Materials and Methods

Patients

Eighteen patients with chronic moderate-to-severe plaque psoriasis were enrolled in Peking University First Hospital. Inclusion criteria for enrolment into the study were the following: 18 years of age or older; diagnosed with plaque psoriasis for more than 6 months; having PASI of 12 or higher and a physician's global assessment of "moderate" or "severe" at baseline; having psoriasis lesions involving at least 10% of their body surface area; and candidates for systemic therapy or phototherapy for psoriasis. Exclusion criteria were as follows: non-plaque or drug-induced psoriasis, or other skin conditions that would interfere with psoriasis evaluation; inability to discontinue current systemic therapy (for at least 4 weeks), topical therapy, or phototherapy (for at least 2 weeks); concomitant oral or injection of corticosteroids; and previous treatment with efalizumab or having participated in studies involving oral tofacitinib. Patients were also excluded from the study if they were pregnant or had immune-deficient diseases or severe systemic disorders.

The patients were randomized to receive placebo or tofacitinib 5 or 10 mg twice daily (b.i.d.) for 16 weeks. Two dermatologists simultaneously evaluated PASI of the patients at baseline, week 8, and week 16. A reduction in PASI of

75% or greater was deemed a significant response to treatment (PASI75). Blood samples were obtained at each visit, and the sera were prepared instantly and stored at −80°C before the ELISA analysis was performed.

Enzyme-Linked Immunosorbent Assay (ELISA)

Serum levels of hBD-2 were measured by using a human hBD-2 ELISA kit (Phoenix Pharmaceuticals, Burlingame, CA, USA) according to the manufacturer's instructions. Briefly, 100 µL of samples or prepared hBD-2 standard solutions were added to each designed well. The immunoplate was sealed and incubated for 2 h at room temperature on the plate shaker. The liquid was completely discarded and the plates were washed 4 times. Then 100 µL of biotinylated anti-hBD-2 detection binding protein, SA-HRP solution, and substrate solution were added successively into each well and incubated for a specified time at room temperature. Finally, 100 µL of stop solution was added into each well to stop the reaction. Optical density was read at 450 nm using a BioTek Synergy H1 Hybrid Reader (BioTek, USA) when the colour of liquid in the well changed from blue to yellow. Duplicate determination was performed. The concentration of hBD-2 was determined according to the standard curve.

Statistical Analysis

We calculated that the sample size of 10 patients (5 in each group) would provide 80% power to detect 55% difference in the proportion of patients achieving PASI75 between the placebo group and the high-dose treatment group at week 16, on the basis of a 2-sided significance level of 0.05 and assuming that 60% of the patients in the high-dose treatment group would achieve PASI75 according to the previous studies.

Data were presented as means and standard deviations for numerical variables, and numbers and percentages for categorical variables. Error bars indicated 95% confidence intervals. One-way ANOVA was carried out to compare PASI and serum hBD-2 concentration among the different study groups. When equal variances were assumed, the least significant difference was used for multiple comparisons. When equal variances were not assumed, multiple comparisons were carried out by the Dunnett T3 test. The Pearson correlation coefficient (denoted by r) was calculated between serum hBD-2 levels and PASI. ROC curve analysis was conducted to assess the diagnostic accuracy of serum hBD-2 levels for differentiating between mild and moderate-to-severe psoriasis in participants. In ROC curve analysis, we defined PASI exceeding 10 as moderate-to-severe psoriasis. The AUC was calculated and the best cut-off point was determined based on the highest sensitivity and specificity. Data from all groups in the 3 visits were pooled for correlation and ROC analyses. All statistical analyses were performed by using

the Statistical Package for Social Sciences (SPSS), version 23. All p values were 2-tailed, and $p < 0.05$ was considered statistically significant.