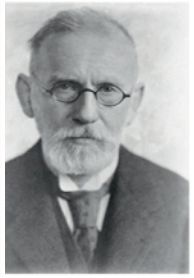
University of Pittsburgh

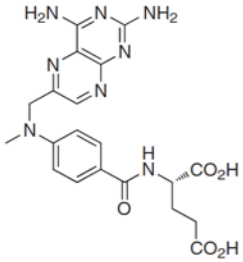
Antibody Drug Conjugates: Principle, Applications, and Future Directions

Raffaele Colombo

Pittsburgh – 11/22/2014

Timeline





1913 — aul Ehrlich described e concept of a agic bullet' and ug targeting (i.e. a eptophore' can eliver a 'toxophore' lectively to a tumor)

1958 — MTX linked to an antibody directed toward leukemia cells

1967 — ADCs proposed; immunoradioactive agent disclosed

1972 — Noncovalent linked ADC tested in animal models

1975 — Production of mAbs using hybridoma-based technology

1983 — Clinical trials w/ ADC vindesine-αCEA

1988 — Covalent linked ADCs tested in animal models

1991 — Humanized mAbs reported

1993 — Immunogenicity of mouse mAbs a serious limitation in development of ADCs

2000 — Curative efficacy in human tumor xenograft models w/ BR96-DOX ADC

2001 — ADC w/ highly potent cytotoxin calicheamicin

2010 — Mylotarg® withdrawn from market

2011 — Mylotarg® clinical failures due to MDR

2013 — First FDA approved ADC (Mylotarg®)

2013 — Kadcyla approve

2011 — Adcetris® approved

Adapted from *Drug Discov Today*. **2014**, *19*, 869-81

ADC facts

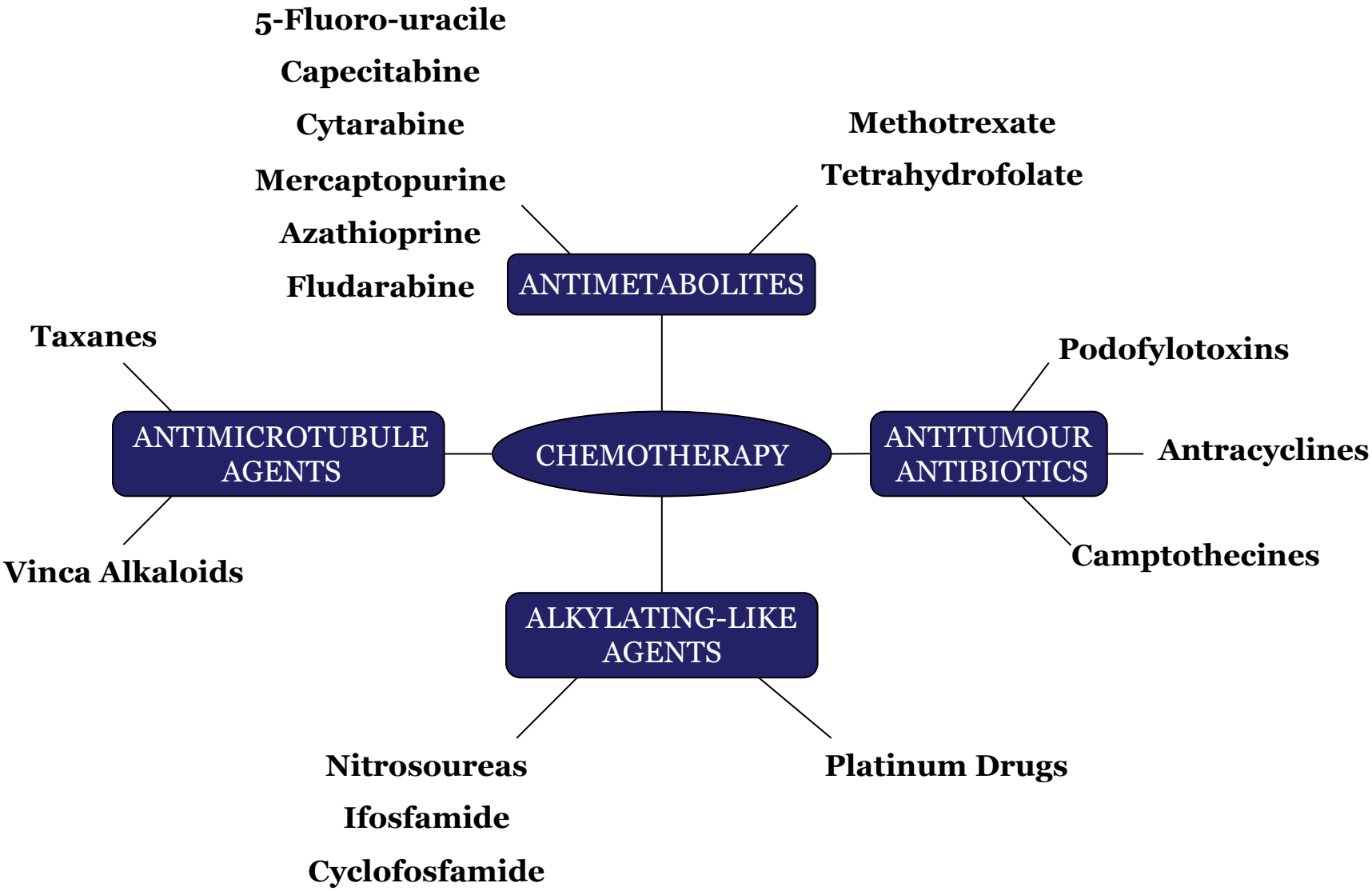
- Leaders of major pharmaceutical companies predict that **> 60% of all existing drugs will be modified** to become **cell specific** in less than 20 years
- The market research firm predicts that **by 2018** sales of launched ADCs products will **exceed $5 billion**
- **More than 30 ADCs** are in **advanced clinical development**
- Industry participants estimate that **between 100 and 150 more ADCs are in the preclinical stage**

Classical types of cancer treatment

- **Surgery:** often the first line of treatment for many solid tumors.
- **Radiation:** kill the cancer cells directly by damaging them with high energy beams.
- **Hormonal Treatments:** designed to inhibit cancer cell growth by preventing the cells from receiving signals necessary for growth and division
- **Chemotherapy:** a term used for a wide variety of drugs used to kill cancer cells. Systemic treatment to try to prevent growth, invasion, and metastasis.

Initially discovered after WW1 when soldiers were exposed to nitrogen mustard. Medical examination of the victims suggested that profound lymphoid and myeloid suppression had occurred after exposure. Louis S. Goodman and Alfred Gilman reasoned that this agent could be used to treat lymphoma

Traditional chemotherapy



Traditional chemotherapy

- Systematic administration of these drugs results in both **tumor killing** and **damage to healthy tissues**
- The balance between these two actions limits the **efficacy** and **tolerability** of single-agent chemotherapy
- The treatments often consist **of combination of chemotherapy agents**, each administered **at or near the maximum tolerated dose** and for a **limited duration**
- The **rapid clearance** of small molecules further reduces the activity of chemotherapy

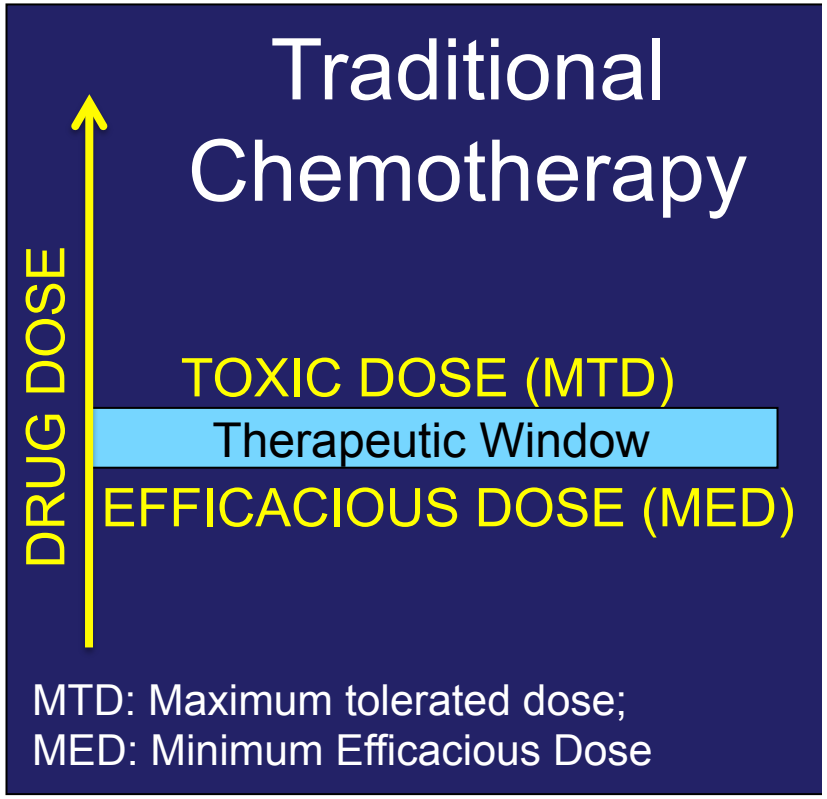
Efficacy of chemotherapy

- Efficacy is limited by their **non-specific toxicity to normal cells** (*especially to rapidly growing cells such as blood, bone marrow, and mucous membrane cells*) **resulting in a low therapeutic index and serious side effects**.

- Efficacy is further limited by the occurrence or development of **drug resistance**:

  because of their genetic **instability**, **heterogeneity**, and **high rate of mutation**, tumor cells can select and overgrow drug-resistant tumor cell populations.

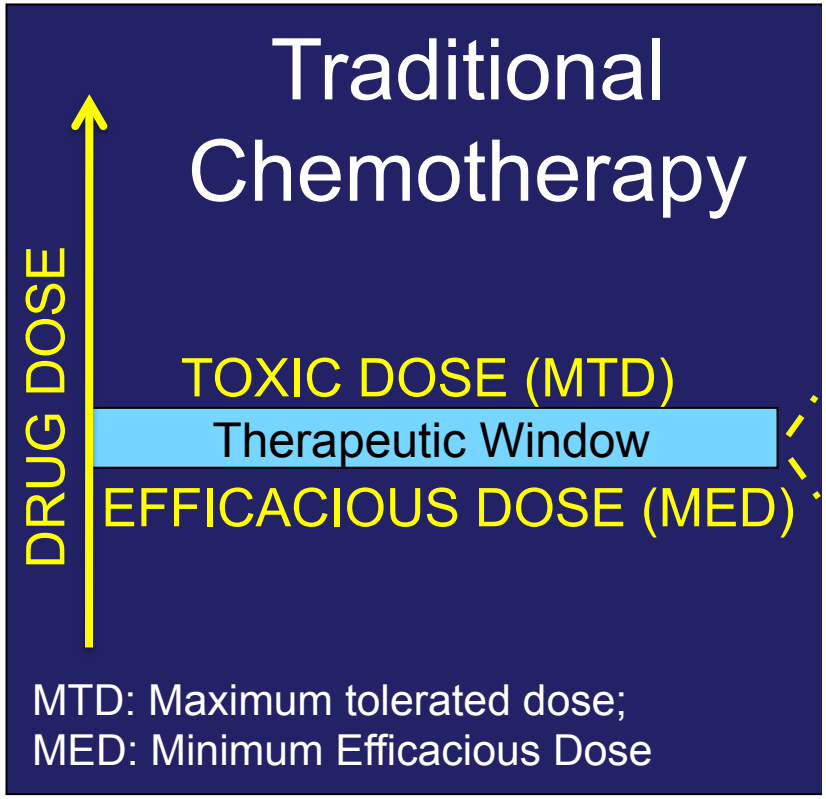
Therapeutic window



Inability to kill a sufficient number of cancer cell without causing toxicity

- **>99%** of the cells in the tumor have to be killed to achieve a **complete remission**
- Significantly **greater** degree of cell kill required to achieve **tumor eradication**

Therapeutic window



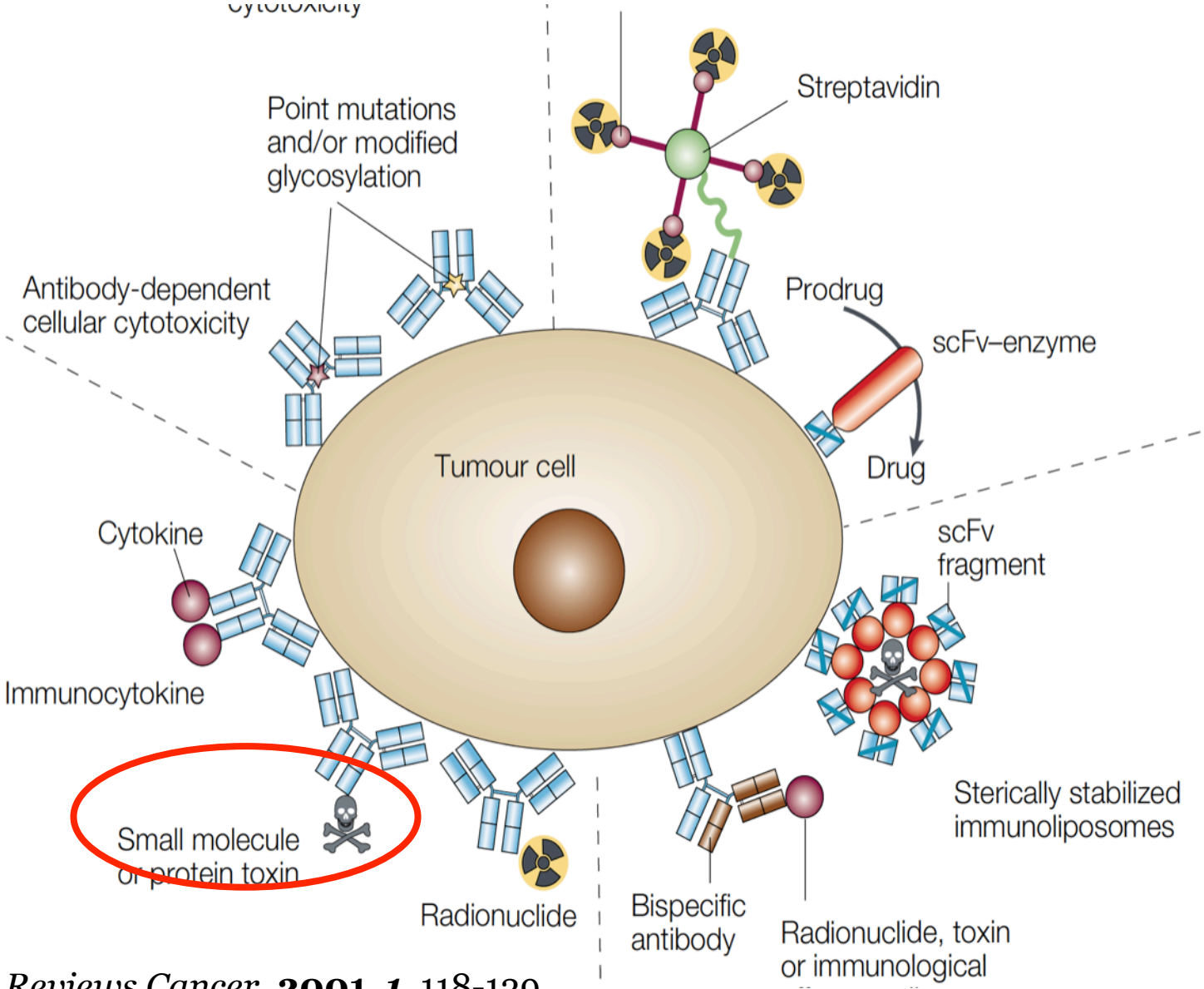
Increase selectivity

Increase potency

ADCs/SMDCs can **selectively deliver a potent cytotoxic drug** to tumor cells via tumor-specific and/or over-expressed antigens/receptors

- Increase drug delivery to tumor
- Reduce normal tissue drug exposure

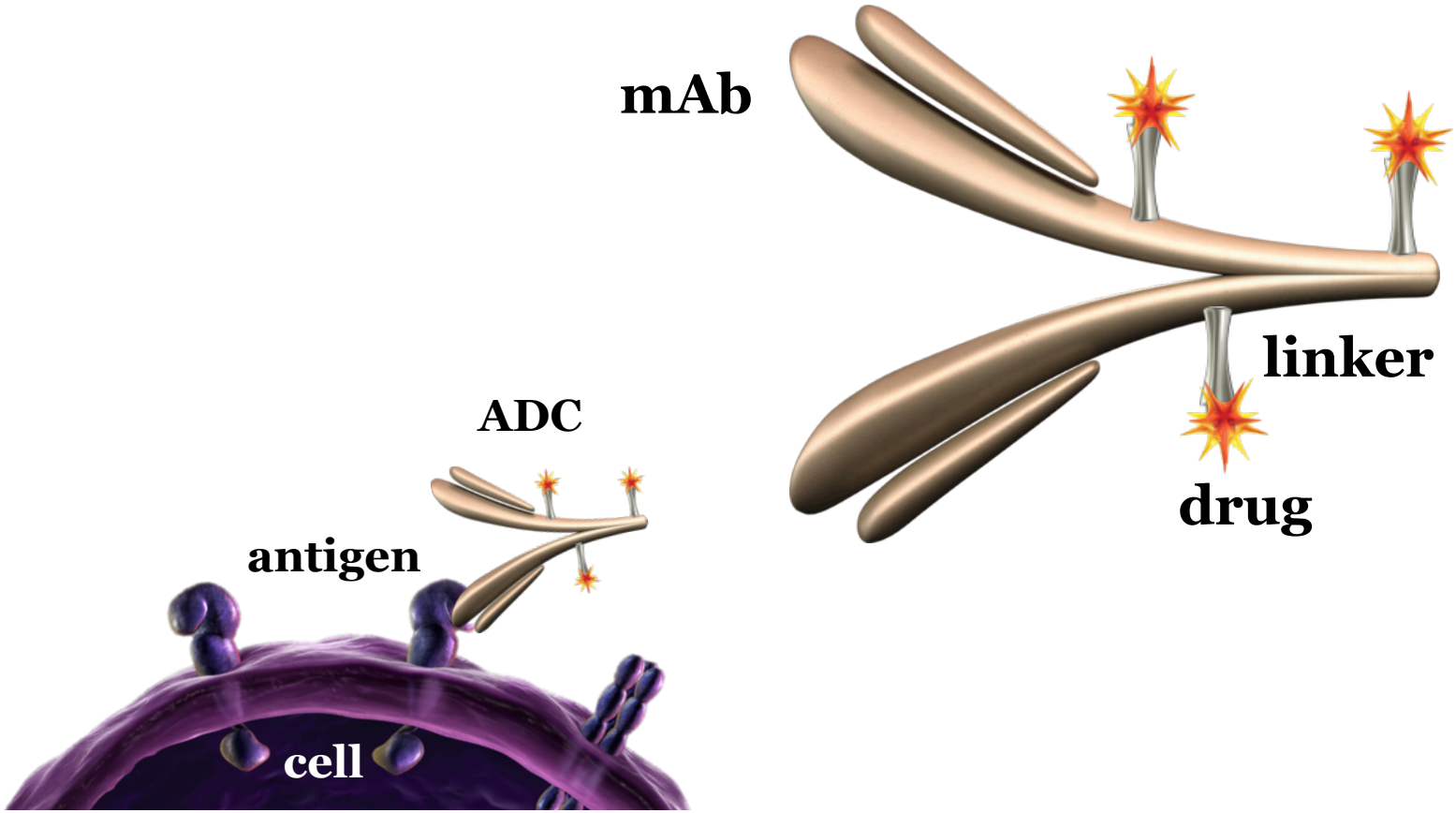
Antibody strategies



Nature Reviews Cancer, **2001**, ***1***, 118-129

What is an ADC?

ADCs consist of a **monoclonal antibody** chemically **coupled** to a **cytotoxic drug** via a **linker**



What is an ADC?

ADCs consist of a **monoclonal antibody** chemically **coupled** to a **cytotoxic drug** via a **linker**

Important aspects are:

- **The molecular target** (antigen)
- **The delivery vehicle** (mAb)
- **Chemically conjugation** (method, site, stoichiometric)
- **The linker** (including the mechanism of release)
- **Cytotoxic agent or payload** (mechanism, potency)

Choosing a good target

Cancer cells possess **specific molecular markers** that play a key role in **tumor growth and progression**

Ideal ADC cancer target should have:

- **abundant** and **uniform** expression on the cell surface
- **low/absent** expression **on healthy tissues**
- **rapid internalization and trafficking** to lysosomes upon antibody engagement
- **accessibility** to intravascular macromolecules

There are a limited number of targets that fulfill all of these criteria

Validated targets are: CD30 and Her2

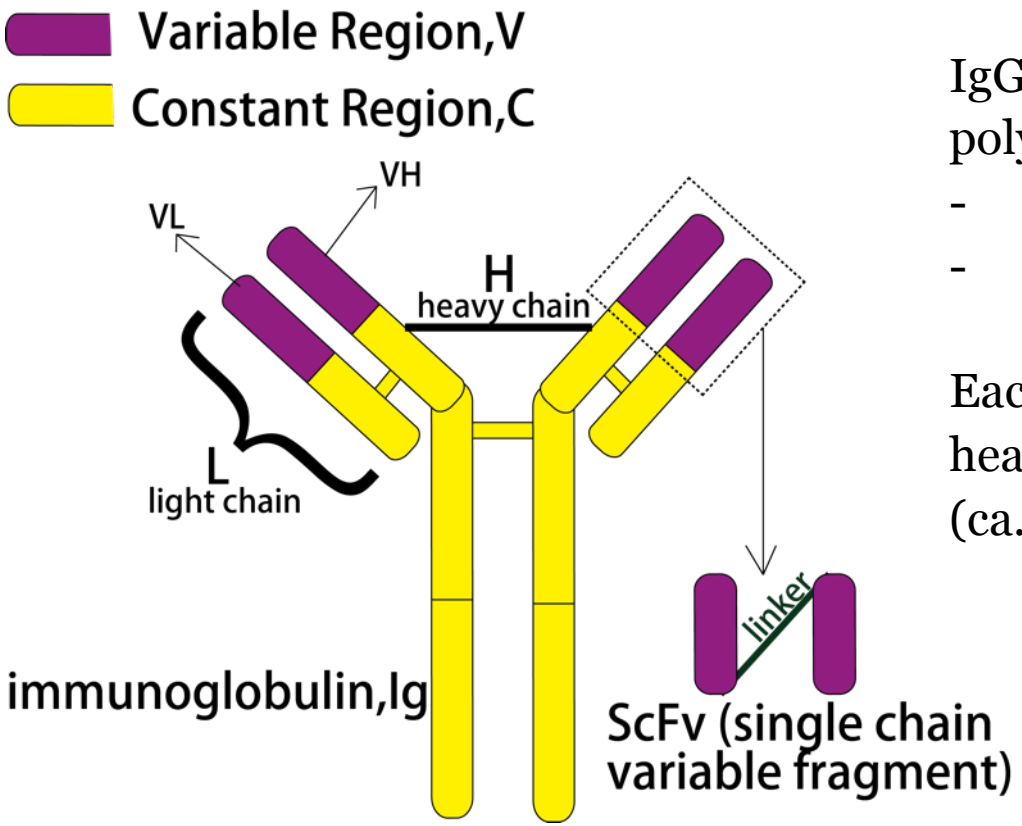
Choosing a good target

A number of new targets are currently being evaluated in clinical stage, for example:

Target antigen	Cancer type
CD20	**B-cell lymphoma, non-Hodgkin lymphoma**
HER2	**HER2-positive breast cancer**
VEGF-A	Colorectal cancer, lung, kidney, glioblastoma
CD52	B-cell lymphoma
CD138	Multiple myeloma
CD22	non-Hodgkin lymphoma
EphA2	Solid tumors
AGS-5	Pancreatic cancer, prostate cancer, epithelial tumors
Nectin-4	Bladder, breast, lung, and pancreatic
GPMNB	Breast cancer, melanoma
CD74	Multiple myeloma
Le	Prostate cancer, ovarian cancer
CD19	non-Hodgkin lymphoma

Antibody structure

The most abundant class of antibody found in the plasma is the Immunoglobulins γ (IgG).



IgG are composed of two different polypeptide chain:

- **Heavy** or H chain (ca. 50 kDa)
- **Light** or L chain (ca. 25 kDa)

Each IgG molecule consists of two heavy chains and two light chains (ca. 150 kDa in total)

Each variants can bind to a different antigen

Antibody structure

The monoclonal antibody represent **more than 90% of the mass** of most ADCs

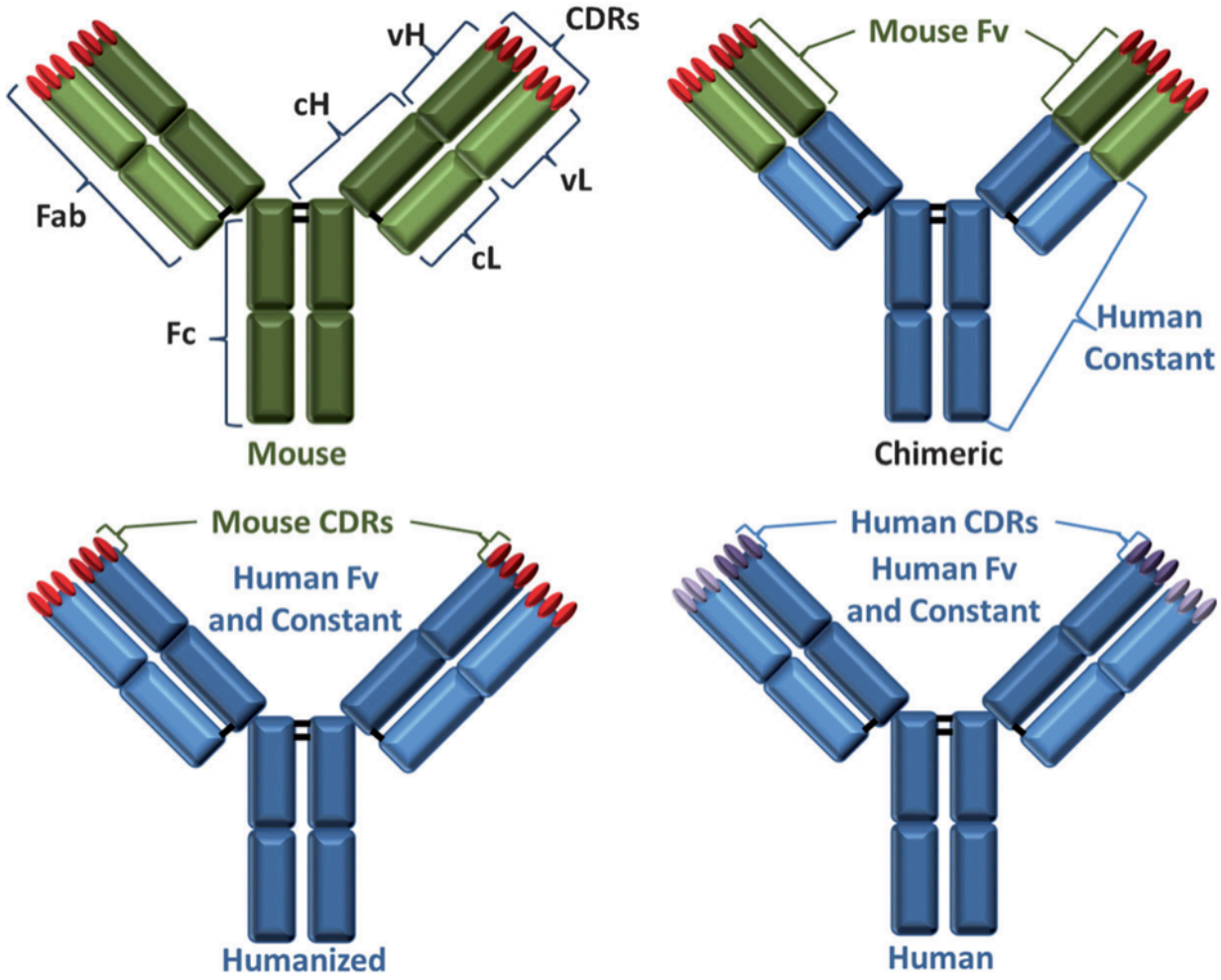
Monoclonal antibodies can be:

Murine **Chimeric** **Humanized** **Human**

HAMA (human anti-mouse antibody)
Rapid clearance

improved efficacy and safety

Antibody structure



Fragment crystallizable region (**Fc**), fragment antigen-binding (**Fab**) heavy-chain variable (**vH**), light-chain variable (**vL**), heavy-chain constant (**cH**), light-chain constant (**cL**), the complementarity determining regions (**CDRs**)

Nude antibody FDA approved

Generic Name	Brand Name	Target	Cancer(s)	Year
Rituximab	Rituxan	CD20	Lymphomas	1997
Trastuzumab	Herceptin	HER-2	Breast	1998
Alemtuzumab	Campath	CD52	Chronic lymphocytic leucemia	2001
Bevacizumab	Avastin	VEGF	Multiple	2004
Cetuximab	Erbitux	EGFR1	Colon, head and neck	2004
Panitumumab	Vectibix	EGFR1	Colon	2006
Ofatunumab	Arzerra	CD20	Chronic lymphocytic leucemia	2009
Ipilimumab	Yervoy	CTLA-4	Melanoma	2011
Pertuzumab	Perjeta	HER-2	Breast	2012

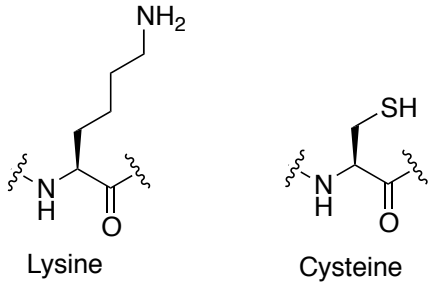
2 chimeric, 4 humanized, 3 fully human mAbs

In 32 years since the first Ab was evaluated, only nine have been approved and mainly in combination with other therapies!

Chemical conjugation

Drugs have been attached to mAb using native aminoacids:

- **the ε -amino terminus of lysine residues**
- **the sulfhydryl portion of reduced cysteine residues**



Conjugation to **lysine** residues results in a **distribution of adducts** with **different stoichiometry and location**

Conjugation to **reduced cysteines** results in **even-numbered** drug additions (2, 4, 6 or 8)

Most ADCs have an average of **4 drugs per antibody**

Chemical conjugation

Drugs have been attached to mAb using native aminoacids:

- **the ε -amino terminus of lysine residues**
- **the sulfhydryl portion of reduced cysteine residues**

In recent year, **site-specific conjugation** methods have been developed by:

- introducing novel **unpaired cysteine** residues
- introducing **non-natural aminoacids** that allow for orthogonal chemistry methods
- enzymatic conjugation

The impact of site-specific conjugation methods has not yet been evaluated in clinical trials

ADC Linker

The nature of **the linker plays an important role** in the bioavailability and activity of the conjugates

Stability of the ADCs before they reach the target cells is the key to **minimize the off-target killing** and **maximize tumor exposure to drugs**

Linker	Release mechanism
Hydrazone	Designed for serum stability and degradation in acidic compartments within the cytoplasm
Peptide	Designed to be enzymatically hydrolysed by lysosomal proteases such as cathepsin B
Disulfide	Designed to be cleaved through disulfide exchange with an intracellular thiol, such as glutathione
Thioether	Non-reducible and designed for intracellular proteolytic degradation

ADC payload

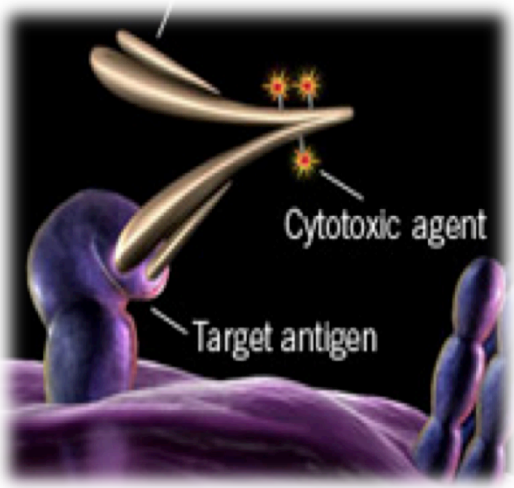
The released drug of an ADC must be present in **sufficient concentration** inside the tumor cells to cause cell death.

The drug is usually **released in the lysosome** but often exerts its cell-killing activity elsewhere, such as the cytoplasm or nucleus.

Identifying cytotoxic agents that are **resistant to lysosomal proteases** and are **able to exit** the lysosome (via diffusion or facilitated transport) are additional considerations for the design of ADCs.

In the last 2 decades, it has become apparent that **highly potent cytotoxic agents** 100-10000 times more potent than standard chemotherapy agents were needed.

Mechanism of action



1. Binding

The monoclonal antibody component of the ADC **binds to the target antigen** on the cell surface.

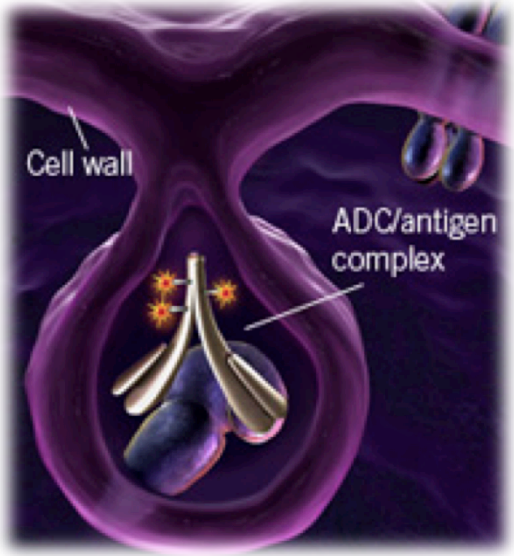
The monoclonal antibody component of an ADC may possess its own anticancer activities, but it's mainly used as **site-directed delivery**

2. Endocitosy

The ADC/antigen complex may be internalized through endocytosis

The efficiency of internalization depends on the type of cell-surface molecule the mAb binds to

The ADC/antigen complex could be quickly, moderately or poorly internalized



Mechanism of action

3. Degradation

Internalization of the ADC/antigen complex is followed by lysosomal degradation of the complex.

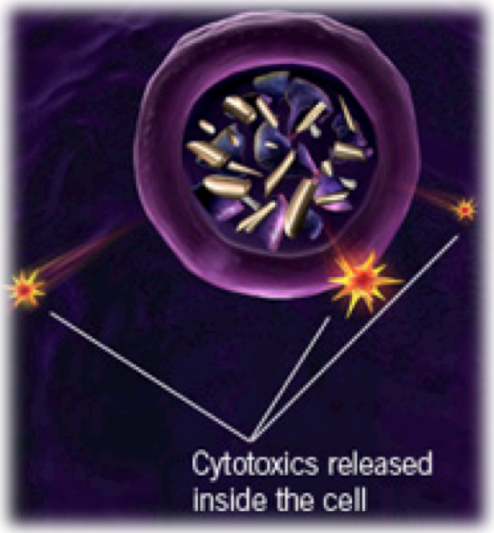
The lysosomes are both acidic and rich in proteolytic enzymes.

4. Release

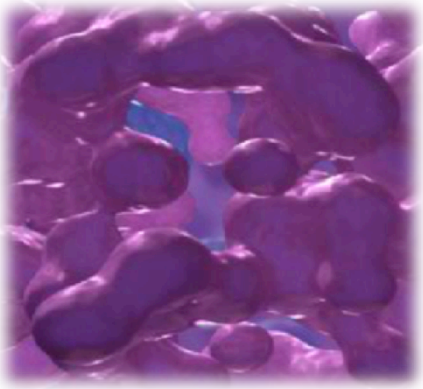
Lysosomal degradation results in release of the cytotoxic drugs

In order for the cytotoxic to be activated, the ADC must be internalized

Internalization and lysosomal degradation activate the release of the cytotoxic inside target tumor cells



Mechanism of action



5. Cell death

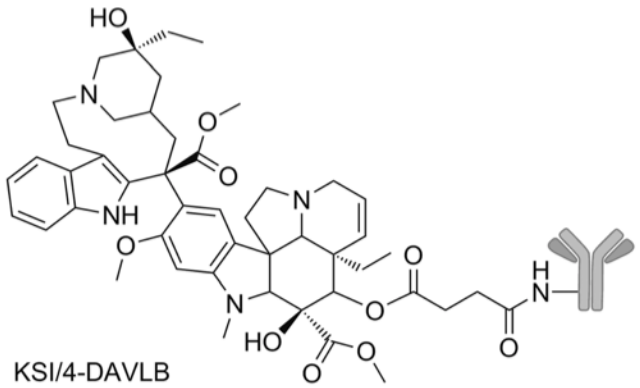
Once the cytotoxic has been released, it then interacts with critical cellular processes to induce cell death

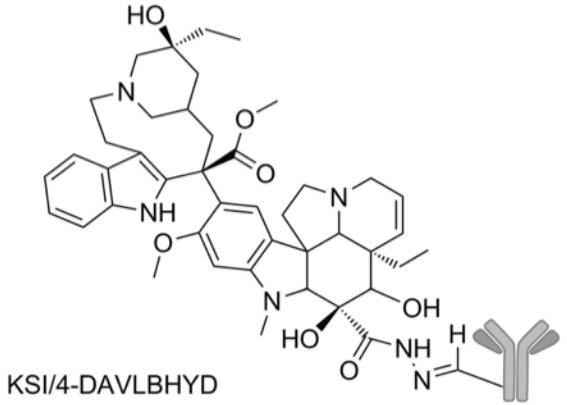
Images from: http://www.biooncology.com/research-education

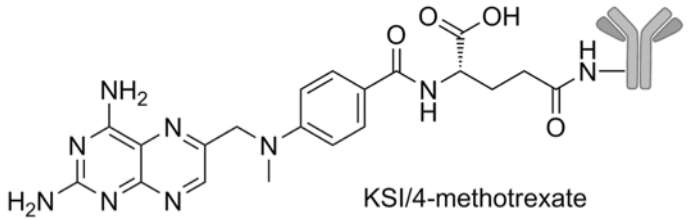
First generation of ADCs

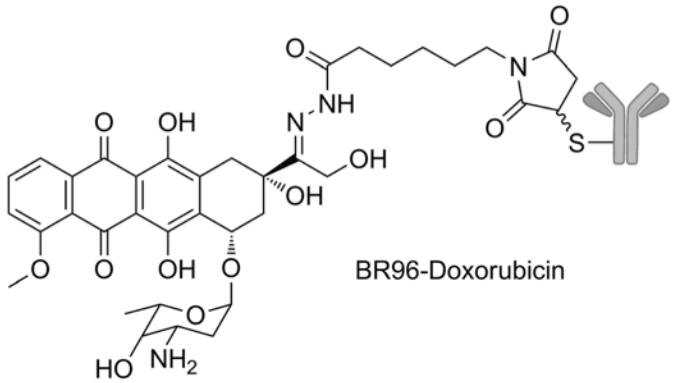
Aim: **improve tumor selectivity** of clinically used anticancer drugs

Drugs: (desacetyl)vinblastine, methotrexate, doxorubicine





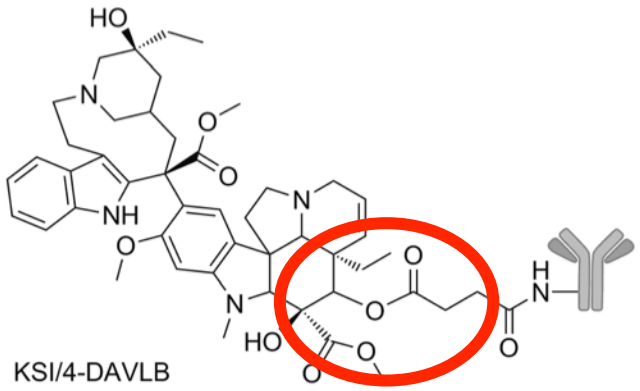


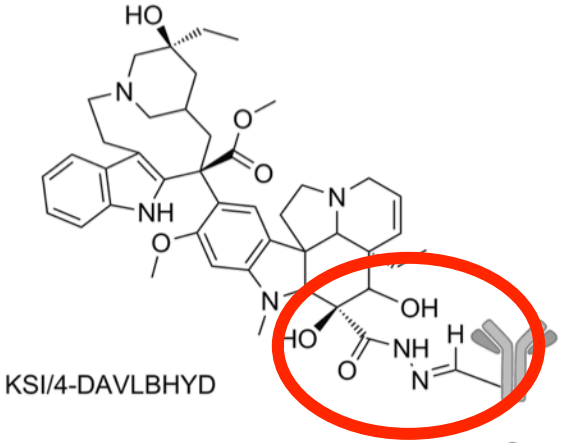


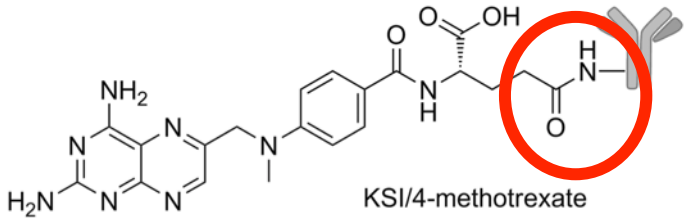
First generation of ADCs

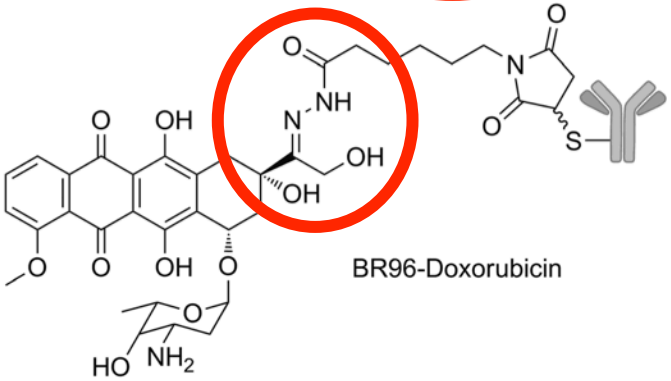
Linker: esters, amides, hydrazones

Release: esterases, peptidases, hydrolysis







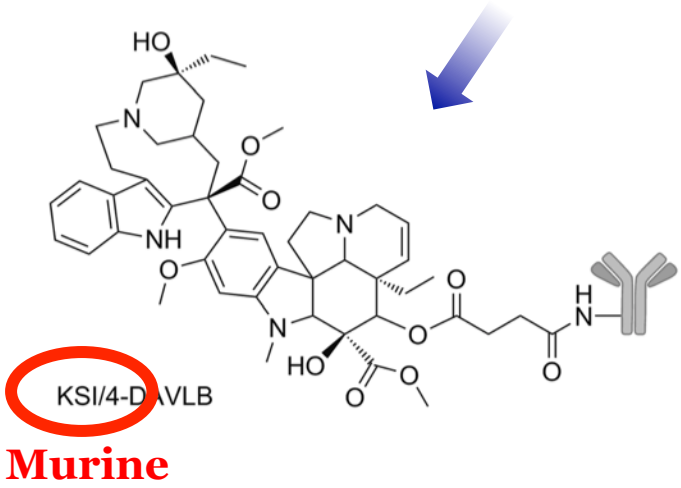


First generation of ADCs

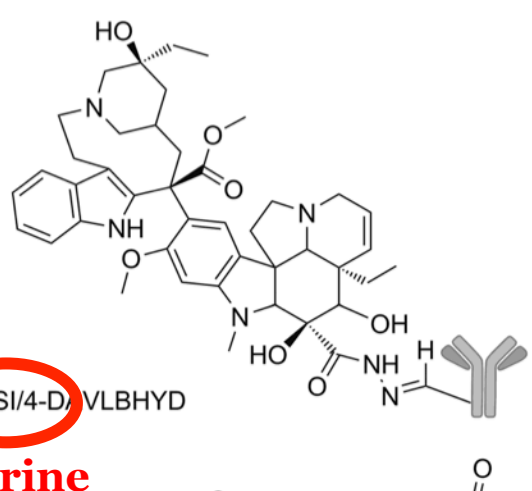
Antibody: murine or chimeric (HAMA in >50% of the patients)

Potency: moderately potent and often less active than the unconjugated drugs

Lung and colon adenocarcinoma

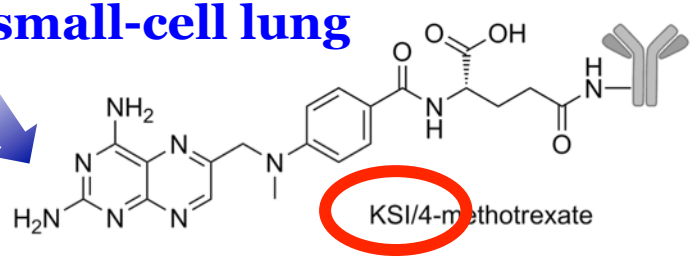


Murine



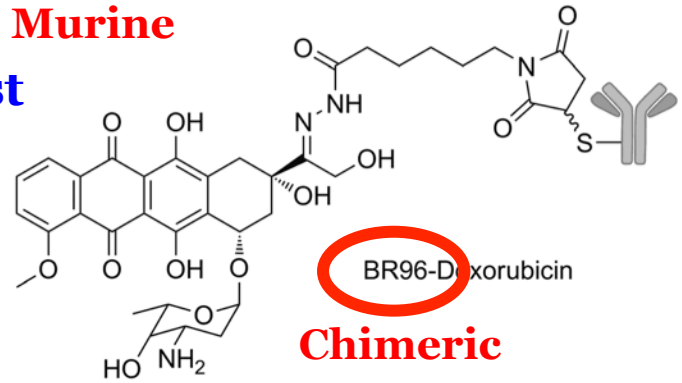
Murine

Non-small-cell lung



Murine

Breast

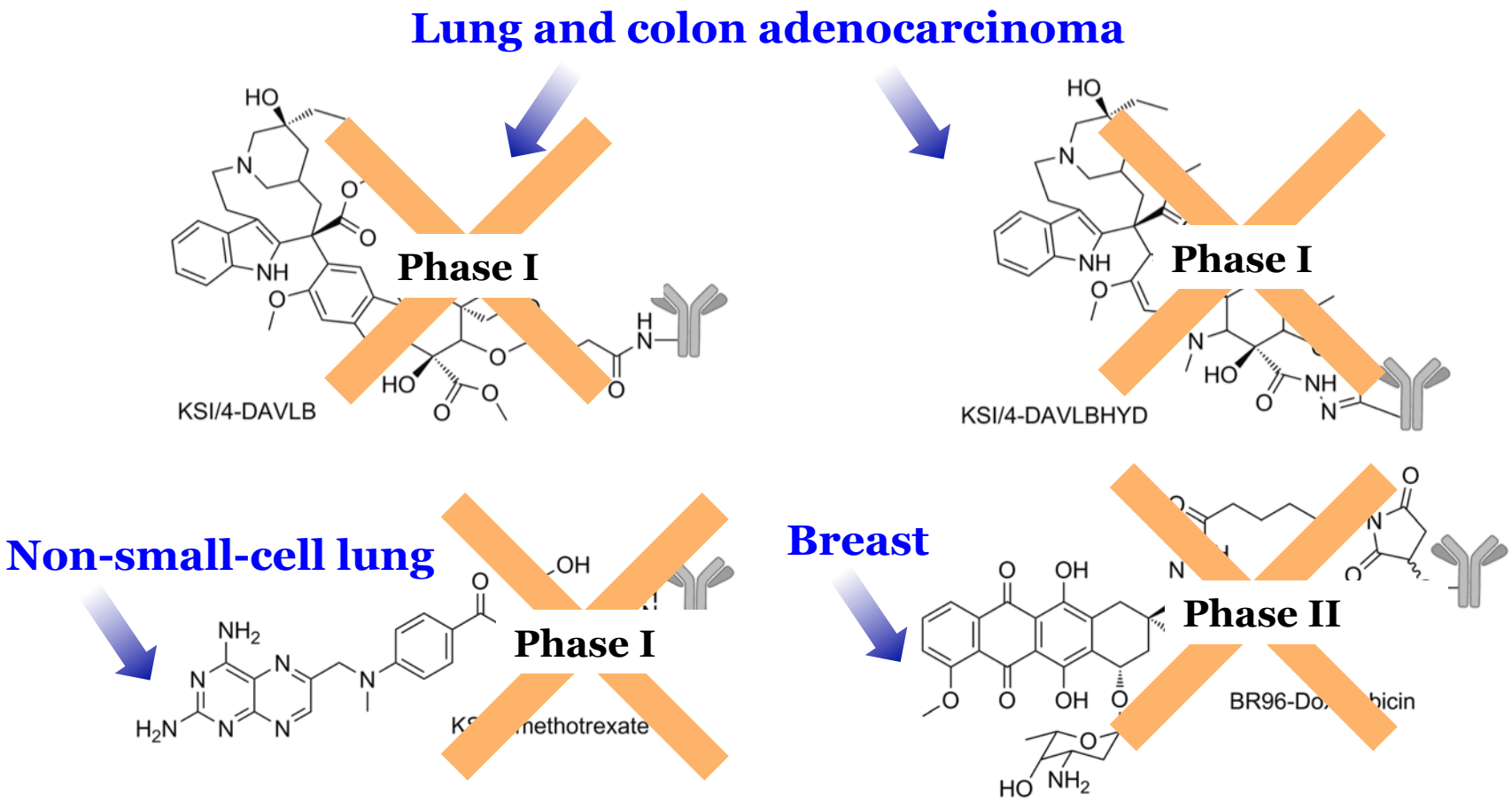


Chimeric

First generation of ADCs

Evidence of tumor localization, BUT:

- little evidence of therapeutic benefit and/or clinical response
- immune response to the mAb and to the drugs (mainly Vincas)



Lessons learned

The linkers were:

- **not stable enough** (esters and hydrazones)
  - → premature release of the drug
    - → systemic toxicity and lower therapeutic index

- **too stable (amides)**:
  - → drug not released
    - → low potency/efficacy

Problem of immunogenicity noted with early ADCs that used murine antibodies: it could be solved using **"humanized"** or **fully human** antibodies

Lessons learned

Lack of sufficient potency:

- **different modes** of cellular uptake of the unconjugated and conjugated drugs
- not **enough concentration** in the tumor cells

The **number of molecules** of a moderately potent cytotoxic drug **required** to effect **cell kill** could be very high (**>10^6** molecules/cell).

Delivery of the **cytotoxic drugs** by an ADC is limited by 2 factors:

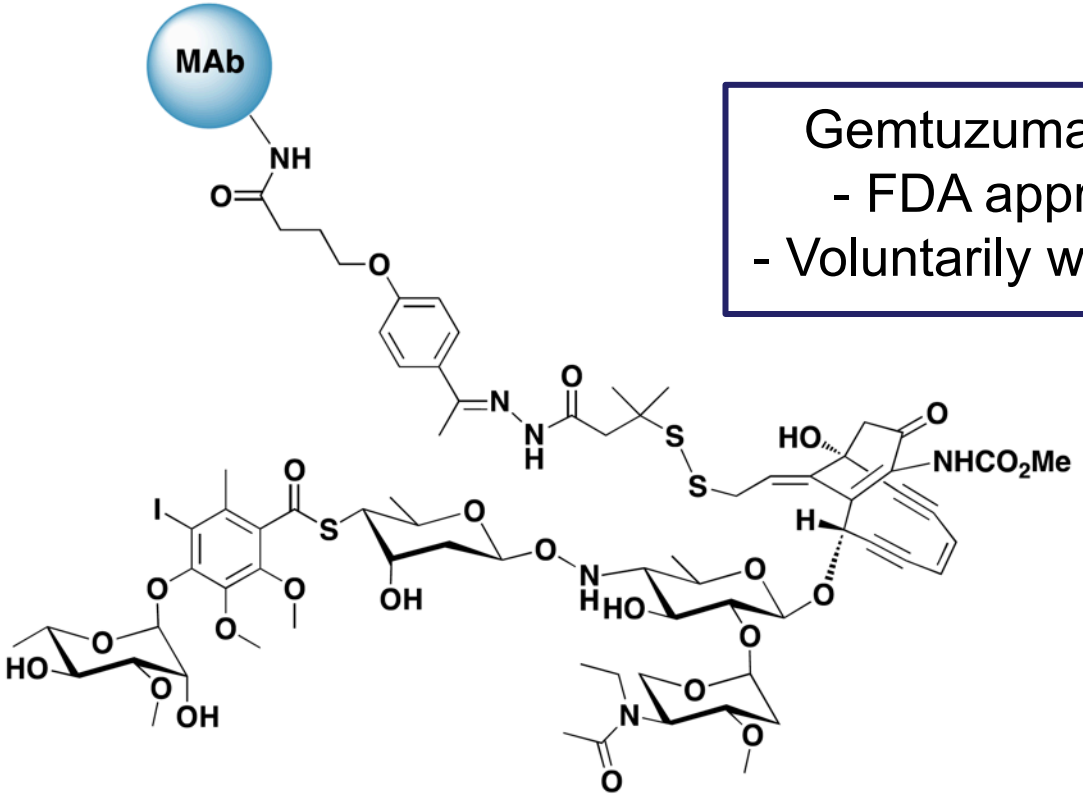
a) **moderate number of antigen** molecules on the cell surface to which the antibody can bind (typically 10^5 receptors/cell)

b) **internalization** of cell-surface bound antigen–antibody complex, or intracellular processing to release the active drug moiety, may not be efficient.

Second generation of ADCs

- "**Humanized**" or **fully human** mAbs

- **More potent drugs**
  (too toxic to be administered as a single agents)



Gemtuzumab ozogamacin
- FDA approved in 2000
- Voluntarily withdrawn in 2010

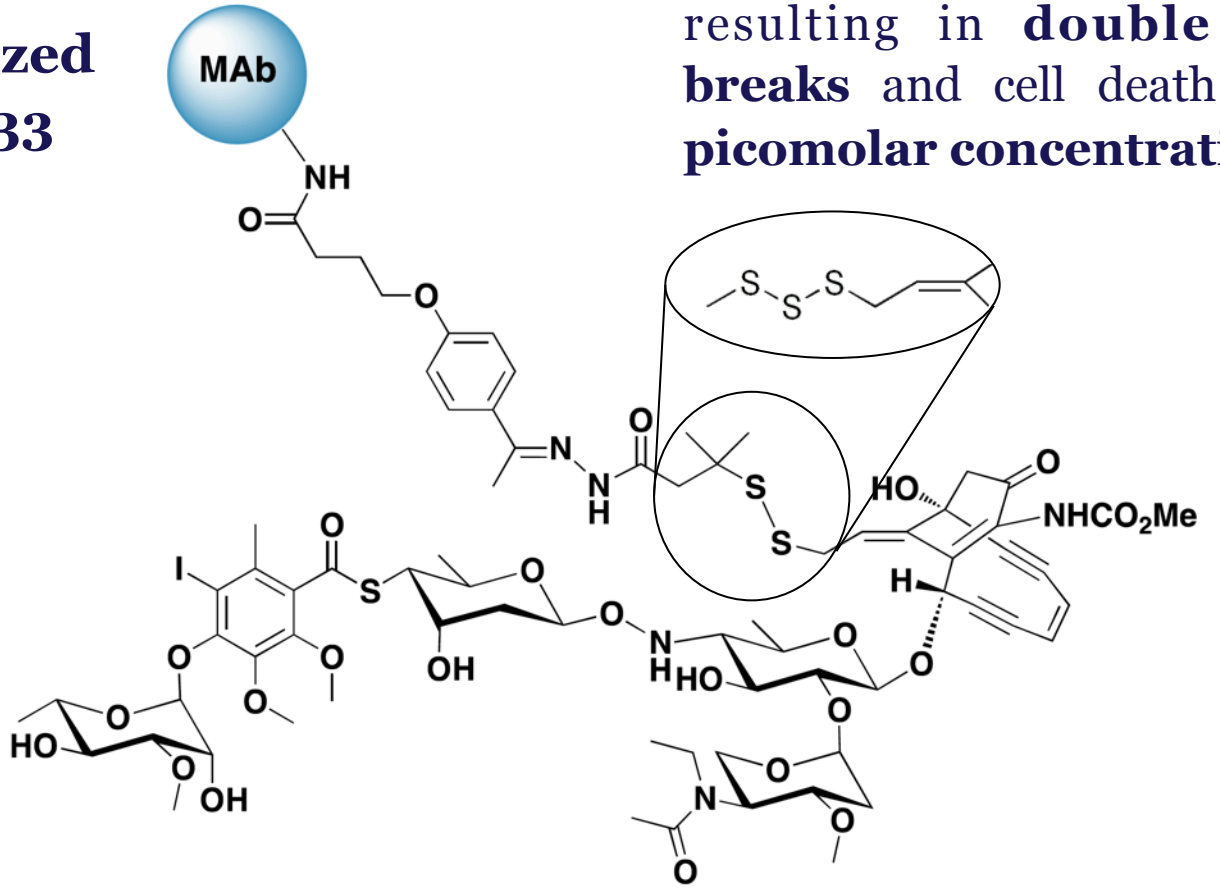
Gemtuzumab ozogamacin

Approved for the treatment of **CD33 positive acute myeloid leukemia (AML)**

Humanized Anti CD33

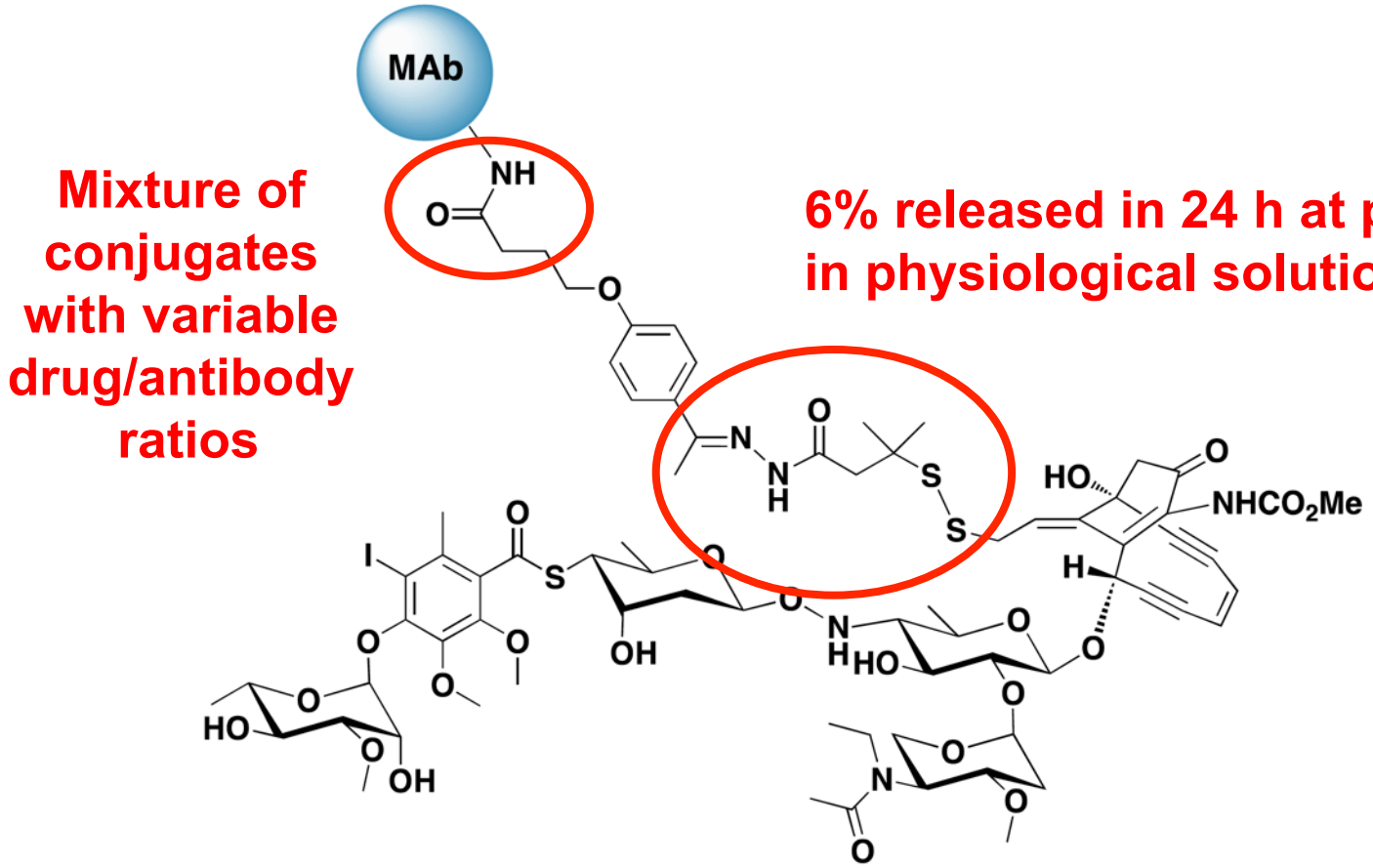
Calicheamicins

Bind the minor groove of **DNA** resulting in **double strand breaks** and cell death in **sub-picomolar concentration**



Gemtuzumab ozogamacin

A phase III started in 2004 after a FDA accelerated-approval process showed ADC **increased patient death** and added **no benefit** over conventional cancer therapies

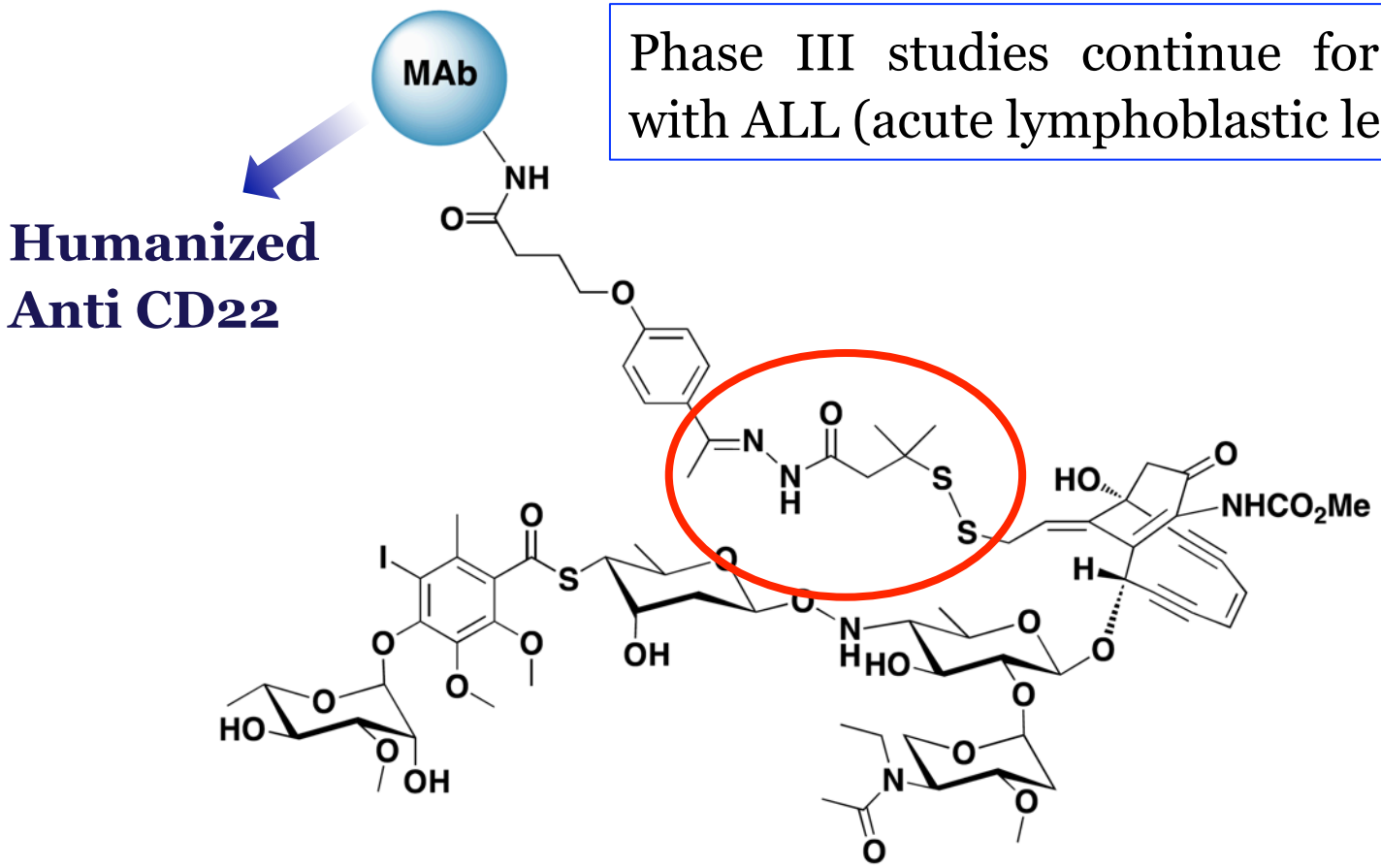


Inotuzumab ozogamacin

Recently failed to demonstrated improved survival in a phase III study for patients with refractory aggressive CD22+ NHL (Non-Hodgkin lymphoma)

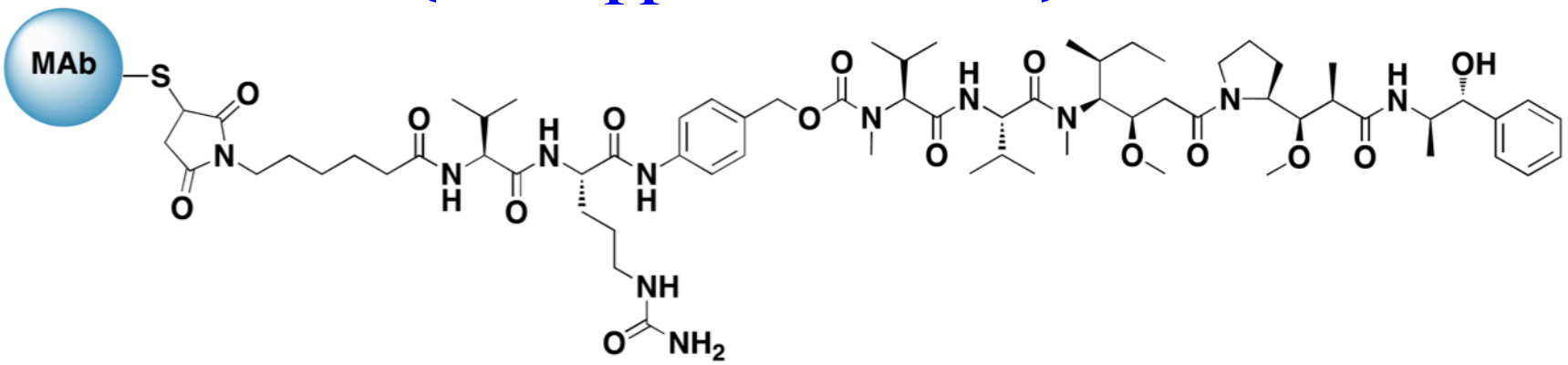
A phase III trial in patients with folicular b-cell NHL has been terminated due to poor enrollment

Phase III studies continue for patients with ALL (acute lymphoblastic leukemia)

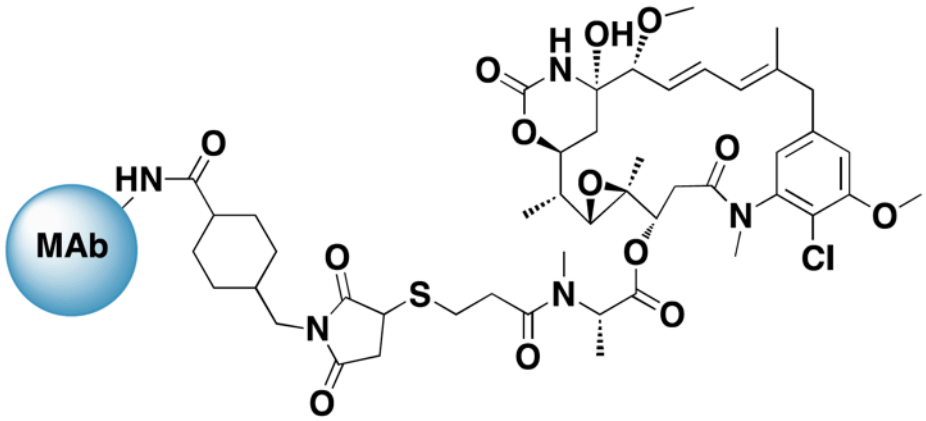


Third generation of ADCs

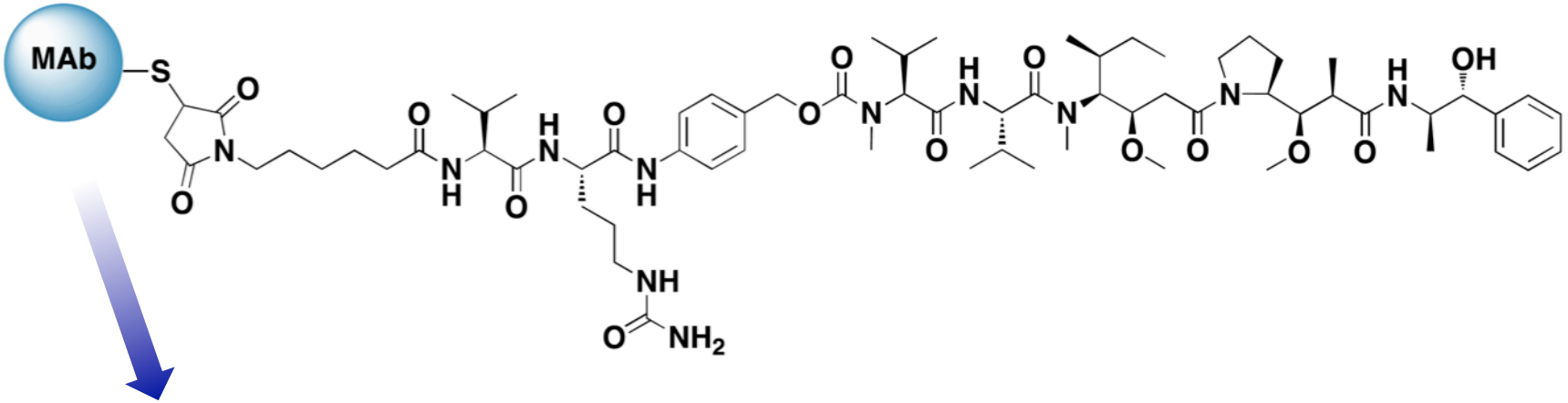
ADCETRIS®
(FDA approved in 2011)



KADCYLA®
(FDA approved in 2013)



ADCETRIS® (Seattle genetics, 2011)

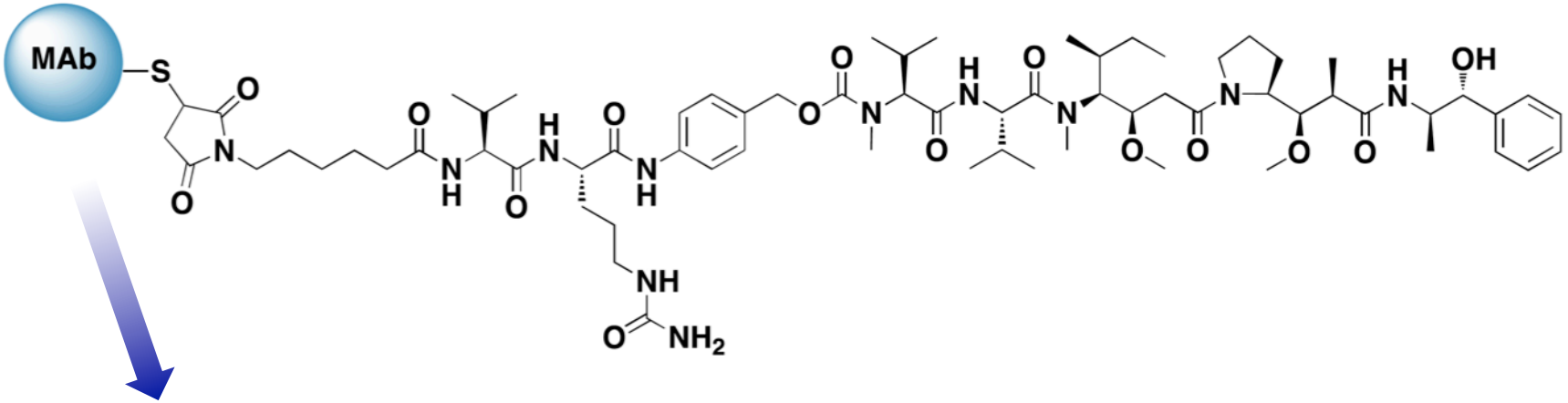


Chimeric monoclonal antibody (cAC10, which targets CD30, a cell mebrane protein of the tumor necrosis factor receptor family)

- Approved for patients with **Hodgkin's** (HL) and **anaplastic large-cell lymphomas** (ALCL).
  (accelerated approval process after phase II)

- In HL, response rate of 75%, with **complete remissions in 34%**. Tumor reductions in 94% of the patients.
- In ALCL, response rate of 86%, with **complete remissions in 53%**. Tumor reductions in 97% of the patients.

ADCETRIS®

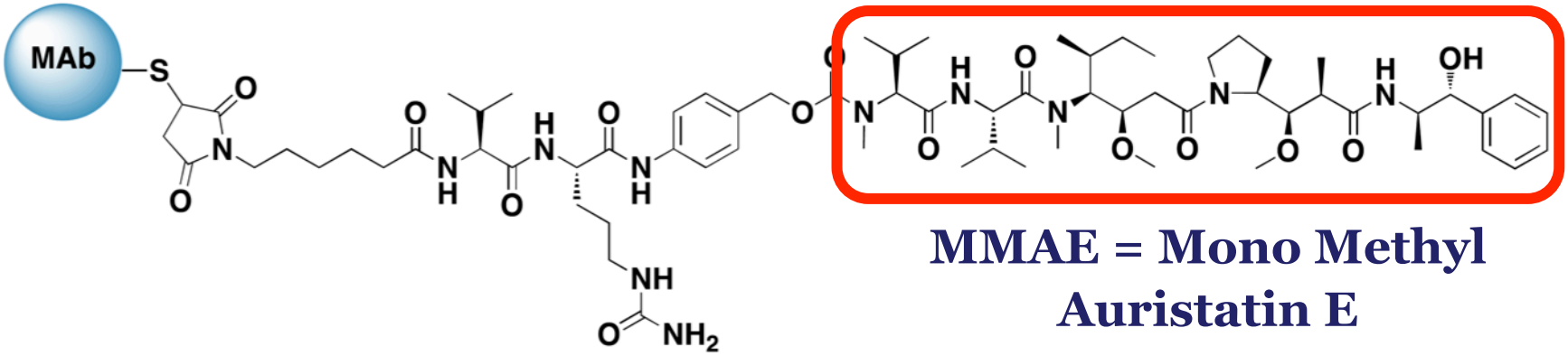


Chimeric monoclonal antibody (cAC10, which targets CD30)

- A **phase III clinical trial** is currently comparing CHOP (Cyclophosphamide, Hydroxydaunorubicin, Oncovin [vincristine], Prednisone) and CHP – ADCETRIS for Non-Hodgkin lymphoma

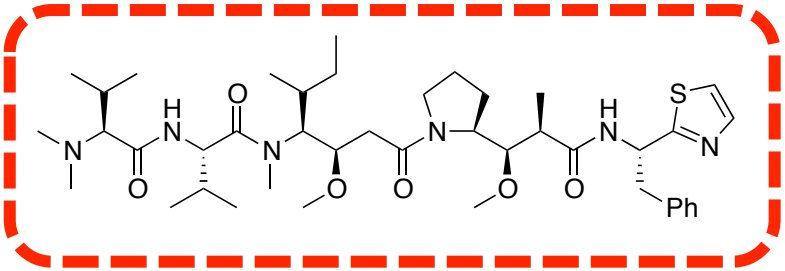
- A **phase III clinical trial** is currently comparing ABVD (doxorubicin, bleomycin, vinblastine, dacarbazine) and AVD – ADCETRIS for Hodgkin lymphoma

ADCETRIS® - auristatin



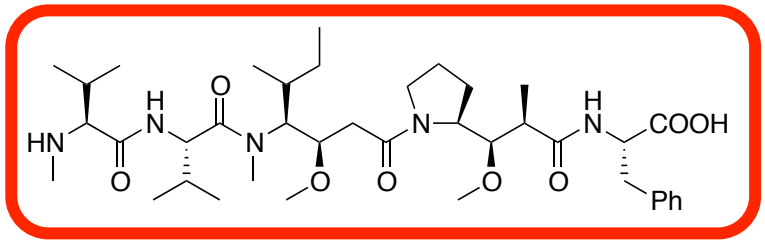
MMAE = Mono Methyl Auristatin E

- **Auristatin agents** are **fully synthetic** compounds that were identified by **SAR studies** of **Dolastatin 10**



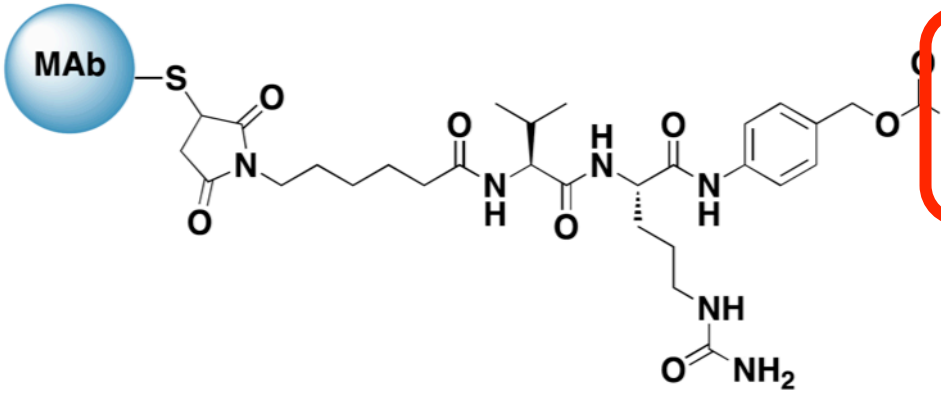
Dolastatin 10

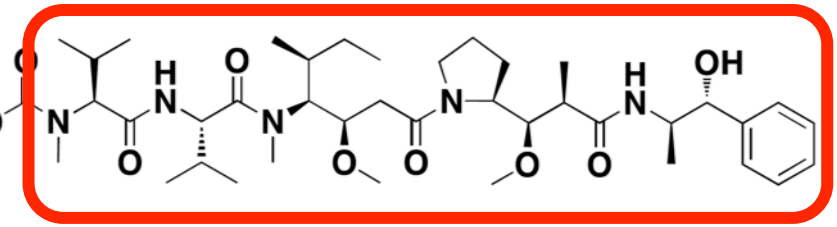
- **Dolastatin 10** is a lipophilic pentapeptide isolated from the mollusk *Dolabella auricularia*



MMAF = Mono Methyl Auristatin F

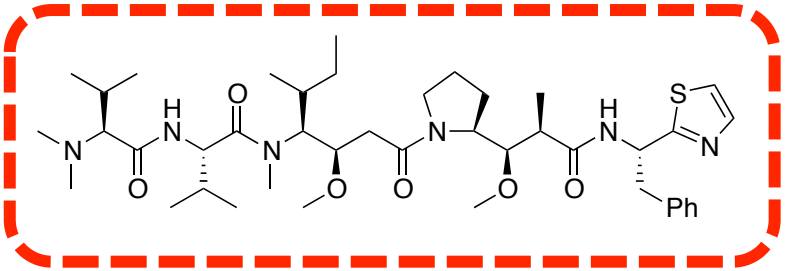
ADCETRIS® - auristatins





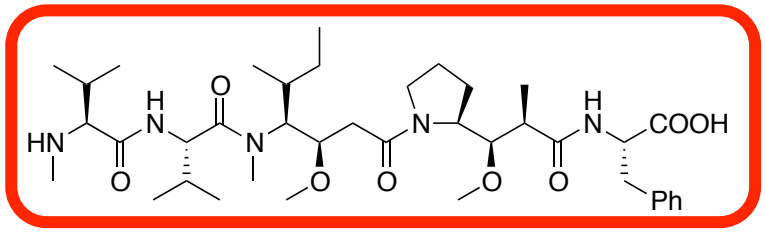
MMAE = Mono Methyl Auristatin E

- Highly potent (nM-pM IC_{50}) **Vinca-domain** binders of β-tubulin that inhibit tubulin polymerization resulting in G2/M arrest and cell death



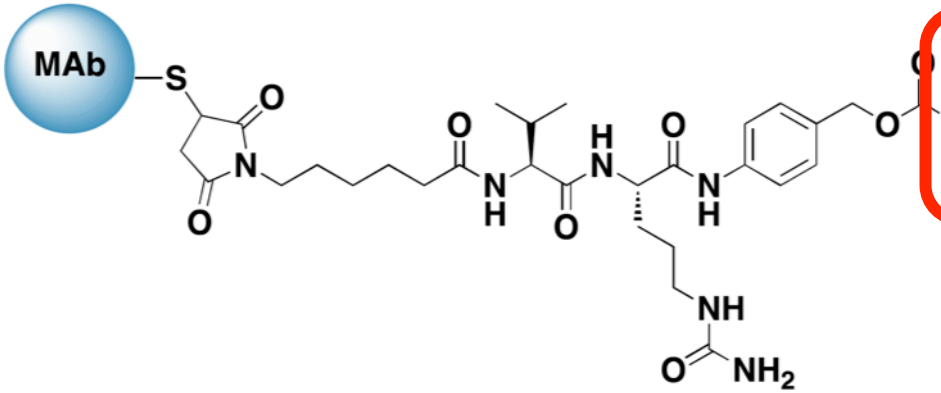
Dolastatin 10

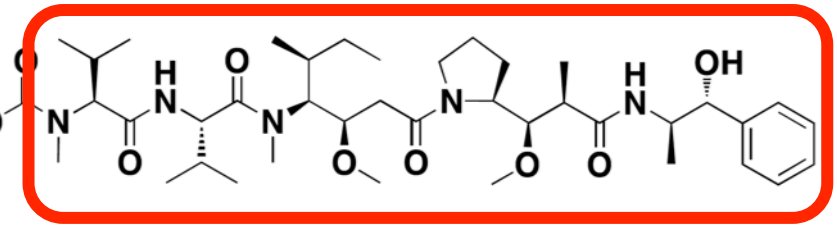
- MMAF is 100 times less cytotoxic than MMAE *in vitro*
- MMAF-OMe is 100 times more cytotoxic than MMAE



MMAF = Mono Methyl Auristatin F

ADCETRIS® - auristatins

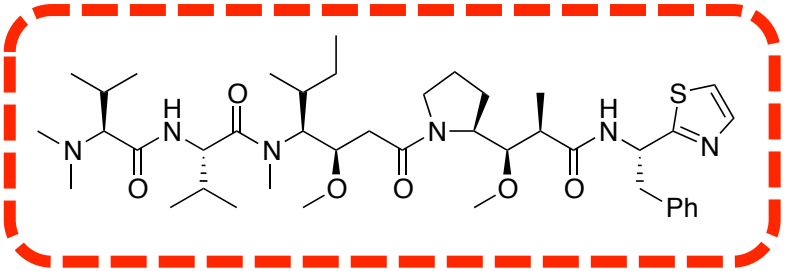




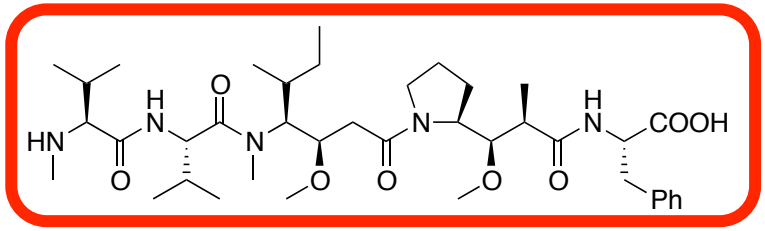
MMAE = Mono Methyl Auristatin E

Auristatins in other ADCs:

- **16 / 43 ADCs in clinical trials use MMAE**
- **2 / 43 ADCs in clinical trials use MMAF**

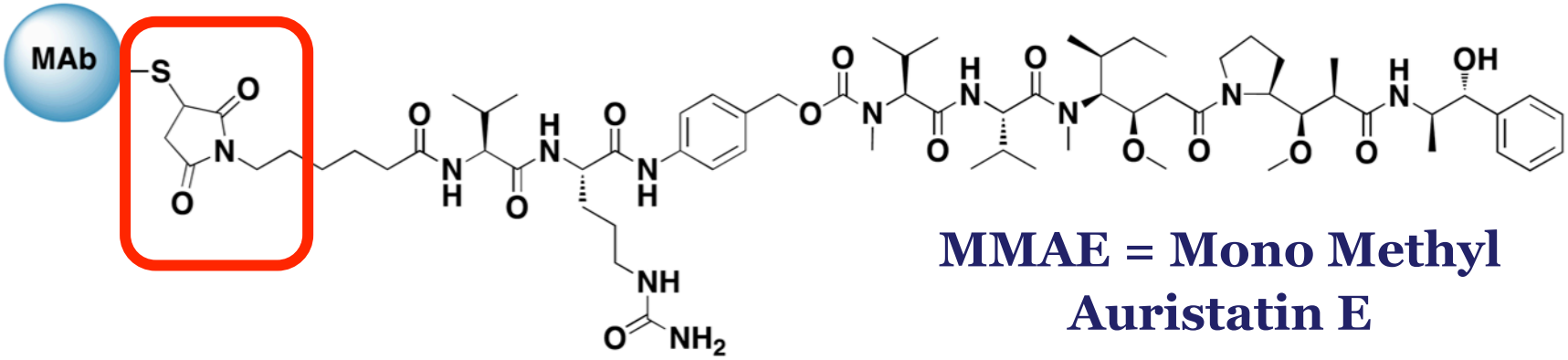


Dolastatin 10



MMAF = Mono Methyl Auristatin F

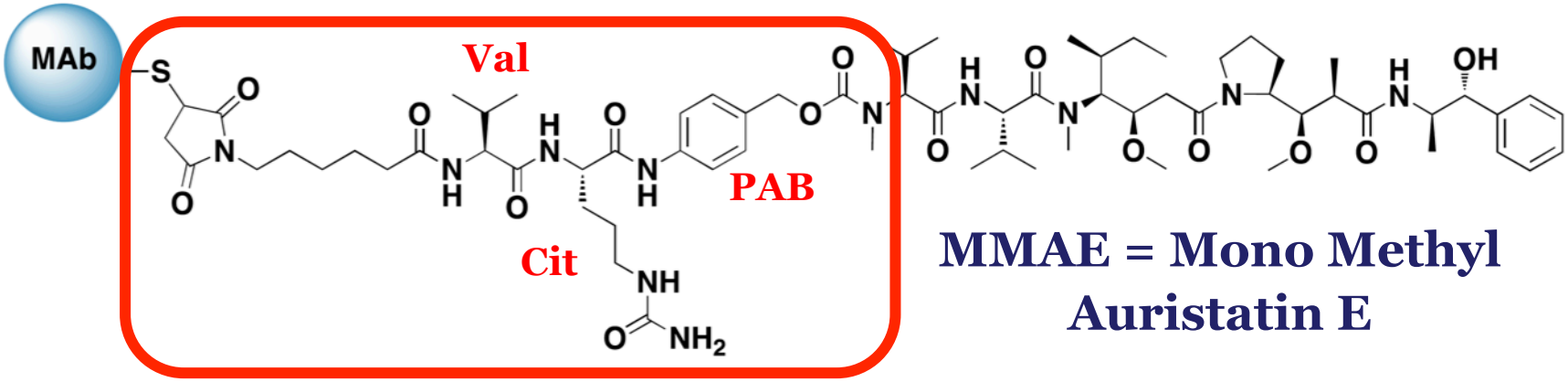
ADCETRIS® - conjugation site



MMAE = Mono Methyl Auristatin E

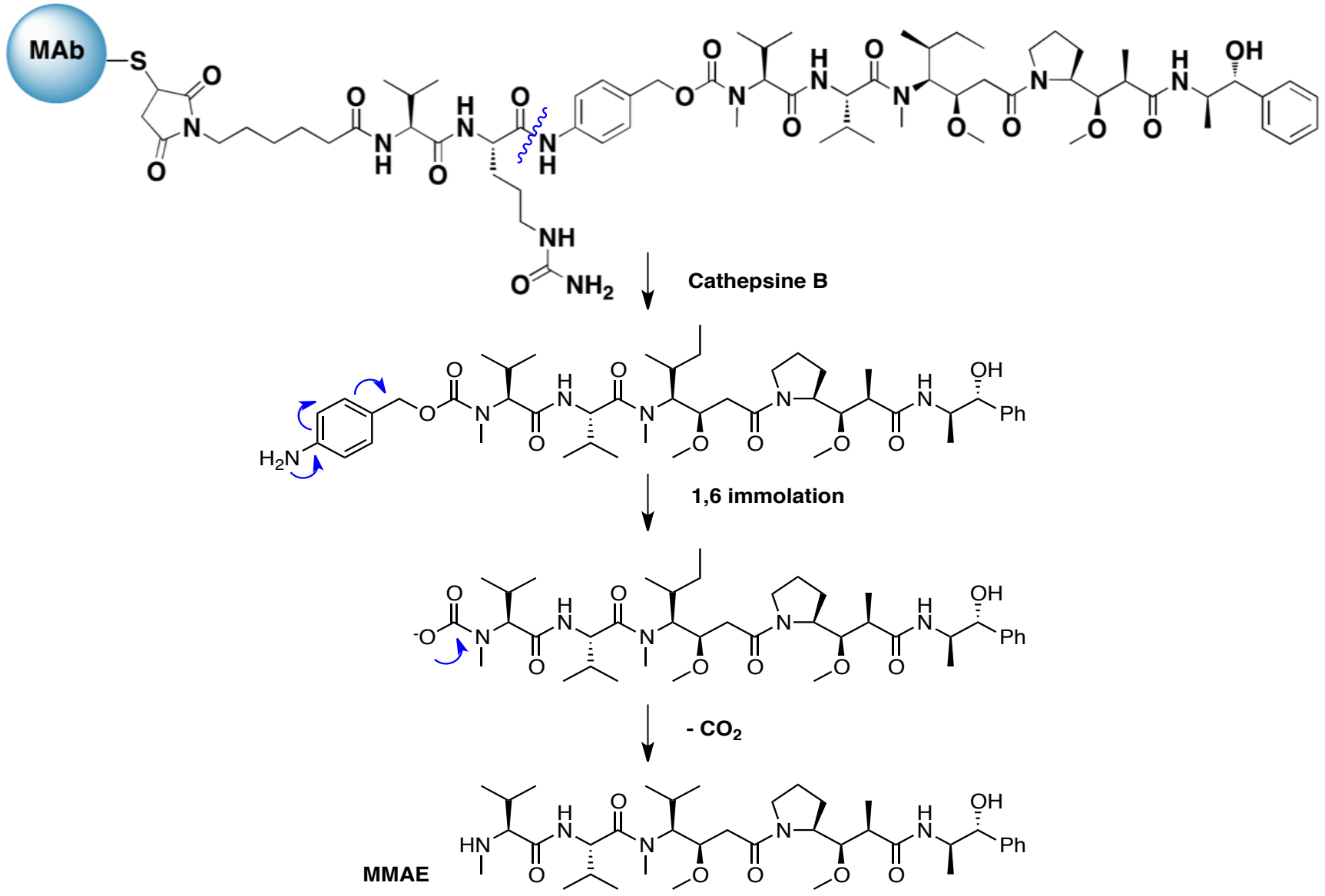
- **Free thiol moieties** on the mAb **are required** to enable the conjugation of the **maleimide**-bearing auristatin derivatives
- mAbs do not generally contain free thiols but they contain endogenous cysteine residues that exist as **disuflide pairs** (**4 interchain** and **12 intrachain** disulfide bonds for a human IgG_1)
- **Interchain disulfide** can be reduced and maintained as free thiols easier than intrachain disulfides (**2 to 8 free thiols**)
- Distribution of conjugates are obtained, with an avarage of **4 auristatins per mAb** (the separated species can be purified by HILIC but have not yet been tested in clinic studies).

ADCETRIS® - smart linker

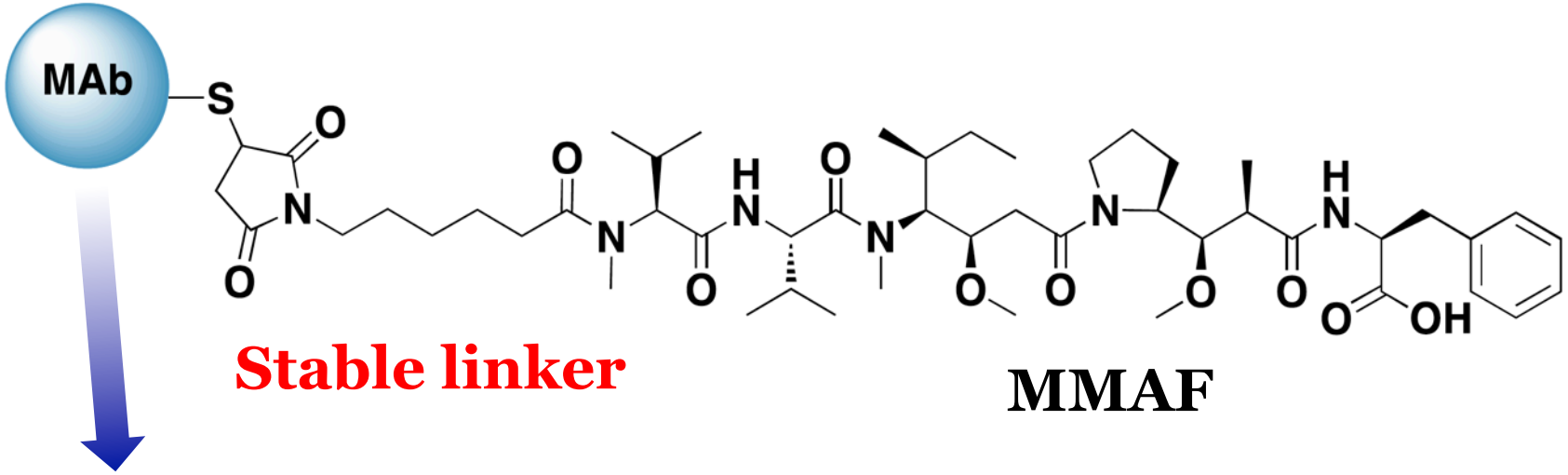


- A **valine-citrulline linker** is designed to provide **high plasma stability** (half-lives 6 days in mice and 10 days in monkey)
- **Upon internalization**, the dipeptide linker is **efficiently hydrolyzed** by the **cathepsin B proteases**
- After enzymatic cleavage, **1,6-elimination** of the strongly electron-donating *p*-aminobenzyl group (**PAB**) occurs, releasing MMAE in its active form.
- The **self-immolative spacer** is needed to **spatially separate the drug from the site of enzymatic cleavage** (the bulky payload has a negative influence on the kinetics of the peptide hydrolysis).

ADCETRIS® - smart linker



Vorsetuzumab mafodotin

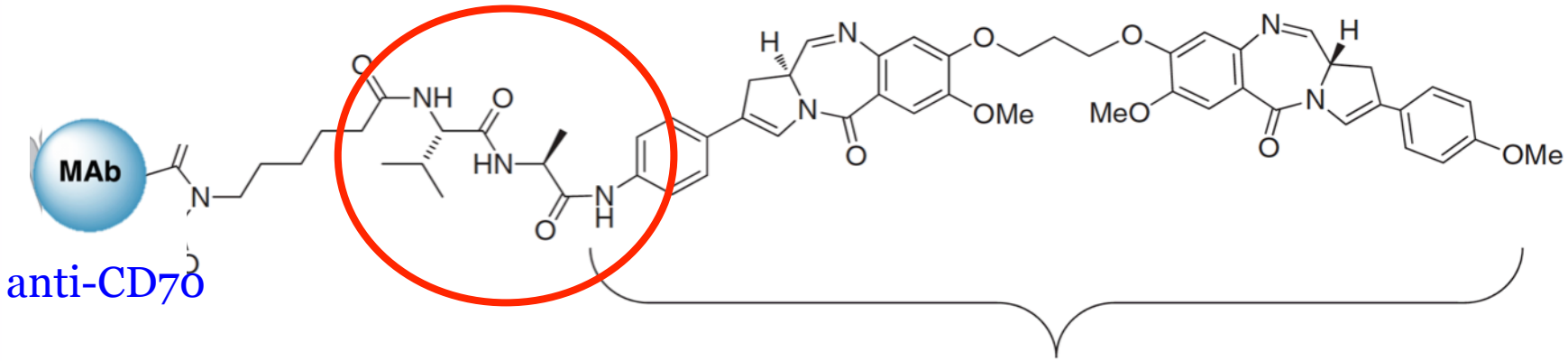


- **Humanized** monoclonal antibody (which targets CD70) molecule, conjugated to the auristatin analogue monomethyl auristatin phenylalanine (MMAF)

- Seattle Genetics has **discontinued** the development of vorsetuzumab mafodotin (SGN-75), for renal cell carcinoma or non-Hodgkin lymphoma in favor of **SGN-CD70A** (same mAb, different drug, *i.e.* **pyrrolobenzodiazepine**, currently in **phase I** for the same tumors)

SGN-CD70A

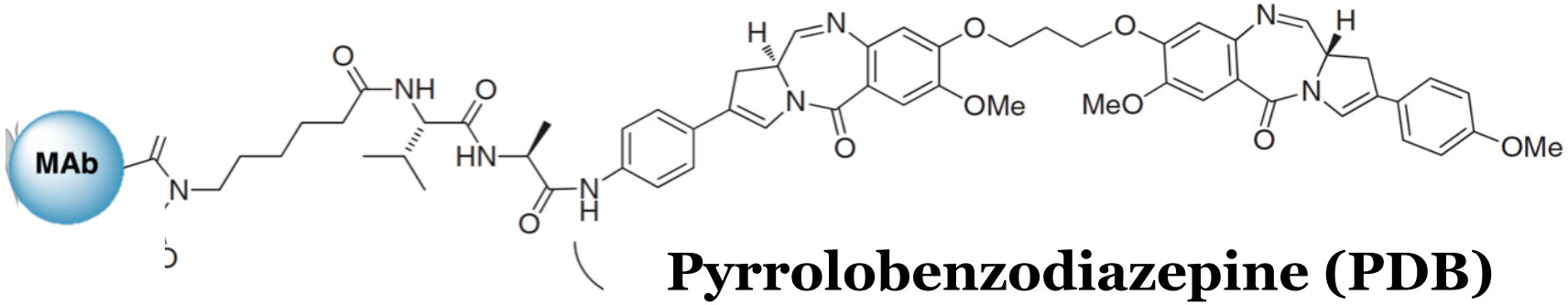
Cleavable linker



Pyrrolobenzodiazepine (PDB)
(DNA-crosslinking agents)

- Seattle Genetics has **discontinued** the development of vorsetuzumab mafodotin (SGN-75), for renal cell carcinoma or non-Hodgkin lymphoma in favor of **SGN-CD70A** (same mAb, different drug, *i.e.* **pyrrolobenzodiazepine**, currently in **phase I** for the same tumors)

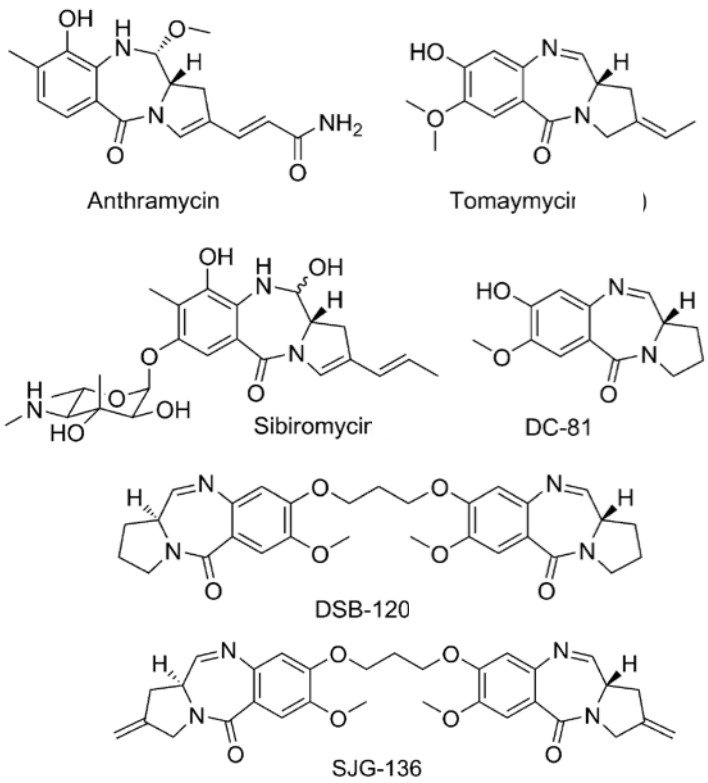
SGN-CD70A



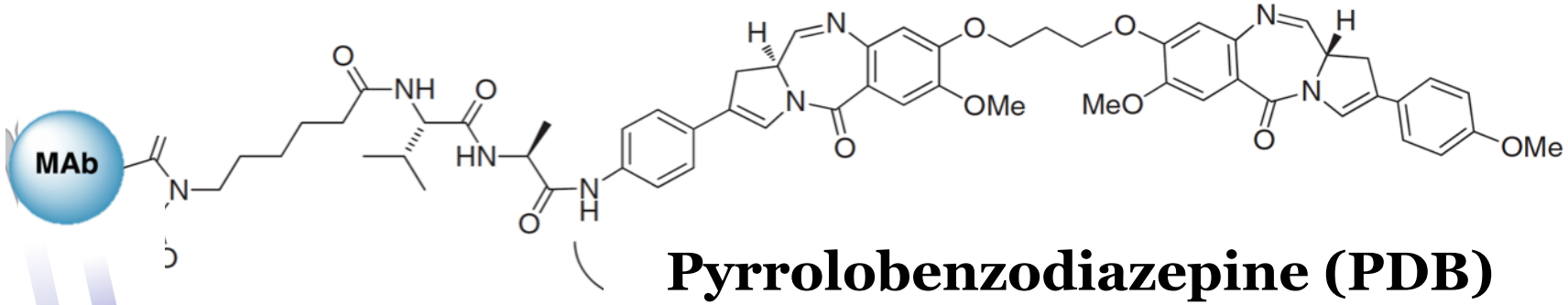
Pyrrolobenzodiazepine (PDB)
(DNA-crosslinking agents)

Pyrrolobenzodiazepines (PBD) were first discovered in the 1960s, when **anthramycin** was isolated as the active constituent of the fermentation broth of *Streptomyces refuineus*

Dimers resulted in significant **increase in potency** (for example DSB-120 is 600 times more cytotoxic than DC-81 in vitro)



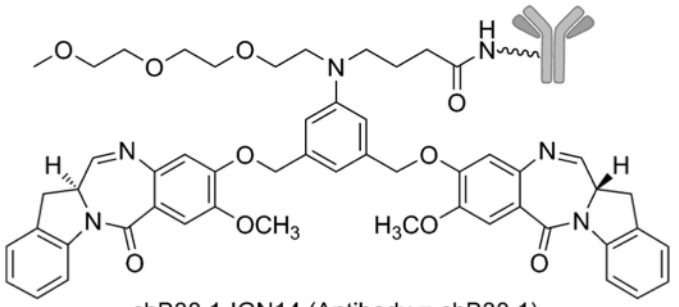
PDB-ADCs



Pyrrolobenzodiazepine (PDB)
(DNA-crosslinking agents)

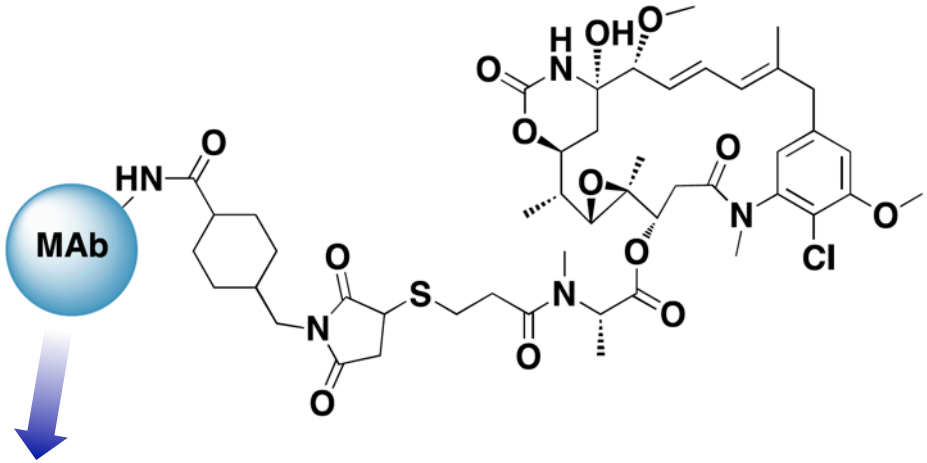
- SGN-CD70A is in **phase I** for patients with **renal cell carcinoma** and with **non-Hodgkin lymphoma**
- SGN-CD33A is in **phase I** for patients with **acute myeloid leukemia** (AML)

- IGNs containing ADCs showed **promising result** *in vitro* and *in vivo* against both normal and multi-drug-resistant cancer cell lines



chB38.1-IGN14 (Antibody = chB38.1)

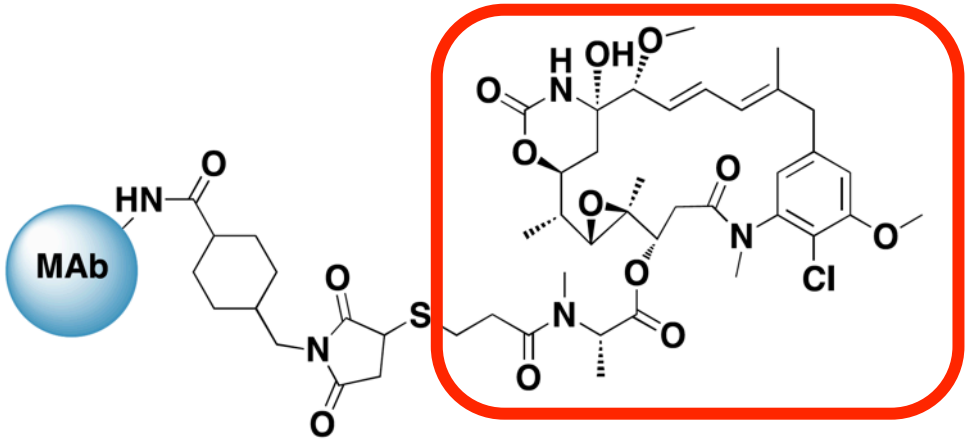
KADCYLA® (Roche, 2013)



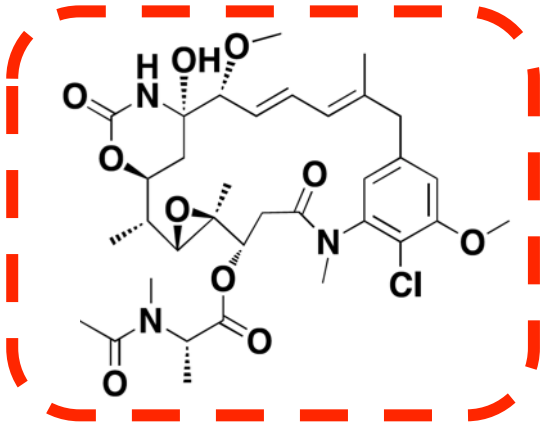
- **Humanized** monoclonal antibody (trastuzumab, clinically approved which targets human epidermal growth factor receptor 2, **HER2**)
- Her2 is an excellent ADC target: it is **amplified** and **overexpress** in 20-25% of human breast cancer and is **quickly internalized** following the binding

- In **breast cancer**, response rate of **43%** with complete remissions in 1%
- First ADC approved for a **solid tumor**, first ADC to complete a phase III

KADCYLA® - maytansine

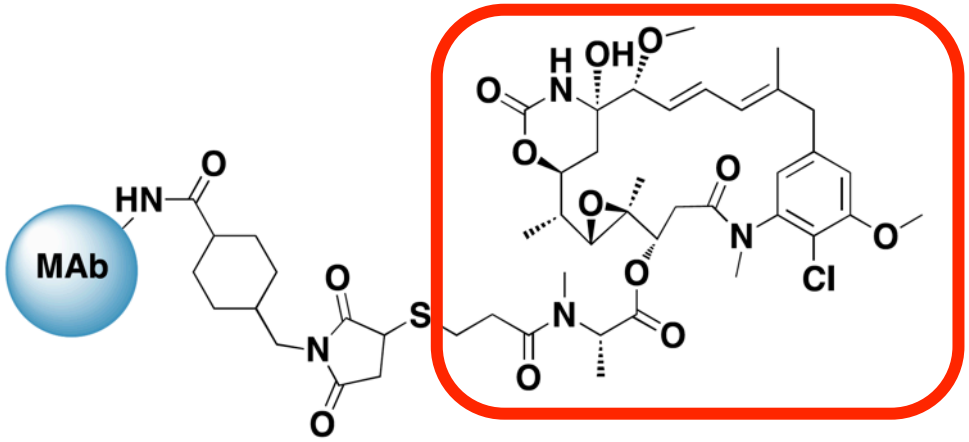


- Maytansine is a **benzoansamacrolide** isolated from the bark of the Ethiopian shrub *Maytenus ovatus* in 1972
- It binds tubulin near the Vinca binding site and has a **high affinity for tubulin** located at the ends of micrutubules
- As single agent, it **failed** to **demonstrate a therapeutic benefit** at tolerable dose

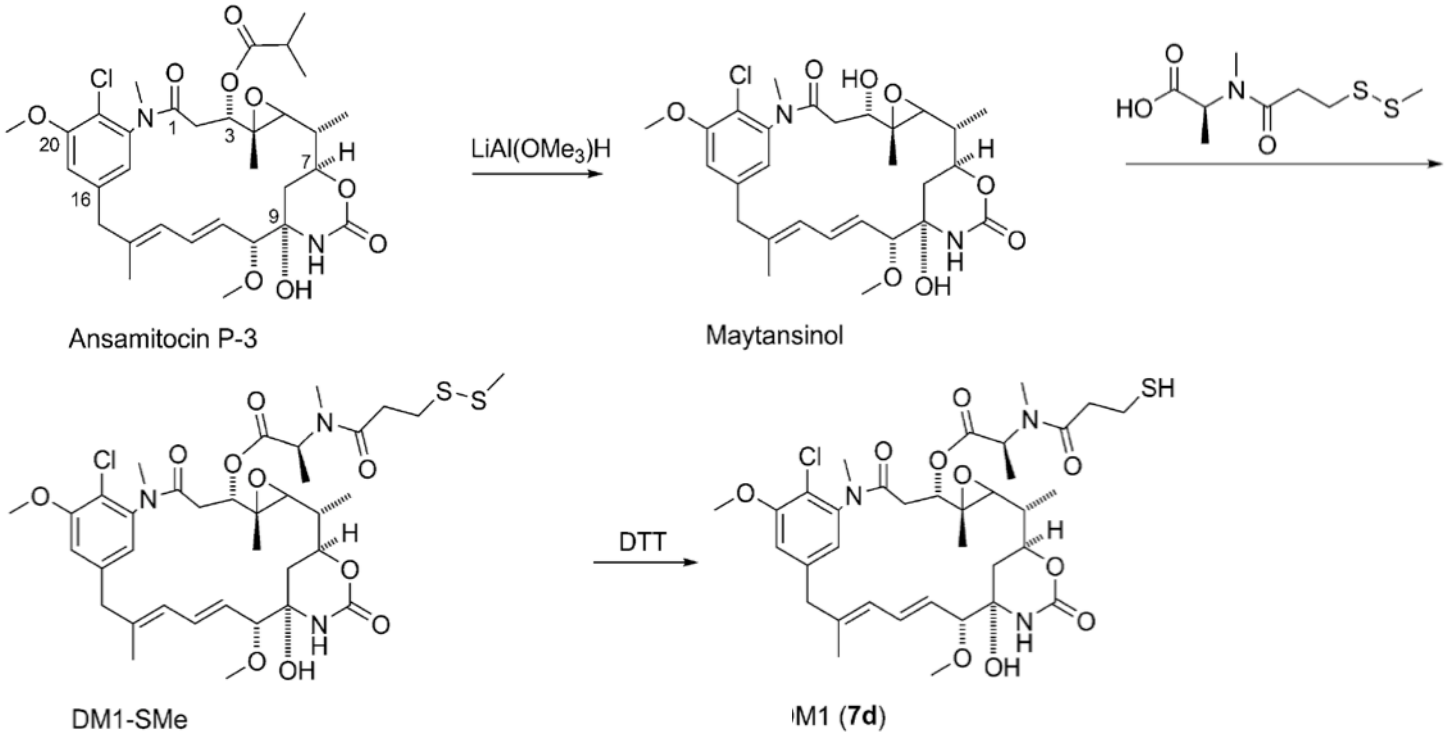


Maytansine

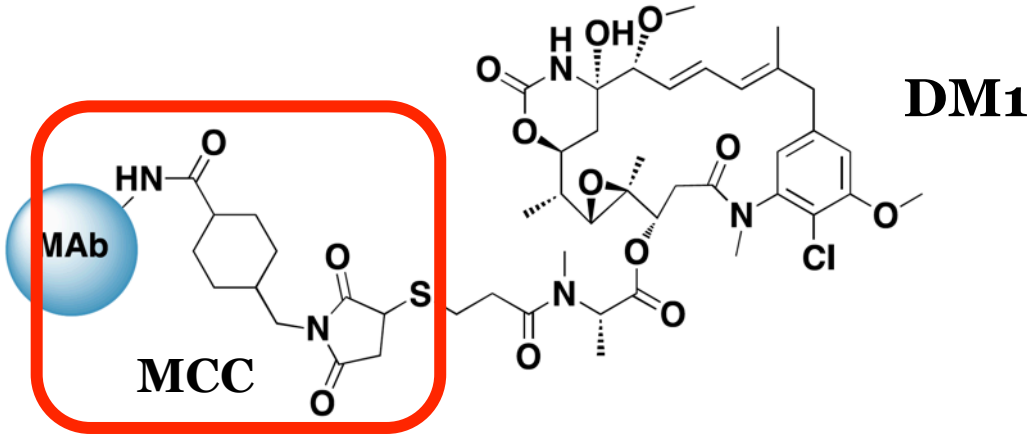
KADCYLA® - maytansine



- Published semi-synthesis

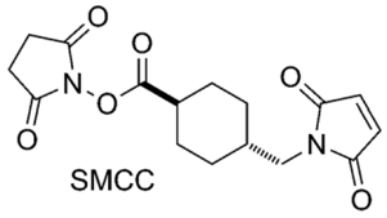


KADCYLA® conjugation & linker



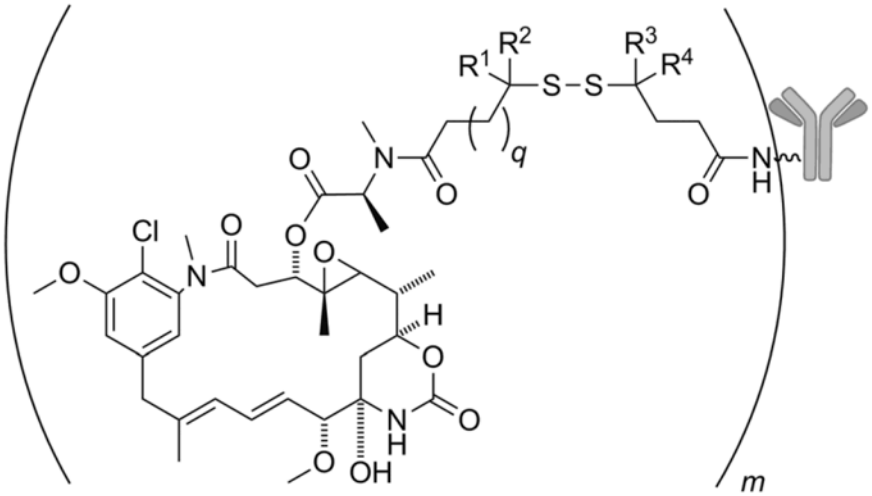
MCC =*N*-Maleimidomethylcyclohexane-1-carboxylate

- The ADCs are prepared by reacting the **antibody lysines** with a bifunctional linker composed of an activated ester and a maleimido group (SMCC), followed by the reaction of the modified antibody with the thiol containing maytansinoid (DM1)



- Average of **3.5 DM1** per antibody
- Non-cleavable linker: drug is released only after **fully degradation of the ADC**

Other maytansinoid ADCs



13 / 43 of ADCs in clinical trials use matynsinoids as paylod (mainly DM1 or DM4)

	DM side		mAB side		*q*	Disulfide reduction rate ($k \cdot M^{-1} \cdot min^{-1}$)*	Relative stability	In vivo PK $t_{1/2}$ (h)
	R1	R2	R3	R4				
DM1	**H**	**H**	**H**	**H**	**0**	**14**	**1**	**15**
	H	H	Me	H	0	2	7	47
	H	H	Me	Me	0	0.8	16	*n.d.*
DM4	**Me**	**Me**	**H**	**H**	**1**	**1**	**14**	**87**
	Me	H	Me	H	1	0.8	170	*n.d.*
	Me	Me	H	Me	1	0.014	980	218
	Me	Me	Me	Me	1	0.0006	22000	*n.d.*

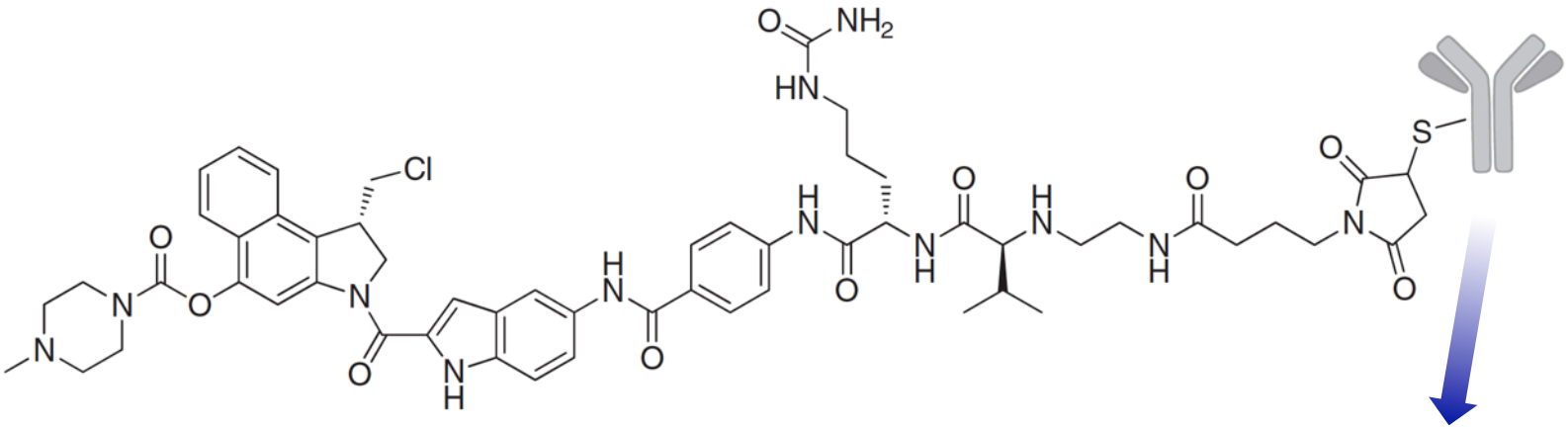
* by DTT at pH = 6.5, at 37°C

Other cytotoxic drugs as payload

Total of 43 ongoing clinical trials with ADCs:

Drug	ADCs in clinical trials	Mechanism of action
Calicheamicin	2	DNA minor groove binder
Doxorubicin	1	DNA intercalating agent
Pyrrolobenzodiazepine dimer	2	DNA minor groove binder
Auristatins	18	*Microtubule disrupting agents*
Maytansinoid	13	*Microtubule disrupting agents*
Duocarmycin analog	**1**	***Alkylating agent***

Duocarmycin

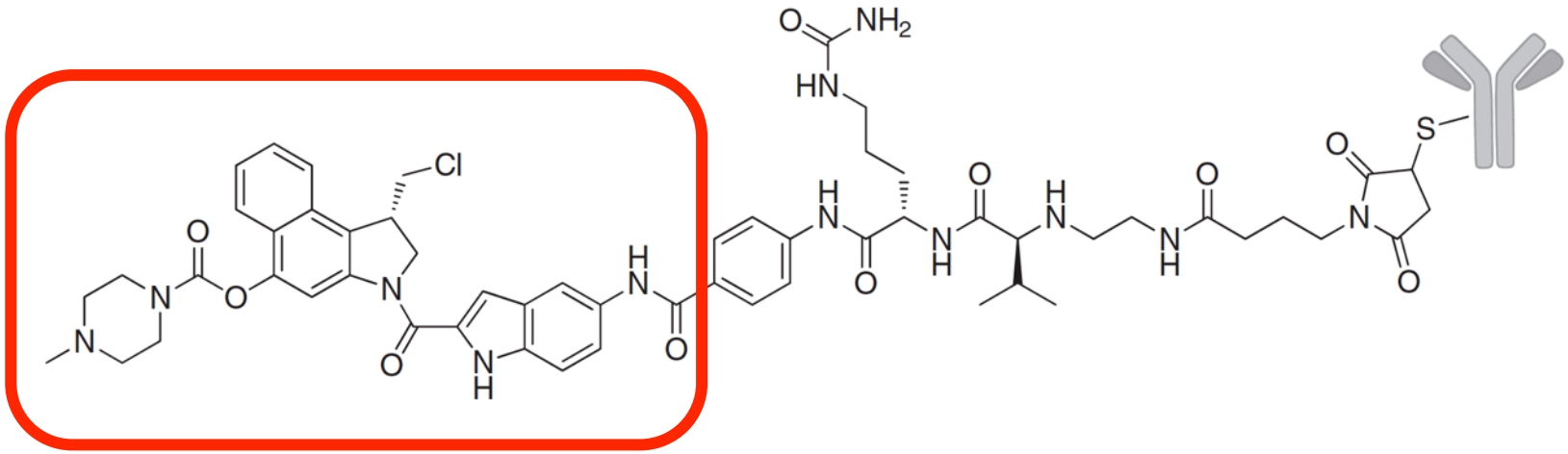


- **Humanized** monoclonal antibody (trastuzumab, clinically approved which targets **human epidermal growth factor receptor 2**, HER2)

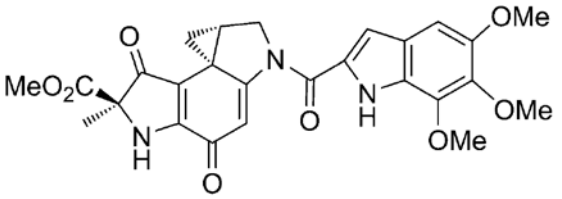
- **Advantage compare to KADCYLA** in preclinical studies

- A **phase I** clinical trial for patients with **locally advanced or metastatic solid tumors** has started one month ago (**October 21, 2014**)

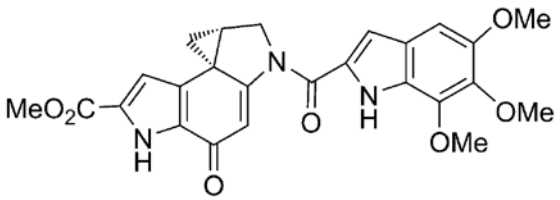
Duocarmycin



- Duocarmycins, first isolated from *Streptomyces* bacteria in 1988, **bind to the minor groove of DNA** and subsequently cause **irreversible alkylation of DNA**
- This **disrupts the nucleic acid architecture**, which leads to **tumor cell death**

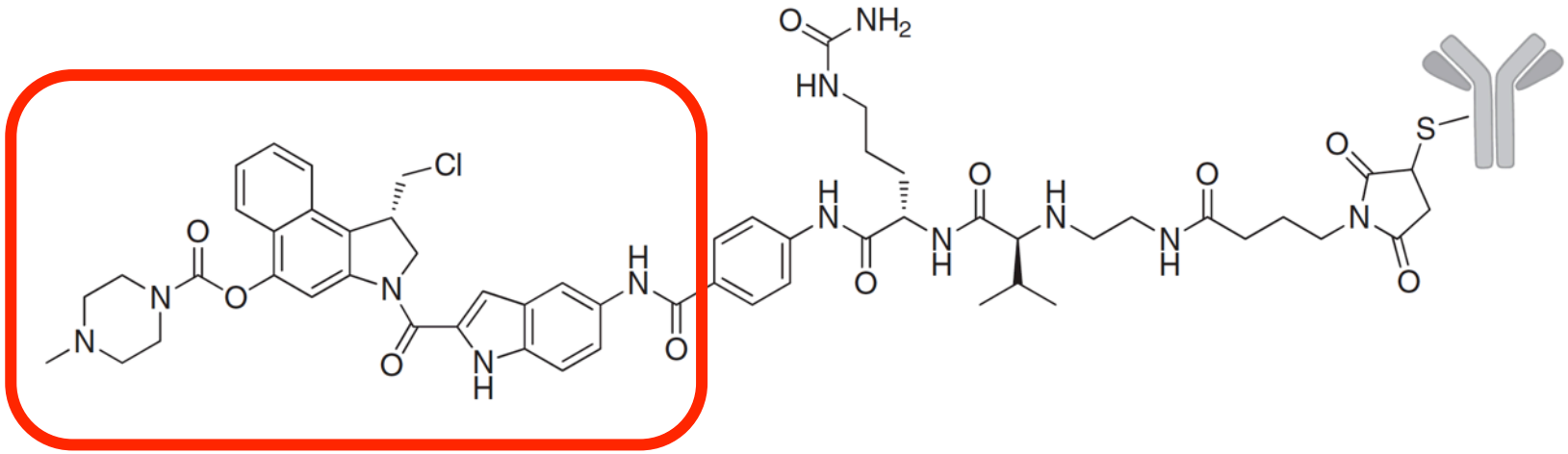


Duocarmycin A

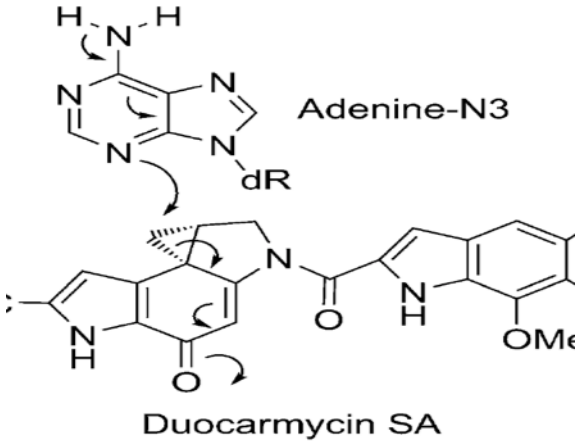


Duocarmycin SA

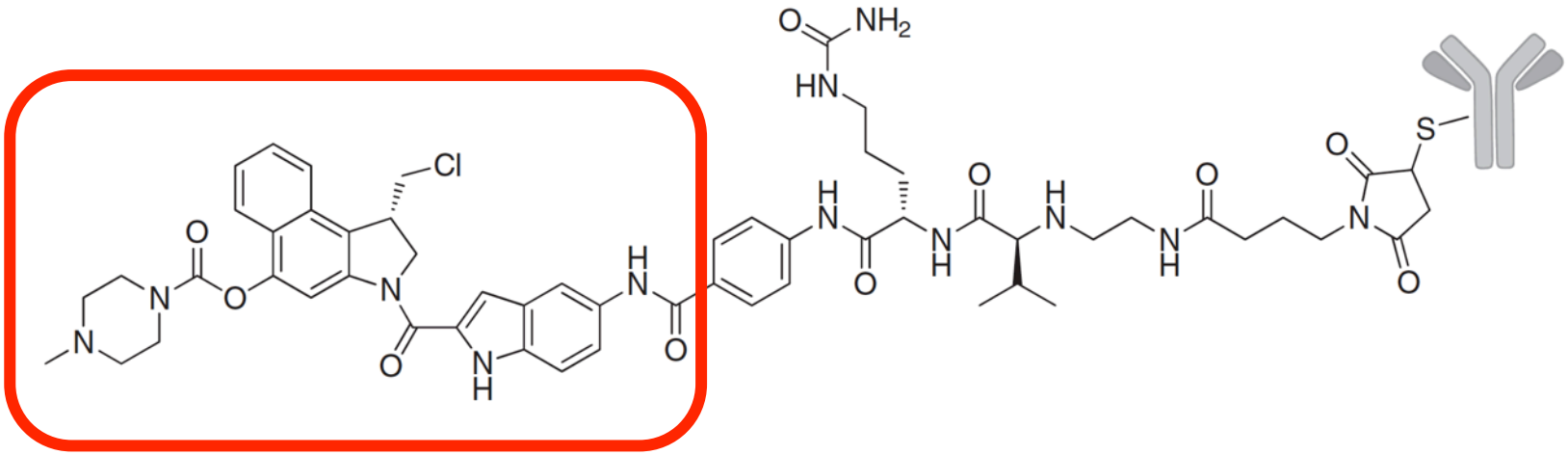
Duocarmycin

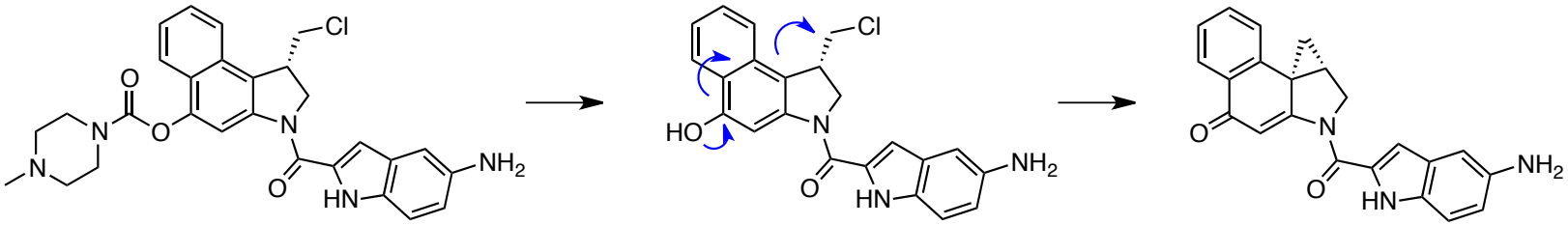


- Duocarmycins are able to **exert their mode of action at any phase** in the cellular cycle (tubulin binders attack tumor cells in a mitotic state)
- **Limit therapeutic activity** in clinic of duocarmycins as a single agent
- Lessons learned in the **SAR** have been essential for developing **duocarmycins as ADC payloads**



Duocarmycin

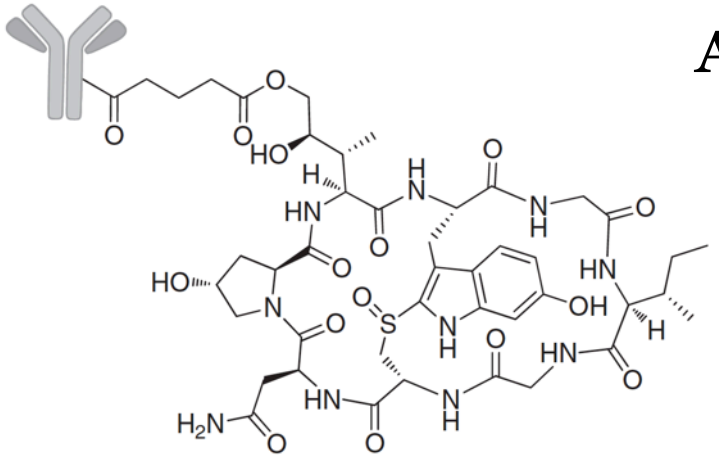




- The cyclopropapyrroloindolo moiety is derivatized in its **ring open chloromethyl form in the phenolic state**, which allowed the preparation of various types of prodrugs.

- **Ring closure**, either chemically or enzymatically, **occurred quickly *in vitro* and *in vivo***

Promising drug (preclinical)

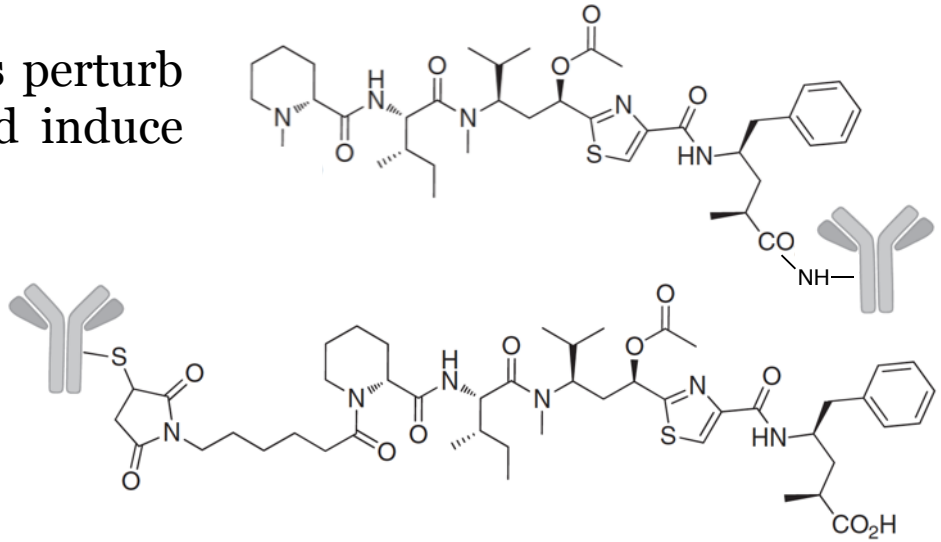


Amanitin

α-Amanitin, a **cyclic octapeptide** found in several species of the *Amanita genus* mushrooms, **strongly inhibits RNA polymerase II**, leading to **inhibition of DNA transcription** and **cell death**

Tubulysins

Tubulysins, **natural tetrapeptides** perturb **microtubule polymerization** and induce apoptosis of various cell lines (including those with multi drug resistance) at **subnanomolar concentrations**



Drawbacks of ADCs

Most of the ADCs:

- have a **restricted ability to cross endothelial cell membranes** by passive diffusion and non-specific uptake by the liver and the reticuloendothelial system.
- can induce **cell surface receptor clustering** (for example with integrins) thereby **enhance tumor angiogenesis**
- may elicit **severe immune responses** in a patient (recombinant proteins in general are immunogenic)
- need to be **rigorously purified** in order to comply with the regulations

The costs for therapeutic applications are very high (for example Kadcyla in the US is $9,800 per treatment. The estimated cost of a course of Kadcyla is approximately $94,000)

What's next?

- Developing new technology to create **well-defined drug conjugates:**
  - PolyTheric's ThioBridge is 3 carbon bisthiol linker that reconnects the cysteines, to which a toxic payload is already attached)
  - Similarly, Igenica's SNAP technology uses bifunctional linkers to maintain the interchain bonding
  - Reedwood's SMARTag technology to introduce new amino acids in the antibody as a linkage points
  - ADC Bio has developed a "lock and release" technology through which it immobilizes the antibody on a solid support, conjugates it, and then releases the ADC in a soluble form.

Greater homogeneity is expected to **improve** ADC **stability** and **performance** while greatly simplifying the demanding analytics needed to characterize their complex structures.

What's next?

- Developing new technology to create **well-defined drug conjugates:**
  - PolyTheric's ThioBridge is 3 carbon bisthiol linker that reconnects the cysteines, to which a toxic payload is already attached)
  - Similarly, Igenica's SNAP technology uses bifunctional linkers to maintain the interchain bonding
  - Reedwood's SMARTag technology to introduce new amino acids in the antibody as a linkage points
  - ADC Bio has developed a "lock and release" technology through which it immobilizes the antibody on a solid support, conjugates it, and then releases the ADC in a soluble form.

The linker **determines the nature of the metabolite produced**. Thus, changes in **linker design** would alter the **metabolite profile**, which in turn would **influence clinical toxicity**

What's next?

- At the same time, **more potent classes of toxins**, having **different mechanisms of action**, are emerging as alternatives to the auristatins from Seattle Genetics and the maytansines from ImmunoGen, all of which act by inhibiting tubulins
  - **pyrrolobenzodiazepines**, **duocarmycins**, **α-amanitin** are the most promising
  - conjugation highly hydrophobic drugs without aggregations

- Develop ADCs outside oncology in areas such as **inflammatory and autoimmune diseases**

- **Identification** of the **new cell surface targets**, based on antigen expressions, heterogeneity and internalization rate

What's next?

- Use of smaller scaffold proteins **or antibody fragments**, instead of whole IgGs, may offer the advantage of **greater tumor penetration**
    - For example, the smallest fragment (single variable domain, sFv), demonstrated maximal tumor penetration at 0.5 hours, while the intact IgG took 48 to 96 hours to achieve the same level of penetration
- **The faster clearance of these fragments and the propensity to accumulate in kidneys requires half-life extension strategies (*e.g.* conjugation with polymers)**

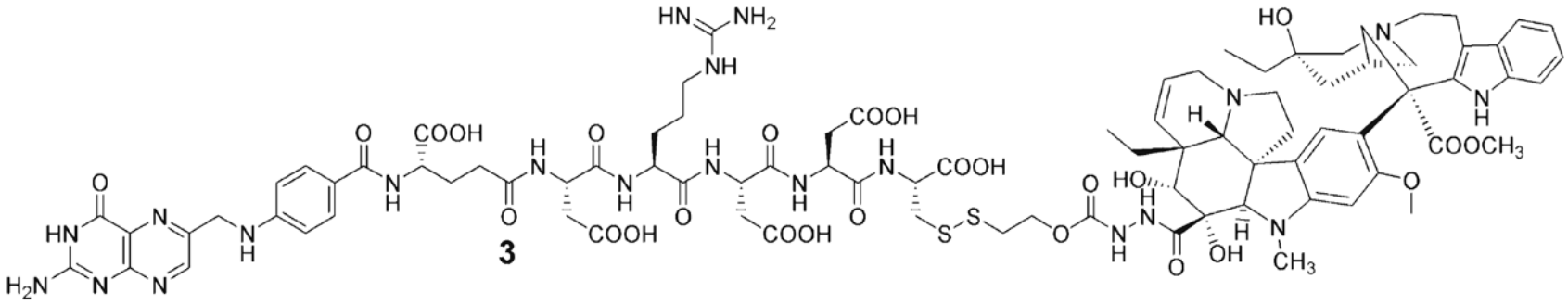
What's next?

- **More preclinical evaluation** prior to advancement to the clinic:
  - For ADC therapy, preclinical models for efficacy studies have to be **carefully selected** such that the antigen expression on the xenograft **reflects the clinical population** to be treated
  - It is important to demonstrate antitumor activity in vivo at doses achievable in the clinic (2–7 $mg \cdot kg^{-1}$ for most ADCs)
- Setting such **higher preclinical bars** may mitigate the chance of failure in the clinic

Small molecule drug conjugates

- **Linker** and **cytotoxic drug** similar to ADCs
- The **targeting ligands** consist of high-affinity small molecules that selectively bind to the diseased cell's receptors
- **The most used receptors are:**
- **Folate receptors**, integrins, hyaluronic acid receptors (CD44), transferrin receptors (CD71), growth factor receptors (EGFR, VEGFR, PDGF)

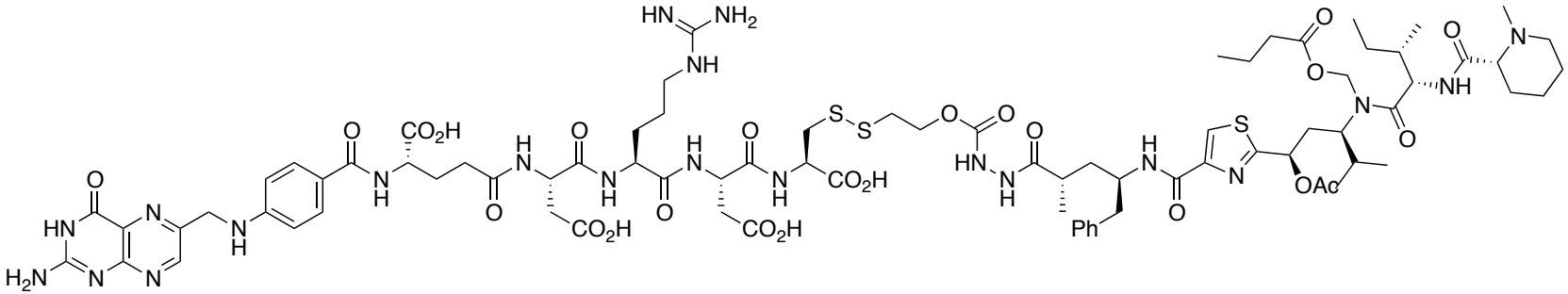
Vintafolide



- **Vintafolide**, the most advanced SMDC, is a derivative of the anti-mitotic drug **vinblastine** which is chemically linked to **folic acid**
- **Phase III study was stopped** in May because vintafolide didn't demonstrate efficiency when treating patients with **platinum-resistant ovarian cancer**
- **Phase IIb study in non-small-cell lung carcinoma** (NSCLC) **is still ongoing**

Other folate conjugates

- **Two folate conjugates are currently in a active phase I clinical trials, sponsored by Endocyte**

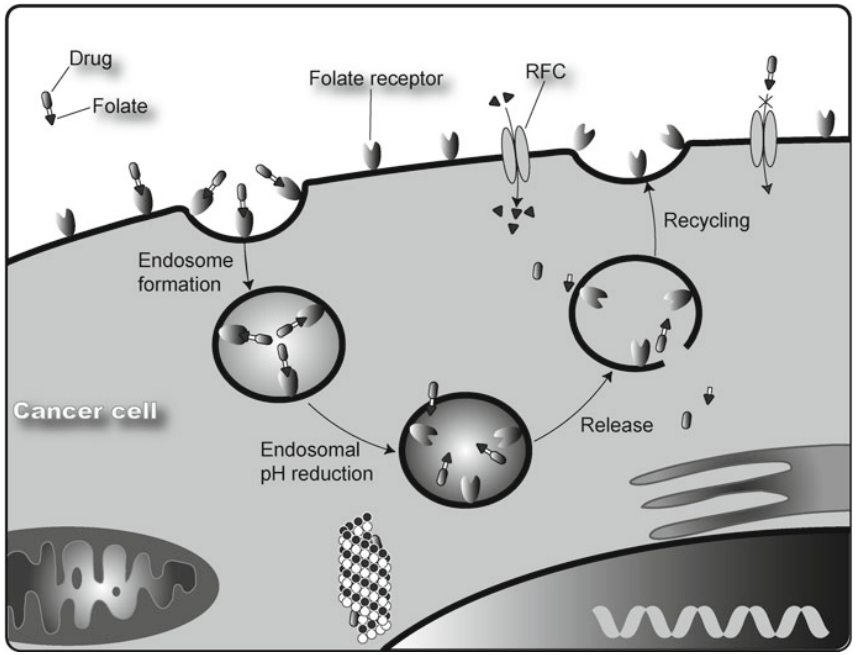


EC1456: patients with Advanced Solid Tumors; Triple-Negative Breast Cancer (TNBC); Advanced Non-Small Cell Lung Cancer (NSCLC); Ovarian Cancer; Hepatocellular Carcinoma (HCC)

EC1456 (structure not released): patients with prostate cancer

Mechanism of folate conjugates

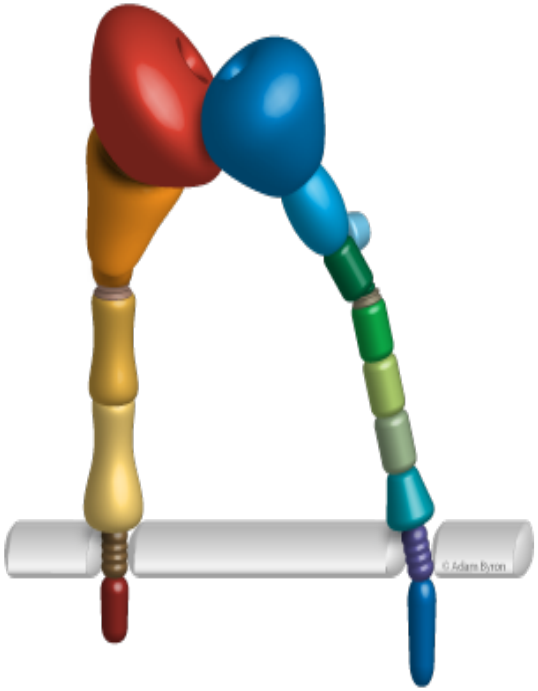
- Folate is required for cell division, and rapidly dividing cancer cells often express folate receptors in order to capture enough folate to support rapid cell growth.
- Elevated expression of the folate receptor occurs in many diseases, including other aggressively growing cancers and inflammatory disorders.
- Folate conjugates bind to the folate receptor and are subsequently internalized via endocytosis
- Once inside the cell, the serum-stable linker selectively releases the vinblastine



Targeted Drug Strategies for Cancer and Inflammation. 2011, pp 135-150

Integrins

Integrins are cell surface receptors that interact with the extracellular matrix and mediate several intracellular signals



The main functions of integrins are:

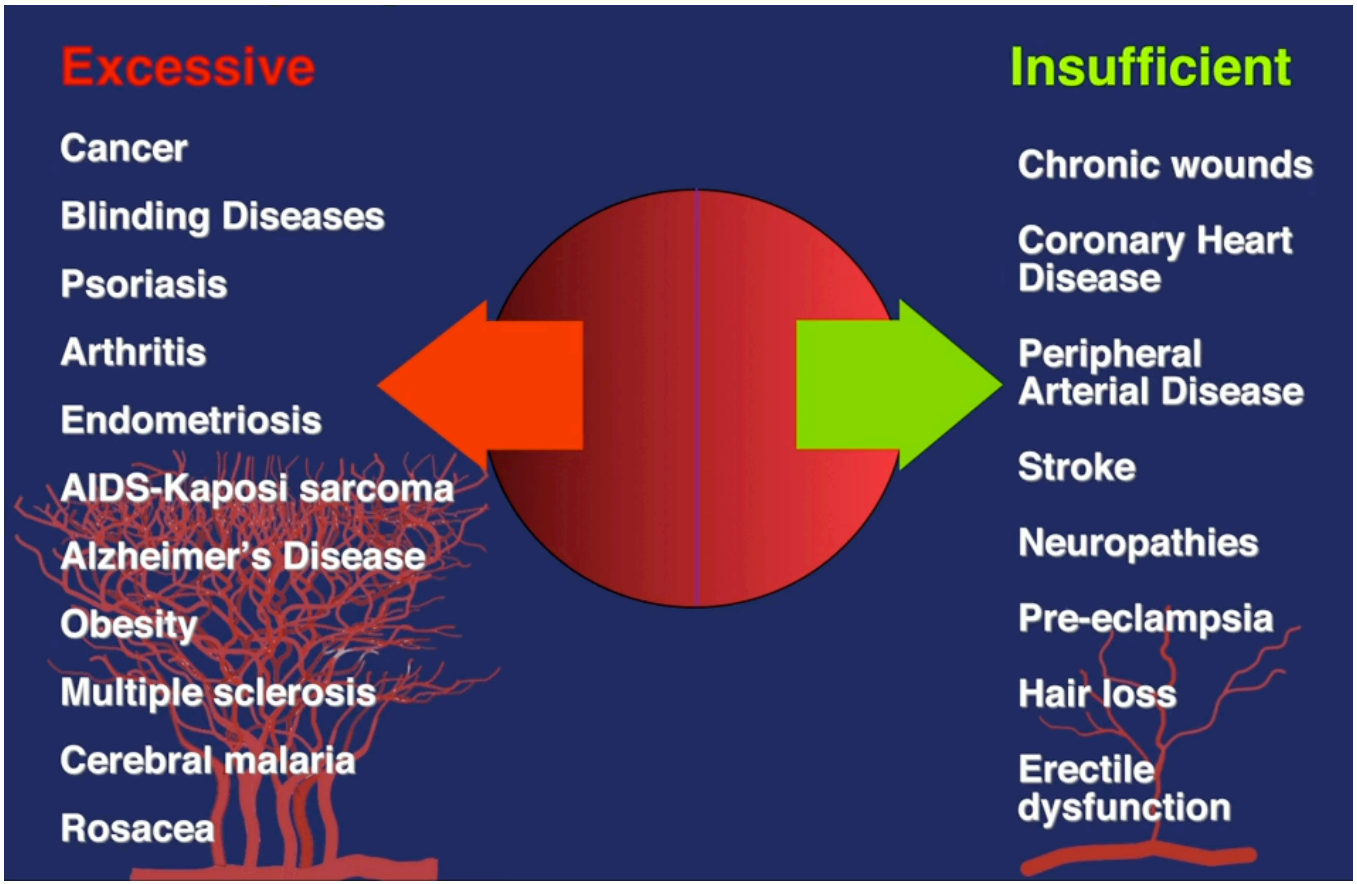
- **Signal transduction** from the extracellular matrix to the cell.

  The signals involve: cell growth, cell migration, cell division, cell survival, cellular differentiation, apoptosis (programmed cell death).

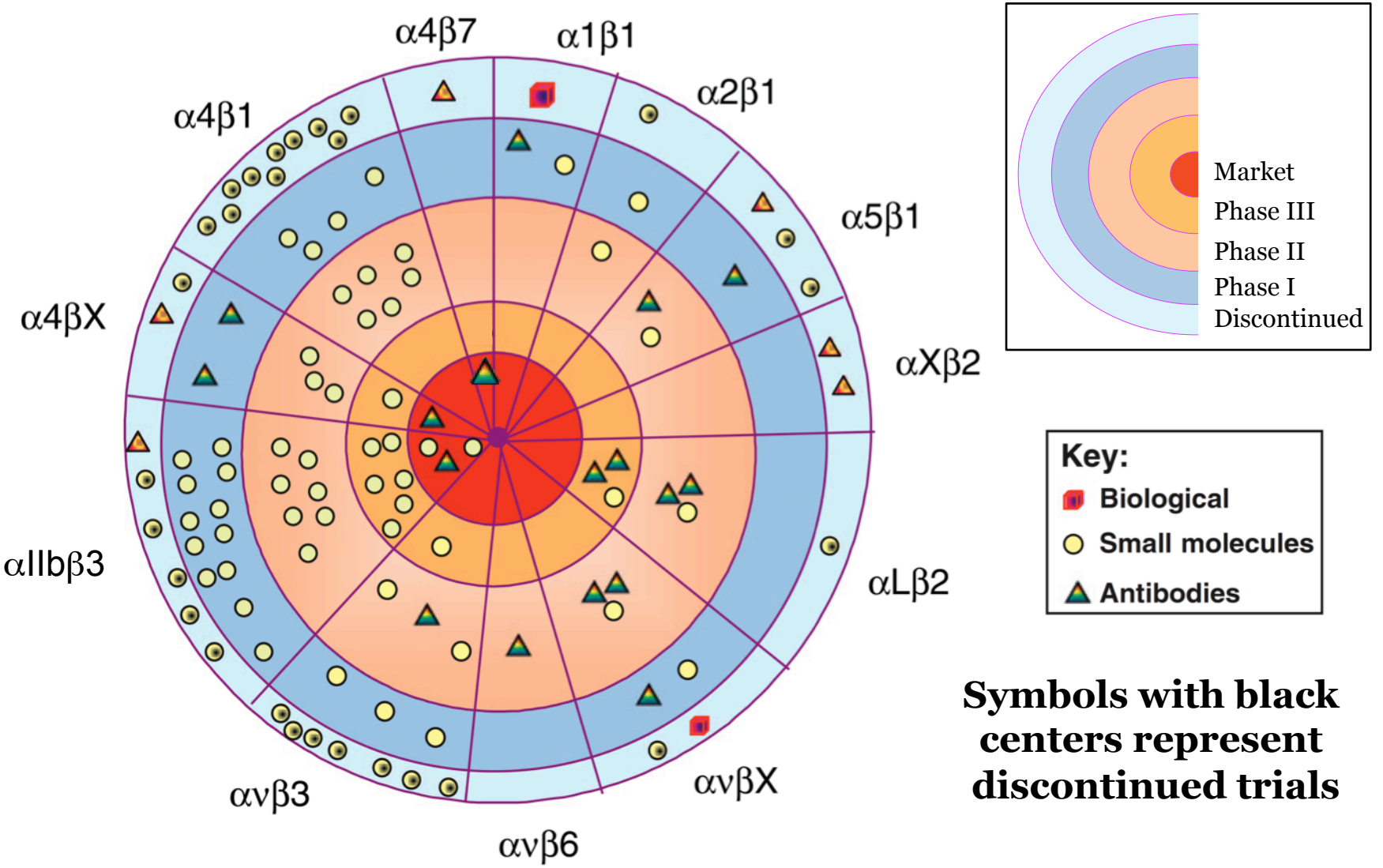
- **Attachment of the cell** to other cells and to the **extracellular matrix**

Integrin disturbance

Disturbance of integrin function is connected to a large variety of pathological processes which makes integrins **attractive targets** for pharmacological research



Integrins as therapeutic targets



Symbols with black centers represent discontinued trials

Adapted from: S. L. Goodman, M. Picard, *Trends in Pharmacological Sciences*, **2012**, *33*, 405-412

References & future readings

https://clinicaltrials.gov/
http://adcreview.com/knowledge-center/

Antibody **2013**, *2*, 113-129
Hematology **2013**, 306-310
Angewandte Chem. Int. Ed. **2014**, *53*, 3796-3827
Biomedicines **2014**, 1-13
Drug Discovery Today **2014**, 869-881
Pharmaceutical Today **2014**, 42-47
Chemical & Engineering News **2014**, *92*, 13-21
AAPS J. **2014**, *16*, 899-913
Bioorganic and Medicinal Chemistry Letters 10.1016/j.bmcl.2014.10.021

and references herein

Thanks!

Prof. Wipf
Wipf group past & present

Prof. Gennari & Gennari group
Eli Lilly – LIFA (Lilly innovation fellowship award)

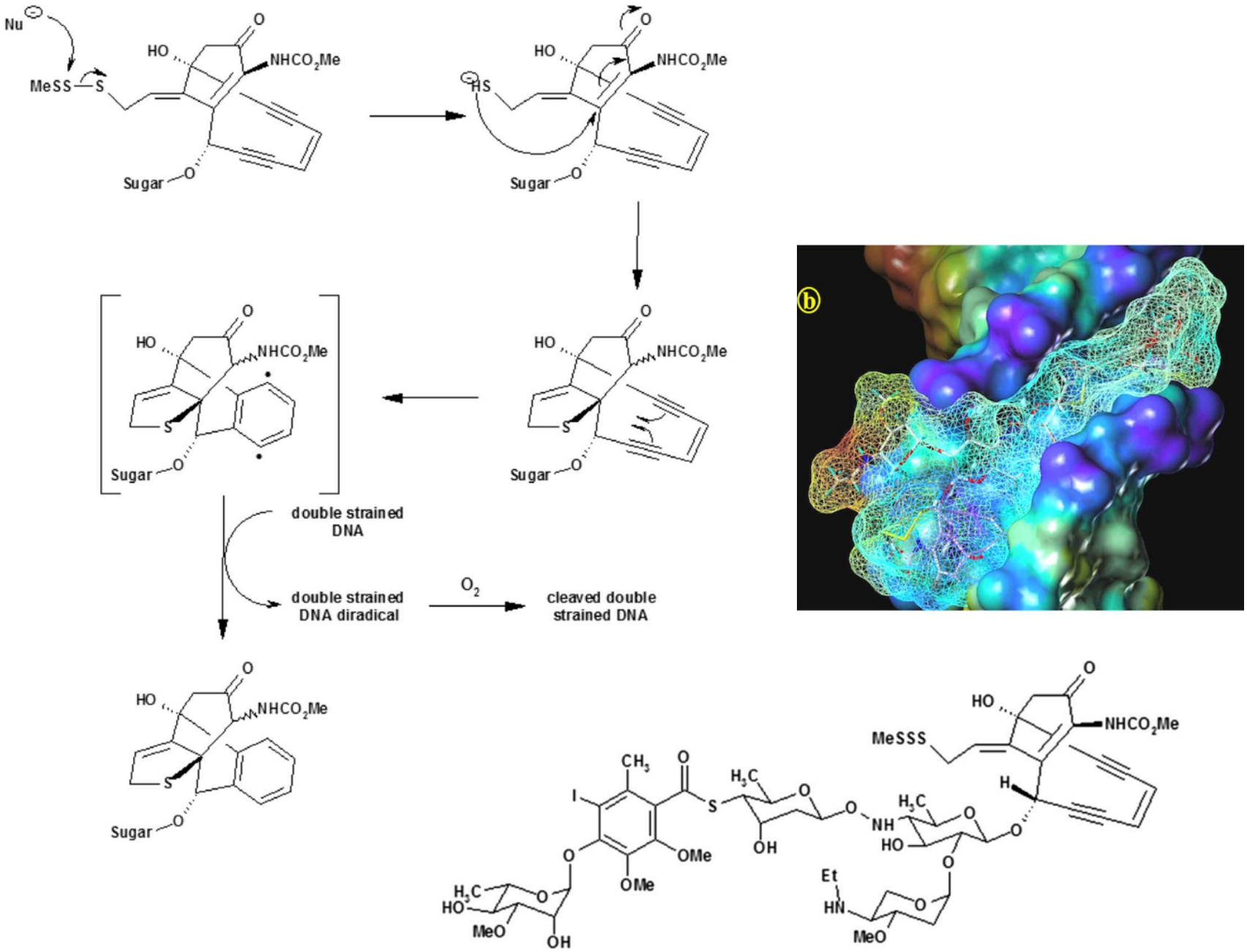
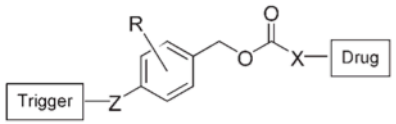
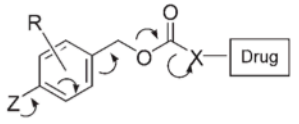
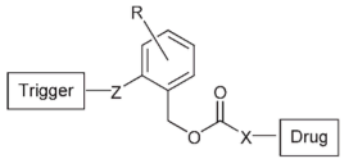
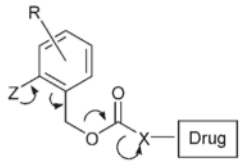
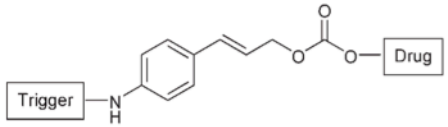
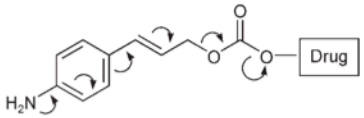
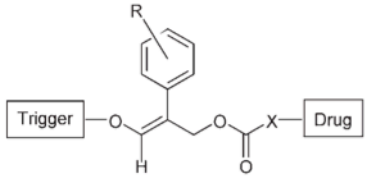
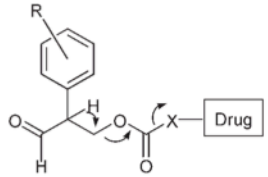
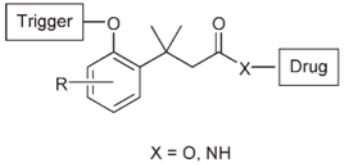
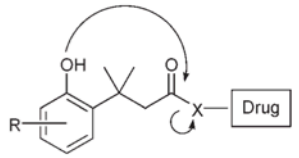
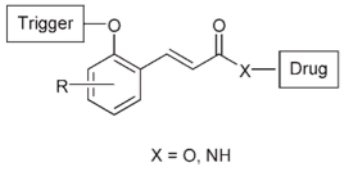
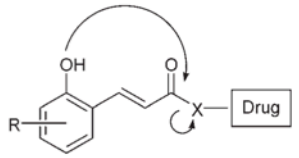
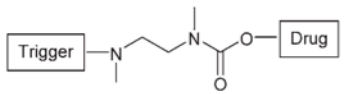
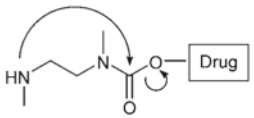
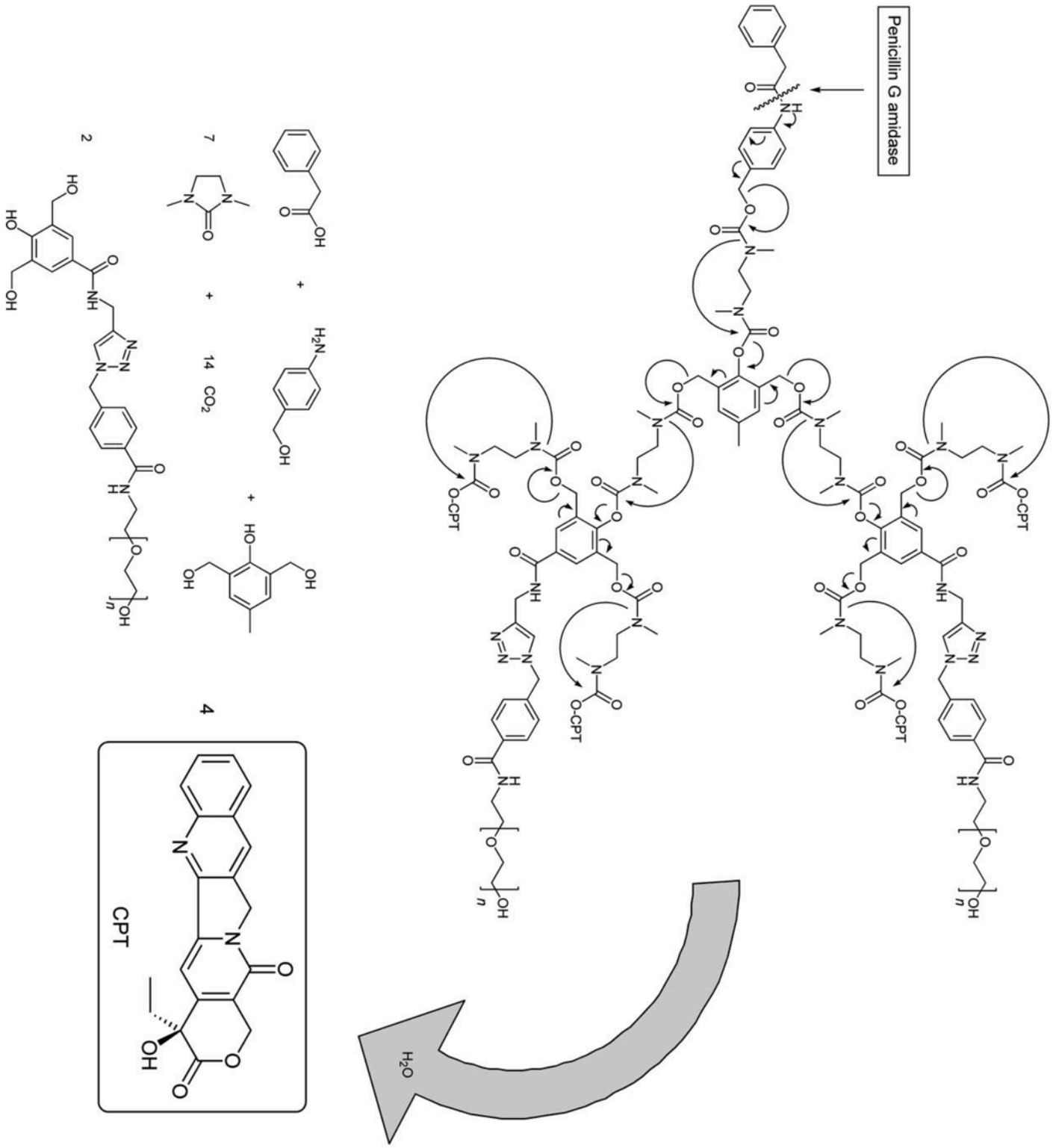
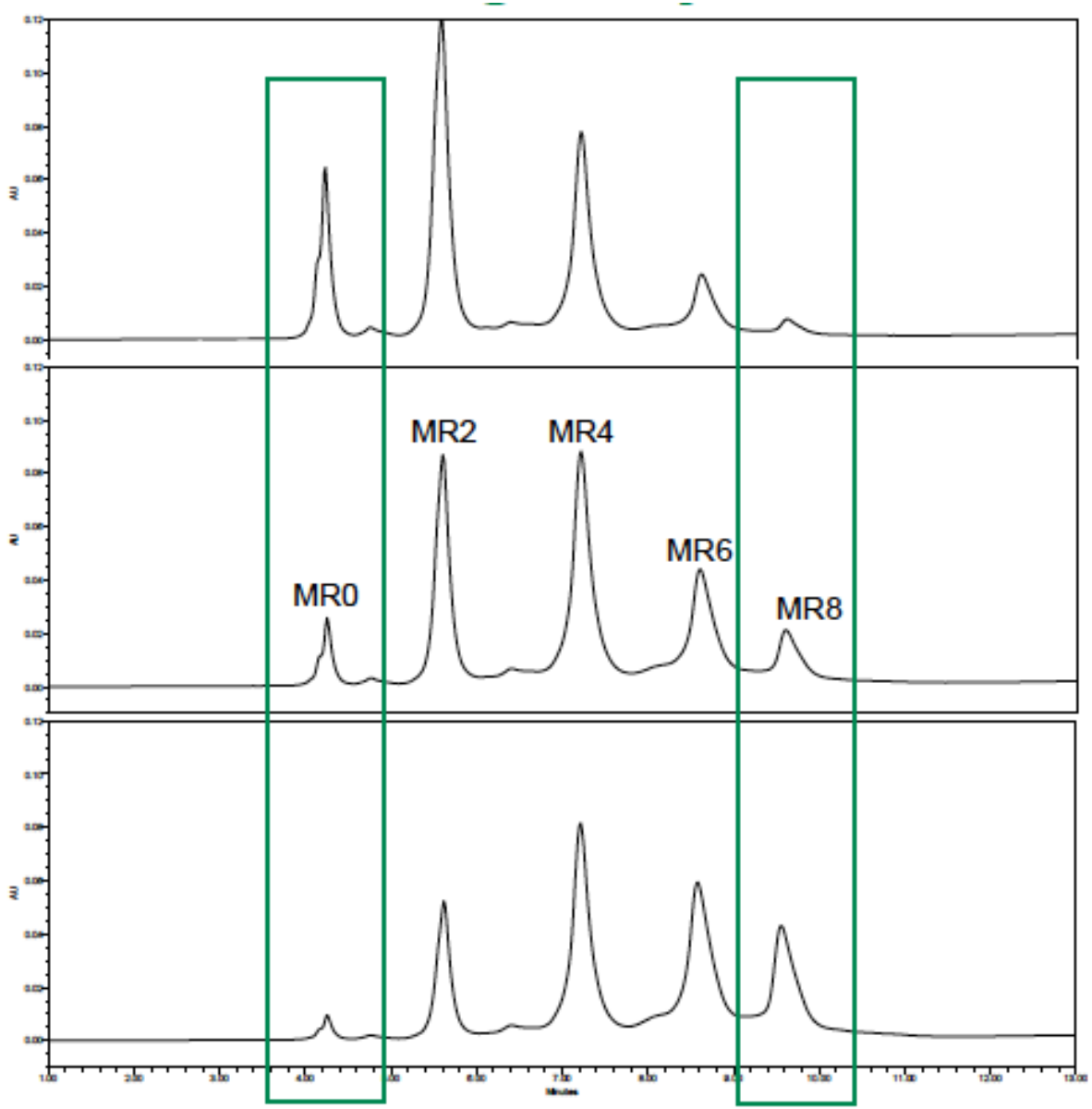


Figure 3. Structure of calicheamicin.

Linker	Reaction type	Release mechanism
 X, Z = O, NH	1,6-Benzyl elimination	 X, Z = O, NH
 X, Z = O, NH	1,4-Benzyl elimination	 X, Z = O, NH
	1,8-Elimination	
 X = O, NH	β-Elimination	 X = O, NH
 X = O, NH	Cyclization (lactonization)	 X = O, NH
 X = O, NH	Cyclization (lactonization)	 X = O, NH
	Cyclization	


Penicillin G amidase
O-CPT
H2O
2
7
14 CO2
4
CPT

Hydrophobic Interaction Chromatography (HILIC) Measures Heterogeneity



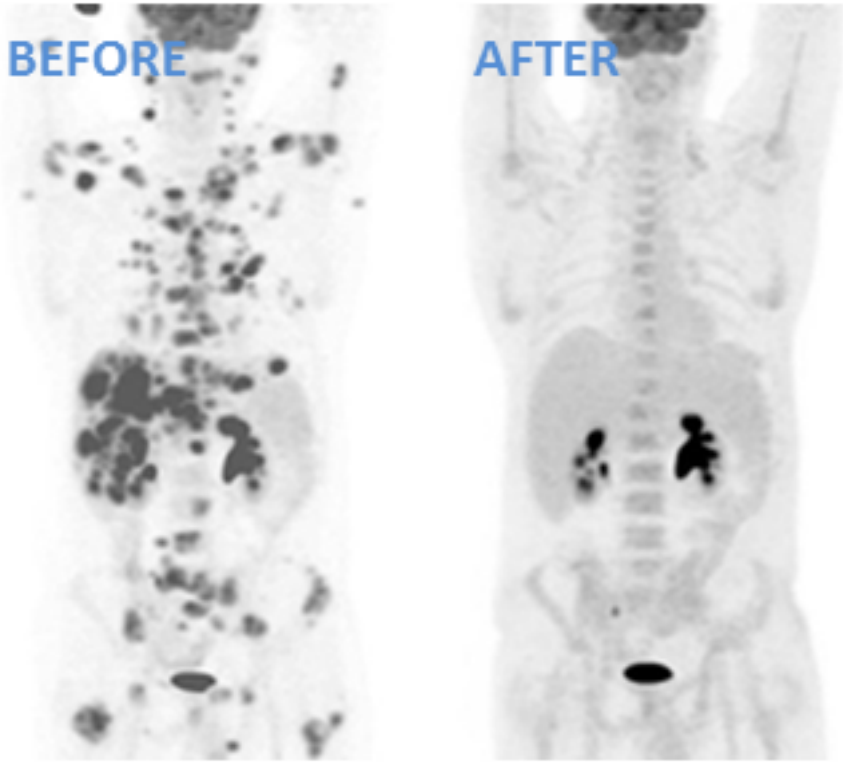
3 drugs/antibody

4 drugs/antibody

5 drugs/antibody

University of Pittsburgh
Department of Chemistry

ADCETRIS



Before (left) and after (right) treatment scans. The image on the left shows the extent of metastatic disease (spread of cancer) in the patient (70 tumours from Non-Hodgkin's Lymphoma).

The scan on the right demonstrates complete elimination of tumours two weeks after treatment (the black blobs in the scan on the right are normal (brain, kidneys, and the bladder).

Trastuzumab – tubulysin ADC

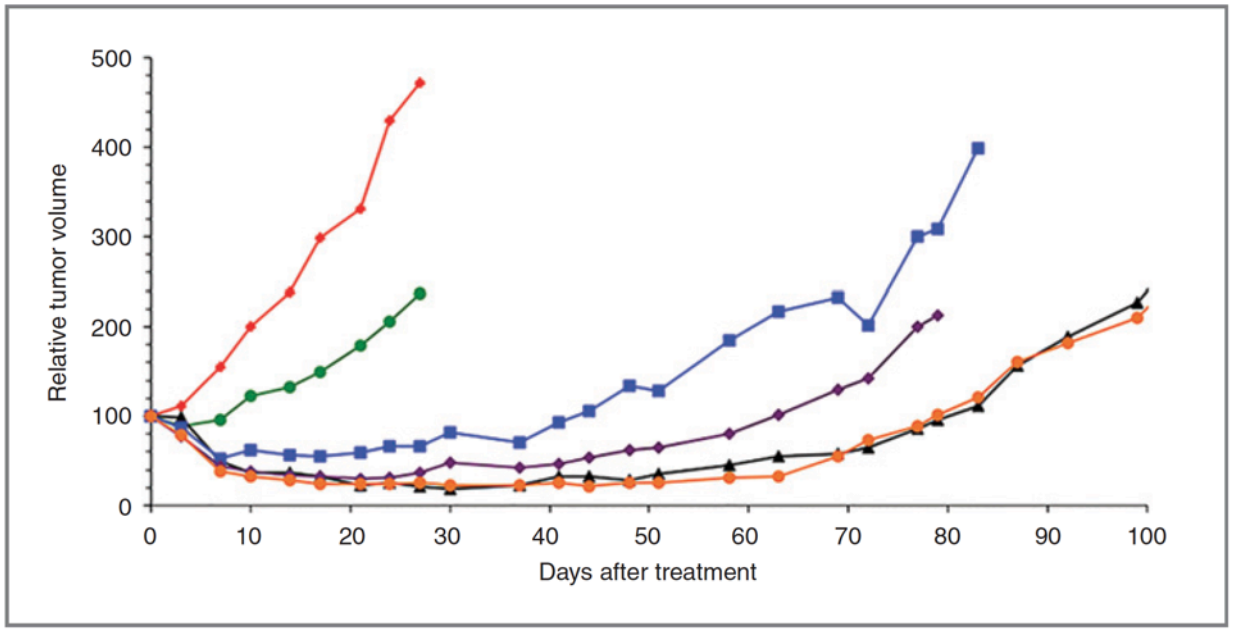
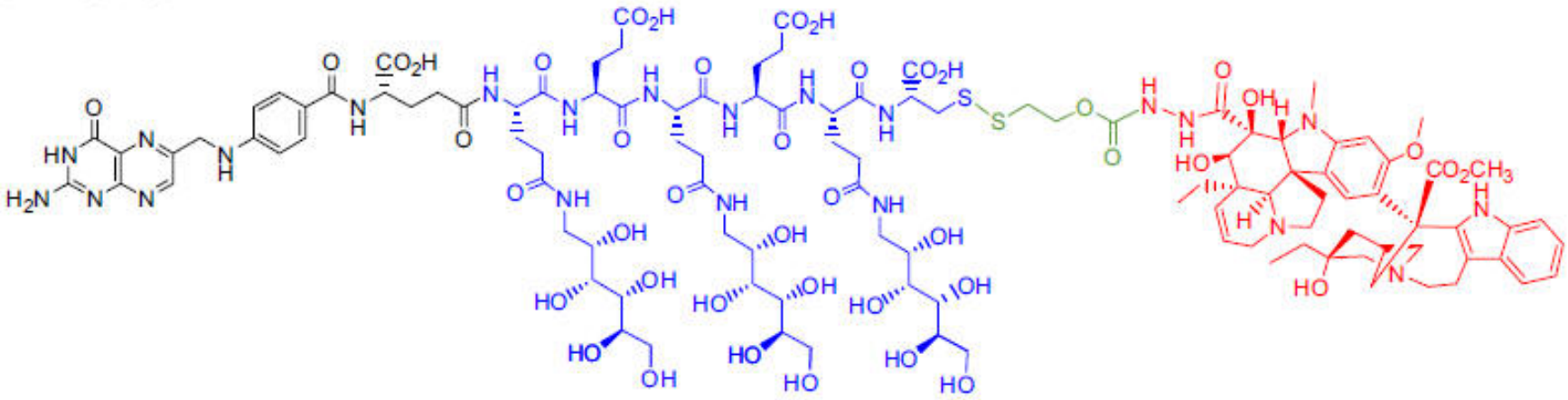


Figure 6. Efficacy of TUB-OMOM–trastuzumab in N87-tumor–bearing mice. The effect on tumor growth after a single i.p. injection of 0.9% NaCl solution (control, red line), trastuzumab (15 mg/kg, green line), 15 mg/kg TUB-OMOM–trastuzumab (blue line), 30 mg/kg TUB-OMOM–trastuzumab (purple line), 60 mg/kg TUB-OMOM–trastuzumab (orange line), or ado-trastuzumab emtansine (T-DM1; 15 mg/kg; black line). SDs have been omitted for the sake of clarity. Of note, cures, defined as no outgrowth of regressed individual tumors during the follow-up period, were seen in mice treated with 30 mg/kg TUB-OMOM–trastuzumab ($n = 1$), 60 mg/kg TUB-OMOM–trastuzumab ($n = 3$) and ado-trastuzumab emtansine ($n = 3$).

Improved folate – vinblastine analogue

c EC0489



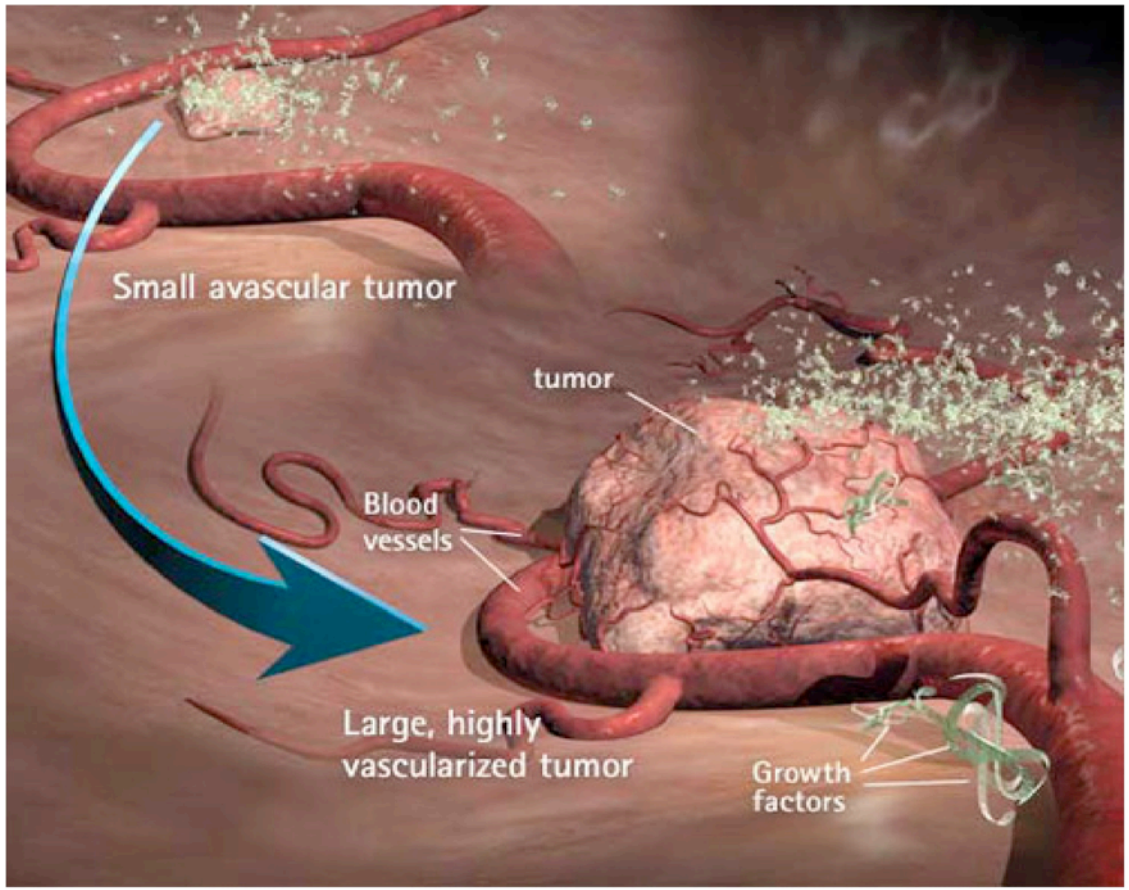
EC0489 is the latest folate-targeted chemotherapeutic to enter clinical trials sponsored by Endocyte, that was designed to have limited non-specific clearance properties through the liver. By reducing hepatic clearance, less drug will transit through the biliary excretion route; as a consequence, less off-target toxicities (predicted from preclinical tests) are expected

University of Pittsburgh

Department of Chemistry

19 Oct 2009

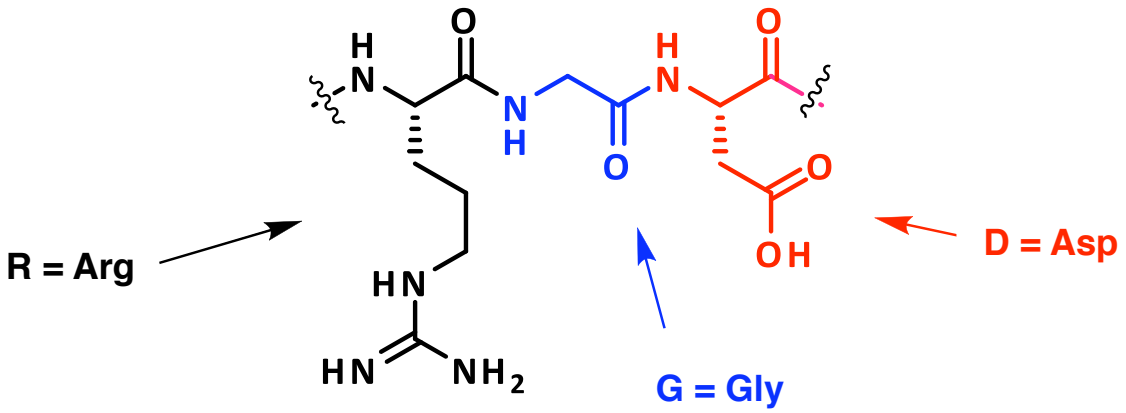
Integrins: angiogenesis key-factors



Of the 24 different heterodimers known, the **RGD-binding integrins** $\alpha_V\beta_3$, $\alpha_V\beta_5$, $\alpha_5\beta_1$ are key-factors of angiogenesis

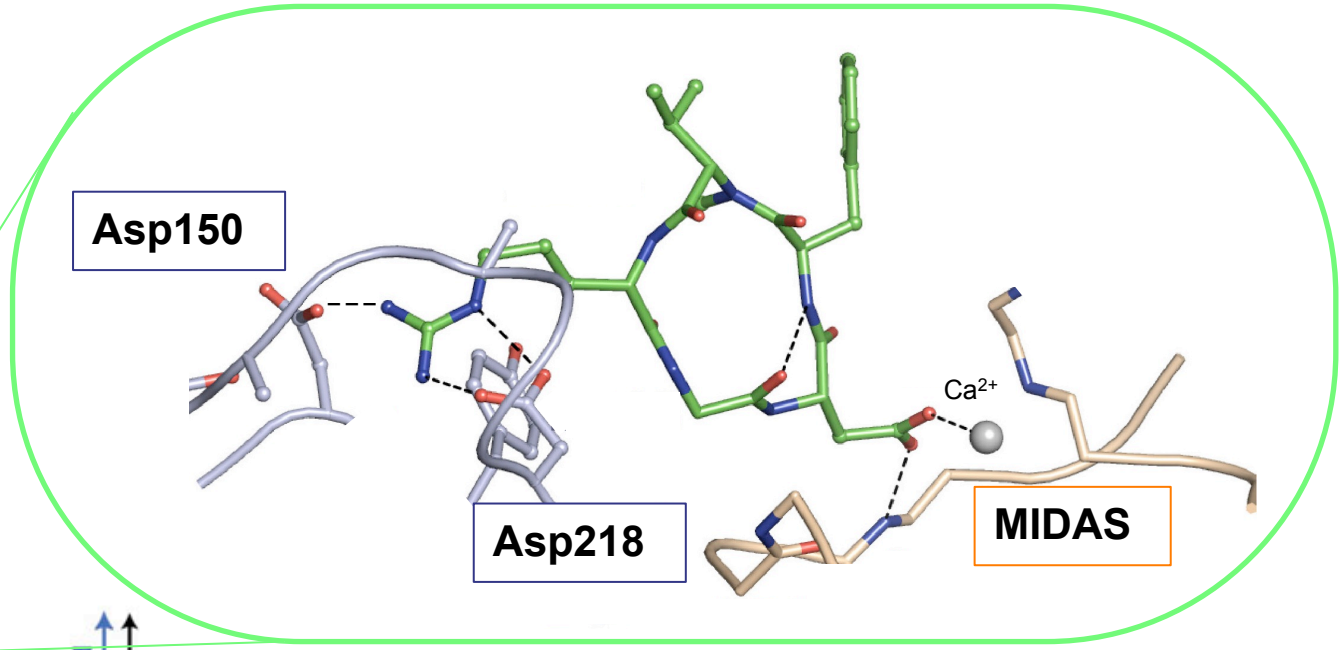
RGD-binding integrins

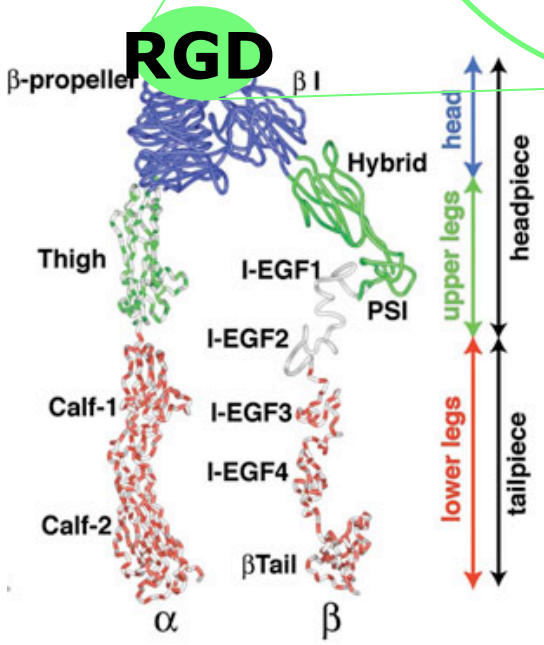
- All five α_V **integrins**, two β_1 **integrins** (α_5, α_8) and $\alpha_{IIb}\beta_3$ share the ability to recognise ligands containing an **Arg-Gly-Asp (RGD)** tripeptide active site

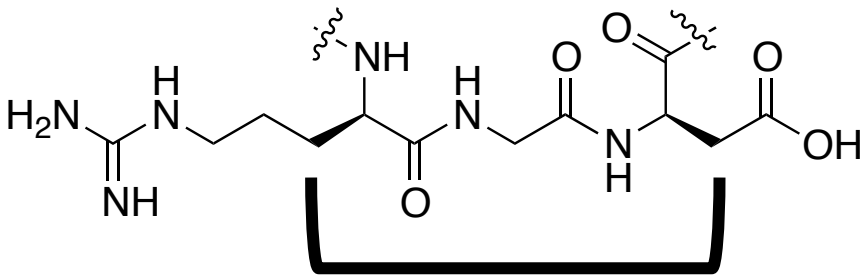


- Synthetic RGD-ligands can bind selectively $\alpha_V\beta_3$, $\alpha_V\beta_5$, $\alpha_5\beta_1$ integrins and significantly **inhibit angiogenesis**, **tumor growth** and **metastasis**.

Integrin electrostatic clamp

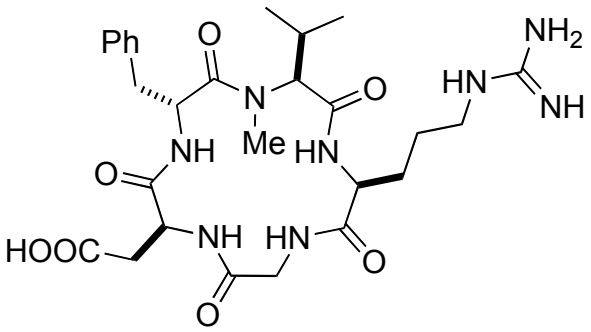






C(β)-C(β) Distance: ≈ 9 Å

RGD-binding integrins



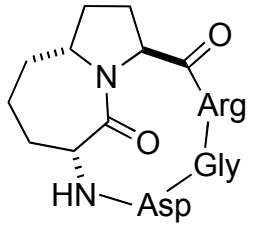
EMD121974

Cilengitide

The potent $\alpha_V\beta_3$ ligand, cyclic peptide
cyclo[Arg-Gly-Asp-D-Phe-N(Me)-Val]
(H. Kessler et al., *J. Med. Chem.* **1999**, *42*, 3033)

Inhibition of biotinylated vitronectin binding to integrin receptor
$\alpha_V\beta_3$ IC_{50} 0.58 **nM**

Cilengitide is currently in phase III clinical trials for patients with glioblastoma multiforme



ST1646

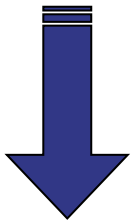
ST1646

Cyclic RGD pentapeptide mimics
cyclo[Arg-Gly-Asp-lactam]
(C. Scolastico et al., *Org. Lett.* **2001**, *3*, 1001)
(C. Scolastico et al., *ChemMedChem* **2009**, *4*, 615)

Inhibition of biotinylated vitronectin binding to integrin receptors
$\alpha_V\beta_3$ IC_{50} 1.0 ± 0.5 **nM**
$\alpha_V\beta_5$ IC_{50} 1.4 ± 0.8 **nM**

RGD ligands conjugate to anticancer drugs

- α_V integrins are overexpressed on the surface of cancer cells
- α_V integrins can be internalized by cells



Conjugate RGD ligands can be employed as **tumor-homing** peptides for **site-directed delivery** of potent cytotoxic drugs (*e.g.* Paclitaxel)

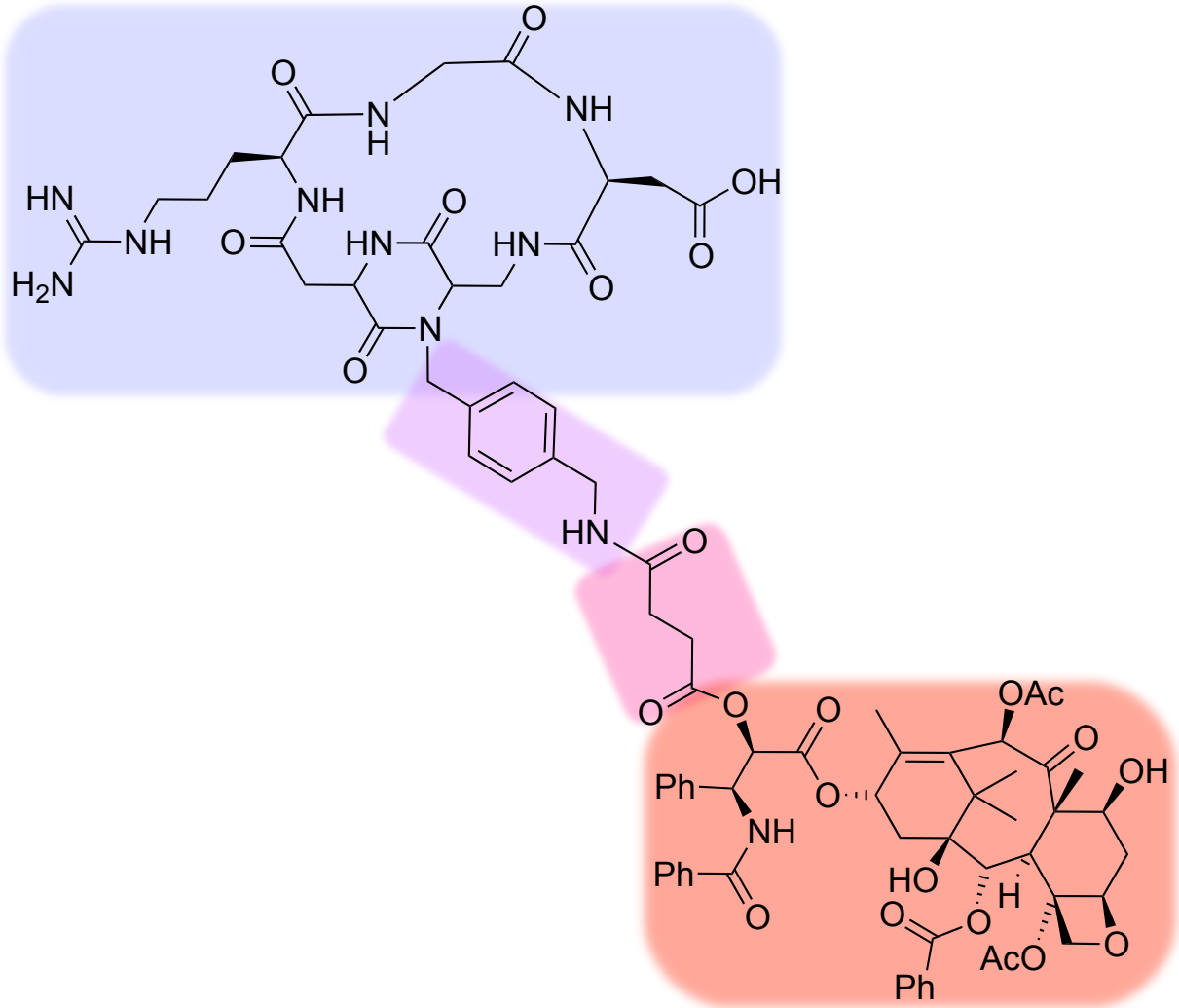
RGD ligands conjugate to anticancer drugs

[DKP-RGD]

LINKER

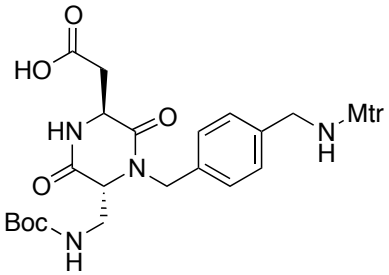
SPACER

CYTOTOXIC DRUG

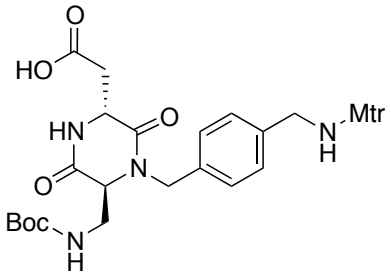


R. Colombo, M. Mingozzi, L. Belvisi, D. Arosio, U. Piarulli, N. Carenini, P. Perego, N. Zaffaroni, M. De Cesare, V. Castiglioni, E. Scanziani C. Gennari. *J. Med. Chem.* **2012**, *55*, 10460-10474.

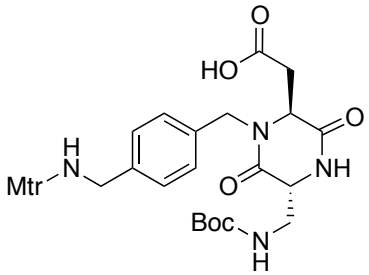
Synthesis of *cyclo*[DKP-RGD] ligand



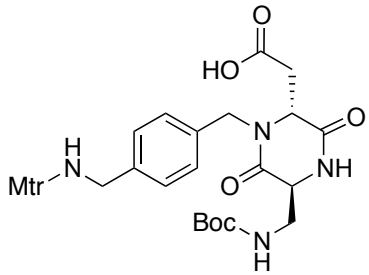
DKP-*f*2



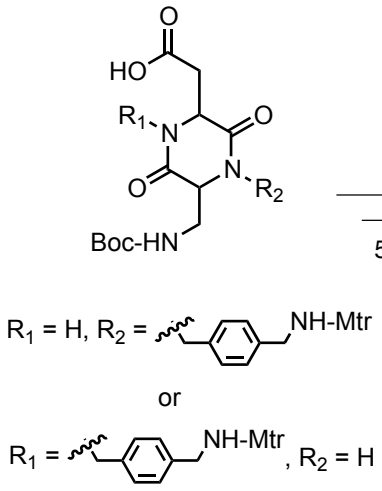
DKP-*f*3



DKP-*f*5

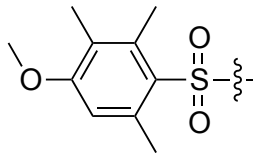


DKP-*f*6



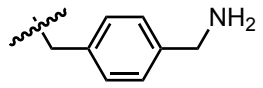
***cyclo*[DKP-*f*2-RGD]: 3*R*, 6*S*, R_1= H, R_2= linker**
***cyclo*[DKP-*f*3-RGD]: 3*S*, 6*R*, R_1= H, R_2= linker**
***cyclo*[DKP-*f*4-RGD]: 3*R*, 6*S*, R_1= linker, R_2= H**
***cyclo*[DKP-*f*6-RGD]: 3*S*, 6*R*, R_1= linker, R_2= H**

Mtr =

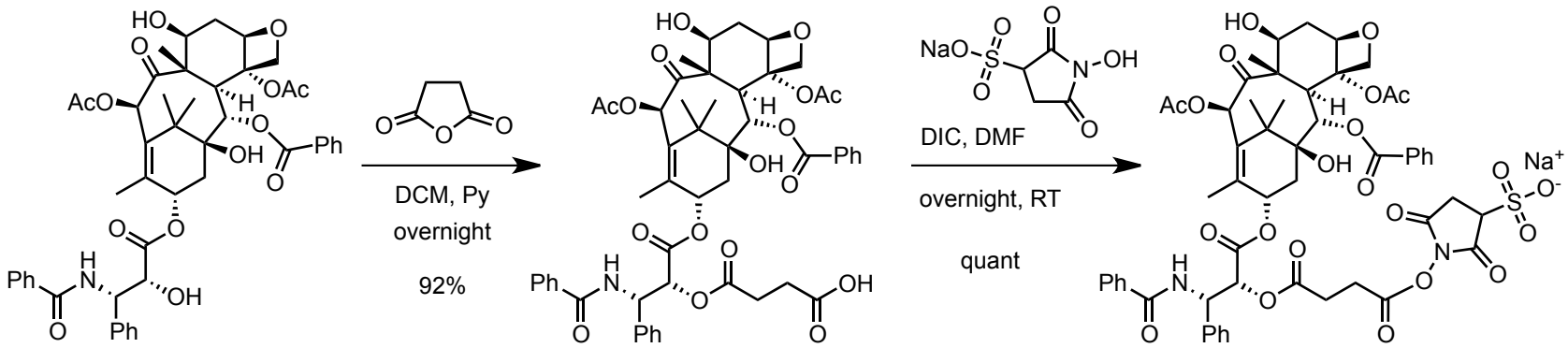


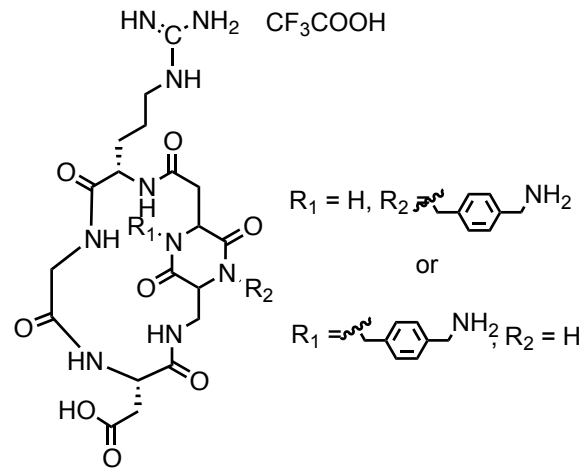
orthogonal to:
methyl, benzyl, allyl, *t*Bu, Boc,
and Cbz protecting groups

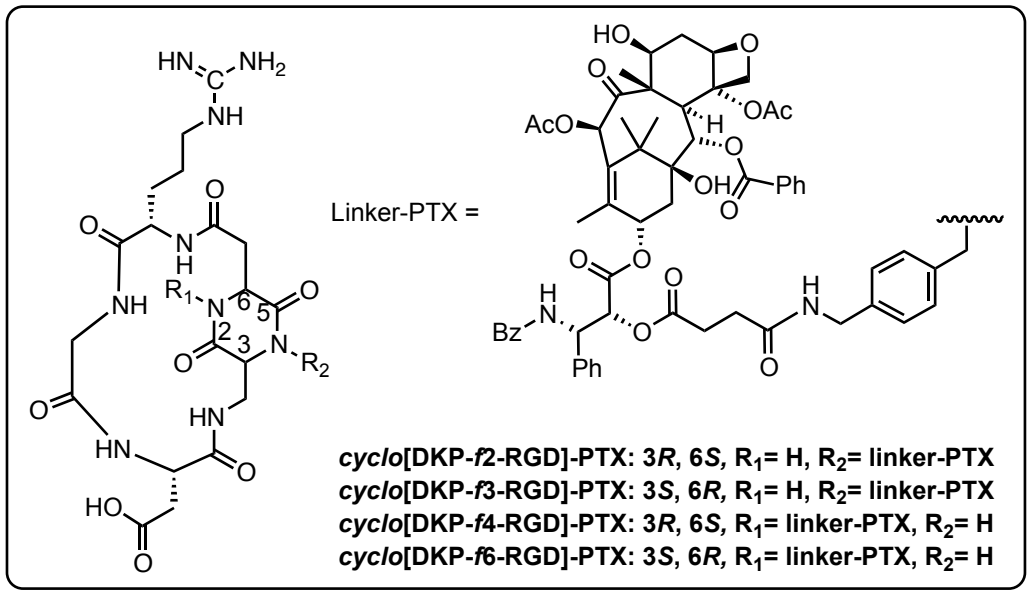
linker =



Synthesis of *cyclo*[DKP-RGD]-Paclitaxel conjugates







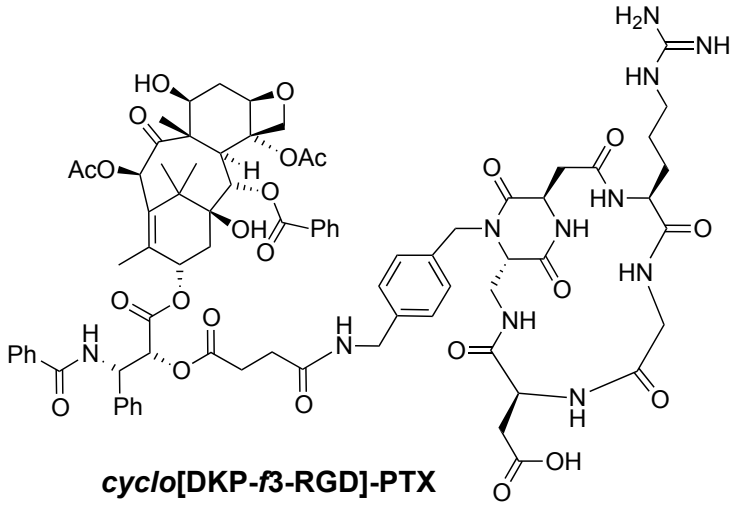
***cyclo*[DKP-*f*2-RGD]-PTX: 3*R*, 6*S*, R_1= H, R_2= linker-PTX**
***cyclo*[DKP-*f*3-RGD]-PTX: 3*S*, 6*R*, R_1= H, R_2= linker-PTX**
***cyclo*[DKP-*f*4-RGD]-PTX: 3*R*, 6*S*, R_1= linker-PTX, R_2= H**
***cyclo*[DKP-*f*6-RGD]-PTX: 3*S*, 6*R*, R_1= linker-PTX, R_2= H**

Inhibition of biotinylated vitronectin binding to $\alpha_v\beta_3$ and $\alpha_v\beta_5$ receptors

Compound	$\alpha_v\beta_3$ IC_{50} [nM]	$\alpha_v\beta_5$ IC_{50} [nM]
cyclo[DKP-*f*2-RGD]-PTX	8.5 ± 0.8	518 ± 10
cyclo[DKP-*f*3-RGD]-PTX	5.2 ± 2.3	219 ± 124
cyclo[DKP-*f*4-RGD]-PTX	0.9 ± 0.6	76 ± 32
cyclo[DKP-*f*6-RGD]-PTX	1.1 ± 0.1	22 ± 3
cyclo[DKP-2-RGD]	3.2 ± 2.7	114 ± 99
cyclo[DKP-3-RGD]	4.5 ± 1.1	149 ± 25
cyclo[DKP-4-RGD]	7.6 ± 4.3	216 ± 5
cyclo[DKP-6-RGD]	2.1 ± 0.6	79 ± 3
cyclo(RGDfV)	3.2 ± 1.3	7.5 ± 4.8

Integrin expression

Integrin	Mean fluorescence intensity (cellular line)					
	IGROV-1	IGROV-1/Pt1	U2-OS	SKOV3	PANC-1	MIA-PaCa2
$\alpha_v\beta_3$	4.8 ± 1.9	23.3 ± 5.0	1.8 ± 0.6	6.4 ± 0.05	7.9 ± 2.8	1.2 ± 0.1
$\alpha_v\beta_5$	3.4 ± 0.9	3.3 ± 0.5	27.4 ± 0.1	4.4 ± 0.5	25.7 ± 6.5	5.6 ± 0.9



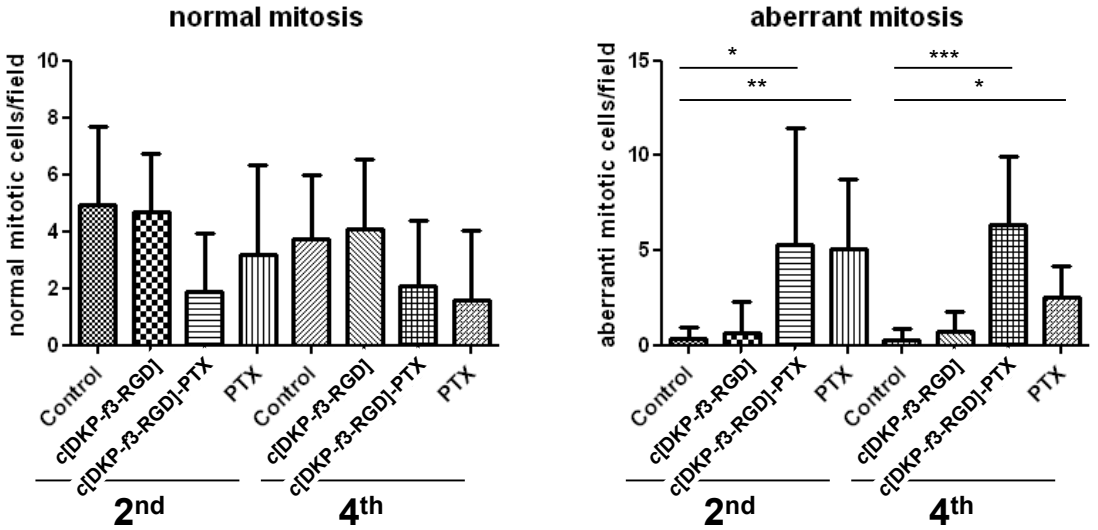
***cyclo*[DKP-*f*3-RGD]-PTX**

IGROV-1/Pt1 was chosen as *in vivo* model becouse of its high expression of $\alpha_v\beta_3$ integrin

***cyclo*[DKP-*f*3-RGD]-PTX** was chosen as lead conjugate mainly because of its straightforward synthetic accessibility on a multi-gram scale

Evaluation of *in vivo* antitumor activity

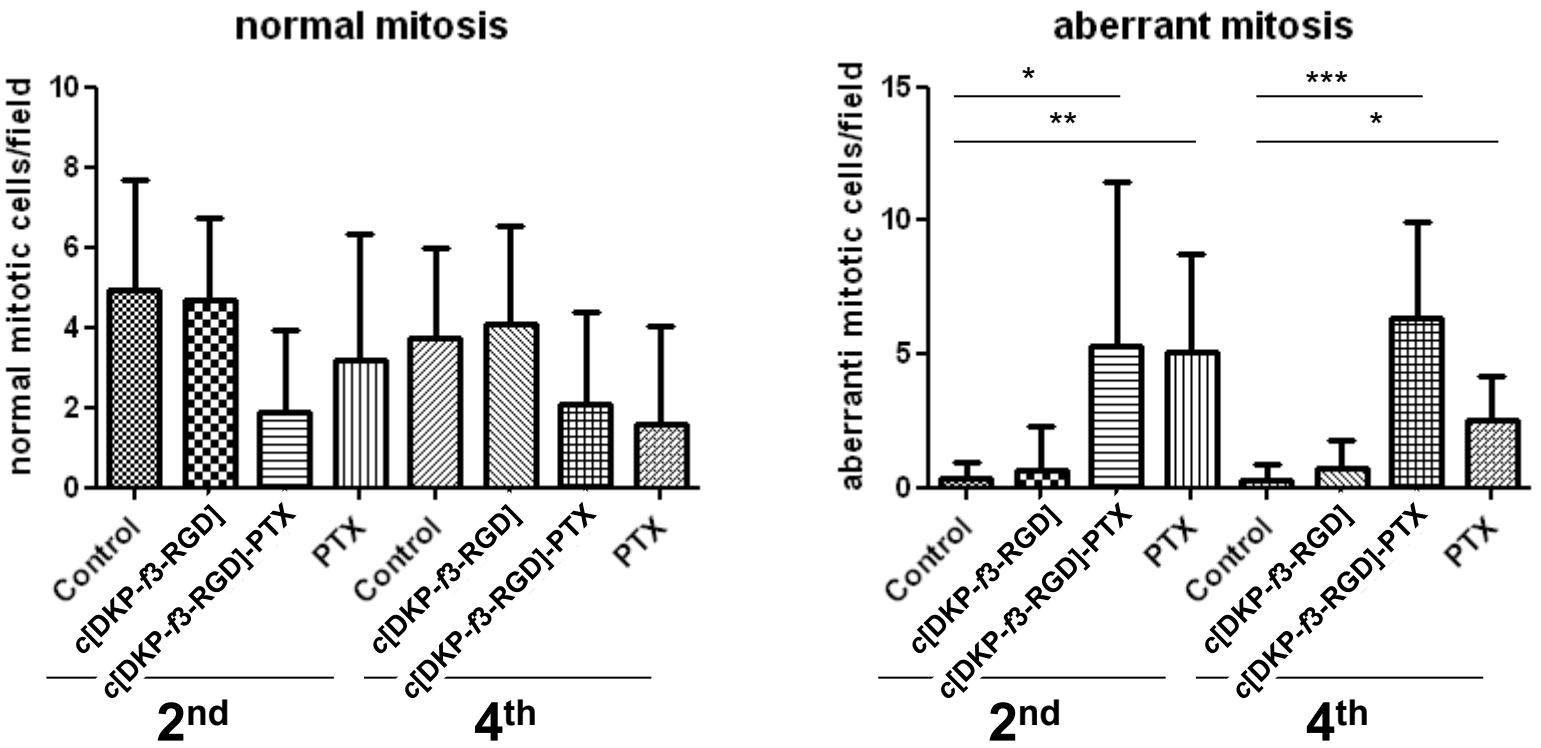
Treatment	Dose (mg/kg)	Dose (mmol/kg)	TVI%	CR	NED	BWL%	D/T
Paclitaxel	30	35.1	76	3/8	0/8	4	0/4
cyclo[DKP-*f*3-RGD]-PTX	15	9.6	64	0/8	-	0	0/4
cyclo[DKP-*f*3-RGD]-PTX	30	19.1	85	2/8	2/8	3	0/4



High levels of aberrant mitoses were observed with ***cyclo*[DKP-*f*3-RGD]-PTX** after the 2nd treatment and persisted after the 4th treatment.

The amount of aberrant mitotic cells observed after treatment with Paclitaxel as single agent decreased over time

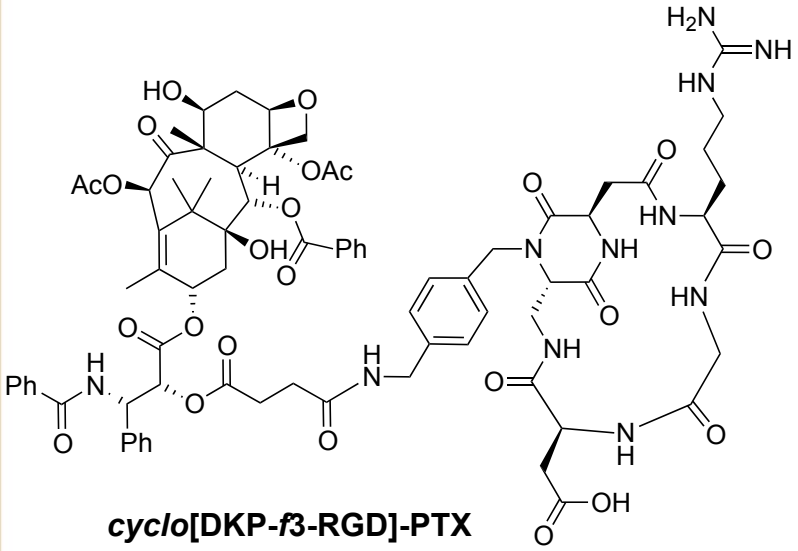
Immunohistochemistry



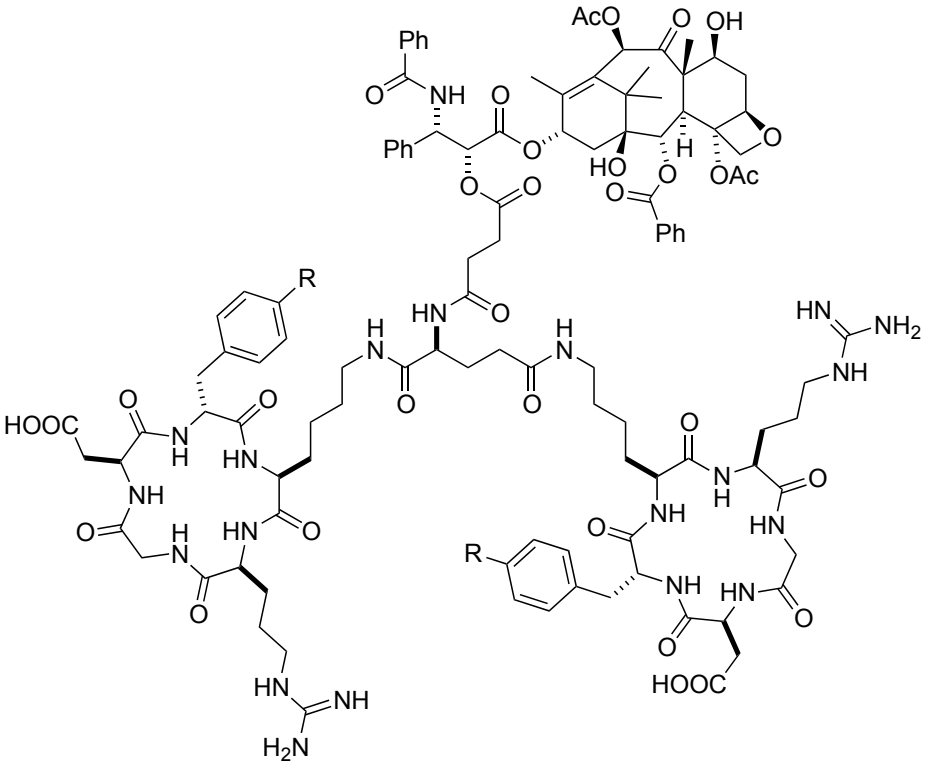
High levels of aberrant mitoses were observed with ***cyclo*[DKP-*f*3-RGD]-PTX** after the 2nd treatment and persisted after the 4th treatment. On the contrary, the amount of aberrant mitotic cells observed after mice treatment with Paclitaxel decreased over time

R. Colombo, M. Mingozzi, L. Belvisi, D. Arosio, U. Piarulli, N. Carenini, P. Perego, N. Zaffaroni, M. De Cesare, V. Castiglioni, E. Scanziani C. Gennari. *J. Med. Chem.* **2012**, *55*, 10460-10474.

Cyclo[DKP-*f*3-RGD]-PTX *vs* PTX-E(*cyclo*[RGDfK]$_2$)



***cyclo*[DKP-*f*3-RGD]-PTX**



PTX-E(cyclo[RGDyK]$_2$) R = OH (Chen and co-workers)
PTX-E(cyclo[RGDfK]$_2$) R = H (Ryppa and co-workers)