Industry Perspective: Experience with MSC Characterization

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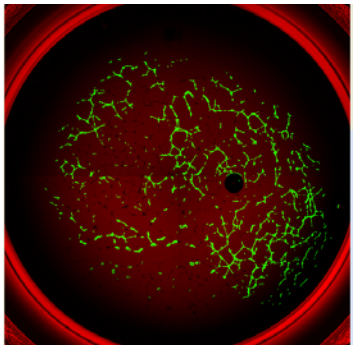
Baltimore MD 5.8.13

Where is Cell Comparability Critical in the Development Path?

- Analytical tools support consistency in product manufacturing and process change towards commercialization
- Rigorous potency assessment is key in designing clinical strata/endpoints for clinical proof of concept
- Donor comparability is key whether comparing master cell banks or large donor sets
- Large scale manufacturing moves towards extended population doublings with need to assess safety and potency
- Ensuring distinction between competing products in adherent stem cell space – enforcing and growing IP boundaries
- Building a Regulatory strategy for implementing major process changes (xeno-free media; alternate bioreactor work; driving down COGS)

Cell Equivalency for Minor Process Change

Cell equivalency assays
Growth kinetics – telomerase activity
Viability, attachment post thaw
Defined cell population in size, granularity
Acceptable expression of CXCL5, VEGF & IL8
Valid flow cytometric profile - identity and purity
Normal karyotype
qPCR marker expression
Acceptable differentiation to osteo, adipo, chondro
Activity in T cell proliferation assay
Activity in angiogenesis assay



Automated quantitation of vascular tube formation assay

Tiered Equivalency Testing

Primary Screen

Growth Kinetics and population doubling
Freezing/thaw viability and attachment
Microscopic morphology and size
ELISA assay for CXCL5, IL-8, VEGF
Flow cytometry for size and phenotype
qPCR for pos/neg markers

Secondary Screen

Replicative senesence endpoint
Cytogenetics
in vitro differentiation assays
immunomodulation assay
Vascular tube formation
Functional CNS assay
Viability and stability (>24 hr) post thaw
Factor immunoblot

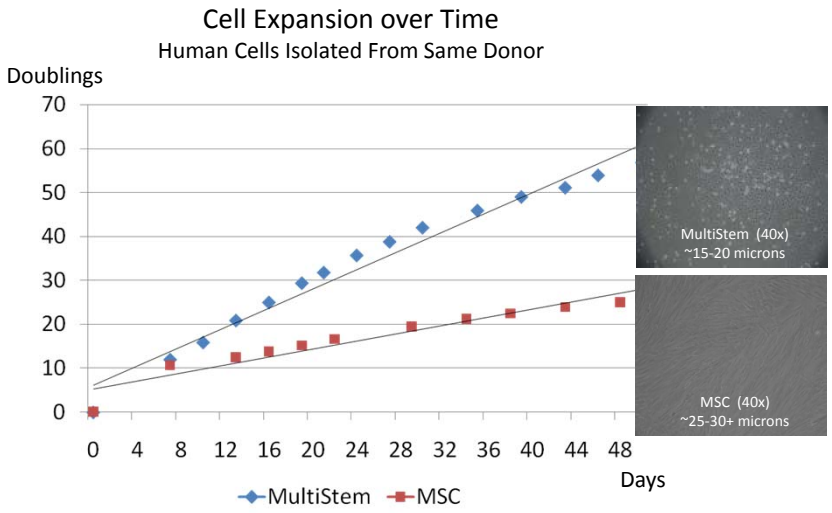
Tertiary Screen

Replicative senescence endpoint
Cell migration assay
Telomerase assay
Transcriptional profiling
miRNA array
Gene methylation array
Proteomics screen
Mesodermal, endodermal, ectodermal assays

Pre-Clinical Studies

Murine diodistribution model
Xenogeneic AMI model
Xenogeneic Stroke/TBI model
Xenogeneic GVHD model
Xiomarker models when available
Acute toxicity in murine healthy animal
Nude mouse tumorigenicity model

Expansion Profile for MultiStem vs. MSC



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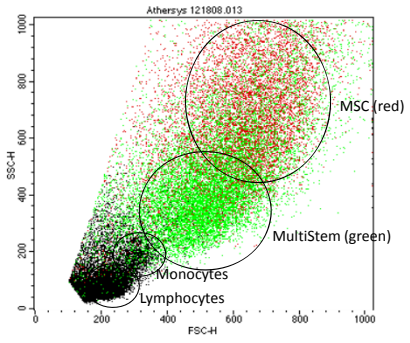
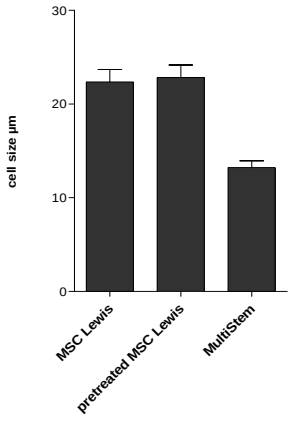
MultiStem Different in Size than MSC and other Bone Marrow MNCs

Flow Cytometric Analysis Of Human MultiStem By Forward / Side Scatter Plot



Microscopic Size Comparison of Rat MultiStem and MSC



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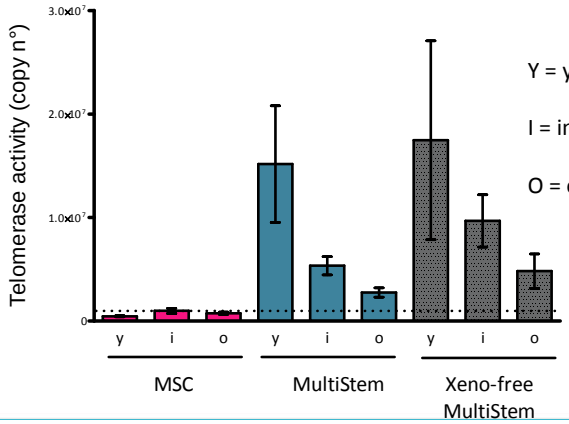
Telomerase activity

- Telomerase activity is preserved in Xeno Free MultiStem

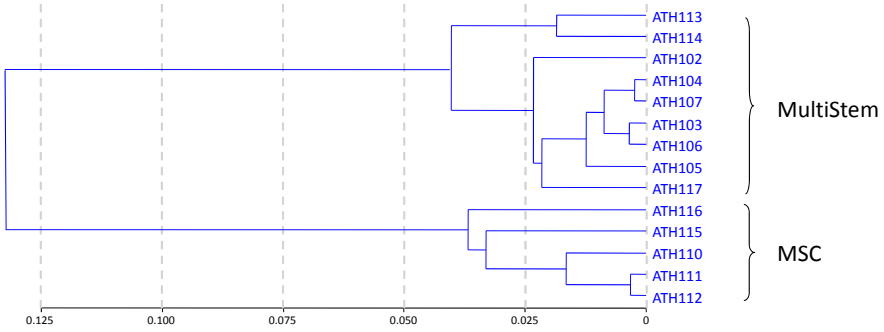


Y = young (15 PD)

I = intermediate (25 PD)

O = old (40 PD)

Distinctive Transcriptional Profiles for MultiStem and MSC



Blinded analysis of gene expression by an independent lab demonstrates that:

(1) MSC gene expression is tightly correlated with other MSC samples;

(2) MultiStem gene expression is tightly correlated with other MultiStem samples; and

(3) MSC and MultiStem gene expression patterns are distinctive.

X-axis represents the correlation value.

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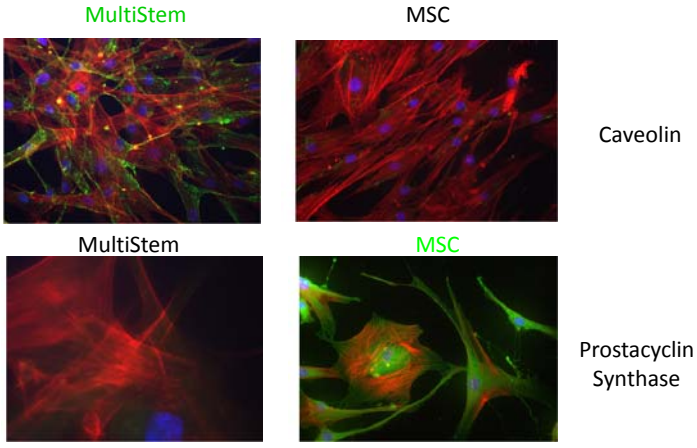
Distinctive Protein Expression – Representative Examples



Target Marker Detection using FITC (green) Fluoresence
Blue Nuclear Counterstain, Red Actin Filament Counterstain

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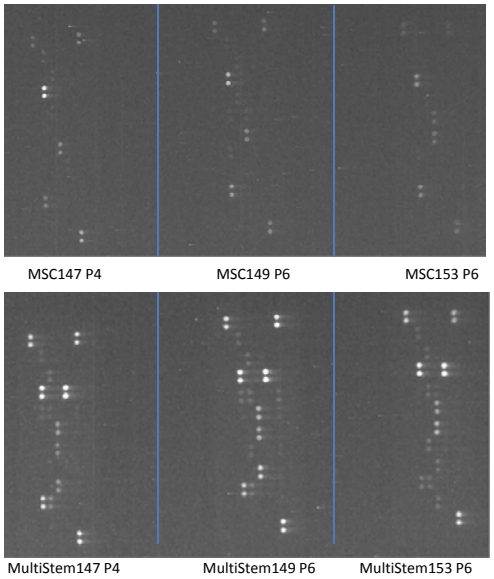
MultiStem and MSC have Different Angiogenic Factor Profiles

- Angiogenic Factor Immunoblot
  - Capture Assay using 60 angiogenic factor mAB
- Comparison of activity from MSC (top) and MultiStem (bottom)
  - MSC / MultiStem from same donor bone marrow
  - Repeated across three donors



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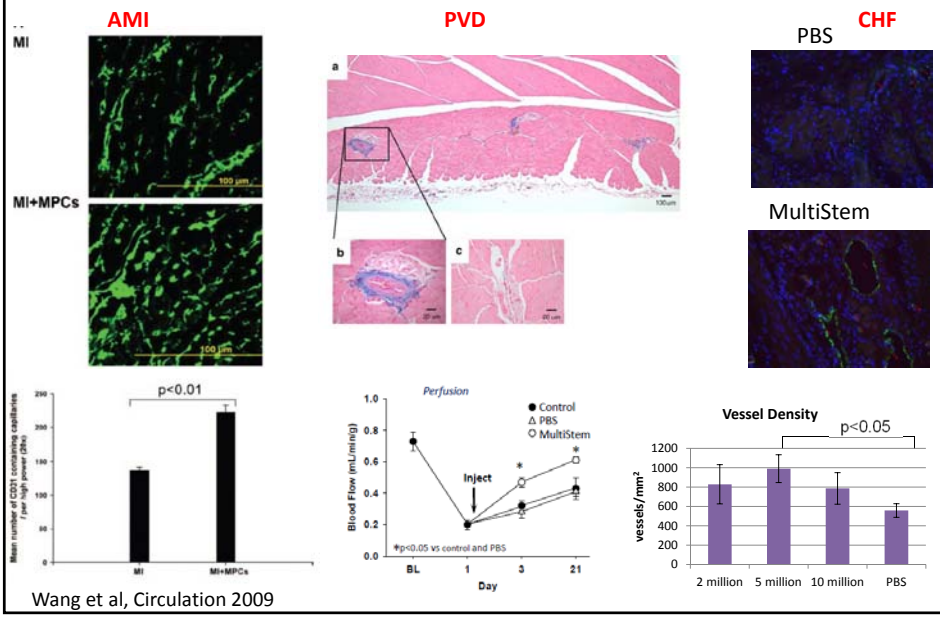
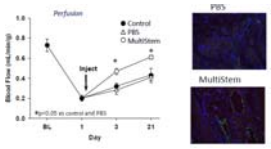
Neo-Angiogenesis Correlates with Response to Ischemia

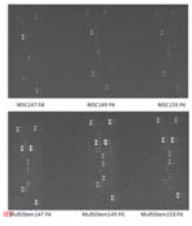


Wang et al, Circulation 2009

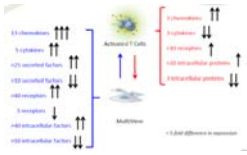
Angiogenesis Potency Assay

- Pre-clinical models show neo-angiogenesis in parallel with recovery benefit
- Screening of conditioned media shows expression of angiogenic factors, increased when exposed to inflammatory environment
- Confirmed by in vivo tissue microarrays



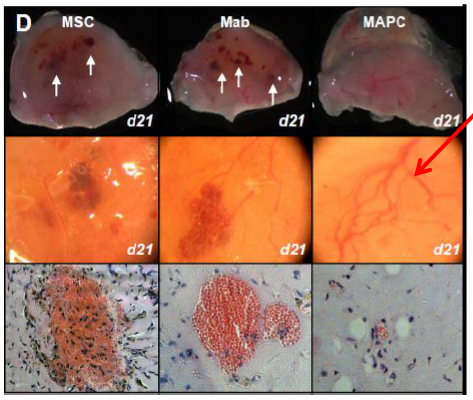


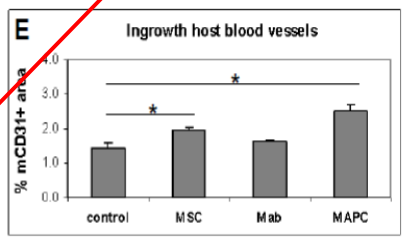
VEGF, CXCL5, IL-8



Multiple Angiogenic Factors Required for Patent Vessel Formation

Undifferentiated MSC, mesoangioblasts (Mab) and MAPC mixed with matrigel and $VEGF_{165}$/bFGF were transplanted under the skin of nude mice. (D) At 21 days, matrigel plugs were removed and examined macro- and microscopically (1nd and 2nd row) (2.5x and 6.6x respectively)





Human MSC and Mab-containing Matrigels harbored leaky vessels, indicated with white arrows, which could be also seen on H&E stained cross-sections (last row) (40x)

Roobrouck et al, *Stem Cells* 2011

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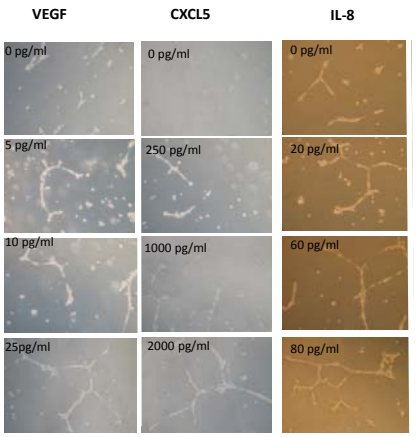
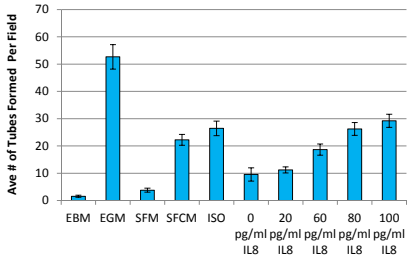
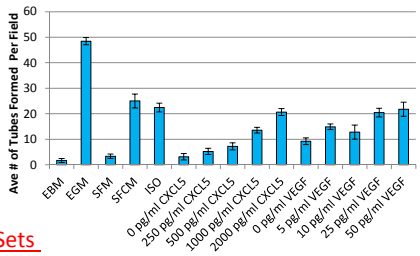
Minimum Levels of VEGF, CXCL5 and IL-8 Required for Induction of Angiogenesis

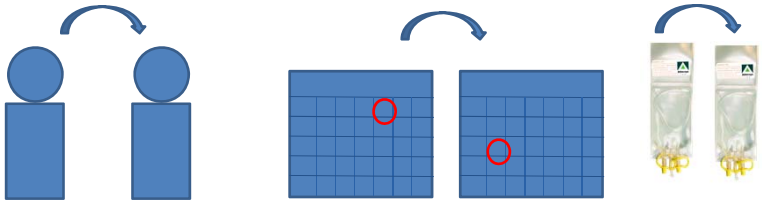






Pass-Fail Criteria: Knockdown and Add-back Sets
Critical Threshold Required for Activity

Qualification of ELISA as a Potency assay

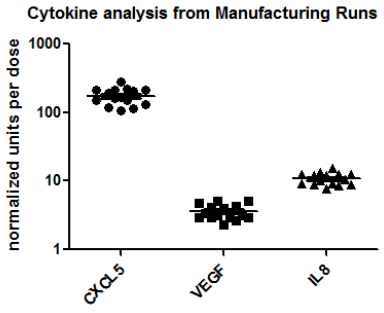


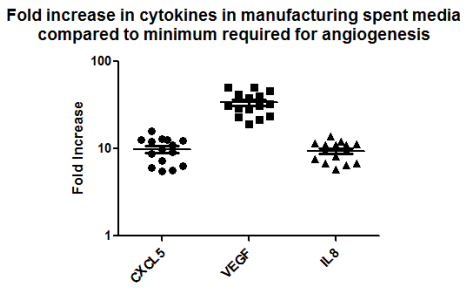
Coefficient of Variance (%)

	Pooled			Lot 1			Lot 2			Lot 3			Lot 4		
	VEGF	CXCL5	IL-8	VEGF	CXCL5	IL-8	VEGF	CXCL5	IL-8	VEGF	CXCL5	IL-8	VEGF	CXCL5	IL-8
Between operators															
Day 1	3.713	4.712	3.895	9.861	6.031	12.558	7.670	4.306	13.779	9.513	2.835	2.877	7.859	4.616	2.862
Day 2	8.074	2.113	6.761	4.279	3.759	5.604	5.933	5.788	4.048	4.391	5.582	10.090	4.862	6.665	16.938
Between Days															
Operator 1	2.482	4.867	4.680	9.730	5.474	10.682	3.809	4.498	9.963	4.185	6.959	11.525	4.868	4.095	8.468
Operator 2	9.727	5.374	5.900	3.189	10.594	7.315	7.825	6.116	6.467	3.105	7.796	17.689	2.778	7.870	14.108
Both Operators and days	6.798	4.940	5.020	7.249	8.352	8.950	6.551	5.205	10.066	7.114	7.209	18.499	6.702	5.790	11.010

Clinical Manufacturing Lots Show Consistent Cytokine Expression

Clinical History Provides Statistical Reference Point for High and Low Threshold





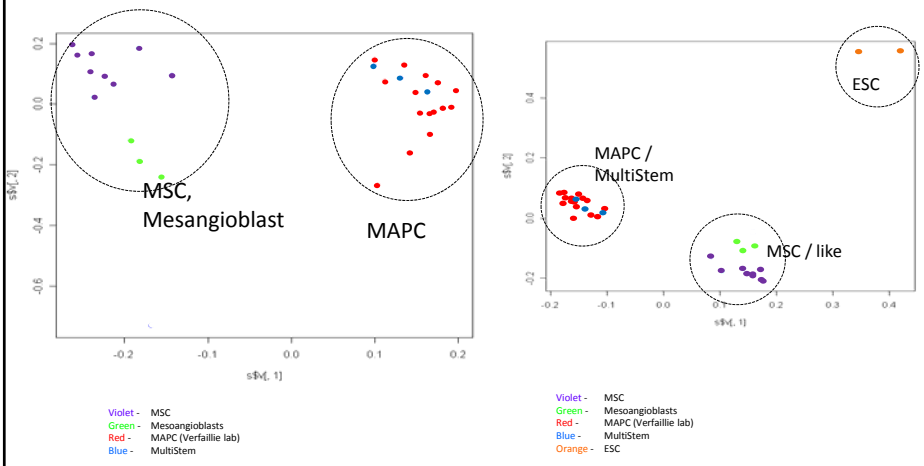
Potency Assay Provides Consistency and Comparability Guidance in Process Improvements for Phase III

Potency Assay Ensures Angiogenic Factor Production is Above Critical Pass/Fail Threshold

Epigenetics and Profiling Comparability Testing

Transcriptome
Proteome
miRNA
Gene methylation

Transcriptional Profiling Creates Identify Assay



Full Genome Transcriptional Profiling of MSC, Mesangioblast and MAPC Culture Conditions
Roobrouck, V Stem Cells 2011

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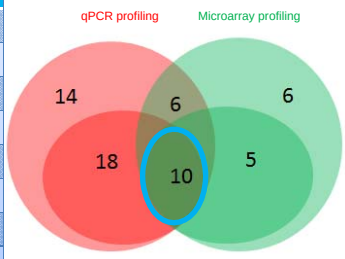
miRNAs as markers

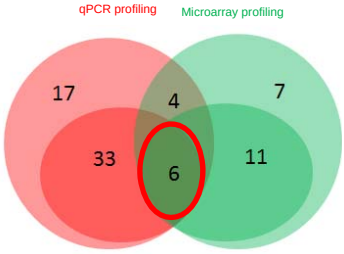
- Key miRNA markers (MS vs MSC)

MS markers
miR-1
miR-2
miR-3
miR-4
miR-5
miR-6
miR-7
miR-8
miR-9
miR-10

MultiStem upregulated miRNAs (MS markers)



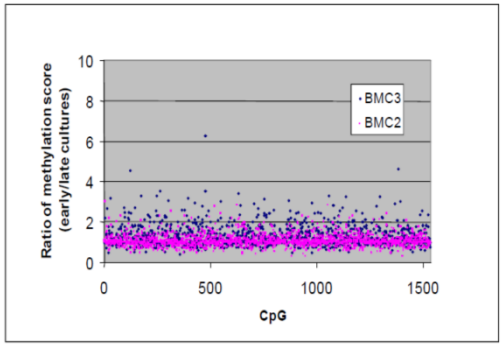
MultiStem downregulated miRNAs (MSC markers)



MSC markers
miR-a
miR-b
miR-c
miR-d
miR-e
miR-f

high stringency : FC > 1,5 in all three donors
low stringency : FC> 1,5 in at least two donors

Stability of Gene Methylation Profiles at Early and Late Expansion Stages



- Methylation profile of early (~20 PD) and late (~40 PD) MultiStem cultures depicted as the ratio of early/late methylation levels for 1536 CpG islands
- x-axis represents the Gene ID corresponding to the alphabetical listing of the genes associates with the CpG islands as shown in supplemental data
- Average ratio for two independent MultiStem lines, BMC2 and BMC 3 was 1.08±0.31 and 1.25±0.48, respectively

Boozer, S, Lehman N, Lakshmipathy et al. Global Characterization and Genomic Stability of Human MultiStem, A Multipotent Adult Progenitor Cell J Stem Cells 2009: 4(1).

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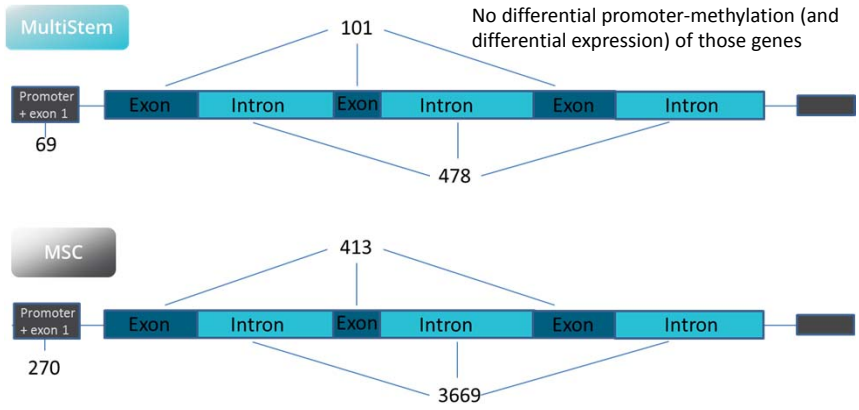
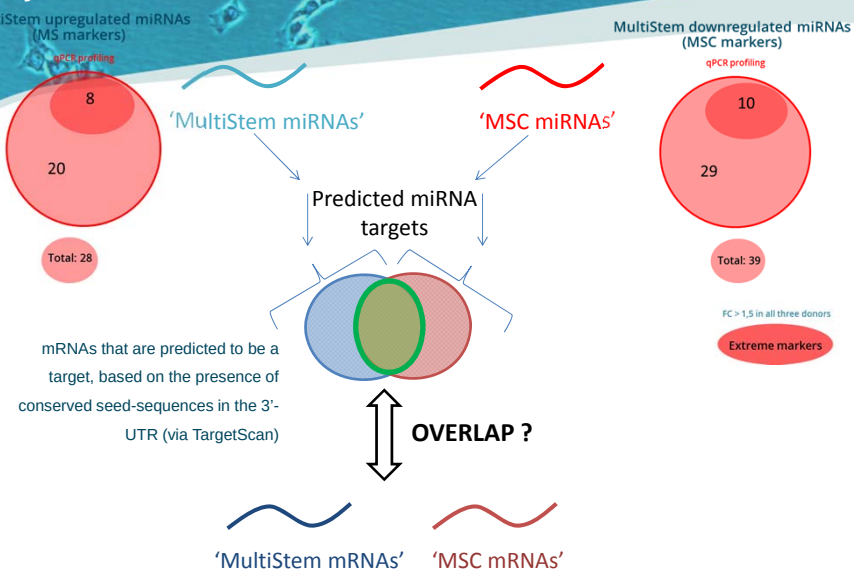
Distribution of methylation cores

Differences between MultiStem and MSC are not due to aberrant methylation of annotated tumor related genes

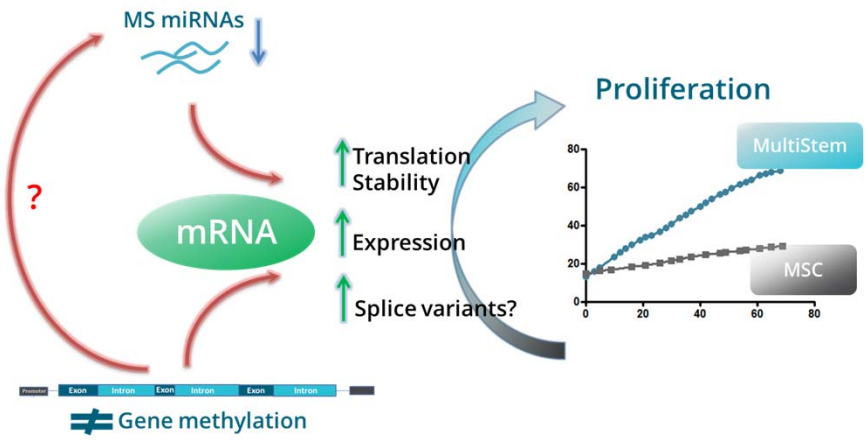
No differential promoter-methylation (and differential expression) of those genes



Analysis overview



Multi-layered epigenetic regulation of MultiStem expansion capacity



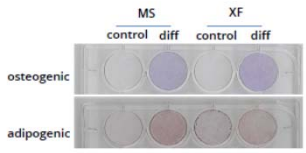
Athersys inc.

Major Process Changes

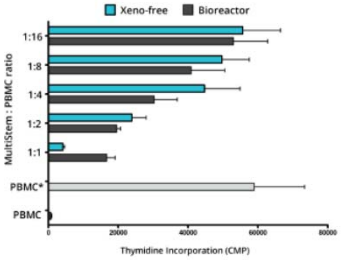
Xeno-Free Expansion and Cryoformulation

Maintenance of biological properties

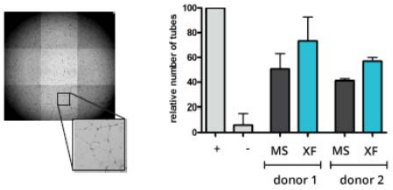
Multipotent differentiation



Inhibition of T-cell proliferation



In vitro angiogenesis



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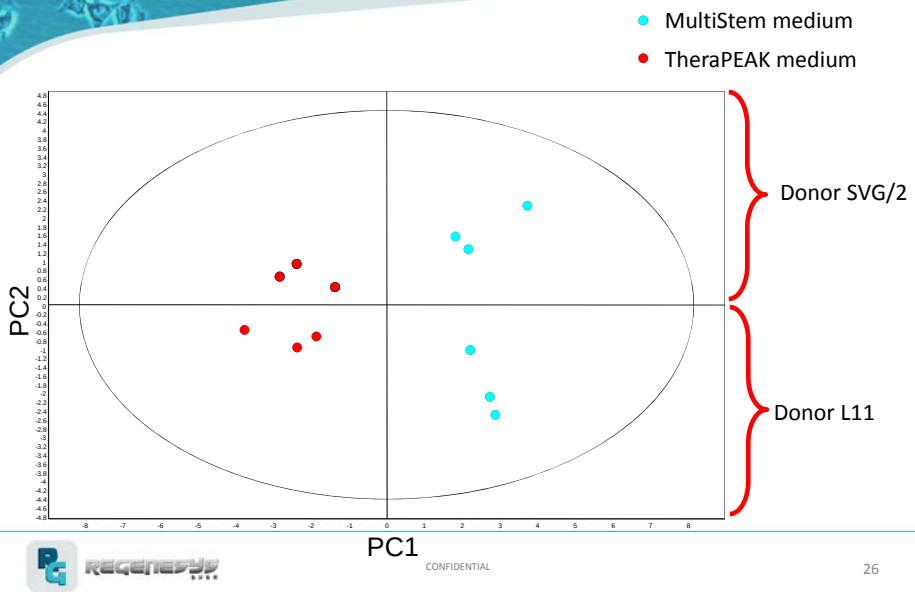
Principle Component Analysis of Serum vs Xeno Free Media



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2D-DIGE workflow

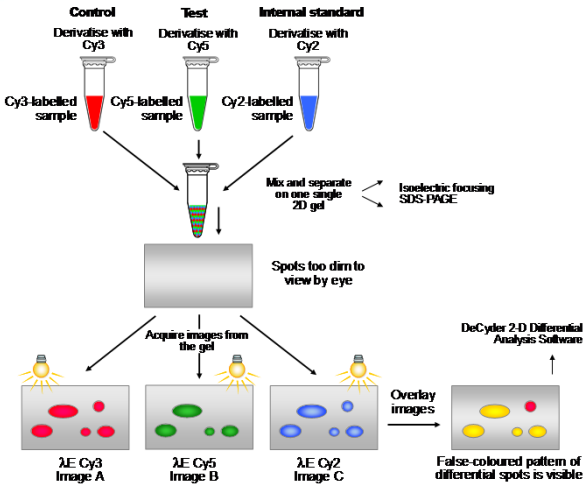
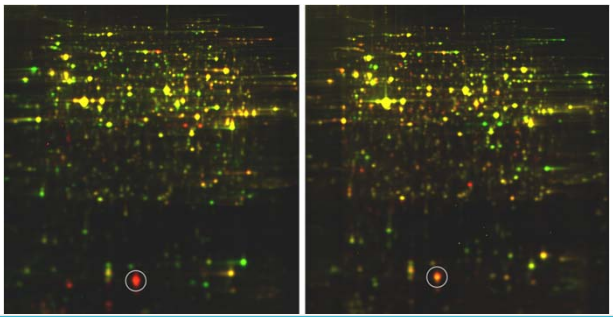


Illustration of 2-DIGE approach

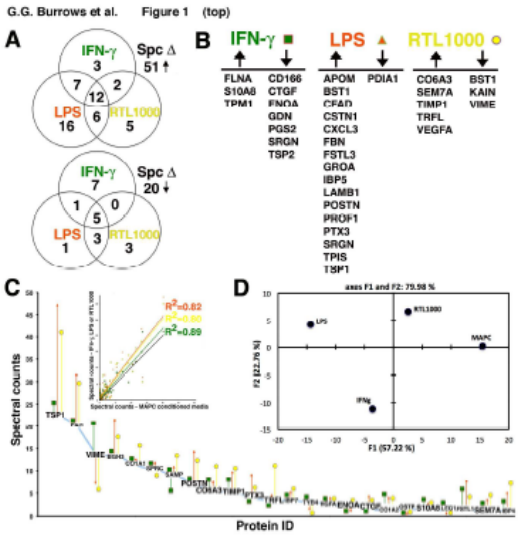
Gel 1: Control 1 Cy3 + Test 1 Cy5 + Internal standard Cy2
Gel 2: Control 1 Cy5 + Test 1 Cy3 + Internal standard Cy2

Gel 3: Control 2 Cy3 + Test 2 Cy5 + Internal standard Cy2
Gel 4: Control 2 Cy5 + Test 2 Cy3 + Internal standard Cy2

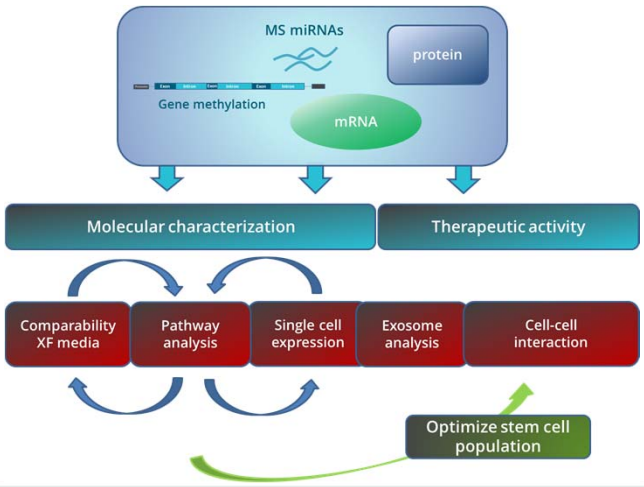
Gel 5: Control 3 Cy3 + Test 3 Cy5 + Internal standard Cy2
Gel 6: Control 3 Cy5 + Test 3 Cy3 + Internal standard Cy2



IFN-γ, LPS and RTL1000 Induce Up and Down Regulation of Secreted Proteins at 48 Hours.



Application of 'omics' data in product development



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