

Treatment failure in squamous cell carcinoma of the anus – real-world data and biological features of recurrences

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Background:

Squamous cell carcinoma of the anus (SCCA) is a rare disease, with an annual incidence of 1-2 per 100.000 habitants in the Nordic countries¹, comprising less than 200 new cases in Denmark per year. The overall survival rate is approx. 67%².

Internationally there are only a few randomized studies investigating treatment in SCCA. The rarity of SCCA necessitate systematic investigation of national data to develop future treatment regimens. In Denmark the treatment of SCCA is centralized in only three oncological departments (Aarhus, Vejle and Herlev), allowing for unique collaboration and data collection, despite our small country.

The primary treatment modality for SCCA is chemo-radiotherapy (CRT)³. High doses of radiotherapy are needed to achieve a complete response. Consequently, there is substantial risk of acute and late side effects in terms of local skin damage, bowel, bladder and bone toxicity in addition to sexual dysfunction and negative effect on patient's quality of life (QoL)^{4 5}. Although the majority of patients are cured by primary therapy, some patients experience treatment failure in the pelvis or systemically to other organs. Both local and distant failure implies a major therapeutic challenge and substantial critical symptoms for the patients.

Standard treatment of pelvic recurrence is extensive salvage surgery with moderate chance of cure (5-Year OS 50-64%), and profound negative effect on patient's QoL. Furthermore, systemic treatment with chemotherapy for inoperable/metastatic disease has sparse effect with only limited number of clinical trials to support treatment decisions. Research to prevent and treat recurrent and advanced SCCA is urgently needed.

There is an obvious lack of useful knowledge of the SCCA biology. However, molecular biological features of SCCA include a strong HPV association, indicating an increased sensitivity for CRT in HPV positive tumors and a positive influence on prognosis⁶. Furthermore, hypoxia is a well-known feature of squamous cell carcinomas, which renders tumors less sensitive to radiation therapy⁷. Recently checkpoint inhibitors has shown promising efficacy in advanced SCCA, indicating a key role of the biological immune system in this disease. The above-mentioned factors are, however sparsely investigated in SCCA and call for further biological and translational studies.⁸

In summary, current treatment decisions are based on clinical staging with no account of the biological differences between tumors that may affect outcomes. There is consequently an obvious lack of data to support more personalized CRT in SCCA. We need predictive and prognostic markers, and data to support optimized treatment options, such as neoadjuvant or concurrent immunotherapy, or new radiation techniques such as proton therapy. Research are needed to lower the risk of treatment failure and improve the outcome.

Patient-derived xenografts (PDX):

PDX are clinical tumor samples implanted directly in immunodeficient mice. PDX is an emerging pre-clinical research model for human cancers. Compared with cancer cell lines or cell line xenografts PDX models may more closely resemble their human original tumors when looking at molecular characteristics and treatment response.^{9 10} PDX models are there for an active platform to study disease biology, treatment and biomarker. PDX models have already been used in oropharyngeal squamous cell carcinoma and have been proven a clinically relevant surrogate model for head and neck cancer.¹¹

Hypotheses:

- There is no real-world data in mapping of local recurrences of SCCA. This will give us important new knowledge in optimizing the treatment strategies
- PDX from SCCA will resemble the original tumor and will be a good preclinical research model for SCCA.
- Molecular biological features of SCCA includes a strong HPV-association indicating an increased sensitivity for CRT in HPV-positive tumors and thereby a positive influence on prognosis.

This PhD project comprises two overall sub studies:

Study 1 – Real-world retrospective data: A large retrospective national dataset from patients having undergone treatment for SCCA to analyze the pattern of failures.

- Reporting of nationwide outcome, and mapping of anatomical location of recurrences in a nationwide data collection from 2000-2018.¹²
- Correlation of recurrences with radiotherapy dose, and building a tumor control probability (TCP) model for recurrence.
- Comparative simulation of alternative treatment planning by IMRT, VMAT and protons
- Simulation of potential re-irradiation by proton beam therapy.

The following questions will be addressed:

- What is the real-world risk of treatment failure?
- What is the pattern of failure in the pelvis?
- How are the failure mapped in relation to radiotherapy dose?
- Would it be possible to increase the dose without increase in side effects by using other radiotherapy techniques?
- Is it technically possible to re-irradiate recurrences without lethal toxicity if using proton therapy, and thereby add to the success of surgery?

Study 2 – Biological data: Experimental studies to explore the underlying biology and risk factors for anal cancer recurrences

- Initiation of a prospective clinical protocol for fresh tissue collection from SCCA in HPV negative and HPV positive tumors
- Establishing 2x5 xenografts with different biological features
- Analyzing radiation sensitivity in tumors with different predefined biological features
- Analyzing radiation sensitivity with hypoxia modification
- Developing biomarker profiles for radiation sensitivity testing of SCCA in vivo

- Validating developed sensitivity markers in a clinical cohort of 400 patients

The following questions will be addressed:

- What characterizes HPV positive and HPV negative tumors in terms of molecular biological features in SCCA?
- What characterizes HPV positive and HPV negative tumors in terms of sensitivity to radiotherapy in SCCA?
- Will testing for hypoxia and other markers in tumors help to predict which tumors are more resistant to radiotherapy, and more at risk for treatment failure?
- Will treatment with hypoxia and others markers in tumors help to predict which tumors are more resistant to radiotherapy, and more at risk for treatment failure.
- Does hypoxia related, HPV related and immune response related biomarkers hold prognostic value in SCCA?

Materials and methods:

Study 1:

Patient material:

- Data from an estimated sample size of approximately 2000 patients diagnosed with SCCA in Denmark during 2000-2018 for which approval to collect data has been granted. Data will be retrieved through national registers and patients' charts.

Statistical analysis plan:

- Primary outcome data in terms of recurrence rate, recurrence free survival and overall survival data will be presented based on KM statistics.
- Prognostic value of pre-treatment and treatment related factors will be analyzed with parametric and non-parametric statistics and cox-regression analysis when appropriate.
- Recurrences will be identified; the number is unknown but estimated to 2-300
- Treatment plans will be collected and mapped with images of the recurrent tumor.
- Tumor control probability (TCP) models will be built by standard statistical methods on recurrent tumors and matching controls
- Planning simulation will compare dose parameters from dose volume histograms (DVH) to the relevant organs at risk (OAR) and target volume parameters by so-called fixed field IMRT, VMAT 2 and 3 arch techniques and proton therapy planning.

Study 2:

Patient material:

- The clinical collection study will include 15 patients newly diagnosed with SCCA. Biopsies will be taken and processed for xenograft establishment. The clinical set-up at AUH allows for collection of fresh biopsies at primary staging during anesthesia (standard routine), for which a clinical protocol will be established.

Laboratory methods:

- The tumor xenografts will be established in mouse models by methods established in Department of experimental clinical oncology (ECO) AUH, for other squamous cell carcinomas. Radiation response will be established with a tumor growth delay assay, and will be correlated to the impact of the relevant biological factors, such as HPV and hypoxic level, and immune parameters. The ECO is an established radiobiology

laboratory, with yearlong experience with experimental radiobiology and employing xenografts models. ECO's facilities include all the necessary equipment for radiobiological studies including mouse facilities and an experimental radiation machine.

- The gene expressions levels in the hypoxia profile and two cancer stem cell markers will be measured in RNA extracted from sections of the formalin fixed paraffin embedded diagnostic biopsy. This method is routinely used at ECO and is tested in a small pilot cohort of SCCA to assess that technically RNA from the 20 involved genes can be measured in the material.
- Further markers will be analyzed at the DNA, RNA and protein levels.
- Markers will be validated in a retrospective cohort of 200 recurrences and a prospectively collected cohort of 200 patients from the Plan-A study treated with CRT for SCCA in Denmark.

Perspectives:

No real-world data exists to describe the pattern of failure in SCCA and the national Danish cohort will provide important new data on these aspects. This will allow for further research into development of optimal treatment methods to lower the risk of treatment failure and side effects from primary treatment. The present studies will also lay the ground for potential re-irradiation of pelvic recurrences from this disease, and hereby patients will potentially avoid mutilating surgery.

Very little is known of the biological features of SCCA and the candidate markers have not been investigated in this disease before. The xenograft studies will provide us with important biological knowledge and allow for testing of different combinations of radiotherapy with systemic drugs. We will, for the first time, potentially be able to identify new tools for precision therapy in SCCA.

Ethical consideration:

Approval by the Danish Patient Safety Authorities and the Central Denmark Region Committees on Health Research Ethics for collecting the retrospective data have already been granted. The prospective clinical protocol on collecting fresh tissue for PDX of SCCA will be conducted according to all relevant regulative approvals and supported by the Clinical Research Unit (KFE) at Department of Oncology.

Collection of fresh tissue will be possible during the weekly staging-procedures done in collaboration with the surgeons where patients who are newly diagnosed with SCCA are examined under light anesthesia (a standard procedure).

All animal testing regulations will be respected. The laboratory has the relevant certifications for conducting animal trials.

My part in the research as a PhD-student:

I will collect the retrospective data on recurrence from medical charts and report the outcome and mapping of anatomical location of recurrence.

I will work closely together with the Department of Medical Physics in terms of TCP-modelling and comparative planning.

I will design, write and initiate the prospective clinical protocol for fresh tissue collection from newly diagnosed patients with SCCA. I will include patients to the protocol and collect the fresh tissue and bring them to fast review at Department of Pathology.

I will take part of the laboratory work and the data analysis.

Research environment and support:

Main supervisor Professor Karen-Lise Garm Spindler is chair of the Danish Anal Cancer Group, DACG, formally established in 2017. All three responsible anal cancer treatment centers in Denmark are represented in DACG facilitating good collaboration possibilities.

Co-supervisor Professor Jens Overgaard is head of Department of Experimental Clinical Oncology and has yearlong experience with translational studies especially in SSC.

The Department of Medical Physics will be engaged in TCP modelling and the comparative planning studies with photons which will be performed by an anal cancer responsible clinical physicist. The proton comparative studies will be done at the Danish Center for Particle Therapy (DCPT).

At Department of Clinical Oncology the setup for the PDX models has been established over the past three years as part of a PhD study in head and neck cancer and the methods have been successfully developed and the radiobiology laboratory has a yearlong experience with a hypoxia profile. Co-supervisor Lector Morten Busk has the necessary experience in PDX models. The clinically and scientific expertise and laboratory facilities are fully available.

Research plan:

Research plan	2020 (year one)	2021 (year two)	2022 (year three)	Writing of thesis
Study 1	Retrospective data collection (approximately 2000 patients treated for SCCA from 2000-2018)	Mapping of the recurrences (the expected number of recurrences is 2-300)	Building a tumor control probability (TCP) model of recurrence Comparative planning studies (IMRT, VMAT, Protons) on the recurrence cases	
Study 2	Writing and initiating the PDX protocol Inclusion of patients and tissue collection	Inclusion of patients and tissue collection Establishing SCCA PDX's Analysing radiation sensitivity	Establishing SCCA PDX's Analysing radiation sensitivity Validation of potential new biomarkers	
Tentative publications: study 1	I: Real-world mapping of anal cancer recurrence – the anatomical location of recurrences in the Danish Anal cancer Cohort		IIIa: The correlation of squamous cell carcinoma of the anus recurrences with radiotherapy dose and tumor control probability modelling of the recurrences IIIb: Recurrence of squamous cell carcinoma of the anus: comparative treatment planning	
Tentative publications: study 2	II: Characterization and radio-sensitivity of HPV-related squamous cell carcinoma of the anus patient-derived xenografts		IV: Validation of biomarkers of squamous cell carcinomas of the anus	

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