PRoF Award abstract – Call 2015

Rarefishmed- Discovering novel pathogenetic cascades in rare disorders

1. Research Outline

Acronym	Rarefishmed
Project name in English	Discovering novel pathogenic cascades in rare disorders
Pitch (1 sentence)	The project relies on the cross-translational use of a fish model and cell-cultured patient fibroblasts to identify novel molecular targets involved in lysosomal storage disorder pathogenesis.
Executive summary (max. 10 lines)	
The project concerns the identification of novel cellular pathways involved in the early onset of rare lysosomal storage disorders (LSDs), by means of combined *in vivo* and *in vitro* approaches. We will focus our investigation on two alternative LSDs, the Gaucher Disease (sphingolipidosis) and the Hunter syndrome (mucopolysaccharidosis), for which current therapeutic approaches suffer from limited efficacy. We will test zebrafish loss of function models for both diseases and compare the results with those obtained from cultured patient fibroblasts analysis. We will finally carry out a systematic screening of novel developed small molecules to assess the recovery of lysosomal function in both *in vitro* and *in vivo* models.	

2. Cause and context of the research

Lysosomal storage disorders (LSDs) represent a group of inborn metabolic disorders affecting almost 1 every 8000 people worldwide (Poupetova et al., 2010). These diseases are generally due to impaired lysosomal enzymatic function, which leads to the progressive lysosomal accumulation of undegraded substrates, ultimately severely affecting cell function and viability. In LSDs several organs are affected by lysosomal dysfunction and in most severe cases infant death may occur.

In recent decades a progressive effort to develop therapeutic interventions by both academic institutions and private pharmaceutical companies has led to the development of the so-called enzymatic replacement therapy (ERT), which relies on the administration of recombinant lysosomal enzymes in affected patients. However, despite encouraging results have prompted clinicians to apply ERT-based protocols in several affected patients, ERT remains of very limited efficacy for the treatment of some organs (heart, bone) or does not recover defects at the CNS level. Moreover, the lack of a complete pathogenetic cascade for most LSDs has hampered the development of alternative therapeutic strategies. In the past few years, researchers have started to finely dissect downstream molecular processes triggered by lysosomal dysfunction. In particular, the discovery that also neurodegenerative disorders (Alzheimer's disease, Parkinson and Lewy body diseases) are associated with aberrant lysosomal function has suggested to revise the paradigm of lysosomal function and LSDs (Appelqvist et al., 2013).

In fact, as several pathways terminate in the lysosome, lysosomal dysfunction may impact several cellular phenomena, including autophagy, vesicle trafficking, cell membrane turnover, and cell signaling transduction.

- Poupetová H1, Ledvinová J, Berná L, Dvoráková L, Kozich V, Elleder M. (2010) The birth prevalence of lysosomal storage disorders in the Czech Republic: comparison with data in different populations. J Inherit Metab Dis. 33(4):387-96
- Appelqvist H1, Wäster P, Kågedal K, Öllinger K.(2013). The lysosome: from waste bag to potential therapeutic target. J Mol Cell Biol. 2013 5 (4):214-26.

3. Innovation results achieved

We have recently demonstrated a novel proof of concept that a well-known lysosomal storage disorder, namely Gaucher disease, is tightly associated with a specific cell signaling impairment (Wnt signaling), which leads to reduced bone mineralization and osteoblast differentiation (Zancan et al., 2015). We have successfully applied a combination of *in vivo* and *in vitro* approaches based on zebrafish loss of function models for the enzyme glucocerebrosidase (GBA1) and concomitant analysis of fibroblasts from Gaucher patients. The zebrafish models enabled us to rapidly trace some of the molecular pathways affected at early life stages by GBA1 dysfunction. In particular, we sorted out that GBA1 impairment

triggers an early cellular oxidative stress and negatively affects the Wnt signaling pathway, which is relevant for the osteoblast differentiation program. *In vitro* analysis of human fibroblasts and sera from a cohort of selected Gaucher patients confirmed the *in vivo* observations. Our published results have been positively judged by the scientific community for the Gaucher disease, thus emphasizing the usefulness of identifying the primary cellular abnormalities triggered by lysosomal dysfunction. A second major achievement was the discovery that an *in vivo/in vitro* parallel approach can be successfully applied for investigations related to LSDs.

- Zancan I, Bellesso S, Costa R, Salvalaio M, Stroppiano M, Hammond C, Argenton F, Filocamo M, Moro E. (2015). Glucocerebrosidase deficiency in zebrafish affects primary bone ossification through increased oxidative stress and reduced Wnt/β-catenin signaling. Hum Mol Genet. 24(5):1280-94.

4. Link to the PRoF values

The innovative aspect of the project stands in the development of an intedisciplinary approach for the discovery of novel treatments for lysosomal storage disorders. The use of a Biobank, of a small-molecule based platform (at NIH) together with the support of a pharmaceutical company holds great promise for the succesful combination of a basic research and a clinical translation of the project. This perspective is in perfect agreement with the PRoF Chair Initiative that encourages the cooperation between the academic world and other healthcare institutions and healthcare stakeholders, such as pharmaceutical companies.

5. Applicable IPR rules

All participants have agreed to share the foreground of the project with the possibility of owning the intellectual property only in the case of novel patents development. It is, however, foreseeable that a potential joint ownership agreement will be established among partners during the project to clarify management issues such as the sharing of the costs arising from legal protection procedures and the exploitation of the jointly owned foreground.

6. Information on the partners

The project is carried out in collaboration with the pharmaceutical company Genzyme Sanofi, which is deeply involved in the development of novel breakthrough therapies for patients affected by lysosomal storage disorders. Genzyme-Sanofi is committed in both preclinical analysis, aimed at discovering novel drugs through the use of in vitro and in vivo-based assays, and in the clinical management of patients, by an interdisciplinary interaction between academic and healthcare institutions.

A second major partner is the Telethon Biobank Institution at the Gaslini Institute (Genova, Italy), held by Dr. Mirella Filocamo, which collects more than 9000 specimens (fibroblasts, lymphoblast transformed cell lines, amniocytes, trophoblast cells, DNAs, RNAs, serum) derived from patients affected by more than 200 different genetic defects (http://dppm.gaslini.org/biobank/). Among the various samples, 1110 come from patients affected by lysosomal diseases. Dr. Fillocamo is currently providing fibroblast cells, serum and RNA samples from Gaucher and Hunter syndrome patients.

We have started a very recent collaboration with Dr. Juan Marugan belonging to the National Center for Advancing Translational Sciences of the National Institutes of Health (NIH). Dr. Marugan is expert in developing and testing small molecules in high-throughput screening. He will provide us with a platform of small molecules to be tested in both *in vitro* and *in vivo* loss of function models for lysosomal function recovery.

Addendum: Contact information

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