Somatic mutations in aplastic anemia: significance for classification, therapy, and outcome

Henry J. Wood, Judith C.W. Marsh
Department of Haematological Medicine, King’s College Hospital/King’s College London, London, United Kingdom

Take home messages
- Somatic mutations occur in 70% of AA patients, and up to a third have myeloid-specific mutations.
- The immune response to somatic mutations contributes to determining their fate and impact in AA.
- The full significance of myeloid-specific somatic mutations in AA requires correlation with cytomorphological and cytogenetic features and future serial sampling in prospective clinical studies.

Introduction
Next generation sequencing has enabled detection of clonal hematopoiesis in many more patients with aplastic anemia (AA) than previously realized by conventional metaphase cytogenetics, FISH or whole genome scanning using SNP-karyotyping and flow cytometric detection of PNH clones. An abnormal clone can expand in an aplastic BM through selection exerted by an autoimmune attack, for example, where there is loss of certain HLA class I alleles, through loss of heterozygosity (LOH) for 6p, or by structural somatic HLA allelic mutations,1,2 or with clonal expansion of GPI-deficient PNH clones that escape the autoimmune attack,3,4 and/or somatic mutations (SM) can also emerge randomly in AA through genetic drift, whereby a mutant clone can more easily expand in a hypocellular bone marrow.5,6 Some SM may be preleukemic, but others may be neutral or even beneficial.

Current state of the art
Impact of the immune signature of AA and MDS on somatic mutations
The immune signature of AA is defined by a proinflammatory environment with combined expansion of T helper (Th)1 (clonal) and Th17 cells, and a reduction in T regulatory cells (Tregs) that are dysfunctional in terms of their ability to suppress autoreactive cytotoxic CD8 T cells.7,5,6 In low-risk MDS Tregs are normal but Th17 cells are increased. In high risk MDS, increased Tregs and myeloid-derived suppressor cells and features of smoldering/chronic inflammation, result in a switch from immune surveillance to immune-subversion and subsequent disease progression (Fig. 1).7,8,9 The immune response (innate and adaptive) likely plays a key role in modulating the fate of abnormal mutated clones in AA, but other factors include the cellular origin and type of mutation, clone size, neoantigen formation,10 ethnicity and age, and possible defective DNA repair mechanisms.

SM that arise through immune escape
PNH clones are detected in up to 50% of AA patients using flow cytometry, which is more sensitive than PIGA sequencing for a clone size of <10%.11,12 SM in PIGA are predictive of response to immunosuppressive therapy (IST), and good prognosis. 6pLOH is relatively specific to AA and occurs in up to 19% of patients, compared to 1% in MDS and is very rare in the general population. 6pLOH favors loss of specific HLA alleles such as HLA-B*40:02, B*54:01. SM in HLA-B40:02 leading to loss of function phenotype have also been detected in patients who show high response to IST and low risk of progression to MDS/AML.1,12

Myeloid-specific SM in AA
Approximately one third of AA patients have a myeloid-specific SM, but they occur less frequently than in MDS.11,12,13,14 Differences in frequencies reflect differences in methodologies, depth of sequencing, age, and stage of AA. Genes mutated commonly involve ASXL1, DNMT3A, or BCOR/BCORL1, and there is underrepresentation of TET2, JAK2, RUNX1, and TP53. ASXL1 and DNMT3A clones do more often expand over time in AA, but not in all cases. Some of the myeloid-specific SM are the same ones that are seen in ARCH, and in AA their incidence increases with age.10 Other differences in SM between AA, MDS and AML are summarized in Fig. 1. In contrast to PIGA, and BCO/BCORL1, patients with DNMT3A, ASXL1, TP53, RUNX1, or CSMD1 treated with IST, show worse response, overall and progression free survival.12 Presence of SM after IST
is associated with increased risk of MDS/AML, especially for ASXL1, RUNX1 and splicing factor SM with high VAF%, higher number of SM per patient and longer duration of AA. In contrast, recent studies failed to show an associated risk of MDS with DNMT3A and TET2.

SM in AA compared to hypoplastic MDS
The distinction between AA and hypoplastic MDS is challenging morphologically. In a recent collaborative King’s College London/University of Pavia study, a new diagnostic score discriminated between 278 patients with hypoplastic MDS and 136 with AA. In hypoplastic MDS the prevalence of SM, their VAF and number of SMs per patient, were intermediate between the patterns seen in AA and normo-/hypercellular MDS (see Fig. 1). Incorporating SM with morphological data enabled separation of hypoplastic MDS patients into 2 groups, one with clinical and genetic features highly consistent with myeloid neoplasm and one with features of non-malignant bone marrow and absence of progression to AML.

Future perspectives
Following IST for AA, late clonal progression to MDS/AML increases to a maximum of 15% to 26% at 10 years. Nineteen percent of patients with refractory SAA treated with eltrombopag, developed abnormal cytogenetic clones (mostly abnormalities of chromosome 7) at a median of 3 months. Key to understanding the significance of SM in AA is serial analysis over time, particularly in a disease where abnormal cytogenetic clones over time may evolve, remain stable, or even disappear. This, and the possible contribution of eltrombopag to later clonal progression in the context of IST, will be addressed in the prospective randomized study, EBMT RACE trial of first line horse ATG, ciclosporin with or without eltrombopag (ClinicalTrials.gov, NCT02099747) currently in progress. Serial samples for SM, and high dimensional immune-phenotyping will examine the immune response to SM. Currently the finding of a myeloid-specific SM in AA in the absence of morphological features of MDS or an MDS-defining cytogenetic abnormality, should not trigger a change in treatment, but the full blood count and SM should be monitored carefully along with an early assessment of potential hematopoietic stem cell transplant donor availability, in the event of subsequent early disease progression.

References
1. Ogawa S. Clonal hematopoiesis in acquired aplastic anemia. Blood. 2016;128:337–347.
2. Zaimoku Y, Takamatsu H, Hosomichi K, et al. Identification of an HLA class I allele closely involved in the autoantigen presentation in acquired aplastic anemia. Blood. 2017;129:2908–2916.
3. Luzzatto L, Risitano AM. Advances in understanding the pathogenesis of acquired aplastic anemia. Br J Haematol. 2018;182:758–776. A recent comprehensive review of pathogenesis focussing on mechanisms of clonal haemopoiesis.
4. Cooper JN, Young NS. Clonality in context: hematopoietic clones in their narrow environment. Blood. 2017;130:2163–2172.
5. Kordasti S, Marsh J, Al-Khan S, et al. Functional characterization of CD4+ T-cells in aplastic anemia. Blood. 2012;119:2033–2043. The first study demonstrating that abnormalities in the CD4+ T cell compartment are the main immune drivers of the immune dysregulation in AA.
6. Kordasti S, Costantini B, Seidl T, et al. Deep-phenotyping of Tregs identifies an immune signature for idiopathic aplastic anemia and predicts response to treatment. Blood. 2016;128:1193–1205.
7. Kordasti SY, Ingram W, Hayden J, et al. CD4+CD25 high Foxp3+ regulatory T-cells in myelodysplastic syndrome (MDS). Blood. 2007;110:847–850.
8. Kintang AO, Kordasti S, Sand KE, et al. Expansion of myeloid derived suppressor cells correlates with number of T regulatory cells and disease progression in myelodysplastic syndrome. Oncotarget. 2015;5:1062208.
9. Barreyro L, Chlon TM, Starczynowski DT. Chronic immune response dysregulation in MDS pathogenesis. Blood. 2018;132:1553–1560. This review highlights recent understanding of abnormalities in innate immunity producing chronic inflammation that drives disease progression in MDS.
10. Coats T, Smith AE, Mourikis A, et al. Neoantigens in MDS are associated with two novel CD4+ T cell subsets and improved overall survival. Blood. 2017;130:2985.

11. Kulasekararaj AG, Jiang J, Smith AE, et al. Somatic mutations identify a subgroup of aplastic anemia patients who progress to myelodysplastic syndrome. Blood. 2014;124:2698–2704.

The first large study demonstrating that myeloid-specific SMs occur in a fifth of AA patients and that they are associated with high risk of progression to MDS/AML.

12. Yoshizato T, Dumitriu B, Hosokawa K, et al. Somatic mutations and clonal hematopoiesis in aplastic anaemia. N Engl J Med. 2015;373:35–47.

This large combined Japanese/NIH study defined the prognostic significance of specific groups of SMs and examined the clonal landscape over a 6-month period.

13. Negoro E, Nagata Y, Clemente MJ, et al. Origins of myelodysplastic syndromes after aplastic anemia. Blood. 2017;130:1953–1957.

14. Huang J, Ge M, Lu S, et al. Mutations of ASXL1 and TET2 in aplastic anemia. Haematologica. 2015;100:e172–e175.

15. Babushok DV. A brief, but comprehensive, guide to clonal evolution in aplastic anemia. Hematology Am Soc Hematol Educ Program. 2018;2018:457–466.

16. Jaiswal S, Fontanillas P, Flannick J, et al. Age-related clonal hematopoiesis associated with adverse outcomes. N Engl J Med. 2014;371:2488–2498.

17. Bono E, McLornan DP, Travagino E, et al. Hypoplastic myelodysplastic syndrome: combined clinical, histopathological and molecular characterization. Blood. 2017;130:588.

18. Desmond R, Townsley DM, Dumitriu B, et al. Eltrombopag restores tri-lineage hematopoiesis in refractory severe aplastic anemia which can be sustained on discontinuation of drug. Blood. 2014;123:1818–1825.

19. Papaemmanuil E, Gerstung M, Malcovati L, et al. Clinical and biological implications of driver mutations in myelodysplastic syndromes. Blood. 2013;122:3616–3627.

20. Haferlach T, Nagata Y, Grossmann V, et al. Landscape of genetic lesions in 944 patients with myelodysplastic syndromes. Leukemia. 2014;28:241–247.