4 STUDY FORMS AND INSTRUCTIONS

This section provides information about two types of forms: data collection forms and administrative forms. Data collection forms are used to collect data from or about the participant. These forms are entered into the AsthmaNet database and submitted to the DCC. Administrative forms facilitate the processing of the participant and the visit flow by the performance sites and the DCC. Administrative forms are not entered into the AsthmaNet database and they are not submitted to the DCC in most cases.

These instructions are divided into two parts—instructions for data collection forms followed by instructions for administrative forms. The instructions for both parts are in alphabetical order based on the full form name found in the header of the form. Forms with a 'P9' prefix are specific to the ALfA protocol.

For each form, the following information is provided: the purpose of the form, who completes the form, when the form should be completed, and form instructions. Most forms have a comments section (Q6000) at the bottom of the form. The coordinator can record additional comments or information related to the form in this section. This information is entered into the AsthmaNet database management system. If you are unable to find the specific information needed to complete a form, please contact the ALfA Data Manager at (717) 531-3663.

4.1 *ALfA Data Collection Forms*

Packet data forms are found in visit-specific packets, and they are submitted to the DCC as packets. Individual data forms (single forms) are submitted on an as-needed basis. Concurrent forms (AECLIN, CMED) are completed at each study visit and can be updated throughout the ALfA study. All concurrent forms should be submitted when the participant concludes his or her participation in the ALfA study. Some forms (e.g., ALfA Scheduled Medications (P9_MED)) can be submitted as part of a visit packet or as a single form, depending on the specific circumstances. The schematic of the ALfA visit structure is posted as a sub-item to this section on the website.

4.1.1 Adult Body Measurements (BODYMEAS_ADULT)

Purpose: To record the height, weight, and circumference measurements of an adult participant.

Who: An AsthmaNet coordinator completes the form.

When: Visit 1

Form Instructions:

Height and weight are recorded in metric units (cm and kg) on this form. Convert the height and weight to US units (in and lbs) and record the values in Q6000. The US units will be used to calculate the participant's eGFR on the P9_LAB form at Visit 1.

For more information related to taking these measurements, please refer to Section 3 of the AsthmaNet General MOP.

4.1.2 Adult Participant Contact Information (CONTACT_ADULT)

Purpose: To record pertinent participant identification information for all adults so that the AsthmaNet coordinator will know how and when to easily contact the participant.

Who: The participant completes the form.

When: Visit 1

Form Instructions:

Highlight the participant's preferred method of contact (phone/e-mail/text) for weekly adherence contacts during the randomized treatment phase.

For use only at the clinical site – DO NOT forward this form to the DCC.

This form will be reviewed during AsthmaNet site visits.

4.1.3 Clinical Adverse Events (AECLIN)

Purpose: To record the details and events that occur each time a participant experiences a clinical adverse event.

Who: An AsthmaNet coordinator completes the form.

When: Visits 1, 2, 3

Note: This form should also be completed if the participant contacts study personnel to report a clinical adverse event outside of scheduled visits. This form should also be updated if the participant reports having an asthma/allergy or adverse event between visits. Questions on other forms may also prompt a coordinator to complete this form.

Form Instructions:

Clinical adverse events that started in between visits but were reported by the participant at the following regular visit should be recorded on the current visit's AECLIN form. For example, events started in between Visit 2 and Visit 3 and reported at Visit 3 would get recorded on the Visit 3 AECLIN form.

If the participant contacts the clinic coordinator between visits, record the new event(s) on the AECLIN form completed at the last regular visit. This new event should be entered in the Participant Data module within the data management application.

For more information on recording Clinical Adverse Events (AECLIN), see Section 10 of the AsthmaNet General MOP.

4.1.4 Concomitant Medications for Asthma/Allergy and Adverse Events (CMED)

Purpose: To record any asthma/allergy and adverse event related concomitant medications that the participant uses during the study.

Who: An AsthmaNet coordinator completes the form.

When: Visits 1, 2, 3

Note: This form should be completed if the participant contacts study personnel to report a concomitant medication used outside of scheduled visits. This form should also be updated if the participant reports taking an asthma/allergy or adverse event related concomitant medication between visits. Questions on other forms may also prompt a coordinator to complete this form.

Form Instructions:

Concurrent medications that were started in between visits but were reported by the participant at the following regular visit should be recorded on the current visit's CMED form. For example, medications started in between Visit 2 and Visit 3 and reported at Visit 3 would get recorded on the Visit 3 CMED form.

If the participant contacts the clinic coordinator between visits, record the new medication(s) on the CMED form completed at the last regular visit. This new medication should be entered in the Participant Data module within the data management application.

If the participant is taking allergy shots or has received any vaccines, these should be noted on the CMED_NON form and not recorded on the CMED form.

For more information on recording Concomitant Medications for Asthma/Allergy and Adverse Events (CMED), see Section 10 of the AsthmaNet General MOP.

4.1.5 ALfA Methacholine Challenge Testing (METHA)

Purpose: To record outcome measurements from the methacholine challenge procedure.

Who: The Pulmonary Function Technician administers the methacholine and pulmonary function tests and completes the form

When: Visits 1, 2, 3

Form Instructions:

If methacholine challenge is completed at a Visit 1 Continuation visit, a single Spirometry Testing (SPIRO) form should be completed and the single Spirometry Testing Report (SPIRO_RPT) should be marked 'No' in the database. The packet Methacholine Challenge Testing form should be entered using the continuation Visit Date. The packet Methacholine Challenge Report (METHA_RPT) should be marked 'Yes' in the database. The spirometry session data is included on the Methacholine Challenge Report (METHA_RPT) and a separate Spirometry Testing Report (SPIRO_RPT) does not need to be printed.

For the **ALfA study ONLY** at Visits 2 and 3 ONLY the participant will use 2 puffs of **ipratropium** instead of albuterol for standard reversal. This is indicated in the prompt below Question 7.

Question 1090. For the **ALfA study ONLY** at Visits 2 and 3 ONLY the methacholine reversal reference value is calculated by multiplying the value in PADVAIR_SPIRO Q1030 by 0.9.

The ALfA version of the METHA form can be found in the following locations with the subheading 'For ALfA Study at Visits 2 and 3 Only':
Home: Forms: Standard Forms and Home: Forms: ALfA: Data Collection Forms.

For more information on the Methacholine Challenge Testing (METHA) form see Section 2 of the ALfA MOP and Section 10 of the AsthmaNet General MOP.

4.1.6 ALfA Additional Treatment Post Methacholine Challenge Testing (METHA_ADD_TRT)

Purpose: This form should be completed following methacholine challenge testing if the participant did not reverse to 90% of baseline FEV_1 after the standard reversal treatment of 2 puffs of **ipratropium**.

Who: The Pulmonary Function Technician administers the additional treatment, pulmonary function tests, and completes the form.

When: If the participant's FEV_1 is not greater than the reference value after standard reversal from methacholine challenge testing.

Form Instructions:

For the **ALfA study ONLY** at Visits 2 and 3 ONLY the participant will use **ipratropium** instead of albuterol for standard reversal. This is indicated in Q1010, Q1020.

Questions 1100 and Q1230. For the ALfA study ONLY at Visits 2 and 3 ONLY the methacholine reversal reference value is calculated by multiplying the value in PADVAIR_SPIRO Q1030 by 0.9.

The ALfA version of the METHA_ADD_TRT form can be found in the following locations with the subheading 'For ALfA Study at Visits 2 and 3 Only':
Home: Forms: Standard Forms and Home: Forms: ALfA: Data Collection Forms.

For more information on the Additional Treatment Post Methacholine Challenge Testing (METHA_ADD_TRT) form see Section 10 of the AsthmaNet General MOP.

4.1.7 Adult Methacholine Challenge Testing Checklist (METHACHK_ADULT)

Purpose: To determine if a participant is eligible to proceed with the diluent (solution #0) pulmonary function testing for methacholine challenge testing.

Who: A Pulmonary Function Technician completes the form.

When: Visits 1, 2, 3

Form Instructions:

If methacholine challenge is completed at a Visit 1 Continuation visit, the packet Adult Methacholine Challenge Testing Checklist (METHACHK_ADULT) form should be entered in the database using the continuation Visit Date.

Question 1050. Refer to the Concomitant Medications for Asthma/Allergy and Adverse Events (CMED) form, the ALfA Significant Asthma Exacerbation (P9_SIGEX) form and the ALfA Asthma Monitoring Log (P9_ASTHMA_LOG) for records regarding systemic corticosteroid use for the treatment of an asthma exacerbation in the last 4 weeks.

Question 1060. Participants with $FEV_1 \geq 50\%$ predicted are eligible to proceed with methacholine challenge in ALfA. If FEV_1 is 50% to 54.9% predicted and ≥ 1.0 L, check 'Yes' to Q1060, and provide comment in Q6000 that $FEV_1 \geq 50\%$ predicted and ≥ 1.0 L.

For more information on the Adult Methacholine Challenge Testing Checklist (METHACHK_ADULT), see Section 10 of the AsthmaNet General MOP. For additional instructions for the ALfA protocol, see the Methacholine Challenge discussion in Section 2 of the ALfA MOP.

4.1.8 Genetics Analysis Blood Draw (GABLOOD)

Purpose: To record information related to a participant's genetic analysis blood draw.

Who: An AsthmaNet coordinator completes the form.

When: Visit 2 or 3 (if necessary)

Form Instructions:

The Genetic Analysis Blood Draw form (GABLOOD) should be completed for most participants at the time of Visit 2 when the blood draw is first attempted. If the genetic blood draw is successful, this form is entered at Visit 2 as a packet form.

If the genetic blood draw is not done at Visit 2, but is instead deferred to Visit 3 in the ALfA study, the Visit 2 packet Genetics Analysis Blood Draw (GABLOOD) form should be marked missing. The Genetics Analysis Blood Draw (GABLOOD) form should be completed and entered as a single form at Visit 3.

If the participant terminates early from the study and never completes a blood draw at Visit 3, submit a data correction to have the Visit 2 Genetics Analysis Blood Draw (GABLOOD) form set to present. Q1000 and Q1010 should be completed, indicating that a blood sample was not obtained. When the data correction is submitted through the AsthmaNet application, complete the form and send a copy of the Genetics Analysis Blood Draw (GABLOOD) form for Visit 2 to the DCC for first and second entry. A subsequent blood draw could also be missing because consent is withdrawn.

If the genetic sample blood draw is attempted at Visit 2 but is unsuccessful, and the participant is unwilling to have another draw attempted at Visit 3, then the Genetics Analysis Blood Draw (GABLOOD) form should be completed and data entered as part of the Visit 2 packet. In that case, Q1000 and Q1010 should be completed, indicating that a blood sample was not obtained, and the participant should provide source documentation.

See the Genetics Blood Draw discussion in Section 2 of the ALfA MOP for more details on the genetics analysis blood draw and Appendix 4 of the AsthmaNet General MOP for more details on the Genetics Analysis Blood Draw (GABLOOD) form.

4.1.9 Serious Adverse Event Reporting Form (SERIOUS)

Purpose: To record the details of each serious adverse event.

Who: An AsthmaNet coordinator completes the form in collaboration with the Principal Investigator.

When: Visits 1, 2, 3

Form Instructions:

Question 1020. Open-label Flovent® Diskus®, Advair® Diskus®, ipratropium inhaler (green RESCUE1), albuterol inhaler (blue RESCUE2), and blinded alendronate capsules given out as part of the study should be considered “study drug” when answering this question. Rescue prednisone is NOT considered a “study drug”.

For more information on the Serious Adverse Event Reporting Form (SERIOUS), see Section 10 of the AsthmaNet General MOP.

For further details on the reporting of adverse events in the ALfA trial, see the Adverse Events discussion in Section 2 of the ALfA MOP.

4.1.10 Spirometry Testing (SPIRO)

Purpose: To record the outcome measurements from the participant's pre-bronchodilator spirometry procedure

Who: The Pulmonary Function Technician completes the form.

When: Visits 1, 2, 3

Form Instructions:

If the Spirometry Testing (SPIRO) form is completed between visits, specify the number of the last visit completed and the current visit date in the upper right-hand corner. This form should be entered as a single form.

If methacholine challenge is completed at a Visit 1 Continuation visit, a single Spirometry Testing (SPIRO) form should be completed and the single Spirometry Testing Report (SPIRO_RPT) should be marked 'No' in the database. The packet Methacholine Challenge Report (METHA_RPT) should be marked 'Yes' in the database. The spirometry session data is included on the Methacholine Challenge Report (METHA_RPT) and a separate Spirometry Testing Report (SPIRO_RPT) does not need to be printed.

If spirometry testing is completed at Visit 2 or 3, the Spirometry Testing Report (SPIRO_RPT) should be marked 'Yes' in the database. This will be its own report with only the Spirometry Testing Report rows present.

If methacholine challenge is completed at Visit 1 (and thus post-albuterol (4 puffs) spirometry testing is **NOT** completed), the Spirometry Testing Report (SPIRO_RPT) and the Post-Albuterol (4 puffs) Spirometry Testing Report (PALB4_RPT) should be marked 'No' in the database. If methacholine challenge is completed at Visit 2 or 3, the Post-Advair® Spirometry Testing Report (PADVAIR_RPT) should be marked 'No' in the database. The Methacholine Challenge Report (METHA_RPT) should be marked 'Yes' in the database. The Post-Advair® spirometry session data is included on the Methacholine Challenge Report (METHA_RPT) and a separate Spirometry Testing Report (PADVAIR_RPT) does not need to be printed.

If post-albuterol (4 puffs) spirometry testing is completed at Visit 1 (and thus methacholine challenge is **NOT** completed), the Spirometry Testing Report (SPIRO_RPT) and the Methacholine Challenge Report (METHA_RPT) should be marked 'No' in the database. The Post-Albuterol (4 puffs) Spirometry Testing Report (PALB4_RPT) should be marked 'Yes' in the database. The spirometry session data

is included on the Post-Albuterol (4 puffs) Spirometry Report (PALB4_RPT) and a separate Spirometry Testing Report (SPIRO_RPT) does not need to be printed.

If Post-Advair® spirometry testing is completed at Visit 2 or 3, and methacholine challenge testing is not performed, the Methacholine Challenge Report (METHA_RPT) should also be marked ‘No’ in the database. The Post-Advair® Spirometry Testing Report (PADVAIR_RPT) should be marked ’Yes’ in the database.

Visit 1 Pulmonary Function Scenarios:

Methacholine Challenge	**Reversibility Testing**	**Reversibility Testing AND Methacholine Challege**	**Not eligible for either Methacholine or Reversibility**
SPIRO	**SPIRO**	**SPIRO**	**SPIRO**
PALB4_SPIRO-mark missing	**PALB4_SPIRO**	**PALB4_SPIRO**	*PALB4_SPIRO-mark missing*
METHACHK_ADULT	*METHACHK_ADULT-mark missing*	**METHACHK_ADULT**	*METHACHK_ADULT-mark missing*
METHA	*METHA-mark missing*	**METHA**	*METHA-mark missing*
METHA_RPT	*METHA_RPT-mark missing*	**METHA_RPT**	*METHA_RPT-mark missing*
PALB4_RPT-mark missing	**PALB4_RPT**	**PALB4_RPT**	*PALB4_RPT-mark missing*
SPIRO_RPT-mark missing	*SPIRO_RPT-mark missing*	*SPIRO_RPT-mark missing*	**SPIRO_RPT**
		SPIRO (single form)	
		SPIRO_RPT (single)-mark missing	

Visit 2 and 3 Pulmonary Function Scenarios:

Post-Advair Spirometry and Methacholine Challenge	**Post-Advair Spirometry only**	**Not eligible for either Post-Advair Spirometry or Methacholine Challenge**
SPIRO	**SPIRO**	**SPIRO**
METHACHK_ADULT	**METHACHK_ADULT**	*METHACHK_ADULT-mark missing*
PADVAIR_SPIRO	**PADVAIR_SPIRO**	*PADVAIR_SPIRO-mark missing*
METHA	*METHA-mark missing*	*METHA-mark missing*
METHA_RPT	*METHA_RPT-mark missing*	*METHA_RPT-mark missing*
PADVAIR_RPT-mark missing	**PADVAIR_RPT**	*PADVAIR_RPT-mark missing*
SPIRO_RPT	**SPIRO_RPT**	***SPIRO_RPT***

For more information on the Spirometry Testing (SPIRO) form, see Section 10 of the AsthmaNet General MOP.

4.1.11 ALfA Asthma Monitoring Log (P9_ASTHMA_LOG)

Purpose: This form records the number of daily green RESCUE1 and blue RESCUE2 inhaler puffs taken by the participant, as well as information on adverse events and concomitant medications.

Who: The participant completes the form.

When: Return Visits 2 and 3

Form Instructions:

Starting the day after Visit 1, the ALfA Asthma Monitoring Log (P9_ASTHMA_LOG) will be completed by the participant on a daily basis.

If the participant loses his or her Asthma Monitoring Log between visits, instruct the participant to return to the clinical center as soon as possible to receive a new one. The participant should only complete the remainder of the days until the next visit; he or she should not try to recall information from the lost days. Date and initial a note in the Comments section (Q6000) on the new Asthma Monitoring Log to indicate why days are missing.

If the participant returns to the clinic with an Asthma Monitoring Log that contains information that appears to be fabricated, instruct the participant on proper completion of the log. The P9_ASTHMA_LOG data should still be entered and submitted to the DCC as usual. Clinical staff should not edit the data on the logs, as it is reported by the participant.

If the participant is unable to complete a scheduled visit at the clinic, it is the coordinator's responsibility to ensure that the participant has enough pages of the log to enter data until coming to the clinic for the next visit.

The first field of the Date column in the .pdf version of the ALfA Asthma Monitoring Log (P9_ASTHMA_LOG) is fillable. Please note that the ALfA Asthma Monitoring Log (P9_ASTHMA_LOG) is not present within the visit packets. It is available on the AsthmaNet secure website via the following path: Forms: ALfA: Data Collection Forms and present as a link under the Visit 1 and Visit 2 ALfA packets. The Visit 1 and Visit 2 P9_ASTHMA_LOGs differ from each other; the Visit 2 log has an additional page for recording rescue use due to a longer visit interval between Visits 2 and 3.

Before printing out this form to give to the participant, the coordinator can prefill all date fields on the form by entering the current date in the first field of the Date column

and then clicking elsewhere in the form. The rest of the fields will prefill with subsequent dates. Please note that a date will already be present in this field – you should be sure to update the date and prefilled dates before giving the form to the participant.

At Visit 1, the coordinator should complete the puffs reference value in Paragraph 2 before giving the ALfA Asthma Monitoring Log to the participant. The value to be entered here is the High Rescue Use value, which is calculated by adding 8 to Q1000 on the P9_BASELINE form. At Visit 2, this value will be updated based on the Visit 2 P9_BASELINE form.

ddate. This field should be auto-populated by the coordinator using the fillable PDF posted on the AsthmaNet website prior to distribution to the participant. This allows the coordinator to prefill the header information, as well. During entry in the Participant Data module, this field will also populate once the first 'ddate' is entered. This field is still available for edit after it has been populated.

Questions 1000 and 1010. The participant should record the total number of green RESCUE1 and blue RESCUE2 inhaler puffs taken each day, excluding preventive puffs. If the participant did not take any puffs on a given day, then '0' should be recorded.

Questions 1020 and 1030. The participant should indicate whether the morning or evening puff was taken from the specified Diskus®. If the puff was not taken the field should be left blank.

The last page of the ALfA Asthma Monitoring Log (P9_ASTHMA_LOG) is designed to record the following information that occurred since the last study visit:

1. Any non-study medications the participant took since the last study visit

2. Details of any medical problems the participant experienced since the last study visit (adverse events)

Review the information found for items 1 and 2 above and update the AECLIN and CMED concurrent forms, if necessary.

Collecting the Asthma Monitoring Log from the participant:

Make sure that the 'Return Visit' and 'Return Visit Date' match the current visit number and current date.

Review the logs for completeness and legibility. The logs must be legible and completed in black ink. If necessary, have the participant re-copy the logs before they are sent to the DCC. (Store the originals in the participant's study folder.)
Puffs taken the day of a visit should be recorded on the subsequent visit's log. If the participant has documented rescue puffs taken the day of a visit (prior to the visit) on the log, instruct the participant to include those puffs in that day's evening assessment on the log he/she is given at the visit. Do not data enter these puffs in the database, as any puffs taken on the day of a visit should be recorded on the subsequent visit's log.

Distributing the Asthma Monitoring Log to the participant:

Access the fillable PDF P9_ASTHMA_LOG file on the AsthmaNet website. Complete the current date (date of the visit) in the first date field at the top of the form. All dates will be completed automatically throughout the rest of the form once the user clicks out of that field.

Complete the upper right-hand corner of each log. Write in the return visit number and the proposed visit date on which the log will be returned.

Remind the participant to complete everything on the log. The log must be legible and completed in black ink.

When collating this form, all pages of the P9_ASTHMA_LOG returned on the same date should be paper clipped together as one form.

A photocopy of the logs should be made and filed in the participant's folder, while the original log should be sent to the DCC.

4.1.12 ALfA Baseline PEF and Rescue Use Values (P9_BASELINE)

Purpose: The participant's baseline peak flow (PEF) and baseline rescue use values are recorded on this form.

Who: An AsthmaNet coordinator completes this form.

When: Visits 1 and 2

Form Instructions:

Record the participant's baseline peak flow (PEF) value in the gray box for reference. This value is obtained from prebronchodilator spirometry at Visits 1 and 2, and converted to L/M. This value will not be entered into the database.

Question 1000. Record the participant's baseline rescue use value in Q1010 according to the following guidelines:

Visit	Baseline Rescue Value
1	Self-reported average daily use of home rescue inhaler during the 7 days prior to Visit 1
2	Average daily use of ipratropium (RESCUE1) during the 7 days prior to Visit 2 based on the values entered on the P9_ASTHMA_LOG

At Visit 2 any days left blank on the P9_ASTHMA_LOG should not be included in the calculation.

For example if Visit 1 occurred on 2/11 and Visit 2 on 2/18 and the P9_ASTHMA_LOG had the following values:

2/11	0
2/12	0
2/13	0
2/14	2
2/15	4
2/16	4
2/17	4

In this case the calculation would be

0+0+0+2+4+4+4 = 14 / 7 days = 2

2/11	
2/12	
2/13	
2/14	2
2/15	4
2/16	4
2/17	4

In this case the calculation would be

2+4+4+4 = 14 / 4 days= 3.5 (rounded to 4)

4.1.13 ALfA Compliance Checklist (P9_COMPLY)

Purpose: The participant's compliance with scheduled Diskus® inhaler and scheduled daily capsules are recorded on this form.

Who: An AsthmaNet coordinator completes this form.

When: Visits 2 and 3

Form Instructions:

Question 1000. The number of scheduled puffs should include all doses the participant should have taken since leaving the last clinic visit. Note that at Visit 3, the puffs withheld the morning of the visit should not be counted in the number of scheduled puffs. An ALfA Scheduled Puffs Calculator is available on the website to help determine this value (Home: Protocols: ALfA)

Question 1010. The value for Q1010 is obtained from the participant's Flovent® (at Visit 2) or Advair® (at Visit 3) Diskus® inhaler. If more than 1 Diskus® was required between visits, record the remaining puffs on Diskus® 1 + remaining puffs on Diskus® 2 for Q1010. This will occur at Visit 3 when the time between visits is greater than 30 days.

Question 1020. Calculate the number of puffs taken by subtracting the number of remaining puffs reflected on scheduled Diskus® counter (Q1010) from 60. If more than 1 Diskus® was required between visits, Q1020 will be calculated by subtracting the remaining puffs (Q1010) from 120.

Question 1030. Calculate the percent compliance by dividing the number of puffs taken (Q1020) by the number of scheduled puffs since the last visit (Q1000) and multiplying by 100. Round to the nearest tenth of a percent and record the value in Q1030..

Questions 1040-1070. Information for these questions is obtained from the MEMS®6 Monitor Report (MEMS_RPT). The information for Q1040-Q1070 is obtained from the participant's MEMS®6 TrackCap for the scheduled daily capsules at Visit 3 and early post-randomization termination visits

Question 6000. If a participant is recounseled on how to improve low compliance, please provide a comment documenting this in Q6000. Recounseling should be provided each time the participant has low medication compliance (< 80%).

See the Dosing Compliance discussion in Section 2 of the ALfA MOP for more details on the compliance calculations.

4.1.14 ALfA Coordinator Study Treatment Questionnaire (P9_CTXQX)

Purpose: This form helps to determine whether the blind on the scheduled medication was effective from the coordinator's perspective.

Who: The AsthmaNet coordinator who was primarily responsible for the participant's ALfA visits completes the form.

When: Visit 3 or early post-randomization termination visits

Form Instructions:

The ALfA Coordinator Study Treatment Questionnaire (P9_CTXQX) form should be completed at Visit 3 or on the day of a randomized participant's last visit if he or she terminates prior to Visit 3 and after Visit 2.

The visit date recorded on the form should be the date the form is completed. If the coordinator who was primarily responsible for the participant's ALfA study visits is not present during a visit when this form is to be completed, it should be completed upon his or her return and dated appropriately.

Question 1000. Q1000 should be answered with the option that most closely represents the coordinator's feelings about which type scheduled capsules the participant received during the treatment period.

Question 1020D. Any comments with respect to any other observations the coordinator may have made that helped him or her make a choice in Q1 should be recorded in Q1020D and entered into the AsthmaNet database (up to 250 characters).

To verify that the information collected on this form is correct, the coordinator who completed the form should initial and date the form in the shaded source documentation box provided (Q1030-1040) at the bottom of the page.

4.1.16 ALfA Eligibility Checklist 1 (P9_ELIG1)

Purpose: This form consists of questions that assist in determining if a participant is eligible to enroll in the ALfA study.

Who: An AsthmaNet coordinator completes the form.

When: Visit 1

Form Instructions:

For detailed information regarding eligibility criteria, see the Eligibility Criteria discussion in Section 2 of the ALfA MOP.

Question 1000. Do not ask the participant this question.

Data can **only** be collected if the participant has signed an informed consent form for the ALfA study. See the Informed Consent discussion in Section 2 for further details.

Question 1010. The signature date should be the date the participant signed the consent document. If the consent was signed prior to the Visit 1, the consent should be reviewed by the participant on the day Visit 1 takes place. The date the consent form was signed should **not** be updated.

Questions 1050 and Q1060. Refer to the ALfA ICS Equivalency Reference Card (P9_ICS_EQUIV).

Question 1140. Show the participant a demo ALfA capsule to be sure that they are able to swallow the blinded study capsule.

Question 1150. If any of the shaded boxes are completed, the participant is ineligible. The visit should be stopped and file the completed forms in the participant's folder. When a participant is ineligible at Visit 1, the packet is not entered into the database or forwarded to the DCC.

If the participant is eligible, continue with the rest of the Visit 1 procedures.

General Instructions:

If an eligibility protocol exception was granted through the DCC, complete the question(s) that the exception was granted for accurately (i.e. complete the shaded

box). Q1150 should be answered 'Yes' to indicate the participant is eligible to proceed and any entry errors that result from the exception should be marked unresolvable. In the unresolvable comment section, indicate that a protocol exception was granted, who granted it, and the justification for the exception. Also, complete the comment field (Q6000) provided on the last page of the form with additional information on the exception.

To verify that the information collected on this form is correct, have the participant initial and date the form in the shaded source documentation box provided (Q1160-1170) on the last page of the form.

4.1.17 ALfA Eligibility Checklist 2 (P9_ELIG2)

Purpose: This form consists of questions that assist in determining if a participant is eligible to enroll in the ALfA study.

Who: An AsthmaNet coordinator completes the form.

When: Visit 1

Form Instructions:

For detailed information regarding eligibility criteria, see the Eligibility Criteria discussion in Section 2 of the ALfA MOP.

Question 1000. If the participant indicates historical evidence of a disease or medical condition listed on the Exclusionary Medical Conditions for ALfA (P9_EXCLMED) reference card, but has no current evidence, Q1000 should be answered 'No'. The participant must have current evidence of one of the medical conditions for Q1000 to be answered 'Yes'.

If a participant screened at Visit 1 has one of these exclusionary medical conditions and is being allowed to progress through the study per a DCC-approved protocol exception, then Q1000 should be answered 'Yes' and Q1330 should also be answered 'Yes' (if no other ineligibity criteria are met). Resulting errors should be marked unresolvable, and the participant's condition and physician approval to proceed should be documented in the comment provided. Such cases will be treated as protocol exceptions.

Questions 1010-1110. Review participant medical records (if available) and responses on the PRIOR_COND_ADULT when answering these questions.

Question 1120. If the participant has taken one of the drugs that are listed as exclusionary on the P9_EXCLDRUG reference card within the specified time periods, but is allowed to progress through the study per a DCC-approved protocol exception, Q1120 should be answered 'Yes' and Q1330 should also be answered 'Yes' (if no other ineligibility criteria are met). Resulting errors should be marked unresolvable, and the participant's ALfA-exclusionary medication and physician approval to proceed should be documented in the comment provided. Such cases will be tracked as protocol exceptions.

Question 1157. The participant must be able to discontinue use of aspirin and/or NSAIDS for the course of the study.

Question 1160. If the participant is currently taking prescription or OTC medications other than those listed on the Allowed Medications for ALfA (P9_MEDALLOW) reference card, the coordinator should confirm through the DCC that the medication is allowed before continuing. If the medication is approved by the DCC, Q1160 should be answered 'No'. If the medication is not approved by the DCC, the participant is ineligible to continue in the study and Q1160 should be answered 'Yes'.

Questions 1170 and 1180. The participant must agree to either adhere to a specific dose of an intranasal steroid **OR** stop use of all intranasal steroids for the duration of the ALfA study, starting at or before Visit 1.

The intranasal steroid should be recorded on the Concomitant Medications for Asthma/Allergy and Adverse Events (CMED) form as an ongoing record.

Question 1190. The participant may enroll in the study if an established maintenance regimen was implemented continuously for a minimum of 3 months prior to Visit 1.

Question 1220. Calculate pack-years by multiplying the number of packs smoked per day by the number of years smoked at that quantity. One pack equals 20 cigarettes. Pack-year history will be recorded on the Adult Asthma and Allergy History (ASTHMA_HX_ADULT) form at Visit 1.

Question 1270. If there is any possibility that the participant is physically able to bear children, Q1270 should be answered 'Yes' (even if the participant indicates she is not currently engaging in heterosexual intercourse).

If the participant is surgically sterile or post-menopausal for at least one year, Q1270 should be answered 'No'. If the participant is male, Q1270 should be answered 'N/A'.

Questions 1280-Q1320. Complete only if the participant is able to bear children.

Questions Q1280-Q1300. If the participant is currently pregnant or unwilling to stop nursing during the study and for 6 months following study completion, she is ineligible to participate in the study at this time.

Questions 1310 and Q1320. Show the participant the Birth Control Methods (BIRTH_CTRL) reference card found on the AsthmaNet secure website in the Standard Forms: Reference Cards folder and ask if she is using one of the listed birth control methods.

A participant who is able to bear children **must** be using a birth control method listed on the reference card during the study and for 6 months following study completion to be eligible for the study. If the participant is not engaging in heterosexual intercourse, abstinence applies as a legitimate birth control method.

Question 1330. If any of the shaded boxes are completed, the participant is ineligible. The visit should be stopped and completed forms filed in the participant's folder. When a participant is ineligible at Visit 1, the packet is not entered into the database.

If the participant is eligible, continue with the rest of the Visit 1 procedures.

General Instructions:

If an eligibility protocol exception was granted through the DCC, complete the question(s) that the exception was granted for accurately (i.e. complete the shaded box). The applicable eligibility question(s) should be answered 'Yes' to indicate the participant is eligible to proceed and any entry errors that result from the exception should be marked unresolvable. In the unresolvable comment section, indicate that a protocol exception was granted, who granted it, and the justification for the exception. Also, complete the comment field (Q6000) provided on the last page of the form with additional information on the exception.

To verify that the information collected on this form is correct, have the participant initial and date the form in the shaded source documentation box provided (Q1340-1350) on the last page of the form.

4.1.18 ALfA Eligibility Checklist 3 (P9_ELIG3)

Purpose: This form consists of questions that assist in determining if a participant is eligible to enroll in the ALfA study.

Who: An AsthmaNet coordinator completes the form.

When: Visit 1

Form Instructions:

For detailed information regarding eligibility criteria, see the Eligibility Criteria discussion in Section 2 of the ALfA MOP.

Question 1000. The participant's prebronchodilator (baseline) FEV_1 (% predicted) value should be obtained from Q1040 on the Spirometry Testing (SPIRO) form completed at the visit. The participant's prebronchodilator FEV_1 value should be obtained from Q1030 on the SPIRO form. Both SPIRO Q1030 and Q1040 are used to answer P9_ELIG3 Q1000.

Question 1020. This question should only be answered if IRB approval for protocol version 2.2 has NOT been obtained. If the participant's FEV_1 % predicted is greater than or equal to 80 percent this question should be left blank and skip to Q1030.

If the participant's FEV_1 % predicted is less than 80 percent this question should be completed and Q1030 should left blank.

To determine the % change in response to albuterol, calculate as follows:

> [(Post-bronchodilator FEV_1 – Pre-bronchodilator FEV_1) / Pre-bronchodilator FEV_1] x 100

Calculations must be based on FEV_1 in liters (not % of predicted scale).

Do not round the result to the nearest full percentage when assessing eligibility.

If the calculation is close, but less than 12.0%, contact the ALfA Scientific Coordinator at the DCC for an exception before assuming the asthma verification criteria have been met. The exception must be documented on P9_ELIG3 as explained below.

Question 1025. This question should only be answered if IRB approval for protocol version 2.2 has been obtained. If the participant's FEV_1 % predicted is greater than or equal to 80 percent this question should be left blank and skip to Q1030.

If the participant's FEV_1 % predicted is less than 80 percent predicted, reversibility and/or methacholine challenge may be completed.

- If only methacholine challenge is performed Q1025 should be answered 'N/A'. If reversibility is performed this question should be completed.
- If only reversibility is performed Q1025 should be completed and Q1030 should be left blank.
- If reversibility is completed and FEV_1 does not improve 12% or more and methacholine challenge is performed at a continuation visit both Q1025 and Q1030 should be completed.

If a Continuation Visit is necessary, PREG_TEST, P9_PULMONARYCHK single form, SPIRO single form, METHACHK_ADULT and METHA forms will be completed first. The rest of the P9_ELIG3 form and Visit 1 forms should then be completed as outlined by the visit procedure checklist, using the visit date of the continuation visit.

To determine the % change in response to albuterol, calculate as follows:

$$[(\text{Post-bronchodilator } FEV_1 - \text{Pre-bronchodilator } FEV_1) / \text{Pre-bronchodilator } FEV_1] \times 100$$

Calculations must be based on FEV_1 in liters (not % of predicted scale).

Do not round the result to the nearest full percentage when assessing eligibility.

If the calculation is close, but less than 12.0%, contact the ALfA Scientific Coordinator at the DCC for an exception before assuming the asthma verification criteria have been met. The exception must be documented on P9_ELIG3 as explained below.

Question 1030. If participant's FEV1 % predicted is greater than or equal to 80, Q1030 will be obtained from Q1050 on the Methacholine Challenge Testing (METHA) form.

Question 1060. If any of the shaded boxes are completed, the participant is ineligible. The visit should be stopped and file the completed forms in the participant's folder. When a participant is ineligible at Visit 1, the packet is not entered into the database.

If the participant is eligible, continue with the rest of the Visit 1 procedures.

General Instructions:

If an eligibility protocol exception was granted through the DCC, complete the question(s) that the exception was granted for accurately (i.e. complete the shaded box). The applicable eligibility question(s) should be answered 'Yes' to indicate the participant is eligible to proceed and any entry errors that result from the exception should be marked unresolvable. In the unresolvable comment section, indicate that a protocol exception was granted, who granted it, and the justification for the exception. Also, complete the comment field (Q6000) provided on the last page of the form with additional information on the exception.

4.1.19 ALfA Eligibility Checklist 4 (P9_ELIG4)

Purpose: This form consists of questions that assist in determining if a participant is eligible to continue in the ALfA study.

Who: An AsthmaNet coordinator completes the form.

When: Visit 2

Form Instructions:

For detailed information regarding eligibility criteria, see the Eligibility Criteria discussion in Section 2 of the ALfA MOP.

Questions 1000-1030. Refer to the P9_LAB form in the Visit 1 packet for responses to these questions.

Question 1040. If the participant had an asthma exacerbation it should be recorded on the AECLIN form using ICD9 code 493.92.

Question 1050. If the participant has taken one of the drugs that are listed as exclusionary within the specified time periods, but is allowed to progress through the study per a DCC-approved protocol exception, Q1050 should be answered 'Yes' and Q1120 should also be answered 'Yes' (if no other ineligibility criteria are met). Resulting errors should be marked unresolvable, and the participant's ALfA-exclusionary medication and physician approval to proceed should be documented in the comment provided. Such cases will be tracked as protocol exceptions.

Question 1060. Refer to Q1030 on the P9_COMPLY form.

Question 1070. This question should only be answered if IRB approval for protocol version 2.3 has NOT been obtained. The participant must be able to provide five 8 mL tubes (for a total of 40 mL) of blood. If the participant is unable to provide the full amount the visit can be rescheduled. If unable to draw this amount at second attempt, the participant is ineligible. Do not continue with the rest of the Visit 2 procedures.

Question 1075. This question should only be answered if IRB approval for protocol version 2.3 HAS been obtained. The participant must be able to provide ten 8 mL tubes (for a total of 80 mL) of blood. If the participant is unable to provide the full amount the visit can be rescheduled. If unable to draw this amount at second attempt, the participant is ineligible. Do not continue with the rest of the Visit 2 procedures.

Question 1080. Refer to Q1050 on the METHA form.

Question 1120. If any of the shaded boxes are selected, the participant is ineligible. If the participant is ineligible based on P9_ELIG4 form, do not continue the Visit 2 procedures and mark the rest of the packet forms missing during data entry. Complete the P9_TERM form single form, and enter it as a Visit 2 single form.

General Instructions:

If an eligibility protocol exception was granted through the DCC, complete the question(s) that the exception was granted for accurately (i.e. complete the shaded box). The applicable eligibility question(s) should be answered 'Yes' to indicate the participant is eligible to proceed and any entry errors that result from the exception should be marked unresolvable. In the unresolvable comment section, indicate that a protocol exception was granted, who granted it, and the justification for the exception. Also, complete the comment field (Q6000) provided on the last page of the form with additional information on the exception.

4.1.20 ALfA Laboratory Results (P9_LAB)

Purpose: This form is completed after the local lab report is received.

Who: An AsthmaNet coordinator completes the form.

When: Visit 1

Form Instructions:

Visit Date. Record the visit date as the date the blood is drawn (should match collection date on lab report).

Questions 1000-1110. Refer to the laboratory results printout generated at each clinical center's local laboratory to answer Q1000 – Q1110.

Question Q1100. Complete this question if the participant's total serum Calcium is less than 8.5 mg/dL, otherwise leave this field blank.

Question 1120. Use on-line calculator at www.mdcalc.com/creatinine-clearance-cockcroft-gault-equation to compute the Cockcroft-Gault estimate. Gender, age, weight (lbs), and height (in) are required to compute eGFR. Refer to Q6000 on the BODYMEAS_ADULT form for the converted units that you noted when completing the form. Following completion of the specified fields on the website, value given for "Creatinine Clearance Modified for Underweight/Normal weight/Overweight patient" should be entered in Q1120.

Round each value to the nearest tenth, where applicable, and record the value on the form. Ensure that measurement units match those on the form; otherwise, make the necessary conversions before submitting the data to the DCC.

Submit the original lab report with the participant's visit packet. The coordinator should record the participant's ID number in the upper right-hand corner of the report.

All identifying information (name, medical record number, etc.) should be blackened-out prior to forwarding the report to the DCC with the packet. If the DCC receives a report for which the identifying information has not been blackened-out, a protocol violation may be assigned.

4.1.21 ALfA Participant Study Treatment Questionnaire (P9_PARTTXQX)

Purpose: Any observations the participant may have made during the ALfA study that may have compromised the study blind on the scheduled medication are recorded on this form.

Who: The participant completes the form.

When: Visit 3 or after Visit 2 if the participant terminates after randomization prior to Visit 3

Form Instructions:

The ALfA Participant Study Treatment Questionnaire (P9_PARTTXQX) form should be completed at Visit 3 or on the day of a randomized participant's last visit if he or she terminates prior to Visit 3.

The visit date recorded on the form should be the date the form is completed.

Question 1000. Check only one box.

Question 1010. The participant should check the box that most closely represents his or her feelings about which type of scheduled capsules he/she used over the past 8 weeks.

Question 1020. If the participant chooses option 2, he or she can comment on the taste of, smell of, or physical sensations produced by the capsules in Q1020D.

To verify that the information collected on this form is provided by the participant, have the participant initial and date the form in the shaded source documentation box provided (Q1030-1040).

This form should not be edited by the study coordinator for any reason. Please review the form with the participant at the visit and if any corrections or clarifications need to be made have the participant make the edit and initial and date any changes.

4.1.22 ALfA Pulmonary Procedure Checklist (P9_PULMONARYCHK)

Purpose: This form assists the coordinator in determining if the participant is eligible to proceed with pulmonary function testing.

Who: The Pulmonary Function Technician or an AsthmaNet coordinator interviews the participant and completes the form. The individual completing the form **must possess ALfA protocol certification**.

When: Visits 1, 2, 3

Form Instructions:

If the ALfA Pulmonary Procedure Checklist (P9_PULMONARYCHK) form is completed in between regular study visits, specify the number of the last visit completed and the current visit date in the upper right-hand corner. This form should be entered as a single form.

If any medications other than the study medications (green RESCUE1 or blue RESCUE2) were used, record the medications on the Concomitant Medications for Asthma/Allergy and Adverse Events (CMED) form.

Questions 1130-1140. These questions are only answered at Visits 2 and 3.

Questions 1150-1155. These questions are only answered at Visit 3.

Question 1180. The participant is ineligible to perform pulmonary function testing if any of the shaded boxes are completed.

If the participant is not eligible to proceed with spirometry and is willing to reschedule the visit, file the collected data in his or her study folder; do not enter the data or forward it to the DCC.

If a spirometry eligibility protocol exception was granted through the DCC, complete the question(s) for which the exception was granted accurately (i.e. complete the shaded box). Q1180 should be answered 'Yes' to indicate the participant is eligible to proceed and any entry errors that result from the exception should be marked unresolvable. In the unresolvable comment section, indicate that a protocol exception was granted, who granted it, and the justification for the exception. Also, complete the comment field (Q6000) provided on the last page of the form with additional information on the exception.

Question 1190. If participant is 18 to 20 years old, complete Q1190 at Visits 2 and 3.

At Visits 1, refer to height recorded on the Adult Body Measurements (BODYMEAS_ADULT) form; do not record on this form.

4.1.23 ALfA Saliva Collection Checklist (P9_SALIVACHK)

Purpose: This form assists the coordinator in determining if the participant is eligible to proceed with saliva collection.

Who: An AsthmaNet coordinator interviews the participant and completes the form.

When: Visits 2 and 3

Form Instructions:

Question 1060. The participant is ineligible for saliva collection if any of the shaded boxes are completed. A saliva sample is required at Visit 2.

If the participant is not eligible to proceed with saliva collection and is willing to reschedule the visit, file the collected data in his or her study folder; do not enter the data or forward it to the DCC.

If the participant is not eligible to proceed with saliva collection and is not willing to reschedule the visit at Visit 3, indicate this in field Q6000.

If a saliva collection eligibility protocol exception was granted through the DCC, complete the question(s) for which the exception was granted accurately (i.e. complete the shaded box). Q1060 should be answered 'Yes' to indicate the participant is eligible to proceed and any entry errors that result from the exception should be marked unresolvable. In the unresolvable comment section, indicate that a protocol exception was granted, who granted it, and the justification for the exception. Also, complete the comment field (Q6000) provided on the last page of the form with additional information on the exception.

4.1.24 ALfA Scheduled Capsules (P9_MED)

Purpose: The dispensation of post-randomization scheduled capsules is recorded on this form.

Who: An AsthmaNet coordinator completes the form.

When: Visit 2

Note: This form must be completed every time scheduled capsules are dispensed at Visit 2 and in the event of backup dispensation for lost or expiring scheduled capsules.

Form Instructions:

The ALfA Scheduled Capsules (P9_MED) form must be completed **every** time scheduled capsules are dispensed.

Following the loss of medications, complete a new ALfA Scheduled Capsules (P9_MED) form with the current date and the visit number corresponding to the last visit completed in the upper right-hand corner.

For more information on backup drug procedures, see Section 5 and the Study Medications discussion in Section 2 of the ALfA MOP.

If backup capsules are dispensed, complete the ALfA Scheduled Capsules (P9_MED) form and enter it as a single form. For example, when scheduled medications are dispensed at Visit 2, complete the packet Visit 2 ALfA Scheduled Capsules (P9_MED) form. If the participant loses the bottle dispensed at regular Visit 2, generate a backup bottle number and complete the ALfA Scheduled Capsules (P9_MED) form for the backup bottle dispensation. Enter this form as a Visit 2 single form at the time of backup bottle dispensation.

Question 1000 and Label. Remove the label from the dispensed capsule bottle and affix to the ALfA Scheduled Capsule (P9_MED) form in the box next to Q1000. Copy the bottle number into field Q1000.

After affixing the label, the coordinator should sign and date the source documentation box provided (Q1010-1020).

Record all dispensations on the Post-Randomization Study Medications (P9_DRG_SCH_ALEN) log.

4.1.25 ALfA Significant Asthma Exacerbation (P9_SIGEX)

Purpose: This form outlines the significant asthma exacerbation criteria to determine if a participant experienced an event during the ALfA study.

Who: An AsthmaNet coordinator completes the form.

When: Visits 1, 2, 3

Form Instructions:

The ALfA Significant Asthma Exacerbation (P9_SIGEX) form is completed **only** if the participant experiences a significant asthma exacerbation as defined in the Significant Asthma Exacerbation discussion in Section 2 of the ALfA MOP.

The ALfA Significant Asthma Exacerbation (P9_SIGEX) form should be entered and forwarded to the DCC within one week of form completion. If this form is completed between visits, specify the number of the last visit completed and the current date in the upper right-hand corner.

Question 1000. To determine this response:

1. Determine the baseline rescue use value (Refer to Q1000 on the last completed P9_BASELINE form)
2. Add 8 to this value to determine the participant's High Rescue Use value
3. Refer to the participant's P9_ASTHMA_LOG. If any combined puffs for RESCUE1 (Q1000) and RESCUE2 (Q1010) is greater than or equal to the participant's High Rescue Use value for two consecutive days, Q1010 should be answered 'Yes'.

Example: After Visit 2 the participant reports an increase in rescue use. The value for Q1000 on the participant's Visit 1 P9_BASELINE form is 4. 4 + 8 = 12
If the sum of the values for Q1000 and Q1010 on the P9_ASTHMA_LOG is greater than or equal to 12 for any two consecutive dates, Q1010 should be answered 'Yes'.

Date (ddate)	Total green RESCUE1 Puffs Used (1000)	Total blue RESCUE2 Puffs Used (1010)	
1/1/2014	6	0	
1/2/2014	12	0	12+0=12
1/3/2014	10	4	10+4=14

*Note that in this example the date of the Significant Asthma Exacerbation is 1/3/14

Question 1010. Refer to the participant's P9_ASTHMA_LOG. If any combined puffs for RESCUE1 (Q1000) and RESCUE2 (Q1010) for a given day is greater than or equal to 16, Q1010 should be answered Yes.

Question 1020. Refer to Q1030 on the participant's spirometry data collected on the Spirometry Testing (SPIRO) form. The baseline value is Q1030 on the Spirometry Testing (SPIRO) form at Visit 1. If the FEV_1 value is below 80% of the baseline prebronchodilator value the criterion is met. If spirometry is not performed, Q1020 should be answered 'N/A.'

Question 1030. Refer to Q1040 on the participant's spirometry data collected on the Spirometry Testing (SPIRO) form. If the FEV_1 value is below 40% of predicted according to Q1040, the criterion is met. If spirometry is not performed, Q1030 should be answered 'N/A.'

Question 1040. If the study or treating physician prescribed the participant oral/parenteral corticosteroids for the treatment of his or her asthma, record the details on the Concomitant Medications for Asthma/Allergy and Adverse Events (CMED) form.

Question 1050. If any of the shaded boxes are completed in Q1000-1040, the participant experienced a significant asthma exacerbation. Complete Q1060 and record the event on the Clinical Adverse Events (AECLIN) form using ICD-9 code 493.92.

If IRB approval for protocol version 2.2 has been obtained, and this is the first exacerbation of the treatment phase, the participant's participation should be extended so that the final visit occurs at least 4 weeks after completion of the prednisone course. If this extension causes the treatment period to be more than 12 weeks, the participant should be terminated and a P9_TERM form should be entered. On the P9_TERM form Q1080 and Q1170 should be answered 'Yes'. A comment should be added to Q1180D noting that the treatment period would exceed 12 weeks due to a prednisone course and the primary reason for termination should be 'Other' (i.e. Q1190 should be 'L').

If IRB approval for protocol version 2.2 has been obtained, and this is the second exacerbation of the treatment phase, the participant should be terminated and a P9_TERM form should be entered. On the P9_TERM form Q1080 and Q1170 should be answered 'Yes'. A comment should be added to Q1180D noting that the participant experienced two significant asthma exacerbations and the primary reason for termination should be 'Other' (i.e. Q1190 should be 'L').

If the completed form indicates the participant did not experience a significant asthma exacerbation, do not complete Q1060 and do **not** enter or submit the form to the DCC.

Question 1060. Record the date when the exacerbation criteria is met. If multiple criteria were met to indicate a significant asthma exacerbation, record the earliest date criterion was confirmed.

Question 1140. If Q1140 is answered Yes, complete a SERIOUS form and forward to the ALfA scientific coordinator within 72 hours.

Question 1180-1230. Participants experiencing a significant asthma exacerbation will be treated with oral prednisone (dispensed at participant's first visit). Record oral prednisone, as well as non-study inhaled corticosteroids, nebulized bronchodilator, oral corticosteroids, IM or IV steroids, antibiotics, or other medications taken for the exacerbation on the Concomitant Medications for Asthma/Allergy and Adverse Events (CMED) form. Link the medication to the significant asthma exacerbation event on the Clinical Adverse Events (AECLIN) form.

Oral prednisone taken for treatment of a significant asthma exacerbation should be recorded on the Concomitant Medications for Asthma/Allergy and Adverse Events (CMED) form and linked to the significant asthma exacerbation event. Do this by recording the significant asthma exacerbation event record ID in Q1020 of the medication record on the Concomitant Medications for Asthma/Allergy and Adverse Events (CMED) form. Any treatment required should also be indicated in the significant asthma event record on the Clinical Adverse Events (AECLIN) form.

If non-study medication was taken for treatment of the significant asthma exacerbation, record the medication on the Concomitant Medications for Asthma/Allergy and Adverse Events (CMED) form and link the medication to the significant asthma exacerbation event. Do this by recording the event record ID in Q1020 on the Concomitant Medications for Asthma/Allergy and Adverse Events (CMED) form.

4.1.26 ALfA Termination of Study Participation (P9_TERM)

Purpose: The date and the primary reason for a participant's termination of study participation are recorded on this form.

Who: An AsthmaNet coordinator completes the form.

When: Visits 1-3

Note: This form is completed at Visit 3 for those participants who complete the entire ALfA study. It may be completed at or between regular study visits (Visits 1-2) when the participant withdraws consent, becomes pregnant, or is terminated by performance site staff.

Form Instructions:

Visit Date: This date should be the date the form is completed. It may not necessarily be the same as the most recent regular visit date. For example, if the participant is being terminated due to loss to follow up, the visit date would be the date the coordinator completed this form to document the termination, NOT the visit date of the last regular visit.

If a participant withdraws consent or is terminated from the ALfA study during a visit, specify the number of the current visit and the current visit date in the upper right-hand corner. For example, if the participant terminates during Visit 2, then the visit number on the form should be '2.' This form will be entered into the AsthmaNet database as a single form.

If a participant withdraws between visits, submit the ALfA Termination of Study Participant (P9_TERM) form with the number of the last visit completed in the upper right-hand corner. For instance, a participant could be terminated from the ALfA study following Visit 2 but before Visit 3 due to moving out of the area. In this case, the ALfA Termination of Study Participant (P9_TERM) form should be entered as a single form with the last visit number completed in the upper right-hand corner of the form, Visit 2.

Question 1010. If Q1010 is answered 'Participant,' complete Q1020 and Q1030D, if applicable, and skip to the signatures section of the form. Otherwise, skip to Q1040 and complete the rest of the form.

Question 1030D. An explanation should be provided for Q1030D if Q1020 is answered 1, 2, 6, 7, 8, or 10. If an explanation is provided, enter the full explanation (up to 100 characters) into the AsthmaNet database; otherwise, leave the field blank during data entry.

Questions 1040-1170. ALL applicable reasons for termination should be noted in these questions, even if not the primary reason for termination (Q1190). For example, if the participant is being terminated due to a medication-related adverse event, but also had an asthma-related adverse event both Q1080 and Q1090 would be answered 'Yes'.

Question 1040. If the participant is male, Q1040 should be answered 'N/A'. If the participant is female and surgically sterile or postmenopausal, Q1040 should be answered 'No'. Q1040 should be answered 'Yes' if the participant becomes pregnant during the course of the ALfA study.

Question 1180D. An explanation should be provided for Q1180D if any of the following is answered 'Yes': Q1050, Q1080-Q1130, Q1160-Q1170. If an explanation is provided, enter the full explanation (up to 100 characters) into the AsthmaNet database. Otherwise, leave the field blank during data entry.

Question 1190. At least one of the questions in Q1040-1170 must be answered 'Yes' if clinical staff terminated the participant. Of the questions Q1040-1170 marked 'Yes', indicate the letter associated with the primary reason for termination in Q1190.

This form requires the signatures of the coordinator and AsthmaNet investigator to verify that all data collected for this participant are correct to the best of their knowledge.

Questions 1200 and 1220. If a signature is not present, this field should be left missing during data entry.

Any AsthmaNet investigator (site director, Principal Investigator, or other) may sign field Q1220 to verify that all data collected for this participant are correct to the best of their knowledge.

4.2 *Administrative Forms*

Administrative forms facilitate processing of the participant and visit flow by the performance sites and the DCC. They are not entered into the AsthmaNet database and they are not submitted to the DCC in most cases. The following is a list of all ALfA study administrative forms and related instructions[1]:

Administrative Form Name	Form Code
ALfA Participant Assignment Log	P9_LOG
ALfA PBMC Sample Log	P9_PBMC_SAMP_LOG
ALfA Phadiatop Serum Sample Log	P9_PHAD_SAMP_LOG
ALfA Phone Contact Form	P9_CONTACT
ALfA Saliva Sample Log	P9_SALIVA_SAMP_LOG
ALfA Visit Procedure Checklists	P9_VISIT1, P9_VISIT2, P9_VISIT_PC, P9_VISIT3
ALfA Weekly Contact Log	P9_CONTACT_LOG

[1]Drug logs and related procedures are covered in the ALfA Pharmacy MOP

4.2.1 ALfA Participant Assignment Log (P9_LOG)

Purpose: This form is a log of all participants enrolled in the ALfA study.

Who: An AsthmaNet coordinator completes the log.

When: Visits 1 and 2

Form Instructions:

The ALfA Participant Assignment Log (P9_LOG) must be used each time a **new** participant ID number is assigned. A new participant ID number is assigned by completing the next available blank entry on the log at Visit 1. The protocol ID, site ID, and participant ID will be pre-filled on the assignment log printed from Forms: ALfA: Admin Forms section on the AsthmaNet secure website.

Participant initials must have three letters. The letter "X" should be used if a participant does not have a middle initial. The participant's initials must be the same initials entered in the AsthmaNet Registry module.

The participant's name should be written last name first, followed by first name on the ALfA Participant Assignment Log (P9_LOG).

At Visit 2, if the participant is randomized, mark the checkbox under the Randomized column.

This log, along with the corresponding medication logs, will be reviewed during AsthmaNet site visits.

For use only at the Clinical Center. This form is not data entered.

DO NOT forward to the DCC.

4.2.2 ALfA PBMC Sample Log (P9_PBMC_SAMP_LOG)

Purpose: To record information regarding a participant's specimen collection for peripheral blood mononuclear cell (PBMC) isolation.

Who: An AsthmaNet Coordinator completes the log.

When: When blood samples are collected for PBMC isolation at Visits 2 and 3.

Form Instructions:

When a blood sample is collected in a CPT tube for PBMC isolation the Coordinator must complete a new row on the log for the participant ID. The blood sample will be processed and separated into six cryotubes used for Western Blot, cAMP-PBS, cAMP-Isoproterenol, cAMP-forskolin, Radioligand binding, and mRNA biochemical assays. The log captures information on the collection of the tube as well as the processing. Storage conditions are also recorded.

ALfA sample barcodes do not contain participant identifiers. **It is important to record the barcodes on the log and enter the samples into Biological Sample Tracking as soon as possible after processing occurs.**

This log will be reviewed during AsthmaNet site visits.

For use only at the Clinical Center. This form is not data entered.

DO NOT forward to the DCC.

4.2.3 ALfA Phadiatop Sample Log (P9_PHAD_SAMP_LOG)

Purpose: To record information regarding a participant's specimen collection for Phadiatop testing.

Who: An AsthmaNet Coordinator completes the log.

When: When serum samples are collected for Phadiatop testing at Visit 2.

Form Instructions:

When a blood sample (red top tube) is collected for Phadiatop testing, the Coordinator must complete a new row on the log for the participant ID. The log captures information on the collection of the tube as well as the processing. Storage conditions are also recorded.

ALfA sample barcodes do not contain participant identifiers. **It is important to record the barcodes on the log and enter the samples into Biological Sample Tracking as soon as possible after processing occurs.**

This log will be reviewed during AsthmaNet site visits.

For use only at the Clinical Center. This form is not data entered.

DO NOT forward to the DCC.

4.2.4 ALfA Phone Contact Form (P9_CONTACT)

Purpose: This form guides the coordinator in completing a scheduled phone contact with the participant. The questions assist in checking the participant's asthma control, scheduled medication usage, and medical care.

Who: An AsthmaNet Coordinator interviews the participant while completing this form.

When: At phone calls between in clinic study visits starting after Visit 2

Form Instructions:

When completing this form, specify the number of the last visit completed in the upper right-hand corner.

Complete the gray box with coordinator ID, date, time, and if contact occurred for each attempt made to contact the participant.

When contact is made with the participant, ask him or her to refer to the ALfA Asthma Monitoring Log (P9_ASTHMA_LOG) and the Advair® Diskus® counter. The coordinator will be asking the participant questions regarding RESCUE medication use and study medication (study capsules and Advair® Diskus®) use and compliance. The coordinator will also ask questions about any changes to medical conditions or medications.

Question 1. Check only one box. If Q1 is answered 'Yes', a description should be provided.

Questions 2 and 3. Indicate if the participant has been using his/her scheduled medications daily and as instructed.

If any of the questions are answered 'No,' review study adherence and capsule instructions with the participant.

Question 4. Indicate if the participant has been taking 1 puff from the Advair® Diskus® every morning and evening.

If the question is answered 'No,' review study adherence with the participant.

Question 7. Indicate if the participant indicates that he or she has used more than his/her High Rescue Use value puffs on any day. The High Rescue Use value is calculated by adding 8 to Q1000 on the P9_BASELINE form at Visit 2.

Question 7a. If Q7 is answered Yes, record the dates and number of puffs of high rescue use.

If participant used greater than or equal to his/her High Rescue Use value for two days in a row or 16 or more puffs per day, he/she has experienced a significant asthma exacerbation. Complete a Significant Asthma Exacerbation (P9_SIGEX) form, and enter records on the AECLIN and CMED forms.

Question 8. If the participant indicates that he or she took any new medications other than those given as part of the study since the last visit, record the medication on the Concomitant Medications for Asthma/Allergy and Adverse Events (CMED) form at the next visit.

Question 8a. If the participant was treated with prednisone or another systemic corticosteroid since the last study contact, he/she has met Significant Asthma Exacerbation criteria. Complete a Significant Asthma Exacerbation (P9_SIGEX) form and update CMED and AECLIN forms.

Question 9. If the participant indicates that he or she experienced a medical problem since the last visit, record the event on the Clinical Adverse Events (AECLIN) form at the next visit.

Question 10. If the participant indicates that he or she had any changes to non-study medications since the last visit, record the medication change on the Concomitant Medications for Asthma/Allergy and Adverse Events (CMED) form at the next visit. Depending on the type of medication that was changed, the Concomitant Medications for Non-Asthma Drugs (CMED_NON) form may instead need to be updated.

This form will be reviewed during AsthmaNet site visits.

For use only at the performance site

DO NOT forward to the DCC.

4.2.5 ALfA Saliva Sample Log (P9_SALIVA_SAMP_LOG)

Purpose: To record information regarding a participant's pre- and post-Advair® saliva specimen collections.

Who: An AsthmaNet Coordinator completes the log.

When: When saliva samples are collected at Visits 2 and 3.

Form Instructions:

When saliva samples are collected, pre- and post- Advair® administration the Coordinator must complete a new row on the log for the participant ID. The log captures information on the collection of the tubes as well as the storage conditions.

ALfA sample barcodes do not contain participant identifiers. **It is important to record the barcodes on the log and enter the samples into Biological Sample Tracking as soon as possible after processing occurs.**

This log will be reviewed during AsthmaNet site visits.

For use only at the Clinical Center. This form is not data entered.

DO NOT forward to the DCC.

4.2.6 ALfA Visit Procedure Checklists (P9_VISIT1, P9_VISIT2, P9_VISITPC, P9_VISIT3)

Purpose: To provide the coordinator with a checklist of all procedures and forms completed during a visit.

Who: An AsthmaNet coordinator completes the form.

When: At the specified visit

Form Instructions:

These checklists serve as guides for the coordinator and should be sent to the DCC, in front of the visit packet, with the other forms in the packet.

For all procedures and forms, indicate whether or not the procedure or form was completed. If it was not completed, indicate the reason in the comment field.

If a visit is missed, complete the checklist indicating the missed visit and document if any other actions were completed (i.e., dispensation of additional study medications). The completed checklist should be filed at the performance site and does not need to be sent to the DCC.

Procedures should be followed in the order they are presented on the visit checklist for applicable visits. If certain procedures, such as pulmonary function testing and questionnaire completion, are performed out of order, a protocol deviation will be assigned.

This form is not entered during data entry. Note: The asterisks present throughout the checklists are to indicate forms that should not be sent to the DCC. The checklists should be sent to the DCC with successful visit packets.

4.2.7 ALfA Weekly Contact Log (P9_CONTACT_LOG)

Purpose: To document weekly participant contacts during the randomized treatment period to encourage adherence with daily dosing schedule.

Who: An AsthmaNet coordinator completes the form.

When: Weekly following randomization at Visit 2.

Form Instructions:

The contacts at weeks 1, 3, 5, 6, and 7 are meant to encourage study capsule and Advair® Diskus® compliance in between study visits.

If the participant reports non-compliance with study medications, review study procedures with the participant.

If the participant reports a significant asthma exacerbation, complete a Significant Asthma Exacerbation (P9_SIGEX) form and update the CMED and AECLIN forms.

If the participant reports any new or changes to medical problems complete and/or update the appropriate AECLIN form.

If the participant reports any new or changes to medications complete and/or update the appropriate CMED form.

At Weeks 2 (Visit 2A) and 4 (Visit 2B) complete the ALfA Phone Contact (P9_CONTACT) administrative form in addition to this log. For more information on the ALfA Phone Contact (P9_CONTACT) administrative form, see Section 4.2.4 of this MOP.

Coordinator ID. Record the Coordinator ID of the research coordinator who makes the contact.

Date. Record the date of the successful contact. If the contact method is e-mail or text record the date that the communication was sent.

Contact Method. Indicate the contact method that was used.

Phone Contact Time. If the participant was contacted via a phone call record the time of the call.

Phone Contact Occurred?. If the participant was contacted via a phone call indicate if the coordinator spoke to the participant or left a message.

Contents

2.1 Adherence Issues

Participants enrolled in the ALfA protocol are involved in study activities throughout the trial. A great deal is asked of participants, and the quality of the study results is a function of the participants' level of protocol adherence. Each participant must be given every opportunity to be compliant and successful.

Factors That Affect Adherence
It is important to be aware of factors that may affect a participant's adherence level.

Participant Characteristics

- ability to comprehend and recall instructions
- support of family members for study participation
- satisfaction with care and caregivers
- degree of concern about health
- perception of disease severity
- perceived costs and benefits of treatment

Performance Site Personnel Characteristics

- consistency of AsthmaNet personnel with whom participants have contact during the study
- demonstration of interest and genuine concern for the participant's health
- warm and caring demeanor; approachable
- engagement in social conversation and active interchange
- presentation of clear instructions
- proficiency in clinical activities
- accessibility when the participant has questions, concerns or emergency needs

Clinic Characteristics

- positive and warm environment (unhurried and comfortable)
- timely appointments
- organized and efficient

Characteristics of Regimen (determined by the protocol)

- most important determinant of adherence
- should not be too complex
- side effects of study drug should not be a big problem/concern
- regimen should be adaptable to participant's life and work, not the other way around

Improving Adherence

A number of approaches can be used to improve adherence in the ALfA trial:

- Associate the regimen with daily activities

  Encourage the participant to associate the required study activities with his/her daily routine to help make these steps automatic. This point can be reinforced while reviewing the Daily Activities handouts at each visit (P9_DAILYACT1, P9_DAILYACT2).

- Educate the participant
  - Make sure the study activities are understood
  - Demonstrate the activities and have the participant do the same
  - Present instructions as clearly as possible
  - Have the participant repeat instructions
  - 'Quiz' the participant on the instructions
  - Teach the regimen in a stepwise fashion (i.e., step 1, step 2, step 3 for AM and PM activities)
  - Review 1 or 2 of the participant handouts at each visit
  - Use phone contacts to reinforce instructions and to ensure that the participant is performing activities correctly

- Provide positive reinforcement for excellent participant adherence

- Encourage support of family and friends during study participation

- Prepare participants for what will happen at upcoming visits

- Run the clinic on schedule and make good use of the participant's time

- Make sure the clinic is accessible with flexible hours and ample, convenient parking

- Avoid no-shows with a reminder phone call in advance of the visit date. Call the participant’s residence and cell phone immediately if there is a no-show

- Ensure that clinic personnel are easily accessible by phone, pager, and e-mail

- Develop a friendly and caring relationship with the participant

  An integral part of the visit is interacting with the study personnel. A feeling of attachment or obligation to an individual improves adherence and reduces withdrawals.

Tools for Monitoring and Improving Adherence during the ALfA Trial
The following tools are in place to improve and/or monitor adherence (form name is given in parentheses, where applicable):

Visit Scheduler Reports
Missed visits and poorly timed visits are forms of non-adherence. In order to allow the participant and the performance site to plan for upcoming visits, visit scheduler reports have been programmed that list the ideal dates and lower and upper regular and extended windows for upcoming visits, phone calls and contacts per the protocol.

See the Visit Scheduler discussion in this section and Section 3 of this manual for further details on the creation of visit scheduler reports.

Visit Handouts and Study Folder
A series of handouts is presented and reviewed with the participant at Visits 1 and 2 as new procedures and concepts are introduced. Because it may be difficult to comprehend and execute all instructions initially, and because activities change during the study according to the study phase, participants are asked to bring this folder to each visit for review and replacement of certain materials. A description of each of the ALfA handouts follows can be found in the Study Handout Folder discussion in this section.

Daily Activities Handouts
ALfA Daily Activities: Visit 1 (P9_DAILYACT1)
ALfA Daily Activities: Visit 2 (P9_DAILYACT2)

These handouts contain simple summaries of the study activities that must be carried out each day, including dosing with the Flovent® Diskus® during the run-in and Advair® Diskus® and scheduled capsules during the randomized treatment phase. These handouts also provide the participant a quick reference for criteria for determination of significant exacerbation conditions. See the Daily Activities Handout discussion in this section for further details.

How to Use Your Metered Dose Inhaler (HTMDI)
This handout provides general instructions for proper inhalation technique for home use of the MDI inhaler.

How to Use Your Diskus® (HTDISKUS)
This handout provides general instructions for proper inhalation technique for home use of the Diskus®.

Participants must demonstrate proper Diskus® inhalation technique as assessed through the Diskus® Inhalation Technique Checklist (TECH_DISKUS) before leaving Visit 1. Diskus® inhalation technique will also be assessed through the Diskus® Inhalation Technique Checklist (TECH_DISKUS) at Visit 2. See the Inhalation Technique Assessment discussion in this section for further details.

ALfA Participant Identification Card (P9_ID)
The ALfA Participant Identification Card (P9_ID) facilitates the identification, treatment, and handling of worsening asthma symptoms by the participant and by healthcare providers. Baseline peak flow, 70% baseline peak flow, baseline rescue use, and high rescue use values are completed on the ID card at Visit 1. These values will be updated at Visit 2. See the Participant Identification Card discussion in this section for further details.

If Your Asthma Gets Worse (P9_ASWORSE)
This handout contains instructions for recognizing and treating asthma attacks. It outlines proper use of the Atrovent® (green RESCUE1) inhaler and the Ventolin® (blue RESCUE2) inhaler. It is important for the integrity of the study for the participant to understand how to recognize asthma attacks, and to use these inhalers as outlined in the protocol. This handout should be thoroughly covered at Visit 1 and reviewed at Visit 2. For further information regarding treatment of asthma exacerbations, see the Significant Asthma Exacerbation and Study Medications discussions in this section.

ALfA Visit Preparation Checklist (P9_VISPRP)
This handout is a tool for improving adherence with respect to the participant's preparation for each visit. The P9_VISPRP handout contains a checklist to help participants to remember to bring all necessary medications and materials to each visit. It also includes reminders to ensure that the participant refrains from using certain medications, foods, and beverages and doing certain things (i.e., vigorous exercise, eating) within protocol-specified periods prior to each visit. Clinic personnel should review this handout with the participant before he/she leaves each visit to be sure the information in the checklist is understood.

Diskus Inhalation Technique Checklist (TECH_DISKUS)
Proper inhalation technique using the Diskus is important to the study. Improper technique is a form of non-adherence with study procedures. Instruction in proper technique and continual coaching serve to improve adherence. The Diskus® Inhalation Technique Checklist (TECH_DISKUS) is used to document that each participant has achieved proper Diskus® inhalation technique at Visits 1 and 2. Proper inhalation technique is an eligibility criterion assessed at Visit 1. See the Inhalation Technique Assessment discussion in this section for further details.

ALfA How to Use Your MEMS®6 Cap (P9_MEMSINST)
This handout instructs participants on proper use of the MEMS®6 event monitoring cap that is placed on their study capsule vial. The MEMS®6 device improves adherence with dosing with daily capsules by monitoring each time the cap is removed from the capsule vial. See the MEMS®6 Cap discussion in this section and the MEMS®6 Manual in Appendix 5 of the AsthmaNet General Manual of Operations for further details.

Counseling for Non-Adherence

At each contact, the participant's level of adherence with study procedures must be assessed. Individuals who have maintained high levels of adherence should be applauded. If adherence levels are low, this should be addressed with the participant.

During each contact, review the necessity of correct study medication use and the importance of avoiding medications that are not allowed during the study. Discuss the importance of rescue use information that is collected at home. Remind the participant that correctly following study procedures is crucial to the study; it is a part of the commitment he/she made when agreeing to participate.

When addressing problems, try to be constructive and helpful:

Acceptable: "I noticed that you have not been taking your Diskus® twice daily regularly. Is there anything we can do to help you?

Unacceptable: "You are not doing what you are supposed to do. What is your problem?"

When dealing with problems it is best to re-explain procedures slowly and thoroughly and to rationalize and persuade logically. Attribute lack of adherence to a misunderstanding between clinic personnel and the participant. Ensure that the participant is aware of the resources available to help him/her understand the study procedures, such as study handouts and the availability and willingness of clinic personnel to answer questions whenever they arise.

2.2 Adverse Events

Definition and Reporting
Adverse events include the following:

- Clinical Adverse Events:

  A clinical adverse event is any unintended worsening in structure or function of the body; any illness that occurs during the trial. These events are documented on the Clinical Adverse Events (AECLIN) form.

  The term 'study drug' on the AECLIN form should be interpreted to mean any drug dispensed as part of the study, including Flovent® Diskus®, Advair® Diskus® and blinded study capsules. If an adverse event is thought to be related to one of these medications, this fact should be documented in Q1080 on the AECLIN form.

  See Section 10 of the AsthmaNet General Manual of Operations for further details on AECLIN form completion and submission.

- Laboratory Adverse Events:

  A laboratory adverse event is the occurrence of abnormal laboratory tests or other test (e.g., lab) results. These events are documented on the Clinical Adverse Events (AECLIN) form.

- Significant Asthma Exacerbation:

  Significant asthma exacerbations should be recorded on the Significant Asthma Exacerbation form (P9_SIGEX). In addition, significant asthma exacerbations should be recorded on the AECLIN form using ICD-9 code 493.92. If a participant experiences a significant asthma exacerbation during the run-in phase, he/she is ineligible for randomization and should be terminated. See the Significant Asthma Exacerbation discussion in this section for further details.

- Serious Adverse Events:

  Any experience that poses a significant hazard to a participant is considered a serious adverse event. With respect to human clinical experience, a serious adverse event includes any experience that meets at least one of the following criteria:

  1. Results in death
  2. Is life threatening (places the participant at immediate risk of death from the event as it occurred)
  3. Results in a significant or persistent disability/incapacity
  4. Requires inpatient hospitalization or prolongation of an existing hospitalization

5. Results in a congenital anomaly/birth defect
6. Any other adverse event that, based upon appropriate medical judgment, may jeopardize the participant's health and may require medical or surgical intervention to prevent one of the other outcomes listed in this definition. Examples include allergic bronchospasm requiring intensive treatment in an emergency department or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or abuse.

Serious adverse events are reported on the Serious Adverse Events Reporting Form (SERIOUS) as well as on the Clinical Adverse Events (AECLIN) form.

If an adverse event is deemed serious by the above definition, a SERIOUS form should be completed and faxed or e-mailed to the ALfA scientific coordinator at the DCC as soon as possible, preferably within 72 hours of clinic notification. Promptly faxing this form to the DCC expedites communicating the details of the adverse event to the Steering Committee, Data and Safety Monitoring Board (DSMB), and Institutional Review Boards (IRBs) if the event was deemed unexpected and possibly related to the study. If the documentation cannot be assembled within 72 hours due to the need for access to medical records or inability to contact the study participant, contact the ALfA scientific coordinator so that the DCC has communications on file and can follow up.

For detailed information on adverse events, see Section 4 in the AsthmaNet General Manual of Operations.

ICD-9 Codes

In general, ICD-9 codes describing an adverse event of any type should be obtained by searching the AsthmaNet ICD-9 Codes Excel spreadsheet. This spreadsheet can be accessed on the secure website in the Applications folder or through a link provided in concurrent forms entry. The spreadsheet includes the ICD-9 code for a particular diagnosis, along with long and short text descriptions of the related diagnosis. Clinical personnel can search the spreadsheet for a specific condition to find an appropriate code. Codes and their associated descriptions were downloaded from the Department of Health & Human Services, Centers for Medicare & Medicaid Services (CMS) website. They are from version 27 of the full and abbreviated code titles of the ICD-9-CM codes effective October 1, 2009. This code library will be used for the duration of AsthmaNet to ensure standardization across trials. Note that no other ICD-9 code references are acceptable.

For AsthmaNet, reported ICD-9 codes should describe the underlying condition or disease that resulted in a particular adverse event. For example, if a participant is hospitalized for a hysterectomy that was necessitated by uterine fibroids, the ICD-9 code for uterine fibroids should be recorded on the Clinical Adverse Events (AECLIN) form. The procedure code for hysterectomy is unavailable in the master spreadsheet and should not be recorded. In general, procedure codes will not be reported.

A significant asthma exacerbation is reported using the ICD-9 code 493.92.

Visit 1

Record any adverse events that have occurred since the participant signed the informed consent on the Clinical Adverse Events (AECLIN) form

If the participant experienced any adverse events between the date he/she signed the informed consent form (original signature date) and the date of Visit 1, record the events on the Visit 1 AECLIN form. If no adverse events are recorded for the participant at Visit 1, check the 'None' box.

A comprehensive medical history is taken during Visit 1. As part of this history it is important to probe for pre-existing conditions, both those related to asthma and those unrelated to asthma. This baseline knowledge is necessary to determine if conditions experienced during the ALfA study should be considered adverse events (i.e., worsening of a chronic condition or a condition that appears for the first time during the study). Pre-existing conditions should not be recorded on the Clinical Adverse Events (AECLIN) form, but they should be noted in the clinic notes that are stored in the participant's study folder.

Visit 2

Follow up clinical and laboratory adverse events from previous visit and record any new events (AECLIN)

The Clinical Adverse Events (AECLIN) form should be updated each time the clinic has contact with a participant, whether for a scheduled visit or phone contact, impromptu visit, or unexpected phone call.

In preparation for each contact, review the participant's file to determine if there were any ongoing adverse events at the last visit/contact. If an ending date for an ongoing adverse event becomes available, update the AECLIN form with this new information. Probe the participant for the occurrence of any new adverse events and record these on AECLIN.

An AECLIN form should be completed for each participant at each visit, even if the participant has not experienced any new adverse events since the previous visit. If no new adverse events are being recorded for the participant at a visit, check the 'None' box. If new information is available, record it and have the participant review it for accuracy.

Visit 3 or other early termination visit

Events that are ongoing at the time a participant leaves the study should be left open for stop dates (i.e., coded as 'ongoing at final visit'). The participant should be probed for any stop dates that are now known to close out previously-recorded events. All AECLIN

forms for a given individual should be forwarded to the DCC following his/her study termination.

See Section 10 of the AsthmaNet General Manual of Operations for further details on AECLIN form completion and submission.

2.3 Appointments: Confirming and Scheduling

Visits 1, 2
Run ALfA Visit Scheduler
Review planned visit schedule
Confirm/Schedule upcoming appointment(s) and review Visit Preparation handout (P9_VISPRP)

At each visit, review the current ALfA Visit Scheduler Report and confirm the date of the next regular visit and any upcoming phone contacts. Write the scheduled date on the participant's copy of the Visit Scheduler Report for his/her reference, and enter the date into the clinic's appointment book or scheduling calendar.

Review the ALfA Visit Preparation handout (P9_VISPRP) with the participant. Remind him/her of the substances and activities that must be avoided prior to each scheduled visit. Also remind the participant to bring his/her study medications (used and not used), ALfA Asthma Monitoring Log (P9_ASTHMA_LOG), and handout folder to each visit. Review the checklist on side 2 of the ALfA Visit Preparation handout.

Visits for a given participant should be scheduled for the same time of day (+/-3 hours of Visit 1 spirometry) to avoid the introduction of circadian variability into the assessment of lung function. If a participant needs to be scheduled outside the 3-hour window, the ALfA scientific coordinator at the DCC should be contacted to obtain an exception.

See the Visit Scheduler and Visit Windows discussions in this section for further details.

2.4 Asthma Control Test

Visits 1, 2, 3
Administer Asthma Control Test

Visits 2A, 2B
Instruct participant to complete Asthma Control Test (ACT)
Ask participant to read his/her responses to Asthma Control Test (ACT) and record responses on ACT form at clinic

General Information
The Asthma Control Test (ACT) is administered at ALfA Visits 1 – 3, as well as at the two phone contacts between Visits 2 and 3. The ACT is designed for adolescents and adults ages 12 and above, and has been validated for use for ages 14 and above.

The administration of the ACT is one of the first procedures performed at a visit. This timing in the visit structure is intentional so that a participant's responses are not affected by other study procedures, such as spirometry. Study coordinators should observe the order of procedures as they are laid out on the visit procedure checklists to ensure that ACT results are not biased by other study activities. At visits where multiple questionnaires are administered early in the visit, including the ACT, the ACT must be the first one administered.

If a given visit has been partially completed and then rescheduled for a later date because of the participant's time constraints on that day, a new ACT form must be completed at the beginning of the rescheduled visit. Do **not** allow the participant to refer to or update his/her previously completed questionnaire. Old copies of the questionnaires should be filed in the participant's study folder and clearly marked or shredded; they should not be entered into the study database or forwarded to the DCC. When administering the ACT questionnaire, request that the participant complete the entire form and provide answers as completely and as accurately as possible. No stated or implied time limit should be set. If the participant requests help with or clarification of any question, the study coordinator should instruct him/her to reread the instructions and to give the best answer possible to each question. The study coordinator should not provide an answer to any question. Providing guidance may bias the responses.

Following are guidelines for ACT administration to ensure the best quality data:

- Provide the participant a quiet place to complete the questionnaire.
- Before the participant completes the ACT, the study coordinator should do the following:
    - Complete the information in the form header.
    - Tell the participant that all questions should be answered.
    - Tell the participant that only one response may be given for each question.

- Remind the participant that he/she is scoring problems experienced due to asthma and not because of any other conditions.
- Remind the participant that the ACT is collecting data about their asthma over the past 4 weeks.

Participants should use a black or blue pen to complete the questionnaire. If the respondent wishes to change a response, the original response should be crossed out with a single line and then dated and initialed. The final response should be circled for clarification. No changes to the participant-completed form may be made by study personnel; changes may only be made by the respondent.

When the participant is finished with the questionnaire, collect it and review it for completeness before proceeding with the visit. If a question has been left blank, ask the participant to do his/her best to answer it. The answers to all of the questions are necessary to score the instrument. Check that the responses are clearly marked.

Asthma Control Test (ACT)

The ACT is a trade-marked 5-item questionnaire that was developed through research by GlaxoSmithKline and is now managed by QualityMetric Incorporated. AsthmaNet has paid a licensing fee for the use of the ACT in the ALfA trial. QualityMetric supplied the version of the form that we are using, and AsthmaNet was refused permission to implement any formatting changes to make it more compatible with our database. See the data management guidelines for this form in Section 10 of the AsthmaNet General Manual of Operations for more information.

The ACT gathers information on asthma control using a 4-week recall window. The form is self-administered and participant completed. The ACT website is: www.asthmacontrol.com.

No source documentation can be provided on this questionnaire due to the constraints imposed by QualityMetric.

Administering ACT at Phone Contacts

At Visit 2, the participant will be given ACT forms to complete at phone contacts. During phone contacts performed 2 and 4 weeks after Visit 2 (Visits 2A and 2B), the participant will be asked to complete the ACT questionnaire while on the phone with the coordinator. After completion, the participant will read responses to the coordinator. If the participant does not have the form with him/her, the coordinator will administer the ACT questionnaire using the script provided with the phone contact visit packet.

2.5 Asthma Monitoring Log

The Asthma Monitoring Log (P9_ASTHMA_LOG) was created to document a participant's rescue use (in puffs) each day. With this, the participant can assess how his/her rescue use may have changed over recent days, possibly signaling the onset of a significant asthma exacerbation. The log also includes fields to document daily Diskus® use as well as space to record any non-study medications that are taken between visits, and any medical problems the participant experiences. This information is useful in recording concomitant medications and adverse events at the participant's next study visit. The participant should be instructed to complete this form and to return it at his/her next visit.

The P9_ASTHMA_LOG form is set up as a fillable PDF file with an auto-populating date field. When preparing a log for a participant, the coordinator should complete the current date (date of the visit) in the first date field at the top of the form. All dates will be completed automatically throughout the rest of the form. The participant should begin completing the log with his/her PM scheduled session on the day of the visit.

Asthma logs serve as a daily log and should be completed by the participant once daily before the participant goes to bed. Total daily Atrovent® (green RESCUE1) and Ventolin® (blue RESCUE2) inhaler puffs for that day (in past 24 hours) should be recorded at that time. In the run-in, the participant should also check the 'Yes' box in "Morning Flovent® Taken?" column if took AM Flovent® dose as instructed (on the Daily Activities handout), and check the 'Yes' box in "Evening Flovent® Taken?" column if took PM Flovent® dose as instructed. During the randomized treatment period, the participant should do the same in the respective Advair® columns.

Visits 1, 2
Complete and distribute Asthma Monitoring Log (P9_ASTHMA_LOG)

At Visits 1 and 2, a new P9_ASTHMA_LOG form should be completed with participant information in the key fields area and dates, starting with the date of the current visit. The participant's high rescue use value will also need recorded in the blank field in the text at Visits 1 and 2, and is defined as follows:

- At Visit 1, it is the average daily use of home rescue inhaler during the week prior to V1 (based on participant's self-report)+ 8 puffs;
- At Visit 2, it is the average daily use of ipratropium during the week prior to V2 (as recorded on Asthma Monitoring Log) + 8 puffs;

The form should be given to the participant to complete until the next regularly scheduled visit.

Explain that non-study medications and medical conditions should be documented on the last page of asthma log. The participant should be instructed that preventive

bronchodilator puffs (taken routinely prior to exercise and other strenuous activities) should not be recorded in daily rescue use counts.

Encourage the participant to record the information each and every day. It is helpful if the recording of the data can be associated with specific daily activities (e.g., brushing teeth). Emphasize that data should not be *made up* or *recalled* more than one day back if days are missed.

Visits 2, 3
Collect Asthma Monitoring Log (P9_ASTHMA_LOG)

Near the beginning of each visit, the participant's completed P9_ASTHMA_LOG form should be collected and reviewed with him/her for any recorded comments, concomitant medications, or adverse events experienced since the last visit.

Review the asthma logs with the participant for completeness and legibility. Make sure the entries are completed in black ink. If they are not, remind the participant to use black ink. Also make sure the entries are easy to read. If numbers seem unclear, request clarification from the participant and have him or her update the values on the asthma log. If a page of the asthma log is difficult to read, ask the participant to copy the information onto a new asthma log before leaving the clinic. Note that clinical personnel should not make changes to the logs; only the participant may alter this information.

Examine the back of the asthma logs. This information is important for the documentation of adverse events and concomitant medications. If appropriate, complete the corresponding form(s) (AECLIN, CMED, and CMED_NON).

2.6 Baseline Peak Flow and Rescue Use Values

Baseline peak flow (PEF) and rescue medication use values are determined at Visit 1 and updated at Visit 2. Values are recorded on the ALfA Baseline PEF and Rescue Use Values (P9_BASELINE) form and entered into the study database. The rescue use value is used by the participant and clinical personnel to identify when the participant meets certain exacerbation criteria, and the baseline PEF is used in the treatment of an asthma exacerbation.

Visit 1
Complete Baseline Peak Flow and Rescue Use Values form (P9_BASELINE)

At Visit 1, the participant is just starting the ALfA run-in. Therefore, no rescue use data has been recorded by the participant at this point in the study. The baseline peak flow and rescue use values are defined at Visit 1 as follows:

Baseline Peak Flow (PEF)
The baseline PEF is the spirometry peak flow value corresponding to the best effort during baseline spirometry (converted to liters/minute) at Visit 1. This value is obtained by multiplying the value from Q1050 (FEF Max) on the Visit 1 Spirometry Testing (SPIRO) form by 60 and rounding to the nearest whole liter/minute. If a participant with FEV_1 < 80% requires a Visit 1 Continuation visit to establish his or her study eligibility (by performing methacholine challenge), the baseline PEF should be calculated from Q1050 on the SPIRO form completed at the initial Visit 1.

The baseline PEF value is recorded in the grey box at the top of the P9_BASELINE form. It is also recorded on the Participant Identification Card (P9_ID) given at Visit 1, along with the 70% PEF reference value.

Baseline Rescue Use Value
At Visit 1, the baseline rescue use value is the participant's self-reported average daily use (in puffs) of albuterol or levalbuterol (common RESCUE medications) during the 7 days prior to the visit. Ask the participant to recall the amount of rescue medication puffs he/she used daily over the previous week. A ballpark average amount of daily puffs of medication is sufficient for monitoring exacerbation between Visits 1 and 2. Round to the nearest puff if calculating the value. The participant should not include preventive puffs (e.g., pre-exercise puffs or puffs taken in advance of allergen exposure) in his/her estimate. Preventive puffs also will not be included in the daily puffs of RESCUE reported on the participant's Asthma Monitoring Log (P9_ASTHMA_LOG) each day.

The baseline rescue use value is recorded in Q1000 on the P9_BASELINE form. The participant's High Rescue Use value is necessary starting at Visit 1, and is calculated by adding 8 to the baseline rescue use value. If the participant uses greater than or equal

to his/her High Rescue Use per 24 hours for 48 hours, then he/she has experienced a significant asthma exacerbation. The baseline rescue use and High Rescue Use values are recorded on the Participant Identification Card (P9_ID) at Visit 1, as well as on several other participant handouts.

Visit 2
Complete Baseline Peak Flow and Rescue Use Values form (P9_BASELINE)

At Visit 2, the participant has several weeks of rescue use data recorded on his/her Asthma Monitoring Log. The baseline rescue use value is updated at Visit 2 based on the participant's recorded use on this Log. The participant's baseline PEF is updated at Visit 2 since he/she has been on a stable dose of Flovent® since Visit 1. These values will be used by the participant and clinical personnel to identify when the participant meets certain exacerbation criteria, and in the treatment of an asthma exacerbation. The baseline peak flow and rescue use values are defined at Visit 2 as follows:

Baseline Peak Flow (PEF)
The baseline PEF is the spirometry peak flow value corresponding to the best effort during baseline spirometry (converted to liters/minute) at Visit 2. This value is obtained by multiplying the value from Q1050 (FEF Max) on the Visit 2 Spirometry Testing (SPIRO) form by 60 and rounding to the nearest whole liter/minute.

The baseline PEF value is recorded in the grey box at the top of the P9_BASELINE form. It and the 70% PEF reference value should be updated on the Participant Identification Card (P9_ID) at Visit 2.

Once established at Visit 2, the participant's baseline PEF value will not change for the remainder of the trial.

Baseline Rescue Use Value
At Visit 2, the baseline rescue use value is defined as the average daily use of Atrovent® (green RESCUE1) inhaler during the last week prior to the visit as recorded on his/her Asthma Monitoring Log. This is further defined as the average daily RESCUE1 inhaler puffs used during the 7 days prior to Visit 2, rounded to the nearest puff. It is possible that a participant's baseline rescue use value may be based on fewer than 7 days' worth of data.

The baseline rescue use value is recorded in Q1000 on the P9_BASELINE form. The participant's High Rescue Use value is calculated by adding 8 to the baseline rescue use value. If the participant uses greater than or equal to his/her High Rescue Use per 24 hours for 48 hours, then he/she has experienced a significant asthma exacerbation. The baseline rescue use and High Rescue Inhaler Use values are recorded on the Participant Identification Card (P9_ID) at Visit 1, as well as on several other participant handouts.

Once established at Visit 2, the participant's baseline rescue use value will not change for the remainder of the trial.

2.7 Blood draw for Biochemical Assays

Visit 2, 3

Obtain 40 mL blood for biochemical assays (five 8 mL heparin CPT tubes) ***(if IRB approval for protocol version 2.3 has NOT yet been obtained)***

Obtain 80 mL blood for biochemical assays (ten 8 mL heparin CPT tubes) ***(if IRB approval for protocol version 2.3 has been obtained)***

Log RB, cAMP, WB, mRNA sample information (P9_PBMC_SAMP_LOG)

Enter Radioligand binding, cAMP-PBS, cAMP-Isoproterenol, cAMP-forskolin, Western Blot and mRNA sample information into Biological Sample Tracking module

Supplies

The following supplies are required to collect biochemical assay samples at Visit 2 and 3:

Item	Vendor	Catalog #	# Per Collection
8 mL heparin CPT tube	Provided by DCC (BD)	362753	5 (10 if have IRB approval for protocol version 2.3)
Fisherbrand Premium Microcentrifuge Tubes: 2.0mL (no substitutes)	Fisher Sci.	05-408-138	6
White Laser Cryo-Babies barcode label (Cryo-Babies 1.28”x0.5”)	Diversified Biotech	LCRY-1700	6
50 mL centrifuge tube			1
100-1000 µL pipette			1
PBS -/- (without calcium or magnesium) (500 ML)	Life	10010-023	
HBSS -/- (500 ML)	Life	14175-095	
IBMX (250 MG)	Sigma	I5879	
Isoproterenol (500 MG)	Sigma	I6504	
Forskolin (10 MG)	Sigma	F3917	
HCl (500 ML)	Sigma	318949[Δ]	
Dimethyl sulfoxide (DMSO) (500 ML)	Sigma	D8418[‡]	

[Δ] Catalog #318965 can be substituted.

[‡] Catalog #D4540 can be substituted.

Due to the processing required for these samples, only one Visit 2 or 3 should be scheduled in one day.

Please follow all the recommendations listed on the above reagents' material safety data sheets while handling them. Abide also by any additional institutional policies that may exist for the handling of these reagents.

Preparing reagents

IBMX (isobutylmethylxanthine) 250mg from Sigma

1. Transfer 2,500µL of DMSO into a 2mL microcentrifuge tube, and place tube in a 37 degree water bath for 10 minutes.
2. Add 1,125 µL of warm DMSO to the 250mg lyophilized IBMX. (This will make a 1M solution—label it as such.)
3. Take 40µL of 1M IBMX and add to a 2mL microcentrifuge tube containing 960uL of warm DMSO to get 1000uL of 40mM IBMX.
4. Make 20 aliquots of 40mM IBMX: take twenty (2)mL microcentrifuge tubes and add 40µL to each. Label each microcentrifuge tube as 40mM IBMX (including the one with the remaining 200µL). Store these and the remaining 1M IBMX in a -20°C freezer.

Forskolin 10mg from Sigma

1. Add 2mL DMSO to the 10mg lyophilized forskolin to get a 12.2mM solution.
2. Make 20 aliquots of this: take twenty (2)mL microcentrifuge tubes and add 40µL to each. Label each microcentrifuge tube as 12.2mM (including the one with the remaining 1200µL). Store these in a -20°C freezer.

ISO (isoproterenol) 0.5g from Sigma

1. Pour 10mL of ultrapure water into a 50mL centrifuging tube.
2. Transfer the 0.5g of ISO powder into it to make a 202mM solution. Mix well by vortexing.
3. Add 493µL of PBS-/- and 7.4µL of 202mM ISO to a 2mL microcentrifuge tube, mix by pipetting up and down. This makes a 3mM ISO solution.
4. Make 20 aliquots of this: take twenty (2)mL microcentrifuge tubes and add 20µL to each. Label each microcentrifuge tube as 3mM ISO (including the one with the remaining 100µL). Store these and the remaining 202 mM in a -20°C freezer.

Hydrochloric acid (HCl) 1M (500mL) from Sigma

1. Pour 22.5mL of ultrapure water into a 50mL centrifuging tube.
2. Transfer 2.5mL of HCl (1M) into the 50mL centrifuge tube for a final volume of 25mL of a new HCl concentration of 0.1M. Mix by vortexing. CAUTION: DO NOT ADD WATER TO ACID; THE RESULTING SOLUTION MAY BOIL AND SPLASH. ADD ACID TO WATER.

3. Make 20 aliquots of HCl (0.1M): take twenty (2)mL microcentrifuge tubes and add 1mL to each. Label each microcentrifuge tube (as well as the 50mL centrifuging tube with the remaining 5mL) as 'HCl (0.1M)'. You may store these at room temperature.

Collection (Performed by Coordinator)

1. ***If IRB approval for protocol version 2.3 <u>has NOT yet been obtained</u>,*** collect 40 mL blood into 5 BD heparin CPT tubes provided by DCC (8 mL blood per tube using 5 BD tubes).

   If IRB approval for protocol version 2.3 <u>has been obtained</u>, collect 80 mL blood into 10 BD heparin CPT tubes provided by DCC (8 mL blood per tube using 10 BD tubes). **<u>NOTE:</u>** <u>If protocol version 2.3 is not IRB approved at the time of a participant's Visit 2 and participant is randomized under protocol version 2.2, proceed with collecting 40 mL blood at Visit 3 IF participant does not re-consent to provide 80 mL blood at Visit 3.</u>

2. Gently mix blood by carefully inverting each tube 6 times (do not shake). Proceed to centrifuging immediately after blood collection. Samples should be kept room temperature.

Processing

1. Ensure that BD heparin CPT tubes properly fit in centrifuge (that they are not too long, to prevent these from shattering).
2. Centrifuge samples at 1600 RCF for 25 minutes at room temperature (21°C). Turn deceleration to zero (otherwise cells WON'T BE ISOLATED), but keep max centrifuge acceleration. Balance with a water-filled BD heparin CPT tube. Stopping time with the brake off is about 5 minutes. Record the time spinning is initiated on P9_PBMC_SAMP_LOG.
3. While centrifuging tubes, label six 2 mL microcentrifuge tubes with the appropriate sample barcode labels (Cryo-Tag) generated through the Biological Sample Tracking (BST) module of the AsthmaNet database management system. The barcode labels include a pre-printed sample type as well as a barcode number. Sample types and what the corresponding barcode starts with is below. Labels should be placed vertically on tube so that the barcode can be scanned. The length of the label is 1.5" so the label should be placed as high as possible (just under the screw top).

   3a. ALfA Western Blot: Barcode starting with 9WB
   3b. ALfA RB: Barcode starting with 9RB
   3c. ALfA mRNA: Barcode starting with 9MRNA
   3d. ALfA cAMP – PBS: Barcode starting with 9CPBS
   3e. ALfA cAMP – ISO: Barcode starting with 9CISO
   3f. ALfA cAMP – forskolin: Barcode starting with 9CFORS

   Place label firmly on microcentrifuge tube.

4. With an alcohol-proof permanent, fine Sharpie marker, write barcode on side of the microcentrifuge tubes. Doing so allows sample to be identified should label come off tube.
5. Also label (without barcode; these 6 tubes will be discarded the day of the visit):
    - a 50 mL centrifuge tube as 'ALfA cAMP –master cell suspension'
    - a 2 mL microcentrifuge tube as 'cAMP-cell dilution'
    - a 2 mL microcentrifuge tube as 'cAMP-cell+Turk's mix'
    - a 2 mL microcentrifuge tube as 'cAMP – PBS'
    - a 2 mL microcentrifuge tube as 'cAMP – ISO'
    - a 2 mL microcentrifuge tube as 'cAMP – forskolin'
6. Add (pour) 25mL PBS -/- to a 50mL centrifuge tube. Using a 1000 µL pipette, carefully transfer the buffy coat layer from each of the 5 BD tubes ***(10 BD tubes if have IRB approval for protocol version 2.3)*** into the 50 mL centrifuge tube containing PBS -/-. Avoid the transfer of the red precipitate in the gel or the white gel itself. Add PBS -/- to increase the volume to 50 mL (max fill).
7. Mix sample by gently inverting tube 5 times.
8. Centrifuge sample at 300 RCF for 10 minutes at 4° Celsius (be sure to have turned brake back on). Balance with a 50 mL centrifuge tube filled with water.
9. Look for a white pellet at the bottom of the centrifuge tube. Pour out the supernatant and flick the tube ~15 times in order to break up the pellet (see instructional video). Using a transfer pipette, add 3.5 mL HBSS -/-. Invert the centrifuge tube 5 times to mix the sample. Quickly proceed to step 10 (to avoid sedimentation of the cells, and allow for an accurate distribution of cell number to each assay).
10. From the cell suspension obtained in step 9, transfer:

    10a. **1000µL** into 2 mL microcentrifuge tube labeled for ALfA RB.

    10b. **500**µL into the 2 mL microcentrifuge tube labeled for ALfA Western Blot

    10c. **250**µL into the 2 mL microcentrifuge tube labeled for ALfA mRNA.

    Place tubes in ice.
11. *For this step, refer to ALfA Cell Counting Worksheet (P9_CELL_COUNT_WKS).* Count the cells remaining in final 1750uL of the cell suspension, and write number down. Use at least 40x magnification to visualize cells with microscope; count only white blood cells (lymphocytes and monocytes)

    11a. If cell concentration ≤17.14 x 10^6/mL, then transfer 1750uL of sample to 'ALfA cAMP –master cell suspension' 50 mL centrifuge tube. Add 1350uL of PBS-/- to increase volume to 3100uL.

    11b. If cell concentration >17.14 x 10^6/mL, then transfer volume B of cell suspension (on cell counting worksheet) to 'ALfA cAMP –master cell suspension' 50 mL centrifuge tube

Add volume C of PBS -/- to fill master mix tube to 3.1 mL.

12. Add 31uL of 40mM IBMX to 'ALfA cAMP –master cell suspension' 50 mL centrifuge tube. Mix sample by inverting 5 times.
13. Incubate at room temperature for 15 minutes.
14. While the IBMX is incubating,

14a. Pellet down the cells in the ALfA WB and ALfA mRNA tubes by spinning at 500 RCF for 5 minutes at room temperature (the tubes may be used to balance one another).

14ai. Discard supernatant (10-20uL always remains in bottom, and is ok). Store pellets at -80°C.

14b. To ALfA RB microcentrifuge tube,

14bi. Add 110 µL of dimethyl sulfoxide (DMSO).
14bii. Mix sample by inverting 3 times.
14biii. Quickly store in -80°C freezer.

15. Returning to the cAMP master cell suspension tube after incubation time is over, transfer 1000µL into the three 2 mL microcentrifuge cryotubes labeled (<u>without</u> barcode) for:

- cAMP – PBS
- cAMP – ISO
- cAMP – forskolin

15a. To each respective tube, add:

15ai. 10 µL of PBS -/- (blank control) to tube labeled for ALfA cAMP – PBS
15aii. 10 µL of 3mM ISO to tube labeled for ALfA cAMP – ISO
15aiii. 8.2 µL of 12.2mM forskolin to tube labeled for ALfA cAMP – forskolin

Mix well by inversion.

15b. Incubate at **37° Celsius** for 10 minutes in a water bath (be careful not to exceed 10 minutes of incubation). Be careful that water doesn't leak into tubes (use Styrofoam buoys to make tubes float).

15c. Pellet cells at **500**RCF for 10 minutes at **room temperature**. Discard supernatant.

15d. Break pellet by tapping 15 times, and add 300 µL of stock 0.1M HCl to each tube (mix by pipetting up and down 3 times when adding), and incubate at room temperature for 20 minutes.

15e. Centrifuge at 10,000RCF for 10 minutes at 4° Celsius. KEEP SUPERNATANT.

15f. Transfer the SUPERNATANT from each tube to its respective 2 mL microcentrifuge cryotube labeled (<u>with</u> barcodes):

15fi. ALfA cAMP – PBS
15fii. ALfA cAMP – ISO
15fiii. ALfA cAMP – forskolin.

15g. Store in -80°C freezer. Discard the old microcentrifuge tubes containing the residual pellet.

16. Make sure tube caps are secure.

17. Access the BST module and scan the barcode to insert record for all the samples. Input the participant ID information to link the barcodes to the correct ALfA participant. It is imperative that all samples be scanned the day of collection so that they are associated with the correct participant ID and are available for inclusion in the next shipment. For details on accessing and interacting with the BST Module in the AsthmaNet Database Application, see the AsthmaNet Computing and Networking Environment details in Section 7 of the AsthmaNet General Manual of Operations.

18. Record sample barcode numbers on P9_PBMC_SAMP_LOG.

19. Store the samples in one 5x5x2 chipboard storage box.

20. Store the samples at -80° Celsius until the shipment day. Record the date/time the sample is placed in the freezer and the current freezer temperature on P9_PBMC_SAMP_LOG.

Sample Shipping

PBMC samples will be shipped priority overnight to Boston the second Tuesday of the month. Only samples for randomized participants should be shipped for analysis. Samples for visits completed since the last shipment will be shipped.

Note: Samples for unsuccessful Visit 2s (i.e., Visit 2 did not result in randomization) can be discarded.

Preparing PBMC Samples for Shipment to Boston

A few days prior to shipment, e-mail Boston to notify them of the shipment:

Xiaofeng Jiang (xjiang@hsph.harvard.edu)
Kristen McIntire (kamcintire@partners.org)
Camille Yongue (cyongue@partners.org).

To create a shipment, scan the barcodes for all samples available to ship into the AsthmaNet BST system. Include a shipment comment detailing the contents of the shipment (i.e., human PBMC). Each shipment (from each site) will receive a unique shipment ID number. A shipment inventory will be generated that contains: date of shipment, shipping tracking number, site of origination, shipment ID, and an inventory detailing all the tubes in the shipment with their barcode numbers and participant information (Participant ID number, initials, visit number and collection date). Print the shipment inventory for inclusion in the shipment.

Once the shipment is confirmed in the BST module, an e-mail will automatically be sent to Boston. The e-mail will include an export file from the database that shows the information from the shipment inventory. A summary of the shipment will be included in the body of the e-mail message.

Packaging PBMC samples for Shipment to Boston

Before packaging available samples for shipment, they must be scanned into the BST system and an inventory of the shipment must be generated and printed as described above. After the samples have been scanned and the shipment has been confirmed by the performance site, the samples should be packaged for shipment. The following materials are required:

Item	Vendor	Catalog #	# Per Shipment
ThermoSafe Styrofoam mailer in corrugated carton	Fisher Scientific	03-525-36	1
FisherBrand Biohazard Polyethlene Transport Bag 8x8” (or larger)	Fisher Scientific	01-800-07 (8x8”)	1
FisherBrand Biohazard Wipes, standard absorbency (4x4”)	Fisher Scientific	06-670-35	2
Packaging tape	Staples	380107	
Exempt Human Specimen labels Therapak 2.5”x2”	Fisher Scientific	22-130-070	1
Therapak Dry Ice Label – UN1845 (5.5x5.5”)	Fisher Scientific	221-30-065	1
Shipment inventory from BST			1

Assembly instructions:

1. PBMC samples will be shipped in chipboard box.
2. Place one sheet of absorbent material on top of the samples inside the chipboard box to be shipped. Close the box.
3. Place the closed box into the plastic transport bag.
4. Place a second sheet of the absorbent material in the plastic transport bag.
5. Seal the transport bag tightly.

6. Fill the bottom of the Styrofoam shipper with approximately 1 inch of cubed/chipped dry ice.
7. Place the plastic transport bag containing the samples on top of the dry ice layer.
8. Cover the transport bag with more crushed dry ice so that the box of tubes cannot be seen. Continue to fill the Styrofoam box with as much dry ice as possible.
9. Place a copy of the shipment inventories for each sample type and the original ALfA Cell Counting Worksheet (P9_CELL_COUNT_WKS) in a plastic Ziploc bag on top of the dry ice and close the Styrofoam mailer tightly.
10. Seal the Styrofoam mailer with tape. Do not completely seal the box so that it is airtight. Carbon dioxide from the dry ice must be allowed to escape.
11. Place the Styrofoam mailer inside a cardboard mailing sleeve (the specified shipper in the table above comes with a cardboard mailer).
12. Attach one “Exempt Human Specimen” sticker and one “DRY ICE – UN 1845” label to the cardboard carton. Mark the appropriate weight of dry ice in kg on the label.

13. Address the shipment to:

    Kristen McIntire
    BWH Asthma Research Center
    75 Francis Street, Surgery Building 1, Suite 155
    Boston, MA 02115
    (617) 732-8259

14. Specify FedEx priority overnight shipment. No other form of shipping is acceptable.

Lu Laboratory Contacts
Primary
Xiaofeng Jiang
Lu Lab - (617) 432-7290

Secondary
Quan Lu
Cell number - (857) 208-6590

2.8 Blood draw for Creatinine, Calcium and CBC

Visit 1
Obtain blood for creatinine and calcium (one tiger-top tube)
Obtain blood for CBC/differential (one purple-top tube)

Creatinine and Calcium Procedures
For eligible participants only, fill one 5 mL tiger-top tube with blood for creatinine and calcium determination. These samples will be analyzed in the clinical center's local lab. Samples should be transported to the lab within **two hours** of the blood draw.

Record the participant's calcium and creatinine values in Q1090 and Q1110 n the P9_LAB form. Note that values must be recorded in mg/dL. No other units are acceptable. Any necessary conversions must be made prior to recording data on the form and entering the data into the study database. Refer to Section 4 of the ALfA MOP for more details on how to complete the P9_LAB form.

Calcium
If the participant's calcium is less than 8.5 mg/dL, ionized calcium determination is required. Before beginning ALfA, contact your local lab to determine the requirements for ionized calcium. It may be possible to run this test with the sample collected in the tiger-top tube. However, it may require another tube. Work with your local lab to decide how this can be done without requiring participant to return to clinic for another blood draw. Ionized calcium should be recorded in Q1100 on P9_LAB.

Participants with calcium less than 8.5 mg/dL and ionized calcium less than 4.4 mg/dL are ineligible for ALfA. This is because alendronate is contraindicated in participants with hypocalcemia (low blood calcium).

Creatinine
Serum creatinine tests are being done at Visit 1 in order to estimate the participant's glomerular filtration rate (eGFR). Roughly, the eGFR corresponds to the percent of kidney function a person has available. Individuals whose Visit 1 eGFR is less than 35 ml/min are ineligible to continue in the study, as they have indications of impaired renal function.

The ALfA trial will employ the Cockcroft-Gault equation to calculate eGFR. This equation uses the participant's serum creatinine measurement, along with age, weight, height and gender to estimate GFR as follows:

$$\text{eGFR (male)} = \frac{(140 - \text{age}) * (\text{weight})}{\text{creatinine} * 72}$$

where age is in years, weight is in kilograms, and serum creatinine is in mg/dl.

eGFR (female) = eGFR (male) * 0.85

For ALfA, eGFR will also be adjusted for BMI. Adjustments are as follows:

- Underweight (BMI < 18.5): Weight uses actual/total body weight (No adjustment)
- Normal Weight (BMI 18.5 - 22.9): Weight uses ideal body weight (with the range using the actual body weight), where:
  - Ideal Body Weight (IBW), Men = 50 + (2.3 * (Height in inches - 60))
  - Ideal Body Weight (IBW), Women = 45.5 + (2.3 * (Height in inches - 60))
- Overweight/Obese (BMI ≥ 23): Weight uses adjusted body weight (with the range using ideal body weight), where:
  - Adjusted Body Weight (ABW) = IBW + 0.4 * (TBW - IBW) where 0.4 is a correction factor, and TBW is total (actual) body weight

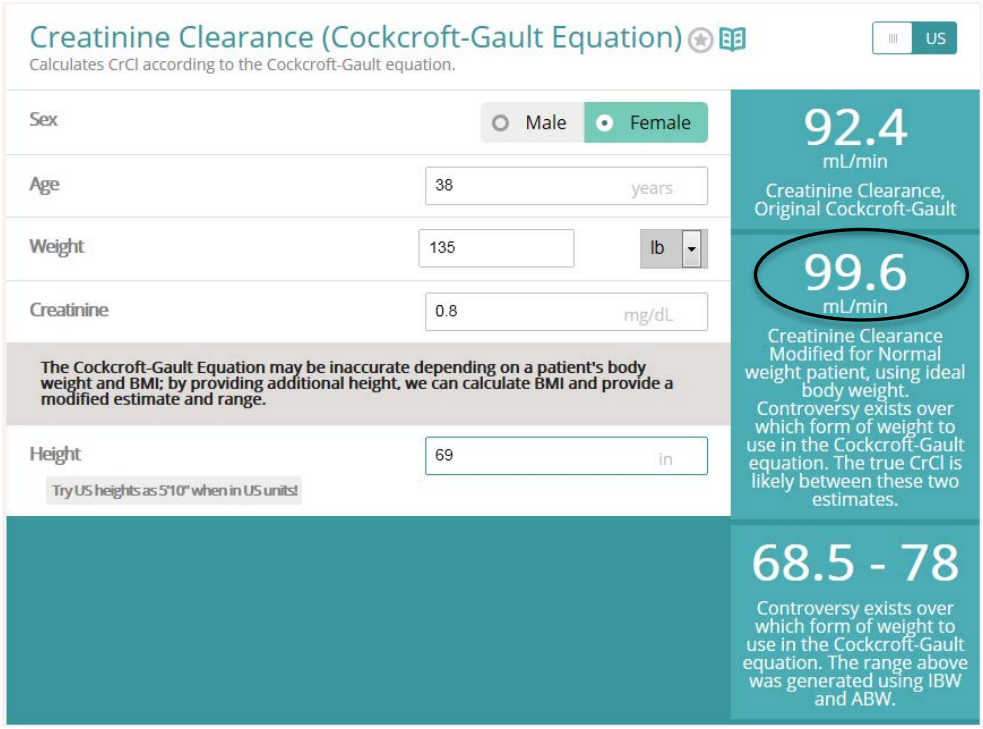
To facilitate the eGFR calculation, the on-line calculator at mdcalc.com should be used. The full link to the calculator is:
http://www.mdcalc.com/creatinine-clearance-cockcroft-gault-equation.

Before using the calculator, the coordinator should first complete the fields in the 'Clinic Use Only' box on page 2 of the P9_LAB form. This box contains spaces to record the participant's gender, current age (in whole years), weight (in pounds) and height (in inches) as required for input into the calculator. Note that the calculator requires weight in pounds and height in inches. The weight measured at Visit 1 and recorded on the Adult Body Measurements (BODYMEAS_ADULT) form should be converted from kilograms to pounds by multiplying by 2.2. The height measured at Visit 1 and recorded on the Adult Body Measurements (BODYMEAS_ADULT) form should be converted from cm to inches by multiplying by 0.39. The converted weight and height should be recorded in the 'Clinic Use Only' box.

The calculator will likely appear in metric units. To switch to US units, click the white half of the box to the left of Creatinine Clearance (Cockcroft-Gault Equation):

The bar should toggle from SI to US, as shown in the screen shot on the following page.

Enter the information from the P9_LAB form into the calculator, and verify that information was entered correctly. The participant's computed eGFR values will appear in the green boxes on the right. Value given for "Creatinine Clearance Modified for Normal weight patient" should be entered in Q1120 on P9_LAB.



If a participant is underweight, it will read “Creatinine Clearance Modified for Underweight patient.” If the participant is overweight, it will read “Creatinine Clearance Modified for Overweight patient.”

CBC/Differential Procedures

The order of the blood draws, as specified on the Visit Procedure Checklists, must be observed.

For eligible participants only, fill one 4 mL purple-top tube with blood for CBC/differential determination. These samples will be analyzed in the performance site’s local lab. Samples should be labeled according to local requirements and transported to the lab within **two hours** of the blood draw.

After the results are available, record the participant’s CBC/differential values on the P9_LAB form in Q1000 to Q1080. When completing the P9_LAB form, review the units on the lab report and form. If different, convert the lab value to the appropriate units on the form. To assist in completion of P9_ELIG4 Q1020, a “Clinic Use Only” box has been provided to record the sum of lymphocytes and monocytes.

Lab Reports

A copy of the local lab reports should be forwarded to the DCC with the P9_LAB form. All identifying information (name, medical record number, etc.) must be blackened out prior to sending the reports to the DCC. Write the participant's ID number at the top of the reports.

Refer to Section 4 of the ALfA MOP for more details on how to complete the P9_LAB form.

2.9 Blood draw for Phadiatop

Visit 2
Obtain 2 mL blood for Phadiatop (one red-top tube)
Log Phadiatop sample information (P9_PHAD_SAMP_LOG)
Enter Phadiatop serum sample data into Biological Sample Tracking module

Supplies
The following supplies are required to collect the serum samples for Phadiatop testing at Visit 2:

Item	Vendor	Catalog #	# Per Collection
3.0 mL red-top vacutainer* (366668)	Fisher Sci.	02-657-27	1
SST label (Avery #5160)	Staples	209882	1
Sterile pipette			1
2.0 mL self-standing Saf-T-Seal screw cap tube, natural (USA Scientific, no substitutions)	Fisher Sci.	1420-9700	2
Fiberboard storage box for cryovials (Fisherbrand Cryo/Freezer boxes, 5x5x2“ with 81 cells; no substitutes)	Fisher Sci.	03-395-464	1 box with 81 cell dividers
White Laser Cryo-Tags barcode label (Cryo-Tags 1.5”x0.75”)	Diversified Biotech	LCRY-1200	2

* Can use red-top vacutainer you have in stock and discard extra serum.

Processing

1. Fill one red-top vacutainer with the participant’s blood. The vacutainer must be labeled with participant ID, initials and visit number. A template for labels for the red-top tubes (Avery #5160) can be found on the AsthmaNet secure website in the Protocols: ALfA: Labels folder.

   Complete an entry for the blood draw on the ALfA Phadiatop Serum Sample Log (P9_PHAD_SAMP_LOG). Complete the participant’s ALfA Participant ID number, visit number and collection date/time.

2. Invert the SST tube 5 times. Allow the blood sample to clot at room temperature for 20 minutes to 1 hour.

3. While the blood is clotting, prepare a 2 mL cryotube (specified in the above table; no substitutions) for the participant's serum sample.

   Label one 2 mL cryotube with an ALfA Phadiatop barcode label (Cryo-Tag) generated through the Biological Sample Tracking (BST) module of the AsthmaNet database management system. The barcode label includes a pre-printed 9-digit barcode number, starting with "9PHAD". Labels should be placed vertically on tube so that the barcode can be scanned. The length of the label is 1.5" so the label should be placed as high as possible (just under the screw top). The sample type associated with this tube in the BST module is "ALfA Phadiatop". The ALfA Phadiatop label appears as follows:

4. At the end of the clotting period, complete the time spinning is initiated on P9_PHAD_SAMP_LOG. Centrifuge the clotted blood at 1000-1300 RCF (*g*) for 10 minutes to separate the serum from the red blood cells.

5. Using a sterile pipette, carefully remove the serum from above the clot and aliquot at least 0.5 mL into 2 mL cryovial with Phadiatop label (barcode starting with "9PHAD")

6. Screw tube shut. Be sure the cap is secure.

7. Access the BST module and scan the barcode to insert record for the sample. Input the participant ID information to link the barcode to the correct ALfA participant. <u>It is imperative that the sample be scanned the day of collection so it is associated with the correct participant ID and is available for inclusion in the next shipment.</u> For details on accessing and interacting with the BST Module in the AsthmaNet Database Application, see the AsthmaNet Computing and Networking Environment details in Section 7 of the AsthmaNet General Manual of Operations.

8. Record sample barcode number and sample volume on P9_PHAD_SAMP_LOG.

9. Store the sample in one 5x5x2 chipboard storage box. These samples will be shipped to ADx labs in Denver at study completion.

10. Store the serum samples at -80°C until the shipment day. Record the date/time the sample is placed in the freezer and the current freezer temperature on P9_PHAD_SAMP_LOG.

Sample Shipping
Phadiatop samples will be shipped priority overnight to ADx Labs at National Jewish Health in Denver on 7/21/2015 and 1/19/2016. Only samples for randomized participants should be shipped for analysis.

Note: Samples for unsuccessful Visit 2s (i.e., Visit 2 did not result in randomization) can be discarded.

Preparing Phadiatop Samples for Shipment to Denver Lab
To create a shipment, scan the barcodes for all samples available to ship into the AsthmaNet BST system. Include a shipment comment detailing the contents of the shipment (i.e., human serum). Each shipment (from each site) will receive a unique shipment ID number. A shipment inventory will be generated that contains: date of shipment, shipping tracking number, site of origination, shipment ID, and an inventory detailing all the tubes in the shipment with their barcode numbers and participant information (Participant ID number, initials, visit number and blood draw date). Print the shipment inventory for inclusion in the shipment.

Once the shipment is confirmed in the BST module, an e-mail will automatically be sent to the Denver Lab. The e-mail will include an export file from the database that shows the information from the shipment inventory. A summary of the shipment will be included in the body of the e-mail message.

Packaging Phadiatop for Shipment to Denver Lab
Before packaging available samples for shipment, they must be scanned into the BST system and an inventory of the shipment generated and printed as described above. After the samples have been scanned and the shipment has been confirmed by the performance site, the samples should be packaged for shipment. The following materials are required:

Item	Vendor	Catalog #	# Per Shipment
ThermoSafe Styrofoam mailer in corrugated carton	Fisher Scientific	03-525-36	1
FisherBrand Biohazard Polyethlene Transport Bag 8x8” (or larger)	Fisher Scientific	01-800-07 (8x8”)	1
FisherBrand Biohazard Wipes, standard absorbency (4x4”)	Fisher Scientific	06-670-35	2
Packaging tape	Staples	380107	

Item	Vendor	Catalog #	# Per Shipment
Exempt Human Specimen labels Therapak 2.5”x2”	Fisher Scientific	22-130-070	1
Therapak Dry Ice Label – UN1845 (5.5x5.5”)	Fisher Scientific	221-30-065	1
Shipment inventory from BST			1

The instructions for assembling shipments below meet the minimum federal standards. Each performance site’s institution may have additional guidelines. Sites should follow their institutional guidelines as long as they are in compliance with the minimum federal standards.

Assembly instructions:

1. Only Phadiatop samples for randomized participants will be shipped for analysis. Samples for non-randomized participants should be removed and placed in another chipboard box.
2. Place one sheet of absorbent material on top of the samples inside the chipboard box to be shipped. Close the box.
3. Place the closed box into the plastic transport bag.
4. Place a second sheet of the absorbent material in the plastic transport bag.
5. Seal the transport bag tightly.
6. Fill the bottom of the Styrofoam shipper with approximately 1 inch of cubed/chipped dry ice.
7. Place the plastic transport bag containing the samples on top of the dry ice layer.
8. Cover the transport bag with more crushed dry ice so that the box of tubes cannot be seen. Continue to fill the Styrofoam box with as much dry ice as possible. Do not ship more than one fiberboard storage box in a Styrofoam shipper.
9. Place a copy of the shipment inventory (in a plastic Ziploc bag) on top of the dry ice and close the Styrofoam mailer tightly.
10. Seal the Styrofoam mailer with tape. Do not completely seal the box so that it is airtight. Carbon dioxide from the dry ice must be allowed to escape.
11. Place the Styrofoam mailer inside a cardboard mailing sleeve (the specified shipper in the table above comes with a cardboard mailer).

12. Attach one “Exempt Human Specimen” sticker and one “DRY ICE – UN 1845” label to the cardboard carton. Mark the appropriate weight of dry ice in kg on the label.

13. Address the shipment to:

    Advanced Diagnostic Laboratories at National Jewish Health
    ATTN: Preanalytical
    1400 Jackson Street
    Room M013
    Denver, CO 80206
    Phone: (303) 270-2663

14. Specify FedEx priority overnight shipment (AM receipt not required). No other form of shipping is acceptable.

ADx Laboratory Contact
Michael AronNational Jewish Health
Advanced Diagnostics Laboratories
E-Mail: AronM@njhealth.org
Phone: (303) 270-2578

2.10 Certification

Study Coordinators and Technicians
Coordinators who carry out ALfA study visits must be certified to do so. That is, personnel who complete pregnancy tests (PREG_TEST form) or any of the protocol-specific ALfA forms (designated by a P9 prefix in the form name) must possess ALfA protocol certification, as well as certification in Human Subjects Protection Training, HIPAA and Good Clinical Practice. Note that protocol-specific forms include completion of the ALfA Pulmonary Procedure Checklist (P9_PULMONARYCHK).

To obtain ALfA coordinator certification, clinic personnel must complete the following steps:

- Thoroughly read the ALfA protocol and this Manual of Operations.
- Pass the ALfA coordinator certification exam. This exam can be found on the AsthmaNet secure website in the Certification: ALfA folder. Exams should be completed, scanned into a pdf file, and e-mailed to the AsthmaNet-Certification alias. Include 'ALfA Exam' and your performance site number on the subject line of the e-mail message to ensure efficient processing and routing at the DCC.

Any individual who performs spirometry, methacholine challenge, or eNO testing as part of an ALfA visit must be AsthmaNet certified in these procedures or be supervised by a certified technician, as applicable. MEMS certification is also required. Certification for these procedures is tracked independently of ALfA study certification. It is acceptable for these procedures to be performed during the ALfA study by technicians who possess only individual procedure certification and not ALfA protocol certification, but it is preferred that technicians review the protocol and take the certification exam, as well. If a technician is only certified in spirometry and not in the ALfA protocol, an ALfA-certified coordinator must complete the ALfA Pulmonary Procedure Checklist (P9_PULMONARYCHK) to qualify participants for spirometry and methacholine challenge testing.

Protocol deviations will be assigned when an uncertified individual performs protocol-related tasks or carries out procedures for which he/she is uncertified. Protocol violations will be assigned if this persists at a given site over a period of time. The AsthmaNet Quality Control Committee (QCC) will be informed of continued neglect of appropriate certification procedures.

The quality of AsthmaNet data is tracked and reported on a regular basis to the individual performance sites, clinical center partnerships, the AsthmaNet Quality Control Committee (QCC), and to the Data and Safety Monitoring Board (DSMB). It is possible to become decertified in some of the procedures (e.g., spirometry, sputum induction) if lack of quality becomes an issue and the study data begins to be affected adversely. The DCC will contact individuals who are in danger of becoming decertified to discuss the situation before they are decertified formally in the certification tracking system. It is

also possible to become decertified if a coordinator or technician leaves the Network and returns later, not having performed spirometry or other procedures for an extended period of time. See the individual procedure MOPs in the AsthmaNet General MOP for details.

Licensed Medical Practitioners (LMPs)
Physicians who are listed on the local IRB application as 'key personnel' must take and pass the ALfA physician certification exam before interacting with study participants. The physician exam is located on the secure website in folder Certification: ALfA.

Non-physician LMPs, such as nurse practitioners and physician's assistants, may perform physical exams for the ALfA study (see the Physical Exams discussion in this section for details). If these individuals will be performing exams for ALfA participants on a regular basis, then they should take either the coordinator or the physician exam and become certified. If they fill in for study physicians only occasionally, then certification is not required. Note that certification requirements for non-physician LMPs will vary from study to study.

Data Entry Personnel
Individuals who are only providing data entry support for the ALfA study and are not collecting data or performing study procedures do not have to meet any specific AsthmaNet certification requirements. However, it should be ensured that local institutional requirements for these individuals (e.g., HIPAA, GCP, and Human Subjects' Protection) have been met and are clearly documented on-site. This documentation may be subject to audit during an AsthmaNet site visit.

2.11 Concomitant Medications

Participants in AsthmaNet protocols are likely to be taking medications for asthma and allergy-related symptoms, both over-the-counter and prescription. It is important to document the medications a participant is taking, or begins to take, throughout the study to ensure that he/she is not taking medications that are excluded during the trial because they may confound the study results. Further, it is important to document any non-study asthma medications the participant begins using during the trial, as such use may indicate that the participant has experienced, or is experiencing, a significant asthma exacerbation.

The ALfA trial will employ the two standard concomitant medications forms: Concomitant Medications for Asthma/Allergy and Adverse Events (CMED) and Concomitant Medications for Non-Asthma Drugs (CMED_NON)**.**

Medications taken for treatment of adverse events, both asthma-related and those unrelated to asthma, should be recorded on the CMED form. Medications taken for treatment of asthma/allergy symptoms, other than dispensed study medications, should also be recorded on this form.

Medications not taken for asthma, allergies or adverse events should be recorded on the CMED_NON form. Examples include multivitamins and herbs the participant is taking for health maintenance and maintenance drugs taken for a pre-existing condition (e.g., Paxil for depression). Other non-asthma, non-allergy drugs the participant takes chronically, such as oral contraceptives, should also be recorded on this form.

Study medications, including Flovent® Diskus®, Advair® Diskus®, rescue medications (i.e., Atrovent® green RESCUE1 and Ventolin® blue RESCUE2) and blinded scheduled capsules, should not be regarded as concomitant medications and should not be recorded on CMED or CMED_NON. Prednisone taken to treat an asthma exacerbation or other adverse event *should* be recorded on the CMED form as a concomitant medication and linked to the appropriate adverse event on the Clinical Adverse Events (AECLIN) form.

Non-study asthma medications are considered concomitant medications and should be recorded on the CMED form if they are prescribed during the study (note that these drugs are excluded during the study and their use should be avoided if at all possible).

The following classes of drugs/solutions/products do not need to be recorded on a participant's CMED or CMED_NON form:

- Anesthesia medications administered during surgery and outpatient procedures
- Sedatives used prior to and during procedures
- Novacaine and other dental anesthetics

- Solutions/drugs taken prior to specialized procedures [e.g., Golytely (Colye, Nulytely), phospho-soda, and sodium phosphate tablets (Osmo-Prep, Visicol) taken prior to colonoscopy, Glucola taken during an oral glucose tolerance test]
- Iodine dye and other contrast materials used for MRIs and other procedures
- Allergy shots (i.e., immunotherapy injections)
- Vaccinations (e.g., flu vaccine)

Visit 1

Record concomitant medications the participant has taken since the informed consent was signed on the appropriate concomitant medications (CMED, CMED_NON) form

Thorough questioning about medication use during the initial study visit will prevent the presentation of unexpected information when it is time to randomize a participant. It also will help to prevent misinterpretation of medications reported at subsequent contacts, particularly if the participant interacts with a different coordinator.

During the first visit, prompt participants with the following questions:

- What over-the-counter medications do you typically take during a given month, including continuous use and as-needed medications, such as laxatives, antacids, stool softeners, ibuprofen, etc.? Inquire about the participant's use of vitamins and herbal remedies. Use of certain herbs, such as St. John's wort or valerian, during study participation should be discouraged.
- What prescription medications do you typically take during a given month, including continuous use and as-needed medications?
- What over-the-counter medications do you typically pack when you go on vacation or away for business? What prescription medications?
- What over-the-counter medications do you keep in your desk drawer or purse? What prescription medications?

If the participant has taken any medications for asthma, allergies or adverse events that have occurred since he/she signed the informed consent (original signature date), record them on the Concomitant Medications for Asthma/Allergy and Adverse Events (CMED) form. Medications taken on the day of Visit 1 should be recorded even if the participant has agreed to stop taking them after completing the visit. List the consent date as the start date for the medication (i.e., when the use of the medication became concomitant with study participation) if the participant started taking the drug prior to his/her original consent signature date.

Note: ALfA requires that participants who will need an intranasal steroid during the study begin using one as of Visit 1. These drugs also must be listed on CMED.

Any medications that were used to treat conditions <u>other than</u> asthma, allergies or adverse events since the participant signed the informed consent should be recorded

on the Concomitant Medications for Non-Asthma Drugs (CMED_NON) form. This includes substances like multivitamins, vitamin D and calcium supplements, and herbs the participant is taking for health maintenance. It also includes maintenance drugs for a pre-existing condition (e.g., Paxil for depression or insulin for diabetes) and other drugs the participant takes chronically, such as oral contraceptives.

Probing for medication use during Visit 1 affords an opportunity to recognize clinically significant medical problems early in the study. For example, a participant may take several medications to treat hypertension. The participant's condition may be deemed unstable and poorly controlled, therefore, ineligible on the basis of the information collected for the concomitant medications form. If a participant is taking medications for a condition that may exclude him/her from study participation, first check the ALfA Exclusionary Medical Conditions (P9_EXCLMED) reference card. If the applicable condition is not listed specifically, contact the DCC for guidance.

When scheduling Visit 1, the potential participant should be asked to bring all over-the-counter and prescribed medications and supplements he/she is currently taking to the visit. Alternatively, the participant may write down the names of the medications and supplements and the date he/she started taking each medication and bring this list to the visit.

Note that participants must wash out of oral corticosteroids, leukotriene modifiers, LABAs, etc. for a period of time prior to Visit 1. See the Eligibility Criteria discussion in this section for more details. Some institutions require that participants read and sign the study informed consent document prior to washing out of medications for purposes of study enrollment.

Visit 2, 3

Follow up medication use from the previous visit and record any new concomitant medications (CMED, CMED_NON)

Each time the clinic has contact with a participant, whether for a scheduled visit or phone contact, impromptu visit, or unexpected phone call, information on concomitant medications should be collected. During these contacts, the concomitant medication information obtained during previous contacts should be updated. If the participant discontinued a medication that he/she was taking, update the stop date on the CMED or CMED_NON form, as appropriate. Probe the participant for any new medications that may have been taken and record these on the appropriate form for the next visit. If the participant began taking a new medication for a condition or disease that existed prior to study enrollment and no adverse event (i.e., worsening of the condition) is associated with the change in medication, record this information on the CMED_NON form. If the participant has not taken any new medications for asthma, allergy or an adverse event, mark the 'None' box on the CMED form for the applicable visit.

Visit 3 and other early termination visits

Medications that are still in use at the time of the final study visit or contact should be left open for stop dates. On the CMED form, these are coded as 'ongoing at final visit' (Q1090 = 1). On the CMED_NON form these are coded as 'ongoing at end of study.' During the participant's final visit or contact with the clinical site, finalize his/her CMED and CMED_NON forms. All CMED forms for a given individual should be forwarded to the DCC following his/her study termination. CMED_NON forms are not sent to the DCC.

2.12 Contact Information

Visit 1
Administer Adult Contact Information form (CONTACT_ADULT)

The Adult Participant Contact Information (CONTACT_ADULT) form is completed by the participant. The purpose is to collect pertinent participant identification information such as full name, address, and telephone number, as well as alternative ways to contact the participant through work, family, or friends. It also includes contact information for the participant's health care provider.

In ALfA, participants will be contacted weekly during the randomized treatment phase to encourage adherence to study drug dosing. There will be scheduled phone contacts 2 and 4 weeks following Visit 2. The remaining weeks (1, 3, 5, 6 and 7 weeks following Visit 2), the participant will be contacted via their preferred method of contact. At Visit 1, the participant should be asked their preferred method of contact for these weekly contacts, and the preferred method of contact highlighted on the CONTACT_ADULT form.

General Information

- This form serves as source documentation proving the existence of the participant. It **must** be completed.
- A space for the participant's social security number has been included on the form for the convenience of the performance site in paying participant stipends. This field may be left blank if institutional policies prohibit recording and storing this information with the clinical records, or if social security number is not needed.
- It is important to obtain complete and accurate phone number information for the participant during Visit 1. The participant will need to be contacted via phone if they miss a visit and for phone contacts as part of the ALfA trial.
- Store the CONTACT_ADULT form in the participant's study folder; do not forward it to the DCC. This form contains the participant's name, address, and other identifying information. A protocol violation may be assigned if this form is misdirected to the DCC or another off-site group affiliated with AsthmaNet (e.g., Lu Lab, ADx Lab, etc.).

2.13 Continuation Visit 1

If a participant with FEV_1 < 80% does not reverse ≥ 12% at Visit 1, Visit 1 will be stopped following post-albuterol spirometry testing and a continuation visit will be scheduled. Continuation visit should try to be scheduled to take place within 24-48 hours, and within 7 days maximum. No Visit 1 data should be entered into the database unless/until the participant's eligibility is confirmed at the continuation visit. Exclusion criteria assessed at the initial Visit 1 apply to the continuation Visit 1 as well (i.e. respiratory infection in past 4 weeks, medication exclusions, etc.). Review Eligibility Checklist 1 and 2 to be sure nothing has changed, and participant still meets eligibility criteria at Continuation Visit 1. Visit will start with completing Urine Pregnancy Test (PREG_TEST) form for all female participants, administering urine pregnancy test if necessary, followed by the completion of the Pulmonary Procedure Checklist (P9_PULMONARYCHK), and spirometry testing (SPIRO).

Participants must pass all of the checks on the ALfA Pulmonary Procedure Checklist (P9_PULMONARYCHK) and the Adult Methacholine Challenge Testing Checklist (METHACHK_ADULT) before proceeding with the challenge. Results of the challenge are recorded on the Methacholine Challenge Testing (METHA) form and are referenced on ALfA Eligibility Checklist 3 (P9_ELIG3). The methacholine challenge report generated through the MedGraphics system must be printed and submitted with the data forms.

If an individual does not meet all the criteria on the Adult Methacholine Challenge Testing Checklist (METHACHK_ADULT) at Continuation Visit 1, the participant is ineligible to continue participation in ALfA. Likewise, participants who qualify for the methacholine challenge but do not meet the PC_{20} criterion for eligibility (PC_{20} ≤ 8 mg/mL) are also ineligible for the ALfA study. In these cases, data collected at Visit 1 should not be entered, and the Visit 1 packet should be filed in the participant's study folder. See the discussion of Withdrawals in this section for further details.

If the participant meets the methacholine challenge criteria (PC_{20} ≤ 8 mg/mL), he or she will continue with Visit 1 procedures. All Visit 1 data collected at the initial Visit 1 and the continuation visit should be entered into the study database. See Section 4 of this manual for information concerning entry of the forms completed at these visits.

2.14 Daily Activities Handout

Visit 1
Complete and distribute Daily Activities Handout (P9_DAILYACT1)

Near the end of Visit 1, review the summary handout "ALfA Daily Activities (Visit 1-2)" (P9_DAILYACT1). This handout summarizes what the study participants must carry out each day of the run-in phase until Visit 2. Daily high rescue use will need to be completed in the blank space provided on page 1. It is calculated by adding 8 to Q1000 on the Baseline PEF and Rescue Use Values form (P9_BASELINE).

Review handout to confirm participant understands the daily activities and how to monitor his/her asthma. The participant should also be instructed not to take his/her Flovent® Diskus® dose the morning of Visit 2.

Visit 2
Complete, distribute and review Daily Activities Handout (P9_DAILYACT2)

Near the end of Visit 2, remove the "ALfA Daily Activities (Visit 1-2)" handout (P9_DAILYACT1) from the participant's folder and discard it. Obtain a copy of the "ALfA Daily Activities (Visit 2-3)" (P9_DAILYACT2) handout. This reference lists what the participant must carry out each day until Visit 3. The High Rescue Use value will be calculated by adding 8 to Q1000 on the Visit 2 Baseline PEF and Rescue Use Values form (P9_BASELINE), and this will be completed on the blank space provided on page 1.

Review handout to confirm participant understands the daily activities and how to monitor his/her asthma. Be sure participant understands how he/she must take the scheduled capsule. It is very important the participant takes the scheduled capsule as directed. Specifically:

- Participant must take capsule just after getting out of bed in the morning, before eating or drinking anything.
- Capsule should be swallowed whole with a full glass (6 to 8 ounces) of plain water. Capsule should not be taken with tea, coffee, juice, milk, mineral water, sparkling water, or anything other than plain water.
- For at least 30 minutes (1 hour recommended) after taking capsule, the participant should not eat, drink or take medication, and should not lie down.
- Participant should sit upright or stand upright until at least 30 minutes have passed since taking capsule and participant has eaten first food of the day.

The participant should also be instructed not to take his/her Advair® Diskus® dose the morning of Visit 3.

Instructions on switching Diskus devices are included on the reverse side.

2.15 Dosing Compliance

Visits 2
Check compliance with run-in Flovent® Diskus® (P9_COMPLY)

Visits 3
Check compliance with Advair® Diskus® (P9_COMPLY)

The Diskus® contains a counter that shows the number of puffs remaining (out of a total of 60 puffs in a new Diskus®). The counter will be used to assess the participant's compliance with dosing from the Diskus® during the study. Participants are instructed to take 1 puff BID from the Flovent® Diskus® during the run-in and 1 puff BID from the Advair® Diskus® during the randomized treatment period.

The number of scheduled puffs should include all doses the participant should have taken since leaving the last clinic visit.

Compliance with dosing from the Diskus® is documented on the ALfA Compliance Checklist (P9_COMPLY) and entered into the study database.

Example Compliance Calculations
The following chart shows the number of Advair® Diskus® puffs a participant should have taken between Visit 2 and Visit 3 (ideal 8-week interval). Visit 2 took place on 5/25/2015 and Visit 3 takes place on 7/19/2014. Two Diskus® devices were returned – one with 0 puffs remaining and one with a counter value of 15.

Date	7/19 Visit day	7/18	7/17	7/16	7/15	7/14	7/13
Scheduled Diskus Puffs	0	2	2	2	2	2	2

Date	7/12	7/11	7/10	7/09	7/08	7/07	7/06
Scheduled Diskus Puffs	2	2	2	2	2	2	2

Date	7/05	7/04	7/03	7/02	7/01	6/30	6/29
Scheduled Diskus Puffs	2	2	2	2	2	2	2

Date	6/28	6/27	6/26	6/25	6/24	6/23	6/22
Scheduled Diskus Puffs	2	2	2	2	2	2	2

Date	6/21	6/20	6/19	6/18	6/17	6/16	6/15
Scheduled Diskus Puffs	2	2	2	2	2	2	2

Date	6/14	6/13	6/12	6/11	6/10	6/09	6/08
Scheduled Diskus Puffs	2	2	2	2	2	2	2

Date	6/07	6/06	6/05	6/04	6/03	6/02	6/01
Scheduled Diskus Puffs	2	2	2	2	2	2	2

Date	5/31	5/30	5/29	5/28	5/27	5/26	5/25
Scheduled Diskus Puffs	2	2	2	2	2	2	1 – PM dose only

Diskus® Compliance assessment (follow P9_COMPLY, Questions #1a-1d):

1a. Number of scheduled puffs (from above table): 109

1b. Number of remaining puffs (from Diskus® counters): 0 + 15 = 15

Remaining puffs on all returned Diskus® devices should be added together to get total number of remaining puffs.

1c. Number of puffs taken:

The number of puffs taken is equivalent to the number of puffs packaged in the Diskus® (or 60 puffs) x number of returned inhalers – the number of puffs remaining in the Diskus®.

The number of puffs taken is equivalent to (60 x 2 inhalers) – 15 = 105

1d. Percent compliance = # puffs taken / # puffs scheduled x 100

= 105 / 109 x 100
= 96.3%.

∴ Because the participant's compliance percentage exceeds the 80% goal laid out for the study, the participant is doing a good job of dosing with his or her Diskus®. He/she should be praised and encouraged to continue being diligent with taking study medications according to protocol.

2.16 Eligibility Criteria for Run-in

Visit 1
Complete Eligibility Checklist 1 (P9_ELIG1)
Complete Eligibility Checklist 2 (P9_ELIG2)
Complete Section 1 of Eligibility Checklist 3 (P9_ELIG3)
Complete Section 2 of Eligibility Checklist 3 (P9_ELIG3)

At Visit 1, participants will have a thorough medical history taken and will undergo a comprehensive physical examination. Findings from these procedures can affect the participant's continued study eligibility. Basic eligibility criteria and eligibility criteria related to the participant's medical condition and medical history are recorded on Eligibility Checklists 1 and 2 (P9_ELIG1 and P9_ELIG2).

If the participant remains eligible at Visit 1 following his/her exam and medical history assessment, he/she will perform spirometry. Based on the participant's spirometry results, he/she will:

- ***Protocol Version 2.1:*** either perform reversibility testing (if FEV_1 <80% predicted) or methacholine challenge testing (if FEV_1 ≥ 80% predicted). For participants with FEV_1 <80% predicted, his/her FEV_1 must improve at least 12% in response to 4 puffs albuterol to be eligible. For participants with FEV_1 ≥ 80% predicted, he/she must have a PC_{20} ≤ 8 mg/mL to be eligible. Source documentation of PC_{20} ≤ 8 mg/mL is <u>not</u> allowed.
- ***Protocol Version 2.2:*** either perform reversibility testing or methacholine challenge (if FEV_1 <80% predicted) or methacholine challenge testing (if FEV_1 ≥ 80% predicted).

  A participant with FEV_1 ≥ 80% predicted at Visit 1 must perform methacholine challenge to qualify, and must have a PC_{20} ≤ 8 mg/mL to be eligible. Source documentation of PC_{20} ≤ 8 mg/mL is not allowed.

  A participant with FEV_1 < 80% predicted at Visit 1 may qualify by demonstrating reversibility <u>or</u> PC_{20} ≤ 8 mg/mL. For participants with FEV_1 < 80% at Visit 1, the decision to perform reversibility or methacholine challenge at the initial Visit 1 will be made at the discretion of the study site's principle investigator, in consultation with their study coordinator. If a participant attempts methacholine challenge at the initial Visit 1and has PC_{20} > 8 mg/mL, he/she **<u>cannot</u>** qualify with reversibility. If a participant with FEV_1 < 80% predicted attempts reversibility at the initial Visit 1and does not reverse ≥ 12%, Visit 1 will be stopped following post-albuterol spirometry testing and a continuation visit will be scheduled to perform methacholine challenge. For participants with FEV_1 <80% predicted, his/her FEV_1 must improve at least 12% in response to 4 puffs albuterol at initial Visit 1, or he/she must have PC_{20} ≤ 8 mg/mL at initial Visit 1 or Continuation Visit 1.

Eligibility criteria related to baseline FEV_1 and PC_{20} are documented on Eligibility Checklist 3 (P9_ELIG3). Participants who remain eligible following completion of P9_ELIG3 will provide blood samples for lab testing.

Participants should review the data recorded on P9_ELIG1 and P9_ELIG2 and initial/date the source documentation box on the forms.

Note: Nightshift workers and others with altered schedules should not be enrolled in the ALfA study.

Visit 1 Inclusion Criteria

- Ability to provide informed consent, as evidenced by the signing of a copy of the ALfA study consent form approved by the study institution's Committee on Human Subjects' Research (i.e., Institutional Review Board).

  The informed consent documents must be signed on or before the Visit 1 date.

  See the discussion of Informed Consent in this section for further details.

  This criterion is documented in Q1000 and Q1010 on P9_ELIG1.

- Male or female, age 18 and older.

  This criterion is documented in Q1020 on P9_ELIG1.

- Physician-diagnosed asthma at least 12 months ago.

  Participant report is sufficient. Medical records and prescriptions for asthma medications are not required, but are helpful if the performance site has routine access to them.

  This criterion is documented in Q1230 on P9_ELIG2.

- Taking the equivalent of > 100 mcg and ≤ 1000 mcg of fluticasone daily for the past month

  This criterion is documented in Q1050 on P9_ELIG1.

- FEV_1 ≥ 50% predicted

  This criterion is documented in Q1000 on P9_ELIG3.

- ***(Protocol Version 2.1 Only)*** If FEV_1 < 80% predicted, FEV_1 improvement ≥ 12% in response to four puffs of albuterol

  At Visit 1, participants with FEV_1 < 80% predicted must demonstrate FEV_1 improvement ≥ 12% in response to four puffs of albuterol. During reversibility

testing, participants perform baseline spirometry followed by the administration of 4 puffs of albuterol and another spirometry session 10-15 minutes later. See the Spirometry discussion in this section and the Spirometry Manual of Operations in Appendix 1 of the AsthmaNet General Manual of Operations for further details on the reversibility testing procedures.

For purposes of eligibility assessment, reversibility is calculated on the basis of the baseline spirometry results (recorded on the Spirometry Testing (SPIRO) form) and the post 4 puffs spirometry session (recorded on the Post-Albuterol (4 puffs) Spirometry Testing (PALB4_SPIRO) form). Reversal is the relative change in FEV_1 expressed as a percentage.

Sample reversal calculations:

> To calculate the participant's % reversal with 4 puffs of albuterol, take the difference in raw FEV_1 values (in liters) (post FEV_1 value – pre FEV_1 value) and divide by the pre FEV_1 value. Multiply the result by 100.
>
> Pre-test FEV_1 (from Q1030 SPIRO form): 3.24 liters
> Post-test FEV_1 (from Q1030 PALB4_SPIRO form): 3.80 liters
>
> Reversal % = (3.80 – 3.24) / 3.24 * 100 = 17.28%

If the participant's reversal % is ≥ 12% (without rounding), he/she meets the criterion. The participant in the example meets the criterion.

This criterion is documented in Q1020 on P9_ELIG3.

- ***(Protocol Version 2.2 Only)*** If FEV_1 < 80% predicted, FEV_1 improvement ≥ 12% in response to four puffs of albuterol or PC_{20} ≤ 8 mg/mL

  At Visit 1, participants with FEV_1 < 80% predicted may qualify by demonstrating reversibility or PC_{20} ≤ 8 mg/mL. The decision to perform reversibility or methacholine challenge at the initial Visit 1 will be made at the discretion of the study site's principle investigator, in consultation with their study coordinator. If a participant attempts methacholine challenge at the initial Visit 1and has PC_{20} > 8 mg/mL, he/she **cannot** qualify with reversibility. If a participant with FEV_1 < 80% predicted attempts reversibility at the initial Visit 1 and does not reverse ≥ 12%, Visit 1 will be stopped following post-albuterol spirometry testing and a continuation visit will be scheduled to perform methacholine challenge. For participants with FEV_1 <80% predicted, his/her FEV_1 must improve at least 12% in response to 4 puffs albuterol at initial Visit 1, or he/she must have PC_{20} ≤ 8 mg/mL at initial Visit 1 or Continuation Visit 1.

  During reversibility testing, participants perform baseline spirometry followed by the administration of 4 puffs of albuterol and another spirometry session 10-15

minutes later. See the Spirometry discussion in this section and the Spirometry Manual of Operations in Appendix 1 of the AsthmaNet General Manual of Operations for further details on the reversibility testing procedures.

For purposes of eligibility assessment, reversibility is calculated on the basis of the baseline spirometry results (recorded on the Spirometry Testing (SPIRO) form) and the post 4 puffs spirometry session (recorded on the Post-Albuterol (4 puffs) Spirometry Testing (PALB4_SPIRO) form). Reversal is the relative change in FEV_1 expressed as a percentage.

Sample reversal calculations:

To calculate the participant's % reversal with 4 puffs of albuterol, take the difference in raw FEV_1 values (in liters) (post FEV_1 value – pre FEV_1 value) and divide by the pre FEV_1 value. Multiply the result by 100.

Pre-test FEV_1 (from Q1030 SPIRO form): 3.24 liters
Post-test FEV_1 (from Q1030 PALB4_SPIRO form): 3.80 liters

Reversal % = (3.80 – 3.24) / 3.24 * 100 = 17.28%

If the participant's reversal % is ≥ 12% (without rounding), he/she meets the criterion. The participant in the example meets the criterion.

If the participant performs methacholine challenge, he/she must demonstrate $PC_{20} \leq 8$ mg/mL. See the Methacholine Challenge discussion in this section and the Methacholine Manual of Operations in Appendix 2 of the AsthmaNet General Manual of Operations for further details.

Source documentation of a $PC_{20} \leq 8$ mg/mL is <u>not</u> acceptable.

This criterion is documented in Q1020 and Q1025 on P9_ELIG3.

- If $FEV_1 \geq 80\%$ predicted, $PC_{20} \leq 8$ mg/mL

  At Visit 1, participants with $FEV_1 \geq 80\%$ predicted must demonstrate $PC_{20} \leq 8$ mg/mL. See the Methacholine Challenge discussion in this section and the Methacholine Manual of Operations in Appendix 2 of the AsthmaNet General Manual of Operations for further details.

  Source documentation of a $PC_{20} \leq 8$ mg/mL is <u>not</u> acceptable.

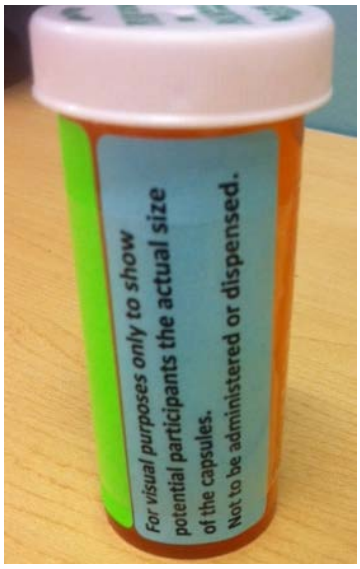
  This criterion is documented in Q1030 on P9_ELIG3.

- Ability to stay upright (sit or stand) for 30 minutes after taking oral medication in the morning

This criteria is documented in Q1130 on P9_ELIG1.

- Ability to swallow capsules like those used in the ALfA study

  You will be provided a vial with two empty capsules like those used in the ALfA study. The capsules are for demonstration purposes only.



  This criteria is documented in Q1140 on P9_ELIG1.

- If intranasal steroids will be needed at any time during the study, willingness of the participant to use a single intranasal steroid at a stable dose continuously for the duration of the study, starting at or before Visit 1.

  Any intranasal steroid may be used, as long as it is used at a constant dose continuously throughout the participant's study participation. The study physician should be consulted if the participant is not using an intranasal steroid at the time of screening (Visit 1) and the need for one is unclear. Examples include: Nasonex, Flonase, Nasacort, Rhinocort, etc. Intranasal steroids are not provided by the ALfA study.

  Use of intranasal steroids must be recorded on the Concomitant Medications for Asthma/Allergy and Adverse Events form (CMED). It is important to the goals of the study to be able to account for all steroid dosing, including intranasal steroids.

  This criterion is documented in Q1170 and Q1180 on P9_ELIG2.

- If currently lactating, willingness not to nurse during the ALfA study and for 6 months following study completion

  This criterion is documented in Q1270, Q1290 and Q1300 on P9_ELIG2.

- If potentially able to bear children, willingness to use one of the approved methods indicated on the Birth Control Methods (BIRTH_CTRL) reference card during the ALfA study and for 6 months following study completion

  This criterion is documented in Q1270, Q1310 and Q1320 on P9_ELIG2.

- Ability of the participant to use the Diskus® properly.

  This criterion will be evaluated objectively for all participants using the Diskus® Technique Checklist. Participants must achieve a perfect score of ten to pass the performance check on the Diskus® Technique Checklist (TECH_DISKUS). Participants will dose from Diskus® training devices for purposes of this assessment. See the Inhalation Technique Assessment discussion in this section for further details.

  This criterion is documented in Q1040 on P9_ELIG3.

Visit 1 Exclusion Criteria

- Plans to move away from the clinical site in the upcoming 3 months such that a participant's ability to complete the study will be jeopardized.

  If a participant is planning to move in the near future to a location that would preclude his/her completion of the study at the original performance site or at another AsthmaNet ALfA performance site, then he/she should not be enrolled. This concern should be discussed with students who tend to relocate during the summer months to determine if they will be able to complete all study visits at the local site or make alternate arrangements. Only participants who have a high likelihood of completing the entire study (through Visit 3) should be enrolled.

  This criterion is documented in Q1030 on P9_ELIG1.

- Use of investigative drugs or enrollment in an intervention trial in the past 30 days, or plans to enroll in such a trial during the ALfA study.

  Good clinical practice dictates that an individual should not participate in multiple intervention trials at the same time, due to possible interactions of study interventions which pose a safety concern and confounding of the resulting data. When screening potential ALfA participants, ensure that they are not currently participating in another intervention trial and, if they participated in one recently, that at least 30 days have elapsed since they terminated from the other study. Do not screen or enroll individuals who indicate that they are interested in participating in other intervention studies while they are still in the ALfA trial.

  While in the ALfA trial, individuals may participate in non-intervention studies that do not interfere with the medications and procedures required for the ALfA trial. Contact the ALfA scientific coordinator at the DCC to discuss individual circumstances as they arise.

This criterion is documented in Q1040 on P9_ELIG1.

- For participants taking the equivalent of >500 mcg fluticasone daily, ACT score < 18

  This criterion is documented in Q1060 and Q1070 on P9_ELIG1.

- Medical contraindication to LABA (salmeterol) or a history of adverse reactions to ICS (fluticasone) or LABA preparations or any of their ingredients

  This criterion is documented in Q1080 on P9_ELIG1.

- History of adverse reactions to anticholinergic inhalers (e.g., Atrovent, Oxivent, Spiriva)

  This criterion is documented in Q1090 on P9_ELIG1.

- History of adverse reactions or allergic reactions to bisphosphonates (e.g., Actonel, Actonel+Ca, Aredia, Boniva, Didronel, Fosamax, Fosamax+D, Reclast, Skelid, and Zometa)?

  This criterion is documented in Q1100 on P9_ELIG1.

- History of a respiratory infection in past 4 weeks.

  A respiratory tract infection is defined as a cough, runny nose plus or minus fever, or sore throat that is not related to allergen exposure. This criterion is evaluated by participant self-report; no specific medications need to have been taken to meet this criterion. At all subsequent visits, the occurrence of a recent infection should be documented on the Clinical Adverse Events (AECLIN) form.

  This criterion is documented in Q1110 on P9_ELIG1.

- Dental extraction or root canal in the past 8 weeks, or anticipate having one in the next 3 months

  This criterion is documented in Q1120 on P9_ELIG1.

- Chronic diseases (other than asthma) that in the opinion of the local investigator would prevent participation in the trial or put the participant at risk by participating, based on physical exam and medical history at Visit 1.

  In particular, individuals with presence of chronic or active lung disease other than asthma or history of unstable significant medical illness other than asthma, including (but not limited to) thyroid disease, diabetes mellitus, Cushing's disease, Addison's disease, hepatic disease, or concurrent medical problems that could require oral corticosteroids during the study or that would place the participant at increased risk, will be excluded.

Note that the majority of the following conditions are exclusionary only if deemed clinically unstable or contraindicated for the protocol in the judgment of the local investigator and the principal investigator for the protocol. If a potential participant's eligibility is in question, contact the ALfA scientific coordinator at the DCC for assistance.

Exclusionary conditions include, but are not limited to:

- Addison's disease
- AIDS
- Benign Prostatic Hyperplasia (BPH)
- Bladder-neck obstruction
- Cardiac arrhythmias or disorders (clinically significant)
- Congenital anomaly, including growth abnormalities (clinically significant)
- Congestive heart failure
- Coronary artery disease (unstable or severe)
- Cushing's disease
- Delayed esophageal emptying (caused by esophageal abnormality)
- Diabetes mellitus (poorly controlled)
- Dyspnea due to cause other than asthma, in judgment of investigator
- Eating disorder (e.g., active anorexia or bulimia)
- Esophageal ulcers (history of)
- Gastro-esophageal reflux disease (GERD; uncontrolled)
- Glaucoma (narrow angle)
- Hematemesis (history of)
- Hematologic disease (unstable, e.g., severe anemia)
- Hepatic disease[1]
- Hypertension (poorly controlled)
- Hyperthyroidism[2]
- Immunologic compromise[3]
- Chronic kidney disease (e.g., glomerulonephritis, polycystic kidney disease, etc.)
- Lactation
- Lung disease other than asthma (e.g., COPD, emphysema, chronic bronchitis, pulmonary embolism, malignancy, cystic fibrosis, among others)
- Lupus (active disease, requiring immunosuppressant)
- Any malignancy other than basal cell skin cancers
- Mental illness (uncontrolled)[4]
- Mental retardation

[1] Nonactive hepatitis B/C is allowable; active hepatitis (including antigen positivity or disease requiring treatment) is exclusionary.
[2] Controlled hypothyroidism is allowable.
[3] Resulting in prior infections and/or susceptibility to new infections.
[4] Anxiety, depression, or bipolar disease well-controlled on allowed medications are allowable conditions for the ALfA trial.

- Neurologic disease (including epilepsy requiring treatment)
- Obesity treated with bariatric surgery
- Osteonecrosis of jaw (history of)
- Peptic ulcer disease (active)
- Pregnancy
- Schizophrenia
- Skeletal disorders, including osteoporosis and rheumatoid arthritis[5]
- Sleep apnea (untreated)[6]
- Substance abuse (including active drug or alcohol abuse)
- Tuberculosis (active disease excluded; history of positive skin test with negative chest X-ray allowed)
- Urinary retention (active symptoms within last 6 months)
- Vocal cord dysfunction (diagnosis of)

These illnesses are listed on the ALfA Exclusionary Medical Conditions (P9_EXCLMED) reference card.

This criterion is documented in Q1000 and Q1000D on P9_ELIG2.

- History of bladder-neck obstruction

  This criterion is documented in Q1010 on P9_ELIG2.

- History of urinary retention

  This criterion is documented in Q1020 on P9_ELIG2.

- History of benign prostatic hyperplasia (BPH)

  This criterion is documented in Q1030 on P9_ELIG2.

- History of clinically relevant urologic disorder that precludes study participation.

  This criterion is documented in Q1040 on P9_ELIG2.

- History of narrow angle glaucoma

  This criterion is documented in Q1050 on P9_ELIG2.

- History of significant cardiovascular disorders or arrhythmias

  This criterion is documented in Q1060 on P9_ELIG2.

- History of esophageal ulcers

[5] Participants who have rheumatoid arthritis and are on excluded medications should not be screened; osteoarthritis is an allowable condition for the ALfA trial. Scoliosis, degenerative disc disease, and spinal stenosis are not exclusionary.
[6] Individuals with an OSA diagnosis who are receiving treatment with CPAP, BiPAP, or APAP are eligible.

This criterion is documented in Q1070 on P9_ELIG2.

- History of hematemesis

  This criterion is documented in Q1080 on P9_ELIG2.

- History of uncontrolled gastro-esophageal reflux disease

  This criterion is documented in Q1090 on P9_ELIG2.

- History of delayed esophageal emptying caused by abnormality such as stricture or achalasia?

  This criterion is documented in Q1100 on P9_ELIG2.

- History of osteonecrosis of the jaw

  This criterion is documented in Q1110 on P9_ELIG2.

- Need for the use of any of the drugs listed in Table 1 (that follows); inability to go off these drugs for the required washout periods prior to Visit 1 and for the duration of the ALfA study. The ALfA Exclusionary Drugs (P9_EXCLDRUG) reference card contains a summary of this table.

  Excluded drugs/substances on P9_EXCLDRUG must be washed out prior to Visit 1, and the participant must refrain from using them for the duration of the trial. If a participant is taking one or more of these medications at the time of Visit 1, the indication for the drug should be discussed with the local investigator to determine if it is safe for him/her to go off the drug to participate in the trial starting with Visit 1.

  It is important to note that <u>any and all changes</u> in a participant's medications must be approved by a study physician and documented in the participant's clinic notes.

  This criterion is documented in Q1120 on P9_ELIG2.

Table 1. Drugs to be withheld throughout the study.

Excluded Drug	Generic Names (may not be inclusive)	Trade Names (may not be inclusive)	Washout Prior to Visit 1
Steroid Medications			
Oral or intravenous steroids for any reason except as provided in study	dexamethasone, prednisone, prednisolone	Decadron, Medrol, Orapred, Prednisone, Prelone	4 weeks
Inhaled steroids, except as provided in study	beclomethasone, budesonide, ciclesonide, flunisolide, fluticasone, mometasone, triamcinolone acetonide	Aerobid, Alvesco, Asmanex, Azmacort, Flovent, Pulmicort, QVAR	None

Excluded Drug	Generic Names (may not be inclusive)	Trade Names (may not be inclusive)	Washout Prior to Visit 1
Intranasal steroids, except at stable drug and dose throughout study	beclomethasone, budesonide, ciclesonide, flunisolide, fluticasone, mometasone, triamcinolone acetonide	Beconase AQ, Flonase, Nasacort AQ, Nasarel, Nasonex, Omnaris, Rhinocort	None
Nonsteroidal Antiinflammatory Medications			
Leukotriene modifiers	montelukast, zafirlukast, zileuton	Accolate, Singulair, Zyflo	2 weeks
Cromolyn/Nedocromil for asthma	cromolyn, nedocromil	Intal, Tilade	1 week
NSAIDs	aspirin, ibuprofen, naproxen, meloxicam, ketoprofen	Advil, Aleve, Anacin, Ascriptin, Bayer, Bufferin, Ecotrin, Midol, Mobic, Motrin	None
Bronchodilators			
Oral β-agonists	albuterol, metaproterenol, terbutaline	Alupent, Brethine, Bricanyl, Metaprel, Proventil, Repetabs, Ventolin, Volmax	1 week
Short-acting inhaled β-agonists	epinephrine	Bronkaid Mist, Duo-Medihaler, Medihaler-Epi, Primatene Mist	24 hours
Intermediate-acting inhaled β-agonists, except study RESCUE2 drug	albuterol, bitolterol, levalbuterol, metaproterenol, pirbuterol, terbutaline	Alupent, Brethaire, Brethine, Bronkometer, Maxair, Metaprel, Proventil, Tornalate, Ventolin, Xopenex	24 hours
Long-acting inhaled β-agonists, except as provided in study	formoterol, salmeterol	Advair, Dulera, Foradil, Serevent, Symbicort	4 weeks
Short-acting inhaled anticholinergics, except study RESCUE1 drug	atropine, ipratropium bromide, pirenzepine, scopolamine	Atrohist, Atrovent, Bellatal, Combivent, Donnatal, Scopoderm, Transderm-Scop	12 hours
Long-acting inhaled anticholinergics	tiotropium	Spiriva	2 weeks
Xanthine Derivatives			
Short-acting theophylline	theophylline	Aminophylline, Slo-Phyllin	12 hours
Long-acting theophylline	theophylline	Slo-bid, Theo-Dur	24 hours
Ultra Long-acting theophylline	theophylline	Theo-24, Uniphyl	48 hours

Drugs to be withheld throughout the study.

Excluded Drug	Generic Names (may not be inclusive)	Trade Names (may not be inclusive)	Washout Prior to Visit 1
Anti-IgE Therapy			
	omalizumab	Xolair	3 months
Cardiac Drugs			

Alpha-beta blockers	labetalol	Normodyne	2 weeks
Beta blockers	acebutolol, atenolol, betaxolol, bisoprolol, carteolol, metoprolol, nadolol, penbutolol, pindolol, propranolol, timolol	Blocadren, Cartrol, Corgard, Inderal, Kerlone, Levatol, Lopressor, Sectral, Tenormin, Visken, Zebeta	2 weeks
Psych or CNS-Related Drugs			
Monoamine oxidase (MAO) inhibitors	harmaline, iproclozide, iproniazid, isocarboxazid, nialamide, phenelzine, selegiline, toloxatone, tranylcypromine	Nardil, Parnate	4 weeks
Antibiotics			
Macrolide antibiotics, chronic use excluded	azithromycin, clarithromycin, dirithromycin, erythromycin, roxithromycin, troleandomycin	Biaxin, Dynabac, Rulid, Surlid, TAO, Zithromax, Zitromax	2 weeks
Miscellaneous Exclusionary Drugs			
Drugs contraindicated when taking ipratropium	dicyclomine, glycopyrrolate, hyoscyamine, orphenadrine, tolterodine tartrate	Anaspaz, Antiflex, Banflex, Bentyl, Cystospaz, Detrol, Disipal, Donnamar, Flexoject, Levsin, Mio-Rel, Myolin, Myotrol, Orfro, Orphenate, Robinul	None
Drugs for urinary hesitancy	oxybutynin, tolterodine tartrate	Detrol, Ditropan	None
Drugs for narrow angle glaucoma	betaxolol, pilocarpine, timolol maleate	Betoptic S, Ocusert Pilo, Timoptic	None
Bisphosphonates	alendronate, etidronate, ibandronate, pamidronate, risedronate, tiludronate, zoledronic acid	Actonel, Aredia, Boniva, Didronel, Fosamax, Reclast, Skelid, Zometa	6 months

Drugs/substances to be withheld prior to Visits 1-3*.

Drug/Substance	Trade Names (may not be inclusive)	Washout Prior to Visits
ipratropium (study RESCUE1 inhaler)	Atrovent, Combivent	12 hours
albuterol (study RESCUE2 inhaler)	Ventolin, Ventolin, Proventil	24 hours
Tricyclic antidepressants and atypical antipsychotics (amitriptyline, clomipramme, desipramine, doxepin, imipramine, nortriptyline, quetiapine)	Anafranil, Elavil, Norpramin, Pamelor, Tofranil, Sinequan, Seroquel	24 hours**
Second-generation oral antihistamines (cetirizine, desloratadine, fexofenadine, levocetirizine, loratadine)	Allegra, Clarinex, Claritin, Xyzal, Zyrtec	24 hours

Drug/Substance	Trade Names (may not be inclusive)	Washout Prior to Visits
First-generation and other types of oral antihistamines (brompheniramine, carbinoxamine, chlorpheniramine, clemastine, cyproheptadine, dexchlorpheniramine, dimenhydrinate, diphenhydramine, doxylamine, hydroxyzine, ketotifen, meclizine)	Atarax, Ala-Hist, Benadryl, ChlorTrimeton, Dayhist, Dimetapp, Dramamine, Nyquil, Palgic, Sominex, Tavist, Tylenol PM, Unisom, Vistaril, Zaditen	12 hours
Ophthalmic antihistamines (azelastine ophthalmic, emedastine difumarate, epinastine ophthalmic, ketotifen fumarate, olopatadine ophthalmic)	Alaway, Elestat, Emadine, Opitvar, Pataday, Patanol, Zaditor	12 hours
Nasal antihistamines (azelastine nasal, olopatadine, levocabastine)	Astelin, Astepro, Livostin, Patanase	12 hours
Methylxanthine-containing food or beverages (caffeinated colas, coffee, tea)	Coke, Barq's Rootbeer, Mello-Yellow, Mountain Dew, Pepsi, Red Bull	6 hours
Methylxanthine-containing medications	Anacin, Darvon, Esgic, Excedrin, Fiorinal, Fioricet, No-Doz, Norgesic, Vivarin	6 hours
Weight loss medications	Belviq, bitter orange, Xenadrine, EFX, Thermorexin, Qsymia	4 hours
Alcohol-containing foods or beverages		4 hours

*These drugs/substances are allowed between visits, but not prior to pulmonary function testing.
**If participant must dose evening prior to visit, last dose should be taken at same time prior to each visit.

- Use of omalizumab (Xolair®) within the past 3 months

  This criterion is documented in Q1130 on P9_ELIG2.

- Use of a LABA in the past 4 weeks

  This criterion is documented in Q1140 on P9_ELIG2.

- Use of bisphosphonates within the past 6 months

  This criterion is documented in Q1150 on P9_ELIG2.

- Use of aspirin or non-steroidal anti-inflammatory medications (NSAIDs) regularly and inability to discontinue use of aspirin and NSAIDs during the course of the study

  This criterion is documented in Q1153 and Q1157 on P9_ELIG2.

- Use of any prescription or over-the-counter medication other than those listed on the ALfA Allowed Medications (P9_MEDALLOW) reference card.

  Chronic use of any medications other than RESCUE1 or RESCUE2 beta-agonist except:

  o analgesics for acute/chronic pain management (with MD discretion)

- antianxiety agents/anxiolytics (e.g., diazepam, chlordiazepoxide, alprazolam, clonazepam, lorazepam, gabapentin, buspirone) at a stable dose
- antibiotics (e.g. penicillins, cephalosporins, quinolones, monobactams, sulfonamides, doxycycline, minocycline, nitroimidazoles (Flagyl), macrolides) for intermittent use
- antibiotics for acne (topical/oral) (macrolides allowed for intermittent use only)
- anti-cholesterol medications (e.g., gemfibrozil, statins, fenofibrate, niacin), except cholestipol and cholestyramine
- specific antidepressants at a stable dose
  - Selective Serotonin Reuptake Inhibitors (SSRI) (e.g., citalopram, escitalopram, fluoxetine, fluvoxamine, paroxetine, sertraline)
  - Selective Serotonin Norepinephrine Reuptake Inhibitors (SSNRI) (e.g. desvenlafaxine, duloxetine, venlafaxine)
  - Non-SSRI/SSNRI antidepressants (except MAOI class drugs) (e.g. amitriptyline, amoxapine, bupropion, mirtazapine, nefazodone, trazodone and others)
- antihistamines (e.g. chlorpheniramine (Chlor-Trimeton), desloratadine (Clarinex), diphenhydramine (Benadryl), fexofenadine (Allegra, Allegra-D), loratadine (Claritin), cetirizine (Zyrtec), and others)
- specific antihypertensive medications
  - alpha blockers (e.g. doxazosin, prazosin, terazosin)
  - angiotensin converting enzyme (ACE) inhibitors (e.g. benazepril, captopril, enalapril, fosinopril, lisinopril, quinapril, ramipril)
  - angiotensin receptor blockers (Sartans) (e.g. candesartan, eprosartan, irbesartan, losartan, olmesartan, telmisartan, valsartan)
  - calcium channel blockers (e.g. amlodipine, diltiazem, felodipine, isradipine, nicardipine, nifedipine, verapamil)
  - diuretics (e.g. amiloride, bumetanide, chlorothiazide, chlorthalidone, furosemide, hydrochlorothiazide, indapamide, methyclothiazide, metolazone, spironolactone, triameterene)
  - mineralocorticoid receptor antagonists (e.g. eplerenone)
  - sympathetic nerve inhibitors (e.g. clonidine, guanabenz, guanfacine, methyldopa)
- antitussives (e.g. benzonatate (Tessalon Perles, Zonatuss), dextromethorphan)
- calcium-based antacids used PRN (e.g. TUMS®)
- calcium supplements at a stable dose throughout study (up to 2500 mg/day)
- CNS stimulants/appetite suppressants (e.g. lisdexamfetamine, methylphenidate (Ritalin), amphetamine preps)
- Cox-2 drugs (e.g. celecoxib (Celebrex))
- decongestants (e.g. pseudoephedrine (Sudafed), oxymetazoline (Afrin), and others)
- Depo-Provera®
- oral diabetes medications (for treatment of stable, controlled diabetes)
- erectile dysfunction medications (e.g. sildenafil, tadalafil, vardenafil)
- estrogen/progesterone replacement therapy for postmenopausal women

- expectorants (OTC only) (e.g. guaifenesin)
- eye preparations for allergic eye symptoms (topical) (e.g. antihistamines, NSAIDS, antiallergic compounds)
- H2 blockers (e.g. ranitidine, cimetidine, famotidine, nizatidine) for GERD
- hair growth preparations (e.g. finasteride (Propecia®))
- hemorrhoid treatments
- herpes medications (e.g. acyclovir (Zovirax), valacyclovir (Valtrex))
- insulin and injectable antidiabetic medications (for treatment of stable, controlled diabetes)
- intranasal steroids (any drug) at a stable dose throughout study
- laxatives
- Librax
- lithium
- migraine analgesics/preventatives (e.g. butalbital, triptans, topiramate)
- nasal antiallergic spray (Cromolyn/Atrovent)
- nasal saline spray
- Norplant®
- oral contraceptives
- proton pump inhibitors (e.g. omeprazole (Prilosec), pantoprazole, lansoprazole (Prevacid), esomeprazole (Nexium)) for GERD
- psyllium
- sleep aids used PRN
- stool softeners
- study medications
- thyroid replacement medication (e.g. Levothroid, Levoxyl, Synthroid)
- tretinoin (Retin-A) for acne
- vitamins, minerals
- Low potency topical corticosteroids (BID) (e.g., alclometasone dipropionate, desonide, dexamethasone, dexamethasone sodium phosphate, fluocinolone acetonide, hydrocortisone, hydrocortisone acetate)
- Medium potency topical corticosteroids (BID) (e.g., betamethasone benzoate, flurandrenolide, betamethasone dipropionate, fluticasone propionate, betamethasone valerate, hydrocortisone butyrate, clocortolone pivalate, hydrocortisone valerate, desoximetasone, mometasone furoate, fluocinolone acetonide, triamcinolone acetonide)

If a participant's use of a specific allowed medication is chronic, a complete clinical assessment should be performed to ensure the participant's safety and his/her ability to complete the entire study. Care should be taken to evaluate any underlying conditions the participant may be treating with these medications, in the event that he/she may have an exclusionary medical condition.

If a participant is taking a medication that does not appear in the above list, but also does not appear on the ALfA Exclusionary Drugs (P9_EXCLDRUG) reference card, first consult the local investigator. If the local investigator feels the participant should be considered eligible, then contact the ALfA scientific

coordinator at the DCC with the details. She will contact the lead study investigators and will document the final decision on the participant's suitability for the study.

This criterion is documented in Q1160 on P9_ELIG2.

- Allergen immunotherapy other than an established maintenance regimen implemented continuously for a minimum of 3 months.

  Allergen immunotherapy (also referred to as hyposensitization therapy or allergy shots) is allowed during the ALfA trial. Participants must be on consistent immunization therapy for at least 3 consecutive months prior to Visit 1 for the program to be considered an established maintenance regimen. Participants must be willing to continue on the same program, and new programs should not be initiated, for the duration of the individual's participation in the ALfA trial.

  Before screening a participant who is receiving allergen immunotherapy *other than allergy shots*, contact the ALfA Scientific Coordinator at the DCC for an assessment of the participant's eligibility.

  This criterion is documented in Q1190 on P9_ ELIG2.

- Smokeless tobacco product (e.g., chew, snuff) in past year.

  This criterion is documented in Q1200 on P9_ELIG2.

- Smoking of any substance (cigarettes, a pipe, cigar, marijuana, electronic cigarettes, other substance, etc.) in the past year (12 months).

  This criterion is documented in Q1210 on P9_ELIG2.

  Note: Participants should not use smokeless tobacco products (e.g., chew, snuff etc.) for the duration of the ALfA study.

- Lifetime smoking history greater than 10 pack-years.

  The pack-year limit applies regardless of when an individual stopped smoking.

  Definition of pack-year: A participant smoked for one pack-year if he/she smoked one pack of cigarettes (i.e., 20 cigarettes) a day for a period of one year. In general, the number of pack-years someone smoked is computed as:

  pack-years = #packs/day * #years smoked that quantity

  A participant with a 10-pack-year history could have smoked one pack of cigarettes per day over 10 years or two packs a day for 5 years, or many other combinations of packs/day and durations.

If a participant smoked an odd number of cigarettes per day, or had a history of smoking variable amounts of cigarettes per day over time, the resulting number of pack-years should be estimated to one decimal place for each part of the calculation.

For example, suppose a participant smoked an average of 8 cigarettes per day for 6 years, and 3 cigarettes per day for 3 years, eventually quitting. His/her pack-year history would be computed as:

(8/20) * 6 + (3/20) * 3 = 2.4 + 0.5 = 2.9 pack-years

This criterion is documented in Q1220 on P9_ELIG2.

Note: Pack-year history is quantified on the Adult Asthma and Allergy History (ASTHMA_HX_ADULT) form completed at Visit 1.

- A life-threatening asthma exacerbation requiring treatment with intubation, mechanical ventilation or resulting in hypoxic seizure in the past 2 years

  This criterion is documented in Q1240 on P9_ELIG2.

- Asthma exacerbation or other condition requiring systemic corticosteroid treatment in past 4 weeks.

  Systemic corticosteroids include oral (e.g., prednisone), injectable (IM), and intravenous (IV) steroids.

  This criterion is documented in Q1250 on P9_ELIG2.

- More than 5 courses of systemic corticosteroids in past year for treatment of asthma exacerbation conditions

  This criterion is documented in Q1260 on P9_ELIG2.

- Pregnancy

  If the participant is a woman of child-bearing potential, she will undergo a urine pregnancy test at Visits 1, 2 and 3. For additional details, see the Pregnancy Test discussion in this section.

  This criterion is documented in Q1280 on P9_ELIG2.

- If potentially able to bear children, not using an acceptable form of birth control.

  Acceptable forms of birth control include:

  - Birth control patches (Ortho Evra™)
  - NuvaRing®
  - Oral contraceptives

- Norplant®
- Depo-Provera®
- IUD
- IUS
- Single and double barrier methods (e.g., condom, spermicidal foam)
- Surgical sterilization (i.e., hysterectomy, tubal ligation, or vasectomy in monogamous partner)
- Post-menopausal (at least 1 year since last menses)
- Abstinence

This list is summarized on the Birth Control Methods (BIRTH_CTRL) reference card.

A history of infertility may not be used as a substitute for appropriate birth control.

This criterion is documented in Q1310 and Q1320 on P9_ELIG2.

- Any condition or compliance issue which, in the opinion of the investigator, might interfere with study participation.

  After the physician interacts with the participant at Visit 1, and the results of the physical exam and medical history are known, it may become apparent that the participant is not an ideal candidate for the ALfA study for a variety of reasons. If this is the case, the participant should be terminated from the study.

  This criterion is documented in Q1050 and Q1050D on P9_ELIG3.

2.17 Eligibility Criteria for Randomization

Visit 2
Complete Section 1 of Eligibility Checklist 4 (P9_ELIG4)
Complete Section 2 of Eligibility Checklist 4 (P9_ELIG4)

Eligibility criteria assessed at Visit 2 are recorded on Eligibility Checklist 4 (P9_ELIG4). Participants who have had one or more asthma exacerbations, have used exclusionary medications, were not able to provide 40 mL of blood for assays, etc. are not eligible to continue and will be terminated. Complete eligibility criteria can be found below. If a participant remains eligible following completion of P9_ELIG4, he/she will proceed with the visit which includes randomization.

Visit 2 Inclusion Criteria

- At least 80% compliance with required puffs from Diskus® during the run-in

  If the participant took less than 80% of the scheduled Diskus® puffs, re-emphasize the importance of maintaining the daily dosing schedule and reschedule visit in 2 weeks. If the participant still took less than 80% of the scheduled Diskus® puffs at the rescheduled Visit 2, the participant is ineligible for the study.

  This criterion is documented in Q1060 on P9_ELIG4.

- Ability to provide 40 mL of blood for biochemical assays ***(if IRB approval for protocol version 2.3 has NOT yet been obtained)***

  If unable to draw this amount, visit should be rescheduled to attempt blood draw again. If unable to draw this amount at second attempt, participant is ineligible.

  This criterion is documented in Q1070 on P9_ELIG4.

- Ability to provide 80 mL of blood for biochemical assays ***(if IRB approval for protocol version 2.3 has been obtained)***

  If unable to draw this amount, visit should be rescheduled to attempt blood draw again. If unable to draw this amount at second attempt, participant is ineligible.

  This criterion is documented in Q1075 on P9_ELIG4.

- Salmeterol-protected $PC_{20} \geq 0.25$ mg/mL and ≤ 16 mg/mL

  This criterion is documented in Q1080 on P9_ELIG4.

Visit 2 Exclusion Criteria

- Serum total calcium < 8.5 mg/dL and serum ionized calcium < 4.4 mg/dL

  Refer to Visit 1 Laboratory Results (P9_LAB) form.

  This criterion is documented in Q1000 and Q1010 on P9_ELIG4.

- Sum of absolute lymphocytes and monocytes < 900 cells/µL

  Refer to Visit 1 Laboratory Results (P9_LAB) form.

  This criterion is documented in Q1020 on P9_ELIG4.

- Estimated Glomerular Filtration Rate (eGFR) < 35 mL/min

  Refer to Visit 1 Laboratory Results (P9_LAB) form.

  This criterion is documented in Q1030 on P9_ELIG4.

- One or more asthma exacerbations since Visit 1.

  A significant asthma exacerbation has occurred if the participant experienced or is experiencing an increase in asthma symptoms (cough, phlegm/mucus, chest tightness, wheezing or shortness of breath) in association with increased rescue use (≥ 8 rescue puffs/day over baseline use for a period of 48 hours or ≥ 16 rescue puffs in a 24 hour period), low Visit 2 FEV1 (FEV1 < 80% of baseline (Visit 1) or < 45% predicted), or treatment with systemic corticosteroids for his/her exacerbation. If a participant is found to have had an asthma exacerbation during the run-in or at the time of Visit 2, he/she is ineligible.

  This criterion is documented in Q1040 on P9_ELIG4.

- Treatment with any excluded medication (P9_EXCLDRUG).

  This criterion is documented in Q1050 on P9_ELIG4.

- Participant wishes to withdraw consent.

  This criterion is documented in Q1090 on P9_ELIG4.

- New information that makes the participant ineligible according to the eligibility criteria.

  This criterion is documented in Q1100 on P9_ ELIG4.

- Any condition or compliance issue which, in the opinion of the investigator, might interfere with study participation.

After the participant is in the study for a couple weeks, it may become apparent that the participant is not an ideal candidate for the ALfA study for a variety of reasons. If this is the case, the participant should be terminated from the study.

This criterion is documented in Q1110 and Q1110D on P9_ELIG4.

2.18 Exhaled Nitric Oxide Procedures

Visits 2, 3
Perform FeNO testing (ENO)

Levels of forced exhaled nitric oxide (FeNO) are known to be elevated in people with asthma. In addition, FeNO may be involved in airway inflammation. FeNO is an important secondary outcome variable in the ALfA study.

FeNO will be collected at Visits 2 and 3 in ALfA. Results are documented on the Exhaled Nitric Oxide (ENO) form. The FeNO collection procedures should precede any pulmonary function testing procedures at a given visit. Clinical personnel should follow the order of procedures outlined on the visit procedure checklists. Any deviation from this order of procedures will result in the assignment of a protocol violation.

General Information
The NIOX MINO will be used to measure FeNO. Any individual who participates in eNO collection must possess AsthmaNet eNO certification or be directly supervised by a certified coordinator. A biological control test must be performed on the NIOX MINO every day before it is used with participants. This must be performed by a QC tester who has qualified for this procedure. See Appendix 8 of the AsthmaNet General Manual of Operations for details regarding eNO certification and QC procedures.

Prior to proceeding with exhaled nitric oxide testing, participants must pass the eligibility checks on the ALfA Pulmonary Procedure Checklist (P9_PULMONARYCHK). If any of the required washouts are not met, the participant should not proceed with collection of FeNO or pulmonary function testing at the visit. The FeNO levels may be affected by eating, drinking and strenuous activity/exercise, so participants should be encouraged to refrain from these activities for 1 hour prior to their visit. Testing will still be performed at the visit if a participant has eaten, drank, or taken part in strenuous activity/exercise in the past hour, but this will be documented on the ENO form. Mobile phones and cordless phones may interfere with the MINO, so they should be kept away from the MINO device.

The FeNO collection process may be attempted up to eight times in an effort to achieve an acceptable measurement. These eight attempts include all blows, even those when the NIOX MINO did not calculate and display a measurement because it did not find the maneuver technically acceptable.

The technician doing the collection should record the FeNO reading on the ENO form. If after eight attempts, no acceptable measurement was attained, the technician should record the time when the maneuver started and write “No acceptable maneuver” in Q6000.

Detailed instructions on using the NIOX MINO to collect exhaled nitric oxide measurements are documented in the Exhaled Nitric Oxide Manual of Operations located in Appendix 8 of the AsthmaNet General Manual of Operations.

See Section 10 of the AsthmaNet General Manual of Operations for specific information on completing the ENO form.

2.19 Genetics Blood Draw

Visit 2

Obtain blood sample for DNA extraction and genetic analysis (three 10 mL purple-top tubes) (optional)
Complete Genetic Analysis Blood Draw (GABLOOD) form
Enter genetics sample information into Genetics Tracking module, if applicable
Record genetic sample information on log (GEN_SAMP_LOG), if applicable

Genetics Consent

Before drawing blood for genetic analysis, verify that the participant has given consent to participate in the genetic analysis component of the ALfA study. The genetic analysis blood draw is optional; as stated in the consent, participants can refuse this blood draw and still participate in every other aspect of the ALfA study. The genetic analysis participation rate for each clinical center partnership and performance site will be summarized on the ALfA Accrual Report.

Blood Draw

The genetic analysis blood draw is scheduled for Visit 2 in the ALfA protocol; however, blood may also be drawn at Visit 3. See below for details on managing data in this case.

AsthmaNet genetics procedures are described in Appendix 4 of the AsthmaNet General Manual of Operations. The standard blood sample for genetic analysis purposes for participants ≥ 12 years old consists of three purple-top 10 mL vacutainers. Make certain that all tubes are as full as possible to ensure sufficient DNA for future genetic analyses. If a participant cannot provide three full purple-top vacutainers of blood, collect as much blood as possible and submit it to the Arizona Genetics Lab in Tucson for DNA extraction and storage.

Genetics Sample Tracking

Blood tubes collected for genetic analysis should be scanned into the AsthmaNet Genetics Tracking module immediately after they are drawn. The scan date is saved in the database and must be interpretable as the blood draw date. This information is forwarded to the Arizona Genetics Lab electronically and is needed for their tracking database and possible future sample submissions to the Biologic Specimen and Data Repository Information Coordinating Center (BioLINCC). Discrepancies between the scan date in the database and the blood draw date written on the blood tubes will be noted by the lab and reported to the DCC.

Information regarding the genetics blood drawn for a given participant must be entered onto the AsthmaNet Genetics Sample Log (GEN_SAMP_LOG) just prior to refrigerating the samples. This log tracks the collection date and time, refrigeration date and time and the volume of blood collected in each tube. The log collects information needed for BioLINCC purposes.

GABLOOD Form

Complete the Genetic Analysis Blood Draw (GABLOOD) form for all participants, regardless of whether or not they consent to provide a genetics blood sample. For those who elect to provide a blood sample, this form records information about their level of consent for future genetic analyses, as well as the total volume of blood drawn.

See Section 10 and Appendix 4 of the AsthmaNet General Manual of Operations and Section 4 of the ALfA MOP for specific information on completing the GABLOOD form. Note that the participant must review the form and complete the source documentation information (initials and date), even if he/she did not provide a blood sample.

Note: If a participant consents to provide a genetic blood sample, but the sample is not obtained at Visit 2 (due to a hard stick, dehydration or another problem), the blood draw may be delayed to Visit 3. If the genetics blood draw is deferred to Visit 3, the Visit 2 packet GABLOOD form should be marked missing. The GABLOOD form should be completed and data entered as a single form for the visit at which the blood draw takes place (e.g., Visit 3). If the blood draw is attempted at Visit 2 but is unsuccessful, and the participant is unwilling to have another draw attempted at Visit 3, then the GABLOOD form should be completed and data entered as part of the Visit 2 packet. In that case, Q1000 and Q1010 should be completed, indicating that a blood sample was not obtained, and the participant should provide source documentation. All individuals who make it past Visit 2 in the study must have a GABLOOD form present in the database.

Unsuccessful Visit 2

If a participant does not qualify for randomization at Visit 2, genetics samples should not be shipped to Tucson. They can be discarded.

2.20 Home Environment Questionnaire

Visit 1
Administer Home Environment Questionnaire (HEQ)

The Home Environment Questionnaire (HEQ) was developed by AsthmaNet. This questionnaire collects information about characteristics of the participant's home in general, his/her bedroom, his/her pets, and exposure to others' pets. Information regarding exposure to potential allergens that might affect the participant's asthma is collected in detail.

The questionnaire is completed by participant interview. The coordinator should provide assistance for any questions when requested. If the participant would rather not answer certain questions, they may be left blank. The participant should initial and date the source documentation box on the last page of the form when he/she is finished.

2.21 Household Socio-Economic Information Form

Visit 1
Administer Household Socio-Economic Information form (HOUSEHOLD_SEI)

Socio-economic status (SES) and health outcomes tend to be positively correlated (i.e., the higher the SES, the better the health outcome in terms of morbidity and mortality). Dr. Sheldon Cohen, affiliated with the Pittsburgh clinical center partnership, is an expert in this field and provided assistance for AsthmaNet to develop a very brief Household Socio-Economic Information (HOUSEHOLD_SEI) form. This form collects the highest level of education attained by members in a participant's household, the combined gross annual income of all members of the household, and the number of individuals supported by the income.

This form is completed by the participant. The respondent can decline to answer any question he/she wishes.

2.22 Informed Consent

Visit 1
Acquire signed ALfA informed consent

Informed consent **must** be obtained before any study information is collected or any study procedures are performed.

The ALfA consent template explains the procedures and time commitment necessary to participate in the ALfA trial, should the potential participant be deemed eligible. The AsthmaNet Data and Safety Monitoring Board reviewed and approved the template language which was prepared and submitted to each performance site's Institutional Review Board (IRB) for consideration. Some IRBs require or request changes to the template language which are reviewed by the DCC for consistency with the intent of the original document and completeness in terms of included information. A copy of the IRB approval memo and an IRB-stamped version of the consent document must be forwarded to the DCC prior to the start of recruitment at a given performance site. Each performance site must use its most recent IRB-approved version of the consent document in obtaining consent. The potential study participant must be given the opportunity to read, understand, and sign the consent document before any study-related activities take place.

Guidelines for obtaining consent:

- At the beginning of Visit 1, or prior to scheduling the visit, provide the potential participant a copy of the informed consent document and ask him/her to read it thoroughly. The participant should not sign the form until after you have discussed its contents with him/her.
- Allow ample time for the potential participant to read the informed consent form thoroughly. This will take some time, as the documents are often lengthy and include very detailed information for full disclosure.
- If the potential participant is unable to read the informed consent form or seems to be struggling, offer to read it to him/her or to help him/her with the more difficult sections.
- Be prepared to answer any questions the potential participant may have. If the person does not appear to understand the study or what participation entails, or if he/she has any other doubts about enrolling, do not ask him/her to sign the informed consent form. This person is not eligible to participate in the study.
- Maintain the signed informed consent form in the participant's study folder. To ensure confidentiality, **do not send this form to the DCC**. This document will be reviewed during data quality site visits.

If the participant fails to qualify during the run-in for a reason that can be remedied (e.g., respiratory tract infection, borderline compliance, etc.), he/she may be re-enrolled starting at Visit 1 at a later date. During the new Visit 1, the participant should be given a clean copy of the performance site's most current, IRB-approved ALfA consent document to review and sign. See the Re-Enrollment discussion in this section for further details.

If modifications are made to the ALfA consent document and approved by the local IRB while a participant is in the study, he/she must be re-consented following local IRB rules. All versions of the ALfA consent document the participant signed must be retained in his/her ALfA study folder and are subject to audit.

Local IRB rules and regulations regarding the consenting and assenting process should be followed at all times.

Note: The ALfA consent template contained language for the ALfA main study and optional genetic analysis participation. Some IRBs required the language for optional sections to be placed into its own consent document. At the participant's first visit, consent should be sought for the ALfA main study and genetics component, regardless of how they are packaged at a given performance site. All signed documents must be retained in the participant's study folder.

The date the participant signed the ALfA study consent is recorded and tracked on ALfA Eligibility Checklist 1. Genetic analysis participation is tracked on the Genetic Analysis Blood Draw (GABLOOD) form which is completed at the blood draw visit (Visit 2). See the Genetics Blood Draw discussion in this section for further details.

Visit 1
Administer BioLINCC consent
Complete BioLINCC Consent Tracking Form (BIOLINCC)

As a network funded by the National Institutes of Health, National Heart, Lung, and Blood Institute (NIH/NHLBI), AsthmaNet is expected to participate in the NHLBI's biobank which is coordinated by the Biologic Specimen and Data Repository Information Coordinating Center (BioLINCC). A biobank is a centralized collection of biological samples and health information that can be used by researchers outside of AsthmaNet for future studies in the areas of asthma and other diseases. At some time in the future, with the acceptance of BioLINCC, leftover samples from the ALfA study (potentially including plasma and DNA) will be transferred to BioLINCC and made available to other researchers. A participant must be asked to give his/her consent to transfer samples to BioLINCC. Samples for participants who refuse to provide consent will be retained by AsthmaNet. Participation is voluntary. See the AsthmaNet Genetics Procedures and BioLINCC manual in Appendix 4 of the AsthmaNet General Manual of Operations for further details regarding BioLINCC.

At Visit 1, after providing consent to be in the ALfA trial, the participant must be given the IRB-approved ALfA BioLINCC consent document to review. If he/she agrees to allow his/her leftover ALfA samples to be transferred to BioLINCC, he/she should sign the document and indicate the level of consent he/she is providing. Two levels of consent are possible: 1) allowing consent for all types of analyses, including genetic analyses, on the transferred samples and 2) allowing analyses with the exception of genetic analyses by researchers outside of AsthmaNet. The participant should indicate his/her preference in the consent document, prior to signing it. If the participant consents to participate in BioLINCC for his/her ALfA samples, then his/her consent document must be retained with the ALfA study consent document in his/her ALfA study folder at the performance site. This consent document is also subject to audit during an AsthmaNet data quality site visit.

Every ALfA participant must have a BioLINCC Consent Tracking Form (BIOLINCC) completed at Visit 1. This form tracks whether or not the participant agreed to donate his/her leftover ALfA samples to BioLINCC and, if so, what level of consent he/she provided. Information submitted to the DCC on the BIOLINCC form must match the participant's consent document. The BIOLINCC form data will be used to determine which samples are transferred to BioLINCC in the future.

2.23 Inhalation Technique Assessment

General Information

In the ALfA study, Diskus® technique assessment will be done at Visit 1. Assessments are done to assure the participant is able to use the device, as well as to document his/her ability to use it during the course of the study. Because placebo MDI is unavailable, MDI technique assessment cannot be performed.

Being as proper medication dosing is crucial for the success of the ALfA study, each participant must demonstrate that he/she can accurately use a Diskus® inhaler. Proper Diskus® technique is an eligibility requirement that is assessed at Visit 1 on ALfA Eligibility Checklist 3 (P9_ELIG3).

To assure that each participant has met the AsthmaNet standards for Diskus® use, a Diskus® Inhalation Technique Checklist (TECH_DISKUS) has been developed. Participants are considered eligible at Visit 1 only after they are able to carry out each of the ten steps (corresponding to ten points) listed on the technique checklist. There is no upper limit on the number of test puffs a participant may take to satisfy these requirements.

During the technique assessment, ten separate criteria are assessed by observing the participant inhale from a demonstrator Diskus® provided by the DCC. These units do not contain any ingredients (not even placebo) and can be used multiple times on a given occasion for purposes of training. Sites will be sent yellow “ALfA Diskus® Demonstrator” labels to adhere to the demonstration units so that they can be assigned to a given participant for reuse at subsequent visits. Coordinators should affix a label to the back of the assigned demo device and complete the participant’s ALfA ID number and initials in the fields provided.

The participant is given one point for each of the following steps that is completed correctly:

1. Uses thumb or finger in thumb grip to open device until the mouthpiece appears
2. Keeps Diskus® horizontal prior to step #3 and until step #7 completed
3. Slides lever once until it clicks
4. Breathes OUT fully
5. When breathing out fully (step #4), does so away from Diskus®
6. Puts lips tightly above and below mouthpiece opening
7. Breathes IN QUICKLY, filling lungs with medicine
8. Holds breath for at least five seconds (with or without Diskus® in mouth)
9. Removes Diskus® before breathing normally
10. Closes Diskus® by placing thumb or finger in the thumb grip and sliding it closed

Results of the technique assessment are recorded on the TECH_DISKUS checklist and stored in the participant's study folder; do not submit these forms to the DCC.

Visit 1

Assign participant an "ALfA Diskus Demonstrator". Affix label to back of device and complete participant's ID number on label.
Instruct participant on how to take run-in Diskus® (HTDISKUS, P9_DAILYACT1)
Assess Diskus® inhalation technique using participant's placebo Diskus® (TECH_DISKUS). Complete as many forms as necessary and store in participant's folder.
Disinfect mouthpiece and store participant's Inhalation Technique Diskus® for use at Visit 2
Instruct participant on how to take ipratropium and albuterol inhalers (HTMDI, P9_ASWORSE)

At Visit 1, the participant will be assigned a demonstator Diskus® to be used throughout the study. Participant ID should be written on device, which will be stored for use during the study. All participants should then be instructed in how to use the Diskus® device, and technique assessment performed.

A participant handout titled "How to Use Your Diskus®" has been developed as a quick reference for the participant to ensure that he/she is using correct Diskus® technique at home. The coordinator should review this handout with the participant at the visit and answer any questions that arise.

Because proper medication dosing is crucial for the success of the ALfA study, each participant must demonstrate that he/she can accurately use the Diskus® at Visit 1. Participants are considered eligible at Visit 1 only after they are able to carry out each of the ten steps (corresponding to ten points) listed on the TECH_DISKUS form. This requirement is documented at Visit 1 on ALfA Eligibility Checklist 3 (P9_ELIG3). Retrain and reassess as many times as needed.

At Visit 1, participants will be instructed on use of an MDI inhaler; however, no technique assessment will be done due to lack of placebo MDI.

Visit 2

Instruct participant on use of Diskus® (HTDISKUS)
Assess Diskus® Inhalation technique using Diskus® Inhalation Technique Checklist (TECH_DISKUS) and placebo Diskus® assigned at Visit 1. Complete as many TECH_DISKUS* forms as necessary

Diskus® Inhalation Technique will be re-assessed at Visit 2. All participants will be re-educated on how to take the Diskus®, and inhalation assessment will be performed. Participants must achieve a score of 10 on their technique assessment. Retrain and reassess as many times as needed.

2.24 Medical History

Visit 1
Complete Adult Asthma and Allergy History form (ASTHMA_HX_ADULT)
Complete Prior Conditions for All Participants form (PRIOR_COND_ALL)
Complete Prior Conditions for Adult Participants form (PRIOR_COND_ADULT)
Complete Prior Asthma/Allergy Treatment form (PRIOR_TRT)

A comprehensive medical history is taken at Visit 1. The medical history is broken into three parts recorded on four data collection forms:

1. The Adult Asthma and Allergy History (ASTHMA_HX_ADULT) form collects information regarding the onset of asthma and family history, recent asthma symptoms and acute episodes of asthma, asthma triggers, allergies, and basic smoking history.

   Note that smoking history is quantified in pack-years. One pack-year is defined as a one-year period when the participant smoked one pack (20 cigarettes per pack) per day. Participants whose smoking history changed over time will have their pack-year history calculated in pieces and summed over the entire history. For example:

   Sam smoked ½ a pack of cigarettes per day (10 cigs per day) while in his last year of college. Following college, he smoked a pack per day (20 cigs per day) for four years, until his employer no longer allowed smoking in the building. At that point he cut back to 5 cigarettes per day (0.25 packs per day) for 6 months while trying to quit. He has been a non-smoker ever since.

   Sam's pack-year history is calculated as follows:

   (1 x .5) + (4 x 1.0) + (.50 x .25) = 4.625 pack-years

   Sam may be eligible for ALfA, given his current non-smoker status and less than 10 pack-year history. Note that pack-year history is assessed for eligibility on Eligibility Checklist 2 (P9_ELIG2). Actual pack-years are recorded on ASTHMA_HX_ADULT.

2. The Prior Conditions for All Participants (PRIOR_COND_ALL) and Prior Conditions for Adult Participants (PRIOR_COND_ADULT) forms collect detailed information on prior diseases, illnesses, conditions and surgeries the participant has had.

3. The Prior Asthma/Allergy Treatment (PRIOR_TRT) form collects detailed information about the medications the participant used to treat asthma and allergies in the past 12 months. This form also collects non-asthma/allergy use of

oral and injectable steroids. Information on this form will be used to determine if the participant meets necessary washouts for spirometry and for entry into the study according to the eligibility criteria.

The medical history is administered early in the visit so that eligibility criteria that are relatively easy to confirm can be checked quickly. All portions of the medical history are obtained by participant interview. Read each question to the participant in a consistent, even tone, exactly as written on the forms. Provide clarification when asked.

When available, information contained in medical records should be considered more accurate than participant reporting. If the coordinator chooses to report interview information rather than information from the participant's medical record (when it is available), the affected item(s) should be dated and initialed to document this override. A notation indicating the override should also appear in the clinic notes. This documentation will be necessary when the data are audited during a site visit.

See Section 10 of the AsthmaNet General Manual of Operations for further details regarding the completion of the medical history forms.

2.25 MEMS

The MEMS®6 Monitor is a special closure that fits on a conventional medicine bottle. The closure/cap records the time and date of each opening/closing of the container from which compliance with medication dosing is inferred. A reader transfers the dosing history data from the MEMS®6 cap to a computer at which time an AsthmaNet Compliance Report can be generated for a given participant. For the ALfA trial, the MEMS®6 cap will be used to estimate each participant's compliance with taking his/her scheduled daily capsules. Capsules should be taken every morning between 5 AM and noon starting the morning after Visit 2.

For information on configuring the MEMS®6 cap, reading the data, and generating compliance reports, see the MEMS®6 Monitor Manual of Operations in Appendix 5 of the AsthmaNet General Manual of Operations.

Before dispensing the MEMS®6 cap with the capsule vial, the cap must be woken up and then initiated on the medAmigo webpage. Brief instructions are provided below; complete instructions can be found in the MEMS®6 Monitor MOP in Appendix 5 of the AsthmaNet General MOP. The MEMS® Monitor Quality Control (MEMSQC) form will also need to be completed.

For ALfA, the number of monitored days between Visit 2 and 3 (not including the day of the prior visit), number of correct days, % of correct days, and % doses in time-window are evaluated. Results are transcribed onto the ALfA Compliance Checklist (P9_COMPLY) in fields Q1040-1070.

Visit 2
Wake-up/initiate MEMS® monitor, attaching to capsule vial

Before programming the MEMS®6 cap, the cap will need to be woken up. See Appendix 5 for instructions on how to do this.

During the wake-up procedure, the MEMS®6 cap will be attached to the participant's capsule vial. Afterwards, go to the medAmigo webpage and select "Initiate a new patient (first reading)". On the patient data form:

- Enter Participant's ID and initials;
- Choose the appropriate Time zone for your location;
- Choose the "Monitoring begin" date. This should be the day following Visit 2, since that is when the participant will begin study capsule dosing.

The cap has now been initiated and is ready to be dispensed. Refer to the MEMS®6 MOP for complete details on configuring the MEMS®6 cap.

Visit 2
Perform MEMS® Monitor Quality Control (MEMSQC)

Quality control (QC) is required prior to dispensing the MEMS® cap to ensure that the cap has enough memory and battery power to last until the participant's Visit 3. The QC process also confirms that the device will not expire prior to Visit 3. If a participant's cap fails the QC process, issue a new cap and complete a new MEMSQC form. Enter both forms with the visit packet.

Alerts regarding battery expiration, battery voltage, and used memory (Q4-Q6) will appear when reading the MEMS®6 cap for the first time (Cap will be read when initiating cap as explained above.). This should be done prior to dispensing MEMS®6 cap for use. If no alerts appear, expiration, voltage, and memory are adequate; Q1040, Q1050 and Q1060 should be answered 'No'.

Visit 2
Instruct participant on use of MEMS® Monitor (P9_MEMSINST*)

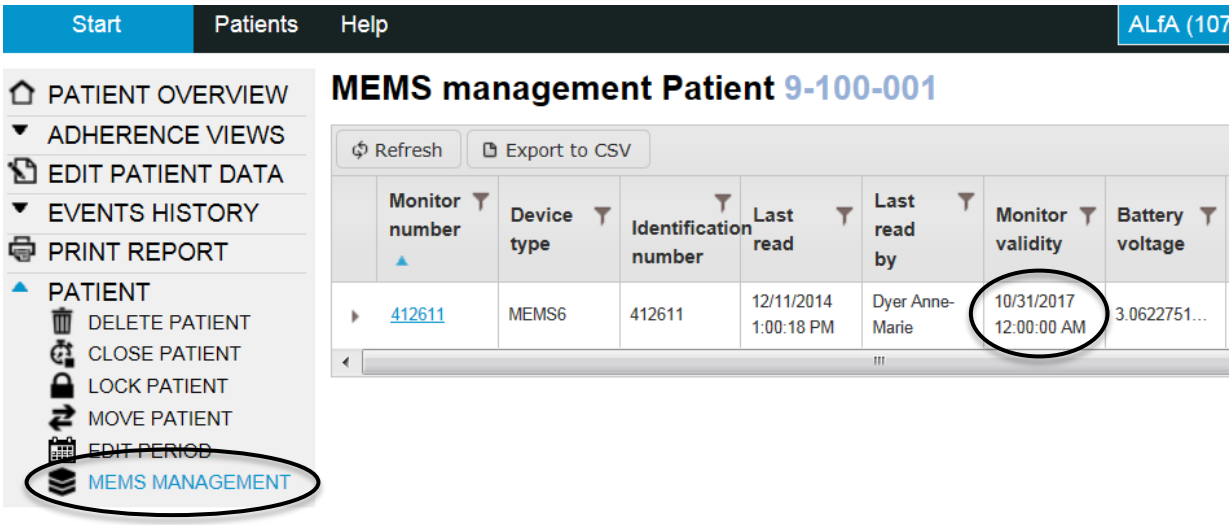
After programming MEMS® monitor, introduce the participant to the MEMS® cap. Ensure that the participant knows that the cap is child resistant and confirm that he/she can remove the cap without a problem. Review appropriate dosing of the blinded capsules and explain that his/her compliance with dosing will be reviewed at Visit 3. The participant should take his/her first capsule from the vial the morning after the visit.

Visit 2
Log/dispense MEMS® Monitor (MEMS_LOG)

Visit 3 or other early termination visit
Collect/log MEMS® Monitor (MEMS_LOG)

Any time a MEMS® cap is given to a participant or collected from a participant, this information needs to be recorded on the MEMS® Monitor Log (MEMS_LOG). One general log is used to track the supply of MEMS® caps across all AsthmaNet studies for a given performance site. Each time a cap is dispensed, decrement the available balance of caps by 1 and record the device serial number, validity date (see below for where this is found), participant ID number, date dispensed, and the dispenser's initials. Each time a cap is returned, record the date returned, collector's initials, QC status of the cap and any related comments. If the cap is viable for use with another participant, increment the balance of caps by 1 and record the serial number and validity date on the next available row. This cap should be cleaned, prepared, and dispensed the next time a cap needs to be assigned to a participant to maximize its use before it expires.

After reading the MEMS® cap for the first time, the validity date can be found under PATIENT > MEMS MANAGEMENT in medAmigo. Validity date should be recorded in format mm/yyyy.



Visit 3
Generate MEMS® Monitor Report
Check MEMS® (capsule) compliance (P9_COMPLY)

At Visit 3, the MEMS®6 monitor will be read to create an AsthmaNet Compliance Report. To read the monitor, select "Read new dosing history data from MEMS monitor (following visits)". After reading monitor, you will see the history of bottle openings. When setting up the MEMS®6 monitor, the "Monitoring Begin" date should have been set to the day after Visit 2, so it should be set to report from the dosing start date to the date of Visit 3.

Report can be generated by selecting "Print Report" and Print. Report should be printed and results transcribed onto the ALfA Compliance Checklist (P9_COMPLY) in fields Q1040-1070.

MWV	**Patient adherence report**
Patient number	**9-100-001**
Monitor number	**412611**
Period	**11/12/2014 - 11/16/2014**

MEMS1 (Alendronate sodium, 10 mg or placebo)
Drug :
Regimen : 1 X per day

Number of monitored days: 4	**Q1040**
Number of correct days 4	**Q1050**
% of days with correct nbr of doses taken : 100 %	**Q1060**
Time window : 100 %	**Q1070**

2.26 Methacholine Challenge

General Instructions

At Visit 1, the methacholine challenge establishes study eligibility for participants with baseline FEV_1 ≥ 80% predicted. In protocol version 2.2, it may also establish eligibility for participant's with baseline FEV_1 < 80% predicted. At Visits 2 and 3, salmeterol-protected methacholine challenge is performed. Only participants with salmeterol-protected PC_{20} ≥ 0.25 and ≤ 16 mg/mL at Visit 2 will be eligible for randomization. The resulting PC_{20} at Visits 2 and 3 will be used to calculate the primary outcome for the ALfA study. At Visits 2 and 3, methacholine challenge procedure should be started prior to 11 AM; any challenge set to start after 12:15 PM must be rescheduled.

Individuals performing methacholine challenges must be AsthmaNet-certified in this procedure or, at minimum, supervised by AsthmaNet-certified personnel.

To maximize supplies, old (unexpired) stock of methacholine should be used before newer lots.

Participants must pass all of the checks on the ALfA Pulmonary Procedure Checklist (P9_PULMONARYCHK) to proceed with spirometry and methacholine challenge. They must also pass all of the checks on the Adult Methacholine Challenge Testing Checklist (METHACHK_ADULT). Note that in ALfA, METHACHK_ADULT Q1050 excludes a participant from performing the challenge if he/she used systemic corticosteroids for 4 or more days for treatment of an asthma exacerbation OR for another indication. Also, participants with FEV_1 ≥ 50% predicted are eligible to proceed with methacholine challenge in ALfA. If FEV_1 is 50% to 54.9% predicted and ≥ 1.0 L, check 'Yes' to METHACHK_ADULT Q1060, and provide comment in Q6000 that FEV_1 ≥ 50% predicted and ≥ 1.0 L.

Respiratory Infection

A participant who has a respiratory infection within the past 4 weeks is not eligible to proceed with methacholine challenge. This is asked on Eligibility Checklist 2 (P9_ELIG2) at Visit 1, and on the ALfA Pulmonary Procedure Checklist (P9_PULMONARYCHK) at Visits 2 and 3. If a participant reports having a respiratory infection in the past 4 weeks at:

- Visit 2: Visit 2 should be rescheduled to allow 4 weeks between resolution of lower respiratory symptoms and Visit 2. If respiratory infection was acute, supervising physician should be consulted. If physician feels methacholine challenge results will be adversely affected even after 4 week delay, the date of the rescheduled visit should be based on the physician's discretion.
- Visit 3: Visit 3 should be scheduled to allow 4 weeks between resolution of lower respiratory symptoms and Visit 3. If respiratory infection was acute, supervising physician should be consulted. If physician feels methacholine challenge results will be adversely affected even after 4 week delay, the date of the rescheduled

visit should be based on the physician's discretion. If 4+ week delay requires more than 12 weeks* on alendronate, participant should be terminated. See Phone Contact and Study Medications discussions in this section for information on respiratory infections before Visits 2 and 3, and ensuring the participant does not continue on alendronate beyond 12 weeks*.

* The standard extended visit window applies, so Visit 3 date can be up to 12 weeks +5 days after randomization (Visit 2).

General procedures for carrying out a methacholine challenge can be found in the Methacholine Manual of Operations in Appendix 2 of the AsthmaNet General Manual of Operations.

Salmeterol-protected Methacholine Challenge
Salmeterol-protected methacholine challenge (SpMCh) is performed at Visits 2 and 3. For this procedure, two puffs Advair HFA 115/21 mcg will be administered during the visit and methacholine challenged started one hour later.

Post-Methacholine Challenge Procedures (Visit 1)
After methacholine challenge has been completed, the participant should be reversed back to at least 90% of baseline (pre-challenge) lung function. At Visit 1, albuterol will be used for reversal. Baseline lung function (FEV_1) is obtained from Q1030 on the participant's Spirometry Testing (SPIRO) form completed at the visit.

Standard reversal for ALfA Visit 1 is two puffs of albuterol. If additional treatment is needed, two additional puffs albuterol should be given. Additional treatment with albuterol is at discretion of physician.

Results of standard reversal are recorded on the Methacholine Challenge Testing (METHA) form. If a participant requires additional treatment to achieve reversal, this information should be recorded on the Additional Treatment Post Methacholine Challenge Testing (METHA_ADD_TRT) form. This form is entered as a single form.

Puffs of albuterol given to reverse the participant from a methacholine challenge should not be counted in the Ventolin® (blue RESCUE2) puffs the participant records on the Asthma Monitoring Log (P9_ASTHMA_LOG) the evening of the visit.

Post-Methacholine Challenge Procedures (Visits 2, 3)
At Visits 2 and 3, ipratropium will be used for reversal. Ipratropium is being used because albuterol may interfere with blood tests performed in the study. However, albuterol will be on hand and may be used if needed. Baseline lung function (FEV_1) is obtained from Q1030 on the participant's Post-Advair Spirometry Testing (PADVAIR_SPIRO) form completed at the visit.

Standard reversal for ALfA Visits 2 and 3 is two puffs of ipratropium. If additional treatment is needed, two additional puffs ipratropium should be given. It is sufficient to

wait 15 minutes following ipratropium administration before performing spirometry. Additional treatment with albuterol is at discretion of physician. If albuterol is administered, it should be recorded on the Concomitant Medications for Asthma (CMED) form.

Results of standard reversal are recorded on the Methacholine Challenge Testing (METHA) form. If additional treatment is required to achieve reversal, this information should be recorded on the Additional Treatment Post Methacholine Challenge Testing (METHA_ADD_TRT) form. This form is entered as a single form.

Puffs of ipratropium and albuterol given to reverse the participant from a methacholine challenge should not be counted in the green RESCUE1 and blue RESCUE2 puffs the participant records on the Asthma Monitoring Log (P9_ASTHMA_LOG) the evening of the visit.

See Section 4 of this MOP for details on the completion of the forms above.

Visit 1
Complete Adult Methacholine Challenge Testing Checklist (METHACHK_ADULT)
Perform Methacholine Challenge Testing (METHA)
Complete Additional Treatment Post Methacholine Challenge (METHA_ADD_TRT) form, if needed

If participant's FEV_1 is ≥ 80% predicted at Visit 1, participant must have PC_{20} ≤ 8 mg/mL to be eligible for ALfA. Source documentation of PC_{20} ≤ 8 mg/mL is <u>not</u> allowed.

Protocol Version 2.2 Only: A participant with FEV_1 < 80% predicted at Visit 1 may qualify by demonstrating reversibility or PC_{20} ≤ 8 mg/mL. The decision to perform reversibility or methacholine challenge at the initial Visit 1 will be made at the discretion of the study site's principle investigator, in consultation with their study coordinator. If a participant with FEV_1 < 80% attempts methacholine challenge at the initial Visit 1and has PC_{20} > 8 mg/mL, he/she **<u>cannot</u>** qualify with reversibility. If a participant with FEV_1 < 80% predicted attempts reversibility at the initial Visit 1and does not reverse ≥ 12%, Visit 1 will be stopped following post-albuterol spirometry testing and a continuation visit will be scheduled. Continuation visit should try to be scheduled to take place within 24-48 hours, and within 7 days maximum. Visit will start with completing Urine Pregnancy Test (PREG_TEST) form for all female participants, administering urine pregnancy test if necessary, followed by the completion of the Pulmonary Procedure Checklist (P9_PULMONARYCHK), and spirometry testing (SPIRO).

Participants must pass all of the checks on the ALfA Pulmonary Procedure Checklist (P9_PULMONARYCHK) and the Methacholine Challenge Testing Checklist (METHACHK_ADULT) before proceeding with the challenge. Results of the challenge are recorded on the Methacholine Challenge Testing (METHA) form and are referenced on ALfA Eligibility Checklist 3 (P9_ELIG3). The methacholine challenge report

generated through the MedGraphics system must be printed and submitted with the data forms.

If an individual does not meet all the criteria on the Adult Methacholine Challenge Testing Checklist (METHACHK_ADULT) at Visit 1, the participant is ineligible to continue participation in ALfA. Likewise, participants who qualify for the methacholine challenge but do not meet the PC_{20} criterion for eligibility ($PC_{20} \leq 8$ mg/mL) are also ineligible for the ALfA study. Data collected at Visit 1 should not be entered, and the Visit 1 packet should be filed in the participant's study folder. See the discussion of Withdrawals in this section for further details.

Visits 2, 3

Complete Adult Methacholine Challenge Testing Checklist (METHACHK_ADULT)
Administer 2 puffs of Advair® 115/21, and wait 1 hour before proceeding with post-Advair® saliva collection, spirometry, and Methacholine Challenge testing
(One hour after administering Advair®) Perform Methacholine Challenge Testing (METHA)
Complete Additional Treatment Post Methacholine Challenge (METHA_ADD_TRT) form, if needed

At Visits 2 and 3, methacholine challenge will be performed one hour after the participant has taken 2 puffs Advair® HFA 115/21 mcg. Methacholine Challenge should begin no later than 70 minutes after Advair® administration. (Post-Advair® saliva collection can occur no earlier than 50 minutes after Advair® administration.) Order of procedures outlined on the visit checklist should be followed to ensure procedures are performed in the proper order.

Participants must pass all of the checks on the ALfA Pulmonary Procedure Checklist (P9_PULMONARYCHK) and the Methacholine Challenge Testing Checklist (METHACHK_ADULT) before proceeding with the challenge. Note that in ALfA, METHACHK_ADULT Q1050 excludes a participant from performing the challenge if he/she used systemic corticosteroids for 4 or more days for treatment of an asthma exacerbation OR for another indication.

- ***Protocol Version 2.1:*** If a participant has taken a systemic corticosteroid within the 4 weeks prior to Visit 3 or meets exacerbation criteria at the time of Visit 3, he/she should not proceed with methacholine challenge testing at the visit and the visit should be rescheduled so that the final visit occurs at least 4 weeks after the participant completes his/her prednisone burst.

- ***Protocol Version 2.2:*** If a participant has taken a systemic corticosteroid after the first 4 weeks of blinded treatment or meets exacerbation criteria at the time of Visit 3, their participation should be extended so that the final visit occurs at least 4 weeks after they complete their prednisone burst.

- If this extension goes beyond 12 weeks* of alendronate, his or her participation in the study will be terminated.
- If a participant requires a second burst of systemic corticosteroids for an asthma exacerbation after randomization, his or her participation in the study will be terminated.
- If a participant meets exacerbation criteria at the time of Visit 3, and the 5-day course of prednisone followed by 4 week washout requires more than 12 weeks* on alendronate, his/her participation in the study should be terminated.

* The standard extended visit window applies, so Visit 3 date can be up to 12 weeks +5 days after randomization (Visit 2).

2.27 Missed Visits

A missed visit is defined as one for which the participant is unavailable to undergo any clinic procedures for purposes of obtaining important outcome data for analysis. If spirometry is attempted during a visit, the visit is not considered missed, even if not all procedures are completed. If the Pulmonary Procedure Checklist (P9_PULMONARYCHK) is completed, the visit is not considered missed, even if the participant does not qualify to perform spirometry at the visit.

ALfA has three visits. Visits 1 and 2 are required for eligibility and randomization, and Visit 3 is required for end-of-treatment measures. As a result, missed visits will only occur if a participant withdraws during the randomized treatment period, and should therefore be rare. If a participant is ineligible to proceed with spirometry or methacholine challenge at Visit 3 (i.e., due to not meeting medication washout), the visit should be rescheduled.

Ideally, all visits for a participant should occur at the same time of day (+/- 3 hours of Visit 1) as measured by the time that baseline spirometry takes place during a visit. Note that Visits 2 and 3 must occur before 11 AM, so Visit 1 should be scheduled with that in mind. More specifically, salmeterol-protected methacholine challenge at Visits 2 and 3 should begin prior to 11 AM, meaning visit procedures prior to methacholine challenge should be completed, and the challenge started by 11 AM; any challenge set to start after 12:15 PM must be rescheduled. When not possible to schedule at the same time of day, it is desirable for all visits to fall within a 4-hour window. Do not skip a visit if it is not possible to maintain these goals. Consistency in spacing of visits is more important for the collection of outcome data. If a participant cannot be seen within the 3-hour time window, contact the ALfA Scientific Coordinator at the DCC to discuss the allowance of an exception. Visits that take place outside the 3-hour window from the time of baseline spirometry at Visit 1 without a pre-approved exception will be assigned protocol deviations.

If it is not possible to schedule a visit within the regular visit window, schedule it in the extended window, if possible. If a participant cannot be seen within the extended windows, contact the ALfA Scientific Coordinator at the DCC to discuss alternate arrangements. See the Visit Windows discussion in this section for further details.

Visits 1, 2
These visits are mandatory. Eligibility assessments take place at Visits 1 and 2, and randomization takes place at Visit 2. Contact the ALfA Scientific Coordinator at the DCC if scheduling issues arise for Visit 2. As mentioned above, Visits 2 and 3 must take place prior to 11 AM, so schedule Visit 1 accordingly.

Visit 3

Visit 3 is the end-of-treatment visit and the termination visit. For this reason, it cannot be missed.

2.28 Participant Assignment Log and Protocol Enrollment

A Participant Assignment Log (P9_LOG) has been developed for ALfA for each performance site. This log includes columns for unique participant ID numbers, participant initials, participant's name, and randomization status.

Participant ID numbers are preprinted on P9_LOG and are comprised of 7 digits:

- The first digit is the number of the AsthmaNet protocol. For the ALfA protocol the first digit is 9.
- The next 3 digits are the AsthmaNet performance site identifier
- The last 3 digits constitute the participant identification (ID) number that is unique within the performance site. Participant IDs start with 001 and increase sequentially for the number of participants who are screened for the ALfA protocol at Visit 1 at a given site.

To assign an individual a participant ID number, select the next available blank entry on the ALfA Participant Assignment Log. This number will be the primary participant identifier used during the ALfA study; it should be used in all communications with the DCC. The participant ID number also should be used to label the participant's ALfA study folder at the performance site.

Once issued, a participant ID number cannot be re-assigned to any other person.

If a participant re-enrolls, a new participant ID number should be assigned. See the Re-Enrollment discussion in this section for further details.

In order to maintain participants' confidentiality, do NOT use participants' names in any communications with the DCC, either written or oral. Provide only participant ID numbers and initials.

The Participant Assignment Log (P9_LOG) is a confidential document because it ties a participant ID number to a name. This document is required when it is necessary to verify a participant's actual treatment assignment, either during or after the study. For this reason, this log should be stored in a secure location and retained indefinitely at the performance site following the close of the study.

Visit 1
Assign participant ID number (P9_LOG)

Immediately following assignment of the participant's ID number on the ALfA Participant Assignment Log (P9_LOG), the protocol enrollment module should be accessed to formally enroll the participant in the ALfA database. Close attention should be paid when entering the participant's information to ensure that the correct ID is entered. If a

participant is enrolled mistakenly under an incorrect participant ID, the DCC should be contacted immediately for assistance in correcting the error.

Visit 2
Randomize participant, if eligible (Check box on P9_LOG)

After accessing the randomization module at Visit 2 to randomize the participant, check the box in the ‘Randomized’ column on the ALfA Participant Assignment Log (P9_LOG).

2.29 Participant Identification Card

The ALfA Participant Identification Card (P9_ID) provides a quick reference for the participant to use to monitor his/her asthma. It includes information for determining when a participant may be experiencing an asthma exacerbation. The ID card also contains instructions for treatment of asthma attacks by physicians and emergency department personnel who may not be familiar with the ALfA study. The ID card should be carried by the participant at all times in a wallet or purse that is readily accessible.

Visit 1
Complete and distribute Participant ID Card (P9_ID)

The ALfA Participant Identification (ID) Card (P9_ID) will be provided by the DCC on card stock. Write the participant's name, ALfA participant ID number, and the names and phone numbers of study personnel on the card. The participant may enter the name and number of his/her primary physician, if applicable. All information should be written in dark ink.

Fill in the participant's Baseline PEF, Baseline Rescue Use and High Rescue Inhaler Use values in the spaces provided on the front of the ID card:

- The Baseline PEF can be found in the grey box at the top of the ALfA Baseline PEF and Rescue Use form (P9_BASELINE).
- The Baseline Rescue Use can be found in Q1000 on the ALfA Baseline PEF and Rescue Use form (P9_BASELINE).
- High Rescue Inhaler Use value is calculated by adding 8 to the Baseline Rescue Use value (Q1000 on P9_BASELINE). This value should be written in the space provided next to "High Rescue Inhaler Use ".

On the back of the ID card, fill in the participant's 70% Baseline PEF value in the space provided:

- 70% Baseline PEF is calculated by multiplying Baseline PEF (in grey box at top of P9_BASELINE) by 0.70.

Review the contents of the ID card with the participant and explain the use of the card. Stress to the participant that the Atrovent® (green RESCUE1) inhaler is the first-line treatment for asthma symptoms, and how to take it for worsening symptoms. If the participant's symptoms have not improved after an hour of Atrovent® treatment as outlined on the "If Your Asthma Gets Worse" handout (P9_ASWORSE), he/she should begin treatment with Ventolin® (blue RESCUE2) and contact performance site. If the participant's symptoms have not improved after an hour of Ventolin® treatment, he/she should contact performance site, personal physician or go to the emergency department for care. Review when and where emergency care should be sought. Remind the

participant that he/she should seek care from study personnel, if possible. However, participants should never delay seeking care if study personnel cannot be reached.

Treatment procedures have been developed with the utmost regard for participant safety. Instruct the participant to contact study personnel if he/she receives emergency treatment outside the study. Document medications, procedures, and other treatments the participant received.

As indicated on the ID card and the "If Your Asthma Gets Worse" handout, the participant should contact study coordinator if he/she has:

- reached his/her High Rescue Inhaler Use value (green RESCUE1 and blue RESCUE2 combined) on each of past 2 days, or
- taken 16 or more RESCUE puffs (green RESCUE1 and blue RESCUE2 combined) in past 24 hours, or
- experienced symptoms that are not satisfactorily relieved after 60 minutes of Atrovent® (green RESCUE1) use.

The first two bullets are ALfA significant asthma exacerbation criteria. If a participant meets either criterion, he/she will receive treatment with prednisone. The third bullet requires the participant to start treatment with Ventolin® (blue RESCUE2) and may necessitate further treatment if symptoms do not improve after 60 minutes.

Visit 2
Update Participant ID Card (P9_ID)

Collect the participant's ALfA Participant ID Card (P9_ID). Obtain a new ALfA Participant Identification (ID) Card (P9_ID), and do the following:

- Complete the participant's name, ALfA participant ID number, site personnel contact information and primary physician contact information.
- The participant may enter the name and number of his/her primary physician, if applicable. All information should be written in dark ink.

Fill in the participant's new Baseline PEF, Baseline Rescue Use and High Rescue Inhaler Use values in the spaces provided on the front of the ID card. Baseline PEF and Rescue Use can be found on ALfA Baseline PEF and Rescue Use (P9_BASELINE) form completed at Visit 2, and the High Rescue Inhaler Use value can be calculated as described above using the Visit 2 P9_BASELINE form.

On the back of the ID card, fill in the participant's 70% Baseline PEF values in the space provided. This value can be calculated as described above using the Visit 2 P9_BASELINE form.

2.30 Participant Status Report

An ALfA Participant Status Report has been developed to communicate important information from the ALfA database to the performance sites on a participant-specific basis. The report shows, in numeric order of participant ID number, all participants enrolled in the ALfA trial at a specific performance site for whom Visit 1 data have been entered, along with the columns of information defined below.

The Participant Status Report is accessed through the AsthmaNet secure website by clicking on the 'Participant Status Reports' link on the homepage and then choosing ALfA from the protocol list. If a coordinator has access to data from more than one performance site, he/she will need to choose the site for which the report is requested from a dropdown list. If a coordinator has access to data from only one performance site, the report request will be submitted automatically.

The Participant Status Report runs in real-time, accessing the current data in the database each time a request is submitted. Because the report is running a program in the background, it may take several seconds (or minutes as the database grows) for the results to appear.

Pre-Rand Term: Indicates if the participant was terminated from ALfA prior to randomization at Visit 2. Sets to 'Yes' if P9_TERM indicates that the participant terminated prior to randomization.

Randomized: Participant's randomization status. Updates to 'Yes' when the participant is randomized at Visit 2.

Visit 2 Scheduled Capsules: Displays bottle number assigned to a participant at Visit 2 (through randomization module). If a backup bottle is ever assigned, its number will show under the original bottle number dispensed at Visit 2.

Post-Rand Term: Indicates if the participant terminated from ALfA after randomization and before completion of Visit 3. Sets to 'Yes' if participant terminates early (when P9_TERM is entered); sets to 'No' when a randomized participant completes the trial.

Completed Study: Indicates if the participant completed the ALfA trial through Visit 3. Sets to 'Yes' when a participant's P9_TERM form is entered indicating study completion. Sets to 'No' for participants with Post-Rand Term status of 'Yes.'

Current Status: The participant's current study status is summarized in the following categories:

1. Enrolled in run-in (individuals who have Visit 1 data entered, no P9_TERM form, and have not yet been randomized at Visit 2)
2. Run-In term (individuals who have a P9_TERM form indicating termination prior to randomization)
3. Randomized and currently active (randomized at Visit 2 and no P9_TERM form entered)
4. Post-randomization drop-out (randomized and P9_TERM is entered with Q1000=0)
5. Completed ALfA (completed study through Visit 3; P9_TERM Q1000=1)

The bottom of the Participant Status Report gives a frequency table for the ‘current status’ variable for all participants at a given performance site.

2.31 Phone Contact

Post-Randomization Phone Contacts – Visits 2A and 2B (Weeks 4 and 6)
The ALfA protocol designates 8 weeks between Visits 2 and 3. Because of the lack of clinic contact during these periods of the study, and the medication being taken, formal phone contacts are scheduled between these visits. Phone contacts allow the coordinator to address the participant's concerns regarding the study medications, his/her asthma control and to schedule an extra clinic visit, if needed. These phone contacts also afford the coordinator an opportunity to ensure that the participant is carrying out his/her home procedures correctly, including taking study medications as instructed and completing the Asthma Monitoring Log.

Phone contacts should be scheduled at Weeks 4 and 6 (2 and 4 weeks following randomization). Ideal contact dates and windows are listed on the Visit Scheduler Report run at Visit 2. If multiple attempts are made to contact the participant within the windows specified on the report and no contact is made, the coordinator should continue to try to get in touch with the participant until his/her next scheduled visit. Document all contact attempts on the ALfA Contact Form (P9_CONTACT).

Refer to Section 4 of this MOP for more details on how to complete this form.

Phone Contact day before Visit 2
Participants should be contacted the day before Visit 2 to remind him/her to take his/her last dose of Flovent® that evening prior to 1 AM (between 5 PM and 1 AM). Flovent® should not be taken the morning of Visit 2. However, if Flovent® is taken after 1 AM the day of the visit, the visit will not need to be rescheduled. Participants should also be reminded of the other withholds prior to the visit as listed on their Visit Preparation (P9_VISPRP) handout, and to bring study materials to visit.

Post-Randomization Contacts – between Visits 2 and 3 (Weeks 3, 5, 7, 8, 9)
Contacts should be made weekly during the post-randomization period to encourage adherence to the daily dosing schedule. Contact can be made by phone, email or text, according to the participant's preferred method of contact. Preferred method of contact for these weekly contacts (excluding the phone contacts 2 and 4 weeks after Visit 2) should be established when completing the Adult Contact Information (CONTACT_ADULT) form at Visit 1, and the preferred method of contact highlighted on the CONTACT_ADULT form. Note that participant must be contacted by phone at Weeks 2 (Visit 2A) and 4 (Visit 2B).

During Weeks 7, 8 and 9, in addition to encouraging adherence, ask participants to report if they have experienced a respiratory infection. If a participant has experienced a respiratory infection, be sure to follow up with the participant at subsequent contacts in order to pinpoint resolution of symptoms and to schedule Visit 3 with the necessary minimum 4 week delay. See Visit Window discussion in this section for important limitations when postponing Visit 3 due to respiratory infection.

Phone Contact day before Visit 3

Participants should be contacted the day before Visit 3 to remind him/her to take his/her last dose of Advair® that evening prior to 1 AM. Advair® should not be taken the morning of Visit 3. If Advair® dose is not taken the evening before the visit (between 5 PM and 1 AM), the visit will need to be rescheduled. If Advair® dose is taken after 1 AM the day of the visit, the visit will need to be rescheduled. Participants should also be reminded of the other withholds prior to the visit as listed on their Visit Preparation (P9_VISPRP) handout and to bring study materials to visit, and asked if they have experienced a respiratory infection. If a participant has experienced a respiratory infection, be sure to follow up with the participant in order to pinpoint resolution of symptoms and to schedule Visit 3 with the necessary minimum 4 week delay. See Visit Window discussion in this section for important limitations when postponing Visit 3 due to respiratory infection.

All of these contacts are included on the Visit Scheduler Report run at Visit 2. Document successful contact attempts on the ALfA Weekly Contact Log (P9_CONTACT_LOG).

2.32 Physical Exams

See Section 3 of the AsthmaNet General Manual of Operations for information regarding the physical exam clinical procedures. ALfA-specific procedures follow.

Adult physical exams are documented on administrative forms that are not entered into the study database. Comprehensive exams are documented on the Adult Long Physical Exam form (LEXAM_ADULT). Brief exams, documented on the Adult Short Physical Exam form (SEXAM_ADULT), will not be performed in ALfA. The LEXAM_ADULT form should be completed at Visit 1 and stored in the participant's study folder at the performance site. This form is subject to audit during an AsthmaNet site visit.

The long physical exam includes measures of resting blood pressure, pulse rate, body temperature, and pulmonary auscultation, as well as documentation of the presence/absence of oral candidiasis and physical findings. A licensed medical practitioner (LMP) must complete the physical findings and pulmonary auscultation portions of the long exam. A LMP is defined as a physician (MD/DO), physician assistant (PA), or nurse practitioner; a registered nurse does not qualify as a LMP. If a non-physician LMP completes the required long exam at Visit 1, the participant still must have interaction with a physician during the visit. The individual performing the long exam should be certified in the ALfA protocol unless he/she is filling in temporarily for personnel who usually conduct these exams. Individuals who will provide these exams on a regular basis should possess ALfA certification (physician or coordinator exam).

In addition to regular physical exams, additional physical measurements including height and weight, and waist, hip and neck circumference, are taken for adults during each study. These measurements are documented on the Adult Body Measurements form (BODYMEAS_ADULT) and entered into the AsthmaNet database. Body measurements can be made by study coordinators, registered nurses, physician assistants, and other individuals who are appropriately trained in these procedures and certified in the ALfA protocol.

Note that height will be measured at all visits for adult participants who are between the ages of 18 and 21, until the participant reaches age 21. For visits where no BODYMEAS_ADULT form is completed, the height measurement will be recorded on the ALfA Pulmonary Procedure Checklist (P9_PULMONARYCHK). Height updates are required for adults in this age range because they may still be growing and height impacts predicted lung function estimates.

Visit 1
Perform Adult Long Physical Exam (LEXAM_ADULT)

A long physical exam is required at Visit 1 in order to ensure that it is safe and appropriate for each participant to enroll in the ALfA study. For the ALfA trial,

participants must have interaction with a physician at Visit 1 even if the physician is not performing the long exam.

The LMP conducting the long physical exam should sign, date and note the time in the gray box on the LEXAM_ADULT form as source documentation.

Visit 1
Complete Adult Body Measurements form (BODYMEAS_ADULT)

Follow the instructions on the form for making the various measurements. Body mass index (BMI) should be calculated and written in the gray box under Q1010. This value is not entered into the study database but it should be available for reference during the trial.

Note that height is captured on the BODYMEAS_ADULT form for adults at Visit 1. Individuals 18-20 years of age will have their heights updated at every visit until the point when they turn 21. Updated heights are recorded on the ALfA Pulmonary Procedure Checklist (P9_PULMONARYCHK) at Visits 2 and 3 for these individuals.

2.33 Pregnancy Test

At all ALfA visits, urine samples will be obtained from female participants of child-bearing potential for assessment of pregnancy by the presence of the beta subunit of human chorionic gonadotropin (HCG). Testing will be performed at the performance site during the participant's visit using the HCG combo stick test approved by each institution. The results of the pregnancy test should be recorded on the Urine Pregnancy Test (PREG_TEST) form and the participant should initial and date the source documentation box to acknowledge the results. If a participant is found to be pregnant at any point during the ALfA study, she must be terminated from study participation immediately. See additional instructions below.

Visits 1, 1Continuation Visit, 2, 3
Complete Urine Pregnancy Test form (PREG_TEST) form for all female participants; administer urine pregnancy test, if necessary

At all ALfA visits, the PREG_TEST form is required for <u>all</u> female participants, regardless of their child-bearing potential. A urine pregnancy test must be administered if the participant is deemed to be of child-bearing potential.

At all ALfA visits, if the participant is potentially able to bear children by the information supplied on the PREG_TEST form, the pregnancy test must be performed and results reported to the participant and to the DCC.

Participants who are post-menopausal (defined as at least one year since last menses) or have undergone a hysterectomy or tubal ligation do not need to be tested. This information is documented on the PREG_TEST form.

Note that a history of infertility does not constitute a valid reason to skip the pregnancy test at a visit, nor does a participant’s insistence that she does not have heterosexual intercourse.

Note that individuals who are transgendered or are transitioning to the opposite gender should be tested for pregnancy in accordance with their biological sex. Biologically female participants who are of child-bearing potential must use birth control and provide urine for pregnancy tests as required by the protocol.

After performing a urine pregnancy test, the participant should be shown the results and asked to initial and date the source documentation box at the bottom of the form as verification that the information on the form is correct and acknowledged by her.

Source documentation should be completed even if a pregnancy test was not performed at the visit.

Visit 1, 1 Continuation Visit, 2, 3
If a participant is considered able to bear children, results of the pregnancy test must be known before she proceeds with the diluent stage of the methacholine challenge at all visits. Pregnant women should not perform methacholine challenges. In addition, pregnant or nursing participants (who plan to continue to nurse) are ineligible for the ALfA protocol.

Pregnancies Discovered during Study Participation
If a woman is found to be pregnant at any time during the study, either through a pregnancy test performed at a study visit or through another means, she is ineligible for continued participation. Pregnant women should be seen at the performance site and terminated from further study participation immediately. Participants who become pregnant during the study should have an ALfA Termination of Study Participation (P9_TERM) form submitted to the DCC as soon as possible recording pregnancy as the primary reason for study termination (Q1040 should be answered 'Yes' and Q1190 should be answered 'a'). Pregnancy should not be reported as an adverse event or as a serious adverse event in the ALfA database.

Disclosure of Blinded Study Treatment after study completion
(for those with IRB approval of ALfA Protocol Version 2.2)
Women of child-bearing potential will be informed of their blinded study treatment when they complete the study. This is being done so participants who took placebo do not need to continue birth control or avoid breastfeeding for the next 6 months.

For women of child-bearing potential only, check the box indented under Alendronate on the ALfA Prescription Order form to indicate the participant is a woman of child-bearing potential. This serves to notify the pharmacist that you need an unblinding envelope for that participant's assigned bottle number. A sealed envelope will then accompany the bottle dispensed by the pharmacist at Visit 2. The participant's ID number will be written on the envelope and the envelope should be stored in a safe, secure location until the participant completes the ALfA study. At the end of Visit 3, the envelope should be given to the participant along with the letter explaining the envelope. A letter template can be found on the AsthmaNet website under Protocols: ALfA: MOP, and can be customized for your site. The letter explains that the participant should open the envelope only after leaving the clinic. The letter also asks that the participant not inform the study doctor, study personnel or study participants of the treatment she received.

2.34 Randomization

Visit 2
Randomize participant, if eligible (Check box on P9_LOG)
Log/dispense capsule vial (P9_DRG_SCH_CAPS)
Confirm capsule vial dispensation (P9_MED)

ALfA is a parallel arm trial during which a participant is randomized at Visit 2 to receive one of the following regimens for 8 weeks:

- alendronate capsule

OR

- placebo capsule.

Dosing is one capsule in the morning.

Target sample size is 76 randomized participants Network-wide.

Randomization balances treatment sequence assignments within the nine partnerships (i.e., Boston, Chicago, Denver, Madison, Pittsburgh, St. Louis, San Francisco, Tucson/Durham, Winston-Salem/Emory), not within a given performance site.

At the end of Visit 2, if the participant meets all of the eligibility requirements to date and documented on ALfA Eligibility Checklist 4 (P9_ELIG4), he/she is eligible to be randomized. The study coordinator should access the ALfA Randomization Module on the secure AsthmaNet website and enter the participant's ALfA ID number, and the performance site at which the participant is being randomized. At this point, the system is assigning the participant to the regimen that he/she will receive for the rest of the study. A capsule vial from the designated performance site that matches the person's randomized arm will be assigned.

After the participant is randomized successfully, the 'randomized' box should be checked on the ALfA Participant Assignment Log (P9_LOG). The assigned capsule vial should be logged on the Participant-Specific Drug Accountability Log for Post-Randomization Scheduled Capsule Vials (P9_DRG_SCH_CAPS). Information on the participant's assigned bottle number at Visit 2 will also be included on the ALfA Participant Status Report.

In order to validate the assigned capsule vial number through the ALfA database, the ALfA Scheduled Medications form (P9_MED) should be completed any time a vial number is generated through the randomization module to be dispensed to a participant. Remove the label from the assigned vial and attach it to Q1000 on the P9_MED form. This form will be data entered as part of the Visit 2 packet.

It should be noted that participants can be randomized in the ALfA randomization module at Visit 2 only if all of the following criteria are met:

1) The participant's ALfA ID number is enrolled in the ALfA protocol.
2) The participant's Visit 1 packet, including Visit 1 eligibility data, has been entered at the performance site (only first entry required). The Visit 1 eligibility forms (P9_ELIG1, P9_ELIG2 and P9_ELIG3) must indicate that the participant is eligible (P9_ELIG1 Q1150=1 and P9_ELIG2 Q1330=1 and P9_ELIG3 Q1060=1).
3) No ALfA Termination of Study Participation (P9_TERM) form has been entered for the participant.

See Section 3 of this manual for details on accessing and interacting with the ALfA Randomization Module.

Note that regimen assignments in the ALfA study are double-blind. That is, neither the participant, nor performance site personnel, will be aware of the contents of the participant's blinded capsules from Visit 2 through 3. The majority of DCC personnel are also blinded to the treatment assignments while the study is ongoing.

Backup Capsules
If a participant loses his/her blinded capsule vial between Visits 2 and 3, then he/she will require the assignment of a new (backup) capsule vial. To generate a new vial number, the study coordinator should access the ALfA Randomization Module on the secure AsthmaNet website and enter the participant's ALfA ID number, and the performance site at which he/she is being seen for the visit. The randomization module will recognize that the participant has already had capsules assigned at Visit 2 and will provide a warning message giving the coordinator a chance to cancel out of the module if a mistake has been made. If the coordinator chooses to proceed, the module will provide the backup vial number

The assigned vial number should be recorded on the Participant-Specific Drug Accountability Log for Post-Randomization Scheduled Capsule Vials (P9_DRG_SCH_CAPS). Information on the participant's assigned vial backup number(s) at Visit 2 will also be included on the ALfA Participant Status Report. An ALfA Scheduled Medications single form (P9_MED) should be completed and data entered any time a backup vial is dispensed to a study participant. Affix label from the capsule vial to the P9_MED form.

Backup randomization procedures
In the rare event that the ALfA Randomization Module is unavailable during any visit when it is required, clinic personnel must contact the DCC for assistance. During week days (Monday through Friday) between 8 AM and 5 PM ET, calls should be made to the AsthmaNet main line at 717-531-3663. The ALfA scientific coordinator, project coordinator, or one of the data management staff will be able to assist with backup randomization procedures. After regular work hours, calls should be made to the

AsthmaNet main line and the option for after-hours emergency contact for ALfA should be chosen. The ALfA scientific coordinator will answer and facilitate backup randomization procedures.

It is extremely important that blinded capsule vials are assigned using the ALfA Randomization Module. Randomly choosing an available vial at Visit 2 and assigning it to a participant in lieu of the randomization module is inappropriate, as it may not contain the participant's assigned treatment regimen. If an incorrect vial is dispensed to a participant, a protocol violation will be assigned.

2.35 Recruitment

ALfA visits will commence on January 7, 2015. Nine clinical center partnerships composed of 11 participating performance sites will recruit for ALfA.

A recruitment period of 10 months has been established for ALfA, with the final randomization visit occurring by November 11, 2015 in order to complete the trial by January 6, 2015.

The gender and minority status of individuals screened and enrolled at Visit 1 and individuals randomized in ALfA will be summarized by clinical center partnership and, within each partnership, by performance site on the ALfA accrual report. This report will be available on the secure AsthmaNet website in the Reports: Accrual: ALfA folder shortly after recruitment begins. Partnerships should strive to screen at least 50% female participants and 33% minority participants over the recruitment period.

Target sample sizes for each partnership are based on the number of participants who are successfully entered into the run-in, and subsequently randomized in the ALfA trial. Each of the nine clinical center partnerships is expected to randomize 8-9 adult participants for a Network total of 76 participants. With a recruitment period of 10 months, that is equivalent to about 1 randomized participant per partnership per month.

Approximate ALfA Timelines

January 7, 2015:	First participant screened (Visit 1)
January 21, 2015:	First participant randomized (Visit 2)
October 28, 2015:	Final screening visit (Visit 1)
November 11, 2015:	Final randomization visit (Visit 2)
January 6, 2016:	Final participant visit (Visit 3)

2.36 Re-Enrollment

Participants who do not successfully complete the ALfA run-in for reasons that may be overcome with time or additional training (e.g., use of excluded medications, respiratory infection, borderline compliance, etc.) may be suitable candidates to re-enroll in ALfA for a second attempt. Randomized participants who drop out early may not re-enroll in the trial.

Visit 1 Failures
Participants who do not qualify for the ALfA study at Visit 1 for reasons that may be overcome with time (e.g., insufficient medication washout, respiratory infection in past 4 weeks, use of excluded medications, etc.) may be invited to repeat Visit 1 at a later date. Data collected during the unsuccessful Visit 1 should not be entered into the AsthmaNet database and forms should not be forwarded to the DCC regardless of whether the participant will re-enroll in the study or not. The Visit 1 packets should be stored at the performance site in a section of folders denoted as 'ALfA Visit 1 Failures.'

Participants who return to the performance site for a second attempt at Visit 1 should repeat all of the Visit 1 procedures as listed on ALfA Visit Procedure Checklist (P9_VISIT1). A new visit packet should be completed.

When re-enrollment occurs, the following procedures apply:

- The participant must be given a new ALfA participant ID number from the Participant Assignment Log (P9_LOG). See the Participant Assignment Log discussion in this section and Section 4 for further details. This new ID will need to be linked to the participant through the protocol enrollment process before data can be entered into the ALfA database. For information on the protocol enrollment process, refer to Section 7 of the AsthmaNet General Manual of Operations.
- The participant must read and sign new copies of the current IRB-approved ALfA and BioLINCC informed consent documents. The documents signed at the initial enrollment should reside in the folder created for the participant's original ID number. The newly signed consent documents should reside in the participant's current study folder. Informed consent documents should not be updated with a new signature and date, as this practice violates institutional procedures at some of the performance sites.
- The Adult Participant Contact Information (CONTACT_ADULT) form should be reviewed and updated by the participant, as necessary. A photocopy should be made and stored with the participant's original Visit 1 packet. The original form with updates should be stored in his/her new study folder.
- A new Visit 1 packet with the participant's new ID number should be completed and submitted to the DCC if the participant is now eligible. Do not attempt to update previously-completed forms with the participant's new information. A new

study folder should be created to house the participant's forms under his/her new study ID number.

After a Successful Visit 1 and Prior to Randomization at Visit 2
Once a participant is deemed eligible at Visit 1, he/she is formally enrolled in the ALfA study. The data collection forms from Visit 1 should be entered into the study database and forwarded to the DCC.

If a participant withdraws consent or is deemed ineligible during the run-in, then he/she must be formally terminated from the study. An ALfA Termination of Study Participation (P9_TERM) form should be completed and entered into the database. All of the forms completed at the termination visit should be entered into the AsthmaNet database and sent to the DCC. If any blood samples were collected during the run-in, they should be sent to the appropriate labs according to the instructions in this section of the MOP.

Such participants should not be invited to re-enroll unless their reason for withdrawing or being withdrawn was such that there is a very high probability that re-entry will result in randomization and full participation in ALfA. For example, if extenuating circumstances caused a participant to miss visits or not be able to carry out daily procedures for a period of time, then he/she may be a good candidate to re-enroll after life settles down and adequate time can be devoted to study procedures. A participant terminated at Visit 2 because he/she was not able to perform methacholine challenge or provide 40 mL blood should not be re-enrolled.

Participants who are good candidates for re-enrollment must re-enter the ALfA study starting anew at Visit 1.

The following guidelines apply when the participant is re-enrolled:

- The participant must be given a new ALfA participant ID number from the Participant Assignment Log (P9_LOG). See the Participant Assignment Log discussion in this section and Section 4 for further details. This new ID will need to be linked to the participant through the protocol enrollment process before data can be entered into the ALfA database. For information on the protocol enrollment process, refer to Section 7 of the AsthmaNet General Manual of Operations.
- The participant must read and sign new copies of the current IRB-approved ALfA and BioLINCC informed consent/assent documents. The documents signed at the initial enrollment should reside in the folder created for the participant's original ID number. The newly signed consent documents should reside in the participant's current study folder. Informed consent documents should not be updated with a new signature and date, as this practice violates institutional procedures at some of the performance sites.
- The Adult Participant Contact Information (CONTACT_ADULT) form should be reviewed and updated by the participant. A photocopy should be made and

stored with the participant's original Visit 1 packet. The original form with updates should be stored in his/her new study folder.

- The Adult Asthma and Allergy History (ASTHMA_HX_ADULT) form, Home Environment Questionnaire (HEQ), Prior Conditions for Adult Participants (PRIOR_COND_ADULT) form, Prior Conditions for All Participants (PRIOR_COND_ALL) form, and Prior Asthma/Allergy Treatment (PRIOR_TRT) forms from the participant's prior enrollment may be reused. These forms must be reviewed with the participant in detail and updated appropriately. The participant's new ID number and visit date must be written on the forms. A photocopy should be made and stored with the Visit 1 packet from the participant's original enrollment. The form with the handwritten updates should be stored in his/her new study folder and sent to the DCC.
- All study procedures must be carried out anew, with the exceptions noted above, beginning with Visit 1. Complete and submit new data collection forms for the participant using his/her new participant ID number and current dates.
- The blood draw for genetic analysis is optional in the ALfA study; however, participants who gave a sample prior to their study termination should be asked to provide a new blood sample upon re-enrollment, if the participant is amenable. New blood samples must be obtained at the applicable visits.

After Randomization in ALfA

Participants who withdraw consent after they have been randomized in the ALfA study at Visit 2 are NOT eligible to re-enroll. Each participant can contribute only one set of data for the analysis.

2.37 Registration

At or prior to first visit
Register participant in AsthmaNet Registry

Before a participant can be enrolled in the ALfA trial, he/she must be present in the AsthmaNet Registry with 'complete' status. ACRN and CARE Network participants who completed Registry forms in those networks already will have 'complete' status in the AsthmaNet Registry. Any participants from the earlier networks who have 'incomplete' status, or individuals who are new to the NHLBI asthma networks, will need to undergo the full AsthmaNet registration process.

All individuals who are enrolled in the ALfA trial will need to have AsthmaNet label sheets and reports printed and stored with the AsthmaNet Registry documentation.

Complete Registry procedures are documented in Section 9 of the AsthmaNet General Manual of Operations.

Visit 1
Complete Registry Checklist (REG_CHK)

Follow the procedures for completing the Registry Checklist (REG_CHK) as outlined in Section 9 of the AsthmaNet General Manual of Operations. Attach one of the participant's "Registry Checklist" labels to the gray box at the bottom of the checklist before submitting the form to the DCC. This label contains the participant's AsthmaNet master ID number and serves as a reference during the protocol enrollment process.

Include REG_CHK behind the Visit Procedure Checklist (P9_VISIT1) in the participant's Visit 1 packet.

2.38 Saliva Sampling

Visit 2
Have participant rinse mouth with water ***(in preparation for saliva collection; must be done at least 10 minutes prior to collection)***
Complete Saliva Collection Checklist (P9_SALIVACHK)
Perform pre-Advair® saliva collection
Enter pre-Advair® saliva sample into Biological Sample Tracking module
(One hour after administering Advair®) Perform post-Advair® saliva collection
Enter post-Advair® saliva sample into Biological Sample Tracking module

General Instructions
Before a participant can proceed with saliva collection, he/she must meet all of the requirements specified on the ALfA Saliva Collection Checklist (P9_SALIVACHK) with "gray box" exclusions. If a participant has failed to meet any one of the requirements within the specified washout period prior to a visit, he/she generally may not proceed with collection at the visit. In this case, the visit should be rescheduled within the visit window for appropriate washouts to be met. If the participant has almost met a required washout period, contact the ALfA scientific coordinator at the DCC. An exception may be allowed. Given that most of the windows are within an hour of collection, it is also possible to wait the time needed to meet the washout before proceeding.

If an exception is granted through the DCC, Q1060 on P9_SALIVACHK should be marked 'Yes' even though one or more of the 'gray boxes' corresponding to washouts is completed. This conflict will result in a data error which the coordinator should mark unresolvable; the exception should be explained in a comment.

Supplies
The following supplies are required to collect the saliva samples at Visits 2 and 3:

Item	Vendor	Catalog #	# Per Collection
Cryovial	Provided by DCC		2
Saliva Collection Aid	Provided by DCC		2
2" Cryostorage box w/81-cell divider	Provided by DCC		1
White Laser Cryo-Babies barcode label (Cryo-Babies 1.28"x0.50")	Diversified Biotech	LCRY-1700	2

Preparing for collection

Participant will rinse mouth with water 10 minutes prior to collection. Wait AT LEAST 10 MINUTES after rinsing to avoid sample dilution before collecting saliva. Participant should not eat, drink or chew gum between first and second collection.

Collection

1. Remove cap from cryovial.
2. Remove Saliva Collection Aid from packaging and place securely into cryovial.
3. Instruct subject to allow saliva to pool into mouth. Some find it helpful to imagine eating their favorite food.
4. With head tilted forward, subject should drool through the Saliva Collection Aid to collect saliva in the cryovial.
5. Record collection start time on Saliva Collection Checklist (P9_SALIVACHK).
6. Repeat until a sufficient sample (1 mL) is collected.
7. Samples visibly contaminated with blood should be DISCARDED and RECOLLECTED (repeat steps 1-5 with a new cryovial and Saliva Collection Aid). Collect 1 mL of saliva. Do not exceed 1 mL so as to reserve air space in the cryovial to accommodate the expansion of saliva during freezing.
8. Replace cap onto cryovial. Be sure the cap is secure.
9. Label final, properly obtained saliva specimen with an ALfA SAA barcode label (Cryo-Tag) generated through the Biological Sample Tracking (BST) module of the AsthmaNet database management system:
   - For pre-Advair® saliva samples, barcode label will includes a pre-printed 9-digit barcode number, starting with “9SAA1”. The sample type associated with this tube in the BST module is “ALfA Pre-Advair saliva”.
   - For post-Advair® saliva samples, barcode label will includes a pre-printed 9-digit barcode number, starting with “9SAA2”. The sample type associated with this tube in the BST module is “ALfA Post-Advair saliva”.

   Labels should be placed vertically on tube so that the barcode can be scanned. The length of the label is 1.28” so the label should be placed as high as possible (just under the screw top). The ALfA saliva labels appear as follows:

10. Access the BST module and scan the barcode to insert record for sample. Input the participant ID information to link the barcodes to the correct ALfA participant and visit number. <u>It is imperative that all samples be scanned the day of</u>

collection so that they are associated with the correct participant ID and visit number, and are available for inclusion in the next shipment. For details on accessing and interacting with the BST Module in the AsthmaNet Database Application, see the AsthmaNet Computing and Networking Environment details in Section 7 of the AsthmaNet General Manual of Operations.

11. Record participant ID number, visit number, collection date and time, sample barcode number and sample volume on P9_SALIVA_SAMP_LOG.
12. Store the samples in one cryostorage box. These samples will be shipped to Salimetrics at the end of the study.
13. Store the saliva samples at -80° C until the shipment day. Freezer must not be self-defrosting. Record the date/time the sample is placed in the freezer and the current freezer temperature on P9_SALIVA_SAMP_LOG.

    NOTE: If not possible to immediately place in -80°C freezer, immediately place cryovial temporarily in 4°C (39 °F) refrigerator FOR A MAXIMUM OF 2 HOURS, then move to -80°C.

Sample Shipping

Saliva samples for randomized participants will be shipped priority overnight to Salimetrics at the end of the study. Shipment date will be coordinated with the DCC. Samples can be shipped Monday, Tuesday or Wednesday ONLY.

Note: Saliva samples for unsuccessful Visit 2's will be shipped priority overnight to Rush University at the end of the study.

Preparing Saliva Samples for Shipment to Salimetrics

To create a shipment, scan the barcodes for all samples available to ship into the AsthmaNet BST system. Include a shipment comment detailing the contents of the shipment (i.e., human saliva). Each shipment (from each site) will receive a unique shipment ID number. A shipment inventory will be generated that contains: date of shipment, shipping tracking number, site of origination, shipment ID, and an inventory detailing all the tubes in the shipment with their barcode numbers and participant information (Participant ID number, initials, visit number and collection date). For saliva samples ONLY, do NOT print the shipment inventory for inclusion in the shipment.

Once the shipment is confirmed in the BST module, an e-mail will automatically be sent to the DCC. The e-mail will include an export file from the database that shows the information from the shipment inventory. A summary of the shipment will be included in the body of the e-mail message.

The DCC will incorporate the samples into a sample roster template provided by Salimetrics. The DCC will email the sample roster to Salimetrics (testing1@salimetrics.com) and the site. The site must print this sample roster and include it with the shipment.

Packaging Saliva Samples for Shipment to Salimetrics

Before packaging available samples for shipment, they must be scanned into the BST system and sample roster received and printed as described above. After the samples have been scanned and the shipment has been confirmed by the performance site, the samples should be packaged for shipment. The following materials are required:

Item	Vendor	Catalog #	# Per Shipment
Bioshipper	Provided by DCC		1
FisherBrand Biohazard Polyethlene Transport Bag 8x8" (or larger)	Fisher Scientific	01-800-07 (8x8")	1
FisherBrand Biohazard Wipes, standard absorbency (4x4")	Fisher Scientific	06-670-35	2
Packaging tape	Staples	380107	
Exempt Human Specimen labels Therapak 2.5"x2"	Fisher Scientific	22-130-070	1
Therapak Dry Ice Label – UN1845 (5.5x5.5")	Fisher Scientific	221-30-065	1
Rubber bands			
Newspaper			
Sample Roster			1
Testing Services Order Form	Completed order form shipped with Salimetrics supplies		1

The instructions for assembling shipments below meet the minimum federal standards. Each performance site's institution may have additional guidelines. Sites should follow their institutional guidelines as long as they are in compliance with the minimum federal standards.

Assembly instructions:

1. Place one sheet of absorbent material on top of the samples inside the cryostorage box to be shipped. Close the box and secure lid with rubberbands.
2. Place the closed box into the plastic transport bag.
3. Place a second sheet of the absorbent material in the plastic transport bag.
4. Seal the transport bag tightly.

5. Fill the bottom of the Styrofoam shipper with approximately 1 inch of cubed/chipped dry ice, covered with crumpled newspaper or other absorbent material on bottom of shipper.
6. Place the plastic transport bag containing the secured cryostorage box on top of the dry ice layer.
7. Fill any remaining space on sides and between cryoboxes with additional packing material (crumpled newspaper, packing peanuts, etc.) to prevent shifting.
8. Cover the transport bag with more crushed dry ice so that the box of tubes cannot be seen. Continue to fill the Styrofoam box with dry ice but do NOT overstuff the box.
9. Place a copy of the Testing Services Order Form and sample roster (in a plastic Ziploc bag) on top of the dry ice and close the Styrofoam mailer tightly.
10. Seal the Styrofoam mailer with tape. Do not completely seal the box so that it is airtight. Carbon dioxide from the dry ice must be allowed to escape.
11. Place the Styrofoam mailer inside a cardboard mailing sleeve (the specified shipper in the table above comes with a cardboard mailer).
12. Attach one “Exempt Human Specimen” sticker and one “DRY ICE – UN 1845” label to the cardboard carton. Mark the appropriate weight of dry ice in kg on the label.

13. Address the shipment to:

    Salimetrics SalivaLab
    Attn: Michelle Wells
    5962 La Place Court, Suite 275
    Carlsbad, CA 92008
    Phone: (760) 448-5397

14. Specify FedEx priority overnight shipment. No other form of shipping is acceptable.

2.39 Satisfaction Questionnaire

Participant's termination visit
Give participant AsthmaNet Satisfaction Questionnaire (SATQX) with preaddressed, postage-paid envelope

The AsthmaNet Satisfaction Questionnaire (SATQX) is a quality control tool that was developed by the AsthmaNet Quality Control Committee (QCC) to solicit feedback from participants when they leave AsthmaNet studies. The questionnaire is anonymous in that no participant or master ID number or other identifying information is recorded on the form. In addition, the participant returns the form directly to the DCC in a pre-addressed, postage-paid envelope. Performance site staff does not review the data on the form, does not see individual results, and does not data enter the information on the form. Data entry takes place solely at the DCC.

The Satisfaction Questionnaire (SATQX) is posted on the secure AsthmaNet website in the visit packet corresponding to the final study visit for a given protocol. For ALfA, it is present in the Visit 3 packet. In addition, the questionnaire is also posted appended to the single ALfA Termination of Study Participation (P9_TERM) form for use with participants who terminate from the study before Visit 3.

Postage-paid envelopes that are pre-addressed to the DCC may be obtained from the DCC as supplies are needed. At least one month's lead time should be allowed for shipment and receipt of the envelopes to ensure an adequate supply at the performance site at all times.

Only ALfA participants who successfully complete Visit 1 should be given a questionnaire at the time of their study termination.

Process: The following steps should be carried out to ensure that all participants who terminate from the ALfA trial have an equal opportunity to provide feedback on their experiences.

1. Distribute a copy of the questionnaire to any participant who successfully completes Visit 1 , then terminates, whether he/she completes the study or terminates early (for his/her own reasons, due to ineligibility, or for other reasons).
2. Download the questionnaire from the secure AsthmaNet website along with the ALfA Termination of Study Participation (P9_TERM) form. Questionnaires in visit packets will have protocol number and site ID pre-completed in the key fields area of the form. Questionnaires appended to single P9_TERM forms will have only protocol number completed. Coordinators should complete the site number before distributing the questionnaire to a participant.
3. Print the questionnaire double-sided and staple the pages together to avoid loss.

4. Complete the participant's final study status in the gray box at the top of page 1 of the form. Individuals who terminate during the pre-randomization phases of the study should be coded as 'Run-in termination.'
5. Give the questionnaire to the participant at the conclusion of his/her final study visit. The participant should be given a pre-addressed, postage-paid envelope with the questionnaire.
6. Instruct the participant to complete the questionnaire, put it in the envelope, seal it, and place it in the US postal mail. If a participant elects to complete the questionnaire at the performance site, clinic personnel should not interact with him/her as the form is completed. In this case, it is preferable for the participant to drop the questionnaire in any postal box himself, but he/she may seal the questionnaire in the envelope and ask clinic personnel to mail it. The questionnaire should not be sent to the DCC with form shipments.

Note: If an individual is not present at the time he/she withdraws from the study, and he/she is unwilling to come to the performance site for a final visit, the Satisfaction Questionnaire should be mailed to his/her home address. Include instructions for completion with the questionnaire and prepaid envelope.

Personnel at performance sites who have access to the secure AsthmaNet website can generate Satisfaction Questionnaire Reports for sites to which they have been granted access in the database. The reports summarize the site's response rate, by study and overall, as well as the frequencies of responses to each of the questions on the questionnaire, by study and overall.

The DCC will provide periodic reports of the data from the questionnaires for all sites for the QCC. Response rates will be compared across the performance sites and clinical center partnerships to ensure that all sites are participating fully in the survey process.

2.40 Significant Asthma Exacerbation

Visits 1 – 3

Definition

Asthma exacerbations are more severe episodes of acute worsening, defined by the development of an increase in symptoms of cough, phlegm/mucus, chest tightness, wheezing or shortness of breath in association with one or more of the following:

1. Use of ≥ 8 rescue puffs/day over baseline use for a period of 48 hours

   Baseline rescue use should be taken from Q1000 on the participant Baseline PEF and Rescue Use Values form (P9_BASELINE). Baseline for the run-in phase will be defined as the average daily use of home rescue inhaler reported during the week prior to Visit 1. Baseline for the randomized phase will be defined as the average daily use of ipratropium rescue inhaler reported during the week prior to Visit 2 as recorded on the participant's ALfA Asthma Monitoring Log (P9_ASTHMA_LOG). The participant's combined rescue use from RESCUE1 and RESCUE2 will be used when evaluating this criterion. The participant should be instructed to monitor his/her rescue use on his/her ALfA Asthma Monitoring Log (P9_ASTHMA_LOG) to pick up the occurrence of this criterion.

2. Use of ≥ 16 rescue puffs in a 24 hour period

   The participant should be instructed to monitor his/her rescue use on his/her ALfA Asthma Monitoring Log (P9_ASTHMA_LOG) to pick up the occurrence of this criterion. The participant's combined rescue use from RESCUE1 and RESCUE2 will be used when evaluating this criterion.

3. FEV_1 <80% of the <u>baseline</u> pre-bronchodilator FEV_1

   The baseline pre-bronchodilator FEV_1 value (in liters) should be taken from Q1030 on the participant's Visit 1 Spirometry Testing (SPIRO) form. This value is used for assessing asthma exacerbation criteria for the remainder of the participant's study participation. If a participant with FEV_1 < 80% requires a continuation visit to establish his or her study eligibility with methacholine challenge, the baseline pre-bronchodilator FEV_1 should be taken from the SPIRO form completed at the initial visit.

   A participant will meet this criterion if he/she experiences pre-bronchodilator FEV_1 value that is <80% of the Visit 1 FEV_1 value.

4. Pre-bronchodilator FEV_1 <45% of predicted

   The pre-bronchodilator FEV_1 % predicted value should be taken from Q1040 on the participant's Spirometry Testing (SPIRO) form for a given visit. A participant will meet this criterion if he/she experiences pre-bronchodilator FEV_1 value that is <45% of predicted.

5. Receives systemic corticosteroids for an exacerbation

Rescue Algorithm
Participants who experience worsening of asthma will be managed according to the following rescue algorithms. Rescue algorithms are based on recommendations from the NAEPP Guidelines for Diagnosis and Management of Asthma and prior ACRN trials.

Atrovent® (green RESCUE1), Ventolin® (blue RESCUE2) and oral prednisone are the principal medications for rescue management. At Visit 1, participants will be given green RESCUE1, blue RESCUE2 and a course of prednisone to keep at home for rescue use. Participants will be instructed in their use for home management. Oral prednisone will be used if increased ipratropium therapy followed by increased albuterol therapy does not resolve the asthma exacerbation, and only as directed by a study physician. For severe acute episodes of asthma, treatment will be administered according to the best medical judgment of the treating physician.

Once an asthma exacerbation has occurred, the participant should contact the study coordinator and/or be evaluated at the performance site or the nearest medical emergency facility as quickly as possible.

Home Care
Asthma exacerbations will be recognized by the criteria described above. Participants will be educated to recognize exacerbations as early as possible to facilitate prompt treatment and to lessen morbidity.

Participants who recognize an exacerbation will be instructed to use Atrovent® by MDI, 4 puffs initially, then 2 puffs every 20 minutes up to 60 minutes, if needed, and then every 4 hours, or less, if needed to reduce symptoms. If symptoms are not improved after the first 60 minutes of Atrovent® therapy, the participant will be instructed to use the same treatment scheme substituting Ventolin® MDI for the Atrovent® and to contact the study coordinator. Participant will take 4 puffs Ventolin® initially, then 2 puffs every 20 minutes up to 60 minutes, if needed, and then every 4 hours, or less, if needed to reduce symptoms. If symptoms are not improved after the first 60 minutes of Ventolin® therapy, the participant should contact the study coordinator, investigator, their primary physician, or seek care in the emergency department.

Failure of albuterol may necessitate the use of oral steroids (see below).

Physician's Office or Emergency Room Treatment
Participants will be assessed by history, physical examination, and by physiological monitoring including spirometry or PEF. If the participant's PEF and/or FEV_1 are less than 25% of predicted or if the participant 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 department, participants should initially be given albuterol by nebulization (0.5 cc of 0.5% solution) every 20 minutes over the first 60-90 minutes.

If the PEF increases to ≥70% of predicted (or of best known PEF value) after the first 60-90 minutes, the participant 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 PEF remains <70% of baseline, nebulized albuterol should be continued as often as every hour and further treatment with oral or parenteral corticosteroids should be considered (e.g. prednisone 40 mg orally; methylprednisolone 40 mg IV bolus). Monitoring of PEF or spirometry should continue every hour. Within 4 hours of treatment, a decision should be made regarding participant disposition.

If PEF increases to ≥70% of baseline within 4 hours, the participant can be discharged to continue treatment at home. Home treatment should include a 5-day course of prednisone (see below).

If PEF remains >40% but <70%, an individualized decision should be made to hospitalize the participant for more aggressive therapy or to continue therapy at home with a course of prednisone.

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

Prednisone Treatment

In this protocol, prednisone will be used when acute exacerbations cannot be controlled by increased ipratropium or albuterol therapy alone. The dose of prednisone used during an acute exacerbation shall consist of 40 mg as a single oral dose every day for 5 days. The decision to initiate or to continue a course of prednisone beyond 5 days is left to the discretion of the physician. Rescue prednisone will be given to the participant at Visit 1 to keep at home to be used only on the advice of study staff.

Study Participation Following a Significant Asthma Exacerbation during Run-In

Participants experiencing an asthma exacerbation during the run-in phase of the study are ineligible to continue in the trial. A significant asthma exacerbation has occurred if the participant experienced or is experiencing an increase in asthma symptoms (cough, phlegm/mucus, chest tightness, wheezing or shortness of breath) in association with increased rescue use (≥ 8 rescue puffs/day over baseline use for a period of 48 hours or ≥ 16 rescue puffs in a 24 hour period), low Visit 2 FEV_1 (FEV_1 < 80% of baseline (Visit 1) or < 45% predicted), or treatment with systemic corticosteroids for his/her exacerbation. If a participant is found to have had an asthma exacerbation during the run-in or at the time of Visit 2, he/she is ineligible. An ALfA Termination of Study Participation (P9_TERM) form should be completed, as well as a Significant Asthma

Exacerbation (P9_SIGEX) form. The P9_TERM and P9_SIGEX forms will be submitted as single forms with the visit number 1. See the discussion of Withdrawal Due to Exacerbation in the Withdrawal section for further details.

Study Participation Following a Significant Asthma Exacerbation after Randomization

Protocol Version 2.1 Only: If a participant has taken a systemic corticosteroid within the 4 weeks prior to Visit 3 or meets exacerbation criteria at the time of Visit 3, he/she should not proceed with methacholine challenge testing at the visit and the visit should be rescheduled so that the final visit occurs at least 4 weeks after the participant completes his/her prednisone burst.

Protocol Version 2.2 Only: If a participant has taken a systemic corticosteroid after the first 4 weeks of blinded treatment or meets exacerbation criteria at the time of Visit 3, their participation should be extended so that the final visit occurs at least 4 weeks after the participant completes his/her prednisone burst.

- If this extension goes beyond 12 weeks* of alendronate, their participation in the study will be terminated.
- If a participant requires a second burst of prednisone for an asthma exacerbation after randomization, his or her participation in the study will be terminated.
- If a participant meets exacerbation criteria at the time of Visit 3, and the 5-day course of prednisone followed by 4 week washout requires more than 12 weeks* on alendronate, his/her participation in the study should be terminated.

* The standard extended visit window applies, so Visit 3 date can be up to 12 weeks +5 days after randomization (Visit 2).

See Backup Dispensation in the Study Medications discussion in this section for additional details.

Documentation

Once the significant asthma exacerbation has been confirmed, in addition to the forms completed above, the following forms should also be completed:

- Clinical Adverse Events (AECLIN)

  All significant asthma exacerbations should be documented on AECLIN using ICD-9 code 493.92.

  The start date recorded should correspond to the date exacerbation criteria were confirmed. For example, if a participant's rescue use is ≥ 8 puffs/day over baseline use for a period of 48 hours, the date corresponding to the second day of rescue use should be recorded as the exacerbation date. If multiple criteria for asthma exacerbation are met, record the earliest date any of the applicable criteria were met.

- Concomitant Medications for Asthma/Allergy and Adverse Events (CMED)

  Oral prednisone and any non-study medications used to treat the exacerbation event should be recorded on the CMED form. Examples include parenteral corticosteroids and nebulized beta-agonist administered in a physician's office or other care facility.

  Medications used for treatment of exacerbations and listed on the CMED form should be linked to the exacerbation adverse event recorded on the AECLIN form.

- ALfA Significant Asthma Exacerbation (P9_SIGEX)

  P9_SIGEX must be completed any time the participant meets the criteria for an asthma exacerbation.

  The significant asthma exacerbation date is recorded in Q1060. It should correspond to the date exacerbation criteria were confirmed for the current event. If multiple criteria for exacerbation are met, record the earliest date any of the applicable criteria were met. Guidelines by exacerbation criterion follow:

  - Use of ≥ 8 rescue puffs/day over baseline use for a period of 48 hours.

    If the participant used ≥ 8 puffs over baseline rescue use per 24 hours for a period of 48 hours, the significant asthma exacerbation date should be the second day the participant uses ≥ 8 puffs/day over baseline. Rescue use represents combined puffs from RESCUE1 and RESCUE2.

  - Use of ≥ 16 rescue puffs in a 24 hour period

    If the participant used ≥ 16 rescue puffs per 24 hours, the significant asthma exacerbation date should be the day the participant uses ≥ 16 puffs per 24 hours. Rescue use represents combined puffs from RESCUE1 and RESCUE2.

  - FEV_1 <80% of the baseline pre-bronchodilator FEV_1

    If the participant experiences an FEV_1 <80% of the baseline (Visit 1) pre-bronchodilator FEV_1, the significant asthma exacerbation date should be the date of the FEV_1 measurement.

  - FEV_1 <45% of predicted

    If the participant experiences an FEV_1 <45% predicted, the significant asthma exacerbation date should be the date of the FEV_1 measurement.

  - Receives systemic corticosteroids for an exacerbation

    If the participant received systemic corticosteroids for an exacerbation, the significant exacerbation date should be the date the participant started the systemic corticosteroids.

If a participant meets asthma exacerbation criteria during the run-in (between Visit 1 and Visit 2), P9_SIGEX should be completed as a single form and data entered. Visit number should be 1. In this situation, the participant is ineligible and must be terminated from further study participation.

If a participant meets asthma exacerbation criteria during the post-randomization phase of the trial, P9_SIGEX should be completed as a single form and data entered. Visit number should be 2.

- Serious Adverse Event Reporting Form (SERIOUS)

  If a participant is hospitalized due to a significant asthma exacerbation event, or the event is considered to be life-threatening or meets other criteria in the definition of a serious adverse event (SAE), a SERIOUS form should be completed. SERIOUS forms should be submitted to the DCC within 72 hours of the notification of a SAE. See the Adverse Events discussion in this section for further details.

2.41 Spirometry

Spirometry procedures are carried out at all ALfA visits. Pulmonary function data are very important, as they confirm the participant's eligibility for the study, and provide data for assessment of significant asthma exacerbation criteria. Spirometry is also used in methacholine challenge to determine PC_{20}, ALfA's primary outcome.

General Instructions
AsthmaNet utilizes the MedGraphics spirometry system. The Spirometry Manual of Operations is located in Appendix 1 of the AsthmaNet General Manual of Operations.

Individuals performing spirometry must be AsthmaNet-certified in pulmonary function testing or, at a minimum, observed and supervised by an AsthmaNet-certified technician. If an uncertified individual is performing any spirometry procedures at a visit, a supervisor ID must be recorded on the applicable form(s), including the Spirometry Testing (SPIRO) form, Post-Albuterol (4 puffs) Spirometry Testing (PALB4_SPIRO) form, and Post-Advair Spirometry Testing (PADVAIR_SPIRO) form, as applicable at a given visit.

A participant's prior spirometry results should not be reviewed with him/her at the current visit. Knowledge of past test results can influence current expectations and bias the resulting data.

In general, before a participant can proceed with spirometry testing, he/she must meet all of the medication and substance holds specified on the ALfA Pulmonary Procedure Checklist (P9_PULMONARYCHK) with "gray box" exclusions. If a participant has taken any of the listed substances within the specified washout period prior to a visit, he/she generally may not proceed with spirometry testing at the visit. In this case, the visit should be rescheduled within the visit window for appropriate washouts to be met. If the participant has almost met a required washout period, contact the ALfA scientific coordinator at the DCC. An exception may be allowed.

If an exception is granted through the DCC, Q1180 on P9_PULMONARYCHK should be marked 'Yes' even though one or more of the 'gray boxes' corresponding to drug or substance washouts is completed. This conflict will result in a data error which the coordinator should mark unresolvable; the exception should be explained in Q6000.

eNO Testing in Relation to Spirometry
Since spirometry testing is known to affect eNO measures, eNO testing must be performed after the ALfA Pulmonary Procedure Checklist is completed and prior to spirometry at Visits 2 and 3. See the Exhaled Nitric Oxide discussion in this section for further details.

Demographics
Care must be taken to enter the participant's identification (i.e., participant ID number with leading '0', initials, etc.) and demographic information into the spirometry software correctly. A technician ID must also be included for each test that is performed.

Height
Participants who are less than 21 years old (i.e., participants who have not yet had their 21st birthday) will have their height measured and recorded at each visit until they turn 21. Height is recorded on different data forms depending on the participant's age.

Heights for individuals who are age 18-20 will be recorded in Q1190 on the P9_PULMONARYCHK form at all spirometry visits with the exception of Visit 1, at which height is recorded for all adult participants on the Adult Body Measurements form (BODYMEAS_ADULT). Participants who are at least 21 years old at enrollment will have their height measured and entered in the spirometry system only once during the study at Visit 1. Once a participant is over the age of 21, he/she should not be re-measured. Height values should be updated in the spirometry system each time they are measured.

Race/Ethnicity
The participant's spirometry race/ethnicity designation should be retrieved from his/her AsthmaNet Registry Report. The participant's spirometry race/ethnicity category corresponds to the primary racial designation that he/she supplied in Q1150 on the Registry (REGISTRY) form. Individuals who specified 'American Indian/Alaskan Native' or 'Other' will use Caucasian predicted lung function equations. Always use the spirometry race/ethnicity designation listed on the participant's Registry report in the MedGraphics software. Race/ethnicity has a large influence on a participant's predicted lung function values.

Gender
Individuals who are transgendered or transitioning to the opposite gender should have their biological sex entered into the AsthmaNet Registry (under 'gender'). Biological sex should be entered into the MedGraphics software for purposes of calculating predicted lung function values.

Visit 1
Complete Pulmonary Procedure Checklist (P9_PULMONARYCHK)
Perform Spirometry Testing (SPIRO)

Baseline spirometry at Visit 1 is used to determine study eligibility. Results are recorded on the Spirometry Testing (SPIRO) form and are referenced on ALfA Eligibility Checklist 3 (P9_ELIG3).

Protocol Version 2.1 Only: Based on the participants Visit 1 FEV_1 % predicted, the participant will either perform reversibility (FEV_1 < 80% predicted) or methacholine challenge (FEV_1 ≥ 80% predicted) at Visit 1 to qualify for ALfA.

Protocol Version 2.2 Only: A participant with $FEV_1 \geq 80\%$ predicted at Visit 1 must perform methacholine challenge to qualify for ALfA, whereas a participant with $FEV_1 < 80\%$ predicted at Visit 1 may qualify by demonstrating reversibility or $PC_{20} \leq 8$ mg/mL. For participants with $FEV_1 < 80\%$ predicted at Visit 1, the decision to perform reversibility or methacholine challenge at the initial Visit 1 will be made at the discretion of the study site's principle investigator, in consultation with their study coordinator. If a participant attempts methacholine challenge at the initial Visit 1and has $PC_{20} > 8$ mg/mL, he/she **cannot** qualify with reversibility. If a participant with $FEV_1 < 80\%$ predicted attempts reversibility at the initial Visit 1and does not reverse ≥ 12%, Visit 1 will be stopped following post-albuterol spirometry testing and a continuation visit will be scheduled. Continuation visit should try to be scheduled to take place within 24-48 hours, and within 7 days maximum. Visit will start with completing Urine Pregnancy Test (PREG_TEST) form for all female participants, administering urine pregnancy test if necessary, followed by the completion of the Pulmonary Procedure Checklist (P9_PULMONARYCHK), and spirometry testing (SPIRO). Spirometry at the Continuation Visit 1 will be used to qualify the participant for methacholine challenge.

Visit 1 (for participants with $FEV_1 < 80\%$ predicted)

Administer 4 puffs of albuterol, wait 10-15 minutes, and perform post-bronchodilator testing

Perform Post-Albuterol (4 puffs) Spirometry Testing form (PALB4_SPIRO)

Protocol Version 2.1 Only: At Visit 1, post-albuterol spirometry is used to determine study eligibility for participants with $FEV_1 < 80\%$ predicted. To qualify the participant, he/she should perform baseline spirometry, then be given 4 puffs of albuterol and be allowed to rest for **10-15 minutes**. After the 10-15 minute wait, spirometry should be repeated and the results recorded on the PALB4_SPIRO form. All participants with $FEV_1 < 80\%$ predicted should complete the pre/post spirometry sessions at Visit 1, regardless of whether or not they provided source documentation from a previous pre/post spirometry test to support their eligibility. If participant reverses ≥ 12%, he/she is eligible to continue with Visit 1.

Protocol Version 2.2 Only: At the initial Visit 1, post-albuterol spirometry can be used to determine study eligibility for participants with $FEV_1 < 80\%$ predicted. To qualify the participant, he/she should perform baseline spirometry, then be given 4 puffs of albuterol and be allowed to rest for 10-15 minutes. After the 10-15 minute wait, spirometry should be repeated and the results recorded on the PALB4_SPIRO form. Source documentation from a previous pre/post spirometry test is not allowed. If participant reverses ≥ 12%, he/she is eligible to continue with Visit 1. If participant does not reverse ≥ 12%, a continuation visit is required. At the continuation visit, new ALfA Pulmonary Procedure Checklist (P9_PULMONARYCHK) and Spirometry Testing (SPIRO) forms must be completed with the current date (using the same visit number as the prior visit). The P9_PULMONARYCHK and SPIRO forms from the continuation visit should be entered into the ALfA database as single forms (in addition to the regular

packet forms). A single METHA_RPT should be printed to document the values on the single SPIRO and METHA forms. All data collected on the P9_PULMONARYCHK and SPIRO forms for both parts of the visit should be entered into the study database.

Note: When the participant returns for the continuation visit, the first procedure performed is the pregnancy test followed by spirometry. Do not have the participant redo previously completed questionnaires at this visit; the questionnaires completed on the original visit date will be submitted with the visit packet.

For pre/post spirometry, participants should dose from albuterol (Ventolin®) inhalers taken from bulk supply at Visit 1. This should be logged on ALfA Drug Dispensing Log: Ventolin® (RESCUE2) Inhaler (P9_DRG_RESC2). Actuators should be sterilized between participants, allowing for multiple participant use.

Albuterol puffs taken as part of the pre/post spirometry testing procedure should not be included in the RESCUE puffs the participant records on his/her Asthma Monitoring Log (P9_ASTHMA_LOG) the evening after the visit.

Visits 2, 3

Complete Pulmonary Procedure Checklist (P9_PULMONARYCHK)
Perform Spirometry Testing (SPIRO)
Administer 2 puffs of Advair® 115/21, and wait 1 hour before proceeding with post-Advair® saliva collection, spirometry, and Methacholine Challenge Testing
(One hour after administering Advair®) Perform post-Advair® Spirometry Testing (PADVAIR_SPIRO)

Spirometry at Visits 2 and 3 is used to determine eligibility for methacholine challenge. Results are recorded on the Spirometry Testing (SPIRO) form. If FEV_1 is 50% to 54.9% of predicted and ≥ 1.0 L, participant is eligible to proceed with Methacholine Challenge. Check 'Yes' to METHACHK_ADULT Q1060, and provide comment in Q6000 that FEV_1 ≥ 50% of predicted and ≥ 1.0 L.

At Visits 2 and 3, following eNO testing, participants should perform baseline spirometry, then be given 2 puffs Advair® HFA 115/21 and allowed to rest for 1 hour. After the 1 hour wait, post-Advair® saliva collection, spirometry, and Methacholine Challenge will be performed (in that order). Spirometry results are recorded on the Post-Advair® Spirometry Testing (PADVAIR_SPIRO) form.

After administering Advair®, one hour must pass before post-Advair® saliva collection, spirometry, and Methacholine Challenge can be performed. Post-Advair® saliva collection should occur no sooner than 50 minutes after Advair® administration, and Methacholine Challenge no later than 70 minutes after Advair® administration.

2.42 Study Handout Folder

At the end of the first visit, ALfA study participants will be given several handouts related to study procedures. Each handout contributes to increased adherence in areas such as dosing with study medications, treatment of worsening asthma, and monitoring for significant asthma exacerbation. Participants should be given an AsthmaNet folder to use for carrying and storing the handouts. The participant should store the study folder in a convenient location, as it will serve as a reference throughout his/her ALfA participation. The folder should be brought to each study visit so that clinical personnel can review and/or update handouts, as necessary.

ALfA Study Handout Folder Contents

- ALfA Daily Activities (Visits 1-2) (P9_DAILYACT1) – distributed at Visit 1
- ALfA Daily Activities (Visits 2-3) (P9_DAILYACT2) – distributed at Visit 2

"Daily Activities" handouts contain simple summaries of the activities the participant should carry out each day during the ALfA study, including how to take study medication(s). They also list the participant's High Rescue Use reference value, and direct the participant to the "If Your Asthma Gets Worse" handout (P9_ASWORSE) if asthma gets worse. The Daily Activities handouts also prompt the participant to contact clinical personnel when they may be experiencing increased symptoms or an asthma exacerbation. See the Daily Activities Handout discussion in this section for further details.

- If Your Asthma Gets Worse (P9_ASWORSE)
- Participant Identification Card (P9_ID)

These references facilitate the identification and treatment of an asthma exacerbation according to the protocol, both by the participant and by healthcare providers. Both handouts are introduced at Visit 1 and reviewed at subsequent visits. Both contain criteria, reference values and treatment for asthma exacerbations, as well as instructions for emergency treatment. The P9_ID card should be carried in the participant's wallet so that it is available at all times. See the Participant Identification Card and Significant Asthma Exacerbation discussions in this section for further details.

- How to Use Your Metered Dose Inhaler (HTMDI)

This is a standard handout that provides information on MDI closed-mouth inhalation technique and instructions for cleaning the inhaler. It is introduced at Visit 1 along with the Atrovent® (green RESCUE1) and Ventolin® (blue RESCUE2) inhalers.

- How to Use Your Diskus® (HTDISKUS)

This is a standard handout that provides information on how to use the Diskus®. It is introduced at Visit 1 along with Diskus® Inhalation Technique training.

- ALfA Visit Preparation Checklist (P9_VISPRP)

This handout is a tool for improving the participant's adherence with respect to keeping scheduled visits and preparing for the visits appropriately. The handout should be photocopied/printed two-sided. The P9_VISPRP handout includes a list of medications and activities that should be avoided for a specified amount of time prior to each visit. The reverse side of handout includes a checklist that itemizes the medications and other study materials the participant should bring to each visit. The participant should check off each item as he/she prepares for each visit to ensure that nothing is overlooked. If clinical personnel notice that the participant is not using the checklist, and he/she is not always prepared for visits, use of the checklist should be reinforced. This handout is introduced at Visit 1 and should be referenced throughout the participant's study participation.

- ALfA Visit Scheduler Report

A copy of the current Visit Scheduler Report should be included in the participant's handout folder for personal reference. Old versions should be discarded to avoid confusion. See the Visit Schedule discussion in this section for further details.

- ALfA How to Use Your MEMS®6 Cap (P9_MEMSINST)

This handout instructs participants on proper use of the MEMS®6 event monitoring cap that is placed on their study capsule vial. The MEMS®6 device improves adherence with dosing with daily capsules by monitoring each time the cap is removed from the capsule vial.

2.43 Study Medications

A general description of the ALfA study medications is given below. See the ALfA Pharmacy MOP for procedures related to drug preparation, logging, and dispensation for clinic staff and pharmacists.

Rescue medications

Visit 1
Log/dispense ipratropium (green RESCUE1) inhaler (P9_DRG_RESC1)
Log/dispense albuterol (blue RESCUE2) inhaler (P9_DRG_RESC2)
Log/dispense rescue prednisone (P9_DRG_PRED)

Visit 2
Log/dispense ipratropium (green RESCUE1) inhaler, if needed (P9_DRG_RESC1)
Log/dispense albuterol (blue RESCUE2) inhaler, if needed (P9_DRG_RESC2)

Visit 3
Collect/log ipratropium (green RESCUE1) inhaler (P9_DRG_RESC1)
Collect/log albuterol (blue RESCUE2) inhaler (P9_DRG_RESC2)

During the ALfA trial, all participants will receive the following study rescue medications:

- Ipratropium rescue drug (Atrovent®), a short-acting muscarinic antagonist to be used as-needed throughout the ALfA trial to treat asthma symptoms as described on the "If Your Asthma Gets Worse" handout (P9_ASWORSE). Ipratropium is the first-line treatment for asthma symptoms.

  Atrovent ® rescue drug will be labeled with a green label supplied by the DCC that includes the ALfA study name and space for the visit number, current date, lot, expiration date, participant initials and participant ID number. Atrovent ® will be dispensed from bulk supplies provided by the DCC at the participant's first visit, and as needed thereafter. This inhaler will be referred to as the green RESCUE1 inhaler (color of label) on participant handouts.

- Albuterol rescue drug (Ventolin®), an inhaled beta-agonist to be used as second-line treatment as described on the "If Your Asthma Gets Worse" handout (P9_ASWORSE) should Atrovent® fail to relieve asthma symptoms. <u>It should only be used when Atrovent® fails to relieve asthma symptoms after an hour of use as specified on P9_ASWORSE.</u> This is necessary because albuterol may interfere with the biochemical assays.

  Ventolin® rescue drug will be labeled with a blue label supplied by the DCC that includes the ALfA study name and space for the visit number, current date, lot, expiration date, participant initials and participant ID number. Ventolin® will be dispensed from bulk supplies provided by the DCC at the participant's first visit,

and as needed thereafter. This inhaler will be referred to as the blue RESCUE2 inhaler (color of label) on participant handouts.

- Rescue prednisone, an oral corticosteroid to be used only in emergencies and under the direction of clinical staff to treat an asthma exacerbation.

  Rescue prednisone will be obtained through the individual performance site pharmacies and dispensed to each participant at their first visit. Packaging should be childproof. See the Significant Asthma Exacerbation discussion in this section for further details regarding use of prednisone for treatment of a significant asthma exacerbation.

Run-in medication

Visit 1
Log/dispense run-in Flovent® Diskus® (P9_DRG_RUNIN)

Visit 2
Collect/log run-in Flovent® Diskus® (P9_DRG_RUNIN)

To qualify for randomization, participants will be evaluated on open-label Flovent® Diskus® 250 mcg. Pharmacy will label the Diskus® device. One Flovent® Diskus® will be dispensed at Visit 1. Flovent® dosing is 1 puff BID.

If Visit 2 is delayed beyond 30 days due to respiratory infection, participant will need a second Flovent® Diskus® device.

Double-blind treatment period medications

Visit 2
Log/dispense capsule vial (P9_DRG_SCH_CAPS)
Log/dispense Advair® Diskus® (P9_DRG_ADVAIR)

Visit 3
Collect/log capsule vial (P9_DRG_SCH_CAPS)
Collect/log Advair® Diskus® (P9_DRG_ADVAIR)

Study capsules are double-blind. Participants, performance site staff, and DCC personnel involved in day to day decision-making for the study and statisticians on the project will not know what treatment regimen (alendronate or placebo) the participant is receiving at any point during the study.

The bottle has a 2-panel label. The perforated label containing the name of the medication and the bottle number should be affixed to the P9_MED form.

Advair® Diskus® 250/50 mcg is open-label. Advair® dosing is 1 puff BID.

Two Advair® Diskus® devices will be dispensed at Visit 2. The Diskus® devices will be dispensed by the pharmacy in their foil pouches in Ziploc bags which include circular labels to be affixed to each Diskus®. All information, with exception to "Do Not Use After" date, should be completed by the pharmacist. Since the Diskus® device expires 30 days after the date the pouch is opened, the coordinator will only open Diskus® "1 of 2" from its foil pouch at Visit 2. The study coordinator will complete the "Do Not Use After" date on the Diskus® label, remove Diskus® "1 of 2" from its pouch, and adhere the circular labels to the front and back of the Diskus® at the time of the visit. The participant will need to complete the "Do Not Use After" date on the Diskus® label, open Diskus® "2 of 2" from its pouch, and adhere the circular labels to the Diskus® at home. Instructions for carrying out these steps are outlined on the back of the Daily Activities handout (P9_DAILYACT2). The coordinator should clearly note the date when the participant should begin using his/her second Diskus® on the P9_DAILYACT2 handout and on his/her Asthma Monitoring Log (P9_ASTHMA_LOG).

If the participant cannot return for Visit 3 until the last day of the extended upper window, he/she will require 3 Diskus devices. In this case, the coordinator will only open Diskus® "1 of 3" from its foil pouch at Visit 2. The study coordinator will complete the "Do Not Use After" date on the Diskus® label, remove Diskus® "1 of 3" from its pouch, and adhere the circular labels to the front and back of the Diskus® at the time of the visit. The participant will need to complete the "Do Not Use After" date on the Diskus® label, open Diskus® "2 of 3" from its pouch, and adhere the circular labels to the Diskus® at home. The participant will need to do the same for Diskus® "3 of 3". The coordinator should clearly note the date when the participant should begin using his/her second and third Diskus® on the P9_DAILYACT2 handout and on his/her Asthma Monitoring Log (P9_ASTHMA_LOG).

Taking study capsule

The Daily Activities handout (P9_DAILYACT2) provides detailed instructions to the participant on how to take their study capsule. It is very important that the participant take it as instructed:

- Participant must take capsule just after getting out of bed in the morning, before eating or drinking anything.
- Capsule should be swallowed whole with a full glass (6 to 8 ounces) of plain water. Capsule should not be taken with tea, coffee, juice, milk, mineral water, sparkling water, or anything other than plain water.
- For at least 30 minutes (1 hour recommended) after taking capsule, the participant should not eat, drink or take medication, and should not lie down.
    - The optimal time to fast after swallowing the capsule is 1 hour, not 30 minutes.
    - NPO for 30 minutes is for safety, NPO for 1 hour is for the sake of study design (increased absorption).
- Participant should sit upright or stand upright until at least 30 minutes have passed since taking capsule and participant has eaten first food of the day.

Backup Dispensation

A backup alendronate bottle may need to be dispensed if the initial bottle is lost, or Visit 3 needs to be delayed due to prednisone use or respiratory infection. For the latter, participant should be able to meet 4-week washout, and complete Visit 3 on or before the stop date, to receive a backup bottle. Stop date is Visit 2 date + 12 weeks + 5 days. The ALfA Prescription Order Form will include a field for Visit 2/Randomization date and STOP DATE; coordinator must complete these fields when ordering a backup alendronate bottle due to a delayed Visit 3. Pharmacy is instructed to add the stop date to the outpatient pharmacy label (and/or a separate label) on these backup bottles. This stop date should be reinforced by the study coordinator in the contacts leading up to the stop date. If participant is unable to meet 4-week washout and complete Visit 3 on or before the stop date, his/her participation in the study should be terminated. Coordinator should instruct the participant to stop taking study capsules and have participant return as soon as possible to return study materials.

If backup bottle is shipped to participant, he/she should be instructed to switch caps on the bottles (i.e., move the MEMS cap from the old bottle to the new bottle, and the regular cap from the new bottle to the old bottle).

If Visit 3 is delayed beyond 60 days and participant was only dispensed two Advair Diskus devices at Visit 2, participant will need a third Diskus device.

2.44 Study Treatment Questionnaires

Visit 3
Have participant complete Participant Study Treatment Questionnaire (P9_PARTTXQX)
Complete Coordinator Study Treatment Questionnaire (P9_CTXQX)

The study treatment questionnaires are used to assess how well the masking of the scheduled inhalers was carried out. The Participant Study Treatment Questionnaire (P9_PARTTXQX) was developed to evaluate the blind from the participant's perspective. The Coordinator Study Treatment Questionnaire (P9_CTXQX) was developed to evaluate the blind from the study coordinator's perspective. Each questionnaire is completed at the participant's final study visit (Visit 3). Questions on the forms address the treatment the participant or study coordinator thought the participant received since starting blinded, randomized treatment.

If a participant withdraws from the study following randomization and prior to Visit 3, both questionnaires should be completed at the time of the participant's final contact with the performance site. If the final contact is by phone, the coordinator may administer the P9_PARTTXQX questionnaire over the phone. In this case, no source documentation will be recorded. Single forms will need to be completed if the participant withdraws in the middle of the treatment period.

Participant Study Treatment Questionnaire
Near the conclusion of Visit 3, the participant should complete a Participant Study Treatment Questionnaire (P9_PARTTXQX). This form is designed to determine how well the blind on the ALfA capsules performed with respect to the participant's perceptions of the study medications he/she received during that treatment period. Clinical personnel should explain the purpose of the questionnaire to the participant and confirm that the participant understands that the form references only the medication taken from his/her blinded capsule vial.

This questionnaire is completed by the participant. It is relatively short and should take no longer than five minutes to complete. Study personnel should not help the participant to answer questions on the form, as such assistance could influence the responses and result in bias. Participants should be asked to answer all questions to the best of their ability; they should not leave any blank. When the form is complete, the participant should initial and date the source documentation box on page 2. Coordinators should check the completed questionnaire to ensure that it has been completed correctly.

Coordinator Study Treatment Questionnaire
Near the end of Visit 3, the study coordinator who was primarily responsible for the participant's ALfA study visits during the randomized treatment period should complete a Coordinator Study Treatment Questionnaire (P9_CTXQX). This form is designed to

determine how well the blind on the ALfA capsules performed with respect to the coordinator's perceptions of the study medication the participant received. The coordinator should complete this form before reviewing the participant's questionnaire (P9_PARTTXQX) and before entering the participant's form into the study database. The participant should not review the coordinator's form, and the coordinator should not discuss his/her perceptions of the study treatment with the participant.

When the P9_CTXQX form is complete, the coordinator should initial and date the source documentation box at the bottom of the page. If the primary study coordinator in charge of the participant's visits during the randomized treatment period is unavailable during the final visit of the treatment period or the participant's early withdrawal visit, the P9_CTXQX form should be completed as soon as possible on his/her return to the performance site, preferably within 1 week of the visit. Only one coordinator should complete the form, and only one form should be submitted per randomized participant.

If a randomized participant is lost to follow-up or withdraws early and is unavailable to complete the P9_PARTTXQX form, the study coordinator still should complete a P9_CTXQX form, as long as the participant had at least one follow-up during the double-blind treatment period. In this case the P9_CTXQX form should be submitted as a single form.

See Section 4 in this manual for further details regarding the completion of the P9_CTXQX and P9_PARTTXQX forms.

2.45 Transfer Participants

Transfer participants are defined as individuals who are enrolled in a trial and successfully complete at least one study visit at one performance site, then transfer to another performance site for a set number of visits or for the remainder of their study participation. General database procedures related to transfer participants are outlined in Section 7.5.2 of the AsthmaNet General Manual of Operations. ALfA-specific considerations follow.

- Participant Assignment Log: Complete the participant ID number and other information on the Participant Assignment Log (P9_LOG) (Not Pre-Filled) version. Maintain this log with the site-specific ALfA log. The participant should retain his/her original ID that was assigned at the originating site.
- Registry Report: Generate a copy of the participant's Registry Report to obtain demographics needed for spirometry reports.
- Randomization: In the ALfA Randomization Module, enter the participant ID and select the location where the randomization is taking place (i.e., 'new' site). If the enrollment site is chosen by mistake, the Randomization Module will return blinded bottle numbers that are physically located at the transfer participant's enrollment site, not the site of the current visit. If this occurs, the DCC should be contacted immediately.
- Study ID Card: A new study ID card should be distributed to the participant (with updated study personnel and primary physician information completed, as necessary).
- Current Dosing Information and Asthma Exacerbation History: The originating site should supply the new site details of the participant's study participation, and a summary of adverse events and any asthma exacerbation events the participant has experienced in the study, along with their dates and any ongoing treatment (i.e., prednisone). The new site may view the data collection forms from the enrollment site within the Participant Data module if appropriate database permissions have been requested/granted.
- Baseline Reference Values: The originating site should provide the new performance site a photocopy of their last Baseline PEF and Rescue Use (P9_BASELINE) form. In addition, the originating site should confirm the participant's baseline (Visit 1) FEV_1 value.
- Physical Measurements: For participants ≥ 21 years old, the new performance site may use the Participant Data module to view the Adult Body Measurements (BODYMEAS_ADULT) form completed at Visit 1. The height and weight recorded on this form should be referenced when entering participant characteristics into the MedGraphics PC.

For participants under age 21, the originating site should supply the new site a copy of the most recent Pulmonary Procedure Checklist (P9_PULMONARYCHK).

- Genetics Blood: If the genetics blood draw was deferred to a later visit and has not yet been completed, the originating site should notify the new site. The new site should confirm the participant's consent for participating in the genetics blood draw based on his/her responses on the local consent documents.
- Visit Schedule: The originating site should supply the new site a copy of the most recently generated Visit Scheduler Report.
- Prednisone Supply: The new site should verify that the participant has a supply of rescue prednisone on hand. If he/she does not, a new supply should be dispensed.

2.46 Visit Schedule

Visits 1, 2
Run ALfA Visit Scheduler
Review planned visit schedule

A visit scheduler program has been included on the AsthmaNet secure website to allow clinical personnel to create a Visit Scheduler Report for a given participant's ALfA study visits. It can be found under Reports: Visit Scheduler Reports: ALfA. The visit scheduler is run at Visits 1 and 2. The visit scheduler creates the participant's visit and phone contact schedule at:

- Visit 1, based on the Visit 1 date, for Visits 2;
- Visit 2, based on the Visit 2 date, for phone calls, contacts and Visit 3.

The visit scheduler at Visit 1 creates the participant's schedule, based on the Visit 1 date, for Visit 2. If a participant with FEV_1 < 80% predicted at Visit 1 performs a Continuation Visit 1 (to perform methacholine challenge for eligibility purposes), date of continuation visit should be used in Visit 1 scheduler.

The Visit 2 scheduler creates the participant's phone contact and contact schedule between Visits 2 and 3, as well as his/her final visit (Visit 3). Phone contacts are performed 2 and 4 weeks following randomization and contacts are performed 1, 3, 5, 6 and 7 weeks following randomization. See Phone Contact discussion in this section for further details.

Instructions for accessing and generating the ALfA Visit Scheduler Reports on the AsthmaNet secure website can be found in Section 3 of this manual.

Copies of the ALfA Visit Scheduler Reports should be included in the participant's study handout folder for personal reference. An additional copy should be placed in the participant's study folder at the performance site. As Visit Scheduler Reports are updated at appropriate visits, be sure to discard outdated copies.

2.47 Visit Windows

The table below summarizes the regular and extended windows allowed around the ideal visit date for each of the ALfA study visits. The run-in is 2 weeks long. The randomized treatment phase is 8 weeks long, with phone contacts 2 and 4 weeks post-randomization.

Visits should be scheduled on the ideal date whenever possible. When this is not possible, the regular windows should be used. The extended windows should be used only to accommodate extenuating circumstances when a visit will otherwise be missed. When extreme scheduling conflicts arise and the extended windows do not provide enough flexibility, the ALfA scientific coordinator at the DCC should be consulted before scheduling the visits to ensure that analysis- and drug-related repercussions of any mistimed visits have been considered.

Note that in addition to the visit windows, the time of day of the visits should also be considered. Because of the circadian variability associated with lung function, all subsequent visits should be scheduled such that baseline spirometry at the visit occurs within +/-3 hours of baseline spirometry at Visit 1. If a participant with FEV_1 < 80% requires a continuation visit to establish his or her study eligibility (by performing methacholine challenge), spirometry completed at the initial Visit 1 is baseline. When scheduling Visit 1, keep in mind that Visits 2 and 3 must occur prior to 11 AM. More specifically, salmeterol-protected methacholine challenge at Visits 2 and 3 should begin prior to 11 AM, meaning visit procedures prior to methacholine challenge should be completed, and the challenge started by 11 AM; any challenge set to start after 12:15 PM must be rescheduled. If a participant cannot be scheduled in the spirometry windows, contact the ALfA scientific coordinator at the DCC to seek an exception.

Regular and Extended Windows for ALfA Study Visits

Visit Number	Study Week	Regular Window (days) Lower	Regular Window (days) Upper	Extended Window (days) Lower	Extended Window (days) Upper
1	0	-	-	-	-
2	2	- 3 days	+ 3 days	- 5 days	+ 5 days
2A PC	4	- 3 days	+ 3 days	- 5 days	+ 5 days
2B PC	6	- 3 days	+ 3 days	- 5 days	+ 5 days
3	10		+ 3 days		+ 5 days

Visit 2 marks the end of the ALfA run-in phase.

Visit 3 marks the end of the randomized treatment period.

Ideal visit dates and regular and extended visit windows have been programmed into the ALfA Visit Scheduler Reports for ease of scheduling participant visits. See the Visit Schedule discussion in this section and Section 3 for further details on these reports.

If a participant routinely fails to keep scheduled visits, he/she should be counseled by the performance site coordinator. If the problem persists, the local investigator should talk with the participant. Participants who have unusual scheduling conflicts or miss/reschedule run-in visits multiple times may not be good prospects for randomization, as all of the ALfA study visits cannot be missed. If counseling by the site coordinator during the run-in phase does not seem to improve the situation, the coordinator should consider terminating the participant from further study participation by filing an ALfA Termination of Study Participation form (P9_TERM).

Postponing Visit 3 due to Prednisone Use or Respiratory Infection
Visit 3 may need to be delayed due to prednisone use or respiratory infection. The maximum treatment length established in the protocol is 12 weeks. The standard extended visit window applies, so Visit 3 date can be up to 12 weeks +5 days after randomization (Visit 2). If participant is unable to meet 4-week washout and complete Visit 3 on or before the stop date, his/her participation in the study should be terminated. Coordinator should instruct the participant to stop taking study capsules after the stop date and have participant return as soon as possible to return study materials.

2.48 Withdrawals

Early Study Withdrawal
Complete ALfA Termination of Study Participation form (P9_TERM)

Participants have the right to withdraw consent for study participation at any time and for any reason. In the case of a serious adverse event, either due to an asthma exacerbation or another medical condition, the study investigator may determine that it is in the best interest of the participant to discontinue participation in the trial.

When a participant is withdrawn from the study or withdraws consent after completing Visit 1 successfully, an ALfA Termination of Study Participation (P9_TERM) form should be completed, entered into the database, and submitted to the DCC as soon as possible. Note that any AsthmaNet investigator at the performance site may approve and sign off on the P9_TERM form.

In addition to the P9_TERM form, participants who are withdrawing or have been withdrawn from ALfA should be asked to complete an AsthmaNet Satisfaction Questionnaire (SATQX). This questionnaire is optional and anonymous in that no participant ID number or other identifying information is recorded on the form. The participant should be given a pre-addressed, postage-paid envelope in which to return the questionnaire directly to the DCC. The Satisfaction Questionnaire is posted on the secure AsthmaNet website appended to the single P9_TERM form and as part of the Visit 3 packet. See the Satisfaction Questionnaire discussion in this section for instructions on the administration of the Satisfaction Questionnaire (SATQX).

The specific termination procedures that should be followed are dependent on when in the trial the participant terminates his/her participation. See below for additional details.

Visit 1 Screen Failures
At any point during Visit 1, a participant may be deemed ineligible or withdraw consent. Information on such participants should be maintained at the performance site in the participant's study folder. Only those participants who pass all of the eligibility criteria at Visit 1 should have data entered into the study database and forms forwarded to the DCC.

If a participant is ineligible for a reason that may change soon, such as a recent respiratory tract infection, he/she may be able to meet eligibility criteria in the near future. If the participant rejoins the study, he/she must be assigned a new study ID number (through the Protocol Enrollment module of the AsthmaNet database application) and repeat Visit 1. See the Re-Enrollment discussion in this section for further details.

Withdrawals during the Run-in Phase (Visits 1-2)

The primary purpose of the run-in phase (Visit 1-2) is to identify an appropriate group of asthmatic participants for randomization in the ALfA trial. This phase gives clinic personnel an opportunity to review eligibility criteria and adherence to study procedures for each participant before he/she is randomized. For the ALfA study, it is extremely important to gauge the participant's ability to maintain high levels of compliance. Participants who cannot accommodate the date/time of the visits, take exclusionary medications, or fail to take study medications correctly and on schedule are non-compliant. These participants should not be randomized at Visit 2, as their lack of adherence can affect the results of the study adversely and may jeopardize their safety if they cannot recognize asthma exacerbation conditions appropriately. The run-in phase is the optimal time to identify and withdraw non-compliant participants.

When a participant is withdrawn from the run-in or withdraws consent prior to randomization at Visit 2, an ALfA Termination of Study Participation (P9_TERM) form should be submitted to the DCC along with any study data that have been collected. In addition to the P9_TERM form, participants who are withdrawn after successfully completing Visit 1 and prior to Visit 2 should also be asked to complete an AsthmaNet Satisfaction Questionnaire (SATQX). The participant's status at the time of termination should be completed by the coordinator at the top of the form as 'Run-in termination.'

Minimum data packet requirements for individuals terminated at Visit 2 include:

- Asthma Monitoring Log (P9_ASTHMA_LOG)
- Compliance Checklist (P9_COMPLY)
- Eligibility Checklist 4 (P9_ELIG4) Termination of Study Participation (P9_TERM)

Early Withdrawals after Randomization

The intention-to-treat principle applies to the ALfA study. Once a participant has been randomized, all efforts must be made to follow the participant and to collect data on his/her progress for the duration of the study. This principle applies even for participants who are discovered to be ineligible (unless the reason for ineligibility presents a safety concern) or who fail to comply with study procedures following randomization. Once a participant leaves the performance site with his/her randomly assigned capsule vial at Visit 2, he/she *must* be followed. Any losses in participant follow-up can lead to bias in the study results. Participant withdrawal during the post-randomization period is permissible only in the following situations:

- Withdrawn Consent (i.e., participant refusal to continue)
- Pregnancy
- Serious Adverse Event or Severe Asthma Exacerbation

  A serious adverse event, either unrelated to asthma or due to a significant asthma exacerbation, may prompt the study investigator to terminate the participant from further study participation because it is in the participant's best interest for safety reasons.
- Loss to Follow-up

Participants who cannot be contacted for an extended period of time qualify as lost to follow-up. Clinic staff should continue to attempt to contact the participant until the time he/she would have completed the trial. At this point, an ALfA Termination of Study Participation (P9_TERM) form should be completed, entered into the database, and sent to the DCC.

Once randomized, participants cannot be terminated from the study solely for non-compliance with attendance at study visits, dosing with study medications, or any other form of non-compliance. Non-compliance may be stated as a secondary reason for participant termination on the P9_TERM form; it may not be used as the primary reason for termination.

Withdrawal between Visits 2 and 3

If a randomized participant withdraws consent by contacting performance site personnel between Visit 2 and 3, he/she should be asked to return to the clinic for a brief termination visit, if possible. The purpose of the visit is to collect study materials, document adverse events and new concomitant medications, and to ensure that the participant has plans for his/her asthma care. At a minimum, the ALfA Termination of Study Participation form (P9_TERM) must be completed. The participant should be asked to complete the ALfA Participant Study Treatment Questionnaire (P9_PARTTXQX). In addition, an ALfA Coordinator Study Treatment Questionnaire (P9_CTXQX) should be completed.

If the participant refuses to return to the performance site for even an abbreviated visit, arrangements must be made to have the participant ship his/her study medications back to the site. Compliance should be estimated as best possible from the returned capsule vial and Diskus® devices and recorded on the ALfA Compliance Checklist (P9_COMPLY). For participants who are unwilling to come to the performance site for an exit visit, the study coordinator may administer the ALfA Participant Study Treatment Questionnaire (P9_PARTTXQX) over the phone, if the participant is agreeable. No source documentation will be available on the form in this case. These forms, including P9_TERM and P9_CTXQX, should be entered as a single form using visit number 2.

The participant should be given or mailed an AsthmaNet Satisfaction Questionnaire (SATQX) with return envelope and instructions for completion.

Withdrawal at Visit 3

If a randomized participant withdraws consent during Visit 3, any data already collected at that visit should be reported on the data collection forms, entered into the database and forwarded to the DCC. An ALfA Termination of Study Participation (P9_TERM) form should be submitted, as well as the Compliance Checklist (P9_COMPLY). The participant should be asked to complete the ALfA Participant Study Treatment Questionnaire (P9_PARTTXQX) and the coordinator should complete the ALfA Coordinator Study Treatment Questionnaire (P9_CTXQX). The participant should be

given an AsthmaNet Satisfaction Questionnaire (SATQX) with pre-addressed, postage-paid envelope to complete and return at his/her leisure.

Withdrawal Due to Exacerbation

Participants who have a significant asthma exacerbation during the run-in phase (pre-randomization) will be terminated from study enrollment and managed as clinically-indicated, with treatment based on clinical standard and initiated by/in accordance with the participant's usual asthma care provider. The participant may be re-screened at Visit 1 for entry into the study at the discretion of the local investigator. Eligibility criteria, such as no asthma exacerbation requiring systemic corticosteroid treatment in past 4 weeks, will need to be met at re-enrollment. See the Re-Enrollment discussion in this section for further details.

Once randomization has occurred at Visit 2, intention-to-treat principles apply. Should a participant receive systemic corticosteroid treatment for an asthma exacerbation after randomization at Visit 2, the study will continue in accordance with the participant's visit schedule. Should asthma exacerbations become too severe following randomization, the principal investigator or site director of the participant's performance site may at any time elect to drop him/her from further study participation for the participant's safety. Study termination procedures will be completed. Any complication resulting from an asthma exacerbation (pneumothorax, pneumomediastinum, etc.) will be recorded as an adverse event in addition to the significant asthma exacerbation event itself.

See the Significant Asthma Exacerbation discussion in this section for details on forms completion and rescue algorithm.

General Note:

After a participant has been terminated from the ALfA trial, no additional data and/or specimens may be collected from the participant with the exception of the AsthmaNet Satisfaction Questionnaire (SATQX) referenced above. If any procedures are performed and/or specimens are collected after the participant's termination date, a protocol violation will be assigned.