Clinical and Genetic Aspects of Myeloma: An overview

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Multiple myeloma is a disorder of dysregulated immunity; what is the origin of the malignant cell?

Figure 1 | The B cell immune response. Walker et al, 2014 Nature Reviews
Walker et al, 2014 Nature Reviews
Myeloma statistics

- incidence 60-70 per million; commoner in Afro-Carribeans
- Approx 4,000 new cases of myeloma per annum in the UK (CRUK 2007)
- median age 70 yrs (Bird et al 2010); 15% <60; 2% <40 (Smith et al 2006)
- OS ranges widely from a few months to, in a small number of cases, >20 yrs.
- The median overall survival of patients treated with conventional intensive regimens followed by autologous stem cell transplantation is 62 months ie 5 years, 2 months (Gulbrandsen et al. 2001).
- Median survival since introduction of thalidomide estimated at approx 7 years in the UK MRC MMIX trial (Morgan 2009).
- This is likely to extend further with the additional use of bortezomib and lenolidomide. (Kenneth Anderson, Dana Faber Cancer Center estimates 10 years, ‘Bari Nov 2011’)
- Recent report from the VISTA study (VMP regimen) in older non transplant pts quotes median survival as 56 months ie 4yrs 8 months

(http://info.cancerresearchuk.org/cancerstats/types/multiplemyeloma/incidence/index.htm)

The prediction is that myeloma will become a chronic illness with complete responses in a significant fraction’ - Kenneth Anderson
Devastating consequences of myeloma bone disease
Prehistoric multiple myeloma – Red Indian artefacts

Calico Hills Burial #1 (AD 2-900)

Calico Hills Burial #2 (AD 2-900)

Calico Hills Burial #2 (AD 2-900)

Sowell Mound Skull (approx AD 610)

Morse, D et al, NY Acad Med, 1974
In May 1840, Sarah Newbury experienced severe back pain while stooping and a strange sensation in her right leg.
She was given an infusion of orange peel and a rhubarb pill.
Sections of the bones revealed ‘a red grumous matter’; the red matter was examined by Dr Solly and Mr Birkett of Guy’s Hospital; the majority of the nucleated cells had a clear, oval outline and one or rarely two bright central nucleoli.
Dr Henry Bence Jones, renowned chemical pathologist at St George’s Hospital, was consulted on a second notable case

- Thomas McBean, ‘a grocer of temperate habits and exemplary conduct’ presented with fatigue and a stooped gait
- While vaulting out of an underground cavern on his country vacation, he suddenly felt as if something had snapped or given way within his chest and for some minutes he lay, unable to stir because of severe pain.
- Treated with acetate of ammonia, camphor julap and compound tincture of camphor.
- Died in 1846 – cause of death ‘atrophy by albuminuria’, according to Bence Jones.

‘Dear Dr Jones,
The tube contains urine of very high specific gravity. When boiled it becomes slightly opaque. On the addition of nitric acid, it effervesces, assumes a reddish hue, and becomes quite clear; but as it cools, assumes the consistence and appearance which you see. Heat reliquifies it. What is it?’ (Dr Thomas Watson, GP, to Dr Henry Bence Jones, 1847)
• Abnormal serum electrophoresis showing immunoparesis and a monoclonal IgG spike

• Normal serum electrophoresis showing normal polyclonal immunoglobulins
Myeloma – the immunoglobulin product

- Myeloma cells usually produce both an intact immunoglobulin product - IgG (2/3), IgA (1/3) or rarely IgD (1.8%), IgM (0.4%) or IgE and monoclonal free light chains (90% of cases); in a minority of cases, myeloma cells produce FLC only (10%) termed ‘free light chain myeloma’
- Rarely, myeloma can be ‘low secretory’ ie secrete only small amounts of either intact Ig (measured in g/l) typically <10g/l or FLC
- Very rarely, myeloma may be completely non-secretory
- IgM monoclones much more commonly seen in lymphoplasmacytic lymphoma formerly known as Waldenstrom’s macroglobulinaemia

IgG

½ life approx 23d

cf ½ life FLC approx 4hrs

IgE

IgD

IgM

½ life approx 6d
Timeline depicting the history and treatment of myeloma from 1844 to the present.

**History**

- **1844**
  - First documented case
- **1845**
  - Abnormal urine protein, later termed Bence Jones protein
- **1895**
  - Description of plasma cells
- **1928**
  - First large case series of myeloma
- **1939**
  - Serum protein spike identified
- **1956**
  - Light chain types (later termed kappa and lambda) recognized
- **2005**
  - International staging system
  - Cytogenetic classification
- **2005**
  - Durie-Salmon staging system

**Treatment**

- **1845**
  - Steel and quinine (T. Watson)
  - Rhubarb and orange peel (S. Solly)
- **1947**
  - Urethane (N. Alwall)
- **1958**
  - Melphalan (N. Blokhin)
- **1962**
  - Corticosteroids (R. E. Maas)
  - Autologous transplantation (T. J. McElwain and R. L. Powles)
- **1983**
  - Thalidomide (S. Singhal and B. Barlogie)
- **1999**
  - Bortezomib (R. Z. Orlowski)
- **2002**
  - Lenalidomide (P. G. Richardson and K. C. Anderson)

Kyle R A, Rajkumar S V Blood 2008;111:2962-2972
Aims of treatment

1. Reduce myeloma tumour burden
2. Prevent and treat bone and tissue damage
3. Improve quality of life and survival
At presentation

• 15% of patients have no symptoms

• 38% emergency presentation
  - Kidney failure
  - Spinal cord compression/loss of movement
  - Fracture

• Remainder have symptoms, though often non-specific
  - Backache or bone pain
  - Tiredness/anaemia/recurrent infections
Treatment approach

[Flowchart diagram]

1. **Diagnosis**
   - Asymptomatic myeloma
     - Regular monitoring
   - Symptomatic myeloma
     - Are you a candidate for stem cell transplant?
       - Yes
         - Induction treatment, stem cell transplant
       - No
         - Non-intensive drug treatment

Clinical study
The problem - myeloma remains almost always incurable; it is a remitting, relapsing condition which inexorably progresses to refractory disease and death.
Current UK chemotherapy regimens for myeloma

- **First line**
  - cyclophosphamide, thalidomide and dexamethasone (CTD)
  - lenalidomide ie revlimid, cyclophosphamide and dexamethasone (RCD)
  - cyclophosphamide, carfilzomib, lenalidomide ie revlimid and dexamethasone (CCRD)
  - bortezomib ie velcade, cyclophosphamide and dexamethasone (VCD), autograft for younger, fitter patients,
  - bortezomib ie velcade, melphelan, prednisolone (VMP); alternative regimen for older patients
  - bortezomib ie velcade, thalidomide and dexamethasone (VTD)

- **Second line**
  - Bortezomib (Velcade) and dexamethasone (VD) +/- 2nd autograft

- **Third line**
  - lenalidomide (Revlimid) and dexamethasone (Rd)

- **Fourth line**
  - pomalidomide and dexamethasone

- **And beyond**
  - experimental therapy eg daratumumab (anti CD38), elotuzumab (anti CS1)
Biggest challenges in multiple myeloma

Myeloma usually (although not always; are their gentler options?) responds well to treatment but relapses - why? And can you stop it?

1. Residual populations – MRD, niche environments, quiescent cells/progenitor populations
2. Primary refractory disease – about 10% of patients with myeloma die within the first few months of diagnosis
3. Bone disease
4. Renal failure
5. ‘Malignant’ MGUS – LLR focus
6. Smouldering myeloma – imaging, Osteolytica
7. Allografts – selection, GvHD (LLR PhD bid)
Genetic Aspects of Myeloma

- Basic myeloma genetics
  - Primary and secondary events
  - Inherited variation and epigenetics
- Relationship of genetic abnormalities
- Genetic complexity and heterogeneity
- Prognostic utility and treatment strategies
- CEQAS survey
The Myeloma Genome

Anything but basic!

Hyperdiploidy

*IGH* Rearrangement

Loss & Gain

Somatic Gene Mutation

Inherited Mutation

Epigenetics

Primary events

- Hyperdiploidy vs *IGH* rearrangement

### Hyperdiploidy

- >46 chromosomes (range 48-75, median 53)
- Non random gains of odd numbered chromosomes
  - 3, 5, 7, 9, 11, 15, 19 & 21
- Seen in 30-40% patients
55,XY,+del(3)(p21),+5,+7,+9,+9,+11,+15,-16,+19,+21,+22
## IGH Rearrangements

<table>
<thead>
<tr>
<th>Translocation</th>
<th>Gene(s)</th>
<th>Frequency</th>
<th>Prognosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>t(4;14)(p16.3;q32)</td>
<td>FGFR3/MMSET</td>
<td>15%</td>
<td>Poor</td>
</tr>
<tr>
<td>t(11;14)(q13;q32)</td>
<td>CCND1</td>
<td>15%</td>
<td>Good</td>
</tr>
<tr>
<td>t(14;16)(q32;q23)</td>
<td>MAF</td>
<td>5%</td>
<td>Poor</td>
</tr>
<tr>
<td>t(6;14)(q21;q32)</td>
<td>CCND3</td>
<td>3%</td>
<td>Good</td>
</tr>
<tr>
<td>t(14;20)(q32;q11)</td>
<td>Mafb</td>
<td>2%</td>
<td>Poor</td>
</tr>
</tbody>
</table>
IGH-MAFB Rearrangement
Secondary events

- Regions of recurrent duplication &/or deletion
  - 1p, 1q, 9q, 11q, 12p, 13q, 15q, 16q, 17p, 19q and 22q

- Loss/deletion of chromosome 13
  - Seen in 50%
  - 85% monosomy, 15% deletion

- Deletion and amplification of chromosome 1
  - Seen in 40% of diagnostic MM, 70% at relapse
  - Deletion of 1p (CDKN2C, FAM46, FAF1)
  - Duplication of 1q (often seen together)

- Deletion of 17p13 (TP53)
  - Associated with shorter survival, aggressive disease, increased hypercalcaemia & extramedullary disease
MYC translocations

- **MYC translocations 8q24**
  - Seen in ~15% of diagnostic MM, but 45% with advanced disease
  - t(8;14) *IGH-MYC* accounts for ~25% of MYC
    - Variant IgK/IgL partners in 10%
    - Non-IG partners in 65%
- **Disease progression marker**
  - Associated with MGUS to MM transition
  - Associated with increased proliferation
  - Stromal independence of plasma cells
  - Increased incidence with t(14;16)
  - Decreased incidence with hyperdiploid and t(4;14)
  - Associated with a decreased survival
**MYC super-enhancers**

- Evidence to suggest that *MYC* is able to recruit super-enhancers
- Super-enhancers are the enhancer elements from highly expressed genes
- Partners include:
  
  - *FAM46C*
  - *KRAS*
  - *LRRTM4*
  - *TOB2*
  - *CCND1*
  - *FOXO3*
  - *CHST15*
  - *XBP1*
  
  - Involved in B cell, plasma cell or myeloma development
  - Confirmed the presence of super-enhancers within the vicinity of most *MYC* partners

Somatic gene mutation

- WES has reported mutations in a large number of consistent genes
- 35 - average number of gene mutations per MM patient
- Compares to 8 in simple leukaemias, & ~540 in genetically more complex epithelial tumours
- Supports a hypothesis of specific pathway deregulation

Pathway Deregulation

- Implicated pathways include NF-κB, WNT, RANK, P13K, JAK and MAPK
- MAPK pathway involved in 55% of MM patients
  - KRAS mutation 32%
  - NRAS mutation 18%
  - BRAF mutation 4%
  - More recently NF1 and RASA2
  - mutually exclusive
- Validated in an extended series of 150 trial patients

UK MRC Myeloma IX trial
Inherited Mutation/Variation

- Evidence to suggest that inherited variation can predispose to MGUS
- Current regions include:
  
<table>
<thead>
<tr>
<th>Chromosome</th>
<th>Region</th>
</tr>
</thead>
<tbody>
<tr>
<td>2p23.3</td>
<td>7p15.3</td>
</tr>
<tr>
<td>3p22.1</td>
<td>17p11.2</td>
</tr>
<tr>
<td>3q26.2</td>
<td>22q13.1</td>
</tr>
<tr>
<td>6p21.33</td>
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- Estimated to account for 13% of the familial risk of myeloma
  - More waiting to be discovered!

Epigenetics in Myeloma

- Epigenetic factors have also been shown to play a part in myeloma genetics
  - Global DNA hypomethylation as well as specific gene hypermethylation associated with MGUS to MM transition
  - 15% of t(4;14) show specific gene hypermethylation
  - Overexpression of MMSET leads to histone modification promoting cell survival and progression

- Deregulations of miRNAs
  - A specific cluster on chromosome 13 associated with MGUS to MM transformation.
Inter-relationships

The number of adverse lesions has an additive effect on overall survival.

Adverse *IGH* Translocations
Total = 145

+1q21
Total = 340

213

213

89

89

38

38

16

16

22

22

2

2

38

38

Del(17p13)
Total = 78

Boyd et al. Leukemia 2011
Genetic complexity and heterogeneity

Genetic pathway of myeloma is likely to be consistent with a Darwinian branching evolutionary model, multiple mutations occurring in parallel, not a linear model.....

...resulting in related clones, and not homogeneous populations
44,X,-Y,i(1)(q10),add(2)(q31),?add(3)(p1?3),add(4)(q3?1),del(6)(q21),
add(8)(q24),-13,t(14;20)(q32;q11)
Prognostic Utility

Adverse *IGH* vs neutral *IGH* group
Median OS of 25 vs 45 months
Prognosis continued

\[ \text{del(1p)} \quad \text{dup(1q)} \]

\[ \text{del(13q) / +IGH} \quad \text{del(17p)} \]

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--- MM cases with normal karyotype

…….. MM cases with the described abnormality

del(1p), dup(1q) and del(17p) all poor prognostic markers

del(13q) alone – neutral prognosis

UK Myeloma IX Trial
Bortezomib has been shown to negate the effect of t(4;14) and del(17p)

Bortezomib treatment
Treatment strategies

CML
- Common BCR/ABL1 change

Myeloma
- Primary translocations/hyperdiploidy
- Multiple additional genetic changes

Solid Tumours
- No common mutations
- No common translocations
- Multiple genetic changes
Copy Number Abnormalities

Primary Translocations/ Hyperdiploidy

Gene expression changes

RAS pathway mutations

Acquired mutations

Secondary Translocations

Epigenetic Modifications

BRAF inhibitors

MYC inhibitors

UTX inhibitors

MMSET/CCNDx inhibitors

Presentation: Brian Walker 2014
CEQAS Survey Results

- Survey undertaken as part of a pilot scheme for the assessment of myeloma genetic diagnosis
- Two cases presented,
  - one as fixed material for in-house FISH
  - one as website images
- Required to complete analysis and reporting according to the current laboratory protocols
MM Survey

- 39 participants for the pilot MM scheme, from across Europe
- 18/39 (46%) completed the survey
- Focussed on three areas
  - The type of lab and location
  - The referral type, samples & TAT
  - The techniques employed and the gene regions examined
The labs are predominantly from the UK, and all are diagnostic genetic laboratories, although some are considered joint diagnostic & research.
16/18 labs employed a CD138+ separation method.

FISH as a sole technique -15 labs
DNA arrays – 2 labs
Karyotyping -2 labs

All 39 labs offered *IGH* testing

95% offered *FGFR3* and *MAF*

*TP53* testing only offered by 37/39 (95%) labs.

Chromosome 1 abnormality testing offered by 30/39 (77%) labs.

13q abnormalities by 24/39 (62%)

A minority of labs offered:
hyperdiploid 8/39 (21%)
*MYC* testing 3/39 (8%)

<table>
<thead>
<tr>
<th>Chromosome / Gene regions tested for</th>
<th>Number of laboratories</th>
</tr>
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<tbody>
<tr>
<td>TP53</td>
<td>37</td>
</tr>
<tr>
<td>IGH</td>
<td>39</td>
</tr>
<tr>
<td>IGH-FGFR3</td>
<td>37</td>
</tr>
<tr>
<td>IGH-MAF</td>
<td>37</td>
</tr>
<tr>
<td>IGH-MAFB</td>
<td>6</td>
</tr>
<tr>
<td>IGH-CCND3</td>
<td>1</td>
</tr>
<tr>
<td>IGH-CCND1</td>
<td>18</td>
</tr>
<tr>
<td>1p/1q</td>
<td>26</td>
</tr>
<tr>
<td>1q only</td>
<td>2</td>
</tr>
<tr>
<td>13q</td>
<td>24</td>
</tr>
<tr>
<td>MYC</td>
<td>3</td>
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<tr>
<td>Hyperdiploid assessment</td>
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Acknowledgements

SMaRT team

Sheffield Diagnostic Genetics Service (SDGS)

Cytogenetic External Quality assessment Service (CEQAS)

Leukaemia and Lymphoma Research

Sheffield Blood Cancer Trust
Myeloma usually (although not always; are their gentler options?) responds well to treatment but relapses - why? And can you stop it?

1. Residual populations –
   1. Plateau, relapse and refractory disease
   2. What is the nature of the residual population; stem cell like, plastic or just quiescent?
   3. The niche – does it exist, probably, but can you define it? Can you study it? Can you target it? The plerixafor story – just because others have done some studies, shouldn’t mean that we shouldn’t look at it; CRUK application.
   4. Minimal residual disease – (MRD); considerable interest at present (2 planned PhD – one on genetic approaches to MRD, one on immunophenotypic approaches to MRD)
   5. Autografts – what is the nature of the post autograft residual plasma cell population (Celgene steer)
   6. Drug resistance and clonal selection, cat and mouse

2. Primary refractory disease – about 10% of patients with myeloma die within the first few months of diagnosis

3. Bone disease
   i. New approaches – our forte, but what next
   ii. Bone pain – massive clinical problem; proposed PhD

4. Malignant MGUS – LLR focus

5. Smouldering myeloma – imaging, Osteolytica (COC)

6. Allografts – selection, GvHD (LLR PhD bid)