Importance of Glycobiology in Medicine

- Recombinant glycoprotein drugs:
  - Influence of carbohydrates on clearance
  - Targeting glycoprotein to site of action
- Therapeutic products based on:
  - Carbohydrate antigens
  - Glyco-enzyme inhibitors
  - Cell adhesion receptors

Recombinant Glycoproteins

- Most successful biotechnology products with annual sales over $5 billion (EPO, TPA, FM-CSF)
- Approximately 60 recombinant glycoproteins in development
- Carbohydrate groups critically important for pharmacodynamics of these drugs
Properties of N-linked Carbohydrates Affecting Bio-activity of Glycoprotein Drugs

- Complete glycosylation is required to prevent rapid clearance by hepatocytes and macrophages (e.g. All)
- Bulky tri- and tetra-antenary chains reduce clearance by the kidney (e.g. EPO, NESP)
- Target glycoproteins to the desired cell type (e.g., glucocerebrosidase)

Incomplete Glycosylation: Problem for Glycoprotein Drug Development

- Overproduction of glycoproteins outstrips the producer cell’s capacity to complete glycosylation
- Complex N-linked carbohydrates typically terminate in NeuAc-Gal-GlcNAc-Man sequence
- Incomplete N-linked oligosaccharides with exposed Gal or GlcNAc/Man result in rapid clearance
  - *Asialo-glycoprotein (Gal) receptor in hepatocytes*
  - *GlcNAc/Man receptor on macrophages*
C-type Lectins Group 2: Clearance of plasma glycoproteins

<table>
<thead>
<tr>
<th>Glycoprotein Receptor</th>
<th>Cell Type</th>
<th>Specificity</th>
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<tr>
<td>Asialo-GP-R</td>
<td>Hepatocytes</td>
<td>Galβ1,4GlcNAc</td>
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<td>Asialoagalacto-GP-R</td>
<td>Kupffer cells</td>
<td>GlcNAc/Fucose</td>
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<td>Macrophage lectin</td>
<td>Macrophages</td>
<td>GlcNAc-R Man-R</td>
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Influence of Terminal Sialylation on Half Life \textit{In vivo}

![Graph showing influence of terminal sialylation on half life](image)
**Importance of Bulk Properties: EPO**

- Under glycosylated EPO (rHuEPO, erythropoietin) is rapidly cleared
- Clearance mediated by:
  - Gal and GlcNAc/Man receptors (minor)
  - Secretion into the urine via the kidney due to small size (major)
- Optimal glycosylation requires:
  - Fully sialylated N-linked chains to prevent clearance by carbohydrate specific receptors
  - Tetra-antennary branches instead of bi- or tri-antennary to reduce renal clearance
Anion Exchange Chromatography of rHuEPO Yields Multiple Sialylated Isoforms

Isoform 1

Sialic Acids = 14

(MW=30,400)

Isoform 2

Sialic Acids = 10

= Sialic Acid

Efficacy of Sialylated Isoforms of EPO in Increasing Red Blood Cells in Mice

Egrie et al. 2001 84:3-10
NESP is Engineered to Contain Two Extra N-linked Sugar Groups

rHuEPO

\[=\text{Sialic Acid}\]

(MW=30,400)

NESP

(MW=37,100)

Comparison of NESP and rHuEPO

Reduced clearance time of NESP allows 1 time/wk dosing instead of 3 time/wk

- Reduced clearance time of NESP allows 1 time/wk dosing instead of 3 time/wk

Egrie et al. 2001 84:3-10
Cerezyme: Enzyme Replacement Therapy for Gaucher's Disease

Disease: Glucocerebrosidase deficiency (Gaucher's disease)

Treatment: Enzyme replacement

Strategy: Target glucocerebrosidase to macrophage lysosomes by engineering carbohydrate to expose mannose residues on N-linked sugars

Opportunity: Use similar strategy to target other drugs to cells (Novazyme)

C-type Lectins Group 2: Clearance of plasma glycoproteins

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<td>GlcNAc-R Man-R</td>
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Controlling Sugar Addition on Cerezyme™

N-linked Oligosaccharide Processing in CHO cells

Targeting of Cerezyme to Macrophage Lysosomes Through Mannose BP
Target Glucocerebrosidase to Macrophages

Example: • Glucocerebrosidase deficiency (Gaucher’s disease)

Treatment: • Enzyme replacement therapy

Strategy: • Target glycocerebrosidase to macrophage lysosomes using terminal Man exposed by glycosidase treatment of N-linked oligosaccharides

Opportunity: • Use similar strategy to target other drugs to hepatocytes or macrophages

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  - Cell adhesion receptors
Recognition of Cell Surface Carbohydrates

Carbohydrate Antigens of Bio-medical Importance

- ABO blood groups
- Xenotransplantation antigen
- Tumor antigens
**Xenotransplantation: The opportunity**

- Over 100,000 patients die each year waiting for organ transplants due to short supply of human organs.
- Use of animal organs, especially pig organs, is viewed by many as a long term solution with $1-3$ billion market potential.
- Overcoming the barriers to xenotransplantation are a major challenge.
- One of the biggest challenges results from a carbohydrate antigen in pigs that is not found in humans.

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**αGal Carbohydrate antigen:**

**Major Barrier to Xenotransplantation**

**Objective:**
- Prevent hyper-acute rejection of porcine organ transplants.

**Problem:**
- High titer antibodies in human serum to **Gal**α1,3Gal epitope on porcine tissues causes acute rejection.

**Solution:**
- Generate KO pigs missing the α1,3 galactosyltransferase. Donor tissues will be devoid of this key Xenoantigen.
**α1,3 Galactosyltransferase KO Pigs**

- Successful KO of α1,3 galactosyltransferase in pigs
- Breeding of heterozygous KO pigs is underway
- Homozygous animals will eliminate acute rejection of pig organs

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**Tumor Antigens**

- Tumor cells often express carbohydrate ‘antigens’ that are normal glycoprotein or glycolipid carbohydrates, but expressed in unusual ways that are immunologically distinct:
  - Oncofetal antigens (expressed only in fetus).
  - Increased density of expression compared to normal tissue
  - Novel ‘glycopeptide’ antigen not expressed in normal tissue
- Opportunity to stimulate immune system to attack cancer cells.
### Carbohydrate Cancer Vaccines in Development

<table>
<thead>
<tr>
<th>Indication</th>
<th>Carbohydrate</th>
<th>Company</th>
<th>Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>Melanoma</td>
<td>GM2</td>
<td>Progenics/BMS</td>
<td>Phase III</td>
</tr>
<tr>
<td>Breast Cancer/Melanoma</td>
<td>GM2/GD2</td>
<td>Progenics/BMS</td>
<td>Phase II</td>
</tr>
<tr>
<td>Breast</td>
<td>SAα2,6GalNAc- (TN-antigen)</td>
<td>Biomira/Chiron</td>
<td>Phase III</td>
</tr>
</tbody>
</table>

### Therapeutic Products Based on Glycobiology Concepts

- Carbohydrate antigens
- Microbial adhesion to host cells
- Cell adhesion receptors
Examples of Microbial Carbohydrate Binding Proteins

<table>
<thead>
<tr>
<th>Binding Protein</th>
<th>Carbohydrate Ligand (host cell)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Influenza virus HA</td>
<td>NeuAcα2,6Gal (human virus) NeuAcα2,3Gal (avian virus)</td>
</tr>
<tr>
<td>H. pylori</td>
<td>Fucα1,2Galβ1,4GlcNAc (Lewis b) Fucα1,3 Galβ1,4Glc-R (GM1)</td>
</tr>
<tr>
<td>Cholera toxin</td>
<td>Galb1,3GalNAcβ1,4 NeuAcα2,3Galβ1,4Glc-R (GM1)</td>
</tr>
</tbody>
</table>

Influenza Virus Binds to Sialic Acids on Host Cells

- Sialic Acid
- Hemagglutinin
- Sialidase (Receptor Destroying Enzyme)
Mechanism of Influenza Virus Sialidase Inhibitors (Tamiflu and Relenza)

- Sialidase prevents destruction of sialic acid receptors and subsequent release of virus

Carbohydrate Specific Cell Adhesion Receptors

**Example:**
- Selectin mediated recruitment of neutrophils (other leukocytes)

**Indications:**
- Acute (and chronic) inflammation

**Approach:**
- Inhibition of selectin function

**Opportunity:**
- Therapeutic benefit in modulating other cell adhesion events mediated by mammalian carbohydrate binding proteins
Therapeutic Products Based on Glycobiology Concepts

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- Microbial adhesion to host cells
- Cell adhesion receptors

Selectin Mediated Neutrophil Trafficking to Sites of Inflammation

[Diagram showing white blood cell rolling and sticking to endothelial cells, progressing to migration through tissue at an inflammation site, with selectins and integrins involved in the adhesion process.]
Pharmaceutical Approaches to Modulating Carbohydrate Protein Interactions

**Inhibit Ligand Binding**
- Carbohydrate or ligand mimic
- Antibody to Selectin

**Inhibit Ligand Synthesis**
- Glycosyltransferase inhibitor

**Selectin Inhibitor Approaches in Development**

<table>
<thead>
<tr>
<th>Indication</th>
<th>Cell type</th>
<th>Approach</th>
<th>Company</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reperfusion Injury</td>
<td>Neutrophils</td>
<td>Glycopeptide (P-selectin ligand inhibitor)</td>
<td>Genetics Institute / Wyeth</td>
</tr>
<tr>
<td>(Acute)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rheumatoid Arthritis</td>
<td>Th-2 / CD4 T cells</td>
<td>Fucosyltransferase Inhibitor</td>
<td>Hoffman La Roche / BioXcell</td>
</tr>
<tr>
<td>Psoriasis Asthma</td>
<td>Leukocytes</td>
<td>Selectin ligand mimetic</td>
<td>Texas Biotech</td>
</tr>
</tbody>
</table>
### Other Glyco-related Therapeutics

<table>
<thead>
<tr>
<th>Indication</th>
<th>Drug</th>
<th>Approach</th>
<th>Company</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacterial Infection</td>
<td>Vancomycin</td>
<td>Glycopeptide antibacterial</td>
<td>Eli Lilly</td>
</tr>
<tr>
<td>Gauchers Disease</td>
<td>Vevesca</td>
<td>Inhibitor of glucosyl-ceramide synthetase</td>
<td>Oxford Glycosciences</td>
</tr>
<tr>
<td>Cancer</td>
<td>GDOO39</td>
<td>Alters carbohydrates on tumor cells to prevent metastasis</td>
<td>Glycodesign</td>
</tr>
<tr>
<td>Cancer</td>
<td>PI-88</td>
<td>Blocks angiogenesis</td>
<td>Progen (Aus)</td>
</tr>
<tr>
<td>Hepatitis C</td>
<td>UT321B</td>
<td>Blocks Hepatitis C virus from infecting cells</td>
<td>United Therapeutics</td>
</tr>
<tr>
<td>Pancreatic Cancer</td>
<td>GCS-100</td>
<td>Blocks binding of sugar binding to tumor cells</td>
<td>Glycogenesis</td>
</tr>
</tbody>
</table>

### Selected References

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