

Medical cannabis, melatonin and oxygen-ozone therapy for neuroblastoma: two case reports

Maria Beatrice Morelli

University of Camerino

Adriana Elisa Colpani

Maria Guarino" Foundation-AMOR No Profit Association

Franco Baldaccini

Maria Guarino" Foundation-AMOR No Profit Association

Massimo Nabissi

University of Camerino

Margherita Luongo

fondazioneamor@gmail.com

Maria Guarino" Foundation-AMOR No Profit Association

Case Report

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Posted Date: June 11th, 2026

DOI: <https://doi.org/10.21203/rs.3.rs-9651000/v1>

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Additional Declarations: No competing interests reported.

1 **Medical cannabis, melatonin and oxygen-ozone therapy for neuroblastoma: two case reports**

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3 Maria Beatrice Morelli¹, Adriana Elisa Colpani², Franco Baldaccini², Massimo Nabissi^{1 *}, Margherita
4 Luongo^{2,3*}

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6 ¹ School of Pharmacy, University of Camerino, Camerino, Macerata, Italy.

7 ² Maria Guarino" Foundation-AMOR No Profit Association, Pozzuoli, Naples, Italy;

8 ³ School of Medicine and Surgery, University of Campania Luigi Vanvitelli, Naples, Italy.

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10 *Correspondence: Margherita Luongo (fondazioneamor@gmail.com), Massimo Nabissi
11 (massimo.nabissi@unicam.it)

13 **Abstract**

14 **Background** Neuroblastoma is the most common and lethal solid tumor in children, with high-risk cases
15 showing only a 50% 5-year survival rate. Increasing attention has been directed toward integrative
16 strategies exploiting compounds with potential anticancer and cytoprotective properties. Medical
17 cannabis has been reported to modulate tumor cell proliferation, apoptosis, inflammation, and immune
18 responses through the endocannabinoid system. Melatonin exhibits tumor-suppressive effects, including
19 antioxidant activity, regulation of circadian biology, mitochondrial protection, and enhancement of
20 apoptosis in tumor cells. Oxygen-ozone therapy has been proposed to improve tissue oxygenation,
21 modulate oxidative stress, and influence tumor metabolism through controlled redox signaling. The
22 rationale for combining these approaches lies in their potential complementary actions on oxidative
23 stress regulation, mitochondrial function, immune modulation, and tumor metabolic vulnerability.

24 **Case presentation** We report two pediatric patients with metastatic stage IV neuroblastoma refractory
25 or poorly responsive to conventional treatments. Both patients subsequently received an integrative
26 regimen based on medical cannabis, melatonin, and oxygen-ozone therapy. Following initiation of the
27 combined treatment, both patients showed progressive clinical improvement, weight recovery, and

complete regression of detectable disease on imaging. They remain in long-term disease-free status, with no significant adverse effects reported during the integrative treatment period.

Conclusions These two cases evidenced as this integrative approach contributed to durable remission and good tolerability in high-risk pediatric neuroblastoma. However, the evidence remains extremely limited. Further preclinical and prospective clinical studies are required to elucidate mechanistic interactions, evaluate potential synergy, and define optimal therapeutic protocols for this integrative approach.

Keywords Neuroblastoma, Pediatric cancer, Cannabinoids, Melatonin, Oxygen–ozone therapy, Case reports, Integrative therapy.

Introduction

Cancer remains one of the leading causes of morbidity and mortality worldwide, and increasing research efforts are being directed toward the identification of novel therapeutic strategies that can complement conventional treatments and improve clinical outcomes. Within this context, growing attention has been given to bioactive compounds with potential anti-tumor properties, including medical cannabis, melatonin, and oxygen-ozone therapy, which may act on complementary biological pathways involved in tumor progression.

The anti-tumor effects of phytocannabinoids have been extensively investigated in preclinical cancer models, highlighting a complex multi-targeted mechanism of action that interferes with nearly all the hallmarks of cancer (Hinz et al. 2022; Shah et al. 2023). Traditionally utilized in palliative care to alleviate chemotherapy-induced nausea, pain, and cachexia, these plant-derived compounds are now recognized for their direct anti-neoplastic properties. Mechanistically, phytocannabinoids exert their effects through both canonical endocannabinoid receptors (CB1 and CB2) and non-canonical targets, including orphan G-protein coupled receptors, transient receptor potential channels, and peroxisome proliferator-activated receptors. The modulation of these receptors triggers intracellular signaling cascades that inhibit key survival pathways, while promoting endoplasmic reticulum stress, intracellular reactive oxygen species accumulation, and mitochondrial dysfunction (Faiz et al. 2024). While Δ^9 -tetrahydrocannabinol (THC) has demonstrated potent anti-proliferative and anti-angiogenic properties

across various malignancies, its clinical development as a primary chemotherapeutic agent is often hindered by its prominent psychotropic effects (Faiz et al. 2024). Consequently, significant research focus has shifted toward non-psychotropic phytocannabinoids. Cannabidiol (CBD) is approved as a breakthrough treatment (Epidiolex) for severe, drug-resistant infantile epilepsies (Abu-Sawwa et al. 2020). In oncology models, CBD has demonstrated the ability to reduce tumor cell viability, inhibit proliferation, and induce apoptosis in neuroblastoma models (Fischer et al. 2016; Alharris et al. 2019). In xenograft models, CBD has also been associated with reduced tumor growth, supporting its potential role as an adjunctive anticancer agent (Fischer et al. 2016). Furthermore, emerging preclinical evidence suggests that minor non-psychoactive cannabinoids, such as cannabigerol and cannabichromene, can exert comparable cytotoxic profiles in cancer cells (Jurga et al. 2024; Aguzzi et al. 2024; Zeppa et al. 2024a; Lah et al. 2021). Interestingly, combinatory approaches utilizing specific ratios of CBD and THC have shown synergistic anti-metastatic potential enhancing therapeutic efficacy while safely mitigating THC-induced psychotoxicity (Twelves et al. 2021). Although clinical evidence remains limited, emerging data suggest that medical cannabis compounds may exert biologically relevant anti-neoplastic and immunomodulatory effects in pediatric oncology (Malach et al. 2022).

Melatonin, an indolamine primarily produced by the pineal gland, has also been widely studied for its anti-tumor properties. It exerts antioxidant, antiproliferative, and pro-differentiation effects across several tumor types, including neuroblastoma (Bonmat-Carrion et al. 2021). Both physiologic and pharmacologic concentrations of melatonin have been shown to promote neuronal differentiation (Pourhanifeh et al. 2021, Lee et al. 2019), reduce VEGF-mediated angiogenesis (González et al. 2017), and induce apoptosis and autophagy in human neuroblastoma cell lines (García-Santos et al. 2006; Qiu et al. 2026). These effects, together with its ability to mitigate chemotherapy-related side effects, make melatonin an attractive adjunct in pediatric oncology (Antonini et al. 2023).

Oxygen-ozone therapy has been proposed as a complementary treatment in various chronic and inflammatory conditions due to its ability to induce a controlled oxidative stress response (Baeza-Noci et al. 2021; Clavo et al. 2018; Li et al. 2024; Clavo et al. 2023; Lei et al. 2025). This effect is thought to modulate redox homeostasis, improve tissue oxygen utilization, and regulate immune and inflammatory pathways. However, current evidence remains limited and largely derived from preclinical studies and

small clinical cohorts, as recently reported by the European Society of Medicine (Rowen et al. 2025). In neuroblastoma, experimental data suggest that ozone exposure can inhibit cell cycle progression and trigger apoptosis (Cannizzaro et al. 2007), possibly by altering the hypoxic microenvironment that supports tumor growth, angiogenesis, and metastasis. In recent glioblastoma case reports, a combination of oxygen-ozone therapy with medical cannabis and melatonin was associated with durable remission and long-term survival (Antonini et al. 2023; Mammone et al. 2025a; Mammone et al. 2025b), providing a conceptual precedent for using similar integrative combinations in other pediatric solid tumors. Retinoids, particularly 13-cis-retinoic acid (RA), have been incorporated into standard adjuvant therapy for high-risk neuroblastoma. They induce differentiation and growth inhibition in neuroblastoma models (Marayati et al. 2020), and their inclusion in high-risk regimens has contributed to improved outcomes, although relapse and resistance remain significant challenges (Makimoto et al. 2024). Palmitoylethanolamide (PEA), an endogenous fatty acid amide and the peroxisome proliferator-activated receptor α agonist, has been shown to reduce survival, proliferation, and clonogenicity of neuroblastoma cells in vitro, acting on intrinsic apoptotic pathways (Camoglio et al. 2024). Its broader anticancer activity across different tumor types, including neuroblastoma-derived lines, supports its potential as part of a multi-compound integrative regimen (Pagano et al. 2021; Di Marzo et al. 2001; Hamtiaux et al. 2012).

Against this background, neuroblastoma represents a particularly relevant model for exploring innovative therapeutic strategies. It is the most frequent extracranial solid tumor in preschool children and represents the most common tumor in newborns as well as one of the most prevalent solid tumors in children overall, accounting for about 7–8% of all childhood cancers (Yadwad et al. 2024). Approximately 90% of cases occur in children under 5 years of age, and neuroblastoma is responsible for about 15% of all pediatric cancer-related deaths. In United State the prevalence is about 1 case per 7,000 live births. The incidence is 8.3 cases per 1 million per year in children younger than 15 years (NIH. Neuroblastoma Treatment (PDQ®)–Health Professional Version). Neuroblastoma arises from neural crest-derived neuroblasts and is classified among the peripheral neuroblastic tumors, which also include ganglioneuromas and ganglioneuroblastomas (Shimada et al. 1999). The most common primary

sites are the adrenal glands and the extra-adrenal sympathetic chain, involving the retroperitoneum, thorax, neck, and pelvis, while central nervous system-primary neuroblastoma is very rare. Abdominal tumors predominate, accounting for about 70% of cases and often correlating with a worse prognosis. Metabolomic profiling of neuroblastoma patients revealed significant alterations in pathways related to tyrosine, methionine, steroid, and leucine metabolism, with classical markers such as vanillylmandelic acid and homovanillic acid and novel combination such as 3-methoxytyramine sulphate, cystathionine, and cortisol (Sawada 1997, Yokota et al. 2021). While most cases are sporadic, a small hereditary fraction (1–2%) is linked to germline mutations in genes such as ALK and MYCN, with MYCN amplification occurring in about 25% of patients and strongly associated with aggressive disease and poor outcome (Goto et al. 2001).

At diagnosis, a large proportion of patients already present with metastatic disease involving bone marrow, bone, liver, and lymph nodes (Yadwad et al. 2024). Clinical presentation is conditioned by the site of the primary lesion and the stage of the disease. Localized forms are discovered incidentally or are revealed by the presence of an abdominal or thoracic mass, which may be associated with abdominal pain and/or distension and/or respiratory problems (more frequent and detectable in infants). Paraspinal tumors of the thorax, abdomen and pelvis are found in 5-15% of patients: these tumors can extend to the intervertebral neural foramina (hourglass neuroblastomas), causing symptoms of compression of the nerve roots and spinal cord. Signs of metastatic disease include bone pain, claudication, paralysis, Horner's syndrome, hepatomegaly (Pepper's syndrome), and proptosis (Hutchinson's syndrome). Bone marrow involvement can lead to anemia and/or thrombocytopenia, and hematological parameters at diagnosis have been shown to influence prognosis. Paraneoplastic syndromes such as Opsoclonus-Myoclonus Syndrome and secretory diarrhea due to vasoactive intestinal peptide hypersecretion are also reported, adding to the complexity of the disease picture (Yan et al. 2020).

Current risk stratification, based on the International Neuroblastoma Risk Group (INRG) system and the Children's Oncology Group (COG) criteria, incorporates age, stage, histology, tumor differentiation, MYCN status, 11q aberrations, and tumor cell ploidy to classify patients into very-low, low, intermediate, and high-risk groups (Cohn et al. 2009).

Standard therapy for high-risk neuroblastoma includes intensive chemotherapy followed by surgery,

autologous stem cell transplantation, radiotherapy, and immunotherapy. However, even with this aggressive regimen, survival for high-risk and relapsed disease remains modest, with 5-year survival rates around 50% and significantly lower in refractory or recurrent settings. Moreover, treatment-related toxicity frequently leads to substantial morbidity and long-term functional impairment, highlighting the need for novel therapeutic strategies that can improve both survival and quality of life (Smith et al. 2018; DuBois et al. 2022; Tolbert et al. 2018; Matthay et al. 1999; Park et al. 2011; Garaventa et al. 2009). The combination of medical cannabis, melatonin, and oxygen-ozone therapy is based on the hypothesis of converging mechanisms, including modulation of oxidative stress, mitochondrial function, tumor metabolism, and immune responses. Although each of these agents has shown individual anti-tumor potential in preclinical studies, their combined use has been scarcely explored. In preclinical cancer models, only one study has reported the antitumoral effect of the combination of CBD, melatonin and oxygen-ozone therapy, demonstrating the reduction of pancreatic cancer growth in an orthotopic xenograft mouse model (Zeppa et al. 2024b). To date, no published studies have systematically examined this combination in neuroblastoma. Therefore, this report presents two pediatric cases of advanced neuroblastoma treated with an integrative protocol based on these compounds, aiming to explore their potential contribution to disease control and clinical stabilization in high-risk settings.

Case report 1

In June 2020, a 3-year-old girl presented to the emergency department following a febrile episode and the appearance of lateral cervical swelling. After ultrasound findings of a calcified lymph node, prescription of antibiotic therapy and a new ultrasound scan, a hematological consultation was requested with an indication for hospitalization for a metaiodobenzylguanidine (MIBG) scintigraphy which highlighted the presence of a left mediastinal mass, for which a biopsy was ordered (Figure 1).

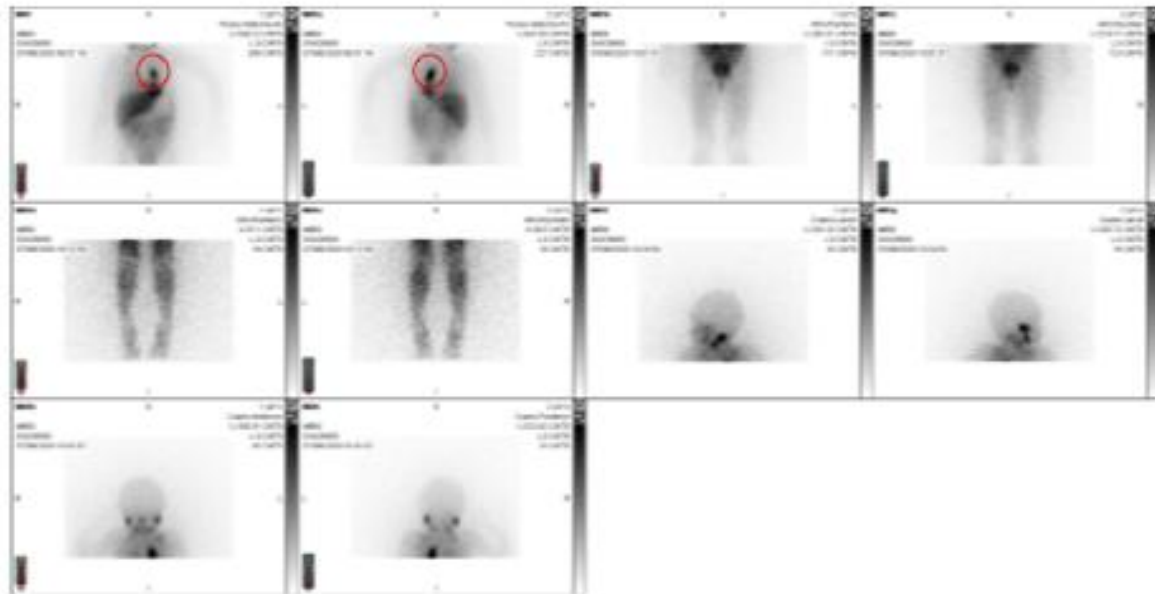


Figure 1. MIBG scintigraphy shows the presence of lesion in the posterior mediastinum.

The red-circles depict the mediastinal mass (left: anterior view; right: posterior view), while the remaining paired images (anterior and posterior projections) show no evidence of additional pathological lesions in other body regions.

The biopsy investigation found the presence of a neuroblastoma. It was classified as stage L2, MYCN negative. The patient was started on the LINES (European Low and Intermediate Risk Neuroblastoma) protocol, for which 6 cycles of therapy were programmed. After 2 months the investigations showed a slight decrease in the mass. After 4 months an increase in urinary catecholamines was detected, with subsequent findings on scintigraphy of metastatic disease. Thus, the neuroblastoma was reclassified as stage IV according to the International Neuroblastoma Staging System (INSS) classification, with a left supraclavicular lymph node localization, and a bone metastasis at the pubic level.

The patient received two cycles of ICE (ifosfamide, carboplatin, etoposide) in January and March 2021, followed by two cycles of TPT/EDX (topotecan and cyclophosphamide) between March and April 2021. In June 2021, debulking surgery of the mediastinal mass was performed, leaving a residual unresectable paravertebral component. This was followed in July by neoadjuvant TEMIRI (temozolomide and irinotecan), and subsequently by high-dose MIBG radiometabolic therapy, administered in two cycles, as well as melphalan in August 2021.

In September 2021, the patient underwent autologous stem cell transplantation, with moderate treatment tolerance. During the same period, high-dose chemotherapy with busulfan/melphalan followed by autologous stem cell reinfusion was initiated. Re-evaluation showed a reduction in the primary tumor and stable metastatic disease.

In October 2021, disease reassessment with total-body computed tomography (CT) scan demonstrated a reduction of the mediastinal mass. Bone marrow aspirate/biopsy was negative for neuroblastoma involvement. MIBG scintigraphy revealed persistent radiotracer uptake in the known residual paravertebral thoracic mass, stable mild uptake in the left supraclavicular region, and a small focal uptake in the left pubic bone.

On October 2021, the patient was admitted for surgical excision of the left supraclavicular lymph node. Histological examination confirmed lymph node metastasis from neuroblastoma. Next-generation sequencing (NGS) analysis of the lymph node tissue was negative for detectable fusion genes, copy number variations, and clinically relevant mutations in AKT1/2/3, ERBB2, and PIK3CA.

In November 2021, the patient underwent radiotherapy (21 Gy) to the tumor bed and mediastinum. Subsequent evaluations showed resolution of lymph node involvement, with persistence of residual disease at the posterior mediastinal level.

In January 2022, immunotherapy with anti-GD2 CAR-T cells was initiated. Treatment was complicated by fever requiring a 20-day hospitalization, severe asthenia, weight loss, and deterioration of general clinical conditions. Approximately one month after fever onset, therapy was resumed following gradual clinical recovery, with a good response of metastatic lesions. At 12-week evaluation, complete remission of metastatic disease and stable metabolically active residual thoracic disease were confirmed (Figure 2).

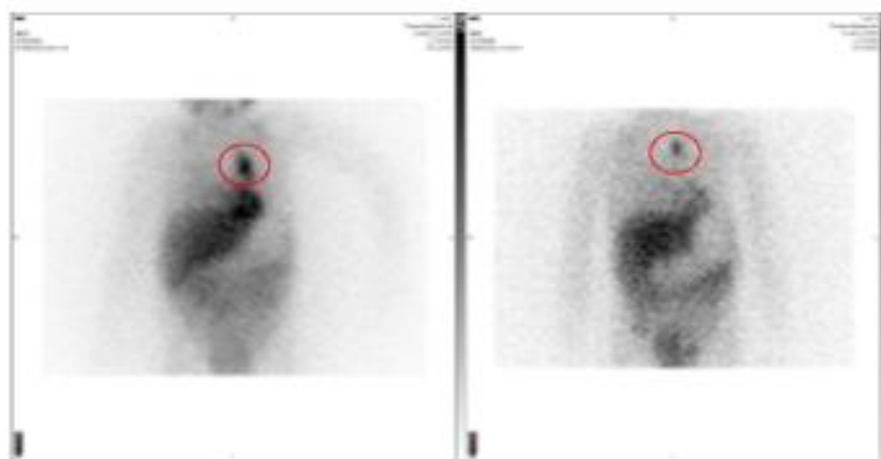


Figure 2. Comparison of MIBG Scintigraphy (March 2022 vs Diagnostic Scan). MIBG scintigraphy from March 2022 (right), compared with the diagnostic scan (left), shows a reduction in the size of the mediastinal lesion.

In April 2022, the patient underwent radical surgery to the primary tumor bed, followed by the initiation of radiotherapy in June 2022 (15 Gy). The scintigraphy performed in May 2022 demonstrated no evidence of disease (Figure 3).

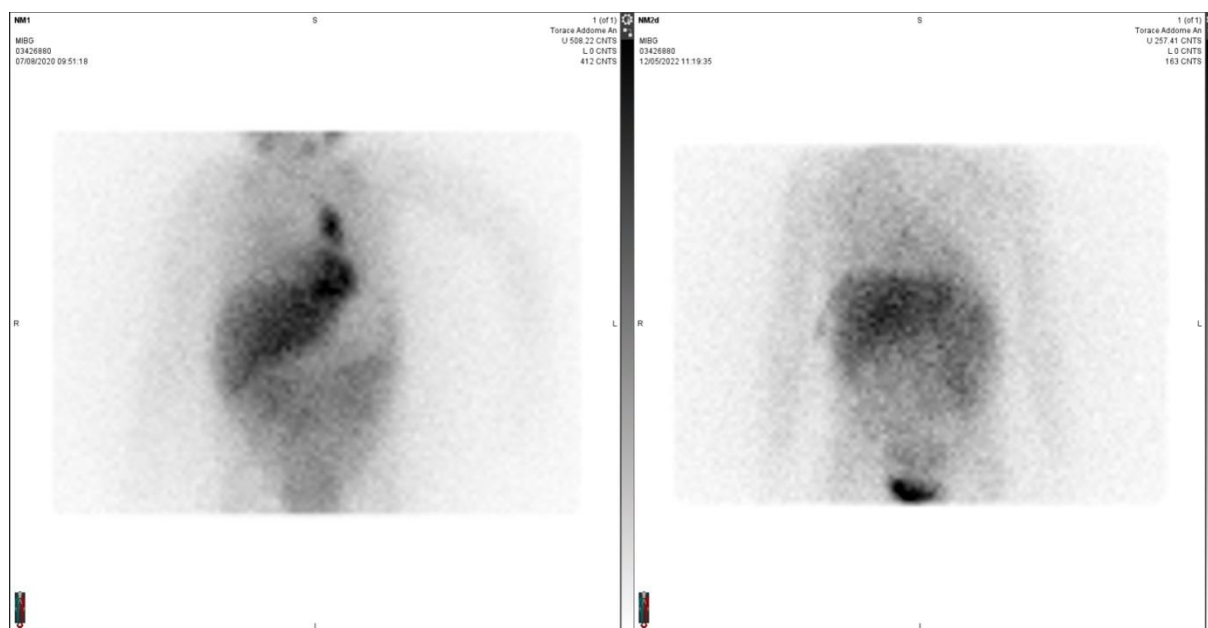


Figure 3. Comparison of MIBG Scintigraphy (May 2022 vs Diagnostic Scan). MIBG scintigraphy from May 2022 (right), compared with the diagnostic scan (left), shows absence of disease.

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During chemotherapy cycles, it was used Filgastrim to stimulate white blood cell production, Acyclovir and Bactrim prophylactically, and levothyroxine replacement therapy in addition to folic acid and iron supplements.

In July 2022 the patient began an integrated treatment approach combining oxygen-ozone therapy and medical cannabis. Oxygen-ozone therapy was administered rectally by insufflation of a gaseous mixture containing 97% oxygen and 3% ozone, at a concentration of 80 µg per 2.5 ml/Kg, once daily for four consecutive days per week during the first three months. This regimen was repeated in cycles of three months of treatment followed by a three-month break, until oncological assessments were negative.

Medical cannabis supplementation consisted of 5 drops of 10% CBD oil, taken four times daily during the initial three months, with the option to increase to 10 drops if well tolerated.

Additionally, the patient was followed by a nutrition biologist, who emphasized adequate hydration and regular physical activity, particularly during the oxygen-ozone therapy breaks.

Monitoring included monthly blood chemistry analyses. At the end of the first trimester of integrative therapy, contrast-enhanced CT and color Doppler echocardiography were performed, considering the location of the primary tumor.

The MIBG scintigraphy performed on August 2022 (Figure 4) and compared with the previous one on August 2020 (Figure 3), demonstrated no tracer uptake in the mediastinum, left supraclavicular region, or left pubic bone. The patient was therefore NED (no evidence of disease).

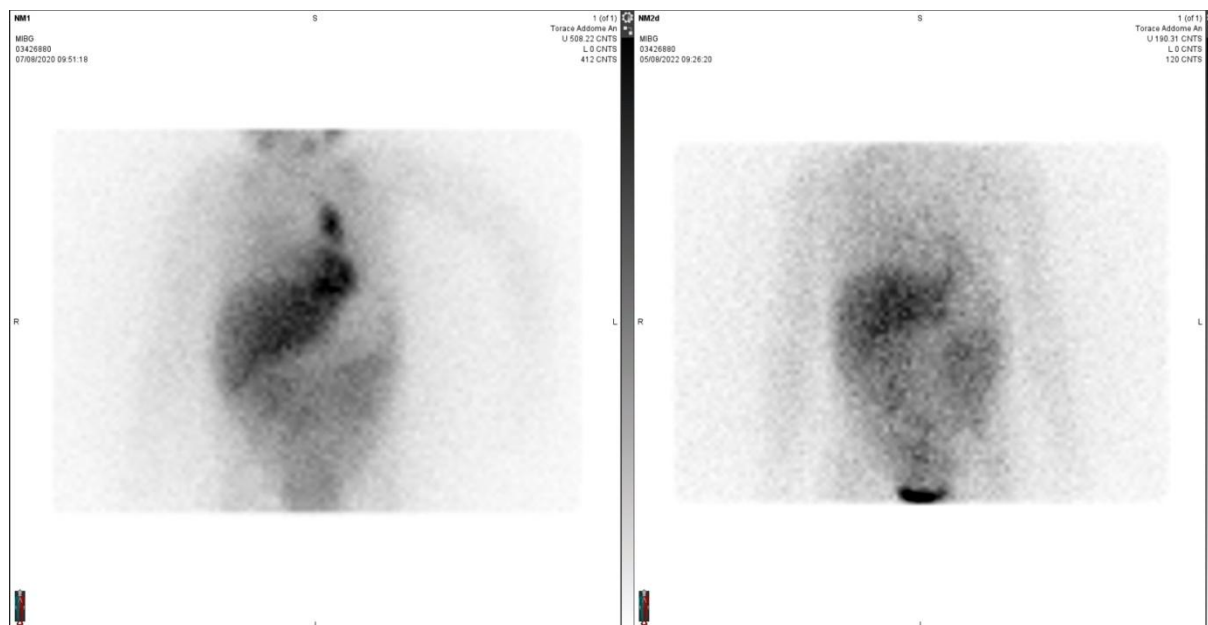


Figure 4. Comparison of MIBG Scintigraphy (August 2022 vs Diagnostic Scan). MIBG scintigraphy from May 2022 (right), compared with the diagnostic scan (left), shows that the patient was still NED.

Follow-up assessments in December 2022, including bone scan, CT scan, and total-body positron emission tomography (PET), confirmed stable results over time. By January 2023, the patient was in good general health, with improved mood, reduced asthenia, and normalized hemoglobin levels. The CBD dosage was increased to 10 drops four times daily due to good tolerance. Additional supportive treatments included melatonin (25 mg once daily). The frequency of oxygen-ozone therapy for the subsequent three-month cycle (April-May 2023) was adjusted from four insufflations per week to two sessions per week, spaced one week apart if previous evaluations remained negative. The gas mixture volume was increased to 60 mL, proportionally to the patient's weight.

To further enhance the integrative pharmacological regimen, PEA (600 mg/day) and retinoic acid (1 teaspoon three times daily; Axerophthol palmitate 0.125 g + trans-retinoic acid 0.125 g + beta-carotene 0.5 g + vitamin E acetate 250 g) were added. Melatonin was gradually increased from 30 to 60 mg in the evening.

Subsequent imaging confirmed no evidence of disease, and the patient continues maintenance therapy with oxygen-ozone insufflations twice weekly, along with melatonin (15 mg once daily), CBD, retinoic acid, and PEA.

Case report 2

In May 2019, a 2-year-old child presented to the emergency department with atraumatic frontal swelling. A CT scan revealed a left adrenal mass suggestive of neuroblastic origin, which was subsequently confirmed by a by MIBG scintigraphy (Figure5).

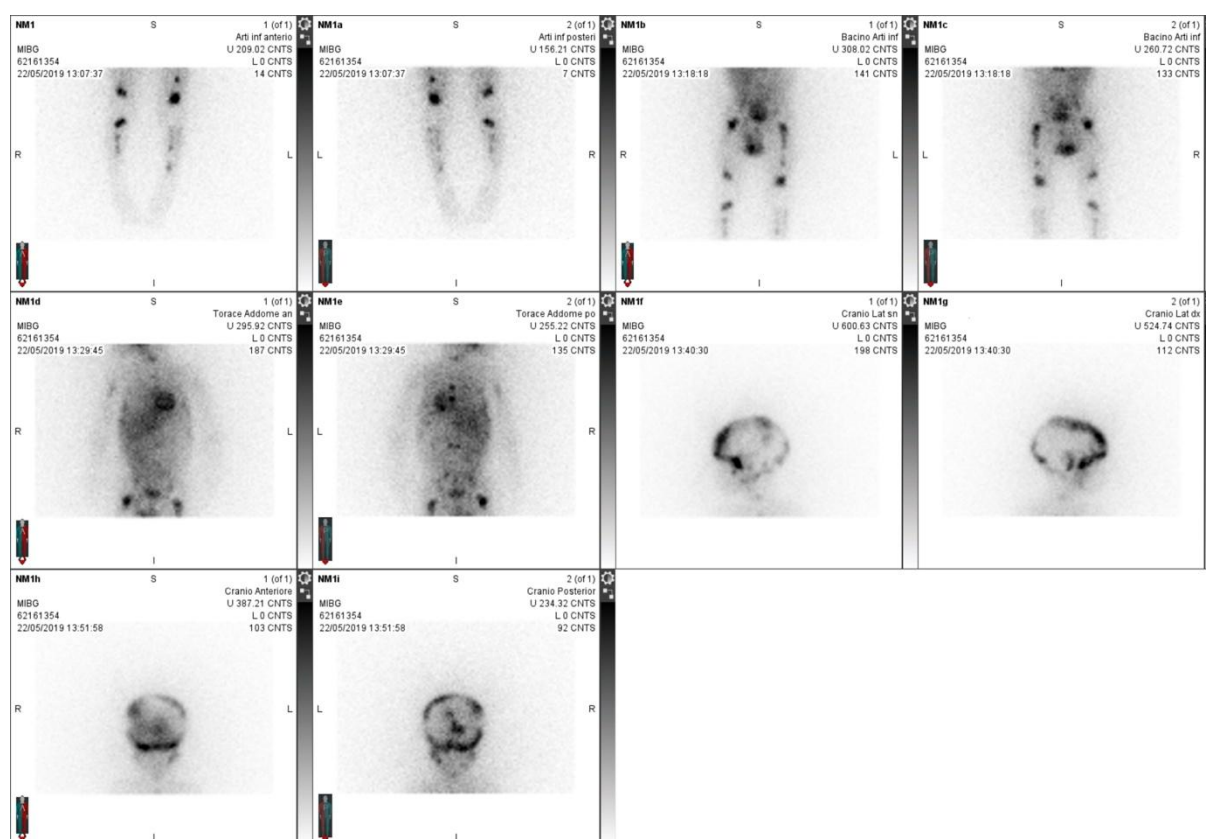


Figure 5. Distribution of Disease on MIBG Scintigraphy. MIBG scintigraphy shows the presence of disease at both bone and adrenal sites.

The patient underwent a left adrenalectomy and a bone marrow biopsy within one week, leading to a diagnosis of metastatic neuroblastoma (INSS stage IV).

Immediately postoperatively, chemotherapy was initiated according to the RAPID COJEC protocol, which included cisplatin, vincristine, carboplatin, etoposide, and cyclophosphamide over a 10-week period. Treatment was discontinued after the fourth cycle due to renal failure.

From July to December 2019, the patient received experimental therapy with eculizumab for atypical hemolytic uremic syndrome. Concurrently, chemotherapy with cisplatin and vincristine was resumed

from July to September 2019. Follow-up MIBG scintigraphy on August 2019, demonstrated a partial response to treatment (Figure 6).

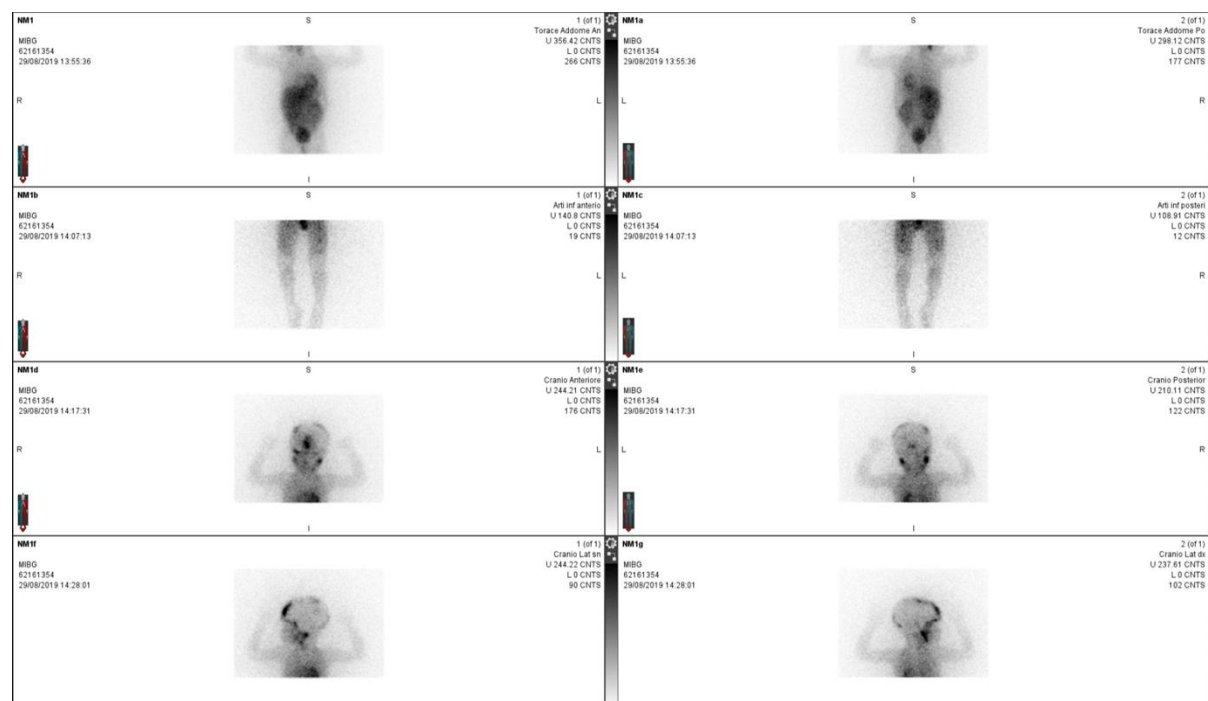


Figure 6. First follow-up MIBG scintigraphy at 3 months. The scan performed three months after the initiation of chemotherapy shows a partial response.

Following subsequent administration of temozolomide and irinotecan, the patient underwent radiotherapy and required an autologous stem cell transplant in December 2019.

In February 2020, chemotherapy with busulfan and melphalan was administered, followed by a second autologous stem cell transplant. From April to July 2020, therapy with temozolomide and irinotecan was resumed but had to be interrupted due to pneumonia. In August 2020, the patient received immunotherapy with anti-GD2 CAR-T cells, which resulted in a poor response.

The patient then resumed the TEMIRI regimen from August to October 2020, which was complicated by toxicity, including diarrhea, vomiting, and significant weight loss. Follow-up MIBG scintigraphy in October and November 2020 consistently demonstrated persistent lesions at the skull base and left femur (Figures 7 and 8).

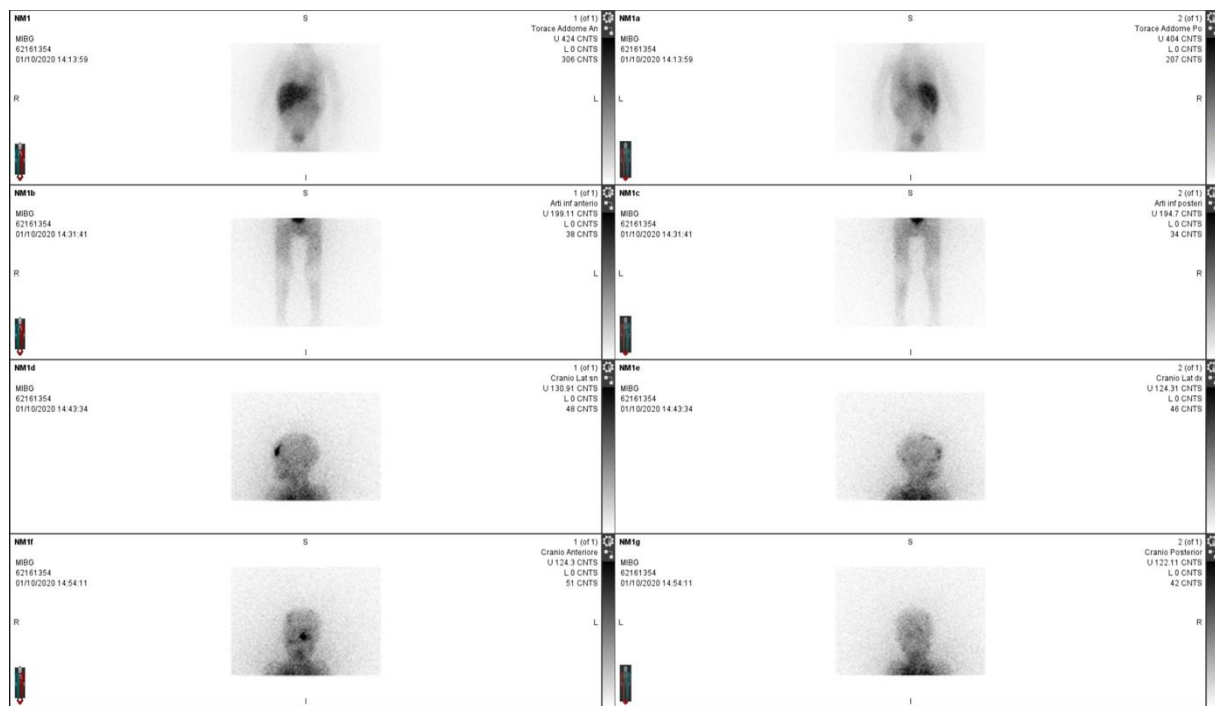


Figure 7. Persistence of disease at 18-month follow-up. MIBG scintigraphy performed in October 2020, 18 months after diagnosis, shows persistent disease in the cranial vault and left femur.

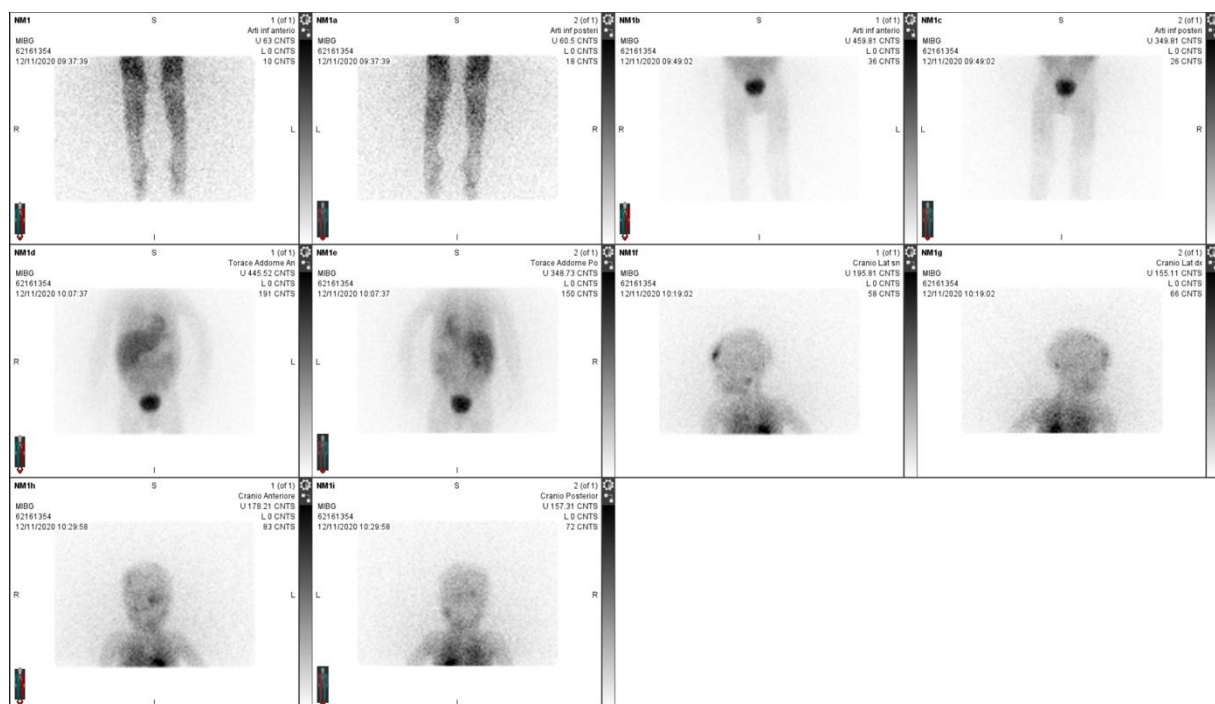


Figure 8. Pre-treatment follow-up MIBG scintigraphy. MIBG scintigraphy performed in November 2020, prior to the initiation of oxygen-ozone therapy.

Following episodes of dehydration and re-hospitalization during chemotherapy, the referring oncologist decided to suspend all therapies in January 2021.

Up to that point, the patient had been on chronic supportive treatment including vitamin C, lactoferrin, probiotics, and vitamin D3, alongside acyclovir (4 mg/day), doxazosin (Cardura, 1 mg twice daily), trimethoprim-sulfamethoxazole (Bactrim, three times per week), carvedilol (6.25 mg daily), amlodipine (Norvasc, twice daily), Calisvit (on alternate days), and levothyroxine (Tirosint, daily).

In February 2021, the patient commenced an integrated therapy regimen. This included rectal insufflation of oxygen-ozone at a concentration of 80 µg/ml, with an initial volume of 30 mL appropriate for the patient's weight (12 kg), administered once daily for four consecutive days per week over three months. Concurrently, CBD oil (10%) was prescribed at an initial dose of 5 drops three times daily, with planned titration up to 20 drops three times daily as tolerated. Nutritional support was provided under the guidance of a nutritional biologist, emphasizing adherence to a suitable diet.

A follow-up scan in June 2021 showed further progression of the known cranial disease, predominantly in the right orbital region, with slight tracer uptake in the femur (Figure 9).

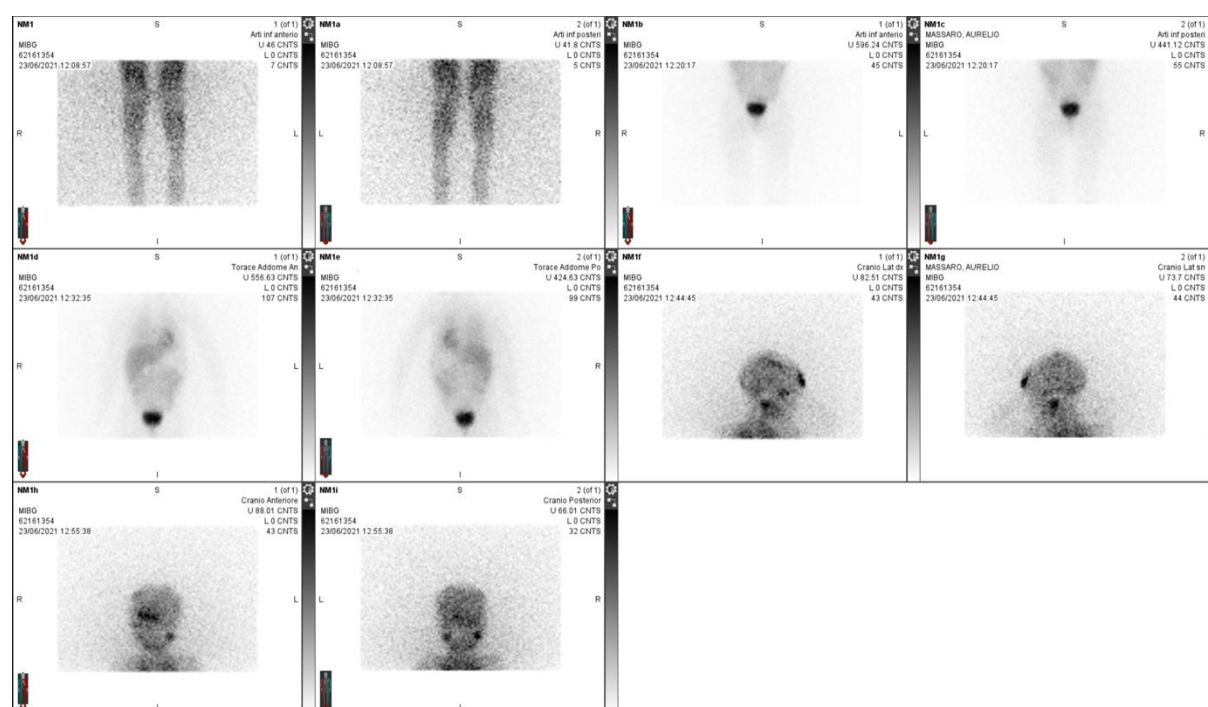


Figure 9. MIBG scintigraphy at 4-month follow-up. Imaging performed four months after the start of integrated therapies shows a partial response, with disease localized exclusively to the skull bones.

At the July follow-up, the CBD dosage was increased to 20 drops four times daily. Boswellia (100 mg, four times daily) and retinoic acid supplementation (Axerophthol palmitate 0.125 g, trans-retinoic acid 0.125 g, beta-carotene 0.5 g, vitamin E acetate 250 g) were also added, at a dose of 1 teaspoon twice daily.

Following the second quarterly cycle of oxygen-ozone therapy, the patient reported a general sense of well-being, resuming physical and social activities, including school attendance, with improved mood. In October 2021, evening melatonin (10 mg) and phycocyanin (100 mg, four times daily) were added to the regimen, and the oxygen-ozone gas volume was increased to 40 mL to account for the patient's weight gain.

A control scan in January 2022 (Figure 10) demonstrated substantial stability, with persistent lesion in the frontal region and reduced extension in the right orbital region. No new lesions were observed.

In May 2022, palmitoylethanolamide (PEA) therapy was initiated at a dose of 300 mg, taken once daily.

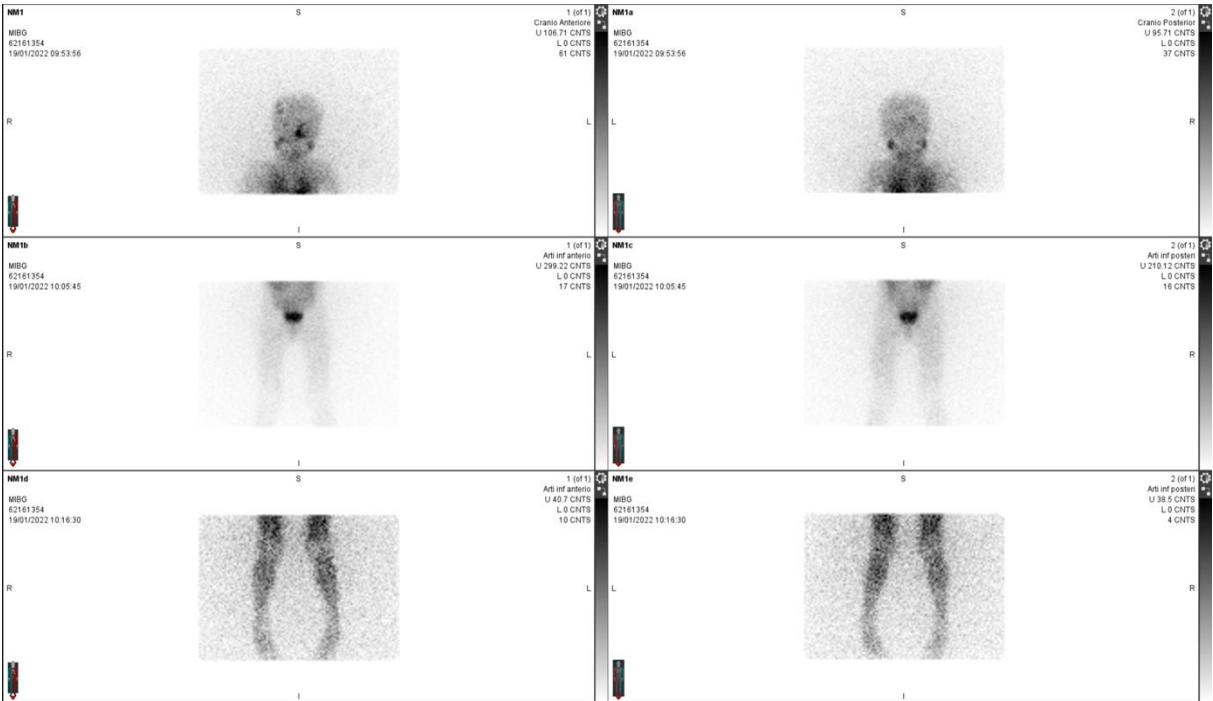


Figure 10. Persistence of disease in the cranial vault. MIBG scintigraphy performed in January 2022 shows persistent disease limited to the cranial skull.

On July 2022, a PET-TC scan showed complete regression of the neoplastic lesions (Figure 11).

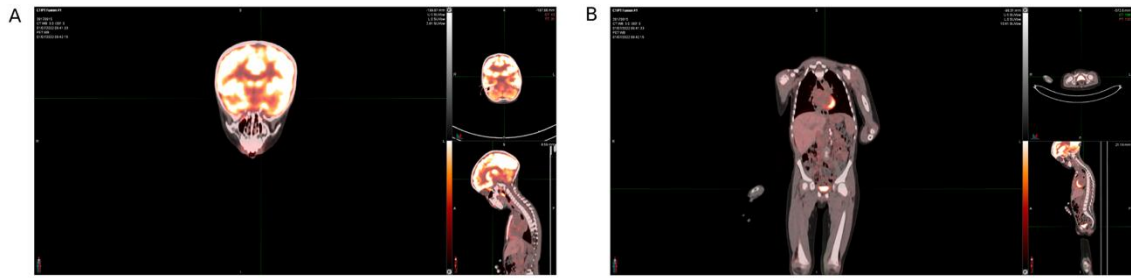


Figure 11. PET scan showing metabolic response (July 2022). PET imaging performed in July 2022 demonstrates complete absence of hypermetabolic areas in both the cranial skull (A) and lower limbs (B).

In April 2024 the patient was still NED (Figure 12). Oxygen-ozone insufflation therapy was further adjusted according to body weight and reduced in frequency, from four weekly sessions to two weekly sessions over three months, while oral therapy with CBD, melatonin, PEA, and retinoids was maintained.

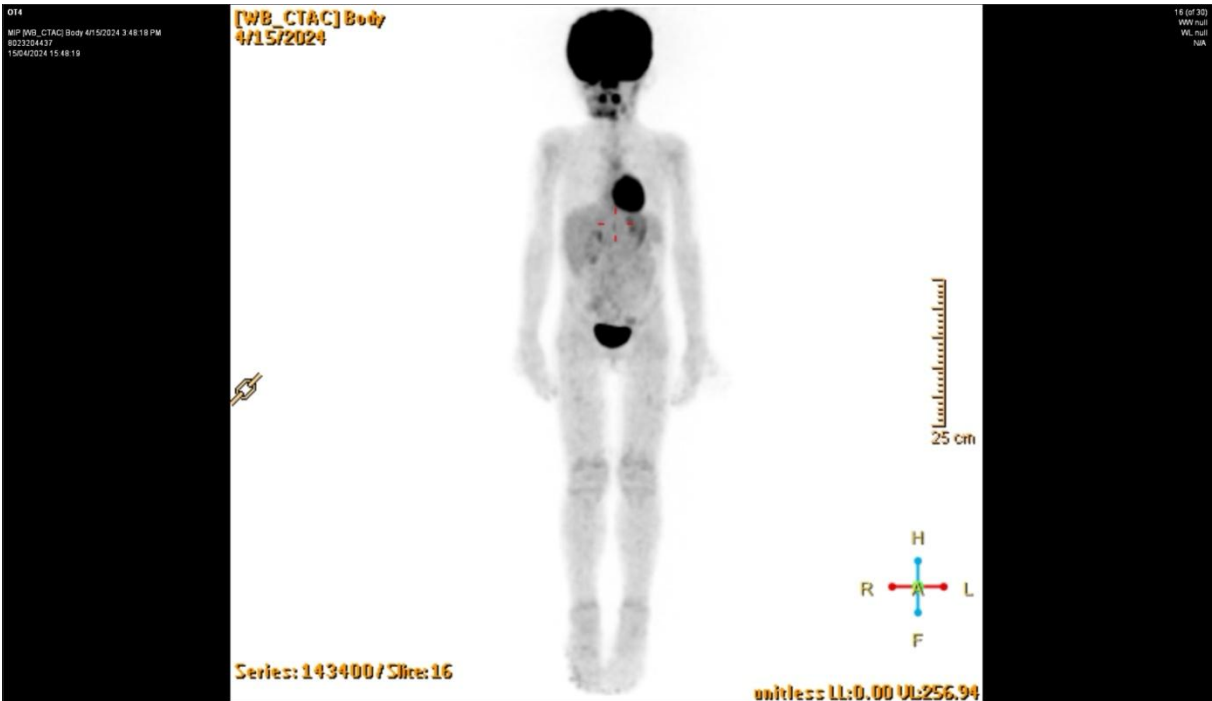


Figure 12. PET/CT Scan (April 2024). PET/CT imaging performed in April 2024.

Discussion and Conclusion

We report two pediatric patients diagnosed with neuroblastoma who, following multiple lines of conventional therapy and in poor physical condition, turned to an integrative supportive therapy. In one case, the patient had shown limited or no response to standard treatments, while in the other, integrative therapy was initiated to maintain remission and improve overall functional recovery. The supportive regimen included oxygen-ozone therapy, melatonin, CBD, RA, and PEA, supplemented with Boswellia acids.

Although most studies have investigated individual components, recent case reports have documented the combined use of CBD, melatonin, and oxygen-ozone therapy in oncology. Mammone et al. reported integrative therapy in oral tongue carcinoma, demonstrating feasibility and safety in adult patients (Mammone et al. 2025a). Antonini et al. described the effects of the same combination on glioblastoma multiforme, showing potential clinical benefits in disease control and quality of life (Antonini et al. 2023). Additionally, Mammone et al. reported a case of anaplastic astrocytoma treated with this integrative approach, further supporting the applicability of this regimen in different tumor types (Mammone et al. 2025b). These reports provide preliminary evidence that such multi-component integrative approaches can be applied safely in oncological settings.

Several completed and ongoing trials continue to investigate individual compounds. RA has been studied in neuroblastoma for its ability to induce differentiation and inhibit tumor growth (Makimoto et al. 2024), while ozone therapy is under prospective evaluation for its palliative and quality-of-life benefits in patients with failed standard treatments (NCT05417737). PEA is being tested in phase II trials for chemotherapy-induced neuropathy (NCT05246670). Melatonin has been investigated for appetite regulation, sleep disorders, fatigue control, chemotherapy toxicity, adjuvant therapy, and antiproliferative effects in adults, with a phase I pediatric study assessing safety in relapsed solid tumors (NCT01858155). Observational studies have been conducted or are ongoing to evaluate medical cannabis in pediatric oncology for symptom management and quality of life, including a completed study at Children's Hospital Colorado (NCT03052738) and the ongoing CAN-RWE trial across Canada (NCT05863910).

Boswellia acids, the active components of *Boswellia serrata*, have demonstrated preclinical anti-

inflammatory and anti-cancer effects, supporting their inclusion in integrative regimens (Sharma et al. 2020).

In our two cases, conventional and integrative therapies were not administered simultaneously. The first patient, already in partial remission, used integrative therapy to maintain disease control and promote functional recovery, whereas the second patient, considered non-responsive to standard therapies and with severe systemic impairment, relied solely on the integrative regimen.

Both patients experienced complete remission of detectable disease and remained without disease progression throughout the treatment period. Importantly, no adverse effects were reported. At the last follow-up, both patients continued to be NED (no evidence of disease).

While these cases, together with previously published reports, suggest potential benefit and safety of combining CBD, melatonin, oxygen-ozone, RA, and PEA in supportive oncology care, current evidence remains limited. Most clinical trials have focused on individual compounds rather than integrated multi-component regimens. Further well-designed clinical studies are warranted to determine whether this approach can serve as a valid supportive or adjuvant therapy in neuroblastoma and other malignancies.

List of Abbreviation

Cannabidiol (CBD)

Children's Oncology Group (COG)

Computed tomography (CT)

ICE (ifosfamide, carboplatin, etoposide)

International Neuroblastoma Staging System (INSS)

International Neuroblastoma Risk Group (INRG)

LINES (European Low and Intermediate Risk Neuroblastoma)

Metaiodobenzylguanidine (MIBG)

NED (no evidence of disease)

Next-generation sequencing (NGS)

Palmitoylethanolamide (PEA)

Positron emission tomography (PET)

402 Retinoic acid (RA)
403 TEMIRI (temozolomide and irinotecan)
404 TPT/EDX (topotecan and cyclophosphamide)
405

406 **Declarations**

407 **Ethics approval and consent to participate**

408 This study was conducted in accordance with the Declaration of Helsinki and was determined by the
409 Institutional Review Board of Fondazione Maria Guarino Amor ETS to meet criteria for waiver due to
410 the retrospective, non-interventional nature of the study and the lack of identifying information or
411 protected health information. Written informed consent was obtained from the patients' legal guardians
412 for participation in the integrative therapy.

413 **Consent for publication**

414 Written informed consent was also obtained from the patients' legal guardians for the publication of
415 clinical data and images.

416 **Data Availability Statement**

417 The data presented in this study are available on request from the corresponding author. The data are
418 not publicly available due to their containing information that could compromise the privacy of patient
419 subject of this case report.

420 **Competing interests**

421 The authors declare no competing interests.

422 **Funding Statement**

423 This research received no external funding.

424 **Author Contributions**

425 Conceptualization, M.L. and M.N.; methodology, A.F.; validation, A.E.C., F.B, and M.L.; formal
426 analysis, A.E.C., F.B, and M.L.; investigation, A.E.C., F.B, and M.L.; resources, M.L.; data curation,
427 M.B.M. and M.N.; writing-original draft preparation, M.B.M.; writing—review and editing, M.N.,
428 A.E.C., F.B, and M.L.; visualization, M.B.M.; supervision, M.L. and M.N.; project administration, M.L.
429 All authors have read and agreed to the published version of the manuscript.

Acknowledgments

To “Maria Guarino” Foundation-Amor No Profit Association.

CARE checklist

The authors confirm that this case report adheres to the recommendations of the CARE (Case Report) Checklist and Guidelines, ensuring completeness and accuracy in reporting.

References

- Abu-Sawwa R, Scutt B, Park Y. Emerging Use of Epidiolex (Cannabidiol) in Epilepsy. *J Pediatr Pharmacol Ther.* 2020;25(6):485-499. doi: 10.5863/1551-6776-25.6.485.
- Alharris E, Singh NP, Nagarkatti PS, Nagarkatti M. Role of miRNA in the regulation of cannabidiol mediated apoptosis in neuroblastoma cells. *Oncotarget.* 2019;10(1):45-59. doi: 10.18632/oncotarget.26534.
- Aguzzi C, Zeppa L, Morelli MB, Marinelli O, Giangrossi M, Amantini C, Santoni G, Sazzad H, Nabissi M. Anticancer effect of minor phytocannabinoids in preclinical models of multiple myeloma. *Biofactors.* 2024 Nov-Dec;50(6):1208-1219. doi: 10.1002/biof.2078.
- Antonini M, Aguzzi C, Fanelli A, Frassinetti A, Zeppa L, Morelli MB, Pastore G, Nabissi M, Luongo M. The Effects of a Combination of Medical Cannabis, Melatonin, and Oxygen-Ozone Therapy on Glioblastoma Multiforme: A Case Report. *Reports.* 2023;6(2):22. doi: 10.3390/reports6020022.
- Baeza-Noci J, Pinto-Bonilla R. Systemic Review: Ozone: A Potential New Chemotherapy. *Int J Mol Sci.* 2021;22(21):11796. doi: 10.3390/ijms222111796.
- Bonmati-Carrion MA, Tomas-Loba A. Melatonin and Cancer: A Polyhedral Network Where the Source Matters. *Antioxidants (Basel).* 2021 Feb 1;10(2):210. doi: 10.3390/antiox10020210
- Camoglio C, Balla J, Fadda P, Dedoni S. Oleoylethanolamide and Palmitoylethanolamide Enhance IFN β -Induced Apoptosis in Human Neuroblastoma SH-SY5Y Cells. *Molecules.* 2024;29(7):1592. doi: 10.3390/molecules29071592.
- Cannizzaro A, Verga Falzacappa CV, Martinelli M, Misiti S, Brunetti E, Bucci B. O(2/3) exposure inhibits cell progression affecting cyclin B1/cdk1 activity in SK-N-SH while induces apoptosis in SK-N-DZ neuroblastoma cells. *J Cell Physiol.* 2007;213(1):115-25. doi: 10.1002/jcp.21097.

Clavo B, Santana-Rodríguez N, Llontop P, Gutiérrez D, Suárez G, López L, Rovira G, Martínez-Sánchez G, González E, Jorge IJ, Perera C, Blanco J, Rodríguez-Esparragón F. Ozone Therapy as Adjuvant for Cancer Treatment: Is Further Research Warranted? *Evid Based Complement Alternat Med.* 2018;2018:7931849. doi: 10.1155/2018/7931849.

Clavo B, Cánovas-Molina A, Ramallo-Fariña Y, Federico M, Rodríguez-Abreu D, Galván S, Ribeiro I, Marques da Silva SC, Navarro M, González-Beltrán D, Díaz-Garrido JA, Cazorla-Rivero S, Rodríguez-Esparragón F, Serrano-Aguilar P. Effects of Ozone Treatment on Health-Related Quality of Life and Toxicity Induced by Radiotherapy and Chemotherapy in Symptomatic Cancer Survivors. *Int J Environ Res Public Health.* 2023;20(2):1479. doi: 10.3390/ijerph20021479.

Cohn SL, Pearson AD, London WB, et al. The International Neuroblastoma Risk Group (INRG) classification system: an INRG Task Force report. *J Clin Oncol* 2009;27:289-97. doi: 10.1200/JCO.2008.16.6785

Di Marzo V, Melck D, Orlando P, Bisogno T Zagoory O, Bifulco M, Vogel Z, De Petrocellis L. Palmitoylethanolamide Inhibits the Expression of Fatty Acid Amide Hydrolase and Enhances the Anti-Proliferative Effect of Anandamide in Human Breast Cancer Cells. *Biochem. J.* 2001, 358, 249–255. doi: 10.1042/0264-6021:3580249

DuBois SG, Macy ME, Henderson TO. High-Risk and Relapsed Neuroblastoma: Toward More Cures and Better Outcomes. *Am Soc Clin Oncol Educ Book.* 2022;42:1-13. doi: 10.1200/EDBK_349783.

Faiz MB, Naeem F, Irfan M, Aslam MA, Estevinho LM, Ateşşahin DA, Alshahrani AM, Calina D, Khan K, Sharifi-Rad J. Exploring the therapeutic potential of cannabinoids in cancer by modulating signaling pathways and addressing clinical challenges. *Discov Oncol.* 2024 Sep 27;15(1):490. doi: 10.1007/s12672-024-01356-8.

Fisher T, Golan H, Schiby G, PriChen S, Smoum R, Moshe I, Peshes-Yaloz N, Castiel A, Waldman D, Gallily R, Mechoulam R, Toren A. In vitro and in vivo efficacy of non-psychoactive cannabidiol in neuroblastoma. *Curr Oncol.* 2016;23(2):S15-22. doi: 10.3747/co.23.2893.

Garaventa A, Parodi S, De Bernardi B, Dau D, Manzitti C, Conte M, Casale F, Viscardi E, Bianchi M, D'Angelo P, Zanazzo GA, Luksch R, Favre C, Tamburini A, Haupt R. Outcome of children with neuroblastoma after progression or relapse. A retrospective study of the Italian neuroblastoma

registry. *Eur J Cancer*. 2009;45(16):2835-42. doi: 10.1016/j.ejca.2009.06.010.

García-Santos G, Antolín I, Herrera F, Martín V, Rodríguez-Blanco J, del Pilar Carrera M, Rodríguez C. Melatonin induces apoptosis in human neuroblastoma cancer cells. *J Pineal Res*. 2006;41(2):130-5. doi: 10.1111/j.1600-079X.2006.00342.x.

González A, González-González A, Alonso-González C, Menéndez-Menéndez J, Martínez-Campa C, Cos S. Melatonin inhibits angiogenesis in SH-SY5Y human neuroblastoma cells by downregulation of VEGF. *Oncol Rep*. 2017;37(4):2433-2440. doi: 10.3892/or.2017.5446.

Goto S, Umehara S, Gerbing RB, Stram DO, Brodeur GM, Seeger RC, Lukens JN, Matthay KK, Shimada H. Histopathology (International Neuroblastoma Pathology Classification) and MYCN status in patients with peripheral neuroblastic tumors: a report from the Children's Cancer Group. *Cancer*. 2001 Nov 15;92(10):2699-708. doi: 10.1002/1097-0142(20011115)92:10<2699::aid-cnrcr1624>3.0.co;2-a.

Hamtaux L, Masquelier J, Muccioli GG, Bouzin C, Feron O, Gallez B, Lambert DM. The association of N-palmitoylethanolamine with the FAAH inhibitor URB597 impairs melanoma growth through a supra-additive action. *BMC Cancer*. 2012;12:92. doi: 10.1186/1471-2407-12-92.

Hinz B, Ramer R. Cannabinoids as anticancer drugs: current status of preclinical research. *Br J Cancer*. 2022;127(1):1-13. doi: 10.1038/s41416-022-01727-4.

Jurga M, Jurga A, Jurga K, Kaźmierczak B, Kuśmierczyk K, Chabowski M. Cannabis-Based Phytocannabinoids: Overview, Mechanism of Action, Therapeutic Application, Production, and Affecting Environmental Factors. *Int J Mol Sci*. 2024 Oct 19;25(20):11258. doi: 10.3390/ijms252011258.

Lah TT, Novak M, Pena Almidon MA, Marinelli O, Žvar Baškovič B, Majc B, Mlinar M, Bošnjak R, Breznik B, Zomer R, Nabissi M. Cannabigerol Is a Potential Therapeutic Agent in a Novel Combined Therapy for Glioblastoma. *Cells*. 2021 Feb 5;10(2):340. doi: 10.3390/cells10020340.

Lee WJ, Chen LC, Lin JH, Cheng TC, Kuo CC, Wu CH, Chang HW, Tu SH, Ho YS. Melatonin promotes neuroblastoma cell differentiation by activating hyaluronan synthase 3-induced mitophagy. *Cancer Med*. 2019;8(10):4821-4835. doi: 10.1002/cam4.2389.

Lei L, Xue X, Feng G, Wang X, Jiang Y. Medical ozone treatment for pain, fatigue, anxiety, and

depression in cancer patients: a scoping review. *Front Psychol.* 2025;16:1687754. doi: 10.3389/fpsyg.2025.1687754.

Li Y, Pu R. Ozone Therapy for Breast Cancer: An Integrative Literature Review. *Integr Cancer Ther.* 2024;23:15347354241226667. doi: 10.1177/15347354241226667.

Makimoto A, Fujisaki H, Matsumoto K, Takahashi Y, Cho Y, Morikawa Y, Yuza Y, Tajiri T, Iehara T. Retinoid Therapy for Neuroblastoma: Historical Overview, Regulatory Challenges, and Prospects. *Cancers (Basel).* 2024;16(3):544. doi: 10.3390/cancers16030544.

Malach M, Kovalchuk I, Kovalchuk O. Medical Cannabis in Pediatric Oncology: Friend or Foe? *Pharmaceuticals (Basel).* 2022;15(3):359. doi: 10.3390/ph15030359.

Mammone A, Zuccoli P, Zeppa L, Morelli MB, Fanelli A, Barozza LG, Boschetti CE, Cornacchini N, Nabissi M, Luongo M. Integrative Use of Medical Cannabis, Melatonin and Oxygen-Ozone Therapy in Oral Tongue Carcinoma: A Case Report. *Adv Complement Alt Med.* 2025 8(5). ACAM 000696.2025 doi: 10.31031/ACAM.2025.08.000696

Mammone, Zuccoli P, Giangrossi M, Morelli MB, Fanelli A, Fedi L, Longo A, Pantolini B, Nabissi M, Luongo M. Medical Cannabis, Melatonin and Oxygen-Ozone as Integrative Therapies for the Treatment of Anaplastic Astrocytoma: A Case Report. *Adv Complement Alt Med.* 2025 8(5). ACAM. doi: 10.31031/ACAM.2025.08.000699

Marayati R, Williams AP, Bownes LV, Quinn CH, Stewart JE, Mroczek-Musulman E, Atigadda VR, Beierle EA. Novel retinoic acid derivative induces differentiation and growth arrest in neuroblastoma. *J Pediatr Surg.* 2020;55(6):1072-1080. doi: 10.1016/j.jpedsurg.2020.02.027.

Matthay KK, Villablanca JG, Seeger RC, Stram DO, Harris RE, Ramsay NK, Swift P, Shimada H, Black CT, Brodeur GM, Gerbing RB, Reynolds CP. Treatment of high-risk neuroblastoma with intensive chemotherapy, radiotherapy, autologous bone marrow transplantation, and 13-cis-retinoic acid. Children's Cancer Group. *N Engl J Med.* 1999;341(16):1165-73. doi: 10.1056/NEJM199910143411601.

Pagano E, Venneri T, Lucariello G, Cicia D, Brancaleone V, Nanì MF, Cacciola NA, Capasso R, Izzo AA, Borrelli F, Romano B. Palmitoylethanolamide Reduces Colon Cancer Cell Proliferation and Migration, Influences Tumor Cell Cycle and Exerts In Vivo Chemopreventive Effects. *Cancers*

(Basel). 2021;13(8):1923. doi: 10.3390/cancers13081923.

Park JR, Scott JR, Stewart CF, London WB, Naranjo A, Santana VM, Shaw PJ, Cohn SL, Matthay

KK. Pilot induction regimen incorporating pharmacokinetically guided topotecan for treatment of

newly diagnosed high-risk neuroblastoma: a Children's Oncology Group study. *J Clin Oncol*.

2011;29(33):4351-7. doi: 10.1200/JCO.2010.34.3293.

Pourhanifeh MH, Kamali M, Mehrzadi S, Hosseinzadeh A. Melatonin and neuroblastoma: a novel

therapeutic approach. *Mol Biol Rep*. 2021;48(5):4659-4665. doi: 10.1007/s11033-021-06439-1.

Qiu QQ, Zhang N, Xu YY, Qin JW, Yu GF, Song XR. Melatonin induces autophagy in neuroblastoma

by alleviating Pak2 mediated endoplasmic reticulum stress. *Mol Med Rep*. 2026;33(2):74. doi:

10.3892/mmr.2025.13784.

Rowen RJ. Ozone Therapy – An Unmatched Approach for Near Universal Prevention and Treatment.

Medical Research Archives. 2025;13(6). doi: <https://doi.org/10.18103/mra.v13i6.6523>.

Sawada T. Measurement of urinary vanillylmandelic acid (VMA) and homovanillic acid (HVA) for

diagnosis of neural crest tumors. *Pediatr. Hematol. Oncol*. 1997;14:291–293. doi:

10.3109/08880019709041587.

Shah D, Shah D, Mohamed Y, Rosas D, Moffitt A, Haynes, TH, Cortes F, Bell Neasman T, Kathari

PK, Villagran A, Zeine R. Anti-Tumor Effects of Cannabinoids in Brain, Lung, Breast, Colorectal,

Pancreatic and Prostate Cancers; A Systematic Review of the Literature. *Medical Cannabis and the*

Effects of Cannabinoids on Fighting Cancer, Multiple Sclerosis, Epilepsy, Parkinsons and Other

Neurodegenerative Diseases. 2023 Zeine R. & Teasdale, B. (Eds). doi: 10.4018/978-1-6684-5652-1.

Sharma T, Jana S. Boswellic acids as natural anticancer medicine: Precious gift to humankind. *Journal*

of Herbal Medicine 2020;20:100313. doi.org/10.1016/j.hermed.2019.100313.

Shimada H, Ambros IM, Dehner LP, Hata J, Joshi VV, Roald B, Stram DO, Gerbing RB, Lukens JN,

Matthay KK, Castleberry RP. The International Neuroblastoma Pathology Classification (the

Shimada system). *Cancer*. 1999;86(2):364-72.

Smith V, Foster J. High-Risk Neuroblastoma Treatment Review. *Children (Basel)*. 2018;5(9):114. doi:

10.3390/children5090114.

Tolbert VP, Matthay KK. Neuroblastoma: clinical and biological approach to risk stratification and

treatment. *Cell Tissue Res.* 2018;372(2):195-209. doi: 10.1007/s00441-018-2821-2.

Twelves C, Sabel M, Checketts D, Miller S, Tayo B, Jove M, Brazil L, Short SC; GWCA1208 study group. A phase 1b randomised, placebo-controlled trial of nabiximols cannabinoid oromucosal spray with temozolomide in patients with recurrent glioblastoma. *Br J Cancer.* 2021 Apr;124(8):1379-1387. doi: 10.1038/s41416-021-01259-3.

Yadwad AR, Babbur , Korikani M, Puligundla KC, Amanchi NR, Kancha RK. Neuroblastoma. In: Kancha, R.K. (eds) *Biomedical Aspects of Solid Cancers.* Springer, Singapore. 2024. https://doi.org/10.1007/978-981-97-1802-3_26

Yan P, Qi F, Bian L, Xu Y, Zhou J, Hu J, Ren L, Li M, Tang W. Comparison of Incidence and Outcomes of Neuroblastoma in Children, Adolescents, and Adults in the United States: A Surveillance, Epidemiology, and End Results (SEER) Program Population Study. *Med Sci Monit.* 2020;26:e927218. doi: 10.12659/MSM.927218.

Yokota K, Uchida H, Sakairi M, Abe M, Tanaka Y, Tainaka T, Shirota C, Sumida W, Oshima K, Makita S, Amano H, Hinoki A. Identification of novel neuroblastoma biomarkers in urine samples. *Sci Rep.* 2021;11(1):4055. doi: 10.1038/s41598-021-83619-w.

Zeppa L, Aguzzi C, Morelli MB, Marinelli O, Giangrossi M, Luongo M, Amantini C, Santoni G, Nabissi M. Cannabigerol Induces Autophagic Cell Death by Inhibiting EGFR-RAS Pathways in Human Pancreatic Ductal Adenocarcinoma Cell Lines. *Int J Mol Sci.* 2024 Feb 7;25(4):2001. doi: 10.3390/ijms25042001.

Zeppa L, Aguzzi C, Morelli MB, Marinelli O, Amantini C, Giangrossi M, Santoni G, Fanelli A, Luongo M, Nabissi M. In Vitro and In Vivo Effects of Melatonin-Containing Combinations in Human Pancreatic Ductal Adenocarcinoma. *J Pineal Res.* 2024;76(5):e12997. doi: 10.1111/jpi.12997. PMID: 39076059.

NIH. Neuroblastoma Treatment (PDQ®)—Health Professional Version. https://www.cancer.gov/types/neuroblastoma/hp/neuroblastoma-treatment-pdq#_534_toc