BCR::ABL1 tyrosine kinase inhibitors hamper the therapeutic efficacy of blinatumomab in vitro

Joseph Kauer1,2,3 · Melanie Märklin3,5 · Martin Pflügler1,2,3 · Sebastian Hörner1,2 · Clemens Hinterleitner4,5 · Claudia Tandler1,3,5 · Gundram Jung1,2 · Helmut R. Salih3,5 · Jonas S. Heitmann1,3,5

Received: 17 November 2021 / Accepted: 22 April 2022 / Published online: 13 May 2022
© The Author(s) 2022

Abstract
Purpose Acute B-lymphoblastic leukemia (B-ALL) is a malignant disease characterized by accumulation of clonal immature lymphocytes in the bone marrow and peripheral blood. The approval of BCR::ABL1 tyrosine kinase inhibitors (TKI) such as imatinib, dasatinib, nilotinib and ponatinib marked a milestone in targeted therapy only for a subset of patients carrying the translocation t(9;22)(q34;q11). Immunotherapy with the bispecific antibody (bsAb) blinatumomab targeting CD19xCD3 revolutionized treatment of all B-ALL cases. The combination of both TKI and bsAb, so-called “dual targeting”, is currently under clinical investigation, although TKI might influence T cell effects.

Methods We here investigated the combination of different TKI and blinatumomab in BCR::ABL1+ and BCR::ABL1− B-ALL cell lines and primary samples regarding T cell proliferation, differentiation, cytokine release and killing of tumor cells.

Results In vitro analysis revealed profound reduction of T cell proliferation, differentiation, cytokine release and killing of tumor cells upon application of BCR::ABL1 TKI with blinatumomab. Inhibition was more pronounced with dasatinib and ponatinib compared to nilotinib and imatinib. T cell signalling after CD3 stimulation was impaired by TKI mirrored by inhibition of LCK phosphorylation. This known off-target effect might influence the efficacy of bsAb therapy when combined with BCR::ABL1 TKI.

Conclusion In conclusion, we propose that nilotinib and imatinib might also be suitable substances for combination with blinatumomab and suggest evaluation in clinical trials.

Keywords Acute lymphoblastic leukaemia · BCR::ABL1 · Blinatumomab · Tyrosine kinase inhibitors

Introduction

Bispecific antibodies (bsAbs) improve treatment of hematological diseases, in particular since blinatumomab entered clinical routine for the treatment of acute B-cell lymphoblastic leukemia (B-ALL), a disease characterized by rapid accumulation of clonal CD19+ immature B cells in the bone marrow and peripheral blood (Franquiz and Short 2020). Blinatumomab binds CD19 and CD3 and thereby engages T cells with leukemic blasts, thus inducing a target cell-restricted tumor cell lysis (Bargou et al. 2008). This bsAb was initially approved for refractory and relapsed disease, but in the following years showed promising results in the frontline setting and combinational regimens (Gökbuget et al. 2018). However, uncertainty still remains regarding the optimal combination and sequence of blinatumomab, cytotoxic chemotherapy...
and other emerging agents. Besides chemotherapy consisting of, e.g., cyclophosphamide and anthracyclines, targeted therapy with tyrosine kinase inhibitors (TKI) was approved for a subset of B-ALL cases (Druker et al. 2001). Initially developed for chronic myelogenous leukemia (CML), which carries the translocation t(9;22) (q34;q11) leading to the BCR::ABL1 fusion protein, inhibitory reagents such as dasatinib (DASA), nilotinib (NILO), ponatinib (PONA) and imatinib (IMA) are used for treatment of BCR::ABL1+ B-ALL, a subtype comprising approximately 30% of cases (Hunger and Mullahan 2015; Komorowski et al. 2020). The first approved BCR::ABL1 inhibitor, IMA (Druker et al. 2006), was later complemented by next-generation inhibitors to provide treatment options for IMA-resistant disease (Druker et al. 2006; Talpaz et al. 2006). The second and further generation inhibitors display higher potency regarding BCR::ABL1 inhibition and have a different toxicity profile than IMA (Rix et al. 2007; Weisberg et al. 2007; Kantarjian et al. 2010; Saglio et al. 2010; Krusch and Salih 2011). Of note, TKIs may, besides affecting tumor cells, also inhibit signaling events responsible for activation of immune effector cells, thus pointing to a novel challenge which arises with increasing treatment options. One example constitutes the affection of other kinases like the stem cell factor receptor (cKIT), Src kinases or platelet-derived growth factor receptors (PDGFR), which are involved in the activation of immune effector cells (Mannaioni et al. 1997; Hantschel et al. 2008). Most importantly, the majority of aforementioned TKIs have been reported to inhibit T cell activation and proliferation (Appel et al. 2005; Chen et al. 2008; Schade et al. 2008a, b). Nevertheless, the combination of blinatumomab and DASA exhibited tremendous success in refractory and relapsed B-ALL with 95% overall survival 18 months after initiation of therapy (Foa et al. 2020). Trials combining blinatumomab with other TKIs, e.g., PONA (NCT03263572) are ongoing, the ideal BCR::ABL1 inhibitor to be used in combination regimes is not yet identified (Assi et al. 2017; Chiaretti et al. 2019; King et al. 2019).

Here, we evaluated the combination of bsAb with four different TKIs to unravel the in vitro potential of the combination to provide further evidence for a clinical trial. In addition, we identified potential drawbacks of the combinational treatment.

**Results**

**BCR::ABL1 inhibitors interfere with blinatumomab-induced T cell activation and proliferation**

Both CD19-directed therapies and tyrosine kinase inhibition targeting BCR::ABL1+ blasts show great efficacy in B-ALL. Combinational therapy therefore might be an option for high-risk patients. However, the effect of BCR::ABL1 TKI on blinatumomab therapy remained unclear. To address this question, we performed flow cytometry-based lysis assays using four ALL cell lines: BCR::ABL1+ Tom-1, BCR::ABL1+ SD-1, BCR::ABL1− Nalm-6 and BCR::ABL1− Nalm-16. Exemplary gating strategies are depicted in Supplementary Figure S1A-D. After 3 days of incubation with blinatumomab, approximately 60–80% of CD8+ T cells were activated as mirrored by CD69 expression. Potent inhibition of T cell activation was seen upon treatment with DASA at plasma peak levels and with PONA even at IC50 levels. IMA and NILO showed only moderate effects and in most cases did not inhibit T cell activation by blinatumomab (Fig. 1). T cell activation in the presence of SD-1 cells might be preserved due to increased unspecific immunogenicity of this cell line. Since T cell expansion is a prerequisite for successful bsAb therapy, the influence of TKI on blinatumomab-mediated T cell proliferation was investigated. Blinatumomab induced a 2 to 5-fold increase in absolute CD4+ and CD8+ T cell numbers upon three day cocultures with PMBC and ALL cell lines. Upon concomitant treatment with DASA and PONA even at IC50 levels, proliferation was significantly inhibited (Fig. 2). NILO hampered T cell proliferation only at plasma peak doses and to a lesser extent, while IMA did not show any negative effect on T cell proliferation. This was confirmed by thymidine incorporation assays (Supplementary Figure S2A).

**BCR::ABL1 inhibitors limit T cell differentiation and cytokine release**

Since the efficacy of blinatumomab is thought to rely on expansion of effector memory T cells (Klinger et al. 2012), we investigated whether the TKIs interfered with blinatumomab-induced T cell differentiation. By staining for CD45RA, CD45RO and CD62L, T cell subsets were identified after 3d stimulation with blinatumomab in the presence of BCR::ABL1+ TOM-1 cells (exemplary gating strategy depicted in Supplementary Figure S1E). Incubation with blinatumomab resulted in T cell differentiation into central
memory T cells (CD45RO+CD62L+), effecter memory T cells (CD45RO+CD62L−), and to a lesser extent effecter T cells (CD45RA+CD62L−). IMA and NILO at peak concentrations had only minor effects on differentiation, as mirrored by a reduction of central memory T cells. DASA led to profoundly reduced differentiation into central memory T cells, whereas effecter memory T cells were not affected. PONA, however, almost completely blocked differentiation into effecter memory cells and reduced the percentage of central memory T cells (Fig. 3A). When looking at the expansion of CD45RO+ memory cells upon blinatumomab treatment, significant reduction in absolute cell counts were observed for DASA and PONA (Supplementary Figure S2B).

To further address the immunomodulatory effects of different TKI, cytokine release of PBMCs after blinatumomab treatment with or without concomitant TKI treatment was measured. Supplementary Figure S3 depicts percent changes in cytokine release by TKI treatment of cocultures of PBMC, target cells and blinatumomab. In accordance with the inhibition of T cell activation and proliferation seen by us, DASA, NILO, and PONA inhibited the release of key cytokines such as TNF-alpha, IFN-gamma, and IL-4, IL-6, and IL-10, whereas IMA had no effect or even increased cytokine release (in case of IL-4 and IL-17a). No inhibition was seen for IL-2 and IL-12p70 release. CCL-2 was only affected by DASA at peak doses.

**BCR::ABL1 TKIs inhibit phosphorylation of LCK upon bispecific antibody therapy**

TKIs inhibiting BCR::ABL1 activity often interfere with kinases of the Src family (Konig et al. 2008). In order to elucidate the underlying mechanism of T cell inhibition by BCR::ABL1 TKIs phosphoflow experiments were performed. Since the Src kinase lymphocyte-specific protein tyrosine kinase (LCK) plays a major role in T cell activation by phosphorylation of ZAP-70 and itself, we analyzed pY394 LCK phosphorylation in T cells upon blinatumomab stimulation. After 5 min, profound phosphorylation of LCK was observed, which was still detectable after 15 min (Fig. 3B). In the presence of DASA or NILO, LCK phosphorylation was markedly reduced. IMA did not affect LCK phosphorylation. In the presence of PONA, background phosphorylation of LCK was almost completely blocked and no phosphorylation was observed upon blinatumomab stimulation. This underlines the profound inhibitory effect of BCR::ABL1 TKI on blinatumomab-induced T cell activation.

**Reduced tumor cell lysis upon treatment with BCR::ABL1 TKIs**

To investigate the effect of TKI on blinatumomab-induced target cell lysis, flow cytometry-based lysis assays were performed. After 3ee days of coculture with PBMCs and blinatumomab, potent lysis of ALL cell lines was observed. Tumor cell lysis was impaired by 50–90% through DASA at peak levels. Likewise, PONA inhibited tumor cell lysis by 50–90% even at IC50 doses. NILO only had minor effects on BCR::ABL1+ cell lines, but did not inhibit tumor cell lysis of BCR::ABL1+ cell lines (Fig. 4). IMA did not exhibit inhibitory properties.

**Tyrosine kinase inhibitors affect blinatumomab efficacy in primary human B-ALL samples**

To mimic the conditions in the peripheral blood of B-ALL patients more closely, autologous lysis assays were performed using PBMCs from B-ALL patients with > 60% blasts. Patients’ characteristics are shown in Table 1. After three days of coculture with blinatumomab, T cell cluster formation due to activation and expansion were seen. DASA and PONA at peak levels inhibited cluster formation, whereas IMA and NILO did not (Fig. 5A). Again, activation and proliferation of autologous T cells was markedly reduced in the presence of DASA and PONA (Fig. 5B, C). IMA only affected T cell proliferation at peak levels, which remained unaffected by NILO at all. Absolute tumor cell lysis was measured by flow cytometry by gating for CD10+CD20+ B-ALL blasts (Exemplary gating strategy depicted in Supplementary Figure S1F). Effective lysis was seen in all patients with lysis ranging from 26 to 60%. However, the efficacy of blinatumomab was moderately inhibited by DASA, NILO and PONA at peak levels (Fig. 5D). Synergistic effects, i.e., a more effective lysis by combining TKIs and blinatumomab were not consistently observed (Fig. 5D).

**Discussion**

While bsAbs such as blinatumomab have clearly revolutionized the treatment of B-ALL (Topp et al. 2015), the effect of potent BCR::ABL1 inhibition, e.g., by PONA is equally impressive, which in combination with chemotherapy in the frontline setting improved the long-term
Fig. 3 Impact of TKI on blinatumomab-induced differentiation of T cells and T cell signaling. A Proportion of different CD8+ T cell subsets after 3 day coculture assays (n = 4 donors) with 100,000 PBMC, 100,000 BCR::ABL1+ SD-1 cells and blinatumomab at 1 ng/ml. B Phosphorylation of LCK (p-Y394) on T cells after 0, 5 and 15 min of stimulation with plate-coated blinatumomab was determined by phosphoflow. Pretreatment with TKI was performed overnight prior to bsAb exposure. Exemplary data from one out of three experiments is depicted. *p < 0.05
overall survival up to 80% (Ravandi et al. 2010; Fielding et al. 2014; Chang et al. 2019), compared to 10% in the pre-TKI era (Kantarjian et al. 2000). In contrast to CML, where TKI monotherapy is highly effective, combination regimes are needed to fight B-ALL. Several studies have identified the combination of TKI with blinatumomab as treatment option for BCR::ABL1 + B-ALL patients (Assi et al. 2017; Chiaretti et al. 2019; King et al. 2019; Foa et al. 2020); however, uncertainty remains regarding drug sequence, combination and dosing.

Furthermore, TKI may potently affect antitumor immunity by inhibiting signaling pathways necessary for activation of immune effector cells. We here demonstrate that targeted therapy with BCR::ABL1 inhibitors hampers T cell function in vitro to different extent with more pronounced effects with PONA and DASA than with IMA and NILO. Our findings are in line with the data recently provided by Leonard et al. (2021). In addition to the findings by Leonard et al. we further investigated the precise in vitro effects of TKIs on T cell differentiation and cytokine release induced by blinatumomab.

In our hands, DASA and PONA elicited persistent inhibition of blinatumomab-induced T cell proliferation, differentiation, cytokine production and effective killing of B-ALL cells. Inhibition of killing was similar in all four tested cell lines, while activation and proliferation was somewhat preserved in SD-1 cells, possibly pointing to background off-target effects.

The other TKIs affected T cells more heterogeneously. Beyond inhibiting oncogenic BCR::ABL1 signaling, it has already been demonstrated that TKIs like IMA, NILO and DASA impair reactivity of different immune effector cells like dendritic cells, T cells, and natural killer cells (Appel et al. 2005; Blake et al. 2008; Chen et al. 2008; Schade et al. 2008a, b; Fraser et al. 2009). In terms of T cell biology, data is controversial as it has been shown that naive T regulatory cells (Tregs) accumulated after exposure to TKIs pointing to an anti-inflammatory and immunomodulatory effect (Marinelli Busilacchi et al. 2018). Other authors proposed an inhibition of immunosuppressive cells like Tregs, which might improve function of effector T cells (Krusch and Salih 2011). Notably, this may even apply for DASA as

**Fig. 4** Blinatumomab-induced T cell-mediated target cell lysis in the presence of TKI. Lysis of different ALL cells during 3 day coculture assays with 100,000 PBMC, 100,000 B-ALL cells and blinatumomab at 1 ng/ml was determined by flow cytometry. The following ALL cell lines were utilized: **A** BCR::ABL1+ TOM-1, **B** BCR::ABL1+ SD-1, **C** BCR::ABL1− Nalm-6, **D** BCR::ABL1− Nalm-16. Statistical analysis with Mann–Whitney U test. *p < 0.05
Table 1 Patients’ characteristics

| Patient | Age | ALL type | Karyotype | FISH | Break point | Risk group | PBB % | Diff | WBC [G/l] | Hb [g/dl] | Plt [G/l] |
|---------|-----|----------|-----------|------|-------------|------------|-------|------|-----------|-----------|-----------|
| #1      | 22  | Pre-B    | 46,XY[30] | Neg  | t(9;22)     | HR         | 95    | 74.14| 13,2      | 13        | 121       |
| #2      | 66  | C        | n.d.      | Neg  | –           | HR         | 88    | 800.95| 13.3      | 11.6      | 31        |
| #3      | 21  | C        | n.d.      | Pos  | m-BCR       | HR         | 87    | 463.01| 13.1      | 13.1      | 28        |
| #4      | 68  | C        | 45,XY,-7,t(9;22)(q34;q11.2),del(11)(p10),+2~3mar,inc[6]/46,XY[19] | Pos  | M-BCR       | HR         | 79    | 29.00 | 13.1      | 13.1      | 28        |
| #5      | 76  | C        | 46,XY,+X,t(2;16)(p11;p11)der(8)t(8;8)(p23;q23),t(9;22)(q34;q11)[8]; 47,XY,t(2;16)(p11;p11),+der(8)t(8;8)(p23;q23), der(8)t(8;8)(p23;q23),m-BCR       | Pos  | m-BCR       | HR         | 82    | 68.75 | 9.6       | 20        |

Methods

Patient samples

All experiments were carried out in accordance with the Helsinki protocol and the Ethics Committee of the University of Tübingen vote (13/2007V) between 2007 and 2020. All experimental protocols were approved by the Ethics Committee of the University of Tübingen (13/2007V). Informed consent was obtained from all patients. Gene nomenclature was carried out in accordance with the HUGO Gene Nomenclature Committee (HGNC) recommendations for the designation of gene fusions (Bruford et al. 2021).
Peripheral blood samples of healthy donors or B-ALL patients were collected and PBMCs were isolated using density gradient centrifugation with Biocoll Cell Separation Solution (Biochrom, Berlin, Germany). The B-ALL cell lines Nalm-6, Nalm-16, SD-1 and TOM-1 were purchased from the German Collection of Microorganisms and Cell Fig. 5 Autologous lysis of B-ALL patient samples in the presence of blinatumomab and TKI. A Exemplary microscopic documentation of T cell cluster formation after 3 day coculture of ALL patient samples treated with blinatumomab and different TKI at peak levels. Scale bar=10 µm. B, C Flow cytometric analysis CD8+ T cell CD69 expression (B) and CD8+ T cell proliferation (C) after 3 day coculture assays using 200,000 cells/well (n=4) and blinatumomab at 1 ng/ml. D Inhibition of lysis of ALL blasts by autologous T cells in the presence of TKI during 3 day coculture assays using 200,000 cells/well (n=4) and blinatumomab at 1 ng/ml. *p<0.05
Cultures (DMSZ, Braunschweig, Germany) and were routinely tested negative for mycoplasma. PBMCs and cell lines were kept in RPMI 1640 (Life Technologies, Darmstadt, Germany) supplemented with 10–20% heat-inactivated fetal calf serum (Biochrom), 1 mmol/l sodium pyruvate (Biochrom), non-essential amino acids (Biochrom), 2 mmol/l l-glutamine (Lonza Group, Basel, Switzerland), 100 U/ml penicillin (Sigma-Aldrich, St. Louis, USA), 100 μg/ml streptomycin (Sigma-Aldrich), and 50 μmol/l beta-mercaptoethanol (Merck, Darmstadt, Germany) at 37 °C and 5% CO₂.

Tyrosine kinase inhibitors (TKI)

Dasatinib (DASA), imatinib (IMA), nilotinib (NILO), and ponatinib (PONA) were purchased from Cayman Chemical Company (Ann Arbor, USA). After dissolving in DMSO, TKI were kept at −20 °C until use. Stock concentrations were 20 mM for DASA, IMA and PONA, and 3 mM for NILO, respectively. Peak and IC₅₀ concentrations were chosen according to the literature: DASA 150 nM (peak) and 10 nM (IC₅₀); IMA 3000 nM (peak) and 600 nM (IC₅₀); NILO 3000 nM (peak) and 30 nM (IC₅₀); PONA 145 nM (peak) and 70 nM (IC₅₀) (Peng et al. 2004; Kantarjian et al. 2006, 2010; Rix et al. 2007; Weisberg et al. 2007; Cortes et al. 2012; Menna et al. 2020). Direct effects of DMSO (maximum level was 0.1% v/v in NILO peak samples) were ruled out by including DMSO only as control throughout all experiments.

Thymidine incorporation assays

For determination of T cell proliferation, ³H thymidine incorporation assays were performed. 100,000 PBMCs, 100,000 irradiated target cells, blinatumomab at 1 ng/ml and TKI were incubated for 2 days in 96-well plates followed by 20 h incubation in the presence of ³H-methyl thymidine (0.5 μCi/well). After harvesting on filter mats, incorporated ³H-methyl thymidine was measured using a liquid scintillation counter (MicroBeta2 2450 Microplate counter, PerkinElmer, Waltham, USA).

Flow cytometry

CD4-PacificBlue (clone OKT4; 1:100), CD8-FITC (clone RPA-T8; 1:100), CD10-APC/Cy7 (clone HI10a; 1:100), CD69-PE (clone FN50; 1:200), CD276-PE/Cy7 (clone MIH42; 1:100) and the respective isotype control antibodies were purchased from BioLegend (San Diego, USA). CD45-AmCyan (clone 2D1; 1:200) was purchased from BD Biosciences (Franklin Lakes, USA).

For flow cytometry-based lysis assays, 100,000 target cells were incubated in 96 well plates together with 100,000 PBMCs, blinatumomab at 1 ng/ml and different tyrosine kinase inhibitors at the indicated concentrations. After 3 days, flow cytometric analysis was performed. Nalm-6 cells were defined as CD45⁻CD110⁺, Nalm-16 as CD45⁻CD10⁺, SD-1 per cell size and as CD22⁺, TOM-1 as CD45⁻CD10⁺, T cells as CD45⁺CD4⁺ or CD45⁺CD8⁺, and activated subsets were identified using CD69 as surrogate marker. Absolute cell numbers were determined by the acquisition of equal amounts of compensation particles (BD Biosciences) per sample, thus allowing for calculation of the absolute degree of tumor cell lysis. Binding of antibodies to Fc receptors was blocked with Flebogamma DIF (Grifols, Barcelona, Spain) at 50 μg/ml. Data acquisition was performed using a FACS Canto II (BD Biosciences). For data analysis, FlowJo V10 software (Tree Star, Ashland, OR) was utilized.

Legendplex cytokine arrays

Legendplex cytokine arrays (Human Essential Immune Response, Biolegend) were performed according to the manufacturer’s protocol by utilizing supernatants from lysis assays as described above.

Phosphoflow

For phosphoflow experiments, T cells were isolated by magnetic-activated cell sorting (MACS) using the Pan T cell isolation kit according to the manufacturer’s protocol (Miltenyi Biotech, Köln, Germany). T cells were then cultured in RPMI 1640 media and different TKI at peak concentrations overnight. 96-well flat bottom high protein binding plates (Nunc Maxisorp, ThermoFisher) were coated overnight with blinatumomab at 100 ng/ml in PBS. T cells were transferred to FCS with different TKI present at peak concentrations and stimulated on 96-well plates. After 0, 5, and 15 min, signalling was stopped by buffer 1 of the Perfix Expose Kit (Beckman Coulter) and T cells were further treated according to the manufacturer’s protocol. Intracellular staining was performed with an anti-LCK(pTyr394) antibody (Genetex, 1:500) and anti-rabbit-PE secondary antibody (Cell Signaling Technologies, 1:200). Data acquisition was performed using a FACS Canto II (BD Biosciences).

Statistical analysis

Data are displayed as bar diagram with mean values overlaid by dot plots. Mann-Whitney U tests were used to compare individual groups. Statistical analyses were conducted using GraphPad Prism 8.1.0 software. P values of < 0.05 were considered statistically significant.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1007/s00432-022-04039-5.
Acknowledgements The authors thank Stefanie Müller for expert technical assistance. This work was supported by grants from Deutsche Forschungsgemeinschaft (SA1360/7-3 to HRS), Wilhelm Sander-Stiftung (2007.115.3 to HRS), Deutsche Krebshilfe (1111828 and 70112914 to HRS, 70113496 to MM) and Germany’s Excellence Strategy (EXC 2180/1).

Author contributions JSH and JK designed the research. JK, JSH, MM, SH, and CT performed and analyzed the flow cytometry experiments. JSH collected clinical data. JK, JSH, MM, and HRS drafted the manuscript. GJ, MP, SH, and CH contributed to experimental designs. All authors read, revised, and accepted the submitted manuscript.

Funding Open Access funding enabled and organized by Projekt DEAL. This work was supported by grants from Deutsche Forschungsgemeinschaft (SA1360/7-3 to HRS), Wilhelm Sander-Stiftung (2007.115.3 to HRS), Deutsche Krebshilfe (1111828 and 70112914 to HRS, 70113496 to MM) and Germany’s Excellence Strategy (EXC 2180/1).

Declarations

Conflict of interests The authors declare no competing interests.

Consent to participate Informed consent was obtained from all individual participants included in the study.

Additional information Data sharing upon reasonable request to the corresponding author.

Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article’s Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article’s Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit http://creativecommons.org/licenses/by/4.0/.

References

Appel S, Balabanov S, Brumendorf TH, Brossart P (2005) Effects of imatinib on normal hematopoiesis and immune activation. Stem Cells 23(8):1082–1088

Assi R, Kantarjian H, Short NJ, Daver N, Takahashi K, Garcia-Manero G, DiNardo C, Burger J, Cortes J, Jain N (2017) 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 17(12):897–901

Bargou R, Leo E, Zugmaier G, Klinger M, Goebeler M, Knop S, Noppeney R, Viardot A, Hess G, Schuler M (2008) Tumor regression in cancer patients by very low doses of a T cell–engaging antibody. Science 321(5891):974–977

Blake SJ, Bruce LA, Fraser CK, Hayball JD, Hughes TP (2008) Dasatinib suppresses in vitro natural killer cell cytotoxicity. Blood 111(8):4415–4416

Bruford EA, Antonescu CR, Carroll AJ, Chinnaiyan A, Cree IA, Cross NC, Dalgleish R, Gale RP, Harrison CJ, Hastings RJ (2021) HUGO Gene Nomenclature Committee (HGNC) recommendations for the designation of gene fusions. Leukemia 35(11):3040–3043

Chang J, Doura D, Aldoss I, Vahdani G, Jeong AR, Ghaznavi Z, Zhang S, Yaghmourn G, Lee KJ, Weissman A (2019) Combination chemotherapy plus dasatinib leads to comparable overall survival and relapse-free survival rates as allogeneic hematopoietic stem cell transplantation in Philadelphia positive acute lymphoblastic leukemia. Cancer Med 8(6):2832–2839

Chen J, Schmitt A, Chen B, Rojewski M, Rubel er V, Fei F, Yu Y, Yu X, Ringhofer M, von Harsdorf S, Greiner J, Gotzz M, Guillaume P, Dohner H, Bunjes D, Schmitt M (2008) Nilotinib hampers the proliferation and function of CD8+ T lymphocytes through inhibition of T cell receptor signalling. J Cell Mol Med 12(5B):2107–2118

Chiairetti S, Bassan R, Vitale A, Elia L, Picicchi A, Puzc olo C, Canichella M, Ferrara F, Lunghi M, Fabbiano F (2019) Dasatinib-blinatumomab combination for the front-line treatment of adult Philadelphia chromosome-positive ALL patients. updated results of the gemima LAL2116 D-alpha trial. American Society of Hematology Washington, DC

Cortes JE, Kantarjian H, Shah NP, Bixby D, Mauro JJ, Fli nn I, O’Hare T, Hu S, Narasimhan NJ, Rivera VM (2012) Ponatinib in refractory Philadelphia chromosome–positive leukemias. N Engl J Med 367(22):2075–2088

Couturier M-A, Thomas X, Raffoux E, Huguet F, Berthon C, Simand C, Gallego-Hernanz M-P, Iecheri Y, Hunault-Berger M, Sallard C (2020) ‘Blinatumomab+ ponatinib for relapsed/refractory Philadelphia chromosome-positive acute lymphoblastic leukemia in adults. Leukemia Lymphoma 2020:1–10

Druker BJ, Sawyers CL, Kantarjian H, Resta DJ, Reese SF, Ford JM, Capdeville R, Talpaz M (2001) Activity of a specific inhibitor of the BCR-ABL tyrosine kinase in the blast crisis of chronic myeloid leukemia and acute lymphoblastic leukemia with the Philadelphia chromosome. N Engl J Med 344(14):1038–1042

Druker BJ, Guine hot F, O’Brien SG, Gathmann I, Kantarjian H, Guer termann N, Deininger MW, Silver RT, Goldman JM, Stone RM (2006) Five-year follow-up of patients receiving imatinib for chronic myeloid leukemia. N Engl J Med 355(23):2408–2417

Fielding AK, Rowe JM, Buck G, Foroni L, Gerrard G, Litzow MR, Lazarus H, Luger SM, Marks DL, McMillan AK (2014) UKALLXII/ECOG2993: addition of imatinib to a standard treatment regimen enhances long-term outcomes in Philadelphia positive acute lymphoblastic leukemia. Blood 123(6):843–850

Foà R, Bassan R, Vitale A, Elia L, Picicchi A, Puzzolo MC, Canichella M, Viero P, Ferrara F, Lunghi M, Fabbiano F, Bonifacio M, Fracchiolla N, Di Bartolomeo P, Mancino A, De Propris MS, Vignetti M, Guarrini A, Rambaldi A, Chiaretti S, Investigators G (2020) Dasatinib-Blinatumomab combination for the front-line treatment of adult Ph+ ALL patients. updated results of the gimema LAL2116 D-alba trial. American Society of Hematology Washington, DC

Foa R, Bassan R, Vitale A, Elia L, Picicchi A, Puzzolo MC, Canichella M, Viero P, Ferrara F, Lunghi M, Fabbiano F, Bonifacio M, Fracchiolla N, Di Bartolomeo P, Mancino A, De Propris MS, Vignetti M, Guarrini A, Rambaldi A, Chiaretti S, Investigators G (2020) Dasatinib-Blinatumomab combination for the front-line treatment of adult Philadelphia chromosome-positive acute lymphoblastic leukemia. Blood 123(6):843–850

Franqi uiz MJ, Short NJ (2020) Blinatumomab for the treatment of adult B-cell acute lymphoblastic leukemia: toward a new era of targeted immunotherapy. Biologies 14:23–34

Fraser CK, Blake SJ, Diener KR, Lyons AB, Brown MP, Hughes TP, Hayball JD (2009) Dasatinib inhibits recombinant viral antigen-specific murine CD4+ and CD8+ T-cell responses and NK-cell cytolytic activity in vitro and in vivo. Exp Hematol 37(2):256–265

Gökbuget N, Dombret H, Bonifacio M, Reichle A, Graux C, Fau l C, Diedrich H, Topp MS, Brüggemann M, Horst H-A (2018) Blinatumomab for minimal residual disease in adults with B-cell precursor acute lymphoblastic leukemia. Blood 131(14):1522–1531
Hantschel O, Rix U, Superti-Furga G (2008) Target spectrum of the BCR-ABL inhibitors imatinib, nilotinib and dasatinib. Leuk Lymphoma 49(4):615–619

Hunger SP, Mullighan CG (2015) Redefining ALL classification: toward detecting high-risk ALL and implementing precision medicine. Blood 125(26):3977–3987

Kantarjian HM, O’Brien S, Smith TL, Cortes J, Giles FJ, Beran M, Pierce S, Huy H, Andreff M, Koller C (2000) Results of treatment with hyper-CVAD, a dose-intensive regimen, in adult acute lymphocytic leukemia. J Clin Oncol 18(3):547–547

Kantarjian H, Giles F, Wunderle L, Bhalla K, O’Brien S, Wassmann B, Tanaka C, Manley P, Rae P, Mietlowski W (2006) Nilotinib in imatinib-resistant CML and Philadelphia chromosome–positive ALL. N Engl J Med 354(24):2542–2551

Kantarjian H, Shah NP, Hochhaus A, Cortes J, Shah S, Ayala M, Moiraghi B, Shen Z, Mayer J, Pasquini R, Nakamae H, Huegut F, Boque C, Chuah C, Bleickardt E, Bradley-Garelik MB, Zhu C, Szatrowski T, Shapiro D, Baccarani M (2010) Dasatinib versus imatinib in newly diagnosed chronic-phase chronic myeloid leukemia. N Engl J Med 362(24):2260–2270

Kim DH, Camel-Reid S, Chang H, Sutherland R, Jung CW, Kim HJ, Lee JJ, Lipton JH (2009) Natural killer or natural killer/T cell lineage large granular lymphocytosis associated with dasatinib therapy for Philadelphia chromosome positive leukemia. Haematologica 94(1):135–139

King AC, Pappacena JJ, Park JH, Geyer MB (2019) Blinatumomab administered concurrently with oral tyrosine kinase inhibitor therapy is a well-tolerated consolidation strategy and eradicates measurable residual disease in adults with Philadelphia chromosome positive acute lymphoblastic leukemia. Leuk Res 79:27–33

Klinger M, Brandl C, Zugmaier G, Hijazi Y, Bargou RC, Topp MS, King AC, Pappacena JJ, Tallman MS, Park JH, Geyer MB (2019) Blinatumomab administered concurrently with oral tyrosine kinase inhibitor therapy is a well-tolerated consolidation strategy and eradicates measurable residual disease in adults with Philadelphia chromosome positive acute lymphoblastic leukemia. Leuk Res 119(26):6226–6233

Komorowski L, Fidyt K, Patkowska E, Firczuk M (2020) Philadelphia chromosome-positive leukemia in the lymphoid lineage-similarities and differences with the myeloid lineage and specific vulnerabilities. Int J Mol Sci 21:16

Kong H, Copland M, Chu S, Jove R, Holyoke TL, Bhatia R (2008) Effects of dasatinib on SRC kinase activity and downstream intracellular signaling in primitive chronic myelogenous leukemia hematopoietic cells. Cancer Res 68(23):9624–9633

Kreutzman A, Juvenon V, Kairisto V, Ekbloom M, Stenke L, Seggewiss R, Porrka K, Mustjoki S (2010) Mono/oligoclonal T and NK cells are common in chronic myeloid leukemia patients at diagnosis and expand during dasatinib therapy. Blood 116(5):772–782

Kreutzman A, Ladell K, Koechel C, Gostick E, Ekbloom M, Stenke L, Melo T, Einsele H, Porrka K, Price DA, Mustjoki S, Seggewiss R (2011) Expansion of highly differentiated CD8(+) T-cells or NK-cells in patients treated with dasatinib is associated with cytomegalovirus reactivation. Leukemia 25:1587–1597

Krusch M, Salih HR (2011) Effects of BCR-ABL inhibitors on anti-tumor immunity. Curr Med Chem 18(34):5174–5184

Lee K, Ouwehand I, Giannini A, Thomas N, Dibb N, Bijlmakers M (2010) Lck is a key target of imatinib and dasatinib in T-cell activation. Leukemia 24(4):896–900

Leonard JT, Kosaka Y, Malla P, LaTocha D, Lamble A, Hayes-Lattin B, Byrd K, Druker BJ, Tyner JW, Chang BH (2021) Concomitant use of a dual Src/ABL kinase inhibitor eliminates the in vitro efficacy of blinatumomab against Ph+ ALL. Blood J Am Soc Hematol 137(7):939–944

Mannioni PF, Di Bello MG, Masini E (1997) Platelets and inflammation: role of platelet-derived growth factor, adhesion molecules and histamine. Inflamm Res 46(1):4–18

Marinelli Busilacchi E, Costantini A, Viola N, Costantini B, Olivieri J, Butini L, Mancini G, Scortechini I, Chiuracci M, Poiani M, Poloni A, Leoni P, Olivieri A (2018) Immunomodulatory effects of tyrosine kinase inhibitor in vitro and in vivo study. Biol Blood Marrow Transplant 24(2):267–275

Menna P, De Grazia U, Marchesi F, Minotti G, Salvatorelli E (2020) Further analytical, pharmacokinetic, and clinical observations on low-dose ponatinib in patients with Philadelphia chromosome–positive acute lymphoblastic leukemia. Chemotherapy 65(1–2):35–41

Mestermann K, Giavridis T, Weber J, Rydzek J, Frenz S, Nerrer T, Mades A, Sadelain M, Einsele H, Hudecek M (2019) The tyrosine kinase inhibitor dasatinib acts as a pharmacologic on/off switch for CAR T cells. Sci Transl Med 11:499

Mustjoki S, Ekbloom M, Arstila TP, Dybedal I, Epling-Burnette PK, Guilhot F, Hjorth-Hansen H, Hoglund M, Kovanen P, Laurinolli T, Liesveld J, Paquette R, Pinilla-Ibarz J, Raahala A, Shah N, Simonsson B, Sinissalo M, Steegmann JL, Stenke L, Porrka K (2009) Clonal expansion of T/NK-cells during tyrosine kinase inhibitor dasatinib therapy. Leukemia 23(8):1398–1405

Palacios EH, Weiss A (2004) Function of the Src-family kinases, Lck and Fyn, in T-cell development and activation. Oncogene 23(48):7990–8000

Peng B, Hayes M, Resta D, Racine-Poon A, Druker BJ, Talpaz M, Sawyers CL, Rosamilia M, Ford J, Lloyd P (2004) Pharmacokinetics and pharmacodynamics of imatinib in a phase I trial with chronic myeloid leukemia patients. J Clin Oncol 22(5):935–942

Ravandi F, O’Brien S, Thomas D, Faderl S, Jones D, Garris R, Sara D, Jorgensen J, Kebrizia P, Chaplin R (2010) First report of phase 2 study of dasatinib with hyper-CVAD for the frontline treatment of patients with Philadelphia chromosome–positive (Ph+) acute lymphoblastic leukemia. Blood 116(12):2070–2077

Rix U, Hantschel O, Dürnberger G, Remsing Rix L, Planyavsky M, Fernbach NV, Kaupe I, Bennett KL, Vaal P, Colinge J (2007) Chemical proteomic profiles of the BCR-ABL inhibitors imatinib, nilotinib, and dasatinib reveal novel kinase and nonkinase targets. Blood 110(12):4055–4063

Rodriguez GH, Ahmed SI, Al-akhrass F, Rallapalli V, Safdar A (2012) Characteristics of, and risk factors for, infections in patients with cancer treated with dasatinib and a brief review of other complications. Leuk Lymphoma 53(8):1530–1535

Rohon P, Porrka K, Mustjoki S (2010) Immunoprofiling of patients with chronic myeloid leukemia at diagnosis and during tyrosine kinase inhibitor therapy. Eur J Haematol 85(5):387–398

Saglio G, Kim DW, Issaragrisil S, le Coutre P, Etienne G, Lobo C, Pasquini R, Clark RE, Hochhaus A, Hughes TP, Gallagher N, Hoeneckop A, Dong M, Haque A, Larson RA, Kantarjian HM (2010) Nilotinib versus imatinib for newly diagnosed chronic myeloid leukemia. N Engl J Med 362(24):2251–2259

Schade AE, Schieven GL, Townsend R, Jankowska AM, Susulic V, Zhang R, Szpurka H, Maciejewski JP (2008b) Dasatinib, a small-molecule protein tyrosine kinase inhibitor, inhibits T-cell activation and proliferation. Leukemia 23(48):7990–8000

Schade AE, Schieven GL, Townsend R, Jankowska AM, Susulic V, Zhang R, Szpurka H, Maciejewski JP (2008a) Dasatinib, a small-molecule protein tyrosine kinase inhibitor, inhibits T-cell activation and proliferation. Blood J Am Soc Hematol 111(3):1366–1377
Sillaber C, Herrmann H, Bennett K, Rix U, Baumgartner C, Böhm A, Herndlhofer S, Tschachler E, Superti-Furga G, Jäger U (2009) Immunosuppression and atypical infections in CML patients treated with dasatinib at 140 mg daily. Eur J Clin Invest 39(12):1098–1109

Talpaz M, Shah NP, Kantarjian H, Donato N, Nicoll J, Paquette R, Cortes J, O’Brien S, Nicaise C, Bleierkardt E (2006) Dasatinib in imatinib-resistant Philadelphia chromosome–positive leukemias. N Engl J Med 354(24):2531–2541

Topp MS, Göktegen N, Stein AS, Zugmaier G, O’Brien S, Bargou RC, Dombret H, Fielding AK, Heffner L, Larson RA (2015) Safety and activity of blinatumomab for adult patients with relapsed or refractory B-precursor acute lymphoblastic leukaemia: a multi-centre, single-arm, phase 2 study. Lancet Oncol 16(1):57–66

Weisberg E, Manley PW, Cowan-Jacob SW, Hochhaus A, Griffin JD (2007) Second generation inhibitors of BCR-ABL for the treatment of imatinib-resistant chronic myeloid leukaemia. Nat Rev Cancer 7(5):345–356

Zitvogel L, Rusakiewicz S, Routy B, Ayyoub M, Kroemer G (2016) Immunological off-target effects of imatinib. Nat Rev Clin Oncol 13(7):431–446

**Publisher’s Note** Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.