Deciphering the impact of toxic antisense oligonucleotide (ASO) gapmer off-target (OT) RNA degradation on OT protein level

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One of the potential safety concerns for antisense oligonucleotide (ASO) gapmers are hybridization dependent off-target (OT) effects. This occurs when ASO gapmers bind unintended (m)RNA transcripts via Watson-Crick hybridization that results in meaningful level of RNase H mediated degradation of the off-target transcript. The current workflow for OT assessment includes *in silico* prediction based on ASO:RNA sequence homology, followed by *in vitro* validation and margin assessment using qPCR and RNA sequencing (RNAseq) methods (1,2). Importantly, despite reduction in protein levels being the main concern for OT effects, the OT assessment strategies described today don't include default assessment on protein level. To better understand if protein level studies add value, we made use of the emerging technology of fast proteomics, where 6000+ proteins are quantified with an improved throughput of 60 samples per day, allowing analysis of many samples in reasonable time (3). We designed a pilot experiment in hepG2 cells combining RNAseq with fast proteomics analysis at different time points after transfection of ASOs with different number of predicted OTs and *in vitro* toxicity profiles. First, we found that a large fraction of *in silico* predicted OTs are differentially expressed by RNAseq (25-70%). Second, analysis of the fast proteomics data showed that only few OTs are downregulated at the protein level (5-33% of OTs reduced by RNAseq). Finally, OT fold changes on the protein level were consistently lower than OT (m)RNA fold changes throughout our experiment. Next, we will confirm these findings with a bigger set of toxic ASO gapmers and adjust our OT assessment strategy accordingly.

References

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