Today's Presentation

- What are Antibody Drug Conjugates (ADCs)?
- ADC technology
- ADME of ADCs
  - Typical PK profile
  - Efficacy and tumor distribution
  - Effect of drug loading on PK
  - Effect of drug-linker hydrophobicity on PK
What are Antibody Drug Conjugates (ADCs)?

- ADCs are novel molecular entities which leverage the specificity of an antibody to deliver a potent cytotoxin to the intended pharmacological target to achieve desired therapeutic effect

- Goals
  - Increase therapeutic activity by targeted delivery of drug
    - Overcome therapeutic insufficiency of some antibodies
    - Limited target delivery of small molecules
  - Decrease toxicity by reducing systemic exposure to drug

- ADCs on the market (or off) and in various stages of clinical trials
  - Adcetris, Kadcyla, Mylotarg
  - 30 others in clinical development (Drug Disc Today 2013)

ADC Technology: Empowering Antibodies

ADCs combine the targeting ability of monoclonal antibodies with the potency of cytotoxic agents

- Designed to improve efficacy and reduce toxicity
- Potent cytotoxic agents and stable linkers with long half-lives
- Readily scalable through simple, reproducible synthesis
- SGEN technology empowers more than 25 of the ADCs in clinical development across the industry, including both proprietary and collaborator programs
The ADME Basics of ADC Activity

- ADCs have distinct ADME characteristics
  - PK and biodistribution are dominated by the properties of the mAb, not the small drug
  - The mAb drives drug internalization and subcellular localization
  - Tumor exposure to the cytotoxic agent is quite different compared to small drug administration
- Important parameters
  - Mechanism and potency of the targeted drug
  - Stability in the circulation and efficient intratumoral drug release
  - Disposition of the ADC in the body: tumor versus normal tissues

ADME Considerations

- Absorption
  - i.v. dosing
- Distribution
  - Initially driven by antibody.
  - Released small molecule will likely have unique distribution after re-equilibration.
  - Released small molecule may residualize in tumor and tissue due to target (tubulin, DNA, etc) binding, resulting in distribution different than if small molecule were not conjugated.
- Metabolism
  - Small molecule after catabolic release.
- Excretion
  - Small molecule after catabolic release.
  - Liver known to catabolize antibodies, so may direct biliary excretion of small molecule.
ADCETRIS® (Brentuximab Vedotin)
Injection for intravenous infusion

• ADC directed to CD30.
• FDA granted accelerated approval in 2011 for two indications.
• Commercially available in more than 55 countries, including the United States, Canada, Japan and members of the European Union.
• Supplemental BLA for post-autologous transplant consolidation treatment of Hodgkin lymphoma patients at high risk of relapse or progression is currently under priority review by the U.S. Food & Drug Administration; PDUFA date August 18, 2015.
• Takeda continues to make additional regulatory submissions in its territory.

Brentuximab Vedotin Active In Vivo

- Brentuximab vedotin or MMAE dosed to mice with CD30 positive L540cy HL tumor xenografts
  - Antigen-dependent activity.
  - Brentuximab vedotin more active than MMAE (MMAE dose 25-fold higher).

Alley et al., PNWBIO, 2013
Typical Concentration Time Profiles

- TAb vs ADC: differences potentially due to unconjugated antibody, but assay isn’t designed to measure this directly.
- Total Antibody (TAb), ADC, Free drug, Conjugated drug.

Younas et al, NEJM, 363, 1812, 2010

Equal Moles of Injected MMAE Yields Different PK

- Mass spectrometry detection in L540cy HL mouse xenograft
- MMAE serum clearance much faster than brentuximab vedotin.
- Peak tumor MMAE concentrations later; higher for brentuximab vedotin than MMAE.

Alley et al, PNWBIO, 2013
**Immunohistochemistry Detection of MMAE in L540cy HL Tumor Xenograft**

- Anti-MMAE mAb used to visualize drug in tumor for both brentuximab vedotin and MMAE treated mice
- This technique is not quantitative for MMAE content

Alley et al., PNWBIO, 2013

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**Clinical DDI**

- Negligible effect of CYP450 3A4 inhibitor on SGN35 PK.
- No effect of SGN35 on midazolam PK.
- Rifampin, an inducer, caused a decrease in MMAE exposures but its effect was negligible on ADC exposures.

Clinical DDI

Ketoconazole, an inhibitor, caused a small, but not significant increase in MMAE exposures.
Clinical trial data showed that the ADCs are potential victims of a DDI and not the perpetrators.


Results from Drug Localization Studies

Brentuximab vedotin demonstrated antigen-specific cytotoxicity.
MMAE delivery to tumor xenografts can be visualized by IHC and quantitated by mass spectrometry.
Tumor drug delivery by ADC substantially better than dosing with free drug.
Clinical data showed that Brentuximab vedotin has low DDI potential. The ADC might be a victim but not a perpetrator of DDI.
Tracking mAB and Drug: Dual Radiolabeled SGN-75

- SGN-75, being evaluated clinically in RCC, is composed of the anti-CD70 mAb h1F6 conjugated to mcMMAF.
- Two radiolabels used to track mAb ($^3$H) and drug ($^{14}$C) biodistribution simultaneously.

mcMMAF: Drug Linker with no Built-in Cleavage Site

- Complete mAb degradation by lysosomal proteases yields cys-mcMMAF as the released drug.
mAB and Drug Accumulated in 786-O RCC Mouse Xenograft Model

- Tumor uptake kinetics are different for mAb and released drug.

Alley et al, J Pharmacol Exp Ther, 330, 932, 2009

Tumor and Tissue Released Drug Distribution

- Peak tumor drug concentration at 2 d, while normal tissues much earlier.
- Tumor drug concentrations more than 100 fold higher than tissues.

Alley et al, J Pharmacol Exp Ther, 330, 932, 2009
Tumor Exposure to Released Drug Higher than Normal Tissues

- Tumor to normal exposure ratios were generally greater than 100:1.

Alley et al, J Pharmacol Exp Ther, 330, 932, 2009

Biodistribution Conclusions

- Auristatin ADCs improve anti-tumor activity and tumor localization of drug compared to dosing with free drug.
- Auristatin ADCs achieve high tumor but low normal tissue drug delivery.
Effect of Drug Loading on PK and Efficacy

Hydrophobic nature of drug-linker increases non-specific interactions.

Hydrophobicity is better predictor of Plasma Clearance

• Auristatin ADCs with 8 drugs per antibody evaluated for apparent hydrophobicity and pharmacokinetics.
• HIC retention time predicts pharmacokinetics reasonably well.
Cells of Mononuclear Phagocytic System may Mediate the Accelerated Clearance of Hydrophobic ADCs

Perfused rat livers stained with anti-human Fc antibody:
(1 hour post-dose)

- No observable MPS uptake of unmodified antibody or hydrophilic Auristatin T ADC.
- Recognition of hydrophobic ADCs by the MPS may underlie the increased clearance from plasma.

Evaluating the Role of Drug-Linker Hydrophobicity

- Auristatin drug-linker engineered to eliminate any unnecessary hydrophobic elements and introduce hydrophilic moieties:
  - Phenylalanine of MMAF replaced with threonine
  - Valine-citrulline cleavage site replaced with hydrophilic sequence
  - Self-immolative PAB group eliminated
  - 
  - MDP group imparts stability and is more hydrophilic than maleimido-caproyl

Lyon et al., PEGS, 2014
‘Mask’ing the Hydrophobicity of an MMAE Drug-Linker

(Jeffrey, S et al., Bioconjugate Chem. 2006, 17, 831-840)

PEG24 as a stretcher

\[
\text{Antibody} \quad \begin{array}{c}
\text{PEG24} \\
\text{glucuronide-MMAE}
\end{array}
\]

PEG24 as a side chain

\[
\text{Antibody} \quad \begin{array}{c}
\text{glucuronide-MMAE} \\
\text{PEG24}
\end{array}\]

Lyon et al., PEGS, 2014

PEG Configuration Greatly Influences Apparent ADC Hydrophobicity and PK

HIC of cAC10 glucuronide-MMAE ADCs with 8 drugs per antibody

ADC pharmacokinetics

3 mg/kg iv dose

Lyon et al., PEGS, 2014
Effect of PEG on PK and Efficacy

PEGylation improves plasma PK, tumor exposure and increases anti-tumor activity.

Effect of PEG Chain Length on PK

- ADC exposure is directly related to PEG size up to PEG8.
- Clearance in rats decreases rapidly around PEG8.
- PEG12 is sufficient to optimize PK properties.
Hydrophobicity and Drug Loading

Conclusions

- Increased drug loading changes PK and efficacy in opposite directions.

- Increased hydrophobicity leads to increased clearance and decreased AUC.

- Masking the hydrophobicity of the drug linker, using PEGs, improved plasma and tumor exposures and led to increased efficacy in xenograft models.

Summary

- ADCs provide the best of antibodies and small molecules.

- The antibody drives the cytotoxin/small molecule PK leading to substantially greater tumor drug delivery with ADCs than dosing with free drug.

- ADCs have low DDI potential.

- Increased drug load and linker hydrophobicity increased the clearance of the ADCs.

- Masking the hydrophobicity using PEG improved the plasma and tumor PK and increased efficacy too.
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