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<table>
<thead>
<tr>
<th>Date</th>
<th>Event</th>
</tr>
</thead>
<tbody>
<tr>
<td>January 26</td>
<td>Fighting Cancer: Targeting ChS Malignancy with Kinase Inhibitors, Timothy P. Hefton - Genentech, Mark Wittman - Bristol-Myers Squibb</td>
</tr>
<tr>
<td>February 23</td>
<td>Fighting Cancer: Epigenetic targets for Oncology, Stuart Conway - Oxford, Sharan Bagai - AstraZeneca</td>
</tr>
<tr>
<td>March 28</td>
<td>Fighting Cancer: Allostery and Targeting Cancer Cell Metabolism, Stefan Gross - Agios, Scott Edmundson - AstraZeneca</td>
</tr>
<tr>
<td>April 20</td>
<td>Cystic Fibrosis: Discovery of CFTR Modulators, Peter Groothuis - Vertex, Nick Meanwell - Bristol-Myers Squibb</td>
</tr>
<tr>
<td>May 25</td>
<td>Anti-Infectives: National Approaches to the Design and Optimization, Jason Sello - Brown University, Courtney Aldrich - University of Minnesota</td>
</tr>
<tr>
<td>June 29</td>
<td>Tuberculosis: An Introduction for Medicinal Chemists, Carl Nathan - Weill Cornell Medicine, Christopher Boyce - Merck</td>
</tr>
<tr>
<td>July 27</td>
<td>Viral Hepatitis: The Search for a Cure, Mike Sofia - Arbusa Biopharma, Stephen Mason - CarisCor Corporation</td>
</tr>
<tr>
<td>September 28</td>
<td>Special Broadcast</td>
</tr>
<tr>
<td>October 26</td>
<td>Psoriasis: Treatment and Novel Approaches, Frank Najjar - AstraZeneca, John Morrison - Bristol-Myers Squibb</td>
</tr>
<tr>
<td>November 30</td>
<td>Lupus: Treatment and Novel Approaches, Laurence Minard - Bristol-Myers Squibb, Mary Struthers - Bristol-Myers Squibb</td>
</tr>
</tbody>
</table>

“Treating Lupus: SLE Pathogenesis and Targeted Therapies”

Outline

• Overview of SLE disease and symptoms

• SLE disease pathophysiology

• Targeted pathways
  – BAFF and B cells
  – IFN pathway
  – TLRs and pDC
  – T cell activation and polarization

• Conclusions
Systemic Lupus Erythematosus (SLE)

• More common in women (9:1 ratio)

• US prevalence: 20-150/100,000
  – Symptom onset typically between 20–40 years of age
  – 2-3 x more frequent, with more severe symptoms, in African American, Hispanic, Native American and Asian individuals than Caucasians
  – Periods of remission and flares

• High economic burden of medical costs, job reduction or loss, and work disability: one-third of people with lupus are on work disability; by 15 years after diagnosis, 51% have stopped working

www.lupusresearch.org

Audience Challenge Question

Answer the question on blue screen in one moment

What are common symptoms of lupus? (multiple answers possible)

• Rash on the face
• Mouth ulcers
• Depression
• Joint pain
• Blue urine
SLE Symptoms and Complications

• Common symptoms:
  – Fever and fatigue
  – Stiffness, swelling, and joint pain
  – Red rashes on the face
  – Sun sensitivity
  – Skin lesions
  – Mouth ulcers
  – Shortness of breath
  – Dry eyes
  – Headaches
  – Seizures
  – Confusion
  – Weight gain or loss
  – Anemia

• Lupus nephritis: main complication, can progress to end stage renal disease

• Abnormal blood tests
  – Autoantibodies: anti-nuclear antibodies (ANA), anti-dsDNA, anti-Sm, anti-RNP
  – Low complement (C3 or C4)
  – Lymphopenia

Etiology

• Combination of genetic and environmental factors

**Etiology: Role of Genetics**

- Genome wide association studies have revealed many implicated loci, most of them shared with other autoimmune diseases.
- Each small nucleotide polymorphism (SNP) confers a relative small risk by itself.

<table>
<thead>
<tr>
<th>Pathway(s)</th>
<th>Loci implicated in SLE and other autoimmune diseases</th>
<th>Loci implicated only in SLE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lymphocyte activation</td>
<td>PTPN2, TNFSF4, IL-10, SPRED2, STAT4, PIK3, AFF1, IL-2A, BANF1, TCF7, SKP1, MHC genes, ILK, IKZF1 and IKZF2, BLK, ARID5B, CD44, LYN, ET51, FLI1, SH2B3, CSK, ELF1, CIRH, IFGAM, TK2</td>
<td>IKZF2</td>
</tr>
<tr>
<td>IFN or Toll-like receptors</td>
<td>IFNHI, PRDM1, LHRF1, IFI1, IFI15, IFI19, IFI75, IFI70, IFI44, IFI63, IFI67</td>
<td>None</td>
</tr>
<tr>
<td>Inflammation</td>
<td>TNIPI</td>
<td>None</td>
</tr>
<tr>
<td>Immune complex or waste clearance</td>
<td>FCGR2A, FCGR2B, FCGR3B, FCGR4B, ATG5, CLEC16A</td>
<td>NCF2, LYST</td>
</tr>
<tr>
<td>Unknown</td>
<td>ABHD6 (may be related to lymphocyte activation), RAD51B (may be related to IFN pathways), RASGRF3, TMEFF2, PRKCI, TNK2, JAK3, XKR6, FAM167A-AS1, WDFY4, unknown genes: rN1167798, rN463128, rN136852, rN1797475</td>
<td>SMG7 (may be related to interferon pathways), DHCR7, NAG3YN1, SLC25A4, PLD2, CXorf21</td>
</tr>
</tbody>
</table>


- A few mono-allelic mutations give higher risk to develop lupus or lupus-like diseases (e.g., complement genes, DNASE1, genes associated with nucleic acid sensing and IFN signaling).

**SLE Pathophysiology**

1. **Apoptotic debris bind autoantibodies and activate plasmacytoid dendritic cells**

2. **Dendritic cells activate autoreactive T and B cells and propagate inflammation**

3. **Tissue injury by cytotoxic T cells and autoantibodies**

Adapted from Nature Medicine 18, 871–882 (2012)
Standard of Care Treatments

- Most commonly used and approved therapies:

<table>
<thead>
<tr>
<th>Drug</th>
<th>Use</th>
<th>Significant Toxicities</th>
</tr>
</thead>
<tbody>
<tr>
<td>NSAID, aspirin</td>
<td>Pain, joint inflammation</td>
<td></td>
</tr>
<tr>
<td>Corticosteroids</td>
<td>Flares, but also took in the long term</td>
<td>infections, bone destruction, osteoporosis</td>
</tr>
<tr>
<td>Antimalarials (e.g. chloroquine)</td>
<td>Milder disease</td>
<td>Retinal toxicity</td>
</tr>
<tr>
<td>Cyclophosphamide</td>
<td>Lung and kidney disease</td>
<td>Bladder bleeding, hair loss, sterility</td>
</tr>
<tr>
<td>Mycophenolate mofetil (MMF)</td>
<td>Lupus nephritis</td>
<td></td>
</tr>
<tr>
<td>Azathioprine</td>
<td>Liver and kidney disease</td>
<td>Pancreatitis, hepatitis</td>
</tr>
<tr>
<td>Belimumab</td>
<td>SLE</td>
<td></td>
</tr>
</tbody>
</table>

- These drugs can have significant side effects and toxicities
- Only one biologic approved (belimumab), need for more targeted therapies

Belimumab is an Inhibitor of the BAFF Pathway

1. Apoptotic debris bind autoantibodies and activate plasmacytoid dendritic cells

2. Dendritic cells activate autoreactive T and B cells and propagate inflammation

3. Tissue injury by cytotoxic T cells and autoantibodies

Adapted from Nature Medicine 18, 871–882 (2012)
**BAFF Pathway**

- BAFF/Blys is required for B cell survival and maturation
- Animal models:
  - BAFF Tg mice develop SLE-like disease,
  - BAFF blockade suppresses lupus in mice
- In patients:
  - Higher BAFF level that correlate with disease activity
  - Autoantibodies secreted by B cells

**Belimumab is the 1st BAFF Inhibitor Approved**

- Belimumab is the 1st approved targeted therapy for lupus (2011)
  - IgG1κ targets soluble BAFF, developed by Human Genome Sciences & GlaxoSmithKline
  - 2 phase III trials showed improvement
  - Patients with high disease activity, high anti-dsDNA and low complement levels showed better response

**Combination of 2 trials:** Proportion of patients showing improvement from baseline at 52 weeks

- **All patients (52 weeks)**
- **Patients with high serologic activity at baseline (52 weeks)**

![Graphs showing improvement from baseline at 52 weeks](Image)
Other Inhibitors of the BAFF Pathway

• More inhibitors of the BAFF pathway have been/are being considered

<table>
<thead>
<tr>
<th>Agent</th>
<th>Type</th>
<th>Target</th>
<th>Clinical Stage</th>
<th>Sponsor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atacicept</td>
<td>Fusion protein</td>
<td>BAFF + APRIL</td>
<td>Phase IIb/ III</td>
<td>EMD Serono</td>
</tr>
<tr>
<td>Blisibimod</td>
<td>Peptibody</td>
<td>Membrane and soluble BAFF</td>
<td>Phase III</td>
<td>Anthera</td>
</tr>
<tr>
<td>Tabalumab</td>
<td>Monoclonal antibody</td>
<td>Membrane and soluble BAFF</td>
<td>Discontinued</td>
<td>Eli Lilly</td>
</tr>
</tbody>
</table>

Adapted from Stohl W et al, 2014

B Cell Inhibition

• B cell depletion:
  – Rituximab is an anti-CD20 antibody that depletes CD20+ B cells
  – Failed to show efficacy in 2 phase III trials (SLE and lupus nephritis)
  – BAFF is elevated after B cell depletion therapy, potentially favoring survival and activation of remaining autoreactive B cells and relapse
  – Rituximab followed by belimumab to be tested in clinical trials

• Inhibition of B cell receptor (BCR) signaling with Burton tyrosine kinase (BTK) inhibitor:
  – BTK required for BCR signaling
  – Irreversible BTK inhibitor ibrutinib used to treat B cell cancers
  – BTK also involved in Fc receptor signaling on myeloid cells
  – BIIB068 (Biogen) completed phase I (SLE)
  – Evobrutinib (EMD Sereno) in phase II (SLE)
What does belimumab target?

- Soluble BAFF
- Membrane BAFF
- B cells
- Type I IFNs
- T cell costimulation

Targeting the Type I Interferon (IFN) Pathway

1. Apoptotic debris bind autoantibodies and activate plasmacytoid dendritic cells

2. Dendritic cells activate autoreactive T and B cells and propagate inflammation

3. Tissue injury by cytotoxic T cells and autoantibodies

Adapted from Nature Medicine 18, 871–882 (2012)
IFN Pathway

• IFN have anti-viral properties
  – I: e.g. IFNα, IFNβ
  – II: IFNγ
  – III: IFNλ

• pDC are the largest producers

• Increased type I IFN in SLE sera

• Increased IFN-induced genes in SLE patients

• Several SLE risk gene variants in loci linked to type I IFN system

• SLE-like syndrome with IFNα treatment

IFN Pathway Inhibitors: Biologics

• Biologics being tested in the clinic

<table>
<thead>
<tr>
<th>Target</th>
<th>Drug Name</th>
<th>Progress</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type I IFN</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anti-IFNAR mAb</td>
<td>Anifrolumab</td>
<td>Phase III—recruiting</td>
</tr>
<tr>
<td>Anti-IFNα mAb</td>
<td>Sifalimumab</td>
<td>Phase II—completed</td>
</tr>
<tr>
<td>Anti-IFNλ mAb</td>
<td>Rontalizumab</td>
<td>Phase II—completed</td>
</tr>
<tr>
<td>Anti-IFNκ mAb</td>
<td>ASG-009</td>
<td>Phase I—completed</td>
</tr>
<tr>
<td>IFN-kinoid vaccine</td>
<td>IFN-K</td>
<td>Phase IIb—ongoing</td>
</tr>
</tbody>
</table>

| Type II IFN     |               |                   |
| Anti-IFNγ mAb   | AMG811        | Phase I—completed  |

Oon S, Wilson NJ and Wicks I, Targeted therapeutics in SLE: emerging strategies to modulate the interferon pathway. Clinical & Translational Immunology, 2016
**IFN pathway Inhibitors: Small Molecules**

- JAK small molecules inhibitors in the clinic

<table>
<thead>
<tr>
<th>Target</th>
<th>Drug Name</th>
<th>Progress</th>
</tr>
</thead>
<tbody>
<tr>
<td>JAK/STAT</td>
<td></td>
<td></td>
</tr>
<tr>
<td>JAK1/3 inhibitor</td>
<td>Tofacitinib</td>
<td>Phase I—recruiting</td>
</tr>
<tr>
<td>JAK1 inhibitor</td>
<td>GSK2586184</td>
<td>Phase II—terminated</td>
</tr>
<tr>
<td>JAK/SYK inhibitor</td>
<td>R333 (topical)</td>
<td>Phase II—completed</td>
</tr>
</tbody>
</table>

Oon S, Wilson NJ and Wicks I, 2016; clinicalTrials.gov

**Tyk2 inhibitor:** phase II in SLE initiated by BMS

---

**Upstream of IFN Pathway: pDC and TLR Targeting**

1. Apoptotic debris bind autoantibodies and activate dendritic cells

2. Dendritic cells activate autoreactive T and B cells and propagate inflammation

3. Tissue injury by cytotoxic T cells and autoantibodies

Adapted from Nature Medicine 18, 871–882 (2012)
**Upstream of IFN Pathway: pDC and TLR Targeting**

- pDC produce the highest amount of type I IFN upon stimulation with TLR7 and TLR9
- pDC constitutively express IRF7
- The pDC/TLR axis offers opportunities for targeting by small molecule inhibitors
- Hydroxychloroquine affects acidification of endosomes and inhibits TLR7/8/9

![Diagram showing IFN pathway](image)

Kirou KA & Gkrouzman E, Anti-interferon alpha treatment in SLE, Clinical Immunology, 2013

---

**pDC and TLR Targeting**

<table>
<thead>
<tr>
<th>Target</th>
<th>Drug name</th>
<th>Progress</th>
</tr>
</thead>
<tbody>
<tr>
<td>pDCs</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anti-BDCA2 mAb</td>
<td>BIIB059</td>
<td>Phase II CLE ± SLE — recruiting</td>
</tr>
<tr>
<td>Bcl-2 inhibitors</td>
<td>ABT-199</td>
<td>Phase I trials in SLE completed</td>
</tr>
<tr>
<td>Anti-CD123 mAb</td>
<td>CSL362/JNJ-473</td>
<td>Preclinical, phase I completed in AML</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>DNA/RNA</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>RNase-Fc fusion protein</td>
<td>RSLV-132</td>
<td>Phase Ia—recruiting</td>
</tr>
<tr>
<td>Recombinant DNAse 1</td>
<td></td>
<td>Phase Ib—completed</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>TLRs</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>TLR7/9 oligonucleotide inhibitor</td>
<td>DV1179</td>
<td>Phase Ib/IIa—completed</td>
</tr>
<tr>
<td>TLR7/9 oligonucleotide inhibitor</td>
<td>IRS-954</td>
<td>Preclinical</td>
</tr>
<tr>
<td>TLR7/9 oligonucleotide inhibitor</td>
<td>IMO-3100</td>
<td>Preclinical in SLE; Phase II completed in psoriasis</td>
</tr>
<tr>
<td>TLR7/8/9 oligonucleotide inhibitor</td>
<td>IMO-8400</td>
<td>Preclinical in SLE; Phase II completed in psoriasis</td>
</tr>
<tr>
<td>TLR7/8/9 small-molecule inhibitor</td>
<td>CpG-52364</td>
<td>Phase I—completed</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>MyD88</th>
<th></th>
<th></th>
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</thead>
<tbody>
<tr>
<td>MyD88 dimerization inhibitor</td>
<td>ST-2825</td>
<td>Preclinical</td>
</tr>
</tbody>
</table>

Oon S, Wilson NJ and Wicks I, 2016; clinicalTrials.gov
**Inhibition of T Cell Activation and Polarization**

1. Apoptotic debris bind autoantibodies and activate plasmacytoid dendritic cells

2. Dendritic cells activate autoreactive T and B cells and propagate inflammation

3. Tissue injury by cytotoxic T cells and autoantibodies

Adapted from Nature Medicine 18, 871–882 (2012)

**Inhibition of T Cell Activation and Polarization**

- Costimulation of T cells by antigen presenting cells (APC) leads to the activation of adaptive immunity and T cell-mediated damage
  - Abatacept (Orencia):
    - CTLA4-Ig, blocks CD80/86-CD28 interactions
    - Approved for RA, did not meet primary endpoints in SLE(IIb) and lupus nephritis (II/III), may be beneficial in arthritis
  - Dapirolizumab
    - Anti-CD40L: promising results in phase I
  - Ustekinumab (Stelara):
    - Anti-IL12/23, blocks T cell polarization (Th1/Th17)
    - Approved in psoriasis, psoriatic arthritis and Crohns’ disease
    - Positive results in phase II SLE (60% response vs 31% placebo)
  - Calcineurin inhibitors:
    - Tacrolimus and cyclosporine A already used as induction therapies in lupus nephritis
    - Voclosporin, an analog of CSA, starting phase III in lupus nephritis

Additional Approaches

- **Targeting the defective cleaning of apoptotic debris:** micro-particles, neutrophil extracellular traps (NETs)

- **Metabolic pathways:**
  - Dysregulated metabolic pathways in CD4 T, B, myeloid cells in SLE
  - MMF, methotrexate, glucocorticoids may have a positive impact on metabolic pathways
  - Rapamycin and PPARg agonists (mTOR inhibitors) in trials

- **Antigen-specific targeting, tolerance induction:** expansion of Treg and deletion/anergy of autoreactive T and B cells
  - Mostly pre-clinical
  - Autoantigens are not known for every patient
  - Dosing is crucial, tolerance induction could backfire

**Audience Challenge Question**

ANSWER THE QUESTION ON BLUE SCREEN IN ONE MOMENT

Which statement(s) do you disagree with? (multiple answers possible)

- One new drug has been approved for lupus in decades
- Targeting more than one pathway at once may be required for maximal efficacy
- We just need to ‘crack the code’ and figure out the drug that will work for everyone
- Personalized medicine may be required for transformational efficacy
- I agree with all of the above
Conclusions

• SLE is a complex heterogeneous systemic autoimmune disease
• Both innate and adaptive immunity are implicated in the disease
• Significant unmet needs remain since only one new drug has been approved in decades
• Targeting of significant pathogenic pathways are being tested in the clinic (type I IFN, TLRs, costimulation)
• Personalized medicine and combination approaches may be required for transformational efficacy

Acknowledgments

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• Nicholas Meanwell
• Jim Burke
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• Steve Nadler
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Stephen Mason - CarGEO Corporation

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Kevin Heddle - Harvard Medical School
Alyson Waldmann - ACS Publications

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Joe Fortunak, Professor of Chemistry, Howard University

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