Biological identification of recurrence after curative intended radiotherapy for carcinoma of the head and neck. A DAHANCA 19 study.

What we want to do

Recurrence of head and neck cancer has a poor prognosis. Most recurrences are found locally in the initial highly irradiated area. By analyzing biological characteristics of the primary tumors, we want to identify which tumors that tend to recur locally. These tumors could potentially be offered more intensive radiotherapy.

Aim

The aim of the present study is to biologically identify squamous cell carcinomas of the oral cavity, pharynx and larynx that tend to recur in the high-dose volume despite of primary treatment with curatively intended radiotherapy.

Hypothesis

We hypothesize that we, based on the selected markers, can construct a biological profile that can identify the tumors that tend to recur in the high-dose radiation volume.

Primary endpoint

Five-year loco-regional tumor control will be used as the primary endpoint.

Background

Head and Neck Squamous Cell Carcinomas (HNSCC) is a loco-regional disease that tends to recur in the high-dose radiation volume, despite treatment with primary curatively intended radiation doses. Recently, we demonstrated in a multi-center study in a cohort of more than 1500 patients that, depending on the treatment margins used, 75-93% of all T-site recurrences were covered by at least 95% of the prescribed radiation dose to the tumor tissue [1].

In other words, despite high-dose radiotherapy (RT), the tumors tend to recur from the highly irradiated sites. Upon recurrence only around 1/3 of these patients are eligible for curative intended treatment.

Some of these HNSCC are persistent after primary RT (i.e. failure prior to two months after RT), whereas the remainder mostly recur within 1-1½ year after end of treatment [2]. The reason for this relative radioresistance is at present only partly known but can be related to

tumor hypoxia, presence of clonogenic radioresistant cancer stem cells, high tumor cell proliferation or malfunction of p53. Contrarily, p16 – a pseudomarker for HPV-infection – is indicative of more radiosensitive tumors, hence better prognosis [3].

Hypoxia is a common feature of the microenvironment in HNSCC and all solid head and neck carcinomas are more or less hypoxic. Hypoxia is associated with resistance to RT, reduced therapeutic response, and a poor clinical outcome [4, 5]. Although, hypoxia is counteracted using the hypoxic radiosensitizer, nimorazole, hypoxia might also influence other factors responsible for tumor control, like the p53 protein level and the radiosensitivity of stem cells [6]. Recently, it was possible to characterize the hypoxic tumors using a 15-gene hypoxic classifier [7] with both prognostic and predictive abilities.

Clonogenic cancer cells or cancer stem cells are tumor cells with an unlimited potential of self-renewal and therefore high quantity of stem cells are correlated to radioresistance [3, 8]. Cancer stem cells cannot (so far) be specifically identified, but it is suggested that they can be characterized by the expression of CD44, MET and SLC3A2. The density of cancer stem cells seems to be crucial for increased radioresistance [8] and it is suggested, that hypoxia can even further promote the number of cells with a stem-like phenotype [9]. The presence of these stem cell markers and large tumor volume have a negative prognostic impact on tumor control after curatively intended primary chemo-radiotherapy. HPV p16-negative tumors, high cancer stem cell density and a more hypoxic profile is correlated with a poor prognosis [6, 10].

Reduction in overall treatment time of RT improves loco-regional tumor control and disease-free survival [2], but the HNSCC tumors that benefit most are the ones with high expression of EGFR in combination with well/moderate tumor differentiation possibly by reducing the time available for accelerated repopulation [11]. It is therefore hypothesized that tumor cell proliferation indicated by EGFR and other proliferation markers might play a role for recurrences – probably not by themselves but in combination with other factor factors [12]. This proliferation is likely to be further accelerated by functional mutations in p53 affecting cell cycle control resulting in decreased tumor control [13]. In addition, there is growing evidence that treatment sensitivity is affected by genetic alterations and mutational events in genes other than p53. This was confirmed in a recent German multi-center study, based on targeted next-generation DNA sequencing (NGS) [14].

Whereas most of the mentioned factors reduces radiosensitivity, overexpression of p16 as a pseudomarker of HPV infection markedly increase radiosensitivity by abrogating p53 and pRb tumor suppressor functions, which ultimately leads to uncontrolled cell cycle progression. However, this is dependent on tumor location to the oropharynx and affected by p53 protein status and smoking-related hypoxia [15-17].

The interplay between these markers is complex and the overall aim of the study is to biologically identify the patients that are prone to recur locally despite appropriate radiation coverage of the tumor volume.

An ideal scenario for exploring the possible impact of the above mentioned factors are the randomized, international multi-center study DAHANCA 19 [18] conducted by the Danish Head and Neck Cancer group, DAHANCA, in collaboration with The Norwegian Radiumhospital in Oslo.

The study was conducted with full GCP monitoring for the entire period of the study (including follow-up) and with central quality assurance monitoring of all treatment planning plans. The aim was to determine if adding zalatumumab to primary treatment would improve loco-regional control. From 2007-2012, 608 patients with HNSCC were enrolled in the study and eligible for evaluation. In brief: patients were treated with primary (chemo-) radiotherapy 66-68Gy/33-34fx, 6fx/wk, weekly cisplatin 40mg/sqm (if tumor were locally advanced) and further randomized to the fully human EGFR-inhibitor zalutumumab or control (Figure 1). The study showed no benefit of adding zalutumumab to (chemo-)radiotherapy in terms of 5-year loco-regional control, disease specific survival or overall survival. During the 5-years period of follow up, we have identified 163 loco-regional recurrences among the 608 eligible patients. All relevant parameters are available and GCP-validated in the DAHANCA research database. Follow up on recurrences and death is continued.

Research plan

Treatment plan evaluation and recurrence CT-scans: The RT treatment plans of the patients are already uploaded in the national DcmCollab doseplan bank. The doseplan bank applied in this study is based on a secure computer network and a secure web-based user interface. No local installation of software is required for the participating centers to upload their dose plans for central evaluation. The platform facilitates storing, arranging, analyzing and sharing of digital RT dose plans and furthermore, it supports the extraction of predefined DVH parameters, mean dose and structure volumes [19]. From these plans, we can calculate primary tumor volume to be used for the cancer stem cell marker evaluations. We will also collect the recurrences scans from the 163 recurrences and upload them to the dose plan bank as well.

Co-registration of scans: The treatment plans will be deformable co-registered with the recurrence scans, using the MIM software (MIM Software Inc. Cleveland, Ohio, USA) previously used by our group[23], in order to identify whether the recurrence is actually within the high-dose irradiation field (the 95% isodose margin) or not. The method for

recurrence point of origin analysis will be automated center of mass analysis. Distance from the high dose field to point of origin of recurrence will be calculated, and hence considered inside the high dose field if distance≤0. Estimated delivered dose to the original tumor will be evaluated for both the patients with and without loco-regional recurrence. Norwegian scans will be analyzed during a planned 3 months stay at Radiumhospital Oslo.

Collecting of Paraffin-embedded formalin fixed diagnostic tumor tissue: Pre-treatment tumor tissue as well as biopsies from recurrences from the Danish part of the cohort have been collected. The Norwegian tissue is collected and ready for processing. The tissue has be identified using the DAHANCA research database and the pathology registries and it has been validated that the diagnostic biopsies actually are taken from the primary tumor sites.

Tissue sections for DNA/RNA extraction: Histology slides have been processed using standard methods. Prior to other analyses, we have already performed H&E stainings to ensure that the biopsies contains a reasonable amount of tumor tissue. When necessary, we have performed modified microdissection of the tissue specimen to reduce the amount of non-malignant tissue. The formalin-fixed paraffin embedded tissue blocks are currently being cut and stored in Eppendorf tubes at -20 degrees Celsius. Total DNA and RNA will be extracted from two 7-µm formalin-fixed paraffin embedded tissue sections by the Tissue Preparation System with VERSANT Tissue Preparation Reagents (Siemens Healthcare Diagnostics, Tarrytown, New York, USA), a fully automated, bead-based method.

Gene analyses: Expression of target genes in the 15-gene hypoxia classifier (summarized as less or more hypoxic), stem cell markers and EGFR will be established by qPCR (TaqMan Gene Expression assays, Thermo Fisher Life Technologies) in RNA extracted from formalin-fixed, paraffin-embedded tissue from the pre-treatment biopsy. [21] The analyses are done on a routine basis at Experimental Clinical Oncology, Dept. of Oncology.

HPV status will be examined with the INNO-LiPA HPV Genotyping Extra II assay (Fujirebio) for high-risk HPV DNA. Tumors will be considered HPV positive if considered high-risk genes are present.

NGS: In order to identify a custom-made panel of 409 cancer-related genes (incl. p53) and possible cancer-related preparation (Ion AmpliSeq Library Kit 2.0, Thermo Fisher Scientific), amplification, and sequencing (using the Ion 540TM Kit-Chef and the Ion 540TM Chip Kit, Thermo Fisher Scientific) will be carried out [22]. Furthermore, mutations and copy-number variations will be identified.

Data analysis: A validated copy of the DAHANCA 19 research database will be combined with the experimental data and analyzed according to preplanned analyses using SPSS and STATA.

Statistical considerations

This is an exploratory study aiming at development of a biological characterization of tumors that recur in the radiation high-dose volume. The primary endpoint is loco-regional control and time will be calculated from end of RT to date of event or censoring. Survival curves will be estimated by the Kaplan–Meier method and the impact of potential prognostic variables on the primary endpoint will be evaluated using the univariate Cox-regression model. Significant parameters will thereafter be included in multi-variate Cox regression. For the point of origin analysis, a 3 mm spherical margin will be added to the point of origin because of scan co-registration inaccuracy.

The stem cell biomarkers will be evaluated as described in [6]. The results of the hypoxic profiling will be divided into more or less hypoxic tumors according to Toustrup et al [7]. The cohort will be evaluated as a whole and in subgroups divided by hypoxia profile and HPV p16 status. Furthermore, for the NGS data, profiles will be build using unsupervised two-step clustering analyses.

For all analyses, two-sided tests will be performed and p-values<0.05 will be considered statistically significant. Descriptive statistics and parametric/non-parametric tests will be used to describe patient population, tumor and treatment characteristics as well as distribution of biomarkers.

The original DAHANCA 19 study was powered to find a difference of 10% in loco-regional control. For the present retrospective study, we do know the size of the endpoint: 163 failures in 608 patients, but we do not know how the biomarkers eventually will be distributed and cannot estimate how clear they will separate tumors that will recur from tumors that do not recur. Therefore, a power calculation cannot be performed. In case it is possible to model a biological characterization of the tumors with recurrences in the high-dose volume, then we will validate this biomarker combination in an independent but equally well controlled cohort of patients [1] treated in the same way as the patients in DAHANCA 19 (except from zalutumumab).

Feasibility

The DcmCollab doseplan bank has been well functioning for almost 10 years and we have from previous studies the necessary experience in copying treatment plans and diagnostic CT scans into the bank. We have previously tested and successfully evaluated the commercial DIR algorithm in the MIM software package for deformable co-registration [20] and point-of-origin analyses [23]. All biomarker procedures in the present study are already established in the laboratory of Experimental Clinical Oncology, Dept of Oncology in Aarhus.

Perspectives

Today, it is not possible to predict which HNSCC that will recur locally in the high-dose volume despite of curatively intended irradiation to the area. We aim to construct a biological characterization that will predict which tumors that will recur in locally. If the identification of a profile is successful, we will further validate this characterization in an independent cohort of patients treated with primary curative RT from Herlev, Odense and Aarhus University hospitals.

An alternative to standard moderately accelerated RT (66-68Gy/33-34fx, 6fx/wk) is the more intensive treatment with hyperfractionated accelerated RT (76Gy/56fx, 10fx/wk). A pilot study (DAHANCA 28) of this intensive RT regimen in combination with low-dose weekly concomitant cisplatin was performed by the DAHANCA group and showed a marked reduction of local recurrences but at the prize of more acute morbidity and potentially more late morbidity [24, 25].

If we succeed with the present study, then selection of patients based on the biological characterization could predict high risk of local recurrence in the high-dose irradiation field and these patients could be offered hyperfractionated accelerated RT instead of standard moderately accelerated radiation, in order to reduce their risk of local recurrence. This has, however, to be validated in a prospective study first. Summarily, the overall goal is to individualize the treatment of HNSCC prone to recur locally.

International contacts

The study group have longstanding and good working relations with translational groups in the US and Europe, including the German Cancer Consortium, DKTK-ROG, and OncoRay – the National Center for Radiation Research in Oncology in Dresden, where previous studies in stem cell markers have been developed and tested in collaboration with Experimental Clinical Oncology, Dept of Oncology in Aarhus. Future collaboration concerning stem cells as biomarkers are already planned (case-control studies between patients treated with surgery and postoperative RT vs. patients from the present study treated with primary RT).

Furthermore, the department and the DAHANCA group have for many years worked with the collaborative groups in head and neck cancer in Europe and the US.

Implementation in the clinic and patient involvement

The present project is based on a previous performed randomized controlled trial and archival tumor material. However, in case we succeed developing a biomarker selection tool for treatment, this should be tested in a prospective randomized trial before being

implemented as a standard in the clinic. For development of such a study, patient involvement would be highly recommended, as not only there will be a potential increased tumor control but also increased morbidity. This balance and how it can be challenged needs to be discussed with a patient focus group before a possible study can be developed and launched.

Ethics

The project is approved by The Danish Data Protection Agency, The Danish Health Data Board and The National Committee on Health Research Ethics. As this study is retrospective in its nature (although performed on prospective collected material), there will be no contact with patients of cancer survivors.

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Publication strategy

Positive, inconclusive as well as negative results will be presented at scientific meetings and published in peer-reviewed international scientific journals. The authors will follow the Vancouver guidelines.

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Figures

Figure 1