Newly diagnosed isolated myeloid sarcoma–paired NGS panel analysis of extramedullary tumor and bone marrow

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Abstract
Isolated myeloid sarcoma (MS) is a rare malignancy in which myeloid blast forms tumors at various locations while the bone marrow (BM) remains cytomorphologically free from disease. We analyzed isolated MS from four patients and their BMs at initial diagnosis and follow-up, using a custom next-generation sequencing (NGS) panel. We observed possible clonal evolution and a clonal hematopoiesis of indeterminate potential (CHIP)-like finding in the BM of one of three cases with detectable mutations. Clinical presentation of one patient suggested extramedullary confined homing of blasts to distal sites in the relapse situation still sparing the BM. In summary, our findings shall motivate future work regarding signals of extramedullary blast trafficking and clonal evolution in MS.

Keywords Myeloid sarcoma · CHIP · Clonal evolution · NGS sequencing · Homing

Introduction
Myeloid sarcoma (MS) is characterized as an extramedullary tumor composed of myeloid blasts. It may manifest simultaneously as a part of acute myeloid leukemia (AML), as a progression of myeloproliferative neoplasms or myelodysplastic syndromes, or it may arise at relapse, especially in patients following allogeneic hematopoietic stem cell transplantation [1]. De novo isolated MS without bone marrow (BM) infiltration is a rare variant of MS, but is usually, although not always [2], a harbinger of subsequent BM blast infiltration and overt AML with a short delay [3].

Isolated MS was historically defined on a cytological or histological level, and one may wonder whether this concept can persist in the time of modern sensitive PCR-based detection methods of minimal residual disease (MRD) [4]. Also, a growing understanding of clonal heterogeneity and evolution in healthy and neoplastic hematopoiesis, such as clonal hematopoiesis of indeterminate potential (CHIP) [5], demands a NGS-based parallel assessment of MS specimen and BM samples in isolated MS cases in order to explore any manifestation of those phenomena in this unique scenario.

Materials and methods
We identified four cases of de novo isolated MS, diagnosed between 2015 and 2017 at our institution suitable for subsequent assessment. For all cases, material for DNA isolation from MS primary sites (formalin-fixed paraffin-embedded (FFPE) tissue) and BM (fresh aspirate or FFPE biopsy samples) at initial diagnosis was available. For two of these patients, follow-up samples (blood or BM aspirate) were available during complete response, and in one patient paired samples from a distant extramedullary site and BM at relapse. DNA was isolated according to standard protocols. Samples at initial diagnosis and relapse were submitted to a custom TruSight myeloid NGS sequencing panel approach covering...
Clinical case descriptions

Patient 1 was a 71-year-old Caucasian male, who was diagnosed with isolated MS in February 2015. PET-CT scan showed an occipital cutaneous tumor with adjacent bone lesions of the left parietal calvaria. Diagnosis of MS was established from an initial attempt of surgical tumor resection. Histopathological examination of BM biopsy showed no evidence of AML. Therapy included two courses “7 + 3” induction and one course of intermediate dose Ara C (IDAC) consolidation (cytarabine 1000 mg/qm every 12 h for 3 days), followed by tomotherapy of the calvaria (50 Gy fractionated). PET-CT scan in October 2015 after completion of therapy was interpreted as PET-negative CR. The patient remained in remission until last follow-up, 54 months after diagnosis.

Patient 2 was a 30-year-old male of Arab descent, who was diagnosed with isolated MS in August 2015. PET-CT scan revealed multifocal bilateral enlarged lymph nodes at cervical, axillary, iliac, and inguinal sites, and definite diagnosis was established from histopathological evaluation of a left submandibular lymph node. BM was unaffected by cytology, flow cytometry and histomorphology. The patient received standard AML induction therapy with “7 + 3” chemotherapy and achieved PET-negative complete response after the second course. The patient was scheduled for allogenic matched unrelated donor stem cell transplantation (HSCT) (10/10 HLA match) after myeloablative conditioning with TBI-CY (12 Gy fractionated + cyclophosphamide 100 mg/kg post-transplantation) in September 2019. PET-CT scan 55 days after HSCT displayed normal global glucose metabolism, interpreted as PET-negative CR. The patient was in remission at last follow-up 31 months after diagnosis and 10 months after HSCT.

Molecular findings from NGS panel sequencing

NGS panel sequencing of DNA isolated from isolated MS specimen of these four patients at initial diagnosis resulted in identification of molecular aberrations in three out of four cases (Table 1; patients 1, 2, and 4) and no mutation in the remaining patient. However, parallel assessment of the cytomorphological unaffected BM did not detect these variants in two out of three cases (patients 1 and 4) by means of sensitive amplicon sequencing. Thus, BM involvement was not detectable on the molecular level in these patients, and especially, no presence of a precursor CHIP lesion could be detected, suggesting that malignant transformation occurred at the extramedullary MS site rather than within the BM.

Patient 1’s MS showed typical mutations described for CHIP, namely DNMT3A and TET2 [8]. As we did not detect...
| Case | Sex/age | Site | History | Gene | Variant | Initial diagnosis | 1. CR Follow-up | Relapse | Therapy | Subsequent clinical history |
|------|---------|------|---------|------|---------|------------------|----------------|---------|---------|------------------------------|
| 1    | M/71 years | Cutaneous lesion, occipital | de novo isolated MS | DNMT3A | c.1180G>A (p.Asp394Asn) | 77.4 | Not detectable" | Not detectable" | NA | 2x DA (7+3). 1x IDAC. Tomotherapy (50 Gy) | Sustained remission at last follow-up, 54 months after diagnosis. |
|      |         |      |         |      |         | Tumor VAF (%) | BM VAF (%) | VAF (%) | Tumor VAF (%) | BM VAF (%) |
| 2    | M/30 years | Multifocal lymph nodes (bilaterally cervical, axillary, iliac and inguinal) | de novo isolated MS | ETV6 | c.1187_1188insCGCTACGGATA (p.Arg396SerfsTer13) | 21.9 | Not detectable | Not detectable | NA | 2x DA (7+3). Allogenic matched unrelated donor HSCT (10/10 HLA match) after myeloablative conditioning with TBI-CY. | PET-negative CR after induction. Dead due to septic shock 3 months after diagnosis/26 days after HSCT. |
| 3    | M/79 years | Subcutaneous lesion, left posterior upper arm | de novo isolated MS | STAG1 | c.151C>T (p.Arg51Ter) | 22.9 | Not detectable | Not detectable | NA | 2x DA (7+3). 2x IDAC. Radiotherapy (30 Gy). | Sustained remission at last follow-up, 17 months after diagnosis. |
| 4    | M/39 years | Bone lesion left ilium involving acetabular portion | de novo isolated MS | FLT3 | c.1834T>C (p.Phe612Leu) | 16.6 | Not detectable" | Not detectable" | 46.4† | Initially: 2x DA (7+3). 3x IDAC. Radiotherapy (50 Gy). At time of relapse: 1x FLAG-IDA. Allogenic sibling donor HSCT (9/10 HLA mismatch) after myeloablative conditioning with FLAMSA + TBI-FLU-post-CY. | PET-negative CR after induction. Disease relapse 19 months after diagnosis (isolated MS with liver and multiple bone lesions). PET-negative CR 55 days after HSCT. Sustained remission at last follow-up, 31 months after diagnosis. |
|      |         |      |         |      |         | Tumor VAF (%) | BM VAF (%) | VAF (%) | Tumor VAF (%) | BM VAF (%) |

M male, MS myeloid sarcoma, VAF variant allele frequency, BM bone marrow, HSCT hematopoietic stem cell transplantation, CR complete remission, DA (7 + 3) daunorubicin 60 mg/qm days 3–5 + cytarabine 100 mg/qm days 1–7 as continuous infusion, IDAC intermediate dosed cytarabine 1000 mg/qm twice daily days 1, 3, and 5, TBI-CY 12 Gy fractionated + cyclophosphamide 120 mg/qm + ATG 60 mg/kg, FLAG-IDA fludarabine 30 mg/qm, cytarabine 2000 mg/qm, each day 1–5, idarubicin 10 mg/qm day 1–3, G-CSF 5 μg/kg from day 6, FLAMSA fludarabine 30 mg/qm, amrascan 100 mg/qm, cytarabine 2000 mg/qm, each day 1–4, TBI-FLU-post-CY 12 Gy fractionated + fludarabine 60 mg/qm + cyclophosphamide 100 mg/kg post-transplantation, NA not applicable

" Blood
" Bone marrow
" Liver biopsy
these variants in the corresponding BM, this may foster the hypothesis that CHIP may also occur extramedullary and be confined locally. For the first DNMT3A variant (variant allele frequency (VAF) 77.4%), an additional TP53 mutation in a subclone (VAF 42.7%) may represent clonal evolution locally in this case, resulting in malignant transformation and clinically overt disease.

In patient 2, an ETV6 mutation was detected in BM and MS. Since the ETV6 mutation in the BM sample had a VAF of only 1.42%, which was much higher in the extramedullary tumor (21.9%), it may represent true CHIP. The original definition of CHIP set a VAF cutoff of ≥2%, but this limit took into account methodological limitations and was arbitrary [8].

Clonal hematopoiesis (CH) defined by a variant of ETV6 is uncommon compared with variants frequently observed in CHIP [8].

In previous studies, several other uncommon variants for CH have been reported in unaffected BM specimen of isolated MS cases (IDH2 [9], NFE2, ODF1, TRAFD1 [10], RUNX1, GATA2, FLT3, and NPM1 [11], suggesting a unique pattern of clonal evolution and pathogenesis of isolated MS. The mentioned studies reported simultaneous NGS-based parallel assessment of MS sites and BM for five, two, and six cases of de novo isolated MS, respectively, and detected evidence for CH in unaffected BM in one (20%), two (100%), and three (50%) of analyzed cases. In our cohort, only one of three patients with detectable mutations and isolated de novo MS (patient 2) had evidence of CHIP in the BM at presentation.

In patient 2’s MS, an additional STAG1 mutation outside the BM was found. This indicates that clonal evolution had occurred at an extramedullary site and that this additional mutation may have resulted in the appearance of an overt clinical manifestation.

After initial therapy with induction chemotherapy and radiation of the solitary extramedullary site, previous variants remained undetectable by amplicon sequencing in blood or BM of patients 1 and 4 in continuous CR.

Patient 4 had a multifocal extramedullary relapse, again without BM involvement. The same mutational profile was found in the distant relapse site compared with initial manifestation. Interestingly, the sites of exclusively extramedullary blast infiltration suggested by PET-CT at relapse (lumbar vertebrae 2 and 4, left inguinal lymph node) and by sonography, confirmed by biopsy (liver), differed from the affected site at initial diagnosis (left ilium). The later showed no significant glucose uptake by PET-CT at relapse and was likely unaffected at this time point. Together, these findings suggested extramedullary homing of myeloid blasts in distant sites at relapse without BM involvement. Alternate homing appears to be the hallmark difference between MS and AML, suggesting an aberrant homing signal between these entities [12]. The documented disease presentation of patient 4 raises the question whether there might be even distinct homing signals for trafficking of myeloid blasts between different extramedullary compartments—a question worth to explore in future studies.

**Conclusion and future prospect**

In some cases, isolated MS might arise exclusively extramedullary without precursor cell populations in the BM (as suggested by molecular results from patients 1 and 4, Table 1).

Alternatively, BM precursor clones of isolated MS might be defined by yet uninvestigated features (e.g., uncommon AML/CHIP-associated mutations not captured by our NGS panel, cytogenetic aberrations). Recently, an uncommon accumulation of NFE2 mutations in isolated MS patients (4/6; 67%) has been carved out via whole-exome sequencing [10], also detectable in one of two unaffected BM specimen. Experimental evidence has indeed established NFE2 aberrations as leukemogenic, MS promoting drivers in a murine model [13].

CH has been mainly assessed on the level of gene mutations, but might be identifiable on the level of chromosomal alterations as well with future methodological improvements. Adding systematic parallel characterization of chromosomal aberrations in isolated MS samples and suspected pre-leukemic BM clones will represent a special challenge for future investigations, given the level of sensitivity needed to reliably elucidate different subclones via cytogenetic information [14] as well as the need to gather such information from FFPE MS samples, the latter of which is already possible [15–17].

**Authors’ contributions** N.W.E and W.F. designed the project concept and developed a first draft of the manuscript. N.W.E and J.R. analyzed patient records and acquired samples. M.H. provided the NGS facility and supervised NGS data interpretation. N.M.B., V.P., R.G., and F.T. performed sample preparation and sequencing. W.F. provided overall project oversight.

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**Data availability** Raw sequencing datasets analyzed in this study can be made available on reasonable request.

**Compliance with ethical standards**

**Conflict of interest** F.T. reports advisory role for Abbvie, Astellas, Daiichi Sankyo, Novartis, Celgene, and Pfizer. M.H. reports Honoraria from Novartis, Pfizer and PrmE Oncology, Consulting or advisory role for Abbvie, Bayer Pharma AG, Daiichi Sankyo, Novartis and Pfizer, and Research Funding to institution from Astellas, Bayer Pharma AG, BergenBio, Daiichi Sankyo, Karyopharm, Novartis, Pfizer, and Roche. W.F. reports advisory role for Amgen, Pfizer, Novartis, Jazz Pharmaceuticals, Celgene, Morphosys and Ariad/Incyte, Research...
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Ethics approval All procedures in this study involving human participants were in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Consent to participate/consent for publication Written informed consent was waived, given the retrospective nature of this study.

Code availability Not applicable.

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References

1. Magdy M, Abdel Karim N, Eldessouki I, Gaber O, Rahouma M, Gheereb M (2019) Myeloid Sarcoma. Oncol Res Treat 42(4):224–229. https://doi.org/10.1159/000497210
2. Meiss JM, Butler JJ, Osborne BM, Manning JT (1986) Granulocytic sarcoma in nonleukemic patients. Cancer 58(12):2697–2709. https://doi.org/10.1002/1097-0142(19861215)58:12<2697::aid-cncr2820581225>3.0.co;2-x
3. Neiman RS, Barcos M, Berard C, Bonner H, Mann R, Rydell RE, Bennett JM (1981) Granulocytic sarcoma: a clinicopathologic study of 61 biopsied cases. Cancer 48(6):1426–1437. https://doi.org/10.1002/1097-0142(19810915)48:6<1426::aid-cncr2820480626>3.0.co;2-g
4. Bewdersdorf JP, Shahil RM, Boddu PC, Wood B, Radich J, Halene S, Zeidan AM (2019) The minimal that kills: why defining and its distinction from myelodysplastic syndromes. Blood 126(1):9–16. https://doi.org/10.1182/blood-2015-03-631747
5. Heuser M, Gabdoulline R, Loffeld P, Dobbernack V, Kreimeyer H, Pankratz M, Flintrop M, Liebisch A, Klesse S, Chaturvedi A, Kloos A, Gohring G, Schlegelberger B, Gaidzik VI, Bullinger L, Fiedler W, Heim A, Hamwi I, Eder M, Kräuter J, Schlenk RF, Paschka P, Dohner K, Dohner H, Ganser A, Heuser M (2018) Measurable residual disease monitoring by NGS before allogeneic hematopoietic cell transplantation in AML. Blood 132(16):1703–1713. https://doi.org/10.1182/blood-2018-02-829911
6. Steensma DP (2018) Clinical consequences of clonal hematopoiesis of indeterminate potential. Blood Adv 2(22):3404–3410. https://doi.org/10.1182/bloodadvances.2018020222
7. Pastoret C, Hout R, Llamas-Gutierrez F, Boulland ML, Marchand T, Tas P, Ly-Sunnarab B, Gandemer V, Lamy T, Rousset M, Fert T (2017) Detection of clonal heterogeneity and targetable mutations in myeloid sarcoma by high-throughput sequencing. Leuk Lymphoma 58(4):1008–1012. https://doi.org/10.1080/10428194.2016.1225208
8. Lazarevic V, Orsmark-Pietras C, Liljebjörn H, Pettersson L, Rissler M, Lubking A, Ehinger M, Juliussson G, Fiorets T (2018) Isolated myelosarcoma is characterized by recurrent NFE2 mutations and concurrent preleukemic clones in the bone marrow. Blood 131(5):577–581. https://doi.org/10.1182/blood-2017-07-793620
9. Werstein B, Dunlap J, Casicio MJ, Oghami RS, Fan G, Press R, Raess PW (2020) Molecular discordance between myeloid sarcomas and concurrent bone marrows occurs in actionable genes and is associated with worse overall survival. The Journal of Molecular Diagnostics 22(3):338–345. https://doi.org/10.1016/j.jmoldx.2019.11.004
10. Fajaj CM, Willemze AJ, Rêvész T, Balzarolo M, Tensen CP, Hoogeboom M, Vermeer MH, van Wering E, Zwaan CM, Kaspers GJ, Story C, van Halteren AG, Vossen JM, Egeler RM, van Tol MJ, Annels NE (2010) Chemokine/chemokine receptor interactions in extramedullary leukaemia of the skin in childhood AML: differential roles for CCR2, CCR5, CXCR4 and CXCR7. Pediatr Blood Cancer 55(2):344–348. https://doi.org/10.1002/pbc.22500
11. Jutzi JS, Basu T, Pellmann M, Kaiser S, Steinemann D, Sanders MA, Hinaia ASA, Zeilmaeker A, Bojtte Kovacs C, Koellicker C, Ostendorp J, Aumann K, Wang W, Raffoux E, Cassinat B, Bullinger L, Schlegelberger B, Valk PJM, Pahl HL (2019) Altered NFE2 activity predisposes to leukemic transformation and myelosarcoma with AML-specific aberrations. Blood 133(16):1766–1777. https://doi.org/10.1182/blood-2018-09-875047
12. Takahashi K, Wang F, Kantarjian H, Song X, Patel K, Neelapu S, Gumbs C, Little L, Tippen S, Thornton R, DiNardo CD, Ravandi F, Bueso-Ramos C, Zhang J, Wu X, Garcia-Manero G, Furtéal PA (2017) Copy number alterations detected as clonal hematopoiesis of indeterminate potential. Blood Adv 1(15):1031–1036. https://doi.org/10.1182/bloodadvances.2017007922
13. Kashofer K, Gornicec M, Lind K, Caraffini V, Schauer S, Beham-Schmid C, Wolfli A, Hoeftler G, Sill H, Zebisch A (2018) Detection of prognostically relevant mutations and translocations in myeloid sarcoma by next generation sequencing. Leuk Lymphoma 59(2):501–504. https://doi.org/10.1080/10428194.2017.1339879
14. Heyer EE, Deveson IW, Wooi D, Selinger CI, Lyons RJ, Hayes VM, O’Toole SA, Ballinger ML, Gill D, Thomas DM, Mercer TR, Blackburn J (2019) Diagnosis of fusion genes using targeted RNA sequencing. Nat Commun 10(1):1388. https://doi.org/10.1038/s41467-019-09374-9
15. Mirza MK, Sukhanova M, Stolzel F, Onel K, Larson RA, Stock W, Ehninger G, Kithan F, Zebisch A, Hambach L, Stadler M, Koenecke C, Flintrop M, Liebich A, Klesse S, Panagiota V, Stadler M, Paschka P, Dohner K, Dohner H, Ganser A, Heuser M (2018) Detection of clonal heterogeneity and targetable mutations in myeloid sarcoma by high-throughput sequencing. Leuk Lymphoma 58(4):1008–1012. https://doi.org/10.1080/10428194.2016.1225208

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