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RESEARCH

Innate CD8 T-cells as a potential predictive biomarker for deep molecular response in chronic myeloid leukemia patients

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Abstract

In chronic myeloid leukemia (CML), the role of immune effectors has been suggested in the achievement of a sustained deep molecular response (DMR) and treatment-free remission (TFR) after tyrosine kinase inhibitor (TKI) discontinuation. A contributory role of the distinct new innate CD8 T-cell pool in control of CML residual disease after TKI cessation was recently highlighted. Here, we evaluated longitudinally whether innate CD8 T-cells predict CML therapy success in a cohort of newly diagnosed CML patients treated in the DASA-PegIFN clinical trial. After 3 months of treatment (M3), we observed a significant increase in innate CD8 T-cell frequency as compared to diagnosis, together with an early shift within the pool of CD8 T-cells toward an innate/memory phenotype. We also found that patients with high innate CD8 T-cell frequency at M3 achieved DMR earlier and at higher rates than patients with low innate CD8 T-cell frequency. Remarkably, this signature pre-existed at the time of diagnosis, suggesting the possible role of the patient's initial individual immune status. High innate CD8 T-cell frequency was also associated with maintaining DMR stability for 2 years. Taken together, our findings highlight innate CD8 T-cells as a potential marker for CML therapy success and TFR eligibility.

Keywords Chronic myeloid leukemia · Deep molecular response · Immune biomarker · Innate CD8 T-cell

Introduction

Since the advent of tyrosine kinase inhibitors (TKIs), chronic myeloid leukemia (CML) has become a chronic disease with life expectancy comparable to that of unaffected individuals. Following treatment by TKI, 5-year probability of achieving a deep molecular response (DMR) ranges from 38 to

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64% [1]. Second-generation TKIs (nilotinib, dasatinib, and bosutinib) have been associated with an earlier and higher rate of patients experiencing DMR. Achieving a sustained DMR represents the most recent goal in CML treatment, both to prevent disease progression and to allow an attempt at TKI discontinuation [2].

Although the immune abnormalities reported in CML-chronic phase (CP) patients at diagnosis are partially corrected after TKI therapy, only a few studies have focused on immunological prognosis factors impacting the depth of the response, which heretofore include NK cells (or NK cell-related markers such as killer-cell immunoglobulin-like receptors (KIR)) and Vδ2 γδ T-cells [3–7]. Considering the main conventional anti-tumoral effector compartment, namely, conventional cytotoxic CD8 T-cells, to date no relationship has been established between prognosis and treatment success.

We recently identified a new subset of CD8 T-cells sharing properties with conventional-memory CD8 T-cells and exhibiting NK-like features. This CD8 T-cell subset is characterized by CD8 expression along with a classical TCRαβ, NK receptors (panKIR/NKG2A), an EMRA (effector memory cells re-expressing CD45RA) phenotype ($CD45RA^+$, $CCR7^-$), and high Eomesodermin (Eomes) expression [8, 9]. Innate CD8 T-cells hold high anti-tumoral potential, as evidenced by their prompt IFN-γ production in response to innate-like co-stimulation by IL-12 and IL-18 [8, 9]. Our previous work has shown that peripheral blood innate CD8 T-cells ($CD8^+$ TCR-$\alpha\beta^+$ $Eomes^+$ panKIR/$NKG2A^+$) are drastically reduced and functionally deficient in CML patients at diagnosis [10]. Moreover, innate CD8 T-cell deficiencies at CML diagnosis have been found to be partially reversed in stable complete cytogenetic remission [10] and major molecular response (MMR) patients on TKI treatment [11], highlighting this CD8 T-cell subset as a new potential effector controlling CML. Consistent with this notion, we demonstrated that innate CD8 T-cells are markedly increased in patients in sustained TFR as compared to patients in MMR under TKI therapy, and even to healthy donors [11]. Finally, in a previous prospective study comparing patients in TFR *versus* in molecular relapse (MR) after TKI cessation, we have shown that innate CD8 T-cells, in combination with NK cells, may be a predictive marker for TFR success in CML treatment [12].

In the present study, we hypothesized that innate CD8 T-cells are closely associated with the achievement of DMR under TKI therapy. To test this assumption, we prospectively and longitudinally analyzed a cohort of 38 chronic phase of CML (CP-CML) patients treated first line with dasatinib for three months and subsequently in combination with small doses of IFN-α [13]. This protocol aimed to achieve high rates of early and sustained DMR, hence offering the possibility to investigate biological markers linked to DMR achievement at one year and/or DMR stability over two years in the same cohort over time.

Methods

Patient and healthy donor characteristics

The present work is a sub-study of the DASA-PegIFN study (EudraCT Number 2012-003389-42, ClinicalTrials.gov. NCT01872442) approved by the National Health regulatory authorities and ethical committee (Poitiers, France). This study is a prospective, nonrandomized phase 2 trial conducted in 22 centers in France. All the participating patients provided their written informed consent for the clinical study and ancillary biological studies.

Briefly, newly diagnosed Ph^+ CP-CML patients were treated with first-line dasatinib at 100 mg/day. At month 3, Peg-Interferon-alpha-2b (PegIntron®, Merck KGaA, Darmstadt, Germany) was initiated at 30 µg/week subcutaneously for eligible patients (in the absence of significant cytopenia or extra hematological adverse event greater than grade 2 with dasatinib alone for the 3 first months) and for a maximum duration of 21 months. The results of this trial and protocol have been reported [13]. During the trial, peripheral blood was collected on heparin at several time points: at diagnosis, and at 3, 6, 12, and 24 months after initiation of treatment. Among the 61 patients eligible for the dasatinib + Peg-IFNα2b therapy from month 3, we analyzed samples from 40 consecutive patients for whom at least four time points were available. Two of them were excluded for technical issues. Patient responses to treatment were classified as conforming to 2013 ELN criteria. Major molecular response (MMR) was defined as a ratio of $BCR::ABL1/ABL1^{IS} \leq 0.1\%$ on the international scale (IS). A ratio of $BCR::ABL1/ABL1^{IS} \leq 0.01\%$ defines a deep molecular response (DMR). Patients were separated into two groups according to whether or not (noDMR) they achieved DMR at 12 months (DMR) (see Table 1 for the whole cohort and group patient's characteristics).

Frozen peripheral blood mononuclear cells (PBMC) from 21 healthy donors (HD) (median age 28 years, range 22–65, sex ratio: 0.5) were obtained from the French Blood Institute (Etablissement Français du Sang, Lyon, France).

PBMC isolation and cryopreservation

PBMCs were isolated from blood samples by density gradient centrifugation (Histopaque®-1077, Sigma-Aldrich, St Louis, MO, USA), resuspended in 90% fetal bovine serum (10270106, Gibco®, Thermo Fisher Scientific, Waltham,

Table 1 Patient's characteristics

	All	DMR at 12 months *	
	($n=38$)	noDMR	DMR
		($n=22$)	($n=16$)
Age (years)	45 ± 12	42 ± 12	48 ± 12
Sex, n (%)			
Female	15 (39%)	6 (27%)	9 (56%)
Male	23 (61%)	16 (73%)	7 (44%)
Sokal score, n (%)			
Low (< 0.8)	25 (66%)	11 (50%)	14 (88%)
Int/high (≥ 0.8)	13 (34%)	11 (50%)	2 (12%)
ELTS score, n (%)			
Low	30 (79%)	14 (64%)	16 (100%)
Intermediate	6 (16%)	6 (27%)	0 (0%)
High	2 (5%)	2 (0.1%)	0 (0%)
CMV serology, n (%)			
Negative	20 (54%)	15 (71%)	5 (31%)
Positive	17 (46%)	6 (29%)	11 (69%)
*EMR**, n (%)*			
no EMR	5 (13%)	5 (23%)	0 (0%)
EMR	33 (87%)	17 (77%)	16 (100%)
Peg-IFN duration, n (%)			
< 1 year	9 (24%)	6 (27%)	3 (19%)
> 1 year	29 (76%)	16 (73%)	13 (81%)
Blast in peripheral blood, n (%)			
No	24 (63%)	12 (55%)	12 (75%)
Yes	14 (37%)	10 (45%)	4 (25%)
BCR::ABL1 transcript, n (%)			
e13a2 (b2a2)	20 (54%)	14 (64%)	6 (40%)
e14a2 (b3a2)	15 (41%)	7 (32%)	8 (53%)
e13a2 (b2a2) + e14a2 (b3a2)	2 (5%)	1 (5%)	1 (7%)

*DMR: deep molecular response (*BCR::ABL1/ABL1*$^{IS} \leq 0.01\%$); **EMR: early molecular response (*BCR::ABL1/ABL1*$^{IS} \leq 10\%$, at 3 months); ELTS: Eutos Long-Term Survival

MA, USA) with 10% DMSO (D2650, Sigma-Aldrich), and cryopreserved at -80°C or in liquid nitrogen until use.

Flow cytometry

Phenotypic analysis of cells from HD and CML patients was performed using ex vivo flow cytometry. All monoclonal antibodies (mAbs) used in this study are listed in Supplementary Table 1. Expression of the different markers was assessed by staining PBMC with appropriate combinations of mAbs. PanKIR/NKG2A referred to staining with a mixture of the following three antibodies from Miltenyi Biotec (Bergisch Gladbach, Germany) KIR2D, KIR3DL1/KIR3DL2 (CD158e/k), and NKG2A (CD159a). Dead cells were excluded using the Live/Dead® Fixable NearIR Dead Cell Stain kit (L10119, Invitrogen™, Thermo Fisher Scientific). For intranuclear Eomes staining, cells were permeabilized using an anti-human Foxp3 staining kit according to the manufacturer's protocol (73-5776-40, eBioscience™, Thermo Fisher Scientific). Flow data were acquired on a FACSVerse flow cytometer (Becton, Dickinson & Company, Franklin Lakes, NJ, US) with FACSuite™ software (Becton, Dickinson & Company) and analyzed using FlowJo™ v10 (Becton, Dickinson & Company). A detailed gating strategy is presented in Supplementary Fig. 1. Results are expressed as frequencies or as mean fluorescence intensity (MFI).

Statistical analysis

All statistical data analyses were performed using GraphPad Prism v7.0 (GraphPad Software, Inc). Friedman's test with Dunn's multiple comparison test and Mann–Whitney two-tailed test were used for unpaired data analysis. Two-way ANOVA with Šídák's multiple comparison and Wilcoxon tests were used for paired data. Logistic regression was used with DMR at 12 months as dependent variable and innate CD8 T-cells frequency in peripheral blood at diagnosis or at 3 months as independent variables. We used bivariate analysis with innate CD8 T-cells and each of the following independent variables: age, gender, Sokal score, ELTS (Eutos Long Term Survival) score, CMV serology, early molecular response (EMR) at 3 months, peg-IFN treatment duration, blast percentages in peripheral blood, baseline *BCR::ABL1* transcript levels, and *BCR::ABL1* transcript-type. A p value < 0.05 was considered statistically significant ($*p<0.05$, $**p<0.01$, $***p<0.001$, $****p<0.0001$). Receiver operating characteristic (ROC) curves were built using the logarithm of innate CD8 T-cell frequency after three months of therapy or at diagnosis. The Youden index was used to determine the cut-off of innate CD8 T-cell frequency. Cumulative incidence was presented as Kaplan–Meier curves. Significant differences between curves were analyzed using a log-rank test statistical analysis.

Results

The first three months of treatment are associated with increased innate CD8 T-cell frequency

We analyzed peripheral blood innate CD8 T-cells (defined as Eomes$^+$ panKIR/NKG2A$^+$ cells among TCR-αβ$^+$ CD8$^+$ cells) by flow cytometry at multiple time points from diagnosis up to 24 months of therapy in 38 CML patients participating in the phase II DASA-PegIFN clinical trial (see Methods and Table 1).

Figure 1a shows a twofold increase in the percentage of innate CD8 T-cells at the three-month (M3) treatment stage

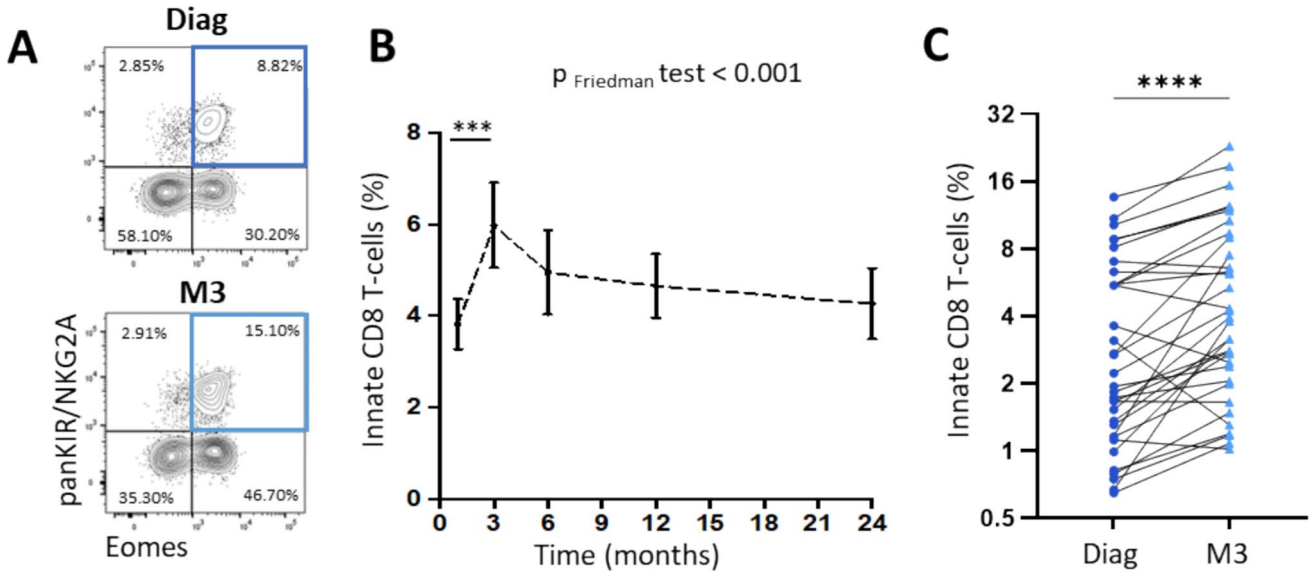


Fig. 1 Innate CD8 T-cells are enhanced after 3 months of CML therapy. **a** Cytograms of one representative CML patient at diagnosis (Diag, upper) and at 3 months (M3, lower) are shown. **b** Kinetics of innate CD8 T-cell frequencies in CML patients analyzed from diagnosis and up to 24 months of treatment (Diag: $n=38$; M3: $n=34$; M6: $n=34$; M12: $n=33$; M24: $n=31$). Data are expressed as mean ± SEM. Statistical analysis: Friedman-test ($n=27$), with Dunn's multiple comparison test, for Dunn's test comparing innate CD8 T-cells frequency at each time point to diagnosis. **c** Frequencies of innate CD8 T-cells (log2 scale) at diagnosis (Diag, $n=34$) and after 3 months of CML therapy (M3, $n=34$). Statistical analysis: Wilcoxon test

(dasatinib alone, Peg-IFN not yet initiated) as compared to diagnosis in a representative patient. Considering the entire cohort, we found a significant increase in the frequency of innate CD8 T-cells (6.0 ± 5.4% vs. 3.8 ± 3.4%, mean ± SEM), as compared to diagnosis (Fig. 1b, M3 vs. diagnosis). After M3, we observed a decline in the percentage of CD8 T-cells (rapid between M3 and M6, and slower thereafter). However, it is worth noting that this rate remains higher than at the time of diagnosis. When individual patients were examined, higher levels of innate CD8 T-cells were observed at M3 in 79.4% of them (Fig. 1c). In accordance with our previous observation in another independent cohort of CML patients [10], we confirmed the specific quantitative deficiency of innate CD8 T-cells at diagnosis as compared to the healthy donor (HD) group (Supplementary Fig. 2). Early effects of TKI therapy on the pool of CD8 T-cells were not restricted to innate CD8 T-cells, as in 85.3% of patients conventional-memory CD8 T-cell (defined as $Eomes^+$ panKIR/$NKG2A^-$) frequency concomitantly increased, whereas naïve CD8 T-cell (defined as $Eomes^-$ panKIR/$NKG2A^-$) frequency decreased after three months of therapy (Supplementary Fig. 3), as previously described [14].

Innate CD8 T-cell frequency at M3 selectively predicts DMR achievement at 12 months

We then searched for a potential relationship between the frequency of innate CD8 T-cells and the achievement of a deep molecular response (DMR) at 12 months. Taking the entire kinetics into account, we evidenced that DMR patients had significantly higher innate CD8 T-cell frequencies throughout the 24 months of therapy compared to the noDMR group (Fig. 2a). Remarkably, the increased frequency of innate CD8 T-cells after three months of therapy, in comparison to diagnosis, was more pronounced in the DMR group than in the noDMR group (Fig. 2b). This phenomenon was specific to innate CD8 T-cells, as conventional-memory CD8 T-cell frequency did not differ between the two groups at diagnosis and at M3 (Supplementary Fig. 4). One explanation could be a shift within the CD8 T-cell pool in favor of innate CD8 T-cells at the expense of the naïve T-cell pool, as suggested by the lower frequency of naïve CD8 T-cells observed concomitantly at M3 (Supplementary Fig. 4). Furthermore, we observed that innate CD8 T-cell frequency at diagnosis tended to be higher in the DMR group than in the noDMR group (p value: 0.0511, Mann–Whitney test, data not shown).

These results provide evidence that innate CD8 T-cell frequency at three months of dasatinib therapy may be a predictive marker for DMR achievement (evaluated at 12 months).

Innate CD8 T-cell frequency as an indicator of DMR achievement and its durability

ROC curves were built to determine a cut-off value for innate CD8 T-cell frequency at M3 (p-value: 0.0044) and

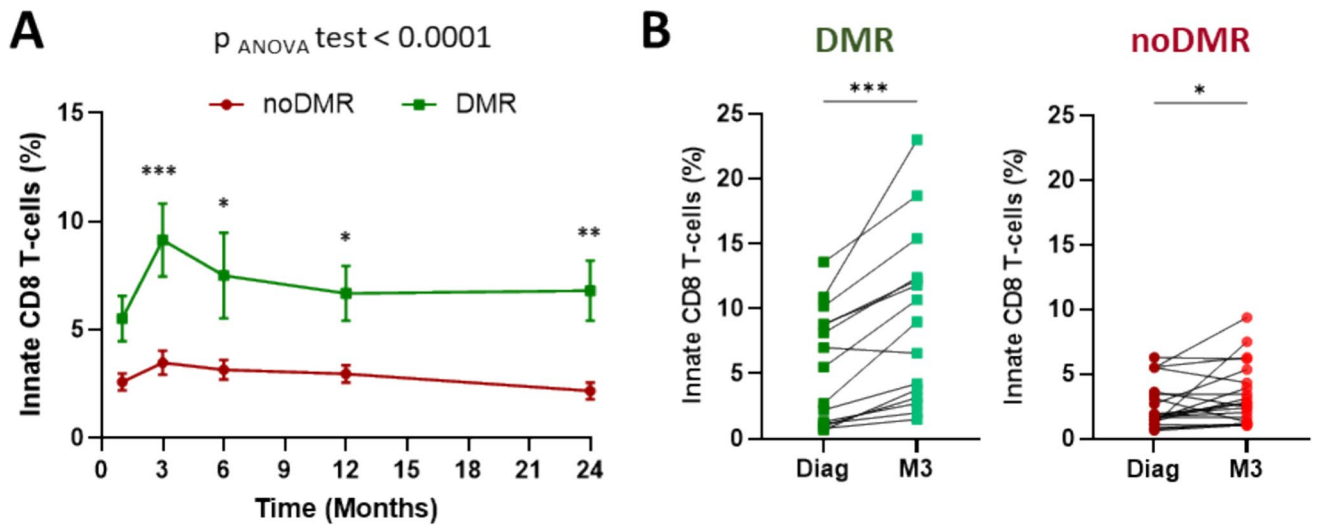


Fig. 2 High innate CD8 T-cell frequency at diagnosis and after three months of treatment is associated with DMR achievement. **a** Kinetics of innate CD8 T-cell frequencies in CML patients having achieved DMR (DMR, green line) or not (noDMR, red line) after 12 months of therapy. Patients were analyzed from diagnosis and up to 24 months of treatment (Diag: noDMR $n=22$; DMR $n=16$; M3: noDMR $n=19$; DMR $n=15$; M6: noDMR $n=20$; DMR $n=14$; M12: noDMR $n=18$; DMR $n=15$; M24: noDMR $n=17$; DMR $n=14$). Data are expressed as a curve of mean ± SEM. Statistical analysis: two-way ANOVA was performed with Šídák's multiple comparisons test comparing each time point between patients groups. **b** Frequencies of innate CD8 T-cells at diagnosis and after 3 months in patients having achieved DMR (DMR, left panel; Diag: $n=15$, M3: $n=15$) or not (noDMR, right panel; Diag: $n=20$, M3: $n=20$) after 12 months of therapy. Statistical analysis: Wilcoxon test

to establish whether this parameter could be used as a biomarker of DMR (Supplementary Fig. 5). Then, we determined the cumulative incidence of DMR over the first 24 months of therapy in patients with low versus high innate CD8 T-cell frequencies at M3 (Fig. 3b). DMR achievement occurred significantly earlier and at higher rates in patients with high innate CD8 T-cell frequency at M3. Patients with low innate CD8 T-cell frequency ($n=25$) exhibited slow kinetics of DMR obtention that never reached the rate of patients with high innate CD8 T-cell frequency ($n=13$): 51.3% during the 24 months of treatment compared to 32.5% (Fig. 3b). These data support the conclusion that high innate CD8 T-cell frequency in peripheral blood after 3 months of therapy may be an indicator of early DMR achievement.

Of note, by applying the same approach based on the frequency of innate CD8 T-cells at diagnosis (Fig. 3a), we observed that 70.9% of patients with high level of innate CD8 T-cells at diagnosis achieved DMR in 24 months of treatment and that only 25.3% of the CML patients with low level of innate CD8 T-cells reached DMR in two years. Consequently, innate CD8 T-cell frequency at diagnosis may also predicts DMR achievement.

Moreover, to study whether innate CD8 T-cells are an independent predictor of DMR at 12 months, we performed bivariate analysis with innate CD8 T-cell percentages and each of the following variables: gender, age, Sokal score, ELTS score, baseline *BCR::ABL1* transcript levels, transcript-type *BCR::ABL1*, CMV serology, EMR, duration of Peg-IFN treatment, and blast percentage in peripheral blood. After adjustment, DMR at 12 months was still significantly associated with innate CD8 T-cell percentages measured at both diagnosis and at 3 months (data not shown).

Alongside DMR achievement, its stability over time (≥ 2 years) is a crucial criterion for CML patients to be eligible for treatment discontinuation [15]. In the DASAPeg-IFN trial, with median follow-up of 54.1 months (range 30.6–69.1), 46% of patients achieved a 2-year sustained DMR [13]. To test whether innate CD8 T-cell frequency was associated with treatment response stability, we separated patients with stable DMR for more than two years ($n=18$) from patients without stable DMR ($n=16$). Remarkably, innate CD8 T-cell frequency after three months of therapy was significantly higher in patients with a sustained 2y-DMR (Fig. 3c). The same conclusion could be applied when considering the frequency of innate CD8 T-cells at CML diagnosis (Fig. 3c), thereby reinforcing the notion that the status of innate CD8 T-cells is closely associated with a stable DMR in CML patients. Finally, this effect was specific to the innate CD8 T-cell compartment, as conventional-memory and naïve CD8 T-cell frequencies were similar between the two groups regardless of the time point (at diagnosis or after three months of therapy) (Supplementary Fig. 6).

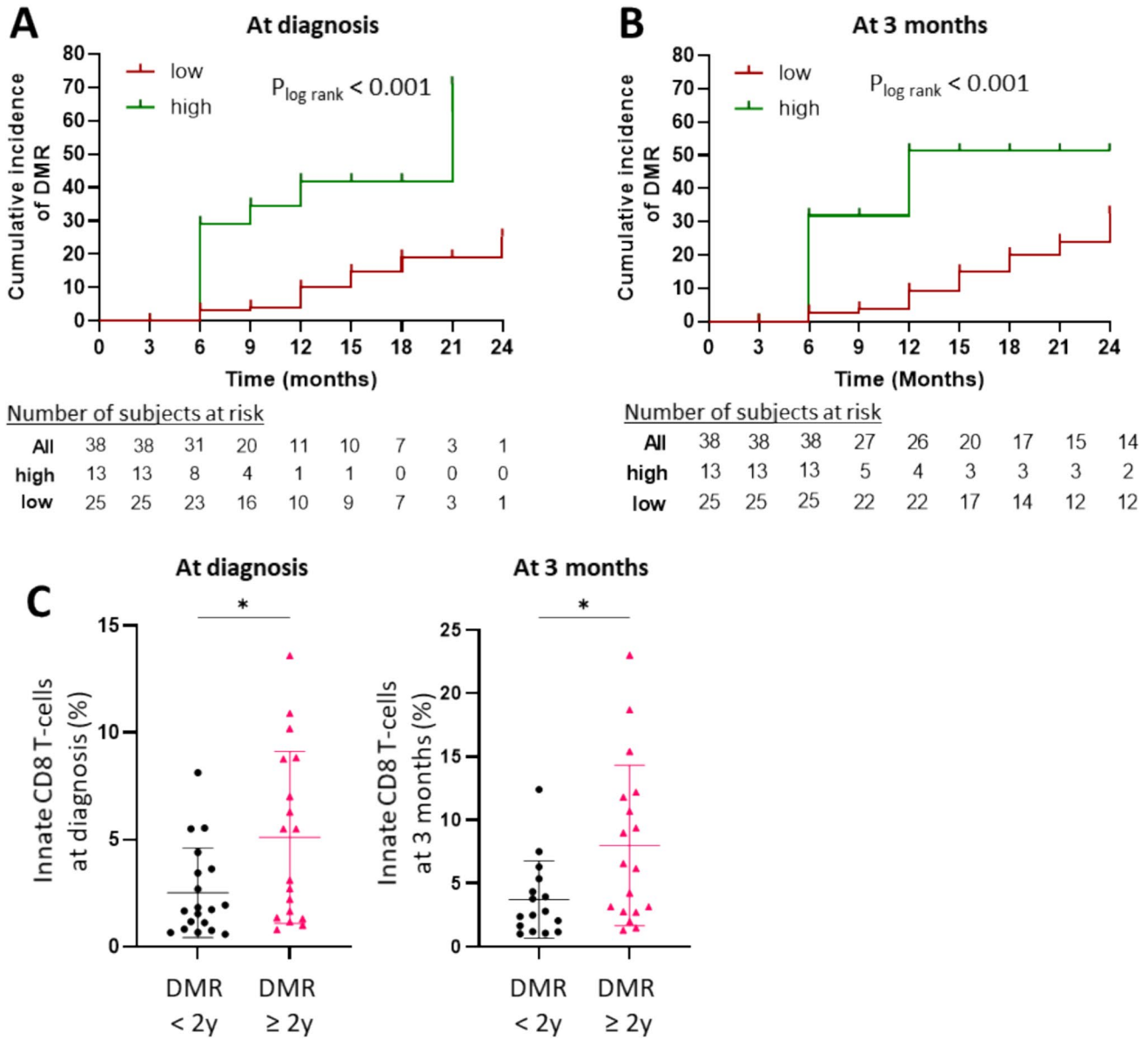


Fig. 3 High innate CD8 T-cell frequency in CML patients at diagnosis and after three months of treatment is associated with both achieved and sustained DMR. **a** and **b** Cumulative incidence of the deep molecular response (DMR) over the first 24 months of therapy in patients with low (red line) or high (green line) innate CD8 T-cell frequency at diagnosis (**a**) or at 3 months (**b**). The number of subjects at risk is shown below the curves. The optimal cut-off values of innate CD8 T-cell frequency (5.5% at diagnosis and 6.4% at 3 months) were calculated using the Youden index. Data are expressed with Kaplan–Meier curves. Statistical analysis: Mantel–cox test. **c** Frequencies of innate CD8 T-cells at diagnosis (left panel) or at 3 months (right panel) in patients achieving a stable DMR over 2 years (DMR ≥ 2y; $n = 18$) or no sustained DMR ($n = 16$). Data are expressed as mean ± SD. Statistical analysis: comparison between groups was done using unpaired Mann–Whitney test

Taken together, our results demonstrate that innate CD8 T-cell frequency at M3 is associated with an early, deep and, stable response to CML therapy. Moreover, this phenomenon may be partially related to the status of this distinct CD8 T-cell compartment at the time of CML diagnosis.

Discussion

A sustained DMR has become a goal in efforts to strengthen CML stability and is a prerequisite for attempts at TKI cessation [2, 15]. Recent data in the literature highlight the potential contribution of immune effectors such as NK cells and γδ T-cells in clinical responses to TKI therapy [5, 7, 16], including achievement of DMR [17–19]. Here,

we focused on new effector CD8 T-cells, specifically innate CD8 T-cells, which consist of unconventional T-cells with innate-like responses. We provided evidence for their close association with the achievement of sustained DMR in a prospective longitudinal study in the DASA-PegIFN clinical trial [13, 20].

We have previously reported deficiencies of innate CD8 T-cells at CML diagnosis that were at least partially corrected in patients having achieved complete cytogenetic remission and MMR following TKI therapy [10, 11]. Here, to reach definitive conclusions on the involvement of innate CD8 T-cells in CML control, we conducted a prospective monitoring from CML diagnosis and through 24 months of treatment. To date, very few immunological longitudinal studies have been carried out [6, 14], particularly taking the patient's initial immune status into account.

In this work, we observed an increased frequency of innate CD8 T-cells in most patients (79.4%) as early as the third month of treatment, i.e., during the initial treatment phase with dasatinib alone. Mechanistically, dasatinib has been described to have immune-mediated effects [7, 16, 21–23], in particular on T-cells. It could act either by driving the proliferation of innate CD8 T-cells or by increasing their number through differentiation from their conventional CD8 T-cell counterparts.

Consistent with the latter hypothesis, we have previously shown in a murine model of dasatinib oral gavage that this TKI induced a drastic decrease in thymic memory CD8 T-cells with a shift toward innate CD8 T-cells [24]. In the present study, we demonstrated that the increased proportion of innate CD8 T-cell compartments is concomitant with increased conventional CD8 memory T-cell frequency, and is also closely associated with decreased frequency of naïve CD8 T-cells, suggesting a shift from naïve to memory T-cells. These results are also congruent with a previous study [14], which demonstrated significant phenotypic changes in immune effector CD8 T-cells toward an EMRA phenotype after three months of treatment with dasatinib. Indeed, we previously showed that the innate CD8 T-cell compartment is primarily composed of memory cells exhibiting an EMRA phenotype and preferentially expressing the surface molecule CD57, a terminal differentiation marker [9].

Following the M3 frequency peak, we observed a decrease in the frequency of innate CD8 T-cells in the whole cohort. This observation is consistent with the decrease in EMRA CD8 T-cells reported by Huuhtanen et al. [14], after administration of IFN-α in combination with dasatinib. They demonstrated that IFN-α broadens the immune repertoire and increases the number of costimulatory intercellular interactions, highlighting the positive immunomodulatory effects of IFN-α. However, in their clinical trial, as in ours, no patients without IFN-α treatment were included, therefore no definitive conclusions can be drawn about the impact of IFN-α. Nevertheless, in our study, given that patients began treatment with Peg-IFN at 3 months, this cannot have had an impact on the frequencies of innate CD8 T-cells measured at diagnosis and at 3 months. Furthermore, we showed that a high level of innate CD8 T-cell frequency was associated with DMR independently of the duration of IFN-α treatment.

As expected, a high proportion of DMR (42.1%) was obtained at 12 months in our DASAPeg-IFN trial. This allowed us to compare the frequency of innate CD8 T-cells between patients achieving DMR at 12 months (DMR, $n=16$) and those who did not (noDMR, $n=22$). Remarkably, although an increased proportion of innate CD8 T-cells was observed in both DMR and noDMR groups between diagnosis and the third month of dasatinib treatment, frequency of these cells measured at three months was significantly higher in the DMR group. Moreover, the higher level of innate CD8 T-cell frequency in DMR patients at 12 months was maintained during the following 12 months (after 24 months of treatment). On the other hand, conventional-memory T-cells did not discriminate between DMR and noDMR patient groups, indicating that innate CD8 T-cells are specifically associated with DMR achievement. Finally, the high proportion of innate CD8 T-cells at M3 was associated with sustained 2y-DMR, suggesting that innate CD8 T-cells influence not only the kinetics and rate of reaching DMR, but also its stability over time.

Another important aspect revealed by our study concerns the potential role of the patient's immune system state at the time of diagnosis in determining the depth of treatment response. Indeed, despite a quantitative deficit in innate CD8 T-cells, at the time of diagnosis, their proportion tends to be higher in patients who achieve/sustain DMR than in those who do not. Moreover, the association of innate CD8 T-cells rates with DMR from diagnostic suggests that it is not strictly dependent on the type of TKI used, but this needs to be confirmed as the association is stronger after the first three months of dasatinib monotherapy. These data allow us to propose a scenario in which the more innate CD8 T-cells are represented at the time of diagnosis, the more efficiently they will be amplified by dasatinib so as to achieve stable DMR.

All in all, our data lead us to suggest that innate CD8 T-cell frequency may be an independent predictive immune marker of the achievement of stable DMR in CML patients.

Of note, this hypothesis was obtained despite the small number of patients who were essentially at low-risk category based on Sokal and ELTS prognosis scores.

Given this particular patients' clinical status distribution in the DASAPeg-IFN trial, with the majority of them classified at Sokal/ELTS low-risk (a consequence of age and Peg-IFN eligibility criteria at M3), our finding need to be replicated using a longitudinal validation cohort including

patients with higher Sokal/ELTS score and treated first line with other TKIs than dasatinib. Also, in the future validation cohort, monitoring of NK and γδ-T cells, already described for their association with clinical response in CML [3–7], will be required to test whether innate CD8 T-cell frequency functions as an independent predictive factor.

Another limitation is that we only analyzed the numerical level of innate CD8 T-cells, without studying their antitumor functions, especially those of the innate type. Further studies focusing on innate CD8 T-cells, including an analysis of their innate-associated transcription factors, effector markers (perforin, granzyme B) and immune checkpoints, may be useful to identify a full DMR immune signature. However, these cells also harbor an adaptative capacity, with IFN-γ secretion in response to a TCR dependent stimulation (CD3/CD28) [8]. Therefore, we cannot rule out a reactivity of innate CD8 T-cells to CML antigens, such as PR-1. Indeed, a previous study showed an enrichment of EMRA CD8 T-cells with an anti-PR-1 phenotype at CML diagnosis and in patients maintaining TFR [25].

In CML, biomarkers are now needed to predict both the achievement of stable DMR and the success of treatment discontinuation. In this respect, a new paradigm would assume that innate CD8 T-cells, alone or in association with other immune populations, especially NK and γδ-T cells, can serve as a longitudinal predictive marker from diagnosis until eligibility for treatment discontinuation. By providing evidence that an individual's innate CD8 T-cell immune profile as early as diagnosis may be a predictive marker of stable DMR, our data support this assumption.

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Author contributions AB, LL, EC, AD and FJ designed the experiments, performed the experiments, analyzed and interpreted the data. NP contributed to sample preparation from patients and healthy controls and performed the experiments. LR, JCC, FG and FN provided clinical samples and contributed to the interpretation of data. SR, AB, AD and LL performed the statiscal analysis. AB, EC, AD and LL wrote the first draft of the manuscript. LR, AB, AH and JMG together were responsible for the overall study design, supervised the project and take primary responsibility for writing the manuscript.

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Data availability The datasets generated and analysed during the current study are available from the corresponding author on reasonable request. Email: andre.herbelin@inserm.fr.

Declarations

Conflict of interest The authors declare no competing interests.

DASA-PegIFN study investigators Lydia Roy, Agnès Guerci-Bresler, Martine Escoffre-Barbe, Stéphane Giraudier, Aude Charbonnier, Viviane Dubruille, Françoise Huguet, Hyacinthe Johnson-Ansah, Pascal Lenain, Shanti Ame, Gabriel Etienne, Delphine Rea, Pascale Cony-Makhoul, Stéphane Courby, Jean-Christophe Ianotto, Laurence Legros, Antoine Machet, Valérie Coiteux, Eric Hermet, Francois-Xavier Mahon, Philippe Rousselot, Franck Nicolini, Emilie Cayssials, and François Guilhot were DASA-PegIFN study investigators.

Ethics approval The present work is a sub-study of the DASA-PegIFN study (EudraCT Number 2012–003389-42, ClinicalTrials.gov. NCT01872442) approved by the National Health regulatory authorities and ethical committee (Poitiers, France). This study is a prospective, nonrandomized phase 2 trial conducted in 22 centers in France.

Consent to participate All the participating patients provided their written informed consent for the clinical study and ancillary biological studies.

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