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AUTHOR CONTRIBUTIONS

SJ and AK designed the study, interpreted data and wrote the manuscript. SoS and KP performed research and generated data. MM and NN performed data analysis. WK, TH, CH and SuS performed diagnostic interpretation of patient samples.

S Jeromin^{1,3}, A Kohlmann^{1,3}, M Meggendorfer¹, S Schindela¹,
K Perglerová², N Nadarajah¹, W Kern¹, C Haferlach¹,
T Haferlach¹ and S Schnittger¹

¹MLL Munich Leukemia Laboratory, Munich, Germany and
²MLL2 s.r.o., Praha, Czech Republic

E-mail: Sabine.Jeromin@mll.com

³These two authors contributed equally to this work.

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OPEN

Inhibiting MEK in MAPK pathway-activated myeloma

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Over the last decade, new drugs have significantly changed the paradigm for treating multiple myeloma (MM), resulting in improved outcomes and reduced toxicity. However, many patients with MM relapse, and those who are refractory to or relapse after therapy with an immune-modulatory drug and a proteasome inhibitor have a dismal prognosis.¹ Improving the outcome of relapsed and refractory MM is a significant clinical challenge. Importantly, in this respect, recently published data have established the frequent mutation of the RAS/mitogen-activated protein kinase (MAPK) pathway,^{2–5} with mutations in *NRAS*, *KRAS* or *BRAF* being present in up to 50% of newly diagnosed MM cases. We routinely perform comprehensive genomic profiling using the FoundationOne Heme assay (Supplementary Methods). Review of these data shows the majority of the *NRAS*, *KRAS* and *BRAF* mutations occur in hotspots causing constitutive activation of the corresponding proteins. This makes the MAPK pathway a significant therapeutic target in MM.

Recent reports have demonstrated that MM cases with *BRAF* V600E mutations can respond to vemurafenib, even in the autologous stem cell transplant (ASCT) double-refractory setting, suggesting that blocking the MAPK pathway can be effective,

even in end-stage, genetically complex cases.⁶ Inhibition of *BRAF* using *BRAF* V600E inhibitors can result in paradoxical activation of the MAPK pathway, due to transactivation of *CRAF*,⁷ a phenomenon that is exaggerated in *KRAS*-mutated cancers.⁸ Inhibition of MAPK kinase (MEK) has emerged as a viable strategy to treat patients with *BRAF*-mutated cancers and to overcome paradoxical activation in the setting of therapy with *BRAF* V600E-directed agents. Trametinib is an oral, allosteric inhibitor of MEK1/2 that has shown early clinical activity in tumors with activating *BRAF* mutations. Preclinical studies have shown potent inhibition of MEK1/2 activation by preventing RAF-dependent phosphorylation of MEK.⁹ Using trametinib *in vitro* resulted in inhibition of growth among most cancer cell lines and tumor xenografts, particularly those with activating mutations in *BRAF* or *KRAS*.⁹

As an index case of *BRAF* wild type, yet with an activating genomic alteration of the MAPK pathway, we report a case of a 52-year-old heavily pretreated man with MM who presented with treatment-resistant extramedullary disease (EMD). He was diagnosed with kappa light-chain MM in 2003, presenting with anemia, hypercalcemia and renal failure requiring hemodialysis. A detailed description of this patient's course of treatment and a timeline of events can be found in Supplementary Material and Supplementary Figure 1. He was initially treated with thalidomide and dexamethasone, followed by high-dose chemotherapy and

Timeline of Treatments and Reasons for Discontinuation of Therapy

Figure 1. Timeline of treatments and reasons for discontinuing therapy for 58 patients. Bar graphs represent days from start of treatment. Bar graphs show time of documented follow-up in aqua, time on multi-agent represented in green, time on single agent in blue, time since previous therapy in gray; discontinuation represented as a cross (X) following the color coding: adverse event in royal blue, progression in teal, physician's choice in yellow, other in pink; deaths are represented as diamonds in red.

ASCT. He relapsed in late 2005 with EMD in the liver and was treated with dexamethasone/cyclophosphamide/etoposide/cisplatin/thalidomide, resulting in a complete remission. In March 2006 he was treated with DT-PACE and tandem ASCT to consolidate his response, which was maintained with TD, keeping him disease free for 2 years. In December 2008 he relapsed with 84 FDG-avid focal bony lesions as well as EMD in the spleen and cervical lymph nodes. Evaluation of the bone marrow at that time showed 52% PC that were high risk by a gene expression

profiling based 70-gene score (GEP70).¹⁰ The patient underwent chemotherapy with PACMED (cisplatin, cytarabine, cyclophosphamide, mesna, etoposide, dexamethasone), resulting in a complete remission.

Between December 2008 and August 2013 the patient experienced multiple relapses and was treated with salvage therapies, which included ASCT, carfilzomib, pomalidomide, multi-agent chemotherapies, metronomic therapy and transarterial chemo-embolization, with varying degrees of responses.

Figure 2. (a) Best response using protein criteria during treatment with trametinib as both single-agent and multi-agent therapies for 40 patients with measurable disease. Best response was determined as the greatest percent change in protein levels for patients with measurable disease. Measurable disease was determined using baseline test results and IMWG criteria, which requires one of the following: M protein (> 1 g/dl), serum protein (> 200 mg/24 h) or free light chain (involving FLC.10 mg/dl, and abnormal ratio). Patient response determined by free light chains are shown in pink, by urine protein shown in green, and serum protein shown in blue. (b) Best response by PET during treatment with trametinib for 24 patients. The bar graph shows patients with at least one focal lesion at baseline. Best response was calculated by the greatest percent change in number of focal lesions. Protein percent change was calculated by the greatest change by serum, urine or free light-chain values. PET results are shown in blue. Protein change is shown in pink.

Over the course of his treatment, the patient developed EMD in the paraspinal muscles and the mesenteric lymph nodes in addition to treatment-resistant EMD of the liver.

In August 2013, comprehensive genomic profiling of CD138+ selected cells from his liver lesion using the FoundationOne assay revealed a KRAS Q61H mutation in 57% of cells. Four weeks after completion of his last salvage treatment at a time when there was positron emission tomography (PET)-proven persistence of disease, the patient was started on 2 mg trametinib daily. A follow-up PET 1 month later revealed complete resolution of all FDG avid lesions. Magnetic resonance imaging carried out 3 months after initiation of trametinib revealed complete resolution of previously identified liver lesion. In August 2014 Mekinist was stopped on account of a decreased left ventricular ejection fraction. The patient was noted to have relapsed disease by PET imaging and serum markers in October 2014.

To understand how this index case represents the RAS-mutated and MAPK pathway-activated population, we identified 58 additional patients who were treated with trametinib as a single agent or in combination with other drugs between August 2013 and May 2014 (Supplementary Figure 2). This retrospective review was approved by the UAMS institutional review board (IRB # 202984). All patients had provided informed consent. Electronic Medical Records and our Multiple Myeloma Data Base were reviewed to obtain demographic information, laboratory results as well as the patient's treatment history. Measurable disease was defined according to the International Myeloma Working Group.¹¹ For those patients with measurable disease, response was measured as the greatest percent change of measurable myeloma protein after initiation of therapy with trametinib. PET response was measured as the greatest percent change of number of FDG-avid focal lesions after initiation of therapy with trametinib. For the measurement of time on trametinib, drug holidays due to adverse events or for dose reduction were not considered as discontinuation of the drug. Lack of trametinib treatment for >3 weeks, that is, even if trametinib was added again at a later time point, was considered definite discontinuation.

Of the 58 patients, 51 patients were treated with trametinib based on the presence of oncogenic mutations of *KRAS*, *NRAS* or *BRAF*. Seven patients were treated based on GEP information suggesting an activation of the MAPK pathway.¹² The GEP information indicating overexpression of the MAPK pathway included overexpression of *ITGB7*, *CCND2* or *CCR1* (Supplementary Methods). Most patients had relapsed or refractory MM and received trametinib on an urgent basis, not allowing for a washout period. Their pre-trametinib features included cytogenetic abnormalities in 61%, while GEP70-defined high risk was present in 35%. PET scans available for all 58 patients showed medullary focal lesions in 30 cases (52%) and EMD in 11 (19%) (Supplementary Table 1). The median number of prior treatments was five, including Total Therapy trials^{13–15} in 34 of 58 patients. Forty-two patients had at least one ASCT, 39 had salvage chemotherapy and 31 had been exposed to pomalidomide or carfilzomib.

Trametinib treatment was well tolerated. Of 58 patients treated, 24 discontinued therapy because of toxicities and 15 discontinued because of disease progression, physician's choice or death (Figure 1). The most significant adverse events were rash, diarrhea and cardiac toxicities. We observed 12 deaths. None of these was attributed to trametinib (Supplementary Table 2). Of the 58 patients treated with trametinib, 48 patients began treatment with monotherapy and 10 began with trametinib in combination with other agents (Supplementary Table 3). Of the 48 patients who began with trametinib as monotherapy, 26 had other agents added during the course of their treatment (Supplementary Table 4). Twenty-two patients received trametinib mono-therapy only (Figure 1).

Of the 40 patients with measurable disease at time of trametinib initiation, 23 patients experienced a reduction of the measurable MM protein by at least 25%. At least 50% reduction of the MM protein was seen in 16 patients (Figure 2a). This number was reduced to four when only considering the time on single agent trametinib (Supplementary Figure 3). Of the 24 patients with ≥ 1 FDG-avid focal lesion on PET imaging at the beginning of treatment and available follow-up studies, 15 showed a >50% reduction in the number of focal lesions. Nine patients achieved complete remission based on positron emission tomography imaging (PET-CR) status, including six who had complete resolution of their focal lesions on single agent trametinib (Figure 2b and Supplementary Figure 4). In general, the PET response correlated well with a reduction of myeloma protein for most patients.

At a median follow-up of 171 days, the median overall survival has not been reached, with 61% estimated to be alive at 260 days (Supplementary Figure 5). Due to the retrospective nature of this review an accurate estimate of progression-free survival (PFS) is not possible. We therefore used 'time to next therapy' (TNT) as a surrogate for PFS. At a median follow-up of 171 days the median TNT was 186 days (95% confidence interval: 106–231 days) (Supplementary Figure 6).

Although this retrospective study may lack the patient uniformity afforded to clinical trials by stringent entry criteria and treatment protocol, it is more representative of the 'real-life' patient population without bias toward benign disease features and better performance status. The trametinib single-drug response rate in a patient population in urgent need of therapy is reminiscent of our early investigations into thalidomide.

Trametinib shows promise as a myeloma therapeutic based on responses seen in this heavily pretreated MM population. The observation of complete responses with trametinib monotherapy supports the continued investigation of targeted therapy of the RAS/MAPK pathway and the use of trametinib as treatment for patients with activating MAPK pathway mutations who have exhausted standard treatments. A prospective trial evaluating the effect of trametinib on outcome in relapsed myeloma has been initiated.

CONFLICT OF INTEREST

Sriraj M Ali, MD, Phil J Stephens, PhD, Jeffrey S Ross, MD, and Vincent A Miller, MD, are employed by Foundation Medicine, Inc. Christoph J Heuck has received speaking honoraria by Foundation Medicine, Inc. Bart Barlogie, MD, is co-inventor of a gene expression risk model, which has been licensed to Signal Genetics, LLC. All other authors declare no conflict of interest.

CJ Heuck¹, Y Jethava¹, R Khan¹, F van Rhee¹, M Zangari¹, S Chavan¹, K Robbins¹, SE Miller¹, A Matin¹, M Mohan¹, SM Ali², PJ Stephens², JS Ross^{2,3}, VA Miller², F Davies¹, B Barlogie¹ and G Morgan¹

¹Myeloma Institute, University of Arkansas for Medical Sciences, Little Rock, AR, USA;

²Foundation Medicine, Inc., Cambridge, MA, USA and

³Department of Pathology, Albany Medical College, Albany, NY, USA
E-mail: cjheuck@uams.edu

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IDH1 and IDH2 mutations in myelodysplastic syndromes and role in disease progression

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Recurrent pathogenic mutations in *IDH1* and *IDH2* at the conserved amino acid sites *IDH1*-R132, *IDH2*-R140 and *IDH2*-R172 occur in ~20% of patients with acute myeloid leukemia (AML).¹ A recent analysis of AML patients at our institution identified *IDH1/2* mutations in 20% ($n=167$) of 826 AML patients, with *IDH1/2* mutations occurring most frequently in the setting of diploid karyotype or other intermediate-risk cytogenetics, particularly trisomy 8 (77 vs 53%, $P < 0.0005$). AML patients with *IDH1/2* mutations were overall less likely to have a diagnosis of therapy-related AML (8 vs 17%, $P = 0.003$).²

Compared with their frequency in AML, *IDH1/2* mutations are less common in myelodysplastic syndromes (MDS), occurring in ~5% of MDS patients, although an incidence as high as 12% has been reported.^{3–8} Although *IDH1/2* mutations are thought to represent early 'driver' events in leukemogenesis with mutational stability over time, reports of *IDH1/2* acquisition at the time of leukemic transformation in patients with myeloproliferative neoplasms and MDS have been described.^{3,9,10}

The purpose of this analysis is to evaluate the overall prevalence of *IDH1/2* mutations in MDS patients treated at our institution, as well as determine the incidence and frequency of *IDH1/2* mutations identified at the time of leukemic transformation in MDS patients.

Eligible patients comprised all adults with histologically confirmed MDS treated at MD Anderson Cancer Center from January 2010 to January 2015. A total of 1042 MDS patients with known *IDH1* and *IDH2* status were included. From January 2010 to September 2012, *IDH1/2* molecular analysis was performed by high-resolution melting curve analysis followed by Sanger

sequencing confirmation (analytical sensitivity: 10–20%) as has been previously described.¹¹ Beginning in September 2012, *IDH1/2* testing was performed within a Clinical Laboratory Improvements Amendments-certified next-generation sequencing platform (analytical sensitivity: 2.5–5%). Statistical analyses were conducted in Statistica v12.0 (StatSoft Inc, Tulsa, OK, USA) and significance defined as $P < 0.05$. Overall survival (OS) was measured as the time from presentation to date of death or last follow-up, and progression-free survival from presentation to date of death, last follow-up, or date of progression to AML. Informed consent was obtained following institutional guidelines and in accordance with the Declaration of Helsinki.

Of the 1042 MDS patients, 60 patients (5.7%) had *IDH1/2* mutations identified. Specifically, 17 patients (1.6%) were *IDH1*-R132 mutated and 43 patients (4.1%) had *IDH2*-R140 ($n=42$) or *IDH2*-R172 ($n=1$) mutations, respectively. The clinicopathological characteristics of patients with and without *IDH1/2* mutations are shown in Table 1. Within this cohort, 701 patients (67%) were untreated and 341 (33%) had received systemic MDS therapy before presentation. MDS patients with *IDH1/2* mutations had a lower absolute neutrophil count ($1.15 \times 10^9/l$ vs $1.71 \times 10^9/l$, $P = 0.02$), higher bone marrow blast percentage (6 vs 4%, $P = 0.001$), and a trend for higher platelet counts ($99 \times 10^9/l$ vs $75 \times 10^9/l$, $P = 0.07$).

Of the 60 *IDH1/2* mutations, 17 (28%) were present in the very low or low-risk IPSS-R groups, 15 (25%) intermediate, and 27 (45%) in the high or very-high IPSS-R prognostic score categories (Table 1). While the distribution of IPSS-R categories among *IDH1/2*-mutants versus wild-type patients was similar, we identified a conspicuously different underlying pattern of cytogenetics and bone marrow blasts. Consistent with karyotypic patterns in *IDH1/2*-mutant AML,² the majority of *IDH1/2*-mutant MDS patients