Complete objective response of neuroblastoma to biological treatment

Giuseppe Di Bella, Biagio Colori

Fondazione Di Bella, Bologna, Italy
"Rizzoli" Institute, Scientific Research and Care Institute, Bologna, Italy;

Correspondence to: Giuseppe Di Bella, MD.,
Fondazione Di Bella
Via Marconi N°51, Post code 40122, Bologna, Italy.
PHONE: 051 239662; 051 230369; E-MAIL: posta@giuseppedibella.it

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Abstract

OBJECTIVES: The combined use (MDB) of Somatostatin, Melatonin, Retinoids, Vitamins E, C, and D_3, with Calcium, Chondroitin sulfate, and microdoses of Cyclophosphamide in a seven-month old baby affected by a voluminous retroperitoneal neuroblastoma measuring 4 × 8 cm produced a 50% objective response in six months, an almost total response in one year and a complete response at 14 months, with cure and absence of disease for over ten years.

RESULTS: This paper discusses the rationale and the molecular mechanisms of action of the treatment which has a differentiating, apoptotic and antiproliferative effect, preserving and enhancing both the trophism and functionality of organs and tissues, and the neuroimmunoendocrine and antiblastic homeostasis. This result is in agreement with the positive results already published on the use of the MDB in lymphoproliferative diseases, in stage III and IV lung cancer, in breast cancer and in cancers of the upper aerodigestive epithelia. Without the need for hospitalisation and with no toxicity, the MDB rapidly reduced and then eliminated the voluminous tumoral mass, allowing a normal quality of life and a perfectly normal psychophysical development.

CONCLUSIONS: We believe it is of use to report this case in order to invite greater interest in the oncological possibilities offered by the immunoneuroendocrine and biological-receptorial properties of the MDB treatment.

Abbreviations :

ATRA	- All Trans Retinoic Acid
BDNF	- Brain Derived Neurotrophic Factor
CCK	- Cholecystokinin
MDB	- Di Bella Method
EGF	- Epidermal Growth Factor
EGFR	- Epidermal Growth Factor Receptor
FGF	- Fibroblastic Growth Factor
GH	- Growth Hormone
GHR	- Growth Hormone Receptor
HGF	- Hepatocyte Growth Factor
IGF 1-2	- Insulin-like Growth Factor 1-2
IGFR	- Insulin-like Growth Factor Receptor
INSS	- International Neuroblastoma Staging System
MRI	- Magnetic Resonance Imaging
MLT	- Melatonin
NBL	- Neuroblastoma
NGF	- Nerve Growth Factor
NT	- Neurotrophin
NHL	- Non-Hodgkin's Lymphoma
PDGF	- Platelet-Derived Growth Factor
PET	- Positron Emission Tomography
RC	- Complete objective response
SST	- Somatostatin
SSTR	- Somatostatin Receptor
TRK	- Tyrosine-kinase
TGF	- Transforming Growth Factor
VEGF	- Vascular Endothelial Growth Factor
VIP	- Vasoactive Intestinal Peptide

CASE REPORT

INTRODUCTION

We present a case of complete remission with biological therapy (MDB) of an unoperable voluminous retroperitoneal neuroblastoma (NBL) in a seven-month old baby. The case has been monitored since 1998. We report the components of the biological therapy (MDB), together with the results of the blood tests and instrumental examinations performed before and after the treatment.

A brief summary is provided of the rationale of the MDB, the scientific bases, the molecular mechanisms of action and the clinical response, with the patient in complete remission for over 10 years.

BRIEF NOTES ON NEUROBLASTOMA

NBL originates from cells of the neural crest, affecting the nerve cells of the sympathetic ganglia and is considered to be hereditary.

It is the most common solid extracranial tumour in children, representing 6–10% of all tumours in infancy with an incidence of 7–13 new cases annually/1,000,000 (children < 15 years).

Neuroblastoma has an extremely variable clinical presentation and biological behaviour. The risk evaluation system drawn up by the *Children's Oncology Group* considers not only histological and histochemical data but also the age of the patient, the volume of the tumour mass and the stage according to INSS criteria (*International Neuroblastoma Staging System):*

Stage 1	Unilateral or midline tumour confined to the area of origin, removed completely with negative or metastatic lymph nodes removed together with the tumour.
Stage 2A	Unilateral or midline tumour, mostly removed but not radically and with negative lymph nodes
Stage 2B	Unilateral or midline tumour, radically removed but with positive lymph nodes
Stage 3	Tumour crossing the midline, infiltrating the surrounding tissues ± regional lymph nodes Tumour that does not cross the midline but with positive contralateral lymph node involvement Midline tumour which cannot be removed with a sufficiently wide margin due to infiltration of the surrounding tissues
Stage 4	Dissemination of the tumour to distant lymph nodes, skeleton, bone marrow, liver, skin and/or other organs
Stage 4S	Localised tumour as for Stages 1 and 2, with dissemination limited to the skin, liver and/or bone marrow (less than 10% infiltration) at age less than 1 year old

NBL is a highly aggressive tumour, with a few exceptions regarding cases which fall within stage 4S of the INSS classification, in which a progressive differentiation up to spontaneous remission may occur. Except for the rare INSS stage 4 cases, a neuroblastoma generally tends, as in the case presented here, to infiltrate the adjacent structures and organs, rapidly spreading to the nearby lymph nodes. As in this case, a single irregular mass with undefined borders is found at diagnosis, consisting of the primary mass and metastatic lymph nodes.

Neuroblastoma often produces catecholamines (this is why it is called a secreting tumour), causing high levels in the urine and blood, useful for diagnosis and monitoring.

In several cases, hypertension and tachycardia occur, although not always together with the increase in catecholamines and the other catabolites of the sympathetic system.

Neuroblastoma can affect the adrenal gland (presenting as an abdominal tumour) or the paraspinal sympathetic ganglia, and can also appear in any part of the vertebral column (abdominal and thoracic). Frequent signs of the disease include fever, anemia, loss of appetite, and bruising around the eyes. Metastases, which are frequent and appear early, affect the bones, the orbital region, the lymph nodes, the liver and bone marrow. Early diagnosis is fundamental as surgical intervention in the early stages increases survival; it is therefore necessary to test for the urinary metabolites of catecholamine (vanillylmandelic acid, homovanillic acid) and neuroendocrine markers (NSE). Ferritin and lactate dehydrogenase (LDH) are also parameters which should be evaluated when NBL is suspected.

The extent of the disease should be assessed with imaging procedures: CT, MRI, PET, and urography. In most cases, neuroblastoma presents with metastases when diagnosed. Cytogenetic analyses in neuroblastoma show a series of breakpoints of chromosome 1p from 1 P22 to 1 P 36. Chromosome 1 P allele deletions and loss are present not only in NBL, but in a wide range of solid and hematological tumours. The distal region of the short arm of chromosome 1 contains one or more genes implicated in the development of tumours**.**

CLINICAL CASE

Neuroblastoma (classification: $T_4N_2M_x$): INSS stage 3

Patient details: Year of birth: 1998 – Sex: M

CASE HISTORY − Born in May 1998 with normal delivery, the child began to suffer continuous problems with his digestive system in November of the same year. As these problems gradually became worse, he was admitted to hospital in Avola, in Sicily, where ultrasound and CT scans, performed on 17/12/98, revealed a voluminous solid tumour in the left mid-lateral retroperitoneal area (4×8 cm) classified as a NBL. According to NBL

classification criteria (International Neuroblastoma Staging System) the case in question was clearly stage 3 (unoperable tumours infiltrating the midline with or without regional lymph node involvement).

INITIAL ROUTINE TESTS

Abdominal CT (17/12/98) – **Report** – "*CT of the upper and lower abdomen, performed in basal conditions, revealed the presence of a large solid formation in the left mid-lateral retroperitoneal area with a maximum width of around* ***4 cm. and a maximum length of around 8 cm****. The structure of this formation is not uniform, and presents numerous calcifications; it has displaced the intestinal loops forward and the aorta towards the right, and is in close contact with the front margin of the spine. The CT appearance of this formation is compatible with neuroblastoma …*"

After the CT scan, the child was transferred to the "Gaslini" Children's Hospital in Genoa (Scientific Research and Care Institute), where MRI of the upper and lower abdomen and urography under general anesthesia were carried out on 24/12/98.

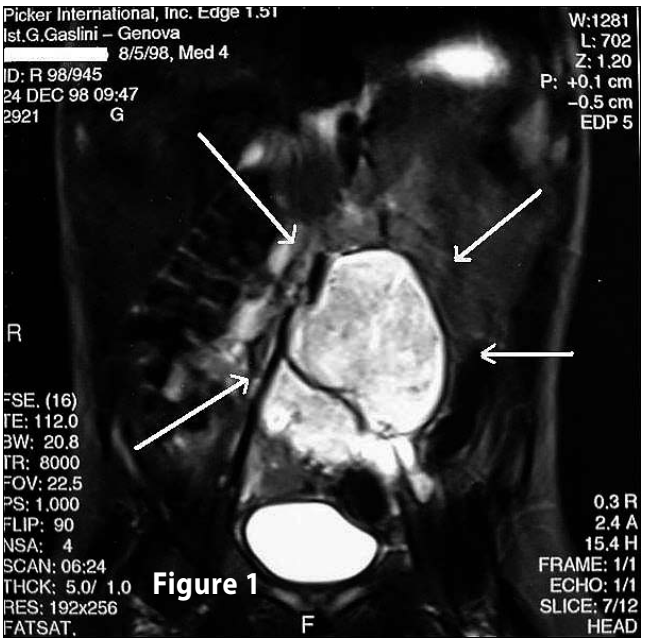


Figure 1

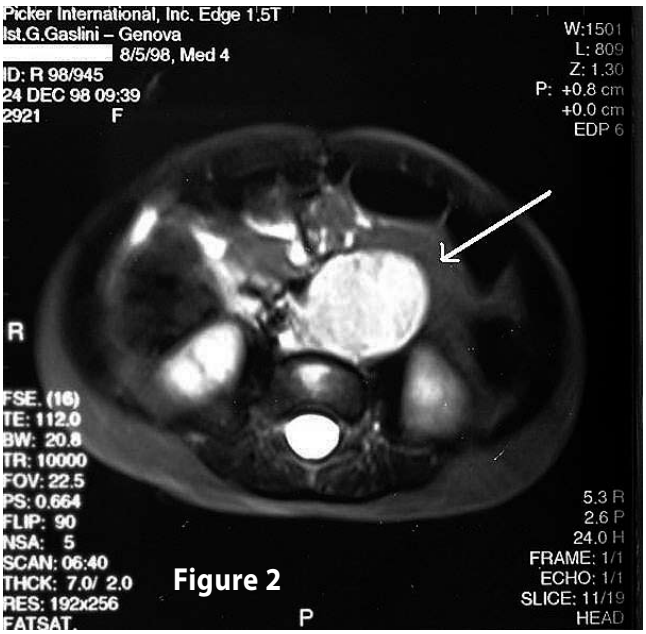


Figure 2

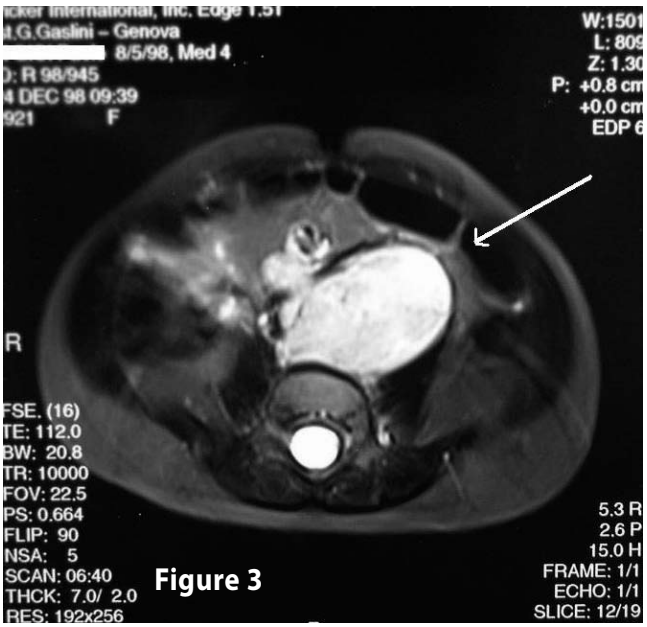


Figure 3

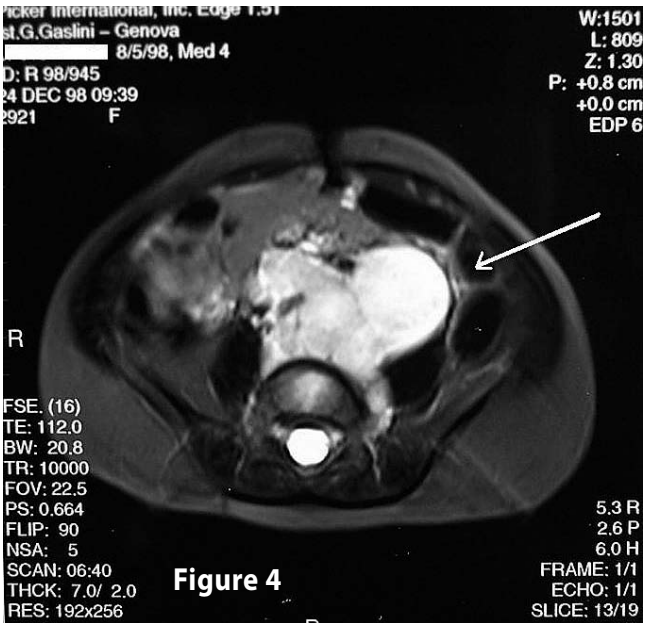


Figure 4

Figures 1–4. MRI (1998, Dec. 24): Confirmed presence of a large expansive process, extending caudally from the renal vascular pedicle to beyond the aorta-iliac division.

MRI (24/12/1998) – **Report**: "*No secondary lesions evident in the liver. No lymphoadenomegaly close to the aorta or vena cava. Normal kidneys and spleen. Confirmed presence of a large expansive process, extending caudally from the renal vascular pedicle to beyond the aorta-iliac division. The mass is midline, extending mainly towards the left (crossing the midline), with displacement of the homolateral psoas. The structure is solid without evidence of necrotic-colliquative phenomena. The signal is clearly hyper-intense in T2 with a hypo-intense diffuse punctiform appearance due to the presence of calcifications. The distal aorta and vena cava, the bifurcation and the iliac veins and arteries (more to the left) are marginally incorporated and displaced towards the right. No liquid in the peritoneum.*"

Urography (28/12/1998) – **Report: "***Prompt elimination and regular concentration of the iodate contrast medium by both renal excretors. On the right, regular calyx-pyelic and ureteral morphology. On the left, normal calyx-pyelic morphology; the ureter presents a normal calibre but an altered course: in the anterior-posterior projection the proximal tract appears to be outwardly displaced, while in the latero-lateral projection the intermediate tract appears to be pushed forward, probably displaced by the retroperitoneal mass.*"

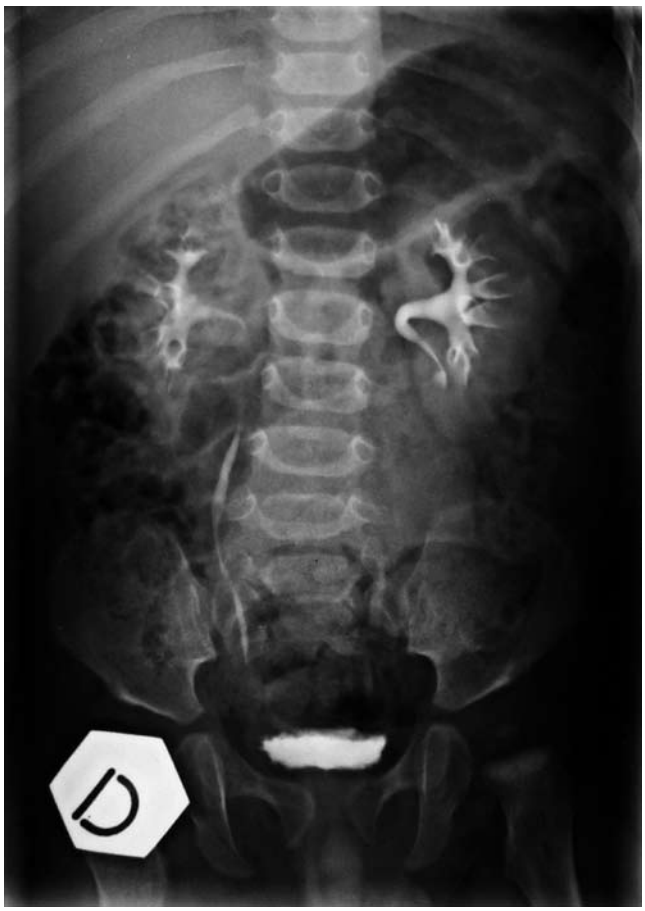


Figure 5. Uroghaphy (1998, Dec.28): The ureter presents a normal calibre but an altered course: in the anterior-posterior projection the proximal tract appears to be outwardly displaced, while in the latero-lateral projection the intermediate tract appears to be pushed forward, probably displaced by the retroperitoneal mass.

The diagnosis of neuroblastoma made at the hospital in Sicily was confirmed at the "Gaslini" Institute.

The parents were informed of the unacceptable risk of surgery, not only because of the child's age but also due to the volume and the marked vascularisation of the tumoral mass, and above all for the difficulty in identifying cleavage planes with the distal aorta and the vena cava, the bifurcation and the iliac veins and arteries, all incorporated by the tumoral mass and displaced towards the left.

The particularly significant volume of the abdominal-pelvic tumour (8 cm × 4 in a 7-month-old baby) with displacement of the ureter and compression of the intestinal loops was causing gastrointestinal problems, colic and vomiting. Chemotherapy was proposed. An urgent solution was necessary due to the persistence of the gastrointestinal symptoms, the deterioration of the child's condition and the rapid progression of the tumour. The parents refused chemotherapy and, in January 1999, the child was therefore discharged from the "Gaslini" hospital with a report to the Pediatric Clinic in Catania, specifying that: "the suspicion of *Neuroblastoma was confirmed by the tests relative to the urinary excretion of sympathetic amines:*

PATHOLOGICAL HEMATO-CHEMICAL VALUES

Vanillylmandelic acid: ***35.6 gamma/mg – creatine***
Homovanillic acid: ***79.7 gamma/mg – creatine***
*NSE****: 30*** *– LDH****: 603***
Hemochrome: *Hb* ***12.9*** *– WBC* ***: 15900*** *(with normal leukocyte formula)*
*Platelets****: 474,000***
Abdominal MRI: *large expansive process below the renal vascular pedicle, mainly on the left*
Intravenous urography: *altered course of the left ureter*

RESULTS

Therapy and clinical course

The parents decided to refuse chemotherapy and to try the MDB.

The treatment prescribed by Prof. Luigi Di Bella and started immediately after the child was discharged from hospital is described below:

1) ***Solution of:***

- **All-Trans-Retinoic Acid**	0.5 g
- **Palmitate axerophthol**	0.5 g
- **Beta carotene**	2 g
- **Alpha-tocopherol-acetate**	1000 g

one 2 ml teaspoonful of Solution every 3 hours together with:

2) ***Dihydrotachysterol*** (one drop in the teaspoon for each administration)
3) ***Bromocriptine*** 2.5 mg tablets (*1/4* of a tablet twice a day, morning and evening)
4) ***Melatonin 12%, Adenosine 51%, Glycine 37%*** (one 5 mg vial diluted in water)
5) ***Chondroitin sulfate*** (one 500 mg vial orally with the evening feed/meal)
6) ***Cyclophosphamide*** 50 mg tablets (*1/4* of a tablet with the main feed/meal)
7) ***Somatostatin*** – 14 amino acids (one 0.25 mg vial injected slowly subcutaneously in the evening).

The treatment was tolerated and blood tests were scheduled every 2 weeks.

The general conditions of the child improved, with a gradual reduction of the colic attacks, the diarrhea, vomiting and anorexia, and an increase in weight. By the end of 1999, the child's weight, height and psychophysical development were within the physiological range. The gradual remission of the symptoms was accompanied by a progressive return to normal of the hematochemical values, with a reduction of the leukocytes, ESR, LDH, Ferritin, catecholamines, NSE, vanillylmandelic acid and homovanillic acid. For the first few months, hemochrome was tested every 2 weeks. Complete hematochemical tests were carried out every 2–3 months since, in addition to a high diagnostic value, these are also useful for monitoring remission and to detect recurrences at an early stage, before any clinical signs become evident. The improvement of the hematochemical values was accompanied by a marked and continuous reduction in volume of the tumoral mass detected by imaging procedures. Forty days after starting the MDB, an abdominal ultrasound scan on 12/02/99 showed a reduction of the tumour, reporting: "*…In the left ilio-pelvic area, close to the vertebral plane, a slightly irregular, egg-shaped neoformation with fairly well-defined borders can be seen. It measures 70 mm (longitudinal) (on 17/12/08 80 mm) × 40 mm …It has a solid and non-uniform structure due to the presence of countless isolated groups of microcalcification echo reflections. Color-doppler, hindered by the movements of the child, showed some intralesional vessels with a low resistance index. Nothing of note regarding the kidneys or the organs in the upper abdomen...*"

The treatment was revised by Prof. Di Bella on 12/03/1999, altering the daily dose of Somatostatin from 0.25 mg/day to 0.5 mg on alternate days for 3 times a week, continuing with 0.25 mg on the other 4 days of the week, and leaving the rest of the treatment unchanged.

Six months after starting the therapy, an MRI on 18/06/1999 showed a regression of the tumour of around 50%.

MRI (18/6/1999**) – Report:** "*…The procedure was carried out with the turbo-spin-echo technique, along the axial and coronal planes, with inversion-recovery sequences and T2-weighted scans, after i.v. injection of gadolinium. Compared with the previous scan on 24/12/98, there is a notable reduction of the retroperitoneal tumour in the left para-aortic and paravertebral region, now measuring 5 cm in length and 2 cm in width. The liver, spleen, pancreas, kidneys and adrenal glands are not affected.*"

At the same time, the values of ESR, LDH, Ferritin, NSE, urinary excretion of Vanillylmandelic acid, Homovanillic acid and Catecholamines had returned to within the normal physiological range.

After one year of treatment, an MRI, carried out on 21/01/2000 in Catania, showed the almost total disappearance of the Neuroblastoma.

MRI (21/1/2000**) – Report**: "*…The MRI was carried out with the spin-echo technique, along the axial and coronal planes, with inversion recovery sequences and T2-weighted scans, after i.v. injection of gadolinium. Compared with the previous scan on 18/06/99, the retroperitoneal neoformation previously visible in the left para-aortic and paravertebral region has almost totally disappeared, now represented by a small residue in the para-aortic region. The other findings are as before.*"

In September 2000 (child's age: 28 months) Prof. Di Bella revised the treatment as follows:

1) ***Solution of:***

- **All-Trans-Retinoic Acid**	0.5 g
- **Palmitate axerophthol**	0.5 g
- **Beta carotene**	2 g
- **Alpha-tocopherol-acetate**	1000 g

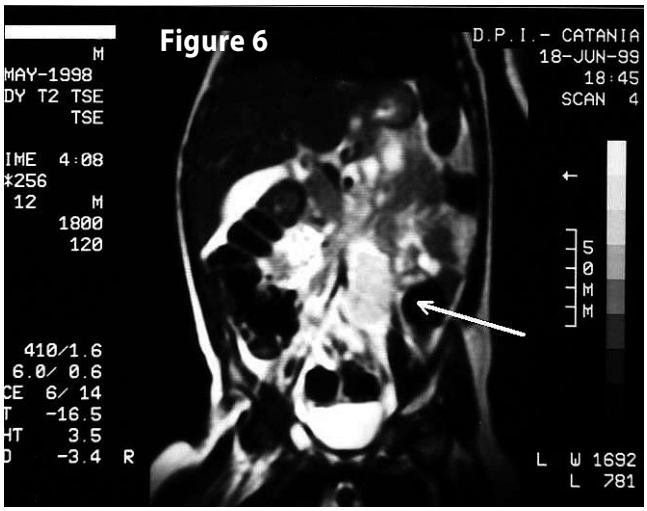
One 2.5 ml teaspoonful of Solution three times a day, morning – mid-day – evening (at least 15 minutes before meals) together with:

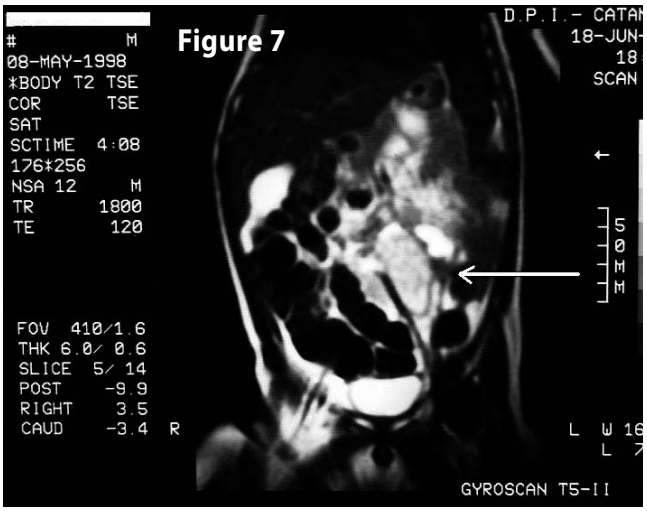
2) ***Dihydrotachysterol*** (4 drops in the teaspoon for each administration)
and the following products orally during the meal:
3) ***Bromocriptine*** 2.5 mg tablets (*1/2* of a tablet, twice a day)
4) ***Melatonin 12%, Adenosine 51%, Glycine 37%*** (2 mg tablets – 2 tablets in the morning and at mid-day, 4 tablets before bed)
5) ***Somatostatin*** – 14 amino acids (one 0.75 mg vial injected slowly subcutaneously in the evening).

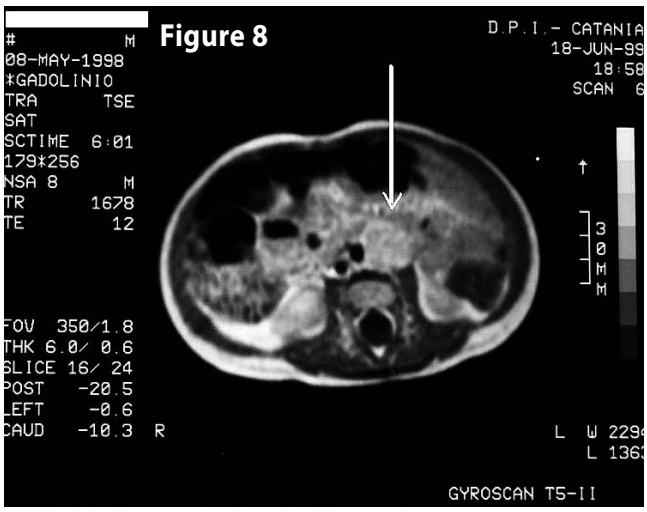
From 2001 the dose of Somatostatin was increased to 1 mg every evening, leaving the rest unchanged.

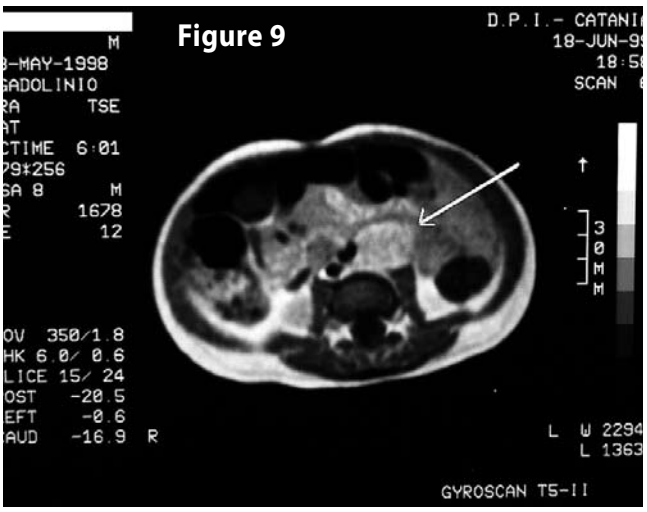
In March 2001 an MRI with contrast medium showed "*the total absence of tumoral traces.*"

The treatment was further reduced by completely eliminating Cyclophosphamide, Bromocriptine, Chondroitin sulfate, and Calcium. The dose of Somatostatin was reduced to 1 mg subcutaneously on alternate days 3 times a week. From 2002 the administration of









Figures 6–9. MRI (1999, Jun. 18): Compared with the previous scan on 24/12/98, there is a notable reduction of the retroperitoneal tumour in the left para-aortic and paravertebral region, now measuring 5 cm in length and 2 cm in width.

Somatostatin was limited to 1 mg a week, from 2003 1 mg every 15 days, and then completely discontinued in 2007, while at the time of writing the administration continues of retinoids with 8 drops of Vit.D_3 (Dihydrotachysterol) every morning before eating, and four 2 mg tablets of MLT after the evening meal.

Since 2001 all the subsequent blood tests and imaging procedures (PET, MRI) have confirmed the absence of disease with complete remission of the NBL.

PET (07/10/2003) – **Report**: "*Absence of areas of pathological accumulation of the metabolic trace agent … The PET scan does not show any alterations that can be attributed to the presence of surrogate disease with high metabolic activity*."

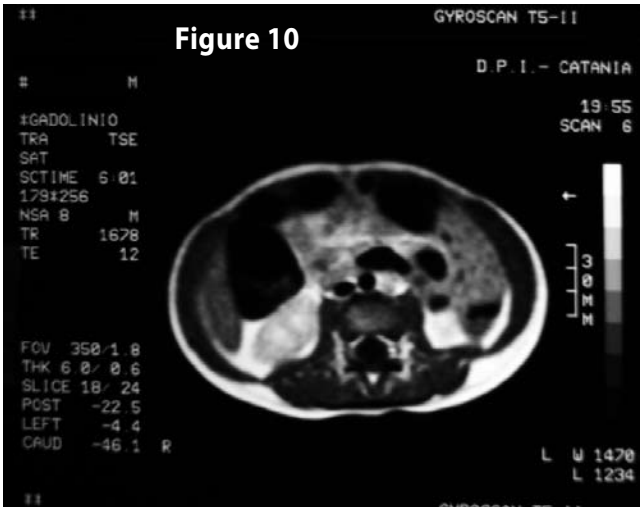
PET (20/09/2004) – **Report: "** *No evidence of pathological accumulation of the metabolic trace agent … The PET scan does not show any alterations that can be attributed to the presence of surrogate disease with high metabolic activity*" (Figure 7)

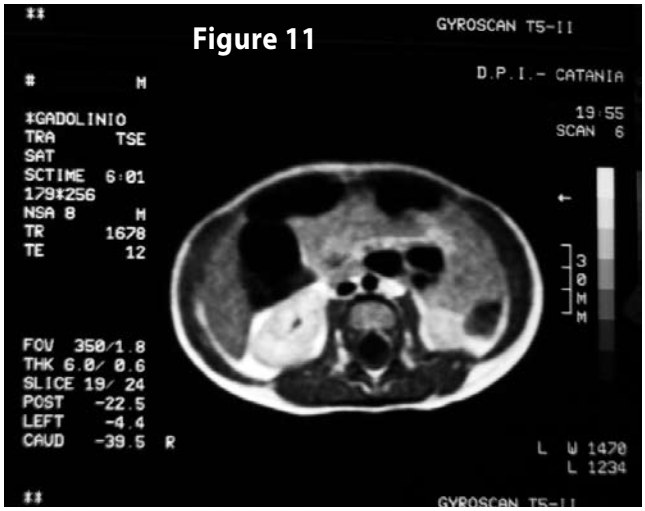
MRI (06/10/2005) – **Report:** " … *Complete macroscopic regression of the expansive tumoral lesion localised in the abdominal-pelvic retroperitoneal area and incorporating the left iliac vessels …*"(Figure 8)

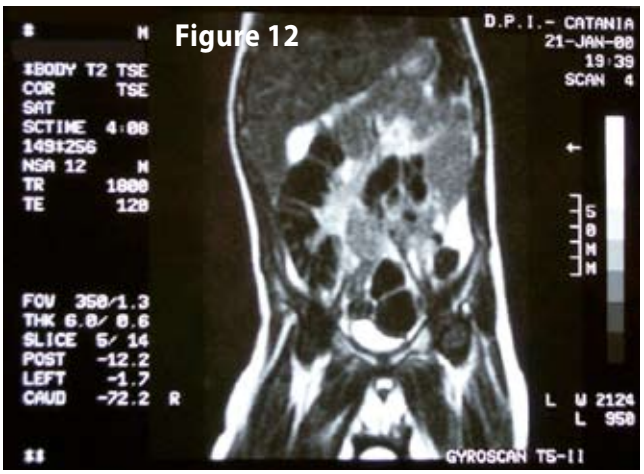
PET (17/3/2008) – **Report:** "*Today's follow-up PET scan, compared with the previous scans in 2003 and 2004, does not show any evidence of pathological accumulation of the metabolic trace agent in the explored area. In particular, nothing to report in the abdomen…*"…"

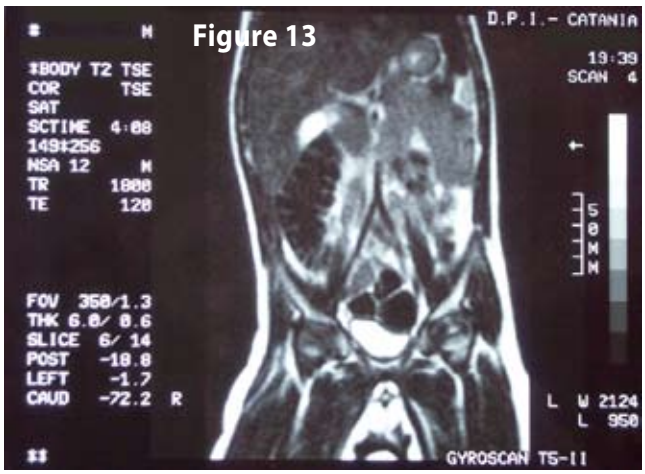
DISCUSSION

The biological neuro-immuno-endocrine therapy prescribed by Prof. Luigi Di Bella (MDB) achieved a complete objective response in fourteen months, with no toxicity. by means of a receptorial, differentiating, apoptotic and antiproliferative mechanism of action, with criteria, methods and mechanisms of action totally different to the usual cytotoxic and cytolytic treatments, proving that it can replace surgery, and radiotherapy









Figures 10–13. MRI (2000, Gen. 21): The retroperitoneal neoformation previously visible in the left para-aortic and paravertebral region has almost totally disappeared, now represented by a small residue in the para-aortic region.

and/or chemotherapy which are in any case unable to provide results that are comparable to surgery but which may precede or follow it.

Rationale of the Therapy

The ubiquitary receptorial expression of Prolactin and GH (De Souza *et al*., l974; Hooghe *et al*., 1998; Tada *et al*. 1999; Ben-Jonathan *et al*., 2002) represents one of the aspects of the direct and generalised mitogenic role of these molecules. The use of SST and/or Octreotide in all tumoral diseases is widely justified by the negative regulation of GH, a hormone with a high mitogenic potential, GH-correlated mitogenic growth factors and neoplastic angiogenesis. This justifies the use of somatostatin and its analogs in all tumors (Di Bella *et al*., 1979; Manni *et al*., 1989; Klijn *et al*., 1996; Pollak *et al*., 1997; Pawlikowski *et al*., 1998; Lachowicz-Ochedalska *et al*., 2000; Friend *et al*., 2000; Schally *et al*. 2001; Schally *et al*., 2003; Massa al, 2004; Arena *et al*., 2007; Guillermet-Guibert *et al*., 2007; Lee *et al*., 2008) with particular efficacy in neuroendocrine tumours such as NBL, characterised by a high receptorial expression for somatostatin (Moertel *et al*., 1994; Sestini *et al*., 1996; Kogner *et al*., 1997; Briganti *et al*., 1997; Borgström, 1999; Friend *et al*., 2000; Steták *et al*., 2001; Orlando *et al*. 2001; Florio *et al*., 2008). SSTRs have also been identified in a wide range of non-neuroendocrine tumours (Schaer *et al*., 1997; Van Eijck *et al*., 1998; Held-Feindt *et al*., 1999; Mishima *et al*., 1999; Pinzani *et al*., 2001; Watson *et al*., 2001; Barnett *et al*., 2003). A causal relationship has been demonstrated between GH (of which SST is the biological antidote) and tumoral induction and progression (Zeitler *et al*., 2000), by histochemically detecting markedly higher concentrations of GHR in tumoral tissue with respect to healthy tissue. The powerful mitogenic role of GH, with a proliferative index and speed of progression of the neoplastic population directly proportional to the receptorial expression of GH, is therefore known and documented H (Lincoln *et al*., 1998). The loss of differentiation and the

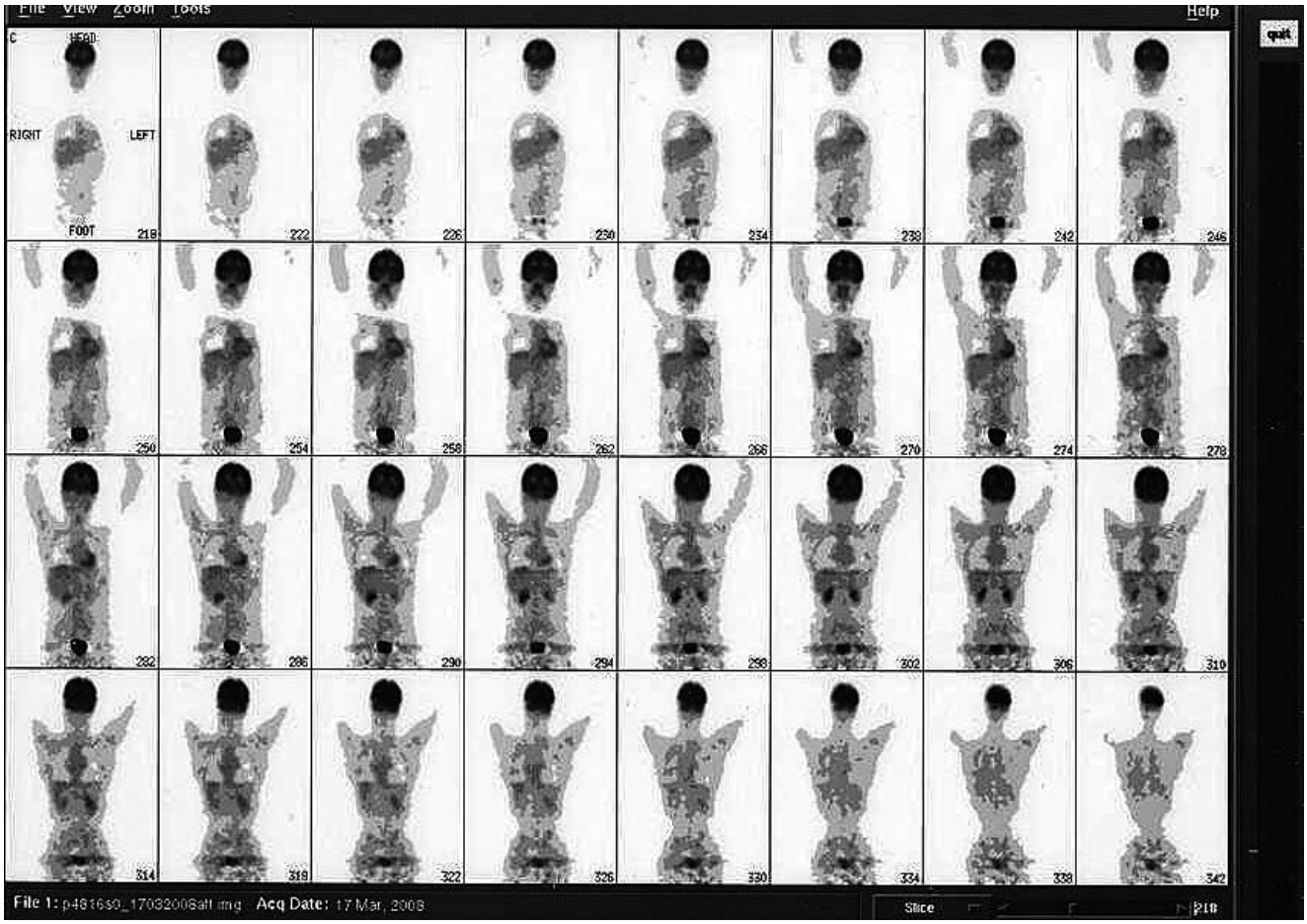


Figure 14. PET (2008, Mar. 17): Does not show any evidence of pathological accumulation of the metabolic trace agent in the explored area. In particular, nothing to report in the abdomen.

uncontrolled proliferation, albeit to different extents, are common denominators of all tumours. Through a differentiating and antiproliferative factorial synergic mechanism, the other components of the MDB reinforce the antitumoral activity of SST.

Interaction of the MDB with the cytogenic and molecular aspects of NBL

In NBL the loss of heterozygotes 1 P (1P LOH) is associated with amplification of the oncogene N- MIC, which is not ascertained in only 3% of cases. The inhibition of various oncogenes, including MIC by some of the components of the MDB, has been documented (Degli Uberti *et al*., 1991; Peverali *et al*., 1996; Sun *et al*., 2002; Gumireddy *et al*., 2003; Durand *et al*., 2008; Aktas *et al*., 2009). The chromosome damage of NBL leads to varying degrees of inactivation of oncosuppresor genes: CD 44, Bcl-2, P53, as well as Caspase 3–8, key elements of the apoptotic cascade. Oncosuppressor inactivation occurs simultaneously with the amplification of oncogenes such as the N-myc gene and the proto oncogene TRK, considered one of the cytogenetic causes of neuroblastoma. The negative regulation of the oncosuppressors is also antagonised by the components of the MDB, such as retinoic acid which inhibits the inactivation of caspase (Piedrafita 1997; Takada *et al*. 2001; Jiang *et al*. 2008) and MLT which protects P53 and Bcl-2 from degradation (Mediavilla *et al*. 1999). One of the pathogenetic factors of NBL is the altered response of the neuroblasts to the differentiating stimulation, effectively countered by the retinoids which induce neuronal differentiation (Hassan *et al*. 1990; Giannini *et al*. 1997; Peverali *et al*. 1998; Voigt *et al*. 2000, Ueda *et al*., 2001; Kulikov *et al*. 2007; Beijersbergen *et al*. 2009; Wu *et al*. 2009; Witzigmann *et al*. 2008). The differentiation is synergically reinforced by other components of the MDB, such as MLT (Cos *et al*. 1996; Garcia-Santos *et al*. 2006; McMillan *et al*. 2007), Vit D3 (Lange *et al*. 2007), Vit E (Turley *et al*. 1995; Swettenham *et al*. 2005), Vit C (Carosio *et al*., 2007) and Chondroitin sulfate (Batra *et al*., 1997; Pumphrey *et al*., 2002).

In NBL there is an alteration of the NGF-TRK receptor-ligand system, consisting of the interaction between families of transmembrane receptors and tyrosine-kinase A-B-C activity, and ligands consisting of trophism and central and peripheral nervous growth systems, represented by nerve growth factor (NGF), neurotrophines (NT3-4-5), and Brain Derived Neurotrophic Factor

(BDNF). Components of the MDB such as STT and retinoids (Giannini *et al*., 1997; Witzigmann *et al*. 2008; Liang *et al*. 2009) antagonise the proliferative stimulus of these molecules. The binding of NGF, NT and BDNF to TRK receptors induces the transactivation and phosphorylation of tyrosine residues with the start of a cascade of intracellular events aimed at cellular proliferation. The neuroblastoma cells contain various levels of expression of tyrosine kinase receptors. The Protein-Tyrosine kinase activity is effectively inhibited by SST and its analogs (Reardon 1996 *et al*.; Pawlikowski *et al*. 1998; Lachowicz-Ochedalska *et al*. 2000; Cattaneo *et al*. 2000; Florio *et al*. 2001; Massa *et al*. 2004; Lee *et al*. 2008; Florio *et al*. 2007). The molecular equivalent of the clinical-prognostic aspects is regulated in the most aggressive NBLs by the reduced or non-expression of TRK –A, the increased expression of TRK-B and amplification of N- Myc negatively regulated by SST (Degli Uberti *et al*. 1991; Sun 2002 *et al*.; Durand *et al*. 2008). In NBLs with the worst prognosis there is little or no expression of CD44 and TRK-A, present due to MRP, together with high telomerasic activity. On the contrary, the decrease of TRK-B and N- Myc, together with the increased expression of TRK-A, is connected with a favourable prognosis, as is a high expression of somatostatin receptors (always present in NBLs).

Antiblastic molecular mechanisms of action of MDB

Cellular proliferation is strictly dependent on prolactin, GH, the main growth factor, and on GH-dependent mitogenic molecules, positively regulated by GH, such as EGF, FGF, HGF, IGF1-2, NGF, PDGF, VEGF and TGF, as well as by growth factors produced by the digestive tract, such as Gastrin, VIP, and CCK, also negatively regulated by SST (Kath *et al*., 2000). Both physiological and neoplastic cellular proliferation are triggered by these molecules, which are used to a much greater extent by the tumour cells than by healthy cells. Biological antidotes of GH, Somatostatin and its analogs, do not merely reduce the expression and transcription of highly mitogenic growth factors, such as IGF1-2 (Sall *et al*., 2004), EGF (Szepesházi *et al*., 1999) and FGF (Held-Feindt *et al*., 1999), but extend their negative regulation to the respective receptors with evident antiproliferative (Mishima *et al*. 1999) and antiangiogenic effects (Barrie *et al*., 1993; Albini *et al*. 1999; Vidal *et al*. 2000; Watson *et al*., 2001;. Bocci *et al*. 2007).

It is known that the GH-IGF1 axis has a determining influence on neoplastic biological development (Murray *et al*. 2004). The IGFRs respond mitogenically to IGF. The suppressive effect of SST and its analogs on serum levels of IGF1 is both direct, through inhibition of the IGF gene, and indirect, through the suppression of GH and thus of its hepatic induction of IGF1 (Sall *et al*. 2004).

It has been widely documented that neoplastic progression is strictly dependent on angiogenesis which represents an obligatory and essential phase. Somatostatin and its analogs negatively regulate all stages of angiogenesis, such as the cascade of monocytes (Wiedermann *et al*., 1993), interleukin 8, Prostaglandin E 2, and endothelial Nitric oxide synthase (e-Nos) (Florio *et al*., 2003), as well as growth factors whose synergy is essential for angiogenesis, such as VEGF (Cascinu *et al*., 2001; Mentlein *et al*., 2001) TGF, IGF1 (Murray *et al*., 2004; Hagemeister *et al*., 2008) FGF, HGF (Jia *et al*., 2003; Hagemeister *et al*. 2008), PDGF (Cattaneo *et al*., 1999), and EGF (Mishima *et al*., 1999; Szepesházi *et al*., 1999; Held-Feindt *et al*., 1999). The inhibition of angiogenesis induced by SST is synergically and factorially reinforced by the other components of the MDB such as MLT (Di Bella *et al*., 2006; Lissoni *et al*. 2001), retinoids (Majewski *et al*., 1994; McMillan K. *et al*.,1999; Kini *et al*., 2001), vitamin D3 (Kisker *et al*., 2003; Mantell *et al*., 2000), Vitamin E (Shklar *et al*., 1996; Tang *et al*. , 2001), Vitamin C (Ashino *et al*., 2003), prolactin inhibitors (Turner *et al*., 2000), and components of the extracellular matrix (Ozerdem *et al*., 2004; Liu *et al*., 2005).

In a similar way, the cytostatic, antiproliferative and antimetastatic effect of Somatostatin (Kogner *et al*. 1997; Orlando *et al*. 2001; Arena *et al*. 2006; Guillermet-Guibert *et al*. 2007; Barbieri *et al*. 2008) is effectively synergised by the other components of the MDB, such as retinoids (Hassan *et al*. 1990; Onogi *et al*., 1998; Piedrafita *et al*., 1997; Voigt *et al*., 2000; Witzigmann *et al*., 2008), MLT (Kvetnoĭ *et al*., 1986; Maestroni *et al*., 1996; Cos *et al*. 2000; Bartsch *et al*., 1999; Mediavilla *et al*., 1999; García-Santos, *et al*. 2006; Pizarro *et al*., 2008), Vit D3 (Celli *et al*., 1999; Barroga *et al*. 2000; Campbell *et al*. 2000; Jensen *et al*. 2001; Stio *et al*., 2001), the prolactin inhibitors Cabergoline and Bromocriptine (Manni *et al*., 1989; Klijn *et al*., 1996; Gruszka *et al*., 2001), Galactosamine sulfate, components of the extracellular matrix (Batra *et al*., 1997; Pumphrey *et al*., 2002),Vit E (Turley *et al*., 1995; Shklar *et al*., 1996; Israel *et al*., 2000; Malafa *et al*. 2002; Neuzil *et al*. 2002) and Vit C (Cameron *et al*., 1979; Murata *et al*. 1982; Head *et al*. 1998; Carosio *et al*. 2007). The literature has therefore confirmed the synergic antineoplastic, differentiating, antiproliferative, antiangiogenetic and antimetastatic mechanisms of action of all the components of the MDB

The objective result, with no toxicity, of the rapid reduction and disappearance of a retroperitoneal mass measuring 4×8 cm in a seven-month-old child, together with the blocking of all metastatic dissemination, demonstrates the efficacy of this treatment and is in agreement with the positive results achieved with the use of the MDB in lymphoproliferative diseases (Todisco *et al*. 2001; Todisco *et al*., 2006; Todisco *et al*. 2009), stage 3 and 4 lung cancer (Norsa *et al*. 2006 ; Norsa et 2007), breast cancer (Di Bella *et al*., 2009). Without the need for hospitalisation or even day hospital, and without in any way impairing the psychophysical development of the child, the MDB avoided difficult radical surgery,

which also involved a high risk since the distal aorta and vena cava, the bifurcation and the iliac arteries and veins were incorporated and displaced by the tumoral mass. It also made it possible to avoid chemo- and radiotherapy with their known and serious contraindications.

The blood tests and the diagnostic procedures showed a progressive decrease in the volume of the tumour, together with a reduction of the amine metabolites of the adrenergic system that are typical of neuroblastoma, such as catecholamine, Vanillylmandelic acid and Homovanillic acid, together with neuroendocrine markers such as NSE, and molecules like Ferritin and HDL which are typical of this disease. The complete objective result in little more than a year, together with the blocking of all metastatic dissemination, frequent in NBL, shows the efficacy of this treatment. The continuous administration of the components of the MDB for over 10 years was perfectly tolerated, allowing immunitary and neuroendocrine homeostasis well within physiological limits, and excellent psychophysical development.

The early application of the MDB as a first line therapy in a patient who was not weakened by the toxic, mutagenic and immunodepressive effects of chemo-radiotherapy was of considerable help to the result. We believe it is of use to report this case to invite greater interest and more detailed research into the possibilities offered in the oncological field by the immunoneuroendocrine, biological and receptorial treatment of the MDB.

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