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Dynamic changes in CCL3 and CCL4 plasma concentrations in patients with chronic lymphocytic leukaemia managed with observation

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The C-C motif chemokine ligands, CCL3 and CCL4, previously called macrophage inflammatory protein-1 alpha and beta (MIP-1α, MIP-1β) are chemokines of the CC subfamily, which are secreted by chronic lymphocytic leukaemia (CLL) cells in response to B cell receptor (BCR) stimulation and upon co-culture with nurse-like cells (NLC) (Burger *et al*, 2009). These chemokines recruit accessory cells, such as T lymphocytes and monocytes, to the tissue microenvironment (Bystry *et al*, 2001; Castellino *et al*, 2006), thereby fostering the assembly of a supportive microenvironment that allows CLL cells to receive survival and proliferation signals from NLC and T cells(Zucchetto *et al*, 2010; Burger, 2012). We previously reported that patients with CLL have elevated plasma CCL3 and CCL4 levels, which rapidly normalize after treatment with kinase inhibitors that target BCR signalling, such as the Bruton tyrosine kinase (BTK) inhibitor, ibrutinib (Ponader *et al*,

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Author Contributions

MS performed the ELISA measurements and analysed the data; LW and DN performed statistical analysis and reviewed the manuscript; LR and TK provided patients' samples and reviewed the manuscript; WW, MK and SOB reviewed the manuscript; JAB designed the research, supervised the study and wrote the paper with MS. All authors reviewed and approved the final version of the manuscript.

Conflict of Interest Disclosure

All authors declare no competing financial interests.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

2012), and the phosphoinositide 3′ kinase delta inhibitor, idelalisib (Hoellenriegel *et al*, 2012). Moreover, in patients with untreated CLL, CCL3 level correlated with established prognostic markers, and high CCL3 levels independently predicted for shorter time-to-disease progression (Sivina *et al*, 2011), emphasizing the clinical relevance of these BCR signalling-related chemokines in CLL.

To characterize the dynamic changes of these biomarkers over time, we analysed plasma CCL3 and CCL4 levels in serial samples collected from untreated CLL patients. We also compared changes in CCL3 and CCL4 over time in patients with progressive CLL who eventually required therapy versus those who did not progress and continued to be managed with observation.

Consent for blood sample collection was obtained in accordance with the Declaration of Helsinki on Institutional Review Board (IRB)-approved protocols. A total of 603 sequential plasma samples from 193 patients fulfilling diagnostic and immunophenotypic criteria for CLL (Hallek *et al*, 2008) were analyzed. The first measurement was the sample collected upon initial patient presentation. Of these 193 CLL patients, 81 (42%) progressed and required therapy after the last plasma sample was collected (treated cohort). The duration between the date of the last sample and the date of the initiation of treatment ranged between less than 1 and 88 months, with a median of 6 months. 112 patients did not progress and continued with observation after the last encounter (observation cohort). Patient characteristics are summarized in Table S1. The sampling interval ranged from 5 months to 12 years (distribution shown in Figure S1). CCL3 and CCL4 plasma levels were measured by enzyme-linked immunoabsorbent assay (ELISA) using Quantikine Kits, accordingly to the manufacturer's instructions (R&D Systems). Data were analyzed in two ways, each using mixed linear model with repeated measures. We modeled mean levels of CCL3, CCL4 and white blood cell (WBC) counts over time, using the restricted maximum likelihood method (REML) to estimate the coefficients; this primary analysis was conducted on the natural logarithm of the CCL3 and CCL4 measurements. An additional model was conducted to summarize percentage change in CCL3, CCL4 and WBC levels from the initial measurements over time, controlling for the patient and adjusting for the initial level.

The median CCL3 level for the initial samples of all 193 patients was 118·pg/ml (range: 2·5–1977·1 pg/ml) and the median CCL4 level was 88·8 pg/ml (range: 6·9–2601·3 pg/ml), which was similar to our previous results in a different cohort of untreated patients with CLL (Sivina *et al*, 2011) (Table S2). The median peripheral WBC count for the initial samples was 21·4 9 × 10^9/l (range: 4·1–330·8 × 10^9/l). Furthermore, in accordance with our previous data, we found higher levels of CCL3 and CCL4 in patients with prognostic markers related to BCR signalling (unmutated *IGHV* and ZAP-70+) (Figure S2). Wilcoxon's rank sum test was used to assess the association of CCL3 and CCL4 levels in the initial samples with IGHV mutation status and ZAP-70 status. CCL3 levels were higher in IGHV unmutated cases (median: 16·9 pg/ml, interquartile range: 11·4–38·1; $n = 70$, $P < 0001$), than in *IGHV* mutated cases (median: 10·5 pg/ml, interquartile range: 6·7–17·8; $n = 120$, $P < 0·001$). Similar differences in CCL3 initial sample concentrations were found for ZAP-70 positive and negative cases (ZAP-70+ median: 17·9 pg/ml, interquartile range: 11·4–33·3, $n = 58$; ZAP-70- median: 11·0 pg/ml, interquartile range 6·7–20·0; $n = 135$, $P < 0001$).

Overall, over time, there were significant increases in CCL3 ($P < 0{\cdot}0001$) and percentage increase over baseline ($P < 0{\cdot}0001$) in both cohorts. Similar observations were found for CCL4 and peripheral WBCs (Fig 1). Coefficients estimated using REML indicated that there was an increase by a factor of 1·11 per year in CCL3, 1·08 per year for CCL4 and 3·75 per year in WBC in the observation group. In the subsequently treated group, a 1·13-fold increase per year for CCL3, a 1·14-fold increase per year for CCL4 and 10·9-fold increase per year for WBC was noted. Consistent results were found using an additional model analyzing percentage changes of CCL3 and CCL4 over time. No statistically significant differences were noted in the rate of increase in chemokine levels over time between the observational and treated cohort, although there was a trend for higher increases in patients that eventually required therapy, presumably reflecting differences in disease activity. For example the median rate of increase for CCL3 was 0·17 (−1·0; 616·0) for patients who did not require therapy and continued with observation versus 0·38 (−1·0; 144·0, $P = 0{\cdot}78$) for the group that progressed and required therapy. Similar, although again not statistically significant, differences were found between the IGHV mutated and unmutated groups.

In summary, these studies demonstrated that plasma levels of CCL3 and CCL4 increase over time in untreated patients with CLL, with a parallel increase in peripheral WBCs over time; this occurs regardless of whether the patients required therapy at a later time or continued without any intervention. The kinetic changes of these chemokines probably reflect BCR signalling activity, which presumably is stable over time, and slow disease progression with resulting changes in leukaemia burden over time. The relative stability of CCL3 and CCL4 plasma levels in individual patients also indicates that these are well-suited markers for indirect assessment and tracking of BCR signalling and disease activity over time. Thereby, these findings provide novel insight into the dynamic changes of CCL3 and CCL4 in patients with untreated CLL.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

Burger JA (2012) The times they are a-changin': prognostic markers in the new era of BCR-targeting therapies for CLL. Expert Opinion on Medical Diagnostics, 6, 49–57. [PubMed: 23480620]

Burger JA, Quiroga MP, Hartmann E, Burkle A, Wierda WG, Keating MJ & Rosenwald A (2009) High-level expression of the T-cell chemokines CCL3 and CCL4 by chronic lymphocytic leukemia B cells in nurselike cell cocultures and after BCR stimulation. Blood, 113, 3050–3058. [PubMed: 19074730]

Bystry RS, Aluvihare V, Welch KA, Kallikourdis M & Betz AG (2001) B cells and professional APCs recruit regulatory T cells via CCL4. Nature Immunology, 2, 1126–1132. [PubMed: 11702067]

Castellino F, Huang AY, Altan-Bonnet G, Stoll S, Scheinecker C & Germain RN (2006) Chemokines enhance immunity by guiding naive CD8 + T cells to sites of CD4 + T cell-dendritic cell interaction. Nature, 440, 890–895. [PubMed: 16612374]

Hallek M, Cheson BD, Catovsky D, Caligaris-Cappio F, Dighiero G, Dohner H, Hillmen P, Keating MJ, Montserrat E, Rai KR & Kipps TJ; International Workshop on Chronic Lymphocytic Leukemia. (2008) Guidelines for the diagnosis and treatment of chronic lymphocytic leukemia: a report from the International Workshop on Chronic Lymphocytic Leukemia updating the National Cancer Institute-Working Group 1996 guidelines. Blood, 111, 5446–5456. [PubMed: 18216293]

Hoellenriegel J, Coffey GP, Sinha U, Pandey A, Sivina M, Ferrajoli A, Ravandi F, Wierda WG, O'Brien S, Keating MJ & Burger JA (2012) Selective, novel spleen tyrosine kinase (Syk) inhibitors suppress chronic lymphocytic leukemia B-cell activation and migration. Leukemia, 26, 1576–1583. [PubMed: 22362000]

Ponader S, Chen SS, Buggy JJ, Balakrishnan K, Gandhi V, Wierda WG, Keating MJ, O'Brien S, Chiorazzi N & Burger JA (2012) The Bruton tyrosine kinase inhibitor PCI-32765 thwarts chronic lymphocytic leukemia cell survival and tissue homing *in vitro* and *in vivo*. Blood, 119, 1182–1189. [PubMed: 22180443]

Sivina M, Hartmann E, Kipps TJ, Rassenti L, Krupnik D, Lerner S, LaPushin R, Xiao L, Huang X, Werner L, Neuberg D, Kantarjian H, O'Brien S, Wierda WG, Keating MJ, Rosenwald A & Burger JA (2011) CCL3 (MIP-1alpha) plasma levels and the risk for disease progression in chronic lymphocytic leukemia. Blood, 117, 1662–1669. [PubMed: 21115978]

Zucchetto A, Tripodo C, Benedetti D, Deaglio S, Gaidano G, Del Poeta G & Gattei V (2010) Monocytes/macrophages but not T lymphocytes are the major targets of the CCL3/CCL4 chemokines produced by CD38(+)CD49d(+) chronic lymphocytic leukaemia cells. British Journal of Haematology, 150, 111–113. [PubMed: 20346010]

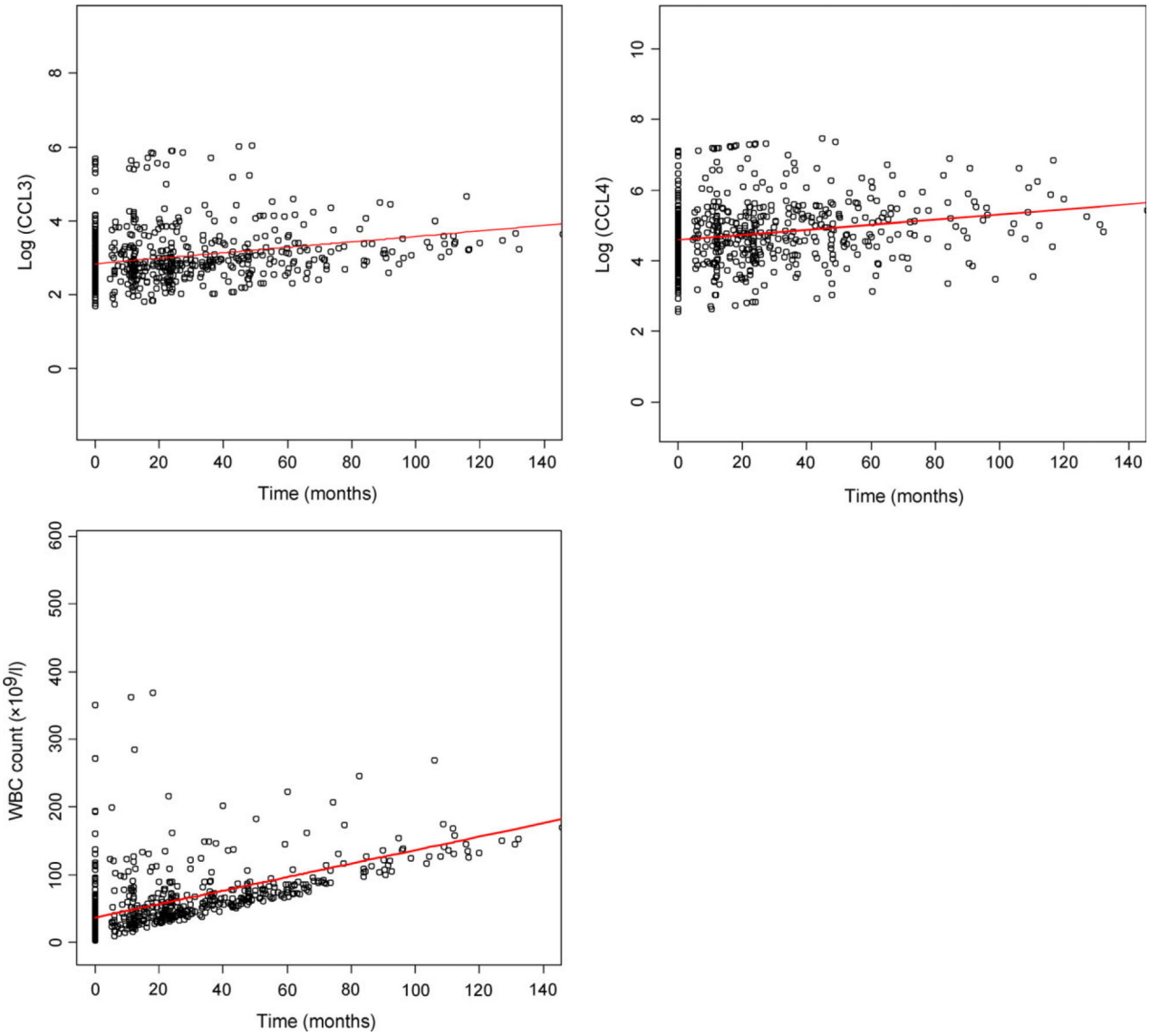

Fig 1.

CCL3/CCL4 plasma levels and WBC counts over time in untreated CLL patients. Graphic representation depicts the changes in the levels of CCL3 (top left), CCL4 (top right) and peripheral white blood cell (WBC) count (bottom) in individual patients at the time displayed on the horizontal axes. CCL3 and CCL4 levels are indicated on the natural logarithm. Each symbol represents an individual patient sample, with the red line showing the accumulative trend for all the patients over time, indicating a slow continuous increase of CCL3 and CCL4 levels, together with an increase in peripheral WBCs in untreated patients with chronic lymphocytic leukaemia.