Antibodies as efficient drug delivery systems

Lecture 5
Outline:

- What is Antibody?
- What is antibody drug conjugates?
- Why they are important?
- How they are used in treatment of cancer?
- What are the important medicinal chemistry considerations for their design and preparation?
- Future directions of the field
What is Antibody?

Antibody (Ab), also known as an immunoglobulin (Ig), is a large Y-shaped protein produced by B-cells that is used by the immune system to identify and neutralize foreign objects such as bacteria and viruses. The antibody recognizes a unique part of the foreign target, called an antigen. Each tip of the "Y" of an antibody contains a paratope (a structure analogous to a lock) that is specific for one particular epitope.

https://www.youtube.com/watch?v=k70mSiHOsgc&feature=player_detailpage

Different classes: https://www.youtube.com/watch?feature=player_detailpage&v=jPqb1_pE41g
What is antibody?

• Large Y-shaped protein naturally produced by immune cells of our body

• Used by the immune system to identify and neutralize foreign objects such as bacteria and viruses

• Recognizes a unique part of the foreign target and binds to it with very high specificity

Copyright: clipartfest.com; wikipedia.org/wiki/Antibody
Monoclonal antibodies (mAbs)

- Dynamic and rapidly expanding class of biotherapeutics capable of targeting multiple human diseases including:
  - cardiovascular
  - respiratory
  - autoimmune
  - oncology disorders

- Several mAbs have achieved extraordinary success as so-called blockbusters

- This success led to the recent emergence of a class of specialist cancer biotherapeutics termed Antibody Drug Conjugates (ADCs).
What is Antibody Drug Conjugates (ADC)?

- Combines excellent specificity of mAb with potency of cytotoxic drugs
  - delivers cytotoxic drug specifically to tumor
  - reduces normal tissue drug exposure + increased concentration in tumor (up 1000 fold)

Works much better than Ab or drug alone!
Antibody Drug Conjugates (ADC)

- Emerging class of targeted cancer biotherapeutics
  - Brentuximab and Trastuzimab ADC
  - 95% percent of patient respond to Brentuximab
  - Nearly half go in complete remission
  - 1B sales in 2015

- Most big pharma and biotech companies have ongoing ADC programs
  - Over 100 molecules are currently in the pipeline

- 10 new drugs projected in the coming years
  - 10B by 2025
Part II: Antibody Drug conjugates for delivery of Therapeutics

- Over 20 antibody-drug conjugates in clinical trials as well as a recently FDA-approved drugs

- Why they are successful?
  - Used for selective delivery of highly cytotoxic agents to tumor cells while sparing normal tissue.

https://www.youtube.com/watch?feature=player_detailpage&v=4NH2ldNPero
How ADCs work?

1. **Receptor binding**
2. **Cellular internalization**
3. **Drug release**
4. **Cell Death**
Anatomy of an Antibody-Drug Conjugate (ADC)

Antibody targeted to tumor
- Humanized monoclonal Ab (IgG1)
- mAb with Fc modifications (modulate ADCC, CDC activity)
- Other mAb fragments

Very potent chemotherapeutic drug
- Tubulin polymerization inhibitors
  - Maytansines (DM1, DM4)
  - Auristatins (MMAE, MMAF)
- DNA damaging agents
  - Calicheamicins
  - Duocarmycins
  - Anthracyclines (doxorubicin)

Linker stable in circulation
- Linker biochemistry
  - Acid labile (hydrazone)
  - Enzyme dipeptides (cleavable)
  - Thioether (uncleavable)
  - Hindered disulfide (uncleavable)
- Site of conjugation
  - Fc, HC, LC
What is Antibody Drug Conjugate?

Elements of an Antibody-Drug Conjugate (ADC)

**Antibody**
Specific for a tumor-associated antigen that has restricted expression on normal cells.\(^1\,^2\)

**Cytotoxic agent**
Designed to kill target cells when internalized and released.\(^1\,^2\)

**Linker**
Attaches the cytotoxic agent to the antibody. Newer linker systems are designed to be stable in circulation and release the cytotoxic agent inside targeted cells.\(^1\,^3\)

References:
Improving the Therapeutic Window

- ADCs can selectively deliver a potent cytotoxic drug to tumor cells via tumor-specific and/or over-expressed antigens
  - Increase drug delivery to tumor
  - Reduce normal tissue drug exposure

Chemotherapy

ADC

TOXIC DOSE (MTD)

Therapeutic Window

EFFICACIOUS DOSE (MED)

MTD: Maximum tolerated dose; MED: Minimum Efficacious Dose
ADC More Efficacious than Free Cytotoxin in Mice

MMTV-HER2 Fo5 mammary tumor (HER2-positive, trastuzumab-insensitive)

Parsons et al, AACR (2007); Modified from S. Spencer
Drug Toleration test: blood cell count

• Neutrophils are a specific kind of white blood cell that help prevent and fight infections.

• A low white blood cell count or “neutropenia” is a condition characterized by abnormally low levels of neutrophils in the circulating blood.

• Chemotherapy-induced neutropenia increases a patient’s risk of infection and disrupts cancer treatment.

• Fortunately, neutropenia can be prevented through the use of white blood cell growth factors.

• The prevention of neutropenia allows patients to receive their scheduled treatment and reduces the risk of infection and hospitalization.

https://www.youtube.com/watch?feature=player_detailpage&v=0TvTyj5FAaQ

From: http://www.texasoncology.com/
ADC Better Tolerated than Free Cytotoxin in Monkeys

- No neutrophil decreases when cytotoxic drug delivered linked to an antibody
- ~2-3 times more cytotoxic drug can be given as an ADC

A. Kim, D. Danilenko, N. Dybdal, K. Flagella, K. Achilles-Poon
Modes of Anti-tumor Activity of ADCs

Tumor cytotoxicity is target-directed
ADC-Ag binding → internalization in lysosomes → ADC degradation → release of toxin intracellularly → tumor cell death

Tumor cytotoxicity is target-enhanced (bystander effect)
ADC-Ag binding → extracellular cleavage of toxin → release of toxin in local tumor environment → diffusion of toxin intracellularly to neighboring tumor cells → tumor cell death
Careful selection of target antigens are an important criterion for both the safety and efficacy of an ADC

• The ‘ideal’ tissue antigen should have:
  – High level of target expression in cancer cells
  – Little to no expression in normal cells
  – Expressed on the cell surface
  – Readily internalized
  – No shedding into the blood by cleavage of the antigen from cancer cell surface

• The number of antigen molecules and antibody binding affinity for the antigen may affect the potency of the ADC
Modes of Toxicity of ADCs

Systemic release of toxin
- Instability of linker
- Catabolism of ADC

Unwanted ADC-mediated cytotoxicity
- Targeted binding to normal tissues expressing antigen
- Off-target (cross reactive) binding to normal tissues
- Non-antigen-mediated ADC uptake (e.g., Fc-mediated uptake, pinocytosis)
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More Stable Linker Reduces Systemic Toxicity of ADC in Rats

Single IV dose given on Day 1:

- **Vehicle**
- **Anti-CD22-MCC-DM1**
- **Anti-CD22-MC-MMAF**
- **Anti-CD22-SPP-DM1**
- **Anti-CD22-MC-vc-PAB-MMAE**

**Change in bodyweight (grams)**

**Days post dose**

Polson, et al., Cancer Res., 69(6), 2009
Why linker is important?
What are the important requirements?

• The exact connection between the cytotoxic agent and the antibody has profound effects on the selectivity, pharmacokinetics, therapeutic index, and overall success of the ADC.

• The linker must be stable in systemic circulation to allow delivery of the intact ADC to the targeted antigen presented on the surface of the tumor cell.

• The linker must then be labile enough to allow efficient release of the cytotoxic drug inside the targeted tumor cell.
Part II: Antibody Drug conjugates for delivery of Therapeutics

- What are the critical aspects of this approach?

- properties of the linker between the antibody and the cytotoxic payload are
  (i) the specifics of attachment to the antibody,
  (ii) the polarity of the linker,
  (iii) the trigger on the linker that initiates cleavage from the drug,
  (iv) the self-immolative spacer that liberates the active payload.
- The N-hydroxysuccinimide ester enables formation of a stable amide bond with the primary amine of one of about 84 available lysine residues on the antibody at pH 7–9.

The maleimide moiety undergoes a Michael addition with sulfhydryl groups at pH 6.5–7.5 to form stable thioether bonds that are used to attach the drug to the antibody.
Modification of Antibodies

N-terminal transamination

Francis MB et al., Chem Biol, 2007, 2, 247-251
**Problem:** The process results in the formation of a mixture of conjugates with drug–antibody ratios (DARs) ranging from 0 to 9.
Important for Clinical Translation!

• **NO** random attachment of drug molecules (easy to characterize)
• **NO** interference with antigen binding site (antibody stays active, able to bind its antigen)
• **NO** mixture of compounds (unlabeled antibodies, antibodies with various numbers of drugs attached)
• **NO** unpredicted toxicity problems
Site-specific modifications of monoclonal antibodies

Laboratory of Bioorganic Chemistry and Molecular Imaging
Trastuzumab (trade names Herclon, Herceptin) is a monoclonal antibody that interferes with the HER2/neu receptor. Its main use is to treat certain breast cancers.

https://www.youtube.com/watch?v=66z6BmeA00I&feature=player_detailpage
Examples: Conjugation of emtansine to trastuzumab

Succinimidyl-4-((N-maleimidomethyl)cyclohexane-1-carboxylate (SMCC) is added to the antibody followed by the addition of emtansine. The process results in the formation of a mixture of conjugates with an average drug antibody ratio = 3.5.

Releasable linker or not?
Polarity of the linker

How can we increase the polarity?

Selectivity

Cytotoxicity

Connection to antibody

Polarity

Trigger

Self-immolative spacer

Drug

(a) (b) (c) (d)

(Polarity)

(PEG)

(Sulfate)

(3)

(4)
Trigger initiates the cleavage of the drug

There are three major trigger release mechanisms:

1. hydrazone hydrolysis (5),
2. disulfide reduction (6),
3. dipeptide linker cleavable by lysosomal proteases (7).
**Hydrazone linkers**

The acid-labile hydrazone linker (5) relies on the increased acidic environment of the endosomes (pH 5.5–6.2) and lysosomes (pH 4.5–5.0) relative to systemic circulation (pH 7.4–7.5) to release the active drug.

78 lysine residues available for conjugation to this antibody.
Hydrazone linkers

Inotuzumab ozogamicin (CMC-544) is currently in Phase III clinical testing for B-cell non-Hodgkin’s lymphoma

(Antibody is for CD22 is a sugar binding transmembrane protein, which specifically binds sialic acid with an immunoglobulin (Ig) domain located at its N-terminus.
Another example: Hydrazone linkers

Doxorubucin antibody conjugates:
There are three major trigger release mechanisms:

1. hydrazone hydrolysis (5),
2. disulfide reduction (6),
3. dipeptide linker cleavable by lysosomal proteases (7).
Example of maleimide/protease chemistry: Adcetris

Brentuximab vedotin (Adcetris) (17) utilizes a direct connection to the sulfhydryl group of a cysteine residue on the antibody liberated after a mild reduction of the interchain disulfide bonds.
Example of: Adcetris

Anti-CD30 mAb attached to the potent antimitotic agent monomethylauristatin E (MMAE). A maleimide (2) is used to selectively add to one of the available eight sulfhydryl groups.

Result: statistical mixture of between zero and eight drugs per antibody, with a DAR of 4 being the most prevalent
Another example of protease linker

- Pyrrolobenzodiazepines (PBDs) are a class of naturally occurring antitumor antibiotics that bind in a sequence specific manner in the minor groove of DNA and have picomolar activity against many human tumor cell lines.

- PBD dimer has been connected to an antibody via a protease-cleavable val.-ala. linker connected to an aniline on the drug.
Trigger initiates the cleavage of the drug

There are three major trigger release mechanisms:

1. hydrazone hydrolysis (5),
2. disulfide reduction (6),
3. dipeptide linker cleavable by lysosomal proteases (7).
Disulfide reduction

Trastuzumab – DM1 conjugates
The self-immolative portion that liberates the drug

Selectivity

Cytotoxicity

Para-aminobenzyl "PAB"

\[ \text{Drug} \]

\[ X = O, S, NH \]
\[ Y = \text{CH}_2, \text{NR}, \text{O} \]
Novel Chemistries might enable new applications

Site-specific modifications of monoclonal antibodies

Laboratory of Bioorganic Chemistry and Molecular Imaging
**Modes of Toxicity of ADCs**

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**Side effects of cetuximab Ab**

**Cetuximab** - is an epidermal growth factor receptor (EGFR) inhibitor used for the treatment of metastatic colorectal cancer and head and neck cancer.

before

after
The Current Challenge

Traditional monoclonal antibodies (mAbs) bind to targets in diseased tissue
The Solution: CytomX Probody™ Platform

Current Monoclonal Antibody
How a Probody Works

Disease-associated protease cleaves substrate and removes mask
How a Probody Works: Selective Binding

Probody only binds to target on diseased tissue
Current Challenges in the field of ADC

Non-specific binding of drugs
Issues with Ab internalization and Drug Release

Receptor binding
Problem: lack of assays for selection of good antibodies

- Current assays are non-reliable and not quantifiable
- No information on internalization efficiently
- No information on delivery of toxin into cytosole
- Not translatable into animal models of cancer
Future directions:

- Continue to produce highly selective and potent ADCs that will target specific tumor antigens.

- To have treatments for a broad group, the antigen expression on individual patients will need to be characterized to personalize the delivery.

- Further advances in linker technology will generate ADCs with improved pharmacokinetic and efficacy/toxicity profiles.

- Understanding the factors that led to this clinical success will help define the next generation of ADCs that will allow the treatment of a wide range of cancers.

- Development of novel chemistries that would allow efficient conjugation of drugs and imaging reagents.
Novel bioluminescence reporter assay

• Real-time kinetic data on uptake and processing

• Cost effective, convenient and easily scalable

• Translation potential – best candidates can be directly validated in animal models of cancer

• Application for high throughput screening of de novo mAb

• Can be expanded to other therapeutic molecules such as RNA, peptides, viruses and nanoparticles
Summary

• An ADC is both a “large molecule” and a “small molecule”.

• **ADCs hold great promise for improving current oncology therapies.**
  
  – Highly potent cytotoxic agents are delivered directly to cancer cells, sparing normal tissues.
  
  – ADCs tend to be better tolerated than standard chemotherapy.
  
  – Increased therapeutic window allows for better balance between safety/efficacy.

• **There is a fine balance between efficacy and toxicity.**
  
  – Choice of linker, cytotoxic drug and mAb are all important determinants of safety, PK, and efficacy.
  
  – Toxicity is usually antigen-independent, ADC/drug-dependent.
  
  – Linker stability, DAR, and site of drug conjugation impacts toxicity.
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Materials used:

   Special Issue-Review
   Cancer Treatment and Personalized Medicine

   Antibody-Drug Conjugates for the Treatment of Cancer

2. NorCal Society of Toxicology Meeting September 27, 2012