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Analysis of the Immunogenicity from Abatacept-Treated Pediatric Patients With Polyarticular-Course Juvenile Idiopathic Arthritis: Findings From Two Phase III Clinical Trials

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Objective. The goal of this article is to present the analysis of anti-abatacept antibody data from children with polyarticular-course juvenile idiopathic arthritis (pJIA), treated with abatacept. The data are from 395 participants with pJIA from two abatacept registrational trials.

Methods. We analyzed immunogenicity data according to age groups, administration route (intravenous [IV] or subcutaneous [SC]), drug treatment interruption, and co-medications (with or without methotrexate [MTX]) to assess impact on the incidence of anti-abatacept antibodies.

Results. The overall immunogenicity incidences observed in both JIA trials ranged between 4.7% and 23.3%. There was a slightly higher immunogenicity incidence in the 2–5-year-old participants (15.2%) compared with 6–17-year-old participants (4.7%). In the study with SC dosing, the overall incidence on treatment was 2.3% (3% if co-dosed with MTX), similar to the incidence for Period A of the IV study (similar duration of treatment as the SC study), which was 2.1% (1.4% if co-dosed with MTX). In the IV study, the period following a 6-month interruption in treatment had comparable immunogenicity incidences (22.9% with interruption vs. 18.2% without interruption, both co-dosed with MTX and 0% for both not co-dosed with MTX). In most cases, participants co-dosed with MTX had higher immunogenicity incidences than those on abatacept alone.

Conclusion. Although some trends were noted in terms of incidence according to age and MTX co-dosing, none where conclusive owing to differences in population size. Drug holiday had no impact on immunogenicity incidence once treatment was resumed, and incidences across SC and IV dosing were comparable. There was no impact of immunogenicity on pharmacokinetics, safety, and efficacy.

INTRODUCTION

Juvenile idiopathic arthritis (JIA) is a form of arthritis of unknown origin that causes inflammation in multiple joints of the hands, feet, and other locations in children less than 16 years of age (1). Polyarticular-course JIA (pJIA), which involves five or more joints, can be a clinical presentation of many of the following subtypes of JIA: including rheumatoid factor positive or negative JIA (resembling adult rheumatoid arthritis [RA]), extended oligoarthritis, psoriatic arthritis, enthesitis-related arthritis, and systemic onset JIA (2).

The short-term goals of therapy in pJIA are to reduce inflammation and relieve pain. Long-range objectives are to prevent disease progression and destruction of joints, bones, and cartilage. Current treatment of pJIA includes nonsteroidal anti-inflammatory drugs, conventional synthetic disease modifying anti-rheumatic drugs (eg, methotrexate [MTX]), and biologics such as tumor necrosis factor (TNF) inhibitors (etanercept, adalimumab),

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anti-interleukin-6 receptor antibody (tocilizumab), and T-cell co-stimulation inhibitors (abatacept). Intravenous (IV) abatacept is approved for the treatment of children with pJIA aged from 6 to 17 years in the United States, Europe, and Japan, and subcutaneous (SC) abatacept is approved for children with pJIA aged from 2 to 17 years in the United States and Europe.

Abatacept is a fully human co-stimulation modulator of T cells (3). It is a recombinant, soluble fusion protein consisting of the extracellular domain of cytotoxic T lymphocyte–associated antigen-4 (CTLA-4) linked to the Fc portion of immunoglobulin G1, modified to prevent complement fixation and antibody-dependent cellular cytotoxicity. CTLA-4 is an endogenous competitive inhibitor of co-stimulation, binding B7-1 and B7-2 ligands on the antigen presenting cell with higher affinity than CD28 on the T cell, preventing the co-stimulatory activation signal. The interaction between CD28 and the B7-1/B7-2 ligands is needed to drive full T-cell activation (4). The CTLA-4 portion of abatacept binds the ligands B7-1 and B7-2 on antigen presenting cells and thereby inhibits their binding to the T-cell costimulatory receptor CD28 on T cells. Thus, by blocking this interaction, abatacept also inhibits activation of other inflammatory effector cells (eg, macrophages), B cells, and synoviocytes (5,6).

As it is the case for other biologics, abatacept has the potential to elicit an immunogenicity response in participants. The consequences of immunogenicity across biologics vary widely from no clinical effect to life-threatening symptoms (7,8). Anti-drug antibodies (ADAs) may form complexes with the drug that result in faster or slower clearance, impacting pharmacokinetics (PK) and consequently receptor occupancy; in some cases, the ADAs may bind to epitopes that prevent the drug from binding to its target. These ADAs are characterized as "neutralizing" antibodies, and depending on their levels in circulation, they may impact the efficacy of the drug (9). ADAs may also result in safety adverse events such as hypersensitivity reactions (10) or more severe events such as thromboembolism (11) and pure red cell aplasia (12). The potential risk to a participant's safety is highly dependent on the mechanism of action of the biologic and its structure (13,14). Hence, it is of great importance to continuously monitor the immunogenicity of a biologic under clinical investigation to understand whether any modifications to the bioanalytical and/or clinical strategy are necessary.

The prescription of biologics for the treatment of JIA has been increasing (15–17), pediatric data from seven biologics has been reviewed (18) in terms of efficacy and safety, and impact of immunogenicity of biologics for treatment of RA has been covered (19), but a deeper analysis of the immunogenicity data would be beneficial to those interested in the field. Here, we analyze the immunogenicity data from two phase III abatacept trials in pJIA participants according to factors known to have the potential to impact immunogenicity: co-medications, route of administration, treatment interruption, and age (20,21).

PARTICIPANTS AND METHODS

Participants and trial design. The abatacept JIA trials "JIA1: AWAKEN" (IV in children aged between 6 and 17 years) and "JIA2" (SC in children aged between 2 and 17 years) with polyarticular disease have been previously reported (22,23) and are briefly described under Supplementary Materials.

Immunogenicity assessments. All immunogenicity assessments followed a three-tier approach: screen, confirm, and titer. In the screening tier, antibodies that bind to the therapeutic protein product are detected using a cutoff value that would result in a 5% false positive rate (this ensures no positive response is missed). Therefore, the confirmatory tier (usually uses competition with the drug in the same assay) is used to ensure the specificity of the response to the drug only. In the third tier, the sample is titrated in the screening assay to characterize the magnitude of the ADA response.

JIA1 (IV) abatacept trial. Blood samples for immunogenicity assessments were collected prior to administration every 3 months for the first 2 years, and every 6 months at the start of year 3. Samples were also collected at the final or early termination visits and then 28, 56 and 85 days after the last dose.

To distinguish ADA specificity (where the antibody binds), two different direct-format validated enzyme-linked immunosorbent assays (ELISAs) were used to evaluate the presence of antibodies against CTLA-4 and the immunoglobulin G (IgG) portion of abatacept. METHOD-1 measures the antibody response to the full drug. Method details are described in the Supplementary Materials and are summarized in Table 1.

JIA2 (SC) abatacept trial. Blood samples for immunogenicity assessments were collected before the dose at Days 1, 57, 85, and 113 of the short-term period (Day 1 through the end of Month 4), at 6-month intervals during the 20-month long term extension period (post month 4 through month 24), and the Final/Early Termination and follow-up visits at 28, 85, and 168 days after the last injection of abatacept.

Drug interference is a challenge in immunogenicity assays because antibodies bound to drug in circulation may not be detected and result in false negatives. To address this, METHOD-3 was developed and validated to improve the drug tolerance compared with METHOD-1 and METHOD-2. Drug tolerance is the amount of drug present in the bioanalytical sample that would not interfere in the detection of the ADAs in the same sample. METHOD-3 is a homogenous bridging assay with a much better drug tolerance than the direct binding ELISAs (24); in addition, the method uses the electrochemiluminescence platform, which improves dynamic range and supports matrix tolerance (25). METHOD-3 details are summarized in Table 1 and additional details in the Supplementary Materials.

Table 1. Summary of critical method parameters for the immunogenicity assays used to support JIA1 and JIA2 sample analysis

	METHOD-1	METHOD-2	METHOD-3
Format and platform	Direct binding (ELISA)	Direct binding (ELISA)	Bridging with acid pre-treatment (ECL)
Analyte	Anti-drug antibodies	Anti-drug antibodies	Anti-drug antibodies
Positive control	Monkey polyclonal	Monkey polyclonal	Monkey polyclonal
Sample dilution	400-fold	25-fold	10-fold
Confirmatory tier	Whole drug, CTLA-4, non-related Ig, ovalbumin	Whole drug, CTLA-4, ovalbumin	Whole drug, CTLA-4
Sensitivity	~20 µg/ml	275 ng/ml	12 ng/ml
Drug tolerance	Significant drug interference	~500 ng/ml Ab PC with ~1 µg/ml of abatacept 975 ng/ml of Ab PC with 5 µg/ml of abatacept	250 ng/ml of Ab PC with 40 µg/ml of abatacept 2 µg/ml of Ab PC with 100 µg/ml of abatacept
Study supported	JIA1	JIA1	JIA2

Abbreviations: Ab,; ECL, electrochemiluminescence; ELISA, enzyme-linked immunosorbent assay; CTLA-4, cytotoxic T lymphocyte–associated antigen-4; JIA1, abatacept trial abbreviation; JIA2, abatacept trial abbreviation; PC, positive control.

Initiated by a health authority request, prior to the start of the JIA2 trial, incurred samples were analyzed in all three assays to assess comparability. Serum samples from 249 disease state participants from an RA study were used in the comparison. Of the 249 participants tested in METHOD-1, five samples (three participants) tested positive with specificity against immunoglobulin. In METHOD-2, two samples (one participant) tested positive with specificity against CTLA-4. In METHOD-3, 24 samples (16 participants) tested positive to at least one of the drug components (26). The comparison of the methods was deemed acceptable by the requesting health authority agency. Therefore, METHOD-3 was used to support immunogenicity assessments in JIA2 and other clinical studies.

Details on the neutralizing antibody, PK, safety, and efficacy assessments are provided in the Supplementary Materials.

Statistical methods. All treated participants with at least one post-baseline immunogenicity result were included in the immunogenicity analysis population.

Participants were defined as immunogenicity positive if they had 1) a missing baseline immunogenicity measurement and a positive analytical laboratory reported immunogenicity response post-baseline, 2) a negative baseline immunogenicity response and a positive analytical laboratory reported immunogenicity response post-baseline, and 3) positive baseline immunogenicity response and a positive analytical laboratory reported immunogenicity response post-baseline that had a titer value greater than the baseline titer value.

Lack of immunogenicity was defined as the absence of a positive response.

No formal statistical tests were performed, and all data were analyzed for descriptive purposes only.

The immunogenicity-evaluable participants for the JIA1 study included 189 of 190 participants who entered Period A, 122 (60 abatacept-randomized; 62 placebo-randomized) participants who entered Period B, and 150 of the 153 participants who entered Period C.

In addition, the following JIA1 subpopulations of 150 immunogenicity-evaluable participants who entered Period C were analyzed: 58 abatacept-randomized and 58 placebo-randomized participants in Period B, and 34 participants who entered Period A and then continued into Period C.

In the JIA2 6- through 17-year-old cohort, 173 participants were assessed for efficacy and safety; 160 participants were immunogenicity evaluable. Forty-six participants were treated in the 2- through 5-year-old cohort and evaluated for efficacy, safety, and immunogenicity. Immunogenicity data analyses for JIA2 study were performed by age cohorts and overall, at study Days 113 and 729.

The proportion of participants who had detectable anti-abatacept or anti-CTLA-4 antibodies was summarized. The interrelationship of a positive serum antibody status was further explored for concomitant MTX use, abatacept minimum steady-state serum concentration (Cmins), disease flare in Period B for JIA1 and loss or maintenance of American College of Rheumatology Pediatric 30% response rate (JIA-ACR30) for JIA2, and occurrence of adverse events during the cumulative period of treatment with IV and SC abatacept. The persistence of an antibody response was explored descriptively.

RESULTS

Overall immunogenicity incidence. In the JIA1 (IV) study, 44 of 189 (23.3%) evaluable participants were positive for ADA at least once during the study, of which 40 of 189 participants (21.2%) had anti-CTLA-4 antibodies, and 6 participants (3.5%) had antibodies for the IgG portion of the abatacept molecule (anti-abatacept). Two participants were positive for both anti-CTLA-4 and anti-abatacept antibodies (Table 2).

In the 2–5-year-old cohort of the pJIA2 (SC) study, 7 of 46 (15.2%) participants were positive for antibodies to abatacept

Table 2. Immunogenicity incidence from the JIA1 (IV) trial summarized by cohort and reactivity by baseline MTX use

Reported Period	Cohort Entering the Reported Period	MTX Use at Day 1	Overall Immunogenicity, Positive/Total[a] (Rate)		
			Anti-CTLA-4	Anti-Abatacept (Anti-Ig)	Total
A[b]	Period A: All participants	Yes	2/140 (1.4%)	0/129	2/140 (1.4%)
		No	2/49 (4.1%)	0/34	2/49 (4.1%)
		Overall	4/189 (2.1%)	0/163	4/189 (2.1%)
B[b]	Period B: Abatacept-treated	Yes	7/45 (15.6%)	0/43	7/45 (15.6%)
		No	0/10	0/6	0/10
		Overall	7/55 (12.7%)	0/49	7/55 (12.7%)
	Period B: Placebo-treated	Yes	19/41 (46.3%)	0/37	19/41 (46.3%)
		No	3/13 (23.1%)	0/9	3/13 (23.1%)
		Overall	22/54 (40.7%)	0/46	22/54 (40.7%)
C[c]	Period B: Abatacept-treated	Yes	5/48 (10.4%)	6/45 (13.3%)	11/48 (22.9%)
		No	0/10	0/10	0/10
		Overall	5/58 (8.6%)	6/55 (10.9%)	11/58 (19.0%)
	Period B: Placebo-treated	Yes	4/44 (9.1%)	4/40 (10.0%)	8/44 (18.2%)
		No	0/14	0/14	0/14
		Overall	4/58 (6.9%)	4/54 (7.4%)	8/58 (13.8%)
	Period A: Non-responders who entered Period C	Yes	4/25 (16.0%)	1/24 (4.2%)	4/25 (16.0%)
		No	3/9 (33.3%)	0/8	3/9 (33.3%)
		Overall	7/34 (20.6%)	1/32 (3.1%)	7/34 (20.6%)
	Overall in Period C	N/A	16/150 (10.7%)	11/141 (7.8%)	26/150 (17.3%)
N/A	Grand overall for Periods A, B, and C	N/A	40/189 (21.2%)	6/171 (3.5%)	44/189 (23.3%)

Abbreviations: CTLA-4, cytotoxic T lymphocyte–associated antigen-4; Ig, immunoglobulin; IV, intravenous; JIA, juvenile idiopathic arthritis; MTX, methotrexate; N/A, not applicable.
[a] Number of subjects who were evaluated.
[b] Reported Period A and B data cut, March 19, 2009.
[c] Reported Period C data cut, May 8, 2012.

during the cumulative period, of which three participants had antibodies to immunoglobulin and/or Junction region and four participants were positive specific to CTLA-4 and possibly immunoglobulin. In the 6–17-year-old cohort, 8 of 172 (4.7%) participants tested positive for antibodies to abatacept in the cumulative period, of which 3 tested positive for antibodies specific to immunoglobulin and/or Junction region and five tested positive specific to CTLA-4 and possibly immunoglobulin (Table 3).

Incidence according to co-medication, age cohort, and administration route. Table 2 summarizes the immunogenicity incidences for participants from the JIA1 (IV) trial according to the different periods in the trial and co-dosing with MTX. The incidences in Period C are very similar between participants who had been in Period B on placebo (18.2%) and on abatacept (22.9%). However, the data from Period B, between the placebo- and the abatacept-treated participants, show that those

Table 3. JIA2 (SC) summary of immunogenicity incidence per age cohort and specificity of the response

Cohort	MTX Use at Day 1	Anti-CTLA-4-Positive/Total[a] (Rate)	Anti-Abatacept (Anti-IgG1): Positive/Total[a] (Rate)	Total: Positive/Total[a] (Rate)
2 to 5-year-old cohort				
Overall, on treatment	Yes	2/36 (5.6%)	3/36 (8.3%)	5/36 (13.9)
	No	0/10	0/10	0/10
Overall, follow-up visits	Yes	2/6 (33.3%)	1/6 (16.7%)	3/6 (50.0%)
	No	0/2	0/2	0/2
Overall	Yes	4/36 (11.1%)	3/36 (8.3%)	7/36 (19.4%)
	No	0/10	0/10	0/10
Grand overall	N/A	4/46 (8.7%)	3/46 (6.5%)	7/46 (15.2%)
6- to 17-year-old cohort				
Overall, on treatment	Yes	1/135 (0.7%)	3/135 (2.2%)	4/135 (3.0%)
	No	0/37	0/37	0/37
Overall, follow-up visits	Yes	4/37 (10.8%)	2/37 (5.4%)	6/37 (16.2%)
	No	0/7	0/7	0/7
Overall	Yes	5/135 (3.7%)	3/135 (2.2%)	8/135 (5.9%)
	No	0/37	0/37	0/37
Grand overall	N/A	5/172 (2.9%)	3/172 (1.7%)	8/172 (4.7%)

Abbreviations: ADA, anti-drug antibody; CTLA-4, cytotoxic T lymphocyte–associated antigen-4; IgG1,; JIA, juvenile idiopathic arthritis; MTX, methotrexate; N/A, not applicable; SC, subcutaneous.
[a] Number of total subjects evaluated in the ADA assay.

on abatacept had a much lower incidence (15.6%) compared with the placebo arm (46.3%). An increase in overall incidence in the abatacept-treated participants in Period B (12.7%) was observed when compared with incidence in Period A (2.1%). Across Periods B and C, participants on MTX had higher immunogenicity incidences than those on abatacept alone. During Period A, there is a marginally higher incidence (4.1% vs. 1.4%) in those with no recorded use of MTX on Day 1.

Table 3 summarizes the immunogenicity incidence for participants from the JIA2 (SC) trial. As reported earlier, the grand overall incidence in the 2–5-year cohort (15.2%) was higher than the 6–17-year-old cohort (4.7%). In the 2–5-year cohort, two out of three participants with CTLA-4-specific ADA positivity were also determined positive for the presence of neutralizing antibodies. In the 6–17-year-old cohort, none of the three participants with CTLA-4-specific ADA positivity developed neutralizing antibodies over 24 months. As shown in Table 3, those participants who did not receive MTX had no ADAs detected.

Data analysis of impact on Cmin. In the pJIA1 study, abatacept Cmins in participants with ADA positivity were within the variability of the participants with ADA negative status (see Figure 1).

The analysis of Cminss by ADA status in the pJIA2 study was performed per age cohort. In the 2–5-year-old cohort, abatacept Cmins on Day 113 exceeded target therapeutic exposure of 10 μg/ml, irrespective of ADA status. At the end of the long-term extension (Day 729), Figure 2 shows impact of ADA positivity on trough concentrations of abatacept compared with those ADA negative; however, analysis of the listing shows that only two participants were ADA positive at this timepoint and JIA-ACR30 status was maintained for one participant despite the drop in abatacept levels. Figure 3 shows no effect of ADA on abatacept Cmins at both timepoints for the 6–17-year-old cohort.

Impact of ADA positivity on efficacy. Supplemental Table 1 shows that, in the JIA1 trial, no flares were reported for ADA-positive participants on treatment. However, one out of two participants with high titers (greater than 20,000) did not achieve responder status after the high ADA levels were detected. There was no association between the presence of ADA positivity and loss of JIA-ACR30 (Period C) (Supplemental Table 1). In JIA2 (see Supplemental Table 2), the number of ADA-positive participants with loss of maintenance of JIA-ACR30 was lower compared with those who were ADA negative and had loss of maintenance of JIA-ACR30. This was consistent for both age cohorts and timepoints analyzed. However, the number of participants with loss of JIA-ACR30 in the 2–5-year-old cohort was eight, making it difficult to draw any inferences from a single ADA-positive participant within those eight.

ADA data can be further analyzed according to the duration of the response. In JIA1 (Supplemental Table 3), there were four participants with ADA-positive results 16 weeks apart or longer (persistent antibody as defined by Shankar et al. [7]) who achieved JIA-ACR30 at the last timepoint with an ADA-positive response. Their highest titer was 102. Two out the four did not have dosing information for Period B.

In JIA2 (Supplemental Table 4), three participants had ADA-positive results 16 weeks apart or close to 16 weeks apart (two did not expand the 16 weeks but were positive at the last

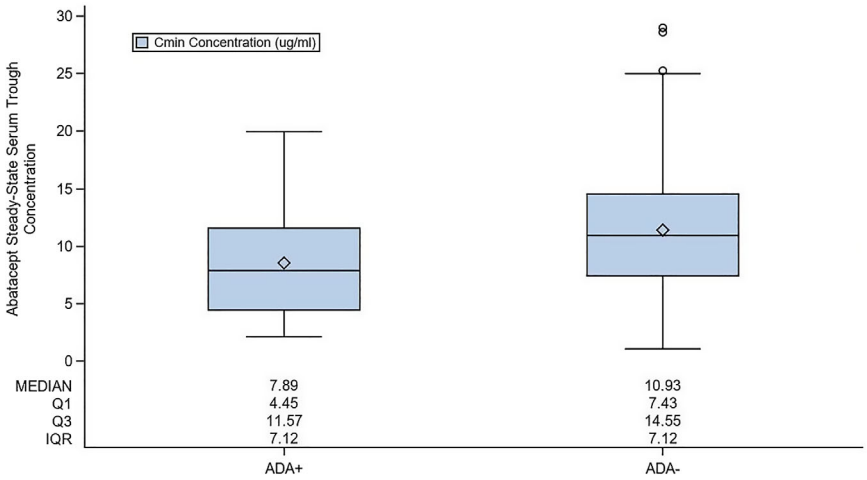

Figure 1. Abatacept steady-state serum trough concentration (Cmin) by ADA status during 2 years of treatment evaluable pharmacokinetics population of JIA1. ADA-positive (ADA+) at a specific post-baseline study day is defined as a participant who satisfies one of the following conditions: a positive laboratory-reported immunogenicity response at the study day of interest and a negative laboratory-reported immunogenicity response at baseline or a positive laboratory-reported immunogenicity response at the study day of interest and at baseline with a post-baseline titer value strictly greater than the baseline titer value. Otherwise, the participant is defined as ADA negative (ADA−) at this study day. The line within each box indicates median value. The diamond within each box indicates mean value. ADA, anti-drug antibody; IQR, interquartile range; Q1, first quartile; Q3, third quartile.

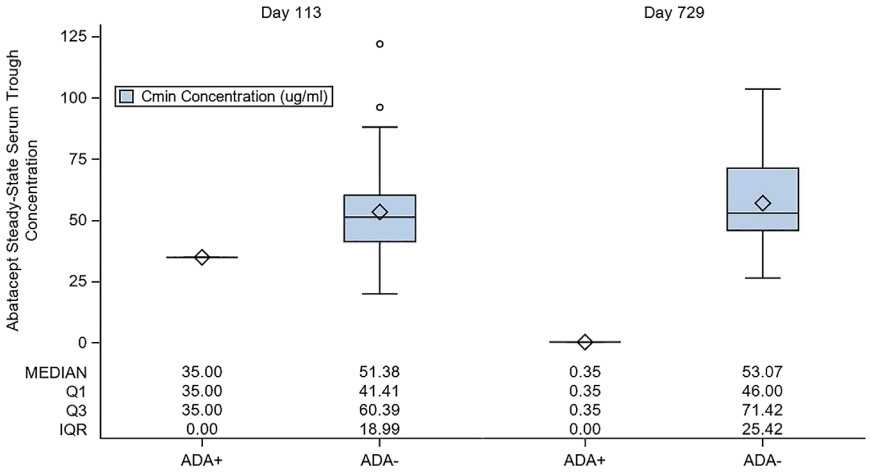

Figure 2. Abatacept steady-state serum trough concentration (Cmin) by ADA status. Evaluable pharmacokinetics population of JIA2, 2–5-year-old age cohort. ADA positive (ADA+) at a specific post-baseline study day is defined as a participant who satisfies one of the following conditions: a positive laboratory-reported immunogenicity response at the study day of interest and a negative laboratory-reported immunogenicity response at baseline or a positive laboratory-reported immunogenicity response at the study of interest and at baseline with a post-baseline titer value strictly greater than the baseline titer value. Otherwise, the participant is defined as ADA negative (ADA−) at this study day. The line within each box indicates median value. The diamond within each box indicates mean value. ADA, anti-drug antibody; IQR, interquartile range; Q, first quartile; Q3, third quartile.

collection): one achieved JIA-ACR30 at the last point with an ADA-positive response, the second participant lost JIA-ACR30 after the first ADA-positive assessment and had a titer of 1,940, and the third never achieved JIA-ACR30 and the highest titer was 63.

Impact of ADA positivity on safety. The safety profile of each participant with at least one ADA-positive test in studies JIA1 and JIA2 was assessed during the cumulative period of treatment with abatacept (IV and SC), respectively (Supplemental Tables 3 and 4). None of the ADA-positive participants from study JIA2 had an adverse event known to be associated with immunogenicity, such as a hypersensitivity reaction, anaphylaxis, anaphylactoid reaction, allergic reaction, serum sickness, and thromboembolism. In study JIA1, three ADA-positive participants were reported with adverse events that potentially could be related to ADA positivity (Supplemental Table 5). However, these adverse events were not temporally associated with ADA positivity, and each of these three participants were ADA negative prior to the occurrence of adverse events. No adverse events associated with

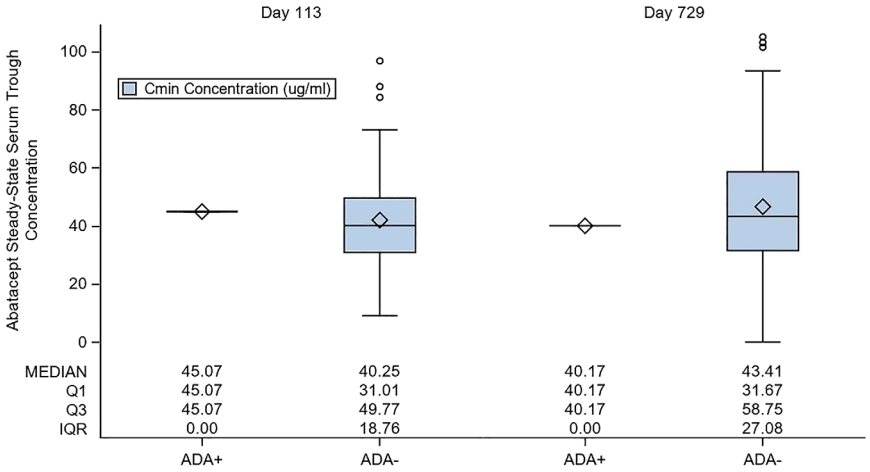

Figure 3. Abatacept steady-state serum trough concentration (Cmin) by ADA status. Evaluable pharmacokinetics population of JIA2. 6–17-year-old age cohort. ADA positive (ADA+) at a specific post-baseline study day is defined as a participant who satisfies one of the following conditions: a positive laboratory-reported immunogenicity response at the study day of interest and a negative laboratory-reported immunogenicity response at baseline. Otherwise, the participant is defined as ADA negative (ADA−) at this study day. The line within each box indicates median value. The diamond within each box indicates mean value. ADA, anti-drug antibody; IQR, interquartile range; Q1, first quartile; Q3, third quartile.

autoimmunity were reported in any participant from studies JIA1 or JIA2.

DISCUSSION

The overall immunogenicity incidence in JIA1 was 23.3%; in JIA2, it was 15.2% for 2–5-year-olds and 4.7% for 6–17-year-olds, with no overall impact on PK, efficacy, or safety. Our detailed analysis facilitates a deeper understanding of these results in terms of factors that may impact immunogenicity, as well as the impact of immunogenicity on PK, safety, and efficacy.

Effect of MTX on immunogenicity. Because of the many reports on the effect of concurrent immunomodulators, such as MTX on immunogenicity across several anti-TNF therapeutics (27–29), enzyme replacement therapies (30) and vaccines (31) in the market, MTX became an important covariate/factor in the analysis of the impact of immunogenicity. Analysis of the published literature shows that MTX reduced immunogenicity incidence when co-dosed with anti-TNFs (adalimumab, golimumab, infliximab, and certolizumab pegol) (28). The ADA incidence with etanercept treatment is low, and there appears to be no information in the literature about the impact of MTX on anti-etanercept antibodies. However, in the JIA1 and JIA2 abatacept trials, most cohorts co-dosed with MTX had a higher immunogenicity incidence (Tables 2 and 3). In the JIA1 trial, only Period A and Period A nonresponders who entered Period C reported lower incidences for the groups co-dosed with MTX, whereas the remaining groups reported higher incidences of immunogenicity for participants co-dosed with MTX. Within the placebo arm of Period B, there was a higher ADA incidence in participants co-dosed with MTX (46.3%) versus not (23.1%). But, as with other periods, there is not a balanced number of participants across groups (41 co-dosed with MTX and 13 not co-dosed); therefore, drawing conclusions on impact is not appropriate. The reason for the higher incidence is unknown and could be confounded by other factors.

In the pJIA2 trial, both cohorts reported higher incidences of immunogenicity for participants co-dosed with MTX. However, because of the smaller number of participants in cohorts with no MTX co-dosed on Day 1, a definitive conclusion cannot be drawn about the association of higher incidence of immunogenicity with MTX co-dosing. For example, for the JIA2 trial, in the 2- to 5-year-old age group with no MTX treatment on Day 1, each participant contributes to approximately 11% of the population, and the addition of an ADA-positive participant in the group would result in an ADA rate comparable to that of the group on MTX. Therefore, caution should be exercised when interpreting the ADA incidence of any cohort with a small number of participants.

There were no interruptions in the MTX treatment in the 2- to 5-year-old cohort. In the 6- to 17-year-old cohort, three of eight ADA-positive participants had interruptions in the MTX treatment. One participant was ADA positive while on MTX, and two other participants had ADA-positive samples at timepoints after MTX was interrupted. It is possible that the interruption of MTX treatment could have influenced the incidence of ADA in these two participants, but it is not conclusive because it is unknown for how long MTX was interrupted.

The aforementioned findings are consistent with those previously reported from phase II and III trials in participants with RA and dosed IV (5) where the incidence of ADAs was 2.3% among those taking MTX versus 1.4% among those not taking MTX.

A SC RA study (32) evaluating immunogenicity to abatacept when used as monotherapy versus combination therapy with MTX reported a marginally higher incidence of immunogenicity at Month 4 (primary endpoint) in the monotherapy arm (4.1%) versus 3.9% for the combination with MTX.

Contrary to the experience with TNF inhibitors, the ADA incidence in participants treated with abatacept has not been reduced by cotreatment with MTX. The reason for this is unknown. This observation has been independent of the participant's age, and it may be related to the mechanism of action. MTX primarily inhibits the activation and proliferation of lymphocytes (to a lesser degree monocytes) and may reduce the interaction of T lymphocytes and synovial fibroblasts because of its effects in reducing cytokine production (33). TNFα activates macrophages and endothelial cells after binding to the TNF receptor (CD120); therefore, TNF inhibitors suppress activation of these cells (33). It is possible that, in combination, they are more effective at suppressing the generation of ADAs. However, with abatacept primarily inhibiting T-cell activation, the immunosuppressant effects of MTX may not be as evident and/or impactful on immunogenicity incidence compared with abatacept alone.

It should be considered that, in JIA1 and JIA2, MTX was dosed as part of the standard of care. In immune tolerance induction protocols (30), which apply a different dosing regimen, there have been favorable results at reduction of ADA incidence, but such protocols have not been tested with abatacept, nor were they applied for studies with TNF inhibitors.

Effect of drug holiday. The assement of effects of a drug holiday in the study was not a planned objective; however, the effect of drug discontinuation can be evaluated in the pJIA1 (IV) study during the 6-month, placebo-controlled duration of Period B. Although there is an increased incidence of immunogenicity during the 6 months of placebo (46.3% for placebo vs. 15.6% for on treatment [both co-dosed with MTX] and 23.1% for placebo vs. 0% for on treatment [no MTX]), the incidences of immunogenicity in Period C (during which time treatment is resumed) are comparable (22.9% for placebo in Period B vs. 18.2% for on treatment in Period B [both co-dosed with MTX] and 0% for both [not co-dosed with MTX]), suggesting that the drug holiday of 6 months did not result in a higher incidence of immunogenicity. In addition, the fact that there was an increase in ADA incidence in the abatacept-treated participants in Period B (12.7%) compared with Period A, for the same group (2.1%), suggests that other factors may have impacted the incidence.

The previously mentioned results are consistent with findings reported for a 3-month interruption of SC administration in adults with active RA (34). SC abatacept responders who had SC abatacept treatment withdrawn for 3 months and subsequently reintroduced had a slightly increased immunogenicity incidence upon drug removal (9.6% placebo vs. 0% abatacept), with a subsequent decrease upon the reintroduction of SC abatacept (2.7% placebo vs. 2.6% abatacept).

Effect of administration route. Study JIA2 was initiated several years after the initiation of JIA1. By the time JIA2 started, a new and more sensitive immunogenicity method was being used for sample analysis. Therefore, any comparison of the immunogenicity incidence between these studies would be confounded by the differences in methods used to assess immunogenicity. When samples from the same study were analyzed by the different methods, a four-fold increase in incidence was observed when the new method was used (26). However, it is worth noting that data from the same age group (6–17-year-old), while participants were on treatment, are very similar across the IV and SC studies. The difference in immunogenicity is also within the four-fold increase observed with changing methods, suggesting that for abatacept, the route of administration had no impact on incidence of immunogenicity. The overall incidence of treatment in JIA2 was 2.3% (3% if co-dosed with MTX), whereas JIA1 incidence for Period A (similar duration of treatment) was 2.1% (1.4% if co-dosed with MTX). This observation aligns with that reported from a phase IIIB noninferiority study in RA participants with an inadequate response to MTX, wherein SC (with IV loading on Day 1) was compared with IV and immunogenicity incidences were 1.1% and 2.3%, respectively (35).

Incidence across age cohorts. Regarding the ADA incidences across age cohorts, the most appropriate comparison is within JIA2, because it removes other variables such as route of administration, immunogenicity assay used, etc. However, because there were considerably more participants in the 6- to 17-year-old cohort ($n = 172$) than in the 2- to 5-year-old cohort ($n = 46$), the comparison between age cohorts is not balanced and not powered appropriately. Therefore, based on the potential variability of the observed rates due to limited sample sizes, it cannot be concluded that the reported immunogenicity rates (19.4% compared with 5.9%) were age dependent.

In a recent review on the experience of monoclonal antibodies and Fc-fusion proteins for pediatric use, Liu et al. (36) compared the immunogenicity incidence in adult and pediatric trials for adalimumab, etanercept, infliximab omalizumab, and tocilizumab. Because in some cases the method may have been improved, they conclude that, for the studies analyzed, immunogenicity incidences were highly variable and that comparisons would not be appropriate. The study results of adalimumab initially appeared to indicate a higher incidence of ADAs within the pediatric populations, but the assay used in pediatric studies for the detection of ADAs had greater sensitivity than that used in the adult studies.

Hence, based on the observations summarized here and those reported for other biotherapeutics (36), more data should be gathered to fully assess impact of age on immunogenicity.

Considerations related to immunogenicity risk assessment. Analyses of factors that impact immunogenicity and its consequent risk are used to generate an immunogenicity risk assessment (IRA) and subsequently drive bioanalytical and sampling strategy in support of clinical trials (13). Given the mechanism of action of abatacept, as an immunosuppressant, and the historical data of low ADA incidence, ADA sample collection and banking could be implemented and only trigger analysis based on safety. However, because of the nature of the patient population (juvenile and the new disease), the approach used in JIA1 and JIA2 provides better characterization of the potential impact and gives prescribing physicians additional information about what to expect in situations such as when using MTX as a concomitant medication and during the implementation of drug holidays. This article focuses on the analysis of immunogenicity data and not on the risk assessment; readers are encouraged to review a good description by Sperinde et al. (37) on how an IRA can be performed for a fusion protein.

Clinical impact of ADA. Consistent with previous abatacept studies, the JIA trials showed that, overall, there was no impact of ADA on PK, safety, or efficacy as measured by flares and JIA–ACRP30. The analysis presented here highlights the importance of aggregating the data and presenting it per ADA status to gain an overall picture of immunogenicity impact on different study outcomes. However, in some cases, analysis of the data at the participant level may be needed. In the 2- to 5-year-old cohort, the impact of immunogenicity on PK is limited because the number of participants is too small to enable a statistically significant observation.

It is important to consider sample size in any analysis. For example, the analysis presented in Supplemental Table 2 (JIA2) is limited because of the following reasons: 1) a low number of participants determined to be positive for the presence of ADAs (a total of 15 subjects with only five with adequate efficacy data) and 2) a lack of relevant efficacy data for participants (6 out of 15) who were positive after the last dose of abatacept (efficacy data not collected). It would not be appropriate to perform an analysis to assess impact without the relevant samples being collected at relevant timepoints to establish a temporal relationship. Therefore, the impact of ADA on efficacy, as determined by JIA–ACR30 response, was performed by review of data at the individual participant level (Supplemental Table 4). Most ADA responses across JIA1 and JIA2 did not expand a period of 16 weeks or longer and would be considered transient by current industry standards. Out of the seven that could be considered persistent (two did not expand the 16 weeks but were positive at the last collection), five achieved JIA–ACR30 at the last

timepoint with an ADA-positive response; two participants with IgG-specific responses with the highest titers ranging between 63 and 1,940 did not achieve JIA–ACR30 at all or lost it after ADAs were detected. The impact of persistent ADA responses on efficacy is not conclusive owing to the small number of ADA-positive participants. Overall, there did not appear to be an association between ADA positivity and lack of JIA-ACR30 response.

This work summarized the immunogenicity incidence to abatacept in pediatric subjects with pJIA. Factors such as route of administration (IV vs. SC), age of subjects, and standard of care concomitant medications were evaluated for their impact on immunogenicity incidence. Even though some trends of impact on immunogenicity were observed for specific age cohorts and MTX co-dosing, a low number of participants in some subgroups prevented us from drawing definitive conclusions.

Drug holiday had no impact on immunogenicity incidence once treatment was resumed, and although a comparison across SC and IV route of administration was not deemed appropriate, due to the differences in the bioanalytical methods used to measure ADAs, similar incidences (less than 1% difference) were observed, which aligned with previously reported comparisons.

The overall immunogenicity incidences observed in both JIA trials (4.7%-23.3%) are slightly higher than those previously reported for participants with RA treated with abatacept (1.1%-10.3%) (32–35,38,39). However, as previously reported in other studies, there was no impact of ADA on PK, safety, and efficacy.

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AUTHOR CONTRIBUTIONS

All authors provided substantial contributions to the analysis and interpretation of data and to the drafting and review of the manuscript. All authors approved the final version to be published.

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