Lactococcus lactis Producing Proinsulin and IL-10 increases Antigen Specific Regulatory T-cells in Monotherapy and in Combination with an anti-CD3 Monoclonal Antibody (teplizumab) in Newly Diagnosed T1D Patients

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PRECIGEN
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Immune Tolerance Network

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

Type 1 Diabetes (T1D)

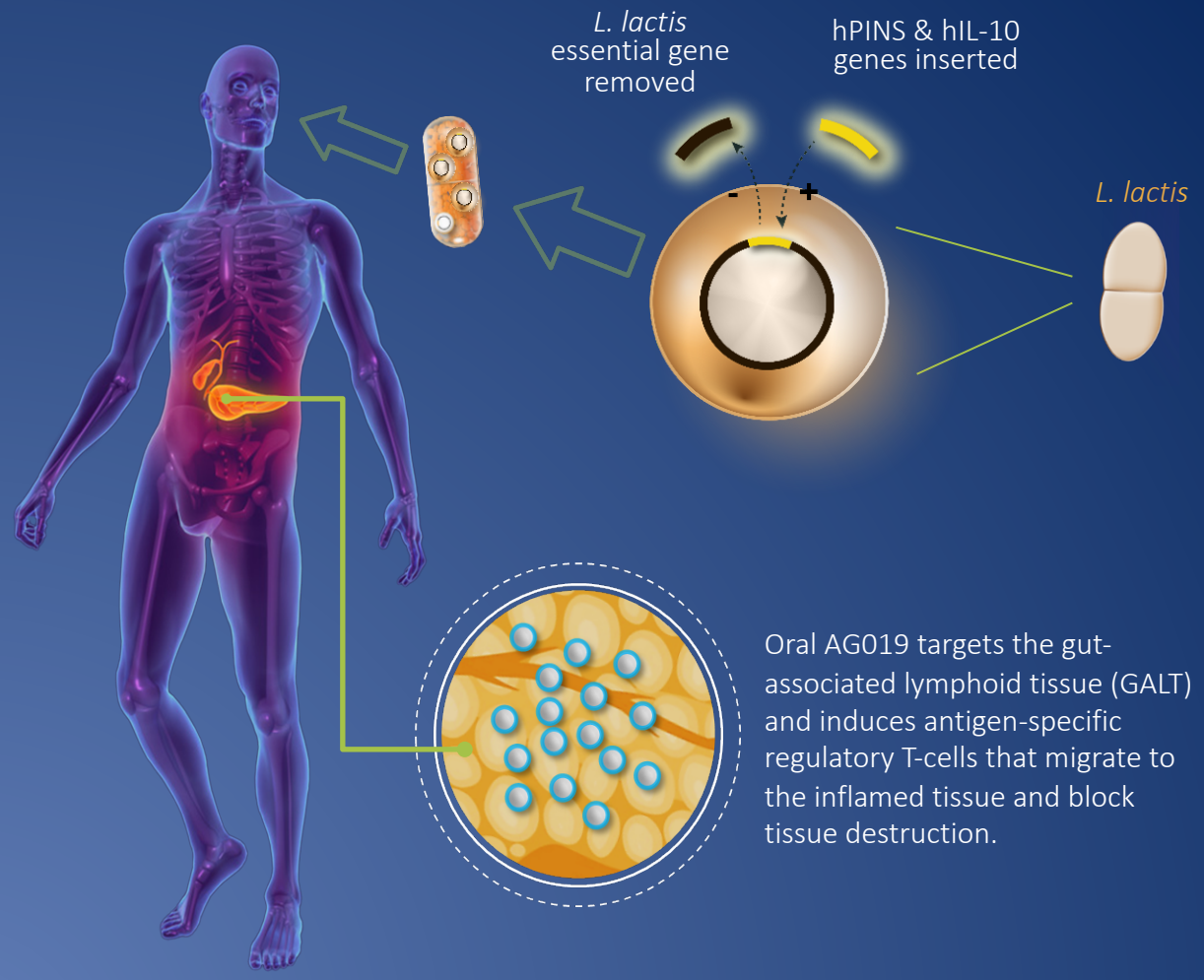
- Current treatment strategies focus on non-disease specific immune suppression with limited efficacy in recent onset T1D
- Antigen-specific immunotherapy represents a promising treatment modality to re-establish long-lasting immune tolerance
- **AG019 is a safe and convenient antigen-specific disease 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 new-onset T1D
- Increased levels of insulin-responsive Treg-cells accumulate and proliferate in pancreatic islet and are essential to maintain active immune-tolerance

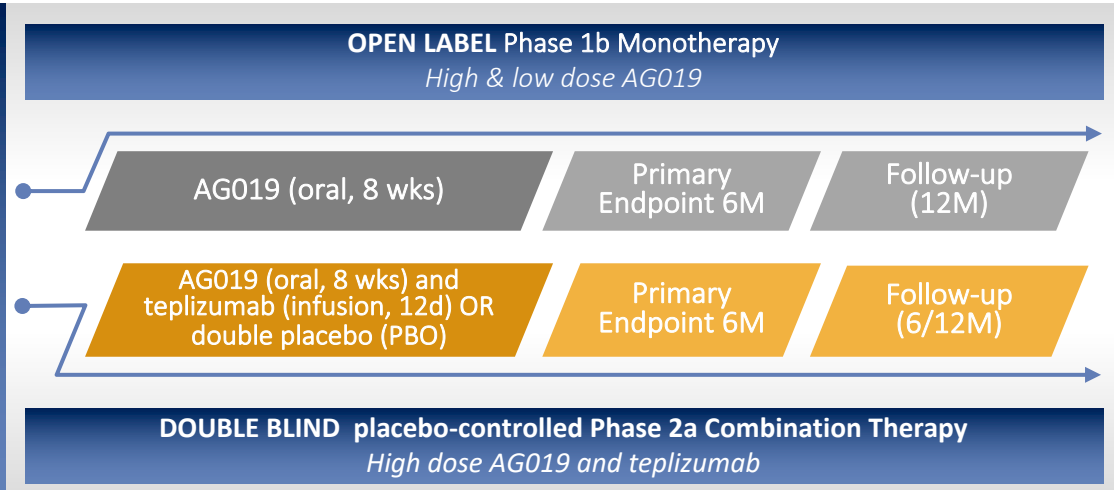
* Takiishi et al., 2012, JCI; Takiishi et al., 2017, Diabetes

AG019 is an oral capsule composed of ActoBiotics™ delivering hPINS and hIL-10



AG019 Phase 1b/2a in recent-onset T1D - Clinical Study Design and Status

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 (##)

AG019 Monotherapy (Open Label Phase 1b)

- Adults (18-42 years) and adolescents (12-17 years): **8 patients in single dose cohorts and 19 patients in repeat dose cohorts (27 patients)**
- Treatment completed
- **12M follow–up of all patients completed**

AG019/teplizumab Combination Therapy (Double-blind placebo-controlled Phase 2a)

- Adults (18-42 years): **10 patients in active group and 2 patients in PBO group (12 patients)**
- Adolescents (12-17 years): **5 patients in active group and 1 patient in PBO group (6 patients)**
- Treatment completed
- **6M follow-up of all adolescent patients completed and 12M follow-up of all adult patients completed**

(*) https://www.diabetes.org/a1c/diagnosis
(**) Recent-onset in this study defined as within 150 days of diagnosis. Other studies (e.g. teplizumab studies PROTECT and Protégé) use other definitions, e.g. 42 days..
(##) In a subset of patients, preproinsulin (PPI) and islet specific CD4+ and CD8+ T-cell responses were measured using a peptide activation assay and Class I MHC tetramers, respectively.

Study Participants

	Phase 1b Monotherapy		Phase 2a Combination Therapy	
	Adults	**Adolescents**	**Adults**	**Adolescents**
Number of patients# (all dosing cohorts)	**10**	**9**	**12**	**6**
Age Years	**24** (18-42)	**14** (12-17)	**26.5** (20-39)	**13.5** (12-15)
Time from diagnosis to treatment Days	**108.5** (35-136)	**118** (70-146)	**82** (58-148)	**132.5** (59-150)
Fasting C-peptide nmol/L	**0.26** (0.10-0.61)	**0.23** (0.17-0.47)	**0.17** (0.03-0.52)	**0.24** (0.16-0.33)
Peak C-peptide nmol/L	**0.96** (0.36-2.99)	**0.91** (0.40-1.37)	**0.79** (0.31-1.17)	**0.66** (0.28-1.13)
HbA1c %	**6.50*** (5.40-8.90)	**6.20** (5.20-7.40)	**6.65** (5.20-9.50)	**7.10** (5.10-11.00)

All values (except number of patients) median and range between brackets

data of 8 single dose patients of the Phase 1b Monotherapy not included

* No baseline HbA1c result available for one patient

Oral AG019 is safe and well-tolerated as monotherapy and combination therapy in recent-onset Type 1 Diabetes

	Phase 1b Monotherapy		Phase 2a Combination Therapy			
			Adults		Adolescents	
	Adults	Adolescents	Active	Placebo	Active	Placebo
Number of patients who completed dosing[#]	10	9	10	2	4 (5[a])	1
Serious Adverse Events (SAEs)	0	0	0	0	0	0
Total severe Treatment-Emergent Adverse Events (TEAEs)	0	0	8[b]	0	1[bb]	0
Total related TEAEs	4[c]	7[cc]	41[d]	22[dd]	1[ddd]	0
Total severe related TEAEs	0	0	2[e]	0	0	0

[#] 8 single dose patients of the Phase 1b monotherapy not included
[a] One patient received one morning dose of AG019 and was subsequently discontinued due to teplizumab infusion withholding criteria, for this patient only baseline through 4 days FUP data is available
[b] Lymphopenia (3), decreased lymphocyte count (1), elevated bilirubin (1), rash (1), diarrhea (1), and vomiting (1), [bb] Decreased lymphocyte count (1)
[c] Mainly diarrhea (3), [cc]Mainly diarrhea (1), and vomiting (1)
[d] Mainly vomiting (11), oropharyngeal pain (5), diarrhea (4), nausea (3), rash (3), and headache (2), [dd] Mainly headache (9), fatigue (3), and oropharyngeal pain (1), [ddd] Blood glucose decreased
[e] Diarrhea (1), and vomiting (1)
The TEAEs reported in the AG019/teplizumab combination cohorts are in line with the safety profile reported for teplizumab in its Investigator Brochure and no unexpected TEAEs were identified

- **AG019 treatment was well tolerated and safe as monotherapy or in combination with teplizumab infusions.**
- **No AG019 treatment discontinuation** due to TEAEs occurred.
- **No serious adverse events** were reported and the TEAEs reported were mostly of mild, sometimes of moderate severity.

Pharmacokinetic analysis demonstrated localized intestinal delivery of AG019 with no systemic exposure

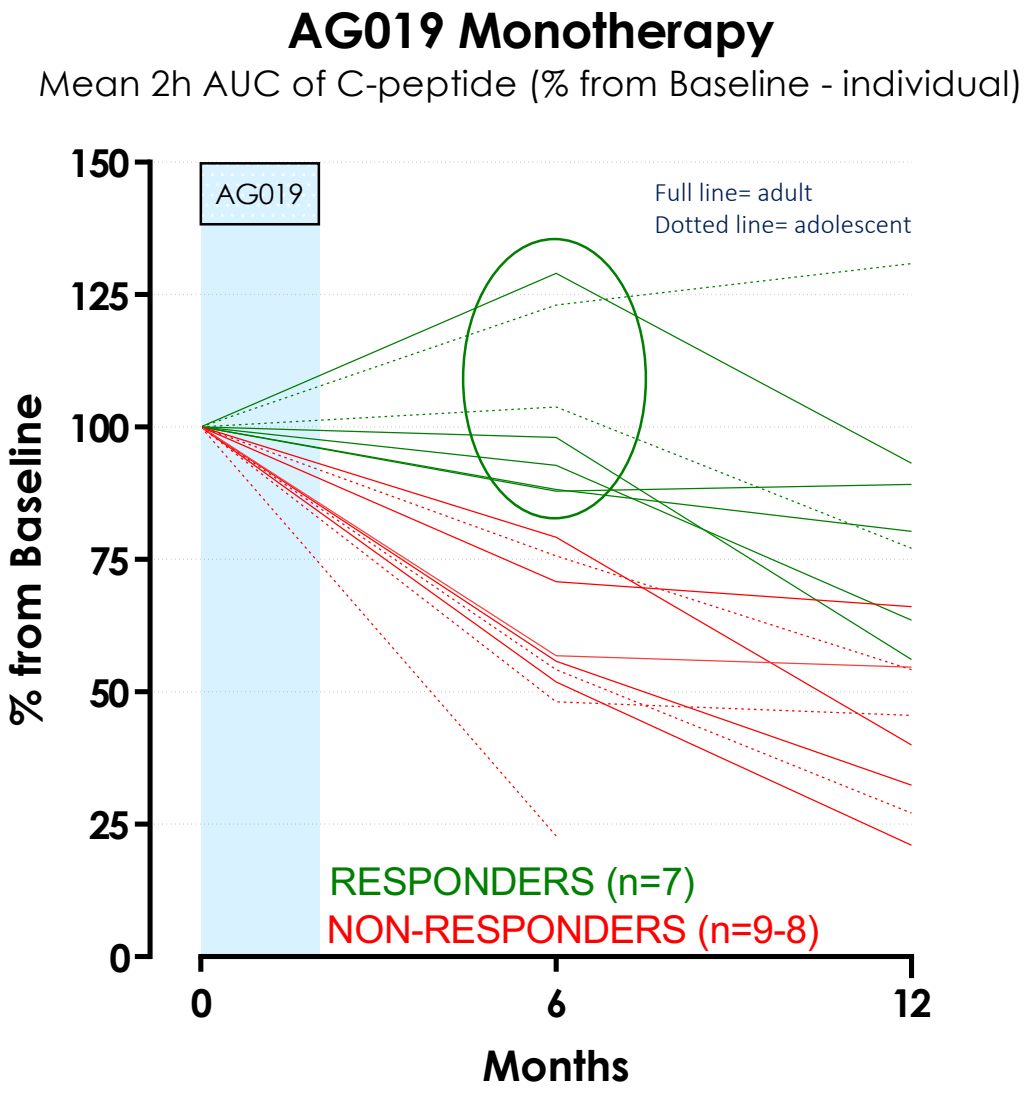
Safety

No systemic exposure of hPINS, hIL-10 (ELISA) and of AG019 bacteria (plating and Q-PCR) in blood of the patients which confirms the safety profile of AG019.

Localized Intestinal Delivery

Fecal exposure of AG019 bacteria (Q-PCR) in the majority of patients (79%). On day 64, clearance of the bacteria from the GI tract was demonstrated in most of the patients (84%), indicating no persistence (no colonization) of the bacteria.

Oral AG019 with a single 8-week treatment cycle resulted in encouraging C-peptide stabilization



Oral AG019 with single treatment cycle resulted in encouraging C-peptide stabilization/increase ('responders'*) during the first 6 months in:

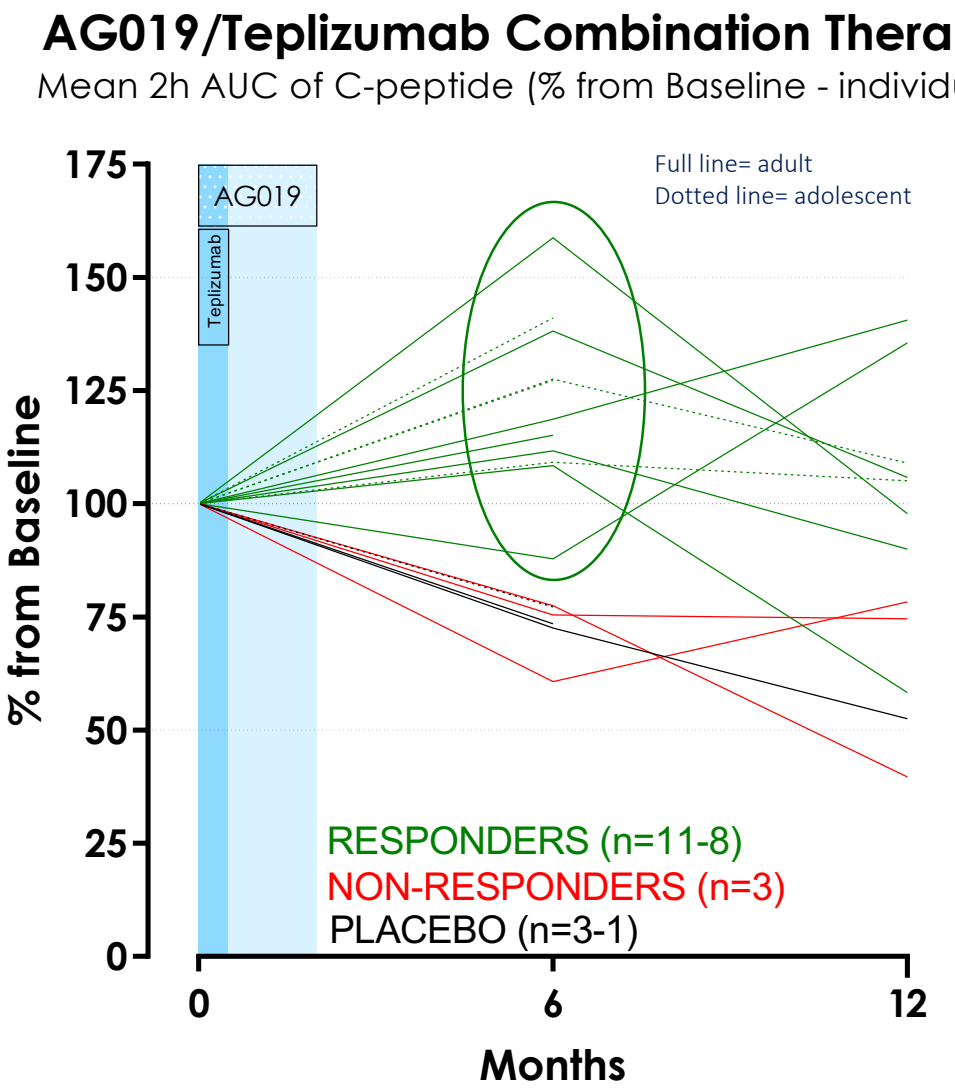
- 44% (7/16**) of all T1D patients, age 12-42 years (median value 98% of baseline)
- 56% (5/9**) of adult patients, age 18-42 years
- 29% (2/7**) of adolescent patients, age 12-17 years

The highest percentage of responders (58%, 7/12) was seen in patients 17 years and above (potential target population for monotherapy).**

* 'Responders' were designated if the coefficient of variance on C-peptide AUC was ≤ 9.7%, or if change from baseline was non-negative

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

Oral AG019 in combination with teplizumab resulted in encouraging C-peptide stabilization/increase



<u>A synergistic effect of oral AG019 treatment in combination with teplizumab</u> resulted in C-peptide stabilization/increase ('responders'*) during the first 6 months in:

- 79% (11/14**) of all T1D patients, age 12-40 years (median value 118% of baseline)
- 70% (7/10**) of adult patients, age 18-40 years
- 100% (4/4) of adolescent patients, age 12-17 years

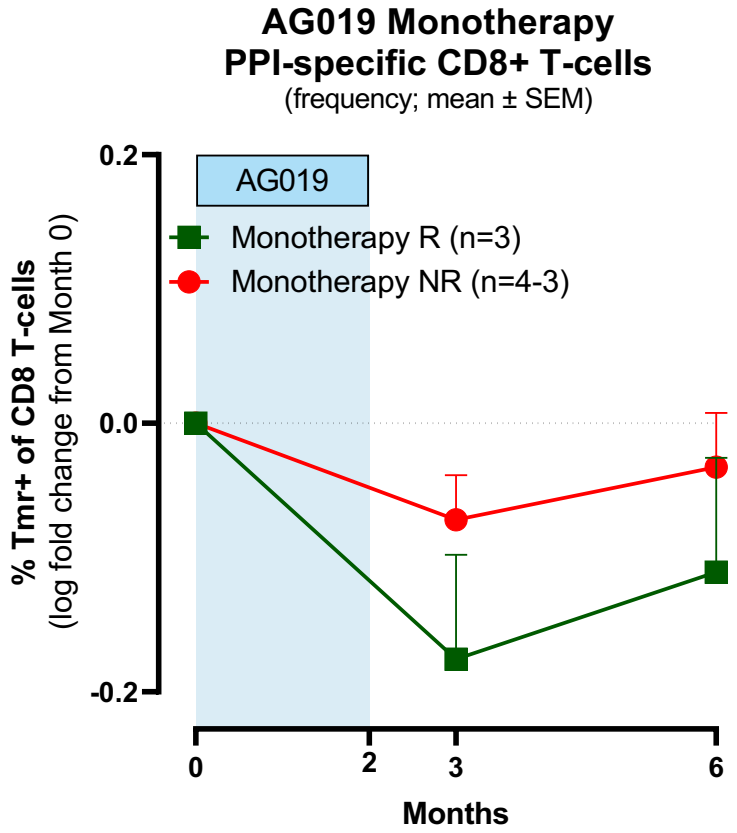
Higher C-peptide levels in AG019 combination therapy-treated patients as compared to placebo at 12 months.

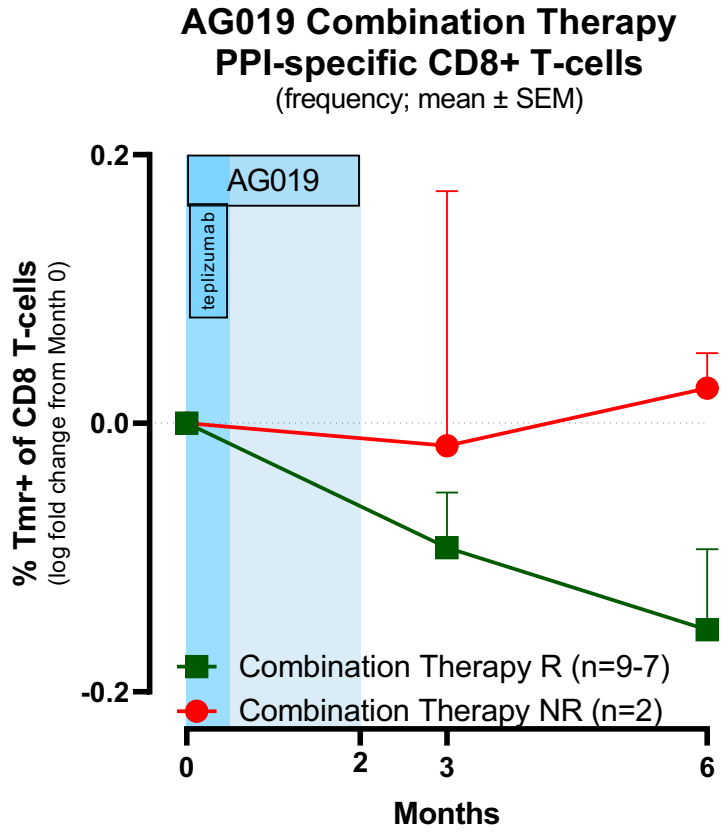
* 'Responders' were designated if the coefficient of variance on C-peptide AUC was ≤ 9.7%, or if change from baseline was non-negative

** PP population

Adolescents combination therapy at 12 months not yet fully available

The reduction in disease-specific CD8+ T-cells correlates with metabolic response

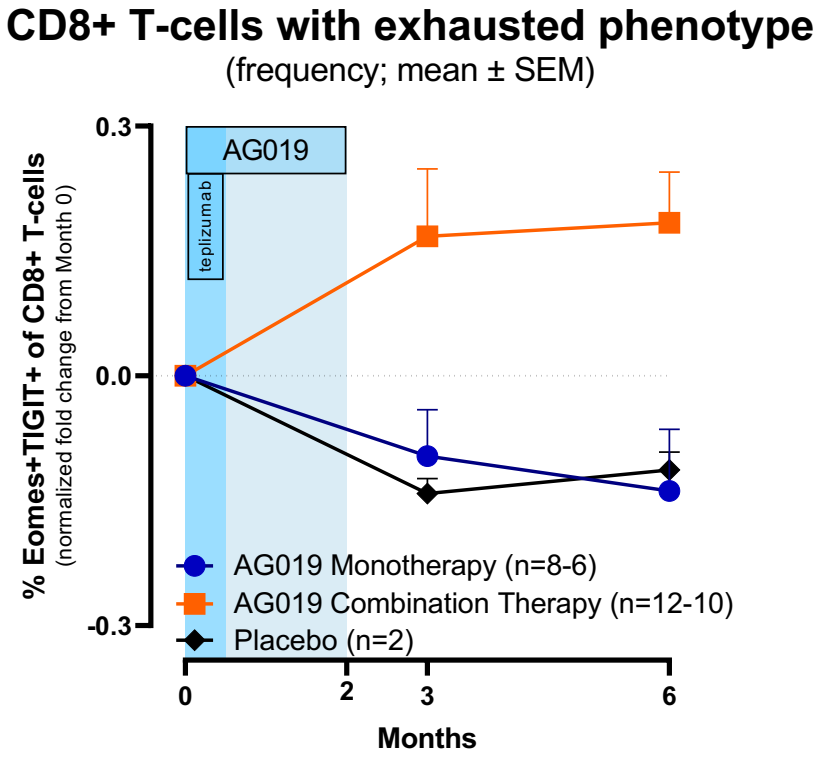


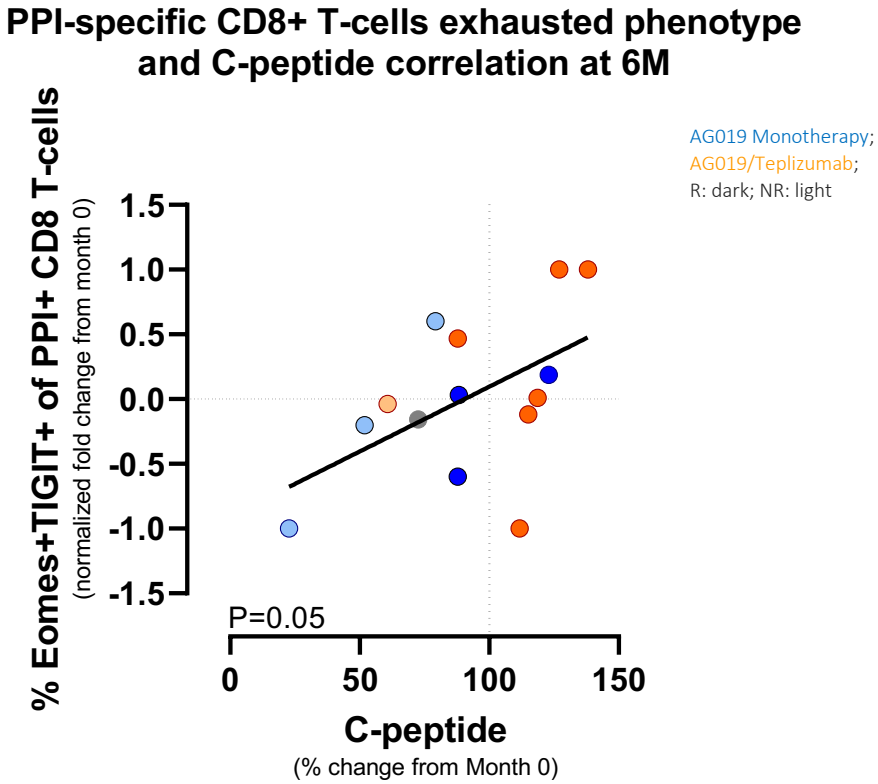


- The **decline in PPI-specific CD8+ T-cells is more substantial for responders** (R) as compared to non-responders (NR), both in AG019 monotherapy and in combination therapy.
- There is a trend towards a correlation between PPI-specific CD8+ T-cells reduction and C-peptide preservation at 6 months (not significant).

Analysis performed in collaboration with ITN/BRI (A. Long / A. Wiedeman)

The combination therapy (AG019 and teplizumab) increased the frequency of exhausted CD8+ T cells, which were associated with preservation of C-peptide

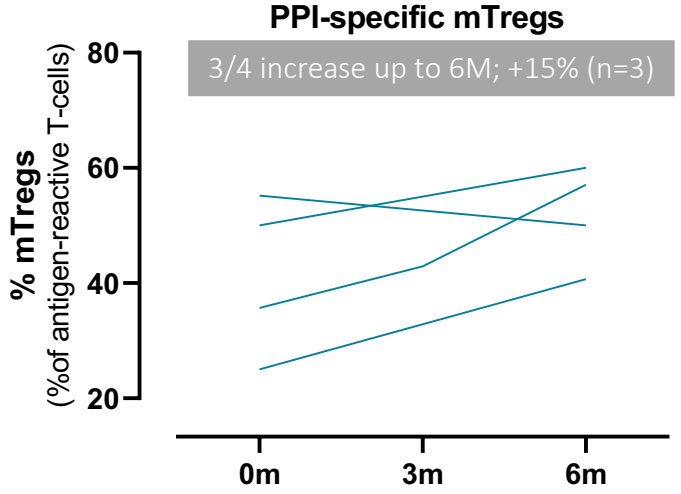


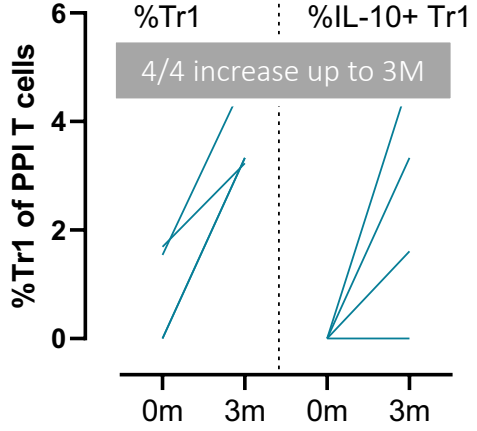


- AG019/Teplizumab combination therapy is associated with an **expansion of total CD8+ T-cells with an exhausted phenotype**.
- At 6 months the frequency of PPI-specific CD8+ T-cells with an exhaustion phenotype is correlated with C-peptide.

There is a trend for increased frequency of antigen-specific Tregs in adult patients treated with AG019

AG019 Monotherapy

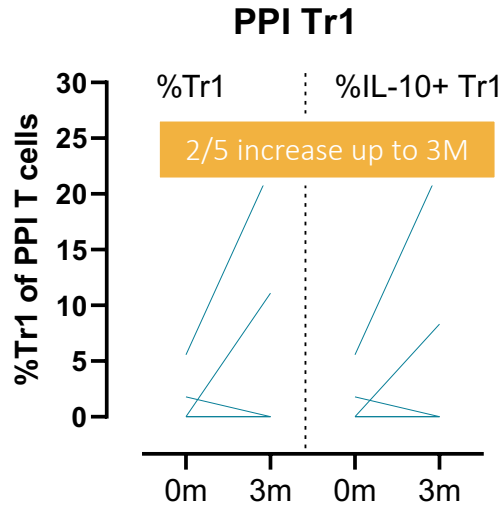




Analysis performed in collaboration with ITN/BRI (K.Cerosaletti).

- In AG019 monotherapy, there is a trend for **increased frequency of antigen-specific memory Tregs** in adult patients (6 months analysis in combination therapy ongoing).
- In AG019 monotherapy and AG019/teplizumab combination therapy there is a trend for **increased frequency of antigen specific CD4+ Tr1 cells*** in adults.

AG019 Combination Therapy



PPI specific T cells (CD4+ CD154+CD69+) were analysed for CD226+LAG3+CD49b+IL-10+; PPI specific Tregs were CD4+CD25hiCD127lowFOXP3+Helios+.

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

Summary and Conclusions

AG019, *L. lactis* producing IL-10 and hPINS, can be administered safely, either as monotherapy or in combination with teplizumab.

⇨ **Opportunity for chronic treatment**

The stabilization of C-peptide in monotherapy with an 8-week treatment cycle of AG019 is encouraging and a synergistic effect was observed between AG019 and teplizumab.

⇨ **Opportunity for sustained treatment effect following prolonged AG019 treatment**

There was a trend towards an increase in islet- and PPI-reactive memory Tregs and Tr1 cells and a decline in the frequency of PPI-specific CD8+ T cells with AG019 therapy in adults.

⇨ **Ability to establish antigen-specific immune modulation opens opportunity to broaden therapeutic applications towards other autoimmune diseases**

AG019, as monotherapy and in combination 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, a clinical trial assessing the efficacy of prolonged treatment of oral AG019 planned.