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Recombinant Interleukin-1 Receptor Antagonist Is an Effective First-Line Treatment Strategy in New-Onset Systemic Juvenile Idiopathic Arthritis, Irrespective of HLA-DRB1 Background and *IL1RN* Variants

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Objective. Human leukocyte antigen (HLA)-DRB1*15:01 has been recently associated with interstitial lung disease (LD), eosinophilia, and drug reactions in systemic juvenile idiopathic arthritis (sJIA). Additionally, genetic variants in *IL1RN* have been linked to poor response to anakinra. We sought to reproduce these findings in a prospective cohort study of patients with new-onset sJIA treated with anakinra as first-line therapy.

Methods. HLA and *IL1RN* risk alleles were identified via whole-genome sequencing. Treatment responses and complications were compared between carriers versus noncarriers.

Results. Seventeen of 65 patients (26%) carried HLA-DRB1*15:01, comparable with the general population, and there was enrichment for HLA-DRB1*11:01, a known risk locus for sJIA. The rates of clinical inactive disease (CID) at 6 months, 1 year, and 2 years were generally high, irrespective of HLA-DRB1 or *IL1RN* variants, but significantly lower in carriers of an HLA-DRB1*11:01 allele. One patient, an HLA-DRB1*15:01 carrier, developed sJIA-LD. Of the three patients with severe drug reactions to biologics, one carried HLA-DRB1*15:01. The prevalence of eosinophilia did not significantly differ between HLA-DRB1*15:01 carriers and noncarriers at disease onset (6.2% vs 14.9%, *P* = 0.67) nor after the start of anakinra (35.3% vs 37.5% in the first 2 years of disease).

Conclusion. We observed high rates of CID using anakinra as first-line treatment irrespective of HLA-DRB1 or *IL1RN* variants. Only one of the 17 HLA-DRB1*15:01 carriers developed sJIA-LD, and of the three patients with drug reactions to biologics, only one carried HLA-DRB1*15:01. Although thorough monitoring for the development of drug hypersensitivity and refractory disease courses in sJIA, including sJIA-LD, remains important, our data support the early start of biologic therapy in patients with new-onset sJIA irrespective of HLA-DRB1 background or *IL1RN* variants.

INTRODUCTION

Systemic juvenile idiopathic arthritis (sJIA) is a complex, severe, and heterogeneous inflammatory disease of childhood onset, still classified as a subtype of juvenile idiopathic arthritis (JIA).[1] It is generally categorized as the most severe subtype. sJIA is distinct from other forms of JIA because of its prominent systemic features, increased risk of developing macrophage activation syndrome (MAS), and specific patterns of refractory disease course.[2,3] The advent of interleukin-1 (IL-1)– and interleukin-6 (IL-6)–inhibiting biologic therapies in the last two decades has brought about a significant improvement in the

treatment outcomes and prognosis of children with sJIA. In addition to reducing glucocorticoid dependency and improving outcomes, the use of these therapies has led to better understanding of the underlying disease mechanisms. It has been recognized that there is a "window of opportunity" early in the disease course, during which autoinflammatory mechanisms appear to be dominant and patients seem more responsive to targeted therapy by IL-1 blockade,[4–6] which may increase long-term chances to develop remission off of medication.

Recently, an increasing number of cases of sJIA-associated interstitial lung disease (sJIA-LD) have been reported as a refractory sJIA disease course, generally developing in the first 2 years of disease.[7–9] Although still poorly understood, suggested risk factors for the development of sJIA-LD are disease onset at young age (≤2 years), persistently high interleukin-18 (IL-18) levels during maintenance treatment, the occurrence of MAS, and adverse drug reactions to initiated biologic therapies (both IL-1 and IL-6 blockers).[7,8,10] Additionally, the association between sJIA-LD and pruritic rashes often accompanied by peripheral eosinophilia raised concerns for some type of drug hypersensitivity. In follow-up of these reports, in a retrospective case series, Saper et al noted that a large number of these patients satisfied the classification criteria for "drug reaction with eosinophilia and systemic symptoms" (DRESS), presumed to be triggered by the biologics used in sJIA.[11] Furthermore, there was a very strong association between this clinical phenomenon and the human leukocyte antigen (HLA)-DRB1*15:01 allele.[11] These findings have spurred a debate among pediatric rheumatologists about the utility of preprescription HLA typing to guide medication decisions for patients with new-onset sJIA. Such decisions may include postponing and even forgoing highly effective biologic therapy in patients with new-onset sJIA who carry the commonly occurring HLA-DRB1*15 haplotypes.

Since the early 2000s, registration trials and longitudinal cohort studies have demonstrated high effectiveness of both IL-1 and IL-6 blockade in sJIA.[12,13] Anakinra, a recombinant IL-1 receptor antagonist (rIL-1Ra) with excellent efficacy and safety profiles,[14] has been implemented as a first-line therapy in the Dutch national protocol for several years. This strategy has significantly reduced the need for glucocorticoids and improved both the percentage of patients and the time to achieve disease remission. However, approximately one-fourth of patients do not achieve or maintain the clinical inactive disease state in the first years after diagnosis on anakinra monotherapy.[4,15] These more difficult-to-treat patients have to switch to other targeted therapies and/or use adjunctive corticosteroids to control their disease.[3,16]

Identifying biomarkers and genetic profiles that predict therapy response at disease onset could pave the way for more personalized therapy in sJIA. Several genetic variants in the (promotor/intronic regions of the) IL1RN gene have recently been shown to modulate the serum levels of IL-1 receptor antagonist,

and the presence of these polymorphisms have been suggested to predict nonresponsiveness to anakinra therapy.[17] These findings, however, were based on a retrospective cohort analysis with a rather rough estimation of treatment response.[17] Attempts to validate these data in other cohorts have produced conflicting results.[6,18]

Here, we present HLA-DRB1 and IL1RN variant genotyping data from our prospective cohort of patients with new-onset sJIA treated in a standardized manner with the rIL-1Ra anakinra as first-line therapy.[4,15] The objectives of the study were to describe the HLA-DRB1 background of our sJIA cohort in relation to disease course and to determine clinical inactive disease (CID) rates in the first 2 years of disease based on HLA-DRB1 background and IL1RN genetic variants.

METHODS

Patients and treatment protocol. We prospectively included all consecutive patients with new-onset sJIA, who presented to the University Medical Center Utrecht (UMCU) between 2008 and 2022 and were diagnosed with sJIA in accordance with the criteria recently proposed by Martini et al (Supplementary Table 1).[19] Patients referred to our tertiary care center from other academic hospitals specifically for a refractory sJIA disease course were not included in the present analysis. In all patients, anakinra was started as the first-line therapy in accordance with our previously reported standardized treatment protocol.[4,15] Briefly, patients who had an unsatisfactory response to indomethacin (or an equivalent nonsteroidal antiinflammatory drug) after 3 to 7 days of treatment, as evidenced by the persistence of fever and/or arthritis, were initiated on anakinra at a dosage of 2 mg per kg per day subcutaneously (with a maximum of 100 mg per day in patients weighing ≥50 kg). If fever persisted after 3 days, the anakinra dosage was increased to 4 mg per kg per day (maximum of 200 mg per day). For patients with persistent disease activity while receiving anakinra monotherapy, prednisolone 0.5 to 1 mg per kg per day was added and/or treatment was switched to alternative biologic agents such as canakinumab 4 mg per kg or tocilizumab 8 mg per kg (for patients >30 kg) and 12 mg per kg (for patients <30 kg).

CID defined by the modified Wallace criteria[20] was set as treatment target in the first 2 years of follow-up. For patients in whom inactive disease was achieved on anakinra monotherapy (without adjunctive corticosteroids), anakinra was tapered and stopped within the first year of disease (some of the patients in this cohort, specifically those diagnosed with sJIA since 2017, were asked to participate in a biomarker based "taper and stop" study when CID was reached with rIL-1Ra alone).

The study has been conducted according to the criteria set by the declaration of Helsinki.[21] Written informed consent for whole-genome sequencing was obtained from all patients and/or

their representatives. Institutional review board approval was obtained by the UMCU ethics committee (08/215, 11/499, and 16/178).

Whole-genome sequencing and genotyping. DNA was isolated from peripheral blood using a Chemagic STAR nucleic acid extraction robot (Hamilton Company, NV, USA) according to the manufacturer's protocol. We performed singleton-based whole-genome sequencing using an Illumina NovaSeq6000 system (Illumina, CA, USA) using 150-bp paired-end reads with an average vertical coverage of 30 unique sequencing reads (30×) per base. Data were processed with an-inhouse–developed workflow, DxNextflowWGS v1.2.0,[22] including GATK v3.8-1-0-gf15c1c3ef[23] according to the best practices guidelines.[24] Briefly, we mapped the read pairs with BWA-MEM v0.7.17[25] against reference genome GRCh37, marked duplicates and merged lanes using Sambamba v0.7.0,[26] and performed base quality score recalibration using GATK BaseRecalibrator. Next, GATK Haplotypecaller was used to create Genome VCF formatted files. Finally, genotyping was performed using GATK GenotypeGVCFs for the following seven variants in the *IL1RN* gene associated with high IL-1Ra expression: rs55663133/–, rs62158854/T, rs62158853/C, rs55709272/T, rs7580634/G, rs4251961/T, and rs555447483/–. HLA alleles were determined using bwakit 0.7.12[25] against reference genome GRCh38.

Statistics. Continuous variables are presented as the median (interquartile range). Differences between two groups for nonnormally distributed data were analyzed by Wilcoxon's and Mann–Whitney's U test. Differences in categorical variables were analyzed by Pearson's chi-square test or Fisher's exact test, as appropriate. RStudio Version 2022.12.0+353 and R version 4.2.2 (2022-10-31) were used to calculate statistical significance. We calculated HLA frequency estimates by dividing the number of times the allele was observed by the total alleles in the cohort (alleles/2n). Percentages from reported allele frequencies were calculated using phenotype frequency = $1-(1-$ allele frequency$)^2$ assuming Hardy–Weinberg proportions. Graphs were created using GraphPad Prism 9. We considered *P* values below 0.05 to be significant.

RESULTS

Overall high response rates to rIL-1Ra as first-line therapy for sJIA. Our prospective cohort was composed of 65 patients with sJIA seen consecutively in our tertiary care center, of whom 54% were male subjects, with a median age of 7.2 years (range: 1–16.6 years) at the start of anakinra treatment. The median follow-up time was 7.8 years. The detailed clinical characteristics of the cohort are shown in Table 1a, 1b. Anakinra was initiated as the first-line therapy in 60 out of 65 patients and as the first-line biologic therapy in the remaining 5 patients (after

short and temporary corticosteroid therapy administered before referral to our center). All patients had at least 12 months of follow-up after initiation of anakinra, and 60 out of 65 patients reached at least the 2-year follow-up time point.

At 6 months, 1 year, and 2 years after starting with anakinra treatment, 89%, 88%, and 88% of patients respectively, achieved CID according to the Wallace criteria (Table 2).[20] These rates were comparable with those in the group of patients who had not received corticosteroids before the start of anakinra (90%, 87%, and 89%, respectively) as well as in line with previously published analyses of our cohort.[4,15] Table 2 also shows specific disease course characteristics of our cohort. Of note, one patient in our cohort developed sJIA-LD in the first 2 years of disease. This patient had digital erythema and clubbing but no pulmonary symptoms (no cough, no tachypnea, and no hypoxia on overnight O_2 saturation assessment). High-resolution chest Computed Tomography revealed pleural and septal thickening and ground glass opacities. This patient has a history of early-onset sJIA (<2 years of age at diagnosis) and two episodes of MAS (no known genetic variants) and was displaying persistently high serum levels of total IL-18, even while on effective maintenance treatment with canakinumab. This patient did not have adverse reactions to biologics.

In the entire cohort, the prevalence of MAS episodes diagnosed in the first 2 years of disease in accordance with the Ravelli criteria[27] was 20% (13 of 65 patients). Three patients had to discontinue anakinra because of (severe) allergic reactions to the drug. One of these patients developed a possible delayed-type drug reaction with high CCL17/TARC[28] and eosinophilia in the first 2 years of disease. The other two patients developed a Gell–Coombs type 4–like hypersensitivity reaction with fever, lymphadenopathy, and localized pruritic skin reactions increasing in severity with blistering or an anaphylactic-like reaction with vomiting, flushing, and globus sensation, respectively (Table 2). The medications used and additional information on the course of the disease in the entire cohort during the first 2 years, including the percentage of patients who were able to taper and stop anakinra, are listed in Supplementary Table 2.

HLA-DRB1*11:01, but not HLA-DRB1*15:01, is a risk allele for the development of sJIA when compared with the healthy control population of European descent. As shown in Figure 1A and Supplementary Table 3, 17 out of 65 patients in our cohort were identified as carriers of at least one HLA-DRB1*15:01 allele. Eighteen of 65 patients were carriers of HLA DRB1*11:01, a previously reported risk locus for sJIA (17 heterozygous, 1 homozygous).[29] The patient with sJIA-LD was a carrier of HLA-DRB1*15:01. Although none of the 16 remaining patients who were HLA-DRB1*15:01 positive developed sJIA-LD, one of them developed a possible delayed-type drug reaction and eosinophilia (Table 3 and Supplementary

Table 1a. Cohort characteristics at diagnosis, before start of anakinra*

Cohort characteristics	Response values
Total number of patients with sJIA in cohort	65
Median age at disease onset, y (range)	6.9 (0.6–15.7)
Median age at start anakinra, y (range)	7.2 (1–16.6)
Median interval between onset of symptoms and start of anakinra in days (IQR)	28 (18–61)
Sex distribution	46.2% female, 53.8% male
Ethnicity, n/total n	
African Arab	4/65
African Black	1/65
Eastern European	1/65
Northern European	0/65
Southern European	1/65
Western European	55/65
South Asian	1/65
Multiethnic ancestry	2/65
Trisomy 21	2/65
Steroid and biologic naive before start of anakinra	60/65
Clinical characteristics before start of anakinra	
Fever, %	100
Arthritis, % (n/total n)	72.3 (47/65)
Rash, % (n/total n)	87.7 (57/65)
Lymphadenopathy, % (n/total n)	60.0 (39/65)
Hepatosplenomegaly, % (n/total n)	29.2 (19/65)
Serositis, % (n/total n)	12.3 (8/65)

* IL = interleukin; IQR = interquartile range; sIL-2R = soluble interleukin-2 receptor.

Tables 4 and 5). A complete overview of the HLA-DRB1 subtypes is provided in Supplementary Table 3. The carriage rate of HLA-DRB1*15:01 in our cohort (26.15%) was comparable with that of the general Dutch population,[30,31] the European population,[32] and the US population of European descent[33] (Figure 1A and Supplementary Table 6). The percentage of individuals carrying HLA-DRB11*11:01 in our cohort was 27.7%. This was significantly higher compared with the available healthy control data in the Dutch population,[30,21] the European population,[32] and the American population of European descent[33] (Figure 1A, Supplementary Figure 1, and Supplementary Table 6). Because only three patients in our cohort were carriers of a different HLA-DRB1*11 subtype (HLA-DRB1*11:04 and HLA-DRB1*11:97), we use only the specific HLA-DRB1*11:01 in the rest of our association analyses.

High rates of CID in the first 2 years of disease, irrespective of HLA-DRB1*15:01 background. Stratification of patients based on the HLA-DRB1*15:01 allele carrier status did not reveal statistically significant differences in the proportion of patients achieving CID at 6 months, 1 year, and 2 years after start of anakinra. Specifically, 88%, 88%, and 80%, respectively, of patients carrying HLA-DRB1*15:01 achieved CID compared with 90%, 88%, and 91%, respectively, in individuals not carrying HLA-DRB1*15:01 (Figure 1B). Similarly, the percentage of patients in CID without medications at 6 months, 1 year, and 2 years after start of anakinra was high and not statistically different between HLA-DRB1*15:01 carriers and noncarriers (Table 3 and Supplementary Table 2). Although the percentage of patients requiring adjunctive corticosteroid treatment (either because of an MAS episode or persistent disease activity) or switching of biologic therapy

Table 1b. Laboratory characteristics before start of anakinra

Laboratory characteristics before start of anakinra	Median (IQR)	Clinical reference values
Hemoglobin, mmol/L	6.2 (5.6–6.8)	7.4–9.0
C-reactive protein, mg/L	135 (74–213)	0–10
Erythrocyte sedimentation rate, mm/h	98 (62–116)	2–8
Ferritin, µg/L	692 (352–1,937)	20–150
Thrombocytes × 10^9/L	578 (389–664)	150–450
Leukocytes × 10^9/L	17.35 (13.20–22.75)	5.00–14.50
Lymphocytes × 10^9/L	2.45 (1.74–3.66)	1.50–6.00
Monocytes × 10^9/L	0.67 (0.51–0.88)	0.00–0.80
Neutrophils × 10^9/L	13.85 (8.76–17.93)	1.50–8.00
Eosinophils × 10^9/L	0.16 (0.10–0.25)	0.00–0.60
Aspartate aminotransferase, U/L	29 (24–40)	0–30
Alanine aminotransferase, U/L	15 (10–24)	0–35
IL-18, pg/ml	9,040 (3,910–23,214)	0–175
sIL-2R, pg/ml	9,864 (5289–15806)	0–7,500

Table 2. Clinical response rates and disease characteristics in the first 2 years after start of first-line treatment with anakinra*

Disease characteristics of the cohort after start of anakinra	% (n/total n)
Clinical response to therapy	
Clinical inactive disease 6 months	89.2 (58/65)
Clinical inactive disease 1 year	87.7 (57/65)
Clinical inactive disease 2 years	88.3 (53/60)
Specific disease courses within first 2 years of starting anakinra	
Macrophage activation syndrome	20 (13/65)
sJIA-LD	1.5 (1/65)[a]
Severe allergic reactions to anakinra	4.6 (3/65)
Anaphylaxis	1.5 (1/65)
Extreme skin reaction	1.5 (1/65)
DDR/DRESS (but no sJIA-LD in FU to 2 years)	1.5 (1/65)[a]
Eosinophilia before start of anakinra (1× >ref value for age)	12.7 (8/63)
Pruritic skin reaction before start of anakinra	26.2 (17/65)
Eosinophilia after start of anakinra (1× >ref value for age)	59.4 (38/64)
Eosinophilia ($\geq 0.5 \times 10^9$/L) >2 occasions	38.1 (24/63)
Liver enzymes >3× the reference value	27.7 (18/65)

* DDR = delayed-type drug reaction; DRESS = drug reaction with eosinophilia and systemic symptoms; FU = follow-up; LD = lung disease; sJIA = systemic juvenile idiopathic arthritis.
[a] These patients are DRB1*15:01 / DRB1*11:01.

because of severe adverse reactions or insufficient response to anakinra was higher among HLA-DRB1*15:01 carriers compared with noncarriers, the differences did not reach statistical significance (53% vs 33%, $P = 0.245$ and 29% vs 17%, $P = 0.299$, respectively) (Figure 1C). Although not statistically significant, the occurrence of at least one episode of MAS within 2 years after the start of anakinra among HLA-DRB1*15:01 carriers (29%) was higher compared with noncarriers (17%, $P = 0.299$) (Figure 1D). Taken together, these data demonstrate that, in our cohort, HLA-DRB1*15:01 was not associated with clinical response to first-line anakinra treatment.

Patients with SJIA carrying an HLA-DRB1*11:01 background had a slightly lower chance to obtain a steroid-free CID state at time point 6 months and 1 year. In our cohort, patients who were carriers of an HLA-DRB1*11:01 allele had a significantly lower CID rate at 6 months and 1 year and a trend towards a lower response at 2 years after the start of therapy (72%, 72%, and 81% compared with 96%, 94%, and 91%, respectively; $P = 0.015$, $P = 0.032$, and $P = 0.370$, Figure 1B). There was also a trend towards a higher proportion of patients requiring addition of corticosteroids (56% vs 32%, $P = 0.095$) or a switch from anakinra to an alternative biologic (39% vs 13%, $P = 0.034$, respectively) (Figure 1C). Furthermore, the percentage of patients with an episode of MAS within 2 years after the start of anakinra seemed also higher

among HLA-DRB1*11:01 carriers (33% compared with 15% noncarriers, $P = 0.162$; Figure 1D).

Finally, although with limited patient numbers per group, we observed that carriers of both an HLA-DRB1*11:01 and an HLA-DRB1*15:01 allele (n = 6) showed the tendency to reach similar rates of CID at 6 months, 1 year, and 2 years after starting anakinra therapy compared with noncarriers (68%, 83%, and 80% vs 92%, 88%, and 89%; $P = 0.123$, $P = 0.561$, and $P = 0.475$, respectively) (Figure 1B). The carriers of both an HLA-DRB1*15:01 and an HLA-DRB1*11:01 allele, however, required addition of corticosteroids more often (83 vs 34%, $P = 0.028$). The need to switch from anakinra to an alternative biologic was also more frequent in this group (67% vs 15%, $P = 0.012$; Figure 1C). Strikingly, although not significantly, the percentage of HLA-DRB1*11:01/DRB1*15:01 carriers with an episode of MAS within 2 years of starting anakinra was 50%, which seemed higher than that of noncarriers (17%, $P = 0.089$; Figure 1D). Altogether, our data suggest that carriership of combined HLA-DRB1*11:01 plus DRB1*15:01 in a patient with sJIA may identify an increased risk for a refractory disease course.

Eosinophilia and transiently elevated liver enzymes are relatively common in patients with sJIA and are not associated with an HLA-DRB1*15:01 background. It was recently suggested that patients with sJIA with an HLA-DRB1*15:01 background have an increased risk of developing DRESS-like reactions in response to biologic therapies.[11] Important indicators of DRESS used in the RegiSCAR scoring system[34] are fever, lymphadenopathy, rash, and organ involvement, which unfortunately overlap with typical characteristics of active sJIA.[1,19,20] Other features such as eosinophilia, nonevanescent pruritic skin rashes, and elevated liver enzymes, although sometimes still present, are less typical for sJIA and may indeed point to the possibility of drug hypersensitivity.[35,36] Because our patients were regularly sampled during the first 2 years (at least every month in the first 3 months and at least every 3 months thereafter), we assessed the occurrence of eosinophilia and liver enzyme elevations, stratified based on the HLA-DRB1*15:01 background (Table 3). The incidence of eosinophilia (based on one measurement above the reference value for age; Supplementary Table 7) before the start of anakinra did not significantly differ and were statistically comparable between HLA-DRB1*15:01 carriers and noncarriers (Table 3). Additionally, eosinophilia and elevated transaminases were relatively common in the disease course (2 years after diagnosis; see Table 2). There was no statistically significant difference in the occurrence of eosinophilia between the groups (69% compared with 56%, based on one measurement above the reference value for age [Supplementary Table 7], and 38% vs 38% when based on >2 measurements of $\geq 0.5 \times 10^9$/L [absolute eosinophil count ≥ 500/μl used in the paper of Saper et al][11]). Similarly, during the first 2 years of treatment, episodes of liver enzymes elevations exceeding three times the reference value
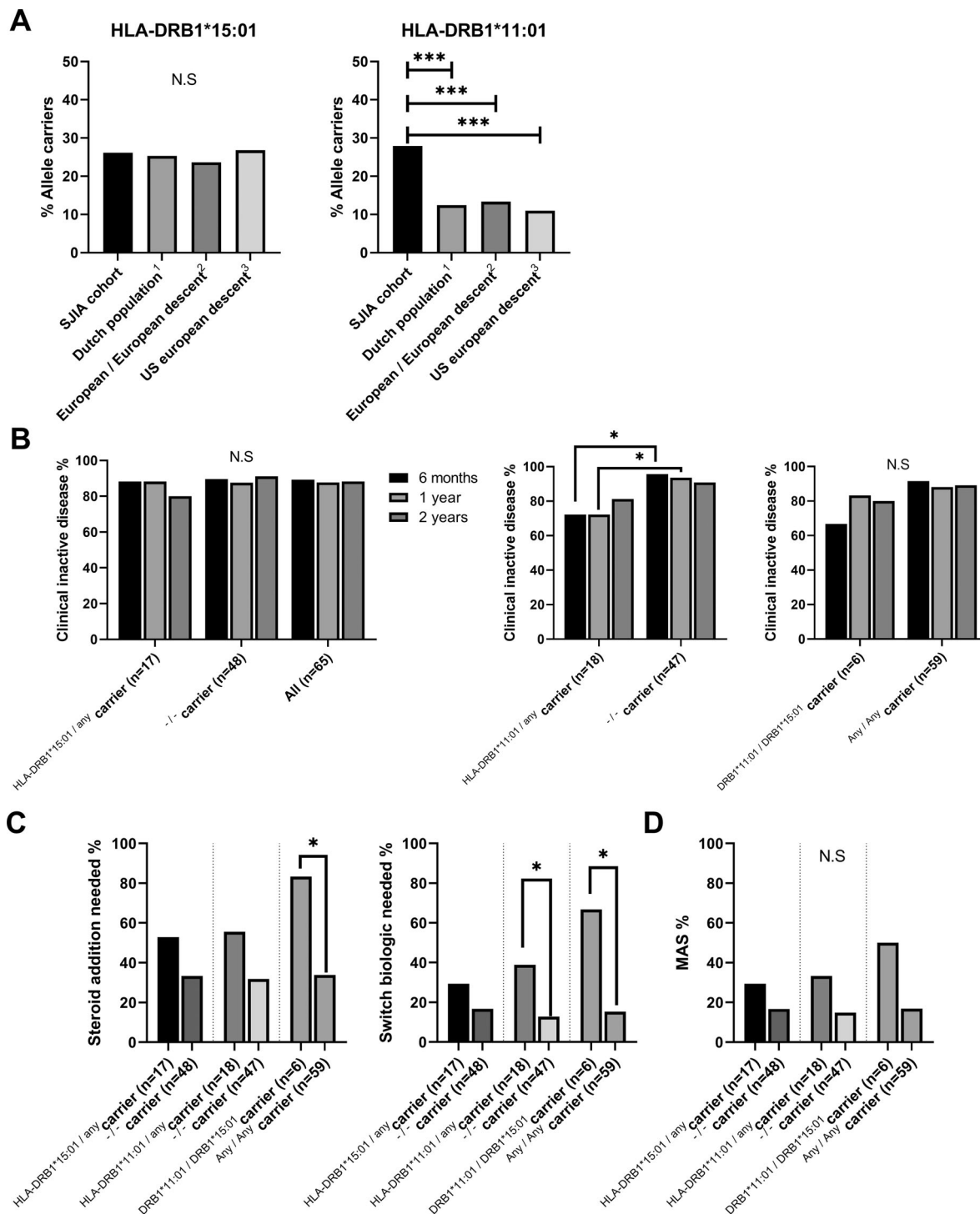
Figure 1. High rates of clinical inactive disease (CID) state irrespective of human leukocyte antigen (HLA)-DRB1 background within the first 2 years after start of first-line anakinra therapy. HLA-DRB1*11:01 with DRB1*15:01 seems to be associated with a higher chance to develop a severe disease course. (**A**) Percentage of patients with at least one allele of HLA-DRB1*15:01 or HLA-DRB1*11:01 in the systemic juvenile idiopathic arthritis (sJIA) cohort compared with the 1) Dutch (n = 1,816),[30] 2) European (n = 5,969,389),[32] and 3) American of European descent (n = 6,396)[33] population is shown. (**B–D**) Percentage of patients with (**B**) CID after 6 months, 1 year, and 2 years of starting anakinra; (**C**) needing steroid addition and needing a switch of biologic because of ineffectiveness or adverse reaction; and (**D**) macrophage activation syndrome (MAS) within the first 2 years in HLA-DRB1*15:01/Any carriers, HLA-DRB1*11:01/Any carriers, or DRB1*11:01/DRB1*15:01 carriers is shown. Chi-square and Fisher's exact tests were used. N.S = not significant.

for age were observed in 31% of HLA-DRB1*15:01 carriers compared with 28% in noncarriers (Table 3). Of note is that age at diagnosis, sex distribution, percentage of patients without arthritis at diagnosis, IL-18 levels, and ferritin levels at the start of treatment did not differ between HLA-DRB1*15:01 carriers and noncarriers.

IL1RN variants are not associated with poor response to anakinra in our cohort. In addition to the HLA-DRB1 background, our whole-genome analysis allowed us to investigate the association between specific IL1RN variants and the subsequent response to anakinra. Because anakinra was started as a first-line biologic treatment in all our patients and because we had a standardized treatment protocol for the first year of disease, our cohort provides a unique opportunity to assess the reported association by Arthur et al.[17]

The percentages of patients who were carriers of one or more of the reported variants in the IL1RN gene that were linked to higher IL-1Ra expression and a decreased response to anakinra therapy[17] are shown in Table 4. The original study demonstrated that the IL1RN 'risk' polymorphisms are inherited as a part of a common haplotype. Indeed, in our cohort, 63% of patients had all the 'risk' variants on at least one of the alleles and 25% of the patients had all the 'risk' variants on both alleles. Our analyses indicate that there were no significant differences in the percentage of patients achieving CID in the first 2 years after

starting anakinra treatment based on their homozygosity or heterozygosity for the risk or reference haplotype (Figure 2A). In addition, stratification of the patients based on the presence of the relevant variants did not reveal significant differences in the proportions of the patients that would require the addition of corticosteroids or switching to an alternative biologic because of an insufficient effect of anakinra (Figure 2B). The seven variants individually were also not associated with nonresponse (data not shown). Pardeo et al[6] showed that homozygosity for at least one of six high-expression variants (excluding rs555447483/–) was associated with an increased risk of nonresponse to anakinra. This study classified response as CID off glucocorticoids at 6 months. We could not confirm a significant association of these variants with the likelihood of achieving CID off glucocorticoids while being treated with anakinra (Supplementary Figure 2).

DISCUSSION

Recent genetic studies have determined putative associations with HLA-DRB1 haplotypes and IL1RN variants potentially impacting disease course and response to biologic treatment in sJIA.[6,11,17] To verify these findings, we performed whole-genome sequencing for all consecutive patients with sJIA enrolled in a prospective, standardized treatment cohort of 65 Dutch patients with sJIA who received anakinra as first-line therapy. A high percentage of patients

Table 3. HLA-DRB1*15:01 background in relation to disease course characteristics*

Clinical information	HLA-DRB1*15.01 (n = 17)	Not HLA-DRB1*15.01 (n = 48)	P value
Before start of anakinra therapy			
Median age, y	5.5	7.3	0.888
Sex distribution, % male	47.1%	56.2	0.514
Steroid naive	82.4 (14/17)	95.8 (46/48)	0.107
Trisomy 21	5.9% (1/17)[a]	2.1 (1/48)	0.458
Arthritis at diagnosis	75.0 (12/16)	72.9 (35/48)	0.870
Eosinophilia (1× >ref value for age)	6.2 (1/16)	14.9 (7/47)	0.667
Pruritic skin reaction	29.4 (5/17)	25.0 (12/48)	0.754
IL-18, pg/ml	8,605 (5,448–23,812)	9,617 (3,123–29,087)	0.822
Ferritin, μg/L	1,139 (501–1,809)	635 (298–1,936)	0.407
After start of anakinra therapy			
Eosinophilia (1× >ref value for age)	68.8 (11/16)	56.2 (27/48)	0.378
Eosinophilia (>2× ≥0.5 × 10^9/L)	37.5 (6/16)	37.5 (18/47)	0.955
Liver enzymes >3× the reference value	31.2 (5/16)	27.7 (13/47)	0.784
Medication free at CID/in remission at the following time points			
6 Months	17.6 (3/17)	25.0 (12/48)	0.741
1 Year	47.1 (8/17)	35.4 (17/48)	0.404
2 Years	50 (8/16)	59.1 (26/44)	0.568
Severe allergic reaction to anakinra	5.9 (1/17)	4.2 (2/48)	1.000
sJIA-LD	5.9 (1/17)	0.0 (0/48)	

* Values are the median (interquartile range) or the % (n/total n) unless indicated otherwise. Continuous variables are presented as the median (interquartile range). Differences between two groups for nonnormally distributed data were analyzed by Mann–Whitney U test. Differences in categorical variables were analyzed by Pearson's chi-square test or Fisher's exact test, as appropriate. We considered P values below 0.05 to be significant.
CID = clinical inactive disease; HLA = human leukocyte antigen; LD = lung disease; sJIA = systemic juvenile idiopathic arthritis.
[a] This patient is DRB1*11:04 / DRB1*15:01.

Table 4. Presence of *IL1RN* variants associated with IL-1Ra high expression and IL-1 pathway–targeted therapy nonresponse in our cohort*

Presumed sJIA risk allele (reference)	Presumed risk allele for nonresponse	GRCh37–Hg19 location	Distribution of the allele in the cohort	
rs55663133/AAT	rs55663133/–	Chr2:113871159–113871173	Homozygous	34%
			Heterozygous	49%
			Reference	17%
rs62158854/G	rs62158854/T	Chr2:113870725	Homozygous	32%
			Heterozygous	51%
			Reference	17%
rs62158853/T	rs62158853/C	Chr2:113869566	Homozygous	32%
			Heterozygous	51%
			Reference	17%
rs55709272/C	rs55709272/T	Chr2:113867288	Homozygous	26%
			Heterozygous	52%
			Reference	22%
rs7580634/T	rs7580634/G	Chr2:113866854	Homozygous	29%
			Heterozygous	52%
			Reference	19%
rs4251961/C	rs4251961/T	Chr2:113874467	Homozygous	43%
			Heterozygous	43%
			Reference	14%
rs555447483/A	rs555447483/–	Chr2:113867341–113867354	Homozygous	29%
			Heterozygous	52%
			Reference	19%
Homozygous for risk haplotype[a]				24.6%

* IL = interleukin; sJIA = systemic juvenile idiopathic arthritis.
[a] Homozygous carrier of all the seven variants associated with failure of IL1β pathway–targeted therapy. Homozygous = homozygous carrier of the presumed risk allele of nonresponse, Reference = homozygous carrier of the sJIA risk allele (reference allele).

reached CID at time points 6 months, 1 year, and 2 years after the start of anakinra (89%, 88%, and 88%, respectively), in line with previous publications of our cohort.[4,15] Although the overall response rate to anakinra was high, the cohort still includes patients with sJIA with a severe disease course, with ∼20% of the patients developing at least one episode of MAS in the first 2 years of disease. In addition, one patient developed sJIA-LD, and three patients had a severe adverse reaction to anakinra.

Our data demonstrate that a complete response (defined as a state of CID) to anakinra as first-line therapy in the first 6 months of disease was high compared with other sJIA cohort studies, irrespective of HLA-DRB1 background. Although the CID rate was significantly lower in HLA-DRB1*11:01 carriers, it was still above 70%. In addition, we did not observe significant differences in treatment response to anakinra as first-line therapy in patients with sJIA stratified to previously reported high-risk *IL1RN* variants for nonresponse to anakinra. Our data therefore strongly support the concept of initiating early targeted treatment in patients with new-onset sJIA regardless of the genetic background.

In our sJIA cohort, we confirmed a significant higher proportion of HLA-DRB1*11:01 carriers compared with the rate in the general population of European descent. Hence, our observations validate the findings of a previous report that HLA-DRB1*11:01 is associated with an increased risk of developing sJIA.[29] The HLA-DRB1*15:01 allele was, however, found in a comparable carrier rate as the general population. Both the

patient who developed sJIA-LD and one of the three patients with a severe drug reaction to anakinra were HLA-DRB1*15:01 carriers. Nonetheless, 16 of 17 DRB1*15:01 carriers did not develop sJIA-LD in the first 2 years of disease, and from three patients with reactions to biologics, only one carried the HLA-DRB1*15:01 allele.

We did not observe any HLA-DRB1*15:01 associations with DRESS-associated symptoms such as increased liver enzymes or eosinophilia in our cohort. We observed that the occurrence of eosinophilia and transaminase elevation was relatively common but generally transient and typically did not require withdrawal of effective biologic therapy. We would like to emphasize that the diagnostic criteria for DRESS used in the RegiSCAR scoring system,[34] including fever, lymphadenopathy, rash, organ involvement, and blood count abnormalities, unfortunately overlap with typical characteristics of active sJIA.[1,20] In our opinion, the RegiSCAR score should therefore be carefully interpreted in the context of sJIA, and prospective validation of this score in patients with sJIA may be needed before conclusions on DRESS-development in these patients can be drawn.

Binstadt and Nigrovic recently coined the cytokine plasticity hypothesis as an alternative explanation for 'DRESS'-like reactions in patients with sJIA using IL-1 or IL-6 inhibitors.[35] This hypothesis proposes that biologics shift the balance of some of the T cells away from the IL-1/IL-6–driven T helper (Th) 17 cell phenotype to Th1, with associated production of interferon
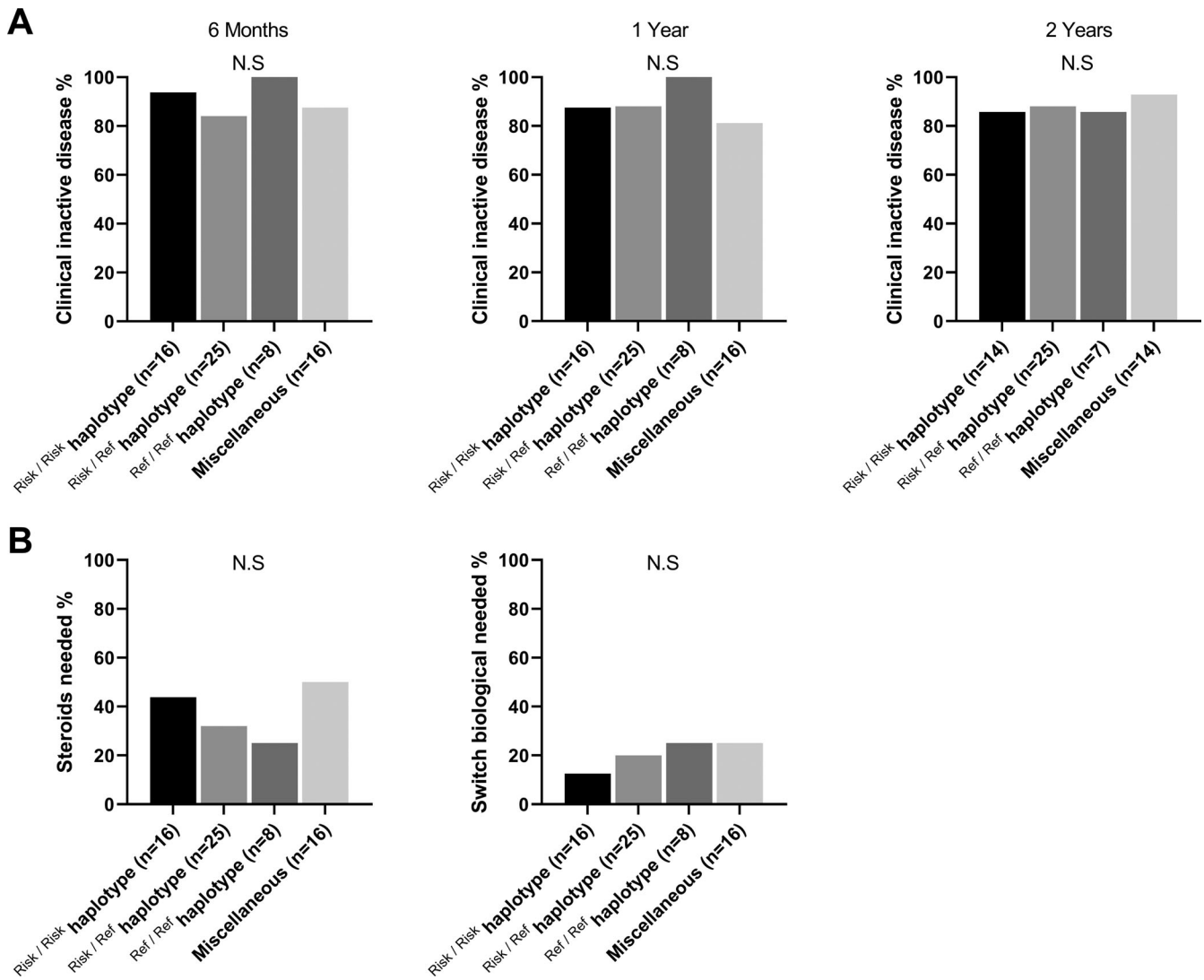
Figure 2. *IL1RN* genetic variants associated with high interleukin-1 (IL-1)Ra expression do not distinguish response to anakinra. The percentage of patients (**A**) reaching clinical inactive disease (CID) at 6 months, 1 year, and 2 years after the start of anakinra or (**B**) needing steroid addition and needing a switch of biologic because of ineffectiveness or adverse reactions based on risk variant haplotype carriers associated with IL-1Ra high expression is shown. Homozygous for risk haplotype (rs55663133/–, rs62158854/T, rs62158853/C, rs55709272/T, rs7580634/G, rs4251961/T, and rs555447483/– on both alleles), heterozygous for risk haplotype (rs55663133/–, rs62158854/T, rs62158853/C, rs55709272/T, rs7580634/G, rs4251961/T, and rs555447483/– on one allele), homozygous for reference haplotype (rs55663133/AAT, rs62158854/G, rs62158853/T, rs55709272/C, rs7580634/ T, rs4251961/C, and rs555447483/A on both alleles), and miscellaneous (individuals carrying a mix) are given. Fisher's exact test was used. N.S = not significant.

gamma and/or promote Th17 to Th2 conversion, leading to a drug-induced hypersensitivity syndrome–type response. Under the right conditions, such clonal skewing might trigger immune responses to exogenous or endogenous antigens. So far, it is poorly understood which epitopes might play a role here.[5,37,38]

Interestingly, our response data show that HLA-DRB1*11:01, but not HLA-DRB1*15:01, is associated with a lower response to our treatment strategy. Moreover, the combination of HLA-DRB1*11:01 and HLA-DRB1*15:01 carriership is associated with the need for adjunctive corticosteroid treatment and an increased rate of switching to alternative biologic

therapies. These findings need to be validated before affecting clinical practice, but altogether, our data point to a heterogenic risk for development of a refractory disease course in sJIA. Therefore, careful monitoring of patients that do not achieve CID in the first months of disease is advised. This may include genetic studies (HLA typing, but not detailed *IL1RN* variant array), but because our data show high (initial) response rates to rIL-1Ra therapy if introduced early, irrespective of HLA-background, these analyses should not delay the start of effective targeted treatment.

Our study has some limitations. Although this is one of the largest single-center sJIA cohorts worldwide with 65 prospectively

included new-onset patients, increased group size might allow identifying differences that this cohort cannot. In this cohort, only 1 of 65 patients developed sJIA-LD, which is less than the reported frequency in some cohorts from the US.[8] Explanations for this might be different ethnicities and therefore genetic backgrounds, differences in inclusion criteria for sJIA (proposed criteria by Martini et al[19] vs International League of Associations for Rheumatology [ILAR][1] criteria), differences in cohort composition (overrepresentation of second opinions or referral of refractory cases), and different (first-line) treatment plans possibly not including early first-line biologics, as well as environmental factors. Our prospective cohort with a standardized treatment plan for new-onset sJIA with first-line biologic therapy targeting the IL-1 pathway is particularly suitable to validate genetic risk factors for nonresponse to rIL-1Ra therapy. However, the single-country, single-center nature of our cohort influences the genetic makeup of our patients, which is likely to be skewed towards individuals with a European ethnic background. For example, all patients carrying an HLA-DRB1*15 allele in our cohort have DRB1*15:01, which predominates in subjects from European descent, whereas 15:03 and 15:06 are more common in subjects from other genetic backgrounds.[33,35] The HLA frequencies are therefore not generalizable to patient populations of African or Asian descent.

In conclusion, we demonstrate that rIL-1Ra treatment is an effective first-line treatment strategy in new-onset sJIA, irrespective of HLA-DRB1 background and variants in the *IL1RN* gene. We do observe a present but significantly lower rate of clinical inactive disease at time points 6 months and 1 year after start of treatment in carriers of an HLA-DRB1*11:01 allele. This finding has to be validated in other cohorts before clinical decisions can be made based on these findings.

Although thorough monitoring for the development of drug hypersensitivity and refractory disease courses in sJIA, including sJIA-LD, remains important and genetic testing for HLA can be a part of a vigilant monitoring scheme in the clinical setting, our data support the early start of biologic therapy in new-onset patients with sJIA irrespective of HLA-DRB1 background or *IL1RN* variants. Further (translational) studies into refractory disease courses, including the development of sJIA-LD, and research into (genetic) characteristics predicting (refractory) disease courses and therapy response in sJIA are needed and will hopefully lead to a more personalized and successful treatment strategy in the future.

AUTHOR CONTRIBUTIONS

All authors were involved in drafting the article or revising it critically for important intellectual content, and all authors approved the final version to be published. Dr. Vastert had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Study conception and design. Erkens, Verwoerd, Sinha, Roth, Schulert, Grom, Vastert.
Acquisition of data. Erkens, Calis, Verwoerd, De Roock, Ter Haar, Den Engelsman, Van der Veken, Ernst, Van Deutekom, Pickering, Scholman, Jansen, Swart, Vastert.
Analysis and interpretation of data. Erkens, Calis, Van Loosdregt, Vastert.

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