Statistics

The confirmatory analysis was based on the full analysis set (FAS) with baseline LOAEL instead of missing values at end of study if applicable and intention-to-treat principle. We focused on two primary endpoints without hierarchy – both $LOAEL_{obj}$ and $LOAEL_{subj}$ as it was unclear in advance how many patients would react with objective signs at DBPCFC. Postinterventional measures after the ingestion of soya-containing CM were used in nonparametric analysis of covariance (ANCOVA) with corresponding baseline measures as covariates (30). The global significance level of the clinical trial was limited to $\alpha = 5\%$. The testwise α-levels were adjusted for multiplicity according to the Bonferroni–Holm method (31) based on the ordered P values observed. The smaller observed P-values $P(1)$ (corresponding with either $LOAEL_{obj}$ or $LOAEL_{subj}$) had to be lower than/equal to $\alpha(1) = \alpha_{global}/2 = 0.025 \geq P(1)$ to identify a significant treatment effect in at least one LOAEL. For the 2nd comparison, $P(2) \leq \alpha(2) = 0.05$ should be observed to establish significance in both endpoints. SAS macros developed and provided by the University of Göttingen within the German Research Foundation (DFG)-sponsored project 'Ordinal Data' were used to compare the treatments without any further covariates in confirmatory analysis. In a planned sensitivity analysis, the same procedures as in confirmatory analysis were applied within the per-protocol population (PPP). Secondary and safety outcomes were analysed by Fishers exact test, repeated-measures ANCOVA and Mann–Whitney U-test, with neither adjustment for multiplicity nor missing value imputations.

Results

Characteristics of screening and study population

A total of 195 patients (63.4% female, mean (SD) age 38.1 (12.8) years) were screened. A total of 138 patients were eligible for DBPCFC, and 82 (59.4%) had positive DBPCFC at baseline (24). Of those 82, 56 patients were randomized (2:1) to interventional AIT with rBet v 1-FV ($n = 38$) or placebo ($n = 18$) (Fig. 1). A total of 19 of 38 (50%) and eight of 18 (44%) had a history of previous reactions to any soya product. A total of 13 of 38 (34%) and eight of 18 (44%) underwent a previous AIT. Table 1 contains major characteristics of the trial population. A total of 54 of 56 randomized patients started the intervention, meaning that only 56% (54/97) of the intended sample size could be recruited within the given time frame. Major protocol violations occurred in nine of 54 subjects: in the active group, two subjects did not fulfil criteria for positive DBPCFC and in three other, cumulative AIT allergen doses applied were below 150 µg. In four subjects of active and two from placebo group, no postinterventional DBPCFC was performed. PPP included 45 subjects (for details, see Table 1).

Allergen immunotherapy and adverse events

Maintenance phase was reached in 31 of 37 (84%) patients of active and in 16 of 17 (94%) of placebo group. Cumulative allergen doses are given in Table 1. During treatment course, 119 injection-related adverse events (AE) occurred in 22 of 37 (60%) patients of the active and in nine of 17 (53%) of the placebo group. AEs were almost exclusively mild: 64 of 119 (54%) consisted of localized injection site reactions, 13 of 119 (11%) were skin reactions with generalized urticaria in one subject, 15 of 119 (13%) had respiratory (nose/lung; three asthmatic responses in two patients), seven of 119 (6%) eye and 20 of 119 (17%) unspecific symptoms. There were no injection-related serious AEs. During AIT, systemic intake of antihistamines was documented in 21 of 54 (39%) and of short-term systemic glucocorticosteroids in eight of 54 (15%) subjects (due to skin lesions in $n = 6$ or asthma in $n = 2$).

Double-blind placebo-controlled food challenges

At baseline, objective signs were present in 45 of 56 (80%): blistering/swelling of oral mucosa 47% (21 of 45), flush 18%, urticaria 2%, angioedema 7%, conjunctivitis 18%, rhinitis 18%, peakflow reduction 9%, heart rate increase 9%, drop in blood pressure 2%, gastrointestinal symptoms 4%. Subjective symptoms occurred in 51 of 56 (91%); most frequently

reported were oral tingling/blistering 34% (19 of 56), dysphagia 23% and itching 14%. Nausea, abdominal pain and dizziness occurred in 7%, respectively, dyspnoea in 4% and perceived lip swelling in 2%. Cumulative doses at occurrence of first symptoms and signs are shown in Fig. 2, and DBPCFC-based outcome measures are listed in Table 2. No relevant dysbalances were seen between both groups at baseline with regard to LOAELs, type of objective signs or type of subjective symptoms.

In confirmatory analyses, in PPP (but not in FAS), $LOAEL_{obj}$ tended to be higher in the active group (treated with rBet v 1-FV) compared with the placebo group ($P = 0.081$). Individual dose-level changes are shown in Fig. 3. With the best/worst case group-related observations (regarding between-group differences, heterogeneity and $LOAEL_{obj}$ as exclusive primary endpoint), we calculated that between 81 and 162 patients (best/worst case scenario) would have been necessary to provide significant test results. A postinterventional increase in one dose level or more occurred in 20 of 26 (77%) subjects of active and in nine of 14 (64%) subjects of placebo group who presented with objective signs both at baseline and postintervention.

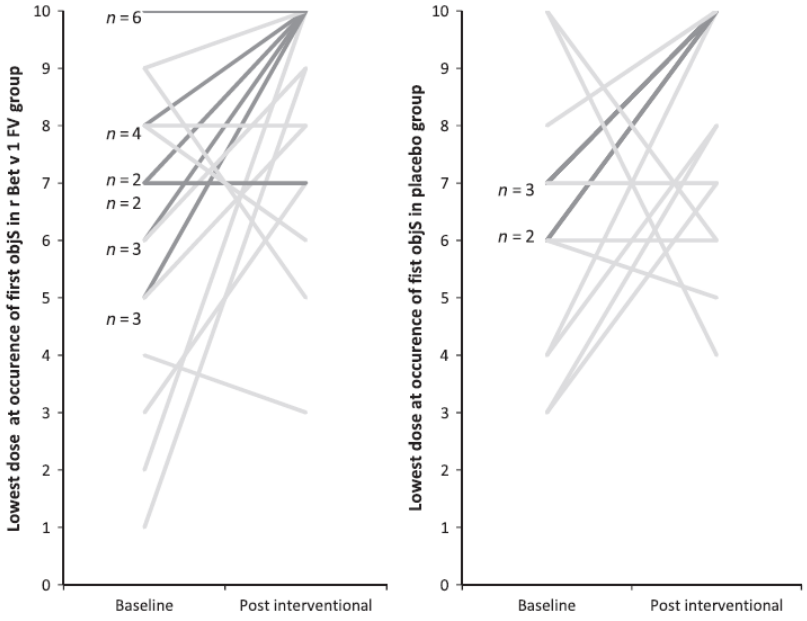

Figure 3 Individual lowest doses at occurrence of first objective signs (objS; LOAELobjective) in per-protocol population at double-blind placebo-controlled food challenge (DBPCFC) (baseline/postinterventional). Left: active group ($n = 30$, Per-protocol population (PPP)), right: placebo group ($n = 15$, PPP). Bold lines indicate same dose levels in more than one patient. Virtual dose level 10 was introduced in case no objS occurred until dose level 9. In six patients, neither at baseline nor postinterventional objective signs appeared.