MINI REVIEW

Do reduced numbers of plasmacytoid dendritic cells contribute to the aggressive clinical course of COVID-19 in chronic lymphocytic leukaemia?

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Abstract
Infections with SARS-CoV-2 have been unduly severe in patients with haematological malignancies, in particular those with chronic lymphocytic leukaemia (CLL). Based on a series of observations, we propose that an underlying mechanism for the aggressive clinical course of COVID-19 in CLL is a paucity of plasmacytoid dendritic cells (pDCs) in these patients. Indeed, pDCs express Toll-like receptor 7 (TLR7), which together with interferon-regulatory factor 7 (IRF7), enables pDCs to produce large amounts of type I interferons, essential for combating COVID-19. Treatment of CLL with Bruton’s tyrosine kinase (BTK) inhibitors increased the number of pDCs, likely secondarily to the reduction in the tumour burden.

INTRODUCTION

Patients with haematologic malignancies have been severely affected by the SARS-CoV-2 infection, those with CLL in particular.1-3 In the Stockholm region, Sweden, during the first wave of the COVID-19 pandemic, 32% of consecutively identified, hospitalized patients with CLL succumbed to the infection, whereas during the second wave, as many as 18% died, despite improved medical care.3 There is currently no clear understanding of the underlying mechanism for the remarkably poor outcome in this patient population.
The risk of death from COVID-19 doubles every five years from childhood and onwards, and elderly men are particularly susceptible.4 CLL is also a disease of the elderly with a male preponderance, but age and sex cannot fully account for the high mortality rates in CLL. While immunosuppressive treatments potentially impair the patients’ ability to overcome the infection, many individuals with early-stage, untreated CLL had similar poor outcome, and some were in fact first diagnosed with CLL at the intensive care unit.1,3

2 | RATIONALE FOR A pDC DEFECT AS A CAUSE OF SEVERE COVID-19 IN CLL

Based on a series of observations explained beneath, we propose that an underlying mechanism for the aggressive clinical course of COVID-19 in CLL is a paucity of pDCs (Figure 1).

First, an international consortium of researchers has identified an essential role for the innate interferon (IFN) system in the protection from severe COVID-19. Autoantibodies against type I IFN or rare loss-of-function variants in genes implicated in viral sensing, type I IFN production, and signalling could explain up to 20% of severe COVID-19 cases.4 Notably, damaging X-linked TLR7 variants were identified in young, previously healthy males with life-threatening COVID-19 pneumonia.5,6 Such rare TLR7 inborn errors of immunity (IEI) account for as many as 1.8% of life-threatening COVID-19 cases among men under 60 years of age.6 TLR7 is a receptor for single-stranded RNA and is predominately expressed by pDCs,7 sensing infection by viruses such as SARS-CoV-2. Furthermore, autosomal recessive variants in IRF7 have been described in several previously healthy adults with life-threatening COVID-19 pneumonia.8 Interferon-regulatory factor 7 (IRF7) is a transcription factor constitutively expressed in pDCs, serving as a master regulator of type I IFN gene transcription.9 It has also been shown that pDCs produce more Type I interferon than any other cell type in blood.10,11 Thus, together, the demonstration that TLR7 and IRF7 deficiency causes severe COVID-19 provides a compelling link to pDCs as a critical source of type I IFN in protection from SARS-CoV-2.

Secondly, there is clear evidence that the number of circulating pDCs is lowered in CLL, as reported by us and others.12–14 In addition, pDC precursors were found to be functionally impaired.15 Moreover, the best experimental model available for CLL, namely the TCL1-transgenic mouse, is also characterized by low numbers/frequencies of pDCs in the spleen, posited to explain an overall high infectious susceptibility in CLL.12 What is the reason for the paucity of pDC in CLL? To answer this question, we describe several key observations that have been made. First, circulating pDCs decline with age.16 Furthermore, differences in TLR7 expression may explain some of the male bias generally observed with respect to severe COVID-19 susceptibility.17 Pioneering studies of mouse models linked gene dosage effects at the Tlr7 locus to autoreactive B cell responses and autoimmunity.18 TLR7 belongs to the

![Figure 1](image-url)

**Figure 1** Plasmacytoid dendritic cell (pDC) and susceptibility to severe COVID-19. Several lines of evidence have highlighted the importance of pDC-derived type I IFN production for protection from severe COVID-19. pDC functional impairment of TLR7-mediated viral sensing in endosomes, or of IRF7-driven transcription, blocks subsequent IFN-α production and occurs by genetic variants causing TLR7 and IRF7 loss-of-function (left). Alternatively, as we propose, in the case of CLL, severe COVID-19 is caused by a reduced number of circulating pDC (right), secondary to the tumour burden (CLL cells are depicted as lymphocytes with antibodies on their surface)
selected group of genes that do not undergo lyonization,\(^1^9\) with higher levels of TLR7 being expressed in females as compared to males.\(^2^0\) Moreover, women seem to have a higher frequency of pDCs, and oestrogen was reported to increase TLR7 activity.\(^2^1\) However, among patients with CLL, there is no overt sex-dependent difference in survival among SARS-CoV-2 infected individuals.\(^2,3\) Thus, the preferential depletion of pDCs in CLL remains enigmatic. We speculate that the tumour burden in CLL and not the treatment, is what may cause the striking reduction in pDCs and susceptibility to severe COVID-19. Even if other haematopoietic cell lineages are also lowered in CLL, as mentioned, multiple lines of evidence suggest that pDCs are essential for the early, innate defence against SARS-CoV-2.\(^5^5\)–\(^1^5\)

It is unclear to what extent the tumour burden in CLL inhibits pDC development. In both CLL patients and in the TCL1 mouse model, pDC precursors were found in normal amounts in the bone marrow. In the periphery, pDC numbers were reduced only in patients with progressive disease and linked to decreased FMS-like tyrosine kinase 3 receptor (FLT3) expression.\(^1^2\) Furthermore, levels of TLR9, another Toll-like receptor highly expressed in pDCs, were reduced in mature pDCs obtained from the TCL1 mouse and from patients with advanced CLL, leading to a reduced IFN-\(\alpha\) response to TLR9 agonists. In the mouse model, inhibition of TNF or TGF-\(\beta\) could increase FLT3 expression and restore pDC numbers.\(^1^2\) Other experimental models have demonstrated that both IFN-\(\alpha\) and IFN-\(\gamma\) can promote pDC development and differentiation synergistically with FLT3.\(^2^2^–^2^4\) The mentioned insights into these cytokine networks also provide clues to how pDC numbers might be enhanced in CLL patients to strengthen viral immunity.

Prior to the COVID-19 outbreak, we and, during the pandemic, others reported that pDCs increase in number in CLL patients under treatment with BTK inhibitors (BTKi), as a likely consequence of the drug-mediated reduction in the tumour burden.\(^1^3,1^4\) Conversely, we observed no changes in plasma IFN-\(\gamma\),\(^2^5\) one of the cytokines influencing the generation of pDCs. BTKi act by inhibiting the intracellular signalling molecule BTK through the binding to its catalytic domain.\(^2^6\) BTKi have during recent years revolutionized the treatment of CLL and other haematopoietic malignancies and, to a great extent, replaced chemotherapy and monoclonals.\(^2^7^–^2^9\) A majority of all clinical trials has been performed using the first approved BTKi, ibrutinib (Imbruvica); both effects and adverse effects may differ depending on the compound, as reviewed.\(^3^0\) Presumably, all BTKi which reduce the tumour burden could promote the generation of pDCs. However, in order to achieve a profound reduction in the tumour mass, combinatorial treatment with other targeted therapies such as BCL-2 inhibitors may be necessary.

### 3 BTK INHIBITOR TREATMENT IN CLL AND COVID-19 SUSCEPTIBILITY

Apart from the effect on pDCs, treatment with BTKi strongly impairs the antibody response to SARS-CoV-2 vaccine.\(^3^1^,3^2\) This is presumably due to the drug affecting not only the tumour population, but also non-malignant, naïve B lymphocytes. From this, it could be deduced that the primary humoral immune response is affected by BTKi not only during vaccination, but likely also in the course of COVID-19.

The overall importance of humoral immunity in COVID-19 is, however, unclear. Treatment of CLL with B cell depleting anti-CD20 monoclonals may aggravate the viral infection, but conclusive evidence is lacking.\(^2\) However, such treatment increases the risk of severe COVID-19 and death in patients with rheumatoid disease.\(^3^3\) Similar to the effect of anti-CD20 therapy, patients with another IIEI, X-linked agammaglobulinemia, XLA, an inherited defect in the BTK gene,\(^3^4\) have essentially no B lymphocytes and cannot mount humoral immune responses.\(^2^9,3^4\) The outcome of COVID-19 in patients with XLA has varied from uneventful to more severe.\(^3^5,3^6\)

Based on all these findings, the increased numbers of pDC during BTKi treatment\(^1^2,1^3\) would improve the prognosis and BTKi could potentially also act as general suppressant of the COVID-19 hyperinflammation itself. To this end, BTKi have been investigated in several clinical trials outside of CLL to elucidate their potential ameliorating effect on severe COVID-19 pneumonia, but without consensus on the outcome.\(^3^7,3^8\) Nevertheless, the time course may be crucial, and there could be a major difference between being on BTKi therapy prior to being infected with SARS-CoV-2 vs being treated after the onset of viral disease. Regarding patients with CLL, it was recently found that continued BTKi treatment may have had a potential, although not statistically significant, clinical benefit upon contracting COVID-19, as compared to those who stopped the drug.\(^2\)

### 4 CONCLUDING REMARKS AND A HYPOTHESIS

In conclusion, based on the existing evidence, we propose that an underlying mechanism for the severe course of COVID-19 reported in CLL is the reduced number of pDCs. Indeed, since these cells are key producers of type I IFN
and, hence, essential for combating COVID-19 at an early stage, any impairment, whether functional or numeric, will negatively affect the individual's capacity to clear an infection with SARS-CoV-2. It could be hypothesized that lack of pDCs also may contribute to the severe COVID-19 observed in other hematological conditions than CLL, as well as following conditioning regimens prior to hematopoietic stem cell or Chimeric Antigen Receptor (CAR) T-cell therapy. Furthermore, it could be envisaged the transfer of pDCs during the early phase of the viral infection may ameliorate COVID-19 in both patients with CLL and those with an impaired pDC function.

CONFLICT OF INTEREST

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AUTHOR CONTRIBUTIONS

CIES involved in conceptualization and writing original draft. RZ, AÖ, MP, PB and YB involved in hypothesis analysis. All authors involved in reviewing and editing.

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REFERENCES

1. Mato AR, Roeker LE, Lamanna N, et al. Outcomes of COVID-19 in patients with CLL: a multicenter international experience. *Blood*. 2020;136(10):1134-1143. doi:10.1182/blood.2020006965

2. Chatzikonstantinou T, Kapetanakis A, Scarfo L, et al. COVID-19 severity and mortality in patients with CLL: an update of the international ERIC and Campus CLL study. *Leukemia*. 2021;35(12):3444-3454. doi:10.1038/s41375-021-01450-8

3. Blick L, Bogdanovic G, Buggert M, et al. Covid-19 in patients with chronic lymphocytic leukemia: clinical outcome and B- and T-cell immunity during 13 months in consecutive patients. *Leukemia*. 2022;36(2):476-481. doi:10.1038/s41375-021-01424-w

4. Zhang Q, Bastard P, COVID Human Genetic Effort, et al. Human genetic and immunological determinants of critical COVID-19 pneumonia. *Nature*. 2022. doi:10.1038/s41586-022-04447-0. Online ahead of print.

5. van der Made CI, Simons A, Schuurs-Hoeijmakers J, et al. Presence of genetic variants among young men with severe COVID-19. *JAMA*. 2020;324(7):663-673. doi:10.1001/jama.2020.13719

6. Asano T, Boisson B, Onodi F, et al. X-linked recessive TLR7 deficiency in ~1% of men over 60 years old with life-threatening COVID-19. *Sci Immunol*. 2021;6(62):eabf4348. doi:10.1126/sciimmunol.abf4348

7. Kadowaki N, Ho S, Antonenko S, et al. Subsets of human dendritic cell precursors express different toll-like receptors and respond to different microbial antigens. *J Exp Med*. 2001;194(6):863-869. doi:10.1084/jem.194.6.863

8. Zhang Q, Bastard P, Liu Z, et al. Inborn errors of type I IFN immunity in patients with life-threatening COVID-19. *Science*. 2020;370(6515):eabd4570. doi:10.1126/science.abd4570

9. Izaguirre A, Barnes BJ, Amrute S, et al. Comparative analysis of IRF and IFN-alpha expression in human plasmacytoid and monocyte-derived dendritic cells. *J Leukoc Biol*. 2003;74(6):1125-1138. doi:10.1189/jlb.0603255

10. Siegal FP, Kadowaki N, Shodell M, et al. The nature of the principal type 1 interferon-producing cells in human blood. *Science*. 1999;284(5421):1835-1837. doi:10.1126/science.284.5421.1835

11. Liu YJ. IPC: professional type 1 interferon-producing cells and plasmacytoid dendritic cell precursors. *Annu Rev Immunol*. 2005;23:275-306. doi:10.1146/annurev.immunol.23.021704.115633

12. Saulep-Easton D, Vincent FB, Le Page M, et al. Cytokine-driven loss of plasmacytoid dendritic cell function in chronic lymphocytic leukemia. *Leukemia*. 2014;28(10):2005-2015. doi:10.1038/leu.2014.105

13. Palma M, Krtsic A, Peña Perez L, et al. Ibrutinib induces rapid down-regulation of inflammatory markers and altered transcription of chronic lymphocytic leukaemia-related genes in blood and lymph nodes. *Br J Haematol*. 2018;183(2):212-224. doi:10.1111/bjh.15516

14. Solman IG, Blum LK, Burger JA, et al. Impact of long-term ibrutinib treatment on circulating immune cells in previously untreated chronic lymphocytic leukemia. *Leuk Res*. 2021;102:106520. doi:10.1016/j.leukres.2021.106520

15. Orsini E, Guarini A, Chiaretti S, et al. The circulating dendritic cell compartment in patients with chronic lymphocytic leukemia is severely defective and unable to stimulate an effective T-cell response. *Cancer Res*. 2003;63(15):4497-4506.

16. ShodellM,SiegalFP.Circulating,interferon-producingplasmacytoid dendritic cells decline during human ageing. *Scand J Immunol*. 2002;56(5):518-521. doi:10.1046/j.1365-3083.2002.01148.x

17. Spiering AE, de Vries T. Why females do better: the X Chromosomal TLR7 gene-dose effect in COVID-19. *Front Immunol*. 2021;11(12):756262. doi:10.3389/fimmu.2021.756262

18. Pisitkun P, Deane JA, Difilippantonio MJ, et al. Autoreactive B cell responses to RNA-related antigens due to TLR7 gene duplication. *Science*. 2006;312(5780):1669-1672. doi:10.1126/science.1124978

19. Souyris M, Cenac C, Azar P, et al. TLR7 escapes X chromosome inactivation in immune cells. *Sci Immunol*. 2018;3(19):eaap8855. doi:10.1126/sciimmunol.aap8855

20. Souyris M, Mejia JE, Chaumeil J, et al. Female predisposition to TLR7-driven autoimmunity: gene dosage and the escape from X chromosome inactivation. *Semin Immunopathol*. 2019;41(2):153-164. doi:10.1007/s00281-018-0712-y

21. Laffont S, Seillet C, Guéry JC. Estrogen receptor-dependent regulation of dendritic cell development and function. *Front Immunol*. 2017;10(8):108. doi:10.3389/fimmu.2017.00108

22. Chen YL, Chen TT, Pai LM, et al. A type I IFN-Flt3 ligand axis augments plasmacytoid dendritic cell development from common lymphoid progenitors. *J Exp Med*. 2013;210(12):2515-2522. doi:10.1084/jem.20130536

23. Laukken A, Bak RO, Krap V, et al. Interferon priming is essential for human CD34+ cell-derived plasmacytoid dendritic cell maturation and function. *Nat Commun*. 2018;9(1):3525. doi:10.1038/s41467-018-05816-y
24. Gardner JK, Cornwall SMJ, Musk AW, et al. Elderly dendritic cells respond to LPS/IFN-gamma and CD40L stimulation despite incomplete maturation. PLoS One. 2018;13(4):e0195313. doi:10.1371/journal.pone.0195313. eCollection 2018.

25. Mulder TA, Peña-Pérez L, Berglöf A, et al. Ibrutinib has time-dependent on- and Oof-target effects on plasma biomarkers and immune cells in chronic lymphocytic leukemia. HemaSphere. 2021;5(5):e564. doi:10.1097/HS9.0000000000000564

26. Zain R, Vihinen M. Structure-function relationships of covalent and non-covalent BTK inhibitors. Front Immunol. 2021;19(12):694853. doi:10.3389/fimmu.2021.694853

27. Advani RH, Buggy JJ, Sharman JP, et al. Bruton tyrosine kinase inhibitor ibrutinib (PCI-32765) has significant activity in patients with relapsed/refractory B-cell malignancies. J Clin Oncol. 2013;31(1):88-94. doi:10.1200/JCO.2012.42.7906

28. Byrd JC, Furman RR, Coutre SE, et al. Targeting BTK with ibrutinib in relapsed chronic lymphocytic leukemia. N Engl J Med. 2013;369(1):32-42. doi:10.1056/NEJMoa1215637

29. Smith CIE, Burger JA. Resistance mutations to BTK inhibitors originate from the NF-kappaB but not from the PI3K-RAS-MAPK arm of the B cell receptor signaling pathway. Front Immunol. 2021;10(12):689472. doi:10.3389/fimmu.2021.689472

30. Estupiñán HY, Berglöf A, Zain R, et al. Comparative analysis of BTK inhibitors and mechanisms underlying adverse effects. Front Cell Dev Biol. 2021;11(9):630942. doi:10.3389/fcell.2021.630942

31. Bergman P, Blennow O, Hansson L, et al. Safety and efficacy of the mRNA BNT162b2 vaccine against SARS-CoV-2 in five groups of immunocompromised patients and healthy controls in a prospective open-label clinical trial. EbioMedicine. 2021;74:103705. doi:10.1016/j.ebiom.2021.103705

32. Herishanu Y, Avivi I, Aharon A, et al. Efficacy of the BNT162b2 mRNA COVID-19 vaccine in patients with chronic lymphocytic leukemia. Blood. 2021;137(23):3165-3173. doi:10.1182/blood.2021011568

33. Andersen KM, Bates BA, Rashidi ES, et al. Long-term use of immunosuppressive medicines and in-hospital COVID-19 outcomes: a retrospective cohort study using data from the National COVID Cohort Collaborative. Lancet Rheumatol. 2022;4(1):e33-e41. doi:10.1016/S2665-9913(21)00325-8

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