

An intranasal Syk-kinase inhibitor (R112) improves the symptoms of seasonal allergic rhinitis in a park environment

Eli O. Meltzer, MD,^a Robert B. Berkowitz, MD,^b and Elliott B. Grossbard, MD^c San Diego and South San Francisco, Calif, and Woodstock, Ga

Background: R112 inhibits Syk kinase, a transducer of signaling through the Fcε receptor of mast cells, blocking mast cell responses to allergic stimuli.

Objective: Examine the efficacy and safety of intranasal R112 in volunteers with symptomatic seasonal allergic rhinitis compared with a placebo in a park setting.

Methods: In this double-blind, placebo-controlled study of 319 volunteers with seasonal allergic rhinitis, 160 were randomized to intranasal R112 and 159 to a vehicle control during 2 days at 2 separate locations in spring 2004. Subjects were evaluated for symptoms of allergic rhinitis (ie, sneezes, runny nose/sniffles, itchy nose, stuffy nose) on the basis of a possible maximum score of 32 for the Global Symptom Complex (GSC) scale. The primary outcome evaluated was the difference in the reduction in GSC (area under the curve over a period of 8 hours) from baseline between R112 and vehicle placebo.

Results: At baseline, the combined GSC was ~18/32 and equal between treatment groups. After 8 hours (dosing 3 mg/nostril every 4 hours × 2), R112 significantly reduced the GSC compared with placebo (7 vs 5.4 units, respectively; $P = .0005$). Each individual symptom combined to form the GSC was also significantly improved in the R112 group compared with control ($P < .05$). As early as 45 minutes after dosing, R112 showed a significant improvement in symptoms over placebo, and the duration of action exceeded 4 hours. Adverse effects were indistinguishable between the groups and clinically insignificant.

Conclusion: Intranasal R112 was effective in this park study and is a promising new treatment for seasonal allergic rhinitis. (*J Allergy Clin Immunol* 2005;115:791-6.)

Key words: Allergic rhinitis, mast cell inhibition, R112, park study, mast cell mediators, Syk-kinase

Given the prevalence of seasonal allergic rhinitis (SAR) in the United States,¹ and the limitations of current therapy to treat some patients successfully,¹⁻³ finding novel interventions that target alternative mechanisms is vital to improving outcomes. Rather than antagonizing individual inflammatory mediators that are released after IgE receptor signal transduction, the new agent R112 inhibits Syk kinase, an intracellular protein tyrosine kinase that plays a key role in mast cell and basophil activation (Fig 1).^{4,5} This activity inhibits the rapid release of histamine, tryptase, and other granule contents as well as the proinflammatory process that causes release of cytokines (TNF-α, IL-4, IL-13) and lipid-derived mediators (cysteinyl leukotrienes, prostaglandin D₂).

In this phase II double-blind, randomized, placebo-controlled study conducted at 2 outdoor sites, the safety and efficacy of intranasal administration of R112 were compared with vehicle placebo in volunteers with symptomatic SAR during 2 days in a park setting. The primary outcome evaluated was the difference in the change from baseline in the Global symptom complex (GSC; area under the curve [AUC] over a period of 8 hours) between R112 and vehicle placebo. Secondary outcomes included evaluation of R112 effects on individual nasal symptom scores, time of onset and duration of effect of R112, and the safety of R112.

METHODS

Study design

This was a double-blind, randomized, placebo-controlled, parallel-group study conducted over a period of 2 days at 2 different outdoor sites, Atlanta and San Diego, in spring 2004. The trial design included a screening period, a placebo run-in period, participation by eligible patients in park day 1, participation by eligible patients in park day 2, and a final follow-up visit. The Sterling Institutional Review Board in Atlanta approved the protocol, and each subject gave written informed consent.

Patients

Volunteers were included if they met the following criteria: male or female; age 12 years or older; history consistent with SAR during

From ^athe Allergy and Asthma Medical Group and Research Center, San Diego; ^bRx Research, Woodstock; and ^cRigel Pharmaceuticals, South San Francisco.

Supported by Rigel Inc, San Francisco, Calif.

Disclosure of potential conflict of interest: E. O. Meltzer is a consultant to Alcon, Altana, AstraZeneca, Sanofi, Aventis, Corixa, Critical Therapeutics, Dey, Genentech, GSK, Greer, Merck, MedPointe, Pfizer, Rigel, Schering, and Wyeth; has received grants/research support from Alcon, Altana, AstraZeneca, Sanofi, Aventis, Genentech, GSK, Novartis, Pfizer, Schering, Rigel, Wyeth, and Merck; is employed by the Allergy and Asthma Medical Group and Research Center; and is on the speakers' bureau for Sanofi, Aventis, AstraZeneca, Genentech, GSK, Merck, Pfizer, and Schering. R. Berkowitz is a consultant for multiple pharmaceutical companies and has received multiple research grants. E. Grossbard has stock/other equity ownership in Rigel and is employed by Rigel.

Received for publication December 15, 2004; revised January 14, 2005; accepted for publication January 24, 2005.

Reprint requests: Eli O. Meltzer, MD, Allergy and Asthma Medical Group and Research Center, 9610 Granite Ridge Drive, Suite B, San Diego, CA 92123-2661. E-mail: eomeltzer@aol.com.

0091-6749/\$30.00

© 2005 American Academy of Allergy, Asthma and Immunology

doi:10.1016/j.jaci.2005.01.040

Abbreviations used

ANCOVA: Analysis of covariance
AUC: Area under the curve
GSC: Global symptom complex
ITT: Intent-to-treat
SAR: Seasonal allergic rhinitis

the spring pollen season for 2 or more years; skin test positive to regional, seasonal spring pollen within the past 12 months with at least a moderate reaction (3 mm wheal greater than the diluent after skin prick test); nasal mucosa consistent with diagnosis of allergic rhinitis; negative urine pregnancy tests in female subjects at visits 1 and 2; and adequate contraception throughout study.

Patients were excluded if they had a clinically significant medical condition (eg, hepatic, neurologic, hematologic, renal, cardiac, gastrointestinal, endocrine, or other major systemic disease) that could interfere with the study; a clinically relevant abnormal laboratory or electrocardiogram result; asthma treated with therapy other than an inhaled, short-acting β -agonist; a nasal structural abnormality; an upper respiratory infection within 3 weeks of randomization; treatment with an investigational drug within 30 days of study entry; or a history of drug or alcohol abuse that could interfere with the study. Also excluded were pregnant or nursing women. Subjects were not allowed to be on any of the following medications after day -7 until visit 5, nor within the time indicated per medication before the start of the placebo run-in period (day -7): systemic or injected corticosteroids (30 days); systemic antibiotics, nasal/inhaled/ocular corticosteroids, nasal/inhaled ipratropium bromide/nedocromil/sodium cromoglycate, leukotriene pathway modifiers, ocular antiallergy medications, oral antihistamines, or intranasal antihistamines (14 days); topical nasal decongestants (7 days); and pseudoephedrine or other oral decongestants or ocular or nasal saline (3 days).

Study sequence

The study sequence is shown in Fig 2. During study visit 1, subjects were screened within 30 days of treatment to determine eligibility and the requirements for exclusionary drugs washout. Before randomization, eligible subjects underwent a 7-day, single-blind, placebo run-in period during which they self-administered placebo twice daily and scored symptoms on run-in diary cards. The completed run-in diary cards were reviewed and scored. Subjects were permitted to participate in park day 1 if they scored ≥ 10 of 24 (symptoms of sneezing, itchy nose, stuffy nose, runny nose/sniffles, postnasal drip/throat clearing, watery eyes, itchy eyes, itchy ears/palate/throat, rated from none [0] to severe [3]) on at least 5 of the 14 recorded time points with $\geq 50\%$ of the total score in the nasal symptom categories (sneezing, itchy nose, stuffy nose, runny nose/sniffles, postnasal drip/throat clearing), and continued to abstain from prohibited medications.

Qualification for randomization on park day 1 was determined by symptom assessment, which was based on nasal symptoms composed of the possible maximum combined GSC score of 32: 0 to 8 points for sneezing and 0 to 4 points for each nostril for congestion, rhinorrhea, and nasal itch at 3 time points (0700, 0730, and 0800 hours). Subjects who had scores of a least 20 of 96 (possible maximum score of 32×3 assessments) qualified. Priority of randomization went to subjects who qualified earlier (at first or second time points) and to those with the highest qualifying symptom scores if they had the same number of assessments. After obtaining symptom scores before dosing at -60 and -5 minutes (averaged as the subject's baseline GSC score), the study drug was assigned and

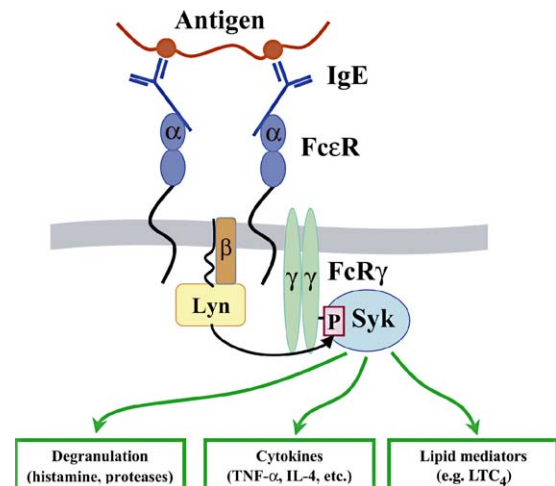


FIG 1. Allergen cross-binding to IgE bound to Fc ϵ receptor on mast cell initiates lyn phosphorylation of receptor and Syk activation with downstream effects that include degranulation as well as lipid mediator and cytokine synthesis and release.

distributed to the subjects qualified for randomization. Group dosing was coordinated so that all subjects had the same baseline time points, 2 dosing times, symptom diary card times, global assessment times, and therapeutic response times. After 8 hours of observation and self-assessment, subjects went home. They returned the next day to repeat the dosing and assessment procedures on park day 2. The total duration of time at the park was about 11 hours on park day 1 and 9 hours on park day 2. At least 6 pollen counts were recorded at each office facility from day -8 through day 3. A final follow-up visit took place within a week of park day 2 to evaluate safety and adverse events.

Treatment

Patients were randomized to receive intranasal R112 or a vehicle placebo. Treatment was administered by a metered spray pump delivering 100 μ L (0.1 mL) per actuation. Each milliliter of drug contained 15 mg R112 and formulation excipients. Patients self-administered 2 actuations (200 μ L) per nostril at 9:00 AM and 1:00 PM on park day 1 and at 8:00 AM and 12:00 PM on park day 2. If rescue medications were needed after park day 2, patients could receive desloratadine or fexofenadine hydrochloride.

Efficacy measures

The prespecified principal endpoint was the effect of treatment on the combined GSC. Secondary endpoints included individual nasal symptom scores, subject global assessment (based on patient scoring from 0 [no symptoms] to 4 [severe symptoms] to the following question: "How would you rate your overall symptoms since taking your first dose of study medication this morning?"), and subject therapeutic response (based on patient scoring from 1 [much better] to 7 [much worse] to the following question: "Compared to before you took your first dose of study medication this morning, how are you feeling now?").

Every subject had a GSC calculated for every time point and the change from baseline for each GSC was computed to generate a curve of change versus time. GSC change from baseline was averaged over time by using the normalized AUC via the trapezoidal method. For each subject at each park day, the AUC was defined as the area under the time profile for the GSC change from baseline score divided by the duration of assessment (8 hours for subjects who completed the

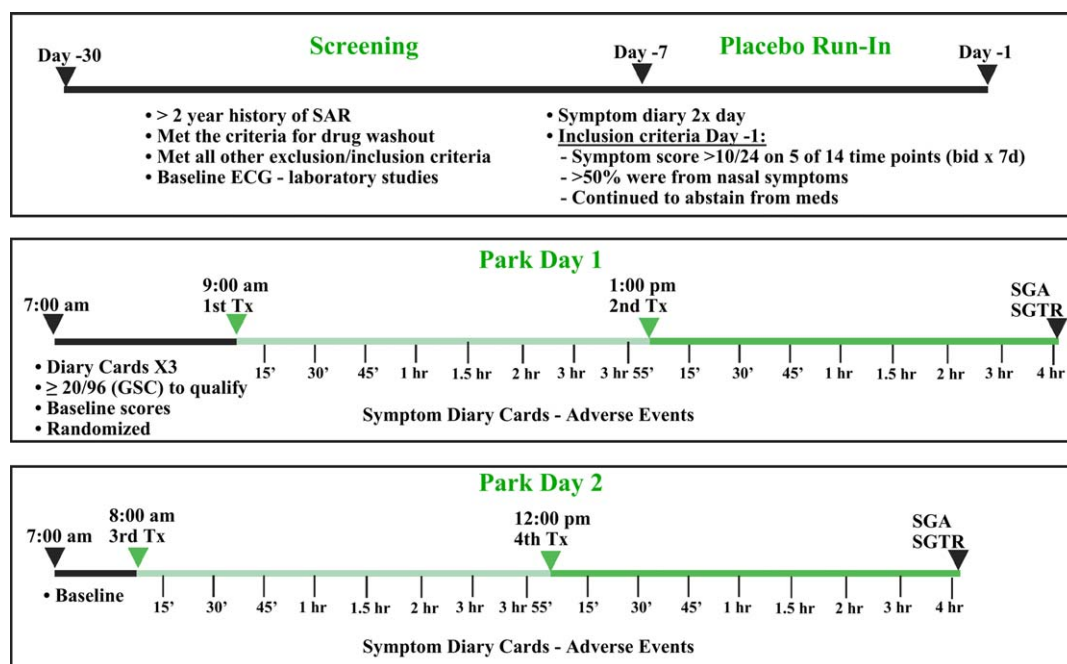


FIG 2. Park study schedule of events. *bid*, Two times daily; ECG, electrocardiogram; SGA, subject global assessment; SGTR, subject global therapeutic response.

observed period). The AUC was a weighted average with the weights determined by the time between assessments.

Secondary efficacy evaluation included (1) change from baseline in the AUC values for each individual symptom for park day 1 and 2, (2) change from baseline at each assessment time for the combined GSC score, (3) change from baseline at each assessment time for the subject global assessment score, and (4) subject global therapeutic response at each assessment time. The time to onset of effect (derived from 2) was prespecified as the earliest time point at which there was a statistically significant difference in the GSC between active and control that was sustained for at least 1 additional consecutive time point.

Safety

Safety was assessed by using adverse events, clinical laboratory evaluations (complete blood count and serum chemistry), vital signs, physical and nasal examinations, and electrocardiograms.

Statistical analysis

Efficacy analyses were based on the intent-to-treat (ITT) population of all randomized subjects who received at least 1 dose of study medication and had at least 1 symptom assessment after the first dose of study medication ($n = 319$), and a per-protocol population of all randomized subjects who completed the 2 park days with no major protocol violations ($n = 310$).

The difference between treatment groups was analyzed based on an analysis of covariance (ANCOVA) model with effects for study site, treatment group, and the GSC baseline score. Park day 1 and 2 results were analyzed separately. A secondary ANCOVA model that included the interaction between treatment group and study site also was assessed.

Individual symptom AUC was analyzed on the basis of an ANCOVA model similar to that for the primary efficacy parameter. Change from baseline in the combined GSC score and each individual symptom score was calculated by using a Cochran-

TABLE I. GSC score*: Change in normalized AUC from baseline

	R112	Placebo	P value	Improvement relative to placebo (%)
Park day 1				
N	160	159		
Mean (SD)	-7.0 (4.67)	-5.4 (4.07)	.0005	23.7
Park day 2				
N	157	153		
Mean (SD)	-5.6 (4.49)	-4.3 (4.14)	.0016	25.1

*Based on AUC for ITT population.

Mantel-Haenszel mean score test stratified by study site, and by using modified ridit scores.

Summary statistics for the change from baseline for the subject global assessment score and numeric subject global therapeutic response were calculated at the end of the day for park day 1 and 2. Treatment groups were compared for both variables by using Cochran-Mantel-Haenszel mean score test stratified by study site, and by using modified ridit scores.

RESULTS

Patients

A total of 462 subjects were screened for the study from 2 study sites, 266 from the site in Atlanta and 196 from the site in San Diego. From these, 320 (69.2%) met study criteria and were enrolled in the study. Across both sites, the mean age was 32.2 years (range, 12-69 years), 35% were male and 65% female, and 48% were black

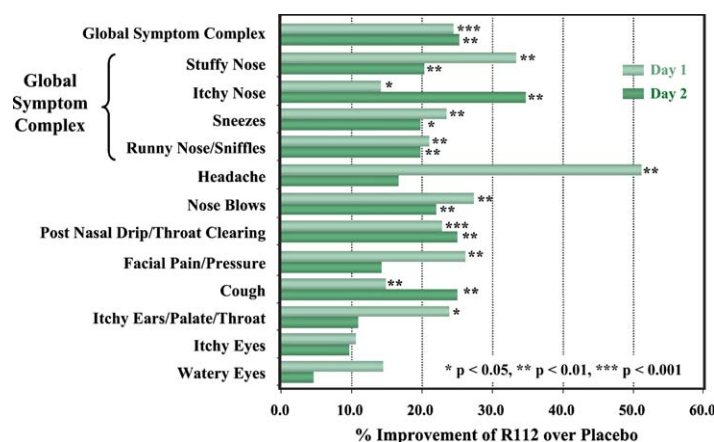


FIG 3. Comparison of improvement (%) in individual symptoms and overall GSC between patients receiving R112 and those receiving placebo.

TABLE II. Subject global assessment score*: Change from baseline, Mean (SD)

	R112	Placebo	P value
Park day 1			
N	160	159	
Mean (SD)	-0.82 (0.85)	-0.58 (0.98)	.015
Park day 2			
N	157	153	
Mean (SD)	-1.04 (0.94)	-0.87 (0.89)	.109

*Responses measured on scale from 0 to 4.

TABLE III. Subject global therapeutic response*: Mean (SD)

	R112	Placebo	P value
Park day 1			
N	160	159	
Mean (SD)	2.96 (1.25)	3.38 (1.36)	.0032
Park day 2			
N	157	153	
Mean (SD)	2.83 (1.22)	3.16 (1.30)	.0279

*Responses measured on scale from 1 (much better) to 7 (much worse).

and 42% white. Of the 320 enrolled subjects, 160 were randomized to R112 nasal spray and 160 to vehicle control.

Pollen counts

Pollen counts were measured at least 6 times from day – 8 to day 3. The mean raw pollen count in April in Atlanta was 523.8 (SD, 382.8) and 39.5 (16.8) in May in San Diego.

Primary efficacy outcome

GSC. The baseline combined GSC on park day 1 was 18.4 (± 4.65) for the R112 group and 18.6 (± 5.43) for the vehicle control group. Fifty percent of subjects had baseline GSCs between 15 and 22, with a range of 6 to 29.5. On day 2, the corresponding baseline GSCs were 15.9 (± 5.27) and 15.9 (± 6.24), respectively. On park days 1 and 2, patients treated with R112 had statistically significant improvement in the GSC compared with placebo ($P = .0005$ and $P = .0016$, respectively; Table I). There were no significant differences in outcome between the 2 treatment sites.

Secondary efficacy outcomes

Individual symptom scores. Individual symptom score as measured by AUC analysis showed a significant improvement for patients receiving R112 compared

with placebo in stuffy nose, itchy nose, sneezes, runny nose/sniffles, nose blows, postnasal drip/throat clearing, and cough on park days 1 and 2 and headache, facial pain/pressure, and itchy ears/palate/throat on park day 1 (Fig 3).

Subject global assessment score. On park day 1, patients treated with R112 had significantly greater improvement in subject global assessment than placebo, with a trend toward greater improvement on park day 2 as well (Table II).

Subject global therapeutic response score. The response was significantly better in the R112 group than the control group on both park day 1 and park day 2 for the ITT population (Table III).

There were no significant differences between the 2 sites for outcome related to global assessment score or global therapeutic response.

Onset and duration of response. On park day 1, R112 was associated with a significantly greater improvement compared with placebo as early as 30 and 45 minutes ($P = .0547$ and $P = .0192$, respectively; Fig 4). At 8 hours on park day 1 and park day 2, the median change from baseline in the R112 group was -7.8 and -6.5, respectively, compared with -5.5 and -5.0 in the control group ($P = .0014$ and $P = .0069$, respectively). This suggested that the duration of effect went well beyond 4 hours after dosing.

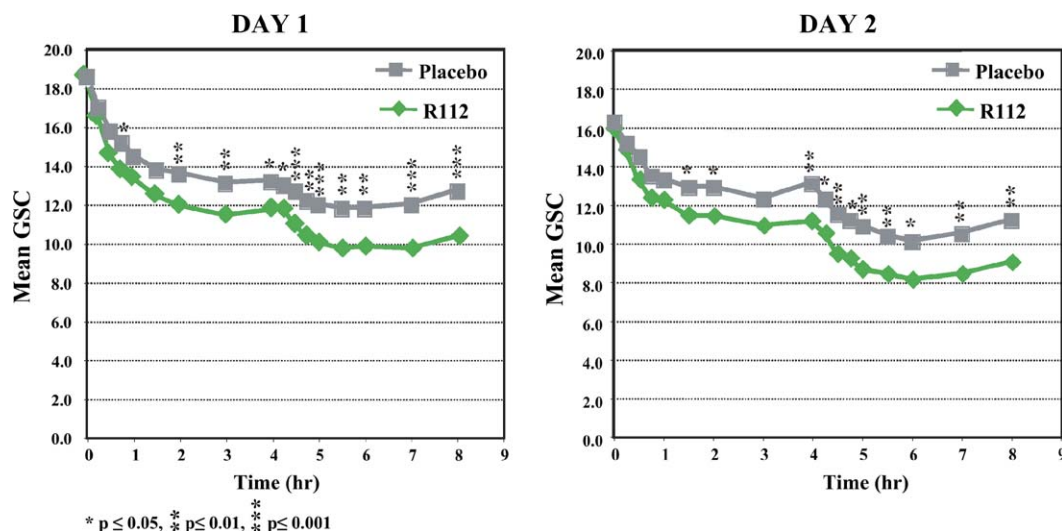


FIG 4. GSC score over time; park day 1 and 2.

Safety

Overall, the treatment was well tolerated, with 1 or more minor adverse events reported in 34 of 160 (21.3%) patients treated with R112 and 33 of 160 (20.6%) of patients treated by placebo. There were no serious adverse events; most events were mild to moderate and resolved uneventfully (Table IV). Discontinuation of therapy because of adverse events occurred in 5 subjects, 3 in the placebo group and 2 in the R112 group. No notable differences were found between treatment groups in clinical laboratory results, vital signs, physical or nasal examinations, and electrocardiogram findings.

DISCUSSION

Although the role of mast cells in the allergic response is well recognized, this is the first clinical demonstration of the importance of the intracellular role of Syk kinase in that pathway. Activation of Syk via the binding of ligand to IgE bound to the IgE receptor leads to an array of responses including degranulation with release of histamine and tryptase, neosynthesis leading to leukotriene and prostaglandin D_2 release, and cytokine production. Recently a nasal allergen challenge study demonstrated that inhibition of that pathway reduces mediator release.⁶

The current study, conducted in a park setting, is the first to demonstrate that R112 provides rapid amelioration of clinical symptoms widely attributed to IgE–mast cell–triggered airway inflammation. A park setting was chosen because of the demonstrated sensitivity of this model for proof of principle to evaluate short-term efficacy and onset of action of drugs used to treat allergic rhinitis.^{7,8} One of the most notable clinical outcomes in the current study is the rapid onset of effect of R112 relative to what may be expected from intranasal corticosteroids.^{9,10} Other notable outcomes are the substantial clinical improvement in the combined GSC as well as in the broad

TABLE IV. Selected adverse events

	R112 (n = 160)	Placebo (n = 160)
Nasal passage irritation	1 (0.6%)	0 (0.0%)
Nasal passage ulceration	1 (0.6%)	0 (0.0%)
Application site burning	2 (1.3%)	1 (0.6%)
Epistaxis	1 (0.6%)	2 (1.3%)
Nasopharyngeal irritation	1 (0.6%)	0 (0.0%)
Rashes	0 (0.0%)	1 (0.6%)
Facial swelling	0 (0.0%)	1 (0.6%)
Palpitations	0 (0.0%)	1 (0.6%)
Somnolence	2 (1.3%)	0 (0.0%)

range of individual symptoms (such as cough, headache, and, importantly, nasal congestion) that are usually minimally benefited by antihistamines.¹¹

Because R112 is not absorbed into the systemic circulation, the duration of effect can be estimated only from the pharmacodynamic effect. In this study, that effect was the duration of symptom relief. Because dosing in the study was every 4 hours during the park day, the most that can be inferred from the very strong effects still observed at the end of the observation period is that the duration of effect significantly exceeds 4 hours. How much beyond 4 hours this effect lasts, however, cannot be determined from this study. It is known from cell-based washout studies that the effect of R112 is rapidly reversible, and thus, the clinical results suggest that tissue concentrations from intranasal dosing are probably well above the concentration needed or that there is a reservoir of drug in the tissue after nasal application.

This current study also confirms the safety of R112 observed in 3 previous phase I studies. All 3 trials demonstrated good tolerability of nasal R112 (Data on file; Rigel Inc).⁶ The results of this and previous studies document that Syk-kinase inhibition can reduce allergic

inflammation. These data also suggest that that R112 is a safe and effective novel agent to treat the symptoms of SAR and is worthy of further clinical investigation.

REFERENCES

1. Bousquet J, Van Cauwenberge P, Khaltaev N, Aria Workshop Group, World Health Organization. Allergic rhinitis and its impact on asthma. *J Allergy Clin Immunol* 2001;108(suppl 5):S147-334.
2. Chervinsky P, Busse W, Casale T, Nayak A, Tripathy I, Fowler-Taylor A, et al. Xolair® in the treatment of perennial allergic rhinitis. *J Allergy Clin Immunol* 2001;107(suppl 2):513.
3. Berger WE, Fineman SM, Lieberman P, Miles RM. Double-blind trials of azelastine nasal spray monotherapy versus combination therapy with loratadine tablets and beclomethasone nasal spray in patients with seasonal allergic rhinitis. *Ann Allergy Asthma Immunol* 1999;82:535-41.
4. Niimi T, Orita M, Okazawa-Igarashi M, Sakashita H, Kikuchi K, Ball E, et al. Design and synthesis of non-peptidic inhibitors for the Syk C-terminal SH2 domain based on structure-based in-silico screening. *J Med Chem* 2001;44:4737-40.
5. Wong BR, Grossbard EB, Payan DG, Masuda ES. Targeting Syk as a treatment for allergic and autoimmune disorders. *Expert Opin Investig Drugs* 2004;13:743-62.
6. Guyer BJ, Shimamoto SR, Bradhurst A, Grossbard E, Dreskin S, Nelson H. The effect of a novel inhibitor of mast cell activation on mediators, symptoms and nasal patency in allergic rhinitis. *J Allergy Clin Immunol* 2004;113(suppl 2):S28-9.
7. US Department of Health and Human Service, Food And Drug Administration, Center for Drug Evaluation and Research (CDER). Guidance for industry, allergic rhinitis: clinical development programs for drug products. April 2000. Available at: <http://www.fda.gov/cder/guidance/2718dft.pdf>. Accessed March 4, 2005.
8. Meltzer EO, Weiler JM, Widlitz MD. Comparative outdoor study of the efficacy, onset and duration of action, and safety of cetirizine, loratadine, and placebo for seasonal allergic rhinitis. *J Allergy Clin Immunol* 1996;97:617-26.
9. Meltzer EO, Rickard KA, Westlund RE, Cook CK. Onset of therapeutic effect of fluticasone propionate aqueous nasal spray. *Ann Allergy Asthma Immunol* 2001;86:286-91.
10. Berkowitz RB, Roberson S, Zora J, Capano D, Chen R, Lutz C, et al. Mometasone furoate nasal spray is rapidly effective in the treatment of seasonal allergic rhinitis in an outdoor (park), acute exposure setting. *Allergy Asthma Proc* 1999;20:167-72.
11. Dykewicz MS, Fineman S, Skoner DP. Joint Task Force summary statements on diagnosis and management of rhinitis. *Ann Allergy Asthma Immunol* 1998;81:474-7.