BIOMARKER DISCOVERIES IN SYSTEMIC RHEUMATIC DISEASES (SRD)

Laurentian Rheumatology Conference
May 11 Biomarker Discoveries in Systemic Rheumatic Diseases (SRD), 2018

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Disclosure: Dr. M. Fritzler

Is or has been a consultant to Inova Diagnostics Inc., BioRad, Euroimmun GmbH, Mikrogen GmbH, Dr. Fooke Laboratorien GmbH, ImmunoConcepts, SKF Canada, Amgen and Pfizer.

Has received gifts in kind from ImmunoConcepts, Inova Diagnostics, Euroimmun GmbH, and Alexon Canada.

Does not own or trade shares in companies associated with medical diagnostics.

Is the Director of Mitogen Advanced Diagnostics Laboratory.

OUTLINE

What are the key drivers of innovative biomarker technologies?

What are some newer autoantibodies of interest?
Goals of Biomarker Discovery

- Early and Accurate Diagnosis
- Intent to PREVENT
- Establish/Confirm DIAGNOSIS
  - Intent to TREAT
  - Guide to further investigations and referrals (biopsy, imaging, etc.)
  - Guide to treatment: Precision Medicine
  - CLINICAL CARE PATHWAYS
- Follow clinical course
- Confirm remission
- Predict or confirm flares
- Understand PATHOGENIC processes
- Indicators of PROGNOSIS/OUTCOMES
- ECONOMICAL: key to adoption

Biomarkers ' OMICS Patient Profiling

- Genomics
  - Epigenomics advancing rapidly
- Proteomics
  - Autoantibody subsets
  - Cytokine/Chemokine subsets
  - Complement
- T and B cell subset profiles
- Markers of cell death: necrosis, apoptosis, NETosis
- Ribonomics: miRNA, CIRCULAR RNA
- Liquid biopsies: Microbody"omics"
- Metabolomics, Metallocins
- Lipidomics, Glycomics
- Bioinformatics, Artificial Intelligence, Big Data!

Biomarker Diagnostic Technologies

- Antigen Arrays on Planar Surfaces: Line Immunoassays (LIA)
- ELISA
- Addressable Laser Bead Assays (ALBIA, BioFlash, SNOW)
- Chemiluminescence (CIA): Bio Flash
- Cell Based Assays (CBA)
- Point of Care Diagnostics: ‘Lab on a chip’
- SOMAScan
- Electrochemiluminescence Arrays
- Nanotechnology — nanobarcodes
- Mass & NMR Spectroscopy: CyTOF
**Newer Autoantibodies**

- Cancer-Associated Autoantibodies
  - RNP C3
  - PUF 60
  - ?MPP-1/KIF20B
  - Bicaudal D2 (BICD2)
  - Survival of Motor Neuron (SMN)/gemins
  - Mup44

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**Age-old question: Biomarkers of cancer in autoimmune rheumatic diseases?**

Dr. Eng M Tan 1995
Dr. Eng M Tan 2016

**SPECIAL ARTICLE**

Dr. Eng. M Tan: a tribute to an enduring legacy in autoimmunity

   When compared to the general population, there is a decreased breast cancer
   Risk in systemic lupus erythematosus (SLE)

   A nucleolytic lupus autoantibody is toxic to BRCA2-deficient cancer cells.
   Lupus derived antibody 5B6 kills BRCA2-deficient cancer cells
Other Autoantibodies Associated with Cancer

Identification of multiple cancer-associated myositis-specific autoantibodies in idiopathic inflammatory myopathies: a large longitudinal cohort study

Haibo Yang1,2, Qinglin Peng1, Liugen Yin1, Zhenghan Li1, Jieli Shi1, Yamei Zhang1, Xin Lu1, Xiaoming Shu1, Ying Zhang1 and Guochun Wang1,2,3

Study of 667 IIM patients

Anti-SAE1, anti-TIF1-γ and anti-NXP2 all associated with a significantly increased risk of cancer in IIM. In some cases, anti-HMGCR, anti-Jo-1 and anti-PL-12 antibodies might also be driven by malignancy.

Other Autoantibodies Associated with Cancer

Systematic autoantigen analysis identifies a distinct subtype of scleroderma with coincident cancer

George L. Schuler1, Matthew A. Neilson2, Marina E. U1,2,3, Qiong Xu1,2,3, Anthony Rosen3, Luis Caceres-Rose4,5, and Stephen J. Etridge1,2,3,4

- Phage screening technologies (Phi-P Seq, PLATO) used to screen for target autoantigens
- RNPC3 identified as the major target in SSc-Cancer
- Minor spliceosome removes introns from pre-RNA
- Confirmed intra- and inter-molecular epitope spreading
  - POLR3A>POLR3F>POLR3H
  - 2 – 4 epitopes on RNPC3
- U11/12 snRNP (SNRNP25, SNRNP35), Programmed Cell Death 7
- Limitations:
  - Sensitivity
  - Linear epitopes
  - Not all proteins in human proteome

318 SSc patients with cancer

- anti-CENP 30.2%; anti-Topo I 17.0%; anti-RNAP III 22.0% (CTP group)
- Anti-RNP-C3 seen in 3.8% of entire cohort; 12.2% of CTP-negative
  - Anti-RNPC-3 had a short cancer-scleroderma interval (median 0.9 years)
  - Relative to patients with anti-CENP, patients with anti-RNPC-3 and those with anti-RNAP III had a >4-fold increased risk of cancer within 2 years of scleroderma onset

Conclusion. Anti-RNPC-3 autoantibodies, like anti-RNAPIII, are associated with an increased risk of cancer at the onset of SSc.
### Anti-RNPC3 biomarker for cancer in SARD?

<table>
<thead>
<tr>
<th>Disease</th>
<th>N</th>
<th>% +ve*</th>
<th>% High +ve*</th>
<th>Comments</th>
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<tbody>
<tr>
<td>SSc Incident Cancer</td>
<td>31</td>
<td>9.7</td>
<td>3.2**</td>
<td>CSRG Cancer</td>
</tr>
<tr>
<td>Adult SS</td>
<td>c</td>
<td>59</td>
<td>6.8</td>
<td>1.7</td>
</tr>
<tr>
<td>Adult SS</td>
<td>c</td>
<td>30</td>
<td>3.3</td>
<td>3.3</td>
</tr>
<tr>
<td>Child SS</td>
<td>c</td>
<td>83</td>
<td>3.6</td>
<td>0</td>
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<td>SLE Cancer</td>
<td>39</td>
<td>23</td>
<td>2.6</td>
<td>Johns Hopkins Cohort</td>
</tr>
<tr>
<td>SLE Random</td>
<td>64</td>
<td>1.6</td>
<td>0</td>
<td>STARLET</td>
</tr>
<tr>
<td>Inflammatory Myopathy</td>
<td>66</td>
<td>1.5</td>
<td>0</td>
<td>CIMS Cohort</td>
</tr>
<tr>
<td>IBM</td>
<td>24</td>
<td>8.3</td>
<td>4.2</td>
<td>McMaster University</td>
</tr>
<tr>
<td>NHS</td>
<td>40</td>
<td>2.5</td>
<td>0</td>
<td>Healthy Controls</td>
</tr>
</tbody>
</table>

* ALBIA using purified recombinant RNPC3:
  - cutoff using normal serum ++ > 200 units
  - high cutoff >=500 units

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### IP anti-RNPC3 sera (ALBIA)

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### Anti-RNPC3 biomarker for cancer in SARD?

- The jury is out
- To do
  - All CSRG
  - Cancers (incident + prevalent)
  - Aab negative (serology gap)
  - SLE-cancer cohort (Sasha Bernatsky)
  - Larger IBM cohort
  - Epitope mapping revealed a single “hot” region of RNPC3
  - Assay developed and evaluation underway
  - Sjögren’s syndrome (40% in small cohort of 10): need larger cohort
Speaking of Sjögren’s syndrome — What about PUF60?

- **PUF60**: Poly(U)-binding-splicing Factor; c-myc repressor

Ref 1:
- 25/94 (30%) SJS; 6/71 (8.5%) SLE; 3/38 (8.9%) controls
- 48/267 (18.0%) DM; 4/45 (8.9%) IBM; 5/45 (11.1%) PM.
- Significantly associated with anti-Ro52/TRIM21 antibodies, rheumatoid factor, and hyperglobulinemia in the primary SJS patients. In DM patients, the antibody was associated with TIF-1y and Caucasian race.

Ref 2:
- Found in 32% of early-stage (pre-op) colon cancer
- Titers decreased after tumor resection


QUESTIONS ??

M-Phase Phosphoprotein 1 (MPP-1)

- Dr. P. Rao (MD Anderson Hospital, Houston) produced monoclonal antibodies to synchronized mitotic cells
- 2 monoclonals designated MPM-1 and MPM-2 of interest
  - IB: 55 – 220 kDa M-phase proteins
  - Labeled with P32 / removed with phosphatase = phosphoproteins
  - 1994 Westendorf identified two cDNAs with unique sequences she named MPP1 and MPP2
  - 1996 MPP1 localized to midbody of dividing cells
  - 1999 immunoscreening an expression cDNA library identifies MPP1 as an autoantibody target in ~25% of idiopathic ataxia*
  - 2003 Abaza et al identify MPP1 as a kinesin related protein: ‘molecular motor’ named KIF20B

Anti-MPP1/KIF20B IIF staining

- 20% of nuclei (star)
- Bright staining of mid-body/intracellular bridge (arrow) of anaphase cells
- Weak cytoplasmic staining
- Nuclei stained blue with DAPI

Anti-MPP1/KIF20B tissue staining

- 20% of pericyte nuclei (arrows)
- Bright staining of Purkinje cell and nuclear layer cell cytoplasm
- Bright staining of nuclei of granular layer cells, testis and ovary

MPP1/KIF20B is a coiled-coil protein
Eptiopes of MPP1/KIF20B are not in coiled-coil regions

Anti-MPP1/KIF20B measured by ALBIA

<table>
<thead>
<tr>
<th>Patient Group</th>
<th>N</th>
<th>% Positive</th>
<th>Cohort</th>
</tr>
</thead>
<tbody>
<tr>
<td>SLE</td>
<td>140</td>
<td>26.0</td>
<td>STARLET</td>
</tr>
<tr>
<td>SLE (high titer*)</td>
<td>140</td>
<td>15.0</td>
<td>STARLET</td>
</tr>
<tr>
<td>SSc</td>
<td>77</td>
<td>10.9</td>
<td>Calgary</td>
</tr>
<tr>
<td>SSc – cancer</td>
<td>31</td>
<td>9.7</td>
<td>CSRG</td>
</tr>
<tr>
<td>SjS</td>
<td>18</td>
<td>11.1</td>
<td>Calgary</td>
</tr>
<tr>
<td>Paraneoplastic</td>
<td>54</td>
<td>13.7</td>
<td>Calgary</td>
</tr>
<tr>
<td>PANDAS</td>
<td>46</td>
<td>0</td>
<td>Missouri &amp; Calgary</td>
</tr>
<tr>
<td>Normal</td>
<td>50</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

* ALBIA using full length recombinant protein (conformational epitopes preserved)
Cutoff >499 median fluorescence units
High titer >1000 MFI)
Clinical Correlates High Titer anti-MPP1 in SLE*

- Higher total SLEDAI-2K score (OR 1.1, [95% CI 1.0, 1.3])
- Serositis (OR 3.0, [95% CI 1.4, 6.6])
- Immunological subscales (OR 2.0, [95% CI 1.4, 2.9])
- Anti-dsDNA (OR 5.5 [95% CI 1.8, 16.6])
- Anti-SSA/Ro60 (OR 3.1 [95% CI 1.0, 8.9])
- Anti-PS/PT complex-IgG (OR 3.6 [95% CI 1.1, 11.5]).

* Univariable analysis

Further studies

- SLE cancer cohort: Sasha Bernatsky
- Larger SLE cohort (SLICC: Ann Clarke, John Hanly)
- NPSLE: Dr. Zahi Touma, U of Toronto
- Epitope mapping

QUESTIONS?

Bicaudal D2 (BICD2)

A study of 451 unselected Canadian Scleroderma Research Group patients
**Anti-BICD2 Serology**

- Anti-BICD2: Addressable Laser Bead Immunoassay
- 25.7% of SSc were anti-BICD2 positive
  - second most common autoantibody in this cohort
  - 19.0% had single specificity anti-BICD2 (ANA negative SSc)
  - 81.0% had other autoantibodies, notably anti-CENP (83/94; 88.3%)

**Anti-BICD2: Clinical Associations**

- 25.7% of SSc were anti-BICD2 positive
  - second most common autoantibody in this cohort
  - 19.0% had single specificity anti-BICD2 (ANA negative SSc)
  - 81.0% had other autoantibodies, notably anti-CENP (83/94; 88.3%)
- Compared to anti-BICD2 negative subjects, single specificity anti-BICD2 subjects had:
  - interstitial lung disease (ILD; 52.4% vs. 29.0%, p = .024)
  - inflammatory myopathy (IM; 31.8% vs. 9.6%, p = .004)
- 40% BICD2+ had anti-Ro52/TRIM21 vs 25% ABN

**Anti-BICD2: Epitope Map**

- Epitope mapping revealed a serine and proline-rich nonapeptide SPSPGSSLSP comprising amino acids 606–614 of BICD2, shared with CENP-A but not CENP-B.
Anti-BICD2 Summary

- Affinity purified anti-BICD2: no identifiable IIF pattern
- Monoclonal anti-BICD2

- Autoantibodies to BICD2 represent a new biomarker as they were detected in patients without other SSc-specific autoantibodies

Moving on… An interesting patient…

- 27-year-old female admitted to hospital with 5 month history of polyarthritits, Raynaud’s, dry eyes, photosensitivity
- Initial laboratory analysis:
  - ANA was positive IIF on HEp-2 cells
    - Titre 1:5120
    - Coarse nuclear speckled and nuclear dots staining pattern
  - ENA: anti-Sm and high titre anti-U1RNP
  - Rheumatoid factor (22 kU/l)
An interesting patient…P/E + Lab

- Complained of myalgias
- Mild proximal muscle weakness 4/5 strength in hip flexors bilaterally
  - CK was 1700 U/L
  - No statin exposure
  - EMG was normal except for area of a single positive sharp wave was noted in the iliopsoas muscle

“Very confident that this may be lupus”?

- SLE vs. undifferentiated or mixed connective tissue disease
  - Hydroxychloroquine and prednisone 15mg PO BID
  - Arthritis and weakness resolved within 4 days so she was discharged
- 30 days later, she returned with worsening symmetrical proximal muscle weakness and shortness of breath
  - Creatine kinase increased to 7805 U/L

Further Investigation

- Cardiac MRI - no myocarditis, no edema
- CT chest/abdomen/pelvis - heterogeneous enhancement throughout the paraspinal, abdominal wall/pelvic musculature and psoas muscles
- MRI hips/pelvis - extensive and symmetric edema in pelvis and thigh musculature
- Muscle biopsy of vastus lateralis "necrosis with minimal inflammation"
Muscle Biopsy of Vastus Lateralis

- Regenerating myofibers
- Edema
- Degenerating and necrotic myofibers

Complement C5b-9/MAC

- Necrotic Fibers

Myositis-Related Autoantibodies

- Myositis Panel: **Negative**
  - Jo-1, Mi2, Mi2-α, Mi2β, MDA5, NXP2, TIF1γ PL7, PL12, PM/Sc170, PM/Sc1100, Ku, SRP, EJ, OJ, Ro52

- Immune Mediated Necrotizing Myopathy/Statin-Related Myopathy Serology: SRP, HMGCR
  - **Negative**
CK Continued to Rise…

- Pulse steroids and IVIG initially
- 1 week later, she developed respiratory failure → intubated

Transferred to ICU

- Multifactorial shock with severe biventricular dysfunction with rising lactate, WBC, and creatinine
- Dialysis, vasopressors, empiric broad spectrum antibiotics
- IV cyclophosphamide and PLEX x 5 days
- Disseminated intravascular coagulation, pulseless electrical activity cardiac arrest x 3 → Passed away on day 14

Challenges in Autoantibody Testing in Autoimmune Myopathies

- Autoantibodies known to be associated with a necrotizing autoimmune myopathy
  - HMG CoA reductase (anti-HMGCR)
  - Signal Recognition Particle (anti-SRP)
- In her case, both were negative, so what else could it be?

*The ANA IIF pattern was a clue!*
WHAT IN THE WORLD IS SMN

- SMN = Survival of Motor Neuron + gemins: 5-8 proteins
- Involved in mRNA splicing
- Nuclear proteins localized to Cajal bodies
- ICAP IIF staining pattern: few nuclear dots = AC-7

ANA was the clue!

- Coarse nuclear speckled and nuclear dots IIF pattern:
  
  **Anti-Survival of Motor Neuron (SMN)/Gemins Antibodies**

- SMN gene deletion/mutation causes spinal muscular atrophy
- The SMN complex (SMN and gemin 3/4 proteins) play a critical role in the assembly of small nuclear ribonucleoproteins (snRNP): Sm and U1RNP
Are anti-SMN/Gemins associated with necrotizing autoimmune myopathy?

- Tested positive for anti-SMN1 / Gemin3 antibodies at Mitogen Advanced Diagnostics Laboratory
- Sera sent to Dr. Minoru Satoh (Japan) for immunoprecipitation

Letter to the Editor (Case report)

Autoantibodies to the survival of motor neuron complex in a patient with necrotizing autoimmune myopathy
Adam Amlani, Glen S. Hazlewood, Leslie Hamilton, Minoru Satoh, Marvin J. Fritscher
Rheumatology (Oxford) 57:199, 2018

SMN: Is it AC-7 or AC-6 or both?
### Anti-SMN pilot data

<table>
<thead>
<tr>
<th>Serum Group</th>
<th>N</th>
<th>% Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>50</td>
<td>0</td>
</tr>
<tr>
<td>AC-6/AC-7 Pattern Sera</td>
<td>80</td>
<td>6.3</td>
</tr>
<tr>
<td>SLE</td>
<td>150</td>
<td>1.3</td>
</tr>
<tr>
<td>SSc (CSRG)</td>
<td>58</td>
<td>17.3</td>
</tr>
<tr>
<td>Myositis (CIMS)</td>
<td>66</td>
<td>9.1</td>
</tr>
</tbody>
</table>

**Is there a consistent clinical phenotype associated with anti-SMN? Overlap SSc/AIM; SLE/AIM?**

### NT5C1/MUP44

- Several decades searching for a biomarker for sporadic inclusion body myositis (sIBM)


### Biomarker for Inclusion Body Myositis?

- Antibodies to Mup44/NT5C1
- Adam Amlani, Mark Tamopolsky, Lauren Brady, Marvin Fritzler
- University of Calgary, McMaster University Medical Center
  - Study of 250 patients:
    - 19 sporadic IBM
    - Controls: healthy (n=28), autoimmune myopathies (n=40), statin-related myopathies (n=4), other SARD (n=97)
  - Anti-Mup44 in sIBM: 11/19 (57.9%).
  - Anti-Mup44 12% in disease controls and 10.7% in healthy controls.
  - Sensitivity 58%, Specificity ~88%
  - sIBM: 1/19 (5%) positive for anti-HMGCR, but negative for all other myositis-related antibodies (Jo-1, OJ, Ti/P1y, PL-12, SAE, EJ, MDA5, PL7, SRP, NXP2, Mi-2).
  - No consistent IIF pattern on HEp-2 seen in anti-Mup44 +ve

* See Poster # 165 this meeting
Biomarker for Inclusion Body Myositis?

- Antibodies to Mup44/NT5C1?
  - Adam Amlani, Mark Tamopolsky, Lauren Brady, Marvin Fritzler
  - University of Calgary, McMaster University Medical Center
  - Study of 250 patients: 19 had sIBM. Healthy controls (n=28); other autoimmune conditions (n=197), autoimmune myopathies (n=40), statin-related myopathies (n=4), osteoarthritis (n=47), other neuromuscular or metabolic disorders (n=13).
  - Anti-Mup44 in sIBM: 11/19 (57.9%).
    - Anti-Mup44 12% in disease controls and 10.7% in healthy controls.
    - Sensitivity 58%, Specificity ~88%
  - sIBM: 1/19 (5%) positive for anti-HMGCR, but negative for all other myositis-related antibodies (Jo-1, OJ, TIF1γ, PL-12, SAE, EJ, MDA5, PL7, SRP, NXP2, Mi-2).
  - No consistent IIF pattern on HEp-2 seen in anti-Mup44 +ve


Acknowledgements

- Dr. May Choi University of Calgary
- Dr. Marie Hamon McGill University
- Dr. Sasha Bernatsky McGill University
- Dr. Yves Trepagnier University of Montreal
- Dr. Ann Clarke University of Calgary
- SLICC Members 14 International Centers
- Dr. Murray Baron McGill University
- CSRG Members
- Dr. Jenny Walker University of Adelaide
- Dr. Shervin Assassi University of Texas (Houston)
- Dr. Michael Meiler Inova Diagnostics, San Diego
- Patricia Swartwood Inova Diagnostics, San Diego
- Dr. Vera Beznakova Alberta Children’s Hospital
- Dr. Marinka Twerd Alberta Children’s Hospital
- Dr. Heike Honekaveling Alberta Children’s Hospital
- Dr. Ron Laser Hospital for Sick Children, Toronto
- Dr. Alan Rosenberg University of Saskatchewan
- Dr. Anne Steven Seattle Children’s Hospital
- Dr. Katie Moore Children’s Hospital of Colorado
- Dr. Kathryn Todor University of Pittsburgh