

### Introduction & Objective

In 2006, the International Society for Cellular Therapy (ISCT) published a position paper proposing a minimal set of standards to define human MSCs. In addition to plastic adhesion properties and multipotent differentiation potential, the ISCT proposed a minimum surface antigen expression profile  $\geq 95\%$  positive for CD105, CD73 and CD90 and  $\leq 2\%$  expression of CD45, CD34, CD14/CD11b, CD79 $\alpha$ /CD19 and HLA-DR (Dominici, 2006). However, this list is by no means exhaustive. Additional markers that have been associated with MSC identification include CD44, CD133, CD49a, LNGFR, CD10, CD13, BMPRIA and STRO-1 antigen molecule, in addition to adhesion molecules VCAM-1, ALCAM, ICAM-1 and CD29. MHC1+, MHCII-, CD40-, CD80-, CD86- markers are linked to MSC immunosuppression and immunomodulation.

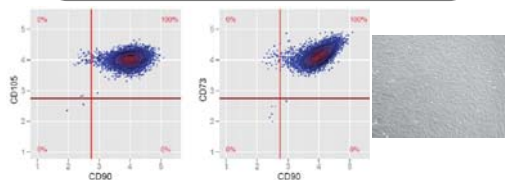
The objective of this investigation was to use high-throughput FACS analysis with 200+ antibodies against cell surface proteins to characterize the MSC receptor profile after *in vitro* culture.

### Methods

Human MSCs were obtained from the iliac crest bone marrow of healthy donors with informed consent and ethically approved procedures. MSCs were isolated and expanded in culture by direct plating. Briefly, 25 mls of bone marrow aspirate was obtained from each donor and diluted with PBS. The bone marrow suspension was pelleted and the supernatant discarded. The cell pellets were combined, resuspended and counted, after which they were plated at appropriate density for the culture flask size. MSC medium ( $\alpha$ -MEM, 10% selected fetal bovine serum (FBS), 1ng/ml FGF-2 and 1% penicillin/streptomycin) was added to the flasks. Cells were incubated at 37°C, 5% CO<sub>2</sub> and 90% humidity. After day 5, the cells were fed twice weekly and cultured when discrete colonies had formed and proliferated. Subsequently, cultures were passaged at 5-7 day intervals and expanded to passage 2 and cryopreserved for shipping to Becton Dickinson (BD) for analysis.

Upon arrival at BD, the cells were thawed and culture expanded for one further passage. After non-enzymatic removal from the tissue culture flask, they were assessed by FACS-CAP analysis as per BD protocols, allowing for 230 cell surface proteins.

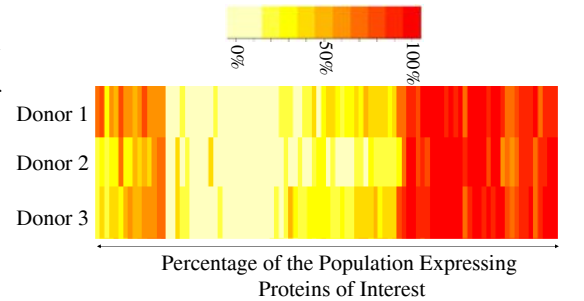
### Results



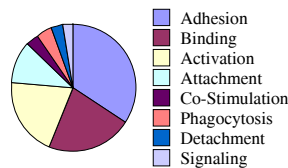
**Figure 1: Confirming an MSC Phenotype.** Supporting the validity of the BD data, the positive expression ISCT markers CD73, CD90 and CD105 and the absence of CD45, CD34, CD14/CD11b and CD79 $\alpha$ /CD19 were confirmed. The fibroblastic morphology was retained upon culturing

### Results

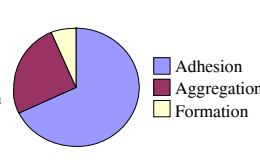
**Figure 2: Comparison of the Expression of 98 Cell Surface Proteins Between Donors.** Heatmap display of FACS analysis where each row represents a cell sample and each column an antigen. 24 proteins were consistently highly expressed (85-100% positive) in all donors, 1 protein was consistently lowly expressed (10-50% positive) and all donors were consistently negative for 107 proteins (0-10% positive). The remaining 98 proteins were regulated between donors.



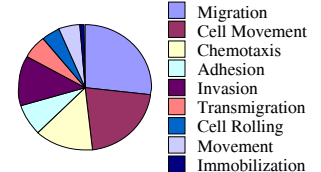
### Intracellular Signaling



### Tissue Development



### Cellular Movement



**Figure 3: Cell Surface Protein Function.** Categorization of protein function using Ingenuity Software indicated the primary functions of cell surface proteins is intracellular signaling, tissue development and cell migration. Each category and representative expression level is here expressed graphically.

| No. Cell Surface Proteins              | Binding Partner                    |
|--|------------------------------------|
| Growth Factor Receptors                |                                    |
| 4                                      | EGF Receptors                      |
| 4                                      | IL Receptors                       |
| 3                                      | Interferon Family Receptors        |
| 4                                      | TGF- $\beta$ Superfamily Receptors |
| 1                                      | HGF Receptor                       |
| 1                                      | Leukotriene Receptor               |
| 2                                      | PDGF Receptors                     |
| 3                                      | Chemokine Receptors                |
| 4                                      | TNF Receptors                      |
| Extra-Cellular Matrix Binding Proteins |                                    |
| 7                                      | Fibronectin                        |
| 1                                      | Vitronectin                        |
| 1                                      | Hyaluronic Acid                    |

**Figure 4: Highly Expressed Receptors.** 26 growth factor receptors and 9 ECM binding proteins were consistently identified on the MSC surface, proteins that may enhance the field's ability to isolate, expand and manipulate cells during *in vitro* culture.

### Discussion

The outcome of this study is a database of cell surface markers expressed on MSCs that will have considerable use in several ways: 1) Providing promising leads for growth factor combinations that will control MSC proliferation and differentiation in serum-free media, 2) Providing ECM targets to enhance cell attachment in serum-free culture condition, and 3) Identifying potential new reagents that can strengthen release criteria for MSCs.

### Funding

This work was conducted as part of the PurStem consortium and supported by the EU Framework 7 program under HEALTH-2007-B-223298. For further information on this collaboration, visit [www.purstem.eu](http://www.purstem.eu) or contact Frank Barry via email at [frank.barry@nuigalway.ie](mailto:frank.barry@nuigalway.ie).