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T CELL EXHAUSTION: SUPREMACY IN LEUKEMIA AS FOR DISEASE AND THERAPY

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In this review, an overview of exhausted T cells in acute and chronic leukemias including myelocytic and lymphoblastic leukemia was made to browse this important subset of cells and highlight circumstantial phenotypic, genotypic, and transcriptional alterations. Ultimately, these changes affect the development and progression of the disease. The process of T cell dysfunction and exhaustion in leukemia is briefly described with special emphasis on the phenotypic state of T cells together with immune checkpoints (ICs) which are frequently altered in this setting. New immune therapeutic approaches are being developed for the treatment of the disease; including IC blockers which are especially designed to block molecular targets specific to leukemia. Furthermore, the interaction of exhausted T cells subset with these therapeutic approaches especially T-cell-based therapeutic strategies such as T cell receptor (TCR)- engineered lymphocytes; and chimeric antigen receptors (CARs) is discussed. This accordingly will improve our understanding of the resistance to therapy and thus may improve our therapeutic strategies

Keyword: *Exhausted T cells, Immune Checkpoints, Chimeric antigen receptor T cells, Myelocytic leukemia, and Lymphoblastic leukemia*

INTRODUCTION

An estimated annual number of new cases of leukemia is about 474,519 and 311,594 deaths in 2020 all over the world according to the Global Cancer Observatory GLOBOCAN[1]. Diagnosis is usually performed by immunophenotyping[2]. The complications are part of a failing immune system with T and B cell dysregulation. This predisposes patients to recurrent infections and autoimmune diseases[2]. Infectious complications in AML patients carry the risk of higher morbidity and mortality specially in limited resources countries. Candida albicans is a common cause of morbidity and mortality for patients with acute myeloid leukemia[3]. Moreover, intensive induction chemotherapy further contributes to increasing the risk of candida infections in AML[4]. Toll like receptors expressions could be important biological markers for the occurrence of invasive fungal infections in AML patients after intensive induction chemotherapy[5].

T cells are chief players in the adaptive immune response. T helper cells are a major subset of cells of the T cells, they are also

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called CD4 cells. These cells act as a maestro to other immune cells, they primarily function by secreting cytokines and chemokines which are chemical mediators to activate other immune cells. T cytotoxic cells also called CD8 cells, are important players in the adaptive immune response, they function mainly by destroying infected or cancer cells. During the course of infection or cancer, T cells are stimulated by foreign antigens which are presented to them by antigen-presenting cells (APCs), leading to subsequent activation of naive T cells ($CD44^{low}CD62L^{hi}$), proliferation, and attaining of effector functions. Following this activation and expansion, 90-95% of T cells die by apoptosis while the rest remain and differentiate into memory T cells which remain in the resting state. Persistent stimulation of T cells to their corresponding antigen results in subsequent upregulation of inhibitory receptors on these cells. Thus, T cells enter a state of dysfunction where effector functions are lost and proliferative capacity is diminished. All together with characteristic transcriptional and metabolic changes. This state of dysfunction was first described for antigen-specific T cells in chronic murine Lymphocytic choriomeningitis (LCMV) infection[6]. T cell dysfunction is usually classified as exhaustion, senescence, and anergy in tumor settings[7]. In addition to the upregulation of inhibitory receptors, T cell exhaustion is also characterized by functional impairments, including reduced cytokine production primarily, interleukin 2 (IL-2), afterward tumor necrosis-α (TNF-α)[8,9] diminished proliferative capacity, and subsequent loss of cytotoxic activity, which further contributes to immune evasion of tumors[10]. The failure of the immune system to defend against tumor, will lead to further progression of the disease. Moreover, genetic and epigenetic alterations frequently occur and differ according to various factors according to disease etiology, cancer stage, host variables, and disease progression, these alterations further lead to alterations in gene expression of upstream and downstream effectors thus, leading to clonal heterogeneity of lymphocytic leukemias, subsequent purifying selection and ultimately a more aggressive disease[11,12]. Therefore, contributes more to the complexity of the treatment plan and host responses. Besides, evidence of the contribution of dendritic cell that is a specialized antigen presenting cells (that is responsible for the 3 signals of T cell activation; antigen presentation to TCR, surface receptor ligand co-stimulation, and cytokine signaling) to exhaustion of T cells due to increased antigen presentation[13-15]. Interestingly, with the aid of clustered regularly interspaced short palindromic repeats **(**CRISPR-Cas9), when tumor cells were gene-edited to remove antigen-presentation capacities of the cells, T cell exhaustion persisted thus, emphasizing the role of APCs[16].

In addition to escaping immune responses by suppressing T cell activity, many mechanisms are utilized by tumors to escape from the host's immune responses and ensure their further progression. Evidence of tumor cells masking their antigens from immune cells together with the failure of co-stimulation has been previously described[17-19]. Besides, a subset of T cells called T regulatory cells (Tregs) is responsible for maintaining immune tolerance to self-antigens and preventing autoimmune diseases from becoming more active and contributing to disease progression[20]. T regulatory cells (T regs) become more active and higher in frequency, thus, further downregulating the activity of lymphocytes in AML and CLL[20-24]. Also, T regs secrete suppressive cytokine interleukin-10 (IL-10)[25] and transforming growth factor β (TGF-β) as auto-regulators[26]. TGF-β1 and interleukin-10[27] are key regulators of immune homeostasis with anti-tumor effect[28].

Exhausted T cells in leukemia

There is increasing evidence that T cell dysfunction and exhaustion develop in acute and chronic lymphocytic leukemia; as a result of persistent stimulation by tumor antigens together with the effect of suppressive tumor microenvironment. Consequently, activation markers become downregulated, inhibitory receptors become overexpressed, and proliferative capacity and cytokine secretion ability become compromised besides impaired degranulation[29]. Different levels of dysfunction were previously described in acute myeloid leukemia (AML)[30]. Exhausted T cells displayed an increased expression of PD-1 and TIM-3, reduced production of IL-2 and interferon- γ (IFN-γ) but increased IL-10 and

reduced proliferative capacity in leukemia[29,31-33]. Correspondingly, a substantial decrease in the expression of IL-2 receptor on T cells in B-Cell chronic lymphocytic leukemia (B-CLL) was also observed[34], which is an important activation marker and has a vital role in immune tolerance by Tregs[35]. Apart from this, highly differentiated T cells displayed a lack of CD28 expression and diminishing replicative capacity in conjunction with reduced telomerase expression[36]. In AML, T cells display phenotypic alterations that are skewed in the direction of terminal differentiation, increased loss of CD28 expression, and decline in naïve T lymphocytes are reported. Besides, there is a reduction in the percentages of stem cell memory T lymphocytes and central memory T lymphocytes accompanied by increased percentages of both differentiated effector memory T lymphocytes and terminally differentiated T lymphocytes[37,38]. This disturbance in T cell distribution subsequently leads to T cell exhaustion[39]. It is repeatedly reported that exhausted T cells are similar characteristically to terminally differentiated T cells and T cells can be categorized into generally and severely exhausted T cells; based on immune checkpoints expression such as; PD-1, T cell immunoreceptor with Ig and ITIM domains (TIGIT) and CD28[40,41]. Despite this, exhausted CD8 T cells were found to originate from memory precursor T cells rather than terminally differentiated CD8 T cells[41]. Previous studies demonstrated an increased expression of signaling lymphocyte activation molecule family 6, SLAMF6 (CD352, Ly108, NTB-A) by exhausted CD8 T lymphocytes in B-cell leukemias and highlighted its role in T helper – B cell interaction[42-44]. Terminally differentiated exhausted T cells expressed T cell-specific DNA-binding protein (TCF-1) in cancer,[45] which is a key player in regulating T cell development and function[46]. TCF1 when not being degraded, TCF 1^+ precursor exhausted T cells were made, which consequently improved therapeutic outcomes during the course of chimeric antigen receptors (CARs) cell therapy of B-cell acute lymphoblastic leukemia (B-ALL) in murine and human xenograft models. Also, TCF 1^+ precursor exhausted T lymphocytes displayed an immune-enhancing property[47]. Exhausted T cells were found greater at the tumor site/bone marrow (BM) than in peripheral blood[48].

The state of T cells is critical to the fate of the disease. As mentioned earlier, disease progression in leukemia in part is due to lymphocytic dysfunction which can be attributed to T cell exhaustion. Accordingly, numerous attempts are conducted in order to restore cell function. Different therapeutic approaches were proposed over the past years and many of these attempts showed promising results. Of these T-cell therapeutic strategies; Adoptive T cell transfer (ACT) with tumor infiltrating lymphocytes (TILs), TCR-engineered lymphocytes, chimeric antigen receptors (CARs) T cell therapy, and vaccines to either treat or prevent cancer. They are designed to stimulate the immune system to respond against cancer; *via* loading tumor proteins to stimulate the host's immune cells to kill tumor cells. In addition to immune-therapeutics that are designed to activate exhausted T cells such as immune checkpoints (ICs) blockers, bispecific T cell engager (BiTE), and improving TCR-ζ expression by cytokines[30,49].

Exhaustion markers in leukemia

Inhibitory receptors or immune checkpoints (ICs) are specific receptors that regulate immune responses to protect against autoimmune reactions. In cancer, these receptors are overexpressed on the surfaces of immune cells and onto tumor cell surfaces, thus, suppressing the functions of effector cells and contributes to tumor evasion of immune responses in AML[50]. The prognosis of the disease determines the ability of the tumor to evade immune responses, and suppress cytotoxic effector functions. Therefore, monoclonal antibodies are designed to block these checkpoints that halt immune responses. And interfere with their killing mechanism.

Programmed cell death molecule PD-1 on T cells is an IC that was first discovered by the Nobel prize winner Professor Honjo[51]. It is an inhibitory receptor that is being expressed by T cells. It regulates T cell activity during an acute infection, chronic infection and cancer[52]. The relative expression of PD-L1 from peripheral blood mononuclear cells PBMNC was significantly higher in myelodysplastic syndrome (MDS) and chronic myelomonocytic

leukemia (CMML) compared with AML[53]. Moreover, Also, its higher expression was associated with poor response to induction therapy in AML patients[54] and with poor prognosis in AML with specific genetic mutations[55]. The PD-1/PD-L1 axis led to T cell dysfunction in CLL[56].

Professor Allison discovered another important checkpoint **cytotoxic T-lymphocyte antigen-4 (CTLA-4)**[57] and was also awarded the Nobel Prize for this discovery. Suppression of T cell activation by PD-1 and/or CTLA-4 is considered one of the major escape mechanisms of cancer cells. **T-cell immunoglobulin– and mucin domain–containing–3 (TIM-3)** is a transmembrane protein that plays a role in immune regulation. Recent studies on leukemias demonstrated an increased expression of PD-1, CTLA-4, and TIM-3 on the surfaces of T lymphocytes in acute and chronic lymphocytic leukemia (CLL) as well as an increased expression of soluble CTLA-4[21, 29, 31, 58-63]. However, in another study, PD-1 was downregulated in CLL[64]. Likewise, CTLA-4 was downregulated in B-CLL[34]. Moreover, TIM-3 was highly upregulated by exhausted T cells in conjunction with PD-1 in acute B-cell lymphoblastic leukemia (B-ALL)[48]. TIM-3 was found overexpressed by exhausted CD8 cells in patients with B-ALL[65], AML, and CLL[31,58,59]. Both TIM-3 receptors and soluble TIM-3 lead to the suppression of T cells and NK cells via increasing galectin-9 (gal-9) and reducing IL-2 in AML, respectively[32,66]. Furthermore, dysfunctional T cells have been shown to overexpress other inhibitory receptors, including; **CD39, TIGIT, and TNu FR2** together with CTLA-4 by exhausted CD8 cells in B-ALL[65]. **The leukocyte immunoglobulin-like receptor sub-family B (LILRBs)** is a family of transmembrane proteins, it is composed of 5 members, bearing extracellular Ig-like domains and intracellular signaling immunoreceptor tyrosine-based inhibitory motif (ITIM)[67,68]. These receptors are expressed on monocytes. They act as inhibitors (ICs) of immune response[69]. Recent studies reported the role of leukocyte immunoglobulin-like receptor B4 (LILRB4) in immune suppression in leukemia[70]. Many recent attempts to design blockers to target this receptor as a suggested strategy to aid in tumor therapy are being conducted. Recently, LILRB has been shown to play an important role in the prognosis of AML; once activated, it leads to an increase (upregulation) of nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB), thus leading to the persistence of the disease. Monoclonal antibodies MAb h128-3 targeting LILRB4. it was demonstrated that h128-3 was able to reverse T cell suppression and improve their proliferation as well as cytokine secretory properties *In vitro.* The study reported that the MAb improved the therapeutic effects of chemotherapy in animal models of AML[71]. **V-domain immunoglobulin suppressor of T cell activation (VISTA)** is a transmembrane protein that is expressed on myeloid and lymphoid cells and has been recently identified as a new immune checkpoint that is exploited by cancer to suppress T cell functions. **V-domain Ig suppressor of T cell activation (VSIR)** is the gene coding for VISTA. Results indicated that patients whose AML cells had higher expression of the gene had shorter survival times than those with lower expression[72]. **Cluster of Differentiation 244 (CD244)** also known as Natural Killer Cell Receptor 2B4 (NKR2B4) is an immunoregulatory transmembrane receptor protein that belongs to the Signaling Lymphocyte Activation Molecule (SLAM) family. Exhausted T cells from AML patients demonstrated high expression of these receptors on their surface.[33]. Another study in CLL indicated that CD244 was overexpressed on $CD8^+$ cells along with PD-1 and CD160[63].

Transcriptional and metabolic changes

The signaling pathways associated with T cell exhaustion and dysfunction such as the forkhead family of transcription factors proteins (FOXO), TGF-β, and mitogen-activated protein kinases (MAPK) are signaling pathways that are found altered in exhausted T cells in acute lymphoblastic leukemia[73]. The nuclear factor thymocyte selection-associated high mobility group box protein (TOX) is a vital regulator of tumor-specific T cell differentiation[74]. Its upregulation is characteristic of exhausted T cells and is frequently reported to contribute to the development of exhausted T cells[75,76]. TOX was found to be highly upregulated in AML and is associated with poor prognosis[77]. Additionally,

it was reported that TOX is important to the initiation and progression of the T-ALL[78]. Moreover, the transcription factor nuclear factor of activated T cells (NFAT) is central to the regulation of T cell activation[79,80], and its role in T cell exhaustion and anergy is documented[81]. Its contribution to the disease progression and persistence in T-ALL[82], as well as, B cell anergy in CLL[83]. The Solute Carrier Family 2, Member 1 gene (*SLC2A1)* is a metabolic gene that encodes transporter protein for glucose, Glucose transporter 1 (Glut1). It is silenced as a consequence of T cell exhaustion in acute lymphocytic leukemias, moreover, it was found that T cell dysfunction was associated/linked with diminished glucose metabolism[31].

Bispecific T cell engager (BiTE) to reverse exhaustion

As mentioned earlier, **bispecific T cell engager (BiTE)** is a monoclonal antibody designed to activate exhausted T cells by binding concurrently to both T cells (by binding to CD3) and tumor cells (tumor antigen). Blinatuzmab, the first approved BiTE, is approved for the treatment of B-ALL[84]. Upon prolonged stimulation, T cells become exhausted, and immune checkpoints such as; PD-1 and TIM-3 become upregulated after relapse upon allogenic hematopoietic stem cell transplant (Allo-HSCT). Treatment free interval gave T cells a chance to revert back from an exhausted state to a functioning state[48].

Effect of T cell exhaustion on chimeric antigen receptor (CAR) T cells

T cell antigen receptor or TCR is a surface receptor on T cells that is responsible for the recognition of antigens presented on major histocompatibility complex (MHC) by APC. TCR role is central for T cell function. Earlier, adoptive T cell transfer (ACT) was a center of focus for many academics in the field. It was performed by isolating tumor-specific T cells and expanding them *ex-vivo*, then reinfusing them back to patients. It became essential to redirect the lymphocyte specificity and function with the aid of chimeric antigen receptors (CARs). Chimeric antigen receptor **(**CAR) T cells are a class of engineered T cells with synthetic chimeric receptors that target specific antigens. They are designed to redirect the lymphocyte specificity and function for the treatment of cancer thus compensating for the diminished T cell function. There are 6 CARs targeting either CD19 or B-cell maturation antigen (BCMA) that are approved by the Food and Drug Administration (FDA) for the treatment of leukemias, lymphoma, and myelomas[85]. CD19-CARs can be potentially used for the treatment of refractory B-ALL patients[86,87]. However, the effect of cell exhaustion on CARs still needs to be thoroughly studied in this setting as it forms a major challenge to treatment. Multiple factors were attributed to the resistance to or limitation of therapy by CARs. Of these T cell-related factors including; altered frequency and distribution of lymphocytes, diminished early lineage cells[88], deviation away from early memory lymphocytes, deviation towards differentiated effector T cells, and aerobic glycolysis, all together with transitioning towards T cell exhaustion which all contributed to resistance to CAR T cell therapy of CLL[89]. Along with tumor microenvironment-related factors **(Fig. 1)** including, the duration of antigenic exposure which determines the extent of exhaustion and whether it can be reversed or not[90], upregulation of ICs, immune suppression by T regs[20-22], faulty signaling of death receptors by lymphoblasts in ALL via Fas-associated protein with death domain (FADD) and BH3 interacting-domain death agonist (BID) [91], leukemic extracellular vesicles (EVs) which drives T cells to a state of dysfunction and exhaustion in ALL and CLL[92] and upregulation of anti-apoptotic signaling proteins[93,94], including; members of B-cell lymphoma 2 (BCL-2) proteins which are chief regulators of apoptosis[95-97] such as myeloid leukemia 1(MCL-1) protein[98], their expression is higher in AML[99] and CLL[100], and resistance to chemotherapy associated with worse prognosis was reported.

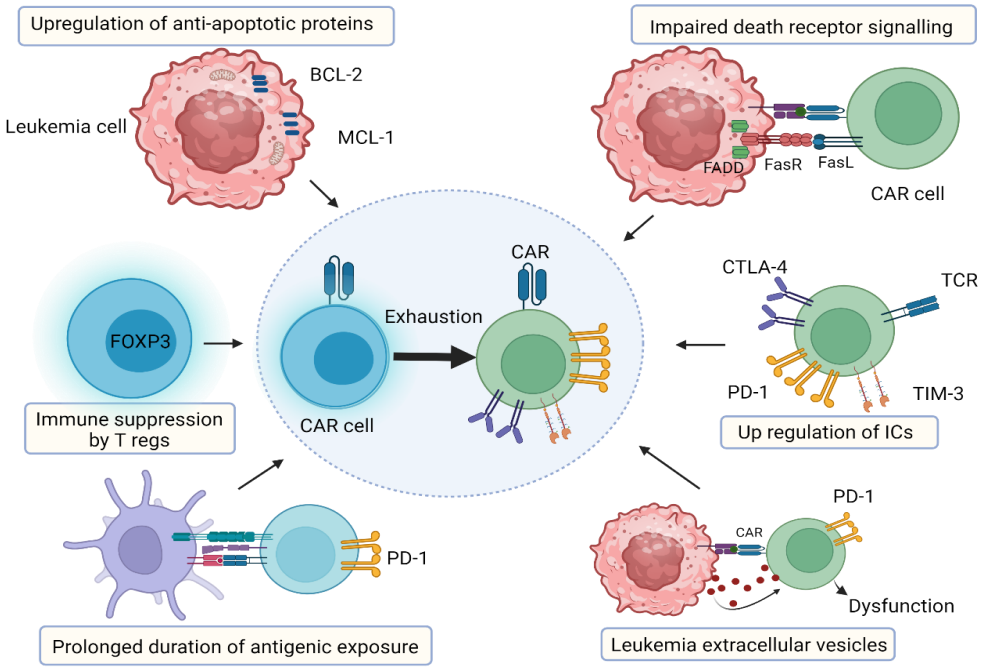

Fig. 1: Demonstrating tumor microenvironment-related mechanisms ultimately leading to CAR T cell dysfunction in leukemia: upregulation of anti-apoptotic proteins BCL-2 and MCL-1. Impaired death receptor signaling via the FasL/FasR/FADD/BID. Immune suppression by T regs. Upregulation of ICs such as PD-1, CTLA-4 and TIM-3. Prolonged duration of antigenic exposure and leukemia extracellular vesicles.

The role of transitioning away from memory cells becomes emphasized as CD19-CARs enter a state of exhaustion where $CD8^+$ CD19-CAR T cells undergo extensive DNA-methylation remodeling predominantly, by repressing memory-associated genes such as; TCF7 and LEF1 in ALL[101], which also were found over represented in CLL cases that had complete remission of the disease and poorly represented by those with worse prognosis[89] implying that exhaustion favors reduction in memory cell potential differentiation[41,102]. The transition of CARs to an exhausted state can be distinctively identified by the upregulation of motif CX3C chemokine receptor 1 (CX3CR1) and repression of Transcription Factor 7 (TCF7) together with lymphoid enhancer binding factor 1 (LEF1) in ALL[101].

Conclusion

Neutralizing T cell exhaustion is a game changer in cancer. Its impact on disease progression and therapy is substantial and detrimental in leukemia. Different therapeutic approaches are considered. Responses to disease and therapy are wide-ranging and could be linked to numerous factors related to patient, disease type, or disease etiology. Exhaustion in CAR T cell therapy should be thoroughly studied; mechanisms leading to exhaustion either related to T cells or tumor microenvironment should be investigated. Extensive research is urged to further understand the progressive process happening in leukemia and molecular mechanisms together with how it interacts with other immune players and immune events.

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استنفاد الخلايا التائية: التفوق فى التأثير في سرطان الدم بالنسبة للمرض والعلاج

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في هذه المراجعة، تم تقديم نظرة عامة على الخلايا التائية المنهكة في حالات سرطان الدم الحادة والمزمنة بما في ذلك سرطان الدم النقوي والليمفاوي، لتصفح هذه المجموعة الفرعية المهمة من الخلايا وتسليط الضوء على التعديلات المظهرية والوراثية والنسخية الظرفية. في نهاية المطاف، تؤثر هذه التغييرات على تطور سرطان الدم. بالإضافة إلى تفاعل مجموعة الخلايا التائية المنهكة مع الاستراتيجيات العلاجية القائمة على الخلايا التائية مثل الخلايا الليمفاوية المعدلة هندسيا لمستقبلات الخلايا التائية ، ومستقبلات المستضد الكيميري والتي ستؤثر وفقًا لذلك على النتائج العلاجية.