Federal Institute for Vaccines and Biomedicines

Paul-Ehrlich-Institut

Regulatory expectations for the characterisation of ADCs

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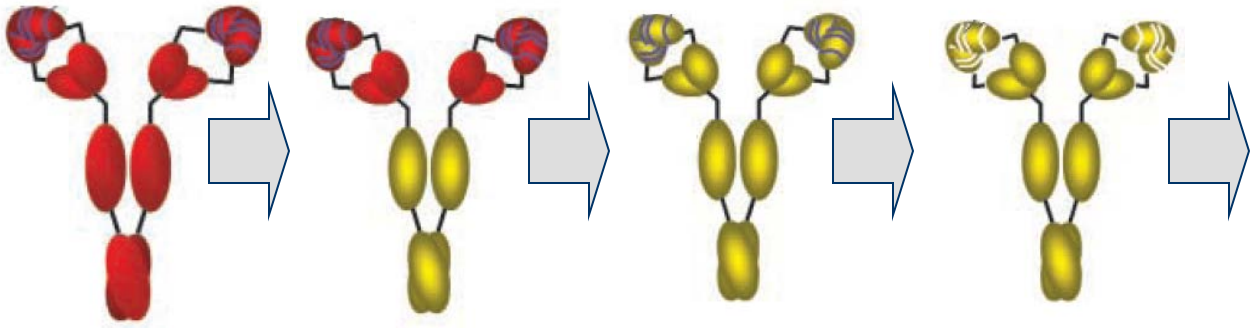
Outline/Points for Discussion

- Molecular particulars and their characterization
- Characterisation of starting material used to manufacture the ADC
- Test methods to use to investigate and quality control ADC
- Testing for residuals and impurities in the ADC DS and DP
- Characterisation of the linker-quality, its mechanism in-vivo and the relevant data requirements

- Non-clinical challenges with ADC

„Evolution“ of therapeutic proteins



= murine
= human

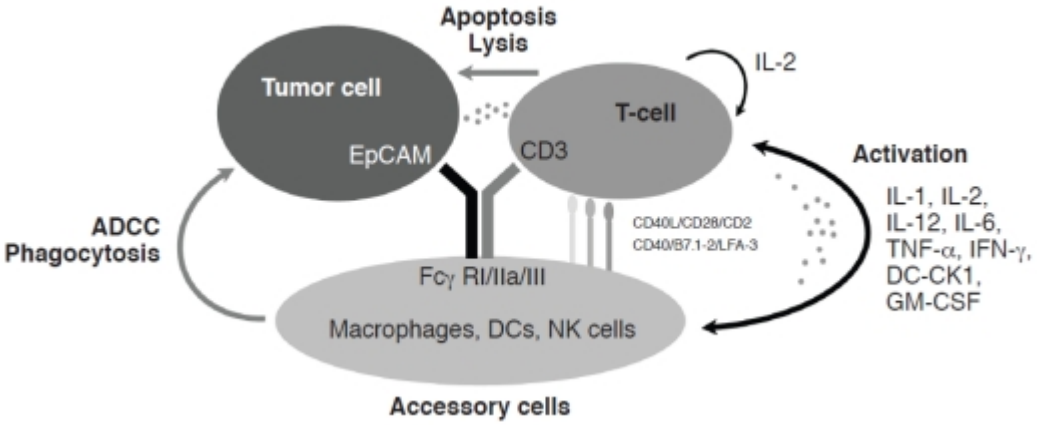
Murine mAb	Chimaeric mAb	Humanized mAb	Fully Human mAb
„-omab“	„-iximab“	„-zumab“	„-umab“
Arcitumomab (CEA-Scan®) (1996)	Infliximab (Remicade®) (1999)	Trastuzumab (Herceptin®) (2000)	Adalimumab (Humira®) (2003)

New constructs
- bispecific antibodies
- diabodies
- single chain fragments
- engineered Fc mAbs
- conjugated mAbs
- ...

Immunogenicity

Monoclonal Antibody Related Products

Source: Cimzia.com



Source: :J Cancer. 2011; 2: 309–316.

- recombinant, humanized, antibody Fab' fragment with specificity for human TNFα
- conjugated via a maleimide group to polyethylene glycol (PEG)
- extend its plasma half-life to that of the whole antibody

Catumaxomab binds to epithelial cell adhesion molecule (EpCAM) on tumor cells, the CD3 antigen on T-cells, and to type I, IIa, and III Fcγ receptors (FcγRs) on accessory cells (e.g. natural killer cells, dendritic cells, and macrophages).

„Evolution“ of ADC discovery

Table 1: Summary of the Antibody-Drug Conjugates

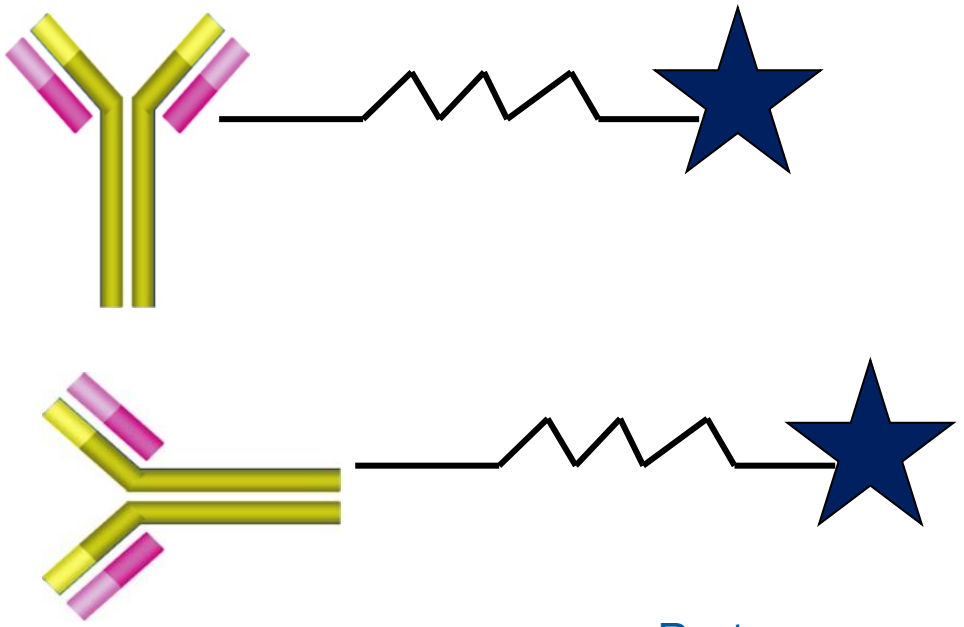
ADC	Brand Name	FDA approved	Target	Antibody	Linker	Toxin	Reference
Combotox	-	No	CD19 & CD22	RFB4 (CD22) & HD37 (CD19)	SMPT (disulfide)	dgRTA	[18]
Moxetumomab Pasudotox	-	No	CD22	Recombinant RFB4	C3 connector	PE38	[54]
Inotuzumab Ozogamicin	-	No	CD22	G5/44	AcBut (acid hydrolyzable)	CalichDMH	[81]
Brentuximab Vedotin	Adcetris™	Yes	CD30	cAC10	Valine-citrulline (dipeptide)	MMAE	[27]
Trastuzumab emtansine	Kadcycla™	Yes	Her2	Recombinant 4D5	MCC (thioether)	DM1	[37]

This is a summary overview of the five ADCs described in more detail in the text.

ADC: Antibody-drug conjugates, FDA: Food and Drug Administration, CD: cluster of differentiation, SMPT: *N*-succinimidyl-oxycarbonyl-a-methyl-a-(2-pyridyldithio)toluene , dgRTA: deglycosylated ricin-A chain , PE38: Pseudomonas Exotoxin a 38, AcBut: 4-(4′-acteylphenoxy)butanoic acid , CalichDMH: *N*-acetyl-gamma-calicheamicin dimethyl hydrazide , MMAE: monomethyl auristatin E , Her2: Human Epidermal Growth Factor Receptor 2, MCC: *N*-[maleimidomethyl] cyclohexane-1 carboxylate , DM1: maytasanoid N(2′)-deacetyl-N(2′)-(3-mercapto-1-oxopropyl)-maytansine

from: Jonathan Feld,*, Stefan K. Barta,*, Carolina Schinke, Ira Braunschweig, Yiyu Zhou, Amit Verma; Oncotarget 2013; 4: 397-412

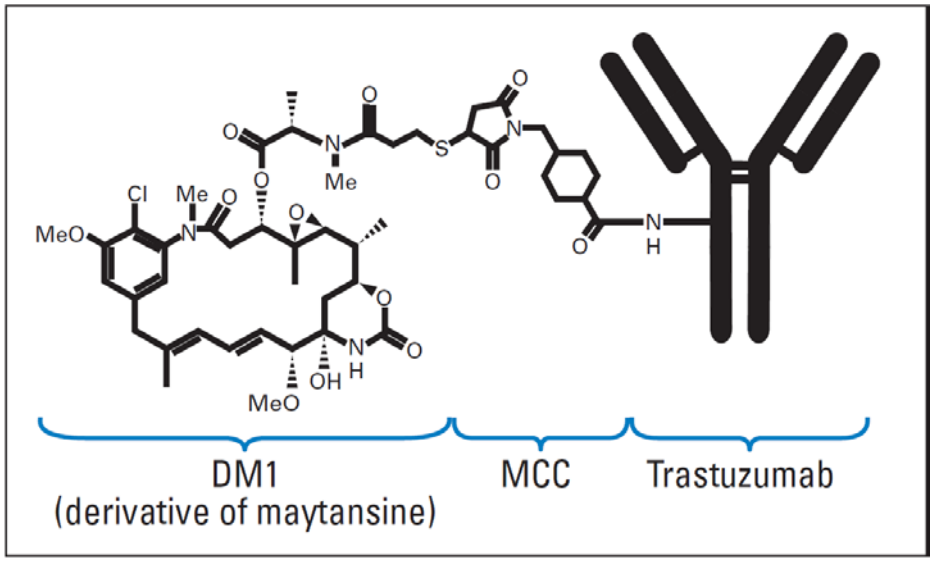
Antibody-Drug-Conjugates(ADC)



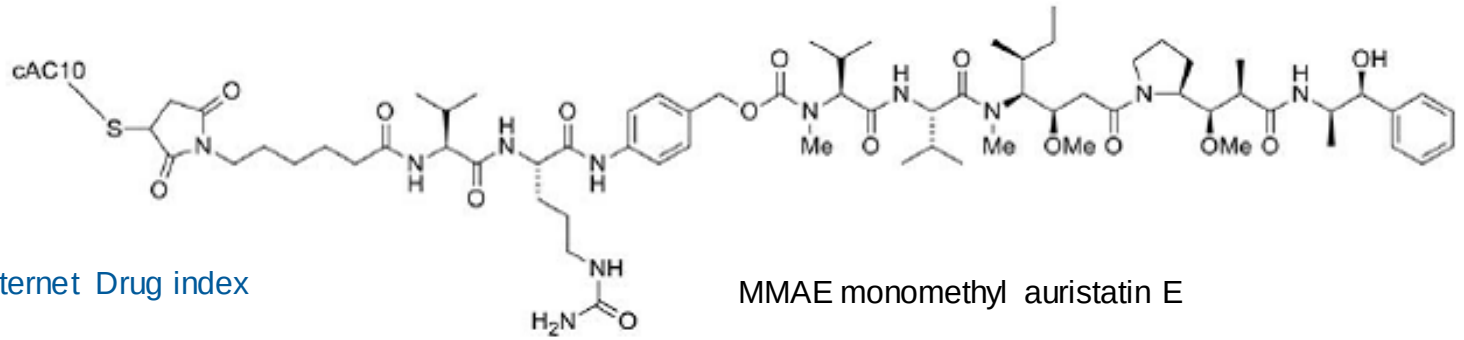
antibody | Attachment group | Protease-cleavable linker | Cytotoxic drug

- antibody-drug conjugate (ADC) composed monoclonal antibody covalently linked, via an enzyme-cleavable linker, to the cytotoxic drug

Antibody-Drug-Conjugates



Source: Krop et al., 2010, Journal of Clinical Oncology



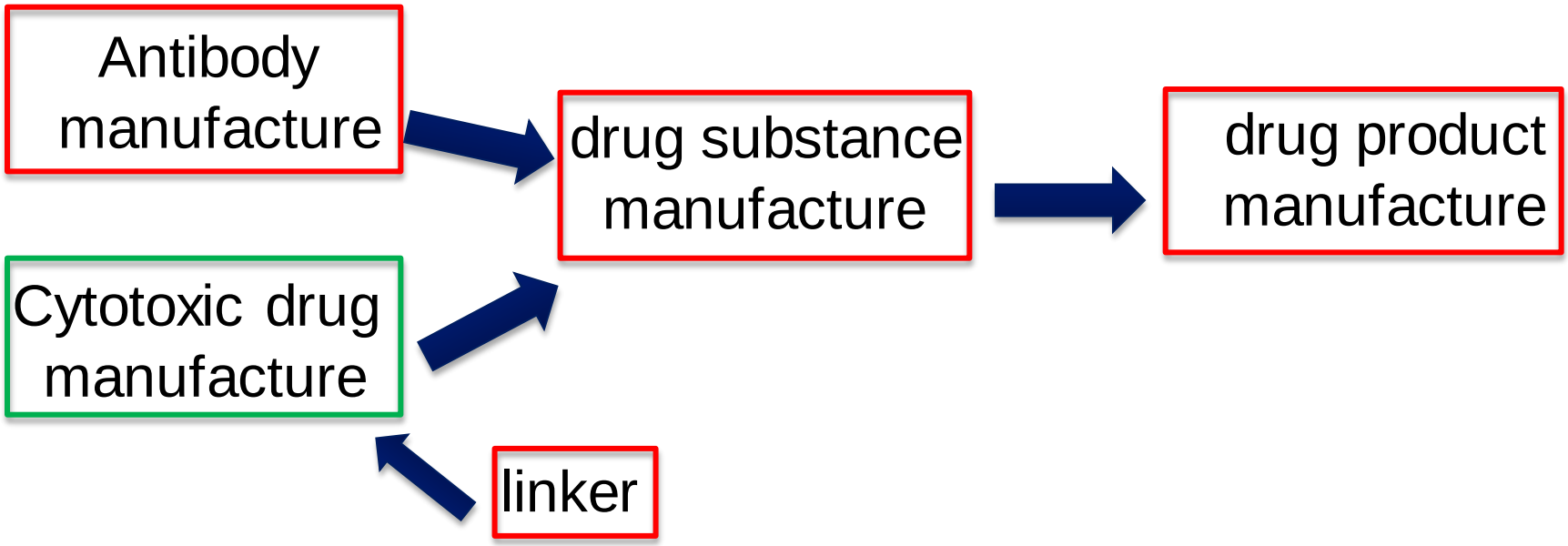
Source: Internet Drug index

MMAE monomethyl auristatin E

Manufacture

4 major manufacturing steps performed at differentmanufacturing sites

1. Manufacture of antibody (Intermediate)
2. Manufacture of drug-linker (Intermediate)
3. Manufacture of antibody-drug conjugate (Bulk Drug Substance (BDS))
4. Manufacture of drug product (DP)



Definition starting material

An “API Starting Material” is a raw material, intermediate, or an API that is used in the production of an API and that is incorporated as a significant structural fragment into the structure of the API. A Starting Material can be an article of commerce, a material purchased from one or more suppliers under contract or commercial agreement, or produced in house.
Starting Materials normally have defined chemical properties and structure. For synthetic processes, this is known as the point at which "API Starting Materials" are entered into the process. It should be noted that from this point on, appropriate GMP as defined in ICH Q7 “Good Manufacturing Practice for Active Pharmaceutical Ingredients” should be applied to these intermediate and/or API manufacturing steps. This would include the validation of critical process steps determined to impact the quality of the API. The description of the process should include all the steps of the process, proceeding from the starting material to the isolated intermediates and ultimately to the final active substance.

Starting material ?

current industry practices for starting material

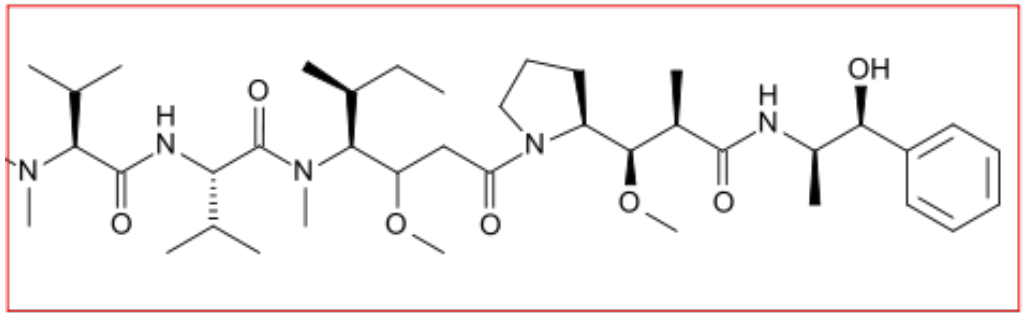
(1) designation for semi-synthetic drug substances,

(2) the process knowledge,

(3) product understanding.

Starting Material-MMAE

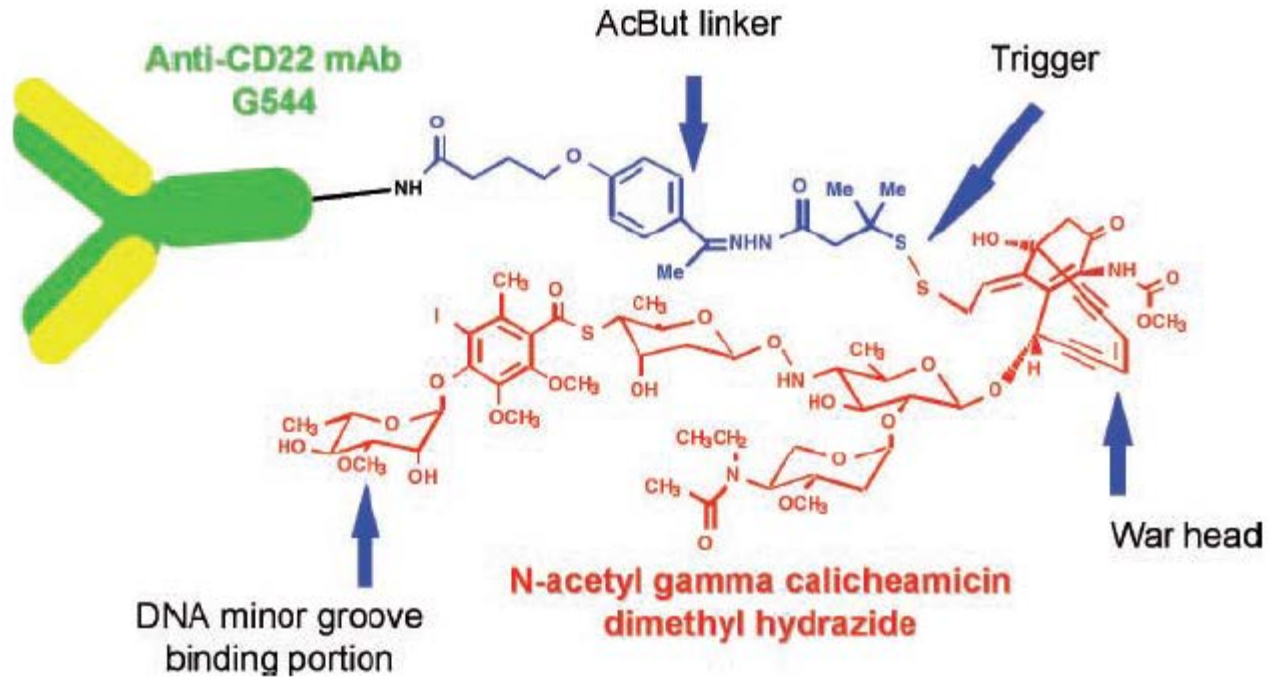
MMAE monomethyl auristatin E



MMAE

convergent, solution phase, fragment-based peptide synthesis.

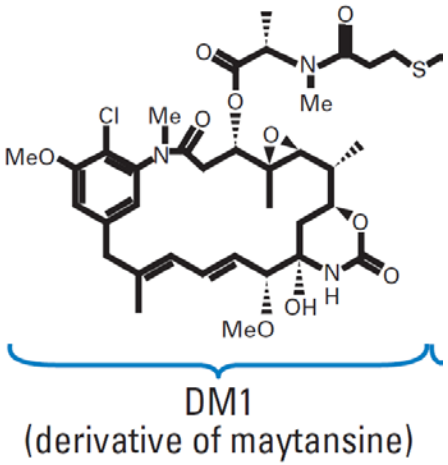
Inotuzumab ozogamicin



Source: DiJoseph et al., 2008, Hematology Meeting Reports 5 (6)

N-acetyl gamma calicheamicin DMH AcBut OSu is obtained by chemical modification of a calicheamicin produced by fermentation

DM1/DM4



• DM1 is synthesized from Maytansinol (MayOH). MayOH is obtained by cleaving the C-3 ester of ansamitocins which are isolated and purified from a fermentation process

• variable product (ansamitocin mixture).

• A consistent purity profile of DM1 is achieved by defined and controlled fermentation process.

•Defining the bacteria working seed lot system/fermentation as starting material

Documentation to be provided

- detailed description of the conversion of MayOH to DM1
- Information on the process as well as the in-process controls and/or in-process tests in place.
- Information on all reagents and catalyst used in the MayOH conversion to DM1

Characterisation/Specifications

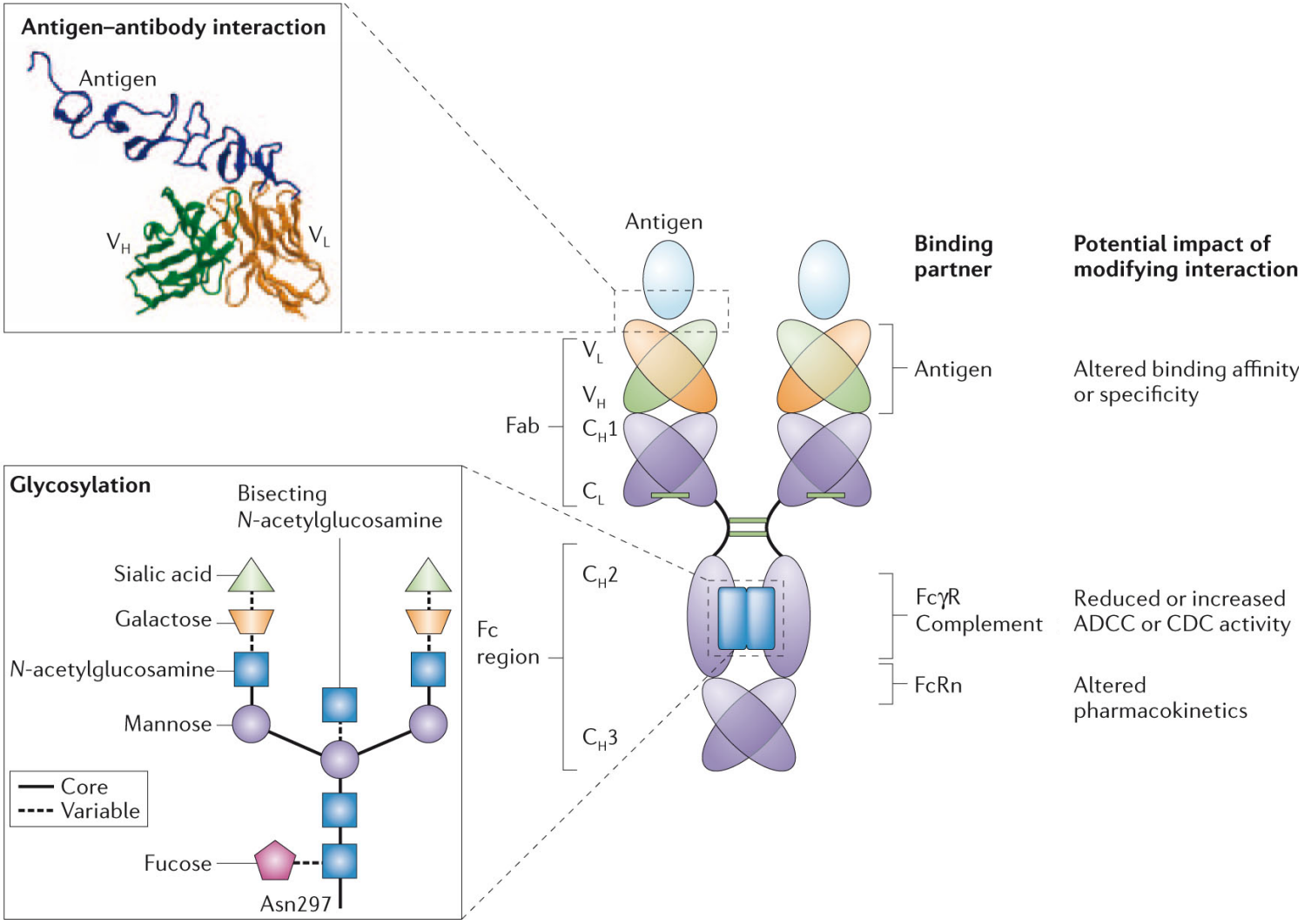
Antibody Specifications
These specifications have to be established based on principles outlined in ICH Q6B.

Maytansinol
• MayOH is a well-characterized, stable compound
• MayOH is a well-defined single compound that is produced and tested for conformance against an appropriate specification using qualified reference standards.
• MayOH has a well-defined and consistent impurity profile

MMAE monomethyl auristatin E
Specifications have been established based on principles outlined in ICH Q6A *Specifications: Test Procedures and Acceptance Criteria for New Drug Substances and New Drug Products: Chemical Substances Specifications.*

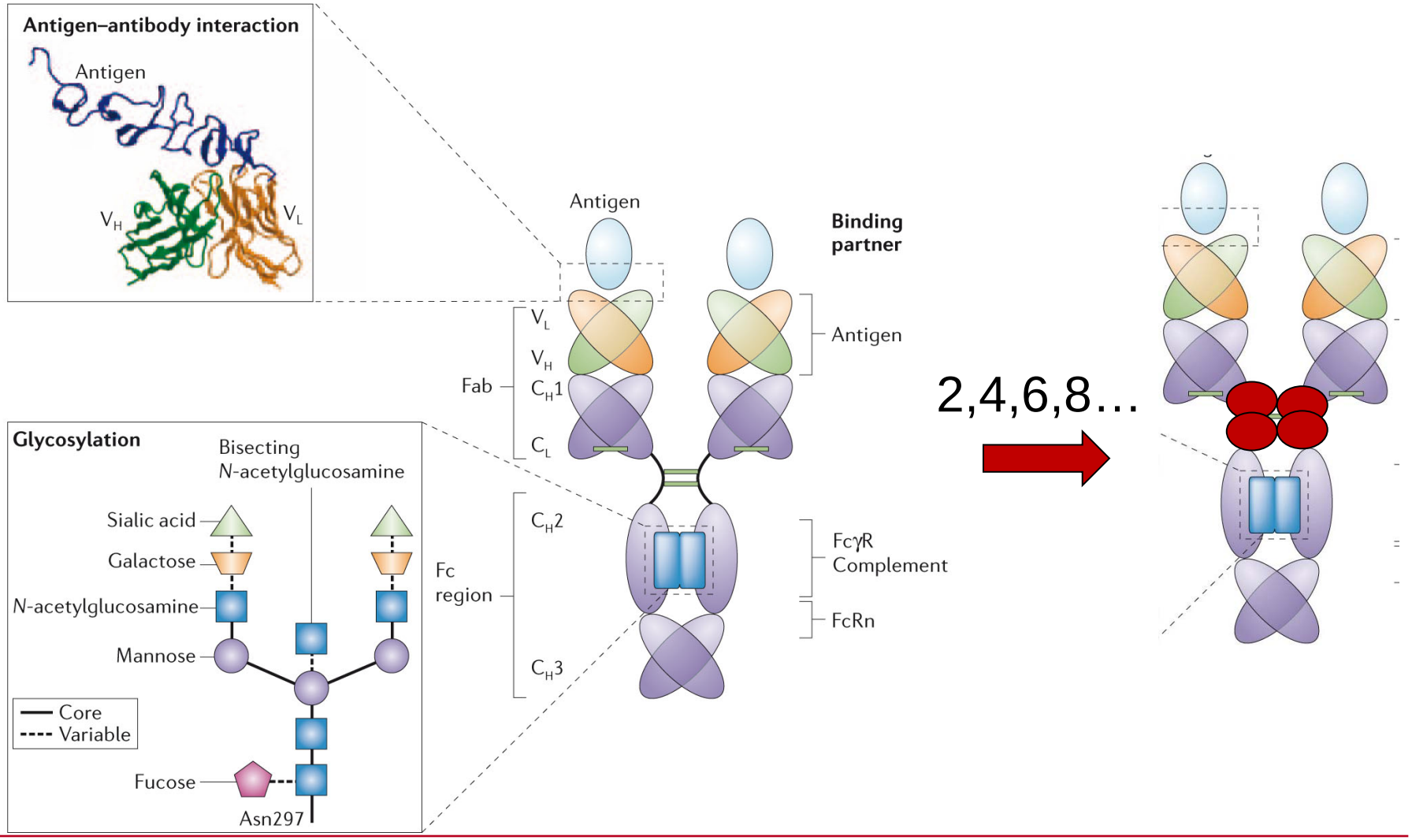
The specifications set for linker and cytotoxic drug should include the recommend acceptable amounts for residual solvents guidance given in the ICH Q3C “Impurities: Guideline for residual solvents” should be followed.

Increased complexity by posttranslational modifications



Adapted from Carter PJ: Potent antibody therapeutics by design, Nature Rev Immunology 6, 343 (2006)

Drug-load variants and isomers



Challenges Drug substance/product

Characterization

1. Drug load variants to be confirmed by the cytotoxicity assay.
2. Contribution of product-related impurities
3. conjugatable and non-conjugatable impurities

Specification

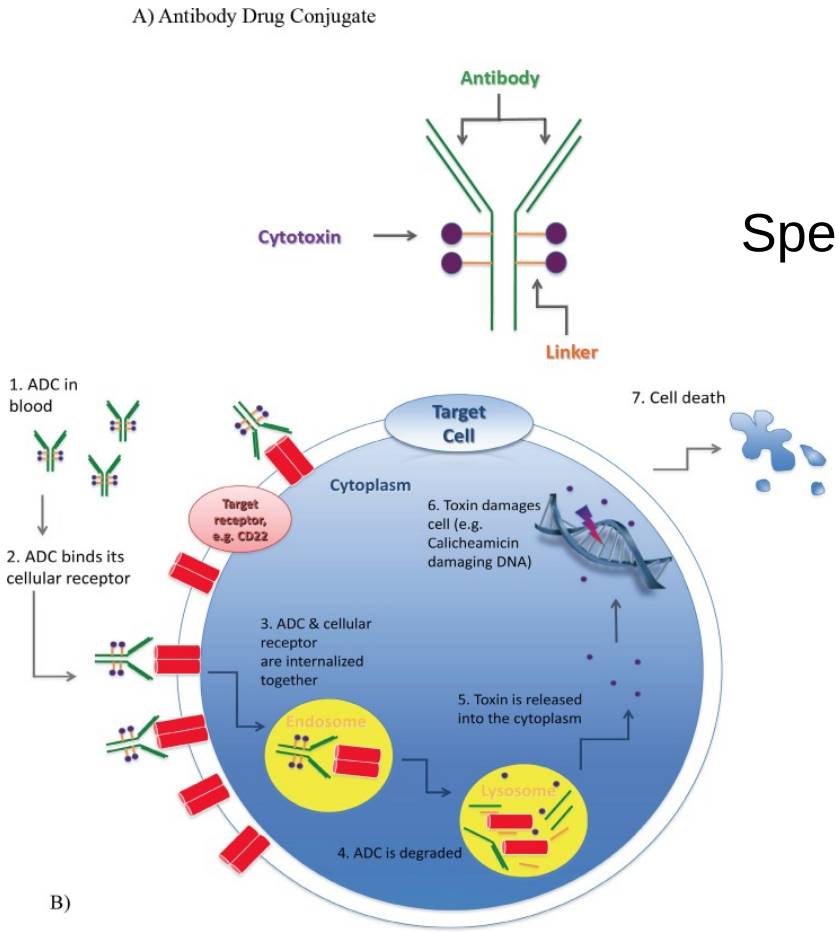
1. Limits for impurities justified in relation to the nature of and amounts of impurities
2. Binding
3. Cytotoxicity assays
4. Batch data

Container closure

1. container and closure are suitable for use with the formulated BDS.
2. Investigation of extractables and leachables

Stability/storage conditions

Non-clinical challenges



Specificity of Ab for target

- conjugation with the toxin does not impair antigen binding
- immunological effector functions of Ab
- Additional MoA?

Source: Oncotarget 2013; 4: 397-412

Objectives of the Pharmacology studies (trastuzumab-emtansine)

- determine the binding characteristics and the kinetics of trastuzumab emtansine
- provide nonclinical proof of activity in trastuzumab-sensitive and trastuzumab-insensitive breast cancer cell lines
- study the compound anti-tumour activity in multiple in vivo mouse tumour models
- establish dose-response relationships in the above mentioned models
- determine the multiple modes of action for trastuzumab emtansine.

Selectivity of ADC for target

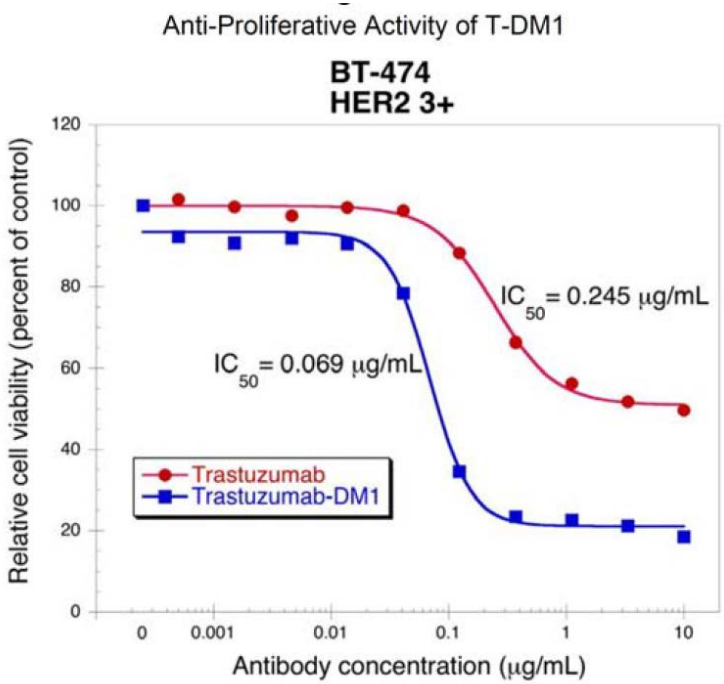
Table 2: Select targets of antibody-drug conjugates (ADC)

Target	ADC	Target Receptor Expression		Normal Function of Target Receptor	Reference
		Normal tissue	Malignant tissue		
CD19	Combotox	B cells, follicular DCs	ALL, CLL, NHL	B cell activation & signaling	[11]
CD22	Combotox, CMC-544, HA22	Normal Pre-B & resting B cells	ALL, NHL, HCL, CLL	Regulates B cell survival & function	[12]
CD25	Denileukin Difitox	Activated T cells, B cells, & monocytes	CLL, ATL, CTCL, HCL, T-ALL	IL-2 receptor α chain-cell activation	[16]
CD30	SGN-35	Activated T, B, and NK cells, monocytes	HL, ALCL, lymphomas, embryonal carcinoma	Enhances B and T cell proliferation	[26]
CD33	Gemtuzumab ozogamicin	Myeloid progenitor cells, basophils, macrophages, DCs, monocytes	AML	Binds sialoconjugates. Regulates innate immunity & inflammation	[10]
Her2	T-DM1	Wide distribution (not hematopoietic cells)	Breast cancer, gastric cancer etc.	Assists in the activation of other EGFR proteins	[13]

CD: cluster of differentiation, ADC: Antibody-drug conjugates, DC: Dendritic cell, ALL: Acute lymphoblastic leukemia, CLL: Chronic lymphoblastic leukemia, NHL: Non-Hodgkin lymphoma, HCL: Hairy cell leukemia, ATL: Adult T cell leukemia, CTCL: Cutaneous T cell lymphoma, T-ALL: T cell acute lymphocytic leukemia, NK: Natural killer, HL: Hodgkin's lymphoma, ALCL: Anaplastic large-cell lymphoma, AML: Acute myeloid leukemia, Her2: Human Epidermal Growth Factor Receptor 2

Additional MoA-Induction of apoptosis

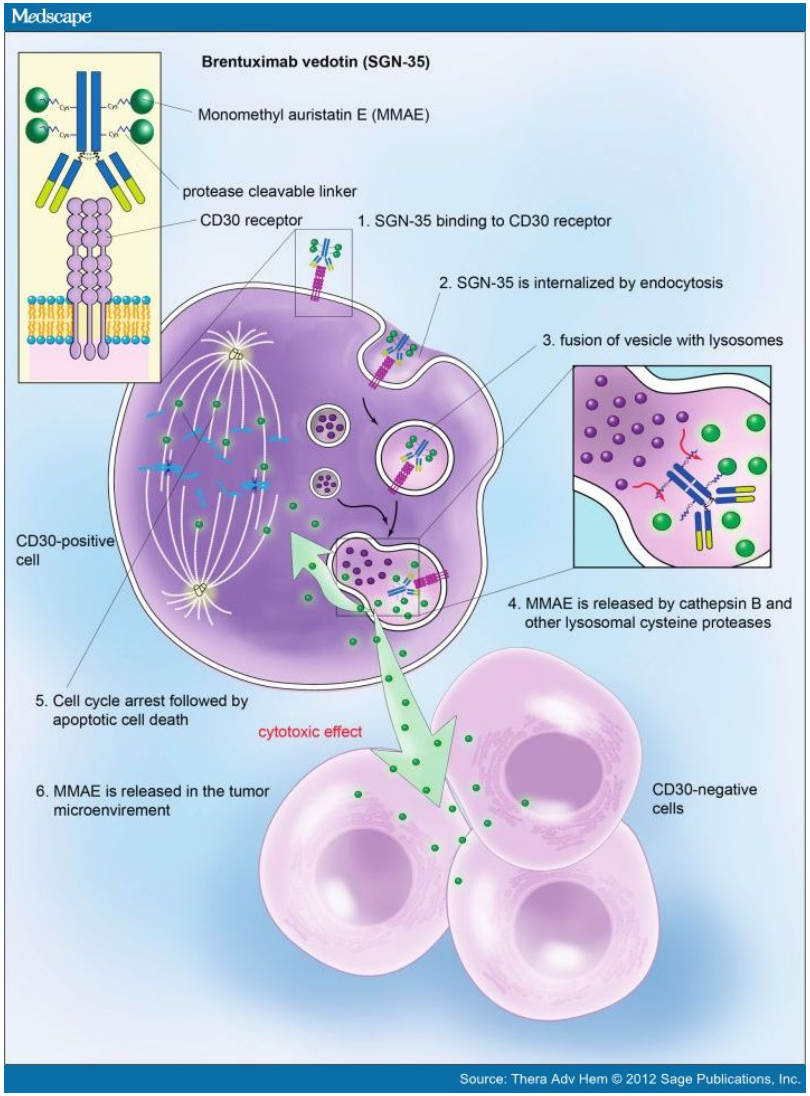
Activation of Caspases 3 and 7



Cell Line	HER2 Expression	IC_{50} TDM1 µg/mL	IC_{50} Trastuzumab µg/mL	IC_{50} TDM1 nM	IC_{50} Trastuzumab nM
BT-474	3+	0.069	0.245	0.46	1.68
SK-BR-3	3+	0.006	0.059	0.04	0.41
KPL-4	3+	0.009	>10	0.06	>70
HCC1954	3+	0.043	>10	0.29	>70
BT-474EE1	2+	0.018	>10	0.12	>70
MCF7	0	>10	>10	>70	>70
MDA-MB-468*	0	2	>10	13.44	>70

httpwww.accessdata.fda.govdrugsatfda_docsnda2013125427Orig1s000PharmR.pdf

Other Non-clinical challenges



Concern free toxin:

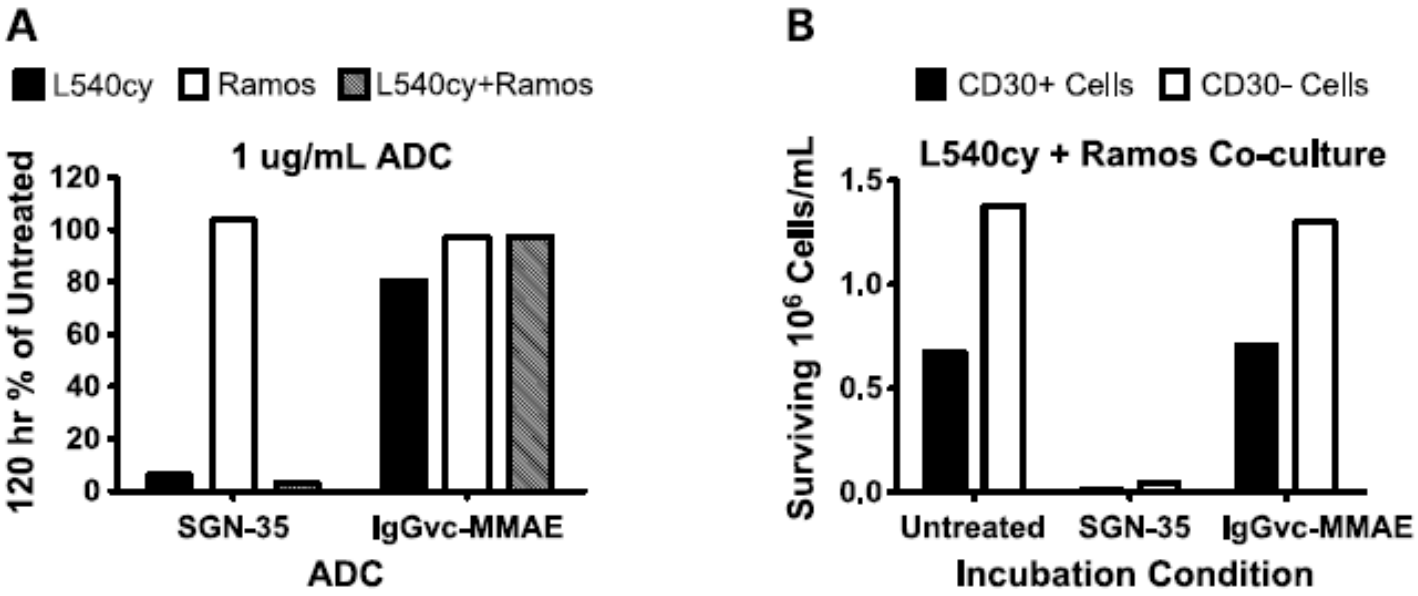
- the toxin is released from the antibody by lysosomal proteases, can the linker can be cleaved by other proteases than the lysosomal proteases?
- Upon endocytosis, antibodies are recycled via binding to neonatal Fc receptor (FcRn) in acidified endosomes.This prevents trafficking to lysosomes and mAb degradation. Can the toxin be released from the antibody during normal recycling? This would lead to release of toxin independent of the target !
- concentration of free cytotoxic drug in circulation
- Cytotoxic Activity of Metabolites

HNSTD: highest non severely toxic dose vs. NOAEL: no observed adverse effect level;

predicted environmental concentration (PEC), assessment of ecotoxicity/environmental risk

Source: http://www.medscape.com/viewarticle/768262_5

Bystander effects
SGN-35



Co-cultures of L540cy and Ramos cells showed that treatment with 1 µg/mL SGN-35 eliminated both populations of cells equally well, whereas a similarly treated mixed cell population with a nonbinding control ADC were unaffected

fromNicole M. Okeley, Jamie B. Miyamoto, Xinqun Zhang, et al., *Clin Cancer Res* 2010; 16:888-897

THANK YOU VERY MUCH!