

Secukinumab Demonstrates Significantly Lower Immunogenicity Potential Compared to Ixekizumab

Sebastian Spindeldreher · Bernard Maillère · Evelyne Correia ·
Maxime Tenon · Anette Karle · Philip Jarvis · Frank Kolbinger

Received: November 30, 2017 / Published online: February 1, 2018
© The Author(s) 2018. This article is an open access publication

ABSTRACT

Introduction: Secukinumab, a fully human monoclonal antibody that selectively neutralizes IL-17A, has been shown to have significant efficacy in the treatment of moderate to severe plaque psoriasis (PsO) and psoriatic arthritis (PsA), demonstrating a rapid onset of action and sustained responses with a favorable safety profile. All biotherapeutics, including monoclonal antibodies (mAbs), can be immunogenic, leading to formation of anti-drug antibodies (ADAs) that can result in loss of response and adverse events such as hypersensitivity reactions. Thus, the

Enhanced content To view enhanced content for this article go to <http://www.medengine.com/Redeem/222DF0607B23F0E9>.

Electronic supplementary material The online version of this article (<https://doi.org/10.1007/s13555-018-0220-y>) contains supplementary material, which is available to authorized users.

S. Spindeldreher (✉) · P. Jarvis · F. Kolbinger
Novartis Institutes for BioMedical Research,
Novartis Pharma AG, Basel, Switzerland
e-mail: Sebastian.Spindeldreher@novartis.com

B. Maillère · E. Correia · M. Tenon
CEA-Saclay, Institut Frédéric Joliot, SIMOPRO,
Université Paris-Saclay, 91191 Gif-Sur-Yvette, France

A. Karle
Integrated Biologics Profiling Unit, Immunogenicity
Risk Assessment, Novartis Pharma AG, Basel,
Switzerland

immunogenicity potential of biotherapeutics is of particular interest for physicians. Of the 2842 patients receiving secukinumab across six phase 3 psoriasis clinical trials, only 0.4% developed treatment-emergent ADAs over 3 years of treatment. Direct comparison of clinical immunogenicity incidence rates is hampered by the nature of clinical immunogenicity assays, differences in study designs, patient populations, and treatment regimens.

Methods: We evaluated side-by-side in the same healthy donors two recently approved IL-17A selective antibodies, secukinumab and ixekizumab, along with adalimumab and ustekinumab, for their capacity to induce anti-drug related T cell responses in vitro and estimated their potential for developing ADAs in patients.

Results: We found that healthy donors show both significantly less frequent T cell responses and lower numbers of pre-existing T cells to secukinumab than to ixekizumab and adalimumab. Although there was a tendency for a lower response to ustekinumab, this difference was not significant.

Conclusion: In summary, this in vitro study confirms the significantly lower immunogenicity potential and provides an explanation for the lower clinical immunogenicity incidence found for secukinumab in comparison to other approved therapeutic antibodies used to treat plaque psoriasis.

Funding: Novartis Pharmaceuticals AG.

Keywords: Anti-drug antibodies; IL-17A; Immunogenicity; Immunogenicity potential; Ixekizumab; Psoriasis; Secukinumab; T cell assay

INTRODUCTION

The pathophysiology of psoriasis is affected in part by the aberrant regulation and secretion of proinflammatory cytokines, including interleukin (IL)-17A [1–3]. Secukinumab (Cosentyx[®], Novartis), a fully human monoclonal antibody (mAb) that selectively neutralizes IL-17A, has been shown to have significant efficacy in the treatment of moderate to severe plaque psoriasis (PsO) and psoriatic arthritis (PsA) [4–11], demonstrating a rapid onset of action and sustained responses with a favorable safety profile. Of the 2842 patients receiving secukinumab across six phase 3 psoriasis clinical trials, only 0.4% developed treatment-emergent anti-drug antibodies (ADAs) over 3 years of treatment [4–10, 12].

Biotherapeutics, including mAbs, are immunogenic to varying extents depending on a range of factors [13–17]. These factors can be product-specific or patient/treatment-specific. Product-specific attributes include the structure and origin of the therapeutic, where fully human antibodies like secukinumab tend to be less immunogenic compared to chimeric or humanized mAbs [18–21]. However, there are also exceptions and fully human antibodies, such as adalimumab, can demonstrate higher levels of immunogenicity that is likely driven by the complementarity-determining regions (CDRs) of immunoglobulin molecules [20, 22, 23]. Patient/treatment-specific factors include the patient's disease and genetic characteristics, the dose frequency, dose amount, and route of administration of the therapeutic. Clinical immunogenicity can range from mild, transient antibody responses with no apparent clinical manifestations to loss of therapeutic efficacy and even life-threatening reactions [24–28].

Secukinumab and ixekizumab (Taltz[®], Eli Lilly) are two therapeutic antibodies targeting IL-17A, while ustekinumab (Stelara[®], Janssen) binds both IL-12 and IL-23 and adalimumab (Humira[®], Abbvie) binds and inhibits tumor necrosis factor alpha (TNF α). Except for ixekizumab, the tested

therapeutic antibodies are approved for treatment of PsO and PsA in EU and US. Ixekizumab is currently approved for treatment of PsO only.

In line with the low clinical immunogenicity observed, secukinumab has previously demonstrated lower potential for immunogenicity compared with other therapeutic antibodies used to treat PsO and PsA in an *in vitro* antigen presentation assay, major histocompatibility complex (MHC)-associated peptide proteomics (MAPPs), and a T cell assay [29]. Similar experimental approaches for rituximab and infliximab have shown that T cell epitopes that are recognized by pre-existing T cells from drug-naïve healthy donors can stimulate T cells collected from treated granulomatous uveitis, Crohn's disease and rheumatoid arthritis patients who developed ADAs [30]. These data emphasize the predictive value of evaluating *in vitro* the pre-existing T cell repertoire in healthy donors to anticipate immunogenicity potential of therapeutic antibodies in patients.

The immunogenicity potential of therapeutic antibodies was compared by evaluating the frequency of mAb-specific pre-existing T cells using an *in vitro* T cell assay [31]. In this study, four mAbs were compared (secukinumab, ixekizumab, adalimumab and ustekinumab).

METHODS

T Cell Assay

The therapeutic antibodies were tested in an *in vitro* T cell assay and the frequency of specific T cells present in the blood of the donors was evaluated for each antibody. Peripheral blood mononuclear cells (PBMCs) were obtained from blood cells collected at the Etablissement Français du Sang (EFS, Rungis, France), as buffy-coat preparations from 16 HLA-DRB1 typed anonymous healthy donors who gave informed consent, in accordance with EFS guidelines. All the samples were genotyped for HLA-DRB1 using the Gold SSP DRB typing kit (One lambda) after DNA extraction from PBMCs with NucleoSpin Blood QuickPure Kit (Macherey-Nagel).

Antibody-specific CD4 T cell lines were generated as described previously [31]. Dendritic cells

(DC) were produced from plastic-adherent cells of PBMCs while CD4 T cells were isolated from PBMCs by using magnetic microbeads (Miltenyi Biotech). The quality of the DCs was assessed by labelling of markers HLA-DR, HLA-A,B,C, CD14, DC-SIGN, CD86 and CD80. DCs were separately loaded overnight at 37 °C with keyhole limpet hemocyanin, KLH (Sigma), used as a positive control (0.25 μ M), or with the therapeutic antibodies secukinumab, ixekizumab, adalimumab or ustekinumab (1 μ M) and matured with lipopolysaccharide (1 μ g/mL). CD4 T cells (200 000/well) were stimulated by protein-loaded DCs (20,000/well) and cultured during 21 days. The number of antigen specific T cells was identified by interferon gamma (IFN- γ) ELISpot assay using an AID ELISpot Reader System. An overview of the T cell assay procedure is presented in Fig. 1.

Antibodies and Control Protein

Four therapeutic antibody samples (secukinumab, 150 mg/mL; ixekizumab, 90 mg/mL;

adalimumab, 50 mg/mL; infliximab 10 mg/ml) were obtained from a pharmacy and stored according to the instructions provided. The four mAbs were reconstituted according to instructions in the package insert to ensure that quality of the material used in this study was equivalent to the quality of the preparations that patients inject to treat their disease. The mAbs were then diluted to the final concentration in cell culture medium. KLH was stored at – 20 °C as a 10 mg/mL stock solution in water. For the studies, an aliquot of KLH was thawed and immediately diluted to the required concentration in cell culture medium.

T Cell Assay Data Analysis

CD4 T cell lines were considered specific when a spot count was 2-fold higher in the presence of the protein than in its absence, with a minimal difference of 25 spots. The frequency of pre-existing, specific CD4 T cells was calculated by considering that the CD4 T cell distribution at

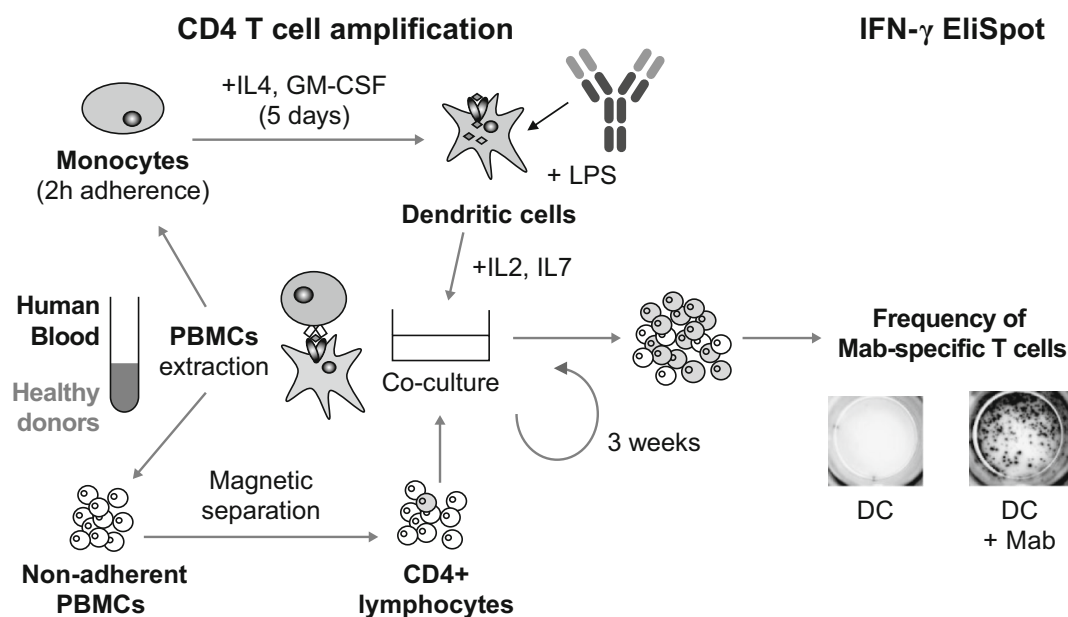


Fig. 1 T cell assay procedure. *IL* Interleukin, *IFN* interferon, *GM-CSF* Granulocyte macrophage colony-stimulating factor, *LPS* lipopolysaccharide, *PBMCs* peripheral blood mononuclear cells, *DC* dendritic cell. Monocyte-derived Dendritic cells (DC) were separately loaded with keyhole limpet hemocyanin, KLH or with the therapeutic

antibodies secukinumab, ixekizumab, adalimumab or ustekinumab and matured with lipopolysaccharide. CD4 T cells were stimulated by protein-loaded DCs and cultured during 21 days. Their antigen specificity was tested by IFN- γ ELISpot assay

Table 1 Levels of immunogenicity potential, discriminated based on previous experiments performed with immunogenic and non-immunogenic proteins [29, 32]

| Immunogenicity potential | Mean frequency (cells/million cells) ^a |
|--------------------------|---|
| High | > 1 |
| Moderate | 0.1–1 |
| Low | < 0.1 |

^a Combines responding donor frequency with the number of pre-existing T cells per donor

the initiation of the culture follows the Poisson distribution and the proportion of culture wells that were reacting to the protein. Calculations were based on the following formula: Frequency = $-\text{Ln}((\text{Number of non-specific CD4}^+ \text{ T cell lines}/\text{Total number of CD4}^+ \text{ T cell lines seeded})/(\text{Number of CD4}^+ \text{ T cells/well}))$. The overall mean frequencies of protein-specific CD4 T cells and of responding donors were calculated with the data collected in all the donors [32].

Levels of immunogenicity potential were discriminated based on previous experiments performed with immunogenic and non-immunogenic proteins [31, 32] or peptides [33] (Table 1).

- Values above $1 \text{ cell} \times 10^6$ CD4 T cells: All the proteins evaluated in the T cell amplification assay reaching this level of response correspond to strongly immunogenic proteins (KLH, murine antibodies, ovalbumin, glutathione S-transferase, LIPO-5 vaccine). The immunogenicity potential is considered HIGH.
- Values between 0.1 and $1 \text{ cell} \times 10^6$ of CD4 T cells: The proteins responding in the T cell amplification assay within this range of response correspond to proteins immunogenic in a selection of patients only (infliximab, adalimumab, rituximab) or proteins rarely immunogenic (hEPo, bevacizumab). The immunogenicity potential is considered MODERATE.
- Values below $0.1 \text{ cell} \times 10^6$ of CD4 T cell: The proteins with this level of response correspond to low or non-immunogenic proteins (insulin, etanercept, trastuzumab). The immunogenicity potential is considered LOW.

All procedures were in accordance with the Helsinki Declaration of 1975. Written informed consent was obtained from each participant before any study procedure.

Statistical Analysis

The T cell data were analyzed using the Quade test [34], the non-parametric version of a two-way analysis of variance (ANOVA), that included donor as a blocking effect in the analysis and weights by the range of specific cell line results within each donor. The positive control KLH data was not included in the statistical analysis. In the analysis of the data listed in Table 2, Donor 12 has the largest range of antibody response (i.e. 0–12) and therefore the biggest influence in the statistical analysis while Donors 11 and 14 with zero antibody response had no influence in the statistical analysis.

RESULTS

In this study, the frequency of pre-existing T cells in a set of 16 healthy drug-naïve blood donors was evaluated. The donors were genotyped for HLA-DR and included the most common HLA-DR alleles found in the ethnically mixed European population (Supplementary Table 1). Minor alleles alone, such as HLA-DRB1*09, HLA-DRB1*10 and HLA-DRB1*12, were not represented in the panel of donors tested.

The highly immunogenic protein KLH was used to assess the capacity of the donors to react to antigens and to demonstrate that they are not immunosuppressed. KLH was tested in 10 parallel cultures (replicates) per donor.

Table 2 Number of specific T cell lines generated by each donor in response to each mAb

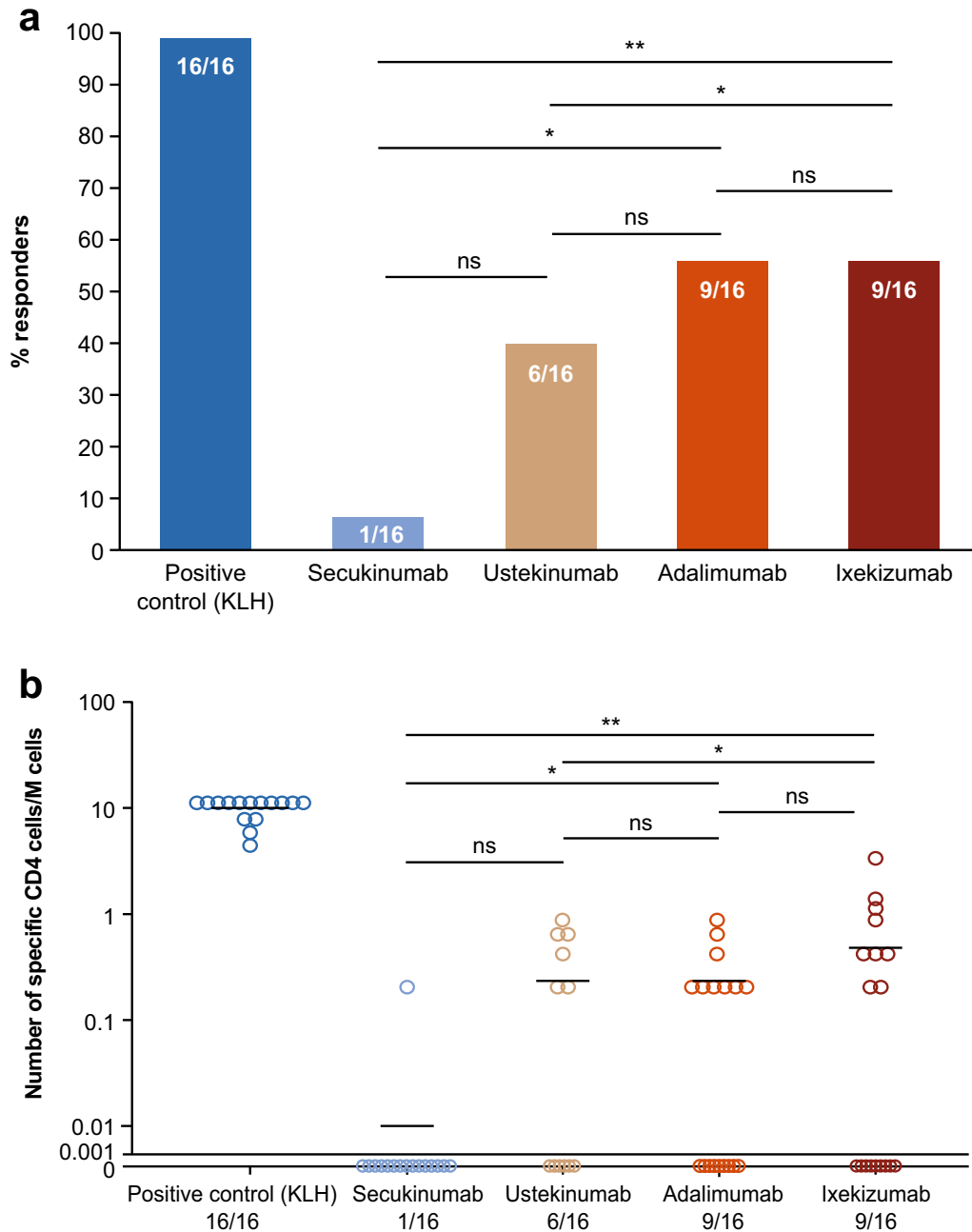
| Donors | KLH | Secukinumab | Ustekinumab | Adalimumab | Ixekizumab |
|------------|---------|-------------|-------------|------------|------------|
| 1 | 10 | 1 | 1 | 0 | 2 |
| 2 | 10 | 0 | 0 | 3 | 4 |
| 3 | 10 | 0 | 0 | 1 | 1 |
| 4 | 9 | 0 | 3 | 0 | 0 |
| 5 | 10 | 0 | 0 | 1 | 5 |
| 6 | 8 | 0 | 0 | 1 | 0 |
| 7 | 9 | 0 | 0 | 0 | 2 |
| 8 | 6 | 0 | 4 | 0 | 1 |
| 9 | 9 | 0 | 0 | 2 | 0 |
| 10 | 9 | 0 | 3 | 0 | 0 |
| 11 | 9 | 0 | 0 | 0 | 0 |
| 12 | 10 | 0 | 2 | 4 | 12 |
| 13 | 9 | 0 | 0 | 1 | 2 |
| 14 | 6 | 0 | 0 | 0 | 0 |
| 15 | 8 | 0 | 0 | 1 | 0 |
| 16 | 7 | 0 | 1 | 1 | 6 |
| Total | 134/160 | 1/384 | 14/384 | 15/384 | 35/384 |
| Responders | 16/16 | 1/16 | 6/16 | 9/16 | 9/16 |

For each donor a total of 24 wells were tested per mAb, 10 wells were tested for the positive control KLH. The number of specific T cell lines generated corresponds to the number of culture wells that were reacting to the respective mAb

All 16 donors responded strongly to KLH with between 6 and 10 T cell lines being raised per donor, demonstrating that all the donors were able to respond to antigenic stimuli and were not immunosuppressed (Table 2 and Supplementary Figure 1). Accordingly, T cell lines from all 16 donors were raised against each of the four antibodies, secukinumab, ustekinumab, adalimumab and ixekizumab, in 24 replicates. The number of T cell lines was used to calculate the frequency of CD4 T cells circulating in the blood of each donor that is specific for the corresponding protein. The frequency of pre-existing T cells was estimated assuming a Poisson distribution as described in the methods.

Only one of the 16 donors responded to secukinumab, generating only one T cell line (Table 2 and Fig. 2a). Hence, the frequency of specific pre-existing T cells for the one donor responding to secukinumab was 0.21 T cells per million T cells. Considering the whole study with all 16 donors, this corresponds to a mean frequency of 0.01 secukinumab-specific pre-existing T cells per million T cells (Table 3 and Fig. 2b).

In comparison, ustekinumab gave rise to 14 specific T cell lines from six of the 16 donors (Table 2 and Fig. 2a). Within these six donors, the response varied from 0.21 to 0.91 specific T cells per million T cells, resulting in a mean frequency of 0.19 ustekinumab-specific T cells



per million T cells for all 16 donors (Table 3 and Fig. 2b).

For both adalimumab and ixekizumab, nine of the 16 donors responded, generating 15 and 35 specific T cell lines, respectively (Table 2 and Fig. 2a). For adalimumab, the individual frequencies of pre-existing T cells ranged from 0.21–0.91 specific T cells per million T cells with an overall mean frequency

of 0.21 adalimumab-specific pre-existing T cells per million T cells for all 16 donors. For ixekizumab, the range was 0.21–3.47 and a mean of 0.54 ixekizumab-specific pre-existing T cells per million T cells for all 16 donors (Table 3 and Fig. 2b).

These differences were significant between secukinumab and ixekizumab ($p < 0.01$), secukinumab and adalimumab ($p < 0.05$), and

◀**Fig. 2 a** Comparison of the frequencies of donors responding to the mAbs in the T cell assay. **b** Size of the pre-existing mAb-specific CD4 T cell repertoire: number of specific T cells per million T cells for each mAb tested. The therapeutic antibodies were tested in an in vitro T cell assay and the frequency of mAb-specific T cells present in the blood of 16 healthy donors was evaluated for each antibody. CD4 T cell lines were considered as specific when a spot count was 2-fold higher in the presence of the protein than in their absence, with a minimal difference of 25 spots. The frequency of pre-existing specific CD4 T cells was then calculated by considering that the CD4 T cell distribution at the initiation of the culture follows the Poisson's distribution and on the basis of the proportion of culture wells that were reacting to the protein. A donor is considered a responder if at least one T cell line could be detected. Figure 2a depicts the percentage of responding donors. Figure 2b presents the frequency of pre-existing T cells for each donor (circle) as well as the overall mean frequency for the respective mAb (line). Nonparametric Quade test was applied to rank the responses. $p < 0.01$ (**); $p < 0.05$ (*); ns = not significant ($p > 0.05$)

ustekinumab and ixekizumab ($p < 0.05$) when analyzed using the non-parametric Quade test.

DISCUSSION

Multiple factors contribute to the immunogenicity of biotherapeutics, including molecular structure and formulation of the biotherapeutics, patient-related factors such as the type of disease and genetic background, e.g. the HLA genotype, as well as treatment-related factors such as route of administration, dose and dosing regimen [14, 16, 17, 20, 35]. Proper comparison of different biotherapeutics is rendered difficult not only by these factors, but also by the nature of clinical ADA assays used, as they may differ in their sensitivity and their tolerance to interfering amounts of biotherapeutics in the sample that is being tested. Since such a direct comparison of clinical immunogenicity incidence rates between biotherapeutics is not possible, the ranges and trends of reported immunogenicity values can only allow for a very rough categorization of clinical immunogenicity such as low, moderate and high

incidence rates. With an immunogenicity incidence rate of 0.4% in psoriasis clinical trials, secukinumab falls into the lower end of the low immunogenicity category [12], while other mAbs used to treat psoriasis, e.g. infliximab, adalimumab and rituximab, show a wide range of clinical immunogenicity incidence rates in the real world setting and could therefore be considered having moderate to high immunogenicity potential [29, 36–49].

In contrast to comparing clinical immunogenicity incidence rates, a comparative assessment of immunogenicity potential of biotherapeutics using in vitro assays might help to understand differences in immunogenicity, as it allows side-by-side evaluation of these biotherapeutics in the same donors under identical and well-controlled conditions [29, 31, 50–54]. It should be noted however, that this type of comparison does not allow conclusions to be drawn on the clinical relevance of ADA, such as secondary failure of a treatment. In a previous study, we used in vitro assays to examine the immunogenicity potential of different therapeutic mAbs based on their antigen presentation as well as their ability to induce T cell proliferation and IL-2 release, which is linked to proliferative capacity, in healthy donors [29]. A challenge for studying immunogenicity in in vitro T cell assays is that responses against mAbs are typically weak, which can be attributed to high sequence similarity between mAbs and endogenous antibodies. In order to address this limitation and to provide biologically meaningful data, we assessed the immunogenicity potential of therapeutic antibodies by determining the frequency of pre-existing T cells in humans, which can respond to the therapeutic antibodies of interest. Rather than investigating T cell proliferation and IL-2 release, we focused on IFN- γ release since the conditions of the in vitro culture promoted Th1 responses. In contrast to IL-2, IFN- γ is also released by non-proliferating memory cells. We recently described such an assay approach by investigating in vitro the predictive value of the mAb-specific pre-existing T cells of healthy blood donors to anticipate immunogenicity potential of the two mAbs infliximab and rituximab in patients [30].

Table 3 Frequency of mAb-specific pre-existing T cells per million T cells within each donor

| Donor | KLH | Secukinumab | Ustekinumab | Adalimumab | Ixekizumab |
|-------|--------------------|-------------|-------------|------------|------------|
| 1 | 11.51 ^a | 0.21 | 0.21 | 0.00 | 0.44 |
| 2 | 11.51 ^a | 0.00 | 0.00 | 0.67 | 0.91 |
| 3 | 11.51 ^a | 0.00 | 0.00 | 0.21 | 0.21 |
| 4 | 11.51 | 0.00 | 0.67 | 0.00 | 0.00 |
| 5 | 11.51 ^a | 0.00 | 0.00 | 0.21 | 1.17 |
| 6 | 8.05 | 0.00 | 0.00 | 0.21 | 0.00 |
| 7 | 11.51 | 0.00 | 0.00 | 0.00 | 0.44 |
| 8 | 4.58 | 0.00 | 0.91 | 0.00 | 0.21 |
| 9 | 11.51 | 0.00 | 0.00 | 0.44 | 0.00 |
| 10 | 11.51 | 0.00 | 0.67 | 0.00 | 0.00 |
| 11 | 11.51 | 0.00 | 0.00 | 0.00 | 0.00 |
| 12 | 11.51 ^a | 0.00 | 0.44 | 0.91 | 3.47 |
| 13 | 11.51 | 0.00 | 0.00 | 0.21 | 0.44 |
| 14 | 4.58 | 0.00 | 0.00 | 0.00 | 0.00 |
| 15 | 8.05 | 0.00 | 0.00 | 0.21 | 0.00 |
| 16 | 6.02 | 0.00 | 0.21 | 0.21 | 1.44 |
| Mean | 9.87 | 0.01 | 0.19 | 0.21 | 0.54 |

The frequency of pre-existing specific CD4 T cells per million T cells was calculated by considering that the CD4 T cell distribution at the initiation of the culture follows the Poisson's distribution and on the basis of the proportion of culture wells that were reacting to the protein

^a Replicates were positive. The frequency of pre-existing T cells is therefore likely underestimated

Although immune responses may be stronger in autoimmune conditions such as psoriasis, the use of healthy donors rather than patients has been proven appropriate for a comparative assessment of immunogenicity potential since patient-derived T cells responded to the very same peptides, which were identified using the assay approach applied here [30].

In the present study, a more sensitive T cell assay format was used to determine the frequency of pre-existing mAb-specific T cells in healthy donors [31]. As seen previously, secukinumab demonstrated the lowest immunogenicity potential. Compared with ixekizumab and adalimumab, the immunogenicity potential was significantly lower, as evidenced by a

significantly lower number of donors responding to secukinumab and a significantly lower frequency of pre-existing T cells responding to secukinumab. According to previous experience, this mean frequency is within the range of low or non-immunogenic proteins such as insulin, etanercept or trastuzumab and is therefore considered overall to represent a low immunogenicity potential, which is consistent with the observed clinical immunogenicity incidence rate [4–10, 12, 31, 33]. In contrast, the mean frequencies of pre-existing T cells found for adalimumab and ixekizumab fall into a range that is typical for mAbs with moderate immunogenicity potential such as infliximab and rituximab [30–32].

CONCLUSIONS

Although secukinumab trended towards a lower response compared with ustekinumab, this difference was not statistically significant, which is in line with the previous study that also showed no significant difference between secukinumab and ustekinumab [29]. In summary, this in vitro study confirmed the significantly lower immunogenicity potential for secukinumab in comparison to adalimumab and demonstrated a significantly lower immunogenicity potential in a direct comparison with ixekizumab. These data indicate that the reason for the low clinical immunogenicity rate observed for secukinumab in comparison to other therapeutic antibodies may be related to the presence of a low number of pre-existing T cells that can respond to the antibody. Whether the potential of the different antibodies to induce a T cell response is based on their unique primary amino acids sequences, and/or levels of humanization is an active area of current research.

ACKNOWLEDGMENTS

Funding. This study was funded by Novartis Pharmaceuticals AG and it was led by Novartis Institutes of Biomedical Research (NIBR). Article processing charges were funded by Novartis Pharmaceuticals AG. All authors have full access to all of the data in this study and take complete responsibility for the integrity of the data and accuracy of the data analysis.

Authorship. All authors meet the International Committee of Medical Journal Editors (ICMJE) criteria for authorship for this manuscript, take responsibility for the Integrity of the work as a whole, and have given final approval to the version to be published.

Medical Writing, Editorial, and Other Assistance. The authors would like to acknowledge the assistance of all investigators. The authors thank Tina Patrick, Ph.D., and Jackie L. Johnson, Ph.D. of Novartis Ireland Ltd

for providing medical writing support/editorial support, which was funded by Novartis Pharmaceuticals AG, Basel, Switzerland in accordance with Good Publication Practice (GPP3) guidelines (<http://www.ismpp.org/gpp3>).

Disclosures. Anette Karle is a full-time employee of and holds shares and stock options in Novartis. Sebastian Spindeldreher is a full-time employee of and holds shares and stock options in Novartis. Philip Jarvis is a full-time employee of and holds shares and stock options in Novartis. Frank Kolbinger is a full-time employee of and holds shares and stock options in Novartis. Bernard Maillère has received grants from Novartis. Evelyne Correia has received grants from Novartis. Maxime Tenon has received grants from Novartis.

Compliance with Ethics Guidelines. The study described in this manuscript was conducted in accordance with ICH-Good Clinical Practice guidelines and the Declaration of Helsinki, and it was approved by the appropriate institutional review committees and regulatory agencies. Written informed consent was obtained from each participant before any study procedure.

Open Access. This article is distributed under the terms of the Creative Commons Attribution-NonCommercial 4.0 International License (<http://creativecommons.org/licenses/by-nc/4.0/>), which permits any noncommercial use, distribution, and reproduction in any medium, provided you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made.

REFERENCES

1. Johansen C, Usher PA, Kjellerup RB, Lundsgaard D, Iversen L, Kragballe K. Characterization of the interleukin-17 isoforms and receptors in lesional psoriatic skin. *Br J Dermatol.* 2009;160:319–24.
2. Lowes MA, Kikuchi T, Fuentes-Duculan J, Cardinale I, Zaba LC, Haider AS, Bowman EP, Krueger JG.

- Psoriasis vulgaris lesions contain discrete populations of Th1 and Th17 T cells. *J Invest Dermatol.* 2008;128:1207–11.
3. Martin DA, Towne JE, Kricorian G, Klekotka P, Gudjonsson JE, Krueger JG, Russell CB. The emerging role of IL-17 in the pathogenesis of psoriasis: preclinical and clinical findings. *J Invest Dermatol.* 2013;133:17–26.
 4. Blauvelt A, Prinz JC, Gottlieb AB, Kingo K, Sofen H, Ruer-Mulard M, Singh V, Pathan R, Papavassilis C, Cooper S, Group FS. Secukinumab administration by pre-filled syringe: efficacy, safety and usability results from a randomized controlled trial in psoriasis (FEATURE). *Br J Dermatol.* 2015;172:484–93.
 5. Langley RG, Elewski BE, Lebwohl M, Reich K, Griffiths CE, Papp K, Puig L, Nakagawa H, Spelman L, Sigurgeirsson B, Rivas E, Tsai TF, Wasel N, Tyring S, Salko T, Hampele I, Notter M, Karpov A, Helou S, Papavassilis C, Group ES, Group FS. Secukinumab in plaque psoriasis—results of two phase 3 trials. *N Engl J Med.* 2014;371:326–38.
 6. Paul C, Lacour JP, Tedremets L, Kreutzer K, Jazayeri S, Adams S, Guindon C, You R, Papavassilis C, group Js. Efficacy, safety and usability of secukinumab administration by autoinjector/pen in psoriasis: a randomized, controlled trial (JUNCTURE). *J Eur Acad Dermatol Venereol: JEADV.* 2015;29:1082–90.
 7. Mrowietz U, Leonardi CL, Girolomoni G, Toth D, Morita A, Balki SA, Szepietowski JC, Regnault P, Thurston H, Papavassilis C, Group SS. Secukinumab retreatment-as-needed versus fixed-interval maintenance regimen for moderate to severe plaque psoriasis: a randomized, double-blind, noninferiority trial (SCULPTURE). *J Am Acad Dermatol.* 2015;73:27–36.
 8. Thaci D, Humeniuk J, Frambach Y, Bissonnette R, Goodman JJ, Shevade S, Gong Y, Papavassilis C, Group Ss. Secukinumab in psoriasis: randomized, controlled phase 3 trial results assessing the potential to improve treatment response in partial responders (STATURE). *Br J Dermatol.* 2015;173:777–87.
 9. Mease PJ, McInnes IB, Kirkham B, Kavanaugh A, Rahman P, van der Heijde D, Landewe R, Nash P, Pricop L, Yuan J, Richards HB, Mpofu S, Group FS. Secukinumab Inhibition of Interleukin-17A in Patients with Psoriatic Arthritis. *N Engl J Med.* 2015;373:1329–39.
 10. McInnes IB, Mease PJ, Kirkham B, Kavanaugh A, Ritchlin CT, Rahman P, van der Heijde D, Landewe R, Conaghan PG, Gottlieb AB, Richards H, Pricop L, Ligozio G, Patekar M, Mpofu S, Group FS. Secukinumab, a human anti-interleukin-17A monoclonal antibody, in patients with psoriatic arthritis (FUTURE 2): a randomised, double-blind, placebo-controlled, phase 3 trial. *Lancet.* 2015;386:1137–46.
 11. Balato A, Scala E, Balato N, Caiazza G, Di Caprio R, Monfrecola G, Raimondo A, Lembo S, Ayala F. Biologics that inhibit the Th17 pathway and related cytokines to treat inflammatory disorders. *Expert Opin Biol Ther.* 2017;17:1363–74.
 12. Reich K, Blauvelt A, Armstrong A, Langley RG, Fox T, Huang J, Papavassilis C, Liang E, Lloyd P, Bruin G. Secukinumab, a fully human anti-interleukin-17A monoclonal antibody, exhibits minimal immunogenicity in patients with moderate-to-severe plaque psoriasis. *Br J Dermatol.* 2017;176:752–8.
 13. De Simone C, Amerio P, Amoroso G, Bardazzi F, Campanati A, Conti A, Gisondi P, Gualdi G, Guarneri C, Leoni L, Loconsole F, Mazzotta A, Musumeci ML, Piaserico S, Potenza C, Prestinari F. Immunogenicity of anti-TNFalpha therapy in psoriasis: a clinical issue? *Expert Opin Biol Ther.* 2013;13:1673–82.
 14. Descotes J. Immunotoxicity of monoclonal antibodies. *MABs.* 2009;1:104–11.
 15. Jullien D, Prinz JC, Nestle FO. Immunogenicity of biotherapy used in psoriasis: the science behind the scenes. *J Invest Dermatol.* 2015;135:31–8.
 16. Leach MW, Rottman JB, Hock MB, Finco D, Rojko JL, Beyer JC. Immunogenicity/hypersensitivity of biologics. *Toxicol Pathol.* 2014;42:293–300.
 17. Purcell RT, Lockey RF. Immunologic responses to therapeutic biologic agents. *J Investig Allergol Clin Immunol.* 2008;18:335–42.
 18. Nelson AL, Dhimolea E, Reichert JM. Development trends for human monoclonal antibody therapeutics. *Nat Rev Drug Discov.* 2010;9:767–74.
 19. Hwang WY, Foote J. Immunogenicity of engineered antibodies. *Methods.* 2005;36:3–10.
 20. Harding FA, Stickler MM, Razo J, DuBridge RB. The immunogenicity of humanized and fully human antibodies: residual immunogenicity resides in the CDR regions. *MABs.* 2010;2:256–65.
 21. Getts DR, Getts MT, McCarthy DP, Chastain EM, Miller SD. Have we overestimated the benefit of human(ized) antibodies? *MABs.* 2010;2:682–94.
 22. van Schouwenburg PA, Kruithof S, Votsmeier C, van Schie K, Hart MH, de Jong RN, van Buren EE, van Ham M, Aarden L, Wolbink G, Wouters D, Rispen T. Functional analysis of the anti-adalimumab response using patient-derived monoclonal antibodies. *J Biol Chem.* 2014;289:34482–8.

23. Malucchi SBA. Clinical aspects of immunogenicity to biotherapeutics. In: van de Weert MME, editor. *Immunogenicity of Biopharmaceuticals*. New York: Springer; 2008.
24. Jahn EM, Schneider CK. How to systematically evaluate immunogenicity of therapeutic proteins—regulatory considerations. *N Biotechnol*. 2009;25:280–6.
25. Bito T, Nishikawa R, Hatakeyama M, Kikusawa A, Kanki H, Nagai H, Sarayama Y, Ikeda T, Yoshizaki H, Seto H, Adachi A, Horikawa T, Oka M, Nishigori C. Influence of neutralizing antibodies to adalimumab and infliximab on the treatment of psoriasis. *Br J Dermatol*. 2014;170:922–9.
26. Carrascosa JM. Immunogenicity in biologic therapy: implications for dermatology. *Actas Dermosifiliogr*. 2013;104:471–9.
27. Farhangian ME, Feldman SR. Immunogenicity of biologic treatments for psoriasis: therapeutic consequences and the potential value of concomitant methotrexate. *Am J Clin Dermatol*. 2015;16:285–94.
28. Hsu L, Snodgrass BT, Armstrong AW. Antidrug antibodies in psoriasis: a systematic review. *Br J Dermatol*. 2014;170:261–73.
29. Karle A, Spindeldreher S, Kolbinger F. Secukinumab, a novel anti-IL-17A antibody, shows low immunogenicity potential in human in vitro assays comparable to other marketed biotherapeutics with low clinical immunogenicity. *MAbs*. 2016;8:536–50.
30. Hamze M, Meunier S, Karle A, Gdoura A, Goudet A, Szely N, Pallardy M, Carbonnel F, Spindeldreher S, Mariette X, Miceli-Richard C, Maillere B. Characterization of CD4 T cell epitopes of infliximab and rituximab identified from healthy donors. *Front Immunol*. 2017;8:500.
31. Delluc S, Ravot G, Maillere B. Quantitative analysis of the CD4 T-cell repertoire specific to therapeutic antibodies in healthy donors. *FASEB J*. 2011;25:2040–8.
32. Delluc S, Ravot G, Maillere B. Quantification of the preexisting CD4 T-cell repertoire specific for human erythropoietin reveals its immunogenicity potential. *Blood*. 2010;116:4542–5.
33. Castelli FA, Szely N, Olivain A, Casartelli N, Grygar C, Schneider A, Besse A, Levy Y, Schwartz O, Maillere B. Hierarchy of CD4 T cell epitopes of the ANRS Lipo5 synthetic vaccine relies on the frequencies of pre-existing peptide-specific T cells in healthy donors. *J Immunol*. 2013;190:5757–63.
34. Conover WJ. *Practical nonparametric statistics*, 3rd Ed. New York: Wiley. 1999. p. 367–86.
35. De Groot AS, Scott DW. Immunogenicity of protein therapeutics. *Trends Immunol*. 2007;28:482–90.
36. de Vries MK, Brouwer E, van der Horst-Bruinsma IE, Spoorenberg A, van Denderen JC, Jamnitski A, Nurmohamed MT, Dijkmans BA, Aarden LA, Wolbink GJ. Decreased clinical response to adalimumab in ankylosing spondylitis is associated with antibody formation. *Ann Rheum Dis*. 2009;68:1787–8.
37. de Vries MK, Wolbink GJ, Stapel SO, de Vrieze H, van Denderen JC, Dijkmans BA, Aarden LA, van der Horst-Bruinsma IE. Decreased clinical response to infliximab in ankylosing spondylitis is correlated with anti-infliximab formation. *Ann Rheum Dis*. 2007;66:1252–4.
38. Hsu L, Armstrong AW. Anti-drug antibodies in psoriasis: a critical evaluation of clinical significance and impact on treatment response. *Expert Rev Clin Immunol*. 2013;9:949–58.
39. Krieckaert CL, Bartelds GM, Lems WF, Wolbink GJ. The effect of immunomodulators on the immunogenicity of TNF-blocking therapeutic monoclonal antibodies: a review. *Arthr Res Ther*. 2010;12:217.
40. Krieckaert CL, Jamnitski A, Nurmohamed MT, Kostense PJ, Boers M, Wolbink G. Comparison of long-term clinical outcome with etanercept treatment and adalimumab treatment of rheumatoid arthritis with respect to immunogenicity. *Arthr Rheum*. 2012;64:3850–5.
41. Mazilu D, Opris D, Gainaru C, Iliuta M, Apetrei N, Luca G, Borangiu A, Gudu T, Peltea A, Groseanu L, Constantinescu C, Saulescu I, Bojinca V, Balanescu A, Predeteanu D, Ionescu R. Monitoring drug and antidrug levels: a rational approach in rheumatoid arthritis patients treated with biologic agents who experience inadequate response while being on a stable biologic treatment. *Biomed Res Int*. 2014;2014:702701.
42. Mok CC. Rituximab for the treatment of rheumatoid arthritis: an update. *Drug Des Dev Ther*. 2013;8:87–100.
43. Paramarta JE, Baeten DL. Adalimumab serum levels and antidrug antibodies towards adalimumab in peripheral spondyloarthritis: no association with clinical response to treatment or with disease relapse upon treatment discontinuation. *Arthr Res Ther*. 2014;16:R160.
44. Ritchlin C, Rahman P, Kavanaugh A, McInnes IB, Puig L, Li S, Wang Y, Shen YK, Doyle MK, Mendelsohn AM, Gottlieb AB. Efficacy and safety of the anti-IL-12/23 p40 monoclonal antibody,

- ustekinumab, in patients with active psoriatic arthritis despite conventional non-biological and biological anti-tumour necrosis factor therapy: 6-month and 1-year results of the phase 3, multi-centre, double-blind, placebo-controlled, randomised PSUMMIT 2 trial. *Ann Rheum Dis*. 2014;73:990–9.
45. Sandborn WJ, Gasink C, Gao LL, Blank MA, Johanns J, Guzzo C, Sands BE, Hanauer SB, Targan S, Rutgeerts P, Ghosh S, de Villiers WJ, Panaccione R, Greenberg G, Schreiber S, Lichtiger S, Feagan BG. Ustekinumab induction and maintenance therapy in refractory Crohn's disease. *N Engl J Med*. 2012;367:1519–28.
46. van Kuijk AW, de Groot M, Stapel SO, Dijkmans BA, Wolbink GJ, Tak PP. Relationship between the clinical response to adalimumab treatment and serum levels of adalimumab and anti-adalimumab antibodies in patients with psoriatic arthritis. *Ann Rheum Dis*. 2010;69:624–5.
47. van Vollenhoven RF, Emery P, Bingham CO 3rd, Keystone EC, Fleischmann R, Furst DE, Macey K, Sweetser M, Kelman A, Rao R. Longterm safety of patients receiving rituximab in rheumatoid arthritis clinical trials. *J Rheumatol*. 2010;37:558–67.
48. Emi Aikawa N, de Carvalho JF, Artur Almeida Silva C, Bonfa E. Immunogenicity of Anti-TNF-alpha agents in autoimmune diseases. *Clin Rev Allergy Immunol*. 2010;38:82–9.
49. Genovese MC, Durez P, Richards HB, Supronik J, Dokoupilova E, Mazurov V, Aelion JA, Lee SH, Codding CE, Kellner H, Ikawa T, Hugot S, Mpofu S. Efficacy and safety of secukinumab in patients with rheumatoid arthritis: a phase II, dose-finding, double-blind, randomised, placebo controlled study. *Ann Rheum Dis*. 2013;72:863–9.
50. Baker MP, Jones TD. Identification and removal of immunogenicity in therapeutic proteins. *Curr Opin Drug Discov Devel*. 2007;10:219–27.
51. Joubert MK, Deshpande M, Yang J, Reynolds H, Bryson C, Fogg M, Baker MP, Herskovitz J, Goletz TJ, Zhou L, Moxness M, Flynn GC, Narhi LO, Jawa V. Use of in vitro assays to assess immunogenicity risk of antibody-based biotherapeutics. *PLoS ONE*. 2016;11:e0159328.
52. Joubert MK, Hokom M, Eakin C, Zhou L, Deshpande M, Baker MP, Goletz TJ, Kerwin BA, Chirmule N, Narhi LO, Jawa V. Highly aggregated antibody therapeutics can enhance the in vitro innate and late-stage T-cell immune responses. *J Biol Chem*. 2012;287:25266–79.
53. Rombach-Riegraf V, Karle AC, Wolf B, Sorde L, Koepke S, Gottlieb S, Krieg J, Djidja MC, Baban A, Spindeldreher S, Koulov AV, Kiessling A. Aggregation of human recombinant monoclonal antibodies influences the capacity of dendritic cells to stimulate adaptive T-cell responses in vitro. *PLoS ONE*. 2014;9:e86322.
54. Wullner D, Zhou L, Bramhall E, Kuck A, Goletz TJ, Swanson S, Chirmule N, Jawa V. Considerations for optimization and validation of an in vitro PBMC derived T cell assay for immunogenicity prediction of biotherapeutics. *Clin Immunol*. 2010;137:5–14.