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This study examines how non-invasive biomarkers of NASH are introduced into the study protocol and whether this has an impact on screen failure. The study will investigate the use of non-invasive biomarkers in the design of study protocols for NASH drug trials and will also examine if screening approaches established using non-invasive biomarkers can reduce screen failure.

Diversified use of Non-invasive Biomarkers in Non-Alcoholic Steatohepatitis Clinical trials

Research Thesis

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TABLE OF CONTENT

LIST OF TABLES

LIST OF FIGURES

CHAPTER I

INTRODUCTION

Non-Alcoholic Fatty liver disease (NAFLD) affects 30% of adults the in United States and has two principal subtypes: 1] Nonalcoholic fatty liver (NAFL) and 2] Nonalcoholic steatohepatitis (NASH). NASH is a progressive form of NAFLD, which is a type of hepatic metabolic syndrome that causes excessive accumulation of triglycerides or fats in the liver followed by inflammation that damages hepatic cells leading to scarring or fibrosis of the liver tissue. The degree of fibrosis associated with NASH indicates the disease's progression, which can lead to hepatic carcinoma or liver failure. Various comorbidities including obesity, type 2 diabetes mellitus, and hyperlipidemia are associated with NASH and NAFLD. NAFL and NASH are asymptomatic until the disease is advanced and is not easy to be diagnosed or distinguished from each other by clinical and routine laboratory tests. Identifying patients at risk for progression, and clinically meaningful adverse outcomes, and developing effective therapies for these patients is a public health priority. Currently there is no approved drug for NASH but there have been extensive clinical trials directed toward it.

The main goal of pharmacotherapeutic intervention is to halt or reverse hepatic fibrosis or to achieve resolution for steatohepatitis. The diagnosis and staging of NASH are determined by liver biopsy (histological assessment of the liver) which is considered a diagnostic reference standard. The selection of subjects or participants via screening biopsy is considered imperfect as many fails meet histopathological criteria for NASH clinical trials (histological samples showing Intra and Inter-variability). In addition, the procedure is invasive, painful, and carries limited but real risks for catastrophic complications and non-compliance. In recent years various non-

invasive biomarkers such as serum biomarkers, imaging markers, and panels or complex algorithms have been developed which help distinguish simple steatosis from NASH and understand stage fibrosis (F1 to F4). It is important to distinguish those with cirrhosis (stage 4 fibrosis) from earlier stage disease because disease biology and clinical course are different requiring different approaches for therapy and assessment. Therefore, it is necessary to develop improved diagnostic and treatment options for patients with NASH, focusing on early-stage illness and advanced liver fibrosis.

Biomarkers often play a significant role in the design and conduction of research protocols targeting various therapeutic indications such as Cancer, Thyroid, etc. They may improve the selection of participants and render more biologically homogeneous sampled populations. Moreover, their use may help demonstrate target engagement by tested intervention, providing evidence of disease modification, informing analytic stratification, and monitoring adverse effects. This study is planning to describe and discuss the state of the art of noninvasive biomarkers or techniques in NASH drug trials by reviewing study protocols and screening failure charts.

CHAPTER II

Background and Literature Review

NASH or Nonalcoholic steatohepatitis is a condition in which fat builds up in the liver which causes inflammation that damages hepatic cells leading to scarring or fibrosis of liver tissue and finally hepatocellular carcinoma. In 1962, the term fatty liver hepatitis first surfaced, and in 1980, the term nonalcoholic steatohepatitis (NASH) was developed. Typically, the rate of NASH disease progression is slow. Approximately 20% of patients with NAFLD will develop NASH within three to seven years, the potentially progressive form of the disease. Over a 10-to-20-year period, up to 25% of individuals with NASH develop cirrhosis. (Heyens, Busschots, Koek, Robaeys, & Francque, 2021) (Gariani & Jornayvaz, 2021). Furthermore, Fat buildup in the liver is associated with insulin-resistant states such as obesity, type 2 diabetes, and/or metabolic syndrome. (Ogawa, Imajo, Yoneda, & Nakajima, 2013), (Dowman, Tomlinson, & Newsome, 2010).

Genetic susceptibility combined with environmental factors like obesity and excessive energy intake can lead to hepatic steatosis, oxidative stress, and activation of inflammatory pathways and in some cases, fibrosis and organ damage. Because of the multifaceted nature of NASH pathogenesis, the influence of multiple contributing factors must be considered and quantified. NASH is traditionally diagnosed through histopathological evaluation of liver tissue via biopsy. In 2005, the NASH clinical research network (NASH CRN) created the NAFLD activity score (NAS), which is now the most popular histological scoring system for NASH clinical trials. Kleiner et al. (2005). The NAS is calculated by adding the histological staging for liver fat, lobular inflammation, and ballooning. A NAS of 4 or greater is generally regarded as indicative of NASH, In addition to having a NAS score of 4, patients must also show fibrosis at

stage 2 or higher, as measured by the Kleiner-Brunt fibrosis scale, which is not included in the NAS 0–4.

Table 1- NASH CRN Scoring System

Histological features	Score	Category definition
Steatosis	0	<5%
	1	5-33%
	2	34-66%
	3	>66%
Hepatocyte ballooning	0	None
	1	Few
	2	Many
Inflammation	1	1-2 Foci per 20 Field
	2	2-4 Foci per 20 field
	3	>4 foci per 20 field
NAS total 0-8		> 3 - No NASH; ≥ 5 -NASH
Fibrosis	0	No fibrosis
	1a	zone 3 mild perisinusoidal fibrosis
	1b	Zone 3 moderate perisinusoidal fibrosis
	1c	Periportal/portal fibrosis only
	2	Zone3+ periportal/ portal fibrosis
	3	Bridging fibrosis
	4	Cirrhosis

Identifying patients who are most likely to meet the NASH Clinical research network [CRN] enrollment criteria based on their biopsy results is an ongoing challenge; increased rate of screening failure after central review of the initial liver biopsy is a common problem in NASH

trials. About 73% and 65% respectively, of the biopsied patients in the PIVENS and CENTAR NASH clinical trials did not meet the requirements for inclusion. (Friedman S. e., 2015) (Dennis, et al., 2020)

It is widely agreed that a liver biopsy is necessary for a conclusive diagnosis of NASH and is also of great value in distinguishing NASH from other diseases for the purpose of making a prognosis and predicting the effects of therapeutic intervention. However, liver biopsies are ineffective in many non-advanced cases and have a number of disadvantages, including sampling error and high cost. In addition, pathologists differ in their interpretation and diagnosis of liver biopsy results. In the recently published guidelines by the American Association for the Study of liver disease (AASLD), liver biopsies are recommended for complications of metabolic syndrome and elevated serum ferritin levels in patients with NASH who are also suspected of having advanced fibrosis. (Sumida, Nakajima, & Itoh, 2014)

A liver sample can be sensitive to sample variability or may not be indicative of the liver's pathology. Ratziu et al. extracted and compared two percutaneous liver biopsy samples from each of the 51 NAFLD patients. They found that the consistency in fatty change was comparatively high (78%), but the fibrosis stage varied between the two samples in 41% of patients. (Ratziu, et al., 2005). In 35% of patients bridging fibrosis was observed in one sample, and only mild or no fibrosis was noted in the other sample. (Larson, et al., 2007)

The discrepancy in the ballooning degeneration of hepatocytes, which is necessary for the diagnosis of NASH, was 18%, indicating that NASH may be missed when only one sample is obtained. In other studies, 30% of patients had outcomes that differed by one or more stages between specimens biopsied from the left and right lobes, with inflammatory findings being more inconsistent than fatty change and fibrosis. (Janiec, Jacobson, Freeth, Spaulding, &

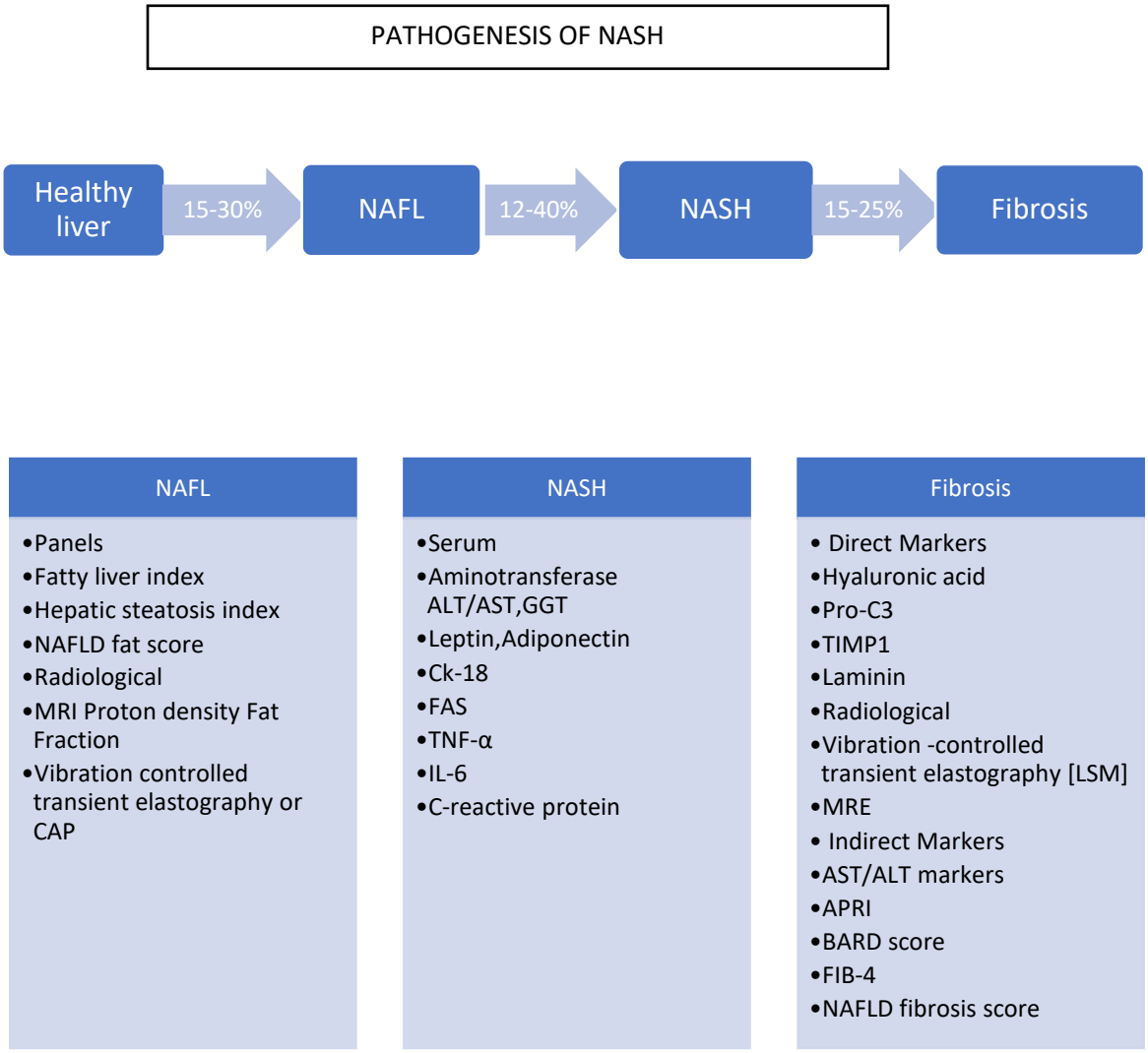
Blaszyk, 2005). Inter and intra-observer variability also presents a fundamental problem for the pathological diagnosis of NASH. A study was conducted that revealed that inter-observer variability occurred even when training in histopathological observation was provided to reduce these inconsistencies. (Younossi, et al., 1998). In addition, the incidence of serious complications and mortality from liver biopsy has been reported to be 0.3% -0.57% and 0.01% respectively. The incidence of pain for biopsy is reportedly 20% but it increases to 84% when a mildly unpleasant feeling is included in the assessment. (Sumida, Nakajima, & Itoh, 2014)

Numerous ineffective biopsies significantly increase the cost and duration of clinical trials. As a result, efforts to reduce the likelihood of screening failure are a top priority for clinical research organizations and pharmaceutical firms developing NASH drugs. There are many compounds in various stages of development as NASH treatments. However, many of them have failed to show an improvement in the surrogate histological endpoints, i.e., resolution of NASH with no worsening of fibrosis and/or at least a 1-point improvement in fibrosis with no worsening of NASH. To diagnose NASH, another marker that is equivalent to a biopsy is certainly needed. Recently, there has been a growing interest in the development and validation of non-invasive biomarkers or tests to facilitate the diagnoses of NASH, stratify risk, predict clinical outcomes, and/or track the disease's progression or regression. Biomarkers are essential to the drug development process and can be helpful in evaluating the effects of investigational compounds during clinical trials. They allow researchers to design smaller, more specific, and more efficient study designs.

Several circulating and imaging-based biomarkers have been developed and have been shown to correlate with liver biopsy histological assessment. Non-invasive biomarkers in NASH

can be classified by type: serological, radiological, and combination panels or algorithms & by the pathogenesis of the disease.

Figure 1 Schematic representation of pathogenesis of NASH



Non-invasive biomarkers for Non-alcoholic steatohepatitis: Biomarkers must address three major domains [1] quantify liver fat to diagnose NASH [2] assess disease severity (degree of inflammation and fibrosis) [3] explore longitudinal changes over time.

1) **Radiological biomarker**: Radiological biomarkers are either ultrasound-based or Magnetic resonance-based, they can identify fibrosis as well as liver fat.

- *MRI Proton Density Fat Fraction*: MRI-PDFF is a quantitative, non-invasive biomarker for assessing liver fat content. MRI scans measure the proportion of triglyceride proton to water proton. This method provides complete coverage of the liver. It identifies different levels of steatosis. MRI-PDFF cut off value 7% to discriminate NAS$<$ 4 and NAS $\geq$ 4 (0.83 sensitivity and 0.70 specificity)
- *Vibration-controlled Transient Elastography (VCTE) or Fibro scan*: An ultrasound-based method for assessing fibrosis and steatosis. Liver stiffness is measured in kPa, which correlates with fibrosis. The controlled attenuation parameter provides the percentage of fatty change in the liver, as measured in decibels per meter. 91% sensitivity and 92% specificity (Vibration-controlled Transient Elastography for Assessment of Liver Fibrosis at a USA Academic Medical Center, 2022) (Siddiqui, et al., 2019). Fibrosis and steatosis were compared between liver biopsy and Fibro scan. In patients with high BMI, inflammation, and congestion, VCTE or Fibro scan liver stiffness measurements differed. (Fang. et.al, 2021)

Table 2 Reference range for VCTE

FIBROSCAN or VCTE					
Fibrosis	Liver stiffness		CAP score		
F0 to F1	2 to 7 kPa	Normal	steatosis grade	CAP score	Percentage of the liver with fatty change
F2	7.5 to 10kPa	moderate scarring	S1	238-260 dB/m	11% to 33% (less than 1/3)
F3	10 to 14 kPa	severe scarring	S2	260-290 dB/m	34% to 66% (between 1/3 and 2/3)
F4	14kPa or higher	Cirrhosis	S3	Higher than 290 dB/m	67% or more (67%)

- *Magnetic Resonance Elastography*: An imaging technique based on MRI that quantitatively measures changes in liver stiffness. It is an excellent indicator of whether or not fibrosis is present in the entire liver (Singh.et.al,2017). In advanced fibrosis, liver stiffness multiplies by several orders of magnitude compared to normal liver tissue. Multiple studies have demonstrated that the MRE stiffness measurement has a high diagnostic accuracy for detecting and staging liver fibrosis. [Wagner.et.al,2017] Area under receiving operating Curve (AUROC): 0.93 (identifying advanced fibrosis ≥ F3), Sensitivity: 85%, and Specificity:92%
- *MRI corrected T1 or Liver Multiscan*: MRI type of scan. It measures the T1 component of the MRI scan, which needs to be corrected for iron, and provides information about fibrosis and inflammation. More validation is required because a handful of studies have proven results during a clinical trial.

2) **Serum Biomarkers**: There are various pro-inflammatory and apoptosis markers that help to discriminate patients with simple steatosis from NASH.

- *Cytokeratin-18*:

  It is the main protein in hepatocyte intermediate filaments. During apoptosis, Ck-18 fragments are cleaved by caspase 3 and released at the extracellular level. A study reported that Ck-18 was able to differentiate simple steatosis from NASH and discriminate patients with different fibrosis stages from healthy controls. [Joke.et.al,2012]. Other studies suggested that Ck-18 fragments may be more effective

in diagnosing NASH when paired with other tests. (Shen, et al.,2012) (Yilmaz, et al., 2007) Sensitivity:66%; Specificity:82% AUROC: 0.70-0.93

- *C-reactive protein*:

  It is an inflammation marker. In patients with NASH and advanced fibrosis, CRP levels are significantly higher than in those with NASH and mild fibrosis. Area under receiver operating curve: 0.833-0.906, Sensitivity:82%; Specificity:88% (Yoneda, et al., 2007)

- *Tumor Necrosis Factor (TNF*-α):

  TNF- α plays an important role in insulin resistance, such as inhibiting tyrosine kinase activity of the insulin receptor. A study reported that patients with NASH had significantly higher serum TNF-α than those with simple steatosis, although exact cut-off value was not provided [Abiru.et.al,2006]. A recent study reported that patients with NASH had higher levels of TNF-α mRNA than healthy subjects. The cut-off value for same is 100 ng/mL predicted NASH. AUROC = 0.68; Sensitivity = 66.7%; Specificity = 74%. (Alaeddine, Sidaoui, Hilal, Abedelrahman, & Khoury, 2012)

- *Interleukin -6 (IL-6):*

  There have been several studies that have examined the role of IL-6 in NASH. A pilot study found a strong association between IL-6 and NASH suggesting there is an increase in levels of IL-6 in NASH patients and decrease with therapy (Vitamin E and exercise) [Kugelmas.et.al,2003]. Another study pointed out the levels of IL-6 correlate with the degree of steatosis but not with NASH [Taratino.et.al,2009]. IL-6 confirmed the absence of NASH at normal levels with a high degree of specificity. Area under receiver operating curve= 0.817, sensitivity: 58.1%, specificity 100%.

- Ferritin –Hyperferrtinemia is a disorder of iron, glucose, and lipid metabolism that is frequently observed in NASH. Several studies have found that NASH patients have significantly higher serum ferritin levels than those with simple steatosis (Kowdley, et al., 2012). The area under receiver operating curve (AUROC): 0.82, sensitivity:91% and specificity:70% (Manousou, et al., 2011). Ferritin level is a useful predictor of advanced hepatic fibrosis among patients with NAFLD. (Kowdley, et al., 2012)
- *Pro-C3*:

  Pro-C3 is the propeptide of collagen type III. An excellent diagnostic marker for fibrosis. It is able to differentiate between patients with simple steatosis, NASH, and advanced fibrosis. (Anwar.et.al, 2012) AUROC: 0.85-0.87

- *Hyaluronic acid (HA):*

  A predictive factor for the presence and stage of liver fibrosis with NASH.

  The area under receiver operating curve= 0.975, Sensitivity: 97.50%, Specificity: 95.70%

- *Laminin*:

  It is an excellent diagnostic marker for fibrosis, but not for staging.

  Area under receiver operating curve =0.789, Sensitivity: 73.90%, Specificity:74%

- *Tissue inhibitor metalloproteinase (TIMP1*):

  It reflects the alteration of tissue matrix remodeling during hepatic fibrogenesis and Fibrinolysis

3.**Combination Panels or Algorithms**:

Table 3: Panels and Combination of Algorithms

Panels or combination algorithms	Analyte involved	AUROC	Diagnostic efficacy
Fatty liver index	BMI + Waist circumference + serum triglycerides +GGT	0.84	sensitivity: 87% specificity: 86%
Hepatic Steatosis index	AST/ALT ratio + BMI+ Sex+ presence of type 2 diabetes +insulin level+ AST	0.67	sensitivity: 92.5% specificity: 92.4%
NAFLD Liver Fat score [37]	Presence of metabolic syndrome + type 2 diabetes + insulin level+ AST+ AST/ALT ratio	0.87	sensitivity: 86% specificity :71%
NAFLD Fibrosis score [Angulo.et.al,2007]	Age + BMI + Impaired Fasting Glucose + Platelet Count + Albumin + AST/ALT	0.88	sensitivity: 89% specificity :89%
AAR	AST/ALT ratio	0.66-0.74	
APRI	AST/Platelet ratio	0.74	Moderate fibrosis: sensitvity:65%, specificity-71%; Advanced fibrosis: sensitivity:75%, Specificity:65%
BARD	BMI + AST/ALT ratio + Presence of diabetes	0.69-0.81	
FIB-4 index	Age+ AST+ PLT +ALT	0.78	sensitivity: 93%; specificity :82%
Enhanced liver fibrosis test	Hyaluronic acid + TIMP1 +Pro-C3	0.86-0.89	sensitivity: 90%; specificity :85%
BAAT score	Age+ BMI+ ALT+ Triglycerides	Mild -0.68 Advanced-0.62	mild - sensitivity 90.4% Specificity :35% advanced- sensitivity 94.9%, specificity- 23.8%

There are newly developed serum biomarkers called Omics, which can be critical in early screening of patients at risk for NASH and fibrosis progression. They need to further validate, due to methodological limitation and reproducibility of results, the identification of omics biomarkers has not yet been widely used.

- **Genomics**: Single Nucleotide Polymorphisms (SNPs) such as PNPLA3 and TM65F2 substantially increase the risk of inflammation and fibrosis progression in NAFLD. PNPLA3 +AST + Insulin is a diagnostic biomarker for NASH. It provides a NASH score (AUROC-0.77 for NASH) Combining genetic data with routine indicators could effectively predict NASH (NASH PT score) (Koo B. K., Joo, Kim, & et.al., 2018)
- **Epigenomics**: The methylation levels of peroxisome proliferator-activated receptor ϒ (PPARϒ) in plasma-free DNA can differentiate the severity of NAFLD and is considered as potential non-invasive biomarker for NAFLD. (Hardy, Zeybel, Day, et. al., 2017) Methylation of 22CpG correlated with the degree of steatosis.
- **Transcriptomics**: Micro RNAs (miRNA) such as miRNA -122 and miRNA- 34a were mostly studied in NASH. The area under curve- miRNA-122 =0.82 and AUC for miRNA -34a was 0.78. They appear to be reliable diagnostic biomarkers for NASH, but these studies are still in the preliminary stages. LeXis is a long non-coding RNA used for diagnosing NASH. AUROC- 0.74; sensitivity-54.3% and specificity 100%. (Park, et.al., 2020). CircRNA overexpression is significantly associated with the inhibitory effect on miR-122 on CPEB1 which is involved in the pathogenesis of NASH (Chien.et.al,2020). Reduced levels of CircRNA are correlated with oxidative stress, lipotoxicity and NAFLD disease severity.
- **Proteomics**: Researchers identified six biomarkers to establish diagnosis of NAFLD (sensitivity-89%; Specificity 83%), upon further analysis it was found that patients with high levels of hemoglobin were more likely to develop NAFLD; thus, hemoglobin is considered as a potential biomarker. (Yu.et.al,2012) Another study revealed that α-2

macroglobulin and coagulation factor V were highly correlated with NASH-related fibrosis. [Younossi.et.al,2018]

- **Metabolomics and Lipidomics**: Metabolomics such as measuring pyroglutamic acid was effective in differentiating between patients with NASH and Steatosis (sensitivity:72% and Specificity 85%; AUROC: 0.82). [Qi.et.al,2017].11 Triglycerides were used to differentiate between healthy controls and steatosis (AUROC = 0.9), and 22 Triglycerides were used to differentiate between steatosis and NASH (AUROC 0.95). It was suggested that lipids have a high diagnostic utility for NAFLD. (Mayo.et.al,2018)

Several studies have been conducted to evaluate the clinical significance of serum, imaging, fibrosis biomarkers, and panels. Serum analyses showed that a single marker has limited specificity and sensitivity, but combinations can improve the accuracy of diagnosing of NASH. [Shen.et.al,2012]. Studies evaluating the effectiveness of direct and indirect fibrosis markers revealed that there is an increase in accuracy in detecting fibrosis when these biomarkers are used in panels such as APRI, FIB-4, NAFLD fibrosis Score, ELF, etc. Pre-biopsy enrichment strategies with non-invasive biomarkers are gaining popularity in some trials to decrease the number of liver biopsy screen failures (Argo.et.al,2009)

In the current NASH drug development paradigm, screening failure is quite common. There are many reasons for this, one of the reasons is the Liver biopsy and NASH CRN scoring system. Predictive biomarkers in clinical trials could refine the clinical trial recruitment process and enable the identification of likely responders e.g., a homogeneous population for biopsy thus decreasing unwarranted biopsy as well as increasing participation compliance. Prescreening with biomarkers will help identify subjects that will be considered preliminary NASH subjects confirmed with a biopsy.

Significance:

The significance of this study is that it will inform how non-invasive biomarkers are currently adopted in drug development, provide more insight into various NASH non-invasive biomarkers that have been developed, and reveal more information on panels or multi-analyte algorithms for Steatosis and Fibrosis that are cost-effective and can be performed in clinical practice, thereby reducing the need for a Liver Biopsy. It is crucial to identify patients with early NASH due to their increased risk of developing liver fibrosis, as many subjects in NASH drug trials are already in Stages 3 or 4. This study will analyze the present usage of non-invasive markers in clinical trials and evaluate their potential applications. In NASH clinical trials, there is a significant rate of Screen failure, which results in adverse outcomes like extended study completion time and cost. Information on the causes of screen failure will help to understand the exact reason behind it as well as give more insight on biomarkers which can be helpful in prescreening subjects for trial enrollment which may have benefit over randomization.

CHAPTER III

Problem and Hypothesis

According to an article published by the FDA on imaging biomarkers in NASH, the use of imaging biomarkers in the diagnostic panel will help to reduce the frequency of screening failure, as well as help to expedite drug development for therapeutic intervention in NASH and will also help to reduce the number of unnecessary liver biopsies. In today's NASH drug development paradigm, screen failure is quite common. Diagnostic use of biomarkers in clinical trials may benefit from refining the clinical trial recruitment process and identifying likely responders, such as the homogeneous biopsy population. This would result in a reduction in unjustified biopsies and an increase in compliance on the part of the subject. Based on current literature, we hypothesize to understand the role of non-invasive biomarkers in NASH clinical trial studies.

Aim 1: To understand the inclusion of biomarkers in designing study protocols.

Aim 2: To analyze screening failure for NASH clinical trials and elucidate the main reason for screening failure to draw a relation between screen failure and non-invasive biomarkers.

Ho - There is no difference in screen failure between invasive and non-invasive biomarkers.

H1- There is a difference in screen failure rate between invasive and non-invasive biomarkers.

CHAPTER IV

METHOD AND STATISTICAL ANALYSIS

For this project, we analyzed NASH clinical trial protocols. All protocols are phase II (2a or 2b) randomized controlled trials or studies, from 2016 to 2021(except for one from 2011) [Table2]

Table 4: Description of RCTs or Study involved in the analysis.

Study/ RCT	Phase of Study	Year
1	2A	2019- 2020
2	2B	2018-2019
3	2A	2018
4	2A	2019-2020
5	2B	2016
6	2A	2020-2021
7	2B	2010-2012
8	2B	2018-2020
9	2B	2019-2020
10	2A	2018-2019

Method for Aim 1:

A total of ten randomized controlled trial protocols of Phase II were retrieved from the Medpace database (SharePoint). Phase II trials are directed at assessing the drug's safety and tolerability, as well as its effectiveness on NASH's underlying pathophysiological mechanisms.

One out of ten RCT protocols (Study 7) did not utilize any biomarkers, instead relying on liver biopsy (an invasive biomarker) to determine eligibility and the primary outcome. Data were collected for two groups of protocols: [1] Those adopting biomarkers for participant selection (Eligibility).[2] Those adopting biomarkers as research outcomes (Endpoints).[3] Those biomarkers both as eligibility criteria and endpoints, (Eligibility and endpoints). A graphical analysis of data from studies was done to evaluate the distinguishing characteristics of non-invasive biomarkers.

Method for Aim 2:

Screen failure is defined as individuals who undergo screening but aren't recruited in a clinical trial. It incurs significant costs without contributing valuable data to the study. In the NASH study trial, screening failure is a core issue, therefore, researchers are attempting to overcome this issue. This study is planning to analyze data on screen failure and ascertain the reasons for screen failure from screening logs of eight randomized controlled trials. [Table 3] This data is obtained from the Medpace database (data on screen failure from two RCTs i.e., 5, 7 was not available).

Table 5: RCTs or studies analyzed for screen failure:8 RCTs.

study	Phase of Study	Type of biomarker used for screening
1	2A	invasive +non-invasive
2	2B	invasive +non-invasive
3	2A	non-invasive
4	2A	invasive +non-invasive
6	2A	non-invasive
8	2B	invasive +non-invasive
9	2B	invasive +non-invasive
10	2A	non-invasive

The screen failure data was classified and summarized for the reason for screen failure. It was analyzed for the gender difference, and age, for both general and particular reasons for screen failure. The screen failure data was analyzed for gender difference from seven randomized controlled trials (except one RCT i.e., 4– only male participants were screened). A chi-square test of independence was conducted to determine whether there is a statistical difference in terms of gender and screen failure. The screen failure data was reviewed for both general and specific reasons.

Table 6: Studies stratification for Screen failure.

Randomized Controlled trials or studies	Stratification for purpose of analysis based on FDA NASH Guidance
RCTs -2,8,9	All are phase 2B trials using both non-invasive and invasive biomarkers. Prescreening logs providing specific reasons were analyzed
RCTs 1,3,4, 6,10	All phase 2A studies only used non-invasive biomarkers to screen subjects for study except for study 4 and study 6 which both invasive and non-invasive biomarkers. Study 4: primarily used liver biopsy as a screening tool. Imaging marker were used just before randomization. Study 6: used historical biopsy results for screening along with non-invasive biomarkers

The graphical analysis along with percentages was calculated to summarize the results.

CHAPTER V

RESULTS

The inclusion and exclusion criteria and primary, secondary, and exploratory endpoints of nine randomized controlled trials (RCTs) were evaluated. Following the tabular presentation of the adaptation of biomarkers in study design (Table 7)

Table 7:Tabular presentation of adaptation of non-invasive biomarkers into 9 RCTs

Non-invasive marker		The study adopted biomarkers or techniques (n=9)		
	Total no. of RCTs	Eligibility	Outcome	Both (Eligibility & Outcome)
MRI-PDFF	9	1	1	7
Cytokeratin -18	9	1	3	
C-reactive protein	9		4	
TNF/IL-6	9		5	
APRI	9		1	
FIB-4 index	9		3	
HA	9		3	
Pro-C3	9		8	
ELF	9		6	3
Fibro scan or VCTE (CAP or LSM)	9	6	1	
MRE	9	4		1
MRI cT1	9		3	2

Protocols adapting non-invasive biomarkers both as eligibility and outcomes.

Most common radiological biomarkers such as MRI-PDFF, MRE, and MRI cT1 were adopted as eligibility as well as the outcome except Fibro scan or VCTE was used more frequently as eligibility compared to outcome (Out of nine studies , six -eligibility & one study- outcome).

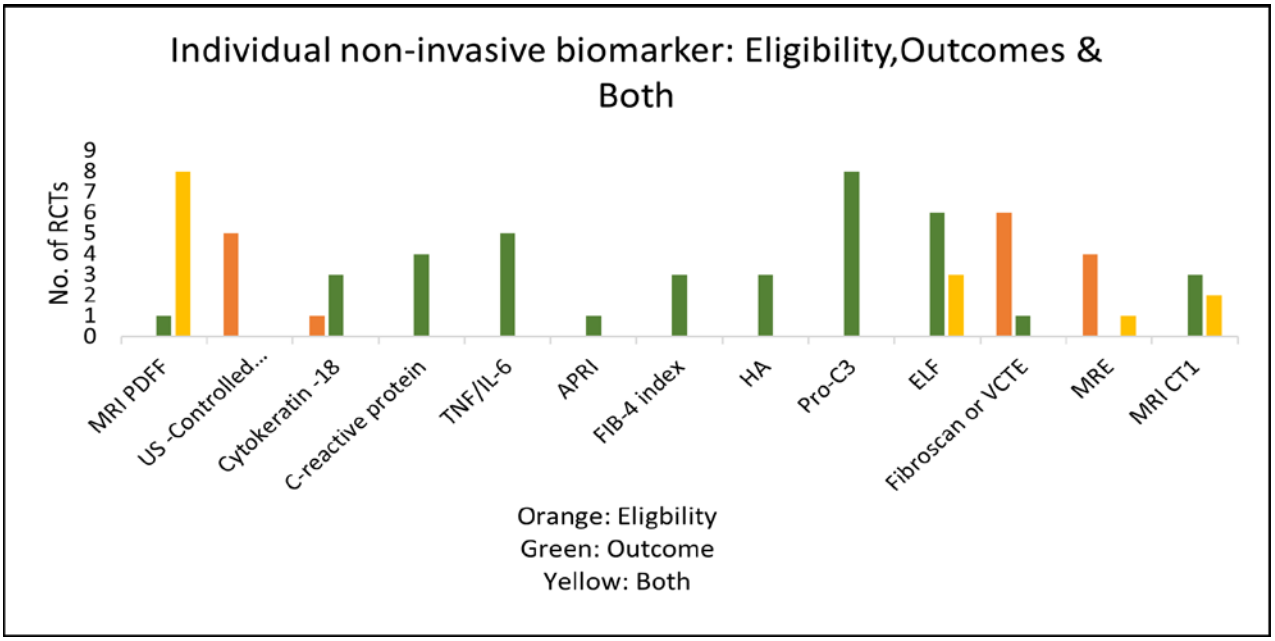
Protocols adapting non-invasive markers either as Eligibility or Outcome:

NASH biomarkers such as Cytokeratin 18, C-reactive protein, and TNF/IL-6 are most frequently used as outcomes except in one study Cytokeratin-18 used for eligibility. Direct fibrosis markers such as Hyaluronic acid and Pro-C3 were mostly adapted as outcomes.

Panels or combination algorithms:

Fibrosis panels such as APRI, FIB-4 index, and ELF panel were adapted into the protocol- APRI, and FIB-4 index was mainly used as outcome while ELF was adapted as follows: Out of nine studies, three used it as eligibility as well as outcome, and six studies used it for – outcomes. None of the Steatosis panels were used in the design of the RCT study protocol. The following is a graphical representation of the biomarkers in the studies. [figure 1]

Figure 2: Non-invasive biomarkers as either eligibility, outcome, or both.



The outcomes were analyzed separately as they were adopted either primary, secondary, or exploratory. The outcome is defined as a result of treatment or intervention that is used objectively to determine progress and efficacy such as safety, clinical improvement, and NASH biological change.

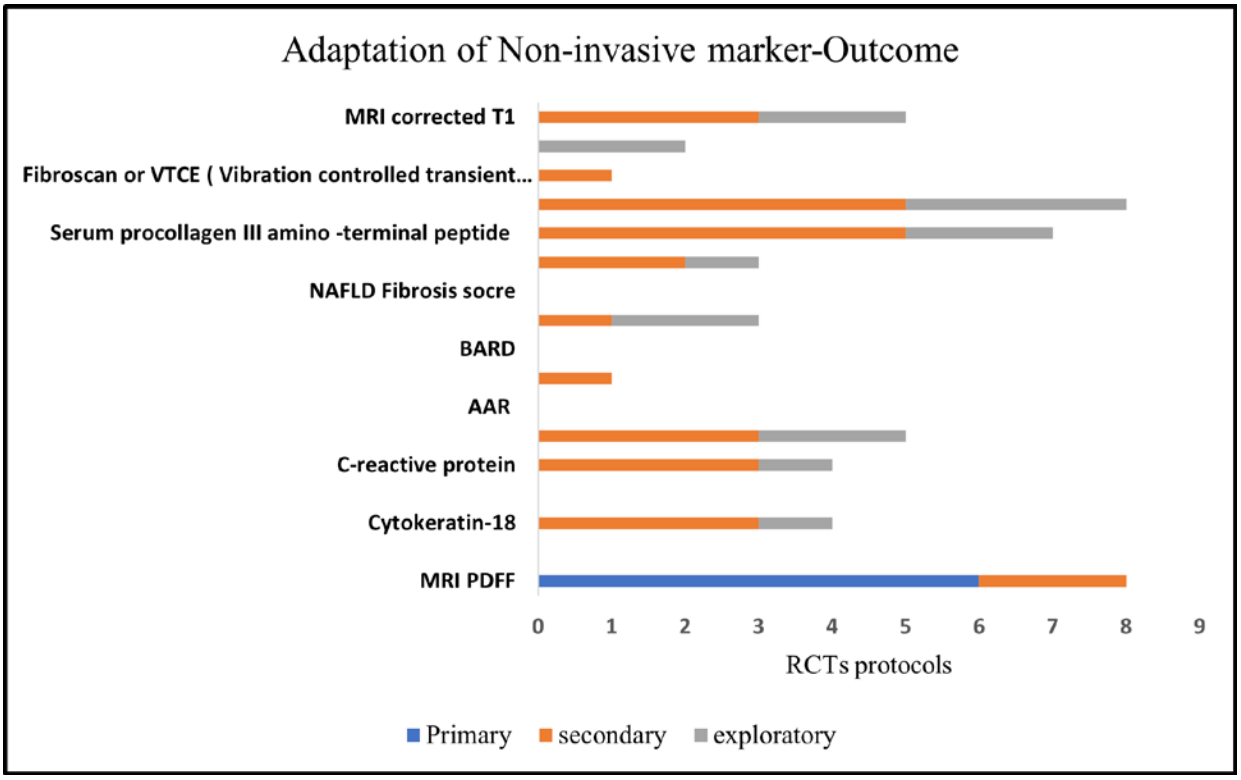
Table 8: Types of Outcomes

Outcomes		
Primary	Secondary	Exploratory
The outcome measure of greatest importance specified in the protocol (≥ 1)	Lesser importance in evaluating the effects of the intervention	Observational studies or post-hoc measures after the trial have started

Table 9: Tabular presentation of non-invasive biomarkers as Outcomes n=9 RCTs

Non-invasive marker	Primary	secondary	exploratory
MRI-PDFF	6	2	
Cytokeratin-18		3	1
FGF-12 & Ck-18			
C-reactive protein		3	1
TNF/ IL-6/IL-1/ IL-1RA		3	2
APRI		1	
BARD			
Fib-4 index		1	2
NAFLD Fibrosis score			
HA		2	1
Serum procollagen III amino-terminal peptide		5	2
Enhanced Liver Fibrosis test (ELF)		5	3
Fibroscan or VTCE (Vibration controlled transient elastography		1	
MRE			2
MRI corrected T1		3	2

Figure 3: Graphical presentation of Outcomes



The above graph signifies that most of the biomarkers (serum or radiological or panels) are commonly used as secondary and exploratory outcomes. Only MRI-PDFF has been adopted as Primary (6 studies) and secondary (2 studies).

Result analysis for Aim 2:

Screening failure was evaluated based on data via prescreening logs. These de-identified logs were obtained from the data team at Medpace. The following analysis will help to review the reasons for screen failure and help to investigate the importance of non-invasive biomarkers for screening patients with NASH.

A total of 5240 were screened for 8 randomized controlled clinical studies. Out of these 1141(21.77%) subjects were enrolled in the study and 4094 (78.13%) subjects were screened failed.

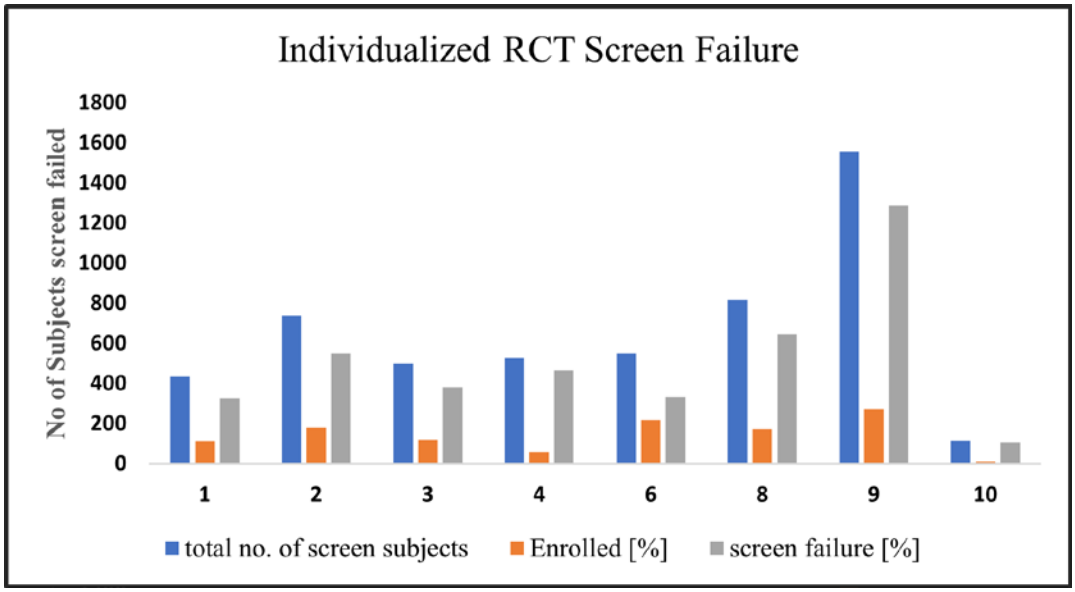
Table 10: Total number of subjects screened outcome across 8 RCTs.

Total number of subjects screened across 8 RCT N= 5240		
Screening outcome	Number of subjects (n)	Percentage of subjects (%)
Enrolled	1141	21.77%
Screen failed	4094	78.13%

Table 11: Individualized study (n= 8 RCT) screening outcome

study	total no. of screen subjects	Enrolled [%]	screen failure [%]
1	436	110[25%]	326 [74.77%]
2	736	181 (24.42%)	557 (75.57%)
3	501	120 (23.95%)	381 (76.04%)
4	526	60 (11.40%)	466 (88.59%)
6	550	219 (39.81%)	331 (60%)
8	817	171 (16.23%)	646 83.76%)
9	1558	271 (17.39%)	1287(82.60%)
10	115	9 (7.82%)	106(92.17%)

Figure 4: Graphical presentation of individual study screening outcomes



Analysis of Screen failure by Gender:

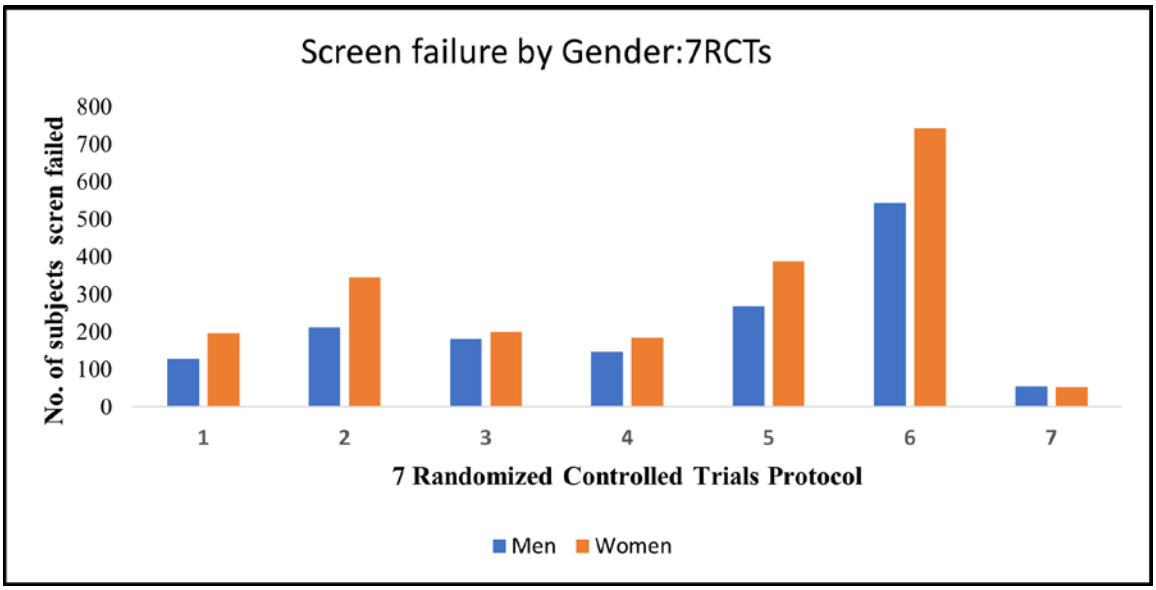
Various research found different associations between NASH and factors like age, gender, and body mass index. Any associations between age and gender and screen failure were also analyzed in the study. The characteristics of subjects who failed screening compared in 7 RCTs (one RCTs enrolled only men)

Table 12: Total number of subjects screen failed:7RCTs n=3645

Study	total screen failed	Men	Women	p-value
1	326	129	197	0.007
2	557	212	345	0.00006
3	381	181	200	0.42
6	331	147	184	0.42
8	657	269	388	0.0117
9	1287	544	743	0.000084
10	106	54	52	0.8907
total	3645	1655	1990	0.000085

Women were more likely to screen fail compared to men. Out of seven studies that screened failed data, only four studies showed significant statistical differences in terms of gender. Total screen failure across all seven studies in terms of gender. (Table12, Figure 5)

Figure 5: Graphical presentation of screen failure by gender



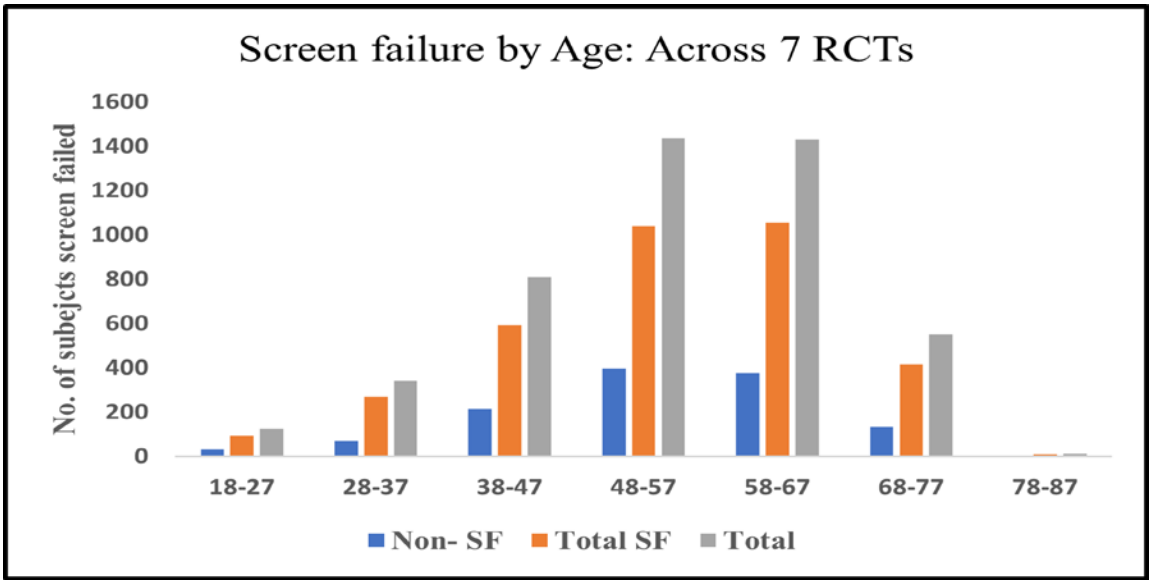
Analysis of Screen Failure by AGE:

The total number of 3485 subjects screened failed consisting of 1439 (41%) men and 2046 (59%) women with mean age of 53.94 ± 12.06 years (range, 18-84 years). There was no difference observed in screen failure in different age groups. The majority of patients are between the ages of 40 and 60, resulting in a higher enrollment or screening rate in those age groups compared to others; however, the failure rate for all age groups was between 70-85%. No trend line was noted.

Table 13: Analysis of Screen Failure by Age

Total number of subjects screened failed by Age				
Age group	Non- SF	Total SF	Total	failure %
18-27	33	94	127	74%
28-37	72	271	343	79%
38-47	215	594	809	73%
48-57	397	1041	1438	72%
58-67	376	1056	1432	73%
68-77	134	417	551	75%
78-87	2	12	14	85%

Figure6: Screen failure by Age n=7 RCT



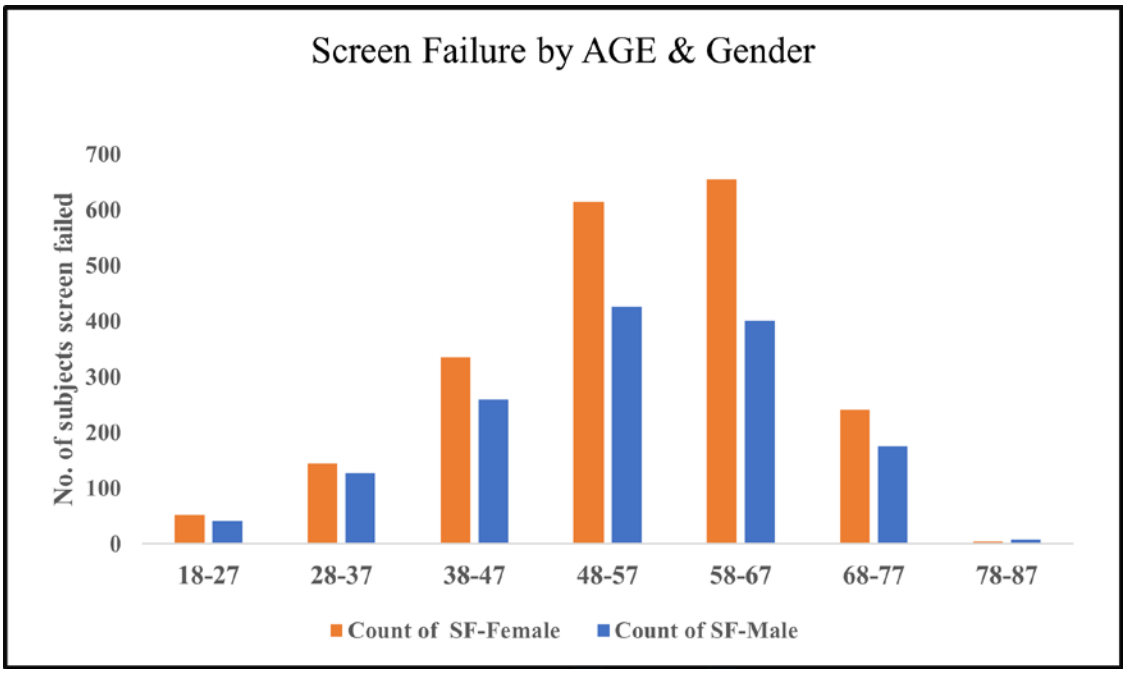
Analysis of screen failure by Gender & Age:

To define any correlation exists between Gender and Age. We compared screen failure across men and women in terms of age.

Table 14:Comparsion between Age and Gender: screen failure

Total number of subjects screen failed by Age and gender				
Age group	total SF	Count of SF-Female	Count of SF-Male	p value
18-27	94	52	42	0.465171
28-37	271	144	127	0.465453
38-47	594	335	259	0.027139
48-57	1041	615	426	0.000032
58-67	1056	655	401	0.00001
68-77	417	241	176	0.024052
78-87	12	4	8	0.407626

Figure 7:Graphical presentation of screen failure in terms of gender and Age



According to Table 14 and Figure 7, the only significant factor in terms of screen failure is gender. Evidently, across all age groups, women are more likely than men to fail a screening.

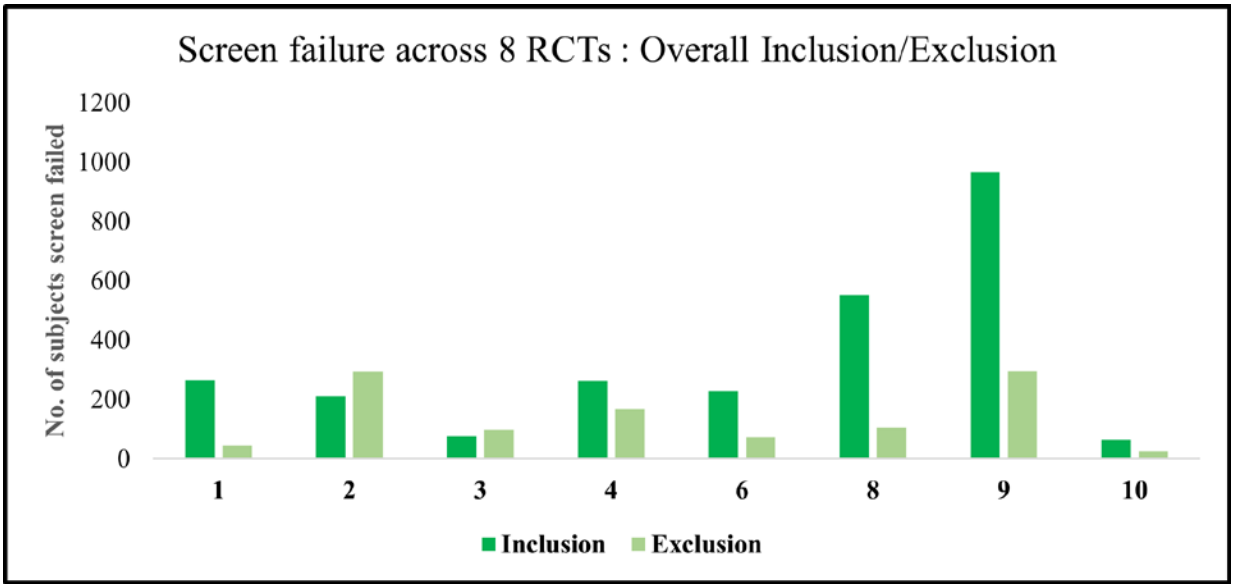
Analysis of Screen failure by generalized and specific reasoning:

All Screen failure data from each study were evaluated for a specific as well as the overall reasons for screen failure. Subject non-compliance and withdrawal were part of the inclusion and exclusion criteria except in one randomized trial study (study 2) where they had separate category called Withdrawal criteria. The most common reason for screen failure was not meeting the inclusion exclusion criteria. The following table shows the details of screen failure by inclusion and exclusion criteria. [Table 15]

Table 15:Analysis of screen failure by overall IE criteria

Total number of patients screened failed across 8 RCTs			
Study	Inclusion	Exclusion	p-value
1	265	46	0.0001
2	212	295	0.008949
3	76	98	0.23733
4	263	169	0.001295
6	228	73	0.0001
8	552	105	0.0001
9	902	325	0.0001
10	64	25	0.0028

Figure 8: Graphical presentation of screen failure: IE criteria



Based on the available data, inclusion criteria is the largest reason for screen failure. Therefore, it is worthwhile to investigate the specific causes of inclusion criteria failure. Exact causes for inclusion criteria failure were primarily separated into four sub-categories: Liver biopsy (invasive marker), MRI-PDFF (the sole extensively used non-invasive biomarker for determining eligibility and outcomes) (Table), AST/ALT levels, Lab abnormalities, concurrent medications, and non-compliance.

For analysis, both inclusion and exclusion criteria were evaluated, as it was found that each trial had a unique method for including IE into its protocol, e.g., few RCTs included AST/ALT as an inclusion criterion, while others included it as an exclusion criterion. Referencing Table 3, we classified eight randomized clinical trials into two groups to determine the key causes of screen failure.

These groups were developed to eliminate any confounding variables and based on FDA NASH guidelines that emphasize the use of solely non-invasive biomarkers and historical biopsy in PHASE IIA studies and the use of both invasive and non-invasive biomarkers in PHASE IIB studies.

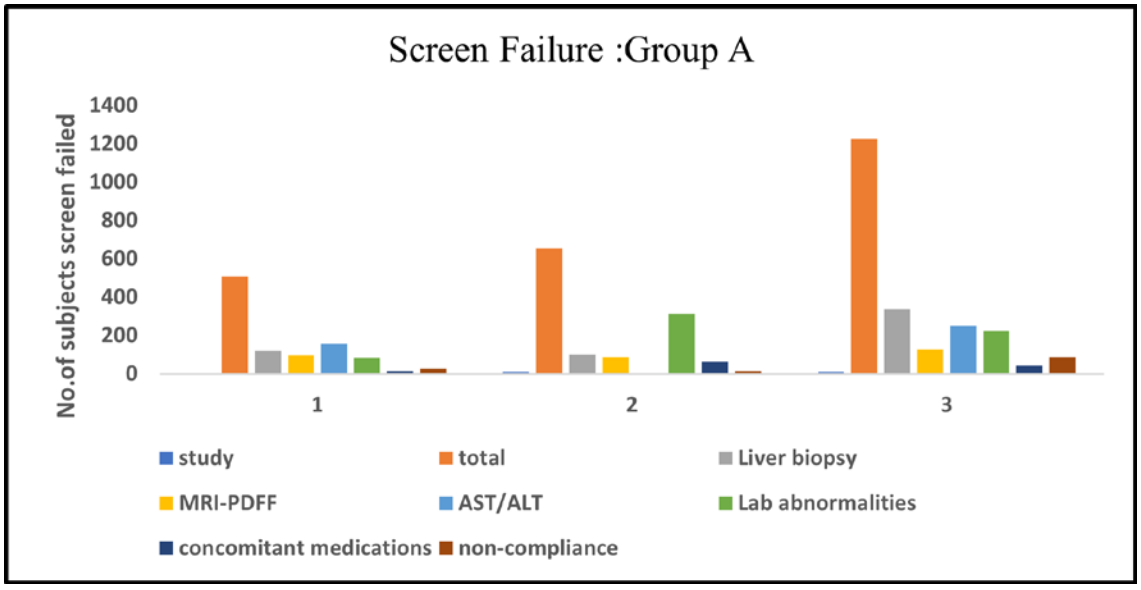
GROUP A	GROUP B
RCTs -2,8,9	RCTs 1,3,4,6,10

Table 16: Screen failure by exact reason: Group A RCTs

Total no. RCT= 4 (study 1,2,8,9)							
Study	Total failure due to IE	Liver biopsy	MRI-PDFF	AST/ALT	Lab abnormalities	Concomitant medications	Non-compliance
2	507	122	98	159	84	16	27
8	657	101	87		312	63	15
9	1227	337	129	251	224	43	87
Total	2391	560	314	410	620	122	129

Reasons for screening failure were classified and summarized, found that the most common reason for screening failure was Abnormal Laboratory results followed by liver biopsy, non-invasive biomarkers (MRI-PDFF), and others. [Table13]. These lab abnormalities accounted for 26% of total screening failures due to inclusion/exclusion criteria. While liver biopsy accounted for 23 %, MRI-PDFF for 13%, concomitant medications accounted for 5% and non-compliance accounted for 5%. Liver function tests such as ALT and AST accounted for 17%. Figure 9 is a graphical presentation of the exact screen failure.

Figure 9:Graphical presentation of screen failure: Group A RCTs



As described in Table 13 and Figure 5, the second most common reason for screen failure is liver biopsy. Screen failure due to invasive (liver biopsy) and non-invasive (MRI-PDFF) biomarkers were statistically analyzed using the Chi-square test [Table 14]

Table 17: Comparison of screen failure and MRI-PDFF

Total no. of RCTs = 4 (Study 1, 2,8,9)			
RCT	Liver Biopsy	MRI-PDFF	p-value
2	122	98	0.2518
8	101	87	0.4654
9	337	129	0.00001

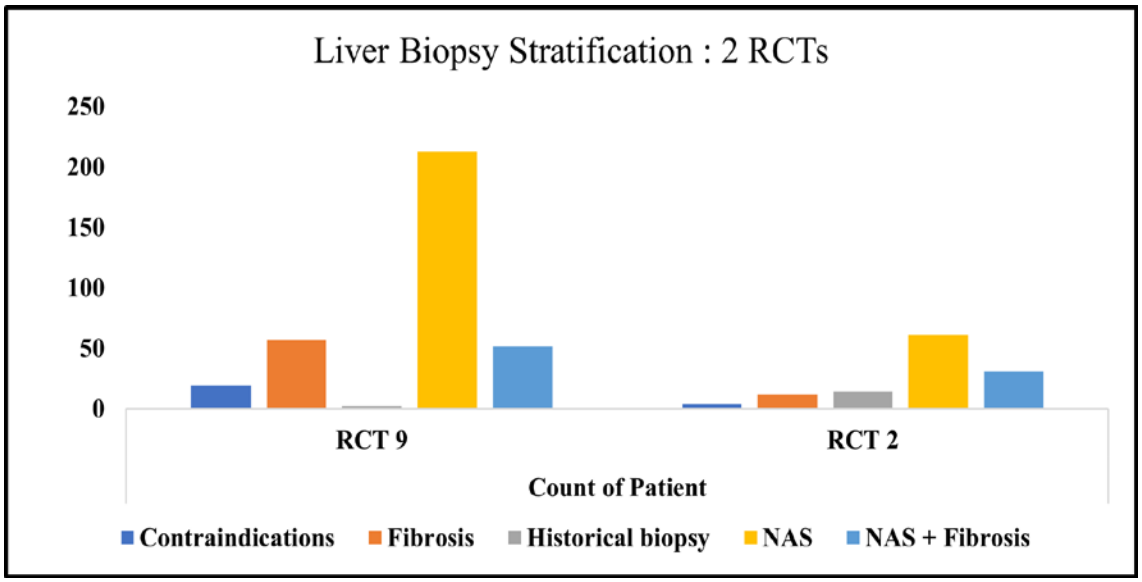
Statistical comparison between liver biopsy and MRI-PDFF showed a significant difference in screen failure in RCT- 9 only.

Table 18 shows liver biopsy-specific reasons for failure in relation NASH CRN system. This data was collected only from two studies as most of the other studies did not clearly define the reason for screen failure in liver biopsy. The most common reason for failure is not meeting NAFLD activity score (NAS) followed by Fibrosis and others.

Table 18: Liver biopsy (NASH CRN system) reason stratification: RCT-9

Liver Biopsy stratification	Count of Patients	
	RCT 9	RCT 2
Contraindications	19	4
Fibrosis	57	12
Historical biopsy	2	14
NAS	213	61
NAS + Fibrosis	52	31

Figure 10 : Graphical presentation of Liver biopsy reasons: RCT 9



Analysis of Screen failure: Group B:

This group primarily used non-invasive biomarkers for eligibility and outcomes with or without historical biopsy. The screen failure reasons were stratified as high, low and average, each study differs in failure range so individually stratified in following table. All the studies most common reason for screen failure was lab abnormalities, invasive or non-invasive biomarkers and ALT/AST abnormalities etc.

Low
High
Average

Table 19 Screen failure reason: RCT 10

Screen failure reasons for RCT 10		
Exact reason	Count of subject	Count of Subject (%)
Abnormal ALT	21	24%
Abnormal Lab values	15	17%
Concomitant medications	2	2%
Fibroscan/MRI/MRE	37	42%
MRI contraindications	1	1%
Non-compliance	4	4%
Other reason	7	8%
site decision to deny	2	2%
Total	89	100%

Table 20: Screen failure reasons for RCT-3

Screen failure reasons for RCT:3		
Reason for Screen failure	Count of Subject	Count of Subject (%)
Abnormal Lab values	55	30%
AST/ALT/Fibroscan/MRI PDFF	59	32%
concomitant medications	6	3%
Lost to Follow-Up	5	3%
MRI PDFF	4	2%
non-compliance	19	10%
other liver disease	1	1%
Other reason	16	9%
Sponsor decision to deny participation	21	11%
Total	186	100%

Table 21 Screen failure reasons: RCT 4

Screen Failure reasons for RCT:4		
Exact reason	Count of Patient	Count of Subject (%)
Abnormal LAB value	91	19.4%
Abnormal LFT	8	1.7%
Liver Biopsy	244	52.02%
MRI	2	0.43%
No reason stated	1	0.21%
Non-compliance	19	4.04%
Other Health condition	42	8.94%
Other liver disease	14	2.99%
Other reason	1	0.21%
site to deny participation	18	3.84%
Withdrawal by Subject	27	5.8%
stable Diabetic medication	2	0.42%
Total	469	100%

Table 22 Screen failure reasons: RCT 6

Screen failure reasons for RCT :6		
Exact Reason	Count of Subject	Count of Subject (%)
ABNORMAL LAB VALUES	37	11%
ALT	123	37%
AST	3	1%
BIOPSY/FIBROSCAN/MRE	27	8%
Concomitant medications	28	8%
FIBROSCAN	4	1%
Lost to follow up	1	0%
MRIPDFF	51	15%
MRIPDFF contraindication	1	0%
NO STATED REASON	1	0%
Non-compliance	29	9%
Other health reason	24	7%
Other liver disease	2	1%
Other reason	5	1%
Grand Total	336	100%

Table 23: Screen failure for RCT-1

Screen failure reasons for RCT:1		
Exact Reason	Count of Patient	Count of Subject [%]
ALT/AST abnormalities	17	5.21%
concomitant medications	16	4.91%
Fibroscan	1	0.31%
investigator decision to deny	1	0.31%
Lab abnormalities	132	40.49%
Liver Biopsy	75	23.01%
MRI PDFF	37	11.35%
no reason stated	3	0.92%
non-compliance	17	5.21%
Other liver disorder	12	3.68%
Other reason	15	4.60%
Grand Total	326	100%

These phase IIA trials didn't point out the exact reason for failure in terms of invasive or non-invasive biomarkers except for RCT -1 and RCT-4. Screen failure between these two studies were compared to analyze hypothesis testing. RCT 1 – that used both invasive and non-invasive biomarkers and RCT-4 that only used invasive biomarker. The results were analyzed.

Comparison of non-invasive and invasive biomarkers between two RCTs		
Exact Reason	Count of patients	
	RCT 1 (invasive + non-invasive)	RCT4 invasive
Lab abnormalities	132	91
AST/ALT abnormalities	17	8
Liver biopsy	75	244
Other reason	28	57
Non-compliance	17	49

The screen failure in study that utilized only invasive biomarkers for selection of subjects showed a high percentage of failure due to failed biopsy compared to other components. Even non-compliance was higher in that study (RCT-4).

Discussion

The aims were:1) ***to understand the inclusion of non-invasive biomarkers in NASH trial design and [2] To understand the screen failure reason in NASH and draw any relation between screen failure and non-invasive biomarkers***. Regarding the first objective, it was discovered that radiological biomarkers were widely used as both (eligibility as well as outcomes). Meanwhile, blood biomarkers and fibrosis panels were commonly utilized for assessing disease progression or treatment efficacy (outcome) in clinical trial design. However, steatosis panels were not included in the study. These include the NAFLD fat score, fatty liver index, and hepatic steatosis index. Due to their low sensitivity and specificity for detection of more than 5% fat and more than 33% fat, these panels are not considered as part of the inclusion criteria. In terms of indirect fibrosis panels such as APRI, FIB-4 & NAFLD fibrosis scores were used only for assessing disease outcomes as secondary endpoints. These can be useful indicator to identify at-risk population & can be considered as screening tool because they are simple, reproducible, inexpensive and widely available. These panels can help pinpoint individuals who would benefit from further examination at an early stage.

NASH is one of the common causes of chronic liver disease with no approved drug therapy. NASH is one of those disease trials that have a high screen failure. The difference observed in screen failure rates based on gender, despite having the same disease suggests that it may have a benign and non-aggressive course in some patients, while in others it may be more aggressive. It may be related to risk factors such as hyperlipidemia, insulin resistance, Obesity, hypertension, etc., but age and gender also influence disease severity. Numerous researchers have studied age and gender prevalence in relation to NASH. One study found that men have a higher risk of

advanced fibrosis than premenopausal women; however, both sexes exhibit equivalent severity of fibrosis after menopause, indicating that estrogen protects against the development of fibrosis (Daryani, et al., 2010). While some studies have shown that men are at a higher risk for NAFLD-related liver-based mortality (Yang , et al., 2014). This study did point to differences in screen failure in terms of gender such as an increase in screen failure in women than men. Based on literature and results the possible explanation would be that screen failure in women may be related to a benign and non-progressive course of disease compared to men, however, this needs more validation.

Screen failure assessment based on the specific cause of failure. To test our hypothesis, screen failure across non-invasive and invasive biomarkers was compared and showed significant differences in the screen failure due to liver biopsy and MRI PDFF. The difference was significant in 1 out of 3 studies and hence needs more validation. In terms of screen failure in liver biopsy, an intercomparison of a study using combination biomarkers (invasive and non-invasive) and a study using only invasive biomarkers revealed a significant difference in terms of screen failure. This finding will help us better understand how non-invasive biomarkers can be helpful in stratifying the population for liver biopsy thus avoiding unwanted liver biopsy (pulling in a more homogenous population for biopsy). The present study does not suggest that liver biopsy should be discarded from the diagnostic investigation of patients with NASH but points to the inclusion of various combinations of biomarkers, at least from two domains related to specific pathophysiologic processes that are associated with NASH which will facilitate the development of better diagnostic criteria for screening.

Chapter VI

SUMMARY AND CONCLUSION

Limitations:

There are notable limitations of this study. The study was designed to analyze data gathered through screen failure and protocols in the form of patient counts and demographic information rather than subject's case report form and laboratory results which would have more effectively pinpointed specific reasons for screen failure. For future study considerations, this would be a much more effective and accurate way to test whether non-invasive biomarkers are more effective than biopsy. The study sample which was recruited for this study was very limited, as only data on 9 NASH study trials and screen failure data from 8 trials were available. Therefore, our results cannot be generalized to other NASH trials data.

Additionally, the study also involved secondary analysis of data which has already been collected. At Medpace, there are two software applications used for capturing screen failure data, review of screen failure data entry is done with one software (IRT) and reasons are entered with another software (EDC). Due to these limitations, specific reasons for screen failure were not always recorded i.e., the reason for failure due to inclusion and exclusion criteria is not available. Data on failure due to liver biopsy was very generalized, not all trials that involved biopsy pointed out exactly which NAFLD activity score (NAS) led to failure. There were some randomized study trials that provided an overall reason for failure but didn't mark a specific reason which would have improved hypothesis testing results.

Conclusion:

In conclusion, we cannot eliminate the screening failure problem but any strategy to reduce the screening failure rate is worthwhile. Non-invasive biomarkers are one to be considered, as screening failure is comparatively less. To accurately diagnose or select a patient for trial, the histological criteria of NAS ≥ 4 and Fibrosis > 2 need to be met; combination of serum, radiological biomarkers or panels should meet these criteria, and this needs more validation over a larger sample size. Liquid liver biopsy, genomics, proteomics, and metabolomics are trending or emerging biomarkers that need more validation. We need to identify such biomarkers or a combination of them that not only reduce the need for unwanted biopsies but also be able to generate quality data in clinical trials as well as bring more subjects into randomization (reduce screen failure rate). In drug development, we need to have verified endpoints to market the drug, in terms of NASH study, liver biopsy is still considered as the primary endpoint which is not feasible. Therefore, devising strategies that will help to reduce screening failure will benefit both the subjects, and researchers.

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Internship Experience

The internship was conducted at Medpace, a contract research organization in Irving Texas, under guidance of site Mentor Mr. Richard Young. It was an office-based internship. Medpace is a full-service contract research organization with over 30 years of experience in designing and executing clinical trials including site feasibility, site qualification, site initiation & site monitoring to close out visit.

During the course of my internship, I was assigned to two studies: 1] AVM003HC- Phase 3 Multicenter Double-blind, Placebo controlled trial of Viralym-M for the treatment of patient with virus -associated Hemorrhagic cystitis after Allogenic Hematopoietic cell transplant.2] Athersys or MASTERS2- Multistem administration for stroke treatment and Enhanced recovery study. I performed the role of In-House CRA in clinical monitoring of these two studies. I also got the privilege to work with different departments such as Clinical monitoring, Patient recruitment and retention, Data management, Regulatory submission & TMF QC within Medpace.

I have acquired knowledge of Clintrak IRT and Clintrak EDC. Medpace has an interactive learning portal (Medpace learning system) which has instructor -led training as well as self-led training in various clinical research and therapeutic areas. Throughout the internship, I was able to understand the designing and execution of clinical research studies from the perspective of a contract research organization.

APPENDIX-A

Daily internship Journal

Week 1: August 22nd – August 31st, 2022

Monday, August 22nd, 2022

- Introduction to Site Mentor Richard Young, CTM, and site orientation
  - Received badge.
  - Medpace campus visit
  - Completed necessary credentialing.
- Laptop set up; office place allotted.
- Introductory meeting with the Site mentor, Medpace portal use was explained.
- Assigned to a few introductory pieces of training.
- Completed corporate compliance training.
- Attended training on Medpace overview.

Tuesday, August 23rd, 2022

- Good Clinical Practice and electronic Trial master file training
- Introduction to Clinical trial management Wednesday, August 24th, 2022
- Medpace-led training on:
  - Effort Reporting
  - Introduction to Rare diseases and pediatric rare diseases
  - Research on Project ideas for practicum thesis

Thursday, August 25th,2022

- More training modules on:
  - Site feasibility visit
  - Operational considerations in Pediatric rare disease
  - Introduction to infectious disease

Friday, August 26, 2022

- Team meeting with site mentor Young Richard
  - Good Clinical Practice principles
  - Functioning of Medpace
- Continuation of training module assigned.

Week-2: August 29, 2022- September 02, 2022

Monday, August 29, 2022

- Continuation of training module assigned.
    - Blinded/unblinded trials.
    - Infectious disease lab overview
    - Pneumonia

Tuesday, August 30, 2022

- Training module on infectious disease training in immunocompromised patients
- Training exercise on Advanced management in orphan disease trial
- Regulatory Considerations for orphan indications

Wednesday, August 31, 2022

- Meeting with Jackie Allison, Data Coordinator, Data management team Discussion about any prospective ideas for the practicum project
- Continuation of more training modules

Thursday, September 01, 2022

- Finalize a few ideas for the project, a quick meeting with Jackie Allison for feedback. Her input was very helpful.
- I was considered for one project – Trail master file -Quality checking. Under this I was tagged for two studies
- Continuation of training module-
    - Managing and monitoring cell and gene therapy

Friday, September 02, 2022

- Attended training on Site Qualification visit.
- Project idea finalization, discussed with Site mentor Richard Young

Week 3: September 06, 2022- September 09, 2022

Tuesday, September 06, 2022

- 1st Advisory Committee meeting
- Meeting with Sireesha, TMF Manager, Medpace
- Medpace- Pace training
    - Oncology certificate program (RECIST 1.1)

Wednesday, September 07, 2022

- Continuation with PACE training
    - Phase 1 oncology
    - Introduction to Leukemia
    - Qc training with Sireesha and Gwyneth for two studies

Thursday, September 08, 2022

- Meeting with Gwyneth for Trial master file- Quality check
- Team meeting with Sara Sampaio, gave more insight into patient recruitment across various sites, an overview of recruitment procedures and tasks
- Pace training-
    - Oncology certificate program- Immunotherapy

Friday, September 09, 2022

- Attended Pace instructor-led training –
    - Source documentation

- Send official mail to all committee members – Research Project
- Pace training – Oncology certificate program
    - Advanced immunotherapy

Week 3: September 12, 2022- September,16,2022

Monday, September 12, 2022

- Pace training-
    - Oncology certificate program completion
    - Stroke Overview & Neuroscience Overview
- Research proposal preparation
- Trial master File -Quality Check for studies

Tuesday, September 13, 2022

- Pace training:
    - Overview of Epilepsy
    - Becker's muscular dystrophy
    - Traumatic brain injury

- Meeting with Clinical monitoring team personnel Erin M for discussion on NASH studies. Collecting and reviewing data.

Wednesday, September 14, 2022

- Trial master file- Quality check for two studies
- Pace training:
    - Dravet syndrome
    - Intensive care unit
    - Sedation analgesia
- Worked on research proposal.

Thursday, September 15, 2022

- Meeting with patient recruitment manager Sara Sampaio about resource collecting for Peripheral artery disease.
- Weekly meeting with site mentor- Richard Young
- Discussion on Data access for practicum report or thesis
- Medpace Team training:
    - Laboratory monitoring

Friday, September 16, 2022

- Started working on patient recruitment assigned work:
    - Collected information on advocacy groups for Peripheral artery disease, complied into an excel sheet.
- For the thesis, requested access to EDC for NASH studies.
- Continued research proposal writing
- Pace training: Muscle biopsy

Week 4: September 19, 2022 – September 23, 2022

Monday, September 19, 2022

- Trial master file- Quality check – focusing on initially submitted documents only (2 studies)
- Advocacy group information for Peripheral artery disease was complied with and sent to the recruitment team.
- Pace training: Nerve biopsy procedure

Tuesday, September 20, 2022

- Assigned to another study- AlloVir.
- Trial master file- Quality check for assigned studies.
- Research work

Wednesday, September 21, 2022

- Trial master file- Quality check
- Pace or MLS training- NASH therapeutic area training

Thursday, September 22, 2022

- Trial master file- Quality check – assigned to a new study.

Friday, September 23, 2022

- Email conversation for granting access to NASH study.
- MLS training or Pace training: SQV (site qualification visit) and SIV (site initiation visit)
- Attended AlloVir study monitoring meeting, responsible for minutes of the meeting. Completed minutes of meeting sent out to Jesse Brown (CRA)

Week 4: September 26, 2022 – September 30, 2022

Monday, September 26, 2022

- Teams training- Patient recruitment and CRA role in recruitment
- MLS or Pace training- RMV (routine monitoring visit)
- Introductory meeting for a new study- Athersys with Hillary (Sr. intern)

Tuesday, September 27, 2022

- MLS or Pace training – Routine monitoring visit basic in Neuroscience
- Trial master file- Quality check for 4 studies

Wednesday, September 28, 2022

- Meeting with Hillary for Athersys study project work and even requested study specific access to Medpace portal (Clintrak and SharePoint)
- MLS or Pace training- Introduction to Radiology
- Trial master file- Quality check for 3 studies

Thursday, September 229, 2022

- Team training- Serious adverse events reconciliation workshop
- Trial master file- Quality check for 3 studies
- MLS or Pace training:
    - Radiopharmaceuticals -Clinical overview and background
    - Radiopharmaceuticals- Chemistry manufacturing & control supply chain
- Weekly meeting with site mentor – Richard Young. Discussed issues with research data, Delinquency related to IRB approval of project.
- Discussion on Effort reporting.

Friday, September 30,2022

- Trial master file- Quality check for 2 studies
- MLS or Pace Training: Clinical imaging for trials in radiopharmaceuticals

Week 5: October 3,2022 – October 7,2022

Monday. October 3,2022

- Trial master file- Quality check for studies, Assigned to new study
- MLS or Pace training
- Thesis Project work

Tuesday, October 4, 2022

- Trial master file- Quality check
- Teams training on Site source documentation process form (SSDPF)
- Thesis Project work

Wednesday, October 5,2022

- Trial master file- Quality check
- MLS or Pace Training- Effective patient management

Thursday, October 6, 2022

- Trial master file- Quality check
- study Meeting with Hillary Gonzales (Sr. Intern)

Friday, October 7, 2022

- Study meeting minutes of meeting completed and submitted.
- Teams met with Dr. Mathew (program director UNTHSC), Nick Salyers (Clinical Director), and Richard Young (site mentor) about discrepancies in current research proposal design, new proposal design was suggested.
- Trial master file- Quality check

Week 6- October 11, 2022- October 14, 2022

Tuesday, October 11, 2022

- Dismissal of first research proposal due to conflicts with data use. Planned new proposal idea.
- Prescreening logs- In-house CRA work for Athersys study site.

Wednesday, October 12, 2022

- Prescreening logs entered into Clintrak for Athersys study site.

Thursday, October 13, 2022

- Trial master file- Quality check for 3 studies
- Finalized new proposal idea.

Friday, October 14, 2022

- Prescreening logs entered into Clintrak & uploaded on SharePoint.
- Committee Meeting for new proposal & finalization
- Trial master file- Quality check

Week 7 – October 17, 2022- October 21, 2022

Monday, October 17, 2022

- Regular Prescreening logs uploaded to Clintrak for the Athersys study site & SharePoint.
- Trial Master File- Quality checking for AiCuris study

Tuesday, October 18, 2022

- Regular prescreening logs uploaded to Clintrak for Athersys study site 105.
- Trial Master File- QC for PQ therapeutics study

Wednesday, October 19,2022

- Regular prescreening logs uploaded to Clintrak for Athersys study site 105.
- Reading protocols for the thesis project.

Thursday, October 20, 2022

- Trial Master file upload Quality checking for AiCuris
- Prescreening logs uploaded to Clintrak for site 110.
- Discussion with designated CRAs on the missing log for site 303, site 202

Friday, October 21, 2022

- Data screening and evaluation for the Thesis project
- Email sent out to different CRAs on missing logs.
- Completed prescreening logs for site 135 & uploaded document on SharePoint.

Week-8 – October 25, 2022 – October 31, 20

Tuesday, October 25, 2022

- Trial master File- Quality check of uploaded documents for the following study.
  - PQ therapeutics
  - NS Pharma
- Weekly meeting with Site Mentor

Wednesday, October 26, 2022

- Trial master File- Quality check of uploaded documents for PQ therapeutics study
- New research proposal submission to Committee for approval
- prescreening logs for site 303 & uploaded them into SharePoint.

Thursday, October 27, 2022

- Trial master file- Quality check of uploaded documents for PQ therapeutics study
- Completed prescreening logs for site 303 & uploaded them into SharePoint.

Friday, October 28, 2022

- Email sent out to different CRAs about missing logs.
- Trial master file- Quality check of uploaded documents

Monday, October 31, 2022

- Trial master file -Quality check of uploaded documents
  - PQ therapeutics study
  - AiCuris
- Prescreening logs for site 303 & uploaded into SharePoint.

Week 9 – November 1, 2022- November 5, 2022

Monday, November 1, 2022

- Weekly meeting with a site mentor
- IP reconciliation exercise -Part 1
- Prescreening logs for site 202 & uploaded into SharePoint.

Tuesday, November 2, 2022

- Completed prescreening logs for site 202 and uploaded them into SharePoint.
- Attended monitoring minutes for AlloVir study, reconciled minutes of meeting for same

Wednesday, November 3, 2022

- Backdated prescreening logs were entered into Clintrak for site 602 [July] and uploaded into SharePoint for the Athersys study.
- Meeting with Jackie Allison for data collection for the research project.

Thursday, November 4,2022

- Backdated prescreening logs were entered into Clintrak for site 602 [August] and uploaded into SharePoint.
- Data management – Meeting with Yara, the Data manager, by work on Lab review
- Trial Master file- Quality check of uploaded documents for N pharma and AiCuris

Friday, November 5, 2022

- Backdated prescreening logs entered into Clintrak for site 602 [September]
- Trial master file -Quality check of uploaded documents for the AiCuris study
- Team training on Site Source documentation process form [instructor-led]
- Data management – Lab review work for audit purposes

Week 10 – November 7, 2022 – November 11, 2022

Monday, November 7, 2022

- Practice exercise for site source documentation process form
- Prescreening logs entered into Clintrak for site 602 [October] and uploaded into SharePoint.

Tuesday, November 8, 2022

- Trial Master file -Quality check of uploaded documents for AiCuris study
- Completion of Lab review work for site 101, sent to data management for review.

Wednesday, November 9,2022

- Completed prescreening logs for site 602 and uploaded into SharePoint.
- Research Project data collection

Thursday, November 10, 2022

- Prescreening logs entered into Clintrak for site 109 for Athersys study.
- Meeting with Jackie Allison for further discussion pending data for the research project.

Friday, November 11, 2022

- Completed prescreening logs for site 109 and uploaded them into SharePoint.
- Research project data collection

Week 11- November 14, 2022 – November 18, 2022

Tuesday, November 15, 2022

- Medpace learning system training:
  - Document Quality Control
  - Naming Conventions

Wednesday, November 16, 2022

- Medpace learning system training:
  - Medpace CRO and CPU Operating models
  - TMF export and transfer
  - Edit Check specifications.
- IP infusion exercise -Part II completed.

Thursday, November 17, 2022

- Completed prescreening logs for site 124 into Clintrak and uploaded them into SharePoint.
- Trial Master file -Quality check for MSTEM2
- Medpace learning system training.
  - Investigator site file
  - Study initiation visit
  - Site essential document collection and review

Friday, November 18, 2022

- Medpace learning system training.
  - Investigator and OHRP compliance checks
  - Unblinded monitoring visit.
  - Informed consent form
- Completed prescreening logs for site 110 [July, August, September] and uploaded them on SharePoint.
- Trial master file quality check for MSTEM2

Week 12- November 21, 2022 – November 25, 2022

Monday, November 21, 2022

- Completed prescreening logs for site 105 into Clintrak and uploaded them into SharePoint.
- Trial master file quality check for study MSTEM2 and AiCuris
- Medpace learning system training.
  - Safety monitoring plan
  - Preparation and submission of safety reports to regulatory authorities, institutional review boards/ Ethics Committees.
  - Serious adverse events processing in clinical trials with an investigational product
  - SSDPF exercise

Tuesday, November 22, 2022

- Trial Master file quality check for study MSTEM2
- Medpace learning system training.
  - Essential documents requirements
  - Maintaining Blind in Clinical studies
  - Emergency Unblinding Process

Week 13- November 28, 2022 – December 2, 2022

Monday, November 28, 2022

- Completed prescreening logs for site 601 for Athersys study [July, August 2022] and uploaded them into SharePoint.
- Trial master file- Quality check for Shockwave study

Tuesday, November 29, 2022

- Completed prescreening logs for site 602 for site 135 [September 2022] and uploaded them into SharePoint.
- Effort reporting for month of November
- Trial master file -Quality check for MSTEM2 and NPHARMA study

Wednesday, November 30,2022

- Completed prescreening logs for site 135 [October 2022] and uploaded them into SharePoint.
- Attended monthly monitoring meeting for Athersys study & compilation of minutes of meeting.
- Teams meeting with TMF Admin for Npharma study.
- Trial master file -Quality check for MSTEM2 study

Thursday, December 1,2022

- Adverse safety event processing in clinical trials
- Prescreening logs for site 105 [November 2022]

Friday, December 2,2022

- Meeting with site mentor
- Overview and discussion about Medpace EDC system
- Trial Master file -Quality check for AiCuris study

Week 14- December 5,2022 – December 9, 2022

Tuesday, December 6, 2022

- Completed prescreening logs for site 134 [November 2022] and uploaded into SharePoint.
- Weekly meeting with Site mentor
- SAE reconciliation exercise
- Outstanding SAE reconciliation mails sent out to CRAs.

Wednesday, December 7,2022

- Outstanding SAE response mail from CRAs complied into one document.
- Attended monthly monitoring meeting for AlloVir study.
- Compilation of minutes of meeting
- Thesis project data work

Friday, December 9 ,2022

- Completed prescreening logs for site 601 [October, November 2022] and uploaded into SharePoint.
- Trial master file -Quality check for AiCuris, MSTEM2 and Npharma studies

Week 15 – December 12,2022 – December 16,2022

December 12, 2022

- Trial master file -Quality check for MSTEM2 study
- Completed prescreening logs for site 107 and uploaded them into SharePoint.
- Completed prescreening logs for site 202 and uploaded them into SharePoint.

Tuesday, December 13,2022

- Weekly meeting with site mentor
- Trial master file -Quality check for MSTEM2

Wednesday, December 14, 2022

- Trial master file- Quality check for AiCuris study
- Thesis project work- Protocol reading.

Thursday, December 15, 2022

- Documents uploaded on the regulatory portal for study MSTEM2 study.
- Trial master file -Quality check for AiCuris study

Friday, December 16, 2022

- Completed prescreening logs for site 134 [July & August 2022] & uploaded them into SharePoint.
- Trial Master file -Quality check for MSTEM2 and AiCuris study

Week 16- December 19, 2022 – December 23, 2022

Monday, December 19, 2022

- Completed prescreening logs for site 134 [September 2022] & uploaded them into SharePoint.
- Trial Master file- Quality check for MSTEM2
- Informed consent reading for study – comparing with ICH guidelines.

Tuesday, December 20, 2022

- Worked on Data for thesis project. Aligning them into proper order for analysis

Wednesday, December 21,2022

- Trial Master file- Quality check for MSTEM2 study
- Completed prescreening logs for site 134 [October 2022] & uploaded into SharePoint.

Thursday, December 22,2022

- Uploaded prescreening logs into Clintrak for site 134 [November 2022] & updated the SharePoint.

Friday, December 23,2022

- Attended monthly monitoring meeting for Athersys study.
- Thesis project work

Week 17 – December 27,2022 – December 30,2022

Tuesday, December 27,2022

- Compilation of minutes of meeting for Athersys study.
- Completed prescreening logs for site 134 [December 2022] & uploaded them into SharePoint.

Wednesday, December 28, 2022

- Thesis data collection & analysis
- Reference scientific paper reading

Thursday, December 29,2022

- Thesis project data analysis & referencing
- Scientific paper reading.

Friday, December 30,2022

- Thesis project data analysis
- Scientific paper reading

Week 18- January 11, 2023 – January 13, 2023

Wednesday, January 11, 2023

- Attended monthly monitoring meeting for AlloVir study.
- Compilation of minutes of meeting
- Addition of two more studies for Trial master file Quality check [NGAM12 & MSP1738]
- Completed prescreening logs for site134 [first half of January 2023] & updated SharePoint.

Thursday, January 12, 2023

- Completed prescreening logs for site 127 [December 2022]
- Trial master file -Quality check for following studies:
  - MSP1738
  - MSTEM
  - NGAM12
- Completed prescreening logs for site 601[December 2022]

Friday, January 13, 2023

- Completed prescreening logs for site 202 [December 2022]
- Trial master file-Quality check for MSP1738 was completed & no longer support needed for study.
- Practicum report writing

Week 19- January 16,2023- January 20,2023

Monday, January 16, 2023

- Trial master file – Quality check for MSTEM2 study
- Meeting with site mentor
- Practicum report writing

Tuesday, January 17, 2023

- Trial master file – Quality check for NGAM12 study
- Practicum report writing
- Regulatory submission exercise

Wednesday, January 18, 2023

- Practicum report writing

Thursday, January 19, 2023

- Practicum report writing

Friday, January 20, 2023

- Assigned to new studies for TMF Qc -Lox43501 and PTG3011
- Trial master file- Quality check for following.
    - Lox43501
    - AiCuris

Week 20 – January 23, 2023 – January 27, 2023

Monday, January 23, 2023

- Trial master file-Quality check for PTG3011 & NGAM12 study
- Completed prescreening logs upload into Clintrak for site 202 [Dec2022] & updated SharePoint.

Tuesday, January 24, 2023

- Attended Informed consent form training module.
- Trial master file-Quality check for NGAM12 study

Wednesday, January 25, 2023

- Medpace IT training
- Completed prescreening logs upload into Clintrak for site 130 & site 103 & updated SharePoint

Thursday, January 26, 2023

- Informed consent exercise
- Trial master file-Quality check for AiCuris study
- Data management – Teams meeting for Lab reference range work.

Friday, January 27,2023

- Attended monthly monitoring meeting for Athersys study.
- Attended Dallas lunch and meet – Various departments interact with each other. PPT – Patient recruitment and retention
- Data management- Lab reference range log verification & update.

Week 21- January 30, 2023 – February 3, 2023.

Monday, January 30, 2023

- Data management -Lab reference range updating for two studies.
- Started new admin assignment- CRA grading assessment. Teams meeting for same with Griffin & Brian
- Trial master file-Quality check for LOX43501 & NGAM12 study

Tuesday, February 01,2023

- Attended monthly monitoring meeting for AlloVir study.
- Teams meeting with Griffin -CRA grading assessment.

Wednesday, February 02, 2023

- Completed lab reference ranges work & sent it to Yara, data management personnel.
- Compilation of minutes of meeting for Athersys study

Thursday, February 03,2023

- Started with CRA grading assessment.
- Quick Team meeting with Griffin – uploading documents on global tracker & SharePoint.

- Completed prescreening logs entry into Clintrak for site 122 & site 134 and updated SharePoint.
- ICF review exercise – Main ICF and site-specific ICF

Week 22- February 06,2023 – February 10,2023.

Monday, February 06,2023

- Compilation of minutes of meeting for AlloVir study
- Trial master file-Quality check for AiCuris study
- Data management- Lab reference work for new study

Tuesday, February 07,2023

- Weekly meeting with site mentor – discussion related to two studies, protocols and other details related to it
- Started writing Daily Internship Journal for submission to University.

Wednesday, February 08,2023

- Data management -Lab reference ranges for site 103
- In person meeting with Regulatory submission coordinator on Informed consent.

Thursday, February 09,2023

- 2nd day of Regulatory submission meeting with Sally Brinkman
- In person meeting with Imaging personnel associated to Medpace Laboratories- insight on radioactive isotopes involved studies.

Friday, February 10,2023

- Trial master file-Quality check
- Teams meeting with Maria, Clinical research associate for 'site personnel training as per protocol' tracker work.

Week 23 – February 13, 2023 – February 17,2023.

Monday, February 13,2023

- Trial master file- Quality check for PTG3011 & LOX43501
- CRA grading assessment- updating global tracker & uploading documents on monitoring SharePoint.
- Team meeting with Maria about updating excel tracker for site personnel training as per protocol.
- Daily Journal writing

Tuesday, February 14, 2023

- CRA grading assessment: 6 in total.
- Trial master file- Quality check for AiCuris study

Wednesday, February 15, 2023

- Team training on Monitoring visit SOP
- Updated site personnel training tracker for site 108/Chae
- Trial master file- Quality check for PTG3011 study
- CRA grading assessment:2.

Thursday, February 16, 2023

- Completed prescreening logs for site 202 & updated the SharePoint.
- Daily journal completion

Week 24- February 20, 2023- February 24, 2023

Monday, February 20,2023

- Team training on General monitoring visit guidelines
- CRA grading assessment:3.
- Completion of excel tracker for site personal training.
- Trial master file- Quality check for LOX43501 study

Tuesday, February 21, 2023

- Team training on Monitoring data cross checks
- CRA grading assessment:1.
- ICF review exercise – amendment v/s ICF for site
- Started ppt preparation for thesis.

Wednesday, February 22,2023

- CRA grading exercise:1.
- ICF review exercise – continued.
- Regulatory submission meeting with Regulatory submission coordinator.
- Attended monitoring meeting for Athersys study.

Thursday, February 23,2023

- CRA grading assessment: 2.
- Trial master file- Quality check for PTG3011 study

Wednesday, February 24, 2023

- CRA grading assessment:4.
- Compilation of minutes of meeting for Athersys study
- PowerPoint preparation for thesis presentation.

Week 25- February 27,2023 – March 10, 2023

Monday, February 27,2023

- Trial master file -Quality check for Lox43501 & PTG3011 study
- CRA grading assessment: 3.

Tuesday, February 28,2023

- CRA grading assessment: 2.
- Trial master file-Quality check for MSTEM study
- Completed prescreening logs and updated SharePoint.

Monday, March 06, 2023

- CRA grading assessment:2.
- Site source documentation process form (SSDPF) for MSTEM study
- Trial master file -Quality check for PTG3011

Tuesday, March 07,2023

- CRA grading assessment:2.
- Trial master file -Quality check for PTG3011
- Compilation of monitoring minutes for the Athersys study

Wednesday, March 08,2023

- Teams training on General monitoring visit guidelines
- CRA grading assessment:3.
- Completion of Excel tracker

Thursday, March 09, 2023

- CRA grading assessment:3.
- Trial master file – Quality check for LOX43501

Friday, March 10,2023

- CRA grading assessment 1.
- Regulatory submission meeting with Regulatory submission coordinator
- Informed consent form exercise

Week 26- March 13, 2023 – March 17, 2023.

Wednesday, March 15, 2023

- Trial Master file -Quality check for NGAM12 and MSTEM2 study

Thursday, March 16,2023

- CRA grading assessment: 4.
- Trial Master file- Quality check for PTG3011.

Friday, March 17, 2023

- Trial master file -Quality check for PTG3011
- CRA grading assessment:1.
- Preparation for thesis defense

Week 27- March 21, 2023 – March 28, 2023.

Tuesday, March 21, 2023

- Meeting with Data management personnel Yara about Case report form development work for one study
- Trial master file- Quality check for PTG3011
- CRA grading assessment 1.

Wednesday, March 22,2023.

- Thesis defense- UNTHSC

Thursday, March 23,2023

- Data management- CRFs template update for a study [CFT7455]
- CRA grading assessment: 1.

Friday, March 24, 2023

- Global tracker updated for CRA grading assessment & uploaded into SharePoint.
- Data management- CRFs template update for CFT7455 study
- Trial Master File- Quality check for PTG3011

Monday, March 27,2023

- Completed prescreening logs for site 601 [Jan 2023] & updated SharePoint.
- CRA grading assessment:1.

Tuesday, March 28,2023

- CRA grading assessment -2.
- Data management- CRFs template update work
- Completed prescreening logs for site 602 [Nov 2022] and updated SharePoint.
- Trial Master file- Quality check for PTG3011.

Week 28- March 29, 2023 -April 05, 2023.

Wednesday, March 29, 2023

- CRA grading assessment 2.
- Data management – CRFs template update work continued.
- Trial Master file- Quality check for NGAM12 study

Thursday, March 30, 2023

- Data management- CRFs template update work continued.
- Trial master file- Quality check for PTG3011 study
- CRA grading Handover training.

Friday, March 31, 2023

- Data management- CRFs template update work.
- Trial master file- Quality check for PTG3011 & NGAM12 study

Monday, April 03,2023

- Data management -CRFs template update work.
- Updated Global tracker for CRA grading assessment.

Tuesday, April 04,2023.

- Completed prescreening logs for site 602 [Feb 2023] & updated SharePoint.
- Completed CRFs template work & updated data management team about same.
- Meeting with Site mentor – End of internship meeting.

Wednesday, April 05, 2023

- Hand over task & Final closure at Medpace.