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
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, MMADF)
- 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

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
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

- TOXIC DOSE (MTD)
- EFFICACIOUS DOSE (MED)

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)

- **Vehicle**
- **Trastuzumab DM1** 15 mg/kg, 817 µg/m²
- **Trastuzumab 15 mg/kg**
- **Trastuzumab 15 mg/kg + Free DM1 817 µg/m²**
- **Free DM1 817 µg/m²**
- **Free DM1 (near MTD) 1947 µg/m²**

Free DM1 (cytotoxin)

T-DM1 (ADC)

IV Dosing

Day

Parsons et al, AACR (2007); Modified from S. Spencer
**Drug Tolerance 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/
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)

Normal 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.
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
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

*Primary Cause of Toxicity*

Traditional monoclonal antibodies (mAbs) bind to targets in diseased tissue.

Traditional mAbs also bind to target in healthy tissue.
The Solution: CytomX Probody™ Platform

Current Monoclonal Antibody

CytomX Probody: Masked antibody activatable only in diseased tissue
How a Probody Works

Disease-associated protease cleaves substrate and removes mask

Mask blocks binding to target on healthy tissue
How a Probody Works: Selective Binding

Probody only binds to target on diseased tissue

Optimally Targeted Medicines

Probody Mask prevents binding to healthy tissue

Copyright: http://www.cytomx.com
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.
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