

## SUMMARY/OVERVIEW

Studies of MS lesions have revealed that spontaneous myelin repair can occur. It remains unclear, however, why repair either does or does not occur, but it is apparent that successful myelin repair is tightly linked to axon health and slowing of disease progression in MS. To harness this endogenous repair capability, it is important to uncover the molecular mechanisms that contribute to successful remyelination, and, ideally, be able to stimulate them using safe and effective therapeutics. With this goal in mind, we have recently investigated the ability of metformin to influence myelin repair. Metformin is a safe and effective drug that is used widely for the treatment of type II diabetes. Metformin is also in clinical trials for several neurodegenerative conditions, as metformin has been shown to have anti-inflammatory properties that are predicted to be beneficial in suppressing neuroinflammation. Recent studies have also found that metformin can significantly improve the ability of aged oligodendrocyte progenitor cells (OPCs), which are normally poor at responding to pro-differentiation cues following myelin damage, to undergo differentiation. Our preliminary findings now indicate that metformin can also significantly influence neonatal and young adult OPCs by altering oligodendrocyte bioenergetics, as well as promoting an accelerated transition from OPC to oligodendrocyte. **We also found that following cuprizone-mediated myelin damage, metformin was able to significantly improve remyelination in young adult mice.** This indicates that metformin may be useful across the broad spectrum of ages in MS patients, which is important as MS is a decades-long chronic disease that is typically diagnosed in young adulthood. Myelin promoting therapies are also being actively sought for neurodegenerative diseases such as AD in which myelin loss is a contributing factor to pathology, increasing the potential for translational benefit from these studies.

**Objective:** Determine the parameters and the underlying cell and molecular basis by which metformin influences myelin repair.

**Specific Aims:** (1) Determine the extent and limitations of metformin's ability to influence myelin repair, (2) determine the cellular mechanisms that underlie the ability of metformin to promote myelin repair, and (3) assess the transcriptomes of oligodendrocyte progenitor cells during myelin repair, +/- metformin treatment.

**Study Design:** **Aim 1.** Our pilot experiment revealed that at an early phase of recovery (1.5 weeks), in which only limited remyelination typically occurs, metformin stimulated a larger degree of seeming repair (increased MBP and increased colocalization of MBP with healthy axons). While this single time point was suggestive of accelerated repair, more work is needed to determine the optimal treatment window as well as the extent of metformin's influence. We therefore propose to examine metformin's effect when administered during ongoing demyelination (during cuprizone administration), or, when administered for different time periods during recovery following demyelination. We will then assess oligodendrocyte dynamics and myelin levels at several intervals during recovery (1, 2, 3, and 4 weeks) to develop a more complete picture of metformin's effect on myelin repair.

**Aim 2.** *First*, mice will be treated with the demyelinating agent cuprizone for 4 weeks as in Aim 1, then allowed to recover for 1, 2, 3, or 4 weeks in the presence or absence of metformin. Mice will be additionally injected with EdU daily for 1-week prior to tissue collection, in order to label all cycling cells, including OPCs. This will allow us to determine the fraction of newly-differentiated oligodendrocytes, as these will be EdU-labelled. *Second*, we will prepare tissue for transmission electron microscopy (TEM) in order to assess myelin ultrastructure. TEM will be used to determine the presence of uncompacted myelin, a hallmark of newly-wrapped axons that is not observed in mature myelin sheaths. In addition, we will also use TEM images to determine myelin thickness and the percentage of myelinated axons (both readouts for the extent of myelin wrapping, which is not appreciated using conventional immunohistochemistry). **Aim 3.** We will assess the transcriptomes of OPCs in response to metformin. RNA Sequencing will be performed on isolated fluorescent OPCs from Cspg4-mEGFP mice following recovery (+/- metformin treatment) from cuprizone-mediated myelin damage for 1, 2, 3, or 4 weeks. The transcripts that are up- or downregulated by metformin during repair will be used to generate hypotheses regarding potential signaling mechanisms, which may lead to further refinement of treatment paradigms and a better understanding of the molecular mechanisms that control myelin repair.

**Innovation:** We propose that a drug currently being used to combat insulin-resistance in type II diabetes may also have benefit as a myelin repair therapy in MS. We will capitalize on the novel finding that metformin not only alters oligodendrocyte cellular metabolism but accelerates the myelin repair process. We will uncover the changes in gene expression that underlie this effect in order to better understand the signaling mechanisms that underlie repair capability, as well as to discover additional targets for potential future therapies. Importantly, it should be noted that current therapies for MS have little or no impact on myelin repair. The proposed study will therefore provide important preliminary data, which we can then leverage for a larger extramural proposal that will lead to a deeper understanding of metformin as a potential myelin repair therapeutic, and hopefully lead to better treatment outcomes for MS patients, including those that do not respond to current therapies.