

Letter to the Editor

Humanized anti-IFN- γ (HuZAF) in the treatment of psoriasis*To the Editor:*

Psoriasis is a chronic inflammatory skin disease in which epidermal hyperplasia occurs because of skin infiltration of activated immune cells. T_H1 cells, defined by their ability to produce IFN- γ , are increased in the peripheral circulation and in skin lesions of patients with psoriasis.^{1,2} IFN- γ has been postulated to be a key cytokine in psoriasis due to several important observations. The number of T_H1 cells in skin lesions is strongly associated with disease activity,¹ IFN- γ mRNA is elevated in skin lesions,³ IFN- γ is increased in serum from individuals with psoriasis,⁴ and large-scale gene expression studies identify increased expression of IFN- γ -regulated genes in lesions.¹ Because of the appreciation of increased IFN- γ in psoriasis, 2 small pilot studies were conducted between 2001 and 2003 using a neutralizing humanized anti-IFN- γ antibody, HuZAF (Fontolizumab; Protein Design Laboratory, Fremont, Calif). It was hypothesized that blockade of IFN- γ with HuZAF could reverse the expression of inflammatory gene products in psoriatic lesions and decrease disease activity.

In this letter, we present the results of both single-dose and multidose HuZAF injections in psoriasis treatment (see additional information in this article's [Online Repository](http://www.jacionline.org) at www.jacionline.org). To test the safety and activity of HuZAF in patients with psoriasis, a single-infusion tolerability study was conducted in 2001. Thirteen men and 7 women (age range, 21-73 years) with moderate-severe stable plaque psoriasis participated in this trial. Cohorts of 4 patients received ascending doses of HuZAF: 0.1, 1, 3, and 10 mg/kg or placebo. Across all dose levels, infusions were well tolerated with only mild/moderate adverse effects noted (transient headache and chills were the most common adverse effects, occurring in 25% and 18% of HuZAF-treated patients, respectively). No severe or serious adverse events were noted.

No significant clinical or histological changes were observed in cohorts receiving less than 10 mg/kg (data not shown). In the highest dose group, 3 of 4 patients showed histologic improvements in index psoriasis lesions from baseline to 29 days of treatment (representative responding patient shown in [Fig 1, A](#)), including reversal of acanthosis (epidermal thickening), and restoration of the granular layer ([Fig 1, A and D](#)). In addition, keratin 16 (K16) immunoreactivity (a marker of keratinocyte hyperproliferation) was reversed in day 29 posttreatment biopsies in 2 of 4 cases (representative responding patient shown in [Fig 1, B](#)); K16 mRNA was also reduced in these patients (data not shown). The Psoriasis Area and Severity Index (PASI) score was decreased in 1 of 2 patients ([Fig 1, C](#)) receiving 10 mg/kg (the PASI score was not available for 2 patients).

Inflammatory parameters were also modified by a single dose of 10 mg/kg of HuZAF. CD3⁺ T-cell counts were reduced in 2 of 4 cases ([Fig 1, E](#)). On average, the expression of inflammation-related genes such as *CXCL9*, *IL12p40*, and *iNOS* was reduced at day 29 versus baseline in 3 of 4 cases ([Fig 1, F](#)). Pooled data and statistics of [Fig 1, D to F](#), are found in [Fig E1](#) in this article's [Online Repository](http://www.jacionline.org) available at www.jacionline.org. Using an antibody specific for the therapeutic antibody (AF2id),⁵ HuZAF was detected in skin lesions of both a responding patient and a

nonresponding patient after treatment with 10 mg/kg. These data showed that short-term HuZAF (at the 10-mg/kg dose) was well tolerated and detectable in lesional skin, with the potential to improve inflammatory parameters and disease-related gene expression.

Based on results above from this single 10-mg/kg dose of HuZAF, a small follow-up pilot study was performed to determine the efficacy of multidose HuZAF; 3 patients were studied at our institution and 7 at a second site. Results from patients at our institution are provided in [Fig 2](#). HuZAF was administered at 10 mg/kg, 4 times within a 10-week period (at weeks 0, 2, 6, and 10) ([Fig 2, A](#)). Nonlesional and lesional skin biopsies from the same index plaque were taken at baseline, as well as at weeks 2, 6, and 12. Reduction in the PASI score was observed in 1 patient (blue), a mild transient reduction in the PASI score was observed in 1 patient (red), and a worsening of the PASI score was observed in 1 patient, who then stopped the study (green) ([Fig 2, A](#)). Quantitative RT-PCR was used to determine changes in the expression of *K16*, *IL23/p19*, *CXCL9*, *IL12/23/p40*, and *iNOS* ([Fig 2, B and C](#)). On average, the expression of these inflammatory markers declined at week 2 postinitiation of HuZAF as was previously observed with a single dose. However, results between patients were highly variable and not sustained. The patient who had the greatest reduction in the PASI score (blue) also demonstrated the greatest reduction in *K16*, *p19*, *CXCL9*, *IL12/23/p40*, and *iNOS*. However, in 2 of 3 patients, additional doses of HuZAF did not improve outcome, and after multiple doses of HuZAF, increased expression of *p19*, *CXCL9*, *IL12/23/p40*, and *iNOS* was noted. Statistical analysis of inflammatory gene expression in all 10 patients from both sites is provided in [Fig E2](#) in this article's [Online Repository](http://www.jacionline.org) available at www.jacionline.org. HuZAF failed to significantly reduce the expression of *K16*, *p19*, and *IL12/23p40*. The expression of *iNOS* was transiently reduced, and the expression of only *CXCL9* was significantly suppressed by HuZAF through week 12. This finding is interesting because *CXCL9* is heavily regulated by IFN- γ , demonstrating that IFN- γ was successfully blocked in these patients despite lack of clinical efficacy. Of all 10 patients treated 4 times with 10 mg/kg of HuZAF, only 1 patient (10%) achieved a significant clinical response. In comparison, treatments such as briakinumab, infliximab, and ustekinumab achieve PASI75 in 60% to 90% of all patients.⁶

Although these studies were limited due to small patient sample size, the results fall short compared with the highly successful therapies targeting the IL-17 axis, which were explored after the discovery of T_H17 cells in psoriasis.⁶ It is possible that IFN- γ is important for the initiation of lesions whereas IL-17 is critical for the chronic nature of psoriasis. Successful resolution of psoriasis after etanercept (anti-TNF- α) correlated more significantly with reduction in IL-17-responsive genes than in IFN- γ -responsive genes.⁷ A recently published study using the IL-23-specific, anti-p19-targeting mAb (guselkumab) demonstrated outstanding clinical efficacy (at the highest dose, all patients achieved PASI75), but without significant reduction in the IFN- γ gene signature.⁸ In addition, anti-IFN- γ antibody (HuZAF) was found to be present in skin lesions of both responders and nonresponders ([Fig 1, G](#)), demonstrating that blocking IFN- γ did not lead to disease amelioration.

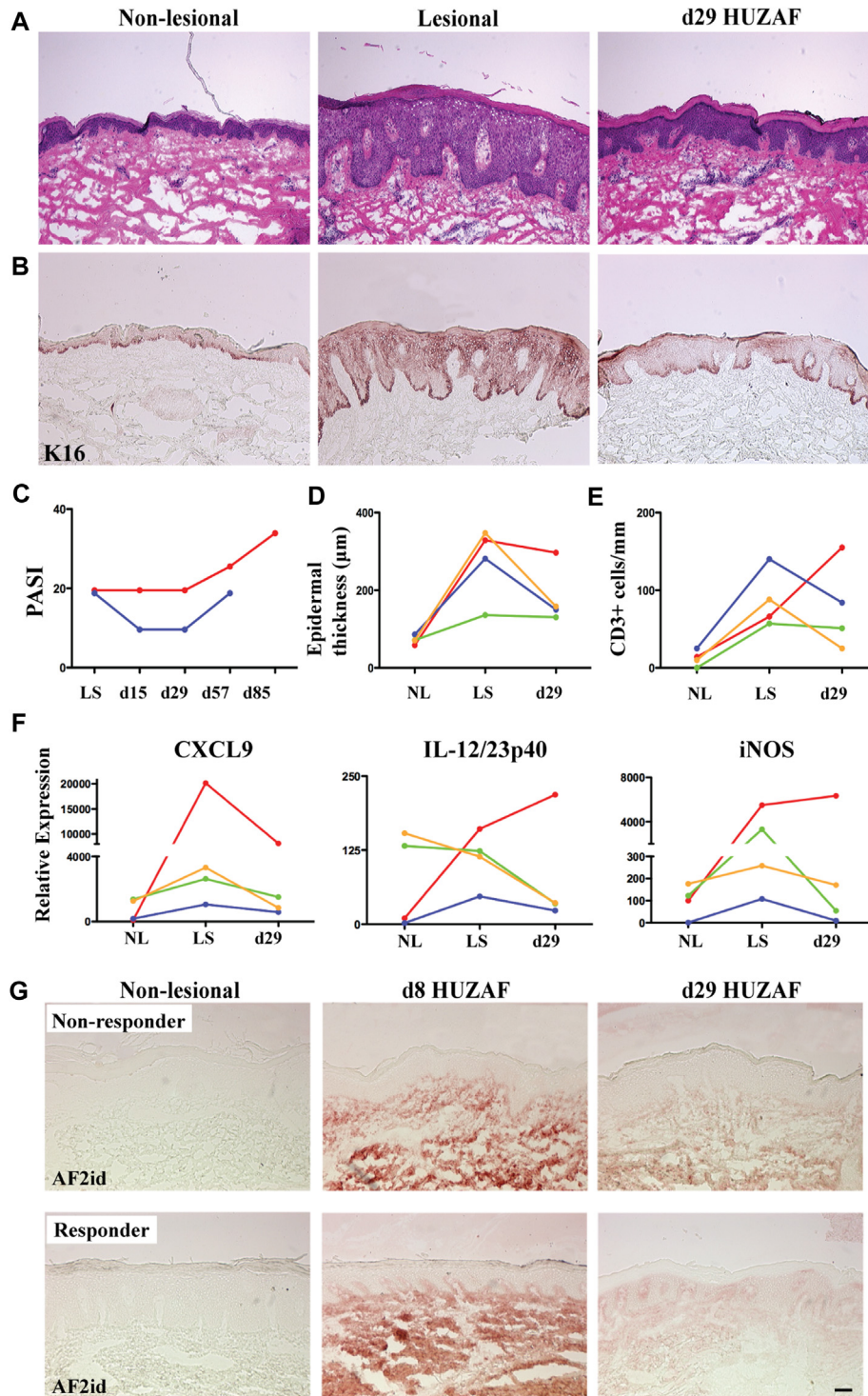


FIG 1. Effect of a single dose of HuZAF (10 mg/kg) for treatment of psoriasis. **H&E (A)** and **K16 (B)** staining of a responding patient. **C**, PASI scores for 2 patients. **D**, Epidermal thickness in baseline nonlesional (NL) and lesional skin (LS), as well as day 29 posttreatment. **E**, CD3⁺ T cells quantified by immunohistology. **F**, Inflammatory gene expression as determined by quantitative RT-PCR. **G**, Staining of HuZAF using AF2id. All histology images shown at same magnification; bar = 10 μm. Each colored line represents a single patient. *H&E*, Hematoxylin and eosin.

These findings suggest that specific targeting and reduction of IL-17 and IL-17-responsive genes is more therapeutic than targeting and reduction of IFN- γ and IFN- γ -responsive genes. This may allude to the initiating and pathogenic importance of

IFN- γ in psoriasis, but reduction is not required for the resolution of chronic psoriatic inflammation. IFN- γ may certainly contribute to inflammation in psoriasis; however, the IL-17 axis appears to be the major driver of this disease. Currently, HuZAF is

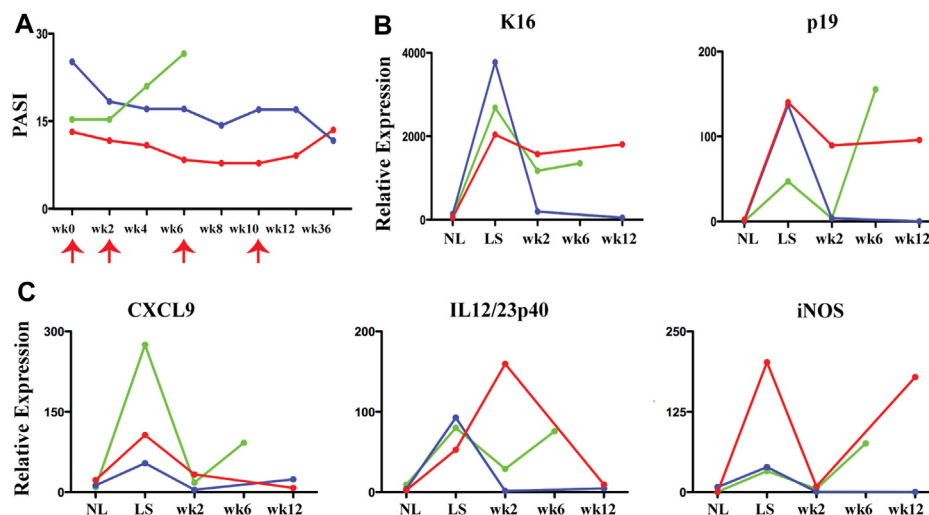


FIG 2. Results of a multiple-dose regimen of HuZAF in the treatment of psoriasis. **A**, PASI scores in patients receiving 4 HuZAF infusions (arrows indicate the administration of treatment). **B** and **C**, Inflammatory gene expression as determined by quantitative RT-PCR, for each patient in nonlesional (NL), lesional (LS), and posttreatment LS skin. Each colored line represents a single patient.

undergoing clinical trials as treatment in Crohn disease, and is showing promise for this particular inflammatory disease.⁹

In conclusion, we have reported here that anti-IFN- γ (HuZAF), while safe and tolerable in patients with psoriasis, had minimal efficacy in the treatment of psoriasis. This suggests that IFN- γ is not a major pathogenic cytokine in chronic psoriasis lesions, leading to the need to redraw the current pathogenic models of this disease.

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Jamie L. Harden, PhD^a

Leanne M. Johnson-Huang, PhD^a

Maria Francesca Chamian, MD^a

Edmund Lee, MD^a

Tillman Pearce, MD^b

Craig L. Leonardi, MD^c

Asifa Haider, PhD^a

Michelle A. Lowes, MD, PhD^a

James G. Krueger, MD, PhD^a

From ^athe Laboratory for Investigative Dermatology, The Rockefeller University, New York, NY; ^bthe Protein Design Laboratory, Fremont, Calif; and ^cthe Department of Dermatology, St Louis University, St Louis, Mo. E-mail: jgk@rockefeller.edu.

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METHODS

Study design

The following studies were Rockefeller University Institutional Review Board–approved protocols. Infusions were intravenous administration over 30 minutes. No significant adverse events were reported for either the single-dose or the multidose HuZAF study.

For single-dose HuZAF studies, 13 men and 7 women (age range, 21–73 years) with moderate-severe stable plaque psoriasis participated in this trial in 2001. Four cohorts of patients received ascending doses of HuZAF (fontolizumab): 0.1, 1, 3, and 10 mg/kg; for each cohort, 1 patient ($n = 1$ out of a cohort of 4–5) was randomly assigned to receive a placebo infusion. Nonlesional and lesional skin biopsies were taken at baseline, as well as on days 8 and 29 from the same index plaque posttreatment.

For multidose HuZAF studies, male and female patients 18 years or older with moderate to severe psoriasis vulgaris could enroll (PASI score > 12). Four doses of 10 mg/kg HuZAF were administered at study days 0, 14 (week 2), 42 (week 6), and 70 (week 10). Nonlesional and lesional skin biopsies were taken at baseline, as well as on week 2, week 6, and week 12 from the same index plaque posttreatment.

For both studies, patients must have adequate organ function, have not received standard systemic therapies other than biologics (methotrexate, cyclosporine A, retinoids, psoralen with ultraviolet A) within 30 days, and have not received antibody or investigational therapies (such as alefacept, infliximab, etanercept, and efalizumab) within 60 days. Patients must test negative in urine pregnancy tests within 48 hours before the first study drug administration. Exclusion criteria include malignancy within 5 years or current malignancies, pregnant or nursing mothers, patients with evidence of infection with HIV, hepatitis B virus, or hepatitis C virus, hematologic abnormalities, previous HuZAF treatment, history of immune deficiency or autoimmune disorders other than psoriasis or psoriatic arthritis, serious infection, hypersensitivity to glycine, histidine, or Polysorbate 80,

tuberculosis or other mycobacterial infection, and patients with guttate psoriasis, pustular psoriasis, or whole-body erythroderma.

Immunohistochemistry

Frozen tissue sections were stained with hematoxylin (Fisher Scientific, Fair Lawn, NJ) and eosin (Shandon, Pittsburgh, Pa), and with murine mAbs to K16 (Sigma Aldrich, St Louis, Mo) and CD3 (BD, Franklin Lakes, NJ) as previously described.^{E1} AF2id, an anti-idiotypic antibody against the HuZAF murine parent antibody AF2 (PDL, 10 ug/mL), was used to evaluate the presence of the therapeutic antibody in skin.^{E2} Epidermal thickness measurements and CD3⁺ T-cell counts were determined through National Institutes of Health software (NIH Image-J 6.1).

RT-PCR

Quantitative RT-PCR was performed on RNAs isolated from skin biopsy samples as previously described.^{E3} Genes of interest were normalized to *human acidic ribosomal protein*, a housekeeping gene.

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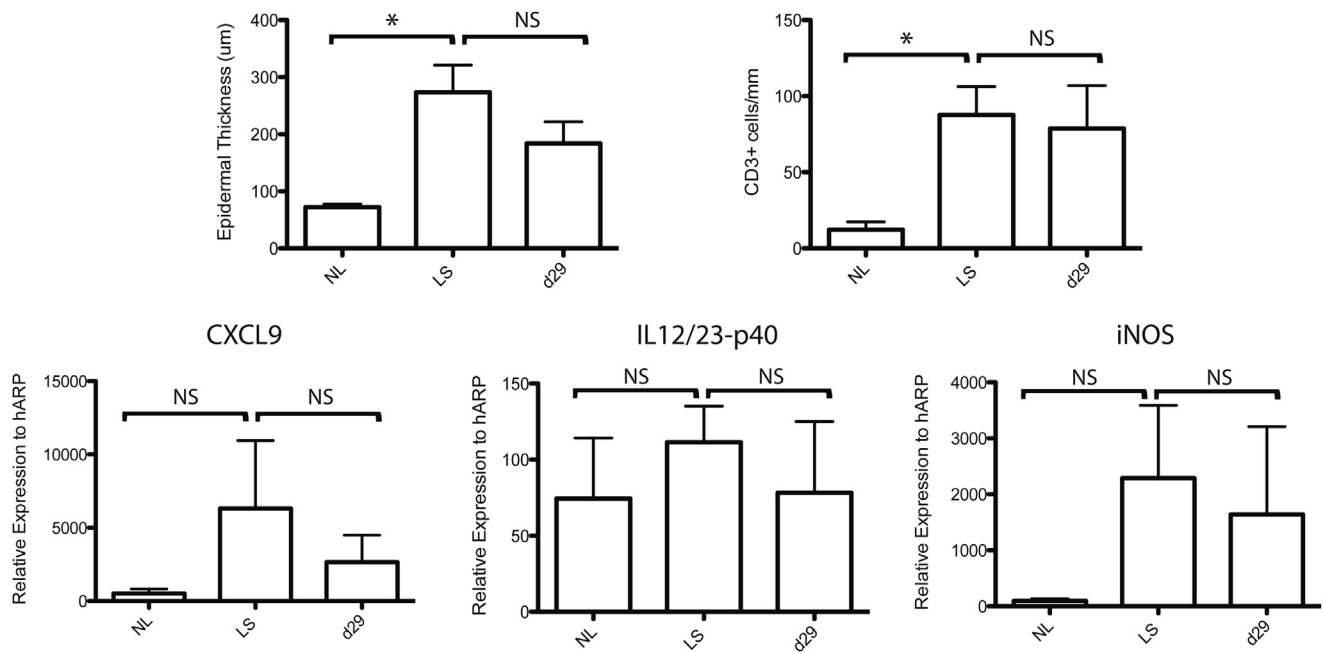


FIG E1. Gene expression of single dose HuZAF (10mg/kg) study. Data from individual patients are pooled and displayed here. Error bars = SE. Data analyzed using paired *t* test. **P* < .05.

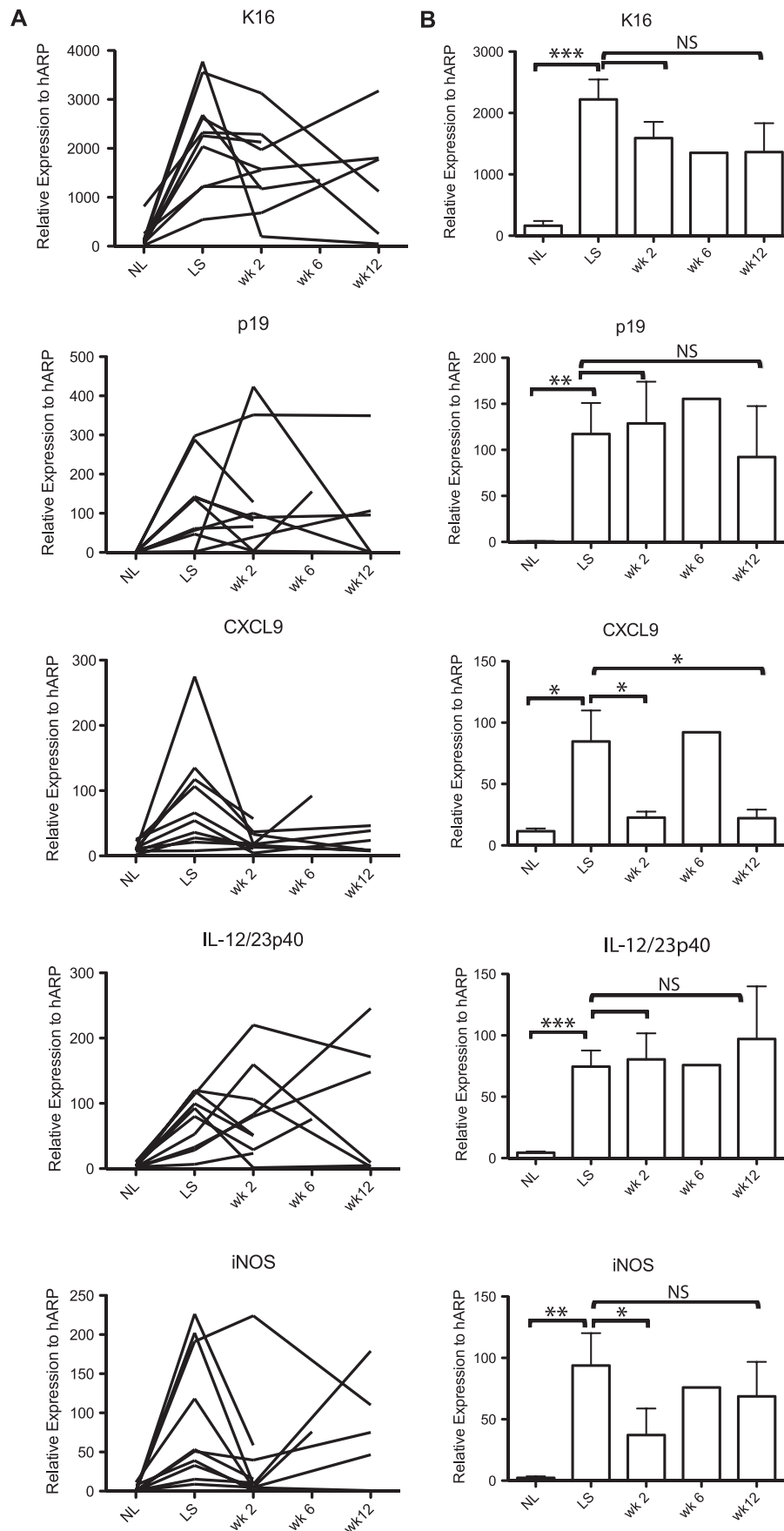


FIG E2. Gene expression of multi-dose HuZAF trial. Inflammatory gene expression determined in biopsies of baseline NL and LS, as well as post-treatment. Data from 10 individual patients (**A**) and combined data (mean) from all 10 patients (**B**); error bars = SE. Data analyzed using paired *t* test. **P* < .05, ***P* < .01, and ****P* < .001.