

Frequent, high density expression of surface CD38 as a potential therapeutic target in adult T-lineage acute lymphoblastic leukemia

Polychemotherapy is the fundamental backbone in the treatment of acute lymphoblastic leukemia (ALL). However, the spectrum of treatment modalities has been expanded in B-cell lineage (i.e., monoclonal antibodies, chimeric antibody receptor T cells), but not in T-cell lineage ALL (T-ALL). This discrepancy is likely due to a limited number of identified potential target antigens in T-ALL. In addition, T-ALL is a less frequent subtype, which further limits the evaluation of novel treatment strategies.¹ Patients with an immature T-ALL subtype exhibit a particular poor prognosis across various studies and age groups.²⁻⁴ Thus, the integration of innovative immunotherapeutic treatment modalities into the treatment strategy of poor-risk T-ALL appears sensible. The CD38 molecule is predominantly expressed on T cells and is associated with activation.⁵ Expression of CD38 is not T-cell lineage-specific, but has also been found on a variety of other hematopoietic and some non-hematopoietic cells. Therapeutic antibodies, which target CD38, have successfully entered clinical routine in the treatment of multiple myeloma. Although CD38 expression is not restricted to multiple myeloma cells, the use of these antibodies is not limited by off-target effects. It is unknown whether this is in part due to differences in cell surface expression on target and off-target cells. However, published data indicate towards improved responses to daratumumab in patients with multiple myeloma and higher expression levels of CD38 on their myeloma cells.⁶

Expression of CD38 has been demonstrated in patients with T-ALL,⁷ and clinical efficacy of daratumumab has been reported in a limited number of ALL patients,⁸ suggesting that CD38 could serve as a useful therapeutic target. In order to broaden the available knowledge about CD38 in ALL, we evaluated the frequency of its expression across various T-ALL subtypes and in a large number of patients. In addition, we compared the densities of CD38 molecules on the surface of T-ALL cells with those on residual, normal T cells. We evaluated diagnostic specimens of adult patients with ALL at the central reference laboratory of the German Multi-center Study Group on Adult Acute Lymphoblastic Leukemia (GMALL). The GMALL studies (*clinicaltrials.gov. identifier: NCT02881086, NCT02872987*) were approved by the ethical committees of the participating centers and patients gave written informed consent for biological research, including research on their archived samples. Study procedures were in accordance with the Declaration of Helsinki of 1975, as revised in 2008.

Cell samples from 401 consecutive patients were investigated

(Table 1). Of these, 121 samples were derived from patients with T-ALL, including 33 patients with an ETP immunophenotype. In addition, 280 samples from patients with various B-cell lineage ALL subtypes were assessed (Table 1).

Expression of CD38 was determined by using the antibody clone LS198.4.3 (Beckmann Coulter, Krefeld, Germany) during routine flow cytometry.⁹ Quantification of CD38 cell surface molecules was done by bead-calibrated flow cytometry using a commercially available kit (QuantiBRITE™ Beads, BD Bioscience, San Jose, California, USA), the antibody clone HB7 (BD Bioscience), and a different flow cytometer (FACSCanto II and FlowJo version 10.1, BD Bioscience), following the manufacturer's instructions (*Online Supplementary Figure S2*).

Expression of CD38 was almost uniformly present on T-ALL leukemic cells with only two (1.7%) negative cases and at least 80% of cells positive for CD38 in 108 (89%) of all tested 121 T-ALL samples (Figure 1A). In B-cell lineage ALL, 266 (95%) of 280 samples were positive for CD38, but the median percentage of CD38-positive leukemic cells was significantly lower compared to T-ALL (84% vs. 98%, respectively; $P < 0.0001$), and CD38 was present on at least 80% of cells in only 161 (58%) of 280 B-cell lineage ALL samples (Figure 1A). In T-ALL, the median percentage of CD38-positive cells was similar in pre- and thymic T-ALL, but lower in mature T-ALL (98%, 98% and 92%, respectively; $P \leq 0.02$; Figure 1B). Eight samples containing $\geq 10\%$ of residual, normal T cells

Table 1. Patient and sample characteristics.

Characteristics	N=121	N=280
ALL subtype	T-cell lineage 53 pre T (29 ETP-ALL) 47 thymic 21 mature (4 ETP-ALL)	B-cell lineage 18 pro B 204 common 47 pre B 11 mature
Sex M/F	90/31	147/133
Age in years range (median)	18-85 (33)	18-86 (55)
Cell sample type		
Bone marrow	92*	202**
Peripheral blood	26	75
Pleural effusion	3	-
First diagnosis/relapse	118/3	280/-

*1 and **3 undeclared specimens, most likely bone marrow aspirates. ALL: acute lymphoblastic leukemia; ETP: early T-cell precursor; M: male, F: female.

were analyzed in more detail. The median percentage of cells with CD38 expression was higher among T-ALL cells compared to normal residual T cells (Figure 1C). In one pre T-ALL sample, the percentage of CD38 expression was slightly lower on T-ALL cells *versus* residual, normal T cells (81% vs. 93%; Figure 1C). Likewise, the median fluorescence intensity (MFI) of T-ALL cells was significantly higher compared to residual, normal T lymphocytes in all 121 analyzed samples (Online Supplementary Figure S1).

The numbers of CD38 molecules per cell were quantified in 21 T-ALL samples (pre T-ALL n=8, thymic T-ALL n=8, mature n=5) using bead-calibrated flow cytometry (11 fresh samples, 10 samples thawed from liquid nitrogen). The number of CD38 molecules on the surface of T-ALL cells widely ranged from 6,406 to 80,122 (median 19,520) with significant lower median numbers in mature T-ALL compared to pre- and thymic T-ALL (8,839 vs. 25,563 and 25,723; $P=0.0194$; Figure 2A). The numbers of CD38 molecules per normal, residual T cell were considerably lower compared to T-ALL cells

across all T-ALL subgroups and ranged from 374 to 5,980 only (median 1,321 vs. 19,520; $P<0.0001$; Figure 2B). The ratios of CD38 molecules on the surface of T-ALL cells *versus* normal, residual T cells in respective samples ranged from 2.4 to 49.9 (median 16.7).

Treatment optimization in T-ALL is urgently needed in patients with high-risk T-ALL or adult patients with advanced age and deemed less suitable candidates for intensification of conventional therapies. The CD38 molecule represents a potential target for integration of respective monoclonal antibodies into the therapy algorithms of patients with ALL. We comprehensively evaluated a large number of T-ALL samples within the framework of the GMALL study group and were able to demonstrate that CD38 is almost uniformly expressed across all T-ALL subtypes. Noteworthy, expression of CD38 was more robust in T-ALL compared to B-cell lineage ALL with higher percentages of positive leukemic cells in T-ALL. Previously published studies investigated lower sample numbers from adult patients and did not

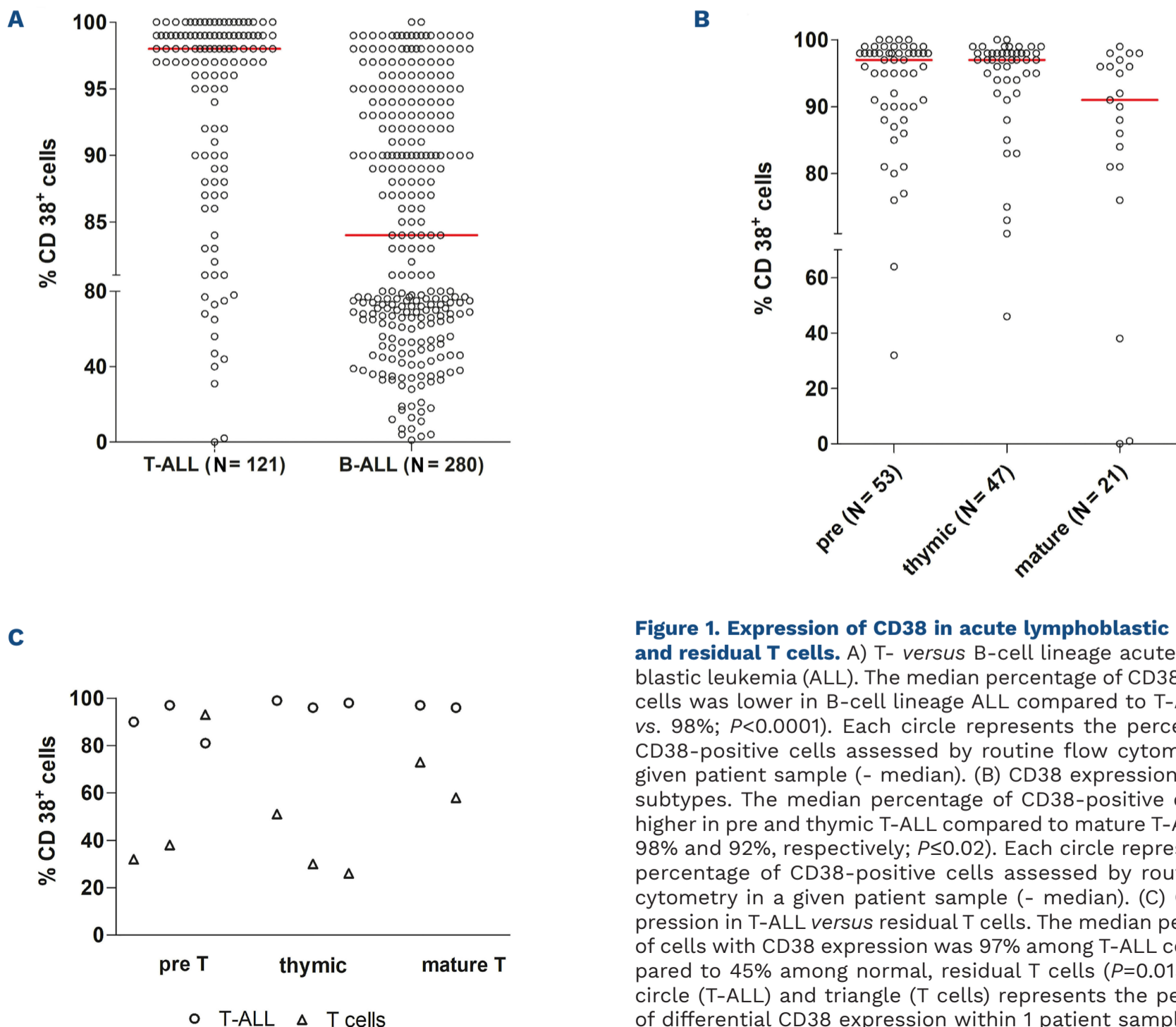


Figure 1. Expression of CD38 in acute lymphoblastic leukemia and residual T cells. A) T- *versus* B-cell lineage acute lymphoblastic leukemia (ALL). The median percentage of CD38-positive cells was lower in B-cell lineage ALL compared to T-ALL (84% vs. 98%; $P<0.0001$). Each circle represents the percentage of CD38-positive cells assessed by routine flow cytometry in a given patient sample (- median). (B) CD38 expression in T-ALL subtypes. The median percentage of CD38-positive cells was higher in pre and thymic T-ALL compared to mature T-ALL (98%, 98% and 92%, respectively; $P\leq 0.02$). Each circle represents the percentage of CD38-positive cells assessed by routine flow cytometry in a given patient sample (- median). (C) CD38 expression in T-ALL *versus* residual T cells. The median percentage of cells with CD38 expression was 97% among T-ALL cells compared to 45% among normal, residual T cells ($P=0.0156$). Each circle (T-ALL) and triangle (T cells) represents the percentage of differential CD38 expression within 1 patient sample.

differentiate the T-ALL subtype beyond ETP and non-ETP ALL. One of these studies evaluated the expression of CD38 in 83 pediatric and 105 adult patients with newly diagnosed T-ALL. Using a similar 20% cutoff for positivity, 184 (98%) of 188 diagnostic samples from these patients were categorized positive for CD38 expression. However, the median percentage of CD38-positive cells in this previously published study was less than 90% with higher numbers of samples in the low range compared to 98% in the present study.¹⁰ The confounding factors for these observed differences are likely diverse (e.g., use of different antibody clones and flow cytometers with variable setups and gating strategies), but these diverging results underscore that use of a more refined T-ALL classification and application of techniques to assess antigen densities on the target cells should be employed before considering CD38 targeted immunotherapies in T-ALL. Published data comparing the densities of CD38 on malignant and non-malignant hematopoietic cells are scarce and evaluated only a limited number of patient samples.¹¹ We observed a remarkable wide range of CD38 expression across T-ALL cells and normal T cells. It has been shown that CD38 expression is associated with T-cell activation and plays a key role in the interaction of T cells with antigen-presenting cells, which might explain at least in part the variable expression in normal T cells.¹² The numbers of cells positive for CD38 in the present study were mostly higher among leukemic cells compared to residual T cells, but there was an overlap in a subset of samples with respect to percentages of positive cells and MFI values. However, the antigen densities measured by bead-calibrated flow cytometry were consistently higher on T-ALL cells. Furthermore, the ratios

of CD38 densities on T-ALL cells *versus* normal T cells were always >2 and ranged up to 49.9. This indicates that targeting of CD38 on T-ALL cells by therapeutic antibodies is likely more efficient compared to normal T cells.

First promising results from a phase II trial evaluating daratumumab together with low-intensity chemotherapy in pediatric patients and young adults with relapsed or refractory ALL (*clinicaltrials.gov. Identifier: NCT03384654*) were released recently. In this study, the rate of complete responses ranged from 60% in young adult patients to 83% in pediatric patients with T-ALL.¹³ Various immune escape mechanisms could limit the efficacy of CD38 directed immunotherapy. Among these, myeloid checkpoint blockade through inhibition of the interaction between CD47 and its ligands has gained interest. It has recently been shown that a combined targeting of CD38 and CD47 could enhance antibody-dependent phagocytosis of T-ALL cell lines and patient derived T-ALL cells *in vitro* and prolong survival in a patient-derived T-ALL xenograft model.¹⁴ In another study, an IgA2-type variant of daratumumab exhibited more effective cell killing by neutrophils, which was further enhanced by blockade of CD47.¹⁵ Interestingly, CD38 expression was shown to be upregulated in the T-ALL cell line HSB-2 after exposure to all-*trans* retinoic acid, which was associated with enhanced cell killing, especially when combined with CD47 blockade. This suggests that the density of CD38 on target cells and the myeloid checkpoint blockade mediated through CD47 are likely of relevance. Our data demonstrate that CD38 is abundantly expressed in adult T-ALL, with very few exceptions, making this molecule an attractive target in adjunct immunotherapeutic approaches in future clinical trials.

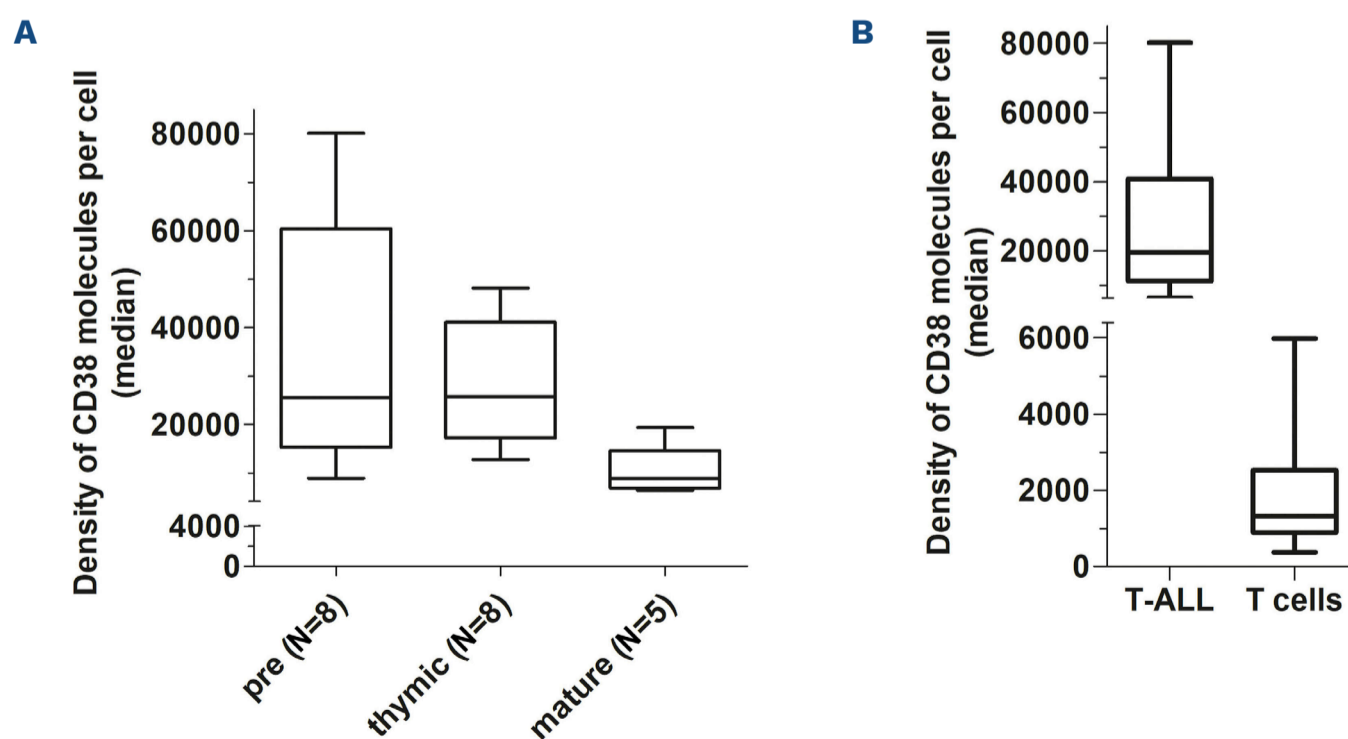


Figure 2. Densities of CD38 molecules in T-cell lineage acute lymphoblastic leukemia and normal T cells. (A) Molecule densities in T-cell lineage acute lymphoblastic leukemia (T-ALL) subtypes. The median densities of CD38 molecules on the surface of pre T, thymic and mature T-ALL cells were 25,563, 25,723 and 8,839, respectively ($P=0.0194$). (B) Molecule densities on T-ALL *versus* normal, residual T cells. The median density of CD38 molecules on the surface of T-ALL cells was 19,518 compared to 1,321 for normal, residual T cells ($P<0.0001$).

Authors

Sebastian Koslowski,¹ Rainer Glauben,² Stefan Habringer,^{1,3} Thomas Burmeister,⁴ Ulrich Keller,^{1,3,5} Monika Brüggemann,^{6,7} Nicola Gökbuget,^{3,8} and Stefan Schwartz^{1,3}

¹Department of Hematology, Oncology and Cancer Immunology (Campus Benjamin Franklin), Charité - Universitätsmedizin Berlin, corporate member of Freie Universität and Humboldt-Universität zu Berlin, Berlin; ²Department of Gastroenterology, Infectious Diseases and Rheumatology (Campus Benjamin Franklin), Charité - Universitätsmedizin Berlin, corporate member of Freie Universität and Humboldt-Universität zu Berlin, Berlin; ³German Cancer Consortium (DKTK) and German Cancer Research Center (DKFZ), Heidelberg; ⁴Department of Hematology, Oncology and Cancer Immunology (Campus Virchow-Klinikum), Charité - Universitätsmedizin Berlin, corporate member of Freie Universität Berlin and Humboldt-Universität zu Berlin, Berlin; ⁵Max-Delbrück-Center, Berlin; ⁶University Cancer Center Schleswig-Holstein (UCCSH), University Hospital Schleswig-Holstein, Kiel; ⁷Department of Medicine II, Hematology and Oncology, University Hospital Schleswig-Holstein, Kiel and ⁸ Medizinische Klinik II, Universitätsklinikum der Johann-Wolfgang-Goethe Universität, Frankfurt/Main, Germany

Correspondence:

S. SCHWARTZ - stefan.schwartz@charite.de

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Disclosures

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Contributions

SK, RG and SS performed research. SK, RG, TB, SH, MB and SS performed data analysis. SK, RG, UK and SS wrote the manuscript. MB and NG provided essential data and have made substantial contributions to the conception and design. NG, UK and SS supervised the study. All authors have drafted the work and approved the final manuscript version.

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Data-sharing statement

The laboratory protocols and original data might be obtained upon request from the corresponding author.

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