RESEARCH

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Conflicts of Interest

- Advisory Board member – Roche
- Clinical trial investigator – Boehringer-Ingelheim
- Clinical trial investigator – Roche
- Hamilton Scleroderma Group
Objectives

- Pre-clinical work
  - Mechanisms of disease, exploration

- Translational ideas
  - Could work

- Clinical trials
  - Should help/Do help
Disruption of Calcium Signaling in Fibroblasts and Attenuation of Bleomycin-Induced Fibrosis by Nifedipine

Subhendu Mukherjee et al

Division of Respirology, McMaster University
Background

Fibroblasts cause fibrosis
Fibroblasts need to be activated and stimulated
TGF-β is well known to be pro-fibrotic
TGF-β activates fibroblasts and causes oscillations
TGF-β signals via SMAD
So do other growth factors
Oscillations are calcium-dependent
Different types of calcium channels
Selective (specific) blockers for these channels
The SMAD proteins are homologs of both the Drosophila protein "mothers against decapentaplegic" (MAD) and the C. elegans protein SMA. It was found that a mutation in the gene MAD in the mother repressed the gene decapentaplegic in the embryo.

The phrase "Mothers against" was inspired by organizations formed by mothers to oppose social problems, such as Mothers Against Drunk Driving (MADD); and based on a tradition of such unusual naming within the gene research community.
Fibroblasts cause fibrosis
Fibroblasts need to be activated and stimulated
TGF-β is well known to be pro-fibrotic
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So do other growth factors
Oscillations are calcium-dependent
Different types of calcium channels
Selective (specific) blockers for these channels
Figure 1. Overnight treatment with 1 nM transforming growth factor (TGF)-β1 evoked recurring Ca2+ oscillations in normal human pulmonary fibroblasts (A). TGF-β1–evoked Ca2+ oscillations in normal fibroblasts are substantially reduced by blocking L-type calcium current using 1 μM nifedipine (B), 1 μM verapamil (C), or 1 mM NiCl2 (D). F510: fluorescence measured at 510 nm. Each tracing is representative of recordings made from batches of cells derived from five donors (at least four cells per batch). Bar diagram indicates mean (± SEM) responses to 1 nM TGF-β1 (number of Ca2+ oscillations) before and during perfusion with different blockers. ***P < 0.0001 versus TGF-β1 alone (n = 5).

Am J Respir Cell Mol Biol, 2015

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Figure 3. Bleomycin treatment dramatically flattened the pressure–volume loop curves, and decreased the K value. More importantly, nifedipine reversed all these changes to nearly normal levels. *Bar diagrams* indicate mean (± SEM) responses to different treatments.

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Figure 5. Nifedipine had little effect on Bleo-induced pulmonary inflammation in mice. Bleo increased total cell counts in bronchoalveolar lavage fluid (A) and increased differential counts for macrophages (B), neutrophils (C), and lymphocytes (D) after 7 and 21 days of administration, except the neutrophil counts were reduced after 21 days. Nifedipine did not significantly alter any of these changes except for neutrophil counts. Similarly, nifedipine had little effect on Bleo-elevated serum levels of TGF-β (total) (E) or IL-6 (F) measured 7 days after Bleo treatment. Levels of TGF-β and IL-6 were undetectable after 21 days. Bar diagrams indicate mean (± SEM) responses to different treatments. *P < 0.05, **P < 0.002, and ***P < 0.0001 versus control; #P < 0.05 versus Bleo + vehicle (n = 5).
Conclusions

Nifedipine reduces fibroblast activation
  - in cells and in a model

May reduce fibrosis if
  - sufficient dose can be delivered
  - discover more potent drug with same effect

Serendipity…..HSG, fibroblasts, fibrosis, calcium
Identification of potential biomarkers for the development of in vitro models of fibrosis using bioinformatic gene expression analysis.

Parthasarathy Pavithra et al

Department of Medicine, McMaster University, Hamilton, ON, Canada
The M2 macrophage: immune cell with fibrosis-promoting properties

Scleroderma patients have higher numbers of M2 macrophages in lung and skin tissue

Identify a gene signature unique to the profibrotic M2 macrophage phenotype, and shared by human and mouse M2 cells
## Results

<table>
<thead>
<tr>
<th>MOUSE</th>
<th>Shared</th>
<th>HUMAN</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ampd2</td>
<td>AP2A2</td>
<td>NFE2</td>
</tr>
<tr>
<td>Arg1</td>
<td>OLR1</td>
<td>P2RY1</td>
</tr>
<tr>
<td>Atic</td>
<td>MYC</td>
<td>SLC4A7</td>
</tr>
<tr>
<td>Auh</td>
<td>PICALM</td>
<td>ST6GAL</td>
</tr>
<tr>
<td>Bmp2k</td>
<td>PTGS1</td>
<td></td>
</tr>
<tr>
<td>Clcn5</td>
<td>CLEC7A</td>
<td></td>
</tr>
<tr>
<td>Egr2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Il6st</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Klf9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ptpre</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sys1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ubl3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acsl1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fpr1</td>
<td>PFKFB3</td>
<td>GBP2</td>
</tr>
<tr>
<td>Pir</td>
<td></td>
<td>GPR183</td>
</tr>
<tr>
<td>Pmaip1</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Now What?

- Find out what these genes code for
  - Could be important for causing disease

- Find out how these genes are regulated
  - Could lead to specific intervention (treatment)

- Determine if down-regulating these genes relates to less fibrosis
  - Motivates development of a treatment
Increased Levels of Circulating CXCL4 in Systemic Sclerosis and the Association with Lung Fibrosis and Pulmonary Arterial Hypertension.

Levels of CXCL4 in the plasma correlate with disease severity.

Although a neutrophil chemokine, it is platelet factor 4 (PF4) which activates platelets and causes clotting.

Hypothesis: that SSc is a microthrombotic disease (with inflammation and fibrosis as consequence rather than cause).

Plan to use rivaroxaban to reduce formation of microthrombi in capillaries.
Clinical Trials

Inflammation causes fibrosis or fibrosis happens

Drug development
anti-inflammation
anti-fibrosis

Underway: nintedanib, pirfenidone, rituximab

Completed: cyclophosphamide, mycophenolate
Anti-fibrotic Activities of Pirfenidone

- Pirfenidone attenuates fibroblast proliferation
- Pirfenidone reduces the synthesis of inflammatory cytokines
- Pirfenidone reduces the production of fibrogenic cytokines and growth factors
- Pirfenidone inhibits collagen production

courtesy K. Kossen, Intermune Brisbane

ASCEND Trial - pirfenidone

Figure S2 – Linear slope analysis of FVC change from baseline to week 52

Absolute Difference, 140 mL
(53.5% relative reduction)
P<0.0001*
Nintedanib Inhibits Fibroblast Migration, Proliferation and Myofibroblast Transformation

Wollin L., Kolb M, Eur Resp J 2015
Annual Rate of Decline in FVC

**INPULSIS-1 and -2 (pooled data)**

Adjusted annual rate of decline in FVC (mL/year)

Δ 109.94 mL/yr 
(95% CI; 75.85, 144.03) 
p<0.0001

Mean (SEM) observed change from baseline FVC (mL)

No. of pts
Placebo 423 417 408 407 403 395 383 345
Nintedanib 638 626 616 613 604 587 569 519

Richeldi L et al, NEJM 2014
Current Anti-Fibrosis Activity

- Pirfenidone for lung disease in SSc
  - LOTUS completed
  - Large RCT underway

- Nintedanib for lung disease in SSc
  - RCT launching
Gilead Terminates Phase 2 Study of Simtuzumab in Patients With Idiopathic Pulmonary Fibrosis
Anti-Inflammation Research

- **Rituximab**
  - Antibodies in serum of SSc stimulate fibroblasts
  - Lymphocyte produce antibodies
  - RTX depletes lymphocytes

- **Cyclophosphamide**
  - Cell poison
  - Lymphocytes are especially sensitive

- **Mycophenolate**
  - Anti-metabolite (aka cell poison)
Scleroderma Lung Study

Blinded oral Placebo

Blinded oral CYC 1mg/kg

1:1 allocation

CBC, urine R&M q2-4weeks

Treatment at discretion of primary physician

Day 0

Month 12

Month 24

Measurements at baseline and q 3months:
- PFTs
- modified Rodnan skin thickness score,
- Mahler BDI and TDI
- modified cough index
- 36-item medical outcomes survey
- HAQ-DI
Adjusted FVC at 24 Months

Tashkin et al. Am J Respir Crit Care Med 2007 176: p1026-1034
Adjusted Mahler TDI at 24 Months

P = 0.074

Tashkin et al. Am J Respir Crit Care Med 2007 176: p1026-1034
Adjusted Rodnan Skin Score at 24 Months

\[ P = 0.23 \]

\[ \text{Tashkin et al. Am J Respir Crit Care Med 2007 176: p1026-1034} \]
No significant difference in FVC at 12 months when the following covariates were added to baseline FVC in Huber Model:

- GGO
- Neutrophilia, eosinophilia or both on BAL
- Honeycombing on baseline HRCT

In 85 patients with diffuse disease, significant decrease in Rodnan Skin Score (-3.06 [CI -3.54 to -0.52]; p=0.008) favoring CYC group
Effects of 1-year treatment with cyclophosphamide on outcomes at 2 years in scleroderma lung disease.

- Question whether the average absolute effect on FVC of 2.5% is worth the price of cyclophosphamide toxicity.

- Benefits of oral cyclophosphamide persist for 6 months after cessation of treatment but are largely lost at 1 year. How to continue prevention?

- Appears that treatment benefits apply more to fibrotic than to inflammatory disease. Were patients with the most to gain underrepresented in this trial?
Mycophenolate mofetil versus oral cyclophosphamide in scleroderma-related interstitial lung disease (SLS II): a randomised controlled, double-blind, parallel group trial

Donald P Tashkin, et al

Lancet, 2016
1 - MMF would have a significantly greater effect on FVC than cyclophosphamide

2 - MMF would be safer and better tolerated than cyclophosphamide
198 participants assessed for eligibility

- 56 ineligible

142 eligible and randomised

69 assigned mycophenolate mofetil

- 20 discontinued treatment
  - 19 withdrew (off drug)
  - 5 died*

53 had 24 month follow-up data available (4 off drug)

73 assigned cyclophosphamide

- 36 discontinued treatment
  - 32 withdrew (off drug)
  - 2 treatment failure
  - 11 died*

53 had 24 month follow-up data available (16 off drug)
Reasons for premature discontinuation of study treatment (N=56).

<table>
<thead>
<tr>
<th>Reason</th>
<th>CYC (%)</th>
<th>MMF (%)</th>
<th>Total (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adverse event</td>
<td>15 (41.7%)</td>
<td>7 (35%)</td>
<td>22 (39.3%)</td>
</tr>
<tr>
<td>Patient request</td>
<td>9 (25%)</td>
<td>8 (40%)</td>
<td>17 (30.4%)</td>
</tr>
<tr>
<td>Non-compliant</td>
<td>6 (16.7%)</td>
<td>3 (15%)</td>
<td>9 (16.1%)</td>
</tr>
<tr>
<td>Lost to follow-up</td>
<td>2 (5.6%)</td>
<td>1 (5%)</td>
<td>3 (5.4%)</td>
</tr>
<tr>
<td>Death*</td>
<td>2 (5.6%)</td>
<td>1 (5%)</td>
<td>3 (5.4%)</td>
</tr>
<tr>
<td>Treatment failure†</td>
<td>2 (5.6%)</td>
<td>0</td>
<td>2 (3.6%)</td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td>36 (100%)</td>
<td>20 (100%)</td>
<td>56 (100%)</td>
</tr>
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</table>

*Pertains only to deaths that occurred while subjects were still in the active treatment phase of study.
†An absolute decrease from baseline FVC of at least 15% of the predicted value occurring at least 3 months after treatment was initiated and lasting for at least one month.
<table>
<thead>
<tr>
<th></th>
<th>Mycophenolate mofetil</th>
<th></th>
<th>Cyclophosphamide</th>
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<tbody>
<tr>
<td></td>
<td>Adverse events</td>
<td>Patients (n=69)</td>
<td>Adverse events</td>
<td>Patients (n=73)</td>
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<tr>
<td><strong>Adverse events</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leucopenia†</td>
<td>5</td>
<td>4 (6%)</td>
<td>51</td>
<td>30 (41%)</td>
</tr>
<tr>
<td>Neutropenia</td>
<td>3</td>
<td>3 (4%)</td>
<td>7</td>
<td>5 (7%)</td>
</tr>
<tr>
<td>Anaemia</td>
<td>18</td>
<td>8 (12%)</td>
<td>26</td>
<td>13 (18%)</td>
</tr>
<tr>
<td>Thrombocytopenia</td>
<td>0</td>
<td>0</td>
<td>7</td>
<td>4 (6%)</td>
</tr>
<tr>
<td>Haematuria</td>
<td>3</td>
<td>3 (4%)</td>
<td>2</td>
<td>2 (3%)</td>
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<tr>
<td>Pneumonia</td>
<td>6</td>
<td>5 (7%)</td>
<td>4</td>
<td>4 (6%)</td>
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<tr>
<td><strong>Serious adverse events‡</strong></td>
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<tr>
<td>Total</td>
<td>42</td>
<td>27 (39%)</td>
<td>36</td>
<td>22 (30%)</td>
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<tr>
<td>Related to treatment§</td>
<td>3</td>
<td>3 (4%)</td>
<td>8</td>
<td>7 (10%)</td>
</tr>
<tr>
<td>Related to underlying disease§</td>
<td>16</td>
<td>9 (13%)</td>
<td>16</td>
<td>13 (18%)</td>
</tr>
<tr>
<td>Due to other causes§†</td>
<td>22</td>
<td>14 (20%)</td>
<td>11</td>
<td>6 (8%)</td>
</tr>
<tr>
<td>Unknown cause§</td>
<td>3</td>
<td>3 (4%)</td>
<td>3</td>
<td>3 (4%)</td>
</tr>
<tr>
<td>Death</td>
<td>‚</td>
<td>5 (7%)</td>
<td>‚</td>
<td>11 (15%)</td>
</tr>
</tbody>
</table>
The graph compares the forced vital capacity (FVC) as a percentage of predicted values over time for two different medications: Mycophenolate mofetil and Cyclophosphamide. The table below shows the number of patients treated with each medication at various follow-up intervals (in months).
**Change in modified Rodnan Skin Score over time**

**A**

![Graph showing change in mRSS over time.](image)

**B**

![Bar chart showing change in mRSS by treatment.](image)

<table>
<thead>
<tr>
<th>Change in mRSS</th>
<th>Mycophenolate mofetil</th>
<th>Cyclophosphamide</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤-13</td>
<td>71.7%</td>
<td>73.6%</td>
</tr>
<tr>
<td>-9 to -12</td>
<td>11.3%</td>
<td>5.7%</td>
</tr>
<tr>
<td>-5 to -8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>-1 to -4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 to 4</td>
<td>17.0%</td>
<td>20.7%</td>
</tr>
<tr>
<td>&gt;4</td>
<td></td>
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</tr>
</tbody>
</table>

**Number of patients**

<table>
<thead>
<tr>
<th></th>
<th>Mycophenolate mofetil</th>
<th>Cyclophosphamide</th>
</tr>
</thead>
<tbody>
<tr>
<td>Follow-up (months)</td>
<td>69 65 60 54 58 51 50 49 53</td>
<td>73 65 58 54 55 45 47 40 53</td>
</tr>
</tbody>
</table>
Discussion – What Happened?

- Negative primary outcome – breathing capacity
- Showing that mycophenolate is better tolerated would not justify a study of this size
- First randomized controlled study showing a positive effect of MMF in SSc related ILD
  - But did it? CYC patients improved also
- Skin scores improved by both drugs
Supplementary Figure 5. Comparison of the changes in FVC% predicted from baseline in the CYC arm of the present trial versus the CYC arm in SLS I.
Discussion – What Happened?

- Negative primary outcome – breathing capacity
- Showing that mycophenolate is better tolerated would not justify a study of this size
- First randomized controlled study showing a positive effect of MMF in SSc related ILD
  - But did it? CYC patients improved also
- Skin scores improved by both drugs
- Did something change at month 21?
Where Now?

- Cyclophosphamide and mycophenolate work
  - Rituximab – on the way

- CYC i.v. instead of oral
- CYC + continuation medication

- More severe disease/longer duration of disease?

- Combination of anti-inflammation + anti-fibrosis
Challenges

- Crackpot vs crackerjack idea?
  - Vulnerable because idiopathic, progressive,
- Uncommon disease
  - Rare, “orphan”, business proposition
- Slow
  - 10 years from candidate to treatment
- Expensive
  - Who should pay?
- Human research is intrusive and involves risk
THANK YOU