Results of the Clinical Study

• Various patients reported **some early exacerbation of symptoms**, which in most cases was **followed by a greater improvement** in symptoms. Three patients decreased their dosage frequency to every second or third day for several days, until they could tolerate the full daily dosage schedule.

• Sixteen of 30 patients (53%) reported an **initial worsening of symptoms**, beginning in most of these cases within 3 or 4 days, but in some cases beginning at up to 2 weeks. **Most of the symptoms were mild**, and none of the patients discontinued usage of the supplements during the first 3 months.
Results of the Clinical Study (continued)

- **Most common side effects**: gastrointestinal (pain, cramps, constipation, or diarrhea), reported by 6 out of 30 patients or 20%; increase in pain, reported by 4 out of 30 or 13%; and increase in fatigue, reported by 3 out of 30 or 10%. Other symptoms, reported by one patient each, were a decrease in appetite, poor sleep, weak legs, flu-like symptoms, and an increase in anxiety and depression.

- For those who experienced improvement, the time to self-reported improvement on the protocol was an average of 5.6 weeks, with a range from immediate improvement (which was rare) to as long as 8 weeks before improvement was experienced.
Study Results at 3 months

• All 30 patients completed the study requirements up to 3 months.

• 25 out of 30 patients reported improvement (83%).

• Among the group that reported improvement, 8 out of 30 reported marked improvement (27%).
Study results at 6 months

• 29/30 patients completed 6 months of treatment. (The patient who dropped out before 6 months reported **100% relief of pain and 60% overall improvement**, but her family insisted that she be treated at another clinic.)

• Two patients reported **complete relief of pain, complete relief of symptoms, returned to work at full capacity** and decided not to continue the study.

• One patient underwent **bilateral hip replacement surgery** and could not return for follow-up.

• One patient was **disappointed with her results** and elected to discontinue her treatment.
After 6 months...

- Dr. Nathan continued the methylation supplementation program and **added individual treatments** based on genomics, visual contrast testing, and evidence of heavy metal toxicity and mold toxicity.

- **25/30 patients completed 9 months of treatment.**
Glutathione: 30 patients initially

<table>
<thead>
<tr>
<th>Duration of treatment (number of patients)</th>
<th>Glutathione (plasma) (micromoles per liter)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial</td>
<td>30 patients initially</td>
</tr>
<tr>
<td>3 mo. (30 pts.)</td>
<td>2.2</td>
</tr>
<tr>
<td>6 mo. (29 pts.)</td>
<td>2.4</td>
</tr>
<tr>
<td>9 mo. (25 pts.)</td>
<td>2.8</td>
</tr>
</tbody>
</table>

Ref. range

- Mean
- Mean ± 2*Std Dev
- Mean ± 1*Std Dev
- Mean ± 0*Std Dev
- Outliers
- Extremes

Duration of treatment (number of patients)
Oxidized glutathione (GSSG): 30 patients initially

<table>
<thead>
<tr>
<th>Duration of treatment</th>
<th>Number of patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial</td>
<td>30 pts.</td>
</tr>
<tr>
<td>3 mo. (30 pts.)</td>
<td>6 mo. (29 pts.)</td>
</tr>
<tr>
<td>6 mo. (29 pts.)</td>
<td>9 mo. (25 pts.)</td>
</tr>
</tbody>
</table>

Ref. range

<table>
<thead>
<tr>
<th>Micromoles per liter</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.16</td>
</tr>
<tr>
<td>0.24</td>
</tr>
<tr>
<td>0.32</td>
</tr>
<tr>
<td>0.4</td>
</tr>
<tr>
<td>0.48</td>
</tr>
<tr>
<td>0.56</td>
</tr>
<tr>
<td>0.64</td>
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<tr>
<td>0.8</td>
</tr>
<tr>
<td>0.88</td>
</tr>
<tr>
<td>0.96</td>
</tr>
</tbody>
</table>

Mean ± 2*Std Dev

Mean ± Std Dev

Mean

Outliers

Extremes
GSH/GSSG: 30 patients initially

Duration of treatment (number of patients)

- Initial
- 3 mo. (30 pts.)
- 6 mo. (29 pts.)
- 9 mo. (25 pts.)

Mean ref. ratio
- 1.5
- 3
- 4.5
- 6
- 7.5
- 9
- 10.5
- 12
- 13.5
- 15
- 16.5
- 18

Mean+2*Std Dev
Mean-2*Std Dev
Mean+Std Dev
Mean-Std Dev
Mean
Outliers
Extremes
S-adenosylmethionine (SAMe): 30 patients initially

<table>
<thead>
<tr>
<th>Duration of treatment (number of patients)</th>
<th>S-adenosylmethionine (SAMe) (micromoles per deciliter)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial</td>
<td>Ref. range: 165, 180, 195, 210, 225, 240, 255, 270</td>
</tr>
<tr>
<td>9 mo. (24 pts.)</td>
<td>Mean: 270, 275, 280, Mean-2<em>Std Dev: 250, 255, 260, Mean+2</em>Std Dev: 290, 295, 300</td>
</tr>
</tbody>
</table>

Outliers and Extremes are indicated on the graph.
S-adenosylhomocysteine (SAH): 30 patients initially

<table>
<thead>
<tr>
<th>Duration of treatment (number of patients)</th>
<th>S-adenosylhomocysteine (micromoles per deciliter)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial</td>
<td>30 pts.</td>
</tr>
<tr>
<td>3 mo.</td>
<td>29 pts.</td>
</tr>
<tr>
<td>6 mo.</td>
<td>25 pts.</td>
</tr>
<tr>
<td>9 mo.</td>
<td></td>
</tr>
</tbody>
</table>

Mean±2*Std Dev
Mean-2*Std Dev
Mean+Std Dev
Mean-Std Dev
Mean
Outliers
Extremes

Ref. range

Initial
3 mo. (30 pts.)
6 mo. (29 pts.)
9 mo. (25 pts.)
Ref. range

Duration of treatment (number of patients)
SAMe/SAH: 30 patients initially

Duration of treatment (number of patients)

<table>
<thead>
<tr>
<th>Initial</th>
<th>3 mo. (30 pts.)</th>
<th>6 mo. (29 pts.)</th>
<th>9 mo. (24 pts.)</th>
<th>Mean ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>2.4</td>
<td>3.2</td>
<td>4</td>
<td>4.8</td>
</tr>
<tr>
<td>Mean+Std Dev</td>
<td>5.6</td>
<td>7.2</td>
<td>8</td>
<td>8.8</td>
</tr>
<tr>
<td>Mean-2*Std Dev</td>
<td>0</td>
<td>-1.4</td>
<td>-2</td>
<td>-2.8</td>
</tr>
<tr>
<td>Mean-Std Dev</td>
<td>4.8</td>
<td>6.4</td>
<td>7.2</td>
<td>8.8</td>
</tr>
<tr>
<td>Mean+2*Std Dev</td>
<td>8.8</td>
<td>10.4</td>
<td>11.6</td>
<td>13.2</td>
</tr>
</tbody>
</table>

Outliers
Extremes
5-methyl tetrahydrofolate: 30 patients initially

<table>
<thead>
<tr>
<th>Duration of treatment (number of patients)</th>
<th>5-methyl tetrahydrofolate (plasma) (nanomoles per liter)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial</td>
<td></td>
</tr>
<tr>
<td>3 mo. (30 pts.)</td>
<td></td>
</tr>
<tr>
<td>6 mo. (29 pts.)</td>
<td></td>
</tr>
<tr>
<td>9 mo. (25 pts.)</td>
<td></td>
</tr>
<tr>
<td>Ref. range</td>
<td></td>
</tr>
</tbody>
</table>

- Maximum
- Minimum
- 75%
- 25%
- Median
- Outliers
- Extremes
Number of symptoms reported: 30 patients initially

Duration of treatment (number of patients)

Initial  6 mo. (29 pts.)  9 mo. (25 pts.)

Number of symptoms reported: 30 patients initially

Number of symptoms

Mean + 2 * Std Dev
Mean - 2 * Std Dev
Mean + Std Dev
Mean - Std Dev
Mean
Outliers
Extremes
Self-rated Energy Level: 30 patients initially

Duration of treatment (number of patients)

Initial 3 mo. (30 pts.) 6 mo. (29 pts.) 9 mo. (25 pts.)

Mean+2*Std Dev Mean-2*Std Dev Mean+Std Dev Mean-Std Dev Mean Outliers Extremes
Self-rated Amount of Sleep: 30 patients initially

Duration of treatment (number of patients):
- Initial
- 3 mo. (30 pts.)
- 6 mo. (29 pts.)
- 9 mo. (25 pts.)

Amount of sleep: 1 = no sleep, 10 = 8 hours of sleep without waking

- Mean
- Mean ± Std Dev
- Mean ± 2*Std Dev
- Mean ± 3*Std Dev
- Outliers
- Extremes
Self-rated Mental Clarity: 30 patients initially

Duration of treatment (number of patients)

Initial 3 mo. (30 pts.) 6 mo. (29 pts.) 9 mo. (25 pts.)

Mean+2*Std Dev Mean-2*Std Dev Mean+Std Dev Mean-Std Dev

Outliers Extremes
Self-rated Freedom from Pain: 30 patients initially

- Initial
- 3 mo. (30 pts.)
- 6 mo. (29 pts.)
- 9 mo. (25 pts.)

Mean + 2*Std Dev
Mean - 2*Std Dev
Mean + Std Dev
Mean - Std Dev
Mean
Outliers
Extremes
Dependence of SAMe + SAH on the CBS C699T polymorphism (Amy Yasko's "bathtub draining" phenomenon): 30 patients initially (number of patients in each group shown at the bottom).
Outcomes after 9 months

• At 9 months, **15 of the 25 patients** remaining in the study at that time reported that they had experienced **>50% improvement**.

• **Three of the 5 patients who had dropped out had also reported >50% improvement.**

• Adding these together, 18 out of the original 30 patients (or **60% of them**) reported **>50% improvement**.
After nine months of study, one additional component was added.

- Nine of the 25 remaining patients still had significantly elevated adenosine levels.

- All agreed to a 2 to 3 mo. trial of Acyclovir. (This was based on advice from Sidney Baker, M.D.)

- Dosage of Acyclovir: 200mg, 5x per day.

- Results: Eight of nine patients reported an additional 20% improvement in overall wellbeing, which held even when Acyclovir was discontinued.
Case Study #1

- D.F. was a 49 year-old white female with a 6-year history of fibromyalgia and ME/CFS.

- Her initial reduced glutathione concentration was 3.0 micromoles per liter (ref. range: 3.8-5.5) and her S-adenosylmethionine level was 217 micromoles per deciliter (ref. range: 221-256).

- Although she felt a little better after the first 3 months of supplements, glutathione was at 2.8 and SAMe was at 226 on her follow-up appointment.

- After 6 months, GSH 3.5 and SAM 240. She still showed minimal clinical improvement.

- Since she had a CBS polymorphism and her 24-hour urine amino acids analysis showed elevated taurine and cysteine, she was given the RNA formulation “Ammonia Support Formula” based on the advice of Dr. Yasko.

- She was also given nucleotides and trehalose, based on the advice of Dr. Amy Yasko.
Case Study #1 (continued)

• After just one month of the additional supplements she felt so much better that she was **able to resume full-time work**, which she had not been able to do for 5 years, successfully.

• She was **free of pain**, and her **energy was back to normal**.

• Levels at nine months were GSH--4.0 (ref. range 3.8-5.5) and SAMe--240 (ref. range 221-256)
Case Study #2

• B.E. was an 84-year-old white female with a 2-year history of fibromyalgia and ME/CFS.

• Her initial GSH level was 2.7 (ref. range: 3.8-5.5); SAMe was 201 (ref. range: 221-256).

• She reported no clinical improvement at 3 months, and had 2 episodes of severe infection, requiring 2 rounds of antibiotics during that time.

• At 3 months her GSH level was 3.2 and her SAMe level was 220.

• At 6 months, there was still no clinical improvement. Her GSH level was only 3.0, but her SAMe level had risen to 233. Since she had a positive visual contrast sensitivity (“FACT”) test (based on the work of Dr. Ritchie Shoemaker), she was evaluated for heavy metal toxicity with a DMPS-provoked urine collection test. An elevated level of mercury was found. She was treated with intravenous DMPS monthly and oral DMSA.

• She was also treated with cholestyramine for mold exposure (based on the work of Dr. Ritchie Shoemaker).
By the completion of the study, she reported marked improvement and was able to join her friends for a week-long visit to Paris, noting that she was pain-free, and her energy had returned to almost normal. At 9 mo, GSH 4.0 (ref. range: 3.8-5.5) and SAM 232 (ref. range: 221-256).
Case Study #3

• S.H. was a 55 year-old white female with a history of fibromyalgia and ME/CFS for 8 years. She was receiving financial support from Social Security Disability Insurance for those conditions.

• Dr. Nathan had discovered an elevated level of mercury on DMPS testing 4 years earlier (level, 34), but she had elected not to treat it, for financial reasons.

• Her initial GSH level was 3.4 (ref. range: 3.8-5.5), and her SAMe level was 207 (ref. range: 221-256).

• These rose over the study period. GSH was 3.9 at 3 months, 4.8 at 6 months, and 4.6 at 9 months.

• SAMe rose to 219 at 3 months, 230 at 6 months, and 267 at 9 months.
Case Study #3 (continued)

• While she reported a 20% improvement in energy, sleep and wellbeing, no additional improvements were noted.

• She continued to decline to treat the elevated mercury level.
The results of the clinical study were consistent with the predictions of the Glutathione Depletion—Methylation Cycle Block hypothesis.

2. The reduced glutathione levels were significantly below normal before treatment was begun.

2. There was a partial methylation cycle block before treatment was begun.

3. The methylation cycle block was partially lifted by treating with bioactive forms of vitamin B12 and folate, together with basic nutritional support, directed specifically at raising the activity of the enzyme methionine synthase, which is the enzyme hypothesized to be partially blocked.

4. Treating to lift the methylation cycle block not only improved the methylation capacity, but also raised glutathione (as well as the ratio of reduced to oxidized glutathione), suggesting that these two phenomena are indeed linked in an interactive mechanism in CFS, as they also appear to be in autism.
Why didn’t all these patients recover completely?

Clearly, treating to lift the partial methylation cycle block alone was not enough to help most of the patients to recover completely.

I suggest that there were stressors such as toxins and pathogens that caused the depletion of glutathione initially, and there were also some that accumulated during the illness after onset of the vicious circle mechanism, and some of these factors need to be treated specifically, in addition to treating to lift the partial methylation cycle block.

Also, a longer methylation treatment duration may be needed in some cases.
Summary

• A hypothesis has been developed to explain ME/CFS.

• It involves a vicious circle mechanism. The key features are—chronic depletion of glutathione, a functional deficiency of vitamin B12, a chronic partial block of the methylation cycle, and significant draining of folate from the cells.

• This hypothesis is able to explain the observed genetic predisposition, most of the observed biochemical abnormalities, and many of the seemingly unconnected symptoms of ME/CFS.

• Lab testing is available to determine whether the hypothesis applies to a particular patient. So far it appears to apply to most ME/CFS patients.
Several treatment protocols are being used. All include forms of folate and high-dose vitamin B12 taken together.

A clinical study was performed using a simplified treatment approach extracted from the full treatment program of Dr. Amy Yasko, and results were found to be consistent with the hypothesis.

This simplified treatment is currently producing significant benefits in most patients who use it, and it has resulted in apparently complete recovery in a small number of patients.

This treatment has not been optimized, and it is likely that it can be improved.

In most cases, it appears that other aspects (such as toxins and pathogens) must also be treated specifically to bring about complete recovery.
General Acknowledgments

- **Paul Cheney**, M.D., **Patricia Salvato**, M.D., **Derek Enlander**, M.D. pioneered use of glutathione in treating ME/CFS.
- **Les Simpson**, Ph.D., **Paul Cheney**, M.D. and **Charles Lapp**, M.D. pioneered use of high-dose B12 in treating ME/CFS. Prof. **Bjorn Regland**’s work showed the importance of folate in addition.
- **Michael Goldberg**, M.D. and Prof. **Malcolm Hooper** noted a connection between autism and ME/CFS.
- **Bernard Rimland**, Ph.D. founded the DAN! Project for study and treatment of autism.
- **S. Jill James**, Ph.D. showed that lifting the partial methylation cycle block also caused glutathione to recover in autism.
- Prof. **Richard Deth** advanced the understanding of methylation in autism.
- **S.R.** (patient) was the first to try the DAN! Treatment for ME/CFS.
- **Amy Yasko**, Ph.D., N.D., developed treatment of methylation-related disorders.
- **S.T.** (patient) was the first to try the full Yasko treatment for ME/CFS.
- **David Bell**, M.D. called for a treatment for ME/CFS based on the GD-MCB hypothesis.
- **K.T.** (patient) helped to formulate the simplified treatment approach.
- **L.D.** (patient) was the first to try the simplified treatment approach.
- **Tapan Audhya**, Ph.D. developed the methylation pathways panel.
Acknowledgments
(Clinical Study)

• The clinical study was supported financially by an anonymous donor as part of the effort of the Ratna Ling Working Group.

• The clinical study was conducted by Dr. Neil Nathan, M.D.

• Drs. Tapan Audhya, Amy Yasko, Jacob Teitelbaum, and Ritchie Shoemaker helped with the planning of the clinical study.

• Management of the clinical study was provided by Kevin Joyce, and nursing support was provided by Neva Dix.