Pharmacogenomics and Molecular Medicine in Application to Rheumatoid Arthritis

Carl K. Edwards, PhD
Inflammation Drug Discovery Research Department

February 12, 2001
NISS Conference on Pharmacogenomics
TA Focused on Specific Target Diseases

- Inflammation
- Asthma
- Rheumatoid Arthritis
- OA
- Acute Injury
- IBD
- Lyme Disease
- CV Disease
- Hepatitis
- MS
- Sepsis
- Osteoporosis
- Rheumatology BU
The Etiology of RA

- Is RA an infectious disease?
- Is RA a disease of hypersensitivity?
- Is RA a nutritional or metabolic disease?
- Is RA an endocrine disease?
- Is the initial synovial lesion of RA a clue to etiology?
- Is RA a psychosomatic disease?
- Is RA a hereditary disease?

Emerging Trends in Rheumatoid Arthritis

- Identification of cells in the rheumatoid joint
- Characterization of cytokines, molecules that communicate between cells
- Distinction between inflammation and joint destruction
- Development of new treatments that prevent joint destruction
Pathophysiology of RA

Arend & Dayer, Arthritis Rheum 1995
Finger Joint in RA

Cartilage

Bone

Synovial Pannus
The initial cause remains unknown

Multiple genetic factors predispose to developing the disease or to increase severity

Perpetuation of the chronic joint disease may involve different mechanisms
Rheumatoid Arthritis 2000: Treatment

- **Inflammation:** Swelling, redness, warmth and pain
  - NSAIDs (COX-2 inhibitors) traditional treatment
    - May improve symptoms but have no effect on long-term outcome
- **Tissue Destruction:** Loss of articular cartilage and erosion of adjacent bone
  - DMARDs (Disease Modifying Anti-Rheumatic Drugs)
    - Use early to arrest the disease process and prevent further joint destruction
    - Lead to improved function and less disability
Arthritis is now a disease that is fought with many drugs. On the whole, these drugs treat inflammation as a symptom, but do not address the actual cause of the disease.
# Selected Companies with Arthritis R&D Programs

<table>
<thead>
<tr>
<th>Company</th>
<th>Program</th>
<th>Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abgenix (Fromont, CA)</td>
<td>Human Mab against IL-6</td>
<td>Phase I/II, 11/98</td>
</tr>
<tr>
<td>Aeterna Laboratories (Québec, PQ, Canada)</td>
<td>Angiogenesis inhibitor (AE-941) derivative for osteoarthritis and rheumatoid arthritis</td>
<td>Preclinical</td>
</tr>
<tr>
<td>Agouron (La Jolla, CA)</td>
<td>Selective matrix metalloprotease (MMP) inhibitor</td>
<td>Phase I, 9/96</td>
</tr>
<tr>
<td>Alexion (New Haven, CT)</td>
<td>Human Mab C9 inhibitor of the complement cascade</td>
<td>Phase II, 8/99</td>
</tr>
<tr>
<td>Anergen (Redwood City, CA)</td>
<td>MHC peptide compound</td>
<td>Phase I, 7/98</td>
</tr>
<tr>
<td>AnorMed (Langley, BC, Canada)</td>
<td>Azaspirane immunomodulators (Atiplimod)</td>
<td>Preclinical</td>
</tr>
<tr>
<td>Autoimmune (Lexington, MA)</td>
<td>Synthetic type II collagen peptide (second-generation Colloral)</td>
<td>Phase I</td>
</tr>
<tr>
<td>Axys Pharmaceuticals (S. San Francisco, CA)</td>
<td>Anti-tumor necrosis factor α (TNFα) Mab</td>
<td>Phase III, 2/2000</td>
</tr>
<tr>
<td>BASF Bioresearch (Worcester, MA)</td>
<td>Humanized anti-TNF antibody</td>
<td>Phase I</td>
</tr>
<tr>
<td>Bayer (Leverkusen, Germany)</td>
<td>Recombinant human γ-interferon</td>
<td>Phase III</td>
</tr>
<tr>
<td>Biogen (Cambridge, MA)</td>
<td>Elastoviscous hyaluron polymer for osteoarthritis of the knee (Synvisc)</td>
<td>Market, 8/97</td>
</tr>
<tr>
<td>Biomatix (Ridgefield, NJ)</td>
<td>Gene therapy to neutralize IL-1 and IL-10</td>
<td>Phase I, 1999</td>
</tr>
<tr>
<td>Boehringer Ingelheim (Ingelheim, Germany)</td>
<td>Oral amnion MSCs to secrete IL-1 and IL-10</td>
<td>PLA or NDA Filed, 6/98</td>
</tr>
<tr>
<td>Boston Life Sciences (Boston, MA)</td>
<td>Anti-TNFα Mab</td>
<td>Phase III, 2/2000</td>
</tr>
<tr>
<td>Cambridge Antibody Technology (Cambridge, UK)</td>
<td>Thalidomide (Thalomid, formally Synovex)</td>
<td>Phase II, 8/99</td>
</tr>
<tr>
<td>Celgene (Warren, NJ)</td>
<td>Human anti-IL-18 antibody from mouse transgenics</td>
<td>Preclinical</td>
</tr>
<tr>
<td>Cellic (Malvern, PA)</td>
<td>Chimeric anti-TNF Mab (Remicade)</td>
<td>Market, 11/99</td>
</tr>
<tr>
<td>Chiron (Emeryville, CA)</td>
<td>Insulin-like growth factor (IGF-1) and IL-2</td>
<td>Lead</td>
</tr>
<tr>
<td>Cortech (Denver, CO)</td>
<td>Orally biocavailable (neutrophil) elastase inhibitor</td>
<td>Lead</td>
</tr>
<tr>
<td>Cypress Bioscience (San Diego, CA)</td>
<td>Protein-A matrix plasma apheresis column (Proserba)</td>
<td>Market, 4/99</td>
</tr>
<tr>
<td>DepoTech (San Diego, CA)</td>
<td>IGF-1 and IL-2 DepoFoam formulations</td>
<td>Lead</td>
</tr>
<tr>
<td>G.D. Searle &amp; Co. (Skokie, IL)</td>
<td>OX-2 inhibitor celipozix (Celebrex)</td>
<td>Market</td>
</tr>
<tr>
<td>Genta (San Diego, CA)</td>
<td>Oral controlled-release formulation of diclofenac (Voltaren)</td>
<td>IND Filed</td>
</tr>
<tr>
<td>IDEC Pharmaceuticals (San Diego, CA)</td>
<td>Second-generation anti-CD4 Mab</td>
<td>Phase I/II, 1997</td>
</tr>
<tr>
<td>Immune Response Corp. (Carlsbad, CA)</td>
<td>Second-generation anti-CD4 Mab</td>
<td>Phase I, 9/92</td>
</tr>
<tr>
<td>Immunex (Seattle, WA)</td>
<td>Vβ3, 14 and 17 T-cell receptor therapeutic vaccine for RA</td>
<td>Market, 11/98</td>
</tr>
<tr>
<td>Inflazyme (Vancouver, BC, Canada)</td>
<td>Soluble TNF receptor (Enbrel)</td>
<td>Preclinical, 1999</td>
</tr>
<tr>
<td>Isis Pharmaceuticals (Carlsbad, CA)</td>
<td>Inflammatory cell activation inhibitor (Bispans)</td>
<td>Terminated</td>
</tr>
<tr>
<td>Kissei Pharmaceutical (Tokyo)</td>
<td>Antisense intercellular cell adhesion molecule-1 inhibitor</td>
<td>Phase II</td>
</tr>
<tr>
<td>Otsuka Pharmaceutical (Osaka, Japan)</td>
<td>Oral small molecule inhibitor of p38 MAP Kinase</td>
<td>Lead</td>
</tr>
<tr>
<td>Peptide Therapeutics (Cambridge, UK)</td>
<td>Orally biocatalytic neutrophil elastase inhibitor</td>
<td>Phase II, 9/97</td>
</tr>
<tr>
<td>SmithKline Beecham (Philadelphia, PA)</td>
<td>HSP-tetrapeptide to split IgA and α-antitrypsin</td>
<td>Phase I/II, 1997</td>
</tr>
<tr>
<td>SuperGen (San Ramon, CA)</td>
<td>IV pentostatin (small-molecule purine analog, Nipent)</td>
<td>Phase II</td>
</tr>
</tbody>
</table>

Source: Biovista (www.biovista.com)

Nature Biotechnology, 2000; 18, IT12/IT14
Increasing understanding of the molecular cascades involved are already producing significantly better drugs than in the past with increased selectivity and fewer side effects.
New Therapeutic Directions in RA

- Cytokine inhibitors or anti-inflammatory cytokines
- Enzyme inhibitors
- Block inflammation pathways inside cells
- Inhibit fibroblast growth
What is Interleukin-1 and Why is it Important in RA?

- **Chemical nature**
  - Small protein, non-structural but active

- **Class**
  - Cytokine, often associated with infection, inflammation and various disease, not health

- **Subclass**
  - Proinflammatory
Prototypical Structure of Several Cytokine Classes
ANAKINRA

Interleukin-1 Receptor Antagonist (IL-1Ra)

- 153 amino acids
- MW 17.3 kd
- Binding Affinity $K_d = 205 \text{ pM}$
- IL-1R Type I ~3300 sites/cell*

*Dripps et al, J. Biol. Chemistry 1991
IL-1Ra Blocks Cellular Activation by Binding to IL-1 Receptor

IL-1β Binding to the IL-1 Receptor

IL-1R1

IL-1R-AcP

Signaling

Nucleus

ACTIVATION

IL-1Ra Binding to the IL-1 Receptor

IL-1R1

IL-1R-AcP

No Signaling

Nucleus

ACTIVATION BLOCKED
The Actions of Interleukin-1 (IL-1) and Interleukin-1-Receptor Antagonist (IL-1Ra)

Dinarello. NEJM, 343:728-730. 2000
Animals Without IL-1Ra Get Arthritis

Balb/cA IL-1Ra^{+/+} Normal  Balb/cA IL-1Ra^{-/-} Affected

Horai et al., J. Exp. Med. 2000 313-320
Effect of IL-1Ra and MTX Treatment: ACR Responses at 24 Weeks

Dose response: $P = 0.0027$

MTX Combination Therapy Study
Effect of IL-1Ra Treatment: Larsen Score

European Monotherapy Study

Change from baseline Adjusted mean ± SE

Placebo vs all IL-1Ra: $P = 0.03$

$P = 0.072$  
$P = 0.149$  
$P = 0.091$

Treatment Group
- Placebo (n = 83)
- 75 mg IL-1Ra (n = 88)
- 30 mg IL-1Ra (n = 89)
- 150 mg IL-1Ra (n = 86)
Pharmacogenomics

Correlating drug response to biological markers

Biological Markers

Germ Line (DNA)

Somatic (DNA/RNA/Protein)

Drug Response

Efficacy

Safety
Why pharmacogenomics, how can it help us?

1. Understand the biology

2. ID surrogate endpoints (predictive)

3. ID patient populations (diagnostic)

4. ID novel gene targets

Support the franchise

- shorten clinical trials
- speed up development
- increase likelihood of success
- reduce unnecessary exposure

Drug Discovery
## Selected Companies with Pharmacogenomics Programs

<table>
<thead>
<tr>
<th>Company</th>
<th>Area</th>
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</thead>
<tbody>
<tr>
<td>Aeiveos Sciences Group (Seattle, WA)</td>
<td>Aging-related genes and gene responses</td>
</tr>
<tr>
<td>AstraZeneca (Cheshire, UK)</td>
<td>Population genomic variability studies</td>
</tr>
<tr>
<td>CuraGen (New Haven, CT)</td>
<td>Integrated genomic and pharmacogenomic platform</td>
</tr>
<tr>
<td>diaDexus (Palo Alto, CA), Joint venture of Incyte (Palo Alto, CA), and SmithKline Beecham (Philadelphia, PA)</td>
<td>Diagnostic and pharmacogenomic kits based on leads from Incyte's, SmithKline Beecham's and Human Genome Science's (Rockville, MD), databases</td>
</tr>
<tr>
<td>EpiDaraus Biotechnologie (Bernried, Germany)</td>
<td>Targeted genomic variability analysis</td>
</tr>
<tr>
<td>Euraona Medical (Upsala, Sweden)</td>
<td>CRC-Retrospective correlations of drug response and genetic profiling</td>
</tr>
<tr>
<td>Gemini Research (Cambridge, UK)</td>
<td>Phenotype-based gene discovery; dizygotic twin studies</td>
</tr>
<tr>
<td>Genaissance Pharmaceuticals (New Haven, CT)</td>
<td>Genetic polymorphism correlations; isogene discovery; breast cancer, vascular lesions</td>
</tr>
<tr>
<td>Genome Therapeutics (Waltham, MA)</td>
<td>Human high-resolution polymorphism database</td>
</tr>
<tr>
<td>Genostic Pharma (Cambridge, UK)</td>
<td>Polymorphisms and allele frequency analysis</td>
</tr>
<tr>
<td>Genentech (Paris, France)</td>
<td>High-density biallelic maps, 60,000 markers</td>
</tr>
<tr>
<td>Hexagen (Cambridge, UK; acquired by Incyte Genomics in 1998)</td>
<td>Single-strand conformational polymorphism detection methodology</td>
</tr>
<tr>
<td>Janssen Pharmaceutica (Beerse, Belgium)</td>
<td>Cytocrome variation analysis</td>
</tr>
<tr>
<td>Lion Bioscience (Heidelberg, Germany)</td>
<td>Proprietary sequencing and analysis software for drug target identification and gene expression data under varying conditions</td>
</tr>
<tr>
<td>Millennium Predictive Medicine (Cambridge, MA, re-acquired by Millennium)</td>
<td>SNP use in pharmacogenomics</td>
</tr>
<tr>
<td>MitoKor (San Diego, CA)</td>
<td>Mitochondrial genom analysis</td>
</tr>
<tr>
<td>Nova Molecular (Montreal, Canada)</td>
<td>CNS disease genetic profiling</td>
</tr>
<tr>
<td>Rosetta Inpharmatics (Kirkland, WA)</td>
<td>&quot;Ink-jet&quot; technology--based oligonucleotide array studies</td>
</tr>
<tr>
<td>Sequana Therapeutics (La Jolla, CA)</td>
<td>High-throughput genotyping</td>
</tr>
<tr>
<td>Variagenics (Cambridge, MA)</td>
<td>Genotyping assays based on haplotypes or SNPs for use in clinical trials.</td>
</tr>
</tbody>
</table>

Source: Biovista (www.biovista.com)
# Selected Companies with Biochip Programs

<table>
<thead>
<tr>
<th>Company</th>
<th>Program</th>
</tr>
</thead>
<tbody>
<tr>
<td>Affymetrix (Santa Clara, CA)</td>
<td>GeneChip Arrays, high-density probes per chip (64-400K spots per chip)</td>
</tr>
<tr>
<td>Amersham Pharmacia Biotech</td>
<td>Cy3 and Cy5 fluorescent dyes for detection by molecular array scanners</td>
</tr>
<tr>
<td>(Uppsala, Sweden)</td>
<td>High throughput single nucleotide polymorphism mapping</td>
</tr>
<tr>
<td>Applied Biosystems (Foster City, CA)</td>
<td>Variable density per chip approach</td>
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<tr>
<td>Axys Pharmaceuticals</td>
<td></td>
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<tr>
<td>(S. San Francisco, CA)</td>
<td></td>
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<tr>
<td>Caliper Technologies (Palo Alto, CA)</td>
<td>Lab-on-a-chip microfluidic technologies</td>
</tr>
<tr>
<td>Cepheid (San Jose, CA)</td>
<td>Microfluidics for clinical diagnostic applications</td>
</tr>
<tr>
<td>Gene Logic (Gaithersburg, MD)</td>
<td>READS microarray technology for expression profiles</td>
</tr>
<tr>
<td>Hewlett Packard (Palo Alto, CA)</td>
<td>Array scanners</td>
</tr>
<tr>
<td>Hyseq (Sunnyvale, CA)</td>
<td>Sequence-by-hybridization chips for sequencing, expression analysis, and diagnostics (8K per chip)</td>
</tr>
<tr>
<td>Incyte Genomics (Palo Alto, CA)</td>
<td>Gene expression microarrays, medium density standardized and/or customized DNA chips (10K spots per chip)</td>
</tr>
<tr>
<td>Micronics (Redmond, WA)</td>
<td>Microfluidics technology development</td>
</tr>
<tr>
<td>Millennium Pharmaceuticals</td>
<td>Expression analysis molecular arrays; surface plasmon resonance array chips</td>
</tr>
<tr>
<td>(Cambridge, MA)</td>
<td>Medium density chips; confocal scanners</td>
</tr>
<tr>
<td>Molecular Dynamics (Sunnyvale, CA)</td>
<td>Acrlylate polyacrylamide gel arrays</td>
</tr>
<tr>
<td>Mosaic Technologies (Boston, MA)</td>
<td>3-D microfluidic chip for genotyping and DNA synthesis</td>
</tr>
<tr>
<td>Orchid BioSciences (Princeton, NJ)</td>
<td>Chips use electronically mediated hybridization to move and concentrate DNA</td>
</tr>
<tr>
<td>Nanogen (San Diego, CA)</td>
<td>Arrayer gel-based biochip for DNA diagnostics</td>
</tr>
<tr>
<td>Packard Instrument Co. (Meriden, CT)</td>
<td>Low density standardized and/or customized DNA chips (1K spots per chip)</td>
</tr>
<tr>
<td>ProtoGene (Palo Alto, CA)</td>
<td>Microfluidics technology development</td>
</tr>
<tr>
<td>Samoff (Princeton, NJ)</td>
<td>Spectrochips for DNA diagnostics by mass spectrometry</td>
</tr>
<tr>
<td>Sequenom (San Diego, CA)</td>
<td>Multiplexed chip for DNA sequencing and fragment analysis</td>
</tr>
<tr>
<td>Soane BioSciences (Hayward, CA)</td>
<td>Gene expression profiling by microarrays</td>
</tr>
<tr>
<td>Xenometrix (Boulder, CO)</td>
<td></td>
</tr>
</tbody>
</table>

Sources: Biovista (www.biovista.com); BioCentury.
The great challenge faced by the pharmacogenomics industry at this point is the systematic correlation between normal versus disease patterns of gene expression in a statistically meaningful way.
Objectives

- Identify patient subpopulations that may be more responsive to one drug versus another
  - PEG sTNF-RI Clinical Experience
- Identify surrogate markers that can be utilized to determine if the drug is efficacious
- Identify novel gene targets that can be utilized for drug discovery
Pharmacogenomics Vision

Today

Number of Patients

% Response

Unpredictable

Future

Number of Patients

% Response

Predictable

Confidential ©2000 Millennium Predictive Medicine, Inc.
Opportunities

Clinical Trials
- Reduce clinical trial size (time and cost)
- Increase likelihood of positive response
- Reduce likelihood of dangerous exposure
- Discover new surrogate markers for drug action and toxicity
- Suggest new targets and strategies for future drug development

Improve Drug Profile
- Define drug for *optimal* population
- Establish new paradigm for drug class
- Avoid toxicities / monitoring requirements
**IL-1Ra Allelic Polymorphisms and Disease**

- An allelic polymorphism is present in intron 2 of the IL-1Ra gene consisting of two to six copies of an 86-bp tandem repeat.

- IL-1Ra allele A2 is associated with various diseases of largely epithelial cell origin, including increased severity of SLE and Sjögren’s syndrome.

- The disease associations of IL-1Ra allele A2 may be secondary to a combination of decreased production of IL-1Ra and increased production of IL-1β.
Different people can have a different nucleotide or base at a given location on a chromosome.

What is an SNP?

What is an SNP map?

How can an SNP map be used to predict medicine response?

Patients with efficacy in clinical trials

Patients without efficacy in clinical trials

Predictive of efficacy

Predictive of no efficacy
Which Genes and Which SNPs

- Genes are biologically plausible
  - Genes in the drug pathway
  - Genes in disease pathway
  - Genes in drug metabolism

- SNPs cause some biologically relevant change
  - Coding region SNPs change amino acids
  - Coding region amino acid changes alter protein structure
  - Promoter region SNPs change gene expression
sTNF-RI is a Novel, High-affinity Soluble TNF Receptor

PEGylation site

Antigenic domains eliminated
When sTNF-RI Was Delivered Weekly, There Was a Clear Dose Response in ACR 20 Scores

When sTNF-RI Was Delivered Weekly, There Was a Clear Dose Response in ACR 20 Scores

Clinical Status

Response Rate (%)

N=61 N=67 N=66

Placebo 400 ug/kg 800 ug/kg

* P=0.022 vs. placebo [CRP or ESR for response], Dose-response (Jonckheere-Terpstra) p=0.007
Treatment with sTNF-RI Resulted in Significant Improvement in Pain

Clinical Status

sTNF990136
Mean Change from Baseline of Pain Assessment
Evaluable for Week 12 Final Analysis

*P< 0.01
sTNF-RI was Tested and Supports Use in Real Life RA Patient Population

Clinical Status

- **sTNF-RI (136)**
  - 38% Enbrel I
  - 22% MTX Alone
  - 23% MTX + Other DMARD(s)
  - 17% No DMARD

- **Enbrel I**
  - 100% of Patients in Trial

- **Enbrel II**
  - 100% of Patients in Trial

- **Anakinra (180)**
  - 100% of Patients in Trial

- **D2E7**
  - 100% of Patients in Trial

- **Remicade**
  - 100% of Patients in Trial

- **Arava**
  - 100% of Patients in Trial
Challenge: Get the Drug to Work Better

To proceed with monotherapy, we need to find an optimized dose that is equivalent to ENBREL®.

To proceed with combination therapy, we need to find a combined dose that is superior to ENBREL®.
NEW ENBREL THE FIRST TNF-RECEPTOR

TNF binds to cell-surface receptors initiating an inflammatory response

Naturally occurring soluble TNF-receptor

Cell-surface TNF-receptor

ENBREL inhibits activation of cell-surface receptors

This patient is representative of responders in clinical studies. In these studies, up to 65% of patients were responders.
Effect of Soluble TNF Receptors on TNF-\(\alpha\)-induced IL-8 in Whole Blood

The graph shows the effect of soluble TNF receptors (p55-PEG and p75-Fc) on the IL-8 production induced by TNF-\(\alpha\) in whole blood. The IL-8 production is expressed as a percentage of TNF. The data is presented for different concentrations of TNF soluble receptors (ng/mL). The significance of the effect is indicated by asterisks (***) for statistically significant differences.
Objectives

• Identify patient subpopulations that may be more responsive to one drug versus another
• Identify surrogate markers that can be utilized to determine if the drug is efficacious
• Identify novel gene targets that can be utilized for drug discovery
Treatment with sTNF-RI Resulted in Significant Improvement in Objective Clinical Measurement (ESR)

Clinical Status

sTNF990136
Mean Change from Baseline of Erythrocyte Sedimentation Rate (ESR)
Evaluable for Week 12 Final Analysis

*P< 0.01
# Acute Phase Reactants and Their General Functions

## Acute Phase Proteins Whose Plasma Concentrations Increase

### Complement System
- C3
- C4
- C9
- Factor B
- C1 inhibitor
- C4b binding protein
- Mannose binding protein (MBP)

### Coagulation and Fibrinolytic System
- Fibrinogen
- Plasminogen
- Tissue plasminogen activator
- Urokinase
- Protein S
- Vitronectin
- Plasminogen-activator inhibitor 1
- Antiproteases
  - α1-Protease inhibitor
  - α1-Antichymotrypsin
- Pancreatic secretory trypsin inhibitor
- Inter-α-trypsin inhibitor

### Transport Proteins
- Ceruloplasmin
- Haptoglobin
- Hemopexin

### Participants in Inflammatory Responses
- Secreted phospholipase A₂ (PLA₂)
- Lipopolysaccharide binding protein (LBP)
- Interleukin-1 receptor antagonist (IL-1Ra)
- Granulocyte colony-stimulating factor (G-CSF)

### Others
- C-reactive protein (CRP)
- Serum amyloid A
- α1-Acid glycoprotein
- Fibronectin
- Ferritin
- Angiotensinogen

## Plasma Proteins Whose Plasma Concentrations Decrease

- Albumin
- Transferrin
- Transthyretin
- α2-HS glycoprotein
- Alpha-fetoprotein
- Thyroxine-binding globulin
- Insulin-like growth factor 1 (IGF-1)
- Factor XII
Objectives

- Identify patient subpopulations that may be more responsive to one drug versus another
- Identify surrogate markers that can be utilized to determine if the drug is efficacious
  - Speed to determine this is essential!
- Identify novel gene targets that can be utilized for drug discovery
Synergistic Effect of IL-1 Plus TNF-α on Induction of IL-8 from COS Cells

Unpublished data, Charles A. Dinarello.
IL-1Ra and PEG sTNF-RI Alone and in Combination Effects on Established Type II Collagen Arthritis in Rats

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Mean±SE histological score (0-5) ankle joints</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arthritis control</td>
<td>4</td>
</tr>
<tr>
<td>IL-1Ra 100mg/kg</td>
<td>3.5</td>
</tr>
<tr>
<td>sTNF-RI 3mg/kg</td>
<td>3</td>
</tr>
<tr>
<td>IL-1ra 100mg/kg + sTNF-RI 3mg/kg</td>
<td>0.5</td>
</tr>
</tbody>
</table>

- **Inflammation**
- **Pannus**
- **Cartilage damage**
- **Bone resorption**

*p*≤0.05, 2-tailed *t*-test to control

Inhibition of Inflammation in Adjuvant Arthritis IL-1ra/PEG sTNF-RI Combination

THE PATHOGENESIS AND PREVENTION
OF JOINT DAMAGE IN RHEUMATOID ARTHRITIS

Advances from Synovial Biopsy and Tissue Analysis

PAUL PETER TAK and BARRY BRESNIHAN
Cell Populations at the Cartilage-Pannus Junction (CPJ) and at non-CPJ Sites
Immunohistologic Analysis of Synovial Cell Population at the Cartilage-Pannus Junction
Immunohistologic Analysis of Synovial Cell Population at sites remote from the Cartilage-Pannus Junction
MODULATION OF INFLAMMATION AND METALLOPROTEINASE EXPRESSION IN SYNOVIAL TISSUE BY LEFLUNOMIDE AND METHOTREXATE IN PATIENTS WITH ACTIVE RHEUMATOID ARTHRITIS

Findings in a Prospective, Randomized, Double-Blind, Parallel-Design Clinical Trial in Thirty-Nine Patients at Two Centers

MAARTEN C. KRAAN, RICHARD J. REECE, ELLA C. BARG, TOM J. M. SMEETS, JACQUI FARNELL, RONALD ROSENBURG, DOUG J. VEALE, FERDINAND C. BREEDVELD, PAUL EMERY, and PAUL P. TAK

Arth. Rheum. 2000; 43, 1820-1830
Mean and SEM change in the Δ matrix metalloproteinase 1 (MMP-1) to tissue inhibitor of metalloproteinases 1 (TIMP-1) ratio after 4 months of treatment in relation to the clinical response.

Arth. Rheum. 2000; 43, 1820-1830
IL-1Ra +/- PEG sTNF-RI
Synovial Biopsy Study

• Provide mechanistic data on protective effects of IL-1Ra +/- PEG sTNF-RI on bone and cartilage in RA patients
  – Support commercialization efforts of IL-1Ra, ie, “bone story”
• Identify prognostic markers for future clinical research (responder vs non-responder)
• Identify potential new drug targets
Objectives

• Primary Objective: Confirm and characterize changes in synovial biopsies to changes in joint architecture in RA patients
  – Biopsies samples => CD+ markers, Cell counting
    • Similar to previous work (560) and recent Arava publication
  – X-ray, bone densitometry changes
• Secondary Objectives:
  – ACR assessments, cytokines, bone & cartilage markers
    • serum, synovial fluid, cell culture
  – Gene expression via microarray (Amgen)
    • Synovial tissue, leukocytes and/or buccal smear
Study Logistics

- Single center study
- Up to 3 biopsies per patient (knee) over 1 yr
- Recruit patients with early active disease (similar to patient population in 0560)
Study Design

- **Two arm, open label study**
  - IL-1Ra 1 mg/kg
  - IL-1Ra & PEG sTNF-RI combination
  - Estimate ~10-15 patients per arm

- **Change from baseline study, with changes assessed at 1, 6, & 12 months.**
Synovial Macrophage Populations Following IL-1Ra

A notable reduction occurred in intimal layer macrophage accumulation and in subintimal macrophage and lymphocyte infiltration following 24 weeks of daily administration of IL-1ra 150mg subcutaneously.

Objectives

Identification of Novel Targets to Accelerate Drug Discovery
Discovery and Analysis of Inflammatory Disease-related Genes Using cDNA Microarrays

(inflammation/human genome analysis/gene discovery)

RENU A. HELLER*, MARK SCHENA*, ANDREW CHAI*, DARI SHALON ‡, TOD BEDILION ‡, JAMES GILMORE ‡, DAVID E. WOOLLEY §, AND RONALD W. DAVIS*

*Department of Biochemistry, Beckman Center, Stanford University Medical Center, Stanford, CA 94305; ‡ Synteni, Palo Alto, CA 94306; and § Department of Medicine, Manchester Royal Infirmary, Manchester, United Kingdom

Contributed by Ronald W. Davis, December 27, 1996

Ninety-six-element microarray design. The target element name and the corresponding gene are shown in the layout. Some genes have more than one target element to guarantee specificity of signal. For TNF the targets represent decreasing lengths of 1, 0.8, 0.6, 0.4, and 0.2kb from left to right.
Expression profiles for early passage primary synoviocytes and chondrocytes isolated from RA tissue

A Human synovial fibroblasts

B. Human articular chondrocytes

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Expression profiles of RA tissue (A) and IBD tissue (B)

A. Rheumatoid arthritis
B. Inflammatory bowel disease

Flow Diagram of a DNA Microarray Tumor Profiling Project

New Technologies for Life Sciences, December 2000
Variation in Expression of 1753 Genes in 84 Experimental samples (17 cell lines and 65 breast tissue samples)
# Family of Matrix Metalloproteases

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Conclusions

• These early studies using Amgen clinical candidates provide value by:
  – establishing “Proof-of-Concept” rationale for Pharmacogenomics and Pharmacogenetics
  – demonstrating platform technologies capabilities to the Drug Development process
  – accelerate drug discovery and target evaluation
# Acknowledgments

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National Institute of Statistical Sciences
PRESENTS

Affiliates Workshop

PHARMACOGENOMICS

February 12, 2001
(8:30 am – 5:00 pm)

&

February 13, 2001
(8:00 am – 1:00 pm)

Building 9 Lecture Hall

Sponsored by

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