Antibody-Drug Conjugates
Design, Development, and FDA Approval of a New Drug Class

Elaine Tsui
Knowles Group
Department of Chemistry, Princeton University
July 9, 2021
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Ehrlich's Magic Bullet

Paul Ehrlich
1908 Nobel Prize in
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Ehrlich's Magic Bullet

"Now, an essential task of the new Institute will be to find substances and chemical groups that have a special relationship to certain organs. It will be of particular importance, however, to equip such substances, acting as trucks so to speak, with chemical groups possessing pharmacological or toxicological effects, so that at the same time they convey the potent load commissioned to them to the appropriate places."

Paul Ehrlich
1908 Nobel Prize in Physiology or Medicine

Evolution of Cancer Therapies

Development of Cancer Therapies

Nitrogen Mustards

\[
\text{Me} \quad \text{Cl} \\
\text{Cl} \quad \text{N} \quad \text{Cl}
\]

chlormethine

for DNA alkylation

Evolution of Cancer Therapies

Development of Cancer Therapies

Nitrogen Mustards \rightarrow Anti-Folates

[Chemical structures of chlormethine and methotrexate]

*Chlormethine* for DNA alkylation

*Methotrexate* for blocking tumor growth

Evolution of Cancer Therapies

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Nitrogen Mustards → Anti-Folates → Vinca Alkaloids

- Chlormethine
  - for DNA alkylation
- Methotrexate
  - for blocking tumor growth
- Vincristine
  - for inhibiting tubulin polymerization

Cancer Therapies

Development of Cancer Therapies

Chemotherapy

cytotoxic agents: cisplatin, nucleoside analogues, cyclophosphamide, Taxol, anthracyclines

lack of selectivity, toxicity to normal cells, limited efficacy

99% of tumor cells have to be killed to achieve remission

## Cancer Therapies

### Development of Cancer Therapies

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Immunotherapy

stimulating immune system to fight cancer: ofatumumab, sipuleucel-T, ipilimumab, pembrolizumab, cemiplimab

generated significant attention but still in early stages of research and development

Cancer Therapies

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Outline

I. What is an ADC?

II. History of the Development of ADCs

III. FDA-Approved ADCs

IV. Outlook and Conclusion
What is an ADC?

Antibody-Drug Conjugate (ADC)

- cytotoxic agent + monoclonal antibody

1) Binds selectively to cancer cell antigen
2) Internalizes through endocytosis
3) Releases payload/warhead/drug
4) Kills cancer cell

What is an ADC?

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ADC Optimization

Antigen Selection

- highly and selectively expressed on the surface of tumor cells with minimal expression in normal cells
- internalizing antigen that can transport drug into cell

ADC Optimization

Monoclonal Antibody (mAb)

- an antibody that targets a specific antigen
- relies on hybridoma technology developed by Kohler and Milstein (1975) for mass production
- considered a key breakthrough
- mAbs themselves do not need to exhibit functional activity in an ADC

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ADC Optimization

Monoclonal Antibody (mAb)

ADC Optimization

Monoclonal Antibody (mAb)

- all ADCs use immunoglobulin G (IgG) antibodies
- different isotypes exist based on heavy chain amino acid sequences
- isotypes determine clearance rates, immune activation, number of disulfide bonds available for modification

ADC Optimization

Monoclonal Antibody (mAb)

- interspecies usage of antibodies provoke harmful immunogenic responses
- humanize antibodies by replacing non-human domains with protein sequences occurring naturally in humans
- advantage: eliminate immune response and longer circulation half-life

ADC Optimization

Cytotoxic Small Molecule

doxorubicin conjugate

8 times less potent

- picomolar potency required
- conjugated drug has decreased potency compared to free drug (e.g. why methotrexate and taxoids don't work)
- drug has to be stable and soluble in aqueous environment of antibody and has to avoid antibody aggregation. Can be easily modified to allow for conjugation

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ADC Optimization

Design and Optimization of Linkers

Requirements:
- stable for several days in circulation
- cleaved upon internalization to release drug
- location of linker should not interfere with function of antibody
- solubilize hydrophobic drug
- drug-to-antibody ratio must be optimized for potency without compromising safety

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ADC Optimization

Drug-to-Antibody Ratio (DAR) Considerations

increased potency, but protein aggregation, increased ADC clearance, toxicity

most commonly 2-4

balance between potency of drug and level of efficacy and toxicity

ADC Optimization

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heterogeneous DAR and drug attachment sites leads to unpredictable pharmacokinetic properties and lower efficacy

ADC Optimization

Drug-to-Antibody Ratio (DAR) Considerations

increased potency,
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decreasing DAR

most commonly 2-4

increasing DAR

balance between
potency of drug and
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heterogeneous DAR and drug attachment sites leads
to unpredictable pharmacokinetic properties and lower
efficacy

ideally, homogeneous ADCs

ADC Optimization

Bioconjugation Strategies

- Goal: to achieve site-selective protein modification and conjugation

often through hydrazone formation or addition to maleimide

Amino Acid Conjugation
natural or engineered residues

ADC Optimization

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Unnatural Amino Acid Incorporation

ADC Optimization

Bioconjugation Strategies

- Goal: to achieve site-selective protein modification and conjugation

Amino Acid Conjugation
- natural or engineered residues

Glycan Modification

Unnatural Amino Acid Incorporation

Peptide Tags
- enzymatic modification of amino acids

often through hydrazone formation or addition to maleimide

most commonly through transglutaminase

Development of ADCs

Early Experimental ADCs

- grew out of a need to improve tumor selectivity
- first ADC reported by Mathe (1958)

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*Speculative Conjugation Pathway*


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Early Experimental ADCs

Yang & Reisfeld (1988)

- first conjugation of intact doxorubicin

Speculative Conjugation Pathway

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Speculative Conjugation Pathway

![Chemical Diagram]

Early Experimental ADCs

Yang & Reisfeld (1988)

- first conjugation of intact doxorubicin

Speculative Conjugation Pathway

2 orders of magnitude higher potency than free drug against tumor cells; suppressed growth of existing tumors in vivo; prolonged life-span of tumor-bearing mice by 81%

Early Experimental ADCs

BMS (1993)
- first ADC to reach phase II

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Early Experimental ADCs

BMS (1993)

- first ADC to reach phase II

_regression and cures of human lung, breast, and colon carcinomas in mouse model; cured 70% mice bearing metastases of human lung carcinoma and 94% of rats with subcutaneous human lung carcinoma
_failed to gain FDA approval due to low efficacy in humans_

Introduction of Calicheamicin $\gamma_1$

discovered in 1987 from *Micromonospora chinospora calichensis*
first synthesis by Nicolaou in 1992
second synthesis by Danishefsky in 1994

component of first FDA approved ADC

## FDA-Approved ADCs

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Mylotarg: gemtuzumab ozogamicin

First-Generation ADC

Mylotarg: gemtuzumab ozogamicin

First-Generation ADC

\[
\text{AcOH (80%) acyl hydrazone formation}
\]

\[
i) \text{AcOH (80%) acyl hydrazone formation}
\]

\[
\text{ii) N-hydroxy succinimide, EDCI (80%) activated ester formation}
\]

Mylotarg: gemtuzumab ozogamicin

First-Generation ADC

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First-Generation ADC

buffer, pH 7.8

Mylotarg: gemtuzumab ozogamicin

First-Generation ADC

payload release via endosomal hydrazone cleavage/
disulfide exchange with glutathione

1,4-conjugate addition
Bergman cyclization

Besponsa: inotuzumab ozogamicin

Second-Generation ADC

- Mylotarg withdrew from market in 2010
- no improvement in survival and higher fatal toxicity rate
- two different internalization mechanisms and off-target effects
- highly heterogeneous mixture with 50% unconjugated antibody
- linker labile toward hydrolysis

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mAb targets CD22
higher average DAR = 6

Adcetris: brentuximab vedotin

cathepsin cleavable
valine-citrulline-PAB

monomethyl auristatin E

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blocks tubulin polymerization

Kadcyla: trastuzumab emtansine

mixture of ansamitocins

$\text{LiAlH}_2(\text{OMe})_3$

$\text{pH 12}$
Kadcyla: trastuzumab emtansine

mixture of ansamitocins

i) LiAlH(OMe)$_3$
ii) pH 12

i) EDCI, ZnCl$_2$
ii) DTT, buffer pH 7.5

Kadcyla: trastuzumab emtansine

i) LiAlH(OMe)$_3$

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mixture of ansamitocins

doubly-activated

i) EDCI, ZnCl$_2$

HS

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MeS$^{-}\text{S}^{-}\text{S}_{\text{Me}}$

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phosphate buffer pH 6

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Future Directions

New Drug Agents

potent small molecule agents with different mechanisms of action

DNA crosslinking with guanine
Future Directions

New Drug Agents
potent small molecule agents with different mechanisms of action

Homogeneous ADCs
homogeneous ADCs require improved site-selectivity and better conjugation methods

DNA crosslinking with guanine
Future Directions

New Drug Agents
potent small molecule agents with different mechanisms of action

Homogeneous ADCs
homogeneous ADCs require improved site-selectivity and better conjugation methods

Beyond Oncology
ADCs explored for the treatment of inflammatory disorders and as an antibiotic

dNA crosslinking with guanine

[Chemical structure of Zynlonta]
[Diagram of homogeneous ADCs]
[Diagram of targeted delivery of glucocorticoid]
Conclusion

A New Era in the Development of ADCs

• Despite struggling in the clinic for most of the last 20 years, ADCs are seeing a comeback. 6 ADCs approved in just the past three years (now 10 total on the market) and >60 in clinical trials
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