Use of Molecular Assays to Assess Cellular Therapies

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Cellular Therapies Section

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Methods for the Characterization of Cellular Therapies

Tests Required by the FDA

- Safety
- Identity
- Purity
- Potency

Testing useful in manufacturing

- Stability
- Consistency
- Comparability

A Centralized Cell Processing Lab

Assays

Cell Counts

Automated
- Cell dyne 3700
- Saphire
- Multi-sizer

Manual

Viability

- Trypan blue (manual)
- 7AAD (flow cytometry)

Flow Cytometry

Surface markers
Intracellular markers

Sterility

Microbial cultures
Endotoxin
Gram stains
Mycoplasma

Gene and MicroRNA Expression Profiling

Advantages

- Simultaneous expression of thousands of gene and hundred's of micro RNA at one time
- Allows for the global analysis of cells
- Few cells are required $1x10^4$ to $1x10^6$ cells
- Can be used with cryopreserved cells

Disadvantages

- Transcription doesn't always reflect protein expression
- Platforms are not fully standardized
- Lengthy assay

Need for Comparability Analysis

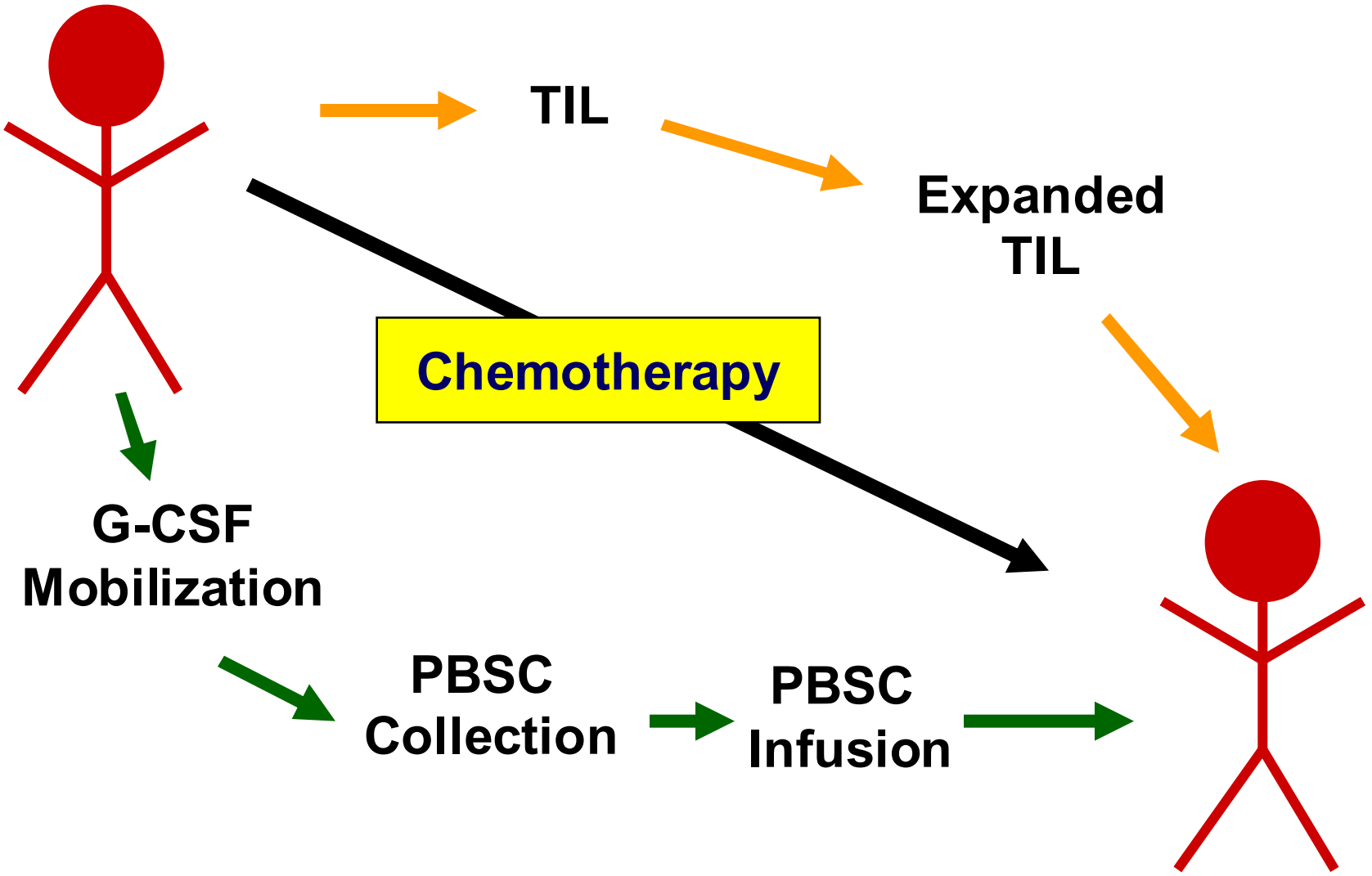
Changes in reagents and supplies

- **Media**
- **Cytokines**
- **Growth factors**
- **Bags**

Changes in starting material

- **Collection procedures**
- **Mobilization agents**
- **Storage duration or conditions**

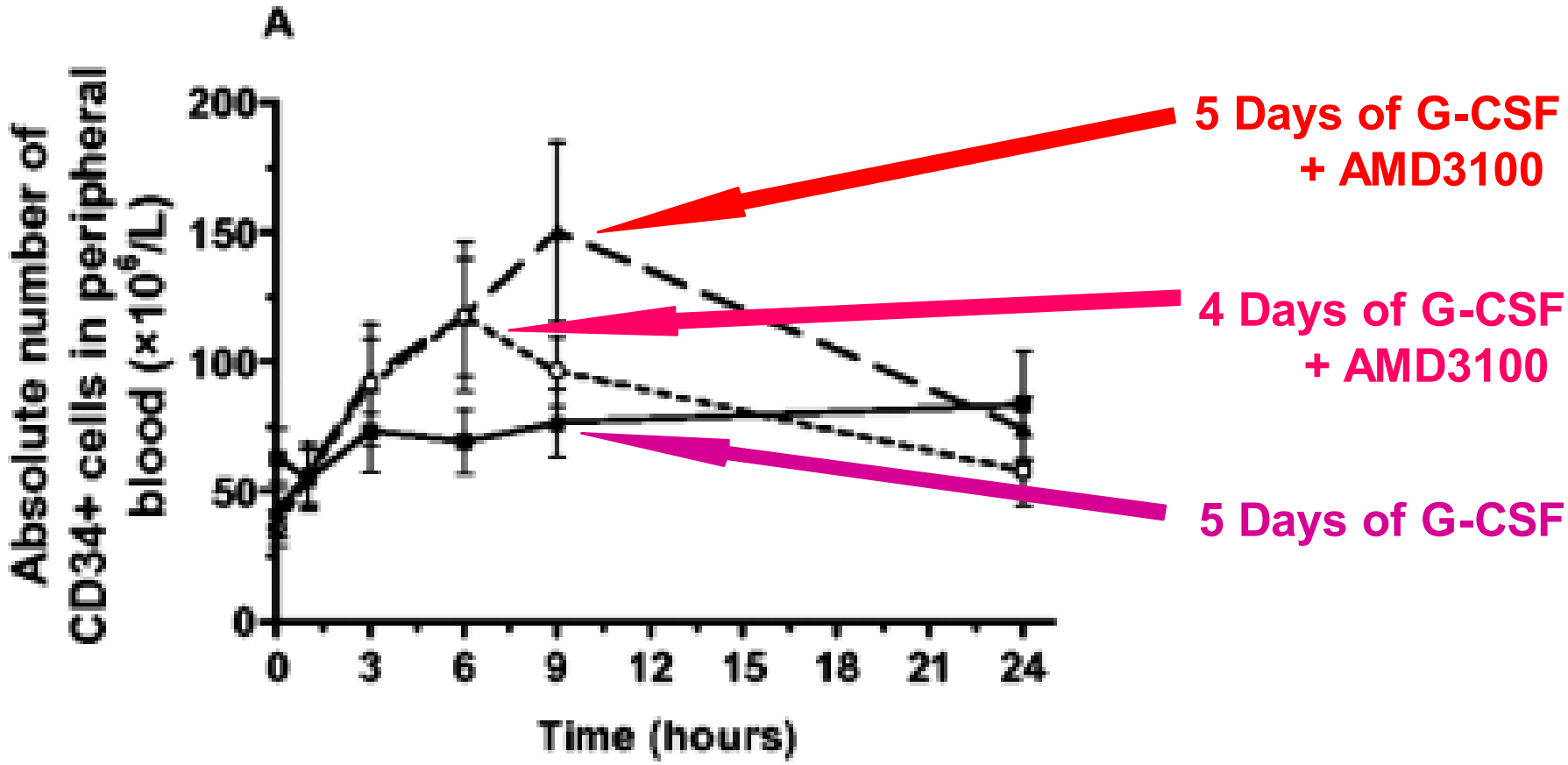
Mobilized Autologous PBSCs in Immune Therapy of Cancer



Plerixafor (AMD3100)

- Disrupts the binding of CXCR4 and CXCL12
- Mobilizes leukocytes and stem cells in 4 to 6 hours
- FDA approved for use with G-CSF for stem cell mobilization for autologous transplantation

G-CSF Plus AMD3100 Mobilization



WC Liles et al. Transfusion 2005; 45:295-300

G-CSF

G-CSF+SCF

AMD3100

AMD3100+G-CSF

Mobilized PBSCs

Mobilized PBSCs

Mobilized PBSCs

Mobilized PBSCs

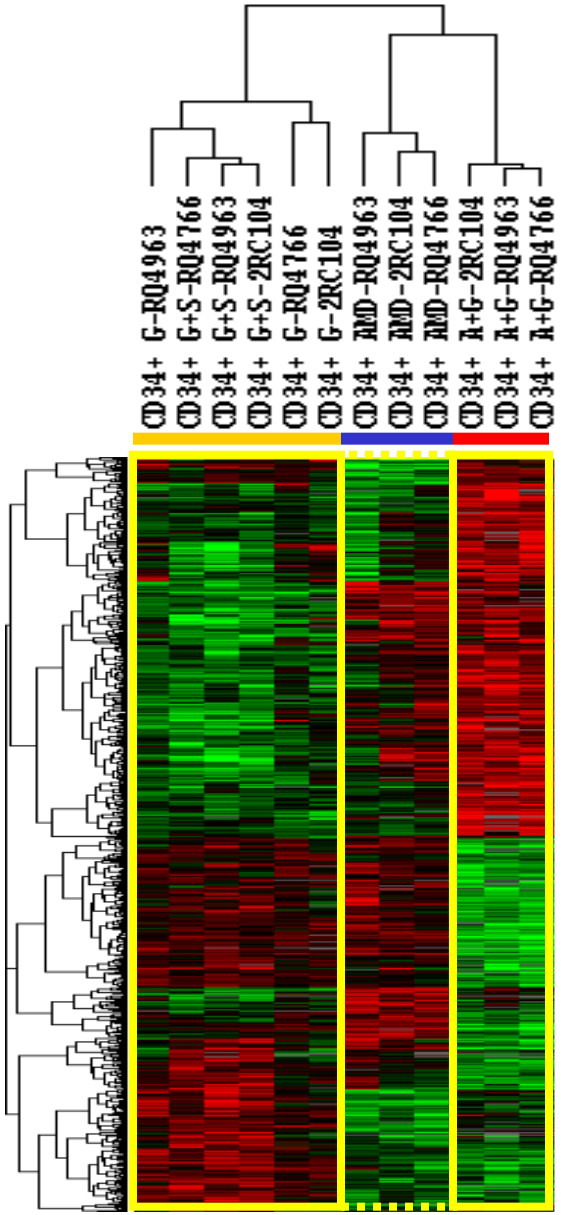
G-CSF-Mobilized CD34+ Cells

G-CSF+SCF-Mobilized CD34+ Cells

AMD3100-Mobilized CD34+ Cells

AMD3100+G-CSF-Mobilized CD34+ Cells

Gene Expression Profiling of Differentially Expressed CD34+ Cell Genes (F-test, p≤0.005, 1,097 genes)



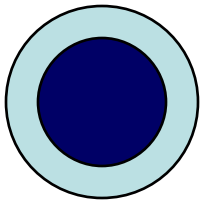
AMD3100 =

AMD3100 + G-CSF =

G-CSF and G-CSF + SCF =

Donahue RE et al. Blood. 2009;114:2530-41

Comparison of Different Types of CD34+ Cells: Summary of Genes Expressed by each type of CD34+ cells



CD34+ cells

G-CSF-mobilized

- Neutrophil
- Mono/Macro/DC
- miR-155 (DC)
- miR-126 (HPC)
- miR-10a (HPC)

AMD3100-mobilized

- B cell
- T cell
- Mast cell
- Progenitor cell

T cells and pre-B ALL

T cells

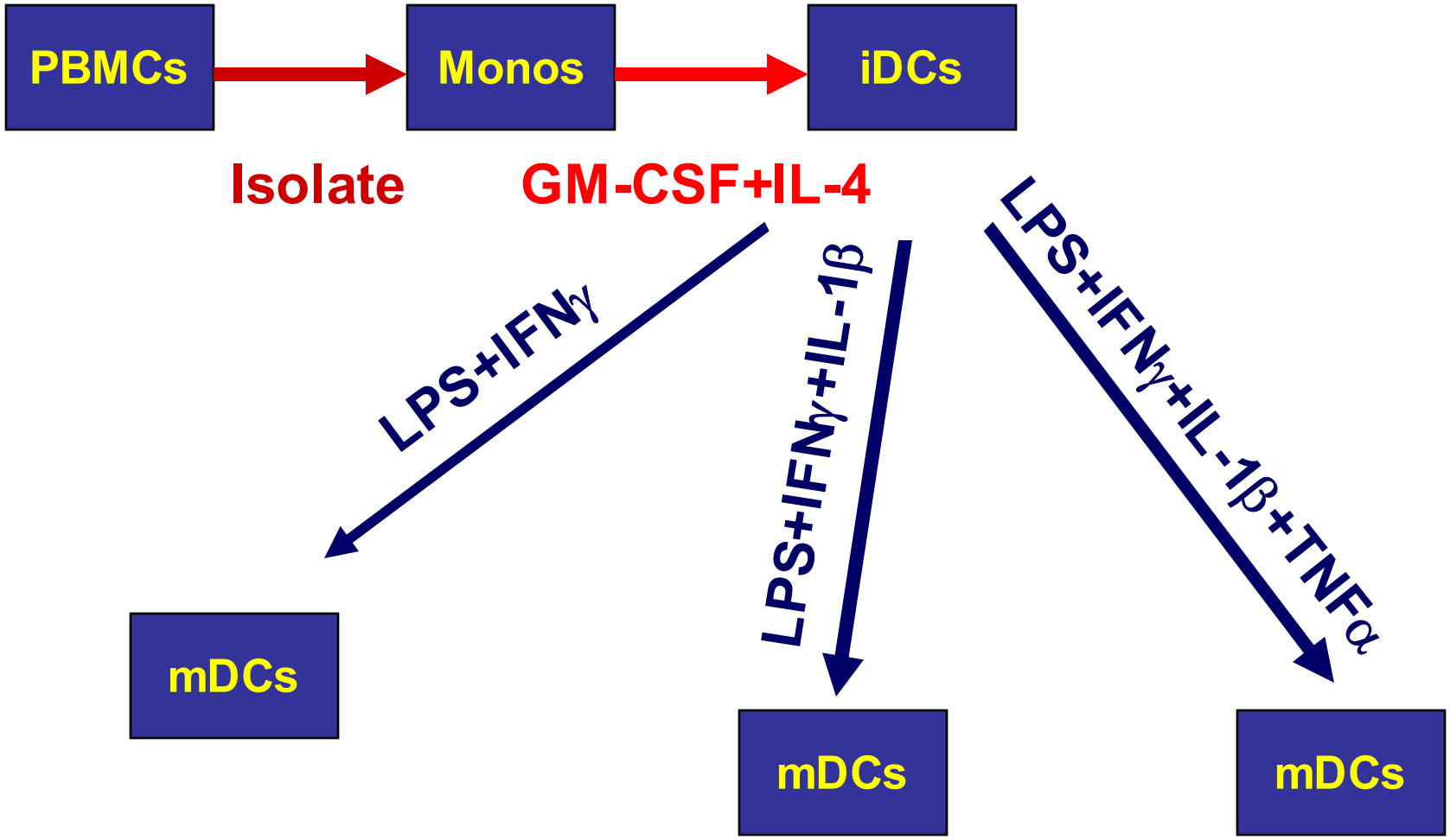
AMD3100+G-CSF-mobilized

- B cell
- T cell
- Progenitor cell
- miR-142-3p
- miR-142-5p

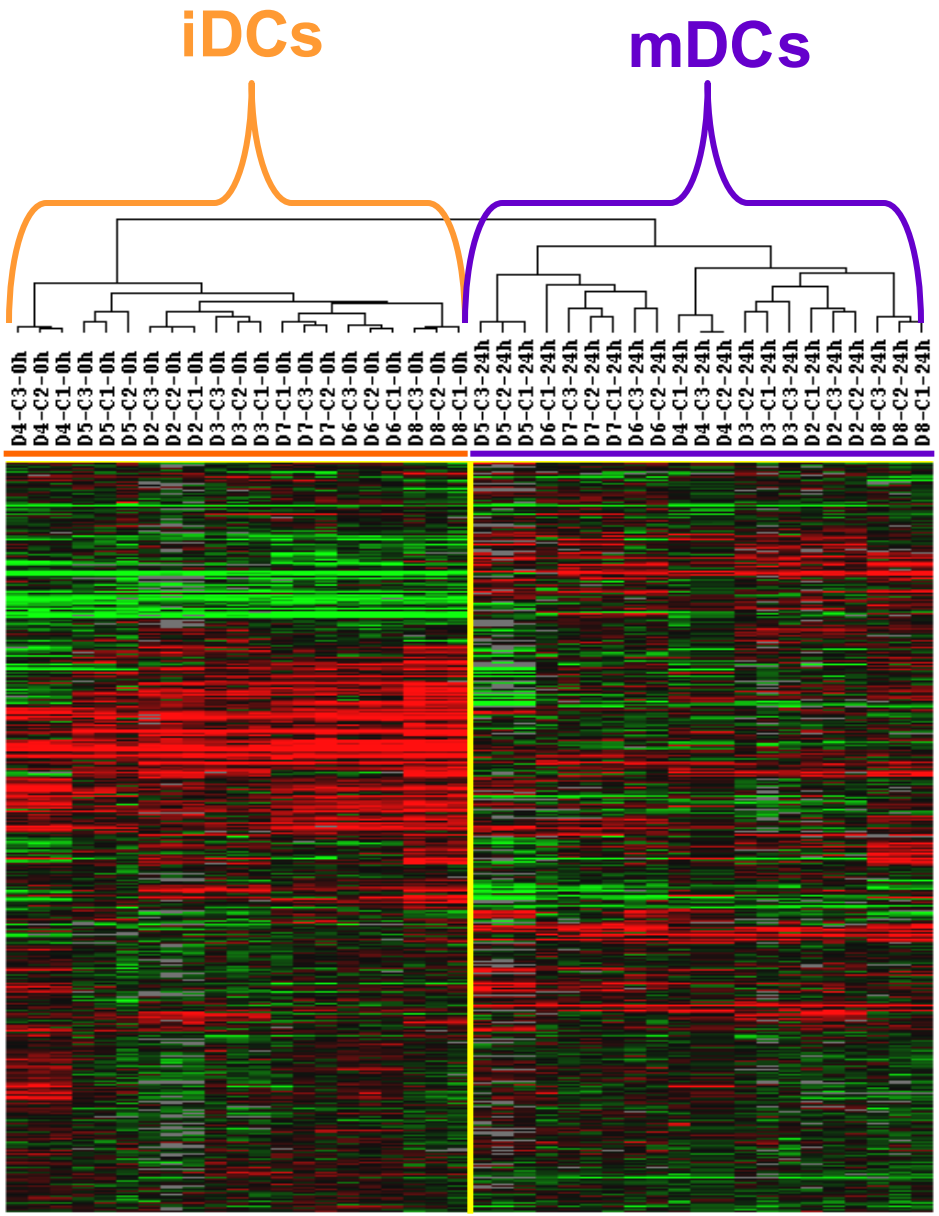
Donahue RE et al. Blood. 2009;114:2530-41

Dendritic Cells

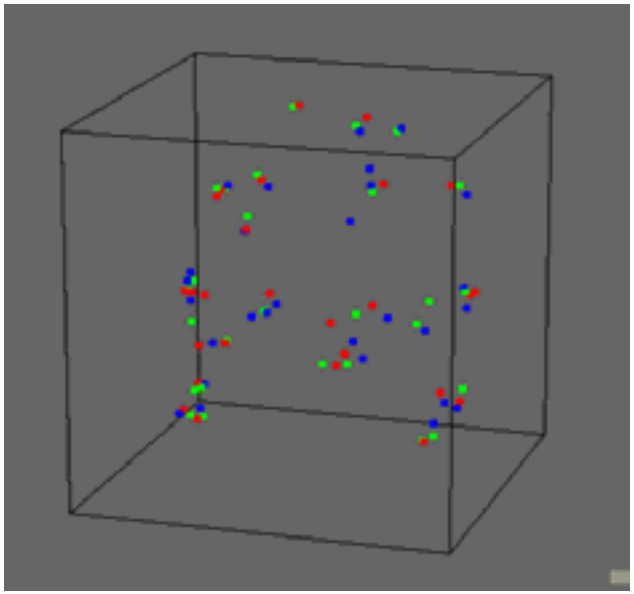
Comparability Testing and DCs: Comparison of DC Maturation Cocktails



Assessed iDCs and mDCs by gene expression profiling



Multidimensional scaling, using centered correlation



The ANOVA model: log expression ~ Donor + Treatment + Time

Table 1: Number of significant genes for testing fixed effects.

Effect	P-value < 0.001	FDR < 0.1
Donor	9590	16103
Treatment	13	0
Time course	9576	15822

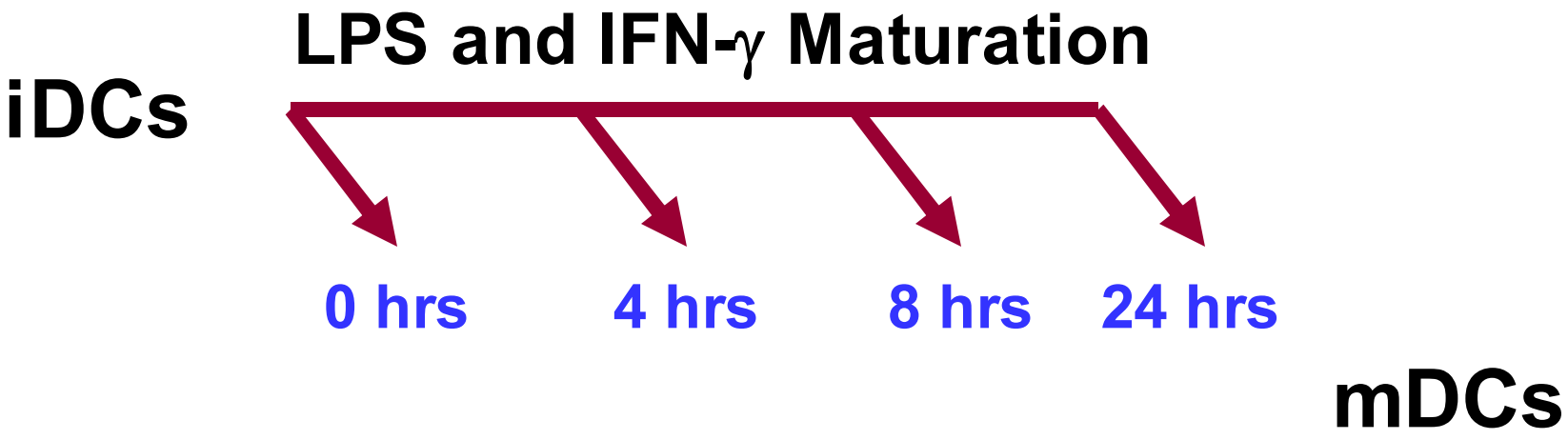
Table 2: Number of significant genes for testing the model.

	P-value < 0.001	FDR < 0.1
Model	12505	18559

C1 = LPS+IFN-γ ●; C2 = LPS+IFN-γ+IL-1β ●; C3 = LPS+IFN-γ+IL-1β +TNF-α ●

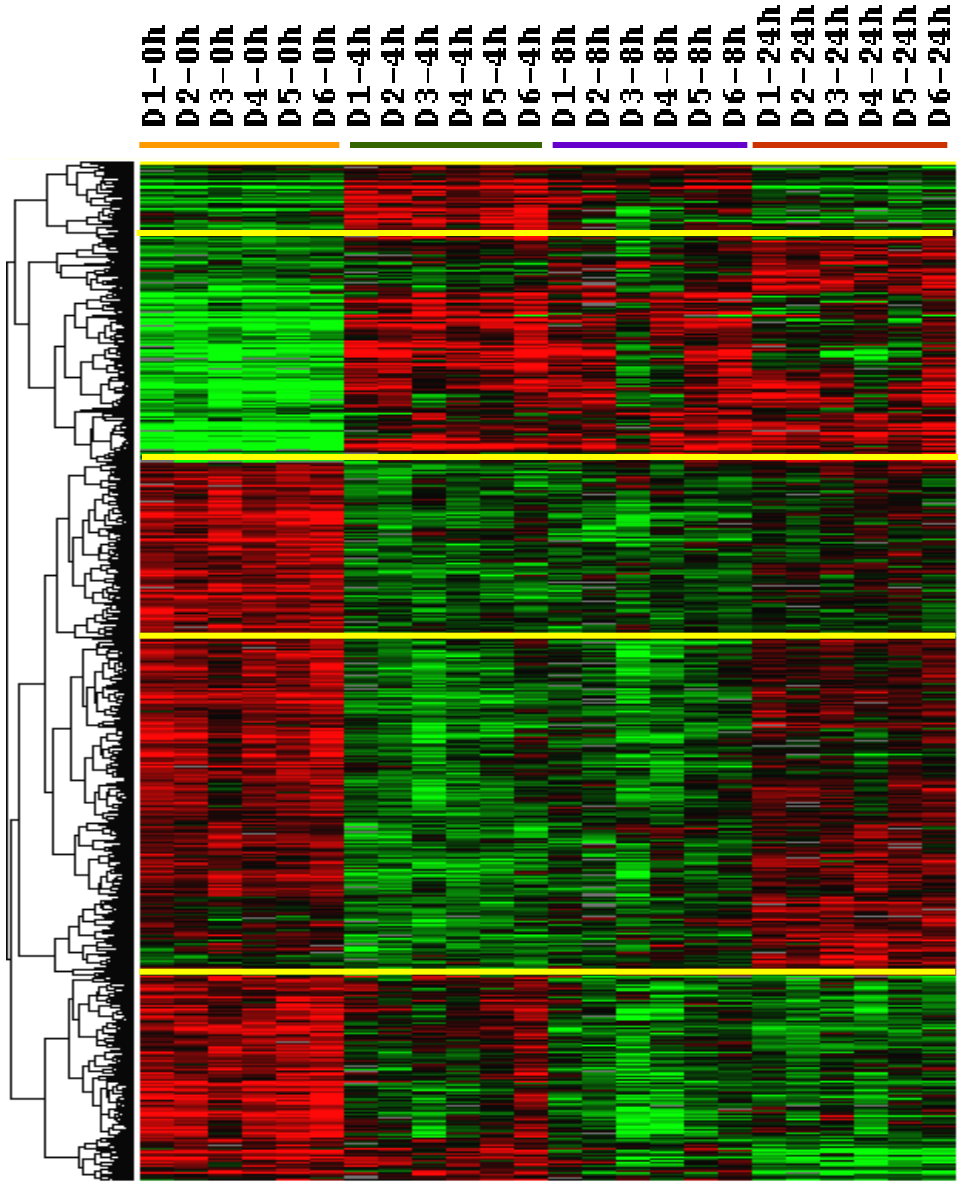
Han TH, Jin P, Ren J, Slezak S, Marincola FM, Stroncek DF. J Immunother. 2009;32:399-407

mDC Biomarker Discovery: Kinetics of the molecular changes associated with DC maturation



Assessed Global Gene and MicroRNA Expression

Kinetics of LPS and IFN-γ Mediated DC Maturation: Transcriptome analysis of DCs from 6 healthy subjects



Cluster 1

CD44, CD48, CXCL1, CXCL2, IL18R1, IL18RAP, IL1A IL1RAP, IRF8, JAK2, MAP3K8, MAP4K4, MAPK4 MMP19, NFKB2, NFKBIZ, TNFRSF18, IKBKA TNFRSF4, TNFSF9

Cluster 2

CCL2, CCL3, CCL4, CCL5, CCL8, CCL17, CXCL1 CXCL2, CXCL9, CXCL10, CXCL11, CCR7, CXCR4, IL1 IL6, IL8, IL10, IL12IL15, IL15R, IL17, IL7R, TNF, IRF4 IRF7, IRF9, NFKB1, NFKBIA, NFKBIB , RELB, SOCS2 STAT1, STAT2, STAT3

Cluster 3

SRM, P4HA1, ALDH3A2, GLUD1, GOT2, CAD ALDH9A1, ALDH9A1, GCSH, AMT, ATIC, PRIM1 DUT DPYD, NUDT9, POLR3E, CTPS, RRM1, RPUSD2 PPP1CC, PLCB2, MAPK1, PPP1R7

Cluster 4

ADSSL1, CRAT, DARS, AARS2, GOT1, AARS POLR1D, RRM2B, DKC1, POLI, DCTD, POLB EIF2AK4, NT5C, NME3, NUDT5, POLR2A, PUSL1 POLR2H, TK1, HARS, EPRS, ERCC1, , ERCC3, XPC ERCC5, SQLE, FDFT1, MVK, HMGCR, TYK2, PIAS1 IFNGR2, IFNGR1, MAPK9, TRAF5, MAP3K4 MAP3K3, MAP2K5, GLB1, MANBA, MAN2A2, CTBP1 PELP1 MAPK1, PCK2,, CTBP2, PHB2, PHKB, GYS1 PHKG2 ITPR3, CALM2, GNB5, ADH5, DGKZ ALDH3A2 CERK, AOAH, ADH5, DGKZ, ALDH3A2 CERK, AOAH GALK1, ACSS1, PGM1, ALDOC

Cluster 5

ATP6V1D, NDUFA10, ATP5A1, NDUFB5, ATP5L COX7A2L, ATP6V1A, NDUFS3, UQCRH, ATP5B ATP5G1, ATP5J2, ATP5F1, ATP6V1B2, ATP5I HSD17B10, PPT1, ACAA2, HADHA, HADH, CPT1A CAT, PSENEN, HLA-DMA, HLA-DRB4, HLA-DRA HLA-DQA2, HLA-DPB1, HLA-DRB5, HLA-DPA1

Criteria for Biomarker Selection for Gene Expression Analysis

Important in DC function

- **Potency, comparability, consistency**

Increased at least 10-fold in mDCs

- **All tests**

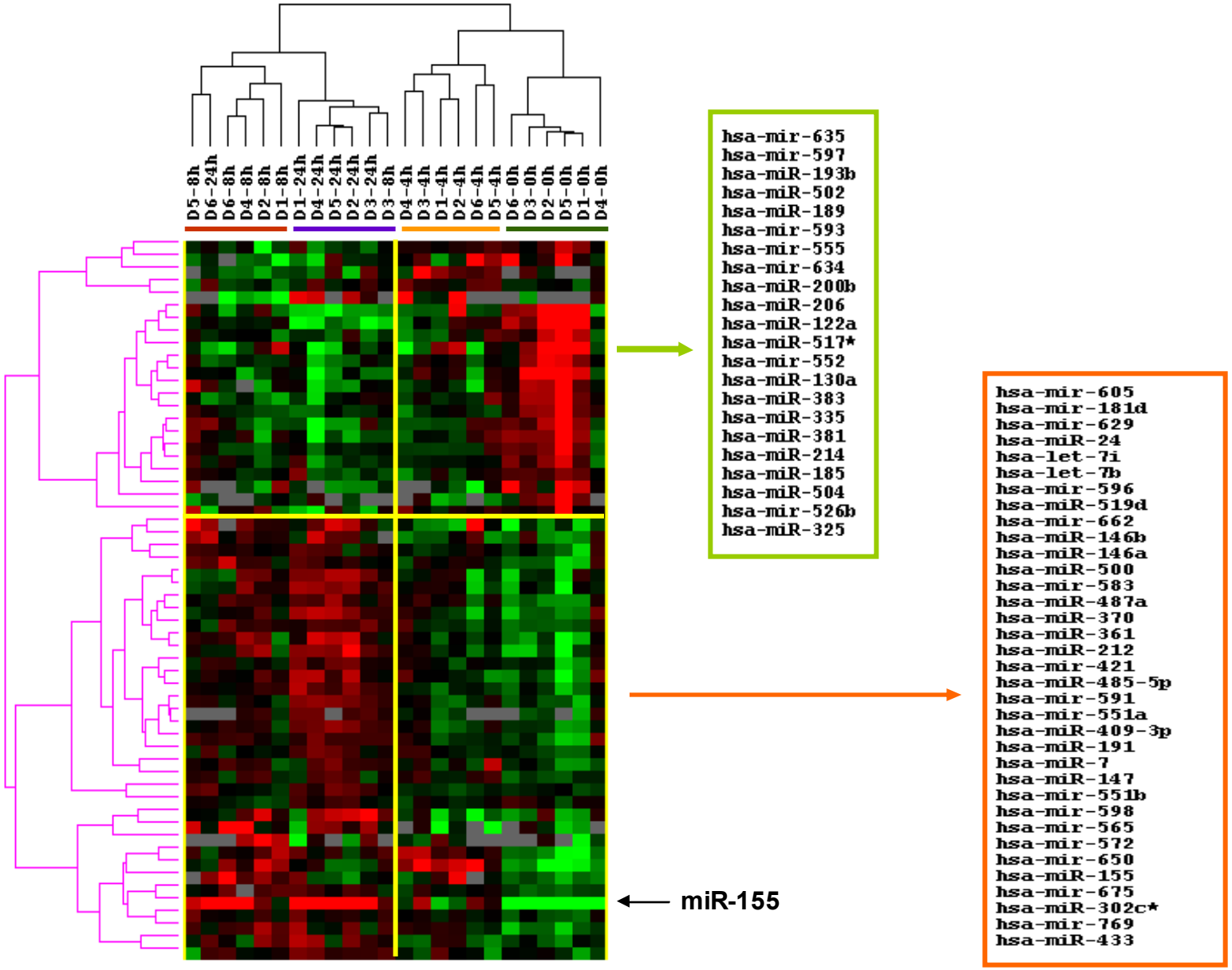
Increased variably during maturation

- **Consistency, comparability**

Potential mDC Biomarkers: Genes up-regulated during DC maturation

	Fold increase in gene expression for each maturation time*						
Gene	4 hours	8 hours	24 hours	Gene	4 hours	8 hours	24 hours
Up-regulated to a similar degree throughout maturation				Up-regulated most early in DC maturation			
CCL5	108	148	94.1	IL6	13.1	11.3	5.15
CXCL10	28.5	31.8	21.2	IL8	78.0	70.3	12.7
CCR7	10.5	11.5	18.2	IL7R	29.1	29.9	10.5
IL15	7.12	5.54	8.13	CCL4	92.3	53.4	6.91
IFI27	6.99	7.62	10.2	TNFAIP6	30.0	18.7	10.7
IFI44L	14.8	16.7	20.5	IFIT3	36.2	22.5	10.5
IFIH1	16.9	9.42	11.3	OASL	68.1	44.1	30.7
IFIT1	29.8	27.0	21.7	GBP1	66.2	35.3	30.2
MX1	18.4	15.6	14.1	HES4	229	115	37.4
ISG15	50.6	58.3	41.8	Up-regulated most late in DC maturation			
ISG20	94.1	87.9	62.9	CCL8	11.3	31.8	31.2
IRF7	9.77	9.38	12.0	EBI3	17.6	21.8	34.6
GBP4	36.3	21.2	20.2	IFITM1	13.2	22.5	48.6
DUSP5	21.7	15.8	22.6	MT1B	10.2	10.5	20.6
NFKBIA	11.7	13.3	10.4	MT1E	NS	1.78	46.1
ATF3	10.2	5.38	11.4	MT1G	NS	2.77	42.3
TNFSF10	19.4	14.8	13.8	MT1H	22.7	20.5	62.6
TNFRSF9	8.13	6.39	10.2	GADD45A	NS	11.6	50.7

MicroRNA changes during DC maturation with LPS and IFN-γ



DC Potency Testing

- **Assay validation**
- **All clinical DCs produced at the Clinical Center**

Luciano Castiello, Marianna Sabatino, and Ping Jin

Acknowledgements

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