Myeloma propagating cells, drug resistance and relapse

Anastasios Karadimitris[1,2], Aristeidis Chaidos[1,2], Valentina Caputo[1], Katerina Goudevenou[1], Kanagaraju Ponnusamy[1], Xiaolin Xiao[1]

[1]Centre for Haematology, Department of Medicine, Imperial College London, Du Cane Road, London W12 0NN, UK
[2]Department of Haematology, Hammersmith Hospital, Imperial College Healthcare NHS Trust, Du Cane Road, London W12 0HS, UK

Abstract 157 words
Text 2390

Correspondence
Anastasios Karadimitris
Centre for Haematology, Department of Medicine
Imperial College London
Department of Haematology, Hammersmith Hospital
Imperial College Healthcare NHS Trust
Du Cane Road, London W12 0NN, UK

e-mail: a.karadimitris@imperial.ac.uk

Abstract

Multiple myeloma (MM) is an incurable tumour of the plasma cells (PC), the terminally differentiated immunoglobulin secreting B lineage cells. The genetic make up of MM has been extensively characterised but its impact on the biology of the disease is incomplete without more precise knowledge of the identity and functional role of cells with multiple myeloma propagating activity (MMPA). We review here recent data that link MMPA with myeloma clonotypic populations organised in a cellular hierarchy that mirrors normal B cell development and also with drug resistance and disease relapse. We further propose a conceptual framework which, with optimal employment of recent technological advances in genomics and phenomics, could allow dissection of the cellular and molecular properties of cells with MMPA, drug resistance and in vivo relapse in an integrated and patient-specific manner. There is real hope that these approaches will significantly contribute to further improvements in disease control, overall survival and possibly cure of patients with MM.

Introduction

Multiple myeloma (MM) is a malignancy of the plasma cells (PC), the immunoglobulin (Ig)-secreting terminally differentiated B lineage cells. Despite recent progress, MM remains an incurable cancer with a median survival of 5-6 yrs[1, 2].

As for the majority of cancers, somatic mutations are required for disease initiation and progression and development of drug resistance. MM is also recognised to establish an intimate cross-talk with its microenvironment(s). Myeloma PC receive pro-survival signals from different cellular populations and in turn, directly or indirectly they incite aberrant osteoclast activation, osteoblast dysfunction and thus development of myeloma bone disease, a complication which is unique to MM amongst haematological malignancies (reviewed in [3-5]). Although MM is primarily a bone marrow (BM)-based malignancy, myeloma PC are nearly always found circulating in peripheral blood (PB) and as the disease progresses, they can metastasize to extra-medullary sites where they can establish solid tumours called plasmacytomas.

A still unresolved and partially addressed question that pertains the biology of MM is the existence, identity and functional role of cells that can propagate myeloma (multiple myeloma propagating cells-MMPC). As per previous suggestions, we use the term MMPC and MM propagating activity (MMPA) operationally for those cells that can propagate MM in animal models[6]. From a clinical perspective, MMPC are expected to identify or at least partially overlap with cells that are resistant to therapy and consequently are the source of disease relapse. To address these concepts one will need to take into account the normal cellular hierarchy and ontogeny that myeloma PC relate to and the presence in malignant PC of somatically re-arranged Ig genes as unique molecular fingerprints of clonality that greatly facilitate study of all of the above.

Finally, understanding of MMPA and drug resistance should include not only the prime role of genetic events in the moulding of the functional profile of MMPC. It should also explore relevant epigenetic mechanisms, dictated either by genetic events or by the interaction of myeloma PC with its different microenvironments and as discussed in detail below by the inherent plasticity of the normal and PC myeloma-associated cellular hierarchy.

Several recent publications, including one in this Journal, expertly reviewed the cellular and molecular processes that relate to MMPA in vitro and in vivo[7-11]. Here, we review and attempt to integrate recent exciting studies which perhaps for the first time linked MMPA directly to clinical drug resistance and relapse. In addition, we propose a blueprint for adopting future strategies for the high resolution characterisation of cells enriched in MMPA, drug resistance and ability to drive disease relapse.

The genetic basis of myeloma initiation and progression
In almost all cases, MM is preceded by monoclonal gammopathy of uncertain significance (MGUS), a pre-malignant condition affecting 1% of individuals over the age of 50, increasing to 5% by the age of 70[1, 12].

Hyperdiploidy (HY) and chromosomal translocations (≈50% of all cases) leading to activation of oncogenes (e.g., *CCND1*, *CCND2*, *c-MAF* and *MMSET*) are the key myeloma initiating genetic events also shared by MGUS[13, 14]. Common secondary driver genetic events that can arise early or late in the natural history of the disease, include activation of proto-oncogenes (e.g. *RAS* and *MYC)*, activation of NFkB and MAPK pathways, tumour suppressor gene inactivation (e.g. *TP53* and *RB*) and copy number variations (deletions and amplifications)[13, 14].

The compendium of genetic lesions in MM was recently expanded by whole genome and exome sequencing of nearly 500 primary human myeloma samples[15-20] and is expected to reach saturation, i.e., identification of all driver mutations with frequency of >1% when ≈2000 samples are sequenced[21]. Although only a small number of new recurrent genetic lesions (e.g., *RIS, FMC34C*) were added by these studies[15-20], the emerging picture suggests a dynamic genetic landscape diversified in space and in time by linear or 'branching' Darwinian clonal evolution patterns [20, 22-24]. This extensive genetic heterogeneity can be observed even at diagnosis and is pertinent not only to MM but to several other types of tumours. Moreover, it suggests that individualized therapy aiming to target specific genetic lesions will be a rather challenging vision to realize in clinical practice calling for a 'post-genomics' understanding of the biology of MM.

Mature B lineage development and MM
After they emerge from BM, B cells localise to secondary lymphoid organs as naïve B cells or they differentiate into marginal zone B cells. These early mature B cells have completed rearrangement of their VDJ and VJ gene segments at the IgH and IgL loci respectively and express surface IgM/IgD **(Figure 1).** Once nominal antigen is encountered, naïve B cells enter the germinal centre (GC) and in a T cell-dependent manner undergo somatic hypermutation required for Ig affinity maturation and class switch recombination (CSR) required for isotype switch (from IgM to IgG or IgA). Such B cells emerge from the GC to join the pool of memory B cells which are required for the recall antigen response or they continue their differentiation into PC[25-28]. This final stage of B cell differentiation proceeds through generation of plasmablasts which leave the secondary lymphoid organs to migrate back to BM where terminal differentiation to mature PC takes place **(Figure 1)**.

The process of PC maturation proceeds with extinguishing of the B cell transcriptional programme instructed by transcription factors (TF) such as PAX5 and by its replacement with a PC-specific programme activated by the TF IRF4 and BLIMP1[29]. XBP1, a critical transcriptional target of BLIMP1, is itself a TF responsible for activation of genes required for the development of the cellular machinery that will anticipate and ensure the efficient translation, folding and secretion by normal or malignant PC of large quantities of Ig. This so called unfolded protein response (UPR) ensures increased capacity for endoplasmic reticulum (ER) protein folding and ER-associated degradation (ERAD) of misfolded and oxidised proteins by the ubiquitin-proteasome system (UPS)[30]. If UPR is overloaded beyond its capacity, terminal ER stress ensues resulting in cell death[31].

Mature PC (normal and myeloma) co-express high levels of CD38 and CD138 while plasmablasts are CD138-CD38+[32]. However, somewhat paradoxically, while 50-80% of normal PC retain expression of CD19[33], a PAX5-dependent gene, >95% of MM cases are CD19-ve[32].

Defining MM propagating cells. Guided by the above outlined differentiation pathway and taking advantage of the unique molecular marker of clonality afforded by the IgH

complementarity determining region (CDR3) fingerprint, investigators searched for putative populations with MMPA as early as in 1970's[34, 35], i.e., well before the current debate about cancer stem cells was resurrected by Dick and colleagues (reviewed in[36]). The weight of evidence, despite earlier reports that MMPA is found in CD34+CD19+ i.e., progenitor cells[37, 38], strongly suggests that MM arises from a cell of post-GC origin and consequently MMPC would be expected to be found in the same pool. Indeed molecular analysis of IgH CSR in the bulk myeloma PC has consistently shown single recombination events and identical clonotypic IgH CDR3 regions shared by mature B cells, plasmablasts and of course the myeloma PC themselves.

Cellular clonotypic hierarchy in MM and myeloma propagating activity

In early work the frequency of clonotypic B cells in myeloma was reported as high as 66% [39]. However, more recent and robust approaches that combine flow-sorting with genomic DNA quantitative PCR highly specific for the clonotypic IgH CDR3 region show that although clonotypic CD19+ cells are easily detected amongst plasmablasts and memory (but not naïve or progenitor) B cells, their frequency is considerably lower (<5%)[40-43]

The question arose whether these clonotypic B cells (memory or plasmablasts) could initiate or propagate myeloma or this was an exclusive property of the myeloma PC which represent the main bulk of the tumour. Closely linked and perhaps of greater importance from a clinical perspective is the question whether these populations are the source of or contribute to drug resistance and disease relapse following initially successful therapy. Cells with the ability to propagate myeloma, as well as displaying MMPA in animal models, at the very minimum, they would be expected to be clonotypic and also harbour myeloma initiating genetic events and some of the secondary driver genetic events.

Before we address these issues in the next section, we will briefly touch upon the biological features of a recently characterised member of the myeloma-related cellular hierarchy, namely the CD138- clonotypic cells we termed pre-PC[43].

While the bulk of the tumour cells in MM express CD138 (an indispensible marker for MM diagnosis), careful high resolution phenotypic analysis reveals the presence of a small fraction (<3%) that is CD138low/- but retains expression of other MM-defining phenotypic features e.g., expression of CD56 and lack of expression of CD19. This CD138- population was previously thought to represent apoptotic PC that had shed their surface CD138[44-46]. Careful re-examination in fresh samples showed that CD138- cells are actually live cells, lack markers of apoptosis and have distinct phenotypic, cellular and molecular features. Specifically, they can be induced to differentiate into mature CD138+ PC in vitro in the presence of MM-promoting growth factors, size-wise and morphologically they resemble more small lymphocytes rather than mature myeloma PC and are mainly resting rather in cell cycle; lastly, they are entirely clonotypic and share the same myeloma-initiating and secondary oncogenic driver genetic events with myeloma PC, at least as defined by FISH analysis[43].

Of note, a population sharing the immuophenotypic features of pre-PC has also been identified in peripheral blood (PB) of normal donors[43, 47] suggesting that the myeloma clonotypic cellular architecture emulates that of normal late B cell development.

Evidence of MMPA in xenograft models. The MMPA of clonotypic B cells, myeloma PC and more recently of pre-PC has been extensively tested in vivo in xenograft models of immunodeficient mice, in some cases grafted with human fetal or rabbit bones[48-51] [52] **(Table 1)**. It should be noted here that because myeloma is by and large a slow proliferative tumour (no more than <4% of PC are in cycle at any time[53]), use of the highly proliferative myeloma cell lines for assessment of MMPA is not appropriate and thus relevant work is not discussed here.

Despite controversy regarding the in vivo potential of memory B cells to propagate myeloma[54-57], the weight of earlier and more recent evidence strongly supports the notion

that clonotypic B cells are devoid of MMPA in animal models[43, 48, 50, 51]. However, all xenograft models have their shortcomings and in particular they have limited ability to support survival of mature human B cells. Perhaps new generations of humanised models such as the HLA-DR4.RagKO.IL2RγcKO.NOD (DRAG) mice[58] which allow expansion of mature B and T cells would provide a more appropriate context to definitively address the MMPA of clonotypic B cells. By contrast, the evidence showing that the bulk of CD138+ myeloma PC can propagate myeloma in vivo is strong and can be regarded definitive [43, 48, 50, 51, 59]. Secondary and tertiary engraftment experiments also allude to self-renewing ability of cells with MMPA present in the myeloma PC [43, 50, 51]. However, exact quantitative analysis by limiting dilution of the MMPC in MM has not been performed, largely because of the generally low rates and levels of engraftment of primary myeloma PC.

Detailed ex vivo analysis of the myeloma BM clonotypic hierarchy that included the pre-PC prompted application of mathematical modeling which sought to fit the observed frequencies of clonotypic, myeloma-related populations[43]. This theoretical approach suggested a bidirectional transition between pre-PC and PC that did not involve the clonotypic B cell fraction. The predictions of the model were confirmed in xenograft assays in which both highly purified PC and Pre-PC myeloma populations engrafted in immunosuppressed mice and importantly both recreated the pre-PC-PC hierarchy seen in the patient BM. Such transitions are highly suggestive of epigenetic plasticity, a notion further supported by the identification in pre-PC of a transcriptional signature enriched in chromatin binding proteins and modifiers including members of the Polycomb complex. Deciphering the exact underpinning epigenetic mechanisms may provide opportunities to therapeutically target the pre-PC-PC transition using the increasingly enriched armamentarium of epigenetic therapies such as Polycomb and BET protein inhibitors[60, 61]. In support of the MMPA activity of pre-PC, Hosen and colleagues had already shown the ability of CD138- as well as of CD138+ PC to engraft myeloma in a rab-SCID model[50].

Clonotypic hierarchy and the origins of the primary and driver genetic events in myeloma

For a clonotypic population to be ascribed with MMPA it would be expected to harbour driver genetic events as well as the specific myeloma initiating event(s) that are present in the myeloma PC. Primary genetic events such as t(11;14) are easily identified by FISH in pre-PC and CD19+ plasmablasts. Aberrant oncogenic transcripts of *MMSET* and *CCND1* as well as *IGH-MMSET* hybrid transcripts have also been reported in CD19+ memory B cells using cell limiting dilution analysis and translocation-specific RT-PCR[62].

Structural analysis of myeloma-associated translocations has shown that in some cases of t(11;14), breakpoints may be found within the D-J gene regions consistent with the translocation arising in progenitor cells (i.e., when D-J recombination takes place during normal B cell development) rather than post-GC B cells[63]. This finding, if experimentally proven, would suggest that B cells carrying oncogenic translocations retain their ability to undergo GC selection. However, even in such a scenario, it is clear from the genetic analysis of the IgH CDR3 region and CSR events, myelomatous transformation is a germinal/post-germinal centre event and MMPA can only be ascribed to clonotypic post-GC cells. These would be expected to carry at least some of the driver mutational events that are present in the myeloma PC themselves. Whether this is the case has not been systematically addressed. The study mentioned above which clearly demonstrated myeloma-associated primary oncogene expression in memory B cells, failed to show presence of oncogenic *K-RAS* in highly purified memory B cells of 7 patients in whom mutated *K-RAS* had been identified as a driver genetic event in the myeloma PC[62].

Correlation of MMPA with drug resistance in vivo and with myeloma relapse

Ultimately, the biological properties of myeloma clonotypic hierarchy and MMPC will have to be viewed from a clinical perspective, namely whether they are relevant to drug resistance and disease relapse. Although drug resistant cells would be expected to overlap with MMPC, this shouldn't necessarily be always the case.

Current therapeutic approaches in myeloma can induce deep remissions, even complete molecular responses in up to 30% of patients. Yet, nearly all patients eventually experience relapse. Genetic analysis of relapse myeloma samples reveals driver genetic events that often pre-existed and were selected by treatment[19, 20, 23, 24]. It would be logical therefore to assume that the pool of myeloma clonotypic cells persisting after therapy, no matter how small, is highly enriched in both drug resistant cells and cells with MMPA and these will be the origin of disease relapse. Recent work has shed some light in the relationship between MM propagating and drug-resistant clonotypic cells.

Chaidos and colleagues studied prospectively 8 patients before treatment and at remission. Independent of treatment type and genetic profile, they found the expected precipitous decrease of the myeloma BM PC at remission[43]. However, this decrease was considerably less pronounced in the pre-PC and clonotypic B cell compartments, thus identifying these populations as relative more drug resistant than myeloma PC. The relative quiescent status of pre-PC might account for their drug resistance while their ability to differentiate to myeloma PC in vivo strongly suggests that these cells are likely to be an additional source of relapse in vivo. Pertinently, in animal models, pre-PC appear to be the dominant population identified in extramedullary sites such as the spleen and liver[43] raising the prospect that drug-resistant cells are favoured by particular types of microenvironment.

Further advances were described in an interesting study in which Leung-Hagesteijn et al found that in some patients, resistance to the proteasome inhibitor (PI) Bortezomib is linked to cells with the pre-PC or plasmablast phenotype sharing the same primary genetic events with the myeloma PC[64]. Mechanistically, bortezomib resistance was due to a lower activity of the IRE-XBP1s axis in pre-PC/plasmablasts reflecting a lower ER stress burden than in mature myeloma PC and thus lower vulnerability to ER stress-induced cell death triggered by bortezomib-mediated UPS inhibition. Strikingly, analysis of BM from some patients in remission following PI treatment or progression while on PI treatment showed selective presence of the myeloma-related pre-PC and plasmablast populations supporting the notion that these populations, like conventional myeloma PC are harbingers of drug resistance and relapse[64].

Highlighting a further level of complexity, a more recent study demonstrated in 30% of MM patients further phenotypic and genetic heterogeneity within the CD138+ myeloma PC linked to differential chemoresistance and in vitro clonogenic capacity[65].

Integrating cellular and genetic approaches to identify and characterise MMPC and drug resistance in MM

Complete remissions in myeloma are associated with improved progression free survival and eventually are likely to lead to improved overall survival[66, 67]. While new drugs and their combinations increasingly achieve this, the focus should shift on maintaining remissions by targeting residual disease which as outlined above is likely to be enriched in drug resistant cells and cells with MMPA driving relapse. While current therapeutic strategies increasingly incorporate long-term maintenance following remission with drugs such as bortezomib and lenalidomide[68, 69], this is not informed by the biological profile of residual myeloma-related cell populations. Therefore, we propose that detailed, multi-dimensional characterisation of residual myeloma cells should provide insights into the biology of drug resistance and MMPA would allow use of patient-specific therapeutic approaches. This approach is increasingly feasible with the advent of the new high throughput technologies in the fields of genomics and phenomics[70] which allow high resolution interrogation of the genetic make up, epigenome, transcriptome and immunophenotype of cancers cells at the population and single cell level. However, this will not be sufficient; it will require to be complemented by data integration and interpretation by means of systems biology and modelling to identify genes and gene regulatory networks that underpin the functional basis of drug resistance and MMPA within the myeloma clonotypic architecture in individual patients and with reference to the non-malignant cellular counterparts. This could inform novel clinical approaches and

guide further experimental work which will include the use of improved xenograft assays in increasingly humanised models.

Another important parameter in this approach would be the ability to interrogate more than one clinical sample in a temporal and spatial manner. Tumour diversification proceeds in 3 dimensions[71, 72] i.e., in time, (diagnosis vs remission/relapse); space (bone marrow vs distant disease) and within the cell architectural space of the tumour itself. Capturing and documenting genetic and functional heterogeneity in myeloma, especially at the stage of post-treatment remission will require sampling of BM from more than one anatomical site and also study of circulating myeloma PC and other clonotypic subsets (these can be readily identified even when the BM appears to be in complete molecular remission[43]). Additionally, facilitated by recent advances in clinical imaging technologies[73], extramedullary disease is becoming increasingly more accessible and safer to sample. If such bold and invasive approaches that exceed current clinical practices were to be adopted they would require not just a clear scientific vision and specific objectives but also a robust partnership between investigators and patients themselves.

Concluding remarks

While MMPA and the closed linked issues of drug resistance and disease relapse are features of myeloma PC, highly analytical approaches have demonstrated the potential of upstream clonotypic populations to contribute to MMPA and drug resistance. These populations comprise a phenotypic and functional hierarchy which mirrors that of the late B cell development and entail important implications for how minimal residual disease (MRD) is assessed in MM[74, 75] and how certain therapeutic approaches might promote their persistence. For example flow-cytometric analysis of MRD status post-treatment may underestimate disease burden if pre-PC cells are not assessed and a therapeutic mAb against CD138 would be predicted to select for the potentially drug-resistant CD138- pre-PC population. By contrast, targeting of the surface molecules CD319 (SLAM7) and CD38 which are stably expressed across the whole myeloma clonotypic hierarchy[43] would be expected to have a significant impact on depth and length of remission after treatment with suitably active mAb that are currently in clinical development[76, 77]. More importantly, the objective of improving further overall survival of patients with MM through a rational use of current and novel therapeutic agents, calls for a detailed, integrated and multidimensional approach in the analysis of the immunophenotypic, genetic, epigenetic and transcriptome profiles of MMPC.

Acknowledgments

Work in the lab of AK has been supported by Leukaemia and Lymphoma Research, the Kay Kendall Leukaemia Fund, Leuka and the NIHR Biomedical Research Centre at Imperial College London.

Conflict of interest

The authors have no relevant conflicts of interest to declare.

Reference List

1. Anderson KC, Carrasco RD. Pathogenesis of myeloma. **AnnuRev Pathol**. 2011;6:249-274.
2. Rollig C, Knop S, Bornhauser M. Multiple myeloma. **Lancet**. 2014.
3. Bianchi G, Munshi NC. Pathogenesis beyond the cancer clone(s) in multiple myeloma. **Blood**. 2015.
4. Fowler JA, Edwards CM, Croucher PI. Tumor-host cell interactions in the bone disease of myeloma. **Bone**. 2011;48:121-128.
5. Terpos E, Sezer O, Croucher P et al. Myeloma bone disease and proteasome inhibition therapies. **Blood**. 2007;110:1098-1104.
6. Valent P, Bonnet D, De Maria R et al. Cancer stem cell definitions and terminology: the devil is in the details. **Nat Rev Cancer**. 2012;12:767-775.
7. Saltarella I, Lamanuzzi A, Reale A et al. Identify multiple myeloma stem cells: Utopia? **World journal of stem cells**. 2015;7:84-95.
8. Kellner J, Liu B, Kang Y et al. Fact or fiction--identifying the elusive multiple myeloma stem cell. **Journal of hematology & oncology**. 2013;6:91.
9. Abe M, Harada T, Matsumoto T. Concise review: Defining and targeting myeloma stem cell-like cells. **Stem Cells**. 2014;32:1067-1073.
10. Hosen N. Multiple myeloma-initiating cells. **International journal of hematology**. 2013;97:306-312.
11. Hajek R, Okubote SA, Svachova H. Myeloma stem cell concepts, heterogeneity and plasticity of multiple myeloma. **Br J Haematol**. 2013;163:551-564.
12. Kyle RA, Therneau TM, Rajkumar SV et al. Prevalence of monoclonal gammopathy of undetermined significance. **N Engl J Med**. 2006;354:1362-1369.
13. Kuehl WM, Bergsagel PL. Molecular pathogenesis of multiple myeloma and its premalignant precursor. **Journal of Clinical Investigation**. 2012;122:3456-3463.
14. Morgan GJ, Walker BA, Davies FE. The genetic architecture of multiple myeloma. **Nat Rev Cancer**. 2012;12:335-348.
15. Chapman MA, Lawrence MS, Keats JJ et al. Initial genome sequencing and analysis of multiple myeloma. **Nature**. 2011;471:467-472.
16. Walker BA, Wardell CP, Melchor L et al. Intraclonal heterogeneity and distinct molecular mechanisms characterize the development of t(4;14) and t(11;14) myeloma. **Blood**. 2012;120:1077-1086.
17. Egan JB, Shi CX, Tembe W et al. Whole-genome sequencing of multiple myeloma from diagnosis to plasma cell leukemia reveals genomic initiating events, evolution, and clonal tides. **Blood**. 2012;120:1060-1066.
18. Keats JJ, Chesi M, Egan JB et al. Clonal competition with alternating dominance in multiple myeloma. **Blood**. 2012;120:1067-1076.
19. Bolli N, Avet-Loiseau H, Wedge DC et al. Heterogeneity of genomic evolution and mutational profiles in multiple myeloma. **Nature communications**. 2014;5:2997.
20. Lohr JG, Stojanov P, Carter SL et al. Widespread genetic heterogeneity in multiple myeloma: implications for targeted therapy. **Cancer Cell**. 2014;25:91-101.
21. Lawrence MS, Stojanov P, Mermel CH et al. Discovery and saturation analysis of cancer genes across 21 tumour types. **Nature**. 2014;505:495-501.
22. Greaves M, Maley CC. Clonal evolution in cancer. **Nature**. 2012;481:306-313.
23. Melchor L, Brioli A, Wardell CP et al. Single-cell genetic analysis reveals the composition of initiating clones and phylogenetic patterns of branching and parallel evolution in myeloma. **Leukemia**. 2014.
24. Walker BA, Wardell CP, Melchor L et al. Intraclonal heterogeneity is a critical early event in the development of myeloma and precedes the development of clinical symptoms. **Leukemia**. 2014;28:384-390.
25. Oracki SA, Walker JA, Hibbs ML et al. Plasma cell development and survival. **Immunol Rev**. 2010;237:140-159.
26. Allman D, Pillai S. Peripheral B cell subsets. **Curr Opin Immunol**. 2008;20:149-157.
27. Pieper K, Grimbacher B, Eibel H. B-cell biology and development. **The Journal of allergy and clinical immunology**. 2013;131:959-971.

28. McHeyzer-Williams LJ, McHeyzer-Williams MG. Antigen-specific memory B cell development. **Annu Rev Immunol**. 2005;23:487-513.
29. Nutt SL, Taubenheim N, Hasbold J et al. The genetic network controlling plasma cell differentiation. **Semin Immunol**. 2011;23:341-349.
30. Hetz C. The unfolded protein response: controlling cell fate decisions under ER stress and beyond. **Nat Rev Mol Cell Biol**. 2012;13:89-102.
31. Auner HW, Cenci S. Recent advances and future directions in targeting the secretory apparatus in multiple myeloma. **Br J Haematol**. 2015;168:14-25.
32. Rawstron AC, Orfao A, Beksac M et al. Report of the European Myeloma Network on multiparametric flow cytometry in multiple myeloma and related disorders. **Haematologica**. 2008;93:431-438.
33. Mei HE, Wirries I, Frolich D et al. A unique population of IgG-expressing plasma cells lacking CD19 is enriched in human bone marrow. **Blood**. 2015;125:1739-1748.
34. Mellstedt H, Hammarstrom S, Holm G. Monoclonal lymphocyte population in human plasma cell myeloma. **Clin Exp Immunol**. 1974;17:371-384.
35. Warner TF, Krueger RG. Circulating lymphocytes and the spread of myeloma. Review of the evidence. **Lancet**. 1978;1:1174-1176.
36. Kreso A, Dick JE. Evolution of the cancer stem cell model. **Cell Stem Cell**. 2014;14:275-291.
37. Pilarski LM, Belch AR. Clonotypic myeloma cells able to xenograft myeloma to nonobese diabetic severe combined immunodeficient mice copurify with CD34 (+) hematopoietic progenitors. **ClinCancer Res**. 2002;8:3198-3204.
38. Szczepek AJ, Bergsagel PL, Axelsson L et al. CD34+ cells in the blood of patients with multiple myeloma express CD19 and IgH mRNA and have patient-specific IgH VDJ gene rearrangements. **Blood**. 1997;89:1824-1833.
39. Szczepek AJ, Seeberger K, Wizniak J et al. A high frequency of circulating B cells share clonotypic Ig heavy-chain VDJ rearrangements with autologous bone marrow plasma cells in multiple myeloma, as measured by single-cell and in situ reverse transcriptase-polymerase chain reaction. **Blood**. 1998;92:2844-2855.
40. Rasmussen T, Jensen L, Johnsen HE. Levels of circulating CD19+ cells in patients with multiple myeloma. **Blood**. 2000;95:4020-4021.
41. Rasmussen T, Jensen L, Johnsen HE. The CD19 compartment in myeloma includes a population of clonal cells persistent after high-dose treatment. **Leukemia & lymphoma**. 2002;43:1075-1077.
42. Rasmussen T, Lodahl M, Hancke S et al. In multiple myeloma clonotypic CD38-/CD19+ / CD27+ memory B cells recirculate through bone marrow, peripheral blood and lymph nodes. **Leukemia & lymphoma**. 2004;45:1413-1417.
43. Chaidos A, Barnes CP, Cowan G et al. Clinical drug resistance linked to interconvertible phenotypic and functional states of tumor-propagating cells in multiple myeloma. **Blood**. 2013;121:318-328.
44. Reid S, Yang S, Brown R et al. Characterisation and relevance of CD138-negative plasma cells in plasma cell myeloma. **International journal of laboratory hematology**. 2010;32:e190-196.
45. Chiron D, Surget S, Maiga S et al. The peripheral CD138+ population but not the CD138- population contains myeloma clonogenic cells in plasma cell leukaemia patients. **Br J Haematol**. 2012;156:679-683.
46. Christensen JH, Jensen PV, Kristensen IB et al. Characterization of potential CD138 negative myeloma "stem cells". **Haematologica**. 2012;97:e18-20.
47. Caraux A, Klein B, Paiva B et al. Circulating human B and plasma cells. Age-associated changes in counts and detailed characterization of circulating normal C. **Haematologica**. 2010;95:1016-1020.
48. Yaccoby S, Epstein J. The proliferative potential of myeloma plasma cells manifest in the SCID-hu host. **Blood**. 1999;94:3576-3582.
49. Epstein J, Yaccoby S. The SCID-hu myeloma model. **Methods in molecular medicine**. 2005;113:183-190.
50. Hosen N, Matsuoka Y, Kishida S et al. CD138-negative clonogenic cells are plasma cells but not B cells in some multiple myeloma patients. **Leukemia**. 2012.

51. Kim D, Park CY, Medeiros BC et al. CD19-CD45 low/- CD38 high/CD138+ plasma cells enrich for human tumorigenic myeloma cells. **Leukemia**. 2012;26:2530-2537.
52. Yata K, Yaccoby S. The SCID-rab model: a novel in vivo system for primary human myeloma demonstrating growth of CD138-expressing malignant cells. **Leukemia**. 2004;18:1891-1897.
53. Drewinko B, Alexanian R, Boyer H et al. The growth fraction of human myeloma cells. **Blood**. 1981;57:333-338.
54. Matsui W, Wang Q, Barber JP et al. Clonogenic multiple myeloma progenitors, stem cell properties, and drug resistance. **Cancer Res**. 2008;68:190-197.
55. Peacock CD, Wang Q, Gesell GS et al. Hedgehog signaling maintains a tumor stem cell compartment in multiple myeloma. **ProcNatlAcadSciUSA**. 2007;104:4048-4053.
56. Matsui W, Huff CA, Wang Q et al. Characterization of clonogenic multiple myeloma cells. **Blood**. 2004;103:2332-2336.
57. Pilarski LM, Seeberger K, Coupland RW et al. Leukemic B cells clonally identical to myeloma plasma cells are myelomagenic in NOD/SCID mice. **ExpHematol**. 2002;30:221-228.
58. Danner R, Chaudhari SN, Rosenberger J et al. Expression of HLA class II molecules in humanized NOD.Rag1KO.IL2RgcKO mice is critical for development and function of human T and B cells. **PLoS ONE**. 2011;6:e19826.
59. Chiron D, Maiga S, Surget S et al. Autocrine insulin-like growth factor 1 and stem cell factor but not interleukin 6 support self-renewal of human myeloma cells. **Blood cancer journal**. 2013;3:e120.
60. Knutson SK, Wigle TJ, Warholic NM et al. A selective inhibitor of EZH2 blocks H3K27 methylation and kills mutant lymphoma cells. **Nature chemical biology**. 2012;8:890-896.
61. Chaidos A, Caputo V, Gouvedenou K et al. Potent antimyeloma activity of the novel bromodomain inhibitors I-BET151 and I-BET762. **Blood**. 2014;123:697-705.
62. Rasmussen T, Haaber J, Dahl IM et al. Identification of translocation products but not K-RAS mutations in memory B cells from patients with multiple myeloma. **Haematologica**. 2010;95:1730-1737.
63. Walker BA, Wardell CP, Johnson DC et al. Characterization of IGH locus breakpoints in multiple myeloma indicates a subset of translocations appear to occur in pregerminal center B cells. **Blood**. 2013;121:3413-3419.
64. Leung-Hagesteijn C, Erdmann N, Cheung G et al. Xbp1s-negative tumor B cells and pre-plasmablasts mediate therapeutic proteasome inhibitor resistance in multiple myeloma. **Cancer Cell**. 2013;24:289-304.
65. Paino T, Paiva B, Sayagues JM et al. Phenotypic identification of subclones in multiple myeloma with different chemoresistant, cytogenetic and clonogenic potential. **Leukemia**. 2014.
66. Kapoor P, Kumar SK, Dispenzieri A et al. Importance of achieving stringent complete response after autologous stem-cell transplantation in multiple myeloma. **J Clin Oncol**. 2013;31:4529-4535.
67. Kumar SK, Lee JH, Lahuerta JJ et al. Risk of progression and survival in multiple myeloma relapsing after therapy with IMiDs and bortezomib: A multicenter international myeloma working group study. **Leukemia**. 2012;26:149-157.
68. Attal M, Lauwers-Cances V, Marit G et al. Lenalidomide maintenance after stem-cell transplantation for multiple myeloma. **N Engl J Med**. 2012;366:1782-1791.
69. McCarthy PL, Owzar K, Hofmeister CC et al. Lenalidomide after stem-cell transplantation for multiple myeloma. **N Engl J Med**. 2012;366:1770-1781.
70. Bendall SC, Nolan GP. From single cells to deep phenotypes in cancer. **Nature biotechnology**. 2012;30:639-647.
71. Gerlinger M, Rowan AJ, Horswell S et al. Intratumor heterogeneity and branched evolution revealed by multiregion sequencing. **N Engl J Med**. 2012;366:883-892.
72. Longo DL. Tumor heterogeneity and personalized medicine. **N Engl J Med**. 2012;366:956-957.

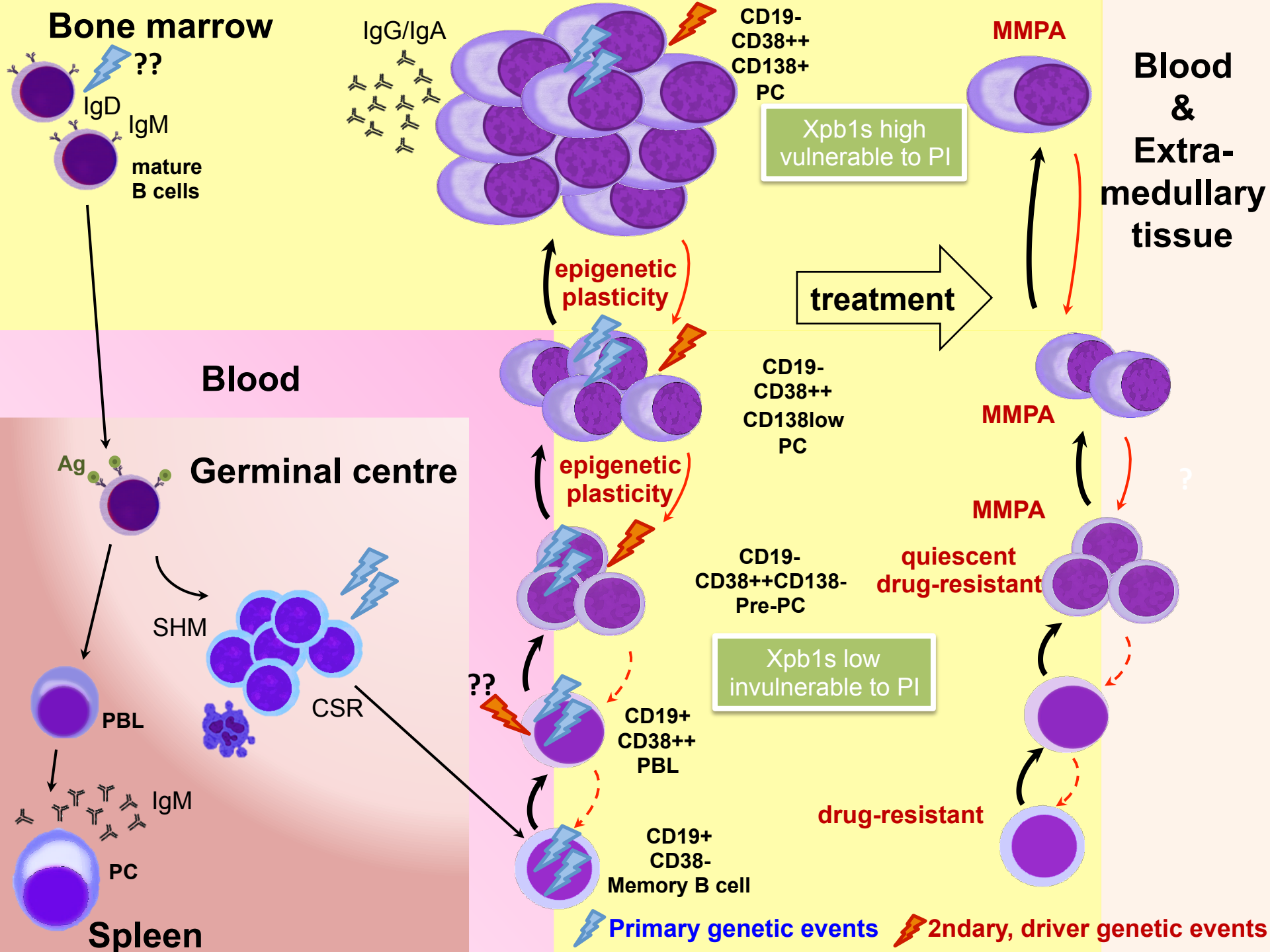
73. Zamagni E, Cavo M. The role of imaging techniques in the management of multiple myeloma. **Br J Haematol**. 2012;159:499-513.
74. Mailankody S, Korde N, Lesokhin AM et al. Minimal residual disease in multiple myeloma: bringing the bench to the bedside. **Nature reviews Clinical oncology**. 2015;12:286-295.
75. Stetler-Stevenson M, Paiva B, Stoolman L et al. Consensus guidelines for myeloma minimal residual disease sample staining and data acquisition. **Cytometry Part B, Clinical cytometry**. 2015.
76. Laubach JP, Richardson PG. CD38-Targeted Immunochemotherapy in Refractory Multiple Myeloma: A New Horizon. **Clin Cancer Res**. 2015;21:2660-2662.
77. Hsi ED, Steinle R, Balasa B et al. CS1, a Potential New Therapeutic Antibody Target for the Treatment of Multiple Myeloma. **Clinical Cancer Research**. 2008;14:2775-2784.

Table 1. Phenoytpes of bone marrow cells with in vivo myeloma propagating activity

Immunophenotype (bone marrow)	MMPA	Mouse Model	Ref
$CD38^{+}CD45^{-}$ PC	Yes	SCID-hu	48, 49
CD34+CD45loCD19-	Yes	NOD/SCID	37
CD138+ PC	Yes	SCID-rab	52
$CD20^{+}CD27^{+}CD34^{-}CD138^{-}$ $CD20^{+}CD27^{+}CD34^{-}CD138^{+}$	Yes No	NOD/SCID	54, 56
$CD19^{-}CD45^{low/-}CD38^{high}CD138^{+}$ CD19+	Yes No	RAG2-/gc- or NSG-hu	51
$CD19^{-}CD38^{+}CD138^{+}$ $CD19\text{-}CD38^{+}CD138^{-}$ $CD19^{+}CD34^{-}$	Yes Yes No	SCID-rab	10
$Lin^{-}CD19^{-}CD4^{5low/-}CD38^{high}CD138^{+}$ $Lin^{-}CD19^{-}CD45^{low/-}CD38^{high}CD138^{+}$ $CD19^{+}CD34^{-}$	Yes Yes No	NSG	43

Figure legend

Figure 1. Integrated view of myeloma clonotypic hierarchy in relation to MM propagating activity and drug resistance. The myeloma clonotypic cellular constituents arise from post-germinal centre mature B cells, having experienced somatic hypermutation (SMH) and class-switch recombination (CSR). Primary genetic events, namely IGH gene rearrangements and/or hyperdiploidy, are thought to occur during SMH and CSR, when cells are more permissive to genetic damage. Genetic analysis suggests that this could also occur at earlier developmental stages-however, it remains to be addressed directly. Secondary driver genetic events, involving Myc, RAS, p53, NFκB pathway genes and copy-number variations become evident at later stages of the disease but whether they can arise upstream of the pre-PC level would require further investigation. CD138+ plasma cells (PC) comprise the bulk of myeloma clone, possess myeloma propagating activity (MMPA) and exist in an equilibrium of bi-directional transition, likely directed by epigenetic mechanisms, with the rarer, immunophenotypically and morphologically distinct CD19-CD138- Pre-PC. Although Pre-PC demonstrate MMPA as well, they are more quiescent and relatively more drug resistant compared to PC. CD19+CD38-/CD38+ cells (i.e., memory B cells and plasmablasts) are at the apex of the myeloma clonotypic hierarchy. Along with Pre-PC, they show lower IRE-XPB1s activity than PC, they are less vulnerable to ER stress and proteasome inhibition (PI), which may result in failure to eradicate the disease and thus lead to relapse.


Bone marrow
??
IgD
IgM
mature
B cells
IgG/IgA
CD19-
CD38++
CD138+
PC
MMPA
Xpb1s high
vulnerable to PI
Blood
&
Extra-
medullary
tissue
epigenetic
plasticity
treatment
Blood
CD19-
CD38++
CD138low
PC
MMPA
Ag
Germinal centre
epigenetic
plasticity
MMPA
?
CD19-
CD38++CD138-
Pre-PC
quiescent
drug-resistant
SHM
CSR
PBL
Xpb1s low
invulnerable to PI
??
CD19+
CD38++
PBL
IgM
PC
drug-resistant
CD19+
CD38-
Memory B cell
Spleen
Primary genetic events
2ndary, driver genetic events