The Treatment of Rheumatoid Arthritis in 2018:
A Triumph of Translational Medicine

Richard Brasington, MD
Professor of Medicine
Rheumatology
Washington University in St Louis School of Medicine
Disclosures

• Speaking:
  – Pfizer (tofacitinib)
  – Abbvie (adalimumumab)
  – Novartis (secukinumab)
  – Mallinckrodt (repository corticotropin)

• Clinical trials (in past)
  – Abbvie (adalimumumab)
  – Amgen (anakinra)
  – Genentech (rituximab and tocilizumab)
  – Bristol Myers Squib (abatacept)
Learning objectives

• Understand the huge change in the treatment of rheumatoid arthritis which has occurred over the last three decades
• Understand a hypothesis-driven approach to identifying targets for new medications
• Recognize the particular toxicities which can occur with these new medication
• Understand the meaning of “biosimilar”
Rheumatoid Arthritis

- Progressive, systemic, inflammatory disorder
- Affects 1% of population
- Women to men 3:1
- Peak incidence 30-40 years
- Unknown etiology
- Characterized by
  - Symmetric synovitis
  - Joint erosions
  - Multisystem extra-articular manifestations
Pathology of the rheumatoid joint

• The normal synovial tissue lining is one or two cell layers thick.
• In RA, the synovium proliferates to many cell layers thick, is infiltrated with lymphocytes, and develops many new blood vessels.
• The activated effector cells within the proliferating synovium include osteoclasts, which degrade the underlying bone, producing erosions and joint space narrowing.
NORMAL SYNOVIIUM
RHEUMATOID SYNOVIIUM
SYNOVITIS OF SMALL JOINTS OF HAND
Clinical appearance of joints in RA

- Synovial proliferation produces swelling of joints
- This feels “squishy” or boggy on examination
- The hands are always involved, especially the MCP and PIP joints. The MTP joints in the forefoot are similarly involved. It is in the hands and feet where deformities are most apparent.
- Large joints may also be involved: wrist, elbow, shoulder, hip, knee, ankle
Rheumatoid synovium growing over bone
The Pathogenesis of Rheumatoid Arthritis

NORMAL RHEUMATOID ARTHRITIS

Synovial membrane

Cartilage

Capsule

Inflamed synovial membrane

Pannus

Synovial fluid

Major cell types: T lymphocytes, macrophages

Minor cell types: fibroblasts, plasma cells, endothelium, dendritic cells

Major cell type: neutrophils

SUBLUXATION OF MCPS WITH ULNAR DRIFT
SUBLUXATION OF MTPS
Course of Rheumatoid Arthritis: Schematic Representation

Severity (arbitrary units)

Duration of disease (years)

- Inflammation
- Disability
- Radiographs

In 1982 I began my rheumatology training at The University of Iowa Hospitals and Clinics.
Evolution of RA Treatment

• 1982 (when I was a first year fellow)
  – Do not start second line therapy until patient has had RA for at least a year, because it might go away
  – The treatments are worse than the disease
  – #1: Injectable gold (Myochrysine, Solganol)
  – #2: D- penicillamine
  – #3: Azathioprine
  – #4: Sulfasalazine and hydroxychloroquine (Plaquenil)
  – MTX: radical option with admission to CRC and liver biopsy and baseline and every six months
My first job after fellowship was as a full time clinician at Marshfield Clinic in Wisconsin.
Evolution of RA Treatment

• 1986 (when I started my first job at Marshfield Clinic)
  – Consider starting MTX even if patient had not failed gold
  – 50% of RA patients disabled at five years
  – Oral form of gold: Ridaura (auranoffin)
  – Recognition that stage IV RA has same mortality curve as 3 vessel coronary artery disease
In 1996, I came to Washington University to be a “clinician educator”
Evolution of RA Treatment

• 1996 (when I started at Washington University
  – Maybe treatments were not working because not started EARLY enough
  – Start DMARDs at time of diagnosis
  – Combination DMARDs, esp. “Triple Therapy”
## DMARDS in RA as of 1999

<table>
<thead>
<tr>
<th>Agent</th>
<th>Time to benefit</th>
<th>Potential for toxicity</th>
<th>Toxicities to monitor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methotrexate</td>
<td>2-3 mo</td>
<td>Moderate</td>
<td>Bone marrow, liver, lung</td>
</tr>
<tr>
<td>Sulfasalazine</td>
<td>2-3 mo</td>
<td>Low</td>
<td>Bone marrow, liver, rash</td>
</tr>
<tr>
<td>Hydroxychloroquine</td>
<td>3-6 mo</td>
<td>Low</td>
<td>Macular damage</td>
</tr>
<tr>
<td>Leflunomide</td>
<td>2-3 mo</td>
<td>Low</td>
<td>Diarrhea, liver, rash, headache, risk of teratogenicity</td>
</tr>
<tr>
<td>Cyclosporine</td>
<td>4–8 wk</td>
<td>High</td>
<td>Kidney, HTN, immunosuppression</td>
</tr>
<tr>
<td>Gold, parenteral</td>
<td>3–6 mo</td>
<td>Moderate</td>
<td>Bone marrow, kidney</td>
</tr>
<tr>
<td>Azathioprine</td>
<td>2–3 mo</td>
<td>Moderate</td>
<td>Bone marrow, liver</td>
</tr>
<tr>
<td>Minocycline</td>
<td>1–3 mo</td>
<td>Low</td>
<td>Hyperpigmentation, dizziness, vaginal yeast infections</td>
</tr>
</tbody>
</table>
Evolution of RA Treatment

• 1998 was the beginning of what I call “The Golden Age of Rheumatology”
  – October: leflunomide (Arava) released - an oral DMARD comparable to MTX!
  – November: etanercept (Enbrel) approved at 25 mg twice a week - the first “biologic DMARD”
    • Initially referred to as “biologic agent” (same category as anthrax...)
  – December: Celebrex (celecoxib) approved - the end of NSAID GI toxicity was in sight...
The major therapeutic advance in the last two decades has been the development of therapies directed at specific individual molecules.
We have participated in trials of:

- Etanercept
- Infliximab
- Anakinra
- Adalimumab
- Abatacept
- Certolizumab pegol
- Golimumab
- Rituximab
- Tocilizumab
- Tofacitinib
Adaptive and Innate Immune Processes within the Joint in Rheumatoid Arthritis.

New RA Targets (1998 and after)

- Tumor Necrosis Factor
- T cell co-stimulation (CD 28)
- B cell depletion (CD 20)
- Interleukin 6 receptor
- Janus kinase
Biologic: From Cloned Gene to Purified Protein

Production Cell Line

Infliximab Genes/DNA

DNA → mRNA → Protein

Perfusion Bioreactor

Purification

Infliximab

TNF α
TNF inhibitors

Etanercept
Infliximab
Adalimumab
Golimumab
Certolizumab pegol
Synthesis and Function of TNF-α
Cytokine Disequilibrium in RA

Proinflammatory

TNF$\alpha$
IL-1

Antiinflammatory

IL-1ra
sIL-1R
sTNFR
IL-10
IL-4
IL-11
ETANERCEPT
a “fusion protein”
Basic Antibody Structure

- **Antigen combining site**
- **Light chain**
- **Fab region**
- **Disulfide bonds**
- **Heavy chains**
- **Fc region**
- **Hinge**
- **Variable domains**
- **Constant domains**

**Heavy chains**

**Fc region**
Chimera
Mouse vs. Chimeric

Mouse A2

V L
Cκ

CH1

CH2

CH3

Remicade cA2

V H

VL Cκ

Cκ

Mouse

Human
Structure of cA2

- Chimeric (mouse/human) IgG₁ monoclonal antibody
- Binds to TNFα with high affinity and specificity
Chimeric A2 (cA2) Monoclonal Antibody

Monoclonal antibody (MAb) anti-TNF

Target cell

Macrophage

TNF
Categories of Therapeutic Antibodies

Murine  Chimeric  Humanized  Human

Mouse  Human
Phage Display

Human B-cells

PCR

VH
VL

Clone

Make Phage

Phage

~10^{10} Unique Specificities

Antibody Fragment

Im mobilized Antigen

Antibody

Secretion

Reformat, Transfect Cell

Recover DNA

Wash, elute specific phage

Specific phage

Reformat, Transfect Cell

Recover DNA
D2E7 contains unique, human CDR regions, allowing specific binding to TNF.
Antibodies from Transgenic Mice

- **Normal Mouse**
  - Immunize with human TNFα
  - Mouse Antibody Genes Suppressed
  - Mouse Antibody

- **Human Antibody Transgenic Mouse**
  - Immunize with human TNFα
  - Human Antibody Genes Inserted
  - Golimumab (human antibody)

---

Golimumab

TNF inhibitors also work in

- Psoriasis
- Psoriatic arthritis
- Ankylosing spondylitis
- Crohn’s disease*
- Ulcerative colitis*
- Uveitis*

*monoclonal antibodies, not etanercept
Unknown stimulus in peripheral tissues

Activation of innate immunity

Dendritic cell

Macrophage

T-cell receptor

MHC-bound peptide

Antigen presenting cell

CD28

CD80/86

Costimulation

Lymph node

Activated T cells

Recruitment of effector cells including macrophages

Mediators
(Interleukin-1, interleukin-6, TNF-α, chemokines, prostaglandins, and proteases)

TNF-α

TNF inhibitors

p75 TNF receptor

Fc portion of IgG

Etanercept

Infliximab

Adalimumab

(anti-TNF antibodies)

Target cells
(immune, inflammatory, and endothelial cells)

p75 TNF receptors

Inflamed synovium

Pannus

Joint space

Cartilage

Bone
Complications of TNF Blockade

- Tuberculosis and other serious infections
- Demyelinating disease
- SLE
- CHF
- ?Lymphoma?
Lymphoma

• FDA has added this to the prescribing information for all drugs on the market
• This is difficult assessment, since RA patients most likely to receive these drugs already have an increased incidence of lymphoma
• These agents are absolutely contraindicated in patients who have had lymphoma
T cell co-stimulation blockade

Abatacept
T Cell Role in RA

• Most of the lymphocytes in the RA synovium are T cells
• So T cells must be important
• This is the first strategy targeting T cells which has been effective
Co-stimulation blockade

• T cell activation requires two signals
  – First signal: antigen binding by T cell receptor
  – Second signal: binding of CD-28 by CD-80/86

• This therapy blocks the second signal, preventing T cell activation
T cell activation plays an important role in the immunopathology of RA.

Activated APC (macrophage) interacts with Activated T cell through Signal 1 and Signal 2.

- Signal 1: MHC-TCR interaction
- Signal 2: CD80/86-CD28 interaction

Cytokine Production:
- IL-1β, IL-6, TNF-α

Clonal Signal 1 and Signal 2 lead to ROS Production, MMP Production, and Cytokine Production, which contribute to Joint Inflammation & Destruction.

(TNF-α)
T cells require 2 signals for activation: Costimulation plays an important role

Signal 1

**APC**

- MHC
- TCR

**T cell**

- CD28

**TCR signal only**

= No activation

Signal 2

**Activated APC**

- MHC
- TCR
- CD80/CD86

**Activated T cell**

- CD28

**TCR + CD28**

= Full activation

Costimulatory signal
CD28 system

• CD28 on T cell surface
  – Member of Ig superfamily
  – Binds to CD 80/CD 86 on APCs
  – Binding stimulates release of IL-2 and promotes T cell survival
CD28 system

- Cytotoxic T-Lymphocyte-Associated Antigen 4 (CTLA4) also on T cell surface
  - Expressed within hours or days of T cell activation
  - Homologous to CD28
  - 500-2500 times the avidity for CD 80 and CD 86
  - Binding transmits a signal that inhibits T cell activation
CTLA4 is the receptor that binds CD80/86 inhibiting T cell activation

CTLA4* has greater avidity for CD80/CD86 than CD28
When CTLA4 (rather than CD28) Binds CD80/CD86, T cell activation does not occur

CTLA4 has greater avidity for CD80/CD86 than CD28
CTLA4Ig (abatacept) is a **Fusion protein**

A human trans-membrane protein

CTLA4

A human antibody

IgG1

Cell Membrane
CTLA4Ig Inhibits T Cell Activation via Costimulation Blockade
Depletion of B lymphocytes

Rituximab
B cell depletion

• In the past, B cells were not thought to be especially important in the pathogenesis of RA
• However, several years ago it was recognized that B cell depletion was very effective
• It is not clear why this treatment works, but it is thought that benefit results from reducing B cell activity other than antibody production
  – B cells act as antigen-presenting cells
  – B cells produce inflammatory cytokines
CD20: An Ideal Target

- 297 AA membrane-associated phosphoprotein (33–37 kD)
- Selective expression
  - NOT expressed on stem cells, pro-B cells, or plasma cells

**Rituximab: Anti-CD20 Monoclonal Antibody**

- **Chimeric** murine/human monoclonal antibody
  - Variable light and heavy chain regions from **murine** model
  - **Human** IgG, \( \kappa \) constant region
Potential Targets in B-Cell Lineage

Antigen Independent Phase

- CD45 (AKA B220) surface marker
- Surrogate light chain
- IgM

Antigen Dependent Phase

- Antigen
- CD40L and cytokines
- IgM, IgD, IgA, or IgE

Secreted IgG, IgA, IgE, or IgM

Targets for rituximab

- Pro-B-cell
- Pre-B-cell
- Immature B-cell
- Mature B-cell
- Activated B-cell
- Plasma cell
Potential Targets in B-Cell Lineage

Antigen Independent Phase

Antigen Dependent Phase

CD45 (AKA B220) surface marker

Surrogate light chain

IgM

IgM

IgM, IgD, IgA, or IgE

CD40L and cytokines

Secreted IgG, IgA, IgE, or IgM

Depleted by rituximab

Pro-B-cell

Pre-B-cell

Immature B-cell

Mature B-cell

Activated B-cell

Plasma cell

B-cell Depletion and Recovery

Median CD19 (Cells x10^3/L)

Time (weeks)

MTX  RTX  RTX+CTX  RTX+MTX

Rituximab also works for

- Immune thrombocytopenia purpura
- Multiple sclerosis
- ANCA associated vasculitis
- Lupus?
IL-6 Blockade

Tocilizumab
Sarilumab
IL-6 is Produced by Multiple Cell Types and Is Associated with Numerous Biologic Activities\textsuperscript{1,2}

Monocytes/macrophages

Endothelial cells

Mesenchymal cells, fibroblasts/synoviocytes

T-cell activation

Hepatocytes

Acute-phase response

Hepcidin, CRP
↓ CYP450

Maturation of megakaryocytes

Osteoclast activation

Bone resorption

B-cells

Auto-antibodies (RF)

Hyper-γ-globulinemia

Thrombocytosis

IL-6 in RA: Elevated Levels of IL-6 Correlate with Disease Activity

- Elevated serum IL-6 correlates with RA disease stage\(^1\)
- IL-6 correlates with severity of joint destruction\(^2\)

Tocilizumab

Complementarity Determining Region (CDR)

- **Humanized** mAb IgG1
  - Human heavy and light chains
  - Only CDR is of murine origin

- Binds soluble and membrane bound IL-6R
Tocilizumab Inhibits IL-6R Membrane-bound Signaling and Trans-signaling

IL-6 cannot bind

Unique Toxicities for Tocilizumab

• Elevated transaminases
• Elevated lipids
• Colonic perforations?
Tocilizumab also works for

• Giant cell arteritis
• Scleroderma?
The “Holy Grail” in drug development has been:

A orally administered small molecule (aka a “pill”) which has the effectiveness of a “biologic DMARD”
Cytokines

• A broad collection of secreted factors of different structural families which use different families of receptors

• The type I and type II cytokine-receptor family binds Interferons, many Interleukins, and Colony Stimulating Factors
  – Erythropoietin
  – Thrombopoietin
  – Growth hormone
  – Prolactin
  – Leptin
Cytokines bind to receptors…

- Cytokine receptor (I, II)
- TNF receptor
- Toll, IL-1 receptor
- IL-17 receptor
- Receptor tyrosine kinase
- TGF-β receptor
- Chemokine receptor
Transmembrane Signalling Results

Janus Kinases (“JAKs”)

• Janus was the Roman god of beginnings and endings, depicted with two faces, looking to the future and to the past

• The Janus Kinases (aka “JAKs”) work in pairs to effect transmembrane signal of cytokines

• There are four:
  – Jak 1
  – Jak 2
  – Jak 3
  – Tyk 2 (tyrosine kinase)
Janus Kinases ("JAKs")

- **Mediators of signal transduction**
- JAKs are **phosphotransferases**: transfer ATP to a substrate when activated by the cytoplasmic domain of cytokine receptors
- The JAKs phosphorylate, and thereby activate, STATs 1-5 (Signal Transducer and Activator of Transcription) when then translocate to the nucleus and regulate gene expression
Rapid membrane to nucleus signaling:

- Cytokines bind transmembrane receptors that are associated with Jaks
- Binding activates Jaks
- Jaks phosphorylate receptors
- STATs bind receptors
- Jaks phosphorylate STATS
- STAT translocate to the nucleus
- STATs bind DNA and regulate transcription
Different Cytokine Receptors Use Different Jaks

\( \gamma_c \) family
IL-2 etc

INF-\( \gamma \)

IL-6 family

IL-12, IL-23

Hormone, Epo, GM-CSF

- Four Jaks: Jak1, Jak2, Jak3, Tyk2
- Work in pairs
Jakinibs Block the Janus Kinase Enzymes

Type I/II cytokines

IL-2, IL-4, IL-7, IL-9, IL-15, IL-21

Jakinibs

Jak1

Jak3

STAT

Nucleus
Jakinibs: Janus Kinase Inhibitors

- Jak are intracellular enzymes, **kinases**, which transfer ATP to substrates
- Jakinibs block ATP binding, so that the signalling cascade is blocked
- The binding of the cytokine to the receptor does not transmit a signal
Jakinibs: Janus Kinase Inhibitors

• Tofacitinib (Xeljanz) inhibits JAK1 and JAK3
  – Fits in the ATP binding site of the JAKs and competes with ATP for the kinase
  – Impact on innate and adaptive immunity
  – FDA approved for the treatment of RA

• Jakanibs seem to have minimal effect on other kinases
Jakinibs compete with ATP
Jakinibs compete with ATP
Jakinibs compete with ATP

Overlay of ATP and tofacitinib in JAK3

Hinge

Overlay of ATP and tofacitinib in JAK3

- Green: ATP
- Pink: Tofacitinib
Clinical Responses over Time.


“Biosimilars”

• This is the term for “generic biologic” meds
• Pathway for development established by The Biologics Price Competition & Innovation Act of 2010
  – “highly similar to the referenced product”
  – “no clinically meaningful differences”
  – must utilize same mechanism of action, route of administration, dose
  – “expected to produce the same clinical result in a given patient”
# Biosimilars Are Not Generics

<table>
<thead>
<tr>
<th>Generics</th>
<th>Biosimilars</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Small molecule drug</td>
<td>• Large complex molecule</td>
</tr>
<tr>
<td>• Chemically synthesized</td>
<td>• Manufactured in living system</td>
</tr>
<tr>
<td>• Fully characterized molecule</td>
<td>• Challenging to fully characterize</td>
</tr>
<tr>
<td>• Typically the mechanism of action is well understood</td>
<td>• Mechanism of action may not be well understood</td>
</tr>
<tr>
<td>• Can be duplicated</td>
<td>• Challenging to duplicate</td>
</tr>
<tr>
<td>• Active ingredient is chemically identical to reference product</td>
<td>• Biological product is highly similar to reference product</td>
</tr>
<tr>
<td>• Automatic substitution can and does occur</td>
<td>• Individual state’s legislation dictate whether substitution is permissible</td>
</tr>
</tbody>
</table>


Monoclonal Antibodies and Fusion Proteins Are Very Complex Structures

<table>
<thead>
<tr>
<th>Small Molecule</th>
<th>Biologics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetylsalicylic acid&lt;sup&gt;1&lt;/sup&gt; ~ 180 daltons</td>
<td>Monoclonal antibodies (mAb)&lt;sup&gt;4&lt;/sup&gt; ~ 1,300 amino acids ~ 150,000 daltons&lt;sup&gt;6&lt;/sup&gt;</td>
</tr>
<tr>
<td>Insulin&lt;sup&gt;2&lt;/sup&gt; ~ 5,700 daltons</td>
<td></td>
</tr>
<tr>
<td>Growth hormone&lt;sup&gt;3&lt;/sup&gt; 191 amino acids ~ 22,000 daltons</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Generic</th>
<th>Small Biologic</th>
<th>Large Biologic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Same Structure&lt;sup&gt;7&lt;/sup&gt;</td>
<td>Highly Similar Structure&lt;sup&gt;7&lt;/sup&gt;</td>
<td></td>
</tr>
</tbody>
</table>

Biologic: From Cloned Gene to Purified Protein

- Production Cell Line
- DNA
- mRNA
- Protein
- Infliximab Genes/DNA
- TNF-α
- Perfusion Bioreactor
- Purification
Biologics and Biosimilars Are Made by Living Cells Through A Very Complicated Processes Which Includes Multiple Stages

DNA = deoxyribonucleic acid.
Typical Biopharmaceutical API Manufacturing Process

Small Scale Cell Culture
- Thaw From WCB
- Expand
- Shaker flask
- Expand
- Spinner flasks
- Expand

Large Scale Cell Culture, followed by Harvest and Capture
- Pre-Culture
- Production
- Remove Cells
- Harvest

Purification
- UF/DF
- Nano Filter
- Virus Inactivation

API = Active Pharmaceutical Ingredient

Data on File; JBI.
Biosimilar: From Protein to Gene, Then From Gene to Protein

Production Cell Line

Infliximab Genes/DNA

Infliximab

DNA → mRNA → Protein

Perfusion Bioreactor

Purification

TNF α
Biosimilars Have Been Approved in Europe Since 2006\textsuperscript{1-5}

More recently, biosimilar anti-TNFs and G-CSFs were approved in Canada and the United States


<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Drug</td>
<td>Somatropin\textsuperscript{1}</td>
<td>Epoetin\textsuperscript{1}</td>
<td>Filgrastim\textsuperscript{1}</td>
<td>Somatropin\textsuperscript{2}</td>
<td>Follitropin\textsuperscript{1}</td>
<td>Insulin\textsuperscript{1}</td>
<td>Filgrastim\textsuperscript{3}</td>
<td><strong>Infliximab</strong>\textsuperscript{2}</td>
<td><strong>Etanercept</strong>\textsuperscript{1,2,4}</td>
<td><strong>Infliximab</strong>\textsuperscript{5}</td>
<td><strong>Infliximab</strong>\textsuperscript{1}</td>
</tr>
</tbody>
</table>
FDA Approved Biosimilars

• Infliximab
• Adalimumab*
• Etanercept*

• Bevacizumab (“Avastin”)
• Trastuzumab (“Herceptin”)
• Filgrastim (“Neupogen”)

*FDA approved but not on the market
Conclusions

• Improved understanding of the fine details of the immune system has lead to identification of promising targets for therapies
• Highly-specific agents have been developed for several of these targets
  – Monoclonal antibodies
  – Receptor-based fusion proteins
  – Small molecule inhibitor
• The future of RA therapies is very promising
RA Highway in The Missouri Ozarks