

## Review Article

# Regulatory T-cells in chronic lymphocytic leukemia: actor or innocent bystander?

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**Abstract:** Regulatory T (Treg) cells are now under extensive investigation in chronic lymphocytic leukemia (CLL). This small subset of T-cells has been, in fact, considered to be involved in the pathogenesis and progression of CLL. However, whether Treg dysregulation in CLL plays a key role or it rather represents a simple epiphenomenon is still matter of debate. In the former case, Treg cells could be appealing for targeting therapies. Finally, Treg cells have also been proposed as a prognostic indicator of the disease clinical course.

**Keywords:** Tregs, chronic lymphocytic leukemia, prognosis

## Introduction

It is well established and generally accepted that several innate and adaptive effector cells and molecules participate in the recognition and destruction of cancer cells. Although some host response may inhibit tumor growth and progression, the immune system can also promote cancer by provoking chronic inflammation and, in turn, elaborating factors that drive tumor growth, survival and angiogenesis [1, 2]. Natural and inducible regulatory T (Treg) cells are a subset of T-lymphocytes able to suppress immune responses by direct interaction with other immune cell types or through immunosuppressive cytokines; they appear crucial in maintaining immune homeostasis, mediating peripheral tolerance and preventing autoimmunity. Emerging evidences suggest that such Treg cells may also modulate host T-cell activity against tumor-associated antigens, thereby facilitating tumor escape from immunological control [3, 4].

Treg cells may be defined as CD4<sup>+</sup> T-cells expressing CD25 (IL2-receptor) at high levels,

cytoplasmic FoxP3, and very low to no CD127 (IL-7 receptor) on their surface [5-9]. Until today, several reports have been published about the elevated number of Treg cells in the peripheral blood of patients with solid tumors and hematologic malignancies as well, but the mechanisms driving Tregs expansion in cancer are not fully understood [10].

Chronic lymphocytic leukemia (CLL) is the most common form of leukemia in Western countries. It is characterized by the accumulation of monoclonal B-lymphocytes in bone marrow, lymphoid organs and peripheral blood [11, 12]. There are now evidences on the possible role of T-cells dysregulation in the pathogenesis and development also of CLL [13]. More recently, attention has been paid on the role of Treg cells [14]. An increased number of Treg cells in peripheral blood of patients with CLL has been reported by several authors in the last recent years [15-20]. Furthermore, a direct correlation between higher Tregs and more aggressive clinical-biological features of the disease, as well as with disease progression, has also been

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**Table 1.** Studies evaluating Treg cells in CLL

Authors	No. of patients studied	Flow-cytometric panel used	Correlation with clinical-biological features
Beyer et al <sup>15</sup>	73	CD4/CD25	Extended disease (Binet stage)
Giannopoulos et al <sup>16</sup>	80	CD4/CD25/FoxP3	Binet stage
Jak et al <sup>17</sup>	21	CD4/CD25/CD127	Not evaluated
D'Arena et al <sup>18</sup>	80	CD4/CD25/CD127	Rai stage, lymphocytosis, LDH
Weiss et al <sup>25</sup>	102	CD4/CD25/FoxP3	Unmutated IgVH, CD38, chromosomal aberrations*
Lee et al <sup>22</sup>	24	CD4/CD25/FoxP3	Not evaluated
Lad et al <sup>20</sup>	32	CD4/CD25/CD127	Binet stage, LDT, autoimmune cytopenias
Biancotto et al <sup>19</sup>	21	CD4/CD25/FoxP3	CD38/ZAP-70

All studies reported an increase number of circulating Treg cells in CLL patients with respect to healthy subjects. \*In this study the Authors observed an influence of Treg cells on the clinical course of the disease only when using their relative but not absolute number. LDT means lymphocyte doubling time.

described. Anyway, the pathogenetic role of Tregs in CLL is still under investigation.

Despite the fact that CLL is a cancer of the B-cell compartment of the immune system, a T-cell deregulation in this disorder is well known. It is generally thought that T-cell immune-surveillance of leukemic cells is derailed and that the increased number of Treg cells observed in CLL patients is directly correlated to the tumor burden. On the other hand, an elevated Treg cell number could result indirectly from intrinsically more aggressive CLL and, thus, be a consequence and not the cause of an aggressive disease or its progression. The elucidation of the mechanisms by which these cells traffic and accumulate in the tumor microenvironment could provide attractive therapeutic targets able to fight tumor-induced immune suppression.

### Treg cells and CLL

Increased circulating Treg cells have been found in untreated CLL patients with respect to healthy subjects in all published study (Table 1) [6-11]. However, as shown in Table 2, a great variability in the percentage of Treg cells has been observed. Generally, the proportion of Treg cells was found greater in CLL patients with respect to controls. Indeed, though two studies have shown a reduced percentage [9, 10], the absolute number of Tregs was always found to be significantly greater in CLL. These discrepancies may be probably due to the use of different markers employed to define Treg cells by means of flow cytometry (i.e. the use of an immunological gate on CD4+ cells combined only with CD25 or, alternatively, with CD127 and CD25 or CD25 and FoxP3).

Jak and co-workers [17] performed experiments aiming to investigate the mechanisms underlying the expression of Treg cells in CLL patients. They showed that in these patients Treg cells displayed the phenotype of primed (CD45RO+) cells. Furthermore, no evidence for a predominant tumor antigen driving Treg cell expansion was found. Furthermore, Treg cells of CLL patients were found more resistant to drug-induced apoptosis than Treg cells in normal controls, probably due to the fact that Bcl-2 levels of the former cells were significantly higher. Taken together, these data permit to speculate that Treg cells in CLL may accumulate both by increased formation, via CD27/CD70 interaction in the lymph node proliferation centers, and by decreased sensitivity to apoptosis [7, 8].

Piper and co-workers, using a [<sup>3</sup>H]-thymidine suppressor assay to assess their activity, showed that Treg cells in CLL patients retained their function and were not influenced by chemotherapy [21]. In addition, a normalization in their number was observed after treatment with fludarabine, despite an initial transient increase. The same phenomenon was reported by Lee et al. in 24 out of 60 patients with CLL in whom the detection of Treg cells was performed at study entry, after 3 and after 15 cycles of treatment with lenalidomide [22]. In particular, a significant increase of Treg cells was observed after 3 cycles of therapy, while a normalization was found after the treatment. These data suggest that lenalidomide is able to modulate cell-mediated immunity in patients with CLL.

Finally, we have recently evaluated Treg cell number in 'clinical' monoclonal B-cell lymphocytosis (MBL) [23]. In this condition, characterized by less than 5,000/μL circulating neoplas-

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**Table 2.** Percentage and absolute number of circulating Treg cells in CLL

Author	Mean age of patients (years)	Tregs (%)	Tregs/ $\mu$ L
Beyer et al <sup>15</sup>	61.2	10.4	-
Giannopoulos et al <sup>16</sup>	63	10.5	-
Jak et al <sup>17</sup>	62.9	0.65	-
D'Arena et al <sup>18</sup>	68.4	0.43	70.8
Weiss et al <sup>25</sup>	69.1	9.7	92
Lee et al <sup>22</sup>	Not reported	2.2	-
Lad et al <sup>20</sup>	64.2	0.98	581
Biancotto et al <sup>19</sup>	66.4	12.8	-

Only in three studies Treg cells were evaluated as absolute cell number ( $\mu$ L) also.

**Table 3.** Partial list of monoclonal antibodies used to inhibit/deplet Treg cells

Antibody	Specificity	Effect on Treg cells
PC61	CD25	Depletion
Daclizumab	CD25	Depletion
Ipilimumab	CTLA-4	Inhibition
Tremelimumab	CTLA-4	Inhibition
P60	FoxP3	Inhibition
Anti-GITR	GITR	Inhibition
Anti-OX40	OX40	Inhibition

Cytotoxic T-lymphocyte antigen-4 (CTL-4), Transcription factor forkhead/winged helix box P3 (FoxP3), Glucocorticoid-induced tumor necrosis factor receptor (GITR).

tic B-cells, in absence of other features of lymphoproliferative disorders, we showed a greater number of absolute Treg cells with respect to healthy subjects, number which was, however, lower in early stage CLL patients.

### Treg cell number as a prognosticator

Contradictory results regarding the prognostic value of the Treg cell number in peripheral blood and/or tumoral tissue of patients with cancer have been reported [24]. Furthermore, little is known on the prognostic role of the number of Treg cells in CLL patients, despite the fact that a general agreement is reported about the association of a higher number of such cells with a more advanced disease [14].

Firstly, Weiss and co-workers reported data suggesting that circulating Treg cell number may contribute to predict the need of treatment in low-intermediate risk CLL patients [25]. These authors divided patients into two groups, according to the percentage of circulating Treg cells (<9,7% and >9,7% of CD4+ T-cells); in this study a higher percentage of Treg cells was associated to a shorter time to first treatment. On the contrary, no significant distinction between the two groups was observed when the absolute number of Treg cells was considered.

Aiming to explore the prognostic role of Treg cells, we evaluated 75 patients with Rai stage 0 CLL, showing that the absolute cell number of Treg cells was able to identify patients with a shorter time to first treatment. The best predictive cut-off level of circulating Treg cell number was in the range from >40 to  $\geq$ 42/ $\mu$ L [26].

### Tregs and targeted therapy

There is accumulating evidence that Treg cells play a critical role in immune evasion mechanisms employed by tumor cells. The manipulation of Treg cells might be a useful tool to contribute to fight cancer.

It is well known that cyclophosphamide is able to induce a depletion of Treg cells [27] and that other conventional antitumor drugs, such as methotrexate, mitoxantrone and gemcitabine, also have modulatory effects on Treg cells. However, which is the best approach (inhibition or depletion?) to Treg cell manipulation is still not clear [28]. In light of this, Treg cells appear as an appealing target for immunotherapy [29, 30].

Several monoclonal antibodies targeting surface cell antigens have been investigated to induce depletion or inhibition of Treg cells [29, 31]. A partial list of these agents is reported in

**Table 3.** Among others, CD25-specific antibody PC61 was used *in vivo* in mice, showing to be able to suppress tumor growth [32, 33]. Daclizumab is another monoclonal antibody targeting CD25 on T-cell surface; it was used to treat patients with breast cancer, achieving contrasting results [34]. Hairy cell leukemia and other hematologic malignancies have been treated with immunotoxin targeting CD25+ T-cells [35]. Finally, ipilimumab and tremelimumab are monoclonal antibodies targeting CTL-4 antigen, a protein constitutively expressed on Treg cells with inhibitory activity on the immune system. They have been used to treat patients with prostate cancer and metastatic melanoma [36-38].

Of interest, the possibility of inducing autoimmune disorders has been reported as a possible consequence of Treg cells depletion in rodents and humans [39-41]. In CLL, a disease with a higher incidence of autoimmune disorders, this aspect appears to be particularly relevant in designing strategies of anticancer therapy with modulation of Treg cells.

### Conclusions

Treg cell number is higher in CLL patients than in age- and sex-matched healthy subjects and correlates with features of progressive disease. Moreover, it appears able to predict the time to first treatment in low-risk patients, thus emerging as a useful biomarker with prognostic power.

Given the role of Treg cells in the progression of cancer and in suppressing tumor-specific immunity, clinical strategies are developing to target such cells, aiming to reduce or to abrogate the antitumor suppression in the context of the treatment of CLL with chemotherapy and immunotherapy. The possible risk of autoimmune disorders must be taken into account in designing such specific strategies.

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