KinCytE- a Kinase to Cytokine Explorer to Identify Molecular Regulators and Potential Therapeutic Opportunities

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ABSTRACT

Cytokines and chemokines are secreted proteins that regulate various biological processes, such as inflammation, immune response, and cell differentiation. Therefore, disruption of signaling pathways involving these proteins has been linked to a range of diseases, including cancer. However, targeting individual cytokines, chemokines, or their receptors is challenging due to their regulatory redundancy and incomplete understanding of their signaling networks. To transform these difficult-to-drug targets into a pharmacologically manageable class, we developed a web-based platform called KinCytE. This platform was designed to link the effects of kinase inhibitors, a well-established class of drugs, with cytokine and chemokine release and signaling networks. The resulting KinCytE platform enables users to investigate protein kinases that regulate specific cytokines or chemokines, generate a ranked list of FDA-approved kinase inhibitors that affect cytokine/chemokine activity, and explore and visualize cytokine signaling network thus facilitating drugging this challenging target class. KinCytE is freely accessible via https://atlas.fredhutch.org/kincyte.

INTRODUCTION

Cytokines and chemokines are a group of proteins that are secreted by cells and play a fundamental role in cellular communication (Fajgenbaum and June, 2020). These molecules can function through autocrine or paracrine signaling mechanisms, and are responsible for a wide range of biological processes, including inflammation, immune response, cell proliferation, differentiation, and survival (Becher et al., 2017). However, when the regulation of these signaling pathways is disrupted, it can lead to the development of several disorders, including autoimmune diseases, allergies, chronic inflammation, and cancer (Fajgenbaum and June, 2020). Inflammatory cytokines, such as interleukin-1 (IL-1), interleukin-6 (IL-6), and tumor necrosis factor-alpha (TNF-α), play a significant role in the pathogenesis of infectious diseases. Additionally, chemokines such as CXCL8 are involved in the recruitment of immune cells to the site of infection or inflammation. In the context of cancer, cytokines and chemokines are involved in the initiation, progression, and metastasis of tumors (Lippitz and Harris, 2016). Tumor cells can produce cytokines and chemokines to evade the immune system and promote angiogenesis, whereas disruption of cytokine and chemokine signaling pathways in cancer cells can lead to resistance to chemotherapy and radiation therapy (Jones et al., 2016). Thus, identifying the molecular regulators and drugs that could inhibit cytokine and chemokine release could result in the development of novel treatments for various disorders.

Delineating the regulation of cytokines and chemokines pose a challenge due to their functional redundancy (Chauhan et al., 2021). Several cytokines may bind to identical receptors and prompt comparable or diverse downstream functions. Moreover, the intricacies of the downstream molecular players that are components of cytokine/chemokine signaling networks remain incompletely understood. As a result, directly targeting a single cytokine or cytokine receptor has been a challenging task. Here, we present KinCytE, a kinase-to-cytokine explorer, a powerful new platform that was designed to allow researchers to explore the downstream molecular regulators and potential drugs that could inhibit the function of one or more cytokines. We anticipate that KinCytE will be highly sought after by biologists from various backgrounds, including immunologists, cancer biologists, virologists, and beyond.

RESULTS and DISCUSSION

Measuring and modeling cytokine and chemokine responses in human macrophages

To model cytokine and chemokine release and responses, we used human macrophages stimulated with lipopolysaccharide (LPS) treatment, a widely used method for activating macrophages and inducing cytokine and chemokine secretion *in vitro* and *in vivo*(Hume, 2015). Therefore, we exposed human monocyte-derived macrophages to LPS and analyzed 191 secreted factors (chemokines, cytokines, and growth factors) in conditioned media using a recently developed nano-ELISA (nELISA) method (Dagher et al., 2023). Out of the 191 secreted factors analyzed, we observed reliable signals for 37 proteins (**Table S1**). Our results indicated significant changes in 34 cytokines, chemokines, and secreted factors following 24 hours of stimulation with LPS. These changes included upregulation of previously known cytokines and chemokines such as IL-1, IL-6, IL-10, CCl3, CCL7, and G-CSF (**Fig. 1A**). Furthermore, we also found significant upregulation of VEGFa and MMP1 (**Fig. 1A**). In addition, we observed significant downregulation of CCL24 (IL-4), a negative regulator of the LPS signaling (Bonder et al., 1999). Therefore, the nELISA assay allowed us to identify a wide range of chemokines and cytokines that are released in response to macrophage activation.

To identify signaling pathways activated in macrophages in response to LPS stimulation and explore kinase inhibitors that could inhibit these pathways, we employed a recently developed strategy called KiR ((Bello et al., 2021b; Chan et al., 2021; Gujral et al., 2014) . The KiR approach uses large scale drug-target profiling data and broadly selective chemical tool compounds to pinpoint specific kinases underlying a given phenotype(Rata et al., 2020). For the purposes of KinCytE development we focused on the release of secreted factors, e.g., cytokines as the phenotype, and quantified the effects of 34 computationally-chosen kinase inhibitors on changes in LPS-mediated release of 37 secreted factors (**Fig. 1C, Table S2**). Using this training dataset, we built elastic net regularization models to predict kinases essential for each of the 37 secreted factors. We evaluated model performance using leave-one-out cross-validation (LOOCV) mean squared error (MSE) between predicted and observed drug response. In LOOCV, each time 37 drugs' activity profiles were used to train the model to predict the remaining drug's effect on LPS-mediated

cytokine release, and MSE between predicted and observed cytokine levels was used to assign an error score to each model (**Fig. S1**). The optimized models with the least normalized root mean squared error of <0.1 and Pearson correlation >0.85 between predicted and measured responses collectively identified 168 most "informative kinases" (out of 298 kinases) that may be involved in the LPS-mediated release of at least one or more factors (**Fig. 1D, Table S3**). These include several kinases known to play a critical role in macrophage survival and activation and cytokine signaling, such TAK1(Irie et al., 2000), Src Family kinases(Smolinska et al., 2008), JAK1, IRAK, GSK3(Noori et al., 2020), FES(Parsons and Greer, 2006), p38 MAPK and CK2(Glushkova et al., 2018). Among these kinases, Src family kinases, GSK3, CK2 and TAK1 were predicted to be important for the release of more than ten cytokines and chemokines. In addition, our model highlighted the importance of various receptor tyrosine kinases including, FGFR, CSF1R, VEGFR and MET. We used siRNA knock-down experiments to validate involvement of specific kinases in regulation of three main cytokines. We observed that in all cases, knockdown of a kinase leads to a decrease in cytokine release (**Fig. 1D**). Moreover, our analysis suggested that a single kinase may play a vital role in the release of several cytokines, and conversely, a given cytokine may be regulated by multiple kinases, indicating a complex interplay between kinases and cytokines.

Given that our analysis implicated multiple kinases in affecting cytokine release, we sought to identify poly-specific kinase inhibitors that could target the release of one or more cytokines and chemokines. To achieve this, we used the optimized KiR models to predict single-agent responses to 427 kinase inhibitors (**Fig. 1E, Table S4**). Our kinase inhibitor collection includes 39 FDA-approved kinase inhibitors and numerous compounds currently undergoing clinical testing. Among these, ponatinib, abemaciclib, and nilotinib were the top hits predicted to inhibit the release of over 15 cytokines and chemokines (**Table S4**). Our findings are consistent with a previous study demonstrating the inhibitory effect of ponatinib on LPS-mediated macrophage activation both *in vitro* and in a mouse model of lung inflammation(Chan *et al*., 2021). Additionally, we experimentally confirmed the efficacy of three inhibitors, cabozantinib, nintedanib, and dovitinib, and observed an accuracy of over 80% in line with the KiR model's predictions (**Fig. 1E**). Consequently, our systems-based modeling efforts enabled us to predict the efficacy

of a vast number of kinase inhibitors in inhibiting the activity of one or more secreted factors. These results hold promise for repurposing existing inhibitors to treat diseases where cytokine release plays a significant role, such as sepsis and viral-mediated infections.

Finally, protein kinases identified through KiR can be used to deduce signaling pathways that are involved in a specific biological process. We have recently developed a new computational approach known as KiRNet, which allows construction of signaling networks based on the KiR(Bello et al., 2021a). We built kinase-centric networks for each of the 33 cytokine, chemokines and other secreted factors by applying pathway analysis tools, including KEGG(Kanehisa and Goto, 2000). These networks can be accessed and explored using the KinCytE tool (see below). Overall, by employing a combination of multiplex measurements, functional screening, and computational methods, we were able to achieve two key outcomes: 1) the creation of network-centric portraits that govern the release of particular cytokines and chemokines, and 2) the generation of ranked lists of kinase inhibitors, including some that contain FDA-approved drugs, that highlight the potential of known drugs to inhibit the function of one or more secreted factors.

Development and use of KinCytE

We designed KinCytE as a publicly accessible online platform that could be used to address fundamental questions in cellular biology, such as "Which signaling pathways are downstream of cytokine and chemokines?", “Which compounds are suitable for inhibiting a specific cytokine or chemokine pathway?", and similar. To begin answering these questions, the first and often most crucial step is to systematically determine the changes in a large number of chemokines and cytokines in response to various perturbations. Leveraging the primary dataset on LPS-induced changes in secreted factors and KiR-model predicted kinases and response to kinase inhibitors, we created KinCytE as a tool for exploring cytokine and chemokine signaling. KinCytE is an intuitive web-based platform that enables users to rapidly determine the essential kinases responsible for releasing particular cytokines or groups of cytokines. Additionally, the platform offers a ranked list of currently available FDA-approved and other drugs that have the potential to

inhibit cytokine/chemokine activity. Finally, the user-friendly graphical user interface facilitates visualization and exploration of cytokine signaling network maps.

The KinCytE app runs entirely in-browser and can be found at https://atlas.fredhutch.org/kincyte/. Upon loading the app webpage, users can: (1) utilize this app to identify drugs that potentially reduce the level of cytokines of interest (COI) via clicking on "Identify Drugs", which will bring them "Drug Discovery" page; (2) view kinases that could be involved in the regulation of the COI via clicking on "Identify Kinases", which would bring them to "Kinase Discovery" page; and (3) explore the connection between COI and its related kinases via clicking on "Explore Cytokines", which leads to "Cytokine Explorer" page (**Fig. 2A**). Each page provides instruction about the expected content and format of input and output.

Kinase Discovery page gives a matrix of kinases that might regulate COI (**Fig. 2B**). The table assigns '1' to a kinase–cytokine combination where kinase could regulate the cytokine and '0' otherwise. Rows of COI are pinned at the top panel. This matrix also summarizes the number of cytokines within COI and among all cytokines that a kinase could affect, with details of its impact on other cytokines that are not included in COI.

Drug Discovery page generates a bar chart and a table based on the given cytokine(s) (**Fig.2C**). The bar chart shows top 10 drugs with highest average efficacy, which is defined by the percentage of the cytokine/chemokine level change compared to the control level. Deep blue color refers to drugs that affect all cytokines while light blue color indicates drugs with narrower scope of targets. Users hover on each bar to get the exact number. The table presents the summary of all 427 drugs from following aspects: average efficacy on COI, percentage of COI affected, drug selectivity and specificity, and FDA approval status. Users clicks drug name on either bar chart or table for a new plot showing the efficacy profile of this drug on COI.

Cytokine Explorer page produces a graphic connection between COI and its potential upstream kinases based on the KEGG analysis (**Fig.2D**). Node represents gene(s), and edge represents interaction between the two nodes (genes) it connects. More detailed annotation is provided in the legend of each

network figure. Users slide the bar to filter out edges of higher weight, which indicates less likely interaction. Users click on a node to drag it, highlight its neighboring network, and obtain additional biological information of gene that this node refers to.

KinCytE links the release of cytokines and chemokines to kinase activity and recommends drugs that target specific cytokine sets, as well as identifies kinases and kinase-related proteins that may be involved. This platform also extends the kinase-cytokine relationship by incorporating information from biological pathways. Its sturdy architecture and standardized data processing facilitate effortless updates to incorporate new cytokines and other immune factors with corresponding experimental data. As a result, we anticipate widespread adoption of this portal.

FIGURES

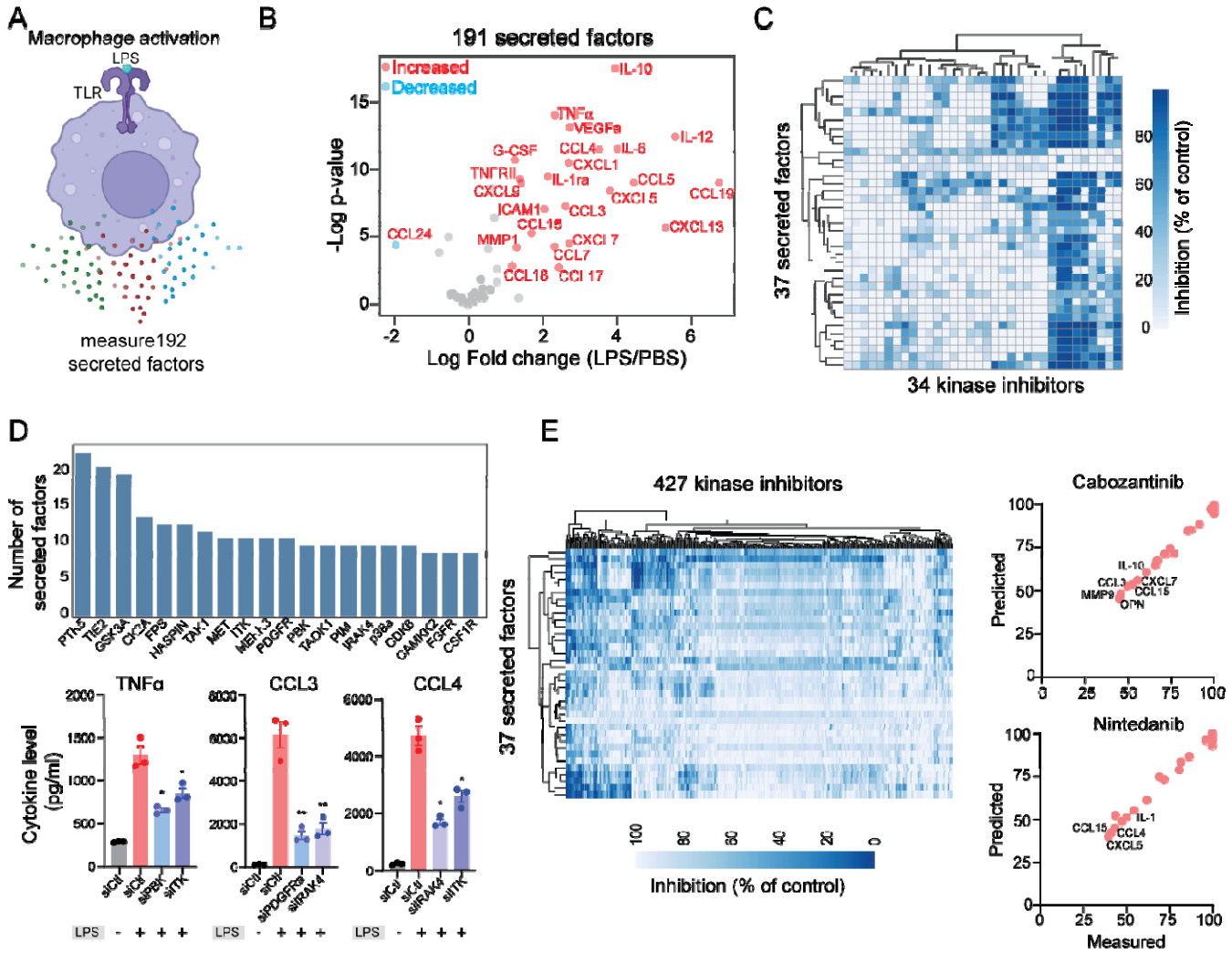

Fig. 1: Modeling LPS-mediated changes in secreted factors in human macrophages. A. A schematic showing LPS-mediated activation of human macrophages. **B.** A plots showing changes in the levels of 191 secreted factors in response LPS stimulation. Significantly increased or decreased factors are also indicated. **C.** A heatmap showing changes in LPS-mediated 33 secreted factor sin response to 34 kinase inhibitors. **D.** Key kinases that regulate cytokines and chemokines. Top, a plot showing the top kinase and the number of secreted factors they regulate. Bottom, experimental validation of change in TNFa, CCL3 and CCL4 upon knockdown of indicated kinases. **E**. A heatmaps showing predicted changes in 33 secreted factors in response 427 kinase inhibitors. Right, experimental validation showing correlation between measured and predicted changes in 33 cytokines in macrophages treated with FDA-approved cabozantinib and nintedanib at 500nM.

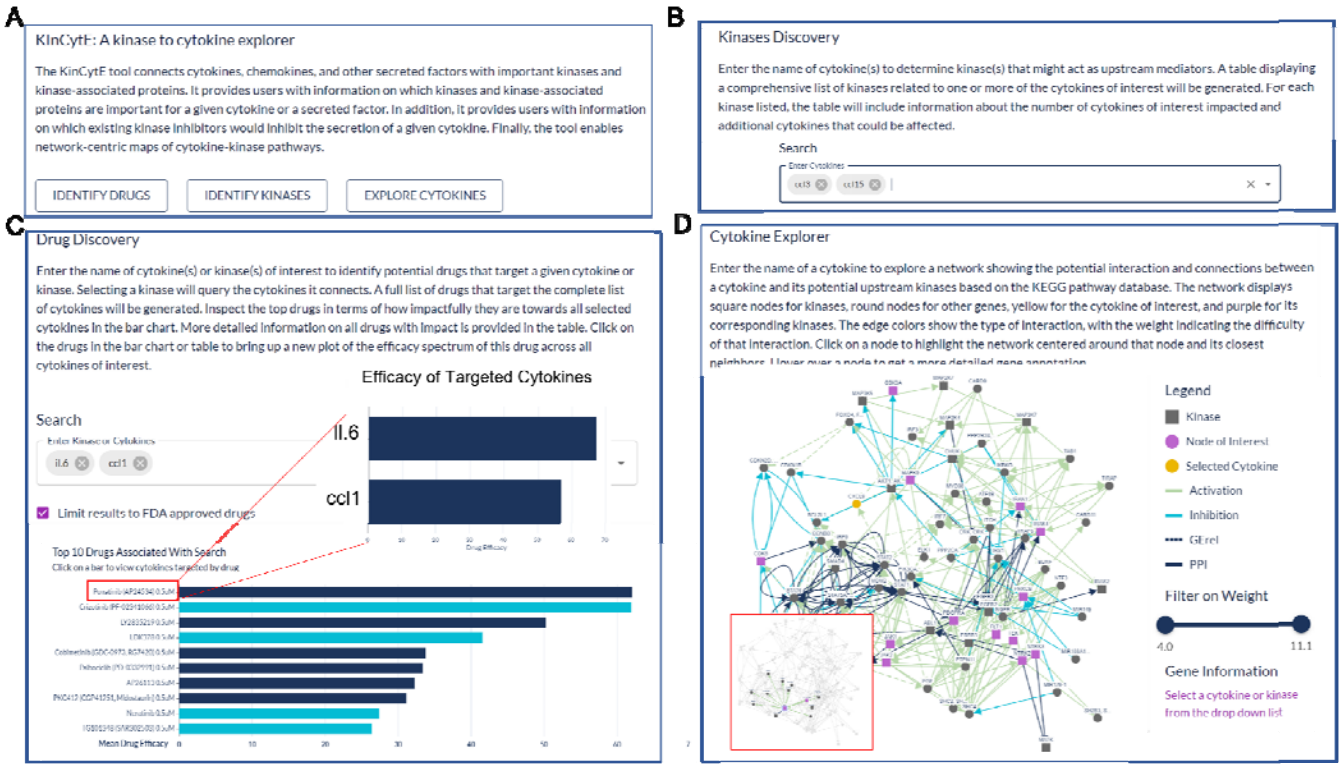

Fig. 2: An illustration showing the steps for using KinCytE. A. KinCytE offers three sections for users to explore potential cytokine(s) or chemokine(s) inhibiting drugs (Identify Drugs), related kinases (Identify Kinase) and cytokine signaling network (Explore Cytokines). Users can click on any of these sections to access the desired information. **B.** Users can obtain information on kinase(s) relevant to the expression of selected cytokines by selecting the desired cytokine(s) from the available options. This will generate a table with the relevant information. **C.** Users select cytokine(s) to view a bar plot showing the top 10 most potent drugs in inhibiting the expression of these cytokine(s), with a table recording the effectiveness of all 427 drugs. Users can click on the drug name in either the table or the bar plot to access a more detailed cytokine efficacy profile of that drug. **D.** To produce a network showing the connection between a selected cytokine and its related kinases, users need to select the desired cytokine. This will generate a network with edges representing the connections between the cytokine and its related kinases. Users can slide the weight bar to prune edges and click on a node to get the annotation of the gene(s) represented by that node. All tables in KinCytE are fully sortable, filterable, and downloadable for the convenience of users.

AUTHOR CONTRIBUTIONS

TSG conceived the study. MC performed all the experiments. YK developed KiR and KiRNET models. SO and MZ developed the KinCytE interface.

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MATERIALS AND METHODS

Cell lines. Human CD14+ monocytes cells were obtained from Bloodworks Northwest. Cells were grown at 37C under 5% CO2, 95% ambient atmosphere and maintained in Roswell Park Memorial Institute (RPMI) 1640 Medium supplemented with 10% FBS (Sigma) and 1% Penn Strep. Macrophages were derived from CD14+ monocytes using M-CSF Recombinant human protein at a final concentration of 50 ng/mL for six days.

Quantitative profiling of the secretome. Conditioned media from macrophages treated with LPS for 24 hours were collected and sent to NomicBio (Montreal, Canada) for nELISA-based cytokine analysis as described previously (Dagher *et al.*, 2023).

Kinase inhibitor screening. Kinase inhibitor screening was performed as described previously(Chan *et al.*, 2021). Monocyte-derived macrophages were plated in 96 well plates. Next days, cells were treated with LPS and a panel of 34 kinase inhibitors at 500nM doses in duplicate. Conditioned media was collected after 2 hours for the secretome analysis as described above.

KiR modeling. Elastic Net regularized models KiR models and the list of predicted key kinases or key functional nodes were generated as previously described(Bello *et al.*, 2021a; Chan *et al.*, 2021; Gujral *et al.*, 2014). Briefly, a panel of 427 kinase inhibitors previously had their pairwise effects on 298 human kinases profiled. The result is a quantitative drug-target matrix, where each entry is a percentage between 0 and 100 that represents that kinases residual activity (as a percent of control, uninhibited activity) in the presence of that inhibitor. The kinase inhibition profiles of each inhibitor and the quantitative responses to those inhibitors were used as the explanatory and response variables, respectively, for elastic net regularized multiple linear regression models. Custom R scripts (available at https://github.com/FredHutch/KiRNet-Public) employing the glmnet package were used to generate the final models. Leave-one-out cross validation (LOOCV) was used to select the optimal value for the penalty scaling factor l. Models were computed for 11 evenly-spaced values of a (the relative weighting between LASSO and Ridge regularization) ranging from 0 to 1.0 inclusive. Kinases with positive coefficients in at least one of these models (with the exception of a = 0, which always has non-zero coefficients for every kinase) were

considered hits. Model accuracy was assessed via the LOOCV error as well as the root-mean-squared error of the predictions for the tested inhibitors.

Small interfering RNA transfection. All small interfering RNA (siRNA) were obtained from Dharmacon (Thermo. Specific sequences and identifiers are provided in Data. siRNA transfections in 12-well plate for measuring changes in secreted factors were carried out using Lipofectamine RNAiMax (Invitrogen) according to manufacturer instructions.

QUANTIFICATION AND STATISTICAL ANALYSIS

All software development, calculations, and analyses were carried out using R 3.3.0 (https://www.r-project.org/). All packages used can be found in the Key Resources Table.

DATA SOFTWARE AND AVAILABILITY

The app portal can be accessed at https://atlas.fredhutch.org/kincyte. The source code and all other files can are listed in the Key Resources Table.

Supplementary Data

Figure S1
Tables S1-S4

RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Chemicals, peptides, and recombinant proteins		
Kinase inhibitors used for KiR model screening	National Center for Advancing Translational Science, NIH	See Table S2
Software and Algorithms		
R 4.2.1	(R Core Team, 2022)	https://www.r-project.org/
glmnet	(Friedman et al., 2010)	https://cran.r-project.org/web/packages/glmnet/index.html
ggrepel	(Slowikowski et al., 2023)	https://github.com/slowkow/ggrepel
tidyverse	(Wickham et al., 2019)	https://tidyverse.tidyverse.org/index.html
ggpubr	(Kassambara et al., 2023)	https://rpkgs.datanovia.com/ggpubr/index.html
ggplot2	(Wickham et al., 2009)	http://ggplot2.org
pheatmap	(Kolde, 2015)	https://cran.r-project.org/package=pheatmap
Python 3.7.11	(Van Rossum et al., 2009)	https://www.python.org/downloads/release/python-3711/
Numpy	(Millman et al., 2020)	https://numpy.org/
Pandas	(McKinney, 2010)	https://pandas.pydata.org/
Scipy	(Virtanen et al., 2020)	https://scipy.org/
Seaborn	(Waskom, 2021)	https://seaborn.pydata.org/
Matplotlib	(Hunter, 2007)	https://matplotlib.org/
UMAP	(Mclnnes, 2018)	https://umap-learn.readthedocs.io/en/latest/
Experimental models: cells		
Human CD14+ monocytes	Bloodworks Northwest	Cat # 4585-18
Oligonucleotides		
siRNA sequences	Horizon ON-TARGETplus siRNA-SMARTpool	See Table S5

CONTACT FOR REAGENT AND RESOURCE SHARING

Further information and requests for resources should be directed to and will be fulfilled by the Lead Contact, Taran Gujral (tgujral@fredhutch.org).

References

Becher, B., Spath, S., and Goverman, J. (2017). Cytokine networks in neuroinflammation. Nat Rev Immunol *17*, 49-59. 10.1038/nri.2016.123.

Bello, T., Chan, M., Golkowski, M., Xue, A.G., Khasnavis, N., Ceribelli, M., Ong, S.E., Thomas, C.J., and Gujral, T.S. (2021a). KiRNet: Kinase-centered network propagation of pharmacological screen results. Cell Rep Methods *1*. 10.1016/j.crmeth.2021.100007.

Bello, T., Paindelli, C., Diaz-Gomez, L.A., Melchiorri, A., Mikos, A.G., Nelson, P.S., Dondossola, E., and Gujral, T.S. (2021b). Computational modeling identifies multitargeted kinase inhibitors as effective therapies for metastatic, castration-resistant prostate cancer. Proceedings of the National Academy of Sciences *118*, e2103623118.

Bonder, C.S., Finlay-Jones, J.J., and Hart, P.H. (1999). Interleukin-4 regulation of human monocyte and macrophage interleukin-10 and interleukin-12 production. Role of a functional interleukin-2 receptor gamma-chain. Immunology *96*, 529-536. 10.1046/j.1365-2567.1999.00711.x.

Chan, M., Vijay, S., McNevin, J., McElrath, M.J., Holland, E.C., and Gujral, T.S. (2021). Machine learning identifies molecular regulators and therapeutics for targeting SARS-CoV2-induced cytokine release. Mol Syst Biol *17*, e10426. 10.15252/msb.202110426.

Chauhan, P., Nair, A., Patidar, A., Dandapat, J., Sarkar, A., and Saha, B. (2021). A primer on cytokines. Cytokine *145*, 155458. 10.1016/j.cyto.2021.155458.

Dagher, M., Ongo, G., Robichaud, N., Kong, J., Rho, W., Teahulos, I., Tavakoli, A., Bovaird, S., Merjaneh, S., Tan, A., et al. (2023). nELISA: A high-throughput, high-plex platform enables quantitative profiling of the secretome. bioRxiv. 10.1101/2023.04.17.535914.

Fajgenbaum, D.C., and June, C.H. (2020). Cytokine Storm. N Engl J Med *383*, 2255-2273. 10.1056/NEJMra2026131.

Glushkova, O.V., Parfenyuk, S.B., Novoselova, T.V., Khrenov, M.O., Lunin, S.M., and Novoselova, E.G. (2018). The Role of p38 and CK2 Protein Kinases in the Response of RAW 264.7 Macrophages to Lipopolysaccharide. Biochemistry (Mosc) *83*, 746-754. 10.1134/s0006297918060123.

Gujral, T.S., Peshkin, L., and Kirschner, M.W. (2014). Exploiting polypharmacology for drug target deconvolution. Proc Natl Acad Sci U S A *111*, 5048-5053. 10.1073/pnas.1403080111.

Hume, D.A. (2015). The Many Alternative Faces of Macrophage Activation. Front Immunol *6*, 370. 10.3389/fimmu.2015.00370.

Irie, T., Muta, T., and Takeshige, K. (2000). TAK1 mediates an activation signal from toll-like receptor(s) to nuclear factor-kappaB in lipopolysaccharide-stimulated macrophages. FEBS Lett *467*, 160-164. 10.1016/s0014-5793(00)01146-7.

Jones, V.S., Huang, R.Y., Chen, L.P., Chen, Z.S., Fu, L., and Huang, R.P. (2016). Cytokines in cancer drug resistance: Cues to new therapeutic strategies. Biochim Biophys Acta *1865*, 255-265. 10.1016/j.bbcan.2016.03.005.

Kanehisa, M., and Goto, S. (2000). KEGG: kyoto encyclopedia of genes and genomes. Nucleic Acids Res *28*, 27-30. 10.1093/nar/28.1.27.

Lippitz, B.E., and Harris, R.A. (2016). Cytokine patterns in cancer patients: A review of the correlation between interleukin 6 and prognosis. Oncoimmunology *5*, e1093722. 10.1080/2162402x.2015.1093722.

Noori, M.S., Courreges, M.C., Bergmeier, S.C., McCall, K.D., and Goetz, D.J. (2020). Modulation of LPS-induced inflammatory cytokine production by a novel glycogen synthase kinase-3 inhibitor. Eur J Pharmacol *883*, 173340. 10.1016/j.ejphar.2020.173340.

Parsons, S.A., and Greer, P.A. (2006). The Fps/Fes kinase regulates the inflammatory response to endotoxin through down-regulation of TLR4, NF-kappaB activation, and TNF-alpha secretion in macrophages. J Leukoc Biol *80*, 1522-1528. 10.1189/jlb.0506350.

Rata, S., Gruver, J.S., Trikoz, N., Lukyanov, A., Vultaggio, J., Ceribelli, M., Thomas, C., Gujral, T.S., Kirschner, M.W., and Peshkin, L. (2020). An optimal set of inhibitors for reverse engineering via kinase regularization. bioRxiv, 2020.2009. 2026.312348.
Smolinska, M.J., Horwood, N.J., Page, T.H., Smallie, T., and Foxwell, B.M. (2008). Chemical inhibition of Src family kinases affects major LPS-activated pathways in primary human macrophages. Mol Immunol *45*, 990-1000. 10.1016/j.molimm.2007.07.026.