Curative Electrochemotherapy in the Head and Neck Area
To Noah, Gabriella, Elias and Mikaela
Curative Electrochemotherapy in the Head and Neck Area
© Fredrik Landström, 2015

Title: Curative Electrochemotherapy in the Head and Neck Area.

Publisher: Örebro University 2015
www.oru.se/publikationer-avhandlingar

Print: Örebro University, Repro 02/2015

ISSN 1652-4063
ISBN 978-91-7529-061-4
Abstract


Electrochemotherapy (ECT) is a local cancer treatment modality in which the local intracellular accumulation of chemotherapeutic agents is enhanced by a local electric field. The effect of ECT is caused by a direct cytotoxic effect on the cancer cells themselves as well as effects of the tumor vasculature. The most common agent used is bleomycin. Today, clinical ECT is mostly used in palliative treatment of skin metastases.

In this thesis the long-term follow up after ECT with intratumoral bleomycin in 26 patients with T1 and T2 head and neck cancer and non-melanoma skin cancer was investigated. The primary outcome was local control and safety of treatment. Secondary outcome was survival and functional assessment. A possible selective effect in vitro of ECT on survival in different human cell-types, normal and malignant, was also investigated.

The local control rate in the 19 head and neck cancer patients treated with curative intent was 100% in the 60 month follow-up period. Six patients were cured by ECT as a mono-modality treatment and six by ECT and adjuvant radiotherapy. Seven patients died, three from intercurrent disease and four from region recurrence making the tumor-specific survival 75%. The safety and functional outcome was very good in the fifteen patients treated with oral tongue cancer but poor in the patients with tumors in the floor of mouth, bucca and tongue base.

Four of the six patients with non-melanoma skin cancer had a complete response 24 months after treatment with ECT alone. The treatment was also organ and function sparing in three patients. One patient had a persistent tumor and one patient had a recurrence 30 months after treatment.

There was also evidence for cell-type selectivity of ECT with bleomycin on cell survival in vitro. The survival was significantly higher in fibroblasts compared to endothelial and squamous cell carcinoma cells.

ECT as a curative treatment merits further investigation.

Keywords: electrochemotherapy, curative, squamous cell carcinoma, basal cell carcinoma, bleomycin, local control, functional outcome, quality of life, cell-type, selectivity.

Fredrik Landström, School of Medicine, Örebro University, SE-701 82 Örebro, Sweden, fredrik.landstrom@regionorebrolan.se
# Table of Contents

**ABBREVIATIONS** .............................................................................................................. 9

**ORIGINAL PAPERS** ............................................................................................................. 11

**INTRODUCTION** .................................................................................................................. 13
The cell membrane .................................................................................................................. 13
The membrane potential ......................................................................................................... 16
Electroporation ....................................................................................................................... 17
ECT in vitro .......................................................................................................................... 21
ECT in vivo .......................................................................................................................... 24
  Effects of ECT on muscle ................................................................................................ 26
  Effects of ECT on the skin ................................................................................................ 27
  Effects of ECT on the vascular system ............................................................................. 27
  Effects of ECT on the heart ............................................................................................. 30
  Effects of ECT on the nervous system .......................................................................... 30
  Effects of ECT on the immune system .......................................................................... 30

**THE TREATED TUMORS** ................................................................................................. 32
Non-melanoma skin cancer in the head and neck area .................................................. 32
Head and neck squamous cell carcinoma ......................................................................... 34
Tumor vascularization and cancer stem cells .................................................................. 37

**CLINICAL ECT** .................................................................................................................. 39
ECT in clinical practice ..................................................................................................... 39
  Clinical efficacy of ECT ................................................................................................. 39
  The ESOPE guidelines ................................................................................................. 39
ECT in non-melanoma skin cancer .................................................................................. 40
ECT in head and neck cancer .......................................................................................... 41
From treatment to healing ............................................................................................... 42
The EU-CCBE-2003 and EU-HNBE-2003 trials ......................................................... 44
  The MedPulser™ system .............................................................................................. 45

**THE PRESENT INVESTIGATION** ...................................................................................... 47
Aims ....................................................................................................................................... 47
  The overall aims of this thesis ....................................................................................... 47
  Specific aims for each paper ........................................................................................ 47
Materials ............................................................................................................................. 48
Study group ........................................................................................................................ 48
### Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>BCC</td>
<td>Basal Cell Carcinoma</td>
</tr>
<tr>
<td>BLM</td>
<td>Bleomycin</td>
</tr>
<tr>
<td>CAL-27</td>
<td>Squamous Cell</td>
</tr>
<tr>
<td>CER</td>
<td>Cytotoxicity Enhancement Ratio</td>
</tr>
<tr>
<td>CR</td>
<td>Complete Response</td>
</tr>
<tr>
<td>CRR</td>
<td>Complete Response Rate</td>
</tr>
<tr>
<td>CRT</td>
<td>Controlled Randomized Trial</td>
</tr>
<tr>
<td>CSC</td>
<td>Cancer Stem Cells</td>
</tr>
<tr>
<td>CSCC</td>
<td>Cutaneous Squamous Cell Carcinoma</td>
</tr>
<tr>
<td>CT</td>
<td>Computed Tomography</td>
</tr>
<tr>
<td>CUP</td>
<td>Cancer of Unknown Primary</td>
</tr>
<tr>
<td>CV</td>
<td>Crystal Violet staining method</td>
</tr>
<tr>
<td>Da</td>
<td>Dalton</td>
</tr>
<tr>
<td>$\Delta V_m$</td>
<td>Induced membrane potential</td>
</tr>
<tr>
<td>DWD</td>
<td>Dead With Disease</td>
</tr>
<tr>
<td>DWOD</td>
<td>Dead Without evidence of Disease</td>
</tr>
<tr>
<td>ECOG</td>
<td>Eastern Cooperative Oncology Group</td>
</tr>
<tr>
<td>ECT</td>
<td>Electrochemotherapy</td>
</tr>
<tr>
<td>EGFR</td>
<td>Epidermal Growth Factor Receptor</td>
</tr>
<tr>
<td>EORTC</td>
<td>European Organisation for Research and Treatment of Cancer</td>
</tr>
<tr>
<td>E</td>
<td>Electric Field Strength</td>
</tr>
<tr>
<td>EP</td>
<td>Electroporation</td>
</tr>
<tr>
<td>EPT</td>
<td>Electroporation Therapy</td>
</tr>
<tr>
<td>ESOPE</td>
<td>European Standard Operation Procedures for Electrochemotherapy</td>
</tr>
<tr>
<td>FB</td>
<td>Fibroblast</td>
</tr>
<tr>
<td>FOM</td>
<td>Floor of Mouth</td>
</tr>
<tr>
<td>G</td>
<td>Electric Conductance</td>
</tr>
<tr>
<td>Gy</td>
<td>Gray</td>
</tr>
<tr>
<td>HNC</td>
<td>Head and Neck Cancer</td>
</tr>
<tr>
<td>HNSCC</td>
<td>Head and Neck Squamous Cell Carcinoma</td>
</tr>
<tr>
<td>HPV</td>
<td>Human Papilloma Virus</td>
</tr>
<tr>
<td>HUVEC</td>
<td>Human Umbilical Vein Endothelial Cells</td>
</tr>
<tr>
<td>I</td>
<td>Electric Current</td>
</tr>
<tr>
<td>IGRT</td>
<td>Image Guided RadioTherapy</td>
</tr>
<tr>
<td>IMRT</td>
<td>Intensity Modulated RadioTherapy</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Definition</td>
</tr>
<tr>
<td>--------------</td>
<td>------------</td>
</tr>
<tr>
<td>IRE</td>
<td>Irreversible Electroporation</td>
</tr>
<tr>
<td>IT</td>
<td>Intratumoral</td>
</tr>
<tr>
<td>IU</td>
<td>International Units</td>
</tr>
<tr>
<td>IV</td>
<td>Intravenous</td>
</tr>
<tr>
<td>NED</td>
<td>No Evidence of Disease</td>
</tr>
<tr>
<td>NMSC</td>
<td>Non Melanoma Skin Cancer</td>
</tr>
<tr>
<td>ORN</td>
<td>Osteoradionecrosis</td>
</tr>
<tr>
<td>PBL</td>
<td>Phospholipid Bilayer</td>
</tr>
<tr>
<td>PDT</td>
<td>Photodynamic Therapy</td>
</tr>
<tr>
<td>PSSHN</td>
<td>Performance Status Scale for Head and Neck cancer</td>
</tr>
<tr>
<td>QLQ-H&amp;N35</td>
<td>Quality of Life Questionnaire-Head and Neck 35</td>
</tr>
<tr>
<td>RR</td>
<td>Regional Recurrence</td>
</tr>
<tr>
<td>RT</td>
<td>Radiotherapy</td>
</tr>
<tr>
<td>SC</td>
<td>Stratum Corneum</td>
</tr>
<tr>
<td>SCC</td>
<td>Squamous Cell Carcinoma</td>
</tr>
<tr>
<td>SCC-4</td>
<td>Squamous Cell Carcinoma Cell Line</td>
</tr>
<tr>
<td>SEM</td>
<td>Standard Error of Mean</td>
</tr>
<tr>
<td>TNM</td>
<td>Tumor, Node, Metastasis classification of malignant tumors</td>
</tr>
<tr>
<td>U</td>
<td>Units</td>
</tr>
<tr>
<td>$V_{rev}$</td>
<td>Threshold for Reversible Electroporation</td>
</tr>
<tr>
<td>$V_{irrev}$</td>
<td>Threshold for Irreversible Electroporation</td>
</tr>
<tr>
<td>$V_m$</td>
<td>Resting Membrane Potential</td>
</tr>
<tr>
<td>VMAT</td>
<td>VoluMetric Arc Therapy</td>
</tr>
</tbody>
</table>
Original papers

This thesis is based on the following papers, which are referred to in this text by their roman numerals.

I. Electroporation therapy of skin cancer in the head and neck area.
Landström FJ, Nilsson CO, Crafoord S, Reizenstein JA, Adamsson GB, Löfgren LA.

II. Electroporation therapy for T1 and T2 oral tongue cancer.
Landström FJ, Nilsson CO, Reizenstein JA, Nordqvist K, Adamsson GB, Löfgren AL.


Submitted to Acta Otolaryngologica.

V. Electrochemotherapy – evidence for cell-type selectivity in vitro.
Landström FJ, Ivarsson M, Koskela A, Magnuson A, von Beckerath M, Möller C.
Submitted to Bioelectrochemistry.
Introduction

Electrochemotherapy (ECT) (1), also known as electroporation therapy (EPT), is a local cancer treatment modality where the intracellular accumulation of a chemotherapeutic agent is enhanced in the presence of an electrical field by the phenomenon known as electroporation (EP) (2). My interest in ECT began in 2005 as a sub investigator in two ECT trials. In this thesis the treatment outcome after ECT in twenty patients with head and neck cancer and in six patients with non-melanoma skin cancer will be reported as well as evidence of cell-type selectivity of ECT in vitro.

The cell membrane

A basic knowledge of the structure and function of the plasma cell membrane is essential to understand the theory behind ECT. The cell membrane surrounding all cells is only 5 nm thick but maintains the cell integrity and hence its survival (3).

The biochemical and biophysical properties of the membrane depends on its molecular composition of which the three basic components are phospholipids, cholesterol and proteins (Figure 1).

![Cell membrane diagram](image)

**Figure 1.** The cell membrane with its main components, phospholipid molecules, proteins and cholesterol (4).
Examples of common phospholipids in human cell membranes are shown in Figure 2; they all share the same basic structure; an aquaphilic “head” and a lipophilic “tail” made up of fatty acids (5).

These amphiphilic chemical properties make the phospholipid molecules spontaneously assemble into a bilayer when placed in water-based solutions, reseal the bilayer when it is torn and also facilitates the transport of some molecules while restricting others. This permeability is determined both by the size and the electrical properties of the molecules.

Nonpolar molecules like oxygen (32 Da) and carbon dioxide (42 Da) and small polar molecules like water (18 Da) can diffuse freely across the phospholipid bilayer (PBL) while the permeability of larger especially polar molecules is restricted (2). The PBL is highly impermeable to even small ions (for instance Na⁺ and K⁺)(3).

The cell membrane is not a static structure; instead the PBL is in a fluid state where the individual phospholipids can diffuse and rotate freely. This fluidity is biologically important and determined especially by the chemical compositions (saturation, chemical bonds) of the fatty acids in the phospholipid molecules (3, 5). Cholesterol helps stabilize the membranes by restricting the movement of the fatty acids thus decreasing the deformability of the membrane (5).

**Figure 2.** Chemical structure of the common phospholipids phosphatidylserine, phosphatidylethanolamine and phosphatidylcholine with lipophilic fatty acids (tails) and hydrophilic groups (heads)(6).
Membrane proteins can somewhat oversimplified be divided into three categories; transport proteins, receptor proteins and glycoproteins (Fig. 1). The receptor proteins are important in cell signalling and the glycoproteins in recognition and adhesion but are, as far as presently known, of limited interest in the understanding of ECT. The transport proteins, on the other hand, are very important in the electrochemical processes and the transport of cytotoxic agents fundamental to understanding ECT.

The transport proteins can be classified as being either channels or carriers. Channels are multipass transmembrane proteins that allow hydrophilic and charged ions and molecules to diffuse through the PBL driven by their individual concentration gradients (Figure 3)(3). For example, even though water can diffuse through the PBL most of its diffusion takes place in special transport protein channels called aquaporins (3, 7).

Carriers, on the other hand, are membrane proteins that actively bind to the molecule and transport it across the PBL, carriers can mediate this transport without energy or it can actively transport molecules against the chemical gradient, these transport proteins are often called pumps and are energy dependent (Figure 3). The Na⁺/K⁺-pump, for instance, transports three Na⁺ ions out of the cell for every two K⁺ ions transported in thus maintaining the osmotic balance and cell volume (3).

Figure 3. The different mechanisms for ion and polar molecule transport across the cell membrane, diffusion, facilitated diffusion via carrier proteins and energy dependent transport (for example the Na/K pump) Modified from Alberts (3).

The cell membrane is asymmetric; the outside and the inside do not have the same composition mirroring the different environments on the inside
and the outside of the cell. For example, the negatively charged phospholipid phosphatidylserine is restricted to the inner, cytoplasmic side of the membrane in normal human cells but is seen in the outer membrane of apoptotic cells serving as a signal to macrophages for phagocytosis (8). The inside of the cell membrane is also in direct contact with the cytoskeleton important in, for instance cell movement and cell division (3).

**The membrane potential**

Due to the semipermeable properties of the PBL and the actions of the membrane transport proteins (channels and carriers) described earlier the intracellular and the extracellular side of the membrane have different chemical compositions. This also leads to a difference in electrical charge over the membrane; the so-called membrane potential $V_m$.

The name is somewhat misleading since it is not a potential but the potential difference (or voltage) between the inside and the outside of the cell membrane, however since the name is used so extensively in the literature we use it in this text as well. The $V_m$ is generated by both the active and passive transport systems of the cell membrane.

$K^+$ is the most important ion in this process although both $Na^+$ and $Cl^-$ ions participate. $K^+$ is pumped into the cells in exchange for $Na^+$ leading to a $K^+$ concentration gradient; the $K^+$ ions can diffuse through specific protein channels (leak channels).

However, since there is an intracellular accumulation of large negatively charged molecules unable to move across the PLM (the fixed anions) there is an electrostatic force that balances the concentration gradient of $K^+$, the membrane potential is the potential difference across the membrane when this electrochemical gradient is zero.

In normal cells the $V_m$ is between -40 and -90 mV (3). The $V_m$ and its very important role in the physiology of the nervous, neuromuscular and cardiovascular systems are well known. There is also a growing knowledge about and interest in the biological significance of the $V_m$ in other areas, for example in cell division and differentiation (9, 10). Proliferating cells, both tumor and non-tumor, seems to have a lower $V_m$ than non-proliferating cells (Figure 4) (9).
**Electroporation**

The cell membrane has two basic electrical properties; it is both an insulator by restricting the flow of charged molecules and a capacitor since it due to its thinness allows electrostatic forces to act between opposite charges on both sides on the membrane (11). In general, the induced membrane potential $\Delta V_m$ when an external electrical field $E$ is applied can be described by the equation: $\Delta V_m = f E r \cos \theta$, where $f$ is a factor related to the form of the cell, $r$ is the cell radius and $\theta$ the angle relative the external electrical field $E$ (Figure 5) (12). It causes hyperpolarization in the pole facing the positive electrode and depolarization in the pole facing the negative electrode.
Figure 5. The induced membrane potential $\Delta V_m$ by an external electrical field $E$ in a cell with radius $r$ and angle $\theta$ causes hyperpolarization in the anode pole and depolarization in the cathode pole of the cell. $\Delta V_m$ varies between a maximal value for $\theta = 0$ and 0 for $\theta = 90^\circ$. The degree of permeabilization is bigger in the depolarized pole allowing for transport of larger molecules like DNA (13, 14).

The induced potential $\Delta V_m$ is superimposed on the resting membrane potential $V_m$. When a certain threshold $V_{\text{rev}}$ for $\Delta V_m + V_m$ is reached the permeability and the conductance (permeability of charged molecules) of the cell membrane suddenly increases (14). This phenomenon is called dielectric breakdown or more commonly electroporation and has been studied for 40 years (15). The threshold $V_{\text{rev}}$ is in the range of 200mV-1V (16-18). $V_{\text{rev}}$ is first reached in the membrane facing the anode since here both $\Delta V_m$ and $V_m$ have the same vectorial direction. At the cathode side these directions are opposite requiring a higher $\Delta V_m$ to reach $V_{\text{rev}}$ (18).

The equation above also shows that a stronger electrical field $E$ is needed to electroporate cells with a smaller radius. Most mammalian cells have a radius between 5 and 15 µm, this could be compared to an axon with a typical diameter of 0.5 µm requiring an electric field at least ten times stronger to achieve EP.

The exact mechanism of EP is not known but is thought to involve rearrangements of the membrane phospholipid molecules leading, within na-
noseconds, to formation of perforating hydrophilic “pores” filled with water molecules (2, 11) (Figure 6).

![Figure 6. Electroporation hypothesis. Changes in the PLM induced by an electrical field leading to pore formation (19).](image)

This leads to a drastic increase in the transmembrane transport of ions and large polar molecules. The area of permeabilization has been shown to be larger in the cell pole facing the anode but that the degree of permeabilization is greater in the pole facing the cathode leading to increased transport of larger molecules, for instance DNA, in this area (Figure 5) (14, 18).

When the external field is removed and if the sum $\Delta V_m + V_m$ is lower than the threshold $V_{irrev}$ the cell membrane, within seconds to minutes depending on the temperature, can recover by membrane resealing with intact cell integrity resulting in so-called reversible EP. The cell survival in reversible electroporation depends on the energy level of the cell, if the energy level is too low the cell will not be able to regulate its ion homeostasis (especially Ca$^{2+}$) leading to cell death by either apoptosis or necrosis (20). If $V_{irrev}$ is reached no resealing is achieved leading to cell death, so-called irreversible EP (21). Different electrical parameters can be used to achieve different biological effects leading to different applications of EP such as gene transfer, Irreversible Electroporation (IRE) and ECT (22) (Figure 7). For instance, pulsed electrical fields with specific lengths and number of pulses instead of static fields can be used to combine efficient “pore” formation (permeabilization) with a high degree of cell survival (23). This is important in both gene transfer and ECT where cell death due to electrocution is unwanted (Figures 7 and 8).
On the other hand, in IRE high field strengths is combined with repetitive pulses to achieve cell death (Figure 7). IRE is a new cancer treatment modality currently investigated for treatment of liver and pancreas cancer, it will not be further discussed in this thesis (24).

![Diagram showing different EP applications in relation to electrical field strength and duration](image)

**Figure 7.** Summary of different EP applications in relation to electrical field strength and duration (22).

![Diagram showing reversible EP with electrical parameters used for ECT (100 μs, 1000 V/cm) and gene delivery (longer pulses, lower field strength)](image)

**Figure 8.** The area of reversible EP with electrical parameters used for ECT (100 μs, 1000 V/cm) and gene delivery (longer pulses, lower field strength). Below the breakdown voltage there is no increased permeability. With higher fields or longer pulses EP becomes irreversible leading to cell death (25).
Also, recently the investigation of very short (nanosecond) pulsed electrical fields (nsPEF) has shown that with sufficient field strengths apoptosis with externalization of phosphatidylserine can be achieved (Figure 7) (8). Cell death due to heating is probably not an important factor in EP with normal parameters, in mathematical models field strengths of 4kV/cm with pulse duration of 100 µs is required to increase the temperature from 37 to 42 °C in vivo (22). This field strength is almost 4 times higher than the usual strength used in ECT (1-1.2 kV/cm)

**ECT in vitro**

One of the applications of EP is to facilitate the transport of low-permeant or non-permeant molecules across the cell membrane of which ECT, the internalization of chemotherapeutic agents, is a special case. In ECT the goal is to permeabilize all cancer cells in order to internalize the chemotherapeutic agents used without killing the cells in the EP process because when \( V_{irrev} \) is reached all exposed cells (cancer and normal) die without selectivity.

Depending on the chemotherapeutic agent used reversible EP can allow for a selective effect where relatively more cancer cells than normal cells die. When considering chemotherapeutic agents suitable for ECT from what we know about the permeability of the cell membrane we could make the assumption that the potentiation of EP for large polar molecules with intracellular targets would be greater than for smaller, non-polar molecules. This is also the case; bleomycin (BLM) and cisplatin are the two chemotherapeutic agents that are enhanced by EP the most (Figure 10) (23, 26). BLM was isolated from the bacteria Streptomyces verticillus in 1966 and is a water-soluble glycopeptide with a molecular weight of approximately 1500 Da (27) (Figure 9). It is currently used in, for instance, the treatment of squamous cell carcinoma (SCC), testicular cancer and lymphomas. It does not pass the blood-brain barrier and is eliminated by the kidneys. Its half-life is approximately 3 hours with intramuscular or intravenous injection. Although the exact mechanism of BLM cytotoxicity is not known DNA is the primary target. BLM causes cleavage in both DNA strands leading to both single and double strand breaks. This process requires oxygen and metals, usually \( \text{Fe}^{2+} \) (27). There are probably other mechanisms of BLM injury, for instance induction of reactive oxygen species (ROS) and lipid peroxidation (28). Cells in the G2 and M phases are more sensitive to BLM cytotoxicity.
Figure 9. Bleomycin and cisplatin molecules. Note the difference in size between the non-permeant bleomycin and the low-permeant cisplatin molecules.

BLM causes two distinct types of cell death depending on the number of intracellular BLM molecules. With a few hundred to a few thousand internalized molecules the cells die of a slow process called mitotic cell death (mitotic catastrophe) (Figure 10). With several thousand or more intracellular BLM molecules the cells die of a much faster (pseudo)apoptotic process (27, 29). If the cell is not killed by BLM its cell cycle can stay in arrest in the G2 or M-phase (Figure 10).

Figure 10. The two types of BLM mediated cell death, the slow mitotic catastrophe and the fast (pseudo)apoptotic death. Cells that survive can stay in cell cycle arrest (G2 or M-phase) (30)
Due to the size and polarity of BLM molecules its diffusion across the plasma membrane is so effectively restricted it can be classified as a non-permeant molecule; instead it is internalized by endocytosis with the help of a transport protein (27, 31). Even with this mechanism less than 0.1% of BLM in medium is transported into the cells (32). EP has been shown to greatly enhance the effect of BLM in vitro by increasing its permeability in several studies (23, 26, 33-35).

The Cytotoxicity Enhancement Ratio for EP is defined by the following formula: (The drug dose needed to kill 50% of cells without EP) / (The dose needed to kill 50% of cells with EP). The CER for BLM in vitro varies between 300 and 5000 and seems to be cell-type dependent as shown in Table 1 (23, 26, 33, 34).

Table 1. Cytotoxicity enhancement ratios in vitro of ECT with bleomycin in three different cell lines. The difference in CER for the DC3-F could probably be explained by the different field strengths used (23, 26, 33, 34).

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Cell type</th>
<th>CER</th>
</tr>
</thead>
<tbody>
<tr>
<td>SCC-25</td>
<td>Human tongue SCC</td>
<td>400</td>
</tr>
<tr>
<td>DC3-F</td>
<td>Chinese hamster fibroblast</td>
<td>300-700</td>
</tr>
<tr>
<td>HMEC-1</td>
<td>Human endothelial cell</td>
<td>5000</td>
</tr>
</tbody>
</table>

SCC, Squamous cell carcinoma

BLM can be inactivated by the intracellular enzyme bleomycin hydrolase; the level of this enzyme is especially low in skin and lung tissue (28).

One of the assumptions of a selective effect of ECT with BLM relies on the fact that highly differentiated cells, for instance muscle and nerve cells, do not divide and the mitotic cell death seen with lower doses of BLM in dividing cells does not lead to death in these non-dividing cells. However, these cells can still be killed by the pseudoapoptotic process with higher BLM doses described above leading to a loss of selectivity (36).

On the other hand, endothelial cells show a high degree of sensitivity to BLM and this is thought to be one of the mechanisms in the often-lethal pulmonary fibrosis seen with intravenous administration of BLM (37). This effect is dose-dependent (> 400,000 IU) and seems to be age-related.

Cisplatin, the drug besides BLM most commonly used in ECT is a platinum-based chemotherapeutic drug that is polar but much smaller than
BLM and hence a low-permeant rather than non-permeant drug (Figure 9). It is, for example, used in combination with RT in treatment of head and neck cancer. Not surprisingly, the potentiation of cisplatin by EP is lower than for BLM with CER 2.3 in vitro (23). On the other hand, the cytotoxic effects of the non-polar lipophilic drugs daunorubicin, doxorubicin, etoposide and paclitaxel are not enhanced by EP with CER = 1(23). BLM was the only drug used in the ECT treatment of the patients in this thesis as well as in the cell studies.

As seen before different electrical parameters can cause different effects in the exposed cells. When using eight 100 µs square wave pulses of an electrical field with 1200 V/cm cell survival and cell permeabilization rates of more than 90 % in vitro can be achieved (23, 38). These are the parameters most commonly used in clinical ECT.

ECT in vivo

The cellular environment in tissues is very different from the environment in solutions. Both the distribution of the chemotherapeutic agents and the electrical field that are fairly uniform in vitro are much more complex in vivo. With ECT in vivo the drug can be administered either intratumorally (IT) or intravenously (IV). With IV administration the drug has to be distributed in adequate concentrations throughout the tumor and the margins before EP, which will be discussed later. The blood vessels also transport the drug from the tumor area thereby limiting the time window for treatment.

The tumor and the surrounding normal tissues have different electrical conductivity with well-vascularized tumors having a higher conductivity than less vascularized normal tissues. A high conductance (G) increases the current (I) flowing between the electrodes and reduces the electrical field strength (E) in accordance with Ohms law (E = I/G) (39).

In order to electroporate all the cells within a volume all cells must be exposed to a field high enough to reach the threshold level $V_{\text{rev}}$ for EP without reaching the threshold $V_{\text{irrev}}$ for irreversible EP. The electrode design for in vivo EP has to take these requirements into consideration to achieve effective treatment results. Plate electrodes adequate for in vitro EP are not as usable in the treatment of solid tumors. This has led to development of needle electrodes capable of penetrating tumors and normal tissue to achieve wide margins on all sides of the tumor. As discussed before, cells that are not electroporated have a very low intracellular BLM
content therefore there is a risk of leaving viable cancer cells if the tumor area is not covered by a sufficiently high electrical field (39, 40).

In Figure 11 a simulation of the electrical field distribution in two planes with a two-electrode model is shown (39). Note that the field immediately outside the needles is lower than $V_{rev}$ threshold thereby avoiding “collateral damage” of normal tissue. Furthermore, the effective electrical field at the depth of the electrodes is actually at a lower depth than the electrode tips themselves (Figure 11). Therefore it is important to insert the needles deep enough to ensure adequate EP of the deep border of the tumor.

Figure 11. Simulation of electrical field distribution ($E$) in two planes with needle electrodes with distance 3 mm and 300 V applied. The tumor area is marked by a circle. The field is highest around the needles. ($E_{rev} = \text{reversible electroporation threshold}, E_{irrev} = \text{irreversible electroporation threshold}$) (39).

If the electrodes are placed in two rows the cells are electroporated in only one direction whereas in a hexagon array the cells can be electroporated sequentially from several directions if the field is applied in opposite electrodes pairwise. The electric field distribution with the hexagon needle electrode array is shown in Figure 12 (41), this was the geometry used in the treatment of all the patients in this thesis. The electrode
polarity was sequentially changed counter-clockwise in a pairwise 2x2 array (Figure 12). This array has been shown to have a significantly better effect than the 3x3 array on tumors in vivo (40).

If the electrode distance increases, the field distribution will become more inhomogeneous leading to inefficient EP (39). With large tumors it is therefore necessary to apply the electrode array in an overlapping sequence to ensure adequate EP in both the tumor tissue and the margin.

![Figure 12. Simulation of the electrical field distribution with a hexagonal needle electrode array with diameter 8.66 mm (d) and 2x2 polarity. The black ring marks a 5 mm treatment area. The model is linear making it scalable to higher values of U. This was the electrode geometry and polarity used in this thesis (41).](image)

**Effects of ECT on the muscle**
The effect of ECT with BLM on normal tissues have been investigated for muscle in vivo in rat hind limbs showing necrosis with higher doses of BLM but with normal limb function (42). EP alone causes muscle contractions related to the field strength and the pulse repetition frequency (43). Traditionally a pulse repetition of 1 Hz (1 pulse per second) was used. Since the field strength cannot be lowered without compromising the efficiency of EP the only parameter that can be changed is the frequency. The frequency of tetanic muscle contractions is 100 Hz and an increase of the pulse frequency above this value results in just one contraction. It has been showed in vivo that an efficient EP can be achieved with frequencies up to 5 kHz (43). This is used in modern clinical EP systems.
Effects of ECT on the skin

The skin consists of three layers, the epidermis, the dermis and the subcutis. The outermost layer of epidermis, stratum corneum (SC), consists of a layer of 15-20 dead cells provides the important barrier function of the skin. The SC also has a much greater resistance to electrical currents than the other parts of the skin (44). This, in accordance with Ohm's law, generates much higher electrical field strengths in the SC than in the underlying layers. This could lead to insufficient EP of tumors penetrating several skin layers. Needle electrodes have been shown to distribute the electrical field more evenly than plate electrodes throughout the skin in an animal model (45).

Effects of ECT on the vascular system

Both EP alone and ECT with BLM have well documented effects on the blood flow in both normal and tumor tissue in vivo (46-48). EP alone causes a 2-phased reduction in blood flow. Phase I is an immediate vasoconstriction of the afferent arterioles (Figure 13). Phase II is a reduction in blood flow measurable within 1-2 hours after EP, then a slow increase in the blood flow with a normalization within 24-48 hours (Figure 13)(46). This is thought to be a combined effect of the vasoconstriction and swelling of the endothelial cells reducing the diameter of the blood vessels.

This reversible effect called the “vascular lock” mechanism leads to capturing of ECT drugs already present in the tumor but also restricts the inflow of drugs given systemically. The critical time window for EP after IV BLM administration seems to be between 8 and 28 minutes (49). The optimal time frame of EP following IT drug injection is not known. For the patients treated in this thesis the time from BLM injection to the start of EP was 10 minutes. EP was completed within an additional 10-15 minutes in all patients. In ECT with BLM there is a dose-dependent decrease in blood flow resulting in a cessation of the blood flow within 12-48 hours with high BLM doses (Figure 13) (47). This irreversible effect called the “vascular disrupting” mechanism of ECT is probably related to endothelial cell death. As seen before, BLM is highly cytotoxic to endothelial cells (37). ECT can enhance this cytotoxicity 5000-fold (33).

Also, because blood has high conductivity the vessel wall where endothelial cells are located is exposed to very high electrical field strengths (50). The antivascular effect is probably of great clinical significance in the overall effect of ECT. It is, for instance, used in treatment of haemorrhaging metastases (51). Since the cytotoxic effect of BLM is dependent on
oxygen, the antivascular mechanisms can also, theoretically, have negative effects on the treatment. A summary of the antivascular mechanisms is shown in Figure 14.

**Figure 13.** Blood flow in subcutaneous Sa-1 tumours in A/J mice measured with laser Doppler flow meter in two different time frames following exposure to BLM, EP alone and ECT with BLM(47, 48, 50).
**Figure 14.** Summary of the antivasculard effects of EP and ECT. EP alone causes a reversible decrease in the blood flow the so-called “vascular lock” effect while ECT with BLM causes an irreversible decrease and cessation of the blood flow, the “vascular disrupting” effect of ECT(50).
Effects of ECT on the heart
Although EP is thought to be an important mechanism in defibrillation of arrhythmias (52) the effect of ECT on heart function has not been thoroughly investigated. In 14 patients treated with ECT ECGs recorded during treatment were analysed. Electroporation drugs (BLM and cisplatin) were injected IT to avoid systemic effects. In six of the patients the treated tumors were located in the thorax. Transient increases in the R-R interval were seen but no manifest pathological changes (53).

In our own experience a case of repeated 10-20 second asystoles in a patient (not in this thesis) treated with ECT in the proximity of the carotid bifurcation was seen. The outcome in that case was good without clinical evidence of myocardial (normal postoperative ECG) or cerebral damage.

Effects of ECT on the nervous system
The nervous system is designed to transmit electrical signals and ECT with its strong electrical fields seems to be able to cause both structural and functional damage to the nervous system. The evidence that electroconvulsive therapy in treatment of depression can cause cognitive side effects seems to support this (54). However no such side effects have been reported with ECT.

In an IRE pig model (90 pulses, 70 µs, 1.5 kV/cm) the sciatic nerve was treated. No histological nerve damage could be detected 2 months after this treatment (55).

In this thesis a case of an epileptic seizure six months after treatment of a BCC in the skin of left temporal area is reported. EP has been proposed as one mechanism in the delayed neurological damage seen in lightning and electrical injury (56).

Effects of ECT on the immune system
In ECT the tumor tissue is not resected but instead left to deteriorate leading to an accumulation of cancer antigens that at least in theory could stimulate an immune response. There is evidence in vivo suggesting a role of the immune system in the efficacy of ECT.

A group of immunocompetent mice had a significantly better outcome than an immunodeficient group in ECT treatment of sarcoma. Furthermore, administration of interleukin-2, an important regulator of leukocyte function, increased the tumor response (57). Also, immunocompetent mice inoculated with a colon cancer cell line treated with ECT showed significantly higher response rates than immunodeficient mice. The immuno-
competent mice also rejected re-inoculation of that specific cancer cell line implying a development of an immune response (58). A case report of the long-term complete response in a patient with advanced malignant melanoma seven years after ECT with BLM and systemic IL-2 administration was published in 2006 (59).
The treated tumors

Non-melanoma skin cancer in the head and neck area

Non-melanoma skin cancer (NMSC) is a very heterogeneous cancer group, from low aggressive basal cell carcinomas (BCC) to highly aggressive tumors with high metastatic potential (i.e. Merkel cell carcinoma).

Basal cell carcinoma BCC is the most common NMSC and the most common cancer in Sweden with approximately 40 000 new cases per year (60). The head and neck region is the site most commonly affected with 70-80% of all cases (61). Intermittent strong UV exposure is the major risk factor in developing BCC and other risk factors include radiotherapy (RT) and immunosuppression (transplant patients, HIV)(62).

BCC usually develops after the fourth decade but there are also hereditary conditions like Gorlins and Bazex-Dupré-Christols syndromes in which the affected patients continuously develop new BCC tumors from an early age. The primary defect in both sporadic and syndrome-related BCC seems to be an up regulation in the membrane based hedgehog signaling pathway that is part of the control system of proliferation and apoptosis(62).

There are four main clinical subtypes of BCC. Nodular (Glas type IA) an superficial BCC (Glas IB) are low aggressive tumors while infiltrative (Glas II) and morpheaform BCC (Glas III) are aggressive tumors usually in the head and neck area with ill-defined tumor borders and infiltrative growth in cartilage, muscle, nerve and bone (62). Metatypical BCC is an infiltrative BCC showing squamous differentiation that have a greater potential to metastasize, something that’s usually very rare in BCC (62).

Surgery is the primary treatment of BCC with, for example, Mohs micrographic technique (63). Other treatment options include photodynamic therapy and radiotherapy, especially in unresectable tumors. There is also a hedgehog pathway suppressor, vismodegib (Erivedge©), available for advanced cases. The recurrence rate after surgical resection is between 1 and 10 % and is especially high in the “mask areas” of the face reflecting the difficulty of getting adequate margins, especially in close proximity to the eyes and the nose (Figure 15)(64).
Figure 15. There is a high risk of recurrence for NMSC in the “mask areas” of the face.

Cutaneous squamous cell carcinoma (CSCC) is the third most common cancer in Sweden with 5718 new cases in 2012 (65). The head and neck area is most commonly affected with approximately two thirds of all cases (62). CSCC usually appears in older age groups.

A high cumulative UV exposure is the main risk factor in the development of CSCC (62). Like in BCC immunosuppression is a risk factor that not only increases the risk of developing CSCC but also seems to lead to more aggressive tumors. Other risk factors include previous RT, chronic inflammation and arsenic exposure. Patients with the hereditary conditions xeroderma pigmentosum and oculocutaneous albinism also have elevated risks of developing CSCC (62).

CSCC often develops in areas with actinic keratosis, skin lesions with chronic UV damage, in a process called field cancerization. The carcinogenesis is thought to involve a UV induced mutation resulting in inactivation of the TP53 gene coding for the tumor suppressor protein p53 and an increased expression of the epidermal growth factor receptor (EGFR) promoting increased cell proliferation (66).

CSCC tumors with high-risk of recurrence and metastasis are; tumors with earlier recurrence, diameter > 2 cm, thickness > 2mm, location in the “mask areas” of the face (Figure 1), poorly differentiated histopathology and perineural invasion(67, 68). The overall risk of metastasis, especially to the regional lymph nodes, is approximately 4-5 %. However in the
high-risk tumors described above the local or/and regional recurrence rate can be as high as 80% in the first two years (68).

The overall prognosis of CSCC is good but decreases dramatically with metastatic disease (34.4% overall 5-year survival) (67). Because of the high incidence of CSCC the total mortality is as high as for malignant melanomas, oropharyngeal cancer and renal cancer (62). Surgery is the first line treatment for primary CSCC resulting in cure rates of 95% (68). Mohs surgery could offer an even better outcome, especially with high-risk tumors (69). RT can be performed as an adjuvant treatment in high-risk tumors or in patients with positive margins after surgery, for salvage or palliation in recurrent disease or as a primary treatment for unresectable tumors. However, in locally advanced CSCC the cure rate with RT alone is only 58% (70).

There are promising results in treating lip SCC with brachytherapy (71). Photodynamic therapy (PDT) is another non-surgical treatment option for superficial lesions. Local treatment of actinic keratosis with 5-fluorouracil and imiquimod (Aldara®) as well as PDT has been shown to prevent field cancerization (68, 72). In summary, although the overall prognosis for NMSC in the head and neck area is good the risk of recurrence and treatment morbidity is strongly related to the tumor location (mask areas). Since the incidence of NMSC is so high the total mortality and morbidity rivals that of more aggressive cancer groups.

**Head and neck squamous cell carcinoma**

Head and neck cancer is a diverse group of tumours often having little more in common than the region in which they develop. For instance, salivary gland malignancies, malignant melanomas in the nasal cavity and sarcomas in the head and neck all have different prognosis and treatment strategies.

However, SCC is by far the most common histopathology in head and neck cancer. Head and neck squamous cell carcinoma (HNSCC) is currently the sixth most common cancer type worldwide with approximately 650,000 new cases and 350,000 deaths annually (73). The sites most frequently affected are the larynx, the oropharynx and the oral cavity. The 2012 incidence of head and neck cancer in Sweden for the most common locales is shown in Table 2 (65).
Table 2. Total incidence of head and neck cancer in the most common locales in Sweden in 2012. (Cancer incidence in Sweden 2012, Socialstyrelsen 2014) (65)

<table>
<thead>
<tr>
<th>Localization</th>
<th>Male</th>
<th>Female</th>
<th>Sum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lip</td>
<td>95</td>
<td>68</td>
<td>163</td>
</tr>
<tr>
<td>Tongue</td>
<td>150</td>
<td>111</td>
<td>261</td>
</tr>
<tr>
<td>Floor of mouth</td>
<td>35</td>
<td>24</td>
<td>59</td>
</tr>
<tr>
<td>Mouth, unspecified location</td>
<td>93</td>
<td>99</td>
<td>192</td>
</tr>
<tr>
<td>Oropharynx</td>
<td>224</td>
<td>84</td>
<td>308</td>
</tr>
<tr>
<td>Larynx</td>
<td>138</td>
<td>37</td>
<td>175</td>
</tr>
</tbody>
</table>

Alcohol and tobacco consumption, especially smoking, have historically been the main risk factors in developing HNSCC. Today, however, there is a worldwide increase in human papilloma virus (HPV)-induced HNSCC in the oropharynx, as well as a decrease in smoking habits especially in industrialized countries (74, 75). The role of HPV in HNSCC of other locations than the oropharynx remains unclear. As with CSCC the carcinogenesis often involves mutations in the TP53 gene leading to a decrease in p53 activity and overexpression of the EGFR-receptor in these tumours leading to loss of control of cell proliferation. The carcinogenesis in HPV-induced cancer is different from alcohol and tobacco-induced cancer; it involves inactivation of the tumor suppressor genes TP53 and the retinoblastoma suppressor gene by viral oncoproteins rather than mutations of the genes themselves (76). The concept of field cancerization described earlier with CSCC is equally applicable in the development of HNSCC, if not more so. It was named by Danely P. Slaughter to describe multiple primary carcinomas arising in the oral cavity from areas with histologically altered mucosa (77).

There is a growing knowledge about the genetic alterations and mutations in these precancerous lesions and their role in the development of multiple primary malignancies (78). There is also a growing knowledge about cancer stem cells and their role in local and regional recurrence (79).

The most important prognostic factor in HNSCC is regional recurrence; the spread of SCC is usually lymphogenic with neck node involvement. Risk factors for regional metastasis in primary tongue cancer is, for in-
stance, a higher T-stage, tumor infiltration depth > 5 mm, poorly differentiated histology and perineural invasion (80, 81).

Although the last decades have seen an increased knowledge about the molecular biology of HNSCC, surgery and RT still remains the primary treatment modalities. The surgical techniques have evolved with introduction of, for example, transoral laser surgery and robotic surgery increasing the tumor access with minimally invasive approaches. The postoperative morbidity has decreased with the introduction of free flap reconstruction (76).

New RT techniques like intensity modulated RT (IMRT), volumetric modulated arc therapy (VMAT), image guided RT IGRT and the development of brachytherapy have found applications in the treatment of HNSCC and combinations of RT and platinum-based chemotherapy have been beneficial in the treatment of advanced tumors and for organ preservation. There is also the emerging field of targeted therapy with antibodies like cetuximab targeting the EGFR receptor.

The progress in imaging techniques has been substantial with the introduction of, for instance, FDG-PET, diffusion-weighed MRI and office-based ultrasound. There is also an ongoing vaccination program for HPV expected to decrease the incidence of HPV-induced HNSCC. All this should have led to a significant increase in the long-term survival and increased quality of life of the patients with HNSCC.

Although there have been a trend of significantly increased survival in patients with tonsil and base of tongue cancer in the last 50 years in Sweden the survival in patients with tongue cancer has improved only modestly (82). Furthermore, patients with oral cavity cancer treated with a combination of surgery and RT show a significant decrease in quality of life measurements even five years after treatment (83). There is also the increased risk of developing second primary tumors in previously treated areas, especially in the oral cavity (84).

The TNM tumor classification system is helpful in predicting the prognosis and useful in treatment decisions. However, in HNSCC there are other important factors such as smoking and HPV status that also determines the outcome and this may lead to more differentiated treatment strategies. Patients with recurrent HNSCC after multimodality treatment remain a group with a very bleak prognosis; there is a need for more treatment strategies for both salvage and palliation.
**Tumor vascularization and cancer stem cells**

A steady supply of nutrients and oxygen are required for cell survival and function. In normal tissues angiogenesis, the development of new blood vessels from existing ones, are controlled by a balance of inhibiting and inducing molecules. Angiogenesis is very important in both primary tumors and metastases; the delicate balance between induction and inhibition seen in normal tissues is lost. The tumor angiogenesis also seems to depend on other cell types, fibroblasts, keratinocytes and monocytes that secrete vascular inducing molecules (85).

This leads to a circulation in solid tumors that is very different from normal tissues in both structure and function. The tumor blood vessels are often irregularly shaped and chaotically arranged leading to poor perfusion and oxygenation. The perimeter of the tumor often has a better perfusion than the interior. This is a challenge in treatment with RT where adequate oxygenation is required and in systemic chemotherapy where inadequate circulation leads to sub lethal doses. It could also be a cause of failure in ECT since oxygen is needed for BLM cytotoxicity.

The perimeter of the tumor is also where the cancer stem cells (CSC) are located (Figure 16)(79).

![Tumor microenvironment](image)

*Figure 16. Cancer stem cells are often found in the “perivascular niche” in the periphery of the tumor where they interact with adjacent normal cells, especially endothelial cells (86).*
These slow-growing cells are less sensitive to RT and chemotherapy than “normal” cancer cells and are thought to be important in local, regional and distant recurrences sometimes even after considerable time. Hypoxia, in addition to reducing the effects of RT and chemotherapy, also appears to promote these cells (87). CSC seems to be dependent on the adjacent stromal cells, especially endothelial cells, for its function and survival. Selective ablation of endothelial cells has been shown to reduce the number of CSCs in vivo (88). Since ECT with BLM seems to have a very strong anti-endothelial effect a hypothesis of a combined effect of ECT on CSC – both directly and indirectly via endothelial cell death – could be formulated.
Clinical ECT

ECT in clinical practice
Today, ECT is probably mostly used for palliative treatment of cutaneous metastases. There are, currently, two systems approved for use in Europe, the Cliniporator™ (IGEA, Carpi, Italy) and the Sennex™ (BionMed, Saarbrücken, Germany) systems. Both offer a variety of electrode applicators for skin treatment but at the present time no flexible applicators are available.

Clinical efficacy of ECT
The efficacy of ECT in the clinical setting has been shown in several small controlled trials (some randomized) where tumors (often in the same patient) were treated with either ECT or drug/EP alone (89-92). Several case studies have shown the efficacy of ECT for cutaneous metastases of different histopathology (HNSCC, malignant melanoma, breast cancer, adenocarcinoma, Kaposi’s sarcoma) (49, 93, 94). Due to its antivascular effect it has also been successfully used in palliation of haemorrhaging metastases of malignant melanoma (51, 95).

In 2013, Mali et al published a meta-analysis of 44 case series, including papers I and II in this thesis, showing that the efficacy was significantly higher with IT than IV drug administration (96-98). There was, however, no statistically significant difference between IT BLM and cisplatin administration on the response rate. Kaposi’s sarcoma and BCC showed the highest response rate, followed by adenocarcinomas, melanomas and SCC (96).

Another meta-analysis of 9 case series, including paper I in this thesis, was published by Mali et al in 2013 (99). There was a significantly lower response rate in skin tumors (both primary and metastases) larger than 3 cm.

The ESOPE guidelines
In 2006 based on the multicentre ESOPE (European Standard Operation Procedures for Electrochemotherapy) study the ESOPE guidelines were published, providing an algorithm for treatment of multiple cutaneous and subcutaneous metastatic nodules (100). In the ESOPE study standard operating procedures for treatment was used and the outcome showed a 73.7% complete response rate (CRR) in 171 cutaneous and subcutaneous me-
tastases of different histopathology in 41 patients after a 5 month median follow-up of period (101).

The ESOPE guidelines provide treatment stratification based on the clinical presentation to electrode selection, drug selection and dose (IV cisplatin or IV/IT BLM) and anaesthesia (local, regional IV or general). Compared to the BLM dose used in this thesis the IT dose used in the ESOPE protocol is significantly lower for the same tumor volumes. This difference is caused by both different equations for the treatment volume and on different BLM doses for different tumor volumes in the ESOPE protocol (1000 IU/cm³ for tumor volumes < 0.5 cm³, 500 IU/cm³ for volumes between 0.5 and 1.0 cm³ and 250 IU/cm³ for volumes > 1.0 cm³) (100). In this thesis the same dose (1000 IU/cm³) was used for all tumor volumes. The tumor volume equation used in the ESOPE protocol is \( V=\pi ab^2/6 \) (\( V= \)treatment volume, \( a=\)the longest tumor diameter, \( b=\)perpendicular diameter). With IV BLM administration the recommended dose in the ESOPE protocol is 15 000 IU/m² surface area.

ECT in non-melanoma skin cancer

Although the skin is easily accessible for ECT relatively few studies of treatment of primary skin cancer have been published. In Table 3 four studies with more than two patients and a median follow-up of at least 5 months are summarized (102-105). BCC was the most common tumor type. However, in one study CSCC and Bowen’s disease were also treated. The complete response rate (CRR) varied between 78 and 100 %. In three studies IT BLM was used. Because of differences in calculating the treatment volume the doses used were lower than in the patients treated in this thesis. In the study by Salwa et al three elderly patients with peri-ocular BCC was treated with complete responses in a follow-up period between 5 and 8 months (104). ECT with intravenous BLM has been tried successfully in three patients with Gorlins-Goltz syndrome showing a CR in 86/99 tumors (106). It has also been tried successfully in treatment of Kaposi’s sarcoma and recurrent Merkel cell carcinomas (93, 107, 108).
Table 3. Studies of ECT treatment in primary NMSC. In the first study there were 54 tumors treated, in the other studies there were only one tumor per patient (102-105).

<table>
<thead>
<tr>
<th>Study</th>
<th>Pats</th>
<th>Histology</th>
<th>Treatment</th>
<th>Follow-up (months)</th>
<th>CRR (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glass, 1996</td>
<td>20</td>
<td>BCC</td>
<td>IT BLM</td>
<td>18</td>
<td>98.1</td>
</tr>
<tr>
<td>Rodríguez-C., 2001</td>
<td>9</td>
<td>BCC</td>
<td>IT BLM</td>
<td>8.6</td>
<td>77.8</td>
</tr>
<tr>
<td>Gargiulo, 2010</td>
<td>15</td>
<td>BCC, SCC, BD</td>
<td>IV BLM</td>
<td>13</td>
<td>80</td>
</tr>
<tr>
<td>Salva, 2014</td>
<td>3</td>
<td>BCC</td>
<td>IT BLM</td>
<td>5</td>
<td>100</td>
</tr>
</tbody>
</table>

BCC, Basal Cell Carcinoma; BD, Bowen’s Disease; BLM, Bleomycin; CRR, Complete Respose Rate; SCC, Squamous Cell Carcinoma

ECT in head and neck cancer

As mentioned before, there are at present no commercially available flexible applicators thereby limiting the use of ECT in the treatment of head and neck cancer. With bendable electrode applicators like the ones used in this thesis access to tumors in the whole oral cavity and the oropharynx can be achieved.

In the first clinical ECT trial published in 1993 by Belehradek et al was 40 cutaneous HNSCC metastases in eight patients was treated with ECT using IV BLM. A CR was achieved in 57% of the tumors with a 36-day (12-250 days) mean follow-up period (89).

As with NMSC there have been few studies investigating the potentially curative aspects of ECT in head and neck cancer. In Table 4, three studies are summarized. In 1998, Panje et al treated 10 patients with either recurrent or primary T1-T4 head and neck cancer RT (109). There was a CR in 5/10 patients with a mean follow-up time of 4 months. These patients were then followed and 4 additional patients were treated. The long-term
follow-up (median 31.5 months) was reported in 2001 with CR in 6/14 patients (110). One case of osteomyelitis and one pharyngocutaneous fistula was reported. Panje et al reported two cases of ECT of sinonasal cancer where one patient had a CR 20 months after treatment (111). In 2003 Burian et al reported treatment of 12 patients with T1-T2 oral cavity and oropharyngeal cancer (112). The treatment area was resected after four weeks with viable cancer in 10/12 patients. In 2014 Campana et al reported the retrospective outcome in 12 patients with cancer in the oral cavity and oropharynx. The CRR was 58.3% and the reported side effects were mycositis in 8/12 patients and ulcerations in 7/12 patients (113).

Table 4. Studies of ECT treatment of primary and recurrent head and neck cancer (110, 112, 113).

<table>
<thead>
<tr>
<th>Study</th>
<th>Pats</th>
<th>Histology</th>
<th>Treatment</th>
<th>Follow-up (months)</th>
<th>CRR (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Allegretti, 2001</td>
<td>14</td>
<td>HNSCC, AC, ACC</td>
<td>IT BLM</td>
<td>31.5</td>
<td>42.9</td>
</tr>
<tr>
<td>Burian, 2003</td>
<td>12</td>
<td>HNSCC</td>
<td>IT BLM</td>
<td>1</td>
<td>83.3</td>
</tr>
<tr>
<td>Campana, 2014</td>
<td>12</td>
<td>HNSCC</td>
<td>IT/IVBLM</td>
<td>14</td>
<td>58.3</td>
</tr>
</tbody>
</table>

AC, Adenocarcinoma; ACC, Adenoidcystic Cancer; HNSCC, Head and neck Squamous Cell Carcinoma

From treatment to healing
After ECT with IT BLM the treated tissues go through three distinct phases with different lengths depending on the tissue treated.

1. A phase of swelling that starts after treatment and continue for days. This phase is more evident in the oral cavity than in the skin.
2. A phase of demarcation and necrosis. In the treated area a distinct border between viable hyper-vascularized tissue and a central necrosis develops. If the necrosis is not resected it will be discharged
within a few weeks. In the skin an ulceration develop followed by a crust formation covering the treatment area for several weeks to months (Pictures 1A and 1B).

3. A phase of healing with minimal scarring which lasts a few weeks in the oral cavity and a few months in the skin (Pictures 1C and 1D).

Figure 17. Necrosis, four weeks after ECT in a patient with ECT for a T2-tongue cancer (A). Ulceration, ten weeks after ECT in a patient with a recurrent cutaneous SCC in the cheek (B). Healing, six weeks after ECT (same patient as in 1A) (C). Healing, two years after ECT (same patient as in 1B) (D).
The EU-CCBE-2003 and EU-HNBE-2003 trials

In 2003 Genetronics, a subsidiary of Inovio Biomedical Corporation (San Diego, CA, USA), started two clinical phase II multicentre trials of ECT in Europe with the MedPulser™ system; the EU-CCBE-2003 trial (ClinicalTrials.gov Identifier: NCT00198276) for treatment of cutaneous and subcutaneous cancer and the EU-HNBE-2003 trial (NCT00198263) for treatment of T1 and T2 head and neck cancer. The Centre for Head and Neck Oncology in Örebro was the only centre in Sweden participating in these studies. Since these were phase II trials there were no control groups and no randomization for comparison to established treatments.

We received approvals from the Regional Board for Ethical Evaluation (decisions 2004: M-296 and 2005:005) and the Medical Products Agency, both in Uppsala, Sweden for enrolment of 10 patients to each trial. In March of 2005 we received approval to enrol 20 additional patients to the EU-HNBE-2003 trial. The primary and secondary outcome measures, planned enrolment, our enrolment and time frame of both studies are shown in Table 5. Beside the inclusion and exclusion criteria, Inovio had no influence over the patient selection; the company also had no part in the treatment of the patients or in the data collection process.

In July of 2007 Inovio stopped the enrolment in both studies due to an increased mortality in the ECT-arm in two other studies comparing ECT to surgery in treatment of primary and recurrent cancer in the oral cavity and the palatine tonsils (HNBE-03-01, NCT00198315) and in the base of tongue, lateral pharynx wall, hypopharynx and larynx (HNBE-03-02, NCT00198328). To our knowledge, no publication of these studies has been published. We applied for and were granted full permission by Inovio to publish our results without any interference from the company in the follow-up or in writing and submission of the manuscripts.

However, we did not have any access to the data from other participating centres but there have, to our knowledge, not been any other publication publications from these aborted studies. We continued to follow all patients for 24 months and the head and neck patients from our county for 60 months; patients referred to us for treatment from other counties were referred back and followed at the referring departments after 24 month. All the follow-up data in all patients with head and neck cancer treated at our centre is reported to our local database.

Papers I, II, III and IV of this thesis is based on the two and five year follow-up data from the 26 patients treated in the framework of these trials.
Table 5. Outcome, enrolment and original time frame of the EU-CCBE-2003 and EU-HNBE-2003 ECT trials.

<table>
<thead>
<tr>
<th></th>
<th>EU-CCBE-2003</th>
<th>EU-HNBE-2003</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Primary outcome</strong></td>
<td>Local tumor recurrence in 6 months after ECT</td>
<td>Local tumor recurrence in 8 months after ECT</td>
</tr>
<tr>
<td><strong>Secondary outcomes</strong></td>
<td>Pharmacoeconomic factors MedPulser system performance Adverse events in 6 months after ECT</td>
<td>Pharmacoeconomic factors MedPulser system performance Adverse events in 4 months after ECT Evaluation of organ function and QoL 4,8 and 12 months after ECT</td>
</tr>
<tr>
<td><strong>Enrolment</strong></td>
<td>95 patients</td>
<td>88 patients</td>
</tr>
<tr>
<td><strong>Start</strong></td>
<td>January 2004</td>
<td>February 2004</td>
</tr>
<tr>
<td><strong>Completion</strong></td>
<td>September 2008</td>
<td>September 2008</td>
</tr>
</tbody>
</table>

**The MedPulser™ system**
The MedPulser system was used in the treatment of all patients in this thesis. It was an EP system intended for clinical use designed and manufactured by Genetronics. It consisted of two main components; the MedPulser Instrument and the MedPulser Applicator (Figure 18). The instrument produced a cycle of six 100 µs square-wave pulses with the field strength 1100 V/cm. The applicators were hexagonal array needle electrodes with a diameter of 0.5 cm or 1 cm and with needle lengths adjustable to 3 cm (Figure 19). The needle angle relative the handle was adjustable between 0° and 90°. All patients in this thesis were treated with this system. The development of the MedPulser system has since been discontinued and it has never been commercially available.
Figure 18. The Medpulser instrument with applicator attached.

Figure 19. The Medpulser applicator.
The present investigation

Aims

The overall aims of this thesis

- To investigate if ECT is an efficacious and safe curative treatment in head and neck cancer and non-melanoma skin cancer in the head and neck area.
- To assess the functional and quality of life outcome in patients with head and neck cancer treated with ECT.
- To investigate if ECT has selective effects in vitro on survival in different cell-types.

Specific aims for each paper

I
Primary outcome: The two-year local tumor control rate and treatment safety in 6 patients with NMSC that treated with ECT with IT administered BLM.
Secondary outcome: The two-year survival and functional outcome after ECT.

II
Primary outcome: The two-year local tumor control rate and treatment safety in 15 patients with oral tongue cancer that were treated with ECT with IT administered BLM.
Secondary outcome: The two-year survival rate and functional outcome after treatment with ECT.

III
Primary outcome: The two-year local tumor control rate and treatment safety in 5 patients with cancer in the oral cavity and the oropharynx that were treated with ECT with IT administered BLM.
Secondary outcome: The two-year survival rate and functional outcome after treatment with ECT.
Primary outcome: The five-year local tumor control rate and treatment safety in nineteen patients with head and neck cancer that were treated with ECT with a curative intent. Quality of life (QoL) outcome assessed at baseline and after twelve months with pairwise comparison of subgroups stratified for adjuvant RT, tumor location and smoking status.

Secondary outcome: The five-year survival rate after treatment with ECT.

Assessment of cell survival in vitro after ECT with BLM in human tongue cancer cells, fibroblasts and endothelial cells in order to investigate possible cell-type survival selectivity. Under the null hypothesis the mean survival was the same in all cell-types.

Materials

Study group

Paper I
Six patients (3 men, 3 women; mean age 76.5) who were diagnosed with non-melanoma skin cancer in the head and neck area were enrolled in the study. The inclusion and exclusion criteria are presented in Table 6. There were three BCC and three SCC tumors. Three of the tumors were recurrences previously treated with surgery. CT scans were performed for correct TNM classification, except in one patient with a low-aggressive BCC of the temporal region. Histological type, localization, and T classification are shown in Table 7. Four of the tumors could be described as complicated by proximity to the buccal and zygomatic branches of the facial nerve and the parotid duct (patient 2); proximity to the floor of the external meatus of the auditory canal, with risk of extensive spread (Patient 3); growth involving the medial rectus muscle of the eye (Patient 5); or proximity to the orbit (Patient 6). All patients gave their written informed consent for treatment with ECT after receiving detailed information about the treatment alternatives, ECT or surgery. The treatment intention was curative in all patients.
Table 6. Inclusion and exclusion criteria for the patients treated with ECT in Papers I, II, III and IV.

<table>
<thead>
<tr>
<th>Inclusion criteria</th>
<th>Exclusion criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biopsy verified CSCC, BCC, melanoma, Merkel cell carcinoma or cutaneous lymphoma (Paper I)</td>
<td>&gt; 50% suspected encasement of major blood vessel</td>
</tr>
<tr>
<td>Biopsy verified cancer in the oral cavity, pharynx, larynx or salivary glands (Papers II, III, IV)</td>
<td>Tumor bone invasion</td>
</tr>
<tr>
<td>Tumors with volumes requiring BLM dose &lt; 80 U</td>
<td>Hypersensitivity to BLM</td>
</tr>
<tr>
<td>Complete tumor and margin accessibility with BLM and electrodes</td>
<td>Lifetime BLM dose &gt; 400 U</td>
</tr>
<tr>
<td>18 years or older</td>
<td>Patients unsuitable for general anesthesia</td>
</tr>
<tr>
<td>Contraceptive methods for men and women with childbearing potential</td>
<td>Emphysema/pulmonary fibrosis</td>
</tr>
<tr>
<td>Baseline performance status: ECOG 0-2*</td>
<td>Patients with pacemakers that cannot be turned off</td>
</tr>
<tr>
<td>Life expectancy of at least 6 months</td>
<td>History of uncontrolled cardiac arrhythmia</td>
</tr>
<tr>
<td>Patients must sign a written Informed Consent</td>
<td>Pregnancy or nursing</td>
</tr>
</tbody>
</table>

*Grade 0: Fully active, able to carry on all pre-disease performance without restriction. Grade 1: Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature, e.g., light housework, office work. Grade 2: Ambulatory and capable of all self-care, but unable to carry out any work activities. Up and about more than 50% of waking hours.

Papers II, IV
Fifteen patients (8 males, 7 females; mean age 54.3 years) diagnosed with T1 or T2 cancers of the oral tongue were enrolled in the study. The inclusion and exclusion criteria are presented in Table 6. The tumors were SCCs in 14 patients and an adenocarcinoma in 1 patient (Table 8). To determine the correct TNM classification a CT scan of the neck and measurement and digital palpation of the tongue tumor under anaesthesia were performed for all patients. All patients gave their written informed
consent for treatment with ECT after detailed information about the treatment alternatives, ECT or laser resection of the tumor, the usual treatment in our department. The treatment intention was curative in all patients.

Papers III, IV
Five patients (all male; mean age 63.8 years) with cancer in the head and neck region were enrolled in the study (Table 9). The inclusion and exclusion criteria are presented in Table 6. Four patients had primary T2 SCC in the oral cavity or the oropharynx and one patient had a metastasis of renal clear cell carcinoma in the right masseter muscle. A CT-scan of the neck and thorax and digital palpation with measurement of the tumor, when needed under general anaesthesia, was performed on all patients to determine the correct TNM classification. All patients gave their written informed consent for treatment with ECT after detailed information about the treatment alternatives, ECT and the standard treatment options in our department (Table 9). In one patient with generalized metastatic disease (Patient 1) the treatment intention was palliative. In the other four patients the treatment intention was curative.

Cells

Paper V
Four different human cell-types were chosen for the investigation, endothelial cells, fibroblasts (FB), and two different HNSCC cell lines. Endothelial cells were selected to investigate a possible endothelial basis for the anti-vascular effects of ECT seen in vivo (46, 47) and fibroblasts because of their important role in wound healing after treatment. HNSCC was chosen because it is the most common type of head and neck cancer.

Human umbilical vein endothelial cells (HUVEC) were prepared from umbilical cords of healthy infants as previously described (114). Normal human fibroblasts (FB) were isolated by explanting pieces of dermis obtained from elective abdominal or chest surgery. The tissue was removed using standard surgical procedures. Two human tongue squamous cell carcinoma cell lines CAL 27 (CRL-2095) (115, 116) and SCC-4 (CRL-1624) (117, 118) were obtained from American Type Culture Collection (Manassas, VA).
**Methods**

**ECT protocol** (Papers I, II, III and IV)

All patients were treated under general anaesthesia with muscle relaxation, using mivacurium chloride to reduce muscle contractions. The tumor was measured, and the treatment volume was calculated with the formula: $0.5(a + 1)(b + 1)^2$, where $a =$ longest tumor diameter and $b =$ maximum perpendicular diameter.

The tumor, including a 1.0-cm margin of tissue with a normal macroscopic appearance, was then infiltrated as uniformly as possible with bleomycin sulfate (Baxter, Halle, Germany) (4,000 IU/mL) using a fanning technique. The dose was 1,000 IU per cm$^3$ tumor volume (0.25 mL/cm$^3$). Ten minutes after the injection, electroporation was performed using a 0.5- or 1.0-cm hexagonal array needle applicator (Genetronics). The applicator was connected to the Medpulser electroporation instrument (Genetronics), which delivered a pulsed electrical field (6 x 100 μs square-wave pulses, 1100 V/cm).

The tumor was treated in overlapping sequences beginning at the margin and ending in the centre of the tumor. The two patients in whom the eyes were involved were treated in cooperation with an ophthalmic surgeon. Except for control biopsies 8 weeks after treatment and removal of obviously necrotic tissue at the revisits in some of the patients treated in the oral cavity, no tissue was resected. Instead, we left the treated area intact to follow the secondary healing process.

**Additional treatment** (Papers I, II, III and IV)

RT after ECT was performed using the same criteria as after surgery based on TNM-stage and estimated depth of tumour infiltration ≥ 5 mm. Neck dissections was performed concurrently with ECT in the patients with neck node disease at the time inclusion and subsequently in patients that developed regional recurrences. Two patients, one with a persistent tumor (pat 3, paper I) and one with a recurrent tumor (patient 5, paper I) after ECT underwent salvage surgery and RT. No primary reconstruction was performed but reconstructive surgery was performed subsequently in two patients that developed osteoradionecrosis (ORN) (patients 2 and 4, paper III) and in one patient that developed a fistula (patient 3, paper III).
Assessment of local control (Papers I, II, III, IV)
Biopsies of the treatment area were carried out in all patients 8 weeks after ECT. To ensure that the biopsies were representative they were performed in general anaesthesia after careful palpation and inspection of the treatment area in all patients with head and neck cancer and in three of the patients with NMSC. In the three remaining patients with NMSC the biopsies were performed in local anaesthesia. The number of biopsies, in each patient, varied between one and five. The patients were then followed clinically every third to fourth month using inspection and palpation.

The patients with NMSC that did not develop recurrences were not followed beyond two years. The patients with head and neck cancer living in our county (Örebro län) were followed for five years. Patients referred to us from other counties were referred back for follow-up after two years. If no recurrence had occurred in five years the patients were declared cancer free. The follow-up data was collected from our local register at the Head and Neck Oncology Center in Örebro.

Assessment of treatment safety (Papers I, II and III)
All treatment malfunctions of the Medpulser system was recorded as well as all serious adverse events (SAE), defined as treatment-related events resulting in death, which was life threatening, required inpatient hospitalization or resulted in persistent or significant disability/incapacity.

Assessment of functional outcome (Paper I)
The patient treated for a tumor in the vicinity of the facial nerve was examined clinically every third to fourth month after ECT including tests of all four facial nerve branches. The two patients that were treated for periocular tumors were assessed by an ophthalmic surgeon after treatment.

Assessment of functional outcome (Papers II and III)
Assessment of eating in public, understandability of speech and normalcy of diet were carried out before and 12 months after treatment using the validated Performance Status Scale for Head and Neck Cancer (PSS-HN scale) (Appendix I)(119). In paper II a voice recording of the patients reading a standard text was carried out between 12 and 15 months after treatment. The voice recordings were assessed by a phoniatrician who were not involved in the treatment.
Assessment of Quality of life (Paper IV)
The validated European Organisation for Research and Treatment of Cancer Head & Neck 35 (EORTC H&N 35) questionnaire was used for QoL assessment at baseline and 12 months after ECT (Appendix II) (120, 121). The questionnaire is specific for patients with head and neck cancer and consists of seven multiple-question subscales for assessment of pain, swallowing, senses (taste/smell), speech, social eating, social contact, and sexuality. It also consists of eleven single-item scales assessing problems associated with teeth, mouth-opening, xerostomia, sticky saliva, coughing, feeling ill, need for painkillers, need for nutritional supplements, need for feeding tube, weight loss and weight gain. The smoking status at the time of treatment was collected from the patient’s journals.

ECT protocol (Paper V)
Fifteen mg (equalling 15 000 IU) of bleomycin (Baxter, Halle, Germany) was dissolved in 1.5 ml sterile 0.9% sodium chloride to a stock solution of 10 mg/ml.

For each cell type, 100,000 well-suspended cells in 200 μL of serum-free cell culture medium were added to electroporation cuvettes with a 2 mm electrode gap (Bio-Rad, Hercules, CA, USA). BLM was then added to final concentrations of 1, 10, 100 and 1000 μg/ml.

Electroporation was performed using the Gene Pulser Xcell™ (Bio-Rad, Hercules, CA, USA). 8 square-wave pulses, each with duration of 0.1 ms and a 0.1 s interval between pulses were used, corresponding to clinical ECT parameters (51). The electrical field strengths were 500, 1000, 1200 and 1500 V/cm. 1.8 ml of cell culture medium was then added to the cuvette. After mixing, the electroporated cells were seeded into a black 96-well plate in quadruplicates.

To separate the effect of electroporation itself from the effect of ECT we also exposed each cell type to EP only. As controls, quadruplicates of each cell-type were added to the cuvette and mixed without BLM and then seeded in a 96-well plate without being electroporated. To account for the background fluorescence (blank) we also added wells with only cell culture medium.

Assessment of cell survival (Paper V)
The survival of all cell types was assessed 24 (day 1), 48 (day 2), 72 (day 3) and 96 hours (day 4) after EP/ECT using both the protease-based Mul-
tiTox-Fluor™ cytotoxicity assessment assay (Promega, Madison, WI, USA) and a crystal violet assay (Sigma-Aldrich Sweden AB, Stockholm, Sweden). All fluorometric readings were done using the FLUOstar Optima™ microplate reader (BMG Labtech, Ortenberg, Germany).

Statistical methods

Paper IV
The EORTC H&N35 QoL questionnaire produces both ordinal and nominal (dichotomous) raw data that were standardized to scores between 0 and 100 by linear transformations according to the EORTC manual where a higher score indicates worse symptoms (122). The Wilcoxon signed rank test was used for paired ordinal data (pain, swallowing, senses, speech, social eating, social contact, sexuality, teeth, mouth-opening, dry mouth, sticky saliva, coughing and feeling ill) and McNemar’s test for the paired nominal data (need for painkillers, nutritional supplements, feeding tube, weight loss and weight gain). For subgroup comparisons the Mann Whitney U-test was used for the ordinal data and Fishers exact test for nominal data. The $\alpha = 0.05$ level was chosen for statistical significance for all the conducted tests. SPSS software (Armonk, NY, USA) was used for all statistical tests.

Paper V
To accept or reject the null hypotheses we were only concerned with the survival after 96 hours. We used the data obtained from the Multitox-Fluor assay because of its very good linear relationship between the fluorescence and the number of cells alive. The fluorescence values of the blanks were subtracted from the data. In order to compare the between group survival independently from cell-type specific factors, for example different proliferation rates, the survival data was then normalized (% of control group from the same cell-type) prior to statistical analysis. The data were tested for normality and equal variance using the Shapiro-Wilk and Leverne’s tests respectively. Welch’s F-test was used to establish if there were any statistically significant within-group differences. In those cases we performed the Games-Howell post-hoc test for between-group comparisons. $\alpha = 0.01$ was chosen as the level of statistical significance. SPSS (Armonk, NY, USA) software was used in all statistical tests. To measure the effect size of the statistically significant survival differences
Cohen’s d formula with pairwise pooled standard deviations to account for unequal variances in the different cell groups was used.

**Results**

**Paper I**

*Primary outcome:* The outcome is summarized in Table 7. There was a persistent tumor in the external meatus after two ECT-treatments requiring extensive salvage surgery and radiotherapy (Patient 3). There were no other local recurrences recorded in the two-year follow-up period and no other patient received adjuvant treatment. The median follow-up time was 24 months. The patient with a metatypical BCC (Patient 5) had a local recurrence 2.5 years after ECT treated with salvage surgery (exenteration of the orbit) and RT.

There was one SAE not reported in the original paper (97); the patient with a BCC in the temporal area (Patient 1) had an episode of epileptic seizure 6 months after treatment. EEG and a CT-scan were normal and the patient did not have any additional seizures in the follow-up period. There was one adverse event, a treatment-related perforation of the nasal septum cartilage. There were no recorded malfunctions of the Medpulser system.

*Secondary outcome:* All patients were alive and tumour-free two years after treatment (Table 7). In the patient with a recurring SCC tumor in the cheek, no sign of facial nerve paralysis or damage to the parotid duct was recorded, even though these structures were located in the ECT treatment area. The two patients with tumors in the medial canthus experienced a period of greater tear fluid but no visual impairment. Within 2 months, the function of the eyelids and the tear apparatus was normal in both patients.
Table 7. Demographics, tumor characteristics, additional treatment and outcome after ECT in six patients with NMSC.

<table>
<thead>
<tr>
<th>Pat</th>
<th>Sex</th>
<th>Age</th>
<th>Tumor type</th>
<th>Tumor size</th>
<th>Localization</th>
<th>Recurrence</th>
<th>Additional treatment</th>
<th>Follow-up (months)</th>
<th>Tumor status</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>M</td>
<td>65</td>
<td>BCC, IA</td>
<td>25x25x5</td>
<td>temporal region</td>
<td>0</td>
<td>None</td>
<td>24</td>
<td>NED</td>
</tr>
<tr>
<td>2</td>
<td>M</td>
<td>81</td>
<td>SCC</td>
<td>28x35x15</td>
<td>cheek</td>
<td>2</td>
<td>None</td>
<td>24</td>
<td>NED</td>
</tr>
<tr>
<td>3</td>
<td>M</td>
<td>70</td>
<td>SCC</td>
<td>20x15x10</td>
<td>external meatus</td>
<td>1</td>
<td>Surgery +RT</td>
<td>3</td>
<td>PT</td>
</tr>
<tr>
<td>4</td>
<td>F</td>
<td>66</td>
<td>SCC</td>
<td>10x10x5</td>
<td>vestibule</td>
<td>0</td>
<td>None</td>
<td>24</td>
<td>NED</td>
</tr>
<tr>
<td>5</td>
<td>F</td>
<td>82</td>
<td>Metatypic BCC</td>
<td>15x15x10</td>
<td>medial canthus</td>
<td>7</td>
<td>None</td>
<td>24</td>
<td>NED</td>
</tr>
<tr>
<td>6</td>
<td>F</td>
<td>95</td>
<td>BCC, II</td>
<td>18x12x5</td>
<td>medial canthus</td>
<td>0</td>
<td>None</td>
<td>24</td>
<td>NED</td>
</tr>
</tbody>
</table>

BCC, Basal Cell Carcinoma; NED, No Evidence of Disease; PT, Persistant Tumor, RT, Radiotherapy, SCC, Squamous Cell Carcinoma.
Pat 3 was treated with ECT two times.

Paper II

Primary outcome: The outcome is summarized in Table 8. The local tumor control rate in the two-year follow-up period was 100% based on 12 surviving patients. All biopsies two months after ECT were negative for cancer and there were no clinical signs of recurrence subsequently. Ten patients received adjuvant RT and five patients were treated with ECT alone. The median follow-up time was 24 months.

There were no recorded malfunctions of the Medpulser system. There was one recorded serious adverse event; the first patient treated was readmitted because of treatment-related pain.

Secondary outcome: The overall survival in the two-year follow-up period was 80 % (12/15) and the disease specific survival was 85.7 % (12/14) (Table 8). Two patients died from regional recurrences (RR) and one patient died from a gastrointestinal bleeding, all without signs of local recurrence. The functional outcome was very good (Table 9). The only notable differences were that after treatment two patients needed liquid assistance when eating a normal diet and one patient that could eat all meats before
treatment could only eat soft foods afterwards (Table 9). All three had been treated with adjuvant RT.

The five patients treated with ECT alone had the highest scores in all three parameters (eating in public, understandability of speech and normalcy of diet) before as well as after treatment (Table 9). In the voice recordings all 12 surviving patients had normal speech intelligibility, although five had minor articulation indistinctiveness but with no impact on the ability to produce any speech sound. The overall communication ability was normal for all patients.

Table 8. Demographics, tumor characteristics, treatment date, additional treatment and clinical outcome after ECT in 15 patients with oral tongue cancer.

<table>
<thead>
<tr>
<th>Sex</th>
<th>Age</th>
<th>TNM</th>
<th>Type</th>
<th>Tumor size(mm)</th>
<th>Treatment date</th>
<th>Neck dissection</th>
<th>Radiotherapy</th>
<th>Last follow-up (month)</th>
<th>Tumor status tongue</th>
<th>Tumor status overall</th>
</tr>
</thead>
<tbody>
<tr>
<td>M</td>
<td>30</td>
<td>T2N0M0</td>
<td>SCC</td>
<td>25x25x5</td>
<td>02-feb-05</td>
<td>No</td>
<td>T+N</td>
<td>24</td>
<td>NED</td>
<td>NED</td>
</tr>
<tr>
<td>M</td>
<td>45</td>
<td>T2N0M0</td>
<td>SCC</td>
<td>25x2x10</td>
<td>07-apr-05</td>
<td>Ipsilateral</td>
<td>T+N</td>
<td>24</td>
<td>NED</td>
<td>RR</td>
</tr>
<tr>
<td>F</td>
<td>74</td>
<td>T1N0M0</td>
<td>SCC</td>
<td>17x17x5</td>
<td>14-apr-05</td>
<td>Bilateral</td>
<td>T+N(ipsilat)</td>
<td>24</td>
<td>NED</td>
<td>RR</td>
</tr>
<tr>
<td>F</td>
<td>65</td>
<td>T2N0M0</td>
<td>SCC</td>
<td>22x14x3</td>
<td>06-dec-05</td>
<td>No</td>
<td>No</td>
<td>24</td>
<td>NED</td>
<td>NED</td>
</tr>
<tr>
<td>F</td>
<td>71</td>
<td>T2N0M0</td>
<td>SCC</td>
<td>35x3x15</td>
<td>01-dec-05</td>
<td>Ipsilateral</td>
<td>T+N</td>
<td>8</td>
<td>NED</td>
<td>DWD</td>
</tr>
<tr>
<td>F</td>
<td>61</td>
<td>T1N0M0</td>
<td>SCC</td>
<td>20x18x3</td>
<td>19-jan-06</td>
<td>No</td>
<td>No</td>
<td>24</td>
<td>NED</td>
<td>NED</td>
</tr>
<tr>
<td>M</td>
<td>60</td>
<td>T2N0M0</td>
<td>SCC</td>
<td>40x20x20</td>
<td>24-jan-06</td>
<td>Ipsilateral</td>
<td>T+N</td>
<td>24</td>
<td>NED</td>
<td>NED</td>
</tr>
<tr>
<td>M</td>
<td>71</td>
<td>T2N0M0</td>
<td>SCC</td>
<td>35x30x20</td>
<td>04-apr-06</td>
<td>No</td>
<td>T+N</td>
<td>8</td>
<td>NED</td>
<td>DWOD</td>
</tr>
<tr>
<td>M</td>
<td>20</td>
<td>T2N2aM0</td>
<td>SCC</td>
<td>25x25x15</td>
<td>18-apr-06</td>
<td>Ipsilateral</td>
<td>T+N</td>
<td>24</td>
<td>NED</td>
<td>NED</td>
</tr>
<tr>
<td>F</td>
<td>47</td>
<td>T1N2bM0</td>
<td>SCC</td>
<td>18x15x5</td>
<td>15-jun-06</td>
<td>Ipsilateral</td>
<td>N</td>
<td>1</td>
<td>NED</td>
<td>DWD</td>
</tr>
<tr>
<td>F</td>
<td>63</td>
<td>T2N0M0</td>
<td>SCC</td>
<td>30x25x10</td>
<td>20-jul-06</td>
<td>No</td>
<td>T+N</td>
<td>24</td>
<td>NED</td>
<td>NED</td>
</tr>
<tr>
<td>M</td>
<td>45</td>
<td>T2N0M0</td>
<td>AC</td>
<td>22x18x5</td>
<td>14-nov-06</td>
<td>No</td>
<td>No</td>
<td>24</td>
<td>NED</td>
<td>NED</td>
</tr>
<tr>
<td>M</td>
<td>64</td>
<td>T2N0M0</td>
<td>SCC</td>
<td>27x20x3</td>
<td>27-dec-06</td>
<td>No</td>
<td>No</td>
<td>24</td>
<td>NED</td>
<td>NED</td>
</tr>
<tr>
<td>F</td>
<td>53</td>
<td>T2N0M0</td>
<td>SCC</td>
<td>30x19x12</td>
<td>01-mar-07</td>
<td>No</td>
<td>T+N</td>
<td>24</td>
<td>NED</td>
<td>NED</td>
</tr>
<tr>
<td>M</td>
<td>46</td>
<td>T2NOM0</td>
<td>SCC</td>
<td>22x17x1</td>
<td>22-mar-07</td>
<td>No</td>
<td>No</td>
<td>24</td>
<td>NED</td>
<td>NED</td>
</tr>
</tbody>
</table>

SCC= Squamous cell carcinoma, AC=Adenocarcinoma T=Tongue, N=Neck, NED = No evidence of disease, RR = Regional recurrence DWD = Dead with disease, DWOD = Dead without disease
Table 9. PSS-HN assessments before and 12 months after ECT in patients with oral tongue cancer.

<table>
<thead>
<tr>
<th>Pat No</th>
<th>Eating in public</th>
<th>Understandability of speech</th>
<th>Normalcy of diet</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before</td>
<td>After</td>
<td>Before</td>
</tr>
<tr>
<td>1</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>2</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>3</td>
<td>75</td>
<td>75</td>
<td>100</td>
</tr>
<tr>
<td>4</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>6</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>7</td>
<td>50</td>
<td>75</td>
<td>100</td>
</tr>
<tr>
<td>9</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>11</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>12</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>13</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>14</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>15</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

PSS-HN = Performance Status Scale for Head and Neck cancer patients
Eating in public: 100=no restriction, 75=no restriction of place but restriction of diet in public, 50=eats only at selected places with selected people. Understandability of speech: 100=always understandable, 75=occasional repetition necessary.
Normalcy of diet: 100=no restriction, 90=full diet (liquid assist), 80=all meat, 50=soft, chewable foods

Paper III
Primary outcome: The outcome is summarized in Table 10. The local tumor control rate in the two-year follow-up period was 100% based on 4 surviving patients. All biopsies at 2 months after ECT were negative for cancer and there were no clinical signs of recurrence subsequently. Three of the five patients received adjuvant RT and one patient were treated with ECT alone. One patient had been treated with palliative RT before ECT (Patient 1). The median follow-up time was 24 months.

There was one recorded malfunction of the Medpulser system; the treatment, however, could be completed successfully. There were four SAEs recorded in the follow-up period; two cases of ORN, one buccal fistula and one nearly lethal bleeding. The three patients that developed
ORN or fistulas were treated with secondary reconstructive surgery (one free flap, two loco-regional flaps).

*Secondary outcome*: Four of the five patients were alive and tumour-free overall at 24 months (80%) (Table 10). The functional outcome was poor. Three of the four surviving patients scored lower in all three parameters (eating in public, understandability of speech, normalcy of diet) after 12 months than before treatment (Table 11). Two of the patients still had feeding tubes one year after treatment.

**Table 10.** Demographics, tumor characteristics, adjuvant treatment, and two-year clinical outcome in 5 patients treated with ECT for cancer in the head and neck area.

<table>
<thead>
<tr>
<th>Sex</th>
<th>Age</th>
<th>Localization</th>
<th>Histology</th>
<th>TNM</th>
<th>Tumor size (mm)</th>
<th>RT</th>
<th>Follow-up (months)</th>
<th>Tumor status</th>
<th>Local</th>
<th>Overall</th>
</tr>
</thead>
<tbody>
<tr>
<td>M</td>
<td>59</td>
<td>Masseter muscle</td>
<td>Clear cell carcinoma</td>
<td>TXNXM1</td>
<td>43 × 26 × 26</td>
<td>Before</td>
<td>4</td>
<td>NED</td>
<td>DWD</td>
<td></td>
</tr>
<tr>
<td>M</td>
<td>65</td>
<td>Floor of mouth</td>
<td>SCC</td>
<td>T2N0M0</td>
<td>30 × 30 × 25</td>
<td>P + N</td>
<td>24</td>
<td>NED</td>
<td>NED</td>
<td></td>
</tr>
<tr>
<td>M</td>
<td>59</td>
<td>Bucca</td>
<td>SCC</td>
<td>T2N0M0</td>
<td>20 × 20 × 25</td>
<td>P + N</td>
<td>24</td>
<td>NED</td>
<td>NED</td>
<td></td>
</tr>
<tr>
<td>M</td>
<td>78</td>
<td>Floor of mouth</td>
<td>SCC</td>
<td>T2N0M0</td>
<td>25 × 25 × 5</td>
<td>P + N</td>
<td>24</td>
<td>NED</td>
<td>NED</td>
<td></td>
</tr>
<tr>
<td>M</td>
<td>58</td>
<td>Base of tongue</td>
<td>SCC</td>
<td>T2N0M0</td>
<td>25 × 25 × 8</td>
<td>No</td>
<td>24</td>
<td>NED</td>
<td>NED</td>
<td></td>
</tr>
</tbody>
</table>

DWD, dead with disease; M, male; N, neck; NED, no evidence of disease; P, primary tumor; RT, radiotherapy; SCC, squamous cell carcinoma.
Table 11. PSS-HN assessments in four patients with cancer in the oral cavity and oropharynx before and 12 months after ECT.

<table>
<thead>
<tr>
<th>Pat No</th>
<th>Eating in public</th>
<th>Understandability of speech</th>
<th>Normalcy of diet</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before</td>
<td>After</td>
<td>Before</td>
</tr>
<tr>
<td>2</td>
<td>100</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>3</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>4</td>
<td>100</td>
<td>75</td>
<td>100</td>
</tr>
<tr>
<td>6</td>
<td>100</td>
<td>25</td>
<td>75</td>
</tr>
</tbody>
</table>

Eating in public: 100 = no restriction, 75 = no restriction of place but restriction of diet in public, 25 = eats only at home in presence of selected individuals, 0 = always eats alone. Understandability of speech: 100 = always understandable, 75 = occasional repetition necessary, 50 = usually understandable; face-to-face contact necessary. Normalcy of diet: 100 = no restriction, 80 = all meat, 30 = pureed foods (in blender), 20 = warm liquids, 0 = non-oral feeding (tube-fed). PSS-HN, Performance Status Scale for Head and Neck Cancer

Paper IV

Primary outcome: The outcome is summarized in Table 12. The local control rate in the five-year follow-up period was 100% based on 12 surviving patients. Six of these patients had received adjuvant RT and six were treated with ECT alone. The median follow-up time was 58 months.

There was one additional SAEs recorded in addition to the five previously reported (pain, ORN, fistula and bleeding) (98, 123) (Table 12). The patient treated for a tongue base cancer (Patient 19) developed severe problems with aspiration and was eventually offered a laryngectomy, which the patient declined.

The QoL data (median values) at baseline and 12 months after ECT are shown in Table 13. The data was based on the 18 patients that choose to participate at baseline and 15 patients a year after treatment. By then three patients had died and one patient chose not to participate. There was a statistically significant increase in problems with senses (p=0.006), speech (p=0.01), opening mouth (p=0.02) and mouth dryness (p=0.005) and a decrease in problems with social contact after treatment for the whole group (p=0.042) (Table 13). There were no statistically significant differ-
ences for the subgroup that were treated with only ECT (6 patients before and 5 patients after) (Table 13).

Three pair of subgroups was compared; patients treated with ECT alone (ECT) vs. patients treated with adjuvant RT (ECT+RT), patients with oral tongue cancer (Tongue) vs. patients with oral cavity cancer in other sites (Non-tongue) and patients that smoked during treatment (Smokers) vs. patients that didn’t (Non-smokers). Twelve months after ECT there were statistically significant unfavourable outcomes regarding swallowing ($p=0.01$) and xerostomia ($p=0.04$) in the ECT+RT group compared to the ECT group. When comparing tumor location, the non-tongue cancer patients had a statistically significantly worse outcome than the tongue cancer patients after ECT regarding need for painkillers ($p=0.03$). Smokers had significantly worse speech problems ($p=0.008$) than the non-smoking group.

Secondary outcome: The overall five-year survival was 63.1% (12/19) and the tumour-specific five-year survival was 75% (12/16) (Table 12). Two patients had already died of RR at the two-year follow-up and two more patients died of RR subsequently. Three patients died of intercurrent disease in the follow-up period. There were no signs of local recurrence in the seven patients that died during follow-up.
Table 12. Demographics, smoking, tumor characteristics, serious adverse events and five-year outcome in 19 head and neck cancer patients treated with ECT.

<table>
<thead>
<tr>
<th>No.</th>
<th>Sex</th>
<th>Age</th>
<th>Localization</th>
<th>Histology</th>
<th>Smoking</th>
<th>THM</th>
<th>Radiotherapy (primary/neck)</th>
<th>Follow-up (months)</th>
<th>Tumor status local</th>
<th>Tumor status overall</th>
<th>Five-year survival/tumor status overall</th>
<th>SAE</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>M</td>
<td>30</td>
<td>Tongue</td>
<td>SCC</td>
<td>No</td>
<td>T2NOM0</td>
<td>57,8 / Bl. 40,8 Gy</td>
<td>63</td>
<td>NED</td>
<td>Alive/ NED</td>
<td>Pain</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>M</td>
<td>45</td>
<td>Tongue</td>
<td>SCC</td>
<td>No</td>
<td>T2NOM0</td>
<td>57,8 / Bl. 40,8 Gy</td>
<td>26</td>
<td>NED</td>
<td>DWOD (06)</td>
<td>None</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>F</td>
<td>74</td>
<td>Tongue</td>
<td>SCC</td>
<td>No</td>
<td>T1NOM0</td>
<td>57,8 / Bl. 40,8 Gy</td>
<td>14</td>
<td>NED</td>
<td>DWOD (07)</td>
<td>None</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>M</td>
<td>65</td>
<td>Hoor of mouth</td>
<td>SCC</td>
<td>Yes</td>
<td>T2H1M0*</td>
<td>57,8 / Bl. 40,8 Gy</td>
<td>57</td>
<td>NED</td>
<td>Alive/ NED</td>
<td>ORN</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>F</td>
<td>65</td>
<td>Tongue</td>
<td>SCC</td>
<td>Yes</td>
<td>T2NOM0</td>
<td>None / None</td>
<td>50</td>
<td>NED</td>
<td>Alive/ NED</td>
<td>None</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>F</td>
<td>71</td>
<td>Tongue</td>
<td>SCC</td>
<td>Yes</td>
<td>T2NOM0</td>
<td>57,8 / Bl. 40,8 Gy</td>
<td>8</td>
<td>NED</td>
<td>DWOD (06)</td>
<td>None</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>F</td>
<td>61</td>
<td>Tongue</td>
<td>SCC</td>
<td>Yes</td>
<td>T1NOM0</td>
<td>None / None</td>
<td>52</td>
<td>NED</td>
<td>Alive/ NED</td>
<td>None</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>M</td>
<td>59</td>
<td>Bucca</td>
<td>SCC</td>
<td>Yes</td>
<td>T1NOM0</td>
<td>57,8 / Bl. 40,8 Gy</td>
<td>62</td>
<td>NED</td>
<td>Alive/ NED</td>
<td>Fistula</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>M</td>
<td>60</td>
<td>Tongue</td>
<td>SCC</td>
<td>Yes</td>
<td>T2NOM0</td>
<td>57,8 / Bl. 40,8 Gy</td>
<td>27</td>
<td>NED</td>
<td>DWOD (lung cancer)</td>
<td>None</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>M</td>
<td>71</td>
<td>Tongue</td>
<td>SCC</td>
<td>Yes</td>
<td>T2NOM0</td>
<td>57,8 / Bl. 40,8 Gy</td>
<td>8</td>
<td>NED</td>
<td>DWOD (GI-bleeding)</td>
<td>None</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>M</td>
<td>20</td>
<td>Tongue</td>
<td>SCC</td>
<td>No</td>
<td>T2H2eM0</td>
<td>57,8 / Bl. 40,8 Gy</td>
<td>64</td>
<td>NED</td>
<td>Alive/ NED</td>
<td>None</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>M</td>
<td>78</td>
<td>Hoor of mouth</td>
<td>SCC</td>
<td>Yes</td>
<td>T2NOM0</td>
<td>57,8 / Bl. 40,8 Gy</td>
<td>35</td>
<td>NED</td>
<td>DWOD (colon cancer)</td>
<td>ORN</td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>F</td>
<td>47</td>
<td>Tongue</td>
<td>SCC</td>
<td>Previously</td>
<td>T1H2eM0</td>
<td>None / Bl. 40,8 Gy</td>
<td>2</td>
<td>NED</td>
<td>DWOD (06)</td>
<td>None</td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>F</td>
<td>63</td>
<td>Tongue (right side)</td>
<td>SCC</td>
<td>Yes</td>
<td>T2NOM0</td>
<td>57,8 / Bl. 40,8 Gy</td>
<td>62</td>
<td>NED</td>
<td>Alive/ NED</td>
<td>None</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>M</td>
<td>45</td>
<td>Tongue</td>
<td>AC</td>
<td>Yes</td>
<td>T2NOM0</td>
<td>None / None</td>
<td>58</td>
<td>NED</td>
<td>Alive/ NED</td>
<td>None</td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>M</td>
<td>64</td>
<td>Tongue</td>
<td>SCC</td>
<td>Previously</td>
<td>T1NOM0</td>
<td>None / None</td>
<td>59</td>
<td>NED</td>
<td>Alive/ NED</td>
<td>None</td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>F</td>
<td>53</td>
<td>Tongue</td>
<td>SCC</td>
<td>Previously</td>
<td>T2NOM0</td>
<td>57,8 / Bl. 40,8 Gy</td>
<td>67</td>
<td>NED</td>
<td>Alive/ NED*</td>
<td>None</td>
<td></td>
</tr>
<tr>
<td>18</td>
<td>M</td>
<td>46</td>
<td>Tongue</td>
<td>SCC</td>
<td>Previously</td>
<td>T2NOM0</td>
<td>None / None</td>
<td>61</td>
<td>NED</td>
<td>Alive/ NED</td>
<td>None</td>
<td></td>
</tr>
<tr>
<td>19</td>
<td>M</td>
<td>58</td>
<td>Base of tongue</td>
<td>SCC</td>
<td>Yes</td>
<td>T2NOM0</td>
<td>None / None**</td>
<td>62</td>
<td>NED</td>
<td>Alive/ NED</td>
<td>Bleeding Aspiration</td>
<td></td>
</tr>
</tbody>
</table>

AC, Adenocarcinoma; Bl, Bilateral; CUP, Cancer of unknown primary; DWOD, Dead with disease; DWOD, Dead without disease; GI, Gastrointestinal; IL, Ipsilateral; NED, No evidence of disease; ORN, Osteoradionecrosis; RT, Radiotherapy; SAE, Serious adverse event; SCC, Squamous cell carcinoma; *Patient died 5.5 years after ECT from a contralateral tongue cancer, **Patient had six years previously received RT for a CU
Table 13. The EORTC H&N 35 quality of life data (median and range) for all patients at baseline and one year after ECT. The p-values are the outcome of the Wilcoxon signed rank test and McNemar’s test. There were significantly increased problems with senses, speech, opening mouth and xerostomia and a significant decrease in problems with social contact. The group sizes are shown at the bottom of the table.

<table>
<thead>
<tr>
<th>QoL items</th>
<th>Baseline</th>
<th>12 months</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Swallowing</td>
<td>0 (0-41.7)</td>
<td>8.3 (0-58.3)</td>
<td>0.17</td>
</tr>
<tr>
<td>Senses (taste, smell)</td>
<td>0 (0-66.6)</td>
<td>16.7 (0-33.3)</td>
<td>0.006</td>
</tr>
<tr>
<td>Speech</td>
<td>0 (0-44.4)</td>
<td>11.1 (0-55.5)</td>
<td>0.01</td>
</tr>
<tr>
<td>Social eating</td>
<td>8.3 (0-66.6)</td>
<td>8.3 (0-75)</td>
<td>0.066</td>
</tr>
<tr>
<td>Social contact</td>
<td>0 (0-66.6)</td>
<td>0 (0-33.3)</td>
<td>0.042</td>
</tr>
<tr>
<td>Sexuality</td>
<td>0 (0-66.6)</td>
<td>0 (0-100)</td>
<td>0.67</td>
</tr>
<tr>
<td>Teeth</td>
<td>0 (0-33.3)</td>
<td>0 (0-33.3)</td>
<td>0.56</td>
</tr>
<tr>
<td>Opening mouth</td>
<td>0 (0-33.3)</td>
<td>0 (0-66.6)</td>
<td>0.02</td>
</tr>
<tr>
<td>Xerostomia</td>
<td>33.3 (0-66.6)</td>
<td>66.6 (33.3-100)</td>
<td>0.005</td>
</tr>
<tr>
<td>Sticky saliva</td>
<td>0 (0-100)</td>
<td>33.3 (0-100)</td>
<td>0.13</td>
</tr>
<tr>
<td>Coughing</td>
<td>0 (0-66.6)</td>
<td>0 (0-33.3)</td>
<td>0.56</td>
</tr>
<tr>
<td>Felt ill</td>
<td>0 (0-33.3)</td>
<td>0 (0-66.6)</td>
<td>0.16</td>
</tr>
<tr>
<td>Painkillers</td>
<td>0 (0-100)</td>
<td>0 (0-100)</td>
<td>1</td>
</tr>
<tr>
<td>Nutritional supplements</td>
<td>0 (0-100)</td>
<td>0 (0-100)</td>
<td>0.62</td>
</tr>
<tr>
<td>Feeding tube</td>
<td>0 (0-0)</td>
<td>0 (0-100)</td>
<td>1</td>
</tr>
<tr>
<td>Weight loss</td>
<td>0 (0-100)</td>
<td>0 (0-100)</td>
<td>1</td>
</tr>
<tr>
<td>Weight gain</td>
<td>0 (0-100)</td>
<td>0 (0-100)</td>
<td>0.51</td>
</tr>
</tbody>
</table>

n=18  n=15

Paper V
The difference in survival day 4 after ECT between the fibroblasts and the other cell types (SCC-4, CAL-27 and the HUVEC) was statistically significant at the α = 0.01 level (Figure 20 and Table 14). The null hypothesis
was therefore rejected for the fibroblasts. Cohen’s d for the differences varied between 18.7 and 42.6 indicating that the survival differences were large. For the HUVEC cells, on the other hand, the null hypothesis was accepted. When compared to the SCC cell lines there were no statistically significant differences in survival after ECT. There were, however, statistically significant survival differences between the HUVEC cells and all the other cell types day 1 after ECT. Cohen’s d values between 9.4 and 14.8 again indicated that these differences were also large (Figure 21 and Table 15).

**Figure 20.** Mean relative survival ± SEM day 1 to 4 for all cell types after ECT with low dose (10 µg/ml) and high dose (100 µg/ml) BLM exposure. Cell survival was assessed with the MultiTox-Fluor assay. The data is based on four independent experiments.
**Table 14.** Result of Welch’s F-test for all cell types 4 days after EP alone, ECT with 10 μg/ml BLM (ECT L) and 100 μg/ml (ECT H) (A). The between cell type comparison with the Games-Howell post-hoc test for the significant results from Welch’s test (ECT L and ECT-H) and Cohen’s d value for these statistically significant differences showing large differences (B). The data was based on four independent experiments. (FB, Fibroblasts; HUVEC, Human umbilical vein endothelial cells; CAL-27 and SCC-4 are two human SCC cell lines).

### A

<table>
<thead>
<tr>
<th>Groups</th>
<th>F-statistic Welch’s test</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>EP</td>
<td>4.79</td>
<td>0.048</td>
</tr>
<tr>
<td>ECT L</td>
<td>343.1</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>ECT H</td>
<td>610.4</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

### B

<table>
<thead>
<tr>
<th>Cell type</th>
<th>ECT L</th>
<th>ECT H</th>
<th>Games-Howell</th>
<th>Cohen’s d</th>
</tr>
</thead>
<tbody>
<tr>
<td>FB - HUVEC</td>
<td>$p &lt; 0.001$</td>
<td>$p &lt; 0.001$</td>
<td>21.8</td>
<td>34.8</td>
</tr>
<tr>
<td>FB - CAL-27</td>
<td>$p &lt; 0.001$</td>
<td>$p &lt; 0.001$</td>
<td>24.5</td>
<td>42.6</td>
</tr>
<tr>
<td>FB - SSC-4</td>
<td>$p &lt; 0.001$</td>
<td>$p &lt; 0.001$</td>
<td>21.8</td>
<td>18.8</td>
</tr>
<tr>
<td>HUVEC - CAL-27</td>
<td>$p = 0.20$</td>
<td>NS</td>
<td>$p = 0.88$</td>
<td>NS</td>
</tr>
<tr>
<td>HUVEC - SCC-4</td>
<td>$p = 0.28$</td>
<td>NS</td>
<td>$p = 0.09$</td>
<td>NS</td>
</tr>
<tr>
<td>CAL-27 - SCC-4</td>
<td>$p = 0.02$</td>
<td>NS</td>
<td>$p = 0.08$</td>
<td>NS</td>
</tr>
</tbody>
</table>
Figure 21. Mean relative survival ± SEM for all cell types day 1 to 4 after EP alone. The cell viability was assessed with the MultiTox-Fluor assay. The data was based on four independent experiments. (FB, Fibroblasts; HUVEC, Human umbilical vein endothelial cells; CAL-27 and SCC-4 are two human SCC cell lines).

Table 15. Result of Welch’s F-test for all cell types day 1 and 2 after EP alone (A). The results from the between group comparison with the Games-Howell post-hoc test for the significant results from Welch test (EP day 1 and day 2) and Cohen’s d value for these statistically significant differences showing large differences (B). The data was based on four independent experiments. (NS=not statistically significant)

<table>
<thead>
<tr>
<th>Groups</th>
<th>F-statistic Welch’s test</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>EP day 1</td>
<td>115.2</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>EP day 2</td>
<td>136.8</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>
### B

<table>
<thead>
<tr>
<th>Cell type</th>
<th>EP day 1</th>
<th></th>
<th>EP day 2</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Games-Howell</td>
<td>Cohen's d</td>
<td>Games-Howell</td>
<td>Cohen's d</td>
</tr>
<tr>
<td>HUVEC - FB</td>
<td>$p = 0.001$</td>
<td>13.4</td>
<td>$p &lt; 0.001$</td>
<td>19.8</td>
</tr>
<tr>
<td>HUVEC - CAL-27</td>
<td>$p = 0.001$</td>
<td>11.2</td>
<td>$p = 0.001$</td>
<td>14.7</td>
</tr>
<tr>
<td>HUVEC - SCC4</td>
<td>$p = 0.007$</td>
<td>9.4</td>
<td>$p = 0.016$</td>
<td>NS</td>
</tr>
</tbody>
</table>
Discussion

For patients with HNSCC or NMSC both the disease and the treatment can cause significant functional impairment and unwelcome changes in physical appearance. In the last decades there has been a tremendous progress in the knowledge about the molecular basis of cancer as well as in diagnostics and treatment (68, 76). Improvements in diagnostic radiology like PET-CT and diffusion MRI could lead to earlier detection of both primary tumors and recurrent disease. Intensity modulated RT, functional neck dissections, transoral robotic surgery and free flap reconstruction have decreased the treatment related morbidity.

However, much remain to be achieved. For example, while the survival in oropharyngeal cancer patients has increased significantly in the last decades in Sweden the survival rate in patients with tongue cancer has increased only modestly (82). Despite an increased knowledge about the molecular basis of HNSCC and NMSC, surgery and RT remain the cornerstones of treatment. There is, however, a need for more safe and efficacious treatment modalities since field cancerization and cancer stem cells increases the risk of second primary tumours and recurrence in previously treated sites (84, 86).

Methodological limitations and strengths

Papers I, II, III and IV

The study design (phase II trial) did not include control groups treated with the standard modalities (surgery and RT). The study groups consisted of 6, 15 and 5 patients, respectively and there were four different cancer types investigated. Obviously, the group sizes, the different tumors involved and the lack of control groups limits the conclusions that can be made. For instance, no direct comparison to surgery and RT could be made. In Paper IV the subgroups assessed for QoL were too small to find all potential significant outcome differences. The significant differences found, on the other hand, were probably large enough to be clinically relevant.

One consequence of treatment with ECT is the lack of a histological specimen. Therefore, potentially important prognostic factors like the depth of infiltration and perineural invasion cannot be adequately determined. It can have potential consequences for the results presented in this study since the depth of infiltration was estimated, not measured, leading to potential misclassification and bias in selection of patients for adjuvant
The adjuvant RT treatment also limits the assessment of efficacy to the patients treated with only ECT. The strengths of these studies were the curative intention, the standardized treatment and follow-up protocols and the length of the follow-up periods (24 and 60 months). No patient was lost to follow up. Also, there have been no selective reporting; the outcome in all treated patients was reported. This was also one of the reasons reason for including Papers I and II in two meta-analyses of ECT efficacy (96, 99).

**Paper V**

There were only three cells groups investigated (SCC, fibroblasts and endothelial cells). There were two SCC cell lines and only one fibroblast and one endothelial cell line. The outcome might be different if other cell lines had been used. The investigation ended 4 days after ECT. Perhaps the long-term outcome would have been different if the experiment had continued since the BLM mediated mitotic cell death is a slow process. The strengths of the study were that two independent methods for cell survival assessment and a conservative statistical test (Welch’s F test) were used. Also, a conservative significance level was used ($\alpha \leq 0.01$). Cohen’s $d$ was calculated for the significant survival differences also indicating the size of these differences.

**Efficacy of ECT**

The previously reported efficacy of ECT (89-92) has been confirmed by this thesis. In Paper I there was a CR in 4/6 patients with NMSC two years after mono modality treatment with ECT (Table 7) (97). Moreover, ECT was an organ sparing treatment in two patients with periocular BCC (Paper I). However, in one of these patients the tumor recurred 2.5 years after ECT and was treated with exenteration of the orbit and RT. The tumor (metatypical BCC) had a long history of recurrence and in this context a delay before exenteration of 2.5 years could actually be considered a success. Salwa et al have reported CR in 3 patients with periocular BCC using the ESOPE protocol. However, the follow-up period in that study was only 5-8 months (104).

In paper IV the, to our knowledge, largest case series (19 patients) with the longest follow-up period (five years) of curative ECT in head and neck cancer was reported. Six patients, five with oral tongue cancer and one with tongue base cancer, were cured by ECT alone (Table 12). Four of these patients had T2 tumors and the histopathology was HNSCC in five patients and an adenocarcinoma in one patient. In addition, six patients
were cured by ECT and adjuvant RT and none of the nineteen patients had any local recurrences (Table 12). The adjuvant dose was 57.8 Gy, which was lower than the dose for macroscopic cancer (68-70 Gy). Therefore, the five-year local control in these patients was probably a combined effect of ECT and RT. There is a need for further investigation since ECT in combined modality treatment has not been extensively reported (124, 125).

The tumor specific five-year survival reported in paper IV was 75% and the over-all survival was 63.1%. This is in agreement with previously reported five-year survival in patients with T1 and T2 tongue cancer treated with surgery and/or RT (62-83.5 %) (126-128).

The outcome in Paper IV (100 % CRR) compares favourably to the outcome reported in the previously largest studies of head and neck cancer treated with curative ECT. In 2001 Allegretti et al reported a series of 14 patients with T1-T4 head and neck cancer treated with ECT with IT BLM. A 42.9% CRR was reported in the median 31.5 month follow-up period (110). In 2014 Campana et al reported a 58.3 % CRR in a retrospective study of 12 patients with oral cavity or oropharyngeal cancer (113). The median follow-up period in that study was 14 months. An important reason for the better outcome seen in our study (Paper IV) could be that the BLM dose was higher due to differences in calculation of the treatment volume; a 1 cm margin was included in the formula used in this thesis (see Methods). The difference in outcome could probably also be explained by the more advanced tumors (T3, T4) included in Allegretti’s study compared to our study of only T1 and T2 tumors (Paper IV).

In 2014 Mali et al published two papers on the efficacy of ECT (96, 99). The first was a meta-analysis of treatment efficacy related to tumor type and the administration route of the drug used. Papers I and II in this thesis were included. IT administration was significantly more effective than IV administration. No differences, however, could be found between cisplatin and BLM (96). The other study was a meta-analysis investigating the relationship between tumor size and ECT efficacy; Paper I in this thesis was included (97). There was a statistically significant lower response rate for tumors ≥3 cm. If anything, the results of the study suggests that the ESOPE guidelines that recommends lower doses of BLM in larger tumors (250 IU/cm³) should be re-evaluated (100).

Eight of 26 patients treated in this thesis had tumors ≥3 cm (Papers I-IV). The local control was 100% in these patients but only two were treated with ECT alone. Considering the very good long-term results re-
ported in this thesis the BLM dose used (1000 IU/cm³ tumor volume) and the calculation of the treatment volume should be further evaluated especially in ECT with curative intention.

Only one patient in this thesis was treated with palliative intention; the patient with a metastasis of renal clear cell carcinoma in the masseter muscle (Paper III). That patient was successfully treated with ECT after palliative RT had failed.

Although it is somewhat rewarding to experience that a method under investigation actually seems to work it is just as important to analyse why and when it doesn’t. In Paper I a patient with CSCC in the external meatus had a persistent tumor after two ECT treatments and was eventually treated with salvage surgery and RT (Table 7). We know that the stratum corneum have a very low conductivity resulting in very high electrical fields in EP. The result could be that the electrical fields in the underlying cartilage are too weak to cause EP. Whatever the reason, treatment in the external meatus cannot be recommended.

Safety of ECT

This thesis has also confirmed ECT as a safe treatment with some exceptions. The EP system (Medpulser) that was used was reliable; all treatments could be completed successfully. Treatment of patients with NMSC (Paper I) and oral tongue cancer (Paper II) was safe with two SAEs in 21 patients (pain and an epileptic seizure) (97, 98). However, in the five patients with cancer in the floor of mouth, bucca and base of tongue there were five SAEs; two ORN, a mucocutaneous fistula, a nearly fatal bleeding and aspiration (Paper III). Both cases of ORN and the fistula required secondary reconstructive surgery. ORN is, of course, caused by RT and this study (Paper III) is obviously very small but none of the 10 patients with oral tongue cancer that received the same RT dose developed ORN (Paper II).

Furthermore, in a retrospective study of 189 patients treated with RT there were no reported cases of ORN in patients that had received the lowest doses, 60-65 Gy (129). The patients in this thesis were treated with an even lower dose 57.8 Gy (Paper II-IV). This might suggest that ECT can be a risk factor for ORN in patients that will receive RT, especially in proximity to the mandible (Paper III). ECT with BLM has a well-documented vascular disrupting effect that may have a role in the pathogenesis (46, 47). There is limited reporting of complications with curative ECT in head and neck cancer. Allegretti et al reported a pharyngocutaneous
fistula and Campana reported mycositis and ulceration after treatment (110, 113).

**Functional and quality of life outcome after ECT**

The patient with a recurring CSCC in the proximity of the facial nerve and parotid duct was successfully treated with ECT (Paper I). There were no signs of facial nerve injury or parotid swelling in the follow-up period. Surgery would almost certainly have led to facial nerve injury.

The patients treated for periocular BCC experienced increased tear fluid a few weeks after treatment but no visual impairment (Paper I). The functional outcome was also very good one year after ECT in the patients with tongue cancer reported in Paper II (Table 9) (98). The patients treated with ECT alone had maximal scores in all three PSS-HN parameters (eating in public, understandability of speech and normalcy of diet) both before and after treatment (Paper II). In the patients with cancer in the floor of mouth, bucca and tongue base reported in Paper III the functional outcome was poor (Table 11). The PSS-HN assessments were worse in all parameters one year after treatment in three of the four surviving patients.

The QoL assessments in the head and neck cancer patients showed a statistically significant increase in problems with sense (taste, smell), speech, mouth opening and xerostomia one year after treatment (Paper IV) (Table 13). These differences were probably caused by RT, not ECT in itself. When comparing three pair of patient subgroups there were a significantly worse outcomes in swallowing in the patients treated with ECT and RT compared to the patients treated with ECT alone, in speech problems in patients that smoked during treatment and in need for painkillers in the patients with oral cavity cancer other than tongue cancer (Table 13). Considering the small group sizes these significant differences probably have to be clinically significant. However, because of the overlap in smoking habits, tumor location and RT exposition in these small groups the results should be with interpreted with caution.

In summary, while ECT seems to be a safe, efficacious treatment with very good functional outcome in NMSC (Paper I) and oral tongue cancer (Paper II) it seems to be less safe in the floor of mouth, bucca and base of tongue (Paper III). The functional outcome was also poor in these patients (Paper III). One conclusion drawn is that for these patients, ECT was not the preferred treatment. With surgical resection and primary reconstruction, these patients in all probability would have had a better outcome. The long-term results in the patients with oral tongue cancer make a
strong case for further investigation of ECT as a curative treatment in T1 and T2 primary tongue cancer (Paper IV).

**Selectivity of ECT**

One of the most important features of ECT is its proposed selectivity. This is especially important in head and neck cancer where sparing of normal tissue often means spared function. There are many theoretical reasons for selectivity. The cell radius is important. If we recall the equation for induced membrane potential the $V_{rev}$ needed for EP is inversely proportional to the cell radius. Since an axon is one tenth of the usual cell radius, EP of the axon would require an electrical field 10 times higher than the cancer cells. This is one selective mechanisms of ECT and could probably explain the lack of facial nerve damage in the patient treated for a CSCC in the cheek (Paper I).

Also, the mitotic cell death seen with BLM is selective towards non-dividing cells (29). Proliferative cells also have lower membrane potentials and are therefore easier to electroporated (Figure 4) (9). Tumor tissue has more irregular vasculature and the antivascular effects of ECT could possibly have a selective effect on tumors with high metabolism (50). There is also some evidence that the immune system could be involved in the effect of ECT (57, 59).

In paper V the possibility of a selective effect of ECT on cell survival between different cell-types was investigated (Figures 19 and 20, Tables 14 and 15). Two significant selective effects were found. The survival of the fibroblasts was significantly higher than the survival of SCC cells and endothelial cells four days after ECT (Figure 19, Table 14). The survival of the endothelial cells was significantly lower than in the fibroblasts and the SCC cells one day after EP alone (Figure 20, Table 15). This suggests that the endothelial cells could have important roles in both the reversible “vascular lock” effect of EP and the irreversible “vascular disrupting” effect of ECT (47, 48, 50). There was no difference in survival between the endothelial cells and the cancer cells after ECT. Endothelial cells also seem to play an important supporting and promoting role for cancer stem cells (88). Hypothetically, ECT could therefore possibly have effects, both direct and indirect, on cancer stem cells. The results presented in Paper V leads to a more complete understanding of ECT and its effect in different cell types. More research is needed to confirm or reject these results in other cell-types. This will hopefully lead to more selective ECT protocols in the future.
**Costs**

The patients in this thesis were all treated in the same setting, under general anaesthesia and admitted in our ward. Because of the few patients treated and the many locales involved the only group that could be used in comparisons were the 15 patients with oral tongue cancer.

The costs can be divided into treatment time in the operating room, admission, number of revisits and the cost for the materials and the pharmacological cost. The treatment time for ECT in tongue cancer is similar to surgery with monopolar diathermia, currently the usual treatment in our department. The admission time was, at least for the first patients, longer than in patients treated with surgery, probably due to our inexperience with this modality.

In the last patients treated the admission times were about the same as for surgery, two to four days. For patients with tongue cancer treated with surgery there is usually only one revisit in the first month after treatment. In the ECT patients, however, there were approximately two to three revisits in the same period. Again, this was done, in part, to study the treatment effect but also to remove necrotic tissue in many of the patients.

The cost of BLM and the needle electrodes for the Cliniporator system currently used is approximately 5 000 SEK per treatment which is a little more than the cost of surgery with monopolar diathermia per treatment. It is fairly obvious that the cost-benefit for treating uncomplicated NMSC with ECT, usually done in an out-patient setting, is not feasible. However, with the 5000 Hz electroporators available today there is possible to treat NMSC in local anaesthesia thereby reducing the costs considerably.

**Treatment recommendations**

So where, if at all, does ECT fit into the armamentarium in the treatment of head and neck cancer? New modalities must be compared to surgery and RT, preferably in randomized control trials, and proven to be at least as safe and efficient to become first line treatments. The situation is different in recurrent head and neck cancer where additional RT or surgery may not be possible or would result in very significant morbidity. In these cases alternative modalities can be especially important. These alternative treatments must of course have been proven to be reliable, safe and efficient. However, the level of evidence needed to become a first line treatment could take years in multicentre studies and this should not exclude patients from safe and efficient treatments, especially when surgery and RT are no longer useful options.
To put ECT in a useful context the author has made a list of when and where ECT could, should and should not be used. This is, of course, a somewhat subjective list based in part on evidence, available treatment alternatives, side effects and the subjective opinion of the author. In all cases there is an underlying assumption that the tumor with adequate margins is fully accessible to the drug and the electrodes.

**When to recommend ECT as a first line of treatment:**

- In palliative treatment of multiple cutaneous metastases with diameters < 3 cm using the ESOPE protocol (protocol 1). There are numerous studies showing the efficacy of ECT in this setting and there are no good treatment alternatives (130, 131).

- In palliative treatment of recurrent NMSC and HNSCC after multimodality treatment where additional surgery is not feasible. If the tumor diameter > 3 cm, the ECT protocol in this thesis should be used (protocol 2). If tumor diameter < 3 cm protocol 1 should be used.(99, 132, 133).

**When to recommend ECT as an alternative to surgery:**

- In primary NMSC with protocol 2 as an organ and function-sparing treatment, especially in BCC in the proximity of the orbit where the alternative is exenteration (with eye muscle involvement but without involving the globe) or in proximity of the facial nerve (97, 104).

- In other primary/recurrent NMSC with protocol 2 in areas with high risk of recurrence (“mask areas” of the face) or with ill-defined tumor borders.

- In primary T1 and T2 oral tongue cancer with ill-defined borders in patients previously treated with RT. Protocol 2 should be used.
When not to recommend ECT:

- In tumors with bone invasion.
- In well-defined NMSC not in the proximity of the facial nerve or the orbit.
- In tumors in the floor of mouth, bucca and base of tongue especially if the patient will receive or have previously received RT.
- In tumors involving the cartilaginous external meatus.

Future perspectives
There are physical limitations to ECT treatment in head and neck cancer. The drug must be reliably administered and the tumour area has to be accessible with electrodes (39). With the limited access of the currently available applicators this excludes treatment of tumours in the oropharynx, the hypopharynx and the larynx. Instead treatment is currently limited to the more accessible parts of the oral cavity. A reason for the lack of suitable applicators could be that, so far, mostly medical oncologists have been involved in the research, development and treatment. The technology has, basically, been developed for palliative treatment of skin metastasis.

This is not a critique of those dedicated oncologists but of head and neck surgeons that so far has shown little interest in the technique and its applications. With more surgeons involved the curative potential of ECT could be investigated more fully and the demand for a wider variety of applicators would increase.

If ECT will have a future as a treatment modality in primary head and neck cancer depends on not yet conducted studies comparing its efficacy and functional outcome to surgery and RT. Based on the results in this thesis the following future studies are proposed:

- A randomized controlled trial of ECT vs surgery in primary T1 and T2 tongue cancer.
- A randomized controlled trial of ECT vs surgery with adjuvant RT in advanced stage tongue cancer (T3, T4).
- A randomized controlled trial of ECT vs surgery in parotid cancer without facial nerve paralysis.
The long-term data presented in this thesis could hopefully increase the interest in ECT as a curative treatment modality and facilitate the realisation of these and many more studies.
Conclusions

- ECT seems to be an efficacious and safe curative treatment for non-melanoma skin cancer in the head and neck area where it can be an organ and function-sparing treatment.

- ECT seems to be an efficacious and safe curative treatment with a very good functional outcome in oral tongue cancer where it should be further investigated as a potential primary treatment.

- ECT with adjuvant RT seems to be an efficacious treatment of oral cavity cancer in the floor of mouth and bucca. In these localizations, however, the safety and functional outcome can be poor.

- There is evidence of survival selectivity in different cell types after ECT in vitro. Fibroblasts seem to have a significantly increased survival when compared to human SCC cells and endothelial cells. In addition, endothelial cells seem to have a significant survival decrease after EP alone.
Acknowledgements

My sincere gratitude to all of you that have helped me with this thesis. I would especially like to thank the following:

- Professor Claes Möller for guiding me through this thesis like a partner, not a parent. Thank you!
- Lennart Löfgren, for introducing me to ECT.
- Johan Reizenstein for your constructive skepticism.
- Mathias von Beckerath, for your contributions to this thesis and for being a friend.
- Hans Gustafsson, for always telling the painful truth.
- Mikael Ivarsson and Anita Koskela, for all your support and patience in the laboratory.
- Stefan Kristiansson, for your words of encouragement.
- Gun-Britt Adamsson for all your help with the data collection. You will be missed.
- To all my colleagues in the ENT department, without your support this thesis would not have been possible.
- To all colleagues and nurses involved in the treatment and follow-up of the patients in this thesis.
- To all my colleagues in the Head and Neck Oncology Centre.
- To all the patients that chose to participate in these studies. Without you there will be no progress.
• My parents Gunnar and Marie, for being my role models.

• My sister Cecilia, for all your good advice.

• Anna, for being in my life. I love you.

• Noah, Gabriella, Elias and Mikaela, you make everything worthwhile. Thank you for your patience during the last month. Now I want to spend my time with you again!
References


11. Chen C, Smye SW, Robinson MP, Evans JA. Membrane electro- 
poration theories: a review. Medical & biological engineering & 


13. Burgain-Chain A SD. DNA Electrotransfer: An Effective Tool for 
Applications 2013.

14. Tekle E, Astumian RD, Chock PB. Electro-permeabilization of cell 
membranes: effect of the resting membrane potential. Biochem Bio-

15. Zimmermann U, Pilwat G, Riemann F. Dielectric breakdown of cell 

16. Gabriel B, Teissie J. Direct observation in the millisecond time range 
of fluorescent molecule asymmetrical interaction with the electropo-

17. Saulis G SR. Comparison of electroporation threshold for different 

18. Teissie J, Rols MP. An experimental evaluation of the critical poten-
tial difference inducing cell membrane electroporemeabilization. Bi-

Kinetics, statistics, and energetics of lipid membrane electroporation 
studied by molecular dynamics simulations. Biophysical journal. 

20. Dong Z, Saikumar P, Weinberg JM, Venkatachalam MA. Calcium in 

21. Deipolyi AR, Golberg A, Yarmush ML, Arellano RS, Oklu R. Irre-
versible electroporation: evolution of a laboratory technique in inter-
ventional oncology. Diagnostic and interventional radiology (Ankara, 


Appendix 1

PERFORMANCE STATUS SCALE FOR HEAD & NECK CANCER PATIENTS - PSS-HN

Suggestions for Administration

These performance scales may be rated by health professionals (e.g., physicians, nurses, nutritionists) or other personnel (e.g., clerks, data managers). Ratings are determined through use of an unstructured interview format.

Normalcy of Diet

Begin by asking the patient what kinds of foods (s)he has been eating. Ask what foods are difficult to eat. Based on the patient's response, choose an item at the low end of the scale. Move up the scale giving examples of foods in each category and asking the patient if (s)he is eating those food items. Even if the patient says that (s)he eats everything, inquire about specific items beginning with 50, soft chewable foods and moving upwards. Stop at the item at, and above which the patient cannot eat. The patient then receives the score below that. If the patient indicates that (s)he is eating a full diet, also inquire whether (s)he needs to drink more liquids than usual with meals; eating a full diet with intake of extra fluids is scored 90. If the patient can take foods orally, but is also using a feeding tube, score based on solid food.

Public Eating

Score the Public Eating scale by asking the patient where (s)he eats (in a restaurant, at home, at friends/relatives' homes, etc.) and with whom (s)he eats (always alone, with family/friends, etc). Ask patient if (s)he chooses different foods (softer, less messy, etc.) when eating with others. When was the last time the patient ate in a restaurant, cafeteria, MacDonald's, picnic, family reunion? Choose the score beside the description that best fits the patient. A patient on a restricted diet, (e.g., tube feeding, pureed foods) who does not eat in public but will join others in a public eating setting should be rated 75. Score 999 for inpatients.

Understandability of Speech

This scale is scored based on the interviewer's ability to understand the patient during conversation (in this case, based on conversation about patient's diet and social activities). Choose the score beside the description that best fits the patient. See if you can understand the patient if you are looking away while (s)he is talking.

Special Considerations for Inpatients: Administration of the PSS-HN varies somewhat for inpatients. Score the Normalcy of Diet and Understandability of Speech Scale as indicated. The Eating in Public Scale is not applicable as inpatients generally have little opportunity to eat with others or leave their hospital rooms. Inpatients receive a score of 999 on the Eating in Public Scale.
## PERFORMANCE STATUS SCALE FOR
HEAD AND NECK CANCER PATIENTS: PSS-HN

---

**Normalcy of Diet / / /**

<table>
<thead>
<tr>
<th>Score</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>Full diet (no restrictions)</td>
</tr>
<tr>
<td>90</td>
<td>Full diet (liquid assist)</td>
</tr>
<tr>
<td>80</td>
<td>All meat</td>
</tr>
<tr>
<td>70</td>
<td>Raw carrots, celery</td>
</tr>
<tr>
<td>60</td>
<td>Dry bread and crackers</td>
</tr>
<tr>
<td>50</td>
<td>Soft chewable foods (e.g., macaroni, canned/soft fruits, cooked vegetables, fish, hamburger, small pieces of meat)</td>
</tr>
<tr>
<td>40</td>
<td>Soft foods requiring no chewing (e.g., mashed potatoes, applesauce, pudding)</td>
</tr>
<tr>
<td>30</td>
<td>Pureed foods (in blender)</td>
</tr>
<tr>
<td>20</td>
<td>Warm liquids</td>
</tr>
<tr>
<td>10</td>
<td>Cold liquids</td>
</tr>
<tr>
<td>0</td>
<td>Non-oral feeding (tube fed)</td>
</tr>
</tbody>
</table>

**Public Eating / / /**

<table>
<thead>
<tr>
<th>Score</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>No restriction of place, food or companion (eats out at any opportunity)</td>
</tr>
<tr>
<td>75</td>
<td>No restriction of place, but restricts diet when in public (eats anywhere, but may limit intake to less &quot;messy&quot; foods (e.g., liquids)</td>
</tr>
<tr>
<td>50</td>
<td>Eats only in presence of selected persons in selected places</td>
</tr>
<tr>
<td>25</td>
<td>Eats only at home in presence of selected persons</td>
</tr>
<tr>
<td>0</td>
<td>Always eats alone</td>
</tr>
<tr>
<td>999</td>
<td>Inpatient</td>
</tr>
</tbody>
</table>

**Understandability of Speech / / /**

<table>
<thead>
<tr>
<th>Score</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>Always understandable</td>
</tr>
<tr>
<td>75</td>
<td>Understandable most of the time; occasional repetition necessary</td>
</tr>
<tr>
<td>50</td>
<td>Usually understandable; face-to-face contact necessary</td>
</tr>
<tr>
<td>25</td>
<td>Difficult to understand</td>
</tr>
<tr>
<td>0</td>
<td>Never understandable; may use written communication</td>
</tr>
</tbody>
</table>

---

Appendix 2

**EORTC QLQ - H&N35**

Patients sometimes report that they have the following symptoms or problems. Please indicate the extent to which you have experienced these symptoms or problems during the past week. Please answer by circling the number that best applies to you.

<table>
<thead>
<tr>
<th>During the past week:</th>
<th>Not at all</th>
<th>A little</th>
<th>Quite a bit</th>
<th>Very much</th>
</tr>
</thead>
<tbody>
<tr>
<td>31. Have you had pain in your mouth?</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>32. Have you had pain in your jaw?</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>33. Have you had soreness in your mouth?</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>34. Have you had a painful throat?</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>35. Have you had problems swallowing liquids?</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>36. Have you had problems swallowing pureed food?</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>37. Have you had problems swallowing solid food?</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>38. Have you choked when swallowing?</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>39. Have you had problems with your teeth?</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>40. Have you had problems opening your mouth wide?</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>41. Have you had a dry mouth?</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>42. Have you had sticky saliva?</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>43. Have you had problems with your sense of smell?</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>44. Have you had problems with your sense of taste?</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>45. Have you coughed?</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>46. Have you been hoarse?</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>47. Have you felt ill?</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>48. Has your appearance bothered you?</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
</tbody>
</table>

*Please go on to the next page*
### During the past week:

<table>
<thead>
<tr>
<th></th>
<th></th>
<th>Not at all</th>
<th>A little</th>
<th>Quite a bit</th>
<th>Very much</th>
</tr>
</thead>
<tbody>
<tr>
<td>49.</td>
<td>Have you had trouble eating?</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>50.</td>
<td>Have you had trouble eating in front of your family?</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>51.</td>
<td>Have you had trouble eating in front of other people?</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>52.</td>
<td>Have you had trouble enjoying your meals?</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>53.</td>
<td>Have you had trouble talking to other people?</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>54.</td>
<td>Have you had trouble talking on the telephone?</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>55.</td>
<td>Have you had trouble having social contact with your family?</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>56.</td>
<td>Have you had trouble having social contact with friends?</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>57.</td>
<td>Have you had trouble going out in public?</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>58.</td>
<td>Have you had trouble having physical contact with family or friends?</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>59.</td>
<td>Have you felt less interest in sex?</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>60.</td>
<td>Have you felt less sexual enjoyment?</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
</tbody>
</table>

### During the past week:

<table>
<thead>
<tr>
<th></th>
<th></th>
<th>No</th>
<th>Yes</th>
</tr>
</thead>
<tbody>
<tr>
<td>61.</td>
<td>Have you used pain-killers?</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>62.</td>
<td>Have you taken any nutritional supplements (excluding vitamins)?</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>63.</td>
<td>Have you used a feeding tube?</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>64.</td>
<td>Have you lost weight?</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>65.</td>
<td>Have you gained weight?</td>
<td>1</td>
<td>2</td>
</tr>
</tbody>
</table>
Publications in the series
Örebro Studies in Medicine


35. Söderqvist, Fredrik (2009). Health symptoms and potential effects on the blood-brain and blood-cerebrospinal fluid barriers associated with use of wireless telephones.


41. Gustafsson, Sanna Aila (2010). The importance of being thin – Perceived expectations from self and others and the effect on self-evaluation in girls with disordered eating.

42. Johansson, Bengt (2010). Long-term outcome research on PDR brachytherapy with focus on breast, base of tongue and lip cancer.

43. Tina, Elisabet (2010). Biological markers in breast cancer and acute leukaemia with focus on drug resistance.


46. de Leon, Alex (2010). *Effects of Anesthesia on Esophageal Sphincters in Obese Patients.*


52. Loiske, Karin (2011). *Echocardiographic measurements of the heart. With focus on the right ventricle.*


64. Nordin Olsson, Inger (2012). Rational drug treatment in the elderly: "To treat or not to treat".


67. Thuresson, Marie (2012). The Initial Phase of an Acute Coronary Syndrome. Symptoms, patients’ response to symptoms and opportunity to reduce time to seek care and to increase ambulance use.


75. Gustavsson, Anders (2012): Therapy in Inflammatory Bowel Disease.


83. Lönn, Johanna (2013): The role of periodontitis and hepatocyte growth factor in systemic inflammation.


96. Sundh, Josefin (2013): Quality of life, mortality and exacerbations in COPD.


98. Palmetun Ekbäck, Maria (2013): Hirsutism and Quality of Life with Aspects on Social Support, Anxiety and Depression.


109. Törös, Bianca (2014): Genome-based characterization of Neisseria meningitidis with focus on the emergent serogroup Y disease


