1. Introduction and Background to Decisions

Discussions of the group were focused on the draft from the multiple sclerosis (MS) Common Data Elements (CDE) document. Text of the neuromuscular group borrows heavily from the antecedent work of the MS group.

Two aspects of this topic were reviewed by the Subgroup – biospecimen collection and clinically relevant biomarkers in neuromuscular diseases derived from those specimens. Specifically not addressed in terms of biomarkers, was the aspect of imaging biomarkers as this is addressed by the Imaging Subgroup.

The Subgroup decided to review, as an initial step, the biospecimen recommendations of the MS biospecimen group, and from other disease areas that have undergone a similar process with NINDS, beginning with procedural issues related to obtaining biospecimens.

* As with other CDE groups, it is strongly advised that no specific biomarker or biospecimen CDE be required as part of future clinical trials or studies.
* Instead, a list of possible specimens and assays are provided as a potential ‘menu’ where menu items can be chosen as deemed relevant.

Each trial or study should examine individual biomarkers in part because the biomarker issues are often situation-specific. The biomarker elements may be, in many instances, different for disease-related studies (looking for markers of disease progression) versus therapeutic studies (looking for markers that demonstrate differential responsiveness to specific therapies but which may not necessarily be markers of disease progression). Many however may serve dual purposes. The Subgroup reviewed available information for standardizing the sample collection methodology and was charged to recommend revisions as appropriate. A problem with Neuromuscular biomarker research is the quality of the laboratory work as many studies/centers do not have the resources to follow Good Laboratory Practice (GLP). Thus, recommendations would by default be targeted towards R01 grantees and would try to adequately address quality control and validation issues.

DNA is important to capture and is of modest burden (although rigorous SOPs must be in place to protect confidentiality). Muscle biopsy, nerve biopsy, Plasma/ serum, and PBMC seem reasonable to collect, depending on the specific trial and endpoints. The Subgroup agreed it makes sense to provide standardized protocols for collecting the various samples but would further discuss mandating such collection while in parallel explore what samples to acquire, how best to handle the samples and provide some input on recommended testing.

1. Mandatory vs. Not Mandatory Collection of Biological Samples in all NIH-funded trials in Neuromuscular Diseases (NMD)

The Subgroup reviewed whether mandatory collection of samples should be recommended in order to foster research in bio-specimens in Neuromuscular diseases both within specific study protocols but also by making samples available widely to neuromuscular researchers. The Subgroup consensus was that bio-specimens (e.g. blood for DNA and/or biomarkers; possibly muscle or nerve biopsy) could ideally be part of every NIH-funded study and that guidance could be given on how to collect and store samples. However, the view was that due to a lack of definitive soluble biomarkers in neuromuscular diseases, it was not appropriate to specify which tests to perform. Which biomarkers to test should remain hypothesis-driven. If the mandatory requirements for obtaining samples deemed as Core elements remained modest, most study sites would be capable of participating.

The Subgroup felt that many or most clinical trials or studies in neuromuscular diseases may require stratification and/or knowledge of genetic diagnosis (e.g. specific gene mutations in one of the 50+ genes causing muscular dystrophy, 20+ genes causing ALS, or 30+ genes causing peripheral neuropathies). Thus, obtaining DNA and banking the DNA samples may be included as part of clinical studies. Banked DNA could be useful for future studies of genetic modifiers (polymorphisms predisposing to treatment response or disease severity).

Another key issue regarding mandating sample collection is funding, which is not trivial, particularly given the current economic and research environment. Mandating collection would require provision of funding to investigators who include sample collection in the protocols. The NINDS noted this could make it more difficult to get grant approval as this aspect may in some cases, in the eye of reviewer, rate a lower study score if not properly incorporated with adequate scientific rigor. An alternate method would be provision of supplementary funding to investigators as a means to encourage voluntary biospecimen collection. In fact there exist already parallel application procedures at NIH for bio-specimen collection as stand-apart from the main grant application which allows for investigators to potentially obtain funds for this purpose, without impacting overall study protocol rating.

The multiple sclerosis (MS) Subgroup raised the idea of mandating collection at the general meeting of all CDE subgroups and while generally favorably perceived, the concept also generated discussion around most of the limitations discussed above. Subsequent to that meeting, the MS Subgroup received feedback from the NINDS on the concept of mandatory specimen collection. The NINDS agrees it is valuable to collect samples and store in a repository; however it must weigh the practical considerations of mandating this and would not be supportive of such a recommendation at this time. Rather NINDS preferred that the Subgroup focus its recommendations on standards for collection, shipping and storage of samples. Decisions regarding NINDS-funded sample collection will continue to be addressed by NIH on a case-by-case basis. The Subgroup agreed in the absence of funds it is not reasonable to mandate this sample collection, while highlighting that this approach may continue to allow the soluble/cellular biomarker field in neuromuscular diseases to languish. It was suggested by the Subgroup that if a study did in fact receive NIH funding, any stored samples from that study should be made available to others in the research community, assuming the request is based on a scientifically valid question, presumably requiring an adjudication/review committee.

1. What to collect, how to handle and what to analyze
   1. Samples

The MS subgroup noted that there are limited numbers of options of samples to collect including blood, other body fluids (CSF, urine), and tissues (biopsies, autopsy). It was felt that some of these are of greater utility than others and some pose greater logistical challenges. Therefore, the MS Subgroup ranked various samples on these two aspects to provide some guidance to investigators wishing to include bio-specimen sampling in their study. The grading system, as currently envisaged, was from 1 to 3, with 1 being most relevant on the utility scale and 1 being the easiest to perform/obtain on the feasibility scale. Based on these rankings, biospecimens could be grouped in 3 overall categories; (i) easy to obtain and highly relevant (serum, plasma, whole blood for RNA/DNA, FACS), (ii) harder to obtain but highly relevant (CSF, CSF cells) and (iii) hard to obtain or less relevant (autopsy material, biopsy specimens [muscle, nerve, skin, bone marrow etc.], urine). A listing of biospecimen samples that could be acquired in clinical studies is provided in Appendix A with a ranking on utility and feasibility. Clearly appropriate specific handling for samples (e.g. PBMC) will depend on the nature of biomarker studies being planned to determine if samples must be processed immediately, or frozen and shipped etc.

The MS Subgroup discussed whether coding and data management is within its purview. Coding of this material is very complicated. The TBI and Stroke groups have provided basic high-level guidance on how the data should be documented. To properly approach this key topic would require agreement on core elements to collect and then agreement on a universal coding system, presumably driven by those maintaining a central repository. This larger effort could be subsequently implemented by individuals collecting samples even if not part of a collaborative group to ensure uniformity should such samples later become more generally available. This effort is beyond the scope of the Subgroup.

The MS Subgroup discussed the importance of collecting associated phenotype data (e.g. demographics, disease measures, MRI data) for each bio-specimen collected. The clinical and para-clinical elements to collect will be aligned with the CDEs proposed by the relevant sub-groups. Demographic data should include age, gender, race while the samples themselves should have recorded key collection information (e.g. date, time, amount collected, number of aliquots, volume per aliquot, storage location, ID number). The MS Subgroup felt strongly that without a well-documented clinical/demographic dataset linked to the specimens, the effort will lose much of its potential.

The Neuromuscular diseases Subgroup concurs with all these careful and well thought-out deliberations of the MS Subgroup, and as stated above, is largely transcribing their report from the MS Subgroup report.

b) Biomarkers

Neuromuscular diseases is a highly heterogeneous and broad category of disorders, encompassing hundreds of genetically defined and acquired disorders. The broadest categories are myopathies (disorders of muscle) and neuropathies (disorders of nerve), and certain biomarkers can be utilized within these groups. For example, in the myopathies, serum phosphocreatine kinase activity levels (CPK) are generally reflective of ongoing muscle damage, and can be used as a surrogate biochemical marker for clinical studies in specific subtypes, such as Duchenne muscular dystrophy. In the neuropathies, many are acquired inflammatory disorders, and standard serum inflammatory markers may be used.

In the Neuromuscular diseases group of disorders, many are single gene (monogenic) inherited disorders, and the primary gene product can be a target of therapeutics (e.g. gene therapy). As such, detection of gene replacement at the gene, mRNA, or protein level in the target tissue (muscle or nerve) becomes an important if not critical biomarker. Regulatory organizations, such as the FDA and EMA, look towards collaborative groups to define standard operating procedures (SOPs) for methods of detection of such biomarkers. The Duchenne muscular dystrophy research community has established an “International Working Group on Biochemical Outcome Measures” to study best practices in detection of dystrophin as a biochemical outcome measure (see <http://www.treat-nmd.eu/downloads/file/newsletter/archive/2010/treat-nmd_newsletter_no87.pdf>). The Neuromuscular Diseases Subgroup encourages similar disease-focused groups to develop similar consensus building efforts towards SOPs.

As with the MS Subgroup, the Neuromuscular diseases Subgroup discussed providing a list of those biomarkers being considered. There are currently no biomarkers that are considered sufficiently validated in Neuromuscular diseases to be considered as Core Data Elements. The Subgroup discussed that if they recommend any biomarkers they would likely be classified as exploratory considering that the biomarkers change quite frequently.

The MS Subgroup noted that investigators should be encouraged to investigate available bio-specimen facilities, both for logistics of obtaining, processing, and storing samples as well as methodological aspects of testing. This information can be obtained at the respective websites of the bio-repositories listed below. Published reviews on this topic related to CSF sample handling have been developed by BioMS*eu* (<http://www.bioms.eu/index.php>) and are available in published articles (Teunissen CE et al, Neurol 2009, 73:1914-1922; Teunissen CE et al, MS International 2011, doi:10.1155/2011/246412; Tumani H et al, Neurobiology of Disease 2009, 35:117-127).

Over time, greater emphasis may be placed on specific biomarkers for disease course/prognosis as well as treatment-response biomarkers.

**4) Examples of Bio-specimen Repositories**

Several examples of existing bio-specimen repositories exist. Among these are the NIH Coriell Institute, Immune Tolerance Network, BioMS (CombiRx Study), National Database for Autism Research (NDAR; <http://ndar.nih.gov/>), Alzheimer's Disease Neuroimaging Initiative (ADNI) as well as Kompetenznetz-multiplesklerose (<http://www.kompetenznetz-multiplesklerose.de/en>) as an MS-specific example in Europe. As is common in many endeavours, the oncology field is often ahead of others and an extensive amount of information on bio-specimens is available via the NCI including a bio-specimen research database (<https://brd.nci.nih.gov/BRN/brnHome.seam>) and a best practices document (<http://biospecimens.cancer.gov/global/pdfs/NCI_Best_Practices_060507.pdf>).

The NINDS has a bio-repository at the Coriell Institute (<http://www.coriell.org/>). Coriell stores specimens along with clinical phenotype data and disperses the samples upon request for a fee. For individuals or groups wishing to bio-bank DNA, one needs to apply to the institute and, if approved, support is provided to investigators to ease the process.

The Immune Tolerance Network (ITN) also has protocols for collection and processing (<http://www.immunetolerance.org/professionals/research/lab-protocols>) that have been vetted extensively and are updated annually. These provide a valuable resource for clinicians involved in clinical trials for which bio-sampling will occur. They have also done a number of gene expression experiments in MS trials using whole blood and have a listing of immune tolerance related genes that can be explored in collaboration with Applied Biosystems and Celera Genomics. ITN is also conducting cell subset and T-cell repertoire studies using an ITN core to do the sequencing.

ITN supported study samples (serum, PBMC) were initially drawn by sites and processed locally (including T-cell activation) before central shipment for further testing. However, due to issues with yield, delivery and specimen consistency, this has evolved to having sites draw samples and ship, without processing, to Rutgers (Rutgers University Cell and DNA Repository) for processing and aliquoting and then storage in liquid nitrogen before subsequent batch shipping to Fisher Bioservices for longer-term storage. PBMCs can be shipped ambient within 24-48 hours while serum, whole blood, and other samples are frozen and shipped on dry ice for processing and storage. The samples (~290,000 currently) have clinical data linked to them within the repository database.

The ITN repository lends itself to four levels of studies to consider.

**Core studies –** conducted by all sites in a study.

**Pooled studies** – subsets of sites, not study-wide, can exchange samples for sub-studies of mutual interest.

**Individual studies** – single-site, specific studies without involvement of Core or other centers.

***Post-facto* external studies (BioShare -** http://itnbioshare.org**/)** – other centers not involved in a specific study can apply for access to samples via ITN and after scientific review, samples, including phenotypic data, can be provided if deemed appropriate. However, there is a moratorium on provision of samples until 18 months after study close to allow investigators first access at pursuing scientific questions. [ed. Note – given the speed of research and value of samples, the subgroup would encourage ITN to consider a reduced length of moratorium]

Informed consent forms are developed for the specific objectives of the various studies. The above serve as examples of what can be done from the perspective of sample collection and storage and provide information on best practices for bio-specimen work. If SOPs for bio-specimen collection and storage were readily available and agreed upon, it is likely that more investigators would obtain samples and that the pharmaceutical industry would also adopt this approach.

The MS Subgroup recommended the ITN model to investigators interested in collecting, and analyzing, biospecimen material from clinical trials, and the Neuromuscular diseases Subgroup concurs with this opinion. Further, the ITN is open to assist investigative groups, including the potential use of ITN resources on a cost-sharing basis.

**5) Conclusions**

Biospecimens most relevant to Neuromuscular clinical studies are DNA (gene mutation), muscle or nerve biopsy (histology, biochemical biomarkers), and serum/plasma (soluble biomarkers such as serum CPK and inflammatory markers). The biospecimens and relevant biomarkers are highly specific to the hundreds of individual myopathies and neuropathies lumped into Neuromuscular diseases. At present no biomarkers achieve status as Core Date Element and very few even achieve the level of Supplemental, but rather most are simply exploratory. To advance the field, a concerted international effort around this topic is required. However, this requires commitment by many individuals and major financing to be done properly.

As noted by the MS Subgroup, given the current lack of central funding support for such efforts, and given the existing experience and facilities of groups such as ITN, investigators considering bio-specimen research should consider leveraging this expertise, including partnering with an existing bio-bank facility when establishing biospecimen activities within study protocols.

Large scale projects could be very expensive. However, if the sample size is kept modest, such as MS Phase 2 studies (~ a log scale smaller than the Phase 3 programs), sampling may be done with a reasonable budget. Output from the CombiRx study will provide useful insights on the larger scale study experience.

**6) Recommendations (from the MS Subgroup):**

a) Urge NIH/NINDS to consider mandating biospecimen collection while at the same time providing financial/logistical support to establish protocols/platforms/facilities for biospecimen sample obtention, retention and analysis within MS studies, including dissemination of this information to researchers.

b) Request NIH/NINDS to establish a working group / workshop of relevant stakeholders to set the ground-rules for such an effort, such as the one established by BioMS*eu*

c) Publicize to Neuromuscular diseases investigators the importance of considering relevant questions to pose in clinical trials that would require and use biospecimen data to enhance understanding of underlying disease course and prognosis as well as treatment-response biomarkers.

d) In the absence of universal guidance, investigators should adhere to already established collection, storage and analytic protocols (such as ITN, BioMS*eu,* BioMS/CombiRx) in an attempt to harmonize data collection while assuring quality control thus potentially allowing for pooling of data and cross-study comparisons

e) As a minimalist first step, encourage all those responsible for Neuromuscular diseases clinical trials to include collection of DNA samples, with broad consent (including use of samples in future by appropriate 3rd parties) to allow the conduct of important disease-related research in future, even if not as part of the specific study. Linking sufficient biographic, demographic and disease characteristics data with the samples is essential. Institutes such as Coriell and potentially ITN (both cited above), could facilitate storage of samples.

f) No recommendations can be given regarding soluble/cellular biomarker testing to conduct given that no biomarker for disease course or treatment response is currently validated.

g) The initial focus of biomarker work may best be directed to Phase 1 and Phase 2 studies as this may be more feasible, allow more in-depth testing and lead to initial progress that could facilitate broader progress in the field subsequently. Ultimately however, validation will require assessment in large-scale, likely pooled, data sets.

**Appendix A – Biospecimen Samples**

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| **Utility Rating**  **1=greatest utility and 3=least utility** | |  | **Feasibility Rating**  **1=most feasible and 3=least feasible** | |
| **Specimen Type** | **Average** |  | **Specimen Type** | **Average** |
| Serum | 1.0 |  | Serum | 1.0 |
| Plasma | 1.2 |  | Plasma | 1.2 |
| PBMC | 1.2 |  | Whole Blood | 1.2 |
| Whole Blood | 1.2 |  | PBMC | 1.3 |
| CSF\* | 1.2 |  | Whole Blood (ABI Tempus tubes - RNA) | 1.3 |
| CSF cells\* | 1.5 |  | Urine | 1.8 |
| Whole Blood (ABI Tempus tubes - RNA) | 1.5 |  | CSF\* | 2.0 |
| Tissue - Autopsy | 1.7 |  | CSF cells\* | 2.3 |
| FACS | 2.0 |  | Tissue - Autopsy | 2.3 |
| Biopsy - Skin | 2.2 |  | FACS | 2.4 |
| Biopsy - CNS | 2.2 |  | Biopsy - Skin | 2.8 |
| Urine | 2.3 |  | Biopsy - BM | 2.8 |
| Biopsy - BM | 2.3 |  | Biopsy - CNS | 3.0 |
| Biopsy - other (specify) | n.r. |  | Biopsy - other (specify) | n.r. |
| Other (Specify) | n.r. |  | Other (Specify) | n.r. |
| \* Teunissen et al, Neurol 2009, 73:1914-1922 | |  | \* Teunissen et al, Neurol 2009, 73:1914-1922 | |
| n.r. Not rated |  |  | n.r. Not rated |  |

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