The myeloma stem cell concept, revisited: from phenomenology to operational terms

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ABSTRACT

The concept of the myeloma stem cell may have important therapeutic implications, yet its demonstration has been hampered by a lack of consistency in terms and definitions. Here, we summarize the current documentation and propose single-cell *in vitro* studies for future translational studies. By the classical approach, a $CD19^-/CD45^{low/-}/CD38^{high}/CD138^+$ malignant plasma cell, but not the $CD19^+/CD38^{low/-}$ memory B cell compartment, is enriched for tumorigenic cells that initiate myeloma in xenografted immunodeficient mice, supporting that myeloma stem cells are present in the malignant PC compartment. Using a new approach, analysis of c-DNA libraries from $CD19^+/CD27^+/CD38^-$ single cells has identified clonotypic memory B cell, suggested to be the cell of origin. This is consistent with multiple myeloma being a multistep hierarchical process before or during clinical presentation. We anticipate that further characterization will require single cell geno- and phenotyping combined with clonogenic assays. To implement such technologies, we propose a revision of the concept of a myeloma stem cell by including operational *in vitro* assays to describe the cellular components of origin, initiation, maintenance, and evolution of multiple myeloma. These terms are in accordance with recent (2012) consensus statements on the definitions, assays, and nomenclature of cancer stem cells, which is technically precise without completely abolishing established terminology. We expect that this operational model will be useful for future reporting of parameters used to identify and characterize the multiple myeloma stem cells. We strongly recommend that these parameters include validated standard technologies, reproducible assays, and, most importantly, supervised prospective sampling of selected biomaterial which reflects clinical stages, disease spectrum, and therapeutic outcome. This framework is key to the characterization of the cellular architecture of multiple myeloma and its use in precision medicine.

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Introduction

The multiple myeloma stem cell (MMSC) is defined as a cell within the malignant tissue that possesses the capacity to self-renew and to differentiate into the predominant lineages of myeloma plasma cells comprising the neoplasm. Self-renewal is cell division without the loss of differentiation potential, at least in some daughter cells. This concept is based on phenomenology, and MMSCs are defined experimentally by their ability to recapitulate the continuous growth of malignant tissue *in vivo* and/or *in vitro*. Experimental approaches underlie the terms "cancer-initiating cells" and "cell of origin", which are used operationally to characterize cancer stem-cell (CSC) compartments. Several studies have analyzed the hematopoietic system by sorting a few or even single cells, tracking acquired genetic changes, and transplanting cells to determine whether subsets within the differentiating hierarchy act as CSCs.[1]

Hierarchical models have been described for hematological malignancies such as acute and chronic myeloid and lymphoblastic leukemia,[2-6] and multiple myeloma (MM),[7-12] suggesting that the malignant clone includes immature progenitors or stem cells present in the proliferating bone-marrow compartment. According to the "multistep-oncogenesis" theory, malignancies develop due to a series of molecular alterations that occur in such cells. Although this theory is appealing and, on the whole, accepted by the scientific community, many aspects of this theory require rigorous scientific questions and answers.

The practical usefulness of the hierarchical model relies on emerging insights into the pathogenesis and identification of new biomarkers. Progress in this area depends on recent technological advances that have enabled researchers to study single-cell gene expression[13] and to address changes in cellular programming in a global fashion by analyzing gene mutations, expression and deregulation.[14-16] Lagging slightly behind this approach is the rapidly developing technology for examining the protein compartment of the cell.[17-19]

In the present review, we have updated the latest scientific reports and propose a revision of the MMSC concept to include operational terms (Table 1), in accordance with recent (2012) consensus statements on the definitions, assays, and nomenclature of cancer stem cells, which is technically precise without completely abolishing established terminology.[20]

We expect this will empower the design of translational research activities based on prospectively sampled biomaterial which reflects the spectrum of clinical disease. We expect such a revision to acquire data that indirectly document the existence of multiple MMSCs and validate its impact via targeted therapy.

The stem-cell concept and the B-cell hierarchy

A normal stem cell is a unique cell type present at low frequency which can renew itself and produce progenitors of one or more specialized cell types. Beginning with the fertilized oocyte, cellular differentiation into specialized cell types, tissues, and organs follows a strict pattern. The classical view is that during such processes, cells gradually lose their capacity for self-renewal, their plasticity, and their ability to develop into different lineages.[21]

Stem cells may be classified into two major classes: 1) pluripotent stem cells derived from early embryos that are able to replenish all cell types in the human body, and 2) multipotent stem cells located in various organs that are dedicated to the replenishment of specific tissues such as blood. Embryonic stem cells, which are derived from the inner cell mass of the blastocyst, can be cultured *in vitro* nearly indefinitely. Unlike embryonic stem cells, multipotent organ restricted stem cells, which may be isolated from a variety of tissues in fetal and adult humans, are lineage specific; hematopoietic stem cells, neuronal stem cells, and hepatic stem cells are all multipotent.

In this review, we consider hematopoietic stem cells and putative CSCs as prototypes of multipotent stem cells. However, not all are multipotent; for example, ´end-stage´ effector B cells may regain self-renewing mechanisms in order to expand and maintain immunity.[22,23] In normal B-cell lymphopoiesis, a number of well-characterized subpopulations have been defined by membrane marker phenotyping, as reviewed and illustrated in the upper part of Figure 1. The very early B-cell precursors develop into pro- and pre-B cells before they migrate as immature B cells into the blood to reach peripheral lymphoid organs as naive B cells.[24-31] Germinal and post-germinal-center centrocytes, centroblasts, memory cells, plasmablasts, and end-stage plasma cells (PCs) are included in the later stages of the mature B-cell differentiation hierarchy. Most malignant B-cell lymphomas, chronic lymphoblastic leukemias, and MMs are considered to originate from these cells following analyses of the somatic hypermutation and class switch-recombination status of the gene encoding the immunoglobulin heavy chain (IgH) which defines the hierarchical status of any clonotypic cell.[32-36] Further understanding of the molecular mechanisms that regulate the malignant B-cell hierarchy requires investigations of purified subpopulations or even single cells.

The phenomenon and its markers

The MMSC concept is based on phenomenology: the outcome of studies in animal and/or humans that rely on *in vivo* and *in vitro* assays. However, these assays address the future potential of the stem cell, while study outcomes address the expression of this potential.[37] Therefore, identifying a stem cell by allowing it to differentiate loses the original cell; at the same time, only a limited range of responses may be evident based on the model used. All stem cell assays reveal an outcome after cells are perturbed, and it is still an open question how the stem cell phenomena should be identified at the single-cell level.

At the cellular level, MM is characterized by uncontrolled expansion of PCs in the bone marrow. In addition, cells belonging to the myeloma IgH-defined clone (clonotypic cells) which precede the PC stage have been detected in peripheral blood, lymph nodes, and bone marrow.[10,33-36,38,39] The earliest clonotypic cells were exclusively identified in the $CD38^-$ B-cell compartment, suggesting a potential precursor and a myeloma hierarchy. The main scientific question was whether these clonal cells were intrinsic to the maintenance of the malignant PC clone defined by membrane markers, as illustrated in the lower part of Figure 1. Functional studies supported this idea,[10,11,38,39] but attempts to identify clonogenic potential in this compartment were unsuccessful,[8,9] and it remains a controversial issue.[12]

Table 1. Terms that define MMSCs and associated information.

Type of cell	Definition
	Conceptual context
Pre-malignant MMSC	Member of a subpopulation of neoplastic stem cells that can propagate clones that may or may not develop into MMSCs over time, but that have no immediate cancer-initiating potential
Malignant MMSC	Member of a subpopulation of neoplastic stem cells within the tumor that indefinitely propagates malignant clones and produces overt myeloma
	Operational context
Cell of origin	A normal cell that acquires the first myeloma-promoting mutation(s); not necessarily linearly related to the MMSC and myeloma populations
Myeloma-initiating cell (*in vivo*)	A cell that regenerates detectable myeloma[a] populations in xenografted immunodeficient mice that are sustained[b]; usually measured via limiting dilution
Myeloma long-term culture-initiating cell (*in vitro*)	A cell that can initiate the sustained[c] production of neoplastic[a] populations when cultured in supportive conditions with or without stromal cells; usually measured via limiting dilution
Neoplastic sphere-forming cell (*in vitro*)	A cell that initiates non-adherent clusters or colonies of neoplastic progeny in *in vitro* cultures; usually measured by counting clusters/colonies that generate secondary units when re-plated.

[a]Defined by obviously abnormal biological features exhibited by cells in the primary sample, for example, the formation of a palpable growth or tumor, the production of myeloma plasma cells, abnormal growth properties, and clonal karyotype or genotype. [b]Based on our experience with normal stem cells, in this context "sustained" usually means ≥16 weeks, with (ideally) demonstrable activity on serial transplantation into secondary mice. [c]Based on our experience with normal hematopoietic stem cells, in this context "sustained" usually means ≥6 weeks with stromal cells ± stimulatory growth factors.

As illustrated in Figure 2, MM is the clinical outcome of a multistep transformation process that includes a pre-malignant state, the monoclonal gammopathy of undetermined significance (MGUS).[40] Two early pathways in MM oncogenesis have been identified: a nonhyperdiploid pathway characterized by translocations involving the IgH locus (14q32), and a hyperdiploid pathway.[41,42] IgH translocations are introduced at the MGUS state; the majority of breakpoints fall within the switch regions of the gene encoding IgH. Thus, the mechanisms and timing of translocation are those of normal IgH class switch-recombination, and define an early oncogenic event or targeting of a gene with oncogenic potential during initiation. In the hyperdiploid pathway, recurrent changes in chromosome number are considered to constitute an early event in MM oncogenesis.

MM cells display stable VDJ joining sequences, an accumulation of somatic mutations and absence of ongoing somatic hypermutation.[43,44] These findings support the idea that some MM cells are derived from a germinal center or post-germinal-center B cell that differentiates into a clonotypic memory B cell or a PC as illustrated in Figure 1.

In search of the MMSC

Although the malignant cells that represent terminally differentiated PCs are readily identifiable via morphological criteria, the phenotype of the MMSC is not yet known with certainty due to several observations that suggest a less-mature clonal precursor. Critically, this idea was supported by data from single-cell analyses.[33,36] More specifically, we identified, sorted and studied $CD19^+/CD27^+/CD38^-$ single-cell libraries of documented clonotypic cells that met all the criteria for a memory B cell, which were present in all 10 patients studied at a median frequency of 0.3% of $CD19^+$ B cells (range 0.001-1 cell per 1000 circulating cells).[45,46]

This observation, together with contradictory MMSC studies[8-11,33-36] and technological progress, motivated detailed multiparametric characterization of MMSCs and related B-cell subsets. To this end, members of the European Myeloma Network initiated a collaborative network of laboratories and scientists (MSCNET) in 2007.

As reviewed above, in 2007, the state of the art of MMSC suggested that post-germinal $CD19^+/CD20^+/CD138^-/CD38^-$ pre-PC B cells constituted the putative MMSC.[10,11,38,39] However, some evidence pointed to a $CD19^-/CD20^-$ $/CD138^+/CD38^+$ plasmablast/PC.[8,9] It was conceivable that both or additional stem-cell compartments could be the cellular basis for the acquired oncogenetic changes that underlie myeloma initiation, maintenance, and evolution.

During the last decade, MSCNET has performed detailed studies to confirm or refute previous studies[47-60] and established protocols[17,61-70] to delineate the phenotypes of subpopulations of cells in randomly selected primary tumor samples and in preclinical disease models. While our investigations did not confirm that pre-PC B cells are myeloma initiating,[50,51,52] several observations suggested that further study of plasmablasts/PCs at the single cell level will be key to elucidating MMSC functions.

This suggestion is in accordance with recent studies documenting that "$CD19^-/CD45^{low/-}/CD38^{high}/CD138^+$ [PCs] are enriched for human tumorigenic myeloma cells" that regenerate detectable myeloma populations in xenografted immunodeficient mice.[12] In a parallel investigation, $CD19^+/CD38^{low/-}$ memory B cells engrafted into human bone grafts, resulting in the repopulation of polyclonal B cells, which supports the hypothesis that memory B cells have the ability to self-renew.[23] However, since few clonotypic B cells were present in these grafts, the specific questions about pre-PC B cells have not been definitively answered; these cells may be present at quantities below the limit of detection. The same limitation may pertain to the first cells of origin that depart from normal B lymphopoiesis and harbor early, but not late,[50] changes in genetic, epigenetic, or other regulatory events that underlie the generation of malignant myeloma-initiating cells.

Another controversy surrounds the $CD138^-$ myeloma PCs studied in our preclinical models. These PCs exhibit engraftment and clonogenic potential *in vitro* (*unpublished data*),[51,52] in accordance with findings from others.[53,54] The nature of the $CD138^-$ and $CD138^+$ PCs in the lineage of

myeloma cells remains to be determined.[55]

Of particular importance, MSCNET has identified a new $CD19^-/CD45^-/CD138^+/CD38^+$ subpopulation associated to the $CD19^+/CD45^-/CD138^+/CD38^+$ normal PC compartment with different gene expression in normal bone marrow, suggesting a differentiation pathway that has not yet been studied in terms of MM pathogenesis.[56] Our next step will be to study the function of these compartments and patients in more detail, guided by novel technological progress in single-cell analysis, a common prospective biobank strategy, and well-characterized preclinical functional *in vitro* and *in vivo* models.

In our search, we propose a revision of the conceptual context of MMSCs to use more operational *in vitro* functions that will enable studies of the origin, initiation, maintenance, and evolution driven by deregulated genetic events. We anticipate that our understanding of MMSC will encompass the multiple dynamic cell compartments that are present from the initial to the final steps of the differentiation, evolution and selection of clonal PCs in myeloma.

Hallmarks: self-renewal, plasticity, and drug resistance

CSC function, a hallmark of cancer in general, is defined to include normal, specific functions such as self-renewal,

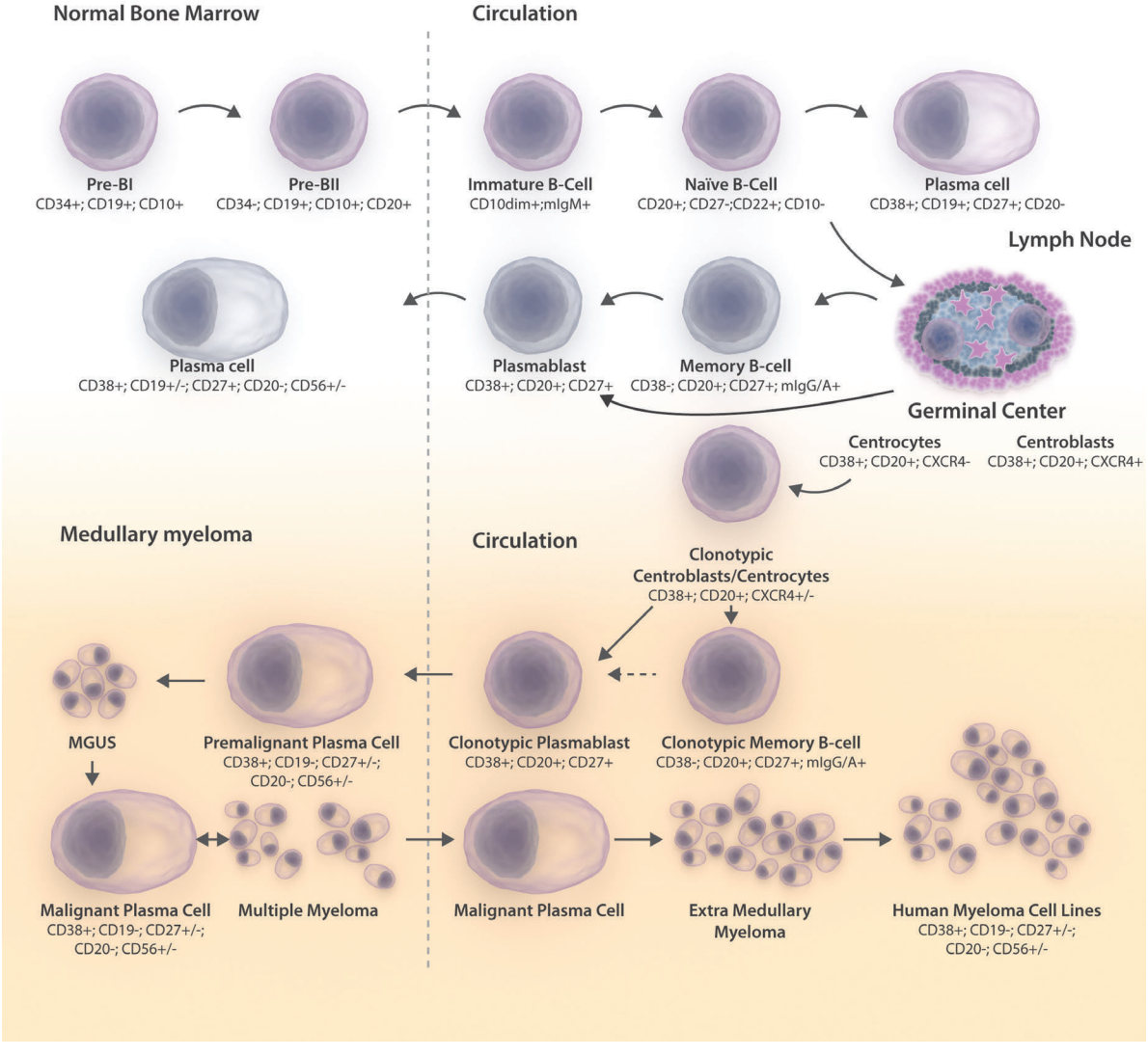

Figure 1. Membrane marker defined subpopulations of the normal B-cell differentiation and the myeloma hierarchy. Upper panel: Cytomic phenotyping of the normal, lineage-specific pro- and pre-B cells in the bone marrow that develops from hematopoietic stem cells and migrates into the blood as immature B cells to reach peripheral tissue as naive B cells. Here, the B-cell receptor is activated and cells develop into short-term PCs during the primary response or enter the germinal center. Germinal-center B cells differentiate from centroblasts and centrocytes into long-term end-stage circulating memory cells or PCs that migrate to tissue survival niches and differentiate into immobile mature PCs. Lower panel: The earliest clonotypic cells were exclusively identified in the CD38- memory B-cell compartment, suggesting a precursor and a myeloma hierarchy that includes circulating memory cells or PCs that migrate to tissue survival niches and differentiate into mature premalignant PCs, giving rise to MGUS. Within this neoplasia, later genetic changes yield a range of myeloma-initiating cells that drives the propagation of a medullary neoplasia at multiple sites that is clinically known as MM. Ultimately, evolution continues to select niche-independent PCs that circulate, resulting in the extramedullary growth of myeloma subclones and advanced disease stages clinically known as extramedullary MM, PC leukemia, and HMCL.

plasticity, and drug resistance.[71,72,68,69] Since most end-stage myeloma cells are short-lived,[73] MMSCs are thought to continuously generate myeloma daughter cells by precisely balancing self-renewal and differentiation. In theory, a CSC can accomplish this balance via asymmetric cell division in which it divides to generate one progeny pool with a stem-cell phenotype (self-renewal) and another progeny pool that differentiates and gives rise to end-stage tumor cells.[74,75] However, stem cells can also use symmetric divisions, defined as the generation of daughter cells that acquire identical phenotypes, to self-renew or to generate differentiated progeny.[75] Stem cells are thus defined by their capacity to generate more stem cells and differentiated daughter cells, rather than by symmetric production of a stem cell and a differentiated daughter at each division. The picture is even more complex in MM as some patients harbor heterogeneous populations of PCs that were most likely initiated from different populations of MMSCs within the myeloma hierarchy after many genetic lesions. Such heterogeneity may be explained by an alternative stochastic model in which all myeloma cells have the potential to self-renew, yet experience a varying probability of entering the cell cycle and finding an environment that supports subclonal evolution and heterogeneity. Future studies should therefore be based on genome-wide fingerprinting of clonal heterogeneity in order to identify factors that drive stepwise malignant transformation from normal PCs to MGUS, medullary MM, extramedullary MM, PC leukemia, and HMCL. Data from analyses of selected tissues and samples[76-79] will qualify biobank material for future studies of the deregulation of normal self-renewal pathways.

Novel technologies and potential strategies include single-cell analyses,[79,80,36,49,55] transgenic mouse models[54,81-82] in addition to the xenogene immuno compromised SCID-hu models,[53] syngeneic BALB/c plasmacytoma or the 5T serie,[83] and investigations of oncogene transformation in primary organoid miniature tissue culture.[84-87] Bmi-1, Notch, Hedgehog, and Wnt, which were initially identified based on their roles in tumor formation, have been shown to be involved in the regulation of self-renewal in normal stem cells in many tissues.[88-90]

Since myeloma cells are influenced by the host, the microenvironment may play a key role in the initiation of myeloma and associated phenotypic changes—a phenomenon called "plasticity", defined as altered cellular phenotype and function during deregulated differentiation. Of interest, this refers to malignant mature B cells that share features of different maturation steps, including precursors. Plasticity in MM is perhaps best illustrated by the subtyping of clinical tumor samples based on B-cell subset-associated gene signatures; tumors previously assigned to PreB-II and memory-cell subtypes of malignant PCs were associated with inferior prognoses.[57-60] This observation provides a new tool for generating insight into the stages of clonal plasticity associated with oncogenesis and deregulated differentiation. The mechanisms of myeloma-cell plasticity should be exploited, and their significance for the concept of MMSCs assessed.

Since a major group of patients suffer from disease recurrence or clinical relapse after chemotherapy, MM is thought to be a consequence of molecular resistance mechanisms that protect the MMSC compartments. The idea that resistant MMSCs are the source of post-therapeutic recurrence is not a new one; it was first described in studies of the stem-cell hierarchy and the self-renewal gene expression signature in leukemia with poor clinical outcome, which was also suggested to be associated with drug resistance.[91-94] In myeloma, it has recently been documented that the level of drug resistance is a function related to the cellular hierarchy[95] and its active or dormant stage[96] that we need to identify and target to overcome it. These findings highlight the potential to develop predictive, drug-specific sensitivity assays. We have taken the first step toward defining gene signatures of drug-specific resistance in pre-clinical models of HMCL.[57-60]

Self-renewal, plasticity, and resistance have been studied in the preclinical model of HMCL, which is considered to be the most advanced and homogenous myeloma tissue available. It is important to recognize that each HMCL reflects the end stage of an individual patient's genetic evolution and selection, but each HMCL also reflects the aggregation of stepwise oncogenic events over time, some of which may have deregulated the hallmarks of MMSCs. Although we acknowledge that this model may be irrelevant for studies of the hierarchical model, it is a tool for identifying potential markers for MMSC. Such findings should be traced back through the myeloma hierarchy in prospective qualified clinical myeloma cases, as exemplified by the MSCNET single-cell approach.[49]

Biological assays of MMSCs

In a conceptual context, classical stem-cell assays capture the phenomenon of a subpopulation that can propagate malignant clones indefinitely, and produce overt myeloma *in vivo* — the MMSCs. In an operational context, these assays indirectly seek to detect MMSCs as engrafting myeloma-initiating cells (*in vivo*), long-term culture-initiating cells (*in vitro*), or short-term sphere-forming cells (*in vitro*) as described in Table 1.

To date, immunodeficient mice have served as the most sensitive recipients for the growth, detection, and quantification of MMSC. Several xenografted mouse models enabled successful detection of the malignant regeneration of myeloma, usually measured via limiting dilution to identify the low frequent cells. However, the ability or failure of a cell compartment to produce myeloma in a transplanted mouse may not directly reflect the function of this compartment in patients. In this regard, the absence of the MMSC niche is a major limiting factor, as most samples include myeloma cells that have not yet completely acquired the ability to grow autonomously. To address this limitation, humanized and genetically modified mice have been designed as the state of the art fundament for functional studies of MMCS in myelomagenesis.[8,12,39,81-83]

Some studies have also evaluated MMSCs on the basis of their presumed self-renewal activity *in vitro* by investigating cells that initiate the sustained production of clonal myeloma cells when cultured in supportive conditions with or without stromal cells; these cells are usually subjected to analysis via limiting dilution. The generation of cellular spheres (as clusters or colonies in non-adherent liquid culture) constitutes a simple yet indirect strategy for identifying MMSCs in cell suspensions from myeloma tissue. It is unlikely that either of these *in vitro* systems fully replicates the three-dimensional structure and environment of myeloma in patients. On the contrary, it is likely that variables important for *in vivo* growth and self-renewal may not be present in *in vitro* investigations; the cells

under study may show no or selected growth, and/or may be anomalously and rapidly induced to differentiate to an end state without prior expansion. Therefore, *in vitro* assays may be useless in terms of clinically meaningful predictions.

Using HMCLs as a source of MMSCs for assays of growth *in vitro* or in transplanted mice is also problematic. Although it is unlikely that these cells reflect the original genotype or origin of MMSCs, HMCLs contain cells that display the functions of MMSCs. Accordingly, considerable caution needs to be taken when formulating questions that will be addressed through the analysis of cells passaged *in vitro*. However, meta-analyses of HMCL responses to known anticancer drugs illustrate how these cells can be used to reveal associations between drug sensitivity and gene-expression profiling in cell lines from individual patients; these gene signatures of resistance have documented prognostic value.[57,58]

Single cells, single genes, single clones

By the time MM is diagnosed, it consists of millions of myeloma cells carrying genetic abnormalities that initiate malignant proliferation, and other mutations are acquired during disease evolution. Some of these secondary mutations emerge due to selective pressure and act as "drivers"; others may be "passengers" resulting from random mutational exposures or genomic instability during many cell divisions. In theory, this instability may yield one MMSC per driver lesion. It is likely that individual MM tumors have multiple MMSCs with different phenotypes that are closely linked to the deregulated functions of self-renewal, plasticity, and drug resistance.

Initial DNA sequencing studies[76-78] have provided insight into mutational profiles in MM and have identified recurrent genes and potential molecular mechanisms responsible for MM initiation, maintenance, and progression. It is becoming clear that complexity beyond the landscape of mutations exists at the level of intraclonal heterogeneity, which directly affects disease progression and treatment resistance during clonal evolution and selection.[69,72,76-78] Understanding these processes and characterizing these subclones will require investigations, of single or few plas-

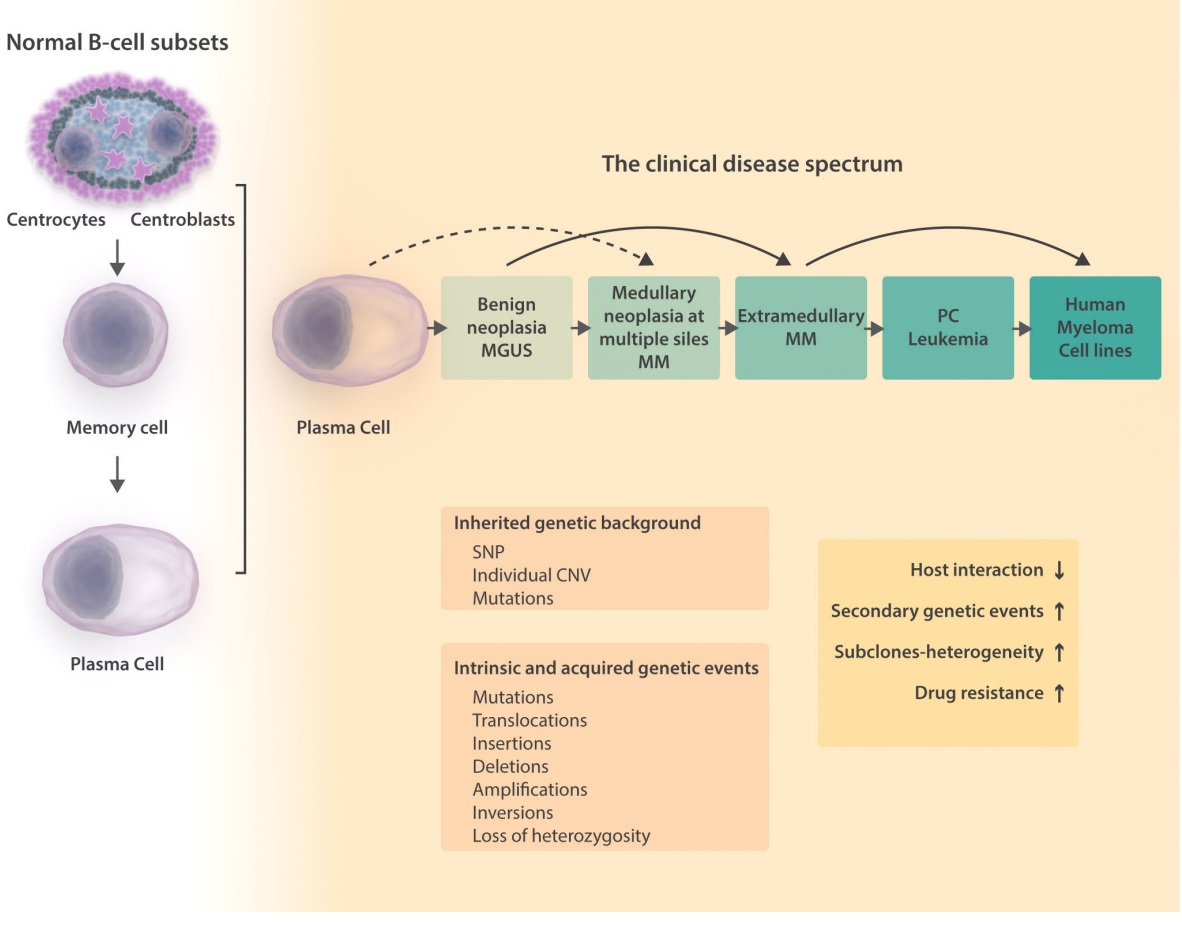

Figure 2 Multi-steps of origin, initiation and the clinical spectrum of multiple myeloma.[40] Left panel: Illustrates the normal B-cell subpopulations of interest in defining the cell of origin or the myeloma initiating cells. Current results support that a detectable, but rare, subpopulation of early oncogene-positive memory-like B cells in lymph node, blood, and bone marrow is descended from the cell of origin in the germinal center. These cells differentiate into premalignant PCs in the bone marrow, propagate through peripheral blood, and give rise to a benign neoplasia, clinically known as MGUS. Right panel: Within this benign neoplasia, later oncogenic events give rise to a range of myeloma-initiating cells that drives the propagation of medullary neoplasia at multiple sites, clinically known as MM. The ultimate selection of niche-independent cells results in extramedullary growth of myeloma subclones and advanced disease stages clinically known as extramedullary MM and PC leukemia. These advanced diseases are the origin of most HMCL. It is thought that transition through this clinical disease spectrum requires an inherited genetic background and the acquisition of genetic events that lead to expression of biological hallmarks that can be used for novel molecular disease classification systems, including drug resistance and plasticity.

ma cells,[97-98] into the functional impact of specific genetic variants.

It will be challenging to combine gene identification and *in vitro* functional approaches in order to separate the true genetic drivers from the many passengers along the tracks of myelomagenesis. There is an increasing interest in defining the exact phylogeny of individual subclonal populations;[80] patient-specific single-cell genetic profiling provides a potential resolution to this problem. Our original work in this area, which included the estimation of subset frequencies,[33 36,45,46] also encompassed studies of differential oncogene expression in cDNA libraries from normal PCs and cases of MGUS, MM, extramedullary MM, and HMCL, as well as the prognostic impact of these differences. In brief, tissue samples were phenotyped via multiparametric flow cytometry, potential subclones were identified, and single cells (or a few cells) were sorted into individual wells containing lysis medium and followed via the global amplification of cDNA from each well. The quality of each library was confirmed via a set of highly targeted QRT-PCRs for chimeric gene fusions, deregulated genes, and single-nucleotide variants (for example, in the IgH locus). These patient-specific libraries were associated with parameters such as tissue type, disease stage, sample site, therapy, and outcome.[97,98] This strategy, which has been automated and optimized to have a low error rate, has been integrated into high-throughput platforms that are useful for DNA and RNA sequencing; this may yield insight into subclonal genetic architectures and phylogenies in MM.[13,75-78]

We propose to combine this state of the art technique with an operational context as summarized in Table 1, in order to better define the phylogenetic relationships among clonal populations in myeloma at clinical presentation, during follow-up, and at relapse. This strategy will enable us to generate quantitative measures of stem-cell activities and functions at the level of single subclones by assessing self-renewal via the analysis of gene-expression signatures,[3,4] plasticity via subtyping by B-cell subset-associated gene signatures,[63,64] and drug resistance via the assignment of gene signatures of drug resistance.[57-60]

Summary and perspective

The term "CSC" captures the idea that a stable, minor, quiescent, and phenotypically definable subpopulation exists within the malignant tissue. This subpopulation has the potential to self-renew and to enhance tumor resistance to toxic stress, thereby propagating the cancer for prolonged or even unlimited periods of time.

This idea is supported by phenomenology, and it plays a major role in our understanding of the pathogenesis of cancer. Although this concept is also widely appreciated for MMSCs, controversy persists regarding the identification and their origin, selection, plasticity, phenotype(s), and heterogeneity in various stages of MM. The existence of MMSCs, and whether they can be documented as a well-defined and characterized entity in MM remains to be demonstrated — and it may never be. However, introducing a more operational context (as suggested in Table 1) into our descriptions may enable the acquisition of data that indirectly support the existence of MMSCs and allow clinical validation of their impact; for example, via targeted therapy. Here we have summarized results from recent experimental work within and outside MSCNET, with a focus on the identification, isolation, and characterization of MMSCs. To date, these results support the hypothesis that myeloma-initiating cells are present in the malignant PC compartment, but the cell of origin is a normal counterpart of a germinal-center B cell that differentiates into a premalignant PC compartment identified in MGUS as indicated in Figure 1. This is consistent with our current understanding of the pathogenesis of MM as a multistep, cellular, hierarchical and linear process of disease initiation, evolution, selection and clinical presentation as illustrated in Figure 2.

A revision of the MMSC concept should include operational terms as described in Table 1 that enable the design of research plans based on prospectively sampled biomaterial that reflects the clinical disease spectrum. We anticipate that such a revision will lead to the acquisition of data that indirectly documents the existence of multiple MMSCs. The ultimate validation of their existence may then be achieved via targeted therapy in clinical trials.

The revision will allow us to identify a range of specific genetic events in various B-cell and PC subsets, and to design research activities focused on targeted therapy,[99,100] based on prospectively sampled biomaterial from all myeloma subtypes classified at diagnosis and during follow-up. This strategy is in accordance with recent consensus statements on the definitions, assays, and nomenclature of CSCs, including a more operational nomenclature that achieves technical precision without completely abolishing established terminology.[1,20]

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