19 - Clonal evolution in multiple myeloma

Summary:
- Multiple myeloma is a clonally diverse disease, with multiple clones present at diagnosis.
- Clones may express intact immunoglobulin, free light chains, both, or rarely, neither.
- Changes in clonal dominance may be reflected by changes in monoclonal immunoglobulin production.
- Free light chain escape is an example of clonal evolution, first characterised over 40 years ago.

19.1. Introduction

Monoclonal gammopathy of undetermined significance (MGUS; Chapter 13) and smouldering multiple myeloma (SMM; Chapter 14) are asymptomatic plasma cell dyscrasias, with a propensity to progress to symptomatic multiple myeloma (MM). Genetic analysis has demonstrated that the pre-malignant conditions share some of the mutations associated with MM, and that multiple genetic “hits” are required for the progression to symptomatic disease [1]. Such mutations may be acquired in a linear fashion (Figure 19.1A), and others through branching, non-linear pathways (Figure 19.1B) [2][3]. Sequencing studies have shown that at MM presentation, the median number of exonic mutations is approximately 60, but this varies greatly between individuals from 10 to more than 500 [3].

During the evolution of MM, tumour clones may acquire further genetic abnormalities, e.g. to allow them to further expand or compete for stromal niches within the bone marrow, such that within an individual patient, multiple genetically distinct clones may be present. Furthermore, the clonal composition changes during the course of the disease, giving rise to “tides” of myeloma clones which compete for dominance in a landscape that is continually changed by therapy [2]. In this chapter we review the current understanding of myeloma clonal evolution and discuss the importance of monoclonal protein measurements in surveying these changes.

19.2. Clonal populations in multiple myeloma
MM patients can be classified according to their monoclonal protein type, i.e. intact immunoglobulin MM (IIMM; Chapter 17), light chain MM (LCMM; Chapter 15) and nonsecretory MM (NSMM; Chapter 16) \[^5\]. At presentation, 95\% of IIMM patients also have an abnormal κ/λ serum free light chain (sFLC) ratio (Section 17.2). The simplest interpretation is that most IIMM patients have a single MM clone producing monoclonal intact immunoglobulin plus free light chains (FLCs). However, specific methods of plasma cell staining have challenged this idea. Ayliffe et al. \[^4\][\[^6\]\] performed double immunofluorescence staining to study immunoglobulin heavy chain and light chain expression by plasma cells in bone marrow biopsies. The majority of patients had a single tumour cell population that expressed either monoclonal intact immunoglobulins and FLCs (42\%), intact immunoglobulins alone (32\%), or FLCs alone (8\%). However, in the remaining 18\% of patients, separate clones expressing either intact immunoglobulin or FLC only were identified (Figure 19.2). These dual clonal populations were the first indicators that, within a single patient, multiple clones expressing different monoclonal proteins could be found.

Subsequently, using array comparative genomic hybridisation (aCGH) and fluorescence in situ hybridisation (FISH), Keats et al. \[^7\]\ elegantly demonstrated the presence of multiple clones in a patient with IgA MM. The impact of these multiple clones is illustrated in the clinical case history below. Further evidence for clonal heterogeneity of MM tumours was demonstrated by Lohr et al. \[^8\]\ who conducted a next generation sequencing study of plasma cell populations from 203 patients with newly diagnosed MM. The authors concluded that most MM patients have at least three minor subclonal populations in addition to the dominant tumour clone. This means that whilst most tumour plasma cells share a common pool of mutations, subclones are present that differ from one another by several mutations \[^3\]\.

The effectiveness of current anti-myeloma therapies on different clonal populations has been investigated by Campbell and colleagues \[^9\]\. The study evaluated 44 patients with biclonal MM enrolled in a series of phase III clinical trials (Myeloma IX, Myeloma XI, and TEAMM). In 32\% (14/44) of patients, the monoclonal proteins corresponding to the dominant and minor clones exhibited a similar response to therapy. However, in 43\% (19/44) of cases, the response of the dominant tumour clone was greater. For example, a very good partial response or higher was achieved for 61\% (27/44) dominant clones but only 45\% (20/44) minor clones (p=0.002). Therefore, anti-myeloma therapies may exert a differential effect against dominant and minor tumour cell clones, this is illustrated in the clinical case history below.

Campbell et al. \[^9\]\ also monitored 31 biclonal MM patients in disease remission. Whilst monoclonal protein concentrations corresponding to the minor clone were initially stable in the majority (90\%) of cases, 50\% of patients had a relapse of their dominant clone. The authors suggest that response of minor clones to anti-myeloma therapy is of greater duration while that of major clones is of greater depth.

19.3. Clonal changes and clonal escape in multiple myeloma

The earliest observation of changes in monoclonal protein production during MM disease evolution was published by J. R. Hobbs in 1969 from his analysis of the first MRC MM trial \[^10\]\. He described the phenomenon of Bence Jones escape (FLC escape) in 15 patients at disease relapse, in which the serum intact immunoglobulin concentration reduced, but Bence Jones proteinuria dramatically increased. This phenomenon is further discussed in Section 18.2.1.
In accord with Ayliffe’s phenotyping of clones based on their protein expression\(^8\), both Hobbs\(^9\) and Brioli\(^13\) used serum protein measurements as indicators of clonal change during the course of a patient’s disease. In an analysis of the MRC VII trial, Hobbs\(^9\) suggested three classifications of clonal evolution: 1) light chain escape - an increase in sFLC concentrations without a corresponding increase in intact immunoglobulins; 2) intact immunoglobulin escape - an increase in monoclonal intact immunoglobulin without an associated increase in sFLC; and 3) clonal change - a change in the relative proportion of monoclonal intact immunoglobulin and sFLCs (Figure 19.3).

In a larger study of 520 IIMM patients at relapse, Brioli et al.\(^13\) reported that 183 (35%) had a significant increase in intact immunoglobulin and sFLC levels, 258 (50%) had an increase in intact immunoglobulin only and 54 (10%) had light chain escape. Similar patterns of serum protein changes were reported by Zamarin et al.\(^11\) in a study of 66 patients (Figure 19.4). Importantly, this study included patients with LCMM. There was no evidence of clonal change in the LCMM patients, suggesting that loss of heavy chain production represented a terminal genetic event.

The case report by Keats et al.\(^7\) and Egan et al.\(^14\) described below provides evidence of the relationship between the genotype and phenotype of the disease.

**Clinical case history**

**Alternating dominance of competing myeloma clones\(^7\)\(^14\).**

Both Keats et al.\(^7\) and Egan et al.\(^14\) described the case of a 67-year-old woman with MM who was monitored closely during the course of her disease using sFLC analysis and total IgA assays. In addition, at 7 time points, genetic analysis of tumour plasma cells was performed using array comparative genomic hybridisation (aCGH) and fluorescence in situ hybridisation (FISH). This enabled the comparison of genetic changes in the competing tumour clones with the serological monoclonal protein changes (Figure 19.5).

At diagnosis, a bone marrow biopsy revealed a plasma cell content of 25%. Tumour cells were comprised primarily of one clone (termed ‘1.1’) but minor sub-clones (1.2 and 2.1) were also detected, both with a frequency of approximately 10% of the myeloma cells. At this point, both total IgA and the κ/λ sFLC ratio were abnormal.

The patient responded to treatment with lenalidomide and low-dose dexamethasone, achieving a partial response. Relapse (R1; Figure 19.5) occurred after 22 months, with a change in clonal dominance (clone 2.1; 64%) and a subtle increase in both monoclonal IgA and FLC. During two subsequent relapses (R2 and R3; Figure 19.5), changes in the clonal composition of the patient’s disease were characterised by an increase in monoclonal IgA, and a gross increase in the sFLC ratio. Interestingly, the dominant clone at R3 was 1.2, which shared a common progenitor with the original tumour clone. A final relapse occurred at 49 months (R4), characterised by the emergence of clone 2.2 and a dramatic increase in the κ/λ sFLC ratio. At 53 months, the patient progressed to secondary plasma cell leukemia (PCL; Chapter 22) with a further increase in the κ/λ sFLC ratio but no further change in the dominant clone present.

The detailed study of this patient revealed that, at each relapse, a clone emerged which was distinct from the previously dominant clone. In some instances the new clone was related to a preceding one, with which it shared a common progenitor. Clones related to sub-clone progenitor 1 were present at diagnosis and relapse R3. Clones related to sub-clone progenitor 2 represented the majority of the tumour population at R1 and R4/PCL progression. The authors termed this
pattern of progression as the “alternating dominance of two major clones”.

In conclusion, for this patient, it can be seen that sub-clones present at diagnosis were responsible for the relapsing pattern of disease and the relative abundance of each clone appeared to be modulated by the therapy received.

Genotypic analysis, although not readily available outside of clinical studies and academic centres of excellence, has revealed changes in the clonal architecture of the disease over time. In some MM patients, the genetic profile of the major clone present at diagnosis and relapse is identical [3]. However, in other cases, the dominant subclone at diagnosis is genetically distinct from the dominant clone at first relapse, which can also differ from those seen at later relapses, as illustrated by the above case study. Corre et al. [3] demonstrated that even if patients are treated with the same initial therapy, each myeloma subsequently evolves in a way that is genetically different from the others.

In conclusion, MM is associated with huge molecular heterogeneity, including at the patient level, which can evolve over time. Although genetic sequencing is not routinely performed, the assessment of monoclonal protein production using sFLC analysis and intact immunoglobulin measurements are simple, widely available techniques that allow patients to be monitored for evidence of clonal evolution.

---

**Figures**

**Figure 19.1.** (A) Linear and (B) branching transformation of MGUS to MM.

Genetic events conferring a selective advantage are shown as diamonds. (Reprinted by permission from Macmillan Publishers Ltd: Nature Reviews Cancer [3], copyright 2012).
Figure 19.2. The same microscope field of an IgGκ MM bone marrow sample showing dual populations stained with anti-IgG fluorescein isothiocyanate (FITC, green) and anti-κ tetramethylrhodamine isothiocyanate (TRITC, red).

In addition, anti-bromo deoxyuridine (BrdU) FITC (green) staining of nuclei has been performed to show cells in S-phase of the cell cycle. Upper panel shows anti-IgG FITC cytoplasmic and anti-BrdU FITC nuclear staining, middle shows the same field with anti-κ TRITC staining and lower shows a double exposure of the 2 upper plates superimposed to demonstrate double stained IgGκ cells (yellow), κ only cells (red) and S-phase cells with green nuclei. Arrows indicate BrdU+ and BrdU- intact immunoglobulin + cells, BrdU+ and BrdU- κ only cells together with non-plasma cells in S-phase [4]. (Obtained from Haematologica Journal website: haematologica.org).

View source:
- 19.2. Clonal populations in multiple myeloma

Figure 19.3. Changes in monoclonal protein expression at MM relapse.
dFLC: difference between involved and uninvolved FLC levels (Reproduced with permission from the British Journal of Haematology and John Wiley & Sons Ltd).

**View source:**
- 19.3. Clonal changes and clonal escape in multiple myeloma

**Figure 19.4. Changes in monoclonal protein type at relapse or progression of disease (R/POD).**

(Reprinted by permission from Macmillan Publishers Ltd: Bone Marrow Transplantation, copyright 2013).

**View source:**
- 19.3. Clonal changes and clonal escape in multiple myeloma

**Figure 19.5. sFLC analysis and total IgA concentrations over the course of treatment and disease relapse.**
The dominant clone present at each disease stage is shown. Red arrows indicate relapse (R1-R4) and remission (Rem). SGN-40: dacetuzemab; MPV: melphalan, prednisone, bortezomib; CyBorDT: cyclophosphamide, bortezomib, dexamethasone; D-PACE: dexamethasone, thalidomide, cisplatin, doxorubicin, cyclophosphamide, etoposide; CyBorP: cyclophosphamide, bortezomib, prednisone. (This research was originally published in Blood © the American Society of Hematology).

View source:

2. Bahlis NJ. Darwinian evolution and tiding clones in multiple myeloma. Blood 2012;120:927-8