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Human Medicines Division

Assessment report for paediatric studies submitted according to Article 46 of the Regulation (EC) No 1901/2006

Kymriah

tisagenlecleucel

Procedure no: EMEA/H/C/004090/P46/012.1

Note

Assessment report as adopted by the CHMP with all information of a commercially confidential nature deleted.

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1. Introduction

On the 16 March 2021, the MAH submitted the final study results from a completed phase IIIb paediatric clinical study for Kymriah (tisagenlecleucel; ATC code: L01XX71) in accordance with Article 46 of Regulation (EC) No 1901/2006, as amended.

The bioanalytical assays used in this phase IIIb clinical study were not available at the time of initial assessment. This report provides an overview of these bioanalytical assays and responses to two comments based on the initial assessment; EMA/H/C/004090/P46/012.

2. Scientific discussion

2.1. Information on the development program

The MAH stated that the Phase IIIb study CCTL019B2001X (hereafter referred to as study B2001X) in paediatric and young adult patients with relapsed/refractory (r/r) acute lymphoblastic leukaemia (ALL) treated with tisagenlecleucel is a stand-alone study.

Study B2001X is a phase IIIb open-label, multicentre, single arm study designed to further evaluate the safety and efficacy of tisagenlecleucel in paediatric and young adult patients with r/r B-cell ALL after the closure of enrolment to the pivotal study CCTL019B2202 (hereafter referred to as B2202). The inclusion of certain patients not studied during study B2202 was also allowed, e.g., < 3-year-olds (3/52 at screening in the paediatric population) or patients with prior blinatumomab exposure of which 21.2% (11/52) were in the paediatric paediatric population and 21.7% in the overall population aged 0 - <26 years (15/69).

Kymriah (INN: tisagenlecleucel, product code CTL019) was approved in the EU via the centralised procedure (Procedure No. EMEA/H/C/004090) on 23-Aug-2018 and is indicated for the treatment of:

- Paediatric and young adult patients up to and including 25 years of age with B-cell ALL that is refractory, in relapse post-transplant or in second or later relapse.
- Adult patients with relapsed or refractory diffuse large B-cell lymphoma (DLBCL) after two or more lines of systemic therapy.

The evidence of efficacy in paediatric and young adult patients with r/r B-cell ALL was at the time of initial marketing authorization (MA) primarily based on data from the pivotal study B2202, which is a phase II open-label, multicentre, single arm study. In addition, the final results from the complete data set of studies B2205J and B2001X which were reviewed during the previous Article 46 procedures (Procedure No. EMA/H/C/004090/P46/011 and EMA/H/C/004090/P46/012 respectively), have provided supportive evidence for the efficacy of tisagenlecleucel in the approved ALL indication (Procedure No. EMEA/H/C/004090/II/0030).

2.2. Information on the pharmaceutical formulation used in the study

Kymriah is an immunocellular therapy containing tisagenlecleucel, autologous T cells genetically modified ex vivo using a lentiviral vector encoding an anti-CD19 chimeric antigen receptor (CAR).

Kymriah comprises cell dispersion for infusion, where 1-3 infusion bags contain a total of $1.2x10^6$ to $6x10^8$ CAR-positive viable T-cells. The concentration of CAR-positive viable T-cells is dependent on

patient body weight for treatment of patients with B-cell ALL. The cellular composition and the final cell number varies between individual patient batches.

The approved dose range for paediatric and young adult patients with B-cell ALL is 0.2 to $5x10^6$ CAR-positive viable T-cells/kg body weight for subjects ≤50 kg and 0.1 to $2.5x10^8$ CAR-positive viable T-cells (non-weight based) for patients >50 kg.

No change in formulation was made for the paediatric population in study B2001X.

2.3. Clinical aspects

2.3.1. Introduction

The MAH has submitted final bioanalytical data reports for the phase IIIb clinical study CCTL019B2001X for r/r pediatric/young adult acute lymphoblastic leukaemia (hereafter referred to as B2001X):

- muCART-19 qPCR assay in support of Novartis Clinical Trial CCTL019B2001X.
  - Finalisation date: 23 September 2021.
  - Sponsor Report Number: DMPK RCCTL019B2001X-pk

- Detection of anti-CTL019 antibodies in human serum samples from clinical study CCTL019B2001X by Flow Cytometry
  - Finalisation date:25 May 2021.
  - Sponsor Report Number: DMPK RCCTL019B2001X-ig

- Determination of CTL019-specific T-cells in human PBMC.
  - Finalisation date: Not given – however, signature date was 20 October 2021.
  - Sponsor Report Number: DMPK RCCTL019B2001X-iga)

2.3.2. A brief overview of Study B2001X

Further details on the B2001X study are provided in the Article P46 procedure EMA/H/C/004090/012. This report will refer only to the cellular kinetic and immunogenicity analysis as appropriate with respect to the bioanalysis reports.

The primary objective of study B2001X was to evaluate the safety of tisagenlecleucel treatment as measured by adverse events (AEs) and laboratory abnormalities.

Secondary objectives included clinical cellular kinetic, and immunogenicity endpoints.

Study design

Study B2001X had the following sequential phases for all patients: screening including leukapheresis, enrolment and pre-treatment (cell product preparation, bridging- and lymphodepleting [LD] chemotherapy), treatment and follow-up, which included a single tisagenlecleucel infusion and follow-up until month 12. After 12 months post-infusion, the patients were transitioned into the long-term follow-up (LTFU) study A2205B for lentiviral vector safety and efficacy follow-up that was run under a separate protocol in accordance with health authority guidelines for patients treated with gene therapies. The main purpose of the study was to assess the safety of tisagenlecleucel for up to 12 months post-infusion.

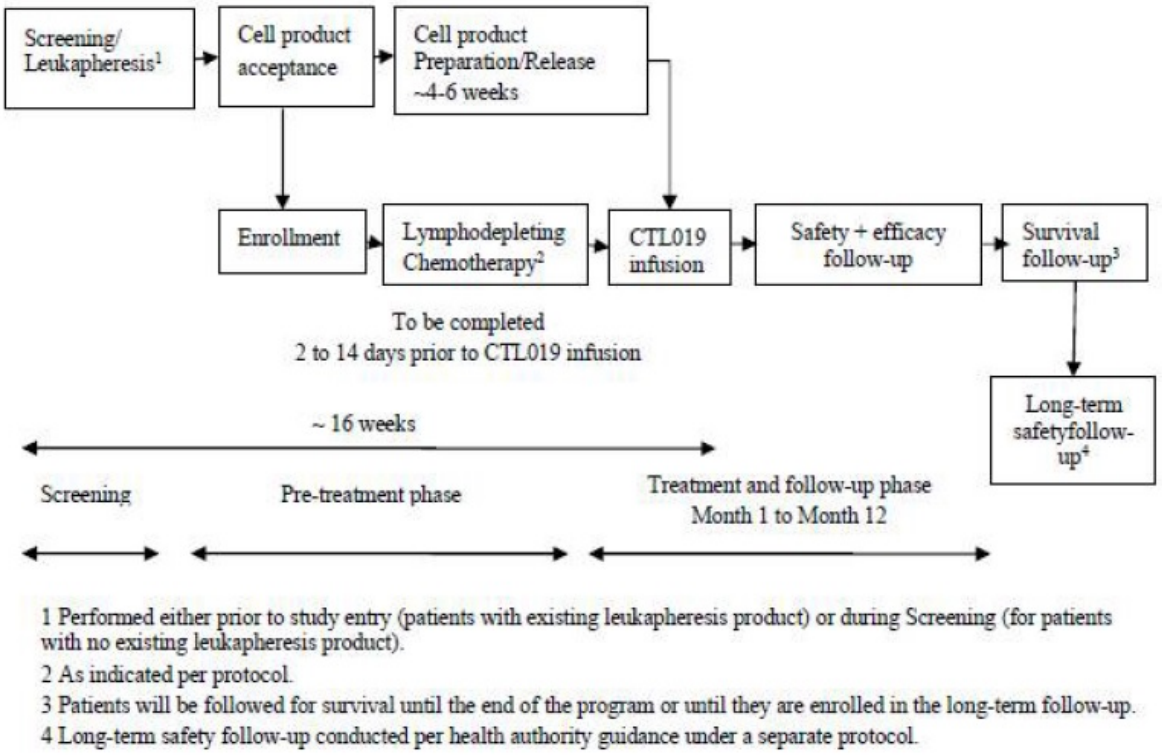


Figure 1: *CTL019 in r/r B-cell pediatric/young adult ALL*

Study population

The target population for enrolment in this study consisted of pediatric and young adult patients with B-cell ALL < 26 years of age at screening who were primary refractory, chemo-refractory, relapsed after allogeneic SCT, or who were otherwise ineligible for allogeneic SCT.

The tisagenlecleucel dose was administered via a single intravenous (IV) infusion.

Results

Study initiation date was 24-Apr-2017 (first patient first visit) and study completion date was 13-Oct-2020 (last patient last visit).

A total of 81 patients were screened, 77 (95.1%) of whom satisfied all eligibility criteria; of these, 74 patients (91.4%) were enrolled in the study.
Table 1: Analysis sets (screened set)

Analysis set	All subjects N=81 n (%)
Screened set	81
Enrolled set (ENS)	74 (100)
Full analysis set (FAS)	69 (93.2)
Safety set (SAF)	69 (93.2)
Cellular kinetic analysis set (CKAS)	69 (93.2)
Per-protocol set (PPS)	61 (82.4)

Percentages are based on the number of subjects in the ENS.

Cellular kinetics

Cellular kinetics was determined in the cellular kinetic analysis set (n=69; 93.2%).

Tisagenlecleucel transgene levels in peripheral blood were determined by qPCR. The cellular kinetic parameters were estimated from the individual concentration versus time profiles using a non-compartmental approach within the modelling program Phoenix® (Pharsight, Mountain View, CA).

Data observed in this study were consistent with the cellular kinetics and exposure results previously observed and reported in paediatric and young adult patients with r/r B-cell ALL in Study B2202.

Cellular kinetics and exposure parameters (AUCs, C_{max}, T_{max}) from the study were summarised by response on Day 28 ± 4 days and by CRS grade.

Assessor's comment:

The data on cellular kinetics are considered acceptable, no comments were raised during the Article 46 procedure EMA/H/C/004090/P46/012.

Immunogenicity

Humoral immunogenicity assessments were performed for the measurement of antibodies binding to murine CAR19 (for simplicity named here anti-mCAR19 antibodies) in human serum. T-cell immunogenicity measured the presence of CAR19-specific CD4+ and CD8+ T-cells against tisagenlecleucel.

Humoral Immunogenicity

The humoral immunogenicity assessment included evaluation of pre-existing (pre-treatment) and post-treatment anti-tisagenlecleucel antibodies to examine the incidence of immunogenicity with treatment, as a secondary endpoint. A validated assay was used to determine presence of anti-mouse CAR19 (m-CAR19) antibodies in serum of patients who received tisagenlecleucel treatment. Antibodies binding to tisagenlecleucel (anti-mCAR19) in human serum were measured using a flow cytometry method. Anti-mCAR19 antibodies in human serum samples were captured by Jurkat cells transfected to express murine CAR19. Untransfected cells were used as reference. The method measures bound IgG/M on viable cells. Subjects were counted as positive if they had one or more positive sample post baseline, otherwise negative if they had at least one negative sample post Baseline and otherwise unknown.

Humoral immunogenicity interpretation data were presented overall for the safety analysis set (SAF) in the initial procedure. The majority of patients were humoral-immunogenicity reactive at baseline (89.9%; 62/69), while 94.2% (65/69) were positive at any time point post-infusion. Humoral immunogenicity data summarised by Day 28 response and time point showed that pre-existing anti-mCAR19 antibodies were detected in similar proportion of patients as those who achieved a BOR of CR/Cri, which is consistent with that previously observed in pediatric r/r ALL patients in study B2202.

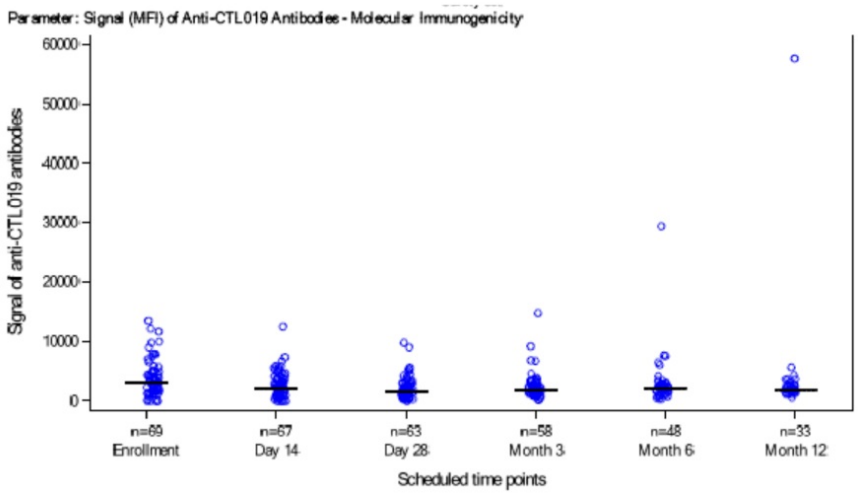
Table 2: *Humoral immunogenicity interpretation by time point, overall (Safety set)*

Time point		All subjects N=69
Baseline	Positive	62 (89.9)
	Negative	7 (10.1)
Day 14	Positive	53 (76.8)
	Negative	14 (20.3)
	Missing	2 (2.9)
Day 28	Positive	50 (72.5)
	Negative	13 (18.8)
	Missing	6 (8.7)
Month 3	Positive	53 (76.8)
	Negative	5 (7.2)
	Missing	11 (15.9)
Month 6	Positive	44 (63.8)
	Negative	4 (5.8)
	Missing	21 (30.4)
Month 12	Positive	32 (46.4)
	Negative	1 (1.4)
	Missing	36 (52.2)
At any time post-baseline *	Positive	65 (94.2)
	Negative	2 (2.9)
	Missing	2 (2.9)

* Summary at any time post-baseline also includes unscheduled assessments. Subjects are counted as positive if they have one or more positive samples post-baseline, otherwise negative if they have at least one negative sample post baseline and otherwise unknown.

Percentages are based on the number of subjects in the SAF (N).

A strip plot of anti-mCAR19 antibodies by time points is shown in Figure 2. Median mean fluorescent intensity signals were observed to be similar at different time points, (i.e. enrollment, Day 14, Day 28, Month 3, Month 6, and Month 12).



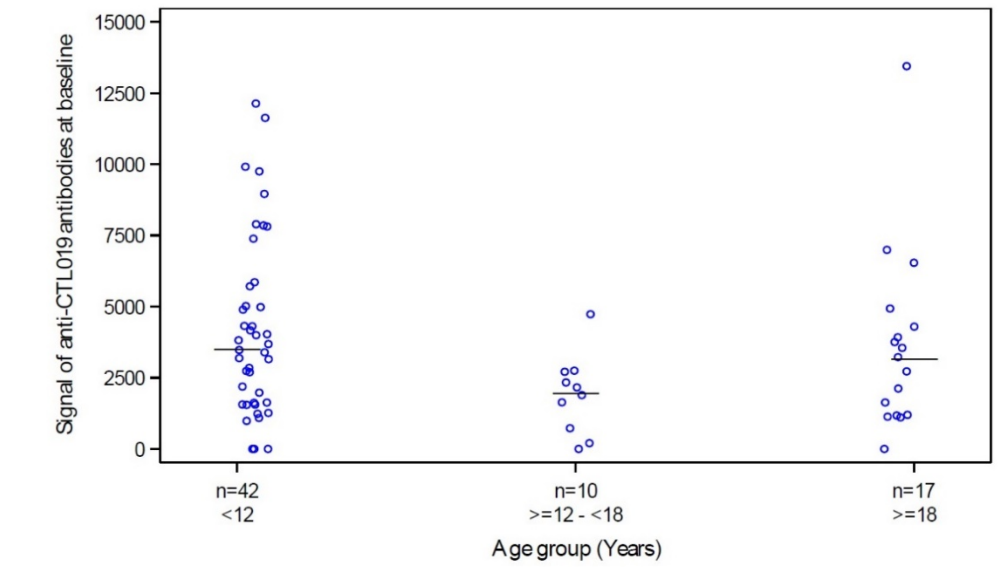
Values below 0 were imputed as 0.

Figure 2: *Strip plot of anti-tisagenlecleucel antibodies by time point – Safety set*

Analysis of ADA in clinical study B2001X according to age group

In the Article 46 procedure EMA/H/C/004090/P46/012, humoral immunogenicity data was only provided for the safety population as a whole (see above). It was therefore not possible to distinguish data from the different age groups in the paediatric population. Although the majority of participants had pre-existing antibody responses, the frequency of antibody responses in the study population was reduced over time and it is not clear if there were differences according to age, for example if all patients <18 years were seropositive, and whether any of those seronegative at baseline did seroconvert. In addition, no indication is provided as to whether any of the antibody responses may be neutralising. The MAH was therefore recommended to discuss these data when submitting the Bioanalysis report

The MAH has provided an updated analysis to investigate the impact of age on humoral immunogenicity (Figure 3 and Table 3). The results suggested no apparent trend for the impact of age on the pre-existing antibody responses, i.e., patients <12 years of age, ≥12 - 18 years of age, ≥18 years of age demonstrated pre-existing antibodies in 92.9%, 70%, and 89.9% of the patients, respectively. In addition to pre-existing antibodies, the percentage of patients with post-baseline antibodies (at any time post-infusion) were similar among these age categories.



Values below 0 were imputed as 0.
Analysis cut-off date: 13Oct2020

Figure 3: *Strip plot (for baseline values) of signal of anti-CTL019 antibodies by age groups (<12, ≥12 - <18, ≥18 years).* Parameter: Signal (MFI) of anti-CTL019 antibodies – Molecular immunogenicity at baseline.

Table 3: *Humoral immunogenicity interpretation by time point. Overall and by age groups (<12, ≥12 - <18, ≥18 years) (Safety set)*

Time point		Age < 12 years N=42 n (%)	Age >= 12 – < 18 years N=10 n (%)	Age >= 18 years N=17 n (%)	All subjects N=69 n (%)
Baseline	Positive	39 (92.9)	7 (70.0)	16 (94.1)	62 (89.9)
	Negative	3 (7.1)	3 (30.0)	1 (5.9)	7 (10.1)
Day 14	Positive	35 (83.3)	6 (60.0)	12 (70.6)	53 (76.8)
	Negative	6 (14.3)	4 (40.0)	4 (23.5)	14 (20.3)
	Missing	1 (2.4)	0	1 (5.9)	2 (2.9)
Day 28	Positive	28 (66.7)	8 (80.0)	14 (82.4)	50 (72.5)
	Negative	10 (23.8)	2 (20.0)	1 (5.9)	13 (18.8)
	Missing	4 (9.5)	0	2 (11.8)	6 (8.7)
Month 3	Positive	33 (78.6)	8 (80.0)	12 (70.6)	53 (76.8)
	Negative	2 (4.8)	1 (10.0)	2 (11.8)	5 (7.2)
	Missing	7 (16.7)	1 (10.0)	3 (17.6)	11 (15.9)
Month 6	Positive	29 (69.0)	7 (70.0)	8 (47.1)	44 (63.8)
	Negative	2 (4.8)	0	2 (11.8)	4 (5.8)
	Missing	11 (26.2)	3 (30.0)	7 (41.2)	21 (30.4)
Month 12	Positive	19 (45.2)	5 (50.0)	8 (47.1)	32 (46.4)
	Negative	1 (2.4)	0	0	1 (1.4)
	Missing	22 (52.4)	5 (50.0)	9 (52.9)	36 (52.2)
At any time post-baseline *	Positive	39 (92.9)	10 (100)	16 (94.1)	65 (94.2)
	Negative	2 (4.8)	0	0	2 (2.9)
	Missing	1 (2.4)	0	1 (5.9)	2 (2.9)

* Summary of at any time post-baseline also includes unscheduled assessments.
- Subjects are counted as positive if they have one or more positive samples post-baseline, otherwise negative if they have at least one negative sample post baseline and otherwise unknown.
Analysis cut-off date: 13Oct2020

Table 4 summarises the proportion of patients that demonstrated either induced or boosted antibody responses post-infusion. Induced and boosted immunogenicity post-infusion responses were observed in 10.1% and 4.3% patients, respectively. No age-related trend was observed for either induced or boosted antibody responses.

Table 4: *Summary of anti-CTL019 induced and boosted humoral immunogenicity interpretation by age groups (<12, ≥12 – 18, ≥18 years) (Safety set)*

	Age < 12 years N=42 n (%)	Age >= 12 - < 18 years N=10 n (%)	Age >= 18 years N=17 n (%)	All subjects N=69 n (%)
Induced	3 (7.1)	3 (30.0)	1 (5.9)	7 (10.1)
Boosted	1 (2.4)	0	2 (11.8)	3 (4.3)
Negative	37 (88.1)	7 (70.0)	13 (76.5)	57 (82.6)
Unknown	1 (2.4)	0	1 (5.9)	2 (2.9)

-Subjects are counted as induced if they have negative interpretation at baseline and one or more positive interpretation post-baseline.
- Subject are counted as boosted if they have positive interpretation at baseline and one or more postbaseline sample greater than 2.8 times baseline value.
- Subjects are counted as negative if they have a positive interpretation at baseline and a positive or negative interpretation post-baseline and did not meet a sample value greater than 2.8 times the baseline value.

Neutralising activity of the generated anti-CTK019 antibodies has not been tested. However, detailed analyses investigating impact of antibody responses following Kymriah administration, indicated no apparent impact on efficacy endpoints in paediatric ALL or DLBCL patient populations (Mueller et al, In Press).

Reference:

Mueller K, Grupp S, Maude S et al., Tisagenlecleucel Immunogenicity in Relapsed/Refractory Acute Lymphoblastic Leukemia and Diffuse Large B-Cell Lymphoma, Blood advances, 2021, In Press.

Assessor's comments

A greater proportion of patients ages <12 years had pre-existing antibodies at baseline (92.9%; n=42) compared to those aged ≥12 - <18 years (70%) of which there were only 10 patients. The age group ≥12 - <18 years is too small to make any firm conclusions. Based on data from patients <12 years and those ≥18 years (94.1%, n=17), it can be anticipated that pre-existing antibody levels were in general similar across all age groups. However, the range of MFI for the youngest age group (<12 years) was more extensive than for the other age groups. This could be due to a greater potential for immune responsiveness at this younger age compared to older patients.

The frequency of patients with positive responses declines over time. The number of missing responses increase over time with data for approximately half of each patient population missing at month 12 [<12 years (22/42; 52.4%); >12 - <18 years (5/10; 50%); >18 years (9/17; 52.9%)]. Nevertheless, the number of seronegative patients remained low throughout the study.

For all subjects, the number of induced immune responses was 10.1% (7/69). However, the greatest number of induced responses appears to be in the age group >12 - <18 years (3/10, 30%) which had a small sample size. The number of induced immune responses was low for the age group <12 (3/42; 7.1%) and for the age group >18 years (1/17; 5.9%).

The number of boosted immune responses across all age groups was low. For all subjects, 3/69; 4.3% showed boosted humoral immunogenicity. For participants <12 years of age, 1/42 (2.4%) showed boosted immune responses, whilst no boosted responses (0/10, 0%) were detected in the age group >12 - <18 years. For participants >18 years of age, 2/17 (11.8%) showed boosted immune responses. However, the latter two groups were small.

It is acknowledged that the number of both induced and boosted immune responses were low. The MAH refers to observations that pre-existing antibodies do not appear to have affected efficacy endpoints for DLBCL or B-ALL indications citing an article that is in Press. (Mueller et al. In Press). The manuscript refers to two single arm studies on B-ALL (ELIANA n=79, data cut-off July 1, 2019; ENSIGN n=64, data cut-off May 24, 2019). The manuscript does not include the clinical study B2001X. Humoral responses were assessed using flow cytometric measurement of anti-murine CAR19(mCAR19) antibodies and cellular responses were determined by measuring T-cell production of interferon gamma in response to two different pools of mCAR19 peptides. Both assays were validated.

The key points from the manuscript:

- Pre- and posttreatment anti-mCAR19 antibodies did not alter tisagenlecleucel cellular kinetics, efficacy or safety in r/r B-ALL or r/r DLBCL.

- T-cell responses to mCAR19 peptides did not influence patient outcomes or cellular expansion in r/r B-ALL or r/r DLBCL.

According to the guideline on immunogenicity assessment of biotechnology-derived therapeutic proteins, (EMA/CHMP/BMWP/14327/2006 Rev 1), if binding antibodies are detected, these should be tested for neutralising activity. It is acknowledged that a high proportion of individuals had pre-existing antibodies.

Although the humoral immunogenicity data suggest no impact on tisagenlecleucel expansion and persistence or efficacy post-infusion, following a single administration of tisagenlecleucel, potential effects of eventual neutralising antibody responses following a second Kymriah administration, which in some cases has been given, is not known.

Cellular immunogenicity results

Activation of T-cells in PBMC collected from patients in response to mCAR19-derived peptides was used to assess the cellular immunogenicity against tisagenlecleucel. T-cell activation was measured by the percentage of interferon gamma (IFNγ)-positive cells detected by intracellular staining and subsequent flow cytometric analysis. Net responses (in %) were calculated for two non-overlapping mCAR19 peptide pools (i.e. Pool 1 and Pool 2), which together span the full mCAR19 protein sequence. Cellular immunogenicity assessment included the percentage of CD4+ and CD8+ T-cells specific to mCART peptides and were measured pre-dose (at enrollment) and up to 12 months post-tisagenlecleucel dose. Cellular responses were consistent over time for all patients with mean values < 0.5% at any time point, demonstrating that cellular immunogenicity does not fluctuate over time.

Analysis of responses to peptide pools corresponding to the mCAR19 protein in the B2001X clinical study

Strip plots in the CSR for the clinical study B2001X were shown for CD4+ and CD8+ T-cells for each pool separately. Although both peptide pools together span the entire mCAR19 protein sequence, further details on which regions of the mCAR19 construct were represented in each peptide pool would

have been useful, since there was slightly greater spread in responses from pool 2 for CD3+CD4+ cells. The MAH was therefore recommended to discuss this in the Bioanalysis report (Q4 2021).

Company Response

A footnote was added under the materials/reagents section of the cellular immunogenicity report to clarify the composition of the 2 peptide pools: "Pool 1: 15-mer overlapping peptides covering the majority of the extracellular domain (251 amino acids) starting at the N-terminus, Pool 2: 15-mer overlapping peptides covering the proximal part of the extracellular domain (61 amino acids), the transmembrane domain and the intracellular portion (signaling domains)".

Assessor's comment:

A footnote has been added to the cellular immunogenicity report (DMPK RCCTL019B2001X-iga) providing details on the peptide pools, which is very helpful, however, no discussion has been provided.

It appears that there was a slightly greater spread in cellular immunogenicity for CD3+CD4+ cells to the transmembrane and intracellular portions of the mCAR19 sequence compared to the extracellular domain.

2.3.3. Bioanalytical assays

2.3.3.1. MuCART-19 qPCR Assay

This report describes the results of the muCART-19 qPCR assay performed on clinical specimens collected during the time period 13 June 2017 to 13 October 2020 for the clinical study B2001X.

The data have been generated and collected under non-GLP conditions whereas the testing was performed in compliance with Navigate BP SOPs, Clinical Laboratory Improvement Amendment (CLIA) regulations, Good Clinical Laboratory Practice guidelines and applicable GxP regulations.

Three Quality Systems audits were performed with outcome reports provided on the 15 Jan 2020, 7 December 2020 and 12 July 2021.

This bioanalytical report summarizes the results of the murine CART-19 qPCR assay (A_WI-01188) performed to measure CART-19 transgene and to evaluate the cellular kinetics of the patients treated with autologous CTL019 cells.

Assay validation is part of a separate report (RPT-01324 and RPT-10016). The original validation was performed using human blood and bone marrow samples collected from healthy donors and spiked with varying amounts of Jurkat cells expressing the murine CART19 construct. The original validation consisted of studies designed to assess accuracy, limit of detection (LOD), lower limit of quantification (LLOQ), precision, robustness, and linearity/dynamic range. The addendum validation consisted of studies designed to assess accuracy, robustness, precision, and linearity/dynamic range using the above-mentioned remnant patient samples and larger sample volumes. The limit of detection of the qPCR assay was determined as the minimum concentration at which the murine CART19 plasmid DNA can be detected with a 95% detection rate. The limit of quantification of the qPCR assay was determined as the minimum concentration at which the murine CART19 plasmid DNA can be quantified with an acceptable recovery rate, precision and 100% detection rate.

A total of 877 specimens were processed for samples collected between 13 June 2017 and 13 October 2020.

Study Objective:

The objective of the testing conducted is to estimate the number of murine CART-19 transgene positive cells in subjects' peripheral blood (PB) and bone marrow (BM) specimens. This assay is also used to monitor, over time, the presence of the murine transgene CART-19 in subjects treated with CART-19 transduced cells.

Methods:

The Murine CART-19 qPCR Assay is a laboratory-developed molecular test using quantitative, TaqMan-based, Real Time PCR.

The Murine CART-19 qPCR Assay for Clinical Trial Testing (AM-0240), A_WI-01188, was used for the testing of all PB and BM specimens received for this trial. A retrospective specimen stability assessment concluded for specimens stored at -70°C to -90°C stability is one (1) year (12 months). DNA extracted from PB stored at -80°C (-70°C to -90°C) is set at two (2) years (24 months).

This testing involved performing the Murine CART-19 qPCR Assay, a laboratory-developed, TaqManbased qPCR assay to quantitate the murine CART-19 transgene in the peripheral blood and bone marrow of patients transduced with CAR positive T-cells. Genomic DNA from patient specimens was tested against murine CART19 calibrator controls (containing genomic DNA spiked with CART-19 plasmid DNA) and an internal reference p21 (CDKN1A) qPCR assay to quantitate the number of murine CART19 transgene copies.

Results:

Validation results showed that the limit of detection was (2.8 copies/200ng DNA with 95% detection rate). The limit of quantification was 10 copies/200ng DNA or equivalent to 50 copies/microgram DNA.

A total of eight hundred and seventy-seven (877) specimens from a total of seventy (70) subjects were processed (Table 5), of which:

• Seven hundred and twenty-nine (729) specimens had evaluable valid results.

• Thirty-nine (39) specimens were not evaluable or not analyzed. Most common reasons for cancellation of testing were insufficient DNA yield and inappropriate specimen type.

• One hundred and nine (109) specimens were reported in the data transfer as "Reported and Unreliable" due to the values being below LLOQ or specimen issues.

• Six (6) deviations (related to assay procedure, other specimen or reporting issues) were observed.

Table 5: *Summary of muCART-19 qPCR Assay Testing*

Method	Collection Period	No. of subjects	Sample type	No. of Specimens – reported & data transferred to NVS			Total No. of Specimens Processed	No. of Deviations*
				Evaluable with valid results	Not Evaluable or Not analyzed	Reported and Unreliable		
Murine CART-19 qPCR Assay, (A_WI-01188)	13Jun2017 to 13Oct2020	70	PB	729	30	108	867	6
			Plasma	0	9	0	9	
			BM	0	0	1	1	
			Totals	729	39	109	877	6

*There were six deviations including incorrect DNA concentration, incorrect concentration of the sample, error in DNA quantitation, incorrect pooling of sample material, insufficient DNA yield for qPCR.

Assessor's comments

In general, the assay system and testing are considered appropriate and sufficient. PBMC were used as the source of cells for analysis with only one sample based on bone-marrow.

2.3.3.2. Detection of anti-CTL019 antibodies in human serum samples from clinical study CCTL019B2001X by flow cytometry

The analyses were performed in accordance with the MAH Standard Operating Procedures and description of methods and according to the basic applicable requirements of Good Laboratory Practices (OECD ENV/MC/CHEM(98)17) and the EMA reflection paper for laboratories that perform the analysis or evaluation of clinical trial samples (EMA/INS/GCP/532137/2010).

The analyses were conducted and reported according to the Study Plan and its Amendments No.01, 02, 03 and 04 approved by Novartis and according to the quality standards and SOPs that had been predefined by PRA Health Sciences based on good laboratory practices (GLP) and conditions. However, GLP compliance was not claimed for this study.

Two audits have been carried out with reporting dates: 4 November 2020 and 29 April 2021.

Objective of the study: To detect anti-mCAR19 antibodies (anti-drug antibodies – ADA) in human serum samples from the clinical study CCTL019B2001X using a validated flow cytometry method.

Methods:

Drug was not available as a specific reagent for this assay, as the drug in the clinical study were modified endogenous T-cells. The modification is Chimeric Antigen Receptor CD19, expressed on the membrane of the modified T-cells (CAR19). To determine presence of anti-drug antibodies in serum from study subjects, a Jurkat cell line, with the same CAR19 modification (CTL019 Jurkat cells) as in study subjects was used.

Anti-mCAR19 antibodies in system suitability control samples (SSCs) or in human serum samples are captured by Jurkat cells (an immortalized line of human T lymphocyte cells) transfected to express murine CAR19. To differentiate the anti-drug antibodies from those specific to the Jurkat cells, samples are also tested separately on wildtype (WT) Jurkat cells. After an incubation step and washing away any unbound antibody, a R-Phycoerythrin (PE) labelled anti-human IgG/M F(ab')2 fragment is added, and in addition a viability dye (efluor 780). After incubation, additional washing steps and fixation, both the CAR19 Jurkat cells and the WT Jurkat cells were analyzed by flow cytometry. The measured median fluorescence intensity (MFI) of the viable cells is increased in proportion to the amount of bound IgG/M present in sample or SSCs.

Samples were analysed with a flow cytometer (BD FACSanto II) for measuring PE and eFluor780 (APC-Cy7). Cell numbers were determined using the Countess II cell counter for monitoring of cell density, splitting of the cells and preparation of the required cell dilutions for the assay.

Specimens were screened for a positive or negative response by reference to the CP. A sample is considered as screening assay positive, if its signal is greater than or equal to the CP. The run specific CP was calculated using the fixed CPF of 2.28 for the CTL019 Jurkat cells, and 1.53 for the Wildtype cells established during validation.

CP = CPF x Run mean NC signal.

Results:

In total 422 samples collected from 70 subjects were screened, and a total of 369 samples from 70 subjects scored positive.

For 6 samples, the backup sample was analyzed in duplicate as the original result showed a CV >30% or the primary sample was measured in single mode due to insufficient sample volume.

Assessor's comment:

The assay system used was validated and considered acceptable.

2.3.3.3. Determination of CTL109-specific T-cells in human PBMC

This study was conducted, and the results were reported in accordance with GCLP guidelines and the Good Laboratory Practice Regulations set forth in Title 21 CFR Part 58 of the Code of Federal Regulations of the United States of America.

Inspections have been performed with interim finding son data analysis reported on the following dates: 31 May 2019, 09 December 2019, 18 November 2020 and 9 June 2021.

Objectives of the study:

The objective of this study was to measure the cellular immune response against infused autologous (CAR-) T cell CTL019 by flow detection of T cells producing IFNγ after ex vivo exposure to peptides derived from chimeric antigen receptor CTL019. IFNγ positive T cells were identified by intracellular staining in human PBMC samples from subjects treated with CTL019 CAR- T cells in support of the clinical trial protocol CCTL019B2001X. The parameters assessed were the number of CD4+ and CD8+

T cells and the percentage of IFNγ+CD4+ T cells IFNγ+CD8+ T cells with respect to their respective parent populations. An increase in IFNγ+ cells represents a cellular response against CTL019.

Method:

Four hundred twenty-six (426) peripheral blood mononuclear cell (PBMC) samples from seventy (70) human subjects enrolled in clinical study B2001X were analyzed. Samples were received frozen in liquid nitrogen and stored at -180°C until analysis. All 426 samples tested in this study were collected between 13Jun2017 and 13Oct2020. These samples were analyzed from 28 Mar 2018 to 14 Apr 2021.

For details on the methods, reference was made to the Method SOP CRO.SOP.00025 and Method Validation Report CRO.Validation.00767 – "Determination of test antigen-specific T lymphocytes in Human PBMC by Intracellular Interferon gamma Staining and Flow Cytometry Method Description and Validation" - Novartis document number: DMPK R1480005-ig, as well as CRO.Validation.01179 – "Partial Method Validation Report: T cell Activation Assay for Peptide Specific Responses in Human PBMCs (BCMA) by Flow Cytometry for Sponsor 187" - Novartis document number: DMPK R1900404-ig. In order to quantify any increase in IFNγ+ cells that may be responsive to peptides derived from chimeric antigen receptor CTL019, the LLOQ values were established based on validation samples with starting material of at least 1 million cells per test obtained from normal healthy volunteers.

During sample testing, clinical study samples yielded far fewer cells on average compared to validation samples. As a consequence, all results were reported even if they were lower than the LLOQ or the LOD to enable plotting of all observed results against other clinical and pharmacokinetic measurements. The number of available cells per gated population differed significantly between individual patients, making a fixed LLOQ difficult to define. More precise definition of an operative LLOQ at the different cell numbers analyzed is currently being investigated and will be reported as an amendment to the Method Validation Report. In addition, the LLOQ values were established to determine the sensitivity of the assay in measuring IFNγ+ T cells, not as a lower limit of cells that are specific for the peptide pools. Analysis of responses to the peptide pools should also take into account analysis of responses to the mock stimulation to take into account any non-specific IFNγ+ T cell responses.

Assessor's comment:

The assay for determining T-cell immunogenicity was validated using cells from healthy volunteers. However, it appears that the sample material from the clinical study was insufficient and too variable to define a fixed LLOQ. A more precise definition of an operative LLOQ at the different cell numbers analysed is currently being investigated and will be reported as an amendment to the Method Validation Report. Consequently, the data on T-cell immunogenicity should be interpreted with caution and cannot necessarily be extrapolated to the two studies ELIANA and ENSIGN) described in Mueller et al., In Press.

2.3.4. Discussion on clinical aspects

Bioanalytical reports were provided that had been used in the analysis of immunogenicity and cellular kinetics of tisagenlecleucel in the B2001X study which was the subject of the Article 46 procedure EMA/H/C/004090/P46/012. Responses to comments raised in this procedure were also addressed.

The assays used were validated, however, GLP was not claimed for all procedures.

Cellular kinetics:

The assay for determining cellular kinetics was considered appropriate and acceptable. Characterisation of the cellular kinetics was a secondary endpoint in the B2001X study. Overall, the observed cellular kinetics was comparable to previous results from studies B2202 and B2205J.

Increasing C_{max}, AUC0-28d and AUC0-84d were associated with higher grade CRS, which is in line with previous findings in B2202 and B2205J trials.

Humoral immune responses to tisagenlecleucel

According to the guideline on quality, non-clinical and clinical aspects of medicinal products containing genetically modified cells (EMA/CAT/GTWP/671639/2008 Rev. 1 – corr) that came into effect in June 2021, immunogenicity testing should be conducted throughout the development. Assessment of immunogenicity needs to take into account clinically relevant immune responses to the transgene product and/or to the transduced cells. The risk for immunogenicity is influenced by the origin of transduced cells (allogeneic versus autologous), the nature of the disease (immune deficient versus immune competent patient population, total absence vs. defective gene product), the type of conditioning regimen, the pre-existing immune response against the transgene product as well as the location of the transgene product (intracellular versus extracellular/secreted).

According to the guideline on immunogenicity assessment of biotechnology derived therapeutic proteins, (EMA/CHMP/BMWP/14327/2006 Rev 1), if binding antibodies are detected, these should be tested for neutralising activity. CAR-T cells express biotechnology-derived proteins on their cell surface, and as such, a determination of neutralising activity for eventual binding antibody responses is of interest.

In the B2001X study, there was a high frequency of participants with pre-existing antibody responses [62/69 (89.9%)] to tisagenlecleucel (anti-mCAR19). No data on neutralising antibody responses have been generated. The MAH refers to observations that pre-existing antibodies do not appear to have affected efficacy endpoints for DLBCL or B-ALL indications citing an article that is in Press. (Mueller et al. In Press). This manuscript refers to two clinical studies on B-ALL that do not include B2001X (ELIANA n=79; ENSIGN n=64). Both ELIANA and ENSIGN are single-arm, open label, multicentre phase 2 studies examining tisagenlecleucel in patients with r/r B-ALL who were 3 (at screening) to 21 years of age at initial diagnosis.

It appears reasonable that pre-existing antibody levels in the B2001X study were similar across all age groups. However, the range of MFI for the younger age group (<12 years) was more extensive than for the other age groups. This could be due to a greater potential for immune responsiveness at this younger age compared to older patients.

The frequency of boosted responses was generally low in the study population as a whole (3/69; 4.3%). The highest frequency of boosted responses was in the age group >18 years [2/17 (11.8%)] although the sample size was small. Similarly, the number of induced immune responses was low in the study population as a whole [10.1% (7/69)]. The highest frequency of induced responses was in the age group >12 - <18 years (3/10, 30%) although the sample size was small. It is not known if any of the boosted or induced immune responses were neutralising.

The number of participants that showed positive anti-mCAR19 antibody responses declined over time whilst the number of missing responses increase over time. At month 12, data for approximately half of each age group assessed was missing at month 12 [<12 years (22/42; 52.4%); >12 - <18 years

(5/10; 50%); >18 years (9/17; 52.9%)]. Nevertheless, the number of seronegative patients remained low throughout the study. The reason(s) for the increase in missing data points has not been discussed by the MAH. Reasons for this could presumably include missed sampling, and patient relapse.

Although the humoral immunogenicity data suggest no impact on tisagenlecleucel expansion and persistence or efficacy post-infusion, following a single administration of tisagenlecleucel, any potential effect of eventual neutralising antibody responses following a second Kymriah administration, which in some cases has been given, is not known.

Cellular responses to tisagenlecleucel

Cellular immune responses in the B2001X study were low. The assay used was validated using human cells from healthy donors, however, it appears that the sample material from the B2001X clinical study was insufficient and too variable to define a fixed LLOQ. A more precise definition of an operative LLOQ at the different cell numbers analysed is currently being investigated and will be reported as an amendment to the Method Validation Report.

The low cellular responses could, in part, explain why there appears to be little or no effect of T-cell responses on patient outcomes or cellular expansion.

3. Request for Supplementary Information

None

4. MAH responses to Request for supplementary information

Not applicable

5. MS comments on the CAT Rapporteur's Preliminary responses assessment report

Not applicable

6. Overall conclusion and recommendation

The MAH has submitted bioanalytical reports for the clinical study B2001X described in the Article P46 procedure EMA/H/C/004090/P46/012.

The efficacy data based on these bioanalytical reports were included in the final analysis of study B2001X and are considered to support the results from the two clinical studies B2202 and B2205J, underlying the approved indication of Kymriah in r/r ALL patients. The amendment to the method validation report for cellular immune responses is to be provided when available.

☒ **Fulfilled:**