EJMO

DOI: 10.14744/ejmo.2023.35138
EJMO 2023;7(4):362–370

Research Article

Disturbed Expression of Memory T-Cell Subsets Could Alter the Outcomes in Adult Acute Myeloid Leukemia

Asmaa M. Zahran,[1] Zeinab Albadry M. Zahran,[2] Doaa A. Gamal,[3] Ibrahim Khalid Ibrahim Elsayh,[4] Sawsan M. Moeen,[5] Mohammed M. Wahman,[6] Marwa A. Sabet,[7] Azza Mostafa Abdelaal,[8] Safinaz Hussein,[5] Khaled Saad,[8] Amal Rayan[3]

[1]Department of Clinical Pathology, South Egypt Cancer Institute, Assiut University, Assiut, Egypt
[2]Department of Clinical Pathology, Faculty of Medicine, Assiut University, Assiut, Egypt
[3]Department of Clinical Oncology, Faculty of Medicine, Assiut University, Assiut, Egypt
[4]Faculty of Medicine, Assiut University, Assiut, Egypt
[5]Department of Internal Medicine, Clinical Hematology Unit, Faculty of Medicine, Assiut University, Assiut, Egypt
[6]Department of Clinical Oncology, Faculty of Medicine, South Valley University, Qena, Egypt
[7]Department of Microbiology and Immunology, Faculty of Pharmacy, Sphinx University, New-Assiut, Egypt
[8]Department of Pediatrics, Faculty of Medicine, Assiut University, Assiut, Egypt

Abstract

Objectives: We compared the percentages of memory T cells in patients with acute myeloid leukemia (AML) to healthy controls and tried to detect any association of these cells to treatment outcome.

Methods: The study involved 34 adult patients with AML and 24 healthy controls. Following the diagnosis of AML, blood samples were collected from patients and controls for flow cytometric detection of CD8+T, CD4+T, T_N, T_{EM}, T_{CM}, T_{EMRA}, and T_{SCM} subsets of both CD4+ and CD8+T-cells.

Results: No significant differences in the mean percentages of CD4+T-cell types between AML patients and controls, with the exception of the total percentage of CD4+T-cells which accumulated in controls, furthermore, significant accumulations of CD8+T_{EMRA}, CD8+CD45+RO, and CD8+T_{EM} were detected in patients compared with controls, while CD8+, CD8+T_{Ns}, CD8+T_{SCM}, and CD8+T_{CM} accumulated in controls compared to patients, moreover, significant elevations of total CD8+T-cells, CD8+T_{EMRA}, CD8+T_{SCM}, CD8+T_{CM}, and CD8+T_{EM} in patients with remission compared to those without remission, on the contrary, CD4+T memory cells did not show any significant differences.

Conclusion: Our results showed that accumulation of CD8+T memory cells in AML patients, especially those who achieved remission, could enhance the immune response, particularly in those at high risk of relapse after bone marrow transplantation.

Keywords: Acute myeloid leukemia, CD4+T memory cells, CD8+T memory cells, flow cytometry, remission.

Cite This Article: *Zahran AM, Zahran ZAM, Gamal DA, Elsayh IKI, Moeen SM, Wahman MM, et al. Disturbed Expression of Memory T-Cell Subsets Could Alter the Outcomes in Adult Acute Myeloid Leukemia. EJMO 2023;7(4):362–370.*

Immunological memory refers to an induced immune response upon re-exposure to an antigen relative to the first exposure. Notably, classic immune memory cells could be induced by different infections. The ability to determine functionally distinct subsets of memory cells has considerable importance as a way to characterize an immune response better.

Memory CD8 T cells are defined as CD8 T cells that respond to primary infection and are maintained for a long

Address for correspondence: Khaled Saad, MD. Department of Pediatrics, Faculty of Medicine, Assiut University, 71516 Assiut, Egypt
Phone: +201097075321 **E-mail:** ksaad8@yahoo.com

Submitted Date: October 14, 2023 **Accepted Date:** November 13, 2023 **Available Online Date:** December 29, 2023

term. Hence, patients who developed memory CD8 T cells achieved a good response against solid tumors and infection. Generally speaking, humans are exposed to variable infections throughout their lives and reencounter the same infections at a later time. Upon antigen exposure, TCM and TEM subsets of memory CD8+T cells can be identified in hosts, along with a terminally differentiated subset that also expresses CD45RA (T_{EMRA}). Initial characterization of these subsets depends on the expression of CD45RA and CD27. [3] However, subsequent reports discriminated TEM and TCM subsets based on the expression of CCR7,[4] and memory cells stained positively by CD45RA, CD27 and CCR7 are identified as naïve cells (TN) i.e. (CD45RA+/CD27+/CCR7+), TEM can be identified as (CD45RA−/CD27−/CCR7−), while TCM as (CD45RA−/CD27+/CCR7+), and finally T_{EMRA} as (CD45RA+/CD27−/CCR7−) CD8 T cells. All these cells accumulate with increasing age. Furthermore, chronic infection such as CMV in humans results in the accumulation of T_{EMRA}.[5]

While memory CD8+ T cells are important for protective immunity against many types of infectious pathogens, CD4+ memory T cells are essential for the production of high-affinity memory B cells and long-lived plasma cells.[6] Following immunization, CD4+ T-cells expand progressively to produce the different specialized effector cells required to defend against the specific antigenic challenge that is followed by a decline in their levels because most of these cells die off. Whereas the remaining memory T cells undergo slow homeostatic proliferation in order to maintain their numbers to respond to previously recognized antigens, likewise to memory CD8+T cells, CD4+ T cells can be divided into different functional subsets such as effector memory (T_{EM}), central memory (T_{CM}), and follicular helper (T_{FH}), each of which has specialized functional capacities and sites of action and may also exhibit different homeostatic and localization characteristics.[7, 8] Recently, a new memory CD4+T cell subset, stem cell memory T (T_{SCM}), which has stem cell-like characteristics, has been identified.[9]

Yao et al. analyzed The percentages of T_{CM}, T_{SCM}, T_{EM}, and T_{EF} cells in CD4+ and CD8+ populations in 20 patients with CML. [10] They found that CD8+ T_{SCM} and CD8+ T_{CM} cells were significantly decreased in the peripheral blood of these patients; however, there was no significant change in the CD4+T cell population. The shift from T_{SCM} and T_{CM} cells to highly differentiated T_{EM} and T_{EF} cells was thought to be due to persistent exposure of T cells to leukemic cells and their microenvironment, leading to T cell exhaustion and/or dysfunction.[11]

To study the influence of memory T cell subsets and their functions in acute myeloid leukemia patients, we collected samples from PB in AML patients at the time of diagnosis. We compared the distributions of memory T cell subsets to healthy controls. Next, we tried to find the impact of their high levels on the response to treatment.

Methods

This prospective cohort study was carried out in Assiut University Hospital (AUH) and South Egypt Cancer Institute (SECI). We recruited a cohort of 34 patients diagnosed with acute myeloid leukemia >18 years old and 24 healthy controls. Patients with microbial-induced inflammation, as detected by cultures and those with autoimmune diseases were excluded.

The diagnosis of acute leukemia was based on peripheral hemogram assessment, morphologic bone marrow (BM) examination, cytochemical studies, and flow cytometry to identify the cell lineages and subsets. At diagnosis, preliminary total and differential CBC, serum albumin (ALB), were used to calculate different inflammatory indicators, including monocyte to lymphocyte ratio, calculated by dividing absolute monocytic count by absolute lymphocytic count; neutrophil to lymphocyte ratio, calculated by dividing absolute neutrophilic count by absolute lymphocytic count; platelet to lymphocyte ratio, calculated by dividing absolute platelet count by absolute lymphocytic count; and the prognostic nutritional index (PNI) was calculated by adding serum ALB (g/L) plus total lymphocytic count per µL after being multiplied by 0.005 (ALB + TLC × 0.005); PNI value ≥ 50 is considered normal; PNI value < 50– ≥ 45 indicates mild malnutrition; PNI value < 45–≥ 40 indicates moderate malnutrition, and PNI value < 40 is considered indicator of serious malnutrition.

Flow Cytometric Detection of Subsets of T Lymphocytes

After diagnosis, peripheral blood mononuclear cells (PBMCs) were isolated from peripheral blood by Ficoll density gradient centrifugation (Biochrom GmbH, Germany).

The cells were washed, and 2×106 cells were stained for 20 min on ice with CD27 FITC, CD4-APC-H7, CD8-PE, CD45RO-PE-Cy7, CD45RA-APC, CCR7-Per-CP-Cy5.5 and CD95-V500 (Becton Dickinson Biosciences, CA, USA).

After washing, the cells were resuspended in PBS and analyzed by FACS Cantor flow cytometry (Becton Dickinson Biosciences, USA). An isotype-matched negative control anti-human IgG was used with each sample. Then, lymphocyte gating was done based on their scatter characteristics on forward and side scatter histograms. First, CD4+ cells and CD8+ cells were gated. Further gating of CD4+ and CD8+ T cells was based on their characteristic expression patterns of CD45RA and CD45RO, followed by gating based on CD27, CCR7 and CD95 expression on CD4+ and CD8+ T cells.

Based on the previous expression patterns, each T cell subset was defined as follows: TCM; CD4+/CD8+ CD45RO+CCR7+.

T_{EM}; CD4+/CD8+ CD45RO+ CCR7−.

T_{EMRA}; CD4+/CD8+ CD45RO− CD45RA+ CCR7− CD27−.

T_{N}; CD4+/CD8+ CD45RO− CD45RA+ CCR7+ CD27+ CD95−.

T_{SCM}; CD4+/CD8+ CD45RO−CD45RA+ CCR7+ CD27+ CD95+ (Fig. 1).

Treatment of AML: The backbone of treatment of most types of AML was chemotherapy, except APL, which was typically given in two divided phases.

1. Remission induction: age, performance status, cardiac status and other risk factors were taken into consideration upon choosing chemotherapy, but mainly depended on Cytarabine (ara C) and anthracyclines (doxorubicin) in what's called 7+3 regimen.

   In some patients, cladribine was added, and intrathecal chemotherapy -/+ craniospinal or cranial radiotherapy were used. Patients with reduced cardiac output as determined by ejection fraction in echocardiography were contraindicated to receive anthracyclines, so they were treated with another chemotherapy drug, such as fludarabine or etoposide.

   Bone marrow biopsy was performed one week after induction, and remission was confirmed by the presence of no more than 5% blasts; if so, they were shifted to the second phase of treatment.

2. Consolidation: For patients younger than 60 years of age, the main treatment options are high-dose Cytarabine (ara-C) given over five days for 3-4 cycles every four weeks, allogeneic stem cell transplant, or autologous stem cell transplant were options. For patients older than 60, options for consolidation included High-dose Cytarabine (usually lower than used in younger patients), standard-dose Cytarabine given together with doxorubicin, or mitoxantrone, non-myeloablative stem cell transplant (mini-transplant) was an option.

Statistical Analysis

All data were analyzed using IBM-SPSS version 26, and all memory T cells were not normally distributed by the Shapiro-Wilk test except CD4+CD45+RA (p=0.2), CD4+CD45+RO (p=0.2), CD8+T_{EM} (p=0.3), CD8+T_{SCM} (p=0.06), & CD8+CD45+RO (p=0.1), the association between scale and two categorical variables were done by Mann Whitney U-test and independent sample t-test, correlations between scale variables were done by Pearson's and Spearman rho according to type of scale variables, because of the presence of multicollinearity between CD8+T memory cells, with condition index >10 and Eigen value approached 0, we chose binary logistic regression with forward likelihood ratio for multivariate analysis. To detect the presence of outliers among T memory in cases we ran Cook's test, we found three outliers of no significant impact, as figured below (Fig. 2)

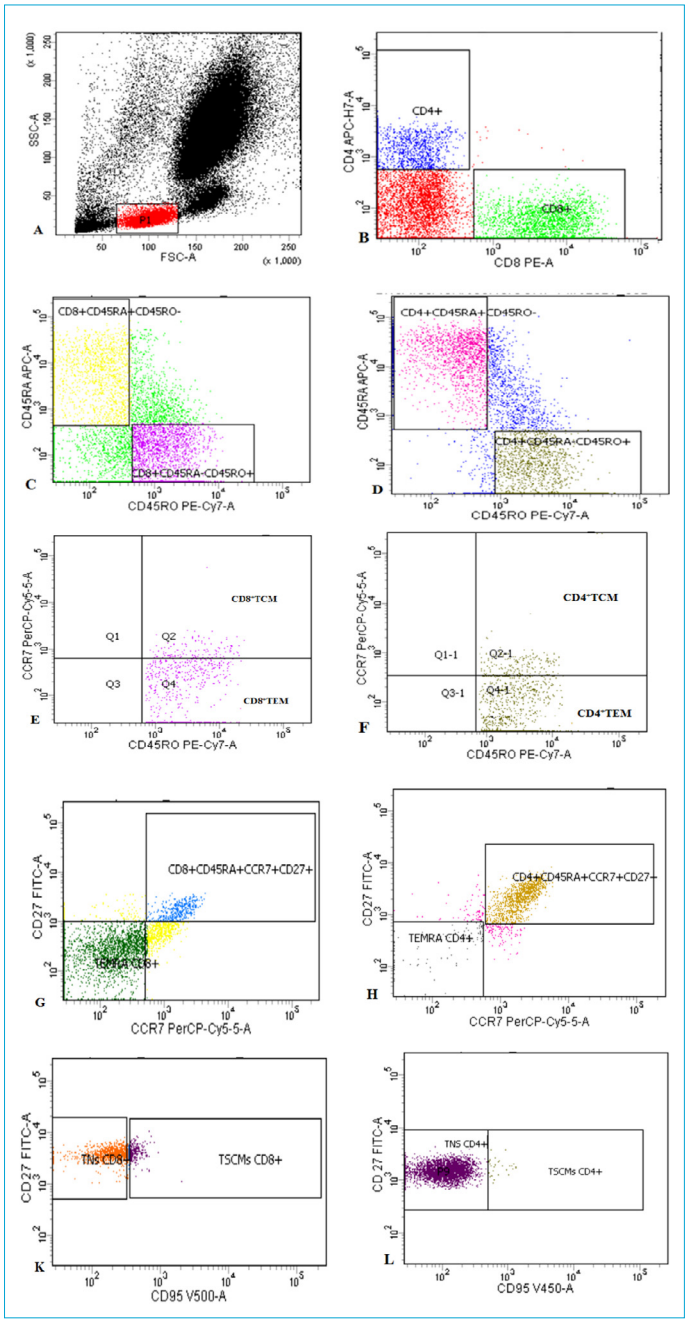

Figure 1. Flow cytometric detection of T lymphocyte subsets: **(a)** Forward and side scatter histogram, Lymphocytes were gated based on their characteristics. **(b)** CD4 and CD8 were assessed on lymphocytes and then gated for further analysis. **(c-l)** CD4+ cells and CD8+ cells were subdivided based on characteristic expression patterns of CD45RA, CD45RO, CD27, CCR7 and CD95 into: T_{CM}; CD8+ CD45RO+CCR7+; T_{EM}; CD8+ CD45RO+ CCR7−; T_{EMRA}; CD8+ CD45RO− CD45RA+ CCR7− CD27−; T_{N}; CD8+ CD45RO− CD45RA+ CCR7+ CD27+ CD95−; T_{SCM}; CD8+ CD45RO−CD45RA+ CCR7+ CD27+ CD95+.

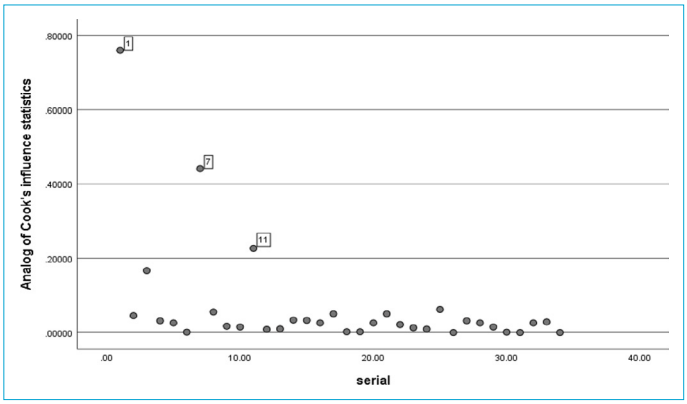

Figure 2. Cook's test among 34 cases of AML.

Results

Table 1 demonstrated different demographic and laboratory characteristics of 34 patients with AML with a mean age of 41.1 years. Most patients were males, the peripheral blast had a mean number of 43.8±5.4, and different hematologic and chemistry profiles, and different electrolyte levels and LDH were tabulated below.

Regarding the inflammatory indices in AML patients, the mean±SE of NLR, LMR, and PNI were 2.0±0.5, 11.15±7.3, and 33.1±1.14.

Notably, there were no significant differences in percentages of CD4+T cell types between AML patients and healthy controls, with the exception of the total percentage of CD4+T cells ($p<0.001$) and CD4+TSCM ($p=0.019$) that accumulated in controls compared to patients, Table 2.

Conversely, significant differences in the mean values of all studied CD8+T memory cells in AML patients compared to healthy controls with the exception of CD8+CD45+RA cells; furthermore, there were accumulations of CD8+T_{EMRA}, CD8+CD45+RO, and CD8+T_{EM} in patients compared with controls, while CD8+, CD8+T_{Ns}, CD8+T_{SCM}, and CD8+T_{CM} accumulated in controls compared to patients as demonstrated in Table 3.

Response to Treatment Among AML Patients

Twenty (58.8%) patients achieved complete remission and 14 patients didn't achieve remission (41.2%).

Univariate analysis of the impact of different T memory cells on the response to treatment

Table 4 demonstrated significant elevations of CD8+T cells, CD8+T_{EMRA}, CD8+T_{SCM}, CD8+T_{CM}, and CD8+T_{EM} in patients with remission compared to those without remission. On the contrary, CD4+T memory cells did not show any significant differences.

Table 1. Demographic and Laboratory characteristics of AML patients

Characteristics	Descriptive (n=34)
Age (mean±SE)	41.1±2.3 years
Range	20-65 years
Sex (male/female)	23/11
Peripheral blasts (mean±SE)	43.8 ±5.4
Range	3-150
Median	36.4
Liver functions	
AST (U/L)	51.6±11.2
ALT (U/L)	46.3±13.7
ALP (U/L)	151.2±17.2
T-bilirubin (mg/dL)	0.8±0.12
Total protein (g/L)	63.6±1.4
Albumin (g/dL)	33.03±1.15
Hematologic data	
HB (g/µl)	8.8±0.4
WBCs (* 10^9/L)	47.4±12.01
Neutrophils (* 10^9/L)	9.5±3.4
Lymphocytes (* 10^9/L)	7.2±2.7
Monocytes (* 10^9/L)	9.9±3.5
Basophils (* 10^9/L)	3.1±1.4
Eosinophils (* 10^9/L)	0.3±0.2
Platelets (* 10^9/L)	56.24±9.7
Renal functions	
Urea (mg/dL)	27.5±2.5
Creatinine (mg/dL)	0.12±0.7
Electrolytes	
Ca (mg/dL)	9.3±0.2
Mg (mg/dL)	2.4±0.1
Ph (mg/dL)	3.5±0.4
Na (mg/dL)	136.2±0.7
K (mg/dL)	3.7±0.2
LDH (U/L)	1399±217.9

Data expressed as mean±SE; Ca: calcium; Mg: magnesium; Ph: phosphorus; Na: sodium; K: potassium.

Table 2. Differential expressions of CD4+ T memory cells between AML patients and healthy controls

	Patients	Controls	p
CD4+T	27.1±1.2	35.7±1.1	<0.001
CD4+CD45+RA[a]	43.5±1.7	48.1±2.3	0.1
CD4+T_{EMRA}	3.19±0.5	3.6±0.45	0.5
CD4+T_{Ns}	37.9±1.7	36.1±2.4	0.5
CD4+T_{SCMs}	1.5±0.1	1.97±0.2	0.019
CD4+CD45+RO[a]	44.04±1.5	41.02±1.4	0.1
CD4+T_{CM}	26.2±1.6	26.2±1.8	0.9
CD4+T_{EM}	17.64±1.1	14.1±1.7	0.06

Data expressed as mean percentages ±SE, analyzed by Mann Whitney test and independent sample t-test.

Table 3. Differential expression of CD8+T memory cells between AML patients and healthy controls

	Mean±SE		
	Patients	Controls	p
CD8+T cells	22.6±0.7	26.6±1.03	0.002
CD8+CD45+RA	43.7±1.4	46.5±2.04	0.2
CD8+T_{EMRA}	19.8±1.45	12.8±1.1	0.001
CD8+T_{Ns}	21.9±1.92	32.3±1.94	<0.001
CD8+T_{SCM}[a]	1.7±0.2	3.7±0.2	<0.001
CD8+CD45+RO[a]	46.6±1.53	42.42±1.3	0.042
CD8+T_{CM}	13.94±0.8	17.2±1.22	0.031
CD8+T_{EM}[a]	31.3±1.6	25.35±1.41	0.008

Data were expressed as mean±SE, analyzed by Mann Whitney test and independent-sample t-test[a].

Correlations between Peripheral Blasts and T Memory Cells

There were no significant correlations between the percentage of pretreatment peripheral blasts and T memory cells except with CD8+T_{EMRA} (r=-0.494, p=0.003) and CD8+T_{EM} (r=-0.395, p=0.021), where a negative association was detected (Fig. 3).

Correlations between Inflammatory Indices and T Memory Cells

LMR was positively correlated with CD8+T, CD8+T_{CM}, and CD4+T_{EMRA} (r=0.39 & p=0.03, r=0.4 & p=0.023, r=0.4 & p=0.02 respectively). while NLR exhibited a negative correlation with CD8+CD45+RO and CD4+T_{CM} (r=-0.4 & p=0.02, r=-0.5 & p=0.006 respectively), and PNI did not show any correlations with T memory cells (Fig. 4 a, b).

Multivariate Analysis of Different Memory Cells

Using binary logistic regression to detect the impact of different memory cells (CD8+, CD8+T_{EMRA}, CD8+T_{SCM}, CD8+T_{EM}, CD8+T_{CM}) and CD4/CD8 ratio in remission status, the correct classification of the model was 82.4%.

The odds of having remission among the studied patients increased significantly for each increase in the level of CD8+T cells but not with other memory cells, as shown in Table 5.

Discussion

Generally, about 70% of adult AML patients achieve complete remission following induction chemotherapy; however, most of them ultimately relapse and die of the disease,[12] according to the National Cancer Institute's SEER (Surveillance, Epidemiology, and End Results) database, only 29.5% of AML patients remain alive for five years.

Table 4. Differential expression of T memory cells according to remission status

T cell memory	Remission=20	No remission=14	p
CD8+T cell	24.61±1	19.7±0.54	<0.001
CD8+CD45+RA	45.32±1.95	41.4±1.9	0.18
CD8+T_{EMRA}	22.28±1.8	16.15±2.2	0.035
CD8+T_{Ns}	23.21±2.74	19.95±2.5	0.4
CD8+T_{SCMs}[a]	2.04±0.26	1.12±0.23	0.017
CD8+CD45+RO[a]	44.13±2.05	50.1±2.02	0.06
CD8+T_{CM}	15.69±1.004	11.4±1	0.007
CD8+T_{EM}[a]	34.01±1.8	27.49±2.5	0.038
CD4+T cell	25.59±1.5	29.19±1.9	0.1
CD4+CD45+RA[a]	42.16±2.6	45.51±2.1	0.3
CD4+TE_{MRA}	2.96±0.6	3.53±0.71	0.5
CD4+T_{Ns}	36.001±2.53	40.5±1.95	0.1
CD4+T_{SCMs}	1.54±0.13	1.47±0.19	0.7
CD4+CD45+RO[a]	45.56±2.04	41.87±2	0.2
CD4+T_{CM}	25.77±2.29	26.75±2	0.7
CD4+T_{EM}	19.03±1.7	15.64±1.1	0.1
CD4/CD8 ratio[a]	1.1±0.1	1.5±0.1	0.002
Pretreatment blasts	28.2±3.2	66.014±9.8	0.002

Data expressed as mean±SE, analyzed by Mann Whitney test and independent-sample t-test[a].

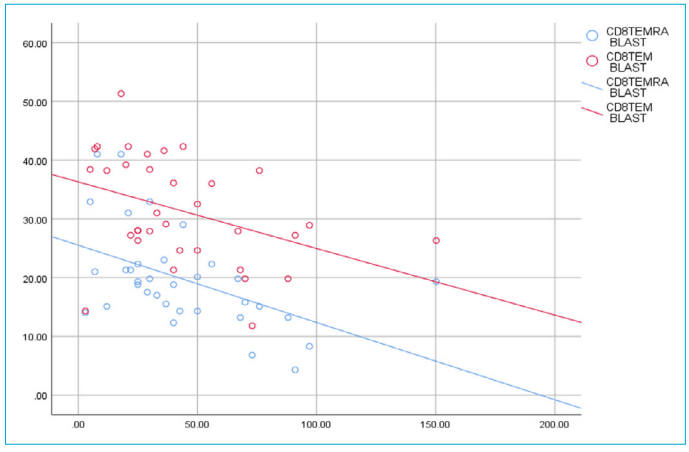

Figure 3. Cook's test among 34 cases of AML.

In spite of being of low immunogenicity, however, AML blasts are gifted several unique immune evasion mechanisms, and their antileukemic responses are mainly related to mutational quality rather than quantity.[13-15] Effector T cells are able to recognize leukemic blasts, evidenced by their susceptibility to T-cell-mediated killing in allogeneic transplantation and donor lymphocyte infusion.[16]

Our results demonstrated that only total CD4+T cells and CD4+T_{SCM} accumulated in controls but not in patients, while significant elevations in CD8+T_{EMRA}, CD8+T_{EM}, and CD8+CD45+RO cells in AML patients compared to controls,

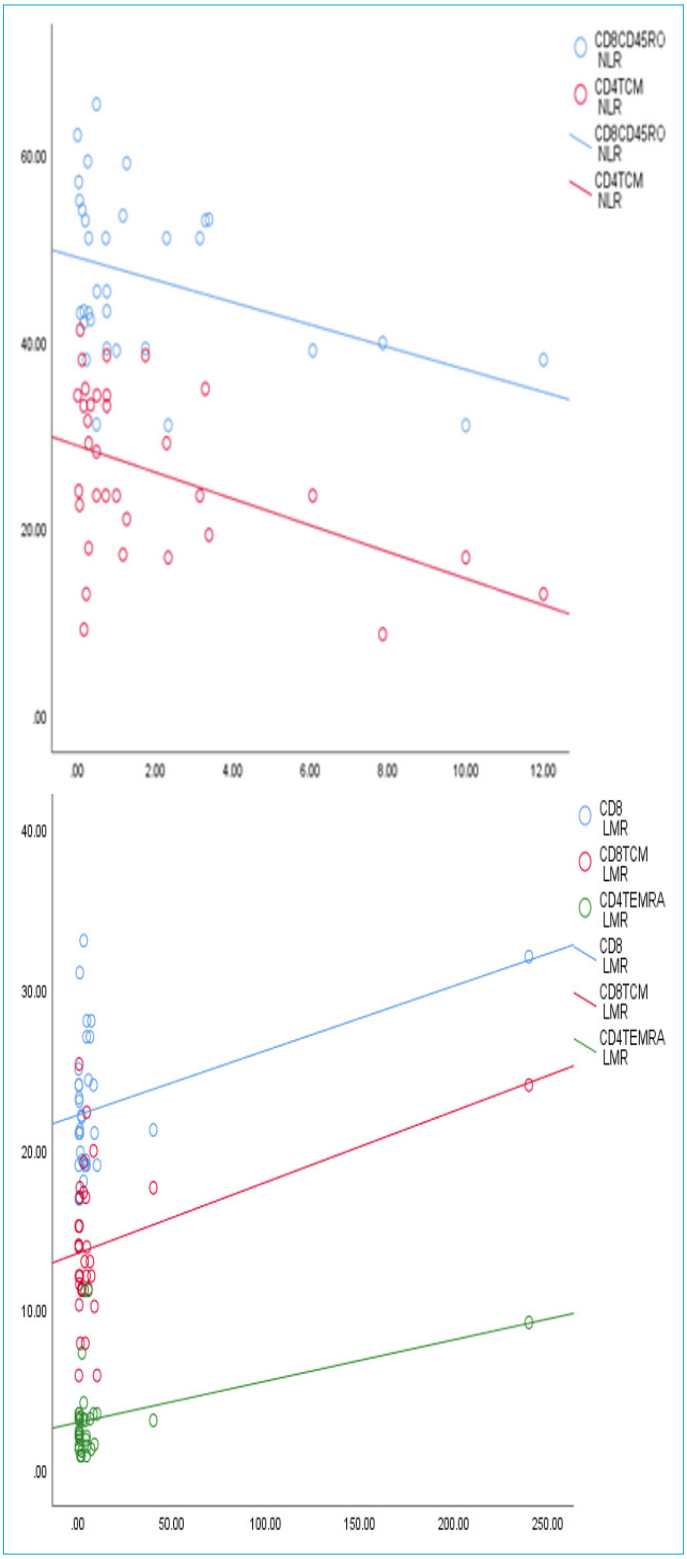

Figure 4. Correlations between inflammatory indices **(a)** NLR & **(b)** LMR and T memory cells.

and the remaining total CD8+T cells, CD8+T_{NS}, CD8+T_{CM}, and CD8+T_{SCM} accumulated significantly in controls compared to patients. Hence, no significant differences in CD4+T memory cells between patients who achieved remission compared to those without remission; however, all CD8+T cell memory accumulated in patients with remission, with the exception of CD8+T_{NS}, CD8+CD45+RA and CD8+CD45+RO.

In secondary lymphoid tissues, CD8+T_{NS} cells are activated by mature circulating dendritic cells (DCs), which present tumor-derived antigens on MHC class I molecules;[17] these cells are activated then differentiated to effector T cells and migrate to peripheral blood where they recognize tumor cells by T-cell receptors, classically, the circulating memory compartment consists of central-memory (T_{CM}) and effector-memory (TEM) CD8+ T cells which are responsible for establishing long-lasting antitumor responses.[4, 18] A subtype of memory T cells persists in lymphoid and non-lymphoid tissues known as tissue-resident memory T cells, where they provide strong innate and adaptive immunity against infectious diseases and tumors in these sites,[19, 20] supporting evidences for the role of memory T cells in immunosurveillance against cutaneous melanoma and other tumor models were eventually explored.[21, 22]

Although we did not test these resident memory cells in our research, but generally speaking, our results might be comparable to previous evidences regarding the significantly higher level of C D8+T_{EM} in patients compared to controls and in patients with remission compared with no remission.

Yasmin et al. evaluated the level of CD8+T_{CM} and CD8+T_{SCM} in positive axillary lymph nodes of breast cancer patients compared to non-tumor-involved lymph nodes. They observed significantly higher levels of T_{CM} in tumor positive LNs. At the same time, T_{SCM} cells were significantly higher in stage II compared to stage I;[23] T_{SCM} were discovered to produce persistent graft versus host disease because they survived for a long time and exhibited significantly better antitumor capability than TEM.[24, 25]

Allogeneic bone marrow transplantation represents the main treatment option for AML. However, disease relapse and progression are responsible for treatment failure.[26] The efficacy of transplantation depends mainly on the ability of donor lymphocytes to eliminate residual tumor tissues via what is called the graft versus leukemia effect. The durable persistence of this effect requires long-lasting maintenance of these reactive T cells, which was explained by the presence of the T_{SCM} pool.[27]

Tumor-infiltrating CD8+T cells were assumed to predict the response and survival in cancer patients treated with immunotherapy,[28-31] in spite, some patients with high levels of CD8+T cells responded. In contrast, others did not that could be partly explained by different phenotypes

Table 5. Multivariate logistic regression of CD8+T memory cells

	B	SE.	Wald	Sig.	Odds ratio	95% C.I.for odds ratio	
						Lower	Upper
CD8[a]	.549	.201	7.486	.006	1.732	1.169	2.566
Constant	-11.484	4.231	7.368	.007	.000		

Only CD8+cells were analyzed, odds=eB.

and functions of tumor-infiltrating CD8+T cells, including memory CD8+T cells; a meta-analysis of nine studies concluded that memory CD8+T cells were closely correlated with progression-free survival and overall survival in cancer patients treated with immunotherapy.[32]

Several studies reported that T_{SCM} cells may play a pivotal role in specific antitumor response and long-term immune surveillance directed against tumors, in addition to their ability to differentiate to T_{CM}, T_{EM}, and terminal effector T cells subsequently proposed to be a key determinant in immune memory with their significance for immune reconstitution and prognosis of patients with hematological malignancies before and after therapy.[33, 34] Consistent with the previous reports, our results revealed elevated T_{SCM}, T_{CM}, T_{EM}, and T_{EMRA} cells in patients with remission compared to those without.

CD4+T memory cells have been reported in many studies to be an important constituent of the tumor microenvironment in many solid tumors, where they infiltrate tumor tissue more than normal tissues in colorectal cancers.[35] However, they are associated with invasion and aggressiveness in triple-negative breast cancer[36] and favorable prognosis and survival in lung adenocarcinoma[37] and gastric cancer.[38] In NHL and CLL, CAR-T cells, generated from both CD4+T and CD8+T memory cell subsets, demonstrated high efficacy in treating these tumors, supporting the role of CD4+T memory cells in inducing and persisting CD8+T memory functions,[39, 40] our data failed to find significant differences between patients and controls in different CD4+T memory subsets with the exception of total CD4+T cell and CD4+T_{SCM} that were accumulated in controls compared to later group, which partially explained comparability of these cells between remitting and non-remitting patients.

The small sample size was a crucial limiting point that led to the loss of multiple relations, partly due to the low flow rate of adult AML presented to our institute; moreover, molecular and cytogenetic markers and karyotypic analysis were not documented for all patients, so we could not analyze their associations with T cell subsets, in addition, because of limited resources, a few numbers of patients could be salvaged with BMT. Hence, the types and percentages of T cell subsets were not evaluated after BMT and their impact on BMT.

Despite significant progress in AML therapy, there is a gap in understanding the role of different T memory cells that requires more effort to clarify further the changes between different subsets of T memory cells, particularly T_{SCM}. To our knowledge, this is the first research to shed light on the role of these cells in AML. Moreover, it is worth noting that a single treatment modality cannot effectively eliminate tumor cells; hence, immune cell therapy should be combined with monoclonal antibody therapy, radiotherapy, chemotherapy, and other treatment modalities so that patients can get better results.

Conclusion

Convincing evidence in preclinical and clinical studies proved that T memory cells are important tools in adaptive immunity in tumor immunotherapy; our results showed that accumulation of CD8+T memory cells in AML patients, especially those who achieved remission, could enhance the immune response, namely those who are at high risk of relapse after bone marrow transplantation.

Disclosures

Ethics Committee Approval: The study was approved by the Assiut University Ethical Committee (IRB; 17300648).

Peer-review: Externally peer-reviewed.

Conflict of Interest: None declared.

Authorship Contributions: A.M.Z., I.K.I.S., D.A.G., S.M.M., M.M.W., S.H., K.S., A.R., A.M.A.: The study's conception, design, patient management, data analysis, and manuscript drafting. A.M.Z., Z.A.M.Z., M.A.S.: The laboratory investigations. All authors participated in evaluation of the final manuscript version.

References

1. Duan S, Thomas PG. Balancing immune protection and immune pathology by CD8+ T-cell responses to influenza infection. Front Immunol 2016;7:25.
2. Schmidt ME, Varga SM. The CD8 T cell response to respiratory virus infections. Front Immunol 2018;9:678.
3. Hamann D, Baars PA, Rep MH, Hooibrink B, Kerkhof-Garde

SR, Klein MR, et al. Phenotypic and functional separation of memory and effector human CD8+ T cells. J Exp Med 1997;186:1407–18.
4. Sallusto F, Lenig D, Förster R, Lipp M, Lanzavecchia A. Two subsets of memory T lymphocytes with distinct homing potentials and effector functions. Nature 1999;401:708–12.
5. Pangrazzi L, Naismith E, Meryk A, Keller M, Jenewein B, Trieb K, et al. Increased IL-15 production and accumulation of highly differentiated CD8+ effector/memory T cells in the bone marrow of persons with cytomegalovirus. Front Immunol 2017;8:715.
6. Prlic M, Williams MA, Bevan MJ. Requirements for CD8 T-cell priming, memory generation and maintenance. Curr Opin Immunol 2007;19:315–9.
7. Zahran AM, Abdel-Rahim MH, Nasif KA, Hussein S, Hafez R, Ahmad AB, et al. Association of follicular helper T and follicular regulatory T cells with severity and hyperglycemia in hospitalized COVID-19 patients. Virulence 2022;13(1):569-577.
8. Sallusto F, Geginat J, Lanzavecchia A. Central memory and effector memory T cell subsets: Function, generation, and maintenance. Annu Rev Immunol 2004;22:745–63.
9. Lichtenegger FS, Krupka C, Haubner S, Köhnke T, Subklewe M. Recent developments in immunotherapy of acute myeloid leukemia. J Hematol Oncol 2017;10(1):142–9.
10. Yao D, Xu L, Tan J, Zhang Y, Lu S, Li M, et al. Re-balance of memory T cell subsets in peripheral blood from patients with CML after TKI treatment. Oncotarget 2017;8(47):81852–9.
11. Tan J, Chen S, Lu Y, Yao D, Xu L, Zhang Y, et al. Higher PD-1 expression concurrent with exhausted CD8+ T cells in patients with de novo acute myeloid leukemia. Chin J Cancer Res 2017;29:463–70.
12. Döhner H, Estey E, Grimwade D, Amadori S, Appelbaum FR, Büchner T, et al. Diagnosis and management of AML in adults: 2017 ELN recommendations from an international expert panel. Blood 2017;129:424–47.
13. Alexandrov LB, Nik-Zainal S, Wedge DC, Aparicio SA, Behjati S, Biankin AV, et al. Signatures of mutational processes in human cancer. Nature 2013;500:415–21.
14. Austin R, Smyth MJ, Lane SW. Harnessing the immune system in acute myeloid leukaemia. Crit Rev Oncol Hematol 2016;103:62–77.
15. Mussai F, De Santo C, Abu-Dayyeh I, Booth S, Quek L, McEwen-Smith RM, et al. Acute myeloid leukemia creates an arginase-dependent immunosuppressive microenvironment. Blood 2013;122:749–58.
16. Kolb HJ, Schattenberg A, Goldman JM, Hertenstein B, Jacobsen N, Arcese W, et al. Graft-versus-leukemia effect of donor lymphocyte transfusions in marrow grafted patients. Blood 1995;86:2041–50.
17. Salmon H, Idoyaga J, Rahman A, Leboeuf M, Remark R, Jordan S, et al. Expansion and activation of CD103+ dendritic cell progenitors at the tumor site enhances tumor responses to therapeutic PD-L1 and BRAF inhibition. Immunity 2016;44:924–38.
18. Reading JL, Gálvez-Cancino F, Swanton C, Lladser A, Peggs KS, Quezada SA. The function and dysfunction of memory CD 8+ T cells in tumor immunity. Immunological reviews 2018;283:194–212.
19. Park CO, Kupper TS. The emerging role of resident memory T cells in protective immunity and inflammatory disease. Nature medicine 2015;21:688–97.
20. Noviello M, Manfredi F, Ruggiero E, Perini T, Oliveira G, Cortesi F, et al. Bone marrow central memory and memory stem T-cell exhaustion in AML patients relapsing after HSCT. Nature Communications 2019;10:1065.
21. Gálvez-Cancino F, López E, Menares E, Díaz X, Flores C, Cáceres P, et al. Vaccination-induced skin-resident memory CD8+ T cells mediate strong protection against cutaneous melanoma. Oncoimmunology 2018;7:e1442163.
22. Enamorado M, Iborra S, Priego E, Cueto FJ, Quintana JA, Martínez-Cano S, et al. Enhanced anti-tumour immunity requires the interplay between resident and circulating memory CD8+ T cells. Nature communications 2017;8:1–11.
23. Vahidi Y, Bagheri M, Ghaderi A, Faghih Z. CD8-positive memory T cells in tumor-draining lymph nodes of patients with breast cancer. BMC Cancer 2020;20:257.
24. Zhang Y, Joe G, Hexner E, Zhu J, Emerson SG. Host-reactive CD8+ memory stem cells in graft-versus-host disease. Nat Med 2005;11:1299–305.
25. Xu L, Zhang Y, Luo G, Li Y. The roles of stem cell memory T cells in hematological malignancies. J Hematol Oncol 2015;8:113.
26. Horowitz M, Schreiber H, Elder A, Heidenreich O, Vormoor J, Toffalori C, et al. Epidemiology and biology of relapse after stem cell transplantation. Bone Marrow Transplantation 2018;53:1379–89.
27. Gattinoni L, Lugli E, Ji Y, Pos Z, Paulos CM, Quigley MF, et al. A human memory T cell subset with stem cell–like properties. Nature medicine 2011;17:1290–7.
28. Jansen CS, Prokhnevska N, Master VA, Sanda MG, Carlisle JW, Bilen MA, et al. An intra-tumoral niche maintains and differentiates stem-like CD8 T cells. Nature 2019;576:465–70.
29. Peranzoni E, Lemoine J, Vimeux L, Feuillet V, Barrin S, Kantari-Mimoun C, et al. Macrophages impede CD8 T cells from reaching tumor cells and limit the efficacy of anti–PD-1 treatment. Proceedings of the National Academy of Sciences 2018;115:e4041–50.
30. Savas P, Virassamy B, Ye C, Salim A, Mintoff CP, Caramia F, et al. Single-cell profiling of breast cancer T cells reveals a tissue-resident memory subset associated with improved prognosis. Nature medicine 2018;24:986–93.
31. Eroglu Z, Zaretsky JM, Hu-Lieskovan S, Kim DW, Algazi A, Johnson DB, et al. High response rate to PD-1 blockade in des-

moplastic melanomas. Nature 2018;553:347–50.
32. Jin Y, Tan A, Feng J, Xu Z, Wang P, Ruan P, et al. Prognostic impact of memory CD8(+) T Cells on immunotherapy in human cancers: A systematic review and meta-analysis. Front Oncol 2021;11:698076.
33. Martin PJ. Reversing CD8+ T-cell exhaustion with DLI. Blood 2014;123:1289–90.
34. Zahran AM, Shibl A, Rayan A, Mohamed MAEH, Osman AMM, Saad K, et al. Increase in polymorphonuclear myeloid-derived suppressor cells and regulatory T-cells in children with B-cell acute lymphoblastic leukemia. Sci Rep. 2021;11(1):15039.
35. Ge P, Wang W, Li L, Zhang G, Gao Z, Tang Z, et al. Profiles of immune cell infiltration and immune-related genes in the tumor microenvironment of colorectal cancer. Biomed Pharmacother 2019;118:109228.
36. Deng L, Lu D, Bai Y, Wang Y, Bu H, Zheng H. Immune profiles of tumor microenvironment and clinical prognosis among women with triple-negative breast cancer. Cancer Epidemiol Biomarkers Prev 2019;28:1977–85.
37. Choi H, Na KJ. Integrative analysis of imaging and transcriptomic data of the immune landscape associated with tumor metabolism in lung adenocarcinoma: Clinical and prognostic implications. Theranostics 2018;8:1956–65.
38. Ning ZK, Hu CG, Huang C, Liu J, Zhou TC, Zong Z. Molecular subtypes and CD4+ memory T cell-based signature associated with clinical outcomes in gastric cancer. Front Oncol 2021;10:626912.
39. Turtle CJ, Berger C, Sommermeyer D, Hanafi LA, Pender B, Robinson EM, et al. Anti-CD19 chimeric antigen receptor-modified T cell therapy for B cell non-Hodgkin lymphoma and chronic lymphocytic leukemia: Fludarabine and cyclophosphamide lymphodepletion improves in vivo expansion and persistence of CAR-T cells and clinical outcomes. Blood 2015;126:184.
40. Turtle CJ, Hanafi LA, Berger C, Sommermeyer D, Pender B, Robinson EM, et al. Addition of fludarabine to cyclophosphamide lymphodepletion improves in vivo expansion of CD19 chimeric antigen receptor-modified T cells and clinical outcome in adults with B cell acute lymphoblastic leukemia. Blood 2015;126:3773.