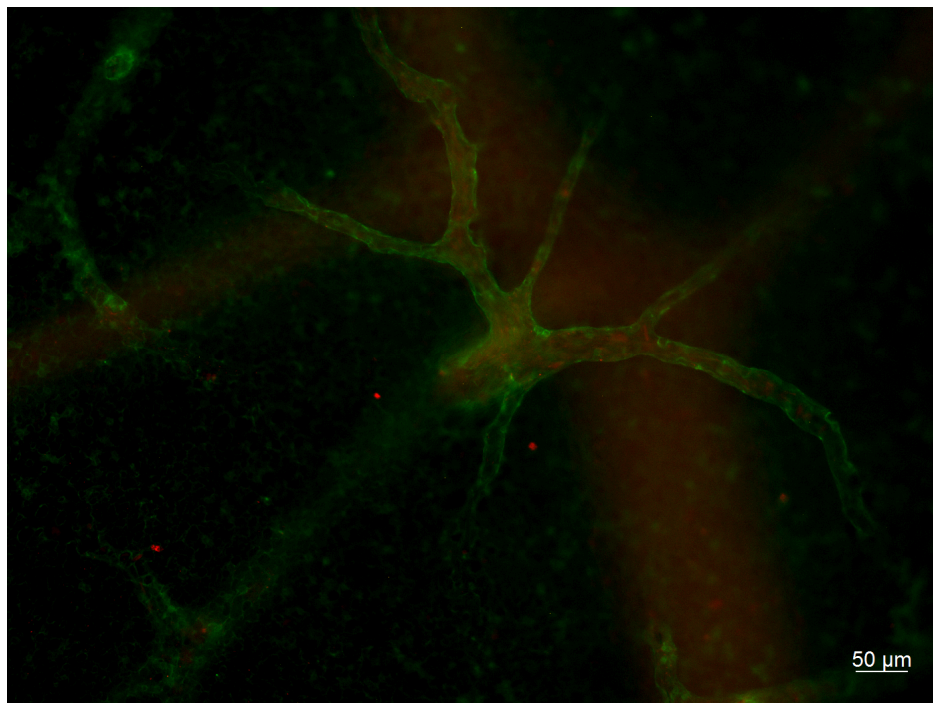
Avian embryo grown *ex ovo*



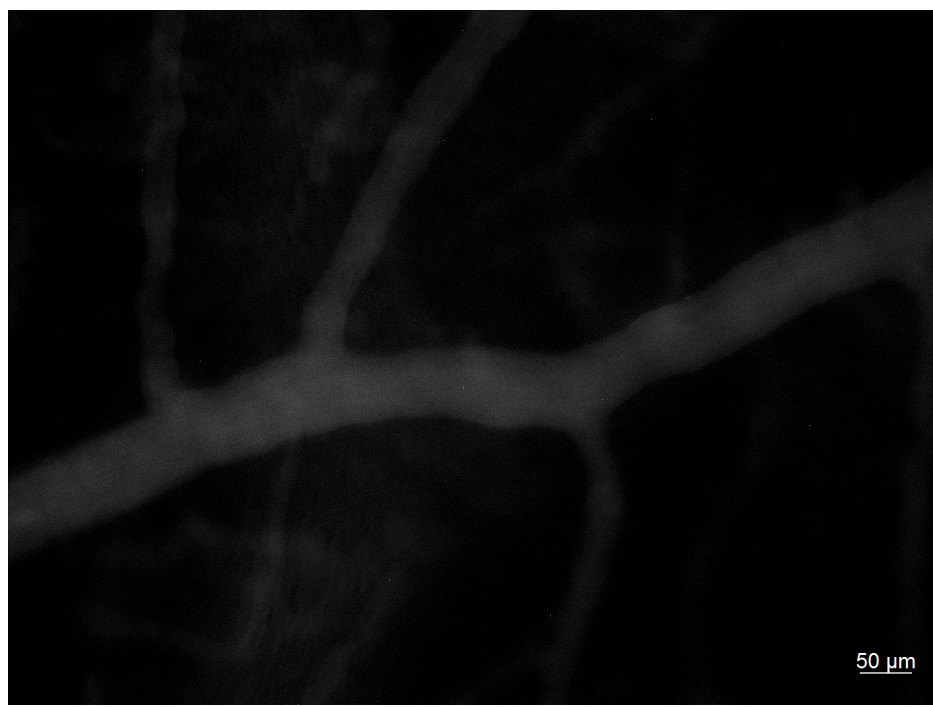
Highly vascularized, easily accessible system to examine protocell flow characteristics, target cell interaction and cargo delivery in real time while under the influence of blood flow utilizing intravital microscopy.



# Protocell behavior in complex, pulsatile blood flow



Protocells (red) flow in the CAM vascular system  
Vessels are stained green



Protocells flow in the CAM vascular system.

CAM has pulsatile flow that allows assessment of flow dynamics of fluorescently labeled protocells in a complex environment including endothelial cells, blood flow and serum proteins.  
Protocells show limited non-specific interaction with vessels and free circulation

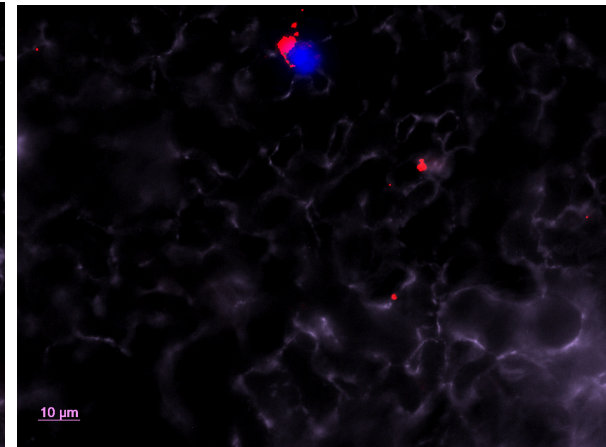
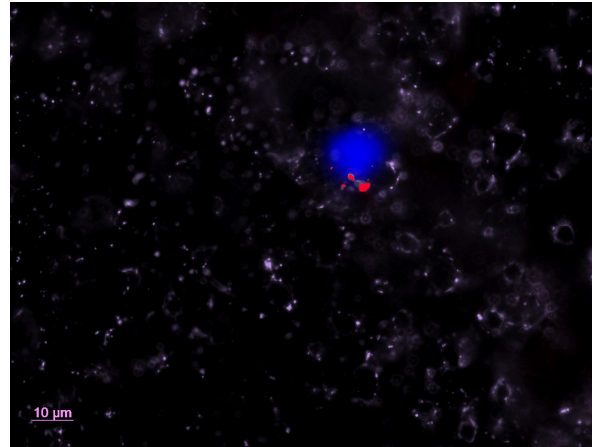
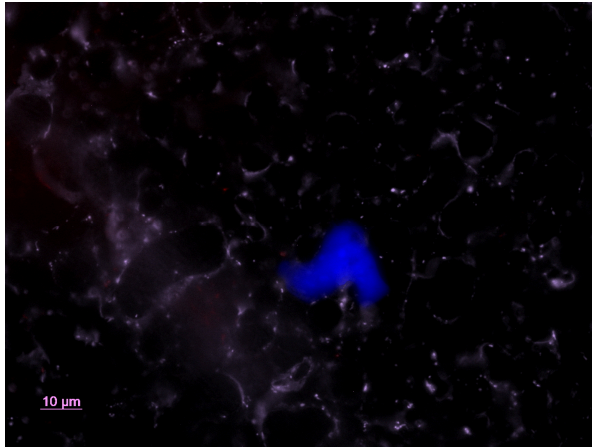
# Both anti-CD19 mAb- and anti-CD19 scFv- targeted protocells exhibit specific binding to natural and engineered CD19 expressing cells in the CAM vascular model at 4 hours post injection

Molt 4 Parental

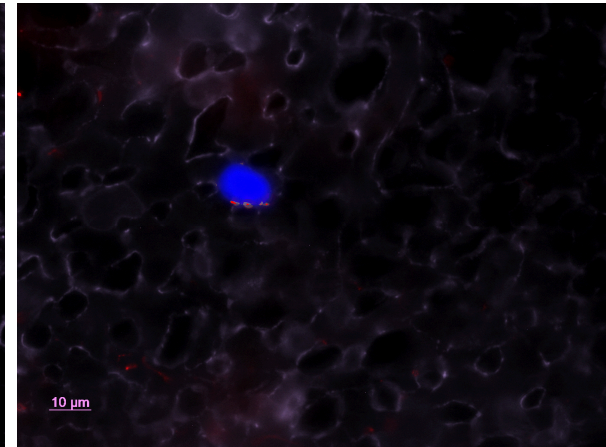
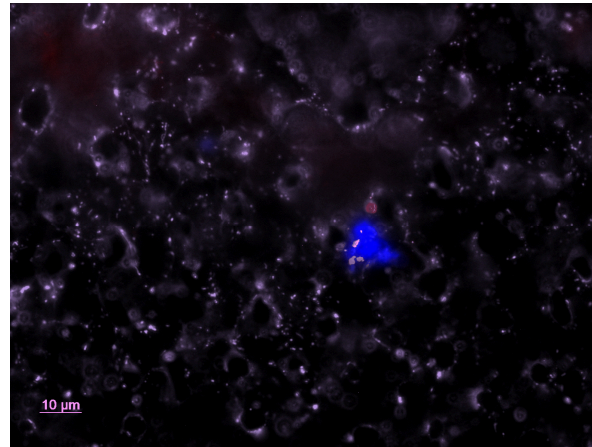
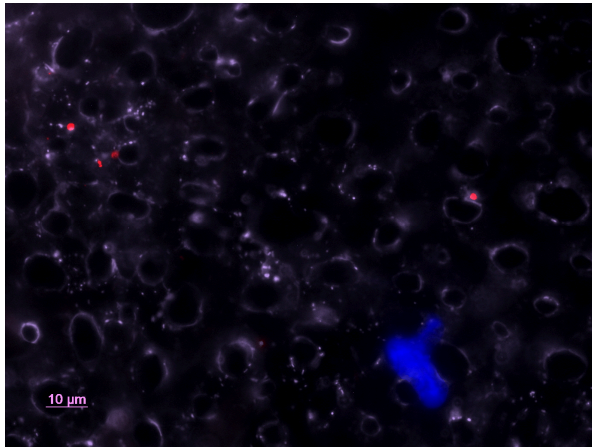
Molt 4 CD19

Nalm 6

CD19 scFv

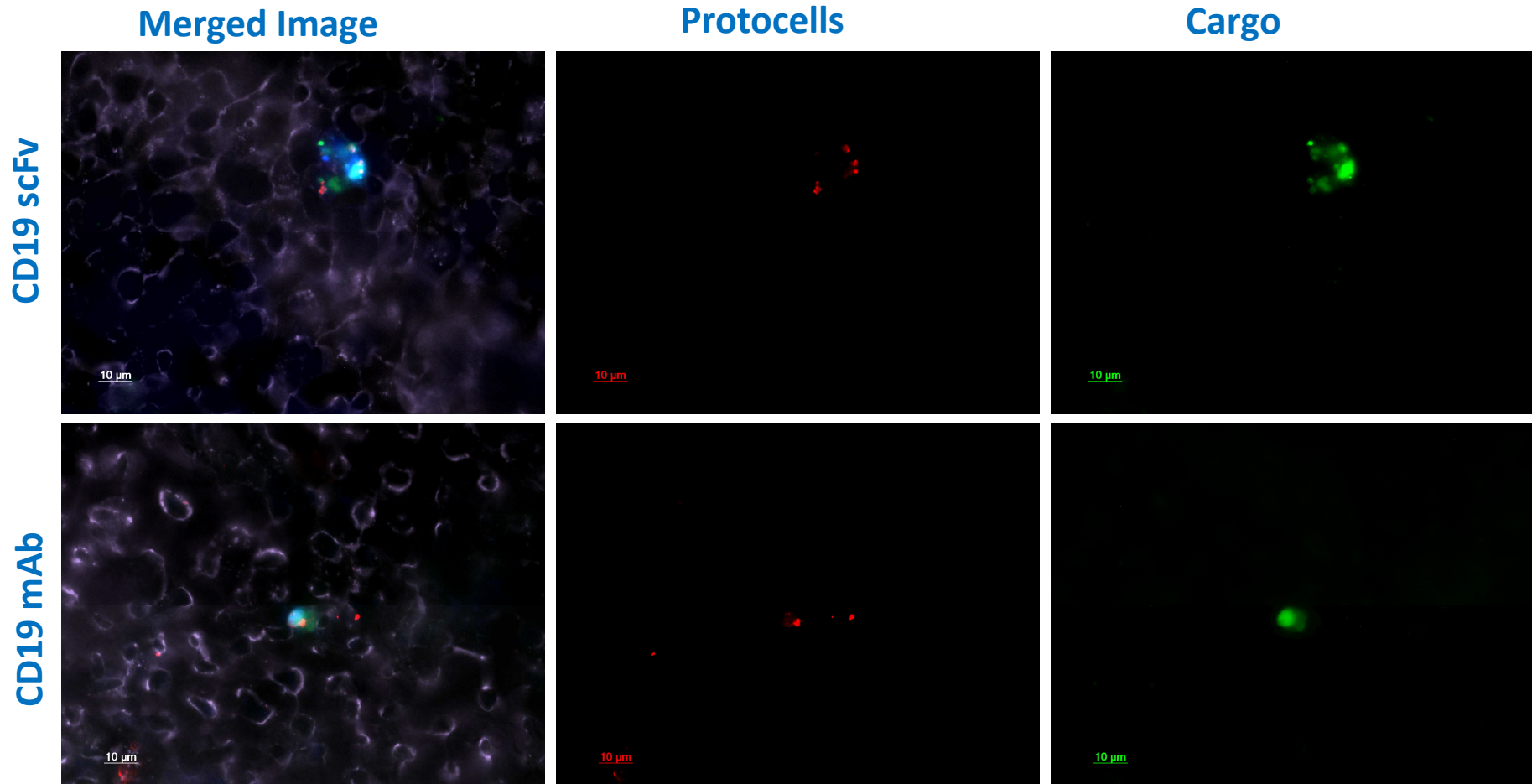


CD19 mAb



CD19 targeted protocells, using either CD19 antibody or scFv, can specifically target CD19 expressing cells with a vascularized system

Both anti-CD19 mAb- and anti-CD19 scFv- targeted protocells deliver a cell impermeant drug surrogate (YOPRO) to the Nalm 6 naturally CD19 expressing cell in the CAM vascular system after 20 hours



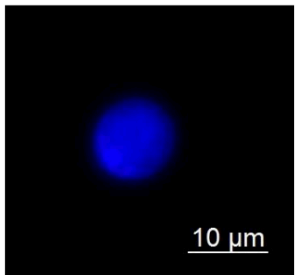
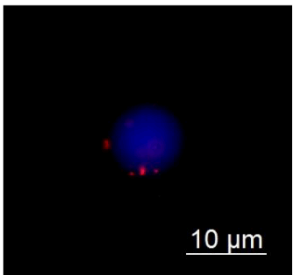
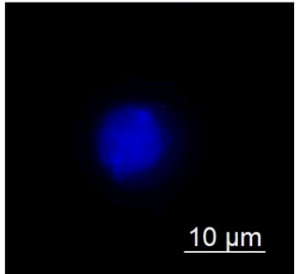
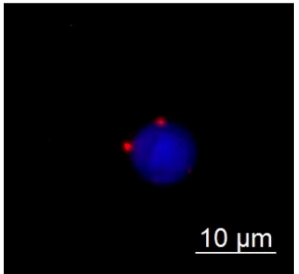
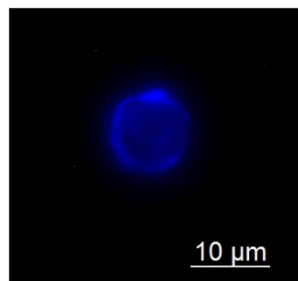
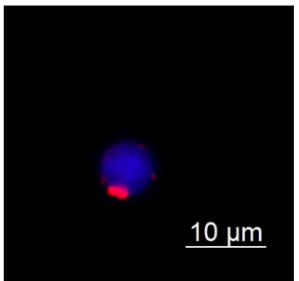
CD19 targeted protocells, targeted with either antibody or scFv, can deliver cargo (fluorescent drug mimic) to cells targeted within the vascular system

# Selective binding of protocells to patient leukemia samples which express CD19 on the cell surface

## Patient 1

Pretreatment  
CD19 positive

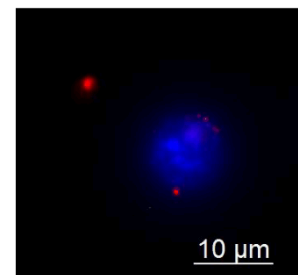
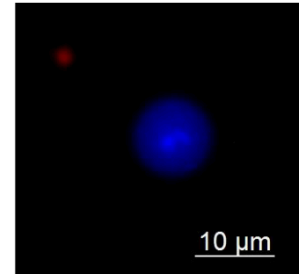
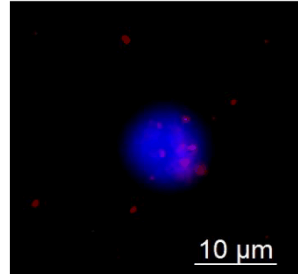
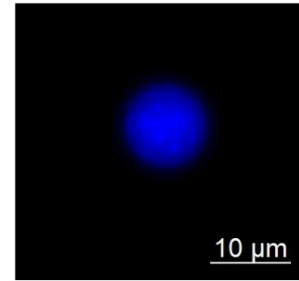
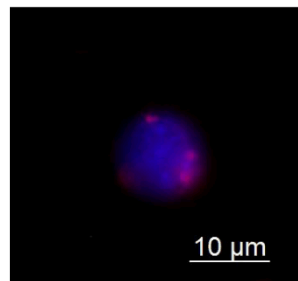
Post-relapse  
CD19 negative



## Patient 2

Pretreatment  
CD19 positive

Post-relapse  
CD19 negative

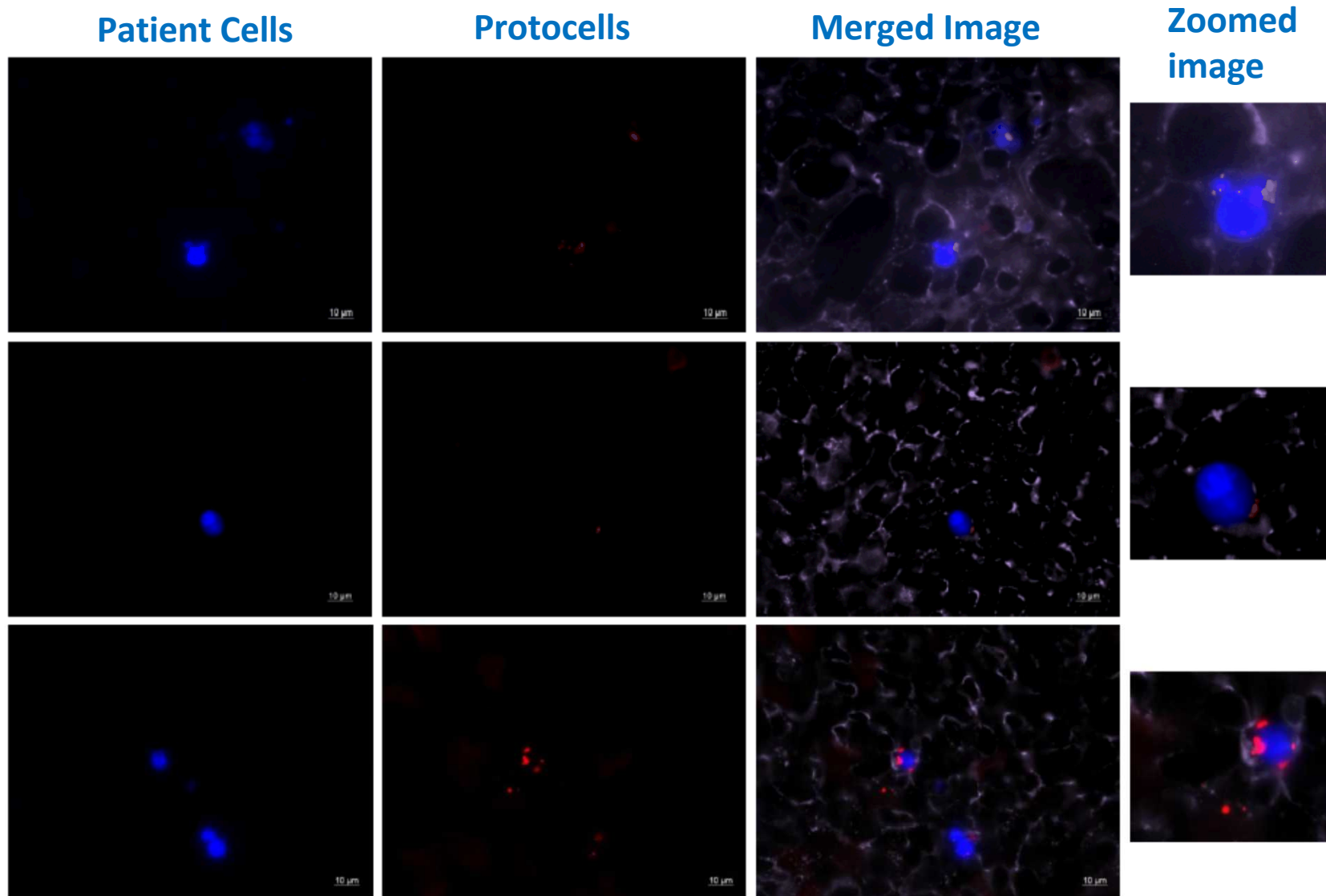


CD19 antibody targeted protocells can selectively bind to primary patient samples that express CD19

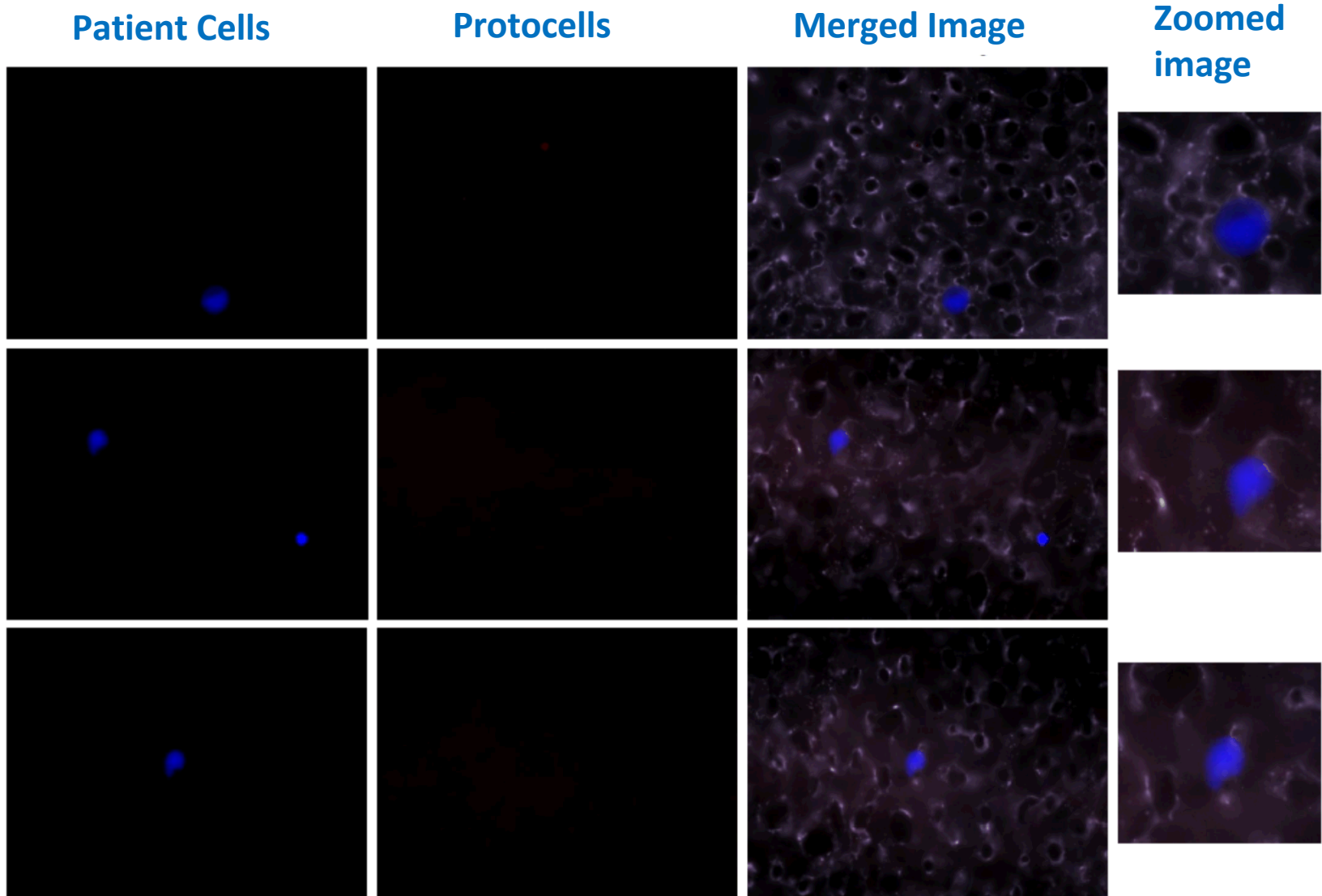
CD19 antibody targeted protocells do not bind primary patient samples that do not express CD19



# Targeted binding of anti-CD19 protocells to primary patient leukemia samples expressing CD19



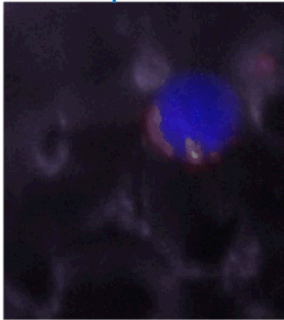
# Anti-CD19 protocells are unable to target primary patient leukemia samples which do not express CD19



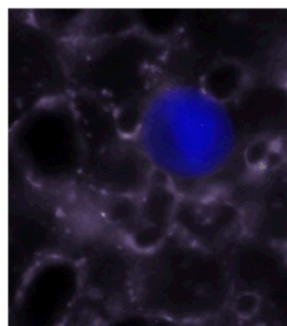
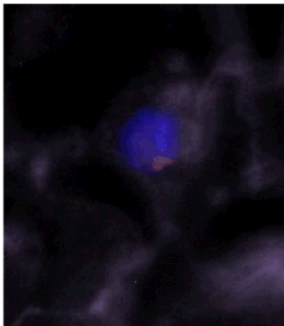
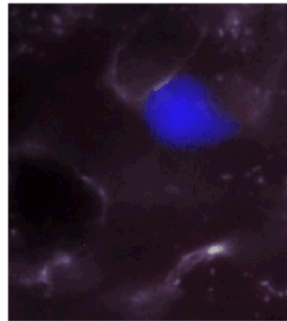
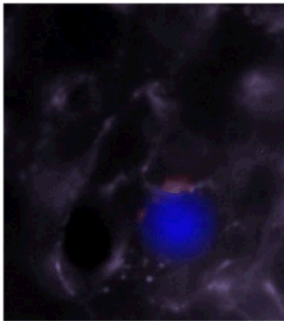
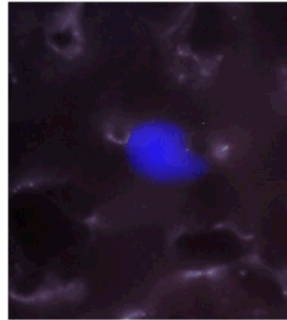
# CD19 antibody targeted protocells show selective binding of patient samples that express CD19 in a vascularized model

## Patient 1

Pretreatment  
CD19 positive

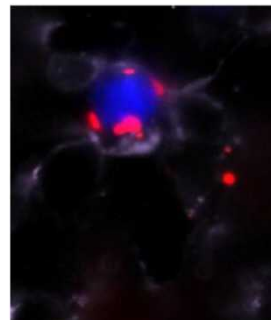


Post-relapse  
CD19 negative

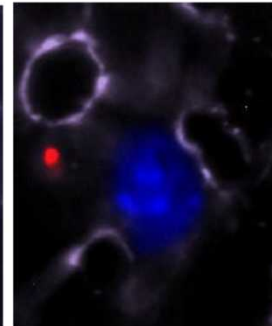
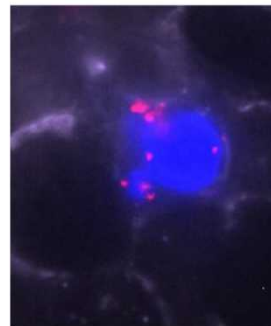
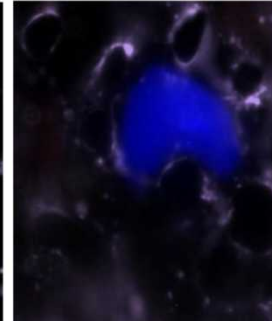
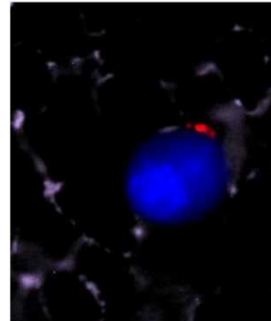
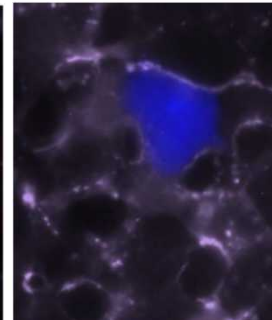


## Patient 2

Pretreatment  
CD19 positive

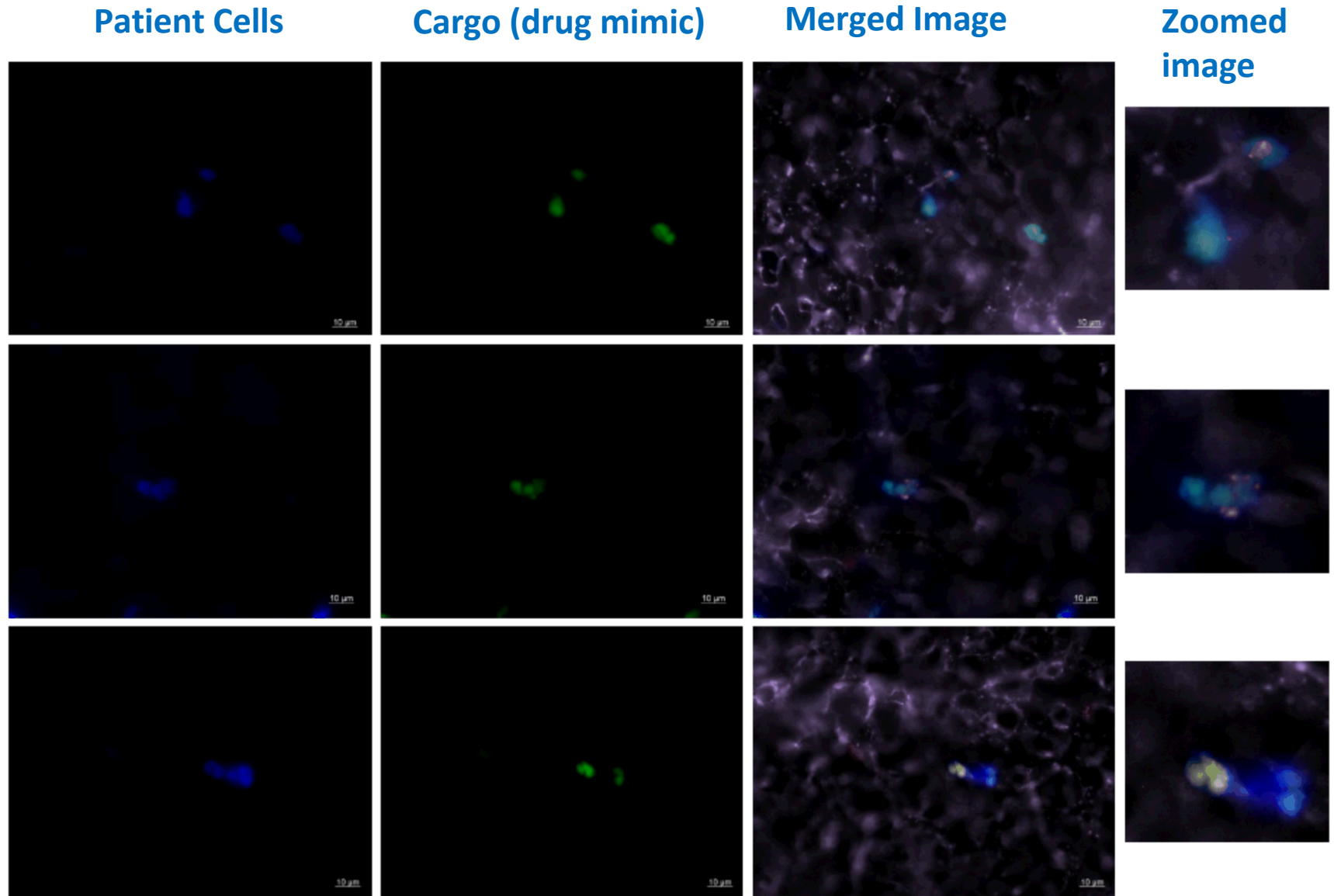


Post-relapse  
CD19 negative



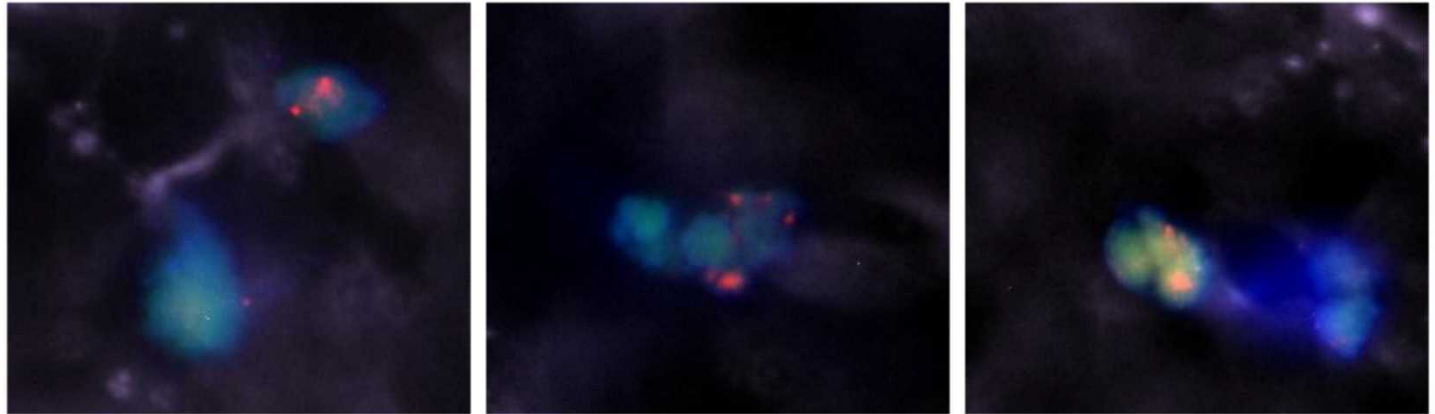
CD19 antibody targeted protocells can selectively bind to primary patient samples that express CD19

# Anti-CD19 protocells are able to deliver fluorescent cargo (drug mimic) to primary patient cells within the CAM system after 30 hours

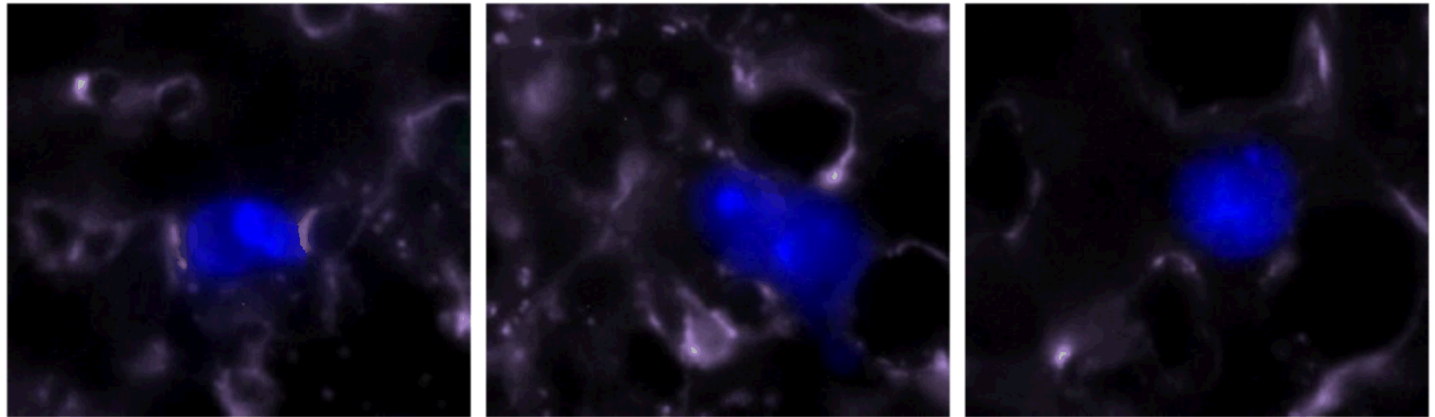


# Anti-CD19 protocells are able to deliver fluorescent cargo (drug mimic) to primary patient cells within the CAM system is selective

Pre-treatment  
CD19 positive

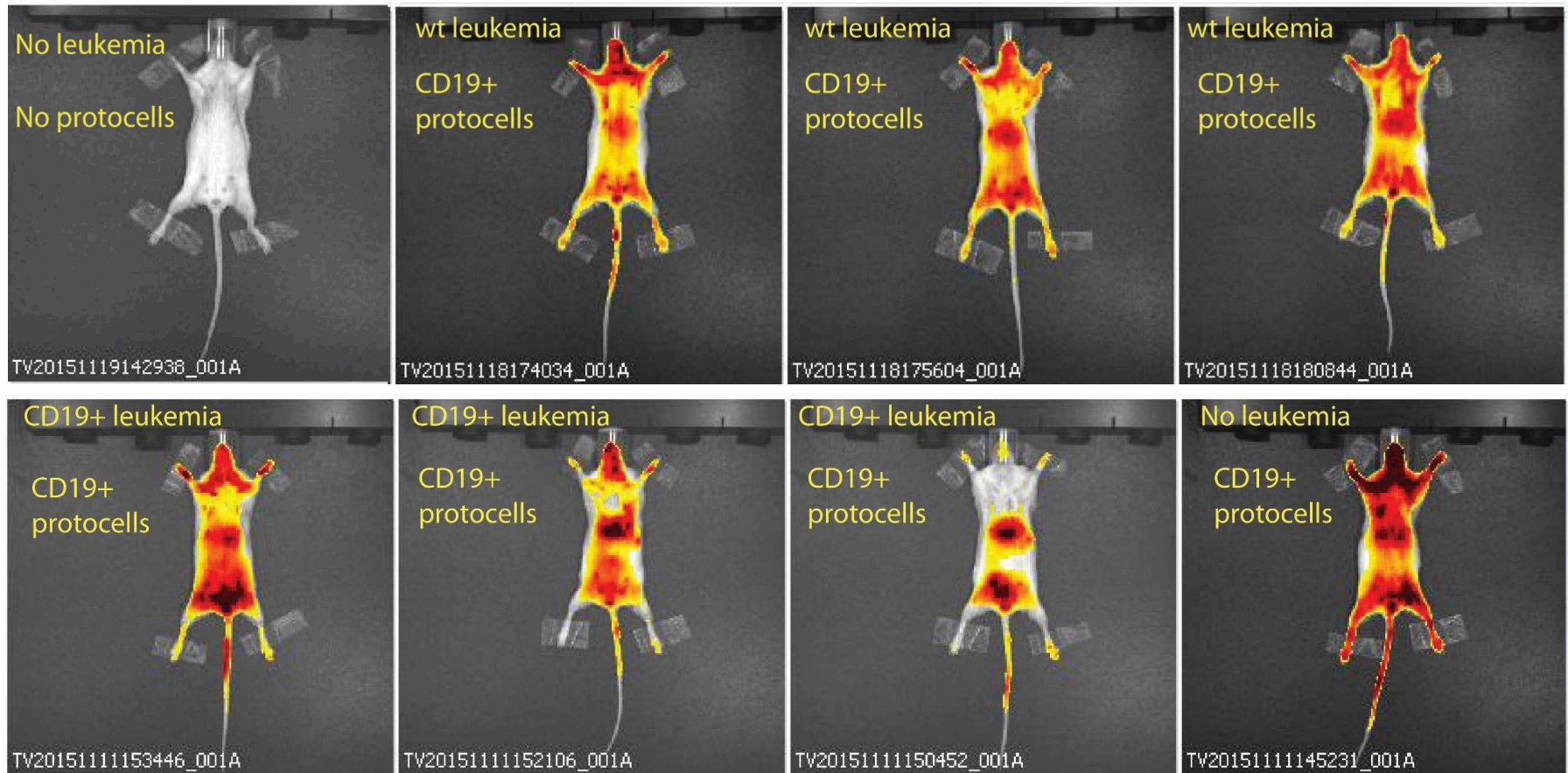


Post relapse  
CD19 negative



Cargo delivery by CD19 antibody targeted protocells is selective or the presence of the CD19 surface protein

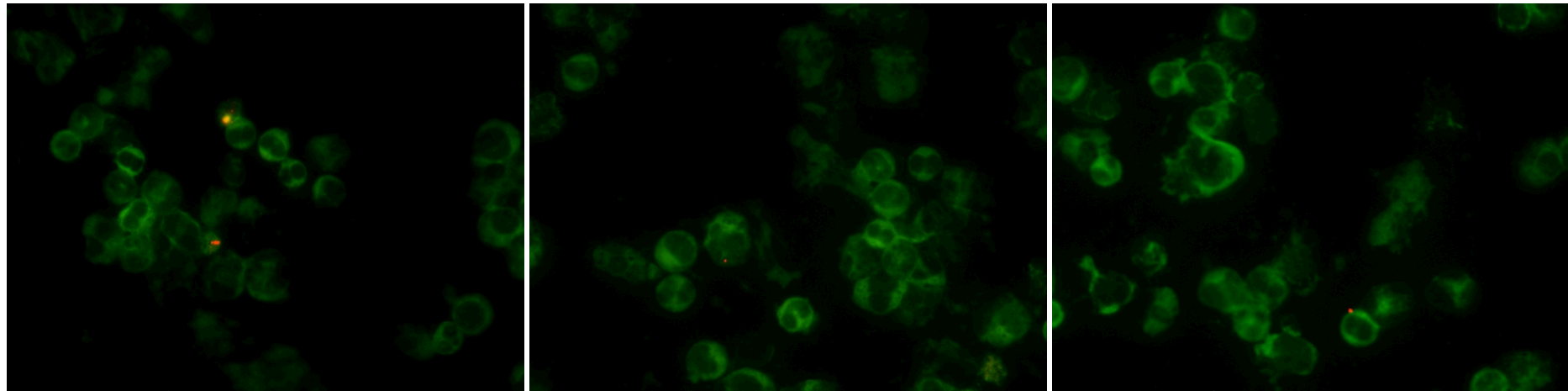
# CD19 antibody targeted protocells have prolonged circulation times in mice



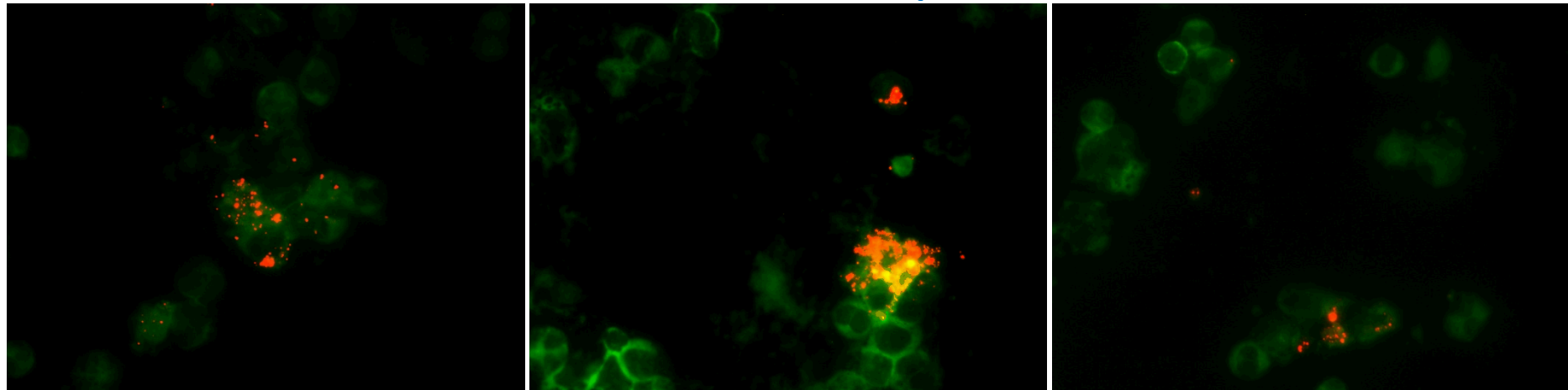
CD19 antibody targeted protocells are still present in circulation and throughout the body >48hours post injection

# Bone marrow samples from mice exposed to CD19 antibody targeted protocells and Click Beetle Green labeled Molt4 and Molt4 CD19 cells

## Molt4 Parental cells, CD19 negative



## Molt4 CD19 cells, CD19 positive



CD19 antibody targeted protocells (red) have enhanced association with Molt4 CD19 expressing cells collected from mouse bone marrow (bottom row)

# Summary and Ongoing work

## Summary

- CD19 targeted protocells can selectively bind and deliver therapeutic cargo *in vitro*
- CD19 targeted protocells flow and do not bind to endothelial cells in the vascularized CAM model
- CD19 targeted protocells can selectively bind and deliver fluorescent cargo to both cell lines and patient samples through the vasculature of the CAM system
- CD19 targeted protocells circulate for prolonged periods in mice and can selectively targeted CD19 expressing leukemia cells in mice

## Ongoing work

- Studies to determine the circulation half life and body clearance of the protocells in mouse models
- Therapeutic efficacy against leukemia cell lines and primary patient samples in mouse models



# Acknowledgements



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- New Mexico Cancer Nanoscience and Microsystems Training Center (CNTC), George D. Montoya Research Scholarship, Edmund J. and Thelma W. Evans Charitable Trust Scholarship, and the Charlotte and William Kraft Graduate Fellowship

## THANK YOU FOR YOUR TIME!

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