

In situ Detection of Islet-Antigen specific CD8 T Cells in Insulitic Lesions of New-onset and Long-term Type 1 Diabetes

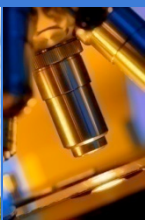
Coppieters & von Herrath

Aims:

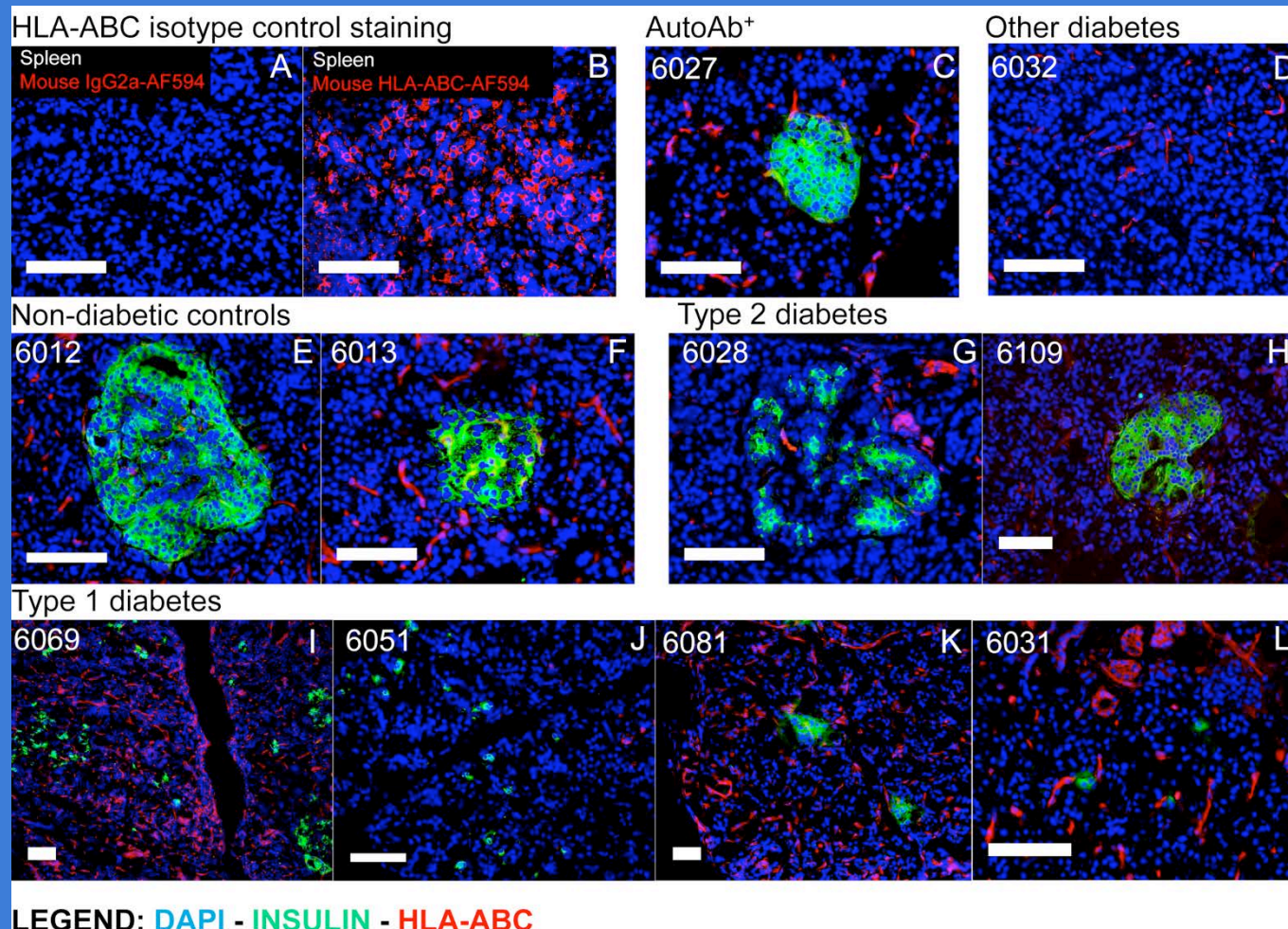
- Persistence of pancreatic insulin
- MHC class I hyperexpression
- CD8 T cell infiltration
- Specificity of the infiltrating CTL

Methods:

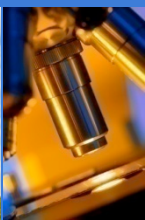
- Frozen sections
- Validated primary antibodies
- Tetramers validated by flow cytometry
- In situ protocol by McGavern et al (Nat. Immunol. 2002)



Evaluation of MHC Class I expression patterns using Dako Clone W6/32

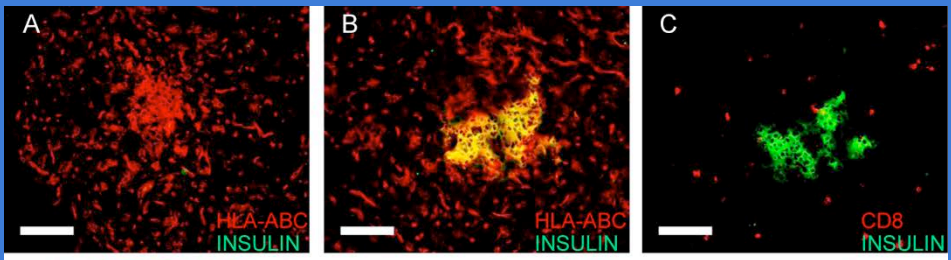


nPOD
Network for Pancreatic Organ
Donors with Diabetes

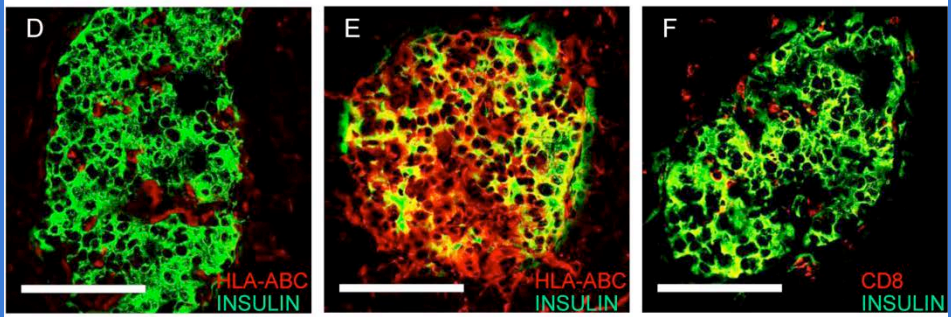


Marked MHC Class I hyperexpression in a subset of T1D cases

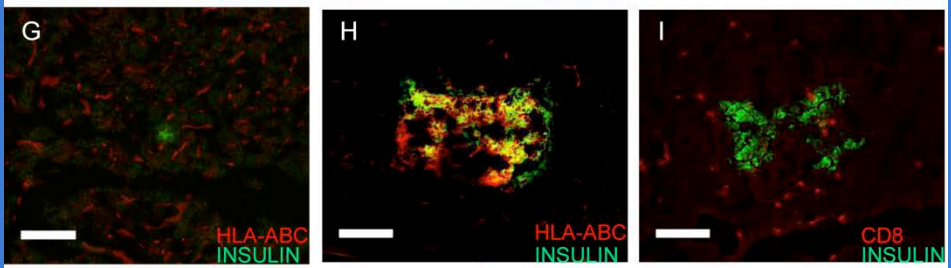
nPOD 6052
1 year



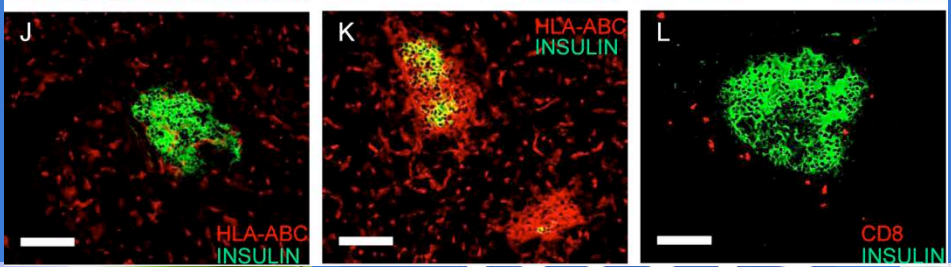
St. Vincent's
3 years



nPOD 6070
7 years



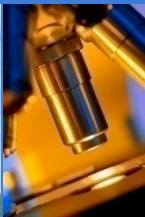
nPOD 6046
8 years



No detectable
C-peptide

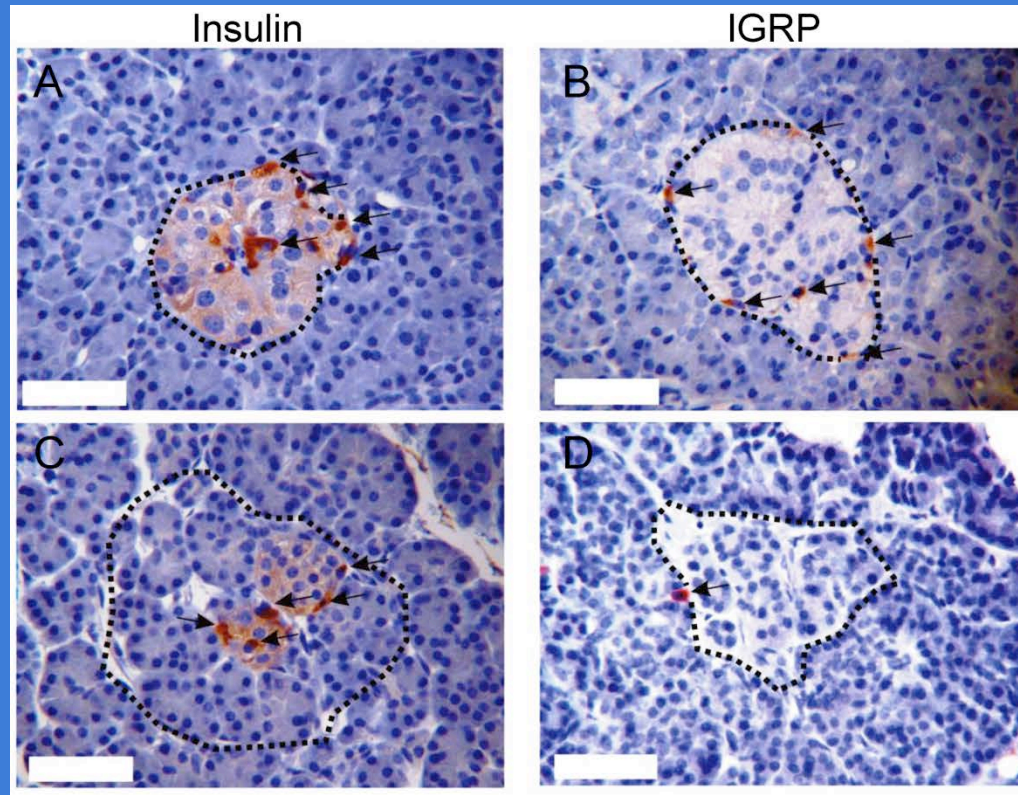


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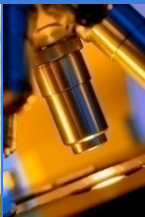


In situ tetramer staining – recent onset patients (Francesco Dotta)

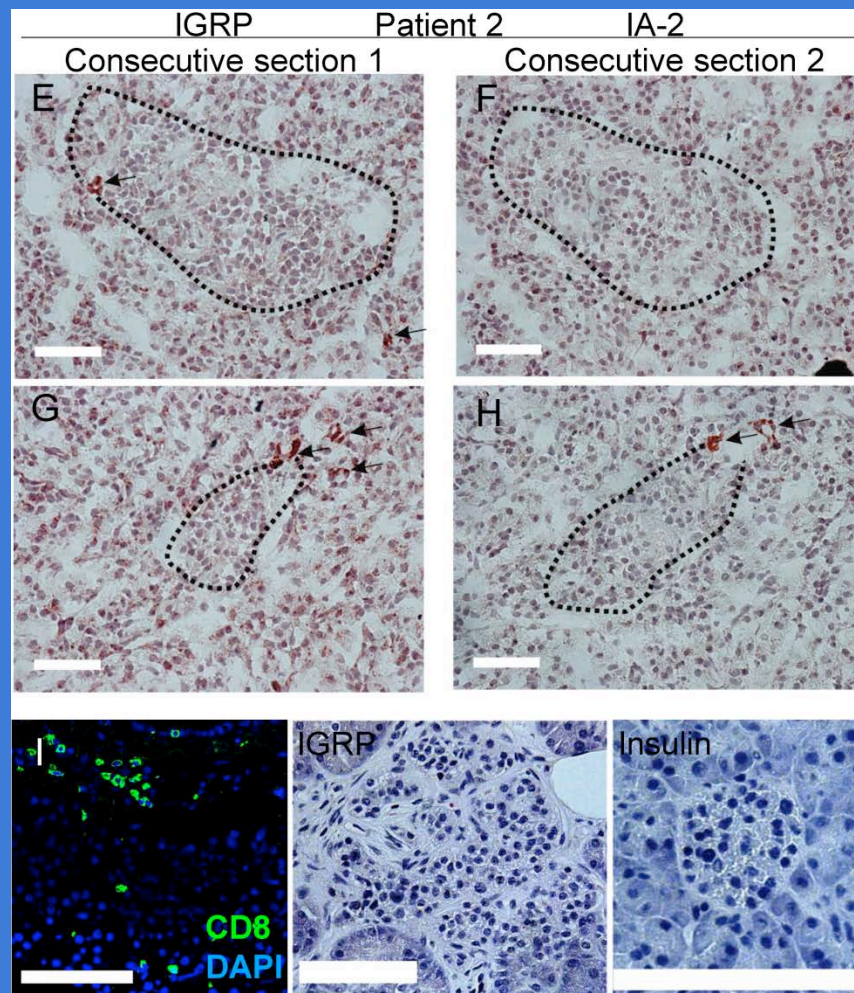
Insulin B10-18
IA-2 797-805
IGRP 265-273
PPI 15-23
GAD65 114-123
ppIAPP 5-13



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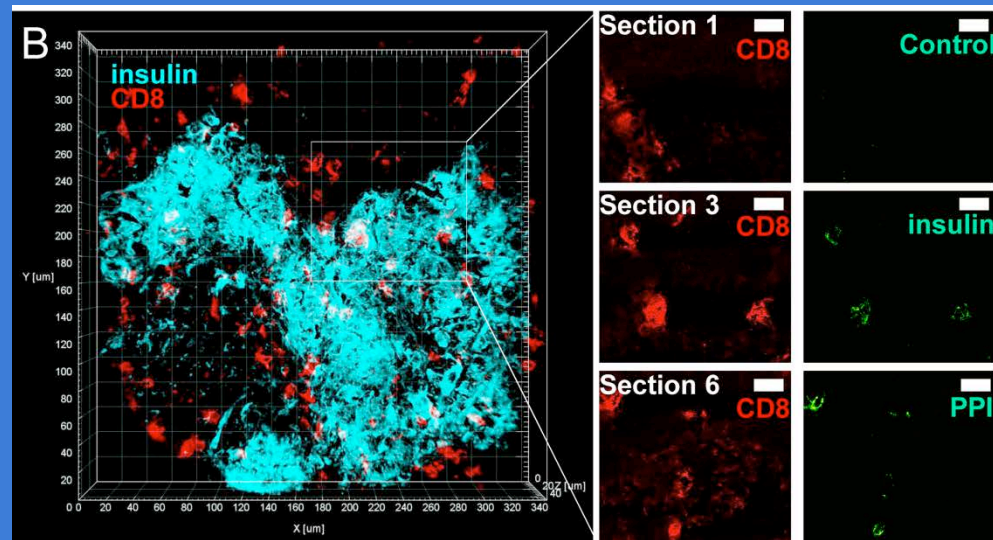
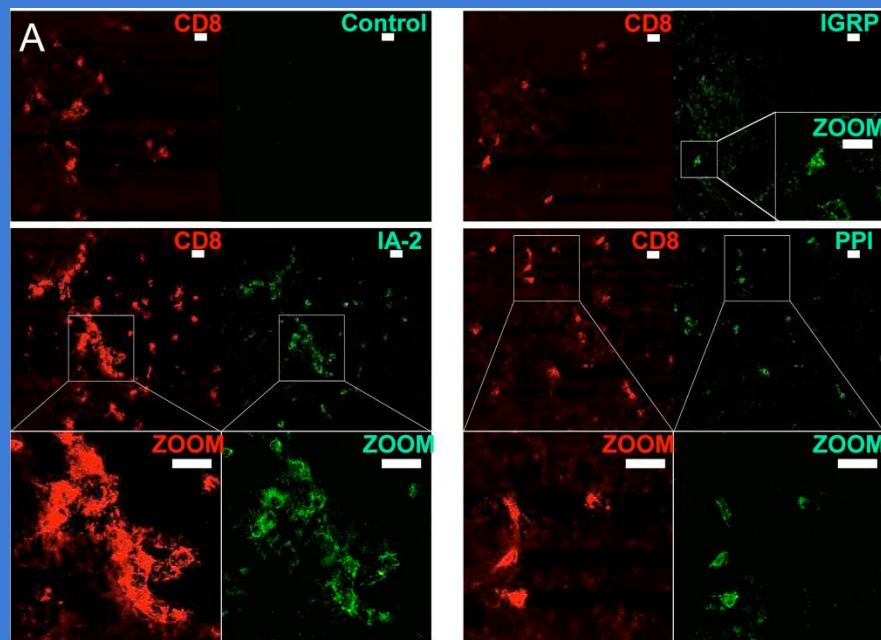
In situ tetramer staining – longstanding patient



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In situ tetramer staining – CD8 colocalization



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Overview of results

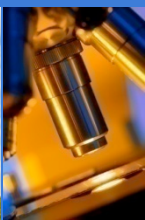
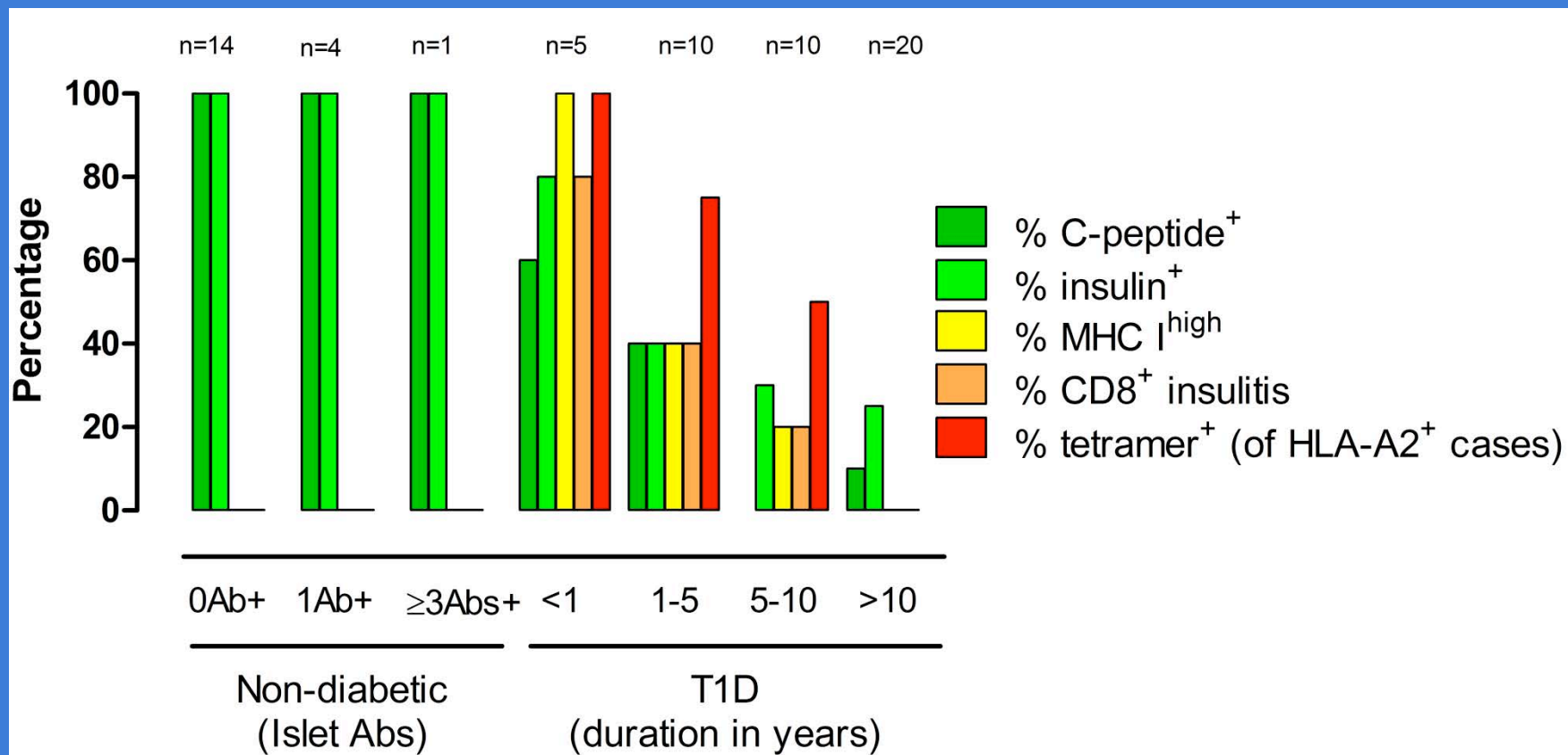
ID	Duration	Insulin+ islets	CD8+ cells/islet (islets counted)	Islet HLA class I (pattern)	Tetramer reactivity (pattern)
Recent Onset Cases					
Patient A	< 1 week	Isolated cells only	8.8 (160)	Positive	Insulin, IGRP (monospecific)
Patient B	1 week	None	8.4 (125)	Positive	Insulin, IGRP (monospecific)
Patient C	10 weeks	Partial loss	<1 (128)	Positive	HLA-mismatched control (no staining)
Patient D	6 months	25%	3.5 (116)	Positive	Insulin
Patient E	9 months	50%	4.1 (150)	Positive	Insulin
Longstanding Cases					
Patient 1	1 year	<50%	3.2 (140)	Positive	IGRP
6052	1 year	10%	4.3 (40)	Positive	IGRP, IA-2, insulin (mono-/multispecific)
Patient 3	3 years	15%	1.5 (70)	Positive	No staining
Patient 4	3 years	39%	6.5 (20)	Positive	IGRP, GAD65 ,pIAPP, insulin (multispecific)
6070	7 years	70%	9.4 (30)	Positive (tail region only)	IGRP, GAD65 ,PPI, insulin, IA-2 (mono-/multispecific)
6046	8 years	70%	3.5 (20)	Positive (lobular)	No staining



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Overview of results



Acknowledgements

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