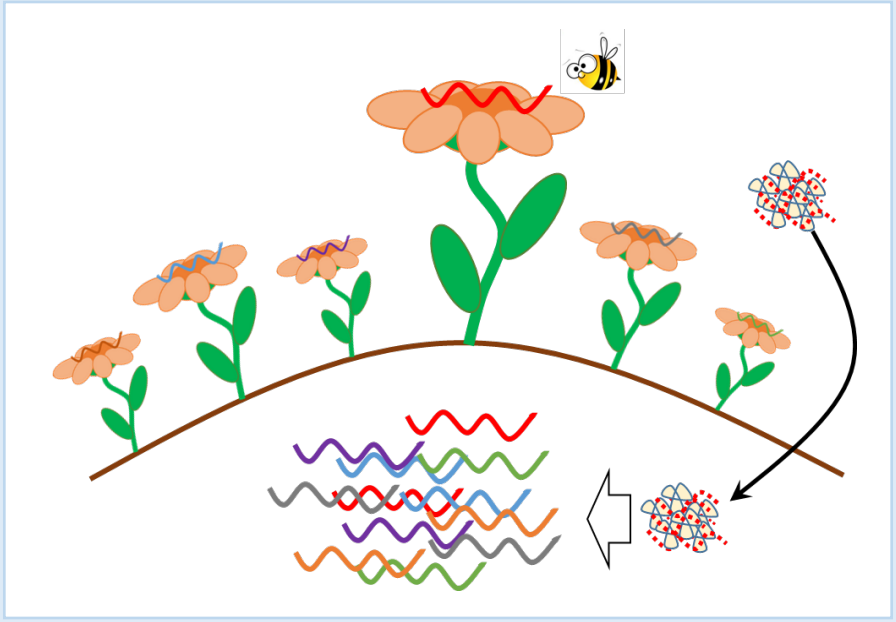
# Endotope

*A platform for the efficient presentation of endogenously encoded epitopes to achieve optimal and balanced immune responses in immunity and tolerance*



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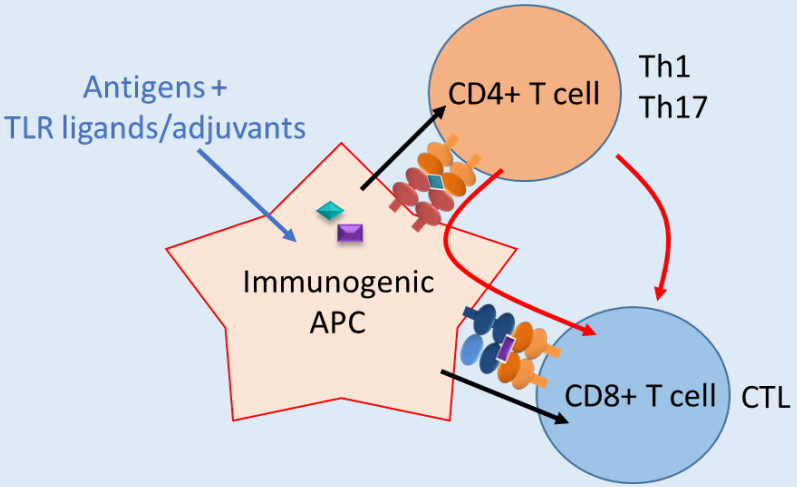
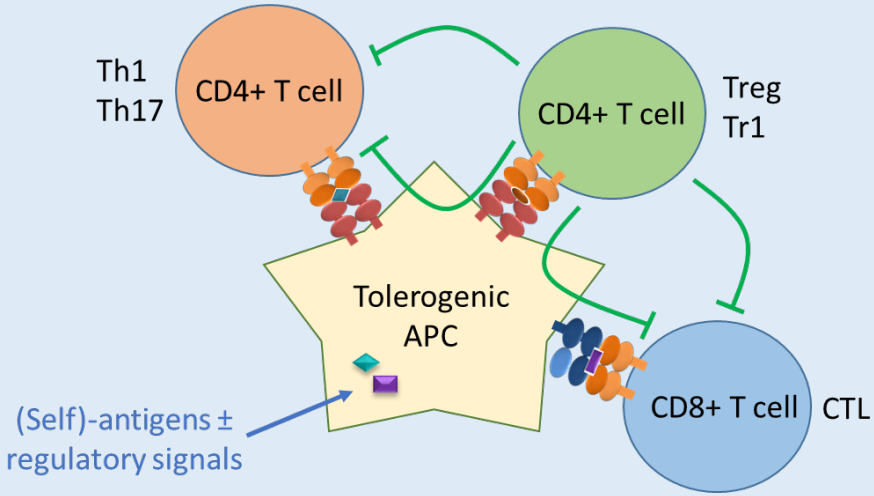
# CD4-CD8 T cell cooperation

In immunity as in tolerance, cooperation between CD4+ and CD8+ T cells is critical.
Cooperation is both direct and indirect (by modulation of antigen-presenting cell (APC) function).
Co-presentation of epitopes from multiple antigens by the same APC is important to achieve this cooperation.




## Immunity

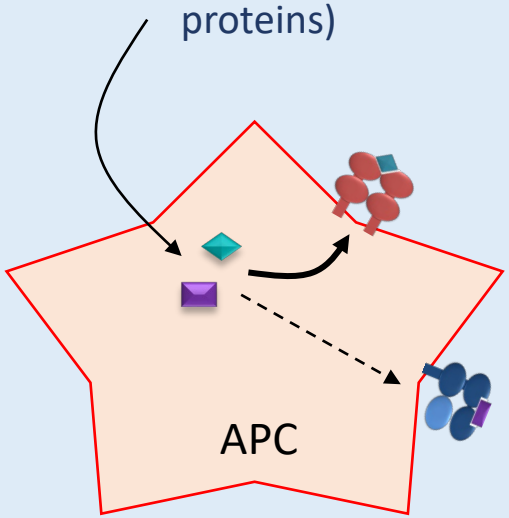
CD4+ T cells provide help to enhance immune responses

## Tolerance

CD4+ regulatory T cells control autoimmune responses

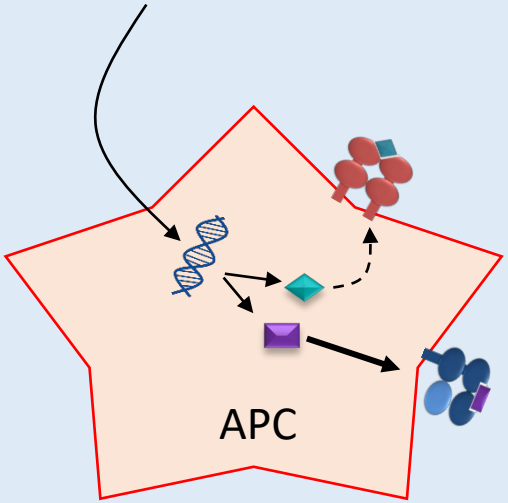
# Types of antigen delivery

Exogenous antigens (peptides, recombinant proteins)

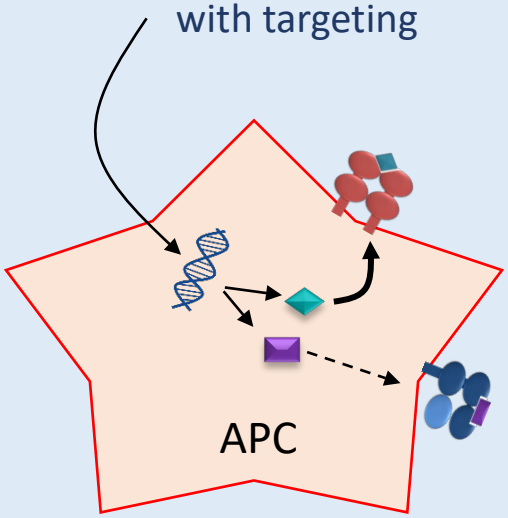



(if peptides, co-delivery not guaranteed)

Endogenous antigens (DNA/mRNA vaccines)




Endogenous antigens (DNA/mRNA vaccines) with targeting

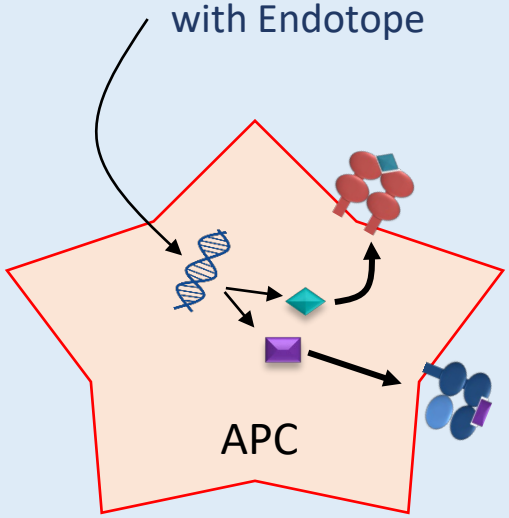



(e.g. UNITE platform, Immunomic)

All types of antigen deliveries can achieve presentation on both class I and class II MHC, but only one type of presentation is optimal. When using soluble peptides/proteins, co-presentation is not guaranteed!

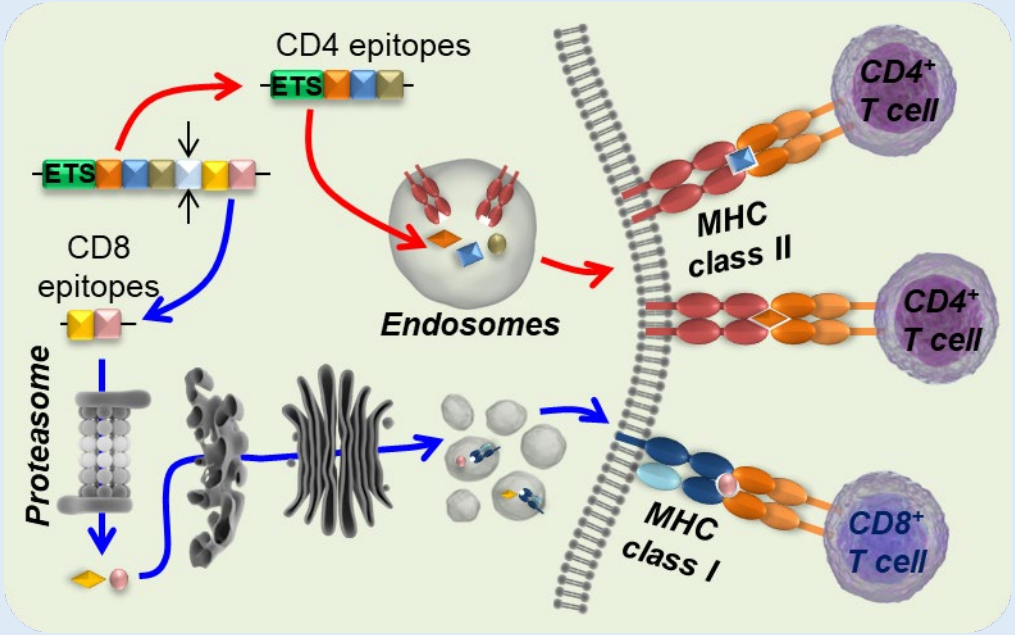
# The Endotope delivery

Endogenous antigens
(DNA/mRNA vaccines)
with Endotope




Patent:
US 10,238,741
(03/2019)

<u>Epitope-based platform:</u>
Selected (major) epitopes from diverse antigens
Neoepitopes not present in native antigen (e.g. PTM epitopes, hybrid peptides, etc)




➔ Co-presentation is guaranteed
➔ Optimal engagement of both CD4+ and CD8+ T cells is achieved

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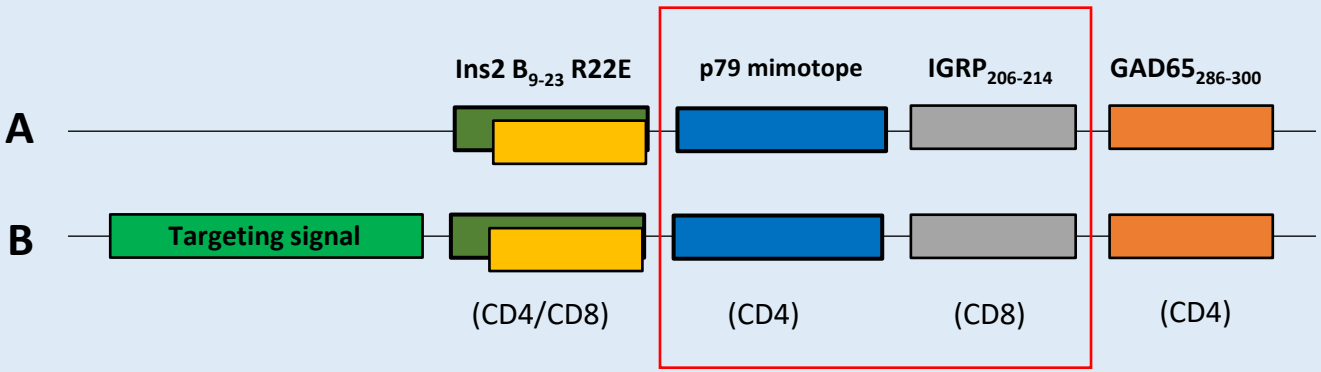
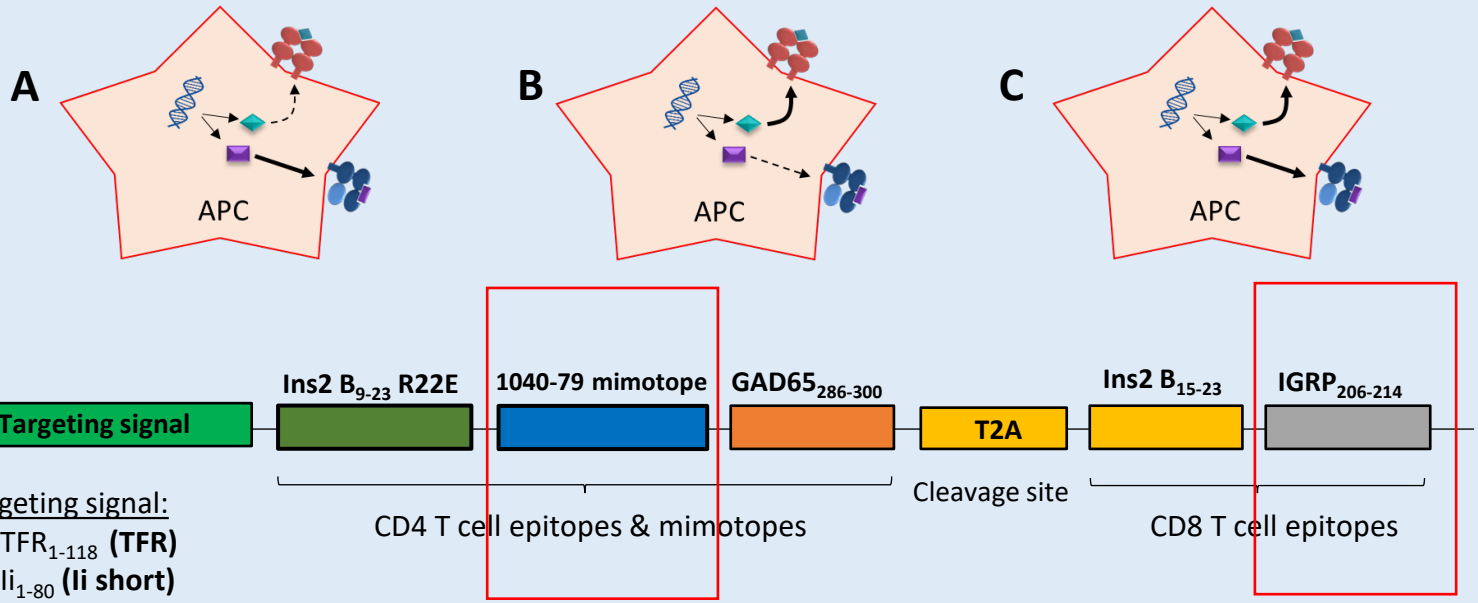
# The Endotope delivery (In vitro studies)




Targeting signal:

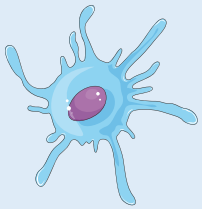
- ➔ $TFR_{1-118}$ **(TFR)**
- ➔ $Ii_{1-80}$ **(Ii short)**
- ➔ $Ii_{1-214}$ **(Ii long)**
- ➔ $CD16_{1-23}$/$LAMP\text{-}1_{166-382}$ **(Lamp1)**



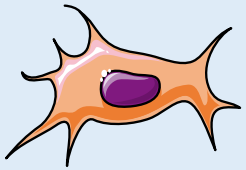
*Dastagir et al., 2017, MTMCD*

# The Endotope delivery

APC



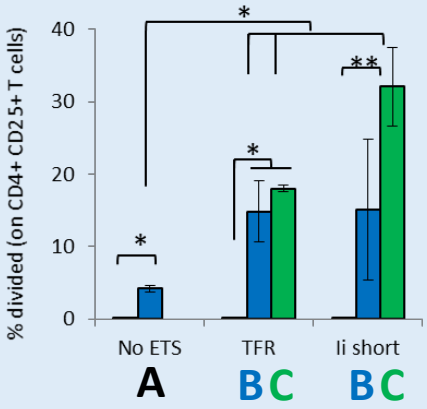
Dendritic cell

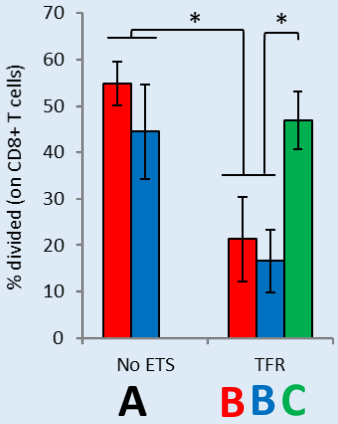


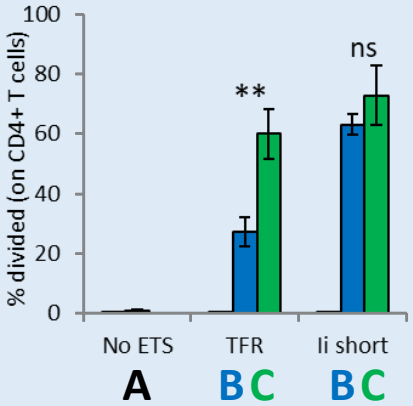
Stromal cell

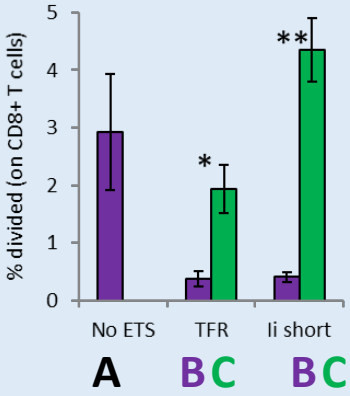
p79 mimotope (CD4)

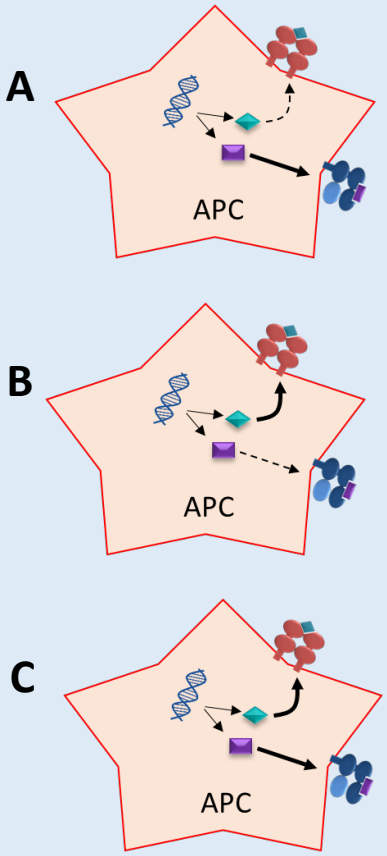
IGRP$_{206-214}$ (CD8)

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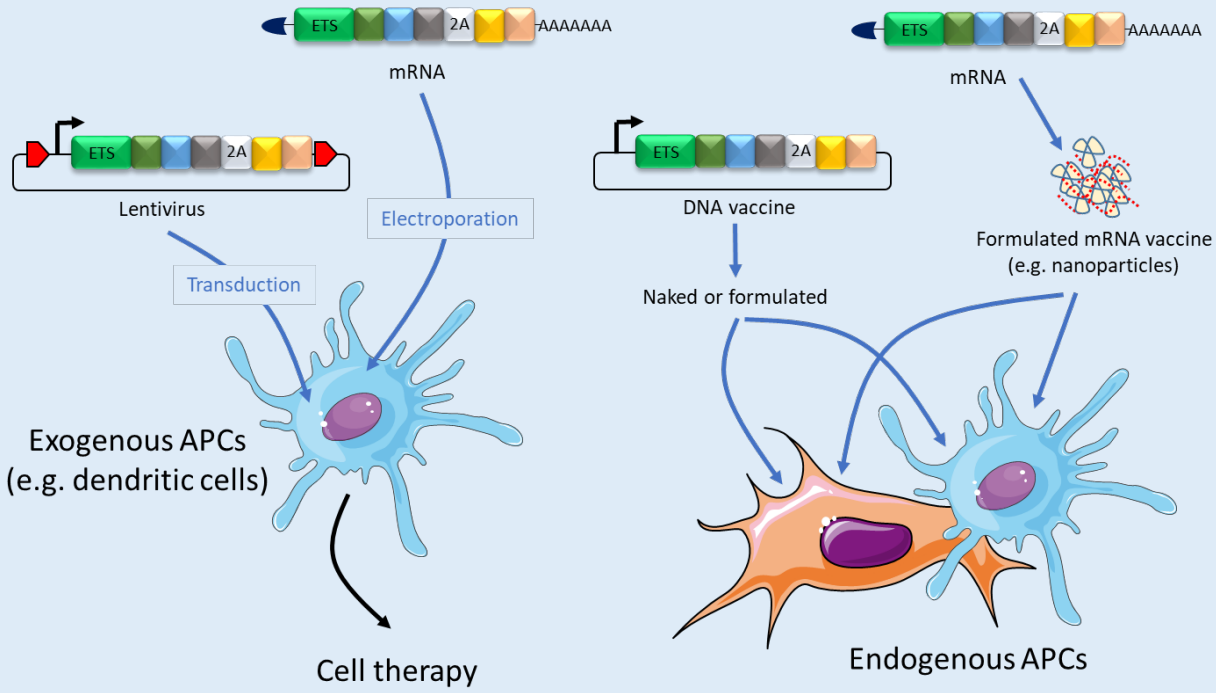
*Reference: Dastagir et al., 2017, PMID: 28344989*

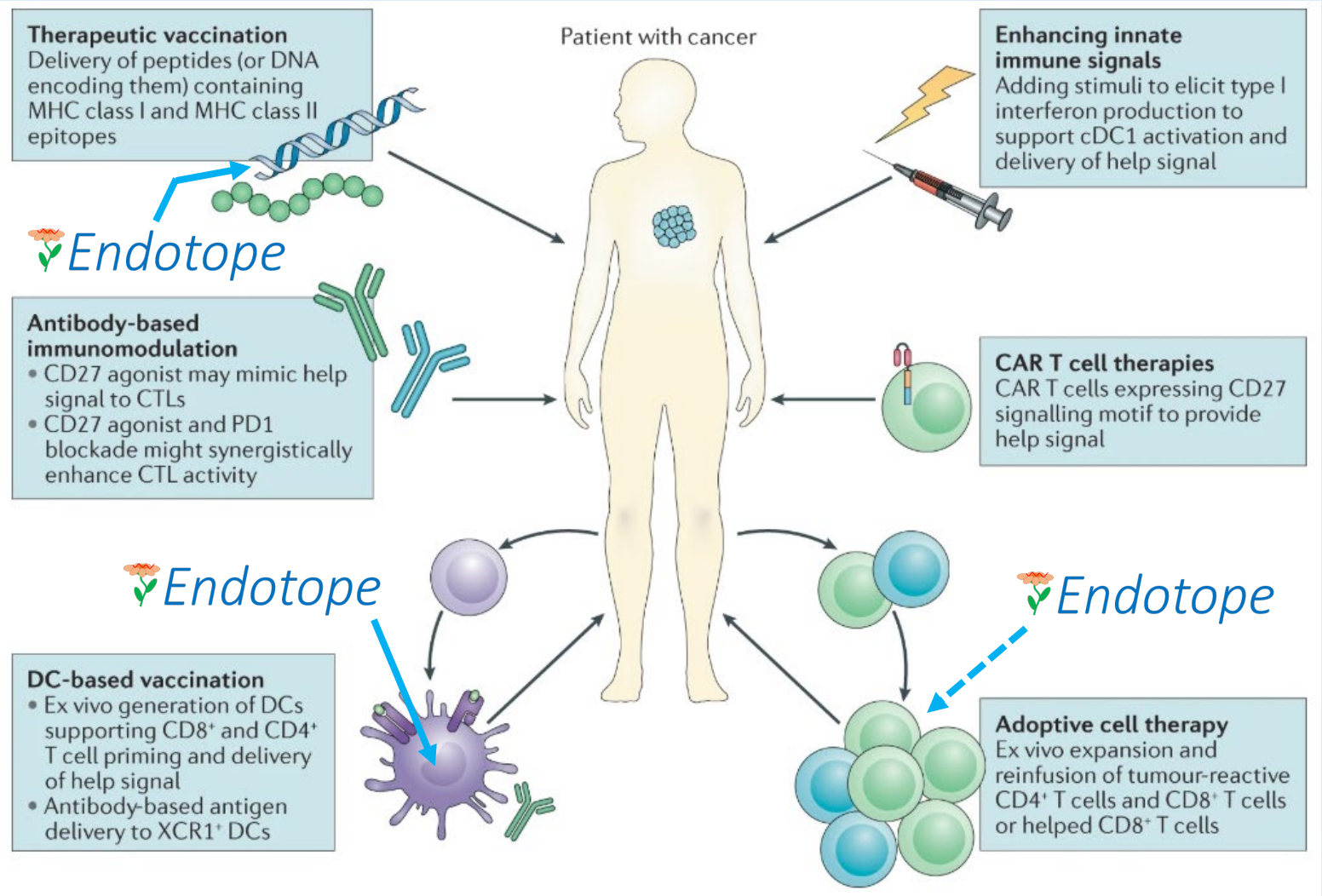
# Different Endotope platforms for immunotherapy




| Delivery system | Cancer immunotherapy | Tolerogenic immunotherapy (T1D) |
|---|---|---|
| DNA vaccine | Preclinical/clinical (full antigens, epitopes) | Preclinical/clinical (full antigen only) |
| RNA vaccine | Preclinical/clinical (full antigens, epitopes) | Not reported |
| DC vaccine (mRNA) | Preclinical/clinical (full antigens, epitopes) | Not reported* |
| DC vaccine (transduced) | Preclinical/clinical (full antigens, epitopes) | Not reported* |

*Preclinical/clinical studies have used full antigen or peptide pulsing.

# Implications / applications




*Borst et al., 2018, Nature Rev. Immunol 18:635-647.*

# Research featuring Endotope

Publications:


● Dastagir SR, Postigo-Fernandez J, Xu C, Stoeckle JH, Firdessa-Fite R, Creusot RJ. Efficient presentation of multiple endogenous epitopes to both CD4+ and CD8+ diabetogenic T cells for tolerance. Mol. Ther. Methods Clin. Dev. 2016; 4: 27-38.

● Postigo-Fernandez J, Creusot RJ. A multi-epitope DNA vaccine enables a broad engagement of diabetogenic T cells for tolerance in Type 1 diabetes. J. Autoimmun. 2018; 98:13-23 (Epub 11/17/18).

● Firdessa-Fite R, Creusot RJ. Nanoparticles versus dendritic cells as vehicles to deliver mRNA encoding multiple epitopes for immunotherapy. Mol. Ther. Methods Clin. Dev. 2019; 16:50-62 (Epub 11/11/2019).

● Gonzalez Badillo F, Zisi Tegou F, Wright S, Scully M, Harwell L, Postigo-Fernandez J, Creusot RJ, Tomei A. Tissue-engineered stromal reticula to study lymph node fibroblastic reticular cells in Type I diabetes. Cell. Mol. Bioeng. 2020; published online (https://doi.org/10.1007/s12195-020-00627-y).

● Firdessa-Fite R, Toussaint-Moreau V, Stock F, Nyamay'antu A, Erbacher P, Creusot RJ. Promising non-viral vector for efficient and versatile delivery of mRNA for antigen-specific immunotherapy. Under revision for Cell & Gene Therapy Insights. 2020

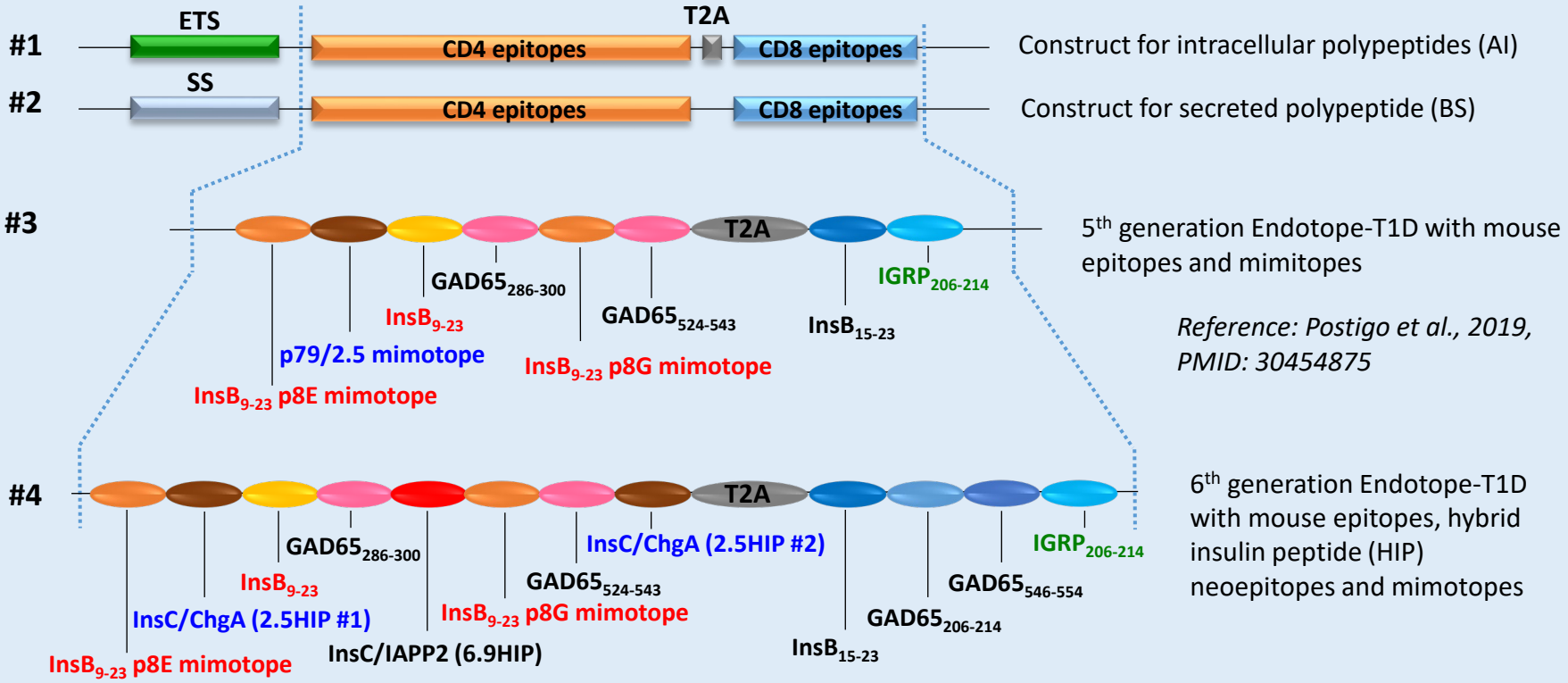

Current collaborators on research featuring Endotope:

UNIVERSITY OF MIAMI

OSSIUM HEALTH

# T1D immunotherapy project

## Epitopes for tolerization in NOD mice




## Epitopes for tolerization in T1D patients

Endotope constructs for human epitopes may be designed based on HLA haplotypes, patient's autoantibody profile; patient's circulating specific T cell profile (multiplexed MHC dextramers analysis), etc. Simplified Endotope construct with epitopes from human antigens are currently used in translational studies (humanized mice) and in vitro studies.

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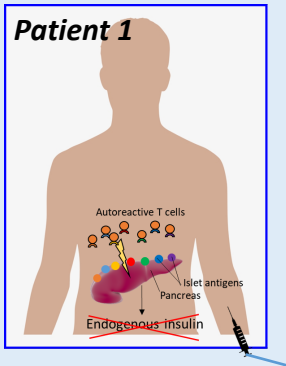
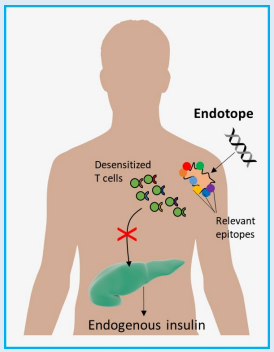
# T1D immunotherapy with precision medicine



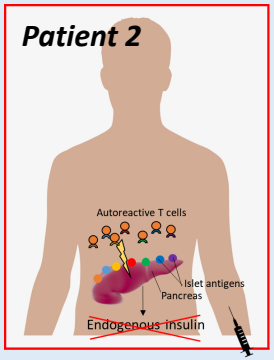

HLA haplotype
Endotype - Autoantibody profile
- Genetic risk score
- Glucose sensitivity

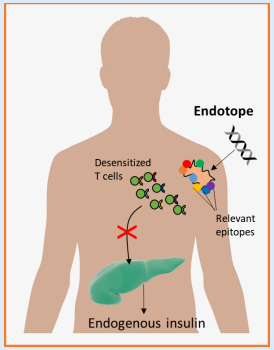
Immune profiling of beta-cell antigen-specific T cells
- Immunoprevalent T cells
- Immunodominant T cells




Blood sample

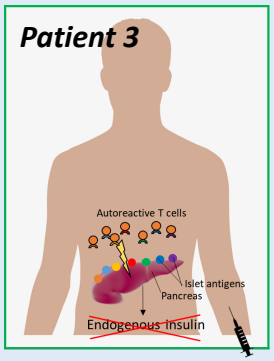
Endotope design

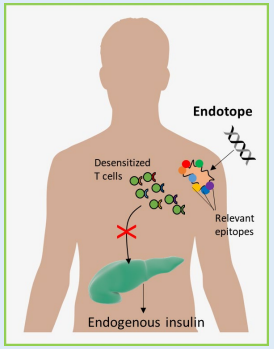



This is so far the only antigen-specific immunotherapy approach with such high level of precision

Current ASIT focusing on peptide have start to restrict their patient population

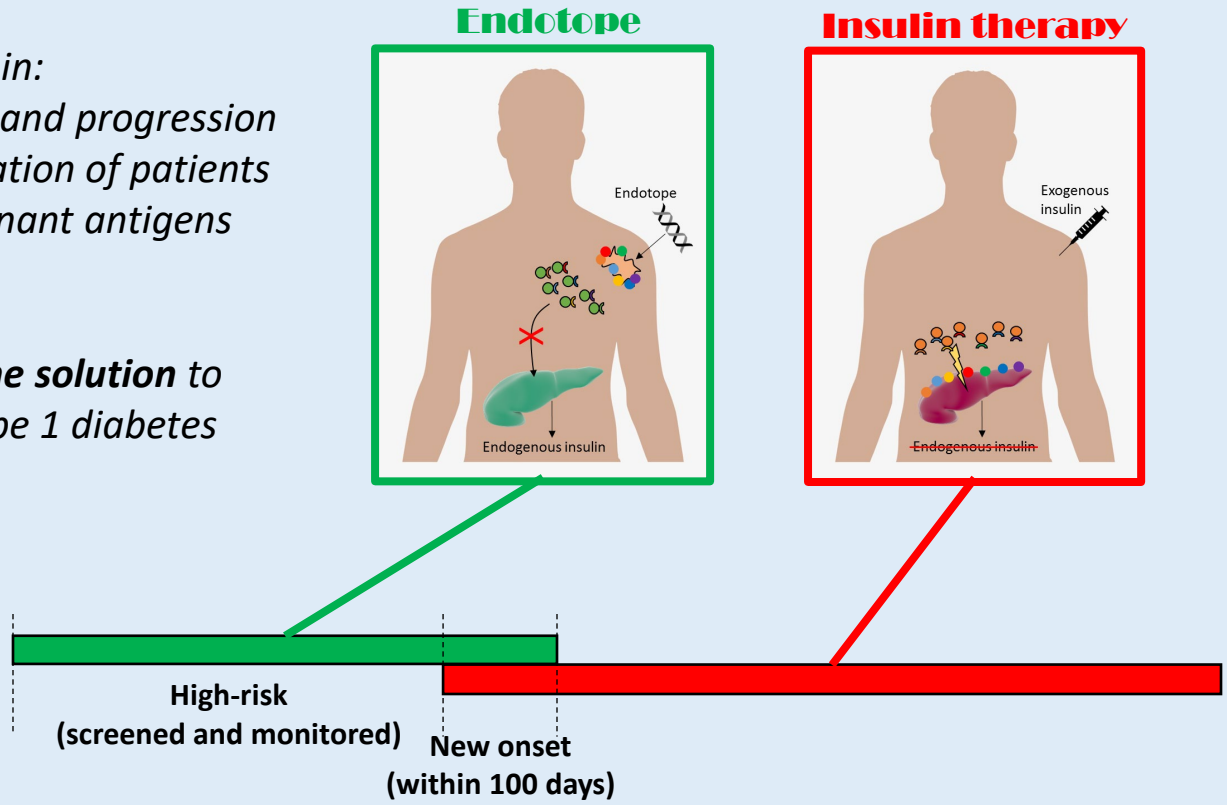


# Endotope-T1D Therapeutic Window

*<u>Endotope</u> leverages advances in:*
*(1) **biomarkers** of disease risk and progression*
*(2) characterization / stratification of patients for specific epitopes and dominant antigens*

*to provide a **precision medicine solution** to antigen-specific therapy of Type 1 diabetes*




| Early events | Phase 1 | Phase 2 | Phase 3 | Phase 4 |
|---|---|---|---|---|
| Genetic risk<br>Trigger<br>Immune response | Normal glycemia<br>≥ 2 autoantibodies | Abnormal glycemia<br>≥ 2 autoantibodies | **Clinical diagnosis**<br>**≥ 2 autoantibodies** | Long-standing T1D |

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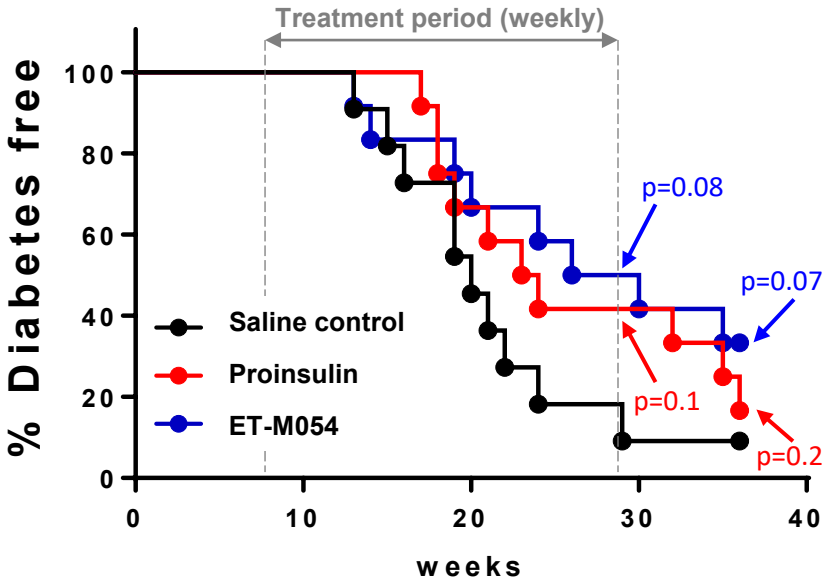
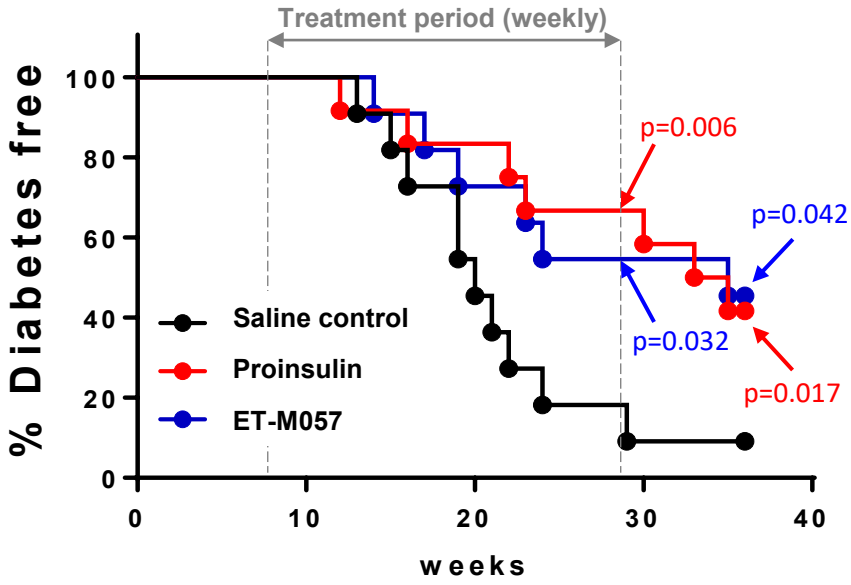
# Tolerogenic DNA vaccine

Comparison of proinsulin DNA vaccine and Endotope DNA vaccine (ET-M054/57) in NOD mice.
Mice received 50 μg per week by intramuscular or intradermal injection.
P values (against control) are shown at the end of treatment period and at the end of experiment.
Protection conferred by Endotope appears more stable than proinsulin pDNA after treatment discontinuation.

## Intramuscular



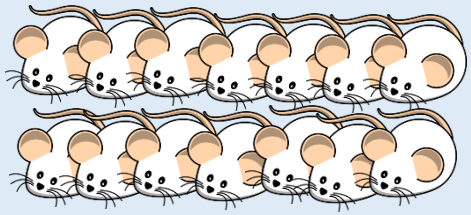

## Intradermal




Note: Endotope ET-M054 and ET-M057 are fifth generation constructs as depicted in construct #3 (slide 10) with hybrid peptides added and designed for expression by DNA vectors. ET-M054 and ET-M057 differ by the residues flanking the epitopes.

# Tolerogenic DNA vaccine

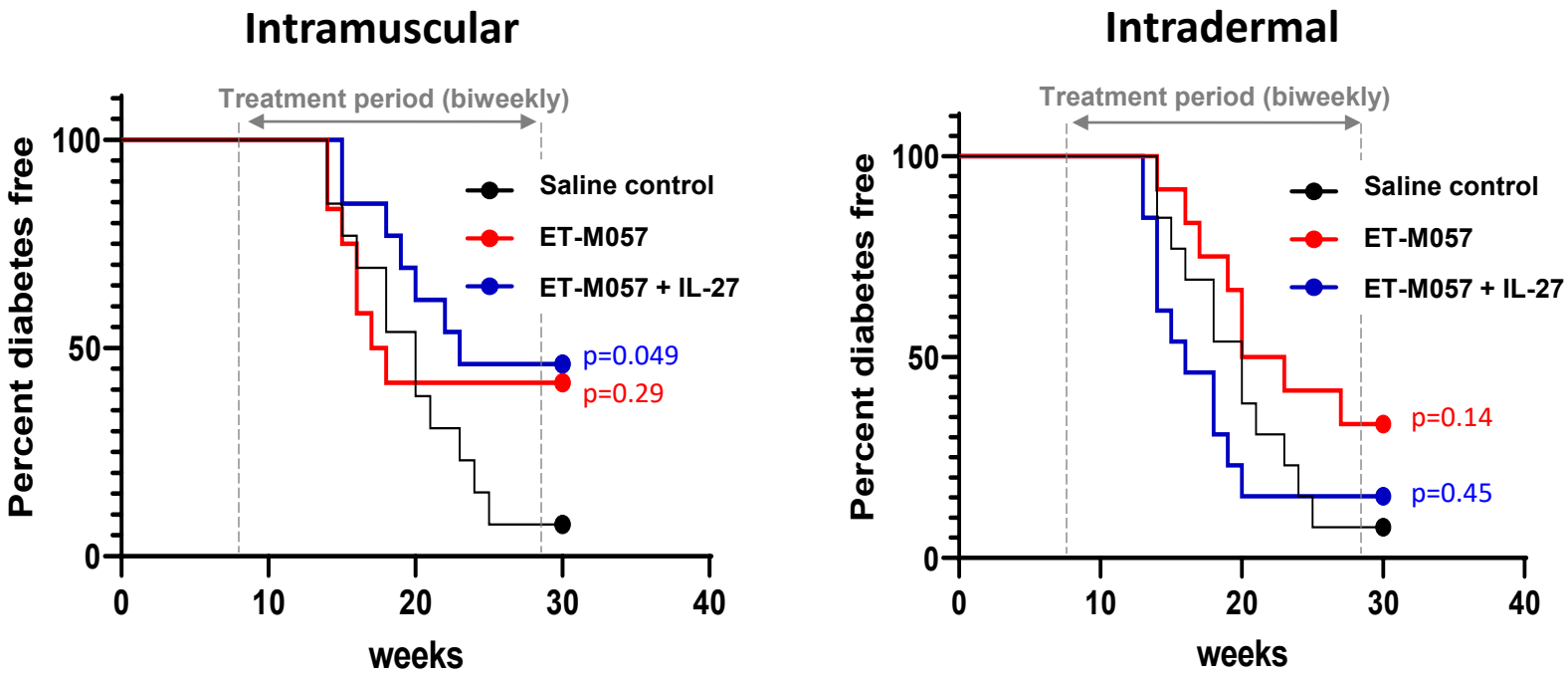
*Endotope DNA vaccine is comparable to proinsulin DNA vaccine in NOD mice but may have an advantage when translated to patients with Type 1 diabetes. Why?*

| Evaluation system / Treatment | **NOD mice** | **T1D patients** |
|---|---|---|
| |  <br>The mice are all genetically identical |  <br>Patients are very heterogeneous genetically and in disease patterns |
|  <br>Proinsulin DNA vaccine* | ✓ Proinsulin is the key driving antigen in NOD mice<br>✓ All possible proinsulin epitopes present | ✘ Proinsulin is NOT a key driving antigen in all patients<br>✓ All possible proinsulin epitopes present |
|  <br>**Endotope** DNA vaccine | ✓ Limited epitopes, but well-characterized and representing multiple antigens, customized for NOD mice | ✓ Customizable epitope representation, selected from multiple antigens based on patient autoantigen / autoantibody profile and HLA haplotypes |

*Currently in clinical trials (Tolerion)

# Tolerogenic DNA vaccine

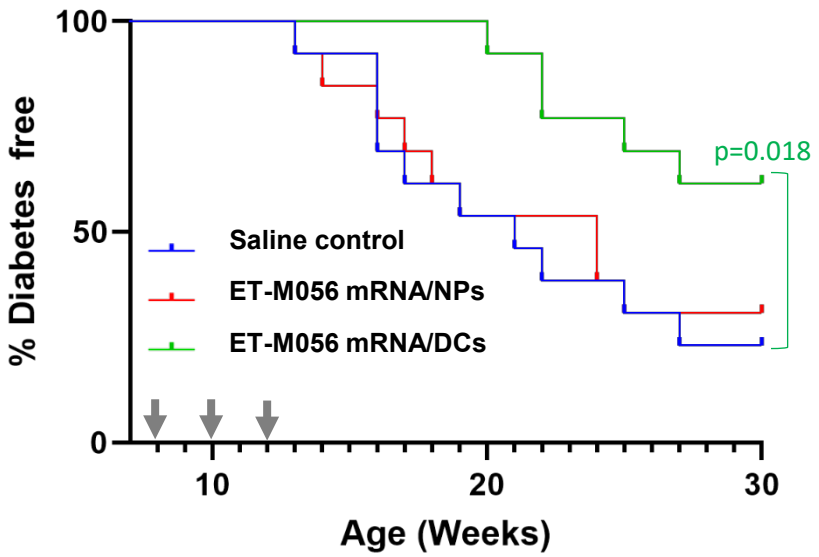
Treatment was changed from weekly to every other week, and the effect of a co-delivered plasmid DNA-encoded immunomodulator (IL-27) was also assessed.
Mice received 50 μg ET-M057 (±IL-27 pDNA) every other week by intramuscular or intradermal injection.

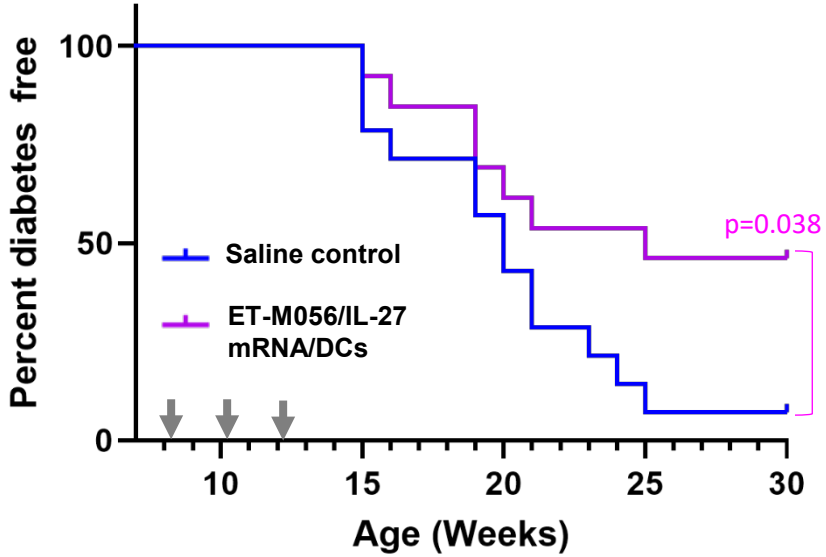



We have shown that Endotope-encoded antigens are presented for at least two weeks, yet reducing treatment frequency from weekly to biweekly reduced therapeutic efficacy.
Surprisingly, immunomodulation with IL-27 improved the efficacy with the intramuscular route but exacerbated disease with the intradermal route.

# Tolerogenic DC therapy

Bone marrow-derived dendritic cells (DCs) were used immature after 6 days differentiation with GM-CSF and IL-4 from bone marrow progenitors.
Treatment was done in three intraperitoneal injections of $1x10^6$ DCs electroporated with 2 μg ET-M056 mRNA (± 4 μg IL-27 mRNA) at 8, 10 and 12 weeks of age (indicated by grey arrows).
The same mRNA administered in nanoparticle formulation did not provide protection in most studies done.

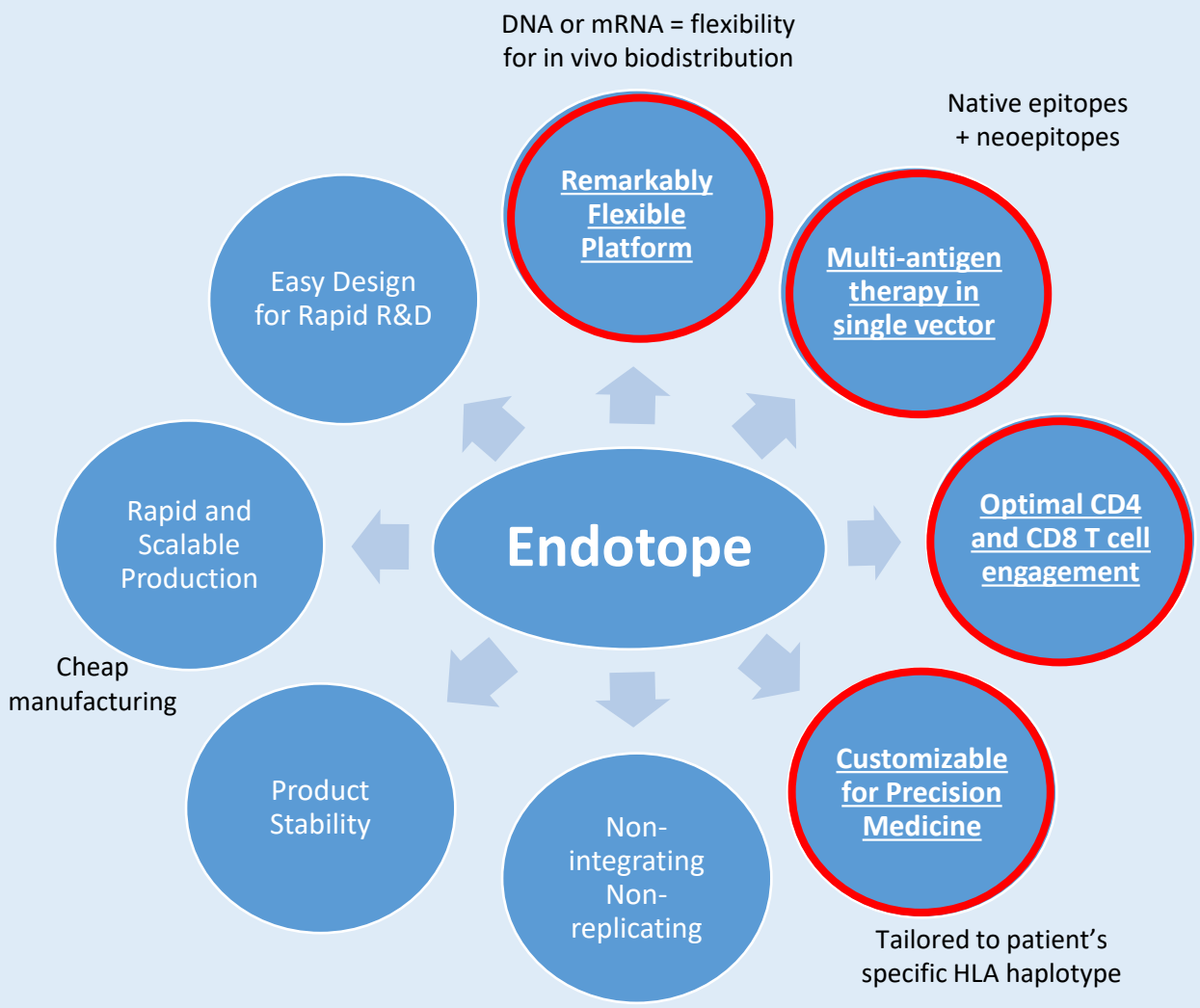



In progress: bone marrow-derived dendritic cells (DCs) with enhance tolerogenic phenotype (after differentiation with GM-CSF, IL-4 ± rapamycin) are evaluated against several DC controls (no relevant antigen).

Note: Endotope ET-M056 is a fifth generation construct as depicted in construct #3 (slide 10) with hybrid peptides added and designed for expression by in vitro transcribed mRNA.

# Value Proposition




Applied to antigen-specific desensitization therapies

**Type 1 diabetes**
Rheumatoid Arthritis
**Multiple Sclerosis**
Psoriasis
Inflammatory Bowel Disease
Allergies

Applied with adjuvants to boost antigen-specific immune responses

**Cancer Immunotherapy**
Chronic Infections

➔ more efficient and targeted engagement of specific T cell populations = more effective desensitization / tolerance induction = *dramatic improvement of living conditions*

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# Competition (T1D)

| | Small molecules | | Biologics | | |
|---|---|---|---|---|---|
| | Long-acting insulin Lantus | Treg-promoting glycolipid RGI-3100 | Neutralizing antibodies Teplizumab | Proinsulin DNA vaccine TOL-3021 | Endotope |
| Restores immune tolerance | NO | YES | YES | YES | YES |
| Antigen-specific tolerance | N/A | NO | NO | YES | YES |
| Flexible & customizable | N/A | NO | NO | To some extent | YES |
| Broad antigen representation | N/A | N/A | N/A | NO | YES |
| Optimal CD4/CD8 T cell targeting | N/A | N/A | N/A | NO | YES |
| Stage | Marketed | Preclinical | Phase III | Phase II | R&D |

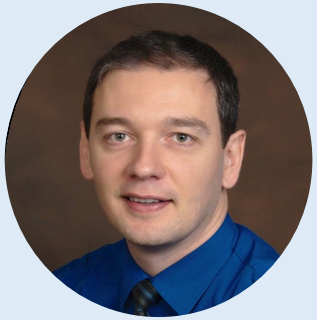
# Competition (cancer)

| | Inovio | Immunomic | Moderna | BioNTech | Endotope |
|---|---|---|---|---|---|
| Platform | DNA | DNA | mRNA | mRNA | versatile |
| Antigens | Multiple antigens | Multiple antigens | Multiplex vaccines | Multiple epitopes | Multiple epitopes |
| Targeting | Unclear | MHC-I or MHC-II | None (MHC-I) | None (MHC-I) | MHC-I **&** MHC-II |
| Broad antigen representation | Up to 3 | Up to 3 | Up to 6 (separate mRNA) | Unspecified, individualized | A dozen epitopes, **on same construct** |
| DC-based option? | No? | Yes | No? | No? | Yes |
| Stage | Phase II-III | PC-Phase II | PC-Phase II | PC-Phase II | R&D-PC |

*Endotope is the only platform offering optimal engagement of both CD4 and CD8 T cells by the same APC, guaranteed (one single construct).*

# The *Endotope* team

**Remi Creusot, PhD**
*Principal Investigator*
*Expert in T1D pathogenesis and immune tolerance*



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