Steno Diabetes Center Copenhagen

β cell dysfunction during T1D development

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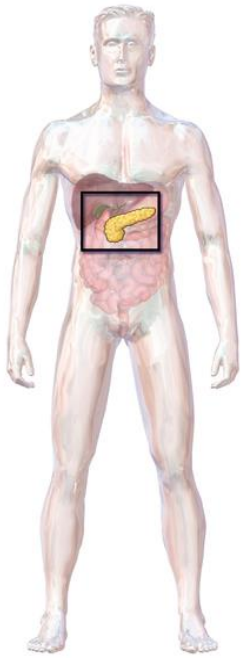
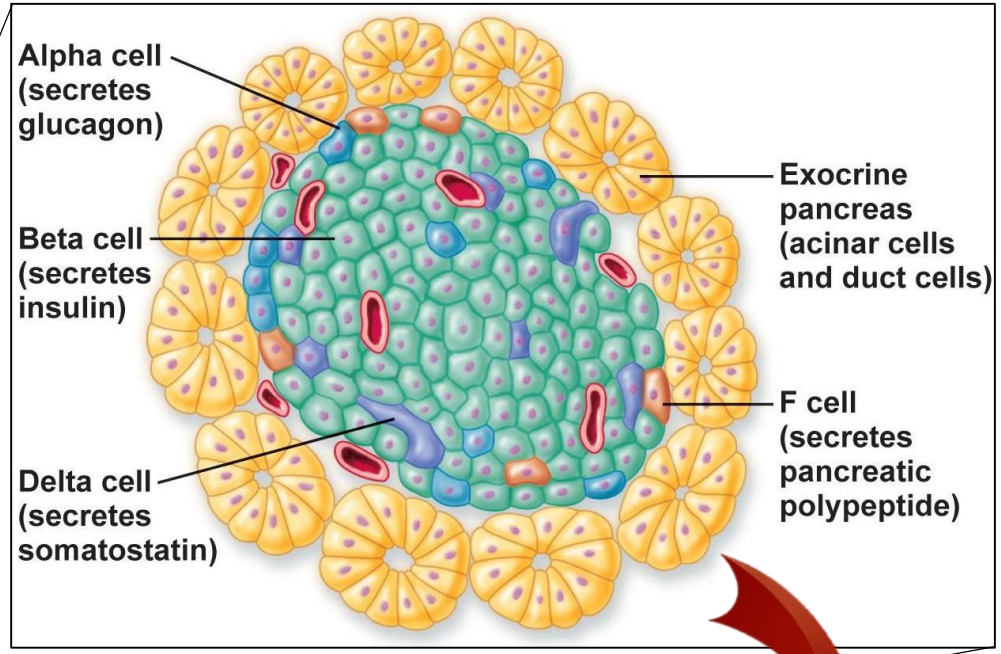
Steno Diabetes Center Copenhagen

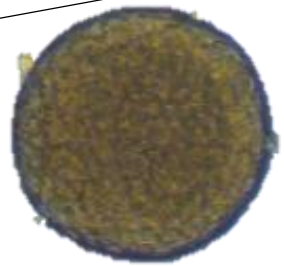
The participants will get familiar with:

- Different **terms in T1D setting**
- Factors contributing to **β cell dysfunction**
- The suggested **mechanisms for β cell dysfunction**
- How can we **study the β cell (dys)function and rescue approaches**

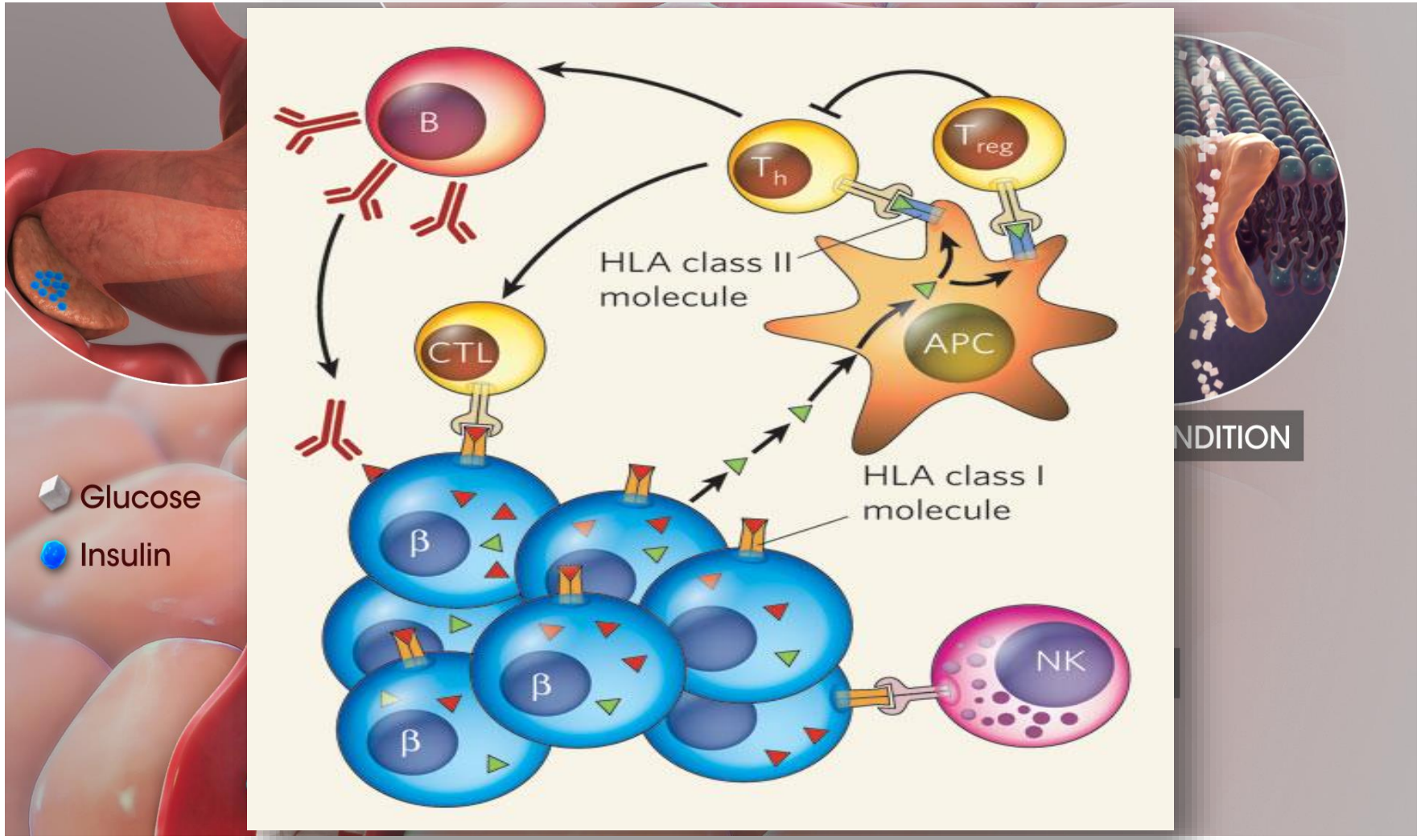
Pancreas islets



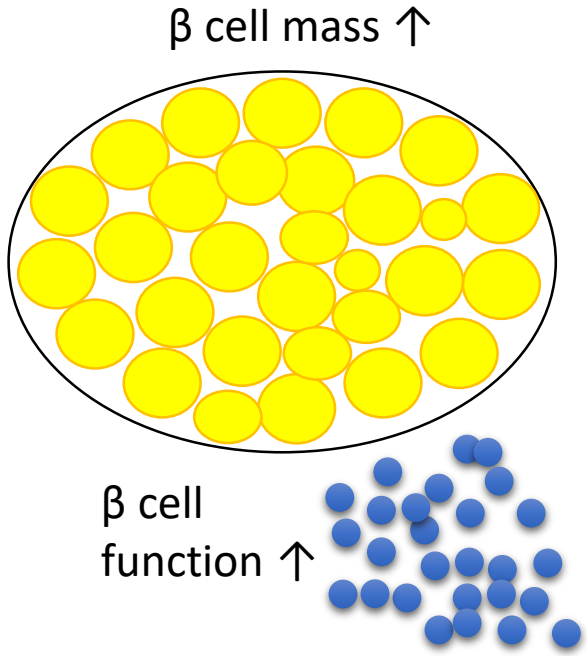




Type 1 Diabetes (T1D)



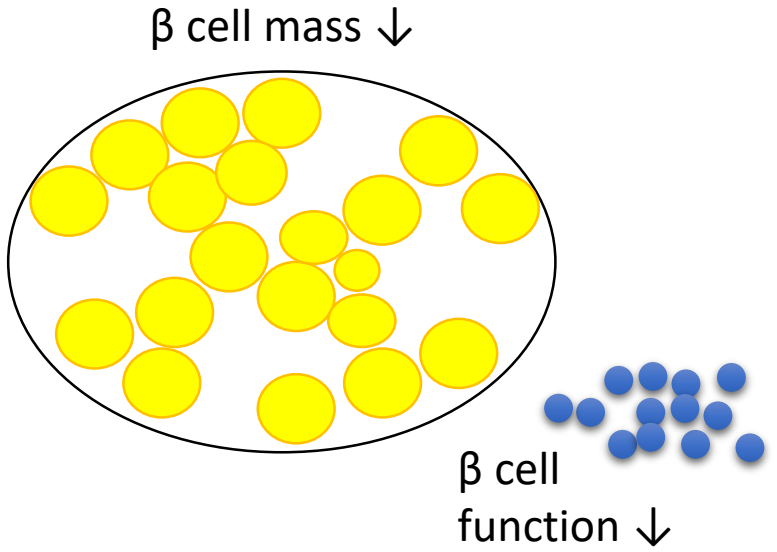
β cell mass and function



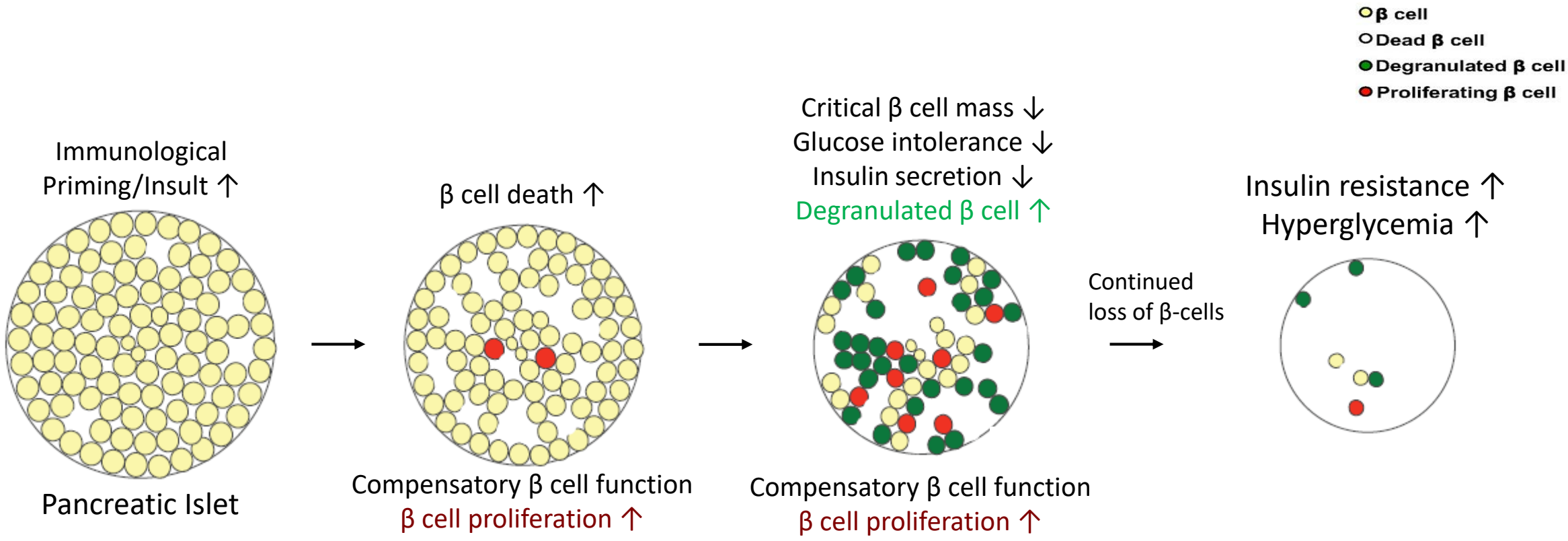
The total amount of released insulin depends on the:

1. Absolute number of BC in the islets (BC mass).

2. Output of each of these cells (BC function).



Changes in β cells mass and function in T1D



Modified from Akirav et al 2008

Pre-Diabetic stage

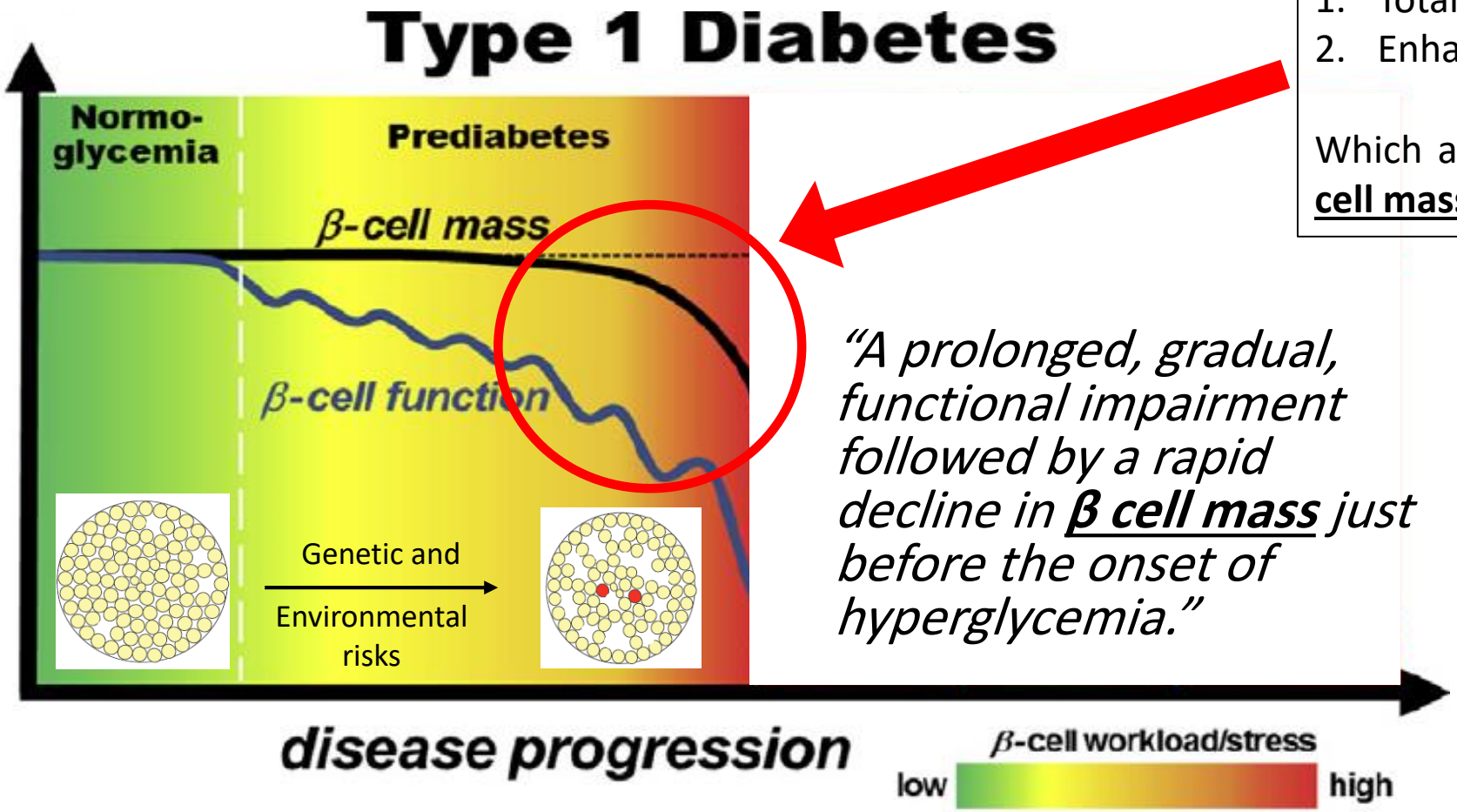
Diabetic stage
(Onset of hyperglycemia)

Prediabetic phase of T1D

Shortly before clinical manifestation of diabetes the prolonged intensified β cell workload and autoimmunity results in:

1. Total cellular exhaustion and
2. Enhanced cell death

Which are leading to a massive decrease in **β cell mass** and the onset of hyperglycemia



*"A prolonged, gradual, functional impairment followed by a rapid decline in **β cell mass** just before the onset of hyperglycemia."*

Black line: β cell mass

Blue line: β cell function.

The color-coded background indicates the intensity of beta cell workload and stress caused by immune infiltration, metabolic demand and hyperglycemia.

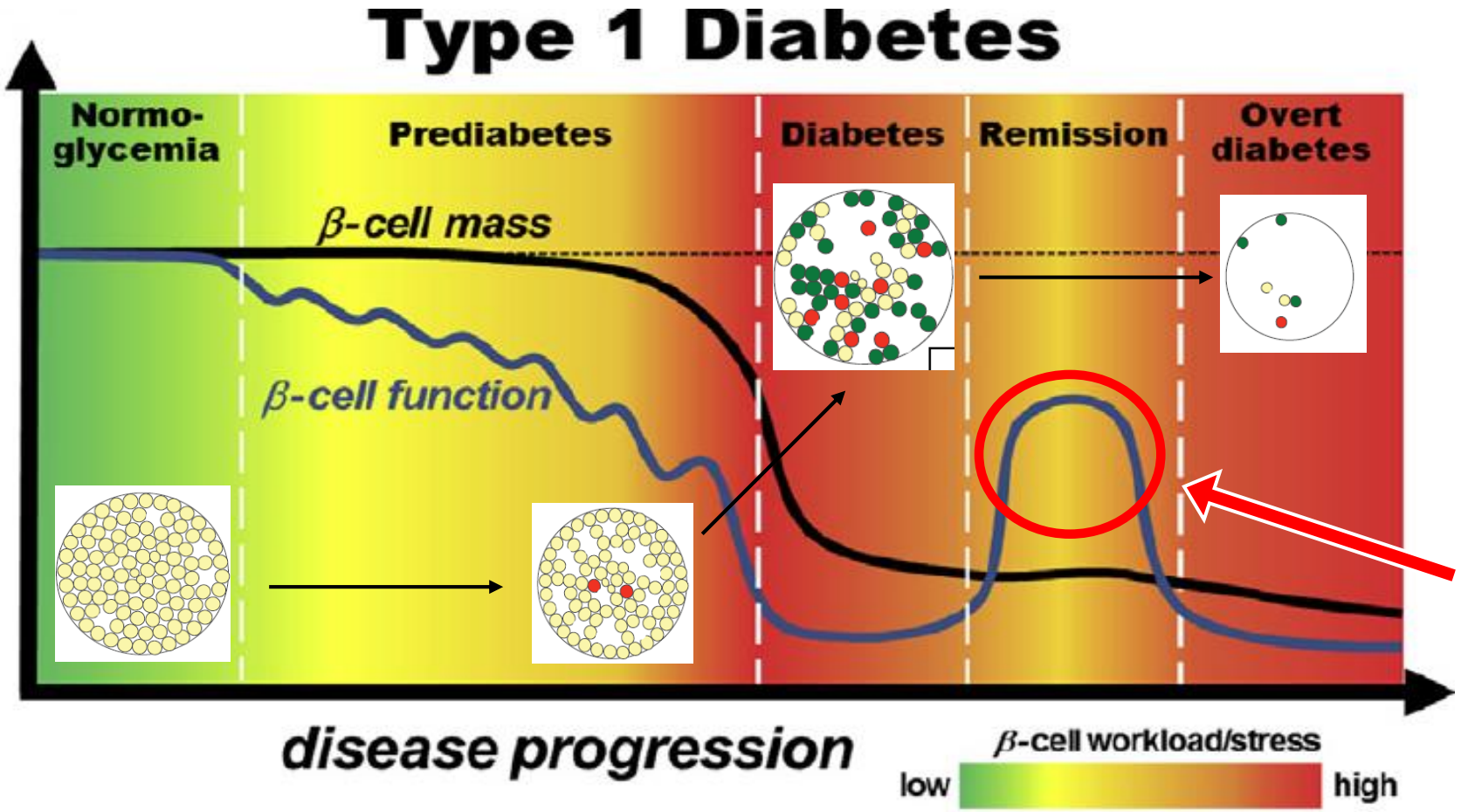
Modified from Chen et al 2017

Onset of hyperglycemia

Black line: β cell mass

Blue line: β cell function.

The color-coded background indicates the intensity of beta cell workload and stress caused by immune infiltration, metabolic demand and hyperglycemia.



"honeymoon phase,"

Modified from Akirav et al 2008 & Chen et al 2017

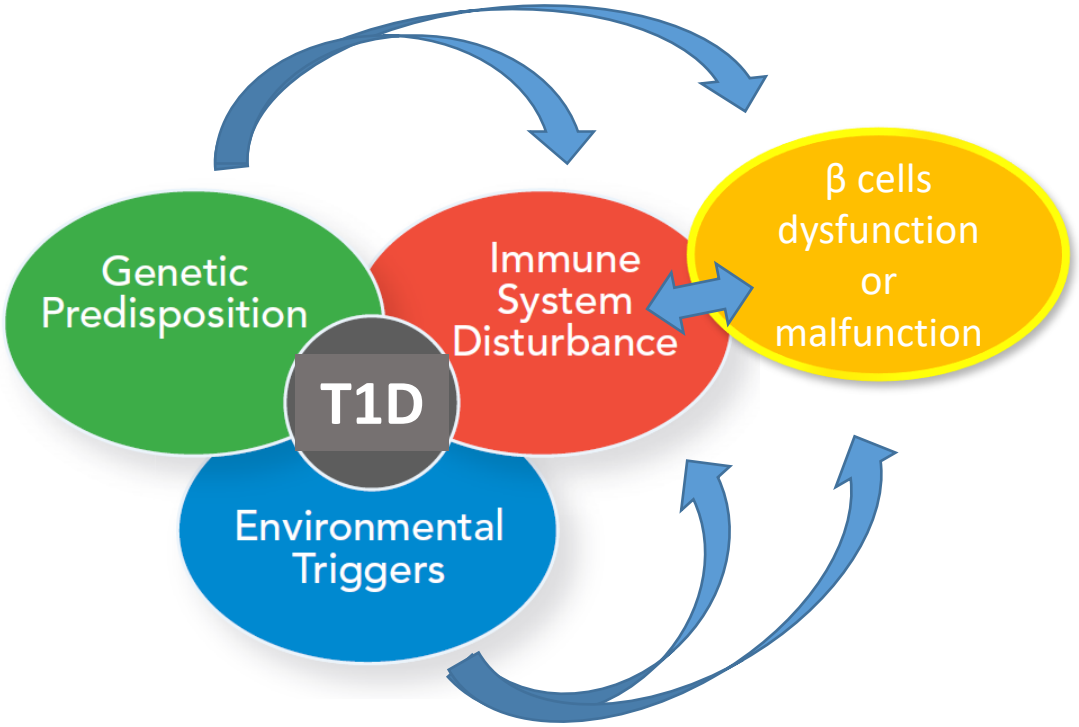
β cell dysfunction

- Complex interplay between:

✓ Genetic predisposition
- HLA
- INS
- …..

✓ Environmental factors
- Infant and adult diet
- Vitamin D
- Trace minerals
- Early-life exposure to virus (enterovirus, rubella, mumps, rotavirus, parvovirus or cytomegalovirus)
- Decreased gut-microbiome diversity

✓ Immune systems and β cells dialogue that vary between individual cases



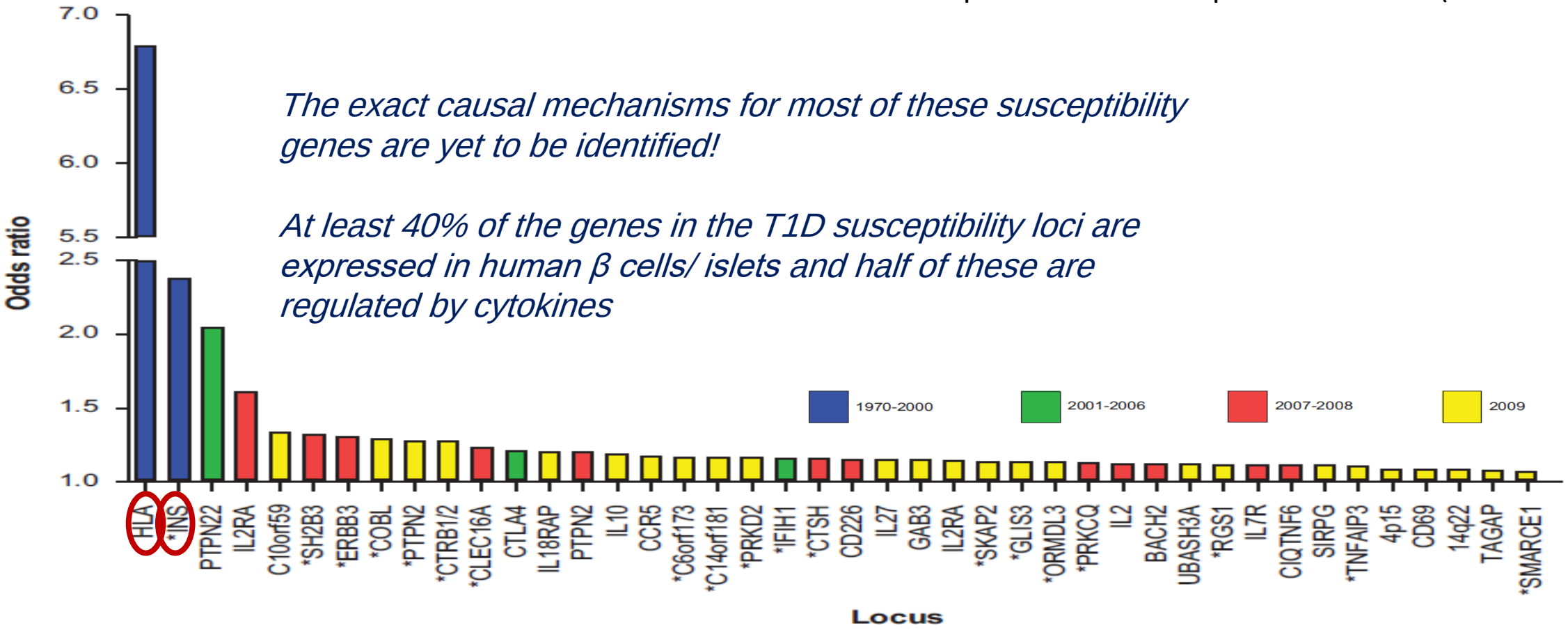
Genetic factors

GWA studies have identified >60
T1D risk loci – most with low ORs

Expressed in human pancreatic islets (marked with *)

The exact causal mechanisms for most of these susceptibility genes are yet to be identified!

At least 40% of the genes in the T1D susceptibility loci are expressed in human β cells/ islets and half of these are regulated by cytokines



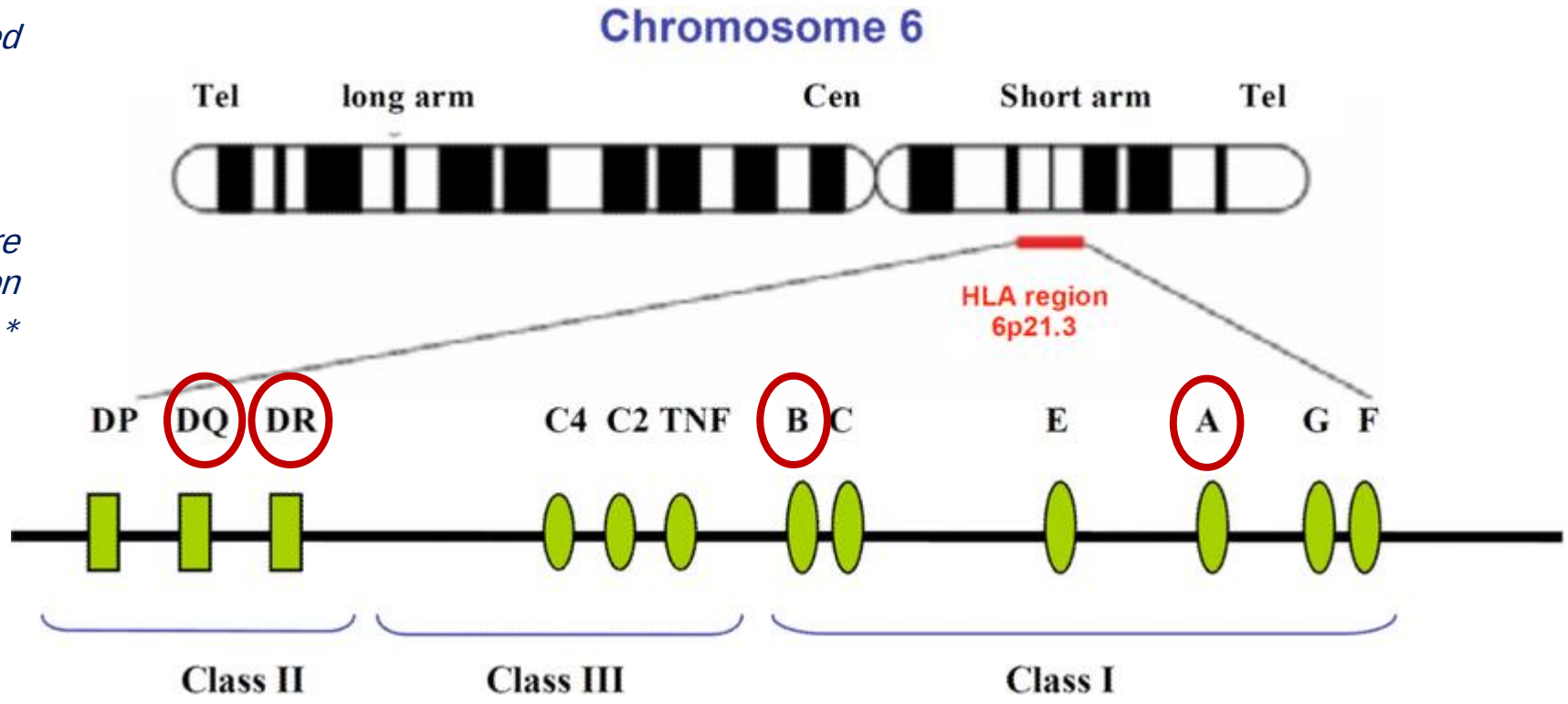
Pociot et al, 2010

HLA

Less than 20% of the T1D cases are associated with mutations in the MHC-I, in which haplotypes HLAB∗ 3906 or HLA-A ∗ 2402 set susceptibility towards T1D

Around 40% of the genetic risk associated to T1D is related to HLA region class II.

Especially HLA-DR and HLA-DQ, where the haplotypes with the greatest association are DRB1 ∗ 0401 or ∗ 0405 and DQB1 ∗ 0301 (DR4-DQ8)

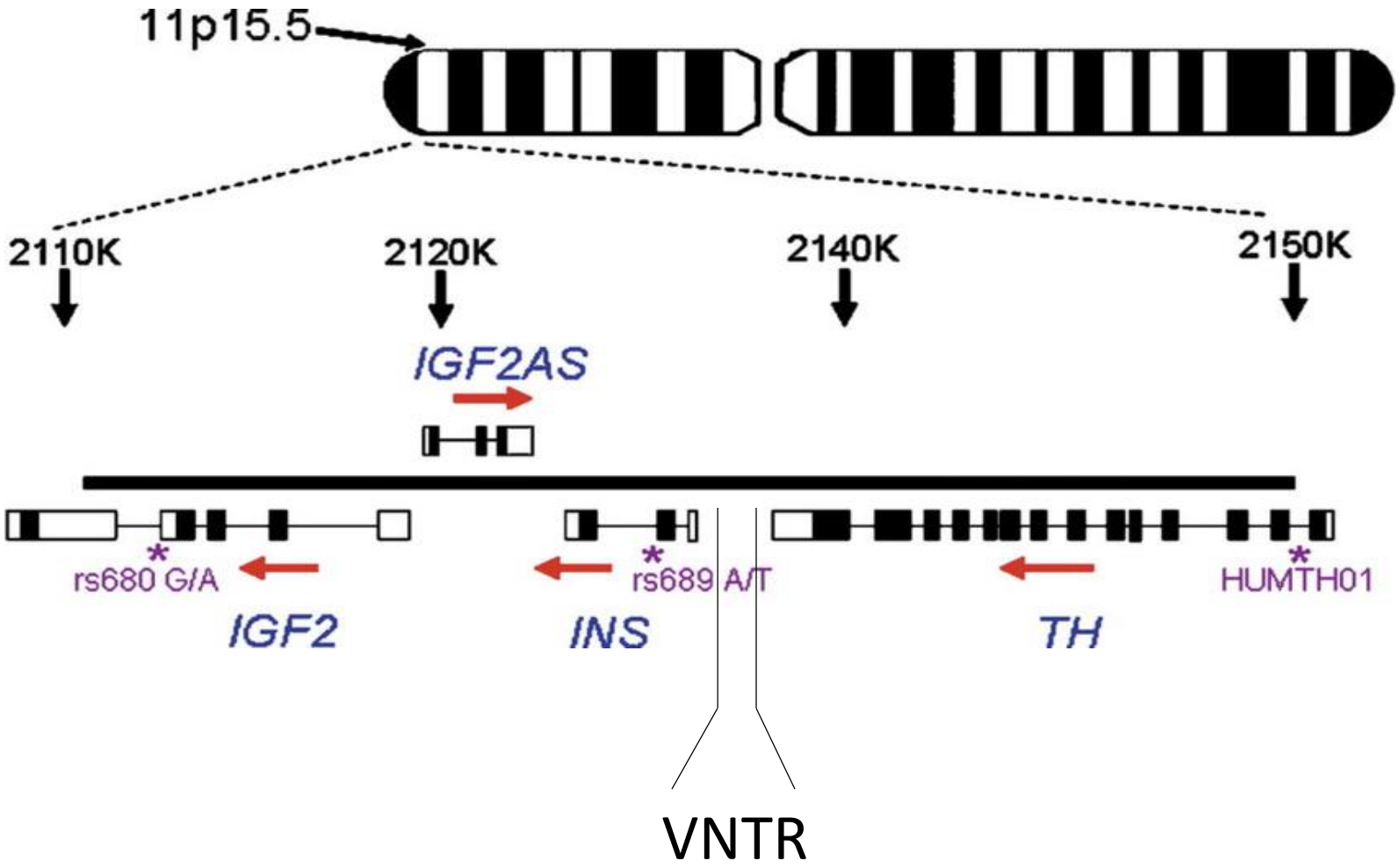


MHC-II +antigenic processing activity

Complement + inflammatory factors

MHC-I

INS


11p15.5
2110K
2120K
2140K
2150K
IGF2AS
rs680 G/A
rs689 A/T
HUMTH01
IGF2
INS
TH
VNTR

Candidate genes affecting β cells mass and function

- **Genomic features:**
  - Expression quantitative trait loci (eQTLs)
  - Transcription factor-binding sites
  - DNase hypersensitive sites
  - Histone modifications
  - ….

Gene (Chromosome)	*Variant(s)*	*Function/pathway affected*
INS (11p15.5)	INS VNTR class I	β-cell expression level
	rs7111341	
	rs11564705[a]	
IFIH1 (2q24.2)	rs1990760	MDA5 signalling
	rs3747517	
GLIS3 (9p24.2)	rs7020673	β-cell development
		β-cell apoptosis
		GLUT2 expression
PTPN2 (18p11.21)	rs1893217	Inflammation and virus-induced β-cell apoptosis
	rs2542151[a]	
CTSH (15q25.1)	rs3825932	Cytokine-induced apoptosis
	rs11856301[a]	
		Insulin transcription
BACH2 (6q15)	rs11755527	Cytokine-induced apoptosis
TYK2 (19p13.2)	rs2304256	Inflammation and virus-induced β-cell apoptosis
CLEC16A (16p13.13)	rs12444268	Autophagy/mitophagy
	rs12708716	
	rs11865121[a]	Insulin secretion

Pociot (2017) Clinical & Translational Immunology

Environmental factors

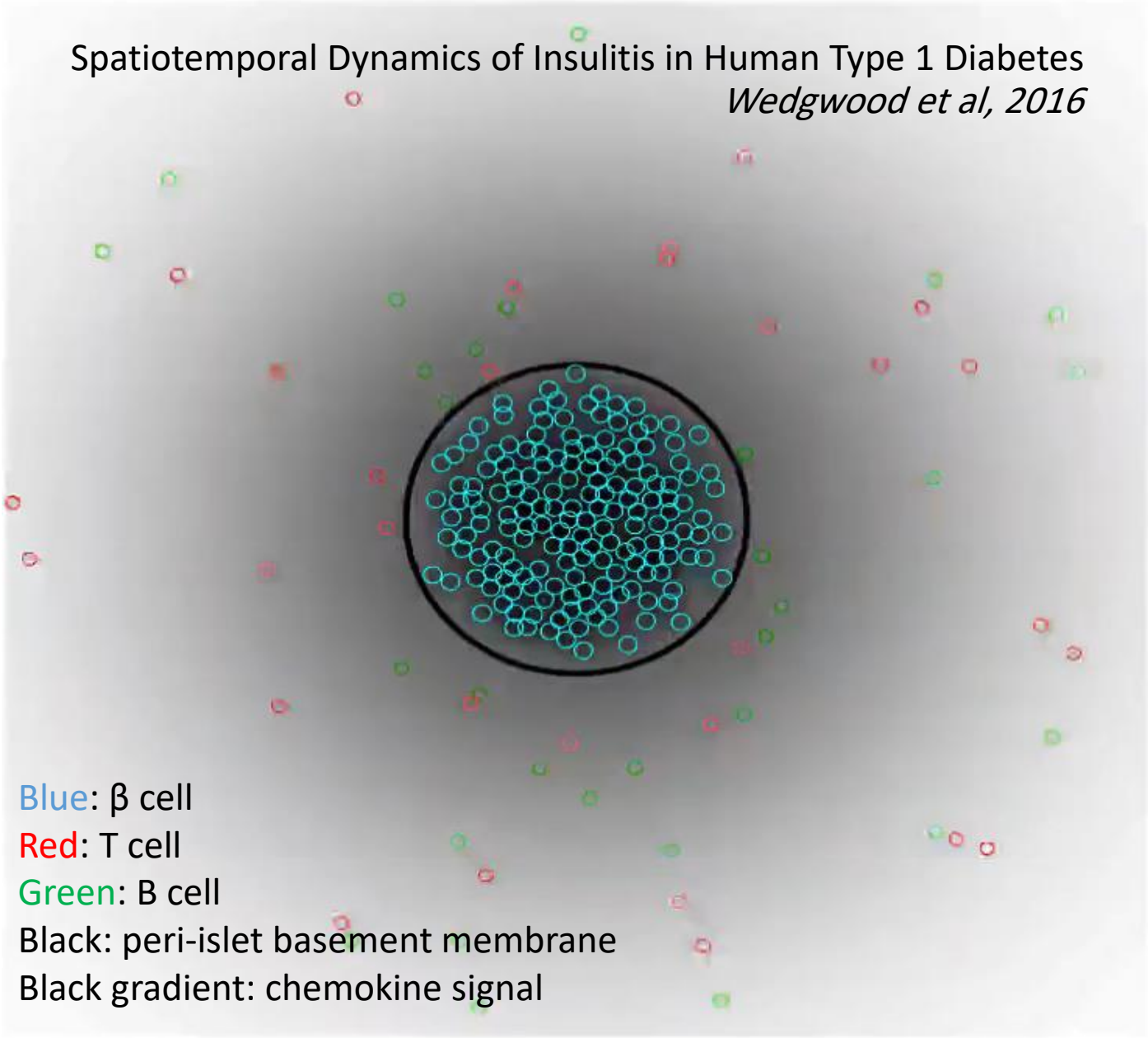
Insulitis

Autoantigens:

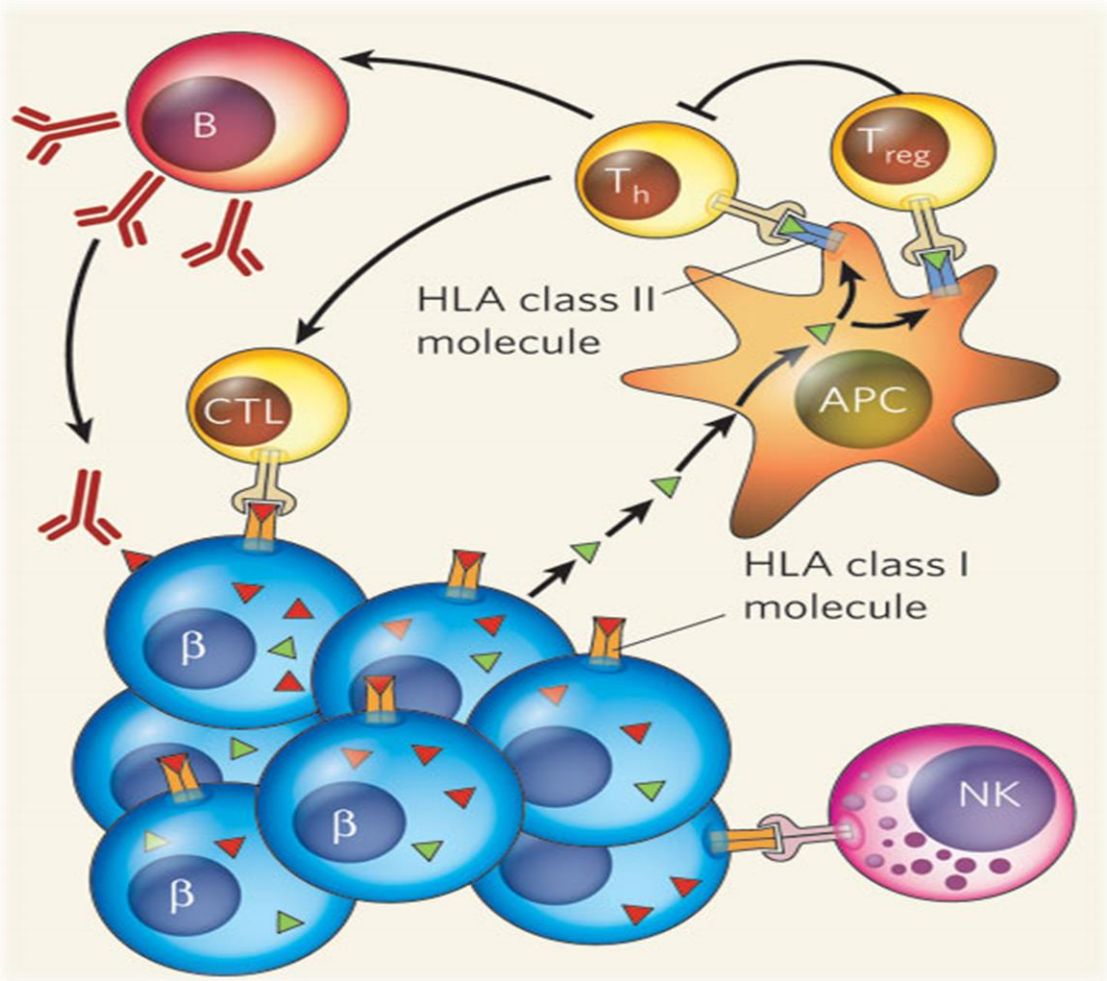
- Insulin
- Glutamate decarboxylase (GAD)
- Protein tyrosine phosphatase
- Insulinoma-associated antigen-(IA-) 2, and IA-2b
- ….

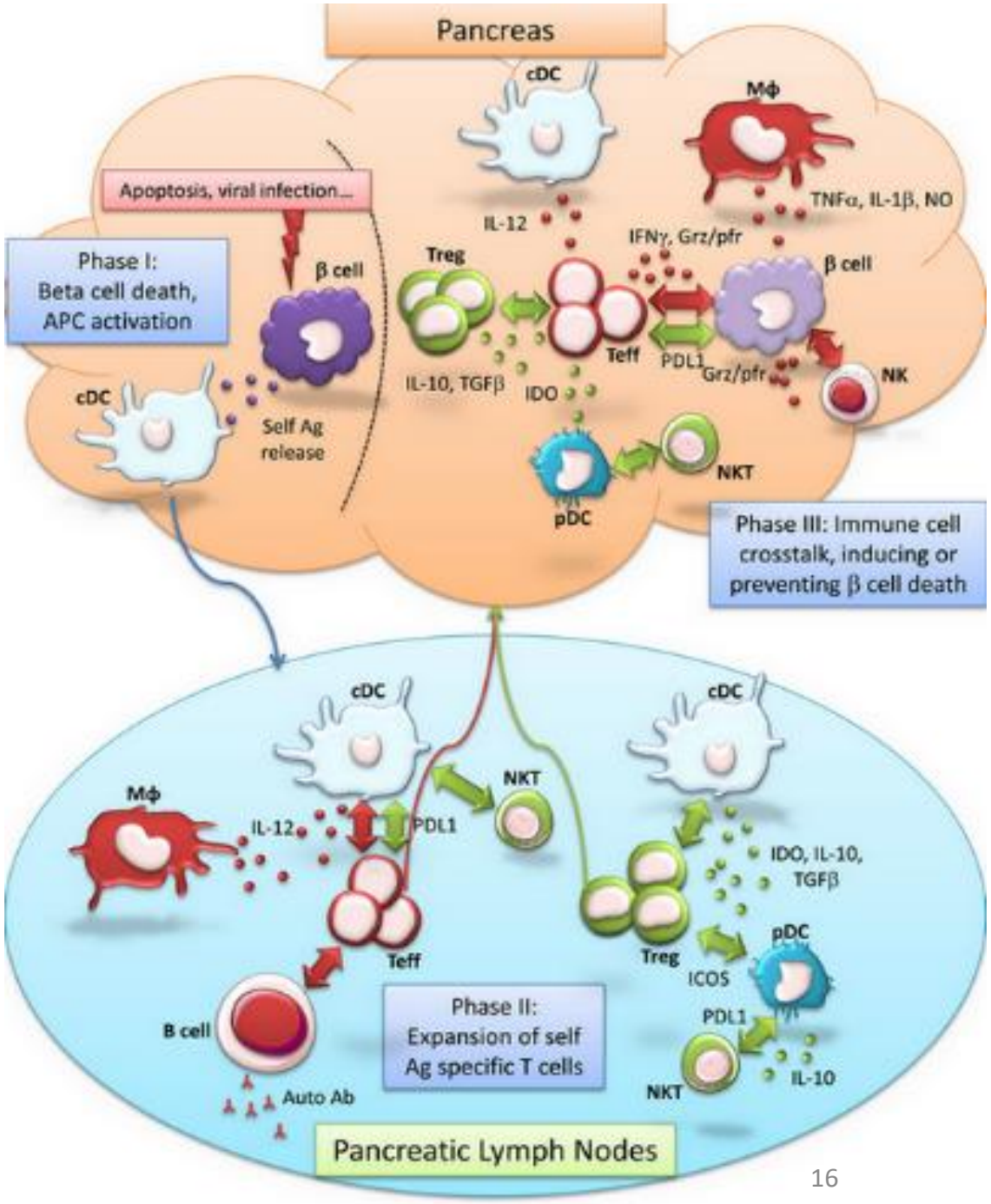
Up to 90 % of newly diagnosed T1D subjects have autoantibodies to one or more of these antigens

Spatiotemporal Dynamics of Insulitis in Human Type 1 Diabetes
Wedgwood et al, 2016



Immunological Priming/Insult





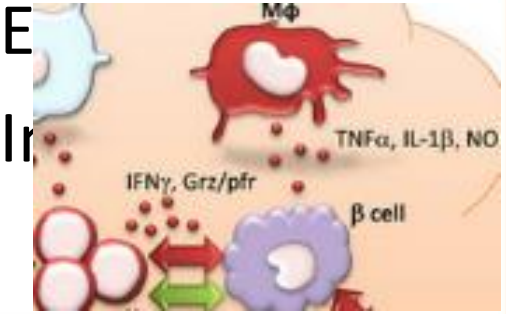
β cell destruction mechanisms

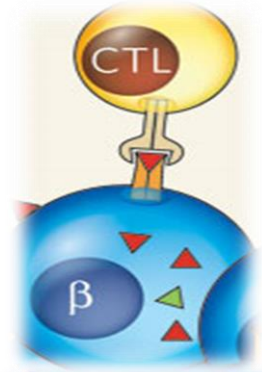
- Apoptosis
  - Fas/Fas-L
  - Il-1β. IL-1β
  - TNF-α
- Necroptosis
- Incomplete Autophagy
- Endoplasmic reticulum stress
  (mainly T2D and later stage T1D)
  - Glucotoxicity
  - Lipotoxicity
  - Amyloid Polypeptide
- Oxidative stress
- Pyroptosis

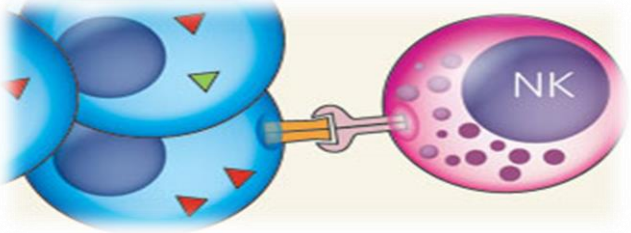
https://nanolive.ch/applications/overview/single-cell-cell-culture-analysis/cell-cycle-analysis/

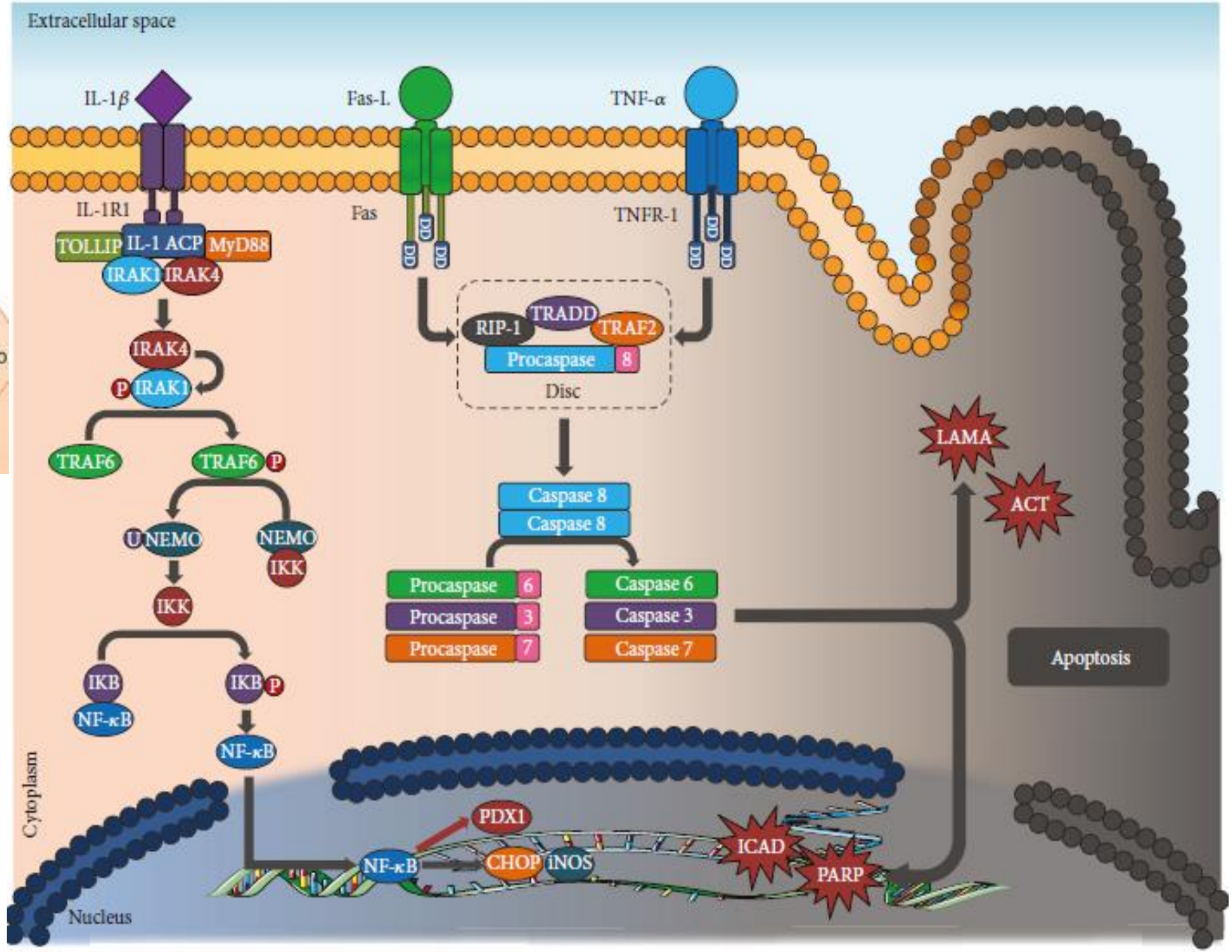
Apoptosis in β cell

- E
- Ir









Necroptosis

The term introduced in the year 2003 by Chan et al

Rojas et al, 2018
Journal of Diabetes Research

Studying the β cell death – *In vitro*

- ***In vitro:***
  - β cell models:
    - EndoC (Human)
    - 1.1B4 (Human)
    - INS-1E (Rat)
    - MIN6 (Mouse)
  - Isolated islets
  - Dispersed β cells
  - hESCs, hiPSCs

→

Assays:

1. **Morphometric analyses**
   - e.g. islet size, proliferation, apoptosis
2. **Hormone secretion**
   - Insulin and amylin, Glucagon, somatostatin, Pancreatic polypeptide, Ghrelin
3. **Intracellular signaling**
   - e.g. Ca2þ, NADPH, exocytosis, mitochondria, electrophysiology
4. **Protein biochemistry**
5. **Omics** (Genomics, transcriptomics, …)
6. ***In vitro* differentiation**

→

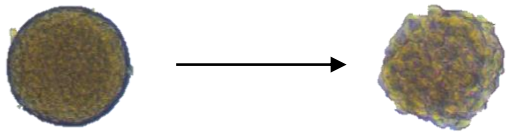
Readout:

β cell mass and **function**

Studying the β cell death – *In situ*

Good

Bad



- ***In situ:***
  - Islet/pancreas organoids
  - Histological analyses
  - Pancreas tissue slices

Assays:

1. **Morphometric analyses**
   - e.g. islet size, proliferation, apoptosis, immune cells infiltration
2. **Pancreas tissue slices**
   - e.g. islet size, proliferation, apoptosis, immune cells infiltration
3. **Hormone secretion**
4. **Intracellular signaling**
   - e.g. Ca2þ, electrophysiology

Readout:

β cell **mass** and function

Studying the β cell death – *In vivo*

- ***In vivo:***
  - Metabolic tests
  - Noninvasive imaging
  - Transplantation

Both T2D and T1D

Assays:

1. **OGTT, MTT, IVGTT, HOMA, HYPERGLYCEMIC CLAMP**
2. **Systemic hormone levels**
3. **PET, SPECT, MRI, bioluminescence, optical coherence tomography**
4. **Morphometric analyses**
   - proliferation, apoptosis in islet grafts
5. **Omics** (Genomics, transcriptomics, …)

Readout:

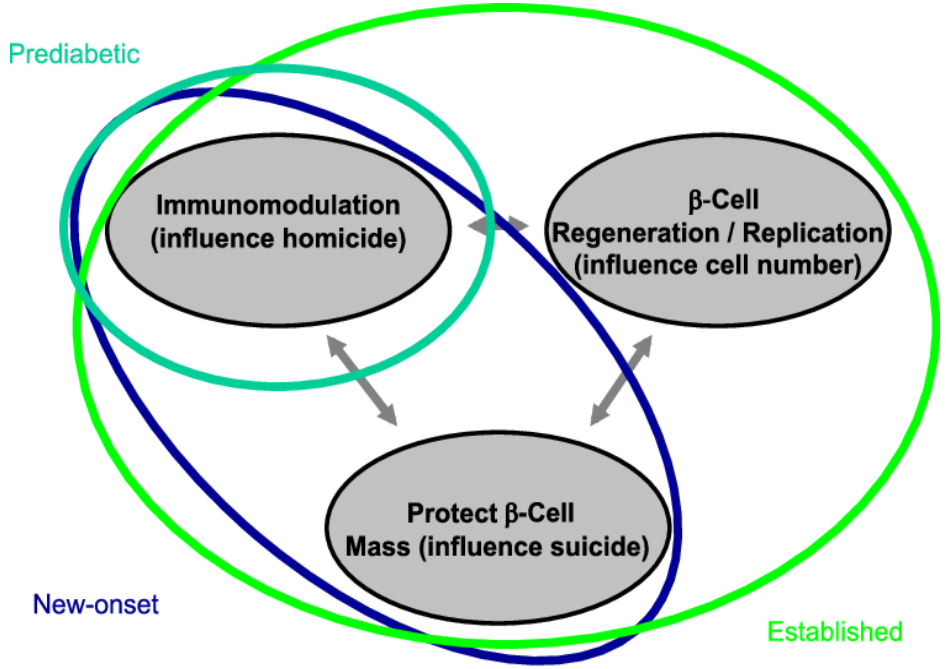
β cell mass and function

Cersosimo et al, 2014

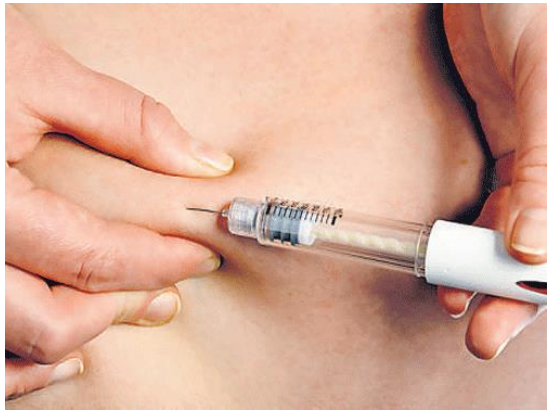
Intravenous glucose tolerance test (IVGTT)
Oral glucose tolerance test (OGTT)
Meal tolerance test (MTT)
Homeostasis model assessment (HOMA)

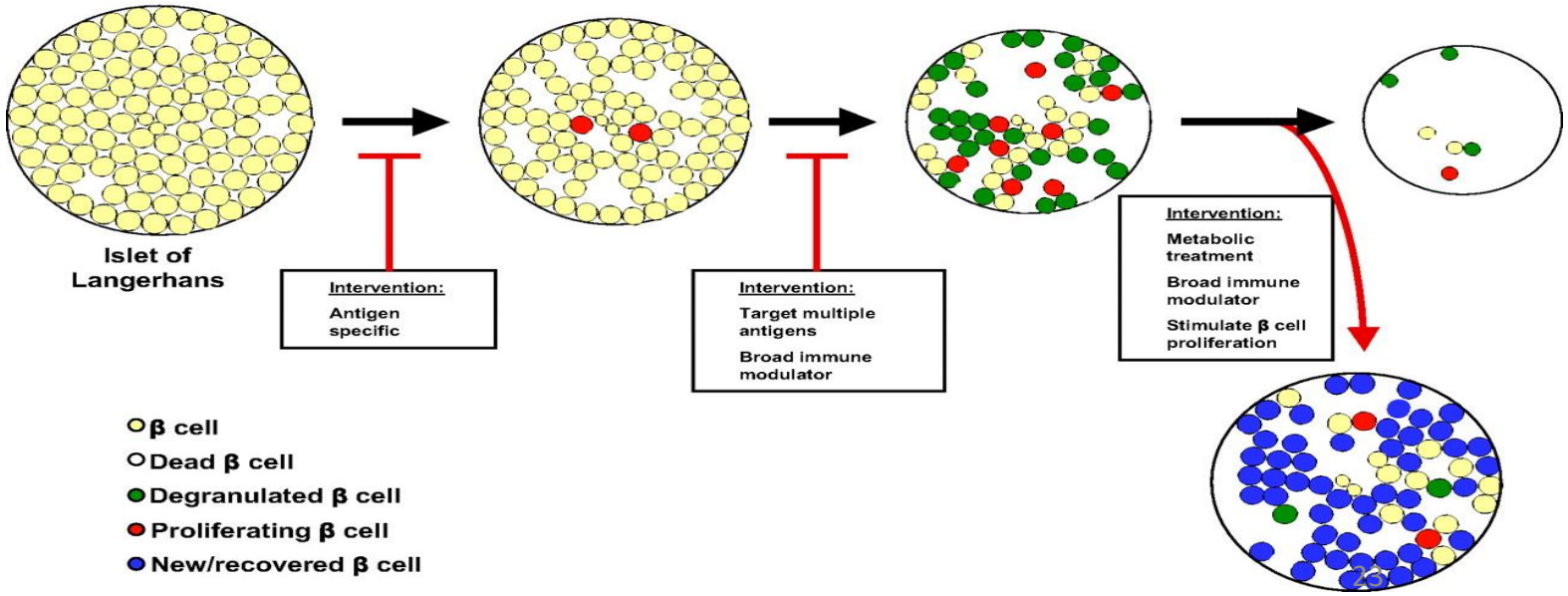
How to rescue β cells?

We are very limited with treatment opportunities!



In most cases **Insulin injection** is the ultimate way!





Application of Stem Cells and Bioprinting for type 1 Diabetes

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PhD Candidate,

Regenerative Medicine and Cellular Pharmacology Laboratory,

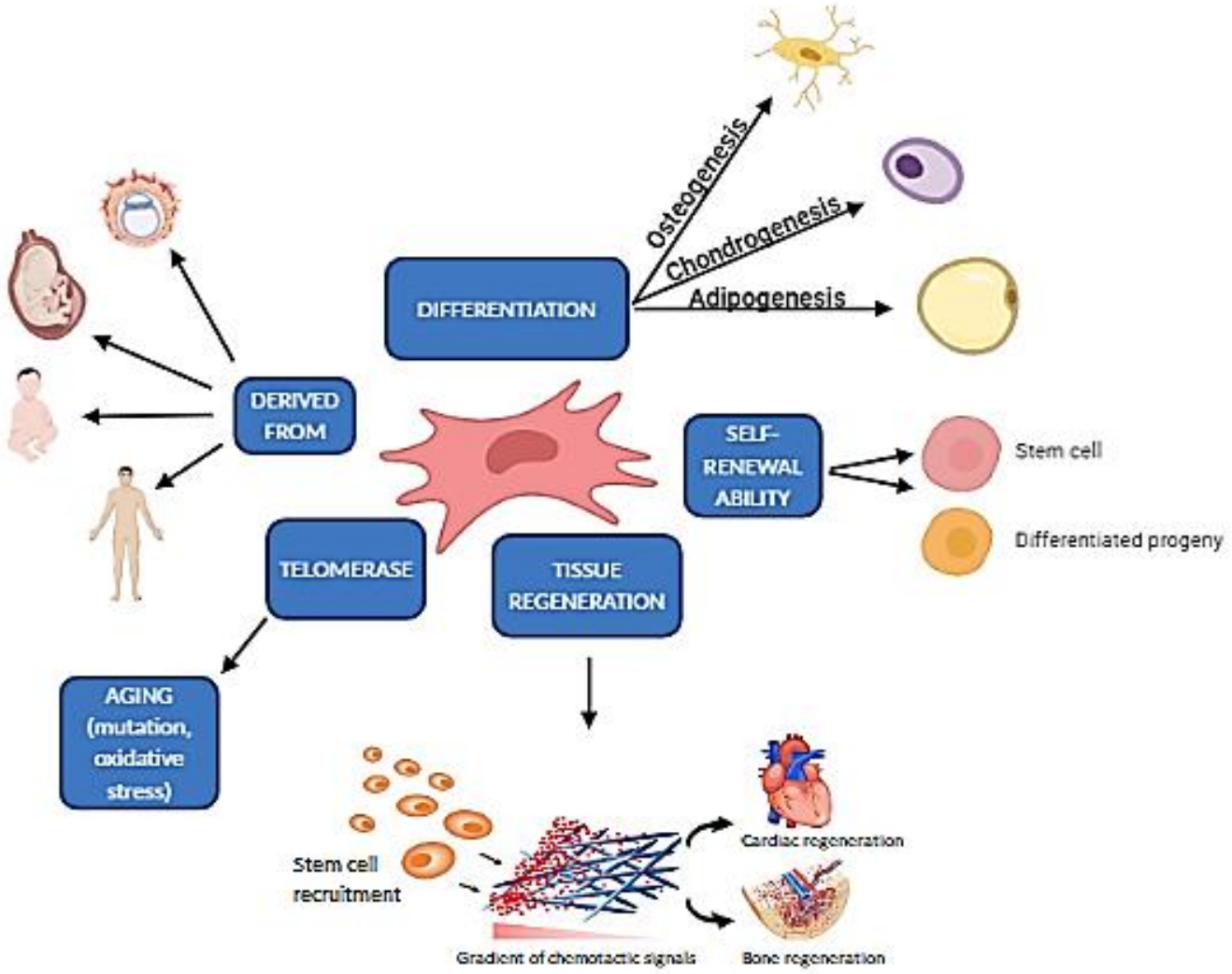
Department of Dermatology and Allergology, Faculty of Medicine,

University of Szeged, Hungary.

Introduction:
Mesenchymal stem cell

1. **Adherence** to plastic
2. **Surface markers** expression (BM-MSC)
3. MSC must **differentiate** to adipocytes , osteoblasts and chondroblasts *in vitro*

POSITIVE MARKERS	NEGATIVE MARKERS
CD73	CD45
CD90	CD34
CD105	CD133
CD146	HLA-DR
CD117 (c-Kit)	CD19



- *Li, N. and Hua, J. 2016. Interactions between mesenchymal stem cells and the immune system , Cell. Mol. Life Sci. 2017*
- *Settimio P., Sayantani B., Jonathan W., Strategies to develop endogenous stem cell-recruiting bioactive materials for tissue repair and regeneration Advanced Drud Delivery Reviews 1 October 2017*

MSCs can act as a “mobile drug store”

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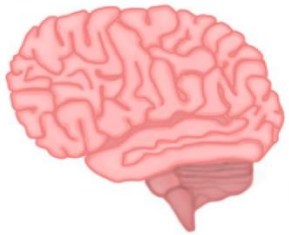
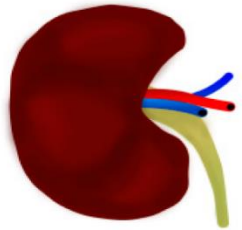


Long-term neuroprotection
Increased cell proliferation
Improved functional recovery
Suppression of inflammation

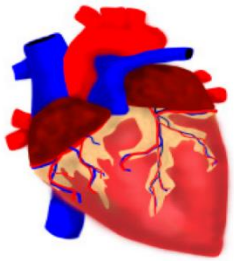


Reduced apoptosis
Increased cell proliferation
Reduced fibrosis
No necrosis
Suppression of inflammation

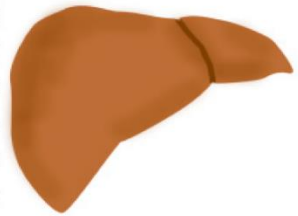
Exosomes

MSC

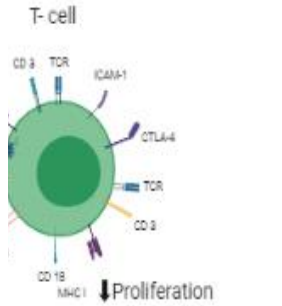
Microvesicles

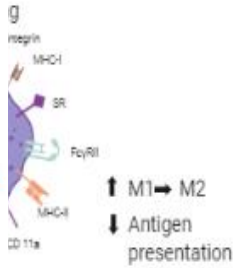


Reduced apoptosis
Increased cell proliferation
Improved functional recovery
Reduced infarct size
Reduced fibrosis
Formation of new blood vessels
Suppression of inflammation



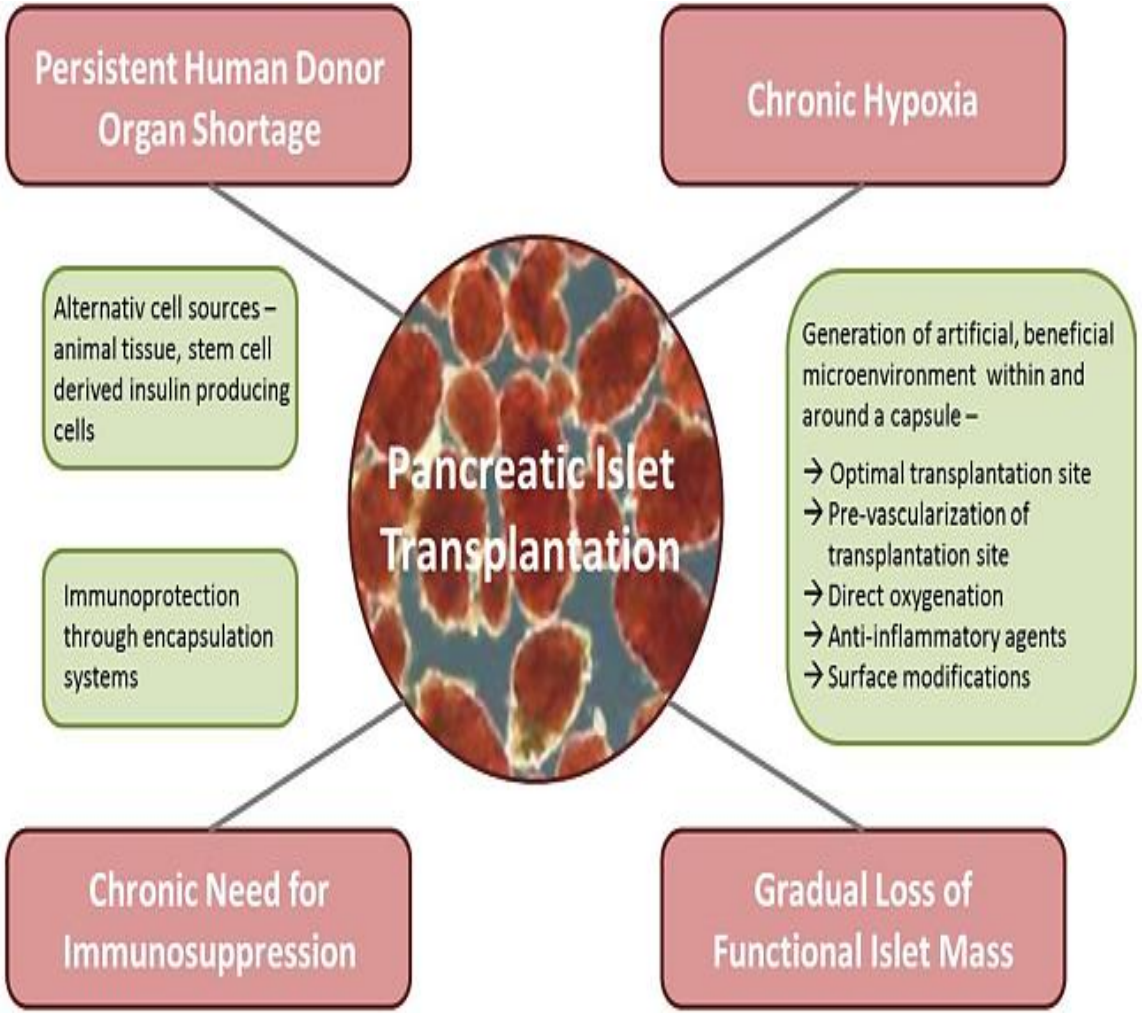
Reduced apoptosis
Increased cell proliferation
Decreased oxidative stress
Improved functional recovery
Decreased liver injury
Suppression of inflammation



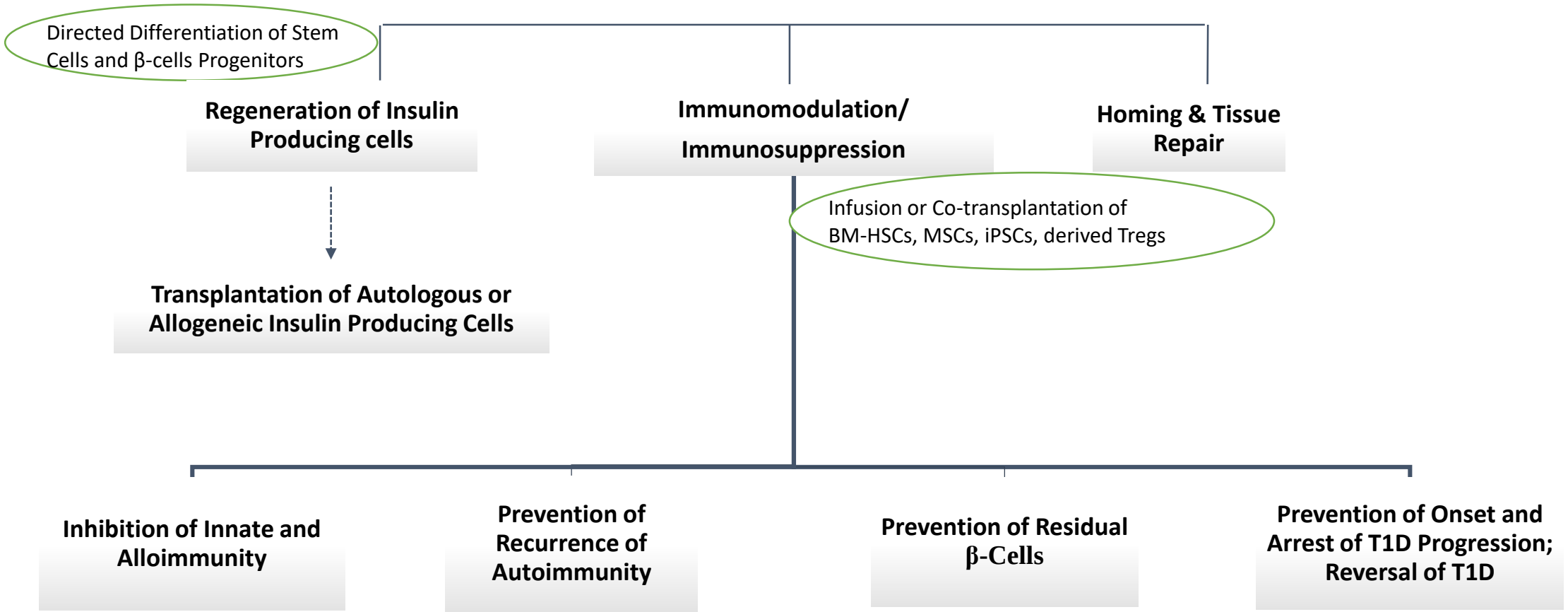


Pancreatic Islet Transplantation In Type 1 Diabetes

- Pancreatic islet cell transplantation is currently the only curative cell therapy for type 1 diabetes.
- Insulin-secreting construct from human sources (allografts) or animal sources (xenografts) have been evaluated.
- Due to lack of donors, whole organ and islet transplantation is not a viable option for diabetes treatment.
- Rejection of transplanted islets by the host immune system is one of the most significant obstacles.



Stem Cell Strategies for T1D



Application of Stem cells in T1D

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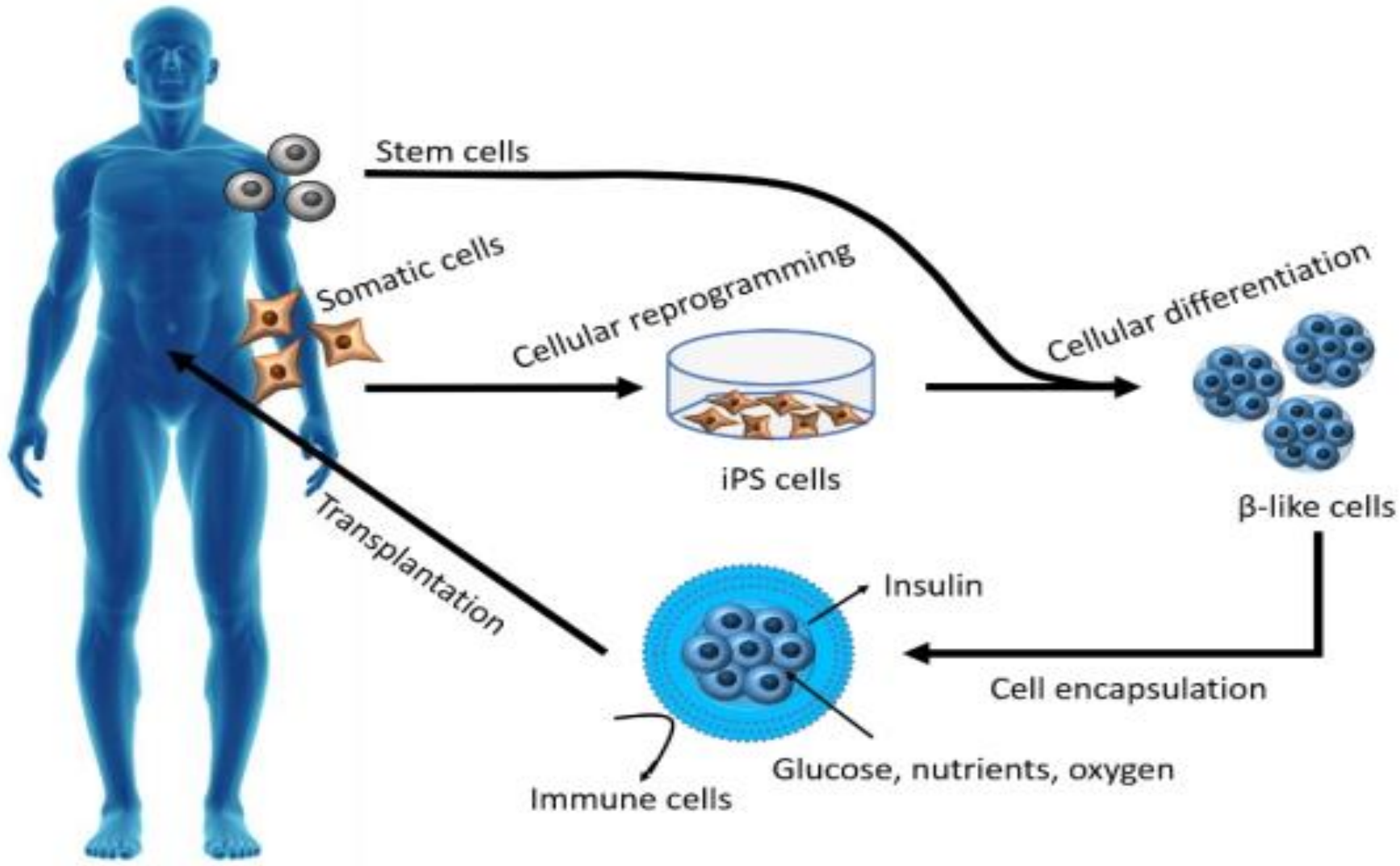

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β-Islet Encapsulation

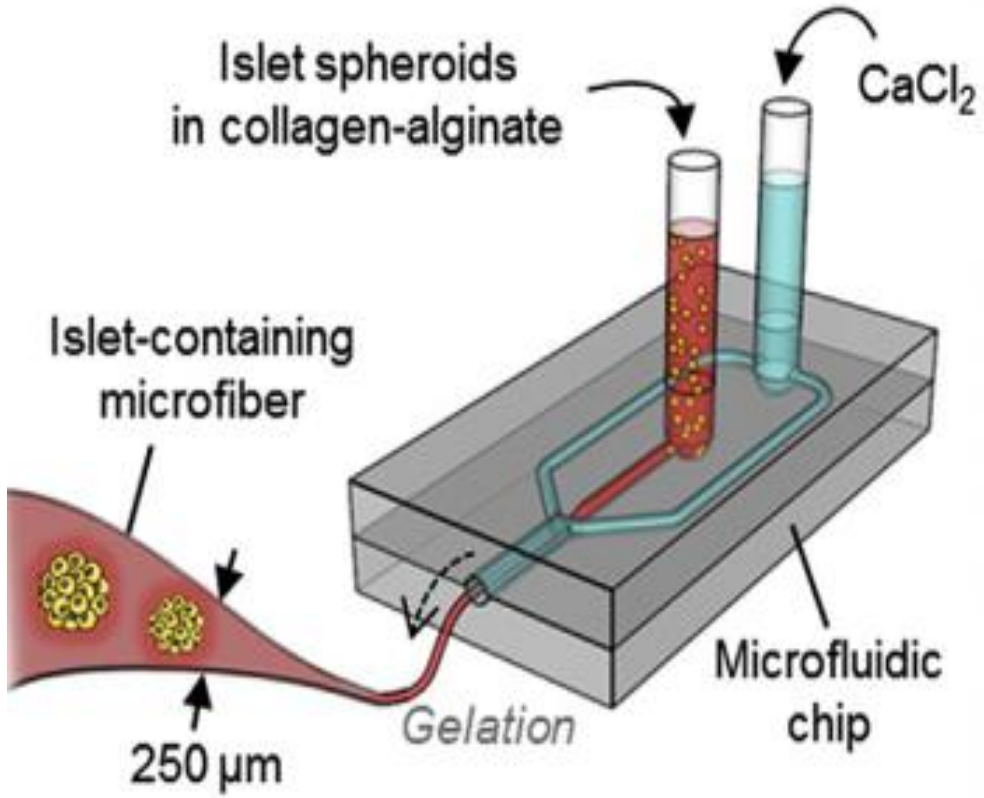
- Encapsulation of cellular grafts within biocompatible scaffolds has been proposed.

- Encapsulation strategy that could create a semi-privilege environment that stimulates natural insulin secretion in response to hyperglycemia preserving cell viability and protec versus immune cells and Ab exchange of nutrients and metabolites.

Applied Materials in encapsulation

- Various types of naturally derived polymers (e.g., alginate, collagen, gelatin, fibrin, and fibronectin).

- Synthetic polymers (e.g., poly lacticco-glycolic acid (PLGA), polysulfone (PSU), polylactic acid (PLA), and polyvinyl alcohol (PVA)) have been evaluated.



Encapsulation Classification

Encapsolated Islets are classified according to their sizes:

a) Microcapsule is typically prepared in the size of 100 µm–1mm and contains one or several islet.

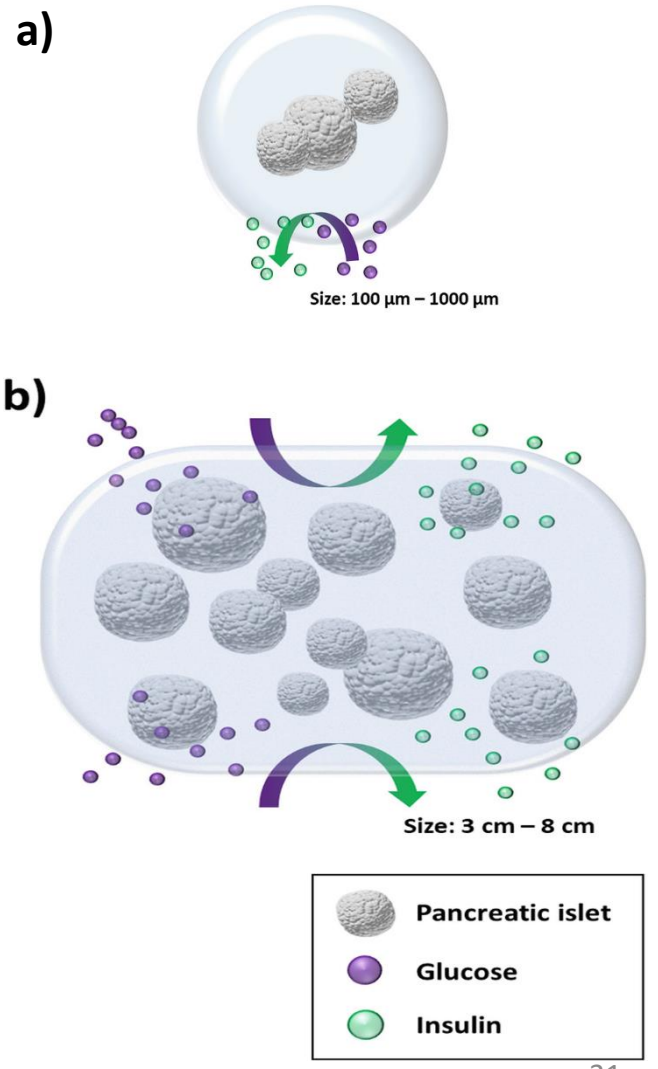
b) Macrocapsule is usually made in 3–8 cm size and contains multiple islet.

Limitation of Microencapsulated Islets:

- Difficult to control the localization of islets during implantation.
- Efficiency of the transplanted microcapsule.

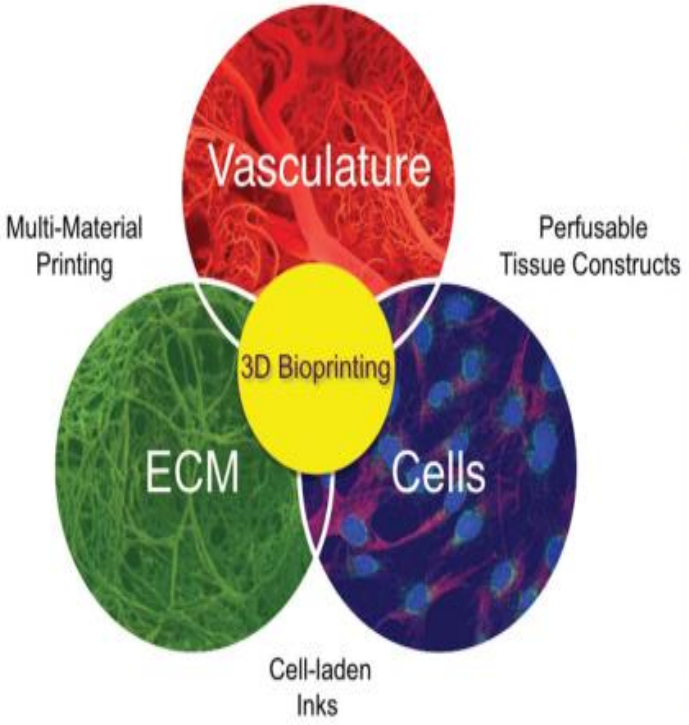
Limitation of Macroencapsulated Islets

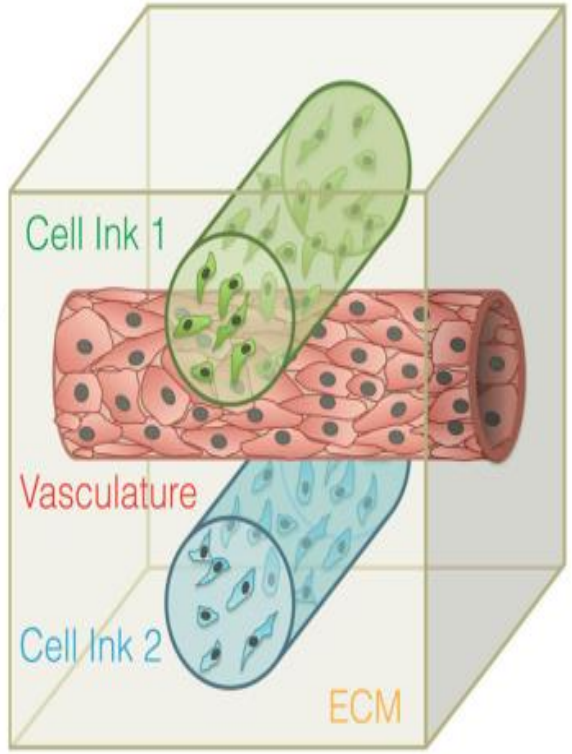
- Hypoxia occurs at the core of capsule
- Limits the Islets loading density and potential for scale up.



Bioprinting (Mimic Human Tissues & Organs)

- 3D bioprinting is a technique for positioning biochemical materials and alive cells in a stacking layer by layer at a desired location.
- 3D structure can be fabricated by controlling the space of the positioned components.
- Can manufacture capsules capable of accommodating cells for a transplantable level and inhibit hypoxia by promoting vascularization through structure and releasing molecules.
- Allows the deposition of a wide array of **cell types, biomaterials (bioink) and bioactive factors** in a precise order to simulate native tissue environment and support cell survival for building artificial tissues and organs.

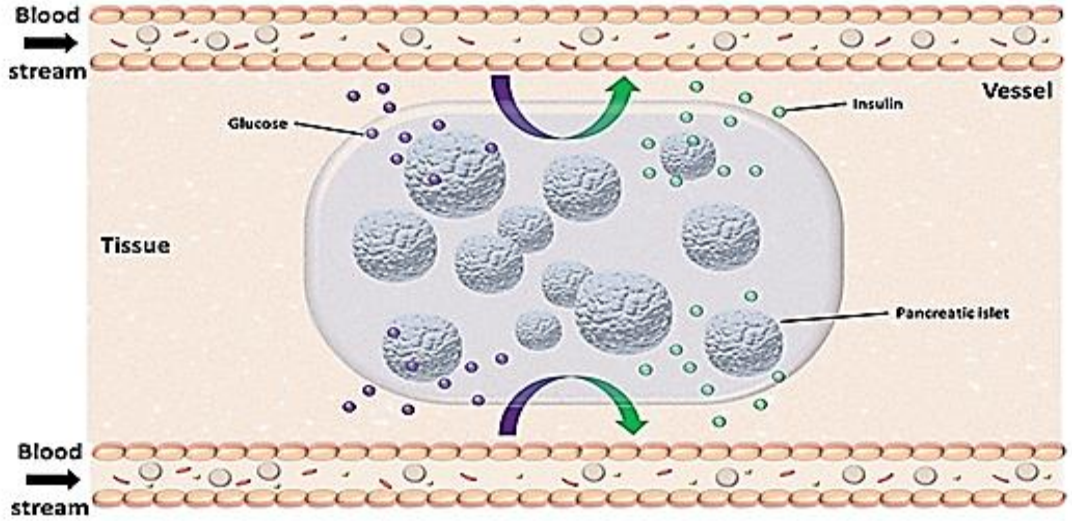




3D bioprinting classified into two systems depending on the materials:

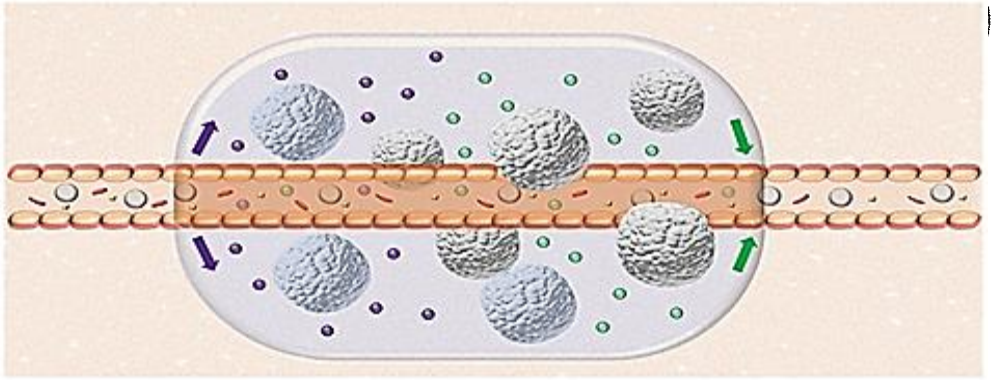
Scaffolding system:

- A synthetic polymer which should has biocompatibility and biodegradability mainly applied to the system.
- For example: polylacitc acid (PLA), poly(Lactide-co-glycolic acid) (PLGA), and polycaprolactone (PCL) approved by FDA are mainly used as synthetic polymers.
- Finally, the 3D structure containing the cells is completed.



Scaffolding free system:

- A **hydrogel** is mostly used for this system.
- The hydrogel can contains biomaterials, alive cells, growth factors and large amount of water that can provide the optimal environment for cells.
- Hydrogel is solidified by physical or chemical crosslinking to stack layer by layer to complete the 3D structure.



Coaxial printing

Now you most probably:

- Are familiar with different **terms in T1D setting**
- Can Identify the contributing factors in **β cell dysfunction**
- Can identify the suggested **mechanisms for β cell dysfunction**
- Know how we **study the β cell (dys)function and what are the available rescue approaches**

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Thanks!

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Learning is a never-ending story

The ones who light up for the others will never remain in darkness