

Clonal hematopoiesis is associated with increased risk of progression of asymptomatic Waldenström macroglobulinemia

Short title: Clonal hematopoiesis increases progression in WM

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Key Points

- Clonal hematopoiesis (CH) is present in at least 14% of patients with Waldenström macroglobulinemia (WM).
- Patients with CH are more likely to progress from IgM MGUS or smoldering WM to symptomatic WM.

Abstract

Clonal hematopoiesis (CH) is associated with adverse outcomes in patients with non-Hodgkin lymphoma (NHL) and multiple myeloma undergoing autologous stem cell transplantation. Still, its implications for patients with indolent NHL have not been well studied. Here, we report the prevalence of CH in patients with Waldenström macroglobulinemia (WM) and its association with clinical outcomes. In order to unambiguously differentiate CH mutations from those in the WM clone, CH was defined by the presence of somatic mutations in *DNMT3A*, *TET2* or *ASXL1* (DTA) and was detected in 14% of 587 patients with IgM monoclonal gammopathy of undetermined significance (MGUS), smoldering WM (SWM) or WM. The presence and size of DTA clones was associated with older age. Patients with CH had an increased risk of progression from MGUS or SWM to WM but not worse overall survival in this cohort. These findings further illuminate the clinical effects of CH in patients with indolent NHL such as WM.

Introduction

Clonal hematopoiesis (CH), a phenomenon in which somatic mutations in hematopoietic stem cells leads to clonal expansion, has been associated with a variety of adverse outcomes including increased risk of developing hematologic neoplasms, including myelodysplastic syndrome (MDS) and acute myeloid leukemia (AML), and cardiovascular disease^{1,2}. CH has also been associated with decreased overall survival (OS) in patients with non-Hodgkin lymphoma (NHL) and multiple myeloma (MM) undergoing autologous stem cell transplantation (ASCT)^{3,4}. Whether CH is associated with similar adverse outcomes in patients with indolent NHL and outside the context of ASCT is unknown.

Waldenström Macroglobulinemia (WM) is an indolent NHL characterized by immunoglobulin-M (IgM)-secreting lymphoplasmacytic cells, and hallmark mutations in genes involved in B-cell signaling including *MYD88*, *CXCR4*, *ARID1A*, and *CD79B*^{5,6}. Patients often present with precursor states such as IgM monoclonal gammopathy of undetermined significance (MGUS) and smoldering WM (SWM) and are observed for years without therapeutic intervention. When patients become symptomatic, standard treatments include cytotoxic chemotherapy (e.g. nucleoside analogs and alkylating agents), proteasome inhibitors, Bruton tyrosine kinase (BTK) inhibitors and anti-CD20 antibodies⁷. Herein, we investigate the prevalence and clinical implications of CH in patients with WM.

Methods

We retrospectively reviewed clinical data of 602 patients with IgM MGUS, SWM and WM who had clinical next-generation sequencing (NGS) performed on bone marrow aspirates or peripheral blood obtained between October 2014 and February 2020 at the Dana-Farber Cancer Institute (DFCI). The study was approved by the DFCI institutional review board (DF/HCC-18-194). It was performed in accordance with the Declaration of Helsinki. Figure S1 summarizes the study workflow.

An Illumina Truseq amplicon-based NGS assay of 95 genes recurrently mutated in myeloid and lymphoid neoplasms was utilized (Table S1)⁸. Each specimen yielded ~2 million reads and ~1500X average coverage with 90% of amplicons having >200X coverage. Pathogenic driver variants were identified based on mutation type, position, and frequency in published reports^{1,3,9} and public databases¹⁰. Table S2 lists the queried variants. Figure S2 summarizes the mutational profile.

Detailed statistical analysis methods are provided in the Supplemental Methods.

Results and Discussion

The cohort included 453 patients with symptomatic WM with a median age of 61 years (range: 22-90) at diagnosis and 68 years (range: 33-93) at time of first NGS assay. Thirteen patients (3%) had a coincident diagnosis of MDS or AML and one had acute lymphoblastic leukemia (ALL) at the time of NGS; these 14 patients were excluded from further analysis. Prior to NGS

testing, 304 patients had received therapy, 145 of whom had received cytotoxic agents (Table S3).

We identified 413 somatic mutations in 258/440 individuals (59%), with the most common being MYD88 p.L265P (46%) and CXCR4 mutations (17.0%) (Figure S3). Because some genes can be mutated in both CH and WM cells (i.e. *TP53*) we restricted our analysis of CH to cases with *DNMT3A*, *TET2* or *ASXL1* (CH-DTA) mutations with a variant allele frequency (VAF) of $\geq 2\%$, where we could unambiguously assign the mutation to the CH clone. DTA mutations represent the three most commonly identified mutations in CH and have been reported primarily in myeloid malignancies. The prevalence of CH-DTA in WM was 14% encompassing 69 mutations in 61 patients with a median VAF of 10.4% (Figure 1A-C). CH-DTA detection was not impacted by BM infiltration by lymphoplasmacytic cells (Figure S4A-B) or MYD88 status (Figure S4C) in our cohort. CH-DTA was positively associated with older age ($p < 0.001$) at time of NGS, with a median age of 72 vs. 67 years for patients with versus those without CH-DTA, respectively^{1,4,11}. Cytotoxic chemotherapy had been administered prior to NGS testing in 43% of patients with CH-DTA compared to 30% of patients without ($p = 0.08$).

CH-DTA prevalence here was higher than that reported in other large healthy cohort studies (5-10% among subjects ≥ 60 years old), particularly when considering the more inclusive CH definition of those studies compared to ours^{1,2,11}. However, those studies utilized whole-exome or whole-genome sequencing and thus had lower sensitivity for mutation detection. In smaller cohorts of cancer patients utilizing targeted NGS, the prevalence of DTA mutations at a VAF $\geq 2\%$ has been similar: 9% in MM⁴, 17% in NHL³ and 21% in patients with metastatic melanoma¹². These differences are likely due to several factors including the cohorts' age distribution, prior cytotoxic therapy exposures and differences in sequencing platforms.

CH-DTA was not associated with inferior OS (Figure 1D) with a relatively short median follow-up from diagnosis and NGS assay of 6.7 (95% CI: 6.1-7.6) and 2.5 (95% CI: 2.2-2.8) years, respectively. The most common cause of death was disease progression with no significant difference between those with or without CH-DTA. Patients with CH-DTA had an increased risk of cardiovascular disease (30% vs. 18%, $p = 0.036$)^{1,13}. Whether survival differences will become apparent with longer follow-up remains unknown.

Patients with CH-DTA had elevated $\beta 2$ -microglobulin at time of NGS (4.0 mg/dL in CH-DTA vs. 3.2 mg/dL in non-CH-DTA patients [$p = 0.004$]), consistent with our prior observation in MM (Table S4)⁴. CH-DTA patients also had a higher frequency of amyloidosis (11% vs. 3%, $p = 0.009$). DTA mutations have been associated with a hyperinflammatory phenotype related to increased IL-6 and IL-1 β mediated by mutant myeloid cells¹⁴. Whether these clinical findings are associated with an inflammatory microenvironment and how this might affect the development and evolution of disease is something that warrants additional study.

Next, we looked at the 31 IgM MGUS and 116 SWM patients who had NGS done. CH-DTA prevalence in MGUS and SWM did not differ significantly from symptomatic WM (13%, 14% and 14%, respectively) (Figure 2A and S5). Among precursor patients, there was an increased risk of progression to symptomatic WM in those with CH-DTA (7/20 patients with vs. 11/116 without CH-DTA progressed over a median of 54 months [$p = 0.002$]) (Figure 2B-C). While

patients with MGUS and asymptomatic WM progress at different rates to WM, we still found that CH-DTA was significantly associated with risk of progression to symptomatic WM when studied separately. Whether this increased risk is due to changes in the bone marrow niche that promote disease, lymphoplasmacytic cells supporting CH expansion or increased risk of defective hematopoiesis and subsequent cytopenias in patients with CH remains unclear.

One-hundred and four patients had samples sequenced at more than one time point, 23 with CH-DTA mutations (Figure S6). Following WM-directed therapy, most patients exhibited a VAF decrease in their WM-related *MYD88* and/or *CXCR4* mutations (Figure 2D). On average, DTA clones expanded 6.2-fold after therapy compared to those without treatment (Figure 2E). These data show that DTA mutations originate from a distinct myeloid clone and do not respond to WM-directed therapy. It is unclear if CH-DTA clones truly expand in response to therapy or this represents pseudo expansion in the setting of a shrinking WM clone. Further analysis of the expansion kinetics and outcomes of CH clones will be important in understanding clonal dynamics during therapy. These changes are important to take into account as NGS use becomes more frequent as a method to track response to therapy.

Increased risk of MDS and AML is well documented in WM¹⁵⁻¹⁸ and CH is associated with increased myeloid malignancy risk³. We performed an exploratory analysis of patients with both WM and a myeloid malignancy. Fourteen patients had a concurrent myeloid malignancy at the time of NGS, developing at a median of 7.9 years from WM diagnosis, 13 of which were MDS (87%) (Figure S7A). During follow-up, one additional patient with *SF3B1* and *SRSF2* mutations developed MDS after receiving bendamustine and rituximab (Figure S7B). All but three patients had received cytotoxic therapy prior to developing MDS or AML (Figure S7C-D). *TP53* mutant CH is among the highest risk lesions for development of a secondary MDS or AML^{19,20}. In this cohort, *TP53* was the fifth most commonly mutated gene (n=19, table S5, figure S3B) and was further enriched in patients with concurrent WM and MDS/AML (4/14, figure S7D). In contrast to a previous report¹⁷, *TP53*-mutated patients did not have an inferior OS or PFS (figure S8A-B), which could be due to the fact that *TP53* mutations may reside in the CH clone rather than the WM clone. Longer follow-up and prospective studies are required to determine which patients with WM and CH have the highest risk of developing a secondary myeloid malignancy.

In conclusion, we demonstrate that CH is common in WM patients and is associated with increased risk of progression from precursor states but not with inferior survival. Further work is needed to determine how the presence of CH might promote progression to WM and whether WM-related microenvironmental changes within the BM niche, leading to immunosuppression, could play a role in CH expansion. Incorporation into risk stratification models will also require further investigation. Importantly, our data do not support changes in clinical management or alterations in therapy for patients with WM and coexistent CH and reinforce the need to interpret NGS results within their specific clinical context.

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Authorship

Contributions: Conceptualization, S.T., T.H.M., A.S.S. and I.M.G.; Methodology, R.R. and L.T.; Investigation, S.T., T.H.M., R.R., L.L., K.I.N., H.E.K., N.K.S., A.H.N., E.A., G.B. and S.A.A.; Writing – Original Draft, ST; Writing – Review & Editing, S.T., T.H.M., H.E.K., A.H.N., E.A., D.P.S., J.J.C., S.P.T., A.S.S. and I.M.G.; Funding Acquisition, I.M.G.; Supervision, A.S.S. and I.M.G.

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Figure legends

Figure 1. Mutational spectrum in patients with Waldenström Macroglobulinemia (WM). (A) The total number of symptomatic WM patients harboring one or more mutations in each gene. (B) Number of symptomatic WM patients harboring mutations in 1, 2, and 3 different genes. (C) Co-mutation plot showing mutations present in all 258 patients: each column represents a single patient. The top row denotes the maximum variant allele frequency (VAF) in each patient, with darker shades of pink indicating higher VAF. The bar graph on the right designates the proportion of the different mutation subtypes for each gene. (D) OS and (E) PFS among WM patients with CH versus those without CH.

Figure 2. Clonal hematopoiesis (CH) in patients with IgM monoclonal gammopathy of undetermined significance (MGUS) and smoldering Waldenström Macroglobulinemia (SWM). (A) Co-mutation plot showing mutations present in all 20 IgM MGUS and SWM patients: each column represents a single patient. The top row denotes the maximum variant allele frequency (VAF) in each patient, with darker shades of pink indicating higher VAF. The bar graph on the right designates the proportion of the different mutation subtypes for each gene. (B) PFS and (C) OS among IgM MGUS and SWM patients with CH versus those without CH. (D) Representative heatmaps for the clonal dynamics of WM-related mutations and DTA mutations in WM. Values depicted in each square represent VAF. (E) Average change in VAF of DTA mutations assessed between consecutive timepoints, with or without intervening therapy.

Figure 1

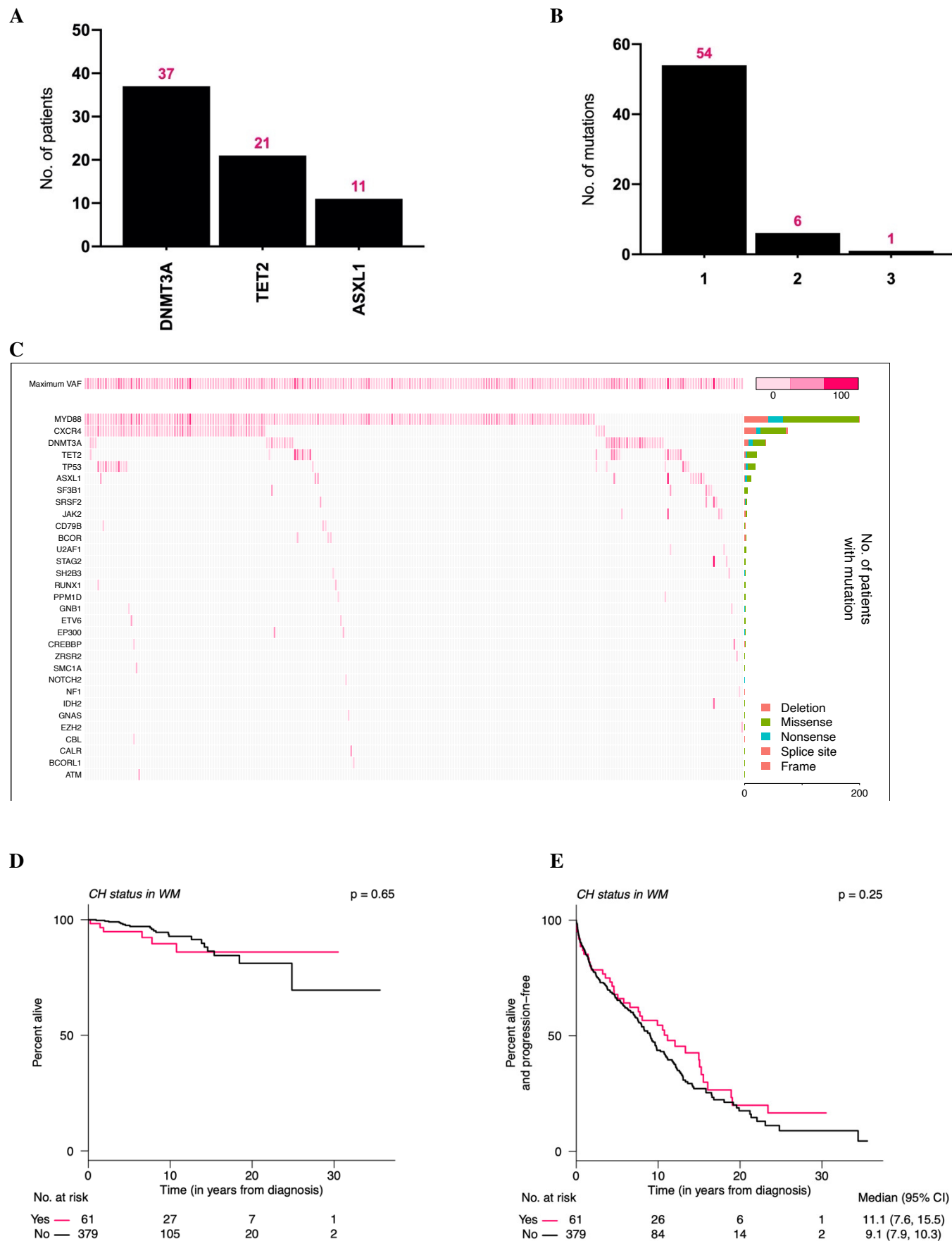


Figure 2

