Innovation in the field of thrombocytopenias: achievements since the beginning of the century and promises for the future

Carlo L. Balduini and Patrizia Noris

Department of Internal Medicine, IRCCS Policlinico San Matteo Foundation and University of Pavia, Pavia, Italy

E-mail: c.balduini@smatteo.pv.it *doi:10.3324/haematol.2015.138149*

Innovation is defined as 'the act or process of introducing new ideas, devices, or methods.' According to this definition, there is no doubt that, in the field of hematology, it is in the area of thrombocytopenias that most innovation has been seen.

The new idea of inherited thrombocytopenias (ITs) that we developed in the last few years is one of the most remarkable innovations. Until the end of the last century, only a few diseases were known; all are characterized by severe bleeding diathesis. The most common and best known IT was the classical, biallelic form of Bernard-Soulier syndrome, a disorder affecting 1:1,000,000 subjects and characterized by recurrent, life-threatening hemorrhages.[1] With the beginning of the new century, the techniques for gene sequencing have become gradually more and more efficient, faster and cheaper, and they have been used in large series of patients with unknown disorders. Reports of this experience have been collected thanks to wide international collaborative efforts that have identified new genes responsible for ITs and increased the number of well characterized diseases to 25. Interestingly, 14 novel disorders have been unveiled since 2010 (Figure 1).[2] This explosion of knowledge has revealed that ITs are actually much less rare than previously thought, affecting at least 2.7:100,000 subjects.[3] Moreover, we realized that the vast majority of patients have a bleeding risk that is mild or even not significantly different from that of healthy people. This is the reason why thrombocytopenia is often discovered incidentally in adult life and many patients are initially misdiagnosed with immune thrombocytopenia (ITP).[4] Although clinically relevant bleeding affects only a minority of patients, ITs still have to be taken seriously because we now know that many affected subjects will acquire additional, life-threatening illnesses. This is the case of *MYH9*-related disease, the most frequent form of IT. After *MYH9* was identified as the causative gene in 2000, the creation of a large international registry (*www.registromyh9.org*) showed that patients usually do not bleed spontaneously, and that their main problem is the high risk during infancy or adult life of developing deafness, pre-senile cataracts and, most importantly, a proteinuric nephropathy that evolves into end stage renal failure and requires dialysis or kidney transplantation.[5] Similarly, the identification in 2013 of *ANKRD26* as the gene responsible for a mild form of IT stimulated a collaborative, international study revealing that this disorder exposes subjects to the risk of myelodysplastic syndromes and acute myeloid leukemia.[6] A similar risk was shown to affect also subjects with familial platelet disorder with predisposition to acute myeloid leukemia due to mutations in *RUNX1*, while, quite recently, it has been suggested that subjects with ETV6-related thrombocytopenia are at risk of lymphoid malignancies.[7] Thus, recognizing patients with these ITs predisposing to additional disorders is mandatory in order to personalize follow up and be ready to give appropriate treatments if new illnesses develop. The use of innovative technologies and international collaborative efforts have provided the basis for this new insight into ITs.

Another important innovation in the field of platelet disorders is represented by the development of techniques for *in vitro* culture of megakaryocytes (Mks) starting from hemopoietic progenitors or multipotent stem cells. The discovery of thrombopoietin (TPO) at the end of the last century was the turning point that made the achievement of this goal possible. Thereafter, further technical innovation resulted in the availability of devices for Mk culture that mimic bone marrow in terms of 3-dimensional structure, cellular composition, extracellular matrix and blood supply.[8] *In vitro* culture of Mks made a big contribution to our understanding of the enigmatic biology of these cells. In brief, it has been shown that Mks originating from

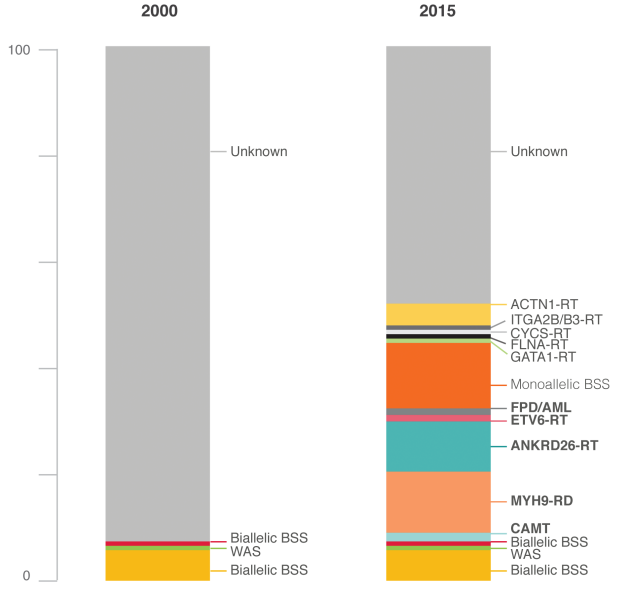

Figure 1. The evolving view of inherited thrombocytopenias. Since the beginning of this century, a number of new inherited thrombocytopenias have been discovered and this advancement of knowledge changed our view of these diseases. A few patients have clinically relevant bleeding, but many subjects are at risk of developing additional, life-threatening disorders (names of diseases in bold). Of note, slightly less than 50% of patients remain without a diagnosis because they do not fit the criteria for any known disease, this indicating that a further effort should be made to improve the knowledge of these diseases. The reported figure is based on the database of our institution comprising 274 consecutive families and 566 patients. This case series does not include all known disorders, since many of them are exceedingly rare. BSS: Bernard-Soulier syndrome; WAS: Wiskott-Aldrich syndrome; GPS: gray platelet syndrome; ACTN1-RT: *ACTN1*-related thrombocytopenia; ITGA2B/B3-RT: *ITGA2B/ITGB3*-related thrombocytopenia; CYCS-RT: *CYCS*-related thrombocytopenia; FLNA-RT: *FLNA*-related thrombocytopenia; GATA1-RT: *GATA1*-related thrombocytopenia.

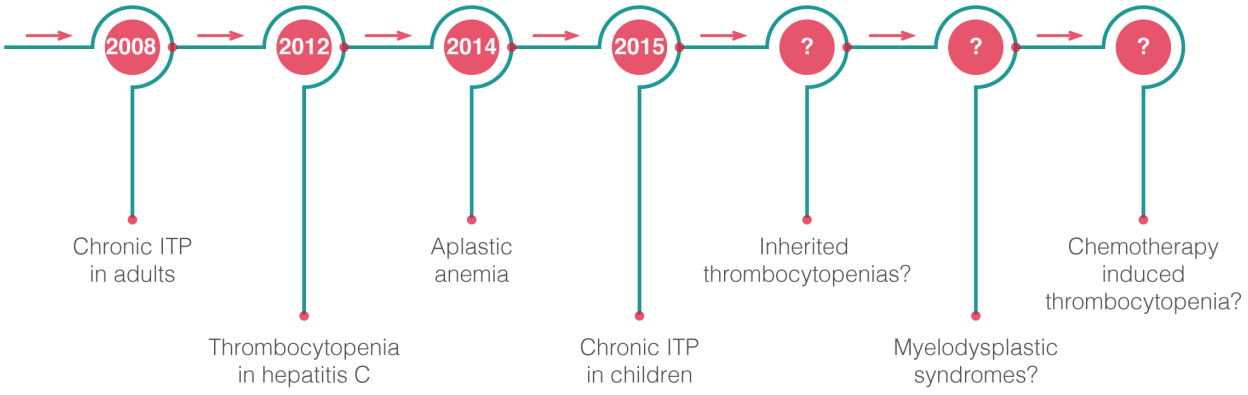

Figure 2. The increasing indications for thrombopoietin mimetics. Thrombopoietin mimetics (eltrombopag and romiplostim) were approved in 2008 for adults with chronic immune thrombocytopenia who did not respond to other treatments. Eltrombopag was also later approved for thrombocytopenia due to hepatitis C, refractory aplastic anemia and refractory chronic immune thrombocytopenia in children. Preliminary evidence indicated that thrombopoietin mimetics increase platelet count also in inherited thrombocytopenias (*MYH9*-related disease and Wiskott-Aldrich syndrome/X-linked thrombocytopenia) and myelodysplastic syndromes. Moreover, it is expected that these drugs might reduce the thrombocytopenia induced by non-myeloablative chemotherapy, but there are still no conclusive data available.

hemopoietic progenitors in the stem cell niche migrate to the vascular niche. Here they extend pro-platelets into the lumen of marrow sinusoids and release pre-platelets directly into the circulating blood, where platelets are finally formed.[9] Moreover, the development of techniques for culturing Mks from circulating progenitors of patients with different forms of IT made it possible, not only to define the pathogenetic mechanisms of these disorders, but also to begin to decipher the sequence of molecular events that are required for effective platelet production.[10]

Platelets formed by cultured Mks resemble blood platelets in terms of morphology and function,[8] and this originated the idea of using them for transfusion purposes. In principle, an amount of human MKs appropriate to the *in vitro* production of platelet concentrates might reasonably be obtained from $CD34^+$ embryonic stem cells, $CD34^+$ umbilical cord blood stem cells, and induced pluripotent stem cells.[11] The most attractive source are human-induced pluripotent stem cells, in that they can be expanded and potentially maintained in culture indefinitely. Besides, they are generated from adult cells and therefore do not give rise to ethical concerns. An attractive feature of platelets derived from stem cell culture is the prospect of genetically manipulating progenitor cells to generate platelets devoid of HLA antigens.[12] This would eliminate the risk of alloimmunization against HLA class I antigens and the resulting refractoriness to subsequent platelet transfusions, which is still a serious clinical problem. Clearly, further progress must be made for this platelet source to become a clinical reality. Particularly relevant challenges remain the need to generate huge numbers of stem cells, ensure that platelets produced with this methodology are safe, and verify whether they are hemostatically effective through clinical trials. There is still a long way to go but the arduous journey has begun.

The discovery of TPO not only represented a turning point for *in vitro* culture of megakaryocytes, but also opened a new chapter in the treatment of various forms of thrombocytopenia. The initial attempts to stimulate *in vivo* platelet formation with molecules structurally similar to native TPO gave promising results in patients with chemotherapy-induced thrombocytopenia. However, clinical trials were stopped in 2001 after a few healthy volunteers developed thrombocytopenia as a result of the formation of alloantibodies cross-reacting with endogenous TPO.[13] Research then shifted to the development of novel agents with the capacity to stimulate the TPO receptor but with no structural homology with human TPO. Two of these molecules, eltrombopag and romiplostim, demonstrated good efficacy and safety in patients with chronic ITP, and were approved in 2008 and 2015 for adults and children, respectively, not responsive to traditional treatments. The experience gained so far indicates that TPO mimetics are the most effective drugs for ITP and questions the current classification of these molecules as second- or third-line agents. The interest in TPO mimetics continued to grow after the recent demonstration that many ITP patients who responded to this treatment can discontinue therapy without relapsing.[14] Investigation into this unexpected phenomenon could provide new insights into the pathogenesis of ITP and the mechanisms of action of TPO mimetics.

Efficacy of TPO mimetics is not limited to ITP (Figure 2). In 2012, eltrombopag was approved to allow patients with chronic hepatitis C and low platelet count to receive interferon and ribavirin therapy. Furthermore, in 2014 eltrombopag was approved for patients with severe aplastic anemia refractory to immunosuppressive therapy after evidence that it was able to produce a hematologic response in at least one cell lineage in 40% of patients, and a trilineage response in nearly half of them. It is interesting to note that, as in ITP, stable blood counts were often maintained after treatment discontinuation.[15] This observation further supports the idea that not all the mechanisms of action of TPO mimetics have been identified. Finally, eltrombopag was proven to be effective in increasing platelet count in *MYH9*-related disease[16] and Wiskott-Aldrich syndrome/X-linked thrombocytopenia.[17]

Interestingly, a few, severely thrombocytopenic patients have already successfully received this drug instead of platelet transfusion as preparation for surgery.[18] An increase in platelet count has been obtained with the TPO mimetics also in myelodysplastic syndromes, but it remains unclear if these drugs favor the progression to leukemia in this clinical setting.[19] On the basis of this success, there is no doubt that the introduction of TPO mimetics represents one the most relevant innovations in hematology since the beginning of this century.

One final, important innovation in the area of thrombocytopenias has been the successful application of gene therapy to Wiskott-Aldrich syndrome, a form of IT associated with immunodeficiency, which is usually associated with death before adulthood due to hemorrhages or infections. Nine of 10 patients receiving lentiviral-transduced hematopoietic stem cells had a stable engraftment resulting in increased platelet count, less bleeding and improved immune function.[20] After this proof of principle, it is desirable that this approach is made available also for a few other life-threatening forms of ITs to provide an alternative to those patients who cannot receive bone marrow transplantation due to the lack of compatible donors.

In conclusion, exciting innovations in the area of thrombocytopenias characterized the beginning of this century, and this is the best promise we have that much more progress will be achieved in the next few years.

Financial and other disclosures provided by the author using the ICMJE (www.icmje.org) Uniform Format for Disclosure of Competing Interests are available with the full text of this paper at www.haematologica.org.

References

1. López JA, Andrews RK, Afshar-Kharghan V, Berndt MC. Bernard-Soulier syndrome. Blood. 1998;91(12):4397-4418.
2. Savoia A. Molecular basis of inherited thrombocytopenias. Clin Genet. 2015 May 7. [Epub ahead of print]
3. Balduini CL, Pecci A, Noris P. Inherited thrombocytopenias: the evolving spectrum. Hamostaseologie. 2012;32(4):259-270.
4. Balduini CL, Savoia A, Seri M. Inherited thrombocytopenias frequently diagnosed in adults. J Thromb Haemost. 2013;11(6):1006-1019.
5. Pecci A, Klersy C, Gresele P, et al. MYH9-related disease: a novel prognostic model to predict the clinical evolution of the disease based on genotype-phenotype correlations. Hum Mutat. 2014;35(2):236-247.
6. Noris P, Favier R, Alessi MC, et al. ANKRD26-related thrombocytopenia and myeloid malignancies. Blood. 2013;122(11):1987-1989.
7. Noetzli L, Lo RW, Lee-Sherick AB, et al. Germline mutations in ETV6 are associated with thrombocytopenia, red cell macrocytosis and predisposition to lymphoblastic leukemia. Nat Genet. 2015;47(5):535-538.
8. Di Buduo CA, Wray LS, Tozzi L, et al. Programmable 3D silk bone marrow niche for platelet generation ex vivo and modeling of megakaryopoiesis pathologies. Blood. 2015;125(14):2254-2264.
9. Kaushansky K. Thrombopoiesis. Semin Hematol. 2015;52(1):4-11.
10. Pecci A, Balduini CL. Lessons in platelet production from inherited thrombocytopenias. Br J Haematol. 2014;165(2):179-192
11. Thon JN, Medvetz DA, Karlsson SM, Italiano JE Jr. Road blocks in making platelets for transfusion. J Thromb Haemost. 2015;13 Suppl 1:S55-62.
12. Feng Q, Shabrani N, Thon JN, et al. Scalable generation of universal platelets from human induced pluripotent stem cells. Stem Cell Reports. 2014;3(5):817-831.
13. Li J, Yang C, Xia Y, et al. Thrombocytopenia caused by the development of antibodies to thrombopoietin. Blood. 2001;98(12):3241-3248.
14. Carpenedo M, Cantoni S, Coccini V, Fedele M, Morra E, Pogliani EM. Feasibility of romiplostim discontinuation in adult thrombopoietin-receptor agonist responsive patients with primary immune thrombocytopenia: an observational retrospective report in real life clinical practice. Hematol Rep. 2015;7(1):5673.
15. McCormack PL. Eltrombopag: a review of its use in patients with severe aplastic anaemia. Drugs. 2015;75(5):525-31.
16. Pecci A, Gresele P, Klersy C, et al. Eltrombopag for the treatment of the inherited thrombocytopenia deriving from MYH9 mutations. Blood. 2010;116(26):5832-5837.
17. Gerrits AJ, Leven EA, Frelinger AL 3rd, et al. Effects of eltrombopag on platelet count and platelet activation in Wiskott-Aldrich syndrome/X-linked thrombocytopenia. Blood. 2015;126(11):1367-1378.
18. Favier R, Feriel J, Favier M, Denoyelle F, Martignetti JA. First successful use of eltrombopag before surgery in a child with MYH9-related thrombocytopenia. Pediatrics. 2013;132(3):e793-795.
19. Prica A, Sholzberg M, Buckstein R. Safety and efficacy of thrombopoietin-receptor agonists in myelodysplastic syndromes: a systematic review and meta-analysis of randomized controlled trials. Br J Haematol. 2014;167(5):626-638.
20. Hacein-Bey Abina S, Gaspar HB, Blondeau J, et al. Outcomes following gene therapy in patients with severe Wiskott-Aldrich syndrome. JAMA. 2015;313(15):1550-1563.