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POSTER ABSTRACTS

321.COAGULATION AND FIBRINOLYSIS: BASIC AND TRANSLATIONAL

Transcriptomic Profiling to Understand Inhibitor Development in Previously Untreated Patients with Severe Hemophilia a

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Background: When starting treatment with factor VIII (FVIII) concentrate in individuals with severe hemophilia A, minimizing the risk of FVIII inhibitor development is an important concern for clinicians, patients and their families. Despite increased understanding of some of the genetic and environmental risk factors for inhibitor formation from epidemiological studies, identification of previously untreated patients (PUPs) at risk of developing inhibitors remains a challenge. Prediction of inhibitor development may help optimize care in PUPs with hemophilia A. RNA sequencing (RNA-Seq) with next-generation sequencing (NGS) could be used to investigate the underlying mechanisms of inhibitor development.
Aims: Toevaluate peripheral blood transcriptomic profiles prior to and during early FVIII treatment in severe hemophilia A patients who did or did not develop inhibitors to simoctocog alfain the prospective NuProtect study.
Methods: The NuProtect study (NCT01712438; EudraCT 2012-002554-23) was a multicenter, multinational, open-label, non-controlled, phase III study that enrolled male PUPs with severe hemophilia A (FVIII:C <1%) of any age and ethnicity. Patients received simoctocog alfa for prophylaxis or on-demand treatment, as well as for surgical prophylaxis. RNA-Seq was performed on blood samples collected from baseline and every 3-4 exposure days (ED) up to 20 EDs or until inhibitor development to assess messenger RNA (mRNA) expression profiles. Bioinformatics analyses were performed for the following timepoints: baseline, early treatment and a later timepoint on treatment. Principal component analysis (PCA) was performed to identify differences in global mRNA expression profiles between inhibitor and non-inhibitor patients. Gene ontology (GO) analyses were performed to detect differences in GO clusters between inhibitor and non-inhibitor patients.
Results: Within this first analysis of the full dataset, transcriptomic profiles from 78 of 108 severe hemophilia A PUPs were analyzed (inhibitors n=22, non-inhibitors n=56). Median (range) age at ED1 was 13 (0.5-146) months; 44.9% of patients had the intron 22 inversion *F8* gene mutation. Although the PCA indicated no strong global signal for differential expression profiles between non-inhibitor and inhibitor patients, GO analyses demonstrated differences between these patient groups. Inhibitor patients demonstrated increased gene expression of metabolic processes (GO:0008152) and cellular processes (GO:0009987) at baseline. Upregulation of immune system processes (GO:0002376) was seen in inhibitor patients at the early timepoint compared with non-inhibitor patients. Expression of ribosomal proteins, including RPL17 and RPS8 were significantly upregulated at baseline in inhibitor patients ($p<0.05$). Inhibitor patients further demonstrated upregulation of proteins involved in adaptive immune responses: IGHV1-69-2 at all timepoints ($p<0.01$) and IGHV2-70D at baseline and the early timepoint ($p<0.01$) compared with non-inhibitor patients. Upregulation of the B-cell related factors DPP4 at baseline and the early timepoint ($p<0.05$) and CD20 at the late timepoint ($p<0.05$) was observed in inhibitor patients compared with non-inhibitor patients.
Conclusion: Differential transcriptomic profiles were seen in severe hemophilia A patients who developed inhibitors compared with those who do not, even before commencing FVIII treatment. Inhibitor patients demonstrated alterations in ribosomal protein expression that could disrupt protein synthesis efficiency, protein folding and quality control processes, and trigger cellular stress responses. Inhibitor patients also showed an upregulation of B-cell mediated factors involved in adaptive immune mechanisms. Additional analyses are ongoing to evaluate dynamic changes in gene expression during commencement of FVIII replacement to further identify patients at risk of inhibitor development.

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