

**RELATIONSHIP BETWEEN  $^{123}\text{I}$ -METAIODOBENZYLGUANIDINE  
( $^{123}\text{I}$ -MIBG) IMAGING FINDINGS AND OUTCOME IN PATIENTS  
WITH NEUROBLASTOMA AT THE RED CROSS WAR MEMORIAL  
CHILDREN'S HOSPITAL**

**By**

**Student:** Yaw Ampem Amoako (AMKYAW001)

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**Supervisor:** Dr. Anita Brink

**Co-Supervisor:** Prof. Mike D Mann

*Nuclear Medicine Division, Department of Radiation Medicine, University of Cape Town*



**UNIVERSITY OF CAPE TOWN**

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## DECLARATION

I, Yaw Ampem Amoako, hereby declare that the work on which this dissertation is based is my original work (except where acknowledgements indicate otherwise) and that neither the whole work nor any part of it has been, is being, or is to be submitted for another degree in this or any other university.

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## ABSTRACT

**Background:** In neuroblastoma, the presence of distant metastases is associated with a poor prognosis.

**Aim:** To assess the relationship between the findings on  $^{123}\text{I}$ -MIBG scan and outcome in patients with neuroblastoma at the Red Cross War Memorial Children's Hospital (RCWMCH).

**Methods:** A single observer reviewed the  $^{123}\text{I}$ -MIBG scans and clinical data of patients who had a histologically confirmed diagnosis of neuroblastoma and a baseline  $^{123}\text{I}$ -MIBG scan and at least one follow up scan after chemotherapy cycles 4 or 7 between January 2001 and May 2015. Follow up extended to June 2016. Disease burden was assessed using the Curie scoring (CS) method.

**Results:** Thirty four stage 4 patients were included in the analysis. Twenty nine (85%) were older than 12 months, with a median age at diagnosis of 32.5 months (range 6 – 93 months). 62% of primary tumours were located in the adrenal gland and half were NMYC amplified. Twenty (59%) patients died, 90% of deaths occurring in patients older than 12 months. No deaths were recorded in the 13 months after recruitment ended. The baseline CS did not predict outcome (alive or dead) or duration of survival. Patients with CS >2 (n = 5) on the cycle 4 scan had a median survival of 19.5 months compared with 29 months for those with a score  $\leq 2$  (n = 17, p = 0.88). Patients with a CS > 2 on the cycle 7 scan (n = 7) had a median survival of 28 months compared with 35 months for those with CS  $\leq 2$  (n = 14, p = 0.93). There was no relationship between the magnitude of the decrease in CS between the baseline and post cycle 4 or 7 scans and outcome.

**Conclusion:** In these 34 high risk patients, the baseline CS and CS at cycle 4 or cycle 7 were not significantly indicative of survival. This is similar to other studies that did not find the pre-treatment score or the post treatment MIBG scan to be a predictor of outcome.

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## LIST OF ABBREVIATIONS

RCWMCH	Red Cross War Memorial Children's Hospital
INSS	International Neuroblastoma Staging System
<sup>123</sup> I-MIBG	Iodine - 123 metaiodobenzylguanidine
<sup>131</sup> I-MIBG	Iodine - 131 metaiodobenzylguanidine
LDH	Lactate dehydrogenase enzyme
CS	Curie score
MCS	Modified Curie score
CT	Computed tomography scan
MRI	Magnetic resonance imaging
UK	United Kingdom
OPEC	Vincristine/ cisplatin/ etoposide/ cyclophosphamide
OJEC	Vincristine/ carboplatin/ etoposide/ cyclophosphamide
CRA	Cis-retinoic acid
CADO	Cyclophosphamide/ adriamycin/ vincristine
CAPE-O	Cisplatin/ adriamycin/ etoposide/ cyclophosphamide/ vincristine
EANM	European Association of Nuclear Medicine
F	Focal uptake
FD	Focal more than diffuse uptake
DF	Uptake more diffuse than focal
D	Diffuse uptake
OS	Overall survival
EFS	Event free survival

## **1.0 RESEARCH PROTOCOL**

This research protocol was submitted to and approved by the Faculty of Health Sciences, University of Cape Town before commencement of the study.

### **Title of study:**

Relationship between  $^{123}\text{I}$ -metaiodobenzylguanidine ( $^{123}\text{I}$ -MIBG) imaging findings at diagnosis and outcome in patients with neuroblastoma at the Red Cross War Memorial Children's Hospital

### **1.1 Background**

Neuroblastomas are common solid tumours of childhood.<sup>1</sup> The presence or absence of metastases is a major factor influencing prognosis.<sup>2, 4, 8, 15, 16</sup> Bone scintigraphy permits visualisation of the entire skeleton and is valuable for assessing bone metastases. However, because the bone scan assesses the metabolic changes in bone due to the neuroblastoma tumour, disease as assessed on the bone scan is not specific. On one hand, uptake on bone scan may be due to new infiltration of bone by neuroblastoma. Conversely, the uptake may be related to enhanced bone metabolic activity occurring in areas of bone healing following treatment. The bone scan is unable to differentiate these two pathologic processes and is therefore not useful for disease follow up or in assessing response to therapy.<sup>2</sup> The radiopharmaceutical  $^{123}\text{I}$ -MIBG is the nuclear medicine agent of choice for staging and assessment of therapy response.<sup>2, 3</sup> While the overall stage of neuroblastoma is seldom different whether bone scan or  $^{123}\text{I}$ -MIBG imaging is used, there may be a difference when assessment is done on a lesion-by-lesion basis.<sup>2</sup> At the Red Cross War Memorial Children's Hospital (RCWMCH), patients with neuroblastoma are currently assessed using both bone scan and  $^{123}\text{I}$ -MIBG imaging at staging and  $^{123}\text{I}$ -MIBG is used for follow up of patients. Nearly three decades ago, Daubenton and co-workers<sup>4</sup> reported on the relationship between

bone scan findings and prognosis in neuroblastoma patients at the RCWMCH. They showed that abnormal bone scan findings at diagnosis were associated with a poor outcome. The presence of  $^{123}\text{I}$ -MIBG avid disease at diagnosis and follow up have been reported to be predictive of poor outcome<sup>16, 19</sup> but to the best of our knowledge, there has been no review of the relationship between the findings on  $^{123}\text{I}$ -MIBG and outcome in the South African setting. The aim of the current study is to assess the relationship between the results of  $^{123}\text{I}$ -MIBG scan and outcome in patients with neuroblastoma at RCWMCH.

## **1.2 Literature review**

Neuroblastomas arise from neuro-ectodermal tissue. Studies from different parts of the world report varying incidence rates for neuroblastoma; in Europe and North America, they account for about 8% of malignant tumors in childhood<sup>1</sup> while a rate of approximately 3% has been reported from Mexico.<sup>5</sup> Stones and co-workers<sup>6</sup>, working in South Africa reported that neuroblastoma accounted for 5.8% of tumours in their paediatric cohort.

The disease can occur anywhere along the sympathetic chain from the base of the skull to the pelvis. Regarding the site of the primary tumour, 35% are found in the adrenal gland with 20% in the posterior mediastinum. About 35% originate in the paraspinal ganglia, with 5% each in the pelvic and neck regions.<sup>21</sup> The clinical presentation is largely dependent on the location of the primary tumour in the sympathetic nervous system and the presence or absence of metastases. The clinical presentation of the disease is variable but patients with metastatic disease are usually quite ill and usually have pain as a prominent symptom.

Patients may also present with cord compression, skin bruising or proptosis. In some cases, affected children present with paraneoplastic phenomena such as intermittent hypertension and diarrhoea. Occasionally, the diagnosis is suspected prenatally following the identification of a mass.<sup>21</sup> Neuroblastoma commonly spreads to cortical bone and bone marrow; up to 70%

of patients have dissemination of disease into bone and/ or bone marrow at presentation.<sup>7</sup>

Less frequently, there is spread to liver, skin, and lungs.

A primary tumour is not detected in up to 10% of children with disseminated neuroblastoma and in those who present with paraneoplastic syndromes.<sup>7</sup> Whether a primary tumour is detected or not does not impact the prognosis of the disease if metastases is already present.

However, a primary tumour when detected may be resected if it is surgically possible to do so as this can result in an improvement in patient symptoms albeit without an impact on overall survival.

The International Neuroblastoma Staging System<sup>2</sup> (INSS) is utilised for disease staging and is summarised in table 1.

Table 1: International Neuroblastoma Staging System<sup>2</sup>

<b>Stage</b>	<b>Description</b>
<b>1</b>	Localised tumour with complete gross excision with or without microscopic residual disease; contralateral and representative ipsilateral regional lymph nodes negative for disease (nodes attached to and removed with primary tumour may be positive)
<b>2A</b>	Localised tumour with incomplete gross resection; ipsilateral and contralateral nodes negative for tumour
<b>2B</b>	Localised tumour with complete or incomplete resection; positive ipsilateral (non-adherent) nodes; contralateral nodes are negative for tumour
<b>3</b>	Unresectable lateral tumour that crosses the midline* with or without regional lymph node involvement; or localised unilateral tumour with contralateral lymph node involvement; or midline tumour with bilateral extension by infiltration or lymph node involvement
<b>4S</b>	Patients younger than 12 months of age with localised tumour (as defined for stages 1, 2A or 2B) and metastases confined to liver, skin, and/ or bone marrow
<b>4</b>	Tumour with distant metastases not fulfilling stage 4S

\*midline is defined as the vertebral column. Tumours originating on one side and crossing the midline must infiltrate to or beyond the opposite side of the vertebral column.

Age at diagnosis and disease stage are important prognostic factors in neuroblastoma; older age at diagnosis and more advanced disease are associated with poor outcome.<sup>8, 9, 10</sup> Patients with stage 1, 2 or 4S tend to have a good prognosis with overall survival of patients with

stage 1 and 2 disease ranging from 80 – 100%.<sup>8,9</sup> In children under age 1 year with stage 4 disease and children older than 12 – 18 months with stage 3 or stage 4 disease, the prognosis is poor.<sup>4, 10, 11</sup>

Screening for neuroblastoma can be done using measurement of urine catecholamines, routine physical examinations including abdominal palpation or abdominal ultrasound. At the present time, general screening for neuroblastoma is not recommended.<sup>33</sup> However, Powell et al<sup>12</sup> reported on the differences in diagnosis of neuroblastoma in children resident in Europe. Their study included details of the manner of presentation (whether the symptoms of neuroblastoma were present at diagnosis or whether the disease was an incidental finding arising during a routine paediatric examination or during investigation of an unrelated disorder such as cryptorchidism). While the children resident in Germany, Austria and UK had information on the manner of presentation, such information was not available for children from France. They found that in countries with routine childhood health screening programmes (Germany and Austria) children were diagnosed with neuroblastoma at an earlier age and with early stage disease. The proportion of early stage disease (stages 1, 2 and 3) was higher in Germany, Austria and France than in the United Kingdom (UK) where no screening programme existed. The proportion with stage 4 disease was higher in the UK (61.5% versus 40%) and UK children aged 1-2 years, had significantly more stage 4 disease than their counterparts in France or Germany.<sup>12</sup>

There are differences in the epidemiology of neuroblastoma in developed and developing countries. In Mexico, a developing country, affected children present at an older age, with more advanced disease and with unfavourable histology.<sup>13</sup> The median age at presentation in the Mexican study was 27 months compared with 14.5 – 21.6 months in a European study.<sup>12</sup> In a previous report from RCWMCH,<sup>4</sup> only 6 of 30 patients with neuroblastoma were younger than 1 year of age. Further, stage 4 disease was more predominant in Mexico than in

some European countries (60% versus 40% respectively).<sup>12,13</sup> Approximately 30% of patients in France and Germany were reported to have either stages 1, 2 or 4S disease<sup>12</sup>, this is in contrast to Mexico where the proportion of children with similar disease stages was only 20%.<sup>13</sup> These differences among children at the time of presentation are largely responsible for the observed differences in disease outcomes between developed and developing countries.<sup>12,13</sup>

Imaging and urine biochemistry for catecholamines or their metabolites (vanillylmandelic acid, homovanillic acid) are usual investigations done prior to surgery or biopsy. Other non-specific markers include thrombocytosis, raised ferritin, neuron-specific enolase, and lactate dehydrogenase. Magnetic resonance imaging (MRI), computed tomography (CT), bone scintigraphy and MIBG scanning are useful imaging modalities for disease staging in neuroblastoma.<sup>21</sup>

MRI and CT are anatomic imaging modalities useful for determining size of primary tumour, extent of regional disease (regional invasion, vascular encasement, lymphadenopathy, and calcification) and to detect distant metastases; MRI is especially helpful for evaluating disease extension to the spinal canal.<sup>29</sup> Although both CT and MRI can detect cortical bone metastases in neuroblastoma, the detection is limited to the area included in the scan. With no exposure to radiation, MRI has an advantage but this is offset by the usual requirement for a general anaesthetic in the majority of patients affected with neuroblastoma. The Radiology Diagnostic Oncology group<sup>29</sup> compared the accuracies of CT, MRI and bone scintigraphy for staging in neuroblastoma. In that study, all patients had a CT of the chest or abdomen and pelvis (depending on the site of the primary tumour) using a fourth generation scanner. MRI was performed using a 1.5T scanner; the included field of view was dependent of the site of the primary tumour: all patients had an MRI of the bone marrow (pelvis, proximal femora and spine) and the chest or abdomen and pelvis; the pelvis was routinely included in the

abdominal examination while the thoracic or lumbar spine was studied depending on the site of the primary tumour. The authors of the study reported CT and MRI to have sensitivities of 43% and 83% respectively for detecting stage 4 disease; the specificities were 97% and 88% respectively. Computed tomography has the advantage of being more widely available and is useful for delineating vascular encasement by tumour in the process providing information about surgical resectability of the primary tumour.

The radiopharmaceuticals  $^{123}\text{I}$ -MIBG and  $^{131}\text{I}$ -MIBG are selectively taken up by tumour cells of sympho-adrenal origin and are known to provide a sensitive method for detecting and evaluating metastases in neuroblastoma.<sup>14, 15, 16</sup> Due to the lower radiation dose and optimal imaging characteristics,  $^{123}\text{I}$ -MIBG (rather than  $^{131}\text{I}$ -MIBG) is the preferred radiopharmaceutical for imaging of neuroblastoma.<sup>3</sup> Imaging with MIBG serves as a useful means of assessing disease extent and severity at diagnosis, thus aiding the choice of therapy.<sup>2</sup> MIBG imaging is also utilised to ascertain the response to therapy, and to detect residual or recurrent disease during follow up.<sup>2, 3</sup> In neuroblastoma,  $^{123}\text{I}$ -MIBG imaging is reported to have sensitivity of 88 – 93% and specificity of 83 – 92%.<sup>32</sup> MRI as a modality has a lower specificity than MIBG imaging for detecting metastatic disease in neuroblastoma.<sup>30</sup> Much of the advantage of MIBG imaging over CT or MRI derives from the fact that it allows functional assessment to help distinguish viable metabolically active disease from post therapy changes.<sup>30</sup> A combination of MIBG imaging with MRI has been shown to improve the sensitivity and specificity of disease detection in neuroblastoma: values of 99% and 95% respectively have been reported.<sup>30</sup>

The presence of extensive MIBG avid disease on the initial staging scan at diagnosis or during treatment usually predicts poor outcome.<sup>16, 19</sup> A positive MIBG scan after induction chemotherapy or just before myeloablative therapy portends poor prognosis.<sup>14, 17</sup> Kusher et al<sup>31</sup> reported on the sensitivities of various surveillance studies for detecting relapse in high

risk neuroblastoma. They found  $^{123}\text{I}$ -MIBG to be superior with a detection rate of 82% compared to 64%, 34% and 36% for  $^{131}\text{I}$ -MIBG scan, bone marrow histology and bone scan respectively. This led them to conclude that  $^{123}\text{I}$ -MIBG scan is essential for valid estimation of duration of relapse free survival of patients with neuroblastoma.

While some children present with only one or two foci of MIBG avid metastases, others present with more extensive disease at diagnosis. Semi quantitative scoring systems have been recommended by the International Neuroblastoma Risk Group Task Force for the assessment of extent and severity of MIBG avid disease at diagnosis and for evaluating treatment response in neuroblastoma.<sup>15</sup> The Curie method, the first developed scoring system, is easy to use<sup>24</sup> and is the most widely used for assessing disease burden at diagnosis and evaluating response to therapy. This scoring system divides the skeleton into 9 segments and utilises a 4-point scoring system to grade uptake. A modification, the modified Curie scoring system, which incorporates a tenth segment for soft tissue uptake<sup>16</sup> has also been used extensively. The modified Curie scoring method has good reproducibility with reported inter-observer concordance rate of 92%<sup>16</sup>. Messina and colleagues,<sup>18</sup> found an inter-observer concordance rate of approximately 95% among users of this modified Curie method. Disease burden as assessed by the Curie or modified Curie score at diagnosis or during induction chemotherapy has been found to correlate with overall response, and with event free survival.<sup>16, 18, 19</sup> Lewington and co-workers<sup>25</sup> published the SIOPEN method in 2009. The SIOPEN method divides the skeleton into 12 segments; the extent of involvement of each segment is scored on a scale of 0 – 6 giving a maximum extension score of 72. Soft tissue involvement is assessed separately in the SIOPEN method. The SIOPEN method was reported to have a concordance of 95% when tested in a blinded review by 6 Nuclear Medicine Physicians.<sup>25</sup> The Frappaz scoring system divides the body into 7 segments, measures an intensity score for each affected site using a 4 point scale and has a maximum

score of 21. The overall semi quantitative Frappaz score has good inter observer concordance rate (over 80%)<sup>26</sup> but is less reliable than the modified Curie score for assessing treatment response partly because it ignores soft tissue involvement in its assessment of disease extent.<sup>15, 16</sup>

Suc et al<sup>27</sup> performed a retrospective review of the pre-treatment <sup>123</sup>I MIBG scans of 86 patients older than 1 year of age with neuroblastoma. They assessed disease burden using a scoring system that ranged from 1 to 7 and divided the patients into two groups: group A with score < 4 and group B with score > 4. They reported that children less than 2 years of age and with pre-treatment score < 4 had a higher remission rate following induction chemotherapy. A score > 4 was associated with a 6.9 fold increased risk of not attaining complete remission (95% CI 2.4 – 19.6) after 4 cycles of induction chemotherapy.

The presence of a Curie score > 2 after induction therapy has been reported to be associated with extremely poor survival (15.4% 3-year event free survival).<sup>19</sup> Similar findings were reported by Katzenstein and colleagues.<sup>28</sup> Furthermore, Yanik et al<sup>19</sup> reported that patients with MYCN-amplified disease had little benefit from stem cell transplantation and subsequent consolidation therapy if they had an Curie score > 0 after induction therapy.

Messina and colleagues<sup>18</sup> used the modified Curie system to assess disease burden in a group of 49 patients with relapsed or refractory neuroblastoma who were treated with <sup>131</sup>I-MIBG. They showed that patients with a lower relative extension score (defined as the absolute post therapy score divided by the absolute pre-therapy score) were more likely to attain complete (CR) or partial response (PR) to therapy. The median relative score for patients with CR or PR was 0.3 compared to 0.98 for those with no response (NR) or progressive disease (PD); this was statistically significant. Further analysis revealed that 68% of patients with a relative

extension score  $\leq 0.5$  had treatment response while only 11% of those with relative extension score  $\geq 0.5$  had a treatment response.<sup>18</sup>

The relationship between the pattern of uptake and disease burden on the MIBG scan and outcome of neuroblastoma has been reported recently.<sup>20</sup> In that study, MIBG avid lesions were categorised as focal or diffuse. Lesions in body segments which showed margins that were clearly distinguishable from background were classified as focal. Lesions with no clearly defined margins were categorised as diffuse. According to the authors of that study,<sup>20</sup> a focal metastatic pattern is associated with better event free and overall survival in patients with N-myc amplified tumours. As the burden of disease is essential in guiding the choice of treatment, an accurate assessment of MIBG uptake and localisation in neuroblastoma tumours is essential both during the initial staging and on follow up.

For neuroblastoma, limited local disease is amenable to surgery and this will lead to complete removal of the tumour. On the other hand, metastatic disease or disease not surgically resectable will require prolonged chemotherapy or irradiation and bone marrow transplantation may benefit some children with this presentation.<sup>21, 22</sup> If post therapy scan indicates absent metastases, then surgery may be done to remove the primary. At the end of treatment, the absence of MIBG uptake on the post treatment scan in conjunction with absent disease on other imaging modalities (such as computed tomography, magnetic resonance imaging and <sup>18</sup>F – FDG positron emission tomography) is an indicator of good treatment response. On the other hand, the presence of MIBG uptake in previous known sites or in new areas indicates non response or a recurrence of disease. It is therefore essential that disease assessment is done accurately both on the initial pre-treatment and on the post therapy <sup>123</sup>I-MIBG scans.

### **1.3 Study questions**

- i. Does disease burden as assessed using the modified Curie score on the pre-treatment  $^{123}\text{I}$ -MIBG scan predict outcome in patients with neuroblastoma?
- ii. Does the form of uptake (focal versus diffuse) on the pre-treatment  $^{123}\text{I}$ -MIBG scan predict outcome in patients with neuroblastoma?
- iii. Does disease burden as assessed using the modified Curie score on the post treatment  $^{123}\text{I}$ -MIBG scan predict outcome in patients with neuroblastoma?
- iv. Does the form of uptake on the post treatment scan predict outcome in patients with neuroblastoma?
- v. What is the effect of an initial  $^{123}\text{I}$ -MIBG scan on the interpretation of the post treatment scan?
- vi. What are the relationships between the modified Curie score, pattern of uptake and age, N-myc status, surgical clearance of disease, bone marrow infiltration at end of treatment and outcome?

### **1.4 Purpose of the study**

This study is being conducted in partial fulfilment of the requirements for the award of an MMed degree in Nuclear Medicine by the University of Cape Town. It will provide information on the impact of  $^{123}\text{I}$ -MIBG on the management of neuroblastoma patients at RCWMCH and form the basis for further research in the area in the future.

## **1.5 Methodology**

### **1.5.1 Study design**

This will be a retrospective review of  $^{123}\text{I}$ -MIBG scans in conjunction with laboratory, clinical and other available imaging data of patients with neuroblastoma who were treated at the Oncology unit of RCWMCH between January 2001 and May 2015.

### **1.5.2 Characteristics of the study population**

The population for the study will comprise all patients with histologically confirmed neuroblastoma. Neuroblastoma patients who have a baseline/ pre-treatment  $^{123}\text{I}$ -MIBG scan and at least one post-treatment scan will be included in the study. The baseline  $^{123}\text{I}$ -MIBG scan should have been done prior to initiation of chemotherapy. Post treatment scan should have been done at the end of chemotherapy.

### **1.5.3 Exclusion criteria**

- i. Poor technical quality images
- ii. Patients with no pre-treatment MIBG scan

### **1.5.4 Research procedures and data collection methods/ method for evaluating the $^{123}\text{I}$ -MIBG scans**

The raw data of  $^{123}\text{I}$ -MIBG scans of all patients with neuroblastoma will be retrieved from the electronic archives of the Nuclear Medicine department of the Red Cross Hospital. The  $^{123}\text{I}$ -MIBG scans will be anonymised by the Supervisor of this dissertation so that the single observer is unaware of the patient name or folder number or the date on which the images were acquired or the report issued. On the first occasion, the single observer will also not be aware of which images are baseline/ pre-treatment or post treatment/ follow up scans. All the anonymised images will be retrieved and re-processed by the observer. The location of any lesion, the body segments involved, and the extent of involvement in a segment will be

recorded according to the modified Curie 4-point scoring system as shown in figure 1. The level of diagnostic certainty and intensity of uptake of involved segments will be recorded using the schema shown in table 2 and table 3 respectively. In addition, the form/ pattern of the uptake seen on the MIBG scan will be assessed and characterised as focal or diffuse using the method described by Bleeker et al.<sup>20</sup> Briefly, lesions in each segment on the scans which show margins that are clearly distinguishable from background will be classified as focal. Lesions with no clearly defined margins will be categorised as diffuse. The number of focal and diffuse lesions in each segment will be counted and recorded.

At least 2 weeks after all scans have been reviewed, the single interpreter will review all the images again. He will be given a matched list of pre-treatment and post treatment scans by the supervisor. The raw data of the matched scans will be retrieved from the archives, reprocessed and findings recorded. Again, the location of any lesion, number of segments involved, and the extent of involvement of a segment will be assessed and recorded according to the scoring scheme as described earlier and shown in figure 1 and the level of diagnostic certainty and intensity of uptake of involved segments will also be recorded using the schema shown in table 2 and table 3 respectively. The burden of disease will be assessed on the pre-treatment and post-treatment scans using the modified Curie 4-point scoring. The modified Curie score on the pre- and post- treatment scans will be computed and recorded. Again, the number of focal and diffuse lesions on each scan will be counted and recorded.

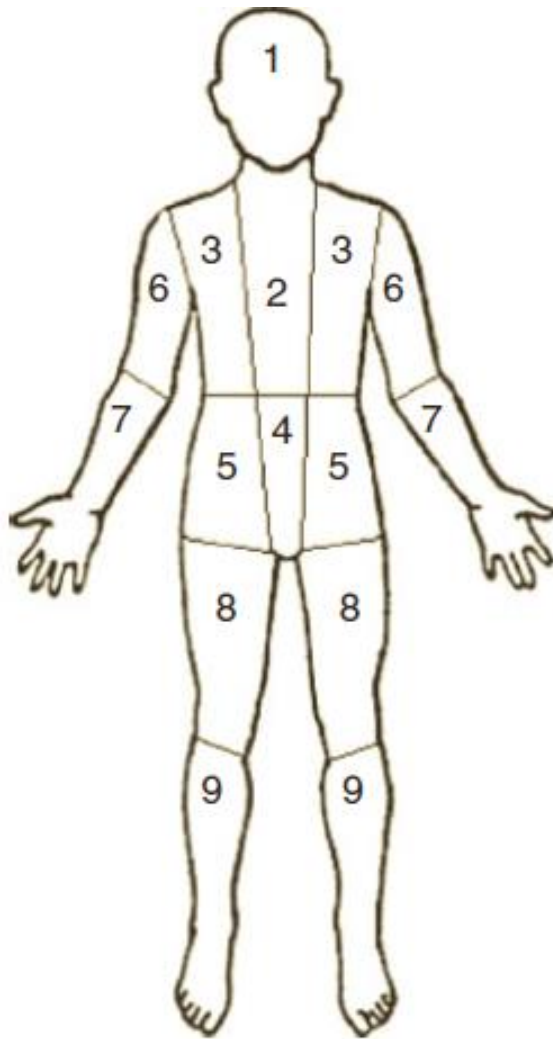
Table 2: Scores for certainty of lesion detection and localisation<sup>34</sup>

Score	Certainty	Description
0	Unknown	Don't know
1	Possible	Might know
2	Probable	Have a good idea
3	Definite	Sure

Table 3: Scores for intensity of uptake<sup>18</sup>

Score	Description
0	No sites of uptake
1	Doubtful uptake
2	Obvious but mild uptake
3	Obvious and intense

Figure 1: Scoring system dividing the skeleton into 9 compartments. A 10<sup>th</sup> compartment (not shown) will be used for soft tissue lesions.<sup>15</sup>



**Schema for scoring for each segment**

- 0 no uptake/ no foci
- 1 one focal lesion
- 2 more than one focal lesion
- 3 diffuse involvement ( $\geq 50\%$  of segment involved)

Soft-tissue lesions will be scored as follows: 0, no MIBG involvement; 1, one MIBG-avid soft-tissue lesion present; 2, more than one MIBG-avid soft-tissue lesion present; and 3, MIBG avidity in a soft-tissue lesion that occupies 50% of the chest or abdomen

\*Maximum attainable score is 30

\*Focal uptake: uptake with clearly defined margins

\*Diffuse uptake: uptake with margins indistinguishable from background

Once all the images have been reviewed and findings recorded as per the data sheets, the observer will be given a list of the folder number associated with each set of images. The oncology folders of all participants will be retrieved from the archives of the Oncology department of the hospital. Patient age, gender, date of diagnosis of neuroblastoma, site of primary tumour, disease stage, histopathology, and bone marrow biopsy results will be recorded on data sheet 3. The urine catecholamine results, N-myc status, routine haematology, serum LDH results and details of chemotherapy will also be abstracted on to

data sheet 3. The clinical outcome/ events and date of each event, date of last clinic visit/ last known contact and duration of follow up will be noted. To ensure that the observer is blinded to the clinical information of patients (other than the diagnosis of neuroblastoma) during the review of the images, the abstraction of data from the clinic folders will only be done after the evaluation of all the MIBG images.

### **1.5.5 Data analysis**

Data will be entered in to a database.

- i. Intra observer agreement of the effect of the pre-treatment scan on the post-treatment review will be assessed using the kappa coefficient
- ii. Data will be analysed for extent of disease as assessed by a semi-quantitative score on the pre- and post-treatment scans.
- iii. Analysis will be made on per lesion and per patient bases for level of certainty of lesion detection/ localisation and extent of segment involvement on the baseline and follow up MIBG scans.
- iv. The numbers of patients with exclusively focal (focal), focal more than/ equal to diffuse (focal  $\geq$  diffuse), more diffuse than focal (diffuse  $>$  focal), and exclusively diffuse (diffuse) lesions during the image evaluation sessions will be analysed.
- v. Data will be analysed for assessment of relationship between the semi quantitative scores on the pre- and post-treatment scans and outcome/ survival of patients.
- vi. The relationship between the form of the uptake (focal or diffuse) and outcome will be assessed.
- vii. Comparison will also be made between the scores and other clinical and imaging results.
- viii. The relationship between the N-myc status and number of involved body segments per patient will be assessed.

Survival will be assessed using the Kaplan-Meier life table method. Event free survival will be calculated as the time from diagnosis to the first event (relapse, progression, death) or last examination/ last known contact if no event occurred. Overall survival will be calculated as the time from diagnosis to death or last examination. Differences in overall survival and event free survival between different groups will be assessed using the log-rank test.

### **1.6 Limitations**

- i. This will be a retrospective study
- ii. A single observer will be involved in interpreting the scans due to constraints of time

### **1.7 Description of risks and benefits**

The images were acquired in accordance with guidelines on MIBG scintigraphy in children issued by the European Association of Nuclear Medicine.<sup>3</sup> There was no deviation from these guidelines for the purpose of this study. As the images to be evaluated are those which have already been acquired and are in the archives of the Nuclear Medicine department at the hospital, there will be no additional risk to the study subjects as no new imaging will be done in the course of this study.

### **1.8 Informed consent process**

The study is retrospective and shall involve analysis of examinations which were recorded as part of the routine management of patients with neuroblastoma at the RCWMCH and which were used in the management of the children.

### **1.9 Privacy and confidentiality**

No patient names will be used in the study or during preparation of the final study report. Paper based records will be kept in a secure location and be accessible to only persons involved in the study. All patient related data will be anonymised at all times to ensure confidentiality and compliance with the Helsinki declaration.<sup>23</sup>

### **1.10 Emergency care and insurance for research-related injuries**

The retrospective nature of the study means that no study subject will be exposed to any injury during the period.

### **1.11 What happens at the end of a study?**

The findings of the study will be submitted as a dissertation to the University of Cape Town in partial fulfilment for the award of the MMed degree in Nuclear Medicine. A final study report will be submitted to the Departments of Nuclear Medicine and Oncology at the hospital. The findings of the study will also be disseminated through presentations at workshops and publication in a scientific journal.

### **1.12 Funding**

All the data to be reviewed in the current study have been previously acquired as part of the routine care of patients with neuroblastoma in the hospital. No funding will be required for the acquisition of any new data. There will be no costs to the hospital during this study. All costs emanating from purchase of stationary, photocopying and printing will be catered for by the researcher's personal funds.

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## **2.0 PUBLICATION READY MANUSCRIPT**

(See appendix IV for Journal of Pediatric Hematology and Oncology author guidelines)

**Title:** Relationship between  $^{123}\text{I}$ -metaiodobenzylguanidine ( $^{123}\text{I}$ -MIBG) imaging findings and outcome in patients with neuroblastoma at the Red Cross War Memorial Children's Hospital

**Authors:**

Yaw Ampem Amoako<sup>1</sup>, FWACP, Anita Brink<sup>2</sup>, MMed, Ann Van Eyssen<sup>3</sup>, FCPaed, Mike Mann<sup>2</sup>, PhD

<sup>1</sup>Nuclear Medicine Division, Groote Schuur Hospital & Red Cross War Memorial Children's Hospital and University of Cape Town

<sup>2</sup>Nuclear Medicine Division, Red Cross War Memorial Children's Hospital and University of Cape Town

<sup>3</sup>Paediatric Oncology Service, Red Cross War Memorial Children's Hospital and University of Cape Town

**Address for correspondence:**

Dr Yaw Ampem Amoako

A3.62 Nuclear Medicine Division, Medical Imaging Department

Red Cross War Memorial Children's Hospital

Klipfontein Road, Rondebosch

Cape Town 7700

Tel: +27846189726/ +27 21 6585367

Fax: +27216585367

Email: [yamoako2002@yahoo.co.uk](mailto:yamoako2002@yahoo.co.uk)

**Conflicts of interest:** We have no competing interests

## 2.1 Summary

To assess the relationship between the findings on  $^{123}\text{I}$ -MIBG scan and outcome in patients with neuroblastoma at the Red Cross War Memorial Children's Hospital (RCWMCH), a single observer reviewed the  $^{123}\text{I}$ -MIBG scans and clinical data of patients who had a histologically confirmed diagnosis of neuroblastoma and a baseline  $^{123}\text{I}$ -MIBG scan and at least one follow up scan after chemotherapy cycles 4 or 7 between January 2001 and May 2015. Follow up extended to June 2016. Disease burden was assessed using the Curie scoring (CS) method. Thirty four stage 4 patients were included. Twenty (59%) patients died, 90% of deaths occurring in patients older than 12 months. No deaths were recorded in the 13 months after recruitment ended. The baseline CS did not predict outcome (alive or dead) or duration of survival. The 5 patients with  $\text{CS} > 2$  ( $n = 5$ ) on the cycle 4 scan had a median survival of 19.5 months compared with 29 months for the 17 with a score  $\leq 2$  ( $p = 0.88$ ). The 7 children with a  $\text{CS} > 2$  on the cycle 7 scan had a median survival of 28 months compared with 35 months for the 14 with  $\text{CS} \leq 2$  ( $p = 0.93$ ). There was no relationship between the magnitude of the decrease in CS between the baseline and post cycle 4 or 7 scans and outcome. In these 34 high risk patients, the baseline CS and CS at cycle 4 or cycle 7 did not predict survival.

**Keywords:** Neuroblastoma, Curie scoring, Outcome, South Africa

## 2.2 Main manuscript

### Introduction

Neuroblastomas are common solid tumours of childhood that arise from neuro-ectodermal tissue.<sup>1</sup> In South Africa, neuroblastoma accounts for 5.8% of paediatric tumours.<sup>2</sup> Poor prognostic factors in neuroblastoma include age at diagnosis, presence of metastases, NMYC amplification and an unfavourable histology.<sup>3, 4</sup>

The radiopharmaceutical <sup>123</sup>I-MIBG is the nuclear medicine imaging agent of choice in the assessment for presence of metastases and therapy response.<sup>5</sup> In view of reports of discordance between results of <sup>123</sup>I-MIBG and bone scan findings in some cases of neuroblastoma,<sup>6- 8</sup> we assess patients with neuroblastoma using both bone scan and <sup>123</sup>I-MIBG imaging at staging and <sup>123</sup>I-MIBG imaging is used during follow up.

The semi-quantitative Curie scoring system was developed to assess disease burden in bone and bone marrow. The Curie score (CS) is reported to have good intra- and inter-observer concordance<sup>9-10</sup> and is useful in assessing patients after treatment.<sup>11-12</sup> It divides the body into 9 skeletal segments (head, cervico-thoracic spine, ribs/ sternum/ scapula, lumbosacral spine, pelvis, upper arms, forearm/ hands, upper legs and lower legs/ feet). Lesions in each skeletal segment are scored on a scale of 0 to 3 as: 0, no uptake in segment; 1, one MIBG avid lesion present; 2, more than 1 MIBG avid lesion present; 3, MIBG uptake involving  $\geq 50\%$  of a particular segment. Patients without disease or with low CS post treatment have a good quality of life and good event free survival. Yanik et al<sup>12</sup> reported that in stage 4 neuroblastoma, patients with a post therapy CS > 2 (more than 2 distinct lesions in skeletal segments) after induction chemotherapy have a poorer outcome compared to those with score  $\leq 2$ . A relative Curie score (calculated as the ratio of post therapy CS to pre-therapy CS), has

been used to identify patients with refractory neuroblastoma who are likely to benefit from  $^{131}\text{I}$ -MIBG therapy.<sup>10</sup>

Nearly three decades ago, Daubenton and co-workers<sup>13</sup> reported on the relationship between bone scan findings and prognosis in neuroblastoma patients at the Red Cross War Memorial Children's Hospital (RCWMCH). They showed that abnormal bone scan findings at diagnosis were associated with a poor outcome. The current study aimed to assess the relationship between the results of  $^{123}\text{I}$ -MIBG scan and outcome in patients with neuroblastoma at RCWMCH.

## Materials and methods

### Patient population

We performed a retrospective review of  $^{123}\text{I}$ -MIBG scans and clinical, laboratory and other available imaging data of patients with neuroblastoma who presented to the RCWMCH between January 2001 and May 2015. The images of patients with histologically confirmed neuroblastoma who had a baseline  $^{123}\text{I}$ -MIBG scan before the start of any chemotherapy and at least one subsequent scan during or after completion of treatment were retrieved from the electronic archives of the Nuclear Medicine department of the hospital. The follow up extended till June 2016 to allow for a follow up period of at least 13 months from presentation.

### Treatment protocol for high risk neuroblastoma at RCWMCH

In most cases, high risk neuroblastoma patients receive 7 cycles of conventional therapy comprising vincristine, cisplatin, etoposide, cyclophosphamide (i.e. OPEC) alternating with vincristine, carboplatin, etoposide, cyclophosphamide (i.e. OJEC) every 21 days. Patients are reviewed with imaging and bone marrow biopsy. If there is no disseminated disease, that is bone and bone marrow disease have resolved after 4 cycles of first line chemotherapy,

surgery is performed to debulk (as much as possible) or remove the primary tumour. Post-surgery, external beam radiotherapy is administered to the residual tumour or surgical bed. A further 3 cycles of chemotherapy are given followed by 6 months of cis-retinoic acid (CRA).

If there is still disseminated disease (on MIBG imaging or bone marrow biopsy) after 4 cycles, chemotherapy is continued for a further 3 cycles. If the review after 7 cycles shows no bone or bone marrow disease, the patient undergoes surgery followed by radiotherapy of surgical bed or residual tumour and 6 months of CRA. However, if there is still disseminated disease after 7 cycles of OPEC/ OJEC, then palliative treatment with cyclophosphamide, adriamycin, vincristine (CADO) is undertaken. Surgical resection of primary tumour is performed only if there is a response to CADO.

Alternatively, patients older than 18 months with NMYC amplified tumours who have medical insurance may receive cisplatin, adriamycin, etoposide, cyclophosphamide and vincristine (CAPE-O) as first line chemotherapy. A small proportion of patients opt for CADO as their preferred first line chemotherapy.

### <sup>123</sup>I-MIBG scanning

All <sup>123</sup>I-MIBG scans were acquired in accordance with the Guidelines on MIBG scintigraphy in children issued by the European Association of Nuclear Medicine (EANM).<sup>5,14</sup>

Administered doses of <sup>123</sup>I-MIBG were in accordance with EANM paediatric dosage guidelines.<sup>15-16</sup> Whole body and static images were recorded 24 hours after injection of <sup>123</sup>I-MIBG (iThemba LABS, Old Faure Road, Faure, Cape Town, SA) using a Phillips Axis dual head camera (previously known as Picker and then Marconi) fitted with a low energy high resolution collimator. Whole body images were acquired using a matrix size of 1024 x 256 while static images were acquired using a 256 x 256 matrix for a duration of 300s. An acquisition zoom as appropriate for age of the child was used (maximum zoom applied was

2). Baseline scans were recorded before the start of any chemotherapy and subsequent scans were performed after 4 or 7 cycles or both of chemotherapy.

#### Interpretation of $^{123}\text{I}$ -MIBG scans

The raw data of the  $^{123}\text{I}$ -MIBG scans of the patients were retrieved from the electronic archives of the Nuclear Medicine department of the Red Cross War Memorial Children's Hospital. The  $^{123}\text{I}$ -MIBG scans were anonymised by <sup>1</sup>AB so that the single observer <sup>2</sup>YA was unaware of the patient's name and folder number or the date on which each set of images were acquired. In addition, the single observer also was blinded to the clinical information of patients other than the diagnosis of neuroblastoma. Each set of images were processed and reviewed twice on a HERMES (version V1.0, 2005, Hermes Medical Systems, Sweden) physicians' workstation. On the first occasion, the single observer was also not aware of whether images were baseline, cycle 4 or cycle 7 scans. All the anonymised images were retrieved and re-processed. The location of each lesion, the body segment involved, and the extent of involvement in each segment were recorded according to the Curie 4-point scoring system<sup>9, 10</sup>. A modified Curie score (MCS) in addition to having 9 skeletal segments, includes a tenth segment for soft tissue involvement. Lesions in soft-tissue were scored as follows: 0, no MIBG uptake in soft tissue; 1, one MIBG-avid soft-tissue lesion present; 2, more than one MIBG-avid soft-tissue lesion present; and 3, MIBG avidity in a soft-tissue lesion that occupies 50% of the chest or abdomen/ pelvis. Each patient's score was calculated as the sum of the individual scores of all involved segments. All scans were scored using the curie and the modified curie score. In addition, the uptake seen in bone on the MIBG scan was assessed and characterised as focal or diffuse using the criteria described by Bleeker et al.<sup>17</sup> Lesions identified as focal were clearly distinguishable from background. Lesions with no clearly

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<sup>1</sup> AB was supervisor for MMed dissertation

<sup>2</sup> YA was the MMed candidate

defined margins were categorised as diffuse. The number of focal and diffuse lesions in each skeletal segment was recorded. The intensity of uptake and level of diagnostic certainty of lesions were recorded.

Three weeks after the first review, a second review of all the images was performed on matched pre- and post-treatment scans. The raw data of the anonymised matched scans were again retrieved from the electronic archives, reprocessed and findings recorded. The location of any lesion, number of segments involved, and the extent of involvement of a segment were assessed and recorded and the level of lesion certainty and intensity of uptake of involved segments were recorded using the same criteria utilised during the first review.

#### Review of clinical data

After all images had been reviewed twice, the anonymisation code was opened and the clinical information of patients was retrieved from the archived folders of the Oncology department of the hospital. Patient age, gender, date of diagnosis of neuroblastoma, site of primary tumour, disease stage, histopathology, and bone marrow biopsy results were recorded. The urine catecholamine results, NMYC status, routine haematology, serum ferritin, serum lactate dehydrogenase (LDH) results and details of chemotherapy were also abstracted. Disease staging was done in accordance with the International Neuroblastoma Staging System criteria<sup>18</sup>. The clinical outcome/ events and date of each event, date of last clinic visit/ last known contact and duration of follow up were noted.

#### Ethical considerations

The study was approved by the Human Research Ethics Committee of the University of Cape Town (HREC: 716/2015) and conformed to the principles set out in the Helsinki Declaration.<sup>19</sup>

## Data analysis

The data was analysed in two parts. The image review data set was analysed to determine if the interpretation of the cycle 4 and 7 scans changed when they were viewed in conjunction with the baseline scans. The number of lesions in the different skeletal and soft tissue segments when the cycle 4 and cycle 7 scans were viewed alone and when the cycle 4 and cycle 7 scans were viewed in conjunction with the baseline scan were compared for any possible changes. The second part of the analysis involved assessment of clinical data for any relationships with the MIBG scan findings.

Clinical data are presented as frequencies and percentages. Overall survival was defined as the time from diagnosis till death or last examination. Survival was assessed using the Kaplan-Meier life table method. Differences in overall survival between different groups were assessed using the log-rank test. Statistical analyses were performed using R statistical package (version 3.2.3; The R Foundation for Statistical Computing) and Microsoft Office 2013 Excel.

## Results

### Clinical characteristics of study participants

Fifty seven (57) patients had at least two MIBG scans. Twelve patients were initially excluded; 3 patients because their clinic folders could not be traced and there was therefore no clinical data and 9 because they had stage 1, 2 or 3 disease leaving 45 patients with stage 4 disease. A further 11 patients with stage 4 disease were excluded from the analysis because 1 had no baseline scan and 10 because their follow up scans were performed at times other than after chemotherapy cycles 4 or 7. Thus, 34 patients with stage 4 disease were included in the analysis. The clinical and pathologic characteristics of the study participants are presented in Table 1. No patient had stage 4S disease. Twenty nine (85%) were older than 12 months and

the median age at diagnosis was 32.5 months (range 6 – 93 months). The adrenal gland was the most common site of the primary tumours and 50% of tumours had NMYC amplification. Twenty eight (82%) patients were treated with OPEC/ OJEC chemotherapy. No deaths occurred in the 13 months after recruitment ended.

#### Timing of <sup>123</sup>I-MIBG scans

All the patients included in this study had a baseline MIBG scan before the start of chemotherapy. Of the 34 patients, 22 had scans acquired after chemotherapy cycle 4 (cycle 4 scans) and 21 had scans after cycle 7 (cycle 7 scans). Nine patients had cycle 4 and cycle 7 scans.

In 8 of the 12 patients who did not have a cycle 4 scan, the scan was not performed because the <sup>123</sup>I-MIBG production plant was closed for maintenance. In 3, the scan was not performed because the bone marrow biopsy was normal while in 1 patient, there was no clear reason recorded.

Thirteen patients had no scans at cycle 7. In 6 of these patients, the scans were not performed because the <sup>123</sup>I-MIBG production plant was shut down for maintenance. Four patients did not have the scan performed because the cycle 4 scans were normal and bone marrow biopsies did not show any infiltration. In 1 patient, the post therapy bone marrow biopsy done after cycle 7 was normal and in 2 patients, the reason for the non-acquisition of the cycle 7 scan was not clear.

#### Effect of baseline scan on interpretation of cycle 4 and cycle 7 scans

Skeletal lesions were most frequently seen in the lower limbs on both the cycle 4 and cycle 7 scans. The forearm/ hands had the least number of lesions.

In the 22 patients who had cycle 4 scans, there were a total of 71 lesions (53 skeletal and 18 soft tissue lesions) in the 10 body segments when the cycle 4 scan was viewed without the baseline scan. Sixty six lesions (53 skeletal and 13 soft tissue lesions) were counted when the post treatment scan was viewed in conjunction with the baseline scan (details in supplementary table S1). There was no change in the assessment of skeletal lesions in the head, cervico-thoracic, lumbosacral, upper arms, forearm/ hands, thighs and lower legs segments. Two patients with lesions in ribs/ sternum/ scapula segment (one with 2 lesions and other with 1 lesion) on the cycle 4 scan were downgraded (classified as having no lesions) when the cycle 4 scan was viewed in conjunction with the baseline scan. One patient was upgraded to have 2 lesions in the pelvis (when the post cycle 4 scan was viewed alone, only a single lesion was seen in the pelvis).

For the 21 patients who had cycle 7 scans, there was no change in the number of skeletal lesions counted when the scan was viewed alone or in conjunction with the baseline scan (supplementary table S2) but in 2 patients there was a change in the skeletal segments involved. There was no change in the assessment of skeletal lesions in the head, upper arms, forearms/ hands, pelvis, thighs and lower legs segments. Upgrading (patients re-classified as having lesions in a skeletal segment when the post cycle 7 and baseline scans were viewed together) occurred in 2 patients: 1 in the lumbosacral segment and 1 in the cervico-thoracic segment. Downgrading (patients re-classified as having no lesions in a skeletal segment when the post cycle 7 and baseline scans were viewed together) occurred in 2 patients for lesions in the ribs/ sternum/ scapula region.

The overall effect of the baseline scan on the interpretation of the follow up scan was that in 19 of 22 patients (86%) with cycle 4 and 17 of 21 patients (81%) with cycle 7 scans, the interpretation was unchanged when the follow up scan was viewed alone or in conjunction with the baseline scan. The certainty of lesion detection and the grading of intensity of lesions

were enhanced when the follow up scan was viewed together with the baseline scan. As expected, there was a very close correlation between the Curie and modified Curie scores ( $r = 0.99$ ). All scores referred to subsequently in this paper are Curie scores.

#### Outcome of stage 4 neuroblastoma patients

Twenty (59%) patients died and 14 were alive when follow up for this study ended. Ninety percent of deaths occurred in patients older than 12 months. The median age at diagnosis for alive patients was 21.5 months (range 6 – 93) vs 37.5 months (range 6 – 72) for those who died (Kruskal Wallis  $H = 1.03$ ,  $p = 0.31$ ). Thirty two patients had NMYC results available. Of the 17 patients with NMYC amplified tumours, 11 died and 6 were alive. Seven patients with NMYC not amplified tumours died: one was diagnosed at age 17 months and the other 6 were  $\geq 36$  months at diagnosis, 4 patients with NMYC not amplified disease had poorly differentiated tumours and 4 had the primary tumour located in the adrenal gland.

The median interval from diagnosis to death was 12 months (range 6 – 123 months). For alive patients, the median interval from diagnosis to the last recorded follow up for the study was 26.5 months (range 14 – 71 months).

There was one treatment related death. This patient had an NMYC amplified primary tumour in the abdomen and died 6 months after diagnosis from disseminated intravascular coagulation 48 hours post-surgical resection of the primary tumour. The cycle 4 scan had a CS of 1.

When the patient data was examined, it was noted that all 9 patients with a diagnosis date before March 2010 had died. All 14 alive patients were diagnosed after March 2010 while 11 of the 25 patients diagnosed after March 2010 died (supplementary figure 1). We were unable to find a reason for this observation despite a review of the clinical notes and treatment protocols. The median overall survival of the 14 alive patients was 26.5 months (range 14 -

71) compared to 11 months (range 6 - 45) for the patients diagnosed after March 2010 who died.

#### Characteristics/ outcome of patients with cycle 4 scans

Ten of 22 (45%) patients with cycle 4 scans died. Nine (90%) of deaths occurred in patients older than 12 months. The median age at diagnosis for patients in this category was 28.5 months (range 6 – 93). For alive patients, the median age at diagnosis was 28 months (range 6 - 93) compared to 30 months (range 6 – 52) for those who died (Kruskal Wallis  $H = 3.84$ ,  $p = 0.82$ ). The patients with NMYC not amplified tumours who died were diagnosed at age  $\geq 17$  months. Nine alive patients had well differentiated tumours compared to 4 in the group who died. Of the alive patients, 5 had no uptake in a skeletal segment while 7 had skeletal disease on the post cycle 4 scan. The clinical and pathologic characteristics of patients with follow up scans at cycle 4 who died or remained alive are presented in table 2.

#### Characteristics and outcome of patients with cycle 7 scans

Thirteen of 21 (62%) of patients with cycle 7 scans died. The median age at diagnosis for patients with cycle 7 scans was 39 months (range 6 – 93). The median age at diagnosis for alive patients was 12.5 months (range 6 – 93) vs 44 months (range 7 – 72) for patients who died (Kruskal Wallis  $H = 2.31$ ,  $p = 0.13$ ). Twelve of 13 deaths were in patients who were diagnosed at age  $\geq 20$  months. The remaining child, diagnosed at age 7 months had a CS = 0 after 7 cycles but died 123 months after diagnosis following disease relapse. Five of the 8 alive patients were age  $\leq 13$  months at diagnosis. Four of 8 alive patients had disease in a skeletal segment and 4 had no skeletal uptake on the cycle 7 scan.

Seven of the patients who died had poorly differentiated tumours compared to 2 in the alive group. Six patients who died had NMYC amplified tumours compared to 2 alive patients. The

clinical and pathologic characteristics of patients with follow up scans at cycle 7 who died or remained alive are presented in table 3.

Curie score (CS) and outcome for patients with cycle 4 scans

Baseline Curie score (CS) and outcome for patients with cycle 4 scans

Five patients did not have any uptake in a skeletal segment on the baseline scan; however, all 5 patients had bone marrow infiltration on biopsy and evidence of cortical bone involvement on the bone scan performed at diagnosis. The baseline CS did not predict outcome (alive or dead) or duration of survival. The survival of the 8 patients with baseline CS  $\leq 2$  was not statistically different from the 14 with CS  $> 2$  (median overall survival median overall 29 vs 13 months,  $p = 0.64$ ).

Post cycle 4 CS and outcome

Patients with post cycle 4 CS  $> 2$  ( $n = 5$ ) had median overall survival of 19.5 months compared to 29 months for those with CS  $\leq 2$  ( $n = 17$ ),  $p = 0.88$ . At 29 months, the proportion of those alive with post cycle 4 CS  $\leq 2$  was more than thrice the proportion of those with CS  $> 2$  (fig 1).

Curie score (CS) and outcome for patients with cycle 7 scans

Baseline Curie score (CS) and outcome for patients with cycle 7 scans

Five patients in this group did not have any uptake in a skeletal segment on the baseline scan. However, all 5 patients had evidence of cortical bone involvement on the bone scan and 3 had bone marrow infiltration on biopsy performed at diagnosis. Again, the baseline CS did not predict outcome (alive or dead) or duration of survival. The survival of patients with baseline CS  $\leq 2$  ( $n = 6$ ) was not significantly different from those with CS  $> 2$  ( $n = 15$ ). The

median overall survival was 23 months for patients with baseline CS  $\leq 2$  compared to 28 months for those with CS  $> 2$ ,  $p = 0.28$ .

#### Post cycle 7 CS and outcome

Patients with CS  $> 2$  on the cycle 7 scan ( $n = 7$ ) had median overall survival of 28 months compared with 35 months for those with CS  $\leq 2$  ( $n = 14$ ),  $p = 0.93$ . At 45 months, the proportion of those alive with post cycle 7 CS  $\leq 2$  was more than thrice the proportion of those with CS  $> 2$  (fig 2).

#### CS and outcome in patients who were diagnosed after March 2010

The predictive value of CS in patients diagnosed after March 2010 was analysed separately. The median OS for patients with CS  $\leq 2$  was 45 months compared to 19 months for those with CS  $> 2$ ,  $p = 0.30$ .

#### Change in baseline and cycle 4 Curie scores and survival/ tumour response

A relative Curie score was calculated for each patient as the ratio of cycle 4 score to the baseline score. This relative CS was used as a measure of tumour response to treatment. The median survival for stage 4 neuroblastoma patients with less than 50% reduction in tumour burden from baseline (relative CS  $> 0.5$ ,  $n = 8$ ) was not statistically different from patients who had at least a 50% reduction (relative CS  $\leq 0.5$ ,  $n = 14$ ) in tumour burden (28 months vs 29 months,  $p = 0.93$ ). At 29 months, the proportion of patients alive with relative CS  $\leq 0.5$  was more than thrice the proportion of those with relative CS  $> 0.5$ . There was no difference in the survival of patients with relative score  $\leq 0.75$  ( $n = 15$ ) and those with score  $> 0.75$  ( $n = 7$ ).

### Change in baseline and cycle 7 Curie scores and survival/ tumour response

The relative score at cycle 7 was calculated as the ratio of the cycle 7 score to the baseline score. The 14 patients with relative CS  $\leq 0.5$  at cycle 7 had a median survival of 28 months compared to 93 months for the 7 patients with relative score  $> 0.5$ ,  $p = 0.71$  (fig 3). There was no difference in the survival of patients with relative score  $\leq 0.75$  ( $n = 15$ ) and those with score  $> 0.75$  ( $n = 6$ ).

Alive and dead patients had decreases in CS on the follow up scan at cycle 4 or 7. There was no relationship between the magnitude of decrease in CS and outcome (supplementary figures 2a and 2b).

### Form of uptake on baseline scan and survival

The patients with no abnormal uptake on the baseline scan were excluded from the analysis of pattern of uptake and survival as there was no pattern of uptake to classify. For the survival analysis, patients with focal uptake (F) on the baseline scan were considered as one category. All patients with more focal than diffuse (FD), diffuse (D) and more diffuse than focal (DF) uptake baseline were also grouped as one category. The survival of patients with F uptake was compared to those with FD or D or DF uptake.

### Form of uptake on baseline scan and survival for patients with cycle 4 scans

While 2 patients with focal uptake died, there were 8 deaths in patients with FD or D or DF uptake. There was no difference in survival between patients with focal uptake ( $n = 6$ ) and those with FD or D or DF uptake ( $n = 16$ ), median survival 26 months vs 28 months,  $p = 0.44$ . Even when the pattern of uptake was categorised as F or FD and D or DF, there was still no difference in survival with respect to the pattern of uptake.

We did not have sufficient patient numbers to enable assessment of the relationship between form of uptake and survival in patients with NYMC amplified tumours who had cycle 4 scans.

Form of uptake on baseline scan and survival for patients with cycle 7 scans

Four patients with focal uptake died with 9 deaths occurring in patients with FD or D or DF uptake. There was no difference in survival between patients with focal uptake (n = 7) and those with FD or D or DF uptake (n = 14), median survival 123 months vs 28 months, p = 0.48. When the pattern of uptake was classified as F or FD and D or DF, there was still no difference in survival with respect to the pattern of uptake.

We did not have sufficient patient numbers to enable assessment of the relationship between form of uptake and survival in patients with NYMC amplified tumours who had cycle 7 scans.

## Discussion

To our knowledge, this is the first review of the relationship between <sup>123</sup>I-MIBG imaging findings and outcome in a neuroblastoma series in South Africa. We found that there was no change in assessment of number of lesions in skeletal segments on the cycle 4 or cycle 7 scans when viewed alone or together with baseline scan. This is in agreement with previous reports of good intra-observer concordance when the semi-quantitative Curie scoring is used in the evaluation of MIBG scans in patients with neuroblastoma.<sup>9-11</sup> The major impact of viewing the matched cycle 4 or cycle 7 and baseline scans was that there was greater certainty of lesion detection.

Eighteen of the 20 patients in our series who died were older than 12 months at diagnosis, 11 of them had NMYC amplified tumours while only 6 patients with NMYC amplified tumours were alive. Nine patients who died had poorly differentiated tumours compared to 3 in the

alive group. This is similar to previous reports of older age at diagnosis, presence of NMYC amplification and poor tumour differentiation being associated with a worse prognosis in neuroblastoma.<sup>3-4, 13</sup>

We found that the baseline CS did not predict outcome in our cohort. This is similar to other reports that the pre-treatment score is not a predictor of prognosis.<sup>6, 12</sup> Yanik and colleagues reported that in patients with stage 4 neuroblastoma, there was no correlation between the pre-treatment CS and treatment outcome.<sup>12</sup> In a study of 30 patients with stage 4 neuroblastoma in the United States, Perel and colleagues<sup>6</sup> found that the 2 year event free survival of patients with initial (pre-treatment) MIBG scores  $\geq 10$  was not different from those with score  $< 10$  ( $27.8\% \pm 11.7$  vs  $59.2\% \pm 14.1\%$ ,  $p = 0.23$ ).

The Curie score after 4 or 7 cycles of chemotherapy did not predict outcome in our high risk neuroblastoma cohort. Previous studies have reported on the prognostic significance of semi-quantitative score post therapy. Yanik and colleagues studied 280 patients with stage 4 neuroblastoma who were on Children Oncology Group (COG) protocol A3973. MIBG scans were evaluated using the same criteria as in our study. They reported that patients with a Curie score  $> 2$  ( $n = 52$ ) had decreased event free survival (EFS) compared to those with Curie scores  $\leq 2$  ( $n = 185$ ) after induction therapy with 3-year EFS of  $15.4\% \pm 5.3\%$  vs.  $44.9\% \pm 3.9\%$ ,  $p = 0.001$ ).<sup>12</sup> Additionally, they found that patients with  $\geq 50\%$  reduction in Curie score from diagnosis to after induction ( $n = 194$ ) had a much better survival when compared to those with  $< 50\%$  reduction ( $n = 43$ ) (3-year EFS:  $42.9\% \pm 3.8\%$  vs.  $17.3\% \pm 5.9\%$ ,  $p = 0.001$ ). In patients with relapsed neuroblastoma, those with relative scores (ratio of post therapy CS to pre-therapy CS)  $\leq 0.5$  are reportedly more likely to have a complete or partial response to <sup>131</sup>I-MIBG therapy.<sup>10</sup> Katzenstein et al<sup>20</sup> studied the prognostic significance of MIBG scan scores in a group of 29 neuroblastoma patients with age at diagnosis  $> 18$  months in Chicago. They used a scoring scheme that divided the skeleton into

10 segments (in contrast to our study, their study divided the head into 2 segments namely calvarium and base of the skull-face). They reported that the post induction scan predicted prognosis as MIBG score  $\geq 3$  after induction therapy was associated with a significantly worse event free survival. Although the pattern of our results is similar to previous reports by Yanik et al<sup>12</sup> and Katzenstein et al,<sup>20</sup> we did not find any statistically significant difference between the survival of patients with cycle 4 or cycle 7 CS  $> 2$  and those with score  $\leq 2$ .

Patients with  $< 50\%$  reduction in tumour burden from diagnosis to post treatment have been reported to have a worse event free survival when compared to those with  $\geq 50\%$  reduction.<sup>12</sup> In our series, the overall survival of these 2 groups was not significantly different. In the study by Perel et al,<sup>6</sup> they reported that an abnormal post induction chemotherapy MIBG scan was associated with a poor outcome: all 5 patients with uptake on the post induction scan had disease relapse while 8 of 16 patients with normal post therapy scans were reported to be in progression-free remission. In contrast, Andrich et al did not find post therapy MIBG imaging findings to be predictive of prognosis.<sup>21</sup> In that study, 8 out of 13 patients with stage 4 disease and normal post therapy <sup>131</sup>I-MIBG scans had disease relapse at one or more sites leading the authors to conclude that normalisation of MIBG scan after therapy was not a predictor of outcome.

Residual MIBG avid disease on the post induction chemotherapy MIBG scan predicts poor outcome after allogenic stem cell transplant<sup>22</sup> and relapse after high dose therapy with peripheral blood stem cell rescue, local radiotherapy, and cis-retinoic acid.<sup>20</sup>

Bleeker et al<sup>17</sup> reported that in patients with NMYC amplified tumours, those with focal lesions had a much better event free survival (EFS) and overall survival (OS) than those in the other metastatic groups (focal  $\geq$  diffuse + diffuse  $>$  focal + diffuse) with 5-year EFS and 5-year OS of  $63 \pm 24\%$  vs.  $21 \pm 15\%$ ,  $p = 0.006$  and  $81 \pm 20\%$  vs.  $28 \pm 17\%$ ,  $p = 0.001$

respectively. In contrast, an exclusively focal pattern of uptake was not associated with a better overall survival in patients with NMYC amplified disease in the present study. The median age at diagnosis of patients in the study by Bleeker et al was similar to those in our cohort (34 months vs 32.5 months respectively); however that study included 84 patients with NMYC amplified disease (total number of study patients was 249) compared to 17 patients with NMYC amplified tumours in the present study. This difference may be responsible for the differences in our results.

While the international recommendations regarding the use of  $^{123}\text{I}$ -MIBG in staging and response assessment of neuroblastoma are clear, there are challenges in the local South African (and probably entire African) setting that impact the use.  $^{123}\text{I}$ -MIBG is cyclotron produced and we rely on iThemba LABS for supply. For several months each year, the radiopharmaceutical is unavailable for use as the production plant is shut down for maintenance. Thus, some patients do not get MIBG imaging at staging while others do not get the follow up imaging. Several patients were excluded from this study because they did not have scans performed at baseline, or at cycle 4 and 7 as dictated by the treatment protocol at RCWMCH due to shutdown of the MIBG production plant. Other stage 4 patients were excluded from the analysis because the follow up scan was only performed after a substantial time interval, again due to production shutdown. This shutdown impacts patient management and also makes it more difficult to establish the beneficial value of MIBG imaging in neuroblastoma in our setting.

Treatment costs for neuroblastoma are high and some specific decisions regarding treatment are impacted by whether or not the affected patient has medical insurance. For instance, colony stimulating factor (GCSF) support for patients who develop neutropenia and autologous bone marrow transplantation are available only to patients with medical insurance.

## Limitations of study

This was a retrospective study and may have had the biases usually associated with such study designs. However, we attempted to reduce the influence of bias by abstracting the clinical data only after all MIBG scans had been reviewed. Only a single observer was involved in the interpretation of the MIBG scans. Any bias on the part of the sole observer was ameliorated by anonymisation of the MIBG scans and allowing an interval of 3 weeks between the first and second image review sessions. The shutdown of MIBG production at iThemba LABS impacted the number of patients included in our study. Not all patients presenting with neuroblastoma to our institution were included in this study. Several patients with neuroblastoma who did not have follow up scans as dictated by protocol were excluded from the study. The small number of patients in our analysis may have precluded our ability to show differences in survival of the magnitude reported by Yanik et al.<sup>12</sup>

This study conducted a review of MIBG scan findings and clinical data of stage 4 neuroblastoma patients in South Africa. There was no change in the assessment of number of skeletal lesions on the follow up scan when viewed alone or in conjunction with baseline scan but the certainty of lesion detection and grading of lesion intensity was enhanced. Similar to previous reports, the baseline CS did not predict prognosis in stage 4 neuroblastoma. However, unlike other reports, the CS on the cycle 4 and cycle 7 scans did not also indicate prognosis in our cohort of high risk neuroblastoma patients. In addition, the magnitude of reduction in CS did not carry prognostic significance in our cohort.

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Table 1: Clinical and pathologic characteristics of all neuroblastoma patients

Characteristic	Age at diagnosis (months)		Gender		Site of primary tumour		NMYC amplification		Bone scan at diagnosis		Bone marrow biopsy at diagnosis		Bone marrow biopsy post therapy		Type of chemotherapy	
All patients, n=34	<12	5	M	17	Abdomen	8	Present	17	Positive	31	Abnormal	28	Abnormal	7	OPEC/OJEC	28
	12-24	9	F	17	Adrenal	21	Absent	15	Negative	1	Normal	6	Normal	26	CAPE-O	5
	>24	20			Thorax Pelvis	2 3	NA	2	NA	2	NA	0	NA	1	CADO	1
All Dead, n=20	<12	2	M	11	Abdomen	6	Present	11	Positive	18	Abnormal	15	Abnormal	6	OPEC/OJEC	16
	12-24	5	F	9	Adrenal	11	Absent	7	Negative	1	Normal	5	Normal	13	CAPE-O	3
	>24	13			Thorax Pelvis	1 2	NA	2	Not available	1	NA	0	NA	1	CADO	1
Dead with diagnosis date after March 2010, n=11	<12	1	M	5	Abdomen	4	Present	7	Positive	9	Abnormal	7	Abnormal	2	OPEC/OJEC	11
	12-24	3	F	6	Adrenal	5	Absent	4	Negative	1	Normal	4	Normal	8		
	>24	7			Thorax Pelvis	0 2	NA	0	NA	1	NA	0	NA	1		
Alive, n=14	<12	3	M	6	Abdomen	2	Present	6	Positive	13	Abnormal	13	Abnormal	1	OPEC/OJEC	12
	12-24	4	F	8	Adrenal	10	Absent	8	Negative	0	Normal	1	Normal	13	CAPE-O	2
	>24	7			Thorax Pelvis	1 1	NA	0	NA	1	NA	0	NA	0	CADO	0

M=male, F=female, NA=not available

Table 2: Clinical and pathologic characteristics of neuroblastoma patients who had follow up scans at cycle 4

Characteristic	Age at diagnosis (months)		Gender	Site of primary tumour		NMYC amplification		Bone scan at diagnosis		Bone marrow biopsy at diagnosis		Bone marrow biopsy post therapy		Degree of tumour differentiation		
All patients, n = 22	<12	3	M	11	Abdomen	4	Present	9	Positive	21	Abnormal	20	Abnormal	3	Poor	7
	12-24	6	F	11	Adrenal	16	Absent	12	Negative	0	Normal	2	Normal	18	Well	13
	>24	13			Thorax Pelvis	1 1	NA	1	NA	1	NA	0	NA	1	NA	2
Dead, n = 10	<12	1	M	6	Abdomen	2	Present	5	Positive	9	Abnormal	8	Abnormal	2	Poor	4
	12-24	3	F	4	Adrenal	8	Absent	4	Negative	0	Normal	2	Normal	7	Well	4
	>24	6			Thorax Pelvis	0 0	NA	1	NA	1	NA	0	NA	1	NA	2
Alive, n = 12	<12	2	M	5	Abdomen	2	Present	4	Positive	12	Abnormal	12	Abnormal	1	Poor	3
	12-24	3	F	7	Adrenal	8	Absent	8	Negative	0	Normal	0	Normal	11	Well	9
	>24	7			Thorax Pelvis	1 1	NA	0	NA	0	NA	0	NA	0	NA	0

M=male, F=female, NA=not available

Table 3: Clinical and pathologic characteristics of neuroblastoma patients who had follow up scans at cycle 7

Characteristic	Age at diagnosis (months)		Gender	Site of primary tumour		NMYC amplification		Bone scan at diagnosis		Bone marrow biopsy at diagnosis		Bone marrow biopsy post therapy		Degree of tumour differentiation		
All patients, n=21	<12	4	M	10	Abdomen	6	Present	8	Positive	18	Abnormal	17	Abnormal	5	Poor	9
	12-24	4	F	11	Adrenal	11	Absent	12	Negative	1	Normal	4	Normal	16	Well	6
	>24	13			Thorax Pelvis	2 2	NA	1	NA	2	NA	0	NA	0	NA	6
Dead, n=13	<12	1	M	7	Abdomen	5	Present	6	Positive	11	Abnormal	10	Abnormal	5	Poor	7
	12-24	2	F	6	Adrenal	5	Absent	6	Negative	1	Normal	3	Normal	8	Well	2
	>24	10			Thorax Pelvis	1 2	NA	1	NA	1	NA	0	NA	0	NA	4
Alive, n=8	<12	4	M	3	Abdomen	1	Present	2	Positive	7	Abnormal	7	Abnormal	0	Poor	2
	12-24	2	F	5	Adrenal	6	Absent	6	Negative	0	Normal	1	Normal	8	Well	4
	>24	2			Thorax Pelvis	1 0	NA	0	Not available	1	NA	0	NA	0	NA	2

M=male, F=female, NA: not available

Figure 1: Overall survival by cycle 4 Curie score for scores  $\leq 2$  vs  $> 2$

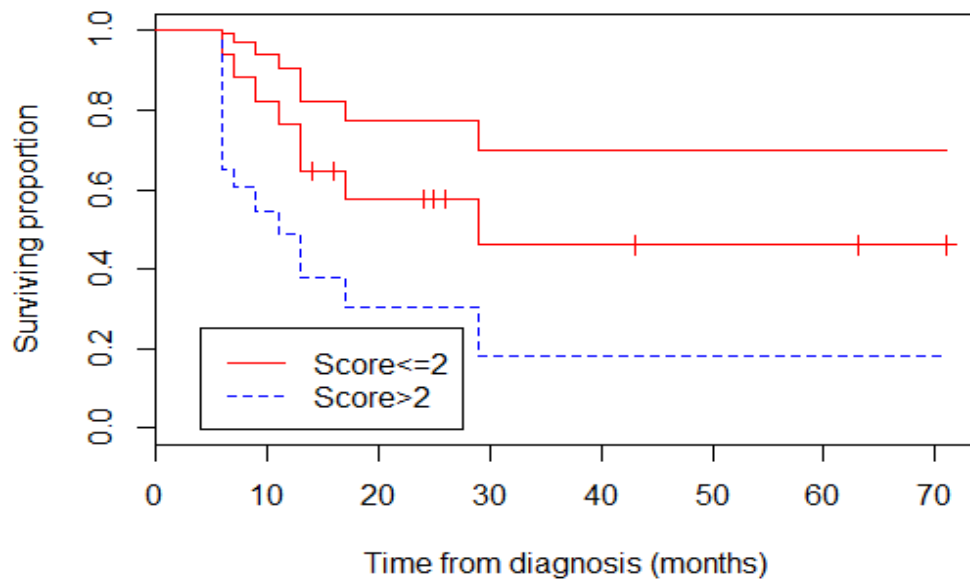


Figure 2: Overall survival by cycle 7 Curie score for scores  $\leq 2$  vs  $> 2$

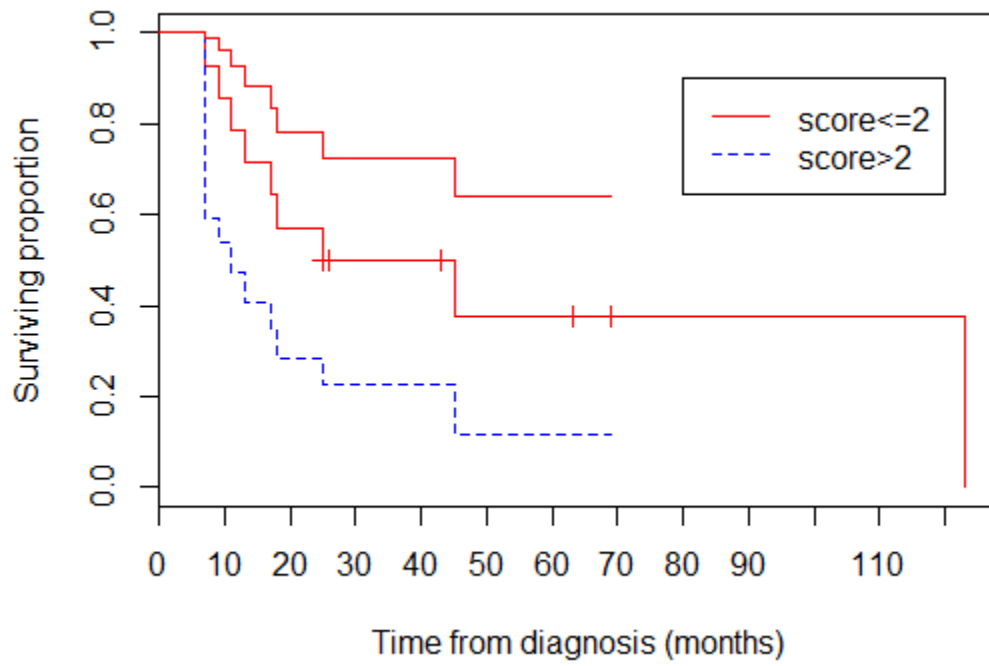
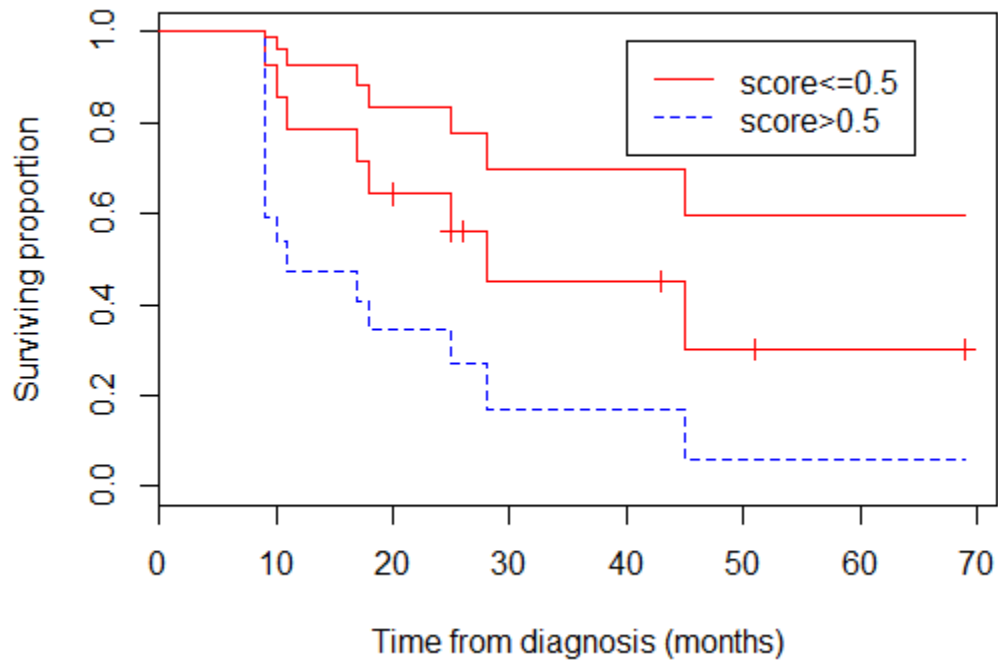


Figure 3: Overall survival by relative Curie score for score  $\leq 0.5$  vs  $> 0.5$  at cycle 7



## SUPPLEMENTARY TABLES AND FIGURES

Supplementary Table S1: Characteristics of skeletal lesions in neuroblastoma patients with cycle 4 scans

		Focal	Diffuse	Total
Cycle 4 scan viewed alone	Number of lesions	42	11	53
	Number of patients	12	4	
	Lesions per patient, median (range)	2 (1 – 9)	2.5 (1 – 5)	
Cycle 4 scan viewed in conjunction with baseline scan	Number of lesions	42	11	53
	Number of patients	10	4	
	Lesions per patient, median (range)	3.5 (2 – 9)	2.5 (1 – 5)	

Supplementary Table S2: Characteristics of skeletal lesions in neuroblastoma patients with cycle 7 scans

		Focal	Diffuse	Total
Cycle 7 scan viewed alone	Number of lesions	62	14	76
	Number of patients	11	4	11
	Lesions per patient, median (range)	6.5 (2 – 13)	2 (1 – 9)	
Cycle 7 scan viewed in conjunction with baseline scan	Number of lesions	59	17	76
	Number of patients	10	3	10
	Lesions per patient, median (range)	6 (2 – 14)	4 (2 – 11)	

Supplementary Table S3: MIBG scan characteristics of patients who had cycle 4 scans

Characteristic	All patients, n=22		Dead, n = 10		Alive, n = 12	
Baseline Curie score	Median	15	Median	15	Median	14
	Range	0 - 25	Range	0 - 21	Range	0 - 25
	IQR	1.25 - 18.75	IQR	1.25 - 18.25	IQR	1.5 - 18.25
Cycle 4 Curie Score	Median	0.5	Median	0	Median	1
	Range	0 - 18	Range	0-18	Range	0-17
	IQR	0 - 2	IQR	-	IQR	0 - 3

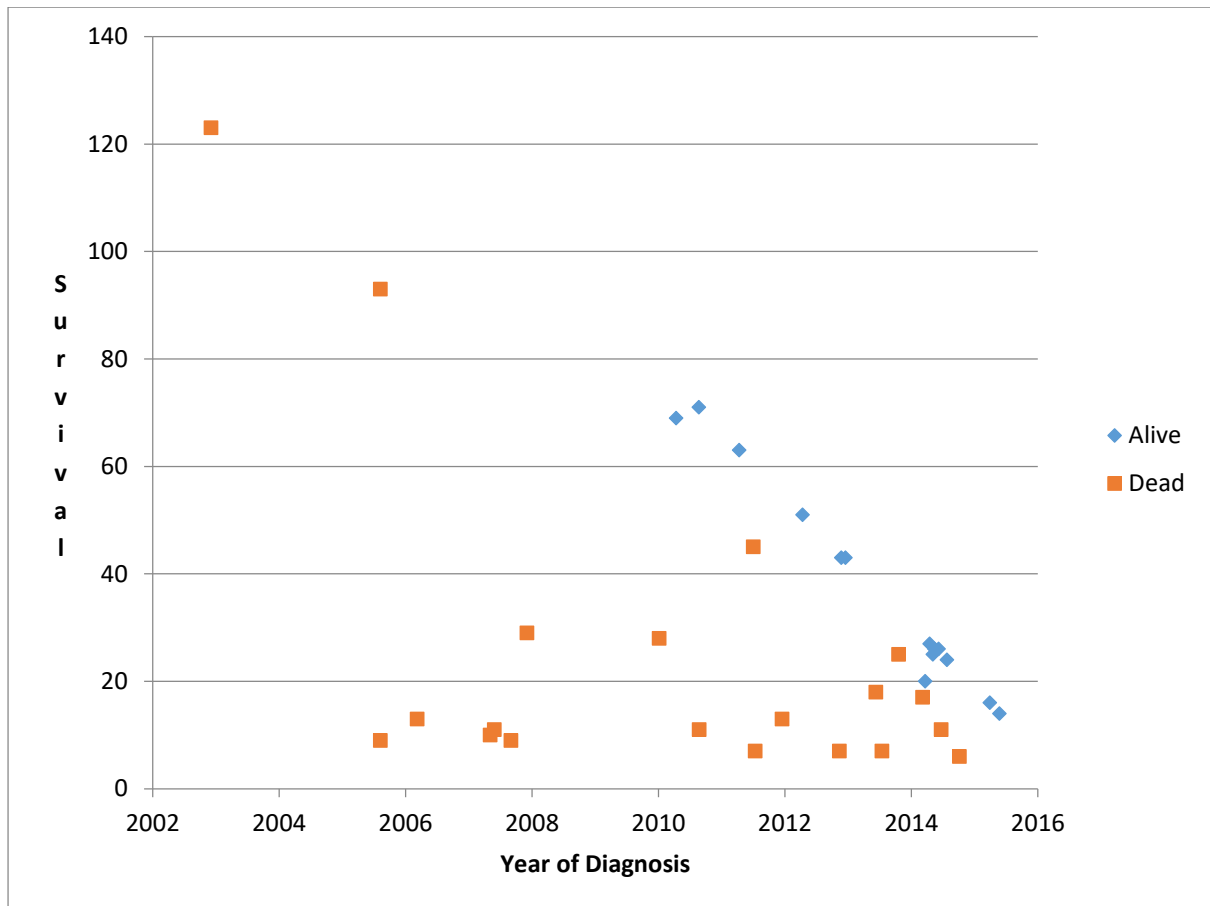
IQR: interquartile range

Supplementary Table S4: MIBG scan characteristics of patients who had cycle 7 scans

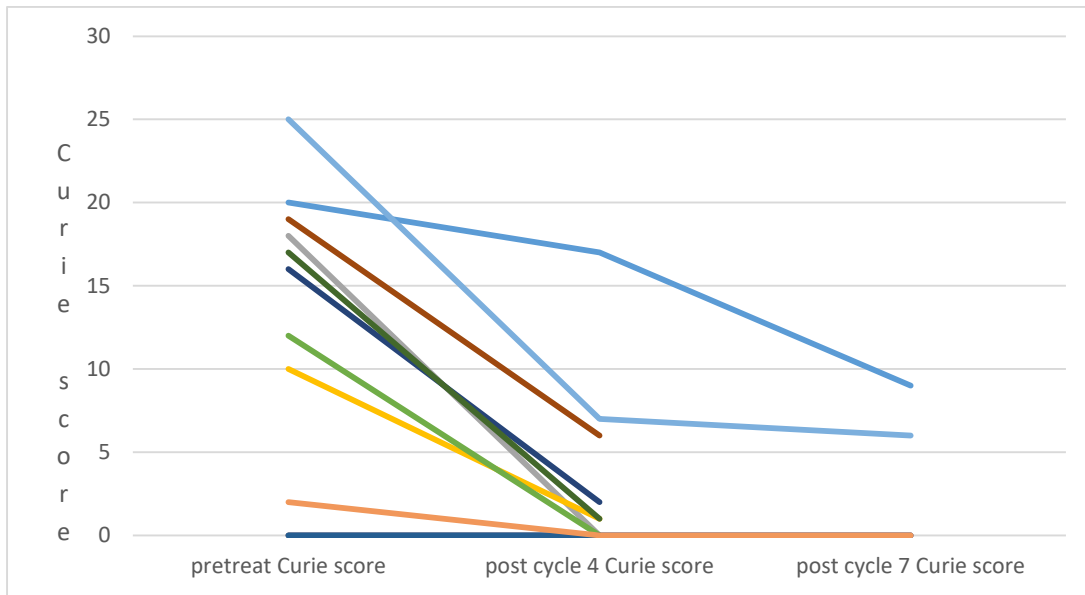
Characteristic	All patients, n=21		Dead, n=13		Alive, n=8	
Baseline Curie score	Median	16	Median	18	Median	7.5
	Range	0 - 25	Range	0 - 25	Range	0 - 25
	IQR	2 - 22	IQR	3 - 23	IQR	1.5 - 18.5
Cycle 7 Curie Score	Median	1	Median	2	Median	0.5
	Range	0 - 24	Range	0 - 24	Range	0 - 9
	IQR	0 - 6	IQR	0 - 6	IQR	0 - 6

IQR: interquartile range

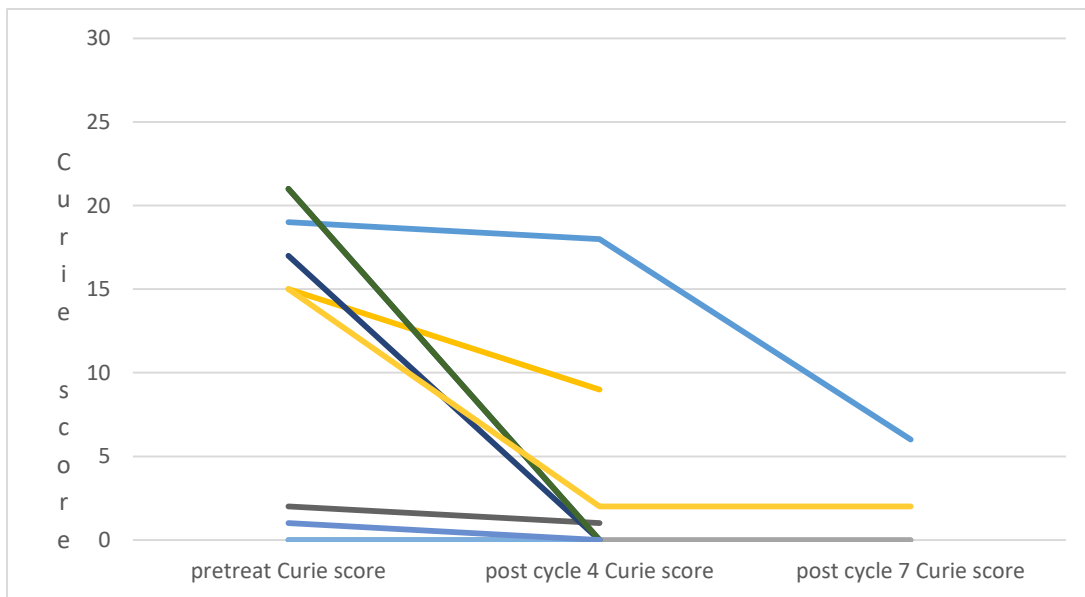
Supplementary figure 1: Survival of neuroblastoma patients by year of diagnosis



Supplementary figure 2a: Change in baseline and follow-up Curie scores and outcome for patients who were alive



Supplementary figure 2b: Change in baseline and follow-up Curie scores and outcome for patients who died



### 3.1 APPENDIX I: DATABASE APPROVAL

UNIVERSITY OF CAPE TOWN



Faculty of Health Sciences  
Human Research Ethics Committee  
Room E52-24 Groote Schuur Hospital Old Main Building  
Observatory 7925  
Telephone [021] 406 6626 • Facsimile [021] 406 6411  
e-mail: [shuretta.thomas@uct.ac.za](mailto:shuretta.thomas@uct.ac.za)

10 January 2013

**REF NO: R009/2012**

**Dr A Brink**  
Nuclear Medicine  
B5  
Red Cross War Memorial Children's Hospital

Dear Dr Brink

**PROJECT TITLE: NUCLEAR MEDICINE DATABASE**

Thank you for registering your database with the Health Sciences Human Research Ethics Committee.

The HREC has approved the registration of your database.

**Please Note:** All research, including that undertaken for a master's or doctoral degree, using registered databases, registries and repositories, requires submission as a new study. It requires an application form (FHS013) and a protocol which has undergone departmental review. The study will receive its own HREC REF number which will be linked to the main database or repository.

The registration of this database is valid until **30 January 2016**.

Please provide the HREC with an update if the database continues beyond this period.

**Please quote the HREC REF in all your correspondence.**

Yours sincerely

**PROFESSOR M BLOCKMAN**  
**CHAIRPERSON, HSF HUMAN ETHICS**

S Thomas

## 3.2 APPENDIX II: ETHICS APPROVAL



**UNIVERSITY OF CAPE TOWN**  
**Faculty of Health Sciences**  
**Human Research Ethics Committee**



**Room E52-24 Old Main Building**  
**Groote Schuur Hospital**  
**Observatory 7925**  
Telephone [021] 406 6338 • Facsimile [021] 406 6411  
Email: [nosi.tsama@uct.ac.za](mailto:nosi.tsama@uct.ac.za)  
Website: [www.health.uct.ac.za/fhs/research/humanethics/forms](http://www.health.uct.ac.za/fhs/research/humanethics/forms)

25 September 2015

**HREC REF: 716/2015**

**Dr A Brink**  
Paediatrics  
Dept of Nuclear Medicine  
Red Cross War Memorial Children's Hospital

Dear Dr Brink

**PROJECT TITLE: RELATIONSHIP BETWEEN <sup>123</sup>I-METAIODOBENZYLGUANIDINE <sup>123</sup>I-MIBG IMAGING FINDINGS AT DIAGNOSIS AND OUTCOME IN PATIENTS WITH NEUROBLASTOMA AT THE RED CROSS WAR MEMORIAL CHILDREN'S HOSPITAL (MMed candidate Dr YA Amoako)**

Thank you for submitting your study to the Faculty of Health Sciences Human Research Ethics Committee.

It is a pleasure to inform you that the HREC has **formally approved** the above-mentioned study.

**Approval is granted for one year until the 30<sup>th</sup> September 2016.**

Please submit a progress form, using the standardised Annual Report Form if the study continues beyond the approval period. Please submit a Standard Closure form if the study is completed within the approval period.

(Forms can be found on our website: [www.health.uct.ac.za/fhs/research/humanethics/forms](http://www.health.uct.ac.za/fhs/research/humanethics/forms))

**Please quote the HREC REF in all your correspondence.**

***We acknowledge that the student Dr YA Amoako will also be involved in this study.***

Please note that the ongoing ethical conduct of the study remains the responsibility of the principal investigator.

Yours sincerely

**PROFESSOR M BLOCKMAN**  
**CHAIRPERSON, FHS HUMAN RESEARCH ETHICS COMMITTEE**

Federal Wide Assurance Number: FWA00001637.

Institutional Review Board (IRB) number: IRB00001938

This serves to confirm that the University of Cape Town Human Research Ethics Committee complies to the Ethics Standards for Clinical Research with a new drug in patients, based on the Medical

HREC 716/2015

3.3: APPENDIX III: DATA SHEETS

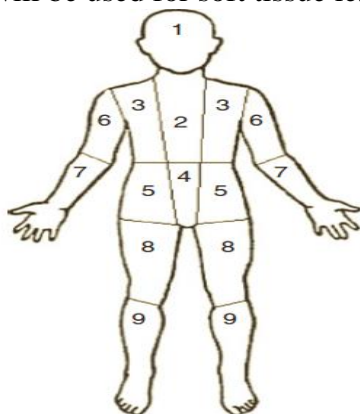
**Data sheet 1**

Study serial number..... Date.....

Type of scan

Unknown  Pre-treatment scan  Post treatment scan

Scoring system dividing the skeleton into 9 compartments. A 10<sup>th</sup> compartment (not shown) will be used for soft tissue lesions.



**Schema for scoring**

- 0 no uptake/ no foci per segment
- 1 one focal lesion per segment
- 2 more than one focal lesion per segment
- 3 diffuse involvement ( $\geq 50\%$  of segment involved)

Soft-tissue lesions will be scored as follows: 0, no mIBG involvement; 1, one mIBG-avid soft-tissue lesion present; 2, more than one mIBG-avid soft-tissue lesion present; and 3,

mIBG avidity in a soft-tissue lesion that occupies 50% of the chest or abdomen

\*Maximum attainable score is 30

\*Focal uptake: uptake with clearly defined margins

\*Diffuse uptake: uptake with margins indistinguishable from background

Location/ segment involved	Certainty	Number of lesions in segment	Extent of involvement/ score	Certainty	Intensity of uptake
Head					
Cervico-thoracic spine					
Ribs/ sternum/ scapula					
Lumbosacral spine					
Pelvis					
Upper arms					
Forearm and hands					
Upper legs/ thighs					
Lower legs/ feet					
Soft tissue involvement					

Lesion certainty: 0=unknown, 1=possible, 2=probable, 3=definite

Intensity of uptake: 0 =no sites of uptake; 1= doubtful uptake; 2= obvious but mild uptake;

3=obvious and intense uptake

Total number of involved body segments:

Number of focal lesions=                      Number of diffuse lesions=

Total number of lesions on scan=

Total Curie score=                      Modified Curie score=

Form of uptake:

Focal                       Focal  $\geq$  diffuse                       Diffuse  $\geq$  focal                       Diffuse

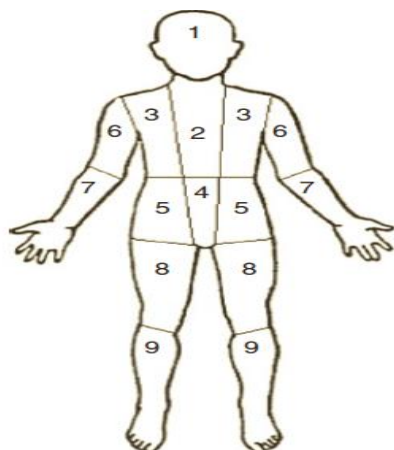
MIBG Uptake in iliac crest    Yes                       No

## Data sheet 2

Study serial number..... Date.....

Type of scan: Pre-treatment scan

Scoring system dividing the skeleton into 9 compartments. A 10<sup>th</sup> compartment (not shown) will be used for soft tissue lesions.



### Schema for scoring

- 0 no uptake/ no foci per segment
- 1 one focal lesion per segment
- 2 more than one focal lesion per segment
- 3 diffuse involvement ( $\geq 50\%$  of segment involved)

Soft-tissue lesions will be scored as follows: 0, no mIBG involvement; 1, one mIBG-avid soft-tissue lesion present; 2, more than one mIBG-avid soft-tissue lesion present;

and 3, mIBG avidity in a soft-tissue lesion that occupies 50% of the chest or abdomen

\*Maximum attainable score is 30

\*Focal uptake: uptake with clearly defined margins

\*Diffuse uptake: uptake with margins indistinguishable from background

Location/ segment involved	Certainty	Number of lesions in segment	Extent of involvement/ score	Certainty	Intensity of uptake
Head					
Cervico-thoracic spine					
Ribs/ sternum/ scapula					
Lumbosacral spine					
Pelvis					
Upper arms					
Forearm and hands					
Upper legs/ thighs					
Lower legs/ feet					
Soft tissue involvement					

Lesion certainty: 0=unknown, 1=possible, 2=probable, 3=definite

Intensity of uptake: Intensity of uptake: 0 =no sites of uptake; 1= doubtful uptake; 2= obvious but mild uptake; 3=obvious and intense uptake

Total number of involved body segments:

Number of focal lesions=

Number of diffuse lesions=

Total number of lesions on scan=

Total Curie score=

Modified Curie score=

Form of uptake:

Focal

Focal $\geq$  diffuse

Diffuse $\geq$  focal

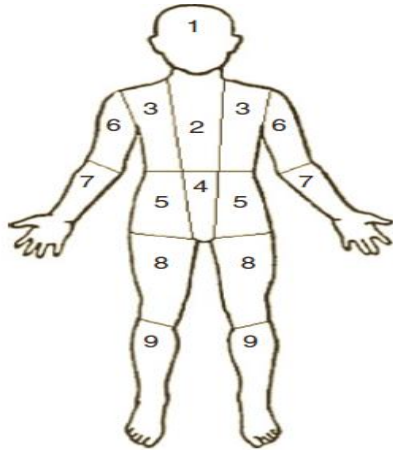
Diffuse

MIBG Uptake in iliac crest Yes

No

Type of scan: Post treatment scan

Scoring system dividing the skeleton into 9 compartments. A 10<sup>th</sup> compartment (not shown) will be used for soft tissue lesions.



**Schema for scoring**

0 no uptake/ no foci per segment

1 one focal lesion per segment

2 more than one focal lesion per segment

3 diffuse involvement ( $\geq 50\%$  of segment involved)

Soft-tissue lesions will be scored as follows: 0, no mIBG involvement; 1, one mIBG-avid soft-tissue lesion present; 2, more than one mIBG-avid soft-tissue lesion present;

and 3, mIBG avidity in a soft-tissue lesion that occupies 50% of the chest or abdomen

\*Maximum attainable score is 30

\*Focal uptake: uptake with clearly defined margins

\*Diffuse uptake: uptake with margins indistinguishable from background

Location/ segment involved	Certainty	Number of lesions in segment	Extent of involvement/ score	Certainty	Intensity of uptake
Head					
Cervico-thoracic spine					
Ribs/ sternum/ scapula					
Lumbosacral spine					
Pelvis					
Upper arms					
Forearm and hands					
Upper legs/ thighs					
Lower legs/ feet					
Soft tissue involvement					

Lesion certainty: 0=unknown, 1=possible, 2=probable, 3=definite

Intensity of uptake: Intensity of uptake: 0 =no sites of uptake; 1= doubtful uptake; 2= obvious but mild uptake; 3=obvious and intense uptake

Total number of involved body segments:

Number of focal lesions=                      Number of diffuse lesions=

Total number of lesions on scan=

Total Curie score=                      Modified Curie score=

Form of uptake:

Focal                       Focal $\geq$  diffuse                       Diffuse $\geq$  focal                      Diffuse

MIBG Uptake in iliac crest    Yes                       No

### Data sheet 3

Folder number: Study ID: Sex: M [ ] F [ ]

Age/DOB: Date of diagnosis of NB: Stage of NB:

Age at diagnosis:

Race: White [ ] Black [ ] Mixed race [ ]

Histopathology result available? Yes [ ] No [ ]

Histopathology report:

Catecholamine level result available? Yes [ ] No [ ]

Urine HMA/VMA result [ ] Elevated (HMA/VMA) Yes [ ] No [ ]

Bone marrow (BM) at diagnosis result available? Yes [ ] No [ ]

Percent (%) of marrow infiltration at diagnosis [ ]

#### Any additional BM results

Date % BM involvement [ ]

N-myc amplification result:

#### Haematologic indices:

Date of test:

WCC Hb Platelets LDH Ferritin

HIV status

#### Site of primary tumor

Cervical [ ] Thorax [ ] Abdomen [ ] Pelvis [ ] Retroperitoneum [ ]

Unknown site [ ]

Bone scan results:

#### Chemotherapy treatment dates

1<sup>st</sup> cycle 2<sup>nd</sup> cycle

3<sup>rd</sup> cycle 4<sup>th</sup> cycle

5<sup>th</sup> cycle

6<sup>th</sup> cycle

\*\*Type of chemotherapy

Did patient undergo bone marrow transplantation? Yes [ ] No [ ]

Intervening events eg relapse, progression, death, hospitalizations, infections, anaemia/  
haemotransfusion/ mucositis

Intervening event? Yes [ ] No [ ]

Type of event: Number of events:

Date of first event:

Event free survival (time from diagnosis to first event):

**Outcome:** Dead [ ] Alive [ ] If dead, date of death:

Cause of death:

Disease related [ ] Treatment related [ ] death unrelated to disease or treatment [ ]

Did patient have surgery Yes [ ] No [ ]

If yes, date of surgery:

Outcome of surgery: Tumour completely removed [ ] Not completely removed [ ]

Need for second line chemotherapy? Yes [ ] No [ ]

**Second line chemotherapy:**

1<sup>st</sup> cycle: 2<sup>nd</sup> cycle:

3<sup>rd</sup> cycle: 4<sup>th</sup> cycle:

5<sup>th</sup> cycle: 6<sup>th</sup> cycle:

**Results of other imaging (CT/MRI) at end of treatment**

Is disease still present at site of primary Yes [ ] No [ ]

Metastatic disease still present? Yes [ ] No [ ]

If yes, site(s) of disease

Is there disease extension into neural foramina? Yes [ ] No [ ]

Date of last clinic visit:

Date of last known contact:

Overall survival (time from diagnosis to death to death or last examination):

AUTHOR GUIDELINES

**Journal of Pediatric Hematology/Oncology  
Online Submission and Review System**

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Conflicts of Interest and Source of Funding: A has received honoraria from Company Z. B is currently receiving a grant (#12345) from Organization Y, and is on the speaker's bureau for Organization X – the CME organizers for Company A. For the remaining authors none were declared.

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**Preparation of Manuscript:** Manuscripts that do not adhere to the following instructions will be returned to the corresponding author for technical revision before undergoing peer review.

**Title Page:** Include on the title page (a) complete manuscript title; (b) authors' full names, highest academic degrees, and affiliations; (c) name and address for correspondence, including fax number, telephone number, and e-mail address; (d) address for reprints if

different from that of corresponding author; and (e) all sources of support, including pharmaceutical and industry support, that require acknowledgment.

The title page must also include disclosure of funding received for this work from any of the following organizations: National Institutes of Health (NIH); Wellcome Trust; Howard Hughes Medical Institute (HHMI); and other(s).

**Unstructured Abstract and Key Words:** Limit the abstract to 200 words. It must be factual and comprehensive. Limit the use of abbreviations and acronyms, and avoid general statements (eg, “the significance of the results is discussed”). List three to five key words or phrases.

**Text:** Organize the manuscript into four main headings: Introduction, Materials and Methods, Results, and Discussion. Define abbreviations at first mention in text and in each table and figure. If a brand name is cited, supply the manufacturer's name and address (city and state/country). All forms of support, including pharmaceutical industry support, must be acknowledged in the Acknowledgment section.

**Abbreviations:** For a list of standard abbreviations, consult the *Council of Biology Editors Style Guide* (available from the Council of Science Editors, 9650 Rockville Pike, Bethesda, MD 20814) or other standard sources. Write out the full term for each abbreviation at its first use unless it is a standard unit of measure.

**References:** The authors are responsible for the accuracy of the references. Key the references (double-spaced) at the end of the manuscript. Cite the references in text in the order of appearance. Cite unpublished data—such as papers submitted but not yet accepted for publication and personal communications, including e-mail communications—in parentheses in the text. If there are more than three authors, name only the first three authors and then use et al. Refer to the *List of Journals Indexed in Index Medicus* for abbreviations of journal names, or access the list at <http://www.nlm.nih.gov/tsd/serials/lji.html>. Sample references are given below:

#### **Journal Article**

1. Ang KK, Price RE, Stephens LC, et al. The tolerance of primate spinal cord to re-irradiation. *Int J Radiat Oncol Biol Phys*. 1993;25:459–464.

#### **Book Chapter**

2. Dimery IW. Chemotherapy in head and neck cancer. In: Myerhoff WI, Rice DH, eds. *Otolaryngology: head and neck surgery*, 2nd ed. Philadelphia: WB Saunders, 1992:1027–1045.

#### **Entire Book**

3. Virchow R. *Cellular Pathology*. Philadelphia: JB Lippincott, 1863.

#### **Software**

4. Epi Info [computer program]. Version 6. Atlanta, GA: Centers for Disease Control and Prevention; 1994.

#### **Online Journals**

5. Friedman SA. Preeclampsia: a review of the role of prostaglandins. *Obstet Gynecol* [serial

online]. January 1988;71:22–37. Available from: BRS Information Technologies, McLean, VA. Accessed December 15, 1990.

#### ***Database***

6. CANCERNET-PDQ [database online]. Bethesda, MD: National Cancer Institute; 1996. Updated March 29, 1996.

#### ***World Wide Web***

7. Gostin LO. Drug use and HIV/AIDS [*JAMA HIV/AIDS Web site*]. June 1, 1996. Available at: <http://www.ama-assn.org/special/hiv/ethics>. Accessed June 26, 1997.

#### ***URL (Uniform Resource Locator)***

8. (J. M. Kramer, K. Kramer [[jmkramer@umich.edu](mailto:jmkramer@umich.edu)], e-mail, March 6, 1996).

### **Figures:**

#### **A) Creating Digital Artwork**

1. Learn about the publication requirements for Digital Artwork:  
<http://links.lww.com/ES/A42>
2. Create, Scan and Save your artwork and compare your final figure to the Digital Artwork Guideline Checklist (below).
3. Upload each figure to Editorial Manager in conjunction with your manuscript text and tables.

#### **B) Digital Artwork Guideline Checklist**

Here are the basics to have in place before submitting your digital artwork:

- Artwork should be saved as TIFF, EPS, or MS Office (DOC, PPT, XLS) files. High resolution PDF files are also acceptable.
- Crop out any white or black space surrounding the image.
- Diagrams, drawings, graphs, and other line art must be vector or saved at a resolution of at least 1200 dpi. If created in an MS Office program, send the native (DOC, PPT, XLS) file.
- Photographs, radiographs and other halftone images must be saved at a resolution of at least 300 dpi.
- Photographs and radiographs with text must be saved as postscript or at a resolution of at least 600 dpi.

- Each figure must be saved and submitted as a separate file. Figures should not be embedded in the manuscript text file.

### **Remember:**

- Cite figures consecutively in your manuscript.
- Number figures in the figure legend in the order in which they are discussed.
- Upload figures consecutively to the Editorial Manager web site and enter figure numbers consecutively in the Description field when uploading the files.

**Figure Legends:** Include legends for all figures. They should be brief and specific, and they should appear on a separate manuscript page after the references. Use scale markers in the image for electron micrographs, and indicate the type of stain used.

**Color Figures:** The journal accepts for publication color figures that will enhance an article. Authors who submit color figures will receive an estimate of the cost for color reproduction. If they decide not to pay for color reproduction, they can request that the figures be converted to black and white at no charge.

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### **Supplemental Digital Content**

**Supplemental Digital Content (SDC):** Authors may submit SDC via Editorial Manager to LWW journals that enhance their article's text to be considered for online posting. SDC may include standard media such as text documents, graphs, audio, video, etc. On the Attach Files page of the submission process, please select Supplemental Audio, Video, or Data for your uploaded file as the Submission Item. If an article with SDC is accepted, our production staff will create a URL with the SDC file. The URL will be placed in the call-out within the article. SDC files are not copy-edited by LWW staff, they will be presented digitally as submitted. For a list of all available file types and detailed instructions, please visit <http://links.lww.com/A142>.

### **SDC Call-outs**

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Example:

We performed many tests on the degrees of flexibility in the elbow (see Video, Supplemental Digital Content 1, which demonstrates elbow flexibility) and found our results inconclusive.

### List of Supplemental Digital Content

A listing of Supplemental Digital Content must be submitted at the end of the manuscript file. Include the SDC number and file type of the Supplemental Digital Content. This text will be removed by our production staff and not be published.

Example:

Supplemental Digital Content 1. wmv

### SDC File Requirements

All acceptable file types are permissible up to 10 MBs. For audio or video files greater than 10 MBs, authors should first query the journal office for approval. For a list of all available file types and detailed instructions, please visit <http://links.lww.com/A142>.

**Style:** In general, style should be patterned after the *American Medical Association Manual of Style* (9th edition), *Stedman's Medical Dictionary* (27th edition) and *Merriam Webster's Collegiate Dictionary* (10th edition) should be used as standard references. Drugs and therapeutic agents should be referred to by their accepted generic or chemical names. The name should not be abbreviated. Code numbers should be used only when a generic name is not yet available. In that case, the chemical name and a figure giving the chemical structure of the drug is required. Copyright or trade names of drugs should be capitalized and placed in parentheses after the name of the drug. Names and locations (city and state in USA; city and country outside USA) of manufacturers of drugs, supplies, or equipment cited in a manuscript are required to comply with trademark law and should be provided in parentheses. Units of measure should be expressed in the metric system, and temperatures should be expressed in degrees Celsius. Conventional units should be written as SI units as appropriate.

**CLINICAL AND LABORATORY OBSERVATIONS:** Clinical observations may include case histories that demonstrate novel findings or associations, important clinical responses when a larger study is not needed to address a specific issue, or a unique laboratory observation linked to clinical care and/or practice. Text should contain 2500 words or fewer, with a brief abstract of 100 words or fewer. Abstracts outline background, observation(s), and conclusions. Include 4 illustrations and/or tables or fewer and 20 references or fewer.

**MEDICAL PROGRESS:** Review articles for this section should highlight what is particularly new and novel in a field related to pediatric hematology/oncology. Text should contain 5000 words or fewer and 100 references or fewer. Shorter reviews are encouraged and preferred. Authors considering submission should consult the Editor-in-Chief.

**MORPHOLOGY CORNER:** This section features photographs of especially interesting blood smears, bone marrow, or other tissue specimens that highlight an important aspect of hematology/oncology. Include an introduction of 200 words or fewer, the figure(s), a conclusion of 200 words or fewer, and 6 references or fewer.

**RADIOLOGY CORNER:** This section features photographs of scans of radiographic studies, such as plain radiographs, bone scans, computed tomography scans, magnetic resonance images, or other modalities highlighting a special feature of a topic or case. Include an introduction of 200 words or fewer, the figure(s), a conclusion of 200 words or fewer, and 6 references or fewer.

**HISTORICAL INSIGHTS:** Historical insights include concise descriptions or analyses of historical importance in the field of pediatric hematology/oncology. These may include personal descriptions of historical figures, important papers, and interesting occurrences that led to advancements in pediatric hematology/oncology. Photographs and artwork are welcome. Text should contain 2500 words or fewer and include 25 references or fewer. All material should be original **or carry permission for publication.**

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**ANNOUNCEMENTS:** Announcements should be submitted 6 months in advance of the event date and may include scheduled meetings, symposia, postgraduate courses, and other announcements of interest to specialists in pediatric hematology/oncology.



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