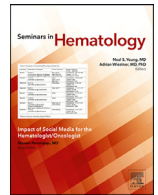




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Aplastic anemia: Quo vadis?

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ABSTRACT

In the last 30 years, the field of aplastic anemia (AA), and more generally bone marrow failure syndromes, has undergone a multitude of new discoveries. The application of modern and sophisticated sequencing techniques unveiled a variety of genes associated with these disorders and contributed to a better understanding of the disease pathobiology. This advancement was paralleled by the discovery, clinical testing and subsequent approval of new drugs for the treatment of AA and associated disorders. Several additional agents are currently under evaluation for possible therapies. Herein, we look at the potential future avenues of research in AA through a brief summary of an intergenerational Socratic dialogue between the mentor, who witnessed and actively contributed to the milestones achieved in the last 30 years, and his fellow, who would himself go on to become the mentor of a new generation of AA researchers.

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“Man cannot search either for what he knows or for what he does not know; He cannot search for what he knows—since he knows it, there is no need to search—nor for what he does not know, for he does not know what to look for...” (*Meno*, Plato [380 BC]) [1]. Socrates and Plato still represent one of the most famous and paradigmatic examples of teacher-student relationship. Through the “dialectic”, the “art of dialogue”, Plato unfolds the Socratic method of teaching, which involves an interactive conversation, a cooperative back and forth between two interlocutors, emphatically aiming at guiding students towards a better understanding of their inner knowledge (a process called “maieutic art”).

As a teacher and a Nestor of the field, Dr Neal Young has cultivated a tradition of scientific disputes about aplastic anemia (AA), engaging with his students, typically after the hours dedicated to the clinical management of patients. About 30 years ago, Dr Jaroslaw Maciejewski (Jarek) had the privilege to be a partner in these dialogues, which Neal used to generate ideas for new research paths [2]. Today, Jarek, now a teacher himself following in the steps of his mentor, conversed with a young student (Dr Carmelo Gurnari) about the future of the AA field, recreating the beautifully instructive moments he has been inspired by during his scientific career. “If the same discussion I had with Neal were to take place today with you, Carmelo, which questions should the

new generation of AA researchers focus on? Remember to appreciate that you may not know future capabilities, as I did not back then, nor even dream about which capabilities will become available!”

The biggest mystery of AA remains unsolved

What triggers this disease [2]? Despite a lot of effort, a successful approach to solve this etiologic mystery has not been found, but this quest will likely constitute the promise of “el Dorado” gold, prompting new explorations. For instance, existing technologies and those of the future should revisit the issue of exogenous etiologic agents, *for example*, by deep sequencing of viral capture libraries or by reverse engineering of T cell receptor (TCR) “antigenomes”. Modern serologic techniques may finally confirm that cell reactivity has to be accompanied by detectable antibody responses, perhaps illuminating the shadowlands of AA diagnosis [3]. Similarly, the old theory of AA viewed as an “over-controlled” leukemia should be re-examined using deep error-corrected and single cell sequencing technologies, possibly enabling a precise definition of its clonal architecture. Should there be some truth to this theory, the latter avenue may have tremendous practical implications. Indeed, if AA represents a hyper-efficient tumor surveillance reaction, can it be redirected taking advantage of the lesson derived from its postulated ontogeny? New classes of TCR-redirection CAR-T cells, with appropriate safety switch technologies and in a proper clinical setting, such as allogeneic stem cell transplant, would answer the question Jarek posted to Carmelo: Would AA

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cytotoxic T cells “take care” of leukemia? After all, without medical countermeasures, they are capable of efficient obliteration of an entire organ: the bone marrow [4]. A provocative question would be: do we even need to know the antigenic triggers? For instance, we use ATG and still do not know how it exactly works. Provision of proof-of-concepts may be a first step and the answers as to the trigger peptide may come sooner than we think.

New treatment agents

ATG in its efficacy and pharmacological crudeness inspires a new series of future tasks, which involve the improvement of the existing therapeutic arsenal. Definitely, following the progresses in other autoimmune diseases, we currently have a multitude of novel, elegant, immunosuppressive agents targeting “old” cytokines such as interferon- γ , but also a seemingly never-ending litany of new anti-interleukins or their receptors. Clearly, design of new trials will be a challenge, especially if we consider the already good overall response rate achieved with the available therapies [5]. Particularly, the improvement made so far in the first-line treatment paradoxically constitutes a major limiting factor for the development of new drugs or the design of a clinical trial in the *de novo* setting [6]. Nevertheless, in the context of the novel opportunities emerging with the advent of modern genetic diagnostic capabilities, we should not forget the insights that may be derived from the traditional immunologic research in AA, which in recent years has been largely overshadowed by the genetic revolution.

Pathobiology of hematopoietic stem cell, germline predisposition and clonal evolution

AA has taught us much about hematopoietic stem cell biology. Especially with regard to induced pluripotent and embryonic stem cells, the progress has been tremendous, and the reward of studies on stem cell compartment in AA may also be very lucrative. For instance, the limits of stem cell re-expansion can be overcome *ex vivo* and *in vivo* (eg, by increasing the probability of symmetric division at a stem cell level) using new drugs. Agents with such a potential may open new opportunities to generate graft libraries for hematopoietic transplantation or to combat aging of the hematopoietic system. What if, in such a scenario, autologous stem cell transplant following *in vitro* expansion of hematopoietic stem cells—similar to what is currently attempted with umbilical cord blood—would not be just a pipe dream?

Another lesson in the setting of stem cell biology and disease mechanisms is represented by the recognition of a strong genetic predisposition, observed in younger patients and associated to a variety of newly discovered germline alterations. Marrow failure is indeed the clinical leitmotiv of a multitude of constitutional genetic syndromes, such as Fanconi anemia or telomeropathies, and modern genome scanning techniques unveiled several new genes associated with the spectrum of AA-related disorders (eg, *GATA2*, *SAMD9/9L*) [7]. An interesting aspect of both constitutional and acquired AA is their dynamics of clonal evolution, characterized by the acquisition of invariant cytogenetic lesions, such as chromosome 7 aberrations, reminiscent of childhood myelodysplastic syndromes and typically recurring in the context of constitutional bone marrow failure defects.

AA and paroxysmal nocturnal hemoglobinuria (PNH): A chicken or egg causality dilemma

PNH is intimately connected with AA as many patients can either present with features of both disorders or evolve from one to the other throughout the disease course. This fluid nosological spectrum historically generated a Sphinx’s riddle: where does PNH stand in the ontogeny of AA [8]? What if PNH is investigated as a trigger of AA exuberant destruction of hematopoietic stem cells? Or is PNH instead a “blessing in disguise” in the relentless autoimmune attack operated by cytotoxic T cells in the marrow [9]? Some considerations on the ontogeny and on the regenerative potential of the PNH clone, which represents a semi-adaptive response to AA immune attack, may stem from the fascinating, rare instance of its spontaneous remission [10]. According to the postulated theory of a “finite life span”, the retraction of the PNH clone would be associated with the recurrence of AA, should normal stem cells be absent [11]. However, a contrasting hypothesis (“neutral drift theory”) posits the absence of a better fitness of the *PIGA*-mutant clone, explaining its expansion without assigning an environmental growth advantage to the PNH clone under immune attack.

As we see today, as it was 30 years ago, a variety of biological and clinical issues in the field of AA still needs to be fully elucidated. Experienced investigators are now relaying the Olympic Torch to the youngsters in a continuum of shared inspiration, passion and commitment, which along with the availability of novel techniques and the pace of modern research capabilities will help us advance towards a brighter future for AA patients.

Disclosure

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

- [1] Plato. *Meno*. In: Grube tbGMA, editor. *Meno*. Indianapolis: Hackett; 380 BC. p. Line 80e.
- [2] Young NS, Maciejewski J. The pathophysiology of acquired aplastic anemia. *N Engl J Med* 1997;336(19):1365–72.
- [3] MT Tiina Kelkka, Lundgren Sofie, Feng Xingmin, et al. Anti-COX-2 autoantibody is a novel diagnostic marker of aplastic anemia. *Blood* 2021;138(Supplement 1):1125 Abstract ASH Meeting 2021.
- [4] Risitano AM, Maciejewski JP, Green S, Plasilova M, Zeng W, Young NS. In-vivo dominant immune responses in aplastic anaemia: molecular tracking of putatively pathogenetic T-cell clones by TCR beta-CDR3 sequencing. *Lancet (London, England)* 2004;364(9431):355–64.
- [5] Young NS. Current concepts in the pathophysiology and treatment of aplastic anemia. *Hematology* 2013;2013(1):76–81.
- [6] Townsley DM, Scheinberg P, Winkler T, et al. Eltrombopag Added to Standard Immunosuppression for Aplastic Anemia. *N Engl J Med* 2017;376(16):1540–50.
- [7] Young NS. *Aplastic Anemia*. 2018; 379(17): 1643–56.
- [8] Young NS, Maciejewski JP. Genetic and environmental effects in paroxysmal nocturnal hemoglobinuria: this little *PIG-A* goes “Why? Why? Why?”. *J Clin Invest* 2000;106(5):637–41.
- [9] Luzzatto L, Bessler M, Rotoli B. Somatic mutations in paroxysmal nocturnal hemoglobinuria: a blessing in disguise? *Cell* 1997;88(1):1–4.
- [10] Gurnari C, Pagliuca S, Kewan T, et al. Is nature truly healing itself? Spontaneous remissions in Paroxysmal Nocturnal Hemoglobinuria. *Blood Cancer J* 2021;11(11):187.
- [11] Hillmen P, Lewis SM, Bessler M, Luzzatto L, Dacie JV. Natural history of paroxysmal nocturnal hemoglobinuria. *N Engl J Med* 1995;333(19):1253–8.