Comprehensive molecular analyses for diagnosis and treatment guidance in an adult neuroblastoma patient

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Abstract

Metastatic cancers of unknown primary (CUP) pose significant diagnostic and therapeutic challenges. We present the case of a 63-year old male patient with a CUP showing neuroendocrine differentiation, metastasized to the iliac bone, bone marrow, supraclavicular and retroperitoneal lymph nodes. Immunohistochemical and molecular profiling revealed strong pan-neurotrophic tyrosine kinase (Trk) expression without NTRK-gene fusion, corroborating the neural cell origin. Following molecular tumor board (MTB) discussion, genome-wide methylation profiling suggested the diagnosis of a neuroblastoma but results were below diagnostic thresholds . Subsequent imaging and laboratory findings confirmed an INRGSS stage M neuroblastoma, a rare finding in older adults. Despite multimodal therapy, including polychemotherapy and immunotherapy according to pediatric GPOH neuroblastoma guidelines, disease progression necessitated an experimental approach. Comprehensive molecular analysis and MTB discussion revealed several potential treatment targets, leading to subsequent treatment including dinutuximab beta, nivolumab, cabozantinib, I-131-mIBG radionuclide therapy and alpelisib, unfortunately, all followed by disease progression.

This case demonstrates the potential of comprehensive molecular analysis including methylation profiling for diagnosis and treatment guidance in rare tumors. Additional research is urgently required to improve outcomes in elderly patients with neuroblastoma.

Key points

- Comprehensive genomic analyses including methylation analysis should be considered for cancer of unknown primary
- Trk expression in the absence of NTRK gene fusion can indicate neural crest origin
- Molecular profiling can identify additional treatment options in advanced malignancies
- Additional research is warranted for adult neuroblastoma patients

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Patient story

A 63-year-old male patient was initially referred to our clinic with complaints of mild fatigue and a swelling in the left cervical region. His past medical history was unremarkable. Physical examination revealed a palpable lymph node in the left cervical area. Initial laboratory evaluations, including a complete blood count and basic metabolic panel, were within normal limits.

Imaging studies commenced with an MRI of the cervical spine, which indicated a mass on the left side. Subsequent CT scans of the neck, thorax, and abdomen revealed a lesion suggestive of malignancy in the left cervical region (**Fig. 1**), suspected retroperitoneal lymphadenopathy, and a 1.7 cm heterogeneous lesion in the middle portion of the left kidney.

The patient underwent a full diagnostic ENT-evaluation including esophagoscopy, laryngoscopy, pharyngoscopy, and rhinoscopy, all without evidence of malignancy, and selective cervical lymphadenectomy at the left level V was performed.

On macroscopic examination, the lymph node measured 45 x 26 x 20 mm. Microscopy revealed an infiltrate of a malignant necrotizing neoplasm composed of pleomorphic cells with enlarged hyperchromatic nuclei with “salt and pepper” chromatin, prominent nucleoli, amphophilic cytoplasm, fibrillary matrix material, and atypical mitosis (**Figure 2A** and **B**). Immunohistochemical staining for synaptophysin, chromogranin A, NB84, NSE (**Figure 2C-J**), and pan-Trk was positive, with negative staining for CD99, panCK, CK7, CK18, TTF1, CDX2, SATB2, NKX3.1, SOX10, HMB45, MelanA, CD20, PAX5, CD21, CD3, CD30, CD34, ERG, Desmin, and Vimentin. The Ki67 index was 50%, indicating high proliferative activity.

Molecular tumor board

DNA (Molecular Health IVD Panel 600+, Agilent Technologies) and RNA (FusionPlex Sarcoma v2, Archer/Invitae) sequence analysis revealed a *PIK3CA* mutation (p.H1047R), a mutation burden of 2.19 Mut/Mb, and no evidence of fusion transcripts, especially no evidence of an NTRK1-3 fusion, which was further excluded by a negative NTRK-FISH and renewed RNA-based sequencing in a second independent laboratory.

Genotyping results and interpretation of the molecular results

After a first interdisciplinary discussion in a molecular tumor board, a neural primary tissue was suggested as a first differential diagnostic consideration, because of Trk expression without an evidence of NTRK-gene fusion. Thus, experimental DNA methylation analysis [1] was performed followed by machine learning methylation-based classification of central nervous system tumors (Version 12.8). Results remained below diagnostic thresholds but suggested a diagnosis of neuroblastoma (classification score 0.82, at a classification threshold of 0.9, No

match result) and showed no chromosome 1p, MYCN, 17q and TERT chromosomal alterations and a broad 11q deletion (**Figure 3A** and **B**).

Clinical significance of the molecular profile

Following this suggested diagnosis, additional diagnostic examinations were performed. Imaging with Iodine-123-mIBG scintigraphy revealed slight MIBG uptake in an individual retroperitoneal lymph node, whereas F18-FDG-PET-CT showed metabolic activity suggestive of metastases in the right oropharynx, retroperitoneal and peri-celiac lymph nodes, and the iliac bone. Focused ultrasound of retroperitoneal lymph nodes was suggestive of a lumbar sympathetic trunk primary.

Urine analysis showed increased levels of vanillyl mandelic acid and homovanillic acid. Bone marrow puncture including 2 biopsies and 2 aspiration cytologies confirmed the presence of discontinuous infiltrates of malignant cells suggestive of neuroblastoma in individual samples. The tumor tissue was additionally sent to two independent reference pathologists (E.W. and C.V.) and discussed in the interdisciplinary German national neuroblastoma board. Thus, other neural-crest derived tumors were ruled out and a diagnosis of poorly differentiated, stroma–poor neuroblastoma with low mitotic and karyorrhectic activity according to INPC, INRGSS stage M was made [2].

Implications for clinical practice

Following German pediatric neuroblastoma guidelines (GPOH), the patient was started on dose-adjusted systemic chemotherapy with a regimen including Vindesine, Cisplatin, Etoposide alternating with vincristine, dacarbazine, ifosfamide and doxorubicin. Imaging after 3 months showed stable retroperitoneal lymph node metastases, and an MRI indicated disseminated osseous metastases.

Patient update

Despite multiple further cycles of chemotherapy, the disease progressed, prompting a treatment switch to immunotherapy with dinutuximab beta combined with irinotecan and temozolomide, again following pediatric protocols. After disease progression, a new tumor biopsy was taken from a rapidly progressing cervical lymph node, confirming metastatic poorly differentiated, stroma–poor neuroblastoma, without morphological changes to the primary diagnosis.

Comprehensive molecular profiling including whole-exome and transcriptomic sequencing was performed within the NCT/DKTK MASTER program, revealing the known activating PIK3CA variant as well as NTRK, RET and ALK upregulation without evidence of gene fusions or ALK mutation. The case underwent repeated discussion at the institutional MTB and additional treatment recommendations were made, that were subsequentially initiated: Based on pediatric case reports, Dinutuximab beta was combined with immune checkpoint inhibition, again followed by disease progression [3, 4]. The patient was then started on cabozantinib, followed by I-131-mIBG radionuclide therapy (**Fig. 4**), both based on biological rationale and available pediatric case reports and followed by disease progression. In the absence of clinical data, last-line therapy with alpelisib was initiated because of the activating PIK3CA variant, again followed by disease progression. The patient ultimately deceased due to disease progression 1 year and 11 months after diagnosis.

Neuroblastoma in adults represents a very rare and diagnostically challenging entity that differs significantly from its pediatric counterpart.

As known for the majority of tumors, TRK-protein expression is a valid screening tool for NTRK-gene fusions [5]. However, physiological TRK expression in neuronal tissue leads to inadequate NTRK-screening in tumors of neuronal origin [6]. Thus, TRK-protein expression in the absence of NTRK-gene fusion, as identified in this case, can be considered a diagnostic hint for a neuronal tissue of origin. However, the discriminatory value of Trk expression alone in the CUP setting is limited because of the broad spectrum of potential tumor types.

Experimental methylation analysis remained below prespecified thresholds but was suggestive of a neuroblastoma diagnosis. Methylation is routinely used for the diagnosis of CNS malignancies [1]. Additional data exist for the use of methylation analysis to correctly assign metastatic tumors [7, 8]. These data are of special interest for the classification of rare tumor types, as previously reported in sinonasal or salivary gland tumors [9, 10]. Consequently, this case report highlights the potential of methylation analysis as an additional diagnostic tool.

The biological behavior, response to treatment, and overall prognosis of neuroblastoma in adults are significantly different from those seen in children [11, 12]. In pediatric patients, neuroblastoma often follows a more aggressive course, frequently associated with genetic aberrations such as *MYCN* amplification, which is a well-known poor prognostic marker [13, 14]. Conversely, neuroblastoma in adults and elderly patients is less likely to present with these high-risk genetic features, potentially contributing to its more indolent behavior [15]. However, the prognosis for adult patients remains poor, largely due to late presentation and the absence of standardized treatment protocols tailored for this age group [11, 12]. In the SEER database review conducted by Esiashvili et al., adult patients with neuroblastoma had a 5-year disease-specific survival rate of approximately 32.6%, which is significantly lower than that observed in

pediatric populations [16]. Furthermore, a review by Franks et al. highlighted the lack of effective chemotherapeutic regimens in adults, with most treatments being extrapolated from pediatric oncology protocols, which may not be suitable for older patients due to differences in disease biology and treatment tolerance [17]. This comparably indolent growth and resistance against standard pediatric neuroblastoma treatment regimens is also mirrored in this case report.

The rarity of neuroblastoma in adults prevents large, randomized controlled trials. Most available data come from case reports and very small case series, making it difficult to draw definitive conclusions but highlighting the importance of reporting individual cases. Treatment efforts in this case were partly guided by available evidence from case series, including data on the use of nivolumab/dinutuximab beta combination [3].

The lack of effective treatment options highlights the need for comprehensive molecular analysis and molecular tumor board evaluation. Activating ALK alterations are potential therapeutic targets in neuroblastoma [18]. In this patient, ALK, RET and NTRK gene overexpression but not amplification or variants were identified by comprehensive molecular analysis. The molecular tumor board therefore favored the use of a tyrosine kinase inhibitor with broad activity. Data from a case series favored the use of cabozantinib, which was ultimately used but did not lead to a clinical response [19]. Additionally, an activating PIK3CA variant but no ALK or MYC alterations were identified. PIK3CA alterations have been described infrequently in neuroblastoma [20]. Activating PIK3CA mutations are established predictive biomarkers for PI3K-inhibitors in other tumor types, including breast cancer [21]**.** In neuroblastoma, only preclinical data exist on the use of PI3K-inhibitors [22]. In the absence of a clinical trial, other treatment options were therefore initially preferred and last line treatment with a PI3K-inhibitor did not yield meaningful clinical benefit in line with the limited activity of PI3K-inhibitors in other solid tumors beyond breast cancer.

Also I-131-mIBG did not achieve a clinical benefit in this patient in a later line of therapy. Combination of radionuclide therapy with chemo- or immune therapy may augment responses as demonstrated in a pediatric population but it is unclear, if this also applies to adult patients [23].

Cellular therapies have shown first encouraging results in neuroblastoma [24]. Unfortunately, no such treatment options were available for our patient, highlighting the need to include rare malignancies in clinical trial designs. Additionally, different toxicity profiles have to be considered when implementing pediatric treatment protocols, such as high-dose therapy, in an older patient population, additionally limiting treatment options.

In conclusion, our case report highlights the impact of advanced molecular profiling to ascertain specific cancer types and tailor treatment strategies for rare tumors . The report further underscores the need for additional research into age-specific treatments for neuroblastoma in adults and continuous reporting of treatment outcomes through case reports. With current knowledge, clinicians should continue to adapt pediatric protocols with caution.

Methods

Consent for publication

The patient provided written informed consent for publication of the case report.

Methylation analysis

Methylation analysis was performed as previously described [1].

Comprehensive genomic sequencing

Whole-exome and -transcriptome sequencing was performed from fresh frozen tumor tissue within the NCT/DKTK MASTER program, as previously described [25]. The NCT/DKTK MASTER program was approved by the participating institutional ethics committees and the patient provided written informed consent for participation in this program. The MASTER program was approved by ethics committees at all participating sites (Lead Heidelberg, S-206/2011).

Data availability statement

The datasets generated are not publicly available due to privacy concerns. Part of the data are available from the corresponding authors upon reasonable request.

Author contribution statement

DTR contributed to case management, diagnostic procedures and analyzed and interpreted patient data and wrote and revised the manuscript. SS, SO, BC, HA, UKeller contributed to case management, analyzed and interpreted patient data and revised the manuscript. HED,

AL, UK, AE, EW, CV, SF, DH and DC acquired, analyzed and interpreted patient data and revised the manuscript. MPD and SS conceptualized the case, acquired, analyzed and interpreted data and wrote and revised the manuscript. All authors read and approved the final manuscript

Conflicts of interest statement

DTR has received consultant and/or speaker fees from Bayer, Eli Lilly, Bristol-Myers Squibb, Johnson & Johnson, Roche, and BeiGene; travel support from Bayer and Johnson & Johnson; and research funding from SeaGen. All other authors have declared no conflicts of interest.

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Figures

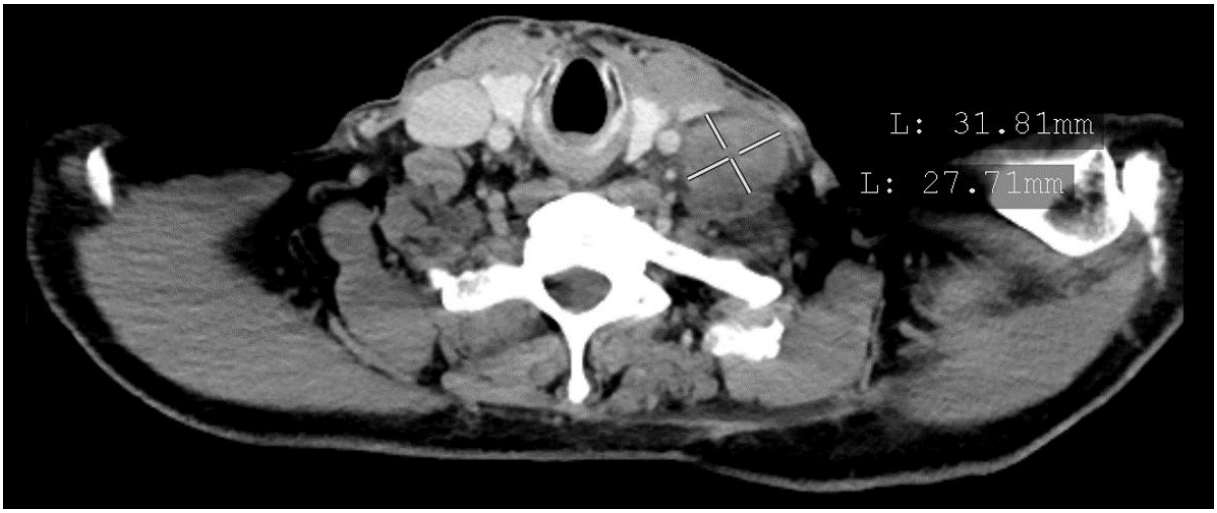

Fig. 1: Initial CT scan revealing a left cervical lymph node metastasis.

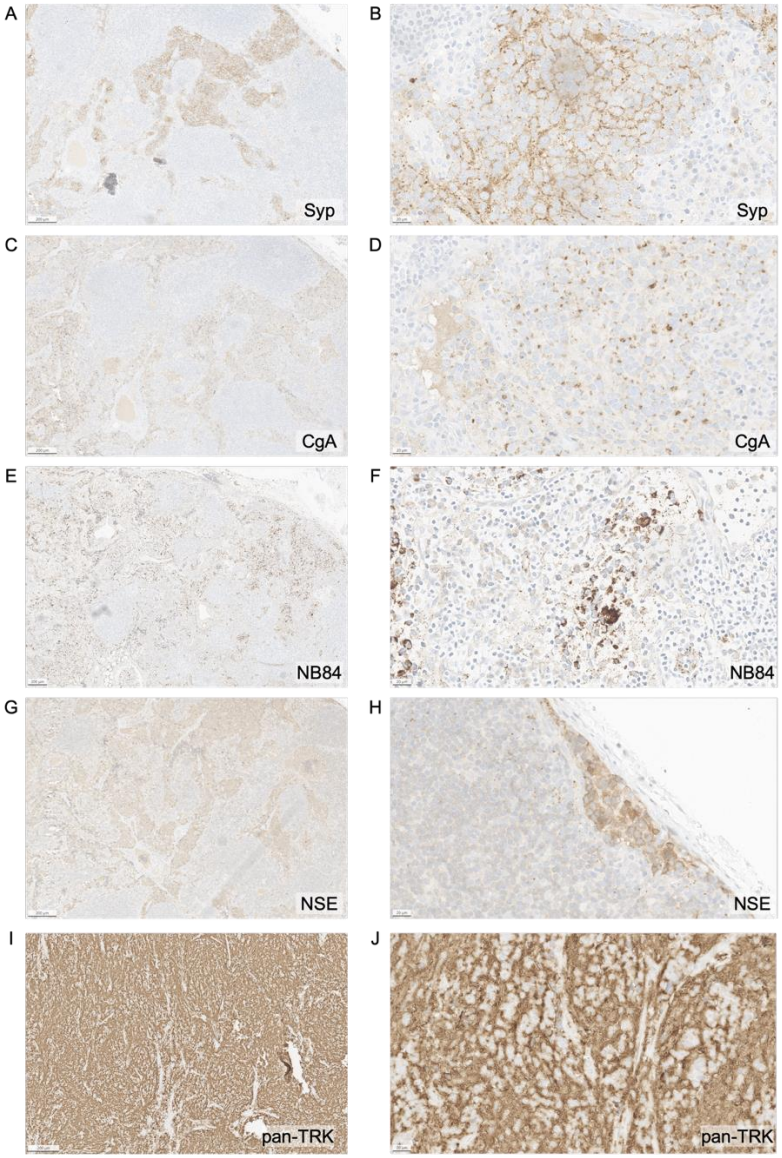

Fig.2 Results from immunohistochemical staining of the lymph node metastasis at two resolutions. Respective antigens are provided in the images.

A

Version 12.8 of the brain classifier results (12.8)

Methylation classes (Highest level >= 0.3, lower levels >= 0.1, all of lowest level)	Calibrated score	Interpretation
Neuroblastoma	0.82	no match ✗
Neuroblastoma	0.82	no match ✗
Neuroblastoma	0.82	no match ✗
Mc Neuroblastoma, Mycn Type	0.40	no match ✗
Mc Neuroblastoma, Alt/tert Tmm Positive	0.31	no match ✗
Mc Neuroblastoma, Tmm Negative	0.10	no match ✗

Legend: ✓ Match (score >= 0.9) ✗ No match (score < 0.9): possibly still relevant for low tumor content and low DNA quality cases.

B

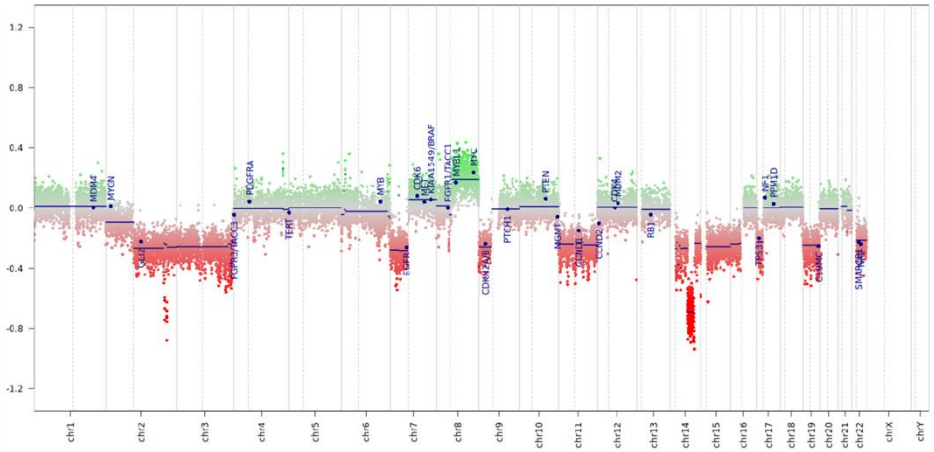

Fig. 3: Experimental methylation classifier showed the highest score for neuroblastoma, albeit below diagnostic cut-off (cut off: 0.9). Calculated Copy number profile from methylation analysis without MYCN amplification is provided in panel B.

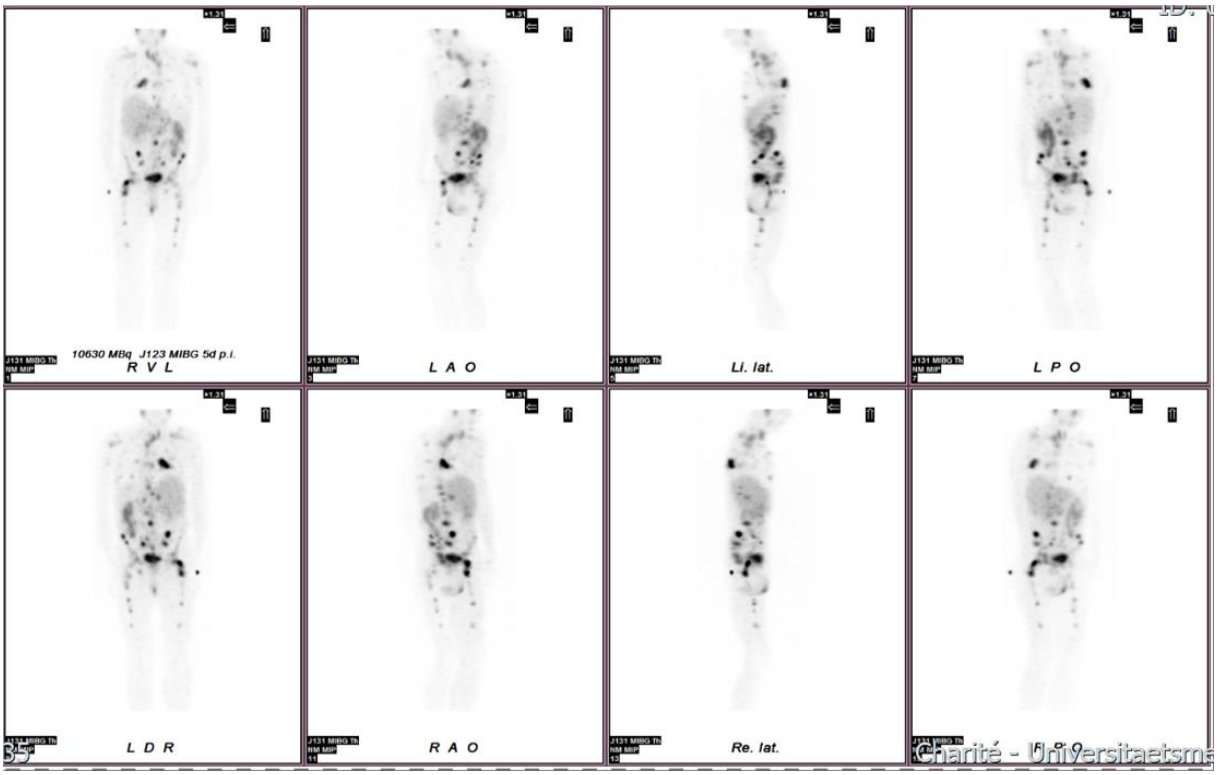

Fig. 4: Posttherapeutic imaging after I-131-mIBG radionuclide therapy