Validated bioanalysis for therapeutic antibodies by LC-MS: Fab-selective proteolysis nSMOL

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Market trend of antibody drugs

<table>
<thead>
<tr>
<th>Market size (2017)</th>
<th>2025 forecast</th>
</tr>
</thead>
<tbody>
<tr>
<td>US M$ 84,500</td>
<td>114,600</td>
</tr>
<tr>
<td>Immune checkpoint inhibitor</td>
<td></td>
</tr>
<tr>
<td>US M$ 10,566</td>
<td>56,530</td>
</tr>
</tbody>
</table>

- **Approval**: 79
- **Clinical trials**: 500
- **Early phase Preclinical**: 2,000
- **R&D Biosimilar**: Over 5,000
Strategy matching of MS technology to medicine and biologics

**Medical**
- Biomarker, surrogate
- Cancer biology
- Clinical trials
- Therapeutic drug monitoring

**Biologics**
- DMPK
- Heterogeneity
- Stability
- Quality control

**Mass spec**
- Integrated hybrid technology
- Physical chemistry
- Proteomics
- Structural biology
- Matrix effect in interface
- Too professional use, or stability issues

Importance of overall method optimization from prep to MS analysis
Development of mAb bioanalysis

- **Clinical demands in antibody treatment**
  - Indicator of drug efficacy
  - Decision of dosing level
  - Drug distribution in plasma and tissue

- **Novel bioanalysis for clinical pharmacokinetics**
  - Structure similarity and sequence specificity for antibody CDR-targeting strategy
  - Independent of a variety of antibodies
  - Structure-indicated MS analysis
  - Clinical PK and discovery for antibody drugs
  - Regulated LCMS bioanalysis
FDA Guidance Finalized on May 24, 2018

- Bioanalytical Method Validation Guidance for Industry have been finalized by FDA

Summary

<table>
<thead>
<tr>
<th></th>
<th>Protein LBA</th>
<th>Small molecule LCMS</th>
<th>Protein LCMS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calibration curve</td>
<td>Same as previously</td>
<td>Almost parameters are same as small molecules.</td>
<td></td>
</tr>
<tr>
<td>Quality Controls</td>
<td>±15% of theoretical concentrations, ±20% at LLOQ for accuracy; and within 15% CV, within 20% CV for LLOQ</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Immunoglobulin structure

Complementarity-determining region: CDR
Antigen binding site ⇒ Antibody specificity
Highest affinity to antigen in CDR3
Frequency of amino acid substitution in Fv region

Concept representation of nSMOL
nano-surface and molecular-orientation limited proteolysis

Trypsin immobilization on the surface

Minimizing sample complexity into LCMS analysis

Large diameter nanoparticle 200 nm

CDR peptides

Accessible surface

Fab orientation to the solution

Small diameter pore 100 nm

IgG collection

GMA layer

Styrene-DVB-GMA Copolymer

Ferrite


Iwamoto N. et.al. Analyst, 2014
Fv-selective detection by nSMOL

Peptide configuration in Nivolumab 3D structure
Detected signature peptides from H-chain and L-chain
# Benefit of nSMOL bioanalysis

<table>
<thead>
<tr>
<th>Method R&amp;D</th>
<th>nSMOL</th>
<th>Affinity capture</th>
<th>ELISA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Collection or detection Ab</td>
<td>No</td>
<td>Individual 6-10 months</td>
<td>Individual 6-10 months</td>
</tr>
<tr>
<td>Cross reactivity</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Effect of ADAs</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Pre validation</td>
<td>1-3 days</td>
<td>1-3 days</td>
<td>2-3 weeks</td>
</tr>
<tr>
<td>Full validation</td>
<td>3-4 weeks</td>
<td>3-4 weeks</td>
<td>3-4 weeks</td>
</tr>
<tr>
<td>Sample prep</td>
<td>Dilution 3-5 hours</td>
<td>Denature, Chemical modification 3-5 hours</td>
<td>Dilution 2-4 hours</td>
</tr>
<tr>
<td>Internal standard</td>
<td>Universal</td>
<td>Individual</td>
<td>Individual</td>
</tr>
<tr>
<td>Dynamic range</td>
<td>Wide</td>
<td>Wide</td>
<td>Narrow</td>
</tr>
<tr>
<td>Selectivity</td>
<td>High</td>
<td>High</td>
<td>Middle-High</td>
</tr>
<tr>
<td>Multiplex assay</td>
<td>Yes</td>
<td>Additional collection Ab</td>
<td>Additional collection /detection Ab</td>
</tr>
</tbody>
</table>
nSMOL advantage in LCMS bioanalysis

BV peptide recovery

Calibration curve in BV bioanalysis

BV proteolysis yield: 97%
(by SDS-PAGE, densitometry test)

Full length Bevacizumab
### Progress of nSMOL project and clinical trials

<table>
<thead>
<tr>
<th>Method setting</th>
<th>Full validation</th>
<th>Paper in progress</th>
<th>Clinical trials</th>
</tr>
</thead>
<tbody>
<tr>
<td>25</td>
<td>20</td>
<td>Accepted: 12</td>
<td>In submission: 3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>In submission: 2</td>
<td>In trials: 11</td>
</tr>
</tbody>
</table>

### Antibodies

<table>
<thead>
<tr>
<th>Antibodies</th>
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</tr>
</thead>
<tbody>
<tr>
<td>Trastuzumab, T-DM1</td>
<td>Infliximab</td>
</tr>
<tr>
<td>(0.06-250 µg/ml, Anal Methods, J Pharm Biomed Anal)</td>
<td>(0.29-300 µg/ml, Curr Pharm Biotechnol)</td>
</tr>
<tr>
<td>Bevacizumab</td>
<td>Biosimilar of Infliximab</td>
</tr>
<tr>
<td>(0.15-300 µg/ml, Drug Metab Pharmacokinet)</td>
<td>(0.29-300 µg/ml, Curr Pharm Biotechnol)</td>
</tr>
<tr>
<td>Cetuximab</td>
<td>Etanercept</td>
</tr>
<tr>
<td>(0.58-300 µg/ml, Bioanalysis)</td>
<td>(0.20-100 µg/ml, Pharmacol Res Perspect)</td>
</tr>
<tr>
<td>Nivolumab</td>
<td>Abatacept</td>
</tr>
<tr>
<td>(0.15-250 µg/ml, J Chromatogr B)</td>
<td>(0.40-100 µg/ml, Pharmacol Res Perspect)</td>
</tr>
<tr>
<td>Rituximab</td>
<td>Tocilizumab</td>
</tr>
<tr>
<td>(0.58-300 µg/ml, Biol Pharm Bull)</td>
<td>(0.78-200 µg/ml, J Pharm Biomed Anal)</td>
</tr>
<tr>
<td>Brentuximab vedotin, multiplex assay</td>
<td>Coexistence with anti-drug antibodies</td>
</tr>
<tr>
<td>(0.58-300 µg/ml, Clin Pharma Biopharma)</td>
<td>(Anal Biochem)</td>
</tr>
</tbody>
</table>
Overall antibody PK for efficacy biomarker

Drug level in disease tissue

Comparative PK
Dosing strategy

Blood monitoring

Effect of anti-drug antibody
Cytokine quantitation
Immune cell profiling
Acknowledgment

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