# The Liver Forum

## Pediatric Working Group Meeting 1: Summary of Proceedings

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**Facilitating Collaborative Research in Drug Development and Health Policy**
1608 Rhode Island Avenue NW, Suite 212, Washington DC 20036
(p) 202-833-4617 (f) 202-872-4316
www.forumresearch.org

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## Part I: Development of a Pediatric Transatlantic Natural History Cohort

**Slides**: http://www.forumresearch.org/storage/documents/LiverForum/PedWG1/00.pdf
**Moderators**: Veronica Miller, Forum for Collaborative Research
Jeffery Schwimmer, University of California, San Diego

### Session I: Overview and Summary of Identified Gaps

**Slides**: http://www.forumresearch.org/storage/documents/LiverForum/PedWG1/01.pdf
**Presenter**: Veronica Miller, Forum for Collaborative Research

- There is an expressed need from regulatory agencies, industry organizations, clinicians, and researchers for better natural history data on non-alcoholic fatty liver disease (NAFLD) and non-alcoholic steatohepatitis (NASH) in the pediatric population. The major identified gaps in pediatric NAFLD/NASH data are:
    - The overall natural history of the disease as it progresses from infant, to child, to adolescent, to young adult.
        - There are different drug development issues associated with each of these sub-populations, including the need for pediatric formulations, the impact of puberty, the role of the maturing immune system, and the impact on liver inflammation.
    - The identification of appropriate endpoint(s) for pediatric clinical trials.
        - Preferable as non-invasive as possible.
        - To what extent can the endpoints and data from adult studies be applied to pediatrics?
        - What is the appropriate length of time for pediatric clinical trials and follow-up? How does disease progression interact with periods of growth (i.e., puberty), and how does this impact the length of follow-up?
    - Children are not simply “small adults”, and they metabolize drugs are different rates. Therefore, the pharmacokinetic (PK) studies will be very important in the different age groups, as will the pharmacokinetic/pharmacodynamic (PK/PD) models.

### Session II: Regulatory Approach to Pediatric NAFLD/ NASH- European Experience

**Slides**: http://www.forumresearch.org/storage/documents/LiverForum/PedWG1/02.pdf
**Presenter**: Elmer Schabel, European Medicines Agency

- European Pediatric Regulation 1901/2006 was established on January 26, 2007 with the objective of improving the health of children by increasing high quality medical research into medicines for children; increasing the availability of authorized medicines for children; avoiding unnecessary studies in children; and not delaying authorization for adults.
- The Pediatric Investigational Plan (PIP) is a binding procedure for all applicants and new substances. PIPs must include information on the quality, safety, and efficacy of the substance.
    - PIPs may include waiver and/or deferral requests
        - Waivers: may apply for full/ partial/ or class waiver if the product is ineffective or unsafe for pediatric populations, or if it applies to a condition only found in adults.
        - Deferrals: may apply for a deferral in order to avoid delaying authorization for products in adults.

  - The submission date for PIPs is at the end of Phase 1, compared with the Food and Drug Administration (FDA) Pediatric Study Plan (PSP), which is due at the end of Phase 2.
    - An agreed PIP is a pre-condition for marketing authorization, and a check for compliance occurs when a marketing authorization application is submitted.
  - The opinion on the PIP is given by the Pediatric Committee, and a final decision is made by the European Medicines Agency (EMA).
- The regulatory experience with PIP applications for NASH is limited. There are currently two agreed PIPs (one for NASH, one for hepatic fibrosis), two ongoing procedures for NASH, and one finalized pediatric Scientific Advice.
  - Elafibranor – Agreed PIP
    - The first agreed PIP, was agreed in July 2016.
    - The proposed indications are NAFLD and NASH.
    - A waiver was applied for and agreed upon for age two and younger.
    - The agreed measures are: development of age appropriate formulation; conduct of juvenile toxicology studies; and clinical studies which include a review of available natural history study data.
    - A deferral was agreed for the PIP to be completed by 2025.
  - Substance XXX – Ongoing PIP
    - The proposed indication for is NASH with stage 2-3 fibrosis.
    - A waiver was applied for patients age 12 and younger.
    - The applicant proposed a deferral pending availability of complete results in adults, noting the difficulty of performing repeated biopsies on children, and the need for additional natural history data on pediatric NASH.
    - The measures proposed included: a PK/PD study in adolescents with doses to be determined by a modeling exercise; this 2-stage PK/PD study would evaluate PK in the first stage and clinical efficacy in the second stage which would be based on non-invasive evaluation of liver stiffness.
    - The proposal was not accepted due to the following:
      - The waiver for children below 12 was not acceptable and the proposal should include patients 2 years and older.
      - Due to the rejection of the age waiver, the proposal should include the development of age appropriate formulation due to lower age.
      - Histology must be included as an endpoint (though did not specific if needed as primary or secondary).
      - Need for the implementation of body weight control in the study.
  - Substance YYY- Ongoing PIP
    - The proposed indication is NASH with stage 2-4 fibrosis.
    - The applicant proposed a waiver for patients less than 8 years old, with no proposal for the generation of natural history data.
    - The applicant proposed a deferral pending the results from adult studies and the availability of natural history data in pediatric NASH. However no proposal was submitted of how the natural history data would be collected.

        - The proposed measures are: no further juvenile toxicology studies, the development of a reduced strength tablet, and a PK/PD efficacy and safety study in patients 8-18 years.
        - This proposal is currently under review.
    - Substance ZZZ- Scientific Advice
        - The proposed indication is NASH, with a waiver requested for patients younger than 2 years old.
        - The applicant proposed a deferral pending the results from the adult studies and the availability of natural history data in pediatric NASH, indicating intent to generate additional natural history data and collaborate with existing registries.
        - The proposed measures are: juvenile toxicology studies, a PK/PD study staggered across age ranges with biomarker endpoints, and a phase 3 trial including the whole age range and basing the evaluation of efficacy on histology of 7-18 year olds, and non-invasive fibrosis analysis in 2-6 year olds.
        - Recommendations from Scientific Advice Working Party (SAWP) and CHMP:
            - Deferral awaiting more comprehensive natural history data is acceptable, but with clear proposal on how to generate this data.
                - Natural history data need to include European population.
                - Natural history data need to be from 2-18 year population.
            - Proposal to determine target population based on results is acceptable.
            - A deferral for patients 2-6 until a clear need to treat these patients is identified is acceptable.
            - The final design and endpoints are not possible to determine at this point, pending natural history data.
- The primary problem identified across these proposals is the need for natural history studies, including: identification of the target population; duration and design of study; and clinical endpoints.
- Natural history data will inform regulatory agencies on:
    - Determining the appropriate age ranges to be included in clinical trials.
    - Identifying the target population: NAFLD vs. NASH; stage(s) of fibrosis; disease activity; differences between type I and II.
    - Determining suitable trial designs including whether a placebo arm is needed.
    - Determining how much adult data needs to be available before beginning pediatric trials.
    - Determining how appropriate extrapolation of data is for the different populations.
    - The justification for and ethical issues around repeated biopsies in children.

## Discussion

- The FDA has not received the same volume of pediatric plans as the EMA because the requirements are different and pediatric plans to the FDA are not due until the end of Phase 2, compared with end of Phase 1 for EMA. The FDA was involved in developing the joint scientific advice.

- Some major questions that the field must consider are: what is the appropriate age range?; how much adult NASH data are needed before moving to pediatrics?; and can a histological endpoint be extrapolated for children without a clinical benefit outcome trial completed in adults?
    - It is possible that a pediatric trial could finish before a clinical benefit outcome trial in adults is completed.
    - Regulatory agencies do not necessarily want to conduct pediatric clinical trials where the outcomes are death or transplant, but do want assurance that histology or other endpoints are predictive of such clinical endpoints.
- Q: *Considering the responses so far to PIP submissions (defer until more data), is it worth the effort and resources for companies to develop a PIP/ PSP?*
    - Yes- both the FDA and EMA agree that the major barrier to doing pediatric trials is the lack of natural history data (what population, what age, how the disease progresses over time). By starting the process early, companies will be able to begin developing age appropriate formulations and generating natural history data, even if they won't be able to start on their pediatric clinical trials.
    - Natural history data is part of both the scientific and regulatory path forward.

  Industry is encouraged to come to the FDA and propose clinical trials in children with NASH, so long as there is a rational argument for prospect of benefit.
- Q: *Are there areas where the FDA and EMA disagree on what's needed for pediatric NASH studies*?
    - The FDA and EMA are generally aligned. Both agencies would like to have more non-invasive biomarkers to use as endpoints with children, but understand that histology is what is currently available.
    - Companies seem to be working separately to collect natural history data, and one of the goals of this meeting is to begin thinking about how companies can work together on this effort.
    - It is important to remember that NASH in children is not a mild form of adult NASH, and children can present with bridging fibrosis or even cirrhosis.
    - There is natural history data from a cohort of pediatric patients undergoing two biopsies that will be available later in the year.
    - There is evidence showing that surrogate endpoints other than histology are inadequate, and so the move away from histology before surrogates are validated may be premature and unethical for trials.
        - There is a common desire to move away from histology; however, there is currently not the data available to support doing so.
    - Using histology as an endpoint influences study design. If children are going to be biopsied, the studies should be longer in duration- not a 6-month study with a repeat biopsy.
- Q: *What evidence is needed to determine what age categories to include/exclude in these studies?*
    - If there is significant evidence and consensus amongst pediatric hepatologists that NASH rarely occurs in patients under 8 and therefore they should not be include in clinical trials, then the field should publish papers and develop practice guidelines supporting that data.
    - It would be almost impossible to conduct a clinical trial in patients under 6, as there are not enough patients to design a trial with this population. Another factor

here is the ability to participate in these types of trials. The field has historically set an age range of 8 and above, as the ability of children to participate in trials increases substantially at around age 8.
    - For the natural history studies (not clinical trials) it is recommended that the ages included should go as low as possible so that we can have as much data as possible.

## Session III: Existing NAFLD/NASH Pediatric Research Across the Atlantic

**Slides**: http://www.forumresearch.org/storage/documents/LiverForum/PedWG1/03.pdf
**Panelists**: Michael Fried, University of North Carolina
Piotr Socha, The Children's Memorial Health Institute
Ingrid Delaet, Intercept Pharmaceutical

### TARGET-NASH: A Longitudinal, Observational Cohort Study

**Presenter**: Michael Fried, University of North Carolina

- TARGET-NASH is a real-world, observational cohort launched in July 2016 that collects both retrospective and prospective data.
    - The goal is to enroll up to 15,000 patients at 50 sites across the US, including 3,000 pediatric patients. Other international sites are being evaluated. As of February 2017, 700 patients have enrolled in the study.
        - Enrolled patients are adults and children over 6 years old that are currently being managed or treated for NASH or NAFL.
    - This project is modeled after HCV-TARGET which was created to understand the impact of new HCV therapies by analyzing high-quality, longitudinal, data from patients undergoing treatment at academic and community medical centers.
- The current paradigm has individual companies trying to obtain the same information about the natural history of a disease, and operating in silos. The TARGET-NASH study design is disease focused and allows for continuous acquisition of natural history and outcomes as new drugs enter the market.
- This is a collaborative effort led by an academic steering committee (K. Cusi, A. Sanyal, B. Tetri, M. Vos, AS. Barritt, S. Klein, R. Lomba).
    - Other components include advisory committees made up of representatives from industry, regulatory agencies, and patient advocates, and a publications committee. Together, these committees develop the research plan.
    - Contracts are in place with investigative sites to provide entire redacted electronic medical records of patients that are being followed, along with bio-sample and patient reported outcome.
    - Using the data collected, TARGET is able to generate quarterly reports on the cohort; create custom data queries to understand the natural history and/or plan for clinical trials; disseminate information through the development of manuscripts and presentations.
    - The primary aim is to establish an understanding of the current natural history of NASH at academic and community medical centers.
    - Goals include:
        - Evaluating NASH treatment regimens that are currently being used in clinical practice

        - Examining populations that may be underrepresented in phase 2-3 clinical trials
        - Evaluating optimal duration and combination of NASH therapies to achieve clinical response and clinical remission
        - Examining staging strategies, including liver histology and other non-invasive markers that are being utilized
        - Estimating adverse event frequency and severity and describing how these are managed in clinical practice
        - Evaluating the impact of NASH therapies on medical co-morbidities including long-term outcomes
- The study design has both retrospective and prospective components.
    - Patients consent to enroll in the study, allowing for collection of biospecimens and patient reported outcomes.
        - Retrospective: data is collected using patient records from the previous three years to establish individual patient baseline characteristics, including disease severity, diagnosis, and follow-up care.
        - Prospective: data is collected on patients for five years, following what happens to the patient over time, and potentially evaluating the safety and effectiveness of new therapies used in clinical practice.
            - Up to 8 years of data possible per patient.
    - The data collected includes patient characteristics (demographics, disease stage, genetic markers, and co-morbidities) and longitudinal data (concomitant medications, patient reported outcomes, medical events, therapies [individual/combination], laboratory results, and findings).
        - The patients entire medical record is provided, which can include x-rays, patient narratives, laboratory data, etc.
        - The type of data collected is not mandated by the study, therefore the data provided allows for understanding of how patients are being treated in routine clinical practice. This allows for identification of treatment patterns.
- The database is 21 CFR Part 11 compliant and meets the guidelines of the Clinical Data Interchange Standards Consortium (CDISC) for data exchange. The database incorporates coding from WHODRUG and Medical Dictionary for Regulatory Activities (MedDRA) so that generated reports are formatted similarly to phase -3 clinical trials.
    - Patient-specific biosamples are collected and retained (DNA and serum) that can be leveraged for future research

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## Diagnostic Approach to NAFLD: The European Experience

**Presenter**: Piotr Socha, The Children's Memorial Health Institute

- A position paper was developed by the European Society for Paediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) and published in the Journal of Pediatric Gastroenterology and Nutrition in 2012[1] on the diagnosis of NALFD in children and adolescents.

[1] Vajro P, Lenta S, Socha P, et al. Diagnosis of nonalcoholic fatty liver disease in children and adolescents: position paper of the ESPGHAN Hepatology Committee. J Pediatr Gastroenterol Nutr 2012;54:700-13.

    - While ALT and ultrasound can be used at a population level to identify those who likely have NAFLD, a liver biopsy is required for diagnosis.
        - For most other liver diseases, biochemical or molecular tests can be used for diagnosis. However, only histology can indicate the severity of NAFLD.
- A 2015 randomized controlled trial tested the use of Omega-3 fatty acids to treat NAFLD using a primary outcome of decrease in ALT, not repeated liver biopsy.
    - In many European countries, ethics committees do not justify repeated liver biopsy even for clinical trials, which is challenging for designing studies.
    - There was no significant change in ALT; however, the study reinforced that surrogate markers for liver steatosis, fibrosis and inflammation are needed.
    - The use of other endpoints for clinical trials is particularly relevant for patients without severe fibrosis.
        - While it makes sense to use histology as an endpoint in populations with severe fibrosis (stage 2-3), it would be difficult to justify in studies where the intervention is a food supplement in a non-severe population.
- Liver Multiscan is a new technique which can describe liver steatosis, fibrosis, and inflammation all together. The images produced show the severity of inflammation and fibrosis, which is calculated as a liver inflammation and fibrosis (LIF) score using a scale of 0-4.
    - Simple method to describe liver damage- the higher the LIF score, the more severe the damage is to the liver.
    - The LIF score corresponds well with the pathological findings in liver biopsy.

## Discussion

- It's clear that the field needs to start formulating a plan now; however, in the absence of really knowing the patient population and the age groups that need to be included in the pediatric plans, a natural history study is truly needed.
- Even though the age group is still unknown for clinical trials, TARGET should start the natural history cohort at age two. Even though there are only a few cases, it would be beneficial to obtain information on the younger age range.
- From an industry perspective, it's important that everyone collaborate to get TARGET-NASH going so that the field can all start from the same level when discussing the pediatric plans. Regulatory agencies recognize the difficulty in writing these plans with the various unknowns and unclear outcomes.
    - The outcomes of these pediatric trials based on histology or a non-invasive may be known before the adult outcome studies are finished.
    - The natural history studies are not what is holding up starting more clinical trials in pediatric NASH. Natural history studies do not need to be completed before sponsors can start, nor do clinical benefit outcome studies need to be completed in adults before starting. The challenge to starting the pediatric studies is having positive outcome results from drugs in adults. It hard to know how much is enough clinical benefit in adults to say that there is a prospect of direct benefit for children. Histology in adults could possibly be enough, and there may be some results from earlier phase trials that can provide enough prospect of benefit to get studies started in children. The field does not need to wait until the natural history studies are done to move forward.

  - At this time placebo-control studies with histologic endpoints for phase 3 studies, to support a marketing application, based on surrogates are necessary for approval. In the future, if noninvasive biomarkers that reliably reflect histology or clinical outcomes are identified they may be used in place of histology.
  - If there are clinical outcome trials in adults, there is potential that a surrogate endpoint can be used for marketing approval for pediatric NASH. However, natural history data in pediatrics will be needed to support the extrapolation of the clinical benefit in adults to pediatrics. Natural history studies that are done well have the potential to substitute as a placebo for the long term clinical outcome trials in pediatrics, if clinical benefit cannot be extrapolated from adults to pediatric populations (e.g., adult clinical outcome data is still pending).
- A natural history cohort has the potential for selection bias. Study patients are selected for follow-up in one or another center and undergo repeated liver biopsy, creating room for bias. It is advisable to agree on surrogate markers and use them consistently in every biopsy across study sites. A reliable and standard surrogate marker will add value to a natural history study.
  - The children who come to clinical attention that are the ones that the field is most interested in. Additionally, there will be substantial misdiagnosis if enrollees have not had a full and complete diagnostic evaluation.
  - TARGET-NASH initially opted to have broader criteria for enrollment to be able to subsequently look back to determine which patient had definite NASH, versus probable NASH, versus unlikely NASH. This allowed Target to collect the whole spectrum of disease, which is important when determining biomarkers because data is needed to understand the negative, as well as the positive predictive values.
    - Everyone in study will have to have a clinical diagnosis, however that is established. This will also be documented, allowing ability to go back and test the accuracy.
  - There are a lot of possible confounders. It's very common to have an anti-nuclear antibody or an anti-smooth muscle antibody positivity in childhood where they have fatty liver on some imaging technique, and then on biopsy, they actually turn out to have autoimmune hepatitis and not NAFLD. It will be difficult to make those distinctions if enrollment is very broad. There may be patients followed in the cohort that actually have a different diagnosis than what is intended or thought to be studied.
    - Many children in the TARGET study will likely be getting biopsied, specifically for the reasons pointed out. This may be very different in the adult population.
- *Q: One of the challenges in adult NASH is that unlike other liver diseases there is much less fibrosis. Is it a similar case in pediatric patients? Are there less collagen deposits in NASH versus HCV or other diseases? This will impact on surrogate markers.*
  - Liver biopsy should be used for diagnosis and to exclude autoimmune hepatitis. But other surrogate markers could be used as a follow-up once diagnosis is confirmed by biopsy.
  - Children can have severe liver disease, including cirrhosis and bridging fibrosis. That said, the proportion of the population in cross-sectional studies show less cirrhosis and bridging fibrosis in children. The proportion of adult NASH patients

with cirrhosis and bridging fibrosis increases with age along with other comorbidities like diabetes. Possibly less than 5% of pediatric patients biopsied have cirrhosis. In young children, about three-quarters have portal fibrotic expansion (stage 1c), and a quarter have bridging fibrosis. Overall, fibrosis looks milder in proportion to what is seen in adults.
    - Aside from worsening histology, a frequent clinical outcome seen in childhood is the development of type 2 diabetes in people that didn't have diabetes at the time of diagnosis. In the TONIC placebo group, over the course of 96 weeks 5 out of 50 patients developed type 2 diabetes.
- *Q: Is NASH specific to the pediatric population that is being biopsied? Is this disease pattern specific to regions or demographics?*
    - Pediatric data from the NASH CRN indicates 17% of patients that are asymptomatic have stage 3 fibrosis on baseline biopsy. Much of the data comes from the West coast, which has a higher population of indigenous Mexican Americans, who may experience more severe disease.
    - In a primarily non-Hispanic cohort from Midwest, there is still a significant amount of fibrosis observed, with 12% of patients having stage 3-4 fibrosis.
- The proposal of using non-invasive measurements is nice and the method presented looks interesting; however from the regulatory side, a strong validation study or several studies would be required that show that the method is capturing the changes that occur with treatment, the inter-rater validity, and consistency of assessments. All these kinds of features will have to be evaluated before this method can replace biopsy. EMA's pediatric committee continues to require repeated biopsy because these new proposed non-invasive measures have not validated. There are ethical concerns to trials using surrogate markers that are not validated and producing results which the field does not know what to do with.
    - Histology samples a small fraction of the liver, so it is important to include multiple endpoints in NASH clinical trials including both histology and imaging.
- The term "validating a biomarker" is being used loosely, but the FDA has a very specific definition for it. Biomarkers do not have to be validated in order to be used in a clinical trial (i.e., histology for NASH is not validated). The biomarkers being used in clinical trials, "1-point improvement in fibrosis", and "resolution of NASH" are not validated and are therefore "surrogates reasonably likely to predict". This is the reason why clinical benefit outcome trials are necessary. For a surrogate to be validated, it needs to be included in multiple clinical trials with different drugs, and over time, data confirms that it predicts an outcome. In hepatitis C, "sustained viral response" is now a validated biomarker and clinical benefit outcome trials are no longer necessary. The accelerated approval pathway is no longer needed once surrogate markers are validated; they basically become the primary endpoint and replace the clinical endpoint.
- In TONIC, the histology endpoints of resolution of NASH and reduction in NAS of 2 or more with no worsening fibrosis was statistically significant for Vitamin E over placebo, but the primary endpoint – change in ALT related to placebo – was not met. In CyNCh, the primary endpoint of histology was not met, but a reduction in ALT and AST – the secondary endpoints – was met. These are examples of how relying on the outcome of a histology surrogate could have been misleading.

    - The FDA takes statistics very seriously, and if a trial fails to meet the primary endpoint, it fails. Unless the statistical analysis has been constructed to allow the study to proceed onto secondary endpoints, then it's not a valid analysis.
- There should be a central review mechanism for the slides before enrolling patients in the TARGET cohort, as interpretations from site pathologists can vary considerably unless there is some type of consensus monitoring. This will be important especially across the Atlantic.
    - TARGET has detailed monitoring practices comparing source data to what is in the database.
    - The diagnosis of NASH in the pediatric population relies heavily on biopsies, perhaps more so than in the adult population. In many circumstances, the impetus to biopsy a pediatric patient is greater than in an adult patient. Seventy-five percent of the pediatric population in TARGET-NASH has been biopsied and will be available for review. Sites are scanning the biopsies and will be available for central review.
- *Q: For the TARGET study, will there be a cost to sample retrieval? And how will the adjudication of sample allocation for different proposed studies work?*
    - To address the requests for samples, the governance structure calls for a review by the steering committee about the scientific merit of each request in order to allocate samples. Scientific merit includes novelty, as well as sample size requested. The goal of such a large database is that a large number of biomarker studies will be able to be accommodated. There will be a cost associated with obtaining the samples. Of the 700 patients currently recruited, 10% are pediatric. As the sites get up and running, the study expects to see a great increase in enrollment.
- After hearing of the challenge in getting ethics committees in Europe to approve studies with biopsies for pediatric NASH, considering the present consensus that biopsy is the gold standard, it would be important for AASLD and EASL to put guidelines out for pediatric NASH that support that use of biopsy.
    - A set of guidelines was recently put out for the diagnosis and treatment of NAFLD in children. These guidelines do not mandate a repeat biopsy, but do state that a repeat biopsy – at the discretion of the physician – is appropriate at two years and up. This is typical clinical practice, because if there is stage 2 fibrosis on the initial biopsy and the patient has continued to have a clinical course that suggests progression, a repeat biopsy can be important to see how much the patient has advanced.
        - This is common clinical practice especially as patients' transition to adult practices.
    - Repeat biopsy in clinical trials should be considered as an option. Patients should have this option to be able to be followed serially to not only potentially impact their own care but the care of others.
    - Currently, the field needs to use histology and trials should be doing repeat biopsy in both treatment and placebo groups. Hopefully these will not be the only options in a few years. There have been two large NIH clinical studies where all children got a biopsy at the beginning and end, and not one IRB raised a red flag. The path forward is to propose clinical trials, and submit them to IRB. If a proposal raises a red flag, then it has to go to a government scientific panel that

can look at the whole picture and say, “because there will never be drug development for these children unless we do this”.
    - It is clear that repeat histology is going to be most helpful, but the literature is really bare on repeat non-invasive testing. When plotting tests against fibrosis level, there are outliers and the diagnostic utility in an individual subject is low. When thinking about therapeutics, it’s all about change over time, and these tests are just looking at single points in time and correlate that with histology at that point in time.
- There may be a more severe population within pediatrics that needs to be targeted, and this might be within the 2-6 year olds. In that case, this possibly could be considered a rare disease, which would be implications for the regulatory pathway. For example, the sample size wouldn’t need to be as large as it is in adults, and trials might need to be a bit more creative.

## Session IV: Advantages and Disadvantages of Natural History Cohort Designs

**Slides**: http://www.forumresearch.org/storage/documents/LiverForum/PedWG1/04.pdf
**Presenter**: Miriam Vos, Emory University

- There are very few existing pediatric NASH trials, and the cohorts from these longer term trials are very small (N=2, 18, and 5). Data from the NASH CRN has been presented in abstract form, and manuscripts are being developed. These studies (4,5) have larger cohorts and high quality data; however, the duration of those studies has been fairly short (1.8-2.2 years).
- The design of a pediatric natural history cohort will be dependent on the gaps in knowledge and will be unique to the disease pathway. Natural history studies can be an important source of key knowledge such as the choice of patients to treat, duration of studies, clinical outcomes, and then validate surrogate markers.
- There are important gaps that can be addressed by a pediatric NASH natural history cohort, including: improving the definition of NAFLD/NASH, defining distinct clinical phenotypes and stratifying by the future risk, identifying the time course of outcomes, identifying variability and progression, and validating histology and/or other markers.
- Key components of natural history studies:
    - Natural history studies fall somewhere in between a clinical trial and a registry. Natural history data needs to be good quality, but does not need to meet the rigor of a clinical trial.
    - Some data quality in monitoring should be included but does no to be as extensive.
    - A prospectively planned natural history study is better than capitalizing on data that’s available because it will generate a higher quality of data.
    - The study design should enable researchers to go back and query the dataset for future research questions. The more data that’s collected, even things that are not currently relevant, can be very beneficial.
    - Natural history studies can evolve over time as knowledge is gained.
- Pros and cons of different types of natural history studies:
    - Medical literature reviews: This is fairly easy to do; unfortunately, they are insufficient for most objectives and are highly biased by clinical care practices.

  - Retrospective chart review: This is an easy starting point and can guide future studies. They are insufficient for most objectives, are biased by clinical care practices, and there is variability in the data available.
  - Prospective cross-sectional: These provide a good idea of the span of disease in childhood; however, they don't provide a timeline of the disease or cross into adulthood.
  - Prospective longitudinal: This is the most comprehensive, and also the most expensive. It sounds great but it is not practical and the data is needed now.
  - Combined approaches: Allows researchers to select methods with the greatest benefits, but then need to watch out for the associated cons.
- Design considerations:
  - There is a pediatric to adult gap in knowledge. There needs to be collaboration between pediatric and adult centers and adult hepatologists in order to know what happens to these children as they become adults.
  - In later adolescence, many patients change or lose coverage and have a gap in healthcare in their 20s and 30s, which is when many of these NAFLD/NASH events are probably happening.
  - The time frame for patients to develop clinical outcomes is very long, and data is needed today, not in 20 or more years.
- Combining two of the study designs is a possible way to develop a natural history study in pediatric NASH. There is a lot of data from children who have undergone liver biopsies, and centers in the United States and Europe have biopsies from 10-15 years ago.
  - This allows us to go back and identify those children, and pull those historic medical records.
  - Framing this as an academic collaboration, a dataset can be developed from a chart review to obtain a historic cross-sectional look at NAFLD. Then, the patients (now young adults) can be located and recruited from that cohort into a prospective assessment.
    - Ideally those young adults would then be enrolled into a longitudinal trial because some of them may be close to clinical outcomes or have improved.
  - The benefits of a combined approach for the natural history cohort include: obtaining data on mortality, central review of liver biopsies, BMI, lab results, and other data included in medical records; identifying pediatric NASH patients who are now adults to analyze the frequency of outcomes, predictors of risk based on their original phenotype and current phenotype, establish time length to outcomes, compare blood-based markers pre and post; following the young adults longitudinally and determine effect of therapies.
- Pediatric Pilot Study
  - An Emory post-doc student did a pilot version of this type of retrospective/ prospective study. All patients within Emory that were diagnosed with steatosis by liver biopsy between 2000 and 2010 were identified. A total of 175 were initially identified. After chart review, 44 patients were confirmed with a diagnosis of NAFLD.
  - Patients (now young adults) were contacted by phone and email, and 23% responded by completing and returning a survey.

## Discussion

- *Q: How would this proposed study differ from the TARGET-NASH study?*
    - This project could capitalize on the resources of TARGET-NASH. Working with TARGET could be very efficient as they already have established sites all over the US, and have engaged a lot of adult centers. Patients for this study are likely to be close to one of the NASH-TARGET centers and could be recruited into that. It is a complex thing to add to a successful ongoing cohort, but it is a great suggestion.
- This approach to finding patients is very targeted, and those responding will be a self-selected group and can lead to selection bias. Something to consider would be to have a specific working group that includes different types of statisticians and taking advantage of causal inference methodology to pull out more inference from such an observational study rather than just using the standard traditional statistical approaches.
    - This is a great suggestion, and there is bias on which patients were biopsied especially 10 and 15 years ago because NAFLD was relatively undescribed in pediatrics. However, it is unlikely that historical patients would be able to be identified in a different way. To develop a historical cohort, biopsy is really the most objective criteria although it is a biased population.
- When analyzing cohorts from observational databases, it is helpful to have different types of cohorts depending on the questions being asked. For example, for questions on the progression of NASH in children who have been previously diagnosed, then the proposed approach would work well. However, if there are questions on distinguishing children who are at risk from those who are not, then different data is needed from before the children were diagnosed with NASH. It is important to keep an open mind to different approaches and the validity of different approaches.
    - By taking data from the different cohorts and clinical trials, and mapping everything out, a broad base of knowledge will be created and researchers will then be able to take an in-depth look at different sections of the disease spectrum. With carefully considered designs, researchers can combine these approaches to gain the most information. As long as there's a mechanism to continue the discussion, particularly around the data analysis part of it, using multiple approaches is a valuable option.
    - Current cohorts include the NASH CRN database, which is a highly detailed characterization of patients who've undergone a liver biopsy, who are primary still within the childhood age range with some limited crossover into adulthood. There is also the TARGET cohort, which is a wide description of disease not requiring a biopsy to come into the cohort, and currently starts at age six. This cohort will be following children of a wide range of disease over time. There are needs that are not met by these two cohorts.
    - It is good to get a broad population into the natural history studies because you can segment them out. This was done in Primary Biliary Cholangitis (PBC) with a global dataset. Using this dataset, researchers were able to segment out a population that matched the population for the trial and accept that the surrogate was predicting outcomes. It wasn't enough to validate the surrogate but was enough to say that it is reasonably likely to predict.

  - More data is better than no data. Presently there is not enough data in NASH. It is unknown whether or how many kids will "grow out" of their disease, for example, how many kids go through puberty and have growth spurts and then don't end up with fatty liver disease. The bigger the data set, the better one is to be able to answer questions.
  - The field is starting to see the benefits of big data. Similar to the global PBC study group in the UK, which has linked data sets that allow data to be mined (with permission) all across the country. These types of approaches where cohorts are developed across a country and observed from a distance are going to be really helpful. They're not going to give as much data as TARGET is because no one's going to be able to review those charts, but there may be able to find tens of thousands of patients with those things. The more data on the table the better it is for the whole community.
- *Q: Is it possible to get another kind of cohort from obesity clinics that have been following pediatric populations and may or may not have biopsy data? Depending on the clinic, patients may be followed more carefully because they need ongoing treatment.*
  - Many hepatologists collaborate with colleagues in the obesity field; however, the quality of the collaboration is dependent on the perception of NASH as an important problem. Another issue is the great variance among hepatology clinics on whether patients are biopsied and how often. If the clinics do not believe the biopsy is important to stage the disease, there will be recruitment issues and data gaps.
  - By necessity, the TARGET cohort would be subject to referral, ascertainment and selection bias. When originally getting patients referred back in 1995 in San Diego, the reason for patient referral was because kids going to junior high had to have a TB test. When the skin test was positive, they were getting put on isoniazid and their liver tests were elevated prior to this. Many of these cases turned out to be fatty liver, and the students would get a biopsy to make sure what stage they were at to know whether they can be put on a drug. Over time, this evolved because the clinic recognized a pattern and then community centers started to refer because patients that were obese, male, 14-year-old, Mexican American children. The reason for whether or not a hepatology center might biopsy them depends on all of those reasons and whether or not the diabetologists or obesity clinics care to refer their patients. This will determine who's going to be in this study.
- *Q: At this point, how many patients are enrolled in the NASH CRN database?*
  - There are 1,500 patients enrolled with the age range starting at two years. NASH CRN has published a large number of studies on a number of questions. If there are specific questions that the field wants to answer that haven't been addressed, the NASH CRN is happy to have partners in ancillary studies to propose to use the data or the samples.
  - There are two longitudinal pediatric studies that are in various stages of being written up. The manuscripts will be sent out for initial publication review in the near future.
  - A possible approach could be for the Liver Forum as a community, to develop and compile a list of regulatory and industry questions, rather than breaking this up by individual sponsors. In reality, everyone likely has the same questions for

the NASH CRN, and these questions will help TARGET-NASH in its early stages of data collection.
    - A collective list of prioritized questions would be a helpful thing for the community to move forward.
- *Q: Regarding the observation of type two diabetes in the pilot cohort study, although that may not appear to be a liver-related outcome, if the liver disease changes the progression of another disease, is that liver related? And should that be something that that's being explored?*
    - The pilot study knew to look at type 2 diabetes ahead of time based on work that has come out of the NASH CRN, including the results from the TONIC clinical that looked at diabetes and pre-diabetes incidence over two years. It does appear that there is a relationship between type 2 diabetes and NAFLD, and also likely NASH. The work so far suggests that NASH is a flag for early onset type 2 diabetes. In the literature, there is a growing swell of evidence around this; however, there is not enough evidence at this point to say it is an outcome of the liver disease, but it certainly appears to be an important clinical progression.
        - Clinical practice to do hemoglobin a1c annually on all patients, and have recently developed guidelines recommending routine screening with either a fasting glucose or a hemoglobin a1c.
    - A paper from the NASH CRN was published in JAMA in October[2] that looked at over 600 children with NAFLD. Of these children, 6% had type 2 diabetes, and 23% had pre-diabetes. These rates of diabetes and prediabetes in children with NAFLD are much higher than the rates in similarly obese children. Researchers believe that NAFLD does have a more direct relationship with diabetes. The data looks like for many children, NAFLD comes first and is the driver for diabetes, though diabetes is complicated so this may be oversimplifying. Additional findings show that having pre-diabetes or diabetes was also strong risk factors for having NASH within the context of NAFLD. There seems to be an overall risk for glucose dysregulation with NAFLD and a bidirectional disease severity relationship between glucose dysregulation and the severity of NAFLD.
    - Lisa VanWagner, Mary Rinella and others are going to be presenting an abstract on a study where they looked at CARDIA data. CARDIA is a long term cardiac focused dataset. Their results showed that 15 years after an imaging-based diagnosis of NAFLD, in patients with NAFLD there's an eight-fold increase in the onset of type 2 diabetes compared to patients without NAFLD. This is a very strong relationship that supports what is being seen in the pediatric population.

## Session V: Pediatric NAFLD/NASH Natural History Inclusion Criteria

**Presenter**: Cynthia Behling, University of California, San Diego

- Early studies of adult fatty liver disease
    - These studies included descriptions of the histology that we now classically know as NASH. Specifically, the livers from these patients had striking fatty chains, lobular hepatitis, necrosis, inflammatory infiltrate, and many had fibrosis.

[2] Newton KP, Hou J, Crimmins NA, Lavine JE, Barlow SE, Xanthakos SA, Africa J, Behling C, Donithan M, Clark JM, Schwimmer JB, for the Nonalcoholic Steatohepatitis Clinical Research Network. Prevalence of Prediabetes and Type 2 Diabetes in Children With Nonalcoholic Fatty Liver Disease. JAMA Pediatr. 2016;170(10):e161971.

These studies eventually led to the recognition that NASH is a pattern that encompasses several histologic features and is not really a single pathognomonic finding. The pattern includes varying degrees of fat infiltrations, lobular inflammation, hepatic injury, and often includes a particular pattern of fibrosis.
    - As the disease was studied, it became apparent that clinical trials were needed and that a better way of measuring histology. As a result, a number of scoring systems have been proposed.
- The NAFLD Activity Score (NAS) was developed as part of the NASH CRN as a way to assess a response to treatment in clinical trials.
    - The NAS score attempts to evaluate the three important features of disease activity: fat, lobular inflammation, and ballooning. And then also evaluate the amount of fibrosis. NAS score provides a grade which indicates how much activity there is, as well as a stage, which indicates how much scarring or fibrosis there is.
    - Components of NAS:
        - Steatosis: the amount of steatosis (accumulation of fat in the liver) is the first thing assessed when evaluating a liver biopsy- both in clinical practice and in a clinical trial setting. This is assessed by the percent of hepatocytes that contain a fat droplet. This can be further divided by large fat droplets, small fat droplets, or micro-fat.
            - Distinct from steatohepatitis, which usually describes accumulation of fat along with the presence of some level of liver injury.
        - Lobular inflammation: can be assessed by counting the number of foci of lobular inflammation, which are collections of lymphocytes or the inflammatory cells in the hepatic parenchyma in a given unit of liver area.
        - Ballooning: the degeneration of hepatocytes is a hallmark feature that is really quite distinctive.
    - Similar features and definitions are used in the FLIP score and the SAF scoring system that is more common in Europe.
    - The staging of fatty liver disease is done by evaluating the amount of collagen deposition. In adult NASH, perisinusoidal collagen is a classic feature. This is typically located around the central vein. Further degrees of fibrosis are evaluated according to the NAS fibrosis stage.
        - Fibrosis stage 1 is perisinusoidal fibrosis.
            - If it is visible on an H&E stain it's considered moderate. If it requires a trichrome stain, it's considered mild.
            - If the fibrosis is isolated to the portal tract, it has a separate category in the NAS fibrosis stage system and is considered as an early stage 1C.
        - Fibrosis stage 2 is when there's portal and perisinusoidal fibrosis together in the same biopsy.
        - Fibrosis stage 3 is bridging fibrosis.
        - Fibrosis stage 4 is cirrhosis which is defined as bridging fibrosis plus regenerative nodule formation.

- These scoring systems apply a quantitative categorization to what is really a dynamic disease that exists in a continuum. Fatty liver disease, in particular, is a bidirectional continuum.
  - Steatosis can develop into steatohepatitis and then regress. Some patients develop fibrosis, which can then regress. Some patients progress to end stage liver disease. It is unclear at this point how many go in each direction.
  - Although useful, the limitation of using these scoring systems is that they attempt to categorize continuous data as a categorical variable.

- Pediatric fatty liver disease:
  - Studies from 1984-2005 demonstrated the occurrence of fatty liver disease in children. In the mid 2000's, there were attempts to characterize the disease in children and a more distinct histologic pattern was identified in pediatric cases.
  - In a study published in 2005[3], the researchers looked at 100 cases of pediatric NASH and performed a cluster analysis of the histologic features. The results showed that certain features grouped together in the pediatric biopsies and it appeared that there were several distinct patterns of NASH.
    - Type 1: Patients demonstrate ballooning degeneration with perisinusoidal fibrosis, which is typical of adult NASH.
    - Type 2: Patients demonstrate portal inflammation and portal fibrosis, but with less ballooning degeneration than what is seen in adults.
    - Additional studies have had similar findings, and it is recognized that biopsies from children can have multiple patterns of fatty liver disease. Some have more steatosis and portal-base inflammation or fibrosis, which is different than what is seen in adults.
      - Variations of pediatric histology: some have a portal-predominant pattern, some have steatosis with little inflammation or fibrosis, and some have similar features as adult NASH.
  - In a typical pediatric pattern is portal-base: the portal tract is banded by fibrosis, which is surrounded by white clear vacuoles that represent the fat droplets in the liver cells. And there is relatively little fat around the central vein.
    - Example of biopsy slide from 14-year-old from San Diego showing expanded portal tracts, bridging fibrosis, and marked inflammatory reactions. Most would agree this patient has severe liver disease and has advancing fibrosis.
  - There have been very few natural history studies in pediatric liver disease which have paired biopsies. A study from the NASH CRN had 102 paired liver biopsies and the resulting data demonstrated differences between paired biopsies in children, and that histology changes over time as reflected by the NAS score.
  - Another study currently in press[4] illustrates that the pediatric pattern can change as children age, with data demonstrating that as children age, the disease patterns move from zone 1 to zone 3 (typical adult pattern).

[3] Schwimmer JB, Behling C, Newbury R, Deutsch R, Nievergelt C, Schork NJ, and Lavine JE. Histopathology of pediatric nonalcoholic fatty liver disease. Hepatology. 2005;42:641–649.
[4] In Children With Nonalcoholic Fatty Liver Disease, Zone 1 Steatosis Is Associated With Advanced Fibrosis

        - This study did not use paired biopsy, but illustrates that pediatric histology patterns change over time.
- Pediatric natural history or longitudinal studies:
    - Some generalities that should be considered are:
        - Believe that the degree of steatosis decreases as children age
        - Lobular inflammation may also decrease
        - Change in ballooning degeneration has not been reported in the literature
        - Portal inflammation decreases
        - The pattern also changes as children age
            - As children age, the histology changes.
    - Importance of liver biopsy:
        - For clinical reasons, liver biopsies confirm diagnosis and exclude confounding diagnoses. Approximately 15% of children have a concurrent or alternate diagnosis even when suspected to have NASH.
        - Biopsies allow the field to:
            - Grade and stage the activity of the liver disease and the fibrosis
            - Assess the effects of treatment
            - Determine the disease tempo, especially in paired biopsy studies
            - Allows in-situ evaluation of the relationship of multiple histologic parameters, each of which might have clinical relevance.
    - Importance of liver biopsy in clinical trials
        - There is not a lot of data on how fibrosis happens, what pattern it takes, or over what period of time fibrosis occurs in fatty liver disease.
        - Biopsy allows researchers to:
            - Examine specific types of collagen, and things that modify the collagen fibrils using special stains.
            - Measure regression and patterns of progression
            - Examine the structural arrangement of the fibrosis, whether it's portal or central.
            - Identify the dynamics of the fat droplets in cells, whether they're located in one particular zone, how they co-localize with various proteins, the endoplasmic reticulum.
            - Refine what is known about the histology of fatty liver disease (for example the refined ballooning score presented recently, and the development of a refined portal inflammation score.)
            - Allow for the study of ultrastructural elements that might relate to the pathophysiology of fatty liver disease such as the mitochondria.
        - General considerations and limitations:
            - Biopsies are a snapshot in time and really only reflective of what is going on in that patient at that moment in time.
            - There will be heterogeneity in biopsies taken from various parts of the liver in one patient, and sampling variability within the liver.

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Africa J, Behling CA, Brunt EM, Zhang N, Luo Y, Wells A, Belt PH, et al. Clinical Gastroenterology and Hepatology. 2017 [In Press].

- Inter-observer variability between pathologists and intra-observer variability with one pathologist is inherent in the interpretation of how we evaluate liver biopsies
- Many unknowns may be reflected in the liver biopsy, including demographic, geographic, genetic, and other variations.
- Important going forward to use digital imaging for pathology, as it is useful for diagnosis as well as clinical trials.

## Session VI: Key Information to Track Disease Progression in Pediatric Trials

**Slides**:http://www.forumresearch.org/storage/documents/LiverForum/PedWG1/06.pdf
**Presenter**: Joel Lavine, Columbia University

- Associations with histology
  - Results from a 2010 publication from the NASH CRN[5] show an association between a number of features of metabolic syndrome and liver histology in children with nonalcoholic fatty liver disease. The most common feature was obesity, though insulin resistance, dyslipidemia, and hypertension were also associated.
  - A 2008 publication in Gastroenterology[6] focused on clinical correlates of histopathology, specifically predictors of fibrosis stage. These predictors included elevation in ALT, AST, GGT, alkaline phosphatase, as well as the presence of definite NASH, and NAS score.
    - Certain groups have an increased risk for pediatric NAFLD, including: obese individuals, boys, indigenous Americans, those with adult-onset diabetes, dyslipidemia, family history of fatty liver disease, obstructive sleep apnea, and hypopituitarism.
    - There have been no prospective pediatric studies that had a reasonable sample size and reasonable follow-up.
  - Lifestyle factors are a major contributor to the development of pediatric NAFLD, and include the modern diet that has too many calories, and not enough antioxidants.
- Baseline predictors of histologic change
  - Data published in a 2012 abstract from the TONIC trial placebo group showed that patients that had a BMI z-score that was less than 2.5 did better in terms of NAS score change than those with a higher z-score.
  - Children that were under 13 did better in terms of the NAS score change over time than those over the age of 13.
  - Non-white patients did better than their white counterparts in regards to fibrosis stage change.

[5] Patton HM, Yates K, Unalp-Arida A, Behling CA, Huang T, Rosenthal P, Sanyal AJ, Schwimmer JB, Lavine JE; and the NASH CRN. Association between metabolic syndrome and liver histology among children with nonalcoholic fatty liver disease. Am J Gastroenterol. 2010;105:2093-2102.

[6] Patton H, Lavine J, Van Natta ML, Schwimmer J, Kleiner D, Molleston J; and the Nonalcoholic Steatohepatitis Clinical Research Network (NASH CRN). Clinical correlates of histopathology in pediatric nonalcoholic steatohepatitis (NASH). Gastroenterology. 2008;135:1961-1971.

    - Patients who had less leisure time and more physical activity did better with fibrosis stage change over time.
        - The study is limited by sample size.
        - Over 96 weeks in the placebo group, 5 children developed diabetes, and 53 did not.
            - Patients who developed diabetes had a higher BMI z-score at the beginning, had higher HbA1c, and had ballooning on their biopsy compared to those that were not diabetic.
        - Over 96 weeks, those who had weight change, or increased ALT, AST, or GGT were more likely to have changed predictors for histology.
        - Placebo group from CyNch trial had a larger placebo group, though treated for a shorter amount of time. This data should be published in the near future. In addition to a longitudinal histology based pediatric study from the NASH CRN.
- Key historical variables to collect for natural history studies
    - Essential
        - How and why the diagnosis is made
            - How did cases come to medical attention (referral and ascertainment bias)
        - Alcohol ingestion history and quantity
        - Past use of medications that could be associated with fatty liver
        - Past use of drugs to treat hypertension, diabetes, or dyslipidemia
        - Past use of drugs to treat fatty liver
        - Sleep patterns, snoring problems or history of sleep apnea
    - Desirable
        - Family member's BMI or BMI z-score
        - Birth weight
        - Gestational age at birth
        - Mode of delivery
        - Weight gain of mother during pregnancy
        - Others in family with known liver disease
        - Antibiotic use prior to age two
        - Whether children were breastfed and duration
- Key demographic variables
    - Essential
        - Age at diagnosis
        - Age at entry in the natural history study
        - Race/ethnicity
        - Location of patient residence.
    - Desirable
        - Patient socioeconomic status
- Key anthropometric variables
    - Essential
        - Height and height percentile
        - Weight and weight percentile
        - BMI z-score
        - Waist circumference

    - Desirable
        - Percent body fat by bioelectrical impedance
        - Localization of fat by DXA scanning
        - Body composition profile by MRI
- Key physical exam variables
    - Essential
        - Blood pressure in percentile for age and gender in terms of systolic and diastolic pressures.
        - Liver span
        - Splenic enlargement, if present
        - Acanthosis nigricans and severity, if present
        - Signs of liver disease (telangiectasia, clubbing, abdominal venous patterns, ascites, jaundice, and signs of excoriation from pruritus)
    - Desirable
        - Tanner stage
- Key laboratory variables
    - Essential
        - Liver function test panel which is ALT, AST, GGT, alkaline phosphatase, and total indirect bilirubin
        - History of autoimmune titers for anti-SMA, ANA, and anti-LKM-1
        - History of having been tested for other causes of liver disease or coexisting causes of liver disease, including the autoimmune panel
        - Fasting glucose and insulin
        - Fasting triglycerides, cholesterol total, HDL and LDL to look for comorbidities that coexist with fatty liver
    - Desirable
        - Prothrombin time, albumin, platelets, WBCs and hemoglobin.
- Key imaging variables
    - Essential
        - Ultrasounds with or without Doppler
        - Fibroscan with a controlled attenuation parameter
        - MR/ MR-PDFF/ MRE
        - CT scan of the liver
- Key histology variables
    - When liver biopsy was done and if it was repeated
    - The pattern (definite NASH, borderline zone I or III, fatty liver with or without inflammation)
    - Grades of:
        - Steatosis
        - Inflammation
        - Cell injury
        - NAS score
    - Stage of fibrosis with location.
        - It would be beneficial to have this information digitized so it could be online to have a separate affirmation of the diagnosis and the scoring severity.
- Key life variables

- Essential
  - Hospitalizations and the reason for those hospitalizations
  - Grade in school the kids are in, and whether they are in regular classes
  - New medications and dosing
- Desirable
  - Quality of life questionnaire
  - Dietary questionnaire
  - Exercise questionnaire.

## Discussion

- *Q: Given what we know about pediatric histology, will the Liver Forum working group focus on disease definitions specific to pediatrics to further clarify the definitions? For example, ballooning is considered a critical feature in adults, but it is lacking in type 2, so it will be a challenge to assign a single endpoint for the two different types.*
  - In regards to the Liver Forum paper on disease definitions, it was not possible to address the complexity of the pediatric challenges with definitions in the current paper as it was being written. A few suggestions were made on the manuscript from the pediatric NASH perspective, but it would be an excellent idea to bring forth the disease definitions in pediatric NAFLD from this group.
- In post hoc analysis of the data from the CyNCh trial, one interesting aspect was that younger children who weighed less and had type 2 or borderline zone and pattern responded and met the primary endpoint. Whereas those that had the other patterns did not. What is the reason for that? Was it a dosing effect? Was it a pattern effect? Currently, there are no answers to those questions.
  - This is a reason why it is important to look at all of these different histologic parameters separately. Having a variety of individual histologic features that are known to be important included in whatever data is monitored is going to be important until such time that the field has decided what to call NASH in a child. Those definitions are something the field debates all the time when making a diagnosis.
  - The approach of the guidelines document was to use a term called pediatric NASH which encompassed both type 1 and type 2. Histology is critical to be able to know what happens based on these subtypes. Medications are ultimately the most helpful in understanding these patterns to see if somebody responds to Drug A with a very clear histologic pattern versus Drug B. The current approach is to be more inclusive regarding patient enrollment, but it is important to characterize the histology in a sufficiently detailed way so that proper analyses can be done.
  - It is important to look at endpoints for drug treatment studies. If a trial has inclusion criteria that require everyone to have NASH, then for children, only a minority of biopsies obtained will be included.
  - If the endpoint is going to be defined by resolution of NASH, then it will depend on what the baseline definition was. The right thing to do may be to make the endpoint related to NAS but in certain components to know whether it's applicable across the board of children with fatty liver or whether it's only good for a subset of them.

- In US clinical practice, a lot of kids are being put on atypical antipsychotic medications to treat depression, bipolar disease. These drugs are being used in increasingly younger children, and children can often gain a lot of weight and develop elevated liver enzymes. Including these medications in a natural history study would be of interest to see what effect, if any, they have on the severity of NASH.
    - Being very meticulous with capturing what medications are being used, not just to treat the disease, but what is going on in the background.
    - That could also potentially be considered a drug-induced liver injury.
- The data capture sheets for the natural history study have to be designed to be able to have uniformity around capturing this data.
- *Q: For clarification on type 1 and type 2, is it safe to say that type 2, eventually becomes type 1?*
    - It has been shown in increasingly more detail that the distribution of zone I or zone III features will differ in a younger child versus an older child. Whether that child's disease starts at a younger age and has zone I predominant features, whether they actually develop zone III predominant features as they age, there is not enough data to really directly answer that question yet.
    - Many of them do, but unsure if all of them do. Any hepatologist could look at a slide not knowing the patient's age and recognize that it is a child because the pattern is not seen in adults. It either has to become the adult-type pattern or it has to go away.
    - Sometimes see children that have both types, is it that one doesn't disappear but that the other emerges?
        - Anecdotally, there are these cases that kind of fall in between having only portal fibrosis, but a zone III distribution of fat and maybe some ballooning. But fibrosis has not yet developed in the middle. There are arguments about which box to put those in because they don't fit cleanly into any box. There aren't that many of these cases, so it's a little bit difficult to see if they fall in the right place because it's all cross-sectional data and maybe some patients have a weird distribution of disease. While the field can make some educated guesses about what happens based on the cross-sectional data, it would valuable to have sufficient numbers of patients who get biopsied as kids at age seven to nine, and again around age 15 to analyze this pattern and track their progression or regression.
- *Q: Have there been efforts to look at genetics?*
    - Yes, the NASH CRN has been collecting DNA for a long time and are genotyping about 850 children.

## Part II: Pediatric Issues in NAFLD/NASH Clinical Trials

**Moderators**: Miriam Benedicta Vos, Emory University
Joel Lavine, Columbia University

## Session VII: Retention and Engagement in Pediatric NAFLD/NASH Clinical Trials

**Panelists**: Dan Peres, Immuron Limited
Reshma Shringarpure, Intercept Pharmaceuticals
Stavra Xanthakos, Cincinnati Children's Hospital Medical Center

## Discussion

- *Q: What do you think that the top problems in engaging children and sites into pediatric trials are?*
    - For parents, it's the safety of the preparation or intervention and the potential for benefit. There has been a lot of discussion today about the invasiveness of the liver biopsy, but from experience as a principal investigator, the procedure is not a major deterrent for enrolling. The major deterrents for most patients are the time required, the distance, or questions about adherence. Patients have not expressed concerns about having to repeat a liver biopsy again at the end of this treatment. There is hesitation about the concept of placebo, and some parents have a difficulty with the fact that their child may not necessarily receive an active treatment. The side effect profile of the study drug is also a paramount issue for most families.
    - The placebo arm in a placebo-controlled trial in adults is simply standard of care, which is not much. In pediatric trials, it makes a great deal of sense to have a little more aggressive version of the standard of care. This allows the field to justify treating and the repeat biopsy of placebo patients. This may be helpful in getting the trials through the IRBs.
    - Ultimately, there needs to be an increase in education and awareness that puts benefit-risk into perspective around these issues. The hepatology field has established the importance of biopsies for diagnosis, but it is really important to clarify the benefit of having a biopsy. On placebo, children receive more frequent monitoring and healthcare management and it is the standard of care as it is available today. There is still a lot of pushback from ethics committees, and so it is important to document and put out literature explaining in the present context, in the absence of validated non-invasive biomarkers, the biopsy is considered essential and regarded by hepatologists as a safe procedure.
        - In adults, the standard of care that's offered is not much and focuses on reminding patients to eat better. But in pediatric trials it may make sense to have a more aggressive standard of care; this allows you to justify that you are treating the placebo patients and they do need a final follow up biopsy.
        - Diet and exercise management could be more robust as a part of the standard of care a bit more robust in pediatric trials. And even considering whether there should be a collaboration or use of what has been used in obesity trials to really make that something that would benefit the patients who are not receiving the drug.
        - Sometimes standard of care that's offered to patients enrolled in the placebo arm of clinical trials is less than what is done in an active clinical program. For example in some well-established NAFLD programs, patients meet a dietician and a psychologist. That's not the case everywhere, and there are also cases where clinical programs see patients only once a year or every six months, and they get minimal to no lifestyle interventions.
    - The safety profile of a drug has a lot to do with recruitment, enrollment, and retention. The repeat biopsy is a hurdle for some parents with younger children, but safety is the leading part of the discussion. By establishing a relationship with

the principal investigator to show the potential benefit these challenges can be minimalized.

- Retention in pediatric trials in possible, the TONIC study enrolled 70 children, and all 70, two years later, had a liver biopsy. CyNCh enrolled 42 children, and 41 of those patients had a liver biopsy as prescribed by the study one year later. There is potential to have extremely high rates- better than is seen in most adult trials. It's important for the field to be aware of that high retention is feasible.
    - In the TONIC trial, the second biopsy wasn't even required. It was a secondary endpoint. Overall the cohort was like 86 percent had both ALT and histology of 170 or so patients.
    - The study coordinators were a key factor in these great retention results. The coordinators were willing to work on Saturdays and schedule appointments beyond normal work hours to be available in the afternoon after school. Additionally, the NIH paid the airfare back to San Diego for those kids who had moved for college so that they could undergo the testing that was needed.
        - Appropriate budgeting for those kinds of things is so important. A significant amount of patients are lower SES, and transportation issues and juggling work and school schedules are big deals. Other key factors are having appointments available in the afternoon and during the weekend, communicating with patients via texting, and developing a good relationship the coordinators and the
- In regard to the second biopsy. In a classic pharmaceutical trial with more advanced patients, a second biopsied is justifiable and can be justified to the parents. Looking at early disease, a second biopsy would be a difficult sell to parents. For example as was mentioned earlier, it would be difficult to justify a biopsy when testing a food supplement.
    - Whether a biopsy is necessary depends on what the purpose of the study is. Early phase studies may not require histology because it's not necessary at the time. A later phase study, whether it's a "drug" or a "supplement", if the purpose is to treat disease, it is necessary to know whether disease improved or not.
    - A food supplement, if used to treat a disease, becomes a drug. If you buy a bag of apples, and are going to give those apples to your patient and tell them it's going to treat their NASH, you have turned that bag of apples into drugs. If food supplements are approved for "liver health" and not to treat a disease, that is under the umbrella of a different regulatory system with different requirements. If the product's intent is to treat, mitigate, cure, diagnose, or prevent a disease, rather than sustain the health of an organ, then it is considered a drug by the FDA.
    - In regards to the second biopsy, even a historical biopsy can be useful and can be used to enter a trial. Another point is to make additional biopsies optional and a choice for patients and their families, which they might choose to do.
- *Q: Is it more challenging to recruit patients for PK/PD studies where the duration of treatment is a lot shorter?*
    - It is not difficult to get patients interested in PK/PD study. It is sometimes easier to do PK/PD studies with older patients than with young patients.
    - It is not difficult to recruit for short-term studies. The first and major factor that parents consider when entering a study is safety. If parents are reassured about the safety of the study for their child, the next concern is how many days of

school the child will miss. The longer the trial, the more visits are needed. An eight visit study is a much bigger deterrent than something that's three visits. For this reason, weekend appointments are necessary for a trials' success.

- *Q: How common are open label extensions after double-blind phases for pediatric trials? If a patient is randomized to the placebo group in a Phase II study and then there is an open label extension, there is some additional safety oversight before patients are rolled-over. Provided there is access to open label drug at the backend of the study, then patients are able to contribute to the study and also benefit from the product in the safest possible way. How often does this happen, and should this be a recommendation to be included for pediatric trials?*
    - There are not a lot of pediatric trials in NASH; however, in rare diseases, the pediatric trials frequently do have open label extensions so that all of the children in the trial are offered a prospect of benefit.
    - There are also have some questions about the post-drug treatment aspect of this and the duration of drug effect. Studies like to have some time when it is still unknown whether a patient was on placebo or active drug to know how long the effect lasts.
- *Q: Is there a lack of public education on the impact of the disease that could be affecting retention and engagement? And how can this be addressed?*
    - There is still a lack of understanding what the disease is. For example, a clinical program uses laminated pictures of histology of a normal liver biopsy and a NASH biopsy and one with no fibrosis and one with advanced fibrosis. Patients don't understand because they feel well and don't understand what's going on inside their liver. It is usually pretty eye-opening for parents when you go over that visual aid.
    - It is important to develop more materials directed to the parents of the patients to highlight the consequences of not treating the liver. While it's in the peer-reviewed literature, it may not be as apparent in the lay media. There needs to be a development of materials to engage and retain these patients and the parents.
- *Q: Is there any role, do you think, for randomized withdrawal studies? If you don't want to put people first on placebo, you can put them all on treatment and then do randomized withdrawal. Is this disease conducive to that?*
    - Theoretically. But first, none of the current studies have been able to answer the question of how long the patient should be treated. Should the patient be treated until their fibrosis resolves and there is complete resolution? And then, should they come off the drug? Or should they stay on it for the rest of their life? It's going to be such a slow process, that a randomized withdrawal study would be difficult. For example, once there is some resolution, and the drug is withdrawn, the study will need to wait a year or two years before re-biopsy.
    - Would want to know the answer about how long someone needs to be on a drug, and it seems like a good way to find out by taking some people off of treatment.
    - Randomized withdrawal is testing something else than the initial treatment. It may be useful to assess the need for a certain time to be treated or assess how long a treatment is necessary, but taking away a treatment is different from initial treatment. Depending on the compound, there might be detrimental effects of taking a treatment away. Whereas, there may not be the beneficial effects when the patient started treatment.

- It seems like this is a perfect or ideal opportunity to use something like a non-invasive assessment alongside both for a recruitment or enrichment strategy to make sure that you are increasing your likelihood of biopsying a patient at baseline that is going to be able to enter your trial. And also in trying to determine the treatment duration, non-invasively at shorter time periods as you evaluate your therapy.
    - Every clinical trial should have both a non-invasive and invasive measure depending on the question being asked. One of the difficult things is that the patients want to know how they're doing, and in an RCT results are not released until the end. But it's very important if possible, to design studies so that patients do get their personal results back at the very end.
    - There is a difference in using a non-invasive measure to assess treatment effect and using a non-invasive measure to enrich the trial population. There are several different sponsors looking at enrichment. Many of the adult trials are enriching their population before they biopsy by using the LIF score or the FIB-4 and then performing biopsies later.
        - How well is that working in pediatrics? Is there an adaptation needed to use that method in pediatric trials?
        - It is dependent on what the inclusion criterion is. If it's a NAS score greater than a certain amount, it's hard because there really aren't any good non-invasive measures for that right now other than maybe ALT. If it's degree of fat reduction, then it could argue for using MRI perhaps to enrich.
- The major difference between adult trials and pediatric trials is that in adult trials, liver biopsies are being done specifically to enroll patients in clinical trials. In pediatrics, liver biopsies are being done for clinical care, not to see whether somebody qualifies for a clinical trial or not. The clinical enrichment strategy doesn't really apply here.
    - It really depends as there are still some variations in clinical care even among pediatric groups. It has held clinical trials back because there might be a site that's not liberally doing biopsies and it slows enrollment at that site.
        - Important to understand when doing multi-center studies.

## Session VIII: Validation of Non-Invasive Biomarkers in Pediatric Clinical Trials

**Panelists**: Keri Hildick, Perspectum Diagnostics
David Kleiner, National Cancer Institute
Dan Peres, Immuron Limited

### Discussion

- Perspectum Diagnostics has a method for assessing MRI proton density fat fraction, the iron content in the liver, and a corrected T1 measure which has been correlated in adults with biopsy. The aim now in the pediatric population is to evaluate healthy subjects across different age ranges to get an understanding of what is normal in a pediatric population. A subset of these subjects will have reproducibility and repeatability analysis similar to what has already been conducted in adults. There's a desire from the field for more of that data to be shared and that data is coming out, though not available in the public domain at this time.
- Presently there is a clear reliance on liver biopsy, but there has been little discussion on the known sampling error, the known variance, the known misdiagnosis as a result of

heterogeneity of disease in the sampling. Liver biopsy is not validated for pediatric subjects, and it is important that opportunities to validate other non-invasive methods are not missed while waiting on the validation of liver biopsy for pediatric patients. Another important point was already made about measuring a continuous variable on a categorical scale. Comparatively, some of the non-invasive measures are in fact able to be measured on a continuous scale.

- It is true that there are categorical variables for a lot of the things that are in histology and it is hard to tell the difference over a year or two on a treatment trial what the difference is between an F2 and an F3. Whereas, if you knew it was an F2.2 and it went to a 2.7, there would be better information on individual subjects rather than a group cohort. It will be valuable to use digital imaging in biopsies, for instance, trichrome stains for fibrosis with a digital number, and then compare that to these types of methodologies that also have continuous variables.
- Liver biopsies offer a different kind of information than what you get from imaging or other biomarker studies. Imaging allows for a window into disease pathophysiology. The tools currently being reported on for these studies are just scratching the surface, and there is so much more that could be done. Image analysis is be a big one, and it can be used to look at collagen proportionate area for fibrosis, which is the proportion of the cross-sectional area of the biopsy that has stainable collagen in it. There are other image analysis techniques that take into account the complexity of the fibrosis. How much is sinusoidal? How much is portal? There are a lot of measurements that could be done that could have bearing on disease progression or disease resolution.
- There is a lot of information content in liver biopsy that is often glossed over because of the need to evaluate things quickly. When the scoring system was developed, the system focused on the things that were thought to be important based on the knowledge and experience at the time. As the field tries to figure out the natural history of this disease, it is important to note that there is room for other kinds of histologic studies that might shed light on what's going on in these patients. These things can then be better correlated with non-invasive markers which are also continuous measurements.
- Biomarker studies are often based on analyses of cross-sectional populations in relatively untreated states. Patient cohorts are formed, and biopsies, biomarkers, and/or imaging are collected, and these are related to the disease at that point in time. In interventions, though, particular pharmacologic interventions can affect the disease in an unpredictable or an irregular way, and that can make a difference for a biomarker. Just because the measurements at the time of the initial assessment correlates with the non-invasive marker, does the subsequent changes in the histology or the diseased state with therapeutic intervention, do the non-invasive markers change in parallel? Do they change ahead of time? Do they lag behind? Just because biomarkers have been studied in a cross-sectional way, doesn't mean they will apply longitudinally during the course of a clinical trial.
    - Oftentimes, the discovery cohort for the non-invasive imaging or serum biomarker may be histology and then, they're just looking to see that it matches up to what it was initially paired to. And maybe that isn't the right thing to validate it against.

- *Q: There is a huge opportunity here for non-invasive biomarkers. How do we address something which is operatively much more complicated to sort of put into something like the natural history cohort, for example Perspectum's technology or the Multiscan? The field is missing out on the opportunity. How would this be implemented?*
    - The Perspectum technology is an exploratory metric to some extent at least in pediatric liver disease as well in adult NASH. The TARGET study is going to collect data where it's being used in clinical care, but there is also the opportunity to capture some of these emerging technologies alongside, at least in maybe a subset of the sites and the clinicians that already have access to these technologies.
        - With serum biomarkers, researchers could store the serum and look back at it and for properties that could be analyzed immediately and prospectively examined. However, this is not the case for imaging.
        - That is correct because the patient does have to be referred for the MR scan; however, the sequences could be acquired and then, batch analyzed if it was of interest. If serum markers at a particular point gave an indication it, then perhaps a deeper analysis would be of interest. Patients are being referred for MR, and the data is being collected.
        - The data would have to be captured by a uniform device in a uniform manner that's retrievable and stored somewhere that's reliable. It's just much more complex.
    - Whatever non-invasive imaging or tests are being done in routine clinical practice, TARGET will be capturing that information. As new tests come online and are utilized in the community more frequently, that information will be captured all that information and go back to see how predictive they are of outcomes.
        - There are two elements here of what TARGET's doing, 1.) the core cohort that is observational in nature and longitudinal, and 2.) the concept presented by Miriam, which is more interventional than the other cohort, and there is more opportunity to be very directive about what is collected and when. These are two separate cohorts. Eventually, the pediatric natural history cohort could flow in back into the larger cohort as well. But they're trying to get at two different questions.
    - The major thing holding back getting the unapproved testing done is funding to get the data collected. The Liver MultiScan technology is a little easier because it is an algorithm that can be performed on many of the machines that are out there. It wouldn't be as difficult as something like the Methacetin Breath Test or the HepQuant tests, where there are limited sites that can perform them or limited abilities. However, these tests are being done under clinical trials and they are out there. Because these non-invasive methods are unapproved, you have to go through the FDA or EMA, but regulatory agencies do want to see the field move forward with the non-invasive biomarkers.
- *Q: We heard that all these techniques may or may not work the same way in pediatrics as they work in adults. So what should we be doing to make sure the data are interpretable even in pediatric patients? And are there companies doing anything separately to validate thresholds or whatever they're putting out there for interpretation separately for pediatrics and adults?*

- It is unreasonable to think that adult biomarkers can be extrapolated to children. Biomarkers have to be examined separately for children and adults.

## Closing Remarks

**Moderator**: David Shapiro, Intercept Pharmaceuticals

## Session IX: Next Steps

- Consensus
  - There is a consensus that there is insufficient information on many aspects related to the natural history of fatty liver disease in pediatric populations, including answers to basic questions.
- Action Items
  - Develop a collective list of priority areas and needs for pediatric populations.
  - Develop recommendations for pediatric trial design and trial conduct for Phase II or Phase III.
  - Develop manuscript/ guidelines on evidence and/or consensus amongst pediatric hepatologists to add to literature on some of the key issues.
    - For example, a review of the prevalence of NASH in young patients (e.g., under 8 years old), to justify their exclusion from clinical trials to regulators.