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Safety and tolerability of a novel inhaled GATA3 mRNA targeting DNAzyme in patients with T_H2-driven asthma

To the Editor:

Asthma comprises several clinical phenotypes associated with different pathophysiologically defined endotypes. There is medical need for novel endotype-specific treatment options that ideally interfere with upstream targets in the respective pathophysiologic pathways. The T_H2-driven endotype represents a prominent subtype, and the transcription factor GATA-3 plays a key role in T_H2 cell differentiation and activation by indispensably controlling T_H2-derived cytokine (IL-4, IL-5, and IL-13)

production.¹⁻³ Sel et al⁴ reported DNAzymes, a new class of antisense molecules with inherent enzymatic activity that specifically bind to and subsequently cleave target RNA,⁵ that cleave GATA3 mRNA (see Fig E1 in this article's Online Repository at www.jacionline.org). No major safety concerns were identified in a rigorous toxicology program for this drug candidate,⁶ no off-target effects were identified,⁷ and intranasal administration to mice resulted in rapid lung tissue distribution.⁸ A human whole blood assay further excluded immunotoxic effects of the novel drug candidate (see the Methods section and Figs E2 and E3 in this article's Online Repository at www.jacionline.org), and an appropriate nebulization system was identified and characterized for inhaled drug application (see the Methods section, Fig E4, and Table E1 in this article's Online Repository). The primary objective of the 3 phase I studies reported here (a first-in-human/first-in-class single-dose study in healthy subjects, a multiple-dose study in healthy subjects, and a single-dose study in asthmatic patients; Fig 1, A) was to assess the safety and tolerability of ascending doses of inhaled SB010, the active pharmaceutical ingredient of which is the GATA-3-specific DNAzyme hgd40 (for details of the drug product and active pharmaceutical ingredient, see the Methods section in this article's Online Repository). Secondary objectives included determination of dose-limiting adverse effects and the pharmacokinetic profile. In addition to strict adherence to standard guidelines on study design, additional expert panel recommendations were implemented to monitor the potential lung toxicity of inhaled oligonucleotides.⁹ The study in asthmatic patients was carried out to exclude drug-induced airway hyperresponsiveness.

The 3 studies were conducted with a total of 108 subjects (75 receiving active treatment and 33 receiving placebo) and 720 single administrations (534 receiving active treatment and 186 receiving placebo). Variation in demographic characteristics between groups and between studies was minimal (see Table E2 in this article's Online Repository at www.jacionline.org for demographic details). As expected, the baseline FEV₁ and forced vital capacity values of the asthmatic patients were less than those of the healthy subjects. All dose-escalation steps went ahead as planned in the 3 studies (Fig 1, A), and no relevant protocol deviations occurred during any of the studies. Safety and tolerability were assessed by means of adverse event reporting, safety laboratory assessments, and pulmonary function testing (see the Methods section and Table E3 in this article's Online Repository at www.jacionline.org for details regarding conduct of clinical studies).

Regarding safety and tolerability, no serious adverse events occurred in any of the studies, and no adverse events led to discontinuation of study medication in the multiple-dose study (Fig 1, B). No significant differences were observed in the total number of adverse events or proportion of subjects with adverse events between the active treatment and placebo groups in any of the studies. In the single-dose and multiple-dose studies in healthy subjects, a causal relationship with study drug was considered either unlikely or, at most, possibly related. Among the asthmatic patients, 1 adverse event was considered certainly related (a subject in the 20-mg dose group [DG] with a 28.6% decrease in FEV₁ compared with predose FEV₁ within 15 minutes of inhalation and spontaneous recovery within an hour) and another adverse event was considered probably related (a subject in the 5-mg DG with subjective bronchospasm 2 hours after administration that resolved spontaneously and caused no discomfort). Although a causal relationship to treatment with SB010 itself is

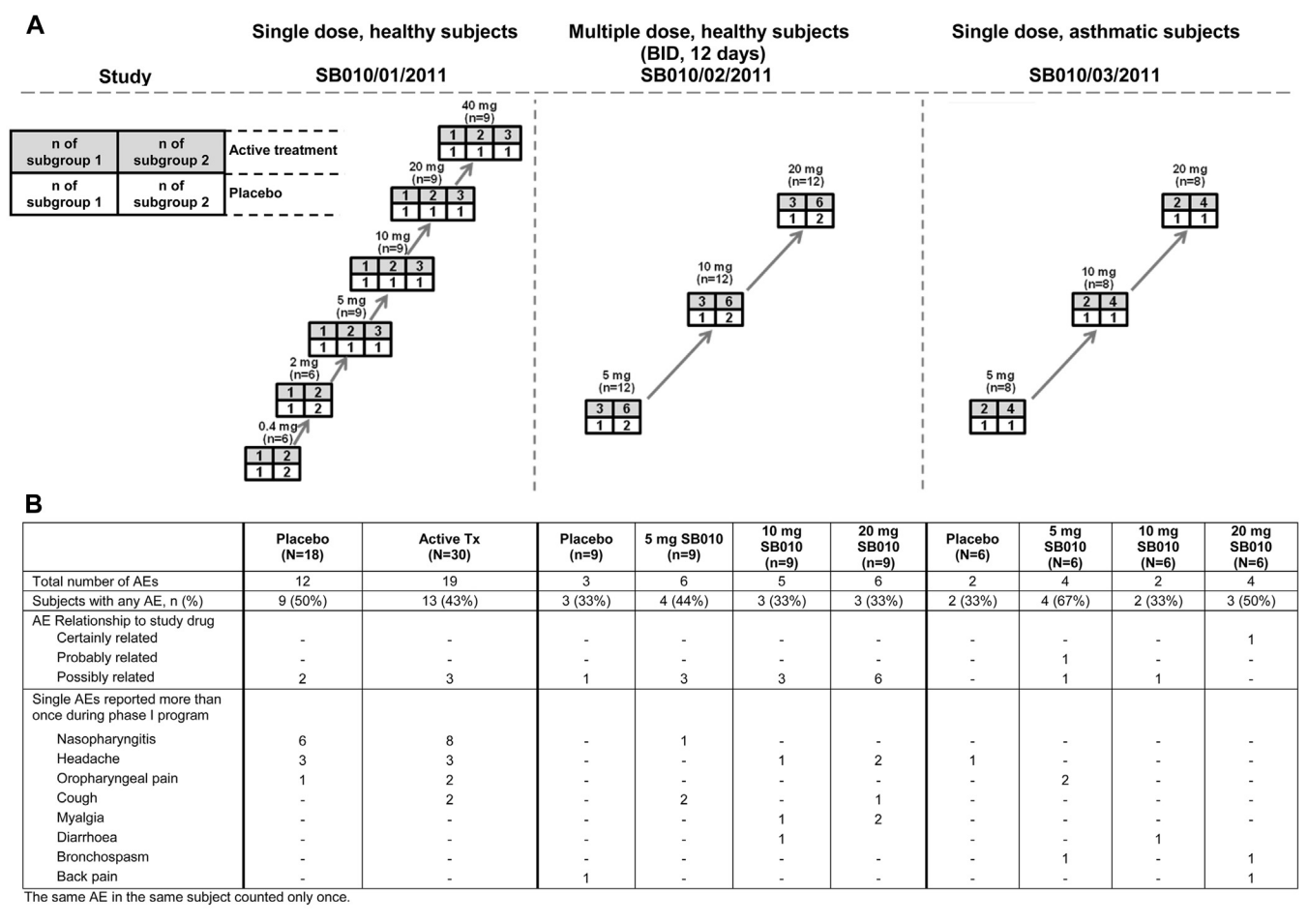


FIG 1. Overview of phase I study dose-escalation designs (**A**) and tabulated summary of treatment-emergent adverse events (**B**). All DGs in all studies were divided into randomized subgroups to minimize the number of subjects per group receiving initial or increased doses of SB010 on the same day. AE, Adverse event.

possible, the observed events might also correspond to mucosal irritation caused by inhalation of the nebulizer formulation. Other respiratory complaints reported were cough (reported only in healthy subjects) and oropharyngeal pain (all were mild and resolved spontaneously). No abnormal findings were reported in electrocardiograms, safety laboratory parameters, or vital signs in any of the studies. The low number of adverse events reported is consistent with the very low systemic exposure and limited off-target effects detected in the toxicology program.^{6,7}

With respect to pharmacokinetics, drug plasma concentrations remained less than the lower limit of quantitation (0.752 ng/mL) in all subjects from the 0.4- and 2-mg DGs and in 4 of the 6 subjects in the 5-mg DG after a single dose of SB010 administered to healthy subjects. In the 20- and 40-mg DGs, all subjects had quantifiable plasma concentrations, with maximums observed between 0.5 and 2 hours after administration (Fig 2, A). After administration of 5 and 10 mg of SB010 to healthy subjects in the multiple-dose study, the maximum plasma concentration (C_{max}) and area under the curve (AUC) were greater on day 1 than on day 12. After administration of 20 mg of SB010, C_{max} and AUC values appeared to increase from day 1 to day 12, although the SD was very high (Fig 2, D and E). The mean (SD) accumulation index at this highest DG was 1.78 (1.74) for AUC and 2.06 (1.84) for C_{max} (calculated for 7 subjects in both cases).

Trough concentrations between day 1 and day 12 were generally less than the limit of quantification (with the exception of 2 subjects, one in the 10-mg DG on the morning of day 6 and one in the 20-mg DG on the evening of day 10). After administration of a single dose to asthmatic patients, 5 of the 6 subjects in the 5-mg DG and all subjects in the 2 highest DGs had quantifiable concentrations, although with high interindividual variability (Fig 2, B). Exposure measured based on C_{max} and AUC values increased nonlinearly with increasing dose in healthy subjects and in asthmatic patients (Fig 2, C). Assuming that the concentration delivered to the lungs is proportional to the dose, the nonlinearity is likely to arise in the systemic absorption step, saturated metabolism/elimination, or both. It could be that local concentrations need to be greater than a certain level for systemic absorption to occur. Comparison of exposure after a single dose and after multiple doses (on day 12) was inconclusive, with contradictory results at the 10- and 20-mg doses (see Table E4 in this article's Online Repository at www.jacionline.org). This discrepancy is probably the result of the small number of subjects and the relatively large individual variation. Asthmatic patients had generally greater systemic exposure than healthy subjects at a given dose, most probably because of compromised epithelium.⁹

In conclusion, the phase I program for SB010, a novel inhaled DNzyme targeting GATA3 mRNA, showed the agent to be safe

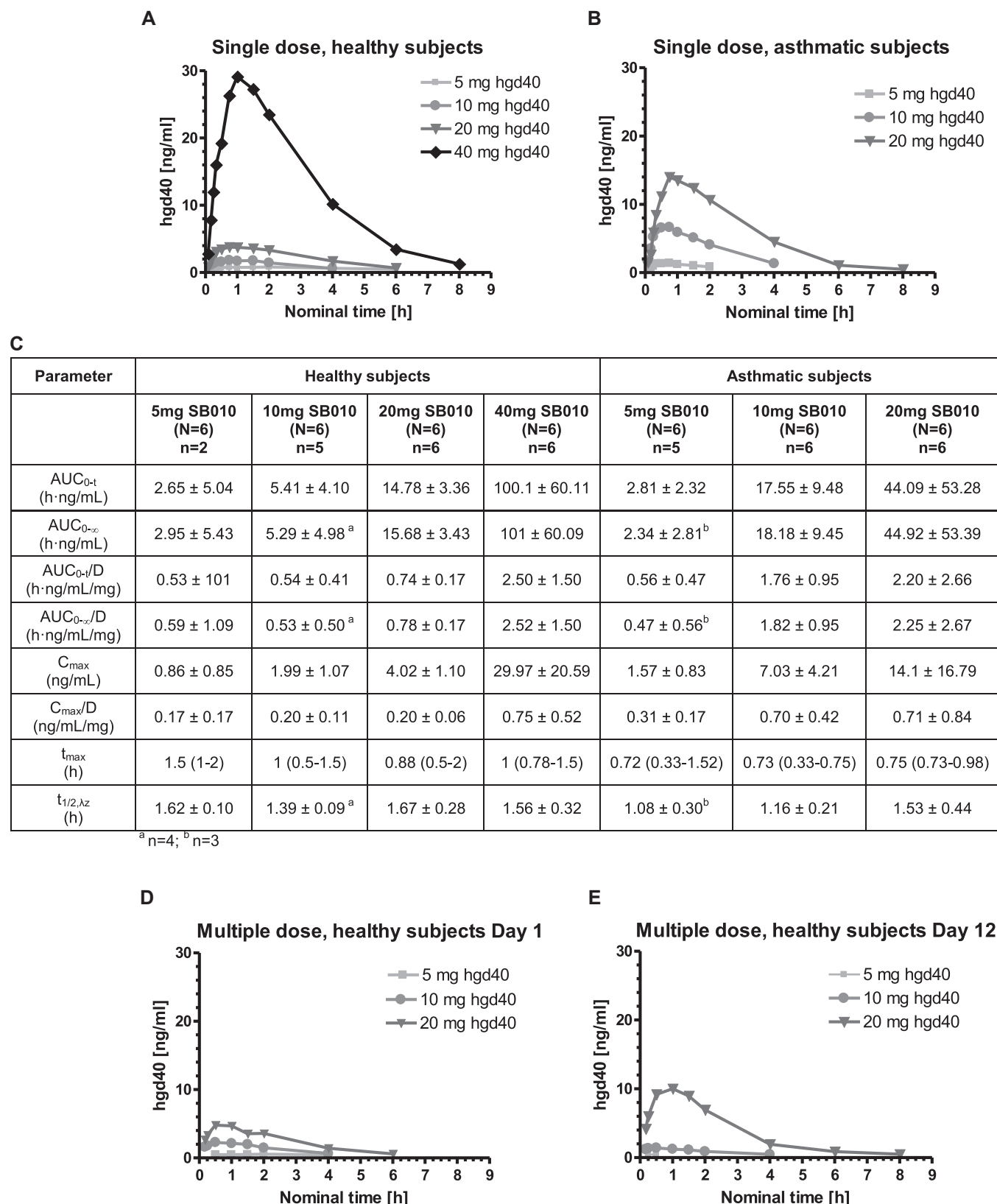


FIG 2. Overview of pharmacokinetic data from single-dose (**A-C**) and multiple-dose (**D** and **E**) phase I studies. Kinetics of mean drug plasma concentrations after single administration to healthy subjects (Fig 2, **A**) and asthmatic patients (Fig 2, **B**) are accompanied by a summary of the main pharmacokinetic parameters (Fig 2, **C**) in these study groups. Mean drug plasma concentrations of healthy subjects in the multiple-dose study are demonstrated after administration on day 1 (Fig 2, **D**) and day 12 (Fig 2, **E**). *N* and *n* refer to the number of subjects dosed and the number of subjects with a valid value available, respectively. AUC_{0-t} , Area under the curve up to the end of sampling; $AUC_{0-\infty}$, area under the curve extrapolated to infinity; *D*, dose (in milligrams); t_{max} , time to maximum concentration; $t_{1/2, \lambda z}$, terminal half-life of elimination.

and well tolerated in the limited number of subjects exposed. In view of the good safety profile and limited systemic exposure found in the phase I program, a phase IIa proof-of-concept study in asthmatic patients (clinicaltrials.gov identifier: NCT01743768) was initiated.

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A novel, nonanaphylactogenic, bispecific IgE-CD3 antibody eliminates IgE⁺ B cells

To the Editor:

IgE, the key pathogenic molecule underlying all allergic disorders, such as asthma and atopic dermatitis, represents an attractive target for therapeutic approaches. The protagonist omalizumab, a humanized anti-IgE mAb, has demonstrated moderate clinical benefit in asthmatic patients, and its effects are mainly characterized by decreased airway inflammation and reduced rate of exacerbation.¹

Recently, IgE⁺ B cells were found in various subsets, such as germinal center B cells and plasma cells, and in addition, IgE⁺ memory B cells were identified as the primary source of IgE.² Initial attempts to eliminate IgE⁺ B cells were made by Lustgarten and Eshhar³ using genetically modified T cells carrying a chimeric anti-IgE T-cell receptor. From the earliest anti-IgE animal studies onward, there has been discussion on whether the administered anti-IgE antibodies not only targeted soluble serum IgE levels but also affected B lymphocytes displaying membrane-bound IgE as an antigen receptor. When vaccinated with syngeneic IgE, long-term IgE suppression was observed in neonatal mice, and in subsequent studies induced anti-IgE antibodies were identified as being responsible for the *in vivo* inhibition of IgE synthesis, as well as for reducing the memory IgE response.⁴ Further evidence came from passive immunization of mice with syngeneic antibodies directed at the extracellular membrane-proximal domain of IgE.⁵ These high-affinity anti-extracellular membrane-proximal domain antibodies suppressed a primary IgE response in mice simultaneously immunized with recombinant Bet v 1a, the dominant birch pollen allergen.

In view of the astounding clinical efficacy of the bsc-CD19/CD3 T cell-engaging antibody, we felt justified to engineer an according bsc-IgE/CD3 construct.⁶ Here we report on the construction and characterization of a bsc-IgE/CD3 antibody as a new therapeutic approach for IgE-mediated diseases (see Fig E1 in this article's Online Repository at www.jacionline.org).