Received: 14 January 2023 | Accepted: 30 August 2023

DOI: 10.1111/bjh.19104

BJHaem
BRITISH JOURNAL OF HAEMATOLOGY

ORIGINAL PAPER

Blinatumomab differentially modulates peripheral blood and bone marrow immune cell repertoire: A Campus ALL study

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Funding information
AIL Bologna ODV; Associazione Italiana per la Ricerca sul Cancro, Grant/Award Number: 21198; Italian Ministry of Health, Grant/Award Number: RC-2022-2773293

Summary

Blinatumomab is the first bi-specific T-cell engager approved for relapsed or refractory B-cell precursor acute lymphoblastic leukaemia (B-ALL). Despite remarkable clinical results, the effects of blinatumomab on the host immune cell repertoire are not fully elucidated. In the present study, we characterized the peripheral blood (PB) and, for the first time, the bone marrow (BM) immune cell repertoire upon blinatumomab treatment. Twenty-nine patients with B-ALL received blinatumomab according to clinical practice. Deep multiparametric flow cytometry was used to characterize lymphoid subsets during the first treatment cycle. Blinatumomab induced a transient redistribution of PB effector T-cell subsets and Treg cells with a persistent increase in cytotoxic NK cells, which was associated with a transient up-regulation of immune checkpoint receptors on PB CD4 and CD8 T-cell subpopulations and of CD39 expression on suppressive Treg cells. Of note, BM immune T-cell subsets showed a broader post-treatment subversion, including the modulation of markers associated with a T-cell-exhausted phenotype. In conclusion, our study

indicates that blinatumomab differentially modulates the PB and BM immune cell repertoire, which may have relevant clinical implications in the therapeutic setting.

KEYWORDS
acute lymphoblastic leukaemia, blinatumomab, immune cells, immune checkpoint receptors

INTRODUCTION

Relapsed or refractory (R/R) B-cell precursor acute lymphoblastic leukaemia (B-ALL) is an entity with no established therapeutic approach and a very poor 1-year overall survival (OS).[1] Bi-specific T-cell engagers (BiTEs) are a class of monoclonal antibodies (mAbs) that by bringing patients' autologous T cells and target tumour cells into close proximity can potently activate cytotoxic T cells.[2] Blinatumomab is the first approved BiTE for the treatment of R/R CD19-positive B-ALL and of minimal residual disease-positive (MRD^+) Philadelphia chromosome-negative (Ph^-) B-ALL. Patients with R/R B-ALL have a superior OS if treated with blinatumomab over standard salvage chemotherapy.[3–5] Of note, blinatumomab can induce a deep response with a high rate of MRD negativity in responding patients, which translates into prolonged and durable complete remissions (CR).[3–5]

Despite these remarkable clinical results, the effects of blinatumomab treatment on the host immune cell repertoire are still poorly elucidated. In patients treated with blinatumomab as maintenance therapy after allogeneic stem cell transplantation, an increase of effector memory (EM) CD8 T cells has been reported[6] and, intriguingly, NK cells have been shown to increase in Ph^+ ALL patients treated frontline with the combination of blinatumomab and dasatinib in the absence of systemic chemotherapy.[7,8] A reduction of T-regulatory (Treg) cells after blinatumomab treatment was shown,[7] whereas the baseline frequency of Treg cells was inversely correlated with the response to blinatumomab.[9] Along with these effects on the number of immune cell subsets, blinatumomab can also modulate the expression of some immune-related receptors, which are associated with immune cell functions. To fully exploit the potential of T-cell-engaging immunotherapies, including blinatumomab, T-cell functionality has been postulated to represent a relevant issue.[10,11] Particularly, it has been shown that in vitro and ex vivo blinatumomab can upregulate the T-cell expression of immune checkpoint receptors, such as PD-1, Lag-3 and Tim-3,[12] the in vitro blocking of which enhances an anti-leukaemia immune response.[12] Indeed, upon blinatumomab treatment, CD8 T cells have been shown to express Lag-3 and Tim-3, suggestive of an exhausted phenotype, which is associated with a reduced response.[11]

These data have been obtained on peripheral blood (PB) samples; to our knowledge, the bone marrow (BM) immune cell repertoire after blinatumomab treatment has so far not been investigated, which limits our understanding of blinatumomab-driven effects on the immune microenvironment of ALL patients. Within the Campus ALL network in Italy, we aimed at expanding the characterization of blinatumomab-driven immune cell changes in the PB by specifically addressing the expression kinetics of immune checkpoint receptors upon the first treatment cycle. Moreover, we evaluated if the changes induced by blinatumomab in the PB immune cell repertoire may differ from those occurring in the BM.

METHODS

Patients

From December 2019 to November 2021, we studied 29 adult patients with R/R B-ALL who received blinatumomab according to normal clinical practice or an expanded access programme. This is a multicentric, prospective study, which was approved by the institutional review boards of each participating centre (Ethical Committee approval code: 217/2019/Sper/AOUBo). PB samples were collected before the start of the first cycle (at baseline), and at Days 14 and 28 (end of treatment). BM samples were collected before treatment and at Day 28. The PCR technique (RQ-PCR) was used to measure MRD levels with a lower limit of detection of 10^{-4}–10^{-5}.

Flow cytometry analysis

The same panel of reagents was purchased from Becton Dickinson Biosciences (BD) and used across the different laboratories of participating centres, where the same protocols for instrument set-up and sample preparation had been preliminarily standardized. Fresh whole blood was used for extra-cellular flow cytometry staining. The mononuclear cells were freshly isolated by Ficoll–Hypaque centrifugation (Cedarlane) from patients' PB and BM samples and used for intracellular staining of Treg cells. Multiparametric flow cytometry was used to provide an extensive characterization of lymphoid subsets such as B, T and NK cells. Percentages and absolute numbers for each antigen were analysed. All mAbs and staining reagents were purchased from BD and used according

to the manufacturer's instructions. The following mAbs were used (clone and catalogue number): CD19 (SJ25C1, 341 113), CD3 (SK7, 332 771), CD4 (SK3, 641 398), CD8 (SK1, 647 457), CD45RA (L48, 337 186), CD197 (CCR7; 150 503, 560 816), CTLA-4 (CD152; BNI3, 562 743), CD279 (PD-1; EH12, 564 494), Gal-9 (9M1-3, 565 890), Tim-3 (CD366; 7D3, 565 568), Lag-3 (CD223; T47-530, 565 616), PD-L1 (CD271; MIH1, 563 738), CD45 (2D1, 655 873), CD3 (SK7, 332 771), CD4 (SK3, 641 398), CD25 (M-A251, 562 442), FOXP3 (259D/C7, 560 045), CD127 (HIL-7R-M21, 558 598), CD45RA (L48, 337 186), CD39 (TU66, 565 469), CD45 (2D1, 655 873), CD3 (SK7, 332 771), CD16 (3G8, 557 744) and CD56 (NCAM16.2, 562 751).

Statistical analysis

Descriptive statistics of sample populations were used. The distribution of immune biomarkers is depicted with their mean and standard deviation (SD), while the absolute and relative frequencies are reported for categorical variables. The paired *t*-test was used to evaluate the modifications of biomarkers' expression over time. The conversion on a log scale guaranteed the normal distribution approximation of each biomarker and allowed us to interpret its expression over time as fold change. All estimates objected of statistical inference are reported with their 95% confidence intervals, and the threshold of 0.05 was considered for statistical significance. Boxplots and the entire analysis were performed using R language and environment for statistical computing (R Foundation for Statistical Computing).

RESULTS

Patients' characteristics

We studied 29 B-ALL patients (11 BCR-ABL$^+$ and 18 BCR-ABL$^-$) treated with blinatumomab according to normal clinical practice in a real-life setting. Eligibility criteria were relapse or refractory disease or MRD positivity after at least one prior therapy. Patients with R/R disease were 10 (34.5%), and patients with MRD$^+$ disease were 19 (65.5%). Among 19 MRD$^+$ patients, 7 were BCR-ABL$^+$ and 12 were BCR-ABL$^-$. Among 10 R/R patients, 4 were BCR-ABL$^+$ and 6 were BCR-ABL$^-$. The demographic and clinical patients' characteristics are summarized in Table 1. The median age was 43 years (range 18–66). Nineteen patients (65.5%) were male and 10 (34.5%) were female. The median number of prior therapies was 1 (range 1–5). At the time of starting treatment with blinatumomab, the median white blood cell count was 5.2×10^9/L, and the median percentage of BM blasts of patients with R/R disease prior to treatment was 24% (Table 1). Five out of 10 R/R patients (50%) achieved a morphological CR, while 15 out of 19 MRD$^+$ patients (78.9%) achieved a MRD negativity. Nine of the 29 patients were non-responders to blinatumomab. Thirteen patients (52%) underwent an allogeneic stem cell transplant after blinatumomab. At the last follow-up, 21 of the 29 patients (72.4%) are in CR.

TABLE 1 Patients' characteristics and demographics.

Characteristics	All patients ($N=29$)
Sex (M/F)	19/10 (65.5/34.5)
Median age	43 (range 18–66)
BCR-ABL pos	11 (37.9)
BCR-ABL neg	18 (62.1)
Risk (BCR-ABL neg)	
Standard	11 (61.1)
High	3 (16.7)
Very high	4 (22.2)
SNC involvement	1 (3.4)
Cytogenetic characteristics	
Wild-type karyotype	9 (2.6)
Complex karyotype	2 (0.6)
t(9;22)	4 (1.16)
Tetraploid	1 (0.3)
i(7)	1 (0.3)
t(5;11)	1 (0.3)
t(9;22), del(7)	1 (0.3)
del(7), der(22), t(9;22), +22	1 (0.3)
t(9;22), t(8;12), delETV6, i (8)	1 (0.3)
del(4), –7p	1 (0.3)
46,XX, dup(3), i(7), t(9;22), +17	1 (0.3)
Molecular characteristics	
KMT2A mutated	None
MYC mutated	1 (0.3)
TCF3–PBX1 gene fusion	1 (0.3)
Number of prior therapies (median)	1 (range 1–5)
At blinatumomab treatment	
Peripheral WBC count ($\times 10^9$/L median)	5.3 (range 2.1–223)
BM blast percentage (% median)	24 (range 2–87)

Blinatumomab induces a transient redistribution of PB effector T-cell subsets and Treg cells with a persistent increase in cytotoxic NK cells

In an attempt to identify early PB modifications in the immune cell repertoire occurring upon blinatumomab treatment, we sought to characterize changes in the immune cell subsets during and after the first cycle of blinatumomab compared to baseline. As expected, total B lymphocytes were reduced at all time points (Table 2). When focussing on T lymphocytes, CD3 and CD8 T cells decreased at Day 14 compared to baseline (1.68- and

TABLE 2 Modulation of peripheral blood (PB) immune cells and checkpoint receptors during blinatumomab treatment.

Cell type	FC Day 14 vs. baseline	Regulation	*p*	FC Day 28 vs. baseline	*p*	Regulation	FC Day 28 vs. Day 14	*p*	Regulation
Total $CD19^+$ B cells (%)	9.128	Downregulated	0.0285	131.063	0.0419	Downregulated			
$CD3^+$ T cells (cells/μL)	1.675	Downregulated	0.0322				1.413	0.0233	Upregulated
$CD8^+$ T cells (cells/μL)	1.646	Downregulated	0.0460				1.885	0.0357	Upregulated
$CD4^+$ TD T cells (cells/μL)	1.419	Downregulated	0.0342				1.472	0.0090	Upregulated
$CD8^+$ N T cells (cells/μL)							1.329	0.0126	Upregulated
$CD8^+$ EM T cells (cells/μL)							2.153	0.0301	Upregulated
$CD8^+$ TD T cells (cells/μL)							2.132	0.0299	Upregulated
Gal-9^+ $CD4^+$ EM T cells (MFI)	1.467	Upregulated	0.0393						
CTLA-4^+ $CD4^+$ EM T cells (MFI)	1.510	Upregulated	0.0130						

Note: Modulation of immune cell subsets and immune checkpoint receptors expression at baseline, Day 14 and Day 28 after the start of blinatumomab treatment. The values are represented as fold change (FC); the reference values 'baseline' or '14 days' were normalized to 1.

1.65-fold decrease; $p = 0.0322$ and $p = 0.046$, respectively) and thereafter increased at Day 28 compared to Day 14 (1.41- and 1.89-fold increase; $p = 0.0233$ and $p = 0.0357$, respectively), whereas total CD4 T cells did not change significantly (Table 2). A more in-depth analysis of T-cell subpopulations based on the CD45RA and CCR7 expression revealed a significant decrease of terminally differentiated (TD) CD4 T cells at Day 14 compared to baseline (1.42-fold decrease; $p = 0.0322$), followed by an increase at Day 28 compared to Day 14 (1.47-fold increase; $p = 0.009$) (Table 2). Moreover, naïve (N), EM and TD CD8 T cells were significantly increased at Day 28 compared to Day 14 (1.33-, 2.15- and 2.15-fold increase; $p = 0.0126$, 0.0301 and 0.0301, respectively), but not to baseline (Table 2). With regard to NK cells, a subset of cytotoxic $CD56^{dim}$ NK cells was significantly increased from baseline to Day 14 (1.37-fold increase; $p = 0.0211$) and Day 28 (1.41-fold increase; $p = 0.0062$) (Figure 1A). Finally, both total and effector Treg cells were significantly decreased at Day 28 compared to Day 14 (1.37- and 1.54-fold decrease; $p = 0.0302$ and $p = 0.0041$) (Figure 1B,C), but not to baseline.

Interestingly, when R/R and MRD^+ patients were analysed separately, some relevant differences in the immune cell repertoire after blinatumomab treatment were observed. In particular, an increase of PB CD4 and CD8 EM T-cell subsets was observed at Day 28 as compared to baseline only in R/R patients (Figure S1A,B). Moreover, effector Treg cells were downregulated at Day 28 as compared to baseline only in MRD^+ patients (Figure S1C,D).

Taken together, these data indicate that blinatumomab induces an early but transient redistribution of PB CD4 and CD8 T-cell subsets, whose frequencies at the end of the first cycle were not different from the baseline. In contrast, circulating cytotoxic NK cells and Treg cells followed different kinetics with a continuous increase and a trend towards reduction respectively. Of note, some effects of blinatumomab on the immune cell repertoire are different in R/R compared to MRD^+ patients.

Blinatumomab induces a transient upregulation of immune checkpoint receptors on PB effector CD4 and CD8 T-cell subpopulations which is paralleled by a transient increase of CD39 expression on suppressive Treg cells

To evaluate the expression of checkpoint molecules on various circulating T-cell subsets, the mean fluorescence intensity was analysed. We first evaluated the expression of immune checkpoint receptors on CD8 and CD4 T cells. Figure 2 shows that a significant increase of PD-1, Tim-3 and Lag-3 was observed on both CD4 and CD8 T cells predominantly at Day 14 compared to Day 28. In particular, PD-1, Tim-3 and Lag-3 were increased on EM CD8 T cells at Day 14 compared to baseline (1.41-, 1.57- and 1.60-fold increase; $p = 0.0026$; $p = 0.0179$ and $p = 0.0181$ respectively).

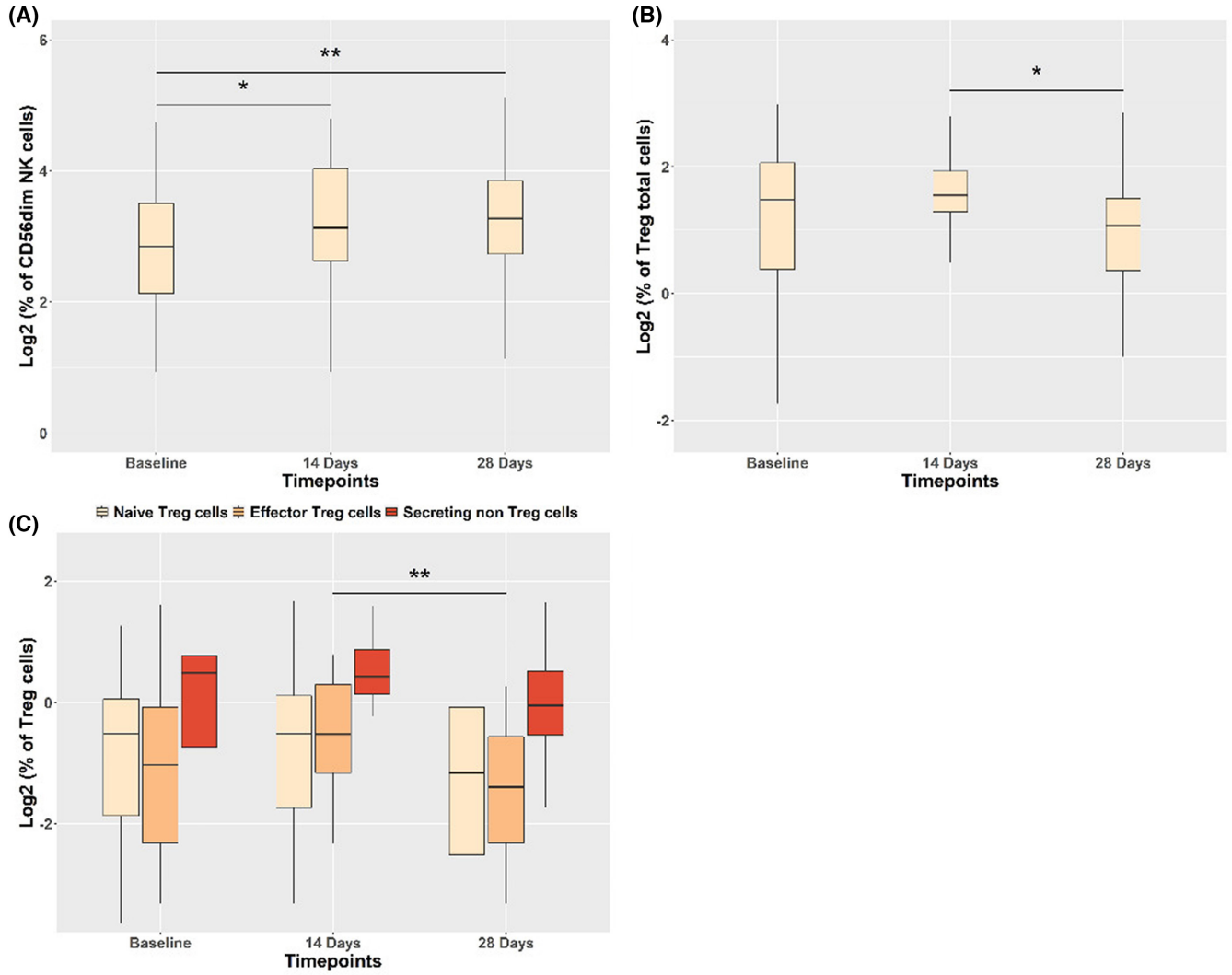

FIGURE 1 Modulation of peripheral blood (PB) immune cell subsets during blinatumomab treatment. Box plot of the comparison of $CD56^{dim}$ NK cells (A; $n = 28$), total Treg cells (B; $n = 28$) and Treg cell subpopulations (C; $n = 28$) at baseline, Days 14 and 28 after the start of blinatumomab treatment. The *y*-axis shows the log2 value of the expression percentage. 95% CI are plotted in the graph. *$p = 0.05$; **$p = 0.01$; CI, confidence interval.

PD-1 and Tim-3 expression was increased on TD CD8 T cells at Day 14 compared to baseline (1.33- and 1.57- fold increase; $p = 0.0059$ and $p = 0.0179$ respectively) (Figure 2A–C). Of note, N CD8 T cells showed an increase of Tim-3 expression at both Days 14 and 28 compared to baseline (3.14- and 1.75-fold increase; $p = 0.0201$ and $p = 0.0382$ respectively) (Figure 2B; Table 3). Regarding CD4 T cells, we found a similar expression pattern to CD8 T cells. In particular, PD-1 was increased on CM CD4 T cells (1.32-fold increase; $p = 0.0473$) (Figure 2D), whereas CTLA-4 and Gal-9 were upregulated on EM CD4 T cells at Day 14 (1.51- and 1.47-fold increase; $p = 0.013$ and $p = 0.0393$, respectively), but not at Day 28 compared to baseline (Table 2). In contrast to CD8 T cells, N CD4 T cells showed an increase of Lag-3 expression at Day 28 compared to Day 14 (1.13-fold increase; $p = 0.0236$) (Figure 2E). We then, tested Treg cells for the expression of immune checkpoint receptors across the different Treg subsets. Interestingly, in the fraction of effector Treg cells, a significant upregulation of CD39 expression was observed at Day 14 compared to baseline (1.42-fold increase; $p = 0.0029$) (Figure 2F), suggesting a transient increase in the suppressive potential of effector Treg cells during treatment.

Furthermore, a separate analysis of R/R and MRD^+ patients revealed some differences in immune checkpoints expression. In particular, in the PB of MRD^+ patients (Figure S2), but not in R/R patients (data not shown), we observed an upregulation of Lag-3 on most of the CD4 and CD8 T-cell subsets (Figure S2A–C) and an increased expression of Tim-3 and PD-1on CD8 N T cells at Day 28 as compared to baseline (Figure S2D).

These findings indicate that treatment with blinatumomab can modulate markers associated with a T-cell exhausted phenotype in the PB, including the upregulation of immune checkpoint receptors, on effector CD4 and CD8 T cells, which is paralleled by a transient increase of CD39 on suppressive Treg cells. Interestingly, in the N CD8 T-cell

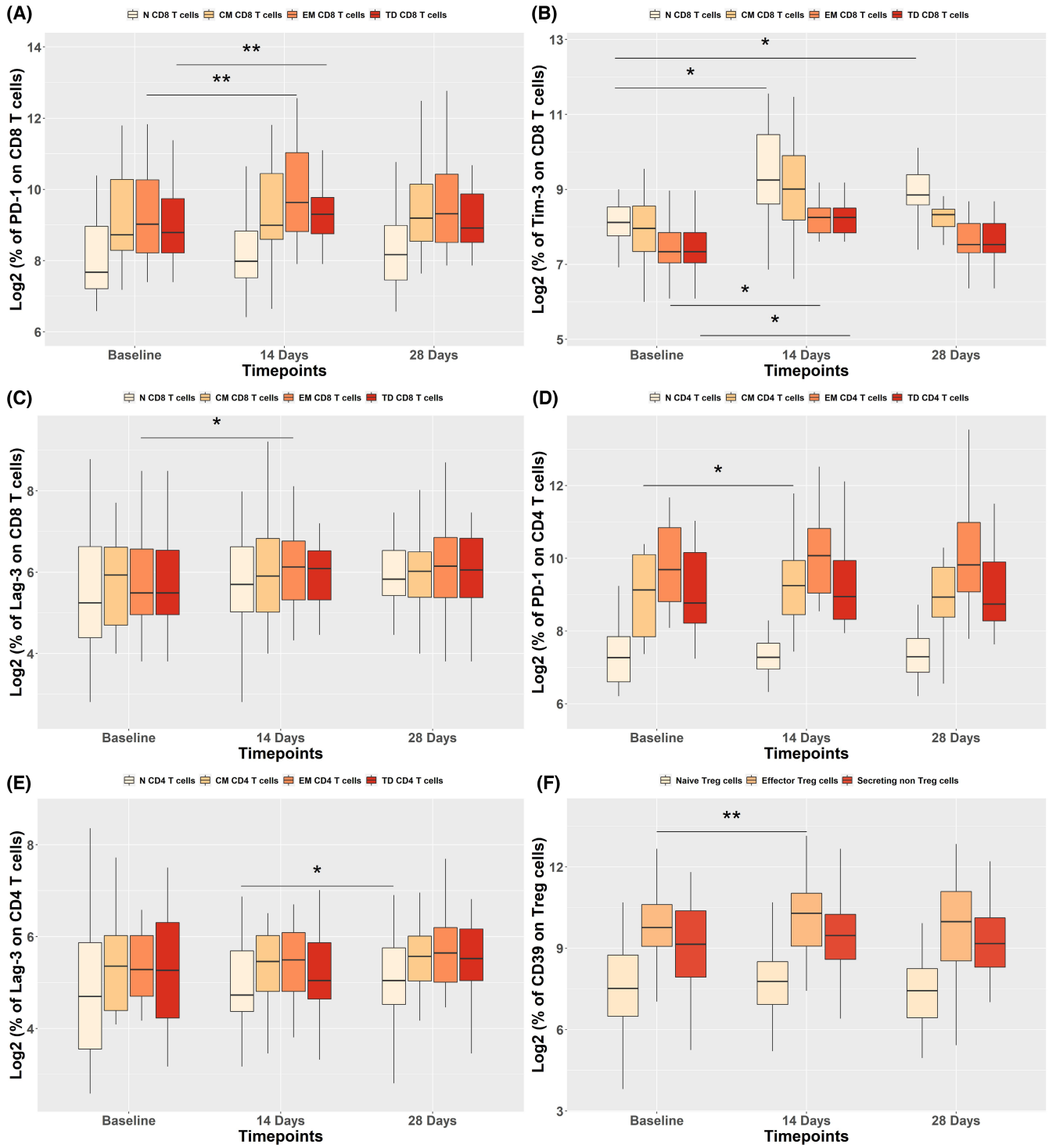

FIGURE 2 Modulation of immune checkpoint receptors on peripheral blood (PB) T cells during blinatumomab treatment. Box plot of the comparison of median fluorescence intensity (MFI) of PD-1 (A; $n = 29$), Tim-3 (B; $n = 14$) and Lag-3 (C; $n = 29$) expression on CD8 T cells and their subsets, and PD-1 (D; $n = 29$) and Lag-3 (E; $n = 29$) expression on CD4 T cells and their subsets at baseline, Days 14 and 28 after the start of blinatumomab treatment. Box plot of the comparison of MFI of CD39 on Treg cells (F; $n = 29$). The y-axis shows the log2 value of the expression percentage. 95% CI are plotted in the graph. Only statistically significant modulations are reported. * $p = 0.05$; ** $p = 0.01$; CI, confidence interval.

subset, a persistent upregulation of Tim-3 during and after blinatumomab treatment was observed. Moreover, a distinct analysis of immunological changes in MRD$^+$ and R/R patients revealed some differences in the kinetics of immune checkpoint expression on CD4 and CD8 T-cell subsets between the two groups.

TABLE 3 Modulation of peripheral blood (PB) and bone marrow (BM) immune cells and checkpoint receptors after blinatumomab treatment.

	PB						BM					
Marker	***n***	**Mean baseline**	**SD**	**Mean Day 28**	**SD**	***p***	***n***	**Mean baseline**	**SD**	**Mean Day 28**	**SD**	***p***
CD3 T cells (%)	**27**	**6.032**	**0.521**	**6.045**	**0.729**	**0.9355**	**10**	**5.233**	**0.739**	**5.974**	**0.646**	**0.0063**
CD4 T cells (%)	27	4.824	0.748	4.720	0.867	0.6494	10	3.944	1.051	4.533	0.907	0.0984
CD4 N T cells (%)	27	4.759	0.724	4.824	1.025	0.7048	9	1.954	1.615	2.365	1.241	0.5042
CD4 CM T cells (%)	26	2.509	1.224	2.360	1.220	0.6150	10	2.236	1.716	3.006	1.558	0.1174
CD4 EM T cells (%)	27	3.468	1.118	3.220	1.179	0.3357	10	2.182	0.677	2.614	0.891	0.2478
CD4 TD T cells (%)	27	3.110	0.744	3.068	1.054	0.8408	9	−0.493	1.840	−0.148	2.244	0.4267
CD8 T cells (%)	**25**	**−0.798**	**2.153**	**−0.929**	**2.116**	**0.6573**	**10**	**4.126**	**0.528**	**4.853**	**0.578**	**0.0060**
CD8 N T cells (%)	27	1.789	1.088	1.761	1.877	0.9089	10	0.478	1.760	1.288	2.367	0.1697
CD8 CM T cells (%)	27	0.409	1.376	0.300	1.188	0.6598	10	−0.456	1.646	0.581	1.353	0.0757
CD8 EM T cells (%)	**27**	**2.983**	**1.260**	**3.147**	**1.351**	**0.5314**	**10**	**2.815**	**0.911**	**3.481**	**0.661**	**0.0297**
CD8 TD T cells (%)	27	3.193	1.084	3.121	1.505	0.7642	10	2.962	0.913	3.430	0.801	0.0940
NK CD56dim cells (%)	**26**	**2.562**	**1.353**	**3.185**	**1.079**	**0.0062**	**10**	**2.419**	**1.148**	**2.825**	**0.923**	**0.1229**
NK CD56bright cells (%)	21	−1.823	1.926	−2.088	2.102	0.6304	9	−2.541	1.311	−2.434	0.985	0.8157
Total_Treg cells (%)	27	1.307	1.428	0.881	1.001	0.1506	11	0.582	1.034	0.558	1.022	0.9345
Naive_Treg cells (%)	23	−0.987	1.405	−1.287	1.428	0.4131	9	−1.074	1.164	−1.032	1.188	0.9315
Effector Treg cells (%)	26	−1.126	1.461	−1.393	1.114	0.3315	8	−1.895	0.843	−1.537	0.594	0.2690
Secreting_non-Treg cells (%)	27	−0.001	1.354	−0.111	1.040	0.7305	11	−0.337	0.967	−0.363	0.782	0.9285
Tim-3 on CD4 N T cells (MFI)	**13**	**7.394**	**0.618**	**7.561**	**0.416**	**0.3561**	**6**	**6.996**	**0.661**	**7.748**	**0.556**	**0.0336**
Tim-3 on CD4 CM T cells (MFI)	13	7.311	0.600	7.489	0.469	0.2586	6	7.025	0.651	7.320	0.601	0.3124
Tim-3 on CD4 EM T cells (MFI)	13	7.313	0.584	7.485	0.806	0.4626	6	7.122	0.857	7.231	0.858	0.7351
Tim-3 on CD4 TD T cells (MFI)	12	7.469	0.613	7.690	0.751	0.4492	6	7.234	0.646	7.168	0.923	0.8340
Tim-3 on CD8 N T cells (MFI)	13	8.032	0.803	8.847	0.742	0.0382	6	7.634	1.040	9.042	0.610	0.0633
Tim-3 on CD8 CM T cells (MFI)	13	7.901	1.018	8.195	0.675	0.4320	6	7.630	1.071	8.669	0.591	0.0895
Tim-3 on CD8 EM T cells (MFI)	**13**	**7.408**	**0.825**	**7.665**	**0.804**	**0.2912**	**6**	**7.190**	**1.026**	**7.686**	**0.771**	**0.0723**
Tim-3 on CD8 TD T cells (MFI)	13	7.408	0.825	7.665	0.804	0.2912	6	7.190	1.026	7.686	0.771	0.0723
PD-1 on CD4 N T cells (MFI)	27	7.294	0.812	7.301	0.642	0.9510	10	7.032	0.771	7.473	1.111	0.0951
PD-1 on CD4 CM T cells (MFI)	**27**	**9.018**	**1.038**	**9.020**	**1.130**	**0.9923**	**10**	**8.988**	**1.403**	**9.432**	**1.476**	**0.0451**
PD-1 on CD4 EM T cells (MFI)	27	9.799	1.061	9.978	1.290	0.2881	10	10.102	1.475	10.41	1.782	0.1393
PD-1 on CD4 TD T cells (MFI)	27	9.087	1.181	9.199	1.167	0.5188	10	8.966	1.167	9.195	1.643	0.3459
PD-1 on CD8 N T cells (MFI)	27	8.080	1.111	8.281	1.048	0.1446	27	8.080	1.111	8.281	1.048	0.1446
PD-1 on CD8 CM T cells (MFI)	27	9.278	1.301	9.390	1.204	0.3700	27	9.278	1.301	9.390	1.204	0.3700
PD-1 on CD8 EM T cells (MFI)	27	9.419	1.334	9.587	1.343	0.2914	27	9.419	1.334	9.587	1.343	0.2914

(Continues)

TABLE 3 (Continued)

	PB						BM					
Marker	***n***	**Mean baseline**	**SD**	**Mean Day 28**	**SD**	***p***	***n***	**Mean baseline**	**SD**	**Mean Day 28**	**SD**	***p***
PD-1 on CD8 TD T cells (MFI)	27	9.039	0.978	9.148	0.857	0.4518	27	9.039	0.978	9.148	0.857	0.4518
Lag-3 on CD4 N T cells (MFI)	26	4.752	1.519	5.150	1.019	0.0926	10	4.321	1.278	4.676	1.871	0.5043
Lag-3 on CD4 CM T cells (MFI)	27	5.466	1.418	5.648	0.877	0.4408	10	5.364	1.079	5.633	1.064	0.0935
Lag-3 on CD4 EM T cells (MFI)	27	5.564	1.511	5.821	1.123	0.3669	10	5.628	1.106	5.978	1.597	0.1758
Lag-3 on CD4 TD T cells (MFI)	25	5.447	1.652	5.575	1.015	0.6735	9	5.640	1.307	5.147	1.848	0.4209
Lag-3 on CD8 N T cells (MFI)	27	5.535	1.418	5.807	1.169	0.1792	10	5.261	1.169	5.998	1.101	0.0618
Lag-3 on CD8 CM T cells (MFI)	27	5.988	1.660	5.997	0.995	0.9749	10	6.033	1.034	6.260	1.167	0.2231
Lag-3 on CD8 EM T cells (MFI)	**27**	**5.838**	**1.502**	**6.196**	**1.228**	**0.1614**	**10**	**5.937**	**1.315**	**6.613**	**1.736**	**0.0297**
Lag-3 on CD8 TD T cells (MFI)	**27**	**5.723**	**1.245**	**6.046**	**1.092**	**0.0856**	**10**	**5.750**	**1.238**	**6.352**	**1.605**	**0.0281**
CTLA-4 on CD4 N T cells (MFI)	13	9.230	0.876	9.198	0.901	0.8636	6	9.100	0.733	9.680	0.650	0.1238
CTLA-4 on CD4 CM T cells (MFI)	13	9.607	1.028	9.637	0.992	0.8950	6	9.562	0.795	10.091	0.423	0.0610
CTLA-4 on CD4 EM T cells (MFI)	13	9.701	0.976	9.794	1.040	0.6990	6	9.773	0.731	10.157	0.549	0.1470
CTLA-4 on CD4 TD T cells (MFI)	12	9.558	1.048	9.573	0.995	0.9496	6	9.688	0.709	10.046	0.479	0.2115
CTLA-4 on CD8 N T cells (MFI)	13	9.497	1.104	9.469	0.911	0.9216	6	9.186	0.759	9.736	0.530	0.0909
CTLA-4 on CD8 CM T cells (MFI)	**13**	**9.825**	**1.067**	**9.661**	**1.098**	**0.5913**	**6**	**9.527**	**0.732**	**10.52**	**0.691**	**0.0191**
CTLA-4 on CD8 EM T cells (MFI)	13	9.468	0.943	9.484	1.016	0.9484	6	9.429	0.697	9.917	0.551	0.1152
CTLA-4 on CD8 TD T cells (MFI)	13	9.468	0.943	9.484	1.016	0.9484	6	9.429	0.697	9.917	0.551	0.1152
Gal-9 on CD4 N T cells (MFI)	13	9.258	1.359	9.241	0.999	0.9678	6	9.106	1.273	9.858	0.736	0.3345
Gal-9 on CD4 CM T cells (MFI)	13	9.393	1.305	9.386	0.965	0.9878	6	9.096	1.230	9.908	0.640	0.2533
Gal-9 on CD4 EM T cells (MFI)	13	9.163	1.223	9.157	0.940	0.9888	6	8.903	1.208	9.623	0.679	0.3087
Gal-9 on CD4 TD T cells (MFI)	12	9.092	1.438	8.948	0.851	0.7695	6	9.052	1.352	9.488	0.638	0.5629
Gal-9 on CD8 N T cells (MFI)	13	9.385	1.330	9.456	0.932	0.8706	6	9.014	1.116	9.891	0.541	0.1986
Gal-9 on CD8 CM T cells (MFI)	13	9.480	1.001	9.461	0.828	0.9479	6	9.093	0.950	9.971	0.550	0.1527
Gal-9 on CD8 EM T cells (MFI)	13	9.190	1.179	9.147	0.988	0.9132	6	8.853	1.235	9.562	0.676	0.3196
Gal-9 on CD8 TD T cells (MFI)	13	9.190	1.179	9.147	0.988	0.9132	6	8.853	1.235	9.562	0.676	0.3196
CD39 on naive Treg cells (MFI)	**26**	**7.670**	**1.660**	**7.084**	**1.991**	**0.0553**	**7**	**7.850**	**2.114**	**7.463**	**2.189**	**0.0432**
CD39 on effector Treg cells (MFI)	27	9.595	1.895	9.599	1.847	0.9870	7	9.583	2.422	10.218	1.823	0.0719
CD39 on secreting non-Treg cells (MFI)	27	9.096	1.627	8.977	1.747	0.6382	8	9.179	2.027	9.669	1.660	0.0620

Note: Modulation of immune cell subsets and immune check point molecules at Day 28 after the start of blinatumomab treatment compared to baseline. Expression of immune checkpoint receptors on T cells are reported as mean of fluorescence intensity (MFI). The values are represented as mean ± SD on Log scale. Statistically significant differences ($p \leq 0.05$) between PB and BM are highlighted in bold.

Blinatumomab induces an increase of BM effector T cells and an upregulation of immune checkpoint receptors on various subpopulations of BM T cells, including Treg cells

The effect of blinatumomab on BM immune cell subsets, including their expression of immune checkpoint receptors, has not been previously investigated. In parallel with PB, we evaluated the changes of the immune cell repertoire in BM samples obtained after the first cycle of blinatumomab. As shown in Figure 3, the percentages of BM CD3, CD8 and CD8 EM T cells were significantly increased at Day 28 compared to baseline (1.61-, 1.67- and 1.49-fold increase; $p = 0.0063$; $p = 0.0060$ and $p = 0.0297$ respectively). The expression levels of immune checkpoint molecules, such as PD-1, Tim-3, Lag-3, and CTLA-4 in T cells, and CD39 in Treg cells, were also evaluated. Interestingly, immune checkpoint receptors were significantly increased after treatment. Particularly, Tim-3 on N and PD-1 on CM CD4 T cells were upregulated at Day 28 compared to baseline (1.66- and 1.59-fold increase; $p = 0.0336$ and $p = 0.0451$ respectively) (Figure 4A,B). With regard to CD8 T cells, an increased expression of Lag-3 on EM and TD subsets (2.48- and 2.38-fold increase, respectively; $p = 0.0297$ and $p = 0.0281$, respectively) and of CTLA-4 on CM subset (1.95-fold increase; $p = 0.0191$) was observed after treatment (Figure 4C,D). With regard to Treg cells, the expression of CD39 was increased on N Treg cells (1.24-fold increase; $p = 0.0432$) after blinatumomab treatment (Figure 4E).

Regarding the separated analysis of BM cells from R/R and MRD^+ patients, a similar pattern was observed as that depicted in the PB. In particular, an increase of CD3, CD4 CM, CD8 and CD8 EM T cells was observed at Day 28 as compared to baseline in R/R patients (Figure S3A), but not in MRD^+ ones (data not shown). Moreover, similarly to what observed in the PB, an upregulation of immune checkpoints on effector T cells at Day 28 was mainly observed in MRD^+ and not in R/R patients. In particular, an upregulation of CTLA-4 on both CD4 and CD8 EM, and on CD8 TD T cells was observed in MRD^+ patients (Figure S3A).

Taken together, these findings indicate that, along with its effects on circulating immune cells, blinatumomab can also modulate the BM immune cell repertoire. Of note, a distinct analysis of the immunological changes in the BM of MRD^+ and R/R patients revealed the same pattern observed in the PB in terms of kinetics of immune checkpoint expression on CD4 and CD8 T-cell subsets in the two groups.

BM and PB show a differential modulation of the immune cell repertoire after blinatumomab treatment, including the expression of immune checkpoint receptors

The results obtained from the analysis of PB and BM at Day 28 after blinatumomab treatment indicate that in the two compartments, a differential modulation of immune cell repertoire is induced by blinatumomab. In particular, CD3, CD8 and CD8 EM T cells were upregulated in the BM, whereas no changes in T cells and their composition were observed in the PB (Table 3). Of note, NK cells, namely the cytotoxic $CD56^{dim}$ NK cells subset, were upregulated only in the PB but not in the BM (Table 3). With regard to immune checkpoint receptors, an increase of Tim-3 on N CD8 T cells was observed in the PB, whereas various checkpoint receptors were upregulated at the end of the first treatment cycle in the BM. Particularly, PD-1 on CM CD4, Tim-3 on N CD4, CTLA-4 on CM CD8, Lag-3 on EM and TD CD8 T cells were significantly increased. These data suggest the modulation of markers associated with an exhaustion phenotype by both CD4 and CD8 T-cell subsets after treatment, the effect of which is observed mostly in the BM (Table 3). Moreover, CD39 was also increased on N Treg cells only in the BM compared to PB, suggesting a predominant impact of blinatumomab on Treg cells in the BM compartment (Table 3).

These data indicate that at the end of the first treatment cycle, the composition of immune cell repertoire in the BM is different from PB. Regarding immune checkpoint receptors, a more pronounced modulation of their expression was observed in the BM as compared to PB.

DISCUSSION

In the present study, we investigated the immunologic repertoire of 29 B-ALL patients who were R/R or had persistent MRD positivity and had received the first cycle of blinatumomab. We have extended previous data regarding the capacity of blinatumomab to modulate effector and regulatory immune subsets by focussing on the expression of immune checkpoint and inhibitory receptors. For the first time, we compared the data obtained by flow cytometry on PB cells with those collected from the BM. Interestingly, our data indicate that, along with its effects on circulating immune cells, blinatumomab can also modulate the BM immune cell repertoire.

Some recent evidence indicates that during treatment, the most evident effect of blinatumomab is the modulation of the PB T-cell subset distribution.[6,7,13] In particular, circulating T cells were shown to decrease within the first day of infusion before recovering to baseline after approximately 1 week. This kinetic has been associated with an increase of T-cell adhesion to blood vessel endothelium as demonstrated by the upregulation of activated cell adhesion molecules, such as lymphocyte function-associated antigen 1, on T cells.[13] Our data confirm and expand these results on the redistribution kinetics of peripheral T cells, showing an on-treatment decrease of CD3, CD8 and TD CD4 T cells, followed by a recovery to baseline levels at the end of treatment (Table 2). Of note, blinatumomab was previously shown to induce an expansion of some T-cell subsets, namely EM CD4 and CD8, and TD CD8 T cells, after the first treatment cycle.[14] Our data also revealed an expansion of CD4 and

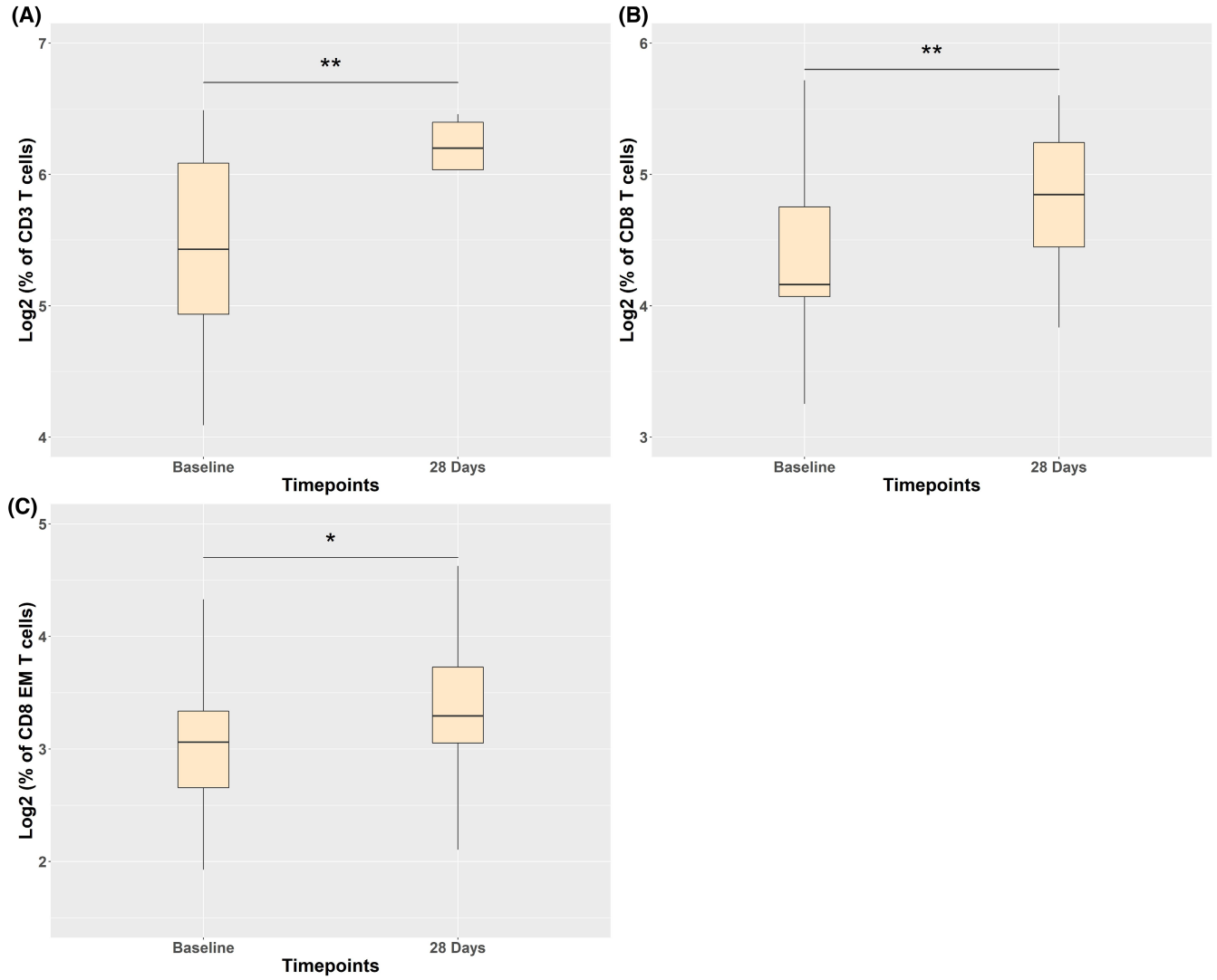

FIGURE 3 Modulation of immune cell subsets in the bone marrow (BM) after blinatumomab treatment. Box plot of the comparison of CD3 T cells (A; $n = 10$), CD8 T cells (B; $n = 10$) and EM CD8 T-cell subpopulation (C; $n = 10$) at baseline and at Day 28 after the start of blinatumomab treatment. The y-axis shows the log2 value of the expression percentage. 95% CI are plotted in the graph. *$p = 0.05$; **$p = 0.01$; CI, confidence interval.

CD8 EM T cells at Day 28 in the PB but only of R/R patients. A possible explanation for this phenomenon could be an increase of leukaemic antigen spread due to the higher levels of tumour burden in R/R patients, as compared to MRD$^+$ patients, which may result in an increased T-effector cell expansion. Since tumour burden is known to affect the immune response to immunotherapy,[15–17] these findings may suggest that the capacity of blinatumomab to affect the composition and kinetics of circulating effector T-cell subsets may vary according to the disease status. Indeed, blinatumomab seems to induce an increase of immune checkpoint expression in both CD4 and CD8 effector T-cell subsets in the PB of patients with low tumour burden (MRD$^+$) compared to R/R patients. These data support the hypothesis that the tumour burden may influence the capacity of blinatumomab to modulate the immune cell repertoire.

Interestingly, our data reveal a gradual increase, which also persists after treatment, of a subpopulation of CD3$^-$ cytotoxic NK cells. A similar pattern has been recently reported in Ph$^+$ ALL patients receiving the frontline combination of dasatinib followed by blinatumomab without systemic chemotherapy.[7,8] Although the mechanisms underlying a NK cell increase after blinatumomab therapy are unknown, an off-target effect of blinatumomab may be hypothesized. Blinatumomab is known to induce an inflammatory milieu, where the T-cell production of cytokines, such as IL-2 and IFN-γ, may enhance NK cell-mediated antibody-dependent cellular cytotoxicity.[18] Future functional studies will help to dissect the contribution of inflammatory signals in regulating NK cell function upon blinatumomab treatment.

Our characterization of the patients' immunological repertoire reveals a scenario where contrasting effects may be in place. Indeed, the above-mentioned T-cell redistribution of EM and TD CD8 T cells also coincided with an upregulation of immune checkpoint receptors, such as PD-1, Tim-3 and Lag-3. These data extend to the

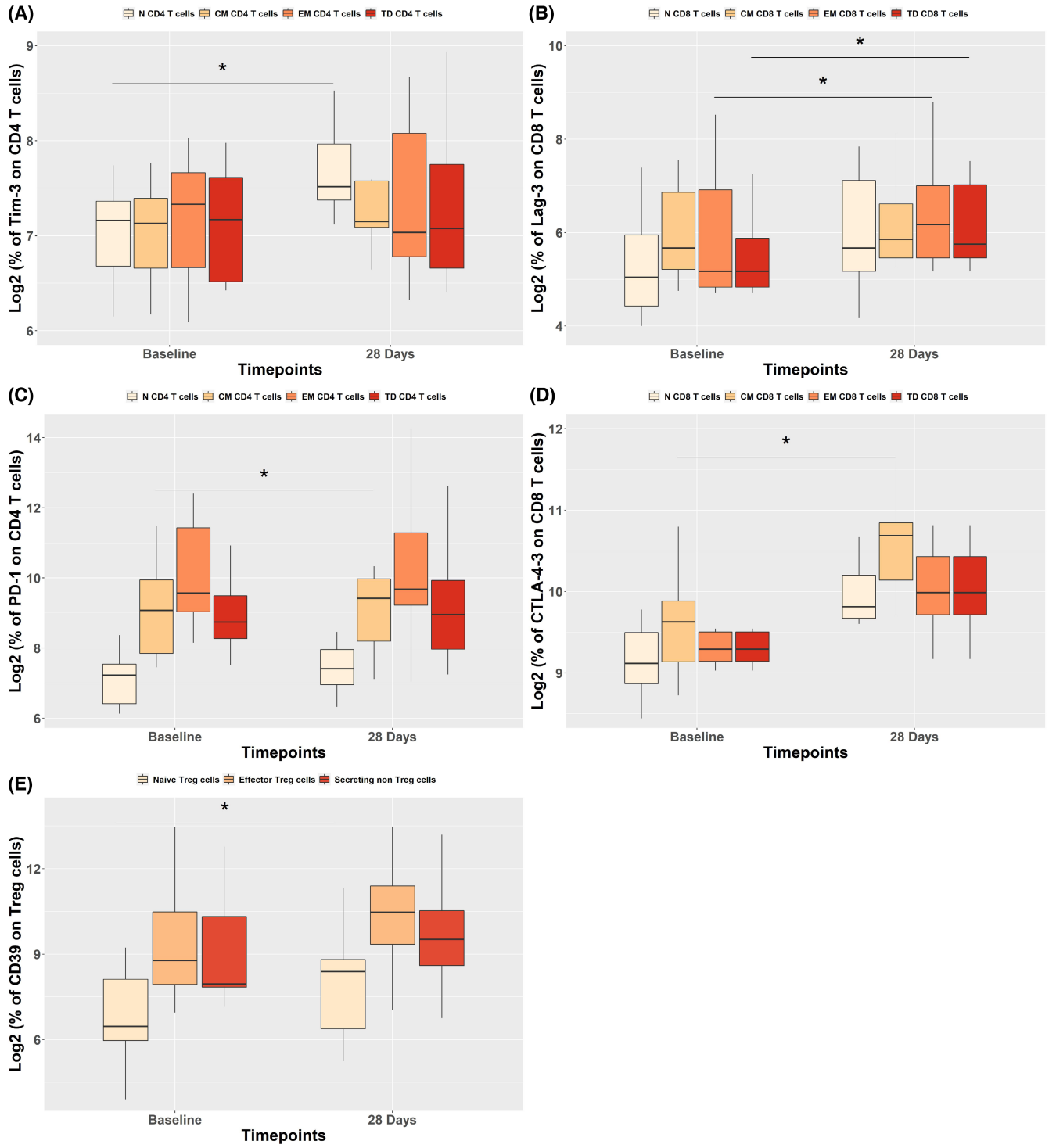

FIGURE 4 Modulation of immune checkpoint receptors on bone marrow (BM) T cells after blinatumomab treatment. Box plot of the comparison of MFI of Tim-3 (A; $n = 6$) and PD-1 (B; $n = 10$) on CD4 T cells and their subsets, and Lag-3 (C; $n = 10$) and CTLA-4 (D; $n = 6$) on CD8 T cells and their subsets at baseline and at Day 28 after the start of blinatumomab treatment. Box plot of the comparison of MFI of CD39 expression on Treg cells (E; $n = 2$). The *y*-axis shows the log2 value of the expression percentage. 95% CI are plotted in the graph. *$p = 0.05$; CI, confidence interval.

setting of blinatumomab therapy the notion that antigen encounter by EM and TD CD8 T cells may be paralleled by the concomitant upregulation of immune checkpoint receptors on antigen-primed effector subsets.[19] Accordingly, blinatumomab-induced recognition of target cells has been shown to drive a dose- and target-cell-dependent increase in the expression of T-cell exhaustion markers, such as CTLA-4, PD-1, Tim-3 and Lag-3.[12] Notably, the blockade of the PD-1–PD-L1 axis can restore T-cell functionality, for example, IFN-γ secretion and proliferation,

and further enhance effector T-cell response against ALL.[12] These and other data suggest that blinatumomab-induced modulation of effector T cells is associated with the induction of exhaustion pathways.[10] Of note, our findings on the Treg compartment indicate that, along with a 'transient' decrease of total and effector Treg cells after blinatumomab, an early, albeit transient, increase of CD39 expression was observed on effector Treg cells. CD39 is an ectonucleotidase that is responsible, together with CD73, for converting ATP into immunosuppressive adenosine released in the tumour microenvironment.[20,21] Adenosine binds A_2A receptors on various immune cells, including Treg cells, thus favouring adenosine-dependent suppression signalling and redirection of pro-inflammatory response.[22] Importantly, adenosine has been shown to increase the number of Treg cells and to further promote their immunoregulatory activity.[22] Taken together, the analysis of immune checkpoint and inhibitory receptors on T-cell subsets, including Treg cells, suggests a potent effect of blinatumomab in modulating immunosuppressive pathways, which may have a relevant impact for the full exploitation of its immunomodulatory activity. Of note, the induction of Tregs was shown to be more evident in R/R patients compared to MRD^+ patients, again suggesting a role of the tumour burden in affecting the effects of blinatumomab on the immune cell composition.

Since leukaemic cells mainly reside in the BM, a broader characterization of the immunological effects of blinatumomab should also take into consideration its capacity to modulate the BM immune cell repertoire. Since no data are available on blinatumomab-induced changes within the immune BM microenvironment,[6,9] we sought to compare the immunological effects induced by blinatumomab after the first cycle of treatment in the PB and in the BM. Our data indicate that the distribution pattern of T cells in the BM was different from that observed in the PB. Particularly, an increase of T cells, that is total and EM CD8 T cells, was observed in the BM but not in the PB. In contrast, a NK cell modulation was shown in the PB but not in the BM. Regarding the expression of immune checkpoint receptors, an increase of Tim-3 on N CD8 T cells was observed in the PB but not in the BM, where a greater modulation of immune checkpoint receptors was observed. Indeed, PD-1, Tim-3, Lag-3 and CTLA-4 were upregulated on a wide variety of BM T cells, mostly belonging to antigen-experienced subsets, and CD39 was increased on BM effector Treg cells. These data point to a different modulation of the PB and BM immune cell repertoire after blinatumomab treatment, which seems to act on the BM compartment through a more profound dysregulation of exhaustion and immunosuppressive signals. This diversity should be taken into consideration for a comprehensive characterization of the changes induced by blinatumomab on the host immune system and may reflect the different cellular compositions of the BM microenvironment. Indeed, it is well known that the immunological tumour microenvironment plays a crucial role in promoting tumour growth and progression and in affecting treatment efficacy.[23,24] The immune microenvironment is emerging as a hallmark for many haematological malignancies, including ALL.[25–27] However, a systematic and extensive characterization of the BM immune landscape correlated to the effects of immunotherapies is still lacking. BiTes, such as blinatumomab, act by bringing patients' autologous T cells and target tumour cells into close proximity, thus activating cytotoxic T cells against tumour antigens.[11] As a consequence of this mode of action, it is not surprising that the changes occurring during and after blinatumomab treatment on immune cells collected from the PB may differ from those observed in the BM, where leukaemic cells mostly reside and grow. Very few studies have made a comparison between the PB and BM immune cell repertoire in response to immunotherapies.[23,24] To our knowledge, this is the first report addressing the differential capacity of blinatumomab to modulate PB and BM immune landscapes.

Our study has some limitations. The study design was not powered to address the potential association of the modifications of immune cell subsets with the clinical response and outcome. However, the reported differences between PB and BM in the capacity of blinatumomab to modulate the composition of immune cell subsets and their phenotype may lead to consider that to fully evaluate the impact on clinical outcome of blinatumomab-induced modifications of immune repertoire, the characterization of both compartments is necessary. Moreover, it will be important to conduct the study when patients receive more than one cycle of blinatumomab to confirm previous results that have shown an increase in T and NK lymphocyte cells after multiple cycles of drug administration.[7,8]

In conclusion, our results provide for the first time a comparison of the impact of blinatumomab on PB and BM immune cells with a special focus on immune checkpoint and inhibitory molecules. Our study indicates that various subsets of NK and T cells, including Treg cells, are differently modulated in the PB and in the BM by blinatumomab treatment. More in particular, the induction of markers associated with a T-cell exhausted phenotype seems to be more evident in the BM. Studies addressing the impact of these immunological changes on clinical response and outcome in a larger cohort of patients after repeated cycles of blinatumomab are warranted.

AUTHOR CONTRIBUTIONS

Antonio Curti and Anna Rita Guarini designed the research study. Darina Ocadlikova, Giulia Corradi, Stefania De Propris, Gian Maria Borleri and Ilaria Tanasi processed patients' samples and performed flow cytometry acquisition and analyses. Darina Ocadlikova, Antonio Curti, Letizia Zannoni and Robin Foà wrote the paper. Antonio Curti, Federico Lussana, Nicola Fracchiolla, Massimiliano Bonifacio, Lidia Santoro, Mario Delia, Sabina Chiaretti, Crescenza Pasciolla, Alessandro Cignetti, Fabio Forghieri, Francesco Grimaldi and Anna Rita Guarini coordinated

patients' sampling, collected clinical data and contributed to manuscript preparation. Sergio Rutella and Jayakumar Vadakekolathu performed RNAseq experiment.

ACKNOWLEDGEMENTS

The authors thank the Becton Dickinson Biosciences for providing all monoclonal antibodies for this study. The authors thank Dr Luca Dozza for his assistance in statistical analyses of the data obtained from flow cytometry. Open access funding provided by BIBLIOSAN.

CONFLICT OF INTEREST STATEMENT

No conflicts of interest to disclose.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article. Supporting Information, further inquiries can be directed to the corresponding author.

ETHICS STATEMENT

Ethical Committee approval code: 217/2019/Sper/AOUBo.

FUNDING INFORMATION

The work reported in this publication was funded by the Italian Ministry of Health, RC-2022-2773293; AIL Bologna ODV (Associazione Italiana contro le Leucemie e Linfomi)/ Bologna Section; Associazione Italiana per la Ricerca sul Cancro (AIRC), Special 5×1000 Program Metastases (21198), Milan (Italy) to RF.

PATIENT CONSENT STATEMENT

All the patients signed the informed consent. I attached the consent form at the moment of the first submission in january.

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REFERENCES

1. Malard F, Mohty M. Acute lymphoblastic leukaemia. Lancet. 2020;395(10230):1146–62.
2. Baeuerle PA, Reinhardt C. Bispecific T-cell engaging antibodies for cancer therapy. Cancer Res. 2009;69(12):4941–4.
3. Kantarjian H, Stein A, Gökbuget N, Fielding AK, Schuh AC, Ribera JM, et al. Blinatumomab versus chemotherapy for advanced acute lymphoblastic leukemia. N Engl J Med. 2017;376(9):836–47.
4. Martinelli G, Boissel N, Chevallier P, Ottmann O, Gökbuget N, Rambaldi A, et al. Long-term follow-up of blinatumomab in patients with relapsed/refractory Philadelphia chromosome-positive B-cell precursor acute lymphoblastic leukaemia: final analysis of ALCANTARA study. Eur J Cancer. 2021;146:107–14.
5. Martinelli G, Boissel N, Chevallier P, Ottmann O, Gökbuget N, Topp MS, et al. Complete hematologic and molecular response in adult patients with relapsed/refractory Philadelphia chromosome-positive B-precursor acute lymphoblastic leukemia following treatment with Blinatumomab: results from a phase II, single-arm, multicenter study. J Clin Oncol. 2017;35(16):1795–802.
6. Gaballa MR, Banerjee P, Milton DR, Jiang X, Ganesh C, Khazal S, et al. Blinatumomab maintenance after allogeneic hematopoietic cell transplantation for B-lineage acute lymphoblastic leukemia. Blood. 2022;139(12):1908–19.
7. Foà R, Bassan R, Vitale A, Elia L, Piciocchi A, Puzzolo MC, et al. Dasatinib-Blinatumomab for Ph-positive acute lymphoblastic leukemia in adults. N Engl J Med. 2020;383(17):1613–23.
8. Puzzolo MC, Radice G, Peragine N, de Propris MS, Mariglia P, Vignetti M, et al. Host immune system modulation in Ph+ acute lymphoblastic leukemia patients treated with dasatinib and blinatumomab. Blood. 2021;138(22):2290–3.
9. Duell J, Dittrich M, Bedke T, Mueller T, Eisele F, Rosenwald A, et al. Frequency of regulatory T cells determines the outcome of the T-cell-engaging antibody blinatumomab in patients with B-precursor ALL. Leukemia. 2017;31(10):2181–90.
10. Philipp N, Kazerani M, Nicholls A, Vick B, Wulf J, Straub T, et al. T-cell exhaustion induced by continuous bispecific molecule exposure is ameliorated by treatment-free intervals. Blood. 2022;140(10):1104–18.
11. Zhao Y, Aldoss I, Qu C, Crawford JC, Gu Z, Allen EK, et al. Tumor-intrinsic and -extrinsic determinants of response to blinatumomab in adults with B-ALL. Blood. 2021;137(4):471–84.
12. Feucht J, Kayser S, Gorodezki D, Hamieh M, Döring M, Blaeschke F, et al. T-cell responses against CD19$^+$ pediatric acute lymphoblastic leukemia mediated by bispecific T-cell engager (BiTE) are regulated contrarily by PD-L1 and CD80/CD86 on leukemic blasts. Oncotarget. 2016;7(47):76902–19.
13. Nägele V, Kratzer A, Zugmaier G, Holland C, Hijazi Y, Topp MS, et al. Changes in clinical laboratory parameters and pharmacodynamic markers in response to blinatumomab treatment of patients with relapsed/refractory ALL. Exp Hematol Oncol. 2017;6:14.
14. Klinger M, Brandl C, Zugmaier G, Hijazi Y, Bargou RC, Topp MS, et al. Immunopharmacologic response of patients with B-lineage acute lymphoblastic leukemia to continuous infusion of T cell-engaging CD19/CD3-bispecific BiTE antibody blinatumomab. Blood. 2012;119(26):6226–33.
15. Kim SI, Cassella CR, Byrne KT. Tumor burden and immunotherapy: impact on immune infiltration and therapeutic outcomes. Front Immunol. 2020;11:629722.
16. Lenci E, Marcantognini G, Cognigni V, Lupi A, Rinaldi S, Cantini L, et al. Tumor burden as possible biomarker of outcome in advanced NSCLC patients treated with immunotherapy: a single center, retrospective, real-world analysis. Explor Target Antitumor Ther. 2021;2(3):227–39.
17. Maleki VS. High and low mutational burden tumors versus immunologically hot and cold tumors and response to immune checkpoint inhibitors. J Immunother Cancer. 2018;6(1):157.
18. Wang Z, Yin C, Lum LG, Simons A, Weiner GJ. Bispecific antibody-activated T cells enhance NK cell-mediated antibody-dependent cellular cytotoxicity. J Hematol Oncol. 2021;14(1):204.
19. Jiang W, He Y, He W, Wu G, Zhou X, Sheng Q, et al. Exhausted CD8$^+$ T cells in the tumor immune microenvironment: new pathways to therapy. Front Immunol. 2020;11:622509.
20. Timperi E, Barnaba V. CD39 regulation and functions in T cells. Int J Mol Sci. 2021;22(15):8068.
21. Deaglio S, Dwyer KM, Gao W, Friedman D, Usheva A, Erat A, et al. Adenosine generation catalyzed by CD39 and CD73 expressed on regulatory T cells mediates immune suppression. J Exp Med. 2007;204(6):1257–65.
22. Ohta A, Madasu M, Subramanian M, Kini R, Jones G, Choukèr A, et al. Hypoxia-induced and A2A adenosine receptor-independent

T-cell suppression is short lived and easily reversible. Int Immunol. 2014;26(2):83–91.
23. Nakou M, Katsikas G, Sidiropoulos P, Bertsias G, Papadimitraki E, Raptopoulou A, et al. Rituximab therapy reduces activated B cells in both the peripheral blood and bone marrow of patients with rheumatoid arthritis: depletion of memory B cells correlates with clinical response. Arthritis Res Ther. 2009;11(4):R131.
24. Safi S, Yamauchi Y, Stamova S, Rathinasamy A, Op den Winkel J, Jünger S, et al. Bone marrow expands the repertoire of functional T cells targeting tumor-associated antigens in patients with resectable non-small-cell lung cancer. Onco Targets Ther. 2019;8(12):e1671762.
25. Forte D, Krause DS, Andreeff M, Bonnet D, Méndez-Ferrer S. Updates on the hematologic tumor microenvironment and its therapeutic targeting. Haematologica. 2019;104(10):1928–34.
26. Lecciso M, Ocadlikova D, Sangaletti S, Trabanelli S, De Marchi E, Orioli E, et al. ATP release from chemotherapy-treated dying leukemia cells elicits an immune suppressive effect by increasing regulatory T cells and tolerogenic dendritic cells. Front Immunol. 2017;8:1918.
27. Meyer LK, Hermiston ML. The bone marrow microenvironment as a mediator of chemoresistance in acute lymphoblastic leukemia. Cancer Drug Resist. 2019;2(4):1164–77.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Ocadlikova D, Lussana F, Fracchiolla N, Bonifacio M, Santoro L, Delia M, et al. Blinatumomab differentially modulates peripheral blood and bone marrow immune cell repertoire: A Campus ALL study. Br J Haematol. 2023;203(4):637–650. https://doi.org/10.1111/bjh.19104