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Evidence Based Medicine in the Treatment of Mitochondrial Disease

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INTRODUCTION

The mitochondria are responsible for energy generation within all cells of the body, with mature red blood cell being the exception. In addition to the critical role in energy production, free radicals, which are critical for normal physiological processes, are generated within the mitochondrion's electron transport chain. Mitochondrial dysfunction can result in variable degrees of both energy deficits and excessive free radical production. Without energy the cell is not able to function, and excessive free radical production can injure the normal structures within the cell, which include the mitochondrion itself. Vitamins and cofactors are the chemical compounds that either speed metabolic reactions or participate in critical steps in metabolism, which include squelching free radicals as they are produced. Vitamins are not made within the body and therefore must be assumed, while cofactors are made within the body. A few examples of vitamins include vitamin C, folic acid, and biotin. Coenzyme Q_{10} and levocarnitine are two examples of cofactors.

The biochemical reactions that are necessary for energy metabolism, and the vitamins and cofactors necessary for the specific enzymes to function, were described decades before the significance of human diseases based on dysfunction of these enzymatic reactions were appreciated. When doctors began discovering mitochondrial diseases, treating patients with vitamins and cofactors was reasonable, with the hope that some patients may have vitamin-responsive disorders. The term "supplements" refers to both vitamins and cofactors used in the therapy of patients with mitochondrial disorders. The purpose of this paper is to introduce the reader to the intended rationale for the use of supplements, to describe basic aspects of clinical trial design, to stress the importance of a properly designed clinical trial, to introduce the concepts of *Evidence Based Medicine, Strength of Evidence, Strength of Recommendations*, to explain the reason clinical trials are difficult to conduct in patients with mitochondrial diseases, and to face the reality that - until we have adequate evidence - common sense has to guide the use of supplements in the treatment of mitochondrial diseases. It is beyond the scope of this article to make any recommendations on which vitamins and supplements are effective.

RATIONALE FOR VITAMIN AND COFACTOR THERAPY

There are both short-term (improved ATP production) and long-term objectives (reduced free-radical production) of vitamin and cofactor therapy. The rationale for vitamin and cofactor therapy in humans is based on enzyme studies *in vivo*, in cell cultures or tissue slice systems, and in some cases on the response of patients to therapy. The most commonly stated rationale for the use of vitamins and cofactors is to stimulate poorly functioning enzymes in energy pathways. If effective, vitamin supplementation should improve enzyme function soon after starting therapy, although cofactors such as levocarnitine and coenzyme Q_{10} may take weeks or months to reach steady state concentrations within mitochondria. Along with stimulating enzyme function, some supplements can also act as alternative energy fuels, or bypass biochemical blocks within the respiratory chain. Other supplements may work by sparing CoA, scavenging toxic free-fatty acids,, organic acids, or excessive free radicals. The beneficial effects or

reducing free radicals is difficult to measure and any beneficial effects may not be realized for months or years.

The goal of increasing ATP production and reducing free radical production is clinical improvement, or at least stabilization of symptoms or signs. For each organ system, the goals of therapy can be further delineated. For example, an effective mitochondrial therapy may improve brain function by reducing seizures, improving the ability to concentrate, improving intellectual function, preventing strokes, lessening abnormal movements, or alleviating headaches. Unlike other diseases, a supplement given to treat a mitochondrial disease may benefit several organ systems. For example, if someone has lung cancer, the single goal of therapy is to shrink the tumor (improving lung function is of little benefit if the tumor does not shrink). Clinical trials involving therapeutic benefit are straightforward to conduct if there is one or two potential benefits that need to be measured. In clinical trials for mitochondrial diseases, there may be several goals. Unfortunately, if there are too many goals of a study, the trial becomes impossible because of prohibitive cost and statistical constraints.

CHALLENGES TO EVIDENCE-BASED TREATMENT TRIALS FOR MITOCHONDRIAL SUPPLEMENTS

To decide if a therapeutic intervention is effective, it must be tested in humans and the results have to be carefully analyzed to define the benefit as well as any toxicity. The process of drug development (taking a medication from the laboratory through animal and human trials to the marketplace) can take years. Most vitamins and cofactors available are not considered "medications" but are labeled by the United States federal regulatory agencies as "foods" and therefore do not require any study of clinical effectiveness, which of course are necessary for any new medication. This leaves to interested investigators the job of studying any supplement with the same type of clinical trial that would be required for any new medication to determine if it is effective. The reason there have been so few trials involving supplements to treat mitochondrial disorders is simply economic. Clinical trials involving one supplement or medication with one study goal can cost hundreds of thousands of dollars (or more). In the pharmaceutical industry, companies support this drug development cost as a calculated gamble. The cost is justified for medications that have a huge market, such as a medication to treat cancer, which will eventually generate billions of dollars of revenue for the company holding the patent. Supplements have no patent rights and many sell for pennies a dose. There is no financial incentive for most corporations to perform clinical testing of these supplements.

Institutional Review Board (IRB)

In the past, medical studies were designed and implemented without formal review of their scientific rationale or ethical considerations. There have been several well-documented human trial investigations that are an embarrassment to the medical system in our country. Fortunately at this time, essentially every hospital and treatment center in the USA conducting human trials has an Institutional Review Board, comprised of

physicians, lawyers, ethicists, and lay persons. The job of the IRB is to review the treatment trial for scientific rationale and ethical concerns, as well as to monitor the study progress. In the last several years, the IRBs have also assumed the responsibility of reviewing potential "conflicts of interests" of the doctors who design and implement these studies. The purpose of this scrutiny is to ensure that any conflicts of interests (usually monetary) are identified and resolved. Often, such potential conflicts of interests need to be disclosed to potential patients before entering the study. Although IRB committees function independently and some have more rigid standards, over the last decade the activities of IRBs have become more standardized.

It has now become a national standard that any researcher intending to report results of a clinical trial must file an application to their IRB, await for IRB approval, and report interim results for monitoring by that IRB. Many IRBs are now requiring researchers to submit an application for approval even to perform a retrospective review of data (looking at results of treatments performed in the past) if the researchers are considering publishing the results. Certainly,
all new therapeutic trials require IRB approval.

Study Protocol

Treatment trials require a formal study protocol. This protocol includes an introduction to the problem or disease being studied, the scientific rationale for the proposed treatment, information about the treatment, and - if the treatment is a medication (or food product like a vitamin) - background information on that substance, including any toxicity information. For drugs that have never been tried in humans, animal data are required. The study protocol will define the exact method of treatment, and the exact measures of therapeutic response and toxicity. There must be a statistical section that defines, using strict mathematical analysis, whether the study outcome is significant or not. Finally, the study protocol includes a copy of the "informed consent" document.

Informed Consent

Patients are recruited to the study by a number of means. The patient (or parents) may hear about the study by word of mouth, find it on the internet (www.clinicaltrials.gov is a government web sites that provide information about therapeutic trials), or be told about the trial by their doctor. The informed consent is a document written in plain English that outlines the study rationale and study design. Patients are instructed that their participation is voluntary. The document outlines all the treatment aspects and the medical tests that the patient must undergo to participate in the study. If there is any cost to the study, this is outlined in the document as well. All possible side effects are listed. There is a phone number to call 24 hours a day if any problem should occur. The informed consent document will also let the patients know they can withdraw from the study at any time without prejudice (meaning the doctor will not be "mad" at them nor will the doctor deny future treatment because of their withdrawal from the study). This document is signed by the patient, a parent if the patient is a minor (or by both if the

patient is able to understand the study), and by a witness. The patient is given a copy of the informed consent.

CLINICAL TRIALS DESIGN

If we knew what drugs or supplements worked in mitochondrial disease, there would be no need for treatment trials (or a need for the UMDF). This is not the case. There are many types of clinical trials, which are outlined **at the end of this document**. There may be some overlap in exactly what type of design can be assigned a particular study. Many IRBs now require the involvement of a biostatistician in designing the study so that the results, whether positive or negative, can be stated with as great a degree of certainty as possible. When interpreting the results of any trial, the trial design must be scientifically sound or the results are useless. It is not easy to design a good trial, and even senior clinical investigators can disagree on the trial design.

EVIDENCE BASED MEDICINE (EBM)

It is very reasonable for patients to expect that the treatments prescribed to them should have a reasonable degree of effectiveness. In addition the safety and side-effect profile should be known. Unfortunately, a single study, even if it is well-designed seldom yields a final determination of a treatment's effectiveness and safety. In many cases, the medical literature contains reports with conflicting results. The term "meta-analysis" refers to a statistical compilation of all results within trials of similar, but rarely identical, treatments for a particular disease or condition. Meta-analysis does not add new data to the literature, only compiles it all together using statistical methods. The goal of EBM is to go one step further than meta-analysis, and in many cases meta-analysis reports are used to develop EBM statements. With regards to medical therapeutics, the purpose of EBM is to interpret the results of all clinical trials available in the medical literature, including case reports. EBM analysis will give extra value to large and properly designed studies and obviously less value to small or poorly designed trials. In addition, EBM does take into consideration expert advise or consensus panel advise. This analysis may involve analyzing 100s of articles, sorting through all results, and writing a single manuscript that summarizes the information in an unbiased fashion. Finally, EBM statements will usually include recommendations, the strength of evidence on which that recommendation is based, and a measure of the strength for which that recommendation should be followed. Sometimes, these reports result in Practice Guidelines that can change the entire approach to caring for patients. One excellent source for these guidelines is at www.guidelines.gov. However, do not expect to find Practice Guidelines for mitochondrial therapeutics, as the field is new and there is not enough literature at this point to form guidelines.

EBM reports, even those that look at hundreds of medical publications, usually can only make a few declarative statements. Each statement includes two key indicators that inform the reader about the strength of the evidence used to reach the conclusion and the strength of the recommendation stated. The strength of evidence (SOE) is given a rating of 'A', 'B', or 'C', with 'A' representing the strongest strength of evidence and 'C'

representing the weakest. An SOE grade of C does not necessarily mean that the declarative statement is incorrect, only that the evidence is not very strong. The strength of recommendation is rated I, IIa, IIb or III. The SOE and SOR tables are adapted from government data and are listed **at the end of this document**.

Interpreting SOE and SOR

In a perfect world, treatments for diseases would come with Category A / Class I recommendations, but we do not live in a perfect world. For most vitamin and cofactor therapy used in mitochondrial diseases, the SOE is class B or C, with the SOR usually falling in the IIb or IIa range. For example, depending on how one interprets the literature, the use of Coenzyme Q10 carries a Category B SOE, based on very few studies, and an SOR of IIb, given its lack of serious side effects. However, several studies, whose design may be considered flawed by today's standards, indicate that coenzyme Q_{10} is of no benefit. At this time, the optimal dose of coenzyme Q10 is far from settled Furthermore, no study has investigated the duration of treatment for those who do (or do not) show a clinical response after a period of time. There are supplements, such as vitamin K_3 and succinate, whose effectiveness was based on a handful of patients and never verified in any controlled trial. One possible flaw is that Category C SOE does not differentiate between "consensus opinion", which will often reflect the clinical experience of a dozen or more experts, and "expert opinion", which would be the opinion of one expert. It is fair to reason that the opinion of a panel of experts is less likely to be flawed than that of one expert, but of course guidance from experts is of less value than evidence from properly conducted clinical trials.

CLINICAL TRIALS IN MITOCHONDRIAL DISEASES – THE OBSTACLES

There are several important reasons why clinical trials are difficult in mitochondrial disease.

1) We are not dealing with one disease. There are over 200 different pathogenic mutations in mtDNA and hundreds of mutations involving over forty mitochondrial-directed nuclear DNA genes that are identified to cause human illness, with more to follow.
2) Although the UMDF wishes to promote the idea that Mitochondrial Disorders are not rare diseases, when compared with major causes of illness such as cancer or heart disease, they are far less common. Recruiting patients for a clinical trial is difficult because of the relative rarity of mitochondrial disorders, and even more difficult because of the extreme rarity of specific mitochondrial disorders.
3) The concept of one genotype (specific genetic mutation) causing one phenotype (well-defined set of clinical symptoms and signs) does not hold true with mitochondrial disorders. For example, the symptom complex is highly variable in many mtDNA disorders, with unpredictable onset and clinical course. For individuals with the A3243G mutation, the most common cause of MELAS, the course can be mild in some family members and devastating in others. Some of this variability can be due to the degree of mutant heteroplasmy but there may be

confounding factors, including the modifying effects of other genes. Measuring an outcome measure when patients entering the trial may be at different stages of their illness is problematic. In the best-studied nuclear DNA gene that causes mitochondrial disease, *POLG*, there are over 150 identified mutations that result in several very different clinical presentations. Mutations in this gene can cause autosomal dominant PEO, autosomal recessive PEO with or without other neurologic problems, the broad spectrum of ataxia-neuropathy syndrome, Alpers syndrome, infantile myocerebrohepatopathy syndrome and the myoclonic epilepsy myopathy sensory ataxia spectrum. The age of presentation for *POLG* diseases ranges from a few weeks of life and 70+ years of life, and some of the disorders are rapidly fatal while others cause relatively mild symptoms.

4) There are fluctuations of symptoms with prolonged and unpredictable periods of disease inactivity.
5) There is no agreed upon paradigm to diagnose most mitochondrial disorders, and this presents a critical problem in developing a rationale approach to investigating the response to any therapy. Many studies are designed to insure that, whatever results are reached, no one will question that all the persons in the study had a mitochondrial disease.
6) Choosing the entry criteria can exclude many patients. Even if a clinical trial is designed to test a medication in one genetic disease, the design of the study could choose very different entry criteria. For example, one study may allow entry without regard to the severity of the illness, requiring only that the person have the mutation. Another study may limit entry only to people who not only have the mutation but also have certain clinical features. Enrolling patients into a study without firm criteria is both medically and morally bankrupt. Designing these criteria has been difficult, in part because it is difficult to diagnose many patients with certainty. (This issue does not exist with lung cancer, for example).
7) Travel Considerations: Because of the complexity of running a clinical trial, most trials are performed at one medical center, or at a few medical centers. However, patients that are eligible for study may live all over the country. Travel and lodging costs are common reasons why patients choose not enter a clinical trial. The use of volunteer private pilots to shuttle patients and their families has been of great help, but does not solve the cost of hotels, time away from work, or the impact on the day-to-day activities of the family. Sometimes the cost of transportation is built into the study design, so that the airfare and housing are covered and the family has minimal out-of-pocket expenses. In most cases, this is simply not possible.
8) Choosing the proper outcome measures and statistical approaches is a big hurdle. The number of outcome measures has to be sufficient to offer the best chance of obtaining a significant result, but not so large as to make the study too expensive .
9) No matter how simple the study design is, clinical trials cost money. The paperwork involved for even simple studies requires management by either a certified medical data manager, or a trained nurse. This is for safety concerns, and regulatory (hospital as well as federal) mandates require close attention to details. As an example of this cost, it is estimated that a full-time data manager is required for every 25-50 patients in a study. Factoring in salary and fringe benefits, this means that the administrative costs (paperwork) of a study may amount to between $1,000-$2,000 per patient. If

the clinical trial involves a medication, many times the company that owns the patent will pay for most or all the costs of the study. However, for vitamins and cofactors, there is no patent owner, so the company may (only) supply that treatment for study participants free of charge. If the researchers cannot find grant funding or other financial support, the cost of the study falls on the medical institution or on the patient.

10) Emotional factors play a huge role in clinical trial consideration. It is critical to remember that - "IF" we knew the outcome - there would be no reason to conduct the clinical trial. For example, if a patient has been on a group of vitamins, and they must come off those vitamins to participate in the trial, there is a fair chance that the patient or family will not agree to participate, especially if they can buy the supplement on their own. If the randomization process does not seem "fair", as in the case of simple randomization between treatment and placebo, then the patient may not agree to participate (the use of the cross-over design has helped somewhat in this regard). It is the burden of the researchers to factor this issue in designing a trial so that the outcome of the trial will mean something, the cost of conducting the trial will be reasonable, and the patient or family will accept the design without feeling unnecessarily burdened by their decision to participate. There have been many excellent clinical trials in medicine that were not completed because the study randomization, although appropriate to answer the question, was perceived to be unfair by patients and referring physicians, so that insufficient patients chose to participate.

DOES EBM HAVE A ROLE IN MITOCHONDRIAL DISEASE TREATMENT TRIALS?

The simple answer to this question is "yes…and no". The issue facing the physicians who take care of people with mitochondrial diseases is that there are inadequate treatments for these diseases. There are no patent rights for any of the vitamins and cofactors, and with rare exception, corporations have no financial incentive to fund clinical studies. Simple clinical trials may cost over $250,000, and without patent protection, any of the hundreds of vitamin companies would benefit from a positive study. It would be very helpful to know what dose of coenzyme Q10 is optimal, what signs and symptoms caused by a mitochondrial disease could improve with therapy, and in what specific diseases it should be used routinely. The trials that are underway may answer the dosing issue and which symptoms improve, although the results may not be able to quantify improvement. (I doubt we will ever get to an SOE of A, and, unless the studies are total failures, the SOR is unlikely to be IIb or III). I do not think we will get answers for most vitamins and supplements. The financial cost and length of time required to perform a good clinical trial of each vitamin and cofactor separately is not reasonable. The cost of testing all the different combinations one could put together is even higher. For many of the vitamins, the decision whether to use one vitamin or not is not a huge issue. Riboflavin, for example, has some limited evidence of effectiveness (C – IIa), especially in headache. It costs pennies a day, has no harmful side effects, and, leaving aside its taste and odor, is tolerated well. An "*n of 1*" trial for an individual patient seems quite reasonable, at least for a period. For other supplements, such as coenzyme Q10, a family may spend

thousands of dollars a year and these people deserve proof of some effectiveness. EBM is a goal to aim for, but there will not be immediate answers to most of our questions.

One of the most common questions asked is "which vitamins and cofactors should I take?". If one looks to the medical literature, then the answer to that question is quite complicated. One of the best clinical studies to date suggests the combination of coenzyme Q10, alpha lipoic acid, and creatine monohydrate results in some modest but real improvements in a number of outcome measures. In this study, only modest dosages of coenzyme Q10 were used, and did not include other "popular" supplements such as levocarnitine and riboflavin (vitamin B2). Although these factors are in no way a criticism of the study, it is reasonable to question (but unreasonable to expect an answer) whether increasing the dose of coenzyme Q10, and/or adding levocarnitine, and/or riboflavin would result in even a better outcome. Given the cost of doing this study, it is not likely that we will have a phase III trial using the initial therapy as one treatment compared in a randomized fashion with a "souped up" treatment. We simply have to accept the results of this well-designed study at face value and wait for another study in the future.

The common use of the so-called "vitamin cocktail" involves the use of many supplements (at one point, I was recommending up to 15 supplements) as part of treatment. These cocktails have been used extensively for the last two decades to treat patients with mitochondrial diseases. The SOE/SOR for this practice is C – IIa or C – IIb, but is based on very limited data for several of these supplements and on theoretical hypotheses for others. There have been no rigorous clinical trials looking at the effectiveness of these many supplements. Although there is seldom serious harm in using them, several factors have made me reconsider this practice. Many of the supplements are expensive. If a physician suggests that a treatment may be helpful, the family will usually make sure that the child gets the treatment, despite the financial and emotional burden. This often results in the parents chasing the child around the house all day. Riboflavin, for example tastes awful and unless a child can swallow a capsule or has a feeding tube, it is not practical to use it in most circumstances (although a recent proprietary riboflavin product gets around this taste/smell issue). Once a theraepeutic program is started, it becomes emotionally difficult to stop it, even if its efficacy is not obvious. For this reason I have, over the last decade, reduced the number of recommended supplements based on personal experience (SOE is level C; "expert opinion"). It is important to remember that my choice for my patients is based on evidence from a few publications and on my experience over time. It is not an opinion based on large scale, well-designed clinical trials --- they have not been performed.

There are some reasonable objections to require EBM for all treatment decisions. Lack of adequate evidence of a treatment's effectiveness is not the same as lack of benefit. For this reason, using a supplement that is inexpensive and free of side effects may be reasonable, even if there is not proof of its effectiveness. This argument however is a slippery slope, and both the physician and patient can get carried away with the number of supplements. It is also problematic for expensive supplements, or if the recommendation to use a supplement is based on a potential conflict of interest. Another

reason EBM can be misleading is that EBM applies to populations of patients and cannot predict the response in an individual patient. For this reason, even if a study shows unimpressive results, it may be reasonable to try the supplement in a given patient. The decision to use a supplement would be easy if we knew 75% of people responded, or only 1 in 10,000 responded. But what if 2 out of 100 patients show real improvement in one outcome measure? The decision to use the supplement should be made with costs, inconvenience and safety in mind. Because mitochondrial disease is understudied for the reasons stated above, there is no choice but to use 'reasonable judgment' when making these decisions.

We can only hope that the marketplace will drive new drug development for mitochondrial diseases. The pharmaceutical industry is actively pursing treatments for disorders that will hopefully benefit those with mitochondrial disorders, and several companies are performing drug development specifically for mitochondrial disorders. It is to be hoped that these corporations will participate in funding properly designed clinical trials so that we can find effective treatments and offer these treatments to those affected. The UMDF has been instrumental in aiding the support of treatment consortiums and fostering this cooperation between large and small mitochondrial centers. One group, NAMDC, has received federal funding that will hopefully result in treatment trials in the near future. Not until these well-designed trials have been performed can we fully expect to rely fully on EBM to guide us, but it is not likely that we will ever have adequate EBM to make all treatment decisions.

SUMMARY

The use of vitamins and cofactors for treating mitochondrial disease has been part of the therapeutic plan since mitochondrial diseases have been identified. Vitamins and cofactors were tried because "it made good sense" based on theoretical considerations. A few clinical trials have provided some evidence that some of these supplements may be helpful in some patients. To develop treatments, properly designed clinical treatment trials must be conducted. Understanding the different types of trials, and how to interpret the results is essential to understand the potential benefit and limitations of these therapies. The use of evidence based medicine, especially the interpretation of "strength of evidence" and "strength of recommendation," adds clarity to the issue of treatment effectiveness. Mitochondrial diseases are illnesses without adequate treatments, and we cannot walk away from scientific principles when deciding therapy. However, when evidence does not exist, emotion (on the part of the doctor, patient or patient's parents) will often drive treatment decisions. Understanding which decisions are based on emotion, which are based on theoretical hypothesis, and which are based on scientific evidence is all part of the relationship between the patient and their doctors.

ADDENDUM

- Types of Clinical Trials
- Categories of Evidence-Based-Medicine

A. Types of Clinical Trials

Uncontrolled Trials

Case Report

A case report is a medical report that describes a single patient's illness, sometimes including the results of a treatment. Case reports are becoming less common in the medical literature because the experience of one patient cannot necessarily be broadened to other patients with the same illness. The medical jargon for reporting clinical improvement in a case report is the term "n of 1" (the term n is used to refer to the number of patients in a study). The term *case series* is used to describe the results of a few patients. Case reports and case series may provide useful information leading to controlled clinical trials, but rarely can be used to document the effectiveness of any medication or treatment. One reason uncontrolled trials have limited usefulness is that negative case reports (stating a drugs uselessness) and negative case series are almost never published. It is possible that 100 patients had been treated by 100 doctors with "compound X" without benefit, but one patient eventually responded --- and his/her improvement was published. It is wise to be skeptical of the presumed effectiveness of a supplement that appeared as being beneficial many years ago in a case report but that has never again appeared in the literature as being a beneficial therapy.

Retrospective Trial

In a retrospective study, the researcher looks back in time at a group of patients treated with a drug (or therapy) and reports the outcome. Publications involving retrospective trials are becoming less common for several reasons. Retrospective studies require the researcher to screen a list of patients that have undergone a therapy, but the list may be incomplete, in which case the results from the retrospective trial may not be valid. Attempting to come up with a list of patients treated in the past is difficult, and trying to obtain medical information from their charts may result in missing data points. In addition, without a formal trial design, the patients usually received similar, but not identical therapy. Retrospective trials are subject to bias, meaning that the treating doctor may have chosen more seriously ill (or less seriously ill) patients to receive that therapy (as opposed to an alternative therapy). This bias may represent the largest factor why results from retrospective trials should be viewed with extreme caution. The results of retrospective trials cannot be used as absolute proof, and like case studies and case series, can only suggest which treatments should be studied with a controlled trial.

Controlled Trials

Pilot Trial

In some circumstances a researcher may have a "good idea" for treatment, but does not have adequate funding to perform a large enough trial that will have statistical significance. Ideas for pilot trials may result from the observation of improvement in a patient's clinical condition after a new treatment with an unstudied drug (or vitamin).

The medical term for reporting on one patient is a "case report" (see above). In this situation, the researcher may design a "pilot trial" whose purpose is to collect enough data on several patients (usually less than 10) by using a standard treatment. If the results are positive, a larger study may follow. Pilot trials require full IRB approval, as well as a consent form. All patients are enrolled and treated only AFTER the project is designed and approved, and researchers are not allowed to report patients treated prior to the trial's approval by the IRB. Results of pilot trials usually do not have statistical significance. Pilot trials involving vitamins and supplements are rare today, and most of the cofactors and vitamins used to treat mitochondrial diseases have been put through formal or informal pilot trials. The results of a pilot trial should not be discounted, only taken in consideration of the small number of patients treated.

Phase I Trial

A phase I trial is usually the first human trial performed with a new medication. The purpose of a phase I trial is to determine the drug's toxicity and safety. These trials are usually performed in people dying of a disease (like cancer) but who are predicted to live long enough to determine the toxic effects of the medication. In addition to the toxicity of the drug, the range of dosing is determined as well as the kinetics of the drug (how rapidly the body can remove the drug). Most phase I studies are designed to treat a group (cohort) of patients (typically 3-5) at a very low dose, and, if this is well tolerated, treat the next cohorts of patients at sequentially higher doses, until toxicity is reached. Phase I trials are not designed to determine the effectiveness of the medication, although in some circumstances a patient may improve. In reality, and this is especially true in cancer treatment trials, the patients enrolled in these trials have such advance diseases that their chance of a long-term improvement is remote. Phase I trials are not common in mitochondrial disorders.

Phase II Trial

Once the optimal dose regimen has been determined, the next step in drug development is the phase II study. In some circumstances, the treatment is so safe (or so dangerous) that researchers design trials that are combinations of phase I and phase II trials. Phase II trials will treat a target population, in which the <u>patients are as similar as possible</u> (see discussion below), with a dose of medication and focus on safety and early signs of efficacy. In general, phase II trials are open label, meaning that the doctors and patients will know they are receiving the drug. All phase II trials have at least one outcome measure, which determines at least one degree of effectiveness. It is critical to understand this aspect of a phase II trial. For example, in a trial looking at the effectiveness of a cancer therapy for patients with brain tumors, that outcome measure could be: time to (of?) radiographic growth; time to death; percent of patients showing at least a 25% shrinkage of the tumor; reduction in one symptom of the tumor. All of these outcome measures are valid, and their choice is based on what the researchers think may be the best measure of the medication's effectiveness. However, the outcome measure must be defined before the study and not changed after the study has started. For example, if the outcome measure for a mitochondrial therapy is improvement in bowel function, and

after entering 5 patients you observe that 3 of the 5 also had seizure improvement (although you have not predetermined a system to measure seizure improvement), you cannot "go back" and add that new outcome measure without redesigning the study. For simple observational measures (like weight gain or seizure improvement) the cost to look at that outcome measure is little. For assessing strength improvement or cognitive improvement, the financial cost is greater. For studies requiring repeated muscle biopsies (to just use an example) the cost can be enormous. It costs money to collect outcome measures and the more outcome measures you include, the more patients you will need to study to determine if the medication is effective (this is not intuitive unless you are a mathematician). For patient's with mitochondrial disease, the outcome measure could be reduction in resting lactate, reduction in seizures, percent of patients showing a weight gain of more than 10%, improvement in cardiac function, improvement in grip strength, change in free-radical injury as measured by compounds found in the urine, and so forth. It is important to remember that each outcome measure has a financial cost and the more outcomes are included, the more patients are needed to determine the significance of the results. Clearly choosing the outcome measures to study is critical, because it impacts on the financial and time costs of the study. In phase II trials, very few outcome measures are chosen because the results are almost never definitive in proving effectiveness, but will usually define those drugs that are worthy of future study there are very few grand slams in clinical trials). In some phase II trials, there may be a control group of usually healthy people to be compared with the study group. This may help us identify if the improvement is specific to the mitochondrial patients, or if the toxicity is different in the two groups.

*Phase III Trial*s

Phase III trials are generally randomized and double-blinded studies aimed at evaluating both safety and efficacy in the target population. "Randomized" means that patients will be treated in one of two (or one of three) ways, as determined by a random computer program. "Double-blind" means that neither the doctor nor the patient knows what treatment is being given. In some circumstances, one-half of the patients are treated with placebo (a sugar pill) or a very low dose of the medication. Because patients do not like the idea of being on placebo, many studies either randomize patients to two different doses of the same treatment or - more commonly - built in a crossover design. In crossover studies, the patients that are initially randomized to receive the treatment will later "crossover" to receive placebo, while those on placebo will crossover to get the treatment. The crossover design is clearly more expensive (outcome measures need to be repeated and the study is twice as long) but the obvious advantages are that it is more acceptable to patients (and their doctors!) and each patient can serve as their own control. In phase III trials, outcome measures are chosen very carefully because of expense and the need of increasing the numbers of patients for each additional outcome measure. The statistical analysis is also critical in phase III trials.

Phase IV Trials

These are post-marketing trials for surveillance, safety and sometimes for new indications. These have not been part of drug development for mitochondrial disorders.

B. CATEGORIES FOR *Strength of Evidence in Evidence-Based Medicine*

Category A - based on data derived from:

- Meta-analyses of randomized controlled trials with agreement regarding the directions and degrees of results between individual studies.
- Multiple, well done randomized clinical trials involving large numbers of patients with agreement in the outcome.

Category B – based on data derived from:

- Meta-analyses of randomized controlled trials with conflicting conclusions regarding the directions and degrees of results between the individual studies.
- Randomized controlled trials that involved small numbers of patients or had significant methodological flaws (e.g., bias, drop-out rate, flawed analysis, etc.).
- Nonrandomized studies (e.g., cohort studies, case-control studies, observational studies).

Category C - Based on data derived from:
-Expert opinion or consensus
-Case reports or case series

Strength of Recommendation

Class I - Recommended
The given test or treatment has been proven to be useful, and is indicated in all cases.
The Class I recommendation should be reserved for tests or treatments for which there is near-universal agreement on their appropriateness. This level of recommendation should be applied to tests or treatments for which there is a large amount of concordant, high-quality evidence proving their usefulness.
An example of a Class I recommendation is the use of aspirin, in the care of patients with an acute heart attack.
In rare circumstances, a Class I recommendation may be applied to tests or treatments for which ethical concerns preclude the performance of randomized controlled studies. An example of such a treatment is the use of oxygen in the care of patients with acute heart attack, or for the layperson, the use of a parachute when jumping out of a airplane.

Class IIa - Recommended, In Most Cases
The given test, or treatment is generally considered to be useful, and is indicated in most, but not all cases.
The Class IIa recommendation should be applied to tests or treatments of demonstrated benefit for which the weight of the evidence, magnitude of benefit, or level of agreement is less than that of a Class I recommendation.

Class IIb - Recommended, In Some Cases
The given test, or treatment is not generally considered to be useful. It may be indicated in some, but not most, cases. The Class IIb recommendation should be applied to tests or treatments of uncertain or little benefit. For such tests or treatments the weight of the evidence, magnitude of benefit, or level of agreement is less than that of a Class IIa recommendation. In general, Class IIb tests or treatments are not indicated; however, they may be appropriate in the care of a given patient in consideration of the specifics of that patient's case.

Class III - Not Recommended
The given test, or treatment has been proven to be harmful, and should be avoided in all cases.

The Class III recommendation should be reserved for tests or treatments for which there is near-universal agreement on their lack of benefit or on their potential to cause harm. This level of recommendation should be applied to treatments for which there is a large amount of concordant, high-quality evidence proving their lack of usefulness or proving their potential to cause harm. An example of a Class III strength of recommendation would be not using valproate in a person with Alpers disease, because of concern of unmasking the liver disease that can occur in such cases. Interestingly the SOE for this recommendation is C; expert opinion a