Executive summaries

D1.1. First List of recruited β-thal patients with characterized genotype/phenotype. Deliverable D1.1 is related to Tasks 1.1 and 1.2 and reports the first THALAMOSS list of recruited patients for future activities employing blood sampling, culturing of erythroid cells and isolation of genomic DNA, RNA and protein. D1.1 contains the list of patients for which the genotype/phenotype was already known or has been determined under the THALAMOSS project. This list includes 107 entries contributed by UNIFE, 680 entries by CING, 22 entries by CU, 100 entries by UNICA, 11 entries by KCL, 173 entries by LGHA and 47 entries by BIOCEP (total 1140 entries). The analysis of the most frequent genotypes and phenotypes allows us to conclude that homozygous patients are 138 $\beta^0$39/$\beta^0$39, 364 β^+IVSI-110/β^+IVSI-110 and 36 β^+IVSI-6/β^+IVSI-6. Double heterozygous patients are 55 $\beta^0$39/β^+IVSI-110, 146 β^+IVSI-6/ β^+IVSI-110 and 65 β^0IVSI-1/β^+IVSI-110. In The list also includes 29 homozygous sickle-cell anemia (SCA, HbS/HbS) patients. The HbS genotype was associated in 24 cases with $\beta^0$39, in 49 cases with β^+IVSI-110, in 9 cases with β^+IVSI-6 and in 12 cases with β^0IVSI-1.

D1.3. Protocol A for ErPC isolation and culture. D1.3 is related to TASK 1.3 of WP1. Three strategies will be used by the THALAMOSS network for in vitro production of erythroid cells. The steps common to all three strategies are: Drawing of peripheral blood (PB, 20–40 ml), Ficoll separation of mononuclear cells and washing. In Protocol A, the mononuclear cells are cultured according to the two-stage procedure first published by the Fibach's group [Blood, Blood, 73:100-103,1989]. Briefly, peripheral blood mononuclear cells are seeded in phase I culture containing alpha minimal essential medium, serum, antibiotics, stem cell factor and cyclosporine, where ErPCs are expanded, and then in erythropoietin-containing phase II, where cells proliferate and differentiate. Protocol A was validated in respect to the major objectives of the THALAMOSS project demonstrating its usefulness (a) in determining different levels of HbF in ErPC from different patients with different genotype (this finding is of interest for WP2) and (b) in determining changes of gene expression levels following treatment with HbF inducers (this finding is of interest for WP3).

D1.4. Protocol B for ErPC isolation and culture. Instruments for the application of automated protocols for the isolation of human hematopoietic stem cells are expected to help in minimizing the variability associated with researcher's activity. In THALAMOSS Protocol B, the isolation of CD34+ cells is performed using the BIOCEP advanced platform for cell separation and its patented Cell Enrichment Process (CEP), yielding larger, purer and less damaged cell populations in comparison with existing technologies; this protocol will allow faster separation of high numbers of CD34+ cells to be used for treatment according to the experiments proposed under WP3. To test this technology, a human CD34+ cell line (KG1-a) was employed. This cell line, which was established in vitro from a patient with myeloid leukemia, was found to carry surface CD34 antigen. To simulate the clinical situation, known numbers of KG1-a cells were added to 10 ml of peripheral blood obtained from normal donors (spiking). Mononuclear cells of these mixtures were purified by Ficoll separation. The cells were then labeled with magnetic beads conjugated with anti-CD34 antibodies. Following incubation, the cells were washed, resuspended in phosphate buffer saline (PBS) and run through the CEP separator. In our experiments with KG1-a cells/blood mixtures, the negative and positive fractions were collected (separately) and KG1-a cells were enumerated microscopically (using a hemocytometer) based on

their unique size and morphology (even when unstained) compared to RBC and WBC. Eventually, purified KG1-a cells were enumerated by flow cytometry of specific antigens. The results demonstrate high level of enrichment of CD34+ cells using the BIOCEP apparatus. These results were confirmed for separation of human ErPC from blood.

D1.5. Protocol C for ErPC isolation and Storage. A protocol (THALAMOSS Protocol C) for efficient culturing, expansion, storage (frozen), subculturing, transduction with therapeutic vectors, differentiation with HbF inducers is provided here. Using this protocol, sample collection and distribution will be coordinated by UNIFE and will take place in at least three additional independent centres (CU, CING and BIOCEP). Dry-ice shipments will be performed for centralized wide-genomics, transcriptomics and proteomics analysis performed under WP2. Protocol C is based on CD34 cell separation using immunomagnetic beads, plating and expanding at low density in StemSpan medium supplemented with StemSpan CC-100 cytokine cocktail, erythropoietin, dexamethasone and penicillin/streptomycin. This protocol is expected to allow freezing of cells from β-thalassemia patients to generate BioBanks.

D1.6. First β-thal cellular BioBank. The first release of the THALAMOSS β-thal BioBank has been completed and validated. At present (June 2014, 18 months of the Project), the BioBank is located at University of Ferrara, Department of Life Sciences and Biotechnology, and consists of a total of 434 vials, comprising cryo-preserved ErPC from 57 patients. The most common genotypes are $\beta^0$39/$\beta^0$39 (19 patients, 142 vials), $\beta^0$39/β^+IVSI-110 (13 patients, 109 vials) and β^+IVSI-110/β^+IVSI-110 (7 patients, 56 vials).

D2.1. Sequencing completed of at least 50 genomic samples. Genomic DNA samples from 52 β-thalassemia patients have been used for identification of β-globin gene mutations, polymorphisms of the β-globin genes, the XmnI polymorphism of the promoter of the fetal γ-globin gene, and polymorphisms of the BCL11A and HBS1L-MYB loci, which are disease modifiers through their role in fetal γ–globin xpression.

D2.7. Report of cell culture standardized. A standardized protocol for the cultures of primary erythroid precursor cells (ErPCs) is critical to avoid centre-to-centre differences leading to differences of results obtained in studies focusing of the transcriptome and proteome. Cell culture and subsequent HbA and HbF quantification show that Cell Culture Protocol C produced quite similar results when implemented at different laboratories. Methods for characterizing the differentiation state of the ErPC cultures are reported.

D2.8. Functional analysis standardized. Standardized protocols for functional assays are critical to avoid centre-to-centre differences, which might create problems in the analysis of results obtained not only in studies based on transcriptomic and proteomic analyses (WP2), but also in studies focusing on the screening and characterization of novel inducers of fetal hemoglobin and of gene-therapy interventions. The standardization of functional analyses includes the isolation of biomaterials for central analyses as well as the common local use of RT-qPCR for mRNA quantification, HPLC for hemoglobin determination and the lentiviral transduction procedure itself as a prerequisite for the analysis of gene therapy.

D2.10. Globin immunization completed. A number of heavy chain-only antibodies have been isolated using peptide immunisations in the proprietary Harbour Antibodies mice. This resulted in the isolation of a number of HCAb specific for the beta globin chain and

some candidate antibodies that also recognize sickle cell beta globin. New immunisations are in progress to obtain a larger panel of antibodies for the development of a dip-stick based diagnostic assay.

D4.1. Analysis of Requirements on Data Management. The goal of this WP4 Deliverable is to provide an analysis on data management in the THALAMOSS project which is required within Task 4.1. We focus on the initial stage which encompasses data acquisition by the partner medical facilities, data de-identification, export from facilities of origin to a central database and distributed data presentation for later research on β-thalassemia. This document describes in the necessary detail all assignments posed by the Task 4.1 and is primarily intended for all THALAMOSS project partners and communities associated with partner hospitals or medical facilities. The following issues have been addressed: (a) External and Internal Requirements, (b) Patient Privacy; (c) Collected Data Structure; (d) Data-collecting Application.

D4.2. THALAMOSS Data Management Platform. This Deliverable extends the previous document, D4.1, which provided an analysis of the requirements on THALAMOSS data model and a proof-of-concept implementation of the data gathering application. Firstly, we proceed to create a fully functional and ready-to-use gathering application. Based on the THALAMOSS workflow, the next step is to centralize the obtained data into one major database and provide a suitable interface for data presentation and distribution. The following issues have been addressed in D4.2: (a) State of the project; (b) Goals in the current stage; (c) Data gathering and centralization; (d) Data presentation.

D5.1. Set-up of the project web-site. The set-up and maintenance of the project's web-site constitutes the main point of collection of the project information, including public deliverables, summary of major scientific achievements, and advertisement of dissemination and training activities. It is constructed to facilitate collaborative exchange and technology transfer between participating laboratories, to provide high-level training and to promote a dialogue with the wider scientific and lay communities across Europe and worldwide on thalassemia and related societal-ethical issues. Maintenance and incremental updates take place on a weekly base, major revisions and restructuring occur monthly or/and every six months. A link to a private, password-protected section of the web-site is reserved for communication internal to the THALAMOSS Consortium, and it is used for communication with EU and the Project Reviewers.

D5.8. Industrial plan for the use and dissemination of the foreground, explaining how knowledge and IP issues will be managed within the consortium between research institutions and industrial partners. The THALAMOSS consortium is planning exploitation of the project results. The exploitation strategy includes the following main points: (a) development of a detailed exploitation plan for the use of the knowledge produced in the frame of the THALAMOSS project; (b) protection of the knowledge produced in the frame of the THALAMOSS project; (c) assessment of the expected socio-economic impact of the knowledge and technology generated in the frame of the THALAMOSS project; (d) carrying out take-up activities to promote the early or broad applications of the state of the art technologies and protocols developed under THALAMOSS. The current deliverable report explains how knowledge and IP issues will be managed within the consortium, between research institutions and industrial partners. The current deliverable report provides an overview of the THALAMOSS exploitation plan and strategy at midterm in the project. This deliverable is linked to deliverables 5.10, 5.11 (to be delivered at month 24) and 5.12 and 5.13 (to be delivered at month 48).

D6.1. Report on management of biological samples from patients. D6.1 deals with the management of biological samples in accordance with the rules outlined in Annex 1 of the GA. All human samples to be processed will remain anonymous. The Helsinki rules will be followed. THALAMOSS partners will also conform to current legislation and regulations in the countries where the research will be carried out. They must seek the approval of the relevant ethics committees prior to the start of the RTD activities that raise ethical issues. D6.1 enlists the EU legislation to which THALAMOSS partners will conform. THALAMOSS members will respect the following international conventions and declarations: (a) Helsinki Declaration in its latest version; (b) Convention of the Council of Europe on Human Rights and Biomedicine signed in Oviedo on April 4, 1997, and the Additional Protocol on the Prohibition of Cloning Human Beings signed in Paris on 12 January 1998; (c) UN Convention on the Rights of the Child; (d) Universal Declaration on the human genome and human rights adopted by UNESCO. The last part of D6.1 describes the management of biological samples regulated by a Material Transfer Agreement (MTA) between partners. The MTA contains management indications concerning the supply of materials, the access to and use of the materials, Finally, D6.1 briefly indicates the strategy for dissemination and Intellectual Property (IP) protection.

D6.2. Report on management of databases on genomic DNA, RNA and proteomic profile. D6.2 is related to TASK 6.1 of WP6 and deals with the management of the results and databases obtained in studies performed with genomic, transcriptomic and proteomic (in brief: OMICS) tools. This activity is covered in the THALAMOSS Project in Task 1.2 of WP1 and, more importantly, in Tasks 2.1, 2.2 and 2.3 of WP2 (Omics analyses). The relevance of these Tasks is due to the fact that novel biomarkers might be identified on the basis of the results obtained. D6.2 describes the flowchart of the collaborative efforts between partners for genomic DNA analyses, the list of partners expected to prepare genomic DNA samples and frozen cellular pellets for transcriptomic and proteomics. Moreover D6.2 identifies the partners involved in the analysis of common polymorphisms associated with high HbF production, in full genomics analysis, in transcriptomic and proteomic analyses. Finally, D6.2 describes how the consortium will deal with the results and databases generated by the OMICS research activity.

D6.3. Report on management of results deriving from experiments on HbF induction. Management of results adheres to the THALAMOSS consortium agreement, in line with the DESCA 3.0 Model Consortium Agreement for FP7, and comprises dissemination and IP protection towards the commercial exploitation of results. The present document summarises the types of results covered, lays out details for the target audience and tools for dissemination, while the pertaining IP protection for any foreground generated by the THALAMOSS project is covered in deliverables D7.6 to D7.8. The present deliverable covers the foreground created by Tasks 3.1 and 3.2. Both tasks are geared towards the stratification of patient groups for the effectiveness of future treatments with HbF inducers and, specifically for Task 3.2, read-through agents. To this end, the consortium has agreed to universally investigate hydroxyurea (HU) as a generally accepted, albeit clinically suboptimal standard, for HbF induction, while additionally investigating further compounds of different chemical classes with potential for future clinical application. The results expected from these investigations are thus (a) novel insights into responsiveness of different clinically and molecularly stratified patients groups to HU and other chemicals and (b) the identification of novel chemicals or the re-employment of known chemicals as HbF inducers that might be effective and save in their application to specific

patient subgroups. Towards management of results, output (a) is suitable for dissemination via the ITHANET web portal and its associated interactive tools as well as through peer-reviewed publications and numerous other dissemination tools, while output (b) is suitable for IP protection and commercial exploitation, coordinated with subsequent dissemination.

D6.4. Report on management of results based on the employment of GT technology. The present document summarises the types of results covered by the research involving Gene Therapy, and lays out details for the target audience and tools for dissemination, while the pertaining IP protection for any foreground generated by the THALAMOSS project is covered in deliverables D7.6 to D7.8. The present deliverable covers the foreground created by Tasks 3.3, based on the stratification of patient groups for the effectiveness of Gene Therapy. The results expected from these investigations are thus (a) novel insights into responsiveness of different clinically and molecularly stratified patients groups to Gene Therapy and (b) the identification of novel lentiviral probes effective and save in their application. The management of the results obtained and the dissemination strategies are presented.

D6.5. Report on regulatory issues associated with cellular THALAMOSS BioBanks. In this deliverable the ethic issues raised by the development of the THALAMOSS Biobanks are presented together with the management of the correlated activities. D6.5 identify the partners involved in the generation of the THALAMOSS Biobanks, the end-users, the general features and the management of the Biobanks, and the interaction with other EU Projects active on this field of applied research.

D6.6. Collection of the approvals from the Bioethic Committees on the use of biological materials from thalassaemic patients. The WP6 deliverable D6.6 reports the overall activity of the THALAMOSS Consortium with respect to the submission to the Bioethic Committees of the THALAMOSS Project for approvals. The official approvals provided by UNIFE, KCL and LGHA are provided. Approvals of activities in common with the THALAMOSS plan of work are also presented for all the other partners involved in collaboration with Hospital Centres.

D6.7. First THALAMOSS report of gender actions. In this first report of the Gender Action Plan for the THALAMOSS project (D6.7), we have established a first estimation of the gender equality among partners and at all levels of the project. We report here the commitment to gender equality at the institutional level among the partners. In the first period (18 months) 8 women have been recruited in a total of 12 recruited researchers. Based on the information obtained, we will explore how progress can be monitored during the project and propose some activities to promote the gender action before giving a brief conclusion.

D7.1. First Periodic Report on Progress, Use of Resources and Financial Statement. This deliverable contains the Core Report of THALAMOSS Project, including a section on Project objectives for the period, a section on Work progress and achievements during the Period and a section on Project management during the period.

D7.5. Guidelines on information exchange of pre-existing know-how. Guidelines for the management of Pre-existing Know-How (PECK) is important in the THALAMOSS network for several reasons, the most important of which is related to the fact that several partners with different background (molecular biologists, geneticians, informaticians, clinicians) are partecipating. In addition four partners from the industrial world (HA,

BIOCEP, IRBM and NOVAMECHANICS) are present in the Consortium. This D7.5 deliverable is associated with D7.6 and D7.8 and reports how the Consortium is going to manage PECK and IP issues.

D7.6. IPR Management Database: Pre-Existing Know-How. This deliverable D7.6 is associated with deliverables D7.5 and D7.7. In D7.6 the Pre- existing Know How (PEKH) to be included and to be excluded are listed for all the beneficiaries (parties). In general most of the partners agrees to disclose only the Pre-existing Know-How previously listed for the Project and to exclude all the other Pre-existing Know-How of its participating institute or organization.

D7.7. IPR Management Database: First Release. The technology and products to be developed in the THALAMOSS project are planned to be exploited in different ways by the various partners, mainly in the fields of discovery of novel therapeutic molecules for thalassemia and in-vitro diagnostics. This D7.7 deliverables presents the first analysis of the possible exploitable research within THALAMOSS.