mean liver volume remained stable at 0.9 MN (non-significant, n=63). In splenectomized-switch patients, mean hemoglobin remained stable: 13.5 to 13.4 g/dL (non-significant, n=36) mean platelet count increased from 295 to 319 x10^9/L (p=0.05, n=34) mean liver volume remained stable: 0.9 to 1 MN (non-significant, n=15). Mean chitotriosidase levels decreased in all groups, with significant reductions in treatment-naïve patients (1325 to 308 nmol/mL/hr, p=0.03, n=6) and non-splenectomized-switch patients (838 to 725 nmol/mL/hr p=0.02, n=86). These real-world results are consistent with those reported in eliglustat clinical trials.

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237
Generation of iPS cells derived from skin fibroblasts of patients with Fabry disease using RNA-reprogramming

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Fabry disease is a lysosomal metabolic disorder with X-linked inheritance caused by a deficiency of alpha-galactosidase which leads to accumulation of globotriaosylceramide (Gb3) in various organs. Clinical features of Fabry disease are renal, cardiac and CNS involvement. However, precise cellular pathogenesis in Fabry disease is still unknown. The iPS technology may be useful for the understanding of pathogenetic mechanism of lysosomal storage disease including Fabry disease. The generation of iPS cells generally have been carried out by the use of Sendai virus vector or episomal vector, but these technologies need the environmental attention and equipment for handling viruses. Therefore, we focused on RNA-reprogramming procedure. Skin fibroblasts from control and Fabry disease were transfected with reprogramming factors (Oct4, Sox2, Klf4, cMyc, Nanog, Lin28), interferon factors (B18, E3, K3), RNA and microRNA and formed iPS colonies were obtained after several cell passages and surveyed for morphological and biochemical studies. Using this method, no virus is used and feeder cells are not required, and also iPS cells can be efficiently obtained in a short period of time. Using this method, the iPS cells were successfully established from healthy human skin fibroblasts and skin fibroblasts derived from three Fabry male sibling patients in same family. Expression of surface markers and undifferentiated markers in these iPS cells showed positive, demonstrated by immunostaining and RT-PCR. Generated iPS cells from these Fabry patients were confirmed as iPS cells by various pluripotency markers. Our RNA reprograming procedure to generate iPS cells could be more widely used for understanding of pathogenetic mechanism of various lysosomal diseases including Fabry disease.

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238
Unexpected genetic findings in a Gaucher disease patient analysed by NGS-based panel sequencing

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A 40-year-old man with a history of mild idiopathic or immune thrombocytopenia since childhood was referred for consultation due to splenomegalia. He had mild normocytic anemia and thrombocytopenia, a markedly enlarged spleen (22 cm) with several focal lesions without lymphadenopathy, and typical Gaucher cells were observed in bone marrow smears. Analysis of genomic DNA by means of the Next-Generation Sequencing (NGS)-based Splenomegalia and Thrombocytopenia resequencing gene panel (STP version 1, 74 genes) and segregation analyses indicated that the patient was compound heterozygous for the known pathogenic mutations p.(Asn409Ser) (formerly N370S) and p.Leu483Pro (formerly L444P) at the GBA gene. Determination of beta-glucosidase activity indicated that only residual levels were present, confirming a diagnostic of Gaucher disease. However, further analysis of panel data revealed that the patient was also hemizygous for a pathogenic mutation, p.(Arg24*), at the G6PD gene on chromosome X. Quantification of glucose-6-phosphate dehydrogenase in blood confirmed reduced activity, well below normal levels. We did not find any evidence of previous episodes of acute anemia, even after exposure to quinine or Vicia faba. Our results indicate that, by simultaneously interrogating many genes involved in lysosomal storage diseases (LSDs) and their differential diagnoses, NGS panel sequencing helps identifying additional mutations that may modulate the phenotypic presentation and therefore suggest different therapeutic approaches. Funding: This work was supported by Sanofi Genzyme.

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239
CRISPR/Cas in iPSCs from sphingolipidoses patients

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Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) were found as an immune adaptive mechanism in bacteria and quickly were applied to various fields as a promising tool for gene editing. Lysosomal storage diseases (LSDs) are a group of metabolic disorders caused by defects in lysosomal proteins leading to accumulation of undigested macromolecules within the cells. The lack of good in vitro models hinders research of the pathophysiologic mechanisms and the development of new therapies. Induced pluripotent stem cells (iPSCs) are patient-specific and can be differentiated in any cell type. The advantage of iPSCs is to enable targeted studies in cells with the patient's own background leading to more straightforward results than other models. Combining CRISPR and iPSCs is, therefore, a promising strategy. We aim to use CRISPR/Cas-mediated gene editing to provide more specific cellular models of disease, to correct causal mutations in LSDs and to create mutants for functional studies. In this work, we generated and characterized iPSCs from human fibroblasts obtained from Gaucher and Fabry patients (through Gaslini Institute) and will edit them with a CRISP/Cas9 approach. Because both gene editing and iPSCs generation require manipulating the cell's genome, we envisage multiple check points along the workflow. It will be useful to compare the "native" mutated cells with the corrected cells that modulate the "disease in a dish". Gene editing is still recent and the methods require improvement, namely increasing transfection rates

and mutagenesis efficiency with less off-targets. Nevertheless, CRISPR/Cas is a promising alternative to other therapies, and every result contributes to the enhancement of this technology, broadening the validation of CRISPR application and making it an accessible option.

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240
Are we missing complex rearrangements by next generation diagnostic approaches: A case report of a complex rearrangement in MPS II

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Mucopolysaccharidosis type II (MPS II) is caused by deficiency of Iduronate-2-sulfatase (IDS) that leads to accumulation of undegraded glycosaminoglycans. So far, over 630 mutations have been described for the IDS gene in the Human Gene mutation database and only 2% of these are classified as complex rearrangements. Here, we present the molecular diagnosis odyssey of an MPS II patient. Non-consanguineos parents had 3 children, the first daughter died with 2.6 years with no confirmed diagnosis but dysmorphic features, the second was a stillbirth with facial dysmorphic features at birth and the third son (proband) died with 6.1 years. He started walking with 2 years of age and had severly impaired speech development limited to few words. He started developing seizures at 4 years of age. Clinical examination revealed: short stature, coarse facial features, neurological impairment, hepatosplenomegaly, proximal muscle weakness, joint rigidity, cardiopathy, dysostosis multiplex, macular degeneration, retinopathy, and hearing loss. The biochemical diagnosis was confirmed at 5.11 years by fluorimetry in dried blood spot. Next generation sequencing of the IDS by Illumina platform and MLPA analyses of the IDS gene did not reveal any clinically significant variant. Sanger sequencing followed by gel electrophoresis revealed a complex rearrangement within intron 3 and intron 7 of the IDS gene and the IDS2 pseudogene. This rearrangement have already been described in the literature. The patient died with 6.1 years of age due to pneumonia. In conclusion, we should be carefull when using solely next generation sequencing approaches for the molecular diagnosis of MPS II patients or MLPA taking into consideration the limitations of these methodologies for the identification of complex rearrangements. The delay in diagnosis of this patient contributed to his premature death due to the lack of treatment that could have helped to delay disease progression if started early.

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241
Accreditation: A challenge for a research laboratory

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A research laboratory focuses its efforts on the development of experiments bringing benefit to the entire scientific community. In fact, current dynamics have led results obtained by several research groups to be of diagnostic value, particularly for those working with low frequency diseases. This situation has allowed research laboratories to incorporate concepts such as quality, quality assurance, certification, and accreditation, among other quality-related concepts. Therefore, promotion and implementation of quality management systems (QMS) has become as opportune as necessary. The international quality standards under which a QMS can be implemented are based on ISO standards. One of them is ISO/IEC17025, which establishes the requirements that must be met by testing and calibration laboratories. This standard is based on ISO 9000 rules but incorporates technical requirements essential to achieve accreditation. The ISO/IEC17025 standard is applied with the objective of demonstrating that testing and calibration laboratories are technically competent and that their results are accurate. For the accreditation process (NTC-ISO/IEC17025:2005-standard) our laboratory selected 6 assays of lysosomal enzymatic activity performed in DBS. This process has been carried out in 2 stages: **1. Implementation: a.Diagnosis:** At initial evaluating laboratory status, it was found compliance in 20% of the standard **b.Planning:** Activities were determine to fill existing gaps **c.Design:** Strategies to comply with the requirements of the standard were defined: process map and 100% of documentary support **d.Implementation:** Disclosure and application of the policy/objectives/documentary support were carried out **e.Evaluation:** An internal audit concluded that the effectiveness of the implementation was 80%. After solving the nonconformities, we proceeded to stage **2. Application:** The application was made to the ONAC. **a.Documentary evaluation:** ONAC's evaluation team declared 95% compliance of documentary support. **b.On-site evaluation:** the conclusion was that efficiency of the QMS accomplished 96%.

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242
CRISPR/Cas9 mediated insertion of α-L-iduronidase (IDUA) and anti-PE receptor in B-lymphocytes for selective activation into long-lived plasma cells for sustainable IDUA expression

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Mucopolysaccharidosis type I is a rare inherited lysosomal storage disorder caused by mutations in the IDUA gene. IDUA deficiency results in the accumulation of glycosaminoglycans (GAGs), which ranges in clinical severity from Scheie syndrome (mild form) to Hurler syndrome (Severe form). At present enzyme replacement therapy (ERT) and bone marrow transplantation (BMT) are the standard of care for this disease[1] . In order to develop new approaches to treating this disease we exploit the unique characteristic that B cells differentiate into antibody producing long-lived plasma cell. We use CRISPR/Cas9 [2] mediated homologous directed repair (HDR) for site-specific insertion to co-express a therapeutic gene (IDUA) and phycoerythrin (PE). CRISPR/Cas9 system will create a double strand break at the endogenous B-cell receptor (BCR) of the B cell genome where homologous directed repair will facilitate the insertion of a therapeutic cassette under the expression of a synthetic promoter. A successfully engineered B cell will produce both IDUA and the PE receptor. A large quantity of IDUA will be secreted into the extracellular environment and the PE receptor will be transported to the cell membrane, which will allow us to