Cognitive Vitality Reports® are reports written by neuroscientists at the Alzheimer's Drug Discovery Foundation (ADDF). These scientific reports include analysis of drugs, drugs-in-development, drug targets, supplements, nutraceuticals, food/drink, non-pharmacologic interventions, and risk factors. Neuroscientists evaluate the potential benefit (or harm) for brain health, as well as for age-related health concerns that can affect brain health (e.g., cardiovascular diseases, cancers, diabetes/metabolic syndrome). In addition, these reports include evaluation of safety data, from clinical trials if available, and from preclinical models.

Methylene blue (and TRx0237)

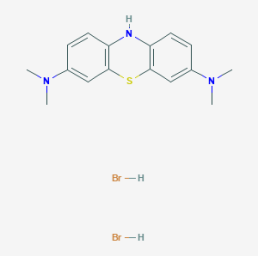
Evidence Summary

Despite several large trials, there is no clear overall benefit of methylene blue or derivatives in patients with dementia. These results may be confounded in part by issues with dosing and difficulty in blinding.

Neuroprotective Benefit: There is theoretical basis of neuroprotective benefit, and preliminary suggestion of benefit for postoperative delirium. However, all large trials have failed to find a clear benefit for dementia, in part because blinding is difficult.

Aging and related health concerns: There is no evidence that methylene blue is beneficial for human aging or age-related disease. Methylene blue extends maximum lifespan in female genetically heterogeneous (UM-HET3) mice.

Safety: Low-dose methylene blue is associated with gastrointestinal and urinary side effects. Higher doses come with side effects such as anemia and serotonin toxicity. Methylene blue should not be taken with serotonergic agents.

Availability: Methylene blue is available in clinical settings or by prescription. Methylene blue derivatives used in clinical trials are not commercially available.	**Dose**: Methylene blue dosing depends on indication and is often dosed by weight. Doses of 2 mg/kg are thought to be well tolerated; doses above 7 mg/kg are associated with adverse events. Methylene blue is typically administered intravenously, but oral formulations are available. TRx0237 is a methylene blue derivative that is an oral formulation; its dosing is still being optimized, and doses of 8 mg to 250 mg a day have been tested	**Methylene blue Chemical formula:** $C_{16}H_{18}ClN_3S$ **MW**: 319.85 g/mol  Source: PubChem **TRx0237 Chemical formula:** $C_{16}H_{21}Br_2N_3S$ **MW**: 447.2 g/mol  Source: PubChem
Half-life: 5 to 6.5 hours	**BBB**: Yes	
Clinical trials: Clinical trials of methylene blue and/or derivatives of methylene blue in MCI or dementia patients have included more than 2,800 individuals.	**Observational studies**: The largest identified observational study involved methylene blue treatment of patients in the ICU; the study included 223 patients.	

What is it?

Methylene Blue (methylthioninium chloride) is phenothiazine drug and dye. It is currently FDA-approved for the treatment of hereditary and acute methemoglobinemia. Methylene blue is used or explored off label for a variety of indications, including for vasoplegic syndrome or septic shock, as methylene blue may help to increase blood pressure; for a neurotoxic side effect of a chemotherapeutic agent called ifosfamide, known as ifosfamide-induced encephalopathy; for certain infections such as malaria, given its antimicrobial properties; and as a dye for intraoperative tissue visualization (Bistas & Sanghavi, 2023). Methylene blue acts as an inhibitor of nitric oxide synthase and guanylate cyclase; this inhibition leads

to vasoconstriction. It is also thought to have antioxidant capabilities and may promote mitochondrial activity, among other proposed roles (Tucker et al., 2018; Saha & Burns, 2020; Bužga et al., 2022).

Methylthioninium chloride is a redox molecule; that is, depending on its environment, it can be present in either its oxidized form known as methylthioninium, or its reduced form known as leucomethylthioninium (LMT). While most applications use methylthioninium chloride, several AD trials have used a modified, stable form of LMT that they have called leuco-methylthioninium bis(hydromethanesulphonate) (LMTM), hydromethylthionine mesylate (HMTM), or TRx0237, depending on the publication. These different versions of methylene blue are thought to have the same mechanism of action, but TRx0237 is thought to have improved absorption, bioavailability, and tolerance (Baddeley et al., 2015; Schelter et al., 2019). This report will largely discuss methylene blue to encompass all versions of the compound and will use the name of the formulation used in each respective publication.

Neuroprotective Benefit: There is theoretical basis of neuroprotective benefit, and preliminary suggestion of benefit for postoperative delirium. However, all large trials have failed to find a clear benefit for dementia, in part because blinding is difficult.

Types of evidence:

- 8 clinical trials
- 1 observational study
- 1 case report
- 2 news articles
- 2 reviews
- Multiple preclinical studies in animal and in vitro models

Human research to suggest prevention of dementia, prevention of decline, or improved cognitive function:

No human studies have tested whether methylene blue prevents dementia or age-related cognitive decline. Preclinical studies suggest that it does cross the blood-brain barrier (Peter et al., 2000).

One randomized clinical trial (RCT) investigated the effects of methylene blue in 26 healthy subjects between the ages of 22 and 62 (Rodriguez et al., 2016). Low dose (280 mg or ~4 mg/kg) methylene blue

increased insular fMRI activity during a sustained attention task as well as during short-term memory tasks, and significantly improved memory retrieval by 7% compared to placebo.

Another RCT examined the effect of low dose methylene blue (260 mg or 4mg/kg per day) on a fear extinction task in 42 subjects with claustrophobia (Telch et al., 2014). Results indicated that methylene blue administration improved fear extinction after 1 month follow up. It also improved contextual memory independent of its effects of fear extinction.

A randomized open-label study assessed the impact of methylene blue administration on postoperative delirium and early postoperative cognitive dysfunction. The study enrolled 248 elderly patients who were scheduled for non-cardiac surgery; they were randomized to receive either 2 mg/kg of methylene blue within the hour after induction of anesthesia or matching saline placebo. There were significantly fewer incidents of postoperative delirium in the methylene blue group as compared to the placebo group (7.3% vs. 24.2%, OR=0.24, 95% CI 0.11 to 0.53, $p < 0.001$); the same was seen with incidence of early postoperative cognitive dysfunction measured at postoperative day 7 (16.1% vs. 40.2%, OR=0.30, 95% CI: 0.16 to 0.57, $p < 0.001$) (Deng et al., 2021).

Human research to suggest benefits to patients with dementia:

Several studies of methylene blue or methylene blue derivatives have been conducted in patients with dementia. The results are controversial in the field and are complicated by the difficulty in blinding these trials. Methylene blue and its derivatives cause urine discoloration; therefore, it has been challenging to find an appropriate true placebo. Many studies have instead used lower doses of methylene blue to achieve the urine discoloration while presumably not achieving a biological effect, though later work indicated that perhaps the lower doses were also biologically active. It is unclear whether the urine discoloration is dose dependent and thus whether a lower dose does sufficiently blind patients, and it is unclear whether lower doses are in fact biologically active. As detailed below, the statistical analyses of the trials have attracted criticism.

A 24-week phase II dose-finding study in 321 patients tested the effect of methylene blue in patients with mild-to-moderate Alzheimer's disease not currently taking an acetylcholinesterase (AChE) inhibitor or memantine; the study was run by TauRX (Wischik et al., 2015). Patients were randomized to either 69, 138, or 228 mg total per day of methylthioninium chloride or matching placebo; doses were administered in three oral doses a day, taken with food. In patients with moderate Alzheimer's, the

group receiving 138 mg/day of methylene blue had reduced cognitive decline compared to those receiving placebo treatment (ADAS-cog scores dropped by 1 point from baseline, 5.42 points better than placebo). Likewise, there were improvements in ADCS-CGIC and MMSE scores compared to placebo and baseline. There were no significant differences between groups in cognitive performance in patients with mild AD, though the treatment group had reduced decline in relative cerebral blood flow than placebo group patients. Cognitive benefits were maintained during a 50-week extension on methylene blue. There were no benefits at the higher nominal dose of 228 mg/day; the authors speculate that this was because of a formulation issue that resulted in less drug being bioavailable in the 228 mg/day dose than in the 138 mg/day dose. Importantly, the authors referred to their placebo as a placebo and did not comment on the composition of the placebo beyond that it was similar visually to the methylthioninium chloride capsules.

TauRx tested TRx0237, a derivative of methylene blue with superior pharmaceutical properties in regards to solubility and PK, in a phase 3 study in 891 patients with mild-to-moderate Alzheimer's. Patients were randomized to either 150 mg TRx0237 (75 mg twice daily), 250 mg TRx0237 (125 mg twice daily), or a control treatment of 8 mg of TRx0237 (4 mg twice daily) in order to control for urine discoloration; dosing lasted for 15 months. The drug failed to have any impact on cognition or function. However, a secondary analysis on patients taking the drug as a monotherapy as compared to as an additional therapy on top of ChEIs and/or memantine showed significantly reduced rates of cognitive decline (ADAS-Cog, ADCS-CGIC, MMSE), functional decline, (ADCS-ADL) and lateral ventricular volume loss. These benefits were seen even when comparing those taking the placebo (8 mg) as a monotherapy versus add-on therapy. It should be noted that there were only small numbers of patients receiving TRx0237 as a monotherapy, with approximately 25 to 35 patients per group receiving TRx0237 as monotherapy and approximately 130 to 220 patients per group receiving TRx0237 plus other AD treatment (Gauthier et al, 2016).

Given the findings in Gauthier et al, 2016 and to further examine these treatment effects, TauRx modified the statistical analysis of a then-ongoing 18-month phase 3 trial of TRx0237 in 800 patients with mild AD. Before unblinding, they amended their statistical plan; their primary analysis was changed to (1) compare 200 mg TRx0237 (100 mg twice daily) as a monotherapy to the control treatment of 8 mg TRx0237(4 mg twice daily), and to (2) compare the control treatment as a monotherapy compared to the control treatment as an add-on therapy. Results from this study were similar to the secondary analyses of the previous study: the authors reported that patients taking 200 mg TRx0237 as a monotherapy had statistically significantly slower decline in cognitive assessments and brain structural

assessments as compared to all patients randomized to the control treatment of 8 mg TRx0237. Patients who received 8 mg TRx0237 as monotherapy also had significantly slower decline in cognitive assessments and brain structural assessments as compared to patients receiving 8 mg TRx0237 as add-on therapy. Exploratory analyses also split the 200 mg TRx0237 group into two subgroups: those taking TRx0237 as a monotherapy, and those taking TRx0237 as an add-on therapy, and also showed a statistically significant slowing of cognitive and structural decline for those receiving TRx0237 as a monotherapy instead of as an add-on therapy. The authors did not find any differences between patients receiving monotherapy of 8 mg TRx0237 compared to those receiving monotherapy of 200 mg TRx0237 (Wilcock et al, 2018).

Several scientists and statisticians at the conference pointed out that there is a fundamental difference between patients who are receiving AD therapies like ChEIs and/or memantine compared to those who are not; those receiving symptomatic AD treatment are likely more ill and declining faster (Schneider et al., 2011). Indeed, the patients who received LMTM as a montotherapy in Wilcock et al, 2018 were less cognitively impaired at baseline and had larger brain volume and glucose uptake on structural imaging than those who were receiving AD treatment; while the authors tried to statistically control for these variables, it is unclear whether the statistical control can replicate a trial of only people not on AD treatments, and using a true placebo. Critics also stated that one of the primary comparisons – that of the higher dose study drug as a monotherapy versus all patients who received the lower dose whether as a monotherapy or add-on therapy – was not an appropriate comparison (Alzforum, 2016).

TauRX then performed pharmacokinetic analysis of samples from the patients in the studies reported in Gauthier et al, 2016 and Wilcock et al, 2018 to assess drug concentrations and efficacy outcomes. They reported a plasma drug concentration-response relationship for the drug at the 8 mg/day dose, and that higher doses were not associated with greater efficacy, as though the drug response had plateaued. They did identify pharmacological action even as add-on therapy, but with a lower effect size. They hypothesized that a monotherapy regimen of 16 mg per dose would achieve the plasma concentrations necessary to be pharmacologically active and that this would be the maximal effective dose (Schelter et al., 2019). A pharmacokinetic analysis of patients from a trial in frontotemporal dementia (FTD) yielded similar results, with significant exposure-dependent differences at 8 mg per day, but worse outcomes at higher concentrations (Shiells et al., 2020).

TauRX proceeded to run a trial in patients with MCI or dementia who were not on ChEIs or memantine. The protocol underwent several significant changes over the course of the study, including changes to

inclusion criteria, outcome measures, and duration of study. The final study protocol was published by Wischik et al., 2022. The trial, known as Lucidity, enrolled 598 individuals with MCI or mild to moderate AD, and tested the same derivative of methylene blue as Gauthier et al, 2016 and Wilcock et al, 2018 used, though the compound was called hydromethylthionine mesylate (HMTM) in the Lucidity trial. The participants were randomized to one of three groups: control, 8 mg a day HMTM, or 16 mg a day HMTM. In order to control for the urine discoloration, the control group received approximately 2 tablets a week of 4 mg methylthioninium chloride, the oxidized version of the compound; the rest of the tablets they received were blank. Therefore, the control dose was lower than the active treatment and a formulation that was hypothesized to be less bioactive than HMTM, but the control dose nonetheless contained a potentially active ingredient. The trial was double-blinded and randomized for 12 months and was followed by a 12-month modified delayed-start open-label treatment phase. The primary endpoints assessed the change in cognitive function from baseline to week 52 in the group receiving 16 mg a day HMTM compared to the group receiving control. The results of this trial have not been published in a peer-reviewed journal.

At CTAD 2022, TauRX announced that the trial failed to meet its primary endpoint; there was no difference in cognitive function from baseline to the end of the double-blind phase between the control group and 16 mg HMTM group. They also announced that a majority of the control group had blood levels of active drug that TauRX believed to be clinically active, and they hypothesized that this was why they didn't see a difference between the intervention group and control group (Medscape). At the Alzheimer's Disease / Parkinson's Disease (ADPD) 2024 conference, the authors announced that at the end of the 12-month doubled blinded phase, participants with MCI in the 16 mg / day group had a 48% lower incidence of progression to dementia diagnosis as measured by CDR score than MCI patients in the control group; some critics have pointed out that many trials use CDR-SB, as CDR-SB is thought to be a more sensitive tool in early stages of dementia. The authors also assessed the cognitive function assessments in the MCI group; while the high dose HMTM group did not have a significant change from their baseline cognitive scores and the end of the full 24-month trial, the control group appeared to decline, and the difference between the high dose and control group was statistically significant. It is worth nothing that at the end of the 12-month randomized phase, there was no difference between these groups in terms of cognitive function; the difference only became significant at 18 and 24 months, when the control patients were then receiving 16 mg of HMTM daily. The investigators posit that this was due to disease progression in the first year of the trial. In terms of biomarkers, the investigators announced that at 12 months, there was a greater increase in levels of neurofilament light levels in control patients as compared to 16 mg daily HMTM patients. This was said to be a pre-specified analysis,

though it was not in the published protocol. Finally, the authors compared the cognitive decline and brain volume decline of all patients in the trial (including control group) with matched controls from ADNI, as well as meta-analytic controls from other trials, and found that patients in the Lucidity trial had significant reduction in cognitive decline and brain atrophy. This, too, attracted criticism at the conference, with some scientists noting that comparisons to ADNI or patients in other trials cannot replace proper controls (Alzforum).

As discussed briefly above, this drug has also been tested in patients with frontotemporal dementia (FTD); as FTD is a tauopathy and LMTM is thought to affect tau levels and/or aggregation, there was interest in whether LMTM might have beneficial effects in FTD patients. The study enrolled 220 patients with FTD and randomized them to either 8 mg LMTM daily, or 200 mg LMTM daily. The trial failed to meet any primary or secondary outcomes; there were no significant differences between the groups; as in the above AD trials, the authors posit that this is because the 8 mg daily dose was pharmacologically active. Posthoc analyses suggested that in the group that received 8 mg LMTM daily, that patients with higher plasma drug concentrations had significantly less decline on cognitive and functional measurements, but the authors caveat that these are hypothesis generating (Shiells et al., 2020). There is also a case report of a single individual with a familial mutation associated with FTD; he received compassionate treatment with LMTM at the typical age of onset of clinical decline for his family and continued for 5 years. Over the course of the 5 years of treatment, the patient remained asymptomatic and experienced less brain volume atrophy than anticipated. However, as this is a case report and a rare mutation, it is difficult to draw any conclusions at this time (Bentham et al., 2021).

The lack of a true placebo and biological activity of lower doses in some trials but not others make it challenging to interpret the findings as a whole. TauRX is pursuing marketing authorization in the UK and is under discussions with regulatory agencies in other countries and regions. They have put forward several possible explanations for why their drug is not effective as an add-on therapy, discussed in the 'Mechanisms of action' section below.

Mechanisms of action for neuroprotection identified from laboratory and clinical research:

Methylene blue and TRx0237 treatment in animal models of Alzheimer's disease and tau suggest mixed results. Some show benefits when treatment is administered before the onset of cognitive deficits but not after. Some suggest they can reverse cognitive deficits. Mixed results suggest that methylene blue and TRx0237 may reduce tau levels, reduce amyloid beta, increase autophagy, increase proteasome

activity, reduce aggregation of α-synuclein, and improve cognition (Medina et al., 2011; Congdon et al., 2012; Spires-Jones et al., 2014; Hochgräfe et al., 2015; Melis et al., 2015; Zakaria et al., 2016; Schwab et al., 2022), while other studies have not replicated these findings; for instance, one preclinical study found that neither methylene blue nor LMTM provided protection against tau-mediated toxicity (Lim et al., 2023).

Methylene blue reduces oxidative stress in AD-like tau and β-amyloid aggregation in *in vitro* models (Wischik et al., 1996). *In vitro*, methylene blue reduces mitochondrial superoxide production and mitigates free radical formation (Poteet et al., 2012). Methylene blue may influence downstream events of mitochondrial respiration including lipid β-oxidation, glycolysis, ATP synthesis, ECM production and Na+/K+ ATPase activity, thereby contributing to increased neuronal oxidative metabolic capacity (Rojas et al., 2012). Methylene blue can readily cycle between oxidized (MB) and reduced (MBH_2) forms, thereby serving as an electron carrier within the electron transport chain in the mitochondrial matrix (Atamna & Kumar, 2010). Further, methylene blue treatment leads to increases in cellular O_2 consumption, ATP production and glucose uptake in primary astrocytes (Roy Choudhury et al., 2015).

Several reasons have been put forward for a mechanistic reason why methylene blue may only have a benefit as a monotherapy instead of as a combination therapy with ChEIs and/or memantine. One study in a tau-transgenic mouse line suggested that HMTM partly normalized expression patterns of proteins that are absolutely required for synaptic vesicle release, known as SNARE proteins, and that this effect was diminished when the mice were given rivastigmine, a ChEI, as well as HMTM (Schwab et al., 2024). Other studies have suggested that HMTM promotes cholinergic signaling in the mouse hippocampus and this effect is abrogated when the mice are pretreated with rivastigmine (Kondak et al., 2022); that in tau transgenic mice, rivastigmine and memantine negatively affect mitochondrial respiration, and HMTM could not compensate for those negative effects (Kondak et al., 2023); and that in tau transgenic mice that show cholinergic impairment, HTMT partly reduced this impairment, but co-administration with rivastigmine was less efficacious at reducing the impairment (Zadrozny et al., 2024). These results all came from the same group; it remains to be seen whether these effects are replicable, and also whether they translate to humans.

APOE4 interactions:

A phase 3 study reported no interactions with ApoE4 (Wilcock et al, 2018)

Aging and related health concerns: There is no evidence that methylene blue is beneficial for human aging or age-related disease. Methylene blue extends maximum lifespan in female genetically heterogeneous (UM-HET3) mice.

Types of evidence:

- 2 meta-analyses or systematic reviews
- 2 observational studies
- 1 case report
- 6 reviews
- 1 lifespan study by the NIA's Intervention Testing Program
- Multiple lab studies in cell culture and 3D-organoid cultures

Methylene blue is known to have benefit and is approved for use for conditions unrelated to age, such as methemoglobinemia. It is also used for a variety of off-label indications and can provide benefit regardless of age, including for vasoplegic syndrome and septic shock; treatment for brain swelling associated with a chemotherapy called ifosfamide, a condition known as ifosfamide-induced encephalopathy; as an intraoperative imaging agent for cancer resections and other surgeries; and potentially as an antimicrobial agent for infections such as malaria (Bistas & Sanghavi, 2023).

Studies have explored or are exploring other potential uses. Methylene blue has been investigated for treatment of pain (reviewed by Lee & Han, 2021), as a combination antimicrobial therapy such as for periodontal infections (Perumal et al., 2024) or superficial fungal infection (Dong et al., 2023), and for hypoxemia (Arastoo et al., 2023), among other indications. It has also been suggested as a treatment for COVID-19 (Emadi et al., 2024).

Longevity and Aging: PRECLINICAL RESEARCH, BUT NO KNOWN BENEFIT IN HUMANS
Although no clinical trials have tested the effects of methylene blue on aging and age-related disorders, several mechanisms have been proposed that provide some evidence for the beneficial effects on aging; some are reviewed by Xue et al., 2021. The NIA's Intervention Testing Program (ITP) tested the effects of 27 mg/kg methylene blue in 4-month-old mice. Although there was no change in median lifespan in either sex, methylene blue significantly increased maximum lifespan by 6% in females compared to placebo (Harrison et al., 2014).

Methylene blue treatment is also shown to induce autophagy, both *in vivo* and *in vitro* (López-Otín et al., 2013).

Recent evidence in human skin fibroblasts from healthy donors and accelerated aging progeria patients showed that methylene blue attenuates reactive oxygen species in both groups. In human 3D skin organoid cultures, methylene blue improved skin viability, improved wound healing, increased skin hydration and dermis thickness (Xiong et al., 2017).

Similar to multiple well-established interventions of aging, methylene blue activates AMPK, thereby inducing macroautophagy *in vitro* and in cortex and hippocampus of methylene blue treated mice, and mitigates autoimmune encephalitis by activating AMPK/SIRT1 axis, inhibiting NF-κB and alleviating helper T-cell and T-regulatory responses (Xie et al., 2013; Wang et al., 2016).

Safety: Low-dose methylene blue is associated with gastrointestinal and urinary side effects. Higher doses come with side effects such as anemia and serotonin toxicity. Methylene blue should not be taken with serotonergic agents.

Types of evidence:

- 3 clinical trials
- 1 review
- 1 professional resource

Methylene blue is on WHO's list of essential medicines with a high safety profile. Methylene blue follows a hormetic response, where it is safe to consume in low doses but can be toxic at relatively higher doses of >5 mg/kg. Since it is a monoamine oxidase inhibitor, intravenous administration can cause serious serotonin toxicity if combined with selective serotonin reuptake inhibitors, including duloxetine, venlafaxine, sibutramine, clomipramine, or imipramine (Gillman, 2006). Clinical trials report gastrointestinal side effects are most common.

Clinical trials have assessed the safety and efficacy of methylene blue or methylene blue derivatives in patients with AD, though it should be noted that some of these trials used a low dose of methylene blue as a control treatment, which confounds assessment of safety events to some extent. One study of 321 patients with mild to moderate AD randomized patients to placebo, 69 mg, 138 mg, or 228 mg of

methylthioninium daily. The authors reported that there were more adverse events and adverse events that led to transient or permanent discontinuation of treatment in the 138 mg and 228 mg treatment groups compared to placebo. There were 8 deaths; 6 were in the treatment groups, though none were attributed to the study drug. Methylene blue was associated with a dose-dependent decrease in red blood cell count (Wischik et al., 2015).

A 15-month study of 891 participants with mild to moderate AD compared 150 mg LMTM daily (75 mg twice a day) or 250 mg LMTM (125 mg twice a day) to control treatment of 8 mg LMTM (4 mg twice a day). The authors found that there was no difference in overall number of treatment-emergent adverse events. Gastrointestinal and urinary related AEs were the most common events and were more common in the higher doses of LMTM. There were no differences in rates of serious adverse events or deaths between groups. There were more discontinuations overall as well as a greater number of withdrawals due to adverse events in the highest dose group; for instance, 9% of the high dose group discontinued for gastrointestinal or urinary adverse events, compared to 2% in the control group (Gauthier et al., 2016). Another study of LMTM enrolled 800 patients with mild AD and tested two doses: 200 mg LMTM (100 mg twice a day) or 8 mg LMTM (4 mg twice a day) as control treatment also reported more gastrointestinal and urinary adverse events in the higher dose group compared to the lower dose group, and adverse events leading to discontinuation were much more common in the higher dose group compared to lower dose group (10% vs 2%, respectively, for gastrointestinal and urinary adverse events). The authors noted that there was a higher incidence of gastrointestinal adverse events in the group receiving both AD treatment in the form of ChEIs and/or memantine compared to those receiving only LMTM (Wilcock et al., 2018).

Individuals with Glucose-6-phosphate dehydrogenase (G6PD) deficiency should not be treated with methylene blue. Additionally, apart from common side effects like headache, sweating, nausea and limb pain, methylene blue can cause weakness and confusion, skin discoloration, blue-ing of urine, high serotonin levels causing hallucinations and fever (Drugs.com – injectable; Medicine.net - oral).

Methylene blue can cause harm to the fetus when administered to a pregnant woman. Epidemiological studies suggest that methylene blue is a teratogen. The use of methylene blue in amniocentesis may be associated with atresia of the ileum and jejunum, ileal occlusions and other adverse effects in the neonate (Drugs.com).

At high doses, methylene blue is shown to disrupt the mitochondrial electron transport chain and can be toxic. Non-pharmaceutical doses of methylene blue contain trace doses of arsenic, cadmium, aluminum, mercury and lead, which in high doses can be extremely toxic.

Drug interactions:

Methylene blue is known to interact with at least 196 drugs. Of these interactions, 129 are major, 65 are moderate, and 2 are minor. Methylene blue should be used with caution or not at all in conjunction with serotonergic drugs, such as selective serotonin reuptake inhibitors (SSRIs), serotonin norepinephrine reuptake inhibitors (SNRIs), monoamine oxidase inhibitors (MAOIs), tricyclic antidepressants, and certain other antidepressants and antipsychotics; use of methylene blue and one of these drugs in close time proximity can lead to serotonin toxicity, also known as serotonin syndrome, due to the MAOI activity of methylene blue. Methylene blue is not recommended for use with prescription pain medication (Drugs.com; Bistas & Sanghavi, 2023).

Methylene blue is contraindicated for use in patients with genetic deficiency of glucose-6-phosphate dehydrogenase (G6PD). As methylene blue can affect renal blood flow, it should be used with caution, if at all, in patients with impaired renal function (Bistas & Sanghavi, 2023). If there are any other specific interactions of TRx0237, they are not yet known.

Research underway:

There are 61 studies on clinicatrials.gov that involve or are investigating use of methylene blue; many of these are exploring use in treatment of infection, pain, or involve using methylene blue as a tracing agent during surgery, such as for detection of sentinel lymph nodes during cancer resections. There are no ongoing studies for TRx0237 registered on clinicaltrials.gov.

One study, NCT05894954, is investigating whether a precision medicine approach can mitigate the decline of cognitive function in patients with mild cognitive impairment or early dementia. The 9-month long study aims to enroll 72 patients who will be randomized to standard of care or to the personalized medicine approach. The personalized medicine approach will involve a battery of testing to create a personalized treatment plan including diet, sleep habits, stress management, and mental and physical

exercise. One of the many potential treatments in the intervention group is treatment with methylene blue, if applicable. The project is predicted to end in fall of 2025.

Search terms:

Pubmed, Google: methylene blue, TRx0237, methylthioninium, LMTM, HMTM

- Alzheimer's, APOE, aging, pain, COVID-19, dementia, antifungal, malaria, cognition, postoperative delirium

Websites visited for methylene blue:

- Clinicaltrials.gov: Methylene blue; TRx0237 (0)
- Examine.com: Methylene blue; TRx0237 (0)
- Drugs.com: Methylene blue; TRx0237 (0)
- PubChem: Methylene blue; TRx0237
- DrugBank.ca: Methylene blue; TRx0237