The Fundamentals of Immunotherapy in AML and MDS

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Moffitt Cancer Center
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Immunology Program
## Conflict of Interest Declaration

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

INFLAMMATION
at the Root of Most Diseases

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

Induction of Myelodysplasia in S100A9 Transgenic Mice


Stimulates immature myelopoiesis

Myeloid Derived Suppressor Cells (MDSCs)

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

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)

T cells

Cytotoxicity

Helper functions

Inflammation

Regulation

Tumor cells

CD8+

MHC class I

CD4+

MHC class II
Three Phases of Cancer Immune Surveillance

Elimination

Equilibrium

Escape

Innate Immunity is an Emerging Driver of MDS Pathogenesis

<table>
<thead>
<tr>
<th>Autoimmune disease</th>
<th>n</th>
<th>%</th>
<th>N=1408; %</th>
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<tr>
<td>Hypothyroidism</td>
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<td>44</td>
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<td>ITP</td>
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<td>Rheumatoid arthritis</td>
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<td>Inflammatory bowel disease</td>
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<td>Vasculitis</td>
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<td>1</td>
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<tr>
<td>Sweet syndrome</td>
<td>13</td>
<td>3</td>
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<tr>
<td>Hyperthyroidism (graves)</td>
<td>12</td>
<td>3</td>
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<tr>
<td>Sweet syndrome</td>
<td>7</td>
<td>2</td>
<td></td>
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<tr>
<td>Hyperthyroidism (graves)</td>
<td>7</td>
<td>2</td>
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<tr>
<td>Fibromyalgia</td>
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<tr>
<td>Systemic Lupus</td>
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<td>Pyoderma gangrenosum</td>
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<td>Sjogren syndrome</td>
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<td>Autoimmune neutropenia</td>
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<td>&lt;1</td>
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<tr>
<td>Immune mediated neutropathy</td>
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<tr>
<td>Miscellaneous</td>
<td>49</td>
<td>5</td>
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</table>

Association of Therapy for Autoimmune Disease with MDS and AML

- 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 neoplasm
- Median time from exposure to myeloid disease 8 years (4-15 yrs)

<table>
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<tr>
<th>Characteristic</th>
<th>No. (%) of Participants</th>
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<tr>
<td></td>
<td>Cases (n = 86)</td>
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<tr>
<td></td>
<td>Controls (n = 172)</td>
</tr>
<tr>
<td>Age, mean (SD), y</td>
<td>72.3 (15.6)</td>
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<tr>
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<td>72.7 (13.8)</td>
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<tr>
<td>Sex, No. (%)</td>
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<tr>
<td>Male</td>
<td>49 (57.0)</td>
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<tr>
<td></td>
<td>98 (57.0)</td>
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<tr>
<td>Female</td>
<td>37 (43.0)</td>
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<td>74 (43.0)</td>
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<td>AID diagnosis (ICD-9 code)</td>
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<td>Rheumatoid arthritis (714)</td>
<td>23 (26.7)</td>
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<td>55 (32.0)</td>
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<tr>
<td>Psoriasis (696.1)</td>
<td>18 (20.9)</td>
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<td>37 (21.5)</td>
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<td>SLE (710)</td>
<td>12 (14.0)</td>
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<td>23 (13.4)</td>
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<td>Crohn disease (555.9)</td>
<td>8 (9.3)</td>
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<tr>
<td></td>
<td>15 (8.7)</td>
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<tr>
<td>Ulcerative colitis (556.9)</td>
<td>6 (7.0)</td>
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<td></td>
<td>13 (7.6)</td>
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<tr>
<td>Multiple sclerosis (340)</td>
<td>4 (4.7)</td>
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<tr>
<td></td>
<td>8 (4.7)</td>
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<tr>
<td>Granulomatosis with polyangiitis (446.4)</td>
<td>4 (4.7)</td>
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<tr>
<td></td>
<td>8 (4.7)</td>
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<tr>
<td>Autoimmune hepatitis (571.42)</td>
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<tr>
<td></td>
<td>2 (1.2)</td>
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<tr>
<td>Psoriatic arthropathy (696)</td>
<td>3 (3.5)</td>
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<tr>
<td></td>
<td>4 (2.3)</td>
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<tr>
<td>Systemic sclerosis (710.1)</td>
<td>2 (2.3)</td>
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<td>1 (0.6)</td>
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<tr>
<td>Dermatomyositis (710.3)</td>
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<td></td>
<td>2 (1.2)</td>
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<tr>
<td>Other</td>
<td>4 (4.7)</td>
</tr>
<tr>
<td></td>
<td>4 (2.3)</td>
</tr>
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</table>

Ertz-Archambault et al. JAMA Oncol.. 3:936-943, 2017
Evolution of the Immune Response in MDS

Lower Risk
- ↑ TNFα-induced apoptosis
- ↑ ROS
- Abnormal ribosomes
- Altered MP localization
- Stromal cell defects
- Suppressed hematopoiesis

Bone Marrow
- Stem cell depletion
- Induction of homeostatic mechanisms
- Telomere erosion and senescence
- Altered T-cell homeostasis
- Inflammatory microenvironment

Progression
- ↑ Bcl-2
- Emergence of abnormal clones with point mutations in NRas and AML1
- Tregs, ↓NK, MDSCs
- Expansion
- Impaired immunosurveillance by NK and T-cells

Higher Risk
- Abnormalities in DNA repair mechanisms with propagation of abnormal cells
- High risk for leukemia transformation

Molecular Model of MDS Progression

Inflammatory Bone Marrow Microenvironment Induced Model of MDS


HSC

iNOS, ROS, Cytokines

MDSC

Immature Myelopoiesis

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

Genomic Instability And Clonal Evolution

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

Ie, TET2, DNMT3A, ASXL1, JAK2, SF3B1, etc.
T cell Inactivation Induces 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)
  - Suppressing molecules
  - Anti-PD1/anti-CTLA-4 antibodies

Imunosuppressive Tumor Microenvironment

- Nutrients
- Suppressing molecules
- MDSC M2-Mac

*Note: The diagram illustrates the various components and interactions within an immunosuppressive tumor microenvironment.*
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
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)1

2007: UK
eATG versus rATG (Stadler)2

2011: MultiCenter Phase III
eATG vs BSC (Passweg)3

2014: MultiCenter Phase II
rATG (Komrokji)4

Improved Survival and Reduced Leukemia Progress in Retrospective Study

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

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

International Myelodysplasia Risk Analysis Workshop (IMRAW)

Hematologic Improvement to rATG

Hemoglobin 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


*New FDA studies in B-cell CLL
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

1950s

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>
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<tr>
<th>Type</th>
<th>Therapy</th>
<th>Endpoint</th>
<th>N=1408; %</th>
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<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>
</tr>
<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>
</tr>
<tr>
<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>
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<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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<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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<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
- Check point blockade (anti-CTLA-4, anti-PD1)
- Exogenous stimulation of T cells
  - Improve T-cell function (CARs)
  - Antibody-based therapies
Tumor Infiltrating Lymphocytes (TIL)
Tumor Infiltrating Lymphocytes (TIL)
Patient Clinical Results – Complete Response

Pre TIL  
1.6 x 1.5 cm

2 months post  
0.8 x 0.6 cm

24 months post  
0 cm

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. Transcription and protein expression
   - 5. CAR cell membrane insertion
   - +/- Lymphodepleting conditioning

3) T Cell Adoptive Transfer

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
**Chimeric Antigen Receptor (CAR)-T**

**Pioneered by Carl June**

**Ectodomain**
- Signal
- Linker
- Vh
- VL
- scFv

**Endodomain**
- Spacer
- Transmembrane
- Intracytoplasmic

**Zap70:CD3ζ**
- Cytotoxicity
- Proliferation/cytokine production
- Survival

**4-1BB**

**CD28**

**First generation**
- Signal 1
- Second generation
- Signal 2
- Third generation
- Signal 7

*Image credit: 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 ozogamicin - (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.