An Industrial Perspective on Nanomedicine Characterization Strategies

2nd Annual FDA/PQRI Conference on Advancing Product Quality
October 5th, 2015
An Industrial Perspective on Nanomedicine Characterization Strategies

- Introduction to Accurins
- Focus on Clinical Translation – Complication versus Complexity
- Identification and Criticality Analysis of Quality Attributes
- Accurin Control Strategy
  - Critical Material Attributes
  - Critical Process Parameters and In Process Controls
  - Finished Product Characterization
- Further Considerations
Targeted Nanomedicines Represent New Therapeutic Class

Most persistent challenge is to control biodistribution of therapeutic agent
Accurins enable control of a therapeutic across a wide range of mechanisms

Conventional Oncology Drugs
Accurins

\[ t_{\text{max}} \text{(tumor)} \]
1 - 2 hours
12 - 24 hours
Accurins Achieve Targeting Through Two Approaches

Hrkach et al; Preclinical Development and Clinical Translation of a PSMA-Targeted Docetaxel Nanoparticle with a Differentiated Pharmacological Profile; Sci Transl Med 4, 128ra39 (2012)
Accurins: What are they and how do they work?

- **Polymeric matrix**
- **Targeting ligands**
- **Stealth PEG layer**
- **Unmodified therapeutic payload**

**Controlled-Release Polymer Matrix**
- FDA approved polymer physically entraps therapeutic payload enabling fine tuning the release rate

**Therapeutic Payload**
- Broad range of therapeutic payloads including small molecules, peptides, proteins & nucleic acids

**Stealth and Protective Layer**
- Protects against immune detection and clearance mechanisms

**Targeting Ligands**
- Active targeting enables particles to bind to specific cell-surface markers, enhancing particle accumulation
Process Flow Diagram for Accurin Production

Upstream Emulsification

- Aqueous Phase → Organic Phase
  - Coarse emulsion
  - High-Energy Emulsification
    - Fine emulsion
  - Particle Quench
    - Hardened NPs
      - Ultrafiltration and diafiltration
        - IPC for concentrations
        - Concentrated NPs
  - WFI or Buffer, Polysorbate 80

Downstream Filtration

- WFI
  - Ultrafiltration and diafiltration
    - IPC for concentrations
    - Concentrated NPs
  - WFI, Sucrose
    - Cryoprotectant Addition
      - IPCs for concentrations, bioburden, and density
      - Formulated NPs

Aseptic Fill/Finish

- Sterile Filtration
  - Bulk Drug Product
    - To liquid fill line for freezing or lyophilization
The BIND Pilot Plant Operates 1-3 kg Clinical Manufacturing Equipment
Outline of the Presentation

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• Further Considerations
Focus on Clinical Translation Eases Characterization Burden

Polylactide is a well understood material used in many approved products

Complicated

Complex

Lupron Depot®
(leuprolide acetate for depot suspension)

Risperdal® CONSTA
risperidone Long-Acting Injection
12.5mg, 25mg, 37.5mg, 50mg

Zoladex®
Goserelin Acetate Implant

Absorb Stent

VICRYL RAPIDE

BYDUREON
Focus on Clinical Translation Eases Characterization Burden

Polylactide – polyethylene glycol block copolymer biodegrades to nontoxic products

Complicated

Complex
Focus on Clinical Translation Eases Characterization Burden

The active ingredient in Accurins is not chemically modified

Complicated

Complex
Focus on Clinical Translation Eases Characterization Burden

A stable nanoemulsion in thermodynamic equilibrium helps ensure a reproducible product.

Stable emulsion droplets
Focus on Clinical Translation Eases Characterization Burden

Targeting is enabled using small molecules as ligands

Complicated

Complex
Focus on Clinical Translation Eases Characterization Burden

Incorporation of targeting ligands via polymeric raw material avoids chemistry on nanoparticles

Complicated

Complex
Focus on Clinical Translation Eases Characterization Burden

Tight control of particle size enables conventional terminal sterile filtration
**Complexity Continuum**

- **IR Oral**
- **SR Oral**
- **Pulmonary, Transdermal**
- **Accurins**
- **Biologics**

**Product complexity; number of CQAs**

**Known CQAs;** well understood

- *in vitro-in vivo* relationship

**Criticality must be assessed;**

- *in vitro-in vivo* relationship may not be clear
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## Criticality Analysis of Quality Attributes

<table>
<thead>
<tr>
<th>Score</th>
<th>Impact</th>
<th>Uncertainty</th>
</tr>
</thead>
<tbody>
<tr>
<td>Catastrophic</td>
<td>Life threatening illness or irreversible injury</td>
<td>Unknown</td>
</tr>
<tr>
<td></td>
<td>Negative efficacy (accelerates disease)</td>
<td></td>
</tr>
<tr>
<td>Major</td>
<td>Major, possibly irreversible impact</td>
<td>Literature reports</td>
</tr>
<tr>
<td></td>
<td>Complete loss of efficacy</td>
<td></td>
</tr>
<tr>
<td>Moderate</td>
<td>Reversible impact, requires medical attention</td>
<td>in vitro data</td>
</tr>
<tr>
<td></td>
<td>Major loss of efficacy</td>
<td></td>
</tr>
<tr>
<td>Minor</td>
<td>Minor, reversible impact</td>
<td>Nonclinical data</td>
</tr>
<tr>
<td></td>
<td>Minor Loss of Efficacy</td>
<td></td>
</tr>
<tr>
<td>Negligible</td>
<td>No AEs</td>
<td>Clinical data</td>
</tr>
<tr>
<td></td>
<td>No Loss of Efficacy</td>
<td></td>
</tr>
</tbody>
</table>

Adapted from ISPE PQLI Guide and ISPE A-Mab Case Study
Criticality Analysis of Quality Attributes

- PEGylation of the Accurin surface is critical to pharmacokinetics

- Acquisition of relevant non-clinical and clinical data can be difficult
  - Univariate alteration of PEG levels without impacting other CQAs is complex
  - Surface characteristics of clinical batches are very uniform (zeta potential of all batches -10 to -15 mV)

- \[ C = I \times U; \] high criticality requires a robust control strategy
Process Knowledge Enables the Control Strategy

Process Knowledge Map relates CQAs to Critical Material Attributes, Critical Formulation Parameters, and Critical Process Parameters

- PLA-PEG CMAs and emulsification CPPs identified as potentially influencing surface characteristics
Impact of Emulsification on Surface PEG Levels

- Accurin components contained within the core have lower mobility; and are not visible in solution NMR spectra
- Surface PEG levels are not affected by relevant emulsion variations
- A focus on the CMAs of PLA-PEG is key to control of surface properties

(Heald et al. 2002, Hrkach et al. 1997)
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• Further Considerations
Nanomedicines rely on complex raw materials to impart many important CQAs

**PLA-PEG CMAs**
- Block architecture
- PEG and PLA $M_n$
- Homo-PLA, free PEG
- Residual monomer, solvent

**Product CQAs**
- Surface characteristics
- Release rate
- Particle size
Quantitatively Linking RM CMAs to Product CQAs

Tunable controlled release is critical to the ability of Accurins to significantly improve therapeutic index

• A quantitative, mechanistic understanding of the relationship between CMA and CQA is critical

• Payload release is diffusion controlled; diffusion rate is controlled by particle $T_g$, which is modulated by PLA molar mass
Can PAT be utilized to enhance control of Accurin particle size?

- In process controls are a critical part of the control strategy
- Particle size is controlled by homogenizer pressure under appropriate emulsion conditions
- Opportunity to apply PAT for CQA control
Process Analytical Technology to Enhance Control Strategy

Organic Phase → Coarse Emulsion Vessel → Homogenizer → Fine Emulsion Vessel → Off-line DLS

Aqueous Phase → Homogenizing Pressure

Off-Line Analysis
Process Analytical Technology to Enhance Control Strategy

- Organic Phase
- Aqueous Phase
- Coarse Emulsion Vessel
- Homogenizer
- Homogenizing Pressure
- At-line DLS
- Sample Line to DLS
- Fine Emulsion Vessel
- At-Line Analysis
On Line Control Successfully Returns PSD to Target

At Line DLS Analyzer

![Graph showing DLS Particle Size and Pressure over sample numbers. The graph depicts a trend where the DLS Particle Size decreases as the Homogenizer Pressure is adjusted. The target is represented by a blue line, and the actual measurement is shown by a red line with a green arrow indicating the pressure adjustment point.](image)
Nanoparticles with high aspect ratio have been reported to have altered circulation times and biodistribution.

Li et al; *Shape design of high drug payload nanoparticles for more effective cancer therapy*; *Chem. Commun.* 49, (2013)
Drug Product Characterization: Particle Morphology

**PSD by Dynamic Light Scattering**

- Normalized Intensity
- Size (nm)

- Lot 1
- Lot 2

**Transmission Electron Micrographs**

- Lot 1
- Lot 2

**in vitro Release Profiles**

- % Released
- Time (h)

- Lot 1
- Lot 2
Cryo-Transmission Electron Microscopy

Ultra-rapid freezing to ensure unaltered morphology

PEG Corona

Improved Contrast from nsTEM
Image analysis can be used to improve quantitative power of images

Original image

Thresholded image

Analyzed image

Feret (Caliper) Diameter

Fh

Fv

Fh

Fv

Feret Dia. Ratio

Sample 1 (elongate)  Sample 2 (spherical)

Algorithm 1, nsTEM

Manual, cryoTEM

Algorithm 2, nsTEM

Algorithm 2, cryoTEM
Field Flow Fractionation – Light Scattering

- Further improvement of sampling and quantitation
- FFF provides improved resolution of particle size
- Static and dynamic light scattering together provide morphological characterization
  - Hydrodynamic radius from DLS diffusion coefficient
  - Radius of gyration from SLS
  - Ratio $\rho$ dependent on particle morphology ($0.775$ for spheres; greater for more elongate)
- Qualitative agreement provides confidence in both measurements

<table>
<thead>
<tr>
<th>Sample</th>
<th>Feret Dia. Ratio</th>
<th>$R_g/R_H$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lot 1 Elongate Particles</td>
<td>3.2</td>
<td>1.01</td>
</tr>
<tr>
<td>Lot 2 Spherical Particles</td>
<td>1.5</td>
<td>0.75</td>
</tr>
</tbody>
</table>
The ability to control and modulate release rate is a differentiating feature of Accurins

- A wide range of release rates is often accessible
- Manipulation of release rate often has significant biological impact

![Cumulative Release vs. Time Graph](image-url)
Optimal Release Rate is Critical to Maximize Therapeutic Index

Release rate may modulate both efficacy and toxicity

Target Coverage

<table>
<thead>
<tr>
<th>Time Post Dose (hr)</th>
<th>pH3 - % control</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>125</td>
</tr>
<tr>
<td>24</td>
<td>100</td>
</tr>
<tr>
<td>48</td>
<td>75</td>
</tr>
<tr>
<td>72</td>
<td>50</td>
</tr>
<tr>
<td>96</td>
<td>25</td>
</tr>
</tbody>
</table>

Formulation B

<table>
<thead>
<tr>
<th>Time Post Dose (hr)</th>
<th>pH3 - % control</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>125</td>
</tr>
<tr>
<td>24</td>
<td>100</td>
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<tr>
<td>72</td>
<td>50</td>
</tr>
<tr>
<td>96</td>
<td>25</td>
</tr>
</tbody>
</table>

Formulation E

<table>
<thead>
<tr>
<th>Time Post Dose (hr)</th>
<th>pH3 - % control</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>125</td>
</tr>
<tr>
<td>24</td>
<td>100</td>
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<tr>
<td>48</td>
<td>75</td>
</tr>
<tr>
<td>72</td>
<td>50</td>
</tr>
<tr>
<td>96</td>
<td>25</td>
</tr>
</tbody>
</table>

Tumor Growth Inhibition

Formulation B & E

Bone Marrow Hypocellularity

Ashton et al; Accurin-AZD1152 hQPA nanoparticles inhibit growth of diffuse large B-cell lymphomas and small cell lung cancer in preclinical models; ACR (2015)

Goodwin et al; Imaging Accurin-AZD1152 hQPA nanoparticle accumulation in preclinical tumours; ACR (2015)
Cross Validation of In Vitro Release

- Ultracentrifugation is used to separate released drug from nanoparticles
  - Avoids adsorption, filter blocking and kinetics issues which can adversely impact membrane barrier methods
  - Dependent on properties of the NP, not the API
  - Cross validation with other separation techniques can provide confidence in the results

Comparative Drug Release – SEC and Ultracentrifugation

![Graph showing comparative drug release over time for different formulations using SEC and UC methods.](image-url)
Drug Product Characterization: In Vitro Release

• Strive for physiological relevance
  – Physiological temperature, pH, buffer
  – Sink conditions are critical for accuracy

• While relevant and predictive, in vitro test does not describe biological behavior completely

**Note**: PK data reflect total (encapsulated + released) AZD2811 plasma concentrations
Drug Product Characterization: In Vitro Release

IVR is an important assessment of product quality – but is not comprehensive for a multifunctional product like an Accurin.

### in vitro Release Profile

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>% Released</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>30</td>
<td>30</td>
</tr>
</tbody>
</table>

- **BIND-510 2.5% GL TNP**
- **BIND-510 PTNP**

### Tumor Growth Inhibition

<table>
<thead>
<tr>
<th>Time (Days)</th>
<th>Tumor Volume (mm³)</th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
<td>500</td>
</tr>
<tr>
<td>25</td>
<td>1000</td>
</tr>
<tr>
<td>30</td>
<td>1500</td>
</tr>
<tr>
<td>35</td>
<td>2000</td>
</tr>
<tr>
<td>40</td>
<td>2500</td>
</tr>
</tbody>
</table>

- **Vehicle - 0.9% Sterile Saline**
- **2 mg/kg BIND-510 2.5% GL-TNP**
- **2 mg/kg BIND-510 PTNP**

TGI: 59% 91%
Drug Product Characterization: In Vitro Release

Product development utility is driven by a combination of discriminatory power and reproducibility.

**In Vitro Release (37 °C)**

![Graph showing In Vitro Release at 37 °C with two batches: Batch 1 and Batch 2.](image)

**In Vitro Release (45 °C)**

![Graph showing In Vitro Release at 45 °C with two batches: Batch 1 and Batch 2.](image)

**Long Term Reproducibility**

![Graph showing long term reproducibility with cumulative release over time.](image)

**Comparison of IVR results**

![Graph comparing IVR results for a CRO and BIND.](image)
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Formulation complexity and method complexity

• Use of conventional, and particularly compendial, methods can lower method development complexity; but bear in mind formulation complexity
• Microbiological methods are a good example

Low Endotoxin Recovery

• Reports of interference of polysorbates with LAL method (Chen PDA 2013)
• Challenge test: spike product prior to dilution and incubate (7 days at 5 °C)
• Compare recovery to positive water control and polysorbate-free product
• No suppression of endotoxin detection observed

Encapsulated Endotoxin

• Determination of MVD for product using USP <85> was successful
• Could LPS be encapsulated during particle production? Would this be detected if present?
• Utilize particle-disrupting solvent during initial dilution phase
• Required additional dilution, and filtration to remove hydrophobic excipients
• Adequate recovery was achieved
Characterization of nanomaterials is a rapidly evolving field – staying current is critical.
Conclusions

- Development of a robust characterization strategy for nanomedicines requires a truly collaborative cross-functional effort
  - Identification and prioritization of CQAs
  - Material and process understanding
  - Analytics
  - Biological evaluation

- It is critical to consider product design with respect as part of characterization complexity

- The Quality by Design framework for product quality is appropriate, effective for and indeed essential nanomedicines
Acknowledgements

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  – Steve Zale

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