Proteomic role in Individualized Medicine: Future Perspectives

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The Need

To provide a compressive assessment of the physiological and pathological status of an individual.

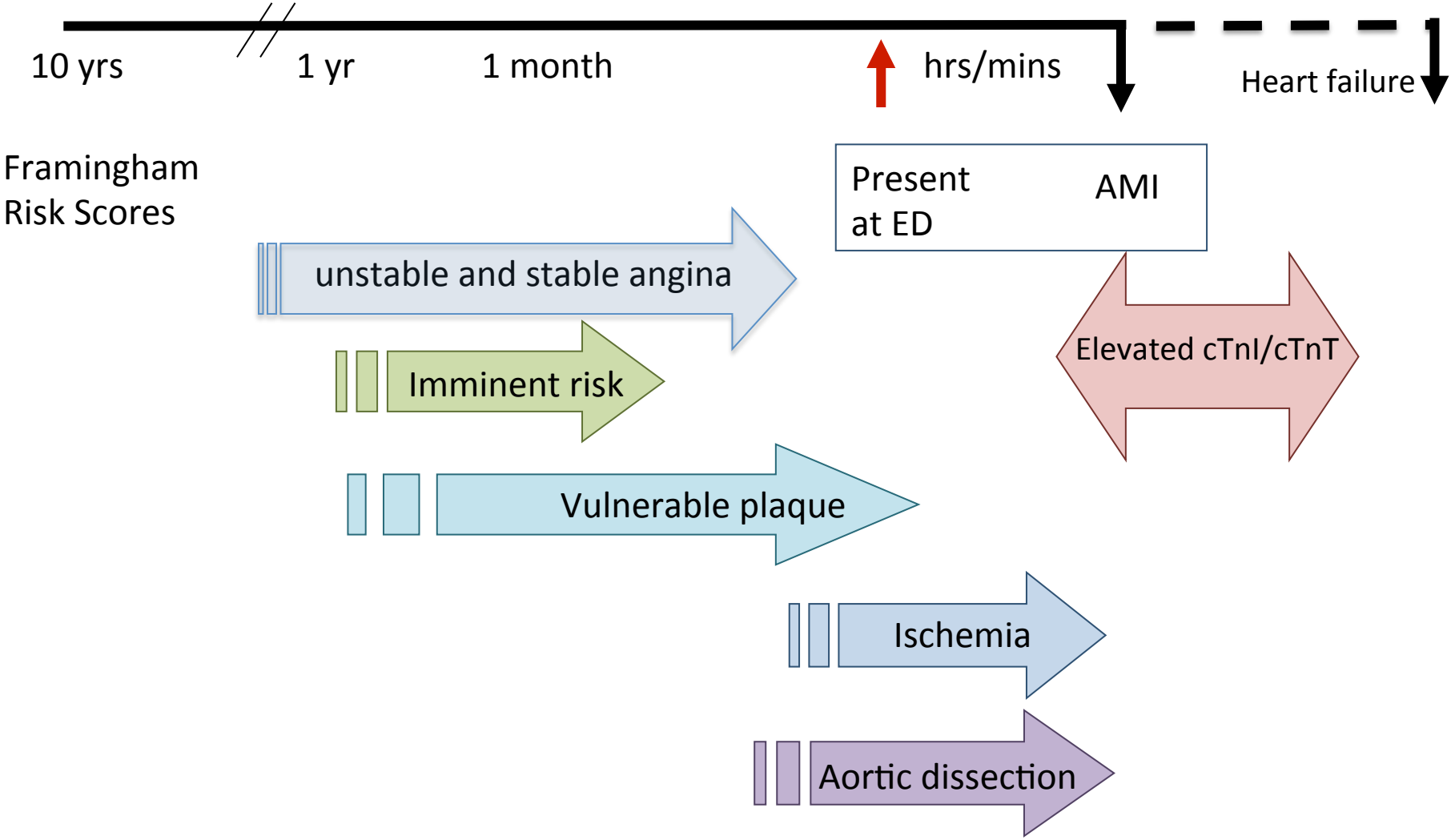
Issues

- Diseases are often multi-factorial and many overlap. Therefore, need to assess the patient within their disease setting. Current markers have a limited focus.

- What is required is to broaden focus and assess many disease pathways, simultaneously. This will provide a more accurate assessment of patient immediate and future health.

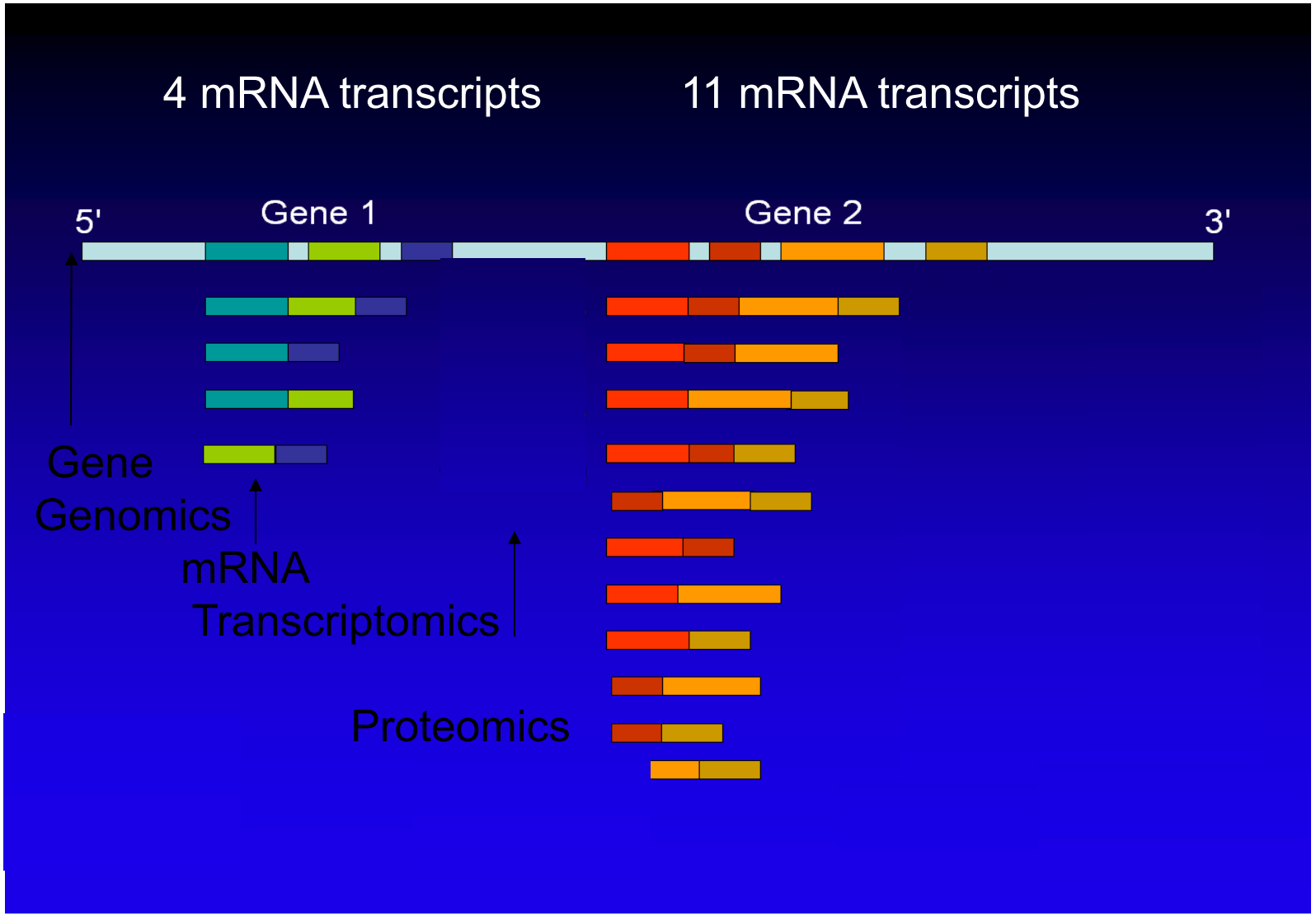
- This requires technical pipelines for discovery and preclinical proteomics with seamless interplay.

Clinical Diagnostic Domains



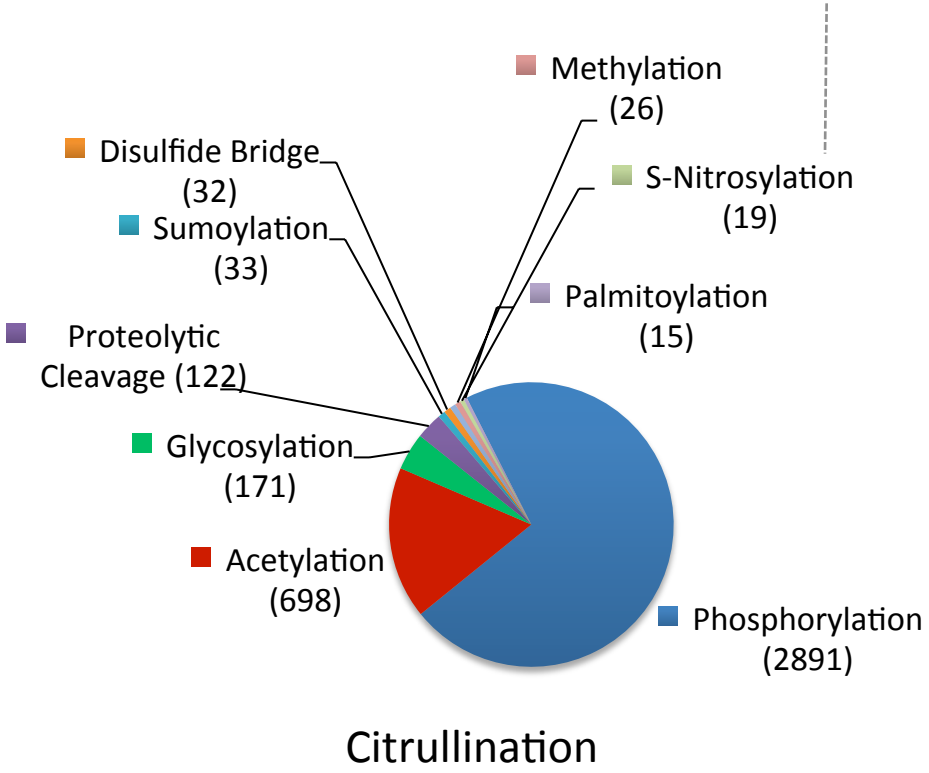
Challenge: The analyte needs to be exclusive for an unique disease attribute.

Solution: Proteins have sufficient diversity to allow for a specific form(s) to be diagnostic to a unique state in the disease.



There are 5892 annotated human cardiac proteins. 68% have a PTM.
~25% have more than one type of modification (Agnetti et al. Circ Res 2011)

Improved method – 83 new SNO sites within the mitochondria. *Murphy et al. MCP 2010*



Citrullination

A PTM newly discovered in the heart

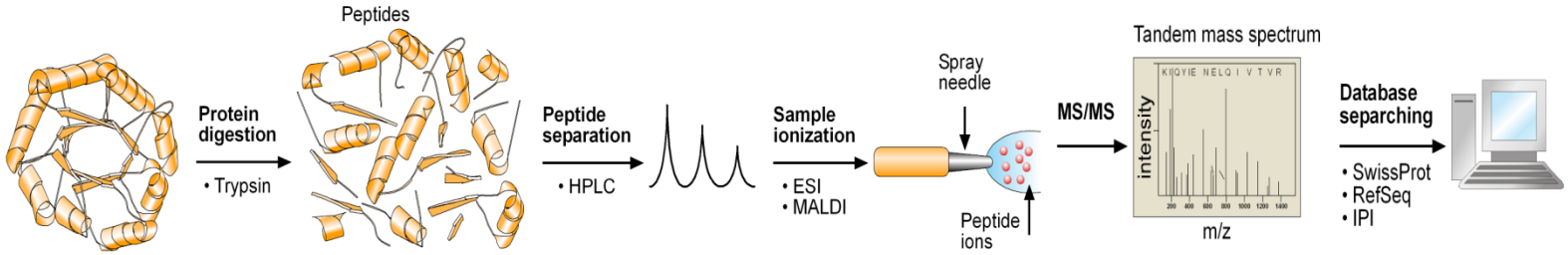
The diversity of proteins and their PTMs drives biology and underlie pathological processes.

The disease-induced protein changes reflect alterations at the gene, mRNA and metabolite level.

Quantitative assessment of disease-induced PTMs should provide disease stage specificity, thus improving individual risk stratification.

Human protein reference database

Proteomics: Multiple needs, technologies and tools



Many separation/enrichment methods
Use depends on goal

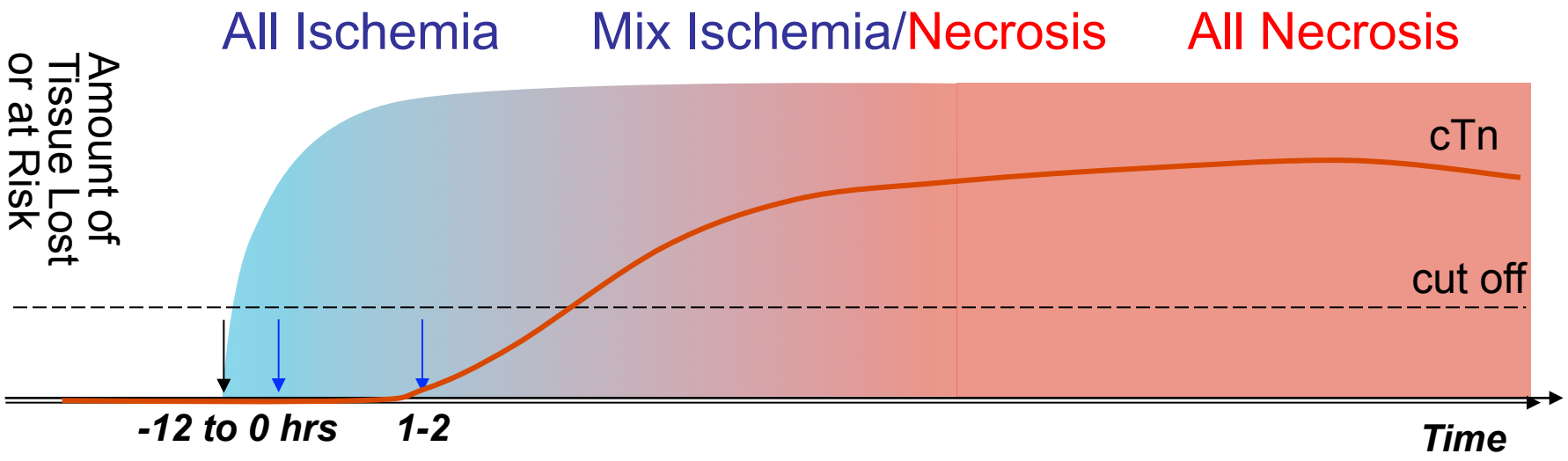
Many mass spectrometers
Use depends on goal

Challenges

1. Large cellular/sample dynamic range.
2. No protein amplification system.
3. Change in single amino acid is sufficient to alter physical and functional properties.
4. The same and different PTMs can be located at multiple sites within one protein.
5. Different PTMs can compete for the same amino acid residue.
6. Small stoichiometric changes can have large functional affects.

To measure organ necrotic/cell death the use of cell specific intracellular protein released into blood stream is sufficient.

Challenge: Measurement of an analyte(s) in body fluid (non-invasive) that allows the assessment of non-cell death disease states.

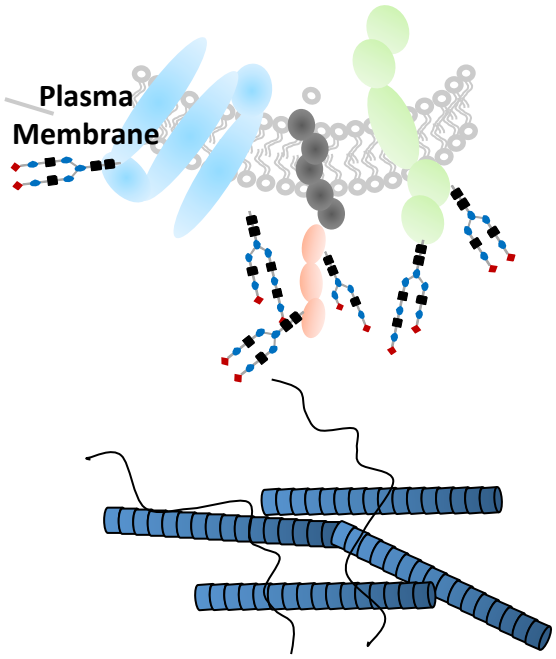


Non-necrosis ischemic markers
Cell secreted proteins, shredded cell surface or extracellular matrix protein may not be organ specific.

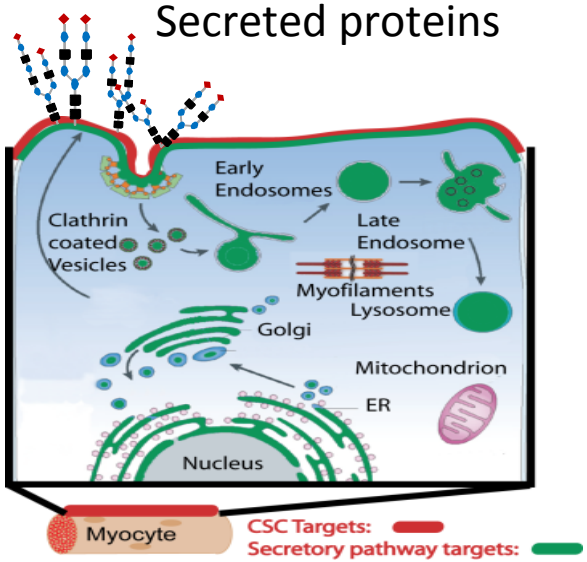
Necrosis markers
Cellular release of cardiac (organ) specific isoform of cTnI/cTnT into blood.

Ischemic/hypoxic-release of protein from isolated cardiac myoctyes and fibroblasts or directly into plasma

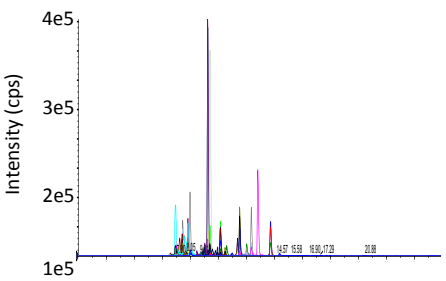
Disease induced PTMs of cTnI

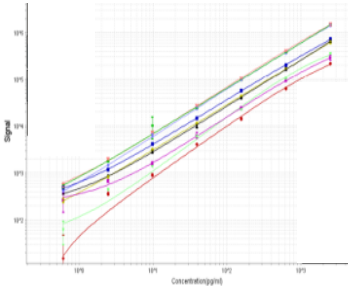


Secreted proteins

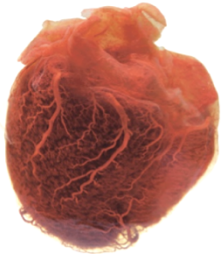


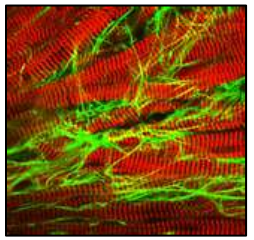
Cell Surface/secreted "Biomarkers"



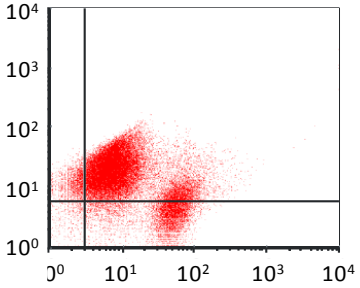


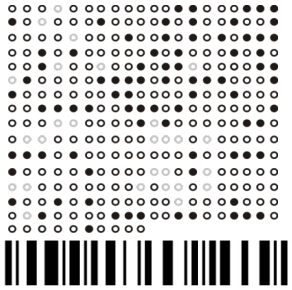
Function/therapeutic intervention



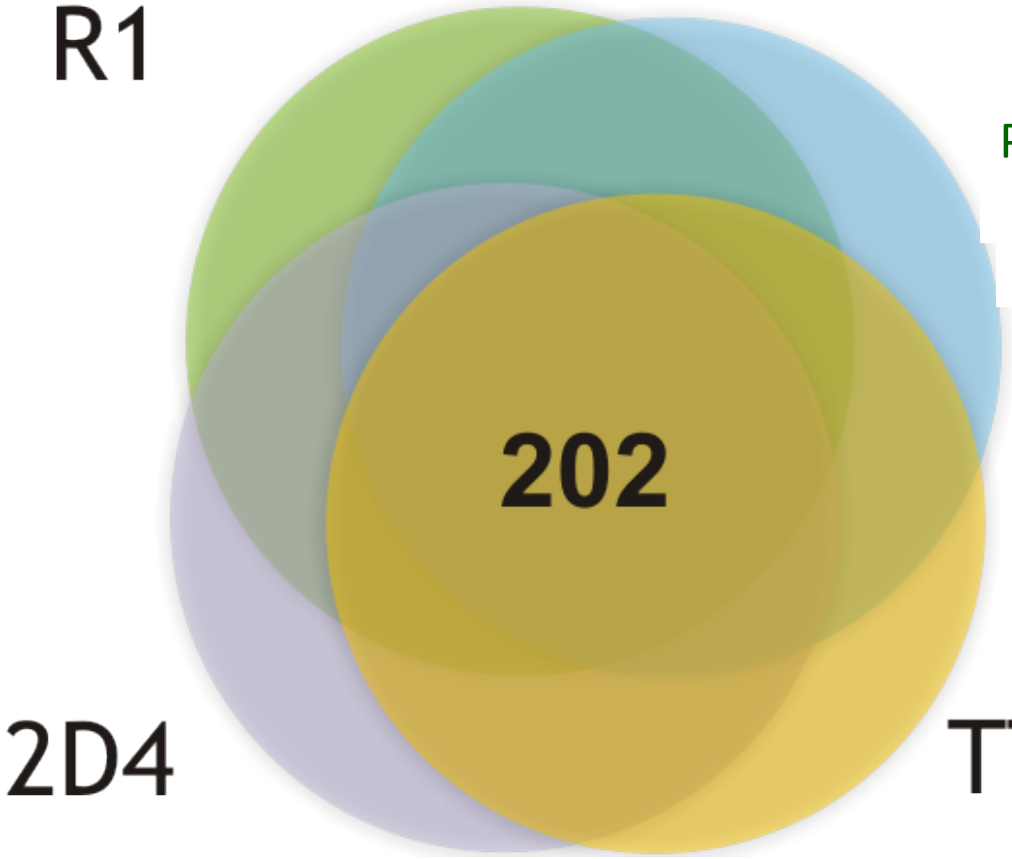


Cell Surface "Barcode" for enrichment



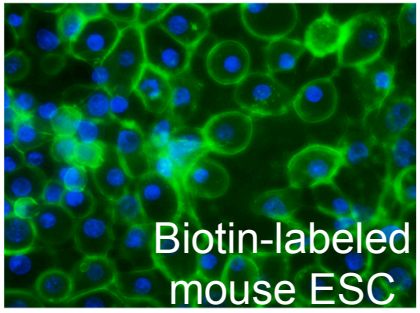


Find unique cell surface markers to differentiate embryonic stem cells (ESC) and induced pluripotent stem cells (iPS)

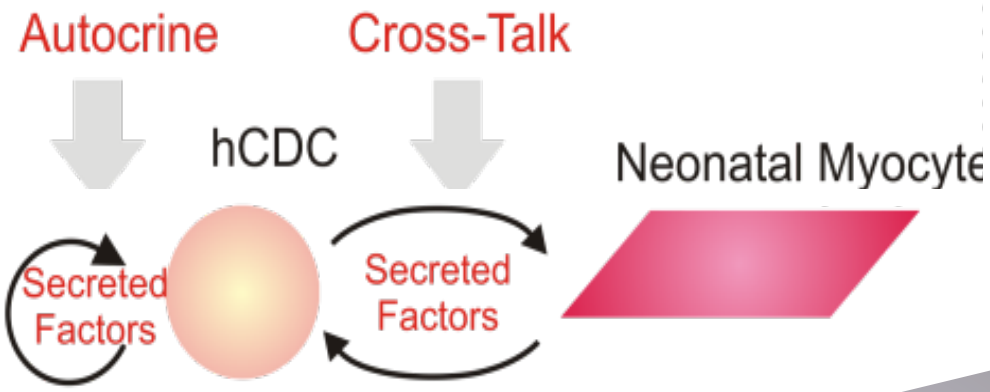


Potentially unique to ES = 56

Potentially unique to iPS = 4



The Secreted Proteome



Discovery, Validation, Quantification of Candidate Secreted Proteins

[Absolute] in large clinical trial & population studies

Diagnostic /prognostic biomarkers

Circulating biomarker for risk stratification

Cellular Function Studies

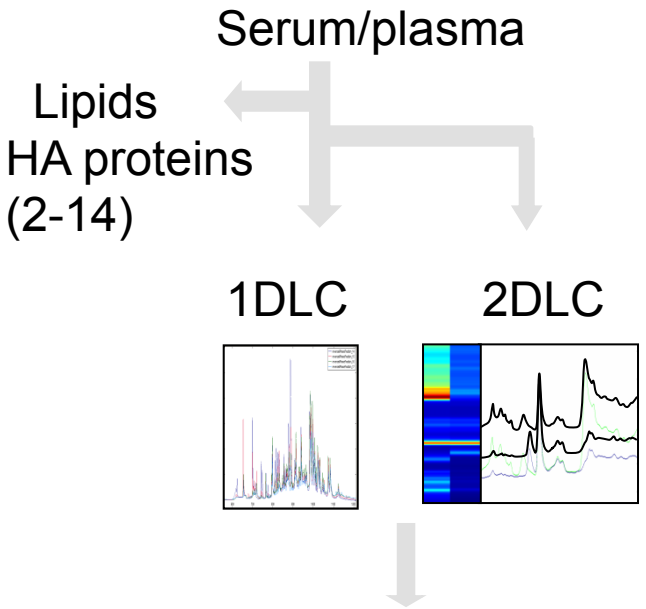
In vivo models

Cocktail for in vivo *cardiac regeneration*

A development pipeline

Review: Stastna *et al* FEBS Lett. 2009;583:1800-7.
Method: Stastna *et al.*, Circ Gen, 2011; in press

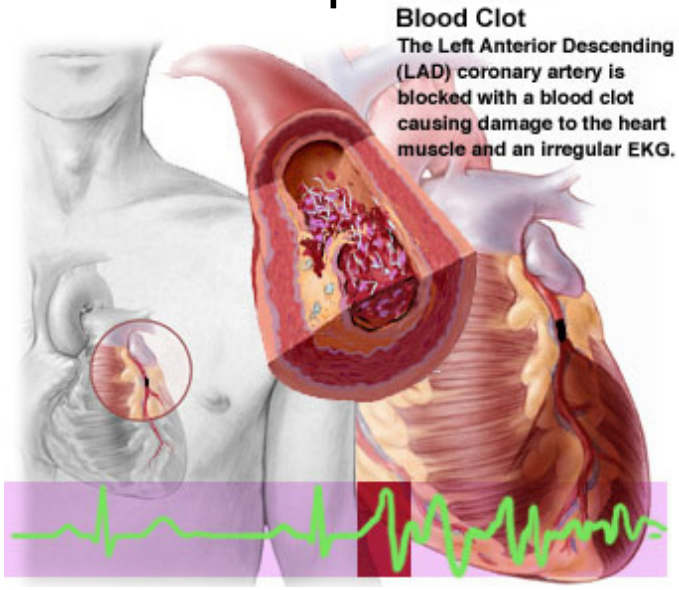
De novo discovery



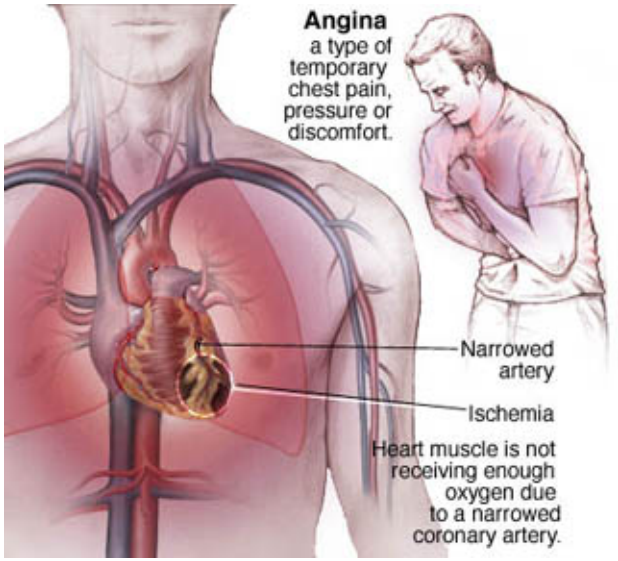
Representative proteins observed	Estimated normal Ref range* [ng/ml]
Beta-2-glycoprotein 1	100,000-200,000
Angiotensin(ogen)	1500
Beta-2-microglobulin	1000-2000
Fatty acid-binding protein	2
Atrial natriuretic factor	0.2-0.1
TGF β1	5
Interleukin 16	0.010-8
Interleukin 3	<50
VEGF	0.03
Myoglobin	42
Troponin I, fast skeletal muscle	1
Glial fibrillary acidic protein	0.45
Cystatin-C	500-1000
Insulin	2000
Insulin-like growth factor II	0.05-20
Myeloperoxidase	50-300

*Can depends on age, gender, race

Finding new markers:
Multiple cohorts used to defined diagnostic space



'SUPPLY ISCHEMIA'



'DEMAND ISCHEMIA'

Discovery Cohort II

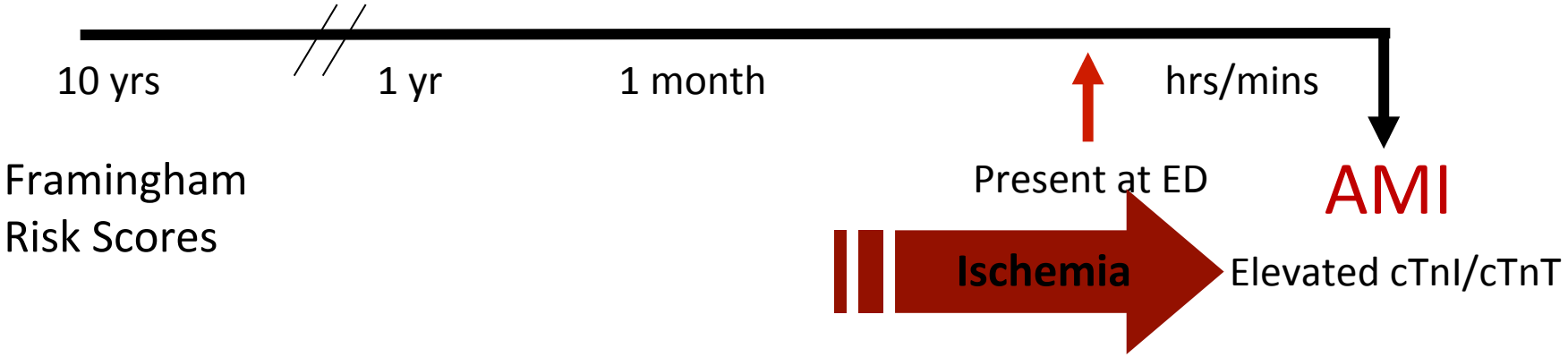
Valve replacement surgery
Dr. Targett, Oxford, UK

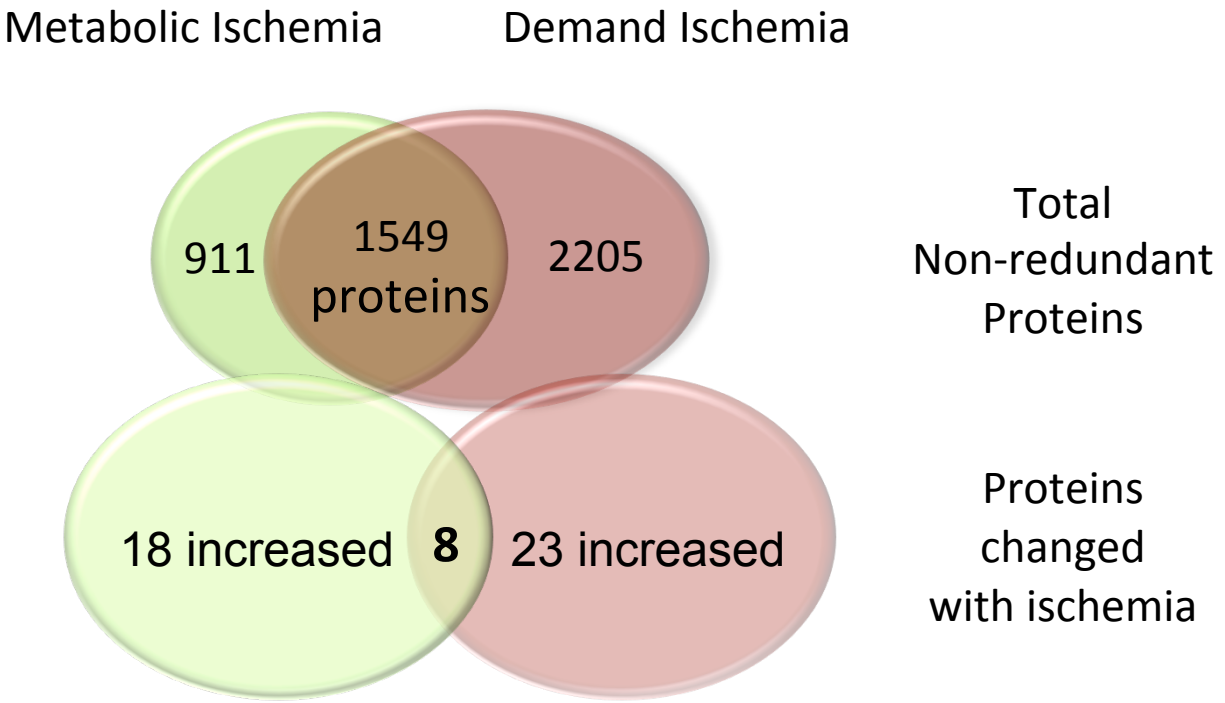
- non-acute patients requiring valve replacement
- controlled time of global ischemia (~90m.)
- 2 coronary sinus (+ 4 periphery) samples
- 20 individuals

Discovery Cohort I

Atrial pacing
Dr. James de Lemos, UT Southwestern

- non-acute stable angina patients referred to cath lab
- demand ischemia due to stepwise pacing
- controlled time of max pacing/until symptoms
- 4 coronary sinus blood samples
- 19 (16) individuals


10 yrs
1 yr
1 month
hrs/mins
Framingham
Risk Scores
Present at ED
AMI
Ischemia
Elevated cTnI/cTnT


Metabolic Ischemia
Demand Ischemia
911
1549
proteins
2205
18 increased
8
23 increased
Total
Non-redundant
Proteins
Proteins
changed
with ischemia

Q. Fu, S. Sheng,

How can one quantify each protein or modified amino acid residue?

Multiple Reaction Monitoring (MRM)

- Relative and absolute quantitation
- Primarily antibody-free assay
- Multiple protein quantified simultaneously
- Differentiation between protein isoforms (splice variants/gene)
- Multiple PTM quantified simultaneously
- Any body fluid and tissue/cells

	Quantitative western	ELISA	MRM
standards	protein	protein	protein or peptide
CV%	>20%	2-20%	2-20%
LLOQ	mg	ng-pg/ml	10 amol - nmol/peptide mg-pg/ml*
multiplex	Not common	<10	<40

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