Why were you inspired into a scientific career dedicated to helping sufferers of β-thalassaemia syndromes?

My scientific career began with studying the regulation of gene expression in eukaryotes, using the globin genes as a very important model system. One of the most important members of the erythroid gene family is represented by genes coding haemoglobin, the oxygen-binding protein in red blood cells. My interest in β-thalassaemia was therefore largely to be expected, since the study of the β-globin gene expression – which encodes haemoglobin subunit β – has a clear impact on the translation of basic science to therapeutic applications in this specific field. The later part of my scientific career has also been aimed at developing patents on small molecules of therapeutic interest for β-thalassaemia.

How many people suffer from β-thalassaemia conditions, and what are their experiences like?

It has been estimated that about 1.5 per cent of the global population (80-90 million people) are carriers of β-thalassaemia, with one aberrant β-globin allele. About 60,000 symptomatic individuals are born annually, the great majority of which live in the developing world.

Can you discuss your project 'THALAssemia MODular Stratification System for personalized therapy of beta-thalassaemia' (THALAMOSS), which aims to develop a universal set of techniques to divide β-thalassaemia patients into treatment subgroups?

The THALAMOSS project could have a huge impact on the lives of patients by pinpointing the modifiers that influence the severity of the disease and a patient’s response to a particular treatment. The transcriptomic and proteomic differences between patients might facilitate the prediction of response to therapy, allowing future personalised treatments. Several of the project’s areas of focus might provide novel clues for the development of approaches leading to improved treatments, too; for example, an increased production of foetal haemoglobin, novel gene therapy protocols using lentiviral β-globin vectors and gene editing approaches.

What have been the biggest challenges you have had to overcome during THALAMOSS, and how did you tackle them?

The biggest challenge we have is the reproduction of experiments involving treatments with foetal haemoglobin inducers and gene therapy vectors in different laboratories, using erythroid precursors from β-thalassaemia patients. We tackled
Modular medicine

**THALAMOSS** is an ambitious and innovative project aimed at developing stratifying biomarkers to place heterogeneous **β-thalassaemia** patients into subgroups across a range of therapies and biological and clinical parameters in order to help prescribe effective, individualised treatments.

**THALASSAEMIA IS A** severe hereditary blood disorder caused by an abnormal haemoglobin production that eventually leads to the destruction of red blood cells. The body attempts to compensate for this disorder by increasing the production of red blood cells, creating other complications in haematopoietic organs, such as the bone marrow.

Typically, thalassaemia is treated by regular blood transfusions, which alleviates the strain on the blood-producing organs. However, it presents an important challenge: frequent blood transfusions cause iron to flood the body, leading to iron accumulations that can reach toxic levels if left untreated.

Fortunately, drugs exist that can remove iron by chelation therapy - a chemical process in which solutions containing chelating agents are administered to patients in order to remove heavy metals and minerals. However, these medications also present challenges. They often have many side effects or must be administered frequently, limiting the patient's quality of life. Treatments that would enable an individual to become independent of blood transfusions are therefore the primary aim of thalassaemia research.

**B-THALASSAEMIA**

Individuals with mutations in their haemoglobin β subunit (β-globin) gene are described as having β-thalassaeemia. Despite the condition itself involving only a single gene, β-thalassaemia patients present a wide range of disease severities and responses to treatment. This is due to the fact that there are more than 300 different known mutations in the β-globin gene, in addition to a small number of other modifiers that have also been identified. Annoyingly, most remain elusive with few conclusive links between genotype and phenotype aside from the primary β-thalassaemia mutations.

As such, there is a major need for more research into the disease modifiers, as a bank of such information would enable medical professionals to reliably classify patients into subcategories and thus provide them with a treatment optimised to their particular needs.

**INTRODUCING THALAMOSS**

The potential for individualised medicine in β-thalassaemia is now under intense investigation, thanks to Professor Roberto Gambi, Ferrara University, Italy. He is the coordinator of the innovative international project ‘THALAssaemia MOdular Stratification System for personalized therapy of beta-thalassemia’ (THALAMOSS), which received substantial funding from the European Commission’s Seventh Framework Programme (FP7) for Research and Technological Development in 2012 to develop markers and techniques for the stratification of β-thalassaemia patients into treatment subgroups.

The four major stratification categories for THALAMOSS are the frequency of blood transfusions, choice of iron chelation medication, induction of endogenous foetal haemoglobin as an alternative to adult haemoglobin and efficacy of gene therapy interventions. The team working on the project strongly believes that integrating these modular therapeutic categories would allow a guided personalised treatment plan to be developed in the future for each patient.

As part of its key milestones, the THALAMOSS project - which comprises 13 participating institutions from across Europe and the US - will analyse blood samples taken from more than 500 patients and perform functional genomic and proteomic analyses to classify patients into subdivisions of thalassaemic mutations and known modifiers. Current treatment regimens will be incorporated into the data, along with information on disease progression. From here the researchers will cluster patients into specific needs, such as transfusion requirements and their foetal haemoglobin inducibility profile, using next-generation sequencing of the different groups to identify patterns of single nucleotide polymorphisms (SNPs) that represent that

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**How would you like to see this project advance in the future?**

Many patients who require frequent blood transfusions develop a dependency on these transfusions in order to survive, which has a significant impact on their quality of life and is associated with substantial clinical and economic consequences. I would like to see my personal research efforts and the THALAMOSS project contribute to society by creating key products and specific protocols targeted towards the final goal of enabling β-thalassaemia patients to become fully or partially independent from blood transfusion.

A second objective is that we hope to investigate the possibility of developing early or noninvasive prenatal diagnostic tools to predict the real clinical status of patients and allow them to start personalised therapy as soon as possible.
THALAMOSS

OBJECTIVE
To develop a universal set of markers and techniques for stratification of β-thalassaemia patients into treatment subgroups for onset and frequency of blood transfusions, choice of iron chelation, induction of foetal haemoglobin and prospective efficacy of gene-therapy.

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ROBERTO GAMBARI has been full Professor of Biochemistry at University of Ferrara since 2001. He was chairman of its PhD course in Biotechnology, and he was the Course Coordinator for the University’s degree in Pharmacy. He is Director of the University’s Biotechnology Center and of the Department of Life Sciences and Biotechnology. He is a member of the Scientific Committee of the Interuniversity Consortium for Biotechnologies. He was coordinator of several research projects, including Target Project Genetic Engineering (1992-1995), ISS-AIDS (1992-1993), Telethon, Target Project ACRO (1992-1995), Fondazione Italiana per la Guarigione della Thalassemia, AIRC (1992-1994, 2003), and currently the FP7 project, THALAMOSS. He has published more than 400 papers and is the co-author of more than 12 patents.

particular set. This enables the stratification of patients by these genetic markers.

One of the key factors of THALAMOSS’s importance is that the sample size is much larger than anything that has been attempted before, in order to capture the wide range of possible genotypes and modifiers and allow a meaningful categorisation of patients. The sample acquisition and characterisation will use a standardised protocol across four independent medical centres with dual testing of samples to control for variability across locations. Cutting-edge technology will be employed to mine the huge and unique dataset for biologically and medically relevant patterns that will reliably predict a specific patient’s subcategory, treatment response and the future progression of the disease.

TESTING PROMISING TREATMENTS
There are several promising new β-thalassaemia treatments whose development has been hindered by the variability of patient response. As such, a key component of the THALAMOSS project is to elucidate the modifiers that affect an individual’s response to these treatments to highlight how they can be more effectively used in the future.

The THALAMOSS project will have a huge impact on the design and planning of individualised medicine

This can be best shown through the example of foetal haemoglobin. Foetal haemoglobin is produced by a developing foetus from seven months before it is born until it is around six months old. Its production sometimes persists in thalassaemic patients and is usually associated with milder forms of the disease. Sickle cell anaemia – another haemoglobin condition – is known to be ameliorated by drugs that induce foetal haemoglobin production. These therapeutics also show great promise as a therapy for β-thalassaemia; however, the response of different patients varies significantly and the drugs show limited efficiency.

The development of more effective foetal haemoglobin inducers has been hampered by a lack of biomarkers to predict patient response, which is something the THALAMOSS team is attempting to tackle. Coordinator Gambari explains: “We recently succeeded in finding a novel foetal haemoglobin-related polymorphism, as well as characterising several foetal haemoglobin inducers exhibiting efficiency comparable or even higher than hydroxyurea, the most commonly used inducer used in clinic. Some of the identified molecules – for instance, Sirolimus – are already employed for the treatment of other pathologies, allowing repurposing for use in β-thalassaemia and applications for orphan drug designation”.

Stop-codon mutations of β-globin are one of the causes of β-thalassaemia; however, treatment with aminoglycosides allows the ribosomes to read past these nonsense mutations, translating a proportion of the affected mRNAs into functional protein. Another aim of THALAMOSS is to assess the efficacy of aminoglycoside treatment in combination with the foetal haemoglobin inducer hydroxyurea to different patient subpopulations, which could be a useful dual therapeutic strategy.

Gene therapy has shown a lot of promise for curing haemoglobin genetic defects in erythroid precursor cells. Genes can be inserted into cells, modified or deleted using vectors derived from lentivirus, enabling researchers to restore haemoglobin production and significantly improve the symptoms of thalassaemia.

Mouse models. The THALAMOSS project has successfully developed and characterised effective gene therapy vectors that are presently under investigation. The project’s members are also looking at them as a combined therapy with known HbF-inducers. Gene editing is also under investigation by THALAMOSS with the goal to correct the defective gene.

TRANSFUSION INDEPENDENT
With a condition as variable as β-thalassaemia, the breadth of information the THALAMOSS project is in the process of producing will have a huge impact on the design and planning of individualised medicine for optimum disease management. “One of our biggest achievements is the generation of datasets allowing the comparison of clinical, genetic, biochemical and molecular parameters in order to stratify patients and contribute to the road of personalised therapy,” Gambari elaborates. “Additionally, the establishment of biobanks of erythroid precursor cells from patients will deeply facilitate future testing of novel therapeutics such as foetal haemoglobin inducers and new gene therapy vectors.”

With one year remaining on the project’s lifespan, THALAMOSS is expected to contribute to the treatment of thalassaemia, with the major objective of enabling patients to live independently of transfusions with the possibility of a permanent cure.