Targeted therapy for pulmonary alveolar proteinosis: the time is now

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Pulmonary alveolar proteinosis (PAP) is a rare syndrome with a complex pathogenesis leading, at the end, to accumulation of surfactant phospholipids and lipoproteins in the alveoli, and causing progressive respiratory insufficiency [1]. Alongside the most common autoimmune form, affecting more than 90% of patients and characterised by the presence of granulocyte-macrophage colony-stimulating factor (GM-CSF) autoantibodies, there are severe forms associated with genetic mutations, which are usually diagnosed in children. The landscape of genetic mutations underlying PAP is rapidly evolving and advances in this field from the past decade are impressive [2, 3]. In terms of frequency, the most common mutations, associated with primary hereditary PAP, affect genes coding for the a- and b-subunit of GM-CSF receptor (CSF2RA and CSF2RB), followed by surfactant (SFTPB, SFTPC, SFTPA1 and SFTPA2), ABCA3 and NKX2.1 gene mutations; PAP syndrome occurs sporadically in the latter two. Further, GATA-2 mutations, associated with a genetic immunodeficiency syndrome (MonoMAC) [4–6], SLC7A7, MARS, FARSB and NPC 2 gene mutations (causing lysinuric protein intolerance, methionine deficit, phenylalanine-tRNA synthetase deficit and Niemann–Pick disease, respectively) [2], and telomerase reverse transcriptase (TERT) mutations [7, 8] can also be revealed by PAP.

Treatment options for PAP are, overall, limited and non-standardised, and there are still no approved drugs. Whole lung lavage (WLL), which only affects the symptoms by removing proteins and phospholipids from the alveoli, remains the first-line treatment but is burdened with complications and high costs. Its use in hereditary PAP is limited by the difficulty of performing this technique in paediatric patients and by its limited efficacy. Results from randomised clinical trials with GM-CSF substitution treatment in autoimmune PAP are promising but need further confirmation [9, 10]. Remarkably, GM-CSF substitution has not yet been tested in hereditary PAP.

The development of new corrective strategies for causative genetic defects underlying PAP have recently been proposed [3]. Gene therapy-based approaches, however, require preclinical toxicology studies before gene transfer can be applied in humans. Another issue is related to the high risk of complications related to the aggressive preconditioning with immunosuppression and myeloablation in the case of transplantation of haematopoietic stem cells. Transplantation of genetically corrected pulmonary macrophages in PAP murine models and patients has shown promising results [11, 12], while development of therapeutic RNA or *in vivo* genetic editing, as seen for other genetic disorders, might also become a concrete perspective in PAP [13].

The original research article entitled "Methionine supplementation for multi-organ dysfunction in MetRS-related pulmonary alveolar proteinosis" by Hadchouel *et al.* [14] reports the results of oral supplementation with methionine in four patients with methionine tRNA synthetase-related PAP. In 2015,

the same group identified recurrent biallelic mutations in MARS that cause a specific type of PAP prevalent on Réunion island, with an incidence of 1 in 10 000 new-borns [15]. Transmission is autosomal recessive, with patients being homozygous or compound heterozygous. MARS encodes the cytosolic methionine tRNA synthetase (MetRS), which belongs to the class 1 family of aminoacyl-tRNA synthetases (ARSs). These enzymes play a critical role in protein biosynthesis by leading to the formation of aminoacyl-tRNA. As for other ARSs, MetRS has also an editing and proofreading function in order to ensure translational fidelity and is involved in immune response, inflammation, tumorigenesis, angiogenesis and neuronal homeostasis [2]. Whereas the association between mutations in MARS and development of PAP is not yet completely elucidated, available evidence from animal models indicates that a deficiency of MetRS activity leads to a reduced aminoacylation and deficient protein translation, with disruption of surfactant composition or homeostasis [2]. Interestingly, in a yeast model of MARS loss of function mutation, addition of methionine in the culture medium completely restores MetRS activity, providing the rationale for a clinical trial [14].

The four patients presented in this study were diagnosed at between 3 and 5 years of age and had systemic disease with lung and liver involvement, haematological and brain abnormalities, inflammation and growth delay. To balance the small number of patients and their heterogeneity at baseline, the authors included patients with the same genotype and compared to those of historical controls. All the patients received WLL as treatment for respiratory insufficiency due to PAP.

Methionine was administered orally or enterally, 20–30 $mg \cdot kg^{-1}$ every 6 h over a period of 2 years. Stable plasma concentrations 1 h after intake were obtained in all patients. The authors provide clear and strong evidence of the efficacy of this standardised methionine supplementation in all these patients, *i.e.* cessation of WLL and oxygen weaning, chest computed tomography improvement, cessation of gastro-intestinal symptoms and improvement in psychomotor delay in all patients. The clinical response was obtained despite phenotypic heterogeneity among the four treated patients, although two patients, who started treatment significantly later, did not achieve complete remission. Noteworthy are the ancillary findings, such as bronchoalveolar lavage fluid examination, showing complete disappearance of the abnormal extracellular lipoproteinaceous material, which never occurred in the past for the historical patients. At a cellular level, the results being shown in the supplementary material, methionine supplementation normalised the production of reactive oxygen species by peripheral monocytes after 3 months. The 20 historical patients included as comparator help us to understand the natural history of MetRS-related PAP. Four of them were weaned from oxygen therapy, 11 died and oxygen supply was still ongoing for five patients at their last follow-up. Fibrosis occurred in about two-thirds of cases, an interesting observation which is not fully understood, as the link between MARS mutation and lung fibrosis has not been studied. It may be hypothesised that the persistent non-resolved inflammation, one of the hallmarks of MetRS-related PAP, or endoplasmic reticulum stress, depending on methionine deficiency, act as drivers of lung (and liver) fibrosis in this disease [16–18]. Considering that the probability of pulmonary fibrosis in MetRS-related PAP has been estimated almost 50% by the age of 14 years [19], long-term methionine supplementation has the potential, if not to be curative, to prolong progression-free survival. The methionine supplementation was well-tolerated, and transient transaminase elevation or gastrointestinal symptoms occurred.

With all the limitations of this study, *in primis* the small sample size, Hadchouel *et al.* [14] pave the way for similar targeted-treatment strategies for other ARS deficiencies. It is encouraging that further research groups recently described successful methionine use in patients harbouring other MARS1 mutations, whereas the administration modality differs across the reports [20, 21].

The present study raises challenging questions about the next steps for this ultrarare disease. Is it ethically acceptable, considering the highly impaired quality of life and growth delay in these children, to design a trial with a blind sequence (with or without treatment) and then a prolonged time with treatment for all patients? Moreover, might an international multicentric trial, whenever feasible, be more efficient in term of patients' recruitment?

Gene therapy, defined as replacing or inactivating a disease-causing gene, has long been considered as the only targeted therapeutic possibility following the identification of specific disease-causing mutations. However, after decades of efforts a very limited number of diseases reached the goal [22]. The breakthrough came in 2016, when the European Commission approved a gene therapy for treating ADA-SCID, a rare immune disorder fatal to children within their first year, followed in 2017 by the approval by the US Food and Drug Administration of a gene therapy to treat a rare form of blindness [23]. There have been recent advances in developing even more effective technologies for gene therapy, such *in*

vivo gene editing (CRISPR) and use of therapeutic RNA (RNA interference), but it is not yet clear whether they will provide a cure. On the other side, the example of cystic fibrosis, and now the study by Hadchouel *et al*. [14], have also taught us that knowing the genetic cause of a disease could lead to the development of "classical" drugs targeting the impaired molecular pathway with dramatic results [24]. What is currently ongoing in the treatment development for MetRS-related PAP almost recalls the story of GM-CSF substitution treatment in autoimmune PAP, as anecdotical reports, the first one in 1996, and observational studies preceded by years the systematic investigation of this approach in randomised clinical trials [9, 10].

Although enormous progress in understanding PAP pathogenesis has been achieved in the past two decades, heterogeneity of phenotypes, delay in reaching a final diagnosis, and lack of approved medications and treatment guidelines inevitably affect patients' quality of life and risk jeopardising stakeholders' investments. The present study underscores once more the importance of awareness and networking for the future of rare and neglected diseases. The creation of international registries is mandatory to avoid dispersion of cases and to facilitate patients' recruitment in clinical trials. We as a PAP community are perfectly aware of the necessity of validating the results of the present study, preferably in a multicentre, controlled fashion, but the feeling we have is that novel pathogenesis-based therapies have already reached clinical practice in PAP, at least in selected subgroups of patients.

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