New Targets and Pathways to Treat Unmet Medical Need in Scleroderma and Fibrotic Disorders

Elma Kurtagic, PhD
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Momenta - Creating Value Through Scientific Capabilities

**Thorough Structural Characterization**
- High resolution physicochemical analytics platform to thoroughly characterize any product

**Control of Manufacturing**
- Understanding the nonlinear chemical and biosynthetic reactions that drive production

**Thorough Biological Characterization**
- High resolution biology applied pre-clinically and in clinical settings

**Biosimilars**
- HUMIRA®
- ORENCIA®
- Five additional biosimilars

**ANDA Generics**
- Generic LOVENOX®
- Glatopa® (Generic daily COPAXONE® 20mg)

**Novel Autoimmune Drugs**
- M281 (Anti-FcRn)
- M254 (hsIVIg)
- M230 (SIF3)
Applying Patient Biocharacterization to Drug Discovery & Development

Indication 1
Indication 2
Indication 3

Identify molecular signatures associated with unmet need using human samples

Replicate and expand findings across indications to identify addressable patient population

Identify drug targets based on molecular signatures associated with unmet need

Identify drug candidate and companion biomarkers

Use the signatures during development for patient selection & outcome measure
Adjuvanted influenza-H1N1 vaccination reveals lymphoid signatures of age-dependent early responses and of clinical adverse events.

* Nature Immunology 17, 204 -213 (2016).

**Individual Patient Data Analysis (IPDA) uncovered the fact that there are several routes to non response. There is no one “non-responder signature”**.
Fibrosis is the process of scarring that manifests itself in many tissues in the body, typically as a result of inflammation or damage.

<table>
<thead>
<tr>
<th>Tissue manifestation</th>
<th>Disease</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Pulmonary Fibrosis</strong></td>
<td>Idiopathic Pulmonary Fibrosis (IPF)</td>
</tr>
<tr>
<td></td>
<td>Cystic Fibrosis (CF)</td>
</tr>
<tr>
<td></td>
<td><strong>Systemic Sclerosis (SSc, Scleroderma) with Interstitial Lung Disease</strong></td>
</tr>
<tr>
<td><strong>Renal Fibrosis</strong> (Tubulo-interstitial fibrosis)</td>
<td>Chronic kidney diseases</td>
</tr>
<tr>
<td></td>
<td>Diabetic neuropathy</td>
</tr>
<tr>
<td><strong>Liver Fibrosis</strong></td>
<td>Autoimmune liver diseases</td>
</tr>
<tr>
<td></td>
<td>• Primary Sclerosing cholangitis (PSC)</td>
</tr>
<tr>
<td></td>
<td>• Primary Biliary Cirrhosis (PBC)</td>
</tr>
<tr>
<td></td>
<td>Non-alcoholic fatty liver disease (NAFLD) e.g. NASH</td>
</tr>
<tr>
<td><strong>Intestinal Fibrosis</strong></td>
<td>Inflammatory Bowel Diseases (Chrohn’s and UC)</td>
</tr>
<tr>
<td><strong>Skin Fibrosis</strong></td>
<td><strong>Systemic Sclerosis (SSc, Scleroderma)</strong></td>
</tr>
</tbody>
</table>
What is Systemic Sclerosis (SSc)?

- Complex autoimmune disease with progressive course, characterized by hardening of the skin and visceral organs
- Pathogenesis is dominated by vascular changes, dysregulation of immunity and fibrosis

Raynaud’s phenomenon  Sclerodactylyl  Telangiectasia

Digital Ulcers
Patients are classified based on the location of skin fibrosis, with high interpatient variability. Current biomarkers along with accompanying complications lack sensitivity to classify the disease early. There is a high need for identification of patients at risk for visceral organ complications and rapid progression.

**ASSESSMENT OF SKIN INVOLVEMENT**

**Modified Rodnan Skin score**

<table>
<thead>
<tr>
<th>Location</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Face</td>
<td>3</td>
</tr>
<tr>
<td>Neck</td>
<td>3</td>
</tr>
<tr>
<td>Anterior chest</td>
<td>3</td>
</tr>
<tr>
<td>Abdomen</td>
<td>3</td>
</tr>
<tr>
<td>Back - upper</td>
<td>3</td>
</tr>
<tr>
<td>Back - lower</td>
<td>3</td>
</tr>
<tr>
<td>Upper arm</td>
<td>3</td>
</tr>
<tr>
<td>Forearm</td>
<td>3</td>
</tr>
<tr>
<td>Hand</td>
<td>3</td>
</tr>
<tr>
<td>Fingers</td>
<td>3</td>
</tr>
<tr>
<td>Thigh</td>
<td>3</td>
</tr>
<tr>
<td>Leg</td>
<td>3</td>
</tr>
<tr>
<td>Foot</td>
<td>3</td>
</tr>
</tbody>
</table>

Maximum (17 site) 51
20 site 60

**Duration of disease in dcSSc at peak skin score**

Royal Free Database analysis – Denton, Black et al 2002
Systemic Sclerosis - High Unmet Need

- 5 year survival rate for SSc with pulmonary hypertension is < 21%
- No disease modifying drugs approved for SSc
  - Symptomatic treatment and immunomodulation
  - High cost of disease burden and morbidities associated with internal organ involvement
## Systemic Sclerosis - Emerging Therapies Based on Pathogenic Hypotheses

<table>
<thead>
<tr>
<th>CANDIDATE THERAPY</th>
<th>TARGET PATHWAY</th>
<th>DISEASE COMPONENT</th>
</tr>
</thead>
<tbody>
<tr>
<td>MACITENTAN</td>
<td>ET&lt;sub&gt;A&lt;/sub&gt;/ET&lt;sub&gt;B&lt;/sub&gt; receptor</td>
<td>VASCULAR</td>
</tr>
<tr>
<td>ZIBOTENTAN</td>
<td>ET&lt;sub&gt;A&lt;/sub&gt; receptor</td>
<td></td>
</tr>
<tr>
<td>SELEXIPAG</td>
<td>IP receptor agonist</td>
<td></td>
</tr>
<tr>
<td>RIOCI GUAT</td>
<td>Guanylate cyclase agonant</td>
<td></td>
</tr>
<tr>
<td>RITUXIMAB</td>
<td>CD20</td>
<td></td>
</tr>
<tr>
<td>BASILIXIMAB</td>
<td>IL-2Ra</td>
<td></td>
</tr>
<tr>
<td>TOCILIZUMAB</td>
<td>IL-6R</td>
<td></td>
</tr>
<tr>
<td>ABATACEPT</td>
<td>CTLA4</td>
<td></td>
</tr>
<tr>
<td>RILANOCEPT</td>
<td>IL-1 ligand</td>
<td></td>
</tr>
<tr>
<td>POMALIDOMIDE</td>
<td>Anti-inflammatory</td>
<td></td>
</tr>
<tr>
<td>DASATINIB, NILOTINIB</td>
<td>c-Abl, c-Kit, PDGF</td>
<td></td>
</tr>
<tr>
<td>GC-1008 (FRESOLIMUMAB)</td>
<td>TGF-β1, -β2, -β3</td>
<td></td>
</tr>
<tr>
<td>FG-3019</td>
<td>CTGF ligand</td>
<td></td>
</tr>
<tr>
<td>IVA337</td>
<td>Pan PPAR agonist</td>
<td></td>
</tr>
<tr>
<td>BIBF1120 (NINTEDANIB)</td>
<td>VEGF, bFGF, PDGF</td>
<td></td>
</tr>
<tr>
<td>ANTI-INTEGRIN ANTIBODIES</td>
<td>Blocking α&lt;sub&gt;v&lt;/sub&gt; integrin activation of TGF-β</td>
<td></td>
</tr>
<tr>
<td>LPA1 ANTAGONISTS</td>
<td>Inhibit myofibroblast differentiation</td>
<td></td>
</tr>
<tr>
<td>CANABINOID RECEPTOR BLOCKADE</td>
<td>Attenuate CB2 mediated fibrosis</td>
<td></td>
</tr>
<tr>
<td>TERGURIDE</td>
<td>Serotonin (5HT) receptor inhibition</td>
<td></td>
</tr>
<tr>
<td>RIOCI GUAT</td>
<td>Guanylate cyclase agonant</td>
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- **VASCULAR**: Treatment targets related to vascular inflammation.
- **INFLAMMATORY**: Therapies that aim to reduce inflammation.
- **FIBROTIC**: Strategies focused on controlling fibrosis.
- **MOMENTA**: Additional treatments targeting specific molecular pathways.
SSc Emerging Therapies were Discovered using Model Systems, not Human Biocharacterization

- Choose human disease
- Create simple model system
- Identify Target to inhibit
- Setup screens
- ID drugs that inhibit Target

- Animal models of human disease
  - Tight skin mouse 1 and 2
  - Avian SSc of the UCD200 chicken
  - Mouse bleomycin-induced lung fibrosis model

- Cellular models of human disease
  - Co-culture human fibroblasts and immune cells
  - 3D organotypic human skin or lung model for fibrosis
  - Fibrosis-on-a-chip microfluidics model

Increasing complexity

- Human Testing Efficacy
- Human Testing Safety
- Tox Testing
- In-vivo models
- In-vitro models

Complex human disease
Challenges in Human Biocharacterization for Drug Discovery

- Design of studies to address heterogeneity of disease and undesired variables
  - Lack of robust definition of disease activity
  - Lack of appropriately matched controls
  - Small sample numbers

- Poor sample collection and processing yields low-quality data
  - Often multi-center with variations in sample collection, storage and processing
  - Samples often processed in batches with minimal batch controls

- Heavy dependence on gene expression/ transcriptomics and genetic analysis
  - Biological relevance of genetic polymorphism and differentially-expressed mRNA unclear
  - Known reproducibility issues with gene expression technologies

- **Result:** Lack of reproducibility across studies and little robustness in identification of biologic pathways associated with disease
20 published patient studies
↓
Sample size assessment
↓
Datasets availability
↓
Quality assessment

**Dataset 1**
Identified the most comprehensive and high-quality dataset

**Dataset 2, 3 and 4**
Findings confirmed with additional datasets

**Novel in-house findings:**
- Key biology pathways associated with disease identified
- > 100 potential targets identified
  - Known targets
  - Novel pathways

**Our Approach to Human Biocharacterization**
Addressed the Challenges
Beyond Differentially Expressed Genes (DEGs) → Well-Associated Proteins (WAPs)

- Typical approach: Identify individual DEGs with significant change in SSc vs. healthy skin

- Large gene-expression data sets on SSc skin samples have been recently released in the public domain [Assassi:15, Taroni:15, Hinchcliff:13, Pendergrass:12, Milano:08]

- MNTA approach: Use STRING analysis (protein function) to identify networks of WAPs that are altered in SSc vs healthy skin

- Combining knowledge about protein function (STRING) to DEGs makes it possible to identify SSc-relevant genes in a statistically sound way, even when they are not themselves differentially expressed (e.g. OSM)

- The identified putative disease culprits are also robust: they are much more reproducible than DEGs across independent data sets
Beyond Differentially Expressed Genes (DEGs) → Well-Associated Proteins (WAPs)

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• The identified putative disease culprits are also robust: they are much more reproducible than DEGs across independent data sets
Well-Associated Proteins (WAPs) Refine and Extend Standard Gene Expression Analysis

WAP scores were developed based on published [Pradines:05,07] and novel computational methods.

- Gene products are scored for their best association (number of interactions) to the top DEGs, without having to choose a threshold for differential expression! Because scoring is fast, false discovery rates (FDRs) can be estimated on WAP scores.
- Application to the largest SSc data set [Assassi:15]:
  - Over 1,000 DEGs (green + red)
  - Only 49 are also WAPs (red): DEGs well connected to other DEGs are more likely to be biologically relevant
  - 89 WAPs are not DEGs (blue): these can only be identified by utilizing the protein network.
WAPs are More Robust than DEGs Across Data Sets

- The two largest SSc data sets yield 54% WAP reproducibility vs. 15% for DEGs, and it’s about 40% vs. 5% over four data sets
- Statistical analysis of >1,000 partitions of large gene-expression studies (SSc, psoriasis, CRC, endometriosis, NSCLC, OSCC, …) shows that WAP scores are much more reproducible than DEG scores when comparing disease versus healthy
- Robustness is obviously a critical criterion for target selection

Each data set was randomly partitioned into two data sets 1,000 times to estimate distributions of overlap statistic maxR for WAPs and DEGs
Top SSc WAPs have Frequent Annotation in Citeline

- Citeline: World’s largest database of drugs in development; 13,718 in 2016

- Not only top WAPs are more robust than top DEGs across the two largest SSc data set, but they are also more enriched in known drug targets (blue versus green)
- Combining the two largest SSc data sets does not improve Citeline representation for DEGs but it does for WAPs

- **Top 100 WAPs**
  - 59 in Citeline
    - Prioritize clinical opportunities?
    - Drug re-purposing opportunity?
  - 41 not in Citeline
    - Novel targets for drug discovery
Heparan sulfate proteoglycans (HSPGs) are essential components of cell surfaces and the extracellular matrix.

Growth factors, cytokines, and chemokines are presented from one cell to another to establish, maintain, and remodel tissue microenvironments.

HSPGs mediate fundamental processes such as cell growth, differentiation, survival, adhesion, migration, and communication.

Many HSBPs have been reported to modulate the fibrotic process.
Multiple Heparin Sulfate Binding Proteins Associated with Systemic Sclerosis

HS-binding proteins in Top WAPs & DEGs

HS-binding proteins with altered expression in SSc

- Interleukin-1
- Interleukin-4
- Interleukin-6
- Interleukin-8
- Interleukin-10
- Interleukin-13
- Interleukin-17
- TGFβ
- MCP1/CCL2
- MCP3/CCL7
- Collagen I
- Tenascin

- Fibronectin
- MMP2 and 9
- TIMP1
- VEGF
- CTGF (CCN2)
- SDF1α
- PDGF
- Endothelin-1
- IGF-II
- Angiotensin II
- Pentraxin-3
- Thrombospondin 1

Could targeting HSBPs be a novel strategy to modulate multiple disease-associated proteins?
Prevention of Bleomycin-induced Lung Fibrosis by Aerosolization of Heparin

Measurement of tissue stiffness

<table>
<thead>
<tr>
<th></th>
<th>Compliance [ml/kg KG/mm Hg]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline (n=11)</td>
<td>3.0 ± 0.2</td>
</tr>
<tr>
<td>Bleo (n=9)</td>
<td>2.5 ± 0.1</td>
</tr>
<tr>
<td>Bleo + early Hep (n=7)</td>
<td>2.0 ± 0.1</td>
</tr>
<tr>
<td>Bleo + late Hep (n=7)</td>
<td>2.2 ± 0.2</td>
</tr>
<tr>
<td>Bleo + early u-PA (n=5)</td>
<td>2.8 ± 0.3</td>
</tr>
<tr>
<td>Bleo + late u-PA (n=7)</td>
<td>3.1 ± 0.4</td>
</tr>
</tbody>
</table>

Measurement of collagen content

<table>
<thead>
<tr>
<th></th>
<th>Hydroxyproline [μg/mg tissue]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline (n=11)</td>
<td>1.5 ± 0.2</td>
</tr>
<tr>
<td>Bleo (n=9)</td>
<td>3.0 ± 0.3</td>
</tr>
<tr>
<td>Bleo + early Hep (n=7)</td>
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<td>2.2 ± 0.2</td>
</tr>
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</table>

Günther, Lübke, Ermert, et al.: Aerosolized Heparin and u-PA Prevent Lung Fibrosis
AMERICAN JOURNAL OF RESPIRATORY AND CRITICAL CARE MEDICINE VOL 168 2003
Several Clinical Trials Suggesting Safety and Efficacy of Heparins in Fibrosis

- **Effect of heparin on liver fibrosis in patients with chronic Hep B** (Shi J et al, 2003)
  - 34 patients treated either with heparin (25mg, IV, bid) or LMWH (6,400 IU, iH, qd) repeatedly over 3 weeks
  - Hepatic function improved significantly and collagen fibers decreased and HA and type IV collagen were reduced

- **Effect of heparin on IPF** (Markart P et al, 2010)
  - Safety and tolerability of heparin in IPF patients tested through inhalation of 37,500 IU heparin inhaled every 12h for 28d
  - No acute deleterious effects on pulmonary function, gas exchange or exercise capability noted following inhalation and no heparin-related side effects observed (median lung function and quality of life scores remained unaltered at this dose)

But Heparin anti-coagulant activity limits dosing.....
Necuparanib: Rationally Engineered to ↓ Anticoagulant Activity and ↑ Anti-HSBP Activity

Reduction of anticoagulant activity relative to LMWH enables delivery of a substantially higher dose of necuparanib to further potentiate anti-fibrotic effects of heparins.

<table>
<thead>
<tr>
<th>Compound</th>
<th>anti-Xa</th>
<th>anti-IIa</th>
<th>FGF</th>
<th>VEGF</th>
<th>SDF-1α</th>
<th>P-sel/PSGL1</th>
<th>Heparanase Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IU/mg</td>
<td>IU/mg</td>
<td>KD</td>
<td>KD</td>
<td>IC₅₀</td>
<td>Kᵯ</td>
<td>IC₅₀ (µg/mL)</td>
</tr>
<tr>
<td>Fragmin</td>
<td>160</td>
<td>60</td>
<td>10.2</td>
<td>112.5</td>
<td>2537</td>
<td>42.4</td>
<td>26.6</td>
</tr>
<tr>
<td>Necuparanib</td>
<td>2-10</td>
<td>2</td>
<td>9.8</td>
<td>99.5</td>
<td>1021</td>
<td>39.5</td>
<td>5.5</td>
</tr>
<tr>
<td>Ratio Fragmin/</td>
<td>16-80x</td>
<td>30x</td>
<td>1.0x</td>
<td>1.1x</td>
<td>2.5x</td>
<td>1.1x</td>
<td>4.8x</td>
</tr>
<tr>
<td>Necuparanib</td>
<td>reduction</td>
<td>reduction</td>
<td></td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

Values represent averages of multiple test runs and lot numbers.
Necuparanib Dose-Dependently Releases HSBPs in Humans

• Completed Phase 1/2 multi-dose studies in pancreatic cancer patients (n=168)
• Safe and well-tolerated
• Reduced anti-coagulant activity confirmed
• Elevation of multiple HSBPs in blood confirming biologic activity

Could Necuparanib represent a Phase 2-ready clinical candidate for SSc?
Summary

• Improving the robustness of human biocharacterization should result in better target selection and optimization of clinical development

• WAP analysis can lead to significantly enhanced robustness across human gene expression studies

• WAP analysis led to a prioritized list of targets for SSc, including existing clinical agents and novel targets

• This approach can be applied to any disease with robust human datasets