The Fundamentals of Immunotherapy in AML and MDS

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Immunology Program
## Conflict of Interest Declaration

<table>
<thead>
<tr>
<th>Conflict Declaration</th>
<th>Company</th>
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<tr>
<td>Grant funding (Epling-Burnette)</td>
<td>Incyte, Corp</td>
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<td>Grant funding (Epling-Burnette)</td>
<td>Celgene, Corp</td>
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<tr>
<td>Grant funding (Epling-Burnette)</td>
<td>Forma Therapeutics</td>
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Overview

- Discuss the fundamentals of immune deregulation in cancer with special focus on MDS and AML

- Immunologically-based therapies for hematological malignancies including MDS and AML
Innate vs. Adaptive Immunity Against Pathogens

- **Innate Immunity** (Neutrophils, macrophages, DCs, NK cells, NKT)
  - Non-specific defense
  - Generic recognition
  - No memory
  - Immediate response to pathogens
  - Evolutionarily older
  - Inherent ability to distinguish self from non-self

- **Adaptive Immunity** (T cells, B cells)
  - Specific defense system (2nd line)
  - Highly specialized cells
  - Activated by the innate immune system
  - Progeny of the cells will inherit the genes (memory)
  - First jawed vertebrates
  - Distinguishes “non-self” antigens in the presence of “self”, during the process of antigen presentation (antigen presenting cells, APCs)
Contributing Events in Cancer Pathology

- Multiple cytokines are increased in MDS
  - Zoumbos N and Georgoulias V. Increased levels of soluble interleukin-2 receptors and tumor necrosis factor in serum of patients with MDS. Blood. 77:413-2, 1991.

  TNFα, IFN-γ, TRAIL, IL-1, TGF-β, IL-4, IL-6, IL-8, IL-32

- Inflammatory mediators
  - Nitric oxide, arginase, DAMPs

How are cytokine and inflammatory mediators induced?

Innate Immunity Activation Through Pattern Recognition Receptors (PPRs)

- Receptors have broad specificity
- Recognize many related molecular structures
  - PAMPs (pathogen-associated molecular patterns)
    - LPS
    - Bacterial Products
  - DAMPs (danger associated molecular patterns)
    - ATP, Uric acid
    - DNA, RNA
    - HMGB1 (chromatin-associated protein)
    - S100 proteins
Pattern Recognition Receptors
Toll Like Receptor Family (TLR)

DAMPs (S100)

Cell expression:
Macrophage and DCs

Cellular localization:
Extracellular – TLR1, TLR2, TLR4, TLR5, TLR6, TLR11
Endosomal-TLR3, TLR7, TLR8, TLR9

*10 total TLRs in humans and 12 in mice that recognize pathogens or damaged cells to activate cytokine release or phagocytosis

*Dimerize during activation, sentinels for detection of microbes

Inflammation and Disease

IS THERE EVIDENCE FOR CAUSATION?
Inflammation Skews HSPC Toward Myelopoiesis and Senescence

Granulocyte

Macrophage

Megakaryocyte

Erythrocytes

G-CSF

M-CSF

TPO

EPO

Induction of Myelodysplasia in S100A9 Transgenic Mice


S100A9 homo or heterodimer

Stimulates immature myelopoiesis

Myeloid Derived Suppressor Cells (MDSCs)

MDSCs increased in MDS

Morphologic cytoplastic features related to MDS pathology with age in S100A9tg mice
NLRP3 Inflammasome and MDS

- MDS cell death pathway
  - Ineffective hematopoiesis, accelerated attrition
  - Inflammatory microenvironment
  - NLR – Pattern recognition receptor
    - NLRP3
    - Recruits adaptor protein ASC (apoptosis-associated speck-like protein containing a caspase-recruitment domain)
    - ASC polymerization
    - Activation of pro-caspase 1, inflammasome assembly
    - Pyroptosis

T cell Activation

Antigen Presenting Cell: Dendritic Cell (DC)

Tumor cells

MHC class I

MHC class II

CD8+

CD4+

T cells

Cytotoxicity

Helper functions

Inflammation

Regulation
Three Phases of Cancer Immune Surveillance

Elimination

Equilibrium

Escape

### Innate Immunity is an Emerging Driver of MDS Pathogenesis

**Autoimmune disease (n=39)**

<table>
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<th>Condition</th>
<th>n</th>
<th>%</th>
<th>N=1408; %</th>
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<td>48</td>
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<td>3</td>
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<tr>
<td>Rheumatoid arthritis</td>
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<td>Inflammatory bowel disease</td>
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<tr>
<td>Vasculitis</td>
<td>19</td>
<td>5</td>
<td>1</td>
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<tr>
<td>Sweet syndrome</td>
<td>13</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Hyperthyroidism (graves)</td>
<td>12</td>
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<td>7</td>
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<tr>
<td>Hyperthyroidism (graves)</td>
<td>7</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Fibromyalgia</td>
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<td>1.5</td>
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<tr>
<td>Systemic Lupus</td>
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<td>1</td>
<td></td>
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<tr>
<td>Pyoderma gangrenosum</td>
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<td>1</td>
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<td></td>
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<tr>
<td>Immune mediated neutropathy</td>
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<td>&lt;1</td>
<td></td>
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<tr>
<td>Miscellaneous</td>
<td>49</td>
<td>5</td>
<td></td>
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</table>

**Graphs**

- OS 60 with vs 45 months without autoimmune disease: p=0.006
- AML transformation 89 (23%) with vs 301 (30%) without autoimmune disease: p=0.011

Multicenter, case-control study of 40,011 patients with primary autoimmune disease

86 with proven therapy-related myeloid neoplasms

Treatment with azathioprine was the only agent associated with significant increased risk (7-fold) for myeloid neoplasms

Median time from exposure to myeloid disease 8 years (4-15 yrs)
Evolution of the Immune Response in MDS

**Lower Risk**
- ↑ TNFα-induced apoptosis
- ↑ ROS
- Stem Cell Depletion
- Abnormal ribosomes
- Altered MP localization
- Suppressed Hematopoiesis

**Progression**
- Induction of homeostatic mechanisms
- Telomere erosion and senescence
- Emergence of abnormal clones with point mutations in NRas and AML1
- Expansion
- Impaired Immunosurveillance by NK and T-cells

**Higher Risk**
- Tregs, ↓NK, MDSCs
- Abnormalities in DNA repair mechanisms with propagation of abnormal cells

**Molecular Model of MDS Progression**

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Inflammatory Bone Marrow Microenvironment Induced Model of MDS


HSC, iNOS, ROS, Cytokines

Age and Inflammation

Immature Myelopoiesis

HSC

Apoptosis and/or Inflammation-associated death

iNOS, ROS, Cytokines

Expanded malignant clone due to clonal selection and effector T cell/NK cell inactivation

Genomic Instability And Clonal Evolution

Ie, TET2, DNMT3A, ASXL1, JAK2, SF3B1, etc.

Expanded malignant clone due to clonal selection and effector T cell/NK cell inactivation
T cell InactivationInduces Immune Evasion by Malignant Cells

Exhausted T cells express inhibitory receptors (PD1/CTLA-4/Lag3)

Tregs****

PDL-1 on tumor

Effector T cells with activating ligands (CD28)

Suppressive molecules

Anti-PD1/anti-CTLA-4 antibodies
Increased Tregs Are Independent Prognostic Markers in Low-Risk MDS

Immune Status in MDS/AML

- Immune Suppression
- Immune Reactivity
Overview

- Theoretical understanding of immune regulation in MDS
- Immune potentiation as therapy for MDS
Revitalization of Immunotherapy

**Immunotherapy: Success at Last**

- Reactivate endogenous T cells
- Increase antigenic recognition (vaccines, TILs)
- Check point blockade (anti-CTLA-4, anti-PD1)
- Exogenous stimulation of T cells
- Improve T-cell function (CARs)
- Antibody-based therapies
Theoretical Immunotherapeutic Approaches

- Suppress innate immune responses
  - Cytokine suppression (single molecules or signaling mechanisms)
  - Differentiation of MDSCs or immature myeloid cells
- Potentiation of effector immune response
  - Remove Tregs or other suppressive populations
  - Break immune tolerance (check point blockade)
  - Enhance signaling mechanisms (Len)
- Multiple therapies????
Shorter Duration of Disease Predicts Response to Immune Suppressive Therapy

Immune Suppressive Therapy in MDS

2002 and 2008: NHLBI
Equine Anti-Thymocyte Globulin (Saunthararajah)

2007: UK
eATG versus rATG (Stadler)

2011: MultiCenter Phase III
eATG vs BSC (Passweg)

2014: MultiCenter Phase II
rATG (Komrokji)

**Improved Survival and Reduced Leukemia Progress in Retrospective Study**

- **N=128**
  - eATG+ Cy response rate 29% in unselected patients

**Fig. 1**
- Clinical outcomes of 89 NIH IST and 55 IMRAW patients.
  - **A.** Mortality rates and their 95% CIs from time in years of IST and time in years from diagnosis.
  - **B.** Leukemia rates and their 95% CIs from time of IST and from time of diagnosis.

**AML**, acute myeloid leukemia

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*International Myelodysplasia Risk Analysis Workshop (IMRAW)*

Hematologic Improvement to rATG

Hemaglobin in MER MCC-06-001


Not currently approved by the FDA
Treatment of MDS with Alemtuzumab

- Alemtuzumab treatment (10 mg/d IV for 10 days)
- Response rate: 17/22 (77%) Int-1; 4/7 (57%) Int-2

Revitalize Endogenous T cells

Pre-Treatment

Week 16 of Lenalidomide

**Immunomodulatory Drugs: Immune Potentiation**

IMiDs = Lenalidomide; Pomalidomide; Thalidomide; CC122 (Treatment of CLL investigational)

**IMiDs Alter Protein Stability and Activate T cells**

Aromatic cage of three Trp residues

Thalidomide

Lenalidomide

Pomalidomide

Erythema Nodosum Leprosum (ENL)

Del5q MDS

## Immune Checkpoint Approaches in AML and MDS: A Next Frontier?

### Ongoing Trials with PD-1 and CTLA-4 Blockade

<table>
<thead>
<tr>
<th>Type</th>
<th>Therapy</th>
<th>Endpoint</th>
<th>N=1408; %</th>
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<tbody>
<tr>
<td>Phase I</td>
<td>Ipilimumab</td>
<td>Toxicity</td>
<td>RR-AML/ high risk MDS</td>
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<tr>
<td>Phase I/Ib</td>
<td>Ipili or Nivolumab</td>
<td>Toxicity</td>
<td>RR-AML after aSCT</td>
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<tr>
<td>Phase II</td>
<td>Nivolumab</td>
<td>EFS</td>
<td>High risk AML in CR</td>
</tr>
<tr>
<td>Phase II</td>
<td>Nivolumab</td>
<td>EFS</td>
<td>AML in CR, MRD+</td>
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<td>Phase II</td>
<td>Pembrolizumab</td>
<td>Toxicity, efficacy</td>
<td>RR-MDS</td>
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<tr>
<td>Phase II</td>
<td>Nivo+Aza</td>
<td>Efficacy-ORR</td>
<td>RR-AML, frontline AML &gt;65 yrs</td>
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<tr>
<td>Phase II</td>
<td>Nivo+7+3</td>
<td>EFS</td>
<td>AML, &lt; 60 yrs</td>
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<td>Phase II</td>
<td>Nivo and Ipi + Aza</td>
<td>Efficacy-ORR</td>
<td>Frontline AML, RR MDS</td>
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<tr>
<td>Phase II</td>
<td>Pembro+Aza</td>
<td>Toxicity</td>
<td>Frontline AML, &gt;65 yrs, RR-AML</td>
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<tr>
<td>Phase II</td>
<td>Lirilumab and Nivo + Aza</td>
<td>Efficacy-ORR</td>
<td>RR-MDS</td>
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<tr>
<td>Phase II</td>
<td>Durvalumab +Aza</td>
<td>Efficacy-ORR</td>
<td>Frontline MDS, AML frontline, &gt;65 yrs</td>
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<tr>
<td>Phase I</td>
<td>Durvalumab + Aza+/tremelimumab</td>
<td>Toxicity</td>
<td>RR-MDS</td>
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<tr>
<td>Phase II</td>
<td>Pidilizumab + DC vaccine</td>
<td>Toxicity</td>
<td>AML in CR</td>
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### Checkpoint Blockade


Not currently approved by the FDA
Immunotherapy: Success at Last

- Reactivate endogenous T cells
- Current therapies
- Checkpoint blockade (anti-CTLA-4, anti-PD1)
- Exogenous stimulation of T cells
  - Improve T-cell function (CARs)
  - Antibody-based therapies
Tumor Infiltrating Lymphocytes (TIL)
## Patient Clinical Results – Complete Response

<table>
<thead>
<tr>
<th>Pre TIL</th>
<th>2 months post</th>
<th>24 months post</th>
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<td><img src="image2.png" alt="Image" /></td>
<td><img src="image3.png" alt="Image" /></td>
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<tr>
<td>1.6 x 1.5 cm</td>
<td>0.8 x 0.6 cm</td>
<td>0 cm</td>
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*Pilon-Thomas, J Immunother 2012*
Genetically Modified T cells for Therapy

1) T Cell Collection
2) T Cell Transfection
   1. Binding
   2. Fusion
   3. Integration
4) Patient Monitoring
   a) Disease response
      - CT scans
      - Bone marrow biopsies
      - Peripheral blood flow cytometry
   b) CAR-T Cell persistence
      - Immunohistochemistry of bone marrow biopsy
      - RT-PCR and flow cytometry of blood and bone marrow aspirate

3) T Cell Adoptive Transfer
   4. Transcription and protein expression
   +/- Lymphodepleting conditioning
   5. CAR cell membrane insertion
Chimeric Antigen Receptor (CAR)-T

Pioneered by Carl June

Ectodomain

Signal

Linker

Vh

VL

scFv

Spacer

Transmembrane

Endodomain

Intracytoplasmic

Zap70:CD3ζ

4-1BB

CD28

Zap70:CD3ζ

- Cytotoxicity
- Proliferation/cytokine production
- Survival

Nature Reviews | Clinical Oncology
CAR-T in CLL

Pioneered by Carl June

CTL019: antiCD19 CAR

4-1BB
CD28
Zap70:CD3ζ
**Antibody Based Therapy**

- Blinatumomab – FDA approved
  July 2017 –

CD19/CD3 bispecific T-cell engager for relapsed or refractory B-cell precursor ALL in adults and children
FDA Approved Immunotherapies

Leukemia:

• Tisagenlecleucel – (Aug) chimeric antigen receptor (CAR) T cell therapy for pediatric and young adults with B-cell precursor acute lymphocytic leukemia
  • Tocilizumab – antibody to treat severe or life-threatening cytokine release syndrome caused by CAR T-cells ≥ 2 years of age

• Gemtuzumab ozogamicin – (Sept) recombinant anti-CD33 drug conjugate for CD33+ AML

• Inotuzumab oxogamicin - (Aug) anti-CD22 monoclonal antibody for treatment of relapsed or refractory B-cell precursor ALL

• Blinatumomab – (July) CD19/CD3 bispecific T-cell engager for relapsed or refractory B-cell precursor ALL in adults and children

• Pembrolizumab (March) classifc Hodgkin lymphoma refractor to treatment or has relapsed after three or more prio lines of therapy

ASH Clinical News, October 2017
Evidence suggests that inflammation and MDS pathogenesis are related events.

- Inflammation suppresses adaptive immune responses.
- Immunotherapy may play an important role in future MDS/AML therapeutics.
The National Myelodysplastic Syndromes (MDS) Natural History Study

Activated April 2016

The National MDS Study website https://thenationalmdsstudy.net/
And The CTSU website http://www.ctsu.org/

The National MDS Natural History study has been supported by US Federal Government Contracts HHSN268201400003I and HHSN268201400002I from the National Heart, Lung, and Blood Institute with additional funding by the National Cancer Institute to its clinical centers.