T-Cells in CLL: Runnin’ on Empty

Presenter: Aaron T. Gerds, MD, MS
Discussant: Edus H. Warren, MD, PhD
Chronic Lymphocytic Leukemia

“More often than not, the finding of such a chronic case leads to considerable alarm, not only in the mind of the patient, but with the family physician as well. Mention of the dread word “leukemia” is often sufficient to set off a violent chain reaction which, by the time it terminated may lead either to neurosis or bone marrow failure, depending upon how the situations is handled.”

- Panels in Therapy, VI. The management of chronic lymphocytic leukemia (moderated by William Dameshek, MD)
CLL: Background

Epidemiology

- 30% of all leukemias in the US
- 0.49% lifetime risk (1 in 202 people)
- In the US during 2013...
  - 15,680 (9,720 men and 5,960 women) will be diagnosed with CLL
  - 4,580 men and women will die of CLL
- Male to female ratio is 1.7:1
- The incidence of CLL varies by race and geographic location

Age-Specific (Crude) SEER Incidence Rates, All Races, Both Sexes, 2000-2010

- Median Age at diagnosis 71
- 90% will are over the age of 50

Incidence per 100,000 per year

Age at Diagnosis

Diagnosis (NCI-WG/IWCLL)

- Lymphocytosis ($\geq 5000$/microL)
  - Morphologically mature-appearing small lymphocytes
- Demonstration of clonality by flow cytometry
  - Extremely low levels of smIg
  - Either kappa or lambda (but not both) light chains
  - Expression of B-cell associated antigens (CD19, CD20, and CD23)
  - Expression of the T-cell associated antigen CD5

Peripheral blood flow cytometry

General concepts

One useful starting point in evaluating specimens for hematopoietic disorders is the CD45 versus side scatter plot:

- CD45 is expressed with increased intensity on hematopoietic cells as they mature.
- Side scatter is a reflection of cytoplasmic granularity.

Using a CD45 versus side scatter plot, one can generate a rough 5 part differential. The image above demonstrates CD45 versus side scatter for a normal bone marrow specimen. Peripheral blood specimens in contrast should consist predominantly of mature granulocytes and lymphocytes. See below.

SSC-H

WBCs

To evaluate for B cell lymphoma, define your B cells by CD19 expression and look for expression of clonality on B cells (the normal kappa to lambda ratio is 1-2:1).

UW Hematology Fellows’ Guide to Flow Cytometry
Peripheral blood flow cytometry

CLL

Normal
# Risk factors in CLL

<table>
<thead>
<tr>
<th>Feature</th>
<th>Favorable</th>
<th>Unfavorable</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clinical stage (Rai/Bient)</td>
<td>Low</td>
<td>High</td>
</tr>
<tr>
<td>Pattern of marrow infiltration</td>
<td>Interstitial or nodular</td>
<td>Diffuse</td>
</tr>
<tr>
<td>Lymphocyte DT</td>
<td>&lt; 12 months</td>
<td>&gt; 12 months</td>
</tr>
<tr>
<td>CD38 expression</td>
<td>Negative</td>
<td>Positive</td>
</tr>
<tr>
<td>Zap-70 expression</td>
<td>Negative (low levels)</td>
<td>Positive (high levels)</td>
</tr>
<tr>
<td>Cytogenetics</td>
<td>Ch 13q14</td>
<td>Del 11q23, 17p-, p53 abnl</td>
</tr>
<tr>
<td>$\beta$ 2 microglobulin</td>
<td>Normal</td>
<td>Elevated</td>
</tr>
</tbody>
</table>

Other unfavorable factors: increased levels of TNF-alpha, IL-6, IL-8, IL-10, LDH, VEGFR-2, CD20, and CD52
Survival in CLL

“… the course of the disease is a very lengthy one. In fact, the leukemia in this instance to coexist peacefully with its host.

“Because of better treatments… high-risk patients who 25 or 30 years ago had a median life expectancy of 2 years, now have a median life expectancy of 5 years. And the intermediate-risk patients, who had a median life expectancy of 7 or 8 years, now have a median life expectancy of 10 or 12 years.”

CLL: Complications

Complications of CLL

• Anemia and thrombocytopenia
  – Marrow infiltration
  – Hypersplenism
  – Autoimmune destruction (AIHA, ITP, PRCA)
  – Chemotherapy-related

• Infections

• Second malignancies
Infections In CLL

- 50% of deaths in patients with CLL
- Patients with CLL have an increased frequency and severity of bacterial infections
  - Encapsulated organisms (*Strep. pneumo, Staph. aureus, and H. influenzae*)
  - Herpes virus reactivation (*Herpes zoster > Herpes simplex*)
- Increases with disease stage and active treatment
  - Bone marrow infiltration
  - Therapy-induced immune dysfunction

Francis S, et al., Cancer. 2006 Sep 1;107(5):1023-33.
Infections In CLL

<table>
<thead>
<tr>
<th>Type of treatment</th>
<th>Spectrum of infection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Single-agent alkylator therapy (+/- corticosteroids)</td>
<td>Common bacterial pathogens (streptococcal/staphylococcal spp., enteric Gram-negative organisms)</td>
</tr>
<tr>
<td>Purine analogs</td>
<td>Candida, Aspergillus, herpesviruses, Pneumocystis</td>
</tr>
<tr>
<td>Monoclonal antibodies</td>
<td>No definitive change in spectrum of infection</td>
</tr>
<tr>
<td>Rituximab</td>
<td>Herpesviruses, including cytomegalovirus, Candida, Aspergillus, Pneumocystis</td>
</tr>
<tr>
<td>Alemtuzumab</td>
<td></td>
</tr>
</tbody>
</table>
Second malignancies in CLL

- 5-10% lifetime risk of transformation
  - Large-cell lymphoma (Richter's transformation)
  - Prolymphocytic leukemia
- Therapy-related MDS/AML
- Higher risk of developing secondary malignancies
  - Inferior survival as compared to those without CLL
Second malignancies in CLL

- 16,367 patients with CLL in SEER database
  - 84,667 person-years of follow-up
  - Mean 5.2 years (2,479 with $\geq$ 10 years)
- 11% developed second malignancy
  - OR = 1.20 (95% CI 1.15 - 1.26)
- The highest excess rates were noted for
  - Kaposi sarcoma (OR = 5.09)
  - Melanoma (OR = 3.18)
  - Laryngeal (OR = 1.72)
  - Lung (OR = 1.66)

Second malignancies in CLL

<table>
<thead>
<tr>
<th>Cancer Type</th>
<th>No. of Patients</th>
<th>No. of Events</th>
<th>HR</th>
<th>95% CI</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breast</td>
<td>568,598</td>
<td>144,141</td>
<td>1.30</td>
<td>1.13 to 1.49</td>
<td>&lt; .001</td>
</tr>
<tr>
<td>Colorectal</td>
<td>394,695</td>
<td>195,621</td>
<td>1.35</td>
<td>1.23 to 1.47</td>
<td>&lt; .001</td>
</tr>
<tr>
<td>Kidney</td>
<td>91,991</td>
<td>36,200</td>
<td>1.15</td>
<td>0.93 to 1.42</td>
<td>.19</td>
</tr>
<tr>
<td>Lung</td>
<td>459,256</td>
<td>375,614</td>
<td>1.01</td>
<td>0.95 to 1.08</td>
<td>.75</td>
</tr>
<tr>
<td>Ovarian</td>
<td>58,986</td>
<td>33,020</td>
<td>0.86</td>
<td>0.63 to 1.18</td>
<td>.36</td>
</tr>
<tr>
<td>Pancreatic</td>
<td>73,117</td>
<td>65,744</td>
<td>0.92</td>
<td>0.76 to 1.12</td>
<td>.40</td>
</tr>
<tr>
<td>Prostate</td>
<td>501,247</td>
<td>99,680</td>
<td>1.21</td>
<td>1.06 to 1.38</td>
<td>.005</td>
</tr>
</tbody>
</table>


All calculated using data from the SEER program. Abbreviations: CLL, chronic lymphocytic leukemia; HR, hazard ratio; OS, overall survival.
# Second malignancies in CLL

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<td>144,988</td>
<td>1.70</td>
<td>1.51 to 1.91</td>
<td>&lt; .001</td>
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<tr>
<td>Colorectal</td>
<td>394,695</td>
<td>196,532</td>
<td>1.65</td>
<td>1.53 to 1.79</td>
<td>&lt; .001</td>
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<tr>
<td>Kidney</td>
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<td>36,415</td>
<td>1.54</td>
<td>1.29 to 1.84</td>
<td>&lt; .001</td>
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<tr>
<td>Lung</td>
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<td>376,476</td>
<td>1.19</td>
<td>1.12 to 1.27</td>
<td>&lt; .001</td>
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<tr>
<td>Ovarian</td>
<td>58,986</td>
<td>33,078</td>
<td>1.04</td>
<td>0.78 to 1.38</td>
<td>.81</td>
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<tr>
<td>Pancreatic</td>
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<td>65,790</td>
<td>0.97</td>
<td>0.81 to 1.18</td>
<td>.78</td>
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<tr>
<td>Prostate</td>
<td>501,247</td>
<td>100,801</td>
<td>1.92</td>
<td>1.73 to 2.13</td>
<td>&lt; .001</td>
</tr>
</tbody>
</table>

Abbreviations: CLL, chronic lymphocytic leukemia; HR, hazard ratio; OS, overall survival.
Second malignancies in CLL

<table>
<thead>
<tr>
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<th>No. of Events</th>
<th>HR</th>
<th>95% CI</th>
<th>P</th>
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<tbody>
<tr>
<td>Breast</td>
<td>567,751</td>
<td>68,646</td>
<td>1.41</td>
<td>1.11 to 1.80</td>
<td>.005</td>
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<tr>
<td>Colorectal</td>
<td>393,784</td>
<td>109,798</td>
<td>1.64</td>
<td>1.45 to 1.85</td>
<td>&lt;.001</td>
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<tr>
<td>Kidney</td>
<td>91,776</td>
<td>20,797</td>
<td>1.41</td>
<td>1.05 to 1.90</td>
<td>.02</td>
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<tr>
<td>Lung</td>
<td>458,394</td>
<td>308,105</td>
<td>1.13</td>
<td>1.05 to 1.22</td>
<td>.002</td>
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<tr>
<td>Ovarian</td>
<td>58,928</td>
<td>26,475</td>
<td>0.88</td>
<td>0.62 to 1.27</td>
<td>.50</td>
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<tr>
<td>Pancreatic</td>
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<td>57,393</td>
<td>0.91</td>
<td>0.74 to 1.13</td>
<td>.40</td>
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<tr>
<td>Prostate</td>
<td>500,126</td>
<td>26,447</td>
<td>1.18</td>
<td>0.87 to 1.60</td>
<td>.28</td>
</tr>
</tbody>
</table>

Complications of CLL

• Why do patients with CLL have an increased risk of second malignancies and fair poor than those without CLL?
  – Genetics
    • Underlying genetic risk
    • Second cancer fundamentally different disease
  – Less able to tolerate preferred treatment strategies
    • Cytopenas resulting in more transfusions and dose adjustments
    • Immune dysfunction resulting in more infections complicating treatment
Primary

- **B-cell defects**
  - Hypogammaglobulinemia
  - Poor response to vaccination

- **T-cell defects**
  - Qualitative
    - Skewed repertoire, decreased CD4/8 ratio
    - Th 2 polarization
    - Abnormal CD30 response
    - Reversible acquired CD40L deficiency
    - Gene expression abnormalities (cytoskeleton, granules)

- **NK cell**
  - Lack of granules
  - Reduced killing activity

- **Neutrophils**
  - Reduced phagocytic and bactericidal function
  - Abnormal migration and chemotaxis

- **Monocytes/macrophages**
  - Reduced cytotoxicity

- **Complement**
  - Reduction in levels and defects in activation and binding
CLL: Exhausted T-cells


CD244; CD160, PD1:
CTLA4, TIM3,LAG3:*
CLL CD8+ T proliferation:
CLL CD8+ cytotoxicity:
Interferon-γ, TNFα:*
IL2:*
T-cells in an acute infection and after resolution

T-cell exhaustion in chronic infections

Naive CD8⁺ T cell → Antigen + costimulation + high inflammation → Effector CD8⁺ T cell → Chronic infection

Antigen persists

Inflammation

IFN-γ | TNF | CTL | IL-2 | Proliferative potential | Apoptosis
---|---|---|---|---|---
+++ | ++ | ++/- | +/- | ++ | -
++ | + | + | - | + | -
+/− | +/− | +/− | - | +/- | +/−
+/− | - | - | - | - | ++

Viral load and/or duration
CD4⁺ T cell help

PD-1
LAG-3
CD244 (2B4)
CD160 (and so on)

IL-10
TGF-β

T cells from CLL patients exhibit features of T-cell exhaustion but retain capacity for cytokine production

John C. Riches,1 Jeffrey K. Davies,1 Fabienne McClanahan,1 Rewas Fatah,1 Sameena Iqbal,1 Samir Agrawal,2 Alan G. Ramsay,1 and John G. Gribben1

1Department of Haemato-Oncology, Barts Cancer Institute, a CR-UK Centre of Excellence, Queen Mary University of London, London, United Kingdom; and 2Academic Haematology Unit (Haematological Oncology), Blizard ICMS, Barts and the London School of Medicine and Dentistry, London, United Kingdom
T cells from CLL patients exhibit features of T-cell exhaustion but retain capacity for cytokine production

John C. Riches, Jeffrey K. Davies, Fabienne McClanahan, Rewas Fatah, Sameena Iqbal, Samir Agrawal, Alan G. Ramsay, and John G. Gribben

• Hypothesis:
  – Chronic stimulation may result in T-cells from patients with CLL becoming functionally “exhausted,” similar to that reported in chronic viral infections

• Questions:
  – Do there appear to be increased numbers of T-cells with an exhausted phenotype?
  – Are the exhausted T-cells functional - do they divide, kill, and produce cytokines?
  – Are the observed defects in T-cell function due to CLL or confounded by CMV?
Study population

• Peripheral blood from 39 untreated CLL patients
  – Median age of 59 years (range 43-86)
  – 31/39 (79.5%) Binet stage A disease
  – 22/39 (56%) CMV seropositive

• Peripheral blood from a group of 20 healthy volunteers (age-matched)
  – Median age of 61 years (range 49-72)
  – 13/20 (65%) CMV seropositive
Expression of PD1 identified a subset of T cells with high expression of BLIMP1 (PD1\(^{+}\)/BLIMP1\(^{HI}\)), that were expanded in both the CD3\(^{+}\)/CD8\(^{+}\)(\(p = 0.0001\)) and CD3\(^{+}\)/CD4\(^{+}\)(\(p = 0.0001\)) compartments in CLL (Figure 1D-E, supplemental Figure 1). Of note, we did not observe increased expression of CTLA4, TIM3, or LAG3 on T cells from CLL patients. A further feature of exhausted T cells in many chronic viral infections is down-regulation of CD127 (IL-7R), as the T cells lose responsiveness to homeostatic cytokines and become dependent on antigen for continued survival. However, we saw no statistically significant decrease in expression of CD127 on CLL CD8\(^{+}\) T cells compared with healthy controls (\(p = 0.079\); Figure 1F).

Previous studies have shown skewing of the T-cell compartment toward activated T-cell subsets in CLL patients and in the E\(^\text{T}-\text{TCL1}\) mouse model. We therefore reasoned that the increased expression of CD244, CD160, and PD1 on CLL CD8\(^{+}\) T cells would be because of an increased proportion of exhausted effector T cells. Consistent with these previous reports, we found both an increased proportion and increased absolute numbers of CD3\(^{+}\)/CD8\(^{+}\)/CCR7\(^{+}\) T cells in CLL patients compared with healthy controls (Figure 2A-B). We therefore investigated expression of CD160, CD244, and PD1 on T-cell subsets from CLL patients as CD8\(^{+}\)PD1\(^{+}\)/BLIMP1\(^{HI}\) p < 0.0001.

<table>
<thead>
<tr>
<th></th>
<th>Naïve T-cell</th>
<th>Central Memory ($T_{CM}$)</th>
<th>Effector Memory ($T_{EM}$)</th>
<th>CD45RA+ Effector Memory ($T_{EMRA}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD3</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>CD8</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>CCR7</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>CD45RA</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>

CD3+ CD8+ CCR7- Effector T cells

Proportion

\[ p = 0.0043 \]

Absolute

\[ p < 0.0001 \]

From we assessed the ability of CD8$^+$ and increased proportion (A) and increased numbers (B) of are increased in CLL.

Controls to proliferate in response to stimulation by anti-CD3 and including loss of proliferative and cytotoxic capacity. T-cell exhaustion also results in progressive loss of T-cell function, proliferation and cytotoxicity on antigen-experienced T cells. Shifted toward an increased proportion of phenotypically exhausted subsets from healthy individuals (supplemental Figure 2). These cells. Similar patterns of expression were seen on lymphocyte defined by their expression of CCR7 and CD45RA, specific for a CCR7$^+$CM$^+$PD1 on CCR7$^+$CM$^+$T cells from patients with CLL show functional defects in and defined related to CCR7 expression (Figure 2E). In contrast, expression of (Figure 2C-E). In particular, expression of CD244 was inversely (Figure 2A).

In contrast, expression of CD160 was higher on T cells from patients with CLL as defined by MFI of CD160, CD244, and PD1 was analyzed on CD8$^+$ cells (Figure 2F) and decreased on T cells from both CLL patients and healthy donors to lyse idiotype-pulsed target cells.

Therefore, the ability of CD8$^+$ T cells capable of killing idiotype-pulsed target cells compared with healthy donors. This defect in target cell killing. Feature of functional T-cell exhaustion is impairment of ex vivo prolongation of the division time of the proliferating cells. Another feature of functional T-cell exhaustion is impairment of ex vivo prolongation of the division time of the proliferating cells.

Summary-1

• Increased expression of the exhaustion markers CD244, CD160, and PD1
  – CTLA4, TIM3, and LAG3 were not increased

• Analysis of the T-cell subsets suggested that the circulating T-cells in CLL have an increased proportion of antigen-experienced T-cells with an exhausted phenotype
CFSE-based assay after stimulation by anti-CD3 and anti-CD28 antibodies for 72 hours.
From expression on CD3 IFN^\gamma^ in infections, CD3^+^ production after stimulation with PMA and ionomycin, and demonstration in vitro.

expression is usually rapidly down-regulated after PMA stimulation and finally IFN^\gamma^ being lost at early stages of exhaustion, followed by loss of TNF^\alpha^.

A further feature of functional T-cell exhaustion during chronic viral infections is failure to produce effector cytokines. This

production of IL2^+^ IFN^\gamma^ CD8^+^ T cells from CLL patients show increased production of IFN^\gamma^ compared with healthy controls. Once again this reflected the

4C) compared with healthy controls. Once again this reflected the

4A-B), without any reduction in IL2 production (p < 0.05)

G H

C D

E F

A B

and TNF^\alpha^.

H9253/H9253/H9253/H9253

P < 0.0005, and increased expression of TBET, but normal

H11545/H11545/H11545/H11545

expression in CD3^+^ T cells from CLL patients had increased

H9251/H9251/H9251/H9251

P < 0.0001) and TNF^\alpha^.

H11001/H11001/H11001/H11001

We therefore analyzed cytokine

H11002/H11002/H11002/H11002

expression in CD3^+^ cells (Figure 4A-C). CD160^+^ cells, which produced more

H9251/H9251/H9251/H9251

p < 0.024

Figure 3. CD8^+^ T cells from patients with CLL show increased production of

H9253/H9253/H9253/H9253

p < 0.001

Figure 4F). Taken together, these findings are consis-

H11005/H11005/H11005/H11005

and expression of CD244, CD160, and PD1

H11001/H11001/H11001/H11001

The impact of CMV serostatus on the distribution of subsets

H11021/H11021/H11021/H11021

differentiate into effector cells with a type-1 cytokine profile.

H9251/H9251/H9251/H9251

of the skew toward effector cell subsets, we examined TBET

H11001/H11001/H11001/H11001

expression was higher in T

H9253/H9253/H9253/H9253

P < 0.0001, in naive or T

H11001/H11001/H11001/H11001

EMRA.

p < 0.001

CM

EMRA.

p < 0.0001

EM

EM

EM

We therefore analyzed cytokine

H11002/H11002/H11002/H11002

activity of CD8^+^ T cells from patients and controls was

H11001/H11001/H11001/H11001

assessed. CD8^+^ T cells from CLL patients fail to lyse

H9251/H9251/H9251/H9251

p = 0.024

idiotype-pulsed target cells in contrast to CD8^+^ T cells reported in

H18528/H18528/H18528/H18528

VOLUME 121, NUMBER 9

BLOOD, 28 FEBRUARY 2013

For personal use only.
CFSE-based assay after stimulation by anti-CD3 and anti-CD28 antibodies for 72 hours
Idiotype-pulsed target cell lysis assay

% Specific lysis

Effector:Target ratio

Healthy (pulsed)

CLL (pulsed)

Healthy (control)

CLL (control)
Functional exhaustion during chronic infections

**Partial exhaustion I**
- No IL-2 production

**Partial exhaustion II**
- No TNF-α production
- Compromised INF-γ production

**Full exhaustion**
- No INF-γ production

From cells were increased in CMV CLL patients and healthy donors. However we also noted that these CD8 CLL patients only in CMV as shown in Figure 5C, PD1 expression was increased in controls irrespective of CMV-serostatus (Figure 5A-B). In contrast, CD160 was increased in CLL patients compared with healthy extensively described in chronic viral infections, it was important pared with controls.

CLL show significantly increased TBET expression compared with controls. (F) CD3 cells, with the highest expression levels in the T subset. (E) The expression of TBET was increased in Tc1 cells. (D) CD160 subsets (T

Because T-cell exhaustion has been investigated of the distribution of lymphocyte subsets provided an

T cells from patients with

EMRA

VOLUME 121, NUMBER 9

Summary-2

- Increased exhaustion markers
- Skewed towards antigen-experienced T-cells
- Decreased proliferative capacity
- Decreased cytotoxicity
- Increase in production of TNF-α and INF-γ, with a lack of reduction in IL-2
  - In contrast to viral infection-associated T-cell exhaustion
CMV and CLL

• Chronic viral infections can lead to T-cell exhaustion

• CMV seropositivity can lead to expanded populations of CMV-specific CD4\(^+\) and CD8\(^+\) T-cells in patients with CLL

We previously demonstrated that CLL T cells exhibit impaired T-cell function because of failure of granzyme localization to the immunologic synapse. This results in disordered, nonpolarized degranulation, and is consistent with failure of effective granzyme B packaging into the cytotoxic vesicles.

In this study we investigated the nature of the T-cell defect in CLL. We demonstrate that T cells from CLL patients exhibit features of T-cell exhaustion, an acquired state of T-cell dysfunction first described in the context of chronic viral infections. T-cell exhaustion is characterized by increased expression of the exhaustion markers, CD244, CD160, and PD1, with expansion of a PD1/CD160/CD244+ T-cell subset with high expression of Blimp1, a transcription factor implicated in the development of exhaustion.

We therefore examined the impact of CMV serostatus on proliferation, cytolytic activity, and actin polymerization and defective immunologic synapse formation on the observed cytolytic defects. The ability of the CD8 T-cell function are because of CMV.

The proportion of CD3+CD8+ T cells that were positive for IFN (p =0.0041) and CMV seropositivity was failure of transport of these molecules within CLL T cells, and granzyme B to the immunologic synapse (polarization), there was failure of local staining of F-actin, CD107a, and CD107a (p =0.0051) (Figure 6C-D). These findings exclude CMV seropositivity as the sole cause of functional defects in CLL T cells. The ability of the CD8 subset to degranulate, and actually showed enhanced cytokine production of CD8

CD244

CD160

PD1

From CD3 are increased in CLL. Controls to proliferate in response to stimulation by anti-CD3 and including loss of proliferative and cytotoxic capacity. Proliferation and cytotoxicity results indicate that the circulating T-cell compartment in CLL is cells. Similar patterns of expression were seen on lymphocyte subsets from CLL patients as defined by CD45RA PD1 on CCR7 lymphocyte subsets from CLL patients as defined by CD160 (D) was increased on T cells as naive (CCR7 (Figure 2C-E). In particular, expression of CD244 was inversely CM/H11001 (E) Expression of CD244 was virtually CD45RA CD45RA/ H11002 CD45RA/ H11002/ H11001 CD45RA/ H11001 (Figure 3A-B). These findings skewed the T-cell repertoire toward a T-em phenotype, specifically EMRA. Expression of CD160 and CD244 MFI: Subsets Naive T CM T EM T EMRA. Therefore, the ability of CD8 T cells from CLL patients was virtually p < 0.001. The proportion of cells able to divide on polyclonal activation, and proliferation index of CD3 cells (Figure 3E-F). This suggests that the proliferative defect seen in CLL patients was because of failure of granzyme localization to the immunologic synapse, which is consistent with failure of effective granzyme B packaging into the immunologic synapse. The relative expression of CD244, CD160, and PD1 on T cells is because of a combination of a reduction in target cell killing. Feature of functional T-cell exhaustion is impairment of ex vivo response to SEB was assessed by analysis of CD107a (LAMP1) chromium release assay. In keeping with our previous demonstration that CLL T cells exhibit impaired cytokine production of CD8 T cells capable of killing idiotype-pulsed target cells at all effector-target cell ratios. This is consistent with failure of effective granzyme B packaging into the immunologic synapse. The relative expression of CD244, CD160, and PD1 on T cells from CLL patients and healthy donors to lyse idiotype-pulsed target cells was significantly lower than healthy controls (Figure 3A-B). There was also a reduction in the proportion of CD8 cells (Figure 3E-F). This suggests that the proliferative defect seen in CLL patients was because of failure of granzyme localization to the immunologic synapse, which is consistent with failure of effective granzyme B packaging into the immunologic synapse. There was also a reduction in the proportion of CD8 cells (Figure 3E-F). This suggests that the proliferative defect seen in CLL patients was because of failure of granzyme localization to the immunologic synapse, which is consistent with failure of effective granzyme B packaging into the immunologic synapse.
Proliferation index

IFNγ

p = 0.0012  p = 0.0036

p = 0.005  p = 0.0008

subsets toward effector differentiation. CD8 T cells from CLL patients also show functional evidence of exhaustion, with impaired proliferative capacity and reduced ability to lyse target cells. The principal way in which CLL T cells differ from the exhausted T-cells described in chronic viral infections is that they retain the capacity to produce cytokines, such as IFN and TNF.

Figure 6. The defects in T-cell function seen in CLL are present irrespective of CMV serostatus. The function of CLL CD8 T cells was compared with CD8 T cells from healthy controls matched for CMV serostatus. Increased production of IFN (A), increased expression of TBET (B), decreased proliferative capacity (C), and decreased cytolytic ability (D) of CLL T cells were all found irrespective of CMV serostatus. Pulsed target cells (closed symbols); unpulsed control target cells (open symbols). The graph representing percentage-specific lysis shows the mean and standard error of results obtained from 13 CLL patients, (5 CMV and 8 CMV), and 12 healthy controls (5 CMV and 7 CMV).

Figure 7. CD8 T cells from CLL patients show defective cytotoxicity because of failure of granzyme localization to the immunologic synapse. The cytotoxic activity of CD8 T cells from CLL patients was assessed further. (A) CD8 T cells from CLL patients retain the ability to degranulate in response to SEB, as shown by their increased ability to transfer CD107a to the cell surface. (B) Healthy CD8 T cells showed effective F-actin (red, rhodamine phalloidin) immunologic synapse formation with colocalization of CD107a (yellow, Alexa Fluor 647) and granzyme B (green, Alexa Fluor 488) at the synapse contact site with healthy B cells (sAg). (C) In contrast, CD8 T cells from CLL patients fail to form effective F-actin immune synapses with autologous CLL cells (sAg) that is associated with strong, nonpolarized expression of CD107a and a lack of granzyme B polarization to the contact site.
Summary-3

• Increased exhaustion markers
• Skewed towards antigen-experienced T-cells
• Decreased proliferative capacity
• Decreased cytotoxicity
• ↑ production of INF-γ / INF-α, no ↓ IL-2
• Skewed T-cell phenotype and dysfunction were independent of CMV infection
From impaired proliferative capacity and reduced ability to lyse target CLL patients also show functional evidence of exhaustion, with CD8 subsets toward effector differentiation.

Figure 6. The defects in T-cell function seen in CLL are present irrespective of CMV serostatus.

Increased production of IFN-γ/hTGF-β obtained from 13 CLL patients, (5 CMV +) and 7 CMV - (A), increased expression of CD107a and a lack of granzyme B polarisation to the contact site. (B) Healthy CD8 T cells showed effective F-actin immune synapses with autologous B cells, whereas in CLL T cells from CLL patients fail to form effective F-actin immune synapses with autologous B cells (C) In contrast, CD8 T cells from CLL patients showed effective contact site localization to the immunologic synapse. (D) Healthy T cells retain the ability to degranulate in response to SEB, as shown by their increased ability to transfer CD107a to the cell surface. (B) Healthy CD8 T cells retain the capacity to produce cytokines, such as IFN-γ, whereas exhausted T-cells described in chronic viral infections are able to produce sAg that is associated with strong, nonpolarised expression of CD107a and a lack of granzyme B with colocalization of CD107a (yellow, Alexa Fluor 488) and granzyme B (green, Alexa Fluor 488) at the synapse contact site with healthy B cells (Fluor 647) and granzyme B (green, Alexa Fluor 488) at the synapse contact site with healthy B cells.

The bottom line

• Skewing of T-cell subsets toward effector differentiation

• T-cells from CLL patients show functional evidence of exhaustion similar to chronic viral infections, except they retain the capacity to produce cytokines

• Why?
Why?

- Chronic stimulation of a high-affinity antigen (not a result of CMV)
- Exhaustion pathways co-opted by CLL to inhibit immune response, but maintain production of cytokines (INF-γ and TNF-α), which have been shown to be pretumoral
- Perhaps due to chronic stimulation by autonomously active CLL cells

Why?

- Novel signal-initiating interaction between the third complementary determining region of the Ig heavy chain variable domain (HCDR3) and an epitope in the second framework region (FR2)

Back to the patient

• “The up-regulation of CD244, CD160, and PD1 is of translational relevance, as these molecules may be useful biomarkers of immune reconstitution, and be potential targets for therapeutic strategies aimed at improving T-cell immunity.”

Safety and Activity of Anti–PD-L1 Antibody in Patients with Advanced Cancer

Julie R. Brahmer, M.D., Scott S. Tykodi, M.D., Ph.D., Laura Q.M. Chow, M.D., Wen-Jen Hwu, M.D., Ph.D., Suzanne L. Topalian, M.D., Patrick Hwu, M.D., Charles G. Drake, M.D., Ph.D., Luis H. Camacho, M.D., M.P.H., John Kauh, M.D., Kunle Odunsi, M.D., Ph.D., Henry C. Pitot, M.D., Omid Hamid, M.D., Shailender Bhatia, M.D., Renato Martins, M.D., M.P.H., Keith Eaton, M.D., Ph.D., Shuming Chen, Ph.D., Theresa M. Salay, M.S., Suresh Alaparthi, Ph.D., Joseph F. Grosso, Ph.D., Alan J. Korman, Ph.D., Susan M. Parker, Ph.D., Shruti Agrawal, Ph.D., Stacie M. Goldberg, M.D., Drew M. Pardoll, M.D., Ph.D., Ashok Gupta, M.D., Ph.D., and Jon M. Wigginton, M.D.

Discussion