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Immunophenotypic Characterization of Normal Bone Marrow Stem Cells

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1. Introduction

Despite of being described more than one decade ago (Pittenger et al., 1999), the immunophenotypic profile of bone marrow mesenchymal stem cells (MSC) still not well documented. The difficulty in achieving a detailed phenotypic characterization is common in less-represented cell populations and/or populations lacking a specific known cell marker, like bone marrow MSC.

The recent advances in flow cytometry technology and the emergence of new high-speed flow cytometers have given a valuable contribute to diminish this problem in two different (but complementary) aspects: 1) by reducing dramatically the acquisition time period, making it more reasonable to study minor cell populations; and 2) by increasing the number of parameters that can be analyzed per cell at the same time, which is critical to improve the immunophenotypic characterization of those not-well characterized cell populations that lack a specific known marker.

A good example of the practical usefulness of such technical developments is the description of different cell compartments in the bone marrow CD34+ hematopoietic stem cell (HSC) population. Detailed studies on this minor bone marrow cell population demonstrated that each compartment is committed to a different hematopoietic cell lineage. An extensive immunophenotypic characterization of those CD34+ compartments allowed the development of protocols to easily and quickly identify, quantify and evaluate phenotypic aberrations and maturational blocks in those cells, which is decisive to the diagnosis, prognosis, or follow-up of a variety of hematological clonal diseases (del Cañizo et al., 2003; Lochem et al., 2004; Matarraz et al., 2008; Orfao et al. 2004).

2. Bone marrow mesenchymal stem cells

After the identification of a plastic-adherent bone marrow stromal cell population in 1976 by Friedenstein and colleagues and the first evidence of their multilineage potential (Pittenger et al., 1999) with subsequent confirmation of their stem cell nature, an increasing interest on these bone marrow MSC has emerged, mainly because of their promising therapeutic applications.

By definition, a stem cell is an undifferentiated cell with the potential ability of self-renewal and the capability of differentiation along different cell lineages (multipotency). MSC can be found on a great variety of adult tissues, where they play an important role in tissue regeneration, such as: bone marrow, adipose tissue, umbilical cord blood, umbilical cord matrix, menstrual blood, endometrium, placenta, dental pulp, skin and thymus, among others (Chamberlain et al., 2007; Ding et al. 2011; Kolf et al., 2007; Martins et al., 2009; Musina et al., 2005; Pittenger et al., 1999).

In addition to their presence in numerous adult tissues, MSC are relatively easy to isolate and have the capability to expand manyfold in culture without lose their stem cell properties. Moreover, when MSC are systemically transplanted, they are able to migrate to sites of injury and promote tissue repair, by producing growth factors or other soluble factors important to tissue regeneration, as well as by undergoing cellular differentiation (Chamberlain et al., 2007, Kolf et al., 2007; Mafi et al., 2011); such features explain the success of MSC transfusion therapy in genetic disorders affecting mesenchymal tissues (Horwitz et al., 2002; Undale et al., 2009). Furthermore, those cells have the ability of suppressing the immune response of a wide variety of immune cells, including T, B and NK lymphocytes, and antigen-presenting cells (Chamberlain et al., 2007; Stagg, 2007), and their importance in patients' clinical outcome has already been proven in severe acute graft-versus-host disease (Remberger et al., 2011; von Bahr et al., 2011). Moreover, the results achieved in animal models of autoimmune diseases are promising and encouraged the beginning of phase I clinical trials in multiple sclerosis (Constantin et al., 2009; Darlington et al., 2011; Siatskas et al., 2009).

2.1 Identification and quantification of bone marrow MSC

As referred previously, the study of minor cell populations with no known specific cell marker toke great advantage on the development of high-speed multi-parameter flow cytometers. The use of an 8-color FACSCanto II (Becton Dickinson Biosciences, BDB) flow cytometer allowed us to identify MSC in bone marrow, quantify them and further characterize their immunophenotypic profile. We employed a monoclonal antibody panel with a backbone of 3 common markers (CD13, CD45 and CD11b) for the identification of MSC (known to be CD13+CD45-CD11b-) in each tube that, at the same time, permitted the study of the expression of five more proteins on MSC per tube.

MSC are rare in bone marrow, being reported that they represent approximately 0,01% of all nucleated bone marrow cells (Chamberlain et al., 2007; Mafi et al., 2011), although is known that their number declines with aging (Caplan, 2007). Our data point to a percentage ranging between 0,01% and 0,03% of all nucleated bone marrow cells (Martins et al., 2009).

2.2 Immunophenotypic characterization of bone marrow MSC

2.2.1 Flow cytometer quality control, compensation setup strategies and other technical issues

According to the manufacturer's recommendations, it is done a daily quality control using the Rainbow Beads (BDB). In what concerns to cytometer's compensation setup, it is made once per month by setting up the Rainbow Beads (BDB) values according to the EuroFlow consortium's guidelines and then by doing a general compensation for stable fluorochromes and a specific compensation for each monoclonal antibody conjugated with tandem

fluorochromes. Although the compensation is automatic, it is always revised by experienced staff at the end of the process.

In order to detect cellular autofluorescence, a negative control was made for each sample, where the bone marrow sample was only stained for CD45 PO and CD34 PerCPcy5.5.

SSC and FSC light dispersion properties allow a good discrimination between viable and dead cells and the doublets were excluded based on FSC-Area *versus* FSC-Height characteristics.

2.2.2 Material and methods

The immunophenotypic characterization of bone marrow MSC were performed in fresh EDTA-collected bone marrow samples from healthy individuals. After collection the samples were stored at 4 ºC and processed within 24 hours.

Whole bone marrow samples were stained for surface cell markers using a stain-lyse-and-then-wash direct immunofluorescence technique. 200 µl of whole bone marrow were aliquoted in different tubes and stained with the following combinations of monoclonal antibodies in an 8-color staining protocol, detailed in table 1.

	FITC	PE	PerCPcy5.5	PEcy7	APC	APCH7	PB	PO
Tube 1	CD49e (SAM1) Beckman Coulter	CD73 (AD2) BD Pharmingen	CD34 (8G12) BDB	CD13 (Immu103.44) Beckman Coulter	CD90 (5E10) BD Pharmingen	HLA-DR (L243) BDB	CD11b (ICRF44) BD Pharmingen	CD45 (HI30) Invitrogen
Tube 2	CD31 (WM59) BD Pharmingen	NGFR (C40-1457) BD Pharmingen	CD14 (M5E2) BD Pharmingen	CD13	CD133 (293C3) Miltenyi Biotec	-	CD11b	CD45
Tube 3	CD15 (HI98) BDB	CD146 (P1H12) BD Pharmingen	CD24 (ALB9) Beckman Coulter	CD13	CD90	CD29 (TS2/16) BioLegend	CD11b	CD45
Tube 4	CD106 (51-10C9) BD Pharmingen	CD105 (1G2) Beckman Coulter	-	CD13	HLA-A, B, C (G46-2.6) BD Pharmingen	-	CD11b	CD45
Tube 5	-	CD73	CD24	CD13	CD90	-	CD11b	CD45

Table 1. Panel of monoclonal antibodies used for the bone marrow MSC characterization. FITC - fluorescein isothiocyanate; PE – phycoerythrin; PerCPcy5.5 - peridinin chlorophyll protein cyanine 5.5; PEcy7 - R-phycoerythrin cyanine 7; APC – allophycocyanin; APCH7 - allophycocyanin H 7; PB - pacific blue; PO - pacific orange

Data acquisition was performed in a FACSCanto II flow cytometer (BDB), using FACSDiva acquisition software (BDB). The total bone marrow cellularity of the whole sample was acquired (5 x 10^6 events, minimum) for each tube. Bone marrow MSC were identified as $CD13^+/CD45^-/CD11b^-$, as shown in Figure 1.

Data analysis was performed using Infinicyt software (Cytognos, Salamanca, Spain).

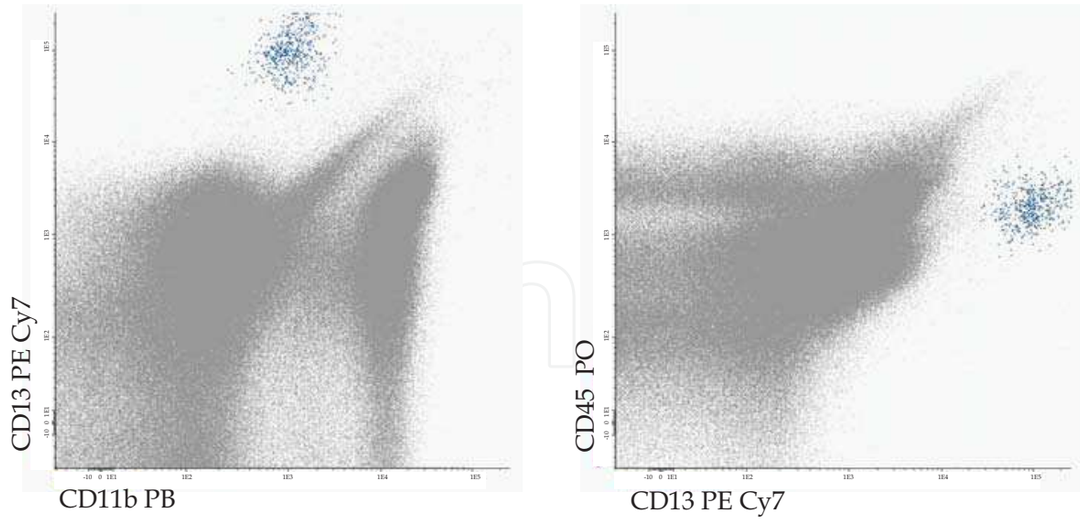

Fig. 1. Identification of bone marrow MSC (blue) present in a whole bone marrow sample, phenotypically characterized as $CD13^{+}CD45^{-}CD11b^{-}$

2.2.3 Results and discussion

Bone marrow MSC showed to be uniformly positive to CD13, CD29, CD49e, CD90, CD106, CD146, CD73, NGFR, CD105 and HLA-A, B, C (Figure 1 and Figure 2); and negative to CD24, CD31, CD11b, CD14, CD15, CD34, CD45, CD133 and HLA-DR, which is in agreement with previous studies described in the literature (Chamberlain et al., 2007; Delorme et al., 2008; Ehninger & Trumpp, 2011; Fox et al., 2007; Jones & McGonagle, 2008; Kolf et al., 2007; Martins et al., 2009; Pittenger et al., 1999; Tormin et al., 2011). Based on the expression profile of these markers, bone marrow MSC behave as one sole cell population, as all the studied markers were homogeneously expressed inside the MSC population.

Several studies on adhesion molecules and chemokine receptors expression have been made in order to shed light on MSC migratory and homing ability. CD29 (integrin β_1-subunit) and CD106 (vascular cell adhesion molecule 1, VCAM-1) seem to be important in the adhesion of MSC to endothelial cells (Chamberlain et al., 2007; Kolf et al., 2007; Stagg, 2007) and CD29, which when dimerized with CD49e (integrin α_5-subunit) forms a receptor that binds to fibronectin and invasin, is likely to promote MSC-extracellular matrix interaction (Gu et al., 2009). CD146 (Muc18) plays an important role in cell-cell and cell-extracellular matrix adhesion and an increased expression of these marker on tumor cells is associated with an increased cell motility and invasiveness/ metastasis capability (Bardin et al., 2001; Zeng et al., 2011). The glycoprotein CD90 (Thy-1) regulates as well cell-cell and cell-extracellular matrix interactions, being involved in adhesion to endothelial cells, migration, metastasis and tissue regeneration (Jurisic et al., 2010; Rege & Hagood, 2006).

The enzyme CD73 is an ecto-5'-nucleotidase that produces extracellular adenosine. In animal tumor models, CD73-generated adenosine inhibits both homing and expansion of T cells via adenosine-receptor signaling. In fact, recent research shows that adenosine suppresses T cell immune response both in activation and effector phases, as well as NK cell immune activity (Wang et al., 2011; Zhang et al., 2010).

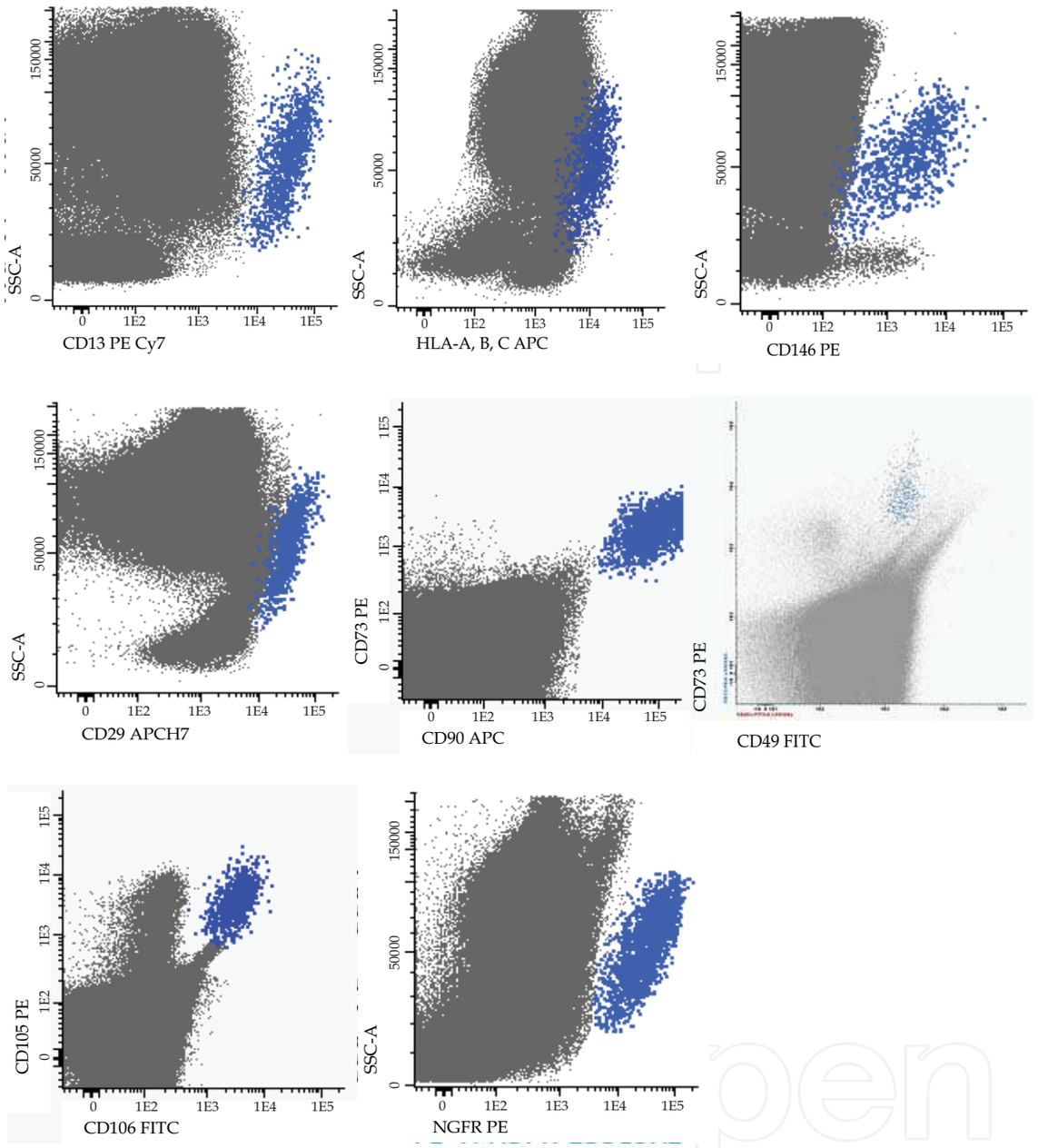

Fig. 2. Immunophenotypic characteristics of bone marrow MSC (blue). The remaining bone marrow nucleated cells are represented as grey events

In what concerns to growth factor receptors, NGFR (nerve growth factor receptor, CD271) is expressed in a wide variety of tissues and, depending on the cell type, signaling through this receptor regulates NF-kB activation, apoptosis, tissue regeneration, immune cell activation, proliferation and cell differentiation (Micera et al., 2007; Rogers et al., 2010). Finally, CD105 (endoglin) is one of the receptors for TGF-β, a growth factor involved in the regulation of development, maintenance and proliferation of MSC (Stagg, 2007), and also known to play an important role in tissue repair.

Some discrepancies described in the expression of adhesion molecules, chemokine receptors and other proteins, may be the reflex of the microenvironmental differences present in different studies. Although there are a great similitude in the phenotypic profile of MSC isolated from different tissues, differences do exist (Chamberlain et al., 2007; Kolf et al., 2007; Martins et al., 2009). As well as different cultures conditions can also change the MSC phenotype (Chamberlain et al., 2007; Halfon et al., 2011; Stagg, 2007; Tormin et al., 2011). This could be a clue of MSC highly sensitiveness to microenvironment alterations, and their potential to change their protein expression profile could be of great importance in giving an appropriate response to physiological or pathological challenges: by changing their migratory pattern, by initiating an immunomodulatory or immunosuppressive response, by modifying the production and release of soluble factors, or by undergoing cell differentiation.

As a minor bone marrow cell population easy to expand in vitro, it is attractive to characterize the MSC immunophenotype after culture cell expansion. Nevertheless, characterizing these cells directly (without previous culture) enables an analysis closest to their physiological conditions, excluding the phenotypic alterations induced by factors present in the culture medium. Moreover, this direct approach allows an accurate quantification of MSC in bone marrow. Also, this same strategy can be applied to MSC from other tissues.

3. Bone marrow hematopoietic stem cells

The multipotent hematopoietic stem cell is mainly located in the bone marrow of adult animals and has the ability to differentiate along all hematopoietic cell lineages. A number of studies based on in vitro cell culture, xeno-transplantation of hematopoietic human cells in immunodeficient mice and in pre-immune animal fetuses, were carried out to identify the human hematopoietic stem cell and unveil the hematopoietic precursors hierarchy (Nimer, 2008; Yin et al., 2007), becoming clear that CD34-positive cells were able to differentiate and give rise to all blood cells. There are evidences that, within this heterogeneous population, the more immature CD34+ HSC expresses CD133 and are CD38-negative/dim. It is also known that the CD34+CD133+ subpopulation can arise from the CD133+CD34-CD38- subset (Goussetis et al., 2006; Nimer, 2008; Yin et al., 1997).

3.1 Identification and quantification of the different bone marrow CD34+ HSC cell compartments

As already referred, CD34-positive cells are an heterogeneous bone marrow cell population, consisting in various cell compartments differing in immunophenotype, size and lineage commitment. The immunophenotypic pattern of each compartment is well described and, with a relatively low number of markers, the majority of those subsets can be accurately and easily identified.

Attending only to the immunophenotypic features, is possible to identify the following bone marrow CD34+ cell subsets by flow cytometry: uncommitted (more immature) precursors, neutrophil precursors, B cell precursors, monocytic precursors, plasmacytoid dendritic cells precursors, erythroid precursors, basophil precursors and mast cell precursors.

A detailed immunophenotypic description of human bone marrow CD34+ cells was published, few years ago, by Matarraz and colleagues (Matarraz et al., 2008) and Lochem and colleagues (Lochem et al., 2004), along with the frequency of each CD34+ cell subpopulation in normal hematopoiesis (Matarraz et al. 2008), presented on table 2.

Bone marrow CD34+ HSC compartments	Mean ± Standard deviation	Range
% Bone marrow CD34+ HSC (of total bone marrow)	0,9 ± 0,3	(0,2-1,6)
Immature CD34+ precursor (%) (within CD34+ cells)	52 ± 12	(19-66)
CD34+ neutrophil precursors (%) (within CD34+ cells)	34 ± 7	(15-47)
CD34+ B cell precursors (%) (within CD34+ cells)	14 ± 10	(1-36)
CD34+ monocytic precursors (%) (within CD34+ cells)	10 ± 7	(0-26)
CD34+ plasmacytoid dendritic cell precursors (%) (within CD34+ cells)	5 ± 2	(0-9)
CD34+ erythroid precursors (%) (within CD34+ cells)	18 ± 8	(1-36)
CD34+ basophil precursors (%) (within CD34+ cells)	0,7 ± 0,4	(0-1,5)
CD34+ mast cell precursors (%) (within CD34+ cells)	0 ± 0,005	(0-0,02)

Table 2. Distribution of the different cell compartments of bone marrow CD34+ HSC. The results are expressed as mean ± standard deviation (range). Adapted from Matarraz et al. Leukemia 2008

The most immature CD34+ subset can be identified based on CD133 expression (Goussetis et al., 2006; Pastore et al., 2008; Yin et al., 1997). When other markers are concerned, these cell are $CD34^{hi}/CD45^{int}/HLA\text{-}DR^{hi}/cyMPO^{-}/nTdT^{-}/CD117^{hi}$ and have intermediate side scatter (SSC) and forward scatter (FSC) light dispersion properties (Matarraz et al., 2008). As previously described by Matarraz and colleagues, the phenotypic profile of CD34+ B cell precursors is $CD34^{int}/CD45^{int/dim}/HLA\text{-}DR^{hi}/cyMPO^{-}/nTdT^{int}/CD117^{-}$ and these cells present the lowest SSC and FSC of all CD34+ subpopulations (Lochem et al., 2004; Matarraz et al., 2008); the CD34+ neutrophil precursors present $CD34^{hi}/CD45^{int/dim}/HLA\text{-}DR^{hi}/cyMPO^{int/hi}/nTdT^{-}/CD117^{hi}$, along with the highest values for SSC and FSC of all CD34+ subsets; the CD34+ plasmacytoid dendritic cell precursors are identified based on the expression of $CD34^{+}/CD123^{hi/int}/HLA\text{-}DR^{hi}$; CD34+ monocytic precursors display $CD34+/HLA\text{-}DR^{hi}/CD64^{hi}/CD45^{hi}/CD117^{-}$ immunophenotype; basophil precursors are described as being $CD34^{+}/CD123^{int/hi}/HLA\text{-}DR^{-/+}$; and CD34+ mast cell precursors are $CD34+/CD117^{hi}/HLA\text{-}DR^{-/int}$ (Matarraz et al., 2008). Finally, CD34+ erythroid precursors are characterized by $CD34^{+}/CD36^{+}/CD64^{-}/CD45^{lo}$ immunophenotype (Matarraz et al., 2008) and by CD105 expression (Buhring et al., 1991; Rokhlin et al., 1995). As a matter of fact, CD105 and TGF-β_1 have a pivotal role in the regulation of the differentiation in the erythroid lineage (Fortunel et al., 2000; Moody et al., 2007).

3.2 A single-tube protocol to identify the different bone marrow CD34+ HSC compartments

Recently, we developed an 8-color single-tube protocol to identify the different bone marrow CD34+ HSC subsets by flow cytometry.

The single-tube protocol we propose here was constructed to allow an accurate, quick and easy identification and quantification of those cellular compartments. Attending to the monoclonal antibodies and fluorochrome-conjugation available on the market and to compensation issues, and based on our experience and knowledge on the hematopoietic maturation dynamics, we elected the best markers to identify with precision the cell populations of interest.

3.2.1 Material and methods

The immunophenotypic characterization of bone marrow CD34+ precursors were performed in fresh EDTA-collected bone marrow samples from healthy individuals. After collection, the samples were stored at 4 ºC and processed within 24 hours. The quality control and compensation strategies are described in detail in section 2.2.1.

A stain-lyse-and-then-wash direct immunofluorescence protocol was used, and the monoclonal antibodies were combined as presented on table 3.

	FITC	PE	PerCPcy 5.5	PEcy7	APC	APCH7	PB	PO
Single Tube Protocol	CD35 (E11) BDB Pharmingem	CD123 (SSDCL Y107D2) Beckman Coulter	CD34 (8G12) BDB	CD117 (PN IM3698) Beckman Coulter	CD133 (293C3) Miltenyi Biotec	HLA-DR (L243) BDB	CD44 (IM7) Biolegend	CD45 (HI30) Invitrogen

FITC - fluorescein isothiocyanate; PE – phycoerythrin; PerCPcy5.5 - peridinin chlorophyll protein cyanine 5.5; PEcy7 - R-phycoerythrin cyanine 7; APC – allophycocyanin; APCH7 - allophycocyanin H 7; PB - pacific blue; PO - pacific orange.

Table 3. Panel of monoclonal antibodies used for the identification and quantification of the different subpopulations found in bone marrow CD34+ HSC

Data acquisition was performed on a FACSCanto II flow cytometer (BDB), using FACSDiva acquisition software (BDB). In a first step of acquisition, the whole bone marrow cellularity was stored (100.000 events). In a second step, only events within the CD34+ electronic gate were acquired (5.000 to 10.000 CD34+ events).

Data analysis was performed using Infinicyt software (Cytognos, Salamanca, Spain).

3.2.2 How to identify the different CD34+ HSC compartments with the single-tube protocol?

1. The most immature (uncommitted) compartment of bone marrow C34+ HSC
   The most immature compartment can be easily identified based on their positivity to CD133 marker ($CD133^{hi}$). To differentiate this subset from CD34+ neutrophil precursors and CD34+ plasmacytoid dendritic cell precursors, also expressing CD133 ($CD133^{int}$),

other important phenotypic characteristics have to be taken into account: CD35^{-}/CD34^{hi}/HLA-DR^{hi}/CD117^{hi}/FSC^{int}/SSC^{int}/CD123^{-}. Figure 3 presents a detailed immunophenotype of this compartment considering all the markers used in this protocol.

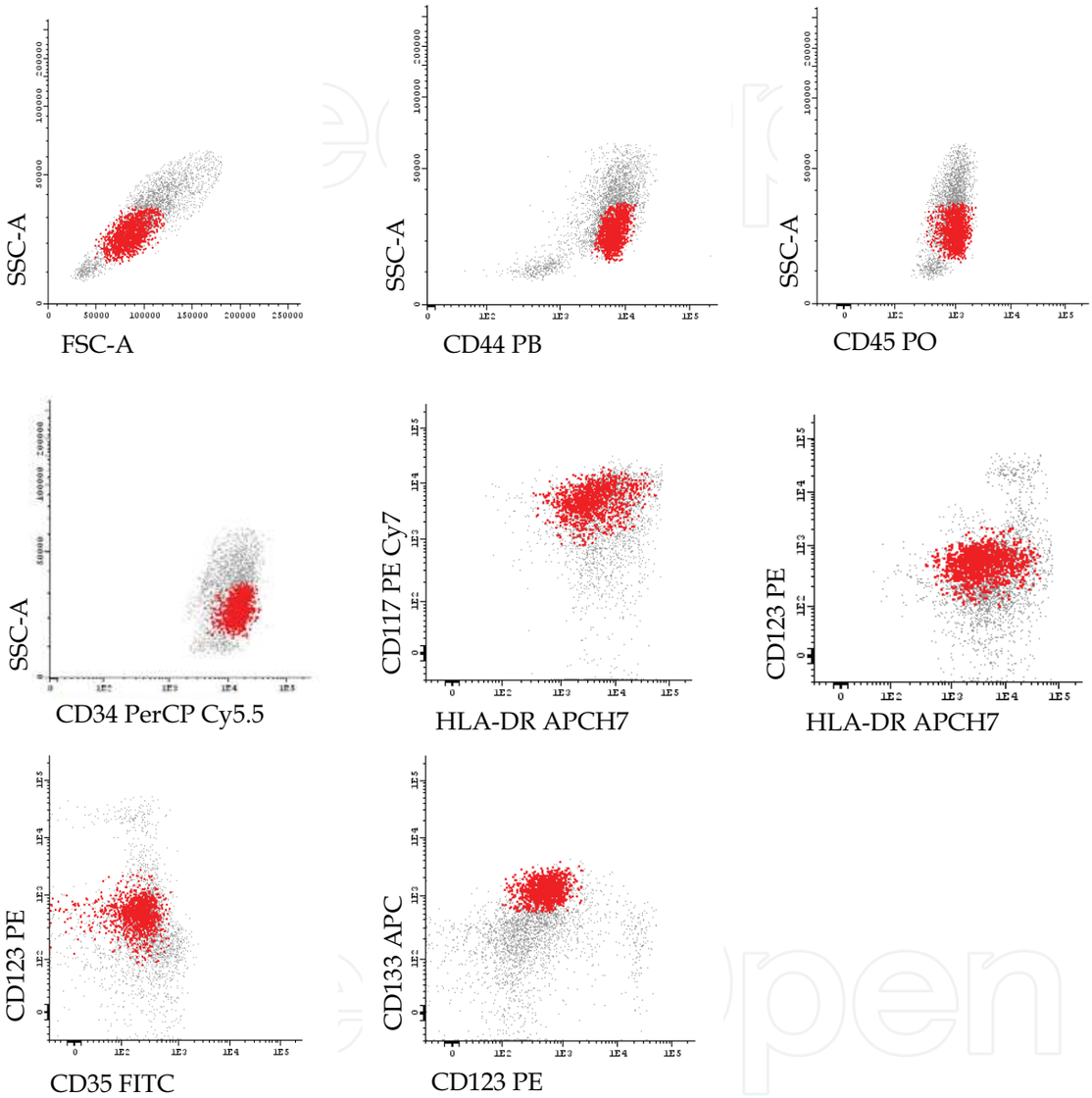

Fig. 3. Uncommitted bone marrow CD34+ HSC (red) immunophenotype. The remaining bone marrow CD34+ cell compartments are presented in grey

2. Bone marrow CD34+ erythroid precursors
   Both CD34+ erythroid precursors and monocytic precursors express CD35. The two CD34+ subpopulations can be distinguished in this protocol by the expression of CD117 and HLA-DR. The erythroid precursors are CD117^{+}/HLA-DR^{int} and the monocytic precursors are CD117$^{dim/-}$/HLA-DR^{hi}. Moreover, the erythroid precursors are characterized by a dim expression of CD34, CD45 and CD44 (Figure 4).

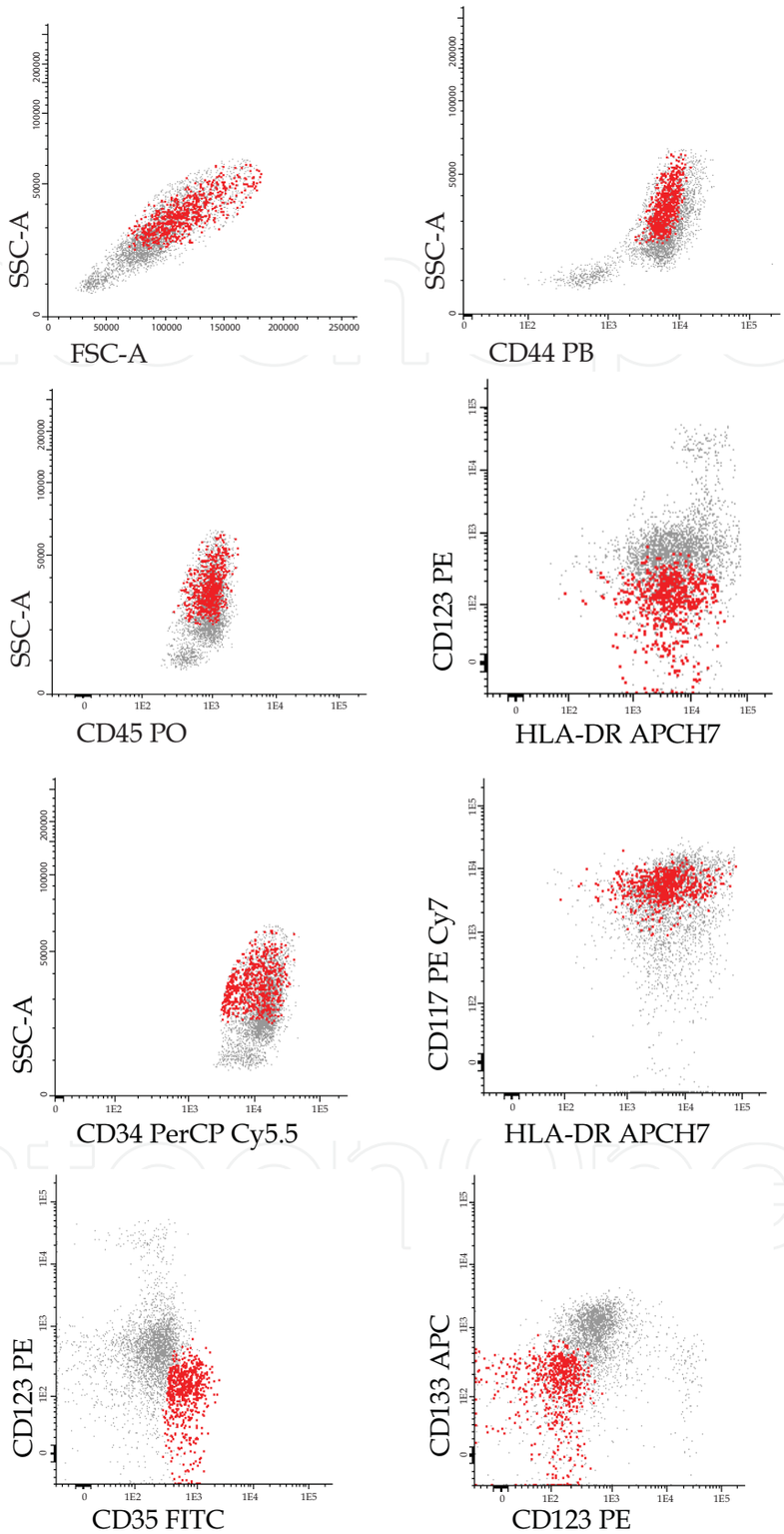

Fig. 4. Erythroid-committed bone marrow CD34+ precursors (red) immunophenotype. The remaining bone marrow CD34+ cell compartments correspond to the grey events

It is worth mentioning that our previous studies with simultaneous staining of CD105 and CD35 proved that the two markers were co-expressed in the same subset of CD34+ bone marrow cells and CD35 appears slightly before CD105 (Figure 5)[1].

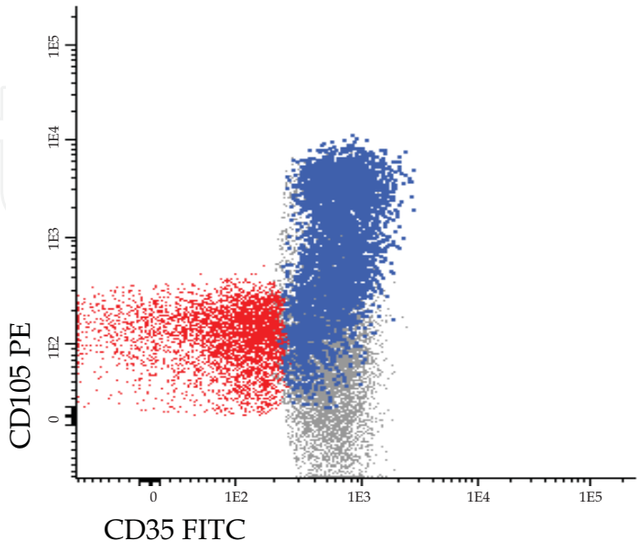

Fig. 5. Expression of CD105 and CD35 in bone marrow erythroid lineage: uncommitted CD34+ cells (red), CD34+erythroid precursors (blue) and CD34- erythroid precursors (grey)

3. Bone marrow CD34+ neutrophil precursors
   Neutrophil precursors show high reactivity to CD44 antigen, as the plasmacytoid dendritic cell precursors ($CD44^{hi}$), but in the absence of CD123 marker. Other important immunophenotypic features of this CD34+ compartment are: $CD133^{int}/CD35^{-}/HLA\text{-}DR^{hi}/CD117^{hi}/CD45^{int/dim}/FSC^{hi}/SSC^{hi}$ (Figure 6).

4. Bone marrow CD34+ monocyte precursors
   Using this single-tube approach, the monocyte precursors are primarily identified by exclusion of all the other myeloid CD34+ precursors. Is noteworthy that a large percentage of monocyte-committed CD34+ precursors express CD35, being discriminated from CD34+ erythroid precursors by their $CD117^{dim/-}/HLA\text{-}DR^{+}/CD45^{hi}$ phenotype. Although classically the identification of this CD34+ subset was made focusing on the expression of CD64, this marker seems to be also present on CD34+ plasmacytoid and myeloid dendritic cell precursors. In line with this, CD35 might be a good option to the identification of CD34+ monocyte precursors. The immunophenotype of this population is depicted in Figure 7.

5. Bone marrow CD34+ B cell precursors
   Even in the absence of an B-cell lineage specific marker, as CD19 or CD79a, CD34+ B cell precursors are clearly identified by the low expression of CD44 and CD45, along with low light scatter properties (Figure 8).

[1] According to our experience, CD35 seems to be expressed earlier than CD105 and CD36 on erythroid committed CD34+ precursors, allowing a more accurate quantification of this subset.

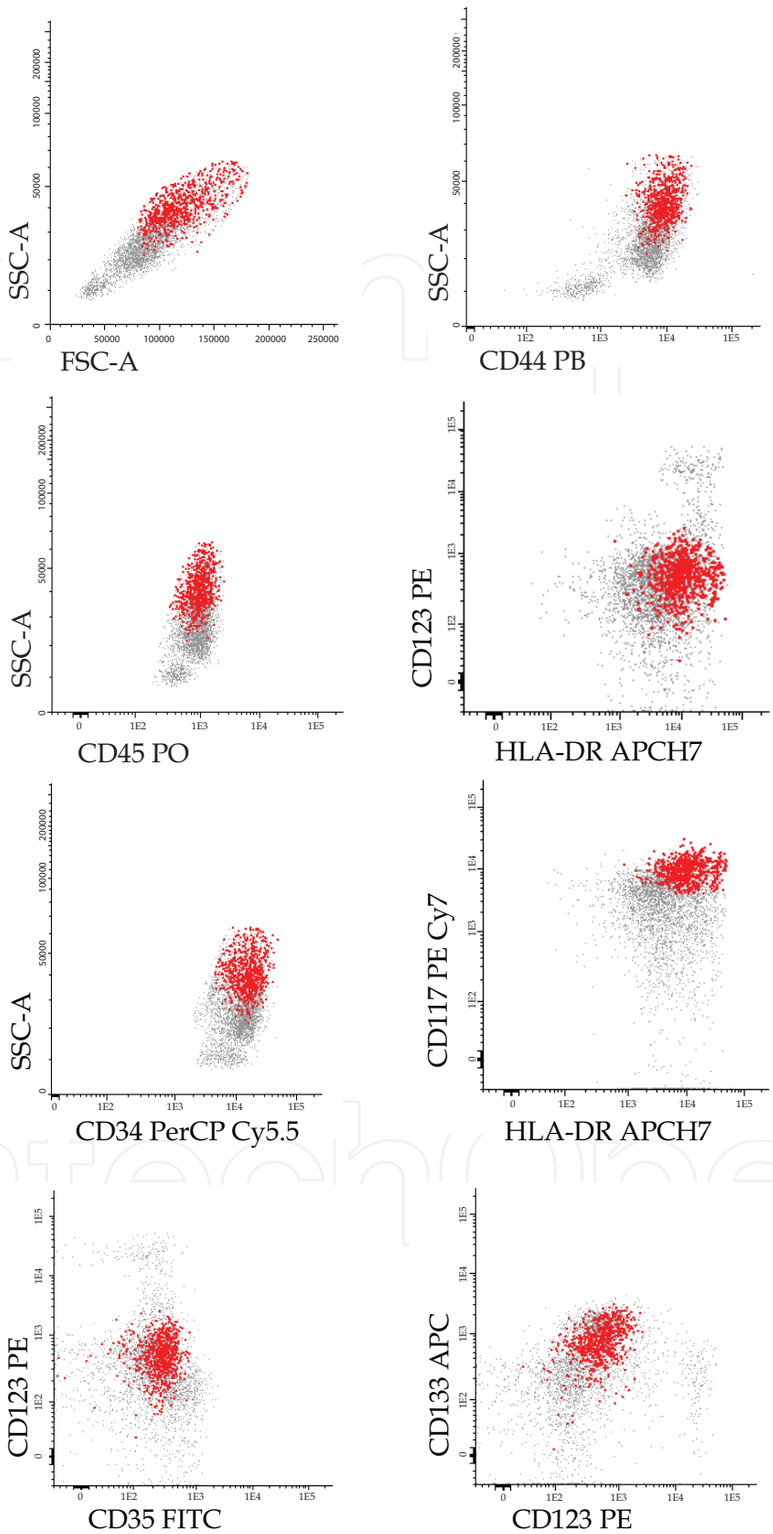

Fig. 6. Neutrophil-committed bone marrow CD34+ precursors (red) immunophenotype. The remaining bone marrow CD34+ cell compartments are presented in grey

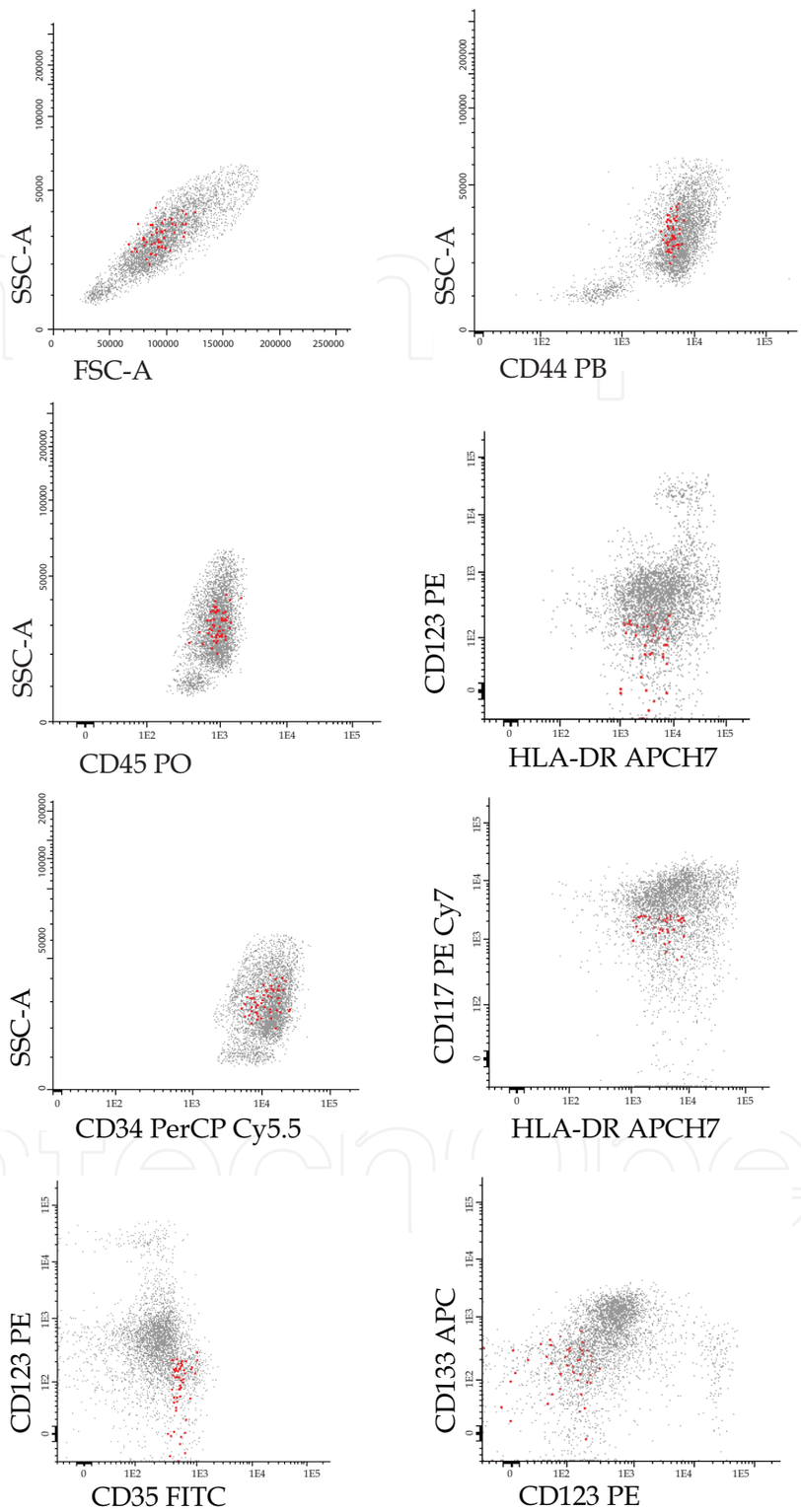

Fig. 7. Monocytic-committed bone marrow CD34+ precursors (red) immunophenotype. The remaining bone marrow CD34+ cell compartments are presented in grey

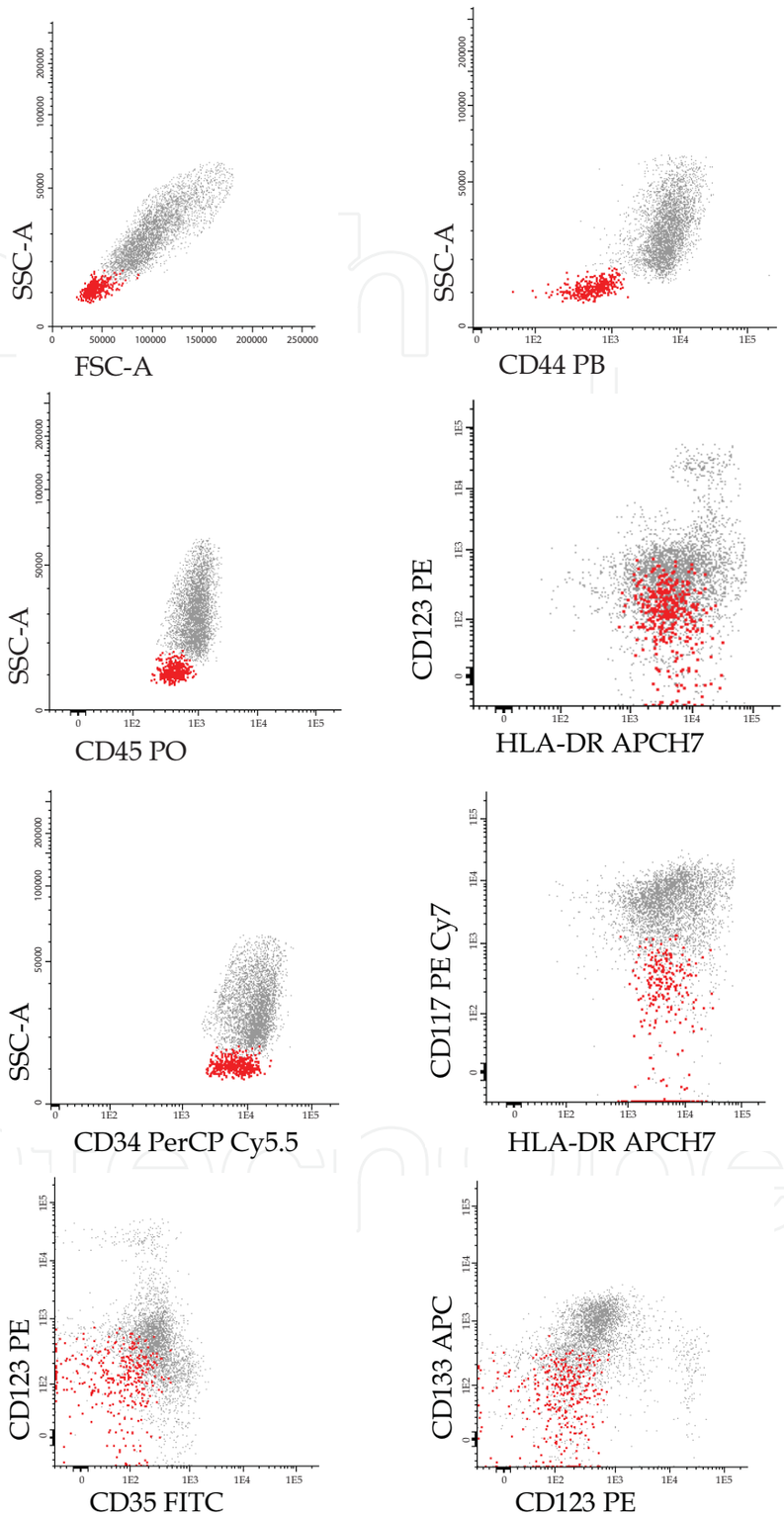

Fig. 8. B-cell-committed bone marrow CD34+ precursors (red) immunophenotype. The remaining bone marrow CD34+ cell compartments are presented in grey

6. Bone marrow CD34+ basophil precursors
   Our protocol allows the identification of basophil precursors using the classical markers and attending to the immunophenotype HLA-DR$^{-/dim}$/CD123$^{int/hi}$. Of note, this CD34+ subset presents the lowest expression of CD44 among all bone marrow myeloid CD34+ cells, being easy to differentiate this precursors from all the other myeloid precursors by using CD44 marker (Figure 9).

7. Bone marrow CD34+ plasmacytoid dendritic cell precursors
   The plasmacytoid dendritic cell precursors are identified using the classical markers, as being HLA-DR^{hi}/CD123$^{hi/int}$. The most immature forms of this precursor express CD133 (CD133^{int}). The immunophenotypic characteristics of this population are represented on Figure 10.

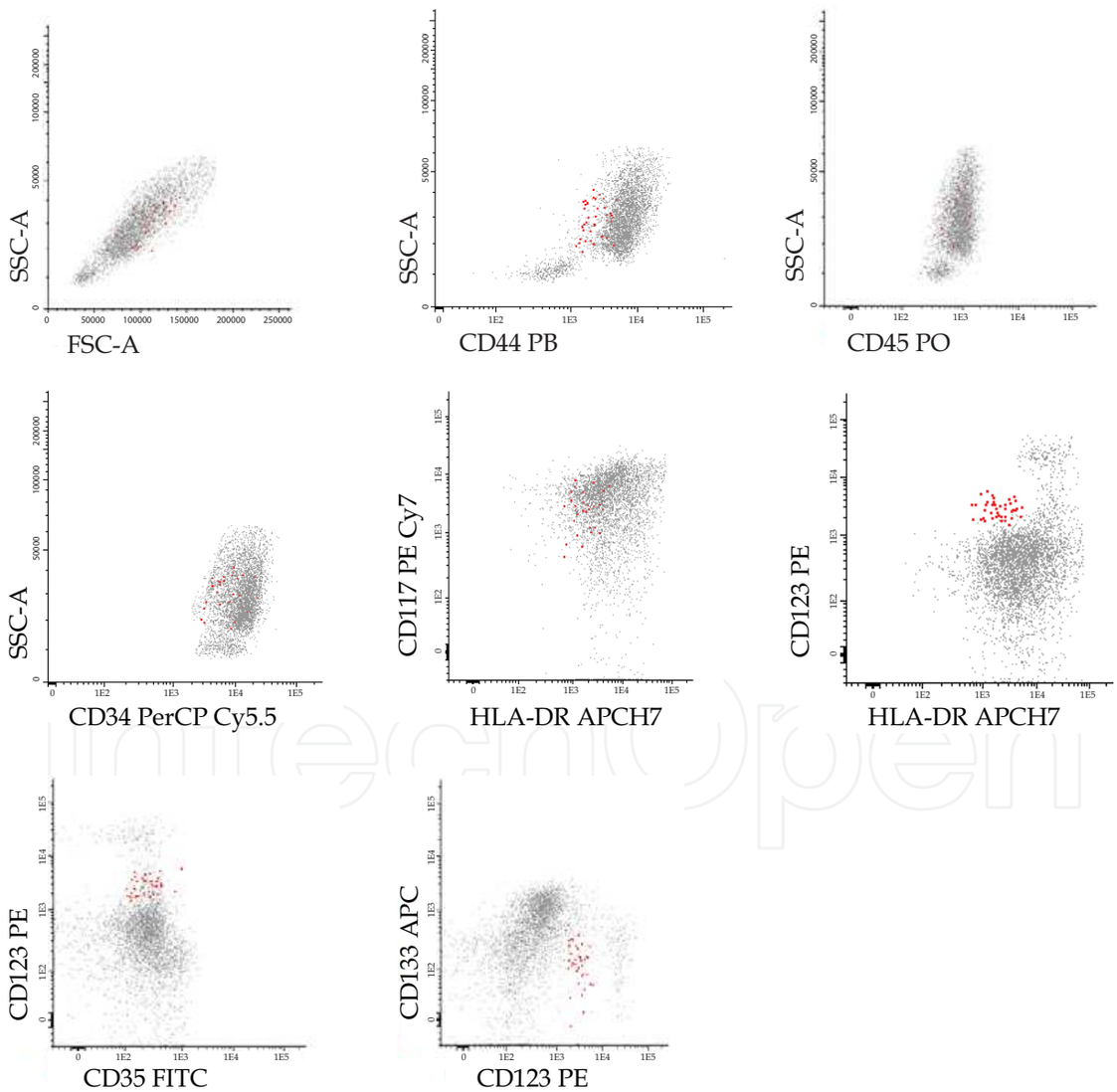

Fig. 9. Basophil-committed bone marrow CD34+ precursors (red) immunophenotype. The remaining bone marrow CD34+ cell compartments are presented in grey

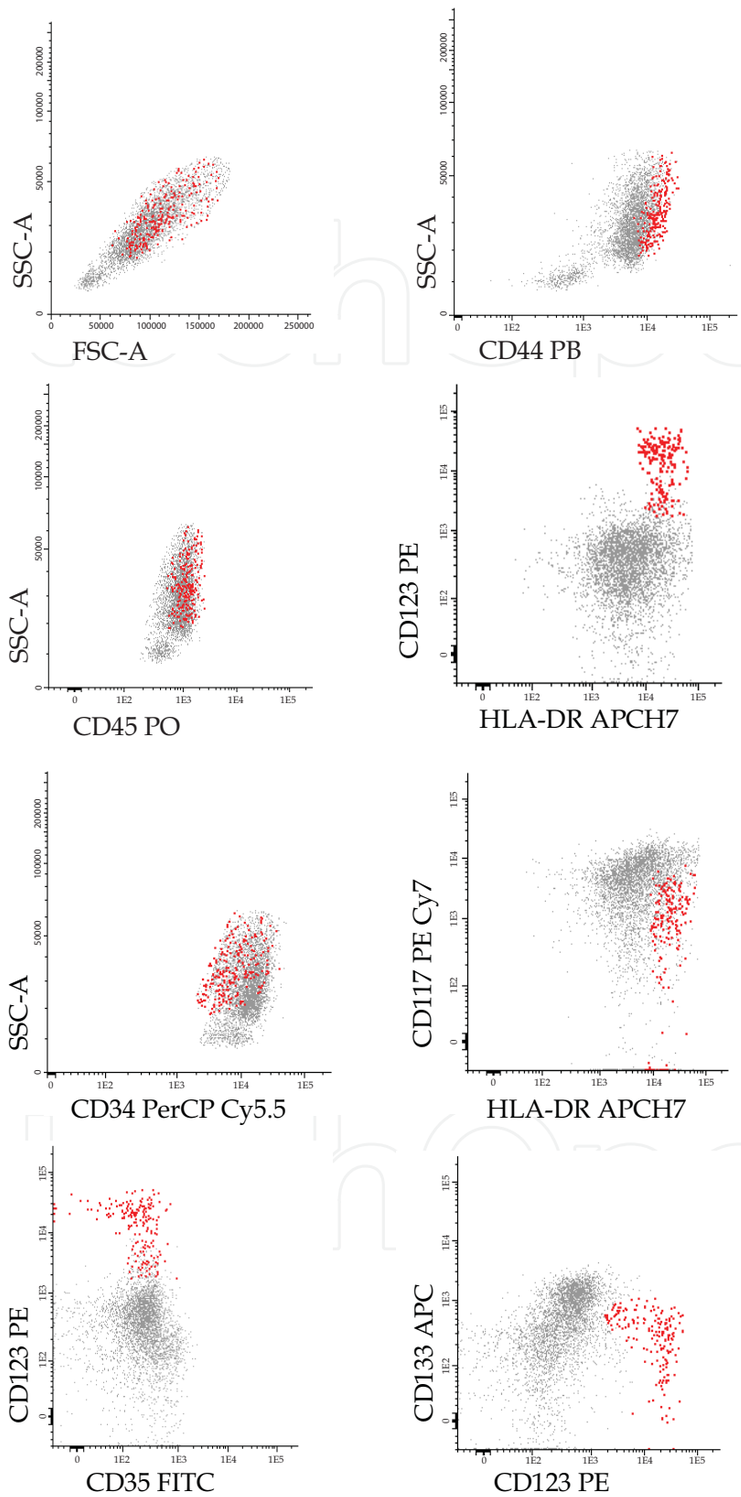

Fig. 10. Plasmacytoid dendritic cell-committed bone marrow CD34+ precursors (red) immunophenotype. The remaining bone marrow CD34+ cell compartments are presented in grey

8. Bone marrow CD34+ mast cell precursors
   The classical markers for the identification of CD34+ mast cell precursors are included in our protocol, and these cells are CD117^{hi}/HLA-DR$^{-/int}$. This subset expresses high levels of CD44. Other immunophenotypic characteristics of this subset are illustrated in Figure 11.

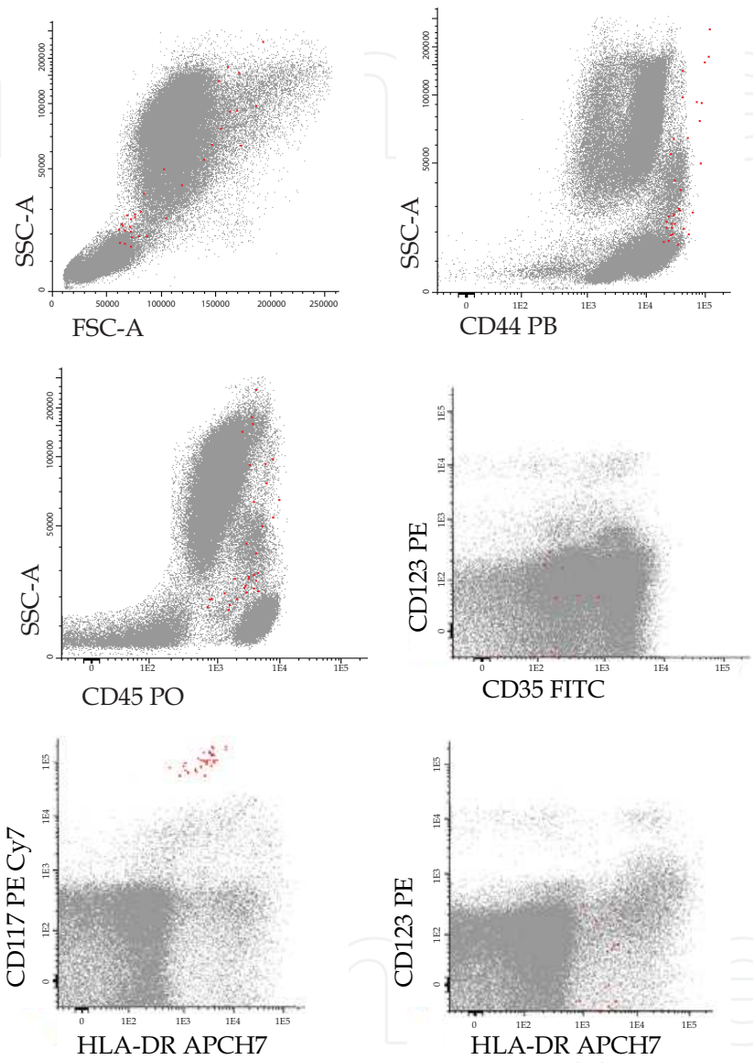

Fig. 11. Mast cell-committed bone marrow CD34+ precursors (red) immunophenotype. The events in grey correspond to remaining whole bone marrow nucleated cells

3.3 The maturation dynamic of bone marrow CD34+ hematopoietic stem cell

The possibility of a multiparameter analysis in a single cell basis conduct to a broader knowledge on the immunophenotypic characteristics of bone marrow CD34+ compartments and how it varies along the differentiation through different hematological cell lineages. Figure 12 depicts the dynamic of the maturation of different bone marrow CD34+ cell compartments.

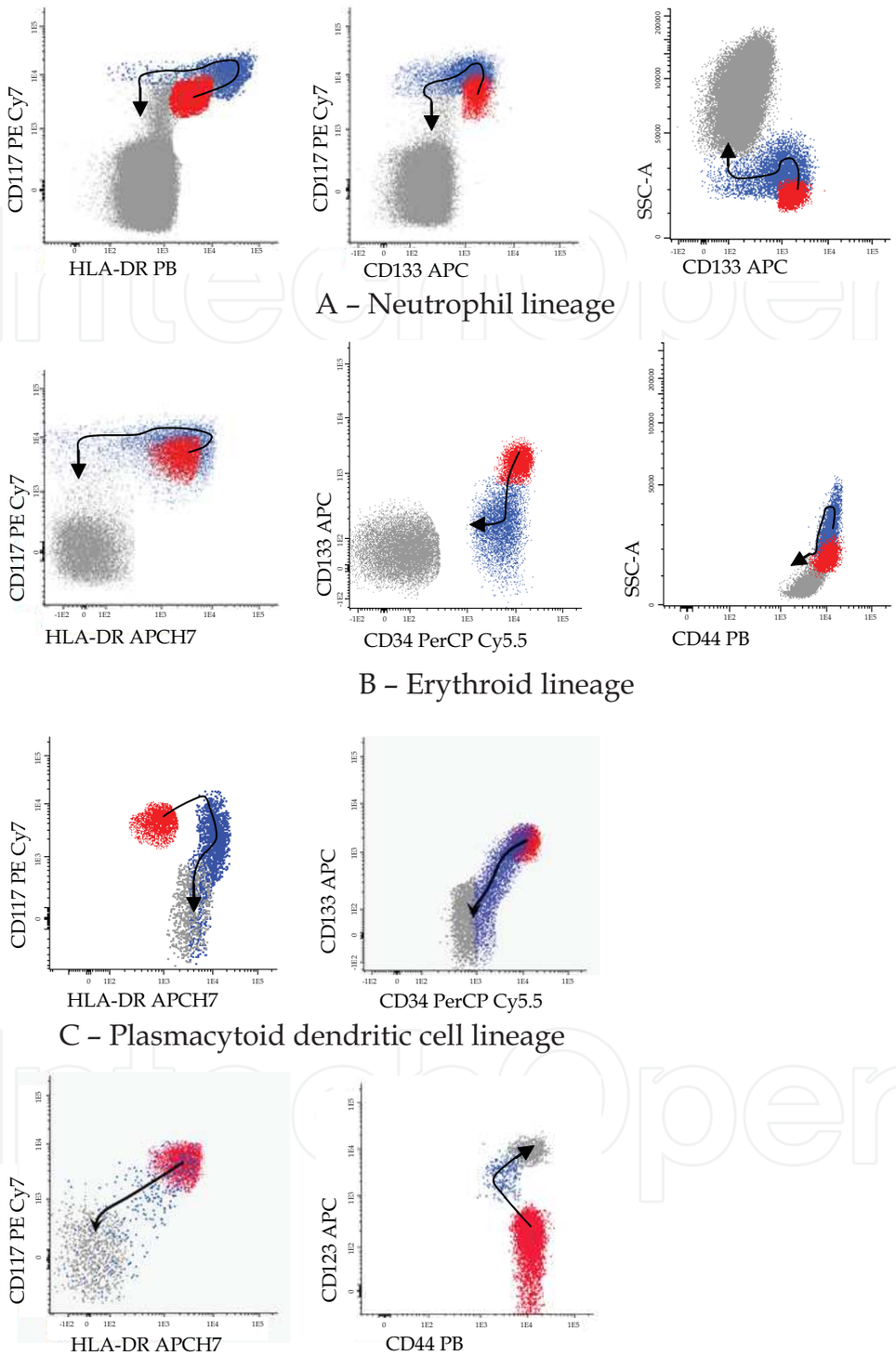

Fig. 12. Maturational dynamic of bone marrow CD34+ HSC. Uncommitted CD34+ cells are presented in red, lineage committed CD34+ cells are presented in blue and the lineage committed CD34- cells correspond to grey events

4. Conclusion

The emergence of high-speed multi-parameter flow cytometers have given an important contribute to unveil the phenotypic characteristics of minor cell populations and/or populations without a known specific cell marker.

Using flow cytometry to characterize bone marrow MSC directly (without in vitro cell culture) represents a great advantage by enabling an analysis closest to the physiologic conditions of the cells, excluding all the phenotypic alterations induced by factors present in the culture medium. Moreover, this direct analysis allows an accurate quantification of these cells in bone marrow. In addition, the strategy used for bone marrow can also be applied in MSC from other tissues, allowing their direct quantification and characterization.

A broader knowledge about the immunophenotypic characteristics of the different compartments of bone marrow HSC could improve their identification, allow a more accurate quantification of those compartments, as well as shed light on the protein expression patterns in the earliest stages of maturation of each hematological cell lineage. Furthermore, a better knowledge of those protein expression patterns might contribute to the development of new strategies to identify aberrant phenotypes in hematological diseases affecting the more immature bone marrow cells compartments, which can be helpful in the classification of acute leukemias, diagnosis of myelodysplastic syndromes and detection of minimal residual disease. A more extensive understanding of the phenotype of CD34+ hematopoietic stem cells in the different maturational stages could also be useful to monitoring and investigate if different mobilization regimens have the capability of mobilizing distinct CD34+ hematopoietic stem cells subpopulations.

Here, we presented a simple, quick and economic approach to identify and quantify the different bone marrow CD34+ HSC compartments.

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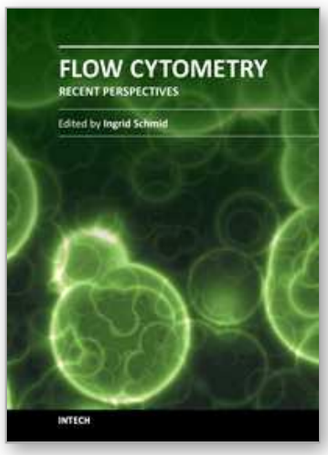

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"Flow Cytometry - Recent Perspectives" is a compendium of comprehensive reviews and original scientific papers. The contents illustrate the constantly evolving application of flow cytometry to a multitude of scientific fields and technologies as well as its broad use as demonstrated by the international composition of the contributing author group. The book focuses on the utilization of the technology in basic sciences and covers such diverse areas as marine and plant biology, microbiology, immunology, and biotechnology. It is hoped that it will give novices a valuable introduction to the field, but will also provide experienced flow cytometrists with novel insights and a better understanding of the subject.

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