Il /la dr./sa Paolo Fiorina dichiara di aver ricevuto negli ultimi due anni compensi o finanziamenti dalle seguenti Aziende Farmaceutiche e/o Diagnostiche:

- Menarini
- Sanofi
- Enthera
- Ascesia

Dichiara altresì il proprio impegno ad astenersi, nell'ambito dell'evento, dal nominare, in qualsivoglia modo o forma, aziende farmaceutiche e/o denominazione commerciale e di non fare pubblicità di qualsiasi tipo relativamente a specifici prodotti di interesse sanitario (farmaci, strumenti, dispositivi medico-chirurgici, ecc.).

Immunoterapia

Paolo Fiorina, MD PhD

International Center for T1D
Romeo ed Enrica Invernizzi
Pediatric Clinical and Research Center
Università degli Studi di Milano, Sacco Hospital

…cosa vi siete persi…

Immunoterapia

1. Introduction
2. Cell-depletion
3. Antigen-specific therapies
4. Anti-inflammatory therapies
5. Cell-therapy
6. Stem cells therapy
7. ImmunoStem
8. Conclusions

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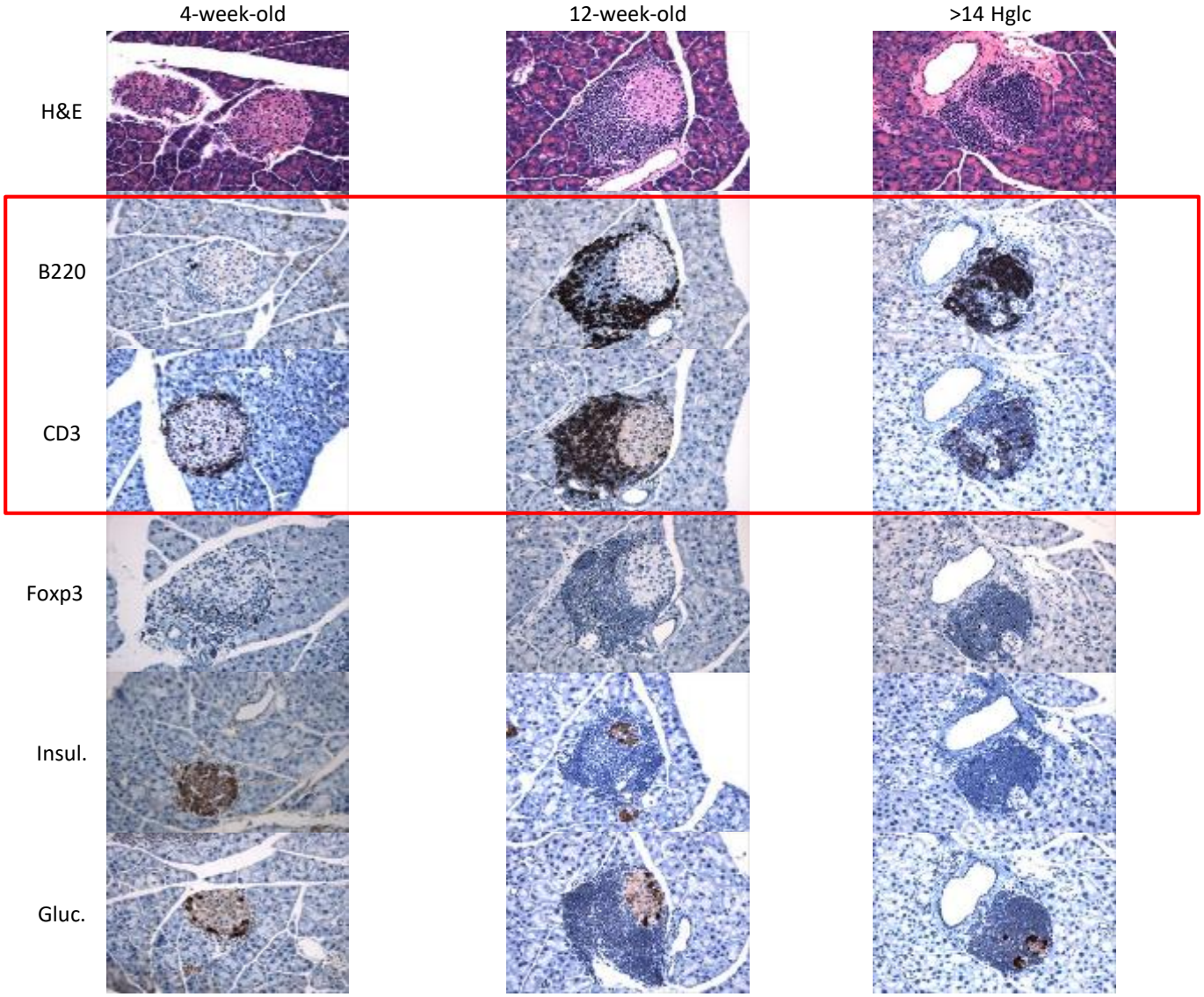
Rationale for Immunotherapy

In order to be considered for immunotherapy
a disorder must be immune-related

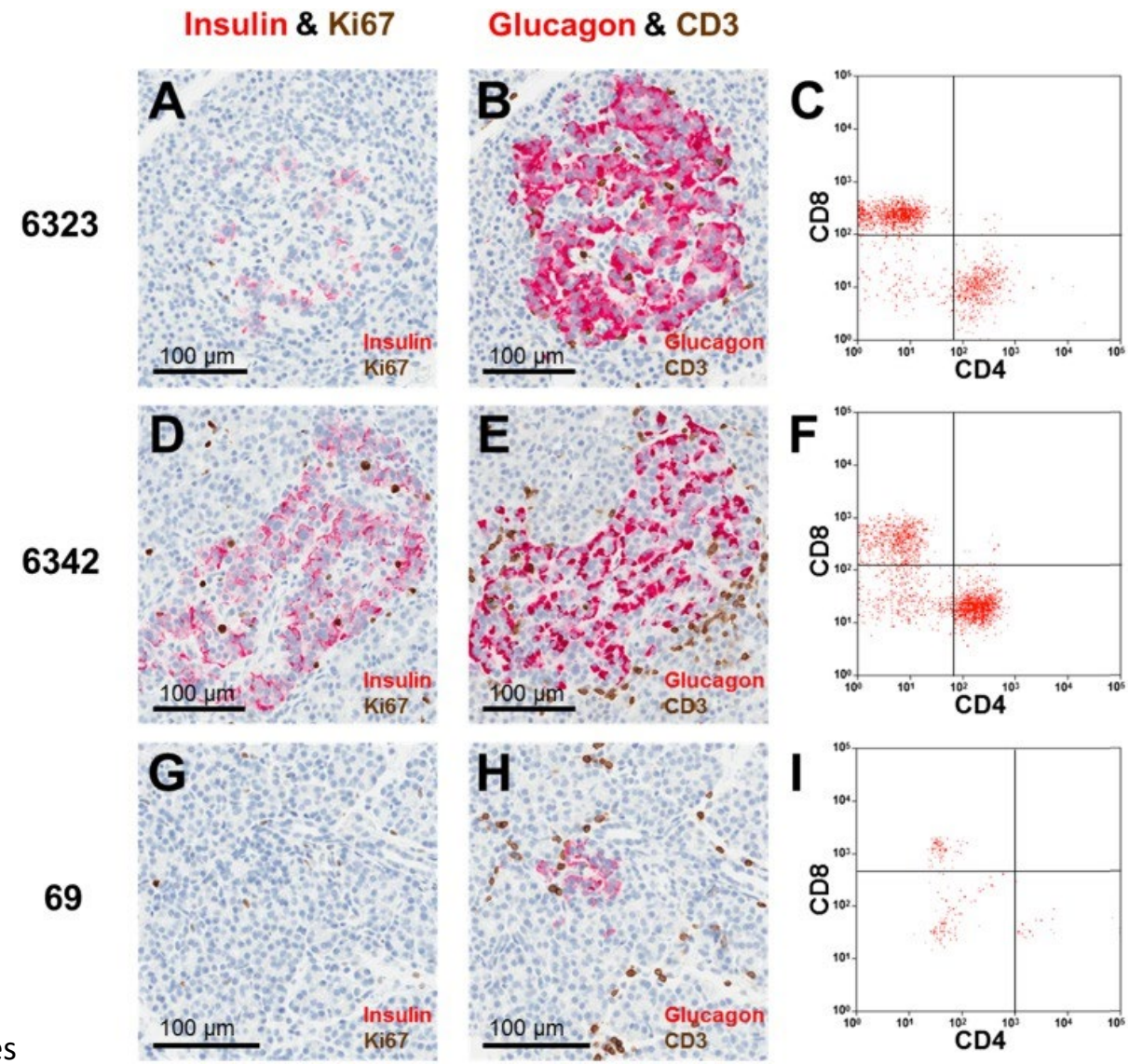
- T1D is characterized by autoimmune destruction of β-cells
- Anti-insulin/islet autoantibodies can be detected
- T lymphocytes response against insular proteins is evident

NOD mice with T1D showed pancreatic islet infiltrate



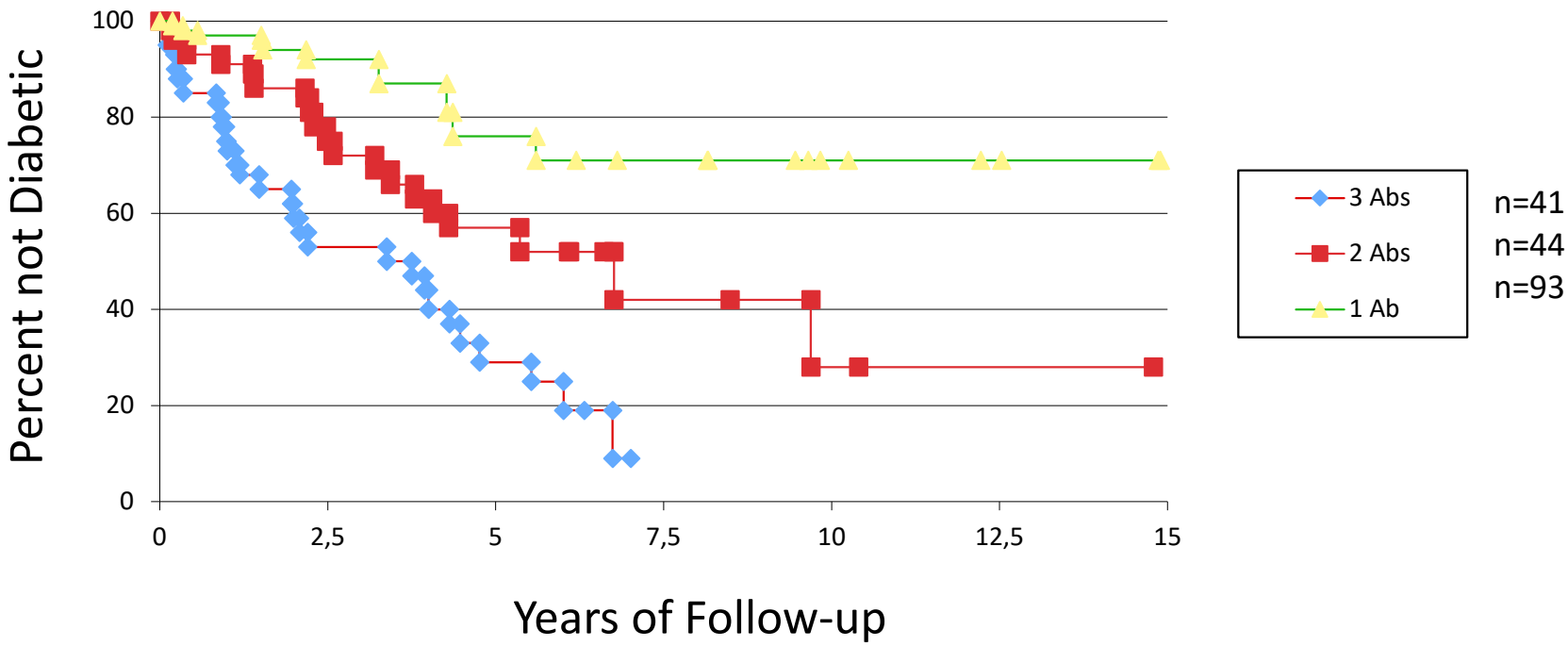
Fiorina et al. Diabetes 2008

Patients with T1D showed pancreatic islet infiltrate



Michels et al. Diabetes

Progression to Diabetes vs. Number of Autoantibodies (GAD, ICA512, Insulin)



Verge et al. Diabetes 1996

Experimental therapies for T1D

- Pancreas transplant
    - Beta cell replacement
    - Major surgery/Immunosupression
    - T1D complications prevention
- Islet transplant
    - Beta cell replacement
    - 20% of residual function after 3 years/Immunosuppression
    - T1D complications prevention
- Immunotherapy

- To deplete autoreactive clones
- To enhance immunoregulation
- To reshape the immune system
- Many side effects

Fiorina et al. Diabetes 2001
Fiorina et al. Diabetes Care 2003
Fiorina P et al. AJT 2011
D'Addio F/Fiorina P et al. Diabetes 2014
Ben Nasr M/Fiorina P et al. Pharm Res 2015

Big hopes for NOD mice on the horizon

Treatments	1st author; Journal; year of publication	Short-term reversal	Long-term reversal	Days of hyperglc before treatment
1. Anti-CD3 mAb 145 2C11	Chatenoud L, PNAS, 1994	100%	64-80%	7 days
2. Ad-hTGF-β1	Luo X, Transplantation, 2005	100%	75%	1 day
3. EGF+ gastrin	Suarez-Pinzon WL; Diabetes, 2005	100%	83%	3-6 days
4. LSF+EX-4	Yang Z, Biochem Biophys Res Commun, 2006	100%	83%	5–7 days
4. Microspheres	Phillips B, Diabetes, 2008	100%	46%	10-18 days
5. B cell depletion (Anti-CD20 and CD22)	Wen L, JCI, 2007; Fiorina P, Diabetes,2008	100%	70%	3 days
6. MSCs	Fiorina P, J Immunology, 2009	100%	30%	3 days
7. HAAT+G-CSF	Ma H, Diabetologia, 2010	100%	50%	0
8. DEF-GAD65	Lin M, Eur J Immunol, 2010	100%	80%	0
9. mATG+ CTLA-Ig	Vergani A/Fiorina P, Diabetes, 2010	100%	100%	2 days

Abbreviations. Ad-hTGF-β1 (adenovirus (Ad) vector encoding active form of human TGF-β1); EGF (epidermal growth factor); LSF (Lisofylline); EX-4 (exendin-4); MSCs (mesenchymal stem cells); hAAT (Human α1-antitrypsin); DEF-GAD65 (a silent monoclonal glutamic acid decarboxylase 65 $GAD65_{217-230}$-specific CD4T-regulatory population upon its activation through a soluble dimeric I-$A\alpha\beta^{g7}$/Fcγ2a/$GAD65_{217-230}$ chimera); mATG (murine anti-thymoglobulin); CTLA4-Ig (Fusion protein made by extracellular domain of CTLA4 and Fc portion of IgG).

Ben Nasr M et al. Pharm Res 2014

But not really for T1D individuals

Treatment(s)	Lead author, Journal, Year of publication	Number of patients	Insulin-independ at 1 yr (%)	C-peptide at 1 yr (nmol/l)	Weeks from diagnosis
Intensive insulin therapy	Shah C; N Engl J Med; 1989	14	0	0.5	2
Vitamin E	Pozzilli P; European J Endocrinol; 1997	84	0	0.2	<4
Nicotinamide	Visalli N; Diab Metab Res Rev; 1999	74	0	0.2	<4
Oral insulin	Chaillous L; Lancet; 2000	131	0	0.1	2
DiaPep277	Raz I; Lancet; 2001	35	0	0.2	<24
DiaPep277 phase3	Raz I; Diabetes Care; 2014	160	0	0.3	16
hOKT3gammal (Ala-Ala)	Herold KC; N Engl J Med; 2002	24	0	0.2	<6
Diazoxide	Örtqvist E; Diabetes Care; 2004	56	0	0.2	1
ATG	Saudek F; Review of Diabetic Studies; 2004	11	18	0.2	<4
Nicotinamide + vitamin E	Crino' A; Eur J Endocrinol; 2004	64	0	0.2	<4
Nicotinamide + intensive insulin therapy	Crino' A; J Pediatr Endocrinol Metab; 2005	25	0	0.1	<4
ChAglyCD3 or otelixizumab	Keymeulen B; N Engl J Med; 2005	80	<5	0.5	3

Ben Nasr M et al. Pharm Res 2014

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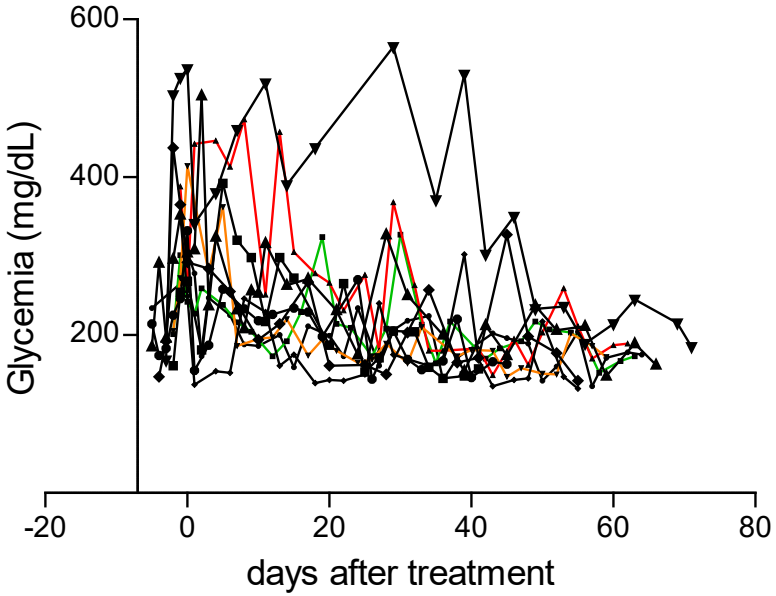
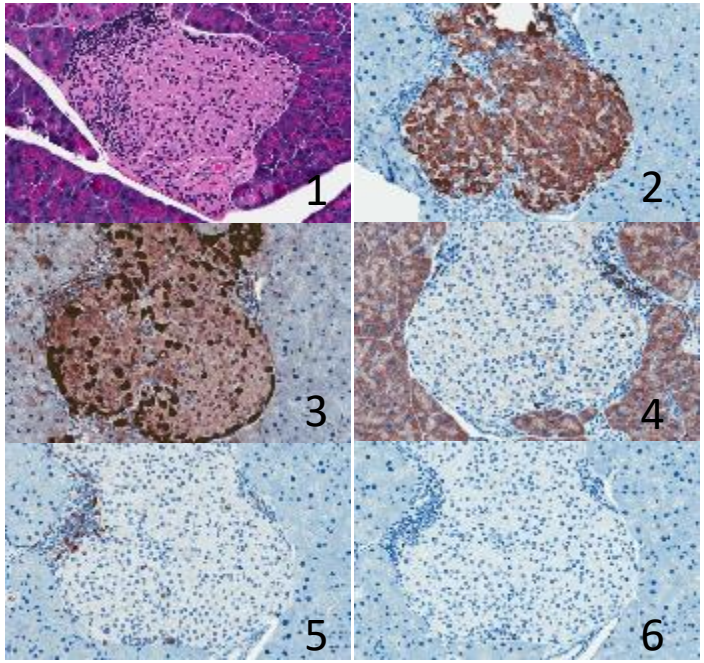
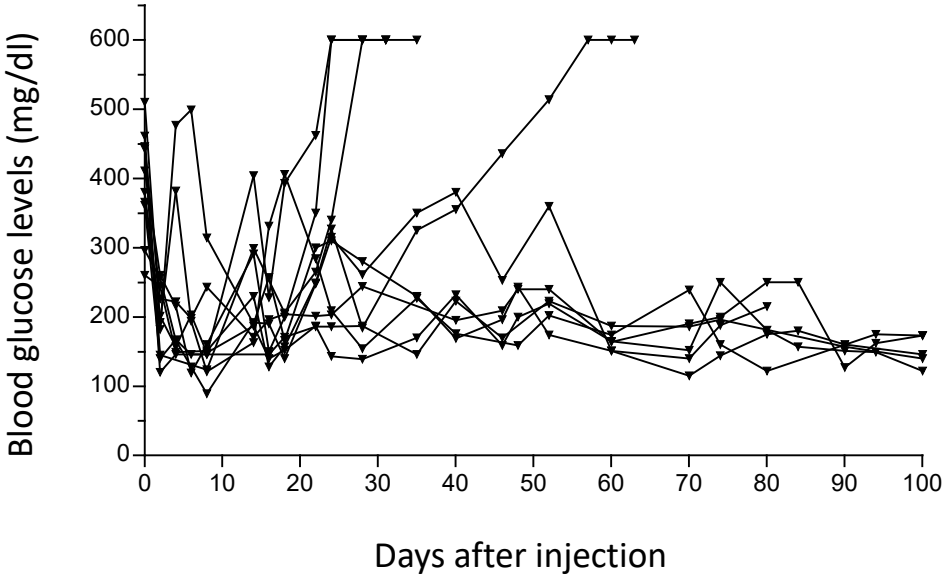
T cell depletion (ATG) cures T1D in NOD mice





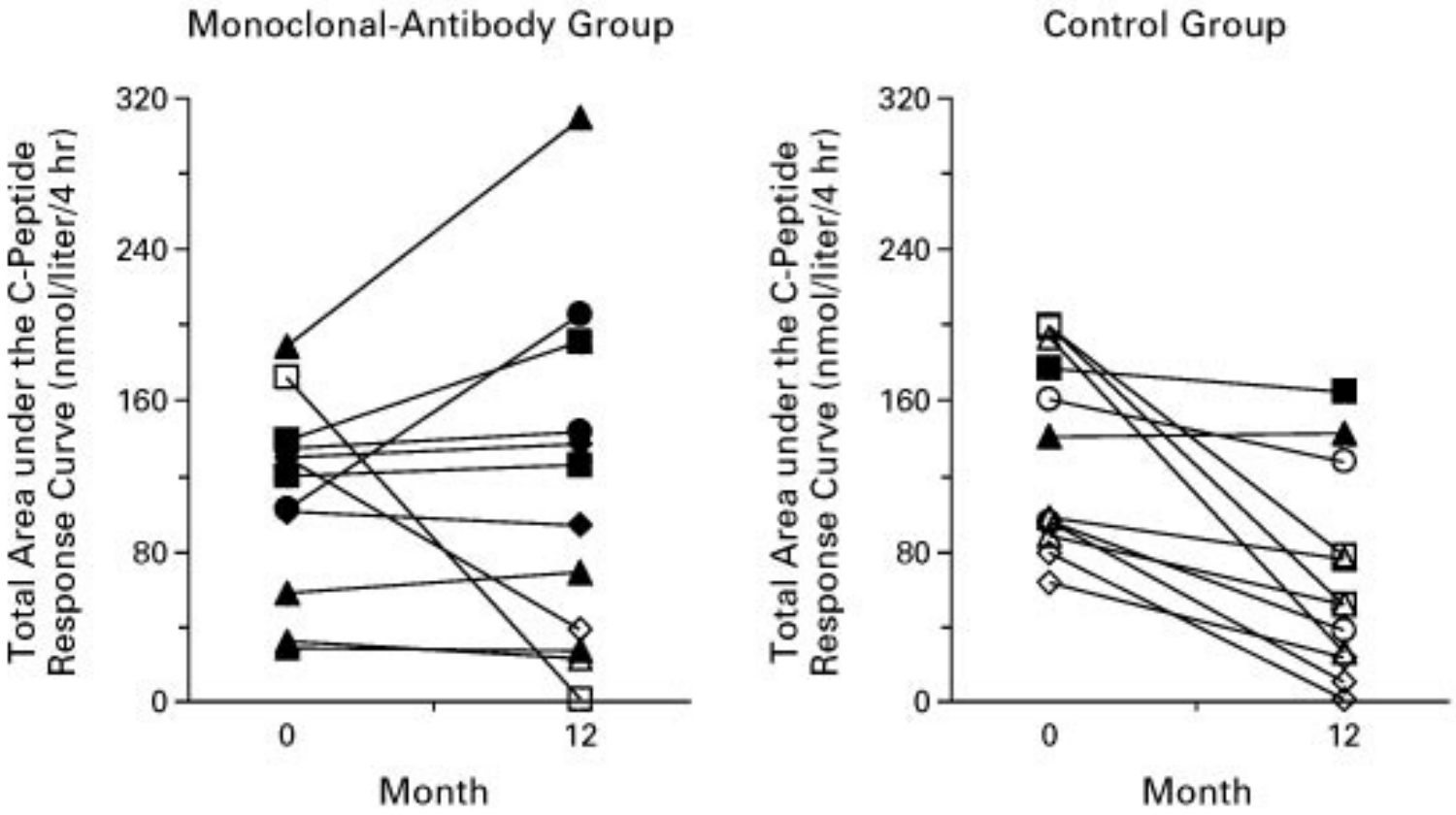
Vergani A/ Fiorina P et al. Diabetes 2010

B cell depletion (anti-CD22) cures T1D in NOD mice



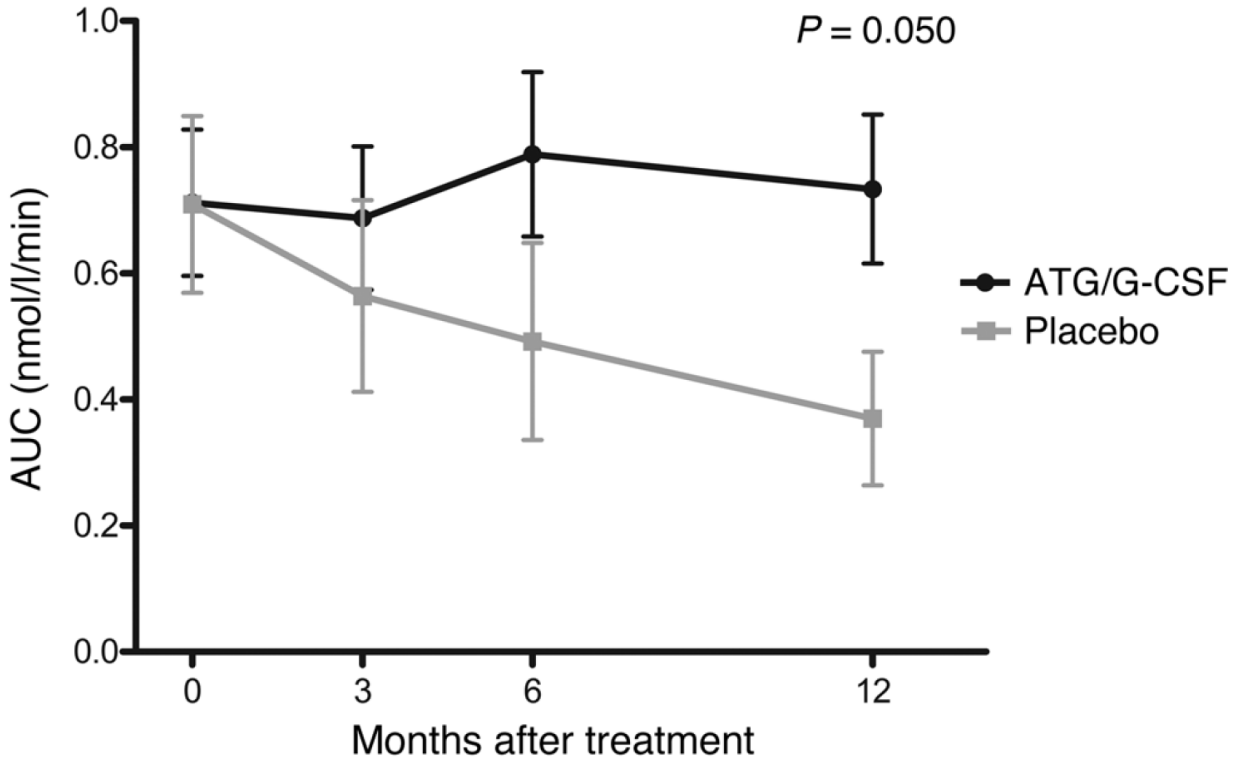
Fiorina P et al. Diabetes 2008

T cell depletion (hOKT3) preserves C-peptide in T1D pts



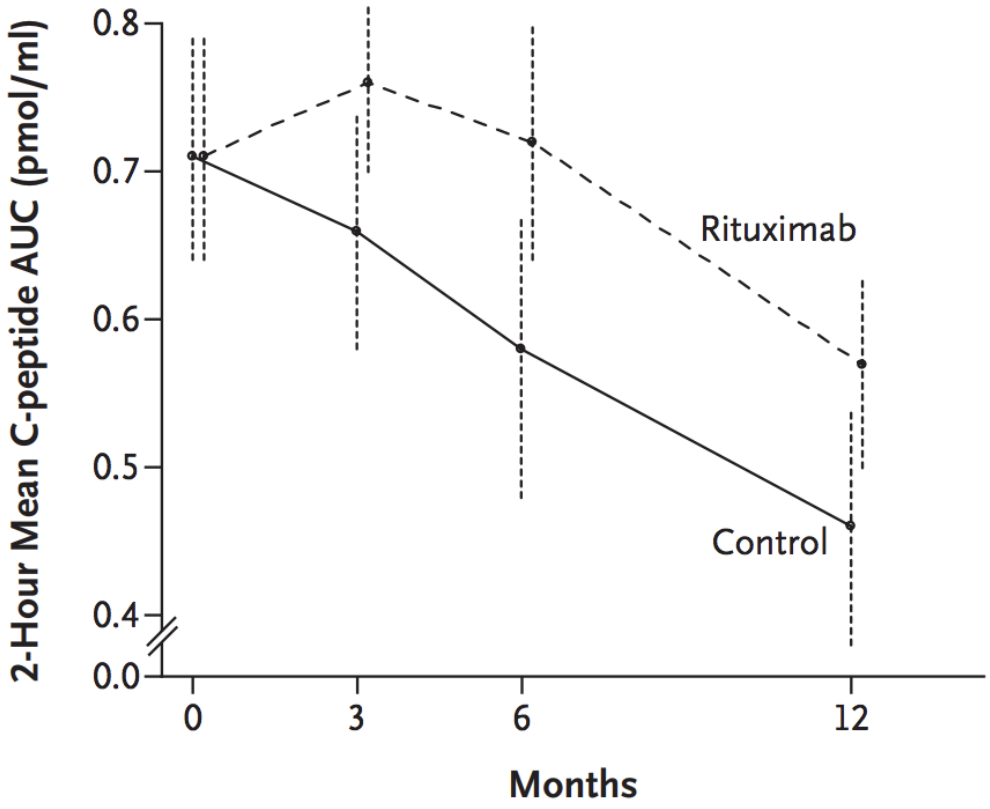
Herold K et al. NEJM 2002

T cell depletion (ATG+CSF) preserves C-peptide in T1D pts



Haller M et al. JCI 2015

B cell depletion (Rituximab) preserves C-peptide in T1D pts



Peskovitz M et al. NEJM 2009

Cell depletion

References	Patients n.	Treatment	Outcomes	C-peptide (AUC-nmol/l/min)
Herold et al. NEJM 2002	12	**hOKT3** 14-day of intravenous hOKT3γ1 antibody	Preserve C-peptide	Treated: 1.28 to 1.27 Control: 1.48 to 0.74
Keymeulen et al. NEJM 2005	80	**Anti-CD3** 6 doses of Otelixizumab	Preserve C-peptide	Treated: 0.85 to 0.80 Control: 0.95 to 0.70
Herold et al. Clin Immunol 2009	10	**Teplizumab** Single course anti-CD3 mAb	Preserve C-peptide	Treated: 0.88 to 0.89 Control: 0.41 to 0.19
Pescovitz et al. NEJM 2009	126	**Rituximab** 4 Rituximab intravenous infusions were given within 22 days	Preserve C-peptide	Treated: 0.75 to 0.59 Control: 0.74 to 0.47
Sherry et al. Lancet 2011	763	**Protégé** 14 daily infusions of Teplizumab	Preserve C-peptide 5% insulin independence	Treated (Δ): -0.06 Control (Δ): -0.14
Herold et al. Diabetes 2013	52	**Teplizumab** 14 doses	Preserve C-peptide	Treated: 0.72 to 0.44 Control: 0.67 to 0.21
Ambery et al. Diabetic Med 2014	179	**Defend-2** 8 doses Otelixizumab iv	Ineffective	Ineffective
Haller et al. J Clin Invest 2015	25	**ATG+GCSF** Iv ATG (1 dose) and sc G-CSF (6 doses)	Preserve C-peptide	Treated: 0.71 to 0.74 Control: 0.71 to 0.43
Gitelman et al. Diabetologia 2016	58	**ATG** Single injection of ATG	Ineffective	Ineffective

Cell depletion - Summary

- T/B cell depletion was promising
- Somehow disappointing clinical results
- Only the achievement of insulin independence can justify the use of immunosuppression
- Adverse effects observed

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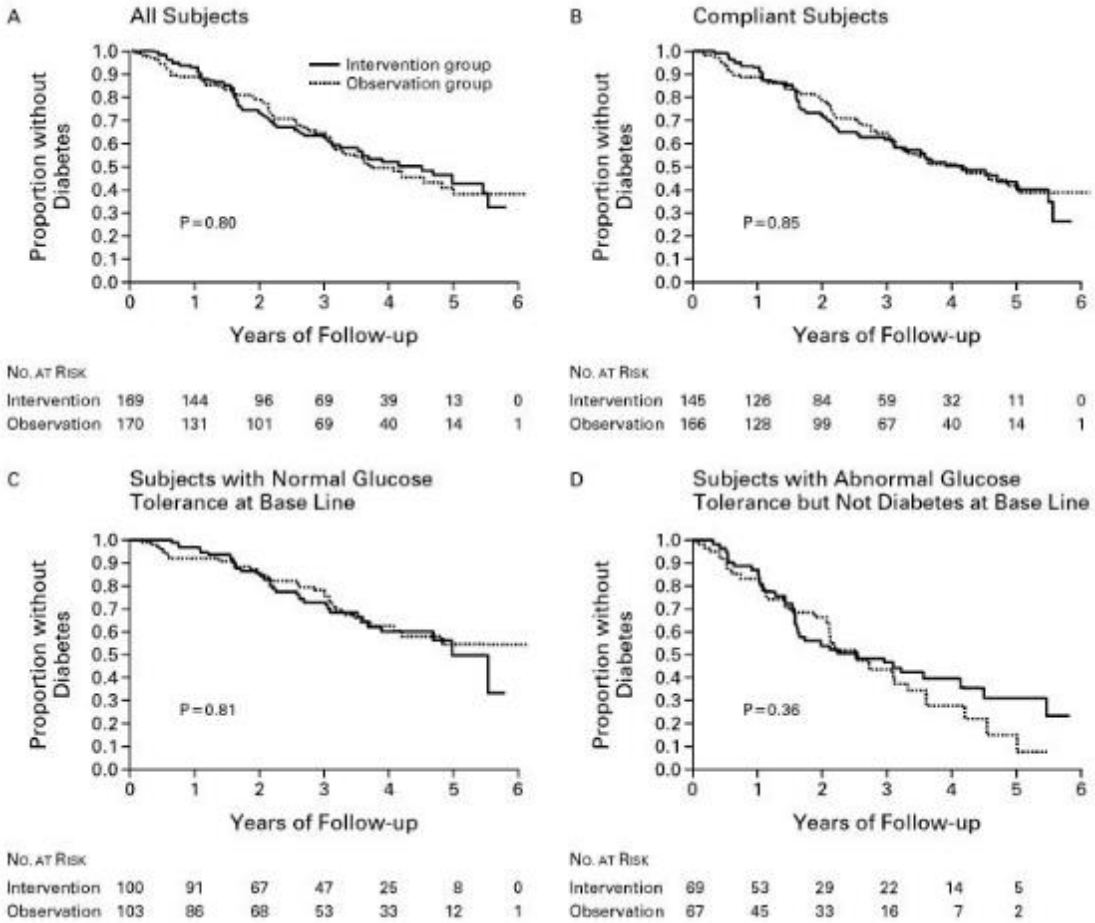
Antigen-specific therapies

- Induction of immunological tolerance
- Insulin administration prevented T1D in NOD mice

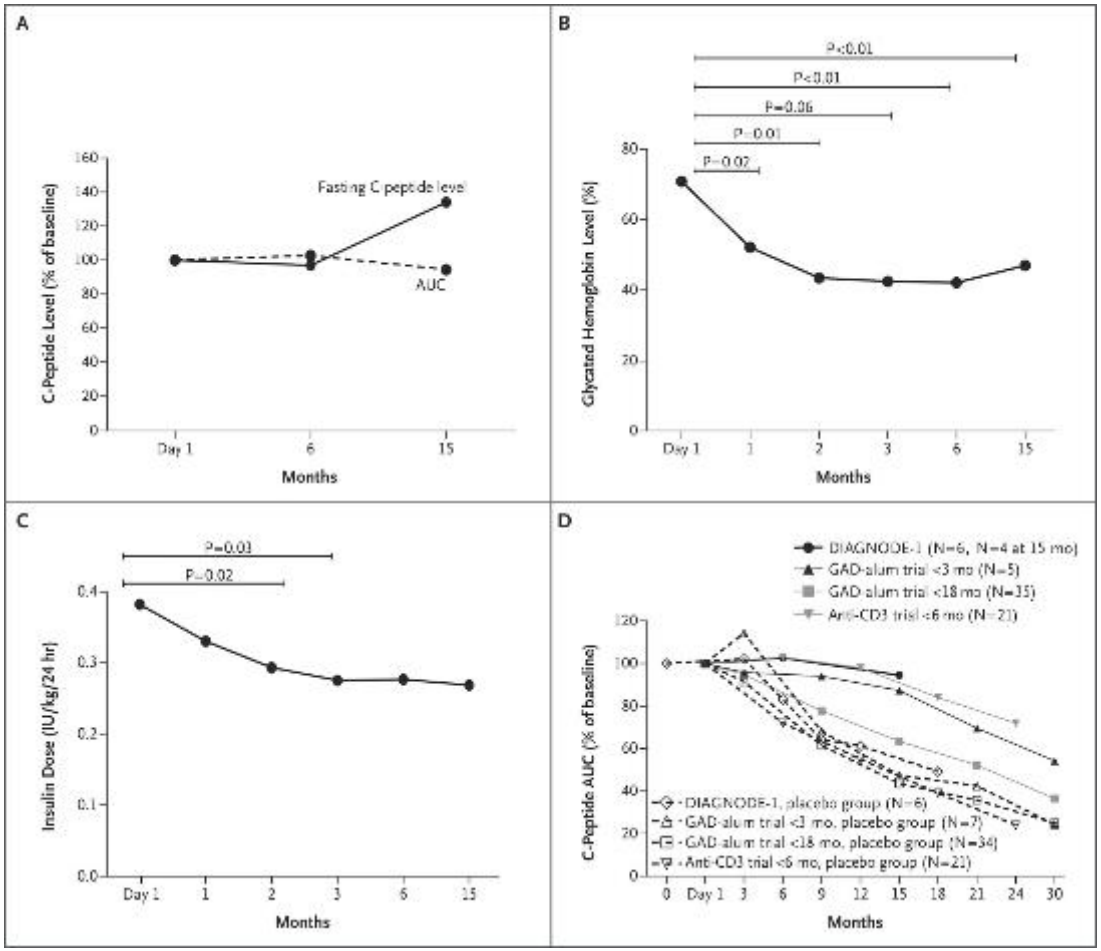
Aspord and Thivolet, Clin Exp Immunol 2002,
Ravanan et al. Clin Exp Immunol 2007

DPT-1 trial:
T1D patients treated with oral and parental insulin



DPT-1, NEJM 2002

GAD-Alum [Diamyd] 4 ug into Lymph Nodes + Vitamin D (2000 UI die) stabilizes C-peptide



Ludvigsson J et al. NEJM 2017

Antigen-specific therapy

References	Patients n.	Treatment	Outcomes	C-peptide (AUC-nmol/l/min)
NEJM 2002	339	**DPT-1** Daily oral and parenteral insulin administration until diagnosis	Ineffective	Ineffective
Wherrett et al. Lancet 2011	126	**GAD** Glutamic acid decarboxylase (GAD) has been injected 3 times	Ineffective	Ineffective
Linköping University et al. NEJM 2017	6	**DIAGNODE-1** GAD Alum+Vit D	Preserve C-peptide	From 0.53 to 0.55

Antigen-specific therapy - Summary

- This approach mat affect the early phase of T1D onset
- Lack of adverse effects makes this strategy attractive

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Anti-inflammatory therapies

- Inflammation predispose autoimmune reaction in T1D

- Preclinical evidence: administration of anti-infiammatory therapies prevent T1D in NOD mice

Preclinical evidences

Rapid Publications

1,25-Dihydroxyvitamin D_3 Prevents Insulitis in NOD Mice

CHANTAL MATHIEU, JOS LAUREYS, HALINA SOBIS, MICHEL VANDEPUTTE, MARK WAER, AND ROGER BOUILLON

The active form of vitamin D, $1,25(OH)_2D_3$, can prevent various forms of experimentally induced autoimmune disorders. The aim of this study was to confirm these findings in NOD mice that spontaneously develop an autoimmune type of diabetes mellitus. Therefore, the effect of a long-term $1,25(OH)_2D_3$ treatment on the incidence of insulitis, the histological lesion preceding diabetes, was studied. Forty-three NOD mice were treated with $1,25(OH)_2D_3$ (5 μg/kg) i.p. every other day from age 21 days on, when no insulitis was present yet. At day 100, 16 control mice receiving the treatment vehicle (arachis oil) had an incidence of insulitis of 75%, whereas only 41% of the $1,25(OH)_2D_3$-treated animals developed insulitis ($P < 0.025$). Calcemia, determined 24 h after the last $1,25(OH)_2D_3$ injection was 2.5 ± 0.3 mM, which was higher than in control animals (2.3 ± 0.1 mM), but was well tolerated. Cellular immunity, as assessed with the mixed lymphocyte reaction performed at day 100, was not impaired significantly. This study demonstrates that long-term treatment with high doses of $1,25(OH)_2D_3$ is able to decrease the incidence of insulitis in spontaneous autoimmune diabetes without major side effects.
***Diabetes* 41:1491–95, 1992**

From the Laboratory for Experimental Medicine and Endocrinology and the Laboratory of Immunopathology, Rega Institute, Catholic University of Leuven, Belgium.
Address reprint requests to Roger Bouillon, Legendo, U.Z. Gasthuisberg, Herestraat 49, 3000 Leuven, Belgium.
Received for publication 15 July 1992 and accepted in revised form 27 August 1992.
$1,25(OH)_2D_3$, 1,25-dihydroxyvitamin D_3; type I diabetes, insulin-dependent diabetes mellitus; FCS, fetal calf serum; SI, stimulation index; IL-2, interleukin 2; IFN-γ, interferon-γ; MLR, mixed lymphocyte reaction; cpm, counts per minute; NS, no significance.

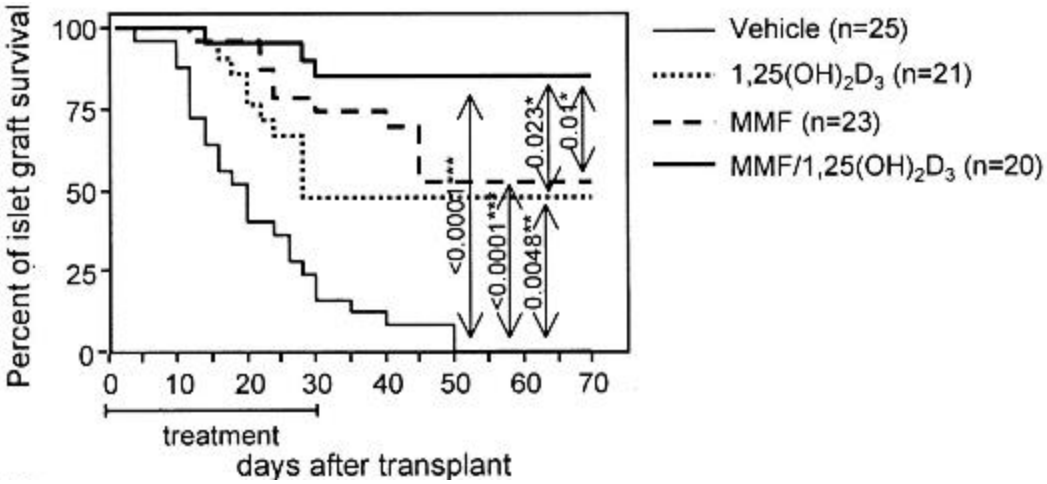
Receptors for the active form of vitamin D, $1,25(OH)_2D_3$, have been discovered in cells of the immune system (monocytes, activated lymphocytes), and $1,25(OH)_2D_3$ has been shown to exert potent immunosuppressive activities in vitro (1–4). It also has been demonstrated in vivo that a short-term treatment with $1,25(OH)_2D_3$ can prevent the occurrence of experimentally induced autoimmune diseases, such as experimental autoimmune encephalitis or experimental autoimmune thyroiditis in mice and a lupus-like syndrome in rats (5–7), when the agent is administered at the time of disease induction. However, few data are available on the efficacy of vitamin D or its analogues in spontaneously occurring autoimmune diseases (8–9). Moreover, in these models, long-term treatment with $1,25(OH)_2D_3$ is necessary, as the exact time of disease onset is unknown, thus increasing the risk for side effects such as hypercalcemia.

NOD mice spontaneously develop diabetes and are a good model for human juvenile type I diabetes (10–12). Clinical disease is preceeded by insulitis, which is the basic histological lesion in the islets of Langerhans of the pancreas. Islets are invaded mainly by $CD4^+$ and $CD8^+$ T-cells and also by monocytes (12,13). The autoantigen remains unknown, and the cell type responsible for the initiation of the autoimmune process is still controversial, but monocytes and $CD4^+$ cells seem to play a major role (12,14,15). The onset of insulitis is observed at 20–40 days of age. In this study, we show that chronic treatment with $1,25(OH)_2D_3$ can prevent the occurrence of insulitis at doses that are clinically well supported, as they do not result in weight loss, severe hypercalcemia, or long-term immunosuppression.

RESEARCH DESIGN AND METHODS

NOD mice that were originally obtained from Professor Wu (1990, Beijing, China), were bred in our animalium

Mathieu C et al. Diabetes 1992



Gregori S et al. J Immunol 2001

Anti-inflammatory therapy

References	Patients n.	Treatment	Outcomes	C-peptide (AUC-nmol/l/min)
Crinò et al. Eur J Endocrinol 2004	64	**Nicotinamide (NA)** alone and in combination with Vitamin E (single injections)	Preserve C-peptide	NA: 0.32 to 0.43 NA+vitamin E: 0.32 to 0.25
Gottlieb et al. Diabetes Care 2010	126	**MMF+Daclizumab** Mycophenolate mofetil alone (given in 2/3 doses within 2 years) and associated with Daclizumab (intravenous infusions at day 0 and 2 weeks later)	Ineffective	Ineffective
Sobel et al. Acta Diabetol 2010	7	**Cyclosporin A+MTX** (daily for 13.5 months) associated with Methothrexate (weekly for 12 months)	Treatment was able to induce the remission of T1D	Parameter not measured
Moran et al. Lancet 2013	69	**Anti-IL1** Monthly injections of Canakimumab (a fully human anti-interleukin-1β monoclonal antibody) for 12 months	Ineffective	Ineffective
Van Asseldonk et al. Clinical Immunol 2015	16	**Anti-IL1** Daily subcutaneous injections of Anakinra for one week	Ineffective	Ineffective

Anti-inflammatory therapy - Summary

- Anti-inflammatory drugs are associated with many adverse effects
- Clinical results mixed
- Some sort of anti-inflammatory strategies may be needed to achieve remission of T1D

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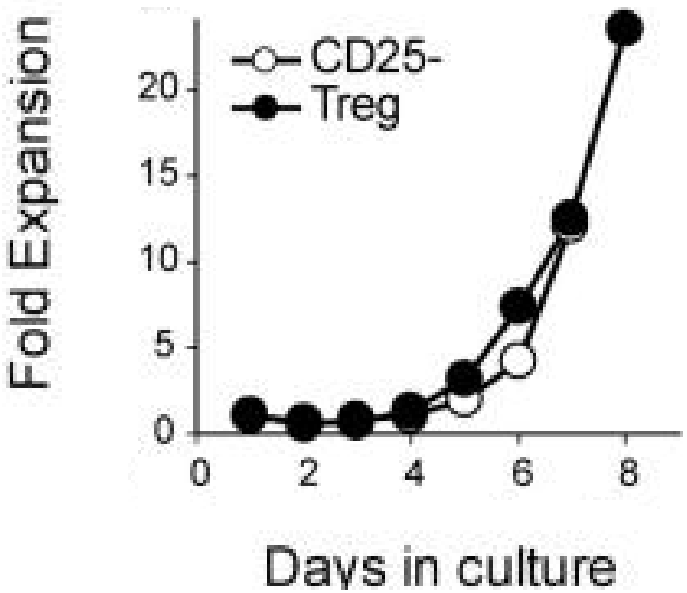
Cell therapy

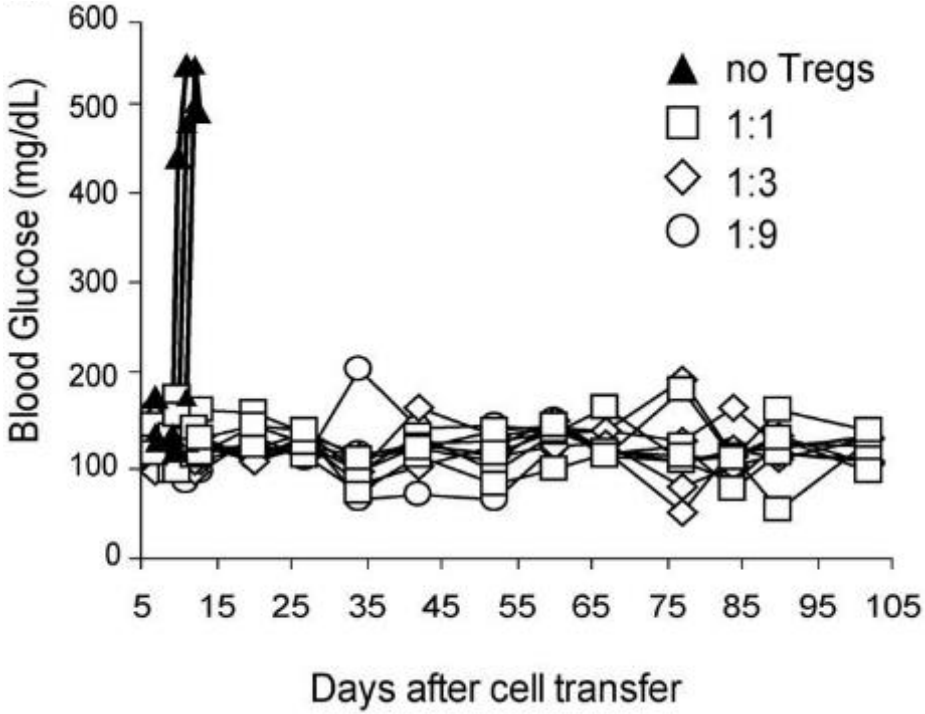
- Regulatory T cells abrogate autoimmune response
- $CD4^+CD25^+Foxp3^+$ cells are immunoregulatory
- Preclinical evidence: Regulatory Dendritic and T cells cells prevent T1D in NOD mice

Feili-Hariri et al. Diabetes 1999,
Harnah et al. Diabetes 2006

Preclinical evidences – In vitro expanded Tregs with IL-2/anti-CD3/CD28 cure T1D in NOD mice



Bluestone J et al. JEM 2001



Gregori S et al. J Immunol 2001

Cell therapy

References	Patients n.	Treatment	Outcomes	C-peptide (AUC-nmol/l/min)
Giannouakis et al. Diabetes Care 2011	10	DC 10 million autologous dendritic cells were injected once every 2 week for a total of 4 times	The treatment induced an increase in peripheral B220+ CD11c- B cells population, but no real effect on glycemia	Treated: ND to 1.10 Control: ND to <0.50
Bluestone et al. Sci Transl Med 2015	14	*Tregs* *Ex vivo*–expanded autologous CD4+CD127lo/−CD 25+ polyclonal T_{Regs} were administered	The treatment induced a good survival of the T regulatory cells, with up to 25% remaining into the bloodstream after 1 year	Values were not significantly different

Cell therapy - Summary

- Regulatory T cells hold great promises
- The work of Bluestone group is moving in the direction of having a product to be used for T1D

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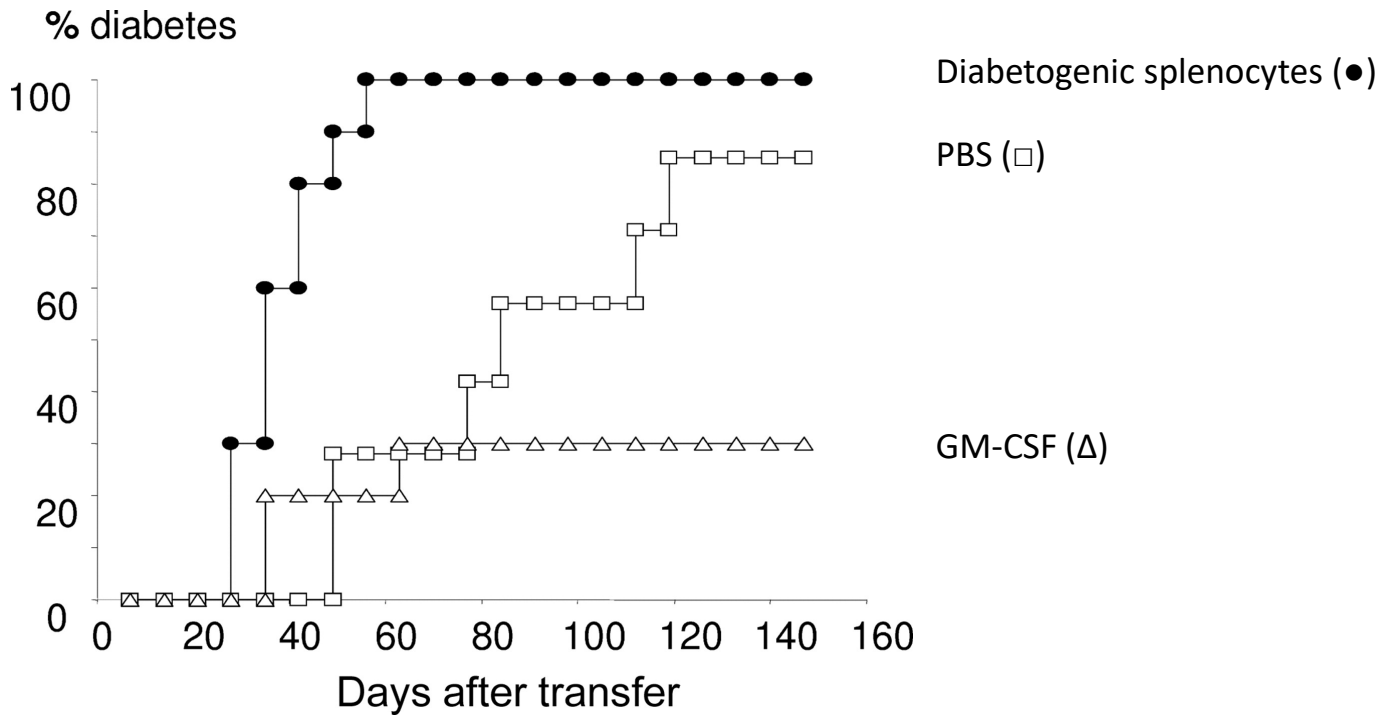
Stem cell therapy

- HSCs are immunoregulatory
- Autologous HSCs cure T1D in NOD mice

Preclinical studies
Autologous HSCs cure T1D in NOD mice

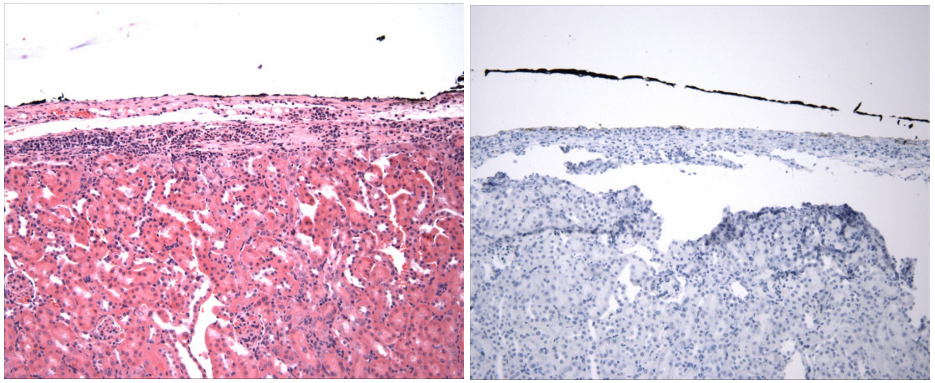


Bachar-Lustig E et al. Nat Med 1995
Kared H et al. Blood 2008

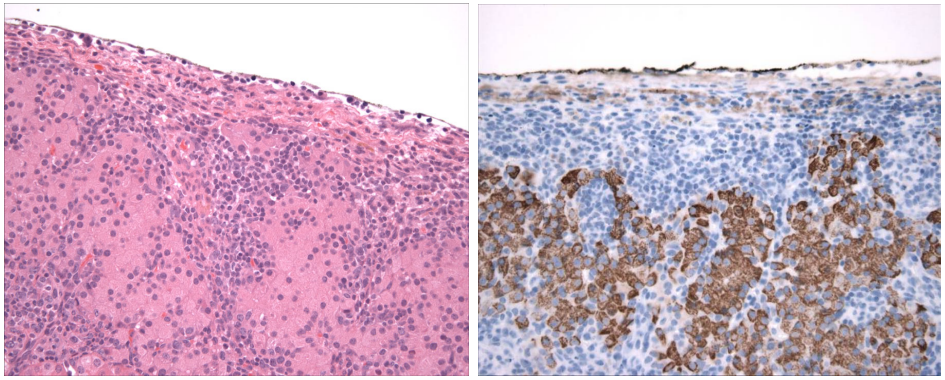
Preclinical studies
Autologous HSCs delay islet tx

H&E | Insulin

Controls



Mobilized HSC



Fiorina P et al. J Immunol 2011

Francesca D'Addio,[1,2] Alessandro Valderrama Vasquez,[2] Moufida Ben Nasr,[1] Edward Franek,[3,4] Dalong Zhu,[5] Lirong Li,[5] Guang Ning,[6] Emilian Snarski,[7] and Paolo Fiorina[1,2]

Autologous Nonmyeloablative Hematopoietic Stem Cell Transplantation in New-Onset Type 1 Diabetes: A Multicenter Analysis

Diabetes 2014;63:3041–3046 | DOI: 10.2337/db14-0295

IMMUNOLOGY AND TRANSPLANTATION

Type 1 diabetes (T1D) is one of the major autoimmune diseases affecting children and young adults worldwide. To date, the different immunotherapies tested have achieved insulin independence in <5% of treated individuals. Recently, a novel hematopoietic stem cell (HSC)–based strategy has been tested in individuals with new-onset T1D. The aim of this study was to determine the effects of autologous nonmyeloablative HSC transplantation in 65 individuals with new-onset T1D who were enrolled in two Chinese centers and one Polish center, pooled, and followed up for 48 months. A total of 59% of individuals with T1D achieved insulin independence within the first 6 months after receiving conditioning immunosuppression therapy (with antithymocyte globulin and cyclophosphamide) and a single infusion of autologous HSCs, and 32% remained insulin independent at the last time point of their follow-up. All treated subjects showed a decrease in HbA_{1c} levels and an increase in C-peptide levels compared with pretreatment. Despite a complete immune system recovery (i.e., leukocyte count) after treatment, 52% of treated individuals experienced adverse effects. Our study suggests the following: *1*) that remission of T1D is possible by combining HSC transplantation and immunosuppression; *2*) that autologous nonmyeloablative HSC transplantation represents an effective treatment for selected individuals with T1D; and *3*) that safer HSC-based therapeutic options are required.

The incidence of type 1 diabetes (T1D) has been significantly increasing worldwide in the last decade, thus becoming the most common autoimmune disorder in children (1). T1D is characterized by a selective and aggressive destruction of insulin-producing β-cells orchestrated by autoreactive T cells (2,3). Unfortunately, exogenous insulin therapy does not always achieve the necessary metabolic control (4), nor does it prevent the occurrence of disease-associated degenerative macrovascular and microvascular complications (5) or halt β-cell decline (6).

The concept of the use of immunotherapeutic strategy to cure T1D has emerged from hallmark data generated using the NOD mouse model and has allowed for a better understanding of the pathogenesis of T1D (7). Several clinical trials—designed based on the preclinical successful targeting of components of innate and adaptive immune responses—performed thus far have failed to cure

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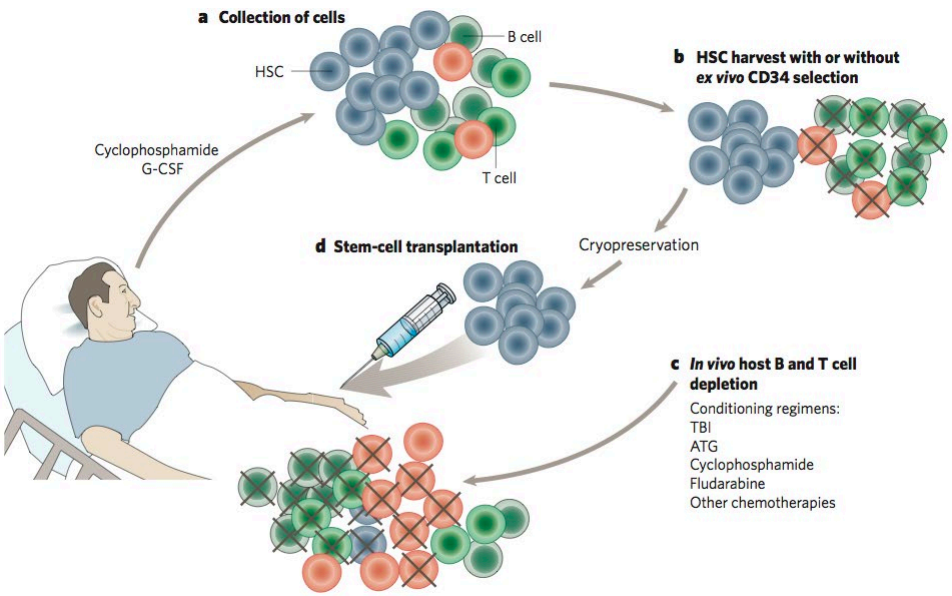
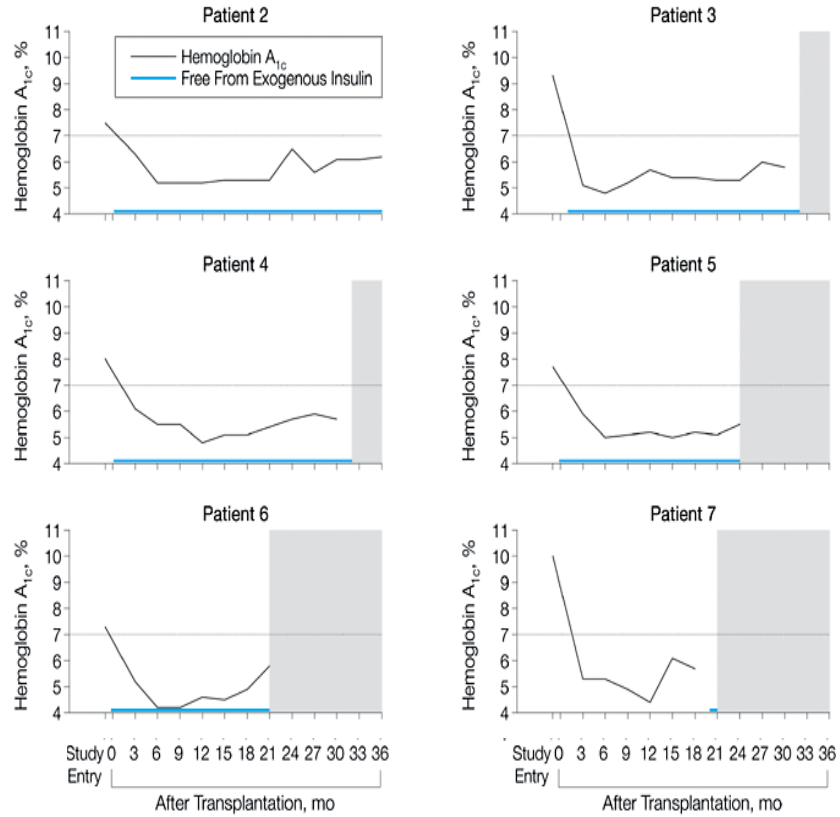
This article contains Supplementary Data online at http://diabetes.diabetesjournals.org/lookup/suppl/doi:10.2337/db14-0295/-/DC1.
F.D. and A.V.V. contributed equally to this study.

D'Addio F/Ben Nasr M/Fiorina P et al. Diabetes 2014

Autologous hematopoietic stem cell transplantation (AHSCT)





Couri CEB et al. JAMA 2009
D'Addio F/Fiorina P et al. Diabetes 2014

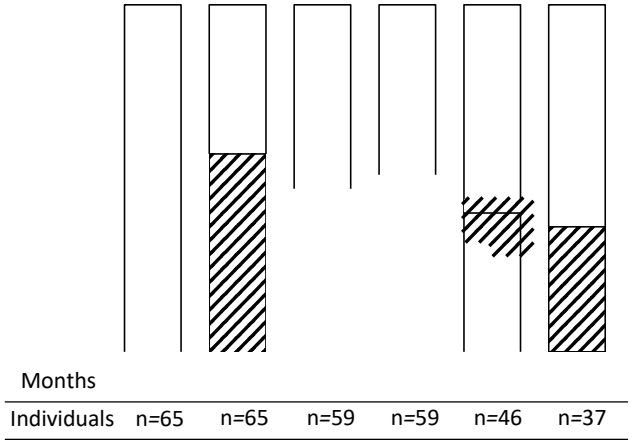
Protocol

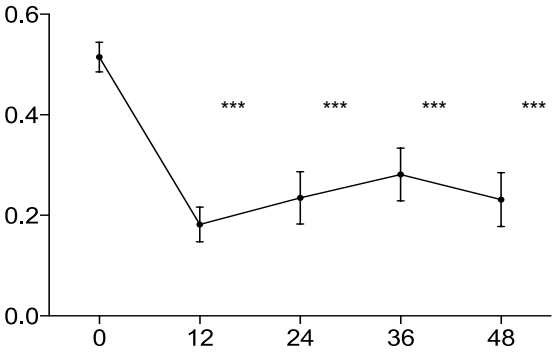
HSC Mobilization regimen	
Cyclophosphamide (g/m^2)	2.0
G-CSF (µg/kg/day)	8.3±2.8
HSC Conditioning regimen	
Cyclophosphamide (mg/kg)	200
Days before transplant	4
Rabbit antithymocyte globulin (mg/kg)	2.7±2.4
Days before transplant	5
HSC Infusion	
CD34$^+$ cells infused (10^6/kg)	5.3±3.9

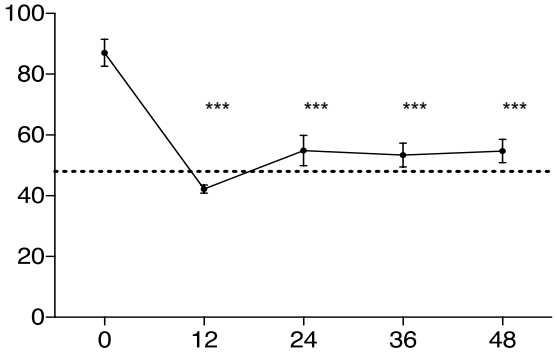
Characteristics	
Number of patients included	n=65
Age (years)	20.4±5.5
Gender (M/F)	41/24
BMI (kg/m^2)	18.1±3.1
DKA or DK history	**n patients/65**
No DKA/DK	43
DKA	21
DK	1

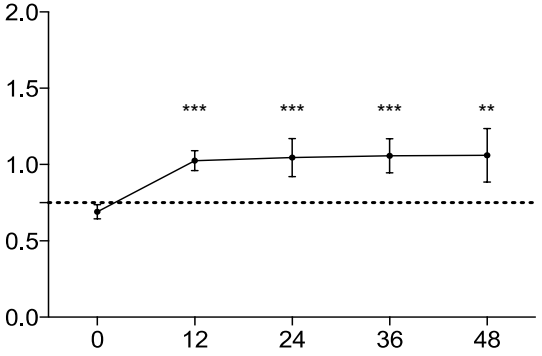
D'Addio F/Fiorina P et al. Diabetes 2014

Outcomes









D'Addio F/Fiorina P et al. Diabetes 2014

Stem cells therapy

References	**Patients n.**	**Treatment**	**Outcomes**	**C-peptide (AUC-nmol/l/min)**
Voltarelli et al. JAMA 2007	**15**	**HSC** Single infusion of Hematopoietic Stem Cells (HSCs**)**	Treatment made all patients but 1 became insulin-independent for at least 6 months, with increased C- peptide levels and decreased anti-GAD auto antibodies	From 0.40 to 1.34
D'Addio et al. Diabetes 2014	65	**ATG-GCSF** were administered prior to a CD34+ single infusion	Among the treated patients, 59% reached insulin-independence and 32% remained insulin independent at the last follow-up (48 months)	From 0.54 to 1.22
Carlsson et al. Diabetes 2015	20	**MSC** Single infusion of autologous MSCs	Treatment reduced decay in C-peptide levels after treatment	Treated: 0.29 to 0.32 Control: 0.28 to 0.29
Cai et al. Diabetes Care 2016	42	**MSC** Single infusion of Umbilical cord blood-MSCs (UC-MSCs)	Treatment induced a decrease in insulin requirements by 30% and AUC C-Peptide increased	Treated: 6.6 to 13.6 Control: 8.4 to 7.7

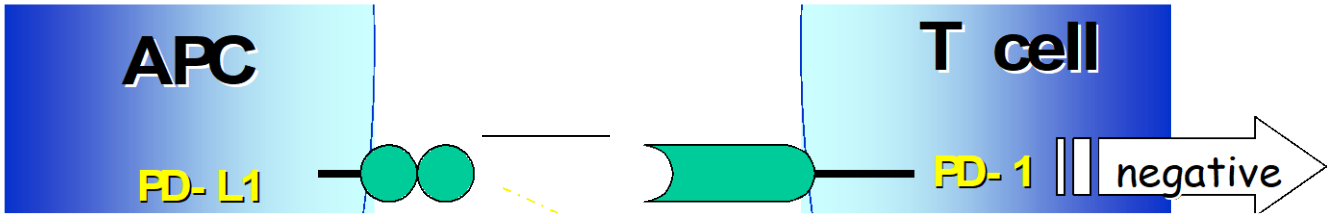
Stem cells therapy - Summary

- Approach limited by adverse events of immunosuppression
- Safer approaches are needed

Immunoterapia

1. Introduction
2. Cell-depletion
3. Antigen-specific therapies
4. Anti-inflammatory therapies
5. Cell-therapy
6. Stem cells therapy
7. ImmunoStem
8. Conclusions

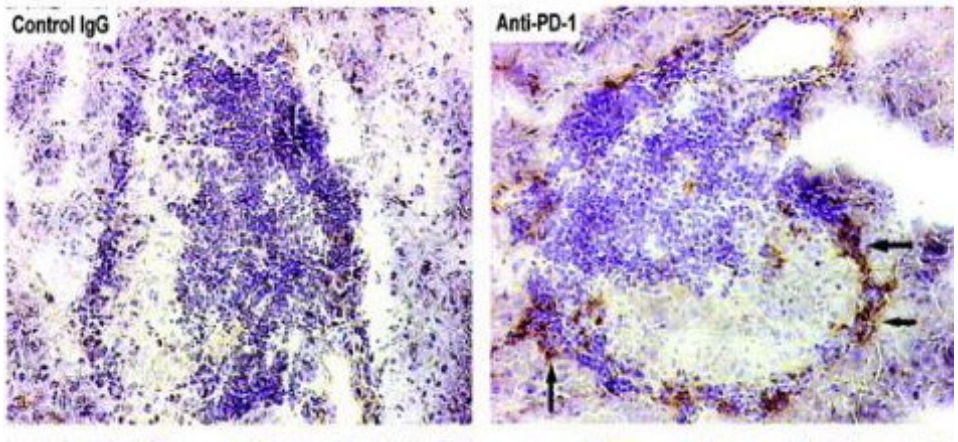
PD-L1 activates negative signals in T cells



Khoury and Sayegh, Immunity, 2004

PD-1 expression in islet-infiltrating T cells

A



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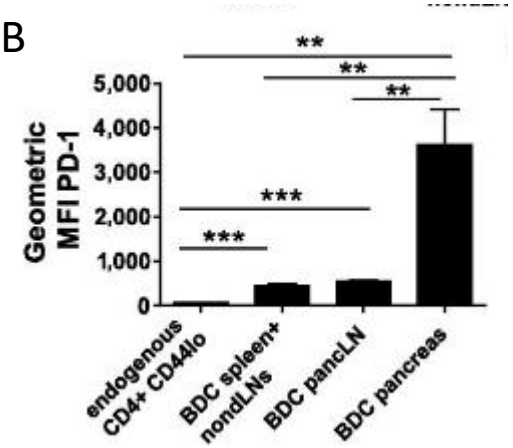
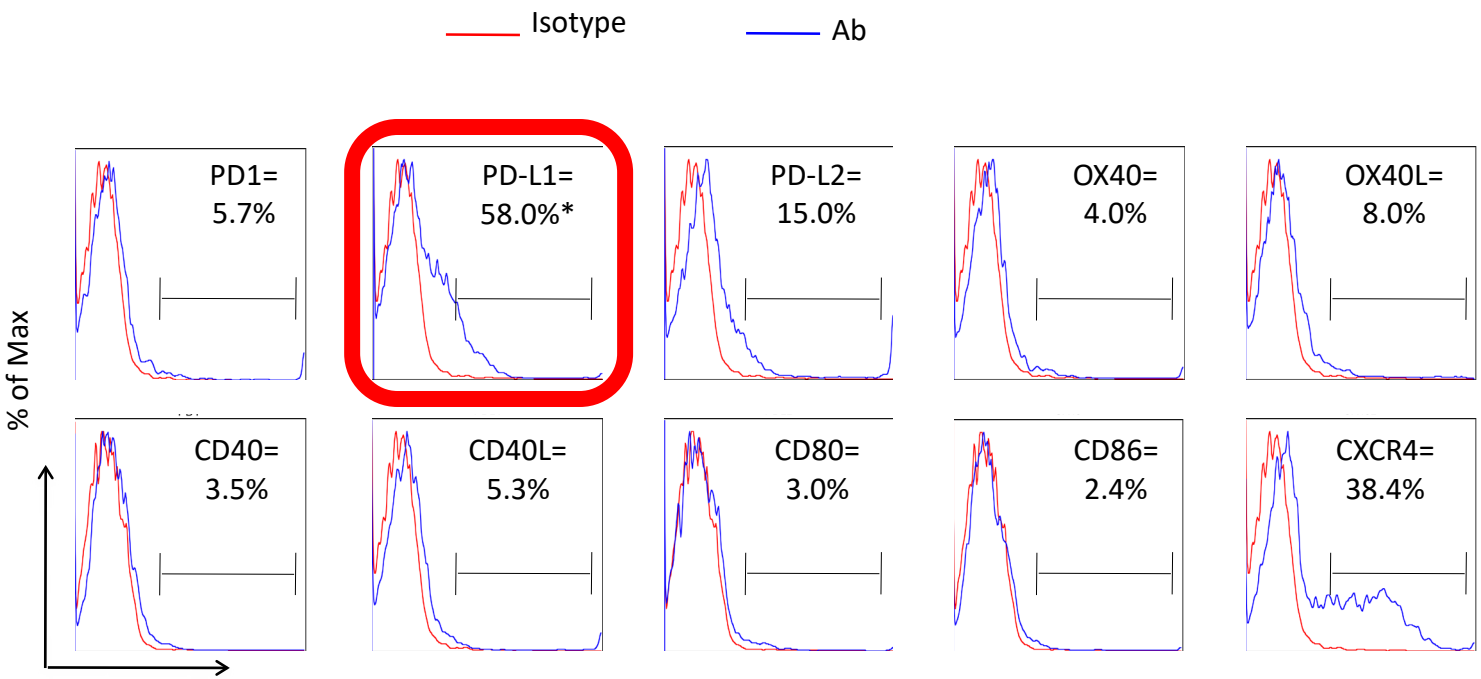


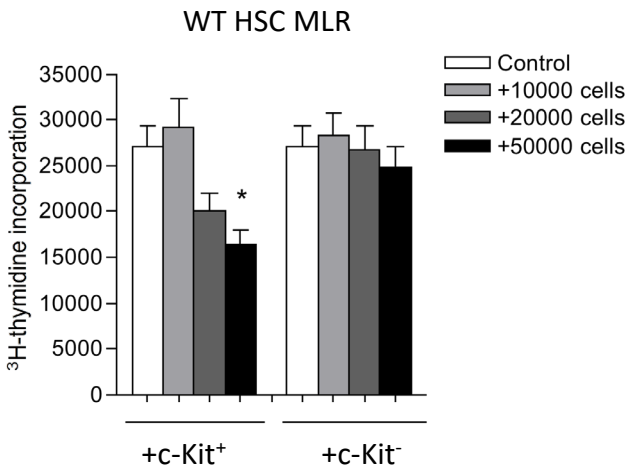
Ansari MJ et al. J Exp Med 2003
Kristen E. Pauken et al. Diabetes 2013

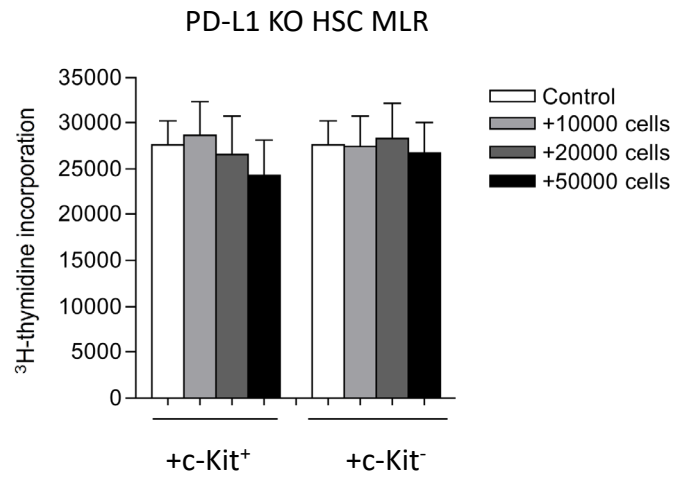
HSPCs are highly positive for PD-L1



Fiorina P et al. J Immunol 2011

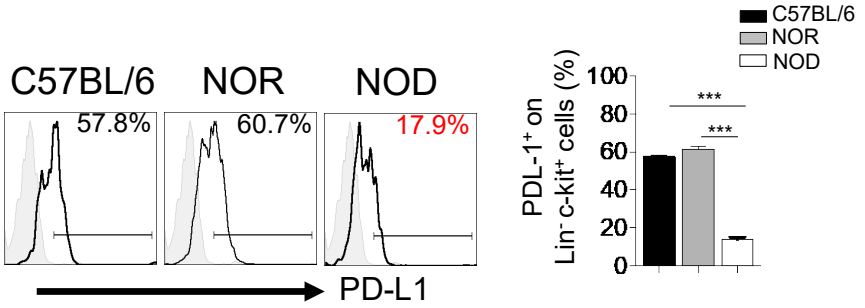
The lack of PD-L1 reduces HSPC immunoregulatory properties

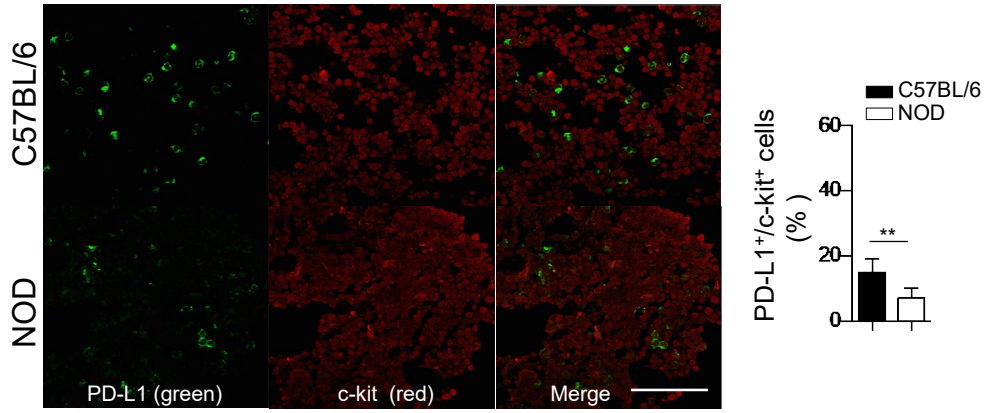




Fiorina P et al. J Immunol 2011

Murine HSPCs from NOD mice are defective in PD-L1 expression





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ImmunoStem

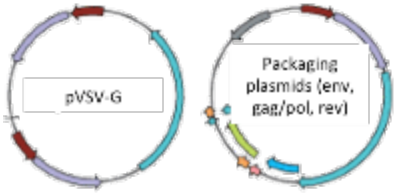
- We created *ImmunoStem* to genetically overturn PD-L1 defect
  - ***ImmunoStem* are PD-L1.Tg HSCs or HSC.Reg with immunoregulatory properties**

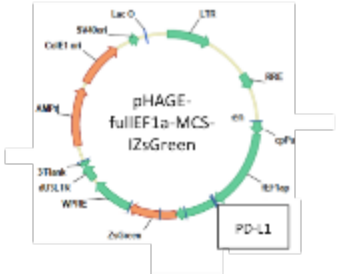
Generation of *ImmunoStem* by lentiviral transduction (genetic approach)

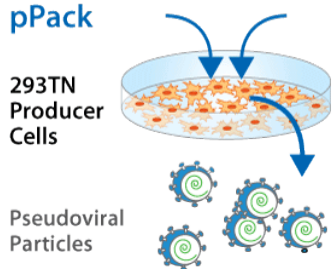
lin⁻ c-kit⁺ KL cells

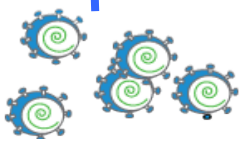
Lentiviral transduction

PD-L1 Transduced KL cells



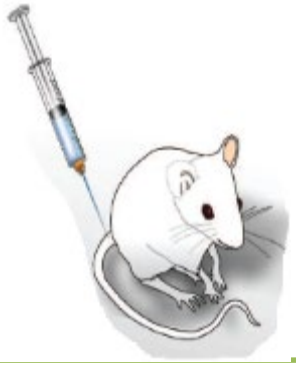






PD-L1 pseudoviral particles

Doxycycline



KL cells Doxa induced expression of PD-L1

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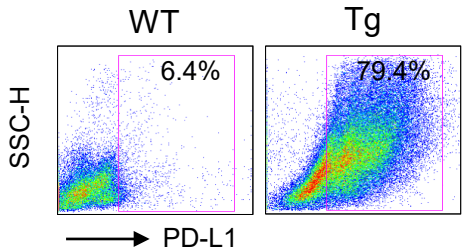
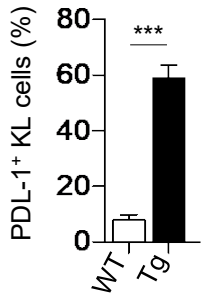
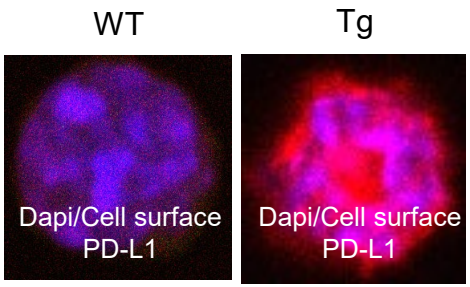
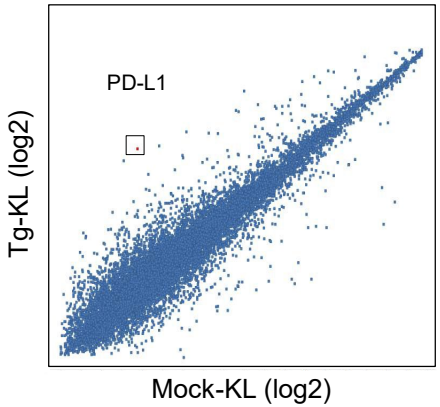
ImmunoStem characterization









Gene Symbol	Tg	Mock	Fold Change
CD274 (PD-L1)	14.12	5.77	**327.59**

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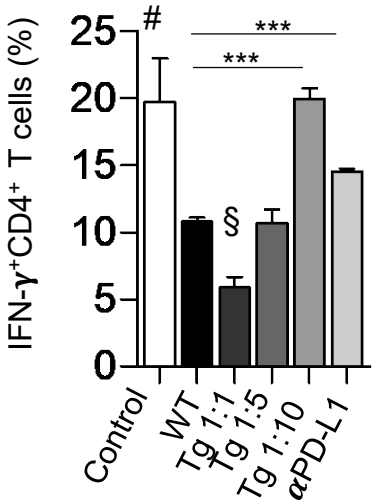
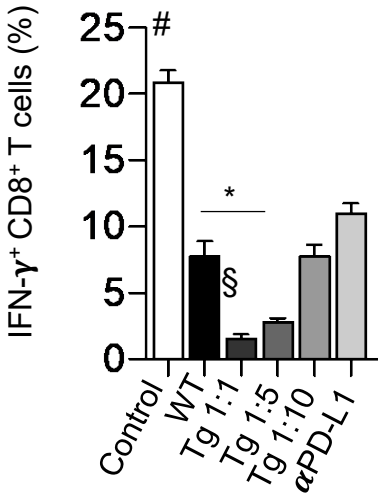
ImmunoStem reduces autoimmune response *in vitro*





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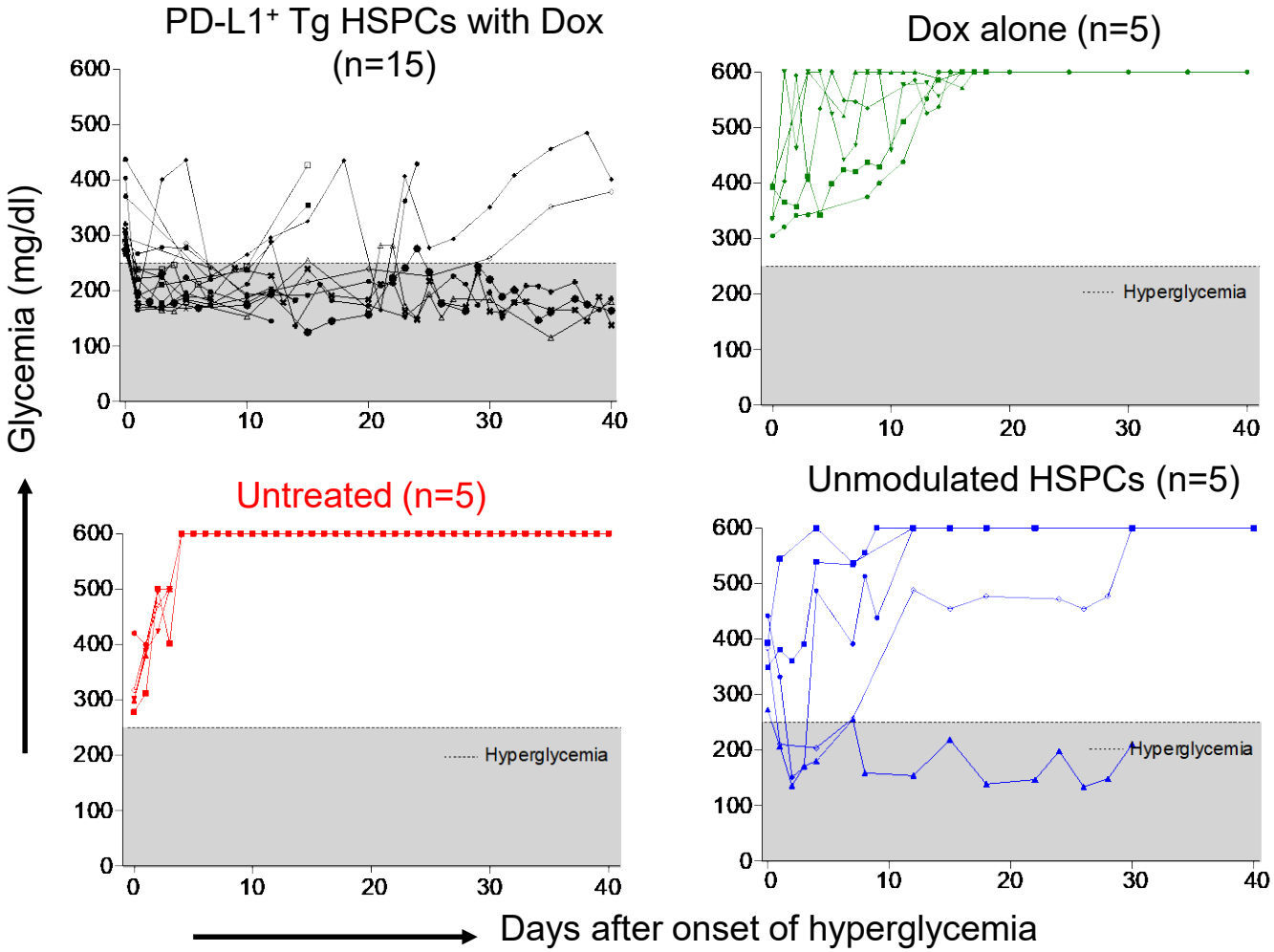
ImmunoStem reverts diabetes in NOD mice *in vivo*



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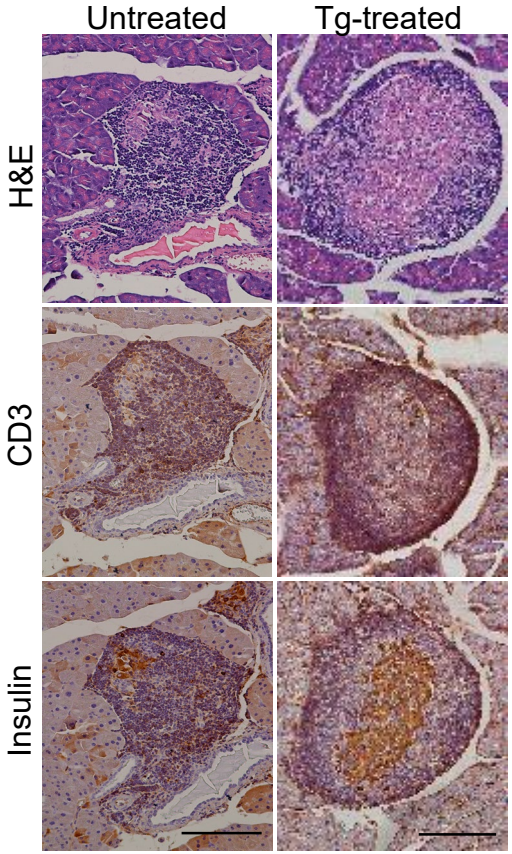
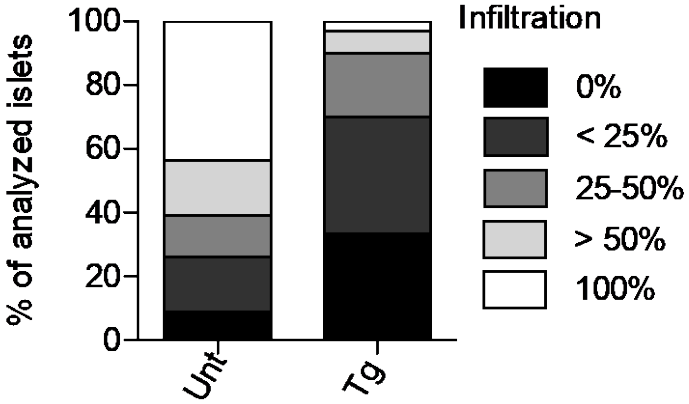
ImmunoStem preserves islet morphology and reduces islet infiltration





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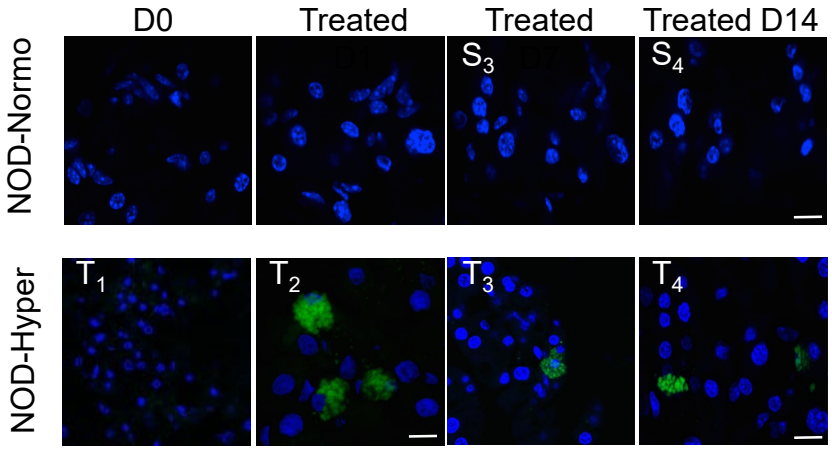
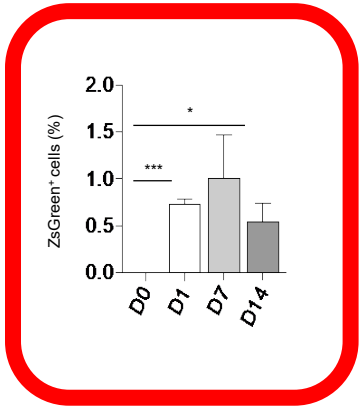
ImmunoStem traffics to the pancreas

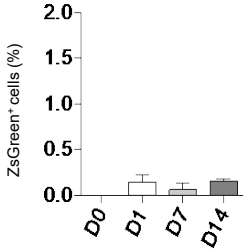


Pancreas

Hyperglycemic NOD



Normoglycemic NOD



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Conclusions

- HSPCs are endowed with immunoregulatory properties due to the expression of PD-L1
- The infusion of autologous newly generated PD-L1^{+}.HSPCs may be a novel therapeutic tool for autoimmune diseases

…grazie…

“Fondazione Romeo and Enrica Invernizzi”