

AG019 ActoBiotics as Monotherapy or in Association with Teplizumab in Recent-Onset T1D Was Safe and Demonstrated Encouraging Metabolic and Immunological Effects

C. Mathieu¹, S. Blomme², S. Caluwaerts², L. Haazen², L. Steidler², K. Van Huynegem², J. Vermeiren², K. Cerosaletti³, A. Wiedeman³, S.A. Long³, E. Serti⁴, AG019-T1D-101 Trial Investigators, P. Rottiers², G.T. Nepom³, K.C. Herold⁵

¹UZ Leuven, Leuven, Belgium, ²Precigen ActoBio, Gent, Belgium, ³Benaroya Research Institute, Seattle, WA, USA, ⁴Immune Tolerance Network, Bethesda, MD, USA, ⁵Yale University, New Haven, CT, USA.



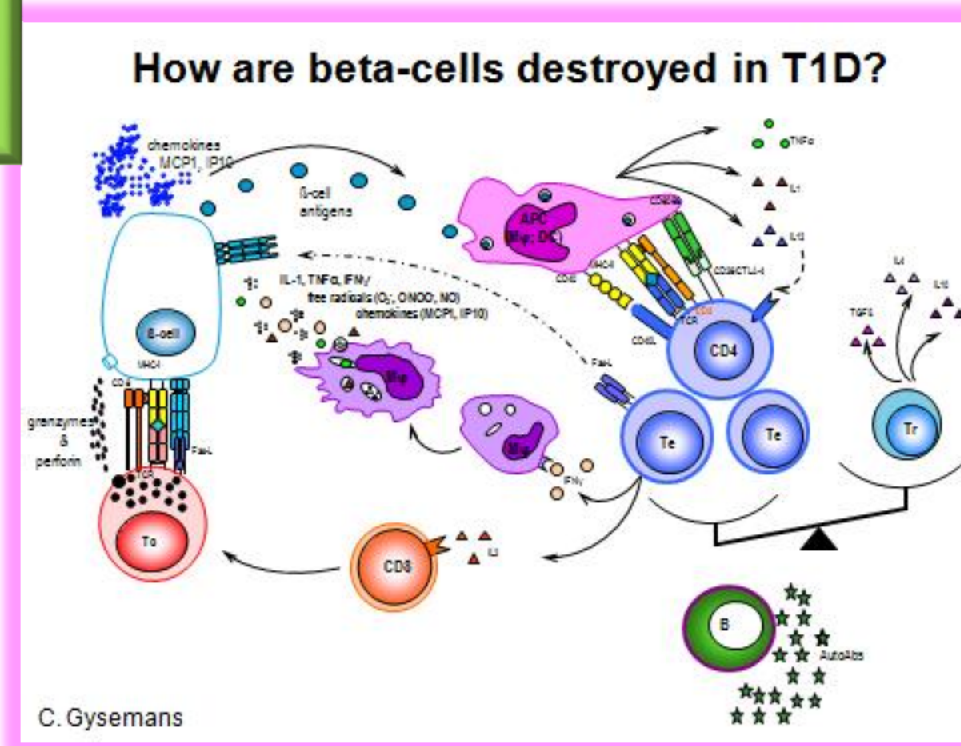
Speaker disclosures

C. Mathieu is or has been consultant for and KU Leuven has received research support or honoraria from: NovoNordisk, MSD, Eli Lilly, GSK, Sanofi, Novartis, AstraZeneca, Boehringer Ingelheim, Janssen Pharmaceuticals, Pfizer, Medtronic, Roche, Precigen ActoBio and Zealand Pharma

The AG019-T1D-101 trial was sponsored by Precigen ActoBio T1D, LLC and registered as NCT03751007 at ClinicalTrials.gov

The concept of antigen-specific tolerance induction in Type 1 diabetes

Antigen-specific tolerance induction



Systemic immune regulation

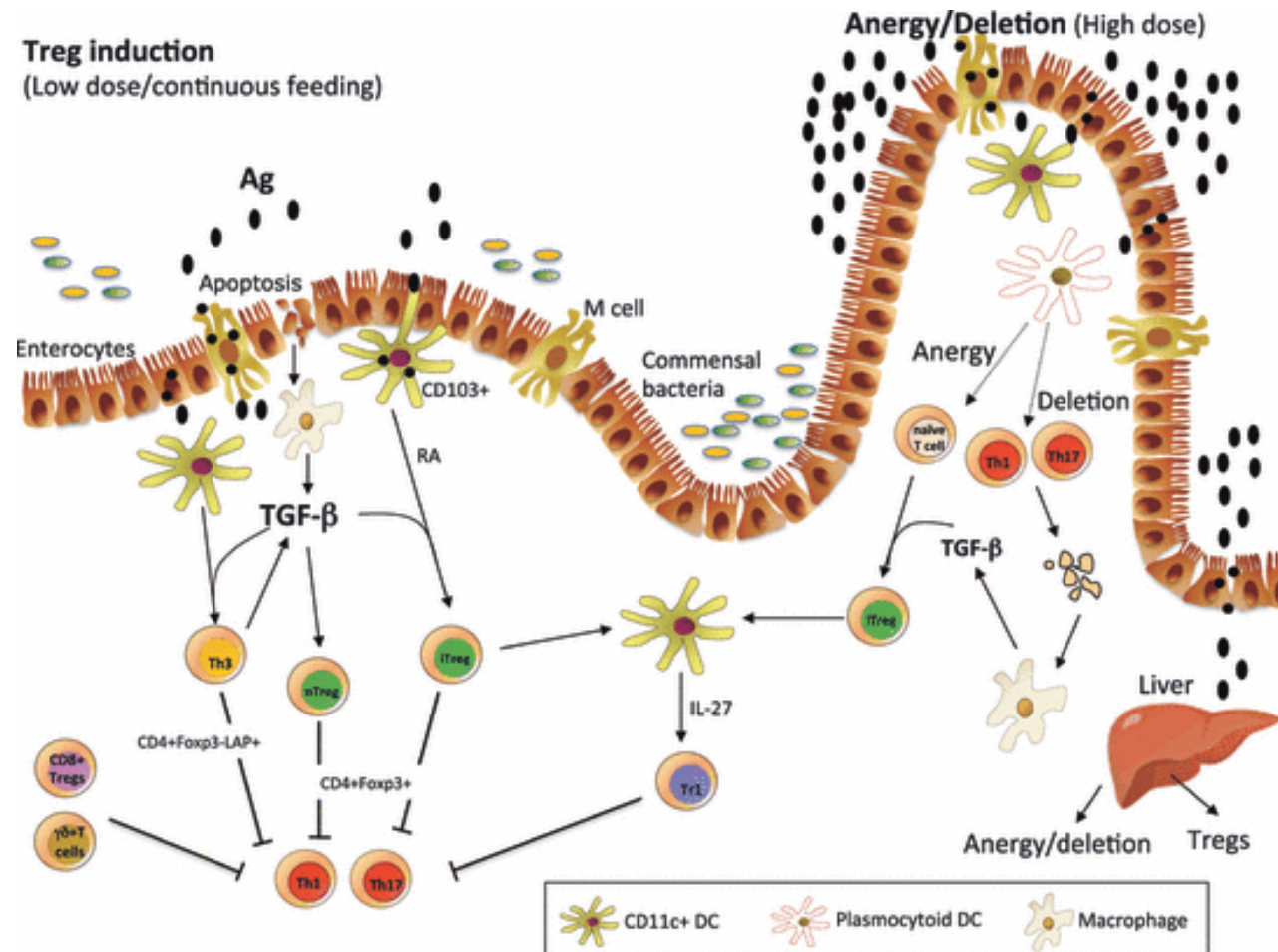
The concept of antigen-specific tolerance induction in Type 1 diabetes

Antigen-specific
tolerance induction



Systemic immune
regulation

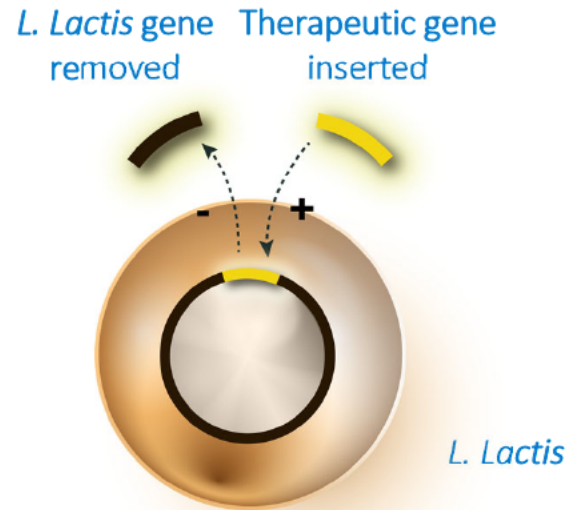
Exploiting the gut for antigen-based tolerance induction



ActoBiotics™, Microbe-based therapeutics platform

Lactococcus lactis: from dairy industry to antigen and therapeutic protein delivery

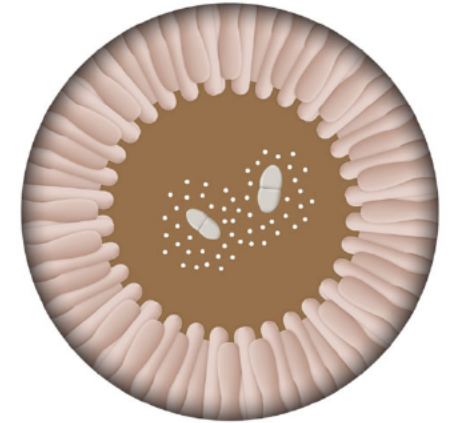
1 Genetic Engineering



2 Oral administration of encapsulated bacteria



3 Direct Delivery to Mucosal Sites



Simultaneous
expression of
multiple genes

Food-grade
bacteria with
history of safe
use

Extensive
regulatory
acceptance

Cost-effective
and scalable
manufacturing

Convenient oral
delivery

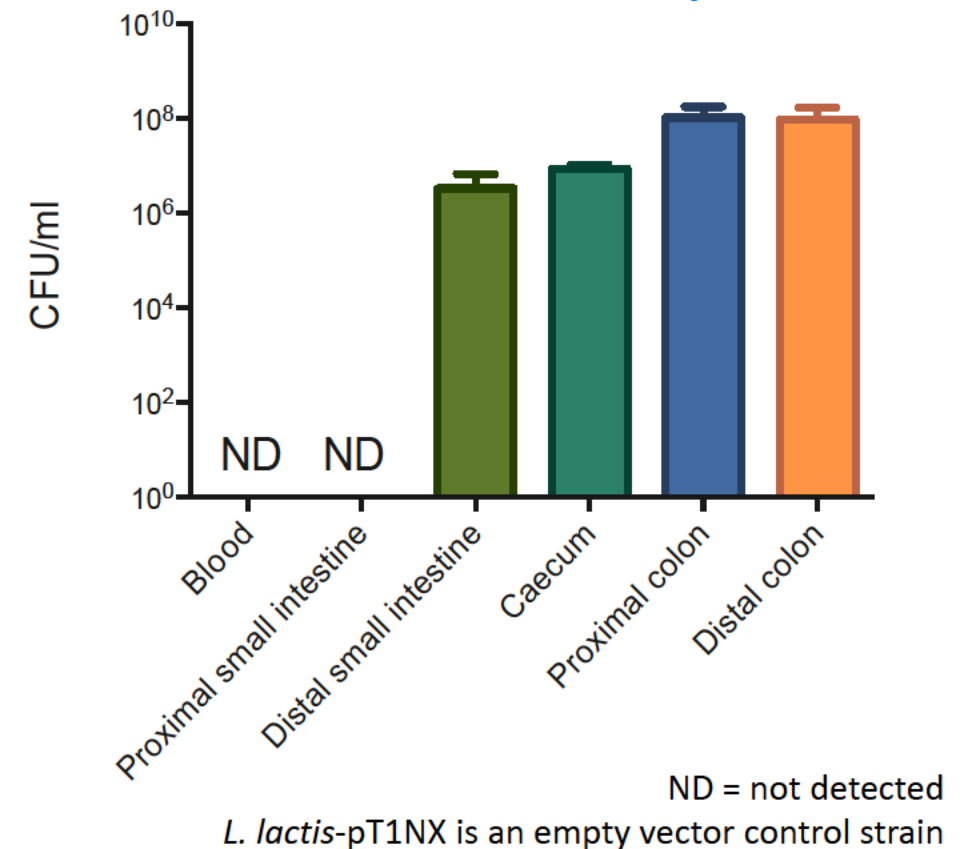
Local gene
expression at
disease site

No known
toxicity or
immunogenicity
observed

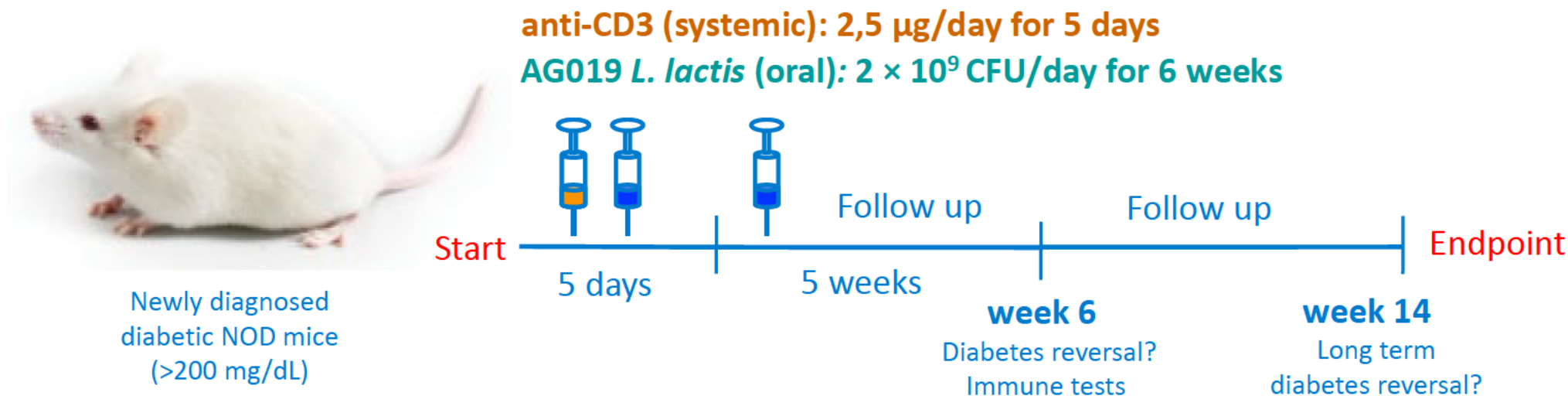
Lactococcus (L.) lactis are non-pathogenic Gram⁺ Lactic Acid Bacteria

- Cannot invade tissues nor cause infection.
- Upon oral administration, the GM *L. lactis* reside in the distal part of the small intestine and in the entire colon.
- The residence time of the GM *L. lactis* bacteria is transient and during the residency, the bacteria produce and deliver therapeutic concentrations of proteins and peptides.
- *L. lactis* are excreted along with feces.

Pharmacokinetic profile of orally administered *L. lactis*-pT1NX



Experimental design: Reversing diabetes in NOD mice

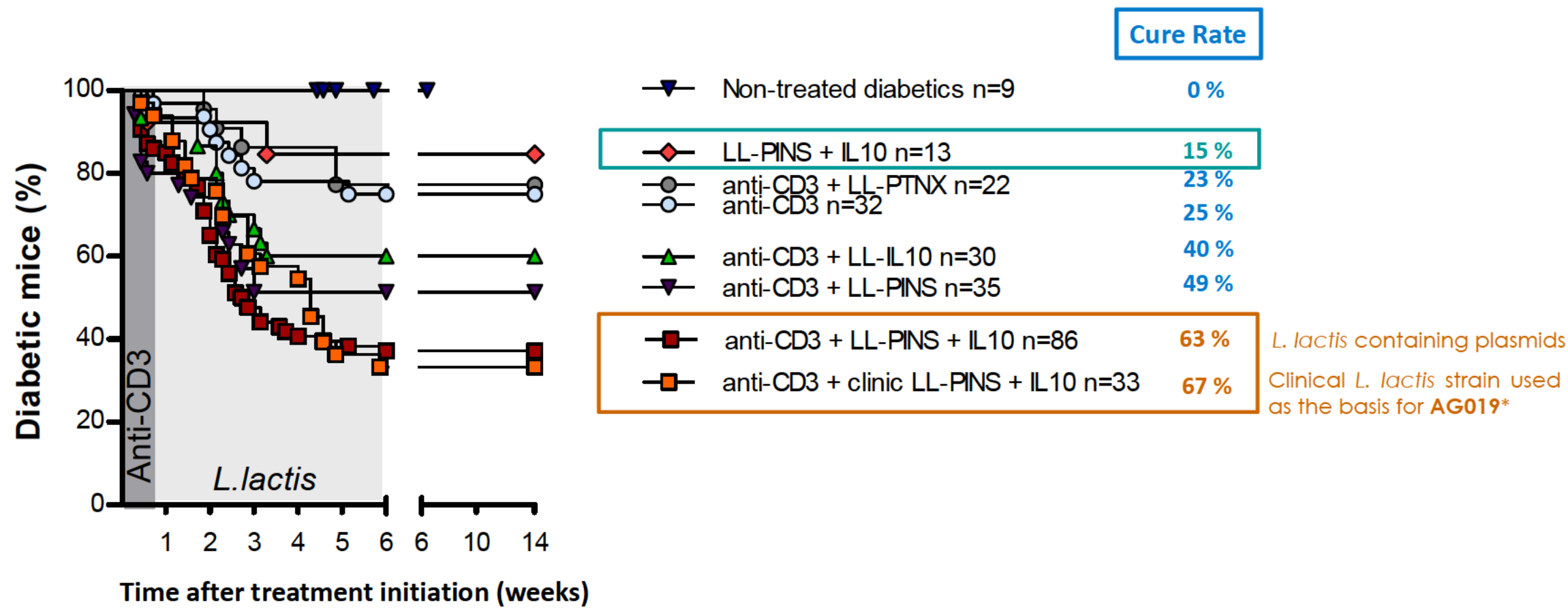


Groups	Treatment
Group 1	anti-CD3
Group 2	anti-CD3 + <i>L. lactis</i> with empty vector (LL-pT1NX)
Group 3	anti-CD3 + <i>L. lactis</i> secreting antigen
Group 4	anti-CD3 + <i>L. lactis</i> secreting IL-10
Group 5	anti-CD3 + <i>L. lactis</i> secreting antigen + IL-10 (LL-PINS+hIL-10)
Group 6	<i>L. lactis</i> secreting antigen + hIL-10
Group 7	Untreated

AG019 Combination Therapy CT

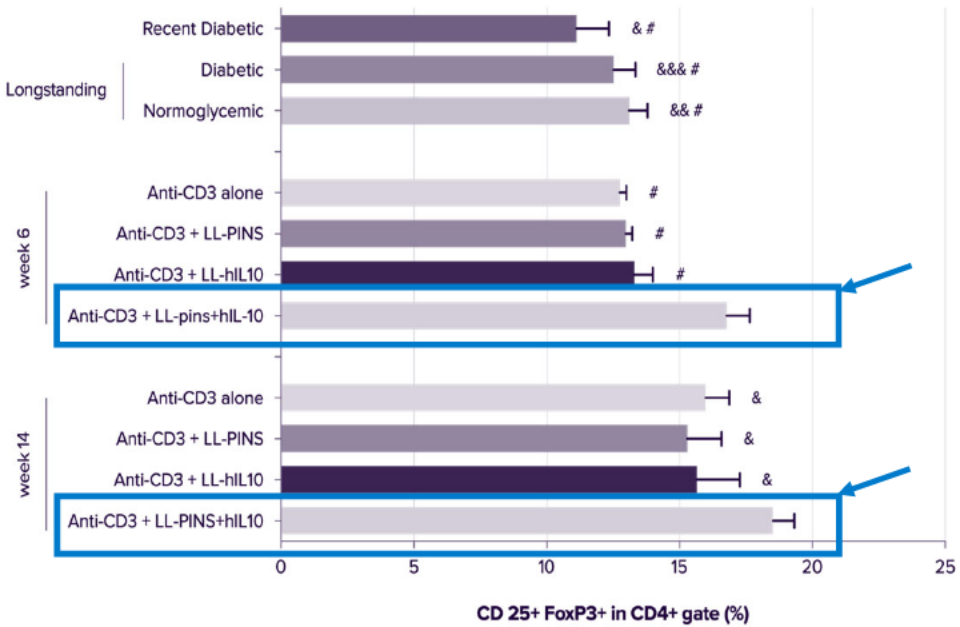
AG019 Monotherapy MT

Reversal of diabetes was demonstrated using *L. lactis* strains secreting proinsulin (PINS) and IL-10



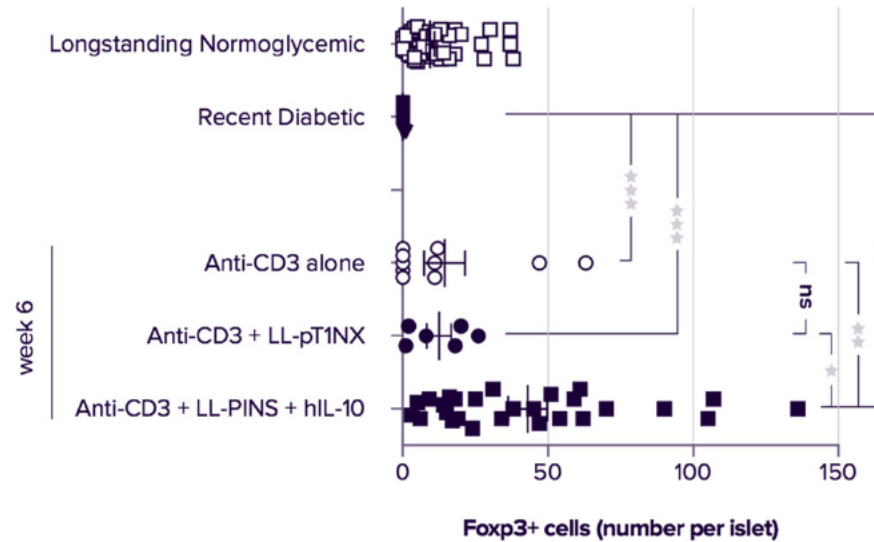
Reversal of diabetes depends on the induction of antigen-specific Tregs

FoxP3+ Tregs in the pancreatic lymph nodes

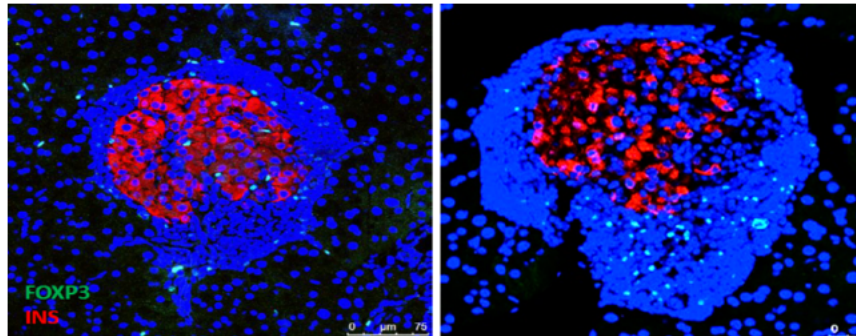


#; &: respectively vs. wk 6 and 14 CT;
& p<0.05; && p<0.01; &&& p<0.001

FoxP3+ Tregs in/around the pancreatic islets

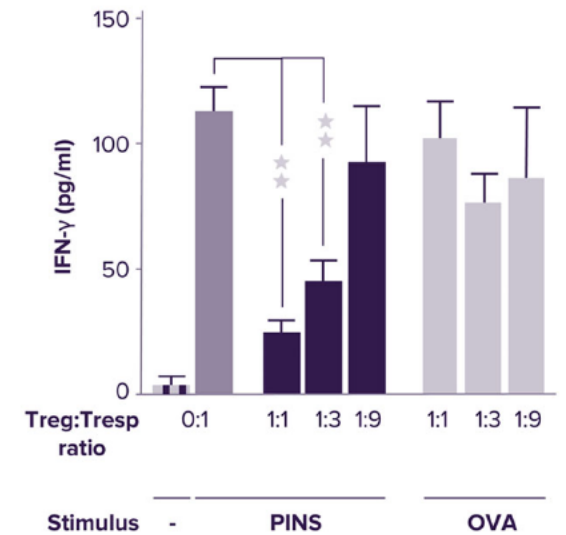


NOD Recent Diabetic



Co-expression of KI67 and FoxP3 reveal local proliferation

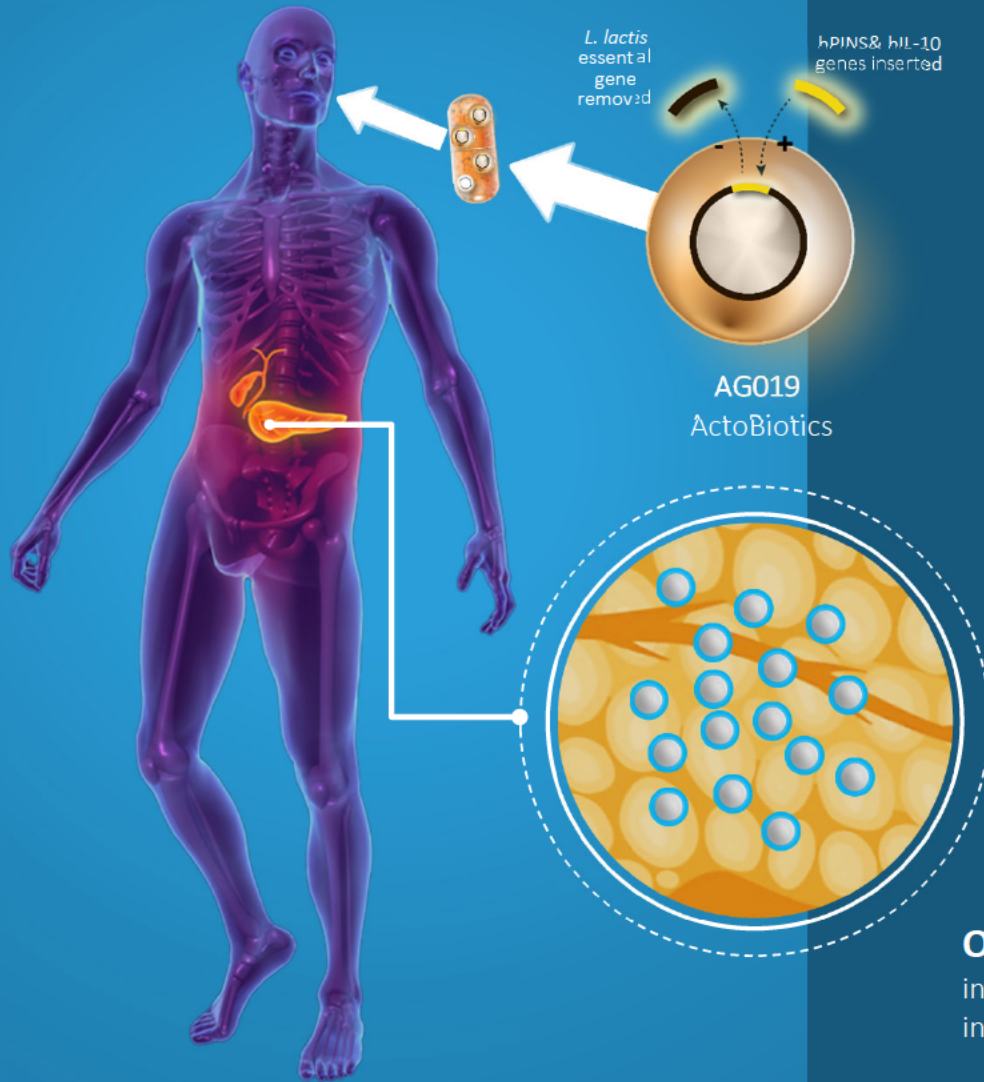
Combination therapy induces antigen-specific Tregs



Tregs suppress T-cell responses to the auto-antigen PINS but not to disease-irrelevant antigens (e.g. ovalbumin, OVA)

AG019 ActoBiotics

A First-in-Class Oral Therapy
in Type 1 Diabetes



AG019 Oral ActoBiotics™, an innovative disease-modifying approach to induce immune tolerance in Type 1 Diabetes

Type 1 Diabetes (T1D)

- Current treatment strategies focus on non-disease specific immune suppression with limited efficacy in recent-onset T1D
- Antigen-specific immunotherapy represents a promising treatment modality to re-establish long-lasting immune tolerance
- **AG019 is a safe and convenient antigen-specific disease modifying treatment currently being developed as mono- and combination therapy in recent-onset T1D**

POC/MOA established in recent-onset T1D animal model*

- AG019 - Genetically modified *Lactococcus lactis* (ActoBiotics™) to deliver autoantigen human proinsulin (hPINS) and human interleukin-10 (hIL-10)
- Oral AG019 in combination with anti-CD3 stably reverted recent-onset T1D
- Increased levels of insulin-responsive Treg-cells accumulate and proliferate in pancreatic islet and are essential to maintain active immune-tolerance

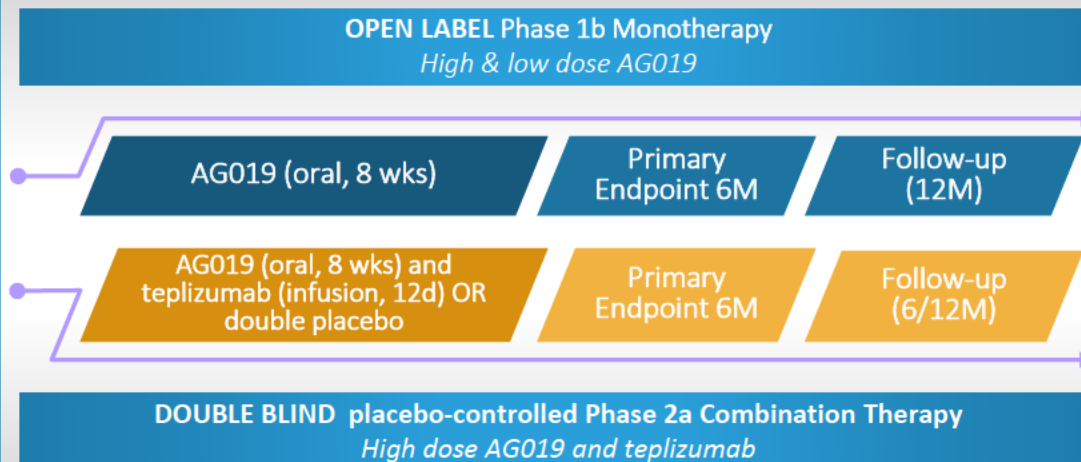
Oral AG019 targets the GALT

induce hPINS-specific regulatory T-cells which migrate to inflamed tissue to block tissue destruction

AG019 Phase 1b/2a in recent-onset T1D

Eligibility:

- 12-42 years of age
- Diagnosis of diabetes according to ADA (*)
- Stimulated C-peptide >0.2 nmol/L
- Treatment start within 150 days of diagnosis
- Autoantibody positivity to at least 1 (Insulin, IA-2, GAD65, ZnT8)
- No active infections



Primary endpoint:

Safety & tolerability at 6 months follow-up

Secondary endpoints:

1. Pharmacokinetics:

- Systemic and local exposure of *L. lactis* and hPINS/hIL-10

2. Pharmacodynamics/metabolics:

- C-peptide preservation
- Mechanistic assessments & biomarkers for immunological changes

Study Status

- Enrolment of 10 adults (18-42 years) and 9 adolescents (12-17 years) in the single and repeat dose AG019 Monotherapy cohorts.
- Enrolment of 12 adults (18-40 years; 10 active and 2 placebo) and 6 adolescents (12-17 years; 5 active and 1 placebo) in the AG019/Teplizumab Combination cohorts.
- 12M follow-up of all repeat dose AG019 Monotherapy patients completed.
- 12M follow-up of all adult Combination Therapy patients completed and 6M follow-up of all adolescent Combination Therapy patients completed.

Study Participant Baseline Characteristics

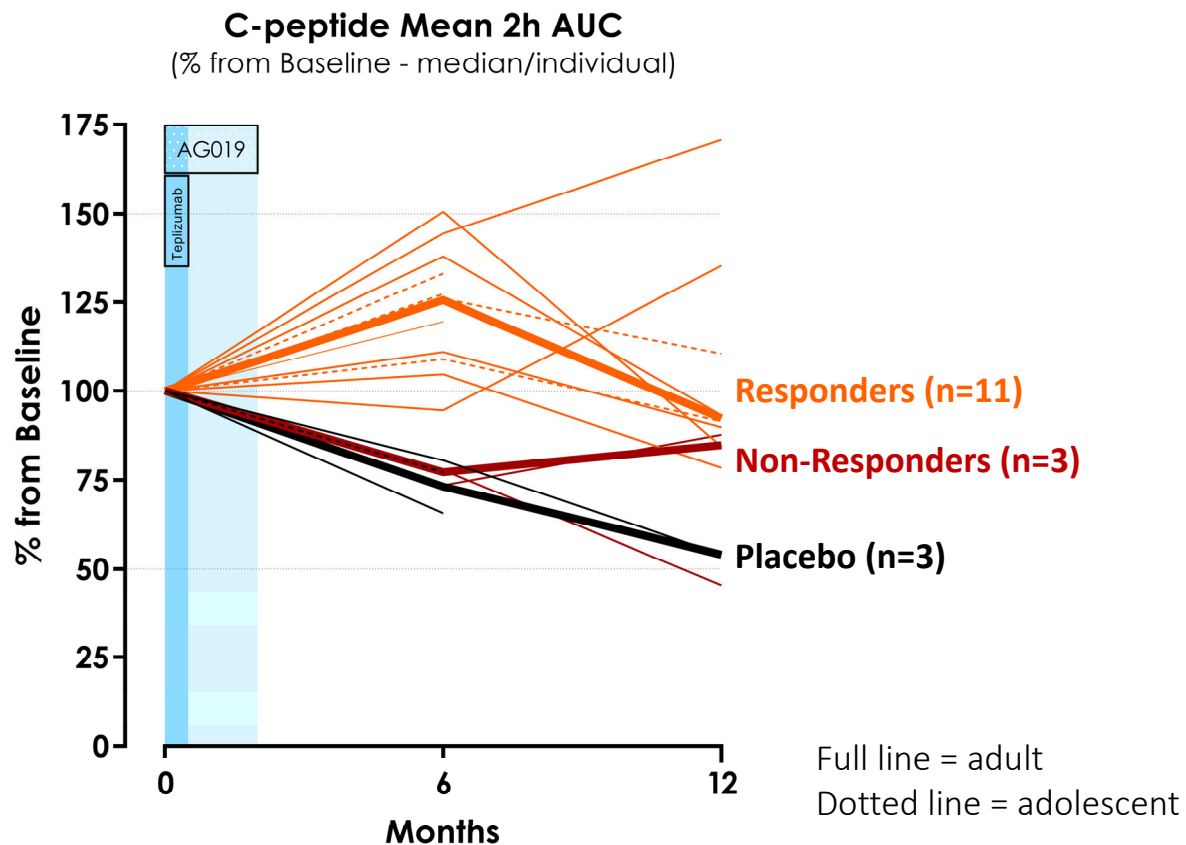
- Similar characteristics for age, gender, fasting and peak C-peptide levels and HbA1c levels.
- Slightly higher insulin-dose adjusted HbA1c levels for adolescents.
- The average time from diagnosis to treatment start (days) is in the second half of the diagnosis window: 75-150 days.

(*) <https://www.diabetes.org/a1c/diagnosis>

Primary endpoint of both Phase 1b AG019 monotherapy and Phase 2a AG019/Teplizumab combination therapy was met

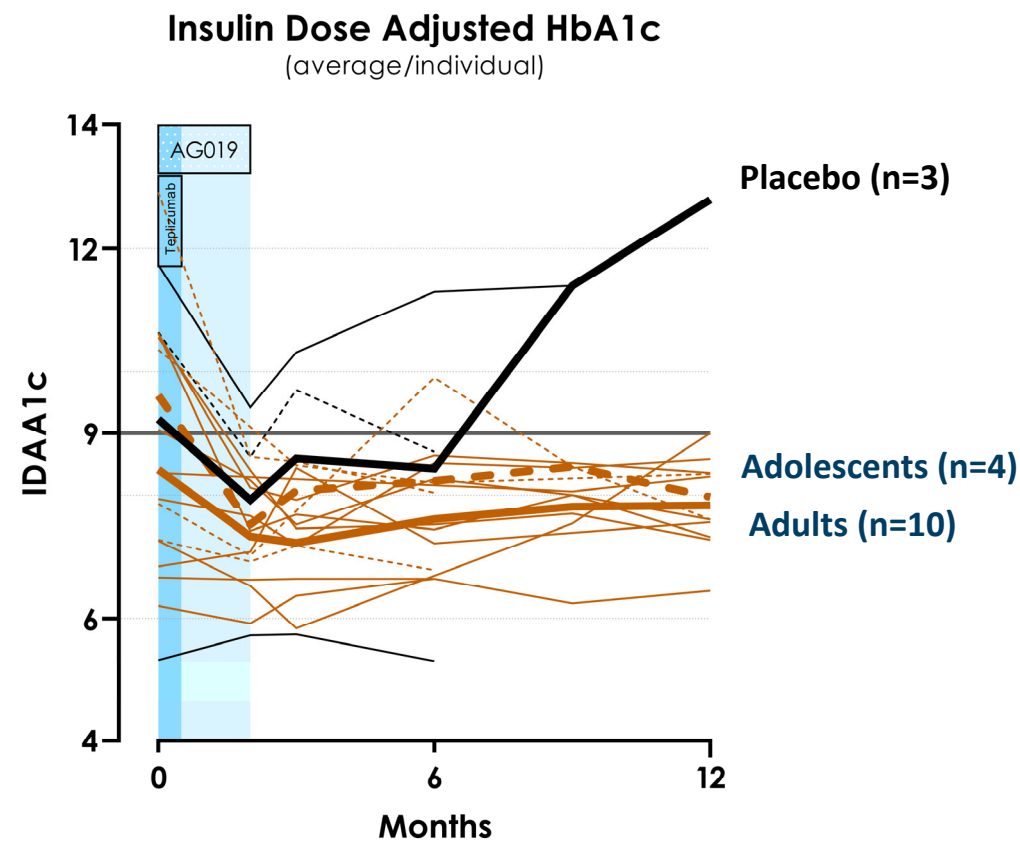
- **AG019 was well tolerated and safe when administered to adults and adolescents either as monotherapy or in combination with teplizumab.**
- **There was no evidence of dose-related adverse events.**
- **No serious adverse events were reported and no AG019 treatment discontinuation occurred due to TEAEs.**
- **No severe TEAEs were reported in patients treated with AG019 monotherapy.**
- **TEAEs reported for both the monotherapy and combination therapy were mostly mild and sometimes of moderate severity.**
- **The TEAEs reported in the combination cohorts are in line with the safety profile reported for teplizumab and no unexpected TEAEs were identified.**

AG019/Teplizumab Combination Therapy: C-peptide and insulin-dose adjusted HbA1c (IDAA1c)



A synergistic effect of oral AG019 treatment in combination with teplizumab resulted in C-peptide stabilization/increase ('responders'*) during the first 6 months in 79% (11/14) of all T1D patients (median value 126% of baseline).**

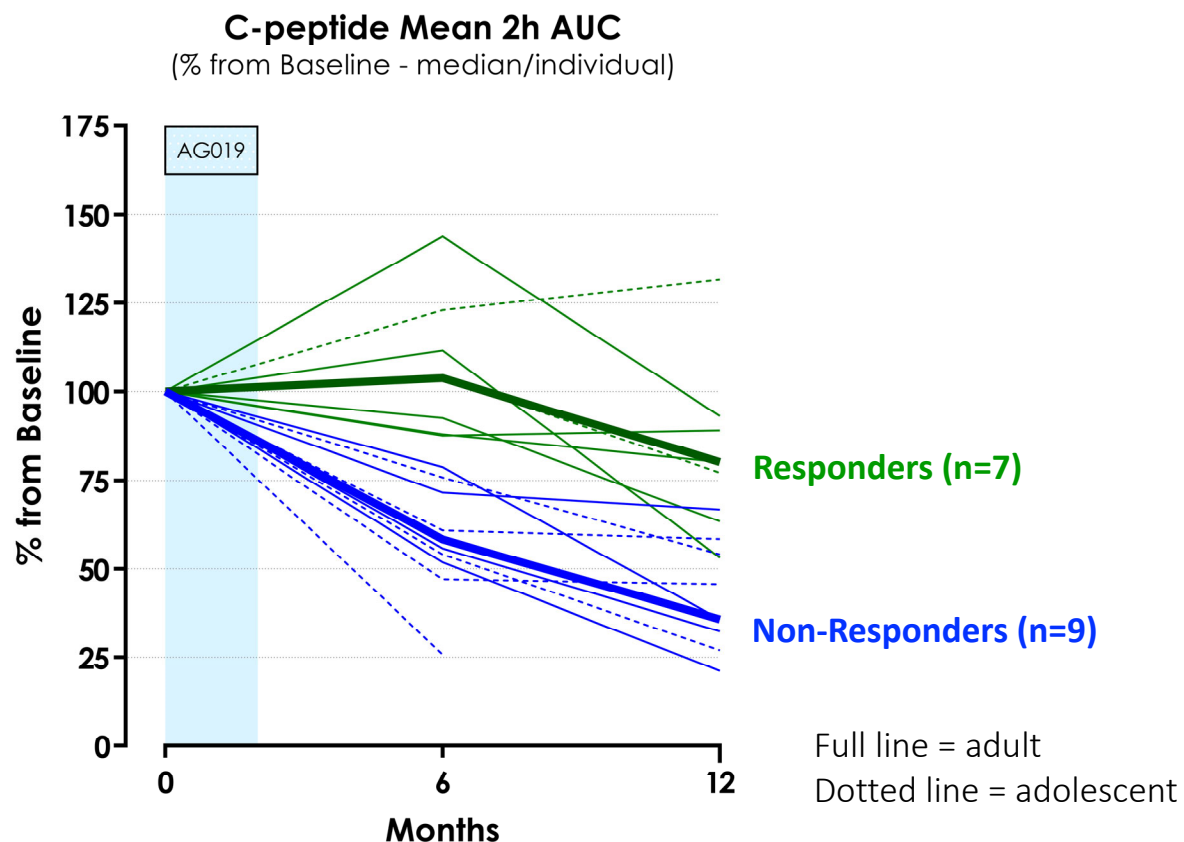
* 'Responders' were designated if the coefficient of variance on mean C-peptide AUC was $\leq 9.7\%$, or if change from baseline at 6 months was non-negative



Oral AG019 in association with teplizumab resulted in stabilization of IDAA1c < 9 (graph) and HbA1c < 7% (data not shown) in adults and adolescent patients.**

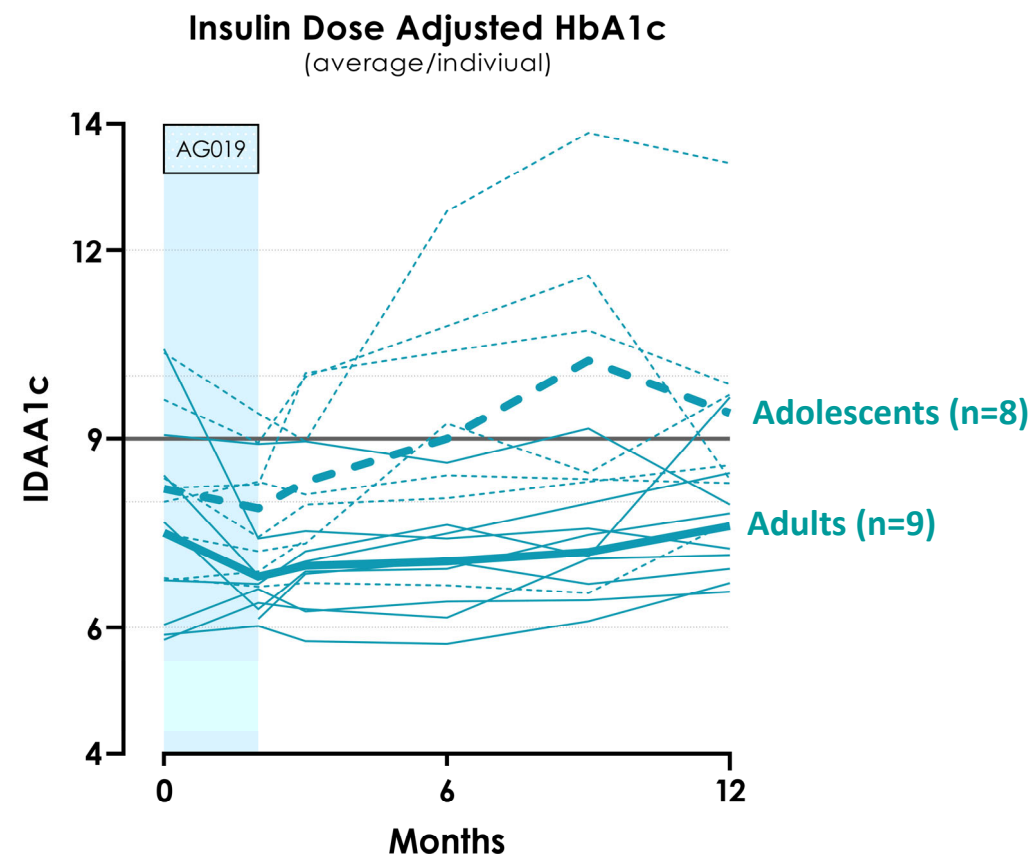
** Per Protocol population.
Adolescent combination therapy at 12 months not yet fully available.

AG019 Monotherapy: C-peptide and insulin-dose adjusted HbA1c (IDAA1c)



Oral AG019 with single treatment cycle resulted in C-peptide stabilization/increase ('responders'*) during the first 6 months in 44% (7/16) of all T1D patients and in 58% (7/12**) of patients aged 17 years and above.**

* 'Responders' were designated if the coefficient of variance on C-peptide AUC was $\leq 9.7\%$, or if change from baseline at 6 months was non-negative

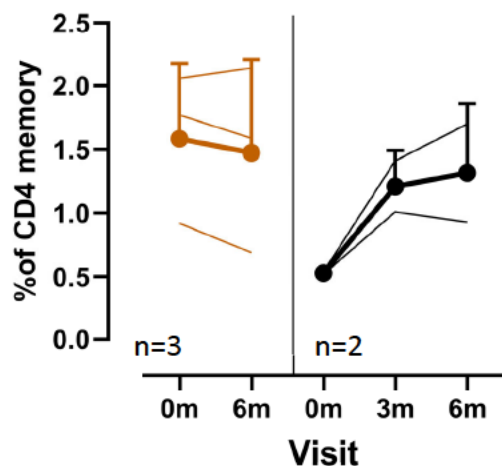


Oral AG019 with single treatment cycle resulted in stabilization of IDAA1c < 9 (graph) and HbA1c < 7% (data not shown) in adults and adolescent patients aged 17 years and above.**

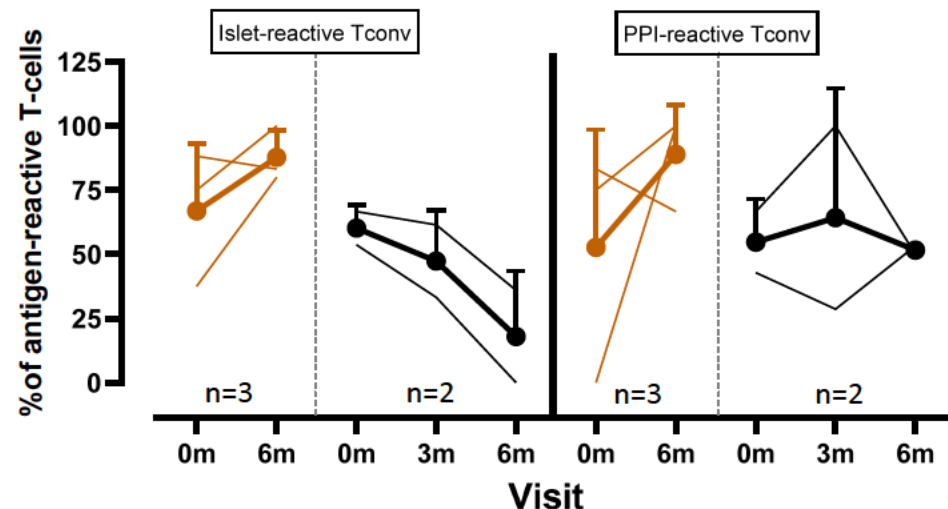
** Per Protocol population and excluding 1 additional patient for which no C-peptide data are available at 6 and 12 month: total analysed n=16

AG019/Teplizumab Combination Therapy: frequency of antigen-specific Tregs

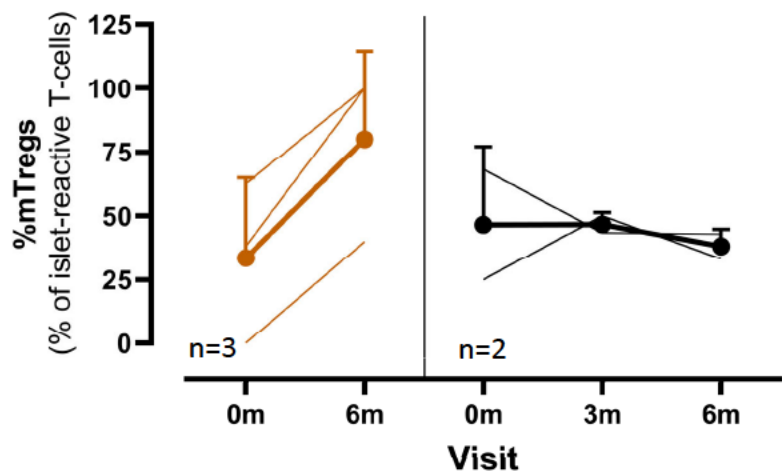
% TNFa+ Conventional T-cells



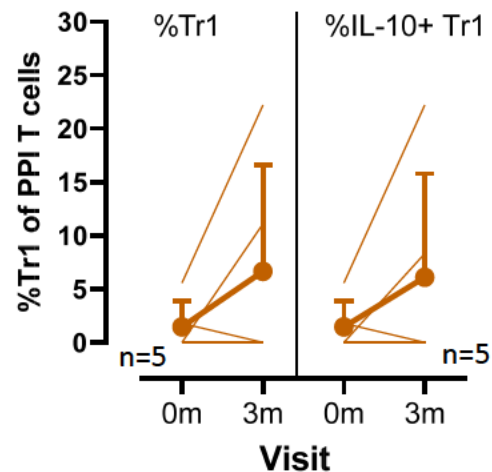
PD-1 expression in antigen-reactive Conventional T-cells



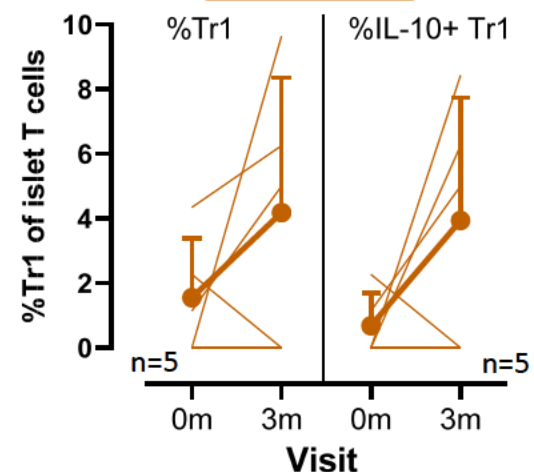
Islet-specific mTregs



PPI Tr1*



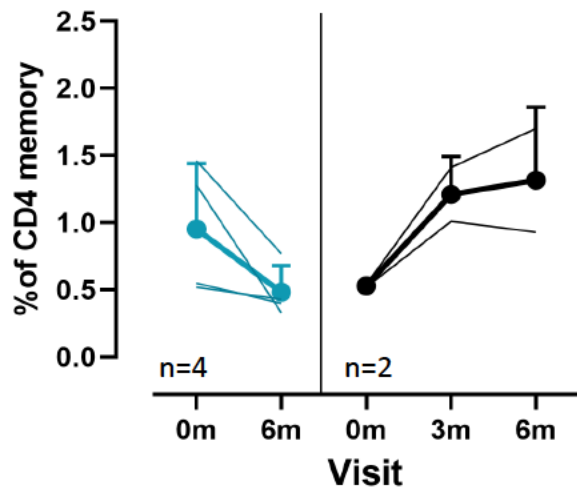
Islet Tr1*



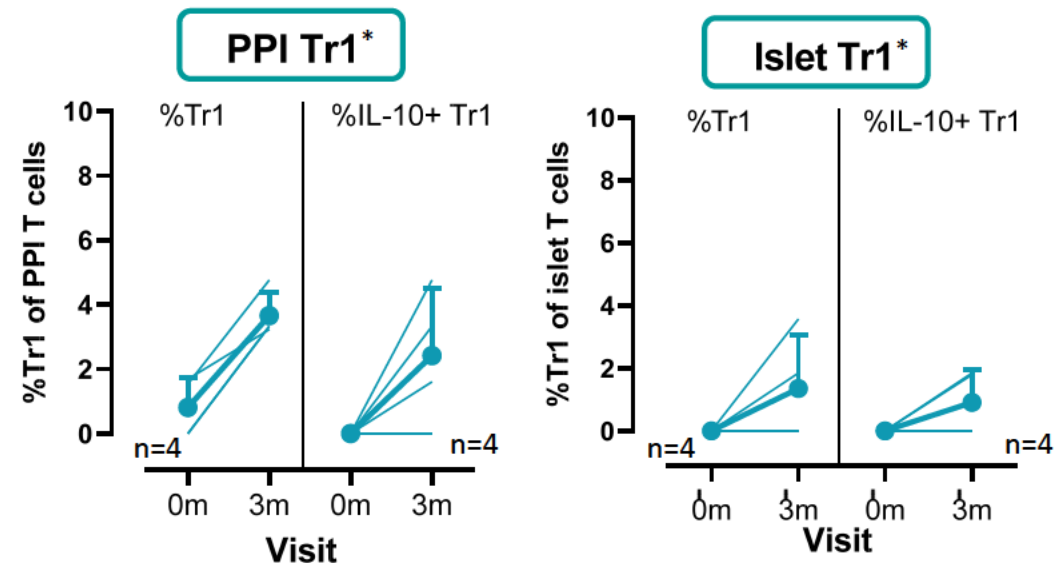
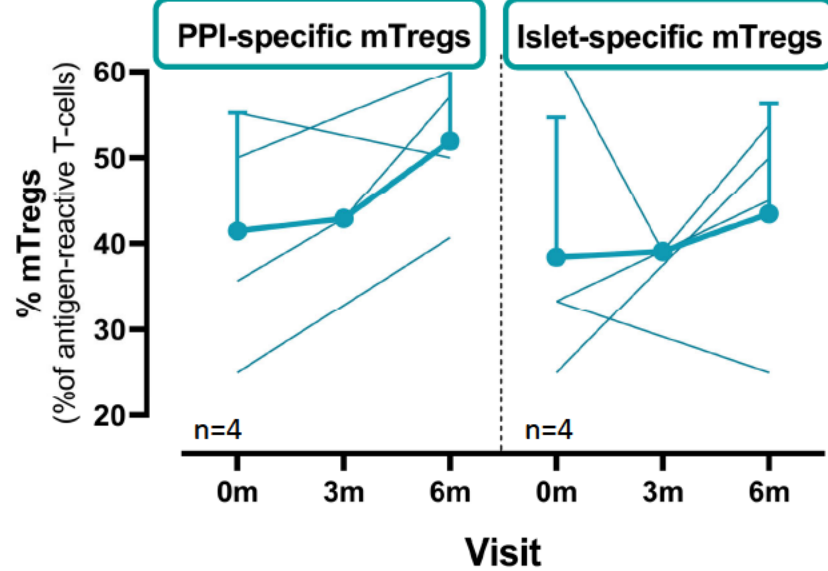
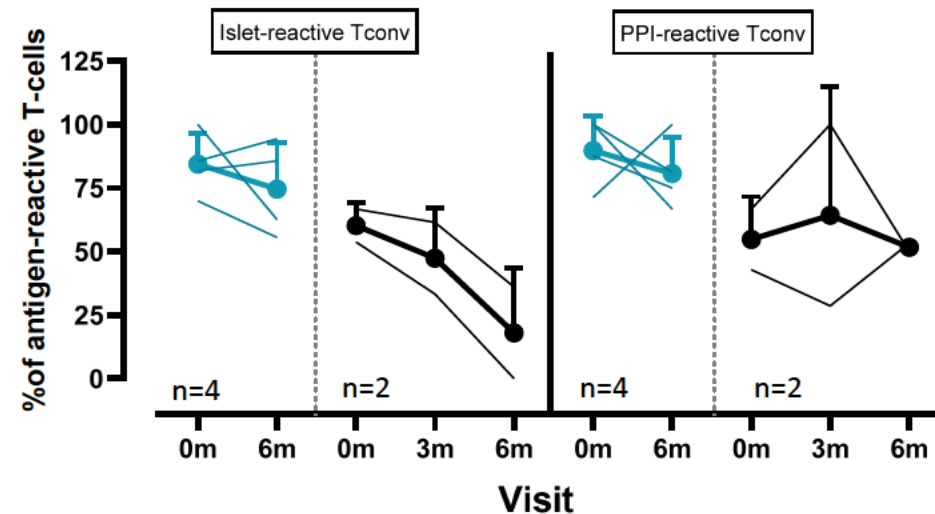
* Data at the limit of detection; needs confirmation with more cells

AG019 Monotherapy: Frequency of antigen-specific Tregs

% TNFa+ Conventional T-cells



PD-1 expression in antigen-reactive Conventional T-cells

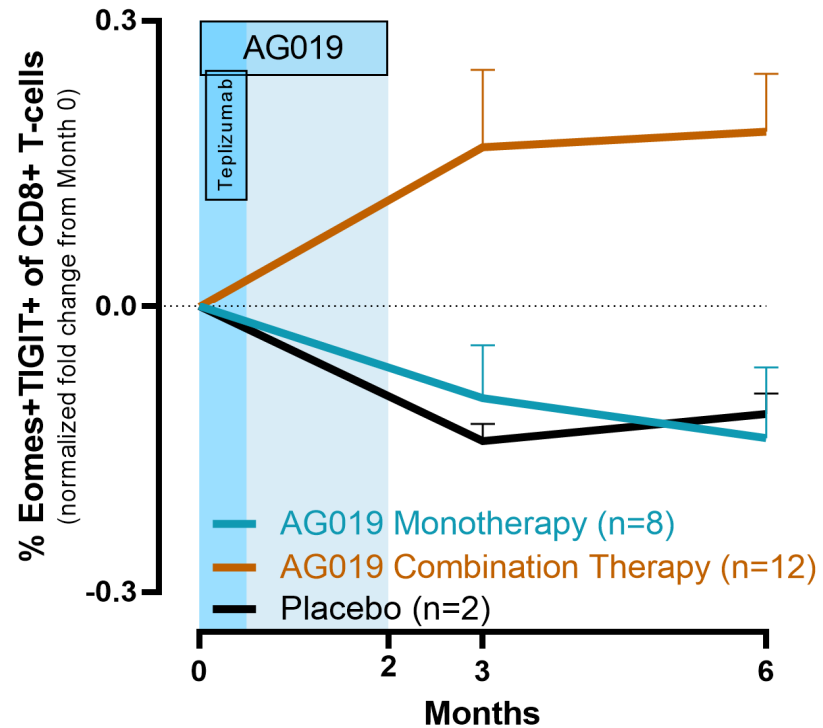


* Data at the limit of detection; needs confirmation with more cells

AG019/Teplizumab Combination Therapy: Increased the frequency of exhausted CD8+ T cells- correlation with preservation of C-peptide

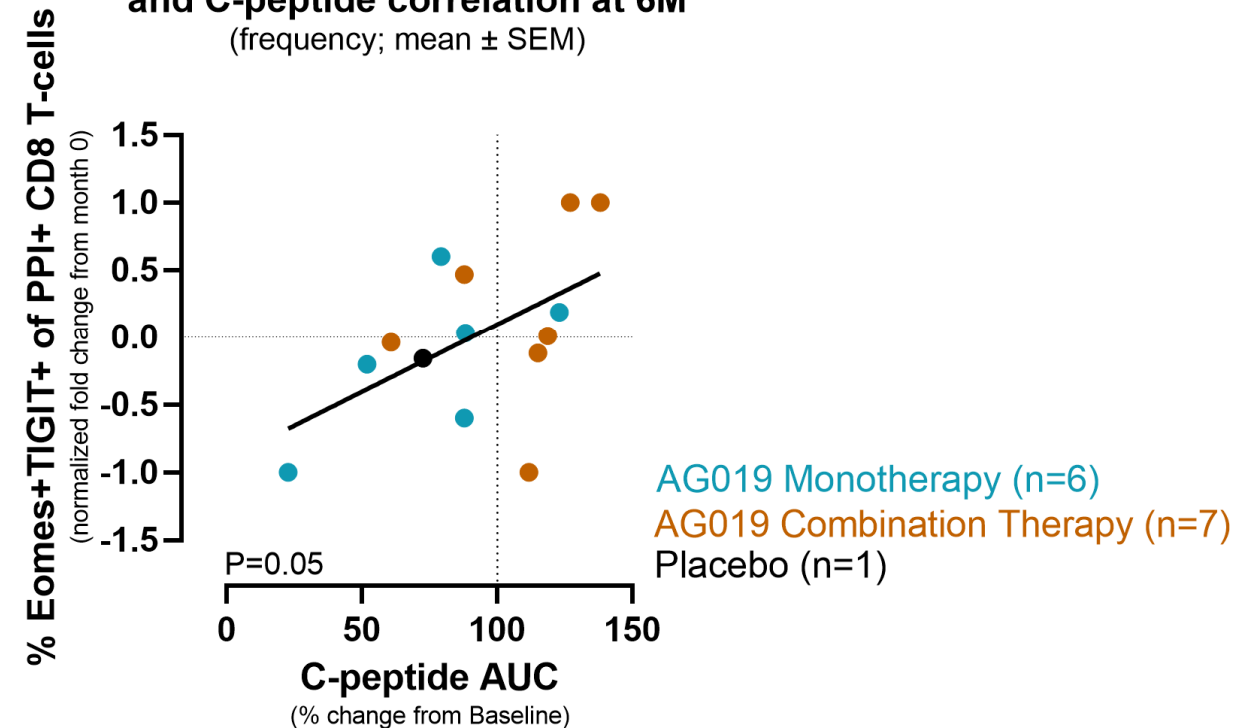
CD8+ T-cells with exhausted phenotype

(frequency; mean $\pm$ SEM)



PPI-specific CD8+ T-cells exhausted phenotype and C-peptide correlation at 6M

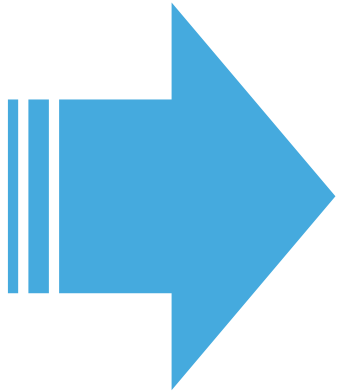
(frequency; mean $\pm$ SEM)



- AG019/teplizumab combination therapy is associated with an expansion of total CD8+ T-cells with an exhausted phenotype.
- At 6 months, the frequency of PPI-specific CD8+ T-cells with an exhaustion phenotype may be correlated with C-peptide.

Summary and conclusions

- AG019, *L. lactis* producing IL-10 and hPINS, can be administered safely, either as monotherapy or in combination with teplizumab.
- The stabilization of C-peptide and HbA1c/IDAA1c levels in monotherapy with an 8-week treatment of AG019 is encouraging and a synergistic effect for C-peptide stabilization was observed between AG019 and teplizumab.
- There was a trend towards an increase in islet- and PPI-reactive memory Tregs and Tr1 cells and a decline in the frequency of % TNF-positive conventional T-cells and PPI-specific CD8+ T cells with AG019 therapy in adults.



AG019, as monotherapy and in association with teplizumab, showed stabilization of C-peptide levels (a biomarker for T1D disease progression) and induced antigen-specific tolerance in conjunction with the reduction of disease specific T cell responses.

Based on the safety and encouraging pharmacodynamic/metabolic data in monotherapy and combination therapy, further clinical assessment of the efficacy of prolonged treatment of oral AG019 is being planned.

Thank you!

Clinical and Experimental Endocrinology

Conny Gysemans

Lut Overbergh

Hannelie Korf

Tatiana Takiishi

Sofie Robert

Tom Van Belle

João Paulo Da Cunha

Dana Cook

Jos Laureys

Martine Gillis

Marijke Viaene

Gabriele Sassi

Pieter Jan Martens

KU LEUVEN



University of Siena

Francesco Dotta

Guido Sebastiani

Francesca Mancarella

Guiliana Ventriglia

University of Florida

Mark Atkinson

Clive Wasserfall



Immune Tolerance Network

Karen Cerosaletti

Alice Wiedeman

Alice Long

Elisavet Serti

Gerald Nepom



Pieter Rottiers
Lothar Steidler
Joan Vermeiren
Silvia Caluwaerts

Sven Blomme
Karolien Van Huynegem

PRECIGEN
ACTOBIO

