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LUNG INJURY PREVENTION WITH ASPIRIN: METHODOLOGY FOR A MULTICENTER RANDOMIZED CLINICAL TRIAL

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ABSTRACT

Introduction: The acute respiratory distress syndrome (ARDS) and the less severe acute lung injury (ALI) are devastating conditions that place a heavy burden on public health resources. Although the need for effective ALI prevention strategies has been increasingly recognized, no effective preventative strategies presently exist. The Lung Injury Prevention Study with Aspirin (LIPS-A) aims to test whether aspirin (ASA) administration could prevent and/or mitigate the development of ALI in patients determined to be at high risk for this life-threatening complication.

Methods and Analysis: LIPS-A is a multicenter, double-blind, phase II randomized clinical trial which aims to test the hypothesis that the early administration of ASA will be associated with a reduced incidence of ALI during the first seven days following hospital admission of adult patients at high risk for ALI. It is anticipated that this investigation will enroll 400 total study participants from 14 hospitals across the United States. Conditional logistic regression will be used to test the primary hypothesis that early ASA administration will decrease the rate of ALI development. A planned interim analysis will be conducted at 50% of study participants enrolled.

Ethics and Dissemination: Safety oversight will be under the direction of a data safety and monitoring board whose members will be independent from the study operations. Safety endpoints will be examined for all eligible patients who sign informed consent and are enrolled in the study on an intent-to-treat basis.

In addition to providing important clinical and mechanistic study results, the findings of this investigation will be informative on the scientific merit and feasibility of a phase III trial on the role of aspirin as an ALI prevention agent. The LIPS-A group will also encourage

investigator-initiated ancillary study proposals that extend or complement the specific aims of the primary LIPS-A trial.

INTRODUCTION

Acute lung injury (ALI) and the acute respiratory distress syndrome (ARDS) are life-threatening syndromes which continue to consume substantial health care resources and profoundly impact patient-important outcomes.[1] Although recent epidemiologic studies suggest the incidence of lung injury may be on the decline,[2] even conservative estimates suggest the associated mortality continues to exceed 25%.[3] Beyond mortality, an episode of ALI/ARDS also substantially influences patient's long-term outcomes with functional deficits persisting up to five years after the episode of respiratory failure.[4]

Importantly, the clinical syndrome of ALI generally occurs as a complication of an initial predisposing acute injury such as pneumonia, aspiration, sepsis, trauma, shock, or massive transfusion.[5] However, only a fraction of patients (10-30%) with these initial injuries develop ALI/ARDS.[6, 7] Only 30% of ALI patients fulfill criteria for ALI within six hours of presentation to the emergency department (ED).[8] The majority of patients develop ALI a median of two days after hospital presentation (IQR 1-4 days). This period of time between hospital presentation and development of ALI presents a window of opportunity for interventions to prevent the development of ALI.

Recently, accumulating evidence suggests an important role for platelets in both ALI pathogenesis [9-11] and resolution.[12-14] Notably, preclinical data suggests that aspirin (ASA) can modulate many of the platelet-mediated processes involved in ALI development [11, 15, 16] and resolution.[17, 18] Proposed mechanisms for these protective effects include reduced thromboxane A_2,[9] P-selectin,[19] and platelet-derived chemokine (e.g. CCL5, CXCL4) [20] production, prevention of the formation of platelet-neutrophil aggregates[9] and neutrophil extracellular traps,[21, 22] and enhanced formation of anti-inflammatory lipid mediators such as

15-epi-lipoxin A4 (Figure 1).[17] Importantly, recent observational studies have also suggested a potential preventive role for antiplatelet therapy in patients at high risk for ALI.[23, 24] However, the evidence remains inconclusive and equipoise remains.

To further enhance our understanding of ASA's role in the prevention and/or mitigation of ALI, the Lung Injury Prevention Study (LIPS) group with the support of the United States Critical Illness and Injury Trials Group (USCIITG) as well as the National Heart, Lung and Blood Institute (NHLBI) have designed the Lung Injury Prevention Study with Aspirin (LIPS-A), a randomized clinical trial that aims to test the safety and efficacy of ASA in the prevention of ALI in patients determined to be at high risk. This paper describes the study procedures and planned analyses for this clinical trial.

METHODS AND ANALYSIS

Administrative Structure

To facilitate the conduct of the present investigation, as well as future ALI prevention studies, three specialized centers were established. The data and statistical coordinating center, responsible for data management, randomization, and pharmacy coordination, will reside at Mayo Clinic in Rochester, MN. The clinical coordinating center (CCC), responsible for the study conduct and safety monitoring, will reside at Beth Israel Deaconess Medical Center in Boston, MA. The biospecimen repository and Knowledge Translation Center, responsible for specimen management as well as the LIPS score and the checklist for lung injury prevention (CLIP) online screening tools, will reside at Montefiore Medical Center in Bronx, NY. The principal investigators from these three centers form the LIPS-A Executive Committee. This committee will collaboratively oversee all aspects of the study design and the protocol implementation.

Study Design

To test the hypothesis that ASA is associated with a reduced rate of incident ALI, the LIPS-A group has designed a multicenter, double-blind, placebo-controlled, parallel group, phase II randomized clinical trial. The ClinicalTrials.gov registration number is NCT01504867. An outline of the study design and study procedures appears in Figure 2.

Study Population

Adult patients aged 18 years and older at high risk for ALI on admission to the hospital will be enrolled. To facilitate the identification of those at high risk for ALI, the LIPS-A study will utilize the recently validated LIPS.[8] Patients will be considered at high risk for development of ALI based on a LIPS score of 4 or greater. Patients who fulfill criteria for ALI on hospital presentation or at any point prior to randomization will be excluded. A full list of exclusion criteria with the justification for each can be seen in Table 1.

Patients will be recruited from 14 clinical sites in the United States with experience in the identification and management of ALI. A full list of the participating institutions as well as each site's primary investigator can be seen in Appendix A and are indexed on ClinicalTrials.gov. The resulting study population is expected to be diverse and representative of the general population of patients at risk for ALI such that the study findings will be externally valid and generalizable to the broader academic community.

To facilitate patient enrollment, study coordinators at each participating institution will screen patients in the ED with a web-based LIPS calculator to determine each potential participant's risk for development of ALI. Eligible patients with a LIPS score ≥ 4 will be approached by study coordinators or study investigators for informed consent. Eligible patients will be enrolled and randomized within 12 hours of hospital presentation. This will allow for

maximal recruitment within the window of opportunity for interventions to prevent ALI development as our preliminary data show median time to ALI is two days after hospital admission.[8]

Interventions

Study drug: The first dose of study drug (ASA versus placebo) will be administered within the first 24 hours after presentation to the hospital, either by mouth or by nasogastric or orogastric tube. For patients randomized to the intervention arm, a generic aspirin 325 mg one-time loading dose on day 1 will be administered followed by generic aspirin 81 mg by mouth once daily for study days 2-7 or until hospital discharge or death, whichever occurs first. The intervention duration of seven days was chosen because > 85% of ALI/ARDS cases were noted to have developed during this time frame in our preliminary studies.[8] In support of the dosing scheme chosen for this investigation, a randomized clinical trial noted low-dose ASA at 81 mg daily was effective in elevating plasma levels of anti-inflammatory lipoxins and inhibiting platelet thromboxane activity with only a slight increase in effect at higher doses of ASA.[25, 26] All study medication doses (active treatment with ASA and placebo) will be in powder form of identical color, contained within capsules that can be opened and administered via a gastric tube.

Co-interventions: Important co-interventions will be standardized in all study patients. To this end, the investigative team has developed a web-based, computerized, interactive tool to standardize essential elements of care delivery such as mechanical ventilation, aspiration precautions, infection control, fluid management and transfusion in patients at risk. This tool is a checklist for lung injury prevention (CLIP).[2] A summary of the CLIP elements is listed in Table 2. Having identified high-risk patients early in the course of the illness with the LIPS calculation and having standardized the important elements of care delivery with the CLIP, we

expect to have optimized our ability to investigate whether ASA is a safe and effective agent in preventing ALI.

Related conditions and variables of interest: Additional conditions and variables of interest including pertinent baseline demographics and clinical characteristics such as age, sex, race, comorbidities, and all LIPS elements will also be recorded. Additional variables of note will include vital signs and laboratory values that are obtained during the course of routine care, APACHE IV scores, coadministration of statins, angiotensin converting enzyme-inhibitors and angiotensin-receptor blocking agents, insulin, amiodarone, or steroids; blood product administration, daily fluid status and vasopressor requirements. A full description of the schedule of events for this study protocol can be seen in Table 3.

Outcomes

Clinical Outcomes: The primary outcome is the development of ALI within seven days of hospital admission. ALI will be defined as requirement for invasive mechanical ventilation and fulfillment of the American-European consensus definition for ALI/ARDS.[27] Patients will be screened daily for respiratory failure and the partial pressure of arterial oxygen (PaO_2) to fraction of inspired oxygen (FiO_2) ratio will be calculated daily for those on mechanical ventilation. Patients ventilated with non-invasive ventilation will not be considered ALI/ARDS as our preliminary data showed that the majority (90%) of ALI patients are eventually intubated.[8] Investigators at each site will review structured online training for assessment of ALI as was used and described in the LIPS.[8] In addition, de-identified chest x-rays of the first five patients enrolled at each site will be sent to CCC for validation by the primary investigators. Any site with significant deviation will be re-trained. Each participating center's principal investigator will adjudicate the diagnosis of ALI/ARDS using standardized definitions. Patients

receiving invasive mechanical ventilation who, within a given 24-hour period, fulfill criteria for $PaO_2/FiO_2 < 300$ mm Hg, bilateral infiltrates consistent with ALI and not completely explained by heart failure, will be determined to have developed ALI. Given prior data suggesting poor agreement in the radiological interpretation of bilateral infiltrates on chest radiographs consistent with ALI,[28] a secondary review of all ALI cases and a random sample of non-ALI cases will be performed by an independent expert investigator who is blinded to the initial ALI/ARDS adjudication. Study participants who die or are discharged from the hospital prior to day 7, and had not met criteria for ALI at the time of death or discharge, will be adjudicated as not having developed ALI.

Secondary clinical outcome assessments will include changes in the lung injury score and sequential organ failure assessment score, as well as the number of ventilator-free days at hospital day 28 and intensive care unit (ICU) and hospital lengths of stay. Mortality will be assessed at discharge from the ICU, from the hospital, and at 28 days. In addition, hospital survivors will undergo a brief follow-up phone survey to assess functional status (Barthel Index), health related quality of life [QOL (SF-12)] and frailty (VES-13) at 6- and 12-months after enrollment.

Mechanistic Outcomes: Secondary analyses will include evaluations of the mechanisms by which anti-platelet agents (e.g., ASA) may modulate the development and progression of lung injury as well as a determination of the value of plasma biomarkers of lung injury in the prediction of ALI development in patients at risk (beyond clinical variables). The study will examine biomarkers previously found to be associated with the *development* of ALI/ARDS in at-risk individuals (Table 4). In addition, to better understand the mechanisms by which ASA may affect the development and progression of ALI, the study will also examine the effect of ASA on

ASA-triggered lipoxins, plasma thromboxane, and platelet-neutrophil aggregates. As it is likely that other important biomarkers in ALI may be identified in the future, plasma from consenting patients will be banked at the biorepository for future studies. Blood samples will be obtained at baseline (after randomization and before initiation of study intervention), on day two of study (approximately 24 hours after the first dose of study drug), and on day four of study (any time during day 4). For patients who provide consent relating to future genetic analyses, appropriate samples will be obtained.

Sample Size Estimation

The primary hypothesis for this investigation is that ASA (when compared to placebo) will result in a lower rate of incident ALI at day 7 following randomization. To adequately address this hypothesis, the sample size is estimated to be 200 participants per group (400 total). The assumptions involved in this calculation include the following: 1) the hypothesized placebo response rate will be 18%,[8] 2) the minimum clinically relevant effect is 10 percentage points, and 3) the type I error rate (alpha) = 0.10 (two-sided) (final alpha=0.0889 after interim analysis at 50% information fraction using O'Brien-Fleming-like alpha spending function). To be conservative during sample size estimation, the null proportion was shifted upwards to 25% (i.e., towards the region of maximum binomial variance) so that the initial sample size estimates are based on 25% vs. 15%. A chi-square test of proportions at the alpha = 0.10 level of significance will have 80% power to detect the 10 percentage point difference with 197 participants per group. Overall recruitment is rounded to 200 participants per group (400 total) to allow for minor attrition, although attrition is not expected to affect the ascertainment of primary outcome. At the hypothesized level of 18% vs. 8% and with the alpha adjusted for multiple interim looks, power with 200 participants per group is 90%. Thus, for the primary analysis 400 total

participants randomized 1:1 to placebo or ASA is anticipated to yield sufficient power to detect a clinically relevant difference in the incidence of ALI.

Randomization and Blinding

Eligible participants will be randomized in a 1:1 ratio to the ASA or placebo treatment arm using dynamic minimization[29] with a second guess probability of 0.2. Randomization will be stratified by center, and the research pharmacist at each center will have electronic access to the unblinded treatment code for study medication preparation and dispensing. The rest of the site investigators and coordinating centers will be blinded to the actual treatment assignment Emergency unblinding is available both electronically and through dispensing records at each pharmacy.

Statistical Methods

Conditional logistic regression will be used to test the primary hypothesis that early ASA administration will decrease the rate of ALI development. Clinical site will be treated as the stratification variable and conditioned out of the estimating equations. This approach is optimal in a clinical trial setting as it provides a test of null hypothesis that the ALI incidence is equal in the two treatment group and estimates the association in the event the null hypothesis is rejected (through the conditional odds ratio estimate). SAS PROC LOGISTIC™ (Cary, NC) will be used for estimation of the primary model.

This analysis will be supplemented by the Cochran-Mantel-Haenszel stratified analysis with odds ratios computed for each site. The Breslow-Day test will be used to examine the data for potential effect modification (i.e., a "site effect"). In the event there is significant site-to-site variability in the estimated effect, stratified results will be reported for this phase II study.

Evidence of heterogeneity of response at this phase of the study will yield invaluable preliminary data for the planning of future changes.

Planned interim analyses will be conducted at 50% of study participants enrolled. With the O'Brien-Fleming-like stopping boundaries, a final adjusted alpha of 0.08885 is anticipated; however, the final value may be changed depending on unplanned interim analyses (conducted at the request of the Data Safety Monitoring Board [DSMB]) or slight deviations from the anticipated information milestones (0.50, 1.0). Stopping boundaries will be estimated using the LD Bounds package for the R system.

For the remaining continuous and dichotomous secondary endpoints, treatment group comparisons will be performed with respect to clinical outcomes as well as important prognostic factors at screening, baseline, and individual follow-up time points during the study duration. For continuous variables (e.g., age, weight, and laboratory assays), linear model techniques including *t*-tests, analysis of variance and analysis of covariance will be applied. Nonparametric procedures (e.g., the Wilcoxon rank sum test), will be used if data are not normally distributed and transformations of the data are not considered useful. Standard techniques for categorical data will be applied, including Fisher's exact test, Pearson χ^2 procedures, weighted least squares, and logistic regression analysis.

Longitudinal (or serially measured) endpoints will be evaluated by generalized linear models and linear mixed. Repeated measure analyses of binary endpoints will be analyzed using generalized estimating equations methods which do not require imputation of missing values, provided the data are ignorable missing.[30] Continuous dependent variables will utilize the mixed model approach with emphasis on evaluating the trajectories of values over time. However, early improvement in these parameters may suggest a supportive, stabilizing role for

ASA as a treatment option in patients at high risk of ALI. For the primary analysis, the clinical center will be treated as a "nuisance" parameter and conditioned out of the estimation routine. For secondary analyses, the clinical center will be used as a fixed covariate to account for differences across sites.

The safety endpoints (see below under "**Adverse Outcomes**") will be examined for all participants in the safety evaluable analysis set. Safety endpoints will include expected clinical events, including death, for this patient population and summarized by treatment group. Also, all serious and unexpected adverse events will be summarized by treatment group. Fisher's exact test will be used to estimate treatment differences in the incidence of each specified adverse event. No adjustments will be made for multiple hypothesis evaluations of safety endpoints. Adverse events will be summarized with groupings by body system. Other safety data (e.g., labs and assay data) will be listed, and when appropriate, summarized in tabular or graphical format.

Data Quality and Management

This investigation will utilize the Medidata Rave™ system for data management and storage as well as to assist with the randomization procedures. This product has been designed to facilitate multicenter clinical trials conducted under 21 CFR Part 11 requirements. This secure, web-based system provides robust data validation routines, custom reporting and straightforward integration with statistical software packages such as SAS (utilized for this investigation). The system is coupled with an integrated randomization module that uses a multidimensional dynamic allocation algorithm to minimize imbalances across multiple dimensions including overall study, sites, factors and cross-factor strata. Specific details regarding the randomization process are given below.

ETHICS AND DISSEMINATION

Adverse Outcomes

Safety data including adverse events such as gastrointestinal ulcers, bleeding from any site, gastrointestinal discomfort, wheezing, rash, hives, angioedema, tinnitus, and mortality will be recorded. Adverse events will be defined as "unexpected," "expected," and "serious." As our patient population is by definition "critically ill," it is expected that they will have a number of unrelated adverse health events during the course of their hospital stay. Therefore, we will limit the scope of our adverse event monitoring and recording to the following:

1) Serious adverse events (SAEs) will be defined as:

- Death, *believed to be related to the study medication or procedures, or a death that is unexpected considering the acuity of a patient.*
- A life threatening experience *believed to be related to the study medication or procedures*
- Persistent or significant disability or incapacity *that is of greater frequency or severity than what would be normally expected in the course of critical illness.*
- An event that jeopardizes the human subject and may require medical or surgical treatment to prevent one of the preceding outcomes *and is not expected in the course of critical illness.*

2) Adverse events possibly related to aspirin administration will be defined as:

- Anaphylaxis / allergic reaction
- Gastrointestinal bleed / bleeding complications
- Transfusion requirements for suspected bleeding

- Acute kidney injury, defined as RIFLE stage "I" or greater
- Tinnitus
- Reye's syndrome

Role of the Data Safety and Monitoring Board

Reporting of SAEs will be conducted through the CCC. All centers will report SAEs within 24 hours of discovering the presence of the SAE. The CCC will report all potentially related SAEs to the DSMB and to NHLBI within 7 days of discovery. A summary report of the events will be provided to the DSMB prior to each DSMB meeting, at least every six months. Safety oversight will be under the direction of a DSMB whose members will be independent from the study operations. The safety endpoints will be examined for all eligible patients who sign informed consent and are enrolled in the study on an intent-to-treat basis. Safety endpoints will include expected clinical events, including death, for this patient population and summarized by treatment group. All serious and unexpected adverse events will be summarized by treatment group as well.

Considerations for Continuation to a Phase III Clinical Trial

The decision to proceed with a phase III trial is formally outlined as follows:

1) Initiate Phase III Study: Demonstrated efficacy signal in addition to adequate safety profile. Criteria: Early termination for benefit at interim analysis or $p < 0.08885$ at final analysis (alpha=0.10 for study). Serious adverse event profile of ASA not statistically worse than placebo (95% confidence interval for the relative risk of any SAE covers the null value of RR=1.0).

2) Further Development Potentially Required: Weak efficacy signal. Criteria: Primary endpoint did not achieve *a priori* level of significance but there were at least a general consistency of

secondary endpoints indicating propensity for efficacy with a larger sample size and/or more specific primary endpoint.

3) Abandon Treatment Platform: Harm (in efficacy or safety endpoints). Criteria: Study terminated early per recommendation by DSMB for safety and/or risk/benefit ratio concerns (i.e., stop for futility, harm, unacceptable risk profile, etc.).

Ancillary Studies

The LIPS-A group will encourage investigator-initiated ancillary study proposals that extend or complement the specific aims of the primary LIPS-A trial. As policy, all proposals will be reviewed by a separate Ancillary Studies and Publications Committee, both to ensure consistency with the goals and conduct of the main study and evaluate scientific merit and validity. Proposed studies may utilize data and/or samples already accrued during the LIPS-A trial or, when feasible, request additional data collection from participating sites. The investigative and statistical plan will be reviewed *a priori*, with committee approval required before analysis begins. Where equivocal, review decisions will be referred to the LIPS-A Executive Committee. All reports, manuscripts or presentations derived from data obtained through the ancillary study process will require review and approval by the Ancillary Studies and Publications Committee prior to submission.

DISCUSSION

We have presented the study protocol and data analysis plans for the first phase II, multicenter randomized clinical trial that will test the efficacy and safety of a promising ALI prevention agent. Specifically, we have hypothesized that early administration of ASA to hospitalized patients who are at high risk for ALI, will be safe and will reduce the likelihood of progression to the full ALI phenotype. Secondarily, this investigation will glean important

mechanistic data on ASA's impact on the pathways believed important in ALI pathogenesis as well as the potential value of relevant biomarkers in the prediction of subsequent development of ALI. Finally, the results of this study will provide essential information on both the scientific merit and feasibility of a larger, phase III trial testing the role of ASA in the prevention of lung injury.

The persistent difficulty in translating promising pre-clinical therapies into the clinical setting has fostered interest in the potential development of effective ALI prevention strategies. Indeed, prevention of ALI has been identified as a key strategic priority for invested parties such as the NHLBI.[31] However, implementation of protocols aiming to test potential ALI prevention strategies have been historically hindered by an inability to accurately predict who is at risk for ALI. Moreover, the typically short interval between risk exposure and development of ALI as well as the small proportion of patients who progress to the full ALI phenotype following an ALI-related exposure has limited the feasibility of ALI prevention studies. In addition, the historic lack of standardization for numerous important co-interventions that confound the associations of interest (e.g., ventilator management, transfusion and resuscitation practices) has also limited our ability to test preventative strategies.

To this end, the recently validated LIPS score is a key element of the herein described study protocol.[8] Specifically, the LIPS score is expected to facilitate the identification of patients at greatest risk of progressing to ALI (a LIPS score ≥ 4 is expected to identify a subgroup of patients who have a risk of progressing to ALI that is greater than 18%). In addition, it is notable that this ALI risk assessment tool was validated using data collected within the first 6 hours after the initial evaluation in the ED. In an ALI prevention protocol such as

described herein, where the time to randomization is limited to 12 hours from presentation to the ED, the ability to accurately determine risk for ALI in such a time-efficient manner is critical.

A second notable strength of the current protocol is expected to be the implementation of the CLIP for standardizing important co-interventions that may otherwise confound our association of interest (ASA and ALI). During the period between hospital admission and the development of ALI, health care delivery factors (timely treatment of infection and shock, appropriate administration of fluid and transfusion therapies, prevention of aspiration, avoidance of large tidal volume ventilation), may be as important as individual biology in determining ALI development and outcome.[32-39] Moreover, a recent survey noted wide variation in clinical practices such as the existence of a sepsis protocol, use of low tidal volume ventilation, positive end-expiratory pressure, and restrictive transfusion practices, between hospitals and among the ED, ICU and operating room within hospitals.[40] Thus, to effectively investigate preventive strategies in ALI, the standardization of care delivery during the early phase of hospitalization would appear critical. Indeed, the ARDSNet investigators have repeatedly shown the value of standardization of clinical processes for ALI patients in clinical trials, allowing for determination of incremental benefit of new interventions.[41, 42] In the current investigation, standardization of care with best practices will help to reduce variability in the rates of ALI and the intensity of lung injury (noise) due to inconsistencies in care delivery. The result is expected to be an increased chance of seeing a beneficial clinical or biological effect from ASA and a better assessment of the potential side effects of ASA in this population.

Though the multicenter randomized clinical trial design, availability of a time-efficient risk assessment tool (LIPS score) and the standardization of important co-interventions with CLIP, as well as the robust study support and quality control offered through Metadata RAVE,

are clear strengths of the current study protocol, several important limitations with the planned investigation deserve note. Lung injury may be present at study entry even as clinical criteria for ALI are not fulfilled. Though a formal diagnosis of prevalent ALI is exclusionary, the molecular machinery will have been clearly set in motion in many of the study participants. Therefore, the study may be more accurately characterized as a prevention/early treatment trial rather than a pure prevention trial. Nonetheless, we have attempted to focus on the early period of ALI development by mandating a short interval from hospital presentation to randomization (12 hours) and a similarly short interval from hospital presentation to administration of the first study dose (24 hours). In addition, the study will exclude patients who presented to an outside hospital ED more than 12 hours before arrival at the enrolling site's facility. The study will also exclude those with ALI on hospital presentation or prior to randomization as well as those who are receiving mechanical ventilation through a tracheostomy tube prior to the current hospital admission (patient who is ventilator dependent) or those with a history of interstitial lung disease with chronic pulmonary infiltrates that may mimic ALI.

A second limitation relates to the intervention of ASA administration. Specifically, it is now well documented that more than 10% of the population will have a variable response to ASA or at least some form of aspirin resistance.[17] These patients may not benefit from ASA, even if ASA can modulate the development of lung injury. However, as part of this study, we will measure plasma thromboxane, a sensitive indicator of ASA resistance, to determine the prevalence of ASA resistance in patients at high risk for ALI. As such, sensitivity analyses, stratifying study participants by ASA resistance (as determined by changes in thromboxane levels), may allow us to determine whether the effect of ASA on ALI development is isolated to those susceptible to the actions of ASA. A related concern is the potential influence of

concomitant medications that may impact aspirin's ability to prevent or mitigate ALI (e.g., statins, corticosteroids). To address this concern, we will be collecting detailed information on concomitant medications and, when necessary, appropriate statistical adjustments will be made.

A third potential limitation with this study relates to a previously recognized major barrier to ALI prevention studies, namely feasibility. First and foremost, a substantial proportion of the target population may be expected to be receiving ASA on presentation to the ED, an exclusion criteria for the current protocol. Notably, however, our preliminary work suggests that upwards of two-thirds of the target population was not on ASA prior to admission. We also note that over the three months of the initial LIPS,[8] there were 800 patients who fulfilled study inclusion criteria of LIPS score ≥ 4 and did not fulfill the exclusion criteria of pre-existing ASA use, prevalent ALI, and elective surgery. Therefore, we believe that with 14 proposed sites and two years of planned enrollment, we will successfully meet our enrollment goals of 400 total patients. Also relating to feasibility, it is possible that some sites will be challenged by the short time interval allowed for patient enrollment as well as the short time to study drug administration. Though a valid concern, we believe the use of the LIPS score and the robust support offered through Metadata™ RAVE will greatly facilitate the enrollment and randomization procedures such that sites will indeed be successful in meeting these time-sensitive challenges.

A fourth and final limitation which deserves mention relates to the potential toxicity of the intervention of interest. Generally, ASA is well tolerated even in acutely ill, hospitalized patients in whom ASA is often continued during the hospitalization. As an example, in a study of ASA use up to the time of cardiac surgery, its continuation was not associated with an increase need for transfusion therapies.[43] Nevertheless, there may be injury associated with

the administration of aspirin. To address this concern, patients at risk for major complications from ASA therapy have been excluded from the study. Multiple stopping criteria for patients who experience adverse events have also been incorporated into the protocol. In addition, the more complete understanding of the safety profile of an intervention of interest is an important goal of all phase II trials. In this regard, the information gleaned from this study, adverse events included, is necessary to help decide on the merits of proceeding to a phase III clinical trial.

CONCLUSION

This manuscript describes the study protocol and analysis plans for the first phase II randomized clinical trial of the promising ALI prevention agent ASA. In addition to providing important information on the safety and efficacy of ASA in patients at high risk for ALI, the results of this trial will also inform the scientific community regarding the merit and feasibility of a more definitive phase III clinical trial. Importantly, the significance of this effort lies not only in the specific results which will be obtained from the study protocol, but equally in the infrastructure that will be created to facilitate the conduct of this trial. Specifically, the development and utilization of innovative methods to facilitate the early identification of high-risk patients with the LIPS and the standardization of potential confounding co-interventions with CLIP will address key barriers to studying ALI prevention measures and is expected to lay a framework for the meaningful conduct of future ALI prevention studies as well.

Table 1. Study Exclusion Criteria

Exclusion Criteria	Justification
Anti-platelet therapy on admission or within 7 days prior to admission	Inability to ethically randomize
Presented to outside hospital emergency department > 12 hours before arrival at site's facility	Inability to enroll within time frame for possible benefit
Inability to obtain consent and randomize within 12 hours of hospital presentation	Inability to enroll within time frame for possible benefit
Admitted for elective or emergency surgery	Aspirin not found to benefit this group in preliminary studies
ALI on hospital presentation or prior to randomization	Inability to adequately assess outcome
Presentation believed to be due to pure heart failure and no other known risk factors for ALI	Inability to adequately assess outcome
Receiving mechanical ventilation through a tracheostomy tube prior to current hospital admission (patient who is ventilator dependent)	Inability to adequately assess outcome
Bilateral pulmonary infiltrates present on admission only if the patient has a history of interstitial lung disease that can reasonably explain the current degree of pulmonary infiltrates present	Inability to adequately assess outcome
Allergy to aspirin or NSAIDs	Intervention contraindicated
Bleeding disorder*	Intervention contraindicated
Suspected active bleeding or judged to be at high risk for bleeding complications	Intervention contraindicated
Presence of acute kidney injury#	Intervention contraindicated
Severe chronic liver disease (Child-Pugh class C)	Intervention contraindicated

Active peptic ulcer disease (within past 6 months)	Intervention contraindicated
Pregnancy or breast feeding	Intervention contraindicated
Inability to administer study drug	Unable to administer study drug
Expected hospital stay < 48 hours	Incomplete study procedures and outcome data
Admitted for comfort or hospice care	Incomplete study procedures and outcome data
Patient, surrogate or physician not committed to full support (exception: a patient will not be excluded if he/she would receive all supportive care except for attempts at resuscitation from cardiac arrest)	Unable to assess primary outcome
Not anticipated to survive > 48 hours	Incomplete study procedures and outcome data
Previously enrolled in this trial	Violates the statistical assumption of sample independence
Enrollment in concomitant intervention study	Potential confounding and co-enrollment interactions

*Any disorder with known associated with increased risk of bleeding. Common disorders may include thrombocytopenia, disseminated intravascular coagulation, hemophilia, von Willebrand disease, oral anticoagulant therapy, or advanced liver disease with associated coagulation disorders. Platelet count < 50,000 or absence of platelet count in the previous 24 hours to allow for assessment of platelet status.

[#]Acute kidney injury defined as "R" or greater according to RIFLE criteria. ALI = acute lung injury, NSAIDs = non-steroidal anti-inflammatory medications

Table 2. Elements of CLIP – Checklist for Lung Injury Prevention

CLIP Elements	Definition
Lung protective mechanical ventilation	Tidal volume between 6-8 mL/kg predicted body weight and plateau pressure < 30 cm H_2O; PEEP ≥ 5 cm H_2O, minimize FiO_2 (target oxygen saturation 88-92% after early shock)
Aspiration precautions	Rapid sequence intubation supervised by experienced providers, elevated head of the bed, oral care with chlorhexidine, gastric acid neutralization in those not receiving tube feeds.
Adequate empiric antimicrobial treatment and source control	According to suspected site of infection, health care exposure, and immune suppression
Limiting fluid overload	Modified ARDSNet FACTT protocol after early shock (first 12 hours)
Restrictive transfusion	Hemoglobin target > 7 g/dL in the absence of acute bleeding and/or ischemia
Appropriate handoff of patients at risk	Providers taking care of patients at risk who require ICU admission will complete a structured handoff to the ICU team to continue with CLIP protocol for the duration of ICU stay

CLIP = checklist for lung injury prevention, PEEP = positive end-expiratory pressure, FiO_2 = fraction of inspired oxygen concentration, ARDSNet = Acute Respiratory Distress Syndrome Network, FACTT = fluid and catheter treatment trial, ICU = intensive care unit

Table 3: Schedule of Events

Event	Time of presentation until first dose (screen / baseline)	First dose until end of that calendar day (Day 1)	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	7 days after last dose	Hospital discharge or study Day 28, whichever comes first	6 Months	12 Months
Informed consent	X											
Inclusion/exclusion criteria	X											
Pregnancy test in women of childbearing potential	X											
Demographics	X											
Medical history	X											
LIPS score	X											
Randomization	X											
Study drug administration		X	X	X	X	X	X	X				
Clinical outcome assessment	X	X	X	X	X	X	X	X				
Safety labs: Cr and Hb	x		X	X	X	X	X	X				
Clinical data as available: labs, ABG	X	X	X	X	X	X	X	X				
CXR / ABG*		X	X	X	X	X	X	X				
CLIP	X	X	X	X	X	X	X	X				
AE/SAE monitoring		X	X	X	X	X	X	X	X	X		

Survival										X		X
Plasma biomarkers of ALI	X		X		X							
SF-12	X										X	X
Barthel Index	X										X	X
Vulnerable Elders Survey	X									X	X	
Brussels / SOFA composite										X		

*Chest x-ray required on days 1-7 ONLY IF patient is intubated, and DOES NOT have ALI / ARDS already, AND there is clinical evidence of worsening respiratory status defined as:

- Previous P/F ratio $\geq$ 300, with current P/F ratio < 300 and no chest x-ray within 24 hours.
- Prior P/F ratio < 300 and the PF ratio has fallen more than 10% AND no chest x-ray within 24 hours.
- In cases where an ABG is not available, the research team should obtain an ABG ***only if*** the S/F ratio falls below 315 consistently. The P/F ratio obtained from that ABG will be used to determine whether a chest x-ray needs to be obtained (as per criteria outlined above).
- If change in P/F ratio triggers the need for a chest x-ray or ABG as above, sites have 24 hours to conduct the necessary procedure. An ABG or chest x-ray obtained by the clinical team during that time period is also acceptable and obviates the need to obtain said procedure for the research study.

LIPS = Lung injury prevention, ALI = acute lung injury, LIS = lung injury severity score, Cr = creatinine, Hb = hemoglobin, ABG = arterial blood gas, CLIP = checklist for lung injury prevention, AE = adverse events, SAE = serious adverse events, SF-12 = 12-Item Short-Form Health Survey, SOFA = sequential organ failure assessment.

Table 4: Plasma biomarkers in ALI/ARDS

Plasma Biomarker	Importance in ALI/ARDS Development	Associated outcomes other than ALI/ARDS
Surfactant protein-D[44-46]	Reflect injury and ↑ permeability of alveolar epithelium	VFD, organ failure
Receptor for advanced glycation end products[47-49]	Reflects endothelial activation and injury	VFD,[49] organ failure,[49] ARDS after lung transplant[47]
Intercellular adhesion molecule-1[44, 50-53]	Reflects endothelial activation and injury	VFD,[51] organ failure[51]
Interleukin-6[44, 54-56]	Inflammation	VFD,[55] organ failure[55]
Interleukin-8[44, 48, 50, 54-56]	Inflammation	VFD, [55]organ failure[55]
Plasminogen activator inhibitor-1[44, 50, 57-61]	Activation of coagulation and inhibition of fibrinolysis	VFD,[61] organ failure[61]
von Willebrand factor[44, 48, 60, 62, 63]	Reflects endothelial activation and injury	organ failure
Protein C[44, 50, 59, 61, 64]	Activation of coagulation and inhibition of fibrinolysis	ARDS after lung transplant,[47] VFD,[61] organ failure[61]

ALI = acute lung injury, ARDS = acute respiratory distress syndrome, VFD = ventilator-free days.

REFERENCES

1. Rubenfeld GD, Caldwell E, Peabody E, et al. Incidence and outcomes of acute lung injury. *N Engl J Med* 2005;**353**:1685-93.
2. Li G, Malinchoc M, Cartin-Ceba R, et al. Eight-year trend of acute respiratory distress syndrome: a population-based study in Olmsted County, Minnesota. *Am J Resp Crit Care Med* 2011;**183**:59-66.
3. Erickson SE, Martin GS, Davis JL, et al. Recent trends in acute lung injury mortality: 1996-2005. *Crit Care Med* 2009;**37**:1574-9.
4. Herridge MS, Tansey CM, Matté A, et al. Functional disability 5 years after acute respiratory distress syndrome. *N Engl J Med* 2011;**364**:1293-304.
5. Hudson LD, Milberg JA, Anardi D, et al. Clinical risks for development of the acute respiratory distress syndrome. *Am J Respir Crit Care Med* 1995;**151**:293-301.
6. Fowler AA, Hamman RF, Good JT, et al. Adult respiratory distress syndrome: risk with common predispositions. *Ann Intern Med* 1983;**98**:593-7.
7. Gong MN, Thompson BT, Williams P, et al. Clinical predictors of and mortality in acute respiratory distress syndrome: potential role of red cell transfusion. *Crit Care Med* 2005;**33**:1191-8.
8. Gajic O, Dabbagh O, Park PK, et al. Early identification of patients at risk of acute lung injury: evaluation of lung injury prediction score in a multicenter cohort study. *Am J Respir Crit Care Med* 2010;**183**:462-70.
9. Zarbock A, Ley K. The role of platelets in acute lung injury (ALI). Front Biosci 2009;**14**:150-8.

10. Zarbock A, Polanowska-Grabowska RK, Ley K. Platelet-neutrophil-interactions: linking hemostasis and inflammation. *Blood Rev* 2007;**21**:99-111.

11. Looney MR, Nguyen JX, Hu Y, et al. Platelet depletion and aspirin treatment protect mice in a two-event model of transfusion-related acute lung injury. *J Clin Invest* 2009;**119**:3450-61.

12. El Kebir D, József L, Pan W, et al. 15-epi-lipoxin A4 inhibits myeloperoxidase signaling and enhances resolution of acute lung injury. *Am J Respir Crit Care Med* 2009;**180**:311-9.

13. Fukunaga K, Kohli P, Bonnans C, et al. Cyclooxygenase 2 plays a pivotal role in the resolution of acute lung injury. *J Immunol* 2005;**174**:5033-9.

14. Maderna P, Godson C. Lipoxins: resolutionary road. *Br J Pharmacol* 2009;**158**:947-59.

15. Chelucci GL, Boncinelli S, Marsili M, et al. Aspirin effect on early and late changes in acute lung injury in sheep. *Intensive Care Med* 1993;**19**:13-21.

16. Sigurdsson GH, Vallgren S, Christenson JT. Influence of aspirin and steroids on acute lung injury after i.v. injection of a sclerosing agent. *Acta Chir Scand* 1989;**155**:163-70.

17. Yasuda O, Takemura Y, Kawamoto H, et al. Aspirin: recent developments. *Cell Mol Life Sci* 2008;**65**:354-8.

18. Jin SW, Zhang L, Lian QQ, et al. Posttreatment with aspirin-triggered lipoxin A4 analog attenuates lipopolysaccharide-induced acute lung injury in mice: the role of heme oxygenase-1. *Anesth Analg* 2007;**104**:369-77.

19. Tabuchi A, Kuebler WM. Endothelium-platelet interactions in inflammatory lung disease. *Vasc Pharmacol* 2008;**49**:141-50.

20. Grommes J, Alard J-E, Drechsler M, et al. Disruption of platelet-derived chemokine heteromers prevents neutrophil extravasation in acute lung injury. *Am J Resp Crit Care Med* 2012;**185**:628-36.

21. Clark SR, Ma AC, Tavener SA, et al. Platelet TLR4 activates neutrophil extracellular traps to ensnare bacteria in septic blood. *Nature Med* 2007;**13**:463-9.

22. Narasaraju T, Yang E, Samy RP, et al. Excessive neutrophils and neutrophil extracellular traps contribute to acute lung injury of influenza pneumonitis. *Am J Pathol* 2011;**179**:199-210.

23. Erlich JM, Talmor DS, Cartin-Ceba R, et al. Prehospitalization antiplatelet therapy is associated with a reduced incidence of acute lung injury. A population-based cohort study. *Chest* 2011;**139**:289-95.

24. Kor DJ, Erlich J, Gong MN, et al. Association of prehospitalization aspirin therapy and acute lung injury: results of a multicenter international observational study of at-risk patients. *Crit Care Med* 2011;**39**:2393-400.

25. Patrono C, Garcia Rodriguez LA, Landolfi R, et al. Low-dose aspirin for the prevention of atherothrombosis. *N Engl J Med* 2005;**353**:2373-83.

26. Chiang N, Bermudez EA, Ridker PM, et al. Aspirin triggers antiinflammatory 15-epi-lipoxin A4 and inhibits thromboxane in a randomized human trial. *Proc Natl Acad Sci USA* 2004;**101**:15178-83.

27. Bernard GR, Artigas A, Brigham KL, et al. The American-European Consensus Conference on ARDS. Definitions, mechanisms, relevant outcomes, and clinical trial coordination. *Am J Respir Crit Care Med* 1994;**149**:818-24.

28. Rubenfeld GD. Interobserver variability in applying a radiographic definition for ARDS. *Chest* 1999;**116**:1347-53.

29. Pocock SJ, Simon R. Sequential treatment assignment with balancing for prognostic factors in the controlled clinical trial. *Biometrics* 1975;**31**:103-15.

30. Diggle P, Liang K, Zeger S. Analysis of Longitudinal Data. New York: Oxford University Press, Inc.; 1994.

31. Spragg RG, Bernard GR, Checkley W, et al. Beyond mortality: future clinical research in acute lung injury. *Am J Respir Crit Care Med* 2010;**181**:1121-7.

32. Gajic O, Dara SI, Mendez JL, et al. Ventilator-associated lung injury in patients without acute lung injury at the onset of mechanical ventilation. *Crit Care Med* 2004;**32**:1817-24.

33. Rana R, Fernandez-Perez ER, Khan SA, et al. Transfusion-related acute lung injury and pulmonary edema in critically ill patients: a retrospective study. *Transfusion* 2006;**46**:1478-83.

34. Gajic O, Rana R, Winters JL, et al. Transfusion-related acute lung injury in the critically ill: prospective nested case-control study. *Am J Respir Crit Care Med* 2007;**176**:886-91.

35. Gajic O, Rana R, Mendez JL, et al. Acute lung injury after blood transfusion in mechanically ventilated patients. *Transfusion* 2004;**44**:1468-74.

36. Gajic O, Frutos-Vivar F, Esteban A, et al. Ventilator settings as a risk factor for acute respiratory distress syndrome in mechanically ventilated patients. *Intensive Care Med* 2005;**31**:922-6.

37. Gajic O, Dzik WH, Toy P. Fresh frozen plasma and platelet transfusion for nonbleeding patients in the intensive care unit: benefit or harm? *Crit Care Med* 2006;**34**:S170-3.

38. Fernandez-Perez ER, Keegan MT, Brown DR, et al. Intraoperative tidal volume as a risk factor for respiratory failure after pneumonectomy. *Anesthesiology* 2006;**105**:14-8.

39. Khan H, Belsher J, Yilmaz M, et al. Fresh-frozen plasma and platelet transfusions are associated with development of acute lung injury in critically ill medical patients. *Chest* 2007;**131**:1308-14.

40. Hou P, Cohen J, Elie-Turenne M, et al. A survey on behalf of USCIITG-LIPS investigators: toward standardization of a checklist for lung injury prevention (CLIP). *Crit Care Med 2010*;**38**:576.

41. Brower RG, Lanken PN, MacIntyre N, et al. Higher versus lower positive end-expiratory pressures in patients with the acute respiratory distress syndrome. *N Engl J Med* 2004;**351**:327-36.

42. Wiedemann HP, Wheeler AP, Bernard GR, et al. Comparison of two fluid-management strategies in acute lung injury. *N Engl J Med* 2006;**353**:2564-75.

43. Gerrah R, Elami A, Stamler A, et al. Preoperative aspirin administration improves oxygenation in patients undergoing coronary artery bypass grafting. Chest 2005;**127**:1622-6.

44. Ware LB, Koyama T, Billheimer DD, et al. Prognostic and pathogenetic value of combining clinical and biochemical indices in patients with acute lung injury. *Chest* 2010;**137**:288-96.

45. Eisner M, Parsons P, Matthay M, et al. Plasma surfactant protein levels and clinical outcomes in patients with acute lung injury. *Thorax* 2003;**58**:983-8.

46. Greene KE, Wright JR, Steinberg KP, et al. Serial changes in surfactant-associated proteins in lung and serum before and after onset of ARDS. *Am J Respir Crit Care Med* 1999;**160**:1843-50.

47. Christie JD, Shah CV, Kawut SM, et al. Plasma levels of receptor for advanced glycation end products, blood transfusion, and risk of primary graft dysfunction. *Am J Respir Crit Care Med* 2009;**180**:1010-5.

48. Fremont RD, Koyama T, Calfee CS, et al. Acute lung injury in patients with traumatic injuries: utility of a panel of biomarkers for diagnosis and pathogenesis. *J Trauma* 2010;**68**:1121-7.

49. Calfee CS, Ware LB, Eisner MD, et al. Plasma receptor for advanced glycation end products and clinical outcomes in acute lung injury. *Thorax* 2008;**63**:1083-9.

50. McClintock D, Zhuo H, Wickersham N, et al. Biomarkers of inflammation, coagulation and fibrinolysis predict mortality in acute lung injury. *Crit Care* 2008;**12**:R41.

51. Calfee CS, Eisner MD, Parsons PE, et al. Soluble intercellular adhesion molecule-1 and clinical outcomes in patients with acute lung injury. *Intensive Care Med* 2009;**35**:248-57.

52. Agouridakis P, Kyriakou D, Alexandrakis MG, et al. The predictive role of serum and bronchoalveolar lavage cytokines and adhesion molecules for acute respiratory distress syndrome development and outcome. *Respir Res* 2002;**3**:25.

53. Covarrubias M, Ware LB, Kawut SM, et al. Plasma intercellular adhesion molecule-1 and von Willebrand factor in primary graft dysfunction after lung transplantation. *Am J Transplant* 2007;**7**:2573-8.

54. Bouros D, Alexandrakis MG, Antoniou KM, et al. The clinical significance of serum and bronchoalveolar lavage inflammatory cytokines in patients at risk for acute respiratory distress syndrome. *BMC Pulm Med* 2004;**4**:6.

55. Parsons PE, Eisner MD, Thompson BT, et al. Lower tidal volume ventilation and plasma cytokine markers of inflammation in patients with acute lung injury. *Crit Care Med* 2005;**33**:1-6.

56. Meduri GU, Headley S, Kohler G, et al. Persistent elevation of inflammatory cytokines predicts a poor outcome in ARDS. Plasma IL-1 beta and IL-6 levels are consistent and efficient predictors of outcome over time. *Chest* 1995;**107**:1062-73.

57. Prabhakaran P, Ware LB, White KE, et al. Elevated levels of plasminogen activator inhibitor-1 in pulmonary edema fluid are associated with mortality in acute lung injury. *Am J Physiol Lung Cell Mol Physiol* 2003;**285**:L20-8.

58. Ware LB, Fang X, Matthay MA. Protein C and thrombomodulin in human acute lung injury. *Am J Physiol Lung Cell Mol Physiol* 2003;**285**:L514-21.

59. Christie JD, Robinson N, Ware LB, et al. Association of protein C and type 1 plasminogen activator inhibitor with primary graft dysfunction. *Am J Respir Crit Care Med* 2007;**175**:69-74.

60. Ware LB, Conner ER, Matthay MA. von Willebrand factor antigen is an independent marker of poor outcome in patients with early acute lung injury. *Crit Care Med* 2001;**29**:2325-31.

61. Ware LB, Matthay MA, Parsons PE, et al. Pathogenetic and prognostic significance of altered coagulation and fibrinolysis in acute lung injury/acute respiratory distress syndrome. *Crit Care Med* 2007;**35**:1821-8.

62. Rubin DB, Wiener-Kronish JP, Murray JF, et al. Elevated von Willebrand factor antigen is an early plasma predictor of acute lung injury in nonpulmonary sepsis syndrome. *J Clin Invest* 1990;**86**:474-80.

63. Ware LB, Eisner MD, Thompson BT, et al. Significance of von Willebrand factor in septic and nonseptic patients with acute lung injury. *Am J Respir Crit Care Med* 2004;**170**:766-72.

64. Matthay MA, Ware LB. Plasma protein C levels in patients with acute lung injury: prognostic significance. *Crit Care Med* 2004;**32**:S229-32.

Appendix A. Lung Injury Prevention Study with Aspirin (LIPS-A) Investigators

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Mayo Clinic – Rochester (Data Coordinating Center)	Wake Forest University Medical Center
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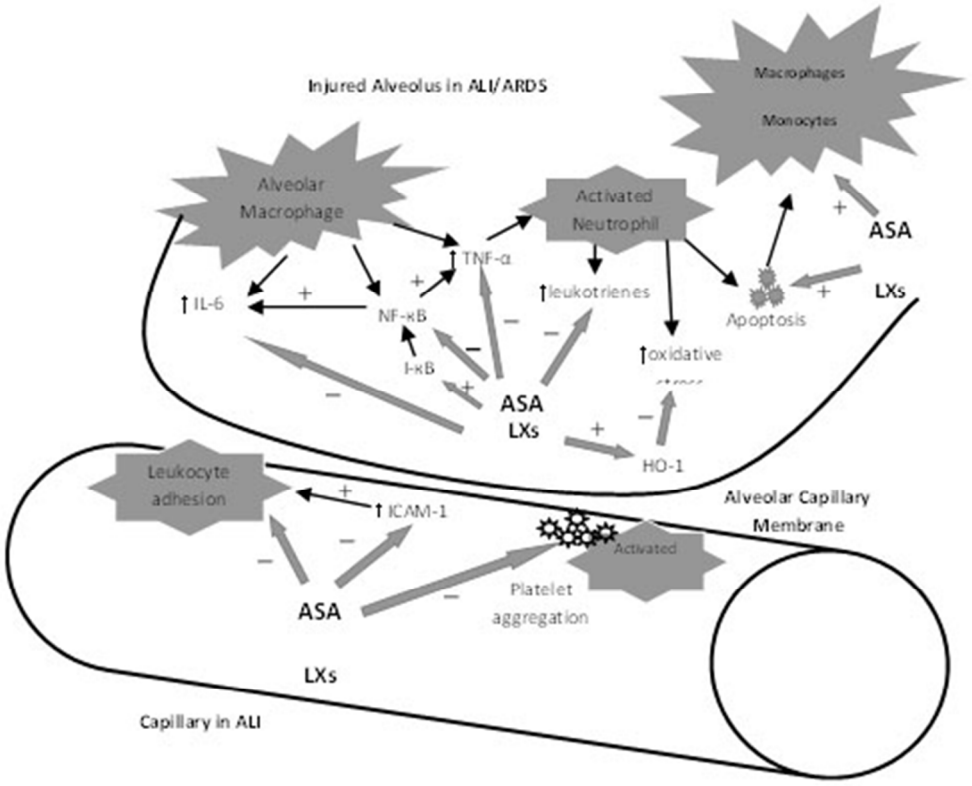

Illustration of the potential role of aspirin, lipoxins, and aspirin-triggered lipoxins on the mediators of ALI development and progression. Black arrows indicate events in ALI. Grey arrows indicate action of ASA, LTXs, or ATLs.

ALI = acute lung injury, ARDS = acute respiratory distress syndrome, ASA = aspirin, LX = lipoxins, ATLs = aspirin-triggered lipoxins, IL-6 = interleukin-6, TNF = tumor necrosis factor, NF-kB = nuclear factor kappa-light-chain-enhancer of activated B-cells, I-kB = nuclear factor kappa-light-chain-enhancer of activated B-cells inhibitor, HO = heme oxygenase, ICAM = intercellular adhesion molecule

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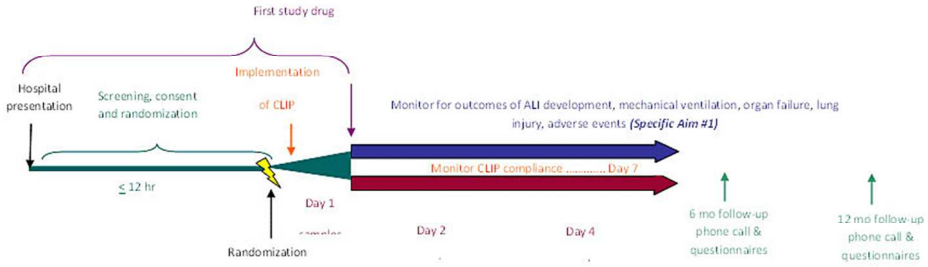

Schematic of the planned study procedures
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Lung Injury Prevention with Aspirin (LIPS-A): Protocol for a Multicenter Randomized Clinical Trial in Medical Patients at High Risk for Acute Lung Injury.

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LUNG INJURY PREVENTION WITH ASPIRIN (LIPS-A): A PROTOCOL ~~METHODOLOGY~~ FOR A MULTICENTER RANDOMIZED CLINICAL TRIAL IN MEDICAL PATIENTS AT HIGH RISK FOR ACUTE LUNG INJURY.

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On behalf of the US Critical Illness and Injury Trials Group: Lung Injury Prevention with Aspirin Study Group (USCIITG: LIPS-A).

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Keywords: acute lung injury, acute respiratory distress syndrome, aspirin, critical illness, prevention, clinical trial.

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ABSTRACT

Introduction: Acute Lung Injury (ALI) is a devastating condition that places a heavy burden on public health resources. Although the need for effective ALI prevention strategies is increasingly recognized, no effective preventative strategies exist. The Lung Injury Prevention Study with Aspirin (LIPS-A) aims to test whether aspirin (ASA) could prevent and/or mitigate the development of ALI.

Methods and Analysis: LIPS-A is a multicenter, double-blind, randomized clinical trial testing the hypothesis that the early administration of ASA will result in a reduced incidence of ALI in adult patients at high risk. This investigation will enroll 400 study participants from 14 hospitals across the US. Conditional logistic regression will be used to test the primary hypothesis that early ASA administration will decrease the incidence of ALI.

Ethics and Dissemination: Safety oversight will be under the direction of an independent data and safety monitoring board (DSMB). Approval of the protocol was obtained from the DSMB prior to enrolling the first study participant. Approval of both the protocol and informed consent documents were also obtained from the institutional review board of each participating institution prior to enrolling study participants at the respective site.

In addition to providing important clinical and mechanistic information, this investigation will inform the scientific merit and feasibility of a phase III trial on aspirin as an ALI prevention agent. The findings of this investigation, as well as associated ancillary studies, will be disseminated in the form of oral and abstract presentations at major national and international medical specialty meetings. The primary objective and other significant findings will also be presented in manuscript form. All final, published manuscripts resulting from this protocol will be submitted to Pub Med Central (PMC) in accordance with the National Institute of Health

Public Access Policy. This trial is registered with ClinicalTrials.gov: NCT01504867. **Introduction:** The acute respiratory distress syndrome (ARDS) and the less severe acute lung injury (ALI) are devastating conditions that place a heavy burden on public health resources. Although the need for effective ALI prevention strategies has been increasingly recognized, no effective preventative strategies presently exist. The Lung Injury Prevention Study with Aspirin (LIPS-A) aims to test whether aspirin (ASA) administration could prevent and/or mitigate the development of ALI in patients determined to be at high risk for this life-threatening complication.

Methods and Analysis: LIPS-A is a multicenter, double-blind, phase II randomized clinical trial which aims to test the hypothesis that the early administration of ASA will be associated with a reduced incidence of ALI during the first seven days following hospital admission of adult patients at high risk for ALI. It is anticipated that this investigation will enroll 400 total study participants from 14 hospitals across the United States. Conditional logistic regression will be used to test the primary hypothesis that early ASA administration will decrease the rate of ALI development. A planned interim analysis will be conducted at 50% of study participants enrolled.

Ethics and Dissemination: Safety oversight will be under the direction of a data and safety monitoring board whose members will be independent from the study operations. Safety endpoints will be examined for all eligible patients who sign informed consent and are enrolled in the study on an intent-to-treat basis. and

In addition to providing important clinical and mechanistic study results, the findings of this investigation will be informative on the scientific merit and feasibility of a phase III trial on the role of aspirin as an ALI prevention agent. The LIPS-A group will also encourage investigator-

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initiated ancillary study proposals that extend or complement the specific aims of the primary LIPS-A trial. The primary objective and other significant findings

INTRODUCTION

Acute lung injury (ALI) and the acute respiratory distress syndrome (ARDS) are life-threatening syndromes which continue to consume substantial health care resources and profoundly impact patient-important outcomes.[1] Although recent epidemiologic studies suggest the incidence of lung injury may be on the decline,[2] even conservative estimates suggest the associated mortality continues to exceed 25%.[3] Beyond mortality, an episode of ALI/ARDS also substantially influences patient's long-term outcomes with functional deficits persisting up to five years after the episode of respiratory failure.[4]

Importantly, the clinical syndrome of ALI generally occurs as a complication of an initial predisposing acute injury such as pneumonia, aspiration, sepsis, trauma, shock, or massive transfusion.[5] However, only a fraction of patients (10-30%) with these initial injuries develop ALI/ARDS.[6, 7] Only 30% of ALI patients fulfill criteria for ALI within six hours of presentation to the emergency department (ED).[8] The majority of patients develop ALI a median of two days after hospital presentation (IQR 1-4 days). This period of time between hospital presentation and development of ALI presents a window of opportunity for interventions to prevent the development of ALI.

Recently, accumulating evidence suggests an important role for platelets in both ALI pathogenesis [9-11] and resolution.[12-14] Notably, preclinical data suggests that aspirin (ASA) can modulate many of the platelet-mediated processes involved in ALI development [11, 15, 16] and resolution.[17, 18] Proposed mechanisms for these protective effects include reduced thromboxane A_2,[9] P-selectin,[19] and platelet-derived chemokine (e.g. CCL5, CXCL4) [20] production, prevention of the formation of platelet-neutrophil aggregates[9] and neutrophil extracellular traps,[21, 22] and enhanced formation of anti-inflammatory lipid mediators such as

15-epi-lipoxin A4 (Figure 1).[17] Importantly, recent observational studies have also suggested a potential preventive role for antiplatelet therapy in patients at high risk for ALI.[23, 24] However, the evidence remains inconclusive and equipoise remains.

To further enhance our understanding of ASA's role in the prevention and/or mitigation of ALI, the Lung Injury Prevention Study (LIPS) group with the support of the United States Critical Illness and Injury Trials Group (USCIITG) as well as the National Heart, Lung and Blood Institute (NHLBI) have designed the Lung Injury Prevention Study with Aspirin (LIPS-A), a randomized clinical trial that aims to test the safety and efficacy of ASA in the prevention of ALI in patients determined to be at high risk. This paper describes the study procedures and planned analyses for this clinical trial.

METHODS AND ANALYSIS

Administrative Structure

To facilitate the conduct of the present investigation, as well as future ALI prevention studies, three specialized centers were established. The data and statistical coordinating center, responsible for data management, randomization, and pharmacy coordination, will reside at Mayo Clinic in Rochester, MN. The clinical coordinating center (CCC), responsible for the study conduct and safety monitoring, will reside at Beth Israel Deaconess Medical Center in Boston, MA. The biospecimen repository and Knowledge Translation Center, responsible for specimen management as well as the LIPS score and the checklist for lung injury prevention (CLIP) online screening tools, will reside at Montefiore Medical Center in Bronx, NY. The principal investigators from these three centers form the LIPS-A Executive Committee. This committee will collaboratively oversee all aspects of the study design and the protocol implementation.

Study Design

To test the hypothesis that ASA is associated with a reduced rate of incident ALI, the LIPS-A group has designed a multicenter, double-blind, placebo-controlled, parallel group, phase II randomized clinical trial. The ClinicalTrials.gov registration number is NCT01504867. An outline of the study design and study procedures appears in Figure 2.

Study Population

Adult patients aged 18 years and older at high risk for ALI on admission to the hospital will be enrolled. To facilitate the identification of those at high risk for ALI, the LIPS-A study will utilize the recently validated LIPS.[8] Patients will be considered at high risk for development of ALI based on a LIPS score of 4 or greater. Patients who fulfill criteria for ALI on hospital presentation or at any point prior to randomization will be excluded. A full list of exclusion criteria with the justification for each can be seen in Table 1.

Patients will be recruited from 14 clinical sites in the United States with experience in the identification and management of ALI. A full list of the participating institutions as well as each site's primary investigator can be seen in Appendix A and are indexed on ClinicalTrials.gov. The resulting study population is expected to be diverse and representative of the general population of patients at risk for ALI such that the study findings will be externally valid and generalizable to the broader academic community.

To facilitate patient enrollment, study coordinators at each participating institution will screen patients in the ED with a web-based LIPS calculator to determine each potential participant's risk for development of ALI. Eligible patients with a LIPS score ≥ 4 will be approached by study coordinators or study investigators for informed consent. Eligible patients will be enrolled and randomized within 12 hours of hospital presentation. This will allow for

maximal recruitment within the window of opportunity for interventions to prevent ALI development as our preliminary data show median time to ALI is two days after hospital admission.[8]

Interventions

Study drug: The first dose of study drug (ASA versus placebo) will be administered within the first 24 hours after presentation to the hospital, either by mouth or by nasogastric or orogastric tube. For patients randomized to the intervention arm, a generic aspirin 325 mg one-time loading dose on day 1 will be administered followed by generic aspirin 81 mg by mouth once daily for study days 2-7 or until hospital discharge or death, whichever occurs first. The intervention duration of seven days was chosen because > 85% of ALI/ARDS cases were noted to have developed during this time frame in our preliminary studies.[8] In support of the dosing scheme chosen for this investigation, a randomized clinical trial noted low-dose ASA at 81 mg daily was effective in elevating plasma levels of anti-inflammatory lipoxins and inhibiting platelet thromboxane activity with only a slight increase in effect at higher doses of ASA.[25, 26] All study medication doses (active treatment with ASA and placebo) will be in powder form of identical color, contained within capsules that can be opened and administered via a gastric tube.

Co-interventions: Important co-interventions will be standardized in all study patients. To this end, the investigative team has developed a web-based, computerized, interactive tool to standardize essential elements of care delivery such as mechanical ventilation, aspiration precautions, infection control, fluid management and transfusion in patients at risk. This tool is a checklist for lung injury prevention (CLIP).[2] A summary of the CLIP elements is listed in Table 2. Having identified high-risk patients early in the course of the illness with the LIPS calculation and having standardized the important elements of care delivery with the CLIP, we

expect to have optimized our ability to investigate whether ASA is a safe and effective agent in preventing ALI.

Related conditions and variables of interest: Additional conditions and variables of interest including pertinent baseline demographics and clinical characteristics such as age, sex, race, comorbidities, and all LIPS elements will also be recorded. Additional variables of note will include vital signs and laboratory values that are obtained during the course of routine care, APACHE IV scores, coadministration of statins, angiotensin converting enzyme-inhibitors and angiotensin-receptor blocking agents, insulin, amiodarone, or steroids; blood product administration, daily fluid status and vasopressor requirements. A full description of the schedule of events for this study protocol can be seen in Table 3.

Outcomes

Clinical Outcomes: The primary outcome is the development of ALI within seven days of hospital admission. ALI will be defined as requirement for invasive mechanical ventilation and fulfillment of the American-European consensus definition for ALI/ARDS.[27] Patients will be screened daily for respiratory failure and the partial pressure of arterial oxygen (PaO_2) to fraction of inspired oxygen (FiO_2) ratio will be calculated daily for those on mechanical ventilation. Patients ventilated with non-invasive ventilation will not be considered ALI/ARDS as our preliminary data showed that the majority (90%) of ALI patients are eventually intubated.[8] Investigators at each site will review structured online training for assessment of ALI as was used and described in the LIPS.[8] In addition, de-identified chest x-rays of the first five patients enrolled at each site will be sent to CCC for validation by the primary investigators. Any site with significant deviation will be re-trained. Each participating center's principal investigator will adjudicate the diagnosis of ALI/ARDS using standardized definitions. Patients

receiving invasive mechanical ventilation who, within a given 24-hour period, fulfill criteria for PaO_2/FiO_2 < 300 mm Hg, bilateral infiltrates consistent with ALI and not completely explained by heart failure, will be determined to have developed ALI. Given prior data suggesting poor agreement in the radiological interpretation of bilateral infiltrates on chest radiographs consistent with ALI,[28] a secondary review of all ALI cases and a random sample of non-ALI cases will be performed by an independent expert investigator who is blinded to the initial ALI/ARDS adjudication. Study participants who die or are discharged from the hospital prior to day 7, and had not met criteria for ALI at the time of death or discharge, will be adjudicated as not having developed ALI.

Secondary clinical outcome assessments will include changes in the lung injury score and sequential organ failure assessment score, as well as the number of ventilator-free days at hospital day 28 and intensive care unit (ICU) and hospital lengths of stay. Mortality will be assessed at discharge from the ICU, from the hospital, and at 28 days. In addition, hospital survivors will undergo a brief follow-up phone survey to assess functional status (Barthel Index), health related quality of life [QOL (SF-12)] and frailty (VES-13) at 6- and 12-months after enrollment.

Mechanistic Outcomes: Secondary analyses will include evaluations of the mechanisms by which anti-platelet agents (e.g., ASA) may modulate the development and progression of lung injury as well as a determination of the value of plasma biomarkers of lung injury in the prediction of ALI development in patients at risk (beyond clinical variables). The study will examine biomarkers previously found to be associated with the ***development*** of ALI/ARDS in at-risk individuals (Table 4). In addition, to better understand the mechanisms by which ASA may affect the development and progression of ALI, the study will also examine the effect of ASA on

ASA-triggered lipoxins, plasma thromboxane, and platelet-neutrophil aggregates. As it is likely that other important biomarkers in ALI may be identified in the future, plasma from consenting patients will be banked at the biorepository for future studies. Blood samples will be obtained at baseline (after randomization and before initiation of study intervention), on day two of study (approximately 24 hours after the first dose of study drug), and on day four of study (any time during day 4). For patients who provide consent relating to future genetic analyses, appropriate samples will be obtained.

Sample Size Estimation

The primary hypothesis for this investigation is that ASA (when compared to placebo) will result in a lower rate of incident ALI at day 7 following randomization. To adequately address this hypothesis, the sample size is estimated to be 200 participants per group (400 total). The assumptions involved in this calculation include the following: 1) the hypothesized placebo response rate will be 18%,[8] 2) the minimum clinically relevant effect is 10 percentage points, and 3) the type I error rate (alpha) = 0.10 (two-sided) (final alpha=0.0889 after interim analysis at 50% information fraction using O'Brien-Fleming-like alpha spending function). To be conservative during sample size estimation, the null proportion was shifted upwards to 25% (i.e., towards the region of maximum binomial variance) so that the initial sample size estimates are based on 25% vs. 15%. A chi-square test of proportions at the alpha = 0.10 level of significance will have 80% power to detect the 10 percentage point difference with 197 participants per group. Overall recruitment is rounded to 200 participants per group (400 total) to allow for minor attrition, although attrition is not expected to affect the ascertainment of primary outcome. At the hypothesized level of 18% vs. 8% and with the alpha adjusted for multiple interim looks, power with 200 participants per group is 90%. Thus, for the primary analysis 400 total

participants randomized 1:1 to placebo or ASA is anticipated to yield sufficient power to detect a clinically relevant difference in the incidence of ALI.

The Data and Statistical Coordinating Center will prepare weekly reports on the accrual process for the trial. The reports, which will be reviewed on the weekly executive committee calls, will include summarization of screening and randomization metrics. Detailed descriptions of exclusion criteria for disqualified study candidates will be provided and reviewed as well. Each clinical center has a target enrollment of 2 randomized participants per month. The reports will include a comparison of observed vs. expected accrual, by clinical center and overall for the trial. The randomization performance of each clinical center will be disseminated monthly to all study personnel through a study newsletter. If site-specific enrollment concerns are identified, methods for addressing these issues will be evaluated by the executive committee working with the site of interest. If a more pervasive and sustained gap between expected and observed participant accrual is identified, potential modifications to the inclusion and exclusion criteria of the protocol will be discussed. Any amendments to the inclusion and/or exclusion criteria deemed necessary by the executive committee will require approval by the DSMB as well as the IRB of each participating institution before implementation. If enrollment remains below plan, the inclusion of additional clinical sites will be considered as well.

Randomization and Blinding

LIPS-A will utilize centralized randomization software hosted by the Data and Statistical Coordinating Center. Randomization through the electronic data management system will be enabled upon electronic verification of inclusion and exclusion criteria and enrollment of the study participant by the clinical site investigators. ~~Eligible~~ Enrolled participants will be randomized in a 1:1 ratio to the ASA or placebo treatment arm using dynamic minimization[29]

with a second guess probability of 0.2. Randomization will be stratified by center. To maintain the double blind for the study, only ~~, and~~ the research pharmacist at each center will have electronic access to the unblinded treatment code for study medication preparation and dispensing. The rest of the site investigators and coordinating centers will be blinded to the actual treatment assignment. Emergency unblinding is available both electronically and through dispensing records at each pharmacy.

In the event the electronic randomization system is not functioning, the research pharmacist at each center has a sealed emergency randomization kit to enable offline randomization. A manual of operation governs the use of the emergency randomization process. Briefly, prior to use of the emergency process, approval of ~~both~~the coordinating centers is required. All attempts will be made to recover the system prior to the use of the offline procedure. Should the offline procedure be used, the electronic data management system will be updated to reflect the treatment assignment using the identification number contained within the randomization kit ~~identification number when it is available~~.

Statistical Methods

Conditional logistic regression will be used to test the primary hypothesis that early ASA administration will decrease the rate of ALI development. Clinical site will be treated as the stratification variable and conditioned out of the estimating equations. This approach is optimal in a clinical trial setting as it provides a test of null hypothesis that the ALI incidence is equal in the two treatment group and estimates the association in the event the null hypothesis is rejected (through the conditional odds ratio estimate). SAS PROC LOGISTIC™ (Cary, NC) will be used for estimation of the primary model.

This analysis will be supplemented by the Cochran-Mantel-Haenszel stratified analysis with odds ratios computed for each site. The Breslow-Day test will be used to examine the data for potential effect modification (i.e., a "site effect"). In the event there is significant site-to-site variability in the estimated effect, stratified results will be reported for this phase II study. Evidence of heterogeneity of response at this phase of the study will yield invaluable preliminary data for the planning of future changes.

Planned interim analyses will be conducted at 50% of study participants enrolled. With the O'Brien-Fleming-like stopping boundaries, a final adjusted alpha of 0.08885 is anticipated; however, the final value may be changed depending on unplanned interim analyses (conducted at the request of the Data and Safety Monitoring Board [DSMB]) or slight deviations from the anticipated information milestones (0.50, 1.0). Stopping boundaries will be estimated using the LD Bounds package for the R system.

For the remaining continuous and dichotomous secondary endpoints, treatment group comparisons will be performed with respect to clinical outcomes as well as important prognostic factors at screening, baseline, and individual follow-up time points during the study duration. For continuous variables (e.g., age, weight, and laboratory assays), linear model techniques including *t*-tests, analysis of variance and analysis of covariance will be applied. Nonparametric procedures (e.g., the Wilcoxon rank sum test), will be used if data are not normally distributed and transformations of the data are not considered useful. Standard techniques for categorical data will be applied, including Fisher's exact test, Pearson χ^2 procedures, weighted least squares, and logistic regression analysis.

Longitudinal (or serially measured) endpoints will be evaluated by generalized linear models and linear mixed. Repeated measure analyses of binary endpoints will be analyzed using

generalized estimating equations methods which do not require imputation of missing values, provided the data are ignorable missing.[30] Continuous dependent variables will utilize the mixed model approach with emphasis on evaluating the trajectories of values over time. However, early improvement in these parameters may suggest a supportive, stabilizing role for ASA as a treatment option in patients at high risk of ALI. For the primary analysis, the clinical center will be treated as a "nuisance" parameter and conditioned out of the estimation routine. For secondary analyses, the clinical center will be used as a fixed covariate to account for differences across sites.

The safety endpoints (see below under "**Adverse Outcomes**") will be examined for all participants in the safety evaluable analysis set. Safety endpoints will include expected clinical events, including death, for this patient population and summarized by treatment group. Also, all serious and unexpected adverse events will be summarized by treatment group. Fisher's exact test will be used to estimate treatment differences in the incidence of each specified adverse event. No adjustments will be made for multiple hypothesis evaluations of safety endpoints. Adverse events will be summarized with groupings by body system. Other safety data (e.g., labs and assay data) will be listed, and when appropriate, summarized in tabular or graphical format.

Data Quality and Management

This investigation will utilize the Medidata Rave™ system for data management and storage as well as to assist with the randomization procedures. This product has been designed to facilitate multicenter clinical trials conducted under 21 CFR Part 11 requirements. This secure, web-based system provides robust data validation routines, custom reporting and straightforward integration with statistical software packages such as SAS (utilized for this investigation). The system is coupled with an integrated randomization module that uses a

multidimensional dynamic allocation algorithm to minimize imbalances across multiple dimensions including overall study, sites, factors and cross-factor strata. Specific details regarding the randomization process are given below.

ETHICS AND DISSEMINATION

Adverse Outcomes

Safety data including adverse events such as gastrointestinal ulcers, bleeding from any site, gastrointestinal discomfort, wheezing, rash, hives, angioedema, tinnitus, and mortality will be recorded. Adverse events will be defined as "unexpected," "expected," and "serious." As our patient population is by definition "critically ill," it is expected that they will have a number of unrelated adverse health events during the course of their hospital stay. Therefore, we will limit the scope of our adverse event monitoring and recording to the following:

1) Serious adverse events (SAEs) will be defined as:

- Death, *believed to be related to the study medication or procedures, or a death that is unexpected considering the acuity of a patient.*
- A life threatening experience *believed to be related to the study medication or procedures*
- Persistent or significant disability or incapacity *that is of greater frequency or severity than what would be normally expected in the course of critical illness.*
- An event that jeopardizes the human subject and may require medical or surgical treatment to prevent one of the preceding outcomes *and is not expected in the course of critical illness.*

2) Adverse events possibly related to aspirin administration will be defined as:

- Anaphylaxis / allergic reaction

- Gastrointestinal bleed / bleeding complications
- Transfusion requirements for suspected bleeding
- Acute kidney injury, defined as RIFLE stage "I" or greater
- Tinnitus
- Reye's syndrome

Role of the Data Safety and Monitoring Board

Reporting of SAEs will be conducted through the CCC. All centers will report SAEs within 24 hours of discovering the presence of the SAE. The CCC will report all potentially related SAEs to the DSMB and to NHLBI within 7 days of discovery. A summary report of the events will be provided to the DSMB prior to each DSMB meeting, at least every six months. Safety oversight will be under the direction of a DSMB whose members will be independent from the study operations. The safety endpoints will be examined for all eligible patients who sign informed consent and are enrolled in the study on an intent-to-treat basis. Safety endpoints will include expected clinical events, including death, for this patient population and summarized by treatment group. All serious and unexpected adverse events will be summarized by treatment group as well.

Ethics Approval

Approval of the protocol was obtained from the data safety and monitoring board as well as from NHLBI prior to enrolling the first study participant. In addition, approval of both the protocol and informed consent documents was required and obtained from the institutional review board of each participating institution prior to enrolling study participants at the respective study site. To ensure that each participating institution's informed consent documentation complied with NHLBI requirements and the Code of Federal Regulations Title 21

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Part 50 Section 50.25, all informed consent forms were reviewed and approved by the CCC. Official documentation of all IRB approvals and all finalized informed consent forms have been collected and stored by the CCC.

Considerations for Continuation to a Phase III Clinical Trial

The decision to proceed with a phase III trial is formally outlined as follows:

1) Initiate Phase III Study: Demonstrated efficacy signal in addition to adequate safety profile. Criteria: Early termination for benefit at interim analysis or $p < 0.08885$ at final analysis (alpha=0.10 for study). Serious adverse event profile of ASA not statistically worse than placebo (95% confidence interval for the relative risk of any SAE covers the null value of RR=1.0).

2) Further Development Potentially Required: Weak efficacy signal. Criteria: Primary endpoint did not achieve *a priori* level of significance but there were at least a general consistency of secondary endpoints indicating propensity for efficacy with a larger sample size and/or more specific primary endpoint.

3) Abandon Treatment Platform: Harm (in efficacy or safety endpoints). Criteria: Study terminated early per recommendation by DSMB for safety and/or risk/benefit ratio concerns (i.e., stop for futility, harm, unacceptable risk profile, etc.).

Ancillary Studies

The LIPS-A group will encourage investigator-initiated ancillary study proposals that extend or complement the specific aims of the primary LIPS-A trial. As policy, all proposals will be reviewed by a separate Ancillary Studies and Publications Committee, both to ensure consistency with the goals and conduct of the main study and evaluate scientific merit and validity. Proposed studies may utilize data and/or samples already accrued during the LIPS-A trial or, when feasible, request additional data collection from participating sites. The

investigative and statistical plan will be reviewed *a priori*, with committee approval required before analysis begins. Where equivocal, review decisions will be referred to the LIPS-A Executive Committee. All reports, manuscripts or presentations derived from data obtained through the ancillary study process will require review and approval by the Ancillary Studies and Publications Committee prior to submission.

Protocol funding and role of the funding sources

This study is supported by the National Institutes of Health-National Heart Lung and Blood Institute (Grant Number U01-HL108712-01), the Mayo Clinic Center for Translational Science Activities (Grant Number KL2 RR024151), and the Mayo Clinic Critical Care Research Committee. Specifically, funding has been provided by each of these entities to support study personnel time and effort, protocol and data management development (Medidata Rave™), sample acquisition, processing and storage, and statistical support. These funding sources have had no specific influence on the scientific content of the study protocol. Similarly, the funding sources will have no direct role in the study conduct, nor data collection, analyses, or interpretation. The funding sources will also have no role in the writing or presentation of study results, nor decisions to submit for publication. The ultimate authority over each of these activities will be the executive committee of the LIPS-A study.

DISCUSSION

We have presented the study protocol and data analysis plans for the first phase II, multicenter randomized clinical trial that will test the efficacy and safety of a promising ALI prevention agent. Specifically, we have hypothesized that early administration of ASA to hospitalized patients who are at high risk for ALI, will be safe and will reduce the likelihood of progression to the full ALI phenotype. Secondarily, this investigation will glean important

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mechanistic data on ASA's impact on the pathways believed important in ALI pathogenesis as well as the potential value of relevant biomarkers in the prediction of subsequent development of ALI. Finally, the results of this study will provide essential information on both the scientific merit and feasibility of a larger, phase III trial testing the role of ASA in the prevention of lung injury.

The persistent difficulty in translating promising pre-clinical therapies into the clinical setting has fostered interest in the potential development of effective ALI prevention strategies. Indeed, prevention of ALI has been identified as a key strategic priority for invested parties such as the NHLBI.[31] However, implementation of protocols aiming to test potential ALI prevention strategies have been historically hindered by an inability to accurately predict who is at risk for ALI. Moreover, the typically short interval between risk exposure and development of ALI as well as the small proportion of patients who progress to the full ALI phenotype following an ALI-related exposure has limited the feasibility of ALI prevention studies. In addition, the historic lack of standardization for numerous important co-interventions that confound the associations of interest (e.g., ventilator management, transfusion and resuscitation practices) has also limited our ability to test preventative strategies.

To this end, the recently validated LIPS score is a key element of the herein described study protocol.[8] Specifically, the LIPS score is expected to facilitate the identification of patients at greatest risk of progressing to ALI (a LIPS score ≥ 4 is expected to identify a subgroup of patients who have a risk of progressing to ALI that is greater than 18%). In addition, it is notable that this ALI risk assessment tool was validated using data collected within the first 6 hours after the initial evaluation in the ED. In an ALI prevention protocol such as

described herein, where the time to randomization is limited to 12 hours from presentation to the ED, the ability to accurately determine risk for ALI in such a time-efficient manner is critical.

A second notable strength of the current protocol is expected to be the implementation of the CLIP for standardizing important co-interventions that may otherwise confound our association of interest (ASA and ALI). During the period between hospital admission and the development of ALI, health care delivery factors (timely treatment of infection and shock, appropriate administration of fluid and transfusion therapies, prevention of aspiration, avoidance of large tidal volume ventilation), may be as important as individual biology in determining ALI development and outcome.[32-39] Moreover, a recent survey noted wide variation in clinical practices such as the existence of a sepsis protocol, use of low tidal volume ventilation, positive end-expiratory pressure, and restrictive transfusion practices, between hospitals and among the ED, ICU and operating room within hospitals.[40] Thus, to effectively investigate preventive strategies in ALI, the standardization of care delivery during the early phase of hospitalization would appear critical. Indeed, the ARDSNet investigators have repeatedly shown the value of standardization of clinical processes for ALI patients in clinical trials, allowing for determination of incremental benefit of new interventions.[41, 42] In the current investigation, standardization of care with best practices will help to reduce variability in the rates of ALI and the intensity of lung injury (noise) due to inconsistencies in care delivery. The result is expected to be an increased chance of seeing a beneficial clinical or biological effect from ASA and a better assessment of the potential side effects of ASA in this population.

Though the multicenter randomized clinical trial design, availability of a time-efficient risk assessment tool (LIPS score) and the standardization of important co-interventions with CLIP, as well as the robust study support and quality control offered through Metadata RAVE,

are clear strengths of the current study protocol, several important limitations with the planned investigation deserve note. Lung injury may be present at study entry even as clinical criteria for ALI are not fulfilled. Though a formal diagnosis of prevalent ALI is exclusionary, the molecular machinery will have been clearly set in motion in many of the study participants. Therefore, the study may be more accurately characterized as a prevention/early treatment trial rather than a pure prevention trial. Nonetheless, we have attempted to focus on the early period of ALI development by mandating a short interval from hospital presentation to randomization (12 hours) and a similarly short interval from hospital presentation to administration of the first study dose (24 hours). In addition, the study will exclude patients who presented to an outside hospital ED more than 12 hours before arrival at the enrolling site's facility. The study will also exclude those with ALI on hospital presentation or prior to randomization as well as those who are receiving mechanical ventilation through a tracheostomy tube prior to the current hospital admission (patient who is ventilator dependent) or those with a history of interstitial lung disease with chronic pulmonary infiltrates that may mimic ALI.

A second limitation relates to the intervention of ASA administration. Specifically, it is now well documented that more than 10% of the population will have a variable response to ASA or at least some form of aspirin resistance.[17] These patients may not benefit from ASA, even if ASA can modulate the development of lung injury. However, as part of this study, we will measure plasma thromboxane, a sensitive indicator of ASA resistance, to determine the prevalence of ASA resistance in patients at high risk for ALI. As such, sensitivity analyses, stratifying study participants by ASA resistance (as determined by changes in thromboxane levels), may allow us to determine whether the effect of ASA on ALI development is isolated to those susceptible to the actions of ASA. A related concern is the potential influence of

concomitant medications that may impact aspirin's ability to prevent or mitigate ALI (e.g., statins, corticosteroids). To address this concern, we will be collecting detailed information on concomitant medications and, when necessary, appropriate statistical adjustments will be made.

A third potential limitation with this study relates to a previously recognized major barrier to ALI prevention studies, namely feasibility. First and foremost, a substantial proportion of the target population may be expected to be receiving ASA on presentation to the ED, an exclusion criteria for the current protocol. Notably, however, our preliminary work suggests that upwards of two-thirds of the target population was not on ASA prior to admission. We also note that over the three months of the initial LIPS,[8] there were 800 patients who fulfilled study inclusion criteria of LIPS score ≥ 4 and did not fulfill the exclusion criteria of pre-existing ASA use, prevalent ALI, and elective surgery. Therefore, we believe that with 14 proposed sites and two years of planned enrollment, we will successfully meet our enrollment goals of 400 total patients. Also relating to feasibility, it is possible that some sites will be challenged by the short time interval allowed for patient enrollment as well as the short time to study drug administration. Though a valid concern, we believe the use of the LIPS score and the robust support offered through Metadata™ RAVE will greatly facilitate the enrollment and randomization procedures such that sites will indeed be successful in meeting these time-sensitive challenges.

A fourth and final limitation which deserves mention relates to the potential toxicity of the intervention of interest. Generally, ASA is well tolerated even in acutely ill, hospitalized patients in whom ASA is often continued during the hospitalization. As an example, in a study of ASA use up to the time of cardiac surgery, its continuation was not associated with an increase need for transfusion therapies.[43] Nevertheless, there may be injury associated with

the administration of aspirin. To address this concern, patients at risk for major complications from ASA therapy have been excluded from the study. Multiple stopping criteria for patients who experience adverse events have also been incorporated into the protocol. In addition, the more complete understanding of the safety profile of an intervention of interest is an important goal of all phase II trials. In this regard, the information gleaned from this study, adverse events included, is necessary to help decide on the merits of proceeding to a phase III clinical trial.

CONCLUSION

This manuscript describes the study protocol and analysis plans for the first phase II randomized clinical trial of the promising ALI prevention agent ASA. In addition to providing important information on the safety and efficacy of ASA in patients at high risk for ALI, the results of this trial will also inform the scientific community regarding the merit and feasibility of a more definitive phase III clinical trial. Importantly, the significance of this effort lies not only in the specific results which will be obtained from the study protocol, but equally in the infrastructure that will be created to facilitate the conduct of this trial. Specifically, the development and utilization of innovative methods to facilitate the early identification of high-risk patients with the LIPS and the standardization of potential confounding co-interventions with CLIP will address key barriers to studying ALI prevention measures and is expected to lay a framework for the meaningful conduct of future ALI prevention studies as well.

Table 1. Study Exclusion Criteria

Exclusion Criteria	Justification
Anti-platelet therapy on admission or within 7 days prior to admission	Inability to ethically randomize
Presented to outside hospital emergency department > 12 hours before arrival at site's facility	Inability to enroll within time frame for possible benefit
Inability to obtain consent and randomize within 12 hours of hospital presentation	Inability to enroll within time frame for possible benefit
Admitted for elective or emergency surgery	Aspirin not found to benefit this group in preliminary studies
ALI on hospital presentation or prior to randomization	Inability to adequately assess outcome
Presentation believed to be due to pure heart failure and no other known risk factors for ALI	Inability to adequately assess outcome
Receiving mechanical ventilation through a tracheostomy tube prior to current hospital admission (patient who is ventilator dependent)	Inability to adequately assess outcome
Bilateral pulmonary infiltrates present on admission only if the patient has a history of interstitial lung disease that can reasonably explain the current degree of pulmonary infiltrates present	Inability to adequately assess outcome
Allergy to aspirin or NSAIDs	Intervention contraindicated
Bleeding disorder[†]	Intervention contraindicated
Suspected active bleeding or judged to be at high risk for bleeding complications	Intervention contraindicated
Presence of acute kidney injury[‡]	Intervention contraindicated
Severe chronic liver disease (Child-Pugh class C)	Intervention contraindicated

Active peptic ulcer disease (within past 6 months)	Intervention contraindicated
Pregnancy or breast feeding	Intervention contraindicated
Inability to administer study drug	Unable to administer study drug
Expected hospital stay < 48 hours	Incomplete study procedures and outcome data
Admitted for comfort or hospice care	Incomplete study procedures and outcome data
Patient, surrogate or physician not committed to full support (exception: a patient will not be excluded if he/she would receive all supportive care except for attempts at resuscitation from cardiac arrest)	Unable to assess primary outcome
Not anticipated to survive > 48 hours	Incomplete study procedures and outcome data
Previously enrolled in this trial	Violates the statistical assumption of sample independence
Enrollment in concomitant intervention study	Potential confounding and co-enrollment interactions

*Any disorder with known associated with increased risk of bleeding. Common disorders may include thrombocytopenia, disseminated intravascular coagulation, hemophilia, von Willebrand disease, oral anticoagulant therapy, or advanced liver disease with associated coagulation disorders. Platelet count < 50,000 or absence of platelet count in the previous 24 hours to allow for assessment of platelet status.

[#]Acute kidney injury defined as "R" or greater according to RIFLE criteria. ALI = acute lung injury, NSAIDs = non-steroidal anti-inflammatory medications

Table 2. Elements of CLIP – Checklist for Lung Injury Prevention

CLIP Elements	Definition
Lung protective mechanical ventilation	Tidal volume between 6-8 mL/kg predicted body weight and plateau pressure < 30 cm H_2O; PEEP ≥ 5 cm H_2O, minimize FiO_2 (target oxygen saturation 88-92% after early shock)
Aspiration precautions	Rapid sequence intubation supervised by experienced providers, elevated head of the bed, oral care with chlorhexidine, gastric acid neutralization in those not receiving tube feeds.
Adequate empiric antimicrobial treatment and source control	According to suspected site of infection, health care exposure, and immune suppression
Limiting fluid overload	Modified ARDSNet FACTT protocol after early shock (first 12 hours)
Restrictive transfusion	Hemoglobin target > 7 g/dL in the absence of acute bleeding and/or ischemia
Appropriate handoff of patients at risk	Providers taking care of patients at risk who require ICU admission will complete a structured handoff to the ICU team to continue with CLIP protocol for the duration of ICU stay

CLIP = checklist for lung injury prevention, PEEP = positive end-expiratory pressure, FiO_2 = fraction of inspired oxygen concentration, ARDSNet = Acute Respiratory Distress Syndrome Network, FACTT = fluid and catheter treatment trial, ICU = intensive care unit

Table 3: Schedule of Events

Event	Time of presentation until first dose (screen / baseline)	First dose until end of that calendar day (Day 1)	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	7 days after last dose	Hospital discharge or study Day 28, whichever comes first	6 Months	12 Months
Informed consent	X											
Inclusion/exclusion criteria	X											
Pregnancy test in women of childbearing potential	X											
Demographics	X											
Medical history	X											
LIPS score	X											
Randomization	X											
Study drug administration		X	X	X	X	X	X	X				
Clinical outcome assessment	X	X	X	X	X	X	X	X				
Safety labs: Cr and Hb	x		X	X	X	X	X	X				
Clinical data as available: labs, ABG	X	X	X	X	X	X	X	X				
CXR / ABG*		X	X	X	X	X	X	X				
CLIP	X	X	X	X	X	X	X	X				
AE/SAE monitoring		X	X	X	X	X	X	X	X	X		

Survival										X		X
Plasma biomarkers of ALI	X		X		X							
SF-12	X										X	X
Barthel Index	X										X	X
Vulnerable Elders Survey	X									X	X	
Brussels / SOFA composite										X		

*Chest x-ray required on days 1-7 ONLY IF patient is intubated, and DOES NOT have ALI / ARDS already, AND there is clinical evidence of worsening respiratory status defined as:

- Previous P/F ratio ≥ 300, with current P/F ratio < 300 and no chest x-ray within 24 hours.
- Prior P/F ratio < 300 and the PF ratio has fallen more than 10% AND no chest x-ray within 24 hours.
- In cases where an ABG is not available, the research team should obtain an ABG ***only if*** the S/F ratio falls below 315 consistently. The P/F ratio obtained from that ABG will be used to determine whether a chest x-ray needs to be obtained (as per criteria outlined above).
- If change in P/F ratio triggers the need for a chest x-ray or ABG as above, sites have 24 hours to conduct the necessary procedure. An ABG or chest x-ray obtained by the clinical team during that time period is also acceptable and obviates the need to obtain said procedure for the research study.

LIPS = Lung injury prevention, ALI = acute lung injury, LIS = lung injury severity score, Cr = creatinine, Hb = hemoglobin, ABG = arterial blood gas, CLIP = checklist for lung injury prevention, AE = adverse events, SAE = serious adverse events, SF-12 = 12-Item Short-Form Health Survey, SOFA = sequential organ failure assessment.

Table 4: Plasma biomarkers in ALI/ARDS

Plasma Biomarker	Importance in ALI/ARDS Development	Associated outcomes other than ALI/ARDS
Surfactant protein-D[44-46]	Reflect injury and ↑ permeability of alveolar epithelium	VFD, organ failure
Receptor for advanced glycation end products[47-49]	Reflects endothelial activation and injury	VFD,[49] organ failure,[49] ARDS after lung transplant[47]
Intercellular adhesion molecule-1[44, 50-53]	Reflects endothelial activation and injury	VFD,[51] organ failure[51]
Interleukin-6[44, 54-56]	Inflammation	VFD,[55] organ failure[55]
Interleukin-8[44, 48, 50, 54-56]	Inflammation	VFD, [55]organ failure[55]
Plasminogen activator inhibitor-1[44, 50, 57-61]	Activation of coagulation and inhibition of fibrinolysis	VFD,[61] organ failure[61]
von Willebrand factor[44, 48, 60, 62, 63]	Reflects endothelial activation and injury	organ failure
Protein C[44, 50, 59, 61, 64]	Activation of coagulation and inhibition of fibrinolysis	ARDS after lung transplant,[47] VFD,[61] organ failure[61]

ALI = acute lung injury, ARDS = acute respiratory distress syndrome, VFD = ventilator-free days.

REFERENCES

1. Rubenfeld GD, Caldwell E, Peabody E, et al. Incidence and outcomes of acute lung injury. *N Engl J Med* 2005;**353**:1685-93.
2. Li G, Malinchoc M, Cartin-Ceba R, et al. Eight-year trend of acute respiratory distress syndrome: a population-based study in Olmsted County, Minnesota. *Am J Resp Crit Care Med* 2011;**183**:59-66.
3. Erickson SE, Martin GS, Davis JL, et al. Recent trends in acute lung injury mortality: 1996-2005. *Crit Care Med* 2009;**37**:1574-9.
4. Herridge MS, Tansey CM, Matté A, et al. Functional disability 5 years after acute respiratory distress syndrome. *N Engl J Med* 2011;**364**:1293-304.
5. Hudson LD, Milberg JA, Anardi D, et al. Clinical risks for development of the acute respiratory distress syndrome. *Am J Respir Crit Care Med* 1995;**151**:293-301.
6. Fowler AA, Hamman RF, Good JT, et al. Adult respiratory distress syndrome: risk with common predispositions. *Ann Intern Med* 1983;**98**:593-7.
7. Gong MN, Thompson BT, Williams P, et al. Clinical predictors of and mortality in acute respiratory distress syndrome: potential role of red cell transfusion. *Crit Care Med* 2005;**33**:1191-8.
8. Gajic O, Dabbagh O, Park PK, et al. Early identification of patients at risk of acute lung injury: evaluation of lung injury prediction score in a multicenter cohort study. *Am J Respir Crit Care Med* 2010;**183**:462-70.
9. Zarbock A, Ley K. The role of platelets in acute lung injury (ALI). Front Biosci 2009;**14**:150-8.

10. Zarbock A, Polanowska-Grabowska RK, Ley K. Platelet-neutrophil-interactions: linking hemostasis and inflammation. *Blood Rev* 2007;**21**:99-111.
11. Looney MR, Nguyen JX, Hu Y, et al. Platelet depletion and aspirin treatment protect mice in a two-event model of transfusion-related acute lung injury. *J Clin Invest* 2009;**119**:3450-61.
12. El Kebir D, József L, Pan W, et al. 15-epi-lipoxin A4 inhibits myeloperoxidase signaling and enhances resolution of acute lung injury. *Am J Respir Crit Care Med* 2009;**180**:311-9.
13. Fukunaga K, Kohli P, Bonnans C, et al. Cyclooxygenase 2 plays a pivotal role in the resolution of acute lung injury. *J Immunol* 2005;**174**:5033-9.
14. Maderna P, Godson C. Lipoxins: resolutionary road. *Br J Pharmacol* 2009;**158**:947-59.
15. Chelucci GL, Boncinelli S, Marsili M, et al. Aspirin effect on early and late changes in acute lung injury in sheep. *Intensive Care Med* 1993;**19**:13-21.
16. Sigurdsson GH, Vallgren S, Christenson JT. Influence of aspirin and steroids on acute lung injury after i.v. injection of a sclerosing agent. *Acta Chir Scand* 1989;**155**:163-70.
17. Yasuda O, Takemura Y, Kawamoto H, et al. Aspirin: recent developments. *Cell Mol Life Sci* 2008;**65**:354-8.
18. Jin SW, Zhang L, Lian QQ, et al. Posttreatment with aspirin-triggered lipoxin A4 analog attenuates lipopolysaccharide-induced acute lung injury in mice: the role of heme oxygenase-1. *Anesth Analg* 2007;**104**:369-77.
19. Tabuchi A, Kuebler WM. Endothelium-platelet interactions in inflammatory lung disease. *Vasc Pharmacol* 2008;**49**:141-50.

20. Grommes J, Alard J-E, Drechsler M, et al. Disruption of platelet-derived chemokine heteromers prevents neutrophil extravasation in acute lung injury. *Am J Resp Crit Care Med* 2012;**185**:628-36.
21. Clark SR, Ma AC, Tavener SA, et al. Platelet TLR4 activates neutrophil extracellular traps to ensnare bacteria in septic blood. *Nature Med* 2007;**13**:463-9.
22. Narasaraju T, Yang E, Samy RP, et al. Excessive neutrophils and neutrophil extracellular traps contribute to acute lung injury of influenza pneumonitis. *Am J Pathol* 2011;**179**:199-210.
23. Erlich JM, Talmor DS, Cartin-Ceba R, et al. Prehospitalization antiplatelet therapy is associated with a reduced incidence of acute lung injury. A population-based cohort study. *Chest* 2011;**139**:289-95.
24. Kor DJ, Erlich J, Gong MN, et al. Association of prehospitalization aspirin therapy and acute lung injury: results of a multicenter international observational study of at-risk patients. *Crit Care Med* 2011;**39**:2393-400.
25. Patrono C, Garcia Rodriguez LA, Landolfi R, et al. Low-dose aspirin for the prevention of atherothrombosis. *N Engl J Med* 2005;**353**:2373-83.
26. Chiang N, Bermudez EA, Ridker PM, et al. Aspirin triggers antiinflammatory 15-epi-lipoxin A4 and inhibits thromboxane in a randomized human trial. *Proc Natl Acad Sci USA* 2004;**101**:15178-83.
27. Bernard GR, Artigas A, Brigham KL, et al. The American-European Consensus Conference on ARDS. Definitions, mechanisms, relevant outcomes, and clinical trial coordination. *Am J Respir Crit Care Med* 1994;**149**:818-24.

28. Rubenfeld GD. Interobserver variability in applying a radiographic definition for ARDS. *Chest* 1999;**116**:1347-53.
29. Pocock SJ, Simon R. Sequential treatment assignment with balancing for prognostic factors in the controlled clinical trial. *Biometrics* 1975;**31**:103-15.
30. Diggle P, Liang K, Zeger S. Analysis of Longitudinal Data. New York: Oxford University Press, Inc.; 1994.
31. Spragg RG, Bernard GR, Checkley W, et al. Beyond mortality: future clinical research in acute lung injury. *Am J Respir Crit Care Med* 2010;**181**:1121-7.
32. Gajic O, Dara SI, Mendez JL, et al. Ventilator-associated lung injury in patients without acute lung injury at the onset of mechanical ventilation. *Crit Care Med* 2004;**32**:1817-24.
33. Rana R, Fernandez-Perez ER, Khan SA, et al. Transfusion-related acute lung injury and pulmonary edema in critically ill patients: a retrospective study. *Transfusion* 2006;**46**:1478-83.
34. Gajic O, Rana R, Winters JL, et al. Transfusion-related acute lung injury in the critically ill: prospective nested case-control study. *Am J Respir Crit Care Med* 2007;**176**:886-91.
35. Gajic O, Rana R, Mendez JL, et al. Acute lung injury after blood transfusion in mechanically ventilated patients. *Transfusion* 2004;**44**:1468-74.
36. Gajic O, Frutos-Vivar F, Esteban A, et al. Ventilator settings as a risk factor for acute respiratory distress syndrome in mechanically ventilated patients. *Intensive Care Med* 2005;**31**:922-6.
37. Gajic O, Dzik WH, Toy P. Fresh frozen plasma and platelet transfusion for nonbleeding patients in the intensive care unit: benefit or harm? *Crit Care Med* 2006;**34**:S170-3.

38. Fernandez-Perez ER, Keegan MT, Brown DR, et al. Intraoperative tidal volume as a risk factor for respiratory failure after pneumonectomy. *Anesthesiology* 2006;**105**:14-8.
39. Khan H, Belsher J, Yilmaz M, et al. Fresh-frozen plasma and platelet transfusions are associated with development of acute lung injury in critically ill medical patients. *Chest* 2007;**131**:1308-14.
40. Hou P, Cohen J, Elie-Turenne M, et al. A survey on behalf of USCIITG-LIPS investigators: toward standardization of a checklist for lung injury prevention (CLIP). *Crit Care Med 2010*;**38**:576.
41. Brower RG, Lanken PN, MacIntyre N, et al. Higher versus lower positive end-expiratory pressures in patients with the acute respiratory distress syndrome. *N Engl J Med* 2004;**351**:327-36.
42. Wiedemann HP, Wheeler AP, Bernard GR, et al. Comparison of two fluid-management strategies in acute lung injury. *N Engl J Med* 2006;**353**:2564-75.
43. Gerrah R, Elami A, Stamler A, et al. Preoperative aspirin administration improves oxygenation in patients undergoing coronary artery bypass grafting. Chest 2005;**127**:1622-6.
44. Ware LB, Koyama T, Billheimer DD, et al. Prognostic and pathogenetic value of combining clinical and biochemical indices in patients with acute lung injury. *Chest* 2010;**137**:288-96.
45. Eisner M, Parsons P, Matthay M, et al. Plasma surfactant protein levels and clinical outcomes in patients with acute lung injury. *Thorax* 2003;**58**:983-8.

46. Greene KE, Wright JR, Steinberg KP, et al. Serial changes in surfactant-associated proteins in lung and serum before and after onset of ARDS. *Am J Respir Crit Care Med* 1999;**160**:1843-50.
47. Christie JD, Shah CV, Kawut SM, et al. Plasma levels of receptor for advanced glycation end products, blood transfusion, and risk of primary graft dysfunction. *Am J Respir Crit Care Med* 2009;**180**:1010-5.
48. Fremont RD, Koyama T, Calfee CS, et al. Acute lung injury in patients with traumatic injuries: utility of a panel of biomarkers for diagnosis and pathogenesis. *J Trauma* 2010;**68**:1121-7.
49. Calfee CS, Ware LB, Eisner MD, et al. Plasma receptor for advanced glycation end products and clinical outcomes in acute lung injury. *Thorax* 2008;**63**:1083-9.
50. McClintock D, Zhuo H, Wickersham N, et al. Biomarkers of inflammation, coagulation and fibrinolysis predict mortality in acute lung injury. *Crit Care* 2008;**12**:R41.
51. Calfee CS, Eisner MD, Parsons PE, et al. Soluble intercellular adhesion molecule-1 and clinical outcomes in patients with acute lung injury. *Intensive Care Med* 2009;**35**:248-57.
52. Agouridakis P, Kyriakou D, Alexandrakis MG, et al. The predictive role of serum and bronchoalveolar lavage cytokines and adhesion molecules for acute respiratory distress syndrome development and outcome. *Respir Res* 2002;**3**:25.
53. Covarrubias M, Ware LB, Kawut SM, et al. Plasma intercellular adhesion molecule-1 and von Willebrand factor in primary graft dysfunction after lung transplantation. *Am J Transplant* 2007;**7**:2573-8.

54. Bouros D, Alexandrakis MG, Antoniou KM, et al. The clinical significance of serum and bronchoalveolar lavage inflammatory cytokines in patients at risk for acute respiratory distress syndrome. *BMC Pulm Med* 2004;**4**:6.
55. Parsons PE, Eisner MD, Thompson BT, et al. Lower tidal volume ventilation and plasma cytokine markers of inflammation in patients with acute lung injury. *Crit Care Med* 2005;**33**:1-6.
56. Meduri GU, Headley S, Kohler G, et al. Persistent elevation of inflammatory cytokines predicts a poor outcome in ARDS. Plasma IL-1 beta and IL-6 levels are consistent and efficient predictors of outcome over time. *Chest* 1995;**107**:1062-73.
57. Prabhakaran P, Ware LB, White KE, et al. Elevated levels of plasminogen activator inhibitor-1 in pulmonary edema fluid are associated with mortality in acute lung injury. *Am J Physiol Lung Cell Mol Physiol* 2003;**285**:L20-8.
58. Ware LB, Fang X, Matthay MA. Protein C and thrombomodulin in human acute lung injury. *Am J Physiol Lung Cell Mol Physiol* 2003;**285**:L514-21.
59. Christie JD, Robinson N, Ware LB, et al. Association of protein C and type 1 plasminogen activator inhibitor with primary graft dysfunction. *Am J Respir Crit Care Med* 2007;**175**:69-74.
60. Ware LB, Conner ER, Matthay MA. von Willebrand factor antigen is an independent marker of poor outcome in patients with early acute lung injury. *Crit Care Med* 2001;**29**:2325-31.
61. Ware LB, Matthay MA, Parsons PE, et al. Pathogenetic and prognostic significance of altered coagulation and fibrinolysis in acute lung injury/acute respiratory distress syndrome. *Crit Care Med* 2007;**35**:1821-8.

62. Rubin DB, Wiener-Kronish JP, Murray JF, et al. Elevated von Willebrand factor antigen is an early plasma predictor of acute lung injury in nonpulmonary sepsis syndrome. *J Clin Invest* 1990;**86**:474-80.

63. Ware LB, Eisner MD, Thompson BT, et al. Significance of von Willebrand factor in septic and nonseptic patients with acute lung injury. *Am J Respir Crit Care Med* 2004;**170**:766-72.

64. Matthay MA, Ware LB. Plasma protein C levels in patients with acute lung injury: prognostic significance. *Crit Care Med* 2004;**32**:S229-32.

Appendix A. Lung Injury Prevention Study with Aspirin (LIPS-A) Coordinating Center Personnel and Site Investigators

Coordinating Centers	
Clinical Coordinating Center	Daniel S. Talmor MD, MPH Valerie M. Banner-Goodspeed, ALB
Data and Statistical Coordinating Center	Ognjen Gajic, MD, MSc, FCCP, FCCM Daryl J. Kor, MD Rickey E. Carter, PhD Richard Hinds, BS, MS, RRT
Biospecimen Repository and Knowledge Translation Center	Michelle N. Gong MD, MS Graciela Soto, MD
Clinical Sites	
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Bridgeport Hospital	David Kaufman, MD
Brigham and Womens Hospital	Peter Hou, MD Bruce D. Levy, MD
Duke University Medical Center	Ian Welsby, BSc, MBBS Heatherlee Bailey, MD, FAAEM, FCCM
Harborview Medical Center	Timothy R. Watkins, MD MSc
Massachusetts General Hospital	Ednan Bajwa, MD, MPH Christopher Kabrhel, MD
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Mayo Clinic – Rochester	Ognjen Gajic, MD Daryl Kor, MD Rahul Kashyap, MBBS Leanne Clifford, MBBS
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University of Illinois College of Medicine	Ruxana Sadikot, MD, MRCP, FCCP
University of Louisville Medical Center	Ozan Akca, MD, FCCM Rodrigo Cavallazzi, MD Melissa Platt, MD
University of Michigan	Pauline Park, MD Jill Cherry-Bukowiec, MD, MS Lena Napolitano, MD Krishnan Raghavendran, MD John Younger, MD, MS
Wake Forest University Medical Center	Jason Hoth, MD

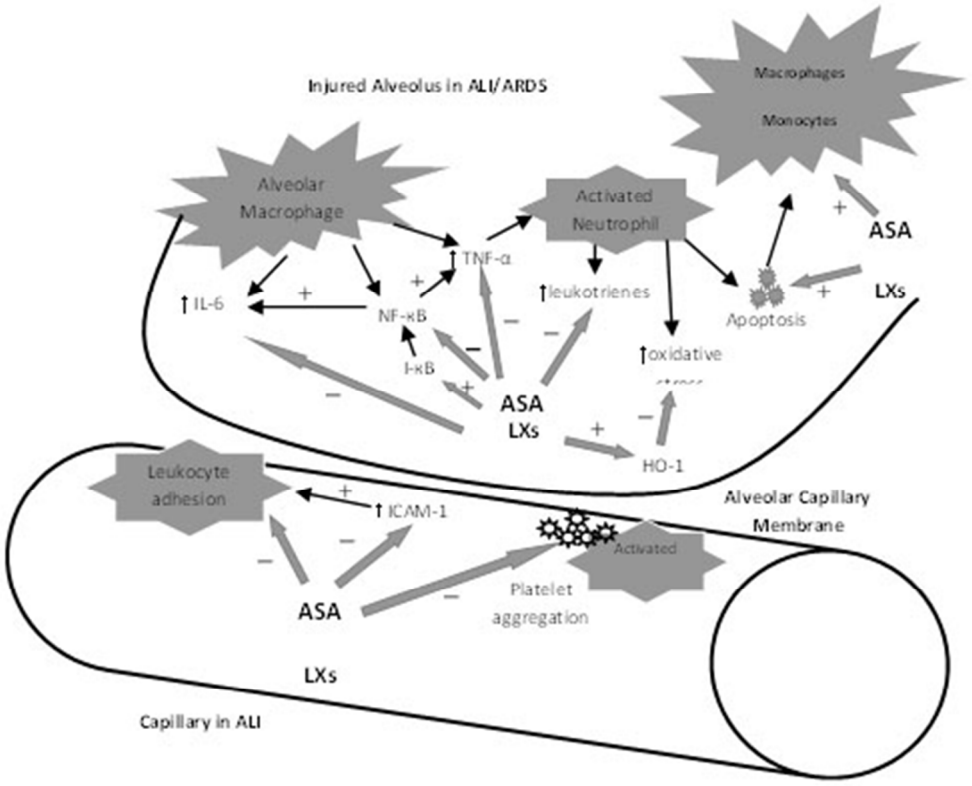

Illustration of the potential role of aspirin, lipoxins, and aspirin-triggered lipoxins on the mediators of ALI development and progression. Black arrows indicate events in ALI. Grey arrows indicate action of ASA, LTXs, or ATLs.

ALI = acute lung injury, ARDS = acute respiratory distress syndrome, ASA = aspirin, LX = lipoxins, ATLs = aspirin-triggered lipoxins, IL-6 = interleukin-6, TNF = tumor necrosis factor, NF-kB = nuclear factor kappa-light-chain-enhancer of activated B-cells, I-kB = nuclear factor kappa-light-chain-enhancer of activated B-cells inhibitor, HO = heme oxygenase, ICAM = intercellular adhesion molecule

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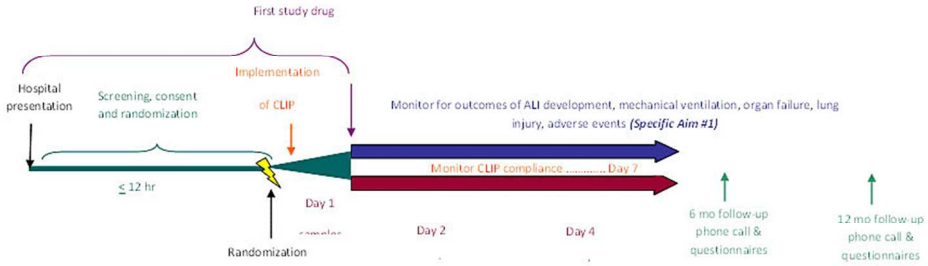

Schematic of the planned study procedures
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LUNG INJURY PREVENTION WITH ASPIRIN (LIPS-A): A PROTOCOL FOR A MULTICENTER RANDOMIZED CLINICAL TRIAL IN MEDICAL PATIENTS AT HIGH RISK FOR ACUTE LUNG INJURY.

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Keywords: acute lung injury, acute respiratory distress syndrome, aspirin, critical illness, prevention, clinical trial.

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ABSTRACT

Introduction: Acute Lung Injury (ALI) is a devastating condition that places a heavy burden on public health resources. Although the need for effective ALI prevention strategies is increasingly recognized, no effective preventative strategies exist. The Lung Injury Prevention Study with Aspirin (LIPS-A) aims to test whether aspirin (ASA) could prevent and/or mitigate the development of ALI.

Methods and Analysis: LIPS-A is a multicenter, double-blind, randomized clinical trial testing the hypothesis that the early administration of ASA will result in a reduced incidence of ALI in adult patients at high risk. This investigation will enroll 400 study participants from 14 hospitals across the US. Conditional logistic regression will be used to test the primary hypothesis that early ASA administration will decrease the incidence of ALI.

Ethics and Dissemination: Safety oversight will be under the direction of an independent data and safety monitoring board (DSMB). Approval of the protocol was obtained from the DSMB prior to enrolling the first study participant. Approval of both the protocol and informed consent documents were also obtained from the institutional review board of each participating institution prior to enrolling study participants at the respective site.

In addition to providing important clinical and mechanistic information, this investigation will inform the scientific merit and feasibility of a phase III trial on aspirin as an ALI prevention agent. The findings of this investigation, as well as associated ancillary studies, will be disseminated in the form of oral and abstract presentations at major national and international medical specialty meetings. The primary objective and other significant findings will also be presented in manuscript form. All final, published manuscripts resulting from this protocol will be submitted to Pub Med Central (PMC) in accordance with the National Institute of Health

Public Access Policy. This trial is registered with ClinicalTrials.gov: NCT01504867.

INTRODUCTION

Acute lung injury (ALI) and the acute respiratory distress syndrome (ARDS) are life-threatening syndromes which continue to consume substantial health care resources and profoundly impact patient-important outcomes.[1] Although recent epidemiologic studies suggest the incidence of lung injury may be on the decline,[2] even conservative estimates suggest the associated mortality continues to exceed 25%.[3] Beyond mortality, an episode of ALI/ARDS also substantially influences patient's long-term outcomes with functional deficits persisting up to five years after the episode of respiratory failure.[4]

Importantly, the clinical syndrome of ALI generally occurs as a complication of an initial predisposing acute injury such as pneumonia, aspiration, sepsis, trauma, shock, or massive transfusion.[5] However, only a fraction of patients (10-30%) with these initial injuries develop ALI/ARDS.[6, 7] Only 30% of ALI patients fulfill criteria for ALI within six hours of presentation to the emergency department (ED).[8] The majority of patients develop ALI a median of two days after hospital presentation (IQR 1-4 days). This period of time between hospital presentation and development of ALI presents a window of opportunity for interventions to prevent the development of ALI.

Recently, accumulating evidence suggests an important role for platelets in both ALI pathogenesis [9-11] and resolution.[12-14] Notably, preclinical data suggests that aspirin (ASA) can modulate many of the platelet-mediated processes involved in ALI development [11, 15, 16] and resolution.[17, 18] Proposed mechanisms for these protective effects include reduced thromboxane A_2,[9] P-selectin,[19] and platelet-derived chemokine (e.g. CCL5, CXCL4) [20] production, prevention of the formation of platelet-neutrophil aggregates[9] and neutrophil extracellular traps,[21, 22] and enhanced formation of anti-inflammatory lipid mediators such as

15-epi-lipoxin A4 (Figure 1).[17] Importantly, recent observational studies have also suggested a potential preventive role for antiplatelet therapy in patients at high risk for ALI.[23, 24] However, the evidence remains inconclusive and equipoise remains.

To further enhance our understanding of ASA's role in the prevention and/or mitigation of ALI, the Lung Injury Prevention Study (LIPS) group with the support of the United States Critical Illness and Injury Trials Group (USCIITG) as well as the National Heart, Lung and Blood Institute (NHLBI) have designed the Lung Injury Prevention Study with Aspirin (LIPS-A), a randomized clinical trial that aims to test the safety and efficacy of ASA in the prevention of ALI in patients determined to be at high risk. This paper describes the study procedures and planned analyses for this clinical trial.

METHODS AND ANALYSIS

Administrative Structure

To facilitate the conduct of the present investigation, as well as future ALI prevention studies, three specialized centers were established. The data and statistical coordinating center, responsible for data management, randomization, and pharmacy coordination, will reside at Mayo Clinic in Rochester, MN. The clinical coordinating center (CCC), responsible for the study conduct and safety monitoring, will reside at Beth Israel Deaconess Medical Center in Boston, MA. The biospecimen repository and Knowledge Translation Center, responsible for specimen management as well as the LIPS score and the checklist for lung injury prevention (CLIP) online screening tools, will reside at Montefiore Medical Center in Bronx, NY. The principal investigators from these three centers form the LIPS-A Executive Committee. This committee will collaboratively oversee all aspects of the study design and the protocol implementation.

Study Design

To test the hypothesis that ASA is associated with a reduced rate of incident ALI, the LIPS-A group has designed a multicenter, double-blind, placebo-controlled, parallel group, phase II randomized clinical trial. The ClinicalTrials.gov registration number is NCT01504867. An outline of the study design and study procedures appears in Figure 2.

Study Population

Adult patients aged 18 years and older at high risk for ALI on admission to the hospital will be enrolled. To facilitate the identification of those at high risk for ALI, the LIPS-A study will utilize the recently validated LIPS.[8] Patients will be considered at high risk for development of ALI based on a LIPS score of 4 or greater. Patients who fulfill criteria for ALI on hospital presentation or at any point prior to randomization will be excluded. A full list of exclusion criteria with the justification for each can be seen in Table 1.

Patients will be recruited from 14 clinical sites in the United States with experience in the identification and management of ALI. A full list of the participating institutions as well as each site's primary investigator can be seen in Appendix A and are indexed on ClinicalTrials.gov. The resulting study population is expected to be diverse and representative of the general population of patients at risk for ALI such that the study findings will be externally valid and generalizable to the broader academic community.

To facilitate patient enrollment, study coordinators at each participating institution will screen patients in the ED with a web-based LIPS calculator to determine each potential participant's risk for development of ALI. Eligible patients with a LIPS score ≥ 4 will be approached by study coordinators or study investigators for informed consent. Eligible patients will be enrolled and randomized within 12 hours of hospital presentation. This will allow for

maximal recruitment within the window of opportunity for interventions to prevent ALI development as our preliminary data show median time to ALI is two days after hospital admission.[8]

Interventions

Study drug: The first dose of study drug (ASA versus placebo) will be administered within the first 24 hours after presentation to the hospital, either by mouth or by nasogastric or orogastric tube. For patients randomized to the intervention arm, a generic aspirin 325 mg one-time loading dose on day 1 will be administered followed by generic aspirin 81 mg by mouth once daily for study days 2-7 or until hospital discharge or death, whichever occurs first. The intervention duration of seven days was chosen because > 85% of ALI/ARDS cases were noted to have developed during this time frame in our preliminary studies.[8] In support of the dosing scheme chosen for this investigation, a randomized clinical trial noted low-dose ASA at 81 mg daily was effective in elevating plasma levels of anti-inflammatory lipoxins and inhibiting platelet thromboxane activity with only a slight increase in effect at higher doses of ASA.[25, 26] All study medication doses (active treatment with ASA and placebo) will be in powder form of identical color, contained within capsules that can be opened and administered via a gastric tube.

Co-interventions: Important co-interventions will be standardized in all study patients. To this end, the investigative team has developed a web-based, computerized, interactive tool to standardize essential elements of care delivery such as mechanical ventilation, aspiration precautions, infection control, fluid management and transfusion in patients at risk. This tool is a checklist for lung injury prevention (CLIP).[2] A summary of the CLIP elements is listed in Table 2. Having identified high-risk patients early in the course of the illness with the LIPS calculation and having standardized the important elements of care delivery with the CLIP, we

expect to have optimized our ability to investigate whether ASA is a safe and effective agent in preventing ALI.

Related conditions and variables of interest: Additional conditions and variables of interest including pertinent baseline demographics and clinical characteristics such as age, sex, race, comorbidities, and all LIPS elements will also be recorded. Additional variables of note will include vital signs and laboratory values that are obtained during the course of routine care, APACHE IV scores, coadministration of statins, angiotensin converting enzyme-inhibitors and angiotensin-receptor blocking agents, insulin, amiodarone, or steroids; blood product administration, daily fluid status and vasopressor requirements. A full description of the schedule of events for this study protocol can be seen in Table 3.

Outcomes

Clinical Outcomes: The primary outcome is the development of ALI within seven days of hospital admission. ALI will be defined as requirement for invasive mechanical ventilation and fulfillment of the American-European consensus definition for ALI/ARDS.[27] Patients will be screened daily for respiratory failure and the partial pressure of arterial oxygen (PaO_2) to fraction of inspired oxygen (FiO_2) ratio will be calculated daily for those on mechanical ventilation. Patients ventilated with non-invasive ventilation will not be considered ALI/ARDS as our preliminary data showed that the majority (90%) of ALI patients are eventually intubated.[8] Investigators at each site will review structured online training for assessment of ALI as was used and described in the LIPS.[8] In addition, de-identified chest x-rays of the first five patients enrolled at each site will be sent to CCC for validation by the primary investigators. Any site with significant deviation will be re-trained. Each participating center's principal investigator will adjudicate the diagnosis of ALI/ARDS using standardized definitions. Patients

receiving invasive mechanical ventilation who, within a given 24-hour period, fulfill criteria for $PaO_2/FiO_2 < 300$ mm Hg, bilateral infiltrates consistent with ALI and not completely explained by heart failure, will be determined to have developed ALI. Given prior data suggesting poor agreement in the radiological interpretation of bilateral infiltrates on chest radiographs consistent with ALI,[28] a secondary review of all ALI cases and a random sample of non-ALI cases will be performed by an independent expert investigator who is blinded to the initial ALI/ARDS adjudication. Study participants who die or are discharged from the hospital prior to day 7, and had not met criteria for ALI at the time of death or discharge, will be adjudicated as not having developed ALI.

Secondary clinical outcome assessments will include changes in the lung injury score and sequential organ failure assessment score, as well as the number of ventilator-free days at hospital day 28 and intensive care unit (ICU) and hospital lengths of stay. Mortality will be assessed at discharge from the ICU, from the hospital, and at 28 days. In addition, hospital survivors will undergo a brief follow-up phone survey to assess functional status (Barthel Index), health related quality of life [QOL (SF-12)] and frailty (VES-13) at 6- and 12-months after enrollment.

Mechanistic Outcomes: Secondary analyses will include evaluations of the mechanisms by which anti-platelet agents (e.g., ASA) may modulate the development and progression of lung injury as well as a determination of the value of plasma biomarkers of lung injury in the prediction of ALI development in patients at risk (beyond clinical variables). The study will examine biomarkers previously found to be associated with the *development* of ALI/ARDS in at-risk individuals (Table 4). In addition, to better understand the mechanisms by which ASA may affect the development and progression of ALI, the study will also examine the effect of ASA on

ASA-triggered lipoxins, plasma thromboxane, and platelet-neutrophil aggregates. As it is likely that other important biomarkers in ALI may be identified in the future, plasma from consenting patients will be banked at the biorepository for future studies. Blood samples will be obtained at baseline (after randomization and before initiation of study intervention), on day two of study (approximately 24 hours after the first dose of study drug), and on day four of study (any time during day 4). For patients who provide consent relating to future genetic analyses, appropriate samples will be obtained.

Sample Size Estimation

The primary hypothesis for this investigation is that ASA (when compared to placebo) will result in a lower rate of incident ALI at day 7 following randomization. To adequately address this hypothesis, the sample size is estimated to be 200 participants per group (400 total). The assumptions involved in this calculation include the following: 1) the hypothesized placebo response rate will be 18%,[8] 2) the minimum clinically relevant effect is 10 percentage points, and 3) the type I error rate (alpha) = 0.10 (two-sided) (final alpha=0.0889 after interim analysis at 50% information fraction using O'Brien-Fleming-like alpha spending function). To be conservative during sample size estimation, the null proportion was shifted upwards to 25% (i.e., towards the region of maximum binomial variance) so that the initial sample size estimates are based on 25% vs. 15%. A chi-square test of proportions at the alpha = 0.10 level of significance will have 80% power to detect the 10 percentage point difference with 197 participants per group. Overall recruitment is rounded to 200 participants per group (400 total) to allow for minor attrition, although attrition is not expected to affect the ascertainment of primary outcome. At the hypothesized level of 18% vs. 8% and with the alpha adjusted for multiple interim looks, power with 200 participants per group is 90%. Thus, for the primary analysis 400 total

participants randomized 1:1 to placebo or ASA is anticipated to yield sufficient power to detect a clinically relevant difference in the incidence of ALI.

The Data and Statistical Coordinating Center will prepare weekly reports on the accrual process for the trial. The reports, which will be reviewed on the weekly executive committee calls, will include summarization of screening and randomization metrics. Detailed descriptions of exclusion criteria for disqualified study candidates will be provided and reviewed as well. Each clinical center has a target enrollment of 2 randomized participants per month. The reports will include a comparison of observed vs. expected accrual, by clinical center and overall for the trial. The randomization performance of each clinical center will be disseminated monthly to all study personnel through a study newsletter. If site-specific enrollment concerns are identified, methods for addressing these issues will be evaluated by the executive committee working with the site of interest. If a more pervasive and sustained gap between expected and observed participant accrual is identified, potential modifications to the inclusion and exclusion criteria of the protocol will be discussed. Any amendments to the inclusion and/or exclusion criteria deemed necessary by the executive committee will require approval by the DSMB as well as the IRB of each participating institution before implementation. If enrollment remains below plan, the inclusion of additional clinical sites will be considered as well.

Randomization and Blinding

LIPS-A will utilize centralized randomization software hosted by the Data and Statistical Coordinating Center. Randomization through the electronic data management system will be enabled upon electronic verification of inclusion and exclusion criteria and enrollment of the study participant by the clinical site investigators. Enrolled participants will be randomized in a 1:1 ratio to the ASA or placebo treatment arm using dynamic minimization[29] with a second

guess probability of 0.2. Randomization will be stratified by center. To maintain the double blind for the study, only the research pharmacist at each center will have electronic access to the unblinded treatment code for study medication preparation and dispensing. The rest of the site investigators and coordinating centers will be blinded to the actual treatment assignment. Emergency unblinding is available both electronically and through dispensing records at each pharmacy.

In the event the electronic randomization system is not functioning, the research pharmacist at each center has a sealed emergency randomization kit to enable offline randomization. A manual of operation governs the use of the emergency randomization process. Briefly, prior to use of the emergency process, approval of the coordinating centers is required. All attempts will be made to recover the system prior to the use of the offline procedure. Should the offline procedure be used, the electronic data management system will be updated to reflect the treatment assignment using the identification number contained within the randomization kit.

Statistical Methods

Conditional logistic regression will be used to test the primary hypothesis that early ASA administration will decrease the rate of ALI development. Clinical site will be treated as the stratification variable and conditioned out of the estimating equations. This approach is optimal in a clinical trial setting as it provides a test of null hypothesis that the ALI incidence is equal in the two treatment group and estimates the association in the event the null hypothesis is rejected (through the conditional odds ratio estimate). SAS PROC LOGISTIC™ (Cary, NC) will be used for estimation of the primary model.

This analysis will be supplemented by the Cochran-Mantel-Haenszel stratified analysis with odds ratios computed for each site. The Breslow-Day test will be used to examine the data

for potential effect modification (i.e., a "site effect"). In the event there is significant site-to-site variability in the estimated effect, stratified results will be reported for this phase II study. Evidence of heterogeneity of response at this phase of the study will yield invaluable preliminary data for the planning of future changes.

Planned interim analyses will be conducted at 50% of study participants enrolled. With the O'Brien-Fleming-like stopping boundaries, a final adjusted alpha of 0.08885 is anticipated; however, the final value may be changed depending on unplanned interim analyses (conducted at the request of the Data and Safety Monitoring Board [DSMB]) or slight deviations from the anticipated information milestones (0.50, 1.0). Stopping boundaries will be estimated using the LD Bounds package for the R system.

For the remaining continuous and dichotomous secondary endpoints, treatment group comparisons will be performed with respect to clinical outcomes as well as important prognostic factors at screening, baseline, and individual follow-up time points during the study duration. For continuous variables (e.g., age, weight, and laboratory assays), linear model techniques including *t*-tests, analysis of variance and analysis of covariance will be applied. Nonparametric procedures (e.g., the Wilcoxon rank sum test), will be used if data are not normally distributed and transformations of the data are not considered useful. Standard techniques for categorical data will be applied, including Fisher's exact test, Pearson χ^2 procedures, weighted least squares, and logistic regression analysis.

Longitudinal (or serially measured) endpoints will be evaluated by generalized linear models and linear mixed. Repeated measure analyses of binary endpoints will be analyzed using generalized estimating equations methods which do not require imputation of missing values, provided the data are ignorable missing.[30] Continuous dependent variables will utilize the

mixed model approach with emphasis on evaluating the trajectories of values over time. However, early improvement in these parameters may suggest a supportive, stabilizing role for ASA as a treatment option in patients at high risk of ALI. For the primary analysis, the clinical center will be treated as a "nuisance" parameter and conditioned out of the estimation routine. For secondary analyses, the clinical center will be used as a fixed covariate to account for differences across sites.

The safety endpoints (see below under "**Adverse Outcomes**") will be examined for all participants in the safety evaluable analysis set. Safety endpoints will include expected clinical events, including death, for this patient population and summarized by treatment group. Also, all serious and unexpected adverse events will be summarized by treatment group. Fisher's exact test will be used to estimate treatment differences in the incidence of each specified adverse event. No adjustments will be made for multiple hypothesis evaluations of safety endpoints. Adverse events will be summarized with groupings by body system. Other safety data (e.g., labs and assay data) will be listed, and when appropriate, summarized in tabular or graphical format.

Data Quality and Management

This investigation will utilize the Medidata Rave™ system for data management and storage as well as to assist with the randomization procedures. This product has been designed to facilitate multicenter clinical trials conducted under 21 CFR Part 11 requirements. This secure, web-based system provides robust data validation routines, custom reporting and straightforward integration with statistical software packages such as SAS (utilized for this investigation). The system is coupled with an integrated randomization module that uses a multidimensional dynamic allocation algorithm to minimize imbalances across multiple

dimensions including overall study, sites, factors and cross-factor strata. Specific details regarding the randomization process are given below.

ETHICS AND DISSEMINATION

Adverse Outcomes

Safety data including adverse events such as gastrointestinal ulcers, bleeding from any site, gastrointestinal discomfort, wheezing, rash, hives, angioedema, tinnitus, and mortality will be recorded. Adverse events will be defined as "unexpected," "expected," and "serious." As our patient population is by definition "critically ill," it is expected that they will have a number of unrelated adverse health events during the course of their hospital stay. Therefore, we will limit the scope of our adverse event monitoring and recording to the following:

1) Serious adverse events (SAEs) will be defined as:

- Death, *believed to be related to the study medication or procedures, or a death that is unexpected considering the acuity of a patient.*
- A life threatening experience *believed to be related to the study medication or procedures*
- Persistent or significant disability or incapacity *that is of greater frequency or severity than what would be normally expected in the course of critical illness.*
- An event that jeopardizes the human subject and may require medical or surgical treatment to prevent one of the preceding outcomes *and is not expected in the course of critical illness.*

2) Adverse events possibly related to aspirin administration will be defined as:

- Anaphylaxis / allergic reaction

- Gastrointestinal bleed / bleeding complications
- Transfusion requirements for suspected bleeding
- Acute kidney injury, defined as RIFLE stage "I" or greater
- Tinnitus
- Reye's syndrome

Role of the Data Safety and Monitoring Board

Reporting of SAEs will be conducted through the CCC. All centers will report SAEs within 24 hours of discovering the presence of the SAE. The CCC will report all potentially related SAEs to the DSMB and to NHLBI within 7 days of discovery. A summary report of the events will be provided to the DSMB prior to each DSMB meeting, at least every six months. Safety oversight will be under the direction of a DSMB whose members will be independent from the study operations. The safety endpoints will be examined for all eligible patients who sign informed consent and are enrolled in the study on an intent-to-treat basis. Safety endpoints will include expected clinical events, including death, for this patient population and summarized by treatment group. All serious and unexpected adverse events will be summarized by treatment group as well.

Ethics Approval

Approval of the protocol was obtained from the data safety and monitoring board as well as from NHLBI prior to enrolling the first study participant. In addition, approval of both the protocol and informed consent documents was required and obtained from the institutional review board of each participating institution prior to enrolling study participants at the respective study site. To ensure that each participating institution's informed consent documentation complied with NHLBI requirements and the Code of Federal Regulations Title 21

Part 50 Section 50.25, all informed consent forms were reviewed and approved by the CCC. Official documentation of all IRB approvals and all finalized informed consent forms have been collected and stored by the CCC.

Considerations for Continuation to a Phase III Clinical Trial

The decision to proceed with a phase III trial is formally outlined as follows:

1) Initiate Phase III Study: Demonstrated efficacy signal in addition to adequate safety profile. Criteria: Early termination for benefit at interim analysis or $p < 0.08885$ at final analysis (alpha=0.10 for study). Serious adverse event profile of ASA not statistically worse than placebo (95% confidence interval for the relative risk of any SAE covers the null value of RR=1.0).

2) Further Development Potentially Required: Weak efficacy signal. Criteria: Primary endpoint did not achieve *a priori* level of significance but there were at least a general consistency of secondary endpoints indicating propensity for efficacy with a larger sample size and/or more specific primary endpoint.

3) Abandon Treatment Platform: Harm (in efficacy or safety endpoints). Criteria: Study terminated early per recommendation by DSMB for safety and/or risk/benefit ratio concerns (i.e., stop for futility, harm, unacceptable risk profile, etc.).

Ancillary Studies

The LIPS-A group will encourage investigator-initiated ancillary study proposals that extend or complement the specific aims of the primary LIPS-A trial. As policy, all proposals will be reviewed by a separate Ancillary Studies and Publications Committee, both to ensure consistency with the goals and conduct of the main study and evaluate scientific merit and validity. Proposed studies may utilize data and/or samples already accrued during the LIPS-A trial or, when feasible, request additional data collection from participating sites. The

investigative and statistical plan will be reviewed *a priori*, with committee approval required before analysis begins. Where equivocal, review decisions will be referred to the LIPS-A Executive Committee. All reports, manuscripts or presentations derived from data obtained through the ancillary study process will require review and approval by the Ancillary Studies and Publications Committee prior to submission.

Protocol funding and role of the funding sources

This study is supported by the National Institutes of Health-National Heart Lung and Blood Institute (Grant Number U01-HL108712-01), the Mayo Clinic Center for Translational Science Activities (Grant Number KL2 RR024151), and the Mayo Clinic Critical Care Research Committee. Specifically, funding has been provided by each of these entities to support study personnel time and effort, protocol and data management development (Medidata Rave™), sample acquisition, processing and storage, and statistical support. These funding sources have had no specific influence on the scientific content of the study protocol. Similarly, the funding sources will have no direct role in the study conduct, nor data collection, analyses, or interpretation. The funding sources will also have no role in the writing or presentation of study results, nor decisions to submit for publication. The ultimate authority over each of these activities will be the executive committee of the LIPS-A study.

DISCUSSION

We have presented the study protocol and data analysis plans for the first phase II, multicenter randomized clinical trial that will test the efficacy and safety of a promising ALI prevention agent. Specifically, we have hypothesized that early administration of ASA to hospitalized patients who are at high risk for ALI, will be safe and will reduce the likelihood of progression to the full ALI phenotype. Secondarily, this investigation will glean important

mechanistic data on ASA's impact on the pathways believed important in ALI pathogenesis as well as the potential value of relevant biomarkers in the prediction of subsequent development of ALI. Finally, the results of this study will provide essential information on both the scientific merit and feasibility of a larger, phase III trial testing the role of ASA in the prevention of lung injury.

The persistent difficulty in translating promising pre-clinical therapies into the clinical setting has fostered interest in the potential development of effective ALI prevention strategies. Indeed, prevention of ALI has been identified as a key strategic priority for invested parties such as the NHLBI.[31] However, implementation of protocols aiming to test potential ALI prevention strategies have been historically hindered by an inability to accurately predict who is at risk for ALI. Moreover, the typically short interval between risk exposure and development of ALI as well as the small proportion of patients who progress to the full ALI phenotype following an ALI-related exposure has limited the feasibility of ALI prevention studies. In addition, the historic lack of standardization for numerous important co-interventions that confound the associations of interest (e.g., ventilator management, transfusion and resuscitation practices) has also limited our ability to test preventative strategies.

To this end, the recently validated LIPS score is a key element of the herein described study protocol.[8] Specifically, the LIPS score is expected to facilitate the identification of patients at greatest risk of progressing to ALI (a LIPS score ≥ 4 is expected to identify a subgroup of patients who have a risk of progressing to ALI that is greater than 18%). In addition, it is notable that this ALI risk assessment tool was validated using data collected within the first 6 hours after the initial evaluation in the ED. In an ALI prevention protocol such as

described herein, where the time to randomization is limited to 12 hours from presentation to the ED, the ability to accurately determine risk for ALI in such a time-efficient manner is critical.

A second notable strength of the current protocol is expected to be the implementation of the CLIP for standardizing important co-interventions that may otherwise confound our association of interest (ASA and ALI). During the period between hospital admission and the development of ALI, health care delivery factors (timely treatment of infection and shock, appropriate administration of fluid and transfusion therapies, prevention of aspiration, avoidance of large tidal volume ventilation), may be as important as individual biology in determining ALI development and outcome.[32-39] Moreover, a recent survey noted wide variation in clinical practices such as the existence of a sepsis protocol, use of low tidal volume ventilation, positive end-expiratory pressure, and restrictive transfusion practices, between hospitals and among the ED, ICU and operating room within hospitals.[40] Thus, to effectively investigate preventive strategies in ALI, the standardization of care delivery during the early phase of hospitalization would appear critical. Indeed, the ARDSNet investigators have repeatedly shown the value of standardization of clinical processes for ALI patients in clinical trials, allowing for determination of incremental benefit of new interventions.[41, 42] In the current investigation, standardization of care with best practices will help to reduce variability in the rates of ALI and the intensity of lung injury (noise) due to inconsistencies in care delivery. The result is expected to be an increased chance of seeing a beneficial clinical or biological effect from ASA and a better assessment of the potential side effects of ASA in this population.

Though the multicenter randomized clinical trial design, availability of a time-efficient risk assessment tool (LIPS score) and the standardization of important co-interventions with CLIP, as well as the robust study support and quality control offered through Metadata RAVE,

are clear strengths of the current study protocol, several important limitations with the planned investigation deserve note. Lung injury may be present at study entry even as clinical criteria for ALI are not fulfilled. Though a formal diagnosis of prevalent ALI is exclusionary, the molecular machinery will have been clearly set in motion in many of the study participants. Therefore, the study may be more accurately characterized as a prevention/early treatment trial rather than a pure prevention trial. Nonetheless, we have attempted to focus on the early period of ALI development by mandating a short interval from hospital presentation to randomization (12 hours) and a similarly short interval from hospital presentation to administration of the first study dose (24 hours). In addition, the study will exclude patients who presented to an outside hospital ED more than 12 hours before arrival at the enrolling site's facility. The study will also exclude those with ALI on hospital presentation or prior to randomization as well as those who are receiving mechanical ventilation through a tracheostomy tube prior to the current hospital admission (patient who is ventilator dependent) or those with a history of interstitial lung disease with chronic pulmonary infiltrates that may mimic ALI.

A second limitation relates to the intervention of ASA administration. Specifically, it is now well documented that more than 10% of the population will have a variable response to ASA or at least some form of aspirin resistance.[17] These patients may not benefit from ASA, even if ASA can modulate the development of lung injury. However, as part of this study, we will measure plasma thromboxane, a sensitive indicator of ASA resistance, to determine the prevalence of ASA resistance in patients at high risk for ALI. As such, sensitivity analyses, stratifying study participants by ASA resistance (as determined by changes in thromboxane levels), may allow us to determine whether the effect of ASA on ALI development is isolated to those susceptible to the actions of ASA. A related concern is the potential influence of

concomitant medications that may impact aspirin's ability to prevent or mitigate ALI (e.g., statins, corticosteroids). To address this concern, we will be collecting detailed information on concomitant medications and, when necessary, appropriate statistical adjustments will be made.

A third potential limitation with this study relates to a previously recognized major barrier to ALI prevention studies, namely feasibility. First and foremost, a substantial proportion of the target population may be expected to be receiving ASA on presentation to the ED, an exclusion criteria for the current protocol. Notably, however, our preliminary work suggests that upwards of two-thirds of the target population was not on ASA prior to admission. We also note that over the three months of the initial LIPS,[8] there were 800 patients who fulfilled study inclusion criteria of LIPS score ≥ 4 and did not fulfill the exclusion criteria of pre-existing ASA use, prevalent ALI, and elective surgery. Therefore, we believe that with 14 proposed sites and two years of planned enrollment, we will successfully meet our enrollment goals of 400 total patients. Also relating to feasibility, it is possible that some sites will be challenged by the short time interval allowed for patient enrollment as well as the short time to study drug administration. Though a valid concern, we believe the use of the LIPS score and the robust support offered through Metadata™ RAVE will greatly facilitate the enrollment and randomization procedures such that sites will indeed be successful in meeting these time-sensitive challenges.

A fourth and final limitation which deserves mention relates to the potential toxicity of the intervention of interest. Generally, ASA is well tolerated even in acutely ill, hospitalized patients in whom ASA is often continued during the hospitalization. As an example, in a study of ASA use up to the time of cardiac surgery, its continuation was not associated with an increase need for transfusion therapies.[43] Nevertheless, there may be injury associated with

the administration of aspirin. To address this concern, patients at risk for major complications from ASA therapy have been excluded from the study. Multiple stopping criteria for patients who experience adverse events have also been incorporated into the protocol. In addition, the more complete understanding of the safety profile of an intervention of interest is an important goal of all phase II trials. In this regard, the information gleaned from this study, adverse events included, is necessary to help decide on the merits of proceeding to a phase III clinical trial.

CONCLUSION

This manuscript describes the study protocol and analysis plans for the first phase II randomized clinical trial of the promising ALI prevention agent ASA. In addition to providing important information on the safety and efficacy of ASA in patients at high risk for ALI, the results of this trial will also inform the scientific community regarding the merit and feasibility of a more definitive phase III clinical trial. Importantly, the significance of this effort lies not only in the specific results which will be obtained from the study protocol, but equally in the infrastructure that will be created to facilitate the conduct of this trial. Specifically, the development and utilization of innovative methods to facilitate the early identification of high-risk patients with the LIPS and the standardization of potential confounding co-interventions with CLIP will address key barriers to studying ALI prevention measures and is expected to lay a framework for the meaningful conduct of future ALI prevention studies as well.

Table 1. Study Exclusion Criteria

Exclusion Criteria	Justification
Anti-platelet therapy on admission or within 7 days prior to admission	Inability to ethically randomize
Presented to outside hospital emergency department > 12 hours before arrival at site's facility	Inability to enroll within time frame for possible benefit
Inability to obtain consent and randomize within 12 hours of hospital presentation	Inability to enroll within time frame for possible benefit
Admitted for elective or emergency surgery	Aspirin not found to benefit this group in preliminary studies
ALI on hospital presentation or prior to randomization	Inability to adequately assess outcome
Presentation believed to be due to pure heart failure and no other known risk factors for ALI	Inability to adequately assess outcome
Receiving mechanical ventilation through a tracheostomy tube prior to current hospital admission (patient who is ventilator dependent)	Inability to adequately assess outcome
Bilateral pulmonary infiltrates present on admission only if the patient has a history of interstitial lung disease that can reasonably explain the current degree of pulmonary infiltrates present	Inability to adequately assess outcome
Allergy to aspirin or NSAIDs	Intervention contraindicated
Bleeding disorder*	Intervention contraindicated
Suspected active bleeding or judged to be at high risk for bleeding complications	Intervention contraindicated
Presence of acute kidney injury#	Intervention contraindicated
Severe chronic liver disease (Child-Pugh class C)	Intervention contraindicated

Active peptic ulcer disease (within past 6 months)	Intervention contraindicated
Pregnancy or breast feeding	Intervention contraindicated
Inability to administer study drug	Unable to administer study drug
Expected hospital stay < 48 hours	Incomplete study procedures and outcome data
Admitted for comfort or hospice care	Incomplete study procedures and outcome data
Patient, surrogate or physician not committed to full support (exception: a patient will not be excluded if he/she would receive all supportive care except for attempts at resuscitation from cardiac arrest)	Unable to assess primary outcome
Not anticipated to survive > 48 hours	Incomplete study procedures and outcome data
Previously enrolled in this trial	Violates the statistical assumption of sample independence
Enrollment in concomitant intervention study	Potential confounding and co-enrollment interactions

*Any disorder with known associated with increased risk of bleeding. Common disorders may include thrombocytopenia, disseminated intravascular coagulation, hemophilia, von Willebrand disease, oral anticoagulant therapy, or advanced liver disease with associated coagulation disorders. Platelet count < 50,000 or absence of platelet count in the previous 24 hours to allow for assessment of platelet status.

#Acute kidney injury defined as "R" or greater according to RIFLE criteria. ALI = acute lung injury, NSAIDs = non-steroidal anti-inflammatory medications

Table 2. Elements of CLIP – Checklist for Lung Injury Prevention

CLIP Elements	Definition
Lung protective mechanical ventilation	Tidal volume between 6-8 mL/kg predicted body weight and plateau pressure < 30 cm H_2O; PEEP ≥ 5 cm H_2O, minimize FiO_2 (target oxygen saturation 88-92% after early shock)
Aspiration precautions	Rapid sequence intubation supervised by experienced providers, elevated head of the bed, oral care with chlorhexidine, gastric acid neutralization in those not receiving tube feeds.
Adequate empiric antimicrobial treatment and source control	According to suspected site of infection, health care exposure, and immune suppression
Limiting fluid overload	Modified ARDSNet FACTT protocol after early shock (first 12 hours)
Restrictive transfusion	Hemoglobin target > 7 g/dL in the absence of acute bleeding and/or ischemia
Appropriate handoff of patients at risk	Providers taking care of patients at risk who require ICU admission will complete a structured handoff to the ICU team to continue with CLIP protocol for the duration of ICU stay

CLIP = checklist for lung injury prevention, PEEP = positive end-expiratory pressure, FiO_2 = fraction of inspired oxygen concentration, ARDSNet = Acute Respiratory Distress Syndrome Network, FACTT = fluid and catheter treatment trial, ICU = intensive care unit

Table 3: Schedule of Events

Event	Time of presentation until first dose (screen / baseline)	First dose until end of that calendar day (Day 1)	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	7 days after last dose	Hospital discharge or study Day 28, whichever comes first	6 Months	12 Months
Informed consent	X											
Inclusion/exclusion criteria	X											
Pregnancy test in women of childbearing potential	X											
Demographics	X											
Medical history	X											
LIPS score	X											
Randomization	X											
Study drug administration		X	X	X	X	X	X	X				
Clinical outcome assessment	X	X	X	X	X	X	X	X				
Safety labs: Cr and Hb	x		X	X	X	X	X	X				
Clinical data as available: labs, ABG	X	X	X	X	X	X	X	X				
CXR / ABG*		X	X	X	X	X	X	X				
CLIP	X	X	X	X	X	X	X	X				
AE/SAE monitoring		X	X	X	X	X	X	X	X	X		

Survival										X		X
Plasma biomarkers of ALI	X		X		X							
SF-12	X										X	X
Barthel Index	X										X	X
Vulnerable Elders Survey	X									X	X	
Brussels / SOFA composite										X		

*Chest x-ray required on days 1-7 ONLY IF patient is intubated, and DOES NOT have ALI / ARDS already, AND there is clinical evidence of worsening respiratory status defined as:

- Previous P/F ratio $\geq$ 300, with current P/F ratio < 300 and no chest x-ray within 24 hours.
- Prior P/F ratio < 300 and the PF ratio has fallen more than 10% AND no chest x-ray within 24 hours.
- In cases where an ABG is not available, the research team should obtain an ABG ***only if*** the S/F ratio falls below 315 consistently. The P/F ratio obtained from that ABG will be used to determine whether a chest x-ray needs to be obtained (as per criteria outlined above).
- If change in P/F ratio triggers the need for a chest x-ray or ABG as above, sites have 24 hours to conduct the necessary procedure. An ABG or chest x-ray obtained by the clinical team during that time period is also acceptable and obviates the need to obtain said procedure for the research study.

LIPS = Lung injury prevention, ALI = acute lung injury, LIS = lung injury severity score, Cr = creatinine, Hb = hemoglobin, ABG = arterial blood gas, CLIP = checklist for lung injury prevention, AE = adverse events, SAE = serious adverse events, SF-12 = 12-Item Short-Form Health Survey, SOFA = sequential organ failure assessment.

Table 4: Plasma biomarkers in ALI/ARDS

Plasma Biomarker	Importance in ALI/ARDS Development	Associated outcomes other than ALI/ARDS
Surfactant protein-D[44-46]	Reflect injury and ↑ permeability of alveolar epithelium	VFD, organ failure
Receptor for advanced glycation end products[47-49]	Reflects endothelial activation and injury	VFD,[49] organ failure,[49] ARDS after lung transplant[47]
Intercellular adhesion molecule-1[44, 50-53]	Reflects endothelial activation and injury	VFD,[51] organ failure[51]
Interleukin-6[44, 54-56]	Inflammation	VFD,[55] organ failure[55]
Interleukin-8[44, 48, 50, 54-56]	Inflammation	VFD, [55]organ failure[55]
Plasminogen activator inhibitor-1[44, 50, 57-61]	Activation of coagulation and inhibition of fibrinolysis	VFD,[61] organ failure[61]
von Willebrand factor[44, 48, 60, 62, 63]	Reflects endothelial activation and injury	organ failure
Protein C[44, 50, 59, 61-64]	Activation of coagulation and inhibition of fibrinolysis	ARDS after lung transplant,[47] VFD,[61] organ failure[61]

ALI = acute lung injury, ARDS = acute respiratory distress syndrome, VFD = ventilator-free days.

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Contributorship Statement:

Daryl J. Kor has contributed to all aspects of the study design. Dr. Kor is the principal investigator at the Data and Statistical Coordinating Center.

Daniel Talmor has contributed to all aspect of the study design. Dr. Talmor is the principal investigator at the Clinical Coordinating Center.

Valerie M. Banner-Goodspeed is the lead study coordinator at the Clinical Coordinating Center. Ms. Banner-Goodspeed has contributed to all aspects of the study protocol design as well as preparations for protocol implementation.

Rickey E. Carter is the lead statistician for this protocol. He has been involved in all aspects of the study design. He is the primary author of statistical descriptions in this manuscript.

Richard Hinds is the lead study coordinator at the Data and Statistical Coordinating Center. Mr. Hinds has contributed to all aspects of the study protocol design and preparations for protocol implementation.

Pauline Park has contributed in a consulting role to all aspects of the study design. Dr. Park is the primary author of the sections of this manuscript which detail the protocol's plans regarding ancillary studies.

Ognjen Gajic has contributed to all aspects of the study design. Dr. Gajic is the principal investigator for the grant mechanism that is the primary funding source for this protocol (Grant Number U01-HL108712-01).

Michelle N. Gong has contributed to all aspects of the study design. Dr. Gong is principal

investigator for the Biorepository and Knowledge Translation Center.

All named authors wrote or revised the manuscript and approved its final submitted version.

REFERENCES

1. Rubenfeld GD, Caldwell E, Peabody E, et al. Incidence and outcomes of acute lung injury. *N Engl J Med* 2005;**353**:1685-93.
2. Li G, Malinchoc M, Cartin-Ceba R, et al. Eight-year trend of acute respiratory distress syndrome: a population-based study in Olmsted County, Minnesota. *Am J Resp Crit Care Med* 2011;**183**:59-66.
3. Erickson SE, Martin GS, Davis JL, et al. Recent trends in acute lung injury mortality: 1996-2005. *Crit Care Med* 2009;**37**:1574-9.
4. Herridge MS, Tansey CM, Matté A, et al. Functional disability 5 years after acute respiratory distress syndrome. *N Engl J Med* 2011;**364**:1293-304.
5. Hudson LD, Milberg JA, Anardi D, et al. Clinical risks for development of the acute respiratory distress syndrome. *Am J Respir Crit Care Med* 1995;**151**:293-301.
6. Fowler AA, Hamman RF, Good JT, et al. Adult respiratory distress syndrome: risk with common predispositions. *Ann Intern Med* 1983;**98**:593-7.
7. Gong MN, Thompson BT, Williams P, et al. Clinical predictors of and mortality in acute respiratory distress syndrome: potential role of red cell transfusion. *Crit Care Med* 2005;**33**:1191-8.
8. Gajic O, Dabbagh O, Park PK, et al. Early identification of patients at risk of acute lung injury: evaluation of lung injury prediction score in a multicenter cohort study. *Am J Respir Crit Care Med* 2010;**183**:462-70.
9. Zarbock A, Ley K. The role of platelets in acute lung injury (ALI). Front Biosci 2009;**14**:150-8.

10. Zarbock A, Polanowska-Grabowska RK, Ley K. Platelet-neutrophil-interactions: linking hemostasis and inflammation. *Blood Rev* 2007;**21**:99-111.

11. Looney MR, Nguyen JX, Hu Y, et al. Platelet depletion and aspirin treatment protect mice in a two-event model of transfusion-related acute lung injury. *J Clin Invest* 2009;**119**:3450-61.

12. El Kebir D, József L, Pan W, et al. 15-epi-lipoxin A4 inhibits myeloperoxidase signaling and enhances resolution of acute lung injury. *Am J Respir Crit Care Med* 2009;**180**:311-9.

13. Fukunaga K, Kohli P, Bonnans C, et al. Cyclooxygenase 2 plays a pivotal role in the resolution of acute lung injury. *J Immunol* 2005;**174**:5033-9.

14. Maderna P, Godson C. Lipoxins: resolutionary road. *Br J Pharmacol* 2009;**158**:947-59.

15. Chelucci GL, Boncinelli S, Marsili M, et al. Aspirin effect on early and late changes in acute lung injury in sheep. *Intensive Care Med* 1993;**19**:13-21.

16. Sigurdsson GH, Vallgren S, Christenson JT. Influence of aspirin and steroids on acute lung injury after i.v. injection of a sclerosing agent. *Acta Chir Scand* 1989;**155**:163-70.

17. Yasuda O, Takemura Y, Kawamoto H, et al. Aspirin: recent developments. *Cell Mol Life Sci* 2008;**65**:354-8.

18. Jin SW, Zhang L, Lian QQ, et al. Posttreatment with aspirin-triggered lipoxin A4 analog attenuates lipopolysaccharide-induced acute lung injury in mice: the role of heme oxygenase-1. *Anesth Analg* 2007;**104**:369-77.

19. Tabuchi A, Kuebler WM. Endothelium-platelet interactions in inflammatory lung disease. *Vasc Pharmacol* 2008;**49**:141-50.

20. Grommes J, Alard J-E, Drechsler M, et al. Disruption of platelet-derived chemokine heteromers prevents neutrophil extravasation in acute lung injury. *Am J Resp Crit Care Med* 2012;**185**:628-36.

21. Clark SR, Ma AC, Tavener SA, et al. Platelet TLR4 activates neutrophil extracellular traps to ensnare bacteria in septic blood. *Nature Med* 2007;**13**:463-9.

22. Narasaraju T, Yang E, Samy RP, et al. Excessive neutrophils and neutrophil extracellular traps contribute to acute lung injury of influenza pneumonitis. *Am J Pathol* 2011;**179**:199-210.

23. Erlich JM, Talmor DS, Cartin-Ceba R, et al. Prehospitalization antiplatelet therapy is associated with a reduced incidence of acute lung injury. A population-based cohort study. *Chest* 2011;**139**:289-95.

24. Kor DJ, Erlich J, Gong MN, et al. Association of prehospitalization aspirin therapy and acute lung injury: results of a multicenter international observational study of at-risk patients. *Crit Care Med* 2011;**39**:2393-400.

25. Patrono C, Garcia Rodriguez LA, Landolfi R, et al. Low-dose aspirin for the prevention of atherothrombosis. *N Engl J Med* 2005;**353**:2373-83.

26. Chiang N, Bermudez EA, Ridker PM, et al. Aspirin triggers antiinflammatory 15-epi-lipoxin A4 and inhibits thromboxane in a randomized human trial. *Proc Natl Acad Sci USA* 2004;**101**:15178-83.

27. Bernard GR, Artigas A, Brigham KL, et al. The American-European Consensus Conference on ARDS. Definitions, mechanisms, relevant outcomes, and clinical trial coordination. *Am J Respir Crit Care Med* 1994;**149**:818-24.

28. Rubenfeld GD. Interobserver variability in applying a radiographic definition for ARDS. *Chest* 1999;**116**:1347-53.

29. Pocock SJ, Simon R. Sequential treatment assignment with balancing for prognostic factors in the controlled clinical trial. *Biometrics* 1975;**31**:103-15.

30. Diggle P, Liang K, Zeger S. Analysis of Longitudinal Data. New York: Oxford University Press, Inc.; 1994.

31. Spragg RG, Bernard GR, Checkley W, et al. Beyond mortality: future clinical research in acute lung injury. *Am J Respir Crit Care Med* 2010;**181**:1121-7.

32. Gajic O, Dara SI, Mendez JL, et al. Ventilator-associated lung injury in patients without acute lung injury at the onset of mechanical ventilation. *Crit Care Med* 2004;**32**:1817-24.

33. Rana R, Fernandez-Perez ER, Khan SA, et al. Transfusion-related acute lung injury and pulmonary edema in critically ill patients: a retrospective study. *Transfusion* 2006;**46**:1478-83.

34. Gajic O, Rana R, Winters JL, et al. Transfusion-related acute lung injury in the critically ill: prospective nested case-control study. *Am J Respir Crit Care Med* 2007;**176**:886-91.

35. Gajic O, Rana R, Mendez JL, et al. Acute lung injury after blood transfusion in mechanically ventilated patients. *Transfusion* 2004;**44**:1468-74.

36. Gajic O, Frutos-Vivar F, Esteban A, et al. Ventilator settings as a risk factor for acute respiratory distress syndrome in mechanically ventilated patients. *Intensive Care Med* 2005;**31**:922-6.

37. Gajic O, Dzik WH, Toy P. Fresh frozen plasma and platelet transfusion for nonbleeding patients in the intensive care unit: benefit or harm? *Crit Care Med* 2006;**34**:S170-3.

38. Fernandez-Perez ER, Keegan MT, Brown DR, et al. Intraoperative tidal volume as a risk factor for respiratory failure after pneumonectomy. *Anesthesiology* 2006;**105**:14-8.

39. Khan H, Belsher J, Yilmaz M, et al. Fresh-frozen plasma and platelet transfusions are associated with development of acute lung injury in critically ill medical patients. *Chest* 2007;**131**:1308-14.

40. Hou P, Cohen J, Elie-Turenne M, et al. A survey on behalf of USCIITG-LIPS investigators: toward standardization of a checklist for lung injury prevention (CLIP). *Crit Care Med 2010*;**38**:576.

41. Brower RG, Lanken PN, MacIntyre N, et al. Higher versus lower positive end-expiratory pressures in patients with the acute respiratory distress syndrome. *N Engl J Med* 2004;**351**:327-36.

42. Wiedemann HP, Wheeler AP, Bernard GR, et al. Comparison of two fluid-management strategies in acute lung injury. *N Engl J Med* 2006;**353**:2564-75.

43. Gerrah R, Elami A, Stamler A, et al. Preoperative aspirin administration improves oxygenation in patients undergoing coronary artery bypass grafting. Chest 2005;**127**:1622-6.

44. Ware LB, Koyama T, Billheimer DD, et al. Prognostic and pathogenetic value of combining clinical and biochemical indices in patients with acute lung injury. *Chest* 2010;**137**:288-96.

45. Eisner M, Parsons P, Matthay M, et al. Plasma surfactant protein levels and clinical outcomes in patients with acute lung injury. *Thorax* 2003;**58**:983-8.

46. Greene KE, Wright JR, Steinberg KP, et al. Serial changes in surfactant-associated proteins in lung and serum before and after onset of ARDS. *Am J Respir Crit Care Med* 1999;**160**:1843-50.

47. Christie JD, Shah CV, Kawut SM, et al. Plasma levels of receptor for advanced glycation end products, blood transfusion, and risk of primary graft dysfunction. *Am J Respir Crit Care Med* 2009;**180**:1010-5.

48. Fremont RD, Koyama T, Calfee CS, et al. Acute lung injury in patients with traumatic injuries: utility of a panel of biomarkers for diagnosis and pathogenesis. *J Trauma* 2010;**68**:1121-7.

49. Calfee CS, Ware LB, Eisner MD, et al. Plasma receptor for advanced glycation end products and clinical outcomes in acute lung injury. *Thorax* 2008;**63**:1083-9.

50. McClintock D, Zhuo H, Wickersham N, et al. Biomarkers of inflammation, coagulation and fibrinolysis predict mortality in acute lung injury. *Crit Care* 2008;**12**:R41.

51. Calfee CS, Eisner MD, Parsons PE, et al. Soluble intercellular adhesion molecule-1 and clinical outcomes in patients with acute lung injury. *Intensive Care Med* 2009;**35**:248-57.

52. Agouridakis P, Kyriakou D, Alexandrakis MG, et al. The predictive role of serum and bronchoalveolar lavage cytokines and adhesion molecules for acute respiratory distress syndrome development and outcome. *Respir Res* 2002;**3**:25.

53. Covarrubias M, Ware LB, Kawut SM, et al. Plasma intercellular adhesion molecule-1 and von Willebrand factor in primary graft dysfunction after lung transplantation. *Am J Transplant* 2007;**7**:2573-8.

54. Bouros D, Alexandrakis MG, Antoniou KM, et al. The clinical significance of serum and bronchoalveolar lavage inflammatory cytokines in patients at risk for acute respiratory distress syndrome. *BMC Pulm Med* 2004;**4**:6.

55. Parsons PE, Eisner MD, Thompson BT, et al. Lower tidal volume ventilation and plasma cytokine markers of inflammation in patients with acute lung injury. *Crit Care Med* 2005;**33**:1-6.

56. Meduri GU, Headley S, Kohler G, et al. Persistent elevation of inflammatory cytokines predicts a poor outcome in ARDS. Plasma IL-1 beta and IL-6 levels are consistent and efficient predictors of outcome over time. *Chest* 1995;**107**:1062-73.

57. Prabhakaran P, Ware LB, White KE, et al. Elevated levels of plasminogen activator inhibitor-1 in pulmonary edema fluid are associated with mortality in acute lung injury. *Am J Physiol Lung Cell Mol Physiol* 2003;**285**:L20-8.

58. Ware LB, Fang X, Matthay MA. Protein C and thrombomodulin in human acute lung injury. *Am J Physiol Lung Cell Mol Physiol* 2003;**285**:L514-21.

59. Christie JD, Robinson N, Ware LB, et al. Association of protein C and type 1 plasminogen activator inhibitor with primary graft dysfunction. *Am J Respir Crit Care Med* 2007;**175**:69-74.

60. Ware LB, Conner ER, Matthay MA. von Willebrand factor antigen is an independent marker of poor outcome in patients with early acute lung injury. *Crit Care Med* 2001;**29**:2325-31.

61. Ware LB, Matthay MA, Parsons PE, et al. Pathogenetic and prognostic significance of altered coagulation and fibrinolysis in acute lung injury/acute respiratory distress syndrome. *Crit Care Med* 2007;**35**:1821-8.

62. Rubin DB, Wiener-Kronish JP, Murray JF, et al. Elevated von Willebrand factor antigen is an early plasma predictor of acute lung injury in nonpulmonary sepsis syndrome. *J Clin Invest* 1990;**86**:474-80.

63. Ware LB, Eisner MD, Thompson BT, et al. Significance of von Willebrand factor in septic and nonseptic patients with acute lung injury. *Am J Respir Crit Care Med* 2004;**170**:766-72.

64. Matthay MA, Ware LB. Plasma protein C levels in patients with acute lung injury: prognostic significance. *Crit Care Med* 2004;**32**:S229-32.

Appendix A. Lung Injury Prevention Study with Aspirin (LIPS-A) Coordinating Center Personnel and Site Investigators

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