Title

Adapting clinical trial design to maintain meaningful outcomes during a multicenter asthma trial in the precision medicine era

Authors

Sorkness, Christine A
King, Tonya S
Dyer, Anne-Marie
et al.

Publication Date

2019-02-01

DOI

10.1016/j.cct.2018.12.012

Peer reviewed

Contemporary Clinical Trials 77 (2019) 98–103

Contents lists available at ScienceDirect

Contemporary Clinical Trials

journal homepage: www.elsevier.com/locate/conclintrial

Adapting clinical trial design to maintain meaningful outcomes during a multicenter asthma trial in the precision medicine era

Christine A. Sorkness[a,*,1], Tonya S. King[b,1], Anne-Marie Dyer[b], Vernon M. Chinchilli[b], David T. Mauger[b], Jerry A. Krishnan[c], Kathryn Blake[d], Mario Castro[e], Ronina Covar[f], Elliot Israel[g], Monica Kraft[h], Jason E. Lang[i], Njira Lugogo[j], Stephen P. Peters[k], Michael E. Wechsler[f], Sally E. Wenzel[l], Stephen C. Lazarus[m], On behalf of the National Heart Lung and Blood Institute's "AsthmaNet"

[a] University of Wisconsin, School of Medicine and Public Health, Madison, WI, United States
[b] Penn State University, Hershey, PA, United States
[c] University of Illinois at Chicago, Chicago, IL, United States
[d] Nemours Children's Health System, Jacksonville, FL, United States
[e] Washington University, School of Medicine, St Louis, MO, United States
[f] National Jewish Health, Denver, CO, United States
[g] Department of Medicine, Brigham and Women's Hospital, Boston, MA, United States
[h] Department of Medicine, University of Arizona, Tucson, AZ, United States
[i] Department of Pediatrics, Duke University, Durham, NC, United States
[j] University of Michigan, Ann Arbor, MI, United States
[k] Wake Forest School of Medicine, Winston-Salem, NC, United States
[l] University of Pittsburgh, Pittsburgh, PA, United States
[m] University of California San Francisco, San Francisco, CA

ARTICLE INFO

Keywords:
Participant recruitment
Multicenter clinical trial
Study design
Precision medicine
Phenotype-stratified
Biomarker-stratified
Induced sputum eosinophilia

ABSTRACT

Precision medicine is expected to impact the care of people with asthma, given its high disease prevalence, heterogeneity of pathophysiologic mechanisms, and consequent clinical phenotypes. A novel phenotype-stratified clinical trial conducted by the NHLBI AsthmaNet Consortium, titled Steroids in Eosinophil Negative Asthma (SIENA), was a randomized, multicenter, clinical trial that prospectively stratified individuals according to their baseline level of sputum inflammation during a screening period. Two phenotypic strata were assigned based on an a priori defined extent of sputum eosinophilia (Eos Low versus Eos High). This article describes: the scientific premise for the trial design, including assumptions used for power calculations; modifications to the analysis plan implemented after the trial started due to a higher than expected prevalence of one phenotypic stratum which impacted the ability to accrue sufficient subjects within the planned budget and study period; investigator alternatives to address the strata imbalance weighing scientific impact and study feasibility; and the final modified SIENA study design and analysis plan. SIENA was successfully completed in a manner that maintained meaningful outcomes. We conclude with recommendations for incorporation of pre-specified contingency plans into phenotype-directed protocols, to address the potential for differences in observed compared to estimated prevalence of different phenotypes in a study population. These approaches can be applied to precision medicine trials for the future.

1. Introduction

Precision medicine refers to the delivery of healthcare interventions tailored to an individual's likelihood of benefits and harms based on genetic predisposition, environment, or lifestyle. Precision medicine is expected to revolutionize the care of people with asthma, given the high prevalence of this disease in both children and adults, and its clinical heterogeneity. The premise of precision medicine relies on well-

* Corresponding author at: PharmD, University of Wisconsin, School of Medicine and Public Health, 750 Highland Avenue, Madison, WI 53705, United States.
E-mail address: sorkness@wisc.edu (C.A. Sorkness).
[1] Drs. Sorkness and King contributed equally to the manuscript

https://doi.org/10.1016/j.cct.2018.12.012
Received 3 October 2018; Received in revised form 19 December 2018; Accepted 25 December 2018
Available online 27 December 2018
1551-7144/

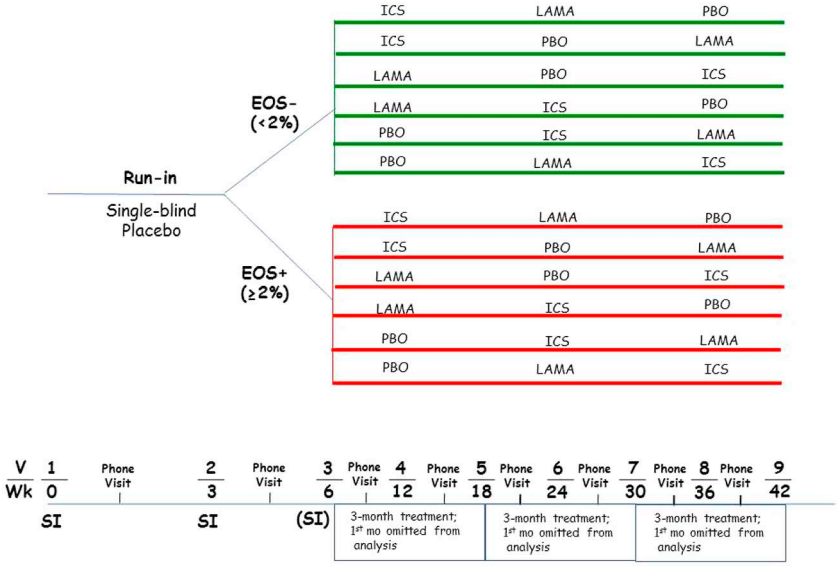

Fig. 1. SIENA study schema.

defined underlying pathophysiologic mechanisms that drive asthma endotypes and their clinical expression (phenotypes) [1]. For example, eosinophilic asthma is an endotype that includes various phenotypes, such as allergic adult-onset asthma and aspirin-sensitive asthma. Endotype/phenotype-directed therapeutic interventions are being developed to identify more precise, evidence-based treatments.

Results of clinical trials indicate that about one-half of patients with asthma do not respond well to treatments, such as inhaled corticosteroids (ICS), which mainly target eosinophilic inflammation [2–4]. Data from various groups suggest that eosinophilic inflammation is not a ubiquitous feature of asthma [5–8]. For example, investigators in the National Institutes of Health/National Heart, Lung, and Blood Institute (NIH/NHLBI) Asthma Clinical Research Network (ACRN) reported that only 36% of 995 individuals with mild to moderate asthma not using ICS have sputum eosinophilia (≥2% eosinophils) [8]. In a subset of these participants undergoing sputum induction procedures repeated over time ($n = 157$), 53% had sputum eosinophilia, and 47% were persistently non-eosinophilic. In a post hoc analysis of the NIH/NHLBI ACRN's Improving Asthma Control Trial (IMPACT) [9], a two-week course of oral prednisone, inhaled budesonide, and oral zafirlukast significantly improved the forced expired volume in 1 s (FEV_1) in participants with persistent sputum eosinophilia, but not in participants who were persistently non-eosinophilic.

These observations led to the design of Steroids in Eosinophil Negative Asthma (SIENA), the only phenotype-stratified clinical trial conducted by the NHLBI AsthmaNet Consortium (ClinicalTrials.govNCT02066298). SIENA was a randomized, multicenter, clinical trial that prospectively stratified individuals according to their baseline level of sputum eosinophilia and was designed to examine whether ICS responsiveness was related to sputum eosinophilia. In this report, we describe: 1) the scientific premise for the stratified randomized clinical trial design based on the eosinophilic phenotype, including assumptions used for power calculations; 2) modifications to the analysis plan that were implemented after the start of the trial due to a higher than expected prevalence of persistently non-eosinophilic asthma among patients screened for SIENA, which impacted the ability to complete SIENA within the constraints of the NHLBI-approved budget and funding period; and 3) the final modified SIENA study design. Our report is intended to inform the planning of future precision medicine trials utilizing phenotype-directed designs, including the need for prespecified contingency plans to accommodate observations after trial initiation.

2. Methods

2.1. Overview of SIENA protocol as originally designed

SIENA was a randomized, stratified, 3-period, double-blind placebo-controlled crossover study of individuals aged 12 years and older with symptomatic mild asthma. Participants not already using ICS underwent sputum inductions on two separate occasions separated by 3–6 weeks during the screening process, to identify individuals who were persistently non-eosinophilic (< 2% sputum eosinophils on both occasions). Participants were then classified 1:1 as eosinophil high (Eos High) if they demonstrated ≥2% eosinophils on at least one sputum sample or as eosinophil low (Eos Low) if both samples showed < 2% sputum eosinophils. Participants in each stratum were treated in random sequence with inhaled medium dose mometasone (an ICS), inhaled tiotropium via Respimat® (a long-acting muscarinic antagonist, LAMA), or placebo.

Critical to SIENA is the four- to six- week single-blind placebo-LAMA run-in period to define level of asthma control and allow characterization of sputum inflammatory cells via the sputum induction. At the end of this run-in period, participants who continued to meet entry criteria and whose adherence to single-blind placebo-LAMA use and diary completion was at least 75% were randomized to the double-blind treatment phase (Fig. 1, SIENA Study Schema). The initial plan was for sites to recruit and randomize all eligible participants. Based on prior ACRN experience, we anticipated that the distribution of eosinophilic phenotype would be approximately 1:1. However, we specified that the AsthmaNet Data Coordinating Center (DCC) would monitor the eosinophil patterns at randomization and possibly restrict enrollment if needed to achieve balanced accrual of the strata.

Each of three treatment periods was 12 weeks in duration and did not include a washout, with outcomes based on the last 8 weeks of each treatment period. Participants were seen every six weeks and assessed by phone calls at the 3-week point between visits to collect outcome data. All participants entered key data (asthma symptoms, medication use, and peak expiratory flow [PEF] measures) into electronic diaries throughout the trial. The primary outcome of SIENA was a hierarchical composite of three measures of asthma control: Treatment Failure (TF), Asthma Control Days (ACD), and FEV_1. The probability of a better response to ICS vs. placebo and LAMA vs. placebo was estimated by comparing these components between relevant treatment periods for each participant. The definition of TF was based on the Symptom-Based

Action Plan that was utilized successfully in ACRN's IMPACT [9] trial, and includes asthma awakenings, frequency and response to rescue use of albuterol for symptoms, and exercise-induced unusual breathlessness. ACDs were documented in daily diaries, and defined as no rescue albuterol use, no non-study asthma medications, no daytime asthma symptoms, no nighttime asthma symptoms, no unscheduled healthcare visits, and a PEF of at least 80% of predetermined baseline. FEV_1 was assessed using standardized procedures. SIENA secondary outcomes included asthma exacerbations and safety.

SIENA's primary objective was to determine if there was evidence of heterogeneity of treatment effects for the primary outcome when comparing the ICS vs. placebo treatment period and LAMA vs. placebo period *across the two strata of participants*. We estimated that a sample size of 384 would yield 90% statistical power with two-sided, 0.025 significance level tests (Bonferroni correction), assuming a 1:1 ratio of Eos High and Eos Low participants and a 15% drop-out rate, to detect a difference of 0.2 in probabilities *between* the two strata. An important secondary objective was to determine if there was a preference for ICS or LAMA within the Eos Low stratum.

3. Results

3.1. Difficulty with accrual and imbalance across the two phenotypic strata

Initiation of SIENA enrollment across the AsthmaNet consortium was delayed by 15 months (July 2014, instead of April 2013), primarily due to delayed availability of donated study medications and placebos from pharmaceutical companies. After the first 8 months of SIENA recruitment (79 total randomized participants), the observed ratio of Eos Low versus Eos High was approximately 3:1 (Figs. 2 and 3). While the number randomized through May 2015 (Table 1) represented a small percentage of the total required sample size (20.5%, 79/384), investigators were concerned that a 1:1 ratio of participants in each of the strata at the end of the study recruitment phase was unlikely. The baseline characteristics of SIENA participants randomized through May 2015 are described in Table 1. Because the sample size calculations for the primary hypothesis assumed an approximately equal ratio of participants in the two strata, the study investigators considered various options to address the likely imbalance in the number of participants in the two strata.

One proposal was to selectively enrich the Eos High stratum by prescreening prospective participants based on blood eosinophils and/or FeNO in order to identify those more likely to be Eos High. Another proposal was to close the Eos Low stratum when 256 participants had been randomized so as not to over-enroll in that stratum. Having 256 in the Eos Low stratum and 128 in the Eos High stratum equated to an achievable 2:1 ratio of Eos Low to Eos High, and would provide 85% power for our primary outcome. Unfortunately, given the actual Eos Low to Eos High ratio at the time, an estimated 725 enrolled participants would have been needed to achieve the 2:1 ratio and an estimated 250 Eos Low participants would have been needed to be excluded following enrollment. Rejecting 250 participants after they had completed the lengthy run-in was highly undesirable to the AsthmaNet Steering Committee, and likely to the IRBs, especially due to the inclusion of pediatric participants. In addition, there were concerns regarding our ability to recruit 725 participants and complete the trial within the NHLBI SIENA budget and project period.

3.2. Approach to modifying the SIENA primary research question and analysis plan

Following consultation with the NIH/NHLBI and the independent NHLBI-appointed Data Safety and Monitoring Board (DSMB), and prior to unmasking study investigators to outcome data, the investigators elected to change the analysis plan to focus the primary objective on what was originally stated as a secondary objective. More specifically, the revised primary objective was to determine whether ICS or LAMA performed better than placebo in the Eos Low stratum. Comparing the differential response to ICS and LAMA *between* the Eos Low and Eos High phenotypes became a secondary objective of the SIENA study.

The revised statistical analysis plan therefore included the same estimation of the probability of a differential response for ICS vs. placebo and LAMA vs. placebo as initially designed, but rather than a two-sided, two-sample frequency test to compare *between* phenotypes, the primary analysis would involve two-sided exact binomial tests at the 0.025-level (Bonferroni correction) for the comparison of ICS vs. placebo and LAMA vs. placebo *within* the Eos Low phenotype. The power calculations required revision to reflect this change in the primary analysis. The revised sample size for the SIENA trial was 336 randomized participants (262 in the Eos Low phenotype and 74 in the Eos

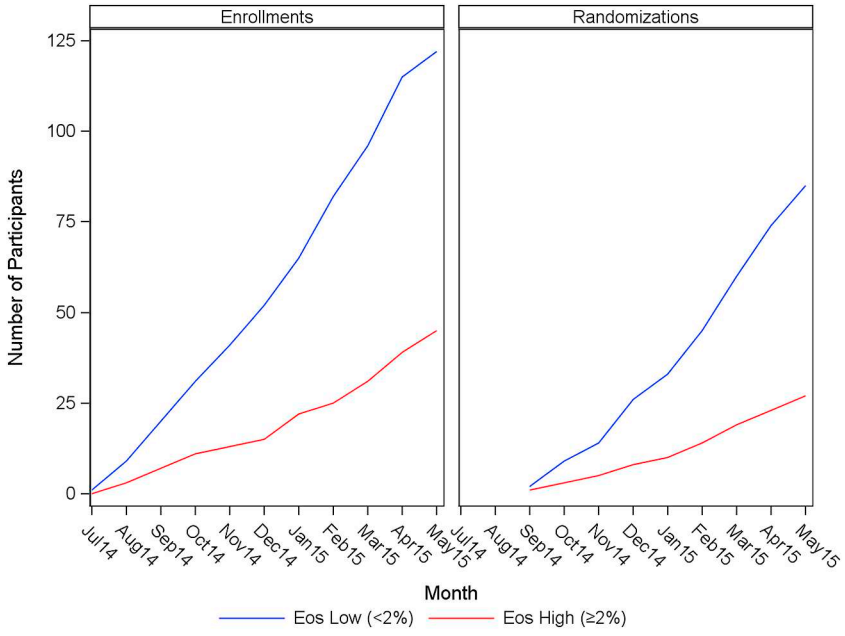

Fig. 2. Number of enrollments and randomizations by Stratum. Fig. 2 describes the number of SIENA particpants enrolled and randomized in the Eos High versus Eos Low strata.

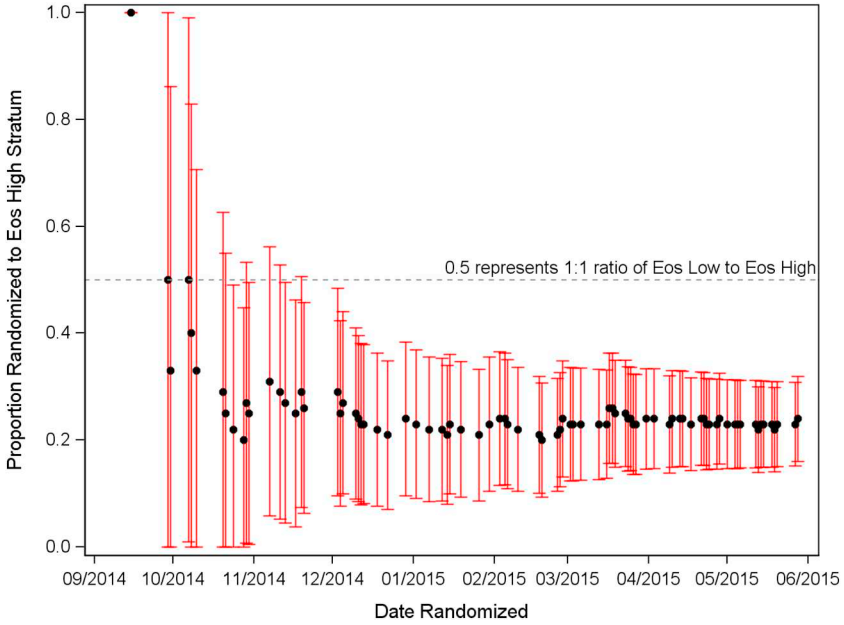

Fig. 3. Proportion of participants randomized to Eos High Stratum. Fig. 3 represents the proportion of SIENA participants randomized to the Eos High stratum throughout the course of the trial.

Table 1
Baseline characteristics of SIENA participants randomized through May 2015.

Characteristic	Eosinophil Low (*N* = 85)	Eosinophil High (*N* = 27)
Demographics		
Age at enrollment	31.6 ± 14.1	29.0 ± 10.8
Male – no. of participants (%)	28 (32.9%)	13 (48.1%)
Race/Ethnicity – no. of participants (%)		
Asian/Pacific Islander	6 (7.1%)	4 (14.8%)
Black	25 (29.4%)	7 (25.9%)
White	51 (60.0%)	11 (40.7%)
Hispanic	3 (3.5%)	5 (18.5%)
Asthma History		
Median age when doctor first diagnosed (interquartile range)	8.0 (3.0–12.0)	7.0 (3.0–13.0)
Duration of asthma (years since doctor first diagnosed)	20.4 ± 9.9	18.6 ± 9.8
Family History of Asthma – no. of participants (%)	59 (72.8%)	17 (63.0%)
Prior Year – no. of participants (%)		
One or more asthma episodes requiring emergency care or unscheduled office visit	18 (21.2%)	1 (3.7%)
One or more overnight hospitalizations due to asthma	1 (1.2%)	0 (0.0%)
One or more courses of systemic corticosteroid therapy taken for asthma	16 (18.8%)	0 (0.0%)
Days of work, school, or housework missed in past year due to asthma:		
0 days	57 (67.1%)	21 (77.8%)
1 to 7 days	21 (24.7%)	4 (14.8%)
> 7 days	7 (8.2%)	2 (7.4%)
ICS (not including combination meds)	18 (21.2%)	3 (11.1%)
ICS/LABA Combination Therapy	6 (7.1%)	1 (3.7%)
Inhaled Muscarinic Antagonist	1 (1.2%)	0 (0.0%)
LTRA/5LO Inhibitors	8 (9.4%)	4 (14.8%)
Clinical and Spirometric Features		
BMI at enrollment (kg/m^2)	28.8 ± 7.6	26.9 ± 5.1
FEV_1% predicted at randomization	91.3 ± 11.4	90.5 ± 11.4
FEV_1/FVC ratio at randomization	0.77 ± 0.07	0.74 ± 0.09
PC_{20} (mg/ml) at enrollment - geometric mean ± CV	2.24 (1.30)	2.37 (1.40)
Bronchodilator Response (4 puffs) at enrollment (relative % change)	10.0 ± 6.7	14.3 ± 9.4
Median eNO (ppb) at enrollment (interquartile range)	25.0 (18.0–48.0)	61.0 (29.0–78.0)
Median Blood Eosinophils (%) at enrollment (interquartile range)	3.0 (2.0–4.1)	4.8 (4.0–7.0)
Median Periostin (ng/mL) at enrollment (interquartile range)	54.0 (46.1–62.2)	55.6 (48.4–64.5)
Median ACT Score at randomization[a] (interquartile range)	22.0 (20.0–23.0)	21.0 (20.0–23.0)

Means ± SD presented unless otherwise noted

[a] Individual ACT questions are scaled 1 to 5, with higher values representing better asthma control. ACT score is sum of questions 1–5.

High phenotype), because we anticipated a revised 3.5-to-1 ratio or less in the number with Eos Low to Eos High phenotype. For the co-primary comparisons within the Eos Low phenotype, the sample size of 262 was estimated to yield statistical power of 0.9 with two-sided, 0.025 significance level tests (Bonferroni correction) to detect a difference in the probability of a better response to ICS vs. placebo and LAMA vs.

placebo of 0.20, while allowing for a 15% drop-out rate. We assumed that 30% of the participants would not display a preference for ICS versus placebo (and that 30% of the participants would not display a preference for LAMA versus placebo). While we initially accounted for a 15% drop-out rate in the estimation of the necessary sample size for the trial, we found a greater than expected withdrawal rate among the participants, as well as continued lagging recruitment with the end of the funding cycle approaching. Therefore, a sample size of 215 Eos Low was determined to be a feasible sample size that would yield an acceptable statistical power of 0.8 with two-sided, 0.025 significance level tests (Bonferroni correction), while allowing for 20% drop-out rate. The key components of the SIENA trial and the earlier and current versions of the protocol are summarized in Table S1. These changes were approved by the site IRBs.

The DCC closely monitored the Eos High enrollments, and when the target of 74 randomized had been met in March 2016, sites were notified that the Eos High stratum was closed. Participant status reports were then updated to identify active participants with EOS High sputum in the run-in who should be terminated. In July 2017, SIENA accrual was successfully completed when 295 participants had been randomized (221 Eos Low and 74 Eos High). The last SIENA participant visit occurred in March 2018, and the main trial outcomes were presented at the American Thoracic Society Annual Meeting in May 2018.

4. Discussion

Successful completion of a clinical trial protocol requires attainment of recruitment goals, protocol adherence, participant retention, and appropriate use of budgeted personnel and financial resources. Ethical guidelines mandate that participants should not be subjected to unjustified risks. Unjustified risks include participating in a study that is statistically underpowered to answer the research questions, and also undergoing procedures that will not ultimately be analyzed. In the phenotype-stratified SIENA trial, we observed a two-fold higher than expected ratio of the Eos Low versus Eos High strata, thus requiring a modification of the primary objective of SIENA.

SIENA was designed to challenge the paradigm that ICS are the appropriate first-line treatment for all patients with mild persistent asthma. Recent data demonstrate different patterns of airway inflammation in asthma, and have shown convincingly that eosinophilic T2 inflammation is not ubiquitous. This may be very important clinically, if patients without airway eosinophilia do not respond to inhaled or oral corticosteroids [5,10]. The exact prevalence of sputum eosinophilia in mild-moderate asthma is unknown. Most studies of sputum eosinophils have been conducted in patients with severe or refractory asthma [11–15], but retrospective data from the NHLBI's ACRN suggested that approximately 50% of patients with mild-moderate asthma, not already treated with ICS, have < 2% eosinophils in induced sputum[8]. ACRN's rigorous analysis of induced sputum in mild-moderate asthma is one of the largest conducted, and provided the best evidence available for SIENA's sample size/power calculation. However, the population recruited for SIENA may have been less severe than that on which our assumptions of sputum eosinophilia were based.

When enrollment for SIENA began, the distribution of Eos Low versus Eos High was much different than the 1:1 ratio we had anticipated. We initially accepted that this deviation from the expected ratio might have been simply due to random variation in a small sample, or perhaps seasonal variation, but after 8 months of recruitment over the Summer-Fall-Winter seasons, the ratio of Eos Low to Eos High was 60:19 (3:1), and seemingly stable. The Steering Committee monitored recruitment carefully and explored possible explanations for this unexpected result: the sputum induction protocol and procedures for performing cell counts were identical to those used in the prior ACRN studies, and in fact the same academic reference laboratory was used in SIENA as for prior studies. Nevertheless, we recounted 100% of all slides, without a change in results. When it became apparent that the proportion of Eos Low was significantly greater than anticipated, the Steering Committee carefully considered the implications and options for addressing this challenge, weighing scientific impact versus study feasibility.

We considered four major alternatives: 1) continue enrollment as planned, which would have extended the accrual phase for much longer and required a larger total number of randomized participants, resulting in major time and financial costs; 2) continue the trial until budgeted time or funding was expended, and accept whatever sample size was achieved (likely to result in very low power for our research questions); 3) screen a larger number of potential participants, to identify a sufficient number of those who were Eos High (increasing costs significantly, and potentially necessitating trial termination of participants in the over-recruited Eos Low stratum, after undergoing the burden of the run-in and sputum Induction procedures); and 4) revise the primary study objective so that the research questions could still be answered with a different (observed) distribution of Eos Low and Eos High strata, without the need for additional participants, and without loss of participants or data already accrued. We selected option 4 which we believed would answer the clinically-important questions while maintaining meaningful outcomes within reasonable expectations and financial resources.

In the original design, the primary objective focused on the differential response to ICS and LAMA *between* the Eos Low and Eos High strata. The sample size assumption for the primary objective required an approximately equal ratio of Eos Low to Eos High participants, as was suggested by the secondary analysis of the NHLBI ACRN studies. The higher than expected ratio of Eos Low to Eos High may have been due to recruitment of individuals with milder asthma than enrolled in ACRN, or to an unidentified explanation. These considerations highlight the importance of re-examining the assumptions that contribute to clinical trial designs, including the expected prevalence of subtypes in phenotype-stratified trials. Further, it is essential to not only monitor for imbalance on an ongoing basis, but to pre-specify corrective actions should strata imbalance emerge.

5. Conclusions

We recommend that investigators incorporate pre-specified contingency plans into phenotype-directed or biomarker-stratified protocols in the future, to address the potential for differences in observed versus expected prevalence of different phenotypes in the study population. Such contingencies could include planned interim analyses, and pre-specified approaches to sample size reassessment based on tabulating potential scenarios with varying study design assumptions (Table S2). These approaches are examples of adaptive trial designs that have been used for many years; such designs will become even more relevant in the precision medicine era. Consideration should be given to designs that adjust the sample size to retain a desired power if the overall event rate is lower than expected, the variability is higher than planned, intervention adherence is worse than expected, or enrollment into phenotypic strata does not follow the assumed ratio, as in this case. In examples such as these, the sample size can be recalculated using the updated information without penalty, as compared to response adaptive or trend adaptive approaches, since there is no preliminary estimation or testing of the treatment effect. Funded by National Heart, Lung, and Blood Institute, SIENA ClinicalTrials.gov number, NCT02066298.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cct.2018.12.012.

References

[1] J. Lotvall, C.A. Akdis, L.B. Bacharier, et al., Asthma endotypes: a new approach to classification of disease entities within the asthma syndrome, J. Allergy Clin. Immunol. 127 (2011) 355–360.

[2] R.J. Martin, S.J. Szefler, V.M. Chinchilli, et al., Systemic effect comparisons of six inhaled corticosteroid preparations, Am. J. Respir. Crit. Care Med. 165 (2002) 1377–1383.

[3] R.J. Martin, S.J. Szefler, T.S. King, et al., The predicting Response to Inhaled Corticosteroid Efficacy (PRICE) trial, J. Allergy Clin. Immunol. 119 (2007) 73–80.

[4] A. Deykin, S.C. Lazarus, J.V. Fahy, et al., Sputum eosinophil counts predict asthma control after discontinuation of inhaled corticosteroids, J. Allergy Clin. Immunol. 115 (2005) 720–727.

[5] I.D. Pavord, C.E. Brightling, G. Woltmann, A.J. Wardlaw, Non-eosinophilic corticosteroid unresponsive asthma, Lancet 353 (1999) 2213–2214.

[6] J.L. Simpson, R. Scott, M.J. Boyle, P.G. Gibson, Inflammatory subtypes in asthma: assessment and identification using induced sputum, Respirology 11 (2006) 54–61.

[7] P. Haldar, I.D. Pavord, Noneosinophilic asthma: a distinct clinical and pathologic phenotype, J. Allergy Clin. Immunol. 119 (2007) 1043–1052 (quiz 53-4).

[8] K.W. McGrath, N. Icitovic, H.A. Boushey, et al., A large subgroup of mild-to-moderate asthma is persistently noneosinophilic, Am. J. Respir. Crit. Care Med. 185 (2012) 612–619.

[9] H.A. Boushey, C.A. Sorkness, T.S. King, et al., Daily versus as-needed corticosteroids for mild persistent asthma, N. Engl. J. Med. 352 (2005) 1519–1528.

[10] H.M. Brown, Treatment of chronic asthma with prednisolone; significance of eosinophils in the sputum, Lancet 2 (1958) 1245–1247.

[11] S.E. Wenzel, L.B. Schwartz, E.L. Langmack, et al., Evidence that severe asthma can be divided pathologically into two inflammatory subtypes with distinct physiologic and clinical characteristics, Am. J. Respir. Crit. Care Med. 160 (1999) 1001–1008.

[12] R.H. Green, C.E. Brightling, G. Woltmann, D. Parker, A.J. Wardlaw, I.D. Pavord, Analysis of induced sputum in adults with asthma: identification of subgroup with isolated sputum neutrophilia and poor response to inhaled corticosteroids, Thorax 57 (2002) 875–879.

[13] L. Jayaram, M.M. Pizzichini, R.J. Cook, et al., Determining asthma treatment by monitoring sputum cell counts: effect on exacerbations, Eur. Respir. J. 27 (2006) 483–494.

[14] P. Haldar, I.D. Pavord, D.E. Shaw, et al., Cluster analysis and clinical asthma phenotypes, Am. J. Respir. Crit. Care Med. 178 (2008) 218–224.

[15] W.C. Moore, D.A. Meyers, S.E. Wenzel, et al., Identification of asthma phenotypes using cluster analysis in the Severe Asthma Research Program, Am. J. Respir. Crit. Care Med. 181 (2010) 315–323.