

Spliceosome mutations: one plus one does not always equal two

Andrea Pellagatti and Jacqueline Boulton

University of Oxford

In this issue of *Blood*, Taylor et al show that two splicing factor mutations can coexist in the same cell in myeloid malignancy patients when their combination includes less common mutant alleles.¹

Mutations in genes involved in pre-mRNA splicing (*SF3B1*, *SRSF2* and *U2AF1* mutations being the most frequent) are common in patients with myeloid malignancies, including myelodysplastic syndromes (MDS), acute myeloid leukemia (AML) and myeloproliferative neoplasms.^{2,3} Several studies involving the analysis of large patient cohorts demonstrated that splicing factor mutations are typically heterozygous, and a striking and consistent finding of these studies is that these mutations are mutually exclusive.⁴⁻⁶ Nevertheless, myeloid malignancy patients harboring two co-occurring splicing factor mutations have been reported, albeit rarely. The existence of such cases represents a conundrum, since the combination of two splicing factor mutations would be expected to be lethal for a cell, unless each mutation occurs in different clones. Indeed, a previous study demonstrated that co-expression of the most common mutations of *SF3B1* (K700E) and *SRSF2* (P95H) *in vivo* in mice is intolerable to hematopoietic cells.⁷

Until now, the characteristics of patients with myeloid malignancies harboring multiple splicing factor mutations had not been systematically investigated. In their study, Taylor et al performed bulk DNA sequencing and single cell sequencing of malignant cell populations from myeloid malignancy patients to determine the frequency and basis for the co-existence of splicing factor mutations.

The authors analyzed genomic DNA sequencing data from more than 4,000 myeloid malignancy patients, and mutations of *SF3B1*, *SRSF2*, *U2AF1* and *ZRSR2* were observed in approximately 23% of all cases. They identified 36 cases with two co-occurring splicing factor mutations. After inclusion of 22 additional patients (from the Mayo Clinic) with two splicing factor mutations, the analysis of the variant allele frequency and cancer cell fraction

showed that the mutations coexisted within the same cell in approximately two thirds of the overall cohort of 58 double mutant samples. Intriguingly, *SF3B1*^{K700} and *SRSF2*^{P95/P96} mutations, which represent the most frequent mutant alleles among splicing factors in myeloid malignancies, were significantly less common in double mutants compared to single mutants, indicating selection against cells with co-occurrence of *SF3B1*^{K700} and *SRSF2*^{P95/P96} mutations. In contrast, selection was observed for less common alleles, such as *SF3B1*^{non-K700E} mutations (e.g. E622, H662, K666), rare amino acid substitutions at SRSF2 P95, and combined *U2AF1*^{S34/Q157} mutations (see figure). These data support the conclusion of the study that mutual exclusivity or co-occurrence of splicing factor mutations is allele-specific rather than gene-specific.

Taylor et al proceeded to perform single-cell DNA sequencing of bone marrow cells from 11 patients that harbored two splicing factor mutations. The data elegantly confirmed the mutual exclusivity of *SF3B1*^{K700E} and *SRSF2*^{P95H} mutations at the single cell level, and demonstrated the potential for co-occurrence of other rare splicing factor mutant alleles.

Functional studies involving the measurement of the extent of missplicing events for SF3B1 mutants and of the binding of SRSF2 mutants to its consensus RNA sequence were then carried out. The proteins encoded by the less common *SF3B1* and *SRSF2* mutant alleles that are enriched in double mutant myeloid malignancy patients were shown to have reduced effects on RNA splicing or binding affinity compared to the most common alleles, providing evidence supporting the concept that the less common mutant alleles may escape from epistasis due to more modest effects on RNA binding and/or splicing.

Interestingly, it was shown that *U2AF1*^{S34} and *U2AF1*^{Q157} mutations co-occurred in myeloid malignancy patients at a significantly higher frequency than expected by chance. Single-cell DNA sequencing analysis of a double mutant patient showed that both *U2AF1*^{S34} and *U2AF1*^{Q157} mutations were present in the same cells, suggesting potential cooperation between the two mutations. Furthermore, these *U2AF1* mutations were found to co-occur in *cis* with preservation of the wild-type allele, a finding in agreement with a previous study demonstrating that the expression of the wild-type *U2AF1* allele is required for survival of cells harboring a *U2AF1* mutation.⁸ The analysis of further double *U2AF1* mutant patient samples is required to establish whether *U2AF1*^{S34} and *U2AF1*^{Q157} mutations are tolerable when co-occurring in *trans*.

This study by Taylor et al has illuminated the genetic and molecular bases for the escape of splicing factor mutations from epistasis in patients with myeloid malignancies, findings that have important clinical and therapeutic implications.

Specific mutant alleles of each splicing factor gene might have different impacts on the clinical features and/or survival of patients with myeloid malignancies. Indeed, this suggestion is supported, for example, by a recent study showing that *SF3B1*^{K666} mutations are associated with some hematological features in MDS and with shorter patient survival and increased progression to AML.⁹ However, the observation by Taylor et al that *SF3B1*^{K666} mutations have a weaker effect on pre-mRNA splicing compared to *SF3B1*^{K700} mutations, likely resulting from distinct structural disturbances at these amino acid locations, might be expected to lead to a milder impact of *SF3B1*^{K666} mutations on patient outcome. Further studies, including functional assessment of aberrantly spliced target genes, are required to elucidate fully the effects of less common splicing factor mutant alleles on clinical features and outcome in patients with myeloid malignancies.

The mutual exclusivity of splicing factor mutations, previous studies showing that these mutations are not tolerated in a homozygous state⁷ and the demonstration that the survival of splicing factor mutant cells depends on presence of the wild-type allele⁸ provided the rationale for the potential therapeutic use of splicing modulators in splicing factor mutant myeloid malignancy patients. The basis of this synthetic lethality strategy is that, unlike wild-type cells, splicing factor mutant cells would be unable to tolerate further disruption to the splicing process by pharmacological inhibition of the spliceosome.¹⁰ The finding by Taylor et al that the most common *SF3B1* and *SRSF2* mutations have more prominent effects on pre-mRNA splicing and RNA binding affinity than less common splicing factor mutant alleles indicates that myeloid malignancy patients with *SF3B1*^{K700E} or *SRSF2*^{P95H/L/R} mutations may be more susceptible to treatment with splicing modulators. Stratification of patients on the basis of specific splicing factor mutant alleles should be considered in clinical trials involving drugs that target the spliceosome.

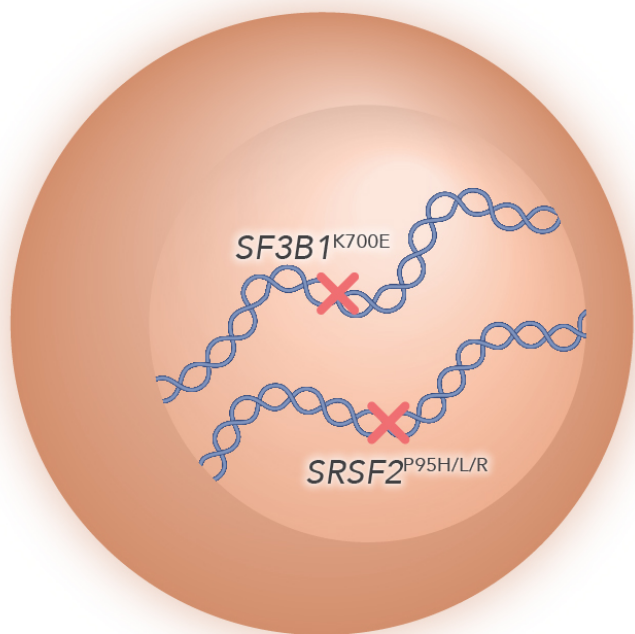
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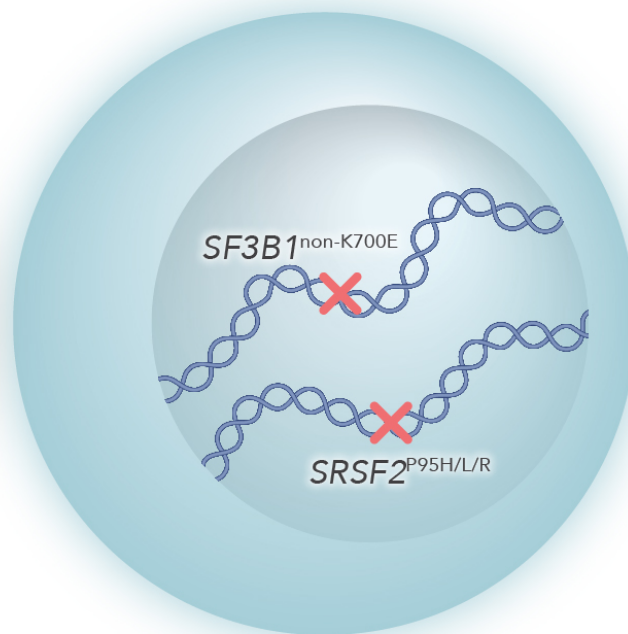
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Figure legend

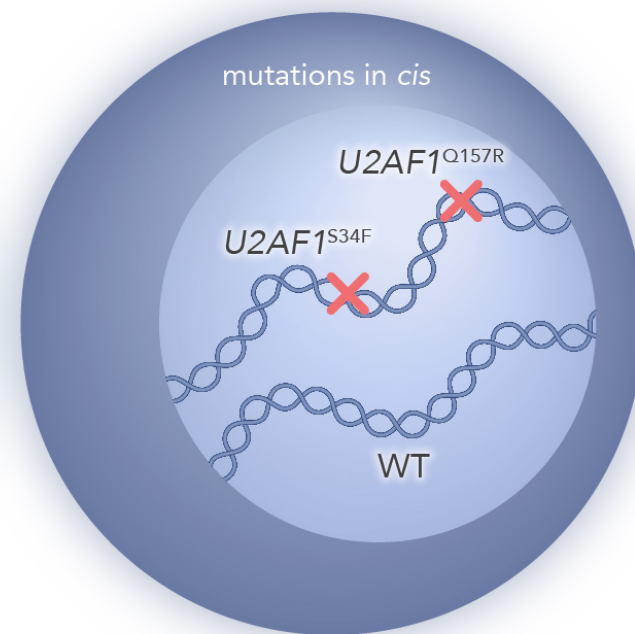
Myeloid malignancy patient cells with two common splicing factor mutations, such as *SF3B1*^{K700E} and *SRSF2*^{P95H/L/R} mutations, are selected against. Selection can occur instead for cells with two splicing factor mutations that include less common alleles, such as *SF3B1*^{non-K700E} mutations (or rare amino acid changes at SRSF2 P95). Selection of cells with combined *U2AF1*^{S34} and *U2AF1*^{Q157} mutations has been shown when the two mutations occur in *cis* with preservation of one wild-type *U2AF1* allele. WT: wild-type.





selected against




selection allowed




selection allowed