

This is an Open Access document downloaded from ORCA, Cardiff University's institutional repository: <https://orca.cardiff.ac.uk/id/eprint/181379/>

This is the author's version of a work that was submitted to / accepted for publication.

Citation for final published version:

Mathieu, Chantal, Wych, Julie, Hendriks, A. Emile J., Van Ryckeghem, Lisa, Tree, Timothy, Chmura, Piotr, Möller, Christopher, Casteels, Kristina, Danne, Thomas, Reschke, Felix, Šmigoc Schweiger, Darja, Battelino, Tadej, Johannesen, Jesper, Rami-Merhar, Birgit, Pieber, Thomas, De Block, Christophe, Evans, Mark, Hilbrands, Robert, Bosi, Emanuele, Willemsen, Ruben H., Basu, Supriyo, Pulkkinen, Mari-Anne, Knip, Mikael, Cnop, Miriam, Nitsche, Almut, Schulte, Anke M., Niemöller, Elisabeth, Peakman, Mark, Wilhelm-Benartzi, Charlotte, Gillespie, David, Overbergh, Lut, Mander, Adrian, Marcovecchio, M. Loredana, Mathieu, Chantal, Overbergh, Lutgart, Evans, Mark, Hendriks, A. Emile J., Marcovecchio, M. Loredana, Peakman, Mark, Tree, Timothy, Morgan, Noel G., Todd, John A., Wicker, Linda, Mander, Adrian P., Dayan, Colin M, Wych, Julie, Gillespie, David, Pieber, Thomas, Eizirik, Decio L., Cnop, Miriam, Brunak, Søren, Pociot, Flemming, Johannesen, Jesper, Mallone, Roberto, Knip, Mikael, Otonkoski, Timo, Veijola, Riitta, Lahesmaa, Riitta, Ziegler, Anette G., Solimena, Michele, Bonifacio, Ezio, Holl, Reinhard, Dotta, Francesco, Chiarelli, Francesco, Marchetti, Piero, Bosi, Emanuele, Cianfarani, Stefano, de Beaufort, Carine, Dahl-Jørgensen, Knut, Jarosz-Chobot, Przemyslaw, Battelino, Tadej, Gotthardt, Martin, Roep, Bart O., Lundgren, Markus, Costecalde, Guillaume, von Herrath, Matthias, Napolitano-Rosen, Antonella, Niemöller, Elisabeth, Keil, Stefanie, Dutta, Sanjoy, Latres, Esther, Agiostratidou, Gina and Koralova, Anne 2025. Minimum effective low dose of antithymocyte globulin in people aged 5–25 years with recent-onset stage 3 type 1 diabetes (MELD-ATG): a phase 2, multicentre, double-blind, randomised, placebo-controlled, adaptive dose-ranging trial. *The Lancet* 406 (10510), pp. 1375-1388. 10.1016/s0140-6736(25)01674-5

Publishers page: [https://doi.org/10.1016/s0140-6736\(25\)01674-5](https://doi.org/10.1016/s0140-6736(25)01674-5)

Please note:

Changes made as a result of publishing processes such as copy-editing, formatting and page numbers may not be reflected in this version. For the definitive version of this publication, please refer to the published source. You are advised to consult the publisher's version if you wish to cite this paper.

This version is being made available in accordance with publisher policies. See <http://orca.cf.ac.uk/policies.html> for usage policies. Copyright and moral rights for publications made available in ORCA are retained by the copyright holders.



A European randomised adaptive dose-ranging trial to identify the Minimum Effective Low Dose of Anti-human Thymocyte Globulin (MELD-ATG) in people 5 to 25 years old with recent onset stage 3 type 1 diabetes

Prof Chantal Mathieu, MD^{1,2*}, Julie Wych, PhD³, A. Emile J. Hendriks, MD⁴, Lisa Van Ryckeghem, PhD¹, Prof Timothy Tree, PhD⁵, Piotr Chmura, MS⁶, Christopher Möller, BSc⁶, Kristina Casteels, MD⁷, Thomas Danne, MD⁸, Felix Reschke, MD⁸, Darja Smigoc Schweiger, MD^{9,10}, Prof Tadej Battelino, MD^{9,10}, Prof Jesper Johannesen, MD^{11,12}, Birgit Rami-Merhar, MD¹³, Prof Thomas Pieber, MD¹⁴, Prof Christophe De Block, MD¹⁵, Prof Mark Evans, MD¹⁶, Prof Robert Hilbrands, MD¹⁷, Emanuele Bosi, MD¹⁸, Ruben H. Willemssen, MD¹⁹, Supriyo Basu, MRCPCH²⁰, Mari-Anne Pulkkinen, MD²¹, Prof Mikael Knip, MD²², Prof Miriam Cnop, MD^{23,24}, Almut Nitsche, PhD²⁵, Anke M. Schulte, PhD²⁵, Elisabeth Niemöller, MD²⁶, Prof Mark Peakman, MD^{5,27}, Charlotte Wilhelm-Benartzi, PhD³, David Gillespie, PhD³, Lut Overbergh, PhD², Adrian P. Mander, PhD³, M. Loredana Marcovecchio, MD⁴ on behalf of INNODIA**

¹Department of Endocrinology, UZ Gasthuisberg, UZ Leuven, Leuven, Belgium

²Department of Chronic Diseases and Metabolism, KU Leuven, Leuven, Belgium

³Centre for Trials Research College of Biomedical and Life Sciences, Cardiff University, UK

⁴Department of Paediatrics, University of Cambridge, and Department of Paediatric Diabetes and Endocrinology, Cambridge University Hospitals NHS Foundation Trust, Cambridge, UK

⁵Department of Immunobiology, School of Immunology & Microbial Sciences, King's College London, UK

⁶Novo Nordisk Foundation Center for Protein Research, University of Copenhagen, Copenhagen, Denmark

⁷Department of Paediatrics, UZ Gasthuisberg, UZ Leuven, Leuven, Belgium

⁸Center for Paediatric Endocrinology, Diabetology, and Clinical Research, Auf Der Bult Children's Hospital, Hannover, Germany

27 ⁹Department of Endocrinology, Diabetes and Metabolic Diseases, University Children's Hospital,
 28 University Medical Center Ljubljana, Ljubljana, Slovenia

29 ¹⁰Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia

30 ¹¹Department of Clinical Medicine, University of Copenhagen, Copenhagen, Denmark

31 ¹²Copenhagen University Hospital Herlev, Department of Paediatrics and Steno Diabetes Center
 32 Copenhagen, Denmark

33 ¹³Department of Paediatric and Adolescent Medicine, Division of Paediatric Pulmonology, Allergology
 34 and Endocrinology, Comprehensive Centre for Paediatrics, Medical University of Vienna, Austria

35 ¹⁴Division of Endocrinology and Diabetology, Department of Internal Medicine, Medical University of
 36 Graz, Austria

37 ¹⁵Department of Endocrinology-Diabetology-Metabolism, Antwerp University Hospital & University of
 38 Antwerp, Antwerp, Belgium

39 ¹⁶Institute of Metabolic Science and Department of Medicine, University of Cambridge, Cambridge, UK

40 ¹⁷Academic Hospital and Diabetes Research Centre, Vrije Universiteit Brussels, Brussels, Belgium

41 ¹⁸Diabetes Research Institute San Raffaele Hospital and San Raffaele Vita Salute University, Milan, Italy

42 ¹⁹Department of Paediatric Diabetes and Endocrinology, The Royal London Childrens' hospital, Barts
 43 Health NHS Trust, London, UK

44 ²⁰Department of Paediatric Endocrinology and Diabetes, Oxford University Hospitals NHS Foundation
 45 Trust, Oxford, UK

46 ²¹New Children's Hospital, Paediatric Research Centre, Helsinki University Hospital and Faculty of
 47 Medicine, University of Helsinki, Finland

48 ²²Research Program for Clinical and Molecular Metabolism, University of Helsinki, Helsinki, Finland

49 ²³ULB Center for Diabetes Research, Université Libre de Bruxelles, Brussels, Belgium

50 ²⁴Division of Endocrinology, ULB Erasmus Hospital, Brussels University Hospital, Université Libre de
 51 Bruxelles, Brussels, Belgium

52 ²⁵Former DCVM Development, R&D, Sanofi-Deutschland GmbH, Frankfurt, Germany

²⁶DCVM Development, R&D, Sanofi-Deutschland GmbH, Frankfurt, Germany

²⁷Sanofi R&D, Paris, France

***Corresponding author:** Professor Chantal Mathieu, Department of Endocrinology, UZ Leuven, Campus Gasthuisberg, Herestraat 49, 3000 Leuven, Belgium. email: chantal.mathieu@uzleuven.be; [tel: +32-16-340623](tel:+32-16-340623)

****A list of INNODIA Principal Investigators and Clinical Investigators is provided in *Supplementary Appendix***

SUMMARY

Background: Type 1 diabetes (T1D) remains an important health care problem, with no disease-modifying therapies available in people with recent onset clinical T1D. Adaptive trial designs, allowing faster evaluation of treatment modalities, remain underexplored in this stage of the disease. We aimed to identify the minimum effective dose of anti-thymocyte globulin (ATG), in 5-25-year-old people with recent-onset clinical T1D.

Methods: In this phase 2, double-blind, randomised, placebo-controlled multi-arm parallel-group adaptive trial design, ATG was given intravenously over 2 consecutive days. Participants aged between 5-25 years diagnosed with clinical, stage 3 T1D between 3-9 weeks before treatment, having random C-peptide levels ≥ 0.2 nmol/L, and at least one diabetes-related autoantibody (GADA, IA-2A or ZnT8) were recruited in 14 hospitals, in 8 European countries. Participants recruited and screened between November 24, 2020, and December 13, 2023 were randomised into 7 consecutive cohorts receiving placebo, 2.5mg/kg, 1.5mg/kg, 0.5mg/kg, or 0.1mg/kg ATG. Additionally, the trial cohorts were stratified into three age groups: 5-9, 10-17, and 18-25 years. Sealed Envelope, a web based randomisation system, was used to generate treatment allocations and execute randomisation, based on block randomisation within age strata. Concealment lists, outlining the treatment allocation, were only available for the pharmacists. The first cohort consisted of all 5 arms, while in subsequent cohorts only placebo and 2.5mg/kg arms remained, with a dose determining committee progressively dropping middle doses. The primary outcome was the area under the C-peptide curve (AUC) during a 2-hour mixed-meal tolerance test (MMTT) at 12-months measured as $\ln(\text{AUC C-peptide}+1)$. Conditional on finding a statistically significant difference at $p < 0.05$ for 2.5mg/kg ATG versus placebo, the minimum effective dose of ATG was determined. All randomised participants were included in the primary and secondary analyses (intention-to-treat population, $n=117$ of whom 63 females), apart from the safety analysis which included only participants who received treatment (safety population, $n=114$ of whom 62 females). The trial was registered at Clinicaltrials.gov (NCT04509791) and is fully executed.

Findings: Participants were mainly European Caucasian, had a median age of 13·0 years (IQR 11·0-16·0), a median duration of diabetes of 51 days (IQR 42-57), a mean HbA1c of 7·79 % (SD 1·20%) and a median AUC of the stimulated C-peptide of 0·82 nmol/L/min (IQR 0·67-1·12). They were treated with placebo (n=30 of whom 10 females), 2·5mg/kg (n=33 of whom 20 females), and 0·5mg/kg (n=34 of whom 22 females) ATG, with progressive dropping of 0·1mg/kg (n=6 of whom 3 females) and 1·5mg/kg (n=11 of whom 7 females) ATG. In the placebo arm, the mean Ln(AUC C-peptide+1) at 12-months was 0·411 nmol/L/min (SD 0·032). In the 2·5mg/kg ATG arm the mean Ln(AUC C-peptide+1) at 12-months was 0·535 nmol/L/min (SD 0·032). The mean difference in the Ln(AUC C-peptide+1) between 2·5mg/kg ATG and placebo at 12-months, as estimated by the repeated measures mixed effects model, and adjusted for baseline C-peptide values, was 0·124 nmol/L/min (95% CI 0·043, 0·205), p=0·003. The mean Ln(AUC C-peptide+1) in the 0·5mg/kg ATG arm, the remaining middle dose, was 0·513 nmol/L/min (SD 0·032) at 12-months, with a mean baseline-adjusted difference in Ln(AUC C-peptide+1) to placebo of 0·102 nmol/L/min (95% CI 0·021, 0·183), p=0·014. Cytokine release syndrome occurred in 11 (33·3%) and 8 (23·5%) participants, and serum sickness in 27 (81·8%) and 11 (32·4%) participants treated with 2·5mg/kg or 0·5mg/kg ATG, respectively, versus none in placebo-treated participants.

Interpretation: In young people with recent-onset clinical T1D, 2·5mg/kg and 0·5mg/kg ATG reduced loss of beta-cell function, opening the potential of an affordable, repurposed agent, ATG, in a low and safe dose, as a disease-modifying agent in children and adolescents with newly diagnosed clinical T1D.

Funding: IMI-JU (115797, INNODIA)

INTRODUCTION

Type 1 diabetes (T1D) is a chronic disease affecting approximately 9·5 million people worldwide.^{1,2} T1D affects both adults and children, but particularly in children and adolescents incidence rates have been rising by more than 2% per annum in the last 20 years.² Growing insight in T1D pathogenesis as an autoimmune disease where destruction of pancreatic insulin-producing beta-cells leads to insulin dependence, has allowed identification of promising disease-modifying interventions.^{1,3} Several interventions have been tested in people with recent-onset clinical T1D (stage 3), and demonstrated relative therapeutic success, with preservation of stimulated C-peptide in the first year after diagnosis.⁴ However, the field is progressing slowly, with sequential testing of new drugs versus placebo.⁵ Doses and administration schedules of these therapies are primarily based on data from animal models, the transplant field or other autoimmune conditions. As such, each trial takes years to complete, and, if the results are promising, the exercise needs to start over to answer additional questions, in particular related to optimal doses and target populations. In T1D, the disease progression is most aggressive in children as reflected by more rapid loss of C-peptide,^{6,7} with sub-analyses of some intervention studies showing a higher potential for immunotherapy agents to preserve endogenous beta-cell function in youngest participants.^{8,9} However, most clinical trials have limited the lower age of the study population to 8 years and above due to safety concerns.¹⁰⁻¹²

Traditional dose-ranging trial designs fix the number of people randomised to several doses at trial initiation. An adaptive trial design has multiple biomarker-based interim analysis points, at which dose selections for subsequent recruitment of participants can be altered.^{13,14} There is growing evidence on the potential of adaptive designs in T1D,¹⁵⁻¹⁷ as it limits experimentation at dose levels that show either a lack of response or unwanted side effects, and can more rapidly and efficiently identify the lowest safe and effective dose.

Polyclonal anti-thymocyte globulin (ATG), a well-known drug in transplantation immunology,¹⁸ has shown disease-modifying effects in T1D, with 2·5mg/kg being able to delay beta-cell destruction in people with recent-onset clinical, stage 3 T1D, in contrast to the higher dose of 6·5mg/kg, which even

showed an accelerated loss of C-peptide.^{12,19-21} These doses, suggested by the transplantation experience, other autoimmune disease therapies and mouse experiments, were tested in adults and adolescents, and showed different immune-modulatory properties, with the lower dose resulting in immune modulation, rather than immune suppression, compared to the higher one.^{12,22} However, no further dose-finding studies, exploring even lower doses, or investigations in young children have been performed.

The objective of the MELD-ATG (Minimum Effective Low Dose: Anti-human Thymocyte Globulin) study was to assess the efficacy on preserving beta-cell function one year after treatment and safety of a wide range of low ATG doses in people with recent-onset clinical T1D, proceeding rapidly to include children as young as 5 years of age, using an adaptive trial design.

METHODS

Study design

The MELD-ATG trial was a phase 2, multi-centre, randomised, double-blind, placebo-controlled, multi-arm parallel cohort trial conducted in accordance with the principles of the Declaration of Helsinki and Good Clinical Practice guidelines.²³ The trial was sponsored by UZ Leuven (Leuven, Belgium), funded by the Innovative Medicine Initiative 2 Joint Undertaking INNODIA (115797) and its execution was guided by a Trial Management Group. The INNODIA Patient Advisory Committee provided guidance in conception, realisation of educational materials for recruitment and retention in the trial. Eight countries were part of the study: UK, Denmark, Germany, Finland, Italy, Belgium, Austria and Slovenia (full list in *Supplementary Appendix*). The trial was registered on October 8, 2020 at Clinicaltrials.gov (NCT04509791, <https://clinicaltrials.gov/study/NCT04509791?term=MELD-ATG&rank=1>). Study results have been published on EudraCT (European Clinical Trials Database, <https://eudract.ema.europa.eu>). A Trial Steering Committee monitored the progress of the study, and an Independent Data Monitoring Committee (IDMC) reviewed efficacy and safety data. At prespecified time-points, being after each cohort was fully recruited and received the intervention, an unblinded

Dose Determining Committee (DDC) selected doses to take forward in subsequent cohorts and approved age expansion to lower ages using prespecified criteria (including metabolic and immune biomarkers, namely stimulated C-peptide levels and CD4/CD8 ratios) (*Supplementary Appendix*). Two polyclonal, rabbit-derived ATG batches were provided by Sanofi (R&D Montpellier, France) through INNODIA. Ethical approval was obtained from all participating clinical centres after approval by the central ethics committee (University Hospitals Antwerp, Ref 2020/32/414). The authors vouch for accuracy and completeness of the data and analysis, and for fidelity of the trial to the protocol.²³

Participants

Participants were recruited in 14 accredited trial centres in hospitals from eight countries in the INNODIA network. The main inclusion criteria were age between 5-25 years at consent, a diagnosis of clinical, stage 3 T1D between 3-9 weeks before treatment day 1, random C-peptide levels ≥ 0.2 nmol/L, and at least one diabetes-related autoantibody (GADA, IA-2A or ZnT8). Insulin antibodies were also measured but considering that participants were receiving insulin injections at the time of screening, they were not taken as criterion for inclusion. Key exclusion criteria were major systemic illnesses, active or chronic infections, malignancies, and treatment with other immunomodulatory agents (Full list in Table S1). Gender and ethnicity were self-reported by study participants or their parents/legal guardian as female or male, and as European, African, Asian, or mixed, respectively. Written informed consent was obtained from all participants older than 16 years in the UK or older than 18 years in other European countries, whereas younger participants gave assent and informed consent was obtained from their parents or legal guardian.

Adaptive design and randomisation

The adaptive trial design included one placebo arm and four arms with active ATG doses (Figure S1). The trial had six planned interim analyses, resulting in seven cohorts and a total sample size of 114 treated participants. The allocation ratios for each cohort were: Cohort 1 (participants 1-30) following

a 1:1:1:1:1 ratio; cohorts 2 and 3 (participants 31-54) a 1:1:1:1 ratio; cohorts 4 to 7 (participants 55-117) a 1:1:1 ratio, to account for three participants randomised but not treated.²³ All cohorts included placebo and 2.5mg/kg ATG, whereas the dose of the other arms was determined based on accruing data and DDC's decision. Additionally, the trial cohorts were stratified into three age groups: 5-9, 10-17 and 18-25 years. Sealed Envelope (web-based randomisation system, <https://www.sealedenvelope.com/simple-randomiser/v1/>), was used to generate treatment allocations and execute randomisation, based on block randomisation within age strata. Concealment lists, outlining the treatment allocation, were only available for the pharmacists and, in case of unmasking, to specific study personnel. ATG doses and placebo were prepared by local unmasked pharmacists/nurses, and infusion bags were delivered to the study teams with a masked label, ensuring masking of participants and study teams (study nurses and principal investigators), who were responsible for collecting source data of participants.

Procedures and dose decisions

Detailed description of the ATG/placebo administration is outlined in the protocol.²³ Briefly, ATG was administered by an intravenous infusion over two consecutive days. On day 1, the infusion lasted at least 12 hours, with a maximal dose of 0.5mg/kg ATG. On day 2, the infusion lasted at least 8 hours, with the remainder of the dose administered.

The trial duration per participant was approximately 13-months, including a 2-3 week screening period, 2-days treatment and 12-months follow up (including follow-up visits at 1, 2, and 4 weeks, 3-, 6-, and 12-months post-treatment) (for assessments at each visit, see *Supplementary Appendix*). An age expansion to include 5-11-year-old participants was implemented after 10 participants aged 12-17 years had been treated and observed for at least 4 weeks after receiving ATG, and data were reviewed by the IDMC and DDC (Figure S1).

At each interim analysis, the middle doses were selected by the DDC using all available clinical, mechanistic and safety data from the previous cohort(s), assisted by a Bayesian model prediction,

using all available Mixed Meal Tolerance test (MMTT) C-peptide measurements and CD4/CD8 T-cell ratios.²³ For this analysis all available data were used in a statistical analysis to determine which of the doses were predicted to give a significant result, from a two-sample t-test, for a 6-month dose effect at the end of the study. The reason for choosing a 6-month comparison for the interim analysis was to reduce the uncertainty in making predictions at the 12-month time point. Each interim analysis required the estimation of a Bayesian repeated measures model including fixed effects for categorical time and a linear (i.e. continuous dose effect) dose level, along with an interaction term between time and dose, and a random effect for participant. Only vague prior distributions were used in this model and no external data or evidence had an influence on the analysis. This model was used to calculate the predictive probability that the mean $\ln(\text{AUC C-peptide}+1)$ was different from placebo for each dose level, given that the remaining participants were allocated to the middle dose. The lowest dose(s) that gave a predicted probability greater than 0.9 was selected for the next cohort. This dose could also be 2.5mg/kg. Middle doses could be dropped or restarted at each dose decision moment. In addition, the interim analyses included information based on the accruing CD4+/CD8+ T-cell count ratio outcome at 1, 2, 4, and 12 weeks using a repeated measures model with fixed effects of dose and nominal days along with an interaction term between time and dose since randomisation. The mean differences between each dose level and placebo was estimated with a 95% confidence interval (CI). CIs that indicated no evidence of change in CD4+/CD8+ T-cell count ratio from baseline (ie, included 0) were used as an indication that this dose may be inactive and dropped from further cohorts. These results were used in conjunction with the C-peptide modelling using a Bayesian model of transformed logarithm ($\ln$) of the area under the curve (AUC C-peptide). Note that the data could only be soft locked a few days prior to the DDC meeting and interim analysis to enable as much data as possible to inform the dose decision. The DDC looked at the predictions, the ratio of CD4 to CD8 T-cells and clinical safety reports to make the decision of which doses to include in the subsequent cohorts. During the interim analyses the DDC committee, as well as trial and senior statisticians were unblinded to treatment

allocation in order to make decision on doses to be dropped/kept. The IDMC were blinded to treatment allocation upon request, data by treatment allocation was coded A, B, C, D & E.

Outcomes

The primary outcome was the AUC of the stimulated C-peptide (nmol/L/min), calculated using the trapezoidal rule, during a 2-hour MMTT, at 12-months post-treatment, and analysed centrally (see *Supplementary Appendix*). Secondary outcomes were MMTT AUC C-peptide at baseline, 3, 6, and 12-months post-treatment; HbA1c (%) at baseline, 3, 6, and 12-months post-treatment; total daily insulin dose (calculated as the average dose over the 3 days preceding study visits, Units/d) at baseline, 1, 2, and 4 weeks, and 3, 6, and 12-months post treatment; continuous glucose monitoring (CGM) metrics (time in range (TIR), time in tight range (TITR), time above range (TAR) and time below range (TBR)) using a blinded Dexcom G6 for 14 days, at 3, 6, and 12-months post-treatment; ratio of absolute counts of CD4/CD8 T-cells at baseline, 1, 2, and 4 weeks, and 3, 6, and 12-months post-treatment; T1D-associated autoantibodies (GAD65, IAA, IA-2A and ZnT8A) at screening and 12-months post treatment; monthly fasting/stimulated dried blood spot (DBS) C-peptide measurements (nmol/L) post-treatment before and 60 min after consumption of a standard liquid meal at home (for more detail on analytical methods, see *Supplementary Appendix*); descriptive analysis of the safety profile of different doses of ATG in different age groups. Additionally, insulin dose-adjusted HbA1c (IDAA1C) was reported. Further, as described in the MELD-ATG protocol, other exploratory outcomes (effects of treatment on other biomarkers related to immunological changes and beta-cell death or survival; multi-dimensional analyses of changes in T1D phenotypes by immunological, proteomic, metabolomic, and lipidomic studies and the relation of these to clinical outcomes and progression; fasting C-peptide, fasting glucose and adjusted ratio of C-peptide and glucose at baseline, 3, 6, and 12-months post-treatment; beta-cell responsiveness (measured as change in C-peptide 0-60mins/change in glucose) calculated separately from MMTT and DBS data at 3, 6, and 12-months post-treatment for MMTT and monthly for DBS) will be reported separately.²³

Adverse events were summarised according to system organ class and preferred term using the *Medical Dictionary for Regulatory Activities* (MedDRA) version 27.0 (<https://www.meddra.org/>). Their severity and relationship to treatment were assessed by the investigators according to the National Cancer Institute Common Terminology for Adverse Events (CACTAE), version 5.0.²⁴ In the case of serious adverse events, masking was maintained, if possible, but investigators could execute an unmasking procedure if deemed necessary. Reporting of adverse events was required during the entire trial participation. Cytokine release syndrome (CRS) and serum sickness, known adverse events of ATG, were defined according to specific criteria (described in Table S2).

Statistical analysis

Sample size calculation was based on the primary hypothesis of comparing 2.5mg/kg ATG to placebo for the primary endpoint, being the ln of the AUC of C-peptide during a MMTT. A between-person standard deviation (SD) estimate of 0.264 nmol/L/min on the transformed $\ln(\text{AUC C-peptide}+1)$ scale was obtained using INNODIA observational data from people aged 5-25 years with newly diagnosed clinical T1D.²³ Assuming this SD and comparing 2.5mg/kg ATG to placebo on the transformed $\ln(\text{AUC C-peptide}+1)$ scale, 32 participants in each arm provided over 90% power at a 5% significance level to detect a change of 0.22 nmol/L/min using a two-sided two-sample t-test.

The primary and secondary analyses were carried out according to the intention-to-treat principle as described in the Statistical Analysis Plan. All participants who were randomised and part of the intention-to-treat population were included in the primary and secondary analyses (n=117), apart from the safety analysis which included only participants who received treatment (Safety Population, n=114).

Continuous variables are summarised as sample size, mean, SD and range or median, maximum, minimum, and interquartile range (IQR) where data were skewed. Frequency and percentages of observed levels were reported for categorical measures. The 2-hour MMTT mean C-peptide level from the AUC was transformed using the function $\ln(\text{AUC C-peptide}+1)$.²⁵

For all key endpoints, a repeated measures mixed effects model was used. We had high levels of data availability for our primary outcome (434/468 (92·7%) data points available, with 99/117 (84·6%) participants having complete data across all time points), with no evidence that missing observations were likely to be systematically different from those available. Thus, we relied on the repeated measures mixed effects model to incorporate missing observations under a missing at random assumption. The model was adjusted for baseline levels of the same endpoint. For the primary analysis the family wise error rate was controlled at 5% by using a gatekeeping procedure for the hypothesis test for 2·5mg/kg ATG versus placebo. Specifically, conditional on finding a statistically significant difference ($p < 0·05$), lower doses were then tested to determine the minimum effective dose. We formally explored differential treatment effects by age group (5-9 years, 10-17 years, 18-25 years) by including a treatment by time by age group interaction for the primary analysis. The secondary outcomes and subgroup analyses were not included in the multiplicity control so individual findings should be interpreted as exploratory (Additional Statistical Methods in *Supplementary Appendix*). We conducted sensitivity analyses exploring differential treatment effects over time by sex and batch, individually, by refitting the primary analysis model that incorporates an interaction term between sex, treatment and time and a second model that incorporates an interaction term batch, sex, treatment and time.

Safety events are described for the safety population. All treatment effect estimates are provided with associated 95% confidence intervals. Stata version 18.5 was used for all analyses.²⁶

Role of funding source

The funder (the European Union's Innovative Medicines Initiative 2 Joint Undertaking) of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. Representatives of Sanofi, as provider of ATG and placebo, and as partners of the INNODIA consortium, approved the protocol before start of the study and reviewed and approved the manuscript for publication.

Post-hoc quantitative response (QR) analysis

In a post-hoc analysis, we calculated the QR C-peptide at 1-year developed by Bundy and Krischer.²⁷ This estimates the expected C-peptide at 1-year (ln (x+1) scale), given baseline C-peptide (ln (x+1) scale) and age at randomisation, and compares it with that observed in the study to provide a standardised response metric, with values >0 representing better than expected response and values <0 representing worse than expected response.

RESULTS

Of the 152 participants recruited and screened between November 24, 2020, and December 13, 2023, 117 were randomised into 7 cohorts (Figure 1-S1). Reasons for screen failure were people not meeting inclusion criteria (C-peptide <0.2 nmol/L (n=7) or absence of autoantibodies (n=7)), meeting safety exclusion criteria (n=7), declining to participate (n=10), known allergy to rabbits (n=1), unable to cannulate (n=1) or other personal reasons (n=2) (Figure 1). Thirty-one participants were assigned to placebo, 6 to 0.1mg/kg, 35 to 0.5mg/kg, 12 to 1.5mg/kg and 33 to 2.5mg/kg ATG (Table 1 and Figure S2). By age category, 21 participants were 5-9 years, 76 were 10-17 years, and 20 were 18-25 years (Table 1 and Figure S2). Cohort 1 included participants on placebo and all four ATG doses. The four arms for cohorts 2 and 3 were placebo, 0.5, 1.5, and 2.5mg/kg ATG, following exclusion of 0.1mg/kg ATG at the first interim analysis by the DDC based on prespecified criteria, including clinical, metabolic (C-peptide), and immune (CD4/CD8 ratio) data. The age expansion occurred after recruitment of cohort 2, which resulted in no participants aged 5-9 years receiving 0.1mg/kg ATG. At the third interim analysis 1.5mg/kg ATG was dropped after interim analysis by the DDC and the remaining cohorts 4-7 included placebo, 2.5mg/kg and 0.5mg/kg ATG (Figure S1). Last follow-up visit of the last recruited participant was executed on December 16, 2024.

Baseline characteristics show a mainly white European population, with similar characteristics of participants receiving the three doses included in all cohorts (placebo, 0.5, and 2.5mg/kg ATG), except

for gender, with more males randomised to placebo (Table 1). Three participants, one randomised to placebo, one to 0.5, and one to 1.5mg/kg ATG, withdrew after randomisation and before treatment day 1 (Figure 1). Of the 114 participants who received treatment, one on 0.1mg/kg, two on the 0.5mg/kg, two on 1.5mg/kg, and one on 2.5mg/kg ATG, discontinued the study during follow up (Figure 1).

In the placebo arm, the mean $\text{Ln}(\text{AUC C-peptide}+1)$ at 12-months was 0.411 nmol/L/min (SD: 0.032). In the 2.5mg/kg ATG arm the mean $\text{Ln}(\text{AUC C-peptide}+1)$ at 12-months was 0.535 nmol/L/min (SD: 0.032). The mean difference in the $\text{Ln}(\text{AUC C-peptide}+1)$ between 2.5mg/kg ATG and placebo, as estimated by the repeated measures mixed effects model, and adjusted for baseline C-peptide values, was 0.124 nmol/L/min (95% CI 0.043, 0.205), $p=0.003$. The mean $\text{Ln}(\text{AUC C-peptide}+1)$ in the 0.5mg/kg ATG arm, the remaining middle dose, at 12-months was 0.513 nmol/L/min (SD: 0.032). The mean baseline-adjusted difference in $\text{Ln}(\text{AUC C-peptide}+1)$ between 0.5mg/kg ATG and placebo was 0.102 nmol/L/min (95% CI 0.021, 0.183), $p=0.014$. The change from baseline in mean $\text{Ln}(\text{AUC C-peptide}+1)$ at 3, 6, and 12-months is shown in Figure 2A and S3-4.

The adjusted mean difference in HbA1c between 2.5mg/kg ATG and placebo at 12-months was -0.36% (95% CI -0.80, 0.08), $p=0.106$, and between 0.5mg/kg ATG and placebo was -0.50% (95% CI -0.93, -0.07), $p=0.024$ (Figure 2B). For daily insulin doses, the adjusted differences were -0.036 units/kg/day (95% CI -0.128, 0.056), $p=0.446$, (2.5mg/kg ATG versus placebo) and -0.013 units/kg/day (95% CI -0.104, 0.079), $p=0.785$ (0.5mg/kg ATG versus placebo) (Figure 2C). At all timepoints, data completeness for insulin requirements was more than 90%. The mean adjusted difference in IDAA1C was -0.576 (95% CI -1.284, 0.133), $p=0.111$ (2.5mg/kg ATG versus placebo) and -0.587 (95% CI -1.295, 0.120) $p=0.104$ (0.5mg/kg ATG versus placebo) (Figure 2D). The treatment effect on metabolic parameters seemed more pronounced in children and adolescents than in adults (Figure S5-7).

CGM metrics showed a TIR of $58 \pm 19\%$ in placebo at 12-months, versus $65 \pm 25\%$ and $64 \pm 20\%$ in 0.5 and 2.5mg/kg ATG, respectively (Figure 3 and Table S3). This difference was not statistically significant. Importantly, insulin delivery systems were not differently used between treatment arms, with at 12-

months 8 (27%), 9 (29%), and 10 (32%) people using insulin pumps in the placebo, 0.5mg/kg and 2.5mg/kg arms, compared with 3 (9.7%), 3 (8.6%), and 4 (12.1%) at baseline respectively. No automated insulin delivery systems were used during the study.

The CD4/CD8 T-cell ratio at baseline decreased one week from treatment start in both 0.5mg/kg and 2.5mg/kg ATG arm, with the decrease in the 2.5mg/kg ATG arm 3-fold greater than in the 0.5mg/kg arm (Figure 4 and S8). In contrast, the placebo arm showed a small increase from baseline. The mean adjusted difference in CD4/CD8 ratio across the 12-month period was -0.585 (95% CI -0.740, -0.430) in those treated with 2.5 mg/kg ATG and -0.033 (95% CI -0.186, 0.120) in those treated with 0.5mg/kg ATG ($p<0.001$ and $p=0.676$ versus placebo respectively) (Figure 4). However, whereas the CD4/CD8 ratio remained reduced relative to baseline at all time points in those treated with 2.5 mg/kg ATG, in those treated with 0.5mg/kg ATG the ratio was not significantly different from baseline at 6 months or different to placebo at the 12-month timepoint (Figure 4 and S8, Table S4).

The overall percentage of people with positive autoantibodies did not substantially change for GAD, IA2, or ZnT8, whereas, as expected due to participants being on insulin therapy, IAA percentage increased (from 71% to 99%) (Table S5). No statistically significant changes were noted between placebo and treatment arms.

Change in fasting DBS-collected C-peptide versus baseline was small and comparable between groups (Figure 5A and Table S6). DBS-collected C-peptide measured 60 minutes after a standard liquid meal declined in all groups, and, partly due to high variability and in the latter 6 months of the trial low data availability was not significantly different between groups (Figure 5B and Table S6).

The safety population included all participants who received the allocated treatment ($n=114$), and all of them experienced at least one adverse event (Table 2 and S7). Most adverse events were Grade 1 or 2, with only 11 participants (9.6%) in the 0.5, 1.5, and 2.5mg/kg ATG arms experiencing Grade 3 events. There were two Grade 4 events (severe hypoglycaemia) in the placebo and 0.5mg/kg ATG arms. Lymphopenia occurred in 39 (34%) of participants, with increasing rates with higher ATG doses, but infection rates were not different.

There were no CRS events in the placebo arm, whereas 11 (33·3%) and 8 (23·5%) of participants in the 2·5mg/kg and 0·5mg/kg ATG arms respectively experienced CRS. Serum sickness occurred in 27 (81·8%) of participants in the 2·5mg/kg compared to 11 (32·4%) in the 0·5mg/kg ATG arm and none in placebo-treated participants.

There were 2 emergency unmaskings (1 serious adverse reaction (hospital admission due to suspected serum sickness) and 1 severe adverse event (subcutaneous infection at infusion site)) for which the investigator deemed necessary to know the treatment allocation, but the rest of the team remained blinded.

In the sensitivity analyses, we found no evidence of any differential treatment effects by sex (p-value = 0·177 for sex by treatment by time interaction) or batch (p-value = 0·939 for batch by treatment by time interaction).

In a post-hoc analysis, the QR was calculated and the between-group difference of QR C-peptide at 1-year (compared to placebo) was 0·12 (95% CI 0·03, 0·21) for 2·5mg/kg and 0·10 (95% CI 0·01, 0·19) for 0·5mg/kg (p=0·009 and p=0·029 respectively) (Table S8).

Tabular data of reported results can be found in *Supplementary Appendix*.

DISCUSSION

The MELD-ATG trial showed that an adaptive trial design for testing an intervention aiming to arrest the loss of functional beta-cell mass in young people with recent-onset clinical, stage 3 T1D successfully identified a minimum effective dose of 0·5mg/kg ATG. In addition, it allowed confirmation of previous efficacy and safety findings of 2·5mg/kg ATG, including in children as young as 5 years of age. Low dose ATG proves to be a safe and effective intervention for arresting or at least delaying progression of T1D. The adaptive trial design allowed for exploration of several questions at once and yielded suggestive answers for the field.¹⁵⁻¹⁷ As such, we were able to explore different doses through dose selection based on prespecified criteria. The unblinded DDC decided on doses retained in such a way that the placebo and 2·5mg/kg arms would remain powered to provide the primary endpoint, but middle doses

could be dropped or restarted during the recruitment period, although the DDC only dropped doses and restarted none. This strategy allowed us to confirm previous observations^{12,19} that 2.5mg/kg ATG slows the decline in stimulated C-peptide levels. The adverse events profile for the 2.5mg/kg dose was in line with previous observations, in all age groups.^{12,19} Importantly, the adaptive trial design provided the power to identify a minimum effective dose of ATG at 0.5mg/kg. This dose not only achieved the primary endpoint, reducing C-peptide decline compared to placebo at 12-months, but also resulted in lower HbA1c levels. CGM suggested a beneficial effect of both ATG doses on glucose metrics, with however great variability. Of note, CGM data were only collected from 3-months post-treatment onwards, to avoid the confounding effect of glucose variability during the first weeks post-diagnosis. Although a similar frequency of total adverse events between ATG doses was observed, a major difference was observed for serum sickness, a reaction of the body against the infusion of xenogeneic (rabbit) antibodies.²⁸ A dose-dependent prevalence and duration of this event over different ATG doses was observed, but this needs to be interpreted with caution due to low numbers in the 0.1mg/kg and 1.5mg/kg ATG groups. Another typical ATG-associated adverse event, CRS, due to release of cytokines upon infusion of an immune-cell targeting antibody, had a slightly lower incidence in the 0.5mg/kg versus 2.5mg/kg ATG.²⁹ Of note, in this lower dose, CRS occurred mostly on the first day of infusion. This can be explained by the fact that both arms received 0.5mg/kg on day 1, whereas on day 2 the 0.5mg/kg arm received placebo and the 2.5mg/kg arm the remaining 2mg/kg ATG. The small difference in CRS occurrence compared to previous reports using 2.5mg/kg ATG may be explained by the criteria used to define CRS, as in our study a minimum of fever together with two other constitutional symptoms was a predetermined criterium. Note that, in particular in the 2.5mg/kg ATG arm, a transient increase in insulin doses was observed in the first weeks of the trial, likely reflecting insulin resistance induced by inflammation and transient steroid use upon occurrence of serum sickness.

Dose decisions were based on safety information, C-peptide predictions as well as CD4/CD8 ratio after infusion. The latter was decided based on earlier reports on effects of ATG.¹⁹ However, the MELD-ATG

study now shows that, although there is a clear ATG dose-dependent effect on CD4/CD8 ratio, the effect of 0.5mg/kg ATG was not maintained at 12-months, whereas its effect on C-peptide and metabolic parameters was. This observation confirms previous data suggesting that the effectiveness of ATG is not strictly related to the level of lymphocyte depletion²² and triggers the need for additional research on the mechanism of action of low dose ATG in T1D, including effects on other immunological changes and their pattern over time.³⁰ Following previous ATG-related findings,²² potential mechanisms explaining the beneficial effect of the 0.5 mg/kg dose could be sparing of regulatory T-cells, exhausted signature of CD4 T-cells or other immunological changes. Future exploratory immunological analyses in our study population are needed to clarify the mechanism of action of the identified lower effective dose of ATG, understand dose–response differences in relation to immunological changes, and determine how these may vary across different age groups. These investigations could offer insights into how dosing might be tailored to individuals based on their clinical or immunological characteristics. In addition, exploration of novel treatment options allowing redosing of ATG, once other forms of ATG than the rabbit ATG are available, may provide additional insights on the contribution of persistent versus transient CD4/CD8 ratio changes.

Our study design allowed us to rapidly lower the age for participants' inclusion and test the efficacy and safety of ATG in children as young as 5 years. Current drug development strategies, driven by regulatory guidance, typically target adults first. Only after safety is proven and efficacy is observed in this age group, new agents can be tested in younger people. This may fail to identify agents potentially effective in younger people but not in adults and might lead to the premature rejection of effective treatments. The MELD-ATG results underscore the need to perform immune modulatory intervention studies directly in young individuals with T1D. Including predefined age categories provided valuable insights, as the primary and secondary metabolic outcomes showed some variation across age groups, with a trend toward more pronounced treatment effects in younger participants; however, the study was not powered to detect statistically significant differences by age. This trend confirms previous observations that effectiveness of immune modulatory interventions in recent-onset clinical, stage 3

T1D may vary by age.^{8,9} This may be related to the more aggressive autoimmune process in children, as reflected by the more rapid C-peptide loss, and carries major implications for drug development in this heterogeneous disease.^{31,32} We included a post-hoc analysis, calculating the QR, that takes into account baseline C-peptide levels, but also age. This analysis fully confirmed the model used in the present trial. Identifying new effective therapies with an acceptable safety profile, such as low dose ATG, able to preserve endogenous beta-cell function in young people, will have significant benefits. The incidence of T1D in this young age group is increasing, and as a result, this will become a growing population in need of disease-modifying interventions.² Such interventions may offer protection against both short- and long-term complications of diabetes, while also improving overall quality of life and life expectancy.

The MELD-ATG trial presents several strengths, including its unique design, inclusion of children as young as 5 years and strict adherence to protocol and statistical analysis plan, allowing solid conclusions based on relatively small group sizes. Nevertheless, some weaknesses need to be acknowledged. Regulatory age stepdown requirements resulted in dropping the first lowest ATG dose (0.1mg/kg) prior to the enrolment of 5-11-year-old children, leading to the absence of data on effect of this lowest dose in that age group. The design forced several interim analyses, making the design complex and losing potentially interesting doses, like the very low (0.1mg/kg) or intermediate (1.5mg/kg) doses, with numbers of participants in these arms that prevent useful conclusions. Another potential weakness is that the adaptive study design may have failed to identify other interesting doses due to the middle dose selection being based, among others, on CD4/CD8 T-cell ratios soon after dosing. Other biomarkers may have provided even better guidance on dose selection. Although the study population covered a wide age range and both genders, most participants were white, reflecting European background,⁷ which may limit the generalisability of our findings to people of other racial or ethnic backgrounds. Finally, although all precautions were taken to make this trial double-blind, we cannot guarantee full blinding of all participants and personnel because of occurrence of expected treatment side effects. This study is expected to generate significant clinical interest, with clinicians in

the field of T1D awaiting these results for several years, as it opens the potential of an affordable, repurposed agent, ATG, in a low and safe dose, in children and adolescents with newly diagnosed clinical T1D. Especially in the youngest age group, the 0.5mg/kg dose was effective with a good safety profile and would be the recommended dose for treatment. Of interest, being able to limit the administration of ATG to 0.5mg/kg would also mean only needing one infusion on one day, instead of the two days of infusion with the previously studied 2.5mg/kg. We need however to acknowledge that ATG did not fully arrest beta-cell destruction, as stimulated C-peptide levels continued to decline, in particular during the latter months of the trial, suggesting that combination treatments or re-administration of ATG, in case humanised ATG forgoing immunisation would be available, may be needed to achieve a more durable beta-cell-protective effect.

In conclusion, the MELD-ATG adaptive trial design allowed the identification of a minimum effective dose of ATG at 0.5mg/kg in people with recent-onset clinical T1D. It also confirmed the effectiveness and safety of previously reported 2.5mg/kg ATG, extending these observations to children as young as 5 years of age. This adaptive trial design can be considered for further exploration of novel therapies in T1D, and even for other fields.

AUTHOR CONTRIBUTIONS

CMa, JW, DG, AM, VWB, AMS, AEJH, LM, TT, LO, AMS, PM conceptualised the study and designed protocol. JW, CWB, DG, AM developed SAP, curated data and performed statistical analyses; PC and CMö developed and managed eCRF database; KC, TD, FR, DSS, TB, JJ, BR-M, TP, CdB, ME, RH, EB, RHW, SB, M-AP, MK, MC collected data; TT, MK, AEJH performed experimental analyses; AN, AMS, EN, MP provided ATG; CMa, JW, DG, AEJH, LM, LVR, TT and LO analysed and interpreted data and wrote manuscript; CMa, JW, DG, LVR, LM directly accessed and verified the underlying data reported in the manuscript; All authors contributed to the execution of the trial, interpretation of the results, reviewing, editing and approval of the manuscript.

DECLARATION OF INTERESTS

CMa serves or has served on an advisory panel for Bayer, Biomea Fusion, Boehringer Ingelheim, Eli Lilly, Abbott, Insulet, Medtronic, Novartis, Novo Nordisk, Roche, SAB Bio, Sanofi, and Vertex. Financial compensation for these activities has been received by KU Leuven. KU Leuven has received research support for CM from Dexcom, Abbott, Novo Nordisk and Sanofi. CM serves or has served on the speakers bureau for Novo Nordisk, Sanofi, Eli Lilly and Company, Medtronic, Roche, Dexcom, Insulet, Vertex and Abbott. Financial compensation for these activities has been received by KU Leuven. CM is president of EASD. All external support of EASD is to be found on www.easd.org.

AEJH has received research support from Dexcom. TT serves on an advisory panel for and has received research support from Sanofi. TD serves or has served on an advisory panel or speakers bureau for Astra Zeneca, Bayer, Boehringer Ingelheim, Abbott, Dexcom, Eli Lilly and Company, Insulet, Medtronic, Menarini, Roche, Sanofi, Vitalaire and Ypsomed.

FR has served on an advisory panel Boehringer Ingelheim. FR serves or has served on the speakers bureau for Insulet, Kyowa Kirin, Novo Nordisk and Sanofi. TB serves or has served on an advisory panel for Eli Lilly, Abbott, Medtronic, Novartis, Novo Nordisk, Roche, and Sanofi. TB has received research support from Dexcom, Abbott, Novartis, Novo Nordisk, Medtronic and Sanofi. TB serves or has served on the speakers bureau for Eli Lilly and Company, Roche, Dexcom, Abbott, Astra Zeneca, Medtronic, Novo Nordisk, Novartis and Sanofi. BRM serves on an advisory panel for Sanofi. BRM serves or has served on the speakers bureau for Eli Lilly, Dexcom, Medtronic, Sanofi Ypsomed. CDB serves or has served on an advisory panel for Abbott, Eli Lilly and Company and Novo Nordisk. University Hospital Antwerp has received research support for CDB from Astra Zeneca, Boehringer Ingelheim, Indigo Diabetes NV and Medtronic. CDB serves or has served on the speakers bureau for Abbott, Astra Zeneca, Boehringer Ingelheim, Eli Lilly and Company, Insulet, Medtronic and Novo Nordisk. ME serves or has served on an advisory panel for Medtronic, Abbott Diabetes Care, Novo Nordisk, Eli Lilly, ITB Medical, Sanofi, Vertex, Provention Bio and Pila Pharma. University of Cambridge has received research support for ME from Medtronic, Abbott, Novo Nordisk, Eli Lilly and Company, ITB Medical and Sanofi. ME serves

or has served on the speakers bureau for Eli Lilly, Abbott and Sanofi. RH has served as speaker for Medtronic. EB serves on an advisory panel for Sanofi. EB is or has been on the speakers bureau for Abbott, Medtronic, Sanofi and Roche. RHW serves on an advisory panel for Sanofi. RHW has been a speaker for Insulet. SB has been a speaker for Sandoz. Financial compensation for these activities has been received by Oxford University. MAP serves or has served on an advisory panel for Sanofi and Novo Nordisk and has been a speaker for Novo Nordisk and Nordic Infucare. AN and AS are former employees of Sanofi. MP and EN are employees of Sanofi. LM has served on an advisory panel for Sanofi and SAB Bio.

DATA SHARING

One year after publication, de-identified trial data will be available upon request to corresponding author. Requests should outline the specified purpose for use of the data and will require a signed data access agreement.

FUNDING STATEMENT

This work was supported by the European Union's Innovative Medicines Initiative 2 Joint Undertaking (IMI-JU) under grant agreement no. 115797 (INNODIA). This Joint Undertaking received support from the European Union's Horizon 2020 research and innovation programme, 'EFPIA' (European Federation of Pharmaceutical Industries and Associations), 'JDRF' (now Breakthrough T1D) and 'The Leona M. and Harry B. Helmsley Charitable Trust'. Views and opinions expressed are those of the authors only and do not necessarily reflect those of the funder who cannot be held responsible for them. Sanofi provided ATG and placebo within the context of INNODIA.

ACKNOWLEDGEMENTS

We thank all participants and parents and the clinical teams. We thank the INNODIA consortium members, in particular the INNODIA Patient Advisory Committee for guidance in conception,

educational materials for recruitment and retention in the trial; and the Ethics Advisory Committee for advising on and evaluating all documents related to ethics. We thank the INNODIA management team (KULeuven and Sanofi) and the logistics team (Cambridge) for their daily support; the laboratories (CBAL (Cambridge), Immune hubs (London, Helsinki, Dresden, Siena, Leiden), Helsinki lab) for their excellent and swift analysis of samples. We thank Monitoring Services and the assigned monitors for trial monitoring. We thank Dexcom for providing continuous glucose monitoring devices. We thank all committees, in particular the Trial Steering Committee, Dose Determining Committee and the Independent Data Monitoring Committee for their expert guidance.

We dedicate this manuscript to the late David Dunger, who was instrumental in the conception of this trial.

RESEARCH IN CONTEXT

Evidence before this study

The efficacy and safety of anti-thymocyte globulin (ATG) in preventing C-peptide loss as a marker of functional beta-cell mass in people with recently diagnosed type 1 diabetes (T1D) has been previously reported in three clinical studies. We searched PubMed for articles published between January 1, 2000, and January 1, 2025, using the terms (“Anti-thymocyte globulin” AND “type 1 diabetes” AND “C-peptide”) as well as (“ATG” AND “type 1 diabetes” AND “C-peptide”). The first study (Gitelman S et al. Lancet DE 2013) reported lack of efficacy of 6.5mg/kg ATG (START trial). In contrast, two subsequent studies demonstrated the efficacy and safety of a lower dose, 2.5mg/kg of ATG, in adolescents and adults with new onset clinical T1D. A preliminary study by Haller M et al. (J Clin Invest. 2015) suggested less C-peptide loss in individuals treated with ATG and Granulocyte-Colony Stimulating Factor (G-CSF). These observations were partially confirmed in a subsequent Trialnet study (Haller et al Diabetes Care 2018), where C-peptide loss was less in adolescents and adults treated with ATG, but not in those treated with ATG/G-CSF. More recently, Haller et al reported 2-year clinical trial outcome data confirming a sustained effect of 2.5mg/kg ATG on C-peptide and HbA1c (Haller M et al Diabetes 2019).

Mechanistic studies on effects of ATG on lymphocyte subsets are emerging, but unclarity exists whether the depletion of lymphocytes and the CD4/CD8 ratio changes observed with ATG are related to the metabolic treatment effect and unclarity on dosing remains.

Added value of the study

The MELD-ATG trial (NCT04509791) investigated the efficacy and safety of different doses of ATG in young people (5-25years) with recently diagnosed clinical T1D using an innovative, adaptive trial design, allowing dropping (or restarting) doses on the basis of prespecified criteria. We not only achieved our primary endpoint, demonstrating prevention of functional beta-cell mass loss, measured as the difference in stimulated C-peptide at 12-months for 2.5mg/kg ATG versus placebo ($p=0.003$), but could also identify a minimum effective dose at 0.5mg/kg ($p=0.014$). Those treated with 0.5mg/kg also had a lower HbA1c compared to placebo (-0.50% , $p=0.024$) and experienced less side effects compared to those treated with 2.5mg/kg, in particular cytokine release syndrome (23.5% versus 33.3%) and serum sickness (32.4% versus 81.8%). The novel, adaptive trial design of this study with progressive age drop-down and its execution in the context of a clinical trial platform with accredited clinical trial sites (INNODIA), allowed to test multiple doses of ATG over a large age span, illustrating that this novel way of testing disease-modifying therapies could allow the field of finally increasing its pace towards finding treatments to arrest T1D progression.

Implication of all the available evidence

Our findings strengthen the potential of ATG as a safe and affordable agent for therapy in children and adolescents with recent-onset stage 3 T1D. Our study also emphasises the feasibility of adaptive trial designs for disease-modifying therapies in T1D. The observations that a therapy like ATG is most effective in the youngest participants, urges for an alteration in the regulatory pathways for testing and approving disease-modifying therapies in T1D.

REFERENCES

1. Quattrin T, Mastrandrea LD, Walker LSK. Type 1 diabetes. *Lancet* 2023; **401**(10394): 2149-62.

2. Ogle GD, Wang F, Haynes A, et al. Global type 1 diabetes prevalence, incidence, and mortality estimates 2025: Results from the International diabetes Federation Atlas, 11th Edition, and the T1D Index Version 3.0. *Diabetes Res Clin Pract* 2025; **225**: 112277.
3. Herold KC, Delong T, Perdigoto AL, Biru N, Brusko TM, Walker LSK. The immunology of type 1 diabetes. *Nat Rev Immunol* 2024; **24**(6): 435-51.
4. O'Donovan AJ, Gorelik S, Nally LM. Shifting the paradigm of type 1 diabetes: a narrative review of disease modifying therapies. *Front Endocrinol (Lausanne)* 2024; **15**: 1477101.
5. Atkinson MA, Haller MJ, Schatz DA, Battaglia M, Mathieu C. Time for changes in type 1 diabetes intervention trial designs. *Lancet Diabetes Endocrinol* 2023; **11**(11): 789-91.
6. Samuelsson U, Lindblad B, Carlsson A, et al. Residual beta cell function at diagnosis of type 1 diabetes in children and adolescents varies with gender and season. *Diabetes Metab Res Rev* 2013; **29**(1): 85-9.
7. Marcovecchio ML, Hendriks AEJ, Delfin C, et al. The INNODIA Type 1 Diabetes Natural History Study: a European cohort of newly diagnosed children, adolescents and adults. *Diabetologia* 2024; **67**(6): 995-1008.
8. Keymeulen B, Walter M, Mathieu C, et al. Four-year metabolic outcome of a randomised controlled CD3-antibody trial in recent-onset type 1 diabetic patients depends on their age and baseline residual beta cell mass. *Diabetologia* 2010; **53**(4): 614-23.
9. Orban T, Bundy B, Becker DJ, et al. Co-stimulation modulation with abatacept in patients with recent-onset type 1 diabetes: a randomised, double-blind, placebo-controlled trial. *Lancet* 2011; **378**(9789): 412-9.
10. Ramos EL, Dayan CM, Chatenoud L, et al. Teplizumab and β -Cell Function in Newly Diagnosed Type 1 Diabetes. *N Engl J Med* 2023; **389**(23): 2151-61.
11. Waibel M, Wentworth JM, So M, et al. Baricitinib and β -Cell Function in Patients with New-Onset Type 1 Diabetes. *N Engl J Med* 2023; **389**(23): 2140-50.
12. Haller MJ, Long SA, Blanchfield JL, et al. Low-Dose Anti-Thymocyte Globulin Preserves C-Peptide, Reduces HbA(1c), and Increases Regulatory to Conventional T-Cell Ratios in New-Onset Type 1 Diabetes: Two-Year Clinical Trial Data. *Diabetes* 2019; **68**(6): 1267-76.
13. Pallmann P, Bedding AW, Choodari-Oskooei B, et al. Adaptive designs in clinical trials: why use them, and how to run and report them. *BMC Med* 2018; **16**(1): 29.
14. Bhatt DL, Mehta C. Adaptive Designs for Clinical Trials. *N Engl J Med* 2016; **375**(1): 65-74.
15. Anderson RL, DiMeglio LA, Mander AP, et al. Erratum. Innovative Designs and Logistical Considerations for Expedited Clinical Development of Combination Disease-Modifying Treatments for Type 1 Diabetes. *Diabetes Care* 2022; **45**: 2189-2201. *Diabetes Care* 2022; **45**(12): 3113.
16. Seelig E, Howlett J, Porter L, et al. The DILfrequency study is an adaptive trial to identify optimal IL-2 dosing in patients with type 1 diabetes. *JCI Insight* 2018; **3**(19).
17. Todd JA, Evangelou M, Cutler AJ, et al. Regulatory T Cell Responses in Participants with Type 1 Diabetes after a Single Dose of Interleukin-2: A Non-Randomised, Open Label, Adaptive Dose-Finding Trial. *PLoS Med* 2016; **13**(10): e1002139.
18. Acharya S, Lama S, Kanigicherla DA. Anti-thymocyte globulin for treatment of T-cell-mediated allograft rejection. *World J Transplant* 2023; **13**(6): 299-308.
19. Haller MJ, Schatz DA, Skyler JS, et al. Low-Dose Anti-Thymocyte Globulin (ATG) Preserves β -Cell Function and Improves HbA(1c) in New-Onset Type 1 Diabetes. *Diabetes Care* 2018; **41**(9): 1917-25.
20. Haller MJ, Gitelman SE, Gottlieb PA, et al. Anti-thymocyte globulin/G-CSF treatment preserves β cell function in patients with established type 1 diabetes. *J Clin Invest* 2015; **125**(1): 448-55.
21. Gitelman SE, Gottlieb PA, Rigby MR, et al. Antithymocyte globulin treatment for patients with recent-onset type 1 diabetes: 12-month results of a randomised, placebo-controlled, phase 2 trial. *Lancet Diabetes Endocrinol* 2013; **1**(4): 306-16.
22. Jacobsen LM, Diggins K, Blanchfield L, et al. Responders to low-dose ATG induce CD4+ T cell exhaustion in type 1 diabetes. *JCI Insight* 2023; **8**(16): e161812.

23. Wilhelm-Benartzi CS, Miller SE, Bruggraber S, et al. Study protocol: Minimum effective low dose: anti-human thymocyte globulin (MELD-ATG): phase II, dose ranging, efficacy study of antithymocyte globulin (ATG) within 6 weeks of diagnosis of type 1 diabetes. *BMJ Open* 2021; **11**(12): e053669.
24. Common Terminology Criteria for Adverse Events (CTCAE) version 5.0. Bethesda, MD: National Cancer Institute, November 27, 2017.
25. Lachin JM MP, Greenbaum CJ, Palmer J, Pescovitz MD, Gottlieb P, Skyler J. Type 1 Diabetes Trial Network. Sample size requirements for studies of treatment effects on beta-cell function in newly diagnosed type 1 diabetes. *PLoS One* 2011; **6**(11):e26471. .
26. StataCorp. 2024. Stata Statistical Software: Release 18.5. College Station, TX: StataCorp LLC.
27. Bundy BN, Krischer JP. A quantitative measure of treatment response in recent-onset type 1 diabetes. *Endocrinol Diabetes Metab* 2020; **3**(3): e00143.
28. Couvrat-Desvergnès G, Salama A, Le Berre L, et al. Rabbit antithymocyte globulin-induced serum sickness disease and human kidney graft survival. *J Clin Invest* 2015; **125**(12): 4655-65.
29. Shimabukuro-Vornhagen A, Gödel P, Subklewe M, et al. Cytokine release syndrome. *J Immunother Cancer* 2018; **6**(1): 56.
30. Mohty M. Mechanisms of action of antithymocyte globulin: T-cell depletion and beyond. *Leukemia* 2007; **21**(7): 1387-94.
31. Leete P, Mallone R, Richardson SJ, Sosenko JM, Redondo MJ, Evans-Molina C. The Effect of Age on the Progression and Severity of Type 1 Diabetes: Potential Effects on Disease Mechanisms. *Curr Diab Rep* 2018; **18**(11): 115.
32. Battaglia M, Ahmed S, Anderson MS, et al. Introducing the Endotype Concept to Address the Challenge of Disease Heterogeneity in Type 1 Diabetes. *Diabetes Care* 2020; **43**(1): 5-12.

FIGURE TITLES AND LEGENDS

Figure 1

Title: MELD-ATG CONSORT Flow diagram

Legend: The flow diagram shows the allocation of participants and indicates the points at which participants discontinued or were lost to follow-up and the associated reason.

Figure 2.

Title: Efficacy outcomes showing change from baseline over 12-months in C-peptide level (A), HbA1c (B), daily insulin dose (C) and Insulin dose-adjusted A1c (IDAA1C) (D) for placebo, 0.5 mg/kg and 2.5 mg/kg ATG.

Legend: Panel A shows the change from baseline of the mean Ln(AUC C-peptide+1) calculated using the trapezoidal rule as the area under the C-peptide concentration-time curve divided by 120 minutes, and transformed to Ln(AUC C-peptide+1); Panel B shows the mean HbA1c level; Panel C shows the

mean daily insulin dose, calculated in clinic typically from the insulin use per kg for the last 3 days prior to clinic visit; and Panel D shows the insulin dose-adjusted A1c (IDAA1C), calculated as $\text{HbA1c (\%)} + [4 \times \text{insulin dose (units/kg/day)}]$. Data for placebo (n=31), 0.5mg/kg (n=35), and 2.5mg/kg (n=33) ATG arms are shown. Mean values and standard error of the mean bars are shown.

Figure 3.

Title: Continuous glucose monitoring (CGM) metrics at 3-, 6-, and 12-months post-treatment in placebo, 0.5 mg/kg and 2.5mg/kg ATG arms

Legend: CGM metrics were analysed using blinded Dexcom G6 for 14 days at 3-, 6-, and 12-months post-treatment. TIR: time in range; TITR: time in tight range; TAR: time above range; TBR: time below range. Data for placebo (n=31), 0.5mg/kg (n=35), and 2.5mg/kg (n=33) ATG arms are shown.

Figure 4.

Title: Change from baseline in the ratio of CD4/CD8 absolute cell counts

Legend: Change from baseline of ratios of absolute counts of CD4 and CD8 positive T-cells are shown for placebo (n=31), 0.5mg/kg (n=35), and 2.5mg/kg (n=33) ATG arms as determined by flow cytometry of peripheral blood mononuclear cells at week 0 (baseline), week 1, week 2, month 1, 3, 6 and 12. Mean values and standard error of the mean bars are shown.

Figure 5.

Title: Dried Blood Spot-measured C-peptide measured monthly in the placebo, 0.5 mg/kg and 2.5mg/kg ATG arms

Legend: Panel A shows the home DBS-collected C-peptide levels in the fasted state. Panel B shows home DBS-collected C-peptide levels 60 minutes after a standardised liquid meal test. Mean values and standard error of the mean bars are shown. Data for placebo (n=31), 0.5mg/kg (n=35), and

753 2.5mg/kg (n=33) ATG arms are shown. Note that after 6 months data capture is falling below 75%
754 completeness, with great variability.