Squamous cell carcinoma of the head and neck arises from the mucosa of the upper aerodigestive tract, including the oral cavity, pharynx, and larynx. Squamous cell carcinoma accounts for more than 90% of all malignant head and neck tumors, and accounts for ~5% of all malignant tumors in Europe and the United States. Worldwide, more than half a million new cases of head and neck cancer are diagnosed, and in The Netherlands, in 2006 more than 2500 patients developed malignancies in the head and neck, whereas more than 800 patients died from this disease.

Two-thirds of the patients with head and neck cancer are diagnosed with advanced stage disease, and their prognosis is poor. During the last three decades, some progress has been made in the locoregional treatment of head and neck cancer, but unfortunately this has not led to substantial improvement of overall survival. First, because head and neck cancer patients frequently develop locoregional recurrences and distant metastases, but also because second primary tumors frequently develop. Retreatment is not always possible, due to side effects on previously treated healthy tissues. Therefore, more specific and precise 'targeted' locoregional treatment should be developed, eradicating malignant tissue while sparing normal tissues. In addition, adjuvant systemic strategies should be developed to eradicate occult distant metastases and minimal residual disease. These 'targeted treatments' are necessary to improve locoregional as well as systemic control and overall survival, while sparing normal tissues and keeping patients' quality of life as best as possible. Targeted therapies can be used as monotherapy as well as in combination therapies with radiotherapy and chemotherapy, and ideally, these treatments have a synergistic effect. The use of multi-targeting instead of mono-targeting strategies should have positive effects on treatment efficacy and avoidance of therapy resistance.

During the last decades, several locoregional treatment options have become available aiming definite local tumor eradication, while at the same time preserving organ functions, relieving symptoms, and avoiding disease-related complications. Bleomycin-electroporation therapy is one of these treatment options, designed to be an alternative for or an addition to surgery in the treatment of solid tumors. This treatment selectively kills cancerous cells and minimizes cosmetic and functional disfigurements. In **chapter 2** a clinical phase IV study is described with bleomycin-electroporation therapy. Patients suffering from malignancies in the head and neck area, who could no longer be treated by surgery or radiotherapy, were treated with bleomycin-electroporation therapy. This therapy consists of injections of bleomycin directly into the tumor, followed by delivery of electrical pulses by a needle-electrode applicator to induce uptake of bleomycin in the tumor cells. Twenty-four tumors in fourteen patients were treated with this locoregional cancer therapy. Local tumor control for more than two years was reached in 10 of the 24 tumors (three out of fourteen patients). These three patients were still alive two years after treatment. In all cases, functions were preserved successfully, and the treatment provided acceptable cosmetic results. Bleomycin-electroporation therapy appeared to be an easy to

perform treatment that causes little burden to the patient and is potentially effective in patients with tumors in the head and neck area. In addition, the procedure is straightforward, and normally takes less than one hour. The treatment can be performed on outpatient basis, but in our hospital all treated patients were cared on the ward for a few days.

Despite the challenging starting point of this study (patients who underwent extensive therapies before, and who could no longer be treated by surgery or radiotherapy), the results with bleomycin-electroporation therapy are encouraging in terms of tissue preservation and tumor control. One problem can be the accessibility of the tumors for the injection of bleomycin and the insertion of the applicator. For the treatment to be effective it is necessary to infiltrate and electroporate the whole tumor area including 0.5 cm margins around. Most patients already had been treated with radiotherapy, and this treatment leads to DNA damage, not only in the cancer cells, but also in the surrounding normal tissues. Because bleomycin leads to similar DNA damage, bleomycin-electroporation therapy resulted in delayed wound healing in previously irradiated areas. Furthermore, some patients already were suffering from distant metastases at the time of treatment, leading to death before reaching the end of study time point. In these cases, palliation was reached, but there was no improved survival. Nevertheless, the results of this study were hopeful.

Bleomycin-electroporation therapy thus far appeared most successful as primary therapy in patients with early stage head and neck cancer, with complete remissions in 83% of the treated lesions four weeks after treatment (1), but also in advanced stage disease, this treatment led to partial (32%) and complete responses (25%) in 57% of the treated lesions. The 30% overall survival rate at one year found in this study was consistent with the overall survival rate of patients with advanced stage head and neck squamous cell carcinoma. Improved local control of the head and neck tumor site did not influence survival. Individual patients demonstrating a positive response to electroporation with bleomycin did not have a longer survival period after treatment. However, by controlling local disease, their quality of life was much improved (2). The routine treatment strategy for early stage oral cavity cancer is surgery, leading to a five-year overall survival of 70-80%. Therefore, changing this treatment strategy to bleomycin-electroporation therapy is not indicated. Advanced stage disease is treated by surgery and/or radiotherapy with or without chemotherapy. In case of local recurrences, second primary tumors, or metastases, prognosis is much worse, and standard treatment strategies are not always available. Bleomycin-electroporation therapy appeared to be a valuable addition to the standard treatments. In our opinion, bleomycin-electroporation therapy is an easy to perform treatment that is potentially effective in selected patients with head and neck or skin tumors, even in patients with advanced stage disease, with recurrences or distant metastases. An enormous advantage of bleomycin-electroporation therapy in comparison to another emerging modality for local treatment, photodynamic therapy (3), is the absence of

photosensitivity that keeps patients inside for several weeks. Low treatment burden is of high importance in the treatment of palliative patients, who have a diminished life expectancy. In addition, bleomycin-electroporation therapy is applicable for treatment of multiple tumor types, not only squamous cell carcinomas of the mucosa and the skin, but also malignant tumors of the salivary glands, basal cell carcinomas, melanomas, adenocarcinomas, Merkel cell carcinomas, sarcomas, cutaneous lymphomas, skin metastases and primary cutaneous tumors. Also larger tumors with invasion depths up to 2.5 cm are eligible for this therapy.

At this moment, there are several groups developing and optimizing electroporation systems. The combination with other cytotoxic drugs is under investigation, for instance electroporation therapy in combination with systemic cisplatin therapy (4-6). Electroporation in combination with simultaneous chemoradiation using cisplatin to improve local drug uptake and improve local tumor control, is interesting and needs further investigation.

During the last decades, systemic selective strategies have become more important to target minimal residual disease and occult metastases. These adjuvant strategies are essential to improve the overall survival of head and neck cancer. Monoclonal antibodies (mAbs) or antibody fragments are agents aimed to selectively target tumor cells, for selective delivery of toxic payloads or for complete neutralization/blockage of critical growth and survival stimulatory pathways. Normal tissues, that are not expressing the specific target, should not be affected by these mAbs. The humanized mAb bivatuzumab is directed against the CD44v6 antigen that is predominantly expressed on squamous cell carcinomas. In previous studies, encouraging progress was obtained with the development of bivatuzumab and other anti-CD44v6 mAbs, in CD44v6-directed radioimmunoscintigraphy studies prior to radioimmunotherapy (RIT) studies in head and neck cancer (7,8). Binding of these mAbs to the target antigen results in internalization of the antibody (9). Phase I dose escalation RIT studies showed promising antitumor effects with consistent stable disease at higher radioactivity dose levels. Myelotoxicity caused by circulating radioimmunoconjugate appeared to be the dose limiting toxicity. In addition, in some patients mild oral mucositis was observed (10-12), while these patients had experienced severe mucositis during previous external beam radiotherapy. These data indicated that cross-reactivity of anti-CD44v6 mAbs with normal mucosa is not a limitation for therapeutic application of these mAbs per se. The use of radioimmunoconjugates resulted in limitations related to logistics and radiation safety. Omission of radioactivity could solve these issues, and therefore, an anti-CD44v6 antibody-drug conjugate was developed. Antibody-drug conjugates are particularly attractive, because no bone marrow toxicity has to be expected, and therefore perspectives might arise for combined treatment with conventional chemotherapeutics.

Bivatuzumab mertansine is a tumor-activated prodrug conjugate, which consists of the super-toxic anti-microtubule drug mertansine (DM1) covalently linked to bivatuzumab. After binding to CD44v6, the complex can be internalized, and the mertansine molecules will be released intracellularly by cleavage of the antibody-mertansine disulfide bonds. Normal tissues, not expressing the CD44v6 antigen will not be affected by this therapy. In **chapter 3**, a phase I dose escalation study with bivatuzumab mertansine is described. The safety, maximum tolerated dose (MTD), and preliminary efficacy of bivatuzumab mertansine were assessed, and the pharmacokinetics and immunogenicity were determined after three weekly i.v. injections of the conjugate.

In this study, no toxicity related to free mertansine as a result of deconjugation in the circulation was observed, and no evidence was found for the non-specific uptake of conjugate by cells not expressing the antigen. The main toxicity of bivatuzumab mertansine was directed against the skin, most probably due to CD44v6 expression in this tissue (cross-reactivity). The majority of skin reactions was reversible; however, one fatal drug-related adverse event had occurred. Toxic epidermal necrolysis (TEN) appeared in one patient treated at a dose of 140 mg/m^2. Toxicity was most probably triggered by the cross-reactivity of bivatuzumab with skin keratinocytes. There was no evidence for a dose-toxicity relationship, and it is therefore difficult to predict when severe skin toxicity will occur (13). It was decided to discontinue further clinical development of bivatuzumab mertansine.

In parallel studies with single infusion of bivatuzumab mertansine, thirty-one head and neck cancer patients had received doses of 25-325 mg/m^2. Thirteen patients received a second infusion after three weeks. The MTD was reached at 300 mg/m^2, with skin toxicity being dose limiting. No immune response was induced by the conjugate in any patient. The principal toxic effects were maculopapular rashes, focal blister formation and skin exfoliation. Three patients had partial responses at doses of 200, 275 and 325 mg/m^2 (14-16). Two patients treated at this latter dose level experienced dose-limiting toxicities, with some signs of epidermolysis, but these toxicities appeared to be totally reversible. The previous dose level of 300 mg/m^2 had been well tolerated.

In another parallel study with bivatuzumab mertansine in patients with CD44v6-positive metastatic breast cancer, twenty-four patients were treated at eight different dose levels (25-200 mg/m^2). Seven of these patients received more than one course of bivatuzumab mertansine. Two dose-limiting toxicities occurred: one patient treated with 125 mg/m^2 developed transient grade 4 elevation of liver enzymes; another patient treated at 175 mg/m^2 experienced grade 3 vomiting. She died from renal failure, which might have been caused by deterioration of pre-existing renal insufficiency. The most common toxicities were transient and mild skin disorders in 75% of patients. No objective responses were observed. Disease stabilization was achieved in 50% of patients independently of dose level (17).

The patient in the study described in chapter 3 who developed TEN had only received two weekly injections of 140 mg/m^2. A total of 70 patients have been treated in different centers, and a total of five dose-limiting toxicities occurred. TEN is considered to be a rare disorder characterized by extensive epidermal death, caused by an idiosyncratic drug reaction, without dose-toxicity relationship. The mortality rate of TEN is high, and no specific treatment regimen has been described. Because it is unpredictable which patient is at risk for developing TEN, the bivatuzumab mertansine conjugate was classified as not save, and clinical development was discontinued before reaching overall MTD.

From this study, it can be concluded that strict tumor-selective expression of the particular target antigen is required, when using supertoxic antibody-drug conjugates for therapy. The expression of CD44v6 was probably not selective enough to allow safe therapy. This may have consequences for the development of other antibody-mertansine conjugates like trastuzumab-mertansine (T-DM1), which is currently evaluated in patients with advanced HER2-positive breast cancer (18). Overexpression of the receptor tyrosine kinase HER2 (erbB2) is correlated with a poor prognosis in breast and ovarian cancer. Treatment with the anti-HER2 mAb trastuzumab improves survival in patients with HER2-positive breast cancer, but it also causes cardiomyopathy (19,20). Anthracycline-trastuzumab-containing regimens are limited by unacceptably high rates of significant cardiotoxicity, particularly upon concurrent administration. Trastuzumab increases the risk of anthracycline-induced cardiotoxicity (21). Myocardial HER2-expression may be transiently upregulated by a compensatory mechanism following cardiac stress (22). Although phase I and II clinical trials with T-DM1 till now did not show cardiac-specific toxicity, results from our trial indicate that care should be taken, especially for patients treated with anthracyclines in the past (23,24).

The selective targeting of tumor angiogenesis and neovasculature is another appealing therapeutic anti-cancer strategy. Angiogenesis is required for tumor progression and metastasis, and the inhibition of this process as well as the eradication of tumor vasculature has become an intensive focus of clinical investigation. The mAb fragment L19-SIP is directed against the extra-domain B (ED-B) of fibronectin, a marker of angiogenesis. **Chapter 4** describes the biodistribution of L19-SIP and its potential for RIT in nude mice bearing the head and neck cancer xenograft lines FaDu and HNX-OE. L19-SIP showed high and selective tumor uptake in the two head and neck cancer xenograft lines tested, with tumor-to-blood uptake ratios ranging from 4.4 ± 1.8 at 24 h to 21.4 ± 1.8 at 72 h after injection, for the FaDu line. Tumor-to-nontumor uptake ratios were in general at least as high, which is encouraging when taken into account that L19-SIP also binds to murine ED-B. RIT with ^{131}I-L19-SIP at MTD level of 74 MBq caused significant tumor growth delay and improved survival in both lines. The efficacy was enhanced by combination of ^{131}I-L19-SIP RIT with cetuximab, without increase of toxicity. When mice with established xenografts were treated with this combination, complete responses without

regrowth during the 3-4 months observation period were observed in two out of eight FaDu tumors and five out of eight HNX-OE tumors.

Selective targeting of tumor angiogenesis and neovasculature can be used in nearly all solid cancers, since elevated ED-B expression is found in most of these tumors (25,26). Recently, a clinical RIT study with ^{131}I-L19-SIP in lymphoma patients showed promising results. ED-B expression was found in tumor biopsies from more than 200 Hodgkin and non-Hodgkin lymphoma patients of nearly all entities, as well as in biopsies from patients with myeloproliferative diseases. In contrast, ED-B expression was nearly absent in normal lymph nodes (n = 10) and bone marrow biopsies (n = 9). The extent of ED-B expression in lymphoma tissues was positively correlated with grade of malignancy. The in vivo accessibility of ED-B for ^{131}I-L19-SIP was confirmed in three lymphoma patients, in whom the lymphoma lesions were visualized by SPECT scanning. These patients had failed multiple therapies, and presented with relapsed disease as documented by ^{18}FDG PET-CT scans. Based on selective uptake into tumor lesions and on adequate bone marrow dosimetry, two patients were selected to receive a therapeutic dose of ^{131}I-L19-SIP of 5.55 GBq (150 mCi), and one patient received a dose of 3.7 GBq (100 mCi). Whole-body and SPECT-CT images were obtained 8 to 12 days after administration of this dose to confirm specific tumor targeting. In two relapsed Hodgkin lymphoma patients, ^{131}I-L19-SIP RIT induced a sustained partial response, qualifying ED-B as a promising target for antibody-based lymphoma therapies (27). In addition, these achievements justify the development of RIT with ^{131}I-L19-SIP in selected patients with solid tumors like head and neck cancer. Extensive immunohistochemical evaluation has shown ED-B expression in the majority of head and neck tumors, with most abundant expression in the more aggressive ones (28). This variability in expression indicates that pretherapy imaging with L19-SIP for the assessment of biodistribution and dosimetry is recommended for the selection of RIT candidate patients. For high resolution imaging and accurate quantification of L19-SIP distribution, PET is a more attractive option than the currently used SPECT procedure (29,30).

Aforementioned encouraging preclinical and clinical data on RIT with ^{131}I-L19-SIP formed the basis for the development of a pretherapy scouting procedure using ^{124}I-L19-SIP as the PET-tracer. Molecular imaging of tumor angiogenesis has clinical perspectives for lesion detection, patient stratification, new drug development and validation, treatment monitoring, and dose optimization (31). **Chapter 5** describes the development of ^{124}I-L19-SIP immuno-PET, not only for imaging of tumor angiogenesis, but also as a scouting procedure prior to clinical ^{131}I-L19-SIP RIT, to confirm selective tumor targeting and to enable dosimetry. Imaging of the expression of ED-B of fibronectin can be important for cancer diagnosis and monitoring of therapeutic efficacy against cancer.

As starting point for establishing ^{124}I-L19-SIP immuno-PET, infrastructure and procedures were developed for efficient GMP-compliant production of large batches of ^{124}I

(> 2 GBq) at high radionuclidic (>99.5%) and radiochemical (>95% as iodide) purity and low tellurium (<1 μg/ml) and pyrogen (<5 EU/ml) content. As a result, clinical grade ^{124}I is now available for worldwide distribution, not only for immuno-PET applications but also for selection of patients with thyroid cancer for ^{131}I therapy (figure 1).

L19-SIP labeled with ^{124}I demonstrated optimal integrity and immunoreactivity, and exhibited the same pharmacokinetic behavior as the corresponding ^{131}I conjugate. Biodistribution studies revealed high and selective tumor targeting, resulting in tumor to blood ratios ranging from 6.0 at 24 h to 45.9 at 72 h after injection. As a result, high tumor to normal tissue ratios were obtained with ^{124}I-L19-SIP, resulting in clear visualization of tumors by PET imaging, even when tumors were small (~50 mm^3). Immuno-PET with ^{124}I-L19-SIP appeared qualified for sensitive imaging of tumor neovasculature and for predicting ^{131}I-L19-SIP biodistribution. This result paves the way for using ^{124}I-L19-SIP immuno-PET in the selection of ^{131}I-L19-SIP RIT candidate patients, on the basis of appropriate tumor targeting and dosimetry (personalized therapy).

Currently, nine mAbs have been approved for cancer therapy, all being intact mAbs. Five of the mAbs have been approved for treatment of hematological malignancies: rituximab, gemtuzumab ozogamicin, alemtuzumab, ibritumomab tiuxetan, and tositumomab. Four mAbs consisting of trastuzumab (anti-HER2), cetuximab (anti-EGFR), panitumumab (anti-EGFR) and bevacizumab (anti-VEGF) have been approved for therapy of solid tumors. One of these mAbs, cetuximab, has been approved for use in head and neck cancer. Despite several clinical successes, it is fair to state that the efficacy of current mAbs is still quite limited, with benefit for just a portion of patients, while costs of mAb therapy are excessive.

Because cancer cells have the inherent ability to use several pathways for growth advantage (e.g. related to proliferation, angiogenesis, invasion and metastasis) selective targeting of just one single tumor target might be insufficient for fully effective cancer treatment. For example, it has been shown that tumors can become resistant for anti-EGFR therapy, and for these tumors the c-Met signaling pathway becomes more important for survival (32). These insights have boosted strategies for the simultaneous blockage of several critical targets. Studies have been started in which combinations of approved mAbs are used, e.g. one mAb directed against EGFR and another mAb directed against VEGF (33). Alternatively, some pharmaceutical companies have started the clinical development of mAb cocktails. Whereas mAbs are considered to be “expensive medicines”, introduction of combination mAb therapy will be even more challenging as far as economic aspects concern.

In this thesis, we introduce the use of nanobodies for inhibition of signal transduction pathways, EGFR mediated signaling included (34). Nanobodies are derived from a unique antibody format, heavy chain-only antibodies that are present in species from the family of Camelidae, including llama, camel, and dromedary. Particularly attractive is

the flexibility of drug formatting whereby it is straightforward to generate multivalent and/or multi-specific single molecule formats and to produce these formats in a cheap way in bacteria or yeast. By doing so, a single "bead chain-like" molecule is obtained consisting of several 15 kDa binding units, which can be directed against the same or against different targets. Very important for the future perspectives of this modular mAb technology is the question whether binding units retain their binding affinity and specificity when placed behind each other within such bead chain. In **chapter 6** we challenged this question by constructing bead chains consisting of anti-EGFR (αEGFR) and/or anti-albumin (αAlb) binding units. In vitro binding studies and in vivo biodistribution studies in tumor bearing mice were performed to test whether both units retained full functionality. An αEGFR-αEGFR nanobody showed rapid blood clearance, very high tumor-to-normal tissue ratios (except for kidney), but moderate levels of tumor uptake (5.0 ± 1.4 %ID/g at 6 h after injection), which rapidly decreased over time. Rapid clearance of this ~35 kDa construct was expected, since molecules smaller than 60 to 70 kDa in general are filtered out by the kidneys very rapidly. A totally different biodistribution profile was obtained when an αAlb binding unit was added to the αEGFR-αEGFR construct. The thus obtained ~50 kDa αEGFR-αEGFR-αAlb molecule showed: (a) rapid, high (up to 35.2 ± 7.5 %ID/g), and selective tumor accumulation, which was similar to that of the 150 kDa anti-EGFR reference mAb cetuximab; (b) prolonged residence time in blood and tumor; (c) homogeneous distribution throughout the tumor, more homogeneous than cetuximab, and (d) apparent saturation of EGFR when administered at a relatively high dose. These data indicate that nanobody technology allows the construction of ligands, which show optimal characteristics for EGFR targeting. In addition, the data show that binding units retain their binding characteristics when placed within a bead chain. Whereas the presence of αEGFR units resulted in selective tumor-targeting of EGFR expressing tumors, the binding of the αAlb unit to serum albumin caused extension of half-life in blood, and in fact similar pharmacokinetics were found for αEGFR-αEGFR-αAlb and murine serum albumin. These findings encourage further exploration of the nanobody toolbox, e.g. for development of biparatopic (two different epitopes on the same target antigen) (35) or multi-specific single molecule nanobody constructs. With respect to the latter, simultaneous targeting of two or more critical tumor targets, e.g. EGFR and VEGF, by one single nanobody molecule (αEGFR-αVEGF-αAlb like formats) comes within reach. Until now, nanobody constructs with up to 5 binding units can be produced. Whether each of these units will contain full binding characteristics has to be demonstrated.

At this moment, three nanobodies are tested in clinical studies. Two therapeutic nanobodies target Von Willebrand Factor, which may reduce the risk of thrombosis in patients with acute coronary syndrome and thrombotic thrombocytopenic purpura. The third nanobody targets tumor necrosis factor alpha (TNF-α), a key drug target in combating inflammation related disorders such as rheumatoid arthritis. Until now, no clinical studies

have been started with nanobodies targeting cancer. One of the important questions in these clinical trials is whether human-anti-llama antibodies will be formed, since such immunological response might hamper efficient targeting. This is particularly true for the slow kinetic constructs containing an αAlb unit.

In conclusion: the results from the studies described in this thesis encourage the further development of targeted therapies for the locoregional and systemic treatment of head and neck cancer. Chemo-electroporation therapy appeared to be a valuable addition in the treatment of patients who could no longer be treated by surgery or radiotherapy, and combination with systemic therapies is interesting and needs further investigation. The selection of target antigens with strict tumor-selective expression needs attention to enable the use of antibody-drug conjugates for therapy. Unacceptable toxicities due to cross-reactivity should be anticipated. The results from the radioimmunotherapy studies for the selective targeting of tumor vasculature pave the way for clinical studies. Appropriate tumor targeting and dosimetry can be obtained by using immuno-PET prior to radioimmunotherapy. The development of next-generation multivalent or multispecific antibody-fragments opens new avenues for multi-targeted therapies by blocking several critical tumor targets simultaneously.

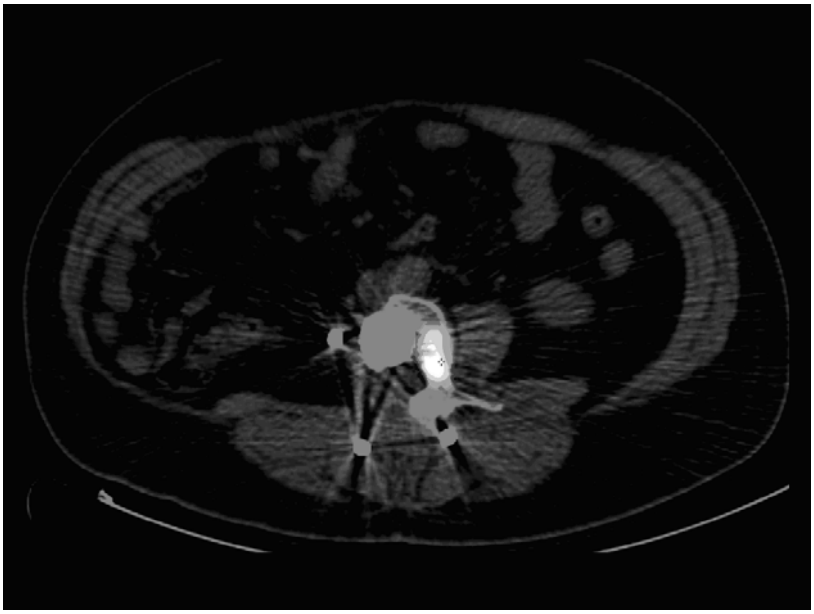

Figure 1. Patient with metastasized thyroid cancer. After extensive vertebral surgery and several treatments with ^{131}I, it remained unclear whether vital tumor tissue was still present. ^{124}I-PET-CT clearly shows iodine uptake (i.e. vital tumor) at the left side of the vertebra. Therefore, adjuvant high dose ^{131}I therapy is recommended for this patient.

References

1. Burian M, Formanek M, Regele H. Electroporation therapy in head and neck cancer. Acta Otolaryngol. 2003;123:264-268.
2. Bloom DC, Goldfarb PM. The role of intratumor therapy with electroporation and bleomycin in the management of advanced squamous cell carcinoma of the head and neck. Eur J Surg Oncol. 2005;31:1029-1035.
3. Copper MP, Triesscheijn M, Tan IB, Ruevekamp MC, Stewart FA. Photodynamic therapy in the treatment of multiple primary tumours in the head and neck, located to the oral cavity and oropharynx. Clin Otolaryngol. 2007;32:185-189.
4. Sersa G, Stabuc B, Cemazar M, Jancar B, Miklavcic D, Rudolf Z. Electrochemotherapy with cisplatin: potentiation of local cisplatin antitumour effectiveness by application of electric pulses in cancer patients. Eur J Cancer. 1998;34:1213-1218.
5. Fulimoto T, Maeda H, Kubo K, et al. Enhanced anti-tumour effect of cisplatin with low-voltage electrochemotherapy in hamster oral fibrosarcoma. J Int Med Res. 2005;33:507-512.
6. Byrne CM, Thompson JF. Role of electrochemotherapy in the treatment of metastatic melanoma and other metastatic and primary skin tumors. Expert Rev Anticancer Ther. 2006;6:671-678.
7. De Bree R, Roos JC, Quak JJ, Den Hollander W, Snow GB, and Van Dongen GAMS. Radioimmunoscintigraphy and biodistribution of ^{99m}Tc-labeled monoclonal antibody U36 in patients with head and neck cancer. Clin Cancer Res. 1995;1:591-598.
8. Stroomer JW, Roos JC, Sproll M, et al. Safety and biodistribution of ^{99m}Technetium-labeled anti-CD44v6 monoclonal antibody BIWA 1 in head and neck cancer patients. Clin Cancer Res. 2000;6:3046-3055.
9. Vrouenraets MB, Visser GWM, Stigter M, Oppelaar H, Snow GB, van Dongen GAMS. Comparison of aluminium (III) phthalocyanine tetrasulfonate- and meta-tetrahydroxyphenylchlorin-monoclonal antibody conjugates for their efficacy in photodynamic therapy in vitro. Int J Cancer. 2002;98:793-798.
10. Colnot DR, Quak JJ, Roos JC, et al. Phase I therapy study of ^{186}Re-labeled chimeric monoclonal antibody U36 in patients with squamous cell carcinoma of the head and neck. J Nucl Med. 2000;41:1999-2010.
11. Colnot DR, Ossenkoppele GJ, Roos JC, et al. Reinfusion of unprocessed, granulocyte colony-stimulating factor-stimulated whole blood allows dose escalation of ^{186}Re-labeled chimeric monoclonal antibody U36

radioimmunotherapy in a Phase I dose escalation study. Clin Cancer Res. 2002;8:3401-3406.

12. Börjesson PKE, Postema EJ, Roos JC, et al. Phase I therapy study with ^{186}Re-labeled humanized monoclonal antibody BIWA 4 (bivatuzumab) in patients with head and neck squamous cell carcinoma. Clin Cancer Res. 2003;9:3961s-3972s.
13. Tijink BM, Buter J, de Bree R, et al. A phase I dose escalation study with anti-CD44v6 bivatuzumab mertansine in patients with incurable squamous cell carcinoma of the head and neck or esophagus. Clin Cancer Res. 2006;12:6064-6072.
14. Riechelmann H, Sauter A, Golze W, et al. Phase I trial with the CD44v6-targeting immunoconjugate bivatuzumab mertansine in head and neck squamous cell carcinoma. Oral Oncol. 2008;44:823-829.
15. Sauter A, Kloft C, Gronau S, et al. Pharmacokinetics, immunogenicity and safety of bivatuzumab mertansine, a novel CD44v6-targeting immunoconjugate, in patients with squamous cell carcinoma of the head and neck. Int J Oncol. 2007;30:927-935.
16. Gronau S, Sauter A, Bogeschdorfer F, et al. Phase I trial with the CD44v6-targeting immunoconjugate bivatuzumab mertansine in squamous cell carcinoma of the head and neck (SCCH&N). Clin Cancer Res. 2005;11(24 Suppl):897s-898s.
17. Rupp U, Schoendorf-Holland E, Eichbaum M, et al. Safety and pharmacokinetics of bivatuzumab mertansine in patients with CD44v6-positive metastatic breast cancer: final results of a phase I study. Anticancer Drugs. 2007;18:477-485.
18. Lewis Phillips GD, Li G, Dugger DL, et al. Targeting HER2-positive breast cancer with trastuzumab-DM1, an antibody-cytotoxic drug conjugate. Cancer Res. 2008;68:9280-9290.
19. Gordon LI, Burke MA, Singh ATK, et al. Blockade of the erbB2 receptor induces cardiomyocyte death through mitochondrial and reactive oxygen species-dependent pathways. J Biol Chem. 2009;284:2080-2087.
20. Fuchs IB, Landt S, Bueler H, et al. Analysis of HER2 and HER4 in human myocardium to clarify the cardiotoxicity of trastuzumab (Herceptin). Breast Cancer Res Treat. 2003;82:23-28.
21. Rayson D, Richel D, Chia S, Jackisch C, van der Vegt S, Suter T. Anthracycline-trastuzumab regimens for HER2/neu-overexpressing breast cancer: current experience and future strategies. Ann Oncol. 2008;19:1530-1539.
22. De Korte MA, de Vries EGE, Lub-de Hooge MN, et al.^{111}Indium-trastuzumab visualises myocardial human epidermal growth factor receptor 2 expression shortly after anthracycline treatment but not during heart failure: a clue to uncover the mechanisms of trastuzumab-related cardiotoxicity. Eur J Cancer. 2007;43:2046-2051.

23. Holden SN, Beeram M, Krop IE, et al. A phase I study of trastuzumab-DM1 (T-DM1), a first-in-class HER2 antibody-drug conjugate (ADC), in patients (pts) with advanced HER2+ breast cancer (BC). J Clin Oncol 2008;26 (May 20 suppl; abstr 1029).
24. Vogel CL, Burris HA, Limentani S, et al. A phase II study of trastuzumab-DM1 (T-DM1), a HER2 antibody-drug conjugate (ADC), in patients (pts) with HER2+ metastatic breast cancer (MBC): Final results. J Clin Oncol. 2009;27:15s (suppl; abstr 1017).
25. Carnemolla B, Balza E, Siri A, et al. A tumor-associated fibronectin isoform generated by alternative splicing of messenger RNA precursors. J Cell Biol. 1989;108:1139-1148.
26. Castellani P, Viale G, Dorcaratto A, et al. The fibronectin isoform containing the ED-B oncofetal domain: a marker of angiogenesis. Int J Cancer. 1994;59:612-618.
27. Sauer S, Erba PA, Petrini M, et al. Expression of the oncofetal ED-B-containing fibronectin isoform in hematologic tumors enables ED-B-targeted ^{131}I-L19SIP radioimmunotherapy in Hodgkin lymphoma patients. Blood. 2009;113:2265-2274.
28. Birchler MT, Milisavlijevic D, Pfaltz M, et al. Expression of the extra domain B of fibronectin, a marker of angiogenesis, in head and neck tumors. Laryngoscope. 2003;113:1231-1237.
29. Verel I, Visser GWM, van Dongen GAMS. The promise of immuno-PET in radioimmunotherapy. J Nucl Med 2005;46:Suppl 1:164S-171S.
30. Van Dongen GAMS, Visser GMW, Lub-de Hooge MN, De Vries EG, Perk LR. Immuno-PET: a navigator in monoclonal antibody development and applications. Oncologist 2007;12:1379-1389.
31. Cai W, Chen X. Multimodality molecular imaging of tumor angiogenesis. J Nucl Med 2008;48:113S-128S.
32. Engelman JA, Zejnullahu K, Mitsudomi T, et al. MET amplification leads to gefitinib resistance in lung cancer by activating ERBB3 signaling. Science. 2007;316:1039-1043.
33. Ciardiello F, Troiani T, Bianco R, et al. Interaction between the epidermal growth factor receptor (EGFR) and the vascular endothelial growth factor (VEGF) pathways: a rational approach for multi-target anticancer therapy. Ann Oncol. 2006;17:vii109-114.
34. Roovers RC, van Dongen GAMS, van Bergen en Henegouwen PMP. Nanobodies in therapeutic applications. Curr Opin Mol Ther. 2007;9:327-335.
35. Neri D, Momo M, Prospero T, Winter G. High-affinity antigen binding by chelating recombinant antibodies (CRAbs). J Mol Biol. 1995;246:367-373.