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Progress toward Fulfilling the Potential of Immunomodulation in Childhood Neurodegeneration?

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In this issue of *Molecular Therapy*, Groh and colleagues[1] have provided further evidence that immunomodulatory approaches may be of value in treating two types of lysosomal storage disorder, CLN1 and CLN3 disease.[1] The study builds upon their previous work in mouse models, which revealed an adaptive immune response mediated by sialoadhesin,[2,3] showing low-level infiltration of the brain by predominantly $CD8^+$ lymphocytes, and that genetically blocking this infiltration partly slowed disease progression and extended life-span moderately.[2] Taken together with evidence of significant activation of the innate immune system in the CNS of these mouse models,[4] these findings raised the possibility that immunomodulatory drugs might have some therapeutic potential in targeting such secondary neuroimmune responses.

The neuronal ceroid lipofuscinoses (NCLs) are a group of fatal inherited neurodegenerative lysosomal storage disorders that predominantly affect children and young adults.[5] Each is caused by a mutation in a different gene that primarily impacts the CNS of affected individuals who suffer visual failure, a progressive decline in cognitive and motor ability, seizures of worsening severity, and premature death. [5]

Groh et al.[1] first provide evidence for a similar adaptive immune response in human CLN2 and CLN3 disease autopsy brain samples. This suggests that the mouse phenotype is likely clinically relevant to the human disease, although we can only study end-stage pathology in such autopsy samples, and the progression of the adaptive immune response may vary between species. The authors then employed two different clinically approved immunomodulatory drugs, fingolimod and teriflunomide, to target $CD8^+$ effector cells within the CNS of mouse models of CLN1 and CLN3 diseases at a pre-symptomatic stage. Although working by slightly different mechanisms, both compounds exhibited robust effects on pathological phenotypes, including axonal pathology, neuron loss, retinal thinning, and brain atrophy, together with a reduced frequency of myoclonic jerks in Cln1 mice. The findings add to the growing body of evidence that innate and adaptive immune responses contribute to disease progression across different forms of NCL.[4] These data also reveal that the course of these disorders can be influenced by means of

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clinically approved immunomodulators. Given the lack of any overt side effects, the authors have proposed that these drugs could be used to help rescue the secondary immune responses seen in these diseases to improve patient quality of life.

Immunomodulation is not an entirely new approach for either of these forms of NCL,[6–8] and this strategy will not treat the underlying gene defect. While attempts to refine enzyme replacement or gene therapy for CLN1 disease are ongoing,[9] a protein replacement approach for CLN3 is not feasible because this transmembrane protein cannot be secreted and taken up by deficient cells. However, a recent pre-clinical study has provided evidence that systemic delivery of a self-complementary AAV9 vector partly corrects pathology in *Cln3*$^{\Delta ex7/8}$ mice.[10] Optimizing and clinically testing this gene therapy strategy will likely take some time, and there is still scope for alternative approaches, such as immunomodulation, to be considered as treatment options.

Until recently, the therapeutic outcome for all forms of NCL has been uniformly bleak. However, based on efficacy data in a non-randomized single arm dose escalation clinical study[11] (https://clinicaltrials.gov/ct2/show/NCT02678689), the Food and Drug Administration (FDA) and European Medicines Agency (EMA) recently approved Brineura (cerliponase alpha) for the treatment of CLN2 disease, which is one of the three most common forms of NCL. This is an enzyme replacement strategy, in which recombinant tripeptidyl peptidase-1, the lysosomal enzyme that is deficient in this form of NCL, is delivered directly to the cerebrospinal fluid (CSF), and is based on promising pre-clinical studies in both mice and dogs.[12] Enzyme replacement therapy (ERT) may not ultimately prove the best long-term means to treat CLN2 disease, since intraventricular gene therapy has also shown great promise in the same dog model;[13] however, the approval of Brineura is nevertheless a major step forward.

Pre-clinical attempts to treat CLN1 by ERT or viral-mediated gene therapy have been less effective than in CLN2 disease.[9] This may partly be due to the earlier onset of CLN1 disease, but also the apparently limited diffusion of PPT1 enzyme, and that other parts of the CNS and body appear to be severely affected in this disorder. There is also a pronounced activation of both astrocytes and microglia before neuron loss in CLN1 disease[4] in addition to the low-level lymphocyte infiltration previously reported by Groh and colleagues.[2] As such, simply replacing the missing enzyme may be insufficient to fully treat this form of NCL. Proof-of-principle for this concept has come from a series of studies demonstrating the additive or synergistic benefit of combining gene therapy with an anti-inflammatory drug (MW151) that attenuates glial cytokine upregulation[7] or with bone marrow transplantation (BMT).[14] MW151 not only demonstrated positive effects upon behavioral and pathological measures of Cln1 disease, but further improved the efficacy of intracranial adeno-associated virus (AAV)-mediated gene therapy.[7] In the case of BMT,[14] immunomodulation by means of gamma radiation may have led to reduced adaptive immune responses, and the reduction of circulating antibodies against the therapeutic vectors may render the latter more effective. However, there is already evidence that glial responses contribute to disease outcome[4] in addition to lymphocyte infiltration. In this respect, while the Groh et al.[1] study is promising, targeting both the adaptive and innate immune responses may be required to improve therapeutic outcome further.

Efforts to devise an effective therapy for CLN3 disease have been even more challenging, given that the defect lies in a transmembrane protein, precluding ERT. Furthermore, the normal function of this protein and how its deficiency results in CLN3 disease remain poorly understood. Before recent gene therapy efforts,[10] investigators have largely relied upon blocking the well-defined downstream effects of the *CLN3* mutation that have been identified in mouse models. This includes attenuating an autoimmune response that is apparently specific to CLN3 disease, and either genetic or pharmacological blockades of antibody production were beneficial in Cln3-deficient mice.[6] This lead to a clinical trial of mycophenylate mofetil in CLN3 disease children (https://clinicaltrials.gov/ct2/show/NCT01399047), but no positive outcomes have been reported. CLN3 disease also shows an early glial response,[4] with localized activation of both astrocytes and microglia occurring before neuron loss. One recent strategy to target such apparently pro-inflammatory[15] neuroimmune responses is the administration of phosphodiesterase-4 (PDE4) inhibitors (rolipram, roflumilast, and PF-06266047) in *Cln3*$^{\Delta ex7/8}$ mice,[7] which produced positive effects on behavior and pathology. Groh et al.'s report of the favorable impact of fingolimod and teriflunomide in targeting CD8 effector cells[1] suggests that targeting other components of the immune response can also be beneficial in CLN3 disease. Given that these compounds are already clinically approved for use in other conditions, there is hope that they could potentially be used in CLN3 disease children.

Until the technical challenges to treat the primary genetic defects in CLN1 and CLN3 disease are successfully overcome, there is still a significant unmet need to provide some form of therapy for these disorders. In this respect, the findings of Groh et al.[1] are significant in showing therapeutic benefit across several disease measures in mouse models of two major forms of NCL. However, approaches that show promise in mice often fail to translate to the clinic, and the absence of side effects in mice cannot be taken as a guarantee that immunomodulatory approaches will not have adverse consequences in children with a life-limiting disease. It will therefore be important to weigh the benefits of such immunomodulatory therapeutic strategies against potential systemic side effects. These therapies were delivered over a period of 5 months,[1] which, although a long time for a mouse, is relatively short-term for a human. In addition, whereas clinical diagnosis would ideally be made as early as possible in patients, the reality is that treatment is more likely to be initiated only once the patients are already symptomatic. This is especially true of the earlier onset forms of NCL, such as CLN1 and CLN2 diseases. It will therefore be of equal clinical importance to study whether

initiating such therapies at later symptomatic time points has any effect on attenuating disease progression and whether there is a stage after which these diseases become refractory to such therapies.

The authors suggest that these drugs may "help to make the orphan CLN diseases more bearable for patients and their relatives at least until safe causal therapies may become available."[1] Such approaches may indeed prove to be useful clinically, but it is becoming more apparent that combinations of therapies may be required to provide better outcomes for these patients. Groh et al.'s findings may therefore provide another important part of the complex therapeutic jigsaw puzzle that is being assembled for these profoundly disabling disorders.

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Unmassked: Single-Cell Profiling of Immune Cell Populations in Tumors

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Extensive preclinical and clinical data accumulated over the last few decades have documented that host immunity can play paradoxical roles in both promoting tumor outgrowth and in promoting tumor control and sculpting the immunogenicity of tumors. T cells and, more specifically, CD8 T cells are considered to be essential for tumor killing, whereas the composition of the innate immune compartment serves to dictate T cell infiltration (secretion of chemokines), activation, phenotype, and functional capacity. Combined mapping of the innate myeloid compartment, primarily the monocytes/macrophages and dendritic cells, and the T cell compartment is thus a powerful method to dissect the immune ecosystem within the tumor microenvironment (TME). This

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