Highly multiplexed and quantitative cell-surface protein profiling using genetically barcoded antibodies

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Human cells express thousands of different surface proteins that can be used for cell classification, or to distinguish healthy and disease conditions. A method capable of profiling a substantial fraction of the surface proteome simultaneously and inexpensively would enable more accurate and complete classification of cell states. We present a highly multiplexed and quantitative surface proteomic method using genetically barcoded antibodies called phage-antibody next-generation sequencing (PhaNGS). Using 144 preselected antibodies displayed on filamentous phage (Fab-phage) against 44 receptor targets, we assess changes in B cell surface proteins after the development of drug resistance in a patient with acute lymphoblastic leukemia (ALL) and in adaptation to oncogene expression in a Myc-induced Burkitt lymphoma model. We further show PhaNGS can be applied at the single-cell level. Our results reveal that a common set of proteins including FLI3, NCR3L1G1, and ROR1 dominate the response to similar oncogenic perturbations in B cells. Linking high-affinity, selective, genetically encoded binders to NGS enables direct and highly multiplexed protein detection, comparable to RNA-sequencing for mRNA. PhaNGS has the potential to profile a substantial fraction of the surface proteome simultaneously and inexpensively to enable more accurate and complete classification of cell states.

Key to the technology we call phage-antibody NGS (PhaNGS) is a collection of defined fragment antibodies (Fabs) previously selected to bind specific targets of interest using high-throughput phage display (SI Appendix, Fig. S1) (4–6). Each Fab-phage was selected from a large synthetic Fab library (~10^16 unique sequences) built from a stable, VH3-Vx1-based Trastuzumab scaffold (7) and validated for affinity and selectivity. The selected Fabs are genetically encoded and displayed on a phage particle that packages its specific Fab gene within (Fig. L4). Taking inspiration from previous work that linked deep sequencing and phage libraries (8), we reasoned that the individual Fab-phage can be distinguished and quantified by NGS of the highly variable complementarity determining region (CDR) H3 loop, which represents both the major binding determinant of the encoded Fab and a unique DNA barcode. The fixed scaffold in which the CDR is embedded allows the use of a common set of primers flanking the H3 region for amplification and sequencing (SI Appendix, Fig. S2) (9). We hypothesized that a pool of preselected Fab-phage specific for a defined set of extracellular proteins would bind their cognate proteins on cells at levels in proportion to receptor abundance and allow for target quantification by NGS (Fig. 1B). The displayed Fab is attached to the phage on the opposite end from its antigen binding site and is known to retain virtually the same affinity for its target as when expressed as soluble Fab (SI Appendix, Fig. S3) (10).

Results

Validation of the PhaNGS Method in Model Experiments. To assess the feasibility of the approach, we used simple phage titering to measure binding of an anti-GFP Fab-phage (GFP-phage) to HeLa cells engineered to express a membrane-anchored GFP or parental (Fig. 1C). After binding and washing, we found 400 times more phage on the GFP-expressing cells than control cells. A control Fab-phage directed to the intracellular transcription factor ZNF2 showed similar low-titer binding to either cell line.

To assess the sensitivity of detection of GFP-phage, we immobilized varying concentrations of GFP on beads ranging from about 2 pM to 20 μM. Eluted phage titers were linear over a 4-log range and showed detectable signal over background (defined as BSA binding) with as little as 30 pM of immobilized GFP (Fig. 1D). We also observed that the fractional recovery of GFP-phage

Next-generation sequencing (NGS) has allowed the comprehensive study of the genome and transcriptome. However, a similarly broad, highly multiplexed, and inexpensive method for proteomics using NGS remains elusive. Here, we describe a phage display-based method using preselected antibodies that are genetically encoded and capable of simultaneous profiling of hundreds of cell-surface targets on cells in culture or singly at low cost and without the need for chemical conjugation to purified antibodies. We use the method to identify cell-surface proteins that change in cancer cells, some of which are coordinately regulated and could lead to new biomarkers and cancer targets.

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Data deposition: The sequencing data that support the findings of this study are available in the Gene Expression Omnibus (GEO), https://www.ncbi.nlm.nih.gov/geo (accession nos. GSE102712 (for PhaNGS data) and GSE102301 (for RNA-sequencing data)). All other data supporting the findings of this study are available in the SI Appendix, Dataset S3.

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Phage antibody clones

![Diagram of the PhaNGS method](image)

**A** Phagemid encoding Fab-pIII

**B** Diagram of the PhaNGS method, which consists of three steps: (i) A collection of phage antibodies is assembled that bind to specific targets of interest; (ii) the library is bound to a cell sample, and nonbinding phage antibodies are washed away; and (iii) the bound phage antibodies are propagated, amplified, and subjected to next-generation DNA sequencing to quantify the retained phage antibodies. (C) One antitarget (GFP.P01, n = 3) and one control phage (ZNF2.P01, n = 3) were profiled against a HeLa line stably overexpressing GFP tethered to the cell surface (green bar), along with its parental line (gray bar) and a no-cell control. Error bars show SD of three replicates. Input titer for GFP/PZN2P01 phage was measured at $3 \times 10^7$ to $1 \times 10^8$ cfu/mL. (D) Signal for each of three CDCP1-phages in the pool increased dramatically to between 50- and 80-fold above background, with significant signal over background, including the tyrosine kinase receptors EGFR, EPHA4, FGFR4, FLT3, INSR, ROR1, and TYRO3. To determine how overexpression affects Fab-phage signal strength, we generated a tetracycline (Tet)-inducible construct for CDCP1, one of the targets basally expressed at background (SI Appendix, File S2). Upon induction with Tet, the NGS signal for each of three CDCP1-phages in the pool increased dramatically to between 50- and 80-fold above background, with significant signal over background, including the tyrosine kinase receptors EGFR, EPHA4, FGFR4, FLT3, INSR, ROR1, and TYRO3. (E) Assembly and Validation of the PhaNGS Library. We then assembled a larger pool of Fab-phages, which grew to encompass 179 clones preselected for high affinity and selectivity to bind 59 purified ectodomain targets broadly associated with cancer and immunology (SI Appendix, Fig. S5, and Dataset S1). We decided to include multiple Fab-phage per target to provide independent measures and to test if certain epitopes were more responsive than others. In the pool, we had an average of four unique Fab-phage per target with a range of one Fab-phage for some targets to up to 13 for others. The Fab-phage pool also includes ZNF2- and GFP-phage background controls, against which the raw NGS counts obtained from antisurface protein Fab-phages can be normalized to determine signal and define nonspecific binding (see Dataset S3).

**Escherichia coli** to saturation followed by PCR (Fig. 1E). We found that propagation and PCR more closely matched the expected results than PCR alone, most likely because fewer rounds of PCR introduce less amplification bias (12). Bradley and coworkers have shown that polyclonal pools of scFv-phage have similar replicative rates independent of the scFv (13). We tested this hypothesis for a pool of 155 Fab-phage clones with roughly equal starting titers and found a small replicative advantage in the pool based on NGS counts (SI Appendix, Fig. S4).

Assembly and Validation of the PhaNGS Library. We then assembled a larger pool of Fab-phages, which grew to encompass 179 clones preselected for high affinity and selectivity to bind 59 purified ectodomain targets broadly associated with cancer and immunology (SI Appendix, Fig. S5, and Dataset S1). We decided to include multiple Fab-phage per target to provide independent measures and to test if certain epitopes were more responsive than others. In the pool, we had an average of four unique Fab-phage per target with a range of one Fab-phage for some targets to up to 13 for others. The Fab-phage pool also includes ZNF2- and GFP-phage background controls, against which the raw NGS counts obtained from antisurface protein Fab-phages can be normalized to determine signal and define nonspecific binding (see Dataset S3).

To test this assembled PhaNGS library, we first profiled HEK293T cells, a standard human expression host derived from human embryonic kidney cells (SI Appendix, Fig. S6). We found that most Fab-phage in the pool showed comparable signal to the nonspecific controls, while about a dozen targets gave significant signal over background, including the tyrosine kinase receptors EGFR, EPHA4, FGFR4, FLT3, INSR, ROR1, and TYRO3. To determine how overexpression affects Fab-phage signal strength, we generated a tetracycline (Tet)-inducible construct for CDCP1, one of the targets basally expressed at background (SI Appendix, File S2). Upon induction with Tet, the NGS signal for each of three CDCP1-phages in the pool increased dramatically to between 50- and 80-fold above background, with small SDs between replicates. In other control experiments with GFP-phages of known affinity, we found that the NGS signals vary in rough proportion to affinity (SI Appendix, File S7). Thus, we expect signal variation between unique Fab-phages to the same target is due to affinity differences.

**PhaNGS Profiling of Diagnosis-Relapse Samples in Leukemia.** We next applied the pool of Fab-phages to profile B cells from an ALL patient before and after chemotherapy. Samples were obtained from the bone marrow of the patient at diagnosis (LAX7D) and after the development of resistance (LAX7R) following a standard 3-wk chemotherapy regimen (see Fig. 2A and Materials and Methods for sample details) (15). PhaNGS profiles on LAX7D and LAX7R were performed in quadruplicate and showed a 1,000-fold signal range (Fig. 2B). Out of the 144 antitarget phages used in this experiment, 125 showed significant signal above background in either the LAX7D or LAX7R conditions. These phages fell into seven groups: About one-third of the Fab-phages showed little change in NGS signal between the LAX7D and LAX7R cells, suggesting little net change in target expression. The remaining clones fell into two groups (Fig. 2C): one where the target protein was high at diagnosis and decreased up to 10-fold in relapse (such as for ROR1 and NCR3LG1), and another where the target was low at diagnosis and increased up to 100-fold at relapse (such as for FLT3 and PDGFRB). FLT3 has previously been observed to be overexpressed and/or mutated in ALL and acute myeloid leukemia (AML) (16, 17). ROR1 also peaked at 0.1, implying that about 10% of total phage particles for this clone display a Fab. This is consistent with earlier estimates of Fab display in monovalent phage format (11).

We next investigated the most efficient means of amplifying phage DNA for NGS over a 4-log range of concentration, either by direct PCR or by first propagating the Fab-phage in...
Fig. 2. PhaNGS profiling of surface proteomes at diagnosis and relapse in a patient with acute lymphoblastic leukemia (ALL). (A) Bone marrow samples obtained from a patient diagnosed with ALL (Ph-negative) at diagnosis (LAX7D) and relapse (LAX7R) were grown as xenografts into immune-compromised NOD/SCID\(\gamma\text{-}_c\)–/– mice, cocultured with OP9 cells, and later frozen as monoculture stocks. Samples were thawed and expanded in culture 1 wk before the PhaNGS profile experiment. Both cell populations were positive for CD10, CD19, and CD45. The LAX7R resistance sample possessed a KRAS\(\text{G12V}\) mutation not detected at diagnosis. (B) PhaNGS profiles for 144 different Fab-phages (SI Appendix, Datasets S1 and S3) directed to 44 different membrane targets were allowed to bind to LAX7D (blue) or LAX7R (red) cells. The average value from four replicates, with SD (gray bars), is shown. (C) Targets are shown that were down- or up-regulated from LAX7D to LAX7R. (D) Experimental scheme for the P493-6 cell line in MycOFF and MycON conditions. Myc was repressed for 48 h with the addition of Tet (100 ng/mL, twice per day). The MycOFF state was harvested, Tet was washed out, and the cells recovered for 6 d before the MycON condition was harvested. (E) The extended bar chart displays the results of the PhaNGS profiling for the MycOFF to MycON experiment (blue and orange bars, respectively). The average value from four replicates, with SD (gray bars), is shown. (F) Targets are presented that were down- or up-regulated when transitioning from MycOFF to MycON.

represents a major target of interest in ALL, chronic lymphocytic leukemia (CLL), and other leukemias (18–20). Despite the variation in NGS signal between Fab-phage against the same target, the ratio of the average fold-change between the LAX7D and LAX7R states for a family of antibodies was consistent, providing additional confidence in the changes observed (SI Appendix, Fig. S8A). The variation in relative NGS signal for antibodies to the same targets is likely due to signal suppression for the weakly bound antibodies, since signal-to-noise is less.

We also performed RNAseq on the LAX7D and LAX7R samples and identified 11 transcripts for which targets were also present in the PhaNGS dataset (SI Appendix, Fig. S9). There was a weak correlation in the fold-change for the common targets (Pearson correlation coefficient, \(R = 0.17\)), possibly reflecting...
differences in receptor translation, trafficking, and stability. Such discrepancies between protein and RNA levels for mammalian cytosolic proteins have been reported and highlight the need for direct cell-surface protein quantitation (3).

**PhaNGS Profiling of Myc-Induced Surface Proteomic Changes.** Oncogenes are known to induce significant changes in gene protein expression. Myc shows especially strong perturbation in expression profiles (21). We wished to use PhaNGS to explore how Myc expression alters the expression of surface targets in our library. We used a model B cell line, P493-6, that has been used to mimic Burkitt lymphoma (22). In these cells, Myc is expressed at high levels but can be repressed by addition of Tet. We cultured these cells, then repressed Myc expression by treating with Tet for 2 d to generate the OFF state (Fig. 2D). The repression of Myc stalled cell growth without inducing apoptosis and led to subtle changes in morphology, as reported (23). Cells were allowed to recover in the absence of Tet for 6 d to re-express Myc, during which time cell growth and morphology returned to that of the ON state. PhaNGS profiles were then generated for cells from the OFF and ON states (Fig. 2E). The general profile for the P493-6 B cells looks remarkably similar to that for the patient-derived ALL cells (Fig. 2B). Myc re-expression lead to down-regulation of NCR3LG1 (for most clones) and ROR1 and up-regulation of FLT3, PDGFRB, and PTK7 (Fig. 2F), the same pattern of expression changes seen between the diagnosis and relapse ALL cells (Fig. 2C and SI Appendix, Fig. S10). The fact that many of the same proteins exhibit similar changes in both the LAX7 and P493-6 experiments suggests that among these B cell subtypes, particular groups of receptors dominate the response to these perturbations, as has been seen for EFG stimulation in other cell types (24). It is noteworthy that the PhaNGS profile for the HEK293T cells is considerably different from the profile seen for the B cells, which suggests PhaNGS can be used as a sensitive probe of cell type.

**Comparison of the PhaNGS Method to Mass Spectrometry and Flow Cytometry.** We next compared fold-change values observed for PhaNGS targets to those collected using more traditional protein detection methods. We used SILAC-based cell-surface capture (CSC) mass spectrometry to profile the fold-changes in cell-surface proteins in P493-6 cells induced by Myc repression or in MCF10A cells induced by KRAS<sup>G12V</sup> and compared 12 common targets to those observed in PhaNGS experiments on the same cells (Fig. 3A). We found a good correlation ($R = 0.66$) despite the sparse overlap from the small target set in the PhaNGS pool and detection of mostly abundant glycoproteins in the CSC experiments. We also expressed and purified two Fabs identified from the PhaNGS experiments that were highly responsive in the Myc-inducible experiment (NCR3LG1 and ROR1) and two that were induced in the KRAS<sup>G12V</sup>-transformed MCF10 cells (ANPEP and CDCP1). All four targets showed the same directional change and roughly the same fold-change by flow cytometry and PhaNGS (Fig. 3B).

**Single-Cell PhaNGS.** The PhaNGS profiles to this point have been on populations containing 1–10<sup>10</sup> cells. Bulk measurements of cancer cell populations can neglect to identify important nuances in cellular heterogeneity revealed by single-cell analysis (25). To extend the reach of the method, we sought to develop single-cell PhaNGS (SI Appendix, Fig. S1A). Here, Fab-phages were added to a population of cells, and then individual cells were flow-sorted into wells and each was profiled by NGS. We tested the single-cell method on HEK293T cells overexpressing cell surface-anchored GFP cells using GFP, CD55, CDCP1, and GHR-Fab phage (SI Appendix, Fig. S1B). For each of the 62 cells tested, we only observed a signal for the GFP-Fab-phage, as expected. We then tested single-cell PhaNGS on P493-6 cells against three targets we found via flow cytometry to exhibit dis-similar distributions of protein abundance (Fig. 4A). Our single-cell PhaNGS experiment found that for 34 single cells, the distributions observed by flow were closely recapitulated by PhaNGS (Fig. 4B).

**Discussion**

We believe that PhaNGS represents an important advance in barcoded antibody technology. This initial PhaNGS demonstration using ~150 antibodies is to our knowledge the largest simultaneous use of defined antibodies yet published, surpassing the theoretical limit for fluorophores using immunoaffluorescence (26), mass tags using CyTOF (27–30), or what has thus far been achieved using oligonucleotide-based barcodes (31). Moreover, we estimate a fixed cost of about $2 per replicate for each PhaNGS profile because the Fab-phages are genetically

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Fig. 3. **Comparison of PhaNGS to established proteomic methods.** (A) Comparison of fold-changes in surface expression of indicated surface proteins pre- and post-2-d suppression of Fabs in P493-6 B cells or for empty vector to KRAS<sup>G12V</sup> transformation of MCF10A cells as assessed by PhaNGS and Stable Isotope Labeling by Amino Acids in Cell Culture (SILAC) mass spectrometry. Dot size indicates spectral counts for mass spectrometry experiments to imply the abundance of each protein. Identity (y = x, gray dashed line) is shown as a benchmark for perfect agreement between MS and PhaNGS. $R = 0.66$ (regression line not pictured, y = 0.98x<sup>0.62</sup>). Where applicable, error bars for PhaNGS fold-change represent SD derived from unique Fab-phages against the same target. (B) Flow data corresponding to mass spectrometry vs. PhaNGS data for ANPEP, CDCP1, NCR3LG1, and ROR1. The fold-change values observed in the flow cytometry experiments closely match those observed for PhaNGS.

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<sup>4</sup>Confidence values for flow data may be found in Supplementary Information.
encoded and renewable (SI Appendix, Table S1). This compares with expensive and perishable antibody reagents needed for probe conjugation using other technologies. Although the PhaNGS experiments presented here were conducted with fewer than 200 unique Fab-phages, we estimate that PhaNGS could profile a target multiplicity of tens of thousands based on experiments using dilute input libraries. Given the growing interest in the development of renewable antibodies (32) and the ability to industrialize their selection (5), we believe the PhaNGS technology will undergo tremendous growth in the size of the probe library in the next few years. Moreover, the application of microfluidic technology to the method will increase its use for single-cell analysis and could allow for simultaneous RNaseq-PhaNGS experiments. The method may also be amenable to alternative display systems such as ribosome display (33). Having identified Fab-phage hits, one can easily transition the recombinant antibody into a multitude of detection or bioengineered formats such as antibody-drug conjugates (ADCs), bispecific T-cell engagers (BiTETs), or chimeric antigen receptor (CAR) T cells. We believe the PhaNGS method will have general utility to profile how cell-surface proteins change in health and disease. Such data will be useful for identifying new combinatorial biomarkers and drug targets.

Materials and Methods

Patient Samples. Patient-derived leukemia samples were collected with informed consent from all participants according to NCI/Cancer Therapy Evaluation Program-approved protocol ECOG E2993T5 and studied with approval of the Institutional Review Boards of the University of California San Francisco (UCSF). The samples were taken as bone marrow biopsies, blast enrichment, and NCR3LG1 Fab-phage antibodies on 84 individual P493-6 cells match observations from flow cytometry.

Panning Phage on Cells. Cells were washed once (to remove media, DMSO) by spinning the cells down at 300 × g for 5 min at 4 °C. Then, cells were resuspended in 1 mL of 0.1 M acetic acid, pH 2–3, allowed to sit for 5 min, spun down, and 800 µL of the acid eluent was transferred to a 1.5 mL Eppendorf tube containing 100 µL of 1 M Tris, pH 7.5, to neutralize. The neutralized solution was propagated as described above for the input pool or by flask (see SI Appendix). After propagation for 16 to 18 h, 50 µL of propagated phage were transferred to a 96-well PCR plate and boiled.

For single-cell experiments, instead of elution, single cells were sorted by forward and side scatter (or fluorescence) into each well of a 96-well plate containing 50 µL of 2xYT broth, then propagated via addition of 100 µL log phase XL-1 culture.

Amplification and Purification. The H3 “barcode” of each phagemid was amplified using boiled propagate template and flanking primers using Phusion polymerase (NEB). See Dataset S4 for primer design. The complete mix was then thermocycled for 12 cycles. Those samples showing bands by agarose gel were combined, gel purified, and submitted to a sequencing facility for analysis on an Illumina HiSeq4000 (Illumina) with a custom sequencing primer (order as shown): TGAGGACACTGCCGTCTATTATTGTGCTCGC (Tm = 67 °C, GC% = 52).

Mass Spectrometry. Cell samples were generated by SILAC as described previously (34). Mass spectrometry work-up was performed as described previously (35). Samples were run on a Q-Exactive Mass Spectrometer (ThermoScientific Inc.). Data were analyzed using MaxQuant (36). Median SILAC ratio values were used to determine fold-change values between conditions.

Code Availability. See https://github.com/sbpollock/PhaNGS-counting for scripts and details on how to convert “.fastq.gz” sequencing files into counts. See Dataset S3 for details on how these counts are interpreted.

Statistics. Error bars represent SD, which was calculated using Excel’s T.TEST function (two-tailed, homoscedastic).

Data Archival. The sequencing data that support the findings of this study are available in the Gene Expression Omnibus (GEO) with the identifier GSE102712 for PhaNGS data and GSE102301 for RNAseq data. All other data supporting the findings of this study are available within the SI Appendix, Dataset S3.

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1. Kolodziejczyk AA, Kim JK, Svensson V, Marioni JC, Teichmann SA (2015) The technology and biology of single-cell RNA sequencing. Mol Cell 58:610–620.
2. Fan HC, Fu GK, Fodor SP (2015) Expression profiling. Combinatorial labeling of single cells for gene expression cytometry. Science 347:1258367.
3. Pascal LE, et al. (2008) Correlation of mRNA and protein levels: Cell type-specific gene expression of cluster designation antigens in the prostate. BMC Genomics 9:246.
4. Huang H, et al. (2015) Selection of recombinant anti-sh3 domain antibodies by high-throughput phage display. Protein Sci 24:1890–1900.
5. Hornby M, et al. (2015) A high throughput platform for recombinant antibodies to folded proteins. Mol Cell Proteomics 14:2833–2847.
6. Garrard LJ, Yang M, O’Connell MP, Kelley RF, Henner DJ (1991) Fab assembly and enrichment in a monovalent phage display system. Biotechnology (NY) 9:1373–1377.
7. Ewert S, Huber T, Honegger A, Pluckthun A (2003) Biophysical properties of human antibody variable domains. J Mol Biol 325:531–553.
8. Matuchko WL, et al. (2012) Deep sequencing analysis of phage libraries using illumina platform. Methods 58:47–55.
9. Persson H, et al. (2013) Cdr-h3 diversity is not required for antigen recognition by synthetic antibodies. J Mol Biol 425:803–811.
10. Sidhu SS, Fairbrother WJ, Deshayes K (2003) Exploring protein-protein interactions with phage display. ChemBiochem 4:14–25.
11. Bass S, Greene R, Wells JA (1990) Hormone phage: An enrichment method for variant proteins with altered binding properties. Proteins 8:309–314.
12. Aird D, et al. (2011) Analyzing and minimizing pcr amplification bias in illumina sequencing libraries. Genome Biol 12:R18.
13. Ferrara F, et al. (2015) Recombinant renewable polyclonal antibodies. MAbs 7:32–41.
14. Mila R, Jorgensen P, Moran U, Weber G, Springer M (2010) BioNumbers—The database of key numbers in molecular and cell biology. Nucleic Acids Res 38:D750–D753.
15. Shojaee S, et al. (2015) Erk negative feedback control enables pre-b cell transformation and represents a therapeutic target in acute lymphoblastic leukemia. Cancer Cell 28:114–128.
16. Kindler T, Lipka DB, Fischer T (2010) Flt3 as a therapeutic target in aml: Still challenging after all these years. Blood 116:5089–5102.
17. Wellmatt S, et al. (2005) Flt3 mutations in childhood acute lymphoblastic leukemia at first relapse. Leukemia 19:467–468.
18. Dave H, et al. (2012) Restricted cell surface expression of receptor tyrosine kinase ror1 in pediatric b-lineage acute lymphoblastic leukemia suggests targetability with therapeutic monoclonal antibodies. PLoS One 7:e52655.
19. Mummery A, Narendran A, Lee KY (2011) Targeting epigenetics through histone deacetylase inhibitors in acute lymphoblastic leukemia. Curr Cancer Drug Targets 11:882–893.
20. Daneshmanesh AH, et al. (2012) Monoclonal antibodies against ror1 induce apoptosis of chronic lymphocytic leukemia (cll) cells. Leukemia 26:1348–1355.
21. Dang CV, et al. (2006) The c-myc target gene network. Semin Cancer Biol 16:253–264.
22. Pajic A, et al. (2000) Cell cycle activation by c-myc in a burkitt lymphoma model cell line. Int J Cancer 87:787–793.
23. Mezaquita P, Parigli SS, Brandvold KA, Ruddle A (2004) Myc regulates vegf production in b cells by stimulating initiation of vegf mrna translation. Oncogene 24:889–901.
24. Rthak DN, Tyanova S, Cox J, Borner GH (2016) Global, quantitative and dynamic mapping of protein subcellular localization. Elife 5:e16595.
25. Maruyuki A, Almedro V, Poljak K (2012) Intra-tumour heterogeneity: A looking glass for cancer? Nat Rev Cancer 12:323–334.
26. Black CB, Duensing TD, Trinkle LS, Dunlay RT (2011) Cell-based screening using high-throughput flow cytometry. Assay Drug Dev Technol 9:13–20.
27. Bandura DR, et al. (2009) Mass cytometry: Technique for real time single cell multitarget immunosassay based on inductively coupled plasma time-of-flight mass spectrometry. Anal Chem 81:6813–6822.
28. Di Palma S, Bodenmiller B (2015) Unraveling cell populations in tumors by single-cell mass cytometry. Curr Opin Biotechnol 31:122–129.
29. Spitzer MH, Nolan GP (2016) Mass cytometry: Single cells, many features. Cell 165:780–791.
30. Kendall T, Lipka DB, Fischer T (2010) Flt3 as a therapeutic target in aml: Still challenging after all these years. Blood 116:5089–5102.
31. Wellmatt S, et al. (2005) Flt3 mutations in childhood acute lymphoblastic leukemia at first relapse. Leukemia 19:467–468.
32. Dave H, et al. (2012) Restricted cell surface expression of receptor tyrosine kinase ror1 in pediatric b-lineage acute lymphoblastic leukemia suggests targetability with therapeutic monoclonal antibodies. PLoS One 7:e52655.