

# Biomarker Discovery for Prediction of Healing vs. Non-Healing Chronic Diabetic Foot Ulcers

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Diabetes and Digestive  
and Kidney Diseases



# No Conflicts of Interest

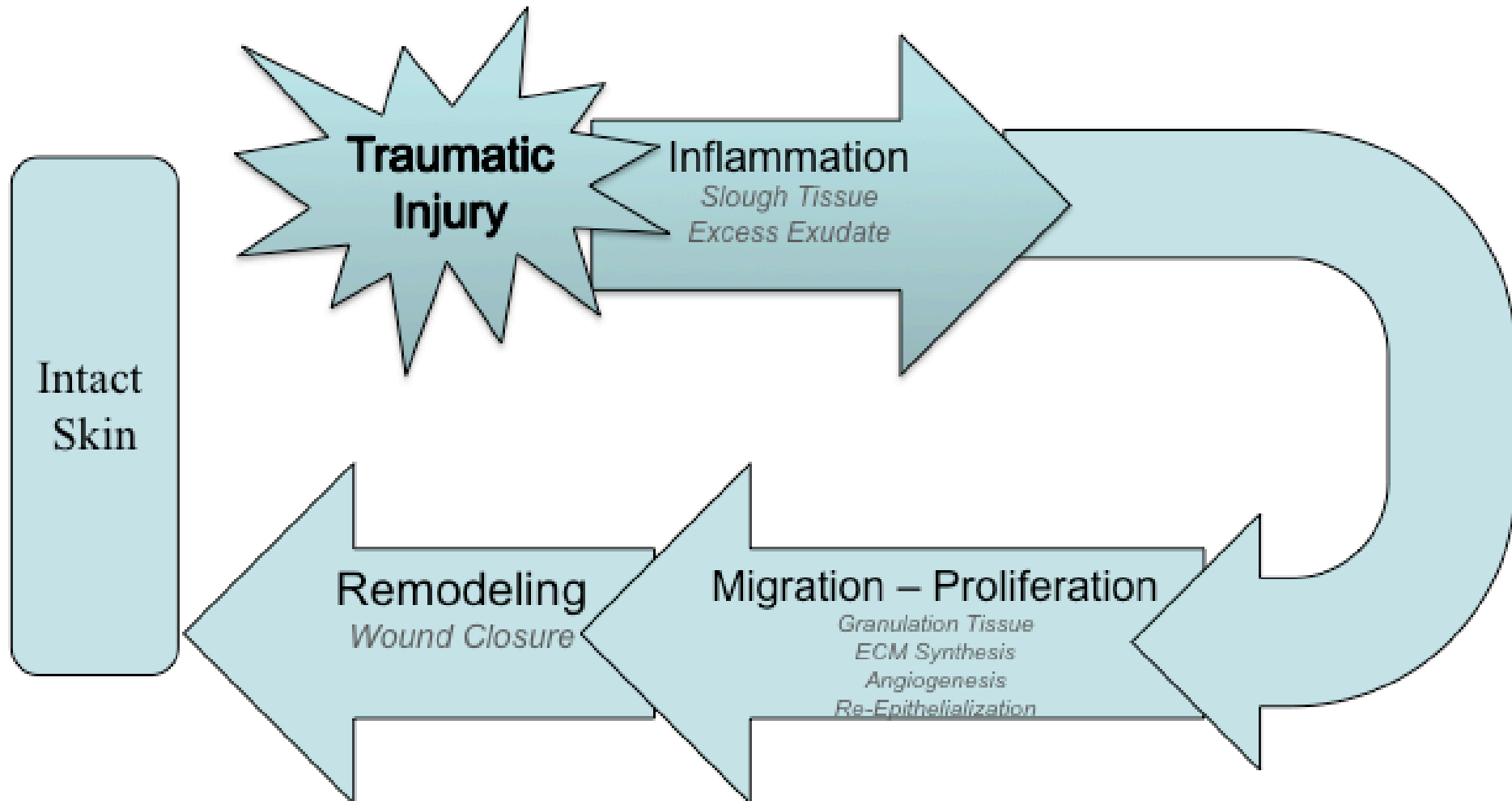


# Diabetic Foot Ulcers (DFU)

- More than 18 million people develop a DFU annually<sup>1</sup>
- DFUs often exhibit impaired healing, increasing chances of infection and/or LE amputation<sup>2,3</sup>
- Approximately 1 in 4 DFUs won't heal within 1 year<sup>4</sup>
- DFU patients have more than double the incremental healthcare costs associated with treatment compared to non-DFU diabetics (\$31,419 v. \$14,536)<sup>5</sup>
- Identification of biomarkers of wound healing in DFU will allow improved clinical diagnosis, prognoses, and targeted treatments



# Wound Healing



# Recruitment

**32 consecutive patients** recruited through a single university health system

Inclusion Criteria: Diagnosed Diabetes Mellitus, peripheral neuropathy, and chronic foot ulcer (>1cm<sup>2</sup> for 4 weeks)

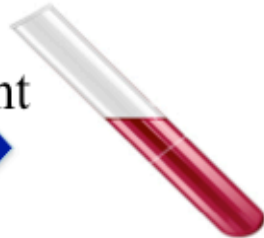
Exclusion Criteria: Infected foot ulcer, untreated osteomyelitis, active Charcot foot, inability to provide informed consent



# Methods



Debridement



Centrifugation



Diabetic Ulcer

EDTA Coated Test Tube

1. Immunodepletion
2. In-solution Digestion
3. UPLC Separation

Processing - Midwest  
Proteome Center

Serum

qPCR

- 84 gene wound  
healing array

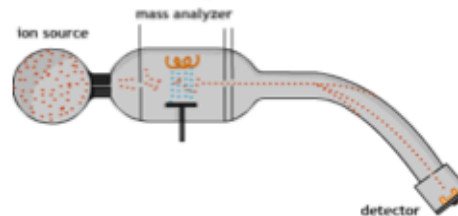
Mass Spectrometer

Luminex

- 18 Cytokines /  
Chemokines

Data Analysis

MS Output



# Cohort Characteristics

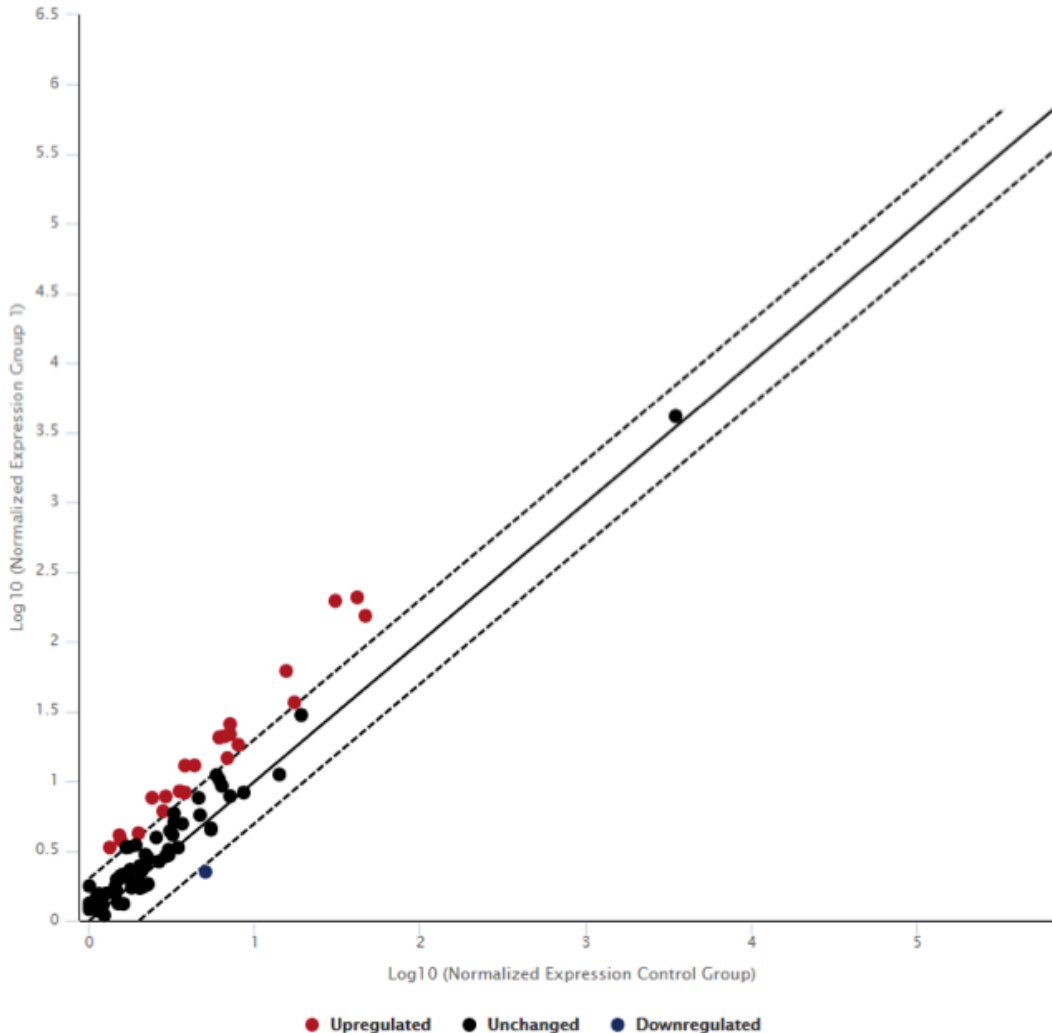
	DM Non-Healers (N = 19)	DM Healers (N = 13)
Males (%)	18 (94.7%)	10 (76.9%)
Age	55.7 ± 14.3	58.4 ± 10.9
BMI	33.4 ± 10.0	36.7 ± 8.2
HbA1C	8.12 ± 2.2	8.56 ± 1.9

Race, Insulin dependency, and tobacco use were also similar between the two groups (data not shown)

**Healing status based on a 12-week follow-up period**



# DM Non-Healers v. DM Healer Genetics



## Upregulated:

Actin Cytoplasmic 1 (ACTB)

Beta-2-microglobulin (B2M)

Collagen Alpha-1 (III) Chain (COL3A1)

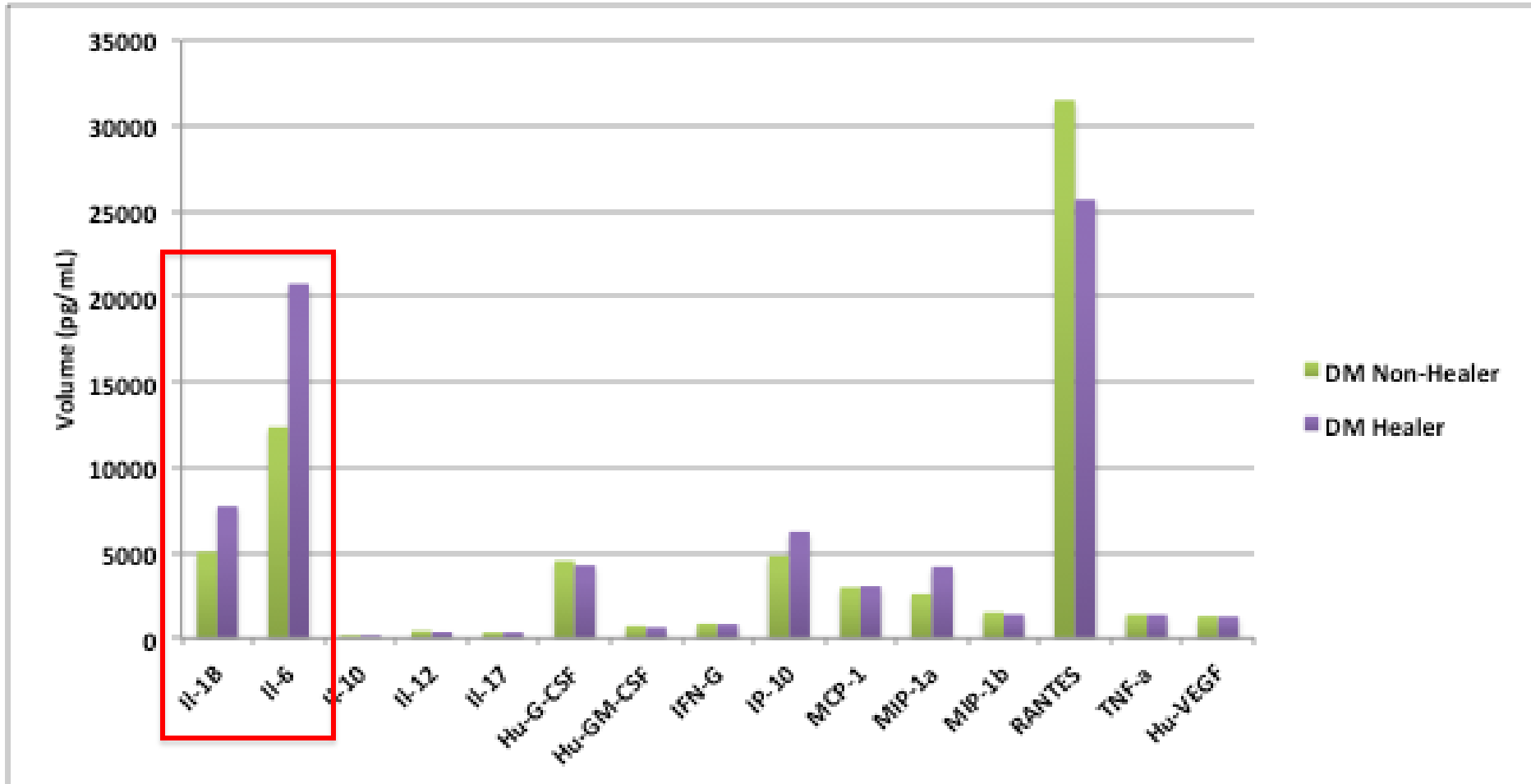
## Downregulated:

Granulocyte-macrophage colony-stimulating-factor (CSF2)





# DM Non-Healer v. DM Healer Cytokines



# Mass Spec

Protein	Function	Non-Healer v. Healer
<b>Annexin A1 (ANXA1)</b>	Plays a role in glucocorticoid-mediated down-regulation of the early phase of the inflammatory response.	<b>3.8-fold decrease</b>
<b>C4b-binding protein (C4BP)</b>	Controls the classical pathway of complement activation.	<b>5.1-fold decrease</b>
<b>Neutrophil gelatinase-associated lipocalin (NGAL)</b>	Iron-trafficking protein involved in multiple processes such as apoptosis, innate immunity and renal development.	<b>3.1-fold decrease</b>
<b>Transketolase</b>	Catalyzes the transfer of a two-carbon ketol group from a ketose donor to an aldose acceptor.	<b>12-fold increase</b>

# Conclusions

- Quantitative differences in gene expression and protein levels exist between diabetic non-healers and healers
- Largest differences were observed in proteins that have been minimally studied in the literature



# Moving Forward...

- Increase sample size to sufficiently power statistical analysis
- Include non-diabetic healer and non-diabetic non-healer control analyses



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# References

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