BIOETHICS AND FUNDAMENTAL PATIENT'S RIGHTS IN THERAPY AND MOLECULAR DIAGNOSIS OF RARE DISEASES: THALASSEMIAS

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Introduction.

Rare diseases are human pathologies which are not frequent in the human population (1/2000)[1]. For this reason, these diseases should be considered "orphan" of the interest of big pharmaceutical industries, which are not prone to sustain research and clinical development. On the contrary, high interest of industry is concentrated on few classes of pathologies with very high number of patients, such as cardiovascular diseases, cancer and infectious diseases[2]. Interventions for these disease is higher that 70% of the all R&D activity of the overall industries[3].This issue is relevant and might prevent development of cures for rare diseases; accordingly, several actions have been recently undertaken stimulating the research and drug development in this applied field. For instance EURORDIS, the European Organisation for Rare Diseases, launched the EURODIS Charter for Collaboration between Sponsors and Patient Organisations for Clinical Trials in Rare Diseases, outlining a number of principles to which sponsors may publicly state their acceptance. EURODIS will then support such sponsors in identifying European patient organisations to co-operate in clinical trials[4]. In this context, patient organizations may faithfully collaborate with sponsors in all phases and on several aspects of clinical trials including the

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[1] A. FISCHER, P. BORENSZTEIN, C. ROUSSEL, *The European Rare Diseases Therapeutic Initiative*, in *PLoS Med*, 2, 2005, e233.

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[3] I. HUDSON, A. BRECKENRIDGE, *The challenges of orphan drugs and orphan diseases: real and immagined*, in *Clin Pharmacol Ther*, 92, 2012, 152-3.

[4] http://www.eurordis.org/about-rare-diseases

following: (a) adapting the design of the study to patients' expectations facilitates their adhesion to the trial; (b) providing early information to potential participants ensures and speeds up their inclusion in the trial; (c) supporting patients during the study reduces number of drop-outs and incomplete files; (d) taking quality of life into consideration and discussing trial results with sponsors contribute to the assessment of clinical and day-to-day benefits of the treatment[5].

The issue of rare diseases is very important also in consideration of the fact that frequently the clinical symptoms are in any case important and therapy is not curative. In most instances, large scale screening, prenatal diagnosis and abortion of affected fetuses are strategies which are preferred to large-scale funding of research focusing on development of novel therapeutic approaches[5].

A Rare Disease: Thalassemia.

Beta-thalassemias are a group of hereditary hematological diseases caused by more than 200 mutations of the human β-globin gene, leading to low or absent production of adult β-globin and excess of α-globin content in erythroid cells, and causing ineffective erythropoiesis and low or absent production of adult hemoglobin (HbA) [6,7,8]. Together with sickle cell anemia (SCA), thalassemia syndromes are the most important problems in developing countries, in which the lack of genetic counseling and prenatal diagnosis have contributed to the maintenance of a very high frequency of these genetic diseases in the population[9]. This contributes significantly to drive changes in the distribution of carriers and affected people in relation to the migration of populations from endemic areas to countries where their prevalence in indigenous populations had been extremely low (USA, Canada, Australia, South America, the United Kingdom, France, Germany,

[5] A. ELEFTHERIOU, *Patients' Rights*, Thalassemia International Federation, 2007.

[6] A.N. SCHECHTER, *Hemoglobin research and the origins of molecular medicine*, in *Blood*, 112, 2008, 3927-38.

[7] D.R. HIGGS, J.D. ENGEL, G. STAMATOYANNOPOULOS, *Thalassaemia*, in *Lancet*, 379, 2012, 373-83.

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[9] D.T. JAMISON, J.G. BREMAN, A.R. MEASHAM, G. ALLEYNE, M. CLAESON, D.B. EVANS, P. JHA, A. MILLS, P. MUSGROVE, *Disease control priorities in developing countries*, Washington (DC), 2006. Second Edition.

Belgium, the Netherlands and, more recently, Scandinavia). These deep changes have encouraged most of the health systems of these countries in facilitating access to the prevention and treatment services available for these hemoglobin disorders. On the other hand, considering limitations and side effects of the currently available therapeutic approaches and management of the thalassemia patients, novel alternative options for therapy are urgently needed[10,11].

As far as relevant numbers concerning these pathologies, 80-90,000,000 carriers (thalassemias and SCA) are present worldwide and 300,000-500,000 children are born each year with severe homozygous states of these diseases (100,000 die early in the post-natal life). In Italy 2,500,000 carriers are present, 4,250 patients affected by thalassemia major, 1,600 by thalassemia intermedia and 600 by SCA[7,8,10,11,12].

The Results of the Human Genomic Project (HGP), Personalized Therapy and Patient's Rights.

The completion of the International Human Genomic Project has a major impact on issues of human and patient rights[13]. It is well accepted that one of the major issue is the equitable access to genetic services. Thalassemia is present in countries exhibiting highly different levels of healthcare. From the "Charter of Fundamental Rights" (Nice, 7 December 2000)[14], article 35 states: "Everyone has the right to access to preventive health and the right to benefit from medical treatment under the conditions established by national laws and practices". These issues are of great relevance, also considering the WHO's global health agenda 2006-2015 ("Engaging for Health")[15], identifying the following priority areas: (a) investing in health to reduce poverty; (b) building

[10] R. GAMBARI, *Alternative options for DNA-based experimental therapy of β-thalassemia*, in *Expert Opin Biol Ther*, 12, 2012, 443-62.

[11] R. GAMBARI, *Foetal haemoglobin inducers and thalassaemia: novel achievements*, in *Blood Transfus*, 8, 2010, 5-7.

[12] R. COLAH, A. GORAKSHAKAR, A. NADKARNI, *Global burden, distribution and prevention of β-thalassemias and hemoglobin E disorders*, in *Expert Rev Hematol*, 3, 2010, 103-17.

[13] http://www.ornl.gov/sci/techresources/Human_Genome/home.shtml

[14] http://www.europarl.europa.eu/charter/pdf/text_en.pdf

[15] http://www.euro.who.int/en/who-we-are/technical-programmes-in-the-european-region/engaging-for-health-eleventh-general-programme-of-work-2006-2015,-a-global-health-agenda

individual and global health security; (c) promoting universal coverage, greater equality and health-related human rights; (d) strengthening health systems and equitable access; (e) harmonizing knowledge, science and technology; (f) strengthening governments, leadership and accountability[5]. Among these WHO priorities, the strength of the health systems and equitable access appears to be very important for thalassemia, especially in consideration of the world-wide distribution of the disease and on the fact that it is present in both industrialized and developing countries, with very different approaches to the management of the patients[5].

The EU's Charter of Fundamental Rights

In 2000, the European Union Member States adopted the 'Charter of Fundamental Rights', signed in Nice on 7 December, setting out the common values of EU Member States, and drawing together rights previously laid down in national laws and international conventions, including those of the Council of Europe, the United Nations and the International Labour Organisation[13]. This Charter of Fundamental Rights contains many provisions that refer either directly or indirectly to patients' rights. Examples include the inviolability of human dignity (Article 1) and the right to life (Article 2); the right to the integrity of the person (Article 3); the right to security (Article 6); the right to the protection of personal data (Article 8); the right to non-discrimination (Article 21); the right to cultural, religious and linguistic diversity (Article 22); the rights of the child (Article 24); the rights of the elderly (Article 25); the right to fair and just working conditions (Article 31); the right to social security and social assistance (Article 34); the right to environmental protection (Article 37); the right to consumer protection (Article 38); and the right to freedom of movement and residence (Article 45) [5]. The issue of patients' rights was finally given full and individual attention with the adoption of the European Charter of Patients' Rights in November 2002[16]. While defining and illustrating the rights specified, the Charter offers guiding principles rather than a handbook for all possible eventualities. The

[16] http://www.activecitizenship.net/patients-rights/projects/29-european-charter-of-patients-rights.html

following patients' rights have been included: (a) Right to Preventative Medicine (every individual has the right to a proper service in order to prevent illness); (b) Right of Access (every individual has the right to access to the health service that his or her health needs require and health services must guarantee equal access to everyone, without discrimination on the basis of financial resources, place of residence, kind of illness or time of access to services); (c) Right of Information (every individual has the right of access to all forms of information regarding their individual health, the health services and how to use them, and all, that scientific research and technological innovation makes available); (d) Right to Consent (each individual has the right of access to all information that might enable them to actively participate in decisions regarding their health); (e) Right to Free Choice (each individual has the right to freely choose from among different treatment procedures and providers on the basis of adequate information); (f) Right to Privacy and Confidentiality (every individual has the right to the confidentiality of personal information, including information regarding their state of health and potential diagnostic or therapeutic procedures, and the right to privacy during diagnostic exams, specialist visits and medical/surgical treatment in general); (g) Right to Respect Patients' Time (each individual has the right to receive necessary treatment within a swift and predetermined period of time, at each stage of treatment); (h) Right to the Observance of Quality Standards (each individual has the right of access to high quality health services, as specified by standard-setting authorities); (i) Right to Safety (each individual has the right to be free from harm caused by the poor functioning of health services, medical malpractice and errors, and the right of access to health services and treatments that meet high safety standards); (l) Right to Innovation (each individual has the right of access to innovative procedures, including diagnostic procedures, according to international standards and independently of economic or financial considerations); (m) Right to Avoid Unnecessary Suffering and Pain (each individual has the right to secure, within the healthcare services, avoidance of as much suffering and pain as possible, in each phase of his or her illness); (n) Right to Personalised Treatment (each individual has the right to diagnostic or therapeutic programmes tailored as much as possible to his or her personal needs); (o) Right to Complain (each individual has the right to complain whenever he or she has suffered harm, and the right to receive a timely response or other

feedback); (p) Right to Compensation (each individual has the right to receive appropriate compensation within a reasonably short period of time whenever he or she has suffered physical, moral or psychological harm caused by a health service treatment) [4,16].

With respect to the major issues of the management of thalassemia, the following Patients' Right appear to be highly relevant: Right to Preventive Medicine, Right to Access, Right to Innovation, Right to Personalized Treatment.

The European Charter of Patients' Rights: Right of Preventive Medicine.

The European Charter of Patients' Rights states that every individual has the right to a proper service in order to prevent illness. With respect to thalassemia, this is a very important issue, involving several procedures of early diagnosis, prenatal diagnosis, newborn screening and diagnosis involving prognostic markers.

As it was already stated, β-thalassemias are caused by more than 200 mutations of the human β-globin gene. This issue should be considered together with the suggestion that, while for some mutations a possible therapeutic approach is expected, for some of them the cure appears to be far away. In this context, the patients have the right to known the genotype, even prenatally. This issue faces with the fact that prenatal diagnosis oftenly requires invasive testing by amniocentesis, chorionic villus biopsy or fetal blood sampling. These diagnostic techniques increase the frequency of fetal loss by about 0.5%[17]. One alternative way for obtaining information on the gestating fetus involves recovery of fetal cells from maternal blood. In this respect, trophoblast cells have not found widespread application in diagnostic studies because they are rapidly cleared by the maternal pulmonary circulation and are likely to exhibit confined chromosomal mosaicism[18]. On the contrary, nucleated red blood cells (NRBC) are the

[17] L. JACKSON, R.J. WAPNER, *Chorionic villus sampling, in Simpson*, in J.L. SIMPSON, S. ELIAS (eds.), *Essentials of Prenatal Diagnosis*, New York, 1993, 45-61.

[18] J.M. HAHNEMANN, L.O. VEJERSLEV, *Accuracy of cytogenetic findings on chorionic villus sampling (CVS)--diagnostic consequences of CVS mosaicism and non-mosaic discrepancy in centres contributing to EUCROMIC 1986-1992*, in *Prenat Diagn*, 17, 1997, 801-20.

most common cells in fetal blood during early pregnancy. Because they have a relatively short half-life, and because they express hematopoietic plasma membrane antigens, such as the transferrin receptor (CD71), the glycophorin A cell surface molecule and intra-cellular markers (epsilon and gamma globin chains), fetal NRBC have become the targets of choice. With respect to analytic molecular biology techniques, the most important molecular techniques that have allowed genetic analysis of enriched fetal cells are PCR and FISH. The ability of PCR to amplify minute quantities of DNA (even single copies) over a billion fold has been exploited for the prenatal diagnosis of monogenic disorders from maternal blood[19,20,21]. Up to date, all of these methods result in the enrichment of fetal cells among larger populations of maternal cells, but they do not enable recovery of pure populations of fetal cells. Experimental approaches that combine fetal cell identification with molecular genetic diagnosis in an in situ technique circumvent these limitations and are especially suited for automation[22].

The European Charter of Patients' Rights: Right of Access.

The European Charter of Patients' Rights states that every individual has the right to access to the health service that his or her health needs require. Health services must guarantee equal access to everyone, without discriminating on the basis of financial resources, place of residence, kind of illness or time of access to services[5].

In this respect, the management of thalassemia and SCA clearly suffers from the fact that the 80-90,000,000 carriers (thalassemia and SCA) and the 300,000 newborns/year (40,000 thalassemia) are

[19] M.C. CHEUNG, J.D. GOLDBERG, Y.W. KAN, *Prenatal diagnosis of sickle cell anaemia and thalassaemia by analysis of fetal cells in maternal blood*, in *Nat Genet*, 14, 1996, 264-68.

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distributed in countries for which even the standard therapeutic approaches (transfusion and chelation therapy) are difficult [Fig.1].

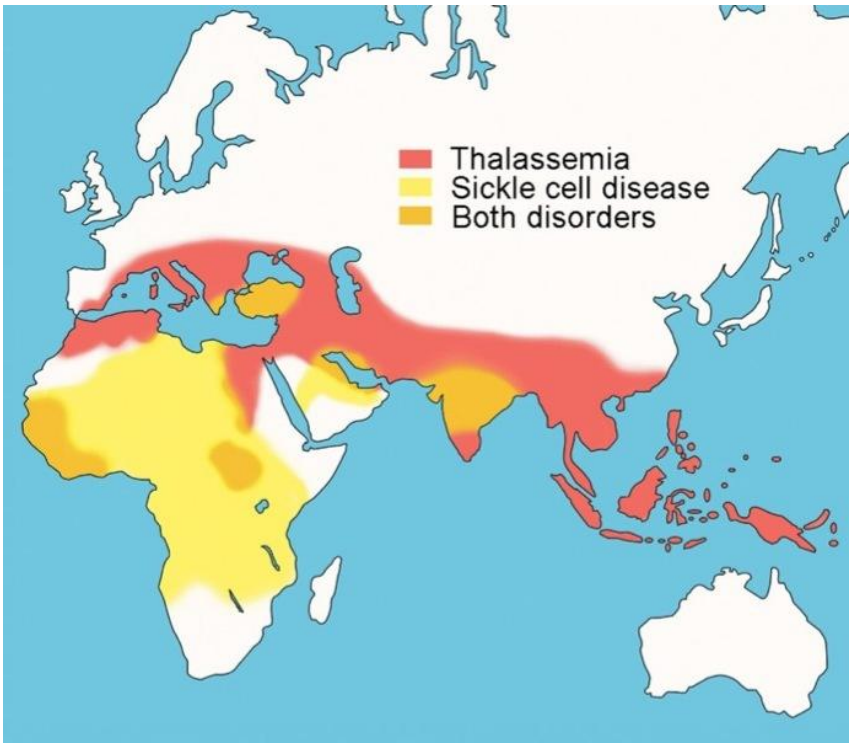

Figure 1. Global Distribution of Thalassemia and Sickle cell disease.

We should take in great consideration the fact that 100,000 newborns die worldwide. The mortality in developed countries is not high, since blood transfusion reaches all patients and chelation therapy is optimized, even using the most recent oral iron chelators. Furthermore, bone-marrow transplantation can be proposed for a sub-set of patients. On the other hand, in developing countries, despite the fact that there are limited data about the true frequency, natural history, and survival of patients with these disorders, it is expected that the mortality is very high. This information is absolutely critical towards providing

governments and international health agencies with accurate information with the objective of stimulating partnerships between rich and poor countries[23,24].

The European Charter of Patients' Rights: Right to Innovation

According to the European Charter of Patients' Rights, each individual has the right of access to innovative procedures, including diagnostic procedures, according to international standards and independently of economic or financial condiderations.

The issue of innovation in diagnostic procedures is very important for thalassemia. For instance, considering the issue of non invasive prenatal diagnosis, this approach using maternal blood appears not to be practicable for approximately 30% of pregnant women, because the NRBC are not recovered[25]. It is then important to optimize enrichment identification and diagnostic protocols. In conclusion, the Right of Preventive Medicine should be considered together the several diagnostic strategies proposed, several of which requires complex instruments and highly trained personnell.

The issue of innovation in therapy of thalassemia is at present very complex. On one hand the Patients' Associations have the right of supporting research groups involved in innovative research trends. On the other hand it become crucial to understand which are the Innovations in Thalassemia possibly leading to novel therapies. In this respect the use of human Embryonic Stem Cells (hESC) including cloned hESC[26,27,28] is a very conflicting field of investigation. It should be considered that embryonic stem cell generation inevitably involves throwing away fertilized embryos, and at the current stage of

[23] D. WEATHERALL, *The inherited disorders of haemoglobin: an increasingly neglected global health burden*, in *Indian J Med Res*, 134, 2011, 493-7.

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[26] Y. STRULOVICI, P.L. LEOPOLD, T.P. O'CONNOR, R.G. PERGOLIZZI, R.G. CRYSTAL, *Human embryonic stem cells and gene therapy*, in *Mol Ther*, 15, 2007, 850-66.

[27] http://cbhd.org/category/issues/stem-cell-research

[28] J.C. CHANG, L. YE, Y.W. KAN, *Correction of the sickle cell mutation in embryonic stem cells*, in *Proc Natl Acad Sci U S A*, 103, 2006, 1036-40.

development many of the embryos selected eventually die. For these reasons, the development of induced pluripotent stem cells (iPSCs) [29,30] from patients' fibroblasts or peripheral blood appears to be very promising, while gene therapy and gene correction by homologous recombination have been demonstrated to be suitable approaches to correct the genetic disease at the level of the genome[31,32][Fig.2].

Some key questions remain still unanswered, including: is cellular therapy feasible with iPSCs? Are iPSCs similar to ESC? Are iPSCs "safe"? In this respect, the use of frozen embryos, available since 1986 from procedures of In Vitro Fertilization (IVF) and no more suitable for embryo transfer should be taken in great consideration. As far as frozen extra embryos, more that 400,000 are present in USA and more that 52,000 in England. Unfortunately, in several European countries, the experimentation on human embryos (even these frozen embryos) is not permitted[33,34].

Human Embryonic Stem Cell's Patent-Eligibility.

At the end of 2011, a judgment of the European Court of Justice [ECJ] ended the ambiguity surrounding that court's position on the patentability of human embryonic stem cells [hESCs] derived from

[29] L. YE, J.C. CHANG, C. LIN, X. SUN, J. YU, Y.W. KAN, *Induced pluripotent stem cells offer new approach to therapy in thalassemia and sickle cell anemia and option in prenatal diagnosis in genetic diseases*, in *Proc Natl Acad Sci U S A*, 106, 2009, 9826-30.

[30] Y. FAN, Y. LUO, X. CHEN, Q. LI, X. SUN, *Generation of human β-thalassemia induced pluripotent stem cells from amniotic fluid cells using a single excisable lentiviral stem cell cassette*, in *J Reprod Dev*, 58, 2012, 404-9.

[31] Y. WANG, C.G. ZHENG, Y. JIANG, J. ZHANG , J. CHEN, C. YAO, Q. ZHAO, S. LIU, K. CHEN, J. DU, Z. YANG, S. GAO, *Genetic correction of β-thalassemia patient-specific iPS cells and its use in improving hemoglobin production in irradiated SCID mice*, in *Cell Res*, 22, 2012, 637-48.

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[33] S. STRÖM, K. RODRIGUEZ-WALLBERG, F. HOLM, R. BERGSTRÖM, L. EKLUND, A.M. STRÖMBERG , O. HOVATTA, *No relationship between embryo morphology and successful derivation of human embryonic stem cell lines*, in *PLoS One*, 5, 2010, e15329

[34] C.B. COHEN, *Ethical and policy issues surrounding the donation of cryopreserved and fresh embryos for human embryonic stem cell research*, in *Stem Cell Rev*, 5, 2009, 116-22.

human embryos. The ruling confirmed the European Patent Office and Boards of Appeal's decision in 2006 interpreting European legislation governing the patent-elgibility of biotechnology. In deciding the case of Oliver Brustle v. Greenpeace eV, the court determined that hESCs are not patent-eligible subject matter because they violate the morality clause of the European Patent Convention. This is the first time that the ECJ has given a plain answer to this patent issue after years of public debate. Thus, the European Union's viewpoint on the patentability of hESCs is no longer blurry, and it is clear that China, the European Union and the United States all have distinct policies toward hESC patent applications[35].

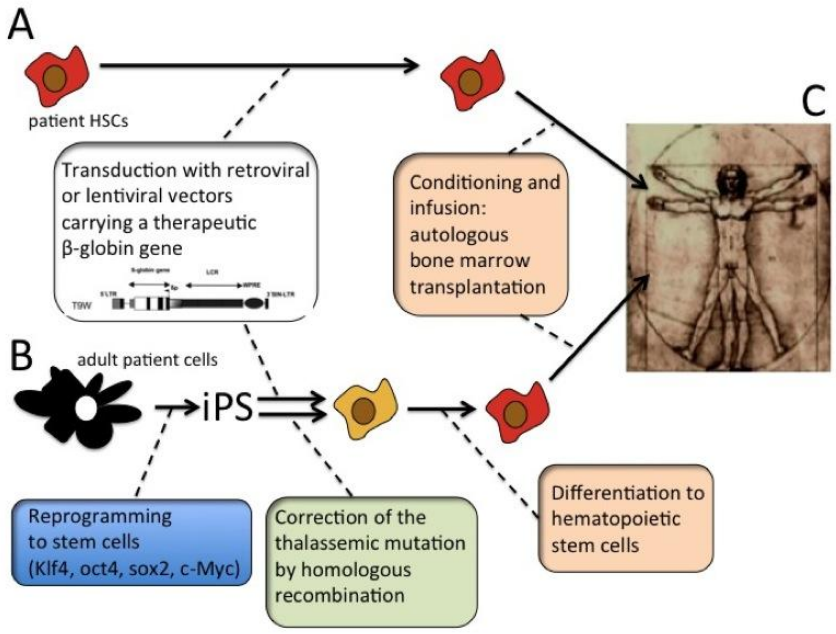

Figure 2. Flow chart of the therapeutic approaches based on gene therapy (A) and correction of induced pluripotent cells (iPSCs) from thalassemic patients (B).

[35] I. ABRAMS, *Embryonic Stem Cells: A Review of the Intellectual Property Landscape*, in *Journal of the Association of University Technology Managers*[TM], Vol XVIII-2, 2006.

The European Charter of Patient's Rights: Right to Personalized Treatment.

According to the Charter definition[5], each individual has the right to diagnostic or therapeutic programs tailored as much as possible to his or her personal needs. With respect to thalassemia, this issue is very important, since evidences demonstrating possible approaches of personalized therapy are already available.

A very exciting possibility linking diagnostics to therapeutic choice is the study by Svasti et al., who described a very interesting approach finalized to the repair of β-globin pre-mRNA rendered defective by a thalassemia-causing splicing mutation, IVSII-654, in intron 2 of the human β-globin gene[36]. This intervention was performed using a mouse model of IVSII-654 thalassemia, and based on the use of the delivery of a splice-switching oligonucleotide (SSO), a morpholino oligomer conjugated to an arginine-rich peptide. The SSO blocked the aberrant splice site in the targeted pre-mRNA and forced the splicing machinery to reselect existing correct splice sites. Repaired β-globin mRNA restored significant amounts of hemoglobin in the peripheral blood of the IVSII-654 mouse, improving the number and quality of erythroid cells. This approach is expected to be used in all the several splicing defects of β-thalassemia which produce large amount of uncorrectly spliced RNA molecules deeply interfering with RNA trafficking and translation[37,38,39]. These studies represent new hopes for specific classes of β-thalassemia patients carrying splicing mutations.

Another example of the need of genomic characterization of β-thalassemia patients is related to the recently proposed read-through approaches for inducing HbA production in erythroid precursors from

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[36] S. SVASTI, T. SUWANMANEE, S. FUCHAROEN, H.M. MOULTON, M.H. NELSON, N. MAEDA, O. SMITHIES, R. KOLE, *RNA repair restores hemoglobin expression in IVS2-654 thalassemic mice*, in *Proc Natl Acad Sci USA*, 106, 2009, 1205-10.

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[39] S. EL-ANDALOUSSI, H.J. JOHANSSON, P. LUNDBERG, U. LANGEL, *Induction of splice correction by cell-penetrating peptide nucleic acids*, in *J Gene Med*, 8, 2006, 1262-73.

patients affected by $\beta^0$39-thalassemia, where the CAG (glutamine) codon is mutated to the UAG stop codon, leading to premature translation termination and to mRNA destabilization through the well-described NMD (nonsense-mediated mRNA decay) [40]. Relevant to this issue, Salvatori et al., after FACS (fluorescence-activated cell sorting) and HPLC (high performance liquid chromatography) analyses, demonstrated that erythroid precursor cells from $\beta^0$39-thalassemia patients are able to produce β-globin and adult hemoglobin after treatment with G418[41]. This study strongly suggests that ribosomal read-through should be considered a strategy for developing experimental strategies for treatment of β^0-thalassemia caused by stop codon mutations, and might be combined with DNA-based strategies to reactivate HbF[41,42]. Accordingly, the identification of patients carrying stop-codon mutations might be relevant to design for them a therapeutic intervention based on the use of read-through molecules. This need does apply also for other pathologies caused by stop-codon mutations, including cystic fibrosis and Duchenne muscular dystrophy[43,44].

The Future of Personalized Therapy of Thalassemia.

In the future, direct targeting of splicing sites, corrections of the effects of stop-codon mutation by using read-through molecole will be

[40] G. NEU-YILIK, B. AMTHOR, N.H. GEHRING, S. BAHRI, H. PAIDASSI, M.W. HENTZE, A.E. KULOZIK, Mechanism *of escape from nonsense-mediated mRNA decay of human beta-globin transcripts with nonsense mutations in the first exon*, in *RNA*, 17, 2011, 843-54.

[41] F. SALVATORI, G. BREVEGLIERI, C. ZUCCATO, A. FINOTTI, N. BIANCHI, M. BORGATTI, G. FERIOTTO, F. DESTRO, A. CANELLA, E. BROGNARA, I. LAMPRONTI, L. BREDA, S. RIVELLA, R. GAMBARI, *Production of beta-globin and adult hemoglobin following G418 treatment of erythroid precursor cells from homozygous beta(0)39 thalassemia patients*, in *Am J Hematol*, 84, 2009, 720-28.

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available. Futhermore, indirect strategies based on patient stratification predicting response to therapy (for instance induction of fetal hemoglobin) might be considered. It is expected that treatment of thalassemia will be managed considering the genetic background and phenotype (personalized therapy); OMICS-diagnosis will be requie (at least transcriptomic and protomi analyses); unfortunately these approaches are complex, expensive and probably not accessible to all patients interested. On the other hand, several of these issues are of great interest (a) for patients who would like to become independent from blood transfusion; (b) for families willing to obtain prenatal diagnosis including information on therapeutic strategies for the newborn; (c) for Healthcare systems (due to the high cost of the management of thalassemia patients).

Conclusion: Stratification of the Thalassemia Patients in the Next Future.

The research on this issue should develop proof of principle of technologies for application in the area of personalised medicine, i.e. tailored medical interventions which are more effective and have fewer undesirable adverse effects in specifically defined patient groups. These technologies should be of use for research, screening, diagnostics and/or guidance of therapeutic interventions. Quality control aspects for data generated and appropriate use statistical tools are important[45] [Fig.3].

[45] R. GAMBARI, A. FINOTTI, *Bioethics and Freedom of Scientific Research in Gene Therapy and Stem Cell Biology*, in R. BIN, S. LORENZON, N. LUCCHI, *Biotech Innovation and Fundamental Rights*, Italy, 2012, 115-30.

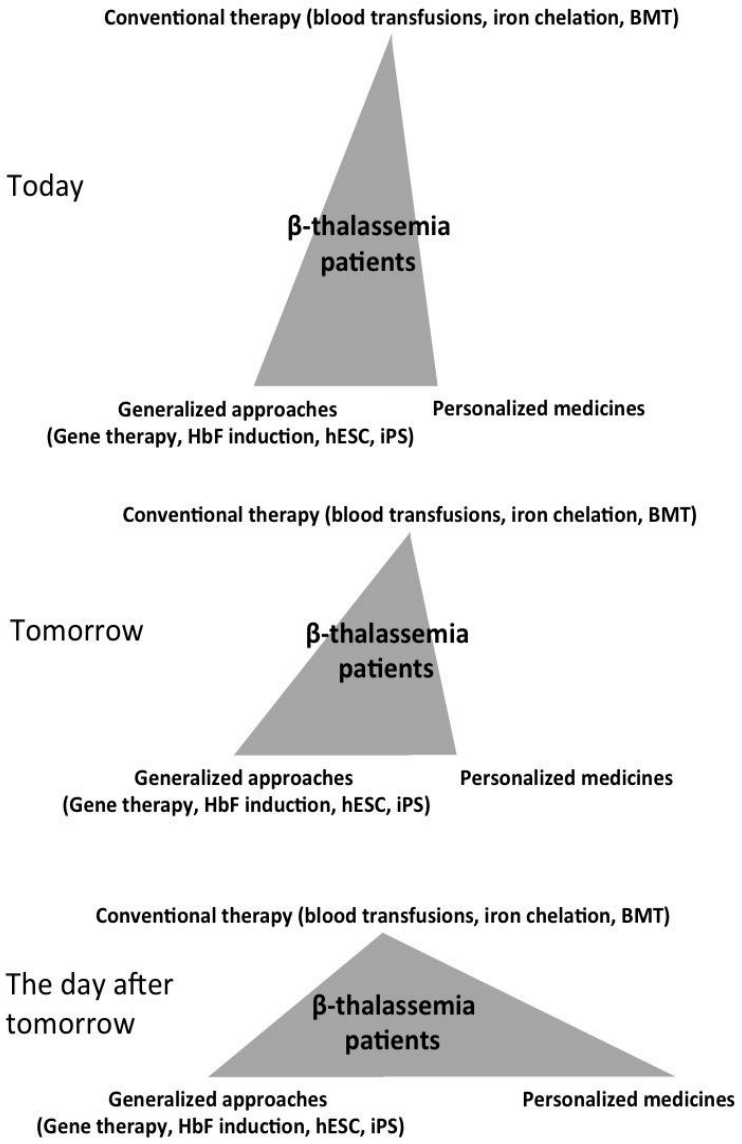

Figure 3. Expected shift from conventional therapy of thalassemia to personalized treatments. In the next few years gene therapy, HbF induction and correction of specific genetic defects will be available to patients and clinicians.

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