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LETTER TO THE EDITOR

CD27 expression and its association with clinical outcome in children and adults with pro-B acute lymphoblastic leukemia

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B-cell precursor acute lymphoblastic leukemia (BCP-ALL) is the most common type of cancer in children, representing up to 80% of pediatric, and ~20% of adult leukemias.[1] BCP-ALL is divided into several genetic subtypes according to acquired chromosomal aberrations with varying prognosis.[2] In children and young adults, BCP-ALL patients have an overall survival (OS) at 5 years around 90%. In contrast, OS in adults is much lower (30–40%), in part due to a higher frequency of ALL subtypes with poor prognosis.[3,4] Hence, identifying new biomarkers for patients with poor prognosis is important as it might allow the development of new approaches to treat high-risk BCP-ALL.

CD27 is a member of the tumor necrosis factor receptor superfamily that regulates lymphocyte function.[5] Expression of CD27 protein and mRNA has been reported in B-cell lymphomas [6] and adult T-cell leukemia/lymphoma.[7] In acute myeloid leukemia CD27 has been shown to be a prognostic biomarker.[8] We previously described a pro-B-cell molecular signature enriched in the *ETV6-RUNX1* subtype, and found that one of these genes is CD27.[9] Consistent with this, previous studies have shown high mRNA and surface levels of CD27 in *ETV6-RUNX1* BCP-ALL.[10,11] However, expression of CD27 in BCP-ALL of other subtypes and its potential clinical relevance is still unclear. Here, we determine the protein and mRNA expression pattern of CD27 during early B-cell development and in BCP-ALL, and investigate the prognostic relevance of *CD27* mRNA expression in pediatric and adult patients with BCP-ALL.

To confirm and extend previous observations,[10–12] we first analyzed CD27 expression during bone marrow (BM) B-cell development using flow cytometry (gating strategy, Supplementary Figure S1a). Pro-B cells ($CD19^+CD34^+IgM^-$) from all five donors expressed CD27, whereas common lymphoid progenitor (CLP, $CD19^-CD34^+IgM^-$), pre-B ($CD19^+CD34^-IgM^-$) and immature B ($CD19^+CD34^-IgM^+$) cells did not (Figures 1a and b, Supplementary Figure S1b). We also noted that only a fraction, on average 25%, of the pro-B cells were positive (Figure 1b). Thereafter, we analyzed six patient samples, of which two expressed CD27 at the time of diagnosis (Figure 1c and Supplementary Figure S1c). The CD27 levels in these two cases, classified as B-other, were much higher than those in the healthy pro-B cells, with the vast majority of the $CD19^+$ leukemic cells being strongly positive. Consistent with the CD27 protein expression pattern, we found that *CD27* mRNA levels were high in pro-B cells as compared to CLP, pre-B and immature B cells (Figure 1d). To determine which BCP-ALL subtypes express *CD27* we analyzed its mRNA levels in public data sets (Supplementary Table S1). In pediatric leukemia samples, for example, data set GSE26281, *CD27* was as expected highly expressed in over 90% of *ETV6-RUNX1,* compared to the mean level of the total samples (M_0) (Figure 1e). In addition, more than half of *BCR-ABL1* and *CRLF2*-rearranged, and a third of B-other BCP-ALL also expressed high levels of *CD27* mRNA. In adult BCP-ALL, for example, data set GSE34861, *CD27* was highly expressed in more than 60% of *BCR-ABL1* and on average in 45% of B-other BCP-ALL compared to the mean level of the total samples (M_0) in each data set (Figure 1f). Thus, *CD27* is not only highly expressed in the *ETV6-RUNX1,* but also in *BCR-ABL1*, CRLF2-rearranged and B-other BCP-ALL.

The *CRLF2*-rearranged subtype was only recently defined[13,14] and, therefore, these patient samples are found within B-other in most public data sets. To determine the *CRLF2*-rearranged subtype in data sets where this has not been defined, the *CRLF2* expression levels in already defined *CRLF2*-rearranged samples (Supplementary Table S1, GSE26281 and GSE11877) were analyzed. We found that the expression levels of *CRLF2* in all *CRLF2*-rearranged samples were at least 10-fold higher than the median levels of total samples (Supplementary Figure S2a). Based on this, samples expressing 10-fold higher level of *CRLF2* than the median are referred to as *CRLF2-high* in other data sets (Supplementary Figure S2b) with the assumption that most of these are *CRLF2*-rearranged. Thereafter, we queried which subtypes were enriched in samples with high *CD27* expression levels, by performing meta-analyses based on eight data sets with over 1500 pediatric patient samples in total and two data sets with over 250 adult patient samples (Supplementary Table S1). Samples in each data set were first divided into four clusters ($CD27^{++}$, $CD27^+$, $CD27^-$ and $CD27^{--}$) according to *CD27* mRNA levels (Supplementary Figure S2c) and thereafter pooled. Subtype distribution analysis in pediatric BCP-ALL showed that *ETV6-RUNX1, BCR-ABL1* and *CRLF2*-rearranged/*high* were proportionally enriched in the $CD27^{++}$ and $CD27^+$ clusters, whereas the opposite was observed for, for example, *KMT2A*-rearranged (Figure 1g). Also in adult samples, the *BCR-ABL1* subtype was enriched in the $CD27^{++}$ and $CD27^+$ clusters, and the opposite was observed for, for example, *KMT2A*-rearranged (Figure 1h). Thus, pediatric and adult BCP-ALL showed similar expression patterns of *CD27*.

Because *CD27* mRNA is highly expressed in the *ETV6-RUNX1* subtype that display a pro-B-cell molecular signature,[9] we hypothesized that BCP-ALL samples with high *CD27* mRNA levels would also display a pro-B signature (Figure 1i). To test this hypothesis, we performed gene set enrichment analyses (GSEA) in leukemia data sets after excluding the *ETV6-RUNX1* subtype. Independent of genetic subtype, pediatric BCP-ALL expressing high *CD27* mRNA levels ($CD27^{++}$) showed a molecular signature similar to pro-B cells (Figure 1j and Supplementary Figure S3). However, we were unable to find a pro-B signature in adult BCP-ALL, which indicates that the molecular signature is different in children and adults.

Since *CD27* mRNA was highly expressed in BCR-ABL1 and *CRLF2*-rearranged/*high* BCP-ALL that are associated with poor prognosis, we asked whether *CD27* has prognostic value. To determine this, we first analyzed clinical data available from 207 high-risk pediatric BCP-ALL patients (Supplementary Table S1, GSE11877). Dividing the patient samples into four clusters according to *CD27* expression levels, we found that the $CD27^{++}$ cluster was associated with poor OS compared with the other clusters (Figure 2a). Moreover, approximately 60% of patients in the $CD27^{++}$ cluster had relapse compared to 25–30% in the remaining clusters (Figure 2b). Thus, *CD27* expression levels correlate with clinical outcome in this cohort with high-risk pediatric BCP-ALL patients. To further confirm this observation, we analyzed the clinical data available from an additional pediatric

cohort including 75 patients (Supplementary Table S1, GSE47051). Because the *ETV6-RUNX1* subtype is associated with good prognosis in pediatric BCP-ALL,[2] we excluded this subtype in the analyses. We did not detect a significant difference in OS between the clusters (Figure 2c), whereas a significantly lower OS was observed in the combined $CD27^{++/+}$ compared to the $CD27^{-/--}$

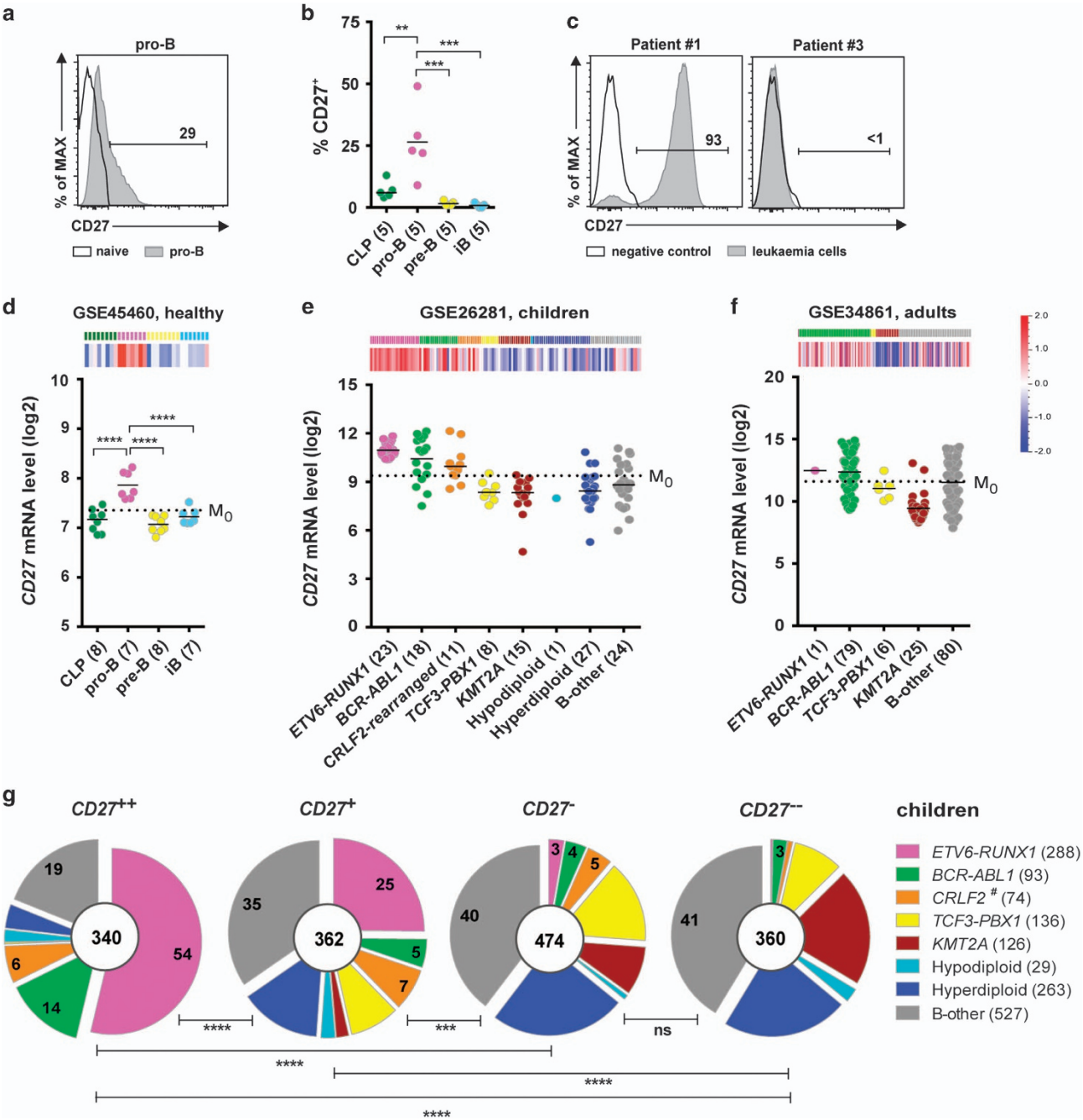

Figure 1. Comparison of CD27 expression and molecular signature between pro-B cells and BCP-ALL. (**a**) Representative histogram shows surface CD27 expression in pro-B cells. (**b**) Scatter plot shows percentages of $CD27^+$ cells in indicated subsets of BM samples from five healthy donors. (**c**) Representative histograms show CD27 surface expression in two BCP-ALL samples ($CD27^+$ and $CD27^-$). (**d**, **e**, **f**) Heat maps and scatter plots show *CD27* mRNA expression levels in (**d**) healthy (GSE45460) BM; (**e**) pediatric (GSE26281) and (**f**) adult (GSE34861) BCP-ALL samples. Dashed line in scatter plots represents M_0. (**g** and **h**) Pie charts show the proportions of BCP-ALL subtypes within each *CD27* cluster based on meta-analyzes of (**g**) eight pediatric data sets (GSE26281, GSE33315, GSE13576, GSE13425, GSE12995, Blood 2003, GSE11877, GSE47051) and (**h**) two adult data sets (GSE34861 and CCR 2005), after classifying BCP-ALL samples in each data set into four groups according to *CD27* expression levels: $CD27^{++}$ ($>M_1$), $CD27^+$ ($<M_1$ to $>M_0$), $CD27^-$ ($<M_0$ to $>M_{-1}$), $CD27^{--}$ ($<M_{-1}$). Numbers in the center of pie charts represent number of patient samples, and those in segments the proportions of the indicated subtype. M_0: mean expression level of *CD27* in all samples; M_1: mean expression of *CD27* in samples with levels above M_0; M_{-1}: mean expression of *CD27* in samples with levels below M_0. (**i**) Heat map shows genes highly expressed in pro-B cells (pro-B signature). Genes are selected according to the criteria: *P*-value (<0.05), *q*-value (<0.1) and fold change (>1.5). (**j**) Heat map (left) and GSEA enrichment plots (right) reveals a pro-B molecular signature in $CD27^{++}$ high-risk pediatric BCP-ALL data set (GSE11877). CLP, common lymphoid progenitor; iB, immature B. #*CRLF2*-rearranged (previously defined) and *CRLF2-high* (defined herein) were pooled. Statistical analysis: (**b** and **d**) one-way ANOVA and (**g** and **h**) χ^2 analysis. $^{**}P < 0.01$; $^{***}P < 0.001$; $^{****}P < 0.0001$.

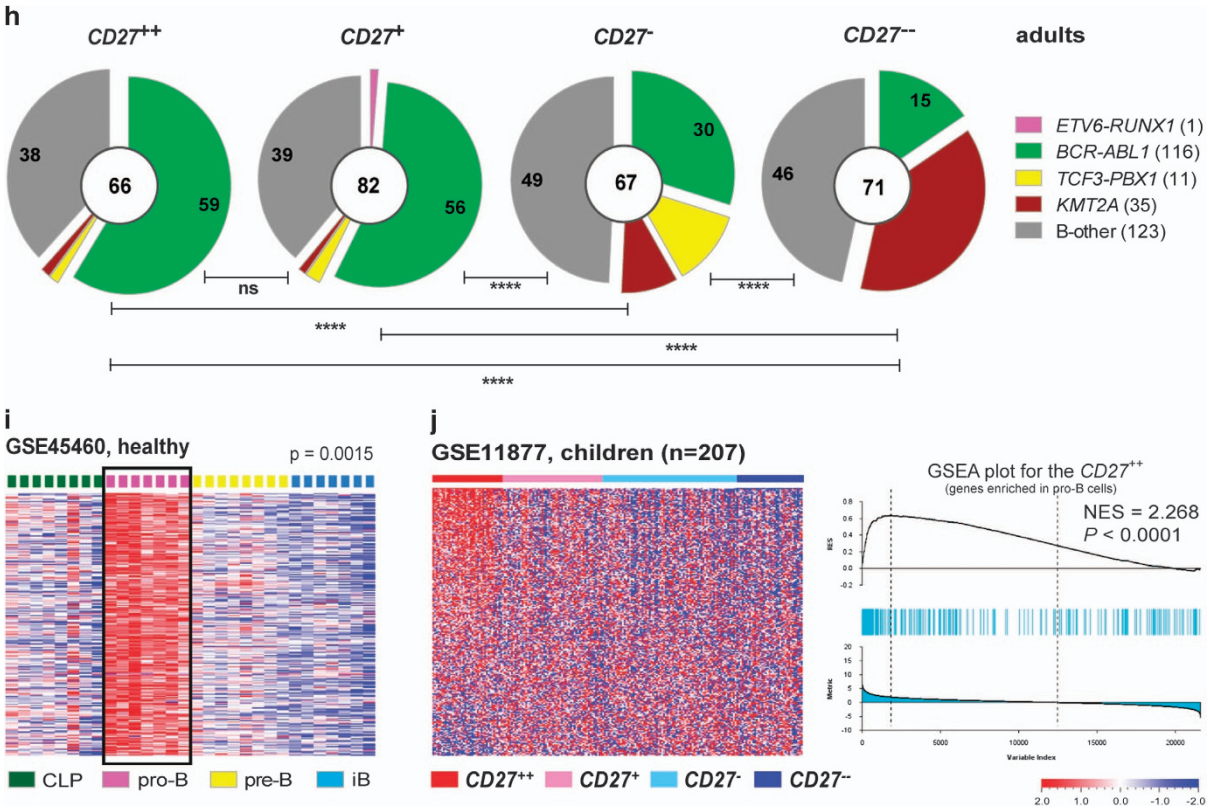

Figure 1. Continued.

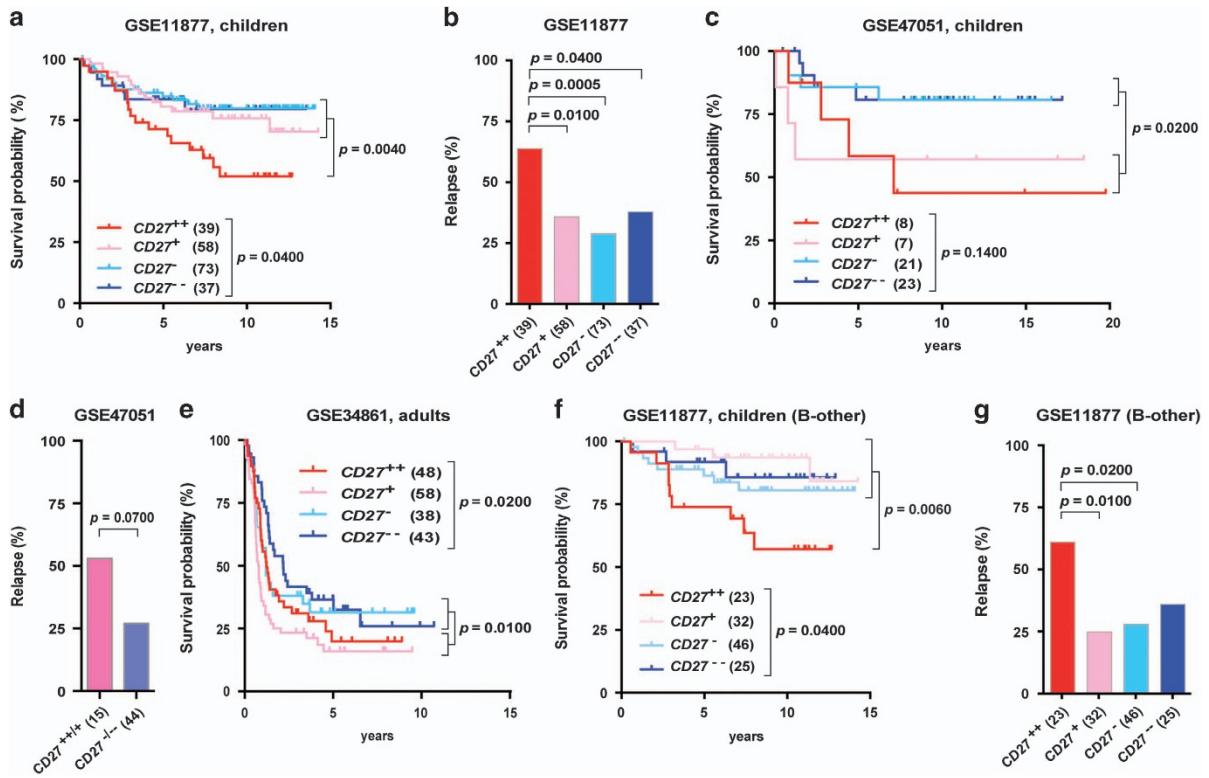

Figure 2. Clinical relevance of *CD27* mRNA levels in patients with BCP-ALL. (**a**, **c**, **e**, **f**) Kaplan–Meier Log-rank survival analysis was used to compare survival of patients within the indicated *CD27* clusters. (**b**, **d**, **g**) Percentages of patients with relapse within indicated *CD27* clusters using Fisher's exact test. (**a**, **b**) High-risk pediatric cohort GSE118877, (**c**, **d**) pediatric cohort GSE47051 (excluding *ETV6-RUNX1*); $CD27^{++/+}$, $CD27^{+}$ and $CD27^{+}$; $CD27^{-/--}$, $CD27^{-}$ and $CD27^{--}$, (**e**) adult cohort GSE34861 and (**f**, **g**) B-other patients within high-risk pediatric cohort GSE118877.

clusters (Figure 2c). There was also a trend for a higher proportion of relapse in the former (Figure 2d). We also analyzed the prognostic relevance of *CD27* expression levels in adults with BCP-ALL in the cohort with available clinical data (Supplementary Table S1, GSE34861). This showed significantly different OS for patients in the *CD27* clusters (Figure 2e). Thus, this suggests that *CD27* could be a marker for poor prognosis for non-*ETV6-RUNX1* BCP-ALL both in children and adults. Our observation is in contrast to a previous study that did not find any significant differences in OS between CD27 positive and negative BCP-ALL.[11] However, this previous study included a low proportion of high-risk patients, and the follow-up time was shorter than 3 years. In our study, all the cohorts included at least a fifth of high-risk BCP-ALL, and the follow-up time was much longer than 3 years.

In B-other BCP-ALL with unknown or not classifying genetic aberrations there are few prognostic biomarkers. As *CD27* showed differential expression pattern in B-other BCP-ALL, we asked whether *CD27* could also be used as a marker for these types of leukemia. To investigate this, the clinical data from patients with B-other BCP-ALL in the above cohorts were analyzed. In the high-risk cohort (GSE11877) with 126 B-other samples, $CD27^{++}$ mRNA levels were associated with poor OS (Figure 2f), and 60% of patients in the $CD27^{++}$ cluster experienced relapse compared to 25–35% in the remaining clusters (Figure 2g). In the other pediatric cohort (GSE47051) with only 23 B-other samples, the $CD27^{++/+}$ cluster showed a trend to poor OS (Supplementary Figure S4a). In addition, in adults with B-other BCP-ALL, the $CD27^{++/+}$ cluster also showed a trend to poor OS (Supplementary Figure S4b). Taken together, these data demonstrate that high *CD27* levels represent a poor prognostic marker for high-risk pediatric B-other BCP-ALL.

In conclusion, our findings that *CD27* is highly expressed in high-risk pediatric and adult BCP-ALL indicates that this molecule might serve as a therapeutic target, especially for the *BCR-ABL1*, *CRLF2*-rearranged/*high* and B-other subtypes. A human anti-CD27 monoclonal antibody has been developed, and *in vitro* and *in vivo* studies have shown that this antibody can exert anti-tumor activity in lymphoma xenograft models.[15] Future studies will concentrate on determining whether CD27 can indeed be used as diagnostic/prognostic markers, and whether anti-CD27 antibodies can be used to directly target BCP-ALL expressing surface CD27.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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REFERENCES

1 Zuckerman T, Rowe JM. Pathogenesis and prognostication in acute lymphoblastic leukemia. *F1000Prime Rep* 2014; **6**: 59.
2 Roberts KG, Mullighan CG. Genomics in acute lymphoblastic leukaemia: insights and treatment implications. *Nat Rev Clin Oncol* 2015; **12**: 344–357.
3 Boer JM, Koenders JE, van der Holt B, Exalto C, Sanders MA, Cornelissen JJ *et al.* Expression profiling of adult acute lymphoblastic leukemia identifies a BCR-ABL1-like subgroup characterized by high non-response and relapse rates. *Haematologica* 2015; **100**: e261–e264.
4 Toft N, Birgens H, Abrahamsson J, Bernell P, Griskevicius L, Hallbook H *et al.* Risk group assignment differs for children and adults 1-45yr with acute lymphoblastic leukemia treated by the NOPHO ALL-2008 protocol. *Eur J Haematol* 2013; **90**: 404–412.
5 Gravestein LA, Borst J. Tumor necrosis factor receptor family members in the immune system. *Semin Immunol* 1998; **10**: 423–434.
6 Dong HY, Shahsafaei A, Dorfman DM. CD148 and CD27 are expressed in B cell lymphomas derived from both memory and naive B cells. *Leuk Lymphoma* 2002; **43**: 1855–1858.
7 Shao H, Yuan CM, Xi L, Raffeld M, Morris JC, Janik JE *et al.* Minimal residual disease detection by flow cytometry in adult T-cell leukemia/lymphoma. *Am J Clin Pathol* 2010; **133**: 592–601.
8 Riether C, Schurch CM, Buhrer ED, Hinterbrandner M, Huguenin AL, Hoepner S *et al.* CD70/CD27 signaling promotes blast stemness and is a viable therapeutic target in acute myeloid leukemia. *J Exp Med* 2016; **214**: 359–380.
9 Chen D, Zheng J, Gerasimcik N, Lagerstedt K, Sjogren H, Abrahamsson J *et al.* The expression pattern of the pre-B cell receptor components correlates with cellular stage and clinical outcome in acute lymphoblastic leukemia. *PLoS One* 2016; **11**: e0162638.
10 Vaskova M, Mejstrikova E, Kalina T, Martinkova P, Omelka M, Trka J *et al.* Transfer of genomics information to flow cytometry: expression of CD27 and CD44 discriminates subtypes of acute lymphoblastic leukemia. *Leukemia* 2005; **19**: 876–878.
11 Kamazani FM, Bahoush GR, Aghaeipour M, Vaeli S, Amirghofran Z. CD44 and CD27 expression pattern in B cell precursor acute lymphoblastic leukemia and its clinical significance. *Med Oncol* 2013; **30**: 359.
12 McWilliams L, Su KY, Liang X, Liao D, Floyd S, Amos J *et al.* The human fetal lymphocyte lineage: identification by CD27 and LIN28B expression in B cell progenitors. *J Leukoc Biol* 2013; **94**: 991–1001.
13 Mullighan CG, Collins-Underwood JR, Phillips LA, Loudin MG, Liu W, Zhang J *et al.* Rearrangement of CRLF2 in B-progenitor- and Down syndrome-associated acute lymphoblastic leukemia. *Nat Genet* 2009; **41**: 1243–1246.
14 Russell LJ, Capasso M, Vater I, Akasaka T, Bernard OA, Calasanz MJ *et al.* Deregulated expression of cytokine receptor gene, CRLF2, is involved in lymphoid transformation in B-cell precursor acute lymphoblastic leukemia. *Blood* 2009; **114**: 2688–2698.
15 Vitale LA, He LZ, Thomas LJ, Widger J, Weidlick J, Crocker A *et al.* Development of a human monoclonal antibody for potential therapy of CD27-expressing lymphoma and leukemia. *Clin Cancer Res* 2012; **18**: 3812–3821.

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