failed to find differences because of these same reasons: inferior inclusion of Ph'+ALL and a reasonable outcome of slow responders in the chemotherapy arm (5-year DFS, 47%) partially explained by the use of our less stringent criteria of failure or slow response to initial treatment. Ph'+ ALL is unlikely to be merged with other ALLs in current and future trials[3,4] and this is probably true for t(4;11)/*MLL-AF4* ALL.[5] Adequate monitorization and interpretation of minimal residual disease will undoubtedly influence the way we approach patients with ALL,[6,7] particularly those experiencing failure to achieve an optimal response to initial therapy, and may further define the exact time point in which an alloSCT is mandatory. International collaboration is essential to redefine the indications of SCT as the definition of VHR-ALL tends to include a further limited number of children.

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AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

The author(s) indicated no potential conflicts of interest.

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CD4+CD25^{high}FOXP3+ Regulatory T Cells in Peripheral Blood Are Primarily of Effector Memory Phenotype

To the Editor: We noted with interest the article by Cesana et al[1] in the March 1, 2006, issue of the *Journal of Clinical Oncology*, entitled "Characterization of CD4$^+$CD25$^+$ Regulatory T Cells in Patients Treated With High-Dose Interleukin-2 for Metastatic Melanoma or Renal Cell Carcinoma." Since the first description of CD4+CD25+ T cells as regulatory T cells (Tregs) by Sakaguchi et al,[2] a number of studies have addressed the frequency of CD4+CD25^{high} Tregs in cancer patients. The increase of Tregs in cancer-bearing patients either in the tumor microenvironment or peripheral blood is now well accepted in the literature.[3,4] How interleukin (IL)-2 therapy influences the frequency of Tregs has been addressed in different settings with similar results.[5-7] A further increase of Tregs after IL-2 administration seems to be associated with progressive disease while a response to IL-2 treatment seems to be associated with decreased Tregs numbers.[1]

However, we would like to draw your attention to the authors' statement that the Tregs were almost exclusively of naïve or central memory phenotype. In 1999, Sallusto et al[8] were the first to describe the differentiation of total CD4+ and CD8+ T cells into CCR7+CD45RA+ naïve T cells, CCR7+CD45RA− central memory and CCR7−CD45RA− effector memory T cells. Since then, this T-cell classification has been applied by many groups confirming the initial observation. For Tregs, however, it was assumed for adults that these cells are exclusively of memory phenotype as defined by surface expression of CD45RO and absence of CD45RA. Only very recently has the existence of human naïve Tregs cells in healthy individuals been demonstrated in two elegant studies.[9,10] Both studies revealed a subset of CD4+CD25+ T cells with full regulatory capacity coexpressing CD45RA. These cells were termed natural naïve Tregs. Unfortunately costainings for CD45RA and CCR7 were not reported in both studies; therefore, not allowing distinction between central and effector memory Tregs cells. However, both studies came to the conclusion that the cell population coexpressing CD4, CD25, and CD45RA constitute approximately one third of the total Tregs population. In addition to the demonstration of the regulatory capacity of the T-cell population under study, it is mandatory to measure the expression of the transcription factor Forkhead Box Protein 3 (FOXP3) to classify the cells as Tregs. In fact, both studies demonstrated a relative increase of FOXP3 mRNA expression in CD4+CD25+CD45RA+ T cells compared with CD4+CD25−CD45RA+ T cells.[9,10] Unfortunately, FOXP3 protein expression was not determined in these studies, so it remained elusive whether few CD4+CD25+CD45RA+ T cells express large amounts of FOXP3 or many CD4+CD25+CD45RA+ T cells little FOXP3. Using the same gating strategy, we assessed 19 healthy donors and confirmed the existence of a CD4+CD25+CD45RA+ T-cell population presenting approximately 30% of the total CD4+CD25+ Tregs population

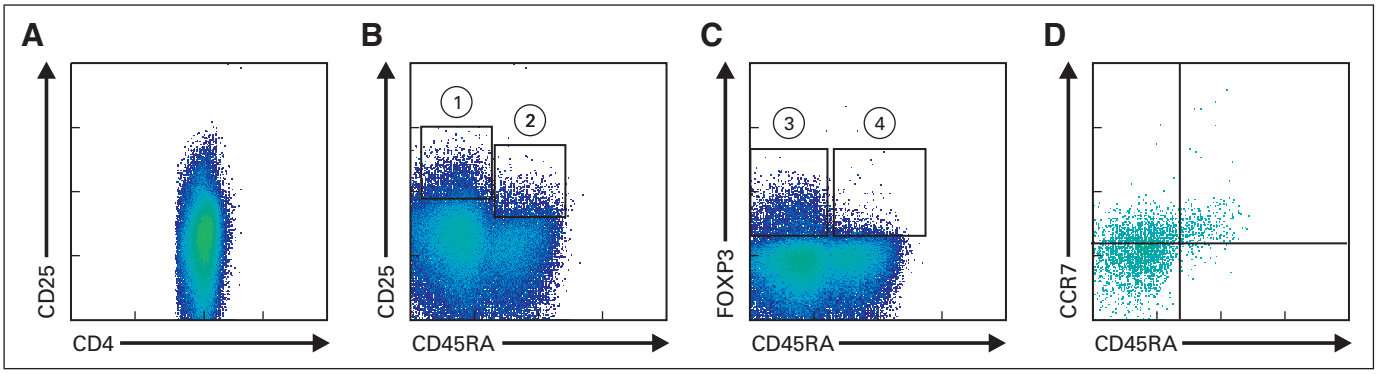

Fig 1. Frequency of naïve CD4+CD25^{high} and CD4+ Forkhead Box Protein 3 (FOXP3)–positive T cells. Flow cytometric analysis of CD25 and CD45RA on peripheral blood derived CD4+ T cells. (A) Expression of CD4 and CD25 gated on CD4+ T cells. (B) Analysis of CD25 and CD45RA on CD4+ T cells demonstrates two distinct CD4+CD25+ T-cell populations, (1) CD4+CD25^{high}CD45RA− and (2) CD4+CD25+CD45RA+ regulatory T cells. (C) Expression of FOXP3 on human (3) CD4+CD45RA− and (4) CD4+CD45RA+ T cells. (D) Expression of CCR7 and CD45RA on CD4+FOXP3+ T cells allows for the identification of CD4+FOXP3+ naïve, central, and effector memory T cells.

(Fig 1B). However, when assessing FOXP3 protein expression in context of CD45RA expression (Fig 1C), only few of the CD4+CD45RA+ T cells were weakly stained for FOXP3 in contrast to a distinct subpopulation of CD4+CD45RA−FOXP3+ T cells. In fact, only 14.6% ± 15.5% of all CD4+CD25+FOXP3+ T cells coexpress the naïve T-cell marker CD45RA (Table 1). This is in sharp contrast to the data reported by Cesana et al[1] who reported almost one half of the Tregs to be of naïve phenotype. To further study the distribution of central and effector memory Tregs, we assessed CCR7 expression in the CD4+ T-cell population in relation to FOXP3 protein expression. Again, this data revealed significantly different results (Fig 1D).[11] While Cesana et al[1] did not find any effector memory Tregs, our results indicate that the majority of CD4+FOXP3+ T cells are indeed of the effector memory phenotype.

To further control for these findings, we applied a second approach (Fig 2). In this case, we first separated CD4+ T cells based on their expression of CD25 into CD25−, CD25^{low}, and CD25^{high} T cells. Next CD25^{high}FOXP3+ cells were determined and CD45RA and CCR7 expression was assessed in comparison with CD25^{high}FOXP3− cells. This approach clearly demonstrated that not all CD4+CD25^{high} T cells are also FOXP3+ as suggested by Cesena et al.[1] Our findings are in line with previous data demonstrating an increase of both FOXP3+ as well as FOXP3−CD4+CD25^{high} T cells after IL-2 therapy.[6] As shown in Figure 2C, within the 19 healthy donors studied, the large majority of CD4+CD25^{high}FOXP3+ T cells are of effector memory phenotype. Only very few Tregs are of a naïve phenotype. In CD4+CD25^{high}FOXP3− T cells, the distribution differs as more cells are of a naïve respectively central memory phenotype (Fig 2D).

As an important control, we also studied CD4+CD25^{low} (Fig 2E) and CD4+CD25− T cells (Fig 2F). Because these cells comprise the majority of human CD4+ T cells, it is not surprising that we can confirm the original observation by Sallusto et al[7] (Fig 2E and F) demonstrating that 20% to 40% of human CD4+ T cells are of naïve phenotype, between 20% and 40% of central memory and the remaining cells of effector memory phenotype. Taken together, we come to the conclusion that human Tregs—in contrast to conventional T cells—are mainly of effector memory phenotype. The discrepancy to the data reported by Cesana et al[1] might be simply due to different technical approaches. Because the distribution of naïve, central, and effector memory T cells within the CD4+CD25− T cell population was not shown by Cesana et al,[1] it is not easy to determine whether the gating strategy applied by Cesana et al[1] would result in data compatible with previous findings[7] concerning the distribution of the different subpopulations within conventional T cells. Clarifying this issue will be of importance in context of malignant disease since the expansion of naïve versus memory Tregs might have significant impact of further therapeutic strategies. In the end, however, the best way to resolve such discrepancies will be the identification of truly specific markers for Tregs, which then will allow better subclassification of these cells and to determine their dynamics during disease or therapeutic intervention.

Table 1. Percentages of Naïve CD45RA+ and Memory CD45RA− Regulatory T Cells As Defined by Expression of CD4 and CD25 in Combination With FOXP3

	CD45RA+ (%)		CD45RA− (%)	
Variable	Mean	SD	Mean	SD
CD4+CD25+	28.4	15.7	71.6	27.4
CD4+CD25+FOXP3+	14.6	15.5	85.4	33.3

NOTE. Numbers depict percentage of either CD4+CD25+ or CD4+CD25+FOXP3+ T cells, respectively.
Abbreviations: FOXP3, Forkhead Box Protein 3; SD, standard deviation.

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AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST
Although all authors completed the disclosure declaration, the following authors or their immediate family members indicated a financial interest. No conflict exists for drugs or devices used in a study if they are not being evaluated as part of the investigation. For a detailed description of the disclosure categories, or for more information about ASCO's conflict of interest policy, please refer to the Author Disclosure Declaration and the

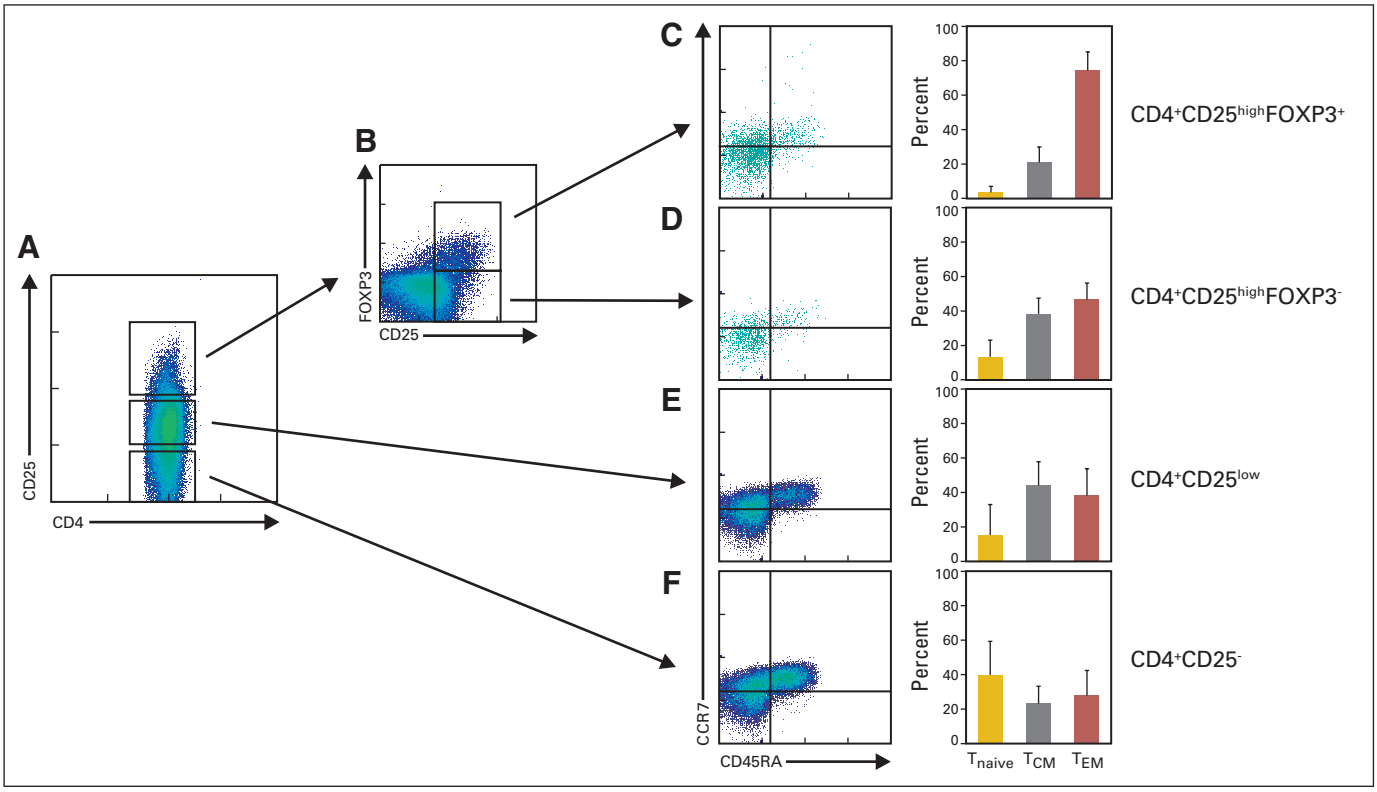

Fig 2. Frequency of naïve CD4+CD25^{high}FOXP3+ regulatory T cells. Frequencies of CCR7^{+}CD45RA^{+} naïve T cells (T_{naive}), CCR7+CD45RA− central memory T cells (T_{CM}), and CCR7−CD45RA− effector memory T cells (T_{EM}) were assessed in peripheral blood. (A) CD4+ T cells were further divided into conventional CD4+CD25−, CD4+CD25^{low}, and regulatory CD4+CD25^{high} T cells according to their CD25 expression. (B) CD4+CD25^{high} T cells were subdivided into CD4+CD25^{high}FOXP3+ as well as CD4+CD25^{high} Forkhead Box Protein 3 (FOXP3) –negative T cells. (C) Frequencies of CD4+CD25^{high}FOXP3+ T_{naive}, T_{CM}, and T_{EM} cells. (D) Frequencies of CD4+CD25^{high}FOXP3− T_{naive}, T_{CM}, and T_{EM} cells. (E) Frequencies of CD4+CD25^{low} T_{naive}, T_{CM}, and T_{EM} cells. (F) Frequencies of conventional CD4+CD25− T_{naive}, T_{CM}, and T_{EM} cells. The left panel of (C) to (F) is one representative example of the 19 healthy individuals studied. The right panels of (C) to (F) represents mean ± standard deviation (n = 19 healthy individuals) of percentages of T_{naive}, T_{CM}, and T_{EM} cells within the respective T-cell subset.

Disclosures of Potential Conflicts of Interest section in Information for Contributors.
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In Reply: High-dose interleukin-2 (IL-2) is an approved treatment regimen for metastatic melanoma and renal cell carcinoma with objective tumor response rate of 16% to 20% and significant durability in selected patients.[1-3] Despite these results, the mechanism of IL-2–mediated tumor rejection is not yet defined.

CORRECTIONS

Author Corrections

The June 20, 2007 correspondence by Beyer and Schultze, entitled, "CD4+CD25^{high}FOXP3+ Regulatory T Cells in Peripheral Blood Are Primarily of Effector Memory Phenotype" (J Clin Oncol 25:2628-2630, 2007), contained an error.

In the Acknowledgment section, the following study support was inadvertently omitted: the German José-Carreras Foundation (M.B. and J.L.S.). The authors apologize for the mistake.

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The January 1, 2009 article by Tarhini et al, entitled, "Prognostic Significance of Serum S100B Protein in High-Risk Surgically Resected Melanoma Patients Participating in Intergroup Trial ECOG 1694" (J Clin Oncol 27:38-44, 2009), contained an error.

The following was inadvertently omitted and should have been acknowledged in the sidebar of the article:

"The project described was supported by Award Number P50CA121973 from the National Cancer Institute. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Cancer Institute or the National Institutes of Health."

The authors apologize for the omission.

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The January 20, 2012 article by Tarhini et al, entitled, "Safety and Efficacy of Combination Immunotherapy With Interferon Alfa-2b and Tremelimumab in Patients With Stage IV Melanoma" (J Clin Oncol 30:322-328, 2012), contained an error.

The following was inadvertently omitted and should have been acknowledged in the sidebar of the article:

"The project described was supported by Award Number P50CA121973 from the National Cancer Institute. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Cancer Institute or the National Institutes of Health. "

The authors apologize for the omission.

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The June 1, 2012 article by Peiffert et al, entitled, "Induction Chemotherapy and Dose Intensification of the Radiation Boost in Locally Advanced Anal Canal Carcinoma: Final Analysis of the Randomized UNICANCER ACCORD 03 Trial" (J Clin Oncol 30:1941-1948, 2012), contained an error.

The following note was inadvertently omitted and should have been included in the sidebar of the article:

"Written on behalf of the Fédération nationale des centres de lutte contre le cancer (UNICANCER), Fédération Francophone de Cancérologie Digestive (FFCD), and the Société Française de Radiothérapie Oncologique (SFRO)."

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The August 20, 2012 article by Ferreri et al, entitled, "*Chlamydophila Psittaci* Eradication With Doxycycline As First-Line Targeted Therapy for Ocular Adnexae Lymphoma: Final Results of an International Phase II Trial" (J Clin Oncol 30:2988-2994, 2012), contained an error.

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