Comparison of intramedullary myeloma and corresponding extramedullary soft tissue plasmacytomas using genetic mutational panel analyses

Multiple Myeloma (MM) is characterized by growth and proliferation of clonal plasma cells in the bone marrow (BM), and these plasma cells depend heavily on this BM microenvironment. In a subset of patients, the myeloma cells can spread hematogenously to soft tissues and these extramedullary myeloma (EM) lesions may be found in up to 30% of MM patients, the majority occurring during the course of the disease. When restricting the definition of EM lesions to infiltration of soft tissue only and excluding the bone-related plasmacytomas, the prognosis is dismal with an overall survival of less than 6 months. It has been suggested that the incidence of EM relapses is increasing, possibly due to novel treatments or allogeneic stem cell transplantation. Therefore novel therapeutic interventions are warranted. Molecular and genetic analysis, we also performed immunohistochemical (IHC) analysis for p53 protein expression as previously described. The characteristics of the patients included in the analysis are shown in Table 1. All patients had been treated with immunomodulatory drugs and 10 with a proteasome inhibitor. In total, 7 out of 14 patients developed the EM relapse after allogeneic stem cell transplantation and/or donor lymphocyte infusion. In total, 12 out of 15 BM biopsies yielded results and 11 out of 14 EM biopsies, demonstrating a success percentage of DNA retrieval in 80% and 79%, respectively. The EM biopsies that were analyzed were located in the lymph node (2), skin (7), orbita (1) and pancreas (1).

Overall a limited number of mutations was found in these samples (Figure 1), most samples containing only a single mutation, with a maximum of three mutations in one sample. Somatic mutations were found in NRRAS, KRAS, Kit c840, ATM, PAC, TP53 and BRAF.

A high prevalence of activating RAS mutations was found both in BM samples in 6 out of 9 patients (67%) and in EM samples in 7 out of 11 patients (64%). The frequency of RAS mutations in this cohort is much higher than the previously reported frequencies of 23–44% in newly diagnosed and relapsed MM patients and is in accordance with the high incidence of RAS mutations reported in plasmocell leukemias from 54.4% at diagnosis to 81% at time of relapse. In five patients with an RAS mutation, we were able to compare the BM and EM samples and demonstrated that in three patients the identical RAS mutation was already present in the diagnostic BM samples. In one patient it was not detectable in the BM at diagnosis, but was present in the BM at relapse and only one patient had a gain of RAS mutation in the EM sample only. This contradicts previous findings that the mutation is acquired during the disease progression from intramedullary to EM disease. The high frequency of RAS mutations does support, however, the previous hypothesis that RAS might be a prerequisite for EM growth, next to a yet- unidentified mechanism.

Further focusing on the seven patients with paired BM and EM analysis available in our cohort, we found no additional mutations that are likely to be causal for EM spread, nor did we find novel treatment targets using this limited gene panel. We did observe an increment in allele frequency in some cases for mutations present in EM compared with BM, which may be due to the clonal
selection, however this may also be attributed to the presence of non-tumor cells in the biopsies.

In our cohort, 1 out of 11 patients had the BRAF V600E mutation in the EM biopsy, which coincide with previously reported prevalence of BRAF mutation in EM. Moreover, we detected three mutations, not previously described in MM patients: KIT C840Y/C844Y, ATM L2877F and APC E1317Q. Mutant KIT has been implicated in the pathogenesis of several cancers including melanoma, acute leukemia and gastrointestinal stromal tumors, but effective treatment strategies are still missing. ATM mutations are commonly found in ataxia-telangiectasia and somatic mutations were recently found to be related to the loss of the 11q23 region in T-PLL, however this is not commonly found in MM. These novel mutations in MM were both found in BM material at

| Patient no | Gender | Age at diagnosis (yr) | Ig-type | % PC in diagnostic BM | Lines of therapy before EM | EM years after diagnosis | Cytogenetics | Allogeneic SCT |
|------------|--------|----------------------|---------|-----------------------|-----------------------------|--------------------------|--------------|---------------|
| 1          | F      | 46                   | IgGλ    | 60%                   | 5                           | 7                        | 48 XX        | Yes           |
| 2          | F      | 49                   | IgGκ    | 40%                   | 10                          | 11                       | 56 XX        | Yes           |
| 3          | M      | 35                   | Light chain | 35%               | 2                           | 1                        | FISH: 1q+    | No            |
| 4          | F      | 63                   | Light chain | NA                 | 0                           | 0                        | FISH: no deletions | No            |
| 5          | M      | 30                   | IgGκ    | NA                    | 2                           | 0.5                      | No           | No            |
| 6          | M      | 61                   | IgAλ    | 90%                   | 7                           | 5                        | Hyperdiploid | Yes           |
| 7          | M      | 49                   | IgGκ    | 40%                   | 2                           | 2                        | FISH: 1q+    | No            |
| 8          | F      | 69                   | IgGκ    | 70%                   | 1                           | 1                        | 46 XY        | No            |
| 9          | M      | 64                   | Light chain | 90%               | 1                           | 1                        | FISH: no deletions | No            |
| 10         | M      | 52                   | IgGκ    | 40%                   | 5                           | 4                        | ND           | Yes           |
| 11         | M      | 69                   | IgGλ    | NA                    | 2                           | 1                        | ND           | No            |
| 12         | M      | 43                   | IgGκ    | NA                    | 9                           | 10                       | 56-56 XY     | Yes           |
| 13         | M      | 55                   | Light chain | 85%              | 6                           | 6                        | ND           | Yes           |
| 14         | F      | 39                   | IgGλ    | 40%                   | 0                           | 0                        | Near tetraploid | No            |

Abbreviations: EM, extramedullary; NA, not applicable; ND, not determined; PC, plasma cells; SCT, stem cell transplantation.

Figure 1. Schematic representation of somatic mutations identified in 14 multiple myeloma patients. The mutant allele frequency of each mutation observed on the IonTorrent PGM using AmpliSeq Cancer Hotspot V2 Panel was plotted for each individual patient indicated, in which sample the mutation was found. ND, not determined, due to technical issues NA, not applicable, unable to retrieve sample for analysis.
diagnosis and in EM tissue, and are therefore unlikely to be related to the development of EM disease. Furthermore, no healthy control tissue of these patients was analyzed and therefore it is not certain that these mutations are involved in the pathogenesis of MM.

TP53 mutations may be associated with the presence of EM disease at diagnosis. In three patients, a TP53 mutation or frameshift was detected in their BM or EM relapse sample. These three patients all showed diffuse and strong nuclear expression of the p53 protein on IHC, also indicative for a TP53 mutation (data not shown). Our IHC analyses also revealed a p53 overexpression in the EM relapse of two patients that did not have a mutation in TP53 and their BM samples had normal and overexpression of TP53, respectively. This is consistent with the general understanding that TP53 mutations are rarely present at the time of diagnosis but occur more frequently in advanced disease as well as in EM disease. We should note, however, that in patient number 1, the TP53 mutation was detected in the BM relapse but not the EM relapse. This indicates that clones with TP53 aberrations do not necessarily have to persist or disseminate in EM disease.

Interestingly, the TP53V197L mutation found in the EM relapse of patient 13 is associated with resistance to radiotherapy (RT) in patients with solid malignancies like glioblastoma, and head and neck cancer. The EM plasmacytoma of this patient was also RT refractory suggesting, for the first time, that the outcome of RT may also be linked to TP53 mutations in MM.

In conclusion, we demonstrate the feasibility of performing next generation sequencing on formalin and decalcified BM biopsy material of MM patients. Patients with an EM relapse have a high frequency of 69% of RAS mutations, in most of them already present at diagnosis. The frequency of TP53 mutations is less and mostly detected in relapsed samples. Therefore, the typical behavior and therapy resistance of EM relapse seems to be mediated by other factors than analyzed in this study and are possibly related to post-transcriptional alterations and/or to the influence of the micro environment.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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REFERENCES

1. Weinstock M, Ghobrial IM. Extramedullary multiple myeloma. Leuk Lymphoma 2013; 54: 1135–1141.
2. Touzeau C, Moreau P. How I treat extramedullary myeloma. Blood 2014; 124: 3212–3221.
3. Minnema MC, van de Donk NW, Zweegman S, Hegenbart U, Schonland S, Raymakers R et al. Extramedullary relapses after allogeneic non-myeloablative stem cell transplantation in multiple myeloma patients do not negatively affect treatment outcome. Bone Marrow Transplant 2008; 41: 771–784.
4. Furukawa Y, Kikuchi J. Molecular pathogenesis of multiple myeloma. Int J Clin Oncol 2015; 20: 413–422.
5. Lohr J, Stoianov P, Carter S, Cruz-Gordillo P, Lawrence M, Auclair D et al. Widespread genetic heterogeneity in multiple myeloma: implications for targeted therapy. Cancer Cell 2014; 25: 91–101.
6. Andrulis M, Lehners N, Capper D, Penzel R, Heining C, Huellein J et al. Targeting the BRAF V600E mutation in multiple myeloma. Cancer Discov 2013; 3: 862–869.
7. Rasmussen T, Kuehl M, Lodahl M, Johnsen HE, Dahl IMS. Possible roles for activating RAS mutations in the MGUS to MM transition and in the intramedullary to extramedullary transition in some plasma cell tumors. Blood 2005; 105: 317–323.
8. Hoogstraat M, Hinrichs NW, Besselink NJM, Rademaker van Loon JM, de Vojis CMA, Peeters T et al. Simultaneous detection of clinically relevant mutations and amplifications for routine cancer pathology. J Mol Diagn 2015; 17: 10–18.
9. Ercan C, van Diest PJ, van der Ende B, Hinrichs J, Bult P, Buerger H et al. p53 mutations in classic and pleomorphic invasive lobular carcinoma of the breast. Cell Oncol (Dordr) 2012; 35: 111–118.
10. Chung WJ, Price-Troska T, Gonzalez-Paz N, Van Wier S, Jacobus S, Blood E et al. Clinical significance of TP53 mutation in myeloma. Leukemia 2007; 21: 582–584.
11. Mulligan G, Lichter DJ, Di Bacco A, Blakemore SJ, Berger A, Koenig E et al. Mutation of NRAS but not KRAS significantly reduces myeloma sensitivity to single-agent bortezomib therapy. Blood 2014; 123: 632–639.
12. Bezieu S, Devilder MC, Avet-Loiseau H, Mellerin MP, Puthier D, Pennarun E et al. High incidence of N and K-Ras activating mutations in multiple myeloma and primary plasma cell leukemia at diagnosis. Hum Mutat 2003; 18: 212–224.
13. Neri A, Baldini L, Trecca D, Cro L, Polli E, Maiolo AT. p53 gene mutations in multiple myeloma are associated with advanced forms of malignancy. Blood 1993; 81: 128–135.
14. Skinner HD, Sandulache VC, Ow TJ, Meyn RE, Yordy JS, Beadle BM et al. TP53 disruptive mutations lead to head and neck cancer treatment failure through inhibition of radiation-induced senescence. Clin Cancer Res 2012; 18: 292–300.
15. Bladé J, Fernández De Larrea C, Rosiñol L, Gibeira MT, Jiménez R, Powles R. Soft-tissue plasmacytomas in multiple myeloma: Incidence, mechanisms of extra-medullary spread, and treatment approach. J Clin Oncol 2011; 29: 3805–3812.

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