Diagnosis and Radioimmunotherapy of Head and Neck Squamous Cell Carcinomas

TOMAS EKBERG
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Abstract

The diagnosis and treatment of patients with advanced tumors in the head and neck is an interesting challenge where there is a need for new approaches in diagnostics and adjuvant treatment. Differences in antigen expression between tumors and normal tissues provide a means for application of antibody-based targeting techniques. By targeting a structure that is abundant on tumor cells and limited on normal cells, radioactivity can be delivered.

The use of positron emission tomography (PET) in patients with head and neck tumors is evaluated in this thesis. PET using the tracer fluorodeoxyglucose (FDG) is found to play an important diagnostic role and often has a direct clinical impact on planned surgery or other treatment. Possible targeting structures are also investigated in this thesis, and it is concluded that the EGFR and CD44v6 stand out as possible antigens for targeting approaches of squamous cell carcinomas in the head and neck (HNSCC). A radioimmunoassay for quantification of EGFR and CD44v6 is validated and concluded to be a valuable complement to immunohistochemistry for the analysis of tumors and for the planning of radioimmunotherapy. Finally, promising results of radioimmunotherapy in tumor bearing mice with the monoclonal antibody U36 labeled with the alpha emitter astatine-211 are presented.

These results demonstrate how differences between tumors and normal tissues can be used to improve diagnostic outcomes and indicate that radioimmunotherapy can be a future adjuvant therapy or treatment of residual disease in HNSCC.

Keywords: head and neck squamous cell carcinoma, tumor targeting, radionuclide targeting, PET, therapy, diagnostics, antibodies

Tomas Ekberg. Department of Surgical Sciences, Otolaryngology and Head & Neck Surgery, Akademiska sjukhuset, Uppsala University, SE-75185 Uppsala, Sweden

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“Most gulls don’t bother to learn more than the simplest facts of flight – how they get from shore to food and back again. For most gulls, it is not the flying that matters, but eating.”

From Jonathan Livingston Seagull, by Richard Bach

To Anna-Karin, Cecilia and Sixten
Original papers

This thesis is based on the following papers, which will be referred to in the text by their roman numbers I-IV:


Reprints were made with permission from Acta Oto-Laryngologica (I), International Journal of Oncology (II), Tumor Biology (S Karger AG, Basel) (III), and Laryngoscope (IV).
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<th>Definition</th>
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<tbody>
<tr>
<td>Ab</td>
<td>antibody</td>
</tr>
<tr>
<td>BIWA</td>
<td>Monoclonal antibody bivatuzumab</td>
</tr>
<tr>
<td>CPM</td>
<td>Counts Per Minute</td>
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<td>CT</td>
<td>Computed Tomography</td>
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<tr>
<td>DNA</td>
<td>Deoxyribonucleic Acid</td>
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<td>EGFR</td>
<td>Epidermal Growth Factor Receptor</td>
</tr>
<tr>
<td>EGFRvIII</td>
<td>Mutant Epidermal Growth Factor Receptor</td>
</tr>
<tr>
<td>ErbB-1</td>
<td>Human Epidermal Growth Factor Receptor 1</td>
</tr>
<tr>
<td>ErbB-2</td>
<td>Human Epidermal Growth Factor Receptor 2</td>
</tr>
<tr>
<td>ErbB-3</td>
<td>Human Epidermal Growth Factor Receptor 3</td>
</tr>
<tr>
<td>ErbB-4</td>
<td>Human Epidermal Growth Factor Receptor 4</td>
</tr>
<tr>
<td>FDA</td>
<td>United States Food and Drug Administration</td>
</tr>
<tr>
<td>FDG</td>
<td>$(^{18F})$Fluorodeoxyglucose</td>
</tr>
<tr>
<td>FLT</td>
<td>$(^{18F})$Fluoro-L-Thymidin</td>
</tr>
<tr>
<td>HACA</td>
<td>Human Antichimeric Antibody</td>
</tr>
<tr>
<td>HER1</td>
<td>Human Epidermal Growth Factor Receptor 1</td>
</tr>
<tr>
<td>HER2</td>
<td>Human Epidermal Growth Factor Receptor 2</td>
</tr>
<tr>
<td>HER3</td>
<td>Human Epidermal Growth Factor Receptor 3</td>
</tr>
<tr>
<td>HER4</td>
<td>Human Epidermal Growth Factor Receptor 4</td>
</tr>
<tr>
<td>HNSCC</td>
<td>Head and Neck Squamous Cell Carcinoma</td>
</tr>
<tr>
<td>huA33</td>
<td>Humanized IgG1 antibody A33</td>
</tr>
<tr>
<td>IHC</td>
<td>Immunohistochemistry</td>
</tr>
<tr>
<td>ip</td>
<td>Intraperitoneal</td>
</tr>
<tr>
<td>iv</td>
<td>Intravenous</td>
</tr>
<tr>
<td>Term</td>
<td>Definition</td>
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<td>-------</td>
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</tr>
<tr>
<td>LET</td>
<td>Linear Energy Transfer</td>
</tr>
<tr>
<td>MAb</td>
<td>Monoclonal Antibody</td>
</tr>
<tr>
<td>MP</td>
<td>Multi point</td>
</tr>
<tr>
<td>MRI</td>
<td>Magnetic Resonance Tomography</td>
</tr>
<tr>
<td>Neu</td>
<td>Human Epidermal Growth Factor Receptor 2</td>
</tr>
<tr>
<td>PET</td>
<td>Positron Emission Tomography</td>
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<tr>
<td>RID</td>
<td>Radioimmunodiagnosis</td>
</tr>
<tr>
<td>RIT</td>
<td>Radioimmunotherapy</td>
</tr>
<tr>
<td>ROI</td>
<td>Region of Interest</td>
</tr>
<tr>
<td>sc</td>
<td>Subcutaneously</td>
</tr>
<tr>
<td>SCC</td>
<td>Squamous Cell Carcinoma</td>
</tr>
<tr>
<td>SD</td>
<td>Single dose</td>
</tr>
<tr>
<td>SPECT</td>
<td>Single Photon Emission Computed Tomography</td>
</tr>
<tr>
<td>SUV</td>
<td>Standardized Uptake Value</td>
</tr>
<tr>
<td>TNM</td>
<td>Tumor, Node, Metastasis</td>
</tr>
<tr>
<td>U36</td>
<td>Chimeric monoclonal antibody U36</td>
</tr>
<tr>
<td>UICC</td>
<td>International Union against Cancer</td>
</tr>
<tr>
<td>USA</td>
<td>United States of America</td>
</tr>
</tbody>
</table>
"Research is what I’m doing when I don’t know what I’m doing.” To me, the words of the German-American rocket scientist Wernher von Braun (1912-1977) speak to the joy of being able to perform tasks without knowing the results in advance. I think this is the essence of research. As a clinically active researcher I have the opportunity to incorporate the results from research into the standardized and evidence based care we provide to our patients.

This thesis comprises different aspects of diagnosis and treatment of patients with cancer in the head and neck. It moves from a retrospective study of the clinical impact of positron emission tomography (PET), which represents the clinical reality of a new diagnostic modality, to the search for new molecular markers for head and neck squamous cell carcinomas (HNSCC) that have potential to be used for diagnosis and treatment. Thereafter a method for quantification of interesting molecular targets is described. Finally, treatment of HNSCC in an in vivo model using radioimmunotherapy (RIT) represents a step towards transforming preclinical science to a clinical reality and providing benefit for our cancer patients.

Head and neck squamous cell carcinoma

The yearly worldwide incidence of cancer in the oral cavity, pharynx and larynx is estimated to approximately 615,000 cases, which is more than 6% of all cancers (excluding skin) \(^1\). It is also a “visible” cancer since the tumor and its treatment might affect the facial appearance of the patient as well as vital functions such as speech, swallowing and breathing. Although cancer can arise in many different tissues and organs in the head and neck region, squamous cell carcinoma (SCC) is the predominant histological type \(^2\). The most important risk factors include life style habits such as alcohol consumption and smoking \(^3,4\); however, viral infections \(^5\) and genetic predisposition \(^6\) can also be part of the etiology.

Advances in treatment methods have been made during recent decades in the following ways: new combinations of radiation and chemotherapy have proven to be more effective \(^7,9\), surgery is often less extensive and mutilating, and reconstructive surgery including microsurgery is routine at most centers. However, while some patients with small tumors have an excellent
prognosis following today’s standard treatment, others with advanced tumors or with tumors in certain sites of the head and neck face high rates of mortality and morbidity. The combined 5-year survival rate for all tumor stages is improving very slowly. Indeed, approximately 50% of patients will die from their disease.

Field cancerization and second primary tumors

HNSCC originates in the epithelial lining the oral cavity, throat, ear, nasal cavity with sinuses and the surface of the tongue. A majority of the carcinomas, particularly in the oral cavity, have a greater linear extension than extension in depth, and the epithelium surrounding the carcinoma is often precancerous. The concept of “field cancerization” was founded in 1953 to describe vast areas of precancerous epithelium that might give rise to multifocal tumors. There is a high rate, in some studies up to 8-10%, of second primary tumors in the oral cavity, airway and esophagus.

There are several possible causes for the high rate of secondary primary tumors and recurrences after surgery. Surgery may not always eradicate all precancerous epithelium, even when it is macroscopically radical; and radiation therapy may not be fully effective on the same areas. The “borderline epithelium” between cancerous and precancerous is also difficult to define, and because of the tumors’ sometimes vast and irregular linear extent there is a risk for residual cancer post surgery. An adjuvant treatment or a treatment for minimal residual disease after surgery would therefore be of great value.

Tumor behavior is influenced by primary site

Even though the head and neck represents a relatively small area of the body and different SCC at first sight may seem alike, this is not always the case. The symptoms and prognosis may differ tremendously depending on which site that hosts the cancer. SCCs originating in some sites (e.g. the vocal cords) cause early symptoms with metastases occurring at a late stage, while SCCs in other sites (e.g. the base of tongue) may not cause any early symptoms from the primary tumor but instead metastasize at an early stage.

The prognosis also differs greatly between small and advanced tumors. Patients with tumors in stage I generally have a good prognosis. On the other hand, many patients with stage III and IV disease develop distant metastases, and are unlikely to be cured with today’s techniques. The five-year survival rate for locally advanced disease, with or without known distant metastases at the time of diagnosis, is low; and the treatment regime for the neck without known metastases (cN0) is a matter of debate.

It also seems as if HNSCCs from different sites express different receptors and other surface molecules in their cellular membranes. Moreover,
antigen expression may serve as a prognostic marker \(^{19-22}\), or possibly the expression patterns change as the tumors become more advanced and possibly metastasize.

**Diagnostic methods**

Currently a series of tests and procedures is used for diagnostic purposes. Patients undergo a physical examination, and endoscopy is used to access the tumor site. Biopsies from suspected or confirmed tumor areas are vital for the diagnosis. Sometimes biopsies guided by ultrasound, computed tomography (CT) or magnetic resonance imaging (MRI) are needed. For imaging of the tumors and areas where metastatic spread is suspected, CT is the preferred modality to assess for bone invasion, including invasion into the skull base. MRI is useful in the assessment of soft tissue involvement, including perineural invasion. Ultrasound is sometimes a good alternative, especially in the neck \(^{23}\), but this method is user dependent and does not produce images for comparison. PET has recently gained acceptance for imaging in the head and neck \(^{24,25}\), and is now part of the standard clinical work up at many centers.

Bearing in mind the dismal prognosis of advanced disease and the reduced chance for cure if the initial treatment fails, a specific diagnosis of HNSCC is especially important in these cases. A clear knowledge of tumor margins is needed, and the presence of distant disease (lymph node- and distant metastases) needs to be verified or denied. Since CT and MRI diagnose lymph node metastases through criteria of size and appearance and fluorodeoxyglucose (FDG) PET (see below) is not tumor specific and has a spatial resolution of at best 5 mm, micrometastases are sometimes missed. An interesting approach to assess for regional disease is “sentinel node” biopsy \(^{26}\). Here the examiner injects a color marker and/or small amounts of a radioactive compound into the primary tumor, and after a waiting time the surgeon can remove the first lymph node draining the tumor, the sentinel node. However, since the standard compounds that are injected are not tumor specific, the technique merely locates the sentinel node and not necessarily a metastatic spread of the tumor. In cases with “skip metastases” \(^{27}\), i.e. metastases distal to the sentinel node, the finding of a tumor free sentinel node using the standard technique yields a false negative result.

**Positron emission tomography**

The current standard clinical PET examination is made with the glucose analogue FDG. The positron emitter fluor-18 is incorporated with FDG, and injected into the bloodstream of the patient. After a waiting time the patient is placed in a scanner, which then detects areas where FDG has accumulated.
Areas with increased glycolysis, such as the heart and brain, accumulate FDG. Thus, FDG PET is not tumor specific but a marker of a metabolic state. Fortunately, many tumors, including most HNSCC, also have an increased glucose metabolism.

To allow for interpretation of results, a standardized uptake value (SUV) may be calculated. Asymmetric or nodular deposits of FDG are considered suspicious for malignancy. In these lesions, a “hot spot” with high FDG uptake is outlined and SUV is calculated as SUV = hot spot activity (Bq/mL) x body weight (g) / injected dose (Bq). However, some tumors do not accumulate FDG while areas with infection or inflammation may which can result in false negative or positive results of the examination. PET images are routinely integrated with CT (or MRI) images. This improves the diagnostic accuracy, but a tumor specific tracer would be advantageous.

**Immunohistochemistry**

Biopsies are vital for tumor diagnosis and specific histologic tumor characteristics often influence the treatment plan. Immunohistochemistry (IHC) is a frequently used and a very important diagnostic tool. In IHC, monoclonal antibodies (MAb) bind to antigens in a specific manner, and a detection system is used to identify the bound primary antibodies. Primary antibodies can be raised against almost any kind of antigen.

IHC plays an important role in distinguishing different tissues and tumors from each other by visualizing tumor antigens within a tissue sample. It is also the most common technique in the search of new targets for targeted therapy (see below). However, IHC has some limitations. It is only semi-quantitative and some caution has to be applied when comparing different studies. The staining intensities may differ from one primary antibody and detection system to another, and criteria for judging intensity and extent of staining may differ between studies. For some purposes, e.g. planning of targeted therapy, a quantitative measure of the number of antigen molecules would be complementary and therefore would add valuable information.

**Treatment**

Treatment of HNSCC includes surgery and external radiotherapy, often in combination with each other and sometimes complemented with chemotherapy. In some cases, photodynamic therapy can be used.

Patients with advanced tumors are often in need of an adjuvant or combination treatment modality if the initial treatment fails. Initial treatment failure can result from an inability to accurately diagnose the transition from precancerous to normal epithelium. Without an exact definition of the tumor’s margin the surgeon or radiotherapist cannot plan the treatment cor-
rectly. If micrometastases are missed in the diagnostic procedure, the follow-
ing treatment might be suboptimal. Another reason for initial treatment fail-
ure can be that surgery or radiotherapy cannot be extensive enough due to
the many vital organs and structures in the head and neck area. Furthermore,
surgery and external radiotherapy cannot cure disease with distant metasta-
ses. Chemotherapy has, in some cases, improved the prognosis when given
in combination with radiotherapy \(33\), however, it is rarely curative on its own.
Finally, these patients have a high risk for second primary tumors in the head
and neck, respiratory organs and esophagus \(12,13\). If full dose radiation ther-
apy already has been given, the treatment choices are limited. Targeted
treatment approaches might be the solution to these problems.

Tumor targeting

The concept of tumor targeting is to specifically locate the tumor cells. To
achieve this, MAbs or antibody fragments are used. In order to be specific,
the target has to be overexpressed on or within the tumor when compared to
normal tissues. The ideal tumor target is only expressed in the tumor cells.
The target antigen must also be accessible to the antibody.

| Radionuclide | Emission of therapeu
tical interest | Physical half-life | Application                |
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<tr>
<td>(^{111})In</td>
<td>Auger cascade</td>
<td>2.8 days</td>
<td>Single cells</td>
</tr>
<tr>
<td>(^{125})I</td>
<td>Auger cascade</td>
<td>60 days</td>
<td>Single cells</td>
</tr>
<tr>
<td>(^{131})I</td>
<td>Low energy beta</td>
<td>8.0 days</td>
<td>Small cell clusters</td>
</tr>
<tr>
<td>(^{186})Re</td>
<td>Medium energy beta</td>
<td>3.7 days</td>
<td>Intermediate clusters</td>
</tr>
<tr>
<td>(^{177})Lu</td>
<td>Low energy beta</td>
<td>6.7 days</td>
<td>Small cell clusters</td>
</tr>
<tr>
<td>(^{211})At</td>
<td>Alpha</td>
<td>7.2 h</td>
<td>Single cells, small cell clusters</td>
</tr>
<tr>
<td>(^{212})Bi</td>
<td>Alpha</td>
<td>1.0</td>
<td>Single cells, small cell clusters</td>
</tr>
</tbody>
</table>

MAbs can work as sole agents to inhibit tumor growth or they can be
“loaded” with cytotoxic drugs or radionuclides \(34\). Radioimmunotargeting
combines radiation delivered by radionuclides with the targeting effect of
MAbs (Figure 1). Depending on the objective, various radionuclides can be
used (Table 1). However, the results do not rely on the binding specificity of
the chosen radionuclide alone. Apart from being specific, several other prop-
erties of the vector are important. One such property is the vector’s kinetics,
which vary with the size of the labeled compound. For radioimmunodiagno-
sis (RID) rapid clearance might be advantageous, while in the case of RIT it
can be a disadvantage. Moreover, the labeling method is of great importance and cannot be allowed to alter the specificity of the antibody.

Figure 1. Radioimmunotargeting is based on a high ratio between targeting antigens in tumor cells and normal cells. Labeled antibodies locate the tumor cells specifically.

Radioimmunotherapy

In RIT the objective is to kill the cancer cells while sparing the surrounding normal tissues. Since radioactivity is the cytotoxic agent, drug resistance can be avoided. RIT is in or is approaching clinical use for several types of tumors, such as hematological, ovarian, prostate, breast, brain, renal, and colorectal cancers (for review see Dearling). HNSCC is likely to be a tumor type well suited for RIT as it is normally sensitive to radiotherapy and expresses elevated levels of some tumor associated antigens.

A variety of radionuclides with different decays and half times can be used depending on the size and histological type of the cancer lesion, the targeting vehicle and the characteristics of the target. Alpha emitting substances have a short range of radioactivity and are highly toxic. These features make them suitable for targeting small tumor cell clusters or single cells. To target larger tumors, beta emitters are suitable. They have a radio-
activity range of several cellular diameters and give rise to a “crossfire ef-
fect” which might be important if intratumoral heterogeneities in hemody-
namics or antigen expression are present. Auger electron emitters require
internalization to deliver their toxicity 48.

Furthermore, the specificity of the vector is of great importance. If large
amounts of radionuclides are emitted in healthy organs such as the skin, liver
or kidneys, the disadvantage of the damage to these organs might override
the advantages of eliminating cancer cells.

Finally, if the vector is a chimeric antibody (partly made up of mouse anti-
tody) there is a risk of a human antichimeric antibody (HACA) response.
The body seems to better tolerate the use of humanized or fully human anti-
bodies 49.

Radioimmunodiagnosis

In RID the objective is to locate the tumor cells and define tumor margins to
receive a tumor specific image. As in the case of RIT, a radionuclide is con-
jugated to a MAb or antibody derivative, and the method relies on a high
ratio between the radioactivity delivered to the tumor and to the surrounding
normal tissues. When combined with other imaging modalities for anatomic
orientation, the result could not only be the basis for the planning of surgery
or radiotherapy but also a surrogate marker for RIT. The imaging mode for
RID could be a gamma camera (images in one dimension unless a dual head
gamma camera is used), single photon emission computed tomography
(SPECT) or PET 50,51. Moreover, an interesting aspect of RID is the possibil-
ity of injecting the targeting compound preoperatively and using a detection
system during surgery to locate tumor cells.

Targets in the head and neck

The epidermal growth factor receptor family

The epidermal growth factor receptor (EGFR) family has four known mem-
bers and consists of the EGFR (HER1/erbB-1), HER2 (erbB-2/neu), HER3
(erbB-3) and HER4 (erbB-4) 52,53. These are transmembraneous type 1 tyro-
sine kinase receptors. Following the binding of a ligand (except for HER2
that has no known ligand) and formation of a hetero- or homodimer with
another receptor in the family they mediate signals to control cell differentia-
tion and proliferation.

EGFR

EGFR is found primarily on cells of epithelial origin, and is overexpressed in
several human malignancies including HNSCC, glioblastoma, non small cell
lung cancer, breast, ovary, prostate and bladder cancer. Not only is EGFR often overexpressed in tumors, but it has been found to play a significant role in the progression of several human malignancies resulting in novel treatment strategies. Cetuximab, a MAb, has demonstrated a direct inhibitory effect on EGFR in HNSCC and recently received an indication for treatment of HNSCC in several countries, including Sweden and the USA.

The proposed overexpression of EGFR in HNSCC might differ between tumors depending on their primary site. EGFR expression is generally low in healthy and normal human tissues, while liver hepatocytes express a high level of EGFR. These factors are important with regard to RIT.

**HER2**

The HER2 receptor is an orphan receptor, which means it does not have a known ligand. Overexpression of the receptor has been found in several types of malignancies, most notably in a subgroup of breast cancers. However, its relevance in HNSCC is still controversial. Most studies indicate variable overexpression rates of the protein, and HER2 expression seems to have a prognostic value particularly for oral SCC. While often overexpressed in malignancies, the receptor has limited expression in normal tissues. However, in some studies of head and neck tumors overexpression has not been seen.

Trastuzumab is a commercially available Mab directed to HER2, and is effective in breast cancer tumors that overexpress the receptor. If the HER2 receptor is overexpressed in some HNSCCs, it would be valuable to identify this subgroup of patients and explore new targeted approaches.

**HER3 and HER4**

The role of HER3 and HER4 in malignant transformation is not fully understood. It has been shown that HER3 can be overexpressed in HNSCC cell lines and is associated with malignant progression. However, both HER3 and HER4 can be significantly expressed also in normal tissues. Possibly coexpression patterns between different members of the EGFR-family is important for malignant transformation. Furthermore, several reports claim that HER3 and HER4 have an intracellular staining pattern in IHC (i.e. are not located in the cellular membrane), which would be a disadvantage in the use of RID or RIT. The possibility of HER3 and HER4 to serve as targeted molecules needs to be further explored.

**CD44v6**

CD44v6 is an isoform of the glucoprotein CD44. It is located on the outer cellular membrane and is frequently expressed in HNSCC and some other human malignancies such as gastric, colorectal, ovarian and prostate cancer. It has been suggested that the glucoprotein is involved in the forma-
tion of tumors and metastases as well as tumor cell invasion\textsuperscript{70,71}. While there is a high expression of CD44v6 in primary HNSCCs and their corresponding metastases, the expression in non-malignant tissues is restricted to a subset of epithelium\textsuperscript{72}.

The chimeric Mab U36 (U36) specifically recognizes the CD44v6 antigen\textsuperscript{73,74}, and has been studied thoroughly. A humanized form of the antibody, bivatuzumab (BIWA), is also available\textsuperscript{49,75}.

What we need, but do not yet have

New approaches to better define of the area to be treated and an effective adjuvant treatment would be valuable. This is especially true for patients with advanced tumors who face an otherwise poor prognosis. Since tumors in the head and neck often differ from each other in growth patterns, prognosis, and expression of surface antigens, it is reasonable to believe that future means of diagnosis and treatment will have to be more personalized. One way to reach the goal of personalized and specific diagnosis and treatment is to use the differences between the tumor and the surrounding normal tissue. This can be achieved with radioimmunotargeting. With greater understanding of the differences between tumors or groups of tumors and introduction of novel techniques in clinical practice, we will be able to tailor the treatment to the individual.
The present study

Tumors in the head and neck; clinical impact of FDG PET (paper I)

Background and aim
Over the last decade the use of FDG PET for imaging of patients with tumors in the head and neck has been gaining acceptance. We experienced numerous cases in which the treatment plan for an individual patient was altered following a PET examination. This retrospective study was conducted to evaluate the efficacy of FDG PET as a diagnostic tool for head and neck tumors and to illustrate the clinical impact of PET exploring the possibility of a reduction in patient morbidity post-treatment.

The study
The case records of the first 80 patients who underwent 104 PET examinations to evaluate tumor extent at the Department of Otolaryngology Head-Neck Surgery, Uppsala University Hospital were studied. The patient’s age, tumor histology, tumor stage (UICC, TNM Classification of Malignant Tumours, 6th ed), and the tumor site were noted. Indications for PET and other imaging methods and their compatibility were recorded. Indications for PET included examinations for staging, to assess for suspicion of recurrent or residual tumor, to assess for an occult primary tumor, or for follow up. The results of the PET examinations were evaluated to determine if they impacted or led to a change in the patient’s staging, work up, treatment plan or follow up.

The oral cavity represented the most common tumor site in our population (\(n=40, 38\%\)), and most tumors were SCCs (\(n=86, 83\%\)). Staging or evaluation was altered after 24 (23%) of the PET examinations. Therapy or follow-up was modified after 33 (32%) examinations, including 12 (12%) where PET directly affected the surgical plan (see Table 2).

The most frequent indication for a PET examination was staging (\(n=39\). Of these, PET detected all but two small tumors (95%), and affected staging in 5/39 cases (39%). Treatment was changed or modified following 6/39 (15%) examinations, including modified or avoided surgery after four examinations (10%).
Table 2. Impact of FDG PET and statistics for different indications in head and neck tumors

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Staging</th>
<th>Recurrence</th>
<th>Occult primary</th>
<th>Follow-up</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Altered staging/evaluation</td>
<td>13%</td>
<td>42%</td>
<td>28%</td>
<td>0</td>
<td>23%</td>
</tr>
<tr>
<td></td>
<td>(n=5)</td>
<td>(n=14)</td>
<td>(n=5)</td>
<td></td>
<td>(n=24)</td>
</tr>
<tr>
<td>Modified therapy/follow-up</td>
<td>15%</td>
<td>52%</td>
<td>39%</td>
<td>21%</td>
<td>32%</td>
</tr>
<tr>
<td>(surgical and non-surgical)</td>
<td>(n=6)</td>
<td>(n=17)</td>
<td>(n=7)</td>
<td>(n=3)</td>
<td>(n=33)</td>
</tr>
<tr>
<td>Modified surgery</td>
<td>10%</td>
<td>21%</td>
<td>6%</td>
<td>0</td>
<td>12%</td>
</tr>
<tr>
<td></td>
<td>(n=4)</td>
<td>(n=7)</td>
<td>(n=1)</td>
<td></td>
<td>(n=12)</td>
</tr>
<tr>
<td>Sensitivity</td>
<td>95%</td>
<td>90%</td>
<td>78%</td>
<td>100%</td>
<td>91%</td>
</tr>
<tr>
<td>Specificity</td>
<td>100%</td>
<td>75%</td>
<td>89%</td>
<td>77%</td>
<td>80%</td>
</tr>
</tbody>
</table>

The most important finding in the study was the high degree of clinical impact found among the 33 PET examinations done on suspicion of recurrent or residual tumor. The results from 17/33 (52%) of the PET examinations influenced further treatment and 7/33 (21%) directly impacted the planned head and neck surgery.

Eighteen patients with metastases from an occult primary tumor underwent FDG PET. The primary tumor was detected by PET in 7/18 (39%) of the examinations and in 4/18 (22%) of the cases PET was the sole modality to do so. One examination was deemed false positive. Among the remaining 10 cases, 2 primary tumors were detected through other diagnostic procedures, and 2 tumors were subsequently regarded as primary malignancies in branchial cysts.

Among the 14 PET examinations done for follow-up, no recurrences or second primary tumors were detected.

SUV was recorded for most tumors. There was a statistically significant difference in SUV between the true positive and true negative examinations, but there was considerable overlap within individual values.

Molecular targets in HNSCC (paper II and III)

Background and aim

Based on the need for a more tumor specific diagnosis and an adjuvant treatment for SCCs in the oral cavity, especially the more advanced tumors, this tumor type was identified as a possible candidate for RIT and RID. The receptors in the EGFR family (EGFR/HER1, HER2, HER3 and HER4) seemed to be reasonable targets, but specific information on their expression in metastatic disease was not available. Furthermore, reports claimed that
HER3 and HER4 might have a predominantly cytoplasmic distribution \cite{63,65,68}, and thus they would not be suitable targets for RIT.

The evidence that the antigen CD44v6 is overexpressed in HNSCC is convincing \cite{46,69}. A reliable method of quantifying antigens in the cellular membrane is needed to better plan RIT and to have a tool complementary to IHC. This applies to CD44v6 as well as EGFR (and other targeting antigens).

The studies in paper II and III aim to further clarify the distribution of possible targets for RIT and RID in advanced cases of HNSCC, and to explore a means of quantifying target antigens. This latter concept could serve as a base for developing more individualized RIT.

The studies

Immunohistochemical studies

In paper II, the expressions of all four receptors in the EGFR family were evaluated in 19 cases of metastatic HNSCC in the oral cavity and base of tongue. The extent and intensity were semiquantitatively judged as proportion of tumor cells stained (0, negative; 1, less than one third; 2, between one and two thirds; and 3, more than two thirds) and intensity of immunostaining (0, negative; 1, weak; 2, moderate (same as epithelium); and 3, intense).

The staining pattern for EGFR and HER2 was membranous, with or without concomitant cytoplasmic staining, while it was mainly cytoplasmic for HER3 and HER4 (Figure 2). The level of expression for EGFR was generally high and consistent, and there was a good agreement between primary tumors and corresponding metastases. Compared to EGFR, HER2 generally had a lower level of staining intensity. Several tumors had less staining than normal epithelium or no staining at all. When HER2 staining was present in a metastasis, it was also present in the corresponding primary tumor.

The expression of HER3 and HER4 varied. It was mainly cytoplasmic and no clear overexpression was found in malignant tissues when compared to normal epithelium.
In paper III, the expressions of EGFR and CD44v6 were evaluated in 10 primary tumors and one metastasis of HNSCC. Nine of the primary tumors originated in the oral cavity. Again, EGFR had a high and consistent level of expression. Expression of CD44v6 was detected in all samples except one. The IHC scores on intensity and extent were almost identical for EGFR and CD44v6 in paper III.
**Cellular experiments (paper II)**

Since immunostaining was found to be mainly cytoplasmic for HER3 (and HER4), cell culture experiments were done in an attempt to determine whether the receptors were present in the cellular membrane. Two cell lines with immunostaining for HER3 (and EGFR) were evaluated. Primary antibodies directed to the extracellular portion of the receptor, and radiolabeled secondary antibodies were used. Parallel experiments with positive (primary antibodies directed to EGFR with known location in the cellular membrane) and negative (without the primary antibodies) controls were performed. The specificity of the secondary antibody was verified by blocking of the binding sites on the primary antibodies by an excess of non radiolabeled secondary antibodies. The results indicate that HER3 is not present at a measurable level in the cellular membrane (Figure 3).

**Quantitative studies (paper III)**

In order to quantify membrane bound antigens a single dose (SD) radioimmunoassay using two MAbs, cetuximab and U36, was validated and then applied to patient samples.

First the *in vitro* binding characteristics of the $^{125}\text{I}$ conjugated MAbs U36 and cetuximab were determined. Then membrane fractions of tumor cells were prepared. When a multipoint (MP) assay was applied to HNSCC cell lines the results did not reveal any statistically significant differences be-
tween cells grown in vitro and in vivo. The MP assay was then used to study CD44v6 and EGFR expression in patient samples. The concentration of antibody where saturation was observed was considered suitable for the SD assay. When the results from the SD and MP assays were compared, no statistically significant differences could be shown. Furthermore, the SD assay was compared to a conventional saturation assay of HNSCC cells. These data were later analyzed in order to identify a relationship between cellular membrane weight and number of cells.

![Antigen quantification using the SD assay in HNSCC tumors and two normal uvula/soft palate samples (UA and UB) for CD44v6 using \(^{125}\text{I}\)-cMAb U36 (shaded bars) and EGFR using \(^{125}\text{I}\)-cMAb cetuximab (white bars). Error bars represent standard deviation. * = not done for EGFR. N = 3.](image-url)

Figure 4. Antigen quantification using the SD assay in HNSCC tumors and two normal uvula/soft palate samples (UA and UB) for CD44v6 using \(^{125}\text{I}\)-cMAb U36 (shaded bars) and EGFR using \(^{125}\text{I}\)-cMAb cetuximab (white bars). Error bars represent standard deviation. * = not done for EGFR. N = 3.
The conversion from membrane weight to number of cells gave an approximation of CD44v6 antigen molecules per cell generally to be between 400,000 and 1,300,000 in the tumor samples, averaging 700,000. The number of EGFR antigen molecules per cell was lower, ranging between 40,000 and 160,000 with an average of 90,000 per cell. This contrasts to the almost identical IHC scores for the same tumors. In normal tissues (uvula and soft palate) the number of antigen molecules per cell was for CD44v6 and EGFR were approximately 200,000 and 20,000, respectively.

Finally a comparison between IHC results (defined as intensity x extent) and antigen quantification values was done. Correlation was found to be significant for both CD44v6 and EGFR (Figure 5).

![Figure 5](image)

Figure 5. Correlation between antigen quantification values using the SD assay and IHC scores for CD44v6 (a) and EGFR (b). In both cases correlation was found to be significant, with \( p<0.001 \) (correlation coefficient = 0.90) and \( p<0.01 \) (correlation coefficient = 0.84), respectively. Error bars represent standard error of mean for the SD analysis.
Radioimmunotherapy of HNSCC (paper IV)

Background and aim
The possibility of using RIT as an adjuvant treatment for HNSCC is appealing. U36 had previously been showed to have good targeting properties and the cell line UT-SCC7 is known to express its target, CD44v6. Clinical phase I studies with U36 in combination with the beta emitting radionuclide rhenium-186 have been completed. The aim was to study the biodistribution and evaluate the therapeutic effect of RIT in vivo using the combination of U36 and the alpha emitter astatine-211 (211At) in nude mice.

The study
The cell line UT-SCC7, derived from HNSCC, was cultured and 10⁷ tumor cells were inoculated subcutaneously (sc) in the left flank of adult female nude mice (balb/c nu/nu) for all experiments. Almost all mice obtained rapid and equal tumor growth, and the tumor doubling time was slightly less than 4 days. U36 was labeled with the alpha emitting radionuclide 211At.

Biodistribution experiments
In the initial biodistribution experiments, 16 tumor-bearing mice were randomized into groups of four. One group (the blocked group) received an excess (100-fold) of unlabeled U36 by way of intraperitoneal (ip) injection. After four hours an intravenous (iv) injection of 211At-labeled MAb U36 was given via the tail vein. Mice were subsequently anesthetized and sacrificed at time points 3, 7 and 21 hours post iv-injection. The mice in the blocked group were sacrificed at 21 hours post iv-injection.

An increasing uptake in tumors with time and a statistically significant difference between the blocked and nonblocked groups at 21 hours demonstrated the specificity of 211At-labeled U36. No significant biodistribution difference between the blocked and nonblocked groups could be discerned in organs.

Therapeutic experiments
To exclude effects by the antibody itself, a 100-fold excess of unlabeled U36 was transplanted sc simultaneously with primary tumor transplantation (n=6). Controls (n=6) were transplanted with tumor cells only. All mice in the two groups formed tumors, and there was no statistically significant difference between the groups.

Finally therapeutic groups with transplanted tumor cells were injected with an excess (120-fold) of unlabeled U36 ip 3 hours before iv injection of the labeled conjugate to block CD44v6 in the tumor cells (n=10), or with labeled conjugate iv only (n=10). The control group (n=6) received no fur-
ther injections after tumor transplantation. 18/20 (90%) cases responded to therapy by decreasing or stabilizing their tumor volumes (Figure 6). No significant difference was seen between the two therapeutic groups.

**Figure 6.** Efficacy of 211At-labeled U36 in the therapeutic experiments. The treated mice are plotted individually and the control group as mean where error bars represent standard deviations. In the experiment with block (top) all mice responded to therapy. In the non blocked group (below) one mouse was a non responder (■) with tumor growth similar to the control group (●) which did not receive therapy. One mouse was a partial responder (♦).
The diagnosis and treatment of HNSCC continues to be an interesting challenge with many possibilities for improvements. As new techniques for imaging and treatment are being explored and implemented in clinical practice it is important to explore recently introduced modalities to be able to use them in the best possible way and to be able to evaluate if any formerly used modalities can be excluded in the future. Naturally, it is equally important to be in the front line of research and development. The present thesis covers different aspects of diagnosis and treatment of HNSCC, with particular emphasis on oral SCC.

Paper I is a retrospective study of the usefulness and clinical impact of FDG PET in head and neck tumors. Most tumors in the population studied, SCCs as well as tumors of other origin, were diagnosed with the PET-technique. This is in accordance with the findings of other authors 25,77,78. Diagnosis was made after considering the FDG PET results in context with an anatomic imaging modality (CT or MRI). SUV, a semiquantitative value, can guide the interpreter, but it involves several variable factors, such as glucose concentration, body weight, time after injection, region of interest (ROI) and spatial resolution of the PET camera 79. The consequence of this variability is that it is difficult to establish a definite SUV that can serve as a marker for malignancy. The generally higher SUV recorded for malignancies is fraught with individual variations resulting in controversy over the use of SUV as opposed to qualitative visual analysis 79,80. The need for good anatomic imaging is not to be ignored. It is also important to remember that FDG is not tumor specific. Mast cells and other inflammatory cells also accumulate FDG. Indeed, some of the FDG accumulation in malignant lesions derives from inflammatory cells within the tumor 81.

The risk for a false positive result calls for a degree of caution during the interpretation of the result from a FDG PET scanning. The ideal PET tracer that provides the pathognomonic sign to distinguish malignant from benign lesions is yet to be found. Attempts to improve the situation are being made in various tumor models. In the head and neck, markers for proliferation (18F-Fluoro-L-Thymidin (FLT)) or apoptosis may prove suitable, and immuno-PET with MAbs could be a possibility 82-84.

The treatment following FDG PET made on the indication recurrent tumor was influenced by the PET results to a high degree. Although a negative PET scan does not exclude residual disease, our data suggest that it is rea-
sonable to adopt a policy of observation in combination with close surveillance when the initial PET examination is negative for tumor. Other authors support this assertion 85. Our results also resemble others’ that support the use of FDG PET in the search of an occult primary tumor 86-88.

Among the patients in the study who were scanned for follow up without clinical suspicion of cancer, no new tumors or recurrences were detected. However, the number of patients in this category is low and no general conclusions can be drawn from these results. Since such patients have a high risk for second primary and recurrent tumors and early detection of these conditions can be the only chance for cure, FDG PET performed for this indication may be warranted in select cases.

Since PET was a new mode of diagnosis when the first patients in our study were referred to the examination, no standard protocol was used. This reflects the clinical reality. A hypothesis prior to the study was that PET had had an impact on clinical decisions and preliminary planned head and neck surgery in numerous cases. This proved to be true, especially when the examination was done for suspicion of recurrent or residual tumor. For selected patients, the results from the PET examination undoubtedly made a difference and in many cases morbidity was reduced (Figure 7). For some patients, the altered planning might even have been the key to cure. However, if FDG PET is to be used in the diagnostic work up for the majority of the patients with tumors in the head and neck, the procedure has to be reasonably cost-effective. The use of FDG PET has proven to be cost effective in terms of savings from preventing contraindicated surgical procedures in contrast to the cost of the PET procedure by a ratio of 2.1:1 in patients with recurrent head and neck cancer 89. Furthermore, it has been proven to be cost effective for the, according to CT, neck without metastases (N0) when the outcome measures were cost per year of life saved and cost per quality-adjusted life-year 90. In our population several cases of costly and unnecessary surgery were avoided, which saved money and spared patients from treatment related inconveniences.
Figure 7. A case study of a 41-year-old woman with a left-sided neck mass. The first biopsy confirmed malignancy but definite histopathological diagnosis could not be made. The primary tumor could not be verified by clinical examination. CT (A left) could not verify a primary tumor, but PET (A middle) revealed a nasopharyngeal tumor which was found to be located in the naso-pharynx when images from CT and PET were combined (A right). CT (B left) could not verify malignancy in the right neck where PET (B middle) showed an increased FDG uptake. The finding of bilateral neck metastases upstaged the metastases from N1 to N2. MRI (C left) made for follow up 4 months post treatment aroused suspicion of residual tumor in the right neck (white arrow). Even though clinical palpation was not conclusive, the patient was scheduled for a neck dissection. Preoperative PET (C middle) proved negative for tumor and the planned surgery was cancelled. MRI 8 months later showed regression of earlier findings.

The use of PET for head and neck tumors can be considered evidence based medicine for N-staging, diagnosis of recurrence and cancer of unknown origin (occult primary tumor) [25]. The evidence for the use of FDG PET in the differential diagnosis between benign and malignant masses however is not conclusive. The majority of the studies on staging of HNSCC are done to
evaluate the usefulness in lymph node staging. The results in the study favor the use of FDG PET in the initial diagnosis and staging of the primary tumor, but the lack of conclusiveness in other reports might correspond to our difficulties to establish a “clear cut” SUV to distinguish benign from malignant. Furthermore, limitations regarding resolution and anatomic detail also add to the equation. Most likely the improved anatomic detail with the use of combined PET-CT (or PET-MRI) will promote the use of FDG PET in initial tumor staging in the future. This procedure is already becoming part of the standard diagnostic work-up at many centers.

The PET study deals with tumors from many sites in the head and neck. As mentioned in the introduction, tumors often differ from each other in a number of ways and in the growing field of tumor targeting one might have to be specific in the selection of tumors. Receptors from the EGFR family could be suitable for RID and RIT since transmembrane receptors usually are located in the cellular membrane, and there are indications that some members of the EGFR family are overexpressed in HNSCC. However, many studies of EGFR family receptor expression in the head and neck include tumors from various sites within the region without differentiation, and only a few evaluate both primary tumors and their corresponding metastases. Since advanced tumors in the oral cavity and base of tongue are difficult to treat using standard treatment modalities, this is an area where better knowledge of the patterns of receptor expression is vitally important. This is especially true if the aim is to perform RIT or RID. Furthermore, oral tumors are easily available for biopsies, and are often large enough to provide material for receptor quantification.

There are commercially available MAbs directed to the EGFR (e.g. cetuximab) and HER2 (e.g. trastuzumab). The findings in the IHC studies with strong and consistent staining for the EGFR are promising. The problems with a high expression of EGFR in the liver hepatocytes need to be overcome. In the case of RID, EGFR could be a possible target for two reasons: the radioactive dose delivered is less than with RIT (therefore causing less damage to the liver); and liver uptake does not obscure the results since HNSCC rarely metastasize to the liver. Regarding RIT, locoregional administration of the targeting conjugate could possibly improve the results. Another interesting targeting approach could be to target EGFRvIII. EGFRvIII is a mutated and truncated form of EGFR, which is reported only to exist in malignancies. It is most extensively studied in gliomas, but expression in HNSCC is reported in 42% of the cases in one study, where IHC staining for EGFRvIII was present exclusively in tumors that also stained positive for wild type EGFR.

In addition to the body of evidence for overexpression of the EGFR, there is evidence that HER2 is overexpressed in a subset of oral SCC. Hence, it would be valuable to identify these patients. However, we had no indication of HER2 overexpression in our study.
The cellular localization and the role of the HER3 and HER4 receptors in malignancies are less clear. Most likely small subsets of the receptors are present in the cellular membrane, and possibly coexpression patterns of the different members of the EGFR receptor family are important. Irrespective of this, the findings with a variable expression and a cytoplasmic staining pattern in the IHC studies in combination with the results from our cellular experiments indicate that these receptors are not suitable for RIT or RID.

In the receptor quantification paper the reason for IHC studies was to confirm the presence of the antigen and provide a basis for comparison. CD44v6 expression was identified in all tumor samples except one. Intensity and extent of staining were strong and similar to that of the EGFR, which is in agreement with previous reports. Since expression of CD44v6 in normal tissues is restricted to a subset of epithelium, our IHC results are in agreement with other authors’ in that the antigen is a suitable candidate for RIT.

Planning of RIT is a complex task, and it would be useful to know not only if the target is “strongly” expressed in a tissue, but also to learn a quantitative value of how many antigen molecules are expressed in that tissue. The design of the therapeutic substance, its physical half life, type of decay, and cellular localization of the decay are obviously important, but the amount of targeting structures also adds to the equation. IHC is a semiquantitative method with many possibilities for fast and accurate diagnosis, but it also has limitations due to subjective scoring and lack of quantification of the results. For diagnostic purposes of HER2 in cases with breast cancer a semiquantitative immunohistochemical immuno assay (HercepTest™) is available. However, this modality does not provide information on the number of antigen molecules, but merely aids in the classification of abnormal cells or tissues and provides a basis for trastuzumab treatment selection.

In the antigen quantification studies, the actual antigen density difference between EGFR and CD44v6 was almost tenfold, with a higher estimated number of antigen binding sites for CD44v6. The almost identical IHC scores for the two antigens serve as an example of a situation where the quantitative assay added information. However, the differences in the results seen between IHC and the quantification assay are not surprising for several reasons. First, staining intensity is governed by a number of factors other than antigen concentration. Furthermore, different antibodies directed towards the same site might give different intensities in staining. Considering the results from the quantitative studies, CD44v6 seems to be a more suitable target than EGFR for RIT of HNSCC.

The aim of the quantification studies was to develop and evaluate the use of the SD and MP assays. The limited number of cases in the study did not allow for correlations of antigen expression to tumor stage, histological grade of differentiation in the tumors, or exposure to previous radiation ther-
apy. In future studies of antigen expression in greater materials, analysis of these factors will be interesting.

In the study the SD assay for antigen quantification was validated and shown to give as reliable results as the MP assay. This finding is promising since less tissue (about 50 mg) is needed, and it makes antigen quantification from a biopsy a future possibility. In this way, the SD assay could be used to personalize cancer treatment. Moreover, the results from the SD assay correlated well with the results from IHC. A combination of unique IHC information on antigen heterogeneity and distribution within the sample with a quantitative value from a SD assay may provide an improved platform for planning of RIT since it would help selecting the right patients.

As mentioned, larger tumors with heterogeneity in the distribution of targeting antigens can be approached by targeting with MAbs labeled with a beta emitting substance. In such cases, the “cross-fire effect” of the relatively long (several cellular diameters) radioactivity range is useful. Since HNSCCs usually are radiosensitive, they are likely to represent a tumor type well suited for RIT. Some studies of HNSCC have been performed with beta emitting substances, such as $^{186}$Re. However, the role of RIT in HNSCC would most likely be that of an adjuvant treatment or therapy for minimal residual disease when other treatment modalities have failed. This means that very small clusters of cancer cells or even single cells would be targeted. If this was performed with a beta emitting substance, a considerable amount of radiation would affect healthy surrounding tissues. An alpha emitting substance on the other hand affects mainly the targeted cells because of its shorter particle path length and high linear energy transfer (LET) that carries a high probability of double strand breaks in the DNA. Such particles have also been well tolerated and have offered significant therapeutic benefits for other tumor types. Limitations with alpha emitters mainly consist of their high price and short physical half-life. Furthermore, the possibility of subclones of tumor cells without expression of the target giving rise to metastases has to be considered since they would not be affected by the RIT.

The findings of a strong and consistent expression of EGFR in both tumors and metastases are promising in this regard, but the high expression in normal hepatocytes is a limitation for an application with therapeutic doses of radioactivity. The target in the study in paper IV, CD44v6, is expressed in most HNSCCs, and in the majority of cases more than 70% of the tumor cells express the target. This makes CD44v6 in HNSCC well suited as a target for therapy with an alpha emitting radionuclide. $^{211}$At has a half-life of 7.2 hours, and might be the most suitable radionuclide for RIT of HNSCC.

In the therapeutic experiments no significant blocking effect of the unlabeled antibodies was present. This result was unexpected and several explanations are possible. The amount of blocking antibodies may have been insufficient to prevent the uptake of labeled antibodies in the tumors; medium-high affinity antibodies like U36 might bind firmly to the periphery of the
tumor; penetration of a relatively large conjugated antibody complex given by ip injection is slow; or the irregular blood flow to the tumor due to a sometimes chaotic vascular system in tumors may have prevented penetration of the blocking antibodies. As a result, some labeled antibodies reached the tumor cells which was enough to suppress the tumor growth. The possibility of the labeled U36 not being specific and accumulating in tumors simply because of an elevated blood flow seems unlikely since in vitro experiments have shown high specific immunoreactivity. Additionally, a statistically significant decrease of binding after saturation of antigen in the biodistribution experiments in this study confirms targeting specificity. Furthermore in recent experiments, tumor uptake of $^{111}$In labeled U36 and huA33 were studied. HuA33 is directed to an antigen not expressed in the transplanted tumors, and had very low uptake in tumors (unpublished results).

As mentioned, RIT with an alpha emitting substance is likely to be used in the treatment of small cell clusters or single tumor cells. Considering the comparably large tumor size in the therapeutic studies, the therapy was surprisingly effective. This might be partly due to the fact that nude mice, unlike humans, do not express CD44v6 in normal tissues. However, there is a high ratio of expression between normal human tissue and tumor (described in the receptor quantification paper). In clinical phase I studies with $^{186}$Re labeled U36 a dose limiting myelotoxicity was the only toxicity observed. In a phase I dose-escalating study the humanized form of U36, BIWA, was conjugated to the very toxic compound mertansine, and one fatal drug related adverse event occurred. It was concluded that targeting of CD44v6 might not be selective enough for approaches with extremely toxic drugs like mertansine. An approach with RIT could prove more suitable, and a cocktail of compounds labeled with beta emitters to get the crossfire effect and alpha emitters to kill single cells could be an interesting approach.

As stated in the beginning of this discussion, diagnosis and treatment of HNSCC continues to be a challenge, but it is indeed a challenge with many possibilities for a solution. There might, however, not be one single solution to the problem, but rather several aspects of the individual tumor have to be exploited in order to obtain an optimal result. During recent decades diagnostic techniques have improved, limited surgery has often proved to be as effective as radical procedures, reconstructive surgery with micro-surgery and free flaps has become standard procedure in advanced cases, radiation therapy has been optimized with improved dose fraction schedules and combination therapies and chemotherapy has proved to be effective in some cases. In patients with advanced tumors, it can be difficult to define the tumors and their borders. We also lack an effective adjuvant treatment and a treatment for minimal residual disease when surgery cannot be more extensive and full dose radiation therapy has already been given. With an in-
creased knowledge of the differences between groups or subgroups of tumors we can achieve a more personalized diagnosis and treatment.

FDG PET is described in paper I as a functional diagnostic tool for most tumors in the head and neck with great clinical impact in many cases. However, the cost-benefit of follow up and identifying the specific subgroup of patients to refer to FDG PET for this indication remains to be determined. The PET technique also has potential to be used for immuno-PET with tracers directed to antigens that can be targeted for therapy. Consequently, a diagnosis based on antigen expression can be achieved. It is possible that labeled tumor specific MAbs could be used in the “sentinel node” technique as well. A reliable quantification of the antigens as described in paper III would be complementary and would add to the degree of personalization. Finally, if the promising therapeutic results from our in vivo experiments with an alpha emitting substance can be refined and incorporated into clinical practice, it would be of great importance to patients with locally advanced, metastatic or residual disease. In conclusion, there is hope for patients with advanced cancers in the head and neck.
Conclusions

The present thesis deals with issues that remain to be solved for patients with cancer in the head and neck. PET is a relatively new diagnostic modality that is now part of the diagnostic work up at many centers. The clinical impact of the most common tracer, FDG was evaluated in this work. Possible targeting structures in HNSCC were assessed and a method for target quantification proposed. Finally, RIT using U36 labeled with the alpha emitting radionuclide $^{211}$At was evaluated in vivo.

Conclusions that can be drawn:

- FDG PET plays an important role in tumor staging, in identification of tumor recurrence and for detection of occult primary tumors in the head and neck.
- In the retrospective study from the Department of Otolaryngology Head-Neck Surgery, Uppsala University Hospital, the results from FDG PET often had a direct clinical impact on planned surgery and further treatment. This was especially pronounced when the indication for the study was suspicion of recurrent tumor.
- In IHC studies of metastatic HNSCC of the oral cavity and base of tongue EGFR stands out as a possible antigen for targeted approaches with a high, membrane associated expression which is similar in primary tumors and metastases.
- HER2 generally had a low expression in the SCC of the oral cavity and base of tongue.
- The expressions of HER3 and HER4 varied, and cellular experiments indicate that only a small amount of HER3 is present in the cellular membrane. Thus, HER3 and HER4 do not seem to be suitable antigens for targeted approaches of tumors in the oral cavity and base of tongue.
- The membrane associated antigen CD44v6 is a suitable target for radioimmunotargeting in HNSCC.
- The SD assay is a reliable method to quantify the antigens CD44v6 and EGFR, and is a valuable complement to IHC for analysis and future planning of RIT.
• $^{211}$At labeled U36 bind specific to HNSCC xenografts expressing CD44v6.
• Non-labeled U36 does not affect tumor growth \textit{in vivo}.
• Alpha emitting $^{211}$At labeled U36 decrease or stabilize growth of HNSCC xenografts in most cases.
PET studies
FDG PET is becoming part of the standardized diagnostic work up for selected patients with tumors in the head and neck. However, for some indications, such as follow up of patients with a high risk for recurrent tumor or a second primary tumor but without clinical suspicion of malignancy, a larger prospective study with a set study protocol should be performed to further clarify the patient selection. The recent introduction of combined PET/CT makes such a study even more important.

Possibilities for the use of tracers other than FDG are being explored. A pilot study is planned to evaluate the use of labeled human EGF as a tracer in PET studies of patients with tumors in the head and neck. The study results will be compared to results from FDG PET, and tumor samples collected during subsequent surgery will be evaluated with the SD radioimmunoassay to acquire quantitative data. Furthermore, alternative tracers as markers for apoptosis might prove suitable for head and neck tumors.

Receptor quantification studies
In paper III, we validated and applied the SD assay for quantification of membrane bound antigens. We intend to apply the method to a larger number of tumors to provide statistically reliable information on antigen expression in relation to previous radiation therapy, histological grade of differentiation and tumor stage.

Radiometals
An alternative to labeling with radiohalogens could be radiometals. A radiometal commonly used for imaging is $^{111}$In, and the beta emitter $^{177}$Lu could be suitable for RIT. In ongoing and future studies we plan to explore the biodistribution, dosimetry, imaging possibilities and ultimately clinical diagnosis and anti-tumor effects of antibody conjugates using radiometals.
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