Title: Should nitrous oxide ever be used in Oncology patients receiving methotrexate therapy?

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Summary

Nitrous oxide (N_2O) is frequently used for short anaesthesia/analgesia in children undergoing painful or repetitive procedures[1]. Children with acute lymphoblastic leukaemia (ALL) require repeated lumbar punctures with direct instillation of intrathecal chemotherapy, usually the anti-folate agent Methotrexate, during their treatment. These procedures are frequently performed under anaesthesia. Concerns have been intermittently raised about a drug-interaction between methotrexate and N_2O that may potentiate the undesirable side effects of methotrexate, including neurotoxicity. However, the clinical evidence consists mainly of isolated case reports leading to a lack of consensus amongst paediatric anaesthetists about the relative risk-benefits of using N_2O in children with ALL. In this article, we review the biochemical basis and scientific observations that suggest a significant interaction between N_2O and methotrexate due to their dual inhibition of the key enzyme methionine

synthase. The possible role of this interaction in potentiating neurotoxicity in children with cancer is discussed, and arguments and counter-arguments about the clinical significance of this largely theoretical relationship are explored. Following comprehensive review of all the available data we make the case for the circumstantial evidence being sufficiently compelling to prompt a review of practice by paediatric anaesthetists and call for a precautionary approach by avoiding the use of N_2O in children receiving concurrent methotrexate.

Introduction

N_2O is still a widely used anaesthetic and is often used for short anaesthesia/analgesia in patients undergoing painful or uncomfortable procedures such as dressing changes, lumbar punctures and intrathecal chemotherapy. Concern has been intermittently raised about N_2O toxicity. In Denmark during the 1950s several patients with tetanus were given N_2O for a period of days to facilitate ventilation and suffered bone marrow toxicity [2]. Haematological toxicity of prolonged N_2O therapy has since been well documented, but a number of questions remain regarding neurotoxic effects, amplified toxicity when co-administered with other drugs, and the impact of short, but repeated, bouts of exposure.

It is increasingly appreciated that children receiving chemotherapy for acute lymphoblastic leukaemia (ALL) may experience adverse neurological and neurocognitive outcomes, both acutely and long-term, with methotrexate implicated as the main causative agent[3,4]. Neurotoxicity takes many forms. Some patients experience devastating acute neurological side effects with highly varied clinical presentations, including stroke-like syndrome, seizures and paralysis. Others have evidence of subclinical white matter changes (leukoencephalopathy) on MRI scanning[5] – a finding that has been linked to long-term adverse neurocognitive and neurobehavioral outcomes[6]. Finally, up to 40% of long-term survivors display some measurable defects in neurocognition, the commonest being impairments in processing speed, memory and executive function[7]. These deficits are persistent, even decades after treatment, and have been shown to have negative impacts on educational attainment and employment[8].

The risk factors for these adverse neurological outcomes are not clearly established and some clinical features of neurotoxicity suggest that environmental factors may be important determinants of risk[9]. Two recent case reports highlighted severe neurotoxicity in children undergoing intrathecal

methotrexate treatment for ALL with a drug interaction between N_2O and methotrexate being postulated as a possible contributing factor[9,10]. Despite this, N_2O is still a popular choice of anaesthetic agent for these children, with a recent paper even discussing the potential benefits of its use in children with leukaemia[11].

In this article, we review the biochemical basis and scientific observations that suggest a significant interaction between N_2O and methotrexate and discuss the possible role of this interaction in potentiating acute and chronic neurotoxicity in vulnerable patient groups. We make the case for the circumstantial evidence being sufficiently compelling to prompt a review of practice by paediatric anaesthetists and call for a precautionary approach by avoiding the use of N_2O in children receiving concurrent methotrexate.

N_2O mechanism of action and use in anaesthesia

N_2O is one of the oldest anaesthetic drugs, it was first synthesised by Joseph Priestly in 1772 and widely experimented upon by Humphry Davy who recognised its analgesic properties in the early 19th century. Its popularity in anaesthesia is credited to Horace Wells, a dentist practicing in the United States of America who began using N_2O clinically in 1844[12]. Today N_2O is supplied as a pressurised liquid in cylinders and used as a gas as it vaporises upon reduction of pressure during administration. It may be supplied as pure N_2O or in a cylinder with 50/50 mixture of Oxygen and N_2O (Entonox).

Despite the advent of many newer anaesthetic agents, N_2O remains extremely popular with anaesthetists today and is used in over 20% of anaesthetics[13-15]. The potentially beneficial properties of N_2O during anaesthesia include:

i) The second gas effect: As N_2O is rapidly absorbed from the alveolus, it has the effect of increasing the relative concentration of other gases and therefore their partial pressure and their own uptake is increased. This creates more rapid induction to the desired state of anaesthesia. Similarly, when N_2O is ceased it will diffuse rapidly out of circulation, expanding the alveolus and create a favourable concentration gradient for the rapid exhalation of volatile agents and speeding up recovery from anaesthesia[16].

ii) Contribution to the depth of anaesthesia; N_2O has a stable profile concerning the cardiovascular system in contrast to some volatile agents, so the incorporation of N_2O in an anaesthetic allows

reduction in the dose of volatile agent and potentially a more stable patient. A large audit on unintentional awareness also pointed towards a role for N_2O in the provision of adequate depth of anaesthesia during Caesarean Section[17,18]

iii) At atmospheric pressure N_2O cannot provide surgical anaesthesia and the ceiling effect on the CNS at a level of 'sedation' have proven a useful property for physicians and other health professionals not wishing to inadvertently administer deeper planes of anaesthesia with their attendant risks of cardiorespiratory depression leading to the requirement for pre-procedural starvation along with a higher degree of both medical supervision and monitoring.

iv) Analgesia; N_2O promotes release of endogenous opiates in the central nervous system, however its principal analgesic action seems to be effected via N-methyl-D-aspartate (NMDA) inhibition [19] unfortunately its effects on prevention of chronic pain are confined to patients with a variant methylenetetrahydrofolate reductase (*MTHFR*) gene [20]

Historically, N_2O has earned a deserved role in as an agent for brief procedural sedation in children due to the combined properties of physiological stability, rapid dose titration, administration by inhalation, analgesia and especially, low potential for acute over-dose. None of the alternative sedative agents be they opiates, benzodiazepines or intravenous anaesthetic agents such as ketamine or propofol can match this and although large comparative outcome studies are lacking[21], expansion of other agents into the widespread role for sedation occupied by N_2O has not occurred. Studies do exist confirming the acceptability and efficiency and extremely low acute complication rate of Nitrous administration in preference to other sedation agents for lumbar puncture[11,22,23]. These are important to recognise as counterbalancing this, it does have a number of undesirable properties, for example:

i) It is a greenhouse gas.

ii) It may reduce fertility in healthcare workers[24]

iii) It causes trapped gas in the gastrointestinal tract to expand and is implicated in postoperative nausea and vomiting.

iv) It can cause megaloblastic anaemia with prolonged or repeated use [25,26].

iv) And most importantly for this article, it has been reported to cause severe neurological injury, in the form of subacute combined degeneration of the cord in patients with occult vitamin B_{12} deficiency[27,28]. The British National Formulary lists the side effects of N_2O as depression of white blood cell formation, hypoxia and neurological toxic effects, linking this to 'interference with the action of vitamin B_{12}' and noting that 'neurological side effects can occur without preceding, overt haematological changes' [29]. The links between methotrexate, vitamin B_{12}, N_2O, and haematological and neurological toxicity are summarised in figure 1.

Methotrexate use in leukaemia

Methotrexate is a common component of paediatric and adult acute lymphoblastic leukaemia and lymphoma treatment regimens. It is administered intravenously, orally and intrathecally, the latter largely replacing cranial and spinal radiotherapy in the 1990's as a method to eradicate leukaemic cells from the CNS, reducing the risk of relapse. However, both acute and chronic neurological side effects are reported following methotrexate use, including seizures, leukoencephalopathy and stroke-like syndrome [5,30-32]. Additionally, long-term neurotoxicity manifesting as neurocognitive impairment is an emerging concern affecting a substantial number of childhood leukaemia survivors [33-35].

Briefly, methotrexate works by inhibiting the enzyme dihydrofolate reductase (DHFR), stopping the cell from processing folate into tetrahydrofolate (THF) required for DNA synthesis, eventually leading to cell death. As with many older chemotherapy agents, this effect is not cancer-cell specific and patients on methotrexate experience drug toxicity, particularly in rapidly-dividing cells such as those of the gut and bone marrow with mucositis and low blood counts being commonly reported side effects. This is a result of inhibition of purine and pyrimidine synthesis – the building blocks for DNA (fig 1). In paediatric leukaemia patients, folate rescue in the form of leucovorin is typically given 24-42 hours after intravenous high-dose methotrexate therapy in an attempt to limit toxicity in normal tissues, whilst still allowing time for the drug to function on leukaemia cells.

Not all patients experience methotrexate neurotoxicity and the range of clinical manifestations is vast and complex, meaning it has been difficult to make progress on identifying risk-factors that predispose to these distressing and sometimes disabling side-effects. Although the intensity of methotrexate therapy[36] and various pharmacokinetic parameters[5] are implicated in some studies, there remains

considerable variation in occurrence of neurotoxicity between patients despite similar drug exposure. Paediatric leukaemia treatment regimens involve multi-agent chemotherapy plus additional drugs to manage treatment-related side effects. We hypothesized in a previous paper that interactions with other drugs and/or low nutrient levels might contribute towards explaining the variability in susceptibility to neurotoxicity. Some drugs have a direct interaction resulting in increased methotrexate drug levels (e.g. fluoroquinolone antibiotics). A more insidious cause could be the interference with the same metabolic pathways as methotrexate and, in particular, additive effects on methionine synthase activity.

The mechanisms of both acute and chronic methotrexate neurotoxicity are incompletely understood but experimental and clinical evidence points to two main pathways. Firstly, by activating microglia resulting in impaired oligodendrocyte maturation and myelination[37], and secondly reduced synthesis of methionine from its precursor homocysteine – a reaction catalysed by the key enzyme methionine synthase. The latter results in excess homocysteine and a reduction in methionine both of which may be neurotoxic as shown in figure 1. Homocysteine excess has been shown in preclinical animal models to result in cognitive impairment via activation of the NMDA receptor[38] and NMDA blockade is able to reverse these cognitive defects[39]. This is supported by human studies confirming a link between elevated homocysteine levels and seizures (acute neurotoxicity) (29) as well as elevated myelin basic protein in CSF (suggesting demyelination and chronic neurotoxicity) [40,41]. The accompanying reduction in methionine levels may also results in downstream effects on myelin sheath homeostasis and regulation of lipid production and thus potentially synergizes with the direct effect on oligodendrocyte myelination. Thus, reduced conversion of homocysteine to methionine appears to be a key factor underlying methotrexate-induced neurotoxicity. It is critical to note that the enzyme catalyzing this reaction– methionine synthase is directly inhibited by N_2O, and N_2O exposure has been shown to significantly increase plasma homocysteine concentrations after surgery[42,43]. Thus, methotrexate and N_2O directly impinge on the same chemical reaction leading to the potential for enhanced toxicity even with low doses or short exposures. The principle of enhanced neurotoxicity by a double hit on this pathway is illustrated by a case report in the New England Journal of Medicine (NEJM) describing the neurological deterioration and death of a child anaesthetised twice with N_2O prior to a diagnosis of methyltetrahydrofolate reductase deficiency[44].

Neurotoxicity may be further compounded by a second interaction with low vitamin B_{12} levels[45]. Methionine synthase requires vitamin B_{12} as an essential co-factor. N_2O prevents formation of active (oxidized) B_{12} and this results in irreversible inactivation of methionine synthase (Figure 1). Restoration of activity requires new synthesis of the enzyme. In the presence of B_{12} deficiency, any impact of methotrexate and N_2O on methionine synthase activity is likely to be significantly amplified. One small paediatric study showed significant correlation between plasma homocysteine rises in response to N_2O and pre-operative vitamin B12 levels[46]. Low B_{12} levels could be dietary or due to other drugs such as proton pump inhibitors, which prevent absorption of vitamin B_{12} via blocking the production of intrinsic factor [9]. Paediatric leukaemia patients may have poor nutritional status secondary to drug side effects such as enteropathy, mucositis and anorexia. Although B_{12} levels are not routinely measured in most centres, one small study, conducted in India, documented low B_{12} levels in 25/80 (31%) of children completing ALL therapy [47].

Much of the heterogeneity in occurrence of neurotoxicity in children given the same intrathecal methotrexate regime could theoretically stem from these additional factors such as interacting drugs, low B_{12} levels and other nutritional deficiencies. Moreover, these factors could significantly influence the sensitivity to N_2O, which would have additive effects by inactivation of vitamin B_{12}. A dose of N_2O that was harmless to a B_{12} replete individual on a low dose of oral methotrexate may have significantly different effects in the presence of directly injected intrathecal methotrexate in a patient with already borderline or low B_{12} levels.

Evidence of a methotrexate/ N_2O **interaction in patients**

Of course, the evidence presented above is largely theoretical and/or circumstantial. The best direct evidence for N_2O potentiating methotrexate toxicity comes from studies in breast cancer. Two studies involving large numbers of breast cancer patients (368 and 2466, respectively), most of whom received N_2O anaesthesia concluded that unpredictable toxic effects of methotrexate such as low blood counts and mucositis were likely due to the combined effect of methotrexate and N_2O on folate metabolism, which was at least partially rescued by the administration of leucovorin [48,49]. Unfortunately, these studies did not evaluate acute or chronic neurological outcomes. However, two case studies have also been published suggesting a link between neurotoxicity and concomitant N_2O and methotrexate use[9,10].

198 Briefly, the first case report involved a 7-year old patient who experienced severe neurotoxicity
199 following her third intrathecal methotrexate dose[10]. Her first LP had been performed under general
200 anaesthesia but the second and third had used N_2O/oxygen gas mix (50:50). She presented with
201 dysarthria followed by a decreasing level of consciousness (Glasgow coma scale 4). No infective
202 cause was found and MRI showed leukoencephalopathy with signs of vasogenic oedema. The most
203 likely cause was methotrexate neurotoxicity possibly potentiated by the concomitant use of N_2O. The
204 patient made a slow recovery over several months and was not exposed to further intrathecal
205 methotrexate.

206 The second case report involved a 12-year old patient who experienced seizures, limb weakness and
207 agitation after her fifth dose of intrathecal methotrexate administered under N_2O anaesthesia[9]. Her
208 serum vitamin B_{12} levels were measured three weeks after her last intrathecal MTX and found to be
209 below the normal range at 154ng/L (normal range 200-1100ng/L) She made a clinical recovery over 2
210 weeks but was left with some residual left upper limb weakness [9].

211 Also of interest, is the perceived lower incidence of neurotoxic effects in adult patients with ALL
212 receiving intrathecal methotrexate treatment. Much of this is anecdotal with little direct supporting
213 evidence in the literature. The best comparison comes from the NOPHO ALL2008 trial, which treated
214 patients up to age 45. Seizures were reported in 6.1% of 10-17 year olds but only 2.4% of 18-45 year
215 olds this did not reach statistical significance [50]. Notably, adult patients usually undergo lumbar
216 puncture without sedation or general anaesthetic so are unlikely to be exposed to the combination of
217 N_2O plus intrathecal methotrexate. This evidence is highly circumstantial but is a possible avenue for
218 further investigation.

219 Although these case reports and clinical observations are clearly not conclusive, we believe they may
220 be indicative of a larger problem, which requires further study and immediately warrants more caution
221 in the field.

222 **Supporting pre-clinical evidence**

223 Although the clinical data above are limited, there are also a number of supporting observations using
224 *in vitro* and *in vivo* models. Interestingly, N_2O has been historically touted as a possible treatment for
225 cancers and a potentiation agent for methotrexate. N_2O was first reported as a possible treatment for
226 CML in 1959 [51]. Tests exposing leukaemia cell lines to N_2O showed a reduction in growth via impaired

5-methyl-THF metabolism. Additionally, N_2O exposure of the leukaemic U937 cell line and primary leukaemia samples revealed a disruption of nucleotide synthesis, which was accentuated by concomitant exposure to methotrexate [52].

A number of animal studies have explored the link between N_2O and methotrexate. One such study exposed rats to N_2O for 12-48 hours before dosing with a single intraperitoneal injection of 10-80mg/kg body weight methotrexate (equivalent to routinely used doses in paediatric leukaemia treatment)[53]. The 50% lethal dose for methotrexate was reduced six-fold from 60mg/kg to 10mg/kg in rats pre-exposed to a mixture of 5-50% N_2O for 48 hours and some increase in lethality was observed with 12-hour exposures. Combining methotrexate and N_2O also showed increase anti-leukaemic effect in rat myeloid leukaemia models [54]. Separate studies using 12-hour exposures showed that methionine synthase activity is completely suppressed and the conversion of methyl-THF to other folate variants needed for purine and thymidylate synthesis is also impacted [55,56]. A 4-hour exposure of N_2O was also shown to reduce 90% of hepatic methionine synthase activity, with a 50% reduction in tetrahydrofolate. In this model, it took 48 hours exposure to normal air for the methionine synthase levels to return to normal [57].

It is difficult to ascertain how these long exposures in rats relate to the normally relatively short exposures experienced by children receiving methotrexate therapy for childhood leukaemia treatment. However, Entonox is 50% N_2O, representing the highest volume used in this study, with other work [58], showing that exposure of a 2% mixture for 15 hours resulted in a 30% inhibition of methionine synthase in rat liver. Most strikingly however was that 15 minutes of exposure to 50% N_2O caused a 55% reduction in methionine synthase activity in the liver, with a 1-hour exposure reducing methionine synthase activity to just 30% of controls. These levels of exposure are clinically relevant, with most children undergoing short durations of anaesthesia in the range 10-30 minutes for lumbar puncture administration of methotrexate. Although methionine synthase activity has been measured in livers of these rats, it is reasonable to hypothesise that neural tissue may be similarly affected, especially since in patients the methotrexate is injected directly into the central nervous system.

Current state of play

As can be seen from the discussion above there is no single definitive piece of evidence that proves a causal link between N_2O and enhanced methotrexate neurotoxicity. This lack of published evidence

has led to some counter arguments that short exposures to N_2O in children are unlikely to be harmful. In addition, there are some perceived advantages in terms of flexibility and ease[11] over alternative approaches. Together this has resulted in continued use of this agent and a lack of general acceptance in the anaesthetic community that an interaction with methotrexate is an important contra-indication to its use. We would like to advance the following arguments in support of our case:

1. The published clinical studies showing that prolonged exposure is needed to cause neurological adverse events in the context of low B_{12} did not include patients receiving methotrexate. Conversely, those investigating methotrexate interactions did not measure B_{12} levels. In addition, in the breast cancer study methotrexate was given IV rather than intrathecally, presumably resulting in significantly lower methotrexate exposure in neuronal cells. Patients with ALL are receiving direct instillation of intrathecal methotrexate and have many reasons to be B_{12} deficient– this “double or even triple whammy” effect may cause neurotoxicity even after brief exposures in paediatric leukaemic patients.

2. The lack of large-scale reporting of neurological adverse drug interactions to regulatory authorities does not necessarily mean that no interaction exists for the following reasons:

i) Neurotoxicity can be subtle and manifest many months or years after initial exposure, making establishment of a causal link problematic.

ii) Anaesthesia is only one part of a multi-agent exposure and many haematologists will be unaware of the anaesthetic agent used – the lack of prescribing of anaesthetic agents on drug charts means they are frequently omitted from lists of concomitant therapy on serious adverse event reports, which are part of mandatory clinical trial reporting. Thus, the opportunity to establish a causal link is lost.

iii) The combination of intravenous induction agents plus N_2O maintenance may well reduce overt neurotoxicity events such as seizures due to the anticonvulsant properties of many induction agents – this may give anaesthetists a false sense of security that neurotoxicity is an extremely rare event, whilst subclinical leukoencephalopathy with resultant effects on long-term neurocognitive outcomes could still be occurring.

Ways to test these arguments are discussed below. In the meantime, we contend that the weight of evidence presented above should lead to implementation of the precautionary principle i.e. ”when an

activity raises threats of harm to human health or the environment, precautionary measures should be taken even if some cause and effect relationships are not fully established scientifically" [59].

Future research directions

A recent single-institution study has highlighted potential neurocognitive adverse effects of repeated propofol and volatile anaesthetic exposure during childhood cancer therapy[60]. This institution did not use N_2O for sedation/anaesthesia during this period (corresponding author personal communication), and therefore does not provide any evidence for or against our arguments above. However, it does raise the possibility that some treatment centres may consider switching to N_2O in place of propofol or volatile based anaesthesia. This makes the need for more research into adverse effects of N_2O more pressing. Definitive proof of a causal role for N_2O in enhanced methotrexate neurotoxicity would require a randomised trial of neurological outcomes following use of N_2O versus alternative anaesthetic agents. However, given the weight of evidence suggesting potential interactions, and no convincing advantage for N_2O use, a prospective study such as this would be likely to be rejected by Ethical review boards. A more feasible alternative is to retrospectively collect more detailed clinical and drug exposure data on patients experiencing acute and chronic neurotoxicity. Data on acute neurotoxic adverse events is currently being collected by the iBFM/Ponte di Legno neurotoxicity working party[3]. This data will enable identification of particular patient groups or countries with high or low rates of neurotoxicity and may enable subsequent cohort studies comparing rates of acute neurotoxicity in patients with and without N_2O exposure. To address the risk of chronic neurotoxicity collection of anaesthetic data on patients that are already participating in long-term neurocognitive outcome studies could be performed. It is also important to establish the prevalence of vitamin B12 deficiency in patients undergoing leukaemia therapy, although retrospectively linking this to N_2O exposure and neurocognitive outcomes will be challenging. The next best approach would be animal studies to investigate any additive effects of N_2O anaesthesia plus methotrexate on cognitive tests or brain histology. These should be conducted as a matter of priority. Finally, innovations in cerebral organoid technology provide an excellent opportunity to studying the effect of methotrexate on white matter volume and structure as well as the relative quantities of various myelin proteins. Such models could then test whether increased toxicity is seen in the context of B_{12} restriction or reduced methionine synthase activity. A recent publication details a very plausible mechanism for long-term, methotrexate-induced cognitive difficulties, concluding that is likely due to tri-glial dysregulation

caused by activated microglia [37]. Although this paper did not specifically look at acute side-effects of methotrexate exposure as in cases of stroke-like-syndrome, the strength of these findings warrant the monitoring of microglial activation in any future studies, including investigations on whether N_2O has any effect on these processes.

Conclusion

Overall, the basic science evidence for a significant interaction between N_2O and methotrexate is compelling. Direct proof of a causal link to enhanced neurotoxicity in patients is lacking, but prospective clinical studies to examine this are likely to be deemed unethical. In our expert opinion, and employing use of the precautionary principle, we support the statement that "N_2O should not be used in acute lymphoblastic leukaemia patients receiving methotrexate". Further retrospective cohort studies and preclinical studies should ensure anaesthetic, haemato-oncological and pharmaceutical input, in order to enhance the evidence base. However, since suitable alternatives exist, it seems sensible and prudent to call for a review of practice and at the very minimum a high level of clinical awareness and pharmacovigilance in patients on oral methotrexate receiving N_2O.

Disclosures

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FIGURE LEGENDS

Figure 1. A summary of the biochemical reactions involving folate and vitamin B_{12} inside an oligodendrocyte and proposed inhibition of myelin production by co-administration of methotrexate (MTX) and N_2O.

MTHF participates in the production of methionine from homocysteine by methionine synthase, catalyzed by $MB_{12}$and zinc, creating THF and methionine. THF participates in the production of purines and pyrimidines for DNA synthesis. Methionine is a vital amino acid involved in myelin production via its conversion to S-adenosyl methionine (SAM). SAM is involved in the methylation of many proteins and intermediates ultimately involved in myelin production, such as phosphatidylcholine, which is important in the production of sphingomyelin, a major component of the myelin sheath. Homocysteine can be converted to homocysteic acid and homocysteine sulfinic acid which are excitotoxic glutamate analogues acting at the N-methyl- d -aspartate (NMDA) receptor, which may be a factor in acute methotrexate-induced neurotoxicity. Methotrexate inhibits the function of DHFR, preventing the conversion of DHF to MTHF. Active vitamin B_{12}contains reduced cobalt (Co+), but N_2O produces irreversible oxidation to Co++ and Co+++, rendering vitamin B_{12} inactive. Any simultaneous compromise of folate and vitamin B_{12} via co-administration of methotrexate and N_2O could result in increased homocysteine and reduced methionine levels both of which may contribute to the neurotoxic effects of methotrexate treatment. Arrows indicate proposed increase or reduction in various relevant pathway metabolites and processes due to methotrexate (orange) and N_2O (blue), respectively. Adapted from Forster *et al,* 2016 (8).

Abbreviations: 5-MTHF (5-methyltetrahydrofolate, levomefolic acid), MB_{12} (methyl B_{12}), THF (tetrahydrofolate, tetrahydrofolic acid), 5,10-MTHF (5,10-methylene THF), DHF (dihydrofolate, dihydrofolic acid), DHFR (dihydrofolate reductase), MTHFR (methylenetetrafolate reductase), met synthase (methionine synthase), SHMT (serine hydroxyl-methyltransferase).