# The Bone Marrow in Type 1 Diabetes

## Feasibility of Flow Cytometric Analysis of nPOD Bone Marrow specimens

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Miami

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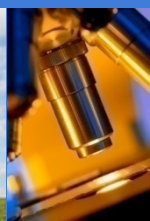
nPOD

Network for Pancreatic Organ Donors with Diabetes

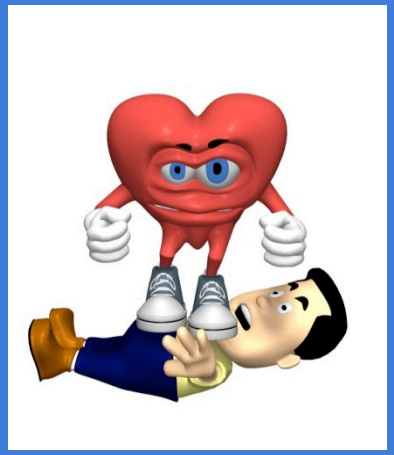



# BACKGROUND

**Cardiovascular complications are responsible for about 50% of deaths in diabetic patients.**

**Clinical outcome is worsened because of impairment of cellular and molecular mechanisms of vascular repair.**

**Unravelling the causes of defective repair mechanisms may lead to new therapies**



***124,000 heart attacks in UK every year***
***In the DDCT/EDIC study, prevalence of myocardial scar was 4.3% by cardiac MRI and 1.4% by clinical adjudication of MI***

***BHF data and Circulation 2011***



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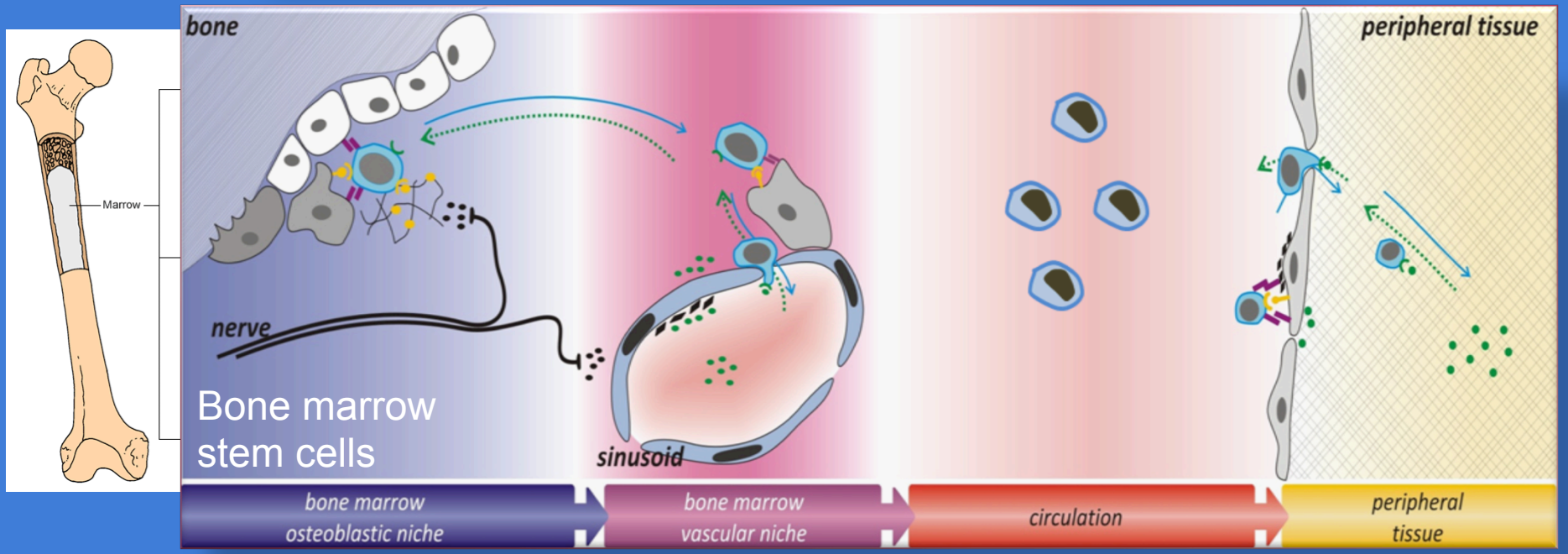
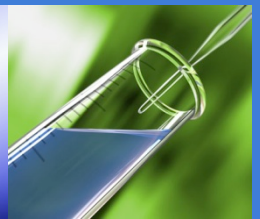
# The central role of bone marrow in cardiovascular repair




1. **Slow, circadian release under homeostasis**
2. **Rapid, protease-dependent following injury**

**Spnetti et al Cardiovasc Res 2011**



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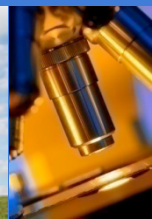
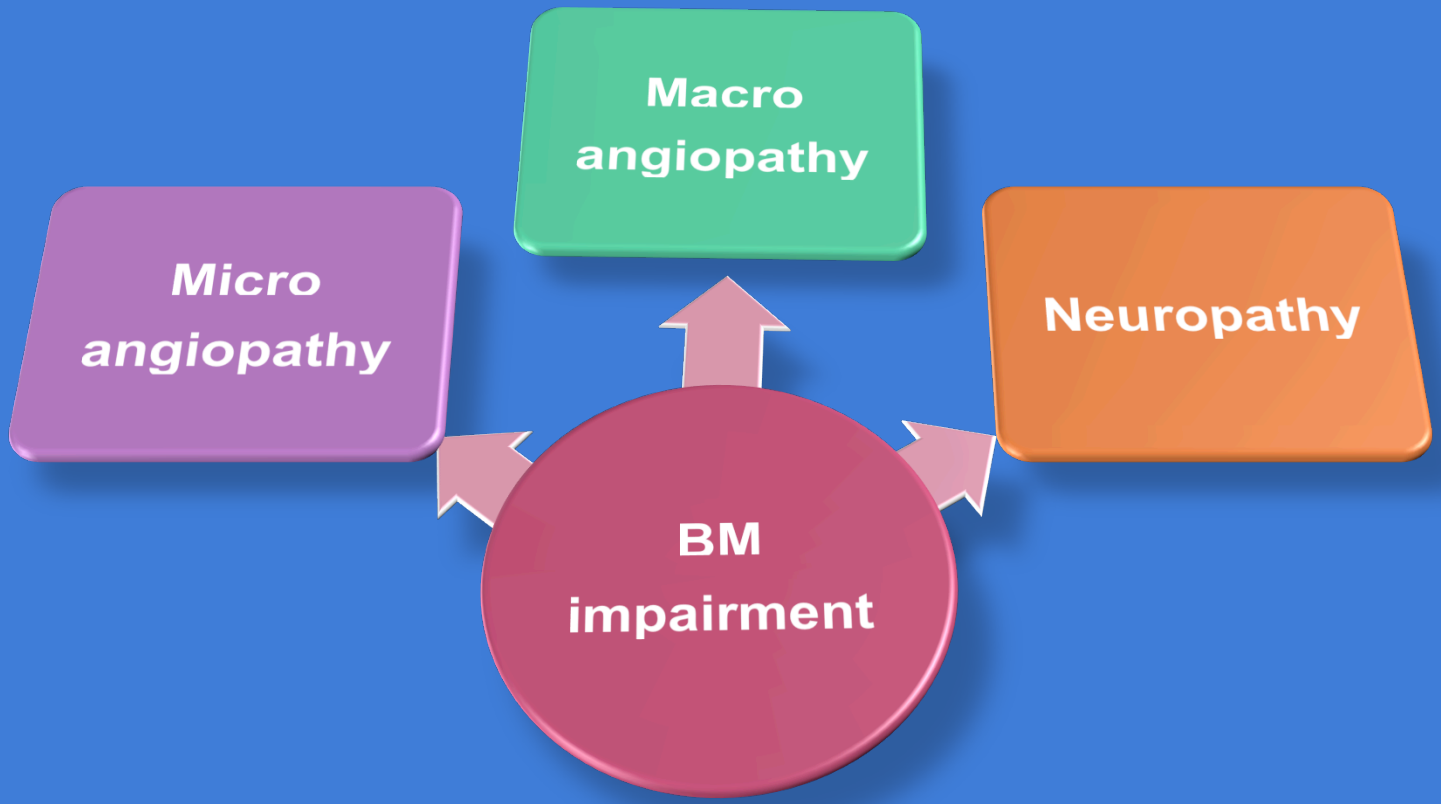


# The cause of all complications?



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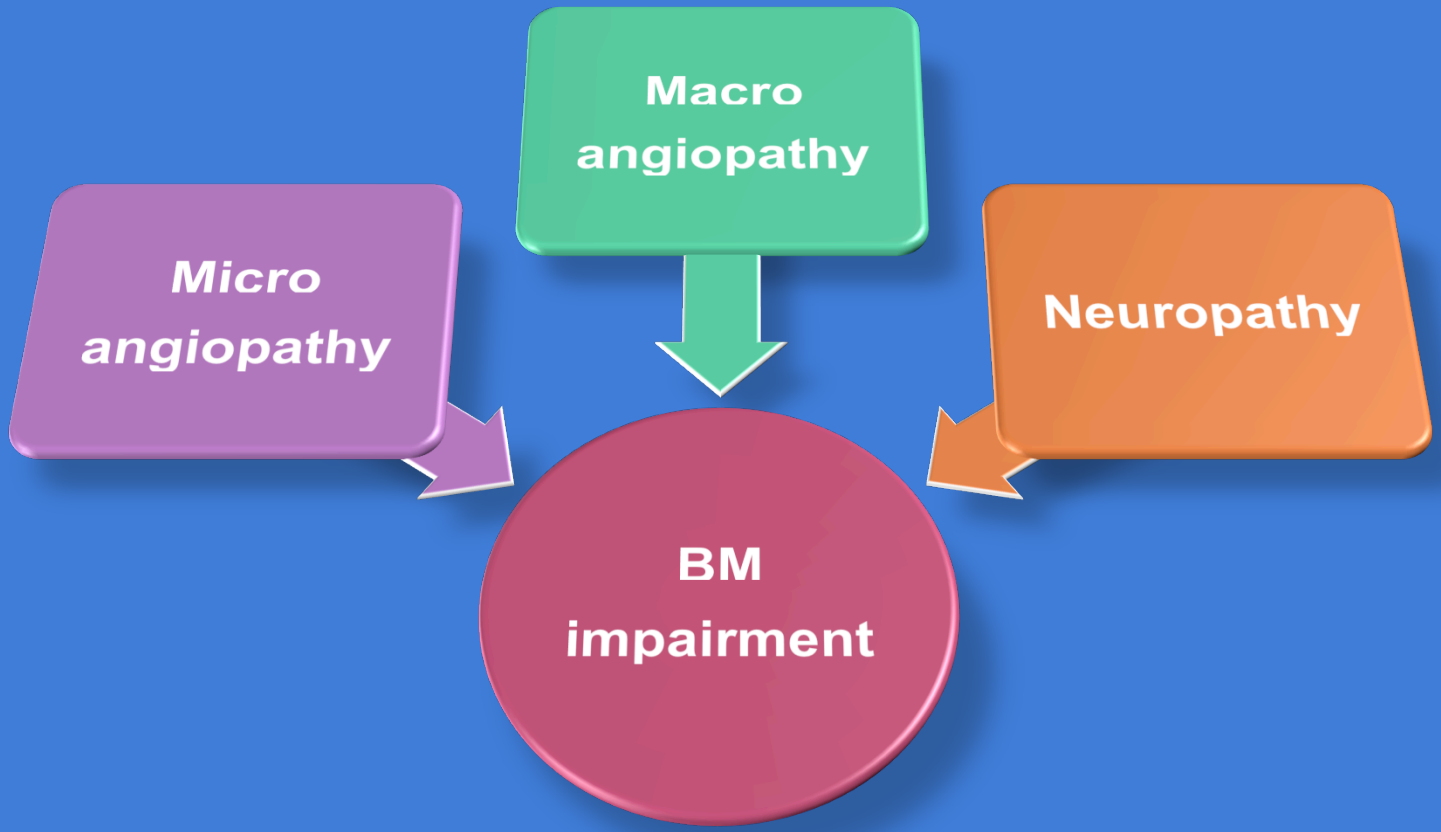
# Reversing the paradigm



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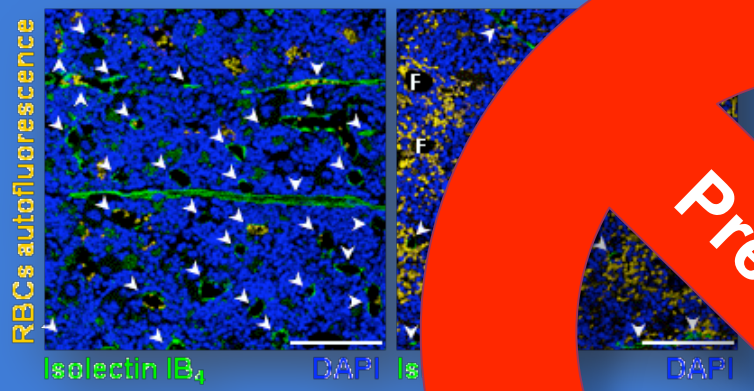
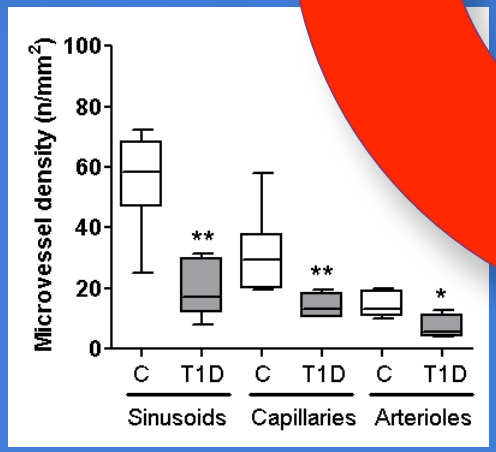
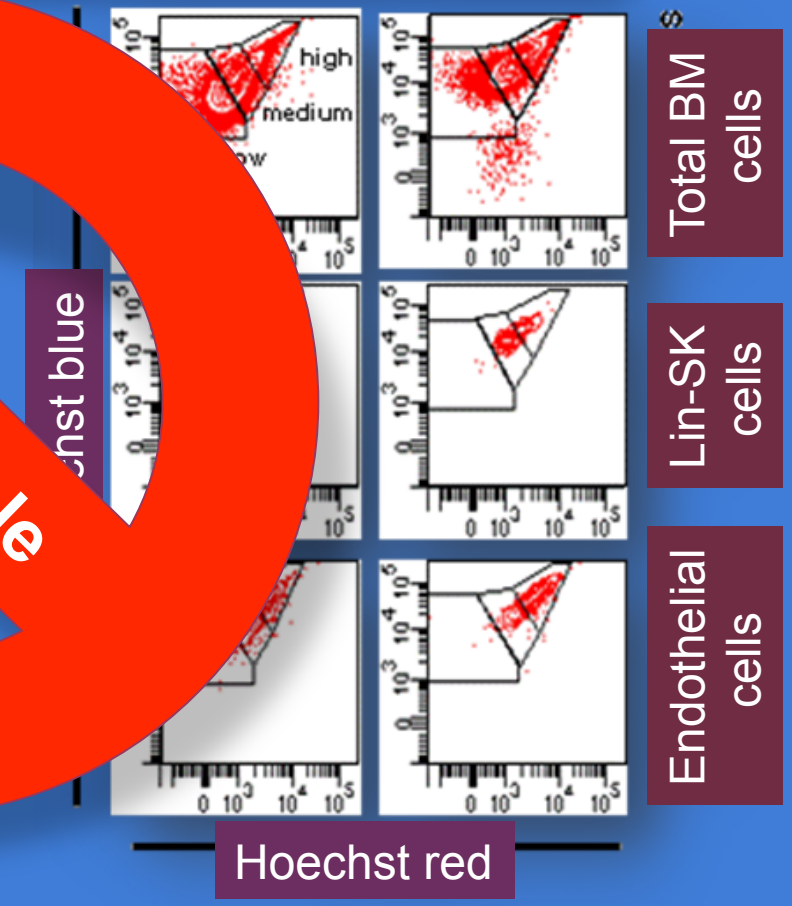
# BM as a target of microangiopathy




Preventable




Oikawa et al, ATVB 2010

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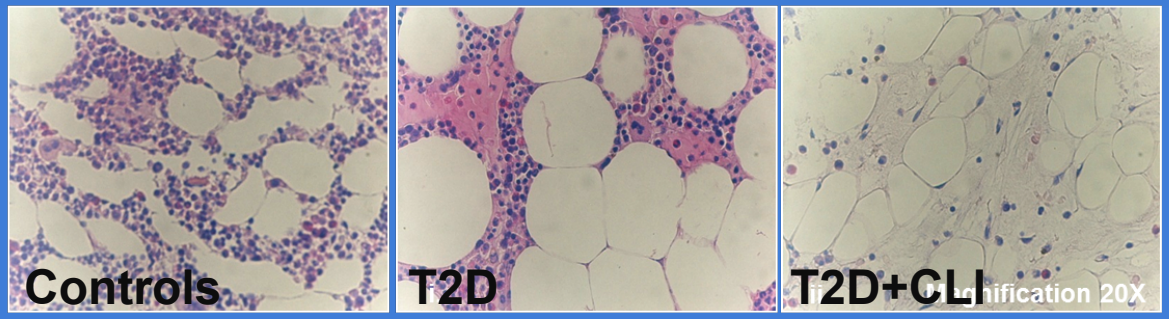
# Human BM as a target of micro-macro-angiopathy (a prospective study on tissue leftovers)

A

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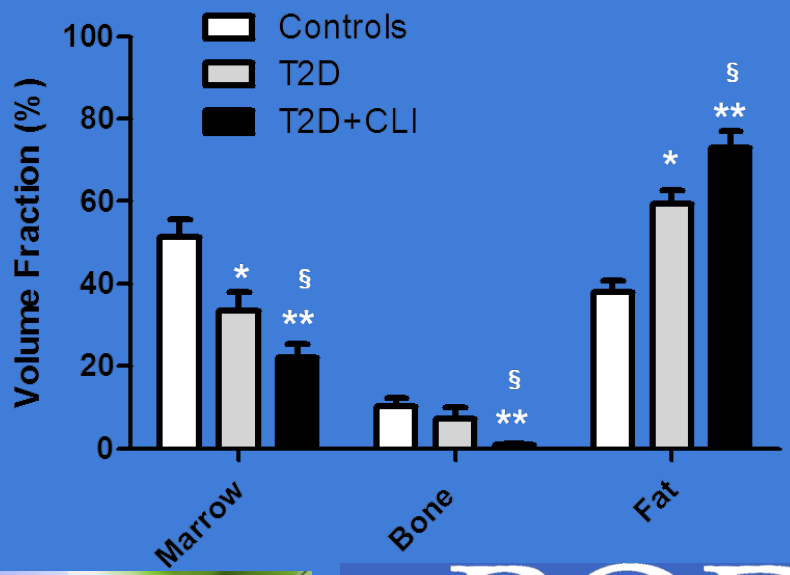
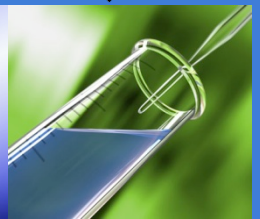



Spinetti et al, unpublished



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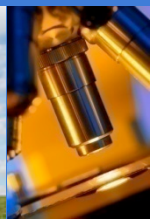
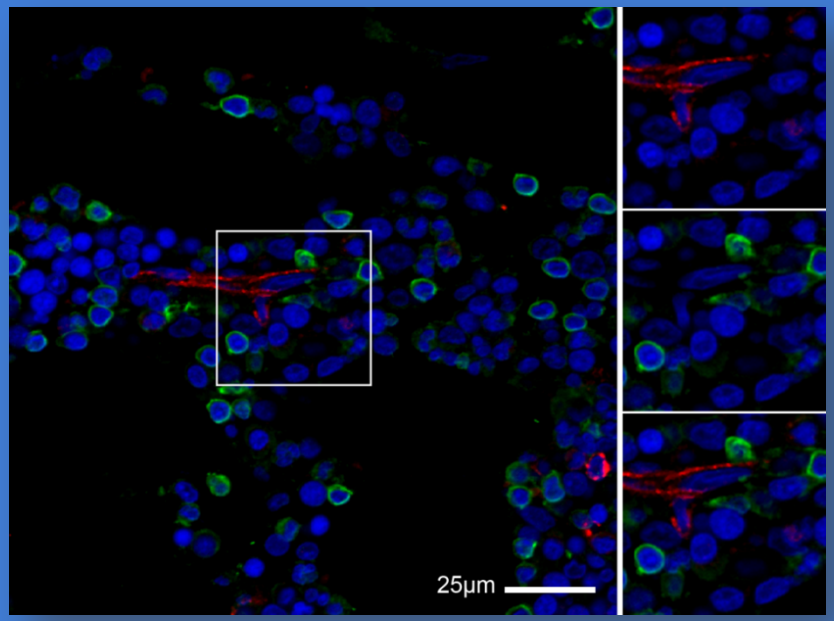
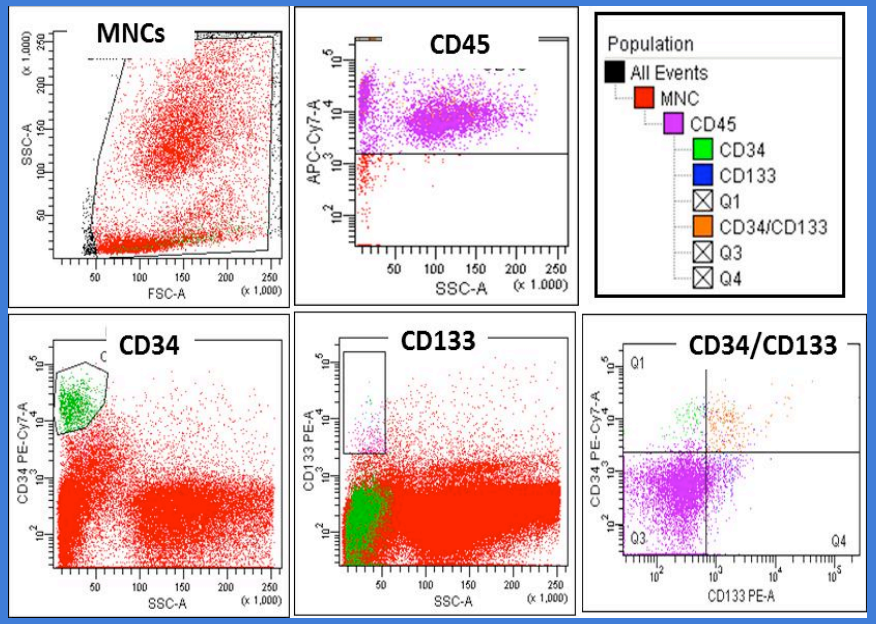
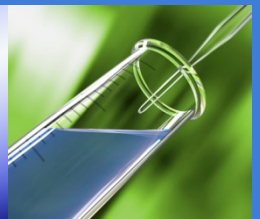


# Enumeration of stem cells in T2D BM




Spinetti et al, unpublished



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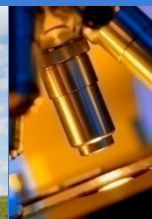
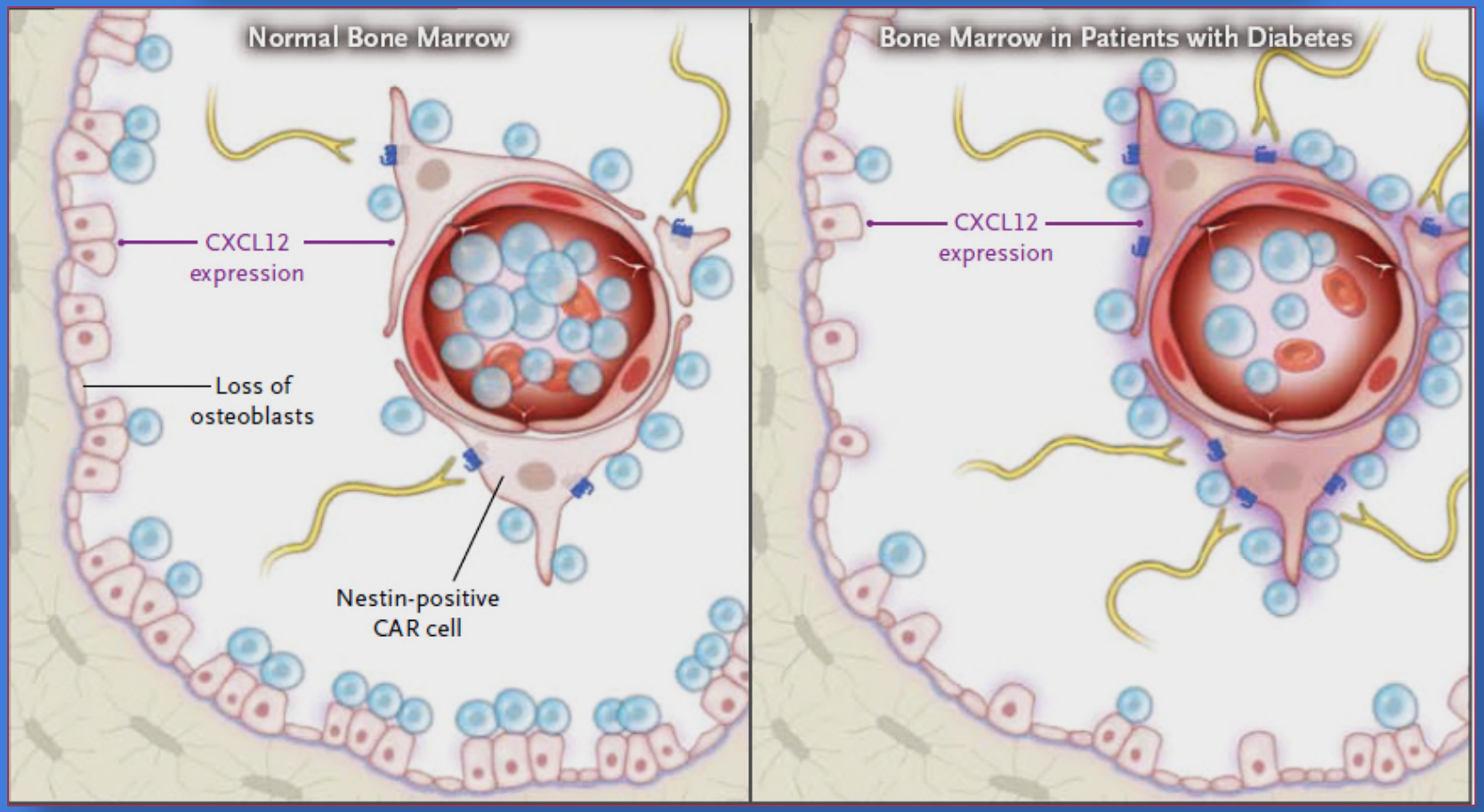


# Diabetic BM neuropathy




Ferraro et al Sci Transl Med 2012
Di Persio NEJM 2012



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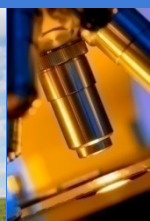
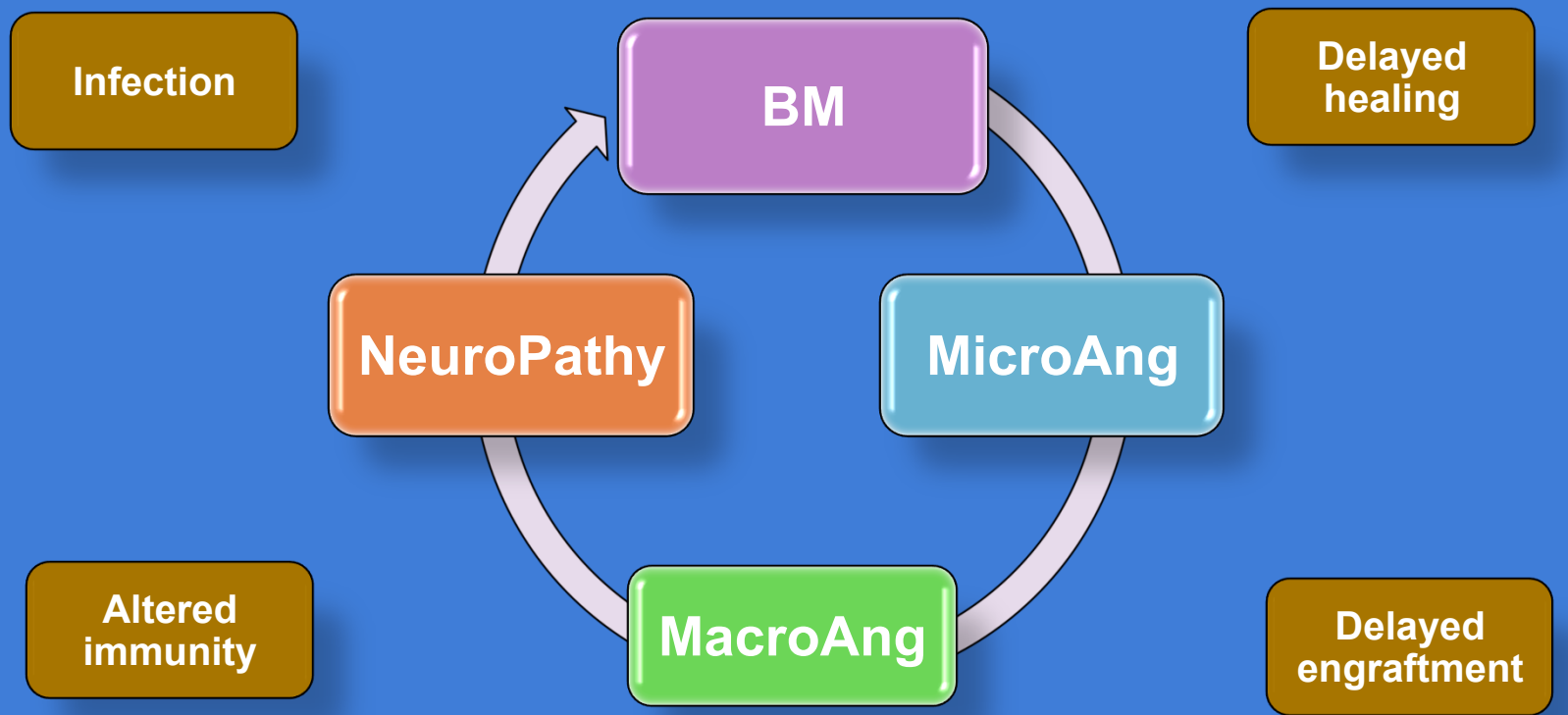


# Consequences of BM remodelling



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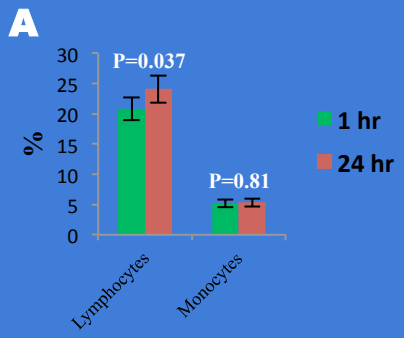
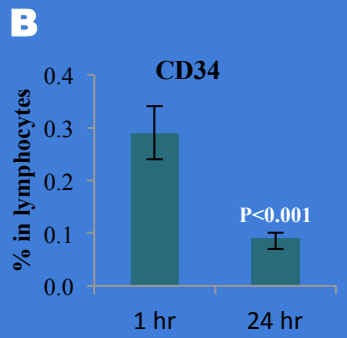
# Objective

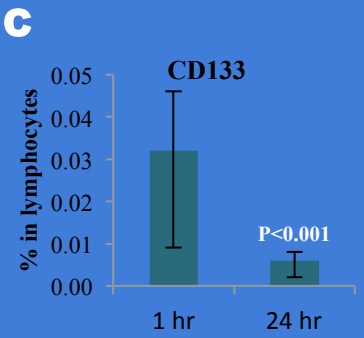
**To characterize BM remodelling at histological cellular and molecular level in nPOD diabetic patients**

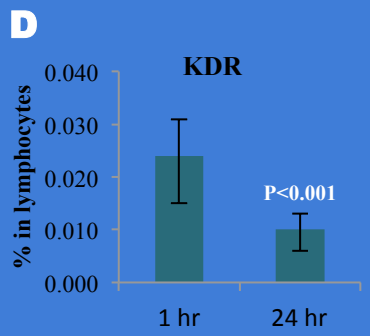


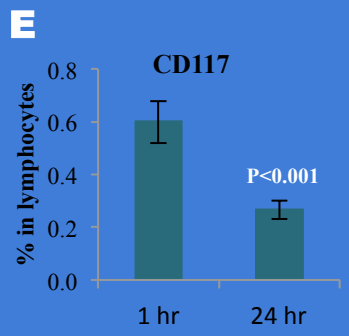

# *The freshness and quality of the samples are relevant for cell enumeration and function*

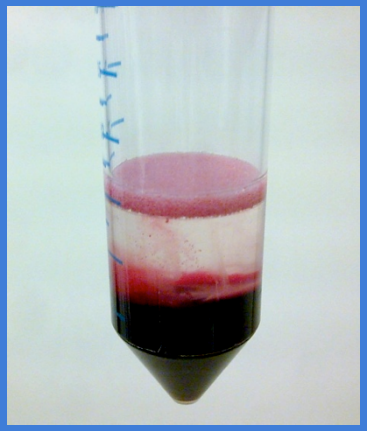


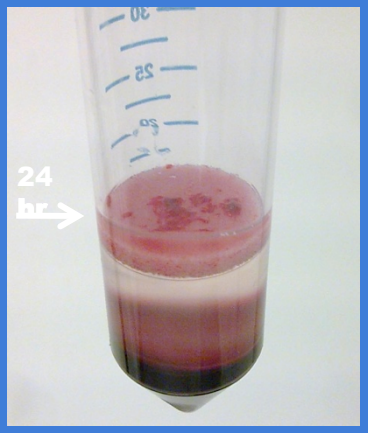



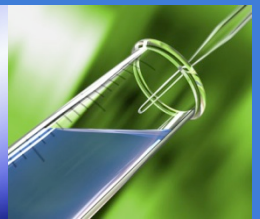






Cui et al PlosOne 2012



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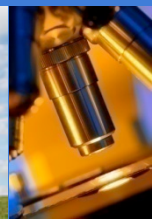
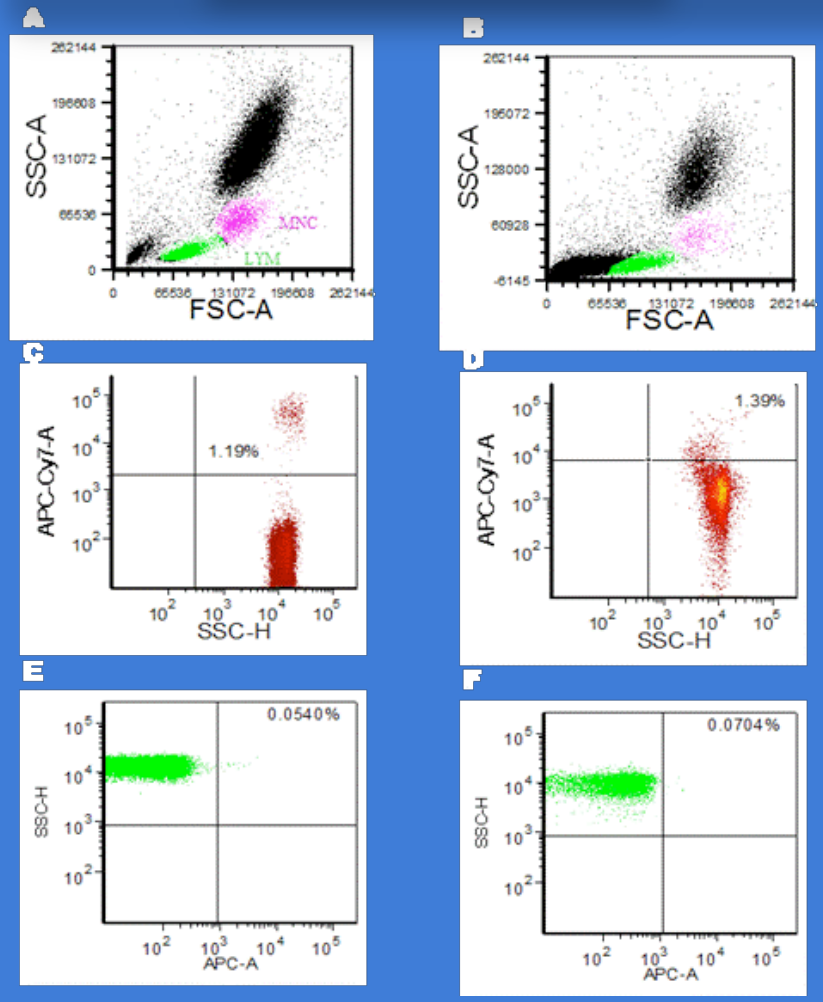


# Use of lysis buffer wash/fixative

Lysis/wash

Lyse/no wash




Fixable Viability Dye eFluor® 780

CD133+ cells

frequently blocked the fluid system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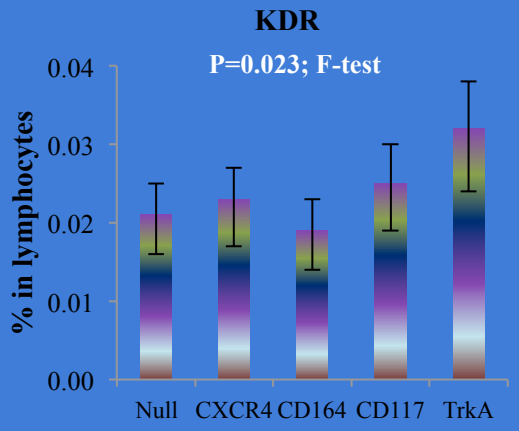
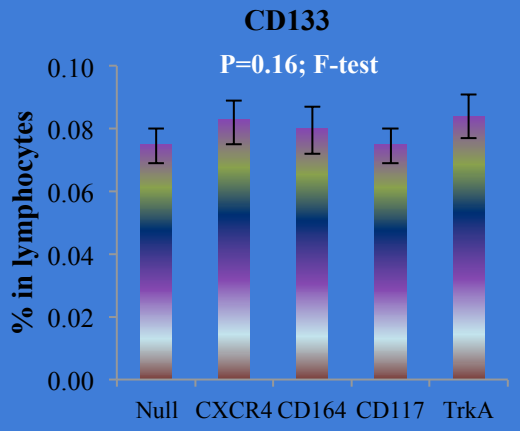
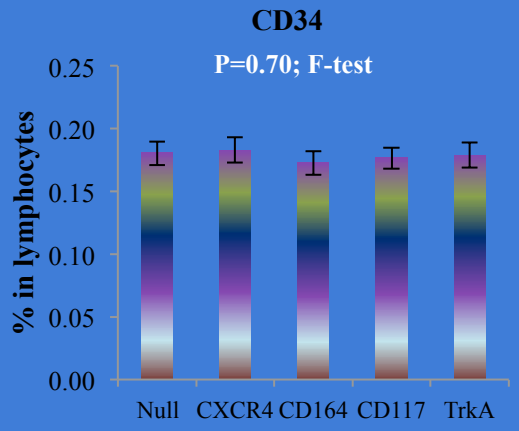
## *Interface of the multiple antibodies conjugated with different fluorophores*




*Tested if different PE-conjugated antibodies(CXCR4, CD164, CD117 and TrkA) have an effect on the enumeration of three commonly-used progenitor cells markers, KDR-FITC, CD133-APC and CD34-PECY7*

**Cui et al PlosOne 2012**

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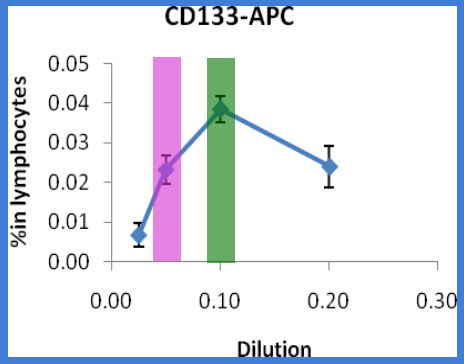
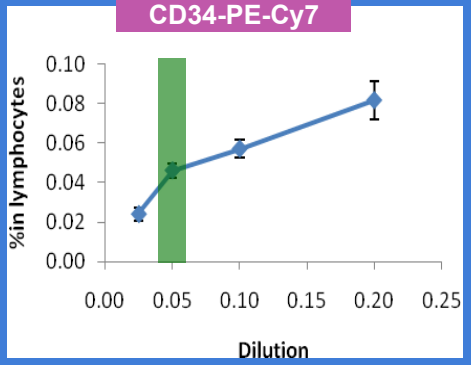
# *Titration of each individual antibody is essential before combination for cell enumeration*

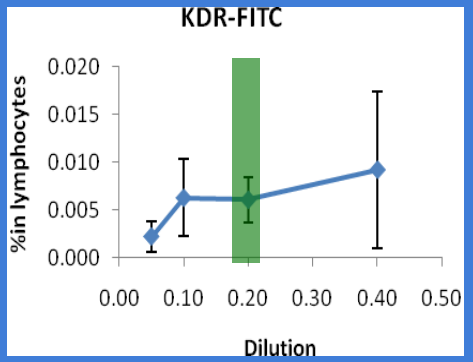
**A**



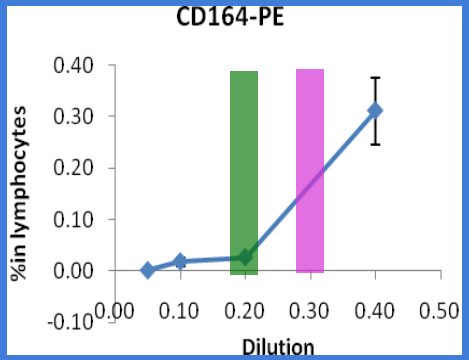

**B**



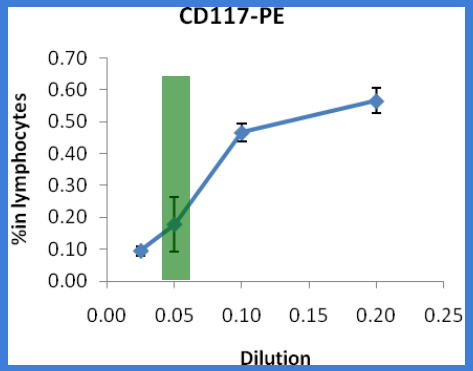

**C**



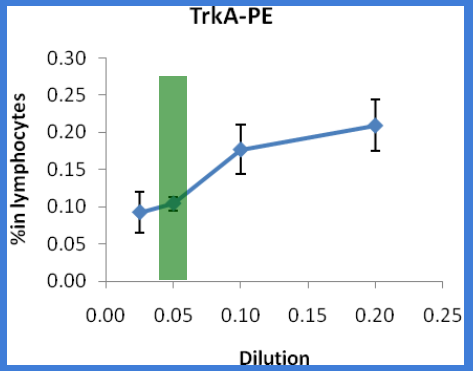

**D**

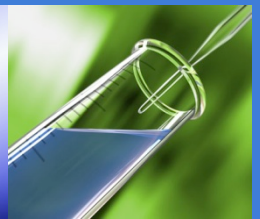



**E**




**F**



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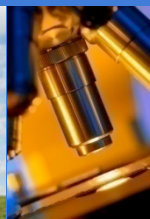


# Methods

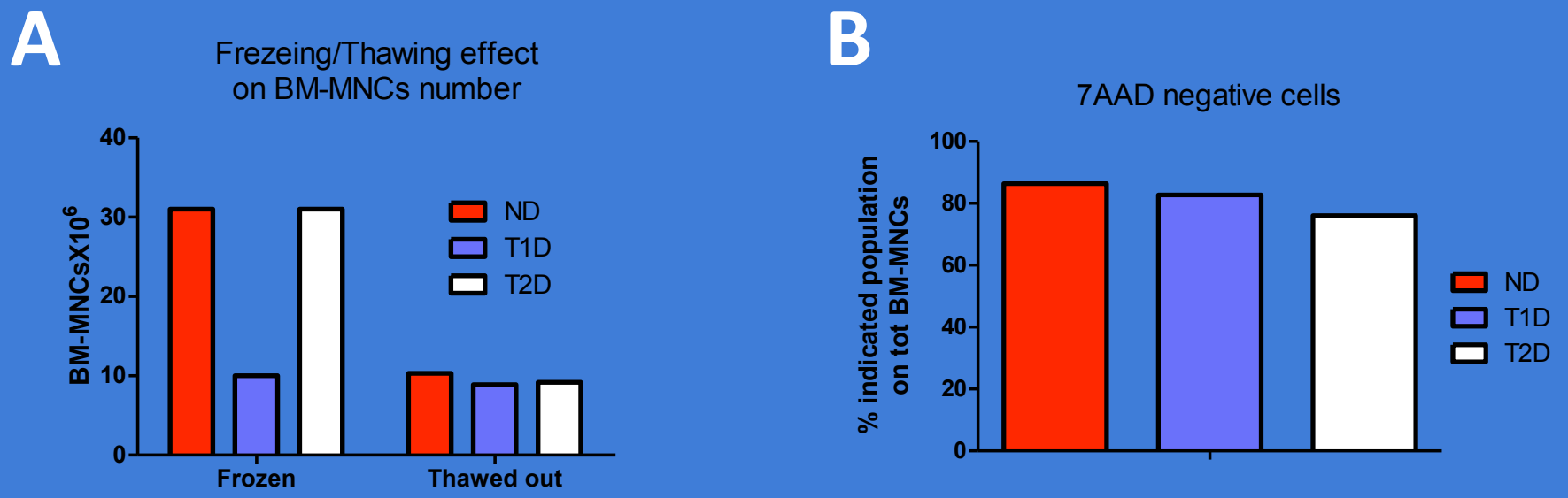
The effect of diabetes on BM-PCs was assessed in three nPOD iliac crest BM cases:

1) case 6126, not diabetic (ND) male (M), age 25 years
2) case 6161, Type 1 diabetic (T1D), M, age 19 years
3) case 6132, T2D, female, age 52 years.

Frozen specimens were thawed following nPOD instructions in DMEM+10% FBS. In addition, cells were left 2 hours at 37C, 5% $CO_2$ to recover from thawing before FACS analysis.




# Recovery after thawing out of shipped frozen nPOD BM-MNC




**A)** number of tripan blue positive BM-MNCs. **B)** Percentage of 7ADDneg-alive BM-MNCs after thawing.



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| Definition | Antigenic profile |
| --- | --- |
| Hematopoietic PCs | $CD34^{pos}$, $CD133^{pos}$, and c-kit $^{pos}$ |
| T-lymphocytes | $CD45^{pos}/CD3^{pos}$ |
| B-lymphocytes | $CD45^{pos}/CD19^{pos}$ |
| Natural Killer (NKs) | $CD3^{neg}/CD56^{pos}/CD16^{pos}$ |
| Mesenchymal Cells (MSCs) | $CD73^{pos}/CD105^{pos}/CD90^{pos}/CD34^{neg}/CD45^{neg}$ |
| Endothelial cells (ECs) | $CD45^{neg}/CD31^{pos}/CD144^{pos}$ |
| Early endothelial PCs | $CD34^{pos}/CD14^{pos}/CD45^{dim}/KDR^{pos}/CXCR4^{pos}$ |
| Late EPCs (lEPCs) | $CD34^{pos}/CD14^{neg}/CD45^{neg}/KDR^{pos}/CXCR4^{pos}$ |




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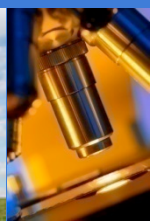
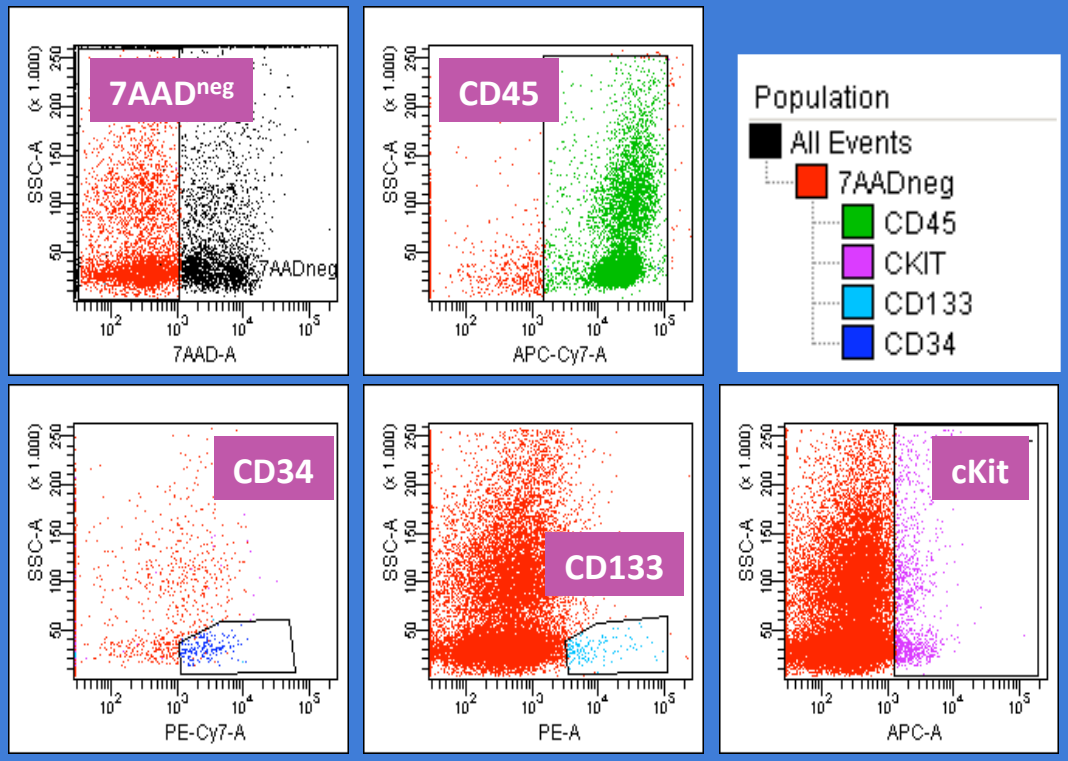
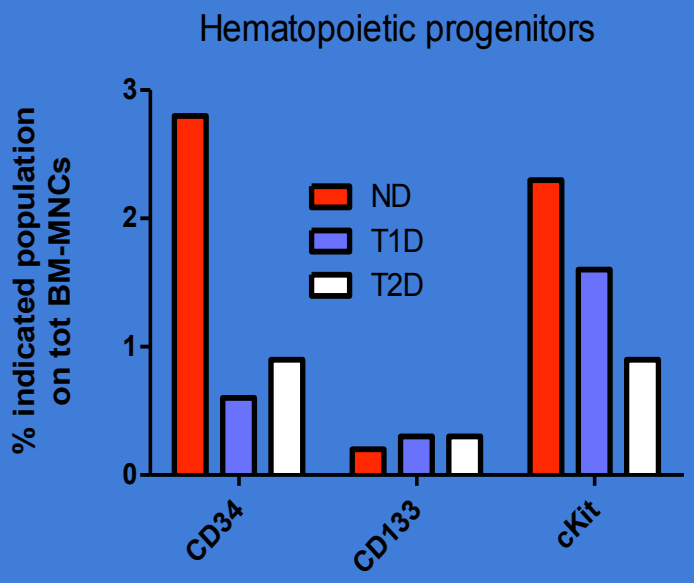


# Hematopoietic stem cells

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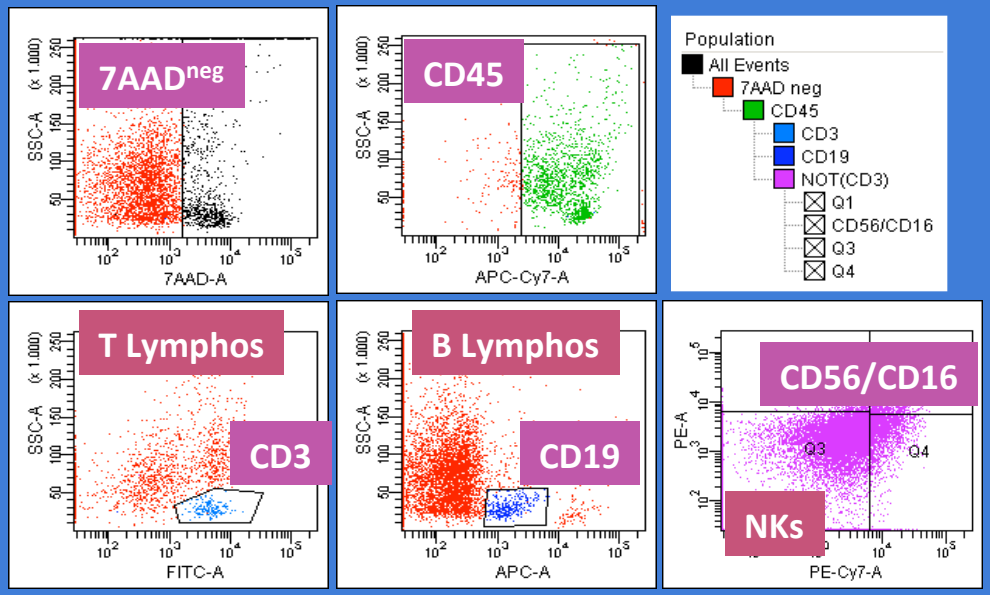
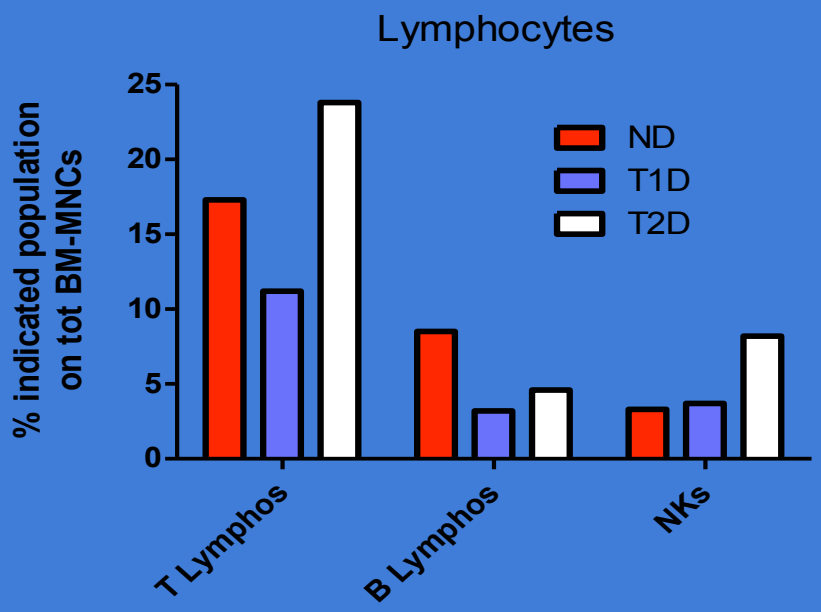
# Lymphocytes

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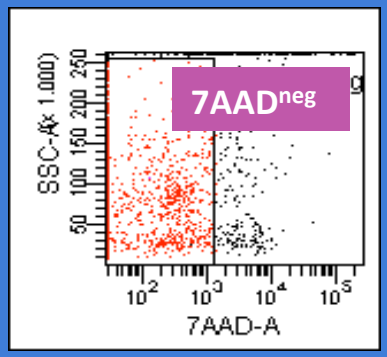
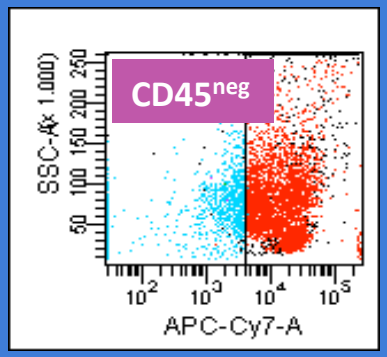
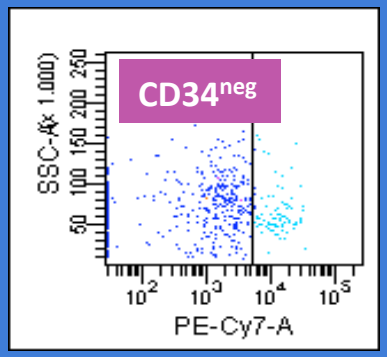
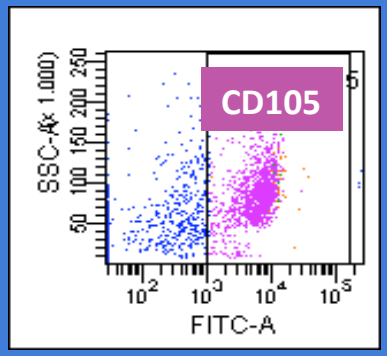
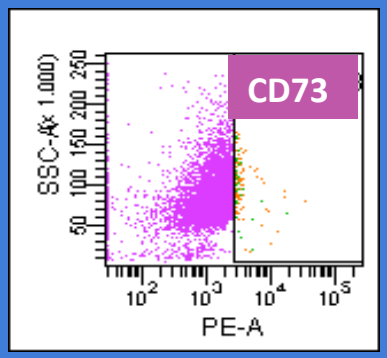
# Mesenchymal stem cells

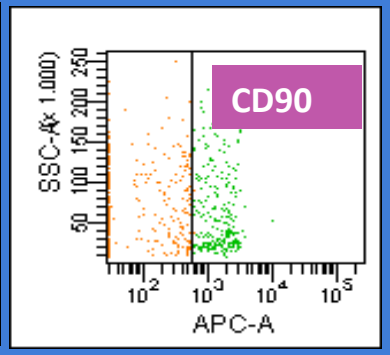


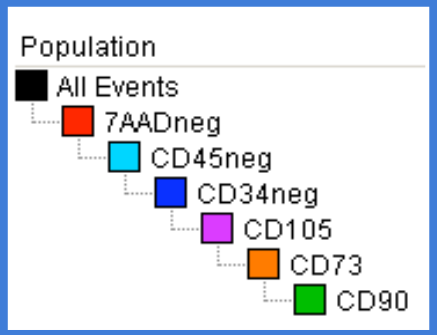



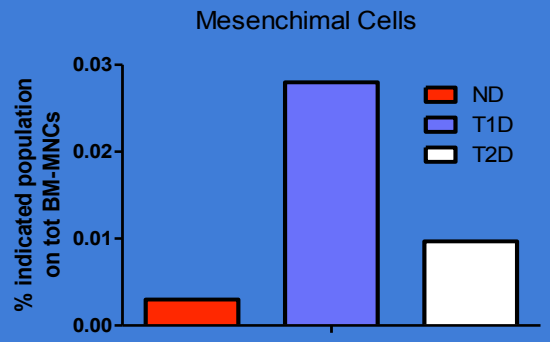

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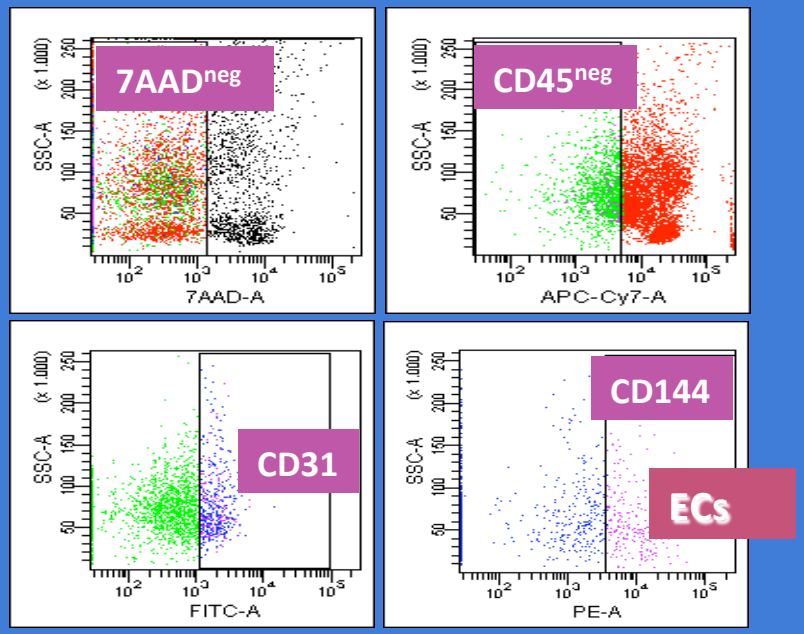
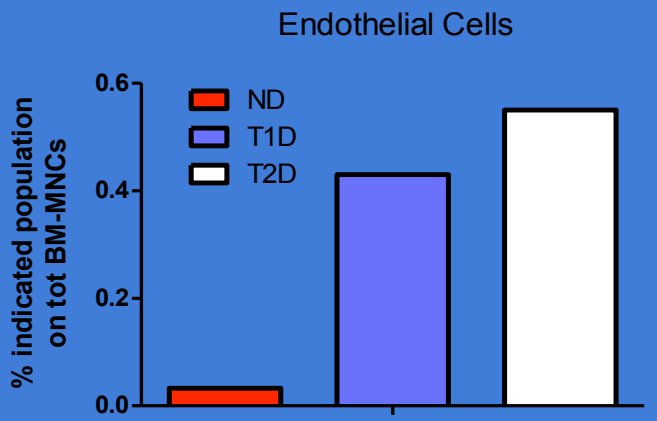
# Endothelial cells





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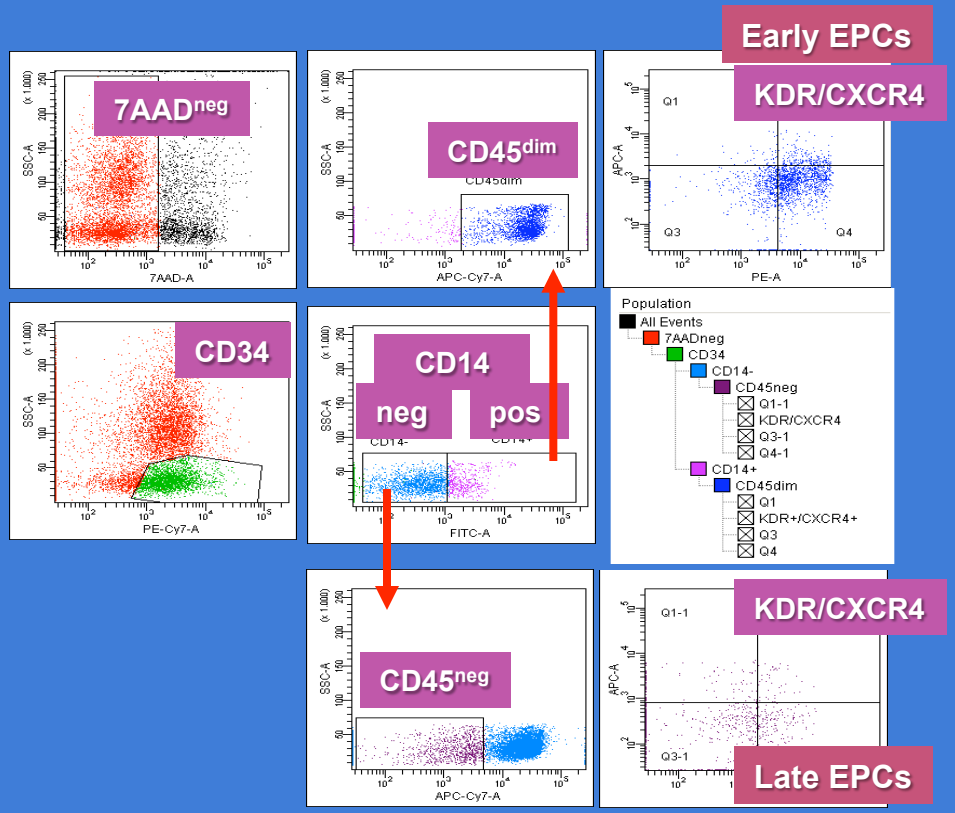
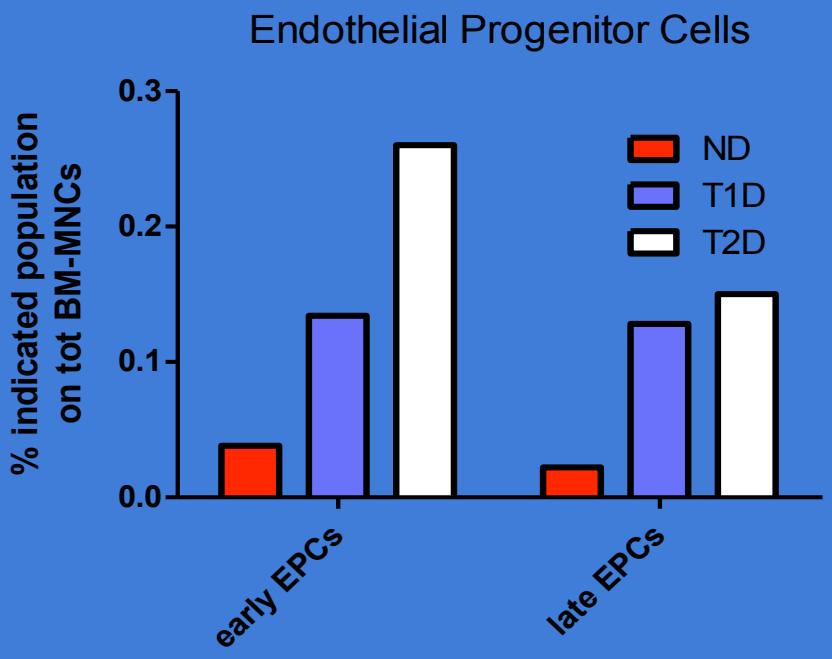
# Early EPCs

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# Conclusions

- ✓ We successfully verified a SOP to thaw nPOD BM specimens for cytometric analysis.
- ✓ Samples were adequate to measure the percentages of different populations of BM cells.
- ✓ However, the proportion of cells can be skewed because of distinctive cell loss during freezing/thawing.



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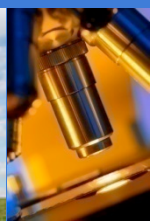


# Perspectives

**BM**

- Collect info on clinical correlates
- Compare with fresh samples
- Focus on certain populations (CD34+, CD34+KDR, lineage) to study cell specification

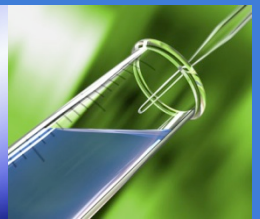
**CVD**

- Extend nPOD to cardiovascular complications (heart, large vessels, limb muscle)
- Focus on similar molecular mechanisms

**Links**

- Connection with other thematic areas





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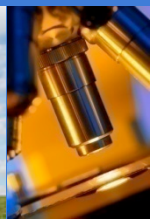

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