Interleukin signaling mitigates the inhibitory effects of combined Src/BCR-ABL1 blockade on T-cell activity in Philadelphia chromosome-positive acute lymphoblastic leukemia

Farnaz Naeemikia,[1] Joshua Reynolds,[1] Cheng Dong[1] and Justin R Pritchard[1,2]

[1]Department of Biomedical Engineering and [2]Huck Institute for the Life Sciences, University Park, The Pennsylvania State University, PA, USA

Correspondence: J.R. Pritchard
Jrp94@psu.edu

Received: August 4, 2025.
Accepted: January 16, 2026.
Early view: February 5, 2026.

https://doi.org/10.3324/haematol.2025.288829

Abstract

Philadelphia chromosome-positive acute lymphoblastic leukemia (Ph$^+$ ALL), driven by the BCR-ABL1 fusion gene, remains a high-risk malignancy despite therapeutic advances. Tyrosine kinase inhibitors (TKI) targeting BCR-ABL1 have significantly improved outcomes, but resistance and relapse persist, necessitating novel strategies such as combining TKI with bispecific T-cell engagers (BiTE) like blinatumomab. Blinatumomab redirects T cells to eliminate CD19$^+$ leukemia cells and has shown impressive clinical activity in Ph$^+$ ALL when combined with SrcBCR-ABL1 TKI. However, this contrasts with preclinical observations reporting that Src kinase inhibition by Src/BCR-ABL1 TKI antagonizes blinatumomab-mediated T-cell activation. Consistent with prior preclinical studies, we demonstrate that dasatinib and ponatinib, unlike SRC-sparing TKI (imatinib, nilotinib), antagonize blinatumomab's T-cell engaging efficacy by potently inhibiting LCK Y394 phosphorylation, a critical step in proximal TCR signaling. This inhibition impairs T-cell proliferation, cytokine production, and NFAT activation. To reconcile this *in vitro* antagonism with favorable clinical combination outcomes, we confirmed that the mechanism of SRC inhibition is T-cell intrinsic, and we explored the impact of interleukins. We show that TKI-induced T-cell suppression and antagonism can be significantly improved by supplementing co-cultures with common γ-chain cytokines, particularly IL-7. IL-7 robustly enhances human T-cell proliferation, reduces exhaustion, and significantly improves blinatumomab's cytotoxic efficacy in the presence of Src/BCR-ABL1 TKI.

Introduction

Philadelphia chromosome-positive acute lymphoblastic leukemia (Ph$^+$ ALL) is an aggressive subtype of B-cell precursor ALL, accounting for approximately 25-30% of adult cases.[1,2] This malignancy is driven by the t(9;22)(q34;q11) translocation, generating the *BCR-ABL1* fusion gene, which encodes a constitutively active tyrosine kinase. Historically, Ph$^+$ ALL carried a poor prognosis, with limited response to conventional chemotherapy and low long-term survival rates.[3,4]

The introduction of tyrosine kinase inhibitors (TKI) targeting *BCR-ABL1* markedly improved outcomes. First-generation TKI like imatinib showed promise but were limited by resistance, often due to BCR-ABL kinase domain mutations.[5,6] Second- and third-generation TKI, including dasatinib, nilotinib, and ponatinib, were developed to overcome these mutations, particularly the T315I "gatekeeper" mutation.[7] Ponatinib plus chemotherapy is now the standard of care, as demonstrated in the phase III PhALLCON study, which showed a significantly higher rate of minimal residual disease (MRD)-negative remission compared to imatinib (34.4% *vs*. 16.7%).[8,9]

Despite the progress achieved, durable remission remains a challenge, necessitating novel approaches. Bispecific T-cell engagers (BiTE), such as blinatumomab, redirect T cells to lyse CD19$^+$ B cells and have shown efficacy in relapsed/refractory B-cell precursor ALL.[10] Blinatumomab is now being explored in chemotherapy-free regimens with TKI, demonstrating encouraging clinical outcomes.[11]

Tyrosine kinase inhibitor-BiTE combinations have yielded remarkable rates of MRD negativity and improved survival.[12] For example, a study combining dasatinib and blinatumomab showed an overall survival (OS) rate of 80.7% at 53 months.[13,14] A separate phase II trial of blinatumomab and ponatinib reported a 3-year OS rate of 88% and 98% of patients achieving MRD negativity.[15] These high rates of durable remission underscore the transformative potential of chemotherapy-free combinations for long-term disease control in Ph$^+$ ALL.[16]

However, the combination of blinatumomab with potent Src family kinase (SFK) inhibitors like dasatinib and ponatinib presents a potential mechanistic conflict.[17] LCK and FYN are pivotal tyrosine kinases in the T-cell receptor (TCR) signaling pathway, and are essential for T-cell activation, proliferation, and effector function.[18] Previous *in vitro* studies have suggested that these TKI can impair T-cell activation and blinatumomab-mediated cytotoxicity by inhibiting LCK activity.[19,20] This evidence suggests a potential antagonistic interaction that could compromise the efficacy of this combination therapy.

This presents a paradox, as the impressive clinical outcomes observed with blinatumomab-TKI combinations[13-16,21] challenge the simple prediction of antagonism based solely on *in vitro* data,[19] which, by their inherent design, often fail to fully recapitulate the complex cytokine milieu critical for sustained T-cell function *in vivo*. This apparent discrepancy highlights a critical need to understand the factors that may modulate or overcome TKI-mediated suppression of T-cell function. A key question arises from the fact that Src/BCR-ABL1 TKI ponatinib and dasatinib are more potent *in vitro* inhibitors of Ph$^+$ALL cells, with IC_{50} in the single-digit nanomolar range — one to two orders of magnitude more potent than nilotinib or imatinib. What if the discrepancy is due to faster killing of CD19$^+$ cancer cells that are required to stimulate T cells *in vitro*, and not due to inhibition of TCR downstream signaling? This result would not be clearly distinguishable in existing literature. So, we aimed to use a modeling approach to test it.

Within this modeling framework, we fit a model and tested two conflicting hypotheses to determine which mechanism best explains the observed T-cell dynamics: (1) that reduced T-cell activity is primarily due to the rapid killing of target B cells, thereby eliminating the T-cell stimulatory signal, or (2) the antagonism arises from direct inhibition of T-cell receptor (TCR)-mediated proliferation, through a direct LCK blockade. Our quantitative analysis resolved this conflict: model simulations demonstrated that the observed T-cell dynamics and the antagonistic effect of Src/BCR-ABL1 TKI could only be accurately recapitulated when the T-cell proliferation rate (parameter p_T) was effectively zeroed out. This result strongly supports direct inhibition of TCR-mediated T-cell proliferation as the dominant factor for the observed antagonism, as simply depleting target cells would not fully explain the T-cell kinetics.

These results led us to investigate whether cytokines could rescue T-cell function by boosting TCR survival signals through a parallel survival pathway. Our findings show that certain interleukins mitigate TKI-induced suppression of blinatumomab activity *in vitro*, enhancing pSTAT5 signaling, which supports T-cell survival and proliferation.[22] These insights potentially explain the favorable clinical outcomes, where such cytokines are naturally present in the body. Moreover, our work provides biological and quantitative evidence to support future testing of TKI-BiTE combinations with interleukins in Ph$^+$ ALL.

Methods

Cell culture and reagents

Experiments were performed using commercially sourced, de-identified peripheral blood mononuclear cells (PBMC) and established human cell lines. Institutional Review Board approval was not required. All procedures complied with institutional and national ethical and biosafety guidelines. Jurkat T cells, and BV173 cells were cultured in RPMI 1640 medium supplemented with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin. TKI were used at clinically relevant concentration (CRC) or peak concentrations (Cmax).[19,20] Blinatumomab was used at 1 ng/mL, and recombinant human IL-2, IL-7, and IL-15 were used at 10 ng/mL.

T-cell functional assays

T-cell signaling and function were assessed using various assays, as previously described.[19,20] LCK phosphorylation was analyzed in Jurkat cells via western blotting and flow cytometry using anti-phospho-LCK (Y394) and total LCK antibodies after TKI pre-treatment and CD3/CD28 stimulation. Luciferase assays were conducted in Jurkat-Luciferase cells co-cultured with BV173 cells and blinatumomab. STAT5 activation was evaluated by western blotting for phospho-STAT5 (Y694) in Jurkat or primary T cells stimulated with interleukins ± TKI. Combination therapy effects were assessed in primary human T cells co-cultured with BV173 cells, blinatumomab, and/or interleukins, with analysis of CD4, CD8, CD19, and PD-1 expression and viability via flow cytometry. Details of the experimental methods are fully described in the *Online Supplementary Methods*.

Modeling method

To generate data for model development, BV173 B cells and primary T cells were co-cultured at an effector-to-target (E:T) ratio of 1:2 in the presence of blinatumomab (1 ng/mL) for six days. Absolute counts of B cells (CD19$^+$), total T cells (CD8$^+$), and exhausted T cells (CD8$^+$PD-1$^+$) were measured daily by flow cytometry.

Two ordinary differential equation (ODE) models were developed to describe co-culture dynamics.

T-B model: basic T–B cell interaction - This model includes

logistic B-cell growth, T-cell-mediated B-cell killing, T-cell proliferation stimulated by B cells, and natural T-cell death. The ODE are:

$$\frac{dB}{dt} = r_B\, B\left(1-\frac{B}{K_B}\right) - a\left(\frac{B}{B^{n_B} + h_B^{n_B}}\right) T$$

$$\frac{dT}{dt} = p_T\left(\frac{B^{n_T}}{B^{n_T} + h_B^{n_T}}\right) T - d_T T$$

where parameters include B(B-cell population), r_B(B-cell growth rate), K_B(B-cell carrying capacity), a(maximum T-cell killing rate), h_B(half-maximal killing constant), n_B(Hill co-efficient for killing), T(T-cell population), p_T(T-cell proliferation rate), hT(half-maximal proliferation constant), n_T(Hill coefficient for proliferation), and d_T(T-cell death rate).

T-B-Tex model: incorporating T-cell exhaustion – The T-B-Tex Model extends the T-B Model to include exhausted T cells (T_{ex}),[23] their generation from active T cells, their suppressive effect on T-cell proliferation, and their own turnover. The expanded ODE are:

$$\frac{dB}{dt} = r_B\, B\left(1-\frac{B}{K}\right) - a\left(\frac{B^{n_B}}{B^{n_B} + h_B^{n_B}}\right) T$$

$$\frac{dB}{dt} = p_T T\left(\frac{B^{n_T}}{B^{n_T} + h_T^{n_T}}\right)\left(\frac{1}{1+ST_{ex}\,T_{ex}}\right) - K_{ex}\left(\frac{T^{n_{ex}}}{T^{n_{ex}} + h_{ex}^{n_{ex}}}\right) T - d_T T$$

$$\frac{dT_{ex}}{dt} = K_{ex}\left(\frac{T^{n_{ex}}}{T^{n_{ex}} + h_{Tex}^{n_{ex}}}\right) T - d_{T_{ex}} T_{ex}$$

Additional parameters are: K_{ex}(rate of exhaustion), s_{TexT}(T_{ex}-mediated suppressive strength), h_{ex}(half-maximal exhaustion constant), n_{ex}(Hill coefficient for exhaustion), and d_{Tex}(T_{ex} death rate).

Models were fit using a Bayesian framework and Markov Chain Monte Carlo (MCMC) to estimate parameters. Model evaluation metrics and posterior estimates are reported in *Online Supplementary Tables S1-S3*. Online Supplementary Figure S1A-C describes the gating strategy for flow cytometry analysis. The modeling method is fully described in the *Online Supplementary Methods*.

Software

Flow cytometry data were analyzed in FlowJo™ v10. Graphs and statistical analyses were performed in Graphpad Prism. The MCMC algorithm was developed and implemented in MATLAB, and all schematic figures were prepared in BioRender.

Results

Src/BCR-ABL1 tyrosine kinase inhibitors antagonize blinatumomab efficacy by suppressing T-cell expansion

Dasatinib and ponatinib are potent inhibitors of both Src and the BCR-ABL protein.[6,8] Blinatumomab, a BiTE, functions by simultaneously binding to CD3 on T cells and CD19 on malignant B cells, redirecting cytotoxic T lymphocytes to eliminate cancer cells through the formation of an immunological synapse and subsequent perforin/granzyme release. Although clinical combinations of these therapeutics have shown promising results in patients with Ph$^+$ ALL, preclinical studies suggest an antagonistic interaction between TKI and T-cell function[13,19,21] (Figure 1A).

We evaluated the direct impact of ponatinib on the Ph$^+$ ALL cell line, BV173. Treatment with ponatinib alone resulted in a dose-dependent reduction in BV173 cell viability. Specifically, we observed an approximately 80% reduction in cell viability at concentrations ≥10 nM, consistent with ponatinib's established anti-leukemic activity via BCR-ABL1 inhibition (Figure 1B). We next assessed the efficacy of blinatumomab using a co-culture system of BV173 cells with primary human T cells. Treatment with blinatumomab at concentrations of 0.5 ng/mL and 1 ng/mL induced significant killing of BV173 leukemia cells (Figure 1C). This potent cytotoxicity was accompanied by a robust increase in CD8$^+$ T-cell proliferation (Figure 1C), characteristic of blinatumomab's mechanism of redirecting and activating T cells against CD19$^+$ targets.

We then investigated the combination of blinatumomab (1 ng/mL) with increasing concentrations of ponatinib. This combination effectively reduced BV173 cell viability (Figure 1D). Moreover, T-cell expansion in the presence of blinatumomab combined with ponatinib (at all tested concentrations) was significantly lower (compare Figure 1D with panels C and E). The expected combination of ponatinib and blinatumomab effects were calculated using the Bliss independence model. Comparison with the experimentally observed killing revealed a significantly reduced response in the combination group (Bliss score: –18.56), consistent with an antagonistic interaction (Figure 1E).

To determine if this antagonism of T-cell function was specific to Src/BCR-ABL1 inhibitors, we compared blinatumomab combinations with SRC-sparing ABL TKI (imatinib, nilotinib) *versus* Src/BCR-ABL TKI (ponatinib, dasatinib). While all combinations reduced B-cell viability, SFK-active ABL inhibitors were less effective, leaving a higher percentage of residual B cells. Crucially, Src/BCR-ABL1 inhibitors significantly impaired T-cell expansion, which remained near baseline (1-1.5-fold increase). In contrast, imatinib or nilotinib did not impair T-cell proliferation when combined with blinatumomab (Figure 1F). These findings strongly suggest that the Src/BCR-ABL1 TKI antagonize blinatumomab's T-cell activity, likely due to off-target inhibition of Src kinases like LCK, which are essential for T-cell activation. This is consistent with previous observations.[19]

Inhibition of Src/BCR-ABL1 tyrosine kinase inhibitors inhibits LCK phosphorylation and downstream signaling

T-cell activation, a complex process essential for adaptive

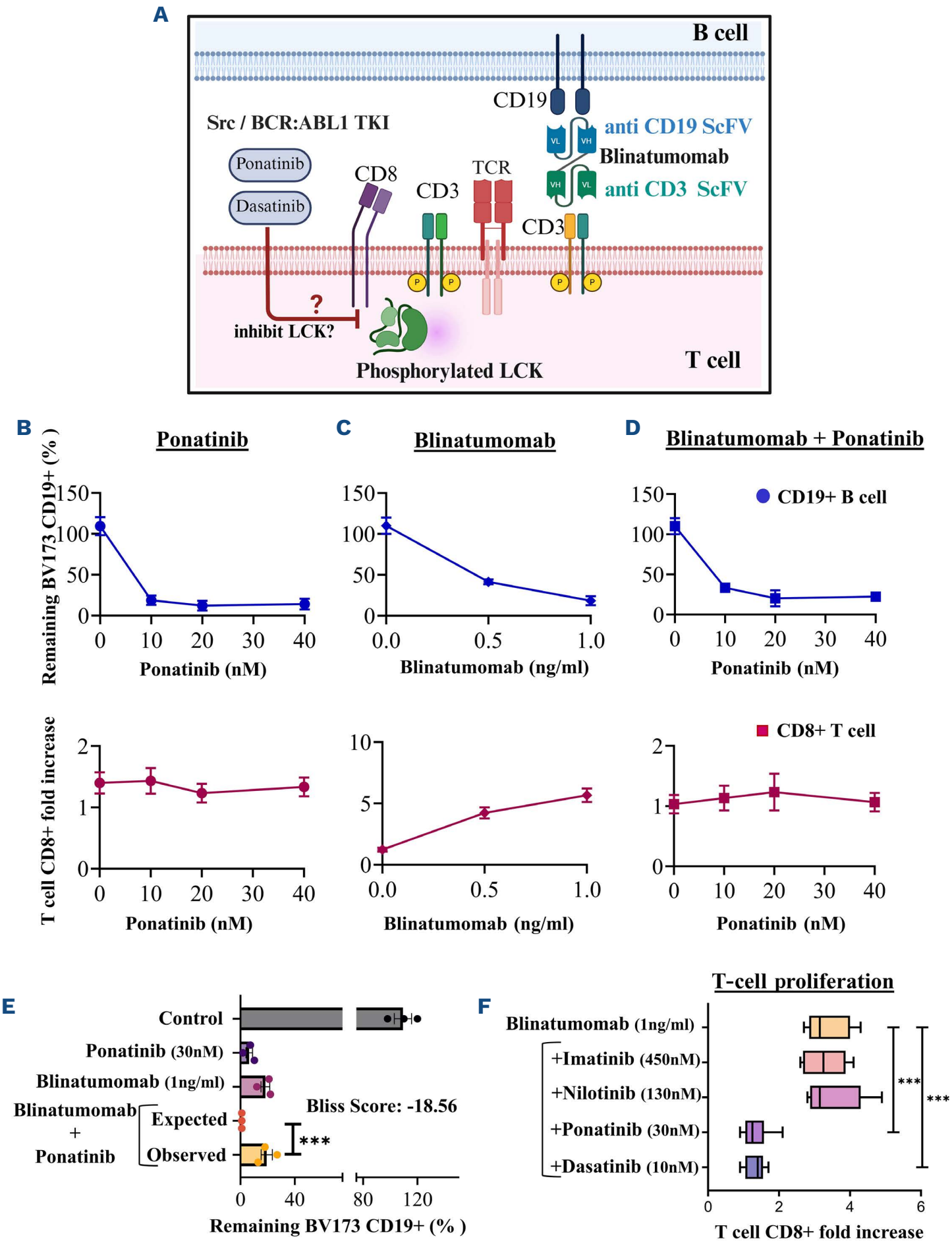


Figure 1. Src/BCR-ABL1 tyrosine kinase inhibitors antagonize blinatumomab efficacy by suppressing T-cell expansion. (A) Schematic illustrating blinatumomab's mechanism of action, in which the CD3–CD19 bispecific T-cell engager links CD3⁺ T cells to CD19⁺ BV173 leukemia cells to promote T-cell-mediated cytotoxicity. (B) BV173 leukemia cell viability (top: blue) following treatment with increasing concentrations of ponatinib, and T-cell proliferation (bottom: red) in response to ponatinib alone. (C) BV173 cell killing induced by blinatumomab alone (top: blue) and CD8⁺ T-cell expansion stimulated by blinatumomab alone (bottom: red). (D) BV173 cell numbers in co-cultures treated with combination of blinatumomab (1 ng/mL) and ponatinib (10–40 nM) (top: blue). Blinatumomab-induced T-cell proliferation in the presence of ponatinib (bottom: red). (E) Comparison of observed *versus* expected BV173 cell killing under blinatumomab + ponatinib treatment, showing an antagonistic interaction (Bliss score: –18.56). (F) T-cell proliferation in co-cultures treated with blinatumomab and Src/BCR-ABL tyrosine kinase inhibitors (TKI) (ponatinib, dasatinib) or BCR-ABL-selective TKI (imatinib, nilotinib). Data represent mean ± Standard Error of Mean from N=3 (for panels B-E) and N=6 (for panel F) from primary human T-cell and BV173 co-cultures at an effector:target ratio of 1:2. Statistical significance was assessed using one-way ANOVA with Dunnett's post-hoc test comparing each treatment condition to blinatumomab alone. ****P*<0.001.

immune responses, is initiated by T-cell receptor (TCR) engagement. Even with stimuli that bypass MHC-antigen presentation, such as CD3/CD28 engagement or BiTE-mediated synapses, the intracellular signaling cascade depends critically on the lymphocyte-specific protein tyrosine kinase LCK.[24] LCK initiates TCR signaling by phosphorylating immunoreceptor tyrosine-based activation motifs (ITAM) within the CD3 complex, facilitating recruitment and activation of ZAP70.[25] LCK activity is tightly regulated by phosphorylation at tyrosine residue Y394 within its activation loop, which stabilizes its active conformation and is essential for robust downstream signaling[18,26] (Figure 2A).

To test the above pathway and the concordance with prior studies,[19] we began by examining their impact on LCK phosphorylation, a critical early event in TCR signaling. Serum-starved Jurkat T-cells were pre-treated with various TKI at both their CRC and peak concentrations (Cmax) for 2-4 hours and then stimulated with CD3/CD28-conjugated dynabeads to activate the TCR pathway. Western blot analysis of LCK phosphorylation at the key tyrosine residue Y394 revealed a significant reduction in phosphorylated LCK (pLCK Y394) in cells treated with ponatinib and dasatinib at both CRC and Cmax (Figure 2B). This inhibitory effect was rapid, becoming evident as early as 30 minutes (min) post stimulation, with the reduction in pLCK Y394 band intensity consistent with the known inhibitory activity of these drugs against Src kinases, including LCK (*Online Supplementary Figure S2A, B*). In contrast, cells treated with the BCR-ABL1-selective TKI imatinib and nilotinib showed no significant change in LCK Y394 phosphorylation compared to stimulated controls (Figure 2B, *Online Supplementary Figure S2B*). These observations were further confirmed and quantified using flow cytometry to analyze intracellular pLCK (Y394) levels in Jurkat T-cells stimulated for 30 mins and 2 hours (hr). Consistent with our western blot results, treatment with ponatinib or dasatinib resulted in a marked and sustained reduction in the mean fluorescence intensity (MFI) of pLCK (Y394) when measured by intracellular flow cytometry on fixed and permeabilized cells (*Online Supplementary Figure S2B*). Specifically, at 30 min, pLCK MFI was reduced by approximately 90% for both ponatinib and dasatinib compared to stimulated control. This significant reduction persisted at 2 hr, with MFI levels remaining approximately 90% lower for both drugs. As observed with western blotting, the selective inhibitors imatinib and nilotinib had no significant effect on LCK phosphorylation under the same condition. These findings provide compelling evidence that ponatinib and dasatinib potently inhibit LCK phosphorylation, while imatinib and nilotinib do not, reflecting their distinct kinase selectivity profiles.

Following this, we assessed the impact of these TKI on T-cell proliferation in a more pharmacologically relevant context. We measured the proliferation of total $CD3^+$ T cells and quantified the absolute counts of specific subsets ($CD4^+$ and $CD8^+$) in co-cultures of BV173 leukemia cells and primary human PBMC after three days of treatment. The treatment with Src/BCR-ABL1 TKI (dasatinib, ponatinib) significantly inhibited the overall expansion of both the $CD4^+$ and $CD8^+$ T-cell populations in the presence of blinatumomab when compared to blinatumomab alone. In contrast, the BCR-ABL1-selective TKI (imatinib, nilotinib) did not show this inhibitory effect on $CD4^+$ and $CD8^+$ T-cell expansion, further supporting the role of Src kinase inhibition in the observed antagonism (*Online Supplementary Figure S2C*).

To investigate the functional consequences of LCK inhibition on downstream T-cell effector functions, we measured IL-2 production using Jurkat-Luciferase reporter cells. These cells, co-cultured with BV173 leukemia cells and treated with blinatumomab in the presence or absence of TKI, report IL-2 promoter activity. We found that the Src/BCR-ABL1 TKI, ponatinib and dasatinib, significantly inhibited blinatumomab-induced IL-2 production. Ponatinib reduced IL-2 production by approximately 30% at its CRC and 80% at its Cmax, while dasatinib reduced it by approximately 50% and 90%, respectively. This demonstrates that the TKI-mediated blockade of LCK phosphorylation directly impairs downstream effector cytokine production (see Figure 2D for IL2 activity). To further evaluate the impact on proximal signaling, we measured NFAT-driven luciferase activity, as NFAT is a critical transcription factor in the TCR signaling cascade that regulates IL-2 and other key activation genes. Consistent with the LCK phosphorylation and IL-2 data, ponatinib and dasatinib significantly inhibited NFAT activation at both CRC and Cmax concentrations. Ponatinib reduced NFAT activity by approximately 30% at its CRC and 50% at its Cmax ($P<0.001$), while dasatinib reduced it by approximately 20% and 50% at these concentrations ($P<0.001$). These results collectively demonstrate that Src/BCR-ABL1 TKI, such as ponatinib and dasatinib, directly inhibit T-cell activation by suppressing phosphorylation of LCK at Y394 (see Figure 2D for NFAT activity). This inhibition effectively abrogates proximal TCR signaling, resulting in impaired NFAT activation, diminished IL-2 production, and ultimately, the functional antagonism of blinatumomab-induced T-cell responses. All of this signaling data were consistent with prior observations[19] and is highly suggestive that SFK inhibition downstream of the TCR is the mechanism of ponatinib / dasatinib-mediated antagonism of T-cell function. However, the assessment of signaling reductions does not precisely specify the mechanism underlying the co-culture effect here. This is because there are two mechanistic paths to reducing TCR signaling in the co-culture experiment.

Mathematical modeling to discern target depletion from direct T-cell inhibition

The clinical success of combining potent Src/BCR-ABL1 TKI (like dasatinib and ponatinib) with blinatumomab presents a paradox when compared to established *in vitro* data, which suggested these agents antagonize T-cell function by inhibiting the proximal TCR kinase, LCK. This conflict is

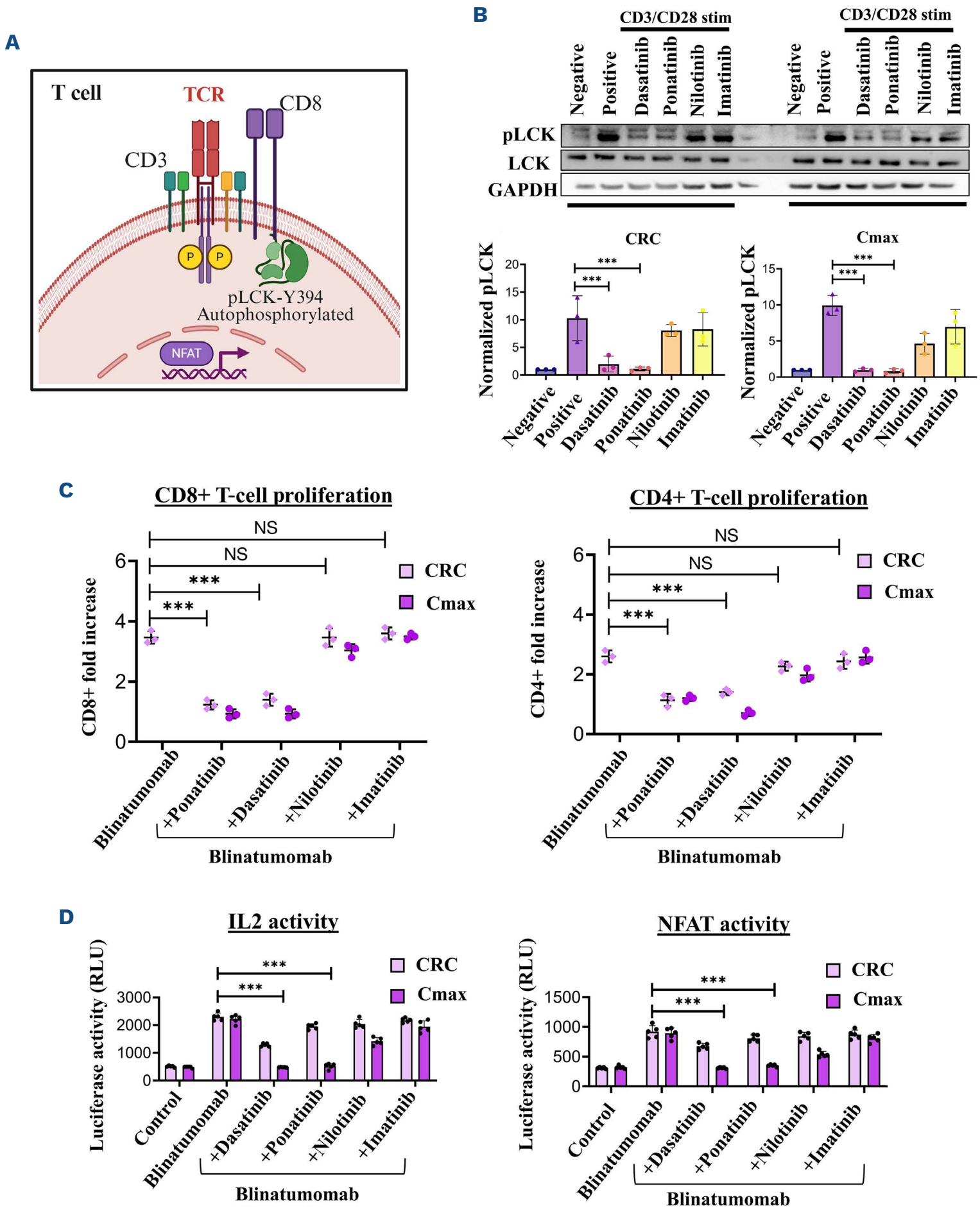


Figure 2. Inhibition of Src/BCR-ABL1 tyrosine kinase inhibitors inhibits LCK phosphorylation and downstream signaling. (A) Schematic depicting the role of LCK phosphorylation at Y394 in initiating proximal T-cell receptor (TCR) signaling and driving downstream activation. (B) Phospho-LCK (Y394) levels in serum-starved Jurkat T cells stimulated (stim) with CD3/CD28 in the presence or absence of tyrosine kinase inhibitors (TKI). Dual Src/BCR-ABL1 inhibitors (ponatinib, dasatinib) reduce pLCK Y394, whereas ABL-selective inhibitors (imatinib, nilotinib) show minimal effect. Bar graphs show pLCK normalized to total LCK at clinically relevant concentration (CRC) and peak concentrations (Cmax). (C) Effects of TKI on blinatumomab-stimulated T-cell proliferation. (Left) $CD8^+$ T-cell proliferation expressed as fold change relative to blinatumomab alone. (Right) $CD4^+$ T-cell proliferation expressed as fold change relative to blinatumomab alone. (D) (Left) Reporter-based assessment of cytokine and transcriptional activity in the presence of TKI. IL-2 production quantified using an IL-2 luciferase reporter in Jurkat–BV173 co-cultures treated with blinatumomab. (Right) NFAT-dependent luciferase activity measured to evaluate TCR-proximal signaling under TKI treatment. Data represent mean ± Standard Error of Mean from N=3 (for panels B and C) and N=5 (for panel D) independent biological replicates. Statistical significance was assessed using one-way ANOVA with Dunnett's post-hoc test comparing each treatment condition to blinatumomab alone. ***P<0.001, NS: not significant.

complicated by a key difference between the TKI: dasatinib and ponatinib are significantly more potent against BCR-ABL cells than imatinib or nilotinib, highlighting an ambiguity in the interpretation of the co-culture results. To definitively resolve the mechanism responsible for the observed T-cell suppression *in vitro*, we formally specified two competing hypotheses, as both are possible consequences of using highly potent Src/BCR-ABL TKI in the co-culture system:

Hypothesis 1: rapid target cell depletion - The mechanism of this is that ponatinib and dasatinib are more potent ABL inhibitors than imatinib and nilotinib. This hypothesis posits that the rapid, TKI-mediated elimination of BCR-ABL-dependent B cells removes the necessary antigenic stimulus for sustained TCR activation, leading to reduced T-cell activity. The clinical significance of this is that, if true, the difference between *in vitro* antagonism and clinical success might be attributed to the persistence of physical "safe harbor" sites *in vivo* where Ph$^+$ ALL cells remain during ponatinib treatment, thereby continuously maintaining T-cell stimulation. In this case, combination with blinatumomab may be critical for clearing these residual disease sites (Figure 3A).

Hypothesis 2: direct inhibition of T-cell activation via LCK/FYN - The mechanism of this is that this proposes the existing hypothesis that SFK inhibition by dasatinib and ponatinib directly impairs TCR-mediated T-cell proliferation and activation through the critical kinases LCK/FYN. This effect is predicted to occur independently of B-cell availability. The clinical significance of this is that, if true, the clinical efficacy suggests that *in vivo* microenvironmental factors — such as naturally occurring cytokines — can overcome or complement the LCK/FYN blockade by engaging a parallel survival or proliferation pathway (Figure 3B).

To evaluate these hypotheses, we reasoned that the quantitative, time-dependent dynamics of the co-culture system would contain sufficient information to unambiguously distinguish between the two mechanisms. We characterized the quantitative dynamics of CD19$^+$ B cells, CD8$^+$ effector T cells, and PD-1$^+$ exhausted T cells in our *in vitro* co-culture system (*Online Supplementary Figure S3A*). We began by constructing a foundational ordinary differential equation (ODE) model (T-B Model) that described B-cell logistic growth, T-cell-mediated killing, T-cell proliferation, and death (Online Supplementary Figure S3B).[18,27] Parameters were estimated by fitting T-B Model to the experimental data using Markov Chain Monte Carlo[28] (*Online Supplementary Table S2*). While this T-B Model successfully reproduced early B-cell depletion and T-cell expansion, it failed to capture T-cell contraction from day 3 onward. An analysis of the residuals revealed systematic deviations during later stages, indicating a clear model misspecification (*Online Supplementary Figure S3C, D*).

Based on experimental evidence of T-cell exhaustion, we extended the model to the T-B-Tex (*Online Supplementary Table S3*) to explicitly include an exhausted T-cell population alongside functional T cells and B cells.[23] T-B-Tex incorporated transitions from functional T cells to an exhausted state, suppression of functional T-cell proliferation by exhausted T cells, and exhausted T-cell death (see Methods). Fitting this final model to the full dataset using MCMC resulted in a markedly improved agreement with the experimental data, accurately reproducing both the early expansion and subsequent contraction of T cells (Figure 3C). Residual analysis further confirmed T-B-Tex's superior fit, showing errors that were more randomly distributed around zero with reduced systematic patterns (*Online Supplementary Figure S4A-C*). Model comparison metrics also supported the T-B-Tex model: AIC and BIC values were lower despite its greater complexity, and log-likelihood, RMSE, and bias were all improved (*Online Supplementary Table S1*).

The validated T-B-Tex model enabled a direct, *in silico* test of the two hypotheses and a comparison to measured T-cell dynamics.

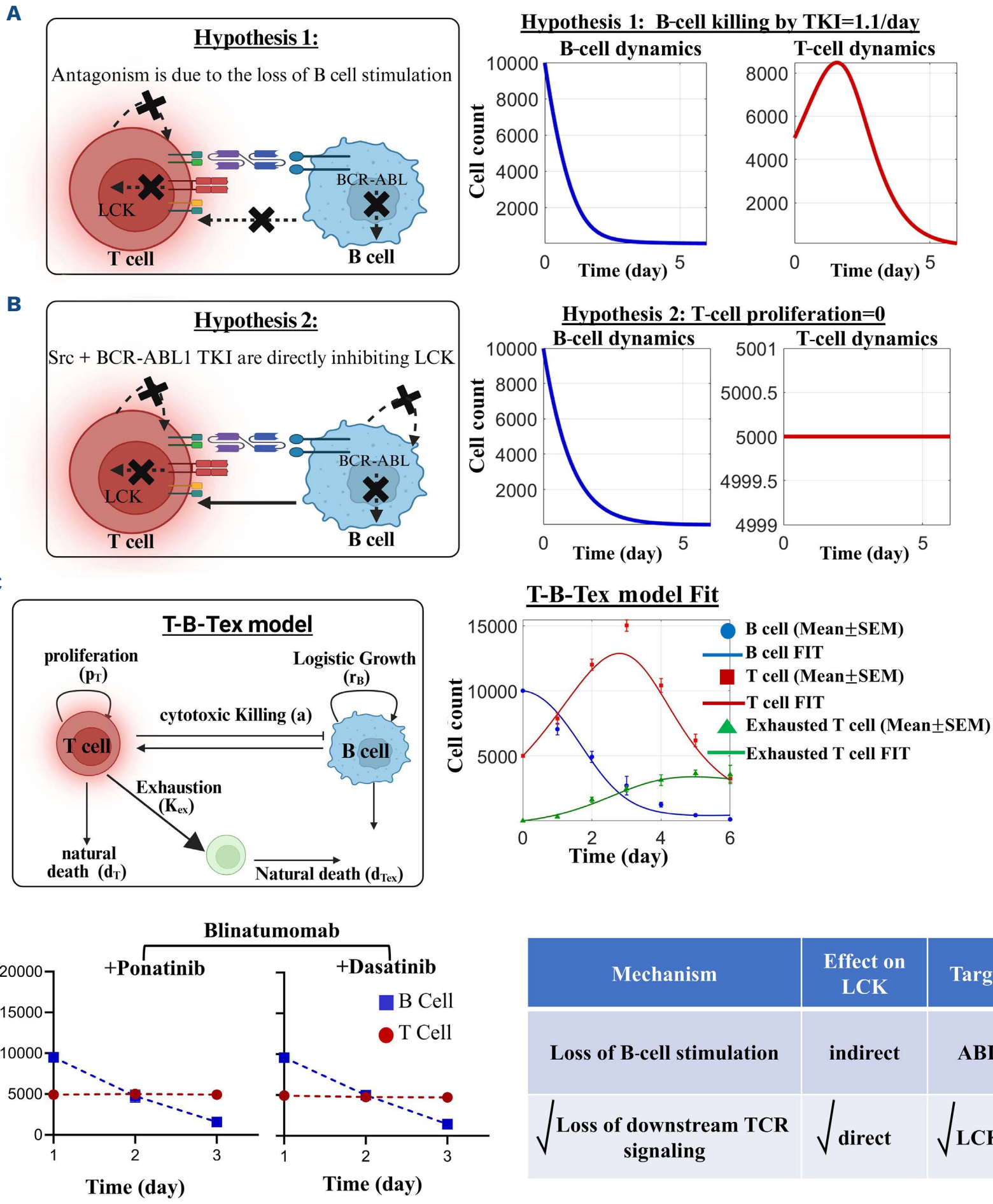
Testing Hypothesis 1 (target cell loss) - When antagonism was simulated solely by the loss of B-cell stimulation — representing rapid target cell depletion — this mechanism could not reproduce the observed T-cell kinetics (Figure 3A).

Testing Hypothesis 2 (direct T-cell intrinsic inhibition of LCK/FYN) - In contrast, when T-cell proliferation was suppressed *in silico* (Model 2), we observed a flat T-cell count at all timepoints (Figure 3B). This was closely recapitulated by the observed T-cell dynamics during combination therapy with Src/BCR-ABL1 TKI (Figure 3D). Given the T-cell count data in Figure 3D, we computed the Poisson log-likelihood for both hypotheses under the blinatumomab + ponatinib condition. Hypothesis 1 produced a log-likelihood of LL of –7407.142, whereas Hypothesis 2 (B-cell killing plus T-cell inhibition) yielded a dramatically higher log-likelihood of LL of –185.142. This more than 40-fold improvement in likelihood strongly supports Hypothesis 2 as the correct explanation for the observed T-cell dynamics (*Online Supplementary Table S4*).

Collectively, these results resolve the mechanistic uncertainty and demonstrate that the observed antagonistic effect of Src/BCR-ABL TKI arises primarily from direct inhibition of proximal TCR signaling through LCK and FYN in the T cell, rather than target cell depletion. This also suggests that the origins of the dramatic *in vivo* activity may be through rescuing lost survival signaling through a parallel survival pathway.

Cytokines IL-2, IL-7, and IL-15 rescue the antagonistic effects of Src/BCR-ABL1 tyrosine kinase inhibitors on blinatumomab efficacy

Based on the concordance between our *in silico* investigation and measured *in vitro* data, which indicated that the antagonistic effect of Src/BCR-ABL1 TKI on T-cell function is primarily due to LCK/FYN inhibition rather than target cell depletion, we explored biological factors that could resolve the apparent discrepancy between preclinical antagonism



Mechanism	Effect on LCK	Target
Loss of B-cell stimulation	indirect	ABL
√ Loss of downstream TCR signaling	√ direct	√ LCK

Figure 3. Mathematical modeling to discern target depletion from direct T-cell inhibition. (A) Hypothesis 1: model implementation in which rapid B-cell depletion (tyrosine kinase inhibitors [TKI]-mediated B-cell killing rate = 1.1/day) reduces antigen availability and indirectly limits T-cell expansion. (B) Hypothesis 2: model implementation in which T-cell proliferation is directly inhibited by TKI (p_T = 0), leading to impaired T-cell expansion and reduced B-cell killing. (C) Schematic and simulation output from the T–B–Tex model showing improved agreement with experimental data, including reproduction of late-phase T-cell decline. The model extends the T–B framework (see *Online Supplementary Figure S3*) by incorporating an exhausted T-cell population (T_{ex}). The schematic depicts transitions among B cells, functional T cells (T), and exhausted T cells (T_{ex}), including the exhaustion rate (k_{ex}) and the suppressive influence of T_{ex} on T-cell activity. (D) Experimental validation of model predictions showing reduced T-cell expansion in combination treatments with Src/BCR-ABL1 TKI (ponatinib, dasatinib). Graphs display absolute BV173 cell counts and $CD8^+$ T-cell counts over three days in co-cultures with BV173 cells treated with blinatumomab alone or in combination with Src/BCR-ABL1 TKI. Data represent mean ± Standard Error of Mean from N=6 independent biological replicates. Model fitting and validation were performed using Bayesian parameter estimation with Poisson likelihood and Markov Chain Monte Carlo (MC-MC) sampling. Model comparison was based on AIC/BIC criteria.

and promising clinical outcomes. This led us to test whether cell-intrinsic pathways might rescue T-cell activity.

We evaluated the impact of three common gamma-chain (γc) cytokines — IL-2, IL-7, and IL-15 — on blinatumomab-TKI combinations. These interleukins are known regulators of T-cell biology, acting through the JAK-STAT pathway and leading to STAT5 phosphorylation (Figure 4A).[29,30] IL-2 supports T-cell proliferation and differentiation,[31,32] IL-7 promotes homeostasis and survival,[33,34] and IL-15 enhances memory CD8$^+$ T-cell function and expansion.[35] We hypothesized that these cytokines might activate compensatory signaling pathways to bypass the impaired LCK-dependent TCR signaling in the presence of Src/BCR-ABL1 TKI.

To assess STAT5 activation, Jurkat T-cells were stimulated with 10 ng/mL IL-2, IL-7, or IL-15 (alone or in combination) for 2-4 hr. Cells were treated with TKI (ponatinib, dasatinib, imatinib, nilotinib) at CRC from the methods and then stimulated for 10 min with CD3/CD28 dynabeads. Western blotting for phosphorylated STAT5 (pSTAT5) showed robust activation by each interleukin (Figure 4B), suggesting that γ-chain cytokine signaling remains active across ABL TKI.

We next tested the functional impact of these cytokines on blinatumomab efficacy in the presence of TKI. BV173 leukemia cells were co-cultured with T cells and treated with blinatumomab (1 ng/mL) plus each TKI at CRC, with or without IL-2, IL-7, or IL-15 for two days. Flow cytometry showed enhanced pSTAT5 signaling with cytokine addition — even in the presence of dasatinib — demonstrating the partial rescue of this key pathway (*Online Supplementary Figure S5A*). Cytokine addition also significantly improved blinatumomab-mediated cytotoxicity, especially with dasatinib or ponatinib (*Online Supplementary Figure S6*). Among the three, IL-7 produced the strongest effect on T-cell proliferation: an approximately 2-fold increase with blinatumomab + dasatinib + IL-7 *versus* blinatumomab + dasatinib alone. The triple combination of blinatumomab, dasatinib, and IL-7 restored CD8 T-cell numbers and restored some of the antagonized killing in CD19$^+$ BV173 cells (Figure 4C), indicating IL-7 can partially restore some of the anti-leukemic T-cell activity suppressed by Src/BCR-ABL1 TKI *in vitro*.

To further validate the rescue effect, we performed Jurkat-luciferase reporter assays for IL-2 and NFAT. Jurkat cells co-cultured with BV173 cells were treated with blinatumomab (1 ng/mL) and dasatinib (10 nM) for 10 hr, with or without the cytokines. All three cytokines significantly increased IL-2 and NFAT luciferase activity, confirming their ability to restore T-cell activation even under Src kinase inhibition at their CRC concentration (Figure 4D).

We then asked whether these cytokines might also protect against T-cell exhaustion, a key contributor to impaired persistence in bispecific T-cell engager therapies[36] and an effect we observed *in vitro* in Figure 3. Figure 4E shows a schematic of the T-B cell synapse and the PD-1 receptor involved in exhaustion.[37,38] PD-1 expression was quantified after 48 hr in blinatumomab co-cultures (BV173 + T cells, E:T 1:1). Blinatumomab alone induced approximately 30% PD-1$^+$ T cells. Supplementation with IL-2, IL-7, or IL-15 reduced PD-1 expression, with IL-7 being the most effective at protecting against exhaustion (Figure 4F). This reduction in exhaustion correlates with enhanced cytotoxicity and T-cell proliferation (see Figure 4C and *Online Supplementary Figure S6*). It further suggests that mitigating exhaustion is a key mechanism by which γc cytokines — particularly IL-7 — might rescue T-cell function in the presence of antagonistic Src/BCR-ABL1 TKI.

Discussion

The treatment landscape for Ph$^+$ ALL has been transformed by targeted TKI[8,9,39] and immunotherapies such as blinatumomab.[11,40,41] While combinations of these potent agents hold significant promise,[13,15] their optimal integration could be improved through a mechanistic understanding of potential synergistic or antagonistic interactions. Our study provides critical insights into the interaction between Src/BCR-ABL1 TKI (dasatinib and ponatinib) and blinatumomab-mediated T-cell function, with direct implications for the design of new combination regimens. We rigorously demonstrate that dasatinib and ponatinib, beyond their potent inhibition of BCR-ABL1, robustly suppress LCK phosphorylation at Y394 — a pivotal step in proximal TCR signaling.[18] This off-target inhibition impairs T-cell proliferation (consistent with prior reports),[19] cytokine production, NFAT activation, and blinatumomab-driven cytotoxicity against leukemia cells. In contrast, SFK-sparing BCR-ABL1$^-$ TKI, such as imatinib and nilotinib, exhibit minimal SFK inhibition at therapeutic concentrations and preserve blinatumomab's ability to stimulate T-cell responses.[19,20] A key question is how, despite this *in vitro* antagonism, clinical trials combining blinatumomab with dasatinib or ponatinib have demonstrated strikingly favorable outcomes.[15]

One remaining question that we addressed in this study is whether the decrease in LCK signaling and antagonism of T-cell activation is upstream or downstream of the TCR. Ponatinib and dasatinib have low single digit nanomolar potency on cells *in vitro*[39,42] while imatinib and nilotinib are significantly less potent. While perhaps less likely, it is formally possible that the kinetics of cell death during a killing assay could deprive the T-cell receptor of stimulation, therefore, inducing antagonism and reducing LCK phosphorylation. Measuring LCK phosphorylation in an orthogonal stimulation experiment does not settle this question, but understanding the dynamics of T-cell growth during co-culture with target cells can resolve it unambiguously. To quantitatively dissect this paradox, we employed mathematical modeling to test two competing hypotheses: (1) that antagonism reflects rapid elimination of target B cells, depriving T cells of stimulatory signals, or (2) that it

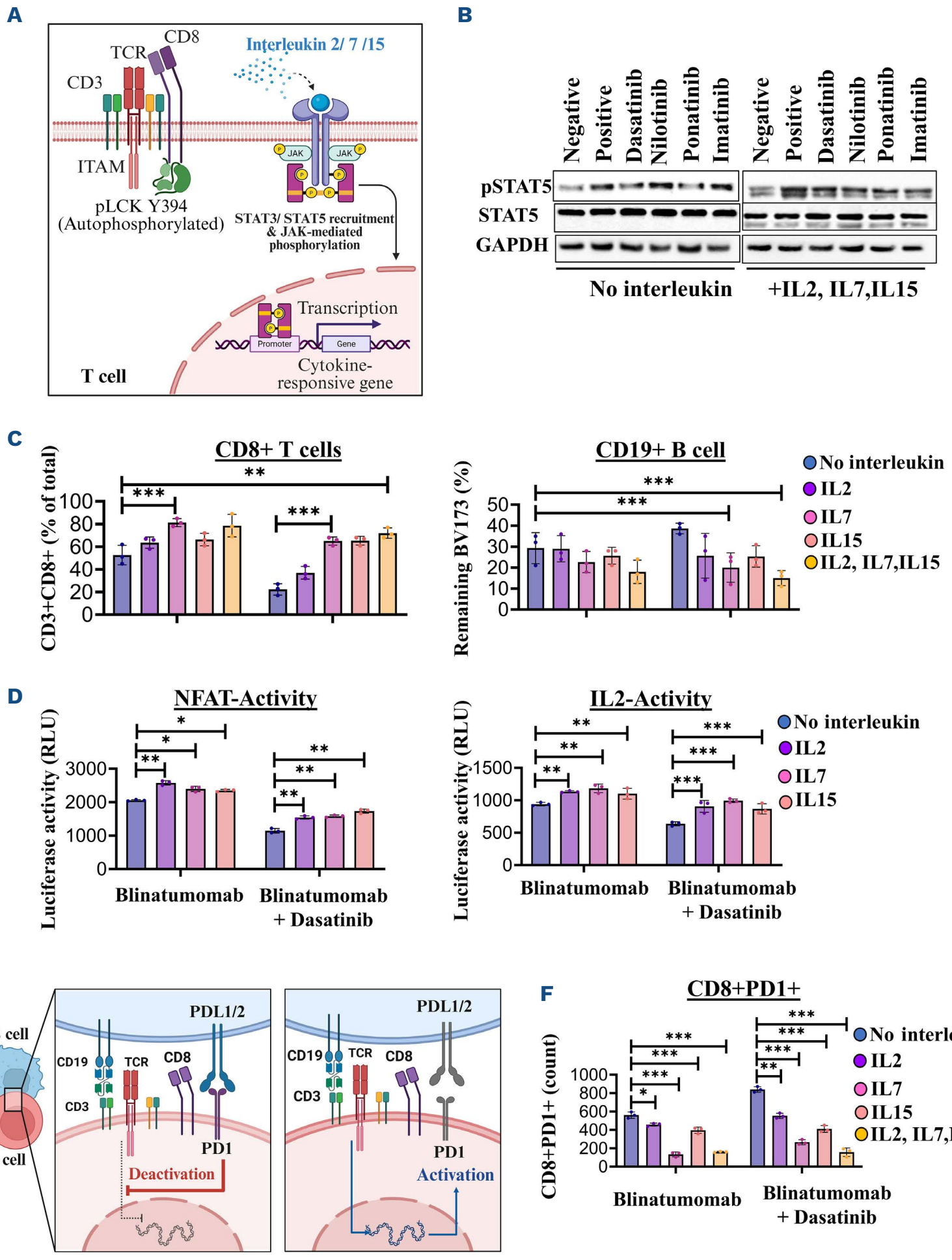


Figure 4. Cytokines IL-2, IL-7, and IL-15 rescue the antagonistic effects of Src/BCR-ABL1 tyrosine kinase inhibitors on blinatumomab efficacy. (A) Schematic illustrating the T-cell activation pathway leading to NFAT and IL-2 production and downstream STAT5 phosphorylation, highlighting the role of LCK Y394 and cytokine receptor signaling (IL-2/IL-7/IL-15). (B) Western blot analysis of pSTAT5 and total STAT5 in Jurkat T cells showing increased STAT5 phosphorylation following addition of IL-2, IL-7, IL-15, and combination of all. (C) T-cell proliferation and BV173 killing in co-cultures treated with dasatinib, with or without IL-2, IL-7, or IL-15. Cytokine addition partially restores CD8⁺ T-cell proliferation and blinatumomab-mediated BV173 killing in the presence of dasatinib. (D) IL-2 and NFAT luciferase reporter activity in Jurkat cells treated with dasatinib, with or without IL-2, IL-7, or IL-15. Cytokines partially restore IL-2 and NFAT promoter activity after 16 hours. (E) Schematic illustrating T-cell activation and deactivation pathways, including PD-1/PD-L1-mediated inhibitory signaling. (F) Frequency of PD-1⁺ CD8⁺ T cells in co-cultures treated with dasatinib, with or without IL-2, IL-7, or IL-15. Data represent mean ± Standard Error of Mean from N=3 (for panels C-F) independent biological replicates. Statistical significance was assessed using one-way ANOVA with Dunnett's post-hoc test comparing each condition with the interleukin to no interleukin (control). **P*<0.05, ***P*<0.01, ****P*<0.001.

results from direct inhibition of TCR-mediated T-cell proliferation through direct inhibition of LCK/FYN, independent of target availability. Our model simulations demonstrated that only the suppression of T-cell proliferation in a cell intrinsic manner could recapitulate the observed dynamics, directly supporting the idea that the direct inhibition of TCR signaling through direct inhibition of LCK/FYN, rather than target CD19 cell depletion, is the dominant mechanism of antagonism. While consistent with prior arguments, this hypothesis of the dynamics involved adds to our understanding. Identifying an unambiguous reading of the mechanism involved has important implications. By showing that the mechanism is unambiguously downstream of TCR, we raise the possibility that other intracellular signals could compensate for part, or all, of the effect.

A major contribution of this work is identifying IL-7 as a potent mitigator of *in vitro* antagonism. IL-7 restored blinatumomab efficacy and T-cell proliferation in the presence of Src/BCR-ABL1 TKI, associated with STAT5 activation and reduced T-cell exhaustion. Thus, the clinical success of TKI-blinatumomab combinations is likely explained by the *in vivo* microenvironment. Blinatumomab infusion is known to induce a transient surge of pro-inflammatory cytokines such as IL2,[43] while separate clinical trials of IL-7-secreting ("armored") chimeric antigen receptor (CAR)-T cells have provided clinical proof-of-principle that IL-7 enhances T-cell efficacy in patients.[44] This aligns with our finding that IL-7 can mitigate the antagonism of Src/BCR-ABL1 TKI on T-cell signaling. These findings suggest that the cytokine-rich *in vivo* microenvironment may help explain the discrepancy between *in vitro* suppression and clinical success.

We acknowledge the limitations of our study, which utilized a simplified *in vitro* system with cell lines and healthy donor PBMC. This approach was necessary for mechanistic clarity but does not fully recapitulate the dynamic clinical context, including patient heterogeneity and the supportive bone marrow microenvironment. Future work could further validate these findings in more clinically relevant primary patient samples.

In conclusion, Src/BCR-ABL1 TKI antagonize blinatumomab by inhibiting LCK-dependent TCR signaling, impairing T-cell activity. IL-7 rescues this effect by restoring proliferation and reducing exhaustion. These results clarify a mechanistic paradox and potentially support cytokine supplementation, particularly IL-7, as a rational strategy to optimize TKI-BiTE combinations in Ph^+ ALL.

Disclosures

JRP reports a consultancy role for Takeda Pharmaceuticals, Versant Ventures, Von Pfeffel Pharmaceuticals, Atlas Biotech, Curie.Bio, Galapagos NV, MOMA Therapeutics, Red Ace Bio, F. Hoffman La Roche, Genentech, Theseus Pharmaceuticals, WuXi Next Code, and Third Rock Ventures, has received travel funding from Roche, Genentech, Theseus, and MOMA Therapeutics, and has received equity in Red Ace Bio, MOMA Therapeutics, and Theseus Pharmaceuticals. All of the other authors have no conflicts of interest to disclose.

Contributions

JRP supervised the project, provided funding, designed the experiments and the data analysis and interpretation, developed the analytical methods, and wrote the manuscript; FN designed and performed experiments, is responsible for data analysis and interpretation, developed and implemented MATLAB MCMC code (Numerical methods), and wrote the manuscript; JAR performed and optimized experiments, and analyzed data; CD provided funding and supervised the project.

Funding

This research was supported by the National Center for Advancing Translational Sciences of the National Institutes of Health (Award Numbers TL1TR002016, 5TL1TR002016-06, and U01CA265709). The content is solely the responsibility of the authors and does not necessarily represent the official views of the NIH.

Data-sharing statement

The custom code and scripts used for data analysis and visualization in this study are available from the corresponding author upon reasonable request. MATLAB Code is available at https://zenodo.org/badge/DOI/10.5281/zenodo.16540528.svg (MCMC code-T-cell and B-cell interaction).

References

1. Faderl S, Kantarjian HM, Thomas DA, et al. Outcome of Philadelphia chromosome-positive adult acute lymphoblastic leukemia. Leuk Lymphoma. 2000;36(3-4):263-273.
2. Paul S, Kantarjian H, Jabbour EJ. Adult acute lymphoblastic leukemia. Mayo Clin Proc. 2016;91(11):1645-1666.
3. Gleißner B, Gökbuget N, Bartram CR, et al. Leading prognostic relevance of the BCR-ABL translocation in adult acute B-lineage lymphoblastic leukemia: a prospective study of the German Multicenter Trial Group and confirmed polymerase chain reaction analysis. Blood. 2002;99(5):1536-1543.
4. Kantarjian H, Aldoss I, Jabbour E. Management of adult acute lymphoblastic leukemia: a review. JAMA Oncol. 2025;11(7):771-778.
5. Soverini S, Hochhaus A, Nicolini FE, et al. BCR-ABL kinase domain mutation analysis in chronic myeloid leukemia patients treated with tyrosine kinase inhibitors: recommendations from an expert panel on behalf of European LeukemiaNet. Blood. 2011;118(5):1208-1215.
6. Cortes JE, Saglio G, Kantarjian HM, et al. Final 5-year study results of DASISION: the dasatinib versus imatinib study in treatment-naïve chronic myeloid leukemia patients trial. J Clin

Oncol. 2016;34(20):2333-2340.
7. O'Hare T, Deininger MWN, Eide CA, Clackson T, Druker BJ. Targeting the BCR-ABL signaling pathway in therapy-resistant Philadelphia chromosome-positive leukemia. Clin Cancer Res. 2011;17(2):212-221.
8. Aldoss I, Ribera J-M, Kantarjian H, et al. Ponatinib versus imatinib in patients with newly diagnosed Ph+ ALL: subgroup analysis of the phase 3 Phallcon study. Blood. 2023;142(Suppl 1):2871.
9. Jabbour E, Kantarjian H, Aldoss I, et al. S110: Phallcon: a phase 3 study comparing ponatinib versus imatinib in newly diagnosed Ph+ ALL. Hemasphere. 2023;7(S3):e68516d0.
10. Bargou R, Leo E, Zugmaier G, et al. Tumor regression in cancer patients by very low doses of a T cell-engaging antibody. Science. 2008;321(5891):974-977.
11. Kantarjian H, Stein A, Gökbuget N, et al. Blinatumomab versus chemotherapy for advanced acute lymphoblastic leukemia. N Engl J Med. 2017;376(9):836-847.
12. Sas V, Moisoiu V, Teodorescu P, et al. Approach to the adult acute lymphoblastic leukemia patient. J Clin Med. 2019;8(8):1182.
13. Foà R, Bassan R, Vitale A, et al. Dasatinib-blinatumomab for Ph-positive acute lymphoblastic leukemia in adults. N Engl J Med. 2020;383(17):1613-1623.
14. Foà R, Bassan R, Elia L, et al. Long-term results of the dasatinib-blinatumomab protocol for adult Philadelphia-positive ALL. J Clin Oncol. 2024;42(8):881-885.
15. Jabbour E, Short NJ, Jain N, et al. Ponatinib and blinatumomab for Philadelphia chromosome-positive acute lymphoblastic leukaemia: a US, single-centre, single-arm, phase 2 trial. Lancet Haematol. 2023;10(1):e24-e34.
16. Assi R, Kantarjian H, Short NJ, et al. Safety and efficacy of blinatumomab in combination with a tyrosine kinase inhibitor for the treatment of relapsed Philadelphia chromosome-positive leukemia. Clin Lymphoma Myeloma Leuk. 2017;17(12):897-901.
17. O'Hare T, Walters DK, Stoffregen EP, et al. In vitro activity of Bcr-Abl inhibitors AMN107 and BMS-354825 against clinically relevant imatinib-resistant Abl kinase domain mutants. Cancer Res. 2005;65(11):4500-4505.
18. Palacios EH, Weiss A. Function of the Src-family kinases, Lck and Fyn, in T-cell development and activation. Oncogene. 2004;23(48):7990-8000.
19. Leonard JT, Kosaka Y, Malla P, et al. Concomitant use of a dual Src/ABL kinase inhibitor eliminates the in vitro efficacy of blinatumomab against Ph+ ALL. Blood. 2021;137(7):939-944.
20. Kauer J, Märklin M, Pflügler M, et al. BCR::ABL1 tyrosine kinase inhibitors hamper the therapeutic efficacy of blinatumomab in vitro. J Cancer Res Clin Oncol. 2023;149(2):679-689.
21. Short NJ, Kantarjian HM, Konopleva M, et al. Combination of ponatinib and blinatumomab in Philadelphia chromosome-positive acute lymphoblastic leukemia: early results from a phase II study. J Clin Oncol. 2021;39(15_suppl):7001.
22. Rochman Y, Spolski R, Leonard WJ. New insights into the regulation of T cells by gamma(c) family cytokines. Nat Rev Immunol. 2009;9(7):480-490.
23. Sahoo P, Yang X, Abler D, et al. Mathematical deconvolution of CAR T-cell proliferation and exhaustion from real-time killing assay data. JR Soc Interface. 2020;17(162):20200747.
24. Courtney AH, Lo WL, Weiss A. TCR signaling: mechanisms of initiation and propagation. Trends Biochem Sci. 2018;43(2):108-123.
25. Hartl FA, Beck-Garcìa E, Woessner NM, et al. Noncanonical binding of Lck to CD3ε promotes TCR signaling and CAR function. Nat Immunol. 2020;21(8):902-913.
26. Horkova V, Drobek A, Paprckova D, et al. Unique roles of co-receptor-bound LCK in helper and cytotoxic T cells. Nat Immunol. 2023;24(1):174-185.
27. Nägele V, Zugmaier G, Goebeler ME, et al. Relationship of T- and B-cell kinetics to clinical response in patients with relapsed/refractory non-Hodgkin lymphoma treated with blinatumomab. Exp Hematol. 2021;100:32-36.
28. Roy V. Convergence diagnostics for Markov Chain Monte Carlo. Annu Rev Stat Appl. 2020;7(1):387-412.
29. Boyman O, Purton JF, Surh CD, Sprent J. Cytokines and T-cell homeostasis. Curr Opin Immunol. 2007;19(3):320-326.
30. Hu X, Li J, Fu M, Zhao X, Wang W. The JAK/STAT signaling pathway: from bench to clinic. Signal Transduct Target Ther. 2021;6(1):115.
31. Malek TR. The biology of interleukin-2. Annu Rev Immunol. 2008;26:453-479.
32. Beadling C, Ng J, Babbage JW, Cantrell DA. Interleukin-2 activation of STAT5 requires the convergent action of tyrosine kinases and a serine/threonine kinase pathway distinct from the Raf1/ERK2 MAP kinase pathway. EMBO J. 1996;15(8):1902-1913.
33. Tan JT, Dudl E, LeRoy E, et al. IL-7 is critical for homeostatic proliferation and survival of naïve T cells. Proc Natl Acad Sci U S A. 2001;98(15):8732-8737.
34. Rathmell JC, Farkash EA, Gao W, Thompson CB. IL-7 enhances the survival and maintains the size of naive T cells. J Immunol. 2001;167(12):6869-6876.
35. Waldmann TA. The IL-2/IL-15 receptor systems: targets for immunotherapy. J Clin Immunol. 2002;22(2):51-56.
36. Philipp N, Kazerani M, Nicholls A, et al. T-cell exhaustion induced by continuous bispecific molecule exposure is ameliorated by treatment-free intervals. Blood. 2022;140(10):1104-1118.
37. Chen L, Flies DB. Molecular mechanisms of T cell co-stimulation and co-inhibition. Nat Rev Immunol. 2013;13(4):227-242.
38. Wherry EJ, Kurachi M. Molecular and cellular insights into T cell exhaustion. Nat Rev Immunol. 2015;15(8):486-499.
39. Gozgit JM, Schrock A, Chen T-H, Clackson T, Rivera VM. Comprehensive analysis of the in vitro potency of ponatinib, and all other approved BCR-ABL tyrosine kinase inhibitors (TKIs), against a panel of single and compound BCR-ABL mutants. Blood. 2013;122(21):3992.
40. Thomas X. Blinatumomab: a new era of treatment for adult ALL? Lancet Oncol. 2015;16(1):6-7.
41. Topp MS, Gökbuget N, Stein AS, et al. Safety and activity of blinatumomab for adult patients with relapsed or refractory B-precursor acute lymphoblastic leukaemia: a multicentre, single-arm, phase 2 study. Lancet Oncol. 2015;16(1):57-66.
42. Zabriskie MS, Eide CA, Tantravahi SK, et al. BCR-ABL1 compound mutations combining key kinase domain positions confer clinical resistance to ponatinib in Ph chromosome-positive leukemia. Cancer Cell. 2014;26(3):428-442.
43. Nägele V, Kratzer A, Zugmaier G, et al. Changes in clinical laboratory parameters and pharmacodynamic markers in response to blinatumomab treatment of patients with relapsed/refractory ALL. Exp Hematol Oncol. 2017;6:14.
44. Lin FY, Stuckert A, Tat C, et al. Phase I trial of GD2.CART cells augmented with constitutive interleukin-7 receptor for treatment of high-grade pediatric CNS tumors. J Clin Oncol. 2024;42(23):2769-2779.