Hindawi
Journal of Immunology Research
Volume 2017, Article ID 1832968, 13 pages
https://doi.org/10.1155/2017/1832968

Review Article

Regulatory T Cells and Their Prognostic Relevance in Hematologic Malignancies

Giovanni D'Arena,[1] Candida Vitale,[2,3] Marta Coscia,[2,3] Agostino Festa,[4] Nicola Matteo Dario Di Minno,[5] Vincenzo De Feo,[6] Michele Caraglia,[4] Gioacchino Calapai,[7] Luca Laurenti,[8] Pellegrino Musto,[9] Giovanni Di Minno,[5] and Daniela Fenoglio[10]

[1]*Hematology and Stem Cell Transplantation Unit, IRCCS Cancer Referral Center of Basilicata, Rionero in Vulture, Italy*
[2]*Division of Hematology, AOU Città della Salute e della Scienza di Torino, University of Torino, Torino, Italy*
[3]*Department of Molecular Biotechnology and Health Sciences, University of Torino, Torino, Italy*
[4]*Dipartimento di Biochimica, Biofisica e Patologia Generale, University of Campania "Luigi Vanvitelli", Napoli, Italy*
[5]*Department of Clinical Medicine and Surgery, Regional Reference Centre for Coagulation Disorders, "Federico II" University, Napoli, Italy*
[6]*Department of Pharmacology, University of Salerno, Salerno, Italy*
[7]*Department of Biomedical and Dental Sciences and Morphological and Functional Sciences, University of Messina, Messina, Italy*
[8]*Hematology Institute, Catholic University of Sacred Heart, Rome, Italy*
[9]*Scientific Direction, IRCCS Cancer Referral Center of Basilicata, Rionero in Vulture, Italy*
[10]*Centre of Excellence for Biomedical Research, Department of Internal Medicine, IRCCS Azienda Ospedaliero Universitaria San Martino-IST-Istituto Nazionale per la Ricerca sul Cancro, University of Genoa, Genoa, Italy*

Correspondence should be addressed to Giovanni D'Arena; giovannidarena@libero.it

Received 17 August 2017; Accepted 14 November 2017; Published 21 December 2017

Academic Editor: Wenxin Zheng

Regulatory T cells (Tregs) have a fundamental function in monitoring the immune homeostasis in healthy individuals. In cancer and, in particular, in hematological malignancies, Tregs exert a major immunosuppressive activity, thus playing a critical role in tumor cell growth, proliferation, and survival. Here, we summarize published data on the prognostic significance of Tregs in hematological malignancies and show that they are highly conflicting. The heterogeneity of the experimental approaches that were used explains—at least in part—the discordant results reported by different groups that have investigated the role of Tregs in cancer. In fact, different tissues have been studied (i.e., peripheral blood, bone marrow, and lymph node), applying different methods (i.e., flow cytometry versus immunohistochemistry, whole blood versus isolated peripheral blood mononuclear cells versus depletion of $CD25^+$ cells, various panels of monoclonal antibodies, techniques of fixation and permeabilization, and gating strategies). This is of relevance in order to stress the need to apply standardized approaches in the study of Tregs in hematological malignancies and in cancer in general.

1. Introduction

Regulatory T cells (Tregs) constitute a small-size subpopulation of $CD4^+$ T cells, accounting for 1–4% of circulating $CD4^+$ lymphocyte in humans, specialized in suppressive functions that control unwanted immune responses not only toward self-antigens but also toward foreign antigens in the context of the immune tolerance [1].

Gershon and Kondo from Yale University first proposed the existence of $CD8^+$ T cells with suppressive activity more than 40 years ago [2]. However, after the initial great interest following this first report, due to the fact that a precise

definition of Tregs lacked for several years, no further advances in the study of this cell population were made for decades. In 1995, Sakaguchi and coworkers identified Tregs in mouse as $CD4^+$ T cells expressing surface interleukin-2 (IL-2) receptor α-chain (CD25) [3]. Baecher-Allan and coworkers, using flow cytometry and analyzing sorted cells *in vitro*, identified a very small subset of T cells with high expression of CD25 and regulatory function in humans [4]. However, CD25 is not exclusively restricted to Tregs, and its surface expression is also seen on effector T lymphocytes after activation [5]. The intracytoplasmic Forkhead helix box P3 (FoxP3), a transcription factor required for the development, maintenance, and function of Tregs was subsequently identified [6, 7]. The central role of this transcription factor is confirmed by the fact that a FoxP3 single gene mutation on the X chromosome induces in Scurfy mice a severe autoimmune/inflammatory disease. In humans, the same mutation causes a disease called IPEX (immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome), characterized by autoimmune manifestations in multiple endocrine organs, such as diabetes and thyroiditis, inflammatory bowel disease, and severe allergies [8]. Finally, the absence of the heterodimeric IL-7 receptor (CD127) combined with CD4, CD25, and FoxP3, has been shown to better identify Tregs, avoiding the contamination from other cell populations such as activated effector T cells [9, 10].

2. Regulatory T Cells and Prognostic Significance in Cancer

The role of Tregs in cancer appears to be relevant by promoting tumor progression and suppressing effective antitumor activity [11–13]. Overall, the large majority of studies report that the frequency and the suppressive function of Tregs are increased in cancer patients as compared to healthy subjects. However, some issues are still a matter of debate, in particular the prognostic significance of this cell subpopulation. In general, Tregs predict poor outcome in cancer patients [12], but some reports have shown that higher Treg numbers and preserved activity are associated with a better prognosis [14–16].

This review stems from the need to reassess the topic of prognostic relevance of Tregs in cancer, focusing on patients with hematologic malignancies. For this purpose, we reviewed a large body of published papers conducting a PubMed literature search (keywords: Regulatory T cells, Hodgkin lymphoma, non-Hodgkin lymphoma, chronic lymphocytic leukemia, chronic myeloid leukemia, acute lymphoblastic leukemia, acute myeloid leukemia, multiple myeloma, monoclonal gammopathies, myelofibrosis, essential thrombocythemia, polycythemia vera, and Ph1-negative chronic myeloproliferative neoplasms).

3. Regulatory T Cells in Chronic Lymphocytic Leukemia

The accumulation of monoclonal B lymphocytes in the bone marrow, lymphoid organs, and peripheral blood is the hallmark of chronic lymphocytic leukemia (CLL), the most common form of leukemia in Western countries [17]. The importance of T cell dysregulation in the pathogenesis and development of CLL is now well established [18, 19], and in this setting, the role of Tregs has also been investigated [20, 21]. As shown in Table 1, several authors reported data on Tregs in CLL showing in the majority of cases an expansion of this population [22–31]. In addition, a correlation between higher Treg numbers and more aggressive clinical-biological features and adverse prognosis of CLL has been described.

As previously discussed [20], the reported percentage of Tregs in CLL is highly variable. According to the majority of reports, the percentage of Tregs is higher in CLL patients than in normal controls, and when the absolute number is considered, Tregs are always found to be significantly greater in CLL as compared to healthy donors.

Interestingly, based on their experimental work, Jak et al. speculated that the accumulation of Tregs in CLL is due to an increased proliferation induced by CD27/CD70 interaction in the lymph node proliferation centers and to a decreased sensitivity to apoptosis [22].

Dasgupta et al. tried to establish an optimal threshold level for prognostic purpose [28]. The cut-off was assessed by receiver operating characteristic (ROC) analysis. A cut-off of 5.7% and 35 cells/μL for percentage and absolute Treg count, respectively, were determined as optimal in patients with CLL, along with a median Tregs percentage of 15.5% used to separate low- and high-risk patients. Using the same approach in the setting of Rai stage 0 CLL patients, our group found that the absolute number of Tregs was an independent predictor of time to the first treatment, with the best predictive cut-off being 41 cells/μL [24]. Overall, these data show that the absolute Treg number is able to identify Rai stage 0 CLL patients at higher risk of requiring therapy.

Rissiek et al., applying a multidimensional scaling analysis to assess the composition of the circulating T cell populations, generated T cell scores showing that suppressive T cell profiles emerge early during monoclonal B cell lymphocytosis (MBL), the well-recognized pre-CLL stage [31–33]. As the disease evolves from MBL to overt and advanced CLL, specific sequential changes in T cells appear, progressively compromising the effector T cells function and contributing to disease progression [30].

In our hands too, the absolute number of Tregs in MBL patients was lower compared to CLL patients, but slightly higher than healthy controls [30]. In addition, the absolute Treg cell number directly correlated with more advanced CLL clinical stages and higher circulating B cell numbers. Of note, the absolute number of Tregs was lower in MBL patients as compared to early-stage CLL patients (0/A according to Rai/Binet stage). In summary, Treg numbers increase gradually from normal subjects to "clinical" MBL patients and are significantly higher in CLL patients as compared to MBL patients.

Regarding the functional properties, some authors reported a reduced inhibitory function of Tregs in CLL [27, 34]. On the contrary, Piper et al. showed that in CLL patients Tregs retain their function and are not influenced by chemotherapy [35]. A correlation between a higher circulating Treg numbers and dysfunctional Vγ9Vδ2 T cells

Table 1: Most relevant published studies investigating frequency, function, and prognostic significance of Tregs in CLL.

Reference	Patients/controls evaluated	Samples tested	Marker panel used in Treg evaluation by flow cytometry	Treg frequency	Functional studies	Impact on prognosis
Beyer et al. [34]	CLL/controls	PB	CD4/CD25	Increased*	Reduced inhibitory function	Extended disease (Binet stage)
Giannopoulos et al. [73]	CLL/controls	PB	CD4/CD25/FoxP3	Increased	Not performed	Binet stage
Jak et al. [22]	CLL/controls	PB	CD4/CD25/CD127	Increased	More resistant to drug-induced apoptosis than controls	Not evaluated
D'Arena et al. [23, 24]	CLL/controls	PB	CD4/CD25/CD127	Increased with a gradual variation from normal subjects to clinical MBL to CLL	Not performed	Rai stage, lymphocytosis, LDH, first time to treatment
Weiss et al. [25]	CLL/controls	PB	CD4/CD25/FoxP3	Increased	Not performed	Unmutated IgVH, CD38, chromosomal aberrations
Lad et al. [26]	CLL/controls	PB and FNA	CD4/CD25/CD127/IL-10	Reduced both Treg and IL-10 expressing Treg; higher absolute number	Not performed	Correlation with LDT (Tregs but not $CD45RA^+$ Tregs and $CD8^+$ Tregs were lower in $CD38^+ZAP70^+$ CLL group (with respect to $CD38^-ZAP70^-$)
Biancotto et al. [27]	CLL/controls	PB	CD4/CD25/FoxP3	Increased	Slightly reduced suppressive activity	Correlation with ZAP-70 and CD38 expression
Dasgupta et al. [28]	CLL/controls	PB	CD4/CD25/CD127/FoxP3	Increased	Not performed	Correlated with ZAP70 and CD38 expression
Mpakou et al. [29]	CLL/controls	PB	CD4/CD25/CD127	Increased	Suppression of T effector cells	Advanced stage
D'Arena et al. [30]	Clinical MBL/ CLL/controls	PB	CD4/CD25/CD127	Reduced as % but increased as absolute number with a gradual variation from normal subjects to clinical MBL to CLL	Not performed	
Rissiek et al. [31]	MBL/CLL/controls	PB	CD4/CD25/CD127/CD39	Expansion. Highly suppressive $CD39^+$ Treg subset increased in all disease stages	Increasingly suppressive regulatory function initiating at MBL stage; effector function impairment after transition to CLL; partially recovered after chemo-immunotherapy	Shorter time to treatment

*Increased at diagnosis; significantly reduced after fludarabine therapy. CLL: chronic lymphocytic leukemia; MBL: monoclonal B cell lymphocytosis; PB: peripheral blood; FNA: fine needle aspiration; LDT: lymphocyte doubling time.

in untreated CLL patients was also shown, thus corroborating the hypothesis that Tregs may not be only bystanders but have a functional role in this setting [36].

A normalization in Treg number was observed after fludarabine therapy [34], and also in CLL patients treated with lenalidomide, suggesting that such drugs are able to modulate cell-mediated immunity in CLL [37].

Finally, we also tested the ability of green tea, a popular beverage in China, Japan, and increasingly used in Western countries, to modulate Treg number in peripheral blood of CLL patients in the early phases of the disease, for which at the present time there is no effective intervention and a "wait and see" policy is generally adopted [38, 39]. We showed that the B cell lymphocyte count and the absolute circulating Treg number were reduced after 6-month consumption of oral green tea extract, suggesting that this compound can modulate circulating Tregs in CLL patients with early stage of disease and delay disease progression.

4. Regulatory T Cells in Lymphomas and Monoclonal Gammopathies

The neoplastic lymph nodes in Hodgkin lymphoma (HL) and non-Hodgkin lymphoma (NHL) contain not only neoplastic B cells but also nontumoral T cells, macrophages, and dendritic cells, constituting the so-called tumor microenvironment. The importance of the microenvironment in the pathogenesis and progression of lymphomas is still a matter of debate and many studies have focused on the role of its different components, including Tregs. Tregs are increased in lymphoma tissues and are able to inhibit cytotoxic $CD8^+$ T cells exposed to lymphoma B cells [40].

Marshall et al. showed that HL-infiltrating lymphocytes are highly enriched in Tregs, which induce a profoundly immunosuppressive environment [41]. This was confirmed by Schreck et al. who demonstrated that in classical HL the microenvironment is dominated by Th2 cells and Tregs [42]. Moreover, a high ratio of Tregs over Th2 cells resulted in a significantly shortened disease-free survival.

However, conflicting results have been reported regarding the prognostic significance of Tregs infiltration in both HL and NHL. In fact, whereas in follicular lymphoma (FL), the most common form of low-grade NHL, germinal center (GC) diffuse large B cell lymphomas (DLBCL), and HL, an intrafollicular infiltration of Tregs, has a positive prognostic significance; this is not true in the case of non-GC-type DLBCL [43]. Moreover, as shown in Table 2, in some reports, a higher number of Tregs correlates with a good prognosis, while in other, it does not [43–49]. Of interest, Kim et al. evaluating Tregs on node biopsy of extranodal natural killer/T cell lymphomas showed that patients with poor performance status and with non-upper aerodigestive tract had a decreased number of Tregs ($<50/0.40\,mm^2$), while an increased number ($>50/0.40\,mm^2$) was associated with prolonged overall survival and progression-free survival [48]. Finally, Carreras et al. reported that the median Treg number in patients with FL at diagnosis had a median cell percentage of 10.5% [49]. Furthermore, patients were classified as having Tregs > 10%, 5–10%, and <5% with a 5-year overall survival of 80%, 74%, and 50%, respectively. Patients with transformed DLBCL showed lower Treg number with respect to patients with grades 1–3 FL.

Regarding the frequency and prognostic significance of Tregs, conflicting results have also been obtained in the field of monoclonal gammopathies (Table 3). In some reports, Tregs were found to be increased in frequency, while in others they were reduced or comparable with respect to healthy subjects [50]. Again, some authors reported a correlation with tumour burden and with worse prognosis, but this was not consistent among different publications [50–57]. We recently published our data on the flow cytometric evaluation of Tregs in multiple myeloma (MM) and monoclonal gammopathies of undetermined significance (MGUS) [51]. We found no differences in Treg frequency in MM and MGUS with respect to normal controls, and no correlations with main clinical and laboratory features in this disease setting were observed.

5. Regulatory T Cells in Acute Leukemias, Chronic Myeloid Leukemia, and Ph1-Negative Chronic Myeloproliferative Neoplasms

Few studies have been published regarding the role of Tregs in acute myeloid and lymphoid leukemias (Table 4) [58–61]. In a study by Bhattacharya et al., an increased number of Tregs was found in patients with B cell acute lymphoblastic leukemia (B-ALL), and a correlation with disease progression was highlighted [58].

Regarding chronic myeloid leukemia (CML), an interesting paper has been published by Zahran and Badrawy, in which Tregs were found increased in the peripheral blood of affected individuals as compared to controls. Moreover, Tregs frequency correlated with the level of BCR/ABL, basophil number, blast cell count, and Sokal score, and Treg number was higher in accelerated and blastic phase with respect to chronic phase [62]. Of note, Treg frequency declined after therapy with imatinib. Rojas et al. found a lower Treg number in patients who achieved a complete cytogenetic response [63], while higher Treg frequencies were found after stem cell transplant compared to normal controls and newly diagnosed patients [64]. Finally, the correlations with Sokal score and basophil number were validated by other studies [65, 66], whereas the impact of treatment has not been confirmed, since no changes in Treg frequency was observed after 6 months of tyrosine kinase inhibitors therapy [65]. Table 5 summarizes the results of studies analyzing Tregs in CML.

Hasselbalch et al. studied patients with Ph1-negative chronic myeloproliferative neoplasms and found that circulating Tregs were significantly expanded in patients treated with IFN-α2 with respect to healthy donors and in patients treated with hydroxyurea [66]. Kovacsovics-Bankowski et al. analyzed patients with polycythemia vera (PV) and essential thrombocythemia (ET) and found increased numbers of circulating Tregs and an enrichment in highly suppressive subsets (defined as $CD39^+/HLA\text{-}DR^+$) in patients treated with PegIFN-α with respect to those treated with hydroxyurea [67].

Table 2: Most relevant published studies investigating the frequency and the prognostic significance of Tregs in lymphomas.

Reference	Patients/controls evaluated	Samples tested	Marker panel used in Treg evaluation by flow cytometry	Treg frequency	Functional studies	Impact on prognosis
Tzankov et al. [43]	Lymphomas	Node biopsy	FoxP3 (IHC)	Increased	Not performed	Correlation with disease-specific and failure-free survival in FL and disease-specific survival in germinal center-like DLBCL and OS and failure-free survival in classical HD, but negative prognostic effect in nongerminal center DLBCL. Independent prognostic significance for failure-free survival in classical HD and of borderline significance for OS in classical HD and disease-specific survival in germinal center-like and nongerminal center DLBCL
Alvaro et al. [44]	Classical HL	Node biopsy	FoxP3 (IHC)	Not reported	Not performed	Small number influenced negatively EFS and DFS
Schreck et al. [42]	Classical HL/hyperplastic tonsils	Node biopsy	FoxP3 (IHC)	Increased	Not performed	Increased DFS and EFS; high Tregs/Th2 ratio correlated with shortened DFS
Garcia et al. [45]	Gastric MALT lymphoma	Gastric biopsy	FoxP3 (IHC)	Increased with respect to DLBCL but similar to chronic gastritis	Not performed	Higher number correlated with response to antibacterial eradication therapy
Koreishi et al. [47]	Relapsed/refractory HL	Node biopsy	FoxP3 (tissue microarray)	Not reported	Not performed	Lower Tregs correlated with poor OS
Chang et al. [46]	DLBCL/normal	Node biopsy	$CD4^+$ $CD25^+$	Increased	Not performed	Higher with poor survival and IPI
Kim et al. [48]	Extranodal natural killer/T cell lymphoma	Node biopsy	FoxP3 (IHC)	Heterogenous expression	Not performed	The decreased number of Tregs was more common in patients with poor performance status or in those presented in nonupper aerodigestive tract. Patients with increased numbers of Tregs showed prolonged OS and PFS.
Carreras et al. [49]	FL at diagnosis and relapse	Node biopsy	FoxP3 (IHC)	Not reported	Not performed	Inversely correlated with OS. Patients with very low numbers of Tregs (<5%) presented more frequently with refractory disease. No correlation with FLIPI. Patients with transformed DLBCL had lower Treg percentages than FL grades 1, 2, or 3

HL: Hodgkin's lymphoma; FL: follicular lymphoma; DLBCL: diffuse large B cell lymphoma; MALT: mucosa-associated lymphoid tissue; IHC: immunohistochemistry; OS: overall survival; DFS: disease-free survival, EFS: event-free survival.

Table 3: Most relevant published studies investigating the frequency and the prognostic significance of Tregs in monoclonal gammopathies.

Reference	Patients/controls evaluated	Samples tested	Marker panel used in Treg evaluation by flow cytometry	Treg frequency	Functional studies	Impact on prognosis
Prabhala et al. [50]	MGUS/MM/controls	PBMC	CD4/FoxP3	Decreased	Unable to suppress anti-CD3-mediated T cell proliferation	Not evaluated
Beyer et al. [52]	MGUS/MM/controls	PBMC	CD4/CD25/FoxP3 (% of $CD4^+$ cells)	Increased in MM versus MGUS (trend without statistical significance)	Strong inhibitory function	Not evaluated
Feyler et al. [53]	MGUS/MM/controls	PBMC and BM	CD4/CD25/FoxP3	Increased in PBMC but not in BM	Not evaluated	Correlation with disease burden (paraprotein)
Gupta et al. [54]	MM	PBMC	CD4/CD25/CD127/ FoxP3 (% of $CD4^+$ cells)	Reduced in untreated which increased after treatment with lenalidomide	Able to inhibit proliferation of $CD4^+CD25^-$ T cells	Increase of Tregs in responding patients to therapy; decrease correlation with ISS I + II
Muthu Raja et al. [55]	MGUS/SMM/MM	PB/BM whole	CD4/CD25/CD127/ CD45RA (% of $CD4^+$ cells)	Increased in MM but not in SMM and MGUS	Able to inhibit proliferation of $CD4^+$ T cells and the secretion of IFN-γ	Correlation with adverse clinical features (hypercalcemia, lower normal PC, and IgA subtype); no correlation with ISS; predict time to progression; MM patients with ≥5% of Tregs had inferior time to progression
Giannopulos et al. [56]	MM/controls	PBMC	CD4/CD25/FoxP3	Increased	Not evaluated	Correlation with shorter overall survival
Foglietta et al. [57]	MM/MGUS/controls	Fresh PB and frozen BM	CD4/CD25/FoxP3	Similar	Effective suppressor function	No correlation with the pattern of BM infiltration
D'Arena et al. [51]	MM/MGUS/controls	PB whole	CD4/CD25/CD127 (% and absolute number)	Similar	Effective suppressor function	No correlation with laboratory and clinical variables; no correlation with outcome

MM: multiple myeloma; MGUS: monoclonal gammopathy of uncertain significance; SMM: smoldering multiple myeloma; ISS: international staging system; PB: peripheral blood; PBMC; peripheral blood mononuclear cells; BM: bone marrow.

Table 4: Most relevant published studies investigating the frequency and the prognostic significance of Tregs in acute leukemias.

Reference	Patients/controls evaluated	Samples tested	Marker panel used in Treg evaluation by flow cytometry	Treg frequency	Functional studies	Impact on prognosis
Bhattacharya et al. [58]	B-ALL	PBMC /BM	CD4/CD25/CD127/FoxP3	Decreased	Higher suppressive capability on $CD4^+CD25^-$ regulatory T cells than controls	Increased frequency with disease progression
Wu et al. [59]	B-ALL/T-ALL/ controls	PB	CD4/CD25	Higher	Not performed	Not evaluated
Wang et al. [60]	AML/controls	PBMC /BM	CD4/CD25	Higher	Inhibition of proliferation and cytokine production (IL2, IFN-γ) of $CD4^+CD25^-$ T cells; improved IL-10 production under coculture of both subsets with stimulation	Not evaluated
Idris et al. [61]	B-ALL/controls	PB and BM	CD4/CD25/CD127	Increased	Not performed	Correlation with age

ALL: acute lymphoblastic leukemia; AML: acute myeloid leukemia; PB: peripheral blood; BM: bone marrow; PBMC: peripheral blood mononuclear cells; IL: interleukin; IFN: interferon.

Table 5: Most relevant published studies investigating the frequency and the prognostic significance of Tregs in chronic myeloid leukemia.

Reference	Patients/controls evaluated	Samples tested	Marker panel used in Treg evaluation by flow cytometry	Treg frequency	Functional studies	Impact on prognosis
Zahran and Badrawy [62]	CML/controls	PB	CD4/CD25/FoxP3	Increased	Not performed	Correlations with the level of BCR/ABL, basophils and blast cells. Significantly higher in accelerated phase and blastic phase than in chronic phase and with high Sokal score. Reduction of Tregs after therapy with IM
Hus et al. [65]	CP CML/controls	PB	CD4/CD25/FoxP3	Increased	Not performed	Correlation with higher basophiles. No change in frequency after 6 months of TKI inhibitors
Bachy et al. [74]	CP CML/controls	CD4+ enriched PBMC cells	CD4/CD25/ CD127/FoxP3	Increased in PB. Increased in BM of patients on IM compared to healthy volunteers.	No difference in inhibition	Correlation with Sokal risk score
Rojas et al. [63]	CP CML/controls	PBMC	CD4/CD25/ CD127/CD62L/ FoxP3	Lower in patients in complete cytogenetic response	Enhanced proliferative response to purified protein derivative	Not evaluated
Nadal et al. [64]	CP CML/controls	PBMC	CD4/CD25/ CD127/FoxP3/ CTLA-4	Higher frequencies after transplant than normal controls and newly diagnosed patients	Purified Tregs from SCT patients had a more potent suppressive activity than those from healthy volunteers	Not evaluated

CP: chronic phase; BM: bone marrow; IM: imatinib; PB: peripheral blood; PBMC; peripheral blood mononuclear cells; BM: bone marrow; SCT: stem cell transplant; IM: imatinib; TKI: tyrosine kinase inhibitor.

Table 6: Most relevant published studies investigating the frequency and the prognostic significance of Tregs in Ph1-negative chronic myeloproliferative neoplasms.

Reference	Patients/controls evaluated	Samples tested	Marker panel used in Treg evaluation by flow cytometry	Treg frequency	Functional studies	Impact on prognosis
Hasselbalch et al. [66]	PV ET PMF Controls	PBMC	CD4/CD25/CD127	Not increased	Inhibitory activity preserved	Marked expansion of Tregs in patients treated with IFN-α2 with than treated with hydroxyurea
Kovacsovics-Bankowski et al. [67]	PV ET	PB	CD4/CD25/FoxP3/Ki-67	Not reported	Not performed	Tregs (including highly suppressive $CD39^+$ $HLA\text{-}DR^+$) increase in patients treated with PegINFα
Massa et al. [68, 69]	PMF	PB	CD4/CD25/CD127/FoxP3	Reduced	Not performed	In patients with CALR mutation genotype association with longer disease duration and hemoglobin concentration

PV: polycythemia vera; ET: essential thrombocythemia; PMF: primary myelofibrosis; PB: peripheral blood; PBMC: peripheral blood mononuclear cells; CALR: calreticulin; IFN: interferon.

Moreover, molecular nonresponder patients showed a trend towards increased frequency of Tregs compared to responder patients, but no changes were observed in terms of absolute numbers of Tregs. Overall, a positive correlation between proliferating Tregs (Ki-67$^+$), highly suppressive Tregs (CD39$^+$/HLA-DR$^+$), and JAK2^{V617F} allelic burden was found, thus suggesting that the lack of ability of PegIFN-α treatment to decrease circulating Tregs predicts a poor molecular response.

Primary myelofibrosis (PMF) is a clonal disease of the hematopoietic stem cell characterized by a variable degree of bone marrow fibrosis, splenomegaly, and an increased risk of leukemic transformation. Contradictory data about Tregs in PMF have been published (Table 6). Massa et al. reported a reduced frequency and absolute number of Tregs in PMF than in normal controls [68]. No association with clinical-biological features of the disease was found, but a correlation between reduced Treg frequency and longer disease duration in patients with CALR mutation genotype was described. In these patients, a higher Treg frequency is significantly associated with advanced disease, higher IPSS/DIPSS score, and lower hemoglobin concentration. The same authors later documented the effect of ruxolitinib on Treg frequency, showing that the treatment with this small-molecule JAK1/2 inhibitor leads to a profound and long-lasting reduction in the frequency of circulating Tregs [69]. Wang et al. found no significant differences in the number of Tregs in patients with primary or post-ET myelofibrosis [70]. However, they reported that ruxolitinib significantly inhibits the release of sIL2-Rα, an inflammatory cytokine produced by Tregs, contributing to the clinical improvement of constitutional symptoms induced by the drug. These data have been further confirmed by an *in vitro* study in which the JAK1/2 inhibition by ruxolitinib was able to prevent Treg differentiation [71]. Table 6 summarizes the results of studies analyzing Tregs in Ph1-negative chronic myeloproliferative neoplasms.

6. Conclusions

Tregs have a fundamental function in maintaining the immune homeostasis in healthy individuals. In cancer and in particular in hematological malignancies, Tregs exert a major immunosuppressive activity, thus playing a critical role in tumor cell growth, proliferation, and survival. Published data on the prognostic significance of the Treg number in hematological malignancies show conflicting results. In our opinion, this variability reported by different groups is most likely explained by the heterogeneity of the experimental approaches that are used. In fact, different tissues have been studied (i.e., peripheral blood, bone marrow, and lymph node) and different analytic methodologies have been applied (i.e., flow cytometry versus immunohistochemistry). Moreover, while some authors studied the whole blood compartment, others evaluated the Treg population in isolated peripheral blood mononuclear cells or in a CD25-depleted subpopulation. Finally, various panels of markers, different techniques of fixation and permeabilization, and several gating strategies have been applied. This is of relevance to stress the need to apply standardized approaches in the study of Tregs in hematological malignancies and in cancer in general.

In perspective, in light of the increasing evidence of the important role of Tregs in immune evasion mechanism exerted by tumor cells, therapeutic interventions targeting intratumoral Treg infiltrates may be conceived in order to fight cancer. Treg inhibition or depletion, the latter uses monoclonal antibodies targeting surface antigens on Tregs such as CD25, is currently under investigation [72].

Conflicts of Interest

The authors declare that they have no conflicts of interest.

References

[1] S. Sakaguchi, K. Wing, and M. Miyara, "Regulatory T cells – a brief history and perspective," *European Journal of Immunology*, vol. 37, Supplement 1, pp. S116–S123, 2007.

[2] R. K. Gershon and K. Kondo, "Cell interactions in the induction of tolerance: the role of thymic lymphocytes," *Immunology*, vol. 18, no. 5, pp. 723–737, 1970.

[3] S. Sakaguchi, N. Sakaguchi, M. Asano, M. Itoh, and M. Toda, "Immunologic self-tolerance maintained by activated T cells expressing IL-2 receptor α-chain (CD25). breakdown of a single mechanism of self-tolerance causes various autoimmune disease," *Journal of Immunology*, vol. 155, pp. 1151–1164, 1995.

[4] C. Baecher-Allan, J. A. Brown, G. J. Freeman, and D. A. Hafler, "CD4$^+$CD25^{high} regulatory cells in human peripheral blood," *Journal of Immunology*, vol. 167, no. 3, pp. 1245–1253, 2001.

[5] R. J. Robb, A. Munck, and K. A. Smith, "T cell growth factor receptors. Quantitation, specificity, and biological relevance," *Journal of Experimental Medicine*, vol. 154, no. 5, pp. 1455–1474, 1981.

[6] S. Hori, T. Numura, and S. Sakaguchi, "Control of regulatory T cell development by the transcription factor *FoxP3*," *Science*, vol. 299, no. 5609, pp. 1057–1061, 2003.

[7] J. D. Fontenot, M. A. Gavin, and A. Y. Rudensky, "FoxP3 programs the development and function of CD4$^+$CD25$^+$ regulatory T cells," *Nature Immunology*, vol. 4, no. 4, pp. 330–336, 2003.

[8] C. L. Bennett, J. Christie, F. Ramsdell et al., "The immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome (IPEX) is caused by mutations of FOXP3," *Nature Genetics*, vol. 27, no. 1, pp. 20-21, 2001.

[9] N. Seddiki, B. Santner-Nanan, J. Martinson et al., "Expression of interleukin (IL)-2 and IL-7 receptors discriminate between human regulatory and activated T cells," *Journal of Experimental Medicine*, vol. 203, no. 7, pp. 1693–1700, 2006.

[10] W. Liu, A. L. Putnam, Z. Xu-Yu et al., "CD127 expression inversely correlates with FoxP3 and suppressive function of human CD4$^+$ Treg cells," *Journal of Experimental Medicine*, vol. 203, pp. 1701–1711, 2006.

[11] M. Beyer and J. L. Schultze, "Regulatory T cells in cancer," *Blood*, vol. 108, no. 3, pp. 804–811, 2006.

[12] T. L. Whiteside, "The role of regulatory T cells in cancer immunology," *ImmunoTargets and Therapy*, vol. 4, pp. 159–171, 2015.

[13] Y. Takeuchi and H. Nishikawa, "Roles of regulatory T cells in cancer immunity," *International Immunology*, vol. 28, no. 8, pp. 401–409, 2016.

[14] J. Wang and X. Y. Ke, "The Four types of Tregs in malignant lymphomas," *Journal Hematological & Oncology*, vol. 4, p. 50, 2011.

[15] P. Farinha, A. Al-Tourah, K. Gill, R. Klasa, J. M. Connors, and R. D. Gascoyne, "The architectural pattern of FOXP3-positive T cells in follicular lymphoma is an independent predictor of survival and histologic transformation," *Blood*, vol. 115, no. 2, pp. 289–295, 2010.

[16] R. Droeser, I. Zlobec, E. Kilic et al., "Differential pattern and prognostic significance of $CD4^+$, $FOXP3^+$ and IL-7^+ tumor infiltrating lymphocytes in ductal and lobular breast cancers," *BMC Cancer*, vol. 12, no. 1, p. 134, 2012.

[17] N. Chiorazzi, K. R. Rai, and M. Ferrarini, "Chronic lymphocytic leukemia," *New England Journal of Medicine*, vol. 352, no. 8, pp. 804–815, 2005.

[18] F. Forconi and P. Moss, "Perturbation of the normal immune system in patients with CLL," *Blood*, vol. 126, no. 5, pp. 573–581, 2015.

[19] M. E. Wallace, M. B. Alcantara, M. Yosuke, G. Kannourakis, and S. P. Berzins, "An emerging role for immune regulatory subsets in chronic lymphocytic leukaemia," *International Immunopharmacology*, vol. 28, no. 2, pp. 897–900, 2015.

[20] G. D'Arena, V. Simeon, F. D'Auria et al., "Regulatory T-cells in chronic lymphocytic leukemia: actor or innocent bystander?," *American Journal of Blood Research*, vol. 3, no. 1, pp. 52–57, 2013.

[21] G. D'Arena, G. Rossi, B. Vannata et al., "Regulatory T-cells in chronic lymphocytic leukemia and autoimmune diseases," *Mediterranean Journal of Hematology and Infectious Diseases*, vol. 4, no. 1, article e2012053, 2012.

[22] M. Jak, R. Mous, E. B. Remmerswaal et al., "Enhanced formation and survival of $CD4^+$ $CD25^{hi}$ $FoxP3^+$ T-cells in chronic lymphocytic leukemia," *Leukemia & Lymphoma*, vol. 50, no. 5, pp. 788–801, 2009.

[23] G. D'Arena, L. Laurenti, M. M. Minervini et al., "Regulatory T-cell number is increased in chronic lymphocytic leukemia patients and correlates with progressive disease," *Leukemia Research*, vol. 35, no. 3, pp. 363–368, 2011.

[24] G. D'Arena, F. D'Auria, V. Simeon et al., "A shorter time to the first treatment may be predicted by the absolute number of regulatory T-cells in patients with Rai stage 0 chronic lymphocytic leukemia," *American Journal of Hematology*, vol. 87, no. 6, pp. 628–631, 2012.

[25] L. Weiss, T. Melchardt, A. Egle, C. Grabmer, R. Greil, and I. Tinhofer, "Regulatory T cells predict the time to initial treatment in early stage chronic lymphocytic leukemia," *Cancer*, vol. 117, no. 10, pp. 2163–2169, 2011.

[26] D. P. Lad, S. Varma, N. Varma, M. U. Sachdeva, P. Bose, and P. Malhotra, "Regulatory T-cells in B-cell chronic lymphocytic leukemia: their role in disease progression and autoimmune cytopenias," *Leukemia and Lymphoma*, vol. 54, pp. 1012–1019, 2013.

[27] A. Biancotto, P. K. Dagur, J. C. Fuchs, A. Wiestner, C. B. Bagwell, and J. P. McCoy Jr., "Phenotypic complexity of T regulatory subsets in patients with B-chronic lymphocytic leukemia," *Modern Pathology*, vol. 25, no. 2, pp. 246–259, 2012.

[28] A. Dasgupta, M. Mahapatra, and R. Saxena, "A study for proposal of use of regulatory T cells as a prognostic marker and establishing an optimal threshold level for their expression in chronic lymphocytic leukemia," *Leukemia & Lymphoma*, vol. 56, no. 6, pp. 1831–1838, 2015.

[29] V. E. Mpakou, H. D. Ioannidou, E. Konsta et al., "Quantitative and qualitative analysis of regulatory T cells in B cell chronic lymphocytic leukemia," *Leukemia Research*, vol. 60, pp. 74–81, 2017.

[30] G. D'Arena, G. Rossi, M. M. Minervini et al., "Circulating regulatory T cells in "clinical" monoclonal B-cell lymphocytosis," *International Journal of Immunopathology and Pharmacology*, vol. 24, no. 4, pp. 915–923, 2011.

[31] A. Rissiek, C. Schulze, U. Bacher et al., "Multidimensional scaling analysis identifies pathological and prognostically relevant profiles of circulating T-cells in chronic lymphocytic leukemia," *International Journal Cancer*, vol. 135, pp. 2370–2379, 2014.

[32] D. Rossi, E. C. Sozzi, A. Puma et al., "The prognosis of clinical monoclonal B cell lymphocytosis differs from prognosis of Rai 0 chronic lymphocytic leukemia and is recapitulated by biological risk factors," *British Journal of Haematology*, vol. 146, no. 1, pp. 64–75, 2009.

[33] G. D'Arena and P. Musto, "Monoclonal B-cell lymphocytosis," *Translational Medicine*, vol. 8, pp. 75–79, 2014.

[34] M. Beyer, M. Kochanek, K. Darabi et al., "Reduced frequencies and suppressive function of $CD4^+CD25^{hi}$ regulatory T cells in patients with chronic lymphocytic leukemia after therapy with fludarabine," *Blood*, vol. 106, no. 6, pp. 2018–2025, 2005.

[35] K. P. Piper, M. Karanth, A. McLarnon et al., "Chronic lymphocytic leukaemia cells drive the global $CD4^+$ T cell repertoire towards a regulatory phenotype and leads to the accumulation of $CD4^+$ forkhead box $P3^+$ T cells," *Clinical & Experimental Immunology*, vol. 166, no. 2, pp. 154–163, 2011.

[36] M. Coscia, C. Vitale, S. Peola et al., "Dysfunctional $V\gamma9V\delta2$ T cells are negative prognosticators and markers of dysregulated mevalonate pathway activity in chronic lymphocytic leukemia cells," *Blood*, vol. 120, no. 16, pp. 3271–3279, 2012.

[37] B. N. Lee, H. Gao, E. N. Cohen et al., "Treatment with lenalidomide modulates T-cell immunophenotype and cytokine production in patients with chronic lymphocytic leukemia," *Cancer*, vol. 117, no. 17, pp. 3999–4008, 2011.

[38] G. D'Arena, V. Simeon, L. De Martino et al., "T-cell modulation by green tea in chronic lymphocytic leukemia," *International Journal Immunopathology Pharmacology*, vol. 26, pp. 117–125, 2013.

[39] G. D'Arena, L. Laurenti, M. Coscia et al., "Complementary and alternative medicine use in patients with chronic lymphocytic leukemia: an Italian multicentric survey," *Leukemia & Lymphoma*, vol. 55, no. 4, pp. 841–847, 2014.

[40] Z.-Z. Yang, A. J. Novak, S. C. Ziesmer, T. E. Witzig, and S. M. Ansell, "Attenuation of $CD8^+$ T-cell function by $CD4^+CD25^+$ regulatory T cells in B-cell non-Hodgkin's lymphoma," *Cancer Research*, vol. 66, no. 20, pp. 10145–10152, 2006.

[41] N. A. Marshall, L. E. Christei, L. R. Murro et al., "Immunosuppressive regulatory T cells are abundant in the reactive lymphocytes of Hodgkin lymphoma," *Blood*, vol. 103, no. 5, pp. 1755–1762, 2004.

[42] S. Schreck, D. Friebel, M. Buettner et al., "Prognostic impact of tumour-infiltrating Th2 and regulatory T cells in classical Hodgkin lymphoma," *Hematological Oncology*, vol. 27, no. 1, pp. 31–39, 2009.

[43] A. Tzankov, C. Meier, P. Hirschmann, P. Went, S. A. Pileri, and S. Dirnhofer, "Correlation of high numbers of intratumoral

FOXP3$^+$ regulatory T cells with improved survival in germinal center-like diffuse large B-cell lymphoma, follicular lymphoma and classical Hodgkin's lymphoma," *Haematologica*, vol. 93, no. 2, pp. 193–200, 2008.

[44] T. Alvaro, M. Lejeune, M. T. Salvado et al., "Outcome in Hodgkin's lymphoma can be predicted from the presence of accompanying cytotoxic and regulatory T cells," *Clinical Cancer Research*, vol. 11, no. 4, pp. 1467–1473, 2005.

[45] M. Garcia, B. Bellosillo, B. Sánchez-González et al., "Study of regulatory T-cells in patients with gastric Malt lymphoma: influence on treatment response and outcome," *PLoS One*, vol. 7, no. 12, article e51681, 2012.

[46] C. Chang, S. Y. Wu, Y. W. Kang et al., "High levels of regulatory T cells in blood are a poor prognostic factor in patients with diffuse large B-cell lymphoma," *American Journal Clinical Pathology*, vol. 144, pp. 935–944, 2015.

[47] A. F. Koreishi, A. J. Saenz, D. O. Persky et al., "The role of cytotoxic and regulatory T-cells in relapsed/refractory Hodgkin lymphoma," *Apllied Immunohistochemistry & molecular Morphology*, vol. 18, pp. 206–211, 2010.

[48] W. Y. Kim, Y. K. Jeon, T. M. Kim et al., "Increased quantity of tumor-infiltrating FOXP3-positive regulatory T cells is an independent predictor for improved clinical outcome in extranodal NK/T-cell lymphoma," *Annals Oncology*, vol. 20, pp. 1688–1696, 2009.

[49] J. Carreras, A. Lopez-Guillermo, B. C. Fox et al., "High numbers of tumor-infiltrating FOXP3-positive regulatory T-cells are associated with improved overall survival in follicular lymphoma," *Blood*, vol. 108, no. 9, pp. 2957–2964, 2006.

[50] R. H. Prabhala, P. Neri, J. E. Bae et al., "Dysfunctional T regulatory cells in multiple myeloma," *Blood*, vol. 107, no. 1, pp. 301–304, 2006.

[51] G. D'Arena, G. Rossi, L. Laurenti et al., "Circulating regulatory T-cells in monoclonal gammopathies of uncertain significance and multiple myeloma: in search of a role," *Journal of Immunology Research*, vol. 2016, Article ID 9271469, 7 pages, 2016.

[52] M. Beyer, M. Kochanek, T. Giese et al., "In vivo peripheral expansion of naive CD4$^+$ CD25^{high} *FoxP3*$^+$ regulatory T cells in patients with multiple myeloma," *Blood*, vol. 107, no. 10, pp. 3940–3949, 2006.

[53] S. Feyler, M. von Lilenfeld-Toal, S. Jarmin et al., "CD4$^+$ CD25$^+$ FoxP3$^+$ regulatory T cells are increased whilst CD3$^+$ CD4$^-$ CD8$^-$ $\alpha\beta$TCR$^+$ double negative T cells are decreased in the peripheral blood of patients with multiple myeloma which correlates with disease burden," *British Journal of Haematology*, vol. 144, no. 5, pp. 686–695, 2009.

[54] R. Gupta, P. Ganeshan, M. Hakim, R. Verma, A. Sharma, and L. Kumar, "Significantly reduced regulatory T cell population in patients with untreated multiple myeloma," *Leukemia Research*, vol. 35, no. 7, pp. 874–878, 2011.

[55] K. R. Muthu Raja, L. Rihova, L. Zahradova, M. Klincova, M. Penka, and R. Hajek, "Increased T regulatory cells are associated with adverse clinical features and predict progression in multiple myeloma," *PLoS One*, vol. 7, no. 10, article e47077, 2012.

[56] K. Giannopoulos, W. Kaminska, I. Hus, and A. Dmoszynska, "The frequency of T regulatory cells modulates the survival of multiple myeloma patients: detailed characterization of immune status in multiple myeloma," *British Journal of Cancer*, vol. 106, no. 3, pp. 546–552, 2012.

[57] M. Foglietta, B. Castella, S. Mariani et al., "The bone marrow of myeloma patients is steadily inhibited by a normal-sized pool of functional regulatory T cells irrespective of the disease status," *Haematologica*, vol. 99, no. 10, pp. 1605–1610, 2014.

[58] K. Bhattacharya, S. Chandra, and C. Mandal, "Critical stoichiometric ratio of CD4$^+$ CD25$^+$ FoxP3$^+$ regulatory T cells and CD4$^+$ CD25$^-$ responder T cells influence immunosuppression in patients with B-cell acute lymphoblastic leukaemia," *Immunology*, vol. 142, pp. 124–139, 2013.

[59] C.-P. Wu, X. Quing, H. Zhu, and H.-Y. Zhou, "Immunophenotype and increased presence of CD4$^+$CD25$^+$ regulatory T cells in patients with acute lymphoblastic leukemia," *Oncology Letters*, vol. 3, no. 2, pp. 421–424, 2012.

[60] X. Wang, J. Zheng, J. Liu et al., "Increased population of CD4$^+$CD25^{high} regulatory T cells with their higher apoptotic and proliferating status in peripheral blood of acute myeloid leukemia patients," *European Journal of Haematology*, vol. 75, no. 6, pp. 468–476, 2005.

[61] S. Z. Idris, N. Hassan, L. J. Lee et al., "Increased regulatory T cells in acute lymphoblastic leukemia patients," *Hematology*, vol. 20, no. 9, pp. 523–529, 2015.

[62] A. M. Zahran, H. Badrawy, and A. Ibrahim, "Prognostic value of regulatory T cells in newly diagnosed chronic myeloid leukemia patients," *International Journal of Clinical Oncology*, vol. 19, no. 4, pp. 753–760, 2014.

[63] J. M. Rojas, L. Wang, S. Owen, K. Knight, S. J. Watmough, and R. E. Clark, "Naturally occurring CD4$^+$ CD25$^+$ FOXP3$^+$T-regulatory cells are increased in chronic myeloid leukemia patients not in complete cytogenetic remission and can be immunosuppressive," *Experimental Hematology*, vol. 38, no. 12, pp. 1209–1218, 2010.

[64] E. Nadal, M. Garin, J. Kaeda, J. Apperley, R. Lechlir, and F. Dazzi, "Increased frequencies of CD4$^+$CD25^{high} Tregs correlate with disease relapse after allogeneic stem cell transplantation for chronic myeloid leukemia," *Leukemia*, vol. 21, no. 3, pp. 472–479, 2007.

[65] I. Hus, J. Tabarkiewicz, M. Lewandowska et al., "Evaluation of monocyte-derived dendritic cells, T regulatory and Th17 cells in chronic myeloid leukemia patients treated with tyrosine kinase inhibitors," *Folia Histochemistry Cytobiologica*, vol. 49, no. 1, pp. 153–160, 2011.

[66] C. R. Hasselbalch, M. K. Jensen, K. M. Brimnes et al., "Increase in circulating CD4$^+$CD25$^+$Foxp3$^+$ T cells in patients with Philadelphia-negative chronic myeloproliferative neoplasms during treatment with IFN-α," *Blood*, vol. 118, pp. 2170–2173, 2011.

[67] M. Kovacsovics-Bankowski, T. W. Kelley, O. Efimova et al., "Changes in peripheral blood lymphocytes in polycythemia vera and essential thrombocythemia patients treated with pegylated-interferon alpha and correlation with *JAK2*^{V617F} allelic burden," *Experimental Hematology & Oncology*, vol. 5, p. 28, 2016.

[68] M. Massa, R. Campanelli, G. Fois et al., "Reduced frequency of circulating CD4$^+$CD25^{bright}CD127^{low}FOXP3$^+$ regulatory T cells in primary myelofibrosis," *Blood*, vol. 128, no. 12, pp. 1660–1662, 2016.

[69] M. Massa, V. Rosti, R. Campanelli, G. Fois, and G. Barosi, "Rapid and long-lasting decrease of T-regulatory cells in patients with myelofibrosis treated with ruxolitinib," *Leukemia*, vol. 28, no. 2, pp. 449–451, 2014.

[70] J. C. Wang, H. Sindhu, C. Chen et al., "Immune derangements in patients with myelofibrosis: the role of Treg, Th17, and sIL2Rα," *PLoS One*, vol. 10, no. 3, article e0116723, 2015.

[71] S. P. Yajnanarayana, T. Stubig, I. Cornez et al., "JAK1/2 inhibition impairs T cell function *in vitro* and in patients with myeloproliferative neoplasms," *British Journal of Haematology*, vol. 169, no. 6, pp. 824–833, 2015.

[72] K. Wang and A. T. Vella, "Regulatory T cells and cancer: a two-sided story," *Immunology Investigation*, vol. 45, no. 8, pp. 797–812, 2016.

[73] K. Giannopoulos, M. Schmitt, M. Kowal et al., "Characterization of regulatory T cells in patients with B-cell chronic lymphocytic leukemia," *Oncology Report*, vol. 20, pp. 677–682, 2008.

[74] E. Bachy, J. Bernaud, P. Roy, D. Rigal, and F. E. Nicolini, "Quantitative and functional analyses of $CD4^+CD25^+FoxP3^+$ regulatory T cells in chronic phase chronic myeloid leukaemia patients at diagnosis and on imatinib mesylate," *British Journal of Haematology*, vol. 153, pp. 129–143, 2011.