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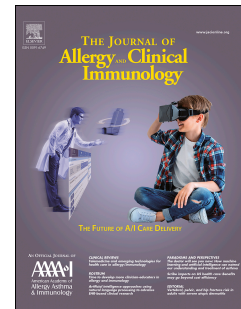
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A Randomized Double-Blinded, Placebo-Controlled Study of Omalizumab for Idiopathic Anaphylaxis

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Conflict of Interest statement:

The authors have no conflicts of interest to report

Abstract:

Background: Idiopathic anaphylaxis (IA) is a diagnosis of exclusion, thus taking away the option of therapeutic management focused on eliminating the inciting agent. Epinephrine and antihistamines followed by systemic corticosteroids are the mainstays of therapy for acute events. There is no prophylactic therapy that reliably prevents anaphylaxis.

Objective: We sought to determine the efficacy of omalizumab in the management of patients with frequent episodes of IA in a DBPC trial.

Methods: We prospectively enrolled 19 patients with frequent IA (≥ 6 episodes/yr) and who then underwent a medical evaluation that included a serum tryptase determination, mutational analysis for *KIT* D816V and bone marrow evaluation to rule out a clonal mast cell disorder. Computer-generated random numbers were provided by the study pharmacist. The primary endpoint was anaphylactic events in the 6 months after baseline. Sixteen patients completed the primary trial.

Results: No statistically significant difference was demonstrated between the placebo and treated groups. There was a trend for efficacy in the treatment group, particularly after 60 days. Overall, the safety profile was favorable without long-term side effects.

Conclusion: Omalizumab was safely administered to a difficult to treat patient population with IA. The efficacy results trended modestly in favor of the treatment group, but no statistically significant differences were detected.

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Key Messages:

1. Administration of omalizumab to patients with recurrent anaphylaxis cannot be expected to prevent subsequent episodes of anaphylaxis and this observation suggests that if omalizumab is administered, careful monitoring is required to evaluate success.
2. Because idiopathic anaphylaxis can decrease in severity and frequency with time, any intervention must be evaluated on a case by case basis for efficacy.

Capsule Summary

Omalizumab administered over six months to patients with idiopathic anaphylaxis in a DBPC trial was found to show no statistically significant difference versus placebo, although there was a trend for efficacy in the treatment group.

Key words

Idiopathic Anaphylaxis
Omalizumab
Randomized
Therapy

Abbreviations

ASqPCR-allele-specific qPCR
bST-baseline serum tryptase
DBPC-Double-blind placebo-controlled
ER-emergency room
IgE-Immunoglobulin E
IA-Idiopathic anaphylaxis
NIH-National Institutes of Health
OMALZ-omalizumab
PLC-placebo

INTRODUCTION

Anaphylaxis is a systemic reaction associated with release of mediators from mast cells and basophils¹. Signs and symptoms of anaphylaxis include generalized flushing, urticaria, nasal congestion, conjunctival irritation, bronchospasm, angioedema of the tongue, throat, palms, and soles, gastrointestinal cramping, lightheadedness, and loss of consciousness. The clinical outcome in anaphylaxis varies from a self-limited episode to fatal circulatory collapse. The occurrence of anaphylaxis reported in epidemiologic studies suggest that 1.2% to 16.8% of the U.S. population suffer from an anaphylactic reaction each year based on the prevalence of allergic patients and that this latter population has a 0.002% annual risk of death². Common causes of anaphylaxis include allergic reactions to foods, drugs, and stinging insects. A causative factor, however, is not identified in up to 50% of the patients with recurrent anaphylactic episodes. Therefore, idiopathic anaphylaxis (IA) becomes a diagnosis of exclusion after other causes have been ruled out in these patients. IA is categorized as frequent (2 episodes in the past 2 months or ≥ 6 episodes in the past year) or infrequent (< 6 episodes in the past year)³.

Management of anaphylaxis consists of administration of epinephrine, protection of airways, and support of circulation. The pattern of IA is unpredictable as well as the response to therapy, and symptoms may cluster with periods of clinical remission. Attempts to prevent or mitigate episodes of anaphylaxis with H1 and H2 antihistamines along with systemic corticosteroids cannot be relied upon in all cases to prevent life-threatening episodes of systemic anaphylaxis and are not without significant side effects. In one retrospective study, high-dose prednisone therapy was administered three times daily for one week or until signs and symptoms were controlled when the prednisone dose was converted to a single dose every other day and then tapered over several months. Patients were also given hydroxyzine with or without ephedrine or albuterol three times daily. Remissions were induced in a substantial number of patients. Patients with infrequent episodes of IA were managed with short term treatment⁴. The reader is referred to the reference for details on drug administration and doses.

The lack of targeted therapy for the treatment of anaphylaxis is, in part, a consequence of the relative absence of data on the entirety of the processes that result in systemic anaphylaxis. Thus, anaphylaxis, in general, has been attributed to activation of mast cells and basophils through antigen-induced aggregation of IgE engaged on the high-affinity receptor for IgE, Fc ϵ RI and subsequent release of potent inflammatory mediators such as histamine. However, blockade of such mediators does not prevent anaphylaxis and does not explain why one person allergic to an antigen has a mild reaction while another suffers a severe systemic reaction. Mast cells are in close proximity to sensory nerves and members of the G-protein receptor family such

as MRGPRX2 can mediate mast cell degranulation may be involved in neuronal excitation⁵. This could be an area of future investigation to identify pathogenic mechanisms associated with IA.

Omalizumab is approved for the treatment of moderate to severe asthma and chronic inducible urticaria or chronic spontaneous urticaria; and acts through a mechanism which targets IgE and is associated with the down-regulation of FcεRI on the surface of basophils, mast cells, and dendritic cells⁶. Omalizumab, an anti-IgE monoclonal antibody, has also been reported to be useful as adjunct therapy in severe food allergy with systemic symptoms (n=84)⁷, eosinophilic gastroenteritis (n=9)⁸ and rush immunotherapy (n=159)⁹. We also have reported that omalizumab prevented episodes of spontaneous anaphylaxis in 2 patients with systemic mastocytosis over a period of 12 years¹⁰. Several case reports have concluded omalizumab prevented anaphylaxis in patients with IA (7)¹¹⁻¹⁷, systemic mastocytosis (1)¹⁸ or monoclonal mast cell activation syndrome (1)¹⁹ over an average time of 11.5 months. The event frequency ranged from weekly to quarterly and several patients demonstrated significant elevations in serum tryptase with an event.

Also, to be considered is a recent report reviewing the data for 132 patients with potential anaphylaxis associated with omalizumab. Seventy-three percent were adjudicated as cases of anaphylaxis related to omalizumab. In this group, 80% were treated for asthma, 84% were female and 43% had a previous anaphylaxis history. This study concluded that patients with a prior history of anaphylaxis unrelated to omalizumab may be more prone to developing anaphylaxis following omalizumab administration²⁰.

The study presented in this report is the first double-blind placebo-controlled trial of the efficacy of omalizumab treatment in preventing episodes of IA in patients where anaphylaxis prior to entry was documented in an ER or doctor's office. Unlike single case reports that may conclude efficacy in one patient, our study evaluated patients in a controlled manner that accounted for a possible placebo effect and spontaneous remission of the disease. As will be presented, we did not demonstrate a statistical difference between the omalizumab and placebo groups, although there was a modest trend for efficacy in the treatment group. Further, we demonstrated a favorable safety profile in a patient population suggested to be at increased risk for anaphylaxis to the study agent.

METHODS

Subjects

Two hundred and sixty individuals were screened to see if they met general criteria for IA. Anaphylaxis was diagnosed by the referral physician using criteria from the summary report on anaphylaxis.²¹ One hundred

and forty-three patients did not meet criteria of anaphylaxis, having only symptoms of flushing or angioedema, or did not complete pre-enrollment paperwork or wish to undergo a bone marrow procedure to rule out clonal mast cell disease. Of the 117 individuals that met criteria for anaphylaxis, 82 patients (age 13-70 years) with a diagnosis of IA defined by current guidelines^{22, 23} and meeting general anaphylaxis protocol entry requirements were enrolled from 22 states, and 1 Canadian province on an IRB-approved NIH protocol (NCT00719719) for further evaluation. Of these enrollees, twelve patients were subsequently diagnosed with either systemic mastocytosis or alpha-gal syndrome (2 with both). Fifty-three of the remaining 70 patients with a diagnosis of IA met the definition of frequent IA, with ≥ 6 episodes of unexplained anaphylaxis within 12 months of study entry, at least one episode in the past two months and at least one event evaluated in a medical facility in proximity to the episode and where the diagnosis of anaphylaxis was confirmed by documenting hypotension and other physical findings.²¹ The additional diagnoses of exercise-induced anaphylaxis and factitious anaphylaxis were ruled out. Thirty-four patients did not enroll for various reasons such as travel, not wanting to be in a DBPC trial and disease improvement with standard of care therapy. Nineteen patients were enrolled on the DBPC trial (NCT00890162), following informed consent (Supplemental Figure). The median age of the patients enrolled was 41 years. Of these, 16 started dosing. Three of these 16 patients were early withdrawals, one after the third dose (OMALZ group) and two after the second dose (OMALZ, PLC) for the following reasons. One patient was seen in the ER at 96 hours after each dose (X3), which at the time we could not rule out attribution to study agent but thought to be associated with food intake. Thus, for patient safety this individual was discontinued in the trial. A second patient 3 weeks after second dose of omalizumab had a prolonged hospital stay unrelated to study agent and was not able to meet the dosing schedule. The third patient experienced a serious adverse event within 24 hours of the second dose consisting of lip edema and flushing and per protocol guidelines, did not receive subsequent dosing. Of the 16 patients that received study drug, 12 were female (75%) and four were male. The majority of patients were White Non-Hispanic (~94%). Prior to enrollment, all patients underwent a complete physical examination, with serum IgE and baseline serum tryptase (bST) measured. A bone marrow aspirate and biopsy performed at the NIH or an outside facility with pathology results confirmed at the NIH could not show evidence of systemic mastocytosis, an exclusion criterion.

Laboratory Studies

Serum tryptase, IgE values and venom-specific IgE

bST levels were determined using a fluoroenzyme Immunoassay (Phadia Immuno CAP, Uppsala, Sweden) at CLIA-approved labs. The normal reference range for this assay is 0.00 – 11.50 ng/ml. The serum IgE level was determined using the Immulite XPI, solid phase chemiluminescence assay (Siemens Medical Solutions, Malvern, PA). IgE levels to honeybee and yellow jacket were screened using a fluoroenzyme immunoassay (Phadia Immuno CAP) with a detection range of 0.35 to >100 IU/ml in Dr. Platts-Mills' laboratory, where values greater than 0.35 IU/ml are considered positive.

Bone marrow examination

Bone marrow trephine biopsies obtained at the NIH were fixed in B-5 fixative, embedded in paraffin and processed for morphology using standard procedures.²⁴ Briefly, immunohistochemical studies with anti-tryptase, anti-CD117 (Cell Marque, Hot Springs, AR) and anti-CD25 antibodies (Vision BioSystems, Norwell, MA) were performed. Bone marrow samples obtained from outside labs underwent immunohistochemical studies only. Multi-parameter flow cytometry was performed after bone marrow aspirates were processed and stained with antibodies for CD2, CD25, CD45 and CD117 as described.²⁴ The detection of the *KIT* D816V mutation was performed by RT-PCR/RFLP in the samples obtained before 2014. The targeted gene region was amplified, and the PCR products subjected to restriction enzymes in order to obtain fragments for analysis as described.²⁴

Starting in 2014, *KIT* D816V allele burden was quantified from the genomic DNA from bone marrow aspirates using real-time quantitative PCR assay (ASqPCR) and primers and probes as described²⁵. Each real-time qPCR reaction was performed in duplicate on the 7500 Real Time PCR System (Applied Biosystems, Foster City, CA) for total of 40 cycles. Results were analyzed using SDS software (Applied Biosystems, Grand Island, NY). *KIT* D816V mutation positive cell fractions of 0.03% and above were considered positive result. Mutation-positive cell fractions between 0.01%- 0.029% were considered trace positive result²⁵.

Study randomization, drug administration and data collection

The randomization sequence was obtained by computer-generated random numbers [36] and was provided by the study pharmacist. The enrollment of patients with serum IgE and height that were within the ranges provided by the US package insert were dosed according to the package insert for asthma. Patients with serum IgE values of <30 IU/mL received 150 mg of omalizumab or placebo every 4 weeks, whereas patients with a higher weight and/or IgE required every 2-week dosing. All dosing (every 2 or 4 weeks) was administered at the NIH Clinical Center. All study agents were prepared by Genentech and the placebo consisted of the vehicle alone. Subjects remained on the assigned regimen (post 24-hour drug administration

period) for 6 months, if any anaphylactic events experienced were not suspected to be related to the study agent. Use of epinephrine and other drugs for treatment of anaphylaxis were allowed as needed for anaphylaxis events. Data was collected on a 2-4-week basis from subjects using the weekly event log and physician reports (both NIH and home) documented at each visit on the physician treatment note.

Study Endpoints

The primary endpoint was the number of anaphylactic events experienced during the 6-month period after randomization. A key secondary endpoint was number of events during the 6 months prior to randomization minus the number of events occurring in 6 months after randomization.

Statistical Analysis

The study was originally designed to have 10 participants per arm; simulations estimated that the study could detect a four-fold event rate ratio with approximately 80% power. All analyses were conducted on the 16 patients who received any study agent. The primary analysis used a Wilcoxon Rank Sum Test on the number of anaphylactic events experienced during the 6-month period after randomization. This test ranks the subjects by sorting them according to the number of events in the 6-month drug period; to break ties between subjects experiencing the same number of events, tied subjects are further sorted such that a subject with an earlier first event is considered to have a worse outcome than a subject with a later first event. The analysis followed the intent-to-treat principle, so that all events counted regardless of whether participants completed the full scheduled drug regimen; however, as pre-specified, the single event that occurred within 24 hours of dosing was eliminated from the analysis. To produce a point estimate and confidence interval for the treatment effect, we estimated the Hodges-Lehmann location shift between the two arms' event rates on the log scale, and this was transformed back to the original scale.

Secondary analyses included: (i) Wilcoxon Rank Sum Test on the number of events recorded in the 6-month period before baseline minus the number of events in the 6-month drug period, and (ii) a recurrent event analysis examining the cumulative incidence rates of the two arms over time via the Wei-Lin-Weissfeld method.²⁶

Several post-hoc exploratory analyses were conducted. Wilcoxon Rank Sum tests compared the arms on the following post-hoc endpoints: (i) percentage decline in events experienced in 6-month drug period as compared to the 6-month period before baseline, (ii) number of events experienced in the 6-month drug period but excluding those events in the first 60 days relative to baseline, and (iii) event rate while on drug. Hodges-

Lehmann confidence intervals were also computed for the median percentage decline. The last of these analyses was not intention-to-treat, as events that occurred after therapy was withdrawn did not contribute to the analysis. As a sensitivity analysis, we performed all primary, secondary, and exploratory analyses again without removing the single event which occurred in the first 24 hours of receiving an injection, and the results, which are not presented, were nearly identical.

All null hypothesis significance testing was done at the two-sided $\alpha = 0.05$ level, and all confidence intervals are reported at the 95% level. All statistical analyses and generation of figures were performed with the use of R, version 3.5.3.

RESULTS

The key baseline variables were generally similar between the omalizumab and placebo groups (Table I). However, despite randomization, the eight patients in the placebo group were female, whereas four of the eight patients in the treatment group were female. There was no significant difference in the average number of events prior to randomization and baseline serum tryptase values were equivalent between both groups (Table). A more detailed account of baseline data for each patient including serum IgE (median 91.8 IU/ml) and tryptase (median 4.2 ng/ml), annual frequency (median 12.0 events/yr) and duration (median 4.0 years) of events in years, symptoms, prior therapy and other diseases are documented in Table II. Sixty-two percent of the patients were atopic by historical and targeted IgE allergen panels and all had been treated with anti-mediator therapy.

A visual representation of the timeline of events over the six months after baseline is shown by arm in Fig. 1. In 13/16 patients (81%), the first event occurred within 30 days of randomization. The figure suggests a trend for better efficacy in the omalizumab group after the 60-day time point.

Comparison of the groups on the primary endpoint (events through 6-months post baseline) is shown in Fig. 2A; the difference was not statistically significant in the pre-specified primary analysis ($p=0.57$, with ties broken by time to first event; exact ordering shown in Table III). Thus, there is insufficient evidence to support efficacy. Analysis of the pre-specified secondary endpoint, the number of events 6 months prior to enrollment minus the number of events during the 6-month treatment period, suggests a possible trend for efficacy of omalizumab (Fig. 2B). Here, larger values are better and visually the omalizumab group is higher, but with considerable overlap with the placebo group ($p=0.26$; Wilcoxon Rank Sum test).

No statistically significant difference was detected on cumulative incidence rates overall ($p=0.92$; Wei-Lin-Weissfeld), but the graphical comparison of mean subject cumulative incidence again illustrates a possible effect for reduced incidence in the omalizumab group after the 60-day time point, which could reflect a delayed

onset of action (Fig. 3A). In a post-hoc analysis where events that occurred in the first 60 days were excluded, the boxplots appear to suggest a greater difference in the two arms than seen in the primary analysis, although still with a non-significant p-value of 0.17 (Fig. 3B).

To examine a possible drug effect from another viewpoint, in a post hoc analysis, we calculated, for each subject, the percent change in number of events experienced during the 6-month drug period as compared to the number of events experienced in the 6-month period prior to baseline. The omalizumab group has a higher median percent change when compared to placebo, 40% vs 13%, respectively (Fig. 4A). The corresponding CIs are wide (omalizumab: (10%, 53%), placebo: (-44%, 49%)). Although this favors the omalizumab group, this did not reach statistical significance. The scatterplot of events pre- and post-baseline is also presented (Fig. 4B), illustrating a similar pattern.

A post-hoc analysis of the female subjects only, repeating the primary analysis, came to the same conclusion as the analysis with all patients (Supplemental Table). This suggests that the failure to detect a treatment effect in the overall analysis was unlikely due to the imbalance in gender between the arms. However, to be clear, there is no basis for evaluating the treatment effect in males, as they are all in one arm.

No patients experienced anaphylaxis attributed to study agent. Although one patient had lip edema and flushing after receiving study agent (<24 hours), objective parameters that would support anaphylaxis such as hypotension, vocal cord edema, hypoxia and syncope were not present. In addition, the patient remained stable for the remainder of the hospitalization (1 day) and after discharge.

DISCUSSION

To our knowledge, this is the only DBPC trial to date to evaluate the efficacy of omalizumab in patients with frequent episodes of IA. This study was designed to detect a large treatment effect (i.e., a four-fold reduction in events), so that the inability to detect a statistically significant differences could be due to a more modest than hypothesized treatment effect. The results in the omalizumab group were numerically better in all efficacy analyses, and indications of a trend favoring omalizumab was most apparent when comparing the arms on (i) the secondary endpoint of number of events prior to therapy minus those on therapy (Fig. 3B), and (ii) the cumulative event incidence which separated after 60 days, extending to the end of the trial (Fig. 3A). In addition, in terms of each individual's percent decline in events from baseline, the results favored omalizumab with a median 40% decline versus 13% in placebo (Fig. 4B).

We documented a favorable safety profile in a patient population that would have been predicted to experience a higher rate of anaphylaxis to omalizumab.²⁰ Only one patient experienced an untoward event to

include lip swelling, mild flushing and anxiety within 24 hours of dosing. However, markers of anaphylaxis such as hypotension, hypoxia, serum tryptase elevation and edema of the vocal cords were not associated with the event. The two remaining patients did not have events associated with study agent administration.

There are several case reports in the literature that relate efficacy of omalizumab for the treatment of IA.^{11-13, 15, 17, 19} These reports are open label and typically involve one index case. However, the natural history of IA is unpredictable, with long periods of remission or complete resolution of the disease²⁷⁻²⁹. In the cases with a single patient, one cannot thus eliminate the influence of the natural disease course or placebo effect that has been shown to be as high as 40%³⁰. Further, as we have shown in a previous study³¹, there may have been an underlying unrecognized condition such as a clonal mast cell disorder, hereditary alpha tryptasemia or alpha-gal syndrome³² that contributed to the symptomatology and responded to omalizumab.

The estimated incidence of anaphylaxis in the general population is about 1.6%³³ and 24-39% are diagnosed with IA^{29, 34, 35}. The ability to detect efficacy in the omalizumab group was likely influenced by the small sample size due to challenges in recruitment of the patients with frequent IA (>6 episodes/yr), which represents a smaller percentage of the total and to the reality that a number of patients with IA in the general population had been placed on omalizumab and therefore were not candidates for this study. Since there is a lag time to decrease the surface IgE from mast cells and basophils, it is possible that better efficacy may have been achieved with a longer study period. We did see a signal for this possibility after 60 days and it has been shown in an urticaria trial³⁶ that maximum efficacy is demonstrated at 120 days.

There were additional limiting factors in this trial that may have contributed to the outcome. All patients had to have a medical facility visit with a diagnosis of anaphylaxis and the degree of disease severity may have had an effect with patients on the milder end of the spectrum undergoing spontaneous resolution. Finally, we cannot eliminate the placebo effect. We would also note that of the 260 screened subjects, 117 met criteria for anaphylaxis and 35 subjects had an antigen identified by the investigators. This finding does imply that care givers as a group may need to expand their differential diagnoses when evaluating patients with anaphylaxis or make sure that they refer to specialists earlier.

We believe that this is an important study in consideration of the evaluation of the efficacy of omalizumab in patients with IA where other treatment options have been exhausted or are not feasible. These patients have significant anxiety and lifestyle-altering changes to their routine that effect their quality of life. Physicians are tasked with finding a treatment that is safe with minimum and efficacious. In patients that appear to demonstrate a response to therapy, there is thus a significant effect benefit. This trial was designed to detect

a large treatment effect of omalizumab, but our results suggest that any treatment effect is more moderate in size. Thus, a larger study with a focus on events occurring after 60 days would contribute to additional insight in defining the role of omalizumab in managing patients with IA.

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Table I: Baseline Demographics and Clinical Characteristics

	OMALZ	PLC	p-value
Median age (Q1, Q3)	43 (30.50, 46.25)	36.5 (23, 58.25)	0.87*
M/F (% Female)	4/8 (50)	8/8 (100)	0.08 [†]
W non-H/total (% White)	7/8 (88)	8/8 (100)	1.00 [†]
Median number of events 6 months prior to baseline (Q1, Q3)	6 (5.75, 7.25)	7.5 (5.75, 8)	0.57*
Median serum tryptase (Q1, Q3)	3.15 (2.98, 5.05)	4.5 (3.69, 5.28)	0.67*

W non-H- White non-Hispanic, IQR-Interquartile range *Wilcoxon Rank Sum Test, [†]Fisher's Exact Test

Table II: Patient Characteristics

Code	Gender	Age	Ethnicity/ Race	IgE IU/L	bST ng/mL	Treatment	Annual Frequency	Duration (yrs)	Symptoms	Prior therapy	Other Diseases
R1	F	23	W-Non-his	165	2.74	PLC	12	8	L, P, W	AH, E	EIB
R2	F	23	W-Non-his	101	5.21	PLC	12	8	A, H, P, W	AH, E, P	DA
R3	M	31	A-Non-his	229	2.39	OMALZ	12	1	H, L, W	AH, E, P, M, AHP	FA, A, AR, HBP, GERD
R4	M	51	W-Non-his	82.6	3.27	OMALZ	6	2	F, H, L, W	AH, E, P, M, A, AD, PSY	DA, RCM, VA, AG
R5	F	44	W-Non-his	12.6	3.02	OMALZ	10	22	A, D, F, P, S	AH, E, M, I	A, AR, VA, POD
R6	M	42	W-Non-his	252	11.2	OMALZ	8	2	D, F, H, P, S	AH, E, AD, MF	A, DB, AT
R7	F	40	W-Non-his	43.4	4.3	PLC	8	2	F, L, P, W	AH, E, M, NCS	A, DA, FA,
R8	F	33	W-Non-his	520	5.5	PLC	12	2	A, L W	AH, E, AS	None
R9	F	58	W-Non-his	30.8	14.4	PLC	10	12	D, P	AH, E, K, P, AD, C	A, IBD, AX, AT
R10	F	19	W-Non-his	73.6	4	PLC	12	2	A, F, L	AH, E, M	SAR, PAR
R11	F	27	W-Non-his	50.9	2.9	OMALZ	20	5	A, D, F, H, P, W	AH, E, M, C, I,	DP, FA, DA
R12	M	50	W-Non-his	112	5.5	OMALZ	6	8	A, DS, F, P	AH, E, AD, AHP, P,	AX, RA, GERD, DP
R13	F	69	W-Non-his	5.1	2	PLC	13	8	A, D, DS, F	AH, E, M	Osteopenia, OAS, SAR
R14	F	59	W-Non-his	102	4.7	PLC	6	3	D, F, H, P	AH E,	DA, IBS
R15	F	29	W-Non-his	2.5	4.9	OMALZ	15	1.5	A, DS, F, P, V	AH, E, AD, AHP, M	AX, HA
R16	F	45	W-Non-his	168	3	OMALZ	12	7	A, D, P	AH, E,	CD, HA, AX
Median		41		91.8	4		12	4			

*-median, †-average; bST-baseline serum tryptase, Non-his-non-Hispanic, A-Non-his-Asian non-Hispanic; PLC-placebo, OMALZ-study drug

Symptoms: A-angioedema, D-diarrhea, DS-dyspnea, F-flushing, H-hives, L-laryngospasm, P-presyncope, S-syncope, V-vomiting, W-wheezing

Prior therapy: A-anticonvulsant, AD-antidepressant, AH-antihistamine, AHP-antihypertensive, AS-aspirin, C-corticosteroids, CR-cromolyn, E-Epinephrine prn, I-inhaled CS, M-montelukast, MF-metformin, PSY-parasympathomimetic

Other diseases: A-Asthma, AG-alpha-gal syndrome, AR-allergic rhinitis, AT-alpha-tryptasemia, AX-Anxiety, CD-celiac disease, DA-drug allergy, DB-diabetes, DP-depression, EIB-exercise-induced bronchospasm, HA-headache, HBP-hypertension, FA-food allergy, OAS-oral allergy syndrome, RA-rheumatoid arthritis, RCM-radiocontrast sensitivity, T-thyroid disease, VA-venom allergy

Table III: Primary Endpoint-Number of events 6-months post-baseline

ID	Treatment	# of Events from baseline to 6-Months post-baseline	# of days between baseline and first event
R8	PLC	1	137
R2	PLC	2	61
R4	OMALZ	2	29
R6	OMALZ	2	3
R12	OMALZ	3	12
R16	OMALZ	3	10
R1	PLC	3	2
R15	OMALZ	5	28
R9	PLC	6	38
R5	OMALZ	6	2
R14	PLC	7	14
R3	OMALZ	7	4
R13	PLC	8	19
R11	OMALZ	8	5
R10	PLC	9	16
R7	PLC	9	10

Wilcoxon Rank Sum Test exact p-value: 0.57, Hodges-Lehman Estimate of (Event Rate OMALZ) / (Event Rate PLC) = 0.85, Confidence Interval for H-L Estimate: [0.33, 2.00]. Ties broken by number of days between baseline and first event, where a patient with a later first event relative to baseline is considered better than a patient with an earlier first event relative to baseline.

Figure Legends:**Figure 1: Timeline of events**

This timeline visually compares the omalizumab group to the placebo group for timing and number of events during 6-month study period. The red line represents the end of dosing period based on 2-4-week dosing, which may fall prior to the end of 6 months. Total events: OMALZ-36, PLC-45.

Figure 2: Number of events during 6-month study period by arm.

The boxplot figures show the number of events each subject experienced during the 6-month study period. (A) The primary analysis is not statistically significant (Wilcoxon rank sum test with ties broken by time to first event, $p=0.57$; complete data shown in Supplemental Table II. The secondary analysis with ties not broken was also not significant: $p=0.46$.) (B) A secondary endpoint analysis compared the number of events prior to study agent minus events on study agent. Here, the larger number is better and box plots show a trend, which did not reach significance (Wilcoxon rank sum test, $p=0.26$). Post-Hoc analysis of the percent change for each subject was calculated comparing the number of events 6 months prior to study agent (baseline) vs the percent change in number of events while on study agent.

Figure 3 A, B: Mean Subject Cumulative incidence by arm across follow-up and post-hoc comparison of post-60-day events

(A) The mean number of events experienced per subject (y-axis) by any given number of days since baseline (x-axis). There event trajectory between the two arms is not significant Wei-Lin-Weissfeld, $p=0.92$), but with some indication of separation after 60 days. (B) A post-hoc analysis of events after 60 days on therapy demonstrate a greater degree of separation between the arms, but still not statistically significant (Wilcoxon rank sum test, $p=0.17$).

Figure 4 A, B: Change in number of events experienced from pre to post baseline

(A) The OMALZ group had a higher median percent decline in events (40%) compared to the PLC group (13%) The p-value of 0.32 and wide 95% confidence interval of the treatment difference of (-29%, 62%) are insufficient to demonstrate efficacy. In a related display (B), regression lines for OMALZ and PLC groups estimate the number of events in the treatment period compared to baseline. The PLC line lies above the OMALZ line again suggesting a trend toward a greater decline in events in the OMALZ arm.

Figure 1: Timeline of Events

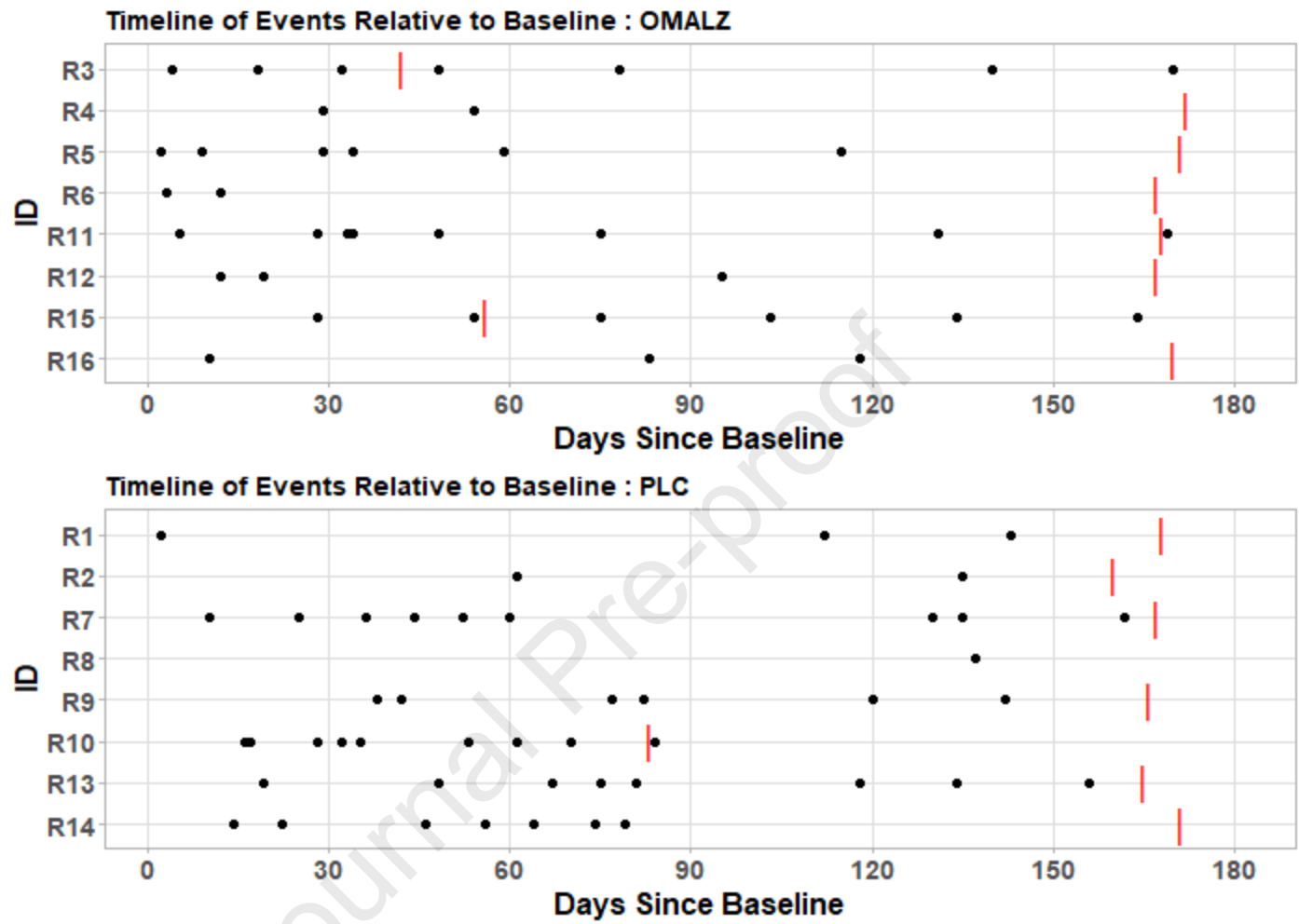
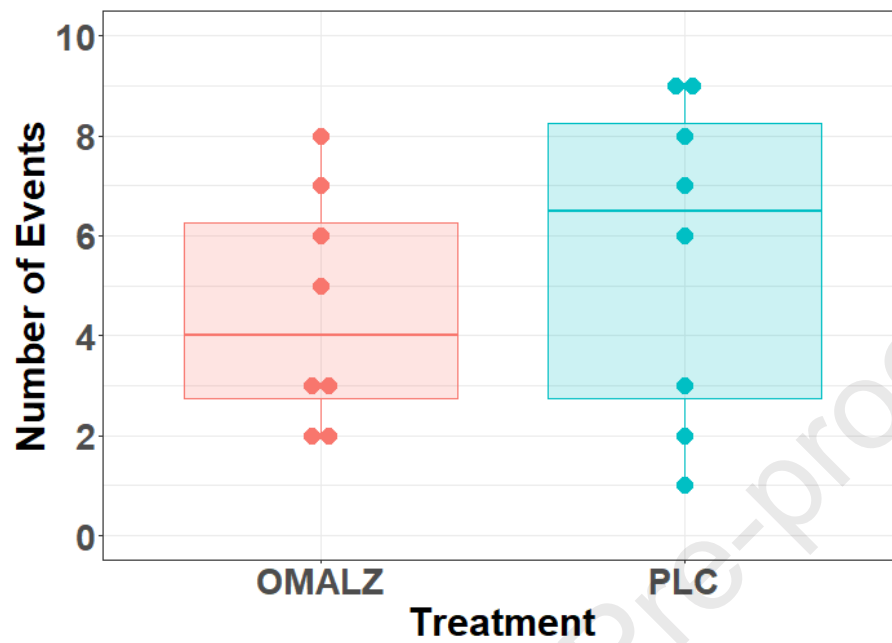


Figure 2: Events during 6-month study period

A.



B.

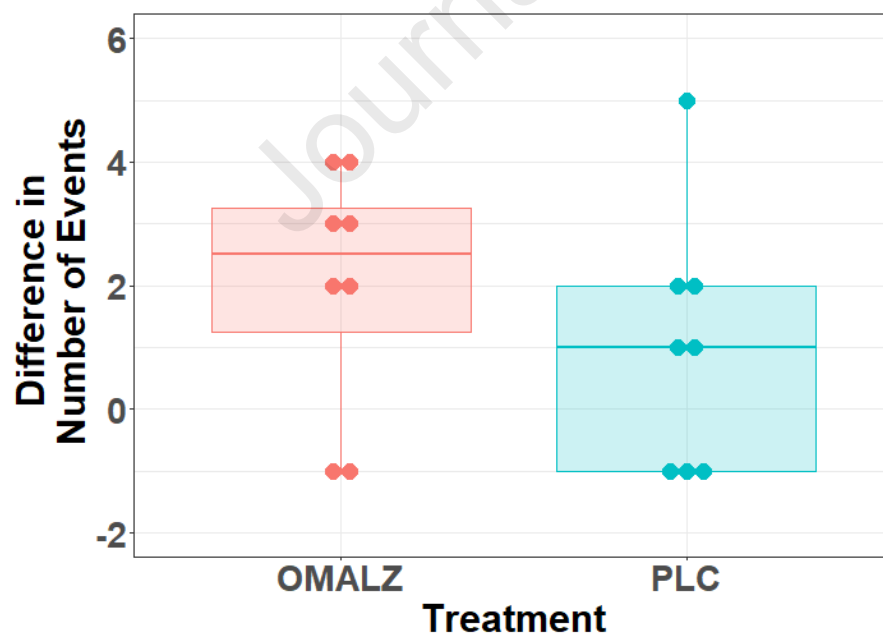
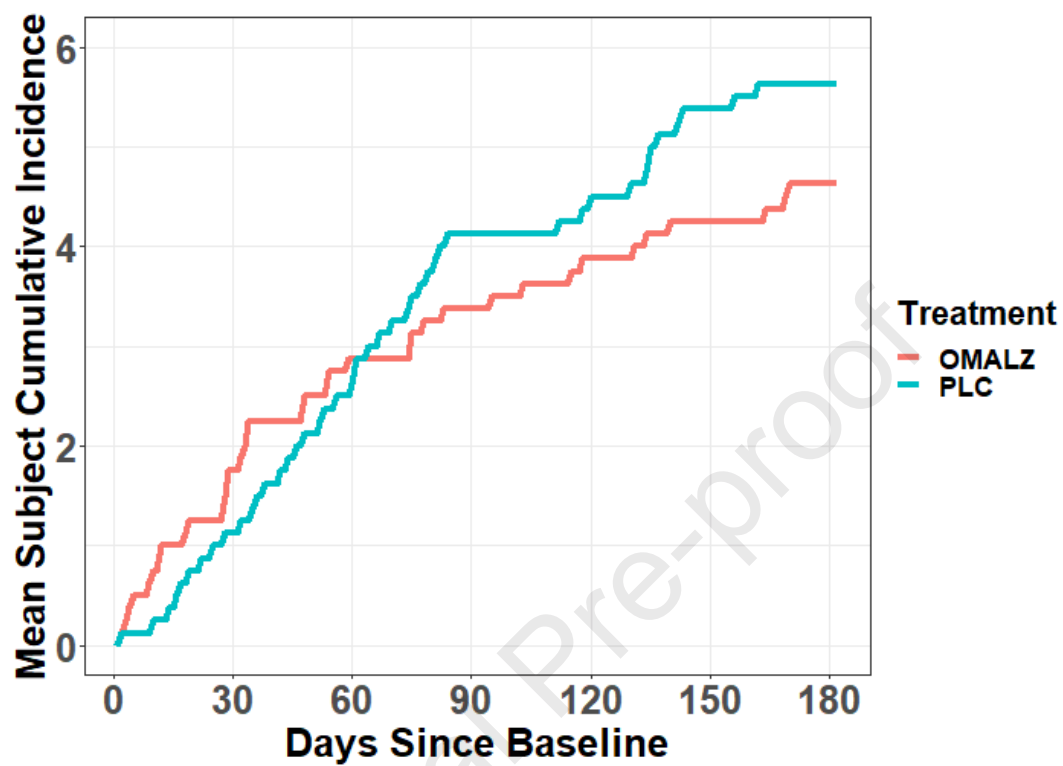
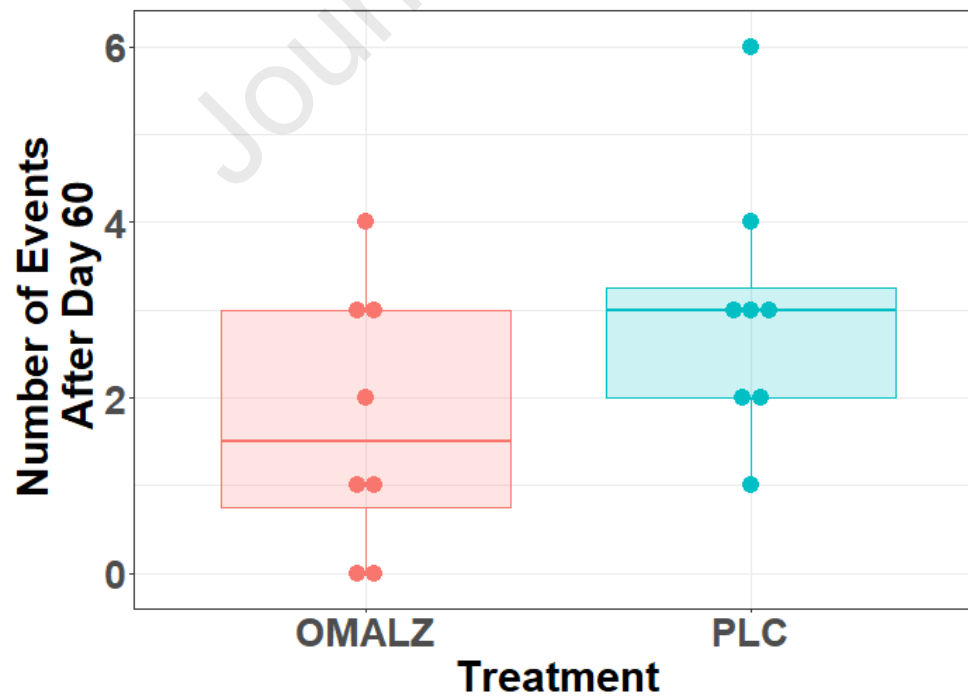


Figure 3: Cumulative incidence of recurrent events

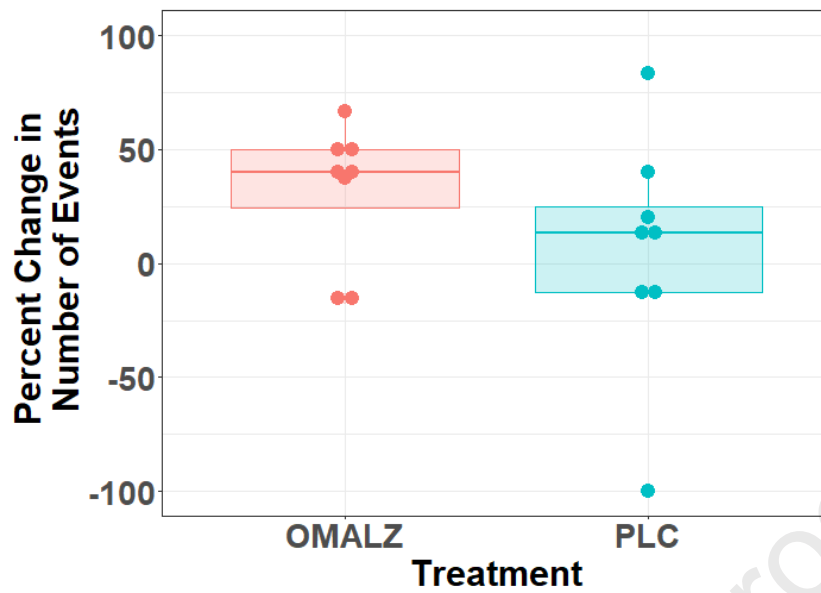
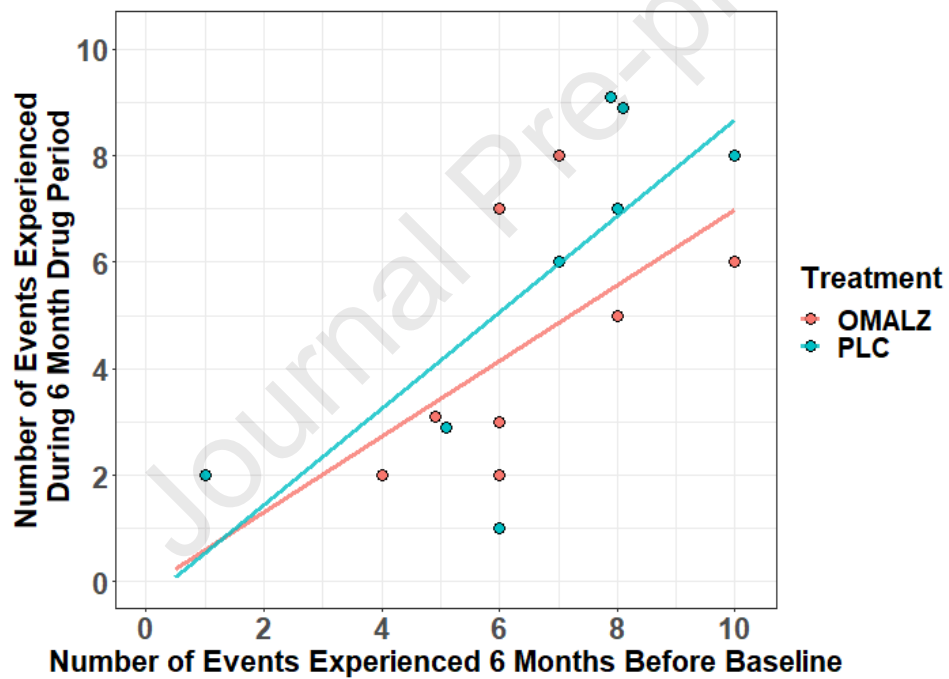
A.



B.

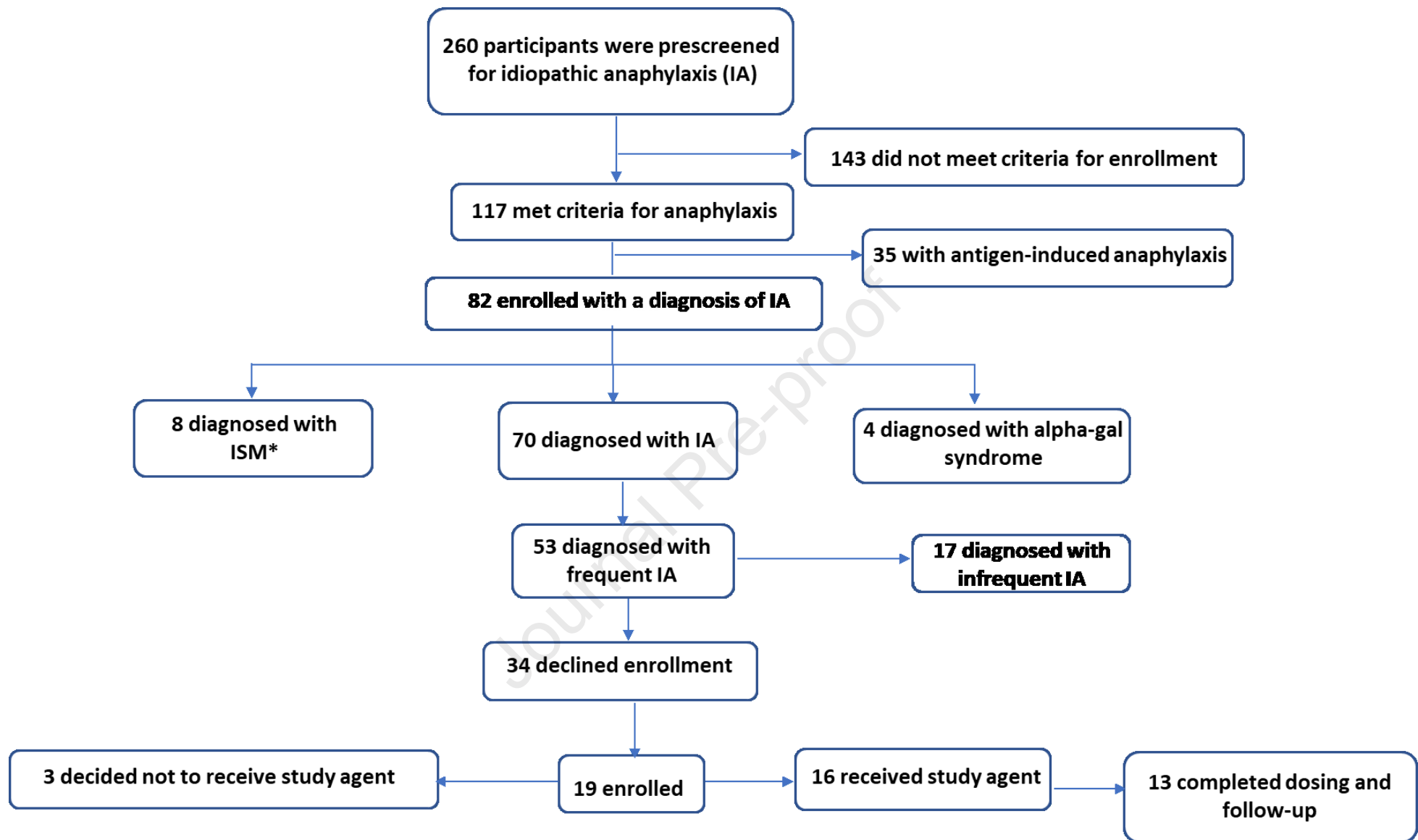


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Figure 4: Change in number of events experienced pre and post baseline**A.****B.**

Supplemental Figure: Enrollment and Randomization

Protocol candidates (n=82) needed a physician referral with a diagnosis of IA to be considered for enrollment for further screening. These individuals were further screened to meet criteria for anaphylaxis using standard accepted criteria, which was documented in a medical facility (emergency room/physician's office). After further stratification to meet criteria for frequent IA (>2 episodes in the past 2 months or ≥ 6 episodes/yr), 53 candidates were diagnosed with frequent IA. Nineteen of 53 patients were enrolled, in part because some candidates did not wish to undergo a bone marrow examination or were unable to commit to the full course of the omalizumab protocol. Sixteen received study agent and 13 completed dosing and all completed follow-up.



Supplemental Table

Table: Post-Hoc Analysis-Treatment effect in female subjects only

ID	Treatment	# of events from baseline to 6-months post-baseline	# of days between baseline and first event
R8	PLC	1	137
R2	PLC	2	61
R16	OMALZ	3	10
R1	PLC	3	2
R15	OMALZ	5	28
R9	PLC	6	38
R5	OMALZ	6	2
R14	PLC	7	14
R13	PLC	8	19
R11	OMALZ	8	5
R10	PLC	9	16
R7	PLC	9	10

Wilcoxon Rank Sum Test exact p-value: 0.93, Hodges-Lehman Estimate of (Event Rate OMALZ)/(Event Rate PLC)[†] = 0.94, Confidence Interval for H-L Estimate : [0.50, 3.00]; Ties broken by number of days between baseline and first event, where a patient with a later first event relative to baseline is considered better than a patient with an earlier first event relative to baseline. In 13/16 patients, the first event occurred within 30 days of randomization.