SID
Società Italiana di Diabetologia
fondata nel 1964

XI EDIZIONE
PARMA DIABETE
19·21 ottobre 2017
Hotel NH PARMA

UNIVERSITÀ CAMPUS BIO-MEDICO DI ROMA

Barts and The London
School of Medicine and Dentistry

Ruolo delle modificazioni ossidative post-traduzionali nella immunopatogenesi del diabete tipo 1

Rocky Strollo, MD PhD

Endocrinologia e Diabetologia,
Universitá Campus Bio-Medico, Roma

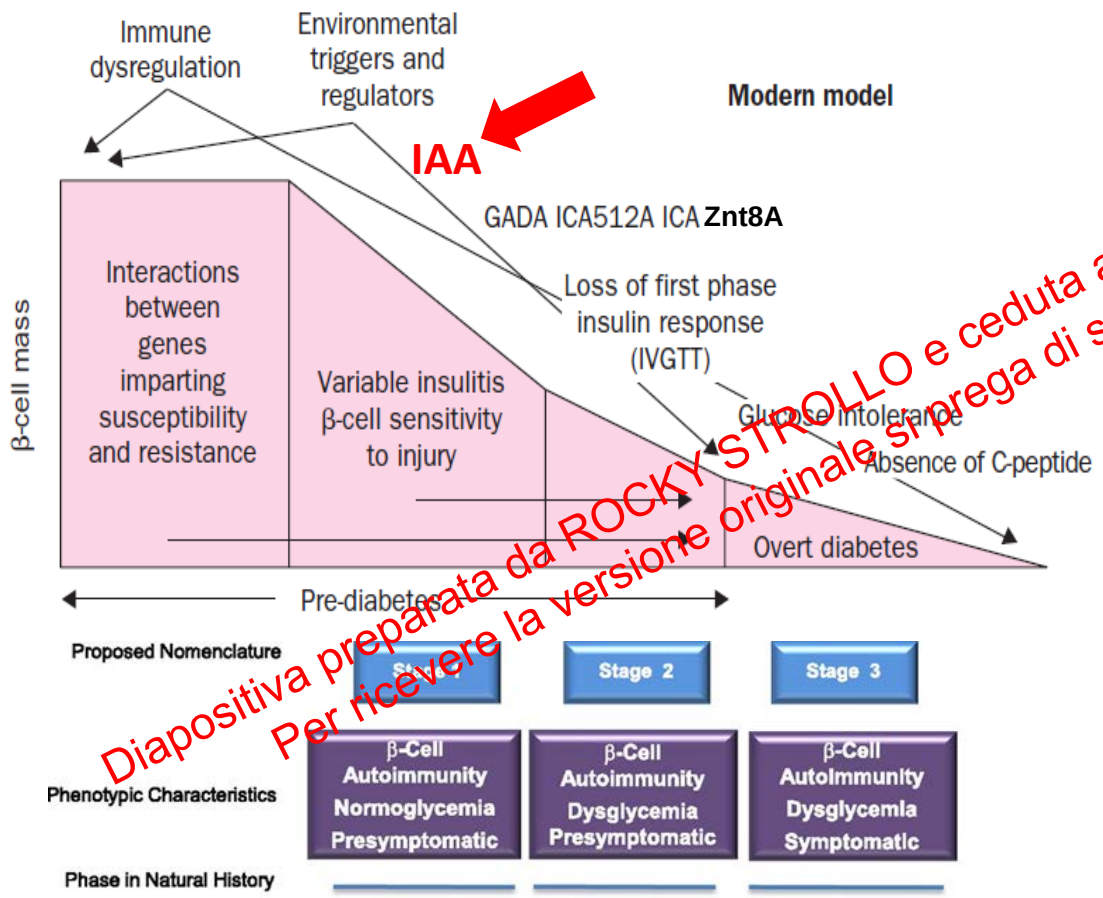
WHRI, Barts and The London,
Queen Mary University, London UK

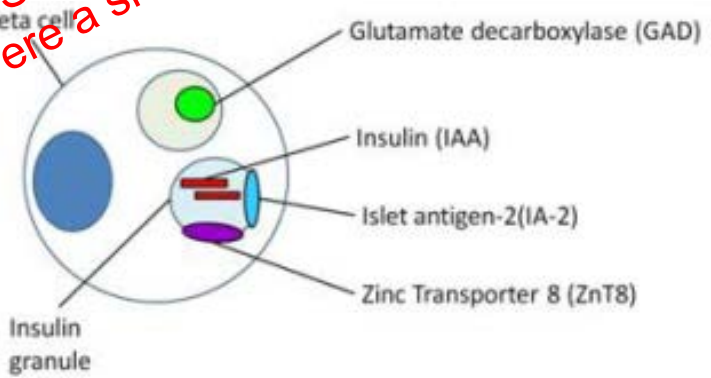
Parma, 20 ottobre 2017

PRESENTER DISCLOSURE

- Rocky Strollo dichiara di aver ricevuto negli ultimi due anni compensi o finanziamenti dalle seguenti Aziende Farmaceutiche e/o Diagnostiche:
- Astrazeneca
- Janssen
- Novartis
- Takeda

Storia naturale del diabete tipo 1





Eisenbarth Lancet 2001
Insel et al. Diabetes Care 2015

Cosa sappiamo del diabete tipo 1?

- **È una malattia autoimmune:**
  - **Il sistema immunitario aggredisce le cellule beta del pancreas che producono insulina e le distrugge.**
  - **La risposta (auto)immunitaria sembra essere diretta principalmente contro l'insulina.**

PERCHÉ?

Diabetologia
DOI 10.1007/s00125-015-3746-x

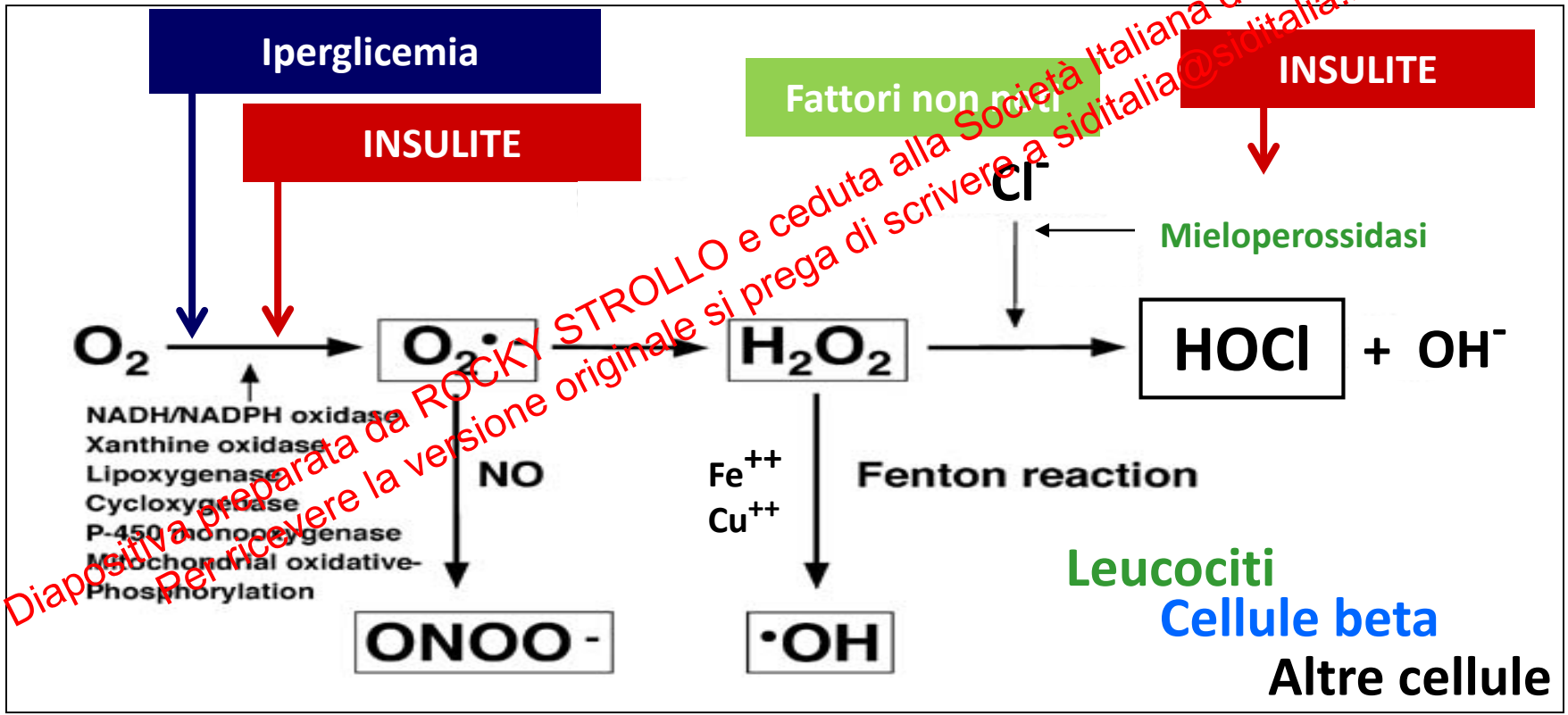
ARTICLE

Antibodies to post-translationally modified insulin in type 1 diabetes

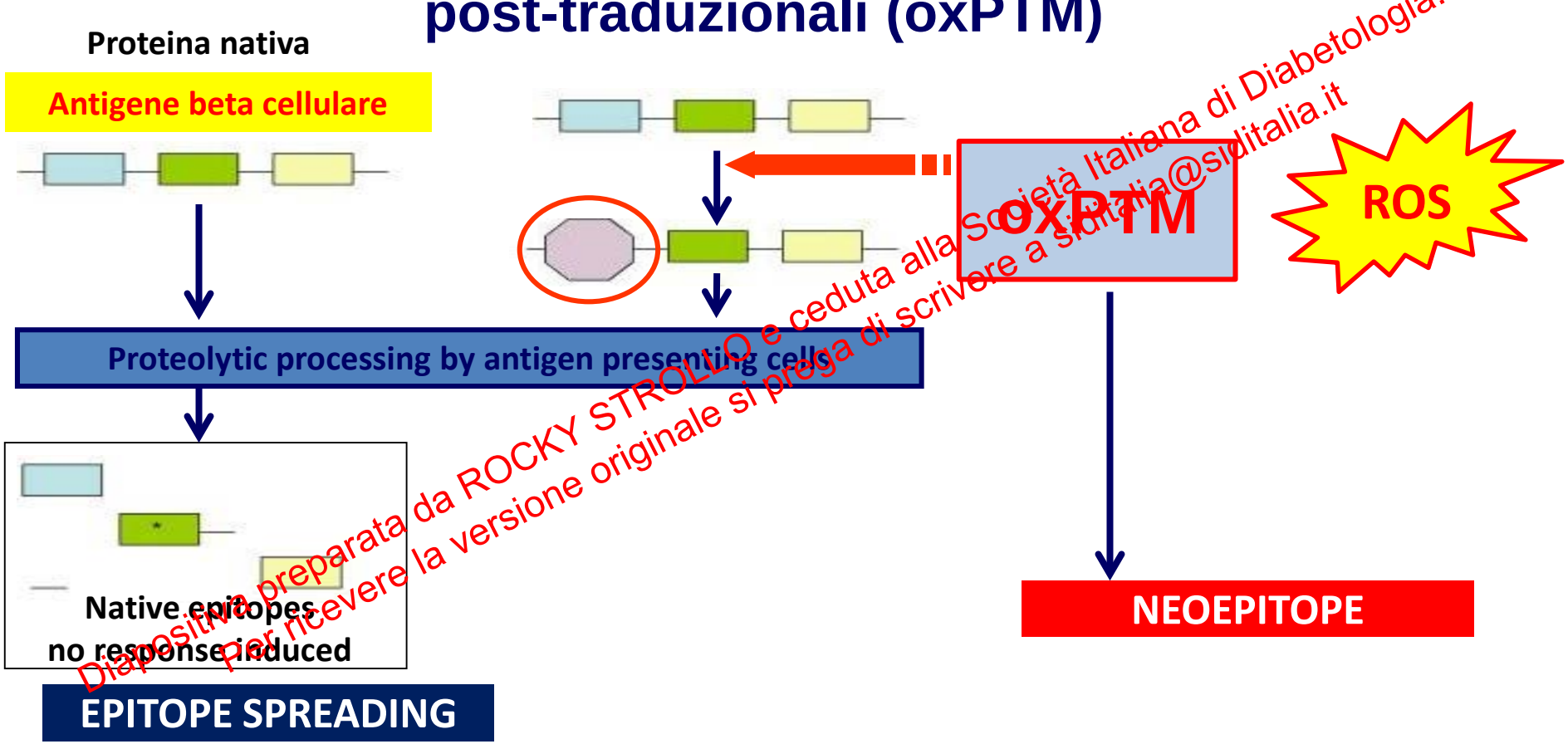
Rocky Strollo[1,2] · Chiara Vinci[1,2] · Mayda H. Arshad[1] · David Pollett[3] · Claudio Tiberti[4] · Francesco Chiarelli[5] · Nicola Napoli[2,6] · Paolo Pozzilli[2,7] · Ahuva Nissim[1]

Un'insulina "alterata" alla base del diabete tipo 1?

La produzione di radicali dell'ossigeno (ROS) è aumentata nel diabete tipo 1



I ROS possono indurre modificazioni ossidative post-traduzionali (oxPTM)



Adapted by **Kurien BJ** et al. Autoimmun Rev 2008; **Griffiths HR**. Autoimmun Rev 2008; **Nissim A** et al 2014

Supporting evidence that oxPTMs are involved in the pathogenesis of autoimmune diseases

Generation of neoantigenic epitopes after posttranslational modification of type II collagen by factors present within the inflamed joint.

Nissim A, Winyard PG, Corrigall V, Fatah R, Perrett D, Panayi G, Chernajovsky Y.
Arthritis Rheum. **2005** Dec;52(12):3829-38.

HLA-dependent autoantibodies against post-translationally modified collagen type II in type 1 diabetes mellitus.

Strollo R, Rizzo P, Spoletini M, Landy R, Hughes C, Ponchel F, Napoli N, Falermo A, Buzzetti R, Pozzilli P, Nissim A.
Diabetologia. 2013 Mar;56(3):563-72. doi: 10.1007/s00125-012-2780-1. Epub 2012 Nov 19.

Autoantibodies to posttranslationally modified type II collagen as potential biomarkers for rheumatoid arthritis.

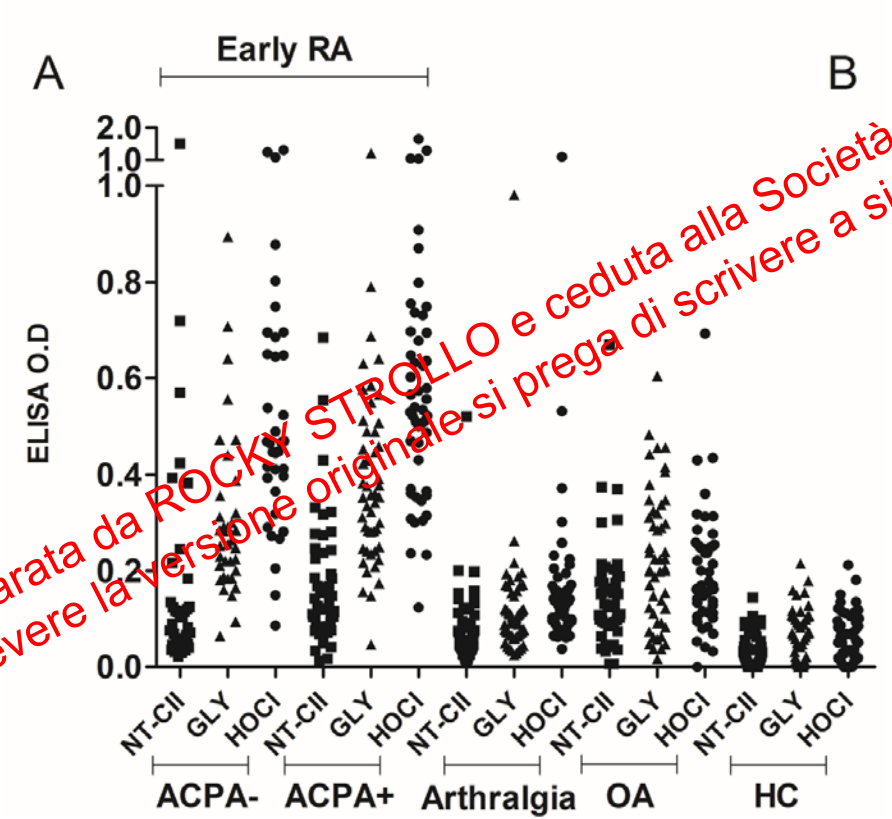
Strollo R, Ponchel F, Malmström V, Rizzo P, Bombardieri M, Wenham CY, Landy R, Perret D, Watt F, Corrigall VM, Winyard PG, Pozzilli P, Conaghan PG, Panayi GS, Klareskog L, Emery P, Nissim A.
Arthritis Rheum. 2013 Jul;65(7):1702-12. doi: 10.1002/art.37964.

Clin Exp Immunol. 2001 Nov;126(2):242-9.

Islet glutamic acid decarboxylase modified by reactive oxygen species is recognized by antibodies from patients with type 1 diabetes mellitus.

Trigwell SM[1], Radford PM, Page SR, Loweth AC, James RF, Morgan NG, Todd I.

Il collageno tipo II modificato dai ROS (ROS-CII) é un autoantigene nell'artrite reumatoide (RA)



Strollo et al. Arthritis & Rheumatism 2013

IPOTESI 1

Iperglicemia e lo stress ossidativo possono favorire lo sviluppo di risposte autoimmuni nel diabete tipo 1

RA and type 1 diabetes (T1D) co-occur within individuals

The risk of developing RA later in life in patients with T1D

Cases: N=1,356 cases; controls N= 863

Type of DM, anti-CCP/RF status	No. exposed cases/ no. exposed controls	OR (95% CI)†		
		Model 0	Model 1	Model 2
Type 1				
All	20/5	4.9 (1.8–13.1)	4.8 (1.8–12.9)	3.5 (1.0–12.1)
Anti-CCP+	18/5	7.3 (2.7–20.0)	7.3 (2.6–20.2)	5.3 (1.5–18.7)
Anti-CCP−	2/5	1.3 (0.3–7.0)	1.3 (0.2–6.9)	1.1 (0.2–6.7)
RF+	19/5	7.1 (2.6–19.2)	7.0 (2.6–19.2)	5.1 (1.5–17.8)
RF−	1/5	0.7 (0.1–5.8)	0.7 (0.1–5.7)	0.6 (0.1–5.6)
Type 2				
All	40/46	1.1 (0.7–1.6)	1.0 (0.6–1.5)	1.1 (0.6–1.8)
Anti-CCP+	22/46	0.9 (0.6–1.6)	0.9 (0.5–1.6)	1.0 (0.6–1.9)
Anti-CCP−	20/46	1.3 (0.7–2.2)	1.1 (0.6–1.9)	1.1 (0.6–2.2)
RF+	27/46	1.1 (0.7–1.8)	1.0 (0.6–1.7)	1.2 (0.7–2.1)
RF−	15/46	1.0 (0.6–1.9)	0.9 (0.5–1.7)	0.9 (0.5–1.9)

MODEL 0: adjusted for AGE, SEX, Smoking
MODEL 1: adjusted for AGE, SEX, Smoking, BMI
MODEL 2: adjusted for AGE, SEX, Smoking, BMI, PTPN22 status

Liao et al. A&R 2009

*HLA-DRB1*04*

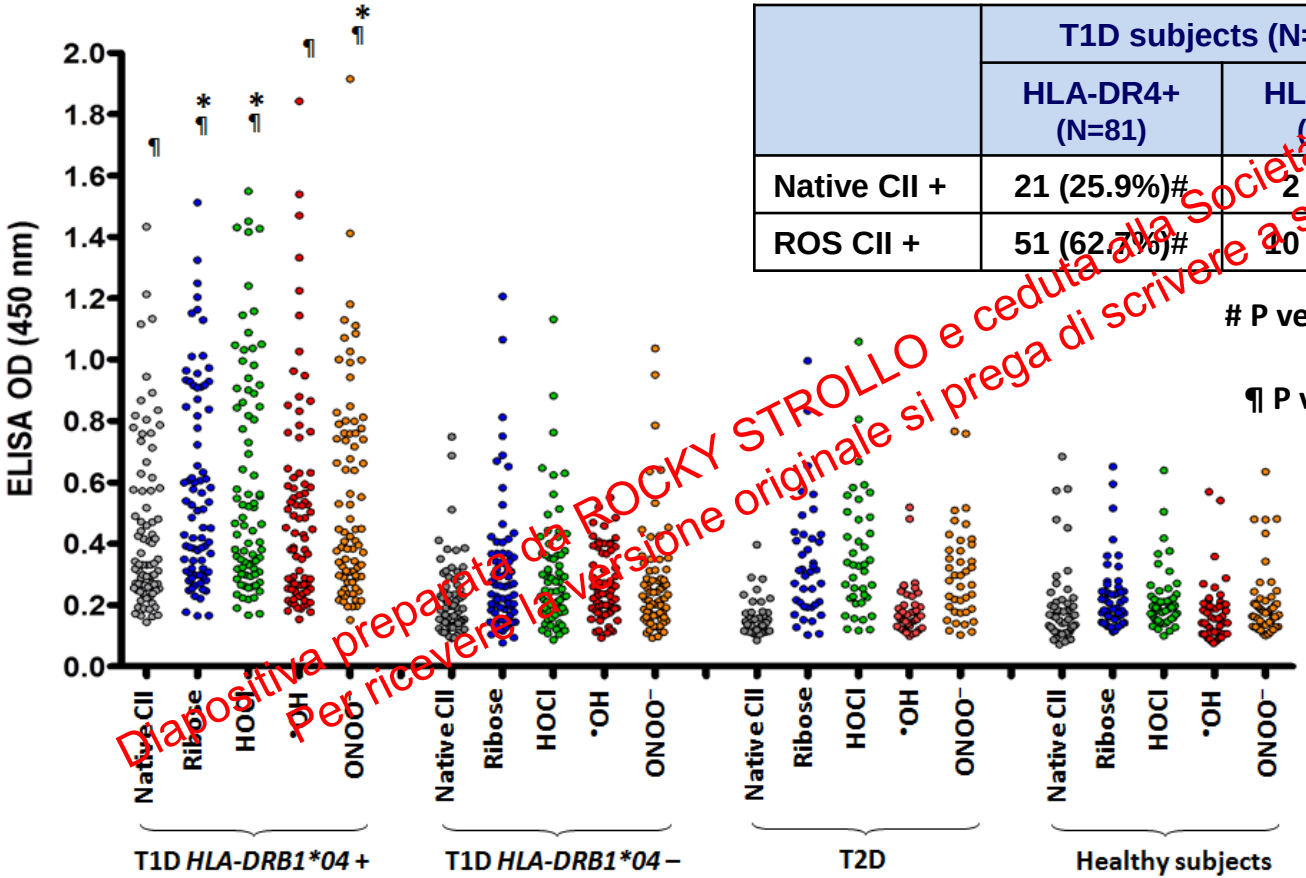
the shared genetics of T1D and RA

	T1D	RA	T1D -Abs	CCP-Abs
Antigen presentation/ specificity	*HLA-DRB1* **0401*; **0405*; **0404*; **0402* **0301* *HLA-DQB1** *0302*	*HLA-DRB1** *Shared epitope* **0401*; **0405*; **0404*; **0101* **0402* is protective.	*DRB1*04* *DRB1*03*	*DRB1*04* *DRB1*03* protective
Innate immunty TNF signalling	*IFIH1*	*IRF-4, TNFAIP3, TRAF1-C4, TNFRSF14*		
Immune cell activation/ signalling	*CTLA4, PTPN2, PTPN22, SH2B3, CD226*	*PTPN22, TNFSF4, BANK1, BLK, LYN*		
T-cell differentiation	*IL2-IL21, IL2RA*	*STAT4*		

RED = Shared genetic susceptibility

GREEN = Genes associated with one diesease/abs but protective for the other one

Auto-anticorpi contro forme modificate di collageno tipo II nel diabete tipo 1: associazione con HLA-*DRB1*04*



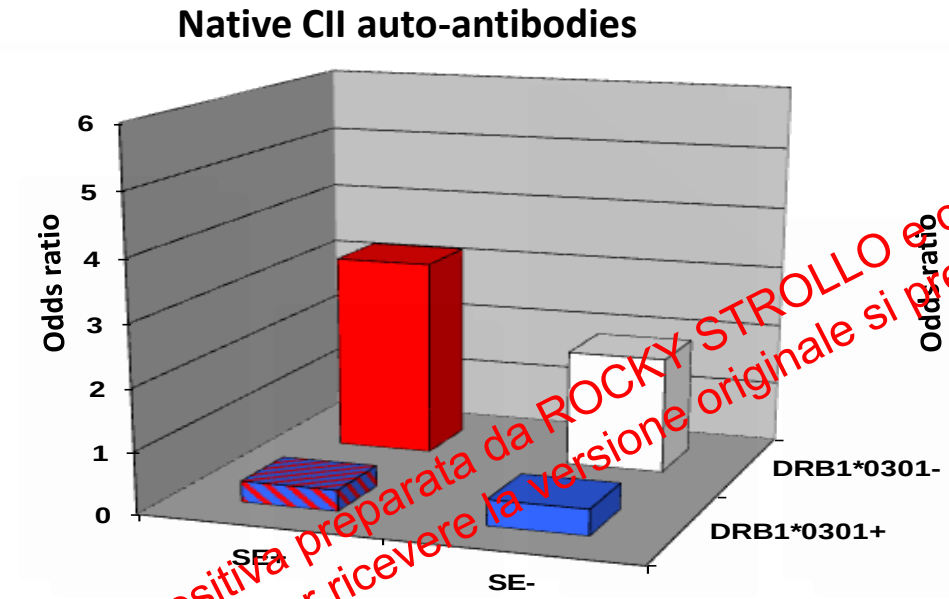
	T1D subjects (N=151)		T2D subjects (N=40)	Healthy subjects (N=85)
	HLA-DR4+ (N=81)	HLA-DR4- (N=70)		
Native CII +	21 (25.9%)#	2 (2.9%)	0 (0%)	2 (2.4%)
ROS CII +	51 (62.7%)#	10 (14.3%)	11 (27.5%)	2 (2.4%)

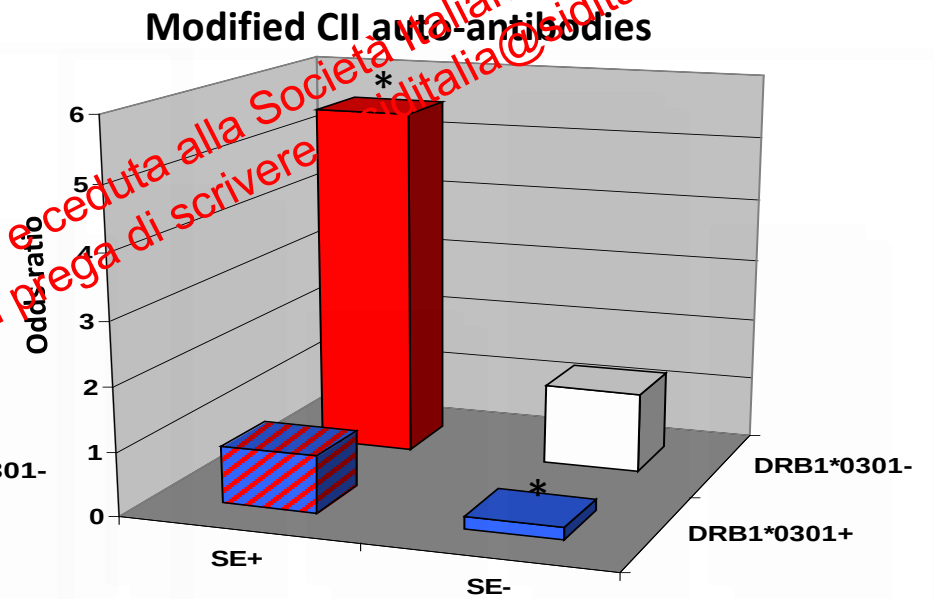
P versus DR4 negative and controls <0.0002
*P vs. binding to native CII <0.001
¶ P vs. T1D DR4 negative and controls <0.05

Strollo et al. Diabetologia, 2013

The association between *HLA-DRB1**SE and CII autoimmunity increased when CII was chemically modified by oxygen species

Interplay between *HLA-DRB1*SE* and *DRB1*0301*





HLA-DRB1*SE only positive
HLA-DRB1*0301 only positive
HLA-DRB1*SE and *0301 positive
HLA-DRB1*SE and *0301 negative

SE refers to at least one DRB1*0401, *0404 or *0405
N=57; * P≤0.02

Diabetologia, 2013

IPOTESI 2

Le modificazioni ossidative (oxPTM) sono coinvolte nello sviluppo dell'autoimmunitá beta cellulare

Study subjects: clinical and biochemical features

Features	Type 1 diabetes at diagnosis (*n*=116)	Type 2 diabetes (*n*=64)	Healthy controls (n=113)	
			Children (*n*=45)	Adults (*n*=68)
Age, years	13.9±0.8	59.5±3.4	14.2±0.6	38.0±1.4
Ratio male:female	0.85	1	0.33	0.66
Blood glucose, mmol/l	21.4±0.7	8.6±0.4	4.2±0.1	
HbA1c, %	11.0±0.3	7.4±0.3	5.4±0.1	
BMI, Kg/m^2	18.7±0.4	31.8±2.1	21.4±0.8	

In vitro chemical modification of insulin

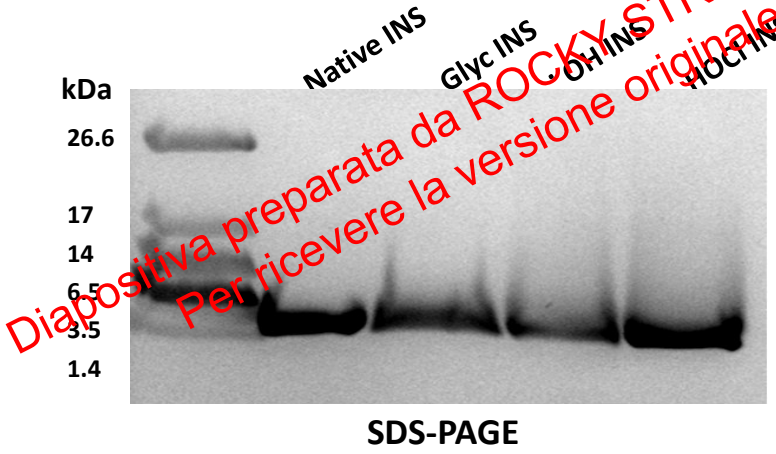
Human recombinant insulin (1 mg/ml) in phosphate buffered saline (PBS) was incubated overnight at 37°C with:

A. **2*M* ribose, to obtain a non-enzymatic glycosylation;**

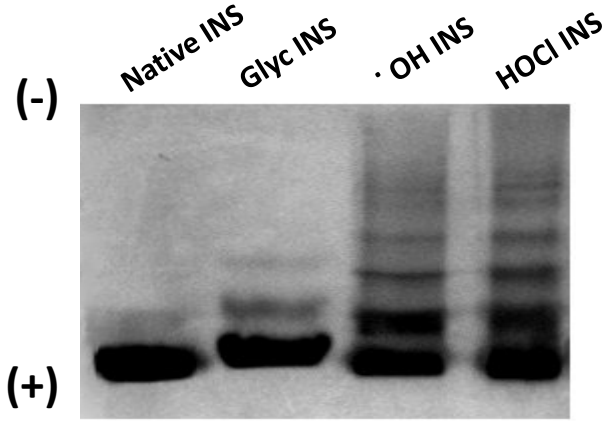
B. **0.1-9 m*M* HOCl;**

C. **0.1 -4.5m*M* $CuCl_2$ and 0.1-9*M* H_2O_2 (Fenton reaction), which were used to modify insulin with hydroxyl radical (•OH);**

PAGE-analysis of Native and ROS-modified Insulin (oxPTM-INS)

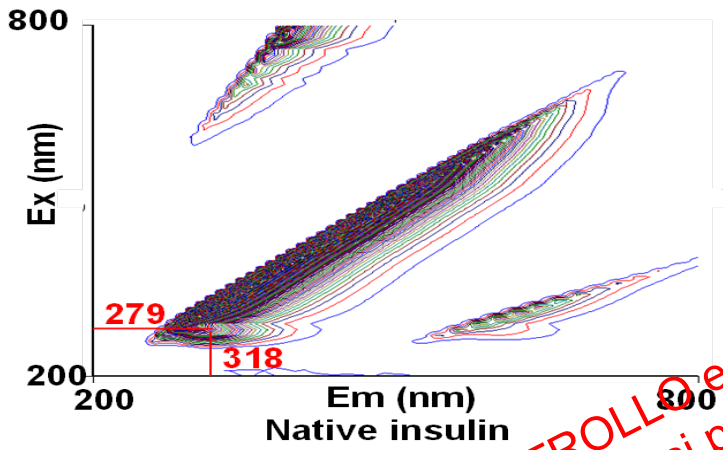


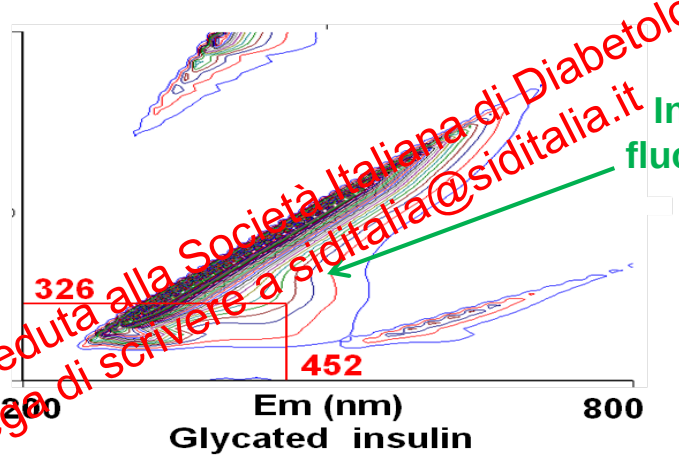
SDS-PAGE



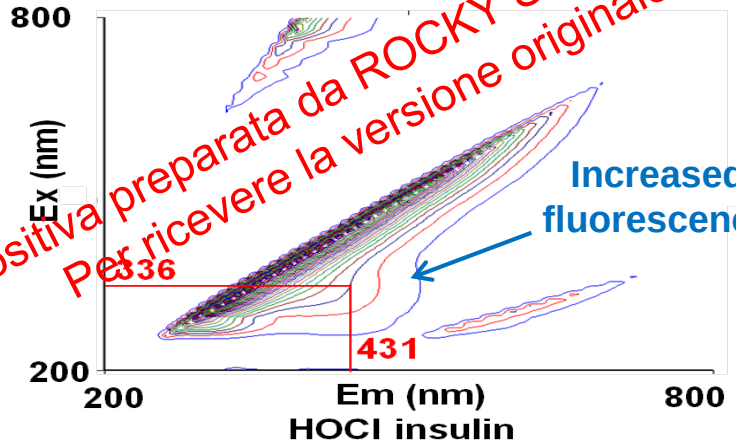
Native-PAGE

3D-fluorescence spectra of oxPTM-INS

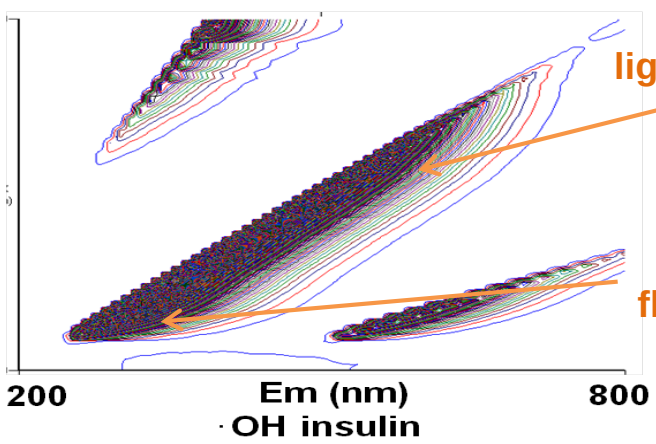




Increased fluorescence



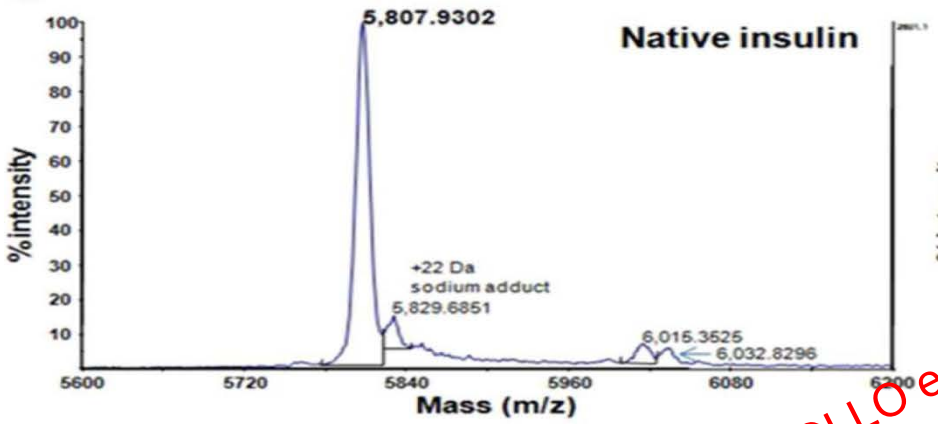
Increased fluorescence

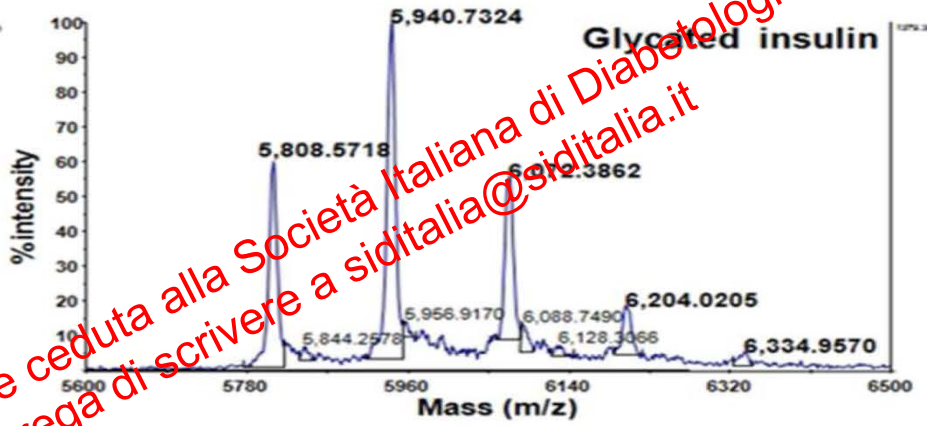


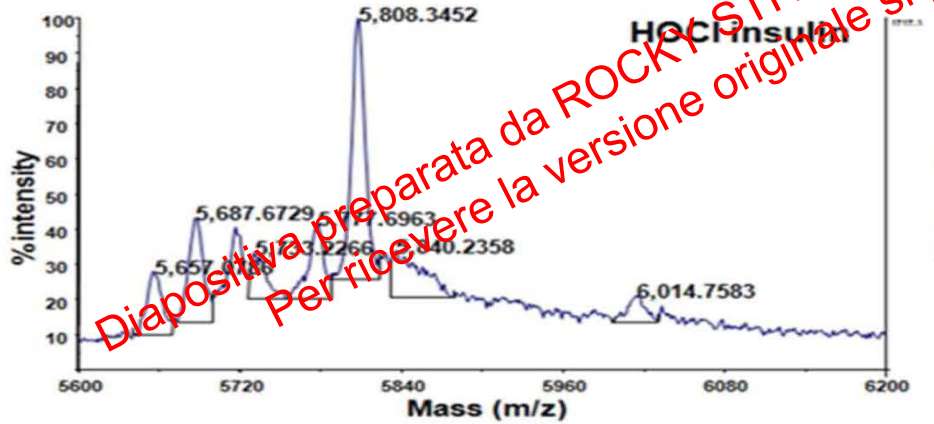
Increased light scattering

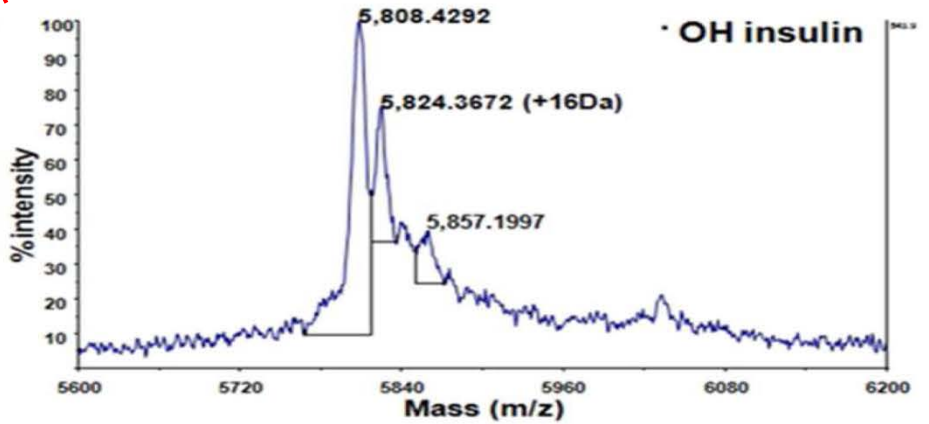
Loss of fluorescence

MS spectrum (MALDI-TOF) of native and oxPTM-INS



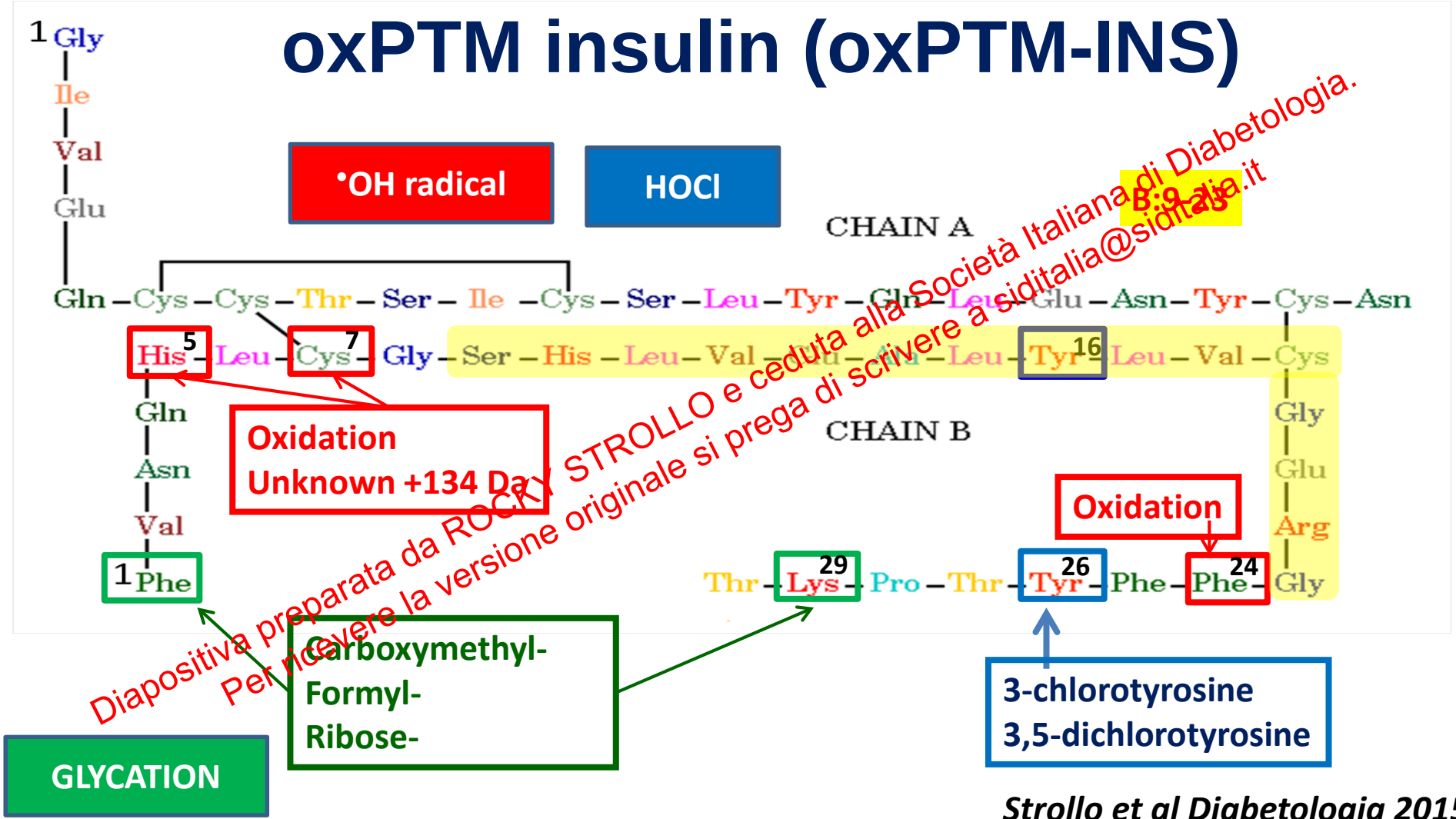






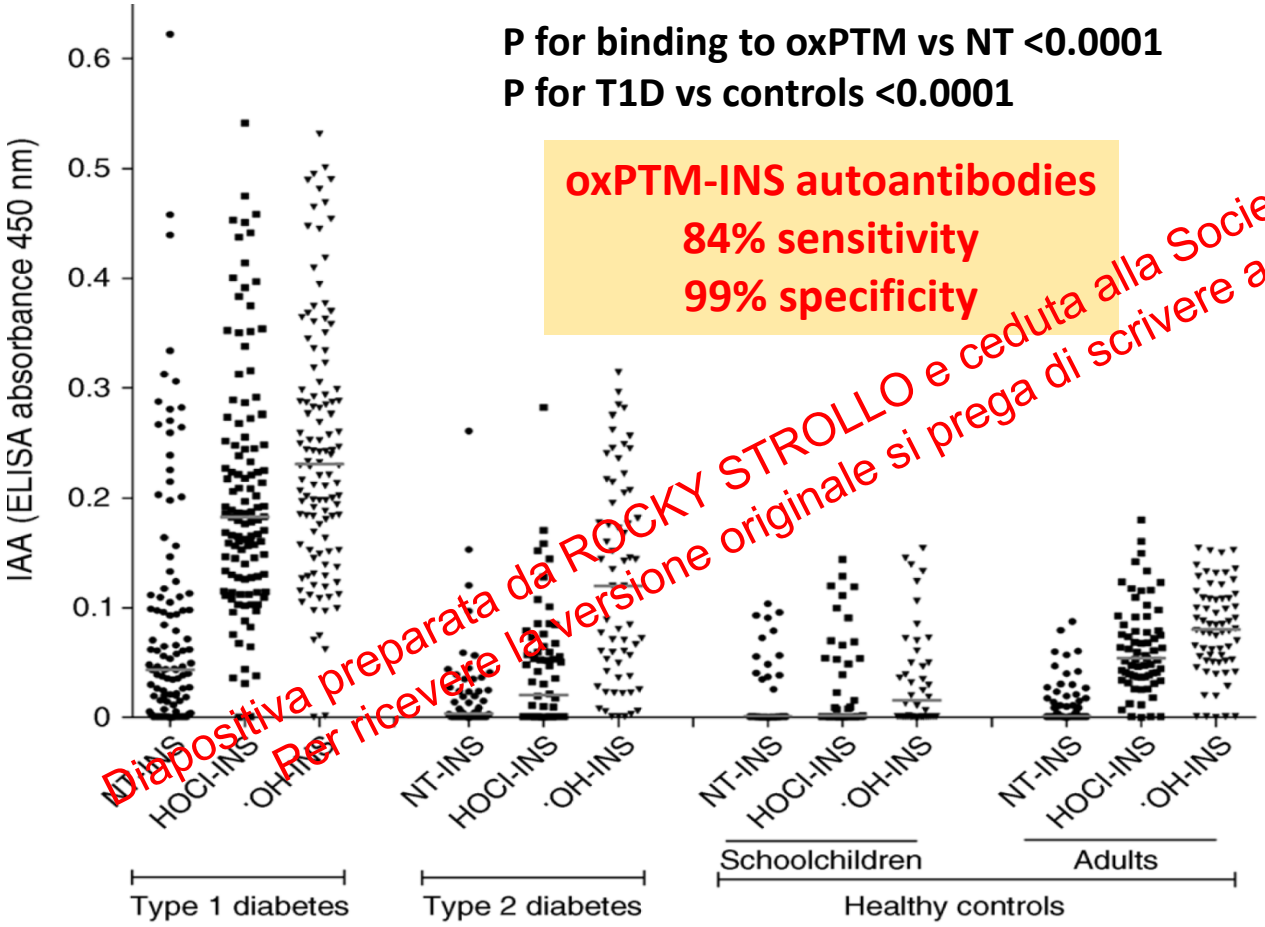
Strollo et al Diabetologia 2015

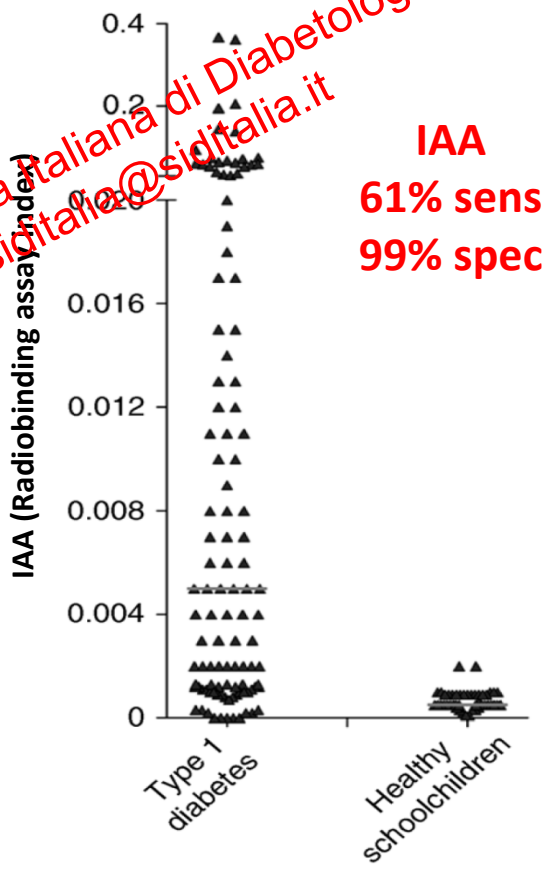
oxPTM insulin (oxPTM-INS)



Strollo et al Diabetologia 2015

T1D serum binding to native and oxPTM-INS

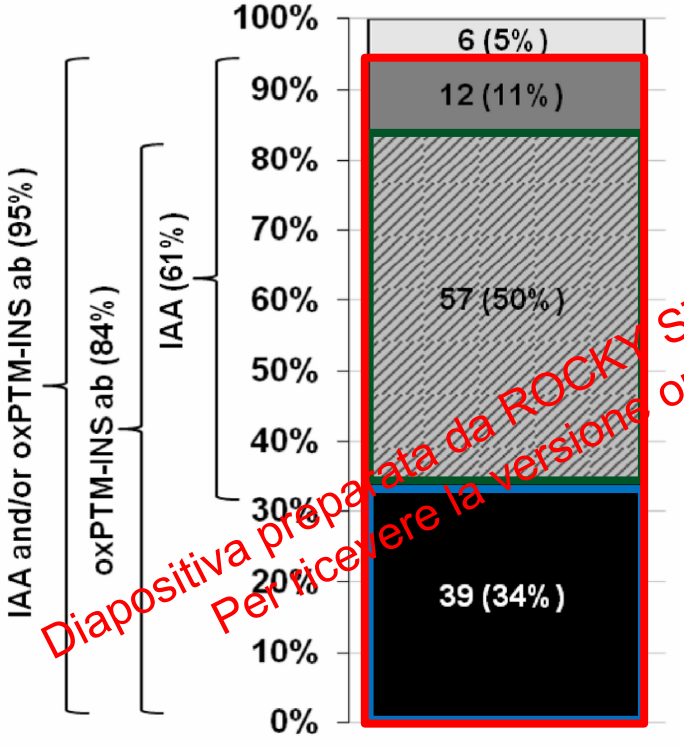




Strollo et al Diabetologia 2015

Anticorpi anti-insulina “ossidata” (oxPTM-INS) bio-marker diagnostico di diabete tipo 1

Superioritá rispetto all’attuale dosaggio per gli autoanticorpi anti-insulina (IAA)



Anti-oxPTM-INS e IAA coesistono nel 50% dei soggetti con diabete tipo 1

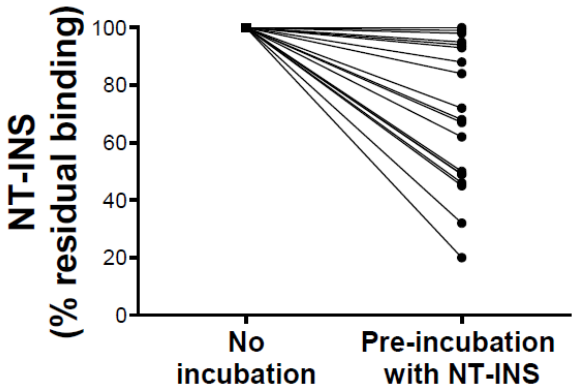
Anti-oxPTM-INS identificano oltre il 30% dei soggetti negativi al dosaggio convenzionale per IAA

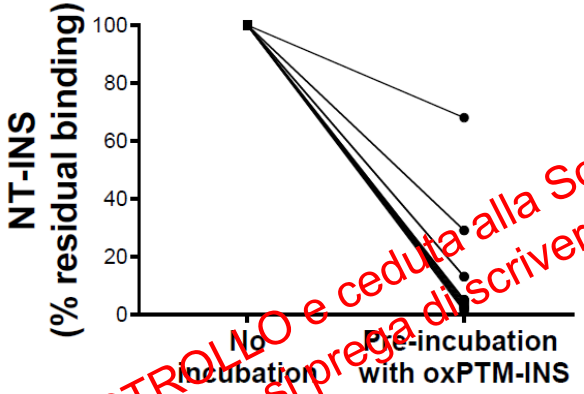
In combinazione, oxPTM-INS e IAA idetificano il 95% dei soggetti con diabete tipo 1

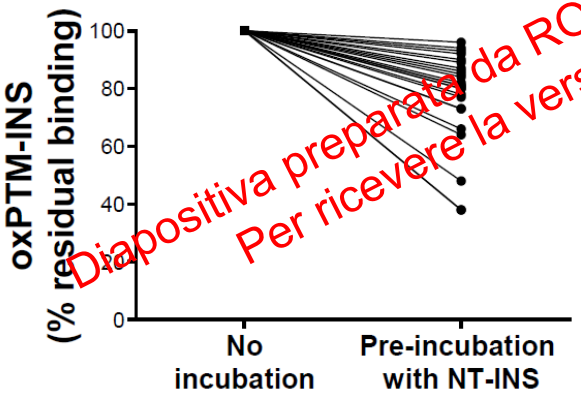
Strollo et al. 2015

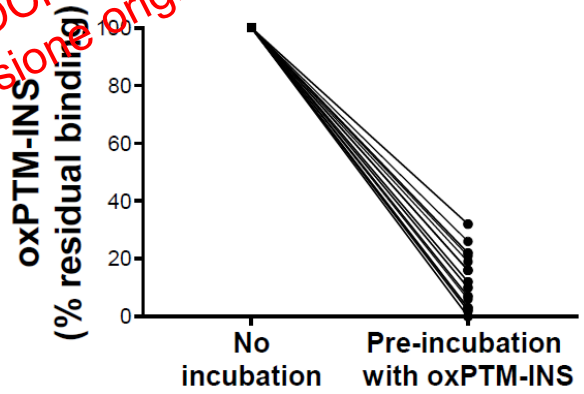
Serum binding specificity to oxPTM-INS

Competitive ELISA

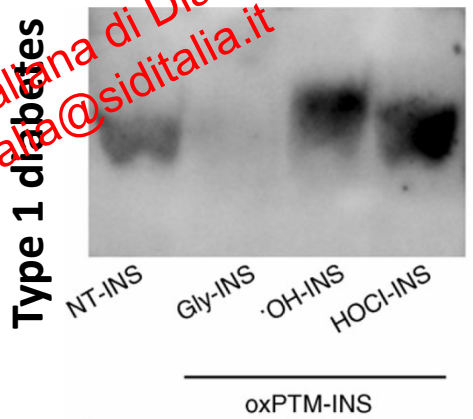


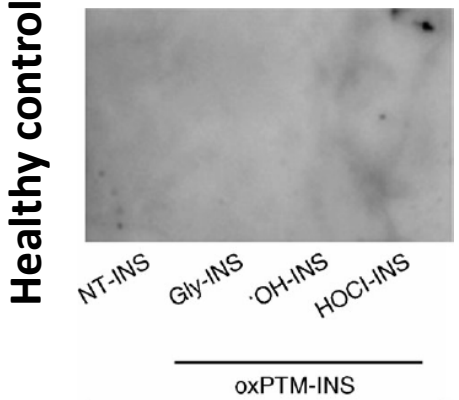






Western blot





HYPOTHESIS 3

oxPTM-INS antibodies (oxPTM-INS-Ab) as biomarker of type 1 diabetes

- **Are oxPTM-INS-Ab present before the clinical onset of type 1 diabetes?**
- **What is the ability of oxPTM-INS-Ab to identify children progressing to type 1 diabetes?**
- **May oxPTM-INS-Ab improve early diagnosis/prediction of type 1 diabetes in comparison with the other islet-autoantibodies: IAA, GADA, IA-2A, ZnT8A?**

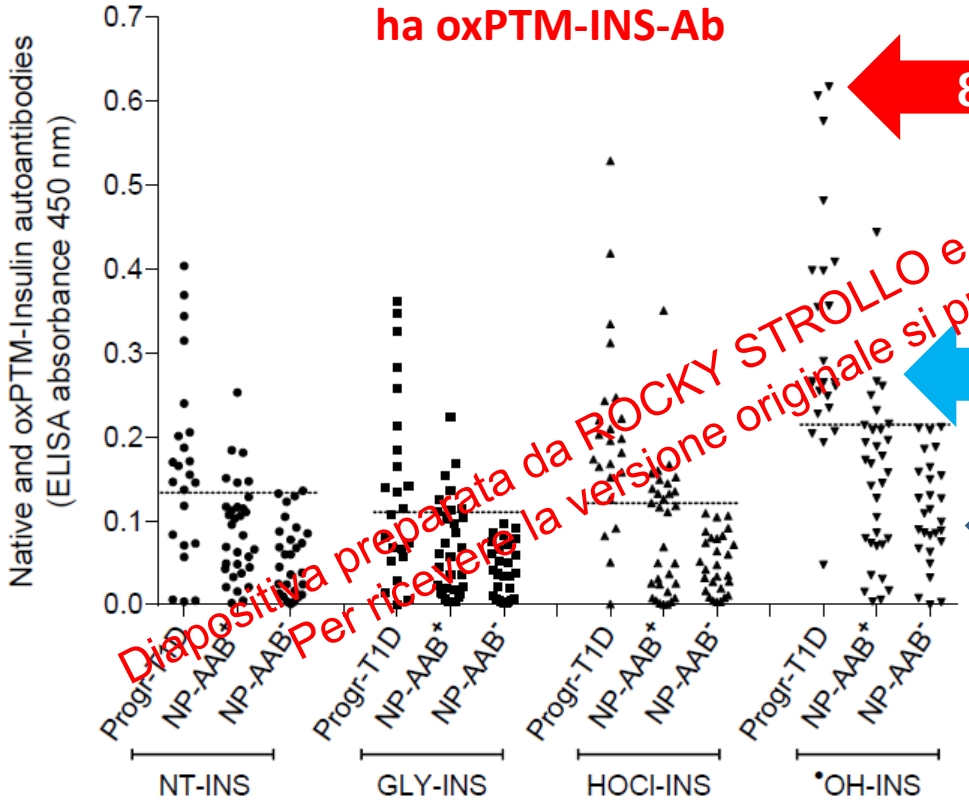
The "*All Babies In Southeast Sweden*" ABIS Study cohort

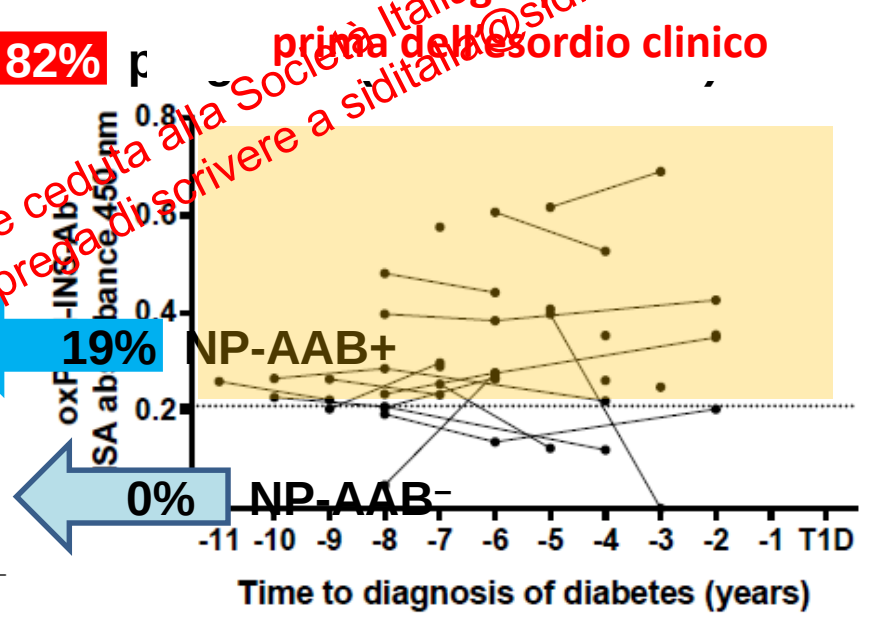
Over 17,000 children from the general population were followed from birth to nearly twenty years old with regular evaluation of islet-autoantibodies: IAA, GADA, IA-2A, ZnT8A

Characteristics	T1D progressors Progr-T1D (n=23)	Children non progressing to T1D	
		NP-AAB^{+} (n=32)	NP-AAB^{-} (n=31)
Age at baseline (years, SD)	6.17±1.49	7.61±2.42	7.13±2.07
Sex, males	15 (65%)	21 (66%)	17 (55%)
Multiple positive islet-autoantibodies	18 (78%)	8 (25%)	NA
HLA susceptible phenotypes	47%	18.75%	0
Years of follow-up (median, IQR)	5.1 (3.2-7.7) prior to T1D diagnosis	10.8 (7.7-12.8)	

Gli oxPTM-INS-Ab precedono l'esordio clinico del diabete tipo 1

La maggior parte dei futuri diabetici ha oxPTM-INS-Ab



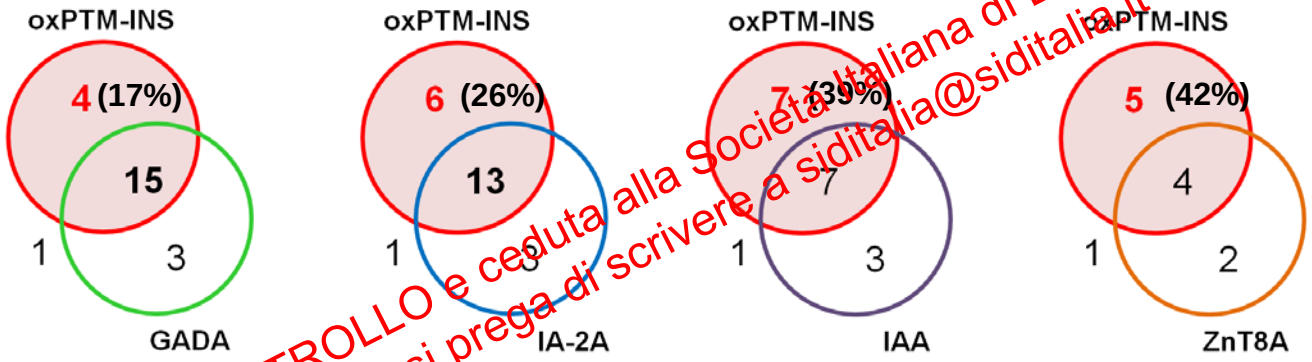


oxPTM-INS-Ab già presenti 10 anni prima dell'esordio clinico

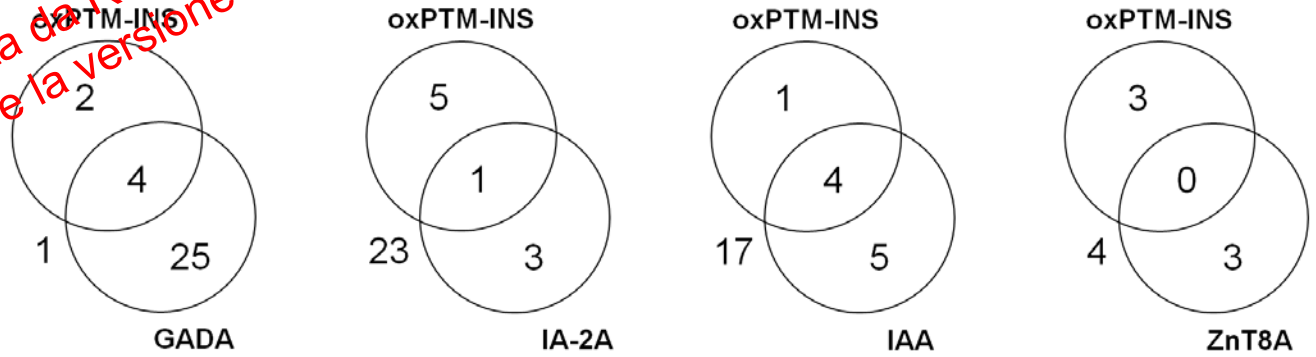
Strollo et al. Diabetologia 2017

oxPTM-INS-Ab in combinazione con gli altri anticorpi beta-cellulari: GADA, IA-2A, IAA e ZnT8A

Progr-T1D

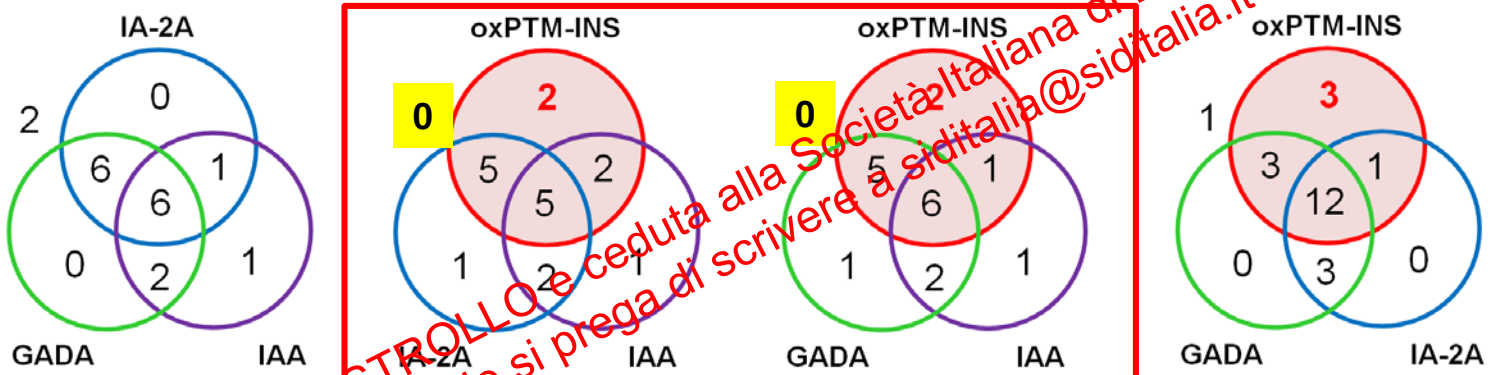


NP-AAB+

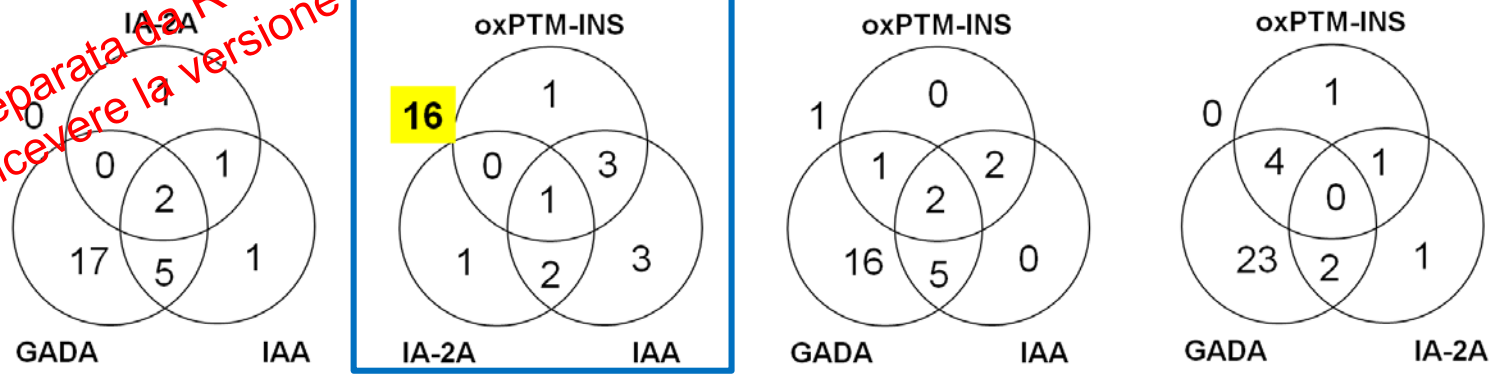


oxPTM-INS-Ab in combinazione con due autoanticorpi “standard”

Progr-T1D



NP-AAB+



Conclusioni (1)

- **Le specie reattive dell’ossigeno possono generare neoepitopi antigenici e favorire risposte autoimmuni nel diabete tipo 1**
- **L’insulina “ossidata” (oxPTM-INS) è un autoantigene nel diabete tipo 1**
- **Anticorpi contro oxPTM-INS (oxPTM-INS-Ab) sono presenti nella maggior parte degli individui con diabete tipo 1**

Conclusioni (2)

- **La immuno-reattivitá contro oxPTM-INS è giá presente vari anni prima dell'esordio clinico della patologia**
- **oxPTM-INS-Ab possono identificare precocemente bambini che svilupperanno il diabete**
- **oxPTM-INS-Ab possono migliorare la predizione del diabete tipo 1 se inclusi nel panel autoanticorpale beta-cellulare**
- **Una nuova visione della immunopatogenesi del diabete tipo 1: "Antigenicentrismo vs. Autoimmunocentrismo"**

Acknowledgments

Ahuva Nissim **Paolo Pozzilli**

Chiara Vinci, Nicola Napoli, Raffaella Buzzetti, Paola Rizzo, Claudio Tiberti, Maria Luisa Spoletini, Johnny Ludvigsson
IMDIAB study group (Rome); ABIS study (Sweden)