Supplementary Figures for:

Modulation of CD22 protein expression in childhood leukemia by pervasive splicing aberrations: implications for CD22-directed immunotherapies
Supplementary Figures S1-S6. 

S1. LSVs in B-cell markers. Quantification of local splice variants (LSVs) across transcripts encoding major B-cell immunotherapeutic targets when normalized by number of exons in gene. 

S2. Detection of CD22 splice isoforms. Immunoprecipitation was performed from a panel of B cell lines to enrich for CD22 isoforms. A polyclonal anti-CD22 antibody (Abcam anti-CD22 – ab25135) was used for pull-down and an N-terminally binding monoclonal anti-CD22 antibody (R&D antiCD22, MAB19681) was used for the western blotting. 

S3. Growth rates of CD22-deleted OCI-Ly8 cells transduced with CD22 splice isoforms. Growth accumulation was measured at 24h, 48h, and 72h, as described in Methods. 

S4. Aberrantly spliced CD22 isoforms and resistance to immunotherapy. Viability assay performed on CD22-deleted OCI-Ly8 cells reconstituted with indicated CD22 isoforms. Cells were treated for 96 hours with increasing concentrations of mouse anti-CD22 antibody (RFB4) followed by treatment with 100 ng/ml anti-mouse-duocarmycin (DMDM) secondary antibody drug conjugate. 

S5. Attempt to detect the CD22 Δex2-6 protein isoform by flow cytometry. Live flow cytometry staining of CD22-deleted OCI-Ly8 cells reconstituted with indicated CD22 isoforms using an anti-VSVg antibody. 

S6. Attempt to detect the CD22 Δex2-6 protein isoform by Western blotting. Uncropped western blots using antibodies directed against CD22 near the N-terminus (left gel) and near the C-terminus (right gel) in CD22-deleted OCI-Ly8 reconstituted with indicated CD22 isoforms. "CD22-no SP" denotes the CD22 construct with selective deletion of the signal peptide.
Supplementary Figures S7-S8. S7. Relative CD22 Δex5-6 expression across primary pediatric B-ALL samples. Quantitations of CD22 exon 5-6-containing and exon 5-6-skipping splice variants within baseline (pre-inotuzumab) AALL1621 B-ALL specimens. Each stack plot represents a single patient (designated by COG unique specimen identifier). S8. Sorting of primary human ALL blasts. Gating strategy used for sorting of blast cells from B-ALL specimens obtained from the AALL1621 trial (PAVDRV and PAWUXD) and for determining CD22 site density.
Supplementary Figures S9-S10. S9. Transcriptional regulation of CD22 protein expression. (A) Flow cytometric analysis and quantitation of CD22 protein density per cell of serial bone marrow biopsies from a recurrently relapsed B-ALL patient treated with inotuzumab. Included in the analysis are one pre-treatment sample (Pre-ino) and two serial post-treatment samples (Post-ino 1 and Post-ino 2). (B) Analysis of CD22 exon 2 splicing in specimens from panel A. S10. Identification of a de novo mutation in the splice junction between exon 4 and intron 4 of CD22 in a post-inotuzumab relapse sample. (A) CD22 mutational analysis based on RNA sequencing of paired Pre-ino/Post-ino PAVDRV specimens. (B) Analysis of CD22 exon 5-6 splicing in specimens from panel A.