Specific Targeting and Delivery of Therapeutics to Cancer Cells Based on the Tumor Microenvironment

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Department of Chemistry

BioE 2 (Sept. 13, 2019)

Anticancer Drugs: Side Effects

- Most anticancer drugs have off-target side effects.
- Severely limit the efficacy of chemotherapy.

Site of toxicity	Cyclophosphamide	Methotrexate	Fluorouracil	Vincristine	Doxorubicin
Bone marrow	■■	■■■	■■■	■	■■■
Digestive track	■■	■■	■■	■	■■
Skin, hair	■■	■	■	■■	■■
Heart					■■■
Kidney		■■		■	
Liver	■	■■			
Nervous System			■	■■■	
Lung	■	■			

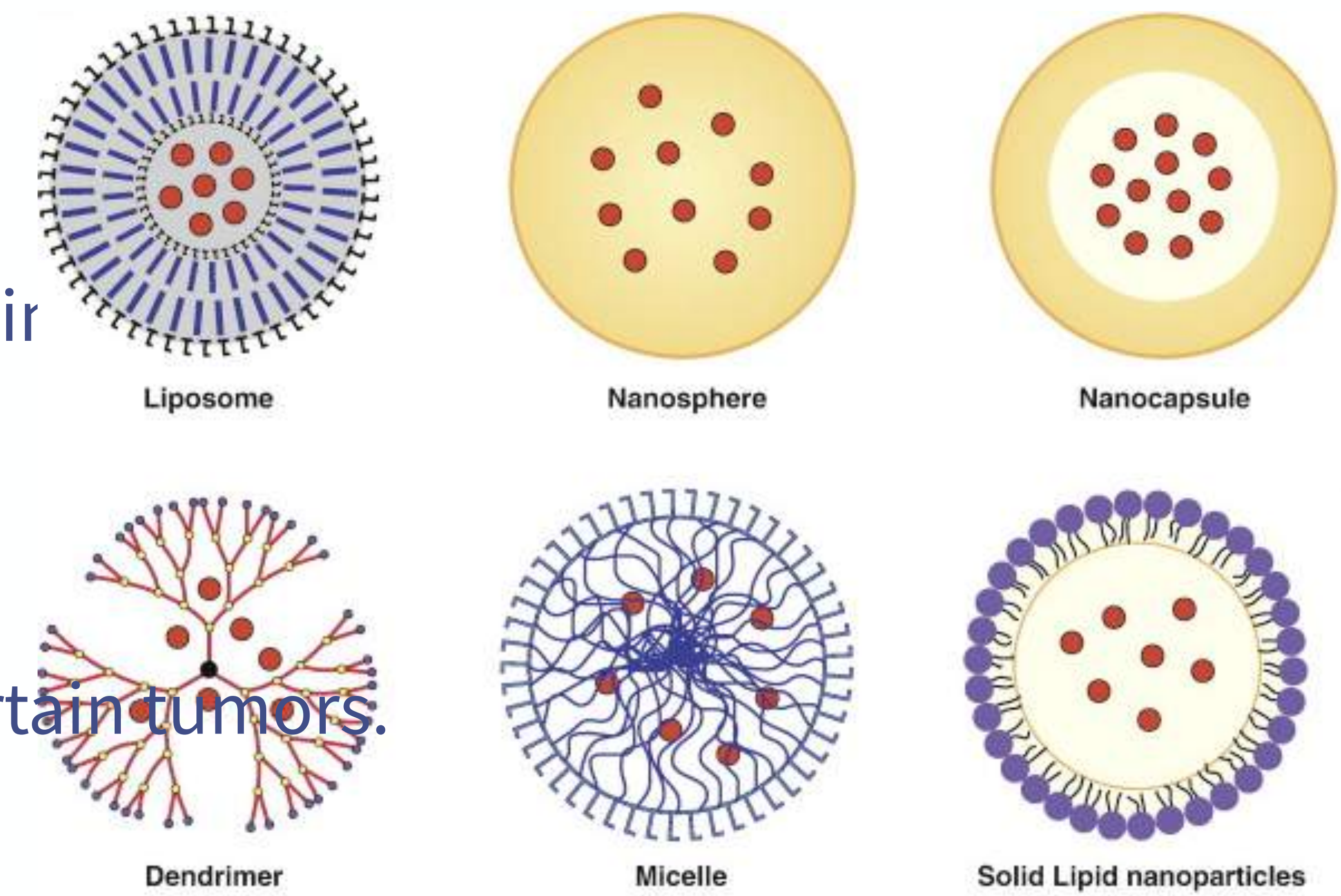
■ occasional ■■ common ■■■ severe, dose-limiting

➡ **Clear needs for targeted therapies**

Drug Carrier Systems

- Can improve the therapeutic index by reducing :
  - ✱ Side effects in healthy tissues.
  - ✱ The overall dose by concentrating the drug in the targeted tissue.

- Carrier systems include:



- Passively target tumors due to the ir
  permeation of many solid tumors.

- However, this effect is small for certain tumors.

➡ **Specific targeting strategies have been developed**

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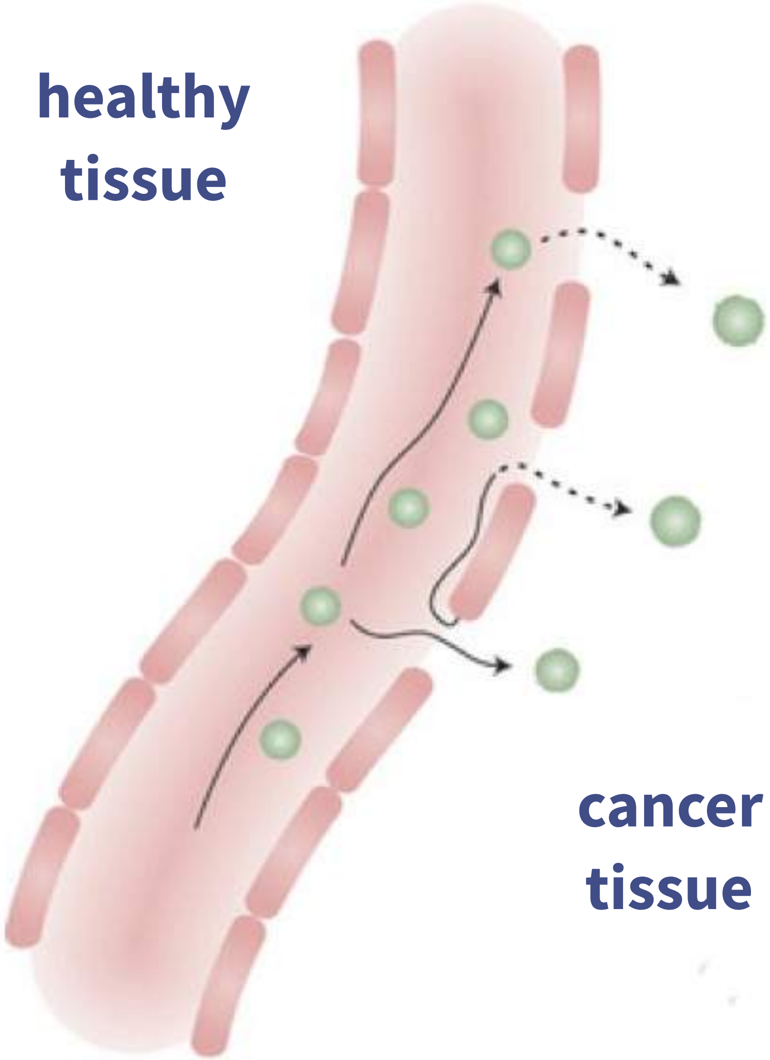
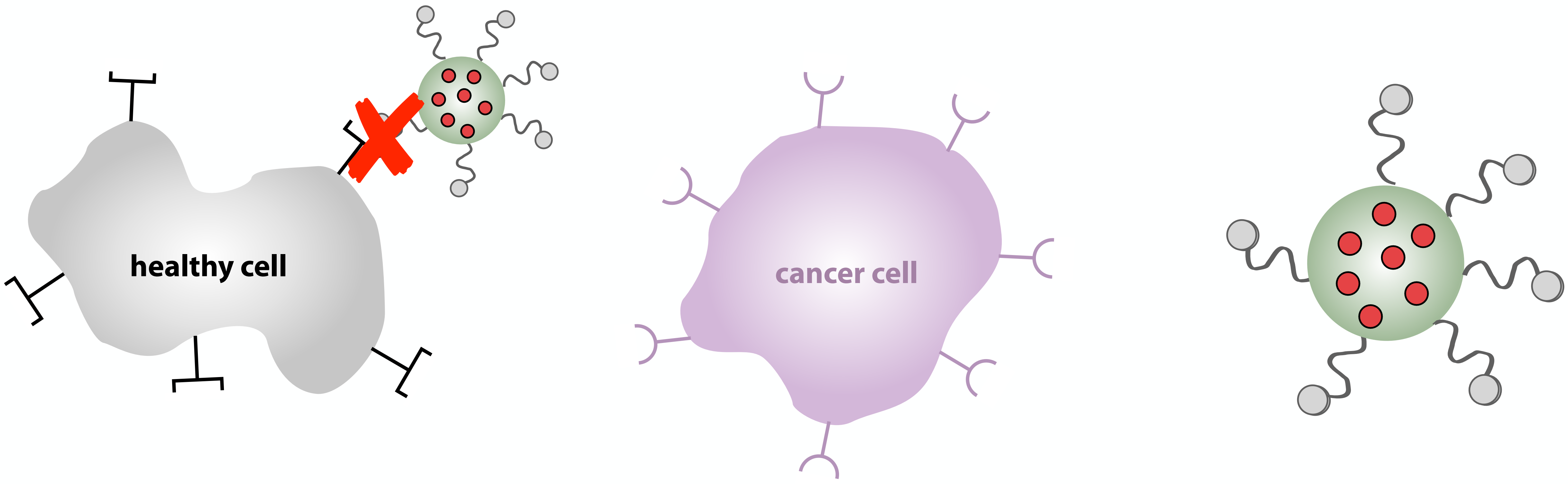


➡ **Specific targeting strategies have been developed**

Current **Targeting** Strategies

- Most take aim at specific cancer cell surface biomarkers.
  - ✱ Example: over-expressed cell surface receptors.
  - ✱ Involve the addition of ligands to the carrier system.

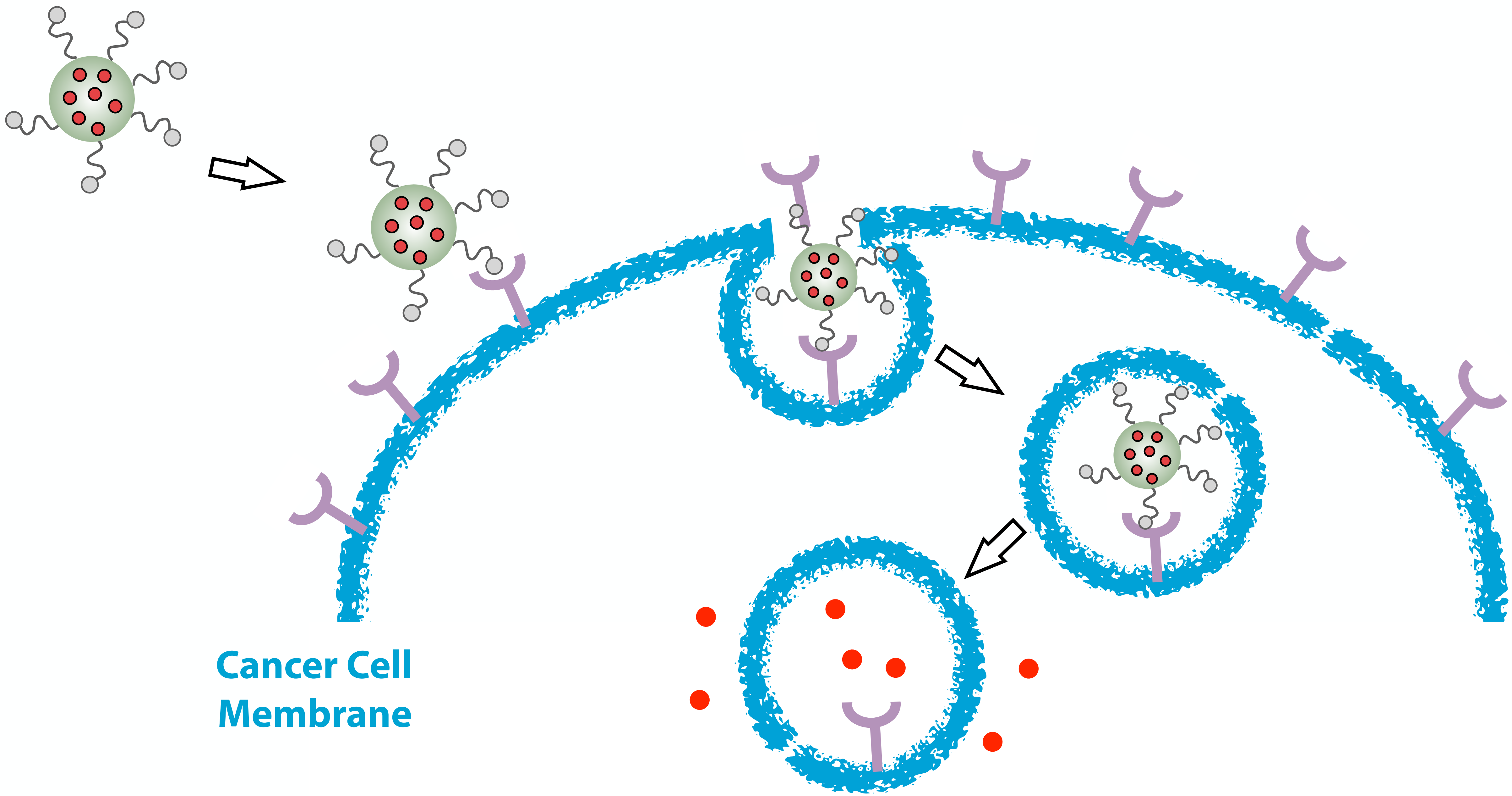


➡ **Allows specific interaction with cancer cells**

How do they get into cells?

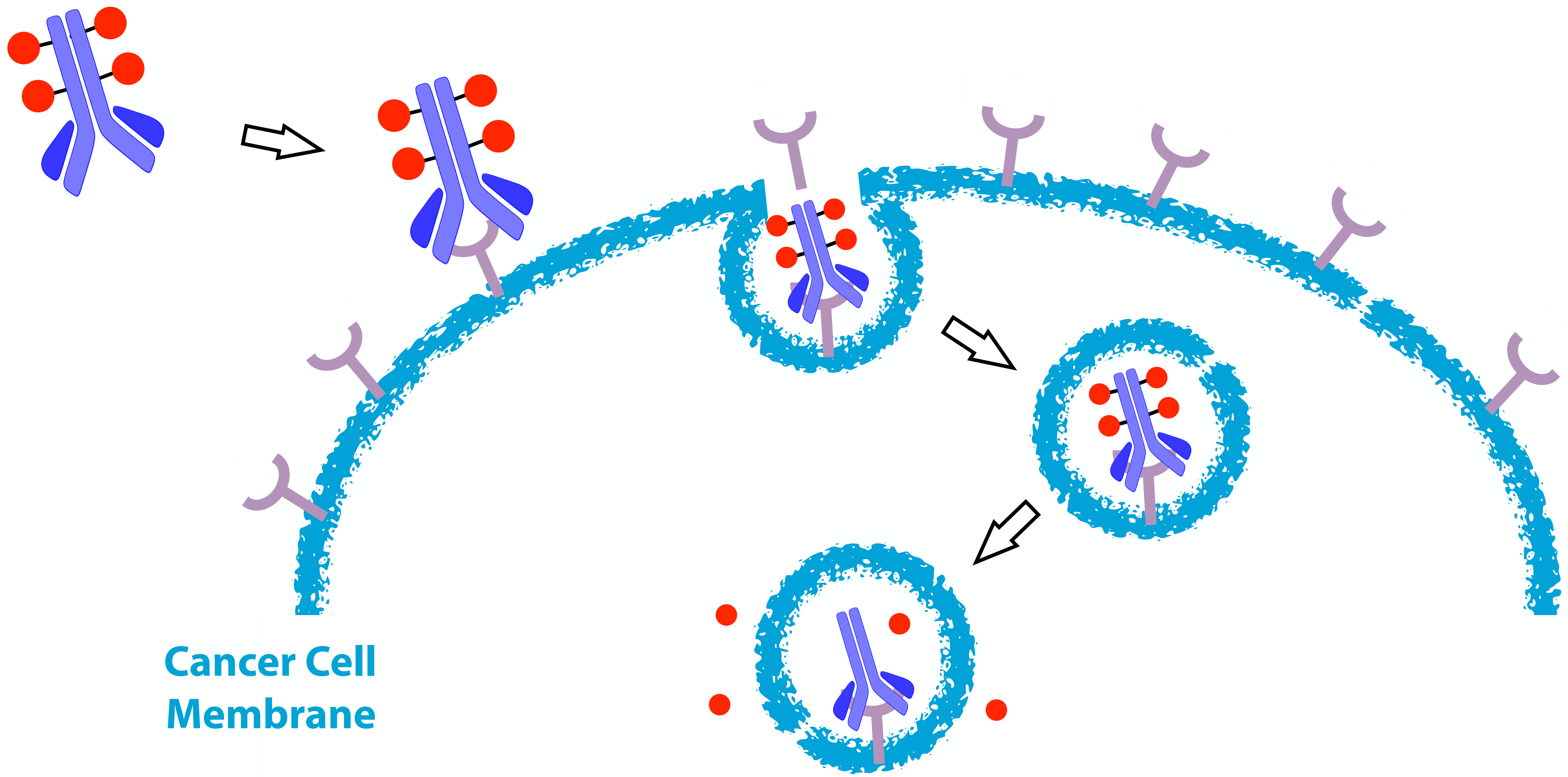
Current Targeting Strategies: Relying on Endocytosis

- Surface receptors are recycled through endocytosis:



Current Targeting Strategies: Monoclonal Antibodies

- Antibodies can be raised against any cell membrane receptors.



Current Targeting Strategies: Monoclonal Antibodies

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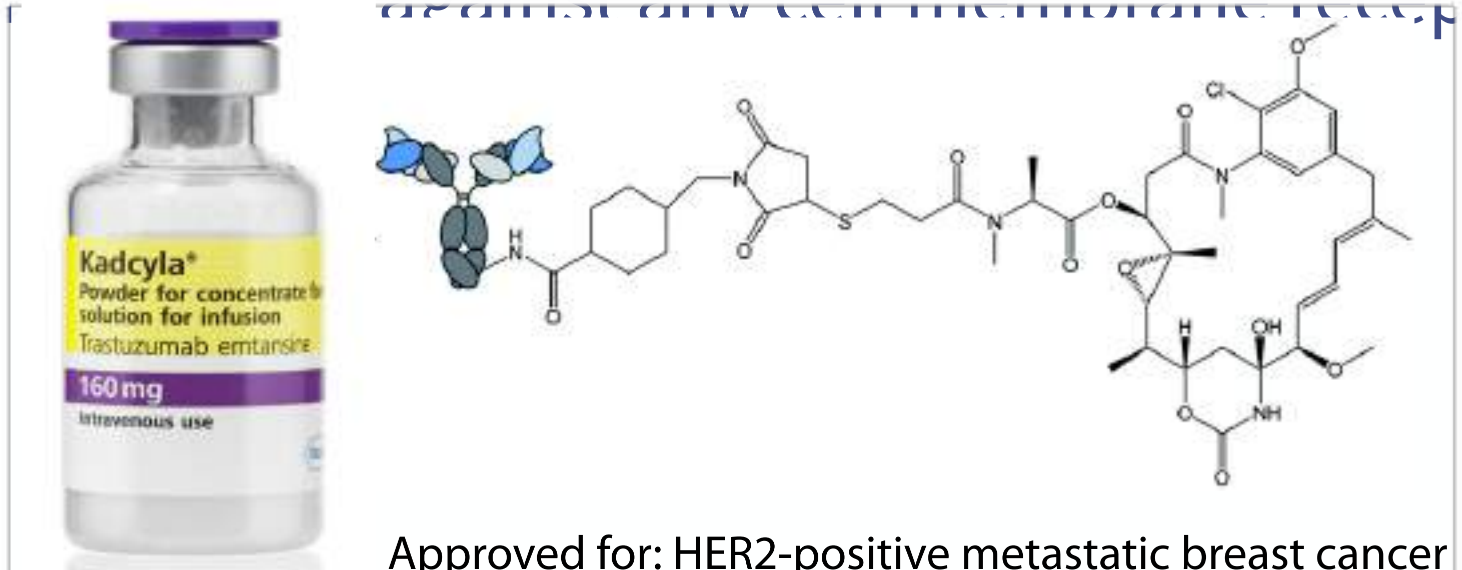
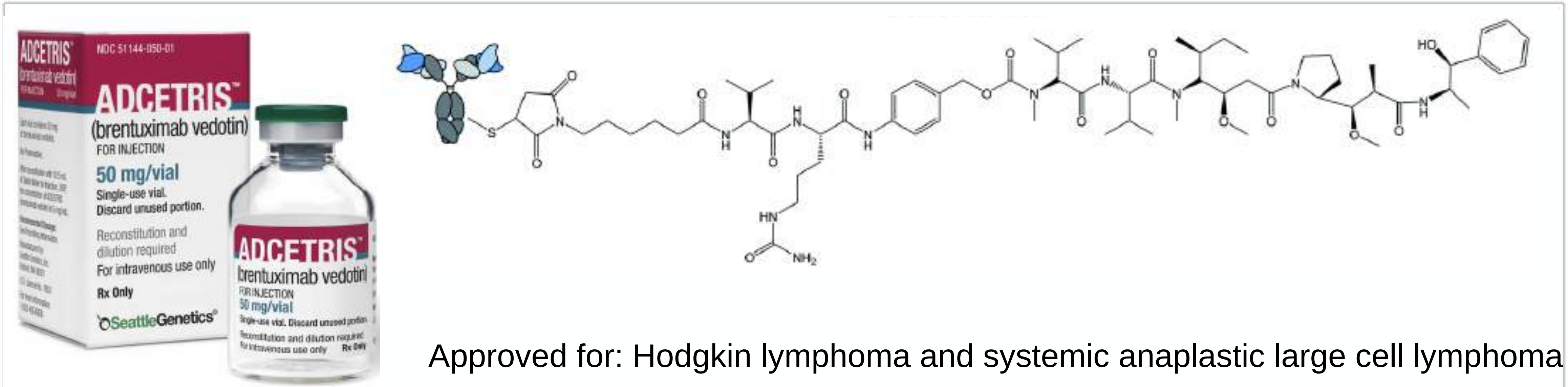


Approved for: HER2-positive metastatic breast cancer



Approved for: Hodgkin lymphoma and systemic anaplastic large cell lymphoma

Hess et al. Med. Chem. Commun. (2014)

Current Targeting Strategies: Drawbacks

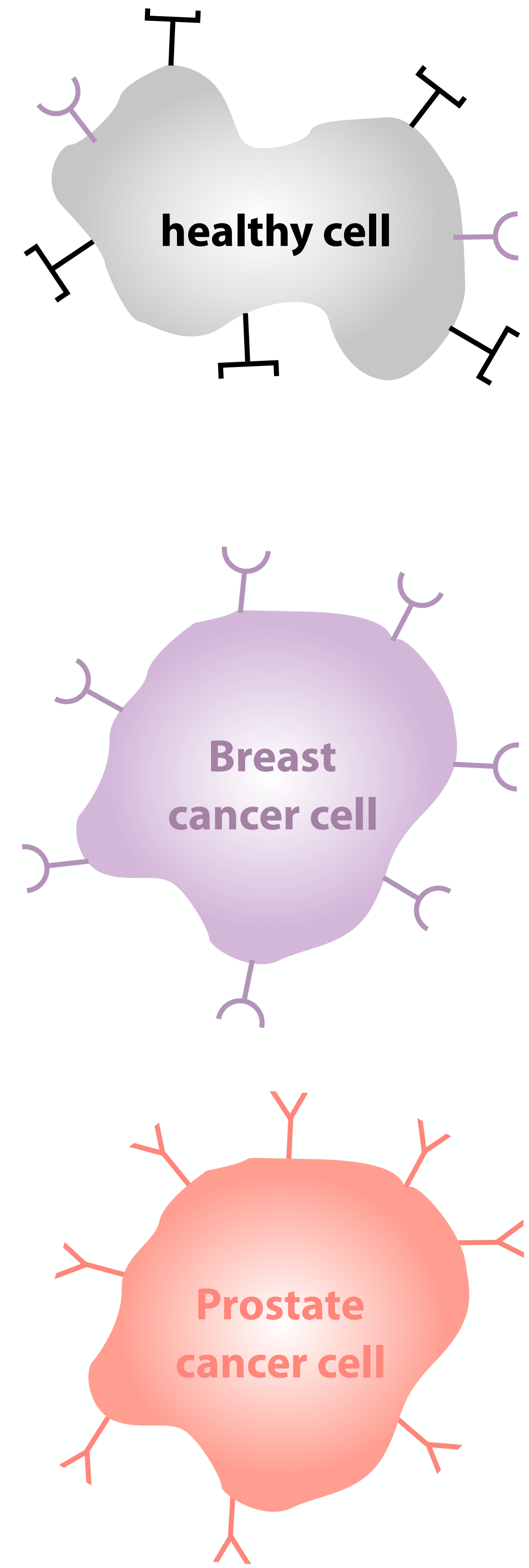
1. Healthy cells also have the same biomarkers.

2. Different cancers have different biomarkers.

3. Even in the same tumor, cancer cells can have different biomarkers.

4. **Fast evolution of cancer cells --> loss of receptor.**

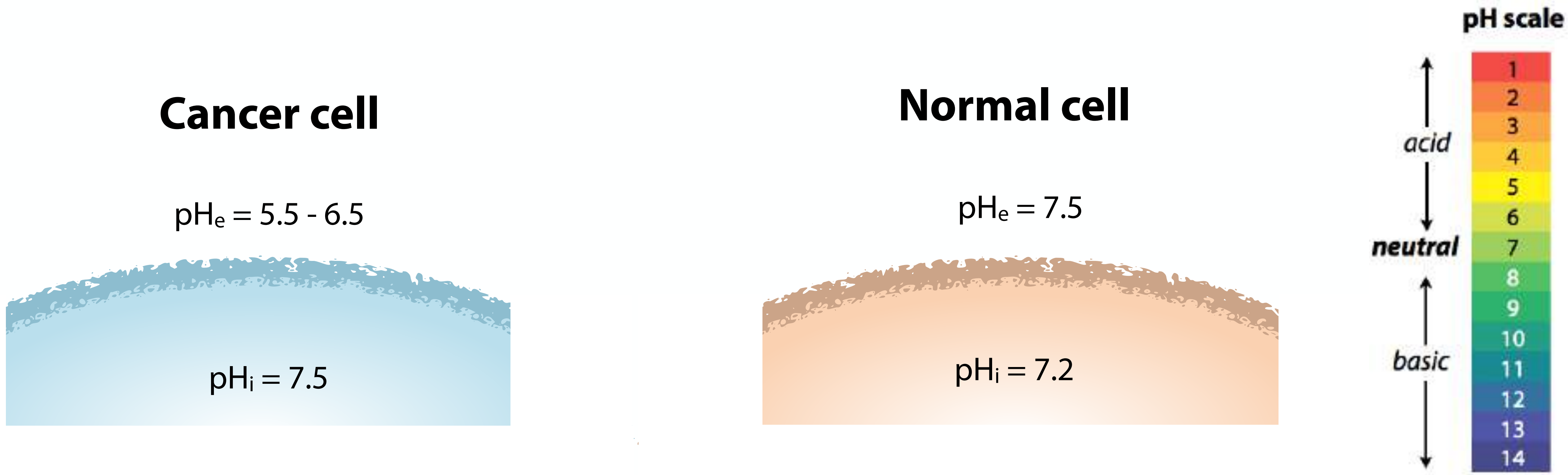
➡ uptake into normal tissues
➡ unacceptable toxicity profiles
➡ therapy resistance and disease progression



➡ **Needs for a more general biomarker**

Acidosis: A General Feature of Tumors

- Tumors: characterized by a lower extracellular pH when compared to healthy tissues.

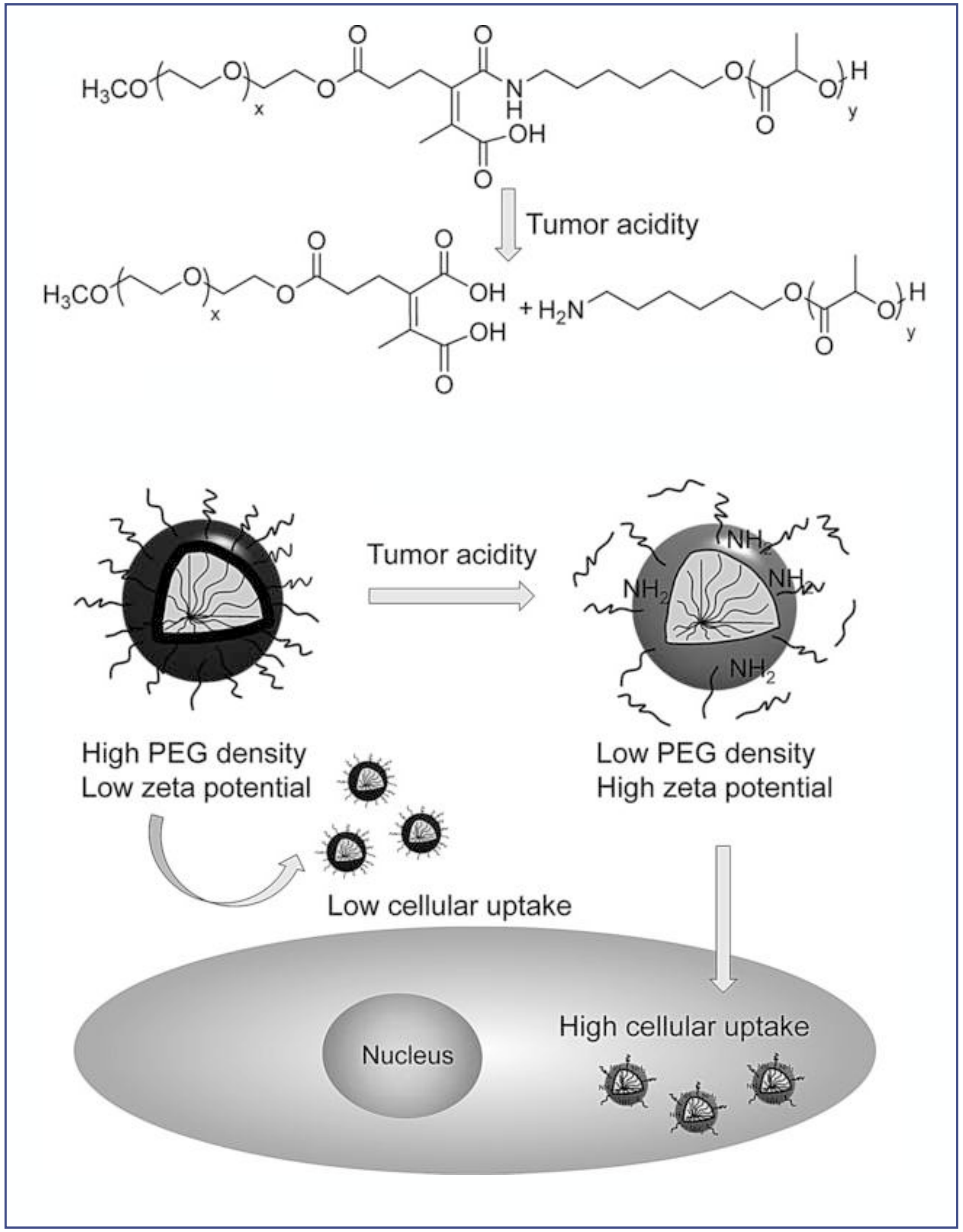


➡ **Acidosis = General biomarker of tumors.**

How can we target acidosis?

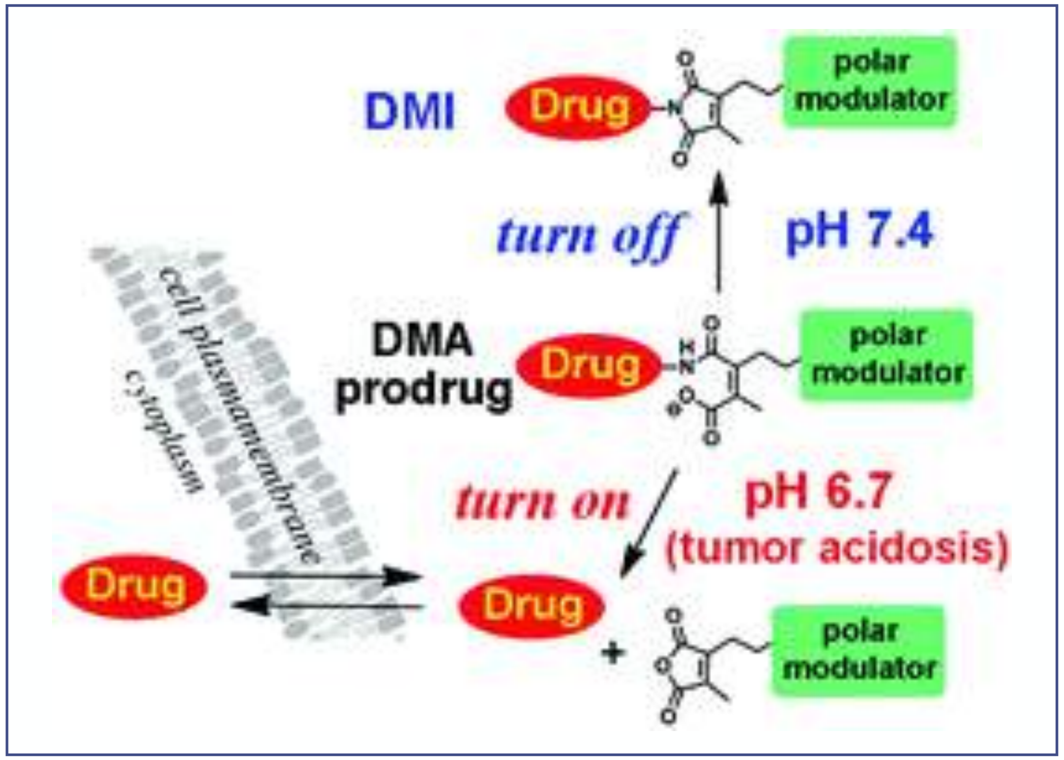
Acidosis: pH-sensitive Pro-drugs and Nanoparticles

Nanoparticle



Angew. Chem. Int. Ed. **2016**, 55, 1010

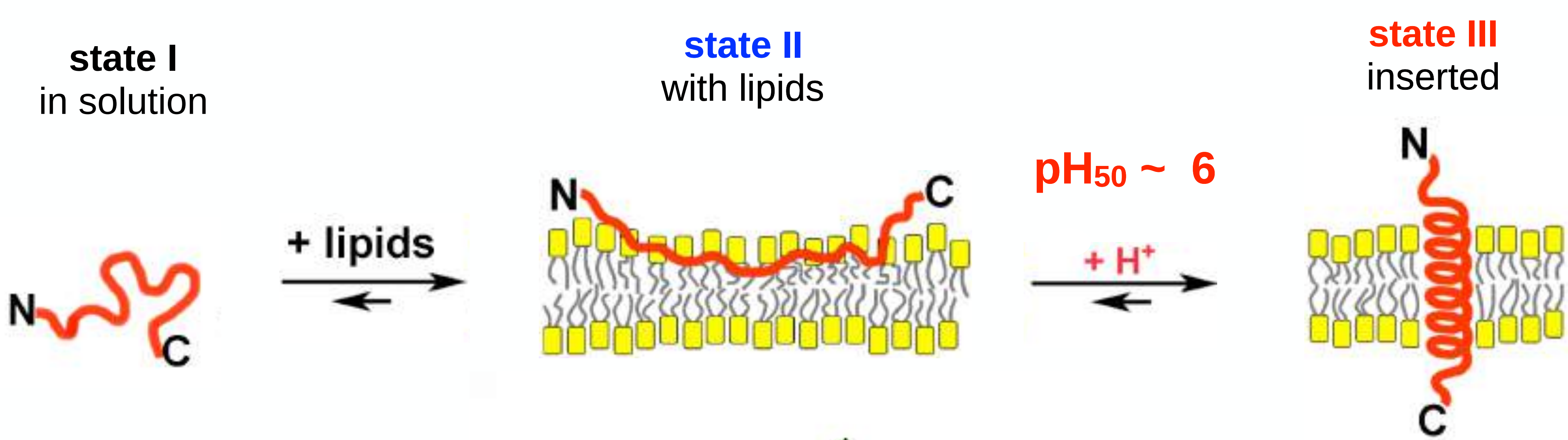
Pro-drug



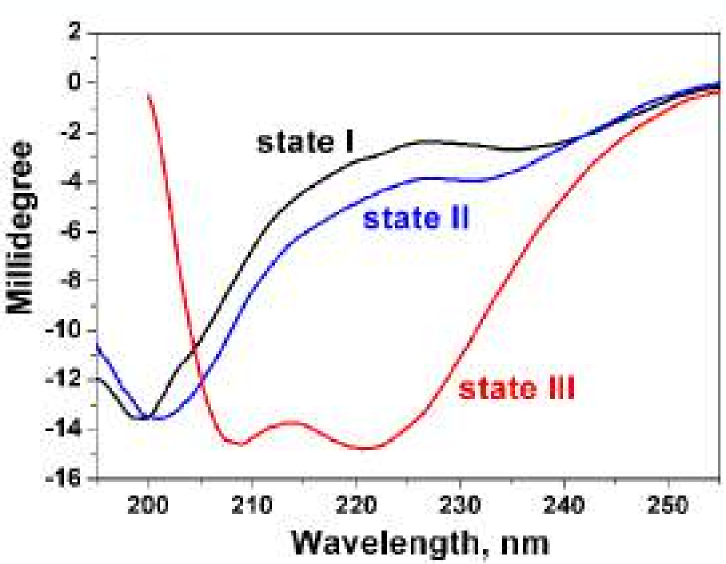
Chem. Commun., **2017**,53, 12826-12829

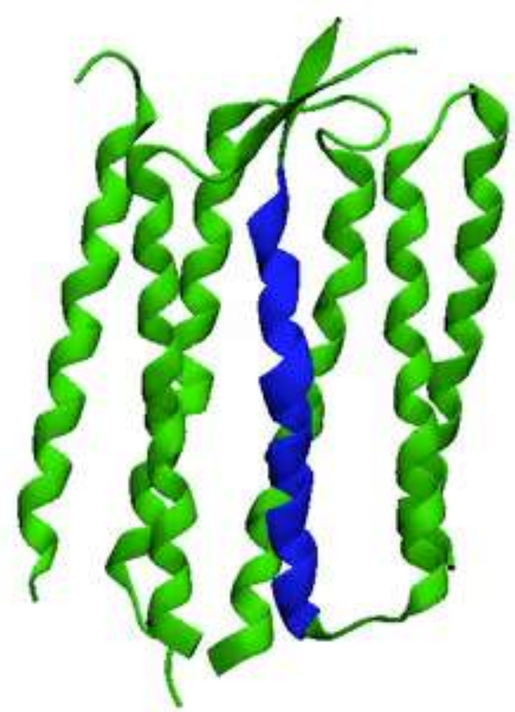
pHLIP: pH(Low) Insertion Peptide

pHLIP: AAEQNPIYW**ARYADWLFTTPLLLLDLALLVDAD**EGTG



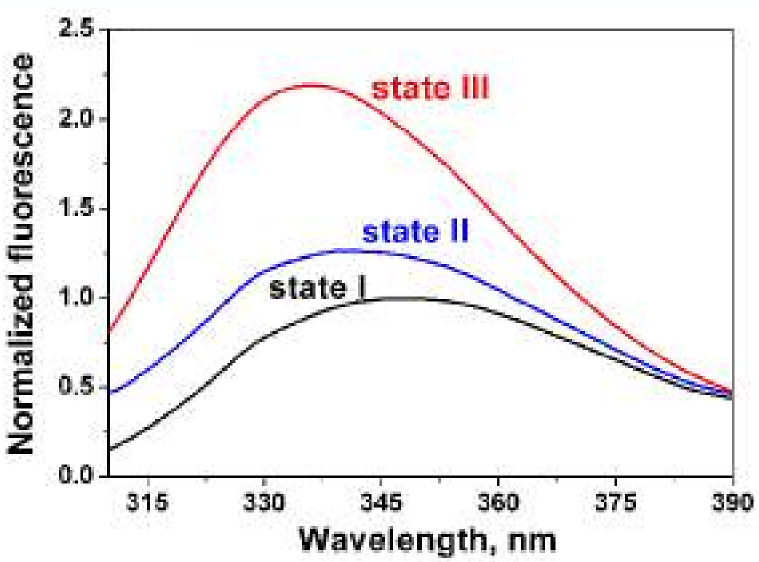
Circular Dichroism





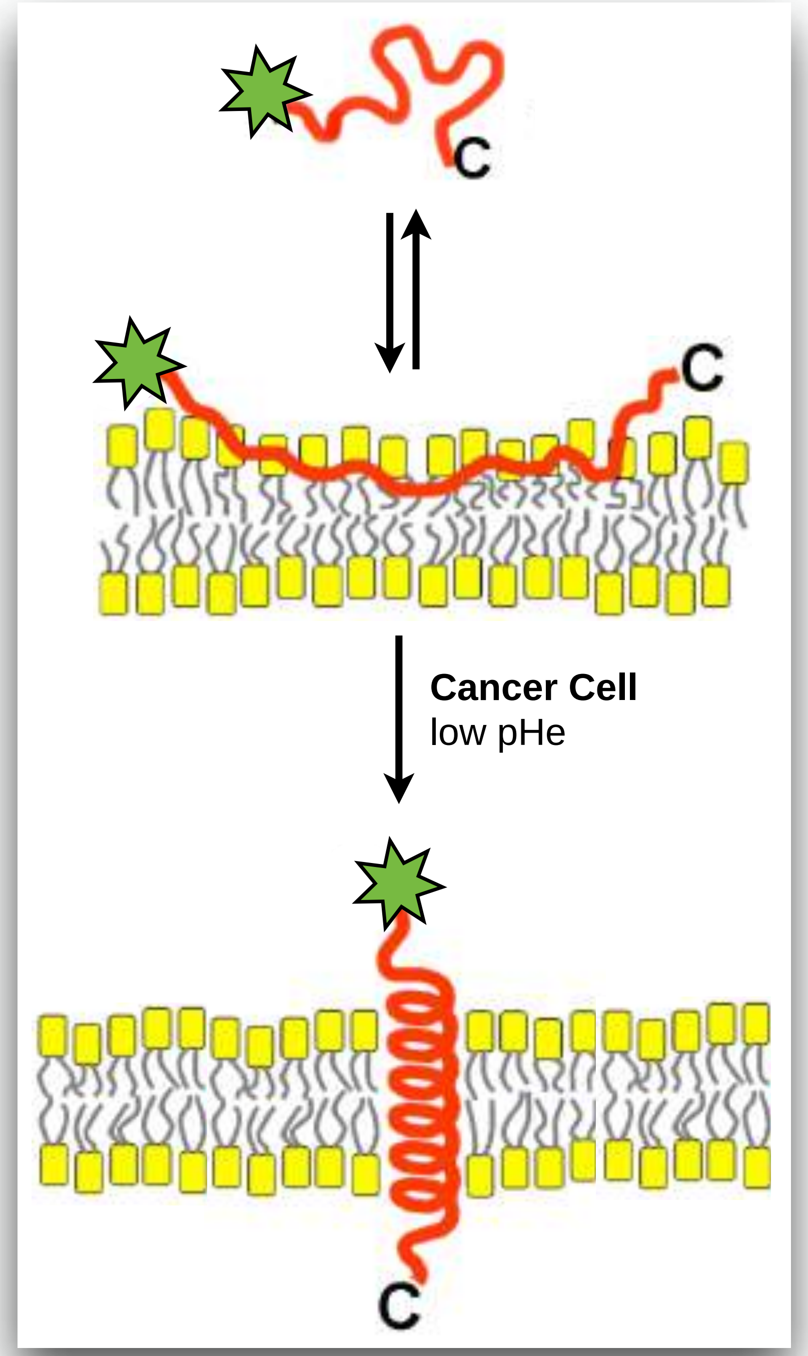
Bacteriorhodopsin
(from *Halobacterium salinarum*)

Tryptophan fluorescence

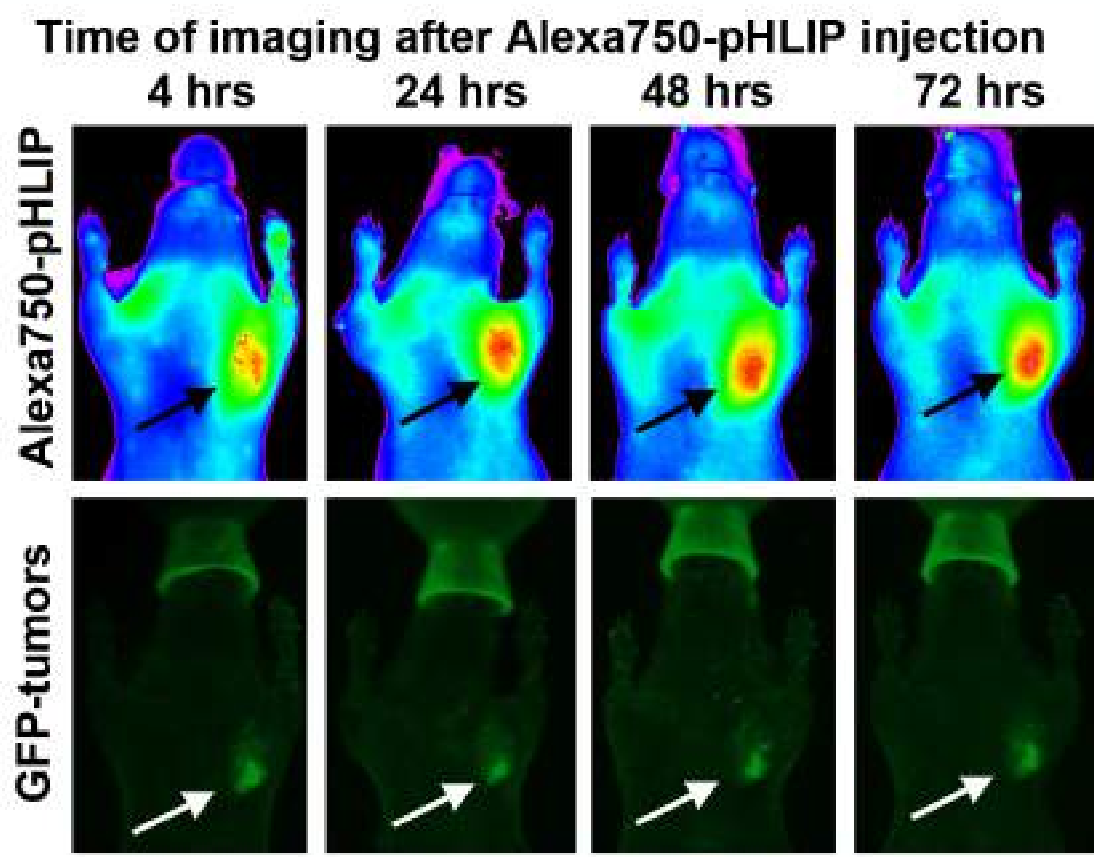


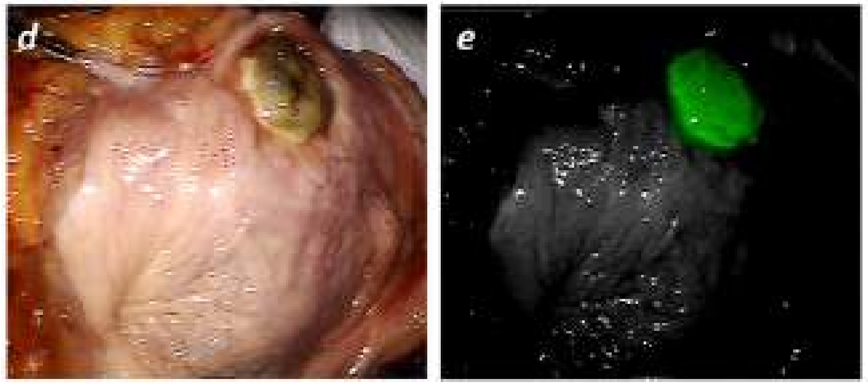
Hunt et al. *Biochemistry* (1997)
Reshetnyak et al. *Biophys. J* (2007)
Reshetnyak et al. *PNAS* (2008)

pHLIP: Imaging Tumors in vivo



Nude mouse with
cancer cells expressing the Green Fluorescent Protein (GFP)





Andreev et al. Chim Oggi. (2009)
Andreev et al. PNAS (2007)
Segala et al. Int J Mol Sci (2009)

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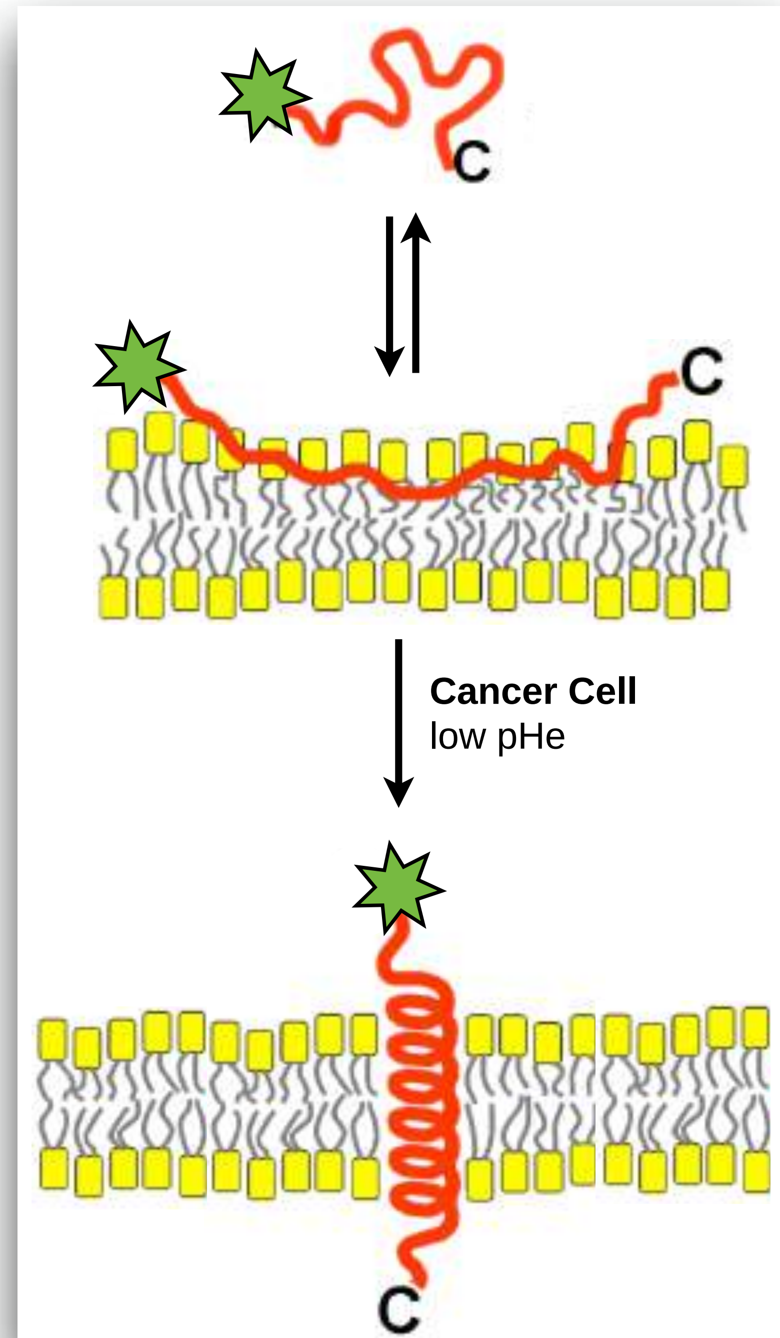
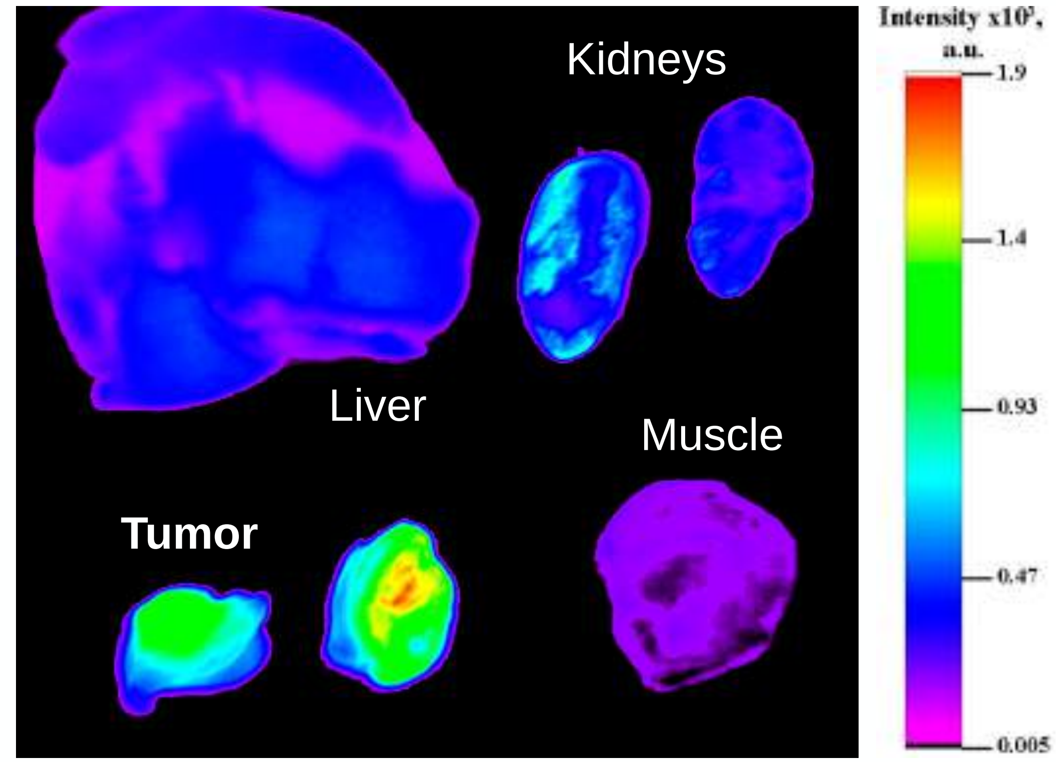
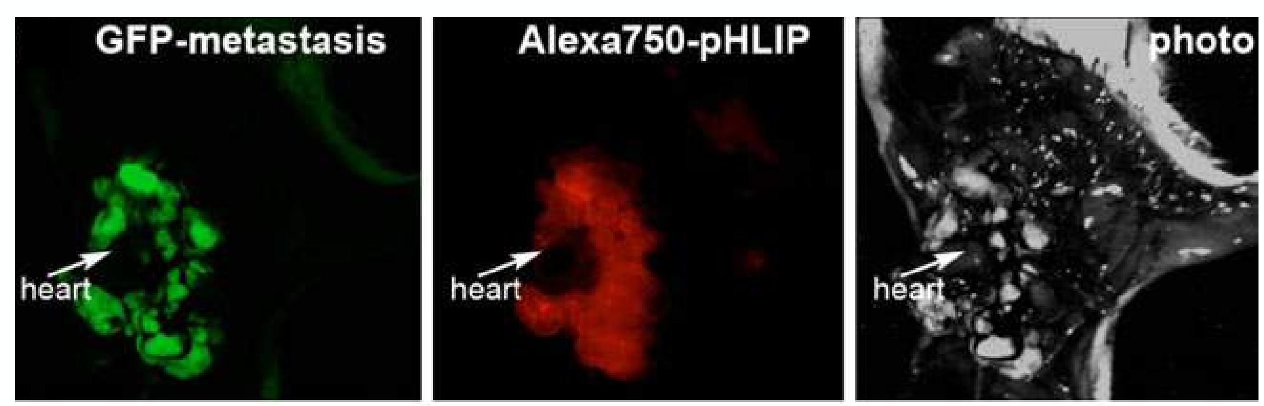


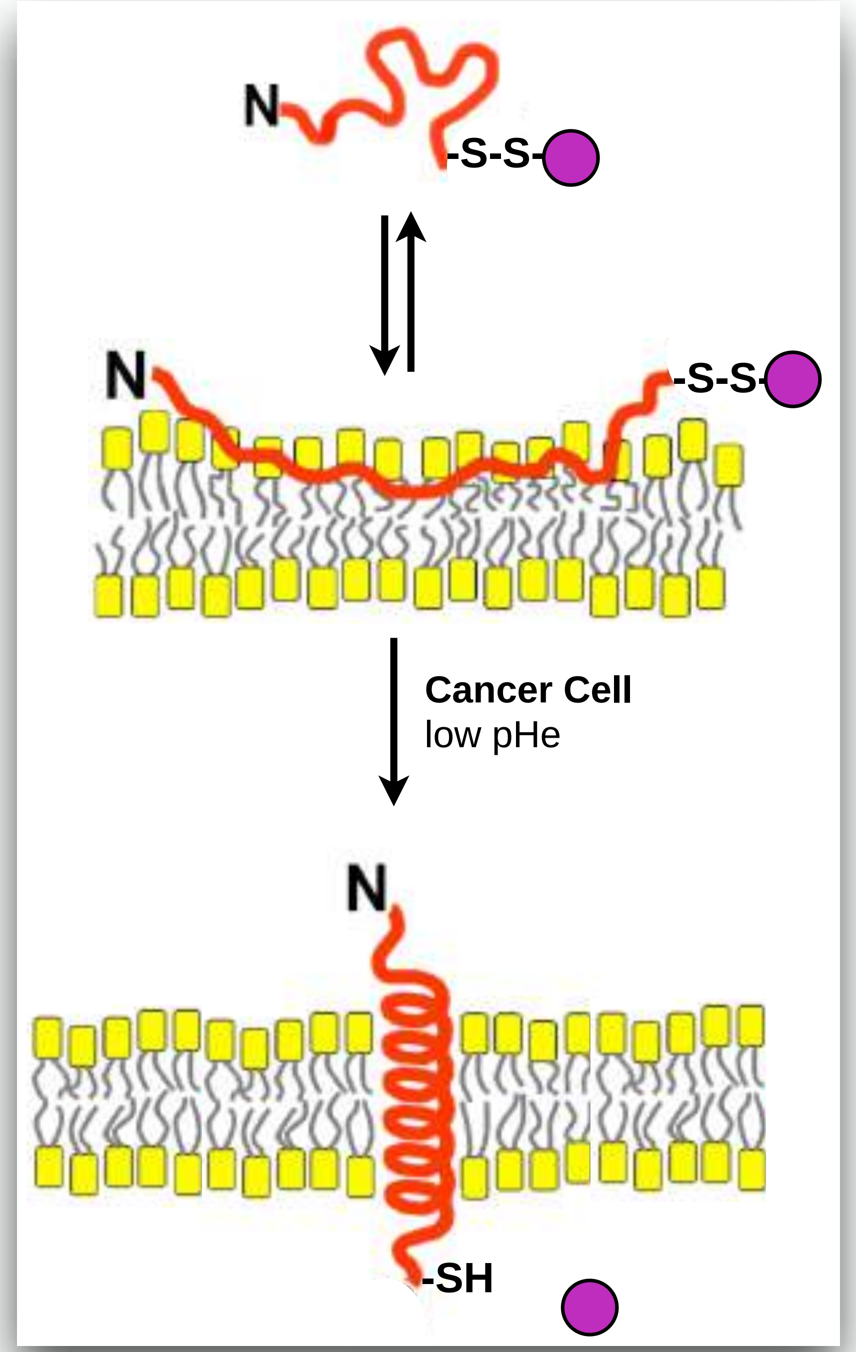


Also identifies metastases



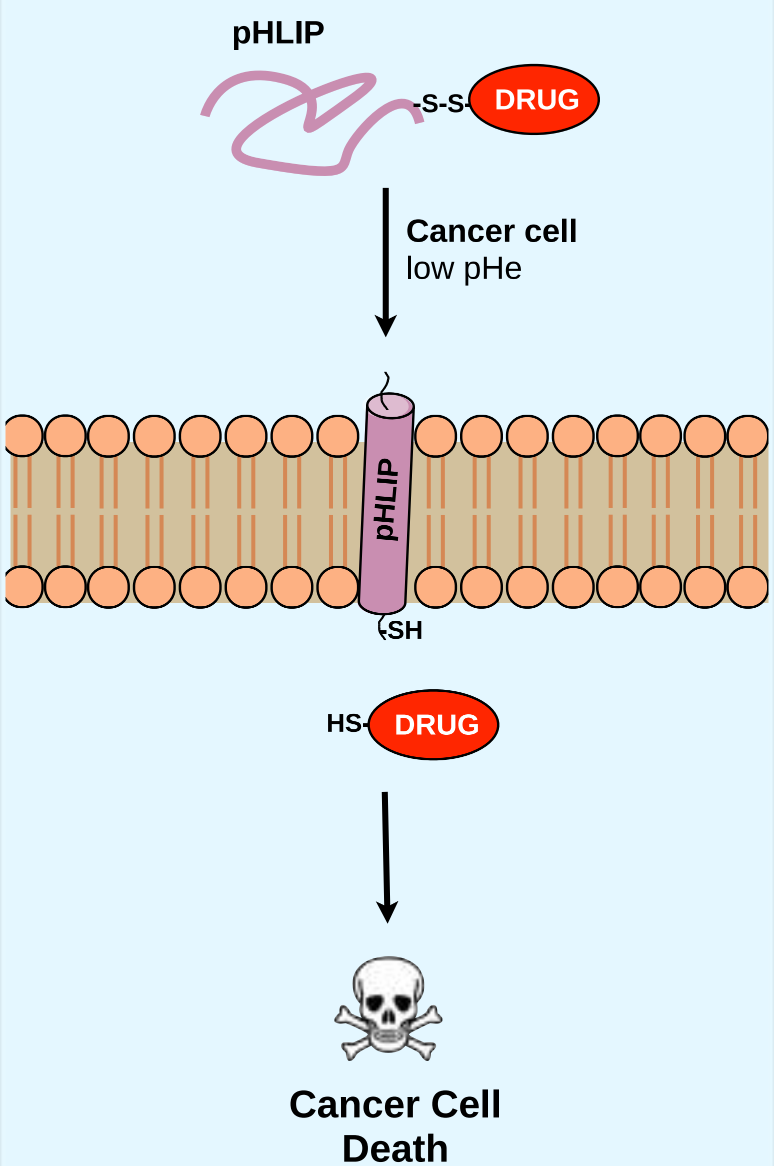
Andreev et al. Chim Oggi. (2009)
Andreev et al. PNAS (2007)
Segala et al. Int J Mol Sci (2009)

pHLIP: A Targeting and Delivery Agent



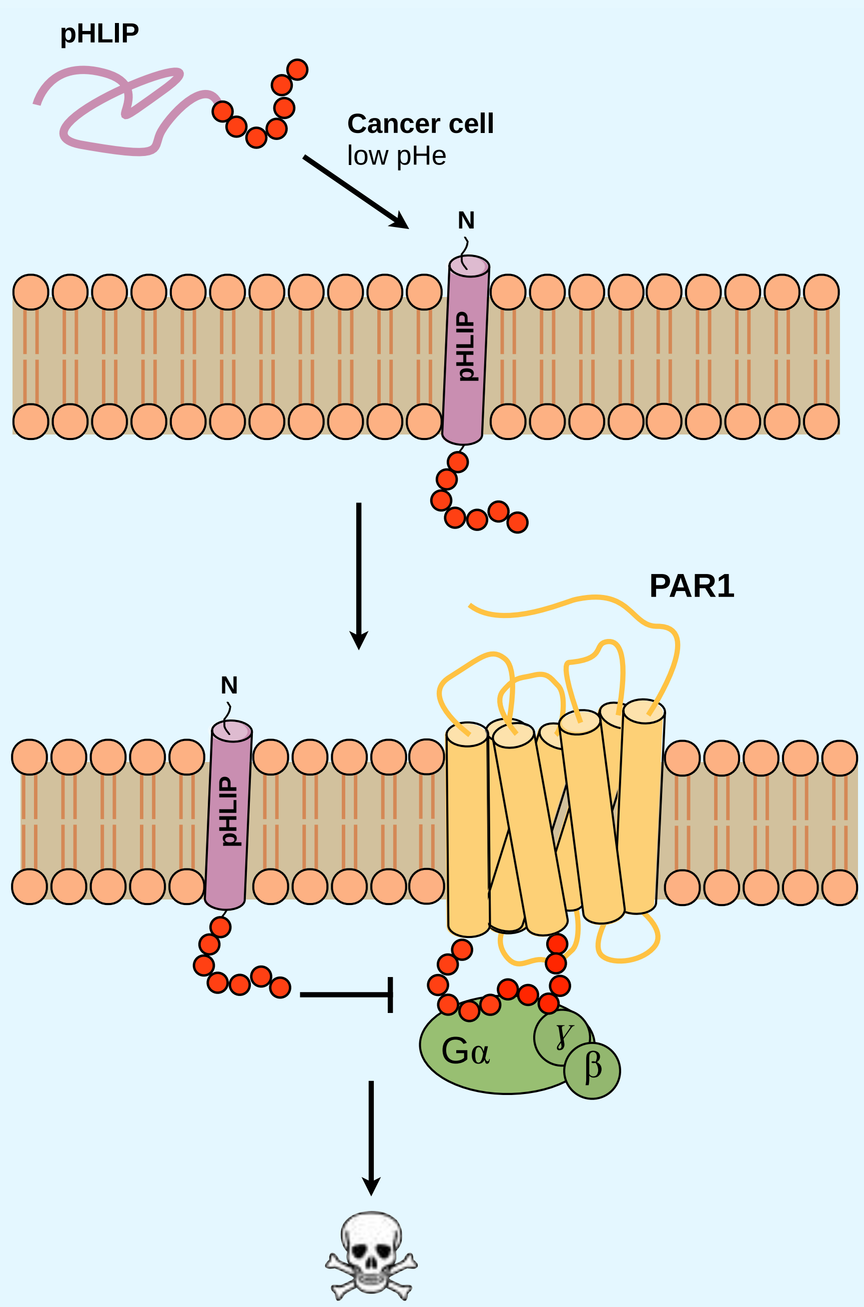
Developing pHLIP as a therapy platform

Delivery of cytotoxics



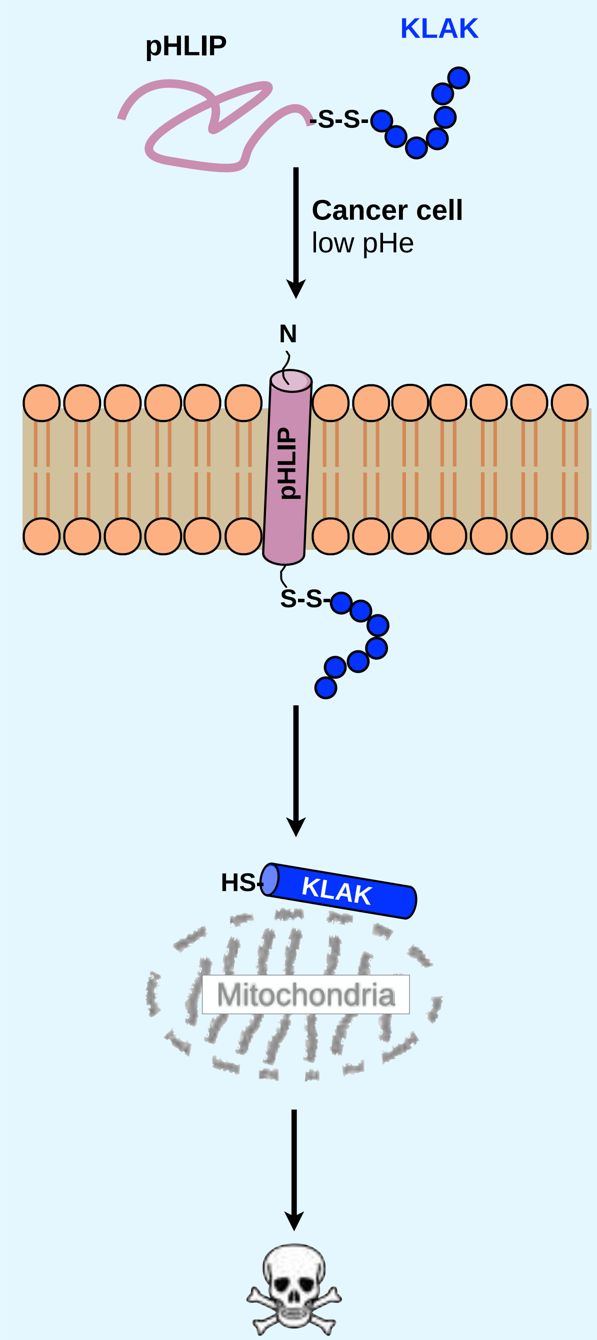
Burns, Thévenin (2015) *Mol. Pharm.*
Burns et al. (2017) *Mol. Pharm.*

Inhibiting Membrane Receptors



Burns, Thévenin (2015) *Biochem. J.*
Gerhart et al. (2018) *ACS Chem. Biol.*

Delivering Antimicrobial Peptides



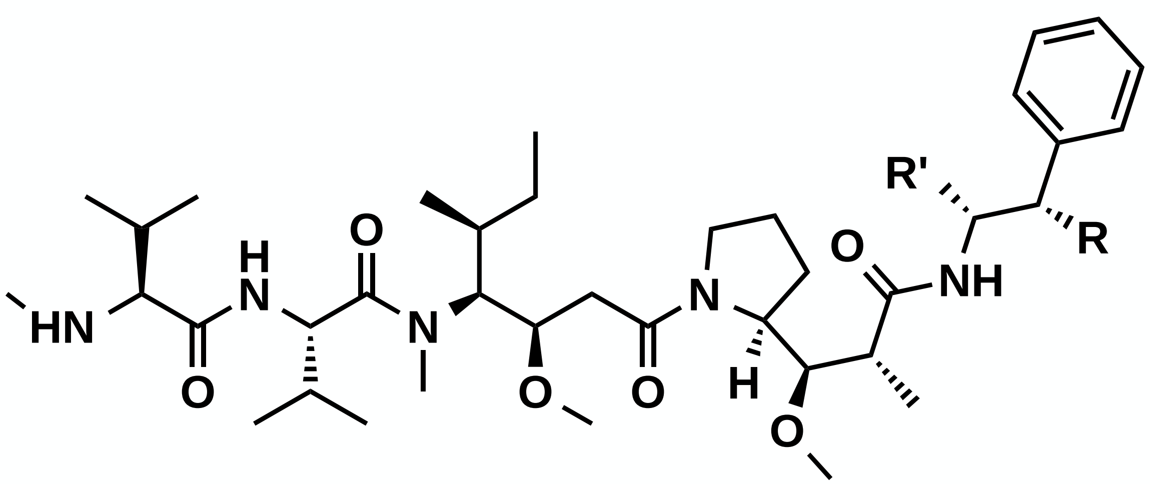
Burns et al (2016) *Sci. Rep.*

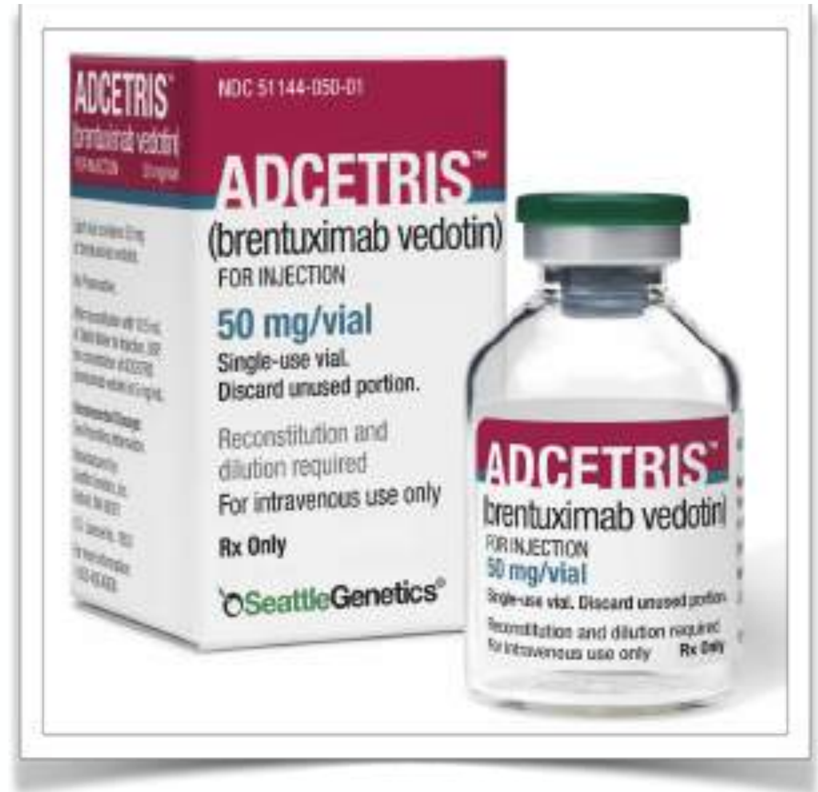
Strategy #1

Specific Delivery of Auristatin Derivatives

Monomethyl Auristatins: Potent Cytotoxics

- **Monomethyl Auristatins**
  - Family of antimitotic agents.
  - Derived from Dolastatin 10.
  - Inhibits tubulin polymerization.
  - Extremely toxic.
  - **Must be delivered specifically.**





Compound	R	R’	Log $P_{o/w}$	IC50 (nM)
Dolastatin 10	H	C_3H_3NS	3.4	0.1
MMAE	OH	CH_3	2.2	0.1 - 2
MMAF	H	COOH	0.7	105 - 250
MMAF-OMe	H	COOMe	2.8	0.001

Burns, Robinson, Thévenin (2015) *Mol. Pharm.*

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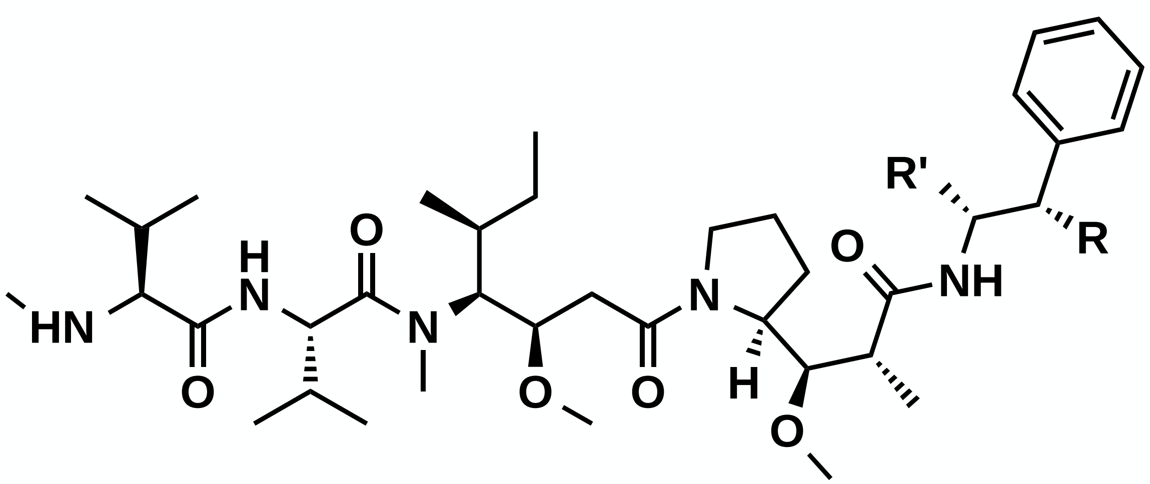

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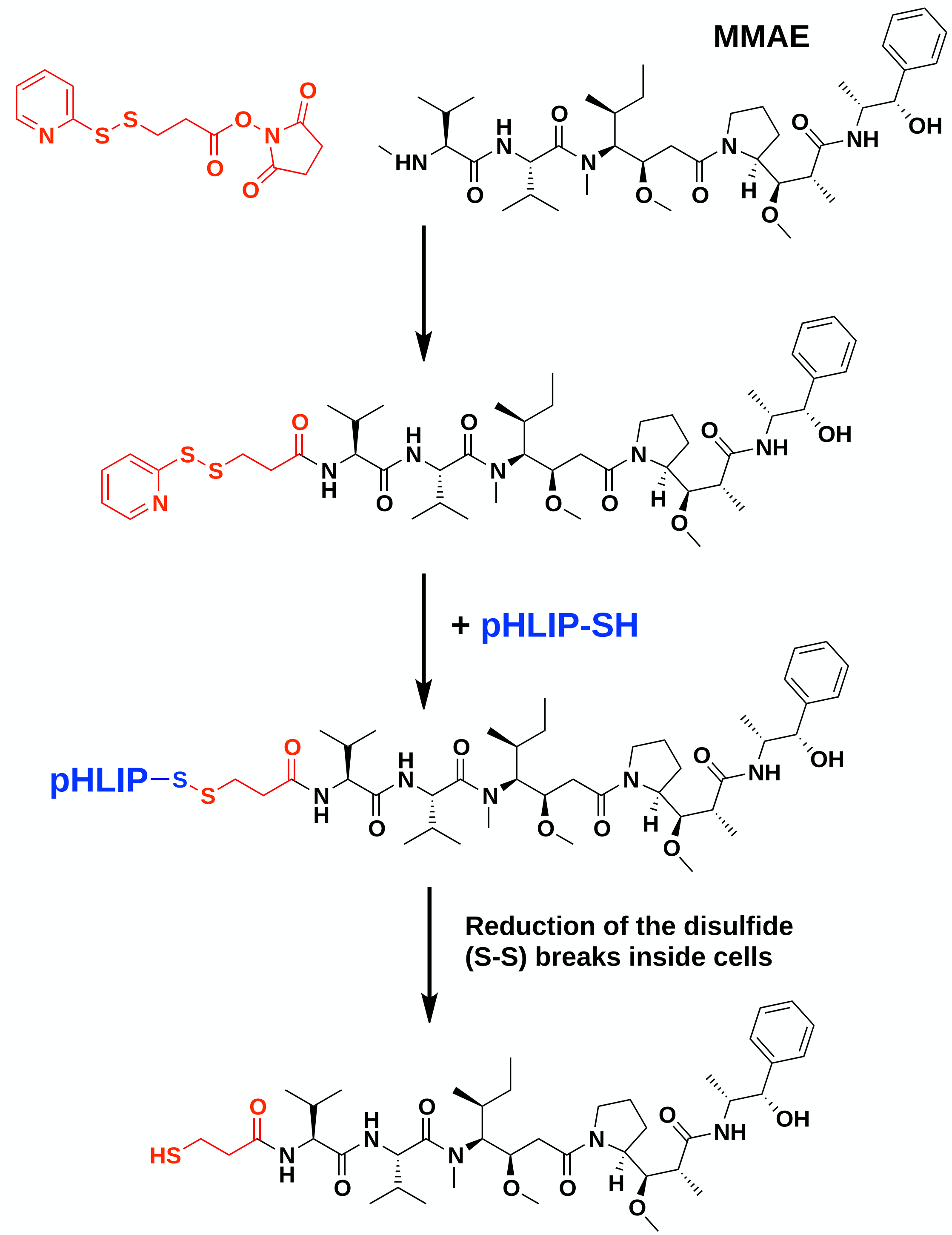
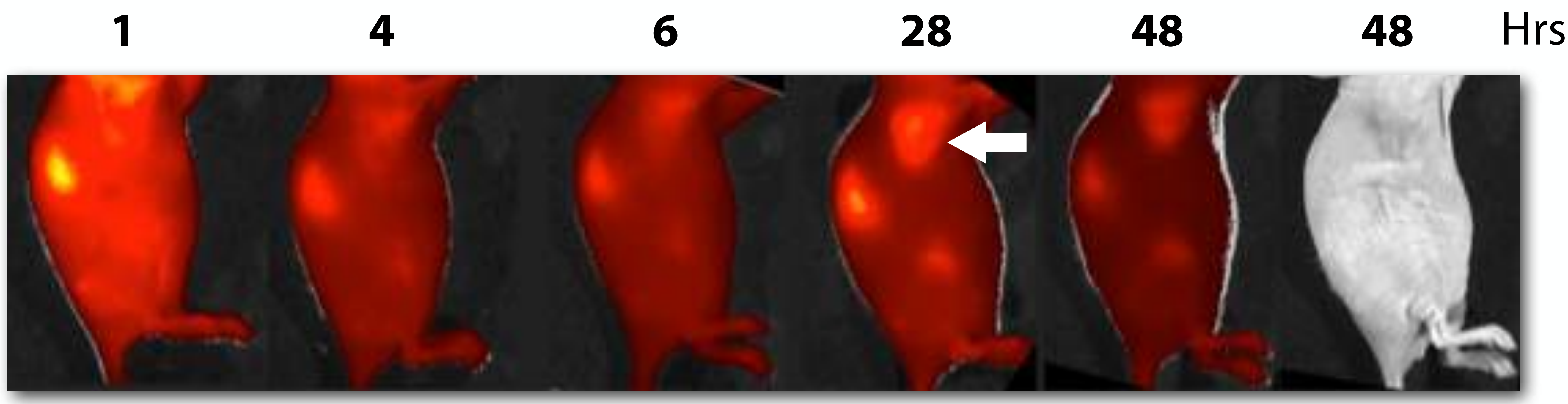


Burns, Robinson, Thévenin (2015) *Mol. Pharm.*

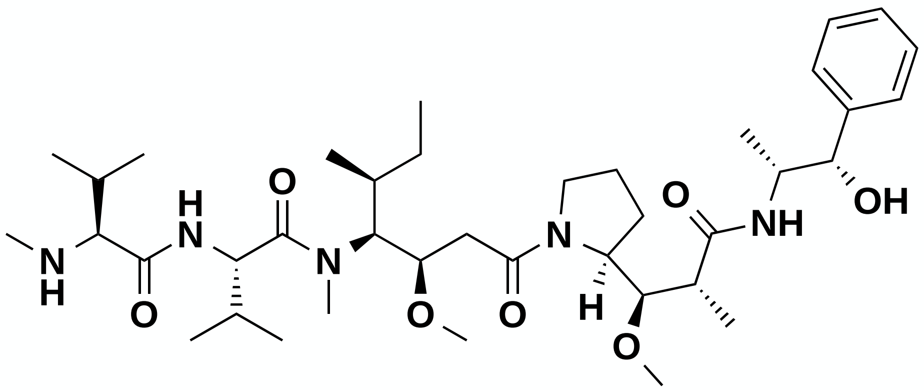
pHLIP-MMAE: In vivo Targeting



Alexa750-pHLIP-MMAE
NCr nu/nu mice
MDA-MB-231 xenograft
Intravenous injection

Burns, Robinson, Thévenin (2015) *Mol. Pharm.*

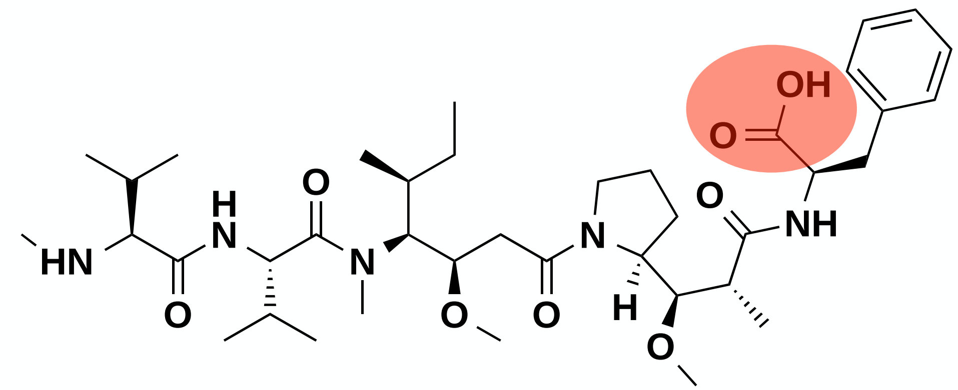
Next Generation Conjugates: MMAF and pHLIP Variants



MMAE

(Log $P_{o/w}$ = 2.2)

VS.



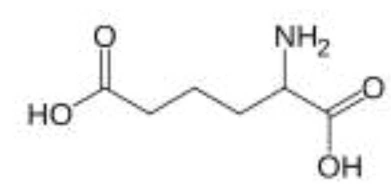
MMAF

(Log $P_{o/w}$ = 0.7)

pHLIP Variant	**Sequence**	**pH_{50}**
WT	AAEQNPIYWARYA**D**WLFTTPLLLL**D**LALLVDADEGTCG	6.1
D25E	AAEQNPIYWARYADWLFTTPLLLL**E**LALLVDADEGTCG	6.5
P20G	AAEQNPIYWARYADWLFTT**G**LLLLDLALLVDADEGTCG	6.8
R11Q	AAEQNPIYWA**Q**YADWLFTTPLLLLDLALLVDADEGTCG	5.8
R11Q + D14Up	AAEQNPIYWA**Q**Y**DA**WLFTTPLLLLDLALLVDADEGTCG	5.6
D14Gla + D25Aad	AAEQNPIYWARYA**Gla**WLFTTPLLLL**Aad**LALLVDADEGTCG	6.8

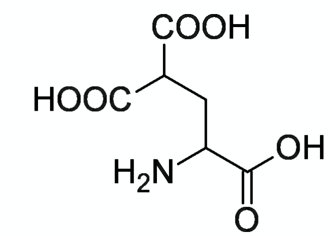
Aad

(α–aminoadipic acid)



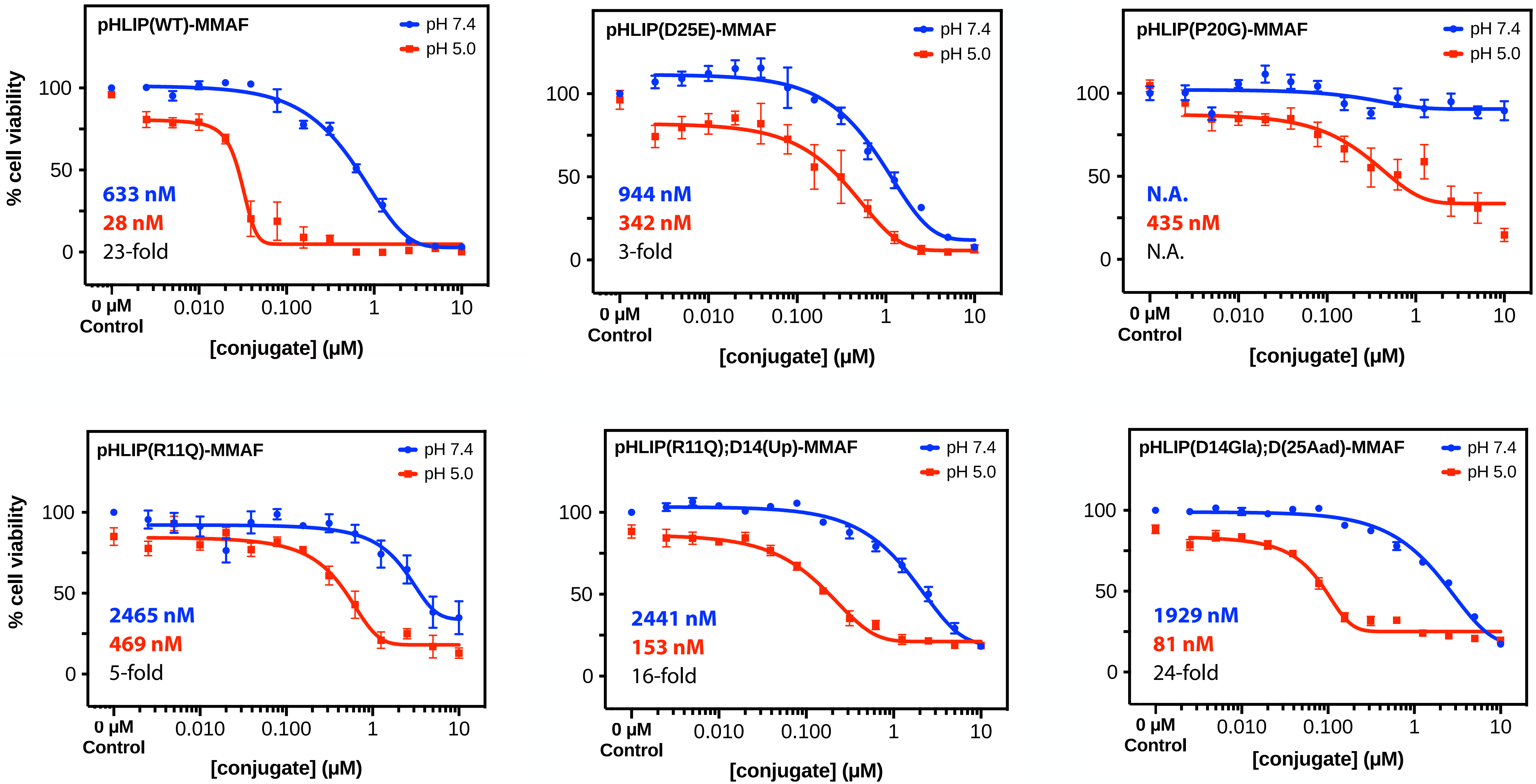
Gla

(ɣ–carboxyglutamic acid)



Onyango et al. (2015) Angewandte Chemie
Fendos et al. (2013)
Biochemistry Barrera et al. (2013) PNAS

Next Generation Conjugates: Cytotoxicity in HeLa Cells



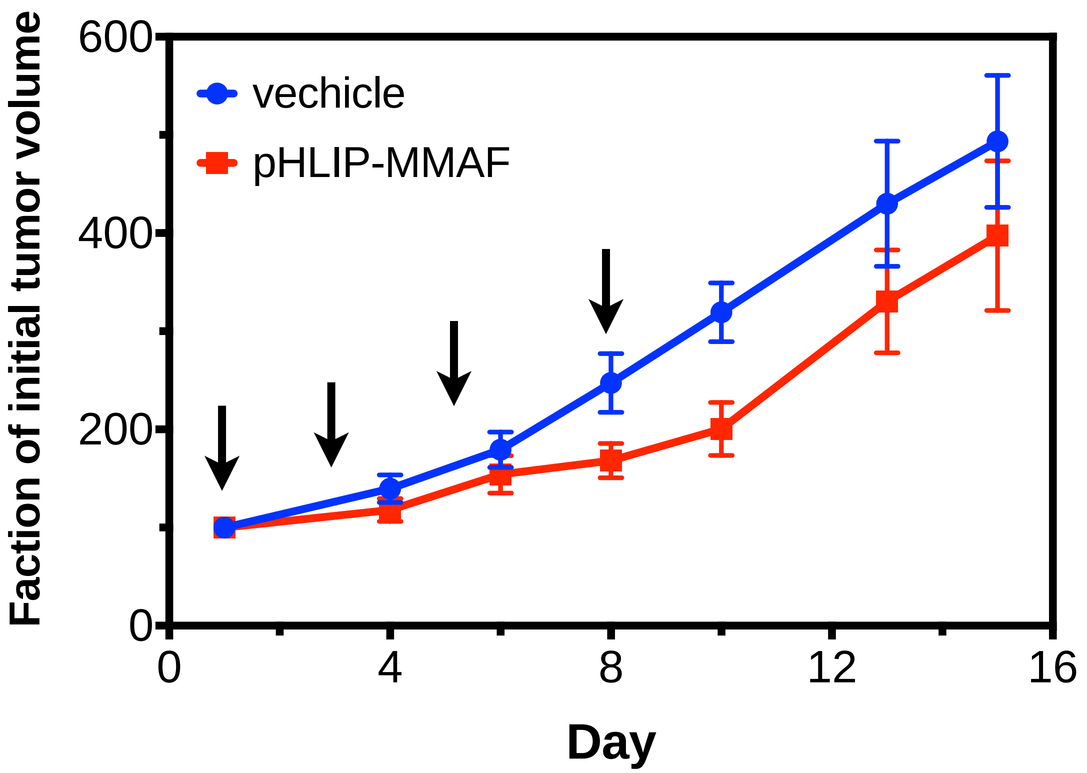
➡ **pHLIP(WT)-MMAF over 100-fold more potent than MMAE conjugate!**

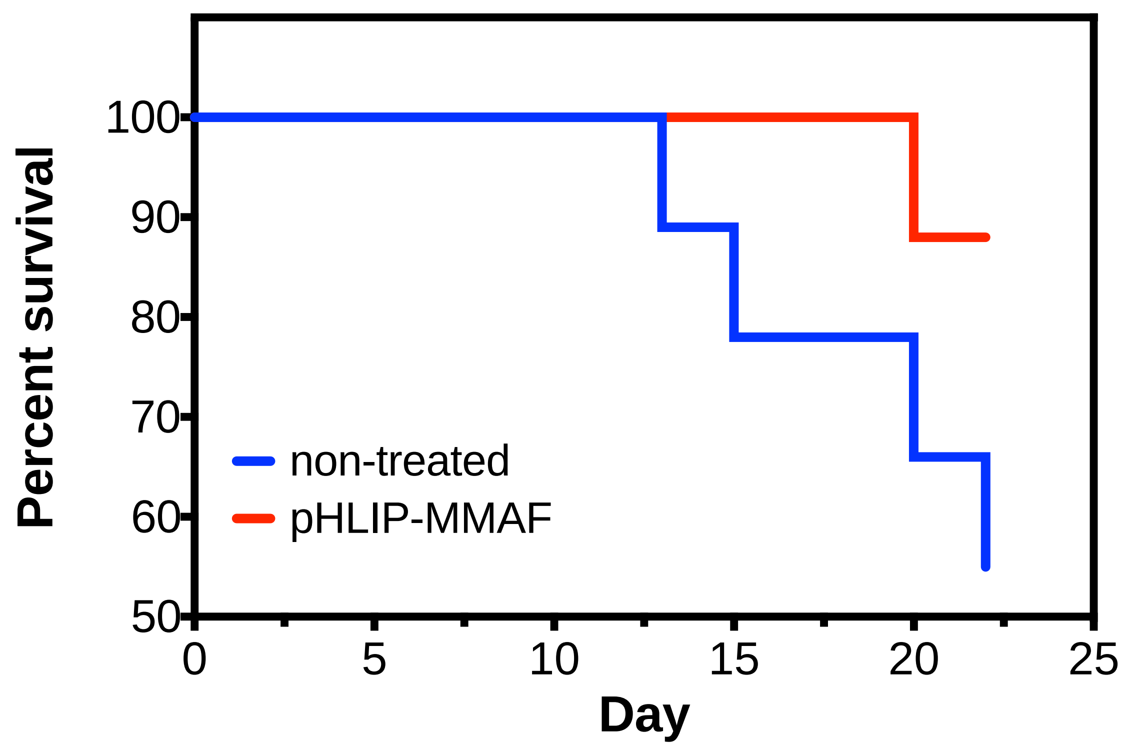
➡ **Lead agent for further in vivo studies**

Burns et al. (2017) *Mol. Pharm.*

pHLIP-MMAF: In vivo Therapy Studies

- NCr nu/nu mice bearing HeLa tumors (injection of with 5x10[6] cells)
- Injection of 1 mg/kg i.v. (Days 1, 3, 5 and 8)
- 10 mice per cohort





- 2 treated mice were excluded from calculations because initial tumors were too small:
  did not end up growing tumors - 1 is certainly cured!!

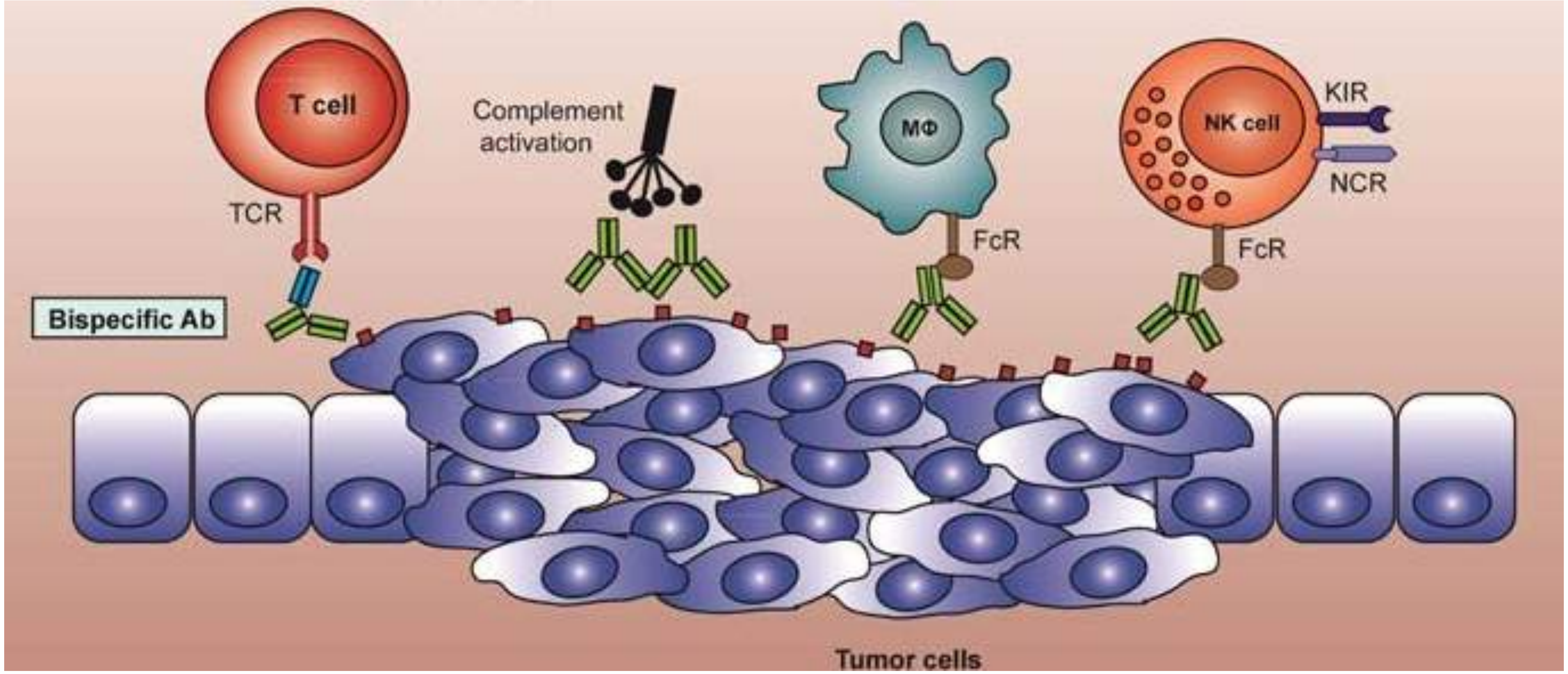
- Histopathology of tumors for Ki-67 (a marker of cellular proliferation)
  - number of cells undergoing cell division is significantly lower in the treated vs non-treated tumors.

Burns et al. (2017) *Mol. Pharm.*

Strategy #2

Immunotherapy Applications

Antibody-mediated Immune Response

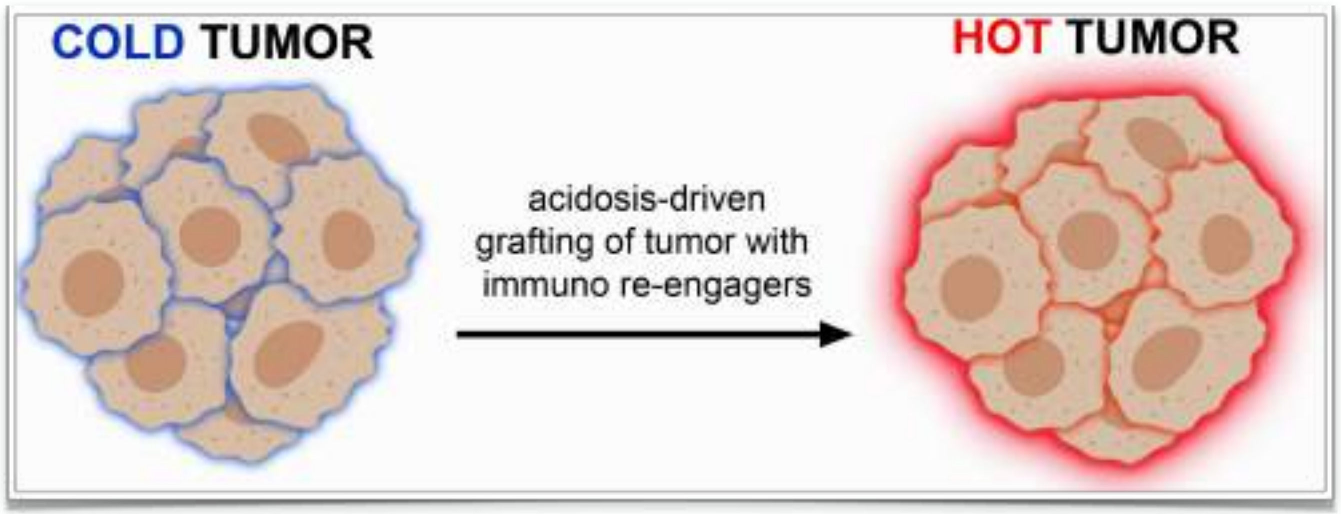


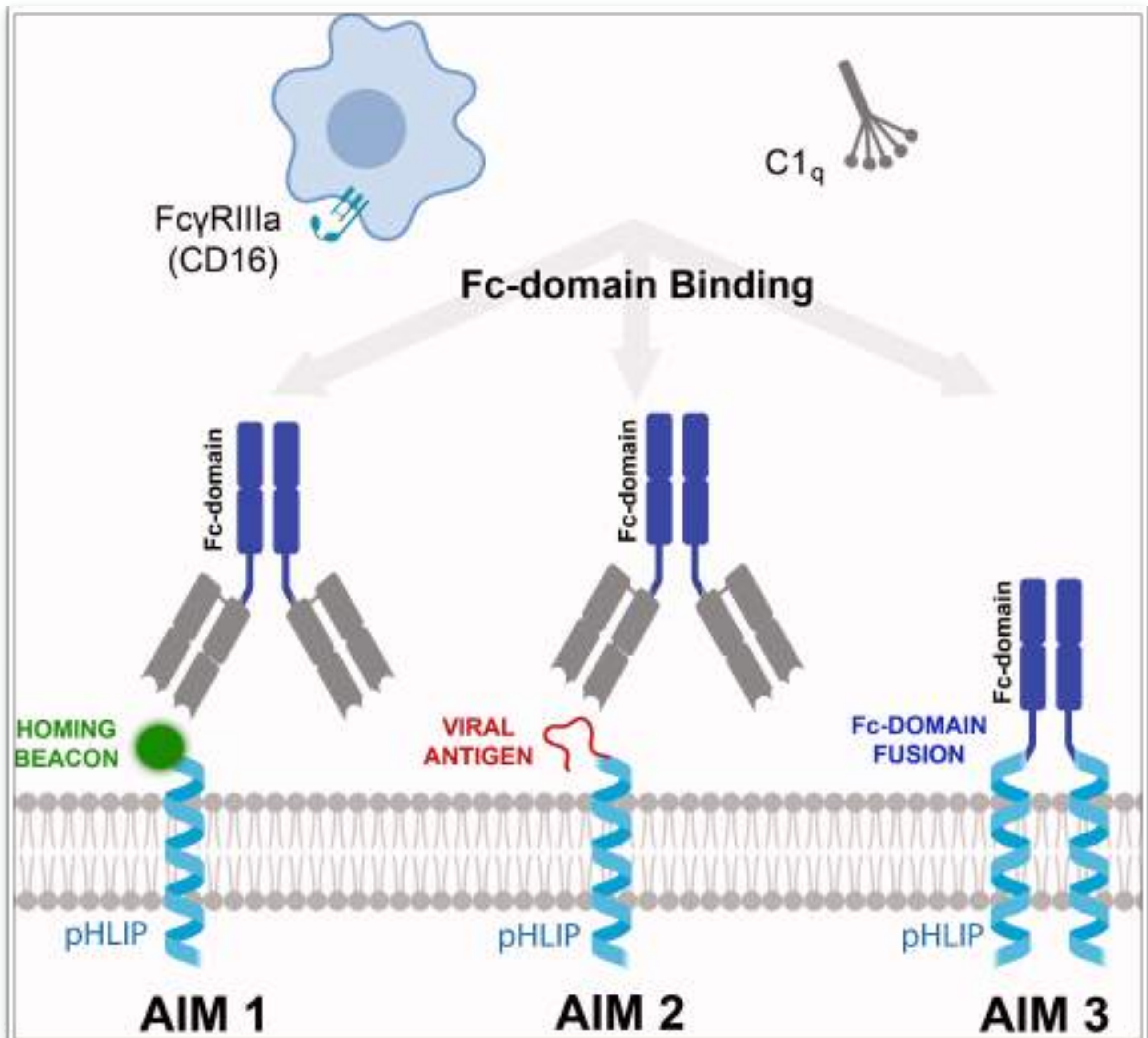
DOI: 10.1177/039463201002300104

One of the major problems in cancer:

Immune system does not recognize cancer cells anymore.

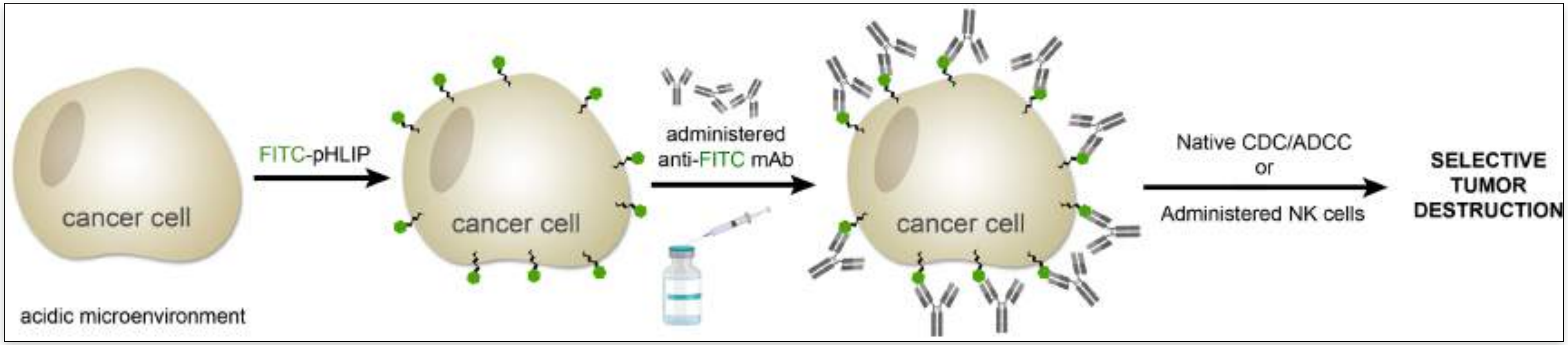
pHIP-related Projects: Immunotherapy Applications



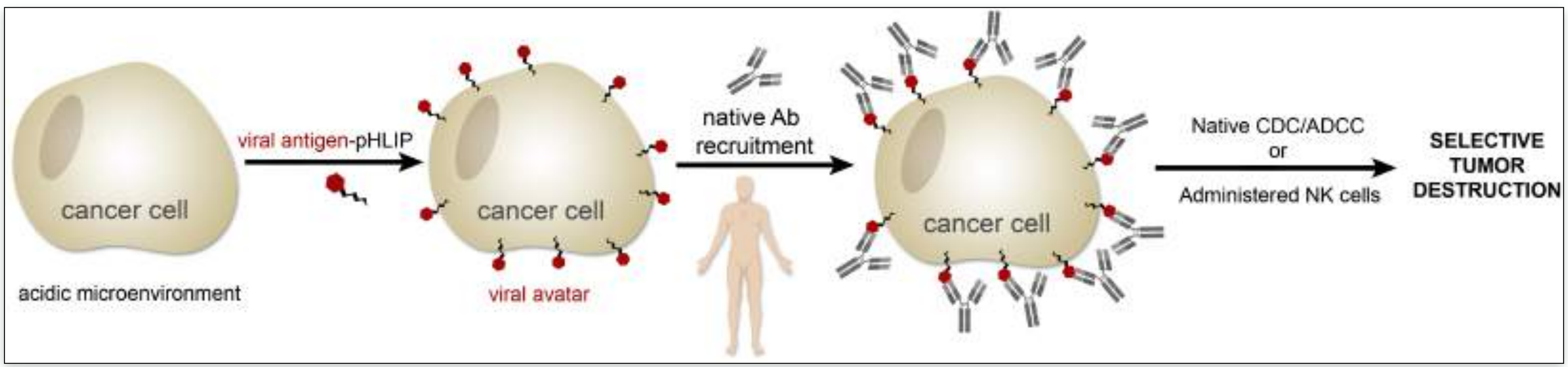


pHIP-related Projects: Immunotherapy Applications

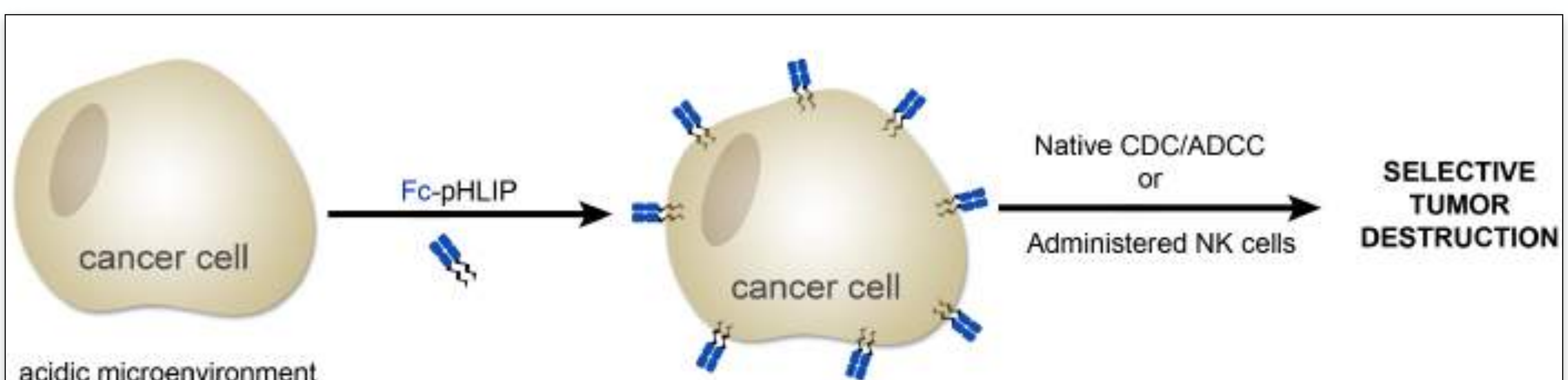
Aim 1



Aim 2

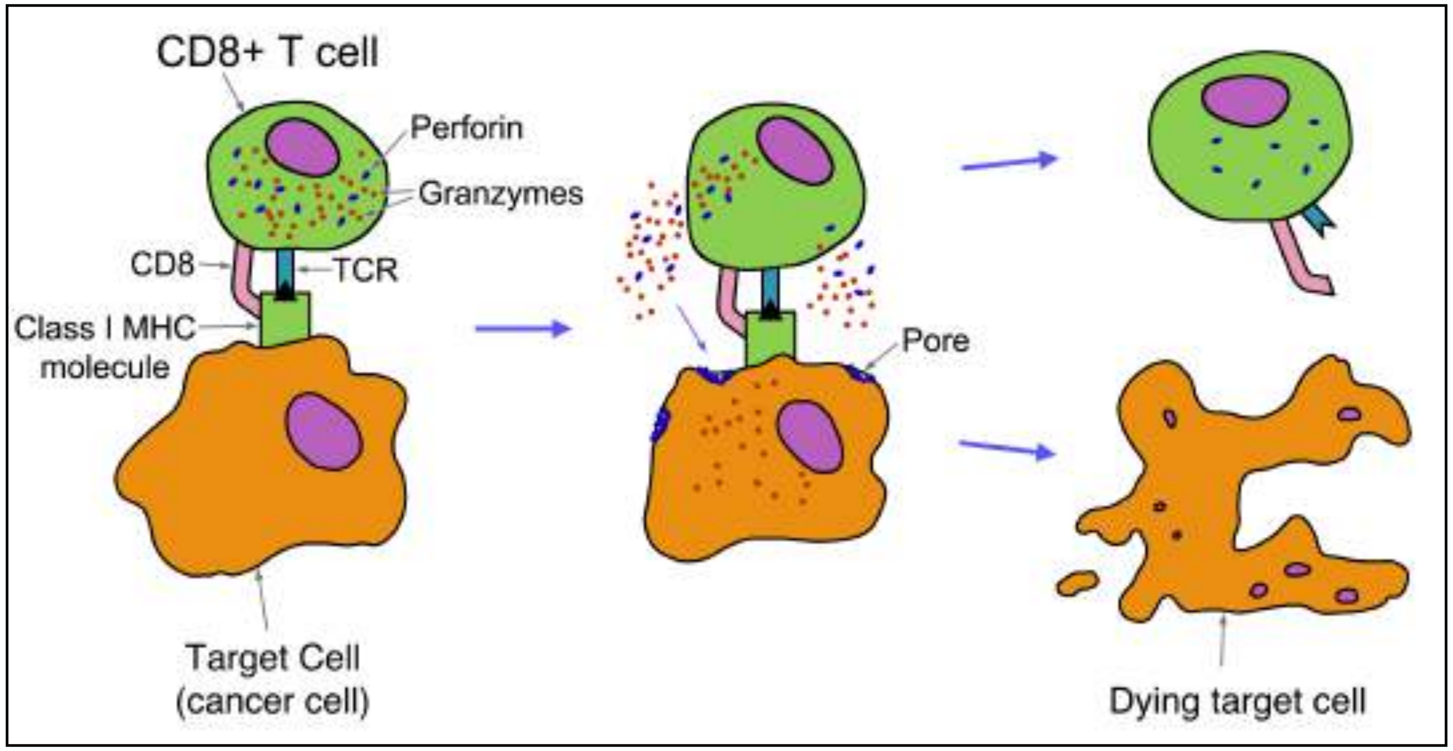


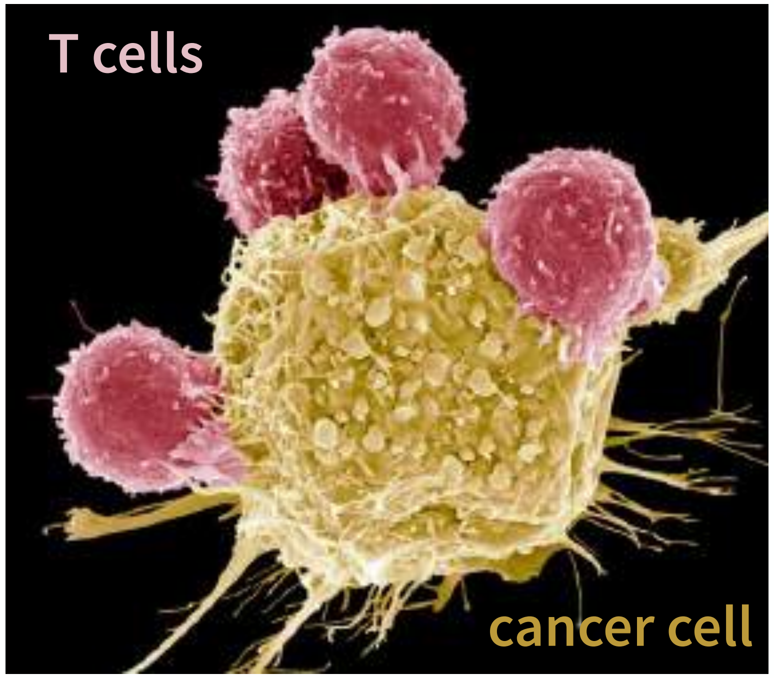
Aim 3



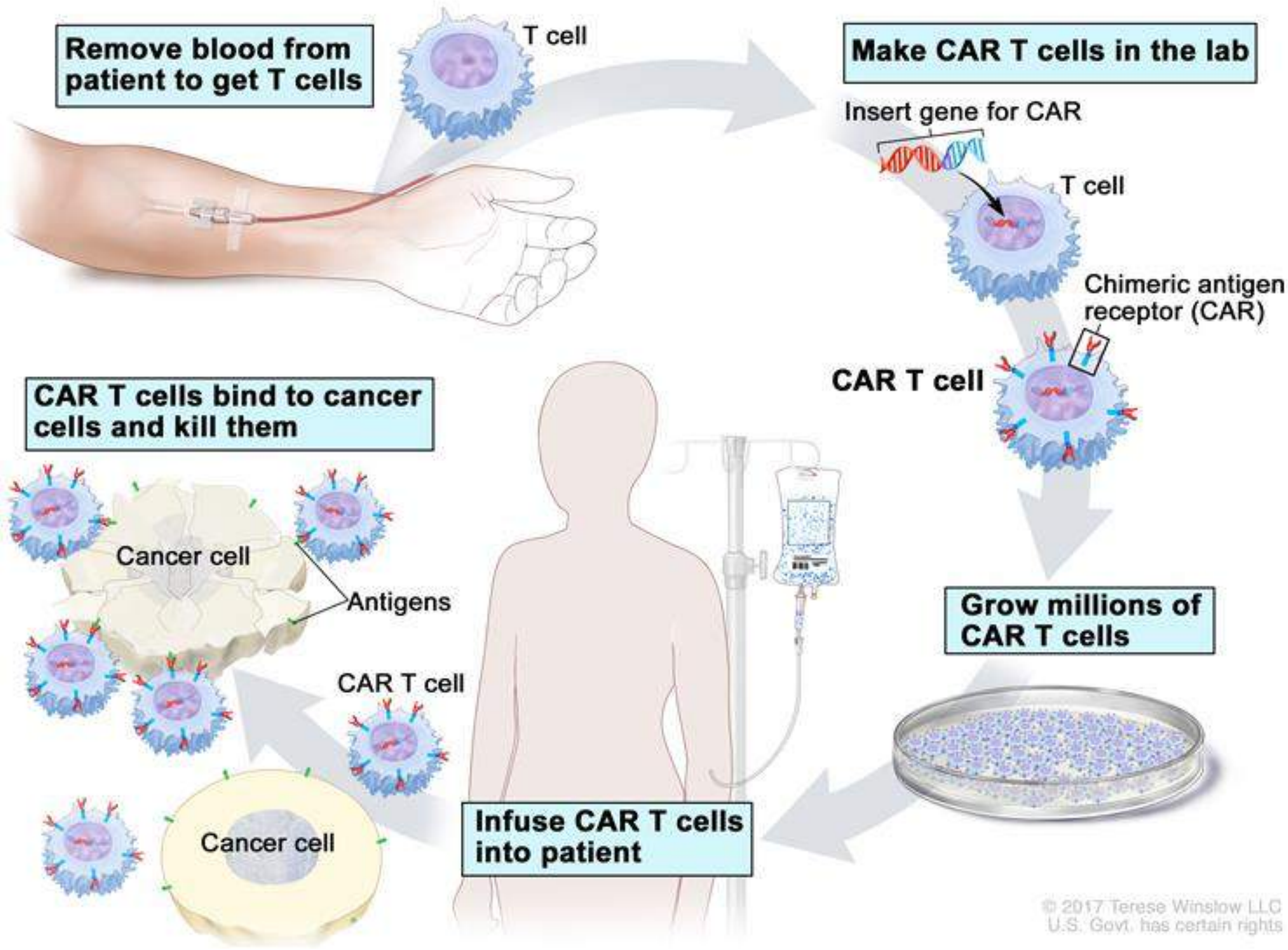
Immune Response: Killer (CD8+) T cells

T cell Attacking Cancer Cell

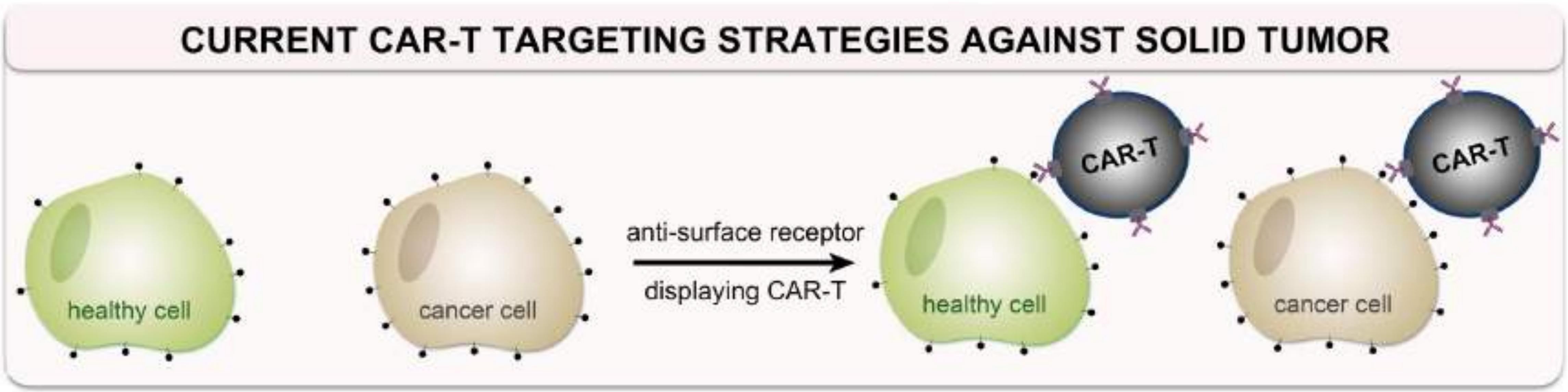


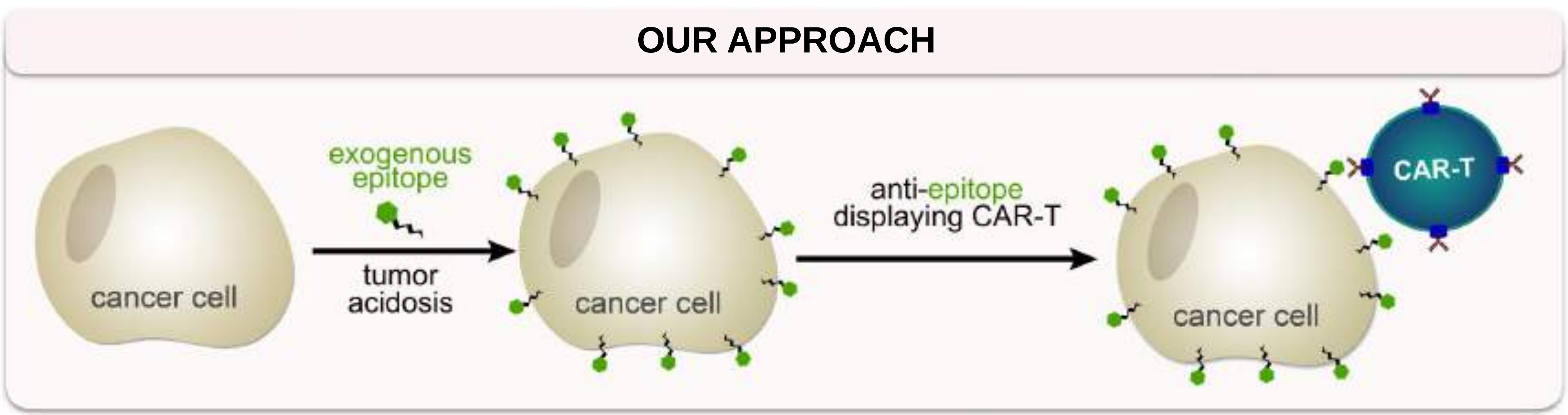


CAR-T: Chimeric antigen receptor T cells



pHLIP-mediated **CAR-T Therapy**





Thévenin Lab: Approaches

a. Chemistry:

- Synthesis and purification of peptides and small molecules
- Stability assay: half-life of peptide in serum determination by HPLC

b. Molecular Biology:

- Cloning and mutagenesis
- Bacterial reporter assay
- Protein expression and purification

c. Biophysical characterization:

- Circular dichroism
- Fluorescence
- Analytical ultra-centrifugation
- Backscattering interferometry

d. Cell Biology

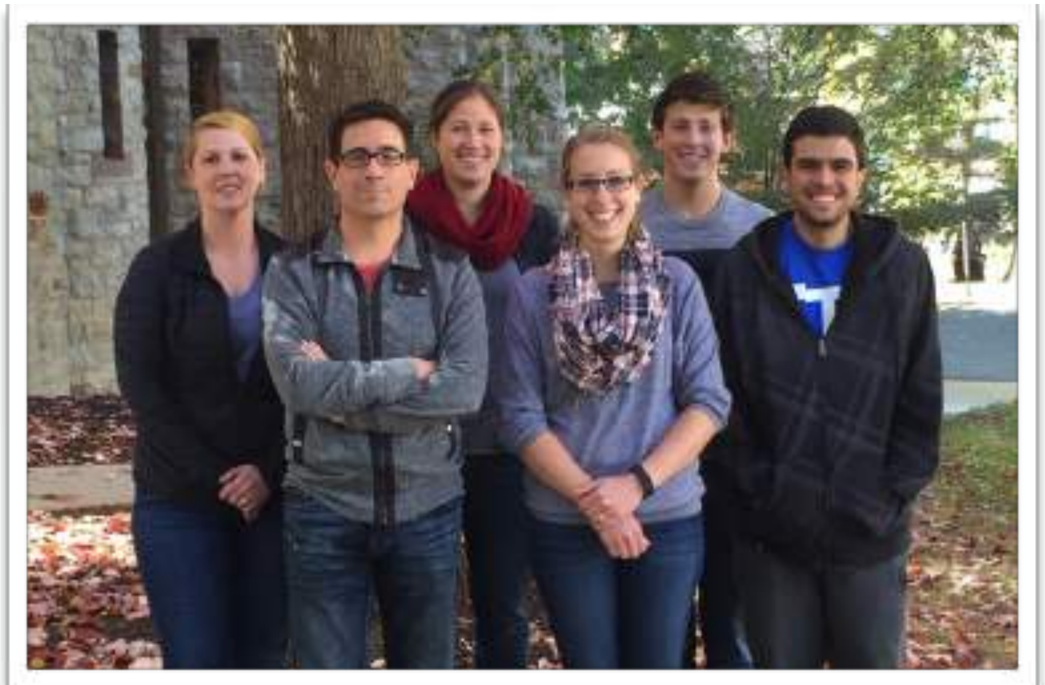
- evaluation of translocation, cell proliferation and migration
- by fluorescence and activity assays
- monitoring of receptor oligomerization (FRET, BRET, PLA).
- assays with immune cells

e. Delivery to tumors in mice:

- in collaboration with Dr. Hensley (Fox Chase Cancer Center, Philadelphia) and Dr. Adam Snook (Thomas Jefferson University, Philadelphia)
- pharmacokinetics and biodistribution
- maximum tolerated dose
- therapeutic efficacy
- tumor measurements by MRI

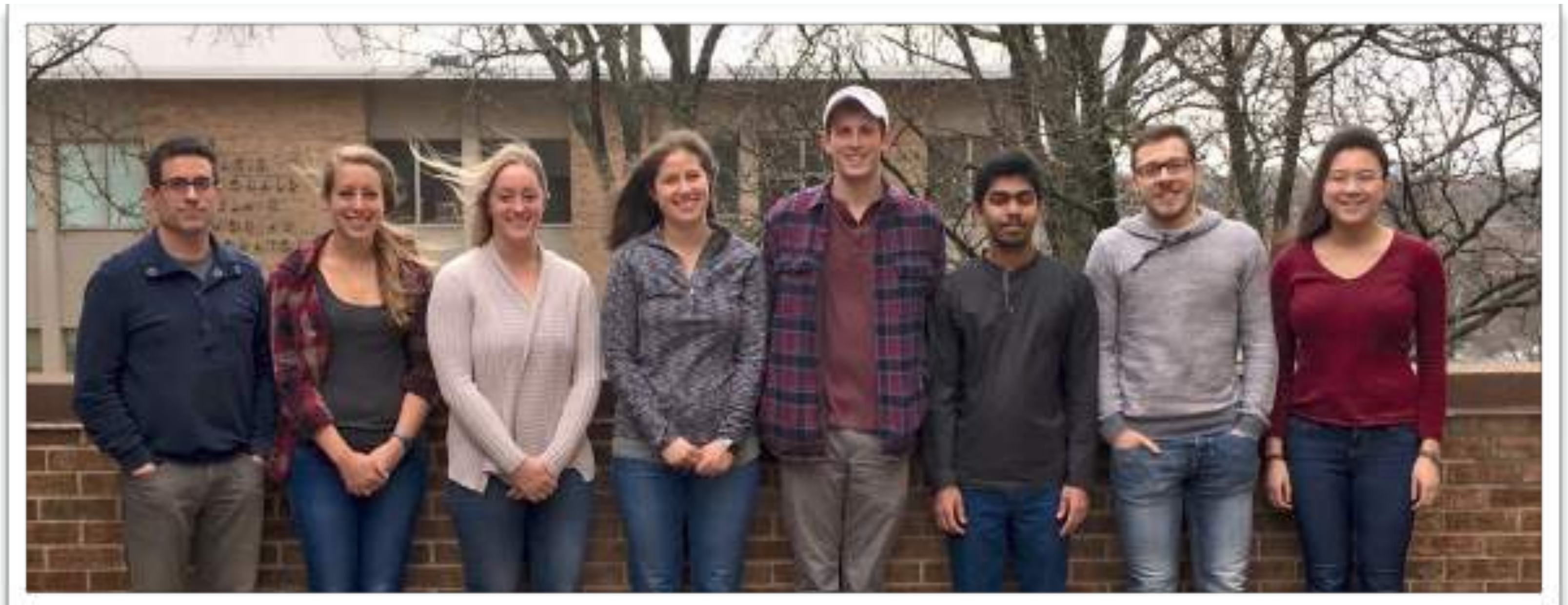
Acknowledgments

LEHIGH UNIVERSITY



Dr. Kelly Burns (former Grad)
Dr. Elizabeth Bloch (former Grad)
Dr. Janessa Gerhart (former Grad)

Eden Sikorski (Grad)
Emily Ankrom (Grad)
Lauren Furst (UG)
Long Gao (UG)



All past members of the lab

FOX CHASE CANCER CENTER TEMPLE HEALTH

Prof. Matt Robinson
Dr. Harvey Hensley

NIH National Institutes of Health

NATIONAL CANCER INSTITUTE