S100B and LDH as parameters to monitor the clinical course of patients with advanced melanoma treated with Vemurafenib

Inaugural-Dissertation
zur Erlangung des Doktorgrades
der Medizin

der Medizinischen Fakultät
der Eberhard Karls Universität
zu Tübingen

vorgelegt von

Abusaif, Sail Nawaf Rashid

2016

Dekan:	Professor Dr. I.B. Autenrieth
1. Berichterstatter:	Privatdozent Dr. T. Eigentler
2. Berichterstatter:	Privatdozent Dr. D. Ketelsen

Table of Contents

List of Figures

List of Tables

Acknowledgement

I would like to show my deepest gratitude for my supervisor, Professor Claus Garbe, for his supervision, guidance and support through the stages of this dissertation. I fully appreciate his willing to teach and help, that exceeded my expectations of support and guidance from a supervisor.

I would like also to thank Dr. Thomas Eigentler for his statistical work on the data collection which was collected by me.

I would like also to dedicate this work to my family. For the accomplishment of this research, their time, strength, and support were essential.

Declaration

I Sail Nawaf Abusaif, declare that the following research and its entire data has been an individual, unaided attempt and have not been published or submitted earlier or by other authors, except me. Additionally, it shows my views and take on the issue and does not describe the view of the University.

Data of this work was published as: Abusaif S, Jradi Z, Held L, Pflugfelder A, et al. S100B and lactate dehydrogenase as response and progression markers during treatment with vemurafenib in patients with advanced melanoma. Melanoma Research. 2013; 23: 396-401.

Chapter 1 Introduction

1.1 Epidemiology

Melanoma is a highly aggressive type of skin cancer with an early potential to metastasize. It is therefore the major cause of mortality among skin neoplasms and accounts for 90% of deaths associated with cutaneous tumors due to metastatic spread.[1]

Melanoma is also a major health issue; it is one of the few malignancies which rapidly increase among Caucasian populations. Even today this trend is still continuing in the industrialized countries.[2] Worldwide approximately 200,000 cases with newly diagnosed melanoma and 46,500 deaths due to melanoma are reported annually. Almost over 80% of the cases occur in general confined to economically developed countries.[3]

In general, high incidence rates are reported in countries with fair-skinned populations. Consequently, the incidence of melanoma has increased over the last 50 years also mostly in fair-skinned populations despite increasing awareness and prevention by the public and health professionals.

The highest incidence of melanoma is present in New Zealand, Australia and the U.S. In Europe, the highest incidence rates were published for the Scandinavian populations, Switzerland, Netherlands and Czech Republic. Lower incidence rates were detected in Southern populations in Europe such as Italy, Spain and Greece.[3] The incidence of melanoma in Germany is currently about 17.1 for men and 16.6 for women per 100,000 inhabitants per year.[4] For many years the incidence rates for female were higher than men.[5] One reason for this change maybe to introduction of a population based screening program for skin cancer in Germany in 2008 (Figure 1-1).

In contrast, despite increasing incidence rates, the mortality rates seem to be stabilized or even decreased due to early detection and effective screening programs and early detection. [6]

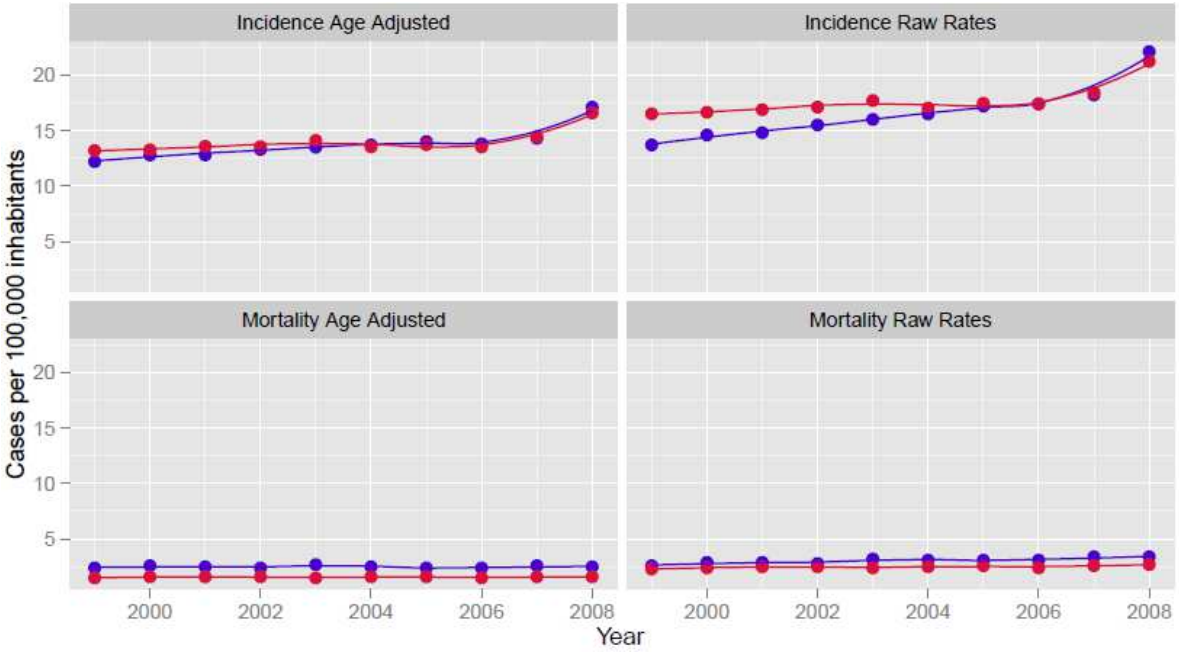

Figure 1-1 German incidence rates (raw rates and age adjusted) and mortalility rates (raw rates and age adjusted) for men (blue) and women (red); data according by the Robert-Koch-Institute, Berlin

1.2 Etiology and risk factors

Generally, the risk for developing melanoma depends on two major principles: Firstly on environmental factors and, secondly on intrinsic factors.

Exposure to UV-radiation is the most important exogenic risk factor for the development of malignant melanoma. The risk to develop malignant melanoma is mostly acquired during childhood and adolescence (before the age of 20 years, mainly in the first decade of life) due to an UV-induced activation of the melanocytic system. This activations leads to the development of benign melanocytic lesions known as nevi:

- The risk to develop malignant melanoma increases with by the total number of melanocytic nevi over the entire body.[7]
- The type of nevi (atypical melanocytic nevi, dysplastic nevi) are additional independent risk factors for melanoma.[7, 8]

- People with skin type I according to Fitzpatrick (fair skin, red or blond hair, tendency to freckle, blue eyes) are more susceptible to develop melanoma than more darkly pigmented people.

- Chronic sun exposure associated with increased incidence of lentigo maligna melanoma, while intermittent sun exposure is associated with superficial and nodular melanoma.[8]

- Familial incidence of melanoma (two or more first degree relatives with the mutations in CDKN2A are detectable diseases [8, 9]) is evident in about 5%-10% of melanoma patients.
  Their lifetime risk to develop melanoma is estimated to reach 67%.[10]

Interestingly, most of the patients detect a suspicious lesion themselves. Only selected individuals, for example, such with a dysplastic nevus syndrome, are regularly screened by dermatologists. In such cases digital dermatoscopy allows analyzing and documenting pigmented lesions reliably. Often diagnostic clues are exclusively detectable using this technique. Experts reach an increase of 20% in the diagnostic sensitivity in contrast to conventional clinical observation. They achieve a sensitivity of about 90% and specify of approximately 80%.[11] Up to date systems are computer based and include automated diagnostic algorithms.

1.3 Genetic factors

Genetic aberrations in melanoma frequently affect cell signaling pathways that play an essential role in normal melanocyte biology. Specific aberrations in melanoma signaling provide treatment targets for molecular therapies. Promising treatment targets are for example genes upregulated in cancer, yet not in normal tissues, or genes with frequent oncogenic mutations that can be specifically targeted.

1.3.1 BRAF

Mutations in the BRAF gene were firstly described by the Sanger Institute in 2002.[12] It is the most commonly mutated oncogene identified in melanoma (approximately 50%) to date and an upstream mediator of the mitogen-activated

protein kinase (MAPK) pathway.[12, 13] In over 80% of the patients this activating mutation results from a substitution of glutamic acid by valine at amino acid 600 (V600E mutation) with most of the remainder consisting of an alternate substitution (lysine for valine) at the V600 locus (V to K); however different other mutations are known meanwhile (Table 1-1).

Table 1-1 Frequency of BRAF mutations [15]

V600E	97.3%
V600K	1.0%
K601E	0.4%
G463A	0.4%
D594G	0.3%
V600R	0.3%
L597V	0.2%

Increased activation of the MAPK pathway is implicated in melanoma tumor genesis and is enhanced in advanced-stage melanoma.[13] Generally, BRAF-mutated melanomas occur in younger aged patients on skin without signs of solar damage and affect less frequently the head and neck area. Therefore, BRAF-mutated melanomas seem to arise early in life at low cumulative UV doses, whereas melanomas without BRAF mutations seem to require accumulation of high UV doses over time.[14]

1.3.2 RAS

Three types of human RAS proto-oncogenes (H-RAS, K-RAS, and N-RAS) have been described. N-RAS mutations are frequent in myeloid leukemias and melanomas.[16] Up to 24% of malignant melanoma have activated RAS gene mutations mutations.[17] N-RAS mutations are detectable more often in melanomas arising from chronic sun exposure and are more common in lentigo maligna melanoma and nodular melanoma than in superficial spreading and acrolentiginous melanoma.[16] RAS mutations are also present in 10% of common acquired nevi and 28% - 56% of congenital nevi.[16]

1.3.3 KIT

C-KIT is a protein that acts as a fundamental growth factor receptor in epidermal melanocytes and is important for differentiation and migration of melanocytic cells during embryonic development.[18, 19] The receptor tyrosine kinase KIT acts on a downstream signaling cascade leading to key intracellular signals controlling cellular proliferation and survival.[20] KIT aberrations were identified in melanomas of mucosal membranes, acral skin and skin with chronic sun-induced damage. KIT aberrations are present in up to 39%, 36%, 26% respectively.[19]

1.4 Management of the Primary Melanoma

Ideally, lesions that are clinically suspicious for melanoma ought to experience an excisional biopsy with slender boundaries (such as 2 mm).[21] Although there is proof that an incisional biopsy does not influence survival,[22] this methodology should ought to be a special case and kept for situations where the tumor is too big to be extracted, or when it is unrealistic to perform a complete excision (for example; the nail unit). The excised sample should be interpreted by an experienced dermatopathologist acquainted with the microscopic diagnosis of melanocytic lesions.

Following histologic diagnosis, the primary melanoma site should be re-excised with a proper margin determined by the Breslow's depth. The basis for extending the excisional margins is the capability of melanoma cells to relocate far from the tumor origin. Melanoma may amplify more extensive or more profound than at first obvious. The major aim is to avert neighborhood repeat or constant infection. Present recommendations are extraction margins of 0.5 cm for *in situ* melanoma, 1 cm for melanoma up to 2 mm tumor thickness and 2 cm for melanomas with more than 2 mm tumor thickness.[1, 23] Margins of excision are also limited by surgically difficult anatomic sites such as the face, the mucous membranes or the distal extremities, and, in numerous examples, an individualized surgical methodology must be undertaken. Typically, lentigo maligna melanoma in the face requires narrower safety margins, and micrographic control of extraction margins may be included so as to safe tissue. Surgical methodologies ought to respect the structure of the face as well as aesthetic and functional aspects.

Lentiginous acral and mucosal melanomas are regularly ineffectively characterized and multifocal with inconsistencies between the clinically visible and histopathologic margins. Local recurrences are more common in these sorts of melanoma. In this manner, elimination can be achieved with expanded safety margins (at least 1cm) or by narrow margins with micrographic control.[24] Likewise, micrographic surgery is indicated for subungual melanoma in order to assure tumor-free resection margins and to achieve better cosmetic and functional results by avoiding amputation.[25]

1.5 Management of the Regional Metastatic Melanoma

In about 70% of the cases metastatic spread of melanoma is primarily regional and confined to the site of the primary melanoma and its draining lymph nodes. Metastasis may manifest as a clinically occult lymph node micro metastasis, as a rapidly growing clinically evident macro metastasis, or as in-transit metastasis.

Approximately 20% of patients with a cutaneous melanoma that is >1 mm in depth plus no evidence (clinically or radiologically) of detectable nodal disease at initial presentation show microscopic involvement.[26] On the premise of an assumed relocation of melanoma cells in an efficient manner towards the draining lymph node, surgical resection of territorial lymph nodes in all patients who suffered from intermediate and high-risk tumors was suggested in the 1980s and alluded to as 'elective lymph node dissection' (ELND). However, four multi-center randomized planned trials in patients with primary melanoma failed to demonstrate a survival benefit for patients treated with ELND plus wide re-excision as compared to wide re-excision alone.[27-30]

As a consequence, a less traumatic strategy to identify regional metastatic disease was presented, assigned as sentinel lymph node biopsy (SLNB).[31] SLNB is based on the finding that the cutaneous site of the melanoma drains to one or more lymph node basins and specially to one (or two yet once in a while more) lymph node, the sentinel node, which is the first site of deposition of metastatic cells. The draining lymph node basins for a given melanoma site and the estimate location of the sentinel node within that basin are identified and

marked on the overlying skin preoperatively during lymphoscintigraphy performed in the nuclear medicine suite. Intraoperatively mainly in conjunction with the wide local excision, technetium sulfur colloid and blue dye are injected into the skin encompassing the melanoma biopsy site. A small cut is made at the beforehand checked site overlying the sentinel node and visual inspection and a hand-held gamma counter are used to identify the 'hot, blue' sentinel node(s) which is selectively biopsied and inspected by serial sectioning using H&E stains joined with immunohistochemistry (S100, HMB45). If melanoma micro metastases are identified, a complete regional lymph node dissection is usually recommended. As the therapeutic benefit of complete lymphadenectomy after positive sentinel node has not been demonstrated in clinical trials, two large multicenter trials (MSLT-2, ADO-LNB1) are presently conducted comparing complete lymphadenectomy versus observation only in patients with lymph node micro metastasis.

Sentinel lymph node biopsy followed by complete lymphadenectomy in case of positive nodes has meanwhile become a standard procedure in treatment and staging of primary cutaneous melanoma of 1 mm tumor thickness or more. Numerous publications identified the status of the sentinel lymph node as a strong prognostic factor for survival and recurrence, and the American Joint Committee on Cancer included it in the latest staging system for cutaneous melanoma.[32] Published in 2006 and 2014 the randomized Multicenter Selective Lymphadenectomy Trial (MSLT-1) confirmed the prognostic value of SLND, and found improved disease free survival for the SLND group, improved survival for SLND positive patients with complete lymphadenectomy as compared to patients developing macroscopic nodal metastasis in the control group, but no difference in overall survival.[33, 34].

If lymph node metastases are diagnosed clinically or by imaging techniques, complete lymph node dissection is considered standard therapy, which consists of an anatomically complete dissection of the involved nodal basin.[1] The extent of complete lymph node dissection is often modified according to the anatomic area of lymph node involvement.

1.6 Adjuvant Treatment of Melanoma

Patients with thick (>2.0 mm) primary melanoma and/or regional lymph node metastases are at expanded danger of repeat and demise.[32] Current recommendations for patients with stage II (as showed by AJCC/UICC classification, yet negative nodes) melanoma are for adjuvant treatment with IFN or enlistment in a clinical trial.[1, 35] Patients with stage III melanoma regularly experience complete lymphadenectomy took after adjuvant treatment with IFN or enlistment in a clinical trial of adjuvant therapy.[1, 35] Over the last 25 years, adjuvant treatment for impending danger (stage II and IIIA) and high-hazard (stage IIIB and in addition resectable stage IV M1a, M1b) patients have stirred from systemic immunostimulants such as pharmacologic immunomodulators such as levamisole, or Bacillus Calmette-Guerin (BCG), Corynebacterium parvum and regional radiotherapy, to recombinant DNA-produced biologic agents such as antibodies, IFN-α, and granulocyte-macrophage colony-stimulating factor that have immunoregulatory function.

Recently, first results from an EORTC trial comparing 10mg/kg Ipilimumab vs. placebo (1:1) in 951 patients with stage III disease were published.[36] After 2.7 years of median follow up Ipilimumab significantly increased recurrence free survival for actively treated patients. However, the majority of the patients receiving Ipilimumab discontinued treatment because of side effects.

1.7 Treatment of Metastatic Melanoma

In previous decades median survival time was estimated to be approximately 8 months (±2 months) for patients suffering from AJCC stage IV metastatic melanoma, and only ~10% of the patients survived more than 5 years from diagnosis of metastatic melanoma.[32] Currently, several agents are acknowledged for the treatment of patients with metastatic melanoma in the US: dacarbazine and high-dose IL-2 were registered in the late 70’s and 90’s. Ipilimumab, a CTLA-4 antibody was approved by the FDA in 2011, followed by the approval of Vemurafenib (BRAF-inhibitor 2011), Dabrafenib (BRAF-inhibitor

2013), Trametinib (MEK-inhibitor 2013) and the combination of Dabrafenib & Trametinib (2014).

1.7.1 Chemotherapy

Chemotherapy is still an accepted palliative treatment for stage IV metastatic disease and dacarbazine is the most broadly utilized sole chemotherapeutic agent for the treatment of metastatic melanoma.[37] Dacarbazine was initially reported to yield objective reactions for almost 25% of patients in older phase II trials, yet current trials in more rigorous, large-scale, supportive group settings have shown response rates of 5%-12%.[37] Unfortunately, most reactions to this agent and its oral analogue temozolomide are transient; only 1%-2% of patients accomplish a robust long-term response to chemotherapy.[37] Other chemotherapies that have been investigated incorporate fotemustine that has essentially enhanced the objective response rate (15.2% vs. 6.8%; p=0.043) and prolonged median overall survival, although non-significantly (7.3 months vs. 5.6 months; p=0.067) comparing with dacarbazine in a phase III trial.[38]

The antitumor activity of mixed chemotherapeutic agents has been assessed as an outcome of the increasingly frequently-held conviction that single agents are unlikely to enhance the result of patients with advanced metastatic melanoma.[37] Other polychemotherapies examined in phase III trials (for example, Dartmouth regimen: vinblastine/ cisplatin/ tamoxifen/ dacarbazine) have failed to exhibit a survival benefit compared with dacarbazine alone.[39]

For patients who are not qualified for current investigational trials, chemotherapy with one of these agents remains a sensible palliative choice; for novel agents being tried in clinical trials, chemotherapy is an acknowledged comparator.[37]

1.7.2 Interleukin-2 and other Immunotherapies

High-dose recombinant IL-2 received its FDA approval in 1998 for the treatment of patients with metastatic melanoma. Objective response rates of up to 16% were seen in a group of phase II trials in patients (N=47) with metastatic melanoma exhibited for administrative audit.

Single-agent therapy was managed utilizing the high-dose regimen of 600,000 U/kg IL-2 at regular interval time of 8 hours for up to 14 doses in inpatient

cohorts.[40] barely 5% of the patients had long-term, durable complete reactions with IL-2, which has been taken as potential cure. However, this therapy has never shown to improve overall survival in a randomized phase III trial.
In addition, IL-2 induced toxicity is severe [40] and normally requires intensive care.[41] Major dose-limiting toxicities include hemodynamic toxicity like hypotension, edema, weight gain, and decreased renal function as well as respiratory insufficiency, and neurotoxicity.[41] In contrast to the US, high-dose recombinant IL-2 has not been permitted in Europe.

1.7.3 Bio-Chemotherapy

Biochemotherapy is the combination of a chemotherapeutic schedule (polychemotherapy) and the addition of cytokines. In a survey of 41 randomized clinical trials of patients receiving several treatment schedules, including many biochemotherapy regimens, none of them enhanced progression-free survival or overall survival.[37] Furthermore, a meta-analysis including 18 trials and over 2600 patients with metastatic melanoma proposed favorable position of biochemotherapy regarding objective response yet discovered no advantage in terms of overall survival (p=0.9).[42]

1.8 Novel Therapies for Patients with Metastatic Melanoma

A continuous improvement in understanding the tumor genesis and biology as well as the nature of immune antitumor response and regulation has led to the development of several novel sophisticated anticancer agents. Different methods to overcome tolerance include inhibition of oncogenic kinase pathways, blockade of inhibitory immune receptors and downregulation of anti-apoptotic proteins.

1.8.1 Antibody Blockade of Cytotoxic T lymphocyte-associated Antigen 4

Basically, a full activation of T-cells requires two major signals: First, an incitement through the T-cell receptor and additionally a Co-stimulatory signal regularly given by the binding of B7 on the antigen-presenting cell (such as dendritic cell) to CD28 on the T cell. Cytotoxic T lymphocyte associated antigen 4 (CTLA4) is an inhibitory T-cell receptor and a homologue of CD28 that is

upregulated following T cell activation. The normal function of CTLA4 is to strive with CD28 to bind B7 and to downregulate T cell activation, acting as a usual “brake" by removing the costimulatory signal. The CTLA4-B7 interaction can be obstructed with an anti-CTLA4 monoclonal antibody (mAb), which has a greater liking for CTLA4 than B7. Accordingly, the inhibitory signal is prevented and the “brake" on T-cell activation released.

Two fully human anti-CTLA4 antibodies were developed in melanoma: tremelimumab and ipilimumab. Target response rates of patients with metastatic melanoma treated with any of the two anti-CLTA4 antibodies as sole agent was quite similar (7%-10%).[43, 44] A randomized study in a phase III of chemotherapy (n=327) with dacarbazine or temozolomide in treatment-naive patients and tremelimumab (15 mg/kg administered once every 3 months, n=328), middle survival was longer (almost 12 months) in patients treated with tremelimumab contrasted with chemotherapy (barely 11 months).[43] However, the distinction was not factually noteworthy (hazard ratio chemotherapy/ tremelimumab 1.04; p=0.729), and the trial was stopped at the second interim analysis.

Ipilimumab was likewise examined in a large phase III trial in patients with advanced melanoma.[44] Results of this randomized phase III trial for the mixture of Ipilimumab treatment versus gp100 vaccination, and versus single ipilmumab and gp100 vaccination have been published demonstrating an enhanced general survival of a median duration of 10 months in the ipilimumab arm and the combined arm, against 6.4 months in the vaccination alone arm. Although objective reaction rates were fairly low with 5.7% in the combined ipilimumab and vaccination arm, 10.9% in the ipilimumab alone arm versus 1.5% in the gp100 vaccination alone arm, highly significant contrasts in hazard rates for overall survival resulted were detected between the combined arm versus vaccination alone (0.68; $p < 0.001$) and between ipilimumab alone versus vaccination alone (0.66; $p = 0,003$).[44] This information prompted the regard of ipilimumab by health authorities for the treatment of advanced melanoma.

Overall, responses to anti-CTLA4 antibodies seem to be durable, yet may take the length of 12 weeks or much more to develop and late-onset objective

reactions are sometimes preceded by months of stable illness or even transient sickness progression. Side effects with CTLA4 barricade are autoimmune-related, however less intense than those detected with exogenous cytokine therapy and are controllable.[44] Most common side effects include diarrhea and rash.[43, 44]

1.8.2 MAPK Signaling and Inhibitors

On the upstream level of receptor tyrosine kinases, 30% to 40% of the acral and mucosal melanomas and melanomas from chronically sun-exposed skin harbor activating mutations or copy number amplifications of the KIT gene.[19] Most frequently affected by constitutively activating mutations are in about 15% to 20% the NRAS gene and in 50%-60% the BRAF gene.[45]
Interestingly, the frequency of BRAF mutations is high in melanomas from intermittently UV exposed skin, yet low in melanomas with histopathologic signs of high UV damage to the skin,[19, 45] i.e. with increasing amounts of mutagenic UV radiation the BRAF mutation frequency drops.

Vemurafenib formerly also known as RG7204 or PLX4032 is a selective inhibitor of the oncogenic V600E mutant BRAF kinase. With evaluated response rates ranging between 60% and 88%, Vemurafenib represents a therapeutic milestone in melanoma patients since decades [3-5]. Patients with solid tumors carrying the V600E mutation showed an improvement up to 70% after phase I dose escalation study according to ASCO 2009.[46] These promising data was confirmed in the consecutive phase II trial including 132 patients with metastatic melanoma carrying a BRAF V600 mutation.[47] The overall response rate in that trial was 53% while the median duration of response, the median progression-free survival and the overall median survival were reported to be 6.7, 6.8 and 15.9 months, respectively.[47] Moreover, an increase in overall survival up to 14 months compared to 9 months with standard dacarbazine treatment was reported whereas some patients are still under treatment after 2 years.[6]

According to the Data which was released in 2011 from the phase III registration trial, 675 patients were randomly assigned to receive vemurafenib (960mg p.o. bid) or dacarbazine (1000/m2 i.v. every 3 weeks). [48] After 6 months, overall

survival was superior in the vemurafenib group compared to the dacarbazine cohort (84% vs. 64%). The hazard ratio for death in the vemurafenib group was 0.37 ($p<0.001$).
Response rates were 48% for the vemurafenib and 5% for the dacarbazine treatment arm. In an updated analysis, median overall survival rates for vemurafenib and DTIC treated patients were 13.2 months and 9.6 months, respectively.[49] In this updated analysis hazard ratios for progression free and overall survival were rendered to be 0.38 ($p<0.001$) and 0.70 ($p<0.001$), respectively.

Other BRAF inhibitors such as dabrafenib and encorafenib are likewise in clinical routine or trials. Recently, data from another registration phase III trial comparing dabrafenib and dacarbazine was published [50]. In this trial, 250 BRAF-(V600E) patients were randomly assigned to receive either dabrafenib 150 mg twice daily p.o. (187 patients) or dacarbazine 1000/m2 i.v. every 3 weeks (63 patients). Median progression-free survival was 5.1 months for dabrafenib and 2.7 months for dacarbazine, hazard ratio 0.30 ($p<0.0001$). Most common observed adverse events in the dabrafenib arm were skin-related toxic effects, fever, fatigue, arthralgia, and headache.

1.9 Aims of this Evaluation

Patients under vemurafenib treatment normally receive CT staging every 6-12 weeks, resulting in 8-16 CT examinations in two years. The potential risk of sequential CT scans to induce cancer is still controversially discussed. In a recent analysis the risk is numbered to be 0.7% which seems to be low, however approximately 29,000 cancers could be related to CT scans in the USA every year.[51] With increasing overall survival time due to effective treatments the reduction of life time radiation and CT scans should also be considered in stage IV melanoma patients.

In this evaluation we were interested in whether S100B and LDH are able to monitor and predict objective tumor responses and tumor progression of

vemurafenib treated patients. Radiologic measurements according to RECIST were taken as gold standard for monitoring patient's course of the disease.

Chapter 2 Material and Methods

2.1 Patients

All patients suffering from unresectable metastatic melanoma who were treated with vemurafenib at the Department of Dermatology, University Hospital Tuebingen, Germany, between May 2010 and September 2012 were included in this retrospective analysis. Data was extracted from the clinical database.

2.1.1 S-100B

Serum S100B concentrations were measured using the commercially available Cobas e411 S100B electrochemiluminescence assay by Roche Diagnostics (Mannheim, Germany). The assay is based on the tow sandwich principle. Total duration of the determination of S100B is 18 minutes.

Analyses were performed according to the manufacturer's instructions and previously published protocols: First, incubation 20(µl) of sample, abiotinylated monoclonal S100-specific antibody, and a monoclonal S100-specific antibody labeled with a ruthenium complex react to form sandwich complex. Second incubation; after expansion of streptavidin-coated microparticles, the composite becomes bound to the solid phase via interaction of streptavidin and biotin. The response mixture is suctioned into the determining cell where the microparticles are magnetically seized onto the surface of the electrode. Unbound substances are then eleminated with ProCell. Use of a voltage to the electrode then prompts chemiluminescent emission which is measured by a photomultiplier. Results are resolved via a calibration curve which is instrument-particularly generated by 2-point calibration and master curve provided through the reagent barcode. Values above 0.100 µg/ml were considered as pathological levels.

2.1.2 LDH

LDH was measured in serum samples at the start of vemurafenib treatment and at the time points of CT imaging. Measurement of LDH activity was done by kinetic enzyme method on an automatic Siemens ADVIA 1800 (Siemens Healthcare Diagnostics, Eschborn, Germany) device. It uses a reversible

oxidation method of lactate to pyruvate with simultaneous reduction of nicotinamide adenine dinucleotide (NAD+) to NADH and H^+ at a pH of 8.55. The change in absorption is measured at 340 nm and considered as proportional to LDH activity.

2.1.3 Radiological Evaluation

All patients received contrast-enhanced CT scans of the brain, head & neck, thorax and abdominal region before start of vemurafenib treatment. Target lesions were defined according to RECIST V1.1 and followed up during regular staging evaluations. RECIST 1.1 defined criteria were used to determine complete response, partial response, stable and progressive disease:

2.1.3.1 Evaluation of Target Lesions

2.1.3.1.1 Complete Response (CR)

- Disappearance of all target lesions.
- Any pathological lymph nodes (whether target or non-target) must have lessening in short axis to <10 mm.

2.1.3.1.2 Partial Response (PR)

- At minimum a 30% reduction in the sum of diameters of target lesions, taking as reference the baseline sum diameters.

2.1.3.1.3 Progressive Disease (PD)

- At least a 20% increment in the sum of diameters of target lesions, taking as reference the smallest sum on study (this includes the baseline sum if that is the smallest on study). Notwithstanding the relative increment of 20%, the whole must likewise exhibit a flat out expansion of no less than 5 mm. (Note: the appearance of one or more new lesions is likewise considered progression).

2.1.3.1.4 Stable Disease (SD)

- Neither sufficient shrinkage to qualify for PR nor sufficient growth to qualify for PD, The smallest diameters was taken as reference while on study.

2.1.3.2 Evaluation of Non-Target Lesions

2.1.3.2.1 Progressive Disease (PD)

- Unequivocal progression of existing non-target lesions. (Note: the existence of one or more new lesions is also considered progression).

All patients had to have unresectable stage III or VI melanoma and were tested to carry the BRAF V600E mutation using the Roche Cobas Assay or by analysing tumor tissue by conventional Sanger sequencing. Cobas tests were performed at Targos Molecular Pathology GmbH, Kassel, Germany.

2.1.3.2.2 Complete Response (CR)

- Disappearance of all non-target lesions and normalisation of tumour marker level. All lymph nodes have to be non-pathological in size (<10 mm short axis)

2.1.3.2.3 Non-CR / Non-PD

- Persistence of one or more non-target lesion(s) and/or maintenance of tumour marker level over the standard limits.

2.1.4 Statistics

R 2.15.2 [52] with the package “caret”[53] was used for all statistical analyses. Figures were plotted using ggplot2 Version 0.95.[54] Accuracy values for predicting RECIST confirmed tumor response and S100B/LDH with 95% confidence intervals were calculated. Correlation was also evaluated using Spearman's rank correlation [rho]. Additionally, polynominal regression models (linear, quadratic and cubic) were established and compared via ANOVA. For all statistical tests a p-value of 0.05 was considered to describe a significant result.

Chapter 3 Results

3.1 Patients Characteristics

44 patients were evaluable and their characteristics at baseline are shown in Table 3.1.1. Almost 75% of the patients had elevated serum S100B protein at baseline while half of the patients had elevated levels of LDH at baseline. The course for RECIST measured target lesions, LDH and S100B is illustrated in Figure 3.3.13 and Figure 3.3.21 respectively.

3.2 Response and Correlation with S100B and LDH

43 patients out of 44 patients showed a RECIST confirmed reduction of target lesions in the first CT imaging (Figure 3.1; Figure 3.13). S100B and LDH elevated above the upper limit of normal (ULN) were both sufficient parameters to correlate with RECIST defined tumor response at time point of first staging (accuracy 81.2% for S100B and 85.7% for LDH, Table 3.2; Figure 3.15 and Figure 3.19). The median decrease for RECIST confirmed response was 30.9% from baseline (25% quartile: 19.3%; 75% quartile: 39.8%), for S100B the median decrease was 79.1% from baseline (25% quartile: 39.6%; 75% quartile: 96.6%) and for LDH the median decrease was 38.5% from baseline (25% quartile: 22.0%; 75% quartile: 55.1%).

In case of normal values at baseline both parameters were not able to predict tumor response adequately (accuracy 63.6% for S100B and 61.4% for LDH, Table 3-2; Figure 3-18 and Figure 3-22). Neither the linear or the quadratic nor the cubic regression model fitted well for correlation (Table 3-2).

14 of 32 patients with elevated serum S100B at baseline showed normalization of serum S100B levels at time point of the first staging. All of these patients showed a RECIST confirmed decrease in target lesions. 11 of 22 patients with elevated serum LDH at start of treatment with vemurafenib showed normalization of serum LDH levels at time point of the first staging. All of these patients showed a RECIST confirmed decrease in target lesions.

Table 3-1 Patient characteristics at baseline

Age (Years)	
Mean	53
Range	29-77
Sex (n)	
Female	19, 43%
Male	25, 57%
Histological type (n)	
Nodular	16, 36%
Superficial spreading	16, 36%
Acrolentiginous	4, 9%
unknown primary origin	8, 18%
Localization of the primary melanoma (n)	
Head	1, 2%
Neck	1, 2%
Back	11, 25%
Trunk	7, 16%
Upper extremity	2, 5%
Lower extremity	14, 32%
Ulceration	
Present	12, 27%
Absent	10, 22%
Unknown	22, 50% (8 cases with no primary melanoma)
Tumor thickness (mm)	
Mean	3.4mm according to Breslow
Range	0.55mm-17mm
Line of therapy (n)	
First	20, 45%
Second	24, 55%

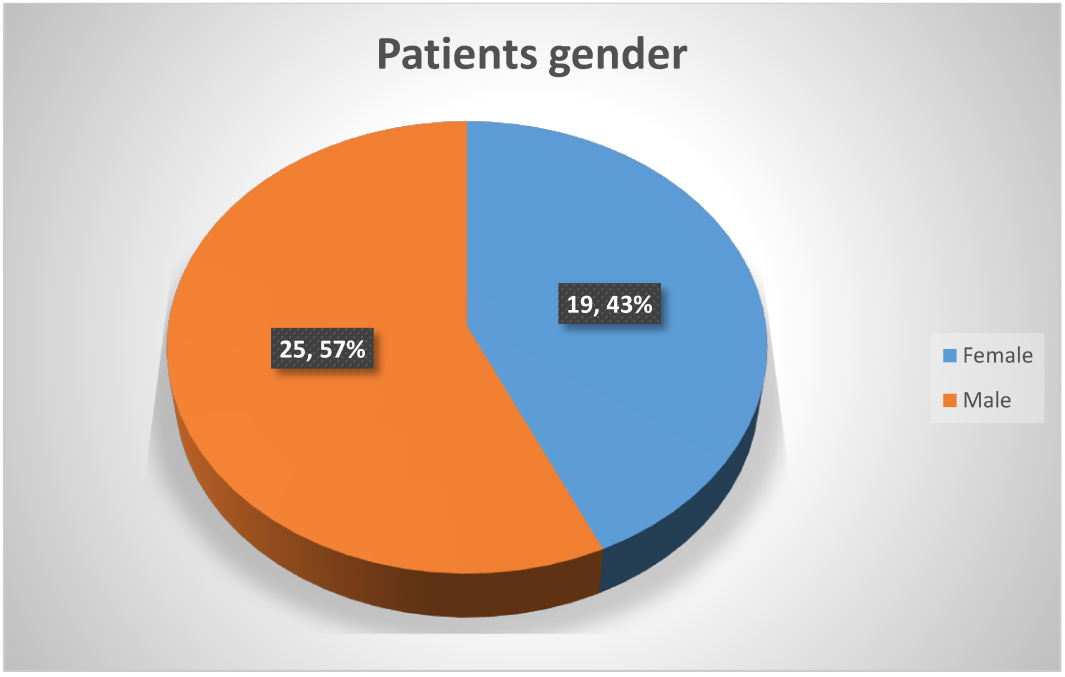

Figure 3-1 Patients gender characteristic

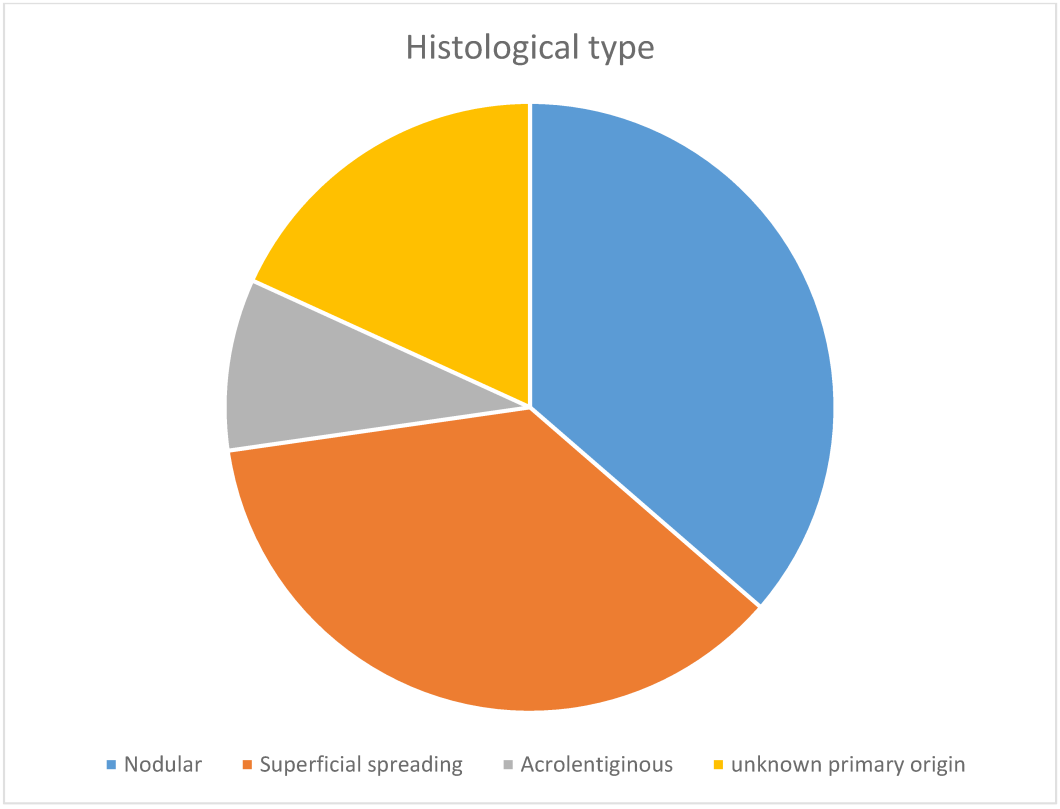

Figure 3-2 Histological type for patients characteristic

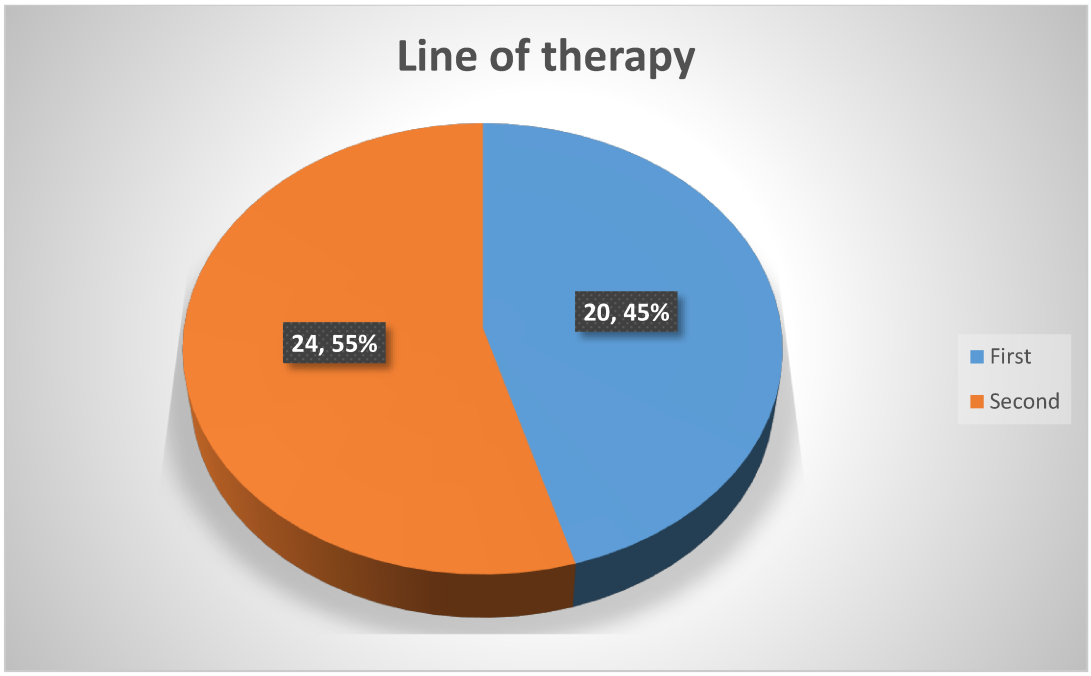

Figure 3-3 Line of therapy for patients characteristic

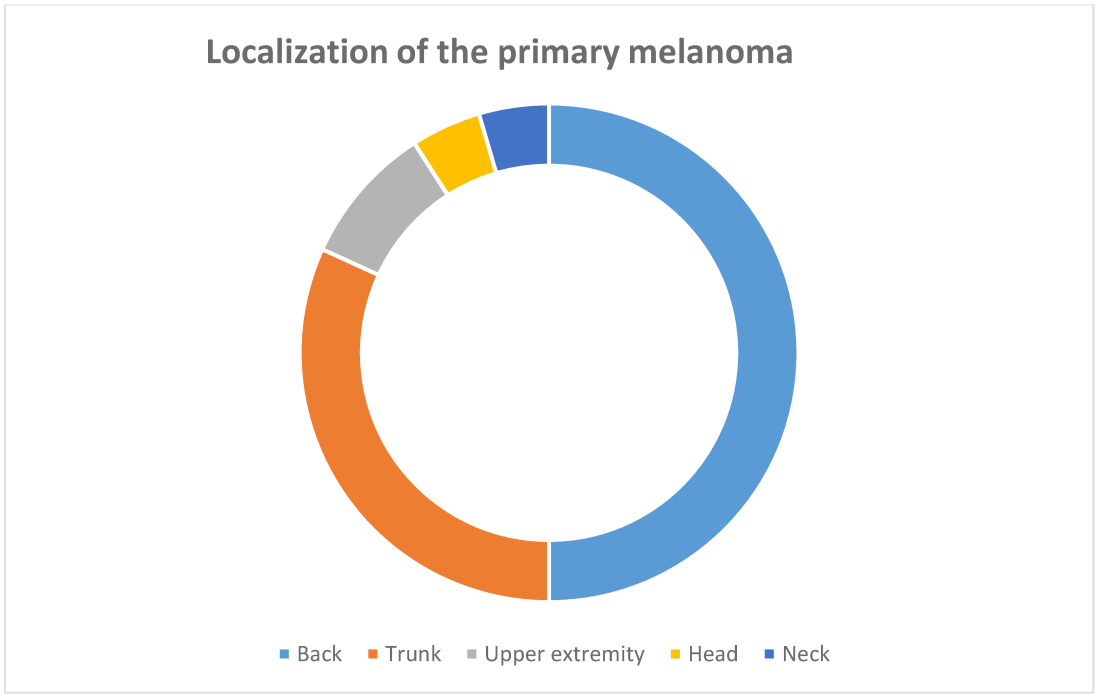

Figure 3-4 Localization of the primary melanoma for patients characteristic

Table 3-2 Accuracy and regression models for correlation between RECIST and S100/LDH

	RECIST vs S100B	RECIST vs S100B evaluated at baseline	RECIST vs LDH	RECIST vs LDH evaluated at baseline
Accuracy	63.6% 95% CI: (47.8, 77.6)	81.2% 95% CI: (63.6, 92.8)	61.4% 95% CI: (45.5, 75.6)	85.7% 95% CI: (63.7, 97.0)
Linear model	p=0.404	p=0.445	p=0.0714	p=0.0831
Quadratic model	p=0.356	p=0.706	p=0.0868	p=0.17
Cubic model	p=0.259	p=0.326	p=0.132	p=0.211
ANOVA	linear vs. quadratic Pr(>F): 0.24 linear vs. cubic Pr(>F): 0.16	linear vs. quadratic Pr(>F): 0.72 linear vs. cubic Pr(>F): 0.10	linear vs. quadratic Pr(>F): 0.20 linear vs. cubic Pr(>F): 0.38	linear vs. quadratic Pr(>F): 0.43 linear vs. cubic Pr(>F): 0.32

3.3 Progression and Correlation with S100B and LDH

Overall, 36 out of 44 patients had progressive disease. At time point of RECIST-confirmed progression 14 patients had increased levels above ULN for LDH and 23 patients had pathologic levels of S100B. Eight patients had both, increased serum S100B values and elevated LDH levels at the same time. 19 patients were judged to have progressive disease because of increased target lesions whereas the other 19 patients had new lesions at time point of progression.

The accuracy to predict progression was 30.3% for S100B and 32.4% for LDH. For patients with increased values at baseline the accuracy rates were 26.9% for S100B and 21.1% for LDH (Table 3-3). After constructing the polynomial regression models for S100B evaluated at baseline, LDH and LDH evaluated at baseline indicated significant p-values for linear, quadratic and cubic evaluations, however, all of them failed in the ANOVA analysis.

Table 3-3 Accuracy and regression models for correlation between RECIST and S100/LDH

	RECIST vs S100B	**RECIST vs S100B evaluated at baseline**	**RECIST vs LDH**	**RECIST vs LDH evaluated at baseline**
Accuracy	30.3% 95% CI: (15.6, 48.7)	26.9% 95% CI: (11.6, 47.8)	32.4% 95% CI: (17.4, 50.5)	21.1% 95% CI: (6.1,45.6)
Linear model	p=0.476	p=0.0382	p=0.0286	p=0.036
Quadratic model	p=0.687	p=0.0394	p=0.0294	p=0.0131
Cubic model	p=0.372	p=0.0416	p=0.024	p=0.0229
ANOVA	linear vs. quadratic Pr(>F): 0.61 linear vs. cubic Pr(>F): 0.13	linear vs. quadratic Pr(>F): 0.13 linear vs. cubic Pr(>F): 0.18	linear vs. quadratic Pr(>F): 0.12 linear vs. cubic Pr(>F): 0.12	linear vs. quadratic Pr(>F): 0.039 linear vs. cubic Pr(>F): 0.297

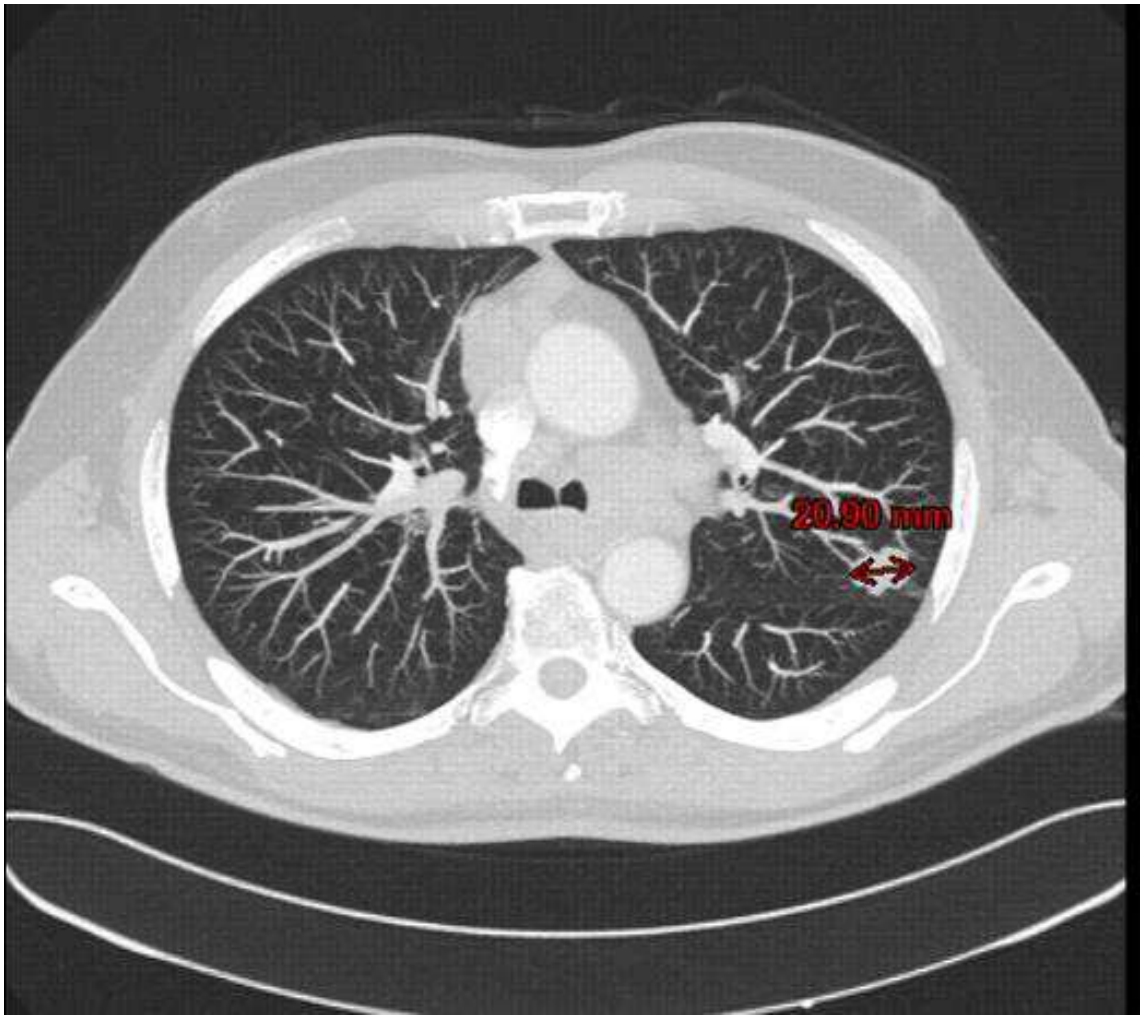

Figure 3-5 Patient 1 - Pulmonary metastasis at baseline

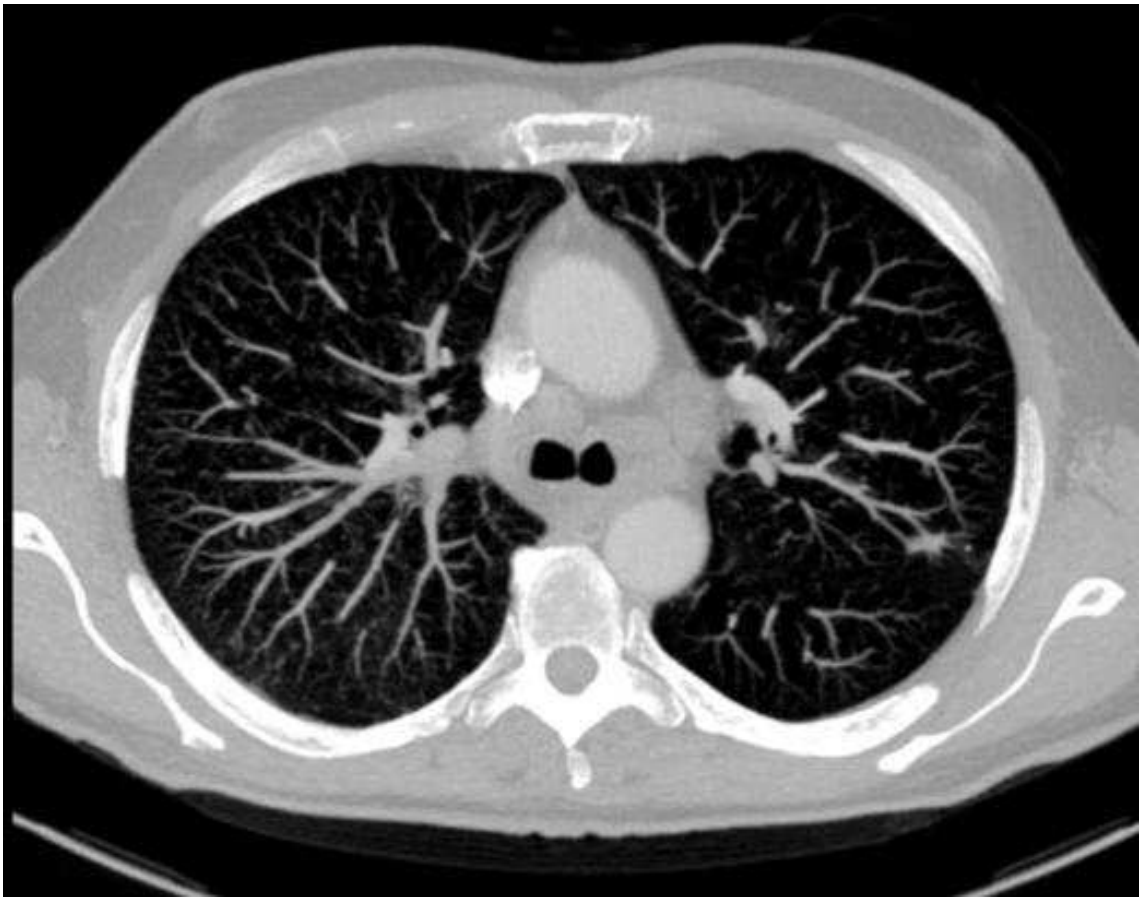

Figure 3-6 Patient 1 - Complete response: Disappearance of pulmonary metastasis after eight weeks of treatment, only small remnants visible

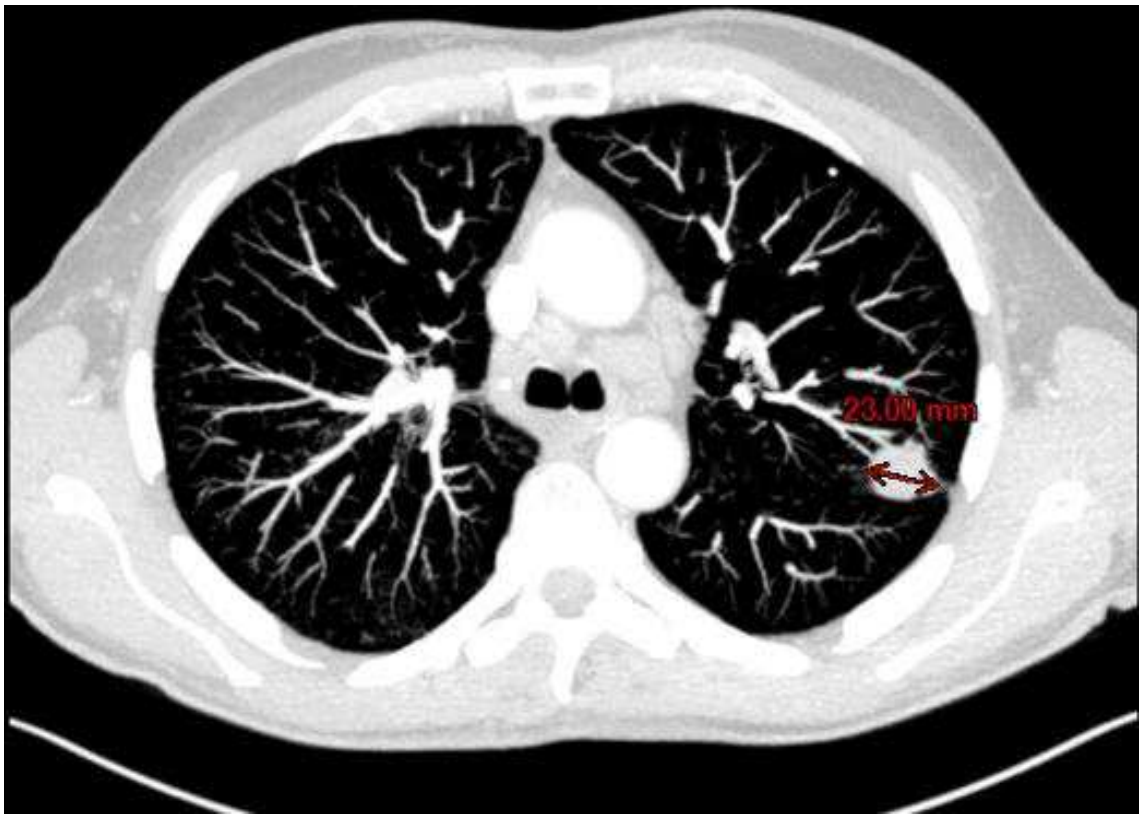

Figure 3-7 Patient 1 - Progressive disease: Progression of pulmonary metastasis at week 48

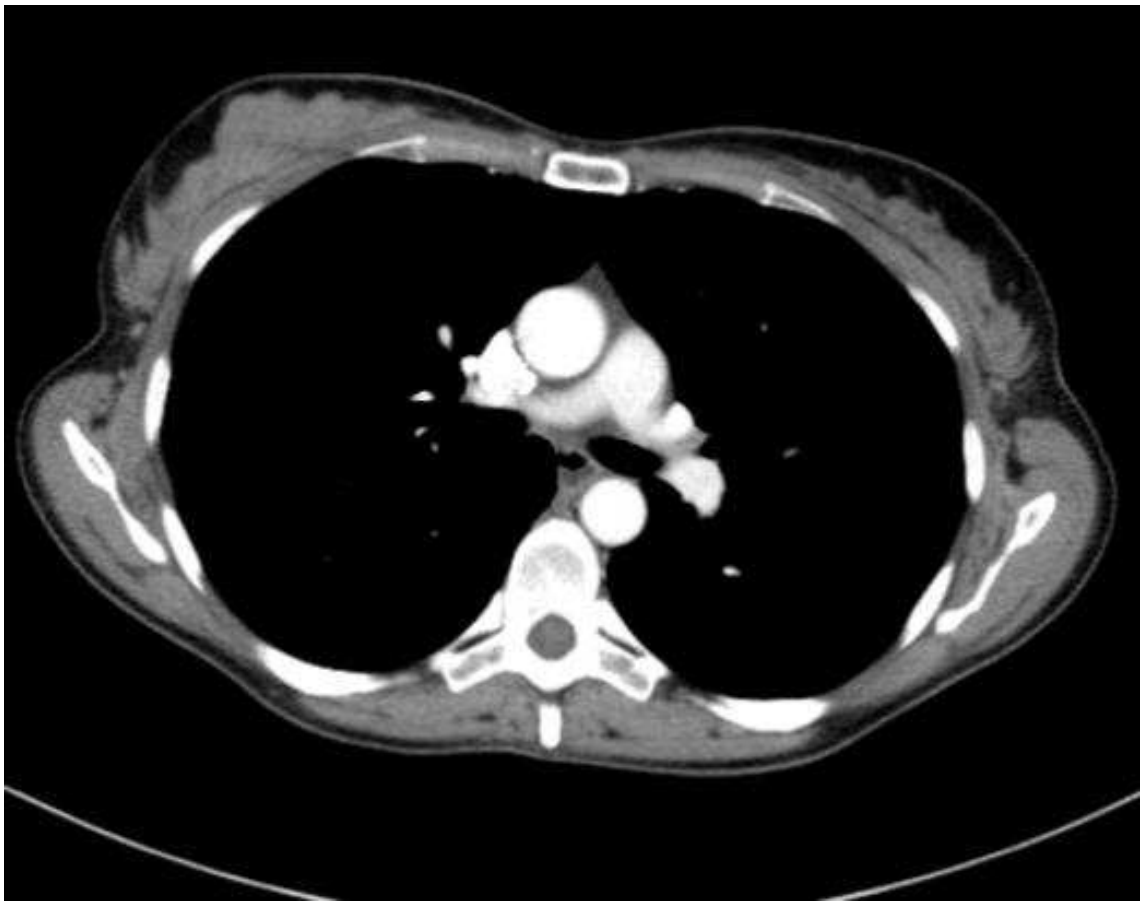

Figure 3-8 Patient 2 - Multiple soft tissue metastases of the right breast at baseline

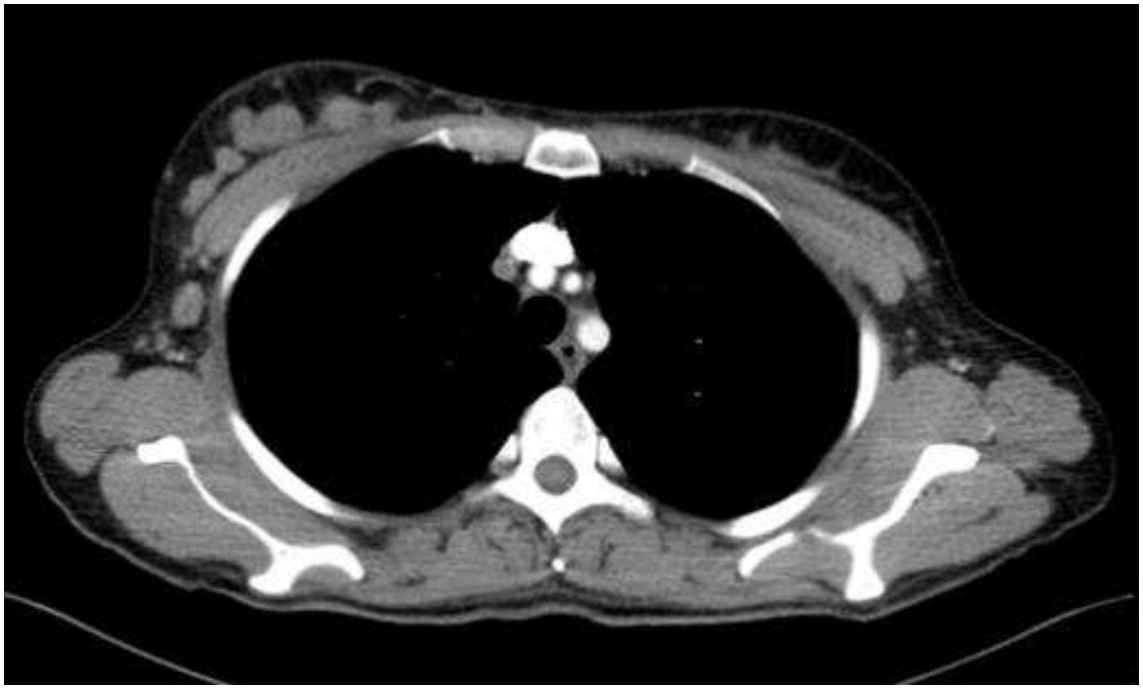

Figure 3-9 Patient 2 - Partial repose after 20 weeks of treatment, still multiple soft tissue

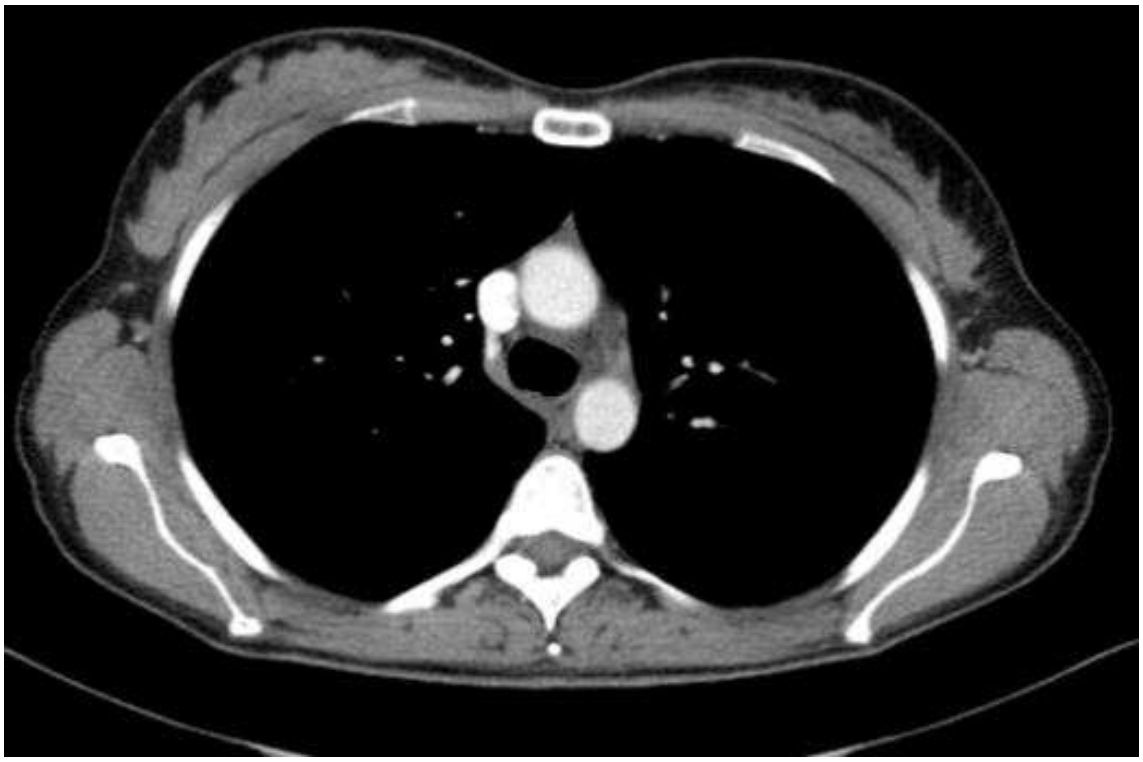

Figure 3-10 Patient 2 - Progressive disease after 24 weeks of treatment of the right breast;

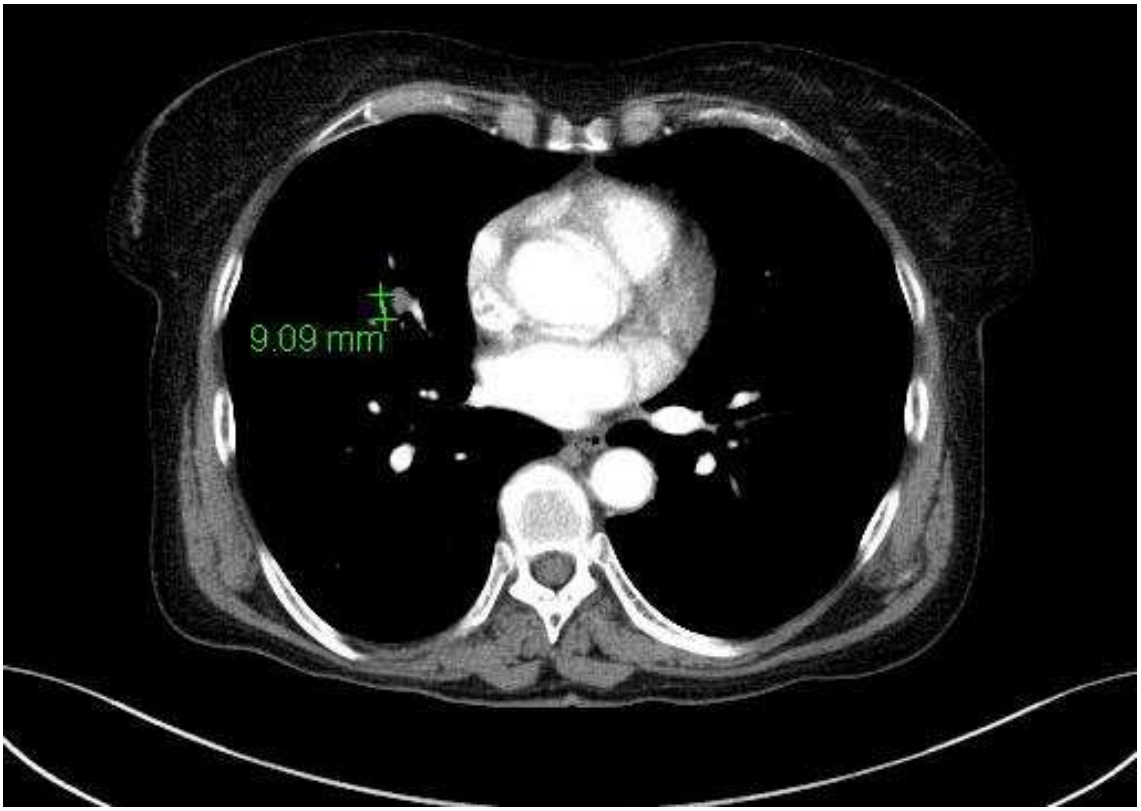

Figure 3-11 Patient 3 - Single pulmonal lymph node metastasis at baseline

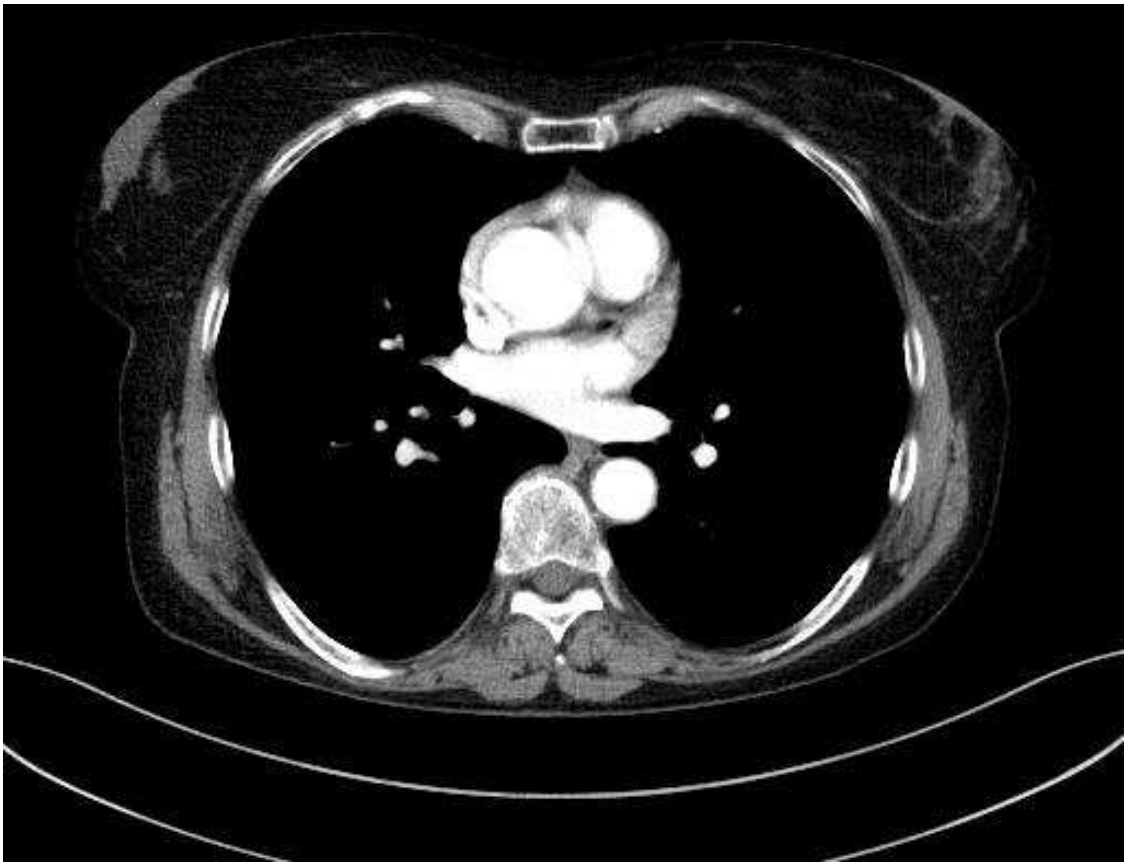

Figure 3-12 Patient 3 - Complete response after 36 weeks of treatment

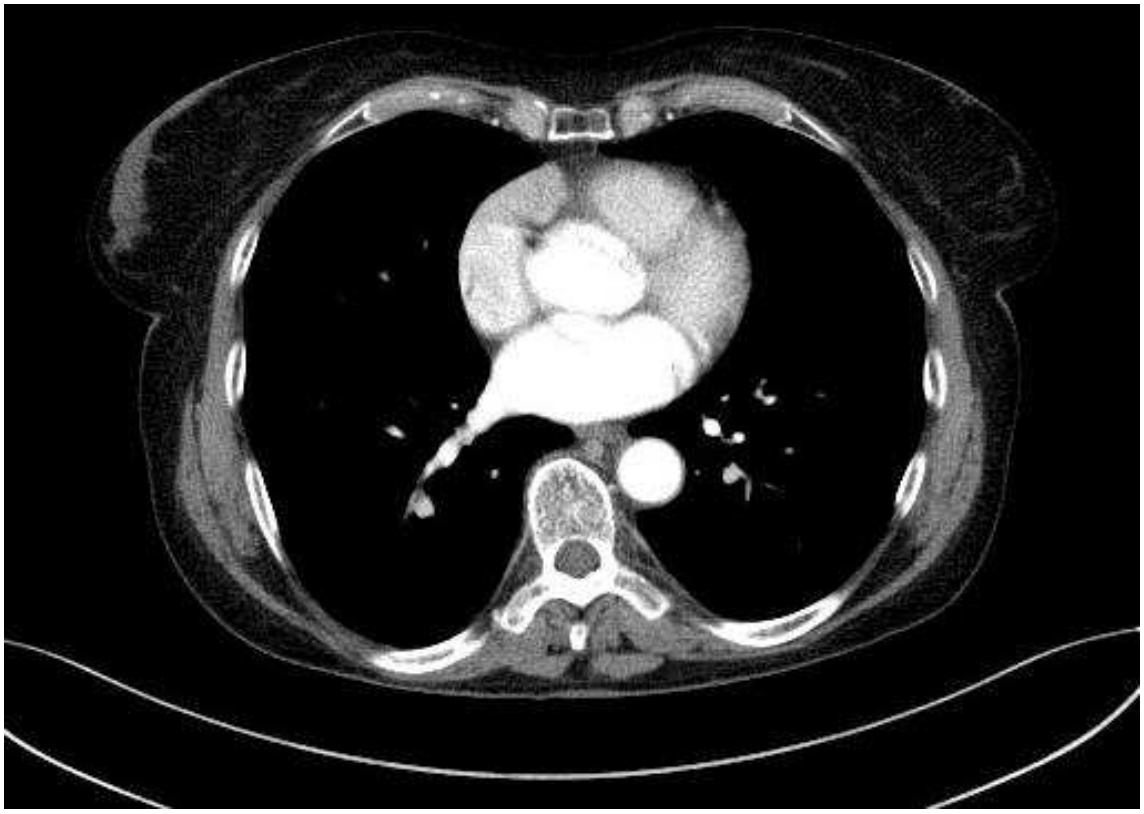

Figure 3-13 Patient 3 - Continuing complete response after 72 weeks of treatment

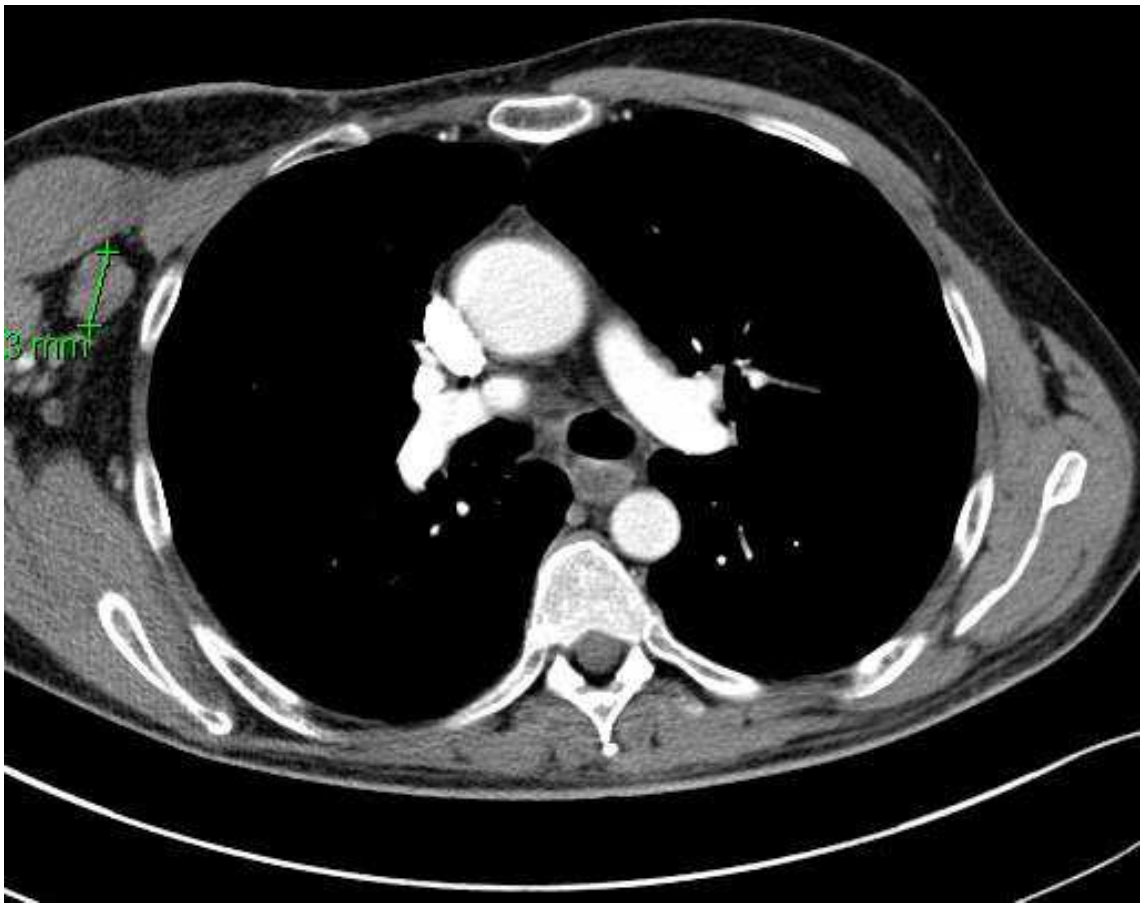

Figure 3-14 Patient 4 - Lymph node metastasis of the right axilla

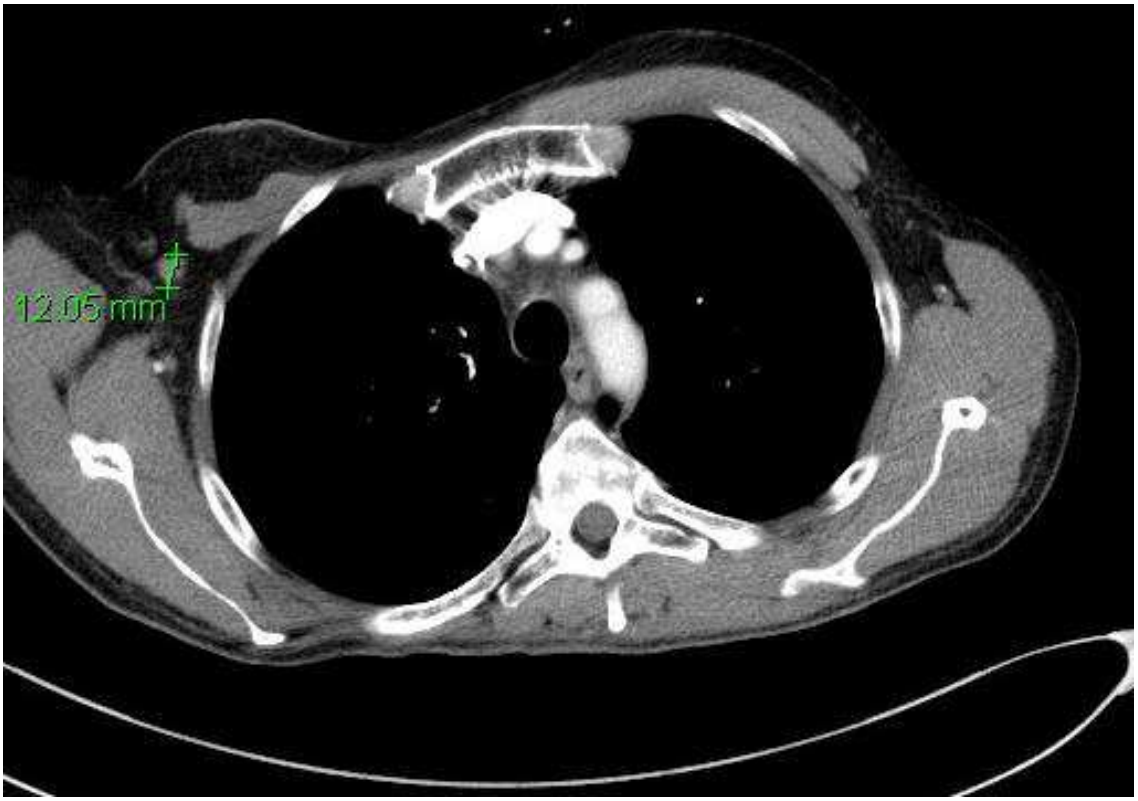

Figure 3-15 Patient 4 - Partial repose after twenty weeks of treatment, still measurable lymph

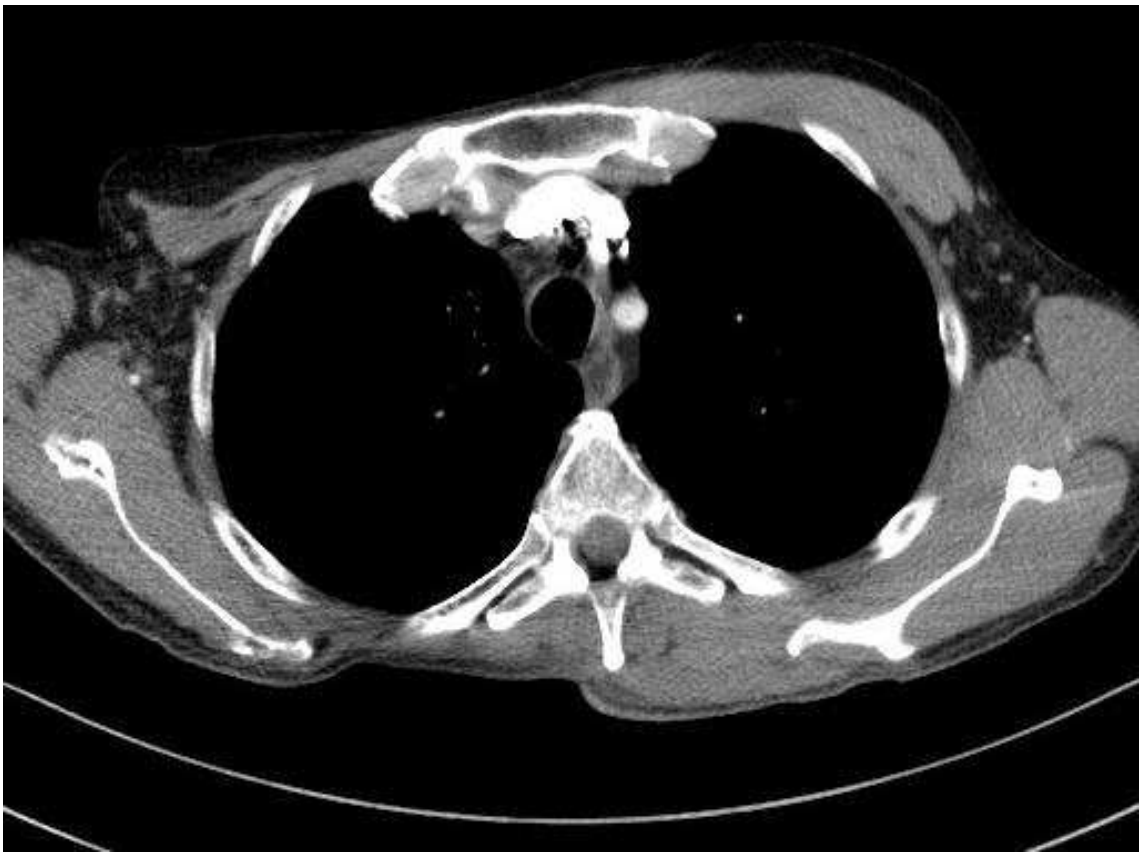

Figure 3-16 Patient 4 - Late complete response after 54 weeks of treatment

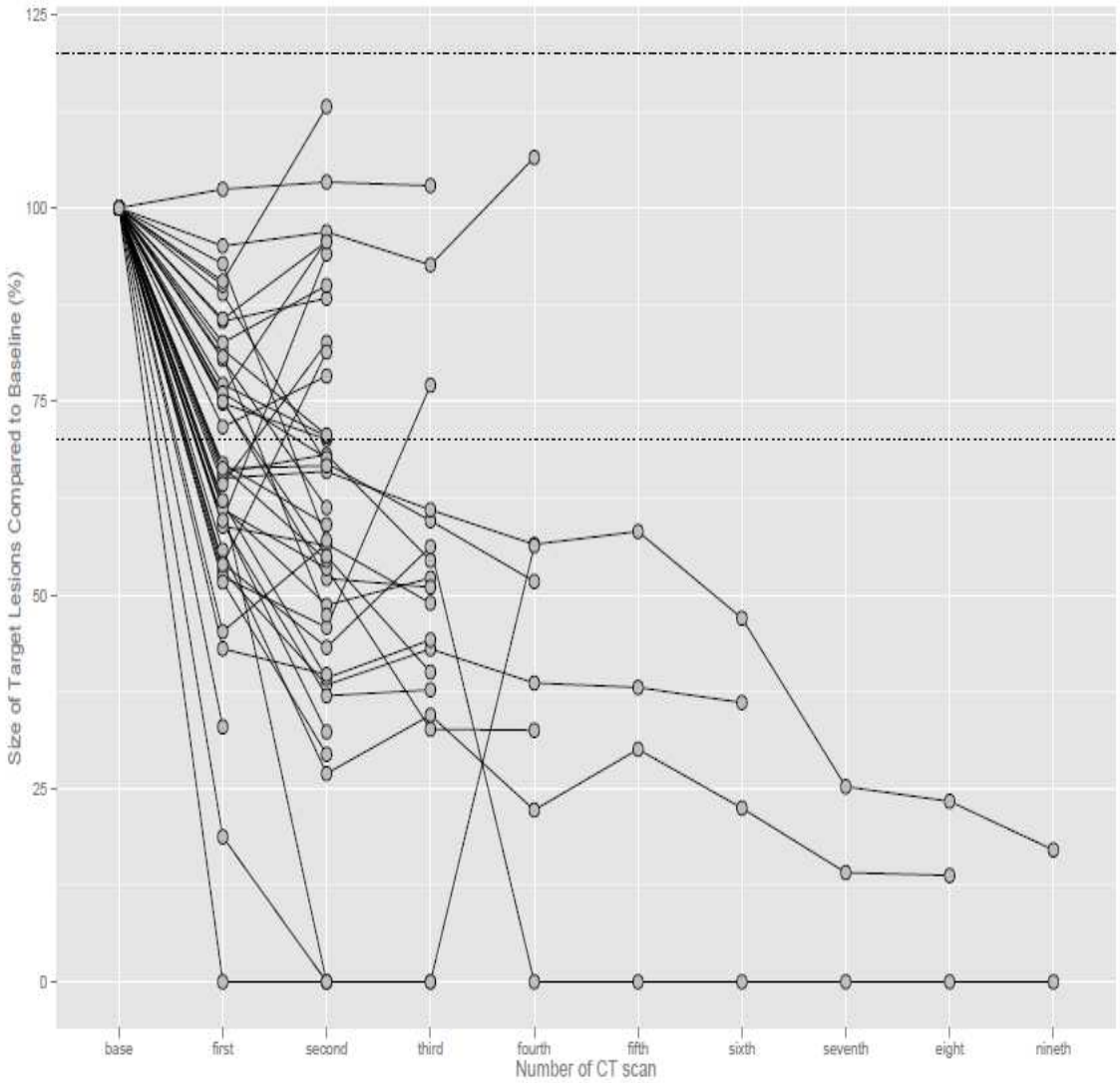

Figure 3-17 Spider plot of the course of target lesions measured by the RECIST criteria. CT, computed tomography; RECIST, Response Evaluation Criteria in Solid Tumors.

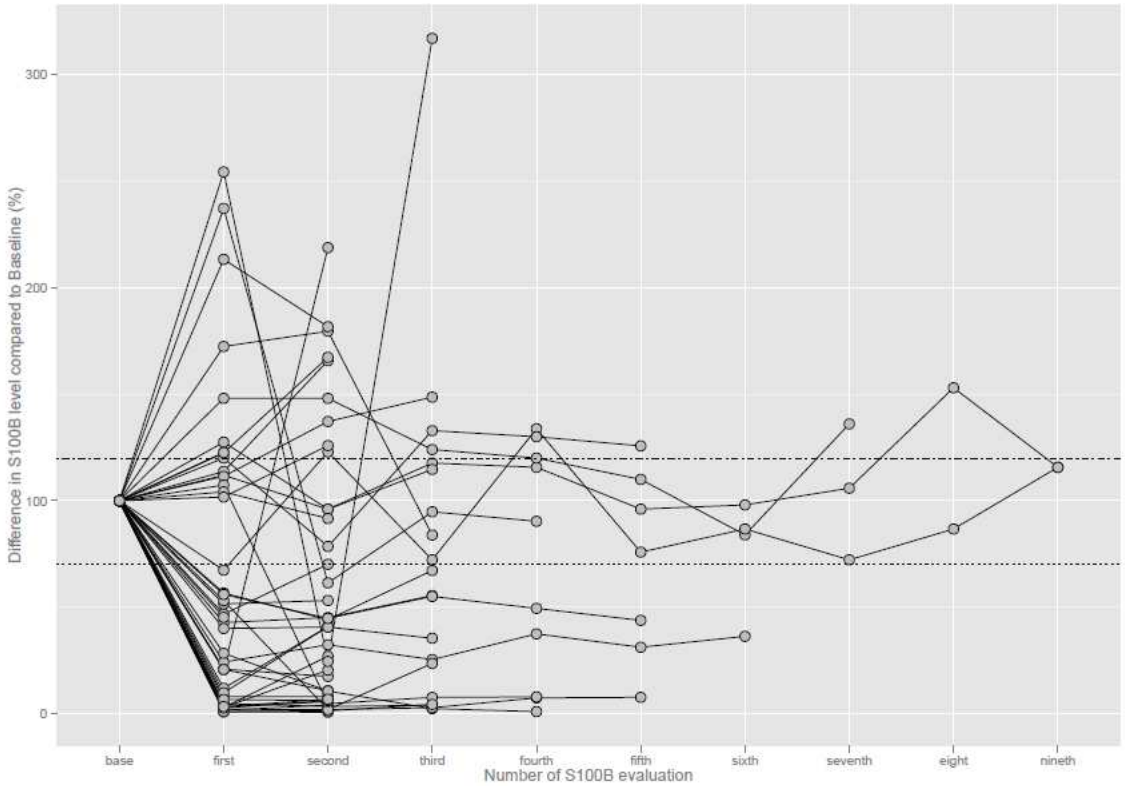

Figure 3-18 Spider plot of the course of S100B.

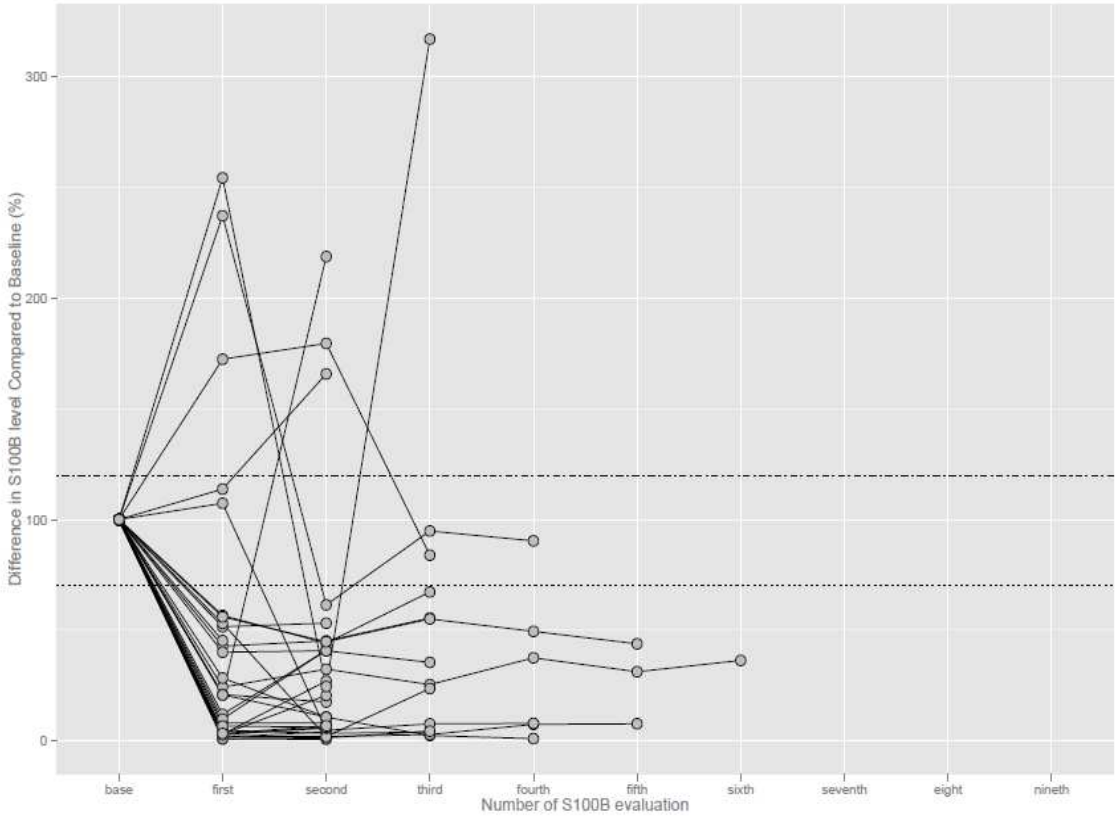

Figure 3-19 Spider plot - course of S100B for those patients with S100B above ULN at baseline.

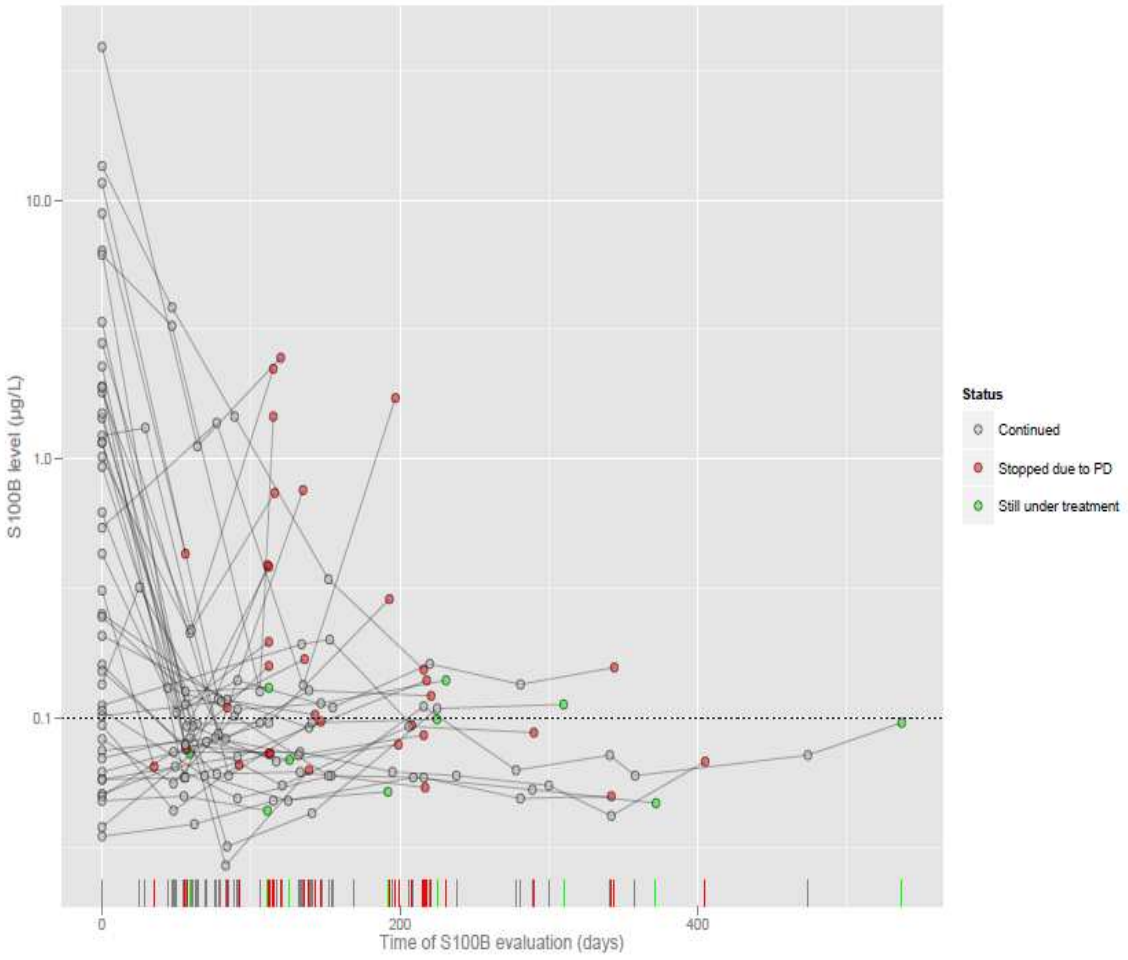

Figure 3-20 Course of S100B levels (logarithmic y-scale). Rug indicates course of action (treatment continued, treatment stopped due to progressive disease, patients still under treatment at end of evaluation period

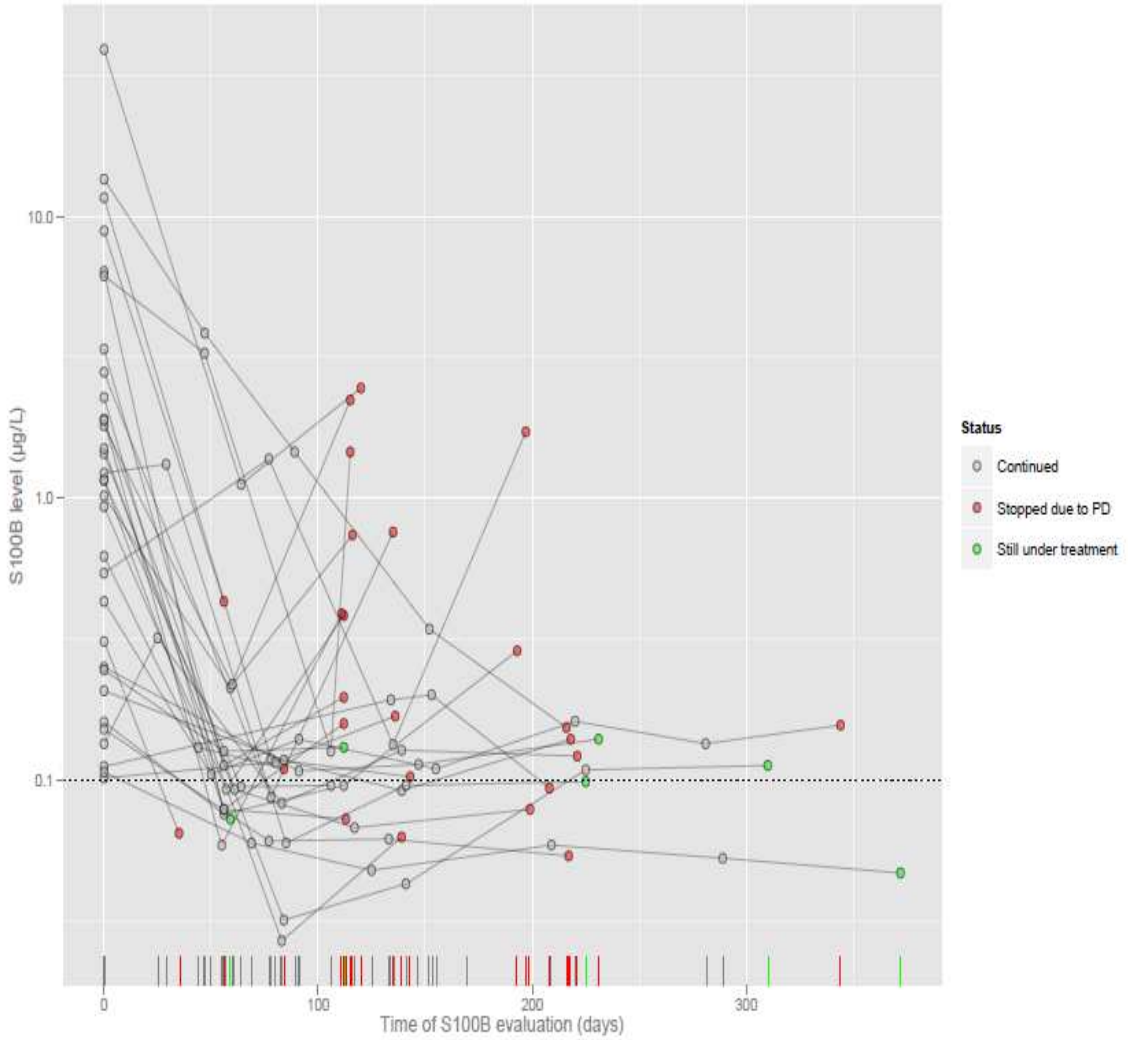

Figure 3-21 Course of S100B levels for patients with S100B above ULN at start of treatment (logarithmic y-scale). Rug indicates course of action (treatment continued, treatment stopped due to progressive disease, patients still under treatment at end of evaluation period)

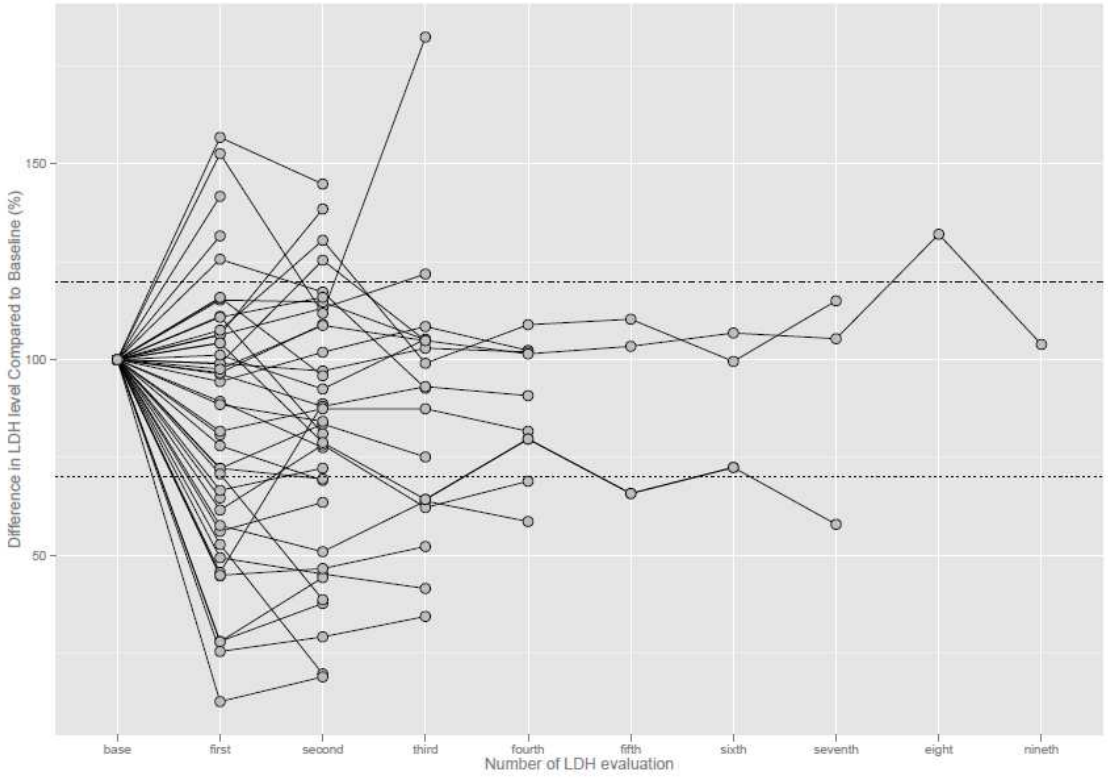

Figure 3-22 Spider plot of the course of LDH.

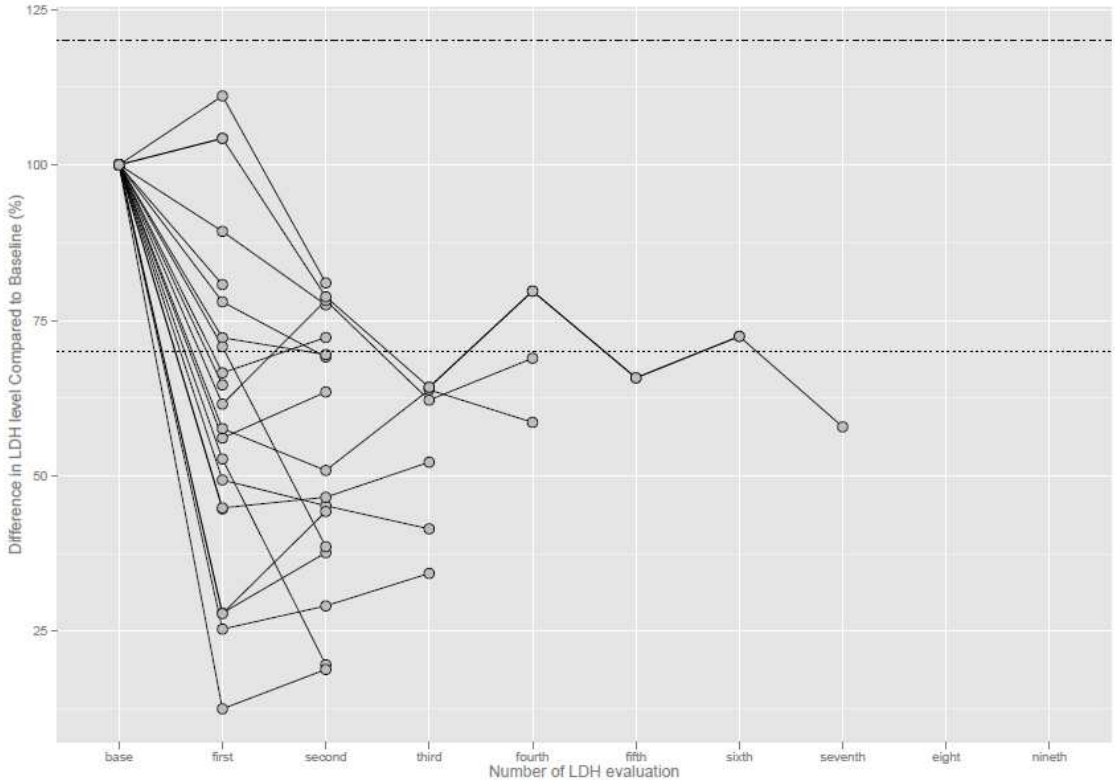

Figure 3-23 Spider plot - course of LDH for those patients with LDH above ULN at baseline.

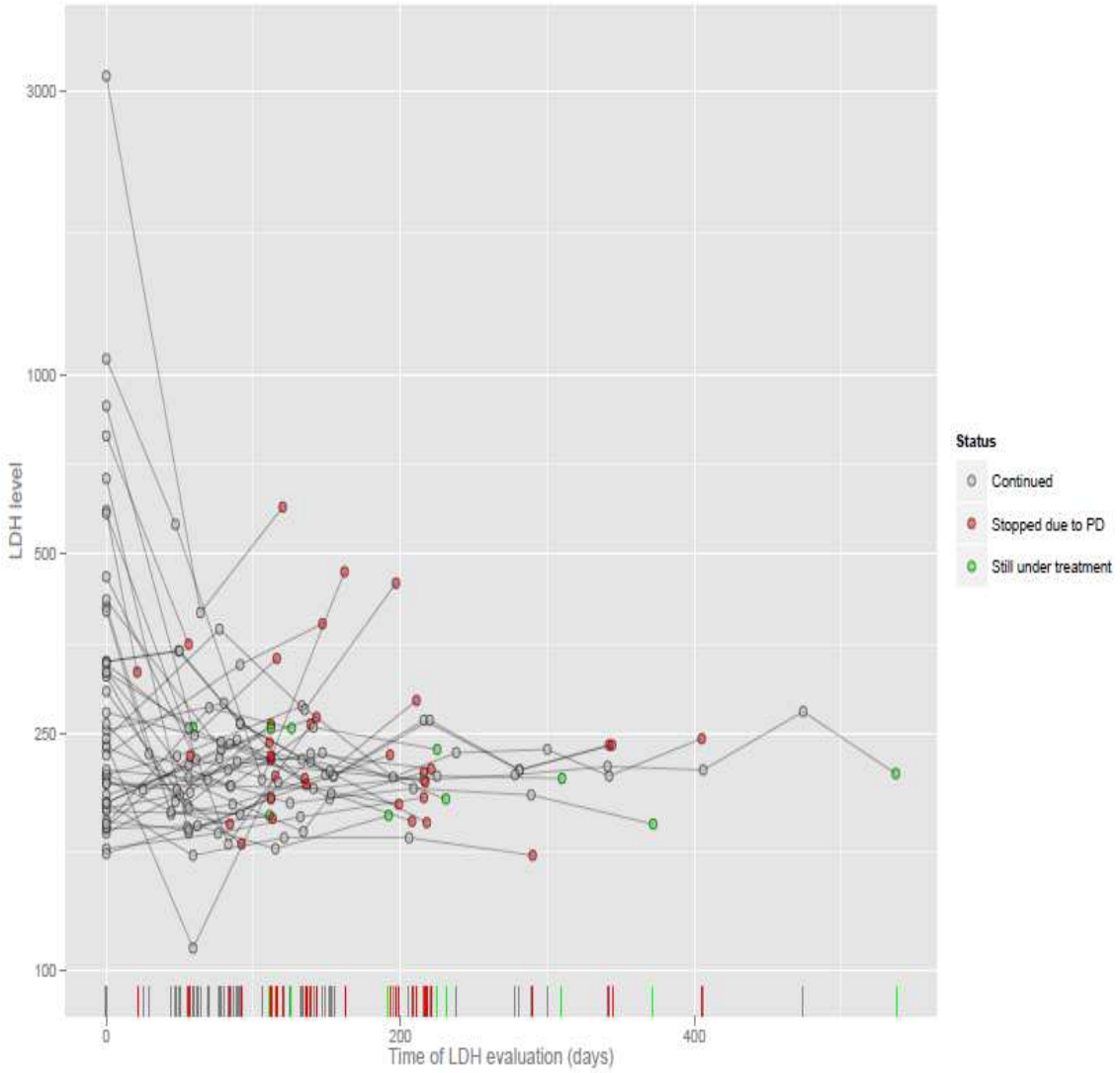

Figure 3-24 Course of LDH levels (logarithmic y-scale). Rug indicates course of action (treatment continued, treatment stopped due to progressive disease, patients still under treatment at end of evaluation period

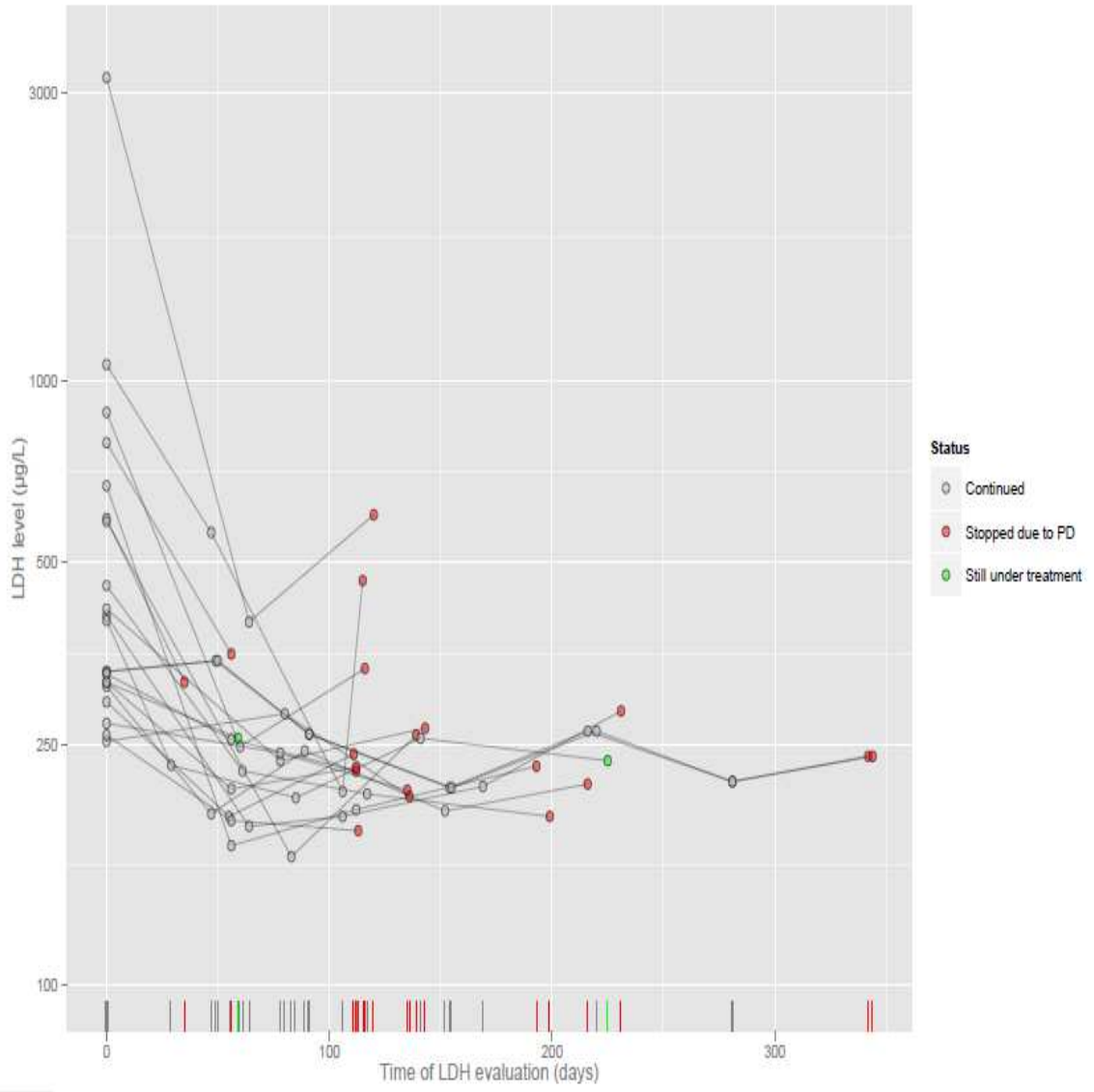

Figure 3-25 Course of LDH levels for patients with LDH above ULN at start of treatment (logarithmic y-scale). Rug indicates course of action (treatment continued, treatment stopped due to progressive disease, patients still under treatment at end of evaluation period

Chapter 4 Discussion

According to this study a decline of S100B and LDH under treatment with vemurafenib indicated initial response, however repeated measurements of S100B and LDH seemed not be sufficient to detect tumor progression. In detail, our analyses we were able to show that for patients with pathologic S100B and elevated LDH at baseline the initial RECIST confirmed tumor response could be correlated with an accuracy of 81.2% and 85.7%, respectively. For patients with normal values at baseline this correlation was much more imprecise.

As reported in several studies, most of the patients which were treated with vemurafenib respond to the drug at least with a stable disease.[46-49] For patients with limited disease S100B and or LDH can be normal at baseline and therefore are probably not suitable to monitor the clinical course of the disease. However, those patients seem to benefit anyway.
In an analysis by Smit and colleagues patients treated with temozolomide and normal S100B values were reported to have an improved overall survival. This was mainly due to their low tumor burden at start of treatment.[55] These findings for S100B were recently confirmed in a cohort of 855 patients by Weide et al. and extended to LDH as well.[56]

Our polynomial models demonstrate that there is neither a lineal, quadratic or cubic correlation of tumor response and LDH/S100B decrease. The reason may be that metastases which are treated with vemurafenib seem to reduce their metabolism almost immediately after start of treatment. This was confirmed by PET scans which were performed 15 days after start of treatment indicating almost no glucose uptake.[57] These metastases are believed not to secret LDH and S100B anymore. However, in the RECIST evaluation the metastases are still present, often with decreased size of course.

Overall, in case of progression the accuracy of S100B and LDH to predict progression was low. One explanation for this may be that half of the patient progressed due to new lesion in case of stable target lesions. The increase of

S100B and LDH however did not correlate in most of those cases either. Another explanation which is likely for S100B is that the tumor loses its ability to secret S100B because of dedifferentiation.[58].

In a recently published report by Sanmamed and colleagues S100B as well as the serum protein melanoma-inhibitory-activity (MIA) were evaluated in terms of their reliability as tumor markers for the treatment of metastatic melanoma with vemurafenib. In contrast to our study, S100B was also judged as good marker to detect tumor progression [59]. One explanation for this difference might be the radiologic evaluations. Whereas in our cohort the extent of the disease was measured according to RECIST regularly, the progression in the cohort of Sanmamed et al. was judged clinically.

Looking at our polynomial models it seems that there is a correlation for S100B evaluated at baseline, LDH, LDH evaluated at baseline with tumor progression. However, in the ANOVA evaluation no model was superior in comparison to the others.

Lactate dehydrogenase is stated pervasively in different healthy tissues. Elevated serum concentrations of the intracellular enzyme are mainly a result of cell lysis. Moreover, increased serum LDH levels occur in different tumor entities and indicate a high turn-over of tumor cells and also necrosis in fast-growing tumors. Increased LDH values are associated with high tumor burden and seem to be particularly elevated in liver metastases for which the reason is not known.[60-62]

S100B is tissue specific and expressed by glial cells of the brainS100B and LDH seem to predict tumor response with acceptable accuracy in early stages of the disease, especially for those patients with increased S100B and LDH levels at baseline. In this early phase of the disease CT scan intervals could be prolonged or scans could maybe omitted completely to reduce the radiation exposure without missing tumor progression. For detecting tumor progression in later phases of the disease both markers cannot substitute CT scans because of their inacceptable accuracy rates, melanocytes, and other cell types, which are derived from the neural crest. Moreover, it is perceptible in dendritic and

chondrocytes cells. Most melanomas strongly express S100B, however a complete lack of S100B expression can be observed by immunohistochemistry in a small proportion of melanoma patients.[63] In cell culture experiments it was shown that S100B is released upon metabolic stress [64] and it is likewise elevated in patients with neural diseases showing metabolic abnormalities like schizophrenia or depression.[65] However, cell death seems to be the major cause for elevated S100B levels.[66]

In conclusion, S100B and LDH seem to predict tumor response with acceptable accuracy in early stages of the disease, especially for those patients with increased S100B and LDH levels at baseline. In this early phase of the disease CT scan intervals could be prolonged or scans could maybe omitted completely to reduce the radiation exposure without missing tumor progression. For detecting tumor progression in later phases of the disease both markers cannot substitute CT scans because of their inacceptable accuracy rates.

Chapter 5 References

1. Garbe C, Peris K, Hauschild A, et al. Diagnosis and treatment of melanoma. European consensus-based interdisciplinary guideline-Update 2012. European Journal of Cancer 2012;48:2375-2390.

2. Leiter U, Buettner PG, Eigentler TK, et al. Hazard rates for recurrent and secondary cutaneous melanoma: an analysis of 33,384 patients in the German Central Malignant Melanoma Registry. Journal of the American Academy of Dermatology 2012;66:37-45

3. Erdmann F, Lortet-Tieulent J, Schüz J, et al. International trends in the incidence of malignant melanoma 1953-2008-are recent generations at higher or lower risk? International Journal of Cancer 2013;132:385-400.

4. Eisemann N, Jansen L, Holleczek B, et al. Up-to-date results on survival of patients with melanoma in Germany. British Journal of Dermatology 2012;167:606-612.

5. Mervic L, Leiter U, Meier F, et al. Sex differences in survival of cutaneous melanoma are age dependent: an analysis of 7338 patients. Melanoma Research 2011;21:244-252.

6. Lin K, Baritaki S, Militello L, Malaponte G, Bevelacqua Y, and Bonavida B. The Role of B-RAF Mutations in Melanoma and the Induction of EMT via Dysregulation of the NF-kappaB/Snail/RKIP/PTEN Circuit. Genes Cancer 2010;1:409-420.

7. Garbe C and Eigentler TK. Diagnosis and treatment of cutaneous melanoma: state of the art 2006. Melanoma Research 2007;17:117-127.

8. Tucker MA and Goldstein AM. Melanoma etiology: where are we? Oncogene 2003;22:3042-3052.

9. de Braud F, Khayat D, Kroon BBR, Valdagni R, Bruzzi P, and Cascinelli N. Malignant melanoma. Critical Reviews in Oncology/Hematology 2003;47:35-63.

10. Rhee JI van der, Boonk SE, Putter H, et al. Surveillance of second-degree relatives from melanoma families with a CDKN2A germline mutation. Cancer Epidemiology, Biomarkers & Prevention 2013;22:1771-1777.

11. Kittler H, Pehamberger H, Wolf K, and Binder M. Diagnostic accuracy of dermoscopy. The Lancet Oncology 2002;3:159-165.

12. Davies H, Bignell GR, Cox C, et al. Mutations of the BRAF gene in human cancer. Nature 2002;417:949-954.

13. Satyamoorthy K, Li G, Gerrero M, et al. Constitutive mitogen-activated protein kinase activation in melanoma is mediated by both BRAF mutations and autocrine growth factor stimulation. Cancer Research 2003;63:756-759.

14. Bauer J, Buttner P, Murali R, et al. BRAF mutations in cutaneous melanoma are independently associated with age, anatomic site of the primary tumor, and the degree of solar elastosis at the primary tumor site. Pigment Cell and Melanoma Research 2011;24:345-351.

15. Flaherty KT and McArthur G. BRAF, a target in melanoma: implications for solid tumor drug development. Cancer 2010;116:4902-4913.

16. Bloethner S, Scherer D, Drechsel M, Hemminki K, and Kumar R. Malignant melanoma- a genetic overview. Actas Dermo-Sifiliogràficas 2009;100 Suppl 1:38-51.

17. Polsky D and Cordon-Cardo C. Oncogenes in melanoma. Oncogene 2003;22:3087-3091.

18. Linos K, Slominski A, Ross JS, and Carlson JA. Melanoma update: diagnostic and prognostic factors that can effectively shape and personalize management. Biomarkers in Medicine 2011;5:333-360.

19. Curtin JA, Busam K, Pinkel D, and Bastian BC. Somatic activation of KIT in distinct subtypes of melanoma. Journal of Clinical Oncology 2006;24:4340-4346.

20. Jiang X, Zhou J, Yuen NK, et al. Imatinib targeting of KIT-mutant oncoprotein in melanoma. Clinical Cancer Research 2008;14:7726-7732.

21. Leiter U, Eigentler TK, Forschner A, et al. Excision guidelines and follow-up strategies in cutaneous melanoma: Facts and controversies. Clinics in Dermatology 2010;28:311-315.

22. Pflugfelder A, Weide B, Eigentler TK, et al. Incisional biopsy and melanoma prognosis: Facts and controversies. Clinics in Dermatology 2010;28:316-318.

23. Pflugfelder A, Kochs C, Blum A, et al. Malignant melanoma S3-guideline "diagnosis, therapy and follow-up of melanoma". Journal der Deutschen Dermatologischen Gesellschaft 2013;11 Suppl 6:1-116, 1-126.

24. Moehrle M, Dietz K, Garbe C, and Breuninger H. Conventional histology vs. three dimensional histology in lentigo maligna melanoma. The British Journal of Dermatology 2006;154:453-459.

25. Moehrle M, Metzger S, Schippert W, Garbe C, Rassner G, and Breuninger H. "Functional" surgery in subungual melanoma. Dermatologic Surgery 2003;29:366-374.

26. Mraz-Gernhard S, Sagebiel RW, Kashani-Sabet M, Miller J R 3, and Leong SP. Prediction of sentinel lymph node micrometastasis by histological features in primary cutaneous malignant melanoma. Archives of Dermatology 1998;134:983-987.

27. Veronesi U, Adamus J, Bandiera DC, et al. Inefficacy of immediate node dissection in stage 1 melanoma of the limbs. The New England Journal of Medicine 1977;297:627-630.

28. Veronesi U, Adamus J, Bandiera DC, et al. Delayed regional lymph node dissection in stage I melanoma of the skin of the lower extremities. Cancer 1982;49:2420-2430.

29. Sim FH, Taylor WF, Pritchard DJ, and Soule EH. Lymphadenectomy in the management of stage I malignant melanoma: a prospective randomized study. Mayo Clinic Proceedings 1986;61:697-705.

30. Balch CM, Soong SJ, Bartolucci AA, et al. Efficacy of an elective regional lymph node dissection of 1 to 4 mm thick melanomas for patients 60 years of age and younger. Annals of Surgery 1996;224:255-263, 255-263.

31. Morton DL, Wen DR, Wong JH, et al. Technical details of intraoperative lymphatic mapping for early stage melanoma. Archives of Surgery 1992;127:392-399.

32. Balch CM, Gershenwald JE, Soong SJ, et al. Final version of 2009 AJCC melanoma staging and classification. Journal of Clinical Oncology 2009;27:6199-6206.

33. Morton DL, Thompson JF, Cochran AJ, et al. Sentinel-node biopsy or nodal observation in melanoma. The New England Journal of Medicine 2006;355:1307-1317.

34. Morton DL, Thompson JF, Cochran AJ, et al. Final trial report of sentinel-node biopsy versus nodal observation in melanoma. The New England Journal of Medicine 2014;370:599-609.

35. Coit DG, Andtbacka R, Bichakjian CK, et al. Melanoma. Journal of the National Comprehensive Cancer Network 2009;7:250-275.

36. Eggermont AM, Chiarion Sileni V, Grob JJ, et al. Ipilimumab versus placebo after complete resection of stage III melanoma: Initial efficacy and safety results from the EORTC 18071 phase III trial. Journal of Clinical Oncology 2014;(suppl; abstr LBA9008):5s.

37. Eigentler TK, Caroli UM, Radny P, and Garbe C. Palliative therapy of disseminated malignant melanoma: a systematic review of 41 randomised clinical trials. The Lancet Oncology 2003;4:748-759.

38. Avril MF, Aamdal S, Grob JJ, et al. Fotemustine compared with dacarbazine in patients with disseminated malignant melanoma: a phase III study. Journal of Clinical Oncology 2004;22:1118-1125.

39. Atkins MB, Hsu J, Lee S, et al. Phase III trial comparing concurrent biochemotherapy with cisplatin, vinblastine, dacarbazine, interleukin-2, and interferon alfa-2b with cisplatin, vinblastine, and dacarbazine alone in patients with metastatic malignant melanoma (E3695): a trial coordinated by the Eastern Cooperative Oncology Group. Journal of Clinical Oncology 2008;26:5748-5754.

40. Parkinson DR, Abrams JS, Wiernik PH, et al. Interleukin-2 therapy in patients with metastatic malignant melanoma: a phase II study. Journal of Clinical Oncology 1990;8:1650-1656.

41. Schwartzentruber DJ, Lawson DH, Richards JM, et al. gp100 peptide vaccine and interleukin-2 in patients with advanced melanoma. New England Journal of Medicine 2011;364:2119-2127.

42. Ives NJ, Stowe RL, Lorigan P, and Wheatley K. Chemotherapy compared with biochemotherapy for the treatment of metastatic melanoma: a meta-analysis of 18 trials involving 2,621 patients. Journal of Clinical Oncology 2007;25:5426-5434.

43. Ribas A, Ke_ord R, Marshall MA, et al. Phase III randomized clinical trial comparing tremelimumab with standard-of-care chemotherapy in patients with advanced melanoma. Journal of Clinical Oncology 2013;31:616-622.

44. Hodi FS, O'Day SJ, McDermott DF, et al. Improved survival with ipilimumab in patients with metastatic melanoma. The New England Journal of Medicine 2010;363:711-723.

45. Curtin JA, Fridlyand J, Kageshita T, et al. Distinct sets of genetic alterations in melanoma. The New England Journal of Medicine 2005;353:2135-2147.

46. Flaherty KT, Puzanov I, Kim KB, et al. Inhibition of mutated, activated BRAF in metastatic melanoma. New England Journal of Medicine 2010;363:809-819.

47. Sosman JA, Kim KB, Schuchter L, et al. Survival in BRAF V600-mutant advanced melanoma treated with vemurafenib. The New England Journal of Medicine 2012;366:707-714.

48. Chapman PB, Hauschild A, Robert C, et al. Improved survival with vemurafenib in melanoma with BRAF V600E mutation. New England Journal of Medicine 2011;364:2507-2516.

49. Chapman PB, Hauschild A, Robert C, Larkin J, Haanen JB, and Ribas A. Updated overall survival (OS) results for BRIM-3, a phase III randomized, open-label, multicenter trial comparing BRAF inhibitor vemurafenib (vem) with dacarbazine (DTIC) in previously untreated patients with BRAFV600E-mutated melanoma.
url: http://meeting.ascopubs.org/cgi/content/abstract/30/15_suppl/8502.

50. Hauschild A, Grob JJ, Demidov LV, et al. Dabrafenib in BRAF-mutated metastatic melanoma: a multicentre, open-label, phase 3 randomised controlled trial. Lancet 2012;380:358-365.

51. Ma S, Kong B, Liu B, and Liu X. Biological effects of low-dose radiation from computed tomography scanning. Int. J. Radiat. Biol. May 2013;89:326-333.

52. R Core Team. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing. Vienna, Austria, 2013. url: http://www.R-project.org/.

53. Kuhn M, Wing J, Weston S, et al. caret: Classification and Regression Training. R package version 5.17-7. 2013. url: http://CRAN.R-project.org/package=caret.

54. Wickham H. ggplot2: elegant graphics for data analysis. Springer New York, 2009. url: http://had.co.nz/ggplot2/book.

55. Smit LHM, Korse CM, Hart AAM, et al. Normal values of serum S-100B predict prolonged survival for stage IV melanoma patients. European Journal of Cancer Feb. 2005;41:386-392.

56. Weide B, Elsässer M, Büttner P, et al. Serum markers lactate dehydrogenase and S100B predict independently disease outcome in melanoma patients with distant metastasis. British Journal of Cancer 2012;107:422-428.

57. Baudy AR, Dogan T, Flores-Mercado JE, et al. FDG-PET is a good biomarker of both early response and acquired resistance in BRAFV600 mutant melanomas treated with vemurafenib and the MEK inhibitor GDC-0973. EJNMMI Research 2012;2:22.

58. Aisner DL, Maker A, Rosenberg SA, and Berman DM. Loss of S100 antigenicity in metastatic melanoma. Human Pathology 2005;36:1016-1019.

59. Sanmamed MF, Fernàndez-Landàzuri S, Rodriguez C, et al. Relevance of MIA and S100 serum tumor markers to monitor BRAF inhibitor therapy in metastatic melanoma patients. Clinica Chimica Acta 2014;429:168-174.

60. Finck SJ, Giuliano AE, and Morton DL. LDH and melanoma. Cancer 1983;51:840-843.

61. Heimdal K, Hannisdal E, and Gundersen S. Regression analyses of prognostic factors in metastatic malignant melanoma. European Journal of Cancer and Clinical Oncology 1989;25:1219-1223.

62. Sirott MN, Bajorin DF, Wong GY, et al. Prognostic factors in patients with metastatic malignant melanoma. A multivariate analysis. Cancer 1993;72:3091-3098.

63. Argenyi ZB, Cain C, Bromley C, et al. S-100 protein-negative malignant melanoma: fact or fiction? A light-microscopic and immunohistochemical study. American Journal of Dermatopathology 1994;16:233-240.

64. Gerlach R, Demel G, König HG, et al. Active secretion of S100B from astrocytes during metabolic stress. Neuroscience 2006;141:1697-1701.

65. Schroeter ML and Steiner J. Elevated serum levels of the glial marker protein S100B are not specific for schizophrenia or mood disorders. Molecular Psychiatry 2009;14:235-237.

66. Ghanem G, Loir B, Morandini R, et al. On the release and half-life of S100B protein in the peripheral blood of melanoma patients. International Journal of Cancer 2001;94:586-590.

Chapter 6 Summary

Vemurafenib is a highly efficient BRAF inhibitor for metastatic melanoma patients carrying the V600 mutation. Progression free survival is prolonged to approximately 6 months and 50 to 80% of the patients show objective tumor responses. S100B and LDH are established tumor markers in routine melanoma follow up. This study evaluated their potential as response and progression markers during vemurafenib treatment.

A cohort of 44 patients with stage IV melanoma disease and treated with vemurafenib was retrospectively analyzed. Staging was performed every 6-8 weeks including CT scans, LDH and S100B. RECIST criteria were used for standardized radiological response evaluation. Correlation between response or progression, LDH and S100B was analyzed by accuracy tests, Spearman's rank correlation rho and polynomial regression analyses.

There was a decent correlation between LDH and S100B decline and RECIST confirmed response especially in case S100B and/or LDH were elevated at baseline (accuracy 81.2% for S100B and 85.7% for LDH). Accuracy in case of RECIST confirmed progression and S100B/LDH was low with 32.4% for LDH and 30.3% for S100B. Neither polynomial regression analyses nor Spearman's rank correlation rho showed a correlation between the clinical course and S100B/LDH.

S100B and LDH seem to predict tumor response with acceptable accuracy in early stages of the disease, especially for those patients with increased S100B and LDH levels at baseline. In this early phase of the disease CT scan intervals could be prolonged or scans could maybe omitted completely to reduce the radiation exposure without missing tumor progression. For detecting tumor progression in later phases of the disease both markers cannot substitute CT scans because of their inacceptable accuracy rates.

Chapter 7 Curriculum Vitae

Sail Nawaf Abusaif, M.B.B.S.

Date of birth:	July, 14, 1975
Place of birth:	Kuwait
Nationality:	Jordanian
Sex:	Male
Marital state:	Married
E-mail:	Sailnawafabusaif@yahoo.com
Home address and telephone:	Street 40, Ibin Sina Al Khandaq Area, Zarqa, Jordan Tel. + 971503765429 (mobile)
Permanent address:	UAE , Abu Dhabi .Delma Street. P.O Box 93750
High school	General secondary education certificate (GSEC) in Jordan June 1994
Present academic rank and position	Dermatologist at New Age Clinic, Abu Dhabi, UAE.

Summary of Medical Qualification	-Jordanian Board in Dermatology and Venereology (March 2008) First part (Feb 2006) Second part (March 2008) -M.B.B.S from the Azerbaijan medical University (June 2001) -Member in the Jordanian Dermatological & venereological Society -Member in the German physicans Society. -Member in the Europian Dermato oncologist Society. - HAAD license. - DHA license - German license.
Postgraduate Medical Training	- Dermatooncology and Dermatosurgery at Eberhard Karls University of Tuebingen – Germany,2011-2013 . - Dermatologist and Venereologist at K.H.M.C. since March 2008 - Jordanian board in Dermatology and Verneology (March 2008) - Resident in Dermatology at KHMC (January 2004-December 2007) - Resident in General Medicine at KHMC (January 2004- December 2004) - Internist at Jordanian university: Medicine (August 2001-Feb 2002) - Surgery (February2002-August 2002)

Professional experience	During my 3 years as senior resident in dermatology I was involved in: - Teaching of junior residents of Dermatology and Venereology at KHMC. - Attending and participating in weekly academic activities and Clinico-pathological Meetings. - Attending patients being admitted to hospital for in-patient treatment, such as severe psoriasis, various Bullous disorders, erythroderma, etc. - Taking part in the management of patients at outpatient clinics. - Taking biopsies for various dermatological disorders. - Using electrocautery and cryotherapy as well as other techniques in the management of suitable skin lesions. - Using Botox and Fillers injections. - Using CO2, PDL, Alexandrite, Diod Laser. - Using surgical procedure for variety of skin disorder such as Hyperhidrosis, Hidradenitis supporativa, Flaps, Grafts. I was involved in the teaching program of the medical students Mu'ta Military University. During my fellowship in Dermatooncology and Dermatosurgery at Tuebingen University _ Germany, attended the melanoma clinics, Non melanoma skin cancer clinics, Dermatoscopy clinics, Dermatosurgery, Lymphoma and transplanted patients clinics, laser and phototherapy.
Scientific participation	Conferences 1. The second international medical conference of the internal medical

department 2006

2. The fourth International Congress of the Jordanian Dermatological and Venereological Society dated 2008 with a paper entitled “Syrigoma case report”.
3. The fourth International Jordanian Conference in Liberia, Monrovia, in a paper entitled “Patterns of cutaneous presentation of river blindness”.
4. The sixth Dermatooncology Congress – Berlin.
5. European Dermatooncology Congress- Barcelona.
6. German Dermatooncologists meeting – Munich.
7. German Dermatosurgery Congress - Tuebingen.

Papers

1. Treatment of post herpetic neuralgia by xylocaine gel 2% in the Royal Medical Services Journal 2008
2. “Vulvar fixed drug eruption - a case report”.
3. Low dose steroid in treatment of Vitiligo.
4. S100B and LDH as parameter in advanced melanoma patients treated with vemurafenib
5. Vemurafenib (Braf inhibitors) published, and printed by Springer.

Languages

-Arabic: Mother language

-English: Fluent

-Russian; Fluent

-German: B2 Level

Abu Dhabi , 8 June, 2015

Chapter 8 Appendices

Appendix A: The concentration of serum S100B protein

Table A- 1 The Concentration of Serum S100 B protein according to the stage and disease activity

PAT_ID	Baseline	Stage 1	Stage 2	Stage 3	Stage 4	Stage 5	Stage 6	Stage 7	Stage 8	Stage 9
5610	1,160	0,095	0,096							
5609	0,094	0,044	0,066							
5608	0,311	0,065	0,034							
5607	0,051	0,065	0,049	0,060	0,059	0,049	0,050	0,054	0,078	0,059
5606	0,431	0,105	0,140	0,110	0,162	0,135	0,157			
5602	0,050	0,074	0,074	0,062	0,060	0,055	0,042	0,068		
5613	0,623	0,131	0,110							
5614	13,520	3,850	1,460	0,344	0,154					
5615	0,083	0,056	0,102	0,060	0,111	0,063	0,072	0,060	0,072	0,096
1	0,208	0,118	0,092	0,14						
2	1,020	0,213	2,230							
3	0,252	0,108	0,114	0,14						
4	11,640	0,119	0,103							
5	0,161	0,073								
6	2,800	0,087	0,759							
7	1,810	0,219	0,741							
8	0,075	0,084	0,072	0,086						
9	8,870	0,431								
10	0,135	0,320	0,083	0,128	0,122					
11	1,910	0,093	0,068	0,079						
12	1,440	0,032	0,030	0,043	0,109	0,113				
13	0,934	0,093	0,384							
14	0,102	0,116	0,169							
15										
16	0,152	0,061	0,062	0,054						
17										
18										
19	0,035	0,039	0,048	0,052						
20	0,112	0,193	0,201	0,094						
21	1,500	0,027	0,063							
22	1,230	1,320	0,060	0,096	0,099					
23	6,370	0,076	0,096	0,288						
24	0,058	0,071	0,097							
25	1,890	0,059	0,390							
26	0,246	0,127	0,131							
27	6,150	3,270	0,127	1,460						
28	0,048	0,050	0,044							
29	0,543	1,380	0,134	1,720						
30	0,107	0,060	0,048	0,059		0,047				
31	0,070	0,084	0,055	0,093		0,088				
32	0,062	0,076								
33	0,058	0,059	0,073							
34	0,038	0,081	0,069							
35	3,380	0,113	0,197							
36	1,160	0,079	0,073							
37	39,000	1,120	2,460							
38	2,280	0,079	0,159							

Appendix B: The concentration of serum LDH

Table B- 1 The Concentration of Serum LDH according to the stage and disease activity

patient no	Base line	stage 1	stage 2	stage 3	stage 4	stage 5	stage 6	stage 7
1	191	240	224	177				
2	239	109	212					
3	185	183	232	194				
4	419	235	266					
5	317	256						
6	271	242	210					
7	887	248	334					
8	160	170	181	195				
9	790	353						
10	213	201	217	231	218			
11	458	226	207	190				
12	206	204	200	212	210			
13	187	199	259					
14	253	281	205					
16	197	227	226	207				
19	173	175	160	182				
20	237	171	198	178				
21	585	163	259					
22	401	231	204	256	235			
23	670	170	195	230				
24	230	326						
25	294	190						
26	191	221						
27	1064	561	209					
28	157	174	182					
29	245	374	274	447				
30	217	209	191	202	197			
31	191	156	167	167	156			
32	174	229						
33	178	172	194					
34	176	276	255					
35	327	255	226					
36	259	187	180					
37	3177	399	600					
38	317	211	229					
5615	206	201	224	216	209	213	220	217
5614	312	192	244	194	215			
5613	209	185	176					
5610	408	183	190	213				
5609	170	197	163					
5608	448	317	173					
5607	330	344	260	212	263	217	239	191
5602	213	229	278	211	232	235	212	245
5606	330	344		212	263	217	239	

Appendix C: RECIST value for each stage

Table C- 1 RECIST value for all patients at each stage

PAT_ID	baseline	Stage 1	Stage 2	Stage 3	Stage 4	Stage 5	Stage 6	Stage 7	Stage 8	Stage 9
5610	27,250	24,230	18,260	16,240	14,100					
5609	32,730	19,490	10,570							
5608	69,640	62,700								
5607	30,690	19,980	20,240	18,720	17,350	17,860	14,420	7,740	7,160	5,230
5606	158,500	84,160	60,790	68,170	61,210	60,290	57,200			
5602	51,710	28,840	13,920	17,820	11,48	15,54	11,61	7,3	7,11	
5613	26,370	15,640	9,750	9,950						
5614	29,340	19,310	19,990	15,980						
5615	10,760	5,820	4,650	6,050	0,000	0,000		0,000	0,000	0,000
1	11,980	7,350	5,830	6,25						
2	96,030	81,970	84,830							
3	73,380	75,160	75,840	75,5						
4	59,250	44,310	41,540							
5	15,750	5,190								
6	70,250	54,250	49,510							
7	85,320	61,190	66,790							
8	43,720	19,790	24,740	21,4						
9	186,130	119,700	153,770							
10	38,920	24,120	15,280	17,2						
11	137,200	103,170	71,490	69,98						
12	52,110	31,830	27,840	17,010	16,94					
13	41,760	27,790	22,750							
14	72,240	54,810	69,240							
15										
16	27,310	16,990	0,000	0,000						
17										
18										
19	6,280	0,000	0,000							
20	70,270	63,620	79,480							
21	66,490	54,330	46,980							
22	27,150	14,210	12,440	20,93						
23	34,130	27,450	20,92							
24	32,210	21,570	19,030							
25	26,940	15,860	15,190							
26	82,770	63,000	56,010							
27	179,370	148,160	161,410							
28	23,460	21,770	13,38							
29	61,920	33,390	50,400							
30	49,580	9,290	0,000	0,000	27,94					
31	43,660	41,520	42,310	40,450	46,51					
32	169,330	72,880	67,270							
33	37,880	22,590	35,63							
34	11,500	9,850	11,000							
35	63,450	42,090	42,32							
36	55,240	28,540	16,25							
37	51,220	41,370	24,27							
38	17,310	12,980	9,520	6,93						

Appendix D: Regression Models correlation before first stage

Table D- 1 Accuracy and regression models for correlation between RECIST and S100/LDH from start of therapy till first staging.

Parameter	S100B	S100B evaluated at baseline	LDH	LDH evaluated at baseline
Accuracy	63.60% 95% CI : (47.8, 77.6)	81.20% 95% CI : (63.6, 92.8)	61.40% 95% CI : (45.5, 75.6)	85.70% 95% CI : (63.7, 97.0)
Linear model	p=0.404	p=0.445	p=0.0714	p=0.0831
Quadratic model	p=0.356	p=0.706	p=0.0868	p=0.17
Cubic model	p=0.259	p=0.326	p=0.132	p=0.211
ANOVA	linear vs. quadratic Pr(>F): 0.24 linear vs. cubic Pr(>F): 0.16	linear vs. quadratic Pr(>F): 0.72 linear vs. cubic Pr(>F): 0.10	linear vs. quadratic Pr(>F): 0.20 linear vs. cubic Pr(>F): 0.38	linear vs. quadratic Pr(>F): 0.43 linear vs. cubic Pr(>F): 0.32