THE CLOËTTA PRIZE 2017
IS AWARDED TO

PROFESSOR

MARKUS G. MANZ

BORN IN 1967 IN STUTTGART, GERMANY
DIRECTOR OF THE DEPARTMENT OF
HAEMATOLOGY AND ONCOLOGY OF THE UNIVERSITY
HOSPITAL OF ZURICH

FOR HIS GROUND-BREAKING CONTRIBUTIONS TO
UNDERSTANDING THE DEVELOPMENT OF DENDRITIC
CELLS ON THE BASIS OF BLOOD STEM CELLS

ZÜRICH, 3TH NOVEMBER 2017

IN THE NAME OF THE FOUNDATION BOARD:

THE PRESIDENT

THE VICE PRESIDENT

MEMBER

Markus G. Manz, M.D.

CURRICULUM VITAE & PUBLICATIONS

Position and Work Address

– Professor/Ordinarius of Hematology University of Zürich (UZH)
– Director Hematology University Hospital Zurich (USZ)
– Director a.i. Oncology USZ
– Director Department of Hematology and Oncology USZ
– Chairman Leukemia-, Lymphoma-, Myeloma-Center USZ

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Personal DOB 03.09.1967, married, 6 children

Education

1974–1987	Freie Waldorfschule, Stuttgart Uhlandshoehe, Germany
1989–1995	University of Tuebingen Medical School, Germany
1994	US Medical Licensing Examination Step II

Public Service

1987–1989	Civil service (alternative to mandatory military service) Karl-Olga-Krankenhaus, Stuttgart, Germany

Clinical Training

1995–1999	Resident Physician, Department of Medicine, and Department of Hematology/Oncology/Immunology University of Tuebingen Medical School, Germany
2004–2006	Clinical Fellow, Department of Hematology/Oncology/Immunology University of Tuebingen Medical School, Germany

Licensure and Board Qualification

2006	Internal Medicine, Germany/European Union
2007	Hematology and Oncology, Germany/European Union
2009	Internal Medicine license recognition Switzerland (FMH)
2009	Hematology license recognition Switzerland (FMH)

Research Training

1992–1996	Thesis (Dissertation): «Tumornecrosis factor ß Gen-Polymorphisms in myasthenia gravis» Department for Hematology/Transplantation Immunology, University of Tuebingen Medical School, Germany Advisor: Prof. Dr. Claudia A. Müller
1999–2002	Postdoctoral Research Fellow Department of Pathology and Developmental Biology Stanford University Medical School, USA Advisor: Prof. Dr. Irving L. Weissman

02/2007	Habilitation (Venia Legendi in Internal Medicine) University of Tuebingen Medical School, Germany

Academic and Hospital Appointments

2002–2004	Research Group Leader Institute for Research in Biomedicine (IRB) Bellinzona, Switzerland
04/2004–09/2006	Associate research group leader Institute for Research in Biomedicine (IRB) Bellinzona, Switzerland
3–9/2006	Physician, Department of Hematology/Oncology/ Immunology University of Tuebingen Medical School, Germany Chairman: Prof. Dr. Lothar Kanz
08/2006	Elected «primo loco» as Director (W3) of the «Department of Cellular Therapeutics and Research» at the Essen University Medical School, Germany (refused 10/2006)
10/2006–08/2009	Research Group Leader Institute for Research in Biomedicine (IRB) Bellinzona, Switzerland
11/2006–08/2009	Attending Department of Hematology, Oncology Institute of Southern Switzerland, Bellinzona Chairman: Prof. Dr. Franco Cavalli
02/2007–08/2009	Privatdozent/PD Medical Faculty, Eberhard-Karls-University Tuebingen, Germany
07/2008–08/2009	Vice-Director IRB, Bellinzona, Switzerland
09/2009–12/2011	Associate research group leader IRB, Bellinzona, Switzerland

09/2009–ongoing	Professor/Ordinarius of Hematology at the Medical Faculty and Director Hematology at the University and University Hospital of Zürich, Switzerland
11/2010–ongoing	Professor with «Promotionsrecht» at the Mathematical and Natural Sciences Faculty at the University of Zürich
01/2012–05/2017	Chairman Steering Committee Cancer Network Zürich (UZH/ETHZ)
06-2017–ongoing	Steering Committee Cancer Network Zürich (UZH/ETHZ)
08/2013–ongoing	Lead Clinical Research Priority Program UZH («Human Hemato-Lymph. Diseases»)
01/2014–ongoing	Director Leukemia-, Lymphoma-, Myeloma-Center USZ
02/2014–ongoing	Cancer Biology PhD Program Steering Committee Member (Life Sciences UZH/ETHZ)
2016–ongoing	Secretary Swiss Society of Hematology
06/2016	Elected “primo loco” as Director (W3) Hematology and Oncology at Charite University Medicine Berlin, Germany (declined 12/2016)
07/2016–ongoing	Director Department of Hematology and Oncology USZ
01/2017–ongoing	Director a.i. Oncology USZ

Honors and Awards

1999 Postdoctoral Research Fellowship, Deutsche Krebshilfe (https://www.krebshilfe.de/)

2002 Swissbridge Award (http://www.swissbridge.ch)

2004 Artur-Pappenheim-Award, German Society of Hematology and Oncology (DGHO; https://www.dgho.de)

2008 San Salvatore Foundation Award (http://www.fondazionesansalvatore.ch/)

2010 Ellermann Award Hematology 2009, Swiss Society of Hematology (SGH; http://www.sgh-ssh.ch/)

2016 Otto Naegeli Award (http://www.otto-naegeli-preis.ch/)

2017 Cloëtta Award (http://www.cloetta-foundation.ch/de/cloetta-preis/)

Professional Organizations and Societies

Swiss Society of Hematology (SGH, Secretary 2016–ongoing), Swiss Medical Association (FMH), Swiss Society for Allergology and Immunology (SSAI), German Society of Hematology and Oncology (DGHO), German Society of Internal Medicine (DGIM), European Society of Hematology (EHA, Sci Committee 2010–2013), European School of Hematology (Scientific Committee 2013–2015), American Society of Hematology (ASH), International Society of Experimental Hematology (ISEH), Henry Kunkel Society (HKS), Swiss Society of the Friends of the Weizmann Institute of Science (Board member), Charles Rodolphe Brupbacher Foundation (Sci Board), Swiss Stem Cell Network (SSCN), Cancer Network Zürich (CNZ)

Scientific Board Member

Swiss Stem Cell Network (SSCN, 2013–ongoing)
Charles Rodolphe Brupbacher Foundation (2011–ongoing)
Justus Müller Stiftung (2015–ongoing)
Swiss Soc. Friends of the Weizmann Institute of Science (2011–ongoing)
International Society of Experimental Hematology (ISEH, 2015–ongoing)
American Society of Hematology (ASH, 2015–ongoing)
Swiss Society of Hematology (SGH, 2016–ongoing)

Editorial Board Member

Blood Editorial Board (2013–2017)
Blood Advances Associate Editor (2016–ongoing)

Ad hoc Editorial Referee

Blood, Cell Stem Cell, European Journal of Immunology, Hematologica–The Hematology Journal, Immunity, Nature, Nature Immunology, Nature Medicine, Nature Biotechnology, PLoS Biology, Proceedings of the National Academy of Sciences, The Journal of Experimental Medicine, The Journal of Immunology

10 MOST IMPORTANT PUBLICATIONS

1. Takizawa H, Fritsch K, Kovtonyuk LV, Saito Y, Yakkala C, Jacobs K, Ahuja AK, Lopes M, Hausmann A, Hardt WD, Gomariz Á, Nombela-Arrieta C, *Manz MG.* Pathogen-Induced TLR4-TRIF Innate Immune Signaling in Hematopoietic Stem Cells Promotes Proliferation but Reduces Competitive Fitness.
Cell Stem Cell. 2017 Jul 19. pii: S1934-5909(17)30239-4. doi: 10.1016/j.stem.2017.06.013. [Epub ahead of print]

2. Boettcher S, Gerosa R, Radpour R, Bauer J, Ampenberger F, Heikenwalder M, Kopf M, *Manz MG.*
Endothelial cells translate pathogen signals into G-CSF-driven emergency granulopoiesis
Blood. 2014 Jul 2. pii: blood-2014-04-570762. [Epub ahead of print]

3. *Manz MG* and Boettcher S.
Emergency Granulopoiesis
Nature Rev Immunol. 2014 May;14(5):302-14. doi: 10.1038/nri3660. Epub 2014 Apr 22. Review.

4. Rongvaux A, Takizawa H, Strowig T, Willinger T,Eynon EE, Flavell RA, *Manz MG*.
Human Hemato-Lymphoid System Mice: Current Use and Future Potential for Medicine
Annu Rev Immunol. 2013 Jan 16. [Epub ahead of print]

5. Takizawa H, Regoes RR, Boddupalli CS, Bonhoeffer S, *Manz MG.*
Dynamic variation in cycling of hematopoietic stem cells in steady state and inflammation.
J Exp Med. 2011 Feb 14;208(2):273–84. Epub 2011 Feb 7.

6. Onai N, Obata-Onai A, Schmid MA, Ohteki T, Jarrossay D, *Manz MG.*
Identification of clonogenic common Flt3(+)M-CSFR(+) plasmacytoid and conventional dendritic cell progenitors in mouse bone marrow.
Nat Immunol. 2007 Nov;8(11):1207–16. Epub 2007 Oct 7.

7. Onai N, Obata-Onai A, Tussiwand R, Lanzavecchia A, *Manz MG.*
Activation of the Flt3 signal-transduction cascade rescues and enhances type-I interferon producing and dendritic cell development.
J Exp Med. 2006 Jan 23;203(1):227–38. Epub 2006 Jan 17.

8. Traggiai E, Chicha L, Mazzucchelli L, Bronz L, Piffaretti J-C, Lanzavecchia A, *Manz MG.*
Development of a Human Adaptive Immune System in Cord Blood Cell Transplanted mice
Science. 2004 Apr 2;304:104–107

9. *Manz MG*,* Miyamoto T*, Akashi K, Weissman IL.
Prospective isolation of human clonogenic common myeloid progenitors.
Proc Natl Acad Sci U S A. 2002 Sep 3;99(18):11872–7. *equal contribution

10. Traver D*, Akashi K*, *Manz M*,* Merad M, Miyamoto T, Engleman EG, Weissman IL.
Development of CD8alpha-positive dendritic cells from a common myeloid progenitor.
Science. 2000 Dec 15;290(5499):2152–4. *equal contribution

HEMATOPOIESIS – A PARADIGMATIC STEM CELL SUPPORTED ORGAN SYSTEM

Markus G. Manz

Summary

The hematopoietic system constitutes one of the largest organs in mammalian bodies. In adults, hematopoiesis is mostly confined to the bone marrow (BM) where red blood cells, platelets and white blood cells are continuously produced from rare, self-renewing hematopoietic stem cells (HSCs) and HSC-derived, highly-proliferative, increasingly lineage-restricted hematopoietic progenitor cells (HPCs). This system was shaped by evolution in a highly regulated and versatile manner, such as it is capable to respond to hemato-immunological stress by increasing its output to circulation from steady state to demand-adapted production of mature hematopoietic effector cells. Adaptability, however, implies risk for instability. Indeed, with ageing, hematopoiesis becomes increasingly frail, oligo- or monoclonal hematopoiesis develops in a substantial fraction of elderly humans, and clinically relevant hematopoietic alterations may occur, which are in worst cases hematopoietic insufficiency or development of HSC-derived leukemia. Hematopoiesis stands as a paradigmatic somatic stem cell-supported organ system, which is, due to its fluid character, highly accessible and scientifically testable. Understanding intrinsic and extrinsic regulation of hematopoiesis in physiology, during hemato-immunologic challenge, and during the process of ageing and malignant transformation will allow to better treat hematopoietic stem and progenitor cell (HSPC) diseases, the ultimate goal in our translational research efforts. In this review, I will focus on our own scientific contributions over the last one and a half decades to understand structure and regulation of mouse and human hematopoiesis in health and disease. These, naturally, are inspired by and connected to many prior and concurrent basic and clinical research achievements from colleagues all over the world.

Introduction

Blood as a renewable and fluid organ is fascinating. This fascination finds its expression in religion, philosophy, art and medicine alike. Since the introduction of blood donation and banking, blood is the only broadly shared, cellular product or "organ", saving millions of lives. Moreover, HSC donation and transplantation for inherited or malignant disease has become routine organ-donation practice. Remarkably, this organ-transplant does not require a scalpel to be successful as HSCs can mobilize from BM to circulation and find their way "home" to bone marrow upon infusion in circulation due to selective adhesion molecule interactions. Moreover, donors re-grow HSCs upon donation, and, likely due to these facts, more volunteer donors have registered than there are recipients in need. Blood is thus connecting humans. However, it also carries critical elements of immune distinction, i.e. the white blood cells that are able to dissect self from non-self.

In this brief piece, I will focus on describing and summarizing our own work (and my fascination) on blood and bone marrow as well as immunology research and thus will mostly cite our own primary and review articles. For a broader and more balanced view on this fast evolving field, I would like to refer to the reference sections of the here cited published work.

Metrics and structural organization of bone marrow

Bone marrow (BM) accounts for 4–5 % of body weight and thus exceeds other organs as brain (2 %), liver (2–3 %) or heart (0.5 %) and lymphoid tissue (1.5 %). BM generates the highest number of cells per day (4–5x10E11 cells in humans, 0.5–1.5x10E6 in mice), outnumbering cellular production of intestinal epithelium or testis. In fact, BM produces about half of its own cellular content per day and, at any given time, about 90 % of total cell numbers in the organism originates from or resides in BM, although only a fraction of these cells are nucleated. In sharp contrast, HSCs only account for a very small fraction of bone marrow hematopoietic cells, i.e. 0.001–0.2 % (about 28–112x10E6 in humans and 11 2000–22 400 in mice) and HSPCs together account for less than 2 % of BM nucleated cells, indicating impressively their replicative and burst forming potential. Table 1 and 2 summarize representative parameters of

BM function and cellularity in humans and mice (Nombela-Arrieta and Manz, 2017; Sender et al., 2016).

Table 1. Representative parameters of BM function in humans and mice

Parameters		Humans	Mice
Total weight of BM (relative to body mass)		4–5 %	1.5–2 %
Weight in standard individual		3 kg	0.4–0.5 g
Total volume BM		1100 cm^3–2000 cm^3	0.35–0.4 cm^3
Total volume blood		70–80 ml/kg	85–95 ml/kg
Blood volume in the BM (% of tissue volume)		N.D	15–30 %
Ratio cortical/ trabecular bone		80:20	65:35
Nucleated BM cells/kg body weight		1.1–2.1 x 10^{10}	1.1–1.8 x 10^{10}
Total nucleated BM cells		0.8–1.5 x 10^{12}	2.8–5.2 x 10^8
Cell production/ day	Total	4–5 x 10^{11} (5–7 x 10^{10}cells/kg)	0.5–1.5 x 10^9 (2–6 x 10^{10}cells/kg)
	RBCs	2.2 x10^{11}	0.45–0.85 x10^9 **
	Platelets	1–1.75 x 10^{11}	0.16–0.7 10^9 **
	Neutrophils	0.45–1.2 x10^{11}	0.7–1.9 x10^7
Major BM cavities (% of total BM content)		Lower Limbs 26–38 % Vertebrae 14–28 % Pelvis 16 %	Vertebrae 32–52 % Lower Limbs 14–20 % Pelvis 25 %
BM adipogenesis (% of BM volume)		50–70 %	1–2 % in 12 week old 18–20 % in 56 week old (measured in proximal tibial metaphysis)
Number of HSCs (functional definition)		28–112 x 10^6*	11200–22400
Myeloid:erythroid ratio		3:1	1.7:1–2.3:1

*Values depicted have been in most cases obtained for, or adjusted to a reference standard individual of 70 kg for humans and 25 g for mice.*Reliable estimations of total HSC numbers in humans are lacking to date. As depicted, for most parameters presented, including weight and blood cell production, the ratio of values between mouse and human species approximately falls with a range of 2500–5000. The theoretical number of HSCs for the human system provided in this table has been estimated assuming that similar ratios would apply for proportional scaling of HSC content between species.*
Table adapted from: Nombela-Arrieta and Manz; Blood Advances, Feb 2017, Volume 1, Number 6

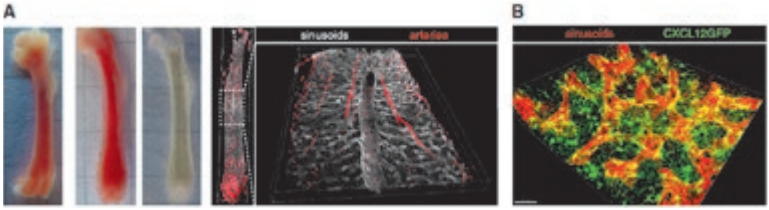
Table 2. Cellular content of the BM as determined by flow cytometry

	Humans		**Mice**	
Populations	**Phenotype**	**Freq. of BMMNC (%)**	**Phenotype**	**Freq. of BMMNC (%)**
HSCs	$Lin^-CD34^+CD38^-$ $CD90^+CD45RA^-$	0.01–0.2	Lin^-c-kit+Sca-1+(LSK) $CD48^-CD150^+$	0.004–0.007
Common Myeloid progenitors	$Lin^-CD34^+CD38^-$ $CD123^+CD45RA^-$	0.2–0.8	Lin^-c-kit+ $CD34^+CD16/32lo$	0.4
Common lymphoid progenitors	$Lin^-CD34^+CD38^+$ $CD127^+$	0.02–0.18	Lin^-c-kit+$CD127^+$	0.02
Erythroid progenitors	$CD235^+CD71^+$	4–16	$Ter119^+$	5–15
Granulocytes	$CD66^+$	57–86	$Gr\text{-}1^+$	30-50
Megakaryocytes	*	0.05	$CD41^+$	0.29
Monocytes	$CD14^+$	2–5.8	$CD115^+$ $C^-11b^+Ly6C^+$	5–7
B cell lineage (including progenitors)	$CD19^+$	1–7	B220	35–45
T lymphocytes	$CD45^+CD66^-$ $CD34^-CD14^-$	8–20	$CD3^+$ $CD8^+$	4–5 2
Endothelial cells	$CD45^-$ Ter119-$CD31^+$	N.D	$CD45^-Ter119^-$ $CD31^+$	0.03–0.4
Fibroblastic reticular stromal cells	$CD146^+$ $CD271^+$ $CD146^+CD51^+$ $CD140a^+$	0.02–0.08 0.03 0.1	$CD45^-Ter119$-CXCL12GFP$^+$ $CD45^-Ter119$-$CD140^+LepR^+$	0.02–0.04 0.3

Estimated ranges of frequencies of different hematopoietic and non-hematopoietic populations in human and mouse BM expressed as percentages of nucleated BM cells.
** Estimation based on microscopic examination of BM, not flow cytometry*
Table adapted from: Nombela-Arrieta and Manz; Blood Advances, Feb 2017, Volume 1, Number 6

In contrast to other organs, spacial organization of BM tissues and relation of this to confined domains of blood formation is only being explored in recent years with the availability of three-dimensional tissue imaging. While mapping of BM is an ongoing effort, it already became clear that there are no clear macro-domains identifiable, but rather the BM is organized in microdomains where few hematopoietic and stromal cells (as vasculature and other mesenchymal cells) built "niches", as HSC-niches, erythroblastic-niches or plasmacell-niches that nurture the respective cell types. Figure 1 shows a resolution of mouse BM and Figure 2 gives a schematic insight of various BM niche organizations.

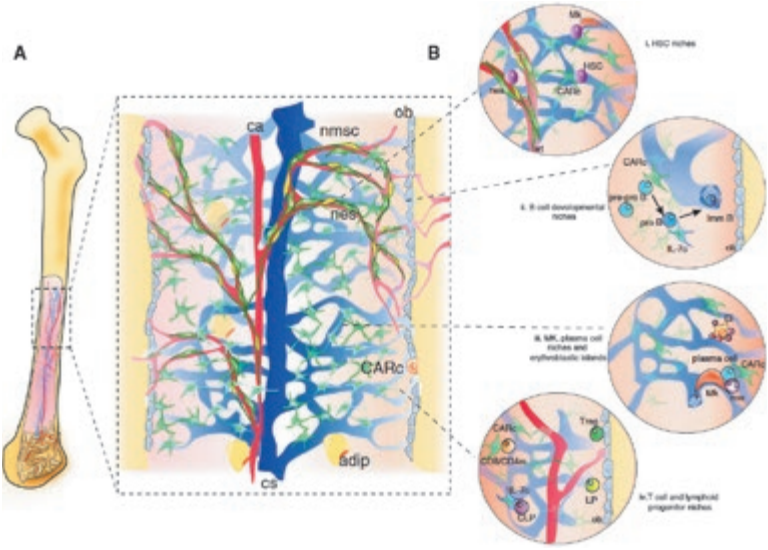
Figure 1. Three-dimensional imaging of BM



(A) Representative stages of murine femoral bones during processing for 3D imaging. From left to right: unsectioned, sliced unprocessed and optically cleared BM slices. The last 2 images to the right correspond to an example of 3D reconstruction of the entire BM microvascular system of the femur (arteries and sinusoids) using multidimensional confocal imaging of a cleared BM slice. A detailed view of the central sinus running along the longitudinal axis of the marrow is provided. (B) High-resolution 3D imaging of a reduced field of view showing sinusoidal vessels (red) and the network of perivascular bodies of CAR cells (green), forming a dense matrix through the emission of abundant cytoplasmic projections.

Figure adapted from: Nombela-Arrieta and Manz; Blood Advances, Feb 2017, Volume 1, Number 6.

Figure 2. Structural organization of BM tissues



(A) BM stromal microarchitecture. The microarchitecture of the vascular system has been studied in detail in the mouse femoral cavity. A central artery (ca) penetrates the cavity through the nutrient canal and splits into ascending and descending branches, which run longitudinally, arborizing into smaller radial arteries that migrate toward endosteal regions. In the proximity of endosteum, arteries give rise to a dense plexus of arterioles that travel along the cortical bone area and eventually develop into venous vessels, of fenestrated endothelium, termed sinusoidal. Sinusoids form a labyrinth that extends inward and merges in a big central collecting sinus (cs) that drains into peripheral circulation (see also Figure 1). Thin periosteal arteries also penetrate the bone and merge with arteriolar vessels in endosteal regions, connecting BM and bone circulation. In trabecular bone areas, multiple smaller arteries enter the marrow cavities and give rise to sinusoidal networks along the endosteal surface of trabeculae. CXCL12-abundant fibroblastic reticular cells (CARc) extend throughout the entire cavity in the form of dense networks. Nestin-GFPhi NG21 (nes) elongated cells run adjacent to arteries and arterioles and bundles of nonmyelinating Schwann cells (nmsc), which ensheath adrenergic nerves. Mature osteoblasts (ob) line endosteal surfaces and are mostly derived from reticular progenitors of osetoadipogenic potential. The emergence of adipocytes (adip) takes place gradually during aging and can be abrupt and prominent in certain pathological conditions. (B) Schematic representation of cell or developmental stage-specific niches. (i) HSC niches: a number of studies have determined that HSCs are found scattered throughout BM tissues adjoining the extraluminal surface of sinusoidal endothelial cells and in contact with stem cell factor–producing, LepR1 CAR cells and, in some cases, to megakaryocytes. In addition, a

minor fraction enriched in quiescent HSCs has been reported to localize in a protective niche adjacent to nonpermeable arterioles, under the regulatory influence of neighboring Nestin-GFPhi, NG2 mesenchymal cells (nes) and nonmyelinating Schwann cells (in yellow). (ii) B-cell progenitors have been suggested to sequentially migrate along different niches as they progress through maturation. Early stage pre-pro B cells are mostly found adjacent to the cell bodies of CAR cells and migrate toward IL-7–expressing CAR cells as they enter the pro B cell stage. Mature B220IgM B cells, in turn, move away from both stromal cell types. The latest stages of B-cell maturation occur intravascularly, within sinusoids, where the dynamic behavior of immature B cells has been visualized in vivo. In addition, early pro B cells (B220CD43) have been shown to accumulate in endosteal zones and gradually decrease in frequency toward bone-distal marrow regions. (iii) Megakaryocytes (Mk) are found in close adjacency to the endothelial surface of sinusoidal vessel wall, which they traverse in the form of protrusions from which proplatelets are continuously shedded into the venous circulatory system. Red blood cell development takes place in so-called erythoblastic islands (Ei), where erythroid precursors proliferate, enucleate, and terminally differentiate into reticulocytes around a central macrophage.108 Plasma B cells are long-lived antibody-secreting cells that have been found in physical association to CAR cells, megakaryoctes, and eosinophils (eos). (iv) Although a significant fraction (30 %) of early lymphoid progenitors (Lin-IL7-ra) has been shown to lie proximal to mature, bone-lining osteoblasts, the vast majority of phenotypically defined CLPs are in contact with IL-7–expressing CAR cells. Quiescent CD4 memory T cells are found scattered throughout the BM in contact with perisinusoidal IL-7–secreting stromal cells. Regulatory T cells (Tregs) have been reported to lodge in close proximity to the endosteum of trabecular bone.

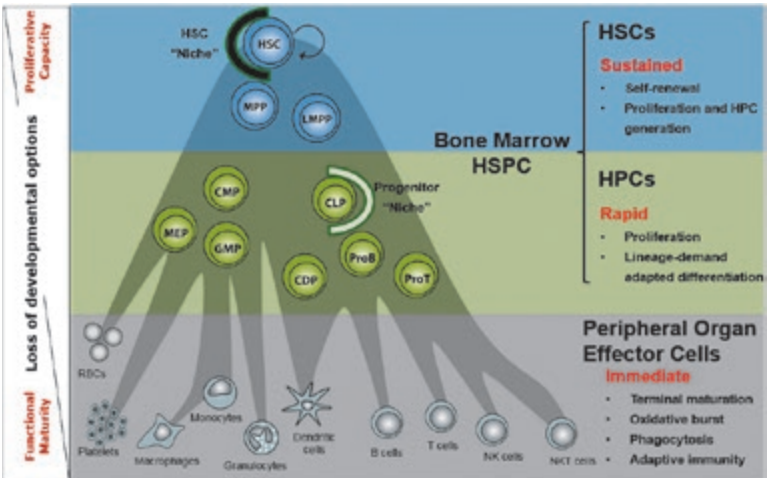
Figure adapted from: Nombela-Arrieta and Manz; Blood Advances, Feb 2017, Volume 1, Number 6

Hierarchical Organization of Hematopoiesis

Hematopoiesis is organized in a hierarchically (Kondo et al., 2001). HSCs stand at the top of the hierarchy, are defined by their capacity to life-long self-renew, and give rise to all mature blood cells. Blood cell production from HSCs occurs in a stepwise process, where HSCs differentiate to HPCs that still have the capacity to produce all blood lineages but that have lost long-term self-renewal capacity. These HPCs then commit via highly proliferative developmental intermediates, increasingly restricted progenitor cells to end-stage differentiated blood cells. Most of these blood cells, with the exception of some lymphocytes and tissue resident phagocytic cells, are post-mitotic and can only be maintained during life by continuous active hematopoiesis from precursors.

With the development of monoclonal antibody technologies and subsequent antibody labeling of cell surface antigens, and with the development of fluorescence associated cell sorting (FACS), it became feasible to isolate rare populations of individual live cells, which subsequently could be molecularly and functionally characterized. This allowed first a population based, and now an increasingly high-resolution single cell, clonal mapping of the hematopoietic tree. A rough, principle model of hematopoiesis is depicted in Figure 3.

Figure 3. Schematic model of hematopoiesis



Schematic model of hematopoiesis depicting the hematopoietic hierarchy and the qualities of cells in the respective developmental sections. HSC, hematopoietic stem cell; HSPC = hematopoietic stem / progenitor cell; MPP = multipotent progenitor; LMPP = lympho-myeloid progenitor; CMP = common myeloid progenitor; MEP = megakaryocyte erythrocyte progenitor; GMP = granulocyte macrophage progenitor; CLP = common lymphoid progenitor; CDP = common dendritic cell progenitor; ProB = pro-B cell; ProT = pro-T cell. Figure adapted from: M.G. Manz, Swiss Medical Forum, 16(18–19); 414–418; 2016

While there is currently agreement on the principle hierarchy, there are still open questions with regard to some of the direct clonal relationships, the existence of additional progenitor populations, as well as the essential versus possible developmental steps in steady state and during hemato-immunological challenges.

We were able to characterize on a clonal level the human counterparts of the initially in mice identified myeloid committed progenitor cells (Manz et al., 2002). In addition, we were able to identify common dendritic cell progenitors in mice on a clonal level and we were able to dissect critical cytokine and signaling pathways that guide dendritic cell development and homeostasis from the earliest progenitors to steady-state mature cells (discussed below).

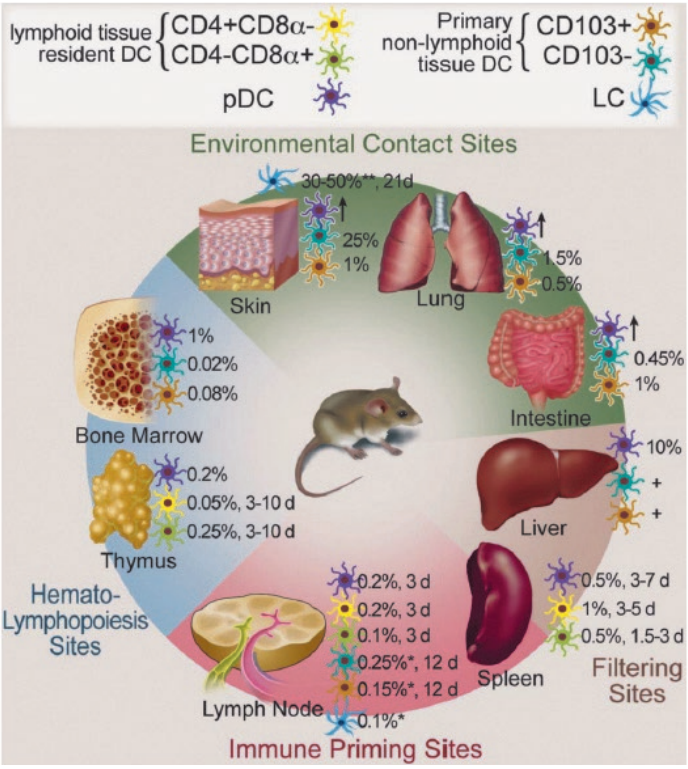
Why is this of relevance? Understanding the fine-tuned hematopoietic hierarchy allows to molecularly interfere, i.e. to support hematopoietic lineage development (e.g. by cytokine or small molecule application as e.g. HSPC-, erythropoiesis-, granulopoiesis-, thrombopoiesis-stimulating agents), or to mobilize HPSCs (e.g. chemokine receptor antagonists). Using this approach, we were able to e.g. show that thrombopoietin-agonists but not granulopoiesis stimulating agents are able to expand HSCs, at least in mice (Kovtonyuk et al., 2016b). In addition, deciphering healthy hematopoiesis helps to characterize the malignant transformed cell counterparts, and to ideally selectively interfere with their molecular machinery (e.g. using fitted kinase inhibitors). Using this approach, we were e.g. able to identify stages and pathways in aggressive transformation of chronic myeloid leukemia (Jamieson et al., 2004).

Dendritic Cell Development from early Hematopoietic Precursor Cells

Dendritic cells (DCs) are hematopoietic-derived, professional antigen presenting cells, key for the guidance of adaptive immunity (Merad and Manz, 2009). DCs are rare cells, comprised of multiple sub-specialized populations that are present everywhere in the body, with a relatively increased frequency at environmental contact sites as skin, lung and gut, and at sites of adaptive immune cell programming, i.e. primary and secondary lymphoid organs. DCs sample and process antigen and move from non-lymphoid to lymphoid tissues to fulfill their effector functions

(one key distinction from organ-resident macrophages). In addition, some DCs are primarily present at lymphoid organs. Figure 4 provides an overview on DC populations and turnover in mice.

Figure 4. Mouse DC populations, location, and turnover in steady state

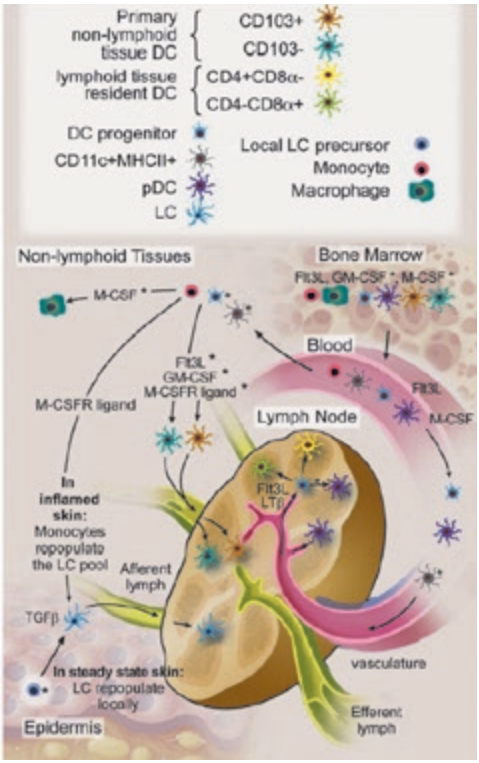


*DCs are distributed throughout the body. The major DC subpopulations at hematopoietic sites, environmental contact sites, filtering sites, and immune priming sites are depicted. Frequencies are given as percentage of total nucleated hematopoietic cells. Time to approximately 50% renewal in steady state is given in days (d). *Skin-draining LN; **epidermis; +present, but exact numbers not known;→ present in inflammation. Professional illustration by Debra T. Dartez.*

Figure adapted from: M. Merad and M.G. Manz, Blood, Volume 113, Number 15, 9 April 2009.

With the isolation of hematopoietic progenitor cells, it became possible to dissect DC developmental pathways. In *in vitro* and *in vivo* experiments, we were able to show that DCs surprisingly develop from both lymphoid and myeloid restricted progenitors (Manz et al., 2001; Chicha et al., 2004; Traver et al., 2000) and that the capacity to develop to DCs in myeloid and lymphoid populations is confined to cells expressing the receptor tyrosine kinase Flt3 (Karsunky et al., 2003). Indeed, we demonstrated that DC-development incompetent progenitors can be instructed to develop to DCs by artificially expressing and stimulating Flt3 (Onai et al., 2006). Moreover, by isolating hematopoietic progenitors on the basis of Flt3 and M-CSF receptor expression, we were able to identify and characterize committed clonal dendritic progenitor cells (CDPs) in mouse bone marrow (Onai et al., 2007), which was confirmed and extended by co-investigating groups in the field (reviewed in [Geissmann et al., 2010; Merad and Manz, 2009]). In accordance with the important role of Flt3-signaling, we found that orally applied small molecule Flt3 inhibitors generate a lymphoid tissue DC depleted phenotype within ten days of application *in vivo* (Tussiwand et al., 2005). Thus, Flt3-ligand is important in steady-state lymphoid tissue DC development and homeostasis (reviewed in [Geissmann et al., 2010; Merad and Manz, 2009; Schmid et al., 2010]). The role of cytokines, progenitors and migration is schematically depicted in Figure 5.

Figure 5. DC migration and homeostasis

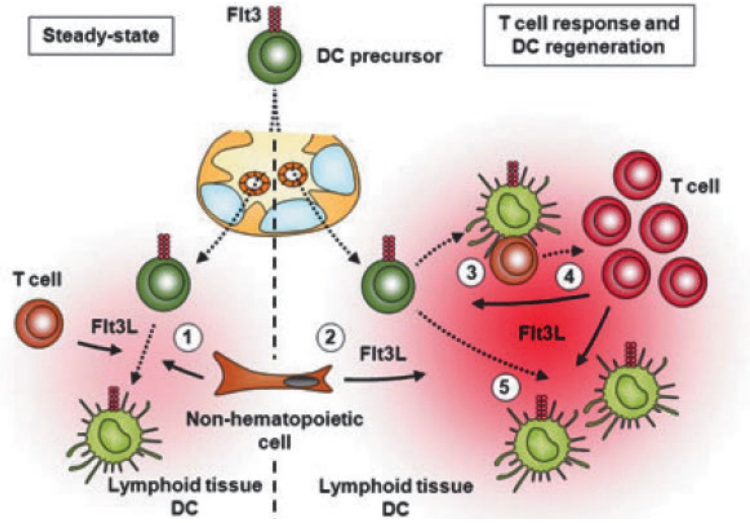


*HSCs produce DC progenitors, pDCs, and DCs in the BM. Flt3 ligand is a nonredundant cytokine for BM DC differentiation, although the exact role of GM-CSF and M-CSFR ligands remains to be determined. BM-derived circulating blood cells maintain, with the exception of epidermal LCs, all known steady-state DC homeostasis in lymphoid and nonlymphoid tissues. We hypothesize that progenitor cells with limited proliferation potential on Flt3 ligand and LT stimulation enter the LNs through high endothelial venules to maintain the majority of LN DCs in steady state. It is also possible that nonproliferating blood DCs follow the same route. In addition, nonlymphoid tissue DCs continuously enter the LNs through afferent lymphatics, but these represent only a minority of steady-state LN DCs. The specific contribution of proliferating DC progenitors, blood DCs, and monocytes to nonlymphoid tissue DCs in the steady state and the relative involvement of cytokines as Flt3 ligand, GM-CSF, and M-CSFR ligands remain to be to be addressed. In contrast to most DCs, LCs repopulate locally in the steady state either through self-renewal or through a local hematopoietic precursor that takes residence in the skin. In inflamed skin, monocytes repopulate the LC pool via a TGF-b and monocyte colony-stimulating factor receptor–dependent pathway. In the steady state, pDCs are recruited to the LN and other lymphoid organs directly from the blood and, with the exception of the liver, enter most nonlymphoid tissues only on inflammation. Whether lymphoid organ pDCs also derive from DC precursors that enter the organs remains to be determined. *Likely, but not formally proven. Professional illustration by Debra T. Dartez.*

Figure adapted from: M. Merad and M.G. Manz, Blood, Volume 113, Number 15, 9 April 2009.

We subsequently investigated which cells are relevant in Flt3-ligand production in DC homeostasis. Interestingly, we found that Flt3-ligand mediated lymphoid tissue DC maintenance is regulated in two ways, through Flt3-ligand production by non-hematopoietic cells and consumption by Flt3-expessing hematopoietic cells, as well as by antigen-mediated T-cell proliferation and Flt3-ligand secretion of these proliferating cells in adaptive immune responses, thereby stimulating local DC replenishment at lymphoid tissue inflammation sites (Saito et al., 2013). This is depicted schematically in Figure 6. Also, we found that DC progenitors from bone marrow are selectively recruited to inflamed lymph nodes via pattern-recognition receptor activation on progenitor cells (Schmid et al., 2011).

Figure 6. Flt3L produced by proliferating T cells contribute to proliferation of cDCs and their precursors in LNs in vivo

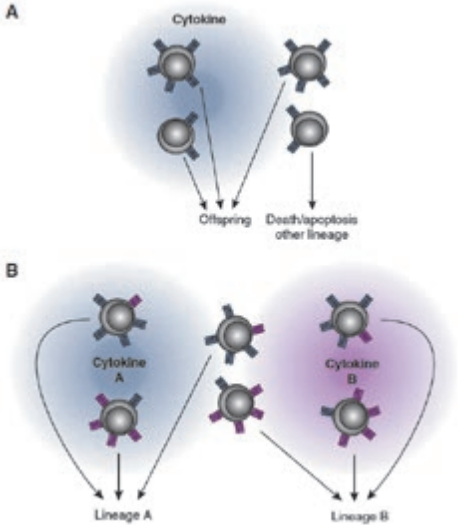


Model for the maintenance of lymphoid tissue DCs by Flt3 ligand. (1) In steady state, Flt3L produced by both nonhematopoietic and hematopoietic cells (mainly T cells) supports lymphoid tissue DC development. (2) During the course of a local immune response, in addition to the production of Flt3L by nonhematopoietic cells, (3) T cells are activated by antigen-presenting DCs, proliferate, and (4) produce overall more Flt3L than in steady state, and thereby (5) enhance local replenishment of lymphoid tissue DCs and their precursors.

Figure adapted from: Y. Saito et al., Eur. J. Immunol. 43:1651–1658. 2013

As with other hematopoietic lineage development, mature DC subtype development is driven by both progenitor cell intrinsic permissive- and responsiveness as well as extrinsic, environmental delivered signals. Consequently, the combination of cytokine concentration and receptor expression determines whether a progenitor receives a sufficiently high signal to differentiate to a particular lineage. This can be achieved in two situations: First, if a high cytokine concentration is available to progenitors (Fig. 7A, left), they will develop irrespective of high or low expression of the respective receptor. This abundance of cytokine will occur at sites or niches where commitment and differentiation typically takes place. Second, if only small amounts of cytokine are available (Fig. 7A, right), progenitors with high receptor expression will still receive sufficient amounts of positive signal to differentiate and give rise to offspring of the corresponding lineage. However, progenitors with low receptor expression will undergo apoptosis or develop into alternative lineages if they are responsive to alternative signals. Usually, more than one cytokine will act in concert on a cell in vivo. Progenitors, which carry the potential to differentiate into two lineages, will express cytokine receptors relevant for both of these. In Fig. 7B, a cell expressing high levels of the receptor for cytokine A and low levels of the receptor for cytokine B (upper cells) and a cell expressing low receptor A and high receptor B (lower cells) are shown to be situated in an environment with overlapping concentrations of these cytokines. When both cells are located in a milieu with high concentrations of cytokine A (Fig. 7B, left), they would differentiate to lineage A, whereas they would both give offspring to cells of lineage B in a milieu with high concentrations of cytokine B (Fig. 7B, right). In the region with overlapping amounts of cytokines A and B (Fig. 7B, center), cells with high expression of receptor A would differentiate to the A lineage, whereas cells with high expression of receptor B would generate cells of lineage B (Schmid et al., 2010).

Figure 7. Hypothesis for cytokine-induced differentiation of progenitors



(A) One cytokine, one receptor scenario: If two progenitors, one with high receptor expression, the other with low receptor expression, are located near high concentrations of the respective cytokine, both cells will acquire sufficient signal to differentiate and give rise to offspring (left). If both cells are exposed to low concentrations of the cytokine, only the cell with high receptor expression will receive sufficient signaling to differentiate, while the cell with low receptor expression will not and will either undergo apoptosis or differentiate into another lineage (right) if it receives signaling through an alternate receptor (not pictured). (B) Multiple cytokines/receptors scenario. As in (A), if two progenitors, one being receptor Ahigh receptor Blow, the other receptor Alow receptor Bhigh, are exposed to high concentrations of cytokine A, both will differentiate into lineage A (left), or conversely, if both progenitors are near very high levels of cytokine B, both cells will give rise to cells from lineage B (right). If both progenitors are exposed to only low concentrations of both cytokine A and cytokine B, the cell expressing receptor Ahigh receptor Blow will preferentially differentiate into lineage A, while the Alow receptor Bhigh cell will differentiate into lineage B.

Figure adapted from: M.A. Schmid et al., Immunological Reviews 234, 2010.

Hematopoietic Stem Cell Cycling and contribution to Blood formation in Steady-State and Inflammatory Challenges

During adult steady-state hematopoiesis, more than 80 % of HSCs are in a resting phase, i.e. are quiescent in G0 of cell cycle at any given time. Therefore, hematopoiesis must be supported by a small fraction of HSCs and downstream, highly proliferative HPCs. We and others thus investigate the questions a) why do we need HSC dormancy; b) which fraction of HSCs engage in hematopoiesis at any time, c) does contribution of different HSCs to hematopoiesis vary over time; d) what factors drive HSCs from quiescence to cycling and lineage commitment versus self-renewal; e) is hematopoietic and self-renewal potential of HSCs determined and correlated to HSC divisional history; and d) how does HSC proliferative history under various conditions influence functional HSC ageing, exhaustion, and possibly malignant transformation?

While others used in vivo labeling or elaborated genetic HSC labeling approaches, we modified a robust and noninvasive cellular labeling technique used largely in immunology research, i.e. labeling with 5(6)- carboxyfluorescein diacetate N-succinimidyl ester (CSFE), a cell-permeable dye that covalently binds to intracellular proteins and equally distributes to daughter cells upon cellular division to address these questions (Takizawa and Manz, 2012). This technique allows steady-state HSC transfer, bone marrow homing, in vivo tracking and high-resolution single-cell re-isolation of zero to more than five times divided live cells, and their subsequent testing *in vitro* and *in vivo* in functional assays (Kovtonyuk et al., 2016b; Pietras et al., 2016; Takizawa et al., 2017; Takizawa and Manz, 2011, 2012; Takizawa et al., 2011).

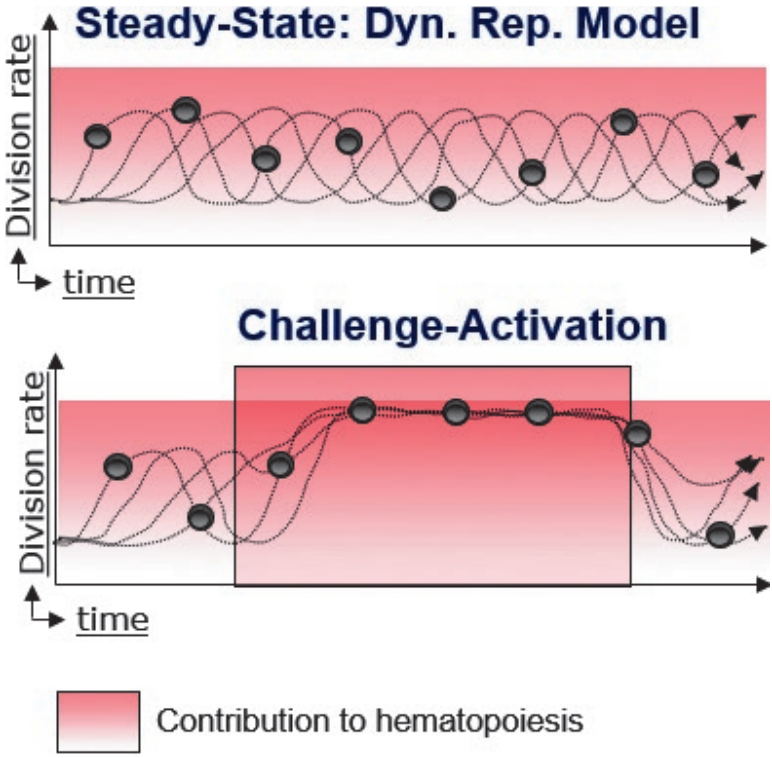
Using this approach, our first finding was that in steady-state, HSCs with equivalent life-long multi-lineage repopulation potential are contained in both frequently cycling cell populations and in quiescent cells that do not divide over 14 weeks. Our second finding was that steady-state fast-cycling HSC populations can slow down over time in steady-state serial transplantation and that HSCs with extensive proliferative history, i.e., HSCs that have gone through extended proliferation in aging or after in vivo challenge by transplantation – are prone to return to quiescence. We also showed that in vivo TLR4 challenge with Lipopolysaccharide (LPS)

recruits in vivo functional quiescent HSCs into proliferation and self-renewal with nonbiased lineage repopulation capacity (Takizawa et al., 2011). Moreover, our mathematical simulation reveals that HSCs with different cycling activity can be contained in one HSC population with relatively broad cycling variation and that, on average, HSCs divide 18× during a 2-yr lifespan of a laboratory mouse (Takizawa and Manz, 2011; Takizawa et al., 2011).

Based on these results, we proposed a dynamically fluctuating HSC cycling model in steady-state, where the turnover of all HSCs would naturally be similar at end of life. This would assure protection of the dormant HSC fraction from cell cycle damaging events, and would allow at the same time overall homogenous use of HSC resources during life. This model is in line with a linear correlation of telomere shortening in human HSCs with aging. Also, it would be in line with an increased risk for the whole HSC population to accumulate genetic events, promoting clonal HSC diseases as myelodysplasia and myeloid leukemia in the aged population.

Steady-state cycling differences of HSCs at any given time in this model would reflect the broad variation of possibilities, but not a static separation of HSCs in distinct classes with different divisional kinetics. Increased cycling of most HSC during hematopoietic challenges via external cues might have developed as an advantage to recruit dormant HSCs in order to better cope with blood cell demand for the necessary time. Broad activation, however, also poses a risk to loose HSCs due to genetic vulnerability and differentiation. Thus, a mechanism to "drive" HSCs to quiescence that increases strength with frequency of total HSC divisions needed to be established. This might be achieved by down-modulation of sensitivity to external stimulation and upregulation of sensitivity to quiescence signals. Indeed, we observed that phenotypically defined HSC populations with extensive proliferative history, i.e., HSCs from aged mice and HSCs that have divided massively after transplantation, subsequently increase quiescence in steady-state environments, likely as an effort to prevent HSC exhaustion (Takizawa and Manz, 2011; Takizawa et al., 2011). Figure 8 shows a graph that illustrates the above-discussed findings.

Figure 8. Schematic model depicting steady-state and challenge-activation of HSCs



HSC repetitively fluctuate in steady-state from quiescence to activation and blood production. At end of life, all HSCs have gone through about the same frequency of divisions. Upon challenge as e.g. massive infection, HSC are recruited from dormancy and contribute to blood formation for the necessary time, to subsequently re-establish fluctuation from quiescence to activation in steady-state.

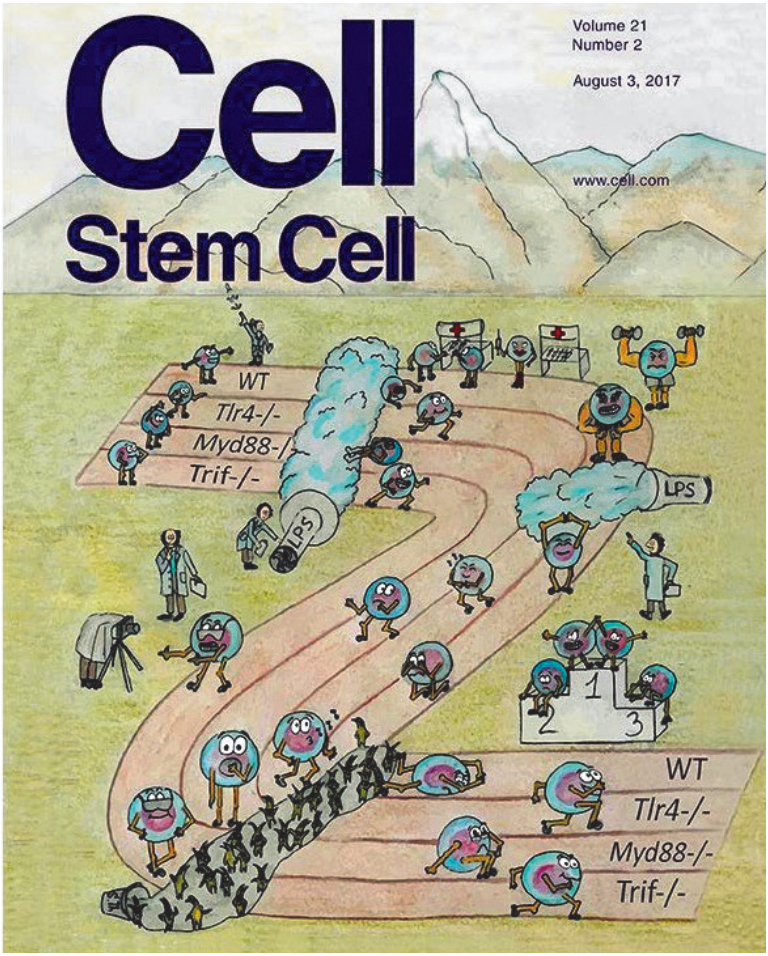
Figure adapted from: H. Takizawa et al., J Exp Med. 2011; 208(2):273-84

To dissect effects of cytokines, chemokine receptor antagonists and direct pathogen-derived stimuli on HSC cycling and function, we further tested *in vitro* and *in vivo* stimulation with thrombopoietin-analoga, granulocyte colony stimulating factor (G-CSF), Flt3-ligand, interleukine-1 (IL-1), and with LPS and life gram-negative bacterial infection (Kovtonyuk et al., 2016b; Pietras et al., 2016; Takizawa and Manz, 2017).

We found that thrombopoietin receptor stimulation on HSCs leads to HSC cycling and expansion without loss of self-renewal capacity (Kovtonyuk et al., 2016b). In contrast, Chronic interleukin-1 exposure drives HSCs towards myeloid differentiation at the expense of self-renewal (Pietras et al., 2016), while G-CSF, Flt3-ligand and CXCR4 antagonists did not affect HSC cycling and potential (Kovtonyuk et al., 2016b). Given the enhancement of multi-lineage regeneration by thrombopoietin-receptor agonists, possibly via cycling and re-expansion of human HSCs in aplastic anemia, we speculate that these also might be tested in clinical settings as an add-on to conditioning chemotherapy before allogeneic HSC transplantation in order to reduce endogenous HSCs. Furthermore, we speculate that, if the thrombopoietin receptor is expressed on malignant counterparts of HSCs, these could also be targeted by thrombopoietin-receptor agonist chemosensitization (Kovtonyuk et al., 2016b).

We also found that self-renewing HSCs directly sense gram-negative bacterial infection through Toll-like receptor 4 (TLR4) activation, which leads to impaired function via proliferative stress. However, this could be prevented by pharmacological blockage of the TLR4-TRIF-ROS-p38 axis (Takizawa et al., 2017). Interestingly, we previously contributed to a collaborative project where we found with our colleagues that very low concentrations of microbial antigens and TLR ligands derived from the intestinal microbiome, well below the threshold required for induction of adaptive immunity, sets the bone marrow myeloid cell pool size (Balmer et al., 2014). Whether inhibition of this pathway as a means of preemptive medicine is able to prevent or delay aging-associated and inflammation-associated HSC defects and malignant transformation will need to be determined in future studies (Kovtonyuk et al., 2016a; Luis et al., 2016; Takizawa et al., 2012; Takizawa et al., 2017; Takizawa and Manz, 2017). Figure 9 shows the related cover of the Cell Stem Cell issue, describing our findings in a cartoon in front of our geographical region.

Figure 9. HSCs in action under inflammation

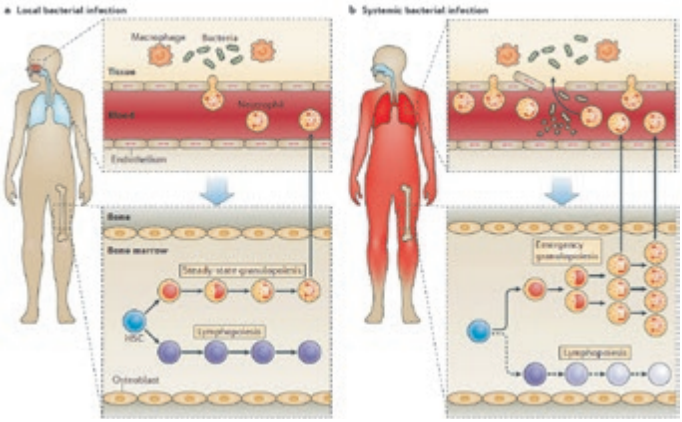


The cover of Cell Stem Cell describes our findings in a cartoon in front of our geographical region. Designed by Larisa Kovtonyuk, Ph.D., member of our laboratory and co-author of the related manuscript. Cell Stem Cell, August 3, 2017

Regulation of Emergency Granulopoiesis

Steady-state hematopoiesis is switched to infection-induced emergency hematopoiesis following detection of systemically disseminated microbial pathogens (e.g. viruses, bacteria, fungi, and parasite). A prototypic and clinically well know response is emergency granulopoiesis, with increase of granulocytes and shift to immature forms in peripheral blood, and release and massively increased production of granulocytes in BM upon lack of local infectious control and systemic spread of bacteria, i.e. bacteriaemia and sepsis (Figure 10).

Figure 10. Local versus systemic bacterial infection



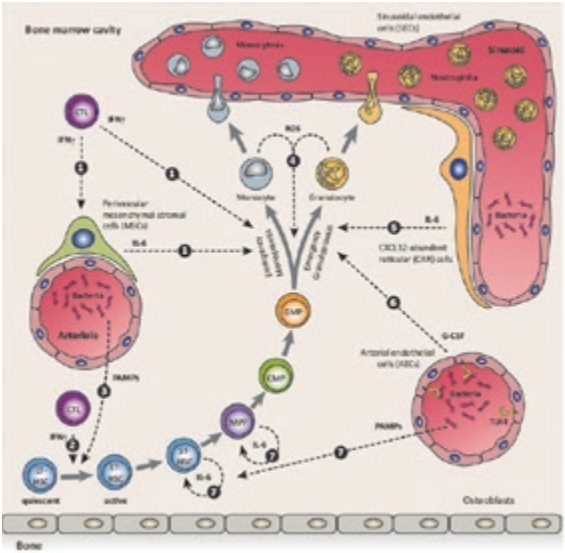
a) During a localized bacterial infection in an immunocompetent individual, several pre-existing antimicrobial effector mechanisms contain bacterial pathogens locally. In most cases, this leads to a rapid resolution of the infection, thereby preventing bacterial dissemination. As a consequence, bone marrow granulopoiesis is unaffected and does not differ from steady-state conditions. b) By contrast, in the setting of severe bacterial infection that overwhelms first-line defence mechanisms, bacterial dissemination occurs and neutrophils are consumed in large quantities, with pre-existing neutrophil pools being used. To counterbalance neutrophil depletion and to provide a supply of urgently needed neutrophils to combat systemic bacterial spread, a haematopoietic response programme termed 'emergency granulopoiesis' is initiated, which is characterized by the large-scale de novo generation of neutrophils from myeloid progenitors in the bone marrow. The expansion of bone marrow granulopoiesis is paralleled by a decrease in bone marrow lymphopoiesis.

Figure adapted from: M.G. Manz and S. Boettcher, Nat Rev Immunol., Vol. 14, May 2014.

How and by which cells sensing of systemic bacterial spread is accomplished, and how this sensing is translated into appropriately, life-saving granulocyte production and subsequent decrease of it upon clearance of infection, was poorly understood. We thus addressed the fundamental question of which cell types act as primary sensors of bacterial dissemination during severe infection and consequently induce the switch from steady-state to demand adapted emergency granulopoiesis.

We approached this by *in vivo* elimination of sensors (TLR4) or downstream signaling molecules (MyD88) in hematopoietic and non-hematopoietic cells using genetic Cre recombinase– mediated tissue-specific ablation approaches. Against our expectations, we found that pathogen sensing by both immature HSPCs and mature hematopoietic cells, including macrophages, is dispensable for the acute process of emergency granulopoiesis. In contrast, we discovered that endothelial cells (ECs) express sensors and, upon ligation of those, are the major producers of G-CSF, which then induces emergency granulopoiesis (Boettcher et al., 2014; Boettcher et al., 2012). Our findings reveal a sophisticated degree of functional interplay between the vascular, the hematopoietic, and the immune systems, which have a common developmental root and might thus be understood as one functional organ. Indeed, ECs are ideally positioned to mark the threshold of local vs systemic infection, and using ECs as safeguards seems a very plausible, danger-adapted defense strategy established in evolution (Boettcher and Manz, 2016, 2017; Manz and Boettcher, 2014). Figure 11 summarizes our proposal for a model of infection-driven emergency hematopoiesis, integrating additional findings from our and other research groups in the field.

Figure 11. Integrated model of emergency hematopoiesis



Multiple distinct and most likely complementary pathways exist and these [284TD$DIF]govern how pathogen-derived signals are detected and translated into emergency hematopoiesis. Of note, the order of mentioning individual pathways does not indicate their importance. (1) Interferon-g (IFNg) released from CD8+ cytotoxic T lymphocytes (CTLs) promotes emergency monopoiesis by stimulating perivascular mesenchymal stromal cells (MSCs) to secrete interleukin-6 (IL-6) as well as by directly acting on granulocyte-macrophage progenitors (GMPs), thereby favoring a monocytic lineage fate. (2) IFNg can also directly induce hematopoietic stem cell (HSC) cycling and recruit quiescent HSCs into the cell cycle. (3) Pathogen-associated molecular patterns (PAMPs) are also able to stimulate HSC proliferation but it remains to be determined whether it is accomplished directly or indirectly. (4) Reactive oxygen species (ROS) released by bone marrow Gr1+ myeloid cells (including monocytes and neutrophils) during Toll-like receptor (TLR) agonist stimulation enhance upstream myeloid precursor proliferation and differentiation. (5) CXCL12-abundant reticular (CAR) cells are a major source of IL-6 that is of importance to sustain emergency granulopoiesis (S.B. and M.G.M., unpublished data). (6) Granulocyte colony-stimulating factor (G-CSF) secreted from predominantly arteriolar endothelial cells (AECs) following lipopolysaccharide (LPS) recognition in an MYD88-dependent manner stimulates emergency granulopoiesis. (7) Direct PAMP recognition by mainly short-term HSCs (ST-HSCs) and multipotent progenitors (MPPs) leads to IL-6 secretion, which acts in an autocrine/paracrine manner to stimulate emergency hematopoiesis. Abbreviations: CMP, common myeloid progenitor; LT, long term.

Figure adapted from: S. Boettcher and M.G. Manz, Trends in Immunology, May 2017, Vol 38, No. 5.

Future studies will need to determine if there are pathogen-specific differences in the activated emergency hematopoietic pathways, what factors determine the threshold for induction of emergency hematopoiesis, what is the contribution of HSC activation versus myeloid progenitor activation to the overall magnitude of emergency hematopoiesis? Is HSC activation during this process a physiologic response or indicative of a failure to control infection, what are the long-term hematopoietic and immunological consequences and the possible underlying molecular mechanisms of chronic infection and inflammation, and what is the molecular mechanism by which chronic infection/inflammation increases the risk for developing hematologic malignancies (Boettcher and Manz, 2017)?

In vivo model Systems to study human Hematopoiesis in Health and Disease

Knowledge on human hematopoiesis and immunology is predominantly gained by clinical observation, cautious, safety-oriented in vivo experimentation, and in vitro surrogate assays (Manz, 2007). Progress in clinical research is mostly slow, and rigorous scientific proof is frequently impossible. Given appropriate ethical consideration, most societies agree on research involving worms, flies, and small vertebrates as mice. Indeed, laboratory mice have become the main model for in vivo basic and applied biomedical science. However, approximately 65 million years of divergence in human and mouse evolution have shaped these two species that differ substantially in size, lifespan, reproductive activity, and exposure to environmental challenges, e.g., species-specific infectious agents that coevolved. Concerning hematology and immunology, many similarities exist, however, mice are not men and some loss in translation of achievements between these two species is observed. Humanized mice, i.e. mice that carry human molecular or tissue elements have been generated over the last few decades with the intention to fill this gap, i.e. to model human physiology and pathology in accessible ways *in vivo*. Achievements in generating human hemato-lymphopoietic mice are summarized in recent reviews by us and colleagues working in the field (Legrand et al., 2009; Manz, 2007; Rongvaux et al., 2013; Shultz et al., 2007; Theocharides et al., 2016; Macchiarini et al., 2005).

In brief, we hypothesized that transplantation of human HSCs into the liver, i.e. a hematopoietic active organ, of newborn T cell, B cell and NK cell deficient mice (Rag2-/-gamma common chain-/-) would lead to human hematopoietic cell development and replacement of lacking mouse components. Indeed, the expanding organism provides a suitable environment for high-level human HSC engraftment and lymphocyte development (Traggiai et al., 2004). We, together with collaborators, could show that human-specific lymphotropic infections with Epstein Barr and Human Immunodeficiency Virus (Baenziger et al., 2006; Cocco et al., 2008; Neagu et al., 2009; Traggiai et al., 2004) are possible and some elements of human disease develop. However, due to insufficient adaptive immune cell education and insufficient cross-reactivity of growth factors in the xenogeneic environment, human adaptive immune reactions are weak, human HSCs are not sufficiently maintained over time, and human myeloid cells developed only at very low numbers and did not populate tissues sufficiently. In order to overcome the limitations of insufficient cytokine cross-reactivity and rejection of human cells via the innate macrophage response, we, in collaboration with Richard Flavell's laboratory at Yale and Regeneron, a company specialized in genetic engineering, generated mice that express human HSC and myeloid cell supporting growth factors under the regulatory element of the mouse genes (Strowig et al., 2011; Takizawa and Manz, 2007). Furthermore, we expressed a human SIRPa transgene on the mouse background to avoid human cell elimination via mouse phagocytic cells (Das et al., 2016; Rathinam et al., 2011; Rongvaux et al., 2014; Rongvaux et al., 2011; Strowig et al., 2011; Willinger et al., 2011). Indeed all engineering steps led to stepwise improvement of human cell engraftment and quality of human hematopoiesis and the combination of human growth factors in mice led to human cell development and distribution that closely resembles distribution in humans (Rongvaux et al., 2014; Theocharides et al., 2016). We now use these humanized mice to model specific human diseases (Figure 12).

Figure 12. Possible human disease-specific, tailored mouse models in future research

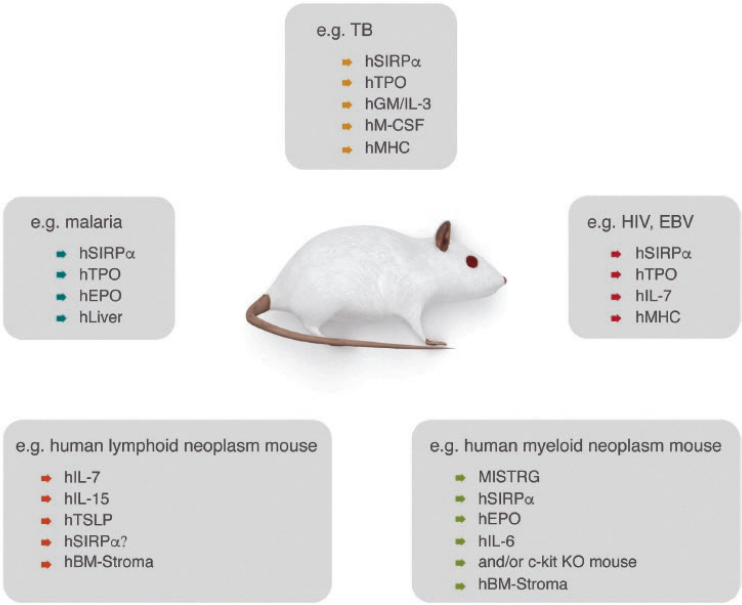


Figure adapted from: A. Theocharides et al., haematologica. 2016; 101(1).

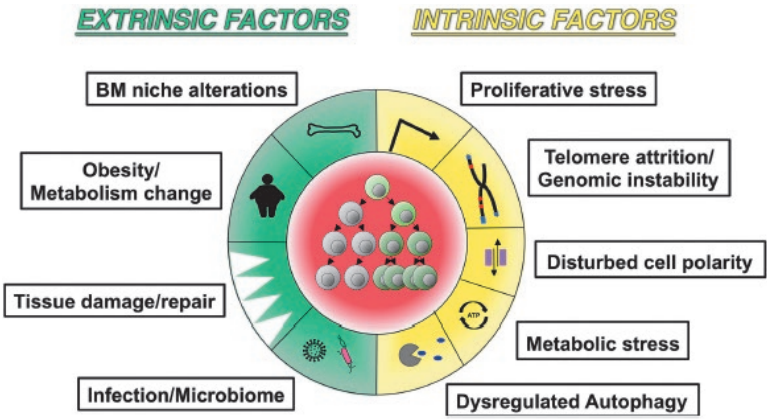
Using these models for human malignancies, we were able to demonstrate a) contribution of human myeloid cells to human melanoma growth (Rongvaux et al., 2014), b) dependence of human favorable-risk AML on macrophage colony-stimulating factor (M-CSF) (Ellegast et al., 2016), and dependence of human clonal plasma cell disease on cytokine combinations including IL-6 (Das et al., 2016). We are hopeful that human-hemato-lymphoid system mice will further be useful for pre-clinically, predictive testing of therapies and thus accelerate development of new successful therapeutic approaches.

Hematopoietic Stem Cells, (Inflamm-)Ageing and Clonal Dominance

With increasing age, HSCs get more numerous (about twofold increased, biologically defined HSCs in aged mice compared to young adult mice), however, their differentiation is biased towards myelopoiesis and their overall self-renewal and output capacity is reduced (Kovtonyuk et al., 2016a). While most of the data on ageing of HSCs is derived from mice, similar observations are made in humans. Biology of aged HSCs, similar as biology of other cells in other tissues, is driven by both cell-intrinsic as well as cell-extrinsic factors. An overreaching theme is the association of ageing and inflammatory signatures, even in a steady-state situation. Indeed, by analyzing BM gene transcription in hematopoietic and non-hematopoietic cells and by measuring expression of bone marrow protein presence in ageing and in inflamed situations, a high level of concordance emerges (unpublished data). We and colleagues in this research field are thus referring to "inflamm-ageing" of hematopoiesis, and we summarized potential hallmarks of this process (Kovtonyuk et al., 2016a) (Figure 13).

Figure 13. Potential hallmarks of inflamm-ageing

In this context, it is highly interesting that the occurrence of HSC neoplasia as AML, MDS and MPN are associated with repetitive infection and inflammatory diseases, and, even more so, with ageing. Surprisingly, benchmark studies in humans without hematopoietic diseases demonstrated during the last years, that clonal dominance of genetically altered hematopoiesis is a frequent event in the ageing population (more than 10 % in the over 70 years old) (Xie et al., 2014; Genovese et al., 2014; Jaiswal et al., 2014). Presence of these clonalities was coined as "clonal hematopoiesis of indetermined potential" (CHiP). Indeed, the genetic alterations that cause clonal dominance are frequently affecting the same genes that have been shown before to be associated or cause AML, MDS and MPN. However, only a small fraction (1–2 % per year) of individuals with CHiP develop these diseases. Thus, in this sense, CHiP shows similarities to other clonal, non-disease defining alterations in the hemato-lymphoid system as monoclonal B cell lymphocytosis (MBL) and monoclonal gammopathy of unknown significance (MGUS) (Steensma et al., 2015). Again, one could speculate that what we observe here in the



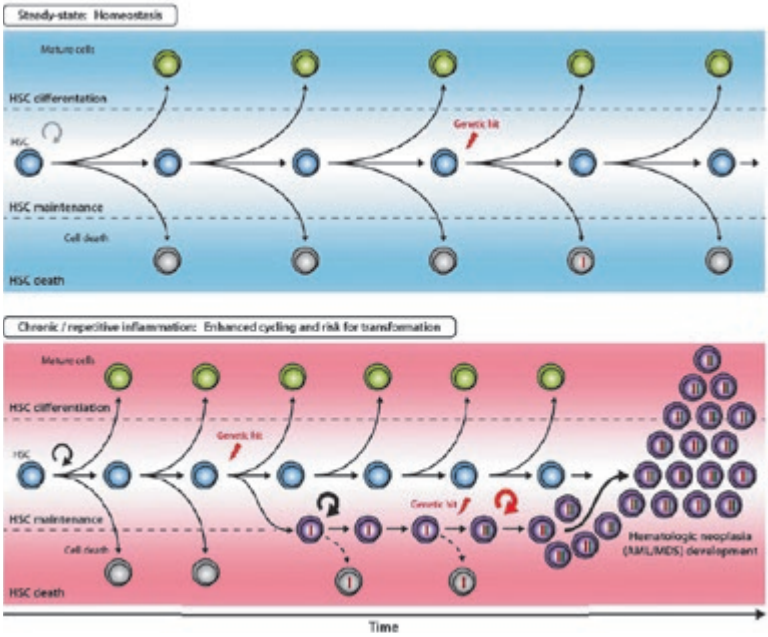
The schematic figure highlights similarities between aging and inflammation-associated changes in hematopoiesis and hematopoietic stem cells: (a) extrinsic factors include bone marrow niche alterations, metabolic changes, tissue damage/repair, and infection/ microbiome; (b) intrinsic factors include proliferative stress, telomere attrition/genomic instability, dysregulated autophagy, metabolic stress, and dysregulated autophagy.

Figure adapted from: L.V. Kovtonyuk et al., Frontiers in Immunology. November 2016, Volume 7, Article 502.

hematopoietic system is similarly occurring in other organ systems without thus far being noticed. While occurrence of genetic CHiP-driver alterations might follow a simple stochastic rule with numbers of cell divisions, we speculate that inflammatory drive in addition might modulate and enhance risk (Takizawa et al., 2012). This is depicted in Figure 14.

Figure 14. Hypothetical model for inflammation-enhanced malignant transformation in early hematopoiesis

A key task is now to understand reasons for progression of CHiP to neoplasia, identify individuals at risk, and develop means to interfere clinically. To this end, we work on a project where we evaluate HSCs and hematopoiesis from one "source" under different environmental conditions. We analyze long-term surviving HSC recipients and their HSC donors for their HSC status on a population and clonal level, and we corre-



The 3 principle outcomes of HSC divisions are self-renewal, differentiation, and death. The balance between these 3 cell fates is closely regulated to ensure balanced blood cell production over the lifetime of an individual. With increased proliferative history in the HSC compartment, the probability of acquiring genetic alterations ("hits") in HSCs increases, but efficient counteractive mechanisms (eg, apoptosis, DNA repair) have evolved to remove or correct genetically altered HSCs from the pool (top). It can be hypothesized that under chronic or repetitive inflammation the likelihood of genetic hit acquisition and subsequent accumulation in HSC is increased by two major mechanisms: First, accelerated HSC cycling and self-renewal enhances the statistical probability of acquiring mutations during DNA replication. Second, genetically altered HSC clones are rescued by signals delivered from the inflammatory environment. Persistent and aberrant self-renewal of the respective HSC clone might enhance occurrence of further genetic hits, eventually, resulting in the development of HSC neoplasia, for example, acute myeloid leukemia/myelodysplastic syndrome (AML/MDS, bottom).

Figure adapted from: H. Takizawa et al., Blood, 29 March 2012, Volume 119, Number 13.

late inflammation/infection, proliferative history and function of HSCs with occurrence and progression of CHiP. We are positive that this unique long-term read-out (in some cases more than 30 years) will shed light on the problem, covering both basic and clinically relevant aspects.

Conclusions and Perspective

Since the beginning of my clinical and scientific career more than twenty years ago, gain of knowledge in molecular understanding of benign and malignant hematology and knowledge-driven capacity to clinically interfere and control or even cure diseases lead to enormous leaps forward. Indeed, few treatment approaches are still the same as they have been then, and because of these developments, being a patient today is in an ever-growing fraction more bearable than before. Further, many basic principles observed in easy to access and to test hematopoietic cells and diseases and respective new treatment approaches have been extended and applied to other organ systems. I am motivated by better care through science and humanities and I am hopeful to contribute with our research on hematopoiesis, hemato-immunology, and hematopoietic malignancies pieces of understanding and insight, ultimately adding to maintenance of well-being of an ageing population and to re-gaining health of patients.

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