Science Webinar Series
Addressing challenges in data collection: The role of automation in complex translational research
September 30, 2015

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Science Webinar Series
Addressing challenges in data collection: The role of automation in complex translational research

September 30, 2015

Participating experts

Francisco J. Quintana, Ph.D.
Brigham and Women's Hospital
Boston, MA

Kristjan Plätzer, Ph.D.
University of Salzburg
Salzburg, Austria
Biomarkers and Immunoregulatory Mechanisms in Multiple Sclerosis

Francisco J. Quintana, Ph.D.

Ann Romney Center for Neurologic Diseases
Department of Neurology
Brigham and Women’s Hospital
Harvard Medical School
Myelin is targeted by the immune response in Multiple Sclerosis (MS) and Experimental Autoimmune Encephalomyelitis (EAE)

Frohman et al., N Engl J Med 354:94202
Immune Status and Disease Course in MS

Innate Immunity

Astrocytes, Microglia, Monocytes

Adaptive Immunity

T cells, B cells

Disability

GD enhancement

Relapsing-remitting → Secondary progressive

Modified from Weiner HL 2009
OVERVIEW

Antigen arrays as biomarkers for Multiple Sclerosis

Regulation of the adaptive immune response

Regulation of local CNS innate immunity
Antigen arrays as biomarkers for Multiple Sclerosis

Regulation of the adaptive immune response

Regulation of local CNS innate immunity
Antigen 1 / Antigen 2
Serum IgM
Serum IgG
αIgM or αIgG detection antibody
IgM or IgG signal

## Performance of Antigen Microarrays

<table>
<thead>
<tr>
<th>Dilution</th>
<th>HSP60-1</th>
<th>HSP60-2</th>
<th>HSP60-3</th>
<th>MBP</th>
<th>PLP</th>
<th>GM4</th>
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<tbody>
<tr>
<td></td>
<td>Array</td>
<td>ELISA</td>
<td>Array</td>
<td>ELISA</td>
<td>Array</td>
<td>ELISA</td>
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<tr>
<td>1:100</td>
<td>MAX 1.98</td>
<td>MAX 1.77</td>
<td>45,693</td>
<td>1.64</td>
<td>MAX 1.51</td>
<td>MAX 2.31</td>
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<td>1:1,000</td>
<td>MAX 1.24</td>
<td>MAX 1.29</td>
<td>23,731</td>
<td>0.73</td>
<td>46,314</td>
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<td>39,837</td>
<td>0.83</td>
<td>5,375</td>
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<td>31,513</td>
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<td>3,489</td>
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<td>742</td>
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<td>6,916</td>
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<td>0</td>
<td>0</td>
<td>0</td>
<td>1,810</td>
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</table>

Antigen Microarray Experimental Setup

**Fig. 1. Antigen microarrays for the study of autoimmune diseases.**

Autoantigen collections (proteins, peptides or lipids) are spotted onto chemically-modified glass slides, and hybridized with patient specimens (serum, plasma, CSF, SF). Antigen–antibody interactions are detected with fluorescent-labeled antibodies (Ab). The bioinformatic analysis of the fluorescent signals results in the identification of autoantibody signatures useful for disease diagnosis, staging and monitoring of the response to therapy.

Modified from Clinical Chemistry 57:1036-44 (2013)
# Variability in Antigen Array Assays

<table>
<thead>
<tr>
<th>Source of Variability</th>
<th>CV</th>
<th>Correlation</th>
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<tr>
<td></td>
<td>IgG</td>
<td>IgM</td>
<td>IgG</td>
</tr>
<tr>
<td>Chip to Chip</td>
<td>13.3 ± 1.2</td>
<td>11.9 ± 2.1</td>
<td>0.94 ± 0.04</td>
</tr>
<tr>
<td>Day to Day</td>
<td>45.6 ± 6.1</td>
<td>22.1 ± 2.1</td>
<td>0.85 ± 0.03</td>
</tr>
<tr>
<td>Batch to Batch</td>
<td>42.2 ± 0.5</td>
<td>44.1 ± 1.3</td>
<td>0.78 ± 0.01</td>
</tr>
<tr>
<td>Intersubject</td>
<td>62.5 ± 1.3</td>
<td>60.2 ± 1.5</td>
<td>0.51 ± 0.03</td>
</tr>
<tr>
<td>Intrasubject over time</td>
<td>38.7 ± 4.6</td>
<td>27.5 ± 6.9</td>
<td>87.7 ± 2.4</td>
</tr>
<tr>
<td>Freezing and Thawing</td>
<td>32.3 ± 5.4</td>
<td>15.4 ± 2.7</td>
<td>0.87 ± 0.04</td>
</tr>
</tbody>
</table>
Identification of antibody patterns of reactivity associated to different types of MS

Controls

\( \uparrow \text{anti-CNS}^1 \)
\( \uparrow \text{anti-HSP} \)

RRMS \( \rightarrow \)

\( \uparrow \text{anti-CNS}^3 \)
\( \downarrow \text{anti-HSP} \)

SPMS  PPMS

\( \uparrow \text{anti-CNS}^2 \)

## Antibody Patterns in MS

<table>
<thead>
<tr>
<th>Comparison</th>
<th>Success rate</th>
<th>Error rate</th>
<th>$P$ value</th>
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</thead>
<tbody>
<tr>
<td>RRMS vs SLE</td>
<td>0.83</td>
<td>0.17</td>
<td>&gt;0.0001</td>
</tr>
<tr>
<td>RRMS vs ALD</td>
<td>0.93</td>
<td>0.07</td>
<td>&gt;0.0001</td>
</tr>
<tr>
<td>RRMS vs AD</td>
<td>1.00</td>
<td>0.00</td>
<td>&gt;0.0001</td>
</tr>
</tbody>
</table>

SLE: Systemic Lupus Erythematosus  
ALD: Adrenoleukodystrophy  
AD: Alzheimer’s Disease

Antigen Microarrays: Serum Antibody signatures

1. **Disease monitoring**: Early diagnosis, disease progression and staging, response to therapy.

2. **Disease Risk**: Characterize environmental factors and immune repertoire for those as risk to get MS

3. **Discovery**: Understanding disease mechanisms and identification of new therapeutic targets.
Antigen arrays as biomarkers for Multiple Sclerosis

Regulation of the adaptive immune response

Regulation of local CNS innate immunity
Astrocytes, Microglia, Monocytes

T cells, B cells

Modified from Weiner HL 2009
Peripheral Lymphoid Tissue

- CD8+ T cell
- Th17 cell
- Th1 cell
- CD8+ cell

Inflammatory Cells

- B cell
- APC
- Thp

Regulatory Cells

- Treg cell
- Tr1 cell
- Th3 cell

Venn Diagram:

- TGF-β (IL-6/IL-21/IL-1β) + IL-23
- IL-12

Central Nervous System

- Axonal and myelin damage
- Initiation of inflammatory cascade

Blood Brain Barrier

- Microglia / Dendritic cells

TGF-β, IL-10, IL-35

Peripheral Lymphoid Tissue

- Microglia / Dendritic cells

TGF-β, IL-6, TNF-α
Zebralfish to study immune regulation

Characterization of zebrafish FoxP3+ Tregs and Th17 cells.

Analysis of CNS autoimmunity in zebrafish.

Zebralfish as a new model to identify genes and molecules that regulate adaptive and innate immunity.

The Aryl Hydrocarbon Receptor (AHR)
Regulation of CNS inflammation by AHR

Th17/Th22 cells
Nat. Immunology (2012) 13:991
Nat. Communications (2014) 5:3753

FoxP3+ regulatory T cells:
PNAS (2010) 107:20768
Nat. Immunology (2013) 14:1054

IL-10+ regulatory T cells (Tr1)
Nat. Immunology (2010); 11:854
Nat. Immunology (2013) 14:1054
Cell (2015) 162:1338
How is the differentiation of Tr1 cells regulated?

- **Diet**
- **Commensal flora**
- **Metabolism**
- **IL-27**
- **AHR ligands**
- **HIF-1α**
- **T cell**

Metabolism of Inflamed tissue
- eATP
- Hypoxia

Dendritic cell

Model 1:
1) \( \frac{dH}{dt} = k_{H0} - k_{HE} \cdot H \cdot E \)
2) \( \frac{dA}{dt} = k_{A0} - k_{HA} \cdot H \cdot A + k_A \cdot A \)
3) \( \frac{dE}{dt} = k_{E0} - k_E \cdot E \)

Model 2:
1) \( \frac{dH}{dt} = k_{H0} - k_{HE} \cdot H \cdot E \)
2) \( \frac{dA}{dt} = k_{A0} - k_{HA} \cdot H \cdot A + k_A \cdot A \)
3) \( \frac{dE}{dt} = k_{E0} + k_{AE} \cdot \frac{A^2}{K_A^2 + A^2} - k_E \cdot E \)

Targets for therapeutic modulation of Tr1 cells

Regulation of CNS inflammation by AHR

**Th17/Th22 cells**
- Nat. Immunology (2012) 13:991
- Nat. Communications (2014) 5:3753

**FoxP3+ regulatory T cells:**
- PNAS (2010) 107:20768
- Nat. Immunology (2013) 14:1054

**IL-10+ regulatory T cells (Tr1)**
- Nat. Immunology (2010); 11:854
- Nat. Immunology (2013) 14:1054
- Cell (2015) 162:1338
Both genetic and environmental factors influence MS

Genes

Environment

- Diet
- Commensal flora
- Infections
- Sunlight
- Pollutants

Dysregulated Immunity

Autoimmune Disease
Environmental control of MS disease activity? Control of Tr1 and Th17 cells by melatonin


Reverse protein arrays
Farez et al, Nat. Immunology (2009)
How does the environment affect the immune response?
Antigen arrays as biomarkers for Multiple Sclerosis

Regulation of the adaptive immune response

Regulation of local CNS innate immunity
Astrocytes, Microglia, Monocytes

T cells, B cells
Blood Brain Barrier Central Nervous System

Initiation of inflammatory cascade

Teff 1

Primary epitope

Epitope spreading

Teff 2

Secondary epitope

Ag release

Activation of the innate system

Cytokines

Direct damage

Danger signals + cytokines

Chronic CNS inflammation

Microglia

Astrocytes

Phagocytosis

Proteolytic enzymes

Glutamate release

NO production

Phagocytosis

Proteolytic enzymes

NO production

Chronic CNS inflammation

Monocyte

MIP-1α

IFN-γ

MIP-1α

IFN-γ

Periphery

Blood Brain Barrier

Central Nervous System

Axonal and myelin damage

B cell

Antibody mediated damage

Peripheral

NAWM Gray Matter

White Matter

Periphery

Blood Brain Barrier

Central Nervous System
What is the role of astrocytes in chronic CNS inflammation?

Depletion of activated astrocytes

NOD model of progressive EAE

Acute phase
Disease worsening

Progressive phase
Disease amelioration

Farez et al, Nat. Immunology 10, 958-64 (2009)
Gene expression profiling of astrocytes during the acute and progressive phases of NOD EAE

B4GALT6 inhibition ameliorates progressive EAE

Brain 137:2271-86 (2014).

Pathogenic activities of astrocytes during chronic CNS inflammation/ progressive MS

Inhibitors

LacCer

B4GALT6

GlcCer

Astrocytes

Recruitment of monocytes

CCL-2

GM-CSF

iNOS

TNFα

Activation

Neurodegeneration

De/Remyelination

MS pathogenesis

Biomarkers and Immunoregulatory Mechanisms In Multiple Sclerosis

Immune Status and Disease Course in MS

- Innate Immunity
- Adaptive Immunity
- Disability
- GD enhancement
- Relapsing-remitting → Secondary progressive

Modified from Weiner HL 2009
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http://brighamandwomens.org/research/labs/quintana/default.aspx
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Salzburg, Austria
Addressing challenges in data collection: The role of automation in complex translational research

Part II: Research on cancer therapies: Monitoring the formation of protoporphyrin IX for photodynamic treatment of malignant cells.

PD Dr. Kristjan Plaetzer

Laboratory of Photodynamic Inactivation of Microorganisms
Department of Materials Science and Physics
University of Salzburg
www.uni-salzburg.at/pdi
Photodynamic tumor therapy (PDT)

(1) Administration of a harmless, photoactive drug = photosensitizer (PS).

(2) Accumulation of the photosensitizer in target cells or tissue.

(3) Illumination of cells or tissue by visible light (no UV) matching absorption of the photosensitizer.

(4) Photophysical generation of reactive oxygen species (ROS) by the PS induces cell death via apoptosis or necrosis due to oxidative damage.
Clinical approvals of PDT

<table>
<thead>
<tr>
<th>approved PS / indications</th>
<th>brain</th>
<th>ophthalmic</th>
<th>head &amp; neck</th>
<th>oesophagus</th>
<th>lung</th>
<th>skin</th>
<th>bile duct</th>
<th>gastric</th>
<th>pancreatic</th>
<th>cervix</th>
<th>bladder</th>
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<td>δ-ALA esters</td>
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excitation wavelength, nm
630 635 652 690


© Kristjan Plaetzer, PDI-PLUS
PDT based on 5-aminolevulinic acid

Challenge: Illumination @ highest intracellular PPIX
Fluorescence diagnosis based on 5-aminolevulinic acid

Papillary bladder carcinoma using white light (left) or blue light (right) during fluorescence diagnosis. Fluorescence of ALA-induced protoporphyrin IX is clearly visible.

Lab workflow of in vitro experiments

Seeding of cells
Growth overnight
Medium change
Addition of compound(s)
Monitoring of response processes
The black box of PPIX formation
Task force workflow automation: using a multimode reader and plate washer

- **Seeding of cells**
  - Use dispenser with heater & stirrer?

- **Growth overnight**
  - Use environmental control features?

- **Medium change**
  - Employ external microplate washer?

- **Addition of compound(s)**
  - Use dispenser with heater & stirrer?

- **Monitoring of response processes**
  - Use environmental control features?
Can one seed living cells using the dispenser with heater & stirrer in a 96-well microplate?
Can one seed living cells using the dispenser with heater & stirrer in a 384-well microplate?

![Graph showing A431 GFP fluorescence 485/535 nm [RFU] comparison between Spark 10M and CO2 Incubator.]
Do cells grow inside the reader as usual after automated seeding in a microplate?
Do cells grow inside the reader as usual after automated seeding in a 384-well microplate?
Manual recording of growth curve and manual medium exchange: issues with cell viability and experimental error

![Graph showing A431 GFP fluorescence intensity over incubation period with manual medium exchange.]
Does automated medium exchange in 384-well MTPs work with a plate washer?
Recording the kinetics of PPIX formation in cancer cells after injection of 5-ALA

- GFP: 485/535 nm Filter
- PPIX: 411/634 nm Monochromator
Recording the kinetics of PPIX formation in cancer cells after injection of 5-ALA
Real-time analysis of endogenous protoporphyrin IX fluorescence from δ-aminolevulinic acid and its derivatives reveals distinct time- and dose-dependent characteristics in vitro

Tobias Kiesslich, a,b Linda Helander, c Romana Illig, d Christian Oberdanner, e Andrej Wagner, b Herbert Lettner, f Martin Jakab, a and Kristjan Plaetzer a

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University of Salzburg, Department of Materials Science and Physics, Laboratory of Photodynamic Inactivation of Microorganisms, Helbrunnerstraße 94, Salzburg A-5020, Austria
Experimental setup in a single 96-well microplate and with manual cell seeding using Tecan’s Infinite 200PRO with Gas Control Module.


$$PPIX \text{ per GFP} = \frac{(FI_{PPIX}^{wells} - FI_{PPIX}^{blank})}{( FI_{GFP}^{wells} - FI_{GFP}^{blank} )}$$
Task force workflow automation: using a multimode reader and plate washer

Seeding of cells

Use dispenser with heater & stirrer?

Use environmental control features?

Growth overnight

Use environmental control features?

Medium change

Employ external microplate washer?

Addition of compound(s)

Use dispenser with heater & stirrer?

Monitoring of response processes

Use environmental control features?
Conclusions

• Photodynamic Therapy based on 5’-ALA-induced protoporphyrin IX represents a safe and powerful approach to cure dermatological cancers.

• PPIX formation in A431 human squamous carcinoma follows distinct kinetics. The time point and level of maximal intracellular protoporphyrin IX depends on the concentration of 5’-ALA.

• Environmental control of the multimode reader allows for continuous recording of signals, which guarantees complete data sets.

• In addition, automatization allows for easy and reproducible handling of 384-well microplates. This maximizes the number of parameters that can be tested in a single experiment and minimizes costs by saving time and reagents.

• Automated washing of cells further helps to minimize the overall experimental error.
Thank you!

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