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

Materials and Methods

Healthy donors and patient samples

Healthy bone marrow cells were isolated from five adult (age 58-85 years) patients undergoing primary total hip arthroplasty at the Sahlgrenska University Hospital, Gothenburg, Sweden. The study was approved by the Regional Ethical Review Board in Gothenburg (627-14), and conducted in accordance with the Helsinki Declaration. All patients provided written informed consent. Six patients diagnosed with BCP-ALL during 2016 from the Affiliated Hospital of Guizhou Medical University (China) were included. Bone marrow samples from BCP-ALL patients were analyzed as part of routine diagnostics. The diagnosis was based on the WHO 2008 criteria [1].

Flow cytometry

Healthy bone marrow samples were filtered using a 40μm filter (BD Biosciences), and thereafter stained with antibodies recognizing: CD27 (PE), CD19 (BV510), CD3 (APC-H7), CD34 (PerCP-Cy5.5), CD24 (PeCy7), CD38 (BV421), CD10 (APC), IgM (APC). BCP-ALL cells were stained with antibodies recognizing: CD27 (PE), CD19 (APC), CD45 (PerCP-Cy5.5), CD20 (APC) (BD Biosciences). The cells were acquired on a FACSVerse or FACSCantoII (BD Biosciences), and data were analyzed using FlowJo software (FlowJo, LLC, USA).

Gene expression microarray data

Gene expression microarray data of BCP-ALL and healthy B cells were gathered from published studies [2-12], and downloaded from the Gene Expression Omnibus (Table S1). All gene expression microarray data are log2 transformed and normalized using the Robust Multichip Average (RMA) method. To assess similarity of molecular signatures between pro-B cells (pro-B signature) and different *CD27*-clusters within BCP-ALL, gene set enrichment analysis (GSEA) was performed. The data were analyzed using Qlucore Omics Explorer 3.2 (Qlucore AB, Lund, Sweden).

Analysis of survival and relapse data

The clinical data from the high-risk pediatric patients (n=207) in data set GSE11877

and adult patients (n=187) in data set GSE34861 were collected from previous studies [10,12]. The "high-risk pediatric patients" in GSE11877 includes for instance children 8 years or older with high WBC, but excludes those with *BCR-ABL1*. Further details can be found at: http://www.ped-onc.org/diseases/ALLtrials/9906.html. The clinical data from pediatric patients (n=75) in data set GSE47051 were obtained from the Nordic Society of Paediatric Haematology and Oncology Registry.

Overall survival (OS) was estimated by the Kaplan-Meier method (Graph Pad Prism). Survival differences were assessed with the log-rank test. OS time was defined as the time from diagnosis until death or the date of last follow-up. Comparison of relapse data was determined with Fisher's exact test.

Table S1. Data sets used in this study

GEO accession	Sample	Country	Platform	Patient #	Clinical data available	Reference
GSE45460	Fetal BM	South Korea	GPL6244	8	No	[2]
GSE12995	Pediatric	USA	GPL96	175	No	[3]
Blood 2003*	Pediatric	USA	GPL96	118	No	[4]
GSE26281#	Pediatric	USA	GPL96	127	No	[5]
GSE13425	Pediatric	Netherlands	GPL96	154	No	[6]
GSE13576	Pediatric	Italy	GPL570	197	No	[7]
GSE33315	Pediatric	USA	GPL96	483	No	[8]
GSE47051	Pediatric	Sweden	GPL570	75	Yes	[9]
GSE11877#	Pediatric	USA	GPL570	207	Yes	[10]
CCR 2005*	Adult	Italy	GPL8300	95	No	[11]
GSE34861	Adult	USA	GPL15088	191	Yes	[12]

* No GEO accession number, herein termed according to journal and year.
# *CRLF2*-rearranged already defined in data set

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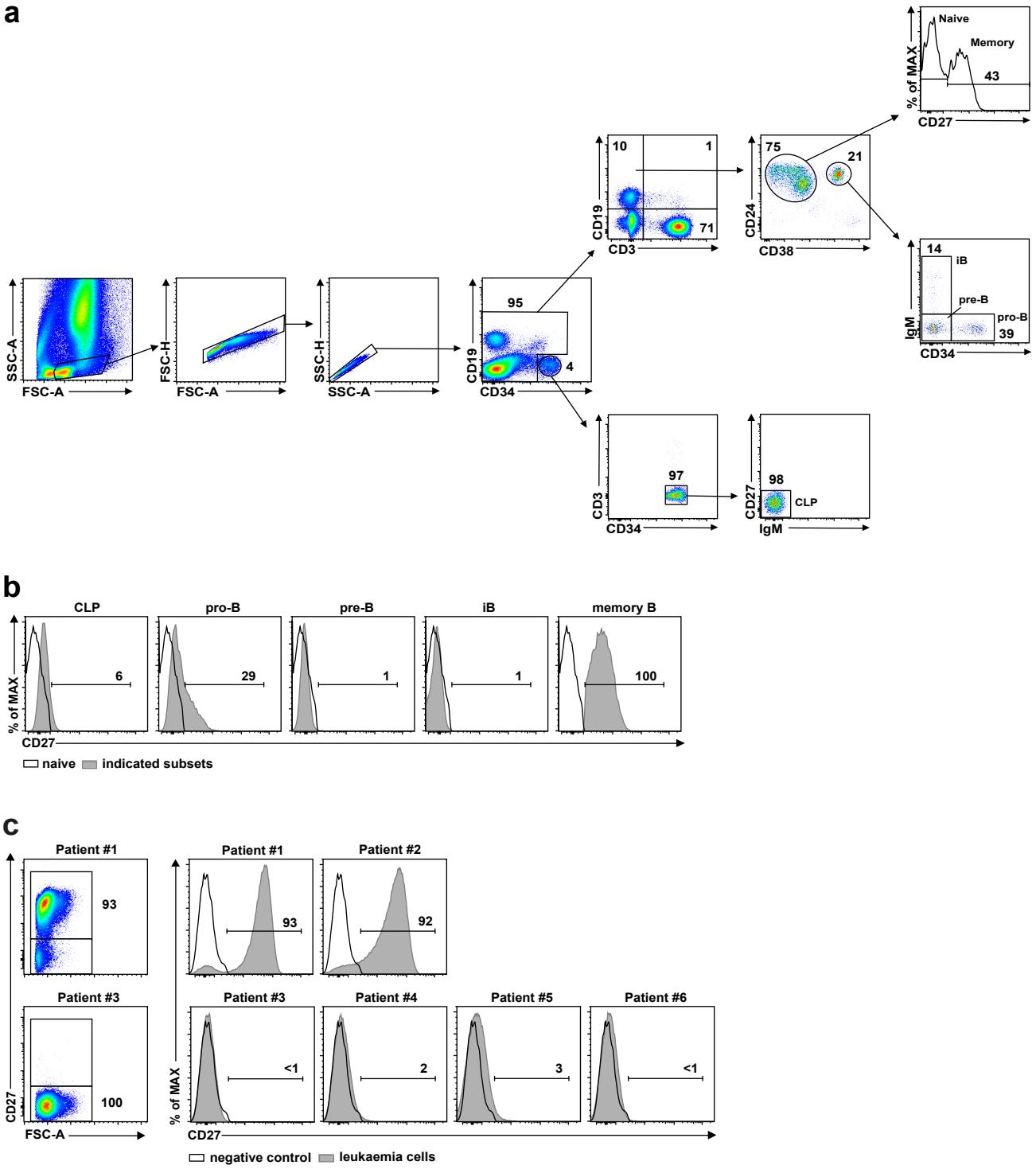
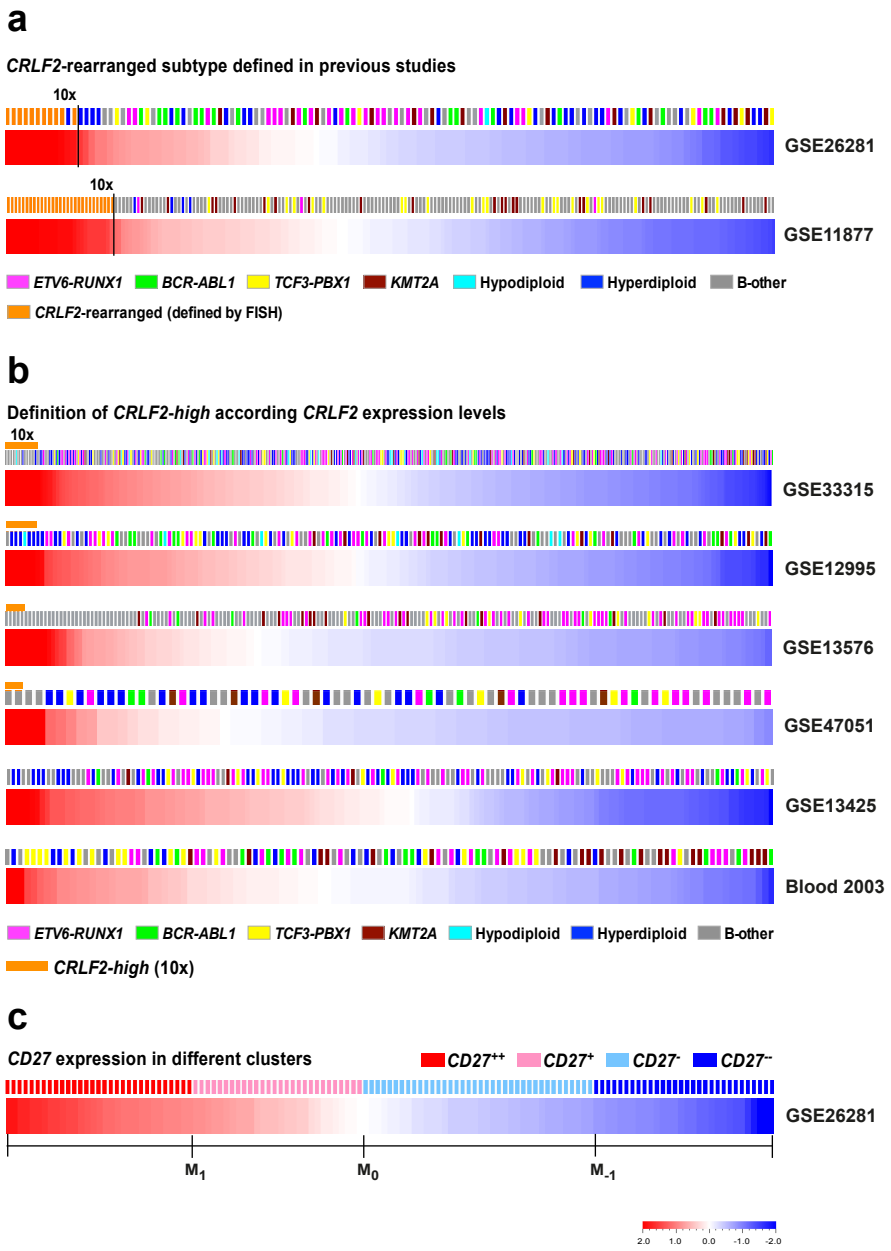
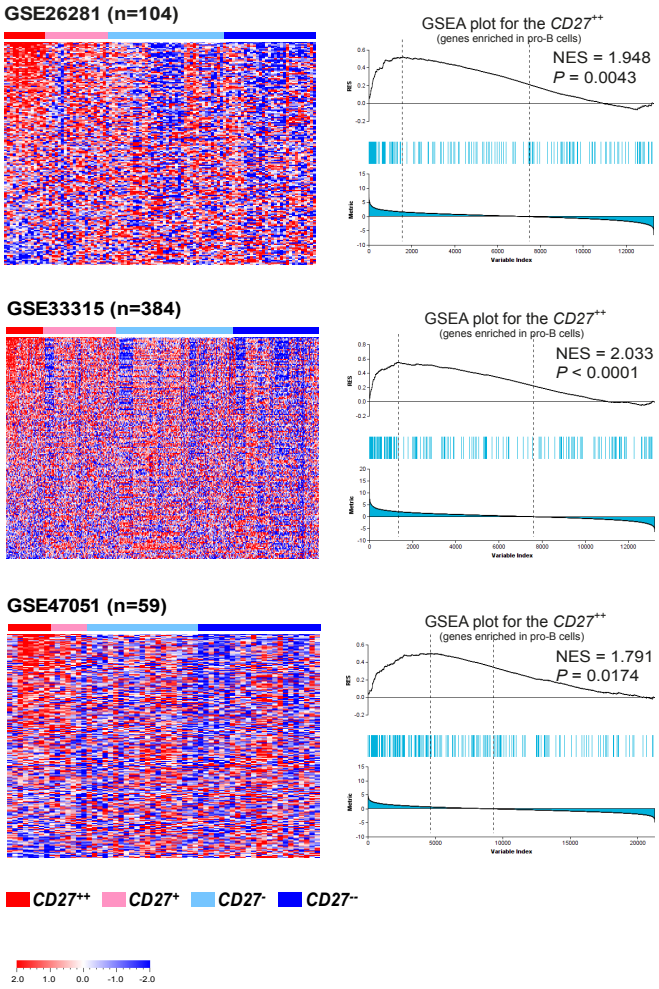


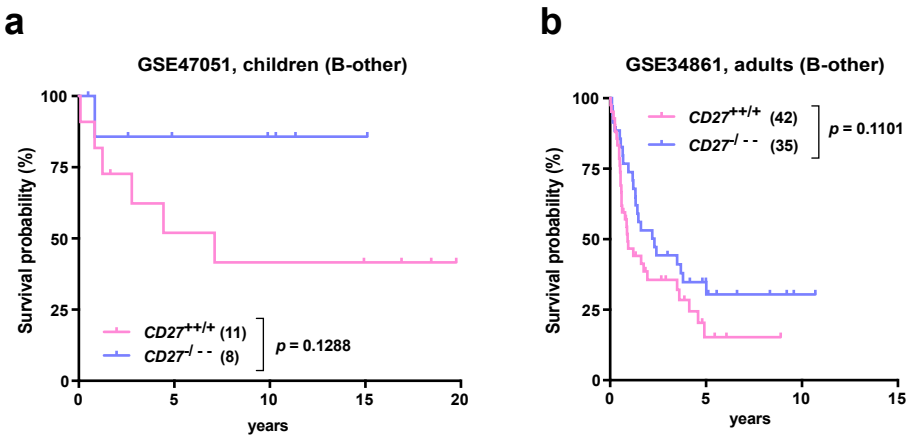
Supplementary Figure S1. (a) Gating strategy for indicated populations in healthy bone marrow, with histogram showing naïve ($CD27^-$) and memory ($CD27^+$) B cells.
CLP, $CD19^-CD34^+CD3^-CD10^-IgM^-$; pro-B, $CD19^+CD3^-CD24^+CD38^+CD34^+CD10^+IgM^-$; pre-B, $CD19^+CD3^-CD24^+CD38^+CD34^-CD10^+IgM^-$; iB, $CD19^+CD3^-CD24^+CD38^+CD34^-CD10^+IgM^+$; naïve B, $CD19^+CD3^-CD24^+CD38^+CD27^-$; memory B, $CD19^+CD3^-24^+CD38^-CD27^+$.
(b and c) Gating strategy for CD27 surface expression on (b) indicated BM populations and (b) leukemia samples ($CD19^+$). CLP, common lymphoid progenitor; iB, immature B.



Supplementary Figure S2. Definition of *CRLF2-high* and *CD27* clusters in BCP-ALL data sets. (a and b) Heat maps show *CRLF2* mRNA expression in indicated BCP-ALL data sets (Table S1). *CRLF2*-rearranged subtype (a) previously defined (b) not defined. (a) Vertical lines indicate *CRLF2* expression 10-fold above the median level. (b) *CRLF2* expression 10-fold above the median level was used as cut-off to define the subtype herein termed *CRLF2-high* (orange bar above samples). (c) Heat map shows the definition of *CD27* clusters in one of the analyzed BCP-ALL data sets. Samples were classified into four groups according to *CD27* expression levels ($CD27^{++}$, $CD27^{+}$, $CD27^{-}$ and $CD27^{--}$), where M_0 is the mean expression level of *CD27* in all samples. $CD27^{++}$ ($>M_1$), $CD27^{+}$ ($<M_1$ to $>M_0$), $CD27^{-}$ ($<M_0$ to $>M_{-1}$), $CD27^{--}$ ($<M_{-1}$).



Supplementary Figure S3. Molecular signature between pro-B cells and *CD27*$^{++}$ BCP-ALL. Heat map (left) and GSEA enrichment plots (right) reveal a pro-B molecular signature in *CD27*$^{++}$ indicated BCP-ALL data sets.



Supplementary Figure S4. Clinical relevance of *CD27* mRNA levels in patients with B-other BCP-ALL. (a and b) Kaplan-Meier Log-rank survival analysis was used to compare survival of patients within the indicated *CD27* clusters. (a) Pediatric cohort GSE47051, and (b) adult cohort GSE34861. $CD27^{++/+}$, $CD27^{++}$ and $CD27^{+}$; $CD27^{-/--}$, $CD27^{-}$ and $CD27^{--}$.