Supplementary Appendix

This appendix has been provided by the authors to give readers additional information about their work.

Supplement to: Zuraw BL, Busse PJ, White M, et al. Nanofiltered C1 inhibitor concentrate for treatment of hereditary angioedema. N Engl J Med 2010;363:513-22.

Supplementary Appendix

This appendix is included to present supplementary data. The appendix is divided into 4 parts as follows:

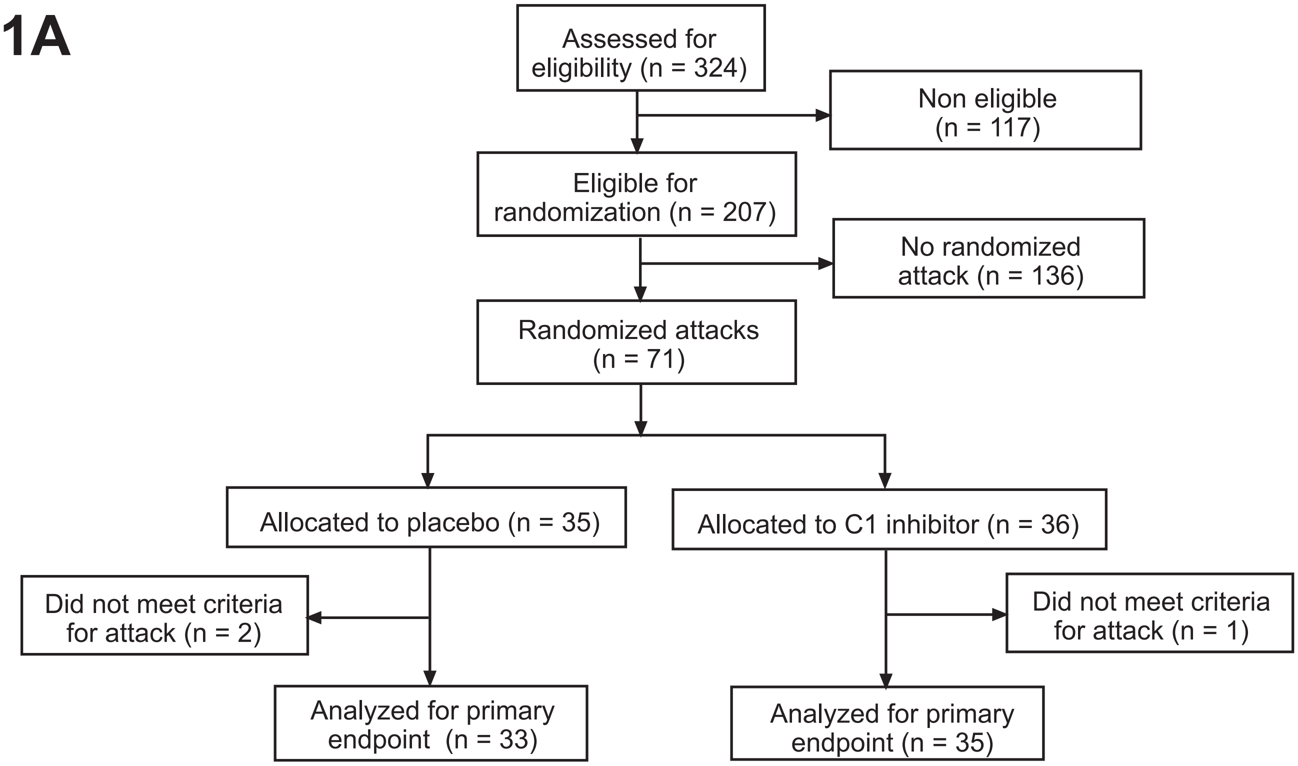
Appendix Part 1: CONSORT Diagrams

Supplemental Figures 1A-1B. CONSORT diagrams of study enrollment and outcomes for:

1A) Acute treatment trial.

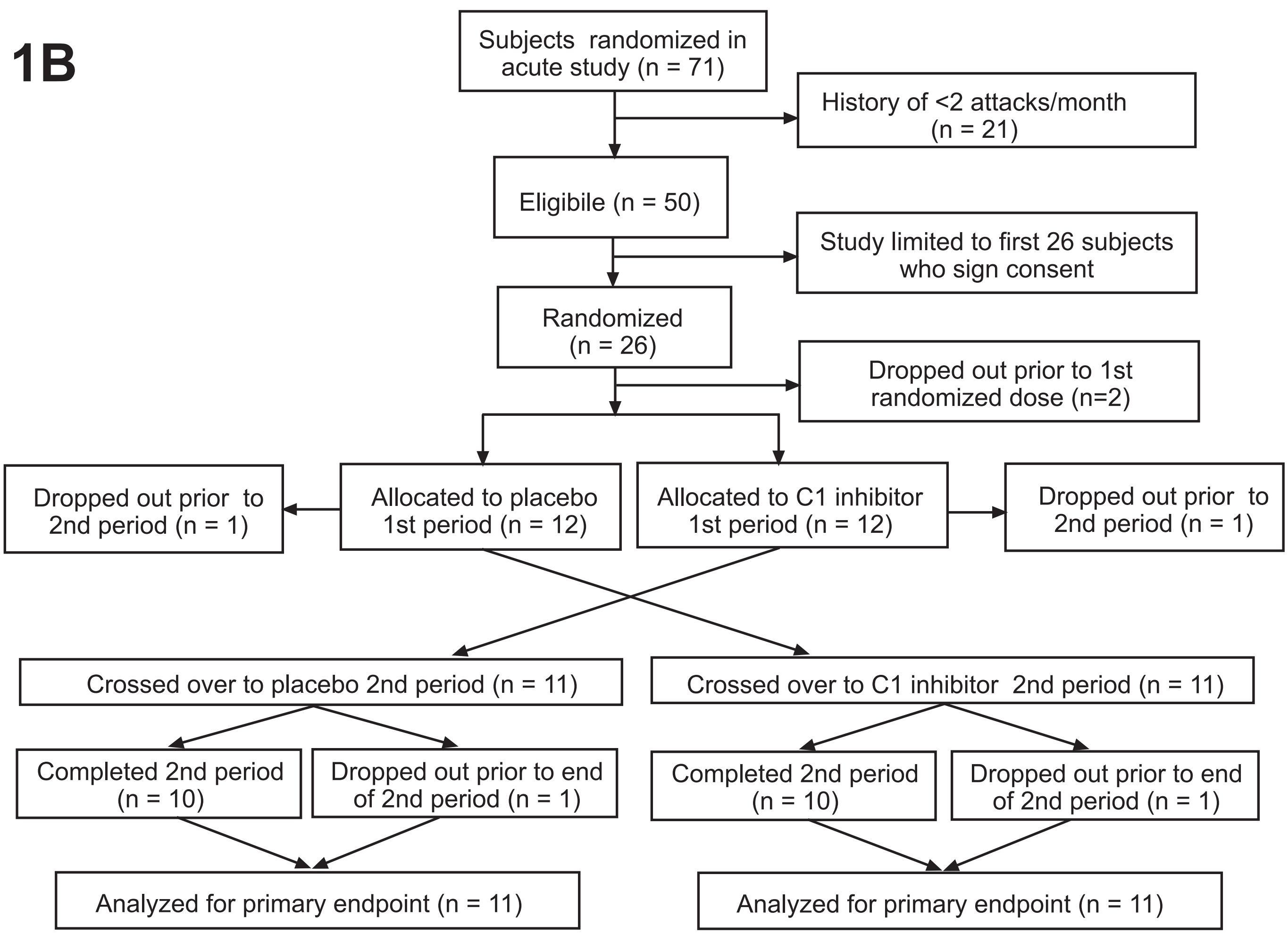
1B) Prophylactic treatment trial

1A



Assessed for eligibility (n = 324)
Non eligible (n = 117)
Eligible for randomization (n = 207)
No randomized attack (n = 136)
Randomized attacks (n = 71)
Allocated to placebo (n = 35)
Allocated to C1 inhibitor (n = 36)
Did not meet criteria for attack (n = 2)
Did not meet criteria for attack (n = 1)
Analyzed for primary endpoint (n = 33)
Analyzed for primary endpoint (n = 35)

1B



Subjects randomized in acute study (n = 71)
History of <2 attacks/month (n = 21)
Eligibile (n = 50)
Study limited to first 26 subjects who sign consent
Randomized (n = 26)
Dropped out prior to 1st randomized dose (n=2)
Dropped out prior to 2nd period (n = 1)
Allocated to placebo 1st period (n = 12)
Allocated to C1 inhibitor 1st period (n = 12)
Dropped out prior to 2nd period (n = 1)
Crossed over to placebo 2nd period (n = 11)
Crossed over to C1 inhibitor 2nd period (n = 11)
Completed 2nd period (n = 10)
Dropped out prior to end of 2nd period (n = 1)
Completed 2nd period (n = 10)
Dropped out prior to end of 2nd period (n = 1)
Analyzed for primary endpoint (n = 11)
Analyzed for primary endpoint (n = 11)

Appendix Part 2: Supplemental Analyses

Supplemental analyses are divided into 3 sub-sections:

2A) Effect Modifier Analyses

2B) Number of Blinded Study Agent Infusions

2C) Analysis of Longitudinal C1INH and C4 Levels

2A) Effect Modifier Analyses

There are a number of factors that could be effect modifiers. A potential effect modifier is a baseline attribute that defines mutually exclusive and exhaustive subgroups having different arm effect sizes, with the possibility of arm effects in opposite directions (across subgroups). Due to the relatively small sample size there is limited statistical sensitivity for assessing for effect modification, however, so the following analyses must be interpreted with caution.

The effect modifier candidates will be analyzed separately using site-stratified proportional hazard regression (PHR) models with pooling of sites without at least one subject in both arms. The covariates in the PHR models include an arm main effect, the potential effect modifier as a main effect and either a categorical covariate or a dichotomous covariate spilt at the median, and interaction term(s) between arm and the effect modifier. The PHR models are estimated using the exact method for handling tied event times.

The criterion for evidence of effect modification is an interaction P value of 0.1 or less. If this criterion is met then separate hazard ratio estimates for each level of the effect modifier will be reported; the arm and effect modifier main effects will not be estimated. When the criterion for effect modification is not met then the model will be refit with only the arm and effect modifier main effects.

Cumulative incidence graphs are also shown. The solid circles on the cumulative incidence graphs prior to 240 minutes are treatment failures. The legend of the cumulative incidence graphs shows the number of subjects and the number of events (subjects meeting the criterion for unequivocal relief) for each subgroup formed by the effect modifier and arm cross-classification.

Body Mass Index (BMI)

The interaction P value for BMI split at the median of 26.6 is 0.0562, suggesting that BMI might be an effect modifier. Therefore separate hazard ratio estimates assessments for low and high BMI will be informative. The hazard ratio estimate (and 95% confidence interval) for low BMI is 1.12 (0.40 to 3.14) and for high BMI is 7.74 (1.54 to 35.90). Thus, since both hazard ratio estimates are greater than unity the interaction appears to be quantitative. Specifically, there is no or minimal C1INH-nf effect in low BMI subjects whereas in high BMI subjects the C1INH-nf effect is large. Figure 2 is the cumulative incidence graph for BMI. The arm-specific cumulative incidence estimates for low BMI are minimally different but are distinctly different for high BMI, and C1INH-nf appears to be favored for each BMI subgroup.

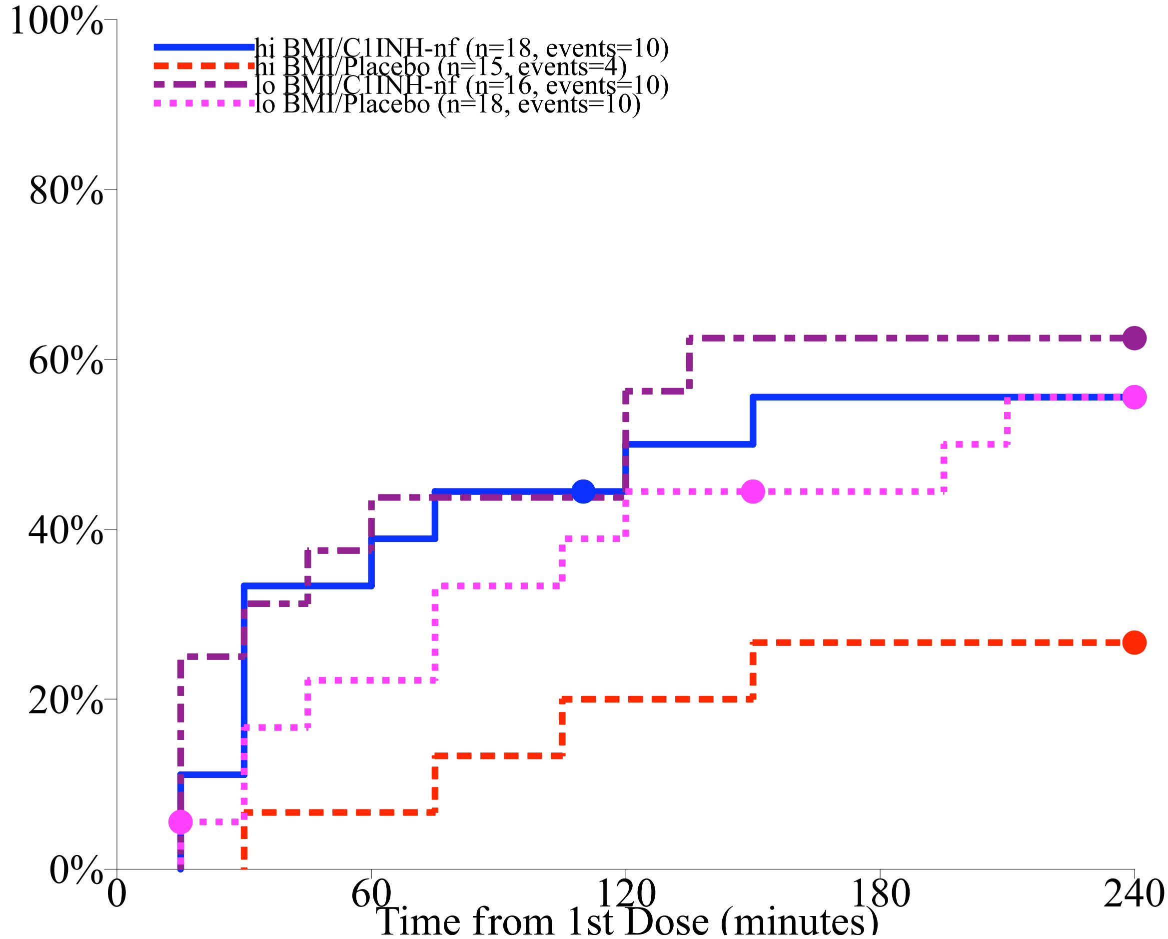


Figure 2: Cumulative incidence estimates for Body Mass Index (BMI) by Arm. The cumulative incidence estimates for low BMI have a smaller between-arm difference than for high BMI, but there is a visual difference favoring C1INH-nf for both levels of BMI.

Gender

The interaction P value for gender is 0.1250 and therefore the criterion for effect modification is not met. Nonetheless, separate estimates of C1INH-nf effect by gender might be informative. The hazard ratio estimate (and 95% confidence interval) for female gender is 1.68 (0.72 to 3.92) and the hazard ratio estimate for male gender is 11.80 (1.18 to 117.60). With no interaction term in the model the P value associated with gender is 0.8148, whereas the P value for arm is 0.0263. Thus, there is no evidence of a gender effect and the C1INH-nf effect is persistent in the model that includes gender. Figure 3 is the cumulative incidence graph for gender. The between-arm cumulative incidence estimates for both genders appear to be different and favor C1INH-nf, but the Between-arm difference for males appears to be larger.

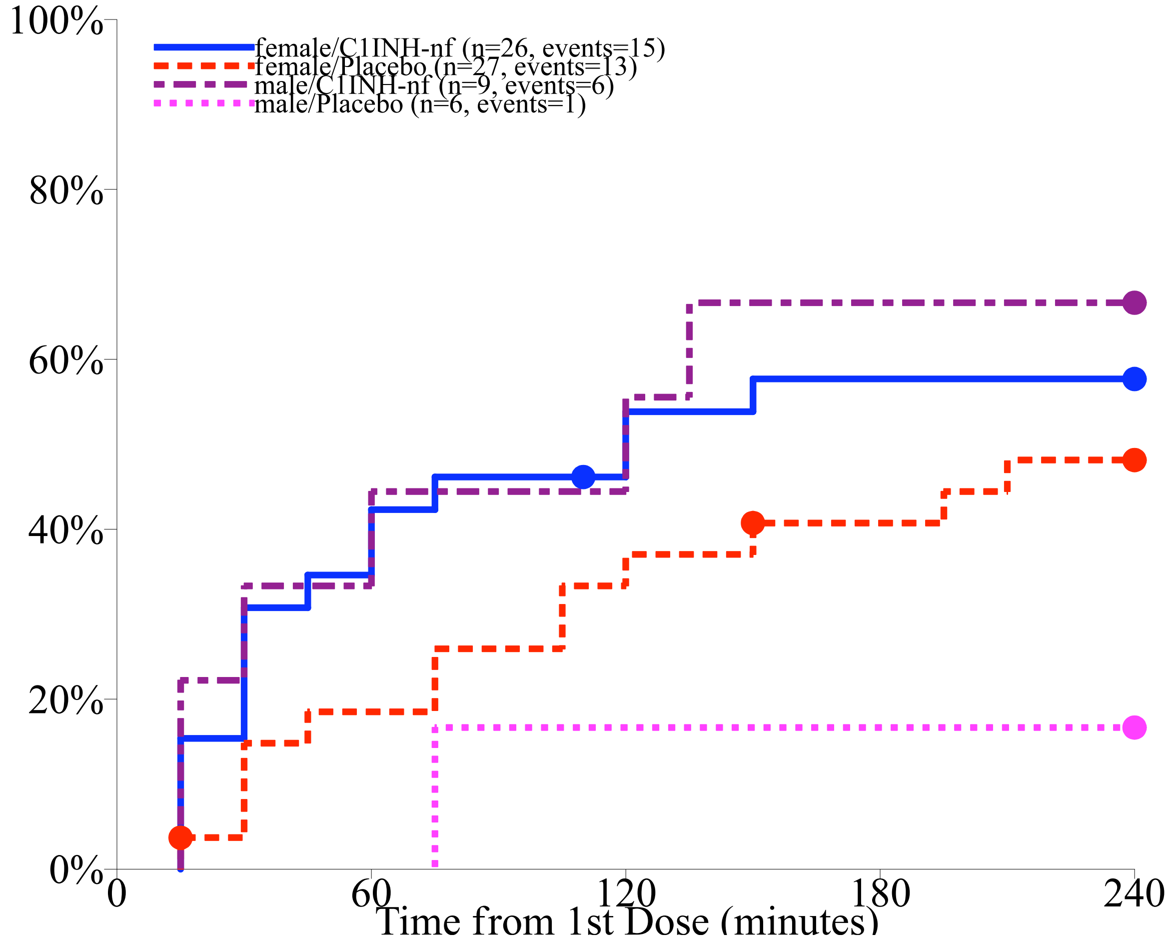


Figure 3: Cumulative incidence estimates for Gender by Arm. The C1INH-nf effect is visually apparent for both genders.

Time Since Start of Attack Until Randomization (TRand)

The time between the start of the attack until the subject was randomized (TRand) is a potential effect modifier. The interaction P value for TRand split at the median of 200 minutes (distant > 200 minutes, recent otherwise) is 0.1486, and therefore the criterion for effect modification is not met. With no interaction term in the model the TRand P value is 0.1678, whereas the P value for arm is 0.0194. Thus, TRand main effect fails to meet conventional criteria for significance and the C1INH-nf main effect is persistent in the model that includes TRand. Figure 4 is the cumulative incidence graph for TRand. The between-arm cumulative incidence estimates for both levels of TRand appear to be different and favor C1INH-nf, but the between-arm difference for attack starting 200 or less minutes prior to the first dose appears to be larger.

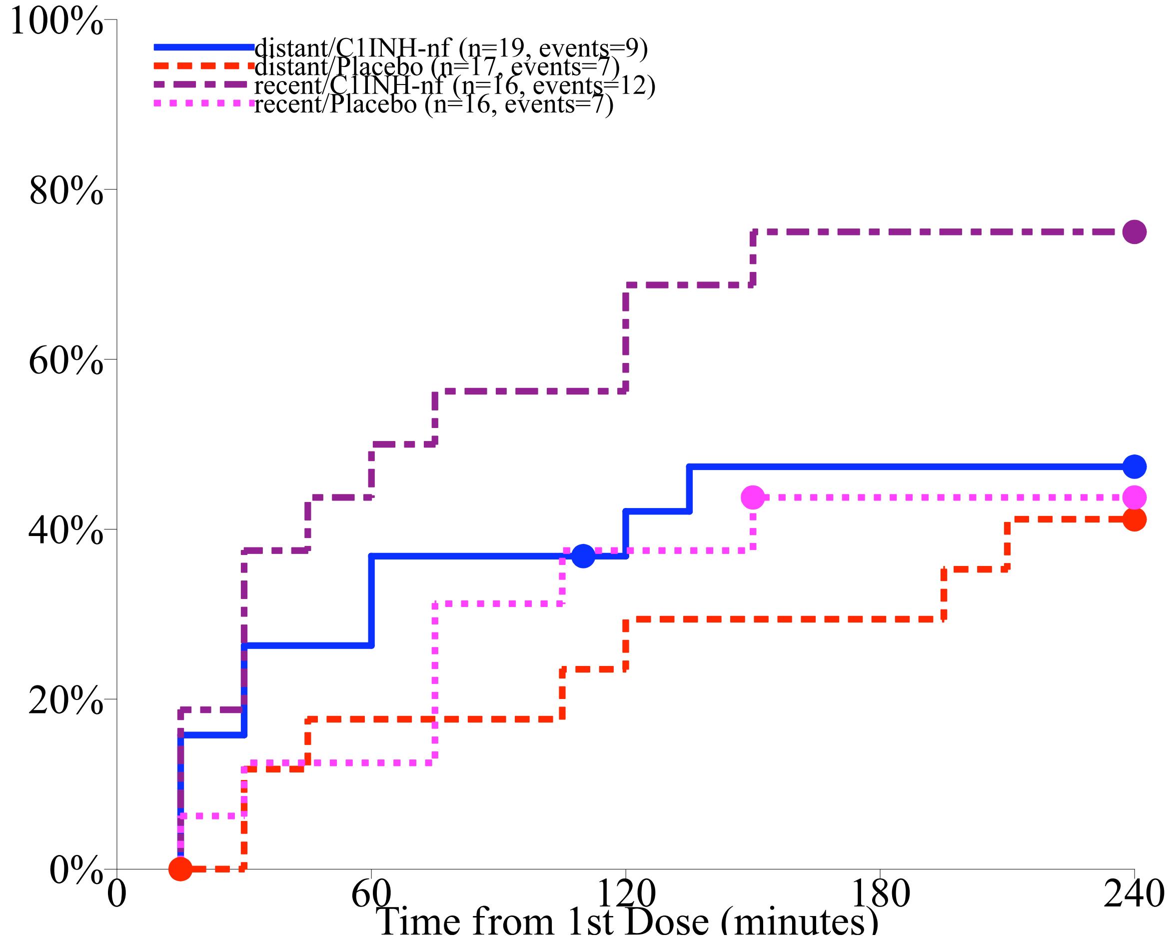


Figure 4: Cumulative incidence estimates for time between the start of attack and randomization (TRand) by Arm. TRand is dichotomized at the median of 200 minutes and labeled as distant (> 200 minutes) and recent (≤ 200 minutes). The arm-specific cumulative incidence estimates favor C1INH-nf for both levels of TRand, but appears to be more visually more pronounced for subjects with recent attacks.

Attack Site

The defining attack site was to be identified for each subject at the time of randomization as facial, gastric, or genitourinary. The P value for the 2 degree of freedom test of the attack site interaction is 0.8760, and therefore the criterion for effect modification is not met. In the no interaction model the P values associated with site are 0.5148 for gastric and 0.5965 for GU (both contrasted to facial), whereas the P value for arm is 0.0178 and therefore the C1INH-nf effect is persistent in a mode that includes site. Figure 5 is the cumulative incidence graph for attack site. The pairs of arm-specific cumulative incidence estimates favor C1INH-nf for each site, but there are only two subjects with GU attacks in the placebo arm and neither had unequivocal relief.

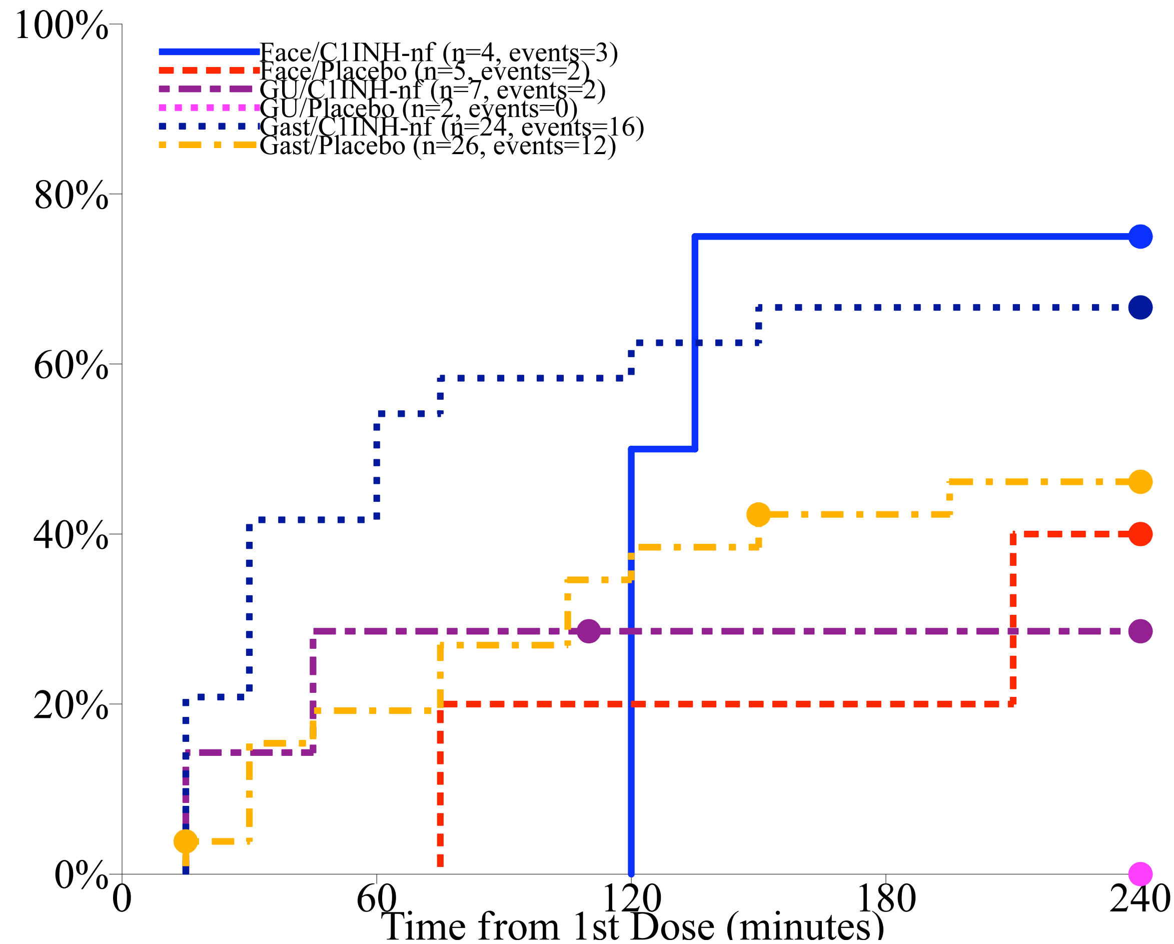


Figure 5: Cumulative incidence estimates for index attack site (Face, Genitourinary [GU], or Gastrointestinal [Gast]) by Arm. Note that C1INH-nf is visually favored for all sites.

Summary of Effect Modifier Analyses

The above effect modifier analyses failed to find evidence contradicting the primary analysis establishing the efficacy of C1INH-nf. Specifically, the direction of the arm effect favors C1INH-nf in all subgroups defined by the potential effect modifiers analyzed, and when the interaction term did not meet the effect modifier criterion the arm main effect has a P value less than two-sided 0.05.

2B) Number of Blinded Study Agent Infusions

Subjects were to be treated with one blinded study agent infusion followed by a second if needed. The number of blinded study agent infusions is related to post-randomization study outcome and therefore does not meet the definition of a potential effect modifier because the subjects segregating into one versus two infusions did so as a result of post randomization events. Assessing the association between the number of infusions of blinded study agent and the primary outcome of time to unequivocal relief has the potential to be informative about whether there is a difference in the primary outcome for subjects receiving only one infusion versus those receiving two infusions. The statistical modeling of this association is problematic, however, because both variables are outcomes and the study size is small. Therefore, only a graphical analysis is presented. Figure 6 is a cumulative incidence graph of arm by whether there were 1 or 2 infusions of blinded study agent. This graph shows that all subjects receiving only one infusion had unequivocal relief, and therefore this dimension of study conduct was met. This graph also suggest that there is very little difference in the time to unequivocal relief outcome for subjects receiving only one infusions (as expected), though there were more subjects in the C1INH-nf arm that achieved unequivocal relief from one infusion [12 of 35 (34.3%) versus 5 of 33 (15.2%)].

Now, assuming that the randomization resulted in minimal between arm differences and assuming that placebo is ineffective, the proportion of placebo arm subjects meeting the criterion for unequivocal relief so as to preclude the second injection, that is, 15.2%, can be taken as an estimate of the proportion of subjects who were on a trajectory for immediate unequivocal relief without intervention. Thus, the expected number of these subjects in the C1INH-nf arm is 15.2% of 35, or 5.3 subjects. However 12 subjects in the C1INH-nf arm, or 34.3% of 35, met the criterion for immediate unequivocal relief and benefited from a single dose of C1INH-nf precluding the second injection.

Also suggested in Figure 6 is a difference in time to unequivocal relief favoring C1INH-nf among subjects requiring two infusions, though it must be remembered there is no basis for assuming that the subjects requiring two infusions from each arm are comparable.

The subjects requiring two infusions can also be analyzed according the proportion meeting the criterion for unequivocal relief on or prior to 240 minutes. In the placebo arm 9 of 28 (32.1%) subjects requiring two infusions met this criterion. If it were assumed that the 23 C1INH-nf arm subjects requiring two infusions are similar to the placebo arm subjects requiring two infusions (likely an untenable assumption), then 32.1% of these 23 subjects, or 7.4 subjects, would meet this criterion. But 9 of the 23 subjects (39.1%) met the criterion.

Thus, while the above analyses are confounded by the possibility of lack of comparability between the subgroups within the arms and can only be regarded as descriptive, no evidence was found contradicting the efficacy of any aspect of the two-dose C1INH-nf intervention algorithm used in the trial.

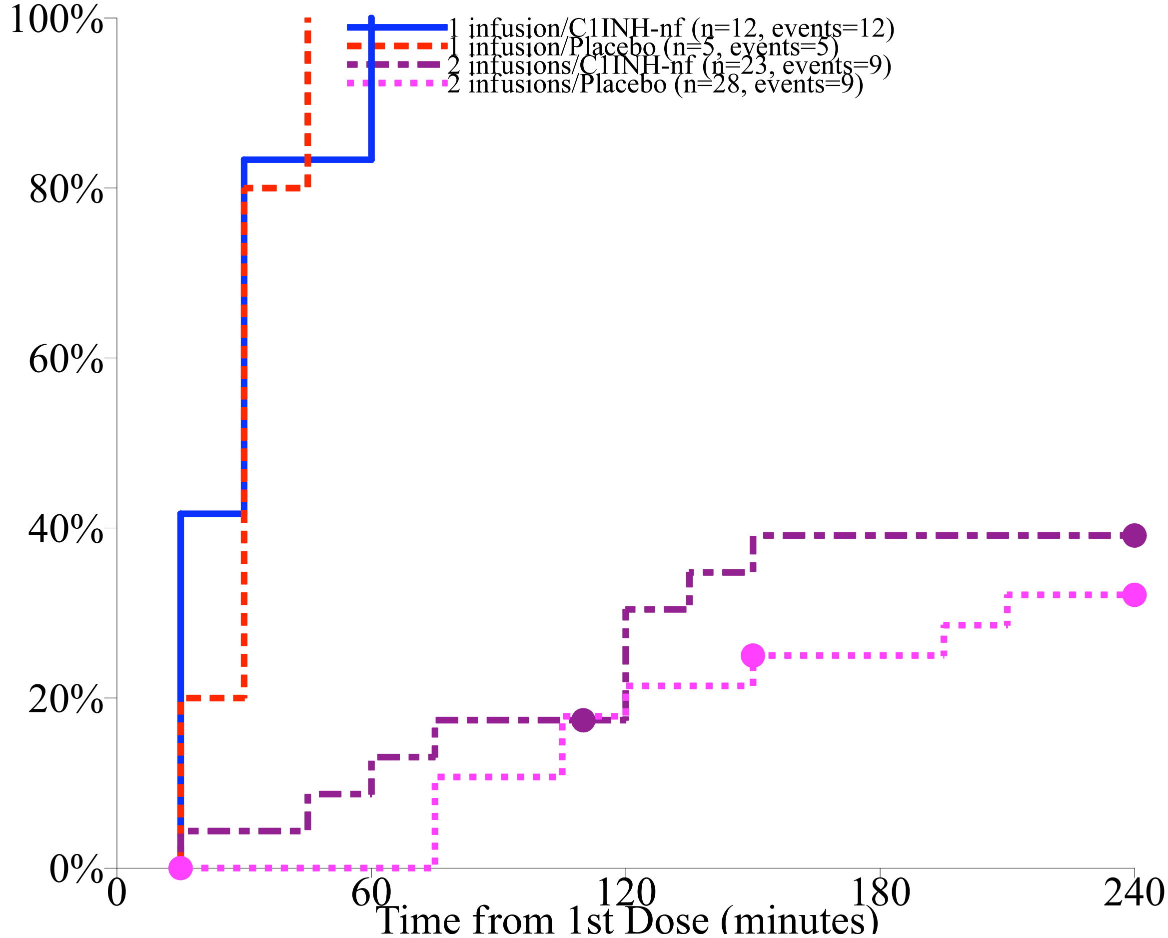


Figure 6: Cumulative incidence estimates for number of infusions (1 or 2) by Arm.

2C) Analysis of Longitudinal C1INH and C4 Levels

Specimens for the C1INH and C4 assays were to be collected at protocol-specified times of pre-infusion and at 60, 120 and 240 minutes following the first infusion. Subjects responding prior to 240 minutes may not have been available for sampling beyond their time of response.

The C1INH antigenic, C1INH functional, and C4 assay results through 240 minutes have been analyzed in a longitudinal linear repeated measures model. The contrast of interest is the difference between the pre-infusion value and the mean of values from the 60, 120, and 240 minute scheduled specimens taken after the first infusion. There was some time variation in when these specimens were actually collected, and also not all subjects had all specimens. The primary hypothesis test is for a between arm difference in the contrast of interest. SAS procedure MIXED was used to test the null hypothesis of no between-arm difference in the contrast.

Table 1 shows the means of the C1INH antigenic, C1INH functional, and C4 assay results and their standard deviations. The pre-infusion, mean of the post-infusion assessments, and contrast between the pre-infusion and mean of the post-infusion values are shown in this table. Note the large standard deviations.

Table 1: Means and standard deviations of C1INH antigenic, C1INH functional, and C4 assay results. The pre-infusion, mean of post-infusion, and contrast between pre-infusion and mean of post-infusion values through 240 minutes are shown by assay type and arm.					
Item	Arm	Time	N	Mean	Standard deviation
C1INH antigenic	placebo	Pre-infusion	33	14.0	18.0
		Mean of post-infusion	31	14.5	17.6
		Contrast	31	0.1	7.2
	C1INH-nf	Pre-infusion	35	14.4	2.0
		Mean of post-infusion	35	23.6	16.9
		Contrast	35	9.2	9.2
C1INH functional	placebo	Pre-infusion	31	32.5	28.2
		Mean of post-infusion	32	34.7	23.4
		Contrast	31	2.9	17.1
	C1INH-nf	Pre-infusion	35	34.5	22.8
		Mean of post-	35	72.6	19.3

		infusion			
		Contrast	35	38.4	17.6
C4	placebo	Pre-infusion	32	6.0	5.0
		Mean of post-infusion	29	5.0	4.5
		Contrast	29	-0.9	1.7
	C1INH-nf	Pre-infusion	34	7.8	8.6
		Mean of post-infusion	31	6.5	3.9
		Contrast	31	-0.9	5.7

The statistical model tests whether the contrast of interest differs between the arms. The P value for this test for the C1INH antigenic and C1INH functional assays are both <0.0001 with estimated contrast differences (and 95% confidence intervals) of 9.2 (5.3 to 13.2) and 37.2 (29.2 to 45.3), respectively. Thus, there is evidence of significant increases in these C1INH levels in the C1INH-nf arm as compared to the placebo arm. It can be seen in Table 1 that the placebo arm contrasts are nearly zero for these C1INH assays ("Contrast" row), whereas these contrasts are greater than zero for the C1INH-nf arm. There is no between-arm contrast difference for the C4 levels P = 0.86 with estimated contrast difference (and 95% confidence interval) of -0.2 (-2.4 to 2.0).

Thus, while there is evidence of C1INH changes in the subjects in the C1INH-nf arm as compared to the subjects in the placebo arm these are based on the group means. The standard deviations in Table 1 show that C1INH is a weak biomarker on which to judge individual response to C1INH-nf intervention.

Appendix Part 3: Treatment-Emergent Adverse Events (TEAEs) in the Prophylactic Study and Open-Label Extension Studies

TEAEs experienced by subjects in the prophylactic study or open-label extension studies are summarized in Table 2. Of the 24 subjects in the prophylactic safety population, 21 (87.5%) had 1 or more TEAEs. One subject (12-002) had only a single TEAE (sinusitis) during the Placebo Arm prior to exposure to open-label or randomized C1INH-nf. The remaining 20 subjects (87.0%) had at least 1 TEAE that followed exposure to open-label or randomized double-blind C1INH-nf.

In subjects treated with C1INH-nf, the most common TEAEs were those that coded to the Infections and Infestations system organ class (14 subjects, 60.9%). Within this system organ class, the most common individual TEAEs were sinusitis (5 subjects, 21.7%), upper respiratory tract infection (4 subjects, 17.4%) and viral upper respiratory tract infection (3 subjects, 13.0%). Gastrointestinal disorders were experienced by 8 subjects (34.8%). The most common TEAEs in this system organ class were gastro-esophageal reflux disease (2 subjects, 8.7%) and vomiting (2 subjects, 8.7%).

Treatment-emergent adverse events that coded to the Skin and Subcutaneous Tissue Disorder system organ class were reported by 6 subjects (26.1%). TEAEs within this system organ class included rash (5 subjects, 21.7%), pruritus (2 subjects, 8.7%), dermatitis contact (1 subject, (4.3%) and erythema (1 subject, 4.3%). Headache was reported by 4 subjects (17.4%). No other individual TEAE was reported by >2 subjects.

Table 2 Treatment Emergent Adverse Events

System Organ Class Preferred Term	Open-label or Double-blind C1INH-nf n (%) (N=23)	Overall n (%) (N=24)
Subjects with at least 1 TEAE	20 (87.0)	21 (87.5)
Blood and Lymphatic System Disorders	2 (8.7)	2 (8.3)
Anemia	1 (4.3)	1 (4.2)
Lymphadenopathy	1 (4.3)	1 (4.2)
Congenital, Familial and Genetic Disorders	2 (8.7)	2 (8.3)
Hereditary angioedema	2 (8.7)	2 (8.3)
Eye Disorders	2 (8.7)	2 (8.3)
Blepharospasm	1 (4.3)	1 (4.2)
Conjunctivitis	1 (4.3)	1 (4.2)
Gastrointestinal Disorders	8 (34.8)	8 (33.3)
Abdominal pain	1 (4.3)	1 (4.2)
Constipation	1 (4.3)	1 (4.2)
Diarrhea	1 (4.3)	1 (4.2)
Gastrointestinal pain	1 (4.3)	1 (4.2)
Gastro-esophageal reflux disease	2 (8.7)	2 (8.3)
Nausea	1 (4.3)	1 (4.2)
Vomiting	2 (8.7)	2 (8.3)
General Disorders and Administrative Site Conditions	4 (17.4)	4 (16.7)
Atrophy	1 (4.3)	1 (4.2)
Chest discomfort	1 (4.3)	1 (4.2)
Pain	1 (4.3)	1 (4.2)
Pyrexia	1 (4.3)	1 (4.2)
Infections and Infestations	14 (60.9)	15 (62.5)
Acute sinusitis	1 (4.3)	1 (4.2)
Bronchitis	2 (8.7)	2 (8.3)
Bronchitis acute	1 (4.3)	1 (4.2)
Ear infection	1 (4.3)	1 (4.2)
Fungal infection	1 (4.3)	1 (4.2)
Gastritis viral	1 (4.3)	1 (4.2)
Gastroenteritis viral	2 (8.7)	2 (8.3)
Herpes simplex	1 (4.3)	1 (4.2)
Influenza	1 (4.3)	1 (4.2)
Nasopharyngitis	2 (8.7)	2 (8.3)
Otitis media	1 (4.3)	1 (4.2)
Pneumonia	1 (4.3)	1 (4.2)

System Organ Class Preferred Term	Open-label or Double-blind C1INH-nf n (%) (N=23)	Overall n (%) (N=24)
Sinusitis	5 (21.7)	6 (25.0)
Upper respiratory tract infection	4 (17.4)	4 (16.7)
Urinary tract infection	2 (8.7)	2 (8.3)
Vaginal candidiasis	1 (4.3)	1 (4.2)
Varicella	1 (4.3)	1 (4.2)
Viral upper respiratory tract infection	3 (13.0)	3 (12.5)
Vulvovaginal mycotic infection	1 (4.3)	1 (4.2)
Injury, Poisoning and Procedural Complications	3 (13.0)	3 (12.5)
Contusion	1 (4.3)	1 (4.2)
Excoriation	1 (4.3)	1 (4.2)
Joint injury	1 (4.3)	1 (4.2)
Limb injury	2 (8.7)	2 (8.3)
Skin laceration	1 (4.3)	1 (4.2)
Thermal burn	1 (4.3)	1 (4.2)
Wound	1 (4.3)	1 (4.2)
Investigations	1 (4.3)	1 (4.2)
Antibody test abnormal	1 (4.3)	1 (4.2)
Metabolism and Nutrition Disorders	1 (4.3)	1 (4.2)
Vitamin B12 deficiency	1 (4.3)	1 (4.2)
Musculoskeletal and Connective Tissue Disorders	5 (21.7)	5 (20.8)
Back pain	2 (8.7)	2 (8.3)
Musculoskeletal pain	1 (4.3)	1 (4.2)
Musculoskeletal stiffness	1 (4.3)	1 (4.2)
Pain in extremity	2 (8.7)	2 (8.3)
Nervous System Disorders	5 (21.7)	5 (20.8)
Carpal tunnel syndrome	1 (4.3)	1 (4.2)
Dizziness	1 (4.3)	1 (4.2)
Headache	4 (17.4)	4 (16.7)
Psychiatric Disorders	1 (4.3)	1 (4.2)
Depression	1 (4.3)	1 (4.2)
Reproductive System and Breast Disorders	1 (4.3)	1 (4.2)
Vulvovaginal discomfort	1 (4.3)	1 (4.2)

System Organ Class Preferred Term	Open-label or Double-blind C1INH-nf n (%) (N=23)	Overall n (%) (N=24)
Respiratory, Thoracic and Mediastinal Disorders	5 (21.7)	5 (20.8)
Cough	2 (8.7)	2 (8.3)
Laryngeal edema	1 (4.3)	1 (4.2)
Nasal congestion	1 (4.3)	1 (4.2)
Pharyngolaryngeal pain	1 (4.3)	1 (4.2)
Rhinorrhea	1 (4.3)	1 (4.2)
Sinus congestion	1 (4.3)	1 (4.2)
Skin and Subcutaneous Tissue Disorders	6 (26.1)	6 (25.0)
Dermatitis contact	1 (4.3)	1 (4.2)
Erythema	1 (4.3)	1 (4.2)
Pruritus	2 (8.7)	2 (8.3)
Rash	5 (21.7)	5 (20.8)
Vascular Disorders	1 (4.3)	1 (4.2)
Poor venous access	1 (4.3)	1 (4.2)

Note: Only 1 subject had a TEAE (sinusitis) after treatment with Placebo and prior to any exposure to open-label or double-blind C1INH-nf.

There were 5 serious adverse events (SAE) in the prophylactic study that resulted in hospitalization of the subjects (4 occurred during the study and 1 occurred after enrollment but before randomization). Three of the 4 consisted of HAE attacks and 1 was for placement of a port for venous access. None of the SAEs were judged related to C1INH-nf. Table 3 summarizes the SAEs.

Table 3. Serious adverse events (SAE) in prophylactic study

Subject	*Serious adverse event*	*Treatment Period*	*Related*
7	Hereditary angioedema attack	Post Study	Not
15	Hereditary angioedema attack	Placebo	Not
15	Lymphadenopathy and infected cyst	Post Study	Not
19	Hereditary angioedema attack	Placebo	Not
25	Hereditary angioedema attack	Pre Study	Not