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Mesenchymal Stromal Cells (MSC) : phenotypical and functional characterizations as tools for immunomodulation in Myasthenia Gravis.

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Background

Myasthenia Gravis (MG)

- MG is a rare neuromuscular disease.
- Pathogenicity is due to autoantibodies directed against proteins of the neuromuscular endplate.
- Common treatments (corticosteroids and azathioprine) trigger severe side-effects, mandating the set-up of novel therapies.

Mesenchymal Stromal/Stem Cells (MSC)

- MSC are non hematopoietic multipotent progenitor cells.
- They can modulate the immune system via soluble mediators and cell-cell contacts.
- Previous studies in our new MG animal model, show that the transfer of MSC conditioned by peripheral blood mononucleated cells (PBMC) improved the clinical status of the animals. (Sudres et al., JCI Insight 2017).

Materials & Methods

MSC Samples

- Adipose derived MSC were obtained from ongoing collaborations: H. Rouard (EFS); J. Larghéro, V. Vanneaux (Hop. St Louis); D. Noël (INSERM UMRS 1183); C. Martinaud (CTSA) .

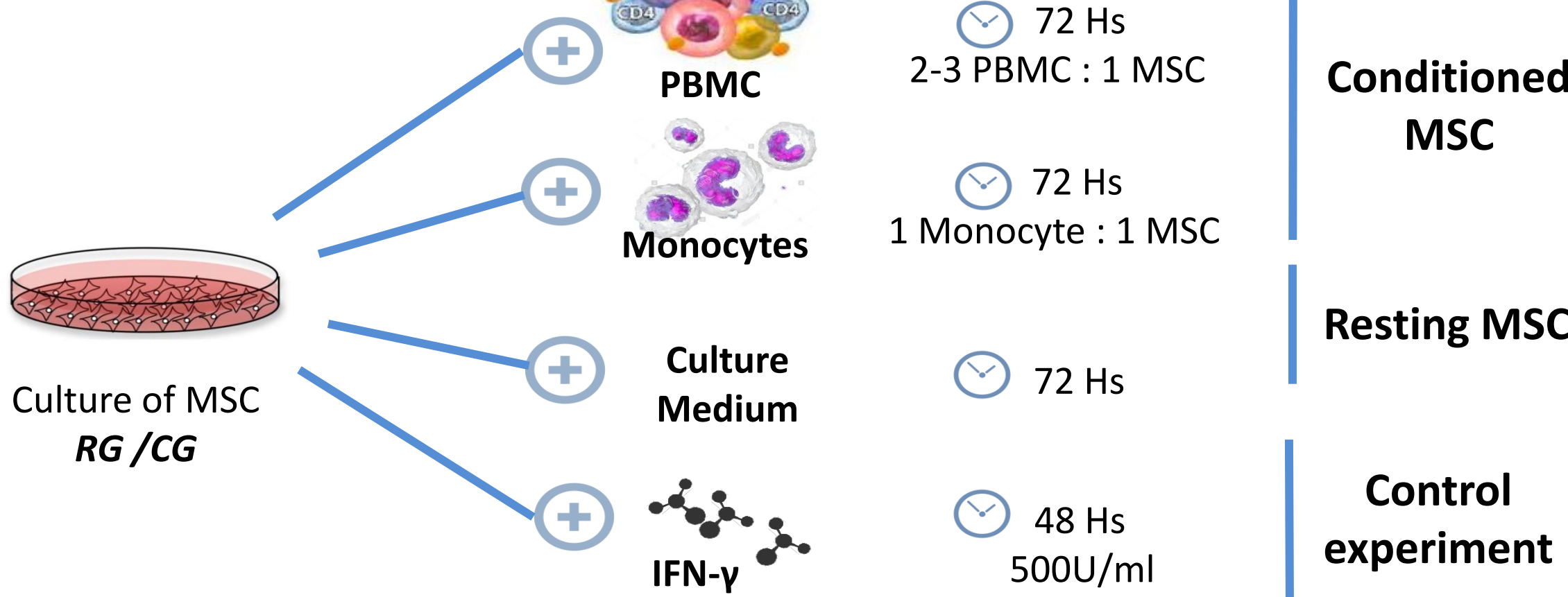
PBMC/ Monocytes samples

- PBMCs were obtained from venous blood from healthy volunteer donors (EFS, Rungis, France) using the Lymphoprep density gradient centrifugation protocol.
- Purified monocytes were sorted from PBMC by immunomagnetic negative selection using anti-human CD14 antibody complexed to magnetic particles.

MSC thawing, cell culture and expansion.

- Cells were seeded at a density of 4,000/cm² cells/
- Media culture for:
 - o **RG** : α-MEM + 10% Fetal Bovine Serum + 1ng/ml bFGF + antibiotics
 - o **CG** : α-MEM + 5% by platelet lysate + 2U/ml heparin + antibiotics
- Cells were kept in a 37 °C humidified incubator containing 5% CO2.

MSC conditioning.



Assessment of markers expression by Flow cytometry and CyTOF

- Resting and conditioned cells (flow cytometry: all conditions; CyTOF: only IFN γ) were trypsinized.
- Cells were incubated with monoclonal fluorochrome-conjugated or metal-conjugated primary Ab against Human antigens.
- 61 antibodies were tested by flow cytometry and 31 by CyTOF.

Category	Antibody	Alternative Name	Category	Antibody	Alternative Name
Miscellaneous molecules	CD10	CALLA	Immunomodulation	CD24	Heat Stable Ag
	CD13	Aminopeptidase		CD55	DAF
	CD34	Hematopoietic progenitor cell Ag		CD59	MAC-IP
	CD45	Leucocyte common Ag (LCA)		CD119	IFN γ Receptor α -chain
	CD45RA	LCA isoform		CD273	PD-12
Cell adhesion molecules (varied)	CD45RO	LCA isoform		CD274	PD-11
	CD9	MRP-1		CD276	B7-H3
	CD36	Platelet glycoprotein 4		CD7	gp40
	CD44	HCAM		CD14	LPS Receptor
	CD47	Neutrophin		CD40	TNFRSF5
	CD54	ICAM-1		CD69	Very early activating factor
	CD56	NCAM		CD80	B7-1
	CD57	HNK1		CD86	B7-2
	CD62E	E-selectin		CD184	CXCR4
	CD62L	L-selectin		CD200	OX-2
Integrins	CD62P	P-selectin	other molecules with immunological implication	HLA-ABC	-
	CD81	Tetraspanin 28		HLA-DR	-
	CD106	MadCAM		CD36	Platelet glycoprotein 4
	CD146	MCAM		CD140a	PDGFR α
	CD166	ALCAM		CD140b	PDGFR β
	CD29	Integrin- β 1	Receptors	CD172a/b	SIRP α
	CD49a	Integrin- α 1		CD221	IGF-R3
	CD49b	Integrin- α 2		CD271	NGFR
	CD49c	Integrin- α 3		CD309	VEGFR
	CD49d	Integrin- α 4	Negative MSC markers	CD14	LPS Receptor
	CD49e	Integrin- α 5		CD11b	Integrin α M
	CD49f	Integrin- α 6		CD45	Leucocyte common Ag (LCA)
	CD49g	Integrin- α 7		CD73	Ecto-5'-nucleotidase
	CD61	Integrin- β 3		CD90	Thy-1
				CD105	Endoglin

PBMC inhibition assay (CFSE test)

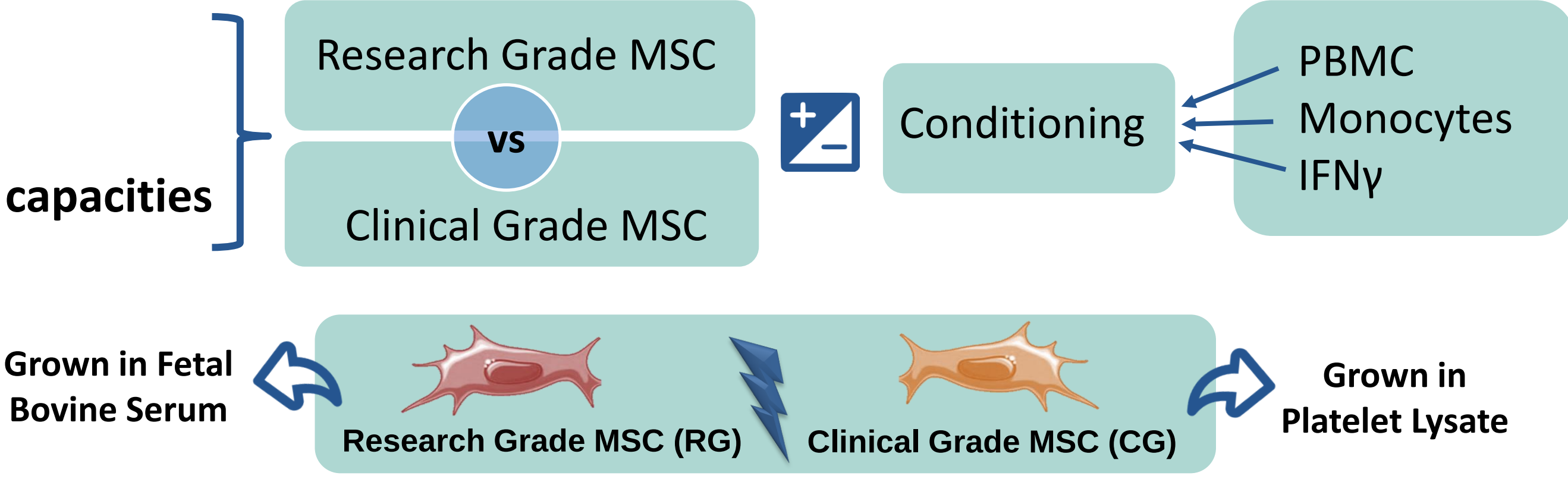
- Activated PBMC were labeled with carboxyfluorescein succinimidyl ester (CFSE), and put in contact with resting or conditioned CG MSC (1:5 ratio)
- After 3 days, the percentage of proliferating PBMC out of the total live cells was assessed by flow cytometry.

Study purpose

To develop an immunomodulating approach in clinical perspective, we compared:

Phenotype

Functional capacities



Results

1 Phenotype comparison between resting Research Grade and Clinical Grade MSC.

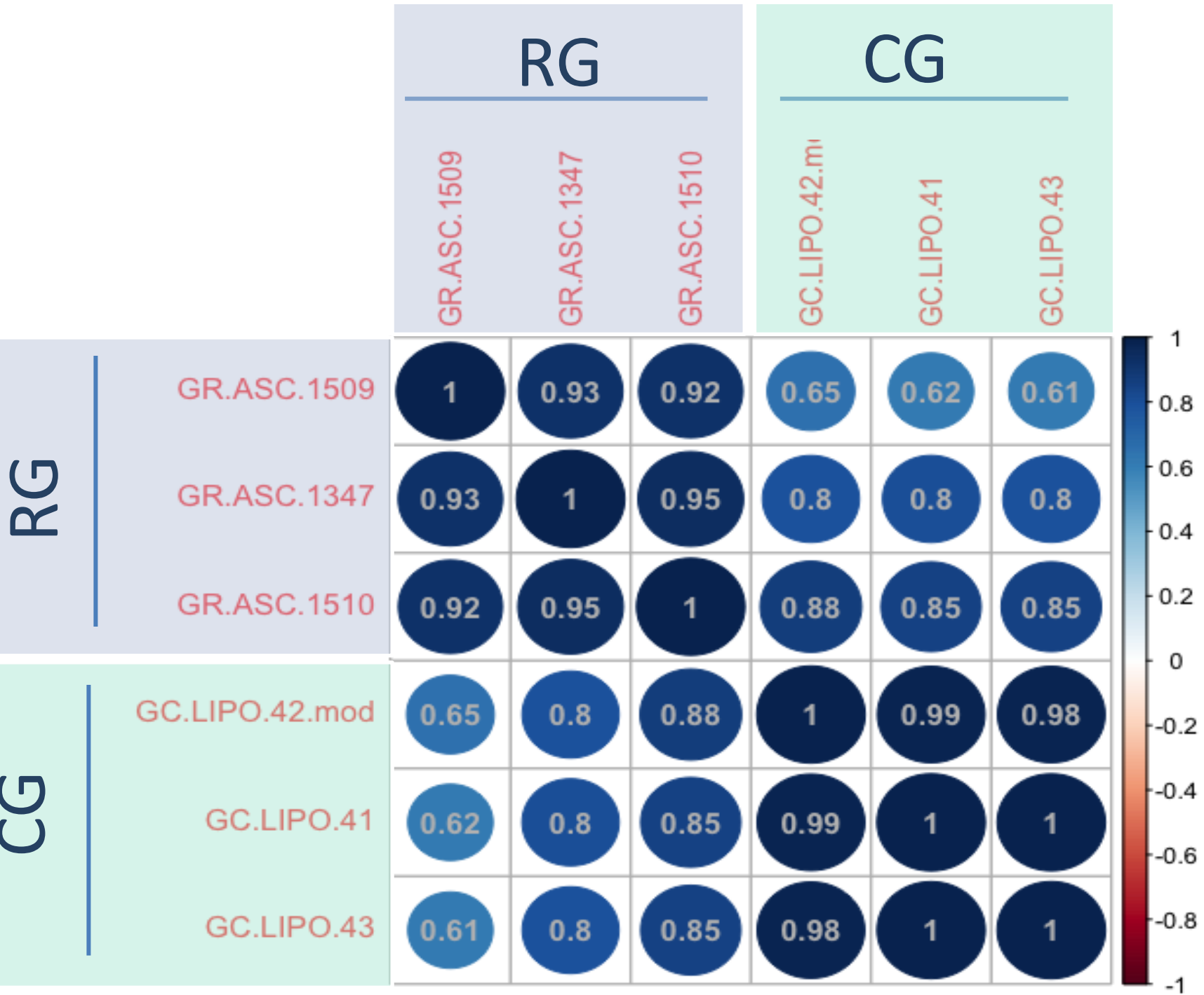
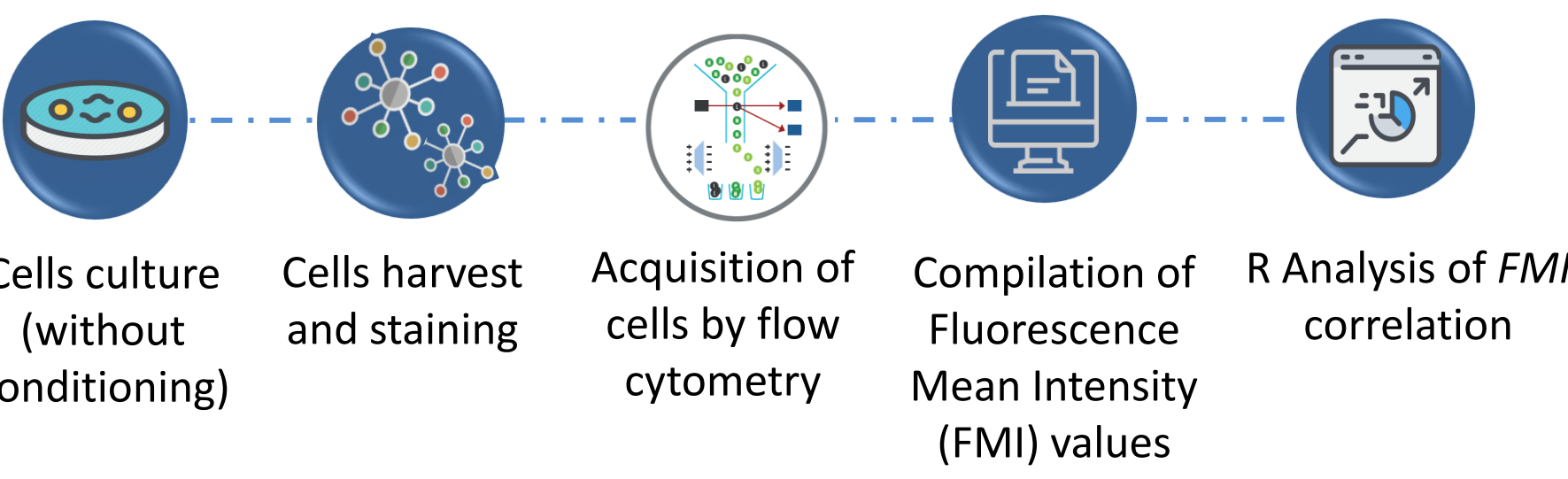
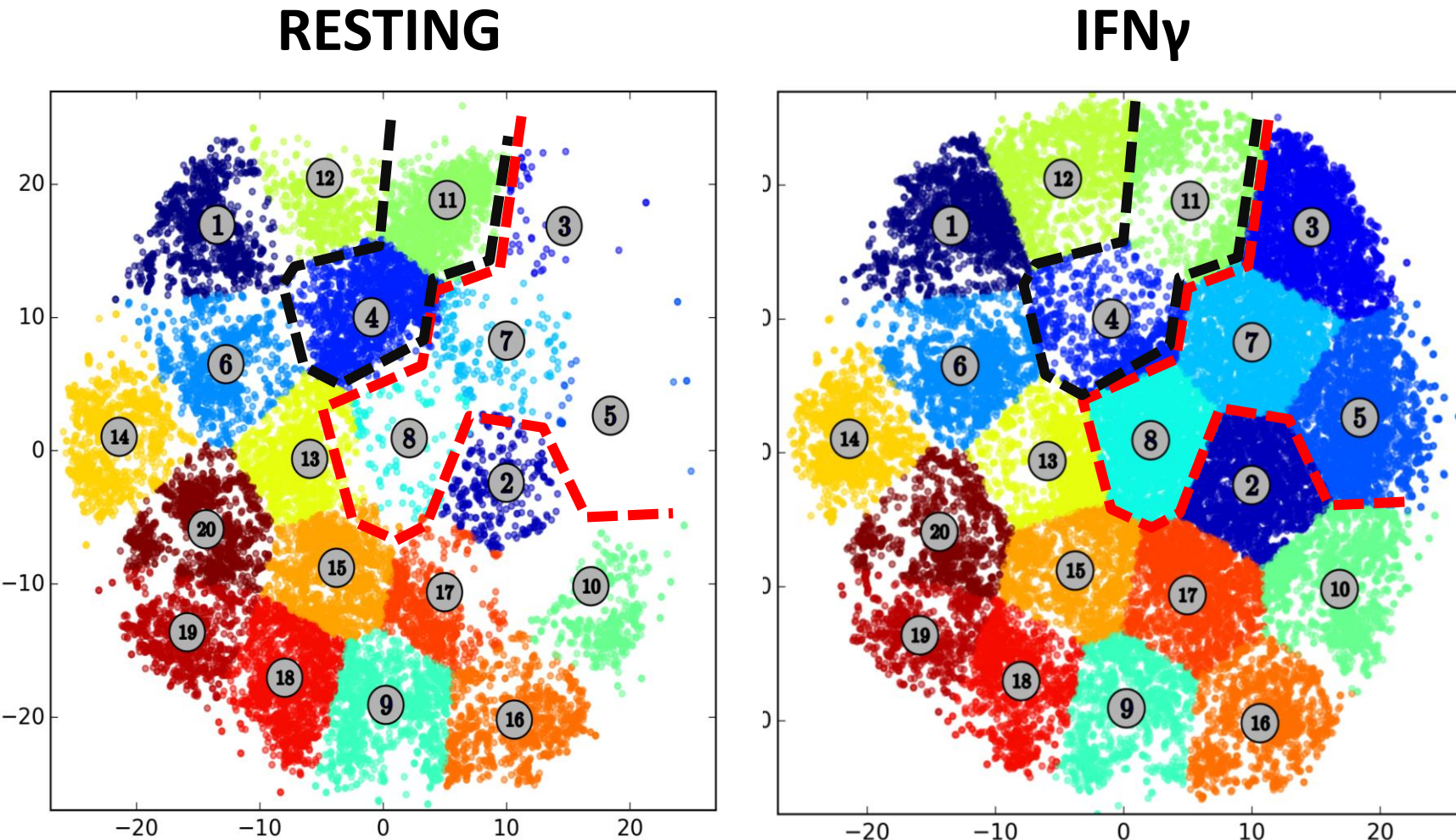


Figure 1: Strong correlation between different RG and CG MSC markers. Correlation between each pair of culture is visualized through a colored circle and the correlation coefficient (color defines the direction of relationship and the correlation coefficient indicates degree of association, ± 1 indicates a perfect association)

3 Phenotype change upon conditioning (CyTOF analysis)



IFN γ modulates sub-populations enrichment.

4 Functional Capacities Assessment

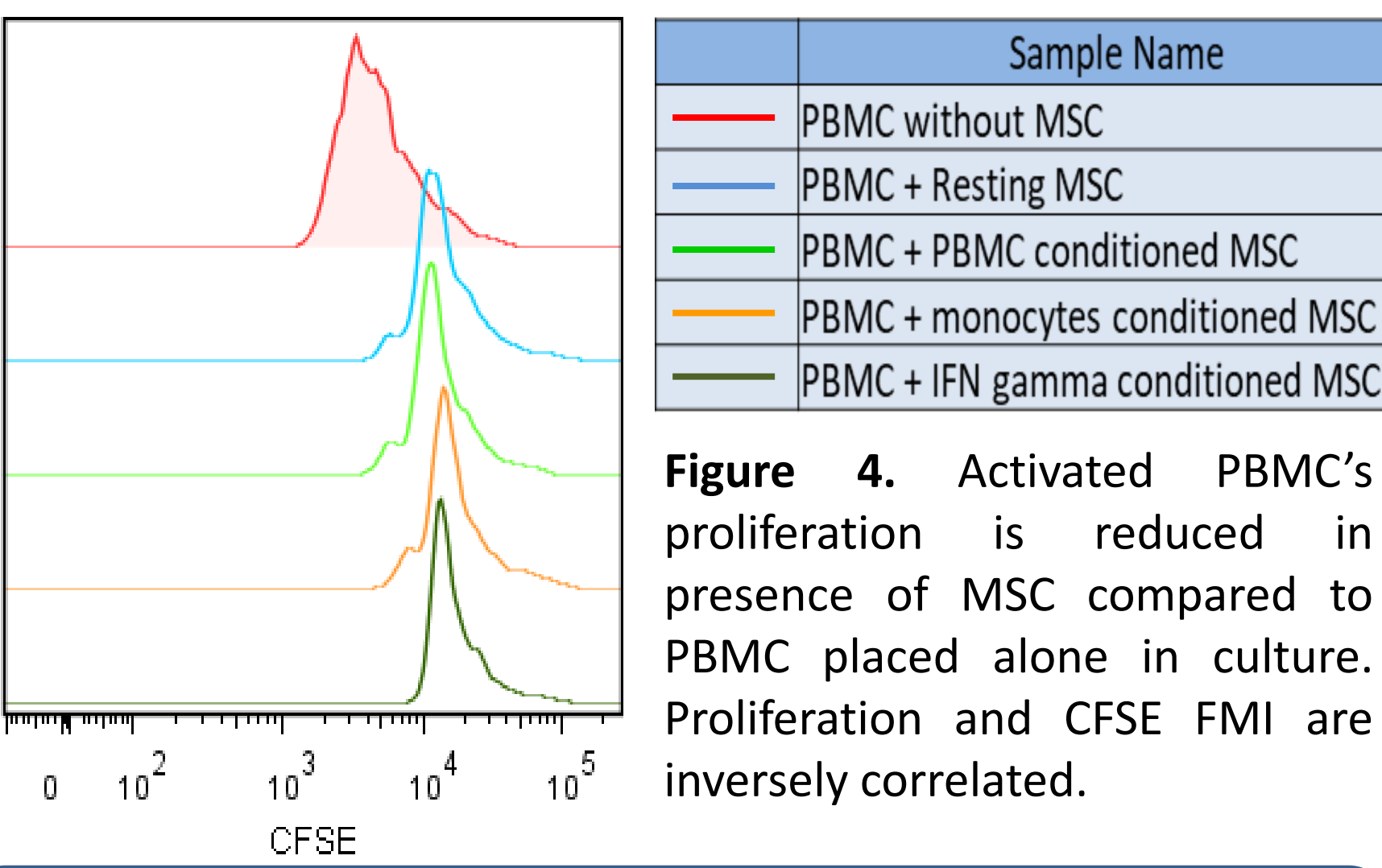


Figure 4. Activated PBMC's proliferation is reduced in presence of MSC compared to PBMC placed alone in culture. Proliferation and CFSE FMI are inversely correlated.

Resting and Conditioned MSCs block PBMC activated in-vitro proliferation.

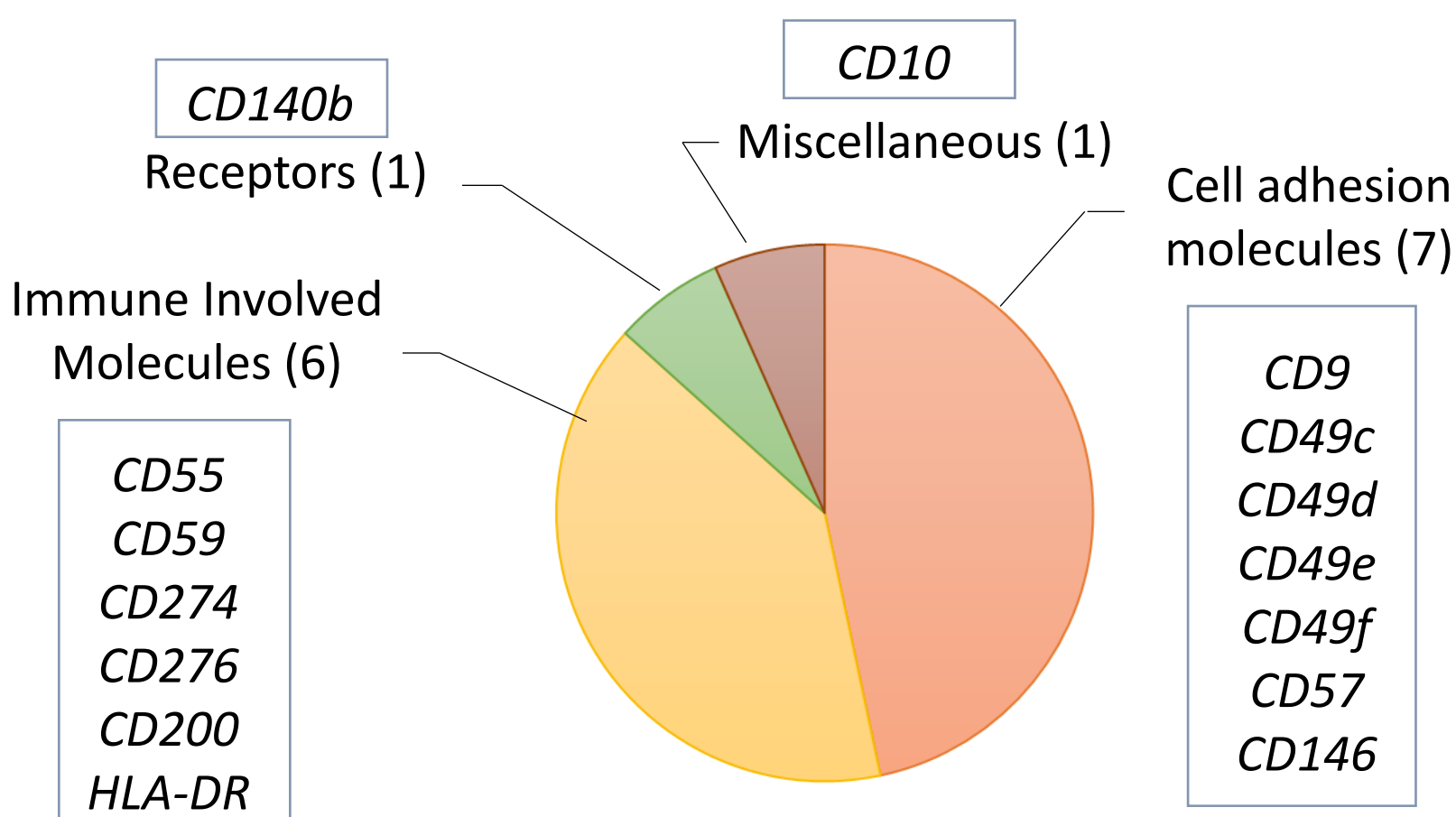
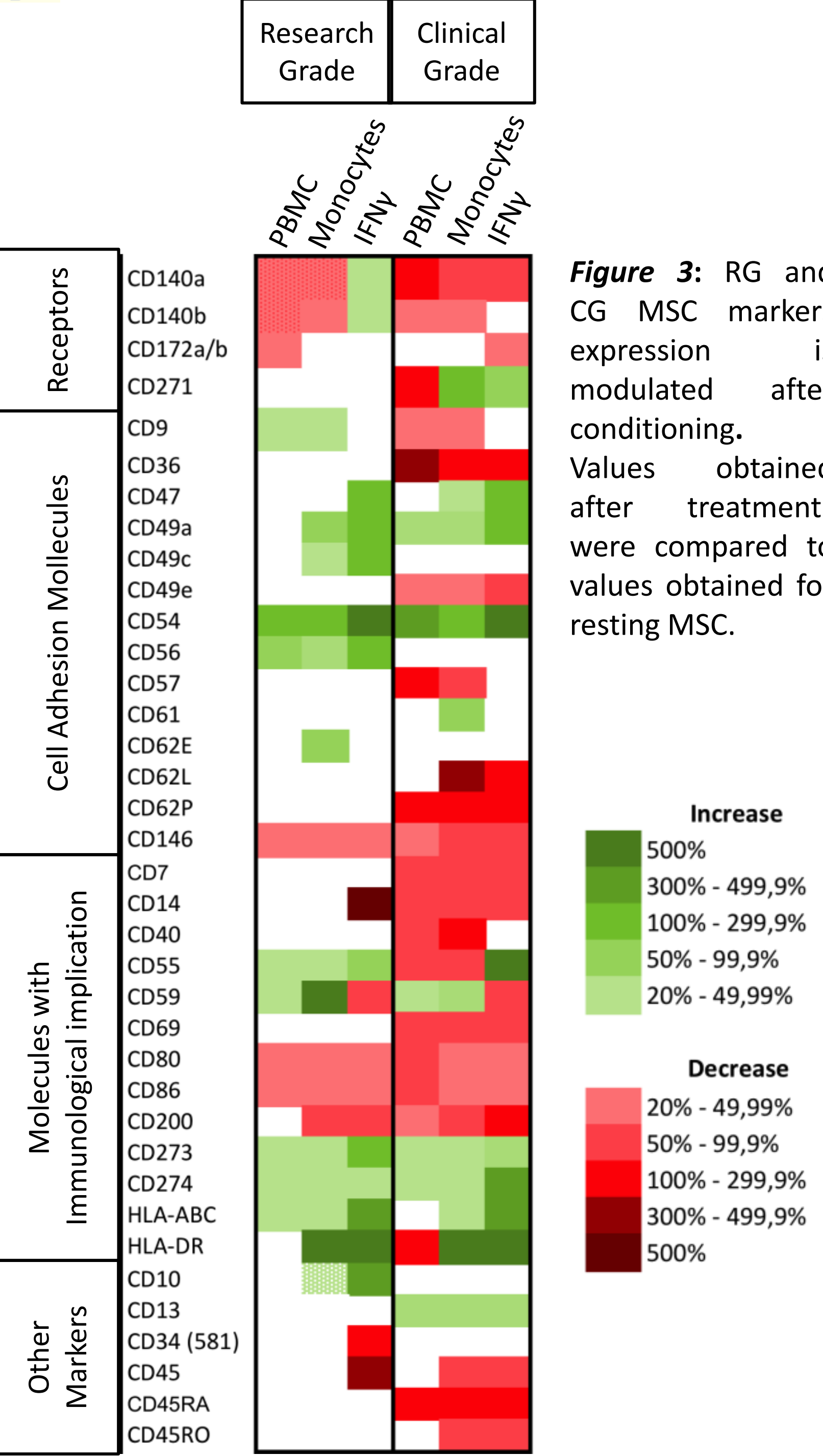


Figure 2: Markers that show significant differential expression between RG and CG cultures

- Strong correlation in markers expression for different CG cultures. Idem for RG cultures
- RG and CG are close in phenotype
- 15/61 markers distinguish CG from RG

2 Phenotype change upon conditioning



- CG MSC are more sensitive to activation than RG
- Some markers are modulated by all treatments
- Some markers are exclusively modulated by 1 tx.

Conclusions

This study first shows that research and clinical grade MSC share close phenotypes. This work also unveils phenotypic and functional markers of MSC along with their modulations according to different treatments. The CyTOF definition of new subpopulations may contribute to validate a cell therapy product for immunomodulation purposes.