Supplementary Methods

Primary B-ALL Cells

The vitally frozen adult B-ALL bone marrow mononuclear cells (BM MNC) obtained from the Finnish Hematology Registry and Clinical Biobank (FHRB) had been extracted with Ficoll-Paque method and frozen in medium with 90% fetal bovine serum and 10% dimethyl sulfoxide. Median age of these patients was 43 years and the average blast percent in the samples, based on the clinical BM analysis, was 86 %. Detailed patient characteristics are found in Supplementary Table 1.

Drug Sensitivity and Resistance Testing (DSRT)

A detailed design of the custom drug plate is found in Supplementary Table 2. The drugs were chosen based on either previously detected anti-leukemic activity in earlier drug-screens[1], or due to an interesting mechanism of action. The drug plate included compounds from glucocorticoids, MDM2 antagonists, and BCR-ABL1, VEGFR, BCL2, BCL-XL, BET, MEK, JAK, Aurora kinase, PI3K, MTOR, IGF1R, ERK, STAT3, STAT5, HSP90, and NAMPT protein inhibitors. Briefly, cryopreserved adult B-ALL BM MNCs were thawed and suspended in mononuclear cell medium (PromoCell) supplemented with 50µg/ml gentamicin. The cells were treated with DNase I (ThermoFisher Scientific) before counting them, and then plated in a concentration equivalent of 5,000 cells per well by using automated microplate dispenser. The cells were incubated with the drug plates for 72 hours in a humid environment at 37˚C and 5% CO_2. Cell viability (CellTiter-Glo, Promega) was measured immediately after plating and after the three-day incubation with PHERAstar FS (BMG Labtech) plate-reader. Data was analyzed using Breeze (https://breeze.fimm.fi) DSRT data

analysis pipeline[2]. DMSO and benzethonium chloride-treated wells were used as negative control and positive controls. A four-parameter dose–response curve was fitted and the drug sensitivity score (DSS) was calculated as previously described

Target Addiction Scoring (TAS)

For TAS, we collected 463 targets for 64 drugs. Of these, 33 drug targets were collected using kinase inhibitor bioactivity (KIBA). [3]The rest of the 31 drug targets were manually curated from literature. In addition, we compared, sample-wise, TAS with gene expression read counts from the same targets.

RNA Sequencing and RNA Sequencing Data Analysis

Briefly, we extracted RNA from primary BM MNC pellets using Qiagen miRNeasy kit. 3 µg of good-quality RNA (RIN 8-10) per sample was used to construct ribo-depletion based RNAseq libraries that were then sequenced using Illumina HiSeq and paired-end 100 bp reads. All patient samples were sequenced in one batch to avoid technical bias.
Processing of RNAseq data was performed as previously described, [4] and included trimming of low-quality reads and bases, mapping of read good quality reads to human reference genome build 38, estimation of expression estimates, and variant calling. R package maftools was used for visualization of variant data. [5]

We used R v3.3.3 [6] for analyzing transcriptomic data. Differential expression (DE) analysis was performed with DESeq2[7], and for DE we used DESeq2-normalized counts. We used alpha value 0.05, and a moderate threshold of 0.32 for log2 fold change. P-values were adjusted with Benjamini-Hochberg method. [8] Lowly expressed genes were pre-filtered

and only protein-coding genes were kept in final results. Groups used in DE comparison were Ph+ ALL versus Ph– ALL. Ph-like samples were not included in the Ph– ALL group, but they were kept in the DE model for analysis.

To study sample purity, RNAseq data was analysed using Cibersort. [9] First, lowly expressed genes were filtered and only protein-coding genes were kept for the analysis. Cibersort recognized naive B-cells as the most prevalent cell type in all samples, as expected. We also compared the results from our cohort to publicly available Cibersort data from Hemap B-ALL cohort (n=1300). Results are listed in Supplementary Table 8.

Gene set enrichment analysis was done with Enrichr. [10,11] When analyzing public expression data, we used R packages ArrayExpress, affy, and limma. [12-14]

Statistical Analysis

We used R v3.3.3 for statistical analyses. [6] R packages ggplot2 and pheatmap were used for data visualization. [15] Multiple testing p-values were adjusted with Benjamini-Hochberg method (q-value). [8] When analyzing drug and public expression data, we used non-parametric Mann-Whitney U test for comparing two groups of continuous variables. Correlation plots were drawn with Prism software version 8 (GraphPad) and tested with non-parametric Spearman correlation.

Archer Fusion Plex Pan-Heme Kit

The samples that were used for this analysis were vitally frozen primary BM MNCs obtained from the FHRB and treated similarly as described above. This commercial fusion screen uses RNA as an input material and covers >199 genes known to relate to hematological malignancies. RNA was prepared using RNeasy Mini Kit (QIAGEN, Hilden, Germany). For the

FusionPlex Pan-Heme Kit, cDNA was synthesized from 250 ng of RNA using random primers to generate random start sites. Sample-specific indexes and specific molecular barcode adapters were ligated to both ends of the end-repaired cDNA molecules. Excess adapters were removed using Ligation Cleanup Beads (ArcherDX, Boulder, Colorado). During the first round of PCR procedure, multiplex gene-specific primers (GSP1) were used. After cleaning, the second round of PCR was performed using nested gene-specific primers (GSP2) to increase specificity. The final libraries were quantified using Ion Library TaqMan™ Quantification kit (Thermo Fisher Scientific, MA, USA) and the final products were sequenced on the Ion Torrent sequencing platform (Ion Proton or Ion GeneStudio S5 system, Carlsbad, CA, USA) to obtain approximately 2.5 million reads per sample. Sequencing data were analyzed in the Suite Analysis 6.0.4 Software (ArcherDX, Boulder, Colorado).

Cell Lines and Drug Combination Assay

Human cell lines were purchased from DSMZ (German Collection of Microorganisms and Cell Cultures) and were grown according to provider's recommendation in RPMI-1640 (Lonza) supplemented with 10%-20% FBS, 2 mM L-glutamine (Lonza), and 100 U/mL penicillin and 100 µg/mL streptomycin (Gibco). Cell lines were plated for the experiments under 15 passages. The cell lines were used for the drug combination assay. The cells were incubated with the combination drug plates for 72 hours in a humid environment at 37˚C and 5% CO_2, and cell viability (CellTiter-Glo, Promega) was measured after the three-day incubation with PHERAstar FS (BMG Labtech) plate-reader. The tested drug combinations are described in Supplementary Table 3. Drug combination synergy data were analyzed with SynergyFinder[16] using ZIP synergy model.

References

1. Pietarinen PO, Pemovska T, Kontro M, Yadav B, Mpindi JP, Andersson EI, et al. Novel drug candidates for blast phase chronic myeloid leukemia from high-throughput drug sensitivity and resistance testing. Blood Cancer J. Nature Publishing Group; 2015;5(5):e309.

2. Potdar S, Ianevski A, Mpindi JP, Bychkov D, Fiere C, Ianevski P, et al. Breeze: an integrated quality control and data analysis application for high-throughput drug screening. Valencia A, editor. Bioinformatics. 2020 Jun 1;36(11):3602–4.

3. Tang J, Szwajda A, Shakyawar S, Xu T, Hintsanen P, Wennerberg K, et al. Making sense of large-scale kinase inhibitor bioactivity data sets: a comparative and integrative analysis. J Chem Inf Model. 2014 Mar 24;54(3):735–43.

4. Adnan Awad S, Kankainen M, Ojala T, Koskenvesa P, Eldfors S, Ghimire B, et al. Mutation accumulation in cancer genes relates to nonoptimal outcome in chronic myeloid leukemia. Blood Adv. 2020 Feb 11;4(3):546–59.

5. Mayakonda A, Lin D-C, Assenov Y, Plass C, Koeffler HP. Maftools: efficient and comprehensive analysis of somatic variants in cancer. Genome Research. 2018 Nov;28(11):1747–56.

6. RStudio Team (2015). RStudio: Integrated Development for R. RStudio, Inc., Boston, MA URL http://www.rstudio.com/.

7. Love MI, Huber W, Anders S. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. Genome Biol. BioMed Central; 2014;15(12):550–21.

8. Benjamini Y, Hochberg Y. Controlling the False Discovery Rate: A Practical and Powerful Approach to Multiple Testing. Journal of the Royal Statistical Society Series B Methodological. 1995;57(1):289–300.

9. Newman AM, Liu CL, Green MR, Gentles AJ, Feng W, Xu Y, et al. Robust enumeration of cell subsets from tissue expression profiles. Nat Methods. Nature Publishing Group; 2015 May;12(5):453–7.

10. Chen EY, Tan CM, Kou Y, Duan Q, Wang Z, Meirelles GV, et al. Enrichr: interactive and collaborative HTML5 gene list enrichment analysis tool. BMC Bioinformatics. BioMed Central; 2013 Apr 15;14(1):128.

11. Kuleshov MV, Jones MR, Rouillard AD, Fernandez NF, Duan Q, Wang Z, et al. Enrichr: a comprehensive gene set enrichment analysis web server 2016 update. Nucleic Acids Res. 2016 Jul 8;44(W1):W90–7.

12. Kauffmann A, Rayner TF, Parkinson H, Kapushesky M, Lukk M, Brazma A, et al. Importing ArrayExpress datasets into R/Bioconductor. Bioinformatics. 2009 Aug 15;25(16):2092–4.

13. Gautier L, Cope L, Bolstad BM, Irizarry RA. affy--analysis of Affymetrix GeneChip data at the probe level. Bioinformatics. 2004 Feb 12;20(3):307–15.

14. Ritchie ME, Phipson B, Wu D, Hu Y, Law CW, Shi W, et al. limma powers differential expression analyses for RNA-sequencing and microarray studies. Nucleic Acids Res. 2015 Apr 20;43(7):e47–7.

15. Wickham H. *ggplot2: Elegant Graphics for Data Analysis*. Cham: Springer-Verlag New York; 2016.

16. Ianevski A, Giri AK, Aittokallio T. SynergyFinder 2.0: visual analytics of multi-drug combination synergies, Nucleic Acids Res. 2020 July 2;48(W1):W488–W493.