Lessons Learned From The Early Development Of Linker-Payloads For Antibody-Drug Conjugates

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Outline

• Discuss how Pfizer classifies HPAPIs
  – Occupational Exposure Band (OEB) values
• What is an ADC?
• Lessons learned from the early development of linker-payloads (LPs) for ADCs
  – Challenges with OEB5 classification process
    • Who is responsible for classification?
    • How do we effectively communicate hazards?
  – Challenges with LP chemistry
    • Use of Synthesis Management Teams (SMTs) to facilitate development of LPs
    • Case studies
Occupational Exposure Limits (OELs)

An 8 hour time-weighted average concentration of a substance in air to which it is believed that employees may be exposed, without personal protective equipment, for eight hours per day, 40 hours per week, without adverse effect.

Occupational Exposure Bands (OEBs)

Occupational Exposure Bands (OEBs) are hazard classifications that correspond to specific [order-of-magnitude] airborne concentration ranges. OEBs are intended to protect workers from the hazardous properties of the compound during handling.

The OEB system separates substances into different hazard categories when the available data are sufficient to do so, but inadequate to establish an Occupational Exposure Limit (OEL).

- Small molecules assigned Occupational Exposure Bands (OEBs),
- Large molecules (Biologics) assigned to Biotherapeutic Occupational Exposure Bands (B-OEBs)
<table>
<thead>
<tr>
<th>Property</th>
<th>OEB 1</th>
<th>OEB 2</th>
<th>OEB 3</th>
<th>OEB 4 (D)</th>
<th>OEB 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Potency (mg)</td>
<td>&gt; 500</td>
<td>50 – 500</td>
<td>5 – 50</td>
<td>5 – 0.5</td>
<td>&lt; 0.5</td>
</tr>
<tr>
<td>Significant Adverse Effects</td>
<td><strong>Lowest Concern</strong></td>
<td></td>
<td></td>
<td></td>
<td><strong>Highest Concern</strong></td>
</tr>
<tr>
<td>Acute Oral Toxicity (mg/kg)</td>
<td>&gt; 2000</td>
<td>300 – 2000</td>
<td>50 – 300</td>
<td>5 – 50</td>
<td>&lt; 5</td>
</tr>
<tr>
<td>Repeat-dose Tox (NOAEL: mg/kg/day)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rat (28 Day)</td>
<td>&gt; 500</td>
<td>50 – 500</td>
<td>5 – 50</td>
<td>5 – 0.5</td>
<td>&lt; 0.5</td>
</tr>
<tr>
<td>Dog (28 Day)</td>
<td>&gt; 300</td>
<td>30 – 300</td>
<td>3 – 30</td>
<td>3 – 0.3</td>
<td>&lt; 0.3</td>
</tr>
<tr>
<td>Rat (90 Day)</td>
<td>&gt; 200</td>
<td>20 – 200</td>
<td>2 – 20</td>
<td>2 – 0.2</td>
<td>&lt; 0.2</td>
</tr>
<tr>
<td>Dog (90 Day)</td>
<td>&gt; 100</td>
<td>10 – 100</td>
<td>1 – 10</td>
<td>1 – 0.1</td>
<td>&lt; 0.1</td>
</tr>
<tr>
<td>Monkey</td>
<td>&gt; 100</td>
<td>10 – 100</td>
<td>1 – 10</td>
<td>1 – 0.1</td>
<td>&lt; 0.1</td>
</tr>
<tr>
<td>Genotoxicity</td>
<td>Negative</td>
<td>Negative</td>
<td>Ames or Other Single <em>in vitro</em> Geneotox Positive</td>
<td>Cat 3 (R68) Cat 2 (R46)</td>
<td>Cat 1 (R46)</td>
</tr>
<tr>
<td>Carcinogenicity</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
<td>Cat 3 (R40) Cat 2 (R45)</td>
<td>Cat 1 (R45)</td>
</tr>
<tr>
<td>Reproductive Toxicity</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
<td>Cat 3 (R62/63) Cat 2 (R60/61)</td>
<td>Cat 1 (R60/61)</td>
</tr>
</tbody>
</table>
Pfizer Definitions

<table>
<thead>
<tr>
<th>Type</th>
<th>Occupational Exposure Level (OEL)</th>
<th>Occupational Exposure Band (OEB)</th>
<th>Biotherapeutics Occupational Exposure Band (B-OEB) based on ADI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Potent Compound</td>
<td>&lt;10 μg/m³ (0.01 mg/m³)</td>
<td>OEB 4</td>
<td>B-OEB 4 (10-100 μg/day)</td>
</tr>
<tr>
<td>Highly Potent Compound</td>
<td>&lt;1 μg/m³ (0.001 mg/m³)</td>
<td>OEB 5</td>
<td>B-OEB 5 (&lt;10μg/day)</td>
</tr>
</tbody>
</table>

- Compounds handled within a research environment are “born” as unclassified from an occupational toxicology perspective unless data indicates otherwise. Unclassified compounds are handled as Occupational Exposure Band 4 (OEB 4s).
- This requirement applies to all Laboratories, Vivarium and Clinical Manufacturing activities.

**ADI** – Allowable Daily Intake
OEB 5 Compounds

- Certain criteria and or concerns drive more conservative handling:
  - Projected potency of < 0.5 mg (or lower end of potency range < 0.5)
  - Analogy to other similar highly potent compounds
  - Mechanism of action – known or suspected to affect rapidly dividing cells
- Classification is usually compound specific but in some cases is made for classes of compounds (Vitamin D analogs, ADCs)
- An OEB 5 or B-OEB 5 classification stays with the compound in all physical states (solid, solution, formulated product), it does not change unless the data set drives a re-classification
- The exposure potential varies depending upon the physical state, amount being handled and effectiveness of controls available
Compound Classification Process

Pfizer employs a Global Compound Classification Process:

- Conducted by EH&S

- Compound classification decisions are made in partnership with Research Project Teams to ensure all relevant data is considered.

- Classification is initiated in line with R&D development stage-gates.

- Pharmacological (or toxicological) data points used (mechanism of action, potency projections, and structural analogy)

- As compounds move through the development process and information is generated on their toxicological profile, further evaluations are undertaken for worker safety endpoints
What is an Antibody-Drug Conjugate (ADC)
What is an ADC?
Highlighting Structural Complexity

<table>
<thead>
<tr>
<th>Small molecule</th>
<th>Linker-payload</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 500</td>
<td>MW</td>
</tr>
<tr>
<td>0-3</td>
<td>Chiral centers</td>
</tr>
<tr>
<td>OEB 4</td>
<td>OEB classification</td>
</tr>
<tr>
<td>Typically &lt;15</td>
<td>Synthetic steps (longest linear)</td>
</tr>
<tr>
<td>Crystalline</td>
<td>Final form</td>
</tr>
<tr>
<td>Crystallization</td>
<td>Purification</td>
</tr>
</tbody>
</table>

Palbociclib
Highlighting Manufacturing Challenges

- MW > 1300
- 9+ Stereocenters
- Longest linear synthetic sequence 17 steps
- Three synthetic amino acids (Dil, Dap, and Doe)
- Reverse phase chromatography for final purification
- Amorphous
- LP and payload classified as OEB5
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*How do we enable rapid process development to achieve FIH?*
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- LP and payload classified as OEB5

How do we enable rapid process development to achieve FIH? …and minimize internal HiPo chemistry? …and ensure that all HiPo work is done safely?
Communication Framework

Medicinal Chemistry

Chemical Research and Development

Environmental Health and Safety
Communication Framework

- CROs
- Biology
- External Research Solutions
- Medicinal Chemistry
- Chemical Research and Development
- Environmental Health and Safety
Synthesis Management Team (SMT)
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How do we make the molecule?
How do we purify the molecule?
How do we analyze for purity, potency, etc?
Synthesis Management Team (SMT)

Where do we manufacture the molecule?
Who performs the non-HiPo work?
Who performs the HiPo work?
How much do we need?
GMP or non-GMP?

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GMP or non-GMP?

How do we make the molecule?
How do we purify the molecule?
How do we analyze for purity, potency, etc?

Which intermediates are OEB5?

How do we ensure work is done safely?
- CRD and WWMC partners in non-GMP SSETS Deliveries
- CRD commits scientific resources on optimizing the synthetic process chemistry for scalable GMP / clinical requirements.
- External partnerships for GMP R1 delivery (and beyond) are initiated shortly after SSETS in preparation for Compound Select (CS) milestone
ADC SMT Roles and Responsibilities

- EH&S Assessment
- Synthetic Route(s)
- Intermediate HiPo Determination
  - Route Development (non-HiPo)
  - Sourcing Activities (HiPo and non-HiPo)
  - Route Development (HiPo)
  - API Manufacture
ADC SMT Roles and Responsibilities

- CRD and Medicinal Chemistry partner to evaluate all known routes to target payload and linker-payload
  - Internal routes
  - External vendor routes
  - Literature routes
- Goal is to target 1 or 2 “most attractive” syntheses
  - Number of steps
  - Overall yield
  - Purifications
  - Number of OEB5 steps
ADC SMT Roles and Responsibilities

**EH&S Assessment**

**Synthetic Route(s)**

- **Route Development (non-HiPo)**

- **Sourcing Activities (HiPo and non-HiPo)**

- **Route Development (HiPo)**

**Intermediate HiPo Determination**

- CRD and Med. Chem. assess route(s) for potential OEB5 intermediates
  - Based on limited data set
- All intermediates that are suspected of being classified as OEB5 will be submitted to *in silico* and biological assays
  - DEREK, SARAH, cytotox assay

**API Manufacture**
ADC SMT Roles and Responsibilities

- EH&S monitor route development activities and assess intermediates based on data gathered from assays.
- CRD and Med. Chem. assess route(s) for potential OEB5 intermediates:
  - Based on limited data set
  - All intermediates that are suspected of being classified as OEB5 will be submitted to *in silico* and biological assays:
    - DEREK, SARAH, cytotox assay
ADC SMT Roles and Responsibilities

- **SMT**
  - **Synthetic Route(s)**
    - **Route Development (non-HiPo)**
      - **Sourcing Activities (HiPo and non-HiPo)**
        - **Route Development (HiPo)**
          - **API Manufacture**

- **Intermediate HiPo Determination**

- **EH&S Assessment**

**Notes:**
- CRD and Med. Chem. develop and optimize non-HiPo chemistry for preparation of tech package
  - Successful route development leads to generation of tech package
  - Unsuccessful route development restarts “Synthetic Route” step
ADC SMT Roles and Responsibilities

- SMT
  - Synthetic Route(s)
  - Sourcing Activities (HiPo and non-HiPo)
  - Route Development (HiPo)
  - Route Development (non-HiPo)
  - Intermediate HiPo Determination

- EH&S Assessment

- Global External Supplies will gather quotes on non-HiPo tech package
- HiPo tech package prepared from best available technology (Med. Chem. Technology)
- Purchase Orders will be awarded for each part
ADC SMT Roles and Responsibilities

- HiPo development performed at external suppliers in preparation for non-GMP early phase tox studies
- Additional Non-HiPo development also performed
ADC SMT Roles and Responsibilities

- **SMT**
  - Synthetic Route(s)
  - Route Development (non-HiPo)
  - Sourcing Activities (HiPo and non-HiPo)
  - Route Development (HiPo)
  - API Manufacture

- **EH&S Assessment**

- **Intermediate HiPo Determination**

- **Linker-Payload manufactured and delivered for early phase tox studies**
- **Lessons Learned from first campaign will drive which “box” we return to**
Case examples of how the SMT process facilitates Linker-Payload manufacture
Payload Manufacturing
Payload Manufacturing

![Diagram of Payload Manufacturing]

1. **Amino Acid 1**
2. **Amino Acid 2**
3. **Amino Acid 3**
4. **Amino Acid 4**
5. **Amino Acid 5**

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**Bottom Diagram**

1. **Amino Acid 1**
2. **Amino Acid 2**
3. **Amino Acid 3**

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**Top Diagram**

1. **Amino Acid 2**
2. **Amino Acid 3**
3. **Amino Acid 4**
4. **Amino Acid 5**
Payload Manufacturing

Payload

Amino Acid 1 ─── Amino Acid 2 ─── Amino Acid 3 ─── Amino Acid 4 ─── Amino Acid 5

Amino Acid 4 ─── Amino Acid 5

1) Peptide Coupling
2) Deprotection

CO₂H

PG
Payload Manufacturing

Amino Acid 1 ─── Amino Acid 2 ─── Amino Acid 3 ─── Amino Acid 4 ─── Amino Acid 5

Payload

Peptide Coupling

De-protection
Payload Manufacturing

Payload

Both products classified as OEB5
Linker-Payload Manufacturing

10 Steps from amino acid building blocks
- 6 non-HiPo steps
- 4 HiPo steps
Linker-Payload Manufacturing

10 Steps from amino acid building blocks
- 6 non-HiPo steps
- 4 HiPo steps

40% of steps require containment
2.7% over all yield
Case Study 1: Linker-Payload Manufacturing – Alternative Route
Case Study 1: Linker-Payload Manufacturing – Alternative Route

**Diagram:**
- Linker A
- Payload A
- Amino Acid 1
- Amino Acid 2
- Amino Acid 3
- Amino Acid 4
- Amino Acid 5
- CO₂H
Case Study 1: Linker-Payload Manufacturing – Alternative Route
Case Study 1: Linker-Payload Manufacturing – Alternative Route

“Tetramer“
- classified as OEB4
- Requires 6 non-HiPo steps
Case Study 1: Linker-Payload Manufacturing – Alternative Route

10 Steps from amino acid building blocks
- 6 non-HiPo steps
- 4 HiPo steps
- 2.6% overall yield

8 Steps from amino acid building blocks
- 6 non-HiPo steps
- 2 HiPo steps
- 27% overall yield

Decreased cost and production time!
Case Study #2

- 31 steps
- 6 HiPo steps
- 0.004% overall yield
Case Study #2

- 25 steps
- 6 HiPo steps
- 27-fold increase in yield up to step 11
CRD and WWMC partners in non-GMP SSETS Deliveries
CRD commits scientific resources on optimizing the synthetic process chemistry for scalable GMP / clinical requirements.
External partnerships for GMP R1 delivery (and beyond) are initiated shortly after SSETS in preparation for Compound Select (CS) milestone
Conclusions

- Pfizer employs a Global Compound Classification Process for Highly Potent APIs (and all NCEs):
  - Compound classification decisions are made in partnership with Research Project Teams to ensure all relevant data is considered.
  - Pharmacological (or toxicological) data points used (mechanism of action, potency projections, and structural analogy)
  - As compounds move through the development process they are frequently evaluated for worker safety endpoints
- Within Pfizer, several types of exposure limits or Occupational Exposure Values are developed:
  - Occupational Exposure Limits (OELs) define the amount (concentration in µg/m³) of compound that you can be safely exposed to over an 8-hour day, 5 days per week without experiencing adverse effects
  - Exposure Bands define an airborne concentration into which the OEL is reasonably expected to fall (once data becomes available)
- Two case example were presented that clearly demonstrate the utility of Synthesis Management Teams as they pertain to the early development of linker-payloads for the ADC program
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Eric Watters
Risk Assessment Process for Containment

• Form
  – Solid or liquid

• Activity
  – Structurally similar compounds with similar potency

• Quantity
  – Small-scale (< 1 g) manufacture
  – Large-scale (500+ g) manufacture

• Quality
  – DSI, DS, or DP

• Available Facilities
  – Research Labs
  – Kilo-lab
  – Pilot Plant
Occupational Exposure Bands

Bands separate compounds into one of five different hazard categories

Occupational Exposure Bands:
- Small molecules
- Airborne concentration range, limited hazard data (LOAEL, NOAEL)
- Exposure controls focused on inhalation exposures

Biotherapeutic Occupational Exposure Bands (B-OEBs)
- Large molecule / biotherapeutic entities
- ADI (μg/day) calculated from Lowest Therapeutic Dose (LOAEL)
- Exposure controls focused on parenteral exposures (sharps) and liquid aerosols

Exposure control and containment strategy = Handling Guidelines
OEB 4 = default in both schemes
Supplier Considerations

- Short-list criteria
  - Capabilities
    - GMP API manufacturing
    - GMP HPAPI manufacturing
    - Chromatography
      - HP GMP
    - Strong analytical
    - Peptide Experience
    - Good standing in Pfizer systems
  - Geography: Not a requirement; L-P is not API so not bound by Import-for-Export limitations
Anatomy of an Antibody Drug Conjugate (ADC)

Pfizer strategy multi-faceted: modify/explore each ADC component

Monoclonal antibody (mAb)
- targets specific receptor/cell type
- weak cytotoxic activity

Biologically active peptide or small molecule
- poor specificity
- exquisite cytotoxicity

Linker molecule (link and spacer)
Antibody Drug Conjugate: The Concept

**Delivery Vehicle**
IgG, antibody fragments or alternative binding platforms

**Goals**
- Enhance anti-tumor activity of antibody
- Reduce systemic toxicity of cytotoxic drug

**Cytotoxic Payload Drug**
(or microbiologic toxin or immunomodulator), linker, conjugation chemistry, stoichiometry

**Targets**
CELL DEATH
Manufacturing Challenges

- “Small molecules” classified as OEB4 (no high potency handling precautions)
- Sourced from several vendors
- All are available in 100g to >1kg quantities
Manufacturing Challenges

- “Small molecules” classified as OEB4 (no high potency handling precautions)
- Sourced from several vendors
- All are available in 100g to >1kg quantities

- “Small molecules” classified as OEB5
- Sourced from vendors capable of HiPo API production
- All are available in 100g to >1kg quantities