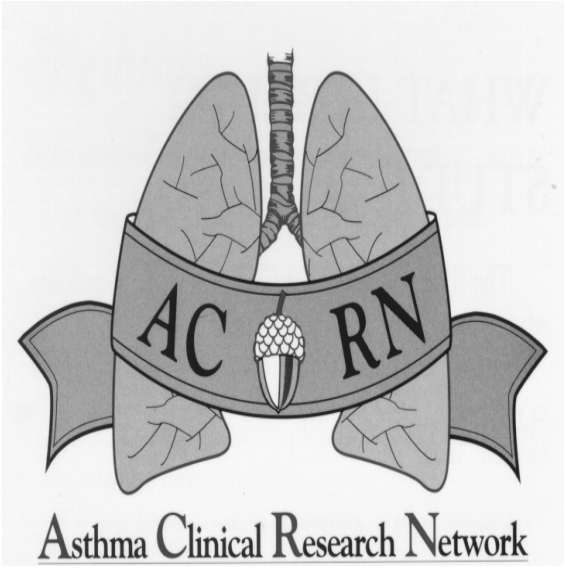


Asthma Clinical Research Network

Predicting Responses for Inhaled Corticosteroid Efficacy (PRICE)

Study Protocol

Version 3.5

I. Purpose:

1. To identify the characteristics (biomarkers) of asthmatic subjects, associated with "poor" and "good" responses to inhaled corticosteroid (ICS) therapy, as defined by the changes in FEV_1 and PC_{20}.

2. To determine whether unresponsiveness to ICS therapy as defined by a $< 5\%$ increase in FEV_1 is associated with loss of lung elastic recoil, and whether unresponsiveness as defined by a < 1 doubling dose increase in PC_{20} is associated with increase in upstream airway resistance.

3. To conduct a pilot project on whether the increase in FEV_1 after 6 weeks of ICS therapy predicts benefit to asthma control from continued treatment.

II. Background and Rationale

A. Introduction

ICS are considered to be the anti-inflammatory treatment of choice for mild, moderate, and severe persistent asthma (1,2). It is, thus, generally assumed by the medical community that all patients respond to ICS. However, Malmstrom and colleagues (3) reported that approximately 35% of patients on inhaled beclomethasone at 400 mcg/d had a FEV_1 response of less than 5%. Although they reported an overall exacerbation rate of approximately 7.5% (3 month study) on ICS, no mention was made as to whether the exacerbation rate was different among those with a good vs. poor FEV_1 response.

The ACRN has recently completed two studies (DICE – Dose of Inhaled Corticosteroids with Equisystemic Effect and MICE – Measuring Inhaled Corticosteroid Efficacy) to determine the mcg dose of ICS that produces similar cortisol suppression (DICE) and using that information to determine efficacy based on equisystemic effect (MICE). In further analyzing the MICE data (4), it became clear that 25-30% of subjects on either beclomethasone dipropionate CFC (CFC-BDP) or fluticasone propionate (FP) had a poor response in regard to FEV_1 or PC_{20} (Figure 1). This held true for both groups, even with maximum ICS dose of FP (dry powder) of 2000 mcg as the final dose schedule. What we do not know is if this has any effect in regard to asthma exacerbations.

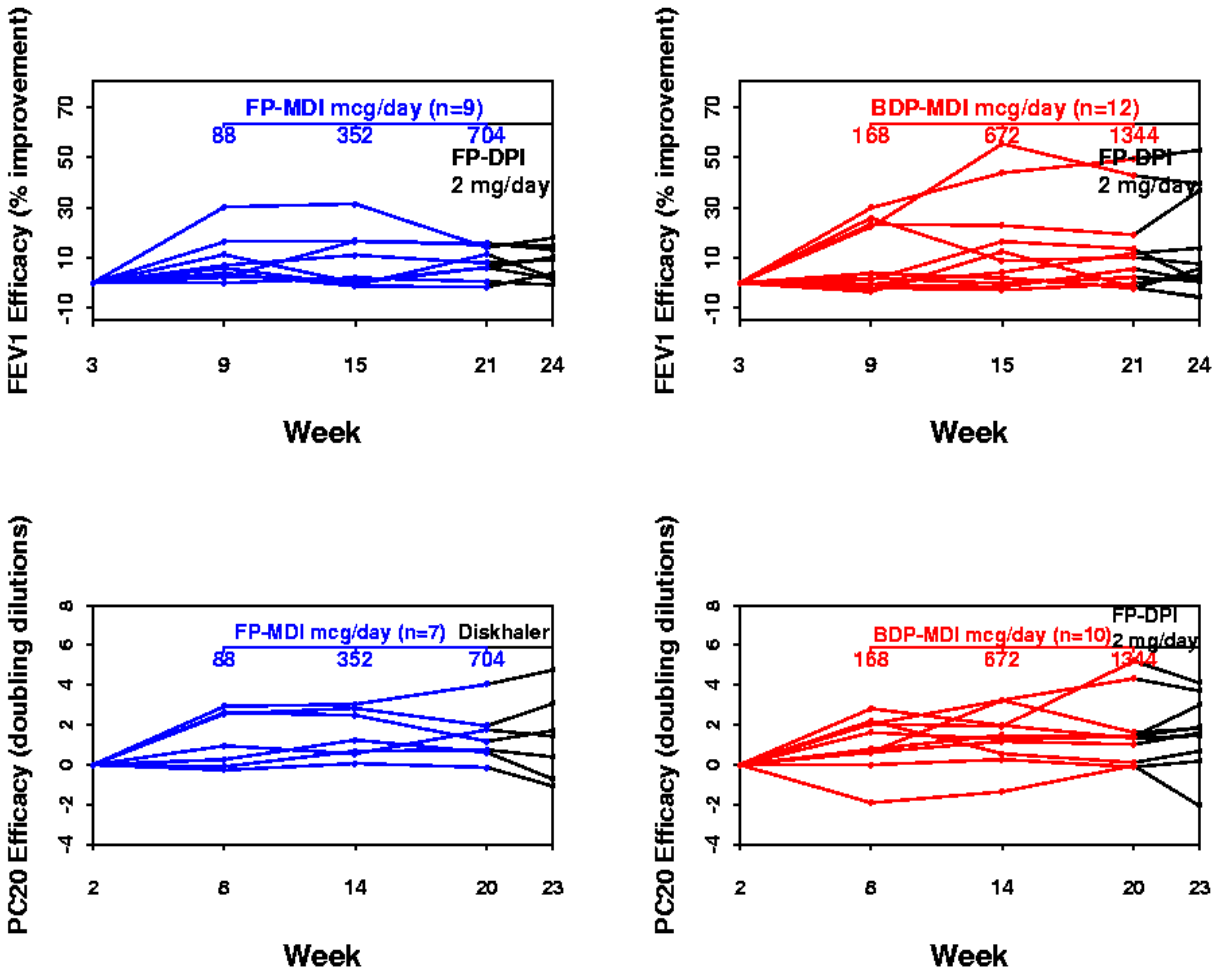


Figure 1. This figure demonstrates the change in FEV_1 (% improvement) and PC_{20} (doubling dilutions) with increasing doses of fluticasone (FP) and beclomethasone CFC (CFC-BDP). From week 3-9 the fluticasone propionate (FP) dose was 88 mcg/d; week 9-15 352 mcg/d; and week 15-21 704 mcg/d. For beclomethasone dipropionate CFC (CFC-BDP) the doses were 168, 672, and 1344 mcg/d, respectively. For both FP and CFC-BDP groups, week 21-24 all subjects were switched to FP dry powder at 2000 mcg (2 mg)/day (4).

What we additionally found in the MICE study was that there may be biomarkers that predict a poor, marginal, and good response (Table 1).

Table 1. Median values (1st quartile, 3rd quartile) of baseline predictors of response and Spearman correlation p-values for the test of association

	% Improvement in FEV_1			3-group P-value	<5% vs. ≥15% P-value
Predictor	**≤5% (n=8)**	**6-14% (n=5)**	**≥15% (n=8)**		
Exhaled nitric oxide (ppb)	11.1 (7.9, 14.2)	21.6 (15.1, 31.5)	17.6 (16.1, 23.0)	0.002	0.002
Maximum reversibility (FEV_1 % change)	8.8 (7.1, 10.7)	9.1 (6.4, 9.5)	25.2 (15.8, 54.5)	0.007	0.002
FEV_1 / FVC ratio	0.73 (0.68, 0.78)	0.68 (0.67, 0.73)	0.63 (0.53, 0.70)	0.025	0.041
	Change in PC_{20}			3 group P-value	<1 vs. >3 P-value
	<1 dd (n=5)	**1 – 3 dd (n=14)**	**>3 dd (n=7)**		
Sputum eosinophils + 0.2 (%)+	0.30 (0.20, 0.55)	1.3 (0.6, 4.7)	3.6 (3.4, 7.8)	0.011	0.013
Duration of asthma++	≥15 (80%)	≥15 (69%)	5-14 (50%)	0.039	0.088

+ The 0.2 for EOS % is added to avoid division by zero errors when evaluating values relative to baseline in additional analyses that were performed (based on 500 cells being read from each slide, adding 0.2 is equivalent to adding one count of an EOS cell to each slide)
+ + Median and percentage reported

Poor, marginal, and good response to ICS in regard to FEV_1 and PC_{20} are seen in Table 2.

Table 2. Baseline Prediction Biomarkers of Response

	FEV_1		PC_{20}	
	ENO*	BD* Response	Sputum EOS*	Duration of asthma
Good	≥15 ppb	≥15%	≥2%	<10 years
Marginal	Only one of above		Only one of above	
Poor	None of above		None of above	

*ENO = expired nitric oxide; BD = bronchodilator; EOS = eosinophils

This relatively new concept that all asthmatic patients do not respond to ICS needs to be investigated by a dispassionate group of investigators. The important issue is how a "response" is defined. Since our data (MICE, see above) support other investigators' findings (3) that approximately 30% of asthmatics do not respond to ICS as measured by FEV_1, does this mean that ICS do not promote a very important outcome, i.e., asthma control in these patients? Or, is the improvement in FEV_1 unrelated to the promotion or maintenance of asthma control produced by ICS treatment?

This protocol will be the first step in understanding one of the most important questions—do all asthmatics need ICS? There is a "ground swell" in the medical community that it is unethical to withhold or withdraw ICS therapy from asthmatic patients enrolled in a clinical trial on ICS. We received multiple letters to the editor for our JAMA publications on the SOCS/SLIC studies claiming that our use of a placebo control group was unethical. The ACRN feels that it may be unethical to place all asthma patients on ICS when, perhaps, one-third do not benefit, but are placed at increased risk of long-term side effects such as osteoporosis, cataracts, and skin thinning/bruising (5).

The proposed protocol will enroll subjects who have been steroid naïve (no inhaled or systemic corticosteroids) for at least 4 weeks (n=80, see below), and then place them on ICS for 6 weeks. This will allow for the baseline biomarkers to be determined and evaluate if they indeed predict "responders" and "non-responders" as defined by change in FEV_1 and PC_{20}. Then, stratified by FEV_1 response, one-half of the subjects will be withdrawn from ICS and one-half continued on ICS for 16 weeks. This final phase of the study, i.e. a pilot study, will enable us to better understand how these groups "respond" in regard to asthma control. We fully realize that 16 weeks is a relatively short interval especially for examining the relationship between the acute physiologic response and the prevention of asthma exacerbations, but we nonetheless believe this study will provide important information upon which future studies can be based.

We specifically propose to examine physiologic disturbances among the change in characteristics for correlation with short-term FEV_1 and PC_{20} responses to ICS. Two tests of physiologic function that are of particular interest are upstream airway resistance and lung recoil. Our interest in upstream resistance stems from observations made by Wagner and coworkers (6). These investigators used a wedged bronchoscopic technique to measure airways resistance in the peripheral lung of asymptomatic asthma patients with near-normal spirometric values and normal subjects (6). Despite their apparent lack of pulmonary impairment, the asthma patients had significantly (7-fold) increased peripheral airway resistance (PAR) compared to controls. Additionally, after removing one outlier the PAR was correlated to bronchial hyperresponsiveness ($r = 0.8$). Wagner and colleagues also examined the hyperresponsiveness in the small airways by measuring changes in peripheral resistance after histamine instillation using a wedged bronchoscopic technique (7). Peripheral resistance doubled at lower average concentrations of histamine in asthma patients compared to normal controls. The small airway response to histamine in the asthma patients was additionally correlated with whole lung responsiveness. While isoproterenol completely reversed the histamine-associated increase in peripheral resistance in normal subjects, it only partly reversed this increase in the asthma patients. This suggests that both central and distal airways are involved in bronchial hyperresponsiveness measurements.

In a model of the human tracheobronchial tree, dynamic changes in airway resistance have been simulated by applying various values for airway smooth muscle shortening and airway wall thickness (8). To establish a relationship between wall area and airway internal perimeter, data from postmortem measurements of lung tissue from asthma patients and from normal individuals, covering a wide range of airway sizes, were utilized (8). The analysis revealed that increases in peripheral airway wall thickness, even in amounts too small to affect baseline airway resistance, were nevertheless capable of dramatically affecting airway narrowing caused by smooth muscle shortening. Particularly revealing was that narrowing of the peripheral airways, and not the large airways, had the most pronounced effect on maximal airway resistance. The results are even more striking given the fact that the contribution of small airway resistance to total pulmonary resistance is relatively low, with estimates ranging from 10% to 29% (9-10).

Our interest in examining lung recoil for a possible relationship to the short-term responses to ICS treatment stems from the findings of Gelb and Zamel (11). These investigators recently demonstrated in asthmatics with fixed (poor beta-agonist responders) airway function that the P-V curve was shifted "up and to the left" as is for emphysema patients, i.e., loss of elastic recoil. However, these asthmatic subjects did not have emphysema based on high resolution CT scans and normal DLCO tests.

In summary, we feel the measurements of lung recoil and upstream resistance measurement will aid in identifying possible reasons for differences in the short-term responses to ICS treatment.

B. Research Hypotheses and Specific Aims

Hypothesis 1 (Primary): The lack of an ICS response defined by (1) change in FEV_1 and (2) change in PC_{20} is correlated with the following baseline biomarkers in the following way (see Table 2 above):

1. Bronchodilator response to a beta-2 agonist <15%
2. eNO <15 ppb
3. Sputum eosinophils <2%
4. Duration of asthma >10 years

Specific Aim 1. To determine predictive biomarkers for ICS response

Hypothesis 2a (Secondary): The lack of an FEV_1 response to ICS is correlated with a decrease in elastic recoil. Thus, the biomarkers of beta-2 agonist bronchodilator response of <15% and eNO <15 ppb are also correlated to a decrease in elastic recoil.

Hypothesis 2b (Secondary): The lack of a PC_{20} response to ICS is correlated with increased upstream resistance. Thus, the biomarkers of sputum eosinophils <2% and duration of asthma >10 years are also correlated to increased upstream resistance.

Specific Aims 2a,b. To determine if loss of elastic recoil or increased upstream resistance is associated with poor responsiveness to ICS therapy, as reflected by changes in FEV_1 and/or PC_{20} and to the associated biomarkers.

C. Pilot Study

We suggest that among subjects who have a poor short-term response to ICS, the rates of poor asthma control and of asthma exacerbations will be similar among those continued on ICS and among those in whom ICS treatment is withdrawn. Conversely, among subjects with a good short-term response to ICS the rates of poor asthma control and of asthma exacerbations will be lower among those continued on ICS therapy versus those in whom ICS treatment is withdrawn and placebo treatment is substituted.

D. Research Questions

Responsiveness to inhaled corticosteroid treatment can be measured in many different ways. Four important dimensions of response are: improvement in lung function (FEV_1); improvement in bronchial hyperresponsiveness (BHR); improvement in asthma control; and prevention of exacerbations. Each of these dimensions may need to be considered in categorizing asthmatic patients as ICS "responders" and "non-responders." It would be a great advantage to practicing physicians if biomarkers could be used to predict which outcomes would be affected by ICS treatment. This would enable physicians to decide quickly whether a patient would benefit from prolonged treatment with an inhaled corticosteroid, a treatment associated with a low but real risk of important toxicities. This study will answer the following questions:

1. Are there biomarkers that can rapidly predict poor and good FEV_1 and/or PC_{20} responses to ICS?
2. Is there a physiologic mechanism to explain the poor and good FEV_1 and PC_{20} ICS response?
3. The pilot study will give insight into the following question. Is a good FEV_1 and/or PC_{20} response to ICS associated with good asthma control and lack of exacerbation while on ICS? Conversely, is a poor FEV_1 and/or PC_{20} response to ICS associated with unchanged asthma control and a lack of asthma exacerbations upon withdrawal of ICS?

III. Protocol

A. Subjects

Eighty asthmatic subjects 18-55 years of age will be enrolled at the six Clinical ACRN Centers. There will be at least 50% female and 33% ethnic minority. Subjects will be recruited from "standing" populations of the participating centers (except randomized MICE study subjects will be excluded), by advertisement, and by physician referral. The ACRN Data Coordinating Center (DCC) will distribute monthly accrual reports of subjects entered by gender, age, and ethnicity for each center.

B. Inclusion Criteria

1. Male and female subjects 18-55 years of age. This age range was used in the MICE study and will be used in this study.
2. History of asthma with baseline FEV_1 55-85% of predicted.
3. Methacholine $PC_{20} \leq 12$ mg/ml at time of study entry.
4. No inhaled (orally) or systemic corticosteroids for 4 weeks prior to initial enrollment.
5. Nonsmoker (less than 10 pack-years and no smoking within the previous year).
6. Ability to provide informed consent, as evidenced by signing a copy of the consent form approved by the Institutional Review Board of the subject's respective study institution.
7. Compliance during run-in period in regard to medication and protocol adherence.
8. For heterosexual females, use of reliable contraception throughout the study.

C. Exclusion Criteria

1. Presence of lung disease other than asthma.
2. Significant medical illness other than asthma.
3. History of respiratory tract infection within the 6 weeks prior to screening visit.
4. History of significant exacerbation of asthma within the 6 weeks prior to screening visit.
5. Receiving hyposensitization therapy other than an established maintenance (continuous for 3 months duration or longer) regimen.
6. Inability, in the opinion of the Study Investigator, to coordinate use of a metered-dose or dry powder inhaler or comply with medication regimens, or inability to comply during the run-in period.
7. Pregnancy or lactation. If potentially able to bear children, not using an acceptable form of birth control (see ACRN MOP).
8. Inability to perform required study procedures.
9. Use of any drugs listed in Table 3 below during the designated washout period prior to Visit 1 or intention to take the drug during the study.
10. Randomized subject in ACRN MICE Protocol.
11. Incomplete/missing data for specific baseline biomarkers measured at Visit 3 and Visit 4. Specifically, subjects will be excluded if they have incomplete data for exhaled Nitric Oxide, maximum reversibility, or sputum eosinophil count at Visit 3 or Visit 4.

Table 3

Drugs to be withheld throughout the study	**Washout prior to Visit 1**
Cromolyn/Nedocromil	≥2 weeks
Leukotriene modifiers (zileuton, zafirlukast, montelukast)	≥2 weeks
Oral beta-adrenergic agonists	>48 hours
Monoamine oxidase inhibitors	≥4 weeks
Tricyclic, heterocyclic, and tetracyclic antidepressants	≥4 weeks
Beta-blockers	>48 hours
Salmeterol, formoterol	≥48 hours
Anticholinergics	≥48 hours
Any theophylline product	≥48 hours
Antihistamines	≥72 hours
Drugs withheld prior to pulmonary function and/or methacholine per MOP	**Specified time period**
Albuterol	≥6 hours
Fexofenadine, loratadine, desloratadine	≥48 hours
Methylxanthine-containing foods or beverages (e.g., coffee, tea)	≥6 hours
Alcohol-containing foods or beverages	≥6 hours

D. Overview (Figure 2)

This protocol is designed to answer the first two important questions discussed above (Section II.D) in regard to how subjects respond to ICS, and to give insight into the third question (pilot study) in order to design a future study.

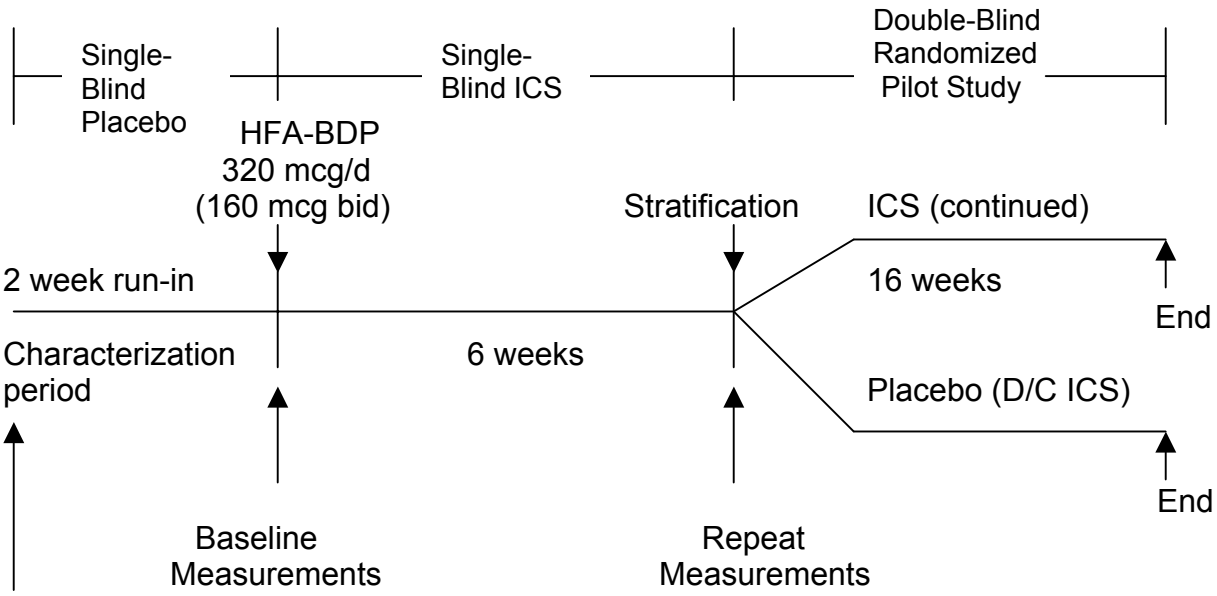
The protocol consists of a 2-week single-blind placebo run-in period for subject characterization. Characterization will be as follows:

- Mean FEV_1 (3 measurements)
- Mean AM and PM peak flow measurements (measured daily)
- Mean Asthma Control Questionnaire score (3 measurements)
- Mean daily ß$_2$ agonist use (measured daily)
- Mean PC_{20} (3 measurements)

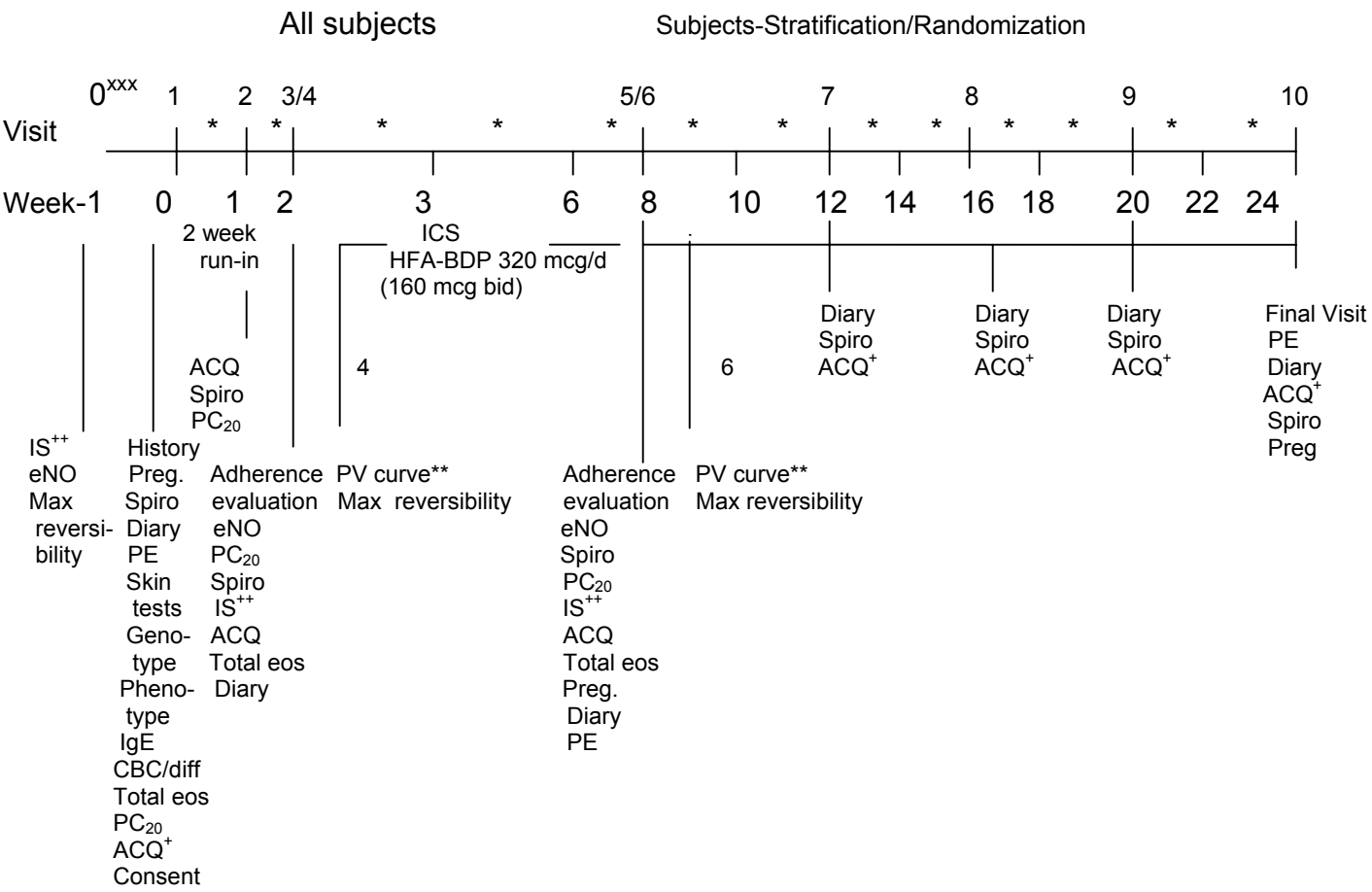
At the end of this 2 -week run-in period, biomarkers (bronchodilator response, eNO, sputum eosinophils, duration of asthma), PV curve, and PC_{20} will be measured, after which all subjects will be placed on single-blind ICS (beclomethasone dipropionate HFA [HFA-BDP] 160 mcg bid) for 6 weeks. At this point all measurements will again be evaluated. There will then be a randomization (stratified based on good, marginal, and poor FEV_1 responders from the 6-week ICS second phase) to double-blind continuation of ICS or complete cessation of ICS with placebo substitution. This is the pilot

portion of this study. Evaluation of asthma control and exacerbations are the outcome measure for this period, which terminates at 16 weeks post-randomization.

Figure 2.
Protocol Overview



Baseline
Characteristics



*AM/PM Peak flows and daily symptoms diary
**PV curves will be performed in a subset of subjects (all subjects at 3 ACRN Centers) at Visits 4 and 6.
+ACQ = Asthma Control Questionnaire
++ IS = Induced sputum
xxx = Optional. Required only for subjects re-enrolling after wash-out period following significant exacerbation during the ICS run-in phase.

Weeks 3, 6, 10, 14, 18, 22 – Phone Calls
Visit 4 occurs 3-7 days after Visit 3
Visit 6 occurs 3-7 days after Visit 5

For both groups, subjects will return to the clinic for their final visit within 3 days of exacerbation or at the 24-week point, whichever comes first.

E. Asthma Control

1. Asthma control will be measured during each of the three study periods: characterization; ICS; and randomization.

2. For specifics of characterization run-in period, See III. D. above. Although these subjects are not on ICS at enrollment, safety criteria will be used to allow progression to the next phase of the study (See X. Asthma Exacerbation)
3. This will include:
    a. Asthma Control Questionnaire (12).
    b. AM peak flow
    c. Peak flow variability = $\frac{PM - AM}{\frac{PM + AM}{2}}$
    d. Symptom-free days (percent)
    e. Symptom-free nights (percent)
    f. Number of beta-2 agonist rescue actuations per day
4. The measurements will be averaged each week of each study period where daily recordings are performed.

F. Asthma Exacerbation

Definition—for this protocol, an asthma exacerbation is defined as the development of an increase in symptoms of cough, phlegm/mucus, chest tightness, wheezing, and/or shortness of breath in association with one or more of the following:

- An increase in "as needed" or rescue albuterol of ≥8 inhalations over baseline use (baseline defined as average daily use during the week prior to Visit 3) for a period of 48 hours or ≥ 16 actuations per 24 hours.

- A fall in PEFR to ≤65% of baseline (baseline is defined as the average am prebronchodilator measurement over the week prior to Visit 3) on 2 of 3 consecutive scheduled morning or evening measurements.

- FEV_1 ≤80% of baseline (baseline is defined as average FEV_1 over placebo run-in period)

- FEV_1 < 40% predicted

- If a subject receives systemic corticosteroids for an exacerbation.

Subjects will be instructed to contact the Clinic Coordinator and/or be evaluated at the study site or the nearest medical emergency facility as quickly as possible.

G. Rationale for ICS Dosing

HFA-BDP at 160 mcg bid was selected based indirectly from the MICE study that demonstrated comparable doses of fluticasone or CFC-BDP would not produce any additional beneficial effect. For PRICE, we additionally have chosen HFA-BDP to demonstrate that even a third

ICS formulation (MICE used fluticasone MDI and DPI, and beclomethasone CFC MDI) produces about 30% non-responders. Also, the initial 6-week treatment period is the same duration as for the MICE trial. One problem may arise with the HFA-BDP in that it has an ultra fine particle size of ~1.1 micron. This has the theoretical possibility of penetrating more distally in the lung than larger particle size ICS. If the percent of non-responders is lower than predicted, the particle size difference may be the reason. However, it would, indeed, be interesting and lead to further investigation.

H. Rationale for Selected Tests

1. Hematocrit from the CBC at Visit 1. To ensure safety and local IRB approval for blood draw.
2. Expired nitric oxide. A biomarker for steroid response from the MICE study (see above).
3. Pregnancy test. For safety.
4. Maximum reversibility to a beta-2 agonist (change in FEV_1 produced by 6-8 inhalations of albuterol). From the MICE study, this was a biomarker of steroid response. In PRICE, FEV_1 response will be analyzed after each 2 actuations to determine if a lower number of actuations can also be a predictor of response. The absolute FEV_1 in liters will be used for calculations as is standard in the ACRN studies. However, we will also evaluate the change as FEV_1 percent predicted.
5. Methacholine PC_{20}. A biomarker of steroid response from the MICE study.
6. Diary cards. A standard ACRN analysis. Also, keeps subjects involved in the study to better ensure compliance.
7. Induced sputum (IS). - % eosinophils from IS was a biomarker of steroid response from the MICE study.
8. Phenotype – demographic, asthma onset and duration, and exacerbation history will allow for further evaluation of prediction of steroid response. Duration and onset were biomarkers of steroid response in MICE study.
9. CBC/diff/total plasma eos – possible biomarkers of steroid response.
10. PV curve/upstream resistance. These measurements will help to determine if loss of elastic recoil and/or more distal resistance separates the ICS responders from the non-responders. See background for discussion of distal lung involvement.
11. Skin tests, IgE, and genotyping. As in all ACRN protocols, these tests are performed to characterize subjects.
12. Asthma control. The Juniper Asthma Control Questionnaire will be used for this measurement (12). Additionally, the measures of morning peak flow, peak flow variability, symptom free days and nights, and beta-2 agonist rescue are commonly used outcomes of asthma control and are used in the guidelines (1).

I. Study Visits

0. Visit 0 (required only for subjects reenrolling after significant exacerbation during the ICS phase, week 2 to 8)
   a. eNO

b. Maximum reversibility (see Section VIII)
c. Induced sputum

1. Visit 1 (Begin run-in) (Single blind, placebo inhaler)
   a. Informed consent (if not already obtained)
   b. Pregnancy test for female subjects
   c. Long physical exam and medical history
   d. Methacholine PC_{20}
   e. Inhaler technique reviewed and rescue medication (albuterol) dispensed (refills dispensed as needed throughout remainder of trial)
   f. Jaeger peak flow monitor dispensed and appropriate technique assured
   g. Diary cards explained and dispensed
   h. Spirometry
   i. IgE/CBC/diff/total plasma eos
   j. Phenotype by age, duration of asthma
   k. Daily AM and PM peak flows and symptom diary to be kept for the 2-week run-in to define asthma stability.
   l. Allergy Skin Test
   m. Asthma Control Questionnaire
   n. Genotyping

2. Visit 2
   a. Subject returns 1 week ($\pm$4 days) after Visit 1
   b. ACQ
   c. Spirometry
   d. Methacholine PC_{20}
   e. Diary

3. Visit 3
   a. Subject returns 2 weeks ($\pm$ 4 days) after Visit 1
   b. eNO
   c. Spirometry
   d. Methacholine PC_{20}
   e. Diary
   f. Induced sputum
   g. Total plasma eos
   h. Asthma Control Questionnaire

4. Visit 4 (Begin ICS run-in) (Singleblind, ICS inhaler)
   a. Subject returns 3-7 days after Visit 3
   b. PV curve/upstream resistance (3 centers only)
   c. Maximum reversibility (see Section VIII)
   d. Beclomethasone dipropionate HFA 160 mcg bid. Appropriate technique taught by the clinical coordinators.

5. Visit 5
   a. Subject returns 6 weeks (± 4 days) after Visit 4
   b. Tests performed as listed for Visit 3.
   c. Pregnancy Test for female subjects.
   d. Short physical examination
   e. Diary

6. Visit 6 (Begin randomized treatment) (Doubleblind, ICS vs placebo inhaler)
   a. Subject returns 3-7 days after Visit 5
   b. PV Curve/upstream resistance (3 centers only)
   c. Maximum reversibility (see Section VIII)
   d. Stratified randomization with approximately the same number of good, marginal, and poor FEV_1 responders in each group (continued ICS and cessation of ICS [placebo])

7. Visits 7, 8, and 9
   a. Subject returns 4 weeks (± 4 days), 8 weeks (± 4 days), and 12 weeks (± 4 days), after Visit 6
   b. Check diary, peak flows for potential unreported exacerbations.
   c. Spirometry
   d. Asthma Control Questionnaire

8. Phone calls made at weeks 3, 6, 10, 14, 18, 22 to reinforce compliance, answer any questions, and to review peak flow records.

9. Visit 10 or Visit 99 (Study Termination)
   a. Subject returns 16 weeks (± 4 days) after Visit 6 for Visit 10 or within 3 days of a significant exacerbation anytime after Visit 6 for Visit 99 (see Part X)
   b. Long Physical exam
   c. Pregnancy test for female subjects
   d. Check diary
   e. Spirometry
   f. Asthma Control Questionnaire

J. Protocol in Tabular Format

Variable	Reenrollment Screening	Run-in (placebo)				Single-Blind ICS		Randomized Treatment				Early Termination For Sig. Exacerbation
Visit	0	1	2	3	4	5	6	7	8	9	10	99 (After Visit 6)
Week	-1	0	1	2	2	8	8	12	16	20	24	After Week 8
Window		±4d	±4d	±4d	+3-7d	±4d	+3-7d	±4d	±4d	±4d	±4d	+3d
Informed Consent	X	X										
Randomization							X					
History		X										
Long Physical Exam		X									X	X
Short Physical Exam						X						
Blood for Genetic Analysis		X										
Blood for IgE		X										
Blood for CBC/diff		X										
Blood for total eosinophils		X		X		X						
Allergy Skin Test		X										
PV Curve*					X		X					
Spirometry		X	X	X		X		X	X	X	X	X
Pregnancy Test		X				X					X	X
Methacholine Challenge		X	X	X		X						
Maximum Reversibility	X				X		X					
Exhaled Nitric Oxide	X			X		X						
Sputum Induction	X			X		X						
Asthma Control Questionnaire		X	X	X		X		X	X	X	X	X
Adverse Events Assessment		X	X	X	X	X	X	X	X	X	X	X
Concomitant Medications Assessment		X	X	X	X	X	X	X	X	X	X	X
Review Peak Flow Data			X	X	X	X	X	X	X	X	X	X
Peak Flow QC			X	X	X	X	X	X	X	X	X	X
Dispense/ Review Diary Cards		X	X	X	X	X	X	X	X	X	X	X
Dispense / Collect Medicines		X			X		X	X	X	X	X	X
Dispense Albuterol Rescue Inhaler		X					X					
Collect Meds											X	X
Phone Contact between previous visit and current visit						At Wk 3, 6		At Wk 10	At Wk 14	At Wk 18	At Wk 22	

* Performed for subjects enrolled at Denver, Madison, and San Francisco.

IV. Recruitment

A. Since this study is based on the results of the ACRN MICE study and recruitment was not difficult in face of a much more difficult study to perform, we feel recruitment for PRICE will run smoothly.
B. Specific plans for each center

Harvard Clinical Center/Boston

The Boston Center has used a variety of recruitment methods to meet and exceed recruitment goals of previous ACRN studies.

1. Over the past five years, we have compiled an internal database of **more than 1320 individuals with asthma** who are interested in participating in asthma studies. All of these individuals contacted us and expressed interest about asthma studies within the past year, and have been evaluated by our staff for participation in ongoing and future asthma clinical research studies.

2. The Boston site actively recruits subjects using a variety of external media. All methods are IRB-approved and include postcard mailings to area zip codes, newspaper advertisements, and broadcast e-mails and internet postings

3. Brigham and Women's Hospital has introduced a new clinical research tool called the **BWH Research Patient Database Registry (RPDR)** that allows researchers with proper IRB approval to query the hospital's patient database for potential research subjects. We recently queried this system and identified **29,204 patients with a diagnosis of asthma.** With permission from their primary care physician, patients may be contacted about current asthma research. We are in the process of developing tools to reach these patients through their physicians. Access to the physician database will further expand our capability to recruit asthmatic patients of differing severities.

National Jewish Medical and Research Center/Denver
Research subject recruitment has been very successful for all types of asthma subjects at the National Jewish Medical and Research Center.

1. National Jewish Outpatient Clinic
   The adult clinic saw 1,079 new asthmatic subjects over the last year with 503 being from the Denver metropolitan area. Another 335 from the Denver area were seen in follow-up. The severity of asthma varies among these subjects, but approximately 50% are in the mild to moderate category. National Jewish has changed markedly over the last decade. It has evolved from a primary inpatient facility with a small clinic to a very active outpatient service. Thus, the Denver Center has access to many more asthmatic subjects of all degrees of severity.

2. National Jewish Asthma Research Pool
There are over 400 asthma subjects (not followed in the National Jewish outpatient clinic) that have participated in research studies conducted at the Denver Center. Many of these subjects have been through various medication studies and bronchoscopies with lavage/biopsies. Their FEV_1s range from 30-110% of predicted.

a. Denver Health Medical Center – Dr. James Fisher, Head of Pulmonary Medicine, is supporting efforts of the Denver Center by helping to recruit from the asthmatic subject population at the Denver Health Medical Center. This is a large county hospital whose subject population comprises mainly Hispanic and African-American people.

b. Denver Veterans Administration Hospital – Dr. Carol Welsh, Pulmonary faculty member, will support this grant. The VA hospital has a large outpatient clinic of patients with asthma, but not chronic obstructive pulmonary disease.

c. Denver Kaiser Permanente HMO – Dr. Timothy Collins is the Director of Pulmonary Medicine and Dr. John Williams is the Director of Allergy at Kaiser. Drs. Collins and Williams have been actively involved in supporting research at National Jewish in the past by referring subjects. Their groups will continue to play an active role in clinical research support.

University of Wisconsin/Madison

The Allergy Research Program of the University of Wisconsin maintains a file of potential subjects with mild to moderate asthma who are interested in future research participation. These individuals have been screened and/or participated in previous asthma studies. The following information is maintained: birth date, gender, ethnic background, age of asthma diagnosis, childbearing status, atopic status (including results of skin testing if performed previously), concurrent medical history, asthma and non-asthma medications. Approximately 85% of subjects in this database have “mild to moderate” asthma. This database of subjects will be used as the primary source of recruitment for this protocol. If additional subjects are needed, they will be recruited via U.W. Human Subjects committee-approved, newspaper advertising and from the U.S. Allergy Clinic subject population. Also, U.W. Sports Medicine Clinic, U.W. Student Health, V.A. Allergy Clinic, Northeast Family Practice Clinic.

Harlem Prevention Center/New York

Central Harlem has a residentially stable population of approximately 115,000 of whom 98% are African American or Hispanic, and 53% are women. The prevalence of asthma in Central Harlem is 3-4 times that in the U.S. population. Harlem Hospital and its network of community-based clinics, together comprise the Northern Manhattan Network. Through the Network, the Harlem Asthma Research Center (HARC) has identified more than 2,000 asthmatic subjects who are in stable primary care relationships, and established collaborative arrangements with their primary care providers.

The Harlem Asthma Research Center will initially recruit participants in ACRN clinical trials through this network of collaborating providers. While the Center will specifically target people of color, it will never turn anyone away.

The investigators anticipate no difficulty in recruitment of women. Accrual of participants will be monitored for all protocols. If targeted approaches are needed, the HARC will consider strategies which have been used successfully to recruit and sustain the participation of women in this community. These have included provision of transportation, meals, child care, home visits, utilizing peer educators, the formation of a woman's support group, culturally appropriate education efforts and linkages to support services.

Primary care physicians from the Northern Manhattan Network will approach their subjects about their willingness to participate in the clinical trials. If they are interested, the screening and all follow-up visits will take place at the Harlem ACRN Clinical Center. Because asthma clinical trials will require procedures that are not performed routinely in primary care offices, appropriate procedures will be followed so that subjects participate fully in ACRN protocols while staying in contact with their primary care providers as needed.

Thomas Jefferson Medical College/Philadelphia

All subjects with a diagnosis of asthma currently cared for in the outsubject offices of the Division of Pulmonary Medicine and General Internal Medicine and the Departments of Family Medicine and Pediatrics are listed in a computerized database. Approximately 85% of 2,600 asthmatics in this database have "mild to moderate" asthma. Terminals located at each clinic site are linked to the ACRN file server located in the study coordinator's office. Subjects fulfilling every criteria for a given study will be identified by the database, and personal contact will be made by the study coordinator for the purpose of explaining the study and enlisting their participation. If on initial contact, the subject agrees, they will return to the study center to verify entry qualifications and further discuss the study. Subjects are also recruited from the local community by radio and newspaper advertising.

University of California/San Francisco

The approach to recruiting subjects with asthma for research studies at the San Francisco Center relies heavily on community advertising. Advertisements are placed in editions of the San Francisco Chronicle and Examiner, in small neighborhood newspapers, and on bulletin boards on the UCSF campus, in community health centers, and at campuses of colleges and universities in the Bay

Area. Advertisements are also placed on radio stations and on local web-based classified advertisements ("CraigsList"). Finally, fliers are placed in the subject waiting areas of the Pulmonary Medicine and Allergy Clinics at the major teaching hospitals of UCSF (Moffitt-Long, San Francisco General Hospital, Ft. Miley V.A. Hospital, and Mt. Zion Hospital). Responses to these advertisements are made to a dedicated telephone number equipped with voice mail. A part-time dedicated recruiter was hired to respond to each inquiry and to obtain basic information about the subject's demographics and about the severity, duration, required treatment, and frequency of symptoms of asthma. To date, over 700 subjects have been screened for the database. These subjects reflect the ethnic diversity of the Bay Area.

V. Drug Supplies

Drug supplies for this study will consist of a placebo inhaler for the run-in period (for adherence check) and during the randomized treatment phase and blinded QVAR™ (beclomethasone dipropionate HFA 80 mcg/actuation) for the ICS phase and randomized treatment phase. These will be supplied by IVAX. Open-label rescue albuterol and open-label prednisone (clinic provided) will also be required.

VI. Compliance and Monitoring

The following mechanisms will be employed to determine compliance and measure outcomes:

1. Diary card: At most visits the symptom diary card will be reviewed with the subject. Limitations are accuracy of subject's recall and honesty in completing the diary.
2. The Jaeger peak flow meter with diary recording will be used to record peak expiratory flows (PEF) and FEV_1, and serve as a check of compliance in general as date and time are electronically recorded.
3. A Doser™ device will be used to record the number and timing of inhaler actuations. The number of actuations used in the beclomethasone (or placebo MDI) inhalers, as recorded in the Doser™, will be tabulated at most clinic visits.

VII. Inhalation Technique

Since the manner in which an inhaled corticosteroid will in part deliver more or less medication to the lungs is critical in reducing variability, the subject's inhalation technique will be reviewed at each visit. Thus, objective feedback can be given to a subject to improve performance.

VIII. Special Study Techniques

Few techniques new to the ACRN are proposed for this study. Standard methods have been developed and described in the ACRN General Manual of Procedures (MOP) for spirometry, physical examination, blood drawing, methacholine challenge, measurement of exhaled NO, sputum induction and analysis, asthma diary instruction, skin testing, and asthma control assessment. Local laboratory methods will be accepted for measurement of total IgE and eosinophil numbers in blood samples. The ACRN also has experience in analysis of DNA extracted from blood samples for genetic variants

thought to be of possible relevance to asthma severity. The ACRN also has experience with methods intended to monitor and assure compliance, and to monitor peak flow. All clinical personnel are certified in each procedure, and new personnel will require certification before participating.

Special study techniques that are not standardized in the ACRN General MOP, are listed below:

1. Maximum Reversibility – To determine the maximal improvement in FEV_1 after albuterol treatment, the standard procedure for spirometry will be followed. Following baseline spirometry, 4 puffs of albuterol will be administered. After waiting 15 minutes, 3 post bronchodilator spirometry maneuvers will be obtained and the best value selected. Two more puffs of albuterol will be administered. After waiting 15 minutes, 3 post bronchodilator spirometry maneuvers will be obtained and the best value selected. The percent difference in FEV_1 between the FEV_1 measured after receiving 360 mcg (4 puffs) albuterol and the FEV_1 measured after receiving 540 mcg (6 puffs) albuterol will be calculated. The test is terminated if the FEV_1 percent difference is <5.0% or if a total of 8 puffs albuterol (720 mcg) have been administered. If the FEV_1 percent difference is <5.0%, the maximal improvement in FEV_1 following albuterol treatments is then identified.

2. PV curve – Lung elastic recoil pressure, flow volume relationship, airway resistance (R_{aw}) and upstream airway resistance (R_{us}) will be determined while the patients are seated in the body plethysmograph. The slope and intercept will also be calculated.

Transpulmonary pressures will be determined by subtracting esophageal pressure from mouth pressure. Esophageal pressure will be measured via a 10 cm PVC balloon on the end of the small-bore polyethylene catheter positioned in the mid-esophagus. A constant-volume history will be established by having the patient perform several maximal inspirations, followed by four normal breaths. The thoracic gas volume (V_{tg}) will then be determined followed by a maximal inspiration to TLC. Static transpulmonary pressures will be measured at the end of interruptions of airflow for 1.5 seconds at differing lung volumes during a slow expiration from TLC. The pressures will be plotted against the absolute volumes as well as the volumes expressed as a percent of predicted TLC. The coefficient of elastic retraction will be calculated by dividing maximal static transpulmonary pressure by 80% TLC. An exponential fit will be applied to the points and a K value depicting the overall compliance of the lungs derived. In addition static transpulmonary pressure and lung compliance at FRC will be recorded.

Upstream resistance will be calculated at isovolume points from 0 to 0.5 L/s by relating the static transpulmonary pressure and maximal expiratory flows at equivalent lung volumes.

$$R_{us} = \Delta P_{EL} / \Delta V_{max}$$

And $$\Delta V_{max} = \Delta P_{EL} / \Delta R_{us}$$

As is indicated by this formula, the maximum airflow at any volume will be lower than expected:

1. When the upstream resistance is increased
2. When the driving pressure is reduced (i.e., a loss of lung elastic recoil), or
3. When both disturbances are present

IX. Risk/Benefit

There is risk related to withdrawing inhaled corticosteroid therapy, however, these subjects were steroid naïve at the start of the study and thus we do not expect this to be a major factor. Additionally, the ACRN has withdrawn ICS from asthmatic subjects in the past without any severe adverse events (13,14). In order to protect this group of patients from undue harm, the following safeguards are provided: (1) patients will be informed of the risks of asthma exacerbation and provided with methods to identify when exacerbations are occurring; (2) patients will be given cards describing names and means of contacting study personnel on a 24 hr/day basis; (3) patients will be given a supply of prednisone to take as instructed by a study physician; (4) specific protocols for treatment of exacerbations are provided in the protocol (see below).

There are no direct benefits to the individual subjects. There is a potential benefit to patients with asthma in general as a more rational basis for therapy is devised.

X. Asthma Exacerbations

A. Definition

The criteria for "exacerbation" status due to poor asthma control are defined in Section III.F. Poor asthma control leading to "exacerbation" status may, depending on severity, also require intervention for patient safety. The following is a description of procedures for medical intervention. For this protocol, an asthma exacerbation is defined as the development of an increase in symptoms of cough, phlegm/mucus, chest tightness, wheezing, and/or shortness of breath in association with one or more of the following:

- An increase in "as needed" or rescue albuterol of ≥8 inhalations per 24 hours over baseline use (baseline defined as average daily use during the week prior to Visit 3) for a period of 48 hours or ≥ 16 actuations per 24 hours.

- A fall in PEFR to ≤65% of baseline (baseline is defined as the average am or pm prebronchodilator measurement over the week prior to Visit 3) on 2 of 3 consecutive scheduled morning or evening measurements. For the run-in phase, the PEFR for the second week will be compared to the mean of the first week.

- FEV_1 ≤80% of baseline (baseline is defined as average FEV_1 over placebo run-in period)

- FEV_1 <40% predicted

- If a subject receives systemic corticosteroids for an exacerbation.

1. If the rescue algorithm dictates, subjects who develop an asthma exacerbation may be given HFA-BDP at 320 mcg/d (160 mcg bid) regardless if on placebo or HFA-BDP and/or prednisone.

2. Patients developing an exacerbation during the initial period (i.e., prior to Visit 6) will be dropped from the protocol and treated according to the judgment of the investigator or primary physician. If the patient wishes to reenroll in the protocol, he/she must complete a washout period after the exacerbation resolves. Additionally, the patient may reenroll only if eligibility criteria are met and if, in the judgment of the investigator, the patient can maintain asthma control with as needed beta-agonists. Patients who have an exacerbation prior to Visit 4 will be required to washout for a period of 6 weeks after the exacerbation resolves, regardless of treatment. Patients who have an exacerbation after Visit 4 and before Visit 6 will be required to washout for a period of 4 weeks after the exacerbation resolves, regardless of treatment. At the time of reenrollment, subjects who developed an exacerbation while in the ICS phase will be tested to assure their sputum eosinophil count is within 1% (absolute) of the count before the significant exacerbation, and their maximum reversibility and exhaled nitric oxide are within 5% (relative) of the levels before the significant exacerbation occurred.

B. Rescue Algorithms

Subjects developing asthma exacerbations not controlled by reinstitution of HFA-BDP or reaching certain criteria during any phase of the study will be managed according to the following rescue algorithms.

Rescue algorithms will be applied in cases where an exacerbation as defined in Section A fails to resolve or PEFR is not improved to >65% of baseline (baseline defined as the average AM or PM pre-bronchodilator PEFR recorded during the study week, just prior to Visit 3) within 48 hours after increasing as needed albuterol use. Rescue algorithms are based on recommendations from the NAEP Guidelines for Diagnosis and Management of Asthma (NHLBI Publication No. 91-3042, 1991). Albuterol and oral prednisone are the principal medications for rescue management and patients will be instructed in their use for home management. Oral prednisone will be used if reinstitution of ICS does not alter the exacerbation. For severe acute episodes of asthma, treatment will be administered according to the best medical judgment of the treating physician.

1. Home Care

Asthma exacerbations will be recognized by an increase in symptoms and by a corresponding drop in PEFR below baseline. Patients will be educated to recognize exacerbations as early as possible to facilitate prompt treatment and to lessen morbidity.

Patients who recognize increased symptoms and/or a fall in PEFR to ≤65% baseline will use albuterol by MDI, 2-4 puffs, every 20 min up to 60-90 min if needed and then every 4 hours, or less, if needed. Patients will be instructed to use the “Rescue MDI” for treatment.

If the PEFR does not increase to >80% baseline or if symptoms are not improved after the first 60-90 min of therapy, the patient should contact the investigator, their primary physician or seek care in the emergency department.

Failure of albuterol to control or maintain PEFR >60% baseline may necessitate the use of steroids (see below).

2. Physician's Office or Emergency Room Treatment

Patients will be assessed by history, physical examination, and by physiological monitoring including spirometry or PEFR. If the patient's PEFR or FEV_1 are less than 25% predicted or if the patient shows evidence of altered mental status, cyanosis, labored breathing, or use of accessory muscles, sampling of arterial blood for respiratory gas analysis is indicated, with appropriate action taken depending on the results obtained.

When treated in the physician's office or the hospital emergency room, patients should initially be given albuterol by nebulization (0.5 cc of 0.5% solution) every 20 min over the first 60-90 min.

If the PEFR increases to >60% baseline after the first 60-90 min, the patient can be discharged to continue treatment at home. Prednisone may be administered at the discretion of the physician to augment therapy.

If symptoms persist and PEFR remains <60% baseline, nebulized albuterol should be continued as often as every hour and further treatment with immediate reinstitution of ICS (open-label HFA-BDP, 160 mcg bid). Oral or parenteral corticosteroids should be considered (60 mg prednisone orally; methylprednisolone 60 mg iv bolus) if ICS do not reverse the exacerbation. Monitoring of PEFR or spirometry should continue every hour. Within 4 hours of treatment, a decision should be made regarding patient disposition.

If PEFR increases to >60% baseline within 4 hours, the patient can be discharged to continue treatment at home. Home treatment should include an 8-day course of prednisone (see below).

If PEFR remains >40% but <60%, an individualized decision should be made to hospitalize the patient for more aggressive therapy or to continue therapy at home with a course of prednisone.

If PEFR is <40% baseline after repeated albuterol treatments, the patient should be admitted to the hospital unless in the physician's best judgment alternative treatment could suffice.

3. Prednisone Treatment

In this protocol, prednisone will be used when acute exacerbations cannot be controlled by albuterol therapy and reinstitution of ICS. Indications for prednisone therapy include the following:

For follow-up management after discharge from the physician's office, emergency room, or hospital for an acute exacerbation.

For home management if the patient is taking ≥ 16 puffs albuterol in 48 hours plus reinstitution of ICS and, despite this therapy, PEFR remains <60% baseline before albuterol use and symptom scores in the same period are > 8.

For home management when symptom scores are > 10 for 48 hours or longer and the patient is taking ≥ 16 puffs of albuterol plus reinstitution of ICS.

When PEFR falls <50% baseline despite albuterol and ICS treatment.

The dose of prednisone used during an acute exacerbation shall consist of 60 mg as a single dose every day for 3 days, followed by a 10 mg/day taper over the next 5 days. The decision to initiate or to continue a course of prednisone beyond 8 days is left to the discretion of the physician.

XI. Anticipated Results

We anticipate that approximately 1/3 of subjects on ICS will be poor responders in regard to FEV_1 and PC_{20}, and 1/3 will be good responders (not all the same subjects for each outcome). We also anticipate that the baseline biomarkers of low eNO and low maximum bronchodilator response will be predictive of poor FEV_1 response, and low IS eosinophils and longer duration of asthma will be predictive of a poor response to PC_{20}, conversely for good responders.

We anticipate that in subjects with $<$ 5% improvement in FEV_1 after 6 weeks of ICS treatment (poor FEV_1 response), change in lung recoil will be significantly lower than in those with a $>$15% improvement in FEV_1. We also anticipate that in subjects with a $<$ 1X doubling dose change in PC_{20}, change in upstream airway resistance (R_{us}) will be significantly greater than in subjects showing a $>$3 doubling dose change in PC_{20}.

For the pilot project, we anticipate that in subjects with $<$ 5% improvement in FEV_1 after 6 weeks of ICS treatment (poor FEV_1 response) there will be little improvement in asthma control over the six weeks of ICS treatment, and that worsening of asthma control and occurrence of asthma exacerbations will be no more likely whether ICS treatment is continued or withdrawn over the 16 weeks thereafter. Conversely, we anticipate that in subjects with a $>$15% improvement in FEV_1 after 6 weeks of ICS treatment (good FEV_1 response) there will be significant concurrent improvement in asthma control. We further anticipate that in these subjects, worsening of asthma control and occurrence of asthma exacerbations will be significantly less likely when ICS treatment is continued, as opposed to withdrawn, over the next 16 weeks. Again, we acknowledge that this proposed study will only provide pilot data on these important outcomes, but this preliminary data on asthma control and exacerbation rates in “poor” and “good” FEV_1 and PC_{20} responders to ICS treatment will be invaluable for future studies.

The physiologic correlate of poor responders will be loss of elastic recoil and increased upstream resistance.

Table 4: Anticipated Biomarker Results at Baseline

ICS Responders	Poor	Marginal*	Good
eNO (ppb)	<15 ppb		≥15 ppb
Max BD	<15%		>15%
Sputum eos	<2%		≥2%
Duration of Asthma	≥10 years		<10 years

*Marginal responders in regard to FEV_1 will have 1 of 2 good responder outcomes for NO and Max BD; similarly for PC_{20} in regard to sputum eos and duration of asthma.

XII. Adverse Events

A. Definitions

An adverse event shall be defined as any detrimental change in the subject's condition, whether it is related to an exacerbation of asthma or to another unrelated illness. Adverse events related to asthma exacerbations will be assigned Treatment Failure status and managed according to rescue algorithms outlined in previous ACRN trials.

An adverse event is deemed serious if it suggests a significant hazard, contraindication, side effect, or precaution. Serious adverse events include any experience that is fatal or life-threatening, is permanently disabling, requires or prolongs inpatient hospitalization, or is a congenital anomaly, cancer, or overdose. Serious adverse events must be reported to the DCC and the National Institutes of Health Project Scientist within 72 hours of notification. Once notified, the DCC will disseminate information about the event to the Data Safety and Monitoring Board and to the Steering Committee.

B. Adverse Events Unrelated to Asthma

Adverse events due to concurrent illnesses other than asthma may be grounds for withdrawal if the illness is considered significant by the study investigator or if the subject is no longer able to effectively participate in the study. Subjects experiencing minor intercurrent illnesses may continue in the study provided that the nature, severity, and duration of the illness are recorded and that any unscheduled medications required to treat the illness are also recorded. Examples of minor intercurrent illnesses include acute rhinitis, sinusitis, upper respiratory infections, urinary tract infections, and gastroenteritis. Medications are allowed for treatment of these conditions in accordance with the judgment of the responsible study physician.

Documentation of an adverse event unrelated to asthma will be recorded on an Adverse Event Report Form and will include the following information:

- Description of the illness
- Dates of illness
- Treatment of illness and dates (medications, doses, and dose frequency)
- Whether emergency treatment or hospitalization was required
- Treatment outcome

C. Adverse Events Related to Asthma Exacerbations (see above description, III F)

XIII. Cost, Liability, and Payment

All tests will be performed without cost to the participating subjects. Since this is a trial of an established asthma treatment, liability for subject care costs incurred by subjects during the course of the trial will in most cases be borne by the subject or their insurer. Details of the National Institutes of Health policies concerning this issue can be found in NIH Documents #5305 and 5352-2, Research Patient Care Costs Supported by NIH Sponsored Agreements, which are in the ACRN Manual of Operations. Each subject will be paid an amount determined by their local center for study reimbursement. For subjects who drop out, reimbursement will be pro-rated for the length of time they stayed in the study.

XIV. Statistical Design and Analysis

A. Data Recording and Data Management

Recording of all data including informed consent, history, physical examination, adverse events, confirmation of medication dispensation, lung function testing, and initial data entry will be done at each Clinical Center and forms will be forwarded to the data coordinating center (DCC) for confirmatory entry. Results from pulmonary function tests and compliance will be transmitted electronically to the DCC where all data will be stored and analyzed.

Each Clinical Center will have a computer configuration that includes a PC, a printer, and a modem. This will give each clinical center the capability of logging directly into the ACRN Network web site with the modem as a back-up if the connection is not possible. Though this set-up is installed primarily to allow for distributed data entry into a centralized and secure database at the ACRN Network web site, menu options will also include sending electronic mail, downloading study documents such as forms and reports, and viewing a calendar of ACRN Network events. A sophisticated security system will limit access to qualified personnel and prevent corruption of the study database.

The DCC will be responsible for generating the data collection forms based on input from the clinical centers. Once the data collection forms have been filled out and reviewed, the Clinic Coordinator will log into the ACRN Network web site and enter the data within 3 days of the patient visit. The advantage of this distributed data entry system is that the Clinic Coordinators will review the data a second time as they are entering it, which serves as another level of quality control. However, the Clinic Coordinators will not be able to query their own data. The data base

management system will have range checks and validity checks programmed into it for a second level of quality control. Forms will then be forwarded to the DCC for the second data entry and filing, which will be performed within 3 days of receipt. The DCC will be responsible for identifying problem data and resolving inconsistencies. Once the quality control procedures are complete, new study data will be integrated into the primary study database. Results from lung function tests will be sent directly to the DCC via a modem in the computer attached to the spirometer.

B. Masking

To minimize the bias due to possible knowledge of the active and placebo treatment arms in the third phase of the study, this part of the study will be double-blinded. Thus, the investigators and the subjects will be blinded to the assigned treatment regimens. Until the time of manuscript preparation, DCC personnel will identify the randomized groups as X and Y, and only limited personnel within the DCC will know the identity of X and Y.

C. Randomization

Randomization occurs in the pilot portion (third phase) of the study. We anticipate there will be an approximately equal number of good, marginal, and poor responding subjects to ICS in regard to FEV_1 during the six-week second phase that will be randomized to continue or discontinue ICS (placed on placebo). This will be done so as to be able to learn more about these responding groups as to asthma control. When a subject at a particular center has completed the characterization and ICS periods (i.e. at week 8), the clinic coordinator will log into the ACRN network server and indicate that a subject requires randomization. Based on the subject's FEV_1 response category, the server will generate a drug packet number, from which all medication for that subject will be dispensed.

D. Sample Size

The precision to estimate whether certain biomarkers can predict response to ICS based on FEV_1 (eNO and BD response) and PC_{20} (sputum eosinophils and duration of asthma) can be determined based on data from the MICE study. These estimates were based on biomarker status (0 biomarkers, 1 biomarker, 2 biomarkers) vs. FEV_1 and PC_{20} response (poor, marginal, good response) as defined in the MICE study. The measures of association between each set of biomarkers and the corresponding response produced Kendall's tau coefficients of 0.75 (sd=0.403) for FEV_1 and 0.73 (sd=0.212) for PC_{20} response. Based on the largest estimate of variability (0.403), a sample size of n = 80 would allow us to estimate a 95% confidence interval for Kendall's tau coefficient with width = 0.20 for each response (tau $\pm$ 0.10). This sample size allows for a maximum of 20% drop-outs and/or missed visits.

The secondary hypothesis deals with evaluating the association between change in elastic recoil and % improvement in FEV_1, and the association between change in upstream resistance and change in PC_{20}. These correlations can be determined by testing the null hypothesis that Kendall's tau coefficient for each association is zero, versus a specified alternative at significance level $\alpha/2$ (for the two comparisons) and statistical power 1 - β. A sample size of n = 40* subjects would provide

approximately 90% power for a two-sided, 2.5% significance level test of the null that tau = 0 against the alternative that tau = 0.4. This sample size allows for a maximum of 20% drop-outs and/or missed visits.

*Note that only half the centers will be contributing PV curve data for testing this hypothesis.

The tertiary hypothesis is descriptive and based on pilot data. Therefore, no power calculations are necessary. If we assume that we will find a similar distribution of poor/marginal/good response defined by % improvement in FEV_1 as we found in MICE, then we would expect to have approximately 30 (38%) poor responders, 19 (24%) marginal responders, and 30 (38%) good responders. This would allow for approximately 15 subjects continuing on ICS within the poor and good responder groups, and 15 subjects discontinuing ICS. The response variables are the rates of asthma control and exacerbation, measured on a continuum from 0.0 to 1.0 for each subject. If we consider estimating rates of asthma control and exacerbation within each of these four groups (good responders continuing ICS, good responders discontinuing, poor responders continuing ICS, and poor responders discontinuing), then a sample size of 15 per group will provide us with the following precision for confidence intervals based on varying estimates of dispersion (no relevant data were available from other studies to use for dispersion):

Width of 95% CI for rates of control/exacerbation	Standard error of rate	Standard deviation of rate
0.3	0.077	0.296
0.4	0.102	0.395
0.5	0.128	0.494
0.6	0.153	0.593
0.7	0.179	0.692

The total sample size of **n=80** would provide adequate power to address the primary and secondary hypotheses, allowing for 20% drop-outs and/or missed visits. This requires 13-14 subjects per center.

E. Statistical Analysis

The primary outcome variables in the PRICE study are:

(1) Percent improvement in FEV_1 during the 6-week treatment phase

$$\frac{\text{Visit 6 } FEV_1 - \text{Visit 4 } FEV_1}{\text{Visit 4 } FEV_1} \times 100\%$$

The absolute FEV_1 in liters will be used for calculations, as is standard in the ACRN studies. However, we will also evaluate the improvement as FEV_1 percent predicted.

(2) Change in PC_{20} during the 6-week treatment phase in terms of doubling dilutions

$$\log_2 (\text{Visit 6 } PC_{20}) - \log_2 (\text{Visit 4 } PC_{20})$$

Percent improvement in FEV_1 and change in PC_{20} from Visit 4 to Visit 6 will be treated as continuous outcomes, as well as categorical outcomes with the following levels:

Level of Categorical Outcome Variable	% improvement in FEV_1	Change in doubling dilutions
Poor	$\leq$5%	<1 dd
Marginal	6-14%	1-3 dd
Good	$\geq$15%	>3 dd

The primary predictor variables measured in PRICE are bronchodilator response to a beta-2 agonist, expired nitric oxide, sputum eosinophils, and duration of asthma. These and other potentially important predictors will be measured during the run-in (Visit 3 and/or Visit 4). **Hypothesis 1** deals with evaluating the association between the predictors and the primary outcomes defined above: (1) % improvement in FEV_1, and (2) change in PC_{20}. From the MICE study, we suspect that bronchodilator response and expired nitric oxide may be biomarkers for response to ICS defined by %improvement in FEV_1. Similarly, we suspect that sputum eosinophils and duration of asthma may be biomarkers for response to ICS defined by change in PC_{20}. Therefore, we will create categorical biomarker variables with levels 0 = no biomarkers, 1 = one biomarker, and 2 = two biomarkers for each of these two sets of predictors. To address the primary hypothesis, we will first evaluate Kendall's tau coefficients between each of the biomarker variables and the relevant outcome variables. We will report the 95% confidence interval for each coefficient, along with the test of whether the coefficient is equal to zero. We will also evaluate whether any secondary predictor variables that are measured during the run-in (Visits 1-4) are significantly correlated with the two responses. In addition, an ordinary linear regression model will be applied for each of the continuous outcomes (%improvement in FEV_1 and change in PC_{20}), which allows for evaluating the association between the predictors and outcomes in the presence of other covariates. In this type of analysis the model function is specified as

$$E(\mathbf{y}) = \alpha + \mathbf{x}^T\mathbf{ß}$$

where
$\mathbf{y}$ = vector of responses for each subject,
α = intercept parameter
$\mathbf{x} = [x_1 \ldots x_k]^T$ is a vector of explanatory variables,
$\mathbf{ß} = [ß_1 \ldots ß_k]^T$ is a vector of unknown parameters.

The vector $\mathbf{x}$ will include the relevant categorical biomarker variable, indicator variables for Clinical Center, and any other relevant baseline covariates.

We will also model FEV_1 and PC_{20} response as categorical outcomes (poor/marginal/good), using proportional odds logistic regression models which can be specified as:

$$\text{Logit}(\boldsymbol{\theta}_j) = \alpha_j + \mathbf{x}^T\boldsymbol{ß}, \quad j=1,2$$

where
$\boldsymbol{\theta}_1$ = vector of probabilities of Good vs. Marginal/Poor response,
$\boldsymbol{\theta}_2$ = vector of probabilities of Good/Marginal vs. Poor response,
α_1 = log odds of Good vs. Marginal/Poor response for reference group,
α_2 = log odds of Good/Marginal vs. Poor response for reference group,
$\mathbf{x} = [x_1 \ldots x_k]^T$ is a vector of explanatory variables,
$\boldsymbol{ß} = [ß_1 \ldots ß_k]^T$ is a vector of unknown parameters.

The vector **x** will include the relevant categorical biomarker variable, indicator variables for Clinical Center, and any other relevant baseline covariates.

Two additional predictor variables that will be measured at Visit 4 and Visit 6 during the ICS Phase are elastic recoil and upstream resistance. **Hypothesis 2** involves evaluating the association between change in elastic recoil and % improvement in FEV_1, and the association between change in upstream resistance and change in PC_{20}. For each of these associations we will first evaluate Kendall's tau coefficients between the predictors and outcome variables. We will report the 95% confidence interval for each coefficient, along with the test of whether the coefficient is equal to zero. In addition, ordinary linear regression and proportional odds regression models will be applied for each outcome (% improvement in FEV_1 and change in PC_{20} considered linearly and categorically), as described above, and the vector **x** will now include explanatory variables for change in elastic recoil and upstream resistance, along with indicator variables for Clinical Center and any other relevant baseline covariates. For this Hypothesis, we will also evaluate the association between the FEV_1 biomarkers (bronchodilator response and eNO) and change in elastic recoil; and between the PC_{20} biomarkers (sputum eosinophils and duration of asthma) and change in upstream resistance. We will apply the same approach as discussed above based on Kendall's tau coefficients and appropriate regression models.

For the pilot project, we will estimate the rates of asthma control and asthma exacerbation, along with measures of variability, for the two treatment arms (continued on ICS vs. switched to placebo) during the randomized phase of the study for the subjects with poor, marginal, and good FEV_1 response to ICS separately. Graphically, we will be able to evaluate Kaplan-Meier curves illustrating time to exacerbation for the two treatment arms stratified by poor, marginal, or good FEV_1 response to ICS. Although the groups are being stratified by the change in FEV_1 during the pilot portion of the study, we expect that a sufficient number of subjects who have had "good", "marginal", and "poor" responses to initial ICS will be assigned to the two arms for us also to examine whether the change in PC_{20} predicts protection against loss of asthma control. Therefore, we will also estimate the rates of asthma control and exacerbation for the two treatment arms for the subjects with poor, marginal, and good PC_{20} response to ICS. Graphically, we will also evaluate Kaplan-Meier curves illustrating time to exacerbation for the two treatment arms stratified by PC_{20} response.

XV. Significance

It is presently suggested that all persistent asthma patients have controller therapy and this is usually ICS. If this study demonstrates, in a cohort of asthmatic subjects, that there are a marked number who are poor responders to ICS in regard to FEV_1 and/or PC_{20}, then this will be new important information to add to our understanding of treatment effects with ICS. Furthermore, if we find that there is loss of elastic recoil in this group then future asthma research can be directed toward distal lung alterations. If biomarkers can predict responders from non-responders, then this could have implications in regard to future decisions about treatment modalities if this also predicts asthma control (future study based on present pilot study). Thus, we feel that this is an exceedingly important protocol to undertake.

XVI. References

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