Speeding Up Monoclonal Antibody Screening and Characterization

Gurmil Gendeh, Ph.D.
Market Development Manager
BioSeparations Group—Dionex Products
Thermo Fisher Scientific
Sunnyvale, CA
Outline

- Monoclonal Antibody Therapeutics
- Challenges in Monoclonal Antibody Analysis
- Shorter Gradients, Smaller Particles and Platform Methods
- Automation to Increase Throughput, Reduce Hands-on Time and Increase Productivity
- Summary
New melanoma drug boosts survival time

By Marilynn Marchione
ASSOCIATED PRESS

CHICAGO — Researchers have scored the first big win against melanoma, the deadliest form of skin cancer. An experimental drug significantly improved survival in a major study of people with very advanced disease.

The results, reported Saturday at a cancer conference, left doctors elated.

“We have not had any therapy that has prolonged survival” until now, said Dr. Lynn Schuchter of the Abramson Cancer Center at the University of Pennsylvania, a skin cancer specialist with no role in the study or ties to the drug’s maker.

The drug, ipilimumab, works by helping the immune system fight tumors. The federal Food and Drug Administration has pledged a quick review, and doctors think the drug could be available by the end of this year.

“People are going to have a lot of hope and want this drug, and it’s not on their doctors’ shelves,” although some may be able to get it through special programs directly from its maker, Bristol-Myers Squibb Co., Schuchter said.

Sen. John McCain, R-Ariz., had a melanoma removed from his nose. Immune-stimulating treatment, or the immune-stimulating treatment alone.

After two years, 24 percent of those given the drug alone or in combination were alive, versus 14 percent of those given just the immune-stimulating treatment.

Average survival was 10 months with ipilimumab versus just over 6
Top Ten Drugs—Emergence of Antibodies

<table>
<thead>
<tr>
<th>Consensus forecasts for 2010:</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Lipitor (cholesterol) Pfizer $11.7B</td>
</tr>
<tr>
<td>2. Plavix (anticlotting) Sanofi/Bristol $9.6B</td>
</tr>
<tr>
<td>3. Advair (asthma/COPD) GSK $9.0B</td>
</tr>
<tr>
<td>4. Remicade (arthritis) Merck/J&amp;J $7.4B</td>
</tr>
<tr>
<td>5. Enbrel (arthritis) Pfizer/Amgen $7.1B</td>
</tr>
<tr>
<td>6. Humira (arthritis) Abbott $6.8B</td>
</tr>
<tr>
<td>7. Avastin (cancer) Roche $6.7B</td>
</tr>
<tr>
<td>8. Rituxan (cancer) Roche $6.1B</td>
</tr>
<tr>
<td>9. Diovan (hypertension) Novartis $6.0B</td>
</tr>
<tr>
<td>10. Crestor (cholesterol) AstraZeneca $5.8B</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Consensus forecasts for 2014:</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Avastin (cancer) Roche $8.9B</td>
</tr>
<tr>
<td>2. Humira (arthritis) Abbott $8.5B</td>
</tr>
<tr>
<td>3. Enbrel (arthritis) Pfizer/Amgen $8.0B</td>
</tr>
<tr>
<td>4. Crestor (cholesterol) AstraZeneca $7.7B</td>
</tr>
<tr>
<td>5. Remicade (arthritis) Merck/J&amp;J $7.6B</td>
</tr>
<tr>
<td>6. Rituxan (cancer) Roche $7.4B</td>
</tr>
<tr>
<td>7. Lantus (diabetes) Sanofi-Avantis $7.1B</td>
</tr>
<tr>
<td>8. Advair (asthma/COPD) GSK $6.8B</td>
</tr>
<tr>
<td>9. Herceptin (cancer) Roche $6.4B</td>
</tr>
<tr>
<td>10. NovoLog (diabetes) Novo Nordisk $5.7B</td>
</tr>
</tbody>
</table>

- World Market for Therapeutic Antibodies expected to top $50B by 2014
MAb Characterization and QA/QC is Challenging
...unlike Aspirin

- This presents some new analytical challenges...
Attributes & Combinatorics

- Pyro-Glu (2)
- Deamidation (3 x 2)
- Methionine oxidation (2 x 2)
- Glycation (2 x 2)
- High mannose, G0, G1, G1, G2 (5)
- Sialylation (5)
- C-term Lys (2)

\[(9600)^2 \approx 10^8 \quad \text{or} \quad 2 \times 6 \times 4 \times 4 \times 5 \times 5 \times 2 = 9600\]

Steven Kozlowski, FDA, WCBP2010
The Challenge in Monoclonal Antibody Analytics

Trends that increase the number of MAb samples requiring analysis:

- Increasing #s of MAb candidates in pipeline
- Advances in automation in cell culture & recovery dev., formulation screening
- QbD guidelines requiring enhanced MAb quality monitoring

MAb Development Workflow

- Drug Discovery
  - Target Identification and Validation
  - MAb Generation
- Preclinical Development
  - Cell Line Development
  - Clone Screening and Selection
- Clinical Development
  - Cell Culture and Purification Process Development
- Pre-Commercialization
  - Formulation
  - Lot Release Testing
  - Stability Studies
- Post-Commercialization
  - Product Improvements
  - Patent Extensions
  - Biobetters

MAb Analytics

- Product Titer
- Purity/Impurities
- Product ID
  - Intact mass
  - Sequence coverage
- Product Quality
  - Charge var.
  - Aggregates
  - Fragments
  - Modifications
- DMPK/Metabolite
- Glycans
Approaches to Increasing Speed, Throughput and Productivity

• Faster separations
  • Speed often trumps resolution
  • Achieved with shorter gradients, shorter columns and/or smaller particle size in LC

• Minimizing method development time
  • The development of “platform” (multi-product) methods
    • A platform method is one that can accommodate most or all samples without further method development, optimization or “tweaking”
    • One definition: “a platform method is one that will accommodate at least 85% of my samples…” without further method development

• Increasing throughput & reducing hands-on time through automation
  • Novel LC hardware configurations that increase throughput and automate multiple steps in MAb analysis

• Better integration of mass spec into the analytical workflow
Approaches to Faster LC Separations

• Faster separations can be achieved by…
  (A) Compressed gradients (in IEC)
    • Can speed up the separation; usually some loss of resolution

  (B) Shorter columns
    • Resolution compromised but often “good enough”

  (C) Smaller particle size resins
    • Speed up the separation, and without loss of resolution

  (D) Combinations of the above
CEX Platform for MAb Charge Variants Analysis

Nonporous Polymeric Beads

Polymeric grafts
WCX, SCX, WAX, SAX

Boundary
(Cross-linked Hydrophilic Layer)

Core
(Highly Cross-linked EVB-DVB)

Untreated MAb

Lysine Variants

KK

Acidic Variants

Basic Variants

MAb After Digestion with CpB

Y (Main Product)

Acidic Variants

Basic Variants

Dionex ProPac WCX-10
Dionex MAbPac SCX-10
Speeding up Analysis with Compressed Gradient

MAbPac SCX-10 Column, 4x150mm
52 min gradient, 70 min analysis time

MAbPac SCX-10 Column, 4x150mm
7 min gradient, 15 min analysis time
Faster Separations Without Loss of Resolution with Smaller Particle Size Resin

- Faster MAb charge variant analysis...by reducing column length, gradient time & particle size

Columns
A: MAbPac SCX-10, 10 μm, 4x250 mm
B: MAbPac SCX, 3 μm, 4x50 mm

Eluents
A: 20 mM MES + 60 mM NaCl pH 5.6
B: 20 mM MES + 300 mM NaCl, pH 5.6

Gradients
A: 15 - 36.44% B in 50min
B: 20 - 35% B in 10min

Flow Rate 1mL/min (0.6 mL/min for B)
Temp 30°C
Sample MAb, A: 10 mg/mL, B: 1mg/mL
Inj Volume A: 5 uL, B: 15 uL
Detection 280 nm
Multiproduct High-Resolution Monoclonal Antibody Charge Variant Separations by pH Gradient Ion-Exchange Chromatography

Dell Farnan* and G. Tony Moreno

Protein Analytical Chemistry, Genentech, 1 DNA Way, South San Francisco, California 94080

In the biotechnology industry, ion-exchange chromatography is widely used for profiling the charge heterogeneity of proteins, including monoclonal antibodies. Ionic strength based ion exchange separations, while having excellent resolving power and robustness, are product specific and time-consuming to develop. In the present work, a pH gradient based separation using a cation exchange column is described and shown to be a multiproduct charge sensitive separation method for monoclonal antibodies.

EXPERIMENTAL SECTION

Developed to-date, an IgG monoclonal antibody molecule is approximately 150 kDa in size and is composed of four interconnected peptide chains. A typical IgG is a dimer comprised of two heavy and two light chains assembled in the recognized “Y” shape motif.

Molecule characterization

Instruments and Equipment. Chromatographic experiments in this work were performed on a U3000 X2 Biocompatible liquid used as part chromatograph (Dionex, Sunnyvale, CA). Components of the chromatograph included dual ternary low-pressure gradient pumps, an autosampler with sample temperature control capability, a thermal compartment to enclose the column, and a four channel UV–vis detector. Instrument control, data acquisition, and compilation of results were performed using Dionex Chromeleon software, version 6.8.

Multiproduct CEX Platform for MAb Charge Variants Analysis—pH Gradient IEC

- Piperazine / imidazole / tris buffer system (PITs) on ProPac WCX-10

Multiproduct CEX Platform for MAb Charge Variants Analysis—pH Gradient IEC

Automation to Increase Throughput, Reduce Hands-on Time and Increase Productivity

• Tandem and parallel LC Configurations
  • To increase sample throughput of validated methods

• Multi-step automation
  • To automated multi-step workflow e.g. MAb purification and analysis on a single LC platform
  • Reduce hands-on time

• Other automation options (software)
  • MAb titer to mass calculation (using extinction coefficient)
  • Titer threshold setting to exclude low titer samples from further analysis
  • Dilutions or adjustment of injection volume to equalize load for subsequent analytical columns
LC Hardware Configurations for Increased Throughput

- **Dual Gradient Pump**
- **“Tandem” Configuration**
  - About 50% increased throughput
- **“Parallel” Configuration**
  - About 100% increased throughput
Case Study: High-Throughput MAb Titer Assay

The Challenge:
- Increased demand to run titer assays using Protein A column
- Average 500 samples per day
- Turn around time to report data is 24 hrs

Method as developed and validated:
- Std HPLC, UV detection, 5 minute cycle time, ternary gradient
- 3 Std HPLC systems were dedicated to running this assay

Solution: Parallel LC Configuration
- Optimization of system setup (lower delay vol) brought the runtime down from 5 to 4.3 minutes
- Running in parallel mode they now have a data point every 2.15 minutes

Net result: about 24 hrs of analysis time for 500 samples on a single LC system
Parallel LC for Dual Assays

Increases throughput, eliminates the need to duplicate sample plates
Configuration for Multi-Step Automation

- Cell Culture Fluid
- Prep Protein A Capture of IgG
- Fraction Collection
- Neutralization
- Aggregate analysis by SEC
- Charge variant analysis by IEC
- Data analysis and clone selection

Dual Gradient Pump

- Pump 1
- Pump 2

Autosampler with Fraction Collection and Re-Injection

- SCX
- SEC

UV

IN

OUT

Waste

TCC

Protein A
Automation of MAb Purification and SEC & IEC Analyses

1. Protein A Purification of IgG (MAb)
   - Purified Antibody

2. SEC Aggregates Analysis
   - High MW

3. IEC Charge Variants Analysis
   - Acidic Variants
   - Basic Variants

- Autosampler with Injection>Fraction Collection>Re-Injection
- Dual Gradient Pump
Reducing Analysis Time by Automatically Rejecting Low Titer Clones

- Cell Culture Fluid
- Prep Protein A Capture of IgG
- Fraction Collection
- Neutralization
- Aggregate analysis by SEC
- Charge variant analysis by IEC
- Data analysis and clone selection

Titer check 2.1x30mm pA column

Post-acquisition mode in Dionex Chromeleneon CDS

Prepurification Analysis

- Check if Ion present?
  - Yes
    - Ion Present Test
      - Passed
      - Add to Purification Sample List
      - Automated adjustment of Injection Volumes
      - Automated sequence creation for pA, SEC & IEC
    - Failed
      - No Purification
  - No
    - Amount Test
      - Passed
      - Add to Purification Sample List
      - Automated adjustment of Injection Volumes
      - Automated sequence creation for pA, SEC & IEC
    - Failed
      - No Purification
Seamless Integration of Salt-Based SEC, IEC, HIC Methods to MS for Characterization of MAb Products and Impurities

**Automated Bio LC-LC/MS**

- 1-D LC
  - SEC, IEC or HIC

**Sample Analysis**

Using HR/AM Mass Spectrometers

**Data Analysis**

Deconvolution of ESI-MS to zero charge accurate mass

- Fraction Collection of MAb Products or Impurities
  - [Automated in Autosampler]

- Automated 2-D LC
  - SPE/Desalting on RP followed by MS

Exact Mass Determination, Bottom-up, and Top-Down Protein Characterization

[Image of Thermo Fisher Scientific equipment]
Several factors are increasing the numbers of MAb samples requiring screening and characterization

- This has driven the need for faster and higher throughput analytical solutions
- There is a desire for methods that are more “platform” (multi-product) in nature
- Solutions need to reduce non value-added time, and minimize hands-on time, enabling labor to focus on value-added activities such as data analysis and in-depth characterization

Solutions in use and under development include:

- Solutions that reduce method development time
- Columns and methods that enable faster separations
- Novel LC and UHPLC configurations that, increasing throughput, automate workflows and reducing hands-on time
- Developments that better integrate various analytical steps

The benefits that accrue from increased analytical throughput are expected to drive continued developments in this area
Thank you—Q&A

Partners in driving value creation