Engineering antibodies beyond affinity: correlation of key biophysical characteristics with antibody specificity and pharmacokinetic properties

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WCBP 2015
27th-29th January 2015
Overview

1. Why antibody developability is important

2. Engineering beyond affinity: anti-NGF mAb case study
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1. Why antibody developability is important
2. Engineering beyond affinity: anti-NGF mAb case study
Lead Selection with Functional Focus

- Kd for target antigen
- In vitro / in vivo potency
- Target clones
- Target specificity, species cross-reactivity

MedImmune
Lead Selection with Developability Focus

- Chemical Stability
- Solution Properties
- Physical Stability

Target clones
Lead Selection with Developability Focus

- Chemical Stability
  - Fragmentation
  - Deamidation
  - Oxidation
  - Asp isomerization
  - Sequence variants
  - O-glycosylation
  - Glycation

- Solution Properties
  - Reversible self-association
  - Solubility
  - Viscosity

- Physical Stability
  - Conformational stability
  - Colloidal stability
  - pI
  - Aggregation

Target clones
Lead Selection with Developability Focus

- Chemical Stability
  - Target clones
- Solution Properties
  - StarGazer 384-well Aggregation analysis ($T_{agg}$)
- Physical Stability
  - Size exclusion Chromatography (SEC)

In Silico aggregation prediction tool

Nanopero HT cIEF

StarGazer 384™ system
Overview

1. Why antibody developability is important
2. Engineering beyond affinity: anti-NGF mAb case study
Anti-NGF Antibody – Medi578

◆ Target
  – Nerve growth factor

◆ Mechanism of action
  – Selectively antagonises binding of NGF to its receptors TrkA and p75

◆ Characteristics
  – Human, derived from phage display library
  – Selective over other neurotrophins
  – Cross-reactivity with cyno and rat NGF
  – $K_D = 69pM$
  – Active in NGF driven \textit{in vitro} assays

◆ Modelling suggested that a higher affinity/potency antibody would suppress serum NGF \textit{in vivo} >90%

Goal to improve the affinity and potency of Antibody 10-fold
Affinity Maturation of MEDI578 → MEDI1912

MEDI-578 Epitope competition assay

% Specific Binding vs. conc (log/M)

Number of CDRs with Mutations vs. Potency improvements relative to MEDI578

K_{off} Ranking vs. IC_{50} Ranking

Spearman r = 0.818
P < 0.0001
Anti-NGF antibody MEDI1912

- Most improved variant, MEDI1912, exhibited ~10 fold improvement
- MEDI1912 contains 12 mutations (compared to MEDI578)

10-fold improved cellular potency

Dorsal root ganglion cell survival  
PC12 cell survival

<table>
<thead>
<tr>
<th>K_{on} (1/Ms)</th>
<th>K_{off} (1/s)</th>
<th>K_{D} (pM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.0 \times 10^7</td>
<td>(20 – 3 \times 10^{-5})</td>
<td>(1.6 – 9.8)</td>
</tr>
</tbody>
</table>
MEDI1912 is specific for NGF

NGF epitope competition assay
MEDI1912 reduces mechanical hypersensitivity in a mouse intra-plantar FCA model

![Graph showing the effect of MEDI1912 on mechanical hypersensitivity](image)

- PBS i.v.
- Isotype control i.v.
- MEDI1912 0.3 mg/kg i.v.
- MEDI1912 1 mg/kg i.v.
- MEDI1912 3 mg/kg i.v.

N=12 per group. Data analysed using 2 way ANOVA with time and treatment as dependant factors. Subsequent statistical significance obtained using Bonferroni's Post Hoc test.
MEDI1912 exhibited significant problematic biophysical and DMPK characteristics

Reversible self-association
Monomer-dimer-trimer equilibrium
Adsorption to surfaces
e.g. SEC column matrix, filter membranes
Low purification yield
Poor solubility
Phase-separation & opalescence issues
High viscosity (when >30mg/mL)
Poor PK in both rat (t½ = 4 days) & NHP
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**In silico** prediction of protein aggregation propensity

- Spatial aggregation propensity (SAP) algorithm developed by Professor Bernhardt Trout, MIT
  - Predicts aggregation propensity based upon atomistic analysis
- SAP algorithm incorporated into Accelrys Discovery Studio

**Chennamsetty et al., 2009**
Engineering MEDI1912 biophysical properties via a structure-based methodology

- Spatial aggregation propensity (SAP) software used to predict aggregation prone regions on IgG surface
  - Three potential positions of interest identified: W30S, F31T (both in VH CDR1) and L56T (VH CDR2)
Engineering MEDI1912 biophysical properties via a structure-based methodology

Spatial aggregation propensity (SAP) software used to predict aggregation prone regions on IgG surface

- Three potential positions of interest identified: W30S, F31T (both in VH CDR1) and L56T (VH CDR2)
Creation & screening of variant IgGs by HPLC-SEC
Mutant STT ameliorates aberrant biophysical properties

HPLC-SEC

Analytical Ultracentrifugation

Dynamic Light Scattering
Mutant STT ameliorates aggregation of MEDI1912
Mutant STT retains affinity and potency of MEDI1912

- STT demonstrates identical affinity for NGF and equipotency in relevant cellular assays
  - The three mutations were not associated with decreased affinity

<table>
<thead>
<tr>
<th>Affinity (BIAcore) for NGF</th>
<th>MEDI1912</th>
<th>1.6 - 9.8 pM</th>
</tr>
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<tbody>
<tr>
<td>STT</td>
<td>1.8 – 8.3 pM</td>
<td></td>
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</tbody>
</table>

**pERK Potency Assay**

- NIP228 IgG1TM YTE
- MED1912 IgG1TM YTE
- MEDI1912_STT IgG1TM

% NGF-induced pERK vs IgG Log [M]
Mutant STT has reinstated favourable DMPK properties

**Rat PK study**

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Dose</th>
<th>T_{max} (days)</th>
<th>CL (mL/day/kg)</th>
<th>T_{1/2} (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MEDI-1912 (WFL)</td>
<td>3.0 mg/kg</td>
<td>0.0104</td>
<td>24.5</td>
<td>4.032</td>
</tr>
<tr>
<td>MEDI-1912 (STT)</td>
<td>3.0 mg/kg</td>
<td>0.0104</td>
<td>7.26</td>
<td>9.43</td>
</tr>
</tbody>
</table>

STT shows no discernible non-specific binding by IHC
Summary

- With higher affinity mAbs can come other challenges
  - MEDI1912 exhibited aberrant CMC/manufacturability and non-linear, impaired PK profile that endangered product development

- *In silico* prediction can be used to enhance developability characteristics

- Mutant STT retains all of the affinity / potency gains of MEDI1912, but with optimal biophysical characteristics and PK profile

- Clear link between biophysical – DMPK – tissue specificity

- Generic engineering approach could be used to improve the biophysical properties of any therapeutic protein
Acknowledgements

- MEDI578 and MEDI1912 project teams
- Claire Dobson
- Andrew Buchanan
- Bojana Popovic
- Chris Lloyd
- Daniel Higazi
- Arthur Lewis
- David Lowe
- Tristan Vaughan
- Biopharmaceutical Development
  - Chris van der Walle
  - David Hayes
  - Catherine Galy
  - Leanne Amery
  - Sofia Ekizoglou
  - Richard Turner

- BSU650
- DMPK Team
  - Jo Goodman
- AstraZeneca Discovery Sciences:
  - Lise-Lotte Olsson
  - Anna aagaard
  - Linda Cederblad
  - Niek Dekker
  - Paul Wan
  - Tomas Akerud
- Biologics Expression Team
  - Neil Brikett
  - Anna Czyz
  - Sonia Raithatha
  - Melanie Medcalf
  - Carolina Casado
  - Nathan Hudson
  - Richard Porter
  - Nicola Forrest-Owen
  - Robin Butler