A synthetic molecule stalls pre-mRNA splicing by enhancing cancer-relevant U2AF2 – RNA complexes

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Dysregulated pre-mRNA splicing is an emerging hallmark of cancers and hematologic malignancies, and renders these malignancies sensitive to spliceosome inhibition. As such, modulators of pre-mRNA splicing may offer as yet unrealized potential as anti-cancer therapeutics. Currently, the pre-mRNA splicing factor SF3b1 is the sole target of the major class of small molecule inhibitors of spliceosome assembly (e.g. sudemycin D6, E7107, H3B-8800). A heterodimer comprising U2AF2 and U2AF1 recognizes the 3´ splice site and initiates pre-mRNA splicing. Whereas U2AF2 is an essential pre-mRNA splicing factor, U2AF1 plays an accessory role for splicing so-called “AG-dependent” sites. As such, a small molecule modulator of the U2AF2 – 3´ splice site complex may have general utility for treatment of cancers and hematologic malignancies.

Here, we report identification and characterization of a new small molecule that inhibits pre-mRNA splicing by modulating U2AF2 – RNA complexes. We screened 1,593 structurally diverse compounds in a fluorescence polarization-based assay. For physiological relevance and statistical robustness of the polarization signal (which is proportional to molecular size), we pre-bound a U2AF2–U2AF1^{S34F}–SF1 heterotrimer to a fluorescein (Fl)-labeled splice site derived from the DEK oncogene. Although three hit compounds dissociated the ribonucleoprotein complex and inhibited pre-mRNA splicing *in vitro*, we considered these compounds lower priority due to nonspecific RNA binding. Remarkably, one hit compound specifically enhanced formation of the U2AF-containing ribonucleoprotein complex, yet inhibited *in vitro* splicing of representative substrates. Orthogonal BIAcore assays identified U2AF2 as the target subunit of this top hit compound. Structure-guided docking followed by mutagenesis established that the compound binds at a site between the tandem RNA recognition motifs of the splicing factor. Native gel electrophoresis further revealed that the compound stalled the pre-mRNA splicing process at the U2AF2-containing stage of spliceosome assembly.

Altogether, our results demonstrate a new means for molecular manipulation of pre-mRNA splicing: Locking spliceosome assembly at an early, inactive stage. Future optimized generations of this U2AF2-targeted compound could amplify the effects of SF3B1 modulators, reduce side effects, and/or increase efficacy in subsets of patients carrying *U2AF1* or *U2AF2* mutations or amplifications.