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# Mutational landscape and complexity in CLL

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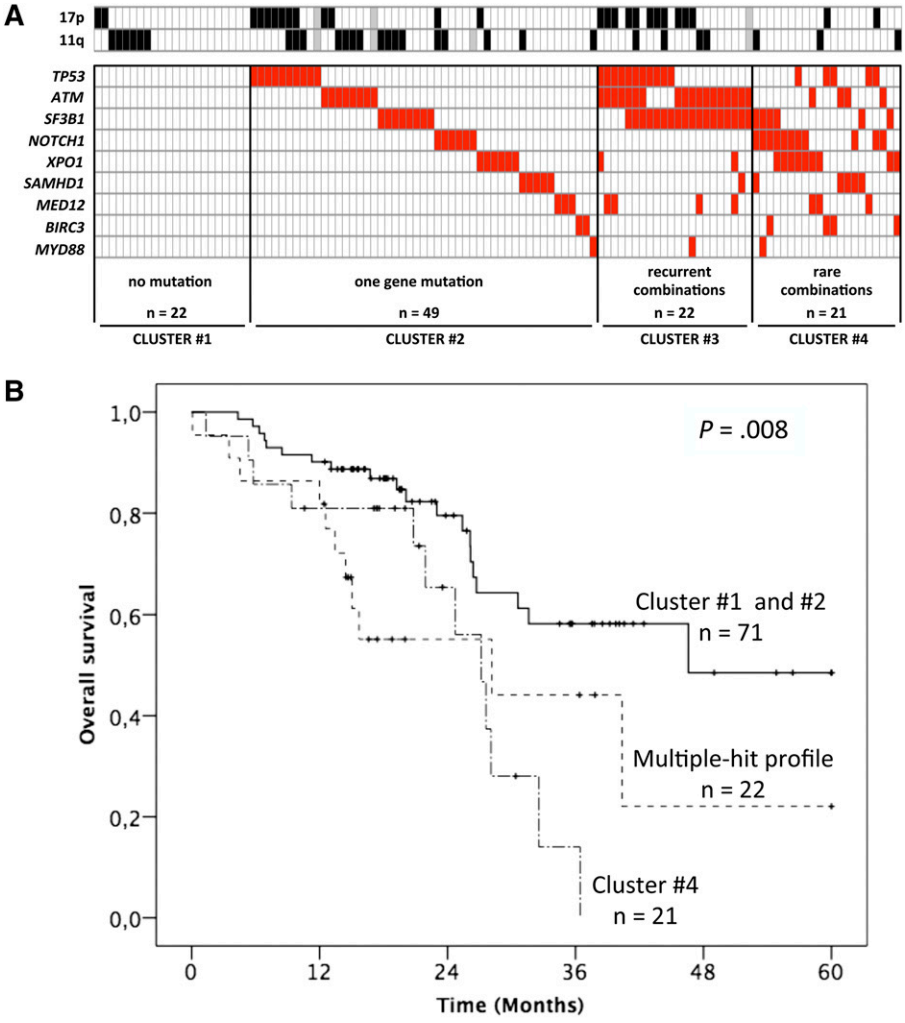
In this issue of *Blood*, Guièze et al show that concurrent driver gene mutations are frequent in patients with relapsed/refractory chronic lymphocytic leukemia (CLL) and associated with worse outcome.[1] Using deep sequencing, the authors analyzed a panel of known recurrently mutated genes from 3 prospective trials investigating chemo(-immuno-)therapy in relapsed/refractory CLL (see figure). Such targeted sequencing platforms are now in reach of any major medical center.

The authors identify patterns of mutations that are in keeping with the previous comprehensive cartography studies.[2-5] Because of the advanced stage of relapsed refractory CLL, the incidence of high-risk mutations is high, with >10% for *XPO1* and *NOTCH1* and >20% for *TP53*, *SF3B1*, and *ATM*. To put these numbers into perspective, a recent study of indolent CLL reported incidences <10% for each of these genes.[6]




Factors influencing outcome in CLL. (A) The incidence of mutations in relapsed and refractory CLL as summarized by Guièze et al. (B) Kaplan-Meier plots for OS of the mutation groups. See Figures 2 and 5 in the article by Guièze et al that begins on page 2110.

In their careful study, Guièze et al classified the patients based on the absence, single, or multiple occurrence of mutations in this 9-gene panel. It is important to remember that the average number of protein coding gene mutations in CLL is 20,[5] and the mutation frequency is associated with *IGHV* mutation status.

The multiple hit group was associated with a poorer prognosis with regard to progression-free and overall survival (OS). In fact, the patients with >1 mutation in the 9 genes (groups 3 and 4 in Guièze et al) had a significantly poorer outcome, with a median OS of 28.2 and 27.1 months, respectively. With the limited sample size of this study, it is difficult to disentangle the genes' individual effects from that of the multiple hit combinations.

What are the implications? We tend to simplify the impact of prognostic factors or gene mutations into single dimensions such as presence or absence. It is becoming clear that clonal size and structure,[4] convergence,[7] and combinations of mutations, as highlighted by this work, will affect outcome. Through advances in sequencing technology, the clonal composition at the genetic level is now relatively simple to investigate, which should not obscure the fact that multiple and much more complex factors including host and additional tumor properties will affect outcome. This immediately raises some of the limitations of the current and comparable studies. The study included controlled trial patients, but treatment was diverse, and the numbers did not allow assessment of the predictive impact of mutations. In general, it is somewhat sobering to acknowledge that, although many prognostic factors have been identified, our arsenal of predictive factors and thus tailored treatments is very limited. In addition, it is more difficult to assess prognostic impact of genetic lesions of patients in relapsed/refractory trials because of sample size and selection bias, which are hard to control for, and also because our definitions of relapsed and refractory disease are relatively crude.

With the emergence of novel treatments targeting the B-cell receptor pathway and the programmed cell death machinery, it will

be particularly important to systematically assess the clonal tides produced in the context of selective pressure. Indeed, the analysis of the genetic fingerprint produced by drug selection is highly informative. In current practice, with most patients receiving chemoimmunotherapy, the emergence of *ATM*, *TP53*, and *SF3B1* mutant cells, as found in the current study, is expected. As we increasingly use drugs with distinctly different mechanism of action, novel mutation targets (eg, phospholipase C-γ and Bruton tyrosine kinase) emerge, which also implies that future platforms will need to cover additional genes.

In summary, the work of Guièze et al casts light on the potential to improve current prognostic models of CLL and should serve as impetus to develop large-scale and meticulous studies of the genetic architecture of CLL, accounting for the dynamic nature of the mutational process.

*Conflict-of-interest disclosure: The authors declare no competing financial interests.* ■

## REFERENCES


1. Guièze R, Robbe P, Clifford R, et al. Presence of multiple recurrent mutations confers poor trial outcome of relapsed/refractory CLL. *Blood.* 2015;126(18):2110-2117.
2. Puente XS, Beà S, Valdés-Mas R, et al. Non-coding recurrent mutations in chronic lymphocytic leukaemia [published online ahead of print July 22, 2015]. *Nature*.
3. Puente XS, Pinyol M, Quesada V, et al. Whole-genome sequencing identifies recurrent mutations in chronic lymphocytic leukaemia. *Nature.* 2011;475(7354):101-105.
4. Landau DA, Carter SL, Stojanov P, et al. Evolution and impact of subclonal mutations in chronic lymphocytic leukemia. *Cell.* 2013;152(4):714-726.
5. Wang L, Lawrence MS, Wan Y, et al. SF3B1 and other novel cancer genes in chronic lymphocytic leukemia. *N Engl J Med.* 2011;365(26):2497-2506.
6. Hurtado AM, Chen-Liang TH, Przychodzen B, et al. Prognostic signature and clonality pattern of recurrently mutated genes in inactive chronic lymphocytic leukemia. *Blood Cancer J.* 2015;5:e342.
7. Jethwa A, Hüllein J, Stolz T, et al. Targeted resequencing for analysis of clonal composition of recurrent gene mutations in chronic lymphocytic leukaemia. *Br J Haematol.* 2013;163(4):496-500.


DOI 10.1182/blood-2015-09-667584



● ● ● PHAGOCYTES, GRANULOCYTES, AND MYELOPOIESIS

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# Oral NETs: the deadly kiss

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In this issue of *Blood*, Mohanty et al have identified a novel mechanism that protects us from pathogens where we encounter them first: in the oral cavity.[1]

Everyday we are exposed to microbes, whether we inhale air, expose our skin, or simply eat. As soon as foreign material enters our body, pathogens get their chance to invade and cause infection. Therefore, our mucosal immune system has evolved specific strategies to defend us at host-environment interaction zones. At the skin, the gut, and the lung, innate immunity has been studied in more detail, but the oral cavity, one of the most heavily colonized parts of the human body, remains surprisingly poorly understood.

Both cellular and noncellular mechanisms shield us from infection. Neutrophils are the first immune cells recruited and employ 3 major weapons to destroy pathogens: phagocytosis, granule release, and the formation of neutrophil extracellular traps (NETs). NETs have attracted increased attention because of the extraordinary nature of this defense mechanism.[2] Using "beneficial suicide" as a defensive measure, neutrophils expel their own DNA to entrap and destroy pathogens extracellularly. By doing so, NETs can combat microbes that are physically larger than the neutrophils, such as fungi.[3] NET formation (NETosis) has been studied in a variety of diseases and NET structures were also found at sites of periodontitis, but their functional role remained enigmatic.[4] As the central entry point to the human body, the oral cavity has one main defense shield, the saliva, which contains a plethora of antimicrobial components, such as lysozyme and mucins.[5] However, how these extracellular salival proteins cooperate with the cellular immune response has not been defined precisely.

Mohanty et al now broaden our view at this intimate host-pathogen interaction area by demonstrating that NETs are present in the oral cavity, are induced by saliva, and are highly bactericidal. Experiments were performed to uncover the factors that were responsible for saliva-induced NETosis, revealing that salival mucins triggered NET formation through sialyl $Lewis^X$– and L-selectin–mediated signaling. This identified pathway turned out to be novel, as it was rapid and did not require reactive oxygen species/reduced nicotinamide adenine dinucleotide phosphate-oxidase or elastase activities that are usually involved in NETosis.[6]

When NETs capture bacteria, two major factors determine how this interaction ends: DNases (destroy NETs, can be produced by certain microbes[7]) and the antimicrobial efficacy of the extruded DNA web. The abundance of NETs present in the oral cavity is somewhat unexpected based on the high DNase activity present in saliva.[8] However, saliva-induced NETs are unique in 2 aspects: (1) they are resistant to DNases in contrast to conventional NETs (induced by phorbol myristate acetate or bacteria), which are degraded, and (2) they destroy bacteria more efficiently than conventional NETs do.

So what is the clinical relevance of these findings? Neutrophils are continuously migrating to the oral mucosa and seem to be important there, since quantitative (neutropenia) or qualitative (functional) neutrophil deficiencies are commonly associated with gingivitis, ulcerations, periodontitis, and a disturbed oral microflora.[9] The authors further showed that saliva-induced NETosis was deficient in patients with Behçet disease (BD), an inflammatory condition characterized by recurrent oral ulcerations, indicating that this cellular mechanism has clinical impact. Nevertheless, the generalizability of this NET-related mechanism for other oral pathologies, such as recurrent aphthous stomatitis independent from BD, needs to be investigated in future studies.

NETosis is becoming more complex the more we learn about it. This study suggests that salival NET formation is essential in maintaining a healthy oral microbiota and pharmacotherapeutic strategies to enhance NET formation could represent a novel approach for oral manifestations of inflammatory diseases. Whereas progress in cell biology in the NETosis field is striking, there is, however, still little progress on how to apply this information therapeutically. In particular, it seems challenging to selectively activate NET

blood 2015 126: 2078-2079
doi:10.1182/blood-2015-09-667584

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Blood (print ISSN 0006-4971, online ISSN 1528-0020), is published weekly by the American Society of Hematology, 2021 L St, NW, Suite 900, Washington DC 20036.