Response comparison of multiple myeloma and monoclonal gammopathy of undetermined significance to the same anti-myeloma therapy: a retrospective cohort study.

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ABSTRACT

Background: Multiple myeloma (MM) is consistently preceded by monoclonal gammopathy of undetermined significance (MGUS). MGUS is usually only treated if it is causing significant disease through deposition of secreted M-proteins, when a form of anti-MM therapy is then employed. However, there are few studies comparing how MGUS and MM plasma cell clones respond to these therapies. In this novel study, we aimed to identify how MGUS and MM plasma cell clones responded to anti-MM therapy in patients newly diagnosed with biclonal gammopathy MM (BGMM). BGMM is characterised by the co-existence of an active MM clone and a benign MGUS clone, and thus provides a unique model to assess the responses of separate clones to the same anti-MM therapy, in the same patient, at the same time.

Methods: We identified BGMM patients by central laboratory analysis of 6,399 newly diagnosed MM patients enrolled in three UK clinical trials (Myeloma IX, Myeloma XI and TEAMM) between 7 July 2004 and 2 June 2015. In addition to the inclusion criteria of these trials, our study necessitated at trial entry the presence of two distinct M-proteins in immunofixation electrophoresis. To exclude confusion with lymphoplasmacytic cell clones, all BGMM patients with an IgM M-protein were excluded (14/6,399). Thus, 44 BGMM patients with IgG or IgA MGUS clones were subsequently identified and then longitudinally monitored. The primary endpoint was difference in response between MGUS and MM clones. Employing international therapy response criteria, we examined differences in the frequencies of different response codes (complete / very good partial / partial / minor responses, or stable / progressive disease) achieved by anti-MM therapy on MGUS and MM clones – overall, within patients, and between therapy types – using chi-squared analyses. Analyses were by intention to treat.

Findings: Longitudinal assessment of BGMM revealed disparate MM and MGUS responses in 30/44 (68%) individual patients. 16/44 (36%) MGUS clones did not respond to anti-MM therapy compared to only 3/44 (7%) non-responsive MM clones ($p<0.01$). In 27/44 (61%) dual responders, the MM response was greater in 5/44 (11%) patients, the MGUS response greater in 9/44 (20%) patients, and the MM and MGUS responses the same in 13/44 (30%) patients - of which 10/44 (23%) were complete responses; 1/44 (2%) were very good partial responses, and 2/44 (5%) were partial responses. Duration of response was better for MGUS with progression in only 1/31 (3%) clones versus 17/31 (55%) MM plasma cell clones.

Interpretation: These results show that, in BGMM, anti-MM therapies exert a greater depth of response against MM plasma cell clones than MGUS plasma cell clones. Whilst some MGUS clones exhibited a complete response, many did

not respond, suggesting that the underlying features that render MM plasma cells susceptible to therapy are present in only some MGUS plasma cell clones. To determine MGUS clone susceptibly to therapy, future studies may seek to identify, using BGMM as an investigative model, the genetic and epigenetic alterations that dictate whether MGUS plasma cell clones are responsive to anti-MM therapy.

Funding: National Institute of Health Research, Medical Research Council, Cancer Research UK.

INTRODUCTION

Multiple myeloma (MM), a cancer of immunoglobulin-secreting plasma cells, is the most common cause of blood cancer deaths worldwide and is consistently preceded by an asymptomatic pre-curser termed monoclonal gammopathy of undetermined significance (MGUS) (1, 2). International guidelines do not recommend treatment of MGUS and, instead, a watch-and-wait approach or clinical study enrolment is advocated until MM arises (3). MGUS prevalence increases with age and is 3-4% in adults over 50 years old in the general population (4-7), is more common in men and twice as common in blacks as whites (7, 8), and progresses to MM at a rate of 0·5 - 1% each year (9, 10).

Smouldering multiple myeloma (SMM) – an intermediate disease stage between MGUS and MM – has a 10% risk of MM progression each year initially but therapy is not recommended because studies of intervention with conventional chemotherapy have shown little benefit (11). More recent studies employing modern therapy modalities in high risk SMM patients have shown variable effects (12-14) and are under continued investigation (15). Studies investigating even earlier intervention in patients with MGUS have thus far been restricted to nutritional compounds (16). However, there is a clinical need to treat MGUS when it is causing significant morbidity by immunoglobulin deposition as monoclonal gammopathy of renal significance (MGRS), polyneuropathy, organomegaly, endocrinopathy, monoclonal gammopathy, and skin changes (POEMS) syndrome or light-chain (AL) amyloidosis (17-19). In these situations, anti-MM therapies are selectively employed to treat each condition, even though the efficacy of these treatments against MGUS compared to MM plasma cell clones is not well characterised. This knowledge gap reflects the difficulty of comparison between two sets of diseases and associated morbidities that are very different, that in themselves are very heterogeneous, and within which patient tolerance of therapy also exhibits great variability.

For further insight, we sought to evaluate responses of both MM and MGUS plasma cell clones to exactly the same anti-MM therapy, in the same patient, at the same time, using multiple myeloma with biclonal gammopathy (BGMM) as an investigative model. We have recently confirmed that up to 1% of newly diagnosed MM patients have two M-proteins in serum immunofixation electrophoresis (IFE), termed BGMM (20). Both MGUS and MM are characterised by M proteins – monoclonal whole antibody and free light chains (FLC) – in blood (MABs), and accordingly, changes in the level of blood MAB provide a unique biomarker of a patient's disease activity and are central to monitoring response to therapy and identifying relapse from remission. As MGUS evolves into MM, intraclonal heterogeneity increases, and at diagnosis

MM has often ten or more parallel sub-clones with different combinations of somatic mutations. These compete and can each manifest as being dominant at different stages of disease (21-24). In some patients, this sub-clonal evolution can be observed by changes in the relative amounts of whole MAB and FLC MAB that are secreted but importantly the heavy and light chain types and their electrophoretic mobilities remain the same and identical between subclones (25). As such, it is proposed that, in the majority of BGMM cases, the largest MAB is a product of the active MM clone and its subclones, and the usually 10 to 20 times smaller MAB represents a separate MGUS clone that is a relic of prior biclonal gammopathy of undetermined significance (BGUS) (26). BGUS is a much more common condition than BGMM (26), and has a prevalence amongst all MGUS of 15·4% in blacks, 6·8% in whites and 12·8% in Hispanics (7). Recently, a study of 539 biclonal gammopathies – that included patients with BGMM and BGUS – diagnosed in one centre from 1980 to 2009 found that, in 23 patients receiving either MM or Waldenstrom's macroglobulinaemia therapy, the MGUS clones in BGMM responded to therapy overall (27). However, to what degree the response of MM and MGUS clones correlate within the same patient, and vary between patients, requires further detailed study in a larger group of patients treated more recently with conventional therapies. Here, following central laboratory analysis and screening of MABs in serum from 6,399 newly diagnosed MM patients entered into three multi-centre UK clinical trials, we have investigated the responses of MM and MGUS plasma cell clones to conventional anti-MM therapies in 44 patients diagnosed with BGMM. Our primary objective was to determine whether anti-MM therapy exerted differences in the depth of response between MGUS and MM clones in BGMM, and our secondary objective was to identify whether there were differences in the frequency of relapse amongst MGUS and MM clones after anti-MM induction therapies. As such, in the largest study of its kind, we provide a comprehensive longitudinal assessment of a BGMM cohort through the course of disease from diagnosis, response to therapy and relapse from remission.

MATERIALS AND METHODS

Patients and trials

Patients included in the present study were enrolled in one of the following multi-centre, phase III trials: the UK Medical Research Council Myeloma IX trial (ISRCTN68454111); the Cancer Research UK Myeloma XI trial (ISRCTN49407852); or, the UK National Institute of Health Research Tackling Early Morbidity and Mortality in Myeloma Trial (TEAMM; ISRCTN51731976). From these trials, 58 patients with a BGMM diagnosis were identified, as described elsewhere (20), and all patients had assessable longitudinal data. Patients whose secondary BGMM MAB exhibited an IgM isotype (N=14/58 patients), were excluded from the study herein on the basis that these IgM MABs are most likely secreted from lymphoplasmacytic clones that may progress to lymphoma rather than the IgG- or IgA-secreting plasma cell MGUS clones that may progress to MM. As such, 44 patients were eligible for inclusion in this study.

Myeloma IX evaluated the effects of bisphosphonate and thalidomide therapy on progression-free survival and overall survival. All trial enrolled patients had newly diagnosed symptomatic MM and were aged >18 years or older. The study protocol and findings have been described in detail elsewhere (28). Patients were assigned to bisphosphonate (oral clodronic acid 1600 mg per day; or intravenous zoledronic acid 4 mg every 21-28 days with induction chemotherapy, and every 28 days thereafter) and induction treatments via an intensive or non-intensive treatment pathway. The intensive pathway consisted of 4 to 6 21-day cycles of either cyclophosphamide-vincristine-doxorubicin-dexamethasone (CVAD; 500 mg oral cyclophosphamide per week, 0·4 mg vincristine daily combined with 9 mg/m^2 doxorubicin daily as a 4-day continuous infusion, and 40 mg dexamethasone daily on days 1–4 and 12–15), or oral cyclophosphamide-thalidomide-dexamethasone (CTD; 500 mg cyclophosphamide per week, 100 mg thalidomide daily and increasing to 200 mg daily as tolerated, and 40 mg dexamethasone daily on days 1–4 and 12–15). After completion of induction therapy, patients underwent peripheral blood stem-cell mobilisation and harvest, intra-venous high-dose melphalan treatment (200 mg/m^2), and autologous stem-cell transplantation. The non-intensive pathway consisted of 6 to 9 28-day cycles of either oral melphalan-prednisone (MP) (7 mg/m^2 melphalan and 40 mg prednisone, both on days 1–4), or attenuated oral CTD (CTDa; 500 mg cyclophosphamide per week, 50 mg thalidomide daily initially and increasing to 200 mg per day as tolerated, and 20 mg dexamethasone daily on days 1–4 and 15–18); until best response. After initial therapy, all eligible patients underwent a second randomization to no maintenance or low-dose thalidomide maintenance therapy given until

disease progression (50 mg daily for 28 days, increasing thereafter to 100 mg daily if well tolerated). 13 eligible BGMM patients from MIX were identified; 7 patients were in the intensive pathway (CTD=4; CVAD=3) and 6 were in the non-intensive pathway (MP=1; CTDa=5).

Myeloma XI completed recruitment in 2017. All trial enrolled patients had newly diagnosed symptomatic MM and were aged >18 years or older. Myeloma XI had two treatment pathways, intensive and non-intensive, which both had induction, consolidation and maintenance therapy components. In the intensive pathway, Myeloma XI compared oral cyclophosphamide-lenalidomide(REVLIMID®)-dexamethasone (CRD; 500 mg cyclophosphamide on days 1 and 8, lenalidomide 25 mg daily for 21 days, dexamethasone 40 mg daily on days 1-4 and 12-15) to oral CTD (cyclophosphamide 500 mg weekly, thalidomide initially 100 mg daily for 3 weeks increasing to 200 mg daily, dexamethasone 40 mg daily on days 1-4 and 12-15) or kyprolis-CRD (CCRD; cyclophosphamide 500 mg on days 1, 2, 8, 9, 15 and 16, carfilzomib 20 mg/m^2 administered on days 1 and 2 of cycle 1 and dose capped at a body surface area of 2·2 m^2, lenalidomide 25 mg daily for 21 days, dexamethasone 40 mg on days 1-4, 8, 9 & 15, 16), repeated every 28 days, for up to six cycles, followed by high dose melphalan and autologous stem cell transplant, as per local practice. In the non-intensive pathway, attenuated oral CRD (CRDa; cyclophosphamide 500 mg on days 1 and 8, lenalidomide 25 mg daily for 21 days, dexamethasone 20 mg daily on days 1-4 and 15-18) was compared to CTDa (cyclophosphamide 500 mg daily, thalidomide initially 50 mg daily for 28 days, increasing every 28 days by 50 mg increments to 200 mg daily, dexamethasone 20 mg daily on days 1-4 and 15-18), repeated every 28 days for ≥ 6 cycles. In patients who demonstrated a sub-optimal response to induction therapy, the use of bortezomib, cyclophosphamide and dexamethasone was investigated. Patients were further randomised to no maintenance or to oral lenalidomide, or oral lenalidomide-vorinostat maintenance therapy (lenalidomide 10 mg daily, vorinostat 300mg daily on days 1-7 and 15-21), on a repeating cycle every 28 days until disease progression. 24 eligible BGMM patients (13 intensive pathway; 11 non-intensive pathway) were found to be eligible for the study herein.

TEAMM was a randomised, double-blind, placebo-controlled, trial assessing the benefits of antibiotic prophylaxis (levofloxacin) and its effects on health care associated infections. All trial enrolled patients had newly diagnosed symptomatic MM and were aged 21 years or older. All anti-MM therapies were eligible for use in TEAMM. Seven eligible BGMM patients from TEAMM were identified, and received the following induction therapies: CTD=4, CTDa=2, and melphalan-prednisolone-thalidomide (MPT)=1. The administration of these therapies was delivered as per local practice.

Outcomes

We hypothesised that MM and MGUS clones would respond differently to anti-MM therapy and so the primary endpoint of this study was difference in response between MM and MGUS clones in BGMM. We used internationally accepted response criteria to define and categorise depth and duration of response according to changes in M-protein levels between BGMM diagnosis and the date of maximum response to anti-MM therapy, and disease progression (29, 30).

Laboratory tests

All serological laboratory testing was undertaken at the Clinical Immunology Service, University of Birmingham, UK. MABs in serum were identified by IFE (Sebia, France) and quantified by protein zone electrophoresis and densitometry (SPE; Interlab, Italy). If accurate quantitation of MABs was not feasible e.g., when a pair of MAB bands shared the same position on SPE (i.e., IgGκ IgGλ), or when the size of the MABs were too small to be detected by densitometry (limit of detection is approximately 1g/L), MAB concentration was estimated from IFE, taking into account the size of the monoclonal bands as a proportion of total immunoglobulin of that HC isotype (i.e., taking into account background polyclonal immunoglobulin). This exercise was carried out by three experienced IFE users, independently, blind of sample timepoint, before agreement was reached per sample. Serum IgG, IgM and IgA, creatinine, β2-microglobulin and FLCs (Binding Site, Birmingham, UK) were measured on a Roche Hitachi Modular analyser. In patients with a LC MAB identified by IFE without a HC component (light chain only myeloma), MAB size was measured and monitored by involved FLC levels (iFLCs) and expressed in g/L.

Statistical analyses

Patient responses to therapy were categorised using international response criteria based on the percentage decrease of MAB size: 100% = complete response (CR), ≥90% = very good partial response (VGPR), ≥50% to <90% = partial response (PR), ≥25% to <50% = minor response (MR), <25% change = stable disease (SD), and >25% increase = progressive disease (PD)(29, 30). Response codes were further aggregated into good (CR/VGPR) vs moderate (PR/MR) vs poor (SD/PD) responses; or responders (CR/VGPR/PR/MR) vs non-responders (SD/PD). Frequency differences between groups (e.g. M1 M2 MABS, treatment pathways) were analysed using the following statistical tests: for 2x2 factors within the same patients (i.e., response/no response x M1/M2) by McNemar's test; for 3x2 factors within the same patients (i.e., good/moderate/poor response x M1/M2) and for 6x2 factors within the same patients (i.e., CR/VGPR/PR/MR/SD/PD x M1/M2) by Stewart Maxwell's test; and for 2x2 factors between independent groups (i.e.,

intensive/non-intensive therapy x M2 response/no response) by Pearson's Chi-Square test. Correlation between change in M1 and M2 levels from diagnosis to maximum response were assessed using Spearman's correlational coefficient. Data are presented as median ± interquartile ranges (IQR) unless otherwise stated. Analyses were by intention to treat. Data were analysed by IBM SPSS (version 24, IBM Corp., Armonk, NY) and R: A Language and Environment for Statistical Computing (version 3·2·1, R Foundation for Statistical Computing, Vienna, Austria).

Role of the funding source

The funders had no role in the design of this study, data collection, data analysis, data interpretation, or writing of the report. The corresponding author (MTD), the first authors (JPC, JLJH), and statistician (DAC) had full access to the data in this study. All authors of this report had final responsibility for the decision to submit for publication.

RESULTS

BGMM demographics

58 BGMM patients were initially identified amongst 6,399 newly diagnosed MM patients. Of these, we found that 14/58 BGMM patients had a secondary biclonal IgM M-protein. On the basis that we could not discern whether these IgM M-proteins were secreted by a lymphoplasmacytic clone – that may progress to Waldenstrom's macroglobulinaemia or lymphoma – or a MGUS plasma cell clone – that may progress to the rare entity IgM MM – these patients were excluded from our analyses. As such, 44/58 patients were eligible for inclusion in this study. Patients were enrolled between 7 July 2004 and 2 June 2015. 28/44 (64%) patients were male and 16/44 (36%) were female, median ± IQR age was 68·7±10·9 years, serum β2-microglobulin was 3·5±2·7 µg/mL and serum creatinine was 92·0±37·0 µmol/L.

MGUS and MM MABs in BGMM

The largest MAB (in g/L) is categorised as the MM MAB and is described as 'M1', and the smaller MAB is categorised as the MGUS MAB and is described as 'M2'. Baseline isotype and size characteristics of M1 and M2 MABS in 44 BGMM patients are outlined in Table 1. M1 MABS were approximately 10 to 15 times larger in size than their M2 counterparts. 4/44 (9%) patients presented with a FLC MAB that did not have an associated intact immunoglobulin MAB of the same LC isotype (i.e., light chain only myeloma); each of these FLC MABs were greater than 500mg/L (range: 0·6-1·9g/L). In each of these patients, the other biclonal MAB – which was a different LC isotype to the FLC MAB – was small in size (range: 2·2-4·5g/L). Comparatively, in 10 other BGMM patients with FLC MAB levels >500mg/L, all had a LC matched intact immunoglobulin M-protein that was >18g/L. As such, in the 4 aforementioned BGMM patients with a light chain only M-protein, the FLC MAB was selected as the M1 MAB and the intact immunoglobulin MAB as the M2 MAB. In 32/44 (73%) BGMM patients, the LC isotype of the M2 MAB was different to that of the M1 MAB, and so it could be discerned which of the M1 and M2 MABs were associated with FLC secretion. 31 of these 32 patients had evaluable FLC data. In only 5 of these 31 patients (16%) was the M2 MGUS clone associated with FLC MAB secretion; this is consistent with MGUS usually having a whole MAB without detectable FLC MAB (31). In contrast and as expected, 89·5% of the non-BGMM patients in the Myeloma IX and Myeloma XI studies secreted FLC MAB (data obtained from N=2823 Myeloma IX patients and N=3154 Myeloma XI patients with evaluable FLC data; data not presented herein), and we found that 28/31 (90·3%) BGMM patients had a FLC MAB secreted by their M1 clone. Serum FLC levels at BGMM diagnosis (42/44 with evaluable data) are illustrated in Supplementary material (Figure 1, page 1).

Comparison of MM and MGUS MAB responses to anti-MM therapy

We next assessed the responses of both the MM and MGUS clones in BGMM to anti-MM therapy via changes to M1 and M2 levels in serum and employing internationally accepted response criteria (29, 30). Response to anti-MM therapy was assessed in 44 patients. Of these, 43/44 (98%) achieved a maximum and stable reduction in levels of M1 MAB whilst one patient's MM progressed during induction therapy. The median duration ± IQR from diagnosis to M1 maximum response was 158±122 days. At diagnosis, M1 levels were much larger than M2 levels (Table 1), yet, overall, fewer M2 clones responded to anti-MM therapy (p=0·0001) (Table 2). Indeed, we found in 27/44 (61%) patients, the M1 response was a VGPR or better, compared to 20/44 (46%) for M2 responses (p=0·002). Insignificant MAB responses (<25% reduction or an increase in MAB levels) were seen in only 3/44 (7%) patients for M1 response but in 16/44 (36%) patients for M2 response (p=0·001). We assessed the effects of intensive versus non-intensive induction therapies (from Myeloma IX and XI trials only) on M1 and M2 levels (Table 2). We observed that M2 responses to non-intensive therapy were inferior than M1 responses to non-intensive therapy (p=0·02); whilst there were similarities in the number of M1s and M2s exhibiting a VGPR or better to non-intensive therapy, there were a greater number of SD/PD responses among M2 clones (p=0·03). With regards to intensive therapies, no significant differences (p>0·05) were observed between M1 and M2 responses, with similar numbers of CRs achieved by M1 and M2 clones, though we note a higher frequency of non-responsive M2 clones (5/20; 25%) than M1 clones (1/20; 5%). In separate analyses, when comparing M2 responses between intensive and non-intensive therapies, we did not find any significant differences (p>0·05), nor when we evaluated M1 responses between intensive and non-intensive therapies (p>0·05).

Comparison of MM and MGUS MAB responses in individual BGMM patients and between different anti-MM therapies

We next assessed whether M1 and M2 clones responded differently within-patients (Figure 1). Maximum response for M2 was always achieved within the time taken to achieve maximum response for M1. The percentage reduction of M1 and M2 MABS from trial entry to time of maximum response to therapy was not significantly correlated and neither were the absolute reductions of M1 and M2 (in g/L). Further analyses (Table 3) revealed that 10/44 (23%) patients achieved a CR of both M1 and M2, and notably, CRs were not limited to LC-matched MABs. Indeed, CRs were achieved in 7/44 (16%) patients in whom the M1 and M2 MABS had different LC isotypes, showing that anti-MM therapy can commonly achieve CRs in two unequivocally independent plasma cell clones in the same patient (Figure 1). In 3/7 of these patients, FLC associated with the M2 clone was elevated at trial entry (77 mg/L, 105mg/L and 186mg/L, respectively [identifiable in Supplementary material - Figure 1, page 1] without evidence of renal damage (serum

creatinine levels all < 100 µmol/L), indicating more advanced neoplastic activity of these particular M2 clones and perhaps particular susceptibility to anti-MM therapies; these therapies were: CVAD=1, and CTD/CRD/CCRD=2. Despite these several dual CRs among BGMM clones, using international response criteria (Table 3) we found in only 14/44 (32%) patients that the M1 and M2 responses were the same; in 11/44 (25%) patients the M2 response was greater than the M1 response, and in 19/44 (43%) patients the M1 response was greater than the M2 response.

In separate analyses, no statistical differences between M1 and M2 responses in the same patient were observed between different types of induction therapy (Figure 2) – though insufficient statistical power, due to the broad range of different treatment regimens implemented, limited these analyses. Finally, M2 responsivity to therapy was not related to the starting M2 size at presentation, as CRs of M2 were achievable from a starting concentration of over 10g/L at disease presentation (Supplementary material, Figure 2, page 2).

Residual MGUS BGMM MAB levels usually remain stable during remission and relapse

We monitored patients whom had follow-up results available during disease remission, to assess M1 and M2 inter-clonal competition and relapse over time. For these 31 patients, the median follow-up duration was 315±397 days (Figure 3). Data in Figure 3 are segregated into patients who achieved a CR of M2 at maximum response to therapy, and those that did not. Follow-up data was available from 15/18 (83%) patients who achieved a CR of M2. 7 of these 15 (47%) patients had a relapse of M1 but no increase in M2, and 8/15 (53%) had no relapse of M1. In the 8 patients with no M1 relapse, 2 patients (25%) had a return of M2: IgGκ 0·5g/L at trial entry and 3g/L at M2 return [153 days after max response]; IgGλ 8g/L at trial entry and 2g/L at M2 return [1183 days after max response]. The remaining 6/15 (40%) patients with no M1 relapse had no M2 increase observed. In 25 patients who did not achieve a CR of M2 at maximum response, subsequent follow-up data was available from 16/25 (64%) patients. 8/16 (50%) patients had a relapse of M1 but no change to M2, and 6/16 (38%) patients had no change in either M1 or M2. 1/16 (6%) patients had a relapse of M1 and a return of M2 (M2 = IgGκ 5g/L at trial entry and 2g/L at return) and 1/16 (6%) patients had a relapse of M1 and a progression of M2 to MM (M2 IgGκ 5g/L at trial entry and 16g/L at progression); M2 responded to subsequent therapy in this patient, before a relapse of M1 occurred and the patient died. Taken together, these results demonstrated that the majority of M2s remained stable after a median follow-up of approximately one year.

DISCUSSION

Results of this study show that anti-MM therapy is more effective against MM than it is against MGUS clones in patients diagnosed with BGMM. Whilst we found that some MGUS plasma cell clones exhibited complete responses to anti-MM therapies, many did not respond. We did not identify features of disease that predicted MGUS responsivity to therapy, yet the response patterns observed may indicate that some MGUS plasma cell clones have genetic and epigenetic alterations more akin to MM – that simultaneously renders them more likely to progress to MM, but also means they are more likely to respond to existing anti-MM therapies.

We conducted this novel study because there is increasing desire to intervene, at an earlier stage, in high-risk asymptomatic monoclonal gammopathies and there is also a requirement to treat MGUS that causes significant disease usually through M-protein deposition in tissues. In these latter circumstances – for example for POEMS, AL amyloidosis and MGRS - variations of current anti-MM therapies are prescribed (17-19), yet there is little knowledge of the relative efficacy of these therapies on MGUS versus MM plasma cell clones. To provide more insight, we conducted this innovative – and the largest ever – evaluation of BGMM through anti-MM therapy, remission and relapse. Whilst prior studies have shown that in a minority of prior BGMM cases, the MAB pairs originate from clonally related plasma cells (32-34), the majority instead arise from two independent plasma cell clones producing unrelated MABs that exhibit either [i] different light chain (LC) isotypes, or, [ii] the same LC-isotype with no clonal relatedness (34). Thus, in most cases, evaluation of BGMM allows investigation of clonally unrelated MGUS and MM responses to therapy, and in doing so enables comparisons within individual patients in whom the two plasma cell clones share the same microenvironment, the same exposure to anti-MM therapy and the great majority of their genes. A profound feature of MM is the broad scale of sub-clonal heterogeneity and the evolution of the sub-clone hierarchy over time and in response to therapy (21-24). In some patients, alterations to the sub-clonal architecture can be observed by changes in the relative amounts of whole MAB and FLC MAB that are secreted, but, importantly, the heavy and light chain types and their electrophoretic mobilities remain the same and identical between sub-clones (25). Accordingly, in this study M1 and M2 MAB levels represent the total clonal substructure of MM and MGUS and do not inform on evolution of intraclonal heterogeneity.

Using international consensus response criteria, we found that anti-MM therapy commonly achieves CRs in both MGUS and MM clones (10/44 patients; 23%) in BGMM. Moreover, we found that 41% (18/44) of MGUS MABs exhibited a complete response; this was a higher proportion of CRs than achieved by MM MABS (14/44; 32%), and this MGUS response was not dependent on MAB concentration at diagnosis, nor its HC or LC-isotype. In a small subset of 3 patients exhibiting elevated FLC secretion (~100mg/L) associated with the M2 clone, CRs were observed in all 3 patients after anti-MM induction therapy, suggesting that monoclonal plasma cells associated with light chain production – akin to observations in AL amyloid, discussed elsewhere (35, 36, 37) – may be more susceptible to anti-MM therapy. However, it may be that in MGUS with AL amyloidosis, the amyloidogenic nature of the FLC, render those plasma cells more susceptible to anti-MM therapies, particularly proteasome inhibitors (37) and none of these BGMM patients had AL amyloid. Importantly, the overall number of MGUS clones responding to anti-MM therapy was inferior than MM clones and insignificant MAB responses (<25% reduction in MAB levels (29, 30)) were observed in 16/44 (36%) patients for MGUS response compared to only 3/44 (7%) patients who elicited an insignificant MM MAB response. This higher proportion of MGUS non-responders compared to MM non-responders is in contrast to the CR rates of 41% (18/44) in MGUS and 32% (14/44) in MM clones and indicates a dichotomous response to anti-MM therapy among MGUS clones in BGMM. This duality of MGUS responsivity contradicts patterns observed for MM responses, which were for the most part (41/44; 93%) responsive to therapy. Together these findings indicate that, unlike MM clones that are entirely malignant, the benign nature of MGUS clones in BGMM is divided into those associated with resistance to anti-MM therapies and those that are susceptible to anti-MM therapies. It would be of future interest to investigate if the latter are also those at greatest risk of progression to MM. BGMM provides a good model to investigate this hypothesis and to assess MGUS resistance to anti-MM therapies, as the nature of the model focuses attention on genomic and epigenetic differences between the MGUS and MM clones in the same patient.

In addition to the comparisons of therapy responses between groups of MM and MGUS clones, our extensive assessment of BGMM enabled the investigation of intra-patient MM and MGUS responses. We observed that the MM clone exhibited a higher response than the MGUS clone in 19/44 patients (43%), and vice-versa in 11/44 patients (25%). MM and MGUS responses were the same in 14/44 (32%) patients (10/44 [23%] complete responses; 1/44 [2%] very good partial responses, 2/44 [5%] partial responses, and 1 [2%] stable disease). The majority (27/44; 61%) of patients had a response of both MM and MGUS, however, a high number (14/44; 32%) of patients had a MM response but no response in their MGUS clone. We found that the baseline size of the MGUS MAB had no effect on the responsivity to therapy, nor did whether the MM and MGUS MABs share the same LC isotypes. 4/44 (9%) patients did not receive a biological

anti-MM agent whilst the other 40/44 (91%) all received either thalidomide or lenalidomide at diagnosis with two patients subsequently receiving Bortezomib after exhibiting <VGPR response to an IMiD therapy. Due to the broad range of anti-MM therapies inducted to patients across the three clinical trials included in our study, we were unable to yield sufficient statistical power to compare the effects of different therapies on MM and MGUS MABs in BGMM. Comparisons between intensive and non-intensive therapies preliminarily indicate that intensive therapies induced deeper responses against both MM and MGUS MABS in BGMM. Future larger studies are needed to confirm this observation. Importantly MGUS response <25% was seen in 16/44 (36%) patients receiving a wide spectrum of therapies including intensive therapy. These findings suggest that the genetic alterations that render a plasma cell clone neoplastic (i.e., transformation from benign MGUS to MM) are the dominant factor in determining response to anti-MM therapy over and above the individual's genetic make-up, the plasma clone's microenvironment and the type of anti-MM therapy used.

Whether treated intensively or non-intensively, we found MGUS MABs levels were stable in 27/31 (87%) BGMM patients with samples available for monitoring throughout MM remission. In one patient, the MGUS MAB progressed to MM; this is broadly in line with known rates of MGUS progression over a one year period in persons of this age (6). In contrast 17/31 (53%) patients suffered relapse of their MM plasma cell clone. These findings indicate that MGUS response to anti-MM therapy is of greater duration than MM responses but greater follow-up time would be needed to confirm duration of remission.

A limitation of this study is that we were unable to characterise and evaluate change at the tumour cell level because bone marrow cells were not available at the time of this retrospective study. Consequently we have not been able to assess two important areas that should be central to future studies of BGMM. These are detailed genetic and epigenetic signatures of MGUS and MM clones.. Secondly that investigation into depth of responses to therapy was limited to MAB levels and not able to assess for existence of low-level minimal residual disease (MRD). Future studies of BGMM should include flow cytometric identification of plasma cell phenotypes including distinguishing MGUS and MM clones through expression of heavy and light chain immunoglobulin isotypes. Subsequent single cell genomic and epigenomic analysis should identify the differences between the two neoplastic clones and normal cells, allowing focus on the differences between the MGUS and MM clones that underlie their different malignancy and response to anti-MM therapy in the same microenvironment.

Collectively, our findings show that anti-MM therapy is more effective against MM than it is against MGUS clones in BGMM. Moreover, anti-MM therapy induced highly variable responses to MGUS MABs in BGMM, indicating that some MGUS clones are highly responsive to therapy, and many are unresponsive; these responses to therapy may indicate that some of these plasma cell clones have genotypes more akin to MM and thus are at higher risk of progression. Future BGMM studies on the neoplastic cells rather than just their secreted M-proteins could provide an understanding of the cause of the high prevalence of MGUS resistance to current anti-MM therapies and reveal alternative therapeutic strategies.

Conflict of Interest Statement

All authors declare no conflict of interest.

Author Contributions

JC, CB and MD wrote the manuscript. MD, JC and JH designed the investigation into BGMM. JC, JH, SP, and ZA conducted the experiments. JC, JH, DC and MTD analysed the data. JC, JH, SP and MD interpreted the data. JAC, GM, WG, MD, RO, GH and MK designed the Myeloma IX/ XI/TEAMM trials. All authors approved the manuscript.

Acknowledgements

We are grateful to the NCRI Haemato-oncology subgroup and to all principle investigators for their dedication and commitment to recruiting patients to Myeloma IX, XI and TEAMM trials. We thank the Clinical Trials Research Unit at The University of Leeds (Myeloma IX and XI) and the Clinical Trials Unit at the University of Warwick (TEAMM). We are grateful to the staff of the Clinical Immunology Service in Birmingham with Tim Plant, Karen Walker, Alison Adkins and Nicola Newnham. Finally, we are grateful to all patients and their clinical teams at centres throughout the UK whose participation made this study possible.

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PANEL: RESEARCH IN CONTEXT

Evidence before this study

Multiple myeloma (MM) consistently arises from a premalignant plasma cell clone called monoclonal gammopathy of undetermined significance (MGUS) that is present in 3-4% of the population aged >50 years. International guidelines do not recommend screening for or treatment of MGUS unless it is directly causing significant morbidity, for example arising from M-protein deposition diseases, such as monoclonal gammopathy of renal significance (MGRS), POEMS or AL amyloidosis. In the treatment of MGUS, anti-MM therapy is employed but there have been few studies comparing responses of MGUS and MM clones to these therapies. Consequently, it is not known if in relatively rare conditions like AL amyloidosis whether the spectrum of response of MGUS plasma cell clones will be similar or different to that of the spectrum of response of MM plasma cell clones - which has already been investigated in much larger studies of this more common disease. The need for randomised studies of different therapies efficacy against diseases like AL amyloidosis are thwarted by small patient numbers and so there is strong reliance on the results of randomised trials in MM on the assumption that the results will largely translate to MGUS plasma cell clones. However, there are few comparisons of anti-MM therapies efficacy against MGUS versus MM. This reflects the difficulty of comparison between two sets of diseases and associated morbidities that are very different, that in themselves are very heterogeneous and within which patient tolerance of therapy also exhibits great variability. Prior to commencement of this study, we conducted literature searches on Medline (US National Institutes of Health) and found limited investigation into the effects of anti-MM therapy on a rare type of MM termed biclonal gammopathy MM (BGMM). BGMM represents the simultaneous presence of a MM plasma cell clone and a MGUS plasma cell clone. As such, BGMM serves as a unique model to assess both MGUS and MM plasma cell clone responses to the same therapy, at the same time, in the same microenvironment - with both clones sharing the great majority of the patient's genes.

Added value of this study

Both MGUS and MM are characterised by monoclonal plasma cells in the bone marrow and monoclonal antibody (MAB) in blood, and accordingly, changes in the level of blood MAB provide a unique biomarker of a patient's disease activity and are central to monitoring response to therapy and for relapse from remission. In contrast to most blood cancers this makes frequent longitudinal measurement of disease activity cheaply and very effectively available by frequent blood sampling. Our blood MAB level results show similar MGUS and MM plasma clone responses in only 14/44 (32%) BGMM patients, highlighting - for the first time that we are aware - the high prevalence of that disparity and by nature of the BGMM model, that the explanation most likely resides in genetic/epigenetic differences between the

MGUS and MM plasma cell clones of the same patient. Further, we have found a dichotomy in MGUS responses: 18/44 (41%) had a complete response whilst 16/44 (36%) failed to respond compared to 3/44 (7%) MM non-responders. Duration of response was better for MGUS with progression in only 1/31 (3%) clones versus 17/31 (55%) MM plasma cell clones.

Implications of all the available evidence

Many MGUS plasma cell clones were unresponsive to current anti-MM therapies including intensive therapy, immunomodulatory drugs and proteasome inhibitors despite the same therapies in the same patients being much more effective against MM plasma cell clones. This highlights the need for caution when translating anti-MM therapy to the uncommon patients with MGUS that require therapy, and the need to find alternative therapies for these patients. The BGMM model whilst clinically uncommon has important and unique facets that will facilitate better understanding of MGUS and MM and the search for new therapies.

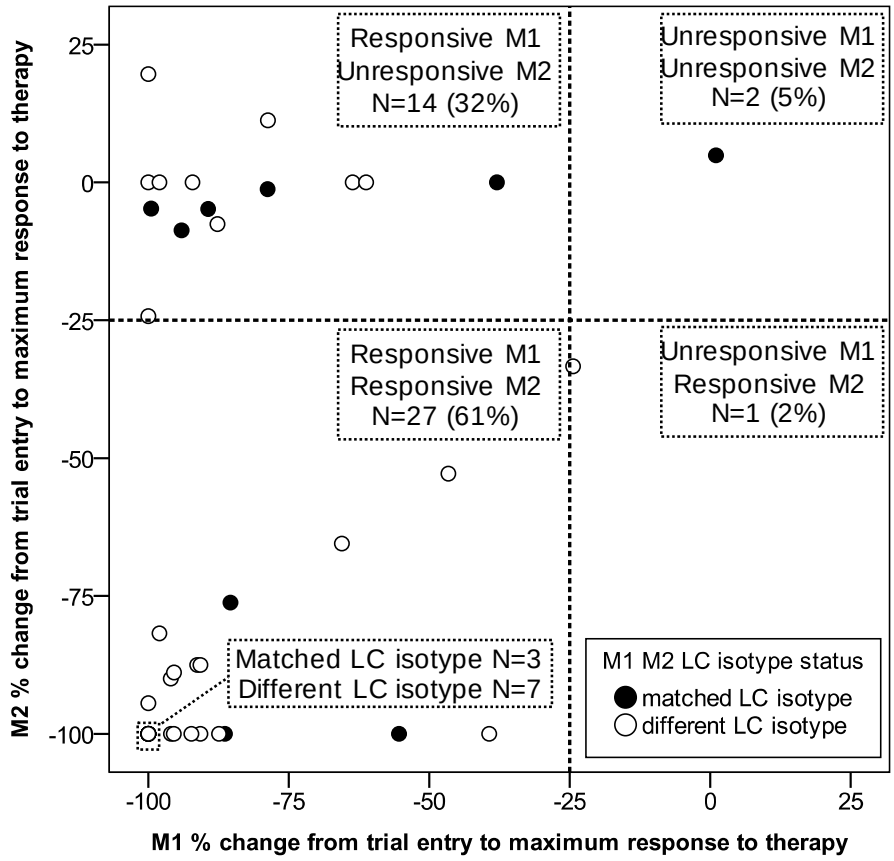
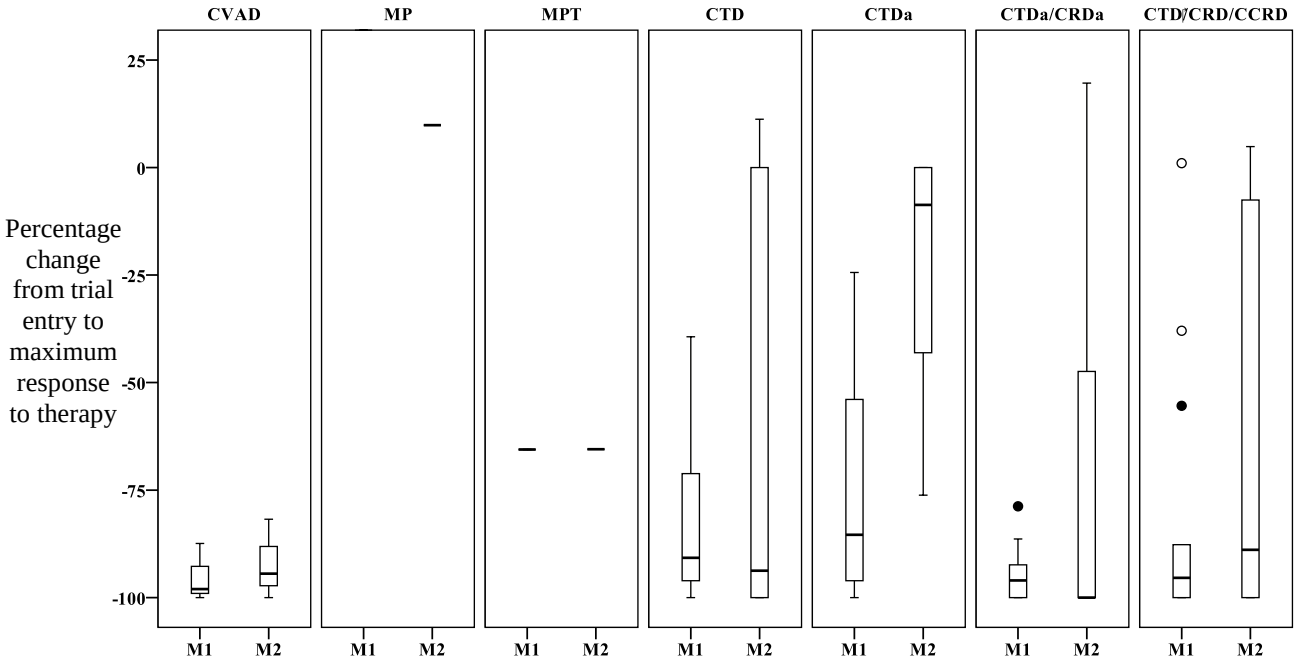


FIGURE 1. Percentage change of M1 and M2 MABS from disease presentation to maximum response to anti-MM therapy. Responses are coloured-coded based on whether the M1 and M2 clone exhibited matched (black) or different (clear) LC isotypes. N=44 patients; note 1/44 patients are not plotted as the patient exhibited progression of M1 and stable M2 in response to anti-myeloma therapy.



	CVAD		MP		MPT		CTD		CTDa		CTDa/CRDa		CTD/CRD/CCRD	
CR	1	1	0	0	0	0	2	4	1	0	5	7	5	6
VGPR	1	1	0	0	0	0	3	0	2	0	3	1	4	0
PR	1	1	0	0	1	1	2	1	2	2	3	0	2	2
MR	0	0	0	0	0	0	1	0	1	1	0	0	1	0
Response	3	3	0	0	1	1	8	5	6	3	11	8	12	8
SD	0	0	0	1	0	0	0	3	1	4	0	3	1	5
PD	0	0	1	0	0	0	0	0	0	0	0	0	0	0
No response	0	0	1	1	0	0	0	3	1	4	0	3	1	5

FIGURE 2. Percentage changes of M1 and M2 MABS from diagnosis to the time of maximum response to different anti-MM therapies in 44 BGMM patients; note 1/44 patients tabulated but only partly plotted as patient exhibited disease progression of M1 and stable disease of M2 in response to MP therapy. Numbers in the table represent frequency of patients achieving response criteria. CVAD = cyclophosphamide-vincristine-doxorubicin-dexamethasone; MP = melphalan-prednisone; MPT = melphalan-prednisolone-thalidomide; CTD = cyclophosphamide-thalidomide-dexamethasone; CTDa = attenuated CTD; CRD = cyclophosphamide-lenalidomide(REVLIMID®)-dexamethasone; CRDa = attenuated CRD; CCRD = kyprolis-CRD. Response codes aggregated into 'response' (CR/VGPR/PR/MR) and 'no response' (SD/PD). Boxplots represent median, $25^{th}/75^{th}$ percentiles and whiskers represent lowest/highest non-outlier value, outliers are represented by black dots (>1.5 x interquartile range) and clear dots (>3.0 x interquartile range).

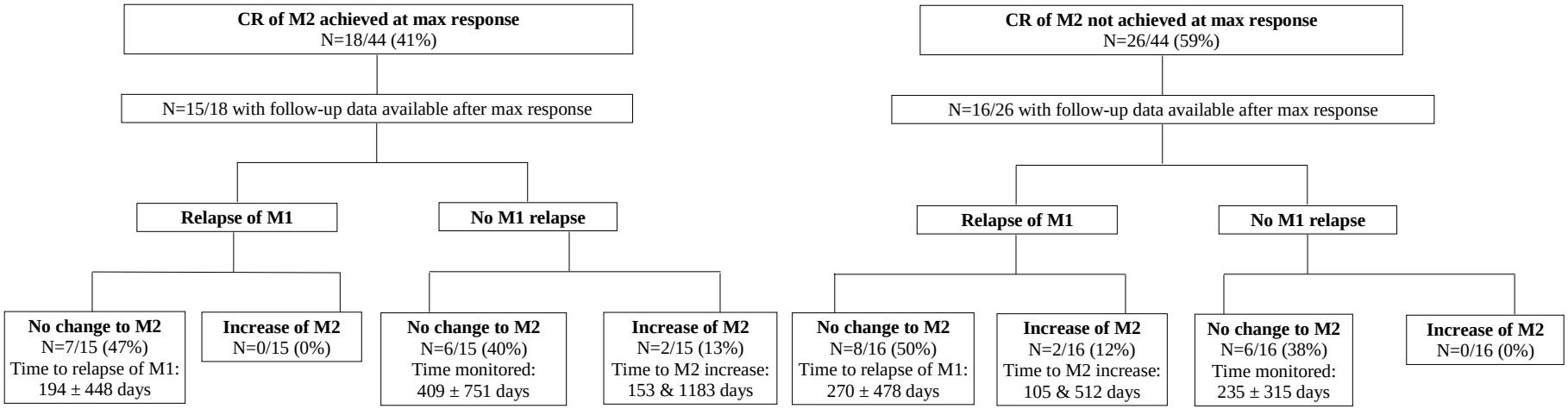


FIGURE 3. Flow diagram illustrating frequency of relapse amongst 44 BGMM patients after maximum response to anti-MM therapy.

TABLE 1. Characteristics and frequencies of M1 and M2 MABS in 44 eligible BGMM patients at trial entry. MAB concentration data represent median ± interquartile range [IQR].

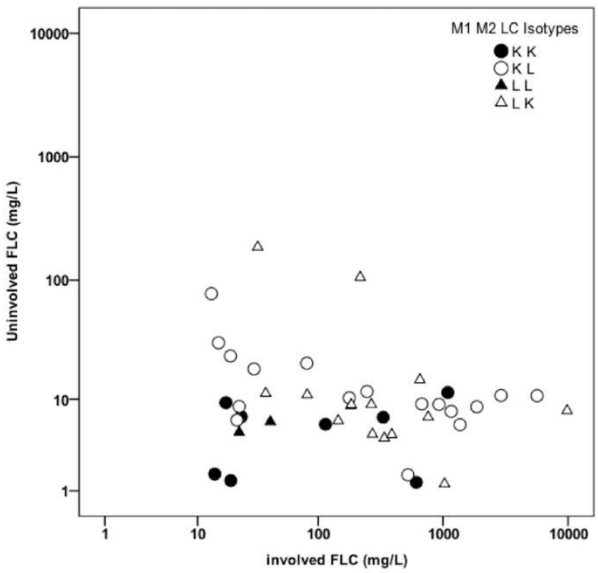
Monoclonal Antibody (MAB) 1				Monoclonal Antibody (MAB) 2			
HC Isotype		Frequency (N)	Conc. (g/L) (median ± IQR)	HC Isotype		Frequency (N)	Conc. (g/L) (median ± IQR)
IgG	Total	27	36.1 ± 34.1	IgG	Total	33	2.6 ± 3.4
	IgGκ	17	36.1 ± 31.5		IgGκ	17	2.5 ± 3.2
	IgGλ	10	33.4 ± 29.4		IgGλ	16	2.8 ± 3.0
IgA	Total	12	29.6 ± 28.2	IgA	Total	11	1.7 ± 3.0
	IgAκ	6	32.6 ± 31.0		IgAκ	5	1.3 ± 2.1
	IgAλ	6	23.2 ± 33.9		IgAλ	6	2.5 ± 4.8
IgD	Total	1	2.6				
	IgDκ	0	-				
	IgDλ	1	2.6				
FLC	Total	4	1.0 ± 1.2				
	FLCκ	3	1.4				
	FLCλ	1	0.7				

TABLE 2. Serological responses of MM ('M1') and MGUS ('M2') MABs at the time of maximum response to anti-MM therapy in all eligible BGMM (N=44 patients, top), and in patients enrolled in Myeloma IX and Myeloma XI trials receiving either intensive (N=20, middle) or non-intensive therapies (N=17, bottom). CR=complete response; VGPR=very good partial response; PR=partial response; MR=minor response (MR); SD=stable disease; PD=progressive disease. §Response codes aggregated into: good (CR/VGPR), moderate (PR/MR) and poor (SD/PD) responses to therapy; or, response (CR/VGPR/PR/MR) and no response (SD/PD) to therapy. Percentages may not add to 100% due to rounding. * Indicates significant differences p=0·0001; χ^2=18·03) observed between M1 and M2 responses to all therapies in all (N=44) patients when responses were assessed using international (CR/VGPR/PR/MR/SD/PD) response codes. ** Indicates significant differences (p=0·002; χ^2=11·34) observed between M1 and M2 responses to all therapies in all (N=44) patients when differences were analysed using good/moderate/poor composite response codes. *** Indicates significant differences (p=0·001) observed between M1 and M2 responses to all therapies in all (N=44) patients when assessed using response/no response composite response codes. # Indicates significant differences (p=0·02; χ^2=10·36) observed between M1 and M2 responses to non-intensive therapy in N=17 patients when responses were assessed using international disease (CR/VGPR/PR/MR/SD/PD) response codes. ## Indicates significant differences (p=0·05; χ^2=6·40) observed between M1 and M2 responses to non-intensive therapy in N=17 patients when responses were assessed using composite good/moderate/poor response codes. ### Indicates significant differences (p=0·03) observed between M1 and M2 responses to non-intensive therapy in N=17 patients when responses were assessed using composite response/no response codes.

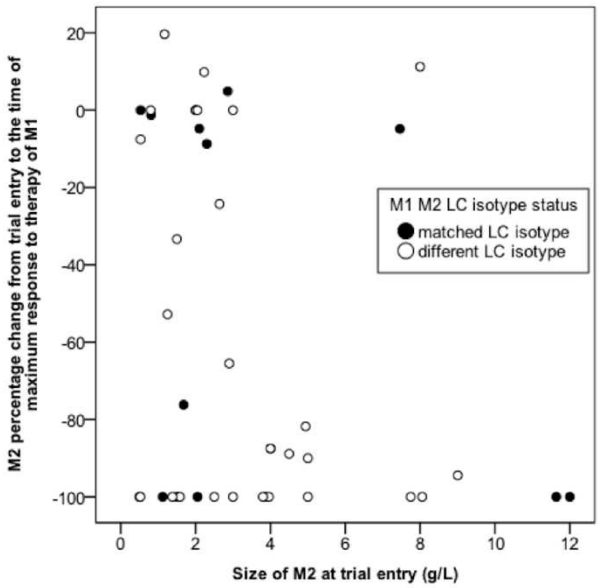
Pathway	M1			M2		
	Serological response code	Frequency (N) Proportion [%]	Composite response codes§ (N [%])	Serological response code	Frequency (N) Proportion [%]	Composite response codes§ (N [%])
All patients (N=44)	CR	14 [32]	Good: 27 [61]	CR	18 [41]	Good: 20 [46]
	VGPR	13 [30]	** Moderate: 14 [32]	VGPR	2 [5]	** Moderate: 8 [18]
	PR	11 [25]	Poor: 3 [7]	PR	7 [16]	Poor: 16 [36]
	MR	3 [7]		MR	1 [2]	
	SD	2 [5]	Response: 41 [93]	SD	16 [36]	Response: 28 [64]
	PD	1 [2]	*** No response: 3 [7]	PD	0 [0]	*** No response: 16 [36]
Intensive therapy (N=20)	CR	8 [40]	Good: 15 [75]	CR	10 [50]	Good: 11 [55]
	VGPR	7 [35]	Moderate: 4 [20]	VGPR	1 [5]	Moderate: 4 [20]
	PR	3 [15]	Poor: 1 [5]	PR	4 [20]	Poor: 5 [25]
	MR	1 [5]		MR	0 [0]	
	SD	1 [5]	Response: 19 [95]	SD	5 [25]	Response: 15 [75]
	PD	0 [0]	No response: 1 [5]	PD	0 [0]	No response: 5 [25]
Non-intensive therapy (N=17)	CR	5 [30]	Good: 10 [59]	CR	7 [41]	Good: 8 [47]
	VGPR	5 [30]	## Moderate: 6 [35]	VGPR	1 [6]	## Moderate: 2 [12]
	PR	5 [30]	Poor: 1 [6]	PR	2 [12]	Poor: 7 [41]
	MR	1 [6]		MR	0 [0]	
	SD	0 [0]	Response: 16 [94]	SD	7 [41]	Response: 10 [59]
	PD	1 [6]	### No response: 1 [6]	PD	0 [0]	### No response: 7 [41]

TABLE 3. Frequency and percentages of responses achieved by M1 and M2 within 44 BGMM patients at the time of maximum response to anti-MM therapies; due to rounding percentages do not add to 100%.

		M2 response					
		CR	VGPR	PR	MR	SD	PD
M1 response	CR	10 [23%]	1 [2%]	0	0	3 [7%]	0
	VGPR	4 [9%]	1 [2%]	4 [9%]	0	4 [9%]	0
	PR	3 [7%]	0	2 [5%]	0	6 [14%]	0
	MR	1 [2%]	0	1 [2%]	0	1 [2%]	0
	SD	0	0	0	1 [2%]	1 [2%]	0
	PD	0	0	0	0	1 [2%]	0



SUPPLEMENTARY FIGURE 1. FLC levels at disease presentation in BGMM patients with FLC results available (N=42 of 44 patients). Symbols depict the different LC-isotypes of the M1 and M2 clones, respectively. The involved FLC was identified by the LC isotype of M1.



SUPPLEMENTARY FIGURE 2. Size of M2 at BGMM diagnosis and percentage change in M2 size from trial entry to the time of maximum response to anti-MM therapy. Responses are colour-coded based on whether the M1 and M2 MABS had matched (black) or different (clear) LC isotypes.