IL-18 and VEGF-A trigger type 2 innate lymphoid cell accumulation and pro-tumoral function in chronic myeloid leukemia

Benedetta Fiordi,[1,2] Valentina Salvestrini,[3] Gabriele Gugliotta,[3] Fausto Castagnetti,[3] Antonio Curti,[3] Daniel E. Speiser,[4] Emanuela Marcenaro,[5,6] Camilla Jandus[1,2#] and Sara Trabanelli[1,2#]

[1]Department of Pathology and Immunology, Faculty of Medicine, University of Geneva, Geneva, Switzerland; [2]Ludwig Institute for Cancer Research, Lausanne Branch, Lausanne, Switzerland; [3]IRCCS Azienda Ospedaliero-Universitaria di Bologna, Institute of Hematology «Seràgnoli», Bologna, Italy; [4]Department of Oncology, Lausanne University Hospital (CHUV) and University of Lausanne, Epalinges, Switzerland; [5]Department of Experimental Medicine (DIMES), University of Genova, Genova, Italy and [6]IRCCS Ospedale Policlinico San Martino, Genova, Italy

[#]CJ and ST contributed equally as senior authors.

Correspondence: S. Trabanelli
sara.trabanelli@unige.ch

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Abstract

Chronic myeloid leukemia (CML) is a hematologic malignancy associated to an unregulated growth of myeloid cells in bone marrow (BM) and peripheral blood (PB), characterized by the BCR-ABL1 translocation. Given the known cytokine impairment in the leukemic niche of CML, we investigated the impact of this microenvironmental dysregulation on innate lymphoid cells (ILC), whose role in cancer has recently emerged. Three ILC subsets are identified based on transcriptional profiles and cytokine secretion. We observed that interleukin 18 (IL-18) and vascular endothelial growth factor A (VEGF-A) are increased in CML patients' sera and that ILC2 are enriched in CML PB and BM. We found that IL-18 drives ILC2 proliferation and that CML ILC2 highly express CXCR4 and CXCR7 BM-homing receptors, potentially explaining their enrichment in PB and BM, respectively. Next, we showed that ILC2 are hyper-activated through a tumor-derived VEGF-A-dependent mechanism, which leads to higher IL-13 secretion. In response to IL-13, leukemic cells increase their clonogenic capacity. Finally, we discovered that the pro-tumoral axis involving VEGF-A, IL-18 and ILC2 was disrupted upon tyrosine kinase inhibitor treatment, normalizing the levels of all these players in CML patients responding to therapy. Overall, our study uncovers the involvement of ILC2 in CML progression, mediated by VEGF-A and IL-18.

Introduction

Chronic myeloid leukemia (CML) is a rare hematologic malignancy associated to an increased and unregulated growth of myeloid cells in bone marrow (BM) and peripheral blood (PB).[1] CML was the first cancer clearly linked to a chromosomal abnormality, namely the translocation between chromosomes 9 and 22, which forms the so-called chimeric "Philadelphia chromosome". This reciprocal process produces a fusion tyrosine kinase oncoprotein (BCR-ABL1), leading to additional genomic instabilities and active proliferation of the malignant precursors.[2] While the introduction of tyrosine kinase inhibitors (TKI) (e.g., Imatinib, Dasatinib, Nilotinib, Ponatinib) has greatly changed the CML treatment landscape, up to 10% of patients still fail to respond because of resistance onset. The only curative option for these patients remains stem cell transplantation, bearing complications such as graft-*versus*-host disease or poor engraftment among others.[3] For these reasons, CML needs a better understanding to provide new therapies to TKI-resistant patients. Innate lymphoid cells (ILC) are a recently described group of innate immune cells, which are characterized by the absence of lineage markers (Lin^-) but positive expression of CD127. Three different subsets of helper ILC, namely ILC1, ILC2 and ILC3, can be distinguished based on transcriptional regulators and cytokine secretion, which functionally mirror helper $CD4^+$ T cells (i.e., Th1, Th2 and Th17, respectively).[4–6] ILC3 are poorly represented in PB, where they are comprised in a $cKit^+$ population of ILC precursors (ILCP).[7,8] ILC have been shown to be involved in different biological processes, both physiological, such as antimicrobial responses, tissue homeostasis, lymphoid organ development, and pathological, such as autoimmunity

and cancer.[9] Previous evidence showed how ILC are phenotypically or functionally altered in several solid tumors and in hematologic malignancies.[10–12] Besides the role of NCR$^+$ ILC3 in graft-*versus*-host disease[13] and ILC1-like cell alterations in acute myeloid leukemia,[14] the two subsets most involved in hematologic malignancies are ILC1, which secrete IFN-α and TNF-γ, thus supporting a type-1 response when functionally effective, and ILC2, that produce a type-2 pro-tumor reaction via IL-4, IL-5, IL-9, IL-13 and amphiregulin secretion.[15–19] In CML, an important regulator of leukemic stem cell survival and proliferation, and disease progression, is represented by the tumor microenvironment of the leukemic niche.[20] Here, immune cells are remodeled in favor of leukemogenesis by the cytokine milieu altered by the blasts.[21] Given the relevance of cytokines and growth factors in driving ILC maturation and fulfilling ILC activities, we aimed at dissecting how these actors were affected in the CML microenvironment.

Methods

Human peripheral blood cell collection

Venous blood was drawn from healthy donors (HD) at the local Blood Transfusion Center, Lausanne, Switzerland and BM samples were obtained from patients undergoing hip joint replacement surgery at the CHUV, Lausanne, Switzerland (EC consents: 2015-00106), under the approval of the Lausanne University Hospital's Institutional Review Board. CML PB and BM samples were obtained from patients at IRCCS University Hospital of Bologna (EC consents: 94/2016/O/Tess) (*Online Supplementary Table S1*), under the approval of the IRCCS University Hospital's Institutional Review Board. Written informed consent was obtained from all subjects and patients, in accordance with the Declaration of Helsinki. Fresh anticoagulated blood diluted at 1:2 ratio in phosphate-buffered saline (PBS) was layered on lymphoprep (ratio diluted blood: lymphoprep 1.5:1). Mononuclear cells were isolated by density gradient centrifugation (1,800 rpm, 20-minute [min] centrifugation without break at room temperature), washed and immediately cryopreserved in 50% RPMI, 40% fetal calf serum (FCS) and 10% dimethyl sulfoxide (DMSO). Serum samples were also collected at the same sampling day after centrifugation of whole blood at 3,000 rpm for 10 min, at room temperature, and immediately frozen.

Flow cytometry analysis

After gating for lymphocytes and singlets, total ILC were identified as living Lineage$^-$CD127$^+$ lymphocytes. Helper ILC were defined by excluding CD56 and CD94 double-positive cells. ILC subsets were identified using CRTH2 and cKit. Dead cells were excluded using the viability dye Live/Dead Zombie Green (Invitrogen). Representative gating strategy is included in the *Online Supplementary Figure S1*. Receptors on cell surfaces were stained with the following antibodies: CD309, CXCR7, CD218a, CXCR4 and NKp30.

Cell culture

For ILC2 expansion, freshly sorted ILC2 were cultured for 2 weeks in supplemented StemSpan SFEM II (Stemcell) with human recombinant IL-2 (200 U/mL, Proleukin Roche) and IL-7 (10 ng/mL, Peprotech). Medium was replaced every 2–3 days and phenotype was checked after 2 weeks of culture. Dasatinib, Imatinib and Nilotinib (Sigma-Aldrich) were resuspended in dimethyl sulfoxide (DMSO) and, where indicated, used at the time and concentration reported in the figure legends.

Quantitative real-time polymerase chain reaction

Transcript levels of *AREG*, NKp30 *(NCR3), IL5RA, IL13RA1, IL13RA2, IL4R1, EGFR* and *VEGFA* were quantified using KAPA SYBR® FAST quantitative polymerase chain reaction (qPCR) Kits (Roche).

Clonogenic assay

K562 cell line (1,000 cells/well) or CD34$^+$ CML sorted cells (10,000 cells/well) were seeded in 6-well plates in 1.5 mL MethoCult Matrix (H4100, StemCell) after treatment for 48 hours with human recombinant IL-13 (50 ng/mL, Peprotech) or medium only. Cells were cultured for 12-14 days to allow the colonies to form. Formed colonies were then scored after incubation at 37°C in a fully humidified 5% CO_2 atmosphere. Counting was performed manually by using an inverted brightfield microscope (Leica) at 10x magnification.

Statistical analysis

Statistical analysis was performed using GraphPad Prism software version 9. According to the Shapiro-Wilk test for normality distribution, we used *t*-tests or Mann-Whitney tests for comparison of two groups and one-way ANOVA for comparison of multiple groups/multiple variables. The data are shown by plotting individual data points and the mean ± standard error of the mean (SEM). A *P* value less than 0.05 (two-tailed) was considered as statistically significant and labeled with $*$. *P* values less than 0.01, 0.001 or 0.0001 were labeled respectively with $**$, $***$ or $****$.

Results

ILC2 are significantly enriched in chronic myeloid leukemia patients at diagnosis and express bone marrow-homing receptors

Given the presence of cytokine alterations in leukemia, and particularly in CML,[22] we investigated whether this

microenvironmental dysregulation was affecting cytokine-responsive cells, such as ILC.[23] In order to do so, we analyzed the total frequency and the prevalence of the different ILC subsets applying our previously established gating strategy[24] (*Online Supplementary Figure S1A-F*) in the PB of CML patients at diagnosis and healthy donors (HD) (Figure 1A-C). Although the overall frequency of ILC was similar in the two groups (Figure 1D), ILC1 were re-

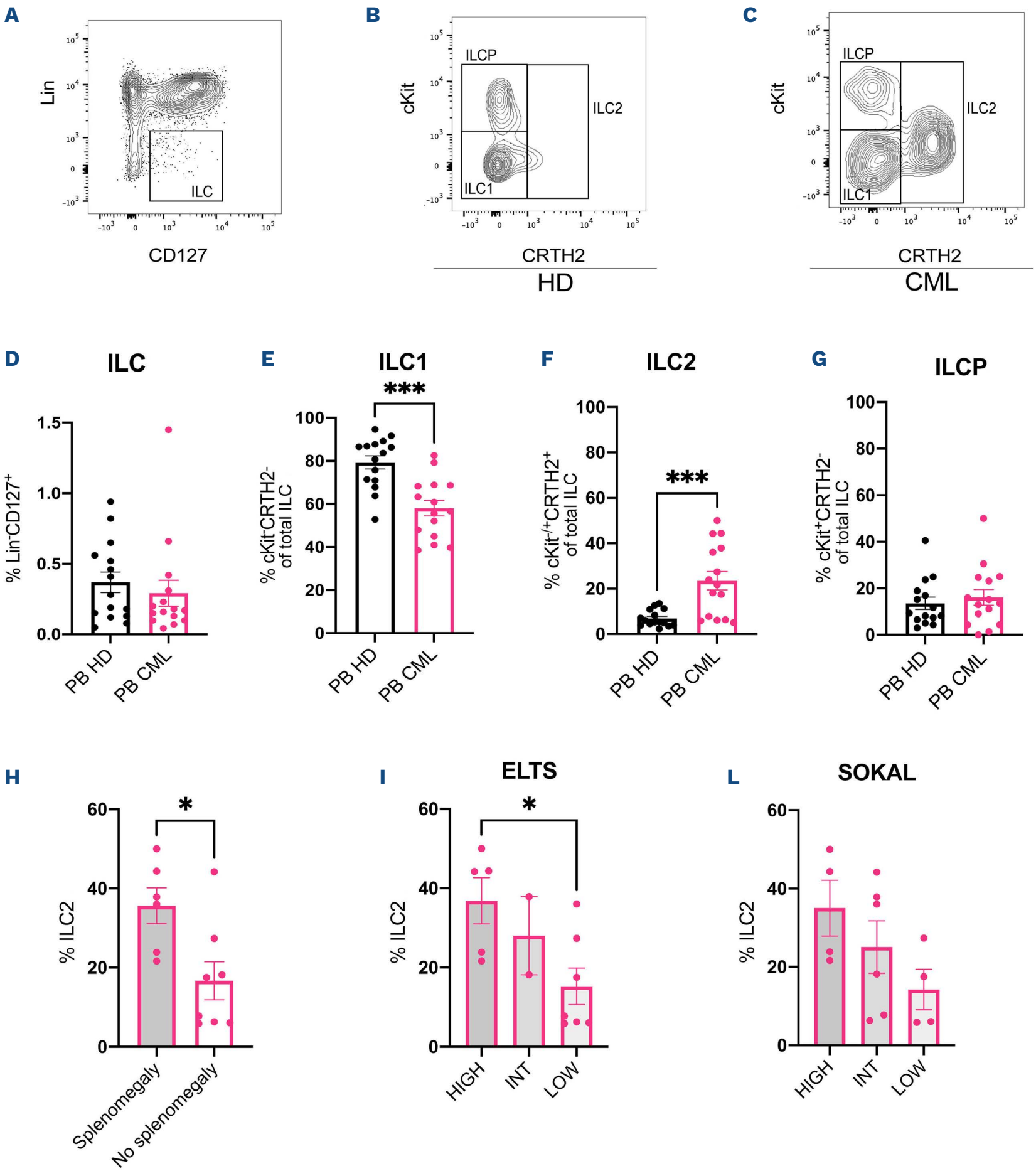

Figure 1. The frequency of innate lymphoid cell subsets in healthy donor and chronic myeloid leukemia peripheral blood at diagnosis, and clinical correlations. (A-C) Representative example of the gating strategy to determine total innate lymphoid cells (ILC) (Lin^-CD127^+ lymphocytes) and ILC subsets ILC1, ILC2 and ILCP in healthy donor (HD) and CML patients according to CRTH2 *vs*. cKit expression. (D-G) Total ILC and ILC subsets' frequency identified in HD and chronic myeloid leukemia (CML) samples (N=15). (H-L) Clinical correlations in CML patients between ILC2 frequency and splenomegaly, ELTS and SOKAL stratification scoring systems (low, intermediate, and high risk). Statistical analysis: Mann-Whitney test and unpaired *t*-test; **P*=<0.05; ***P*=<0.01; ****P*=<0.001; *****P*=<0.0001.

duced in CML patients (Figure 1E) and ILC2 significantly increased (Figure 1F), while no difference was observed for ILCP (Figure 1G). In order to investigate whether the ILC2 increase could be linked with clinical manifestations, we correlated ILC2 frequencies with different clinical parameters (*Online Supplementary Table S1*) and found that ILC2 frequency positively correlates with the presence of splenomegaly (Figure 1H), with the EUTOS long-term survival (ELTS) survival stratification score, and showed a correlation trend with the SOKAL score (Figure 1I-L). Then, since CML arises in the BM, we wondered if the accumulation of ILC2 was present only in the periphery or if this subset could be also recruited/retained into the BM. Thus, we studied the surface expression of the two major BM-homing receptors (i.e., CXCR4 and CXCR7) in all ILC subsets. We found that the expression of these molecules was significantly higher in CML ILC2 (Figure 2B; *Online Supplementary Figure S1G, H*), but neither in ILC1, where only CXCR4 was upregulated, nor in ILCP (Figure 2A-C). Since these chemokine receptors are essential for the recirculation and retention of immune and tumor cells in the BM niche where CXCL12 is enriched, we first measured CXCL12 levels in both BM and PB of CML and HD samples, and then we assessed whether ILC2 could migrate along its gradient. In CML, we found a trend for higher CXCL12 levels in BM compared to the PB, as observed in HD (Figure 2D). By performing a chemotaxis assay (transwell assay) we demonstrated the functionality of CXCR4 and/or CXCR7, since ILC2 were chemoattracted along the CXCL12 gradient (Figure 2E, F). Therefore, these results confirm that in ILC2, the CXCR4 and CXCR7 receptors are functional allowing the migration along a CXCL12 gradient and suggest the possibility of ILC2 retention/recruitment into the BM where CXCL12 is enriched.

In line with our hypothesis, ILC2 were increased in the BM of leukemic patients (Figure 2I), while no difference was detected for ILC1 and ILCP (Figure 2G-L). Lastly, we analyzed cKit expression on ILC2 to discriminate $cKit^{high}$ and $cKit^{low}$ subsets, the latter being a hallmark of fully lineage committed ILC2[25,26]. In both PB and BM of HD $cKit^{low}$ ILC2 were enriched, while in CML, the $cKit^{low}$ population was prevalent only in the PB. When comparing BM of CML and HD, we found an opposite distribution of these two subpopulations, since in CML the $cKit^{high}$ ILC2 were increased and the $cKit^{low}$ ILC2 were decreased in comparison to HD, suggesting reduced differentiation in the BM of CML patients (*Online Supplementary Figure S2A, B*). This finding is in line with the pathogenesis of the disease, in which immature cells can accumulate in the BM. These findings suggest that in CML patients the increased frequency of ILC2 in the circulation and in the BM might contribute to supporting leukemic cell persistence and/or progression.

Vascular endothelial growth factor A and interleukin 18 concentrations are elevated in chronic myeloid leukemia patients' sera at diagnosis

In order to determine the soluble effectors altered in the CML landscape, we measured the concentrations of different cytokines and growth factors in the sera of CML patients at diagnosis as compared to HD. Among the factors under investigation, IL-18 and vascular endothelial growth factor A (VEGF-A) were significantly increased in CML patients (Figure 3A, B), in line with what has been previously reported in CML.[27,28] In order to test whether IL-18 and VEGF-A could be considered as prognostic factors and knowing that the b2a2 transcript has an impact on treatment response to tyrosine kinase inhibitors,[29–31] we correlated the concentration of IL-18 and VEGF-A with the presence of two BCR-ABL1 transcripts. We found a trend, though not significant, between higher levels of IL-18 and VEGF-A and the presence of the b2a2 transcript (Figure 3C, D), suggesting that both IL-18 and VEGF-A might be involved in the progression and resistance to therapy of CML. In order to understand whether these effectors could have a role in ILC regulation in CML patients, we first assessed their receptors' expression on ILC1, ILC2 and ILCP by analyzing the CD218a (IL-18Rα) and CD309 (VEGFR2) expression. We found low expression of CD218a in ILC1 and ILCP, with no significant difference when comparing CML and HD. Instead, ILC2 expressed high levels of IL-18R1 in HD, and intermediate ones in CML (Figure 3E; *Online Supplementary Figure S1I*). Therefore, we hypothesized that the high concentration of circulating IL-18 in patients, and the subsequent binding to its receptor, were resulting in CD218a downregulation in CML. In order to test this hypothesis, we stimulated short-term-expanded ILC2 with IL-18 and analyzed the expression of its receptor. As shown in the *Online Supplementary Figure S3A, B*, upon IL-18 binding, CD218a was downregulated from the cell surface, suggesting that the downregulation observed in patients was likely due to the binding to the circulating IL-18 in CML. With regards to CD309 expression, while no statistical difference could be found in ILC1 and ILCP, we could see a significant upregulation of VEGFR2 on ILC2 from CML patients (Figure 3F; *Online Supplementary Figure S1L*). These results suggest that in CML ILC2 could preferentially respond to IL-18 and VEGF-A.

Interleukin 18 stimulation results in innate lymphoid cell subset 2 proliferation

In order to understand whether the increase of circulating IL-18 in CML (Figure 3A) had a functional impact on ILC2, we generated *in vitro* short-term-expanded ILC2, that maintained CD218a expression at steady state (*Online Supplementary Figure S3C, D*). Activation of ILC2 is characterized by secretion of several cytokines. We

thus hypothesized that IL-18 could stimulate ILC2, resulting in the release of type-2 mediators. We stimulated ILC2 for 48 hours with IL-18 and then analyzed the supernatants for IL-5, IL-13, IL-9, IL-10 and IL-4, but we could not find a relevant difference to medium only (Figure 4A). We then analyzed by CellTrace Far Red staining

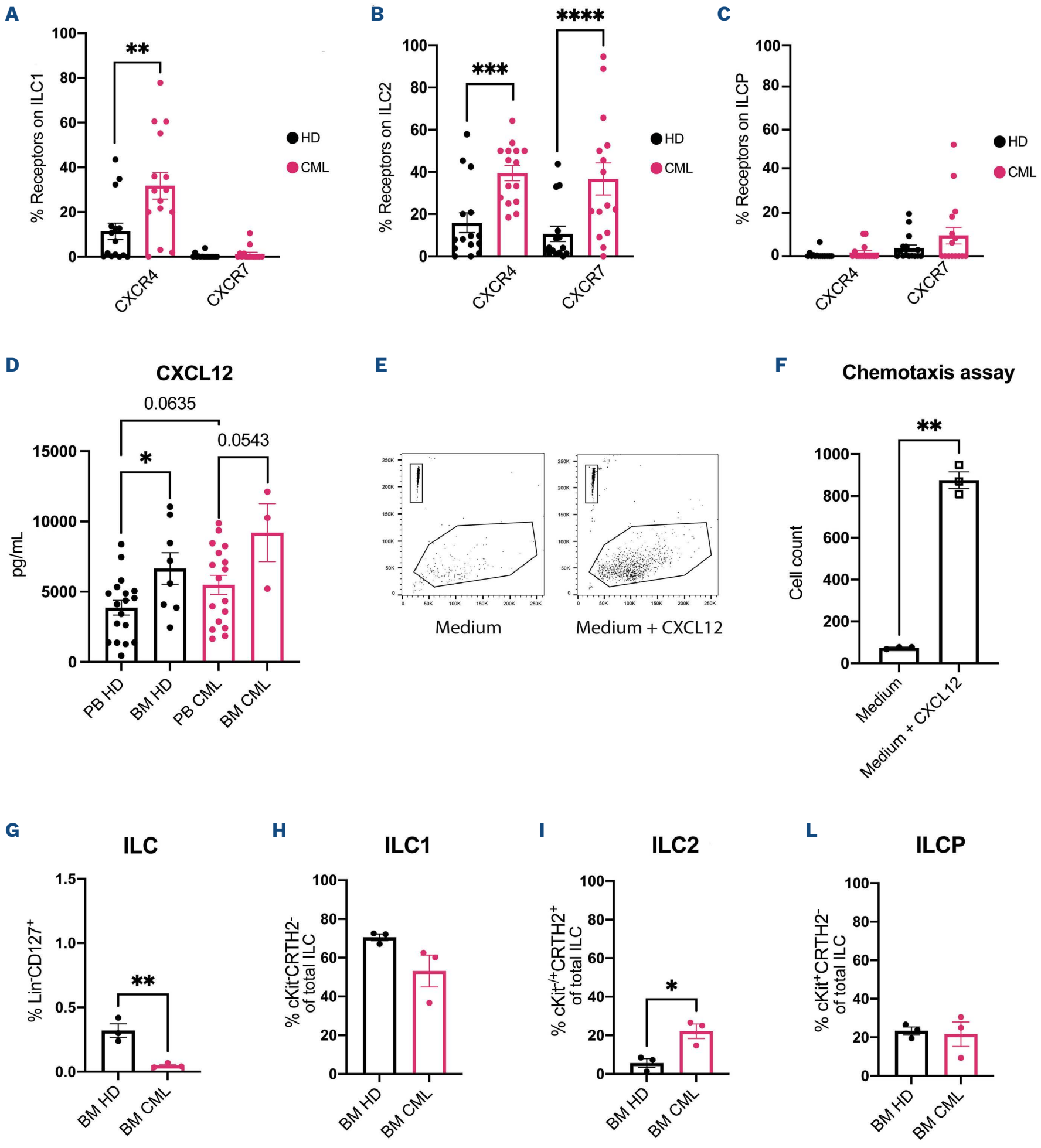

Figure 2. Bone marrow-homing receptor frequencies and function on innate lymphoid cells. (A-C) Frequency of innate lymphoid cell (ILC) subsets ILC1, ILC2 and ILCP positive for the bone marrow (BM)-homing receptors CXCR4 and CXCR7 (N=15). (D) CXCL12 levels (pg/mL) measured in the peripheral blood (PB) and BM of chronic myeloid leukemia (CML) patients and healthy donors (HD) (PB CML N=17; PB HD N=18; BM CML N=3; BM HD N=8). (E, F) Chemotaxis assay of ILC2 toward medium only or with CXCL12 (N=3). (G-L) Total ILC and ILC subsets' frequency in BM samples (N=3 for both HD and CML patients). Statistical analysis: Mann-Whitney test, paired or unpaired *t*-test; **P*=<0.05; ***P*=<0.01; ****P*=<0.001; *****P*=<0.0001.

the proliferation capacity of ILC2 when stimulated with IL-18. We found that IL-18 promotes the proliferation of ILC2, which was previously reported during allergic responses,[32] but not in cancer (Figure 4B, C). Considering IL-18 increase in CML onset, we assessed its level in patients' sera after different TKI therapies at early (early F-U) or late follow-ups (late F-U). Interestingly, IL-18 levels significantly dropped in the first year of therapy irrespectively of the treatment (Figure 4D). Given the observed IL-18 promotion of ILC2 proliferation, we assessed the frequency of ILC in CML patients after therapy. We found that ILC1 and ILC2 were restored to HD levels (*Online Supplementary Figure S3E; Figure 4E*), while no significant changes were found in ILCP (*Online Supplementary Figure S3F*). Interestingly, CD218a levels on ILC1 and ILCP were constant in the follow-ups (*Online Supplementary Figure S3G, H*), while the receptor was recovered on ILC2 after treatment in parallel to the normalization of the IL-18 serology (Figure 4F). These data strongly support the role of IL-18 in promoting ILC2 proliferation in CML.

Tumor-derived VEGF-A stimulates innate lymphoid cell subset 2 effector functions

Given the increased VEGF-A levels in CML patients' sera (Figure 3B) and its relevance in ILC2 regulation in allergy and asthma,[33,34] we analyzed whether this factor could also have a role in the interaction between ILC2 and cancer cells. We first confirmed VEGF-A receptor (CD309) protein expression in *in vitro* short-term expanded ILC2 (*Online Supplementary Figure S4A, B*).

The main *in vitro* model to study CML is the K562 cell line[35] that is often compared to the CD34$^+$ cells of CML patients, given the fact that the malignant blast orig-

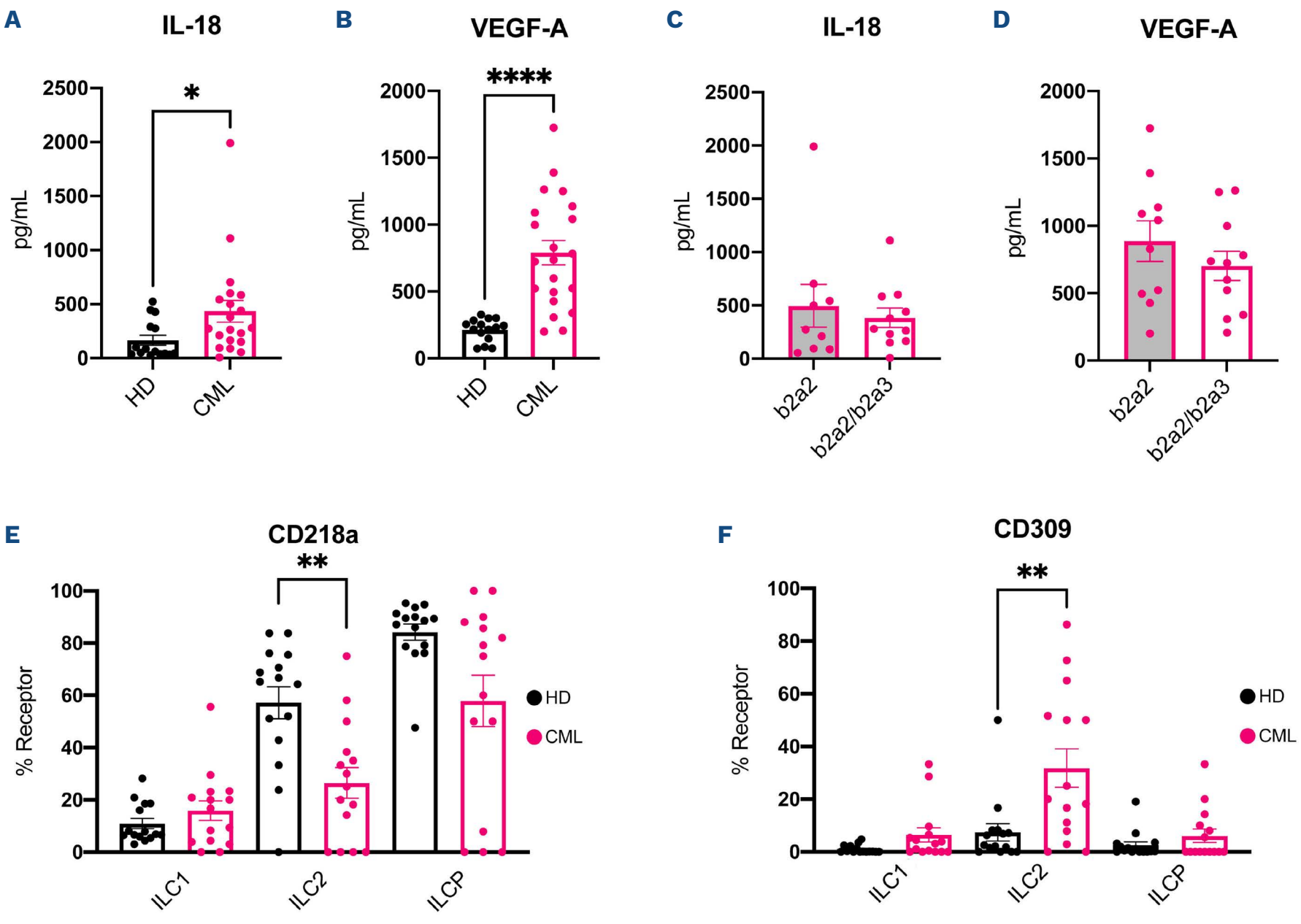

Figure 3. Vascular endothelial growth factor A and interleukin 18 levels are higher in chronic myeloid leukemia at diagnosis compared to healthy donor and innate lymphoid cells express their receptors. (A, B) Interleukin 18 (IL-18) and vascular endothelial growth factor A (VEGF-A) concentrations (pg/mL) in chronic myeloid leukemia (CML) patients' sera at diagnosis (N=21) *vs.* healthy donors (HD) (N=15). (C, D) Correlation between patients' BCR-ABL1 transcripts and IL-18 and VEGF-A levels. (E, F) Frequency of IL-18Rα (CD218a) and VEGF-A (CD309) receptors on innate lymphoid cell (ILC) subsets ILC1, ILC2 and ILCP in HD and CML patients (N=15). Statistical analysis: Mann-Whitney test or unpaired *t*-test; *P=<0.05; **P=<0.01; ***P=<0.001; ****P=<0.0001.

inates from stem cell progenitors. In line with that, to define the source of VEGF-A, we analyzed its transcript levels in primary CD34⁺ cells sorted from both patients and HD and compared them to the ones in the K562 cell line. Interestingly, CML CD34⁺ cells expressed higher *VEGFA* transcripts compared to HD and the results were similar with the expression levels in K562 (Figure 5A). Also, we analyzed the secreted VEGF-A in K562 supernatant and detected high levels of protein (*Online Supplementary Figure S4C*). Thus, we confirmed that the K562 cell line was a good model also in our setting and used it for further analysis. We then hypothesized that the VEGF-A secreted by the leukemic cells in CML patients was contributing to ILC2 activation. In order to in-

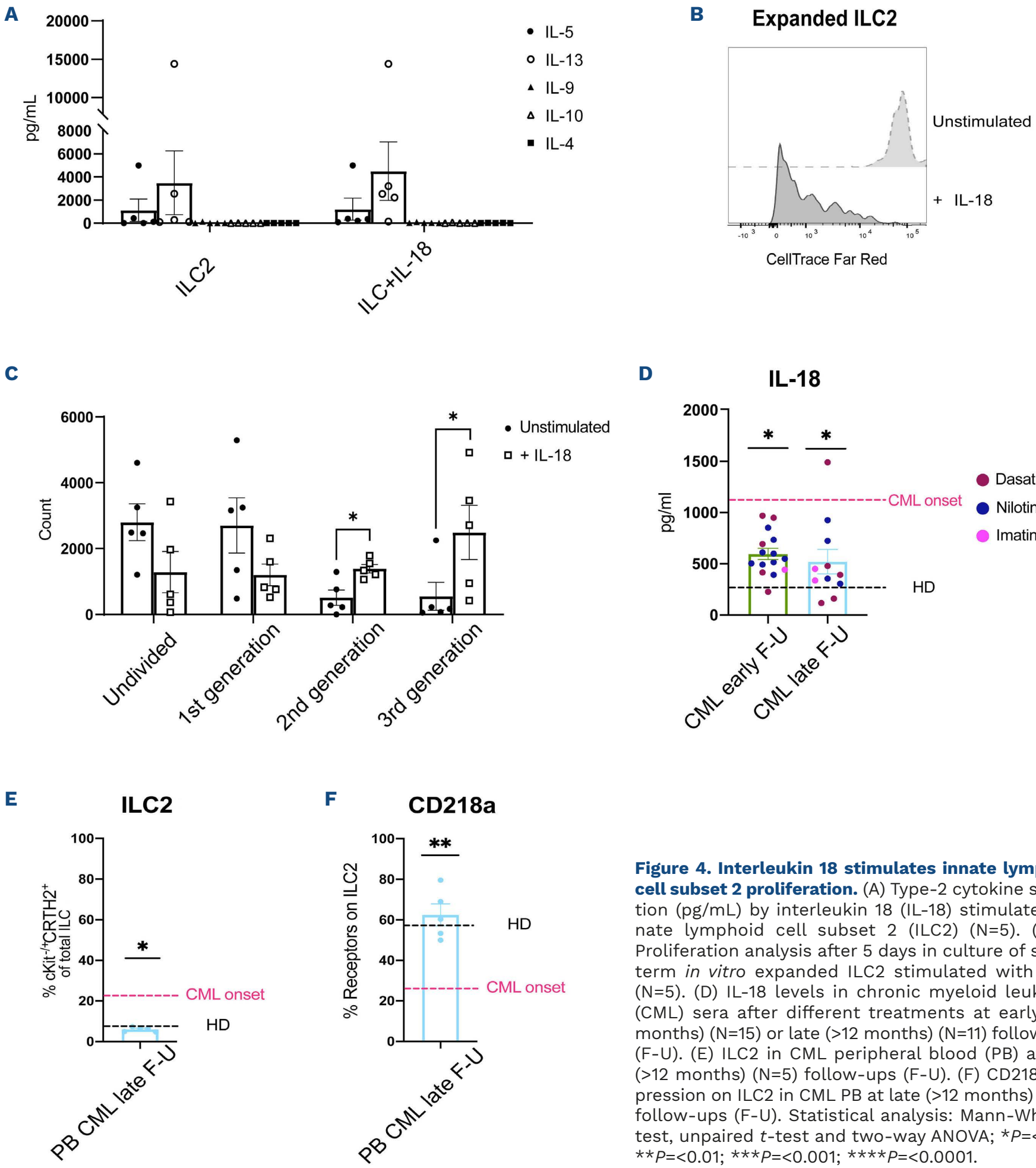

Figure 4. Interleukin 18 stimulates innate lymphoid cell subset 2 proliferation. (A) Type-2 cytokine secretion (pg/mL) by interleukin 18 (IL-18) stimulated innate lymphoid cell subset 2 (ILC2) (N=5). (B, C) Proliferation analysis after 5 days in culture of short-term *in vitro* expanded ILC2 stimulated with IL-18 (N=5). (D) IL-18 levels in chronic myeloid leukemia (CML) sera after different treatments at early (<12 months) (N=15) or late (>12 months) (N=11) follow-ups (F-U). (E) ILC2 in CML peripheral blood (PB) at late (>12 months) (N=5) follow-ups (F-U). (F) CD218a expression on ILC2 in CML PB at late (>12 months) (N=5) follow-ups (F-U). Statistical analysis: Mann-Whitney test, unpaired *t*-test and two-way ANOVA; **P*=<0.05; ***P*=<0.01; ****P*=<0.001; *****P*=<0.0001.

vestigate this, we co-cultured ILC2 with K562 cell line for 48 hours and analyzed type-2 cytokine secretion. IL-5 and IL-13 were significantly increased in the co-culture condition (Figure 5B). In order to confirm the role of VEGF-A in the interplay between K562 cell line and ILC2, we pre-treated ILC2 with an inhibitor of the VEGF-A receptor 2 (VEGFR2) and measured IL-5 and IL-13 secretion upon co-culture with the K562 cell line or the K562 conditioned medium (CM). Interestingly, IL-13 levels dropped in the presence of the VEGFR2 inhibitor (Figure 5C), while a trend of reduction was found for IL-5 (Figure 5D), suggesting that tumor-secreted VEGF-A stimulated ILC2 secretion of both IL-13 and IL-5. ILC2 activation is not only characterized by the secretion of type-2 cytokines and IL-10, but also by the specific production of amphiregulin (AREG), a ligand of the epidermal growth

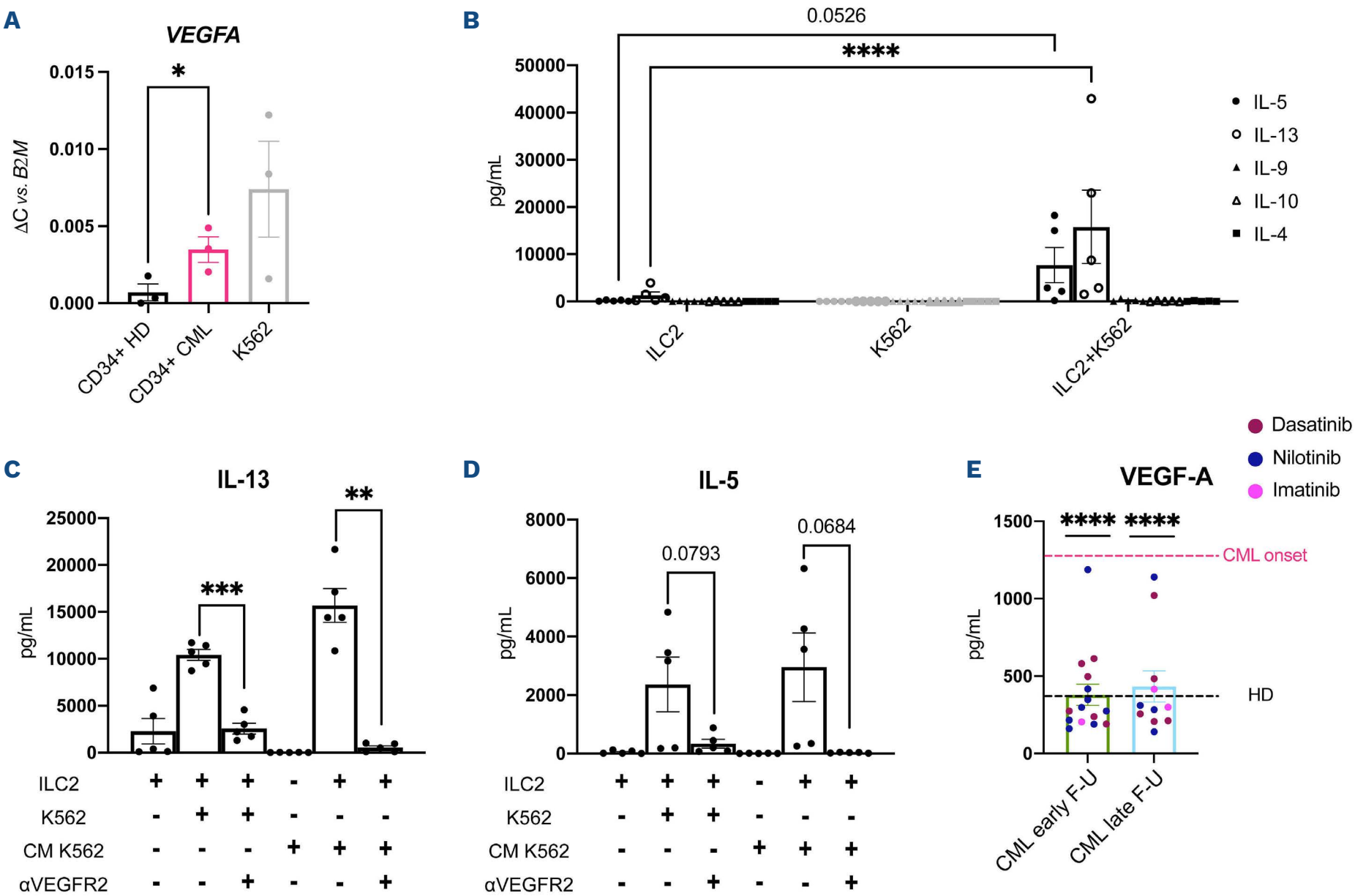

Figure 5. K562 cell line contributes to innate lymphoid cell activation via vascular endothelial growth factor A secretion. (A) Real-time quantitative polymerase chain reaction (RT-qPCR) analysis of vascular endothelial growth factor A (*VEGFA*) gene expression on $CD34^+$ of both chronic myeloid leukemia (CML) patients and healthy donors (HD) (N=3). (B) Type-2 cytokine secretion (pg/mL) in innate lymphoid cell subset 2 (ILC2) and K562 cell line co-cultures, as compared to each cell type in monoculture (N=5). (C) Interleukin 13 (IL-13) secretion (pg/mL) by ILC2 in co-culture with K562 cell line or K562 conditioned medium (CM) with the VEGFR2 inhibitor (10 μM, SU-1498) (N=5). (D) IL-5 secretion (pg/mL) by ILC2 in co-culture with K562 or K562 CM with or without VEGFR2 inhibitor (10 μM, SU-1498) (N=5). (E) VEGF-A levels in CML sera after different treatments at early (<12 months) (N=15) or late (>12 months) (N=11) follow-ups (F-U). (F) CD309 expression on ILC2 in CML PB at late (>12 months) (N=5) follow-ups (F-U). Statistical analysis: Mann-Whitney test, paired and unpaired *t*-test, ordinary one-way ANOVA and two-way ANOVA; **P*=<0.05; ***P*=<0.01; ****P*=<0.001; *****P*=<0.0001.

factor receptor (EGFR), which has been shown to have multiple roles in cancer progression and inflammatory responses.[36–38] For these reasons, we quantified AREG in CML sera at onset and observed a slight but significant increase in the patients (*Online Supplementary Figure S4D*). Moreover, in CML patients' sera after TKI therapies at late follow-ups, AREG concentration significantly decreased (*Online Supplementary Figure S4E*) together with VEGF-A levels at both early and late follow-ups (Figure 5E). Even so, *AREG* transcripts in ILC2 were not increased upon stimulation with the K562 CM and VEGFR2 inhibition did not lead to AREG downregulation (*Online Supplementary Figure S4F*) suggesting that, in CML, tumor-derived factors, including VEGF-A, are not involved in AREG production by ILC2. After TKI therapies, no significant difference was found in CD309 expression on ILC1 (*Online Supplementary Figure S4G*), ILC2 (Figure 5F) or ILCP (*Online Supplementary Figure S4H*). Overall, these observations support a direct effect of tumor-derived VEGF-A on IL-13 and IL-5 secretion by ILC2, that is normalized upon treatment.

In order to understand the relevance of IL-13 and IL-5 in CML, we measured their concentrations in PB sera of HD and of CML patients at disease onset and after TKI treatment. Even though not significant, CML patients showed a trend for increased cytokine levels at diagnosis compared to HD and the levels were normalized upon treatment (*Online Supplementary Figure S5A-D*). In order to verify a potential direct effect of the TKI on ILC2 viability, leading to decreased type-2 cytokines, ILC were treated *in vitro* with different concentrations of Dasatinib, Imatinib and Nilotinib, the drugs used for the treatment of our cohort of patients. Our results showed no major effects of the three drugs on ILC viability (*Online Supplementary Figure S5E*). In order to decipher whether the ILC2 function was impaired by the different TKI, we stimulated ILC2 with the K562 CM in the absence or the presence of Dasatinib, Imatinib and Nilotinib. ILC2 function was not affected by Dasatinib and Imatinib, while Nilotinib showed a trend in reduced cytokine production (*Online Supplementary Figure S5F-G*). In parallel, we tested the cytotoxicity of the same TKI on the K562 cell line. As expected, the viability of K562 was impaired when the cells were treated with the drugs (*Online Supplementary Figure S5H*). Consequently, when ILC2 were cultured in contact with the K562 cell line in the presence of TKI, IL-13 levels dropped, while there was no difference for IL-5 (*Online Supplementary Figure S5I-L*). In order to verify whether the drop in IL-13 production was linked with an impaired K562 stimulation capacity upon TKI treatment, we measured K562 VEGF-A secretion upon TKI treatment and confirmed that the levels were decreased upon administration of the drugs, even though this was only statistically significant with Nilotinib (*Online Supplementary Figure S5M*). Together, our results strongly suggest that Dasatinib, Imatinib and Nilotinib, three different TKI used in clinics for the treatment of CML patients, do not act directly on ILC2 viability, but target tumor cells. Only Nilotinib, a second generation TKI, seems to have an effect on ILC2 function.

NKp30-B7H6 axis is not involved in innate lymphoid cell subset 2 activation in chronic myeloid leukemia

As shown above, when treating ILC2-K562 cell line co-cultures with a VEGFR2 inhibitor, the ILC2-mediated type-2 response was decreased, but not completely abrogated. This led us to hypothesize that other mechanisms could be involved in ILC2 activation via tumor cells, e.g., the NKp30-B7H6 axis. This axis has been reported to hyper-activate ILC2,[39] supporting a tolerogenic pathway involving IL-13 secretion in acute promyelocytic leukemia (APL).[17] Thus, we assessed B7H6 expression on K562 cell line and we confirmed its presence on the tumor cell line (Figure 6A). Then, we confirmed NKp30 expression on *in vitro* short-term-expanded ILC2 (Figure 6B, C). In order to understand whether the binding between NKp30 and its ligand B7H6 on tumor cells could be involved in the K562 cell line-mediated activation of ILC2, we treated the ILC2 with an anti-NKp30 antibody[40] (able to mask the receptor) alone or in combination with the VEGFR2 inhibitor. We tested both contact-mediated and soluble factor-mediated activation of ILC2 by culturing them in direct contact with K562 cell line or with its CM, to analyze the involvement of both membrane-bound and secreted B7H6.[41] We found that NKp30 inhibition with the masking antibody did neither hamper ILC2 secretion of IL-13 (Figure 6D) nor the secretion of IL-5 or transcription of *AREG* and *NCR3* (*Online Supplementary Figure S6A, B*), not in direct contact with K562 cell line nor with the K562 CM. Since in APL the pro-tumoral axis is supported by the combined engagement of B7H6-NKp30 and the binding of the tumor-derived prostaglandin D2 (PGD2) to CRTH2 on ILC2, we analyzed PGD2 levels in CML sera. We found that, in CML, PGD2 is not enriched in PB compared to HD (Figure 6E). Even though ILC2, and not ILC1 and ILCP (*Online Supplementary Figure S6C, D*), show a trend for higher NKp30 expression in CML patients as compared to HD (Figure 6F), this receptor is not involved in ILC2 activation in CML. This was also supported by the lack of any significant difference on NKp30 expression on patients' ILC after TKI treatment (Figure 6G; *Online Supplementary Figure S6E, F*).

Innate lymphoid cell subset 2-derived interleukin 13 enhances chronic myeloid leukemia cell clonogenic capacity

Given the ILC2 activation mediated by the K562 cell line, which led to the secretion of IL-13 and IL-5, we asked how

these effectors could be involved in leukemia progression. First, we assessed the expression of IL-13, IL-5 and AREG receptors (*IL-13Rα1, IL-13Rα2, IL-5Rα* and *EGFR* but also *IL-4R*), considering its known interplay with IL-13Rα1 in reacting to IL-13,[42] on K562 cell line to understand whether the ILC2 secretion could act directly on the tumor cells. We found that K562 cell line expressed high levels of *IL13RA1* transcripts (Figure 7A). Therefore, we focused our next analysis on IL-13 and investigated its effects on the K562 cell line clonogenic capacity. K562 cells were stimulated with IL-13 and then seeded in the matrix. After 2 weeks of incubation, we counted the number of colonies formed per condition. We found that IL-13 stimulation enhanced the clonogenic potential of the K562 cell line (Figure 7B, C). We validated these results by checking whether primary CD34⁺ cells isolated from CML patients at diagnosis had the same property. First, we found that *IL13RA1* is expressed in CD34⁺ cells in CML, with a trend for increased expression as compared to the healthy controls (Figure 7D). Then, we performed the clonogenic assay with the primary cells and confirmed that CML CD34⁺ cells responded to IL-13 by showing a trend for increased numbers of colonies formed (Figure 7E). All together our data suggest a model in which IL-18 and VEGF-A increase in CML leads to ILC2 accumulation and activation, resulting in IL-13-mediated tumor cell growth (Figure 8).

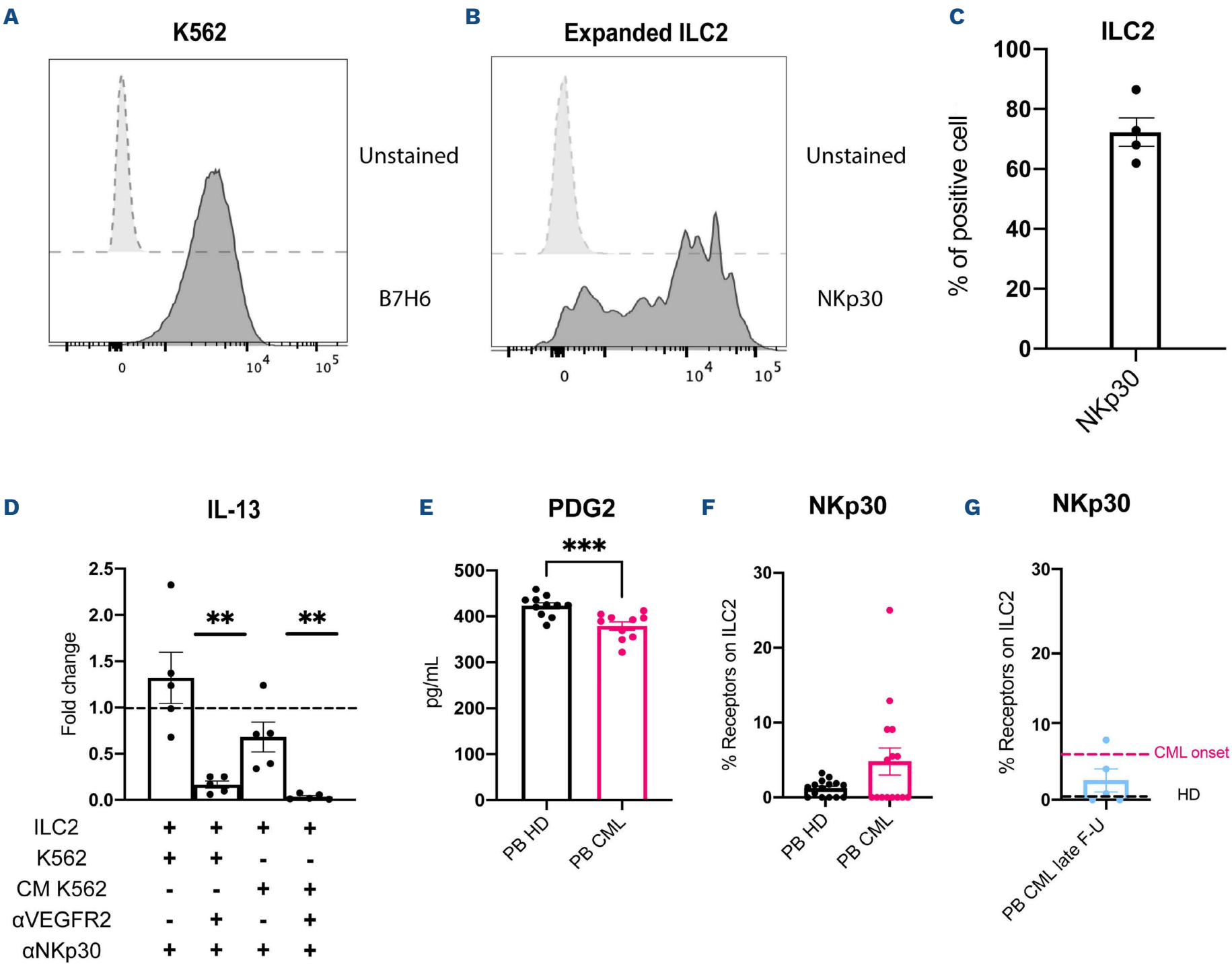

Figure 6. NKp30 is not sufficient in promoting innate lymphoid cell subset 2 triggering via tumoral B7H6 engagement. (A) Expression of NKp30 ligand B7H6 on K562 cell line. (B, C) Expression of NKp30 on expanded innate lymphoid cell subset 2 (ILC2) from healthy donors (HD) (N=4). (D) Fold change of interleukin 13 (IL-13) expression after NKp30 inhibition alone or in combination with SU-1498 (10 μM, VEGFR2 inhibitor) in ILC2 in co-culture with K562 or K562 conditioned medium (CM). Statistics were calculated compared to fold change =1 (N=5). (E) PGD2 concentrations (pg/mL) in chronic myeloid leukemia (CML) patients' sera at diagnosis (N=10) *vs.* HD (N=11). (F) Expression of NKp30 on ILC2 on HD and CML peripheral blood (PB) cells (N=15). (G) NKp30 expression on ILC2 in CML PB at late (>12 months) (N=5) follow-ups (F-U). Statistical analysis: Mann-Whitney test, paired and unpaired *t*-test; **P*=<0.05; ***P*=<0.01; ****P*=<0.001; *****P*=<0.0001.

Discussion

In this study, we report the involvement of IL-18 and VEGF-A in ILC2 activation in CML, which, by secreting IL-13, regulate the capacity of the leukemic cells to produce colonies, supporting tumor growth. This is different in APL, another hematologic malignancy characterized by an increase of ILC2, which are hyperactivated through PGD2-CRTH2 and B7H6-NKp30 pathways.[17] Here, by analyzing the sera of CML patients at diagnosis and excluding the NKp30 involvement, we found that the main ILC2-triggering factors were represented by IL-18 and VEGF-A.

It has been shown that ILC can express the IL-18 receptor and thus, can respond to IL-18. In particular, IL-18 was shown to increase the proliferation of ILC2 in patients with allergic rhinitis,[32] and to induce ILC3 proliferation and production of IL-22 in human tonsils.[43] Here, we show that human circulating ILC2 can respond to IL-18, not by secreting cytokines, but by enhancing their proliferation rate. This result suggests that in CML patients ILC2 proliferation can be stimulated, at least in part, by the high concentration of IL-18 present in their circulation. Indeed, we found an increase of ILC2 in CML patients compared to HD, expressing lower levels of IL-18 receptor (i.e., CD218a). In fact, we showed that CD218a was reduced upon IL-18 binding. In line with these findings, in CML patients responding to TKI, IL-18 is downregulated and ILC2 frequency is restored, together with the IL-18 receptor expression. In addition, we found that ILC2 are also increased in the BM of CML patients, and we showed that this could be explained by the high expression of the BM-homing receptors CXCR4 and CXCR7 on ILC2, that can mediate ILC2 chemotaxis toward CXCL12. This finding is not surprising, since it has been previously shown that ILC can recirculate between tissues in response to chemokines.[44] However, whether IL-18 is also enriched in the BM of CML patients and can provide a proliferative stimulus also in the BM is yet to

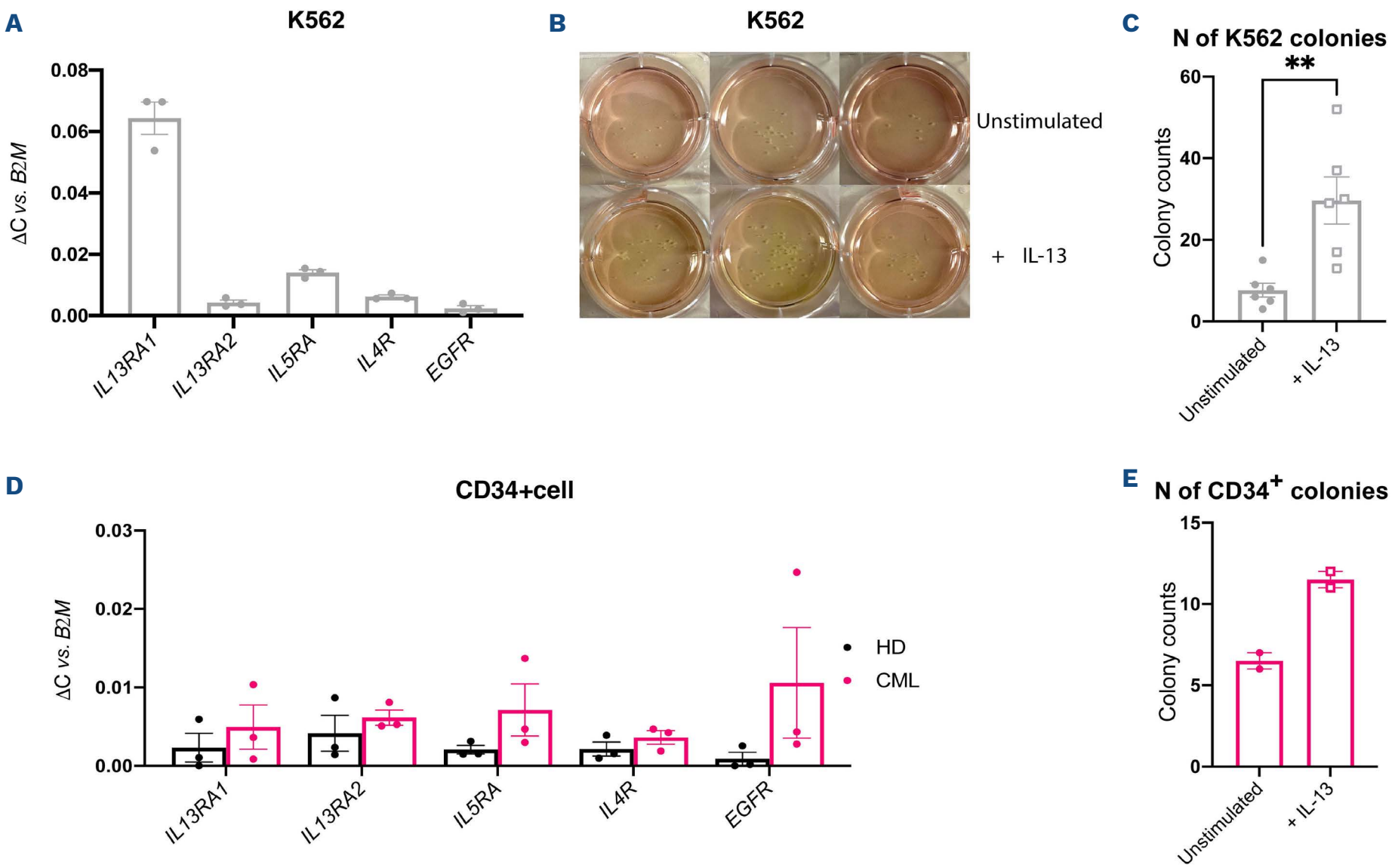

Figure 7. Interleukin 13 stimulation leads to tumor cell survival and proliferation. (A) Real-time quantitative polymerase chain reaction (RT-qPCR) analysis of *IL13RA1, IL13RA2, IL5RA1, IL4RA1* and *EGFR* gene expression on K562 cell line normalized on *B2M* (N=3). (B, C) Colony count after performing a clonogenic assay of K562 cell line treated with interleukin 13 (IL-13) (50 ng/mL) compared to controls after 12 days of culture in methylcellulose matrix (MethoCult H4100, StemCell) (N=6). (D) RT-qPCR analysis of *IL13RA1, IL13RA2, IL5RA1, IL4RA1* and *EGFR* gene expression on $CD34^+$ of both chronic myeloid leukemia (CML) patients and healthy donors (HD) (N=3). (E) Colony count after performing a clonogenic assay on $CD34^+$ of CML treated with IL-13 (50 ng/mL) compared to controls after 12 days of culture in methylcellulose matrix (MethoCult H4100, StemCell) (N=6). Statistical analysis: unpaired *t*-test; **P*=<0.05; ***P*=<0.01; ****P*=<0.001; *****P*=<0.0001.

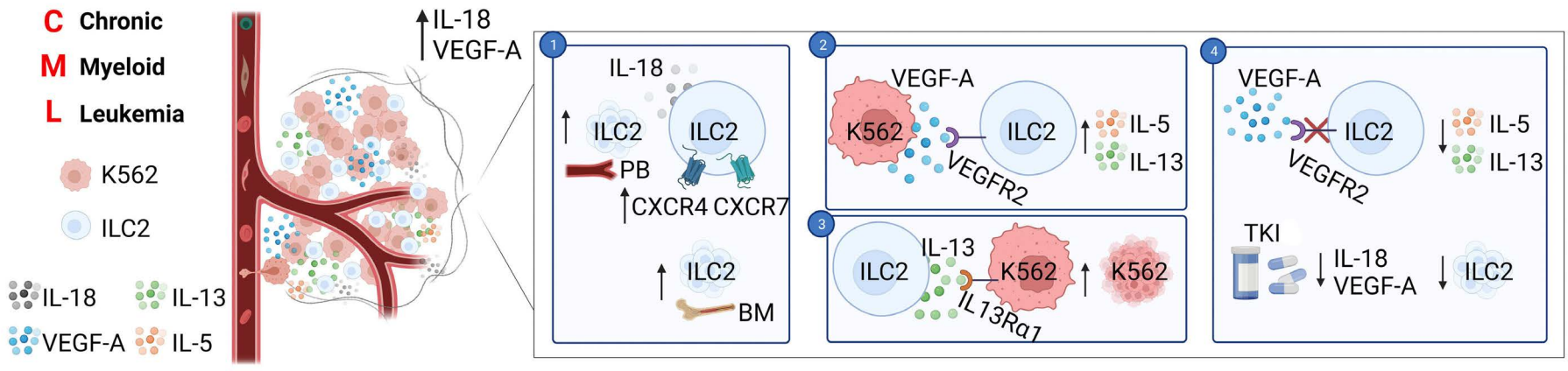

Figure 8. Model for innate lymphoid cell subset 2 involvement in chronic myeloid leukemia. Created with BioRender.

be determined. Interestingly, the observed ILC2 enrichment correlates with the ELTS risk score (and shows a positive trend with the SOKAL index) and with splenomegaly, suggesting that ILC2 frequency might be a prognostic parameter, although additional long-term studies in larger patient cohorts are needed to confirm this hypothesis. The fact that ILC2 frequency correlates with disease aggressiveness is in line with what was already shown for other cancer types. In prostate cancer patients, for instance, ILC2 enrichment is stage dependent and is not present in patients with benign prostate hypertrophy.[17]

The second ILC2 triggering factor we identified was VEGF-A. Angiogenesis plays a major role in both solid tumors and hematologic malignancies, since this process allows malignant cells' dissemination and metastasis. VEGF-A upregulation has been observed in several tumors and hematologic malignancies, leading to increased angiogenic activity promoting cancer cells.[45] In ILC, VEGF-A production has been established for natural killer cells (NK) in hypoxic conditions, moreover it has been found that VEGF-A mediates migration of ILC3, suggesting a pro-angiogenic activity.[34,46] On this ground, we confirmed the capacity of the CML tumor cell line K562 to secrete VEGF-A and of primary CD34$^+$ cells isolated from CML patients to have more abundant *VEGFA* transcripts than their HD counterpart. We found that the major player in the K562 cell line-ILC2 crosstalk is indeed VEGF-A, since upon administration of a VEGFR2 inhibitor the levels of IL-13 and IL-5 produced by ILC2 dropped. Interestingly, the blocking of VEGF-A signaling is recapitulated in CML patients after TKI, since in follow-up sera we found a decreased VEGF-A concentration. These results suggest that in CML, tumor-secreted VEGF-A stimulates ILC2 production of IL-13 and that TKI can hamper this crosstalk by either acting directly on tumor cells secretion or on circulating VEGF-A, blocking the access to ILC2.

We also checked whether the accumulation of IL-18 and VEGF-A could correlate with prognostic markers, such as the BCR-ABL1 transcripts.[29–31] In particular, the most common forms of BCR-ABL1 transcripts derive from the BCR break in exons e13 (b2) or e14 (b3) and the ABL1 break in exons a1 or a2, giving rise mainly to e13a2 (b2a2) and e14a2 (b3a2) transcripts. Here, we found an increasing positive trend between IL-18 and VEGF-A upregulation in patients with the b2a2 transcript, suggesting that with a larger cohort of patients it might be possible to confirm the link between IL-18, VEGF-A and CML prognosis.

Given that, besides the conventional type 2 cytokines, ILC2 also specifically produce AREG, a soluble factor linked with tumor cell aggressiveness and chemoresistance, we hypothesize a role of this other ILC2 effector in CML. Indeed, it was shown that cells of CML patient could release exosomes containing AREG that in turn was acting on stromal cells enhancing the expression of annexin A2 and consequently the adhesion of tumor cells to stromal cells.[47] However, despite the fact that we found that AREG levels in patients' sera at disease onset were elevated and restored after long-term follow-up, we could not prove that ILC2 contribute to AREG production in the CML setting.

Lastly, since ILC have been previously shown to support tumorigenesis via cytokine secretion,[48–50] we checked whether the IL-13 secreted by ILC2, upon stimulation with VEGF-A-producing tumor cells, could support leukemic cell proliferation. Interestingly, we reported that IL-13 stimulation of the K562 cell line increased tumor cells' clonogenic ability and showed a positive trend to increase it also in primary CD34$^+$ cells isolated from CML patients, showing that in this disease IL-13 acts directly on tumor cells supporting their survival and proliferation.

We focused our work on understanding why ILC2 are increased in CML and on characterizing their role in this disease. However, we also found a significant drop of ILC1 frequency in CML patients at disease onset that was recovered after TKI treatment. This finding suggests that the partial loss in ILC1 could play a role in the establishment of the pro-tumoral microenvironment and that their recovery could contribute to the favorable outcome of the TKI treatment. Other experiments are needed to verify this hypothesis.

In summary, we show a new mechanism in CML in which

IL-18 and tumor-derived VEGF-A activate ILC2, leading to their increased proliferation and IL-13 secretion, which in turn sustains tumorigenesis.

Disclosures

No conflicts of interest to disclose.

Contributions

BF performed and analyzed the experiments. VS, GG, FC, AC and DES provided patients' samples and clinical data and critically revised the manuscript. EM provided antibodies and critically revised the manuscript. BF, CJ and ST designed the research, discussed the results and wrote the manuscript.

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Data-sharing statement

All the data are available on request to the first author.

References

1. Cortes J, Pavlovsky C, Saußele S. Chronic myeloid leukaemia. Lancet. 2021;398(10314):1914-1926.
2. Deininger MWN, Goldman JM, Melo JV. The molecular biology of chronic myeloid leukemia. Blood. 2000;96(10):3343-3356.
3. Osman AEG, Deininger MW. Chronic myeloid leukemia: modern therapies, current challenges and future directions. Blood Rev. 2021;49:100825.
4. Spits H, di Santo JP. The expanding family of innate lymphoid cells: Regulators and effectors of immunity and tissue remodeling. Nat Immunol. 2011;12(1):21-27.
5. Ercolano G, Wyss T, Salomé B, Romero P, Trabanelli S, Jandus C. Distinct and shared gene expression for human innate versus adaptive helper lymphoid cells. J Leukoc Biol. 2020;108(2):723-737.
6. Artis D, Spits H. The biology of innate lymphoid cells. Nature. 2015;517(7534):293-301.
7. Lim AI, Li Y, Lopez-Lastra S, et al. Systemic human ILC precursors provide a substrate for tissue ILC differentiation. Cell. 2017;168(6):1086-1100.
8. Ercolano G, Moretti A, Falquet M, et al. Gliadin-reactive vitamin D-sensitive proinflammatory ILCPs are enriched in celiac patients. Cell Rep. 2022;39(11):110956.
9. Chung DC, Jacquelot N, Ghaedi M, Warner K, Ohashi PS. Innate lymphoid cells : role in immune regulation and cancer. Cancers (Basel). 2022;14(9):2071.
10. Ercolano G, Falquet M, Vanoni G, Trabanelli S, Jandus C. ILC2s: new actors in tumor immunity. Front Immunol. 2019;10:2801.
11. Salomé B, Jandus C. Innate lymphoid cells in antitumor immunity. J Leukoc Biol. 2018;103(3):479-483.
12. Jacquelot N, Seillet C, Vivier E, Belz GT. Innate lymphoid cells and cancer. Nat Immunol. 2022:23(3):371-379.
13. Marius Munneke J, Björklund AT, Mjösberg JM, et al. Activated innate lymphoid cells are associated with a reduced susceptibility to graft-versus-host disease. Blood. 2014;124(5):812-821.
14. Salomé B, Gomez-Cadena A, Loyon R, et al. CD56 as a marker of an ILC1-like population with NK cell properties that is functionally impaired in AML. Blood Adv. 2019;3(22):3674-3687.
15. Trabanelli S, Curti A, Lecciso M, et al. CD127+ innate lymphoid cells are dysregulated in treatment naïve acute myeloid leukemia patients at diagnosis. Haematologica. 2015;100(7):e257-e260.
16. de Weerdt I, van Hoeven V, Munneke JM, et al. Innate lymphoid cells are expanded and functionally altered in chronic lymphocytic leukemia. Haematologica. 2016;101(11):e461-e464.
17. Trabanelli S, Chevalier MF, Martinez-Usatorre A, et al. Tumour-derived PGD2 and NKp30-B7H6 engagement drives an immunosuppressive ILC2-MDSC axis. Nat Commun. 2017;8(1):1-14.
18. Lordo MR, Scoville SD, Goel A, et al. Unraveling the role of innate lymphoid cells in acutemyeloid leukemia. Cancers (Basel). 2021;13(2):1-15.
19. Li Z, Ma R, Ma S, et al. ILC1s control leukemia stem cell fate and limit development of AML. Nat Immunol. 2022;23(5):718-730.
20. Torres-Barrera P, Mayani H, Chávez-González A. Understanding the hematopoietic microenvironment in chronic myeloid leukemia: a concise review. Curr Res Transl Med. 2021;69(3):103295.
21. Camacho V, Kuznetsova V, Welner RS. Inflammatory cytokines shape an altered immune response during myeloid malignancies. Front Immunol. 2021;12:772408.
22. Hoermann G, Greiner G, Valent P. Cytokine regulation of microenvironmental cells in myeloproliferative neoplasms. Mediators Inflamm. 2015;2015:869242.
23. Ducimetière L, Vermeer M, Tugues S. The interplay between innate lymphoid cells and the tumor microenvironment. Front Immunol. 2019;10:2895.
24. Trabanelli S, Gomez-Cadena A, Salomé B, et al. Human innate lymphoid cells (ILCs): toward a uniform immune-phenotyping. Cytometry B Clin Cytom. 2018;94(3):392-399.
25. Hochdörfer T, Winkler C, Pardali K, Mjösberg J. Expression of c-Kit discriminates between two functionally distinct subsets of human type 2 innate lymphoid cells. Eur J Immunol. 2019;49(6):884-893.
26. Bernink JH, Ohne Y, Teunissen MBM, et al. c-Kit-positive ILC2s exhibit an ILC3-like signature that may contribute to IL-17-

mediated pathologies. Nat Immunol. 2019;20(8):992-1003.
27. Krauth MT, Simonitsch I, Aichberger KJ, et al. Immunohistochemical detection of VEGF in the bone marrow of patients with chronic myeloid leukemia and correlation with the phase of disease. Am J Clin Pathol. 2004;121(4):473-481.
28. Sillaber C, Mayerhofer M, Aichberger KJ, Krauth MT, Valent P. Expression of angiogenic factors in chronic myeloid leukaemia: role of the bcr/abl oncogene, biochemical mechanisms, and potential clinical implications. Eu J Clin Invest. 2004;34(2):2-11.
29. Jain P, Kantarjian H, Patel KP, et al. Impact of BCR-ABL transcript type on outcome in patients with chronic-phase CML treated with tyrosine kinase inhibitors. Blood. 2016;127(10):1269-1275.
30. Castagnetti F, Gugliotta G, Breccia M, et al. The BCR-ABL1 transcript type influences response and outcome in Philadelphia chromosome-positive chronic myeloid leukemia patients treated frontline with imatinib. Am J Hematol. 2017;92(8):797-805.
31. Greenfield G, McMullan R, Robson N, McGimpsey J, Catherwood M, McMullin MF. Response to Imatinib therapy is inferior for e13a2 BCR-ABL1 transcript type in comparison to e14a2 transcript type in chronic myeloid leukaemia. BMC Hematol. 2019;19:7.
32. Li C, Xu Y, Luo X, Chen F. The effect of IL-18 on group 2 innate lymphoid cells in allergic rhinitis. Iran J Immunol. 2021;18(3):188-194.
33. Shen X, Pasha MA, Hidde K, et al. Group-2 innate lymphoid cells promote airway hyperresponsiveness through production of VEGFA. Xiaofei. J Allergy Clin Immunol. 2018;141(5):1929-1931.
34. Shikhagaie MM, Björklund ÅK, Mjösberg J, et al. Neuropilin-1 is expressed on lymphoid tissue residing LTi-like group 3 innate lymphoid cells and associated with ectopic lymphoid aggregates. Cell Rep. 2017;18(7):1761-1773.
35. Lozzio BB, Lozzio CB. Properties of the K562 cell line derived from a patient with chronic myeloid leukemia. Int J Cancer. 1977;19(1):136.
36. Monticelli LA, Sonnenberg GF, Abt MC, et al. Innate lymphoid cells promote lung-tissue homeostasis after infection with influenza virus. Nat Immunol. 2011;12(11):1045-1054.
37. Trabanelli S, Ercolano G, Wyss T, et al. c-Maf enforces cytokine production and promotes memory-like responses in mouse and human type 2 innate lymphoid cells. EMBO J. 2022;41(12):e109300.
38. Howard E, Lewis G, Galle-Treger L, et al. IL-10 production by ILC2s requires Blimp-1 and cMaf, modulates cellular metabolism, and ameliorates airway hyperreactivity. J Allergy Clin Immunol. 2021;147(4):1281-1295.
39. Salimi M, Xue L, Jolin H, et al. Group 2 innate lymphoid cells express functional NKp30 receptor inducing type 2 cytokine production. J Immunol. 2016;196(1):45-54.
40. Pesce S, Tabellini G, Cantoni C, et al. B7-H6-mediated downregulation of NKp30 in NK cells contributes to ovarian carcinoma immune escape. Oncoimmunology. 2015;4(4):e1001224.
41. Gutierrez-Franco J, Hernandez-Gutierrez R, Bueno-Topete MR, et al. Characterization of B7H6, an endogenous ligand for the NK cell activating receptor NKp30, reveals the identity of two different soluble isoforms during normal human pregnancy. Immunobiology. 2018;223(1):57-63.
42. Bhattacharjee A, Shukla M, Yakubenko VP, Mulya A, Kundu S, Cathcart MK. IL-4 and IL-13 employ discrete signaling pathways for target gene expression in alternatively activated. monocytes/macrophages. Free Radic Biol Med. 2013;54:1-16
43. Victor AR, Nalin AP, Dong W, et al. IL-18 Drives ILC3 proliferation and promotes IL-22 production via NF-κB. J Immunol. 2017;199(7):2333-2342.
44. Ricardo-Gonzalez RR, Schneider C, Liao C, Lee J, Liang HE, Locksley RM. Tissue-specific pathways extrude activated ILC2s to disseminate type 2 immunity. J Exp Med. 2020;217(4):e20191172.
45. Aguayo A, Kantarjian H, Manshouri T, et al. Angiogenesis in acute and chronic leukemias and myelodysplastic syndromes. Blood. 2000;96(6):2240-2245.
46. Hawke LG, Whitford MKM, Ormiston ML. The production of pro-angiogenic VEGF-A isoforms by hypoxic human NK cells is independent of their TGF-β-mediated conversion to an ILC1-like phenotype. Front Immunol. 2020;11:1903.
47. Corrado C, Saieva L, Raimondo S, Santoro A, de Leo G, Alessandro R. Chronic myelogenous leukaemia exosomes modulate bone marrow microenvironment through activation of epidermal growth factor receptor. J Cell Mol Med. 2016;20(10):1829-1839.
48. Han X, Huang T, Han J. Cytokines derived from innate lymphoid cells assist Helicobacter hepaticus to aggravate hepatocellular tumorigenesis in viral transgenic mice. Gut Pathog. 2019;11:23.
49. Kirchberger S, Royston DJ, Boulard O, et al. Innate lymphoid cells sustain colon cancer through production of interleukin-22 in a mouse model. J Exp Med. 2013;210(5):917-931.
50. Bie Q, Zhang P, Su Z, et al. Polarization of ILC2s in peripheral blood might contribute to immunosuppressive microenvironment in patients with gastric cancer. J Immunol Res. 2014;2014:923135.